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María Fernanda Moreno Aguilar

Genómica y evolución de gramíneas Loliinae y ecología de especies de Festuca de los páramos norteandinos. Genomics and evolution of Loliinae grasses and ecology of Festuca species from the northern Andean paramos

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Tesis Doctoral

GENÓMICA Y EVOLUCIÓN DE GRAMÍNEAS LOLIINAE Y ECOLOGÍA DE ESPECIES DE FESTUCA DE LOS PÁRAMOS NORTEANDINOS. GENOMICS AND EVOLUTION OF LOLIINAE GRASSES AND ECOLOGY OF FESTUCA SPECIES FROM THE NORTHERN ANDEAN PARAMOS

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Departamento de Ciencias Agrarias y del Medio Natural
Escuela Politécnica Superior de Huesca
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DOCTORADO INTERNACIONAL

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Huesca, Noviembre de 2022

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Memoria presentada por

Dña. María Fernanda Moreno Aguilar

para optar al Grado de Doctora por la Universidad de Zaragoza

Directores de tesis:

Dra. Pilar Catalán Rodríguez

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Dr. Aminael Sánchez Rodríguez

La Dra. Pilar Catalán Rodríguez, Catedrática de Botánica del Departamento de Ciencias Agrarias y del Medio Natural de la Universidad de Zaragoza, la Dra. Itziar Arnelas Seco, Docente Investigadora de la Universidad Técnica Particular de Loja, y el Dr. Aminael Sánchez Rodríguez, Docente Investigador de la Universidad Técnica Particular de Loja, hacen constar que el trabajo plasmado en la presente memoria de tesis doctoral ha sido desarrollado bajo su dirección y autorizan su presentación y defensa.

Huesca, Noviembre de 2022

La presente Tesis Doctoral se ha llevado a cabo bajo la dirección de la Dra. Pilar Catalán Rodríguez, Dra. Itziar Arnelas Seco y Dr. Aminael Sánchez Rodríguez en el laboratorio del grupo de investigación Bioflora de la Escuela Politécnica Superior de Huesca (Departamento de Ciencias Agrarias y del Medio Natural), Universidad de Zaragoza. El procesamiento e identificación de especímenes colectados en los páramos andinos ecuatorianos se llevó a cabo en el Herbario de la Universidad Técnica Particular de Loja, Ecuador. Los análisis bioinformáticos de captura génica se desarrollaron mediante estancia virtual con el Dr. Juan Viruel, en Kew Gardens, Reino Unido. El resto de los estudios taxonómicos, citogenéticos, genómicos, bioinformáticos, evolutivos y ecológicos se desarrollaron en la Escuela Politécnica Superior de Huesca.

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A mis hermanos Paolo y Katty.

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RESUMEN

Festuca, uno de los géneros más importantes de gramíneas pascícolas y forrajeras de distribución mundial, y otros géneros próximos de la subtribu Loliinae, son analizados filogenómicamente por primera vez empleando datos del plastoma completo y de genes nucleares copia simple, genes ribosomales 45S y 5S y elementos del repeteoma. Las filogenias de las diversas fuentes genómicas apoyan un marco evolutivo que muestra la divergencia de dos linajes principales de Loliinae de hojas anchas (BL) y de hojas finas (FL) y 24 sub-linajes. Las variaciones de los tamaños genómicos de las Loliinae dependen de las abundancias de sus elementos repetitivos, detectándose indicios de paleo-poliploidización en linajes diploides BL y pérdidas masivas de repeteoma en algunos linajes hibridógenos. Los elevados niveles de discordancia intragenómica nuclear en Loliinae son consecuencia del barajeo incompleto de linajes (ILS) y sus altas tasas de introgresión. La hibridación y la alopoliploidía han ocurrido frecuentemente en linajes tanto ancestrales como recientes de Loliinae y han contribuido a su diversificación. Estos estudios abren el camino a la investigación de nuevos procesos de especiación utilizando genomas completos de estas plantas.

ABSTRACT

Festuca, one of the most important genera of pasture and forage grasses with a worldwide distribution, and other related genera of the Loliinae subtribe, are analyzed phylogenomically for the first time using data from the complete plastome and single copy nuclear genes, 45S and 5S ribosomal genes and elements of the repeatome. Phylogenies from all these genomic sources support an evolutionary framework showing the divergence of two main lineages of broad-leaved (BL) and fine-leaved (FL) Loliinae and 24 sub-lineages. The variations of the genome sizes of Loliinae correlate with the abundances of their repetitive elements, detecting signatures of paleo-polyploidizations in diploid BL lineages and massive losses of repeatome in some highly hybridogenic lineages. The high levels of nuclear intragenomic discordance in Loliinae are a consequence of incomplete lineage sorting and their high rates of introgression. Hybridization and allopolyploidy have frequently occurred in both ancient and recent lineages of Loliinae and have contributes to their diversifications. These studies open the way to the investigation of new speciation processes using complete genomes of these plants.

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APARTADO A.

TESIS POR COMPENDIO DE PUBLICACIONES

(THESIS BY COMPENDIUM OF PUBLICATIONS)

A. TESIS POR COMPENDIO DE PUBLICACIONES (*THESIS BY COMPENDIUM OF PUBLICATIONS*)

La presente Tesis Doctoral se presenta en la modalidad de Tesis por compendio de publicaciones. La tesis consiste de siete publicaciones, cuatro artículos, un artículo en revisión, y dos artículos en preparación.

This PhD thesis is presented in the form of Thesis by compendium of publications. The thesis consists of seven publications, four published articles, one article under review, and two articles in preparation.

Artículos publicados (*Published articles*):

- **Moreno-Aguilar MF,** Arnelas I, Sánchez-Rodríguez A, Viruel J and Catalán P (2020) Museomics Unveil the Phylogeny and Biogeography of the Neglected Juan Fernandez Archipelago *Megalachne* and *Podophorus* Endemic Grasses and Their Connection With Relict Pampean-Ventanian Fescues. Front. Plant Sci. 11:819. doi: 10.3389/fpls.2020.00819. IF: 4.402, Q1, D1.
- **Moreno-Aguilar MF,** Inda LA, Sánchez-Rodríguez A, Arnelas I and Catalán P (2022) Evolutionary Dynamics of the Repeatome Explains Contrasting Differences in Genome Sizes and Hybrid and Polyploid Origins of Grass Loliinae Lineages. Front. Plant Sci. 13:901733. doi: 10.3389/fpls.2022.901733. IF: 6.627, Q1, D1.
- **Moreno-Aguilar, MF,** Inda, L.A.; Sánchez-Rodríguez, A.; Catalán, P.; Arnelas, I. Phylogenomics and Systematics of Overlooked Mesoamerican and South American Polyploid Broad-Leaved *Festuca* Grasses Differentiate *F.* sects. *Glabricarpae* and *Ruprechtia* and *F.* subgen. *Asperifolia, Erosiflorae, Mallopetalon* and *Coironhuecu* (*subgen. nov.*). Plants 2022, 11, 2303. https://doi.org/10.3390/plants11172303. IF: 4.67, Q1.
- **Moreno-Aguilar MF**, Inda LA, Arnelas I, Catalán P. 2022. IAPT chromosome data 36/2. *Festuca andicola*, *F. caldasii*, *F. chimborazensis* subsp. *micacochensis*, *F. subulifolia*. *Taxon* 71:1132-1134. IF:2.586, Q2.

Artículo en revisión (*Article under review*):

- Arnelas I, **Moreno-Aguilar MF**, Catalán P. 2022. Second-step lectotypifications of two names of *Festuca* subgenus *Erosiflorae* (Loliinae, Pooideae, Poaceae). *Correspondence. Phytotaxa (under review).*

Artículos en preparación (*Articles in preparation*):

- **Moreno-Aguilar MF**, Viruel J, Sánchez-Rodríguez A, Probatova N, Ospina JC, Martínez-Segarra G, Devesa JA, Stewart A, Arnelas I, Catalán P. 2022. A nuclear single-copy-gene phylogeny of Loliinae (Poaceae): unraveling hybridization episodes. *(in prep.).*
- **Moreno-Aguilar MF**, Benito B, Vicioso A, Sánchez-Rodríguez A, Arnelas I, Catalán P. 2022. Climatic niche conservatism of American I and American II fescue grasses from the North-Andean páramos (*Festuca*, Poaceae) (*in prep.).*

Contribución de la doctoranda a otras publicaciones relacionadas con el tema de la tesis durante el periodo de desarrollo de su tesis (*Contribution of the doctorate student to other publications related to the topic of the thesis during the period of development of her thesis*):

- Kergunteuil A, Humair L, Maire AL, **Moreno-Aguilar MF**, Godschalx A, Catalán P, Rasmann S. 2020. Tritrophic interactions follow phylogenetic escalation and climatic adaptation. *Scientific Reports* 10: 2074.

Grass Phylogeny Working Group III (several authors, incl. **Moreno-Aguilar MF**). 2022. Grass nuclear phylogenomics (*in prep*.).

APARTADO B.

INTRODUCCIÓN GENERAL

B. INTRODUCCIÓN GENERAL

B.1. Contexto sistemático, geográfico y evolutivo de la subtribu Loliinae y del género *Festuca*

Los ecosistemas naturales terrestres más abundantes del planeta agrupan a los prados, pastos, sabanas y pajonales paramunos dominados por gramíneas (Gibson, 2010), incluyendo a especies de la subtribu Loliinae objeto de estudio en esta tesis. Las gramíneas domesticadas como *Triticum aestivum* L. (trigo), *Zea mays* L. (maíz), *Oryza sativa* L.(arroz), *Avena sativa* L.(avena) y *Sorghum vulgare* Pers. (sorgo) se han convertido en los cultivos de mayor relevancia agrícola para la humanidad, constituyendo los alimentos más consumidos por el hombre y sus ganados, mientras que *Hordeum vulgare* L. (cebada) es el cereal más utilizado para producir una de las principales bebidas alcohólicas bajo procesos de fermentación. Estos procesos de domesticación de las gramíneas cerealícolas, desarrollados en distintos momentos del Neolítico en diversos centros mundiales de origen de la agricultura (Cresciente Fértil, SE de Asia, Mesomérica), también afectaron a algunas Loliinae, especies pascícolas y forrajeras consideradas semi-domesticadas en la región Euroasiática-Mediterránea, y cuya dispersión neolítica en el Mediterraneo y Europa pudo verse facilitada al ser transportadas como malas hierbas de los cultivos o por los ganados (Catalán 2006 y referencias en este artículo). Las gramíneas son utilizadas cada vez con mayor frecuencia como materias primas para la producción de biocombustibles, incrementando su importancia a nivel global (Blair, 2014). Los ecosistemas dominados por gramíneas permiten una mejor circulación del agua, aportan nutrientes y secuestran grandes cantidades de carbono edáfico, estimándose que aproximadamente 800 millones de personas utilizan los pastos para su sustento cotidiano. Estos biomas ocupan aproximadamente el 30-40% de la superficie terrestre cubierta por plantas (Blair, 2014). Las Poáceas constituyen una parte fundamental de los ecosistemas de pastos y prados, siendo la cuarta familia más numerosa y diversa de las angiospermas, contando con ~11.500 especies y alrededor de 768 géneros (Kellogg, 2015; Soreng et al., 2015, 2017). Su importancia se basa en su relevante aportación ecológica y económica en forma de pastos y forrajes, tanto para los hervíboros silvestres como para el ganado, y de céspedes en jardines y campos deportivos, así como su contribución a la retención y la formación del suelo, y otras funciones ecosistémicas (Catalán, 2006; Hand et al., 2013; Byrne et al., 2015).

Loliinae y el resto de las gramíneas templadas se clasifican en la subfamilia Pooideae, la más amplia de las Poaceae a nivel mundial (Soreng et al., 2017, 2022). Estudios evolutivos

han evidenciado la existencia de un grupo recientemente evolucionado de pooideas denominado "core pooids" que alberga a las tribus hermanas Poeae s. l. (incluyendo Aveneae) y Triticeae-Bromeae (+ Littledaleae), que muestran altas tasas de diversificación y un incremento considerable de sus tamaños genómicos como consecuencia de la proliferación de DNA repetitivo en su genoma nuclear (Kellogg, 2015; Minaya et al., 2015, 2017). Dentro de las Poeae, Loliinae y Poinae son las dos subtribus con mayor número de taxones (Catalán, 2006; Catalán et al., 2007; Rodionov et al., 2017). Loliinae está compuesta por 14 géneros (*Ctenopsis* De Not., *Dielsiochloa* Pilg., *Hellerochloa* Rauschert, *Festuca* L., *Lolium* L., *Megalachne* Steud., *Micropyropsis* Romero Zarco & Cabezudo, *Micropyrum* (Gaudin) Link, *Narduroides* Rouy, *Podophorus* Phil., *Pseudobromus* K. Schum *Psilurus* Trin., *Vulpia* C.C. Gmel., *Wangenheimia* Moench) y ~659 especies distribuidas en los cinco continentes con excepción de la Antártida (Catalán, 2006; Soreng et al., 2017). Las especies de Loliinae y de otras subtribus próximas (Ammochloinae, Cynosurineae, Dactylidinae, Parapholiinae) presentan espiguillas con flores múltiples, glumas más cortas que la primera lema, lema redondeada en el dorso, con arista terminal o mútica, callo glabro y cariópside con hilum lineal (Catalán, 2006; Catalán et al., 2007; Soreng et al., 2015). Estudios citogenéticos de Loliinae han corroborado la existencia de un número básico de cromosomas único en la subtribu, x =7, y de un amplio rango de niveles de ploidía, que oscila desde especies diploides (2n=2x=14) hasta dodecaploides (2n=12x=84) (Catalán, 2006; Šmarda et al., 2008) con tamaños genómicos entre 4.1 Gb/2C y 23.6 Gb/2C (10x) que varían con la ploidía según los grupos taxonómicos (Loureiro et al., 2007; Šmarda et al., 2008).

Festuca es el género más amplio de las Loliinae y está compuesto por especies con inflorescencias paniculadas y espiguillas con glumas subiguales (Catalán, 2006). Sus taxones, de hojas anchas y hojas finas, se distribuyen geográficamente en ambos hemisferios, siendo un componente importante en comunidades vegetales graminoides de zonas frías y templadas, y en sistemas montañosos en climas tropicales (Catalán, 2006; Stančík and Peterson, 2007) (Figura 1). El género comprende >500 especies, con niveles de ploidía 2x, 3x, 4x, 6x, 8x, 10x y 12x presentes en diversas regiones del hemisferio norte, no habiéndose detectado especies diploides el hemisferio sur, donde se conocen taxones con ploidías 4x, 6x y 8x (Dubcovsky and Martínez, 1992; Catalán, 2006; Stancik and Peterson, 2007; Catalán and Muller, 2012; Ospina et al., 2015).

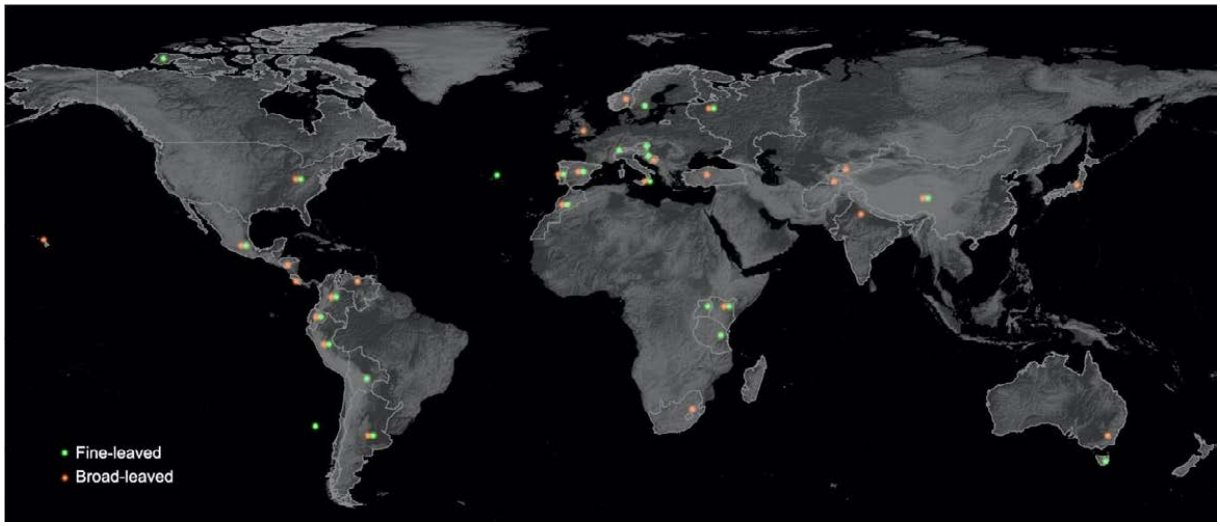

Figura 1. Distribución de especies de la subtribu Loliinae (*Festuca* y géneros próximos) a nivel global. Los puntos anarajandos representan las Loliinaes de hojas anchas y los puntos de color verde las Loliinae de hojas finas.

Los tratamientos sistemáticos propuestos para *Festuca* han variado a lo largo de los tres últimos siglos desde la descripción del género por Linneo en 1753, con la incorporación o segregación de diversos taxones (Catalán et al., 2007). Hackel clasificó a las festucas europeas en seis secciones y diversos rangos subseccionales en 1882, estableciendo el método para su estudio taxonómico basado en la anatomía foliar, la forma y dimensiones de las vainas, aurículas, espiguillas y órganos florales, la pubescencia del ápice del ovario, la inserción de los estilos, la adherencia de la cariópside a la pálea y la longitud del hilum. Este método fue ampliamente aceptado por los agrostólogos y ha sido empleado por prácticamente casi todos los autores posteriores, cuyas aportacions sucesivas han contribuido a incrementar el número de nuevos taxones de *Festuca* descritos en diversos continentes. Entre los botánicos que contribuyeron a las nuevas descripciones de especies y taxones supraespecíficos de *Festuca*, Piper (1906), St-Yves (1927), Krechetovich & Bobrov (1934), Krivotulenko (1960), Tzvelev (1971), y Alexeev (1977, 1978, 1980, 1981, 1986) dividieron el género entre varios subgéneros y secciones (Catalán, 2006, Catalán et al., 2007, y referencias de estos estudios). Los géneros *Vulpia* (Gmelin 1805), *Schedonorus* (Palisot de Beauvois 1812), *Leucopoa* (Grisebach 1852–1853), *Helleria* (*Hellerochloa*) (Fournier 1886) y *Drymochloa* (Holub 1984) han sido segregados de *Festuca* en distintos momentos. *Vulpia* ha sido reconocido como género independiente de *Festuca* por la mayoría de los autores (Cotton and Stace 1977; Stace 1981)

mientras que los otros géneros han sido sinonimizados a *Festuca* en las Floras más recientes. Sin embargo, propuestas relativamente recientes apoyan la segregación de *Leucopoa* (Holub 1984) y *Schedonorus* (Holub 1998; Soreng and Terrell 1998, 2003; Tzvelev 1999, 2000) como géneros independientes (Catalán et al., 2007, y referencias de estos estudios). Las revisiones taxonómicas de las festucas del mundo, llevadas a cabo por Alexeev entre 1977 y 1986, le permitieron reconocer hasta 11 subgéneros y diversas secciones; aportaciones posteriores de otros autores incrementaron el número de secciones de *Festuca* a 25 (Lu, 1992; Müller and Catalán, 2006).

La sistemática de las gramíneas, y las respectivas circunscripciones taxonómicas de sus subfamilias, tribus, subtribus y géneros, se han visto modificadas con el advenimiento de modernos estudios evolutivos basados en datos moleculares. Las diferentes publicaciones del Grupo de estudio filogenético de las gramíneas (*Grass Phylogeny Working Group*) han ampliado y refinado los conocimientos de sus linajes (GPWG I 2001; GPWG II 2012), corroborando la solidez de la monofilia de algunos grupos, como la subfamilia Pooideae, y el origen polifilético del metabolismo C4 en diversos linajes de gramíneas tropicales (Kellogg 2015, y referencias de este estudio). Todos los estudios filogenéticos moleculares desarrollados hasta la fecha han demostrado que *Festuca* es un género parafilético que incluye a los demás géneros de Loliinae en su topología (Inda et al., 2008; Minaya et al., 2017) y que las subtribus hermanas Dactylidinae y Cynosurinae–Parapholiinae son los parientes más próximos de Loliinae (Catalan et al. 2007; Inda et al., 2008; Minaya et al., 2017). Los análisis de tasas mutacionales en Loliinae y sus parientes cercanos mostraron una enorme variación, observándose la tendencia de los linajes perennes a tasas de evolución lenta y la de los linajes anuales a tasas aceleradas. Las diferencias significativas en las tasas de susbtitución nucleotídica de estos linajes mostraron correlación con la hipótesis del tiempo de generación (Catalán et al. 2006). No obstante, las secuencias más heterogéneas mostraban también mayores tasas de homoplasía, lo que podría causar efectos negativos de atracción de ramas largas y de saturación de substituciones, incrementando el riesgo de recobrar relaciones evolutivas potencialmente espúreas (Catalán et al. 2006, 2007). Las reconstrucciones filogenéticas de Loliinae basadas en caracteres morfológicos mostraron en general una baja resolución debido a la alta homoplasía y plasticidad de estos caracteres fenotípicos (Catalán et al. 2007); pese a ello, algunas divergencias dentro de los principales linajes de Loliinae mostraron congruencia con los obtenidos con los marcadores moleculares, apoyando la aplicación del principio de evidencia total para la evaluación del origen de ciertos caracteres morfotípicos en Loliinae.

Los resultados de los estudios filogenéticos de Loliinae de Catalán et al. (2004) y Catalán et al. (2007) permitieron proponer cuatro escenarios evolutivos alternativos para la clasificación de las Loliinae: 1- *Festuca sensu latissimo*. Este escenario estaba basado en un criterio monofilético y en un esquema taxonómico sintético según el cual todas las Loliinae serían sinonimizadas a *Festuca*. Este criterio estaba apoyado en la monofilia de Loliinae y algunos precedentes históricos (por ejemplo, la clasificación de *Vulpia* dentro de *Festuca* de Hackel (1906) y Piper 1906 (1906), pero conllevaba algunos inconvenientes como aceptar la complejidad de un género tan amplio, difícil de diagnosticar mediante un conjunto de caracteres morfológicos. 2- *Festuca sensu lato*. Este escenario se fundamentaba en un criterio sistemático evolutivo que era nomenclaturalmente conservador. Se mantenía la circunscripción tradicional de *Festuca*, reconociendo sus subgéneros, así como la del resto de los géneros separados de él (existiendo incertidumbre en cuanto a la polifilia de *Vulpia*). Este escenario presentaba la ventaja de preservar la estabilidad nomenclatural de *Festuca* hasta completar el estudio filogenético de la mayoría de sus taxones, y la desventaja de reconocer a un elevado número de géneros segregados de *Festuca* evolutivamente emplazados dentro de este taxon ampliamente parafilético. 3- *Festuca sensu stricto*. Este escenario se basaba en el empleo de un criterio monofilético para una clasificación menos conservadora, según la cual el género *Festuca* quedaría restringido a las Loliinae de hojas finas, tratando a los linajes de Loliinae de hojas anchas como géneros independientes. Esta aproximación sistemática se basada en el relativamente alto apoyo del clado de Loliinae de hojas finas, tanto por marcadores moleculares como por algunos caracteres morfológicos (como hojas plegadas o setáceas, sin bloques de esclerénquima abaxiales y con bloques adaxiales predominantemente ausentes o incompletos, y lema frecuentemente aristada). Sin embargo, surgían muchas dificultades para circunscribir varios linajes y para clasificar a los linajes de Loliinae hojas anchas y a los filogenéticamente intermedios entre ambos. La mayoría de las controversias actuales sobre la clasificación de Festuca y sus segregados afectan a este escenario. El reconocimiento de *Schedonorus*, *Leucopoa* y *Drymochloa* como géneros independientes (tal como han propuesto algunos autores anteriores como Palisot de Beauvois 1812; Grisebach 1852–1853; Holub1984, 1998; Soreng & Terrell 1998, 2003, Tzvelev 1999, 2000) implicaría aceptar su no-monofilia, de acuerdo con las filogenias basadas en marcadores moleculares. La búsqueda de una clasificación cladística para el linaje *Schedonorus-Lolium* llevó a algunos autores (Darbyshire, 1993) a sinonimizar a todas las especies de *Festuca* subgen. *Schedonorus* a *Lolium*, que tiene prioridad nomenclatural. Sin embargo, las conspicuas diferencias morfológicas que separan a ambos géneros llevaron a otros autores a clasificar a *Schedonorus* como un género parafilético distinto de *Lolium* (Soreng &

Terrell 1998, 2003). 4- *Festuca sensu strictissimo*. Este escenario se fundamentaba en un criterio sistemático evolutivo o cladístico para una clasificación aún menos conservadora, según la cual el género *Festuca* se restringiría únicamente a los miembros de *Festuca* sect. *Festuca*, reconociendo a otros linajes de *Festuca* y Loliinae de hojas finas y hojas anchas como géneros distintos. Según esta interpretación restringida, *Festuca* sería un género monofilético y muy bien caracterizado morfológicamente; sin embargo, este escenario implicaría el reconocimiento de multiples linajes, actualmente clasificados como *Festuca*, como géneros independientes. Esto también requeriría la necesidad de múltiples combinaciones nomenclaturales nuevas. De estas posibles opciones, (Catalán et al., 2007) propusieron un tratamiento taxonómico para las Loliinae basado en un criterio de clasificación sistemático evolutivo, nomenclaturalmente conservador, que mantiene al género *Festuca* con sus subgéneros y secciones y al resto de los géneros de Loliinae tradicionalmente reconocidos (escenario 2- *Festuca sensu lato*). Tras los avances filogenéticos habidos desde entonces, este escenario y los criterios que lo sustentan sigue siendo el más convincente para la clasificación taxonómica de las Loliinae (Ospina et al. 2016; Minaya et al. 2017). Pese a ello, otras propuestas taxonómicas recientes, basadas en un criterio cladístico filogenético proponen circunscribir *Festuca* a *F*. subgen. *Festuca* y los géneros de Loliinae emplazados evolutivamente en este linaje (Loliinae de hojas finas), y separar al resto de los subgéneros divergentes de *Festuca* (y sus respectivos géneros próximos de Loliinae) (Loliinae de hojas anchas) como géneros independientes (e. g., POWO; https://powo.science.kew.org). Sin embargo, este último tratamiento desprovee de caracteres diagnósticos tanto a *Festuca* como al resto de sus géneros afines (Catalán et al., 2007).

Las especies de *Festuca* muestran una elevada tendencia a la hibridación y a la poliploidización (Catalán, 2006; Loureiro et al., 2007; Šmarda et al., 2008; Ezquerro-López et al., 2017). Si bien la mayor parte de las hibridaciones han ocurrido entre especies evolutivamente próximas, habiéndose detectado eventos de especiación por hibridación homoploide entre especies hermanas (Marques et al., 2016), ciertos linajes se han hibridado con géneros próximos originando diversos híbridos intergenéricos (e. g., *Festuca x Vulpia* = *Festulpia*, *Festuca x Lolium* = *Festulolium*) (Catalán, 2006; Catalán et al., 2007; Kopecký et al., 2010; Kopecký and Studer, 2014). Se estima que un elevado porcentaje de las especies de *Festuca* (>60%) son probablemente alopoliploides (Dubcovsky and Martínez, 1992; Catalán, 2006; Ospina et al., 2015); sin embargo se desconoce la magnitud del fenómeno al no disponer todavía de datos cromosómicos ni de tamaños genómicos para un elevado número de sus taxones.

Los estudios filogenéticos moleculares de Loliinae llevados a cabo hasta la fecha han utilizado secuencias de DNA de diversos loci nucleares y plastídicos y un incremento gradual del muestreo taxonómico y geográfico de sus especies. Las primeras reconstrucciones filogenéticas de la subtribu emplearon marcadores *barcoding* nuclear (ITS) y plastídicos (*trn*TL, *trn*LF) y una representación de taxones fundamentalmente Mediterráneo-Europeos (Torrecilla and Catalan, 2002; Torrecilla et al., 2003; Catalán et al., 2004). Estos análisis se ampliaron posteriormente a especies de otros continentes (America, Africa, Asia, Australia-Nueva Zelanda) (Inda et al., 2008; Minaya et al., 2017) y al empleo de genes nucleares copia simple clonados (*beta-amilasa, GBSS*) y otros genes plastídicos (*mat*K) que permitieron la detección de alelos homeólogos en especies alopoliploides de Loliinae y la identificación de algunos de sus genomas progenitores diploides (Díaz-Pérez et al., 2014; Minaya et al., 2015). Los genes nucleares copia simple también facilitaron la detección de duplicaciones genómicas, recombinaciones, selección no-purificadora y polifilia en genes y linajes (Díaz-Pérez et al., 2014; Minaya et al., 2015), y la distribución y dinámica evolutiva de ciertos elementos transponibles (MITEs) en sus intrones (Minaya et al., 2013). La congruencia obtenida en los diversos estudios filogenéticos de las Loliinae ha permitido establecer un marco evolutivo general para estas gramíneas. Todos los análisis han demostrado la monofilia fuertemente sustentada de Loliinae y la divergencia de sus dos linajes principales, las Loliinae de hojas-anchas (*broad-leaved*, BL) y de hojas-finas (*fine-leaved*, FL). En algunas topologías se distingue un tercer clado de Loliinae de hojas intermedias que incluye especies con características morfológicas similares a las Loliinae de hojas anchas pero filogenéticamente más cercanas a las Loliinae de hojas finas (Torrecilla and Catalan, 2002; Catalán, 2006; Minaya et al., 2017).

Las especies del clado BL Loliinae son por lo general plantas altas, predominantemente perennes, fuertemente rizomatosas, con hojas anchas, planas y convolutas, y panículas grandes (Catalán, 2006); muestran tasas de mutación relajada y crecen en hábitats estabilizados, como bosques mésicos o en pastos (Catalán, Pilar; Torrecilla, Pedro; Lopez, Jose Angel;Muller, 2006). Por el contrario, el clado FL Loliinae lo forman especies en general de pequeño tamaño, perennes o anuales, con presencia o ausencia de rizomas delgados, con hojas estrechas y plegadas, y panículas cortas o pequeñas (Catalán, 2006); presentan tasas de mutación aceleradas y viven en pastos mésicos, xéricos y alpinos o en hábitats alterados (Catalán et al., 2006). El clado BL incluye a representantes de *Festuca* subgen. *Drymanthele* (sects. *Phaeochloa, Muticae, Banksia*), *Leucopoa* (sects. *Leucopoa, Amphigenes,*

Breviaristatae, Obtusae), *Schedonorus* (sects. *Schedonorus, Plantynia*) y *Xanthochloa, F*. sects. *Lojaconoa*, *Pseudoscariosa, Scariosae* y *Subbulbosae,* linajes Tropical Africa y South Africa, y los géneros *Lolium, Micropyropsis* y *Pseudobromus*, y el clado FL (+ intermediate) a representantes de *Festuca* subgen. *Festuca* (sects. *Eskia, Dimorpha, Festuca* (subsect. *Festuca, Exaratae*) y *Aulaxyper*), *Subulatae* (sects. *Subulatae, Longiglumis*), y *Subuliflorae*, linajes American I, American II (incluido *F*. subgen. *Mallopetalon*), American-Vulpia-Pampas, y American-Neozeylandic, y los géneros *Ctenopsis*, *Dielsiochloa, Hellerochloa, Micropyrum, Narduroides, Psilurus, Vulpia* y *Wangenheimia* (Inda et al., 2008; Minaya et al., 2017). Dentro de estos dos clados principales se emplazan 20 linajes de Loliinae, de los que 8 corresponden a linajes BL y 12 a linajes FL (Minaya et al., 2017).

Las dataciones mediante reloj molecular relajado de los tiempos de divergencia de los linajes de las Loliinae estimaron que el ancestro de la subtribu se originó ~22.5 Mya, entre el Oligoceno tardío y el Mioceno temprano, mientras que los ancestros de los clados BL y FL divergieron, respectivamente, hace 18.9 Mya y 17.5 Mya, en el Mioceno temprano, y los de los linajes más recientes de ambos grupos entre el Mioceno tardío y el Plioceno (Minaya et al., 2017). Los análisis biogeográficos de las Loliinae basados en modelos bayesianos de dispersión-extinción-cladogénesis (DEC) infirieron un origen de los ancestros de los linajes de hojas anchas y hojas finas en el hemisferio norte, en la región Mediterránea-SW Asia, y un alto número de dispersiones recurrentes a larga distancia de linajes BL y FL hacia otros continentes y la colonización del hemisferio sur (Minaya et al., 2017). Estos modelos de dispersión norte-sur han sido también deducidos en linajes particulares de Loliinae (e. g., Schedonorus) (Inda et al., 2014); aunque algunos patrones de colonización inferidos para otras Loliinae contradicen las dispersiones preferentes propuestas para varias angiospermas (por ejemplo, el origen de las festucas afroalpinas a partir de ancestros suramericanos, por dispersión transatlántica, en lugar de ancestros euroasiáticos) (Minaya et al., 2017). La región Mediterráneo-Eurasiática constituye hoy en día el área con mayor diversificación de Loliinae y mayor número de especies diploides, siendo Suramérica la segunda región en diversidad de Loliinae conteniendo únicamente especies poliploides (Dubcovsky and Martínez, 1992; Catalán, 2006; Šmarda et al., 2008). Estudios filogeográficos han constatado la elevada capacidad de dispersión a larga distancia de poblaciones de Loliinae, en procesos de colonización glacial/postglacial de islas de montaña (*sky islands*) (Mairal et al., 2021) y de islas oceánicas, seguidas en este último caso por mecanismos de especiación *in-situ* (Díaz-Pérez et al., 2008, 2012).

B.2. Análisis evolutivos de angiospermas basados en datos genómicos

Durante las últimas décadas, el resurgimiento e incremento de tecnologías y métodos de secuenciación de alto rendimiento se han convertido en herramientas útiles que han permitido obtener datos genómicos de angiospermas a gran escala (Besnard et al., 2013; Dodsworth, 2015). El análisis apropiado de los datos de secuenciación de genomas completos (o casi completos) han ayudado a resolver ciertos problemas filogenéticos hasta el momento intratables (Gao et al., 2010), a inferir eventos de reticulación y poliploidización y descifrar los orígenes de los genomas progenitores conocidos y desconocidos de los poliploides (Sancho et al., 2022), a corroborar si la abundancia regional de especies está determinada en gran medida por factores históricos relacionados con procesos biogeográficos y macroevolutivos (Pillar and Duarte, 2010; Besnard et al., 2014), y a interpretar la estructura de las comunidades vegetales en diferentes escalas espaciales y temporales (Besnard et al., 2013), entre otros.

Otra de las ventajas asociada a la secuenciación de alto rendimiento, es la posibilidad de obtener información filogenética a partir de muestras de herbario (museómica) (Besnard et al., 2014). Las colecciones depositadas en los herbarios han cobrado recientemente un rol importante para profundizar en el conocimiento no sólo taxonómico sino también evolutivo de las plantas, pues a partir de pequeñas cantidades de materiales de sus especímenes se han podido obtener valiosos datos que han revelado nuevas relaciones evolutivas de especies tanto existentes como extintas (Sebastian et al., 2010; Silva et al., 2016; Malakasi et al., 2019), y de la dinámica de poblaciones anterior a la intervención antrópica y al cambio climático global (Wandeler et al., 2007; Zedane et al., 2015). Además, las nuevas metodologías de secuenciación genómica permiten expandir la investigación filogenética a colecciones depositadas en diversos herbarios, logrando con éxito el procesamiento de DNA de especímenes antiguos que solo se conocen de colecciones históricas o resultan difíciles de colectar en el campo, o para dilucidar las asociaciones co-evolutivas entre plantas y plantas-simbiontes a través del tiempo (Besnard et al., 2014; Baker et al., 2021; Thomas et al., 2021).

Una de las tecnologías de secuenciación genómica de nueva generación de baja cobertura es *genome skimming* (Straub et al., 2012), que permite obtener información genómica a partir de una pequeña cantidad de DNA total (Dodsworth, 2015). Los datos genómicos obtenidos de esta secuenciación (secuencias -*paired-end reads*- de ~100 bp) son procesados mediante diversas tuberías informáticas, y utilizados para el ensamblaje y la anotación de genomas organulares (plastomas) y familias de genes ribosomales nucleares de plantas (gen

45S rDNA genes (y su cistrón 35S), y genes 5S) (Garcia et al., 2020; Nevill et al., 2020), elementos de DNA repetitivo nuclear (Novák et al., 2020), y algunos genes nucleares copia simple, que deben ser filtrados, seleccionados y ensamblados empleando datos de ortología génica (Besnard et al., 2014; Crowl et al., 2017).

El DNA repetitivo (o repeteoma) contribuye con un elevado porcentaje al tamaño del genoma nuclear en la mayoría de eucariotas y puede llegar a constituir hasta el ~80 % del mismo en algunas plantas (Macas et al., 2015; Weiss-Schneeweiss et al., 2015; Pellicer et al., 2018; Vitales et al., 2020), lo que convierte a las angiospermas en un grupo idóneo para estudiar la evolución de sus repeteomas y de su impacto en los procesos de especiación (Dodsworth et al., 2015; Pellicer et al., 2018). El repeteoma se compone de elementos transponibles dispersos, tales como retrotransposones, transposones de DNA y repeticiones en tándem, formando muy diversas familias que muestran una elevada tasa de mutación (Macas et al., 2015). El repeteoma consiste en diferentes tipos de DNA, clasificados en función de su origen, función, estructura y distribución genómica. Dentro de estos elementos se pueden identificar dos grandes familias; la primera está formada por las repeticiones en tándem (agrupadas) que se divide a su vez en tres subfamilias: DNA satélite (satélites, minisatélites y microsatélite), genes parálogos en tándem, y genes codificadores de RNAs ribosomales (rRNA). La segunda está compuesta por repeticiones dispersas (a lo largo de todo el genoma), y también se dividide en tres subfamilias: parálogos y familias de genes, genes que codifican RNAs de transferencia (tRNA), y elementos transponibles (*transposable elements*, TEs), tales como transposones, retrotransposones y retrovirus, LINEs y SINEs (Sperling and Li, 2013). Las secuencias de DNA nuclear ribosomal (rDNA) constituyen una de las mayores repeticiones en tándem en los genomas de los eucariotas y están compuestas por secuencias evolutivamente conservadas que codifican RNA ribosomal y regiones espaciadoras intergénicas (IGS) de rápida evolución. El locus 5S rDNA se localiza en las regiones peri-centroméricas y está compuesto por una región génica de 120 pb de longitud y espaciador de 100-1000 bp. Estos loci pueden mostrar menos sensibilidad a la homogeneización en algunos alopoliploides, manteniendo la capacidad diagnóstica con respecto a sus especies progenitoras. Los loci 45S rDNA (genes que codifican 18S, 5.8S y 25S rRNA y las regiones espaciadoras (ITS1-ITS2-IGS-ETS)) se localizan en las regiones organizadoras nucleolares (Perumal et al., 2017; Volkov et al., 2017; García et al. 2008, 2020). Los loci 5S – 45S proporcionan los rRNA necesarios para el ensamblaje de los ribosomas funcionales, que representan más del 90 % del RNA celular total (Volkov et al., 2017). Las dos familias de retrotransposones más abundantes en los genomas nucleares de angiospermas son

los elementos Ty3/gypsy y Ty1/copia, si bien distintos linajes presentan diversas composiciones de otros elementos repetitivos (Macas et al., 2011). La innovación experimentada por las tecnologías de nueva generación (NGS) han permitido desarrollar análisis del DNA repetitivo que hasta hace poco eran difíciles de llevar a cabo debido a la abundancia y la complejidad de las secuencias del repeteoma de las plantas (Macas et al., 2011). La secuenciación genómica de baja cobertura *genome skimming* (cobertura 0.1x a 5x), constituye una metodología óptima para la identificación y la caracterización del repeteoma de plantas. El desarrollo de herramientas bioinformáticas para el ensamblaje *de novo* de elementos repetitivos por agrupaciones de secuencias (reads) mediante análisis de topología de gráficos, como Repeat Explorer2 (Novák et al., 2020), ha demostrado que los agrupamientos basados en la similitud de las secuencias son proporcionales a la abundancia genómica y la longitud de dichos elementos repetitivos y puede ser empleados para anotarlos y cuantificarlos (Macas et al., 2011; Pellicer et al., 2018). Repeat Explorer lleva a cabo una comparación por pares entre todas las secuencias y agrupa las que comparten mayores similitudes en grupos o "clusters". Las secuencias repetitivas presentes varias veces en el genoma que producen una cantidad suficiente de similitudes constituyen los grupos o familias principales de los elementos repetitivos (Macas et al., 2015). Estudios basados en el análisis de datos *genome skimming* mediante estos procedimientos permiten la identificación y caracterización del repeteoma en las especies, así como recuperar las relaciones filogenéticas entre taxones empleando métodos de construcción de redes basados en árboles de distancias o en árboles máximo verosímiles según las abundancias de las familias de elementos repetitivos que contengan (Dodsworth et al., 2015; Vitales et al., 2020).

Otro método de secuenciación de alto rendimiento que permite la obtención de secuencias diana y el ensamblaje de genes copia simple o baja copia nucleares, difíciles de conseguir a través de secuenciación no-dirigida (*genome skimming*) en especies con genomas plagados de elementos repetitivos, es la captura génica (*gene capture*) (Johnson et al., 2019; Züst et al., 2020). Este método emplea sondas específicas (*baits*) de genes copia-simple de referencia, y la secuenciación de estas librerías enriquecidas ha demostrado su eficiencia en la recuperación de cientos o miles de genes de copia simple en diversos grupos de plantas (Soto Gomez et al., 2019; Züst et al., 2020) con un alto contenido de señal filogenética (De Smet et al., 2013; Soto Gomez et al., 2019; Maurin et al., 2021). El método ha mostrado una elevada eficiencia en la obtención de ortólogos suficientemente conservados para poder ser identificados sin ambigüedad y suficientemente variables para clarificar las relaciones de parentesco entre linajes. Recientemente, el desarrollo de un kit universal para angiospermas que

permite la captura de 353 genes nucleares copia simple en todas las plantas con flores (Angisoperm353) ha demostrado su utilidad en la resolución filogenética desde superclase a población (Baker et al., 2021). Esta fuente de datos proporciona información complementaria a la de la variación genética neutra y permite la exploración de la potencial discordancia intragenómica nuclear (Züst et al., 2020), el impacto de la hibridación en la filogenia (Pérez-Escobar et al., 2021), y la detección de variaciones selectivamente importantes en la adaptación de las poblaciones al medio (Primmer, 2009).

El grupo de Loliinae que cuenta con mayores recursos genómicos lo forman especies de *Festuca* subgen. *Schedonorus* y *Lolium*, dado su alto valor económico al constituir los principales tipos de gramíneas forrajeras (*Festuca pratensis*, *F. arundinacea*, *x Festulolium* spp) y de céspedes (*Lolium perenne*, *L. multiflorum*) comercializadas a nivel mundial (Catalán, 2006; Kopecký and Studer, 2014). El género *Lolium* comprende un número reducido de especies fundamentalmente diploides, mientras que *Festuca* subgen. *Schedonorus* incluye a especies tanto diploides (*F. pratensis*) como alopoliploides (*F. fenax* 4x, *F. arundinacea* 6x) (Cheng et al., 2016). Se han desarrollado mapas genómicos y atlas transcriptómicos para estas especies (Czaban et al., 2015; Teshome et al., 2019) y borradores genómicos sinténicos de *L. perenne* (Byrne et al., 2015; Copetti et al., 2021) y *F. pratensis* (Samy et al., 2020). Estas plantas también disponen de plastomas anotados (Hand et al., 2013) y diversos marcadores citogenéticos de regiones repetitivas del genoma nuclear (Kopecký et al., 2010; Křivánková et al., 2017), así como de un estudio piloto del repeteoma de ocho de sus taxones (Zwyrtková et al., 2020). Especies de *Festuca* de otros importantes grupos pascícolas y formadores de céspedes, como *F.* sect. *Festuca* (grupo ovina) y *F.* sect. *Aulaxyper* (grupo rubra) cuentan igualmente con atlas transcriptómicos (Wang et al., 2019; Qiu et al., 2021b, 2021a). Pese a estos avances, no se ha obtenido todavía ningún genoma nuclear completo de Loliinae, dada la dificultad de ensamblar y anotar estos genomas de gran tamaño y con elevadas proporciones de DNA repetitivo, y existe una enorme carencia de datos sobre la composición y la variabilidad genómicas de la mayoría de las especies de la subtribu. Este hecho, unido a la baja resolución filogenética obtenida para algunos linajes de Loliinae con la actual información proporcionada por los escasos loci nucleares y plastídicos estudiados hasta la fecha, nos ha llevado a abordar en esta tesis un amplio análisis genómico de todos los linajes conocidos de las Loliinae y a una ampliación del tamaño muestral, incluyendo nuevas especies no estudiadas molecularmente con anterioridad. Empleando secuenciaciones basadas en *genome skimming* y captura génica, nuestro objetivo ha sido obtener bases de datos sólidas del plastoma, de los genes ribosomales nucleares, del

repeteoma y de genes nucleares copia simple que nos permitan explorar en mayor profundidad las relaciones evolutivas en una gran representación de taxones de las Loliinae y de inferir los procesos que han intervenido en su diversificación y especiación.

B.3. Festucas meso-suramericanas y norteandinas, sistemática evolutiva, adaptación ecológica y conservatismo de nicho

Mesoamérica y Suramérica constituyen el segundo gran centro de diversificación de Loliinae, contando con >100 especies, la mayor parte de las cuales pertenecen al género *Festuca* (Tovar, 1972; Catalán, 2006; Stancík and Peterson, 2007; Catalán and Muller, 2012; Ospina et al., 2015). Las *Festuca* suramericanas se distribuyen preferentemente a lo largo de la cordillera andina, y en zonas llanas y esteparias de Patagonia y otras regiones australes donde la gran variedad de hábitats a escala latitudinal y altitudinal (Salariato et al., 2022) ha propiciado su diversificación (Catalán, 2006; Minaya et al., 2017). Pese a que especies de tres subgéneros (*F.* subgen. *Asperifolia, Erosiflorae, Mallopetalon*) y dos secciones (*F.* sects. *Glabricarpae, Ruprechtia*) de *Festuca* se distribuyen únicamente en esta zona, las especies mesoamericanas y suramericanas han sido muy poco estudiadas hasta la fecha, tanto taxonómica como filogenéticamente. Por ello otras de las metas planteadas en esta tesis doctoral han consistido en analizar genómica y taxonómicamente una amplia representación de estos grupos, y de otros géneros suramericanos de Poeae de dudosa adscripción taxonómica (*Megalachne, Podophorus*) para cubrir el vacío de conocimiento sobre su biología, sistemática y evolución.

Dentro de la región Andina, la zona de páramos norte-andina (Ecuador, Colombia, norte de Perú, Venezuela) alberga una de las mayores tasas de endemicidad e hiper-diversificación biológica, asociadas a su historia biogeográfica y a los numerosos microecosistemas que se suceden en un amplio rango altitudinal de 2800 - 5000 msnm (Sklenar and Ramsay, 2001; Antonelli and Sanmartín, 2011; Pérez-Escobar et al., 2022; Salariato et al., 2022). La existencia de una variada orografía y de diferentes pisos altitudinales en las regiones de las montañas norte-andinas, han incrementado sus tasas de biodiversidad y endemicidad (Karami et al., 2020). Entre las diversas gramíneas pooideas que componen los pajonales de páramos, uno de los géneros que incluye a las especies más representativas es *Festuca* (Sklenár and Ramsay, 2001). En los páramos de los Andes septentrionales se han reconocido 53 especies de *Festuca* (Stancik and Peterson, 2007) que muestran adaptaciones a diversas condiciones climáticas y ecológicas, y una distribución actual en distintos biomas y pisos de vegetación

paramunos (subpáramo, páramo, superpáramo) (Sklenar and Ramsay, 2001; Stancik and Peterson, 2007). Por ello, estas especies pueden ser consideradas modelos idóneos para analizar sus características de nicho ambiental y probar la hipótesis de conservatismo filogenético de nicho (Warren et al., 2008; Mccormack et al., 2009; McCormack et al., 2010; Smith and Donoghue, 2010).

La modelización de nicho ecológico realizado, que combina información de datos de ocurrencia puntual de cada especie y variables ambientales-climáticas referenciadas en un periodo de tiempo establecido (e. g., tiempo actual, Último máximo glacial, LGM), constituye una herramienta muy útil para analizar factores evolutivos y adaptativos que hayan podido determinar la distribución geográfica actual de las especies y sus preferencias de nicho (McCormack et al., 2010). La teoría de conservatismo de nicho propuesta por Warren *et al.* (2008) incluye pruebas de dos hipótesis: la *similitud* de nicho, que implica que los nichos de las dos especies comparadas son más similares entre sí de lo esperado aleatoriamente, y la *equivalencia* de nicho, que constata si los nichos de esas especies son indistinguibles o no. Las teorías sobre conservatismo filogenético de nicho predicen que especies próximamente emparentadas comparten un nicho fundamental similar que ha sido retenido a lo largo del tiempo (Peterson et al., 1999; Wiens, 2004; Pyron et al., 2015). Por el contrario, las teorías sobre divergencia de nicho postulan que las adaptaciones a nuevos ambientes mediante cambios en el nicho ancestral promueven la diversificación de las especies (Levin, 2003; Givnish, 2010; Liu et al., 2020). Aunque el conservatismo de nicho ha sido considerado el modelo prevalente en largas escalas evolutivas para especies estabilizadas (Wiens and Donoghue, 2004; Pyron et al., 2023), la divergencia de nicho ha sido propuesta para especies diversificadas con tasas de evolución aceleradas que muestran especiación ecológica (Donoghue and Edwards, 2014; Hu et al., 2015). El conocimiento profundo de las relaciones de parentesco entre especies hermanas o próximas de linajes de *Festuca* que habitan en los páramos norte-andinos, tras el análisis filogenético de los nuevos datos genómicos, nos permitirá enfocar y abordar el estudio de sus tendencias de evolución de nicho. Por ello, otro de los objetivos de esta tesis doctoral se propone utilizar grupos recientemente evolucionados de *Festuca* norteandinas como modelos ideales para probar hipótesis sobre conservatismo y divergencia de nicho (McCormack et al., 2010), y elucidar la potencial señal filogenética de los caracteres (variables) de nicho y sus atributos (Grossman, 2021).

B.4. Objetivos

El objetivo general de esta tesis es incrementar el conocimiento sobre la evolución, la sistemática y la ecología de un elevado número de taxones representativos de la subtribu Loliinae de las gramíneas templadas, en especial de grupo geográficos apenas investigados, para inferir sus orígenes y dilucidar sus procesos de especiación, incluidos eventos frecuentes de hibridación y poliploidización, y probar hipótesis sobre la potencial especiación ecológica, empleando nuevas aproximaciones de secuenciación genómica y de análisis filogenómicos y citogenéticos, junto a revisiones taxonómicas, estudios biogeográficos y de pruebas de conservatismo de nicho ecológico.

Para lograr este objetivo general, la Tesis plantea los siguientes **objetivos específicos**:

1. Desarrollar estudios taxonómicos, citogenéticos, evolutivos, -mediante nuevos datos de secuenciación genómica-, y biogeográficos de linajes de hojas anchas y hojas finas de Loliinae, en especial de taxones no investigados de Suramérica.
2. Analizar el origen y la evolución de los altamente variables tamaños genómicos de los linajes de Loliinae mediante estudios filogenéticos comparados de plastomas, de familias de DNA ribosomales, y de elementos repetitivos nucleares y su dinamismo.
3. Analizar el impacto de la hibridación y la alopoliploidización en la evolución de las Loliinae empleando una amplia representación de sus linajes y pruebas de discordancia intragenómica nuclear y de incongruencia filogenética de genes nucleares copia simple y genes plastídicos.
4. Desarrollar análisis de modelización de nicho ecológico y pruebas de conservatismo *vs* divergencia de nicho en especies de *Festuca* endémicas de los páramos norteandinos.

Objectives

The general objective of this thesis is to increase the knowledge about the evolution, systematics and ecology of a large number of representative taxa of the Loliinae subtribe of temperate grasses, especially of scarcely investigated geographic groups, to infer their origins and elucidate their processes of speciation, including frequent hybridization and polyploidization events, and to test hypotheses about potential ecological speciation, using new approaches of genomic sequencing and phylogenomic and cytogenetic analyses, together with taxonomic revisions, biogeographical studies and ecological niche conservatism tests.

To achieve this general objective, the Thesis proposes the following **specific objectives**:

1. Develop taxonomic, cytogenetic, evolutionary, –using new genomic sequencing data-, and biogeographical studies of broad-leaved and fine-leaved lineages of Loliinae, especially uninvestigated taxa from South America.
2. To analyze the origin and evolution of the highly variable genome sizes of the Loliinae lineages through comparative phylogenetic studies of plastomes, ribosomal DNA families, and nuclear repetitive elements and their dynamism.
3. Analyze the impact of hybridization and allopolyploidization in the evolution of the Loliinae using a broad representation of lineages and nuclear intragenomic discordances and topological phylogenetic incongruence tests of nuclear single-copy genes and plastid genes.
4. Develop ecological niche modeling analyses and test for conservatism vs niche divergence in *Festuca* species endemic to the Northern Andean páramos.

B.5. Trabajos presentados y organización en la Tesis

Para llevar a cabo esta investigación se realizaron salidas de campo en las que se colectaron nuevas muestras vivas de especies de *Festuca* y Loliinae de Ecuador y otros países de Suramérica, así como de otras regiones del planeta, y se trasplantaron al jardín experimental de la Escuela Politécnica Superior de Huesca para su estudio, se seleccionaron y analizaron muestras secas de tejidos foliares conservados en gel de sílice, provenientes de colectas llevadas a cabo previamente por el equipo de investigación Bioflora de la Universidad de Zaragoza en diversos continentes y el de la UTPL en Ecuador, y se estudiaron muestras de especímenes de herbario procedentes de préstamos de los Herbarios AAU, HUTPL, K, OSC, MO, CONC, US, UZ y VBGI. Los trabajos de biología molecular y los análisis bioinformáticos, genómicos y evolutivos, así como la identificación taxonómica de los especímenes colectados en los Andes ecuatorianos, que fueron posteriormente procesados para su estudio genómico, se llevaron a cabo en el laboratorio del grupo de investigación Bioflora.

Para cumplir con las metas establecidas en el objetivo general y en los objetivos específicos de la tesis, esta investigación se estructura en los siguientes seis capítulos, que contienen siete publicaciones (cuatro artículos científicos publicados, uno en revisión, y dos en preparación).

Capítulo 1. *Museomics unveil the phylogeny and biogeography of the neglected Juan Fernandez archipelago Megalachne and Podophorus endemic grasses and their connection with relict Pampean-Ventanian fescues (published, Frontiers in Plant Sciences 2020).*

El objetivo de este estudio consistió en determinar si los dos únicos géneros de gramíneas endémicos del archipiélago chileno de Juan Fernández, *Megalachne* y *Podophorus*, de incierta adscripción taxonómica (clasificados tanto en Bromeae, como en Duthieinae, Aveneae/Poeae, o Loliinae), formaban parte o no de la subtribu Loliinae, y en caso de pertenencia, dilucidar sus orígenes y linajes próximos, datar sus divergencias y reconstruir sus patrones biogeográficos y los procesos de colonización y especiación en las islas del archipiélago. Este estudio estaba relacionado con el objetivo específico 1 de la tesis. Para ello se emplearon aproximaciones museómicas, empleando materiales de herbario de las dos especies principales de *Megalachne*, *M. berteroniana,* localizada en la isla Masatierra o Robinson Crusoe, y *M. masafuerana*, localizada en la isla Masafuera o Alejandro Selkirk, y

del espécimen tipo de *Podophorus bromoides*, una especie considerada extinta desde el siglo XX, aparentemente presente únicamente en la isla Masatierra antes de su extinción, y de 33 especies representantes de los principales linajes BL y FL de Loliinae, obteniendo sus datos genómicos mediante *genome skimming*. Se construyeron filogenias máximo verosímiles plastómicas y del gen nuclear ribosomal 35S para establecer su emplazamiento filogenético y posible incongruencia topológica entre uno y otro árbol. El filtrado de los loci nuclear ITS y plastídico trnTLF a partir de estos datos, junto con los de una amplia representación de otras especies de Loliinae, permitió llevar a cabo dataciones filogenéticas para inferir sus edades y análisis biogeográficos de áreas ancestrales para estimar la procedencia de sus ancestros continentales y la secuencia de colonizaciones y especiaciones que originaron estos taxones en estas islas oceánicas.

Capítulo 2. *Evolutionary dynamics of the repeatome explains contrasting differences in genome sizes and hybrid and 26olyploidy origins of grass Loliinae lineages (published, Frontiers in Plant Sciences 2022).*

La finalidad de este estudio fue probar la hipótesis de si las sorprendentes variaciones observadas en el mayor tamaño genómico de las BL Loliinae con respecto a las FL Loliinae y en la acusada reducción de tamaños genómicos en ciertas especies con elevado nivel de ploidía eran debidas a diferentes composiciones y abundancias de elementos repetitivos de sus genomas nucleares y a las potenciales dinámicas evolutivas de estos elementos. Este estudio estaba relacionado con el objetivo específico 2 de la tesis. Para ello, empleando datos *genome skimming* de 47 representantes de todos los linajes de Loliinae y el análisis individual de sus repeteomas mediante las herramientas de Repeat Explorer 2 (RE2), caracterizamos las identidades y las abundancias de sus elementos repetitivos y estimamos las contribuciones de estos elementos a los respectivos tamaños genómicos de las especies. Mediante análisis comparados del repeteoma para cuatro grupos evolutivos diferentes (Loliinae, BL, FL, Schedonorus) obtuvimos reconstrucciones de redes filogenéticas a partir de árboles Neighbor-Joining computados con matrices de distancias de abundancias de los elementos repetitivos, y probamos la potencial señal filogenética de estos elementos con respecto a la filogenia combinada del plastoma y el gen ribosomal nuclear 35S. Mediante cálculos de similaridades intra- vs interespecíficas de los principales elementos repetitivos analizamos la variabilidad global de estos elementos en el paisaje genómico y sus grados de conservatismo o

diversificación en las Loliinae. Identificamos posibles familias ribosomales 5S con RE2 para determinar la potencial naturaleza poliploide de algunos taxones.

Capítulo 3. *Phylogenomics and systematics of overlooked Mesoamerican and South American 27olyploidy broadleaved Festuca grasses differentiate F. sects. Glabricarpae and Ruprechtia and F. subgen. Asperifolia, Erosiflorae, Mallopetalon and Coironhuecu (subgen. Nov.) (published, Plants 2022).*

El objetivo de este trabajo fue analizar la sistemática y la evolución de un grupo de festucas Meso y Suramericanas de hojas anchas poco conocido y apenas investigado genómicamente, que incluía especies de tres de los nueve subgéneros de BL *Festuca* y dos secciones endémicas de la región, más dos taxones de adscripción taxonómica incierta, con el fin de reconstruir sus relaciones evolutivas, inferir sus orígenes y circunscribir su taxonomía. Este estudio estaba relacionado con los objetivos específicos 1 y 2 de la tesis. Se analizaron muestras de 22 especies representativas de estos taxones en las que se revisaron siete caracteres fenotípicos considerados diagnósticos para su clasificación, y se analizaron datos *genome skimming* obtenidos de ellas y de otras 13 especies de Loliinae representativas del resto de los linajes de la subtribu. Los análisis taxonómicos y los filogenómicos basados en secuencias de plastomas, de genes ribosomales nucleares 35S, espaciador IGS, y 5S, y elementos repetitivos nucleares permitieron establecer la correcta adscripción de especies a rangos taxonómicos supraespecíficos, probar la consistencia monofilética de los mismos, y dilucidar los orígenes de los genomas progenitores materno y paternos de estas especies poliploides.

Capítulo 4. *Nuclear single-copy gene phylogenies reveal hybridization episodes in temperate Loliinae grasses (unpublished).*

La finalidad de este estudio fue investigar los niveles de discordancia intragenómica existentes en el genoma nuclear de las Loliinae y descifrar los episodios de hibridación (y alopoliploidización) que han intervenido en los procesos de especiación de sus taxones. Este estudio estaba relacionado con el objetivo específico 3 de la tesis. Para ello se construyeron las primeras filogenias de la subtribu utilizando genes nucleares copia-simple en una amplia representación de 133 especies de todos sus linajes, y se ampliaron las filogenias del plastoma y del gen rDNA 35S al total de las muestras.

Tras el procedimiento de captura génica con el kit Angiosperm353, se recuperaron 241 genes nucleares copia simple, cuyos datos fueron procesados con la tubería HybPiper, y se sumaron nuevos datos *genome skimming* empleados para ensamblajes de *novo* de plastomas y por mapeos del gen 35S. Se utilizaron análisis filogenéticos por coalescencia para los genes copia simple mediante métodos basados en cuartetos (SVDquartets) y árboles (Astral-III) ante la posible existencia de altos niveles de barajeo incompleto de linajes (*incomplete lineage sorting*, ILS) en grupos recientemente diversificados. Se estimaron los niveles de discordancia intragenómica nuclear mediante el apoyo de las topologías de los genes copia simple a la topología principal del árbol de especie

o Se detectaron los casos de hibridación aplicando una prueba de co-filogenia mediante análisis Procrustes entre árboles bootstrap de genes nucleares copia simple y árboles bootstrap del plastoma empleando PACo. Se analizaron los niveles de hibridación del grupo interno utilizando pruebas de tripletes implementadas en HyDE.

Capítulo 5. *Climatic niche conservatism of American I and American II fescue grasses from the North-Andean páramos (Festuca, Poaceae) (unpublished).*

El interés de este estudio consistió en probar, una vez elucidadas las relaciones de parentesco de los linajes y las especies de Loliinae estudiadas de Suramérica (véanse capítulos anteriores) si la diversificación de especies próximas de *Festuca* se había producido por procesos evolutivos o por especiación ecológica. Este estudio estaba relacionado con el objetivo específico 4 de la tesis. Para ello se seleccionaron especies de la región norte-andina, un hotspot de diversidad global y el segundo centro de endemicidad de *Festuca* en el mundo, pertenecientes a dos linajes del clado FL Loliinae. Se modelizaron los nichos ecológicos de dos especies del linaje American I (*F. chimborazensis, F. vaginalis*), endémicas de Ecuador y distribuidas simpátricamente en los pajonales del superpáramo, y dos especies del linaje American II (*F. asplundii, F. subulifolia*), endémicas del norte de los Andes y distribuidas simpátricamente en los pajonales del páramo, empleando variables climáticas bioclim y el método de máxima entropía Maxent implementado en Maxnet. Se reconstruyeron modelos de nicho para el tiempo presente y último máximo glacial (LGM) y se calcularon las amplitudes de nicho para cada especie en cada periodo temporal. Se desarrollaron análisis de conservatismo filogenético de nicho mediante pruebas de identidad y similitud de nicho empleando las opciones de ecospat.

Se analizó la potencial señal filogenética de las variables empleadas en la construcción de los nichos y de la amplitud de nicho. Se evaluaron las expansiones o contracciones de nicho de las especies desde el LGM a la actualidad y su relación con los cambios climáticos habidos en el páramo y el superpáramo.

Capítulo 6. *IAPT chromosome data 36/2 (published, Taxon 2022). Second-step lectotypifications of two names of Festuca subgenus Erosiflorae (Loliinae, Pooideae, Poaceae) (Correspondence Phytotaxa 2022).*

Este capítulo incluye dos artículos cortos o notas breves sobre estudios cariológicos y nomenclaturales de algunas de las especies de *Festuca* suramericanas estudiadas en la tesis (véanse capítulos anteriores). Estos estudios estaban relacionados con el objetivo específico 1 de la tesis. Debido a la amplia carencia de datos citogenéticos existente para un elevado número de especies de *Festuca*, en especial las menos conocidas especies suramericanas, durante el desarrollo de la tesis nos propusimos analizarlas y proporcionar información sobre sus números cromosómicos y sus tamaños genómicos. Dado que una gran parte de las muestras de *Festuca* analizadas en la tesis provienen de materiales secos en gel de sílice o de herbario, se optimizó el protocolo de estimación de tamaños genómicos mediante citometría de flujo a partir de materiales secos (véase Capitulo 2), y los datos de nuevos tamaños genómicos de especies *Festuca* se indican en los capítulos 2, 3 y 4. Los recuentos cromosómicos sólo se pudieron llevar acabo en placas metafásicas de meristemos radiculares de semillas viables procedentes de nuevas colecciones vivas (siguiendo el protocolo indicado en el Capítulo 2); los nuevos datos se aportan en la primera nota breve. Durante la revisión sistemática y el estudio de los especímenes tipo de las especies de *Festuca* subgen. *Erosiflorae* (véase Capitulo 3) se observó que los tipos de los nombres de dos de las tres especies de este subgénero necesitaban una segunda lectotipificación, de acuerdo con el artículo 9 del Código Internacional de Nomenclatura; las nuevas segundas lectotipificaciones se aportan en la segunda nota breve.

B.6. Referencias

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APARTADO C.

CAPÍTULOS DE LA TESIS

C. CAPÍTULOS DE LA TESIS

C.1. Museomics unveil the phylogeny and biogeography of the neglected Juan Fernandez archipelago *Megalachne* and *Podophorus* endemic grasses and their connection with relict Pampean-Ventanian fescues

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Museomics Unveil the Phylogeny and Biogeography of the Neglected Juan Fernandez Archipelago *Megalachne* and *Podophorus* Endemic Grasses and Their Connection With Relict Pampean-Ventanian Fescues

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Oceanic islands constitute natural laboratories to study plant speciation and biogeographic patterns of island endemics. Juan Fernandez is a southern Pacific archipelago consisting of three small oceanic islands located 600–700 km west of the Chilean coastline. Exposed to current cold seasonal oceanic climate, these 5.8–1 Ma old islands harbor a remarkable endemic flora. All known Fernandezian endemic grass species belong to two genera, *Megalachne* and *Podophorus*, of uncertain taxonomic adscription. Classical and modern classifications have placed them either in Bromeae (*Bromus*), Duthieinae, Aveneae/Poeae, or Loliinae (fine-leaved *Festuca*); however, none of them have clarified their evolutionary relationships with respect to their closest *Festuca* relatives. *Megalachne* includes four species, which are endemic to Masatierra (Robinson Crusoe island) (*M. berteroniana* and *M. robinsoniana*) and to Masafuera (Alejandro Selkirk island) (*M. masafuerana* and *M. dantonii*). The monotypic *Podophorus bromoides* is a rare endemic species to Masatierra which is only known from its type locality and is currently considered extinct. We have used museomic approaches to uncover the challenging evolutionary history of these endemic grasses and to infer the divergence and dispersal patterns from their ancestors. Genome skimming data were produced from herbarium samples of *M. berteroniana* and *M. masafuerana*, and the 164 years old type specimen of *P. bromoides*, as well as for a collection of 33 species representing the main broad- and fine-leaved Loliinae lineages. Paired-end reads were successfully mapped to plastomes and nuclear ribosomal cistrons of reference *Festuca* species and used to reconstruct phylogenetic trees. Filtered ITS and *trn*TLF sequences from these genomes were further combined with our large Loliinae data sets for accurate biogeographic reconstruction. Nuclear and plastome data recovered a strongly supported fine-leaved Fernandezian clade where *Podophorus* was

resolved as sister to *Megalachne*. Bayesian divergence dating and dispersal-extinction-cladogenesis range evolution analyses estimated the split of the Fernandezian clade from its ancestral southern American Pampas-Ventanian Loliinae lineage in the Miocene-Pliocene transition, following a long distance dispersal from the continent to the uplifted volcanic palaeo-island of Santa Clara-Masatierra. Consecutive Pliocene-Pleistocene splits and a Masatierra-to-Masafuera dispersal paved the way for *in situ* speciation of *Podophorus* and *Megalachne* taxa.

Keywords: ancestral range reconstruction, endemic Loliinae grasses, Fernandezian clade, genome skimming, phylogenomics, taxonomically neglected species

INTRODUCTION

Genomic data are increasingly called upon to elucidate evolutionary and taxonomic challenges posed by several cryptic or ambiguously related organisms, which could not be resolved using traditional approaches, such as morphometrics or standard molecular methods (Harrison and Kidner, 2011; Straub et al., 2012; Carter et al., 2020; Larridon et al., 2020). The advent of the high-throughput sequencing (HTS) methods have outpaced classical molecular barcoding and phylogenetic procedures based on few molecular markers that have served to build phylogenies with constrained resolution limits (Diaz-Perez et al., 2018; Sancho et al., 2018). While the results obtained from the genomic-based approaches are overall congruent with previous findings based on reduced sets of genes and genetic markers (Saarela et al., 2018), the thoroughly dissection of genomes have untapped large sets of taxonomically informative gene copy variants or single nucleotide polymorphism (SNPs) and have allowed the reconstruction of better resolved and more strongly supported phylogenies (Soltis et al., 2018). These new metadata have facilitated the identification of previously neglected cryptic taxa (Spriggs et al., 2019) and the construction of more robust phylogenetic trees where the evolutionary positions of previously unknown, doubtful, or ambiguous lineages have been elucidated in some cases (Leebens-Mack et al., 2019; Li et al., 2019).

The application of HTS methods to the analysis of museum collections, defined as museomics, has revolutionized the study of the organismic diversity (Besnard et al., 2014; Nevill et al., 2020). Plant herbarium specimens were occasionally used in traditional phylogenetic and population genetic studies due to the poor preservation of the specimens or their low quality DNA. Herbarium specimens have been progressively incorporated to taxonomic and evolutionary studies using HTS methods thanks to the simultaneous generation of a large quantity of sequences for the different genomes present in an organism (Straub et al., 2012; Besnard et al., 2014). Among the HTS approaches used with both herbarium and fresh collections, genome skimming (Dodsworth, 2015; Richter et al., 2015) has been successfully applied to reconstruct DNA genomes and regions that exist in multiple copies, such as plastomes, mitomes and the nuclear ribosomal cistron, and even some nuclear single copy genes (Besnard et al., 2014). Among other advances, museomics has untapped the placement of recently extinct taxa in phylogenies (Sebastian et al., 2010; Welch et al., 2016; Zedane et al., 2016; Silva et al., 2017). Thus, the combined use of current and extinct plant species samples, and of herbarium and recently collected samples allows to uncover largely sampled phylogenetic trees of plant lineages (Malakasi et al., 2019).

Oceanic archipelagos have been recognized as hotspots of diversity and natural laboratories for long distance colonization and plant speciation events (Triantis et al., 2016). Juan Fernandez is one of the smallest oceanic archipelagos. It consists of three small islands located in the southern Pacific, 580–730 km offshore of the western Chilean coast [Masatierra or Robinson Crusoe (47.94 km^2, 0–915 masl), Masafuera or Alejandro Selkirk (49.52 km^2, 0–1,319 masl), and Santa Clara (2.21 km^2, 0–350 masl)] (Stuessy et al., 1992, 2017). The two main islands have similar sizes but differ in plant communities and diverse grassland extensions due to their different ages and erosional patterns (Greimler et al., 2017), and are separated each other by 181 km (**Figure 1**). The current Fernandezian volcanic islands are relatively young (Stuessy et al., 1984). Despite its total small area (100.2 km^2), the archipelago harbors one of the richest endemic floras (60% vascular species, 11% genera, 1 paleoherb family; Stuessy et al., 1992). Floristic studies indicate that 55 grass species grow in Juan Fernandez archipelago; most of them are invasive taxa except five endemic species that belong to the Fernandezian *Megalachne* Steud. and *Podophorus* Phil. genera (Baeza et al., 2007; Peña et al., 2017; Penneckamp-Furniel and Villegas, 2019). *Megalachne* and the monotypic genus *Podophorus* have been historically assigned to different temperate grass tribes. *Megalachne* was originally described by Steudel in 1854 as close to *Bromus* (it was also described as *Pathantera* by Philippi in 1856), though they differ in the number and disposition of the stigmas (three apical in *Megalachne*, two subapical in *Bromus*) and the shape of the glume apex (aristulate in *Megalachne*, mutique in *Bromus;* Hackel, 1887). However, Pilger in 1920 and Skottsberg in 1922 transferred, respectively, *Megalachne* and *Pathantera* to *Bromus*, based on the sharing of laterally compressed spikelets and keeled lemmas, such as those in *Bromus* sect. *Ceratochloa* (Peña et al., 2017) thus classifying them within tribe Bromeae. In 1954, Pilger recognized *Megalachne* as a separate genus from *Bromus* (Peña et al., 2017); Tateoka (1962) using evidences from the morphology and apical hairiness of the ovary, apical emergence of stigmas, and type of starch grains and serology, suggested the proximity of *Megalachne* to *Festuca*, thus attributing it to tribe Poeae (subtribe Loliinae). The taxonomic adscription of *Megalachne* and *Podophorus* to

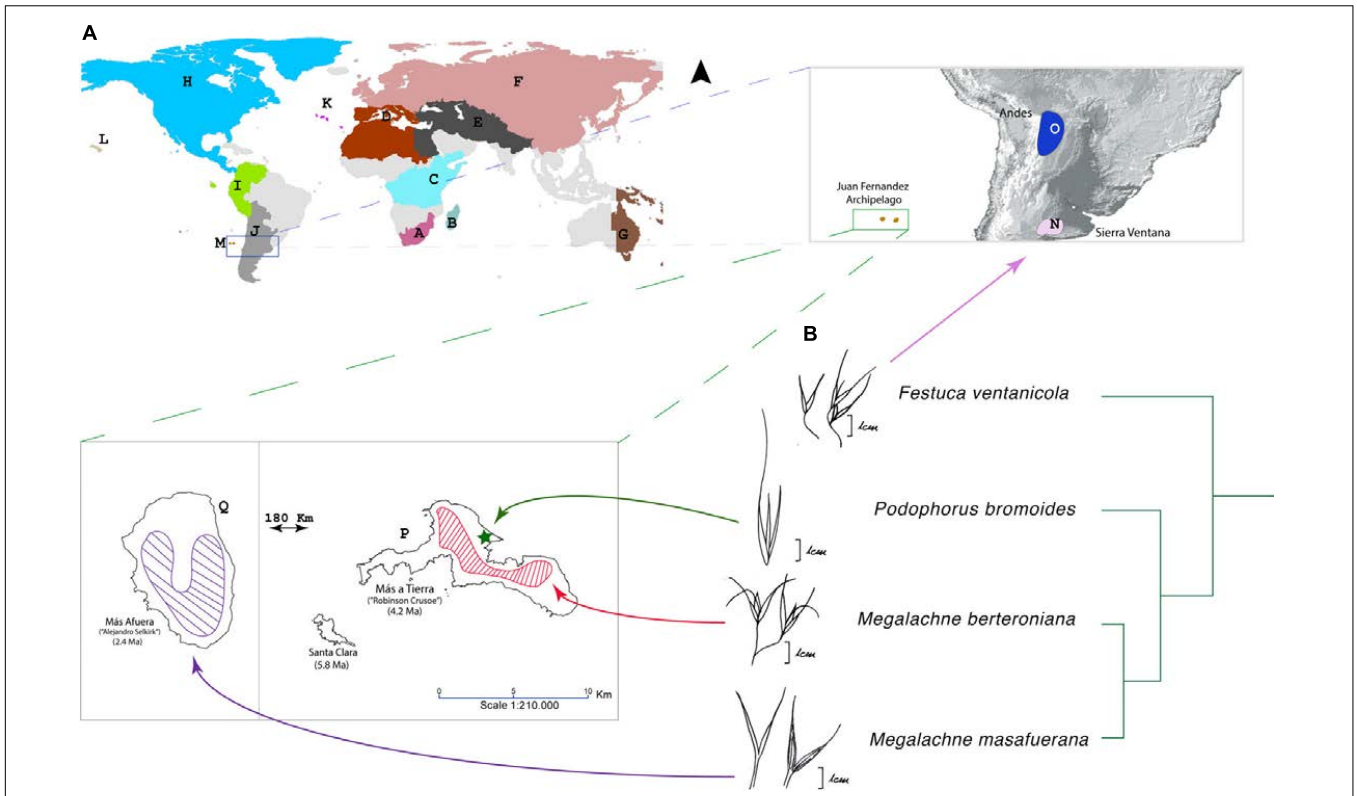

FIGURE 1 | (A) Distribution map of the Juan Fernandez archipelago *Megalachne* and *Podophorus* species included in this study [*M. berteroniana* and *M. masafuerana* distributions have been obtained from Peña et al. (2017) and Penneckamp-Furniel and Villegas (2019); *P. bromoides* is tentatively mapped in the Cumberley bay of Masatierra; cf. Baeza et al. (2007)] and of the Pampean-Ventanian *Festuca ventanicola*, showing the world and the American-Vulpia-Pampas Operational areas (OAs) used in the biogeographical analyses of Loliinae. OAs: Loliinae DEC model: A – South Africa; B – Madagascar and Mascarenes; C – Tropical Africa; D – Mediterranean; E – Irano-Turanian-Himalayan region; F – Eurasia (Eurosiberian region); G – Australasia (New Zealand, south-western Australia, Papua-New Guinea); H – North and Central America; I – Northern Andes; J – Southern Andes and Southern South America; K – Macaronesia; L – Hawaii; M – Juan Fernández; American-Vulpia-Pampas DEC model: H – North and Central America; N – Pampas-Ventania; O – Andes; P – Masatierra; Q – Masafuera. **(B)** Phylogenetic ITS-TLF subtree showing the relationships of the Fernandezian *Podophorus bromoides*, *Megalachne berteroniana*, and *M. masafuerana* grasses and its close relative *Festuca ventanicola*; drawns of the floral "vulpioid" phenotype are shown for each species.

tribe Poeae was accepted in most grass classifications though Soreng et al. (2003) assigned them initially to tribe Stipeae subtribe Duthieinae based on the overall habit resemblance. Nonetheless, the comprehensive morphological and molecular study of the newly delimited tribe Duthieeae of Schneider et al. (2011) demonstrated, using ITS sequences, that *Megalachne* and *Podophorus* were not part of this early diverging pooid lineage, and suggested that they likely belonged to the Aveneae/Poeae complex. In recent studies, phylogenetic analyses conducted by Schneider et al. (2012) and Tkach et al. (2020) using, respectively, nuclear ITS and plastid *mat*K sequences and nuclear ITS and ETS and plastid *mat*K, *trn*K, and *trn*LF sequences corroborated it, showing that *Megalachne* was nested within the fine-leaved Loliinae clade.

Megalachne and *Podophorus* differentiate from each other in the number of florets per spikelet [3–6 in *Megalachne*, 1–(+1 sterile) in *Podophorus*), the type of lemma (keeled vs. rounded], the length of the glumes (equal vs. shorter than anthecium) and the prolongation of the rachilla apex (shorther vs. equal than anthecium; Baeza et al., 2007; Kellogg, 2015; Peña et al., 2017). *Megalachne* consisted until recently of two species, *M. berteroniana* Steud. and *M. masafuerana* (Skottsb. & Pilg. ex. Pilg.) Matthei, endemic to the Masatierra and Masafuera islands, respectively (Baeza et al., 2007). Both species grow in coastal and mountain cliffs in their respective islands (Danton et al., 2006). Recent morphological studies have identified two new species, *M. robinsoniana* C. Peña, endemic to Masatierra (Peña et al., 2017), and *M. dantonii* Penneck. & Gl. Rojas, endemic to Masafuera (Penneckamp-Furniel and Villegas, 2019). The four *Megalachne* species differ in the lengths of the lemma and glume awns and the number of florets per spikelet (Peña et al., 2017; Penneckamp-Furniel and Villegas, 2019). The systematic and evolutionary fate of *Podophorus* is more enigmatic. Its single species *P. bromoides* Phil. is only known from its type specimens, collected in Masatierra and described by Philippi in 1856, and is currently considered to be extinct (Baeza et al., 2007).

Loliinae is one of the largest subtribes of the temperate pooid grasses and contains pasture and forage species of high ecological and economic importance. Its largest genus *Festuca*

is formed by ∼600 worldwide distributed species inhabiting cool seasonal regions of both hemispheres and high tropical mountains (Catalan, 2006). Molecular phylogenetic studies have shown that *Festuca* is largely paraphyletic (Catalan et al., 2007; Inda et al., 2008; Minaya et al., 2017). Recent studies, based on the Schneider et al. (2011, 2012) ITS and *mat*K data and previous morphological findings, reclassified *Megalachne* and *Podophorus* within subtribe Loliinae (Soreng et al., 2015, 2017), however, they did not identify the closest relatives of these Fernandezian grasses. The phylogenetic relationships obtained by previous authors for the three studied *Megalachne* and *Podophorus* taxa were also taxonomically incongruent, showing a closer relationship of *M. berteroniana* to *P. bromoides* than to its congener *M. masafuerana* (Schneider et al., 2011).

Here we have used a museomic approach based on genome skimming data to uncover the phylogenetic and biogeographical history of the neglected Fernandezian *Megalachne* and *Podophorus* grasses. The aims of our study were to (i) infer the phylogeny of *Megalachne* and *Podophorus* within a large sample representation of Loliinae lineages; (ii) identify the closest relatives of the Fernandezian grasses; (iii) reconstruct the relationships among the *Megalachne* and *Podophorus* taxa; (iv) estimate divergence times of the Fernandezian lineages; and (v) infer the colonization patterns and speciation events of the ancestors of *Megalachne* and *Podophorus* in the Juan Fernandez islands.

MATERIALS AND METHODS

Sampling

Representative samples of *Megalachne*, *Podophorus*, and other Loliinae genera were included in the study (**Figure 1** and **Table 1**). Herbarium samples of *Megalachne berteroniana* and *M. masafuerana* provided by the Oregon State University Herbarium (OSC11751 and OSC9150 collections; **Table 1**) were used to isolate high quality and quantity DNA for genome sequencing and downstream evolutionary analyses. A herbarium sample of *M. robinsoniana* provided by the Concepción University Herbarium (CONC40598 collection) failed to generate good quality DNA for the study. The recently described *M. dantonii* species (Penneckamp-Furniel and Villegas, 2019) could not be included in our study. A 164 years old sample of the currently considered extinct *Podophorus bromoides* Phil., only known from its three type specimens, was provided by the Royal Botanic Gardens Kew's Herbarium (Philippi 1861, isotype collection; **Table 1**)[1] and was successfully used for genome skimming sequencing and downstream analysis. In our aim to identify the closest relatives of the Fernandezian *Megalachne* and *Podophorus* grasses, DNA was also isolated from 33 Loliinae samples (**Table 1**) representing all the known broad-leaved, intermediate, and fine-leaved Loliinae lineages (Inda et al., 2008; Minaya et al., 2017) and used for genome skimming sequencing and phylogenomic analyses. Some of these samples were collected from poorly explored geographical areas, including four new *Festuca* samples from South America, the putative region of origin of the ancestors of *Megalachne* and *Podophorus* (Stuessy et al., 2017) and two from Tropical Africa and South Africa. In addition, two new Loliinae samples from South America and one sample from South Africa not studied before (**Table 1**) were sequenced for the nuclear ITS (ITS1-5.8S-ITS2) and the plastid *trn*TL (*trn*T-*trn*L intergenic spacer) and *trn*LF (*trn*A-Leu, *trn*L-*trn*F intergenic spacer, *trn*A-Phe) loci, together with 97 samples from a wide-sampling of all currently known Loliinae lineages (Inda et al., 2008; Minaya et al., 2017). Although species of *Megalachne* and *Podophorus* and other fine-leaved Loliinae genera have been synonymized to *Festuca*, and those of broad-leaved Loliinae to different festucoid genera in recent studies (Soreng et al., 2017; Tkach et al., 2020), we follow the *Festuca* sensu lato classification of Catalan et al. (2007) which is based on an evolutionary systematic criterion that is nomenclaturally conservative and maintains a paraphyletic *Festuca* (with subgenera and sections) and other traditionally recognized genera until more complete phylogenetic studies of Loliinae are conducted. We have selected this scenario because of present uncertainties about the phylogeny of several Loliinae lineages and taxonomic and nomenclatural instability of the *Festuca* sensu stricto (i.e., fine-leaved Loliinae lineages) classification, that would leave some broad-leaved Loliinae lineages without name or with unclear adscription (e.g., some broad-leaved "*Festuca*"). It could be possible, however, that genera phylogenetically embedded within the large Loliinae clade or its fine-leaved subclade would be subordinated to *Festuca*, once all or most of the Loliinae taxa are phylogenetically analyzed and consistent synapomorphies are defined. At this respect, nomenclatural combinations have been proposed for the fine-leaved *Megalachne* (*Festuca megalachna* Röser & Tkach; *F. masafuerana* (Skottsb. & Pilg. ex Pilg.) Röser & Tkach; *F. robinsoniana* (C.M.Peña) Röser & Tkach; *F. dolichathera* Röser & Tkach) and *Podophorus* (*F. masatierrae* Röser & Tkach) species synonymized to *Festuca* (Tkach et al., 2020). Sixteen additional species were added as outgroups to provide reliable fossil calibration points for molecular dating (**Supplementary Table S1**).

DNA Extraction and Sequencing

The 36 samples used in this study were obtained from herbarium specimens (AARHUS, K, MO, US, OS, CONC, HUTPL, University of Zaragoza), silica gel dried leaf tissues collected in field trips, and fresh leaves collected from plants growing in the Universidad de Zaragoza – Escuela Politécnica Superior de Huesca common garden (**Table 1** and **Supplementary Table S1**). Total DNA from fresh and silica gel dried samples was isolated following the DNeasy Plant Mini kit (Qiagen, Valencia, CA, United States) protocol using 20–30 mg of dry leaf tissue or 20 mg of fresh tissue ground to powder with liquid nitrogen. Total DNA from herbarium samples was extracted using a modified CTAB protocol (Doyle and Doyle, 1987) using ∼20 mg of tissue. DNA concentration was quantified with a Qubit fluorometer (Invitrogen by Life Technologies, Carlsbad, California, United States) and DNA quality was evaluated with Biodrop (Harvard Bioscience). The integrity of the DNA was

[1]https://apps.kew.org/herbcat/getImage.do?imageBarcode=K000433684

TABLE 1 | List of taxa included in the phylogenomic study of the Fernandezian and other Loliinae grasses.

Taxon	Source	Ploidy	No. reads	Genbank/Phytozome accession No.	
				Plastome	rDNA cistron
Festuca abyssinica	Tanzania: Kilimanjaro	4x	12041	SAMN14647043	MT145276
Festuca africana	Uganda: Bwindi forest	10x	13549	SAMN14647044	MT145277
Festuca amplissima	Mexico: Barranca del Cobre	6x	12058	SAMN14647045	MT145278
Festuca arundinacea var. *letourneuxiana*	Morocco: Atlas Mountains	10x	16839	SAMN14647059	MT145292
Festuca asplundii	Ecuador: Saraguro	6x	25088	SAMN14647046	MT145279
Festuca caldasii	Ecuador: Las Chinchas -Tambara	?	9863	SAMN14647047	MT145280
Festuca capillifolia	Spain: Cazorla	2x	13430	SAMN14647048	MT145281
Festuca chimborazensis	Ecuador: Chimborazo-Cotopaxi	4x	10913	SAMN14647049	MT145282
Festuca durandoi	Portugal: Alto do Espinheiro	2x	12688	SAMN14647050	MT145283
Festuca eskia	Spain: Picos de Europa	2x	24041	SAMN14647051	MT145284
Festuca fenas	Spain: Madrid	4x	16112	SAMN14647052	MT145285
Festuca fimbriata	Argentina: Apóstoles	6x	15741	SAMN14647053	MT145286
Festuca fontqueriana	Morocco: Rif, Outa-El-Kadir	2x	22187	SAMN14647054	MT145287
Festuca gracillima	Argentina: Tierra de Fuego	6x	13888	SAMN14647055	MT145288
Festuca holubii	Ecuador: Saraguro	?	10264	SAMN14647056	MT145289
Festuca francoi	Portugal: Azores	2x	17592	SAMN14647057	MT145290
Festuca lasto	Spain: Los Alcornocales	2x	21581	SAMN14647058	MT145291
Festuca mairei	Morocco: Atlas Mountains	4x	19134	SAMN14647060	MT145293
Festuca molokaiensis	United States: Hawaii, Molokai	?	12188	SAMN14647061	MT145294
Festuca ovina	Russia: Gatchinskii Raion	2x	11364	SAMN14647062	MT145295
Festuca pampeana	Argentina: Sierra Ventana	6x	14862	SAMN14647063	MT145296
Festuca paniculata	Spain: Puerto de los Castaños	2x	35808	SAMN14647064	MT145297
Festuca parvigluma	China: Baotianman	4x	15872	SAMN14647065	MT145298
Festuca pratensis	England: USDA/283306	2x	30021	SAMN14647066	MT145301
Festuca procera	Ecuador: Riobamba	4x	12189	SAMN14647067	MT145299
Festuca pyrenaica	Spain: Pyrenees, Tobacor	4x	40669	SAMN14647068	MT145300
Festuca pyrogea	Argentina: Tierra de fuego	?	16835	SAMN14647069	MT145302
Festuca quadridentata	Ecuador: Chimborazo	?	15091	SAMN14647070	MT145303
Festuca spectabilis	Bosnia-Hercegovina: Troglav	6x	12960	SAMN14647071	MT145304
Festuca superba	Argentina: Jujuy, Yala	8x	12193	SAMN14647072	MT145305
Festuca triflora	Morocco: Rif, Ketama	2x	24472	SAMN14647073	MT145306
Megalachne berteroniana	Chile: JuanFernandez, Masatierra	?	5288	SAMN14647074	MT145307
Megalachne masafuerana	Chile: JuanFernandez, Masafuera	?	6134	SAMN14647075	MT145308
Podophorus bromoides	Chile: JuanFernandez, Masatierra	?	6694	SAMN14668162	–
Vulpia ciliata	Spain: Mar de Ontígola	4x	11801	SAMN14647076	MT145309
Vulpia sicula	Italia: Sicilia, Madone	2x	11327	SAMN14647077	MT145310
Outgroups					
Brachypodium distachyon	Iraq: near Salakudin	2x	–	NC_011032.1	phytozome.jgi.doe.gov, Bd21 v.3.1
Oryza sativa subsp. *japonica*	cv. PA64S; cv. Nipponbare	2x	–	AY522331.1	AP008215

Taxon, source, ploidy level, number of Illumina reads, and plastome and rDNA cistron Genbank codes are indicated for each sample. Newly generated Loliinae plastome data and sequences have been deposited in Genbank under BioProject PRJNA626668 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA626668) and at Github [https://github.com/Bioflora/Loliinae_plastomes (unfiltered, filtered) and https://github.com/Bioflora/Podophorus_plastome (unfiltered, filtered)].

further checked in a 1% agarose gel. Overall the qualities and quantities of the DNAs were appropriate for genome skimming (∼5 μg, 50 ng/μl), except that of *P. bromoides*, which had <1 ng/μl.

DNAs obtained from three *Megalachne* and *Podophorus* samples plus 33 Loliinae samples were used to construct a genomic library for shotgun sequencing using Illumina technology. The library from freshly and herbarium collected materials DNAs was prepared with KAPA Hyper Prep Kit for PCR-free workflows (Roche Kapa Biociences) with some minor modifications. In brief, 1.0 μg of genomic DNA was sheared in a Covaris^{TM} E220 focused-ultrasonicator into Covaris microTUBE AFA Fiber Pre-Slit Snap-Cap tubes with the following parameters: sample volume 55 μl, duty cycle 15%, intensity 450, cycles/burst 200, time 100 s, temperature 4°C, in order to reach the fragment sizes of ∼200–400 bp.

The sheared DNA was end-repaired, adenylated and ligated to IDT adaptors with unique dual-matched indexes (Integrated DNA Technologies) for paired end sequencing. The adaptor-modified end library was size selected and purified with AMPure XP beads (Agencourt, Beckman Coulter) in order to eliminate non-ligated adapters and adapter dimers. Final library size was confirmed on an Agilent 2100 Bioanalyzer with the DNA 7500 assay. The *Podophorus bromoides* library yielded 13 ng/μl and two normally distributed fragment size distributions of 200 and 500 bp. The PCR free library was quantified by Library Quantification Kit for Illumina Platforms (Roche Kapa Biosystems). The library was multiplexed with other libraries and the pool of libraries was then partly sequenced on a HiSeq4000 and partly on a HiSeq 2500 (TruSeq SBS Kit v4, Illumina, Inc) in paired-end mode (2 × 100 bp) in the Centro Nacional de Análisis Genómicos (CNAG, Barcelona). Primary data analysis, image analysis, base calling and quality scoring of the run were processed using the manufacturer's software Real Time Analysis (RTA 2.7.7) for HiSeq4000, and RTA1.18.66.3 when using HiSeq2500, followed by generation of FASTQ sequence files.

Additionally, four Loliinae samples (**Supplementary Table S1**) were used for Sanger sequencing of the nuclear ribosomal ITS locus and the plastid *trn*LF and *trn*TL loci using the primers and procedures indicated in Inda et al. (2008) in Macrogen and were added to the 97 Loliinae data set obtained from previous studies (Inda et al., 2008; Minaya et al., 2017).

DNA Sequence Data Assembling and Multiple Sequence Alignments

Illumina paired-end (PE) reads of the Fernandezian and other Loliinae samples were checked using FASTQC[2] and the adapters and low quality sequences were trimmed using TRIMMOMATIC (Bolger et al., 2014) at the CNAG. Plastome assembly was performed with Novoplasty v.2.7.1 (Dierckxsens et al., 2017) using the published plastomes of *Festuca ovina* (JX871940.1) for fine-leaved taxa and of *F. pratensis* (JX871941) for broad-leaved taxa (Hand et al., 2013) as reference, and the following parameters: k-mer: 27 or 39, insert size: ∼300 bp, genome range: 120,000–220,000 bp, and PE reads: 101 bp. Assembled plastomes were aligned using MAFFT v.7.031b (Katoh and Standley, 2013) followed by visual inspection using Geneious R11[3]. Because Novoplasty failed to assemble the whole plastome of *P. bromoides* due to the low number and quality of total PE reads, we used a Geneious mapping and readmerging strategy to map its reads to three phylogenetically close plastomes (*Megalachne berteroniana*, *M. masafuerana*, *Festuca pampeana*).

For the assembly of the nuclear ribosomal cistron we used a two-step read mapping and merging approach. Due to the lack of any published Loliinae rDNA cistron, we employed the *Brachypodium distachyon* rDNA cistron (reference genome Bd21, Vogel et al., 2010)[4] as reference and mapped to it the PE reads of the studied Loliinae taxa. Readmerging allowed us to align reads and their reverse complements to create a single consensus read. This step also allowed improving the sequence quality of overlapping parts. In cases of non-overlapping PE reads, the reads were used independently. The integrity of the cistron locus was examined visually for read mappings using Geneious R11.

Forward and reverse ITS, *trn*LF, and *trn*TL Sanger sequences were checked, corrected and merged using Sequencher v. 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, United States)[5]. Each data set was aligned separately, visually inspected using Geneious R11 and manually corrected if necessary. The assembly of the *P. bromoides trn*LF and *trn*TL loci was done through several read mapping iterations with Geneious using as reference the closest *M. berteroniana*, *M. masafuerana*, *F. ventanicola* and *F. pampeana trn*LF and *trn*TL sequences.

A multiple sequence alignment (MSA) of 35 newly assembled *Megalachne* and Loliinae plastomes with *Oryza sativa* (AY522331.1; Genbank) and *Brachypodium distachyon* (NC_011032.1; Genbank) outgroups was performed with MAFFT v.7.215 (Katoh and Standley, 2013). The length of this full Loliinae plastome MSA without *Podophorus* was 146,172 bp length. The short *P. bromoides* consensus plastid sequence was subsequently aligned to the Loliinae full plastome MSA in Geneious R11. The multiple plastome alignment was filtered to remove poorly aligned regions and missing data in *P. bromoides* and other taxa through the automated option of trimAl v.1.2rev59 (Capella-Gutiérrez et al., 2009). The length of the filtered Loliinae plastome MSA with *Podophorus* was 55,872 bp length. A nuclear MSA of 35 newly assembled *Megalachne* and Loliinae rDNA cistrons and of *Oryza sativa* (AP008215; Genbank) and *Brachypodium distachyon* (Bd21; Phytozome) outgroups was also conducted with Geneious R11, rendering a 6,455 bp alignment. Independent MSAs were also produced for the ITS, *trn*LF, and *trn*TL loci of 135 Loliinae species and 16 outgroups, which included the *Megalachne* and *Podophorus* samples in Geneious R11. The *trn*LF and *trn*TL plastid loci were combined into a single plastid TLF MSA; separate phylogenetic analyses of the two loci gave congruent topologies with that recovered for the concatenated TLF haploid data matrix and only results from the latter analysis will be explained further. The nuclear ITS and the plastid TLF data set were further combined into a ITS-TLF MSA after obtaining congruence results from contrasted topological tests.

Phylogenetic Reconstruction and Divergence Time Analysis

Maximum likelihood phylogenetic analysis of the plastome (full and reduced), the rDNA cistron, and the independent and combined ITS, and TLF data sets were conducted with IQTREE (Nguyen et al., 2015) imposing the best-fit nucleotide substitution model to each separate data set that was automatically selected by the ModelFinder option of the program (Kalyaanamoorthy et al., 2017) according to the Bayesian Information Criterion (BIC) [plastome (full and reduced): TVM + F + R3; rDNA cistron: GTR + F + R2; ITS: SYM + I + G4; TLF: K3Pu + FR4]. Each search was performed through the automated computation of 20 Maximum Likelihood (ML) starting trees from 98 alternative

[2]http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

[3]https://www.geneious.com

[4]http://phytozome.jgi.doe.gov, v3.1Phytozome

[5]http://www.genecodes.com

randomized Maximum Parsimony (MP) trees, searching for best-scoring ML trees and estimating branch support for the best tree from 1,000 bootstrap replicates (BS) using the ultrafast bootstrap option (Minh et al., 2013; Chernomor et al., 2016) implemented in the software.

Ancestral divergence ages of the Fernandezian and other Loliinae grasses were estimated from the concatenated ITS-TLF data set with BEAST 2 (Bouckaert et al., 2014). We imposed independent site substitution models, lognormal relaxed clock and Yule tree models (Minaya et al., 2017). Two nodes of the Poaceae tree were calibrated using secondary age constrains for the crown nodes of the BOP clade (*Oryza* + Pooideae) (normal prior mean = 51.9 Ma, *SD* = 1.9) and the *Brachypodium* + core pooids clade (*Brachypodium* + Aveneae-Poeae) (normal prior mean = 30.9 Ma, *SD* = 3.5), following the grass-wide plastome based dating analysis of Sancho et al. (2018) and a third node was calibrated using a minimum age constrain (16 Ma) for the crown node of the fine-leaved Loliinae (lognormal prior mean = 19.5 Ma, *SD* = 0.101) based on a *Festuca* leaf macrofossil from Poland dated to the Early Miocene showing *Festuca* sect. *Festuca*-type adaxial and abaxial epidermises (Juchniewicz, 1975). We also imposed a broad uniform distribution prior for the uncorrelated lognormal distribution (ucld) mean (lower = 1.0E-6; upper = 0.1) and an exponential prior for ucld standard deviation (SD). We ran 600 million Markov chain Monte Carlo (MCMC) generations in BEAST2 with a sampling frequency of 1,000 generations. The adequacy of parameters was checked using TRACER v.1.6[6] with all the parameters showing Effective Sample Size (ESS) >200. A Maximum clade credibility (MCC) tree was computed after discarding 10% of the respective saved trees as burn-in.

Ancestral Range Estimation

We used the parametric dispersal-extinction-cladogenesis (DEC) approach implemented in Lagrange v. 20130526 (Ree and Smith, 2008) to infer global extinction and dispersal rates and ancestral range inheritance scenarios for each node representing the ancestors of the Fernandezian and other Loliinae grasses in the maximum clade credibility (MCC) tree obtained from BEAST. We defined 13 Operational Areas (OAs) (A-M), selected according to the current distribution ranges of the species and the potential historical distributions of their ancestors, delimited by geographical features that could have acted as barriers to dispersal (Minaya et al., 2017) (**Supplementary Table S2A**). Specifically, we selected four American OAs: North America (H), northern South America (I), southern South America (J), and Juan Fernandez (M), aiming to recover the areas of origin of the ancestors of *Megalachne* and *Podophorus* that presumably colonized the Juan Fernandez archipelago from the American mainland through long-distance dispersal (LDD). The ancestral ranges were built imposing a maximum of two ancestral areas (AA), considering that ancestors were not more widespread than their extant descendants (Sanmartín, 2003). Ancestral range inheritances and biogeographic events were inferred from a stratified model with four temporal windows (TSI: Late Oligocene to Middle Miocene, 28.4–16.0 Ma; TSII: Middle to Late Miocene, 16.1–7.2 Ma; TSIII: Late Miocene to Pliocene, 7.3–2.6 Ma; TSIV: Quaternary, 2.61–0 Ma). This model included the different temporal paleogeographical configurations of the Americas and other continents that might have affected the evolution and the distribution of the main Fernandezian and other Loliinae lineages (**Supplementary Table S2B**). In order to obtain a more detailed fine-scale reconstruction of the biogeographic events that resulted in the Fenandezian grasses, a second DEC analysis was performed for the lineages of the American-Vulpia-Pampas clade where the Fernandezian subclade was nested within (see section Results). This second analysis was performed using a pruned MCC dated subtree for the American-Vulpia-Pampas clade and five OAs representing the current and paleo-geographical distributions of the lineages (H-North-Central America, N-Pampas-Ventania, O-Andes, P-Masatierra, Q-Masafuera; **Supplementary Tables S2C,D**).

RESULTS

Loliinae Genome Sequence Data, Plastomes, and Nuclear rDNA Cistrons

Most of the studied Loliinae genome-skimming sequenced samples, including the newly studied *Festuca asplundii*, *F. caldasii*, *F. holubii*, *F. procera*, and *F. quadridentata*, yielded a large number of PE reads, ranging from 9,863 to 40,669 kbp (**Table 1** and **Supplementary Table S1**). The two *Megalachne* samples were below that threshold (*M. berteroniana* 5,288 kbp; *M. masafuerana* 6,134 kbp) but showed high quality reads. The 164 years old *Podophorus bromoides* type specimen sample rendered 6,694 kbp poor quality PE reads (**Table 1** and **Supplementary Table S1**).

Most plastid assemblies produced a single plastome contig with a deep coverage of >50x per sample that contained its two inverted repeat regions (IRa, IRb). However, Novoplasty assemblies of *Festuca durandoi, F. spectabilis, F. superba*, *F. molokaiensis*, *F. abyssinica*, and *Megalachne berteroniana* gave several small contigs and their full plastome assemblies were constructed with these contigs and the read mapping approach using Geneious and plastomes of their closest species as references. Plastome lengths of broad-leaved Loliinae ranged from 134,231 to 134,734 bp and those of fine-leaved Loliinae from 132,599 to 133,869 bp; these values agreed with the plastome lengths retrieved by Hand et al. (2013) for their two main Loliinae group taxa. The lengths of the *Megalachne berteroniana* (132,812 bp) and *M. masafuerana* (132,826 bp) plastomes fell within the fine-leaved Loliinae range. The PE reads of the newly assembled plastomes were deposited in GeneBank under BioProject PRJNA626668[7] with accessions numbers SAMN14647043–SAMN14647077 and SAMN14668162 (**Table 1** and **Supplementary Table S1**). The full Loliinae plastome MSA is available in Github[8]. The *Podophorus bromoides* plastid

[6]http://beast.bio.ed.ac.uk/Tracer

[7]https://www.ncbi.nlm.nih.gov/sra/PRJNA626668

[8]https://github.com/Bioflora/Loliinae_plastomes

consensus sequence (total length ∼69,238 bp) covered different non-overlapping fragments of the aligned Loliinae plastomes (∼40.7%) with a low coverage depth (10x to 1x). The plastid *P. bromoides* sequence (with its nucleotide positions mapped against the full Loliinae plastome MSA) is available in Github[9].

We obtained a single contig of 6,453–6,455 bp for the rDNA cistrons of the studied *Megalachne* and other Loliinae samples. Coverage depth was relatively constant across the rDNA cistron sequences in most cases (>10x). The newly sequenced rDNA cistrons were deposited in GeneBank with accessions numbers MT145276–MT145310 (**Table 1**). The low quality genomic sequence available in the DNA obtained from the *P. bromoides* specimen resulted in a low number of PE reads, which precluded the readmerging of its full rDNA cistron; however, it allowed the assembly of its entire ITS region (**Table 1** and **Supplementary Table S1**). The nuclear rDNA cistron of the studied *Megalachne* and other Loliinae grasses had a conserved structure along its transcriptional unit of 6–6.5 kb length, containing the 5'-ETS (724 bp), the 18S gene (1,818 bp), the ITS (585 bp), and the 25S gene (3,408 bp) regions of similar mean length to those of other grasses.

The nuclear ITS locus and the plastid *trn*LF and *trn*TL loci were filtered, respectively, from the assembled rDNA cistrons and plastomes for the *Megalachne* and Loliinae samples (**Table 1** and **Supplementary Table S1**). For *P. bromoides*, the complete ITS sequence was recovered with a coverage depth ranging from 10x to 1x and was deposited in Genbank under accession code MT022522 (**Supplementary Table S1**). Up to 60 and 70% of, respectively, the *P. bromoides trn*LF and *trn*TL sequences were recovered with a coverage depth of 10x (MSAs available in Github) (see footnote 9). The ITS and TLF sequences of the newly analyzed *F. andicola*, *F. longipes*, *F. vaginalis*, and *F. valdesii* were incorporated to the study and were deposited in Genbank under accession codes EF584922-EF592955-EF585009; KY368804-KY368856-KY368907; EF584977-EF584977-EF585111; MT022522-MT040974 – MT040975 (**Supplementary Table S1**).

Loliinae Plastome and Nuclear Phylogenomic Trees

The full plastome data set (two *Megalachne* and 33 additional Loliinae samples) included 133,894 filtered positions of which 7,480 were variable and 4,160 potentially informative. The best plastome ML phylogenetic tree (**Figure 2A** and **Supplementary Figure S1A**) recovered a fully resolved and highly supported topology with most branches having 100% bootstrap support (BS), and only three (94–99% BS) and one (77% BS) branches having strong to relatively good support. This Loliinae phylogenomic tree based on plastome data showed a main split of broad vs. fine-leaved Loliinae lineages, and successive splits within both the broad-leaved (Central-South American, Lojaconoa, Drymanthele/Tropical and South African, Leucopoa, Subbulbosae, Schedonorus) and the fine-leaved (American-Neozeylandic I, Eskia/American I, American-Vulpia-Pampas, Psilurus-Vulpia/Exaratae-Loretia (with intermediate Subulatae-Hawaiian nested within), Festuca, Aulaxyper, American II, Afroalpine) clades. *Megalachne berteroniana* and *M. masafuerana* plastome sequences formed a Fernandezian clade, sister to *F. pampeana* and nested within the southern American American-Vulpia-Pampas clade. Newly sequenced South American plastome samples fell within the fine-leaved American II [*F. fimbriata*, (*F. asplundii*, *F. procera*)] and American I [(*F. holubii*, *F. chimboracensis*)] clades, and within a Central-South American broad-leaved clade [(*F. caldasii*, (*F. superba*, (*F. quadridentata*, *F. amplissima*)))]. Fuegian *F. pyrogea* fell within the fine-leaved Festuca clade and the broad-leaved *F. fenas* clustered within the European Schedonorus clade (**Figure 2A** and **Supplementary Figure S1A**).

The reduced plastome data set, which included the *Podophorus* sample, had 55,872 positions of which 5,989 were variable and 823 potentially informative. The optimal ML tree (**Figure 2B** and **Supplementary Figure S1B**) recovered a topology that was also fully resolved and almost identical to that of the complete plastome data, though the branch support was slightly lower across the phylogenomic tree [all braches with full support except seven branches with strong (90–99%), three with good (70–89%), and one with weak (60%) BS]. In this phylogenomic tree, *P. bromoides* was resolved as sister to the *Megalachne* subclade (90% BS) and formed a fully supported Fernandezian clade, which was nested within the American-Vulpia-Pampas lineage (**Figure 2B** and **Supplementary Figure S1B**).

The nuclear rDNA cistron data set (two *Megalachne* and 33 additional Loliinae samples) included 6,455 positions of which 502 were variable and 321 potentially informative. The best ML tree (**Figure 3A** and **Supplementary Figure S1C**) retrieved a fully resolved topology; however, some internal branches were very short and showed very low support [21 branches with strong (90–99%), seven with good (70–89%), and seven with weak (60%) or very weak (<50%) BS]. The rDNA cistron-based phylogenetic tree showed the successive divergences of early diverging paraphyletic broad-leaved lineages (Tropical and South African, Drymanthele, Lojaconoa, Leucopoa, Central-South American, South-American, Schedonorus, Subbulbosae), which were in most cases poorly supported and included the intermediate Subulatae-Hawaiian nested within, and the more recent split of the strongly supported fine-leaved clade (97% BS). The topology of the fine-leaved group showed successive weakly to strongly supported lineage splits [(Eskia, ((Aulaxyper, Exaratae-Loretia, Festuca), (American-Vulpia-Pampas, Psilurus-Vulpia, Afroalpine, American-Neozeylandic I, American I, American II)))]. *Megalachne berteroniana* and *M. masafuerana* formed a fully supported Fernandezian clade based on the cistron sequences; this clade was close to other species of the American I (*F. holubii*, *F. chimborazensis*) and American II (*F. asplundii*, *F. fimbriata, F. procera*) assemblages, which together with the American-Neozeylandic I *F. gracillima* formed a well-supported fine-leaved South American clade (91% BS). *Festuca pyrogea* was reconstructed as sister to *F. ovina* within the strong Festuca clade. Within the broad-leaved lineages, the strongly supported Central-South American (*F. amplissima*, *F. quadridentata*) and (*F. superba*, *F. caldasii*) clades were resolved in different positions across the broad-leaved subtree, and *F. fenas* clustered

[9]https://github.com/Bioflora/Podophorus_plastome

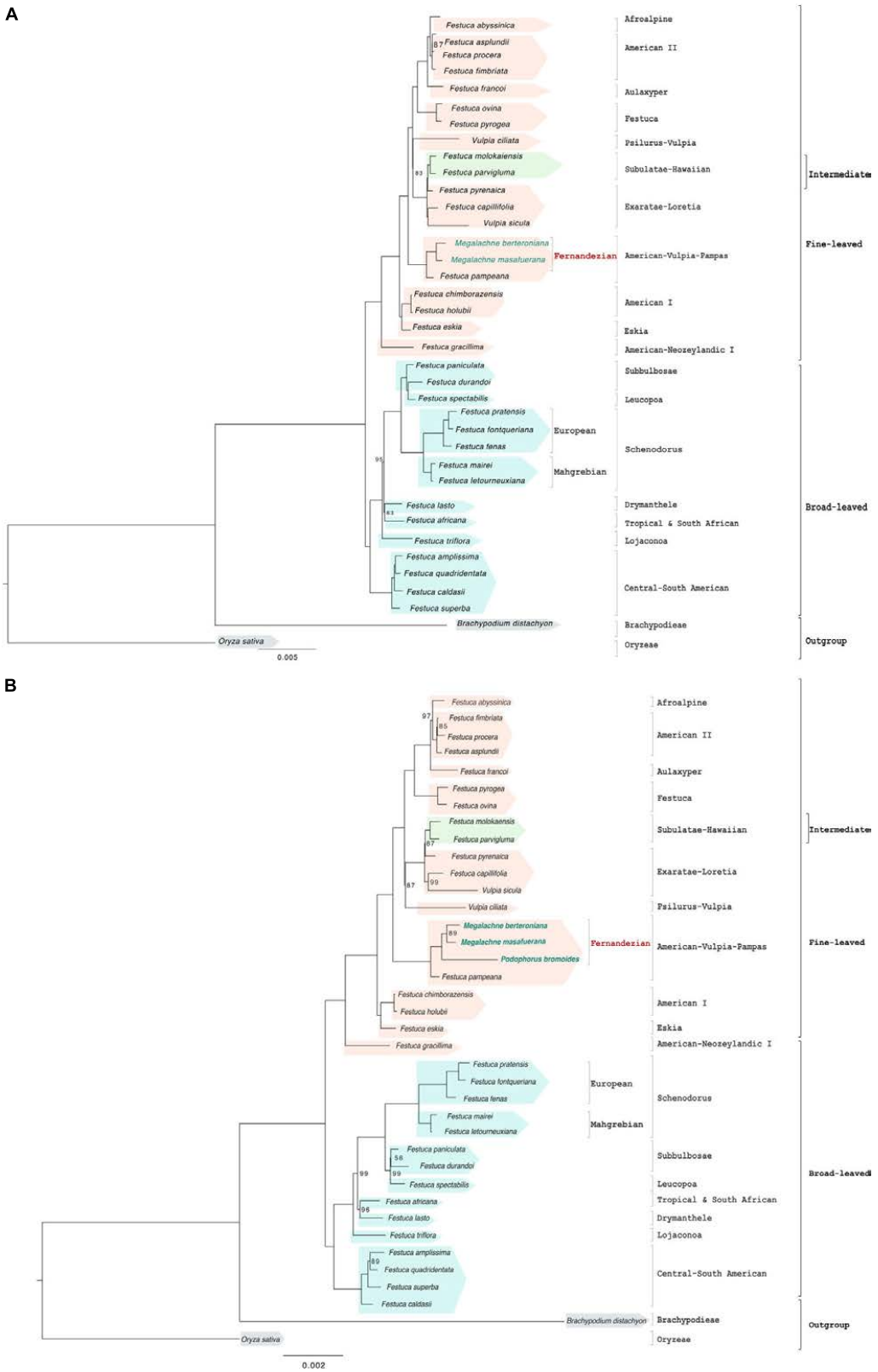

FIGURE 2 | Maximum likelihood full plastome **(A)** and reduced plastome **(B)** trees constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) <100%; the remaining branches have 100% BS value.

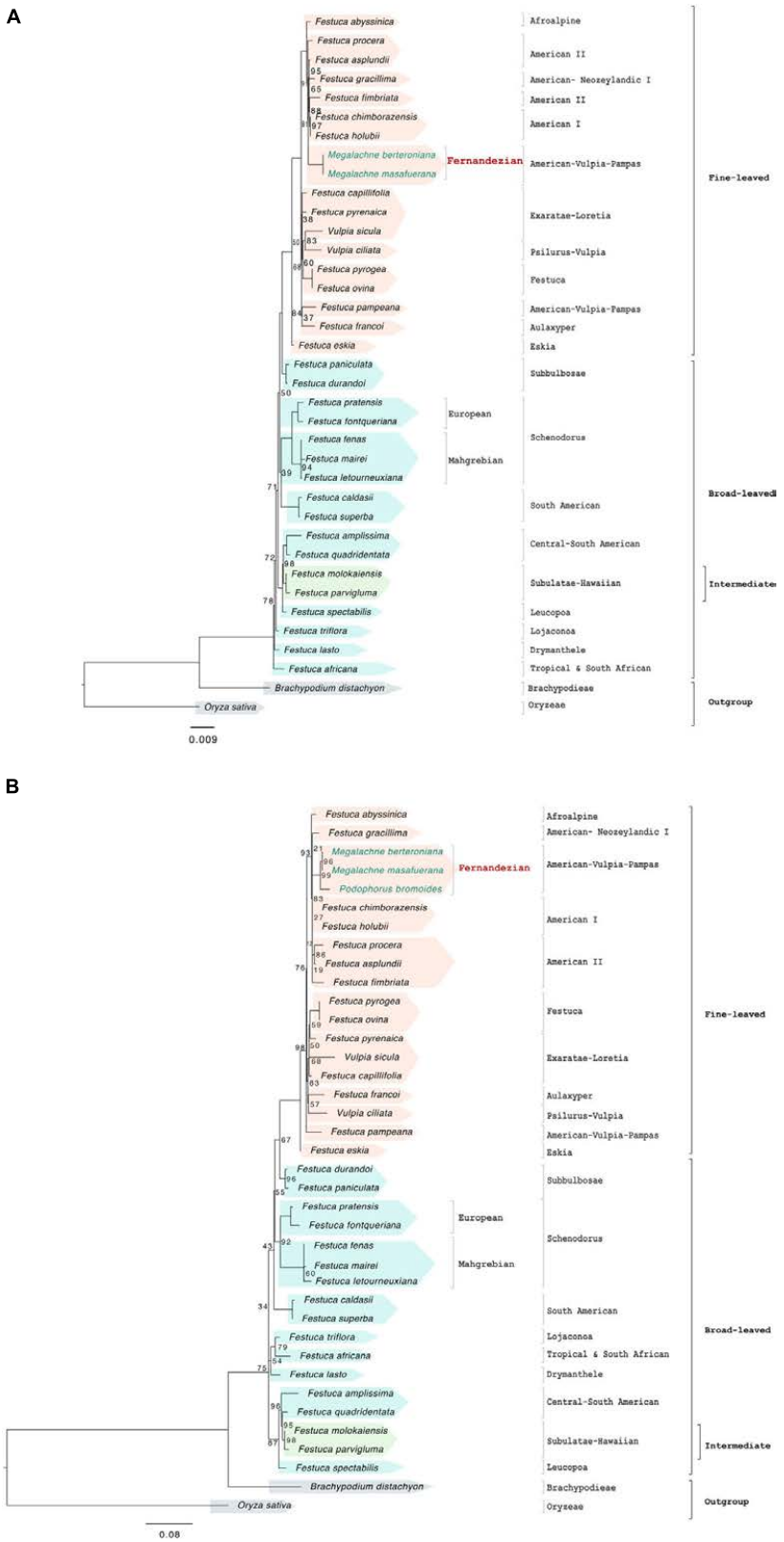

FIGURE 3 | Maximum likelihood nuclear rDNA cistron **(A)** and ITS **(B)** trees constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) <100%; the remaining branches have 100% BS value.

within the Mahgrebian Schedonorus subclade (**Figure 3A** and **Supplementary Figure S1C**). Phylogenetic reconstruction of filtered rDNA cistron sequences for the ITS region, together with that of *P. bromoides*, recovered the same overall tree topology, which showed a strong sister relationship of *P. bromoides* to the *Megalachne* clade (99% BS) (**Figure 3B** and **Supplementary Figure S1D**).

Plastid TLF, Nuclear ITS, and Combined ITS-TLF Phylogenetic Relationships

The separate and combined TLF (2,205 positions, 501 variable, 240 informative), ITS (645 positions, 285 variable, 193 informative) and ITS-TLF analyses of 135 Loliinae and outgroup samples retrieved phylogenies (**Supplementary Figures S2A–C**) highly congruent with those obtained in previous studies. Additionally, these trees showed the evolutionary placements of the three Fernandezian species and of six South American and one South African newly studied *Festuca* taxa. Both the nuclear ITS and the plastid TLF recovered a highly supported Fernandezian clade (99% BS) where *P. bromoides* was sister to the *M. berteroniana*/*M. masafuerana* subclade. Nonetheless, whereas the Fernandezian group was nested within a clade of American-Vulpia-Pampas taxa (69% BS), clearly separated from the American I (82% BS), and American II+Afroalpine (78% BS) clades in the TLF tree (**Supplementary Figure S2A**), it was nested within a large clade of American I + American II + Afroalpine taxa (99%) that also included some (*F. ventanicola*) but not all the American-Vulpia-Pampas species in the ITS tree (**Supplementary Figure S2B**). The combined ITS-TLF analysis placed the fully supported Fernandezian clade within a highly supported American-Vulpia-Pampas clade (97% BS) and resolved *F. ventanicola* as the strong sister lineage of the Fernandezian grasses (100% BS) (**Supplementary Figure S2C**). The TLF and ITS evolutionary placements of the newly sequenced South American taxa agreed with those of the plastome and rDNA trees and were overall congruent to each other. The fine-leaved *F. asplundii* and *F. procera* were nested within the American II + Afroalpine clade and *F. holubii* within the American I clade in the TLF tree (**Supplementary Figure S2A**), whereas the three of them fell within the large American I + American II + Afroalpine clade in the ITS tree (**Supplementary Figure S2B**). The sister *F. asplundii*/*F. andicola* (69% BS) and *F. holubii*/*F. glumosa* (87% BS) relationships observed in the ITS tree and their phylogenetic placements in the combined ITS-TLF tree (**Supplementary Figure S2C**) agreed with those of the plastid tree. The broad-leaved *F. quadridentata* and *F. caldasii* were nested within a large Central-South American-Eurasian-South African clade (97% BS) in the TLF tree (**Supplementary Figure S2A**) and in separate Central-South American (74% BS) and Eurasian-South American (62% BS) clades in the ITS tree (**Supplementary Figure S2B**). Their positions in the combined ITS-TLF tree (**Supplementary Figure S2C**) agreed with those of the nuclear tree. The South African *F. longipes* was resolved as sister of South African *F. scabra* (99% BS) in the TLF tree (Central-South American-Eurasian-South African clade) and of Tropical-South African *F. costata* in the ITS (100% BS) and combined ITS-TLF (88% BS) trees (Tropical-South African clade) (**Supplementary Figures S2A–C**).

Dating Analysis and Ancestral Range Inheritance Reconstruction

The Bayesian ITS-TLF MCC tree constructed with Beast2 (**Figure 4** and **Supplementary Figure S3**) yielded a similar topology to that retrieved in the ML analysis (**Supplementary Figure S2C**). The age of stem and crown Loliinae nodes were estimated to Late-Oligocene (median 21.47 Ma) and Early Miocene (19.4 Ma), respectively, whereas Early and Mid-Miocene divergences were inferred for the splits of the broad (16.31 Ma) and fine-leaved (16.83 Ma) lineages. An older Mid-Miocene origin was estimated for the ancestor of the American-Vulpia-Pampas clade (7.74 Ma) than for the younger Late-Miocene-to-Pliocene ancestors of the remaining fine-leaved [American II+Afroalpine (5.39 Ma); American I (3.89 Ma)] and broad-leaved [South-American (5.04 Ma); Central-South American (3.32 Ma)] South American Loliinae lineages (**Figure 4** and **Supplementary Figure S3**). The ancestor of the Fernandezian clade was inferred to have originated between the Late-Miocene (5.15 Ma; stem node) and the Pliocene (2.72 Ma; crown node), corresponding to the estimated split of *Podophorus* and *Megalachne*, whereas the split of the two *Megalachne* species was estimated to have occurred in the Pleistocene (1.02 Ma, Calabrian). The estimated ages of the Fernandezian ancestors predated those inferred for the ancestor of other oceanic endemic Loliinae lineages [e.g., Canarian fine-leaved Aulaxyper (4.11–2.84 Ma; Pliocene); Hawaiian *F. aloha*/*F. molokaiensis* (1.89–1.16 Ma; Lower-to-Recent Pleistocene); and recent Pleistocene Madeiran broad-leaved *F. donax* (1.23 Ma, Calabrian) and Reunion Island fine-leaved *F. borbonica* (0.3 Ma, Ionian)] (**Figure 4** and **Supplementary Figure S3**).

The ancestral range inheritance scenarios of Loliinae inferred from our Lagrange stratified Loliinae DEC model (-ln likelihood 404.6) had a global estimated dispersal rate (*dis*: 0.09385) 5.5 times higher than the estimated extinction rate (*ext*: 0.01536) (**Figure 5A**). The ancestors of Loliinae and of the broad-leaved and fine-leaved clades were inferred to have originated in uncertain widespread areas of the northern hemisphere (Mediterranean basin, Northern-Central America, Eurasia) in the transition between the Late Oligocene and the Early Miocene. Most of the transcontinental LDDs of both fine-leaved and broad-leaved Loliinae ancestors were estimated to have occurred during the Miocene and the Pliocene (time slices TSII-TSIII), and a few more during the Pleistocene (time slice TIV) (**Figure 5A**). According to our DEC model, the South American subcontinent was simultaneously colonized by broad and fine-leaved Mediterranean ancestors, which arrived, respectively, to the northern and southern South American ranges around the Mid-Miocene (**Figures 4**, **5A**). Within the fine-leaved lineage, a Mid-Miocene vicariance was inferred to have originated the American-Vulpia-Pampas ancestor in

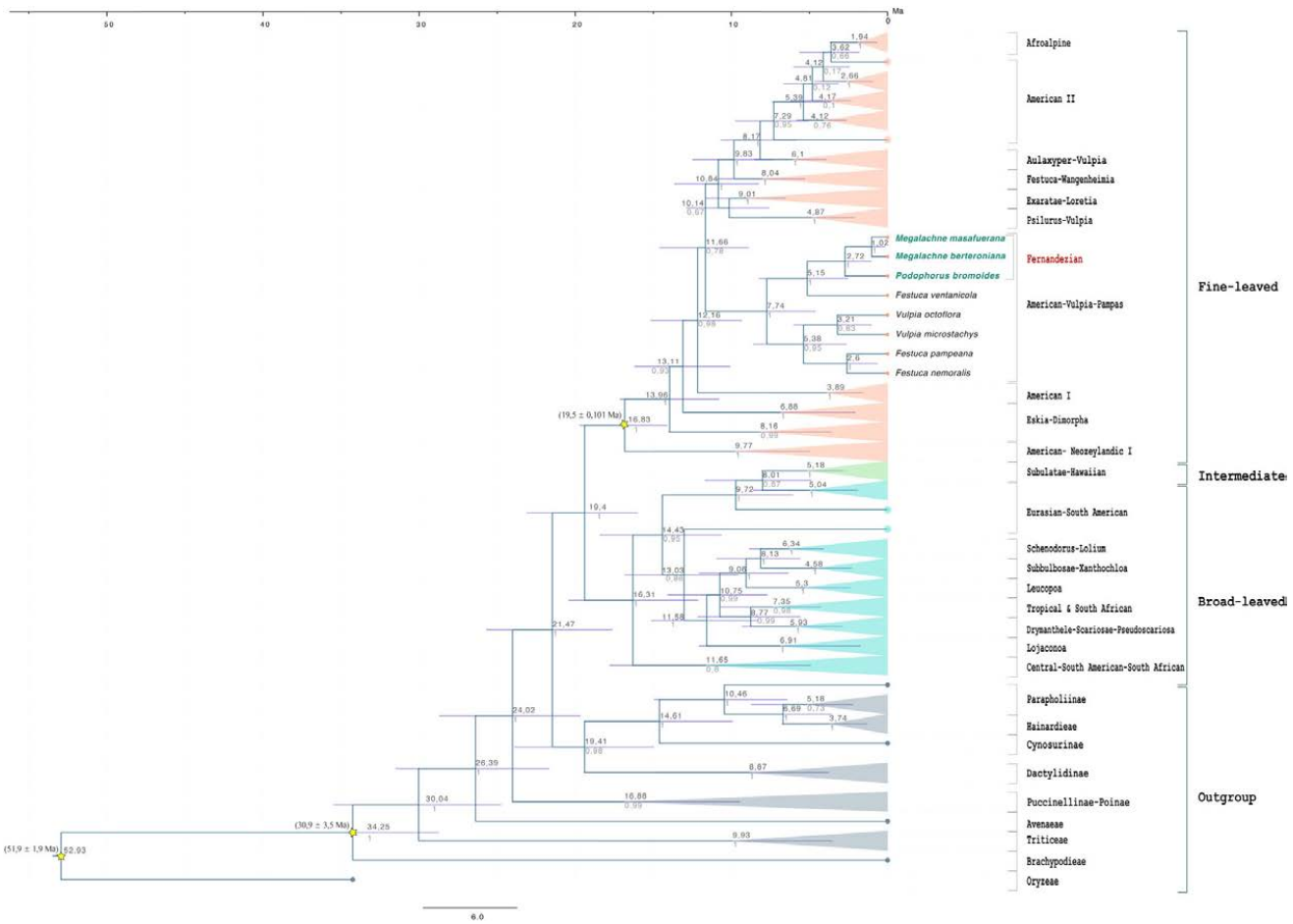

FIGURE 4 | Schematic Bayesian maximum clade credibility dated chronogram of 135 Loliinae taxa constructed with BEAST2 using nuclear ITS and plastid TLF loci showing estimated nodal divergence times (medians, in Ma) and 95% highest posterior density (HPD) intervals (bars) above branches and Posterior Probability Support (PPT) values below branches. Stars indicate secondary nodal calibration priors (means ± SD, in Mya) for the crown nodes of the BOP, *Brachypodium* + core pooids, and fine-leaved Loliinae clades.

southern South America ∼7.74 Ma. This ancestor would have then experienced range expansions to either North-Central America originating the southern American Pampean-Andean and the North-Central American Vulpia clade and to the Juan Fernandez archipelago originating the Pampean-Fernandezian clade at the end of the Neogene. Our stratified Loliinae DEC model suggested that the colonization of the Juan Fernandez archipelago from a mainland ancestor in southern South America could have occurred in the Mid-to-Late Miocene (7.74–5.15 Ma) (**Figures 4**, **5A**). According to this hypothesis, the ancestor of *F. ventanicola* and the Fernandezian *Podophorus* and *Megalachne* grasses was distributed in a widespread southern South America-Juan Fernandez area during the Late Miocene (5.15 Ma). A vicariance event was invoked to explain the split of the common ancestor into the current mainland Pampean-Ventanian endemic lineage and the Fernandezian ancestor, which was inferred to be present in the archipelago in the mid-Pliocene (2.72 Ma) (**Figures 4**, **5A**). A more detailed reconstruction of the biogeography of the Fernandezian grasses within their archipelago was obtained in our second American-Vulpia-Pampas DEC model (-ln likelihood 406.2; dis: 0.08232; ext: 0.0497) (**Figure 5B**). According to this model: (i) the ancestor of the American-Vulpia-Pampas could have been distributed in the Pampas-Ventanian range during the Miocene (7.74 Ma); (ii) this ancestor presumably experienced a range expansion to Masatierra and was present in a widespread Pampas-Fernandezian area during the Late Miocene (5.15 Ma); (iii) after a Pampas-Ventanian/Masatierra vicariance, the ancestor of the Fernandezian grasses was present in Masatierra during the Late Pliocene (2.72 Ma); (iv) an *in situ* speciation event originated the *Podophorus* lineage in Masatierra at that time; (v) a range expansion from Masatierra to Masafuera placed the ancestor of the *Megalachne* clade in the two main Juan Fernandez islands during the Pleistocene (1.02 Ma); (vi) a recent vicariance would explain the respective speciations of *M. berteroniana* in Masatierra and of *M. masafuerana* in Masafuera during the last million years (**Figures 4**, **5B**).

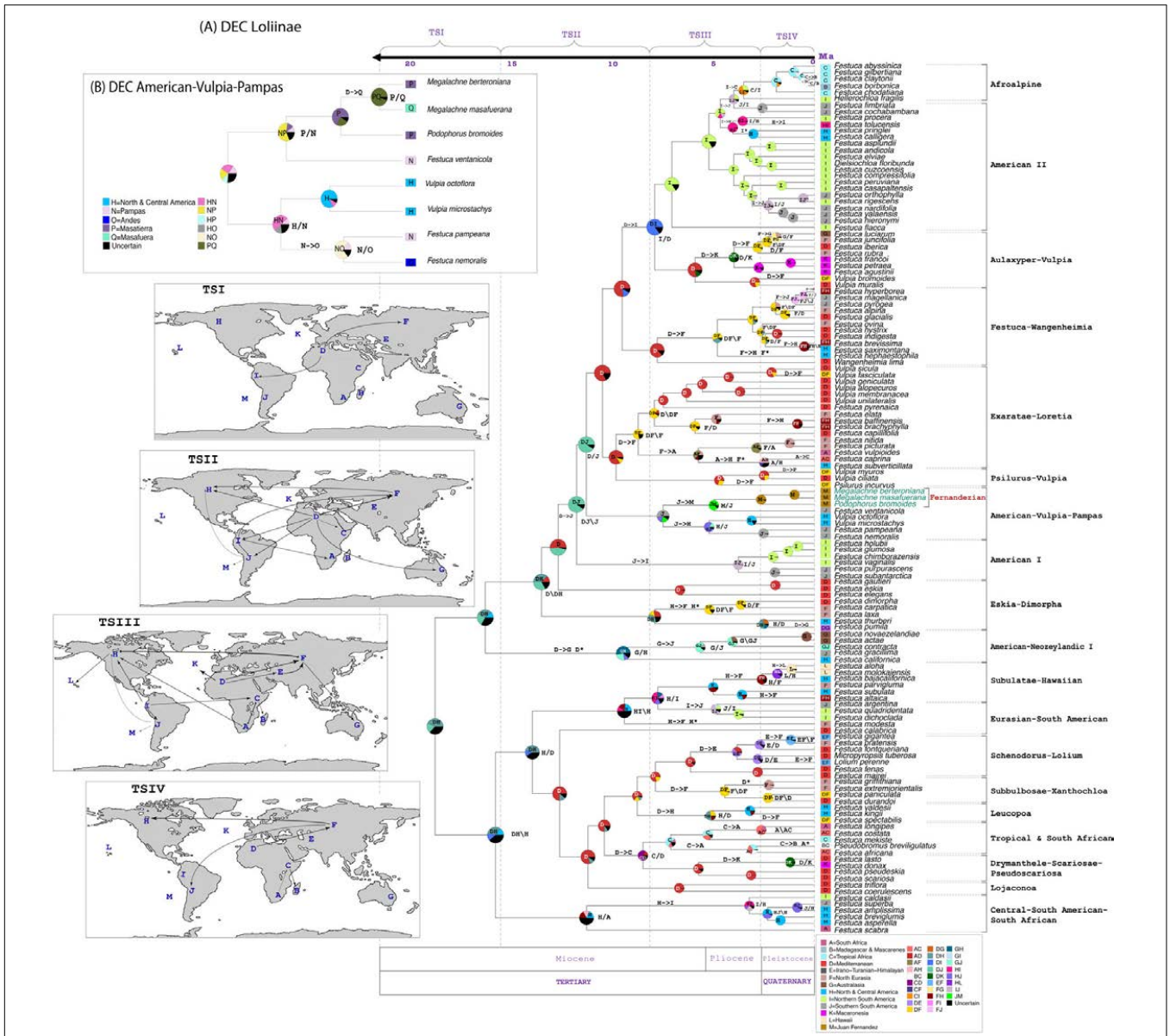

FIGURE 5 | Estimated ancestral ranges and biogeographical events of the Fernandezian and other Loliinae grasses inferred from LAGRANGE under the stratified DEC models mapped on the BEAST2 maximum clade credibility tree with outgroups pruned from it. **(A)** Loliinae DEC model; **(B)** American-Vulpia-Pampas DEC model. The vertical dashed lines separate the four time slices (TSI-TSIV) used in the Lagrange analyses. The maps on the left represent the palaeogeographical configuration of the world in these four time periods and the arrows represent the dispersals between areas that reflect change in continental connectivity over time. The pie charts at the nodes indicate the relative probabilities for alternative ancestral ranges (with their color legends indicated at the respective inset charts). The inferred biogeographic events are indicated at the nodes (X/Y vicariance; X\Y peripheral isolation) and branches (X->Y dispersal; X* extinction) of the tree. The Operational Areas assigned to the species are indicated to the right of the trees.

DISCUSSION

Phylogenetics of *Megalachne* and *Podophorus*: The Loliinae Fernandezian Clade

Our museomic approach, based on the combined use of old and recent herbarium samples and of genome skim data, have allowed us to disentangle the evolutionary origins of the neglected *Megalachne* and *Podophorus* grasses. Complete and partial plastomes as well as the nuclear rDNA cistron and ITS data supported the phylogenetic placement of the studied Fernandezian *Podophorous bromoides*, *Megalachne berteroniana*, and *M. masafuerana* species within the American-Vulpia-Pampas fine-leaved Loliinae clade (**Figures 2**, **3** and **Supplementary Figures S1A–C**). Our results corroborate the early suggestions of Tateoka (1962) who indicated a close

affinity of *Megalachne* to *Festuca* based on shared morphological and serological traits, and the recent phylogenetic findings of Schneider et al. (2011, 2012) and Tkach et al. (2020) who placed them within the fine-leaved Loliinae, and definitively discard its classification within either Bromeae or Duthieinae. Our results have also contributed to enlarge the paraphyly of *Festuca*, which now accounts to up to 14 Loliinae genera nested within its main fine-leaved (*Ctenopsis, Dielsiochloa, Hellerochloa, Megalachne, Micropyrum, Narduroides, Podophorus, Psilurus, Vulpia, Wangeheimia*), intermediate (*Castellia*), and broad-leaved (*Lolium, Micropyropsis, Pseudobromus*) lineages (**Supplementary Figure S2C**; Inda et al., 2008; Minaya et al., 2017).

Our study has demonstrated the utility of museomics to disentangle the evolutionary history of the extinct *Podophorus bromoides* from its 164 years old type specimen. This adds a new extinguished species to the tree-of-life, resolving its phylogenetic position within the grasses, as done before for other exterminated plants, such as *Sicyos villosus* within Cucurbitaceae (Sebastian et al., 2010) *Hesperelaea palmeri* within Oleaceae (Van de Paer et al., 2016; Zedane et al., 2016), *Haplostachys linearifolia* and *Stenogyne haliakalae* within Lamiaceae (Welch et al., 2016) and *Chasechloa egregia* within Poaceae (Silva et al., 2017). Moreover, our phylogenetic analyses based on plastome and rDNA-based data have demonstrated that *P. bromoides* is strongly resolved as sister to the *Megalachne* clade (*M. berteroniana*, *M. masafuerana*) (**Figures 2B**, **3B** and **Supplementary Figures S2A–C**), rejecting thus the moderately supported sister relationship found for the Masatierra taxa (i.e., *P. bromoides* and *M. berteroniana*, 72% BS) in a previous phylogenetic analysis based on partial ITS sequences from some samples (*Podophorus bromoides* ITS1 only) (Schneider et al., 2011).

Our Loliinae-wide phylogenomic analyses have further identified the relict Pampean-Ventanian fescues as the closest relatives of these fine-leaved endemic Fernandezian grasses. Phylogenies based on complete and partial plastome data indicate that *Megalachne* and *Podophorus* are strongly related to the American-Vulpia-Pampas lineage, represented by *F. pampeana* (**Figure 2** and **Supplementary Figure S2A**). By contrast, the nuclear rDNA cistron and the ITS phylogenies place them within a large American I + American II group (**Figure 3** and **Supplementary Figure S2B**), an assemblage that also includes other American-Vulpia-Pampas species, such as *F. ventanicola* (**Supplementary Figure S2B**). However, the phylogenetic tree reconstructed with the combined ITS-TLF data strongly supports nesting the Fernandezian clade within the American-Vulpia-Pampas clade and its sister relationship to the Pampean-Ventanian endemic *F. ventanicola* (**Figure 4** and **Supplementary Figures S2C, S3**). The incongruent placements of the Fernandezian grasses in the maternal plastome (plastid) vs. paternal rDNA cistron (ITS) Loliinae trees is a general feature of many Southern Hemisphere Loliinae species that reflect their hybrid allopolyploid nature (Inda et al., 2008; Minaya et al., 2017). Evolutionary studies have illustrated the different topological placements of known allopolyploid Loliinae species in plastid vs. nuclear trees (e.g., allotetraploid *F. fenas*, allohexaploid *F. arundinacea*, Inda et al., 2014; allotetraploid *F. simensis*, Inda et al., 2014; Minaya et al., 2015; allohexaploid *F. nigrescens*, Kergunteuil et al., 2020). Karyological and genome size reports have further shown that all southern hemisphere Loliinae species studied so far are polyploids (Dubcovsky and Martínez, 1992; Connor, 1998; Namaganda et al., 2006; Smarda and Stancik, 2006). Therefore, the incongruent positions shown by the American I clade polyploids *F. chimborazensis* (4*x*), *F. vaginalis* (4*x*), *F. glumosa* (4*x*), *F. purpurascens* (6*x*), American-Vulpia-Pampas clade *F. ventanicola* (4*x*) and the putative South African polyploid *F. longipes* in our plastid and nuclear trees (**Supplementary Table S1** and **Supplementary Figures S2A,B**) indicate that these taxa probably originated from interspecific hybridization followed by genome doubling. Although genome size or chromosome counting data are lacking for the Fernandezian *P. bromoides* and *M. berteroniana* and *M. masafuerana* species, their equivalent contrasting positions in the plastid and nuclear trees suggest that these endemic grasses are also allopolyploids. It is further supported by the fact that most of the remaining members of the American-Vulpia-Pampas clade are also polyploids [e.g., *F. pampeana* (8*x*), *F. nemoralis* (8*x*), *V. microstachys* (6*x*); Dubcovsky and Martínez, 1992; Smarda and Stancik, 2006; Díaz-Pérez et al., 2014]. Further investigation of these genomic data using the methodology described in Viruel et al. (2019) together with customized genome size analyses from fresh or herbarium samples (Smarda and Stancik, 2006) might reveal the ploidy level of these rare taxa.

Megalachne and *Podophorus* show a "vulpioid" phenotype, having lax panicles and long awned lemmas (**Figure 1**). These are characteristic traits of *Vulpia* and few other Loliinae lineages (Catalan et al., 2007). *Vulpia* and other ephemeral Loliinae genera, such as *Ctenopsis*, separate from *Festuca* based on their annual habit, four or less fertile florets per spikelet, largely unequal glumes, and long awned lemmas, which together distinguish them from the typical festucoid phenotype of *Festuca* and other robust Loliinae, characterized by their perennial habit, four or more fertile florets per spikelet, subequal glumes, and muticous or usually shortly awned lemmas, though none of them is absolute (Catalan et al., 2007). The origins of the polyphyletic *Vulpia* lineages are still intriguing although analysis of cloned single copy genes have demonstrated that some allopolyploid *Vulpia* species bear heterologous copies derived from morphologically close diploid relatives (Díaz-Pérez et al., 2014). The homoplasic "vulpioid" inflorescence phenotype has also appeared in other perennial Loliinae lineages, like the northern South America *Dielsiochloa floribunda* (American II clade), and in some species of *Festuca*. Interestingly, the slender cespitose Pampean-Ventanian endemic *F. ventanicola* shares its "vulpioid" phenotype with its sister Fernandezian *Megalachne* and *Podophorus* taxa (**Figure 1**), suggesting that they could have inherited it from their common ancestor. The long awn is an important dispersal trait in several annual grasses, including the invasive *Vulpia* species (Catalan et al., 2007; Díaz-Pérez et al., 2014), allowing the caryopsis to attach to the feathers or furs of animals and to be dispersed to long distances (Linder et al., 2018). It could be thus hypothetised that the

presumed "vulpioid" ancestor of the Fernandezian grasses could have migrated to the isolated Juan Fernandez archipelago transported by epizoochory or endozoochory through pelagic birds. Interestingly, *Podophorus bromoides* shows an extremely reduced spikelet (**Figure 1**), being the only Loliinae taxon, together with *Vulpia fontquerana* Melderis & Stace (Torrecilla et al., 2004) having a single fertile floret (with a reduced sterile floscule) per spikelet. This, together with its apparent ephemeral habit might be associated to an overall trend toward an annual habit after its speciation in the Masatierra island (**Figures 1**, **5**).

Biogeography and Conservation of the Endemic *Megalachne* and *Podophorus* Grasses

Our Loliinae and American-Vupia-Pampas biogeographic DEC analyses have elucidated the most likely colonization routes of the Fernandezian ancestors, and the speciation events that originated *Podophorus* and *Megalachne* taxa in Masatierra and Masafuera (**Figures 1**, **5**). Our ancestral range analyses identified the Pampean-Ventanian region to be the most likely place of origin for the common ancestors of the Fernandezian endemic grasses (**Figures 5A,B**). The closest relatives of *Podophorus* and *Megalachne* are relict endemic species of the Ventanian region (*F. ventanicola*, *F. pampeana*; Catalan and Müller, 2012) a hotspot of plant and animal diversity (Crisci et al., 2001). The formation of the Ventanian range in the Paleoproterozoic-Ordovician time span (∼2,200–475 Ma; Ramos et al., 2014) largely preceded the Oligocene-Miocene uplifting of the North American (31–28 Ma) and Central-Southern Andean (10–5 Ma) cordilleras (Crisci et al., 2001; Wakabayashi and Sawyer, 2001) as well as the emergence of the volcanic Juan Fernandez archipelago islands (5.8 Ma) (Stuessy et al., 1984). Although the inferred ages of the American-Vulpia-Pampas clade (7.7 Ma), *F. ventanicola* + Fernandezian clade (5.1 Ma) and Fernandezian clade (2.7 Ma) ancestors (**Figure 4** and **Supplementary Figure S3**) are younger than those of the Central-Southern Andes, the altitude and disposition of the austral Andean mountains was probably lower than in the present (Crisci et al., 2001). The geological time layout could have facilitated the hypothetical LDDs of the Ventanian ancestors to other American ranges and to the Juan Fernandez archipelago (**Figures 1**, **5**). Our Loliinae and American-Vulpia-Pampas DEC models support a colonization of the Fernandezian archipelago from a southern South American Pampean-Ventanian ancestor in the late-Miocene 7.7–5.1 Ma (**Figures 4**, **5**). The most recent estimate for that colonization concurs with the radiometric dating of the oldest Fernandezian islands (Santa Clara, 5.8 ± 2.1 Ma; Masatierra, 4.23 ± 0.16 Ma) (Stuessy et al., 1984) which could have been united in the past (Sanders et al., 1987). We could thus infer that the Ventanian Fernandezian ancestors likely arrived at the paleo-island formed by Santa Clara and Masatierra during the Late Miocene (**Figure 5**), probably transported by birds. The estimated split of the *Podophorus* lineage from the *Megalachne* ancestor at 2.7 Ma suggest a late-Pliocene *in situ* speciation event in Masatierra for the origin of the endemic *P. bromoides* (**Figure 4**). Our regional DEC model and our dating analyses infer that the colonization of the Masafuera island occurred from Masatierra during recent Pleistocene times (1.02 Ma), supporting *in situ* speciation events for *M. berteroniana* in Masatierra and *M. masafuerana* in Masafuera (**Figures 4**, **5B**). The westward inter-island colonization likely took place after the emergence of the young Masafuera island in the early Pleistocene (2.44 ± 1.14 Ma) (Stuessy et al., 1984) and was probably favored by the short distance separating them (i.e., 180 km, **Figure 1**). This distance has acted, however, as a strong geographic barrier to gene flow since the divergence of both species. Our biogeographic reconstruction for the Fernandezian Loliinae taxa agree with the hypothesis of higher levels of plant endemism in Masatierra compared to Masafuera, which are related to their respective distances to the closest mainland and their estimated ages (Stuessy et al., 2017). Our study has also identified the previously unknown South American ancestors of these endemic Fernandezian grasses, pointing to the relict Pampean-Ventanian region as their cradle (**Figure 5B**).

The rich endemic flora of Juan Fernandez archipelago is one of the most threatened on earth (Stuessy et al., 1998; Bernardello et al., 2006). Human impact on these islands, such as the introduction of environmentally aggressive herbivores, has probably caused the extinction of at least two endemic Fernandezian endemic plants during the last two centuries (*Santalum fernandezianum* Phil. and *Podophorus bromoides*; Bernardello et al., 2006; Danton et al., 2006). The latter extinct species was extremely rare; collected by Germain in 1854 and described by Philippi in 1856 from Masatierra (without a specific locotype), its existence was later mentioned by Johow in 1896 (Baeza et al., 2007). However, the plant was never seen again, even after exhaustive searches, and was therefore considered extinct (Stuessy et al., 1998, 2017; Baeza et al., 2007). All four *Megalachne* species are classified as threatened according to the IUCN categories of threat (Danton et al., 2006; Danton and Perrier, 2017; Penneckamp-Furniel, 2018; Penneckamp-Furniel and Villegas, 2019): *M. berteroniana* as Vulnerable, *M. masafuerana* as Endangered, *M. dantonii* as Critically Endangered, and *M. robinsoniana* as Endangered. Nonetheless, these IUCN assessments did not include a description of the employed IUCN criteria to classify the plants in their respective categories of menace. Several authors, however, have severe concerns about the threats posed to these endemic grasses by the introduced herbivores and by invasive plants (Stuessy et al., 1998; Bernardello et al., 2006; Danton et al., 2006; Danton and Perrier, 2017) and their survival in some inaccessible places to overgrazing pressure (Danton et al., 2006; Danton and Perrier, 2017). Rigorous populations censuses and population genetic studies of the more largely distributed *M. berteroniana* and *M. masafuerana* species, and of the recently described and still poorly known *M. robinsoniana* and *M. dantonii* species would be required to establish their adequate category of threat and to design appropriate conservation strategies. Historical collections have an enormous value for biogeographical studies. Several plants have gone to extinction in a few decades after human arrival due their high sensitivity to perturbation of their

habitats and their low competitiveness, especially in oceanic islands (Sebastian et al., 2010; Van de Paer et al., 2016; Welch et al., 2016; Zedane et al., 2016; Silva et al., 2017). Regrettably, *Podophorus bromoides* sums up to the list of recently extinct plants although its museomic analysis has unveiled its historical biogeography.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

PC designed the study. MM-A, IA, JV, and PC collected the samples. MM-A and JV developed the experimental work. PC, MM-A, JV, IA, and AS-R analyzed the data, interpreted the results, and revised the manuscript. PC and MM-A prepared the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00819/full#supplementary-material

FIGURE S1 | (A) Maximum likelihood full plastome cladogram (35 Loliinae taxa, *Podophorus* excluded) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS). **(B)** Maximum likelihood reduced plastome cladogram (36 Loliinae taxa, *Podophorus* included) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS). **(C)** Maximum likelihood nuclear rDNA cistron cladogram (35 Loliinae taxa, *Podophorus* excluded) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS). **(D)** Maximum likelihood nuclear ITS cladogram (36 Loliinae taxa, *Podophorus* included) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).

FIGURE S2 | (A) Maximum likelihood nuclear TLF tree (135 Loliinae taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) <100%; the remaining branches have 100% BS values. **(B)** Maximum likelihood plastid ITS tree (135 Loliinae taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) <100%; the remaining branches have 100% BS values. **(C)** Maximum likelihood combined ITS-TLF tree (135 Loliinae taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. *Oryza sativa* was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).

FIGURE S3 | Fully expanded Bayesian maximum clade credibility dated chronogram of 135 Loliinae taxa constructed with BEAST2 using nuclear ITS and plastid TLF loci showing estimated nodal divergence times (medians, in Ma) and 95% highest posterior density (HPD) intervals (bars) above branches and Posterior Probability Support (PPT) values below branches. Stars indicate secondary nodal calibration priors (means ± SD, in Mya) for the crown nodes of the BOP, *Brachypodium* + core pooids, and fine-leaved Loliinae clades.

TABLE S1 | List of taxa included in the phylogenetic study of the Fernandezian and other Loliinae grasses. Taxon, source, ploidy level, nuclear ITS, plastid *trn*TL and *trn*LF, plastome and rDNA cistron Genbank codes, average alignment insert size, total number of pair-end reads, number of plastome assembled reads, and number of rDNA cistron assembled reads are indicated for the corresponding samples.

TABLE S2 | (A) Operational areas used in the stratified Loliinae DEC Lagrange analysis. **(B)** Dispersal rate matrices reflecting the palaeogeographic connectivity among the study areas in each historical scenario (time slices TSI, TSII, TSIII, TIV). **(C)** Operational areas used in the stratified American-Vulpia-Pampas DEC Lagrange analysis. **(D)** Dispersal rate matrices reflecting the palaeogeographic connectivity among the study areas in each historical scenario (time slices TSIII, TIV).

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C.2. Evolutionary Dynamics of the Repeatome Explains Contrasting Differences in Genome Sizes and Hybrid and Polyploid Origins of Grass Loliinae Lineages

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Evolutionary Dynamics of the Repeatome Explains Contrasting Differences in Genome Sizes and Hybrid and Polyploid Origins of Grass Loliinae Lineages

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The repeatome is composed of diverse families of repetitive DNA that keep signatures on the historical events that shaped the evolution of their hosting species. The cold seasonal Loliinae subtribe includes worldwide distributed taxa, some of which are the most important forage and lawn species (fescues and ray-grasses). The Loliinae are prone to hybridization and polyploidization. It has been observed a striking two-fold difference in genome size between the broad-leaved (BL) and fine-leaved (FL) Loliinae diploids and a general trend of genome reduction of some high polyploids. We have used genome skimming data to uncover the composition, abundance, and potential phylogenetic signal of repetitive elements across 47 representatives of the main Loliinae lineages. Independent and comparative analyses of repetitive sequences and of 5S rDNA loci were performed for all taxa under study and for four evolutionary Loliinae groups [Loliinae, Broad-leaved (BL), Fine-leaved (FL), and Schedonorus lineages]. Our data showed that the proportion of the genome covered by the repeatome in the Loliinae species was relatively high (average ∼ 51.8%), ranging from high percentages in some diploids (68.7%) to low percentages in some high-polyploids (30.7%), and that changes in their genome sizes were likely caused by gains or losses in their repeat elements. Ty3-gypsy Retand and Ty1-copia Angela retrotransposons were the most frequent repeat families in the Loliinae although the relatively more conservative Angela repeats presented the highest correlation of repeat content with genome size variation and the highest phylogenetic signal of the whole repeatome. By contrast, Athila retrotransposons presented evidence of recent proliferations almost exclusively in the *Lolium* clade. The repeatome evolutionary networks showed an overall topological congruence with the nuclear 35S rDNA phylogeny and a geographic-based structure for some lineages. The evolution of the Loliinae repeatome suggests a plausible scenario of recurrent allopolyploidizations followed by diploidizations that generated the large genome sizes of BL diploids as well as large genomic rearrangements in highly

hybridogenous lineages that caused massive repeatome and genome contractions in the Schedonorus and Aulaxyper polyploids. Our study has contributed to disentangling the impact of the repeatome dynamics on the genome diversification and evolution of the Loliinae grasses.

Keywords: diploidized paleo-allopolyploids, genome size diversification, Festuca, Lolium, phylogenetic signal, repeatome, transposable elements, 5S loci

INTRODUCTION

Comparative genomic studies have demonstrated that the repetitive DNA fraction is largely present in the nuclear genome of most plants (Pellicer et al., 2018). It is composed of diverse families of mobile elements (retrotransposons and transposons), which constitute the bulk of the predominant repeats, and of tandem satellite repeats, which can make up 10–20% of the genome (Macas et al., 2015). Although the constitution of the repetitive elements is complex and differs, sometimes by some orders of magnitude, among taxa (Hidalgo et al., 2017), there is an overall agreement on the impact that the dynamics of the repetitive elements have had in the variation of the genome size and its evolution across the angiosperms (Dodsworth et al., 2015; Pellicer et al., 2018). Alternative hypotheses have been launched to explain both the causes and the mechanisms of the plant repeatome turnovers. The "polyploid genome shock" hypothesis that postulates genomic reshuffling and mobility of the repetitive elements in hybrid and polyploid plants as a response to the sudden combination of distinct genomes and multiple copies of them (McClintock, 1984) has resulted, in some cases, in a rapid increase of repeats in the genomes after rounds of polyploidizations. The resulting polyploid genomes show additive patterns and equivalent genome size expansions (McCann et al., 2018). However, other plants do not show a proliferation of the repetitive elements in the allopolyploids, or only a gradual and low increase or decrease in their derived subgenomes (Chen et al., 2020). In contrast, other plant groups have experienced the opposite trend, with high-level polyploids exhibiting a drastic reduction in genome size and a considerable shrinkage of their repeatome relative to that of their diploid and low-level polyploid relatives (Chen, 2007; Parisod et al., 2010). The removal of the repetitive elements from the genome, attributed to several recombination mechanisms, and the driven forces that balance the expansions and contractions of the repeatome are still poorly known (Fedoroff, 2012; Drouin et al., 2021). In some exhaustively studied plants (*Gossypium*, *Brachypodium*) the abundance of some retrotransposon families and their apparent facility to proliferate (e.g., centromeric transposons) are interpreted as causing increased genome size, while the ability of other families to recombine and lose repeats are considered potential mechanisms for maintaining reduced genome size (Chen et al., 2020; Stritt et al., 2020). The dynamics of some repetitive elements, especially transposable elements (TEs) insertions, has been also related to the expression of some core or dispensable genes, although their mobility does not seem to substantially affect their regulation (Gordon et al., 2017) but can be affected by epigenetic effects (Chen, 2007; Fedoroff, 2012; Negi et al., 2016).

A comprehensive repetitive DNA analysis of plant genomes is still hampered by the unavailability of assembled and annotated genomes for many groups with complex and large genomes (Michael, 2014). In most cases it has been circumvented by using genome skim approaches and repeatome graph-topology analysis (Weiss-Schneeweiss et al., 2015; Garcia et al., 2020). Several studies have demonstrated that similarity-based clustering of low coverage genome sequencing reads, which confidentially represent 0.50–0.01× of the total haploid genome coverage, is proportional to the genomic abundance and longitude of the corresponding repeat-types (Macas et al., 2015; Pellicer et al., 2018) and could therefore be used to quantify them. The utility of the Repeat Explorer 2 bioinformatics tools for the quantification and annotation of repeats in plants (Novák et al., 2020) has been implemented by phylogenetic and distance-based network methods and by multivariate statistical methods that have corroborated the phylogenetic signal of the repeatome in various groups of angiosperm (Vitales et al., 2020a,b; Herklotz et al., 2021). It has also been supplemented by 5S rDNA graph-based clustering methods which have successfully corroborated the identity of the ancestral progenitor genomes of several polyploid plants (Garcia et al., 2020; Vozárová et al., 2021).

The grass subtribe Loliinae (*Festuca* and other close genera, like *Lolium*) constitutes one of the main lineages of the temperate pooids, both in number of species and in ecological and economic importance (Catalán, 2006; Kopecký and Studer, 2014). The Loliinae include more than 600 accepted species, Catalán (2006; Plants of the World On-line[1], accessed 3rd May 2022) which are distributed in cool seasonal and tropical mountainous regions of the five continents (Minaya et al., 2017; Moreno-Aguilar et al., 2020). The Loliinae species have large genomes ranging from 4.1 Gbp/2C to 23.6 Gbp/2C (Loureiro et al., 2007; Šmarda et al., 2008). Although these taxa show a uniform chromosome base number of $x = 7$ and ploidy levels ranging from diploids to dodecaplois, they exhibit striking differences in monoploid genome sizes, showing a 2.5-fold range decrease in chromosome size and C-values from more ancestral BL lineages (Drymanthele, Scariosae, Subbulbosae) to more recently evolved FL lineages (Festuca, Aulaxyper) (Catalán, 2006; Šmarda et al., 2008). In contrast, the heterochromatin pattern is inversely correlated with the genome size pattern, showing a rank increase of 7.5 between the same groups. However, this pattern is not homogeneous, as the early diverging fine-leaved Eskia lineage and the recently evolved broad-leaved Schedonorus-Lolium lineage

[1]http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:328907-2

revealed independent intermediate karyotype patterns between the BL and FL groups (Catalán, 2006). Genome size analyses of Loliinae and other close Poeae suggested that the ancestor of Loliinae probably underwent a two-fold genome size enlargement (and parallel GC enrichment) relative to its close relatives, which was later followed by dramatic reductions, especially in the rapidly evolving FL Loliinae group (Šmarda et al., 2008). Nonetheless, alternative scenarios could involve large genome size increase only in the BL lineage or parallelisms in the most ancestral BL and FL lineages (Catalán, 2006). A genome downsizing trend has been detected in the fine-leaved Loliinae and in the polyploids, for which more pronounced genome losses have been hypothesized to have occurred in allopolyploids with large progenitor genomes than in autopolyploids with small progenitor genomes (Loureiro et al., 2007; Šmarda et al., 2008). However, none of these hypotheses have been tested yet through genomic analyses. There is a general lack of knowledge on the repetitive elements of the Loliinae genomes except for some chromosome barcoding markers in meadow fescue (Křivánková et al., 2017; Ebrahimzadegan et al., 2019) and the characterization of repeats and centromeric elements in eight species of tall fescues and relatives (Zwyrtková et al., 2020). Apart from these works, no other study has exhaustively explored the composition and dynamics of repetitive elements through a complete representation of the Loliinae.

Here, we have investigated the repeatome of 47 representatives of all the phylogenetic lineages recognized so far within the Loliinae (Inda et al., 2008; Minaya et al., 2017; Moreno-Aguilar et al., 2020) aiming to elucidate the potential role of repeats in the striking differences in genome size and in the evolution of both genomes and species. The objectives of our study are: (i) to characterize and quantify the repetitive elements of representatives of the BL and FL Loliinae and identify single or preponderant repeats in some groups; (ii) to test the plausible correlation between genome size and abundance of the repeats; (iii) to identify repeat types that could have contributed to the expansions or contractions of genomes and their relationships with the ploidy levels, the nature of the polyploidy and the phylogenetic positions of the groups; (iv) to assess the phylogenetic value of repeats using phylogenetic reconstructions and phylogenetic signal approaches; and (v) to test alternative hypotheses about which lineages were affected by repeat proliferation or contraction and the putative paleo-hybrid origin of BL diploids with large genome sizes using mobile and satellite repeat data analysis.

MATERIALS AND METHODS

Sampling, Cytogenetic Data and Genome Skim Sequencing

Forty-seven samples of diploid and polyploid taxa of Loliinae, representing its main broad-leaved (BL, 13 samples), fine-leaved (FL, 17) and Schedonorus (17) groups, were used in the study [**Table 1** and **Supplementary Table 1** (taxonomic ranks and authorships)]. Classification of samples into groups was based on previous phylogenetic frameworks (Minaya et al., 2017; Moreno-Aguilar et al., 2020). The sampling included taxa analyzed genomically for the first time within the BL (*Festuca scabra*, South African lineage; *F. mekiste*, Tropical Africa lineage) and FL (*F. rubra*, Aulaxyper lineage) groups plus the genome skim data generated in a previous study for representatives of other BL and FL lineages (Moreno-Aguilar et al., 2020). We obtained a large taxonomic representation of the Schedonorus group through the additional sequencing of species not studied molecularly (*F. dracomontana*, *F. gudoschnikovii*, *Lolium saxatile*) or genomically (*F. gigantea*, *F. simensis*, *Micropyropsis tuberosa*) before, and from a wide coverage of other tall fescues (*F. arundinacea*, *F. atlantigena*) and raygrasses (*L. canariense, L. perenne, L. persicum, L. rigidum*) (**Table 1** and **Supplementary Table 1**). The 47 selected taxa represent the 20 evolutionary lineages currently recognized within the Loliinae (Minaya et al., 2017; Moreno-Aguilar et al., 2020). They constitute a suitable test-bed case for investigating the putative role of repeat type dynamism in the genomic evolution of the major Loliinae lineages and their contrasting changes in genome size (Catalán, 2006; Šmarda et al., 2008). They could be also used to assess the potential phylogenetic value of the repeat elements at the subtribal level.

Cytogenetic knowledge of Loliinae taxa varies enormously. Besides relatively well scrutinized groups of economic importance, like some members of the Schedonorus, Aulaxyper, and Festuca lineages (Catalán et al., 2004; Šmarda et al., 2008; Minaya et al., 2017), cytogenetic data are missing for other species, especially for taxa from poorly studied taxonomic groups or less explored areas (Catalán, 2006). Chromosome number ($2n$) and genome size (2C/pg) data were estimated for some of the studied samples using DAPI-stained meristematic root cells and flow cytometry analysis following the protocols of Jenkins and Hasterok (2007) and Doležel et al. (2007), respectively. Chromosome staining was performed with the DAPI fluorescent marker (4′,6-diamino-2 phenylindole) and counts were done using a Motic BA410 fluorescence microscope. The nuclear DNA content of *F. asplundii, F. caldasii, F. chimborazensis, F. fontqueri* and *F. procera* were calculated from silica gel dried leaves using nuclei isolated from similarly processed leaves of *Pisum sativum* L. "Ctirad" (9,09 pg/2C) as standard. Nuclei were stained with propidium iodide and samples were analyzed using a CyFlow Ploidy Analyser SYSMEX. At least 5,000 nuclei were analyzed per sample and each sample (two replicates) was analyzed three times. Only measurements with coefficient of variation $< 3.5\%$ were recorded. Ploidy levels were inferred from chromosome counts ($2n$) and GS estimations performed in the same accessions used in our genomic study and through contrasted GS and $2n$ values obtained in conspecific accessions that showed similar values. However, cytogenetic data is still lacking for some unstudied species that could only be analyzed genomically using museomic approaches (Moreno-Aguilar et al., 2020; **Table 1** and **Supplementary Table 1**).

Total DNA for the 15 newly sampled Loliinae taxa was extracted from herbarium specimens (MHU, PRE, UZ, VLA) and silica gel dried leaf tissues from plants growing in the University of Zaragoza – High Polytechnic School of Huesca common garden (**Supplementary Table 1**). Isolation of DNA and its

concentration quantification and quality evaluation for genome skimming sequencing was performed following the procedures indicated in Moreno-Aguilar et al. (2020). PCR free libraries were quantified by Library Quantification Kit for Illumina Platforms (Roche Kapa Biosystems). Genomic sequencing of a multiplexed pool of KAPA libraries was performed on a HiSeq4000 or HiSeq 2500 (TruSeq SBS Kit v4, Illumina, Inc.) in paired-end mode (2 × 100 bp) in the Centro Nacional de Análisis Genómicos (CNAG, Barcelona) as described in Moreno-Aguilar et al. (2020). Illumina paired-end (PE) reads were checked using FASTQC and the adapters and low quality sequences were trimmed and removed using TRIMMOMATIC (Bolger et al., 2014). The Loliinae genomic samples used in downstream analysis contained between 6.1 and 40.6 million reads (average 18.0 million reads) with insert sizes ranging between 190 and 300 bp (**Supplementary Table 2**).

Repeat Clustering and Annotation, and 5S rDNA Graph-Clustering Analysis

Identification of the composition and proportion of repetitive elements in the 47 Loliinae species studied was performed from similarity graph-based clustering analysis of filtered PE reads using the Repeat Explorer pipeline of RepeatExplorer2 (RE2)[2]. It was performed through the Galaxy platform as described by Novák et al. (2020). The clustering analysis of individual samples was fed with 500000 PE reads per sample in order to attain the recommended genome coverage (0.1–0.5×) of each taxon (**Supplementary Table 2**). The clustering was conducted employing default RE2 settings (90% similarity, minimum overlap = 55; cluster size threshold = 0.01%) and long queue (max runtime). Automated RE2 annotation of clusters was used to quantify the clusters and to calculate the proportions of repetitive elements in each sample. Plastid and mitochondrial DNA clusters were removed prior to downstream analyses. Comparative clustering analysis was performed for four evolutionary groups (Loliinae, BL, FL, Schedonorus) due to the impossibility of computing it for all the studied samples (47) in a single run of Galaxy employing the same RE2 configuration used for the individual analyses. The Loliinae group was reduced to 38 samples, representing all its main lineages, while the BL, FL and Schedonorus groups contained the same samples used in the individual analysis except the BL group which had two additional Schedonorus samples (**Table 1** and **Supplementary Tables 1, 2**). The comparative clustering analyses were conducted using the maximum number of randomly sampled PE reads that could be processed, representing ∼0.08–0.2× of genome coverage for each species (**Supplementary Table 2**). Automated RE2 repeat annotation was used to quantify the clusters and to estimate the proportions of repeats among the compared samples within each group. Plastid and mitochondrial DNA clusters were also removed from each group prior to downstream analyses.

Sequences of 5S ribosomal DNA genes from 43 out of the 47 studied Loliinae samples were searched using the TAREAN pipeline of RE2 (Garcia et al., 2020; Novák et al., 2020). The input for the 5S rDNA clustering analysis consisted of 500000 PE reads per sample, covering the expected lengths of the 5S rDNA for most of the Loliinae genomes ranging 4.2–20.7 Gbp (**Supplementary Table 1**). The clustering was performed using default TAREAN tool settings (BLAST threshold of 90%, similarity across 55% of the read to identify reads to each cluster, minimum overlap = 55, cluster threshold = 0.01%, minimum overlap for assembly = 40). The 5S rDNA clusters were found in the TAREAN tandem reports. Their shapes were characterized by a connected component index parameter (C) and their k-mer score was calculated as the sum of frequencies of all k-mers used for consensus sequence reconstruction (Garcia et al., 2020). The 5S rDNA cluster graph topologies were visually inspected and classified into graph groups (type 1, simple circular-shaped graph; type 2, complex graph with two or more loops where the interconnected loops represent IGS spacers) (Garcia et al., 2020). We examined the 5S graphs to detect potential variation of 5S rDNA loci and to identify presumable hybrids and allopolyploids. A RE2 5S rDNA sequence of *Festuca pratensis* (360 bp) was used as reference for a Geneious Prime read-mapping assembly of the 5S rDNA of the four Loliinae species (*F. caldasii, F. gigantea, F. gracillima, F. gudoschnikovii*) that could not be retrieved directly from TAREAN due to insufficient number of reads in the cluster for graphical analysis (see **Table 4**). Newly generated 5S rDNA sequences of Loliinae were deposited in GenBank under accessions codes ON248974–ON249019.

Plastome and Nuclear rDNA Phylogenies of Loliinae

Genome skimming PE reads were used to assemble and annotate the plastomes and the nuclear 35S rDNA of the newly sequenced Loliinae samples (**Table 1**). Plastome assembly was performed with Novoplasty v.2.7.1 (Dierckxsens et al., 2017) following the procedures indicated in Moreno-Aguilar et al. (2020) and using as reference the *Festuca pratensis* plastome sequence (JX871941). The 35S rDNA cistron (transcribed region ETS-18S-ITS1-5.8S-ITS2-25S) was assembled using the read-mapping and merging strategy of Moreno-Aguilar et al. (2020) using Geneious Prime and the *F. ovina* 35S rDNA sequence (MT145295) as reference. Newly generated plastome and 35S rDNA sequences of Loliinae were deposited in Genbank under accessions codes SAMN27777779–SAMN27777788 and ON243855–ON243864 (**Table 1**). Multiple sequence alignments (MSAs) of these sequences, together with those of the previously studied Loliinae samples and the *Oryza sativa* and *Brachypodium distachyon* outgroups (**Supplementary Table 1**), were performed with MAFFT v.7.031b (Katoh et al., 2002), visually inspected with Geneious Prime and debugged with trimAl v.1.2rev59 (imposing parameter-*automated*1) (Capella-Gutiérrez et al., 2009). The filtered plastome (133552 bp) and 35S rDNA cistron (6431 bp) MSA data sets were used to compute Maximum likelihood (ML) phylogenetic trees with IQTREE (Nguyen et al., 2015). Independent ML searches were performed imposing the best-fit nucleotide substitution model selected by ModelFinder for each partition, according to the Bayesian Information Criterion (BIC), and branch support for the best tree was estimated from

[2]https://repeatexplorer-elixir.cerit-sc.cz

1,000 ultrafast bootstrap replicates (BS) (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017).

The well resolved plastome and 35S ML trees were topologically contrasted to each other using the Kishino-Hasegawa (KH), Shimodaira-Hasegawa (SH), and Shimodaira Approximately Unbiased (AU) tests with resampling estimated log-likelihood (RELL) optimization and one million bootstrap replicates in PAUP* (Swofford, 2003). As all the pairwise tests showed that each topology did not significantly differ ($p < 0.001$) from the other topology, we constructed a combined ML plastome + 35S tree with IQTREE imposing the respective nucleotide substitution model to each partition and the procedures indicated above. To account for potential incomplete lineage sorting (Kubatko and Degnan, 2007) and to investigate the possibility that a single concatenated plastome + 35S data set could generate topological errors in the phylogeny, we run a parallel phylogenetic analysis with the same data set but modeling the coalescence process using the Singular Value Decomposition quartets (SVDq) approach implemented in Paup*, which uses a variant of Quartet FM (Reaz et al., 2014) to combine quartet trees into a species tree. We imposed the SVDQuartets nquartets = all seed = 2 nthreads = 4 bootstrap = 1000 options with a multispecies coalescent tree model and the quartet assembly algorithm QFM. Bootstrap support of branches was shown on the tree obtained from SVDquartests + Paup* analysis. Since the topology of the SVDq tree (**Supplementary Figure 1A**) was equal to that of the ML tree (**Supplementary Figure 1B**), we selected the strong to relatively well supported ML tree for downstream analysis. Different ML subtrees were computed from the whole combined plastome + 35S data matrix using the respective subsets of taxa of each of the four Loliinae evolutionary groups employed in the repeatome analyses (Loliinae, BL, FL, Schedonorus). These ML tree cladograms were used to estimate the phylogenetic signal of the repeats of each partition (see below). A MSA was also generated for the 5S rDNA sequences of Loliinae and close outgroups (**Supplementary Table 1**) and a ML phylogenetic tree was computed with this data set following the procedures indicated above.

Repeatome Trees and Evolutionary Networks of Loliinae, Phylogenetic Signal of Repeats

Evolutionary analyses were performed with the repeat data obtained from the comparative clustering of repeats for the Loliinae, BL, FL and Schedonorus groups. Distance-based phylogenetic trees and networks were computed from pairwise genetic distances between the repeat contents of the species included in the datasets. First, calculated repeat sequence similarity matrices for the observed/expected number of interspecies edges for each of the most abundant repeat clusters selected by RE2 were converted to Euclidean distances via the *dist* option of the *proxy* package in R (Euclidean matrices). Second, the same repeat sequence similarity matrices were transformed into distance matrices by calculating the inverse of their values as described by Vitales et al. (2020b) (inverse matrices). In both cases, the clusters with incomplete information (NA or zero values) for the similarity comparisons between species pairs were discarded from the analysis. Next, Neighbor-Joining phylogenetic trees were constructed for each repetitive element using either the Euclidean or the inverse distance matrices and the *NJ* function of *ape* package (Paradis et al., 2004) in R. Finally, consensus networks were built from all the repeat NJ trees with SplitsTree4 (Huson and Bryant, 2006) for each group.

The combined plastome + 35S ML subtrees were used to test the potential phylogenetic signal of different types of repeats of each group using Blomberg's *K* (Blomberg et al., 2003) with the *phylosig* function of the package *phytools* (Revell, 2012) in R. For these tests, *K* values > 1 indicate that the repeatome traits have more phylogenetic signal than expected, values ∼1 that traits are consistent with the tree topology (phylogenetic signal), and values ∼0 that there is no influence of shared ancestry on trait values (phylogenetic independence).

Correlations of Repeat Amounts and Genome Size Variation and Global Diversity Analysis of Repeat Types in Loliinae

The potential contribution of the various groups of repeat types and the repeatome to the variation in genome size (1Cx) observed between and within Loliinae lineages was tested using the data from the comparative analysis and by linear regression model analyses (Pearson correlation coefficient) with the *ggscatter* function from the *ggpubr* package in R. The respective contributions of repeats to pairwise differences in genome sizes were estimated following Macas et al. (2015). To correct for potential phylogeny-based bias, phylogenetically independent contrasts (PIC) methods were previously applied to the data using the *pic* option of the *ape* package in R. Correlations could be only performed for the 23 Loliinae species with known genome size (**Table 1**), representing all the main subtribal groups, and using absolute amounts (Mbp) of repeats calculated for individual species (**Supplementary Table 1**). In addition, we also tested whether there were significant differences in repeat amount for different repeat families obtained from the individual analysis through Kruskal–Wallis rank tests using the *multcompView* and *ggpubrr* packages in R. Furthermore, to investigate the levels of conservatism or diversity of the repeat types that most contributed to genome size variation in Loliinae (23 species with known genome sizes) we performed a genome landscape search for the global variability of these individual repeat types across the Loliinae genomes. We pooled the pairwise similarity values of reads, retrieved from the RE2 outputs (hitsort files), for each species and repeat type in a separate dataset and evaluated their similarities with respect to similarities of reads from the same repeat in all other species following Macas et al. (2015). We calculated intraspecific versus interspecific similarity hit ratios (Hs/Ho ratios) considering that conservative sequence repeats will produce similarity hits with about the same frequency for Hs and Ho, while diversified sequence repeats will generate similarity hits with different frequencies. We also calculated similarity hit ratios for the 5S tandem-repeat rDNA to compare its gene-conserved vs.

IGS-variable Hs/Ho ratios with those obtained from the other repeat elements analyzed.

RESULTS

Multiple Polyploidizations and Genome Size Diversification Across the Phylogeny of Loliinae

Chromosome counts and genome size data obtained for, respectively, 41 and 23 out of the 47 Loliinae taxa studied (**Table 1**) corroborated previous records but also revealed new findings about contrasting genome sizes between and within the BL and FL Loliinae lineages when mapped to the combined Loliinae tree (**Figure 1** and **Supplementary Figure 1B**). The inferred ploidy levels for the newly analyzed South American *F. asplundii* (6x), *F. caldasii* (4x), *F. chimborazensis* (subsp. *micacochensis*, 6x) and *F. procera* (4x) species (**Table 1**) confirmed the lack of Loliinae diploids in the southern hemisphere (Dubcovsky and Martínez, 1992; Catalán, 2006). Genome sizes ranged from 4.3 Gbp (*L. canariense*-2x; Schedonorus) and 4.82 Gbp (*F. ovina*-2x; FL) to 21.23 Gbp (*F. asplundii*-6x; FL), representing a near 5-fold (x4.9) increase within the Loliinae and the FL group. Monoploid genome sizes ranged from 2.02 Gbp (*V. ciliata*-4x; FL) to 4.98 Gbp (*F. caldasii*-4x; BL), representing a ×3.7 increase within the Loliinae (**Table 1** and **Supplementary Table 1**). Within the diploids, the broad-leaved species showed 2C genome sizes (*F. triflora*, 7.67 Gbp; *F. paniculata*, 7.48) 1.5x larger than those of the fine-leaved *Festuca* (*F. ovina*, 4.71) and some *Lolium* (*L. perenne*, 4.2) species, while the early diverging fine-leaved *F. eskia* (5.57) and other Schedonorus species (*F. fontqueri*, 5.52; *F. pratensis*, 6.36; *L. perenne*, 5.39; *L. rigidum*, 5.4; *L. persicum*, 6.26) displayed intermediate GS values between them (**Table 1** and **Supplementary Table 1**). A general trend of reduction in monoploid genome size was observed in some polyploid FL and Schedonorus taxa, showing lower values as ploidy level increased (FL: Aulaxyper: *F. rubra*-6x, 2.23 Gbp; American I: *F. chimborazensis*-6x, 2.2; Schedonorus: *F. arundinacea*-6x, 2.84; *F. atlantigena*-8x, 2.0; *F. letourneuxiana*-10x, 1.93). However, large 1Cx sizes were also detected among polyploid South-American Loliinae species nested either within the BL (Central and South American: *F. caldasii*-4x, 4.98) or the FL (American II: *F. procera*-4x, 3.64; *F. asplundii*-6x, 3.46) clades (**Table 1**, **Supplementary Table 1**, and **Figure 1**).

The combined plastome + 35S rDNA ML tree (**Figure 1** and **Supplementary Figure 1B**) was overall congruent with the phylogenies of Minaya et al. (2017) and Moreno-Aguilar et al. (2020) for the divergences of the main Loliinae lineages. The combined tree retrieved a robust topology which was also congruent with those of the well supported plastome and less supported 35S rDNA trees (**Supplementary Figures 1B–D**). The Loliinae phylogeny showed the split of the sister BL and FL clades (**Figure 1**) and divergences within the clades similar to those indicated in Moreno-Aguilar et al. (2020) except for the position of the BL Subulatae-Hawaiian lineage which was nested within the FL clade in the current tree (**Figure 1**). The largely sampled Schedonorus clade showed the branching-off of the 'Mahgrebian' and 'European' sister clades; the latter included the split of the *Festuca* gr. *arundinacea* allopolyploids from the rest, although their respective nesting positions swapped between their 'European' plastome and 'Mahgrebian' 35S rDNA trees (**Figure 1** and **Supplementary Figures 1B–D**). The remaining Schedonorus lineages of the 'European' clade showed the early divergences of diploids followed by those of polyploids and a reversal trend to diploidization in the recently split *Lolium* clade (**Figure 1**). Diploid and polyploid lineages were spread across the BL and FL clades of the Loliinae tree (**Figure 1**). Although several of the early diverging BL lineages are predominantly or uniquely made up of diploids (Drymanthele, Lojaconoa, Subbulbosae), other early splits contain exclusively low-to-high polyploids (South African, Central-South American). A similar trend of more ancient to more recent origins of polyploids could be observed within the Schedonorus and FL clades. Low-to-high polyploids have evolved in all FL lineages and several of them are formed exclusively by polyploids (American-Neozeylandic, American I, American-Pampas, Psilurus-Vulpia, Subulatae-Hawaiian, American II, Afroalpine) (**Figure 1**).

The Loliinae Repeatome

The annotated repeats found by RE2 in the individual analyses showed large differences in repeat types and amounts among the 47 Loliinae samples and lineages (**Table 2**, **Supplementary Table 2**, **Figure 1**, and **Supplementary Figure 1E**). The proportion of the holoploid genome occupied with repeats ranged from 30.69% (*F. letourneuxiana*-10x) to 68.8% (*L. persicum*-2x), with a mean across Loliinae of 51.8% (**Table 2**, **Figure 1**, and **Supplementary Figure 1E**). The highest percentages corresponded to diploid taxa of the Schedonorus group (e.g., *Lolium* spp., *M. tuberosa*, *F. simensis*; >60%) and diploid or polyploid taxa of the BL group (e.g., *F. lasto*-2x; *F. triflora*-2x, *F. scabra*-4x, Central-South American spp.-4x-6x, *F. africana*-10x, plus FL *F. molokaiensis*; >57%) and the lowest to high-polyploid taxa of the Schedonorus group (Mahgrebian-4x-10x, *F. arundinacea*-6x; <40%) and to diploid and high-polyploid species of the FL Aulaxyper group (*F. francoi*-2x, *F. rubra*-6x; <46%) (**Table 2**; **Figure 1**, and **Supplementary Figure 1E**). LTR-Gypsy and LTR-Copia retrotransposons represented the major fractions of repeatome in the studied genomes followed by Class II TIR-transposons and Satellite repeats (**Table 2** and **Supplementary Figure 1E**).

LTR-Gypsy Retand elements were the most represented repeats in almost all genomes, especially within the BL and Schedonorus groups, where they covered >10% and up to 20% of several Subbulbosae, Leucopoa, Central-South American, 'European,' *F.* gr. *arundinacea* and *Lolium* genomes, as well as two genomes of the BL and FL groups (*F. molokaiensis*, *V. ciliata*). Only the BL Tropical-South African and the FL American II and Aulaxyper genomes showed low coverages (<2%) of Retand repeats (**Table 2** and **Figure 1**). The more heterogeneous LTR-Gypsy Tekay and Athila elements were also well represented in some genomes, the former in the BL genomes (*F. scabra* 14%,

TABLE 1 | Taxa included in the repeatome analysis of Loliinae.

Taxon	Group	Locality	2*n*	Ploidy	2C(pg)	1Cx (pg)	1Cx (Mbp)	GenBank accession no.		
								Plastome	35S rDNA	5S rDNA
Festuca africana	BL	Uganda: Gahinga	70	10x	–	–	–	SAMN14647044	MT145277	**ON248974**
Festuca amplissima	BL	Mexico: Chihuahua	42	6x	–	–	–	SAMN14647045	MT145278	**ON248975**
Festuca caldasii	BL	Ecuador: Catamayo	**28**	4x	**20.36**	5.09	4978.02	SAMN14647047	MT145280	**ON248977**
Festuca durandoi	BL	Portugal: Serra Arga	14	2x	14.66 (4x)	3.66	3584.86	SAMN14647050	MT145283	**ON248980**
Festuca lasto	BL	Cádiz: Jerez	14	2x	–	–	–	SAMN14647058	MT145291	**ON248989**
Festuca mekiste	BL	Kenya: Mt. Elgon	–	–	–	–	–	**SAMN27777779**	**ON243855**	**ON248992**
Festuca molokaiensis	BL	United States: Hawai: Molokai	–	–	–	–	–	SAMN14647061	MT145294	**ON248993**
Festuca paniculata	BL	Spain: Caceres	14	2x	7.65	3.83	3740.85	SAMN14647064	MT145297	**ON248996**
Festuca parvigluma	BL	China: Baotianman	28	4x	–	–	–	SAMN14647065	MT145298	**ON248997**
Festuca scabra	BL	S Africa: Cathedral P.	28	4x	–	–	–	**SAMN27777781**	**ON243857**	**ON249003**
Festuca spectabilis	BL	Bosnia-H: Troglav	42	6x	–	–	–	SAMN14647071	MT145304	**ON249004**
Festuca superba	BL	Argentina: Jujuy	56	8x	–	–	–	SAMN14647072	MT145305	**ON249005**
Festuca triflora	BL	Morocco: Rif Mnts.	**14**	2x	**7.84**	3.92	3833.76	SAMN14647073	MT145306	**ON249006**
Festuca abyssinica	FL	Tanzania: Kilimanjaro	28	4x	–	–	–	SAMN14647043	MT145276	**ON248973**
Festuca asplundii	FL	Ecuador: Saraguro	42	6x	**21.23**	3.54	3460.49	SAMN14647046	MT145279	**ON248976**
Festuca capillifolia	FL	Morocco: Ifrane	14	2x	–	–	–	SAMN14647048	MT145281	**ON248978**
Festuca chimborazensis	FL	Ecuador: Chimborazo	**42**	6x	**13.48**	2.25	2197.24	SAMN14647049	MT145282	**ON248979**
Festuca eskia	FL	Spain: Picos de Europa	14	2x	5.7	2.85	2787.3	SAMN14647051	MT145284	**ON248981**
Festuca fimbriata	FL	Argentina: Apóstoles	42	6x	–	–	–	SAMN14647053	MT145286	**ON248983**
Festuca francoi	FL	Portugal: Terceira	12	2x	–	–	–	SAMN14647057	MT145290	**ON248984**
Festuca gracillima	FL	Argentina: Trra.Fuego	42	6x	–	–	–	SAMN14647055	MT145288	**ON248986**
Festuca holubii	FL	Ecuador: Saraguro	–	–	–	–	–	SAMN14647056	MT145289	**ON248988**
Festuca ovina	FL	Rusia: Gatchinskii Ra.	14	2x	4.82	2.41	2356.98	SAMN14647062	MT145295	**ON248994**
Festuca pampeana	FL	Argentina: Ventana	56	8x	–	–	–	SAMN14647063	MT145296	**ON248995**
Festuca procera	FL	Ecuador: Chimborazo	28	4x	**14.88**	3.72	3638.16	SAMN14647067	MT145299	**ON248999**
Festuca pyrenaica	FL	Spain: Tobacor	28	4x	–	–	–	SAMN14647068	MT145300	**ON249000**
Festuca pyrogea	FL	Argentina: Trra.Fuego	–	–	–	–	–	SAMN14647069	MT145302	**ON249001**
Festuca rubra	FL	Argentina: Trra.Fuego	42	**6x**	13.68	2.28	2229.84	**SAMN27777780**	**ON243856**	**ON249002**
Megalachne masafuerana	FL	Chile: Masafuera	–	–	–	–	–	SAMN14647075	MT145308	**ON249018**
Vulpia ciliata	FL	Spain: Ontígola	28	4x	8.28	2.07	2024.46	SAMN14647076	MT145309	**ON249009**
Festuca a. arundinacea	Sch	Spain: Ferrol	42	6x	17.46	2.91	2845.98	**SAMN27777774**	**ON243850**	**ON249007**
Festuca a. atlantigena	Sch	Morocco: Atlas Mnts	56	8x	16.22	2.03	1982.895	**SAMN27777775**	**ON243851**	**ON248990**
Festuca a. letourneuxiana	Sch	Morocco: Atlas Mnts	70	10x	19.7	1.97	1926.66	SAMN14647059	MT145292	**ON249010**
Festuca dracomontana	Sch	SAfrica:Haernertsburg	–	–	–	–	–	**SAMN27777776**	**ON243852**	**ON249011**
Festuca fenas	Sch	Spain	28	4x	10.48	2.62	2562.36	SAMN14647052	MT145285	**ON248982**
Festuca fontqueri	Sch	Morocco: Rif Mnts	14	2x	**5.54**	2.77	2709.06	SAMN14647054	MT145287	**ON249008**
Festuca gigantea	Sch	Norway	42	6x	20.75	3.46	3382.25	**SAMN27777777**	**ON243853**	**ON248985**
Festuca gudoschnikovii	Sch	Russia: Yermakovskii	28	4x	–	–	–	**SAMN27777778**	**ON243854**	**ON248987**
Festuca mairei	Sch	Morocco: Atlas Mnts	28	4x	10.04	2.51	2454.78	SAMN14647060	MT145293	**ON248991**
Festuca pratensis	Sch	United Kingdom: England	14	2x	6.5	3.25	3178.5	SAMN14647066	MT145301	**ON248998**
Festuca simensis	Sch	Kenya: Mt. Kenya	28	4x	–	–	–	**SAMN27777782**	**ON243858**	**ON249012**
Lolium canariense	Sch	Spain: Canary Islands	14	2x	4.3	2.15	2102.7	**SAMN27777783**	**ON243859**	**ON249013**
Lolium perenne	Sch	United Kingdom: Wales	14	2x	5.51	2.76	2694.39	**SAMN27777784**	**ON243860**	**ON249014**
Lolium persicum	Sch	Georgia	14	2x	6.4	3.2	3129.6	**SAMN27777785**	**ON243861**	**ON249015**
Lolium rigidum	Sch	Turkey	14	2x	5.49	2.75	2684.61	**SAMN27777786**	**ON243862**	**ON249017**
Lolium saxatile	Sch	Spain: Fuerteventura	14	2x	–	–	–	**SAMN27777787**	**ON243863**	**ON249016**
Micropyropsis tuberosa	Sch	Spain: Almonte	14	2x	–	–	–	**SAMN27777788**	**ON243864**	**ON249019**

Loliinae group (BL, broad-leaved Loliinae; FL, fine-leaved Loliinae; Sch, Schedonorus), chromosome number (2n), ploidy level, genome size (2C, pg), monoploid genome size (1Cx, pg; 1Cx, Mbp) and GenBank accession codes for plastome and nuclear ribosomal 35S and 5S genes are given for each sample. Values in bold correspond to new data generated in this study. Hyphens indicate lack of 2n and/or 2C/1Cx data for some taxa. See ***Supplementary Table 1*** *for additional information on taxonomic ranks and taxon authorship, detailed localities and vouchers, and sources of cytogenetic and genomic data.*

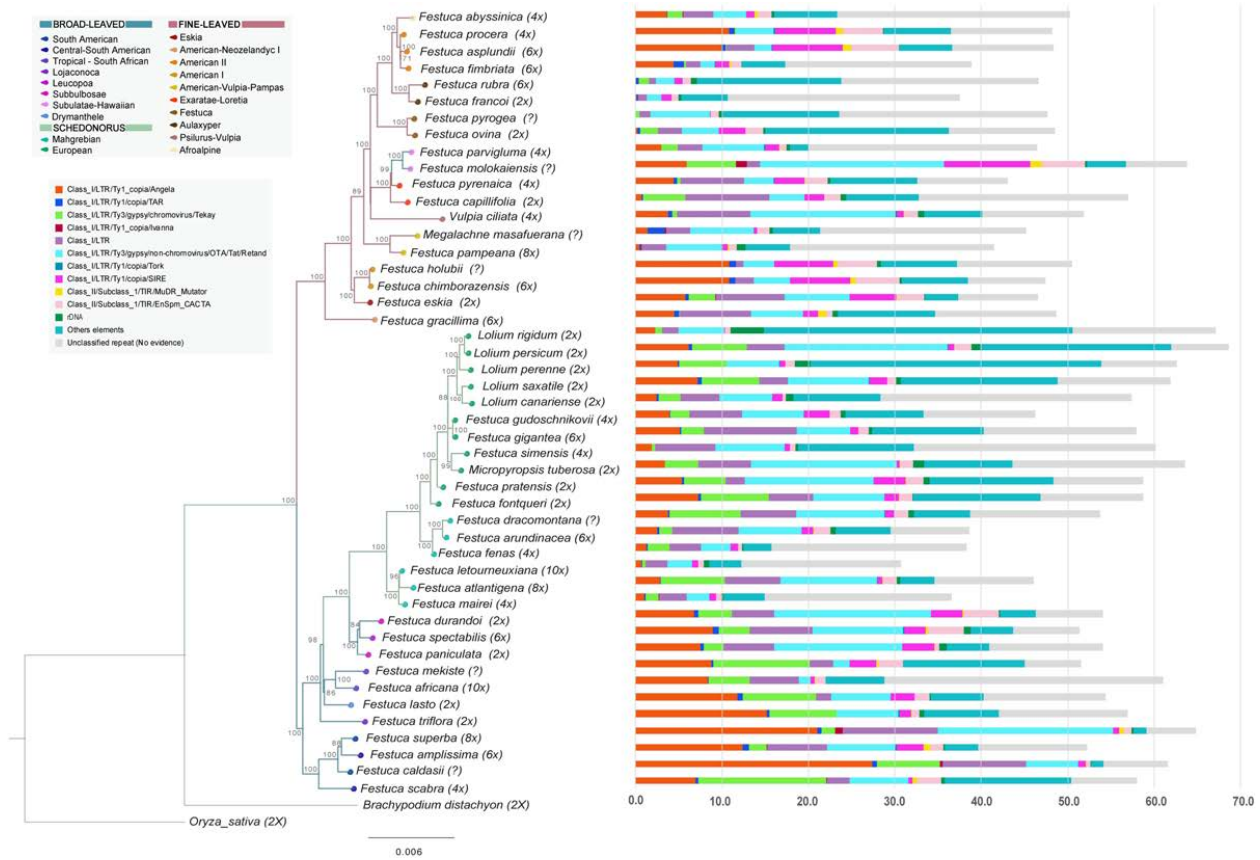

FIGURE 1 | Histograms of repeat contents per holoploid genome (1C) retrieved from the individual Repeat Explorer 2 analyses of the studied Loliinae samples mapped onto the Maximum Likelihood combined phylogenomic tree (plastome + nuclear 35S rDNA cistron) of Loliinae (color codes of Loliinae lineages are indicated in the chart). Color codes for repeat families are indicated in the corresponding inset charts. Scale bar: number of mutations per site.

F. mekiste 11%) and the latter in the *Lolium* genomes (*L. perenne*, 25%; *L. rigidum* 23%). In contrast, those elements generally had low coverages (<2%) in FL genomes (**Table 2** and **Figure 1**). Other LTR-Gypsy families were only moderately represented in some groups, such as Ogre in the Tropical and South African genomes (e.g., *F. mekiste*, 7.9%; *F. africana*, *F. scabra*, 4.6%) and *L. rigidum* (4.8%), and CRM in several Schedonorus genomes (e.g., *L. persicum* 5.1%, *F. pratensis* 4.3%) although they showed low coverages (<2%) in most of the remaining genomes. The LTR-Gypsy OTA, Reina and Tat families were only residually present in a few genomes (**Table 2**).

LTR-Copia Angela elements were the second most frequent repeat family in all Loliinae genomes. They were highly represented in the genomes of Central-South American taxa in both the BL (12–27%) and FL (9.8–10.8%) groups, relatively abundant in all remaining BL genomes (6.6–8.8%), moderately abundant in Schedonorus genomes (except the 'Mahgrebian' taxa, <2%) and in FL *F. eskia* and BL *F. molokaiensis* (5.7–7.2%), and poorly represented in the remaining FL genomes (<2%) (**Table 2** and **Figure 1**). LTR-Copia SIRE elements showed moderate to low frequency in all genomes except in *F. molokaiensis* (10%) and FL Eskia, American I and American II genomes (5.4–7.5%). Other LTR-Copia families (Ale, Ikeros, Ivanna, TAR, Tork) were only residually represented in a few Loliinae genomes (**Table 2** and **Figure 1**). TIR Class II transposons were found less frequently in Loliinae genomes; only CACTA elements were present in all taxa although they were only moderately represented in some FL American I, American II and Hawaiian genomes and in BL Subbulbosae and Leucopoa genomes (4–5.5%). Representation of other transposon elements (Mutator, Harbinger, hAT) in Loliinae genomes was only residual (**Table 2** and **Figure 1**). Some of the less frequent Class I and Class II repetitive elements were only represented in a very small fraction of some particular genomes (e.g., Reina in *L. saxatile*; hAT in *M. masafuerana*; Tat in *F. simensis*; **Table 2**). Tandem satellite repeats were generally moderately to poorly represented in most Loliinae genomes, except for their relatively high representation in FL *F. procera* and *F. pyrogea* (13.3%) and Schedonorus *F. simensis* (12%) and its moderate representation in FL Exaratae, Festuca and Aulaxyper genomes (4.2–5.9%). Kruskal–Wallis rank tests performed for each of the Loliinae repeat elements found significant differences for Retand, CRM, Tekay, Angela, Ivanna, Ale, LTR, CACTA, Mutator, Harbinger, rDNA

TABLE 2 | Genome proportion of repeats estimated by Repeat Explorer 2 for individual Loliinae samples (estimations per holoploid genome, 1C).

Loliinae taxon and phylogenetic group	Class I/LTR/Ty1_copia								Class I/LTR/Ty3_gypsy								Class II/Subclass_1/TIR											
	Ale	Angela	Ikeros	Ivanna	SIRE	TAR	Tork	Ty1_Copia	OTA	Athila	Tat	Ogre	Retand	CRM	Tekay	Reina	Ty3_Gypsy	EnSpm_CACTA	Hat	MuDR-Mutator	PIF–Harbinger	rDNA (5S-45S)	Satellite	Mobile element	Class I/LTR (conflict evidence)	Class I/LINE Repeat (conflicting evidences)		Unclassified (No evidence)	Total (%)
Broad-Leaved																													
Festuca africana Tropical-South African	0	8.42	0	0	0.46	0.06	0	0	0	0.3	0	4.63	1.32	0.14	4.75	0	0	1.14	0	0.13	0	0.04	1.7	0	5.74	0	0	32.26	61.1
Festuca amplissima Central-South American	0	12.43	0.05	0.11	3.04	0.71	0.23	0	0	0.26	0	0.02	7.87	0.67	2.05	0	0	1.54	0	0.79	0	0.12	2.72	0	6.89	0.16	0	12.58	52.25
Festuca caldasii Central-South American	0.03	27.45	0	0.33	0.82	0.48	0.02	0	0	0.26	0	0.13	6.03	0.17	7.32	0	0	0.49	0	0.11	0	0.06	0.88	0	9.64	0	0	7.4	61.63
Festuca durandoi Subbulbosae	0	6.81	0	0	3.6	0.47	0	0	0	0.17	0	0.16	18.2	1.84	3.87	0	0	4.04	0	0.19	0.09	0.1	0.96	0	4.9	0.02	0.87	7.81	54.12
Festuca lasto Drymanthele	0	11.83	0.09	0	2.73	0.59	0.02	0	0	3.42	0	0	6.85	1.76	8.51	0	0	1.72	0	0.03	0	0.17	0.84	0	1.76	0.01	0	14.11	54.46
Festuca mekiste Tropical-South African	0	8.79	0	0.01	3.08	0.24	0	0	0	2.77	0	7.92	1.91	0.35	11.14	0	0	2.86	0	0.27	0	0.03	3.01	0	2.68	0	0	6.5	51.57
Festuca molokaiensis Subulatae-Hawaiian	0	5.94	0.03	1.26	9.96	0.03	0	0	0	0.01	0	1.55	21.35	0.26	5.71	0	0	4.95	0	1.37	0	0.25	1.12	0	1.49	0.02	1.49	7.09	63.85
Festuca paniculata Subbulbosae	0	7.51	0	0	3.75	0.43	0.02	0	0	0.45	0	0	14.83	0.81	2.3	0	0	0.51	0	0.02	0.03	0.89	0.82	2.77	5.82	0.03	0	13.16	54.14
Festuca parvigluma Subulatae-Hawaiian	0	2.99	0.12	0	1.57	0.04	0.15	0	0	0.03	0	0.01	7.16	0.65	1.93	0	0	0.82	0	0.07	0	0.38	1.34	0	2.79	0	0	26.43	46.47
Festuca scabra South African	0	6.95	0.09	0.11	0.47	0.36	0	0	0	5.69	0	4.6	6.86	1.28	14.78	0	0	2.81	0	0.54	0	0.4	2.96	0	2.57	0	0	7.61	58.08
Festuca spectabilis Leucopoa	0	8.94	0	0	2.47	0.73	0.1	0	0	0.07	0	0.12	10.48	2.38	3.54	0	0	4.13	0	0.35	0.05	0.77	2.04	0	7.32	0.2	0	7.71	51.41
Festuca superba Central-South American	0.03	21.07	0	0.9	0.68	0.49	0.01	0	0	0	0	0.01	20.31	0.05	1.54	0	0	0.92	0	0.51	0	0.26	1.4	0	11.01	0	0	5.71	64.9
Festuca triflora Lojaconoa	0	15.24	0	0	1.34	0.33	0.14	0	0	5.98	0	0.11	7.15	0.78	7.71	0	0	0.85	0	0.13	0	0.51	1.78	0	0	0	0	14.93	56.98
Schedonorus																													
Festuca a. arundinacea F.gr.arundinacea	0	2.52	0.06	0	1.36	0.29	0.01	0	0	3.07	0	0.08	7.31	1.27	1.47	0	0	1.92	0	0.03	0.07	0.63	1.53	0	7.66	0.09	0.24	9.09	38.67
Festuca a. Atlantigena F.gr.arundinacea	0	2.84	0.02	0	0.6	0.08	0.01	0	0	0.14	0	0	11.16	1.49	7.5	0	0	1.62	0	0.06	0.03	0.37	2.13	0	6.42	0.08	0.09	11.43	46.09
Festuca dracomontana F.gr.arundinacea	0	3.79	0.03	0	1.05	0.18	0.01	0	0	1.58	0	0.13	10.24	2.45	8.23	0	0	1.68	0	0	0	0.59	1.49	0	6.44	0.04	0.79	15.09	53.82
Festuca fenas Mahgrebian	0	1.29	0.02	0	0.83	0.16	0	0	0	1.15	0	0	3.4	0.8	2.5	0	0	0.45	0	0	0	0.21	1.21	0	3.69	0.02	0	22.64	38.38
Festuca fontqueri European	0	7.31	0.09	0	1.65	0.28	0.01	0	0	7.55	0	0.63	8.21	2	7.9	0	0	1.54	0	0.08	0.01	0.09	3.25	0	5.12	0.03	1.11	11.96	58.82
Festuca gigantea European	0	5.16	0	0	0.98	0.13	0.01	0	0	0.98	0	0	6.19	3.73	2.62	0	0	1.19	0	0	0.03	0.4	8.06	0	10.76	0.1	0	17.62	57.96
Festuca gudoschnikovii European	0	3.96	0	0	3.02	0.12	0	0	0	0.19	0	0	7.17	3.37	2.22	0	0	1.24	0	0	0.02	0.59	5.32	0	6.04	0.07	0	12.93	46.25
Festuca a. letourneuxiana Mahgrebian	0	0.73	0.01	0	0.71	0.08	0	0.12	0.01	1.13	0	0	2.85	0.8	0.43	0	0.01	0.62	0	0	0.01	0.63	1.61	0	2.53	0.02	0	18.39	30.7
Festuca mairei Mahgrebian	0	1.02	0.03	0.02	0.82	0.18	0	0.1	0	1.32	0	0	2.59	0.99	1.51	0	0	0.62	0	0	0	0.3	2.28	0	3.19	0	0	21.58	36.57
Festuca pratensis European	0.04	5.41	0.01	0	3.77	0.19	0	0	0	7.18	0	0.66	14.88	4.26	4.89	0	0	2.02	0	0.01	0	0.69	1.81	0	2.17	0.01	0.4	10.29	58.72
Festuca simensis European	0	1.9	0	0	0.62	0.01	0	0.02	0.02	0.37	0.02	0	8.04	0.91	0.4	0	0	0.66	0	0	0	0.3	12.01	0	6.95	0.01	0	28.01	60.23

(Continued)

TABLE 2 | (Continued)

Loliinae taxon and phylogenetic group	Class I/LTR/Ty1_copia								Class I/LTR/Ty3_gypsy									Class II/Subclass_1/TIR											
	Ale	Angela	Ikeros	Ivanna	SIRE	TAR	Tork	Ty1_Copia	OTA	Athila	Tat	Ogre	Retand	CRM	Tekay	Reina	Ty3_Gypsy	EnSpm_CACTA	Hat	MuDR-Mutator	PIF – Harbinger	rDNA (5S-45S)	Satellite	Mobile element	Class I/LTR (conflict evidence)	Class I/LINE	Repeat (conflicting evidences)	Unclassified (No evidence)	Total (%)
Lolium canariense Lolium	0.11	2.49	0	0	1.22	0.23	0	0	0	0.05	0	0.39	6.04	2.6	2.53	0	0	0.41	0	0.02	0	0.81	6.93	0	4.53	0	0	29.09	57.46
Lolium perenne Lolium	0.07	4.9	0	0	0.64	0.17	0.01	0	0	25.26	0	2.79	6.12	1.75	5.47	0	0	1.09	0	0.04	0.09	1.83	2.03	0	0	0.04	1.64	8.68	62.63
Lolium persicum Lolium	0.11	6.2	0	0	0.73	0.41	0.04	0	0	9.18	0	1.15	18.86	5.15	6.34	0	0	1.97	0	0	0.19	1.02	4.87	0	4.33	0	1.52	6.65	68.71
Lolium rigidum Lolium	0.1	2.29	0	0	0.14	0.04	0	0	0	23.1	0	4.86	5.3	2.68	0.79	0	0	0.63	0	0	0.06	3.83	2.53	0	1.85	0	2.42	16.53	67.15
Lolium saxatile Lolium	0.18	7.25	0.04	0	2.13	0.44	0.02	0.01	0	7.23	0	0	9.39	1.57	6.67	0.01	0	1.03	0	0	0.65	0.56	1.76	0	3.29	0.02	6.64	13.03	61.91
Micropyropsis tuberosa European	0.05	3.38	0	0	0.33	0.01	0	0	0	0.02	0	0.05	16.89	3.58	3.93	0	0	1.5	0	0.02	1.02	1.3	3.99	0	6.07	0	1.47	20.01	63.64
Fine-Leaved																													
Festuca abyssinica Afroalpine	0	3.65	0	0.07	0.96	0.08	0	0	0	0.15	0	1.85	3.83	0.34	1.78	0	0	1.56	0	0.41	0	0.31	4.93	0	3.45	0	0	26.92	50.27
Festuca asplundii American II	0	9.97	0.52	0	8.17	0.53	0.13	0	0	1.31	0	0.82	1.96	0.67	0.02	0	0	5.52	0	0.93	0.01	0.04	1.83	0	3.27	0	0.97	11.75	48.41
Festuca capillifolia Exaratae	0	0.75	0	0	2.32	0.14	0.01	0	0	1.58	0	0.22	4.03	1.41	4.87	0	0	1.86	0	0	0	0.65	5.16	0	9.77	0	0	24.23	57.02
Festuca chimborazensis American I	0	10.89	0.06	0.02	6.98	0.67	0	0	0.01	1.06	0	0.06	4.17	1.16	0	0	0	5.04	0	0.65	0.09	0.3	4.09	0	2.15	0	1.07	8.99	47.45
Festuca eskia Eskia	0	5.77	0.02	0.06	5.36	0.4	0	0	0	0.73	0	0.18	7.51	0.72	3.13	0	0	3.18	0	0.09	0.03	0.11	1.11	0	7.92	0.06	1.04	9.18	46.59
Festuca fimbriata American II	0.01	4.47	0.15	0.04	1.6	1.18	0.12	0.43	0	0.04	0	0.34	1.66	0.09	0.21	0	0	1.18	0	0.2	0	0.05	3.91	0	1.62	0.02	0	21.54	38.87
Festuca francoi Aulaxyper	0	0.13	0.02	0	1.17	0.16	0.01	0.02	0.09	0.85	0	0.07	1.62	0.23	0.03	0	0	0.81	0	0.02	0	0.27	4.16	0	1.07	0	0	26.83	37.56
Festuca gracillima American-Neozeylandic	0	4.5	0.15	0.02	1.74	0.61	0	0	0	0.87	0	1.22	6.05	1.54	0.01	0	0	0.76	0	0.92	0	0.61	1.45	0	8.23	0	6.04	13.95	48.68
Festuca holubii American I	0	10.87	0.07	0	6.86	0.76	0	0	0	0.61	0	0.02	3.52	1.1	0.01	0	0	4.54	0	0.54	0.01	0.42	5.96	0	0.9	0.01	0.99	13.32	50.52
Festuca ovina Festuca	0.03	0.26	0.02	0	3.16	0.33	0	0	0	7.18	0	0.59	4.26	1.04	2.04	0	0	2.01	0	0	0.01	0.28	5.22	0	2.75	0.03	7.1	12.28	48.58
Festuca pampeana American Pampas	0	0.52	0	0.06	0.63	0.1	0.06	0	0	0	0	0.33	6.47	0.03	0	0	0	0.85	0	0.19	0	1.02	4.77	0	2.85	0	0	23.59	41.48
Festuca pirenaica Exaratae	0	10.88	0.36	0	7.1	0.6	0.12	0	0.01	0.87	0	0.34	4.32	1.48	0.21	0	0	4.67	0	0.8	0	0.05	2.66	0	0	0	2.09	11.66	48.21
Festuca procera American II	0.01	4.47	0.11	0	3.53	0.36	0.03	0	0	1.31	0	0.26	3.46	1.41	0.42	0	0	2.62	0	0.01	0	0.37	5.94	0	7.32	0.04	0.96	10.43	43.08
Festuca pyrogea Festuca	0.04	0.02	0	0	0.08	0	0	0.03	0	0	0	0	6.91	0.02	0.49	0	0	0.95	0	0	0	0.42	13.4	0	1.24	0	0	24.13	47.73
Festuca rubra Aulaxyper	0	0.08	0	0	0.96	0.3	0.01	0	0	8.55	0	0.61	2.11	0.69	1.24	0	0	0.95	0	0.01	0	0.7	6.56	0	0.76	0	0.32	22.79	46.65
Megalachne masafuerana American Pampas	0	1.44	0	0.18	0.38	1.98	0	0.02	0	0.1	0	0	7.4	3.31	0	0	0	1.4	0.03	0	0	0.4	1.88	0	2.74	0.04	0.14	23.85	45.28
Vulpia ciliata Psilurus-Vulpia	0.14	3.81	0	0	0.78	0.49	0.16	0	0.01	0.22	0	0.08	16.79	0.86	0.56	0	0	1.6	0	0.11	0	0.7	2.76	0	8.45	0.33	2.33	11.74	51.91
Mean±SD	**0.02**	**5.94**	**0.05**	**0.07**	**2.26**	**0.35**	**0.03**	**0.02**	**0.00**	**2.86**	**0.00**	**0.79**	**7.68**	**1.42**	**3.31**	**0.00**	**0.00**	**1.84**	**0.00**	**0.21**	**0.05**	**0.53**	**3.41**	**0.06**	**4.43**	**0.03**	**0.89**	**15.61**	**51.85**
Kruskal Wallis test	**30.99**	**37.17**	19.01	**35.24**	21.49	21.30	19.04	20.78	9.81	19.63	12.89	20.52	**31.43**	**31.25**	**30.49**	8.40	14.67	**24.84**	22.50	**32.30**	**23.49**	**24.54**	**23.56**	14.67	**28.22**	14.33	22.30	21.92	
Kruskal Wallis test *p.value*	**0.01**	**0.00**	0.16	**0.00**	0.09	0.09	0.16	0.11	0.78	0.14	0.53	0.11	**0.00**	**0.01**	**0.01**	0.87	0.40	**0.04**	0.07	**0.00**	**0.05**	**0.04**	**0.05**	0.40	**0.01**	0.43	0.07	0.08	

Kruskal–Wallis tests for significant differences in repeat proportions for each repetitive element across the studied samples. Significant values are highlighted in bold.

and satellite repeats when examined in the entire group of samples (**Table 2**).

Regression model analysis of repeat content and monoploid genome sizes differences among the 23 Loliinae species with known 2C data, after PIC correction, showed a strong correlation when data from all main repeats were combined (R^2 = 0.83, p = 1.8E-09), accounting for 65.2% differences in genome size between species (**Table 3** and **Figure 2**). Angela repeats presented the highest correlation (R^2 = 0.71, p = 5.44E-07), followed by TAR (R^2 = 0.54, p = 5.85E-05), Tekay (R^2 = 0.38, p = 0.0018), Ivanna (R^2 = 0.35, p = 0.002), LTR (R^2 = 0.27, p = 0.011) and Retand (R^2 = 0.21, p = 0.02) repeats, while the other repetitive elements did not show significant correlations. The Angela family also showed the highest contribution to pairwise differences in genome sizes (19.6%), followed by Retand (10.7%), Tekay (6.47%) and LTR (5.49%), while the contributions of the other families were <5% (**Table 3** and **Supplementary Figure 2**). Our genome landscape analysis of global variability of these individual repeat types across the Loliinae genomes showed different histogram profiles of Hs/Ho hit ratios (**Figure 3**). The histogram of control 5S rDNA sequences comprised a narrow major peak near zero on the log(Hs/Ho) x-axis, indicating that the ratios of intraspecific Hs to interspecific Ho hit frequencies were close to one, and thus reflected the high sequence conservation of the 5S genes. In contrast, this 5S rDNA histogram also included a wide right-hand tail of log(Hs/Ho) hit values ranging from 0.1 to 3, accounting for the high divergence of intergenic spacer sequences (IGS) of 5S rDNA. However, the histogram patterns of the ten repeats analyzed showed general Gaussian distributions for log(Hs/Ho) hit values (**Figure 3**). Among the repeats that contributed the most to genome size variation (**Table 3** and **Supplementary Figure 2**), Angela elements generated main peaks of log(Hs/Ho) values closer to zero in the histogram than those of Retand, LTR and Tekay elements (**Figure 3**), suggesting a slightly higher conservatism of the Angela sequences and a higher diversification of the Retand, LTR and Tekay sequences in the Loliinae genome landscape.

Repeatome Phylogenies of Loliinae and Phylogenetic Signal of Repeats

The results of the RE2 comparative analysis of Loliinae repeats recovered different types and numbers of shared or sample specific repetitive elements in each of the four

TABLE 3 | Pearson linear correlation of repeat abundance with genome size variation (1Cx) in Loliinae, after PIC correction, and contribution of individual repeats to the genome size differences between species.

Repeat type	Correlation to genome size		Abundance in the analyzed genomes [Mbp/1Cx]		Average contribution to pairwise differences in genome sizes [%]
	R^2	p-Value	Min	Max	
Angela	**0.71**	**5.44E-07**	1.775	1366.503	**19.6**
TAR	**0.54**	**5.85E-05**	1.172	24.058	**0.642**
Tekay	**0.38**	**0.00187**	0	364.516	**6.47**
Ivanna	**0.35**	**0.00281**	0	16.597	**0**
LTR	**0.27**	**0.0111**	0	480.094	**5.49**
Retand	**0.21**	**0.0265**	46.947	652.52	**10.7**
Tork	0.16	0.0566	0	5.454	0.0376
SIRE	0.14	0.0784	3.791	282.611	2.8
MuDR_Mutator	0.11	0.131	0	32.148	0.0986
EnSpm_CACTA	0.09	0.165	8.715	190.978	2.27
Ty1_Copia	0.08	0.18	0	2.514	0
Ty3_Gypsy	0.08	0.197	0	0.208	0
Mobile_element	0.06	0.257	0	103.646	0
Ikeros	0.05	0.285	0	17.96	0
LINE	0.05	0.314	0	6.74	0
OTA	0.03	0.397	0	0.379	0
Unclassified	0.03	0.438	197.426	611.73	4.08
CRM	0.03	0.443	8.348	161.049	0.751
Repeat	0.01	0.61	0	167.43	0
Ale	0.01	0.716	0	3.465	0
PIF_Harbinger.	0.01	0.737	0	5.893	0
rDNA_5S-45S	0.00	0.789	1.446	102.852	−0.152
Athila	0.00	0.852	1.146	680.565	0.778
Satellite	0.00	0.863	30.69	272.468	−0.0164
Ogre	0.00	0.93	0	130.467	0.183
All repeats	**0.83**	**1.8E-09**	591.539	3067.826	**65.2**

Only the most represented repeat types of Loliinae are shown. Significant values are highlighted in bold.

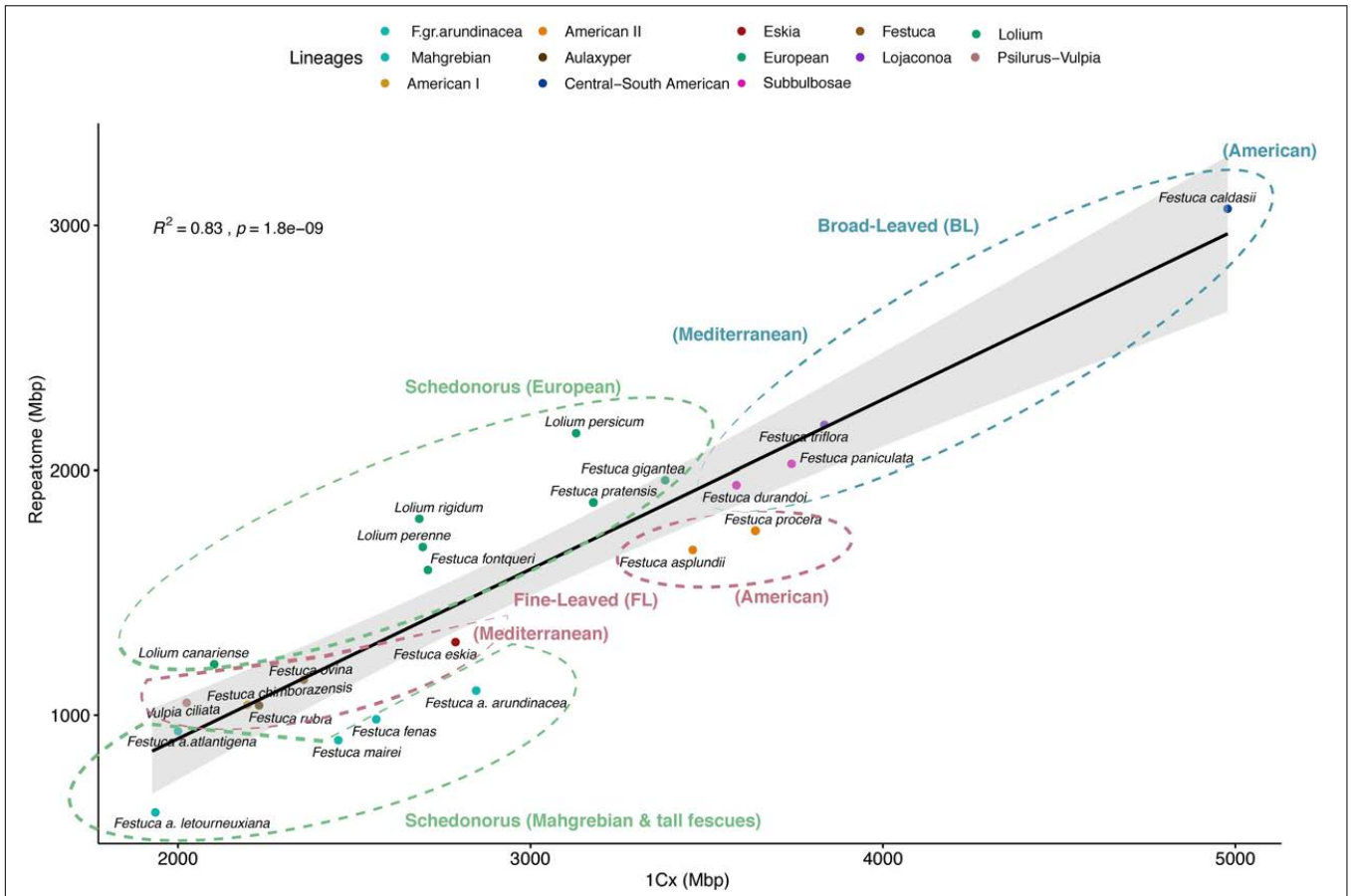

FIGURE 2 | Correlation plot of repeat content and genome size variation (1Cx) for the 23 Loliinae taxa with known genome sizes. Summed abundance values of the most represented repeat types obtained from the individual RE2 analysis. Pearson correlation analysis (R^2 = 0.83, p = 1.8e–09). Ellipses with dashed lines encircle the main Loliinae groups and subgroups [broad-leaved Loliinae (BL), blue; Schedonorus, green; fine-leaved Loliinae (FL), magenta]. Color codes of Loliinae lineages correspond to those indicated in **Figure 1**.

Loliinae evolutionary groups studied (**Supplementary Table 3**). RE2 annotated different numbers of tops clusters in each group [Loliinae: 337 clusters (total number of reads 2,659,145 (57%); minimum number of reads 468); FL: 308 (2,245,911 (57%); 395); BL: 336 (2,841,940 (64%); 443); Schedonorus: 270 (1,771,749 (65%); 274)] (**Supplementary Tables 3A–D**) representing presumably orthologous repeat families from different samples that were grouped together due to their high repeat sequence similarity (Macas et al., 2015). The number of top clusters used to build the NJ trees and networks was reduced in all groups after discarding clusters with NA or zero read values for some samples (Loliinae: 38 clusters; BL: 96; FL: 122; Schedonorus: 167) (**Supplementary Tables 4A–D**). Networks constructed from distance-based NJ trees computed with the Euclidean distances (**Figures 4A–D**) showed better resolutions than those obtained from NJ trees computed with the inverse distances (**Supplementary Figures 3A–D**); therefore, descriptions of repeatome phylogenies were based on the Euclidean networks. The unrooted Loliinae network showed three divergent groups corresponding to each of the main BL, FL and Schedonorus lineages (**Figure 4A**). In this network, the Schedonorus group was highly isolated from the others and, in contrast to its position in the Loliinae tree (**Figure 1**), it was closer to the FL group than to the BL group. Similarly, the fine-leaved *F. eskia* was closer to the BL group than to its own FL group. The unrooted BL network (**Figure 4B**) inferred a topology congruent with that of the BL lineage in the Loliinae tree except for the sister relationship of South African *F. scabra* with the other Tropical and South African taxa and the sister relationship of the two Subbulbosae species (*F. paniculata*/*F. durandoi*), resolutions that, however, matched those recovered from the 35S Loliinae tree (**Supplementary Figure 1C**). The unrooted FL network (**Figure 4C**) was generally consistent with the combined Loliinae tree except for the positions of the American I and American-Pampas taxa, which were closely related to the American II taxa; Afroalpine *F. abyssinica* was also close to them (**Figure 4C**). These phylogenetic topologies were also congruent with those retrieved in the 35S Loliinae tree (**Supplementary Figure 1C**).

The potential phylogenetic signal of the abundance of the repeat clusters (**Supplementary Tables 4A–D**) evaluated in different Loliinae subtrees, rendered significant K values for distinct clusters in each group (**Supplementary Table 5** and

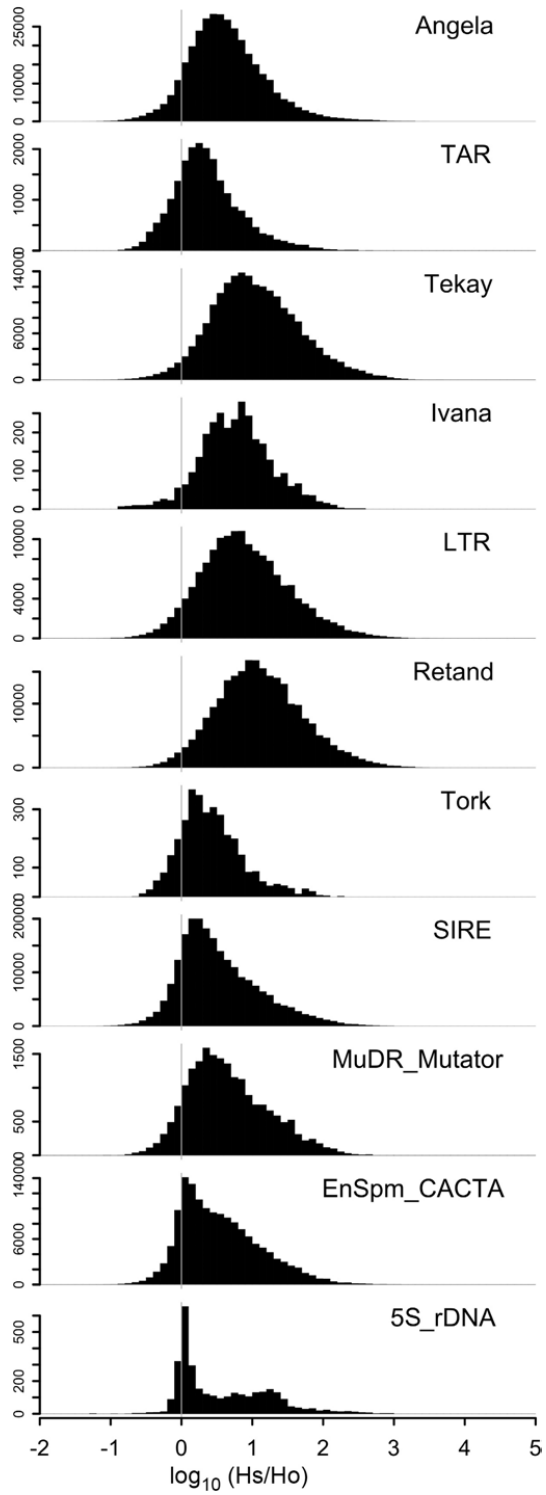

FIGURE 3 | Global variability of main repeat types and their sequence conservation across the Loliinae genome landscapes. Histograms show distributions of read similarity Hs/Ho hit ratios [frequencies of read similarity hits to reads from the same species (Hs) or to reads from all other species (Ho) (log scale, *x*-axis) and number of reads (*y*-axis)]. Hs/Ho ratios close to one (0 on the logarithmic scale) indicate sequence conservatism while larger values indicate sequence diversification.

Supplementary Figure 4). Within the Loliinae group, nine clusters (1 LTR, 4 Angela, 1 SIRE, 3 CACTA) had significant *K* values on the Loliinae tree cladogram, although only the *K* values of the four Angela clusters were >0.5. In contrast, within the FL group only four clusters (1 Angela, 2 Tekay, 1 repeat) had significant *K* values on the FL tree cladogram but all of them were ∼1. The BL and Schedonorus groups had 17 clusters that carried phylogenetic signal on their respective tree cladograms; however, whereas all the BL clusters (1 LTR, 3 Angela, 8 Tekay, 4 Athila, 1 Mutator) had *K* values close to 1, only nine out of the 17 Schedonorus clusters had *K* values ∼1 (3 LTR, 3 Repeat, 1 CRM, 1 Mutator, 1 Tekay) while the remaining eight cluster (6 LTR, 1 Athila, 1 Mutator) carried more phylogenetic signal than expected (*K* values > 1) (**Supplementary Table 5** and **Supplementary Figure 4**).

5S rDNA Graph-Clusters of Loliinae

The Loliinae 5S rDNA region ranged from 245 to 316 bp in the Loliinae [a 120 bp 5S gene conserved in all taxa plus a variable IGS for specific taxa (range 125–196 bp); **Supplementary Table 1**]; the 5S MSA consisted of 316 bp (120 bp 5S gene; 196 bp IGS). The Loliinae 5S ML tree (**Supplementary Figure 5**) had poor support for most of its branches and was topologically incongruent with both the combined Loliinae tree (**Figure 1** and **Supplementary Figure 1B**) and the separate plastome and nuclear 35S rDNA trees (**Supplementary Figures 1C,D**). The only supported lineage was the Schedonorus clade (**Supplementary Figure 5**) although its internal resolution also departed from those of the other trees and was not considered further.

Analysis of the 5S rDNA clusters of 47 Loliinae species studied produced different types of simple and complex graphs that did not always match the expected shapes for their respective ploidy levels (**Table 4** and **Figure 5**). As expected, most graph topologies of diploid taxa corresponded to a simple circular graph that likely represents a single 5S gene family and locus. This was observed for most FL (*F. eskia, F. capillifolia, F. ovina*) and Schedonorus (*F. pratensis, F. fontqueri*, *M. tuberosa*, all five *Lolium* species) diploids. However, within the BL diploids one species showed a simple graph (*F. lasto*) but two species (*F. triflora, F. paniculata*) had complex graphs with two IGS loops interconnected by a junction section (coding region of the 5S gene), suggesting that the latter species could have two 5S ribotypes (**Figure 5**). Within Loliinae polyploids, 5S graph topologies ranged from those taxa showing complex graphs with a number of loops corresponding to their assumed number of 5S loci (tetraploid *F. pyrenaica*, two loops), to high polyploids with lower number of loops than expected based on their ploidy levels (decaploids *F. africana* and *F. letourneuxiana*, two loops), and low-to-high polyploids showing a simple graph (tetraploids *V. ciliata*, *F. parvigluma, F. procera, F. abyssinica, F. simensis, F. fenax, F. mairei, F. mekiste*; hexaploids *F. rubra, F. chimborazensis, F. asplundii, F. fimbriata, F. amplissima*; octoploids *F. pampeana, F. spectabilis, F. atlantigena, F. superba*). Loliinae species from the southern hemisphere with unknown ploidy level displaying complex 5S graphs (e.g., *F. pyrogea*, *M. masafuerana*; two loops) were identified as polyploids, while those displaying a single

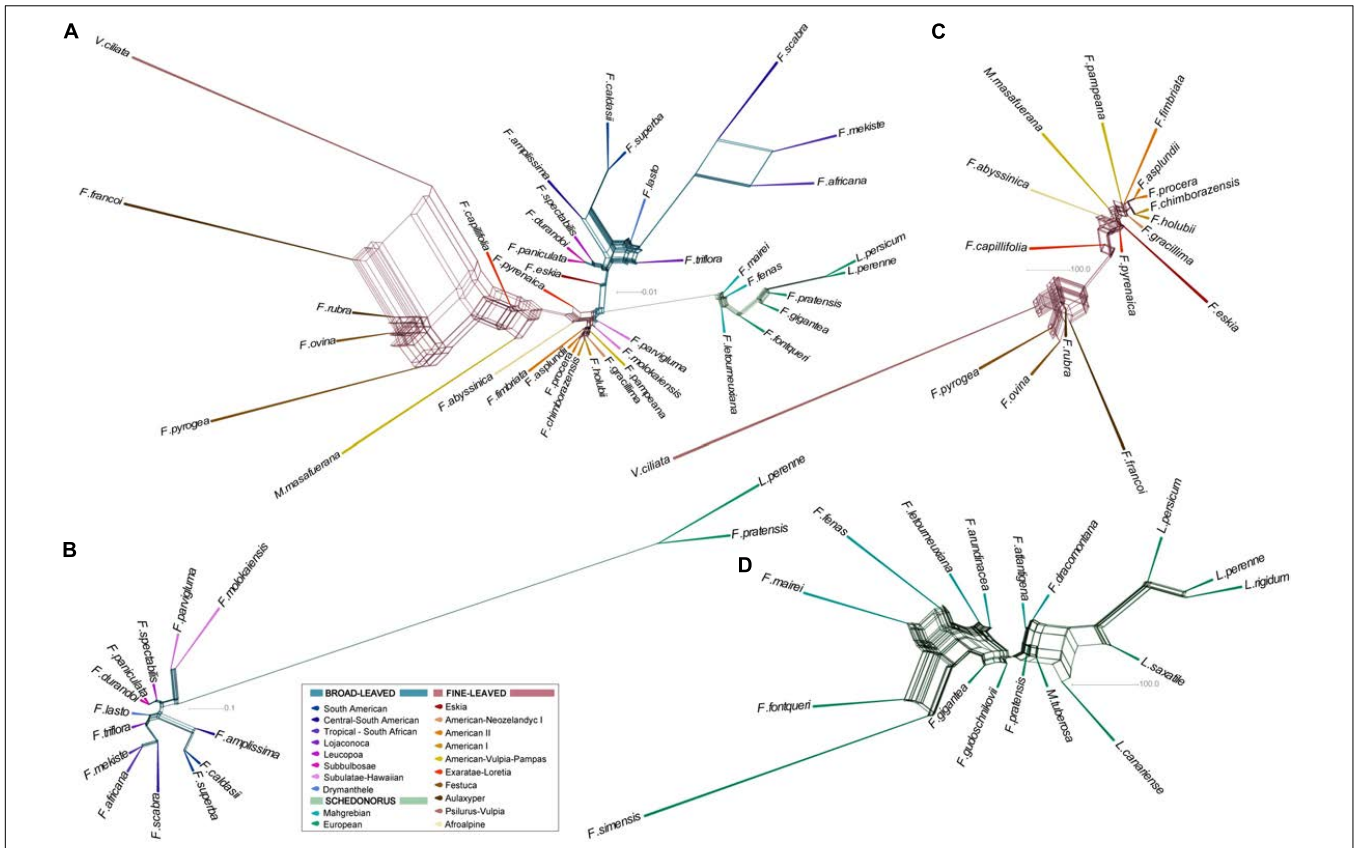

FIGURE 4 | Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the four Loliinae evolutionary groups: **(A)** Loliinae, **(B)** broad-leaved (BL) Loliinae, **(C)** fine-leaved (FL) Loliinae, and **(D)** Schedonorus. The networks were constructed from distance-based NJ trees computed with pairwise Euclidean distances between samples (see text). Color codes of Loliinae lineages are indicated in the respective charts. Scale bar: number of mutations per site.

graph (e.g., *F. dracomontana, F. holubii, F. molokaiensis*) could not be classified as such (**Figure 5**).

DISCUSSION

Characterization of the Loliinae Repeatome and Its Impact on the Diversification of the Genome Size of Its Lineages

Our large-scale exploratory analysis of the Loliinae repeatome has uncovered the abundance and composition of the repetitive DNA across the genome landscape of all the subtribal lineages, confirming the substantial contribution of the repeatome to the genome size diversification of the studied Loliinae genomes (**Table 2**, **Figure 1**, and **Supplementary Figure 1E**). The repetitive elements represent more than half of the holoploid genome of most surveyed Loliinae taxa and accounted for the largest percentages (>60%) in the BL and *Lolium* genomes (**Table 2**, **Figure 1**, and **Supplementary Figure 1E**). Our data has demonstrated that the 1.5- to 3-fold downsizing monoploid genome trend observed by previous authors between BL and FL Loliinae lineages (Catalán, 2006; Šmarda et al., 2008) can be attributed to proportional amounts of their respective repetitive elements (**Tables 2**, **3**, **Figure 1**, and **Supplementary Figure 1E**). Unlike other studies that found no evidence of repeat activity causing large variation in genome size among diploid species (e.g., *Anacyclus*; Vitales et al., 2020a), our analyses have corroborated that striking differences in the 1.5-fold increase in genome size between BL and FL Loliinae diploid genomes was caused by significant differences in the repeat contents of the more abundant Retand and Angela retrotransposons (**Tables 2**, **3**, **Figures 1**, **2**, and **Supplementary Figure 2**). In general, the Loliinae diploid genomes, -either BL, FL or Schedonorus-, showed higher proportions of repeats than the allopolyploid genomes except for some of the South American BL and FL polyploid genomes (**Tables 1**, **2**, **Figure 1**, and **Supplementary Table 1**). Thus, our data partially rejects the "polyploid genome shock" hypothesis that predicts increased genome sizes (and correlated repeat expansions) in polyploids, as well as the additive pattern of diploid repeat contents in the derived allopolyploids (e.g., *Melampodium*; McCann et al., 2018). In contrast, it supports the alternative hypothesis that predicts a trend for genome (and repeatome) reduction after polyploidization due to genomic losses of duplicated genome

TABLE 4 | Ploidy levels and genomic pair-end read features of 5S rDNA loci and cluster graph parameters of the studied Loliinae taxa.

Taxon	Ploidy level	N. reads in cluster	Genome proportion (%)	Repeat size (bp)	k-mer coverage	Connected component index	Graph shape (type)
Festuca abyssinica	4x	180	0.036	316	0.885	0.967	1
Festuca africana	10x	214	0.043	317	0.488	0.879	2
Festuca amplissima	6x	158	0.032	318	0.744	0.994	1
Festuca a. arundinacea	6x	369	0.074	315	0.78	0.987	1
Festuca a. letourneuxiana	10x	532	0.11	307	0.721	0.974	2
Festuca a. atlantigena	8x	428	0.086	307	0.791	0.981	2
Festuca asplundii	6x	110	0.022	318	0.894	0.982	1
Festuca caldasii	4x	–	–	–	–	–	–
Festuca capillifolia	2x	340	0.068	318	0.9	0.976	1
Festuca chimborazensis	6x	179	0.036	319	0.845	0.899	2
Festuca dracomontana	–	629	0.13	307	0.75	0.936	1
Festuca durandoi	2x	520	0.1	318	0.812	0.994	2
Festuca eskia	2x	525	0.1	319	0.873	0.989	2
Festuca fenas	4x	222	0.044	307	0.781	0.973	1
Festuca fimbriata	6x	104	0.021	317	0.8	0.923	1
Festuca fontqueri	2x	470	0.094	296	0.824	0.977	2
Festuca francoi	2x	632	0.13	317	0.748	0.981	2
Festuca gigantea	6x	–	–	–	–	–	–
Festuca gracillima	6x	–	–	–	–	–	–
Festuca gudoschnikovii	4x	–	–	–	–	–	–
Festuca holubii	–	179	0.036	318	0.863	0.944	2
Festuca lasto	2x	470	0.094	296	0.824	0.977	1
Festuca mairei	4x	330	0.066	315	0.791	0.921	1
Festuca mekiste	–	109	0.022	317	0.619	0.917	1
Festuca molokaiensis	–	208	0.042	316	0.666	0.861	2
Festuca ovina	2x	331	0.066	316	0.952	0.985	1
Festuca pampeana	8x	402	0.08	317	0.812	0.98	1
Festuca paniculata	2x	269	0.054	318	0.781	0.978	2
Festuca parvigluma	4x	190	0.038	316	0.711	0.884	1
Festuca pratensis	2x	447	0.089	545	0.832	0.911	2
Festuca procera	4x	165	0.033	317	0.863	0.976	2
Festuca pyrenaica	4x	204	0.041	316	0.62	0.941	2
Festuca pyrogea	–	850	0.17	326	0.602	0.955	2
Festuca rubra	6x	338	0.068	316	0.737	0.87	2
Festuca scabra	4x	232	0.046	301	0.782	0.978	2
Festuca simensis	4x	412	0.082	296	0.675	0.951	2
Festuca spectabilis	6x	1128	0.23	316	0.791	0.99	2
Festuca superba	8x	184	0.037	316	0.772	0.995	1
Festuca triflora	2x	217	0.043	262	0.498	0.982	2
Lolium canariense	2x	306	0.061	294	0.842	0.974	1
Lolium perenne	2x	447	0.089	307	0.868	0.982	1
Lolium persicum	2x	1154	0.23	307	0.832	0.976	1
Lolium rigidum	2x	892	0.18	307	0.809	0.983	1
Lolium saxatile	2x	157	0.031	308	0.914	0.975	2
Megalachne masafuerana	–	690	0.14	224	0.438	0.997	2
Micropyropsis tuberosa	2x	911	0.18	307	0.865	0.98	1
Vulpia ciliata	4x	414	0.083	315	0.916	0.993	2

Graph shape types (type 1, simple circular-shaped graph with one loop; type 2, complex graph with two loops where the interconnected loops represent IGS spacers). 5S clustering analysis of F. caldasii, F. gigantea, F. gracillima and F. gudoschnikovii could not be performed due to insufficient number of 5S reads in the clusters. Hyphens, missing data.

fragments (e.g., *Spartina* and several sequenced plants; Chen, 2007; Parisod et al., 2010; Michael, 2014). The significantly lower genome sizes and correlated lower repeat contents of Old World Loliinae polyploids relative to diploids (**Tables 1**, **2**, **Figures 1**, **2**, and **Supplementary Figure 2**) could be attributed to the relatively ancestral DNA ages of some of these polyploid lineages [e.g., Schedonorus Mahgrebian (6.3 Ma) and FL Aulaxyper (6.1 Ma) clades; Moreno-Aguilar et al., 2020], which might have eliminated duplicated repeats over time. Furthermore, the high level of ploidy (6x-8x-10x) of these allopolyploids, which have apparently lost more redundant repeats compared to their closely related diploids or lower polyploids, could have resulted from

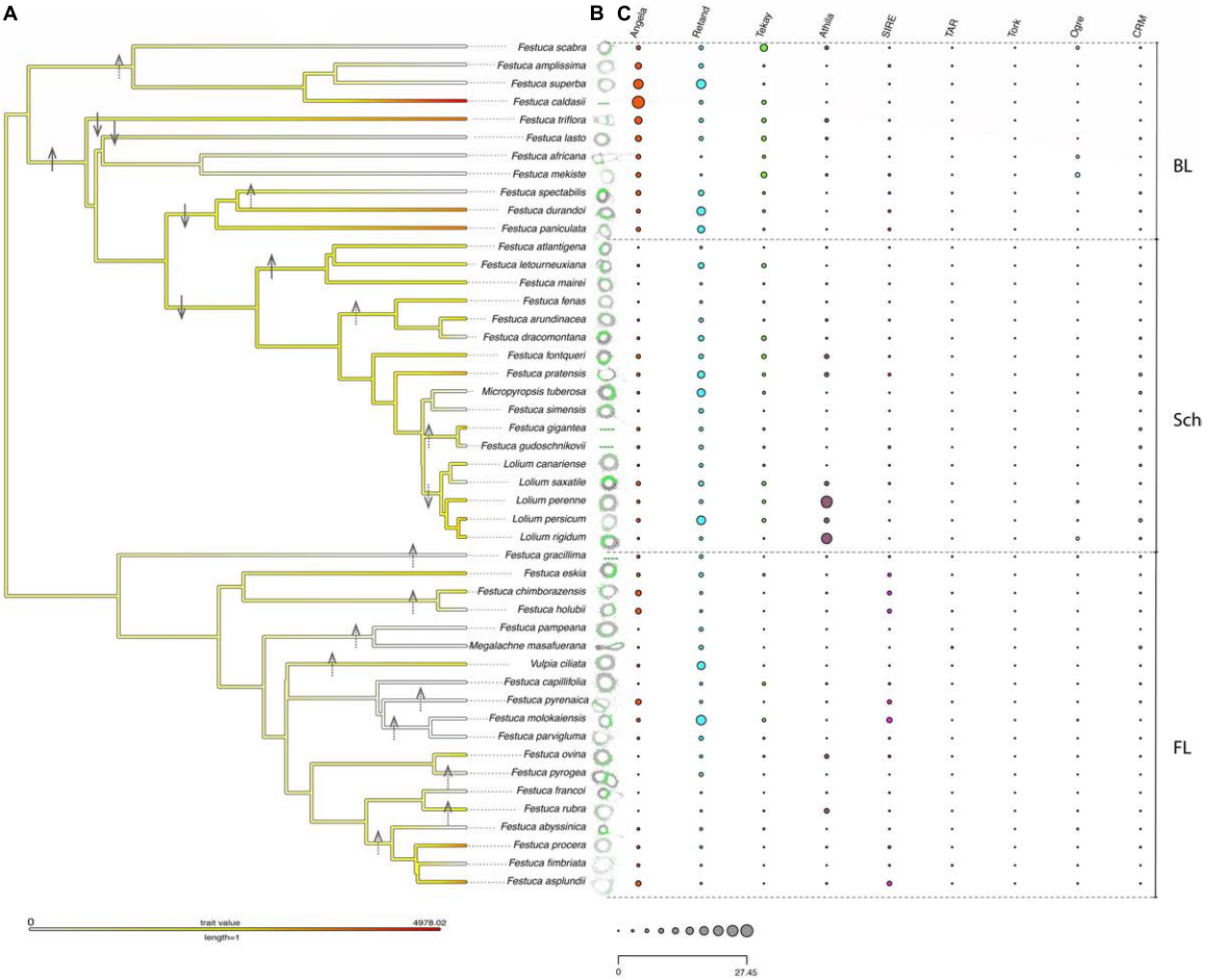

FIGURE 5 | (A) Maximum Likelihood phylogenetic tree (combined plastome + nuclear 35S rDNA data) of the 47 studied Loliinae samples showing their genome sizes by the colors of the terminal branches (color gradients indicate inferred genome size changes); white, missing data. **(B)** 5S clustering graph plots generated by RE2. **(C)** Proportions of the most abundant repeat elements (standardized values) obtained from the individual RE2 analysis of repeats are shown for each taxon. Hypothesized scenarios of allopolyploidization and diploidization events mapped onto the tree branches (ancestral allopolyploidization: solid arrow up; ancestral diploidization: solid arrow down; recent allopolyploidization: dashed arrow up; recent diploidization: dashed arrow down). BL, broad-leaved Loliinae; Sch, Schedonorus; FL, fine-leaved Loliinae.

a selective process to limit repetitive DNA damaging activity (Wang et al., 2021). Alternatively, some of these high polyploids could have originated through autopolyploidy or a combination of autopolyploidy and allopolyploidy; those scenarios would better explain the simple 5S graph patterns observed in many of these taxa (**Figure 5**). However, all thoroughly investigated Loliinae polyploids have been shown to be allopolyploids (Catalán, 2006, and references therein). The considerable reductions in retrotransposon and transposon contents detected in high polyploid Loliinae species are consistent with parallel losses of 35S rDNA loci in the same taxa (e.g., BL *F. africana*-10x, Namaganda, 2007; Schedonorus *F. atlantigena*-8x and *F. letourneuxiana*-10x, Ezquerro-López et al., 2017), suggesting that the two types of repetitive DNA reductions might have occurred after large genomic rearrangements in these high polyploids. In contrast, the large repeat contents of some Old World Loliinae diploids could be explained by the dynamic activity of young repeat types that have proliferated in recent diploid lineages (e.g., Athila in *Lolium*; **Table 2** and **Figures 1**, **2**; Zwyrtková et al., 2020).

As in many angiosperms (Eickbush and Malik, 2002), the retrotransposons LTR-Gypsy Retand (1.6–21.3%) and LTR-copia Angela (0.02–27.5%) were the most widely represented repeat family in the Loliinae genomes (**Table 2** and **Figure 1**). The Tekay, Athila and SIRE elements followed, while other retrotransposons (Ogre, CRM) and transposons (CACTA) were less common (**Table 2** and **Figure 1**). Together, they showed a strong correlation with genome size (R^2 = 0.83, p = 1.8E-09) and a considerable contribution to the differences in genome sizes (65.2%) between Loliinae lineages (**Table 3** and **Figure 2**),

although these contributions varied for the most abundant types. The Retand repeats contributed significantly to the larger genome sizes of the BL and Schedonorus genomes compared to the FL genomes (**Table 2**), while the Angela repeats also contributed to the large sizes of the BL genomes and, notably, to some relatively large genomes of FL American I and American II genomes (**Table 2**). The Angela elements showed the highest correlation of repeat content with genome size (R^2 = 0.71) and also explained the greatest differences in genome size between species (19.6%), in contrast to the Retand repeats that presented lower correlation and contribution values (R^2 = 0.21; 10.7%) (**Table 3** and **Supplementary Figure 2**). The important role of Angela retrotransposons in genome size diversification of Loliinae genomes is likely related to the relatively higher conservatism of these repeats, compared to the more variable behavior of Retand and other repeat elements (**Figure 3**). In agreement with other studies that have also detected older and less active Angela copies in Fabaceae (Macas et al., 2015) and Triticeae (Wicker et al., 2017, 2018), but in contrast to the finding of a high turnover of Angela families in *Brachypodium distachyon* (Stritt et al., 2020), our data indicated that Angela repeats also tend to be relatively conserved in Loliinae and have probably better fitted long-term genomic diversification trends of their ancestral genomes (19.4 Ma; Moreno-Aguilar et al., 2020). In contrast, young and highly heterogeneous Athila families likely experienced a recent burst within the *Lolium* clade and especially in the allogamous *L. perenne* and *L. rigidum* genomes (23–25%) and were moderately abundant in other studied ray-grasses and their close *F. pratensis* and *F. fontqueri* relatives (7–8%) (**Table 2** and **Figure 1**). Noticeably, Athila elements also proliferated in recent FL *F. rubra* (8.5%) and *F. ovina* (7.1%) genomes, constituting the best represented annotated family in the red and sheep fescues (**Table 2** and **Figure 1**).

Phylogenetic Value of the Loliinae Repeatome and Deconvolution of the Origins of Some Genomes From 5S Cluster Graphs

In agreement with previous studies from other angiosperms (Dodsworth et al., 2015; McCann et al., 2018, 2020; Vitales et al., 2020b; Herklotz et al., 2021), the different amounts of shared repeats retrieved from comparative RE2 analyses of Loliinae have been shown to contain phylogenetic information at different systematic levels across the four Loliinae evolutionary groups. All evolutionary analyses have confirmed their ability to recover deep-to-shallow evolutionary relationships that were highly or relatively consistent with those based on the 35S rDNA and the plastome and combined data sets, respectively (**Tables 1**, **4**, **Figures 4**, **5**, **Supplementary Tables 3, 4**, and **Supplementary Figures 1, 3**). Some of the networks have, however, uncovered repeatome-specific topological features, which were not observed in the MSA trees (**Figure 4**).

The unrooted Loliinae and BL repeatome networks have demonstrated the high isolation of Schedonorus from the remaining Loliinae lineages (**Figures 4A,C**). This large divergence was based on the uniqueness of the Schedonorus repeat amounts within the representatives of the subtribe (**Supplementary Table 3**). Although Schedonorus has traditionally been considered a recent split within the broad-leaved Loliinae in all previous evolutionary studies (Minaya et al., 2017; Moreno-Aguilar et al., 2020, and references therein), and in the current combined tree of Loliinae (**Figure 1** and **Supplementary Figure 1B**), this position is mostly based in the strong plastome topology (**Supplementary Figure 1C**) and its large sequence dataset. By contrast, the weak nuclear 35S ML topology showed extremely low support for the potentially basal paraphyletic divergences of the BL lineages and an unclear position for Schedonorus within them (**Supplementary Figure 1D**). The repeatome network placed Schedonorus more closely related to the FL than to the BL group (**Figure 4A**). More reliable phylogenies based on single-copy nuclear genes would be needed to decipher the evolution of Schedonorus and other Loliinae nuclear genomes. Here, the phylogeny of tall fescues and ray-grasses has been enriched with three new taxa, showing the sister relationships of the eastern Canary Islands endemic *Lolium saxatile*-2x (Scholz and Scholz, 2005) to *L. canariense*-2x, of Siberian *F. gudoschnikovii*-4x (Stepanov, 2015; Probatova et al., 2017) to its morphologically close Eurosiberian relative *F. gigantea*-6x, and of previously unstudied South African *F. dracomontana* (Linder, 1986) to *F. arundinacea*-6x (plastome tree) or to the 'European' clade (35S tree) (**Figure 1** and **Supplementary Figures 1A–D**). A notable geographical signal of the repeatome was observed in the close relationships of NW African *F. fontqueri*-2x and Tropical African *F. simensis*-4x with Mahgrebian *F. mairei*-4x (**Figure 4D**), in contrast to their nesting positions within the predominantly diploid "European" clade in the plastome, 35S and combined trees (**Supplementary Figures 1B–D**). Also, the position of *F. dracomontana* in the repeatome network suggest that this austral Schedonorus species could be a polyploid close to the tall fescues (**Figure 4D** and **Supplementary Figures 1B–D**).

Geographically based evolutionary patterns of repetitive elements, congruent with those of the nuclear 35S rDNA tree, have been also observed in the FL and BL repeatome networks (**Figures 4B,C** and **Supplementary Figure 1D**). Within the FL network group, South American representatives of the American I, American-Pampas and American II lineages are closely related to each other (**Figure 4B** and **Supplementary Figure 1D**), while interspersed with other FL lineages in the plastome and combined Loliinae trees (**Supplementary Figures 1B,C**). These lineages are characterized by similar levels of Angela, Retand and LTR repeats (**Table 2** and **Figure 1**) and were inferred to be of similar age (late Miocene_Pliocene transition, 3.4–5.4 Ma; Minaya et al., 2017). They are probably the descendants of the same paternal lineage, which probably evolved *in situ* but crossed with distinct maternal FL lineages giving rise to these close but separate allopolyploid clades (**Supplementary Figures 1B,C**). Within the BL group, the close relationships between South African *F. scabra* and Tropical and South African *F. africana*/*F. mekiste* and between Mediterranean-European *F. spectabilis* (Leucopoa) and *F. paniculata*/*F. durandoi* (Subbulbosae) based on shared repeat contents are more similar to those recovered in the 35S tree than in the plastome tree (**Figure 4C** and

Supplementary Figure 1A–C), also suggesting a concerted evolution of nuclear repetitive DNA families and different hybridizations or chloroplast capture events with other BL lineages. In contrast, the close relationship of Central-American *F. amplissima* to the South American *F. superba*/*F. caldasii* lineage shown in the repeatome network is more similar to that observed in the plastome and combined Loliinae trees than in the 35S tree, probably due to the lower resolution of the nuclear topology (**Figure 4C** and **Supplementary Figures 1A–C**). Interestingly, these Central and South American taxa show some of the highest Loliinae genomic repeat contents (**Tables 1**, **2**, **Figure 1**, and **Supplementary Figure 1E**) despite their high 6x-8x ploidy-levels. It could be a consequence of their relatively young ages (∼5 Ma; Moreno-Aguilar et al., 2020) and the lack of a time course to purge the excess of repetitive DNA (Michael, 2014), or a recent bloating of repeats. The phylogenetic value of the Loliinae repetitive elements has been further corroborated by the significant phylogenetic signals carried by different repeat clusters when tested on the respective tree cladograms of each of the four Loliinae groups (**Supplementary Table 5** and **Supplementary Figure 4**). In most of the groups, the conservative Angela clusters had significant *K* values above 0.5 and close to 1, indicating their strong phylogenetic signal at different taxonomic levels.

Although tandem-repeated 5S rDNA did not retrieve a congruent evolutionary history for Loliinae (**Supplementary Figure 5**), their cluster graph topologies revealed their presumable number of loci (**Figure 5**), indicative of their potential hybridization events (Vozárová et al., 2021) and ploidy levels (Garcia et al., 2020). In contrast to the instability of 35S rDNA loci, the maintenance of 5S rDNA loci in high allopolyploid Loliinae species (Ezquerro-López et al., 2017) is consistent with their conserved patterns in other angiosperm allopolyploids (Garcia et al., 2017). Studies of allopolyploids with known subgenomes have demonstrated that species showing complex graphs with two IGS loops correspond to allotetraploids and those showing three loops to allohexaploids (Garcia et al., 2020), while in highly hybridogenous diploid rose species graphs with two loops probably correspond to ancient 5S rDNA families (Vozárová et al., 2021). Within the Loliinae studied, several polyploid taxa displayed 5S graphs with fewer loops than expected for their ploidy level (**Figure 5**), suggesting the existence of convergent evolution to one or few ribotypes. In contrast, three diploid species, BL *F. triflora* and *F. paniculata* and FL *F. francoi*, showed a 5S graph pattern typical of allotetraploids (**Figure 5**), supporting the hypothesis of their putative paleo-polyploid hybrid origin.

Recurrent Rounds of Allopolyploidizations and Diploidizations Within Loliinae Lineages Revealed by Their Repeats

The widely accepted evolutionary scenario for the origin of the angiosperms, consisting of several rounds of hybridizations and allopolyploidizations followed by a return to the diploid state (Soltis et al., 2016) has been also inferred for the grasses and their main lineages. Evidence suggests that protograss whole genome duplication (WGD) was likely followed by later diploidizations that ended in current paleo-ancestral diploid karyotypes for temperate and tropical grasses (Salse et al., 2008). These involved distinct and profound genomic rearrangements, such as nested chromosome fusions, chromosome inversions and paleocentromere inactivation, along with differential losses of heterologous duplicated copies in subgenomes of divergent lineages (Murat et al., 2010). In contrast, new allopolyploidization events apparently led to the emergence of grass mesopolyploids, originated some million years ago, and grass neopolyploids, considered to have emerged during or after the Quaternary glaciations (Stebbins, 1985; Marcussen et al., 2014). Our data allow us to hypothetize that the evolution of Loliinae could have resulted from relatively rapid recurrent rounds of allopolyploidizations and diploidizations during the last 19–22 Ma (Minaya et al., 2017; Moreno-Aguilar et al., 2020) that have leaved their signatures on their repeats (**Figure 1** and **Supplementary Figure 1E**) and 5S graph topologies (**Figure 5**). We postulate that the large genomes of the early diverging BL diploids (Lojaconoa, Drymanthele, Subulbosae; 7.5–5 Ma, Minaya et al., 2017) likely resulted from WGD of ancestral interspecific hybrids that later reverted to the diploid state with large chromosomes (Catalán, 2006), relatively large monoploid genome sizes and repeat contents (**Table 2**, **Figures 1**, **2**, and **Supplementary Figure 1E**) and complex 5S graphs indicative of putative allotetraploids (**Figure 5**). This polyploid hybrid origin could also explain the potential heterosis of these robust broad-leaved fescues (Catalán, 2006). We also hypothetize that the large genomes and repeatomes of the basal BL polyploid lineages (Central-South American, South African) may have resulted from more recent allopolyploidizations (5–2.5 Ma, Minaya et al., 2017), with genomes that still maintain large sizes and proportions of repeats, and retain traces of more than one 5S ribotype (**Table 2**, **Figures 1**, **2**, **5** and **Supplementary Figure 1E**).

Our findings are not fully compatible with the hypotheses of drastic genome contractions from a hypothetical large-genome Loliinae ancestor to the FL Loliinae lineage and in allopolyploids with large progenitor genomes but not in autopolyploids with small progenitor genomes (Loureiro et al., 2007; Šmarda et al., 2008). The observed reduction in repeat content and correlated genome size from the large BL Loliinae, through intermediate Schedonodorus and *F. eskia*, to the small FL Loliinae genomes (**Figures 2**, **5**) could have resulted from independent genome size diversifications along the major Loliinae lineages (**Figures 1**, **5** and **Supplementary Figure 1**). Our data also support an alternative scenario of independent hybridization and polyploidization events across FL Loliinae, which are similar in age (∼16 Ma, Minaya et al., 2017) to BL Loliinae. Their small chromosomes and genome sizes (Catalán, 2006), especially for the taxa of the core Eurasian and Mediterranean Vulpia, Festuca and Aulaxyper (plus Exaratae) lineages (**Tables 1**, **2** and **Figures 1**, **2**, **5**), are similar to those of the close subtribes Parapholiinae, Cynosuriinae, and Dactylidiinae with which they also share 35S rDNA families (Catalán et al., 2004). Therefore, it could be hypothesized that the ancestor of these FL Loliinae did not undergo the same double

genome enlargement as the ancestor of BL Loliinae. In addition, the various polyploid New World FL lineages (American I, American-Pampas, Subulatae-Hawaiian, American II), which show larger genome sizes and geographically structured repeat contents (**Tables 1**, **2**, **Figures 1**, **4A,C**, **5**) are probably the results of recent allopolyploidizations (5–2.5 Ma, Minaya et al., 2017) that have not yet experienced considerable purging in their repeats.

The isolated Schedonorus lineage emerges as a highly dynamic repeat-driven evolving group, also accumulating evidence of various allopolyploidizations and diploidizations. A distinctive feature is the bloating of Athila repeats in the recently evolved diploid clade *Lolium*, especially in allogamous ray-grasses (**Table 2**, **Figures 1**, **2**, and **Supplementary Figure 2**; Zwyrtková et al., 2020). In contrast, the Mahgrebian clade constitute a relatively ancestral lineage with unknown diploid relatives (Inda et al., 2014), although it shows signatures of ancient hybridizations in its 5S graph topologies (**Figure 5**). The Schedonorus Mahgrebian and the FL Aulaxyper allopolyploid lineages have experienced the most pronounced reductions in their repeats and genome sizes of all Loliinae studied (**Table 2** and **Figures 1**, **2**, **5**). Interestingly, these two lineages also exhibit the highest and most extensive hybridization rates among the Loliinae, producing both intra- and intergeneric hybrids (Catalán, 2006). Schedonorus *Festuca* taxa spontaneously hybridize with each other and with close species of *Lolium* (x *Festulolium*) while Aulaxyper *Festuca* taxa (*F.* gr. *rubra*) also interbreed with each other and with close species of *Vulpia* (x *Festulpia*) (Catalán, 2006, and references therein). Therefore, it might be plausible that these two highly hybridogenous allopolyploid lineages have undergone large genome reshufflings to accommodate their highly divergent heterologous subgenomes and avoid DNA damage (Michael, 2014; Wang et al., 2021). These genomic rearrangements would have caused more severe losses in their respective repeats and genome sizes than those of other high polyploid American BL and FL Loliinae of similar ancestry that resulted from crosses of genomically similar progenitor species and presumably did not experience large repeat contractions (**Table 2** and **Figures 1**, **2**, **5**).

DATA AVAILABILITY STATEMENT

The newly studied grass plastome and 35S and 5S rDNA cistron sequences have been deposited in the Genbank data base under accession numbers SAMN27777779–SAMN27777788, ON243855–ON243864 and ON248974–ON249019, and at the Github repository (https://github.com/Bioflora/Loliinae_Repeatome).

AUTHOR CONTRIBUTIONS

PC designed the study. MM-A, IA, LI, and PC collected the samples. MM-A and LI developed the experimental work. PC, MM-A, LI, IA, and PC analyzed the data and interpreted the results. PC and MM-A prepared the manuscript. PC, MM-A, LI, IA, and AS-R revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.901733/full#supplementary-material

Supplementary Table 1 | Taxa included in the repeatome analysis of Loliinae. Taxonomic rank, taxon authorship, detailed localities and vouchers, and source of cytogenetic and genomic data. Group: BL, broad-leaved Loliinae; FL, fine-leaved Loliinae; Sch, Schedonorus. Chromosome number (2n), ploidy, genome size (2C, pg), monoploid genome size (1Cx, pg; 1Cx, Mbp) and GenBank accession codes for plastome and nuclear ribosomal 35S and 5S genes are given for each sample. Values in bold correspond to new data generated in this study. Outgroups used in the phylogenomic analyses: *Oryza sativa, Brachypodium distachyon*.

Supplementary Table 2 | Loliinae samples used in the repetitive DNA analysis. Genome skimming paired-end (PE) reads per sample and PE reads selected by Repeat Explorer 2 per sample in each of the comparative analyses of the four Loliinae groups: Loliinae, BL (broad-leaved Loliinae), FL (fine-leaved Loliinae), Schedonorus.

Supplementary Table 3 | Repeat Explorer 2 comparative analysis. Repeat content data for top clusters (repeat families) in each of the four evolutionary

groups of Loliinae: **(A)** Loliinae; **(B)** broad-leaved (BL) Loliinae; **(C)** fine-leaved (FL) Loliinae; **(D)** Schedonorus.

Supplementary Table 4 | Repeat Explorer 2 comparative analysis. Repeat content data for phylogenetically analyzed clusters (repeat families) in each of the four evolutionary groups of Loliinae: **(A)** Loliinae; **(B)** broad-leaved (BL) Loliinae; **(C)** fine-leaved (FL) Loliinae; **(D)** Schedonorus.

Supplementary Table 5 | Phylogenetic signal based on Blomberg's *K* values of repeat cluster contents obtained from the comparative RE2 analysis of Loliinae samples assessed in each of the four Loliinae groups: **(A)** Loliinae (38 samples, 38 clusters), **(B)** Broad-leaved (BL) Loliinae (15 samples, 96 clusters), **(C)** fine-leaved (FL) Loliinae (17 samples, 122 clusters), **(D)** Schedonorus (16 samples, 167 clusters), using the *phylosig* option of the *phytools* R package. Cluster abundance values (number of PE reads) are indicated in **Supplementary Table 4**. *K* values close to one indicate phylogenetic signal, values close to zero phylogenetic independence, and values >1 more phylogenetic signal than expected. *p*-Values based on 1000 randomizations. Significant values are highlighted in bold.

Supplementary Figure 1 | (A) Combined (plastome + 35S rDNA) Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. **(B–D)** Maximum Likelihood phylogenomic trees of 47 Loliinae samples based on **(B)** Combined (plastome + 35S rDNA) data, **(C)** plastome data, **(D)** nuclear 35S rDNA data, **(E)** Histograms of repeat contents per holoploid genome (1C) retrieved from the individual Repeat Explorer 2 analyses of the studied Loliinae samples mapped onto the Maximum Likelihood combined phylogenomic tree (plastome + nuclear 35S rDNA cistron) of Loliinae. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.

Supplementary Figure 2 | Correlation plots of repeat content and genome size variation (1Cx) for the 23 Loliinae taxa with known genome sizes. Individual plots for the most represented repeat types found across the 23 Loliinae taxa with known genome size data (see **Table 2** and **Figure 2**). Color codes of Loliinae lineages correspond to those indicated in **Figure 1**.

Supplementary Figure 3 | Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the four Loliinae evolutionary groups: **(A)** Loliinae, **(B)** broad-leaved (BL) Loliinae, **(C)** fine-leaved (FL) Loliinae, **(D)** Schedonorus. The networks were constructed from distance-based NJ trees computed with pairwise inverse distances between samples (see text). Color codes of Loliinae lineages are indicated in the respective charts. Scale bar: number of mutations per site.

Supplementary Figure 4 | Maximum Likelihood Loliinae tree cladograms (combined plastome + nuclear 35S rDNA cistron) showing the relationships among the studied samples in each of the four evolutionary groups of Loliinae and phyloheatmaps of normalized values for different sets of repeat clusters retrieved by RE2 from the comparative analysis of each group: **(A)** Loliinae (38 samples, 38 clusters), **(B)** broad-leaved (BL) Loliinae (15 samples, 96 clusters), **(C)** fine-leaved (FL) Loliinae (17 samples, 122 clusters), **(D)** Schedonorus (16 samples, 167 clusters). Repeat clusters showing significant phylogenetic signal are highlighted with dotted lines.

Supplementary Figure 5 | Maximum Likelihood nuclear 5S rDNA cistron tree showing the relationships among the 47 studied Loliinae samples. Ultrafast bootstrap support values are indicated on branches. *Oryza eichingeri* and *Brachypodium distachyon* outgroups were used to root the tree. Color codes of Loliinae lineages are indicated in the chart. Scale bar: number of mutations per site.

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C.3. Phylogenomics and Systematics of Overlooked Mesoamerican and South American Polyploid Broad-Leaved *Festuca* Grasses Differentiate *F.* sects. *Glabricarpae* and *Ruprechtia* and *F.* subgen. *Asperifolia*, *Erosiflorae*, *Mallopetalon* and *Coironhuecu* (subgen. nov.)

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Abstract: Allopolyploidy is considered a driver of diversity in subtribe Loliinae. We investigate the evolution and systematics of the poorly studied Mesoamerican and South American polyploid broad-leaved *Festuca* L. species of uncertain origin and unclear taxonomy. A taxonomic study of seven diagnostic morphological traits was conducted on a representation of 22 species. Phylogenomic analyses were performed on a representation of these supraspecific taxa and all other Loliinae lineages using separate data from the entire plastome, nuclear rDNA 45S and 5S genes, and repetitive DNA elements. *F.* subgen. *Mallopetalon* falls within the fine-leaved (FL) Loliinae clade, whereas the remaining taxa are nested within the broad-leaved (BL) Loliinae clade forming two separate Mexico–Central–South American (MCSAI, MCSAII) lineages. MCSAI includes representatives of *F.* sect. *Glabricarpae* and *F.* subgen. *Asperifolia* plus *F. superba*, and MCSAII of *F.* subgen. *Erosiflorae* and *F.* sect. *Ruprechtia* plus *F. argentina*. MCSAII likely had a BL Leucopoa paternal ancestor, MCSAI and MCSAII a BL Meso-South American maternal ancestor, and Mallopetalon FL, American I–II ancestors. Plastome vs. nuclear topological discordances corroborated the hybrid allopolyploid origins of these taxa, some of which probably originated from Northern Hemisphere ancestors. The observed data indicate rapid reticulate radiations in the Central–South American subcontinent. Our systematic study supports the reclassification of some studied taxa in different supraspecific *Festuca* ranks.

Keywords: allopolyploid speciation; Mexico–Central–South American broad-leaved *Festuca*; phylogeny; plastome; rDNA 45S and 5S genes; repeatome; taxonomy

1. Introduction

Despite considerable debate about the evolutionary fate of allopolyploids, alternatively viewed as drivers of biodiversity [1] or evolutionary dead ends [2], accumulating evidence suggests that hybridization and whole genome duplication (WGD) has been a preeminent evolutionary mechanism of speciation in the eukaryotic kingdom [3–6]. This is especially remarkable in seed and angiosperm plants, which are all considered descendants of paleopolyploid ancestors [7,8]. Allopolyploids are predominant in the grass family, accounting for 70% of the current species [9,10]. Despite genome duplication being considered generally irreversible in the short term [11], evidence suggests that

the protograss whole genome duplication was likely followed by subsequent diploidizations that originated the respective ancestors of the Bambusoideae–Oryzoideae–Pooideae (BOP) and Panicoideae–Arundinoideae–Centothecoideae–Chloridoideae–Micrairoideae–Aristidoideae–Danthonioideae (PACCMAD) clades [12,13]. The evolutionary scenario of successive rounds of plant hybridizations and allopolyploidizations followed by the return to the diploid state [14] was also inferred for grasses. Grass mesopolyploids and neopolyploids were estimated to have originated some million years ago (Miocene–Pliocene) or during or after the Quaternary glaciations, respectively [11,15,16]. These allopolyploid speciation processes resulted in their current overwhelming representation within the grasses [10], with some genera consisting exclusively of hybrid allopolyploids (e.g., *Elymus* L., *Calamagrostis* Adans. [10,17]) and others containing a large number of them (e.g., *Festuca* L., [18]; *Poa* L., [19]). Molecular phylogenies have helped unravel the hybrid allopolyploid origin of some grass species for which their contrasting plastid vs. nuclear-based topologies have uncovered their respective maternal and paternal lineages [20], while their nuclear single-copy-genes-based topologies have uncovered phased alleles from the distinct progenitor lineages [21].

Subtribe Loliinae, one of the main lineages of the temperate Pooideae, is formed by the large paraphyletic genus *Festuca* and several closely-related genera nested within it [22–27]. Throughout the manuscript, the taxonomic names of *Festuca* are indicated in italics and the phylogenetic lineages of Loliinae in plain text. Phylogenetic analyses have consistently inferred two main clades within the subtribe, the broad-leaved (BL) and fine-leaved (FL) Loliinae, characterized by distinct genomic and phenotypic features [18,23,26,27]. *Festuca* contains approximately 600 species distributed worldwide, inhabiting cool seasonal regions of both hemispheres [18]. *Festuca*'s main center of diversity is the Holarctic region, which harbors nearly 500 species, including all known diploid species of the genus and different polyploids, ranging from tetraploids to dodecaploids [28]. It is also the inferred area for the origin of the BL and FL Loliinae ancestors, which later colonized the Southern Hemisphere according to DEC biogeographic models [25], a hypothesis consistent with the absence of Loliinae diploids in the Southern Hemisphere [18,27,29]. Nearly 80 species of *Festuca* occur in South America [30–34], an area that constitutes a secondary center of diversification of Loliinae and which was colonized several times from different regions [25,26]. Taxonomically, the *Festuca* species have been ranked into eleven subgenera according to the worldwide classification system of Alexeev [35–44]. Of these, the largest subgenus *Festuca*, which encompasses most of the fine-leaved taxa of both hemispheres, makes the bulk of the FL clade. It also includes the small subgenus *Helleria* E.B. Alexeev, also treated as a separate genus *Hellerochloa* Rauschert, and several other genera nested within [18,24–26]. The remaining nine *Festuca* subgenera, except the FL *Mallopetalon* (Döll) E.B. Alexeev, contain species of the BL clade, some of which have been also treated as separate genera. Two of them are native to the Old World (*Schedonorus* (P. Beauv.) Peterm., *Xanthochloa* (Krivot.) Tzvelev), five to the New World (*Asperifolia* E.B. Alexeev; *Subulatae* (Tzvelev) E.B. Alexeev, *Subuliflorae* E.B. Alexeev, *Erosiflorae* E.B. Alexeev, *Mallopetalon*) and two are native to both areas (*Leucopoa* (Griseb.) Hack., *Drymanthele* V.I. Krecz. & Bobrov). The BL clade also includes three additional separate genera nested within [22,25]. The species of the *Festuca* subgenera have been classified in different sections and subsections based on morphological traits ([18,23] and references therein). However, while some of these taxonomic ranks constitute robust lineages of both FL (Festuca (+Wangenheimia), Aulaxyper (+Vulpia 2x), Exaratae (+Loretia)) and BL (Schedonorus (+Lolium and Micropyropsis), Lojaconoa) Loliinae clades, others do not form monophyletic groups or mix with taxa from other *Festuca* ranks [24–27].

Although a large amount of biological and genomic resources has been generated for some economically important forage and grassland *Festuca* species (e.g., *F. pratensis* Huds., *F. arundinacea* Schreb.; [45]), other species of the genus have not been properly analyzed yet. Among the least phylogenetically and systematically studied, Loliinae species are polyploid taxa of six main broad-leaved *Festuca* groups (*Festuca* subgenera *Asperifolia*, *Drymanthele* (sect. *Ruprechtia* E.B. Alexeev), *Erosiflorae*, *Mallopetalon*, *Subulifolia* (sect. *Glabricarpae* E.B. Alexeev), and *F. argentina* (Speg.) Parodi), endemic to Mexico, Central America or South America, some of which constitute the basal-most BL lineages but have uncertain taxonomic adscriptions and evolutionary circumscriptions [24–27]. All of them, except *F. argentina*, include tall fescues that show extravaginal (or mixed) innovations, flat leaves, and open and lax panicles. In a series of successive taxonomic studies, Alexeev described *Festuca* sect. *Ruprechtia* (type specimen *F. amplissima* Rupr.) [37], *F*. subgen. *Asperifolia* (type specimen *F. lugens* (E. Fourn.) Hitchc. ex Hern.-Xol) [38], *F*. sect. *Glabricarpae* (type specimen *F. breviglumis* Swallen) [43], *F*. subgen. *Mallopetalon* (type specimen *F. fimbriata* Nees) [44] and *F*. subgen. *Erosiflorae* (type specimen *F. quadridentata* Kunth) [42] based on the types of innovation leaves, ligules and lemmas, and the presence or absence of ciliate lodicles and of plant and ovary induments. *Festuca argentina*, initially assigned to *F*. subgenus *Festuca* [46], was also considered close to *F*. subgen. *Mallopetalon* [47]; however, it is morphologically different [32] and phylogenetically divergent [24,25] from both taxa. The five subgeneric and sectional *Festuca* ranks described by Alexeev were expanded with other close species described from Mesoamerica and South America by the same or later authors (Table 1). Stančík and Peterson [31] and Stančík and Renvoize [48] extended the concept of *F*. subgen. *Erosiflorae sensu* Alexeev including new broad-leaved South American *Festuca* species within this taxon (e.g., *F. superba* Parodi ex Türpe, *F. venezuelana* Stančík) and transferring taxa from *F*. sect. *Glabricarpae* (e.g., *F. steinbachii* E.B. Alexeev) to it but without strong morphological or phylogenetic arguments.

Despite the importance of previous taxonomic work, the broad-leaved species belonging to these groups have been little studied, and the morphological characters used to delimit their taxonomic ranks remain poorly understood. The high uncertainty about the taxonomic circumscriptions and the evolutionary placements of the five Mesoamerican–South American taxonomic *Festuca* ranks described by Alexeev plus *F. argentina* are of high interest as these polyploid taxa may constitute some of the ancestral lineages of the broad-leaved Loliinae [25,27]. Therefore, the objectives of our study were to: (i) evaluate past classifications and identify diagnostic morphological characters that could serve to circumscribe the taxa; (ii) use genomic data to reconstruct a solid phylogenomic framework to reveal their evolutionary position within the phylogeny of subtribe Loliinae; (iii) detect the putative maternal and paternal origins of these lineages using plastome-based vs. nuclear-based phylogenies; and (iv) propose a reclassification for these taxa based on morphological and molecular evidence.

Table 1. Morphological diagnostic traits used to classify species within *Festuca* subg. *Erosiflorae*, *F*. subg. *Drymanthele* sect. *Ruprechtia*, *F*. subg. *Subulatae* sect. *Glabricarpae*, *F*. subg. *Asperifolia* and *F.* subgen. *Mallopetalon sensu* Alexeev and other authors, plus the newly described *F*. subgen. *Coironhuecu subgen. nov*. (*F. argentina*) and *F*. subgen. *Drymanthele sensu lato* (*F. superba*) analyzed in this study. The type of species of each subgeneric or sectional taxa are highlighted in bold. The asterisks indicate the species used in the phylogenomic analysis.

***Festuca* Subgenera, Sections, Species/Morphological Diagnostic Traits**	**Subgen. *Erosiflorae* *sensu* Alexeev:** ***F. dichoclada* *** ***F. horridula* *** ***F. quadridentata**** ***sensu* Stančík & Peterson:** ***F. carrascana*** ***F. chuquisacae*** ***F. urubambana***	**Subgen. *Drymanthele* Sect. *Ruprechtia* *sensu* Alexeev:** ***F. amplissima**** ***F. jaliscana*** ***sensu* Gonzalez-Ledesma et al.:** ***F. valdesii* ***	**Subgen. *Subulatae* Sect. *Glabricarpae sensu* Alexeev:** ***F. breviglumis**** ***F. chiriquensis**** ***F. dentiflora*** ***F. steinbachii*** ***F. caldasii**** ***F. woodii*** **This Study:** ***F. venezuelana* ***	**Subgen. *Asperifolia* *sensu* Alexeev:** ***F. lugens*** ***F. asperella* *** ***F. tancitaroensis***	**Subgen. *Mallopetalon* *sensu* Alexeev:** ***F. fimbriata* ***	**Subgen. *Coironhuecu* *subgen. nov.* *This Study:*** ***F. argentina* ***	**Subgen. *Drymanthele* *sensu lato* (Without Sectional Assignation) This Study:** ***F. superba* ***
Reproduction	Monoecious	Monoecious	Monoecious	Monoecious	Monoecious	Dioecious	Monoecious
Habit	Largely tussocked or rhizomatous or mixed	Rhizomatous or caespitose	Rhizomatous or loosely tufted	Densely tussocked or rhizomatous	Rhizomatose	Caespitose	Laxely caespitose to rhizomatose
Innovations	Extravaginal or intravaginal	Extravaginal or/and intravaginal	Extravaginal	Extravaginal or intravaginal	Extravaginal	Intravaginal	Mixed
Ligule	Membranaceous, apex acute, erose or lacerate, 5.5–21 mm long	Non-membranaceous, apex truncate shortly ciliate, or short membranaceous, apex truncate and ciliate; 0.1–0.5 (1) mm long	Membranceous or hyaline, apex truncate or rounded, lacerate or dentate; or shortly ciliate; 0.3–4 mm long	Membranaceous, apex truncate or slightly rounded and lacerate or dentate, 1.4–8 mm long	Membranaceous, apex truncate, erose and ciliate, 0.5–1.5 mm long	Membranaceous, apex truncate and densely ciliate, 0.4–1.5 mm long	Hyaline, apex truncate, erose and dentate, 2.7–5.5 mm long
Leaf blade	Flat, involute in the middle and subconvolute at the apex	Flat, involute in the middle and subconvolute at the apex	Flat, involute in the middle and subconvolute at the apex	Flat, involute in the middle and subconvolute at the apex	Largely flat	Plicate, junciform	Largely flat, subconvolute
Inflorescence	Erect	Erect	Nutant or erect with nutant branches	Erect or scarcely nutant	Erect, lax	Erect, contracted	Erect, branches flexuous
Lemma apex	Dentate or entire, unawned	Entire, unawned	Entire or bifid, awned	Bifid, shortly awned or unawned	Entire, scariose, rolled and fimbriate, unawned, muticous	Entire, unawned, muticous or mucronulate	Entire, unawned, muticous
Ovary tip	Glabrescent	Glabrous or hispid	Glabrous or sparsely hispid	Glabrous or hispid	Densely hairy	Sparsely hispid	Densely hairy

2. Results

2.1. Taxonomic Study

The analysis of seven morphological traits used by Alexeev to diagnose the studied *Festuca* subgeneric and sectional ranks (plant habit, type of innovation leaves, ligule type and apex shape, leaf blade type, inflorescence type, lemma apex shape, ovary tip hairiness) plus an additional reproductive trait (monoecy vs. dioecy) (Table 1, Figure 1 and Supplementary Figure S1) on the species classified within these ranks allowed us to identify the taxa proposed by Alexeev and decide on a new supraspecific taxon of *Festuca*.

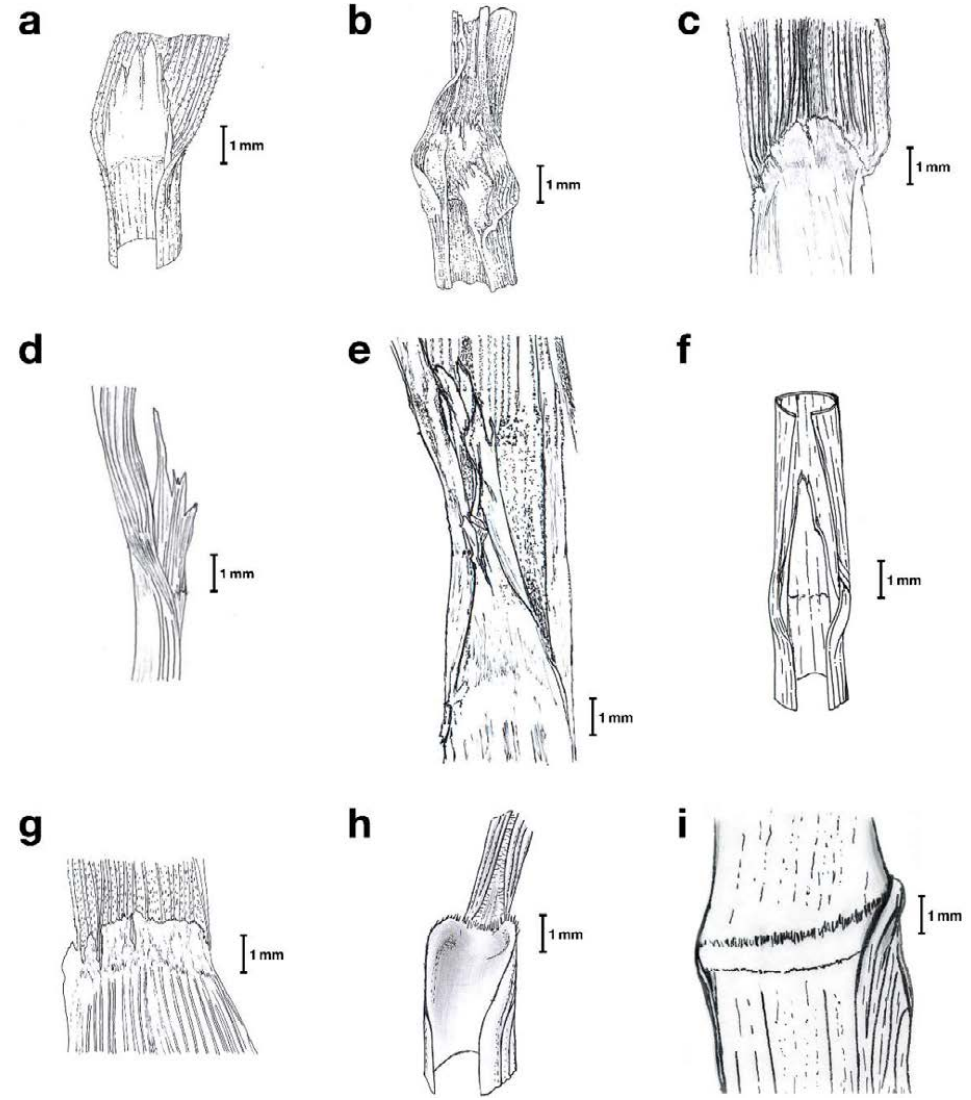

Figure 1. Ligule shape of representative species of Mesoamerican and South American broad-leaved *Festuca* taxa analyzed morphologically in this study. *F.* subgen. *Subulatae* sect. *Glabricarpae*: *F. venezuelana* (**a**); *F.* subgen. *Drymanthele s. l.*: *F. superba* (**b**); *F.* subgen. *Subulatae* sect. *Glabricarpae*: *F. brevi-glumis* (**c**); *F.* subgen. *Asperifolia*: *F. asperella* (**d**); *F.* subgen. *Erosiflorae*: *F. quadridentata* (**e**,**f**); *F.* subgen. *Drymanthele* sect. *Ruprechtia*: *F. amplissima* (**g**); *F.* subgen. *Conrhuecu* (*subgen. nov.*) *F. argentina* (**h**); *F.* subgen. *Multiflorae*: *F. jamesonii* (**i**). Drawings by José Alfredo Hidalgo-Salazar (**a**–**h**) and María Fernanda Moreno-Aguilar (**i**). (**a**,**f**): modified from Stančík and Peterson [31]; (**b**): modified from Türpe [49]; (**c**): Peterson P. M. and Rosales O. 16117, US-3524155; (**d**): Dziekanowski et al., 2022, MO-2107299 (isotype); (**e**): Laegaard S. 55567, AAU; (**g**): Peterson P. M. and Herrera-Arrieda Y. 16150, US-3524157; (**h**): modified from Ospina [34]; (**i**): Kostling M. UZ 498.08).

Species included in *F*. subgen *Erosiflorae sensu* Alexeev [42] are characterized by their monoecy, rhizomatous, tussocked or mixed habit, displaying extravaginal and intravaginal innovation leaves, a long membranous ligule with erose or lacerate apex, flat leaf blades, partially involuted at apex, erect panicles (without nutant branches), unawned dentate or entire lemma apex, and glabrescent ovary tip. These features are present in the type species *F. quadridentata*, endemic from the Ecuadorean paramos, and in two other species distributed in the northern Andes, *F. dichoclada* Pilg. and *F. horridula* Pilg., incorporated into this subgenus by Alexeev [42] (Table 1 and Supplementary File S1; Figure 1 and Supplementary Figure S1). Stančík and Peterson [31] and Stančík and Renvoize [48] expanded the circumscription of *F*. subgen. *Erosiflorae* to six new South American species of which two fulfilled all the main diagnostic characteristics proposed by Alexeev (*F. carrascana* Stančík & Renvoize, *F. chuquisacae* Stančík & Renvoize), one differed from them due to its shortly awned lemma (*F. urubambana* Stančík), another due to its partially nutant panicles and awned lemma (*F. venezuelana*), the fourth for its densely hairy ovary tip, shorter hilum and hyaline ligule with dentate apex (*F. superba*), and the fifth for its short ligule with ciliate apex, nutant panicles and awned lemma (*F. steinbachii*) (Table 1 and Supplementary File S1). Species classified within *F*. subgen *Drymanthele* sect. *Ruprechtia sensu* Alexeev [37] differentiated from those of *F*. subgen. *Erosiflorae* in their short non-membranous ligule with truncate and shortly ciliate apex and in their entire non-dentate lemma apex. It includes the type species *F. amplissima*, distributed in Mexico, Central America and northern South America, and two additional species endemic to Mexico, *F. jaliscana* E.B. Alexeev and *F. valdesii* Gonz.-Led. & S.D. Koch. (Table 1, Supplementary File S1 and Figure 1). The species classified within *F*. subgen. *Subulatae* sect. *Glabricarpae* [43] are separated from *F*. subgen. *Erosiflorae* in their shorter ligules with truncate or rounded and lacerate or dentate apex, nutant panicles or panicle branches, and their entire or bifid and awned lemma apex, and from *F*. subgen. *Drymanthele* sect. *Ruprechtia* in their membranous ligule, nutant panicles and awned lemma apex (Table 1 and Figure 1). Alexeev classified within this section the species type *F. breviglumis*, distributed in Central America and Mexico, and other Mesoamerican and northern South American species, *F. chiriquensis* Swallen, *F. caldasii* (Kunth) Kunth and *F. steinbachii* [41,43]. Stančík and Peterson [50] added to *F*. sect. *Glabricarpae* the North Andean species *F. dentiflora* E.B. Alexeev ex Stančík & P.M. Peterson and *F. woodii* Stančík, which matched the sectional diagnostic features except for the sparsely hairy ovary tip of *F. woodii* (Table 1 and Supplementary File S1). The species classified in *F*. subgen. *Asperifolia sensu* Alexeev [38] departed from the previous taxa in their densely tussocked habit, medium-length membranous ligule with truncate or slightly rounded and dentate or lacerate apex, bifid and short-awned (or awned) lemma apex, and glabrous to sparsely hispid ovary tip. The subgenus includes the type species *F. lugens*, endemic to Mexico and Central America, and other species endemic to Mexico, *F. asperella* E.B. Alexeev and *F. tancitaroensis* Gonz.-Led. & S.D. Koch (Table 1, Supplementary File S1 and Figure 1). *F*. subgen. *Mallopetalon* was described by Alexeev [44] based solely on the type species *F. fimbriata*, which shows some diagnostic traits shared with one or the other previously described taxa, such as the possession of a long rhizomatous habit, a short membranous ligule with erose and ciliate apex, and erect multispiculate panicle, but differentiated from all of them in its fimbriated lodicles, scarious, rolled and fimbriated lemma apex, and densely hairy ovary tip (Table 1, Supplementary File S1 and Figure 1).

We have examined taxonomically and phylogenomically two other species evolutionarily close to the five supraspecific *Festuca* lineages mentioned above. *Festuca superba*, a narrow endemic species from northwestern Argentina, was classified within the *F*. subgen. *Erosiflorae* by Stančík and Renvoize [48] based on general gross morphological traits shared with this taxon. However, it separates from the species of this rank and from the other taxa on the basis of its broad flat leaves with subconvolute vernation, multispiculate inflorescences with flexuous branches, muticous lemma apex and densely hairy ovary tip (Table 1 and Supplementary File S1; Figure 1 and Supplementary Figure S1). *Festuca argentina*, endemic to Patagonia and the southern Andes, is the most phenotypically distinct

species of all taxa analyzed. It has been attributed to fine-leaved *F*. subgen. *Festuca* by some authors [46] due to its caespitose habit and plicate and junciform leaves (Table 1 and Supplementary File S1; Figure 1 and Supplementary Figure S1). However, *F. argentina* shows unique traits, such as dioecy, a narrowly contracted lanceolate panicle and a sparsely hispid ovary tip (Table 1).

2.2. Phylogenomic Analyses

Phylogenomic analyses of a selection of 11 *Festuca* species, representing the five supraspecific *Festuca* ranks of Alexeev and the two close phylogenetic taxa (Table 1), plus 23 additional Loliinae species, representing the 20 evolutionary lineages detected within the subtribe [25,26], were performed using assembled nuclear rDNA 35S and IGS, nuclear rDNA 5S and plastomes retrieved from genome skimming sequencing data (Table 2). New genome skims obtained 10 species, including three species not investigated molecularly before (*F. chiriquensis, F. horridula, F. venezuelana*) and seven species characterized only for a few loci (*F. argentina, F. asperella, F. breviglumis, F. dichoclada, F. gautieri* (Hack.) K. Richt., *F. kingii* (S. Watson) Cassidy, *F. valdesii*), along with genome skimming data on five species of the supraspecific *Festuca* taxa under study (*F. amplissima*, *F. caldasii*, *F. fimbriata, F. quadridentata*, *F. superba*) and 21 species of other Loliinae lineages and two outgroups obtained in previous works [26,27] were used in the analyses. Additionally, nuclear repetitive DNA element frequency data, extracted from the genome skimming data, were used to investigate the evolutionary placement of representative species of the taxa under study within a Loliinae-wide repeatome phylogenetic framework and to compare its topology with those obtained from the plastome and rDNA sequence data sets. Although polyploidy can have a large impact on phylogenies, haploid plastomes are maternally inherited in Loliinae and are not sensitive to ploidy level. In contrast, rDNA genes may be affected by convergent evolution to one or another subgenome and/or by gene loss, or may be missed by genome skimming approaches if some of the subgenomic ribotypes are present at low frequencies in the nuclear genome. The subgenomic repetitive elements may be balanced or may have dominant/submissive contents between subgenomes, although this could not be clarified with genome skimming data alone. However, all these approaches together allowed us to infer the evolutionary history of the species under study.

Genome skimming data from newly sequenced samples ranged from 5683 (*F. asperella*) to 32,808 (*F. horridula*) million Illumina pair-end (PE) reads (Table 2). The sequences of the assembled nuclear rDNA 45S region were split into a transcribed 35S cistron data set and an untranscribed intergenic spacer (IGS) data set. The length of the 35S cistron sequence ranged from 6521 (*F. kingii*) to 6532 bp (*F. chiriquensis*), with a total length of 6589 bp in the multiple sequence alignment (MSA) (894 variable sites, 381 parsimony informative sites). This region showed a conserved structure along its aligned transcriptional unit, composed of the 5′-external transcribed spacer (ETS) (~715 bp), the 18S gene (1818 bp), the internal transcribed spacers and the 5.8S gene (ITS1-5.8S-ITS2) (577 bp), and the 25S gene (3392 bp), which had similar average lengths in the samples studied. The highly variable IGS region, studied for the first time in Loliinae, ranged from 977 (*F. pratensis*) to 1992 bp (*F. gracillima* Hook. f.), producing an MSA 2496 bp in length (1439, 919). The newly assembled sequences of the nuclear rDNA 5S region ranged from 298 bp (*F. kingii, F. valdesii*) to 319 bp (*F. gautieri*). The 5S region consisted of a conserved 5S gene (120 bp in all species) and a 563 bp intergenic variable spacer (IGS) in the MSA (158, 109). The newly assembled plastomes ranged from 131,438 bp (*F. superba*) to 133,638 bp (*F. chiriquensis*), matching the plastome length values obtained in previous studies [26,27] for the respective Loliinae FL and BL clades. Most of the newly assembled plastomes showed good read coverage (>40×) except *F. breviglumis* and *F. valdesii*, which had lower read coverage (13×–26×). The MSA of the plastomes was 134,265 bp in length (14,397, 4776). Newly obtained sequences from each data set were deposited in GenBank under accession codes OP120917-OP120926 (35S), OP158132-OP158167 (IGS), OP142676-OP142686 (5S), SAMN30029287-SAMN30029296 (plastomes) (SRA data under BioProject PRJNA863311) (Table 2).

Table 2. Taxa included in the phylogenomic analysis of Mesoamerican and South American polyploid broad-leaved *Festuca* grasses. Taxon name and authorship, Loliinae phylogenetic lineage, ploidy level, locality of collection and voucher information, number of genomic Illumina pair-end read sequences, and GenBank accession codes for nuclear rDNA 35S cistron, (45S) IGS and 5S gene regions, and plastome sequences are given for each sample. Values in bold correspond to new data generated in this study. Ploidy levels are based on chromosome counts from previous studies (all *Festuca* species show the same chromosome base number of x = 7) [18,22–27] and references therein. Question mark: unknown ploidy level.

Taxon	Phylogenetic Lineage	Ploidy	Locality/Voucher	Illumina PE Reads (Millions)	GenBank Accession No.			
					35S	IGS	5S	Plastome
Broad-Leaved (BL) Loliinae								
Festuca asperella E.B. Alexeev	Asperifolia (MCSAI)	?	Mexico: Mexico DF; MO 2744225	**5683**	**OP120918**	**OP158136**	**OP142677**	**SAMN30029288**
Festuca breviglumis Swallen	Glabicarpae	?	Mexico: Mexico DF; P, M, Peterson 21366; US s.n	**12,181**	**OP120919**	**OP158139**	**OP142678**	**SAMN30029289**
Festuca caldasii (Kunth) Kunth	Glabicarpae (MCSAI)	4×	Ecuador: Catamayo, Chinchas-Tambara; HUTPL14055	9863	MT145280	**OP158140**	ON248977	SAMN14647047
Festuca chiriquensis Swallen	Glabicarpae (MCSAI)	4×	Costa Rica: Cartago, Cantón Turrialba; MO 5175763	**8653**	**OP120920**	**OP158143**	**OP142679**	**SAMN30029290**
Festuca superba Parodi ex Türpe	Drymanthele s. l. (MCSAI)	8×	Argentina: Jujuy, Yala, Laguna Rodeo; PC 356.08 UZ	12,193	MT145305	**OP158163**	ON248977	SAMN14647072
Festuca venezuelana Stančík	Glabicarpae (MCSAI)	6×	Venezuela: Tachira, La Grita; AAU-4262	**7957**	**OP120926**	**OP158166**	**OP142686**	**SAMN30029296**
Festuca dichoclada Pilg.	Erosiflorae (MCSAII)	?	Peru: Cuzco, Quispicanchi; P, M, Peterson 20603; US s.n.	**12,466**	**OP120921**	**OP158144**	**OP142680**	**SAMN30029291**
Festuca horridula Pilg.	Erosiflorae (MCSAII)	?	Peru: Junín, Yauli; Tovar, O, and H, Soplín 6607	**32,417**	**OP120923**	**OP158150**	**OP142682**	**SAMN30029293**
Festuca quadridentata Kunth	Erosiflorae (MCSAII)	?	Ecuador: Chimborazo, Alao; US 1911313	15,091	MT145303	**OP158160**	**OP142684**	SAMN14647070
Festuca amplissima Rupr.	Ruprechtia (MCSAII)	6×	Mexico: Nuevo Leon; Peterson 21097, US s.n.	12,058	MT145278	**OP158134**	ON248975	SAMN14647045
Festuca valdesii Gonz.-Led. & S.D. Koch	Ruprechtia (MCSAII)	?	Mexico: Coahuila; P, M, Peterson 21456; US s.n.	**10,937**	**OP120925**	**OP158165**	**OP142685**	**SAMN30029295**
Festuca argentina (Speg.) Parodi	Coironhuecu (MCSAII)	4×	Argentina: Rio Negro, Bariloche; PC, 0210	**22,928**	**OP120917**	**OP158135**	**OP142676**	**SAMN30029287**
Festuca kingii (S. Watson) Cassidy	Leucopoa	8×	USA: California: San Bernardino Mnts, Leg: Quibell 149; LE	**12,397**	**OP120924**	**OP158151**	**OP142683**	**SAMN30029294**
Festuca spectabilis Bertol.	Leucopoa	6×	Bosnia-Hercegovina: Troglav, Sajkovacko zdrlo, UZ	12,960	MT145304	**OP158162**	ON249004	SAMN14647071
Festuca africana (Hack.) Clayton	Tropical and South African	10×	Uganda: Gahinga; Namaganda 190Vg; MHU1603	13,549	MT145277	**OP158133**	ON248974	SAMN14647044
Festuca mekiste Clayton	Tropical and South African	?	Kenya: Mt, Elgon National Park, Kambi Mtamaiwa; Carvalho 4521	16,245	ON243855	**OP158153**	ON248992	SAMN27777779
Festuca durandoi Clauson	Subbulbosae	2×	Portugal: Serra Arga Alto do Espinheiro; UZ s.n.	12,688	MT145283	**OP158145**	ON248980	SAMN14647050
Festuca paniculata (L.) Schinz & Thell	Subbulbosae	2×	Spain: Caceres, Puerto de los Castaños; UZ 40.07	35,808	MT145297	**OP158157**	ON248996	SAMN14647064
Festuca triflora J.F. Gmel.	Lojaconoa	2×	Morocco: Rif Mountains, Bab Barret-Ketama; PC 39.17 UZ	24,472	MT145306	**OP158164**	ON249006	SAMN14647073

Table 2. *Cont.*

Taxon	Phylogenetic Lineage	Ploidy	Locality/Voucher	Illumina PE Reads (Millions)	GenBank Accession No.			
					35S	IGS	5S	Plastome
Festuca lasto Boiss.	Drymanthele (Phaeochloa)	2×	Spain: Cadiz, Los Alcornocales; UZ 29.08	21,581	MT145291	**OP158152**	ON248989	SAMN14647058
Festuca pratensis Huds.	Schedonorus	2×	UK: England; USDA PI 283306	12,189	MT145301	**OP158158**	ON248998	SAMN14647066
Festuca arundinacea subsp. *atlantigena* (St.-Yves) Auquier	Schedonorus	8×	Morocco: Atlas mountains; ABY BN 807	15,091	ON243851	**OP158138**	ON248990	SAMN27777775
Festuca molokaiensis Soreng, P.M. Peterson & Catalán	Subulatae-Hawaiian	?	USA: Hawaii: Molokai, BISH 728771	12,188	MT145294	**OP158154**	ON248993	SAMN14647061
			Fine-leaved (FL) Loliinae					
Festuca fimbriata Nees	American II	6×	Argentina: Misiones, Dpto, Apóstoles; UZ 498.08	15,741	MT145286	**OP158146**	ON248983	SAMN14647053
Festuca asplundii E.B. Alexeev	American II	6×	Ecuador: Loja, Saraguro; HUTPL14046	25,088	MT145279	**OP158137**	ON248976	SAMN14647046
Festuca procera Kunth	American II	?	Ecuador: Riobamba, Chimborazo; HUTPL14079	40,669	MT145299	**OP158159**	ON248999	SAMN14647067
Festuca chimborazensis E.B. Alexeev	American I	6×	Ecuador: Riobamba, Chimborazo; HUTPL14066	10,913	MT145282	**OP158142**	ON248979	SAMN14647049
Festuca holubii Stančík	American I	?	Ecuador: Saraguro, to Cerro de Arcos; HUTPL14071	10,264	MT145289	**OP158149**	ON248988	SAMN14647056
Festuca pampeana Speg.	American Pampas	8×	Argentina: Buenos Aires, Sierra de la Ventana; PC 428.08	14,862	MT145296	**OP158156**	ON248995	SAMN14647063
Festuca gracillima Hook. f.	American–Neozeylandic I	6×	Argentina: Tierra de Fuego, E. San Pablo; UZ 482.08	13,888	MT145288	**OP158148**	ON248986	SAMN14647055
Festuca abyssinica Hochst. ex A. Rich.	Afroalpine	4×	Tanzania: Kilimanjaro; Afroalp O-DP-42737	12,041	MT145276	**OP158132**	ON248973	SAMN14647043
Festuca rubra L.	Aulaxyper	6×	Argentina: Tierra de Fuego, Cabo Annicolta; UZ 03.09	25,260	ON243856	**OP158161**	ON249002	SAMN27777780
Festuca ovina L.	Festuca	2×	Germany: Thüringen; Müller 10789	11,364	MT145295	**OP158155**	ON248994	SAMN14647062
Festuca capillifolia Dufour ex Roem. & Schult.	Exaratae	2×	Morocco: Middle Atlas, Ifrane National Park; PC 77.17	13,430	MT145281	**OP158141**	ON248978	SAMN14647048
Vulpia ciliata Dumort.	Psilurus–Vulpia	4×	Spain: Toledo, Mar de Ontígola; UZ 109.07	11,801	MT145309	**OP158167**	ON249009	SAMN14647076
Festuca gautieri (Hack.) K. Richt.	Eskia	2×	Spain: Granada, Huéscar; UZ 232.07	**13,941**	**OP120922**	**OP158147**	**OP142681**	**SAMN30029292**
			Outgroups					
Brachypodium distachyon (L.) P. Beauv.	—	2×	Spain: Caceres; UZ 28.07	—	Phytozome Bd21 v.3.1	—	—	NC_011032, 1
Oryza sativa L.	—	2×	China: National Rice Research Center, cv	—	AP008215	—	—	AY522331, 1

The 35S maximum likelihood (ML) phylogenetic tree recovered the expected topology for the Loliinae as previously presented by Moreno-Aguilar et al. [26], consisting of a fully supported FL clade and a series of strongly to weakly supported basal paraphyletic BL lineages (Figure 2a). In this tree, *F. fimbriata* (Mallopetalon lineage) was nested within a strongly supported FL American I–American II clade, whereas the remaining species under study fell into two separate BL groups. Representative species of *F*. sect. *Glabricarpae* (*F. breviglumis, F. cladasii, F. chiriquensis*) and *F*. subgen. *Asperifolia* (*F. asperella*), together with *F. venezuelana* and *F. superba*, formed a robust Mexico–Central–South American I (MCSA I) clade, while the representative species of *F*. sect. *Erosiflorae* (*F. dichloclada, F. horridula, F. quadridentata*), *F*. sect. *Ruprechtia* (*F. amplissima*, *F. valdesii*) and *F. argentina* formed a Mexico–Central–South American II (MCSA II) group integrated into a robust clade that also included representatives of Leucopoa (*F. kingii*, *F. spectabilis* Bertol.) and Subulatae-Hawaiian (*F. molokaiensis* Soreng, P.M. Peterson & Catalán) (Figure 2a). The (45S) IGS ML tree, first computed for the Loliinae in the present study, showed two fully supported FL and BL sister clades (Figure 2b). *F. fimbriata* (Mallopetalon) was also nested within a robust FL American I–American II clade, whereas the other taxa fell within the BL clade. The robust MCSA I clade (Glabricarpae–Asperifolia–*F. superba*–*F. venezuelana*) was resolved as a sister to the also robust tropical–South African clade, although this relationship was weakly supported, and the strongly supported MCSA II clade (Erosiflorae–Ruprechtia–*F. argentina*) was resolved as a sister to a weakly supported Leucopoa clade, although this relationship was well supported (Figure 2b). The 5S ML tree was congruent with the 45S (35S, IGS) ML trees for some but not all lineages (Figure 2c). The 5S-based tree topology also recovered a relatively well supported MCSAI clade, which was resolved as a sister to an Old World Drymanthele/Lojaconoa clade, although this relationship was poorly supported. In contrast, the MCSAII group split into two separate lineages on this tree; in one of them, Erosiflorae species formed a strongly supported clade together with Old World Subbulbosae species, and in the second lineage Ruprechtia and *F. argentina* species joined in a relatively well supported clade together with American and European Leucopoa species. In this 5S-based topology, *F. fimbriata* (Mallopetalon) was also nested within the FL Loliinae clade but close to representative species of American Pampas, Subulatae-Hawaiian and Exaratae lineages and not to those of American II, American I and American–Neozeylandic lineages, which formed a nested group within the BL Loliinae clade (Figure 2c). The plastome-based ML tree also recovered two fully supported FL and BL sister Loliinae clades (Figure 2d). In this matrilineal phylogeny, *F. fimbriata* was nested within a fully supported FL American II lineage, and the remaining species under study within different groups of the BL clade. Species from the MCSAI (all) and MCSAII (pro parte) groups formed a clade, sister to another clade that included two species from the MSCAII group and representatives of the remaining BL lineages, with all these relationships showing full support. Within the MCSA superclade, Glabricarpae, Asperella and *F. superba* (MCSAI group) species were resolved as basal paraphyletic lineages, while *F. venezuelana* formed a fully supported clade with most elements of the more recently evolved and well supported MCSAII pro parte clade. The two species of the MCSAII group that departed from the MCSA superclade, *F. horridula* (Erosiflorae) and *F. valdesii* (Ruprechtia), formed a fully supported subclade together with American *F. kingii* (Leucopoa); this subclade, in turn, joined other Eurasian species of Leucopoa and of Subbulbosae in a fully supported lineage (Figure 2d). To account for potential incomplete lineage sorting, we performed parallel phylogenetic analyses with the same data sets but modeling the coalescence process using the Singular Value Decomposition quartets (SVDq) approach implemented in Paup *, which combines quartet trees into a species tree. Since the topologies of the 35S, IGS, 5S and plastome SVDq trees (Supplementary Figure S2a–d) were the same as those of the ML trees, or recovered similar lineages, only the latter were described. The (45S) IGS ML tree was used to map the diagnostic morphological traits of the supraspecific *Festuca* ranks under study on its branches (Supplementary Figure S3).

II and American I lineages (Figure 3b). The representative species of fine-leaved *F.* sect. *Eskia*, *F. gautieri*, clustered closer to the BL core group than the FL core group, as previously observed for other species in this section (*F. eskia* Ramond ex DC. [27]).

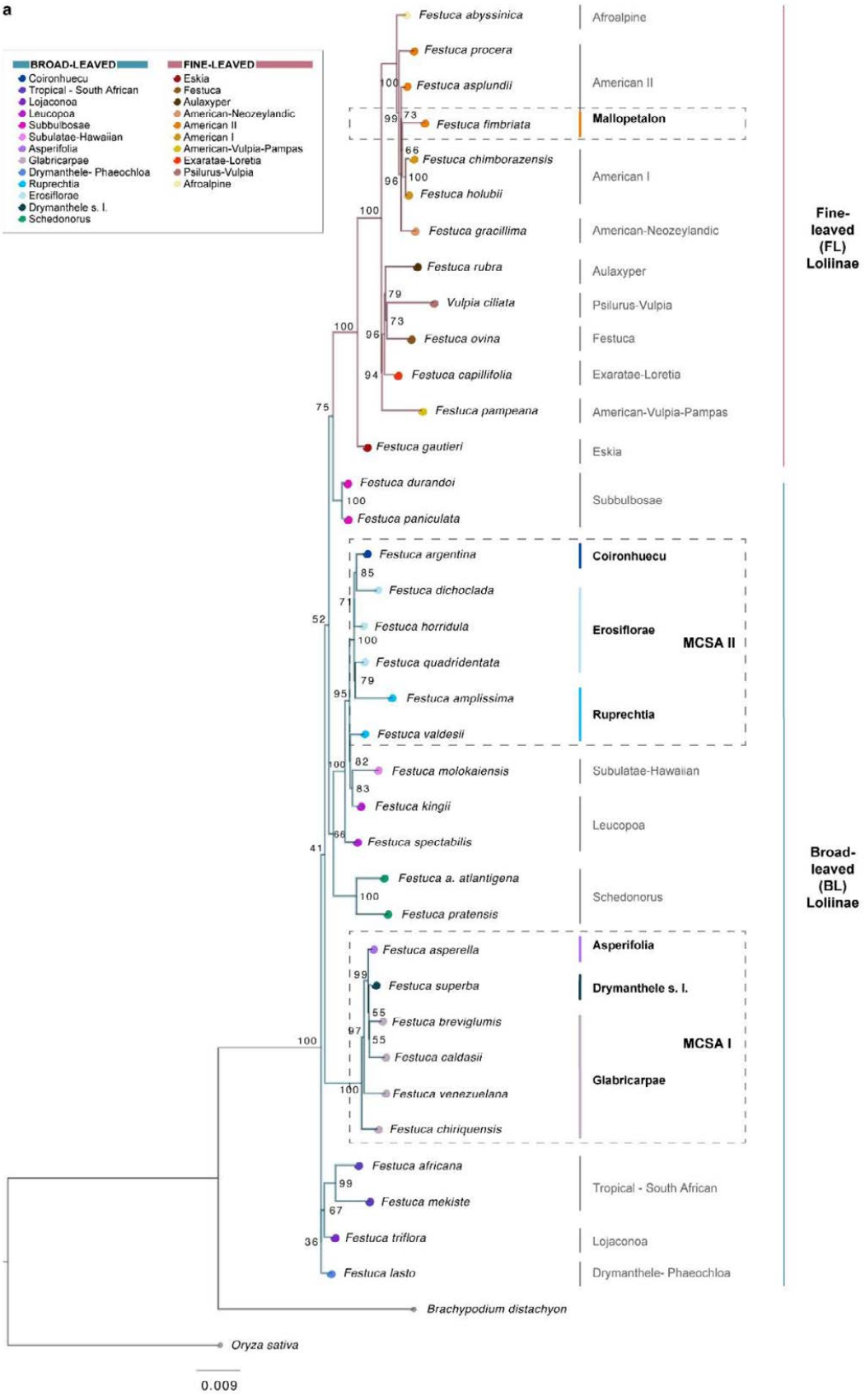

Figure 2. *Cont.*

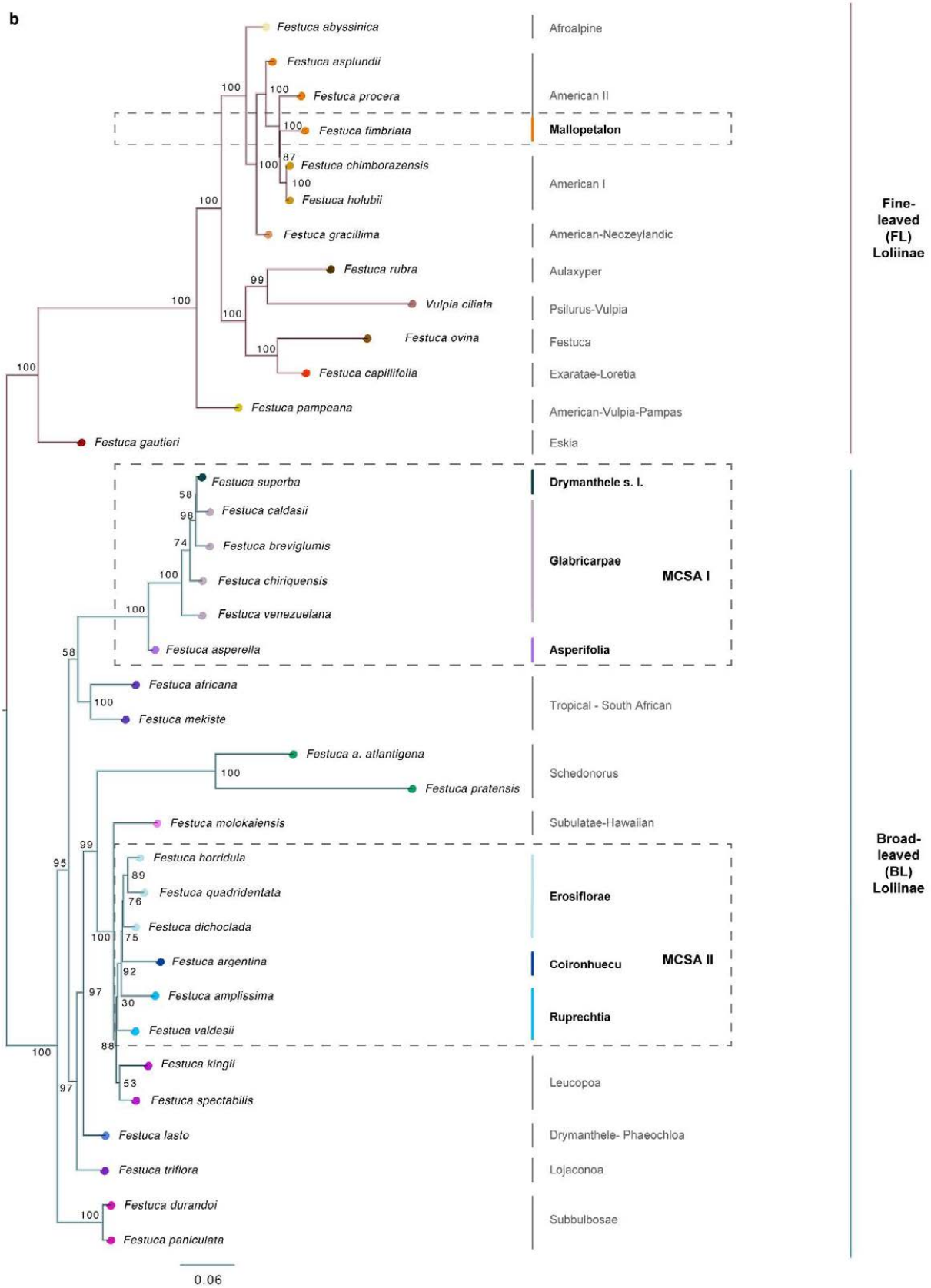

Figure 2. *Cont.*

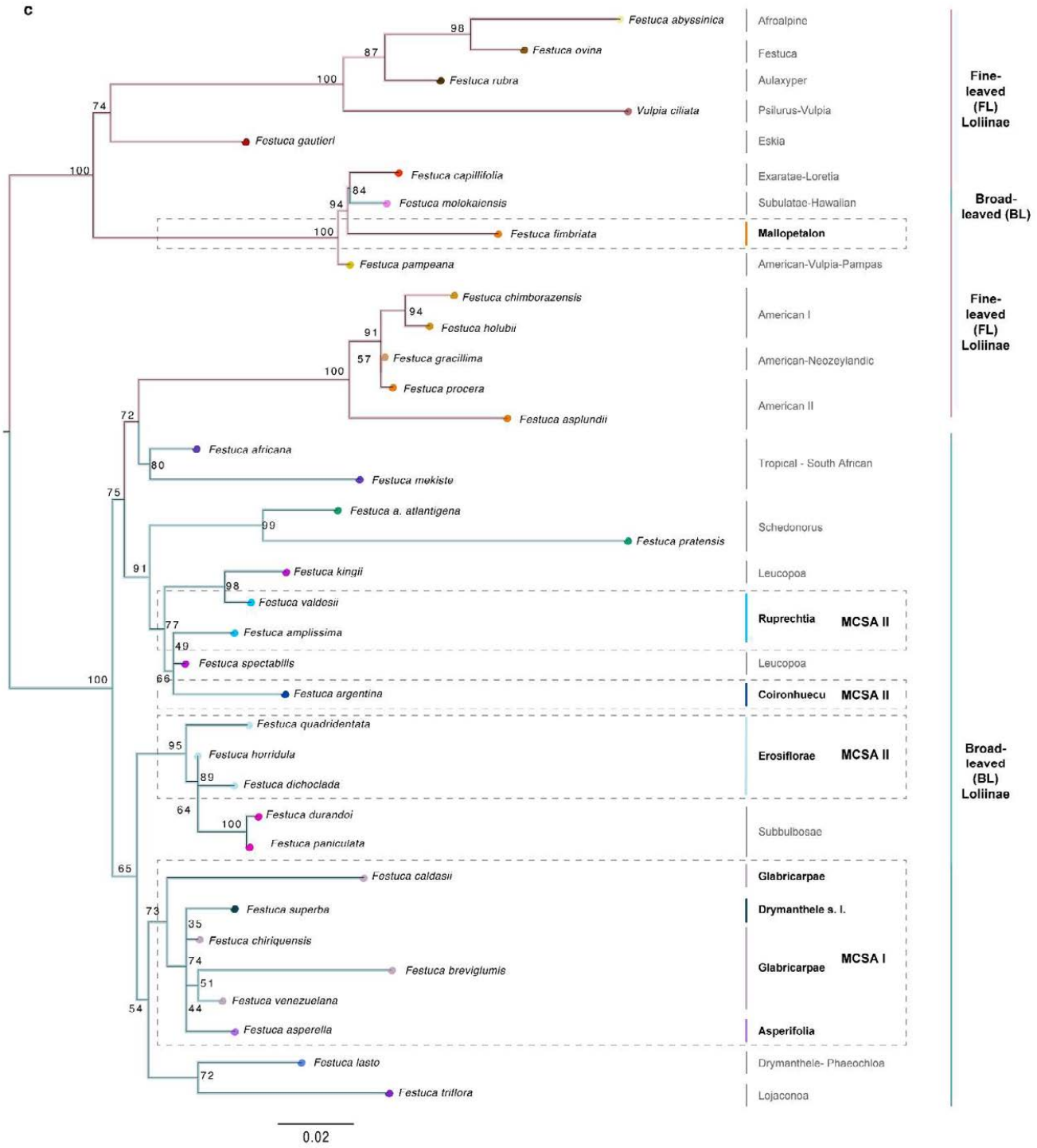

Figure 2. *Cont*.

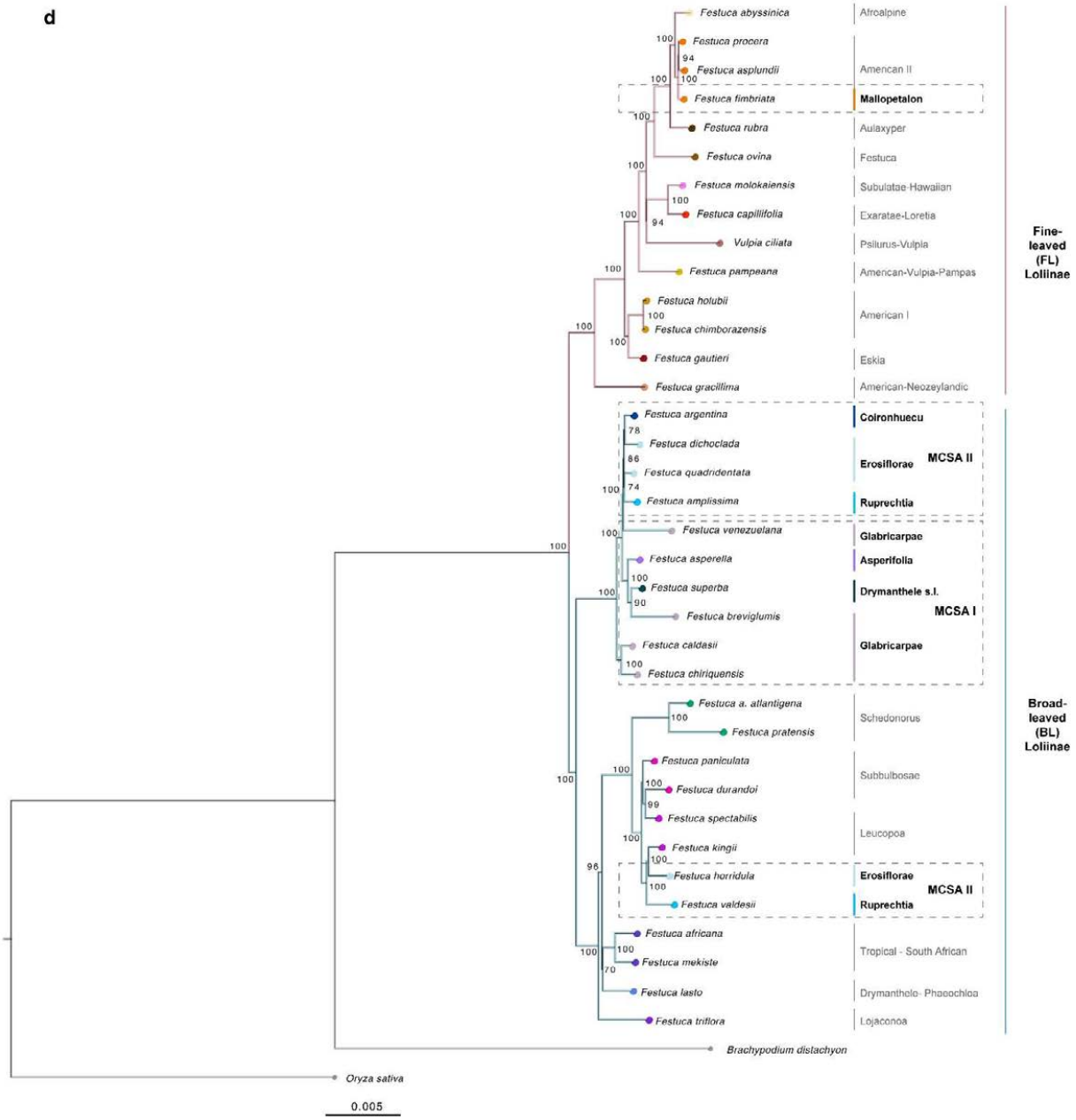

Figure 2. Maximum likelihood phylogenomic trees of the Mesoamerican and South American broad-leaved *Festuca* taxa studied and other representative species of the broad-leaved (BL) and fine-leaved (FL) Loliinae lineages. (**a**) Nuclear rDNA 35S cistron tree. (**b**) Nuclear rDNA (45S) IGS tree. (**c**) Nuclear rDNA 5S tree. (**d**) Plastome tree. Mexico–Central–South American (MCSAI, MCSAII) and Mallopetalon groups are indicated by discontinuous-line rectangles. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees except for the IGS and 5S trees that were rooted at midpoint. Color codes of the lineages are the same as in Figure 2a. Scale bars: number of mutations per site.

The annotated nuclear repetitive elements found by Repeat Explorer 2 (RE2) in the individual analysis of the newly sequenced samples (Supplementary Table S1 and Figure 3a) were consistent with data from a previous study of representative groups of Loliinae [27]. Repeat elements contributed to large proportions of the MCSAI and MCSAII haploid genomes (mean 56.8%; ranging from 49.0% (*F. quadridentata*) to 67.5% (*F. chiriquensis*) (Supplementary Table S1). Interestingly, *F. fimbriata* (Mallopetalon) showed the lowest percentage of repeatomes (38.8%) among the studied species, differing from the relatively high values shown by the American II and American I species (Supplementary Table S1) but being close to the observed values in other high-polyploid Loliinae species (e.g., *F. arundinacea*; [27]). LTR-Copia and LTR-Gypsy retrotransposons represented the major fractions of the repeatomes followed by Class II TIR-transposons and satellite repeats in the newly studied genomes. Of them, LTR-Copia Angela and LTR-Gypsy Retand elements were the most frequent repeat families in all the BL species studied (Supplementary Table S1; Figure 3a). Glabricarpae and *F. superba* showed high coverages of Angela elements, and Erosiflorae, Ruprechtia, *F. breviglumis* (Glabricarpae), *F. argentina* and *F. superba* of Retand elements. *F. fimbriata* had a low coverage of Retand elements, as in some FL American II species (e.g., *F. asplundii* E.B. Alexeev), although unlike the American II and American I species, it showed a much lower coverage of Angela elements (Supplementary Table S1; Figure 3a). A total of 37 top repeat clusters, annotated by RE2 in the comparative analysis of all 36 Loliinae genomes, were used to construct a combined phylogenetic network from the respective distance-based Neighbor-Joining (NJ) trees. The topology of the unrooted Loliinae repeatome network showed the divergence of three main groups, BL (core), FL (core) and Schedonorus lineages, with representatives of the American I, American II, American Pampas, American–Neozeylandic, Subulatae-Hawaiian and Afroalpine lineages occupying an intermediate position between the core FL and BL subnetworks (Figure 3b). The MCSAI and MCSAII species clustered into their respective divergent groups and formed a large MCSA supergroup within the core BL subnetwork; North American *F. kingii* (Leucopoa) was resolved as the closest relative of this MCSA supergroup (Figure 3b). *F. fimbriata* (Mallopetalon) fell within the expanded FL group in this repeatome-based network, nesting in an intermediate position between the American II and American I lineages (Figure 3b). The representative species of fine-leaved *F*. sect. *Eskia*, *F. gautieri*, clustered closer to the BL core group than the FL core group, as previously observed for other species in this section (*F. eskia* Ramond ex DC. [27]).

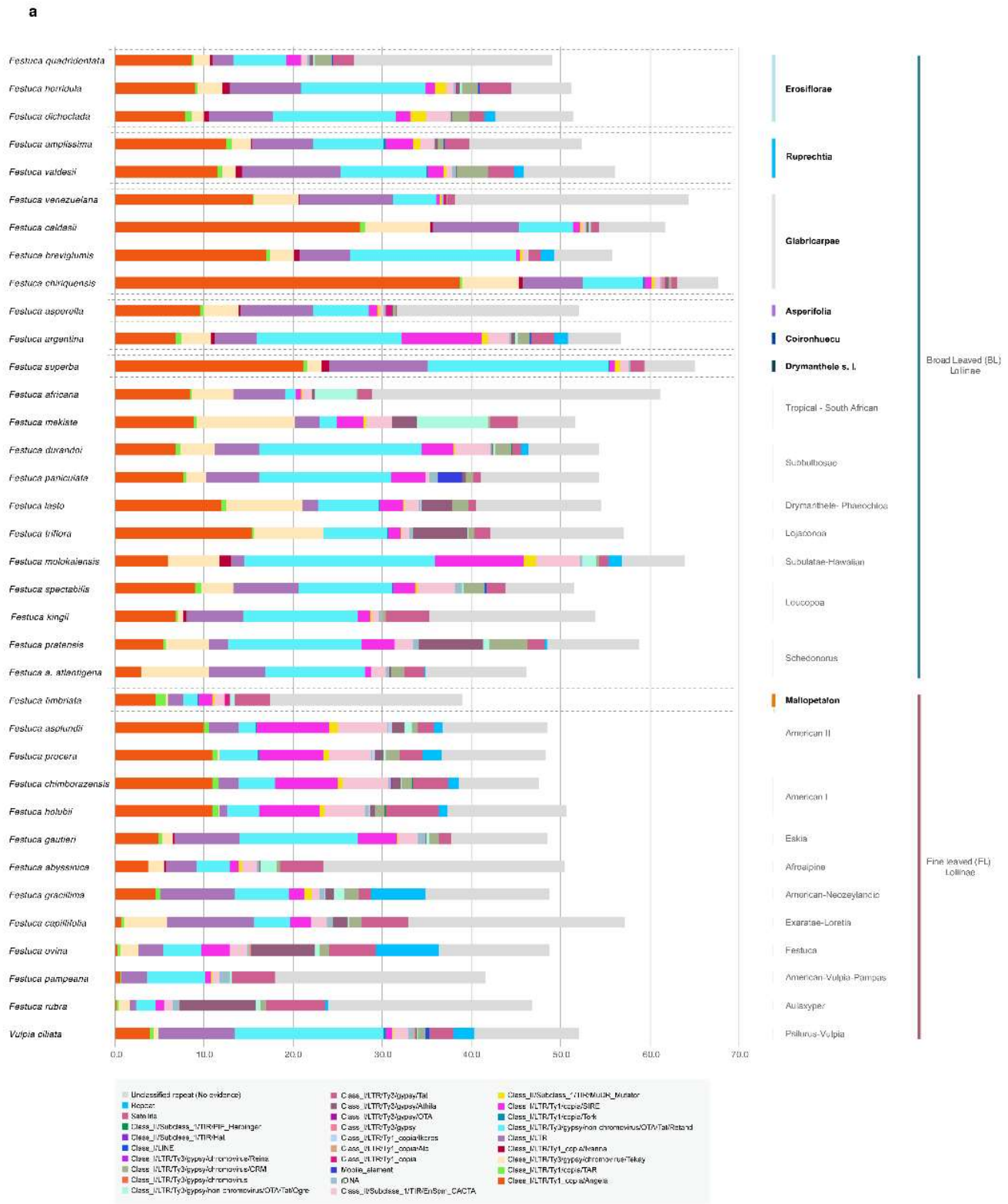

Figure 3. *Cont.*

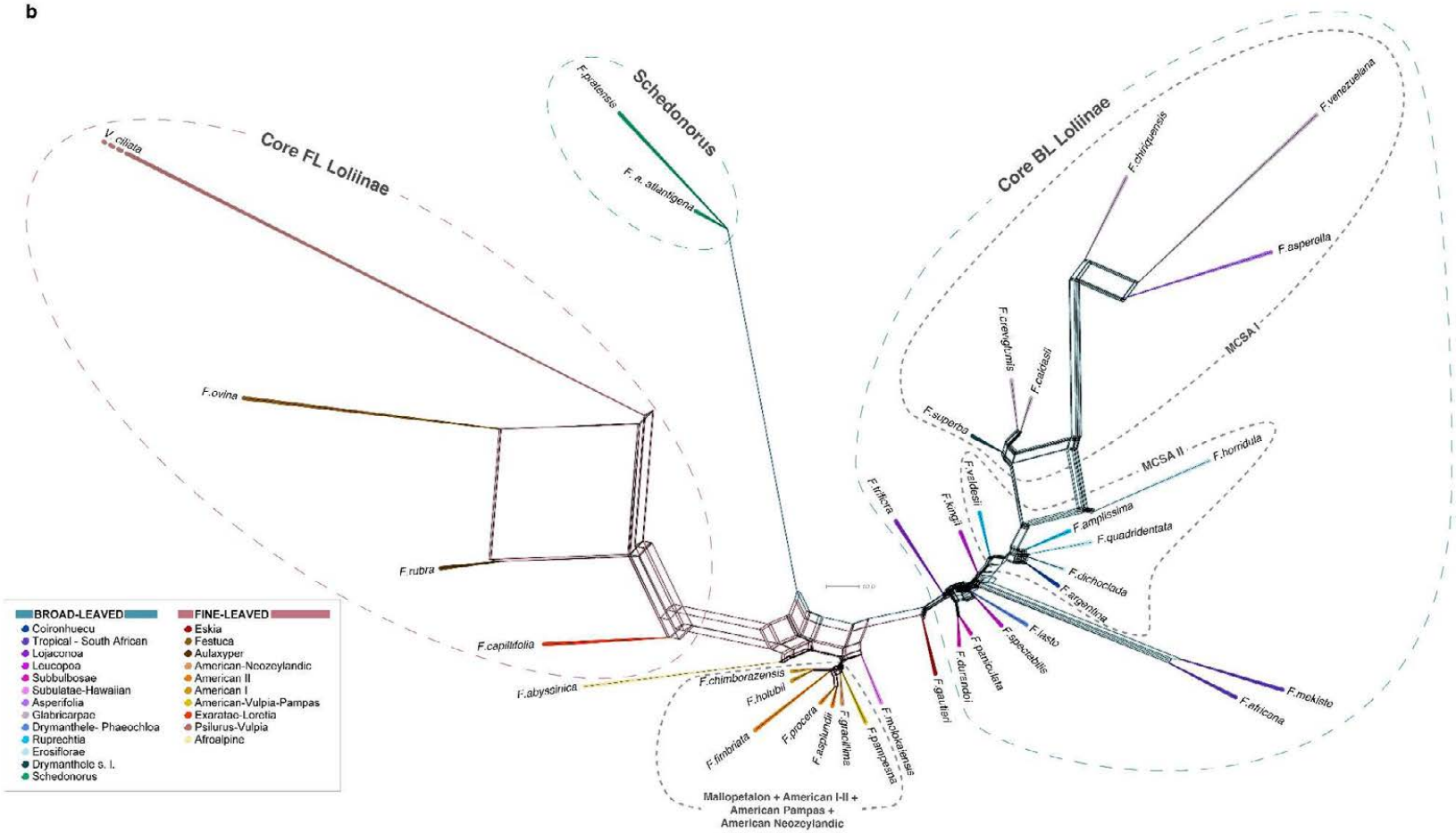

Figure 3. (**a**) Histograms of repeat contents per holoploid genome (1C) retrieved from the individual Repeat Explorer 2 (RE2) analyses of the studied Mesoamerican and South American broad-leaved *Festuca* taxa and other Loliinae samples. Color codes for repeat types are indicated in the charts (**b**) Phylogenetic network based on standardized repeat data sets retrieved from the comparative RE analysis and constructed from distance-based NJ trees computed with pairwise Euclidean distances between samples. Core BL, core FL, Schedonorus, Mexico–Central–South American (MCSAI, MCSAII), and Mallopetalon + other American Loliinae groups are surrounded by dashed lines. Color codes of Loliinae lineages are indicated in the chart.

3. Discussion

3.1. Evolutionary History of Allopolyploid Broad-Leaved Mexico–Central–South American Festuca Lineages (Erosiflorae, Ruprechtia, Glabricarpae, Asperella, Mallopetalon, F. argentina, F. superba)

Our taxonomic and phylogenomic analyses of overlooked Mexico–Central–South American broad-leaved *Festuca* lineages have been instrumental in unravelling the origins and systematics of the seven Loliinae groups studied (Figures 1–3 and Supplementary Figure S1, Tables 1 and 2 and Supplementary Table S1). Our results indicate that *F. fimbriata* (*F*. subgen. *Mallopetalon*) originated from ancestors of FL Loliinae, while species in the other six groups derived from ancestors of BL Loliinae (Figures 2 and 3). This highly divergent evolutionary position of *F. fimbriata* with respect to its morphologically close congeners might be associated with the recent reticulated radiation of polyploid South American *Festuca* species within the FL clade from the early Pliocene to the Pleistocene [25,26]. The "broad-leaved syndromes" that *F. fimbriata* presents in its habit, innovation leaves and inflorescence (Table 1 and Supplementary Figure S3) are also shared by other robust "BL-type" *Festuca* species, which have also originated within the large and phenotypically variable American II (e.g., *F. peruviana* Infantes) and American I (e.g., *F. purpurascens* Banks & Sol. ex Hook. f.) "fine-leaved" lineages [25,26]. However, some of the private morphological features characteristic of *F. fimbriata*, such as the possession of fimbriated lodicles and lemma apex (Table 1), support its classification in the separate *F*. subgenus *Mallopetalon* [44]. *F. fimbriata* is also unique in its adaptation to an exceptional ecological habitat for Loliinae, the flooded swamps of southern South America [32,47]. This allohexaploid species (Table 2) likely originated from an American II maternal ancestor (plastome tree; Figure 2d) and an American I paternal ancestor (nuclear 35S, IGS trees; Figure 2a,b). Its allohexaploidy is corroborated by its asymmetric and heterogeneous karyotype [47], characteristic of polyploid hybrid plants derived from progenitor species with different chromosomal complements [51,52]. Its relatively low percentage of repetitive elements per haploid genome (Supplementary Table S1 and Figure 3a) agrees with those observed in other allohexaploid species of *Festuca* [27]. Despite some morphological similarities with *F. argentina* (Table 1), both species occupy widely divergent positions in opposite Loliinae lineages (the robust *F. fimbriata* nested within the FL clade and the more slender *F. argentina* within the BL clade), as shown in the nuclear, plastome and repeatome phylogenies (Figures 2 and 3b), thus ruling out any close relationship between them and confirming the great plasticity of some of the morphological traits used to separate *Festuca* taxa [23].

Species from the other six broad-leaved Loliinae groups studied fell into two separate BL lineages (MCSAI, MCSAII) in the 35S, IGS, 5S (MCSAI) and repeatome-based nuclear phylogenies (Figure 2a,b and Figure 3b), while in the plastome-based phylogeny, almost all species of both groups shared a common ancestor (Figure 2d). The relatively more ancestral MCSAI clade includes representative species of *F*. subgen. *Asperifolia* (*F. asperella*) and *F*. sect. *Glabricarpae* (*F. breviglumis, F. caldasii, F. chiriquensis*) plus *F. venezuelana* and *F. superba* (Figures 2 and 3). Asperifolia and Glabricarpae taxa share morphological features such as the possession of a membranous ligule with a truncate apex and awned lemma (except in *F. tancitaroensis*), while they differ in their erect (Asperifolia) vs. nutant (Glabricarpae) panicles (Table 1, Figure 1 and Supplementary Figure S3). *F. venezuelana* and *F. superba* were classified by Stančík and Renvoize [48] within *F*. subgen. *Erosiflorae*. However, *F. venezuelana* is morphologically closer to Glabricarpae than to Erosiflorae for the diagnostic traits examined (e.g., nutant panicle, awned lemma; Table 1), which together with its phylogenetic placement within the Glabricarpae lineage (Figure 2, Figure 3 and Supplementary Figure S3), supports its taxonomic transference to *F*. sect. *Glabricarpae*. *F. superba* is morphologically separated from the Erosiflorae and the Glabricarpae–Asperifolia groups (Table 1), although its taxonomic classification is still unclear (see comments below). The expanded Glabricarpae group, therefore, shows a relatively consistent evolutionary history, although it is made up of paraphyletic lineages in most trees and the nuclear phylogenetic network (Figures 2a–c and 3), with Asperifolia and *F. superba* nested in its clade. Glabricarpae is also

reconstructed into a series of basal and subbasal lineages in the MCSA superclade of the plastome tree (Figure 2d).

The relatively more recently evolved MCSAII clade integrates representative species of *F*. subgen. *Erosiflorae* sensu Alexeev (*F. dichoclada, F. horridula, F. quadridentata*) and *F*. sect. *Ruprechtia* (*F. amplissima, F. valdesii*) plus *F. argentina* (Figures 2 and 3). The Erosiflorae and Ruprechtia taxa share common morphological traits, both presenting erect panicles, unawned lemmas and mostly glabrous ovary tips, while differing in the overall long erose or lacerated membranous ligule with an acute and dentate lemma apex of Erosiflorae vs. the overall short non-membranous ligule with a truncate and non-dentate lemma apex of Ruprechtia (Table 1 and Figure 1). In the IGS nuclear phylogeny, the three species of Erosiflorae are reconstructed as a monophyletic group (Figure 2b and Supplementary Figure S3), reinforcing the classic taxonomic circumscription of this taxonomic rank proposed by Alexeev [42]. Although not studied genomically, other species included within *F*. subgen. *Erosiflorae* by Stančík and Renvoize [48], such as *F. steinbachii*, did not fit the diagnostic traits of Erosiflorae but rather those of its earlier *F*. sect. *Glabricarpae* classification [41], as this species has nutant panicles, a short ligule with a truncate and ciliate apex, and an awned lemma (Table 1). Therefore, the taxonomic circumscription proposed by Stančík and Renvoize [48] for *F*. subgen. *Erosiflorae* has been shown to be morphologically and phylogenetically artificial. In the nuclear 45S and 5S and repeatome network phylogenies, the two Ruprechtia species studied are resolved as paraphyletic, although they are closely related to each other (Figures 2a–c and 3b). Of these, *F. amplissima* is more morphologically and phylogenetically related to Erosiflorae + *F. argentina* than *F. valdesii* (Table 1, Figures 2a–c and 3b and Supplementary Figure S3). *Festuca valdesii*, classified within *F*. sect. *Ruprechtia* by González-Ledesma et al. [53], differs from the two species assigned to the section by Alexeev (*F. amplissima*, *F. jaliscana*) in its non-rhizomatous caespitose habit, longer membranous ligule with a truncate and short ciliate apex and hispid ovary tip (Table 1), raising doubts about its definitive systematic classification. Although deeply nested within the MCSAII clade in all nuclear and plastome-based phylogenies (Figures 2 and 3b), *F. argentina* differs morphologically from Erosiflorae and Ruprechtia, as well as from the MCSAI Asperifolia and Glabricarpae taxa (Table 1, Figure 1 and Supplementary Figure S3), and therefore deserves an independent taxonomic classification (see comments below). Interestingly, in the nuclear rDNA 35S and IGS phylogenies, the Erosiflorae, Ruprechtia and *F. argentina* lineages fall into a larger, fully supported clade that also includes closely-related species of the *F*. subgen. *Leucopoa* (*F. kingii*, *F. spectabilis*) and Subulatae-Hawaiian (*F. molokaiensis*) lineages (Figure 2a,b), while in the plastome phylogeny, one species of Erosiflorae (*F. horridula*) and one species of Ruprechtia (*F. valdesii*) split from the MCSA superclade and fell within a separate BL lineage, nesting with the North American Leucopoa *F. kingii* in a strongly supported clade (Figure 2d). The closeness of the MCSAII group to *F. kingii* was also recovered in the repeatome network (Figure 3b).

The different topological positions of the MCSAI and MCSAII lineages in the nuclear vs. plastome trees and in the repeatome network (Figures 2 and 3b) confirm the putative hybrid origins of these polyploid BL *Festuca* species [25,27]. The origins of these allopolyploids could be partially unraveled from our phylogenomic data. Thus, the MSCAII lineages (Erosiflorae, Ruprechtia, *F. argentina*), probably derived from a *Leucopoa* ancestor, which likely acted as the paternal parent for most of these species (nuclear 35S and IGS trees; Figure 2a,b), and from an unknown maternal MCSA parent (plastome tree; Figure 2d). Furthermore, *F. horridula* (Erosiflorae) and *F. valdesii* (Ruprechtia) likely had both paternal and maternal Leucopoa-type parents (Figure 2a,b,d). However, the origins of the MCSAI lineages (Glabricarpae, Asperifolia, *F. superba*) are less clear. The nuclear topologies do not retrieve strongly supported relationships of these slightly older MCSA lineages with any of the remaining BL lineages (Figure 2a–c), while the plastome phylogeny indicates that the MCSAI group shared the same maternal parent as most of the MCSAII taxa (Figure 2d). This would imply three potential colonizations of Eurasian and/or North American *Festuca* lineages to Central and South America. One of them probably contributed

as the maternal parent of most of the MCSAI and MCSAII species and the other two probably contributed as respective paternal parents of the MCSAI and MCSAII (Leucopoa-type) groups. This hypothesis agrees with the proposed DEC biogeographic models for colonizing ancestral BL *Festuca* lineages from the Northern Hemisphere to Mesoamerica and South America [25,27]. The MCSAI and MCSAII nuclear and plastome phylogenies show a trend of more ancestral Mesoamerican and northern South American lineages and more recently evolved southern South American lineages within both clades (Figure 2a–d), which support the North-to-South stepwise colonization pattern proposed for the American *Festuca* ancestors [25]. The absence of diploid species of *Festuca* in these regions and throughout the southern hemisphere [18,29] allows us to speculate that the ancestral colonizers that originated the MCSAI and MCSAII lineages may have been polyploids; however, the lack of supported sister relatives precludes the inference of their putative ploidy levels. The studied species also comply with the observed trend of increasing ploidy level with latitude in *Festuca* [18], with Mesoamerican and northern Andean MCSA species showing lower ploidy levels (4×, and few 6×) and central and southern Andean species showing higher levels of ploidy (6×, 8×; except tetraploid *F. argentina*) (Table 2). Similar patterns of polyploid radiations have been reported for other angiosperms (e.g., C4 grasses, *Silene* L. [16,54]). This latitudinal change, also observed in species of *Festuca* from the Northern Hemisphere, has been related to the drastic effect of the Pleistocene glaciations and the successful postglacial colonization of high latitudinal and altitudinal territories by high polyploids [18]. For the MCSAI Glabricarpae, Asperifolia and *F. superba*, and MCSAII Erosiflorae, Ruprechtia and *F. argentina* lineages, the variations observed within clades in ploidy levels probably involved successive rounds of hybridizations and allopolyploidizations between these and/or other unstudied species that should be investigated through comparative genomic analyses.

3.2. Systematics of Broad-Leaved MCSA and Mallopetalon Loliinae Taxa

The morphological differences observed for the main diagnostic characters (Table 1) of MCSAII *F. argentina*, and MCSAI *F. superba* (Figures 2 and 3b) with respect to the subgeneric or sectional *Festuca* ranks ascribed previously [31,46,48], motivated us to reclassify them (Table 1). *Festuca argentina*, traditionally classified within FL *F*. subgen. *Festuca* [46], shows a caespitose habit containing only intravaginal innovations, and plicate and junciform leaves with conduplicate vernation, which are different from those of all other broad-leaved taxa studied (Table 1, Figure 1 and Supplementary Figure S1). Dubcovsky [47] discussed the similarities between *F. argentina* and *F. fimbriata* (*F*. subgen. *Mallopetalon*), which share muticous or mucronulate lemma apices and hairy ovary tips (Table 1), and ciliate or fimbriated lodicles, 3-veined lower glumes and asymmetric and heterogeneous karyotypes. However, the same author indicated that *F. argentina* differed from *F. fimbriata* based on its intravaginal innovations, plicate leaves, smaller panicles and scabrid lemmas, and suggested a separate subgeneric classification for *F. argentina* [47]. *F. argentina* is nested within or sister to strongly supported Erosiflorae lineages in most nuclear and plastome phylogenies (Figure 2a,b,d and Figure 3b), supporting common ancestry with these taxa despite their disparate morphological traits (Table 1, Figure 1 and Supplementary Figure S1). This tetraploid species has a strongly asymmetric and heterogeneous karyotype, with two extremely discordant chromosomes sets [47], indicative of its allotetraploidy [51,52]. The species is, however, a low polyploid in its austral latitudinal distribution [32], which points to its relatively ancestral hybrid origin [25] and its plausible glacial survival and adaptation to the harsh climate conditions of the Patagonian steppe. One of its main distinguishing features, dioecy (Table 1), is shared with other species of its putative paternal Leucopoa ancestor, such as the North American *F. kingii* (Figure 2a,b and Figure 3b) and various Asian *F*. subgen. *Leucopoa* species [55,56]. As in the close genus *Poa* L., where hermaphroditism is the plesiomorphic state and dioecy has evolved in certain geographically distributed lineages in North and South America [57], the rare dioecy is restricted only to a few species of *Festuca* from Central and East Asia (e.g., *F. olgae* (Regel) Krivot., *F. sibirica* Hack. ex Boiss.)

and their American descendants (*F. kingii*, *F. argentina*) ([55,56], this study). It is plausible to postulate that dioecy and chromosomal sex determination could have been maintained through allopolyploid speciation in *F. argentina*, as demonstrated in other angiosperms [58]. Based on the unique morphological characteristics displayed by *F. argentina* and its strong phylogenetic nesting within the Erosiflorae lineage of the MCSAII lineage, we propose to classify it within a new *Festuca* subgenus *Coironhuecu* Moreno-Aguilar, Arnelas & Catalán (see Taxonomic section below).

Festuca superba was misclassified into the artificially expanded *F*. subgen. *Erosiflorae* by Stančík and Renvoize [48]. However, this species differs morphologically from the species in this taxonomic rank as well as from the species of *F*. subgen. *Asperifolia* and *F*. subgen. *Subulatae* sect. *Glabricarpae* of the MCSAI clade where *F. superba* is evolutionarily positioned in all phylogenetic reconstructions (Table 1, Figures 1, 2a–d and 3b). The morphological features that characterize *F. superba*, such as the possession of broad and flat leaves with subconvolute vernation, entire and unawned lemmas, and a densely hairy ovary tip (Table 1 and Supplementary Figure S3), together with a shorter caryopsis hilum than the Erosiflorae taxa [32], approximate it to *F*. subgen. *Drymanthele* [35,55]. However, some private traits, such as the possession of a long hyaline ligule with an erose-dentate and ciliate apex (Table 1 and Figure 1), differentiate it from species of the sections described so far within this subgenus, namely European species of *F*. sect. *Phaeochloa* Griseb., Asian species of *F*. sect. *Muticae* S.L. Lu, and American and Australian species of *F*. sect. *Banksia* E.B. Alexeev [35,38,39,44,59]. Phylogenetically, some species of *F*. sect. *Banksia* were nested within either the FL clade (e.g., *F. purpurascens*, American I lineage) or within the BL clade (e.g., *F. muelleri* Vickery, Leucopoa-Amphigenes), while the studied species of *F*. sects. *Phaeochloa* (*F. altissima* All., *F. drymeja* Mert. & W.D.J. Koch, *F. lasto* Boiss., *F. donax* Lowe) and *Muticae* (*F. modesta* Nees) always nested within the BL clade [25]. *F. superba* is presumably an allooctoploid, based on its perfectly paired bivalents observed at meiosis [47]. Its high repeat content (Supplementary Table S1 and Figure 3a) and its recently evolved phylogenetic position in the nuclear and plastome trees (Figure 2a,b,d and Figure 3b) corroborate its plausible recent origin and lack of evolutionary time to purge its abundant repeatome [27]. Based on its particular morphological features, which approximate it to *F*. subgen. *Drymanthele* but not to currently described sections of this rank, and because of its strong phylogenetic nesting within the Glabricarpae–Asperifolia clades of the MCSAI lineage, we propose to tentatively classify it within *F*. subgen. *Drymanthele sensu lato* without a sectional assignment until other close broad-leaved Meso-South American taxa are also phylogenomically studied.

The systematics of Loliinae has undergone multiple classifications since the description of its main genus *Festuca* by Linné [23], resulting in the incorporation and segregation of new taxa to it. *Festuca* and fourteen close genera constitute the monophyletic subtribe Loliinae. Phylogenetic analysis has shown that fine-leaved *F*. subgen. *Festuca* species and some broad-leaved fescues (*F*. subgen. *Mallopetalon*, *F*. subgen. *Drymanthele* pro parte) plus ten annual genera (*Ctenopsis* De Not., *Dielsiochloa* Pilg., *Hellerochloa, Megalachne* Steud., *Micropyrum* (Gaudin) Link, *Narduroides* Rouy, *Podophorus* Phil., *Psilurus* Trin., *Vulpia* C.C. Gmel., *Wangenheimia* Moench) make up the FL clade, while taxa of eight broad-leaved *Festuca* subgenera (*F*. subgen. *Asperifolia*, *Drymanthele*, *Erosiflorae*, *Leucopoa, Schedonorus*, *Subulatae*, *Subuliflorae*, *Xanthochloa*) plus three annual or perennial genera (*Lolium* L., *Micropyropsis* Romero Zarco & Cabezudo, *Pseudobromus* K. Schum.) form the BL clade ([23,25–27], this study). The taxonomic distinction of these generic and infrageneric (*Festuca*) taxa is based on several diagnostic vegetative and reproductive morphoanatomical traits ([23], and references therein). Although none of the individual characteristics is absolute to identify a particular taxon, the combination of them has been used successfully to classify all these taxa in various floras and taxonomic treatments. In their systematic approach to subtribe Loliinae based on phylogenetic evidence, Catalán et al. [23] contemplated four potential scenarios for the classifications of the Loliinae (*Festuca sensu latissimo, sensu lato, sensu stricto, sensu strictissimo*). We propose to apply the *Festuca sensu lato* classification scenario, which

is based on a systematic evolutionary criterion that is nomenclaturally conservative and maintains a paraphyletic *Festuca* (with subgenera and sections) and other traditionally recognized genera. Our current study has demonstrated the applicability of our systematic approach in the group of studied broad-leaved MCSA and Mallopetalon species, for which their phylogenetic resolution does not always coincide with their taxonomic classification as a consequence of the high reticulation of the Loliinae but has helped to disentangle their hybrid allopolyploid evolutionary history.

3.3. Description of Festuca subgen. Coironhuecu subgen. nov.

Festuca subgen. *Coironhuecu* Moreno-Aguilar, Arnelas & Catalán, *subgen. nov.*

Description: Perennial dioecious caespitose plant presenting intravaginal innovations, plicate and junciform leaves, short membranous ligule with a truncate and densely ciliate apex, erect narrowly lanceolate and contracted panicle, tri-nerved lower glume, muticous or mucronulate lemma apex and sparsely hispid ovary tip.

Typus: *Festuca argentina* (Speg.) Parodi, Physis (Buenos Aires) 11: 498. 1935. Basionym: *Poa argentina* Speg., Revista de la Facultad de Agronomía y Veterinaria 3 (30–31): 584–585. 1897. Ind. loc.: "Argentina: Hab. ad margim orientalem Lago Argentino, anno 1884". Type specimen: Lago Argentino, 1884, *Sr. Tonini del Furia* s.n. (holotype, LP 001626; isotypes, BAA 2455, US 81670).

The subgenus is integrated only by *Festuca argentina* (Speg.) Parodi. It differs from the rest of the subgenera by the combination of its dioecy, caespitose habit, plicate leaves, tri-nerved lower glume, unawned lemma apex and sparsely hairy ovary tip. Etymology: *Coironhuecu* is based in the common Patagonian native name of *F. argentina* (Coirón huecú) due to its toxicity caused by its fungal endophytes.

4. Material and Methods

4.1. Morphological Study of Herbarium Festuca Specimens

Fifty herbarium specimens from AAU, BAA, MO, SI, US and UZ and 13 digital specimens (Supplementary File S1) from BAA, C, COL, IEB, K, LIL, LPB, MO and US were examined morphologically in search of the diagnostic characters provided by Alexeev and other authors to classify the Mesoamerican and South American *Festuca* species in the subgeneric and sectional taxa under study [30,31,37–39,41,42,44,46,48,50,60–62] and in other close morphological [32,33] and phylogenetic [24,25] taxa. We also evaluated 10 additional quantitative traits (culm height, ligule length, innovation leaf length, inflorescence length, inflorescence width, spikelet length, lower glume length, upper glume length, lemma length, awn length); however, none of them had a robust diagnostic value compared to the qualitative traits studied (Table 1). Ploidy levels were obtained from chromosome counts based on previous studies [18,22–27] and references therein. All *Festuca* species have a chromosome base number of x = 7; ploidy levels of the Meso and South American species studied (Table 2) fall within the expected range of known polyploid levels in the genus [18].

4.2. DNA Sampling of Festuca Species, Genome Sequencing, Data Assembling and Phylogenomic Analysis

Total DNA sampling was performed on representative species of all Mesoamerican and South American supraspecific *Festuca* ranks under study (Tables 1 and 2). We also added a representative species of FL *F*. sect. *Eskia* (*F. gautieri*) to the analysis. DNA was isolated from herbarium specimens or silica gel dried samples using a modified CTAB protocol [63] with ~20 mg of tissue. Genome skimming sequencing was performed from PCR-free libraries through the Illumina technology at the Spanish Centro Nacional de Análisis Genómicos (CNAG) and Macrogen, and the Illumina pair-end (PE) reads were processed following the procedures described in Moreno-Aguilar et al. [26].

Assembled plastomes for most of the newly sequenced samples were obtained with Novoplasty v. 2.7.1 [64] using the *F. pratensis* plastome (JX871941) as a reference and standardized parameters (k-mer: 29–39, insert size: ~95–200 bp, genome range:

120,000–220,000 bp, PE reads: 101–150 bp). The plastomes of four samples with low number of PE reads (*F. asperella*, *F. breviglumis*, *F. valdesii*, *F. venezuelana*,) were assembled using a read-mapping strategy to, respectively, closely related *Festuca* plastomes using Geneious Prime 2022 (Table 2). The plastome sequences of another 14 representative Loliinae lineages were retrieved from previous studies [26,27].

The nuclear rDNA 45S region (transcribed cistron 5′-ETS-18S gene- ITS1-5.8S gene-ITS2-25S gene, plus intergenic sequence (IGS) region) of 27 of the 36 new Loliinae samples studied was extracted with the TAREAN tool of the Repeat Explorer2 (RE2) software [65,66] through the Galaxy platform on the ELIXIR public server (https://repeatexplorer-elixir.cerit-sc.cz accessed on 30 May 2022). Clustering was performed using default TAREAN tool settings (BLAST threshold of 90%, similarity across 55% of the read to identify reads to each cluster, minimum overlap = 55, cluster threshold = 0.01% input reads) and an input of 500,000 PE reads per sample. 45S rDNA sequences were found in the TAREAN tandem reports of each sample. The 45S region was divided into its 35S and IGS regions using the *Brachypodium distachyon* (L.) P. Beauv. 45S sequence as reference (Table 2). The nuclear rDNA 5S gene of most of the newly sequenced samples was also obtained with the RE2 TAREAN tool. The 45S sequences of nine species (*F. abyssinica* Hochst. ex A. Rich., *F. asperella*, *F. asplundii*, *F. capillifolia* Dufour ex Roem. & Schult., *F. fimbriata*, *F. kingii*, *F. pampeana* Speg., *F. quadridentata*, *F. venezuelana*) and the 5S sequences of two species (*F. asperella*, *F. venezuelana*) that could not be recovered by TAREAN were assembled employing a read-mapping strategy using, respectively, *F. triflora* J.F. Gmel. and *F. pratensis* as reference sequences in Geneious Prime 2022. Additional 35S and 5S sequences from other Loliinae lineages were retrieved from previous studies [26,27].

Entire plastomes and nuclear 35S, IGS and 5S sequences were aligned separately with MAFFT v. 7.031b [67]. TrimAl software v. 1.2rev59 [68] was used to remove low quality regions from each of the multiple sequence alignments (MSA) by imposing the *-automated*1 parameter. Maximum likelihood (ML) phylogenetic trees were reconstructed for each separated data set with Iqtree imposing the best-fit nucleotide substitution model, according to the Bayesian Information Criterion (BIC), and estimating 1000 ultrafast bootstrap replicates (BS) for the branch support of the best tree [69–71]. The Singular Value Decomposition quartets (SVDq) approach was implemented in Paup * [72], imposing nquartets = all seed = 2 nthreads = 4 bootstrap = 1000 options with a multispecies coalescent tree model and the quartet assembly algorithm QFM. Bootstrap support of the branches was shown in the tree obtained from SVD quartet analysis.

The composition and proportion of repetitive elements of the studied *Festuca* species were obtained from similarity graph-based clustering analysis of filtered PE reads using the Repeat Explorer pipeline of RE2 [66]. Previous studies have demonstrated that similarity-based clustering of low coverage genome sequencing reads, confidentially representing 0.50–0.01 of the total haploid genome coverage, is proportional to the genomic abundance and longitude of the corresponding repeat types in several angiosperm lineages and the Loliinae, and thus could be used to quantify them ([27], and references therein). The individual and comparative analyses of the studied samples was conducted following the procedures described in Moreno-Aguilar et al. [27]. Briefly, automated RE2 cluster annotation was used to quantify clusters and calculate the proportions of repetitive elements in each sample in the individual analysis (Supplementary Table S1). Comparative clustering analysis was performed for all the 36 samples studied in a single Galaxy run using the maximum number of randomly sampled PE reads that could be processed (~0.08–0.2 genome coverage for each species). Neighbor-Joining phylogenetic trees were computed for the top clusters selected in the comparative RE2 analysis with the NJ function of the ape package in R [73] using pairwise Euclidean genetic distances between the repeat contents of the species. Clusters with incomplete information (NA or zero values) for some samples were discarded from downstream analysis. A consensus network was constructed from all the repeat NJ trees with SplitsTree4 [74].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11172303/s1, Figure S1: Anatomical leaf blade section of representative species of Mesoamerican and South-American broad-leaved *Festuca* taxa analyzed morphologically in this study. *F*. subgen. *Subulatae* sect. *Glabricarpae*: *F. venezuelana* (**a**); *F*. subgen. *Drymanthele s. l.*: *F. superba* (**b**); *F*. subgen. *Subulatae* sect. *Glabricarpae*: *F. breviglumis* (**c**); *F*. subgen. *Asperifolia*: *F. asperella* (**d**); *F*. subgen. *Erosiflorae*: *F. quadridentata* (**e**), *F. dichloclada* (**f**); *F*. subgen. *Drymanthele* sect. *Ruprechtia*: *F. amplissima* (**g**); *F.* subgen. *Coironhuecu* (*subgen. nov.*): *F. argentina* (**h**); *F*. subgen. *Mallopetalon*: *F. fimbriata* (**i**). Drawings by José Alfredo Hidalgo-Salazar (**a**–**h**) and María Fernanda Moreno-Aguilar (**i**). [**a**: modified from Stančik & Peterson [31]; **b**: modified from Türpe [49]; c: Peterson PM. & Rosales O. 16117, US- 3524155; **d**: modified from Alexeev [38]; **e**: modified from St. Yves [46]; **f**: Smith et al. 10782, AAU; **g**: modified from Stančik & Peterson [31]; **h**: modified from Catalán & Muller [32]; **i**: Kostling M. 44, UZ 498.08]; Figure S2: Loliinae coalescent species trees computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. (**a**) nuclear rDNA 35S tree; (**b**) nuclear rDNA (45S) IGS tree; (**c**) nuclear rDNA 5S tree; (**d**) plastome tree. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root some trees. Color codes of Loliinae lineages correspond to those indicated in the chart in Figure S2a. Scale bar: number of mutations per site; Figure S3: Morphological diagnostic traits mapped onto a Maximum Likelihood IGS cladogram tree of the Mesoamerican and South-American broad-leaved Festuca taxa studied and other representative species of the broad-leaved (BL) and fine-leaved (FL) Loliinae lineages. Traits codes: 1. Reproduction: monoecious (0), dioecious (1); 2. Habit: rhizomatous or caespitose or mixed (0), rhizomatose (1), caespitose (2); 3. Innovations: Extravaginal or intravaginal (0), intravaginal (2), extravaginal or/and intravaginal (3); 4. Ligule: membranaceous, apex acute, erose or lacerate, long (0), non- membranaceous, apex truncate shortly ciliate, or short membranaceous, apex truncate and ciliate, short (1), membranceous or hyaline, apex truncate or rounded, lacerate or dentate, or shortly ciliate, medium (2); membranaceous, apex truncate or slightly rounded and lacerate or dentate, medium-long (3); membranaceous, apex truncate, erose and ciliate, short (4); membranaceous, apex truncate and densely ciliate, short (5); 5. Leaf-blade: Flat, involute in the middle and subconvolute at the apex (0), largely flat (1), plicate, junciform (2), largely flat, subconvolute (3); 6. Inflorescence: erect (0), nutant or erect with nutant branches (1), erect or scarcely nutant (2), erect, laxe (3), erect, contracted (4), erect, branches flexuous (5); 7. Lemma apex: dentate or entire, unawned (0), entire, unawned (1), entire or bifid, awned (2), bifid, shortly awned or unawned (3), entire, scariose, rolled and fimbriate, unawned, muticous (4), entire, unawned, muticous or mucronulate (5), entire, unawned, muticous (6); 8. Ovary tip: glabrescent (0), glabrous or hispid (1), densely hairy (2), sparsely hispid (3); File S1: List of 65 specimens examined taxonomically of the species under study [*Festuca* subgen. *Erosiflorae*, *F*. subgen. *Drymanthele* sect. *Ruprechtia*, *F*. subgen. *Subulatae* sect. *Glabricarpae*, *F*. subgen. *Asperifolia* and *F*. subgen. *Mallopetalon* sensu Alexeev, plus the newly described *F*. subgen. *Coironhuecu subgen. nov*. (*F. argentina*) and *F.* subgen. *Drymanthele sensu lato* (*F. superba*)], ranked in alphabetical order; Table S1: Genome proportion of repeats estimated by Repeat Explorer2 for individual Loliinae samples (estimated percentages per holoploid genome, 1C). Values in bold correspond to new data generated in this study.

Author Contributions: P.C., I.A. and M.F.M.-A. designed the study. M.F.M.-A. and I.A. collected samples from Ecuador and P.C. from Argentina. M.F.M.-A. and L.A.I. developed the experimental work. M.F.M.-A., L.A.I., A.S.-R., I.A. and P.C. analyzed the data and interpreted the results. P.C., M.F.M.-A. and I.A. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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C.4. A nuclear single-copy-gene phylogeny of Loliinae (Poaceae): unraveling hybridization episodes

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Nuclear single-copy gene phylogenies reveal hybridization episodes in temperate Loliinae grasses

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Abstract

Resolving the phylogeny of recently evolved groups that have experienced recurrent introgressions and polyploidizations throughout their history is challenging. Evolutionary reconstruction based on a large set of biparentally inherited single-copy nuclear genes is considered a suitable approach to recover relationships that might otherwise be obscured by nuclear ribosomal genes in concerted evolution or biased by maternally inherited plastomes. Here we present the first single-copy gene phylogeny of the ecologically and economically important grass subtribe Loliinae (*Festuca* and related genera) using 241 nuclear coding loci captured from the Angisoperm353 probe set and a large sample of 138 representative taxa of Loliinae, covering all of its 22 evolutionary lineages. We have further completed genome-skimming sampling of other nuclear (35S rDNA gene) and organellar (plastome) sequences to elucidate the reticulate history of the Loliinae from multiple and complementary genome sources. Concatenated maximum likelihood and multispecies coalescent trees of single-copy genes showed well-supported relationships that were generally consistent across analyses and with previous taxonomic and phylogenetic findings but also revealed high levels of gene discordance. Hybridization and topological incongruence tests between the nuclear and plastome-based trees confirmed the rampant introgression experienced by Loliinae at deep and shallow nodes, detecting hybridization in four broad-leaved (Subulatae-Hawaiian, Schenodorus, Tropical-South African, Mexico-Central-South American) and five highly-diversified fine-leaved (American II, Exaratae-Loretia, Aulaxyper, Afroalpine, American-Neozeylandic) Loliinae lineages. The levels of intragenomic discordance could have been magnified by the prevalence of allopolyploids in the Loliinae and by methodological bias in the selection of orthologs; however, our nuclear and plastome trees have revealed key hybrid origins in these grasses.

Key words: allopolyploidization, intragenomic discordance, intergenomic topological incongruence, *Festuca* and related genera, nuclear single-copy genes, plastome, 35S rDNA gene.

Introduction

Reconstructing the phylogenetic relationships of any group of organisms is challenging due to the existence of biological and evolutionary events, such as hybridization and

polyploidization, incomplete lineage sorting (ILS), gains and losses of genome fractions, and lateral gene transfers, which prevent or confound inferences retrieved from simple bifurcating trees (Marcussen et al., 2015; Dunning et al., 2019; Liu et al. 2019). The complexity is exacerbated in highly reticulate lineages where the number of speciation events resulting from introgressions and autopolyploidizations or allopolyploidizations (and subsequent diploidizations) have occurred frequently and recurrently in recent times (Soltis et al., 2016; Sancho et al., 2022). Although the construction of evolutionary species networks from multilabelled trees is considered a suitable alternative to incorporate potential hybridizations and allopolyploidizations in the phylogeny (Huber et al., 2006), successfully finding the correct network would depend on whether the available set of multilabelled gene trees contains all the homeologs representing all subgenomes in the allopolyploid (Marcussen et al., 2015). However, incomplete homeologous sampling is common in ancient polyploids in which many duplicate gene copies have been removed due to genome re-structuring and excess genetic load (Michael, 2014) and even in recent polyploids in which chromosomal instability has promoted rapid genome rearrangements and gene losses (Chen and Ni, 2006). Recently, the use of coalescence-based methods and models applied to multigene data that take into account the potential existence of ILS to produce a single species tree (e. g., Astral, SVDq) has been considered a straightforward approach to infer the true phylogeny of organisms at infra and supraspecific levels (Baker et al., 2022).

Major advances in whole genome and transcriptome sequencing have provided a wealth of genome data for high-precision phylogenetic inference in plants (Leebens-Mack et al., 2019; Soltis and Soltis, 2021). However, phylogenies based entirely on re-sequenced genomes or whole transcriptomes are still rare and are only available for model crops or systems, or for specific case studies. In contrast, other high-throughput genome sequencing methods, such as genome skimming (Dodsworth, 2015; Richter et al., 2015) and gene target capture (Johnson et al., 2019), have emerged as useful tools for building phylogenies for non-model plant groups with limited genomic information and increase the availability of their DNA regions for evolutionary inference (Pérez-Escobar et al., 2021; Baker et al., 2022). Gene-targeted sequence capture, or target enrichment, can recover hundreds to thousands of low-copy nuclear genes with a high content of phylogenetic information (De Smet et al., 2013; Soto Gomez et al., 2019a; Maurin et al., 2021), and thus has become an optimal approach for plant phylogenetics. The method has shown efficiency in retrieving single-copy orthologs sufficiently conserved to be

unambiguously identified and sufficiently variable to help clarify relationships between lineages (Maurin et al., 2021; Zuntini et al., 2021). Furthermore, gene capture sequencing has facilitated phylogenetic investigation of taxa from herbarium materials, successfully achieving DNA processing and gene sequencing of specimens that are only known or available in historical collections (Thomas et al., 2021; Zuntini et al., 2021; Baker et al., 2022). Complementary to unique bait set designs specific for particular plant lineages and their genes (Soto Gomez et al., 2019b; Züst et al., 2020), the recent development of a universal angiosperm kit has enabled the capture of up to 353 nuclear single-copy genes present in all flowering plants, which have proven useful in resolving superclass-to-populations phylogenies (Baker et al., 2021).

The Loliinae subtribe constitutes one of the most diversified lineages of temperate pooid grasses (Catalán, 2006). It consists of more than 600 species of pasture and forage plants, some of which are of considerable ecological and economic importance (e. g., meadow, tall, red and sheep fescues, and ryegrass). Loliinae are distributed in temperate and tropical mountainous regions on all continents except Antarctica (Minaya et al., 2017; Moreno-Aguilar et al., 2020; Moreno-aguilar et al., 2022a). The subtribe comprises the large genus *Festuca,* with more than 500 species, plus 13 closely related genera, totalling about 50 species (Catalán, 2006; Catalán et al., 2007; Moreno-Aguilar et al., 2022b). *Festuca* and four genera (*Megalachne*, *Micropyropsis*, *Podophorus*, *Pseudobromus*) contain perennial species, and the remaining 9 genera contain either exclusively (*Ctenopsis, Dielsiochloa, Hellerochloa, Micropyrum, Narduroides, Psilurus, Vulpia, Wangenheimia*) or predominantly (*Lolium*) annual species. Loliinae species show a uniform chromosome base number of $x = 7$ and different levels of ploidy, ranging from diploid to dodecaploid. Hybridization and polyploidy are common in *Festuca*, whose species are mostly allopolyploids. *Festuca* species from the southern hemisphere are exclusively polyploid (Dubcovsky and Martínez, 1992; Catalán, 2006; Šmarda et al., 2008; Moreno-Aguilar et al., 2022b). This geographic pattern is consistent with Dispersal Extinction Cladogenesis (DEC) biogeographic models that suggest that the broad-leaved (BL) and fine-leaved (FL) lineages of Loliinae originated in the northern hemisphere, where diploids and polyploids currently coexist, and later colonized the southern continents (Minaya et al., 2017). *Festuca* species have been classified into 12 subgenera, according to Alexeev's worldwide taxonomic treatment (Supplementary Table S1; Moreno-Aguilar et al., 2022b, and references therein), and some of them into various sections and subsections according to their phenotypic traits (Catalán et al., 2007;

Moreno-Aguilar et al., 2022b). Previous phylogenetic studies based on few nuclear (ITS, *b-amylase*, *GBSS*) and plastid (*trn*TL, *trn*LF) loci (Inda et al., 2008; Díaz-Pérez et al., 2014; Minaya et al., 2015, 2017) provided an evolutionary framework for Loliinae. These studies demonstrated that *Festuca* is paraphyletic and that Loliinae divided into two main BL and FL lineages, each with several sub-lineages recovered in nuclear and plastid-based topologies. More recent phylogenetic work using genome skimming approaches inferred nuclear 35S cistron, IGS, and 5S Loliinae nuclear rDNA and plastome trees that were generally consistent with earlier phylogenies, but detected more topological incongruences for some specific subclades, and also expanded the sampling of new lineages of Loliinae (Moreno-Aguilar et al., 2020; Moreno-Aguilar et al., 2022b). Evolutionary investigation of the Loliinae repeatome inferred a consensus network topology that was highly congruent with that of the 35S nuclear rDNA tree (Moreno-aguilar et al., 2022a). Despite these advances, phylogenomic information for Loliinae based on a substantial sample of single-copy nuclear genes and their potential congruence with data from plastomes, nuclear rDNA, and repetitive elements remain to be evaluated and tested.

We present here the first phylogenomic study of Loliinae based on target capture data generated with the Angiosperms353 nuclear probe set (Johnson et al., 2019) to analyze primarily DNA samples from herbarium specimens. The aims of our study were: 1) to obtain individual trees and coalescence-based species trees of Loliinae from a large sample of nuclear single-copy genes and taxa; 2) analyze the intragenomic evolutionary congruence of the nuclear loci; 3) compare the phylogenetic resolution of the nuclear single-copy genes (biparentally-inherited) with those of plastome (maternally-inherited) and nuclear 35S nuclear rDNA genes (biparentally-inherited but prone to concerted evolution) and tests their respective pairwise congruence; and 4) evaluate the impact of hybridization and allopolyploidization on the taxa and phylogenetic trees analyzed.

Materials and Methods

Sampling

Samples of 138 species from six genera of Loliinae (*Festuca*, 124; *Lolium*, 5; *Vulpia*, 4; *Micropyorpsis*, 1; *Megalachne*, 1; *Wangenheimia*, 1; *Psilurus*, 1; *Hellerochloa* 1) and three related outgroup grasses (*Oryza sativa, Brachypodium distachyon, Hordeum vulgare*) were included in the study (Supplementary Table S1; Figure 1). All 138 Loliinae samples were first analyzed for capturing single-copy nuclear gene targets, including 81

taxa analyzed with genome skimming data for the first time, and 30 taxa not previously included in phylogenetic studies (Supplementary Table S1). The sampling was carried out from fresh materials collected in the field, from collections of silica-dried leaves stored at the High Polytechnic School of Huesca – University of Zaragoza, and from herbarium samples from different herbaria (AAU, HUTPL, OSC, MO, CONC, US, UZ, VBGI) (Supplementary Table S1). The selected taxa represent the 22 currently recognized evolutionary lineages within the Loliinae (Minaya et al., 2017; Moreno-Aguilar et al., 2022b).

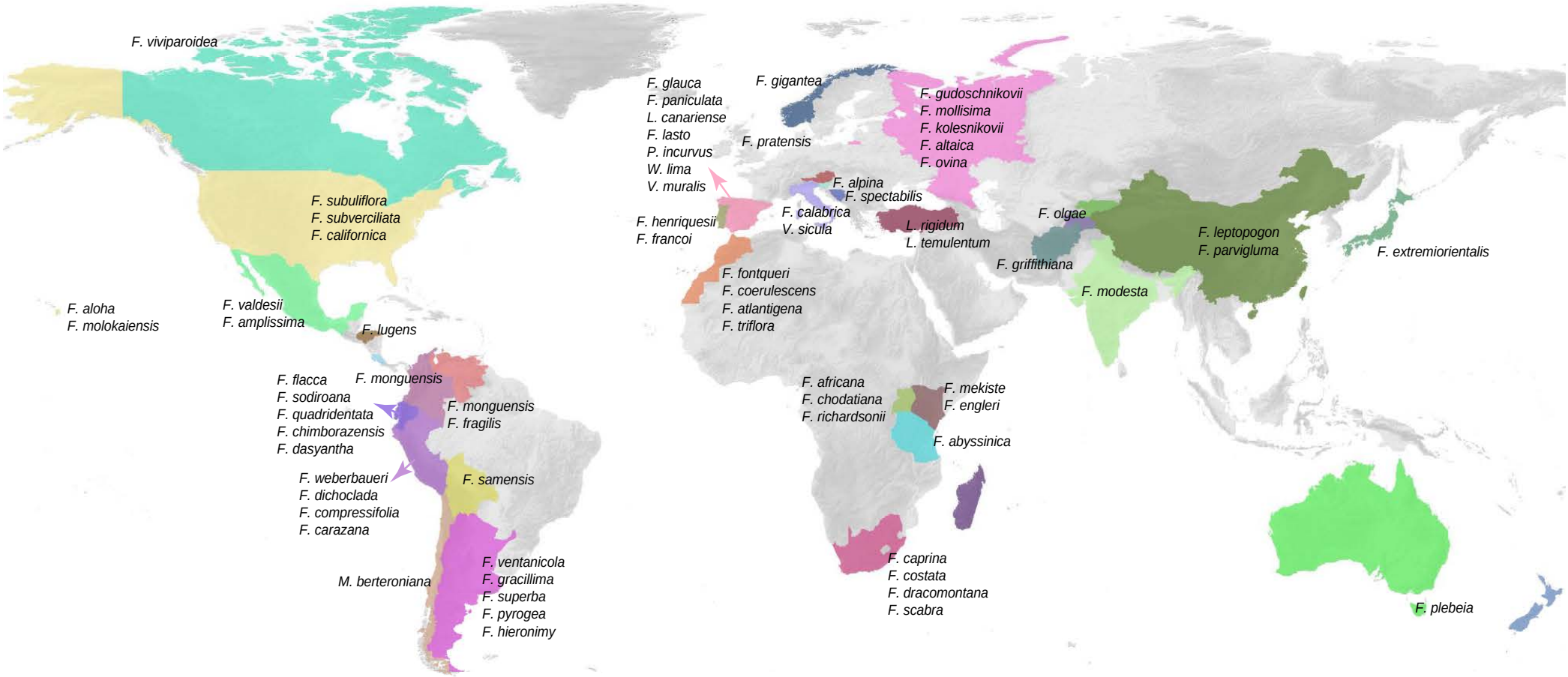


Figure 1. World map showing the geographic distributions of some of the Loliinae representative samples analyzed in this study by country.

DNA extraction, single-copy gene target enrichment and sequencing, and genome skimming sequencing

Total DNA was extracted using ~20 mg of tissue and a modified CTAB protocol (Doyle and Doyle, 1987). The concentration and quality of each DNA sample was estimated with a Qubit fluorometer (Invitrogen by Life Technologies) and with a Biodrop system (Harvard Bioscience), respectively.

Genomic library preparation and target enrichment of Loliinae single-copy nuclear genes were performed with the Angiosperm353 kit at Arbor Biosciences (Michigan, USA), following the protocols outlined in Johnson et al. (2019). Briefly, the quality of the DNA samples was verified in a subset of samples through an intercalating dye assay and visualized with Bioanalyzer (Agilent Technologies). Double-indexed libraries were prepared for each sample using up to 500ng of DNA (obtained from up to 4µg of sonicated DNA), with insert sizes of ~500bp using a blunt-end adapter and a protocol optimized for degraded DNA with up to 6 amplification cycles (Brewer et al., 2019). Hybridization capture was performed with Angiosperms353 baits (https: //arborbiosci.com/genomics/targeted-sequencing/mybaits/mybaits-expert/mybaits-expert-angiosperms-353/) in pools of 12 libraries following the manual myBaits v5 (https://arborbiosci.com/mybaits-manual/). Capture reactions were pooled in equimolar ratios to form a sequencing pool, which was sequenced on a partial lane of the Illumina NovaSeq 6000 platform in paired-end (PE) mode (2 x 150 bp). Demultiplexed data of ~14.8 Gbp (~30 million PE reads) was returned in FASTQ format (Supplementary Table S1). Target enrichment sequencing output reads were checked with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed with Trimmomatic (Bolger et al., 2014) to remove adapters and reads with a quality lower than Phred score of 30 preserving reads with a minimum length of 50bp (trailing:30 minlen:50) to reduce the risk of potential misalignments of short reads to genes.

Genome skim sequencing was performed for 81 Loliinae DNA samples (Supplementary Table S1). PCR-free libraries were quantified using the Library Quantification Kit for Illumina Platforms (Roche Kapa Biosystems). Genomic sequencing of a multiplexed pool of KAPA libraries was performed on a HiSeq4000 or HiSeq 2500 (TruSeq SBS Kit v4, Illumina, Inc) in PE mode (2 x 100 bp) in CNAG and Macrogen as described in Moreno-Aguilar et al. (2020). The quality of the Illumina PE reads was checked with FastQC and adapters and low-quality sequences were trimmed and removed with Trimmomatic.

Skimmed genome data for another 64 Loliinae species were retrieved from earlier studies (Moreno-aguilar et al., 2022a, 2022b). The Loliinae genomic samples used in downstream analysis contained between 1.5 – 35.0 million reads (average 16.0 million reads) with insert sizes ranging between 69 – 260 bp (Supplementary Table S1).

Sequence assemblies and alignments

Loliinae single-copy nuclear genes were recovered from the target-enriched PE reads using the HybPiper v.1.3.1 pipeline (Johnson et al., 2016). This was done by mapping the filtered reads against the template sequences of the 353 low-copy nuclear genes (available at https://github.com/mossmatters/Angiosperms35) using the Burrows-Wheeler Alignment (BWA v.0.7) (Li et al., 2009), and then through *de novo* assembly of mapped reads for each gene separately using SPAdes v. 3.13 (Bankevich et al., 2012), with a minimum coverage threshold by default of 8×. We then used the HybPiper script retrieve_sequences.py which generates a single sequence per gene that is selected based on the criteria of length, similarity, and depth of coverage (Supplementary Table 1). Individual genes were aligned with MAFFT v.7.490 (Katoh & al., 2002) using the iterative refinement method *--maxiterate* 1000. Empty genes for any Loliinae sample were removed from downstream analysis. Phyutility 2.2.6 (Smith & Dunn, 2008) was used to remove sequences with insufficient coverage (<30%, *-clean* 0.3) in well-occupied columns from each individual gene alignment. Gene alignments were visually inspected with Geneious Prime for potentially misaligned sequences. To improve alignment quality, we estimated summary statistics of gene alignments using AMAS v.0.98 (Borowiec, 2016), including alignment length, missing data, and number of parsimony informative sites (Supplementary Table S2). Sequences with less than 60% of the alignment length were then removed to improve the quality of the data sets, filtering out species with potentially low phylogenetic information. To identify and extract putative paralogs, we consecutively run the scripts *paralog_investigator.py* and *paralog_retriever.py* in HybPiper obtaining the fasta paralog files.

Entire plastome and 35S nuclear rDNA cistron sequences were assembled for 81 new Loliinae samples from their respective genome skimming PE reads, following the procedures indicated in Moreno-Aguilar et al. (2022b). Plastome assembly was performed with Novoplasty v.2.7.1 (Dierckxsens & al., 2017) using the *Festuca pratensis* plastome (JX871941) as reference sequence and standardized parameters (k-mer: 30-39, insert size: ~69-200 bp, genome range: 120,000–140,000 bp, and PE reads: 101-150 bp).

Furthermore, to retrieve plastome sequences in data with a low number and quality of PE total reads, plastome assembly was performed using a read-mapping strategy to, respectively, closely related *Festuca* plastomes using Geneious Prime (Supplementary Table S1). The transcribed 35S nuclear rDNA cistron [5′-external transcribed spacer (ETS), 18S gene, internal transcribed spacer ITS1, 5.8S gene, internal transcribed spacer ITS2, 25S gene] of the 81 new Loliinae samples was assembled employing a read-mapping strategy using *F. triflora* as reference sequence in Geneious Prime (Supplementary Table S1). Additional plastome and 35S cistron sequences were retrieved from, respectively, another 49 and 28 representative Loliinae lineages from previous studies (Moreno-Aguilar et al., 2020; Moreno-Aguilar et al., 2022a, 2022b) and incorporated into the study. Whole plastomes and 35S nuclear sequences were aligned separately with MAFFT v.7.031b, and trimAl v. 1.2rev59 (Capella-Gutiérrez et al., 2009) was used to remove poor quality regions from each of the multiple sequence alignments (MSA) by enforcing the -*automated*1 parameter.

Phylogenetic reconstructions and intragenomic discordance

Trimmed MSAs of single-copy nuclear genes were concatenated into a supermatrix with FASconCAT-G_v1.05 (Kück & Longo, 2014) and analyzed phylogenetically according to a maximum likelihood approach using IQtree v. 1.6.12 (Nguyen & al., 2015) with model selection implemented via ModelFinder (Kalyaanamoorthy & al., 2017) for each partition, according to the Bayesian Information Criterion (BIC), and branch support calculated from 1000 replicates of UltraFast Bootstrap (Hoang & al., 2018). In addition, individual ML trees of each nuclear single-copy gene, as well as ML trees of the entire plastome and the 35S nuclear rDNA MSAs were also computed separately using the same IQtree procedure. *Oryza sativa* and *Brachypodium distachyon* (plus *Hordeum vulgare*, single-copy gene dataset) were used to root the trees.

To account for potential incomplete lineage sorting (ILS) events between closely related Loliinae lineages and putative topological incongruences between nuclear single-copy gene trees, we inferred a species tree for the Loliinae samples studied by analyzing the single-copy genes under multispecies coalescent (MSC), using sequence alignments and quartets analysis (SVDq) and partially resolved gene trees analysis (ASTRAL). The Singular Value Decomposition quartets (SVDq) approach was implemented in Paup* (Swofford, 2003), which uses a variant of Quartet FM (Reaz & al., 2014) to combine

quartet trees into a species tree. The analysis was performed by imposing nquartets = 1,000,000 seed = 2 nthreads = 20 bootstrap = 1,000 with a multispecies coalescent tree model and the QFM quartet assembly algorithm. The bootstrap support of the branches was shown in the tree obtained from SVD quartet analysis. The ASTRAL approach was performed with ASTRAL-III v.5.7.8 (Zhang et al., 2018) using the individual IQtree gene trees to estimate the MSC species tree. Gene trees were first rooted using *Oryza sativa, Brachypodium distachyon,* and *Hordeum vulgare* and the *pxrr* function in Phyx (Brown & al., 2018); however, four genes showing nested outgroup samples within the ingroup were rooted using the early diverging samples of *F. lasto* and *F. drymeja* as external species. Branches with likelihood bootstrap support values <30% in gene trees were collapsed using *nw_ed* from Newick Utilities 1.6.0 (Junier and Zdobnov, 2010). ASTRAL-III 5.7.8 was executed by imposing a *-t2* flag parameter obtaining the ASTRAL species tree annotated with quartet support values (Sayyari and Mirarab, 2016) for the main topology (*q1*), the first alternative topology (*q2*), and the second alternative topology (*q3*). To estimate the intragenomic discordance in the nuclear dataset, we analyzed in R the normalized quartets scores generated by ASTRAL when inferring the single-copy gene species tree. The magnitude of the quartets score (proportion of quartets in the gene tree that agrees with the species tree) is inversely proportional to incongruence (1 indicates absence of gene tree discordance) (Pérez-Escobar et al., 2021).

Nuclear vs plastome topological incongruence testing and hybridization detection

The well resolved single-copy nuclear gene MSC species tree, the plastome ML tree, and the 35S nuclear rDNA tree were topologically contrasted with each other by visual inspection and using the Kishino-Hasegawa (KH), Shimodaira-Hasegawa (SH), and Shimodaira Approximately Unbiased (AU) tests with estimated log-likelihood (RELL) resampling optimization and 1 million bootstrap replicates in PAUP* (Swofford, 2003). To refine the assessment of the degree to which the phylogeny retrieved by the nuclear-encoding genome tracked that of the organelle (plastome) genome, we also applied the Procrustean Approach to Cophylogenetics (PACo) pipeline implemented in R (Balbuena et al., 2013; Pérez-Escobar et al., 2016) to the nuclear single-copy gene supermatrix ML trees vs the plastome ML supermatrix gene trees of 129 common Loliinae species. For this, phylogenetic reconstruction was performed using IQtree, supplying the program with partition information from each nuclear and plastome alignment, executing 1,000 ultrafast bootstrap replicates and saving the respective bootstrap trees (ufboot). This

procedure evaluates the similarities between any pair of topologies by comparing the Euclidean distances that separate the terminals in both trees through the Procrustean superimposition (Balbuena et al., 2013). The sum of the squared residuals (the disparity between an observed and a fitted value derived from a model) for each association and each pair of topologies evaluated can be interpreted as a concordance score because it is directly proportional to the magnitude of the topological conflict for the pair of terminals considered (Pérez-Escobar et al., 2016; Hendriks et al., 2022). The differences in the position of terminals between the nuclear single-copy gene ML trees and the plastome ML trees were summarized in barplots using the R package *ggplot2* (Wickham, 2016). The sum of squared residuals for each pair of terminals across nuclear and plastome genes was ranked into quartiles, and the magnitude of the discordance was assessed by the proportion of genes binned in quartiles 3 and 4 (50% and 75%) in each terminal; the more genes binned in quartiles 3 and 4, the more discordant the terminals are (Pérez-Escobar et al., 2021; Hendriks et al., 2022).

To further search for evidence of hybridization, we applied HyDe v0.4.3 (Blischak et al., 2018) which detects hybridization using phylogenetic invariants arising under the coalescent model with hybridization, and tests sets of triples across the whole phylogeny. HyDe requires the input file as a nucleotide alignment, so we used the trimmed concatenated supermatrix of single-copy nuclear genes. The final concatenated alignment of 241 genes (see Results) was employed to generate the input using the *concatenate_matrices.py* script. A high frequency of gamma scores (hybridization proportion) is indicative of a high prevalence of hybridization across all ingroup taxa.

Results

Nuclear single-copy-gene recovery in Loliinae, and plastome and 35S nuclear rDNA datasets

On average, 2.95 M PE reads per sample were sequenced, ranging from 0.87 M in *F. subulifolia* to 0.64 M in *F. californica* (Supplementary Table S1) for target capture data. The average recovery of reads per genus was similar for *Festuca* (3.35 M), *Vulpia* (3.35 M), *Wangenhemia* (3.31 M), *Micropyropsis* (3.69 M), and *Psilurus* (3.62 M), and slightly less for *Megalachne* (2.59 M) and *Lolium* (2.42 M) (Supplementary Table S1). The total number of nuclear Loliinae target genes obtained from the Angiosperms353 kit was 351. The mean number of genes recovered with >50% of the target length for the Loliinae

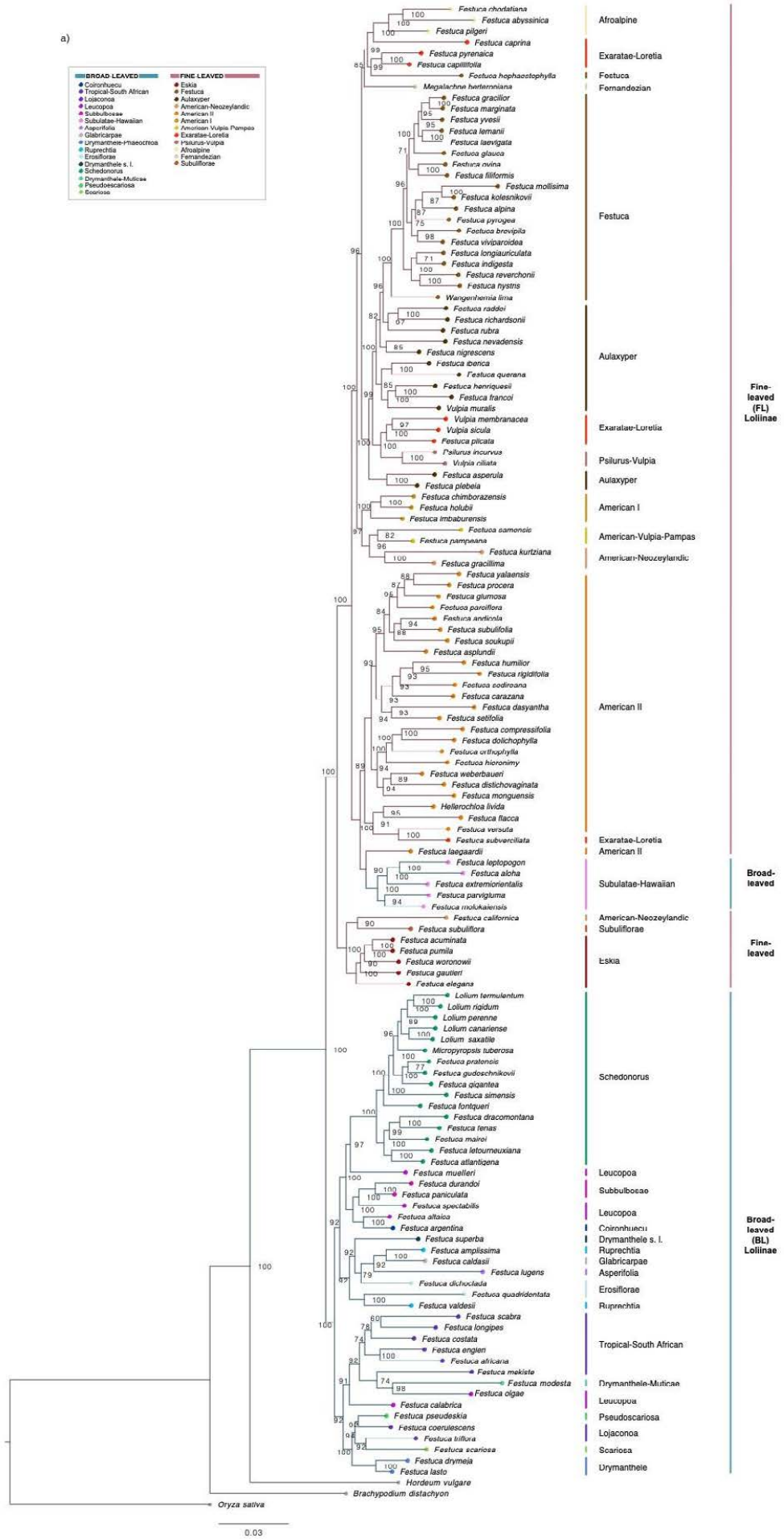
samples in the entire sampling was 248, which represents 70.29% of the target loci, while the mean number of genes with >75% of the target length was 173 (49%) (Supplementary Table S2). We detected 107 potential paralog instances via HybPiper's paralog warnings. After excluding paralog copies, three genes with sequences for less than 35 species, two species that failed to recover any target gene (*F. sibirica, F. ventanicola*), and three species that recovered less than 25% of target genes (*F. breviglumis*: 14 genes, 5.85%; *F. fimbriata*: 61, 25.3%; *F. kamstachica*: 18, 7.5%), the final data set consisted of 241 genes and 133 species, representing on average 68.2% of the 353 reference genes (Supplementary Figure S1; Supplementary Table S2). Individual nuclear single-copy gene alignments ranged from 75bp to 3,339bp, with a mean length of 659bp (Supplementary Table S2). The final alignment of 241 concatenated nuclear genes for 133 species was 158,876bp in length. The percentage of missing data was 25.93% and the percentage of parsimony informative sites 28.9% (45,953bp) (Supplementary Table S3).

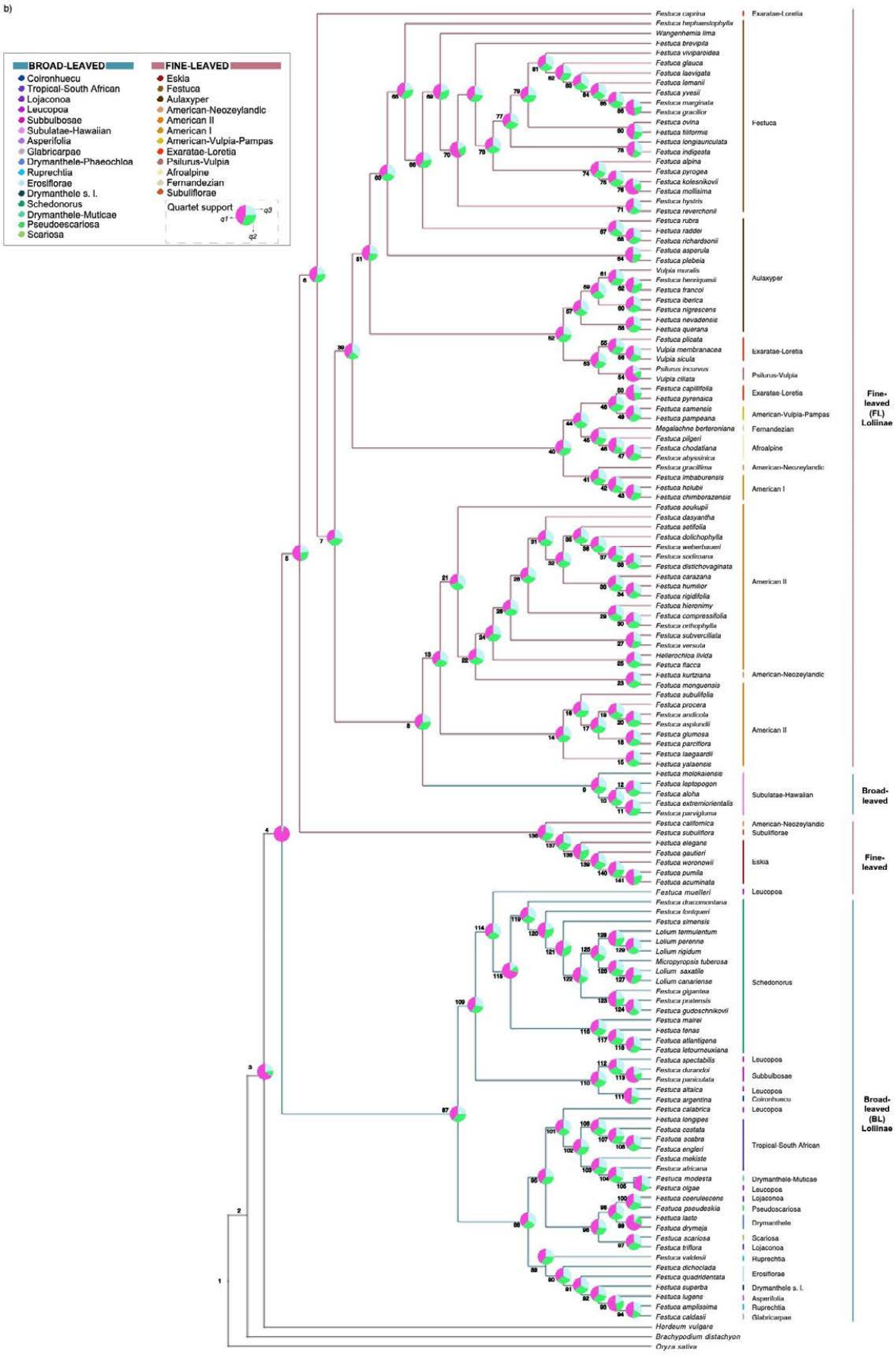
Genome skimming data from 81 newly sequenced samples ranged from 1,56 M (*F. livida*) to 35,89 M (*F. glauca*) Illumina pair-end (PE) reads (Supplementary Table S1). The length of the 35S nuclear rDNA cistron sequence ranged from 6,509 (*F. plicata*) to 6,513bp (*F. caprina*), with a total length of 6,556bp in the multiple sequence alignment (MSA) [1093 (16.7%) variable sites, 611 (9.31%) parsimony informative sites] (Supplementary Table S3). This region showed conserved structure along its aligned transcriptional unit, showing similar average lengths and coverages in the studied samples. Newly assembled complete plastomes ranged from 103,079 (*F. livida*) to 134,746bp (*F. costata*), which is consistent with plastome length values obtained in previous studies of Loliinae for the respective FL and BL clades (Moreno-Aguilar et al., 2022b). Most of the newly assembled plastomes showed good read coverage (>40×). The MSA of the complete plastomes was 135,487bp in length [19,598 (14.46%) variable sites, 8165 (6.02%) parsimony informative sites], being *F. livida, V. muralis, P. incurvus* the samples with the highest percentage of missing data (23%). Newly obtained sequences from each data set were deposited in GenBank (Supplementary Table S1). The plastome-MSA was employed to generate 1,000 bootstrap plastome trees used in the PACo nuclear *vs* plastome topological incongruence test due to the high degree of intragenomic congruence of the Loliinae plastid-encoding genes (Minaya et al., 2017; Moreno-Aguilar et al., 2020).

Nuclear and plastome-based phylogenies of Loliinae and nuclear intragenomic incongruence

Phylogenetic trees retrieved from single-copy genes using concatenated ML and multispecies coalescent (ASTRAL, SVDq) approaches (Figure 2; Supplementary Figure S2) recovered topologies that were relatively highly congruent with each other and with those obtained from other molecules. All three phylogenies supported the main split of BL and FL Loliinae clades as well as divergences from most (BL: Drymanthele-Phaeochloa + Scariosae + Lojaconoa + Pseudoscariosa; Tropical–South Africa; Subbulbosae + Leucopoa, Schedonorus; FL: Eskia; American II; American-Neozeylandic; American I; Psilurus-Vulpia(px); Festuca + Wangenheimia; Afroalpine) but not all (BL: Mexico-Central American-South American (MCSA) I and II; FL: 'intermediate' Subulatae-Hawaiian; American–Vulpia Pampas; Loretia + Exaratae; Aulaxyper) main sublineages of broad- and fine-leaved Loliinae. The main topological discordances were related to the different locations of the BL MCSA I (Glabricarpae, Asperifolia, Drymenthele s. l.) and MCSA II (Erosiflorae, Ruprechtia, Coironhuecu) groups, forming an intermediately evolved sister clade of Subbulbosae-Leucopoa / Schedonorus in the ML tree (Figure 2a), being sister to an ancestral Drymanthele-Phaeochloa + Scariosae + Lojaconoa + Pseudoscariosa / Tropical-South Africa clade in the ASTRAL tree (Figure 2b), or splitting into two non-related clades in the SVDq tree (Supplementary Figure S2). In all these trees, *F. argentina* (Coironhuecu) was separated from the MCSA taxa, nested within the Subbulbosae + Leucopoa clade and resolved as sister of *F. altaica* (Figures 2a, 2b, Supplementary Figure S2). The Exaratae-Loretia group was divided into a clade ((*V. membranacea* / *V. sicula*), *F. plicata*) sister to Psilurus-Vulpia (px), and other lineages (*F. capillifolia* / *F. pyrenaica*, *F. caprina*) which showed disparate resolutions in the FL clade (Figures 2a, 2b, Supplementary Figure S2). Our largest sampling of taxa (Supplementary Table S1) enriched the phylogenetic circumscriptions of several clades or groups with newly studied species (Festuca: *F. gracilior, F. kolesnikovii, F. marginata, F. mollisima, F. reverchonii, F. yvesii*; Aulaxyper: *F. plebeia*, *F. raddei, F. richardsonii*; American-Vulpia-Pampas: *F. samensis*; American-Neozeylandic: *F. kurtziana*; American I: *F. imbaburensis*; American II: *F. carazana, F. dasyantha, F. distichovaginata, F. dolichophylla, F. glumosa, F. humilior, F. laegaardii, F. monguensis, F. procera, F. parciflora, F. rigidifolia, F. setifolia, F. sodiroana, F. soukupii, F. subulifolia, F. versuta, F. weberbaueri*; Subulatae-Hawaiian: *F. leptopogon*; Eskia: *F. acuminata, F. woronovii*; Asperifolia: *F. lugens*).

Most of these species clustered in the same groups in the ML and MCS trees although their relationships differed in some cases from tree to tree (Figures 2a, 2b, Supplementary Figure S2). The strongly supported Australian *F. asperula* / *F. plebeia* clade was closely related to the Aulaxyper group (Figures 2a, 2b, Supplementary Figure S2). The taxonomically and phenotypically diverse American II lineage included species classified within the fine-leaved *Festuca* sect. *Festuca* (e. g., *F. andicola, F. orthophylla*) or within different broad-leaved supraspecific *Festuca* ranks; for example, *F. versuta* (*F*. subgen. *Drymanthele* sect. *Texanae*) / *F. subverticillata* (*F*. subgen. *Obtusae*), *F. flacca* (*F*. subgen. *Subulatae* sect. *Subulatae*), plus fine-leaved *Hellerochloa livida* formed a clade in the ML tree (Figure 2a), and nested in close positions in the ASTRAL (Figure 2b) and SVDq (Supplementary Figure S2) trees. The broad-leaved *F. californica* (*F*. subgen. *Leucopoa* sect. *Breviaristatae*) and *F. subuliflora* (*F*. subgen. *Subuliflora*) were resolved as sister taxa (Figure 2a, Supplementary Figure S2) or closely related species (Figure 2b) in the ML and MCS phylogenies, respectively, nesting in early divergent lineages within the FL clade. *F. muelleri* (*F*. subgen. *Drymanthele* sect. *Banksia*) was resolved as a sister lineage to the Schedonorus clade, strongly supported in all three trees (Figure 2; Supplementary Figure S2). Eurasian broad-leaved *F. modesta* (*F*. subgen. *Drymanthele* sect. *Muticae*) and *F. calabrica* and *F. olgae* (*F*. subgen *Leucopoa*), and South African *F. scabra* fell within an expanded Tropical-South African clade in the ML, ASTRAL and SVDq (all but *F. calabrica*) trees (Figures 2a, 2b, Supplementary Figure S2).


a)
BROAD LEAVED
Coironhuecu
Tropical-South African
Loiaconoa
Leucopoa
Subbulbosae
Subulatae-Hawaiian
Asperifolia
Glabricarpae
Drymantheie-Phaeocmoa
Ruprechtia
Erosiflorae
Drymantheie s. l.
Schedonorus
Drymantheie-Muticae
Pseudoescariosa
Scariosa
FINE LEAVED
Eskia
Festuca
Aulaxyper
American-Neozeylandic
American II
American I
American Vulpia Pampas
Exaratae-Loretia
Psilurus-Vulpia
Afroalpine
Fernandezian
Subuliflorae
Festuca chodatiana
Festuca abyssinica
Festuca pilgeri
Festuca caprina
Festuca pyrenaica
Festuca capillifolia
Festuca hephaestophylla
Megalachne berterroniana
Festuca gracilior
Festuca marginata
Festuca yvesii
Festuca lemanii
Festuca laevigata
Festuca glauca
Festuca ovina
Festuca filiformis
Festuca mollisima
Festuca kolesnikovii
Festuca alpina
Festuca pyrogea
Festuca brevipila
Festuca viviparoidea
Festuca longiauriculata
Festuca indigesta
Festuca reverchonii
Festuca hystrix
Wangenheimia lima
Festuca raddei
Festuca richardsonii
Festuca rubra
Festuca nevadensis
Festuca nigrescens
Festuca iberica
Festuca querana
Festuca henriquesii
Festuca francoi
Vulpia muralis
Vulpia membranacea
Vulpia sicula
Festuca plicata
Psilurus incurvus
Vulpia ciliata
Festuca asperula
Festuca plebeia
Festuca chimborazensis
Festuca holubii
Festuca imbaburensis
Festuca eamonsis
Festuca pampeana
Festuca kurtziana
Festuca gracillima
Festuca yalaensis
Festuca procera
Festuca glumosa
Festuca pareiflora
Festuca andicola
Festuca subulifolia
Festuca soukupii
Festuca asplundii
Festuca humillor
Festuca rigidifolia
Festuca sodiroana
Festuca carazana
Festuca dasyantha
Festuca setifolia
Festuca compressifolia
Festuca dolichophylla
Festuca orthophylla
Festuca hieronimy
Festuca weberbaueri
Festuca distichovaginata
Festuca monguensis
Hellerochloa livida
Festuca flacca
Festuca versuta
Festuca subverciliata
Festuca laegaardii
Festuca leptopogon
Festuca aloha
Festuca extremiorientalis
Festuca parvigluma
Festuca molokaiensis
Festuca californica
Festuca subuliflora
Festuca acuminata
Festuca pumila
Festuca woronowii
Festuca gautieri
Festuca elegans
Lolium temulentum
Lolium rigidum
Lolium perenne
Lolium canariense
Lolium saxatile
Micropyropsis tuberosa
Festuca pratensis
Festuca gudoschnikovii
Festuca gigantea
Festuca simensis
Festuca fontqueri
Festuca dracomontana
Festuca tenas
Festuca mairei
Festuca letourneuxiana
Festuca atlantigena
Festuca muelleri
Festuca durandoi
Festuca paniculata
Festuca spectabilis
Festuca altaica
Festuca argentina
Festuca superba
Festuca amplissima
Festuca caldasii
Festuca lugens
Festuca dichoclada
Festuca quadridentata
Festuca valdesii
Festuca scabra
Festuca longipes
Festuca costata
Festuca engleri
Festuca africana
Festuca mekiste
Festuca modesta
Festuca olgae
Festuca calabrica
Festuca pseudeskia
Festuca coerulescens
Festuca biflora
Festuca scariosa
Festuca drymeia
Festuca lasto
Hordeum vulgare
Brachypodium distachyon
Oryza sativa
0.03
Afroalpine
Exaratae-Loretia
Festuca
Fernandezian
Festuca
Aulaxyper
Exaratae-Loretia
Psilurus-Vulpia
Aulaxyper
American I
American-Vulpia-Pampas
American-Neozeylandic
American II
Exaratae-Loretia
American II
Subulatae-Hawaiian
American-Neozeylandic
Subuliflorae
Eskia
Schedonorus
Leucopoa
Subbulbosae
Leucopoa
Coironhuecu
Drymantheie s. l.
Ruprechtia
Glabricarpae
Asperifolia
Erosiflorae
Ruprechtia
Tropical-South African
Drymantheie-Muticae
Leucopoa
Pseudoscariosa
Lojaconoa
Scariosa
Drymantheie
Fine-leaved (FL) Loliinae
Broad-leaved
Fine-leaved
Broad-leaved (BL) Loliinae


b)
BROAD-LEAVED
Coironhuecu
Tropical-South African
Lojaconoa
Leucopoa
Subbulbosae
Subulatae-Hawaiian
Asperifolia
Glabricarpae
Drymanthele-Phaeochloa
Ruprechtia
Erosiflorae
Drymanthele s. l.
Schedonorus
Drymanthele-Muticae
Pseudoescariosa
Scariosa
FINE-LEAVED
Eskia
Festuca
Aulaxyper
American-Neozeylandic
American II
American I
American-Vulpia-Pampas
Exaratae-Loretia
Psilurus-Vulpia
Afroalpine
Fernandezian
Subulliflorae
Quartet support
q1
q3
q2
Festuca caprina
Festuca hephaestophylla
Wangenheimia lima
Festuca brevipila
Festuca viviparoidea
Festuca glauca
Festuca laevigata
Festuca lemanii
Festuca yvesii
Festuca marginata
Festuca gracilior
Festuca ovina
Festuca filiformis
Festuca longiaurriculata
Festuca indigesta
Festuca alpina
Festuca pyrogea
Festuca kolesnikovii
Festuca mollissima
Festuca hystrix
Festuca reverchonii
Festuca rubra
Festuca raddei
Festuca richardsonii
Festuca asperula
Festuca plebeia
Vulpia muralis
Festuca henriquesii
Festuca francoi
Festuca iberica
Festuca nigrescens
Festuca nevadensis
Festuca querana
Festuca plicata
Vulpia membranacea
Vulpia sicula
Psilurus incurvus
Vulpia ciliata
Festuca capillifolia
Festuca pyrenaica
Festuca samensis
Festuca pampeana
Megalachne berteroniana
Festuca pilgeri
Festuca chodatiana
Festuca abyssinica
Festuca gracillima
Festuca imbaburensis
Festuca holubii
Festuca chimborazensis
Festuca soukupii
Festuca dasyantha
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Festuca weberbaueri
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Festuca carazana
Festuca humilior
Festuca rigidifolia
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Festuca orthophylla
Festuca subvercillata
Festuca versuta
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Festuca flacca
Festuca kurtziana
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Festuca procera
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Festuca muelleri
Festuca ducomontana
Festuca lindquistii
Festuca simensis
Lolium termulentum
Lolium perenne
Lolium rigidum
Micropyropsis tuberosa
Lolium saxatile
Lolium canariense
Festuca gigantea
Festuca palmensis
Festuca gudoschnikovii
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Festuca durandoi
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Festuca scabra
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Festuca olgae
Festuca coerulescens
Festuca pseudeskia
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Festuca drymeja
Festuca scariosa
Festuca triflora
Festuca valdesii
Festuca dichoclada
Festuca quadridentata
Festuca superba
Festuca lugens
Festuca amplissima
Festuca caldasii
Hordeum vulgare
Brachypodium distachyon
Oryza sativa
Exaratae-Loretia
Festuca
Aulaxyper
Exaratae-Loretia
Psilurus-Vulpia
Exaratae-Loretia
American-Vulpia-Pampas
Fernandezian
Afroalpine
American-Neozeylandic
American I
American II
American-Neozeylandic
American II
Subulatae-Hawaiian
American-Neozeylandic
Subulliflorae
Eskia
Leucopoa
Schedonorus
Leucopoa
Subbulbosae
Leucopoa
Coironhuecu
Leucopoa
Tropical-South African
Drymanthele-Muticae
Leucopoa
Lojaconoa
Pseudoscariosa
Drymanthele
Scariosa
Lojaconoa
Ruprechtia
Erosiflorae
Drymanthele s. l.
Asperifolia
Ruprechtia
Glabricarpae
Fine-leaved (FL) Loliinae
Broad-leaved
Fine-leaved
Broad-leaved (BL) Loliinae

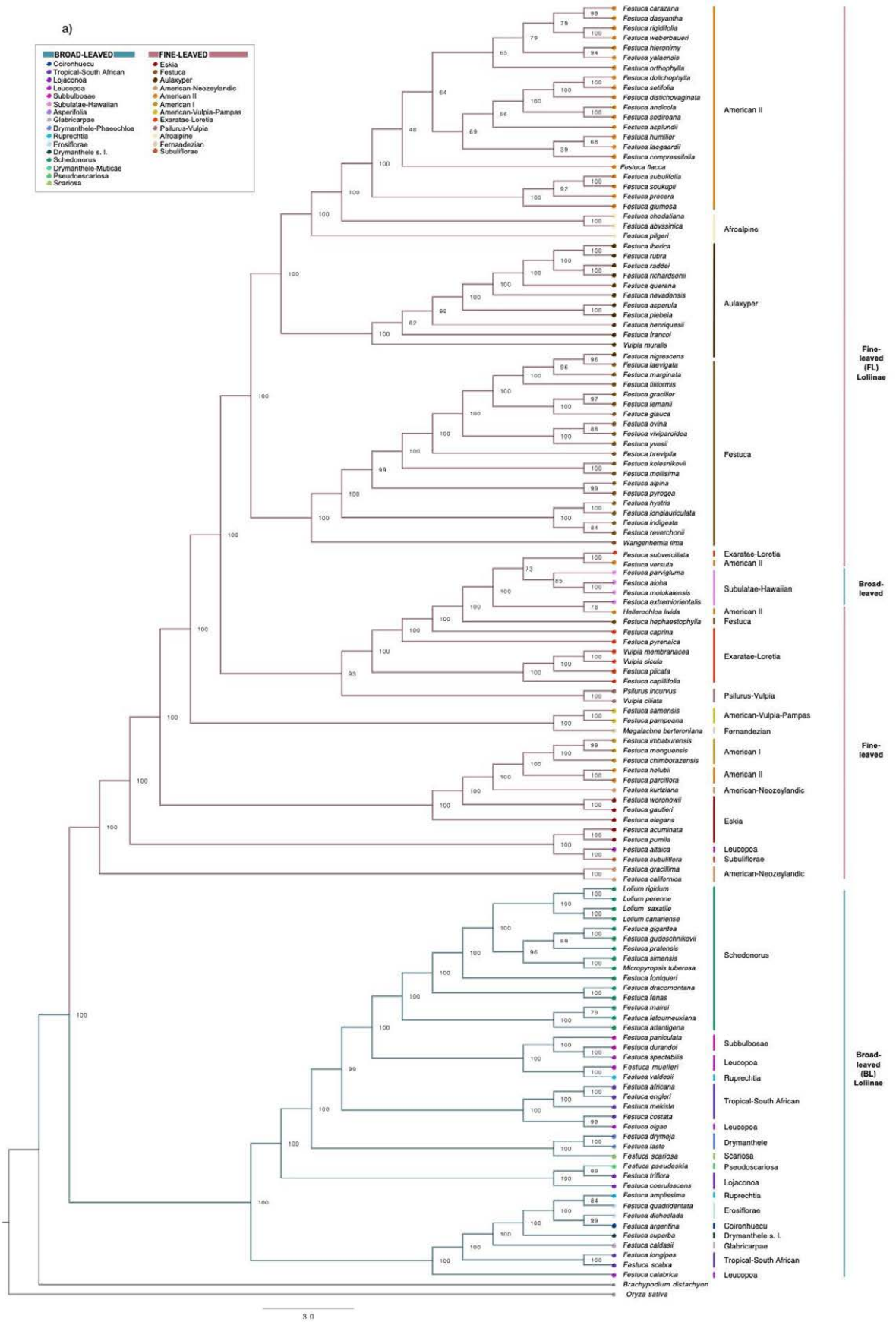
Figure 2. Loliinae single-copy nuclear gene phylogenies based on 241 genes and 133 species. (**a**) Maximum Likelihood (ML) phylogeny of Loliinae constructed from a concatenated supermatrix of 241 genes. Numbers on branches indicate UltraFast Bootstrap supports (BS). Scale bar: number of mutations per site. (**b**) ASTRAL species-coalescent tree of Loliinae inferred from 241 ML gene trees. The branches of the tree show consecutive numbering. Pie diagrams at nodes correspond to quartet support values for the alternative branch topologies (*q1*, *q2*, and *q3*, see color codes in the chart; Supplementary Table S4). *Oryza sativa* was used to root the trees. Color codes of Loliinae lineages are indicated in the charts.

Genomic discordance between nuclear single-copy genes used to reconstruct the phylogeny of Loliinae was high for most lineages (Figure 2b; Supplementary Table S4). Estimation of the proportion of gene tree quartets that agree with the ASTRAL species tree through normalized quartet scores (Figure 2b) indicated considerable intragenomic incongruence. The proportion of gene tree quartets concordant with the species tree was 89%. The MSC analysis revealed that the branches obtained values between 32.06 – 94.64% for the main topology (*q1*), 2.62 – 36.67 % for the first alternative topology (*q2*), and 2.73 – 36.79 % for the second alternative topology (*q3*) (Supplementary Table S4). The highest intragenomic concordances were found for the topological resolution of the Loliinae crown node (94.64% *q1*), followed by those of the Schedonorus (72% *q1*), Psilurus-Vulpia (px) (63% *q1*) and Drymanthele – Phaeochloa (59% *q1*) nodes. Other topological resolutions that showed moderate concordance with the Loliinae ASTRAL species tree were those of the Subbulbosae (40% *q1*), broad-leaved MCSA I-II (41%, *q1*), FL Loliinae *sensu lato* (including Subulatae-Hawaian) (37%, *q1*) and fine-leaved Eskia (34%, *q1*) nodes.

The topology of the strongly supported plastome ML tree (Figure 3a) showed general agreement for the major BL and FL clades with that of the nuclear single-copy gene ML tree (Figure 2a), although the composition and relationships between some lineages differed. In the BL clade, most of the MCSA taxa plus South African *F. scabra* and *F. longipes* and Eurasian *F. calabrica* formed a clade sister to the remaining broad-leaved taxa (Figure 3a). Within the latter clade, successive splits separated the Lojaconoa-Pseudoscariosa, Drymanthele (Phaeochloa)-Scariosae, Tropical-South African (plus *F. olgae*), Subbulbosae-Leucopoa (plus *F. valdesii*) and Schedonorus lineages. In the FL *sensu lato* clade, the "intermediate" American-Neozeylandic (*F. californica* / *F. gracillima*) lineage split first, while the Eskia group split into Subuliflorae + Breviaristatae (*F. altaica*) + Eskia p. p. I (*F. acuminata* / *F. pumila*) and Eskia p.p. II (*F. elegans*, *F. gautieri, F. woronowii*) + American I + American II p.p. (*F. monguensis, F.*

parciflora, F. kurtziana) lineages. The divergence of two sister clades followed, one including the successive splits of the American-Vulpia-Pampas, Psilurus- Vulpia(px), the Exaratae-Loretia grade, and Subulatae-Hawaiian plus Obtusae+ lineages, and the other the Festuca-Wangenheimia, Aulaxyper-Vulpia(2x), and American II + Afroalpine lineages (Figure 3a).

The 35S nuclear rDNA tree showed well-supported branches for major clades and little support for recently evolved intraclade lineages (Figure 3b). This phylogeny also retrieved a topology mostly consistent with the major divergence of the BL and FL Loliinae groups inferred in the nuclear single-copy gene (Figure 2a) and plastome (Figure 3a) ML trees. However, in the 35S topology, the BL lineages were resolved as a basal paraphyletic grade, the fine-leaved Subulatae-Hawaiian lineage sister to the broad-leaved MCSA II clade (plus *F. altaica*), and the broad-leaved Lojaconoa lineage sister to the FL clade (Figure 3b). The 35S tree also recovered an early split for the MCSA I lineage (plus *F. olgae* and *F. scabra*), a close relationship of the Tropical-South African and Drymanthele-Phaeochloa + Scariosae + Lojaconoa + Pseudoscariosa groups, basal paraphyletic divergences from the Eskia and Subuliflorae + Breviaristatae lineages within the FL clade, close relationship for monophyletic Aulaxyper-Vulpia(2x), Psilurus-Vulpia(2x) and Festuca+Wangenheimia plus paraphyletic Exaratae-Loretia lineages, and nesting of American I, American II and American-Neozeylandic taxa in a sister clade to the Afroalpine clade (Figure 3b).


a)
BROAD-LEAVED
Coironhuecu
Tropical-South African
Lojaconoa
Leucopoa
Subbulbosae
Subulatae-Hawaiian
Asperifolia
Glabricarpae
Drymanthele-Phaeochloa
Ruprechtia
Erosiflorae
Drymanthele s. l.
Schedonorus
Drymanthele-Muticae
Pseudoscariosa
Scariosa
FINE-LEAVED
Eskia
Festuca
Aulaxyper
American-Neozeylandic
American II
American I
American-Vulpia-Pampas
Exaratae-Loretia
Psilurus-Vulpia
Afroalpine
Fernandezian
Subuliflorae
Festuca carazana
Festuca dasyantha
Festuca rigidifolia
Festuca weberbaueri
Festuca hieronimy
Festuca yalaensis
Festuca orthophylla
Festuca dolichophylla
Festuca setifolia
Festuca distichovaginata
Festuca andicola
Festuca sodiroana
Festuca asplundii
Festuca humilior
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Festuca compressifolia
Festuca flacca
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Festuca procera
Festuca glumosa
Festuca chodatiana
Festuca abyssinica
Festuca pilgeri
Festuca iberica
Festuca rubra
Festuca raddei
Festuca richardsonii
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Festuca nevadensis
Festuca asperula
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Festuca henriquesii
Festuca francoi
Vulpia muralis
Festuca nigrescens
Festuca laevigata
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Festuca ovina
Festuca viviparoidea
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Festuca breviplla
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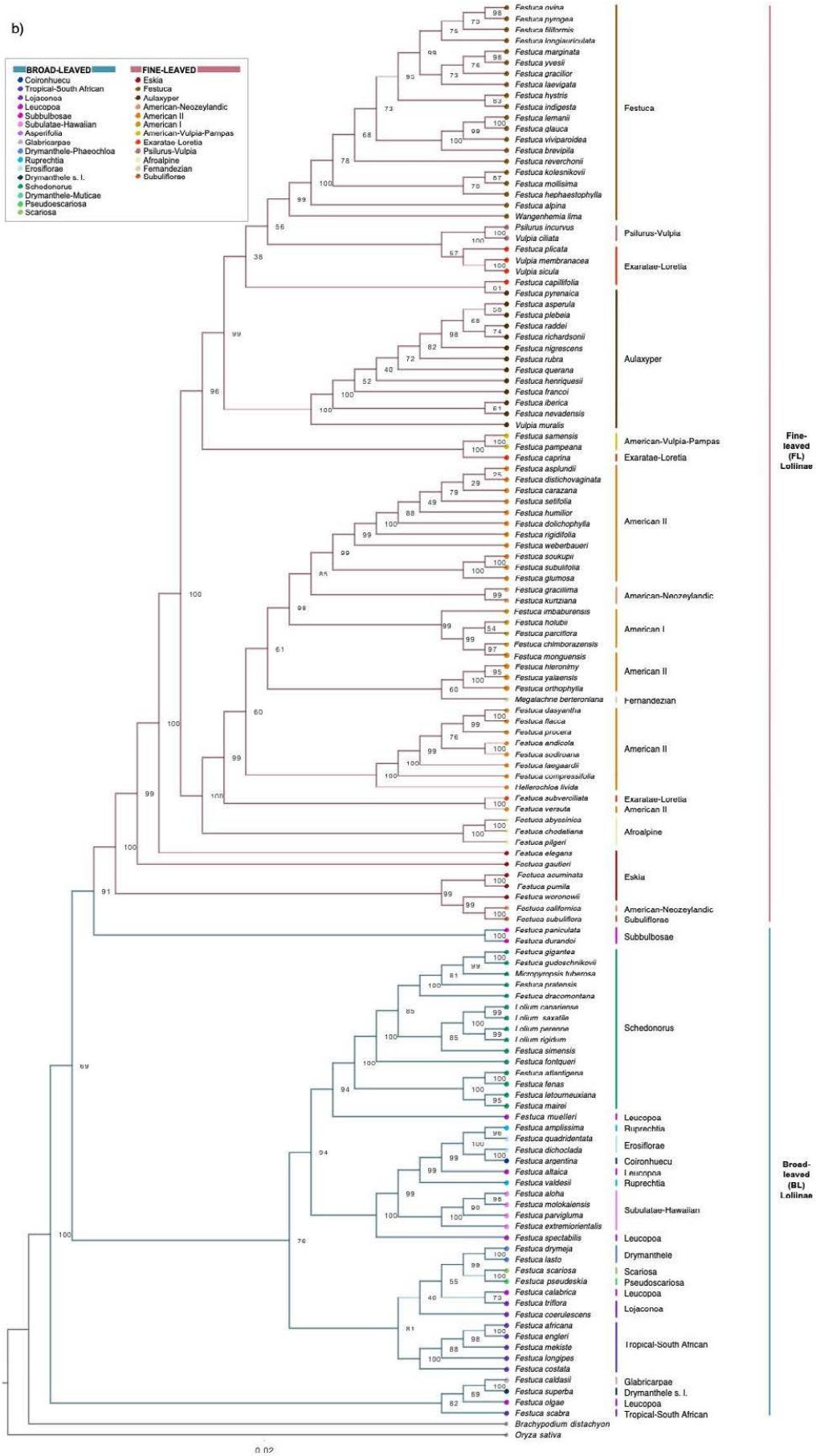
Festuca hystrix
Festuca longiauriculata
Festuca indigesta
Festuca reverchonii
Wangenheimia lima
Festuca subverciliata
Festuca vansuta
Festuca parvigluma
Festuca aloha
Festuca molokaiensis
Festuca extremiorientalis
Hellerochloa livida
Festuca hephaestophylla
Festuca caprina
Festuca pyrenaica
Vulpia membranacea
Vulpia sicula
Festuca plicata
Festuca capillifolia
Psilurus incurvus
Vulpia ciliata
Festuca samensis
Festuca pampeana
Megalachne berteroniana
Festuca imbaburensis
Festuca monguensis
Festuca chimborazensis
Festuca holubii
Festuca parciflora
Festuca kurtziana
Festuca woronowii
Festuca gautieri
Festuca elegans
Festuca acuminata
Festuca pumila
Festuca altaica
Festuca subuliflora
Festuca gracillima
Festuca californica
Lolium rigidum
Lolium perenne
Lolium saxatile
Lolium canariense
Festuca gigantea
Festuca gudoschnikovii
Festuca pratensis
Festuca simensis
Micropyropsis tuberosa
Festuca fontqueri
Festuca dracomontana
Festuca fenas
Festuca mairei
Festuca letourneuxiana
Festuca atlantigena
Festuca paniculata
Festuca durandoi
Festuca apectabilis
Festuca muelleri
Festuca valdesii
Festuca africana
Festuca englerl
Festuca mekiste
Festuca costata
Festuca olgae
Festuca drymeja
Festuca lasto
Festuca scariosa
Festuca pseudeskia
Festuca triflora
Festuca coerulescens
Festuca amplissima
Festuca quadridentata
Festuca dichoclada
Festuca argentina
Festuca superba
Festuca caldasii
Festuca longipes
Festuca scabra
Festuca calabrica
Brachypodium distachyon
Oryza sativa
American II
Afroalpine
Aulaxyper
Festuca
Exaratae-Loretia
American II
Subulatae-Hawaiian
American II
Festuca
Exaratae-Loretia
Psilurus-Vulpia
American-Vulpia-Pampas
Fernandezian
American I
American II
American-Neozeylandic
Eskia
Leucopoa
Subuliflorae
American-Neozeylandic
Schedonorus
Subbulbosae
Leucopoa
Ruprechtia
Tropical-South African
Leucopoa
Drymanthele
Scariosa
Pseudoscariosa
Lojaconoa
Ruprechtia
Erosiflorae
Coironhuecu
Drymanthele s. l.
Glabricarpae
Tropical-South African
Leucopoa
Fine-leaved (FL) Loliinae
Broad-leaved
Fine-leaved
Broad-leaved (BL) Loliinae
3.0


b)
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Coironhuecu
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Subbulbosae
Subulatae-Hawaiian
Asperifolia
Glabricarpae
Drymanthele-Phaeochloa
Ruprechtia
Erosiflorae
Drymanthele s. l.
Schedonorus
Drymanthele-Muticae
Pseudoescariosa
Scariosa
FINE-LEAVED
Eskia
Festuca
Aulaxyper
American-Neozeylandic
American II
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American-Vulpia-Pampas
Exaratae-Loretia
Psilurus-Vulpia
Afroalpine
Fernandezian
Subuliflorae
Festuca ovina
Festuca pyrnaea
Festuca filiformis
Festuca longiaurita
Festuca marginata
Festuca yvesii
Festuca gracilior
Festuca laevigata
Festuca hystris
Festuca indigesta
Festuca lemanii
Festuca glauca
Festuca viviparoidea
Festuca brevipila
Festuca reverchonii
Festuca kolesnikovii
Festuca mollissima
Festuca hephaestophylla
Festuca alpina
Wangenheimia lima
Psilurus incurvus
Vulpia ciliata
Festuca plicata
Vulpia membranacea
Vulpia sicula
Festuca capillifolia
Festuca pyrenaica
Festuca asperula
Festuca plebeia
Festuca raddei
Festuca richardsonii
Festuca nigrescens
Festuca rubra
Festuca querana
Festuca henriquesii
Festuca francoi
Festuca iberica
Festuca nevadensis
Vulpia muralis
Festuca samensis
Festuca pampeana
Festuca caprina
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Festuca distichovaginata
Festuca carazana
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Festuca dolichophylla
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Festuca hieronimy
Festuca yalaensis
Festuca orthophylla
Megalachne berteroniana
Festuca dasyantha
Festuca flacca
Festuca pruvera
Festuca andicola
Festuca sodiroana
Festuca laegaardii
Festuca compressifolia
Hellerochloa livida
Festuca subverciliata
Festuca versuta
Festuca abyssinica
Festuca chodatiana
Festuca pilgeri
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Festuca acuminata
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Festuca extremiorientalis
Festuca spectabilis
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Festuca pseudeskia
Festuca calabrica
Festuca triflora
Festuca coerulescens
Festuca africana
Festuca engleri
Festuca mekiste
Festuca longipes
Festuca costata
Festuca caldasii
Festuca superba
Festuca olgae
Festuca scabra
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Psilurus-Vulpia
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American-Vulpia-Pampas
Exaratae-Loretia
American II
American-Neozeylandic
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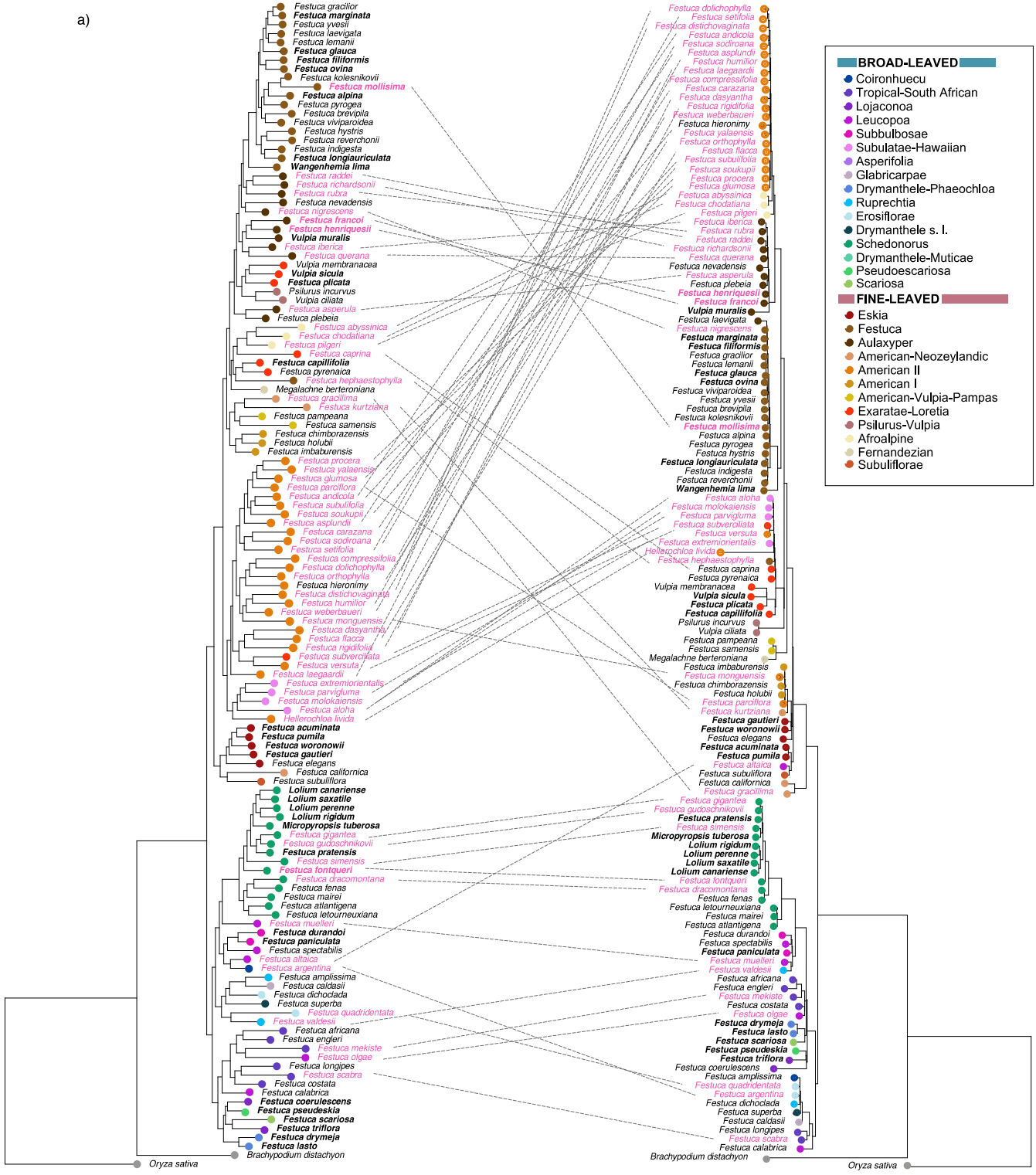
Eskia
American-Neozeylandic
Subuliflorae
Subbulbosae
Schedonorus
Leucopoa
Ruprechtia
Erosiflorae
Coironhuecu
Leucopoa
Ruprechtia
Subulatae-Hawaiian
Leucopoa
Drymanthele
Scariosa
Pseudoscariosa
Leucopoa
Lojaconoa
Tropical-South African
Glabricarpae
Drymanthele s. l.
Leucopoa
Tropical-South African
Fine-leaved (FL) Loliinae
Broad-leaved (BL) Loliinae
0.02

Figure 3. Maximum likelihood phylogenies of Loliinae showing the relationships among the studied species. **(a**) complete plastome tree. (**b**) 35S nuclear rDNA gene tree. Numbers on branches indicate UltraFast Bootstrap supports (BS). *Oryza sativa* was used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bars: number of mutations per site.

Topological congruence/incongruence tests and levels of hybridization

The Kishino-Hasegawa (KH), Shimodaira-Hasegawa (SH), and Shimodaira Approximately Unbiased (AU) pairwise topological congruence tests between the nuclear single-copy gene ML tree, and the 35S nuclear rDNA and plastome ML trees showed that each topology did not differ significantly ($p<0.001$) from any other topology. However, topological incongruence analysis performed in PACo on the nuclear single-copy gene ML trees vs the plastid ML gene trees suggested 61 terminals as potentially conflicting (Figure 4a). The squared residual values of these terminals, computed individually for each nuclear gene tree and with ~50% of their values assigned to quartiles 3 and 4, were overall higher compared with non-conflicting terminals (Figure 4b).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Coironhuecu
Tropical-South African
Lojaconoa
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Subbulbosae
Subulatae-Hawaiian
Asperifolia
Glabricarpae
Drymanthele-Phaeochloa
Ruprechtia
Erosiflorae
Drymanthele s. l.
Schedonorus
Drymanthele-Muticae
Pseudoescariosa
Scariosa
FINE-LEAVED
Eskia
Festuca
Aulaxyper
American-Neozeylandic
American II
American I
American-Vulpia-Pampas
Exaratae-Loretia
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Subuliflorae
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Oryza sativa
Brachypodium distachyon

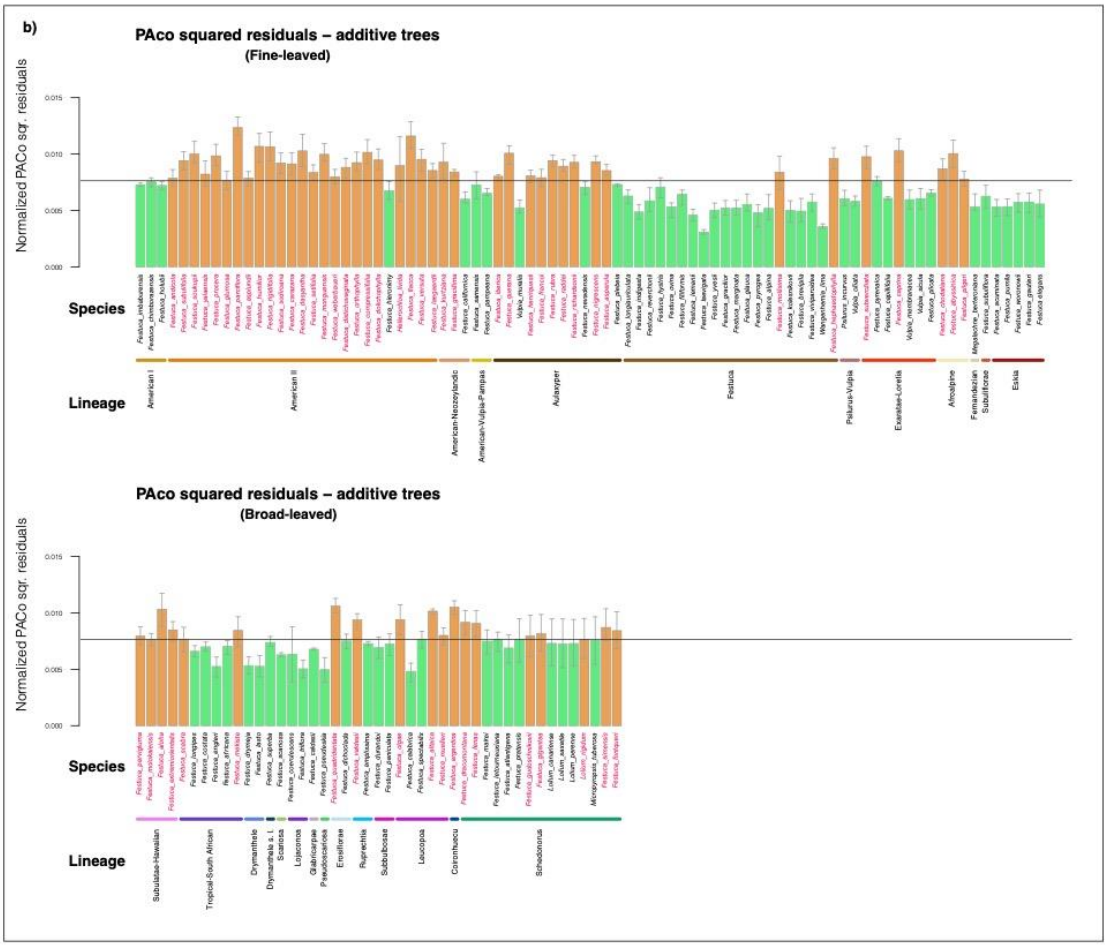

Figure 4. Topological incongruence analysis of single-copy nuclear genes and plastome-based phylogenies of Loliinae using the Procrustean Approach to Cophylogeny (PACo). (**a**) Nuclear phylogeny (left) and plastome phylogeny (right). Terminals that were found to be incongruent between the topologies and placed with robust support in nuclear and plastid trees are highlighted in red and connected with dashed lines. Species diploids are highlighted in bold. (**b**) Boxplot of normalized squared residual values from individual nuclear-plastome associations using phylograms from 1,000 bootstrap replicates. The horizontal black line equals 1/n=0.0077, where *n*= 129 is the number of nuclear-plastome terminal-associations, which equals the number of species under consideration. Median values above this threshold are expected to be linked to species that show incongruence between nuclear and plastome-derived trees, and associated boxes are highlighted in orange.

Estimates of hybridization levels obtained from our HyDE analysis provided gamma scores of 0.3 to 0.7 with high frequency (>400) (Figure 5) indicating the high occurrence of hybridizations in all groups studied.

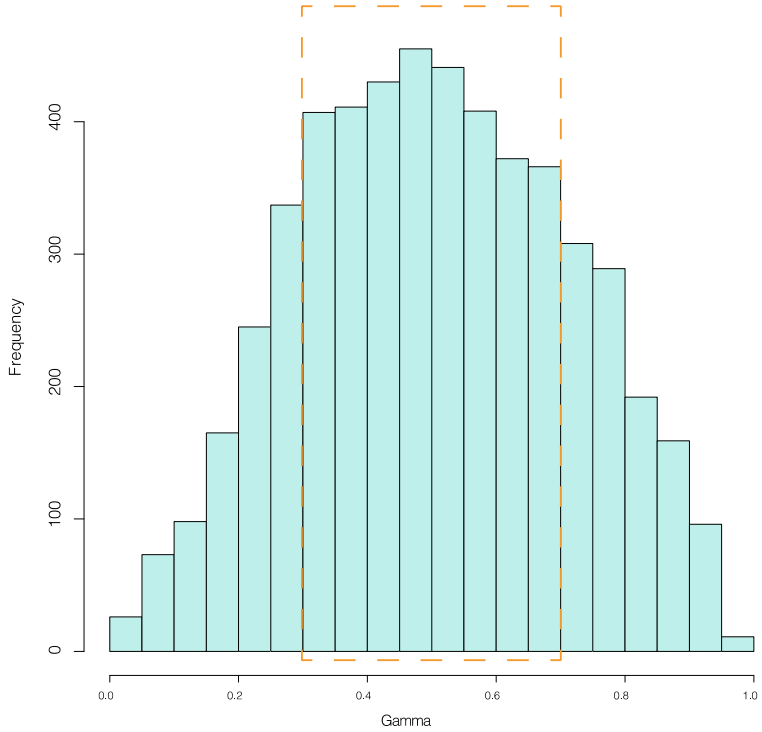

Figure 5. Hybridization levels among 133 Loliinae species estimated by HyDE. Histogram of frequencies of gamma values.

Discussion

Limitations of phylogenetic analyses in Loliinae: advantages and disadvantages of single-copy nuclear genes

Our evolutionary analysis has provided the first phylogeny of Loliinae based on a large number of nuclear-coding genes (Figures 2a, 2b; Supplementary Figure S2). The retrieved phylogeny of the 241 single-copy genes shows high resolution and strong support for the BL and FL Loliinae lineages in the concatenated ML tree (Figure 2a). This topology is in general agreement with topologies inferred from the plastome (Figure 3a) and the 35S nuclear rDNA gene (Figure 3b) for the major lineages of broad and fine-leaved Loliinae, as well as with earlier phylogenies based on a few nuclear and plastid loci (Inda et al., 2008; Díaz-Pérez et al., 2014; Minaya et al., 2015, 2017) and nuclear repetitive elements (Moreno-Aguilar et al., 2022a). Therefore, we can conclude that all compartments of the nuclear (nDNA) and organellar (cpDNA) genomes reconstruct a congruent evolutionary scenario for the divergences of the main lineages of Loliinae, which allows the phylogenetic and statistical test of specific hypotheses about their potential origins.

However, branch support decreases and some relationships differ between recently evolved BL and FL Loliinae sublineages in the single-copy gene MSC ASTRAL and SVDq species trees (Figure 2b; Supplementary Figure S2). The topological incongruences detected between the concatenated ML tree and the ASTRAL and SVDq species trees can be attributed to the severe impact of ILS on the evolutionary history of the younger Loliinae groups (e. g., BL MCSA and FL American II taxa; Figures 2a, 2b; Supplementary Figure S2). In particular, the support of the quartets scores for the main topology of the ASTRAL species tree was generally moderate to low relative to its first and second alternative topologies (Figure 2b; Supplementary Table S4). For most of the ingroup nodes the *q1* topology had higher quartets scores, indicating that the possible gene trees were concordant between them, while for some nodes the three alternative topologies (*q1, q2, q3*) had similar values (~0.33), which reflects that the possible gene trees were present with almost the same frequency for each topology (Figure 2b; Supplementary Table S4). This indicates that many internal branches had lengths close to zero (Züst et al., 2020), giving rise to polytomies that could not be resolved with the 241 single-copy gene sampling used in this analysis. These high levels of intragenomic discordance could have been caused by extensive ILS in the more recently evolved

Loliinae lineages, which likely diverged in Late Pliocene – Quaternary, based on our conservative dating estimates (Minaya et al., 2017; Moreno-Aguilar et al., 2020), and also due to the high frequency of hybridizations and polyploidizations (see discussion below).

Although the Angiosperms353 set of single-copy nuclear genes has proven to be an invaluable tool for reconstructing the phylogeny of supraspecific plant groups (Baker et al., 2021, 2022, and references therein), its resolving power at the species and intraspecific levels is less clear. While some studies have demonstrated the ability of these genes to resolve intricate relationships of recently evolved lineages (Thomas et al., 2021), others have emphasized the problems encountered in reconstructing coalescing phylogenies in lineages prone to short- or long-branch attractions and introgressions (Maurin et al., 2021). Even extensive sampling of thousands of nuclear orthologous genes failed to recover quartets support for the species tree in young, highly hybridogenic angiosperm groups due to large intragenomic discordance (Züst et al., 2020), or ruled out most of gene trees that were topologically incongruent with the diploid species tree (Sancho et al., 2022). Our results are in agreement with these and other works (Philippe et al., 2011; Maurin et al., 2021) stating that adding more single-copy genes may not resolve the phylogenies of recently radiated and hybridogenic groups, although a careful gene selection, refinement of analytical processes, and use of concatenation and coalescent approaches (Smith et al., 2020) can help address the problem. Despite the high levels of intragenomic discordance and the low agreement of individual single-copy gene trees with the species trees, the main topologies of the concatenated ML and the ASTRAL species tree (Figures 2a, 2b) revealed clades adjusted to taxonomic circumscriptions and/or geographic distributions of most of the studied Loliinae species (Catalán, 2006; Catalán et al., 2007; Minaya et al., 2017; Moreno-aguilar et al., 2022b).

Kishino-Hasegawa (KH), Shimodaira-Hasegawa (SH), and Shimodaira Approximately Unbiased (AU) topological congruence tests performed between the concatenated ML tree of single-copy genes and the 35S nuclear rDNA gene tree indicated the lack of incongruence. However, visual topological comparison between the two trees (Figures 2a, 3b) shows some incongruence between these phylogenies and also greater differences of the 35S ML tree than concatenated single-copy gene ML tree with respect to the plastome tree (Figure 3a). The lower resolution and poor support of the 35S ML tree is likely a consequence of the convergent evolutionary trend experienced by these ribosomal DNA genes toward particular ribosomal sequences (ribotypes) in polyploids (Borowska-

Zuchowska et al., 2020), making them particularly prone to phylogenetic bias in groups with high numbers of allopolyploids, such as the Loliinae (Table 1; Catalán, 2006). Although the ITS region of the 35S cistron has been widely used as a nuclear barcode for plants (Hollingsworth, 2011), its value as a phylogenetic marker is questionable in reticulate and polyploid lineages. However, the 35S cistron is a suitable target for ribosomal subgenome dominance studies in polyploid plants (Borowska-Zuchowska et al., 2020).

Rampant introgressions and allopolyploidizations framed the evolutionary history of the Loliinae

Resolving the phylogeny of Loliinae is challenging, as the subtribe experienced a relatively recent radiation that resulted in a large number of hybridizing species and a prevalence of allopolyploidization (Catalán, 2006; Moreno-Aguilar et al., 2022b). Our topological congruence tests between the concatenated nuclear single-copy gene ML tree and the plastome ML tree using the KH, SH and AU approaches ruled out the existence of conflicts, although these highly conservative tests should be taken as inconclusive. In contrast, comparison of the nuclear-encoding ML gene trees and plastome ML trees detected topologically conflicting terminals of presumed hybrid origin from different paternal (nuclear) and maternal (plastome) ancestors (Figures 2a, 3a, 4). The incongruence discordance test performed with PACo confirmed the hybridogenic nature of the 61 mispaired terminals showing strong support in the nuclear and plastid trees (Figure 4). These terminals included lineages or tips with both ancestors evolving within the BL (Subulatae-Hawaiian, Tropical-South African, Mexico-Central-South American, Schedonorus) or FL (American II, Aulaxyper, Afroalpine) clade, and even 'transclade' species originating from distantly related BL and FL ancestors (*F. altaica*) (Figures 4a, 4b). In contrast, the most congruent lineages between the two topologies were those of the BL Drymanthele (Phaeochloa) and Lojaconoa, and FL Eskia, Festuca+Wangenheimia and American-Vulpia-Pampas groups. Rampant introgressions in Loliinae have been further corroborated by the high levels of hybridization detected in the ingroup taxa via HyDE tests (Figure 5). Many of the discordant hybrid terminals correspond to polyploids (Supplementary Table S1), thus reaffirming the pervasiveness of allopolyploidy in the Loliinae, which was also confirmed by the strong asymmetric karyotypes of some of these taxa (Moreno-Aguilar et al., 2022b) and by the finding of recent "transclade" hybrids

(e. g., FL *Festuca rubra* x BL *Lolium perenne*) in the wild, suggestive ongoing disparate introgression (Catalán, 2006, and references therein).

The abundance of allopolyploid species in the Loliinae phylogeny (70% of samples with known ploidy level; Supplementary Table S1) could have aggravated the levels of discordance. However, discordance could also be introduced by the methodological approach used (HyPiper), which selects orthologs according to the length and sequence similarity of the contigs to the reference sequence based on a coverage depth cutoff, and classifies other contigs as putative paralogs (Johnson et al., 2016). These paralogs are generally discarded from evolutionary analysis to avoid phylogenetic bias. However, "paralogs" may correspond to true "homeologs", inherited from different subgenomes in allopolyploids. Tracking the evolutionary history of allopolyploids from unknown or currently extant progenitor species requires identifying and grafting their homeologs onto the subgenomic tree (Sancho et al., 2022), a task accomplished with whole-genome or transcriptome data currently missing for Loliinae. While we have not reconstructed the exact evolutionary history of Loliinae, our single-copy gene species tree and plastome tree have revealed crucial aspects of their phylogenetic relationships and hybridizations (Figures 2a, 2b, 3a, 4, 5) that open the way to future genomic and phylogenomic research of Loliinae.

Author Contributions: P.C., M.F.M.-A. and J.V. designed the study. M.F.M.-A., N.P., J.C.O., G.M.-S., J.A.D., A.S., I.A. and P.C. collected the samples. M.F.M.-A. developed the experimental work. M.F.M.-A., J.V., A.S. and P.C. analyzed the data and interpreted the results. P.C. and M.F.M.-A. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Supplementary Table S1. List of taxa included in the nuclear single-copy gene phylogenetic study of Loliinae. Taxon, code, source, ploidy level, number of Illumina reads obtained from gene target capture and from genome skimming. Plastome and 35S nuclear rDNA cistron Genbank codes are indicated for each sample.

No.	Taxon	Ploidy	Source	Raw Reads Gene capture	Total million Genome skim	Genbank accession No.	SRA accession No.
						35S rDNA Cistron	Plastome
1	*Festuca abyssinica* Hochst. ex A. Rich.	4x	Tanzania: Kilimanjaro; Afroalp O-DP-42737	4,614,899	12,041	MT145276	SAMN14647043
2	*Festuca acuminata* Gaudin	2x	Switzerland:Neuchatel	2,795,919	25,027		
3	*Festuca africana* (Hack.) Clayton	10x	Uganda: Gahinga; Namaganda 190Vg; MHU1603	2,692,853	13,549	MT145277	SAMN14647044
4	*Festuca aloha* Catalán, Soreng & P.M.Peterson	?	USA:Hawaii: Kauai, Waimea, R. Wood 2471	5,390,793	27,133		
5	*Festuca alpina* Suter	2x	Slovenia:Kammiske Alpe: . B. Frajman & P. Schönsvetter.	1,707,739	19,017		
6	*Festuca altaica* Trin.	4x	Russia:Republic of Altai, , № 12264, A. Gnutikov, PC32	2,346,319	16,817		
7	*Festuca amplissima* Rupr.	6x	Mexico: Chihuahua; Barranca del Cobre; PC. 17573	4,160,251	12,058	MT145278	SAMN14647045
8	*Festuca andicola* Kunth	4x	Ecuador:Loja; Saraguro; F90_i	2,033,048	10,178		
9	*Festuca argentina* (Speg.) Parodi	4x	Argentina:Rio Negro: Bariloche, P. Catalan 02.10	2,461,718	22,928	OP120917	SAMN30029287
10	*Festuca arundinacea* subsp. *atlantigena* (St.-Yves) Auquier	8x	Morocco:Mahgrebian, ABY-BN 807	3,444,233	15,091	ON243851	SAMN27777775
11	*Festuca arundinacea var. letourneuxiana* (St.-Yves) Torrecilla & Catalán	10x	Morocco:Atlas Mountains; ABY BN400, UZ 155.07	2,332,917	16,839	MT145292	SAMN14647059
12	*Festuca asperula* Vickery	?	Australia:NWS Australia, CANB 502693	4,684,992	12,030		
13	*Festuca asplundii* E.B. Alexeev	6x	Ecuador: Loja; Saraguro; HUTPL14046	2,290,543	25,088	MT145279	SAMN14647046
14	*Festuca breviglumis* Swallen	?	Mexico:Mexico DF, P. M. Peterson 21366, US s.n.	95,772	12,025		
15	*Festuca brevipila* R. Tracey	6x	Switzerland:Neuchatel	3,202,502	12,712		
16	*Festuca calabrica* Huter, Porta & Rigo	?	Italy:Calabria, Leg Jochen Müller 10836	2,212,093	9,863	MT145280	SAMN14647047
17	*Festuca caldasii* (Kunth) Kunth	4x	Ecuador: Catamayo; Chinchas-Tambara; HUTPL14055	1,465,186	11,919		
18	*Festuca californica* Vasey	8x	USA:California: Napa Co, , MO 4050497.	8,643,128	13,430	MT145281	SAMN14647048
19	*Festuca capillifolia* Dufour ex Roem. & Schult.	2x	Morocco: Middle Atlas; Ifrane National Park; PC 77.17	1,820,969	11,430		
21	*Festuca caprina* Nees	4x	South Africa:Eastern Cape: Stuttenheim, JACA s.n., SA035	3,032,260	29,422		
20	*Festuca carazana* Pilg.	?	Peru:Cerro Huiso, 2618957	3,716,007	10,937	OP120925	SAMN30029295
23	*Festuca chimborazensis* subsp. *Michacoensis* Stančík	6x	Ecuador: Riobamba; Chimborazo; HUTPL14066	1,330,099	10,913	MT145282	SAMN14647049
24	*Festuca chodatiana* (St.-Yves) E.B.Alexeev	?	Uganda:Mt. Elgon National Park, Namaganda 1732a.	3,950,771	10,362		
25	*Festuca coerulescens* Desf.	2x	Morocco:Rif Mountains, PC 34.17	4,145,191	12,481		
26	*Festuca compressifolia* Presl.	?	Peru:Huancavelica, MO-283604	4,409,347	11,308		
27	*Festuca costata* Nees	4x	South Africa:Eastern Cape: Stuttenheim, JACA s.n., SA024	3,843,435	14,693		
28	*Festuca dasyantha* Kunth	?	Ecuador:Imbabura, US-2125643	4,092,718	14,204		
29	*Festuca dichoclada* Pilg.	?	Peru:Cuzco: Quispicanchi, P. M. Peterson 20603. US s.n.	4,078,082	12,466	OP120921	SAMN30029291
30	*Festuca distichovaginata* Pilg.	?	Peru:Puna, ; US- 2180803	3,898,537	16,098		
31	*Festuca dolichophylla* J.Presl	6x	Peru:Lima, Prov. Canta,Gonzáles, P. & E. Navarro 1303c (USM)	5,142,446	11,952		
32	*Festuca dracomontana* H.P. Linder	?	South Africa: TVL; Haernertsburg; PRE 66429	3,601,332	15,835	ON243852	SAMN27777776
33	*Festuca drymeja* Mert. & W.D.J.Koch	2x	Russia:Azerbaidzhancoll. N. Probatova & V. Seledets. PC41	3,426,473	16,225		

34	*Festuca durandoi* Clauson	2x	Portugal: Serra Arga; Alto do Espinheiro	3,781,121	12,688	MT145283	SAMN14647050
35	*Festuca elegans* Boiss.	4x	Spain:CC03; 32.07;	3,071,001	10,765		
36	*Festuca engleri* Pilg.	?	Kenya: Mt. Kenya, Sirimon; Namaganda 1739	3,012,262	16,534		
37	*Festuca extremiorientalis* Owhi	4x	Japan: Tohoku: Fukushima; MO 4628161	5,875,021	15,252		
38	*Festuca fenas* Lag,	4x	Spain: W Mediterranean; PI289654	2,660,548	16,112	MT145285	SAMN14647052
39	*Festuca filiformis* Pourr.	2x	Switzerland: Neuchatel	2,372,826	10,768		
40	*Festuca fimbriata* Nees	6x	Argentina: Misiones; Dpto. Apóstoles; UZ 498.08	1,854,995	-----	-----	-----
41	*Festuca flacca* Hack. ex E.B.Alexeev	4x	Ecuador: Pichincha; US-3428939	1,950,954	14,188		
42	*Festuca fontqueri St.-Yves*	2x	Morocco: Rif Mountains; Talassemtane National Park, PC 59.17	2,686,917	22,187	MT145287	SAMN14647054
43	*Festuca francoi* Fern. Prieto, C. Aguiar, E. Días & M.I. Gut	2x	Portugal: Açores; Terceira; MS4403	1,923,931	17,592	MT145290	SAMN14647057
44	*Festuca gautieri* (Hack.) K.Richt.	2x	Spain: Granada: Huéscar UZ 232.07. FG 30	3,420,343	13,941	OP120922	SAMN30029292
45	*Festuca gigantea* (L.) Vill.	6x	Norway:12/P2007	2,088,065	20,914	ON243853	SAMN27777777
46	*Festuca glauca* Vill.	2x	Spain: Barcelona: Montseny	1,783,800	35,888		
47	*Festuca glumosa* Hack. ex E.B.Alexeev	4x	Ecuador: Cotopaxi; Pujilí; IAS781	2,170,353	15,202		
48	*Festuca gracilior (Hack.) Markgr.-Dann*	2x/4x	Spain: Barcelona: Montserrat	2,593,769	13,184		
49	*Festuca gracillima Hook. f.*	6x	Argentina: Tierra de Fuego; Estancia San Pablo; UZ482.08	3,291,849	13,888	MT145288	SAMN14647055
50	*Festuca gudoschnikovii* Stepanov	4x	Russia:Krasnoyarskii Krai; Yermakovskii Raion; PC 87	2,795,488	13,994	ON243854	SAMN27777778
51	*Festuca henriquezii* Hack.	2x	Portugal: Torre, Sierra de la Estrella COFC 62030	2,560,289	12,574		
52	*Festuca hephaestophila* Nees ex Steud.	4x	Mexico:Nuevo Leon, P. M. Peterson 21460, US s.n.	3,189,816	10,690		
53	*Festuca hieronymi* Hack.	6x	Argentina:Cordoba: Yacanto-Linderos;P. Catalan 403.08(5)	4,080,374	14,242		
54	*Festuca holubii* Stančík	?	Ecuador:Saraguro; route to Cerro de Arcos; HUTPL14071	2,014,431	10,264	MT145289	SAMN14647056
55	*Festuca humilior* Nees	?	Peru:Tarma; US- 2381305	2,883,239	14,100		
56	*Festuca hystrix* Boiss.	2x/4x	Spain:Almeria: Srra. Gador: Nuevo Mundo, UZ 185.07	3,277,551	13,029		
57	*Festuca iberica* (Hack.) K.Richt.	6x	Spain:Granada: Srra. Nevada: Collado Diablo, UZ 218.07	3,816,394	11,016		
58	*Festuca imbaburensis* Stančík	4x	Ecuador:Chimborazo; Riobamba; F68_i	1,780,378	19,836		
59	*Festuca indigesta* Boiss.	6x	Spain:Almería: Sierra de Gádor; COFC 61405	1,892,280	15,233		
60	*Festuca kamtschatica* (St.-Yves) Tzvelev	?	Russia:Kamchatka, Tigil'skii Raioncoll; PC 47	253,560	-----	-----	-----
61	*Festuca kolesnikovii* Tzvelev	?	Russia:Primorskii Krai,. PC 48	3,561,231	18,310		
62	*Festuca kurtziana* St.-Yves	6x	Argentina:Mendoza, Septo Margüe, (SI088616)	4,043,339	8,555		
63	*Festuca laegaardii Stančík*	4x	Ecuador:Azuay, Parque Nacional Cajas; F102_i	2,064,736	15,489		
64	*Festuca laevigata* Gaudin	8x	Spain:Cataluña:Girona; Albanyá	2,811,015	15,693		
65	*Festuca lasto* Boiss.	2x	Spain:Cadiz: Jerez de la Frontera; Los Alcornocales; UZ 29.08	2,353,759	21,581	MT145291	SAMN14647058
66	*Festuca lemanii* Bastard	6x	Spain:Girona: Macąnet de la selva	1,864,653	13,309		
67	*Festuca leptopogon* Stapf	4x	China:Xizang; Linzhi county; N.C. 669; 3876	1,472,141	-----	-----	-----
68	*Festuca longiauriculata* Fuente, Ortúñez & Ferrero Lom.	2x	Spain:Almería: Sierra de los Filabres: Calar Alto, UZ- 59.2000 y UZ192.07	2,792,617	15,218		
69	*Festuca longipes* Stafp	?	South Africa:SA 031; E Cape, Mogsback, Gaika´s Kop	1,637,913	15,404		
70	*Festuca lugens* (E.Fourn.) Hitchc. ex Hern.-Xol.	4x	Honduras:Morazán; Uyuca; MO 3127500	2,558,095	-----	-----	-----
71	*Festuca mairei* St.-Yves	4x	Morocco: Atlas Mountains; PI-610941	4,423,842	19,134	MT145293	SAMN14647060
72	*Festuca marginata* (Hack.) K. Richt.	2x	Spain: Montsec2	1,980,381	14,637		
73	*Festuca mekiste* Clayton	?	Kenya:Mt. Elgon National Park, Kambi Mtamaiwa; Carvalho 4521	2,570,539	16,245	ON243855	SAMN27777779
74	*Festuca modesta* Steud.	2x	India:NW Himalaya, US 1299512.	3,735,505	-----	-----	-----
75	*Festuca mollissima* V.I. Krecz. & Bobrov	2x	Russia:Primorskii Krai, Dal'negorskii Raion, PC 51	4,383,423	13,510		
76	*Festuca molokaiensis* Soreng, P.M. Peterson & Catalán	?	USA:Hawai: Molokai, BISH 728771	2,703,379	12,188	MT145294	SAMN14647061
77	*Festuca monguensis* Stančík	?	Colombia:Boyacá, Satnacik, D. & M. Galvis 2028 (FBM y COL)	6,603,137	13,545		
78	*Festuca muelleri* Vickery	?	Australia:Australia Capital Territory, CBG 67177	3,806,324	14,886		

79	*Festuca nevadensis* (Hack.) K. Richt.	10x	Spain:Almeria: Gador, UZ 189.07	6,011,636	13,223		
80	*Festuca nigrescens* Lam.	6x	Switzerland: Neuchatel	2,205,457	23,337		
81	*Festuca olgae* (Regel) Krivot.	?	Kazakhstan:West Tyan-Shan mts., N Tzvelev, LE	2,771,621	18,527		
82	*Festuca orthophylla* Pilg.	8x	Argentina:Jujuy: La Quiaca; 368:08(1)	3,615,539	13,484		
83	*Festuca ovina* L.	2x	Germany: Thüringen; Müller 10789	3,117,984	11,364	MT145295	SAMN14647062
84	*Festuca pampeana* Speg.	8x	Argentina:Buenos Aires; Sierra de la Ventana; PC 428.08	2,867,938	14,862	MT145296	SAMN14647063
85	*Festuca paniculata* (L.) Schinz & Thell	2x	Spain: Caceres, Puerto de los Castaños, UZ 40.07	5,807,680	35,808	MT145297	SAMN14647064
86	*Festuca parciflora* Swallen	4x	Ecuador:Cotopaxi; Ambato, F10 ii	2,315,801	16,413		
87	*Festuca parvigluma* Steud.	4x	China:China: Baotianman; Henan; Neixiang Xian; MO 4922557	4,354,758	15,872	MT145298	SAMN14647065
88	*Festuca pilgeri* St.-Yves	?	Kenya:	4,257,594	20,003		
89	*Festuca plebeia* R.Br.	?	Tasmania:Proctors Road, HO 329947.	2,646,006	15,479		
90	*Festuca plicata* Hack.	2x	Spain:Granada: Sierra Nevada: acceso a Dornajo, UZ 204.07	2,499,030	14,451		
91	*Festuca pratensis* Huds.	2x	England:UK: England, USDA PI 283306	2,431,565	30,021	MT145301	SAMN14647066
92	*Festuca procera* Kunth	4x	Ecuador:Chimborazo; Riobamba; HUTPL14079	2,280,684	40.669	MT145299	SAMN14647067
93	*Festuca pseudoeskia* Boiss.	2x	Spain:Granada: Srra Nevada: Veleta, UZ 221.07	2,551,426	14,767		
94	*Festuca pumila* Chaix	2x	Austria:Steiermark, Nordöstliche Kalkalpen UZ 17.08	3,045,899	19,264		
95	*Festuca pyrenaica* Reut.	4x	Spain: Huesca; Pyrenees, Tobacor, UZ/PC ...	1,298,678	30.021	MT145300	SAMN14647068
96	*Festuca pyrogea* Speg.	?	Argentina:Argentina: Tierra de fuego, Cabo San Pablo; PC 494.08	2,921,816	16,835	MT145302	SAMN14647069
97	*Festuca quadridentata* Kunth	?	Ecuador:Chimborazo; AUU:55411	3,161,560	15,091	MT145303	SAMN14647070
98	*Festuca queriana* Litard.	4x	Spain:Zamora: Sierra de la Culebra; COFC 61511	1,837,449	18,691		
99	*Festuca raddei* Enustsch. & Prob.	?	Russia: Far East;Isotype № 178308 – VLA.. PC84	1,501,208	14,121		
100	*Festuca reverchonii* Hack.	2n	Spain:Jaén: Sierra de Cazorla, COFC 61363	1,302,842	14,565		
101	*Festuca richardsonii* Hook.	6x	Uganda:Chukotskii Natsional'nyi Okrug, PC 63	2,005,109	14,041		
102	*Festuca rigidifolia* Tovar	?	Peru:Lima, Huarochiri, MO-1344249	2,440,444	14,379		
103	*Festuca rubra* L.	6x	Argentina:Argentina: Tierra de fuego; Cabo Annicolta; UZ 03.09	1,379,087	25,260	ON243856	SAMN27777780
104	*Festuca samensis* Joch.Müll.	?	Bolivia:Santa Cruz, Vallegrande,JRI 106232 (LPB)	3,238,719	18,985		
105	*Festuca scabra* Vahl	4x	South Africa: South Africa: KwaZulu Natal; Cathedral Peak; UZ-SA047	3,132,091	21,174	ON243857	SAMN27777781
106	*Festuca scariosa* Lag. ex. Willk.	2x	Spain: Córdoba: Cabra: Srra. Horconera, UZ 103.07	4,343,876	16,170		
107	*Festuca setifolia* Steud. ex Griseb	?	Peru: Ancash, Carhuaz Prov,MO-1364632	3,192,887	19,074		
108	*Festuca sibirica* Hack. ex Boiss.	4x	Russia:Irkutskaya Oblast', PC68	23,419	-----	-----	-----
109	*Festuca simensis* Hochst. ex A.Rich.	4x	Kenya: Mt. Kenya, Meteorological station; Namaganda 1750	2,097,137	14,159	ON243858	SAMN27777782
110	*Festuca sodiroana* Hack. ex E.B. Alexeev	4x	Ecuador:Azuay; Parque Nacional Cajas, US-3237112	2,220,817	17,431		
112	*Festuca spectabilis* Bertol.	6x	Bosnia-Hercegovina:Bosnia-Hercegovina: Troglav; Sajkovacko zdrlo	2,399,570	18,189		
113	*Festuca subuliflora* Scribn.	4x	USA: Marion county: Canyion Creek park	2,500,554	12,960	MT145304	SAMN14647071
111	*Festuca subulifolia* Benth.	4x	Ecuador:Cotopaxi; AmbatoF14_i	867,054	17,089		
114	*Festuca subulifolia Benth.*	4x	Ecuador:Chimborazo; Riobamba; F60_ii	2,742,422	20,276		
115	*Festuca subverticillata* (Pers.) E.B.Alexeev	4x	USA:Missouri: Carter Co, MO 5029476.	3,251,197	19,399		
116	*Festuca superba* Parodi ex Türpe	8x	Argentina:Jujuy; Yala; Laguna Rodeo; PC 356.08	1,746,583	12,193	MT145305	SAMN14647072
117	*Festuca triflora* J.F. Gmel.	2x	Morocco:Rif Mountains, Bab Barret-Ketama; PC 39.17	3,566,291	24,472	MT145306	SAMN14647073
22	*Festuca valdesii* Gonz.-Led. & S.D.Koch	?	Mexico:Coahuila,. P. M. Peterson 21456, US s.n.	4,088,715	10.937	OP120925	SAMN30029295
118	*Festuca ventanicola* Speg.	6x	Argentina:Buenos Aires: Sierra de la Ventana, 418.08	1,698	-----	-----	-----
119	*Festuca versuta* Beal	?	USA:Texas: Kendall Co: MO 814432	3,378,862	3,938		
120	*Festuca viviparoidea* Krajina ex Pavlick	8x	Canada:Northwest territories; Banks island	3,549,531	18,988		
121	*Festuca weberbaueri* Pilg.	?	Peru:Recuay, Ancash; Patavilea Huaraz, US-3097922	3,962,222	16,083		
122	*Festuca woronowii* Hack.	2x?	Russia:Dagestan [Daghestanskaya ASSR], PC 78	2,901,261	14,067		

123	*Festuca yalaensis* Joch.Müll. & Catalán	?	Argentina:Jujuy: Yala: Laguna Rodeo, P. Catalan 358.08 & J. Müller.	3,283,076	18,550		
124	*Festuca yvesii* Sennen & Pau	8x	Spain:Girona: Coma Morera	2,914,782	19,772		
125	*Hellerochloa livida* (Kunth) Rauschert	?	Mexico:Veracruz: La Perla, MO 3500730	2,573,854	1,563		
126	*Lolium canariense* Steud.	2x	Spain:Canary isles, PI 320544 82i USDA_Pullman	1,837,344	16,359	ON243859	SAMN27777783
127	*Lolium perenne* L.	2x	United Kingdom:UK: Wales; USDA PI 619001	2,387,236	28,103	ON243860	SAMN27777784
128	*Lolium rigidum* Gaudin	2x	Turkey: USDA PI 545604	2,693,803	16,730	ON243862	SAMN27777786
129	*Lolium saxatile* H. Scholz & S. Scholz	2x	Spain: Canary islands; Fuerteventura	2,910,153	16,001	ON243863	SAMN27777787
130	*Lolium temulentum* L.	2x	Turkey:PI 545635	2,273,535	-----	-----	-----
131	*Megalachne berteroniana* Steud.	?	Chile: Juan Fernandez archipelago, Masatierra, 11751 (05)	2,594,653	5,288	MT145307	SAMN14647074
132	*Micropyropsis tuberosa* Romero-Zarco & Cabezudo	2x	Spain:Huelva; Almonte	3,692,416	19,803	ON243864	SAMN27777788
133	*Psilurus incurvus* (Gouan) Schinz & Thell.	4x (x=7)	Spain:Caceres: Malpartida de Plasencia, UZ 31.07	3,620,406	15,885		
135	*Vulpia ciliata* Dumort.	4x	Spain: Toledo; Mar de Ontígola; UZ 109.07	5,087,200	21,775		
136	*Vulpia membranacea* (L.) Dumort.	2x	Spain:Huelva: Almonte: El Porretal, UZ 80.07	2,112,044	11,801	MT145309	SAMN14647076
134	*Vulpia muralis* (Kunth) Nees	2x	Spain:Caceres: Ctra. Navalmoral, UZ 23.07	2,615,655	27,825		
137	*Vulpia sicula* (C. Presl) Link	2x	Italy:Sicilia: Piano Battaglia - Battagliete, Madone	3,592,248	11,327	MT145310	SAMN14647077
138	*Wangenheimia lima* (L.) Trin.	2x	Spain:Zaragoza: Vedado de Peñaflor, UZ 113.07	3,318,538	24,316		

Supplementary Table S2. Statistics of the Loliinae single-copy nuclear genes analyzed with HybPiper. (**a**) Total number of genes captured per species, total (and percentage) gene length, and associated data. (**b**) Filtered gene alignment length, percentage of missing data, number and proportions of variable and parsimony informative positions and associated data. Available at Github/Bioflora/Loliinae Gene_target.

Supplementary Table S3. Information of sequence alignment length, percentage of missing data, number and proportions of variable and parsimony informative positions (PIS) of the Loliinae single-copy nuclear gene supermatrix, the plastome matrix and the 35S nuclear rDNA gene matrix.

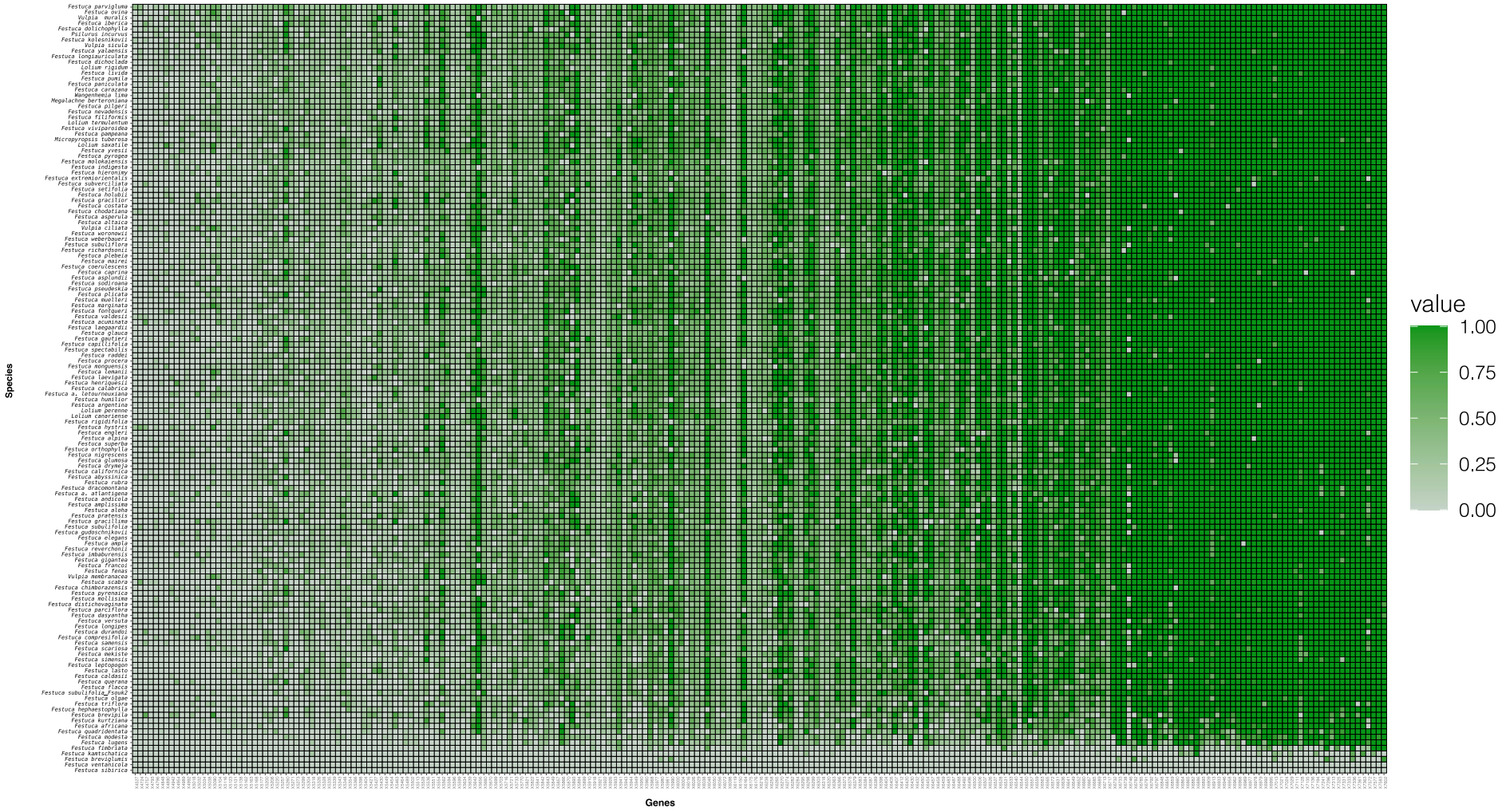
Alignment	No. taxa	MSA length	No. matrix cells	Undeter. characters	Missing percent	No. variable sites	% variable sites	No. PIS	% PIS
Loliinae single-copy genes	136	158876	21607136	5602945	25.931	82844	52.1	45953	28.9
Loliinae plastome	131	135487	17748797	413930	2.332	19594	14.5	8164	6.02
Loliinae 35S rDNA	131	6557	858967	7442	0.866	1093	16.7	611	9.31

Supplementary Table S4. Discordance metrics for the ASTRAL phylogeny of Loliinae nuclear single-copy genes (Figure 2b). Quartet support values (proportion of quartets in the gene tree that agrees with the species tree) for the main topology (*q1*), the first alternative topology (*q2*), and the second alternative topology (*q3*).

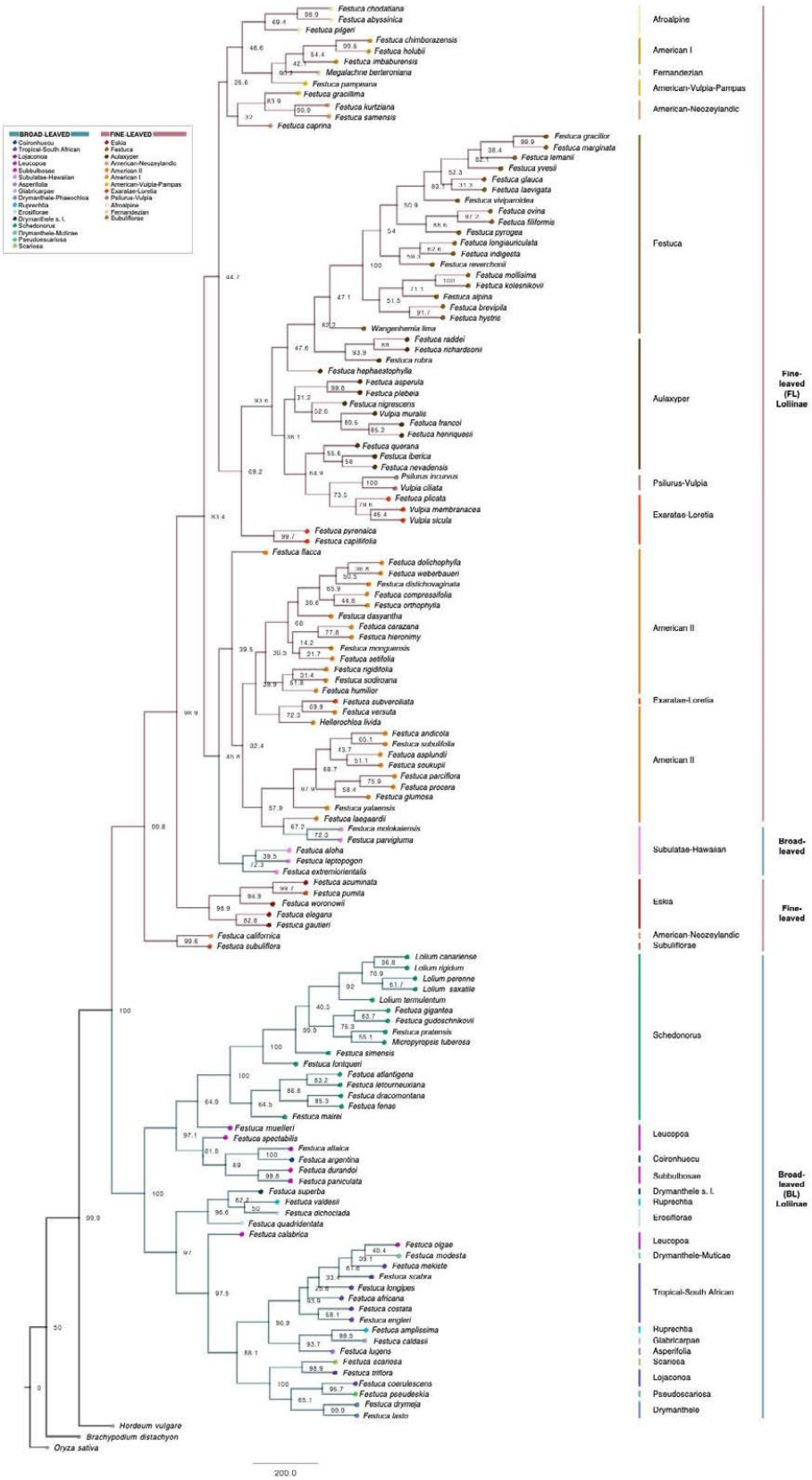
Branch No.	*q1*	*q2*	*q3*
1	NA	NA	NA
2	NA	NA	NA
3	65	13	22
4	95	3	3
5	52	27	22
12	44	32	24
13	35	37	29
14	40	36	24
15	38	32	30
16	37	28	35
17	41	32	27
18	36	31	33
19	37	32	32
20	37	32	32
21	35	31	34
22	37	35	28
23	36	33	31

24	39	29	32
25	35	32	33
26	34	35	30
27	32	32	35
28	34	34	32
29	37	31	32
30	37	34	29
31	35	33	32
32	35	34	31
33	45	25	29
34	35	34	31
35	33	34	34
36	35	32	32
37	35	33	33
38	35	32	33
39	36	32	32
40	39	29	33
41	35	33	33
42	34	32	34
43	38	31	31
44	35	30	35
45	40	24	36
46	39	34	27
47	36	32	32
48	41	28	32
49	42	30	28
50	34	32	35
51	40	31	29
52	44	22	34
53	40	30	30
54	35	35	29
55	40	31	30
56	52	22	26
57	46	26	28
58	38	36	27
59	42	27	31
60	63	18	19
61	39	33	28
62	41	28	31
63	36	29	35
64	37	33	30
65	34	30	36
66	37	31	31
67	38	33	29
68	45	35	20
69	35	32	33
70	48	27	25
71	42	35	23
72	41	34	24
73	35	30	35
74	39	30	32
75	46	34	21
76	58	24	19
77	40	26	34
78	40	33	27
79	35	33	32
80	40	28	32
81	35	35	30
82	63	20	17
83	37	34	30

84	45	21	33
85	36	37	27
86	47	26	27
87	36	30	34
88	43	31	26
89	34	32	34
90	35	31	34
91	35	33	32
92	53	25	23
93	39	35	25
94	38	27	34
95	41	34	26
96	36	31	33
97	36	33	32
98	39	32	29
99	53	27	19
100	47	20	32
101	41	34	25
102	44	28	28
103	36	33	31
104	38	29	34
105	70	18	11
106	40	31	29
107	37	27	35
108	40	35	26
109	37	34	30
110	35	32	33
111	48	18	34
112	36	34	30
113	40	35	25
114	37	30	33
115	41	30	29
116	40	31	29
117	48	23	29
118	33	32	34
119	61	18	22
120	37	29	34
121	72	13	15
122	38	33	30
123	38	33	29
124	41	28	32
125	36	30	33
126	47	32	20
127	46	35	18
128	45	23	32
129	50	28	23
130	40	23	37
131	37	30	33
132	37	33	30
133	44	32	24
134	48	29	23
135	37	27	36
136	32	27	142
137	33	30	143
138	34	20	144
139	29	31	145
140	33	26	146
141	29	21	147



Supplementary Figure S1. Density statistics plot of single-copy nuclear genes captured in the 138 Loliinae samples stu



Supplementary Figure S2. Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* was used to root the trees. Color codes of Loliinae lineages are indicated in the chart.

C.5. Climatic niche conservatism of American I and American II fescue grasses from the North-Andean páramos (*Festuca*, Poaceae)

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Climatic niche conservatism of American I and American II fescue grasses from the North-Andean páramos (*Festuca*, Poaceae)

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Abstract

Evolutionary speciation that retains the fundamental niche and ecological speciation that changes the ancestral niche have been considered key mechanisms of species diversification. We analyzed recently evolved northern Andean *Festuca* grass species from the fine-leaved American I and American II lineages to test the alternative hypothesis of niche conservatism versus divergence using environmental niche modeling (ENM) approaches. Current and LGM climate envelopes were constructed for American I *F. chimborazensis* and *F. vaginalis* and American II *F. asplundii* and *F. subulifolia* distributed sympatrically, respectively, in the superpáramo and parámo-puna belts. Niche identity tests indicated that the respective ENMs of the American I and American II species-pairs were not equivalent. Niche similarity tests revealed niche conservatism for American I and American II taxa, respectively, and those niche comparisons showed large niche overlap. Evolutionary divergence rather than selective adaptation to heterogeneous

niches was hypothesized for *F. chimborazensis* and *F. vaginalis* and for *F. asplundii* and *F. subulifolia*. *F. asplundii*, a species adapted to flooded páramo grasslands, and *F. subulifolia*, a dominant species in wet to dry páramo grassland communities, showed the greatest niche breadth. Only one climatic variable (Annual temperature range) of the five used to build the ENMs carried a phylogenetic signal. LGM and current projections indicated that the areas suitable for the presence of these species were wider in the past; the stronger range contractions experienced by *F. subulifolia* and *F. asplundii* could be related to the aridification of the lower puna belt during the Holocene. Our results illustrate how these North Andean fescues responded to environmental changes in the last 20 kya across different niche dimensions.

Keywords: climate niche modeling, niche conservatism, North-Andes, páramo-puna *Festuca* grasses, phylogenetic signal, range shifts.

Introduction

Evolutionary hypotheses about speciation imply processes of genetic isolation and drift as a consequence of disruption of gene flow, with the subsequent divergence of populations and lineages and the emergence of new taxa (Sobel et al., 2010). The high rates of divergence of some organisms have caused considerable radiation and diversification of closely related species in relatively short time spans (Linder, 2008). Among the hypothesized factors that drove such diversifications are mutation and migration, dispersal, selection for local adaptation and population sizes (Gavrilets, 2003), and the rapid adaptation of new lineages to different habitats, a process also called ecological speciation (Levin, 2003; Pyron et al., 2015, 2023). Phylogenetic niche conservatism theories predict that closely related species would share a similar fundamental niche that would be retained over time (Peterson et al., 1999; Wiens, 2004; Wiens et al., 2010; Peterson, 2011; Pyron et al., 2015). In contrast, niche divergence theories support that adaptations to new environments through ancestral niche shifts also promote species diversification (Levin, 2003; Givnish, 2010; Liu et al., 2020). Rapid changes in genomic and adaptive traits of closely related taxa in different environments prevent competition for the same niche and displacement (McIntyre, 2012; Lopez-Alvarez et al., 2015). Although niche conservatism has been considered prevalent at long-term evolutionary scales for stabilized species (Wiens, 2004; Wiens and Donoghue, 2004;

Pyron et al., 2015), niche divergence is predicted for diversified species with accelerated evolution (Hu et al., 2015), as well as for hybrids and polyploids that may be outcompeted by their parents or diploids relatives in the native niche (Treier et al., 2009; López-Alvarez et al., 2015). Ecological speciation could therefore be an important mechanism of evolutionary speciation, which may act in parallel with other ongoing processes, such as dispersals, genetic isolation and drift (Donoghue and Edwards, 2014).

Recently evolved groups are ideal models to test hypotheses about conservatism and niche divergence (Salariato et al., 2022) and to assess the impact of environmental variables on the shaping of the species' ecological niches (Hu et al., 2015). They could also serve to test relationships between phylogenies and niche attributes and the phylogenetic signal of niche traits (Grossman, 2021), which could be used to unravel the extent of ecological adaptation in the process of speciation and divergence. We have investigated the environmental niche variation, and niche conservatism versus divergence, in recently diverged species of temperate *Festuca* grasses endemic to the North-Andean páramos. *Festuca* is the largest genus of subtribe Loliinae and contains nearly 600 worldwide distributed species, growing at high altitudes in tropical and subtropical regions (Catalán, 2006; Minaya et al., 2017; Moreno-Aguilar et al., 2022a, 2022b). Some 53 *Festuca* species are endemic to the North-Andean region, a secondary center of diversification for the genus (Stančik and Peterson, 2007). Most of these species are phylogenetically nested within the fine-leaved Loliinae clade, a large group consisting predominantly of the northern hemisphere *Festuca* subgen. *Festuca* taxa plus other lineages from the southern hemisphere, such as the American I and American II clades (Minaya et al., 2017; Moreno-Aguilar et al., 2020, 2022a). These latter groups have been reconstructed as related lineages on single-copy nuclear and plastome-based trees, showing evidence of hybrid origins for their polyploid species (Moreno-Aguilar et al., unpubl. data). Dating analysis indicated that the MRCA of the fine-leaved (FL) Loliinae began to diversify in the early Miocene (17.5 Mya) (Minaya et al., 2017); however, the estimated ages for the ancestors of the North Andean FL lineages were relatively young, having diverged within the early Quaternary (Moreno-Aguilar et al., 2020). Biogeographic studies inferred that the Loliinae originated in the northern hemisphere and evolved through recurrent long-distance dispersals (Inda et al., 2008; Minaya et al., 2017). The ancestors of the North Andean American I and II lineages were hypothesized to have derived from Mediterranean colonizers, which later dispersed to southern South

America, North America and Tropical Africa in the Late-Miocene – Pliocene (Minaya et al., 2017; Moreno-Aguilar et al., 2020).

Fescues in the northern Andean region (Ecuador, Colombia, northern Peru, Venezuela) grow in páramo-type (and puna-type) grassland communities that dominate the upper vegetation belts of the Cordillera (Sklenář and Ramsay, 2001; Stančik and Peterson, 2007). These territories are home to a great biological diversity, associated with their biogeographic history (Antonelli et al., 2009; Antonelli and Sanmartín, 2011) and high environmental heterogeneity throughout a wide altitude range (2800 – 5000 masl) (Antonelli et al., 2018; Salariato et al., 2022). The predominant plant communities in the páramo and superpáramo belts are pajonales, extensive grassland communities dominated by temperate grasses adapted to the harsh climate of these high Andean regions (Sklenar and Ramsay, 2001). Among the dominant species of pajonales, *Festuca* includes some of the most representative species (Sklenař and Jørgensen, 1999; Sklenař and Ramsay, 2001; Stancik and Peterson, 2007). We selected species from the two main lineages American I (*F. chimborazensis*, *F. vaginalis*) and American II (*F. asplundii*, *F. subuliflora*) of fine-leaved *Festuca* (Moreno-Aguilar et al., 2020, 2022a). All four taxa show a native geographic distribution in the Northern Andes, growing sympatrically and allopatrically in different páramo (American II taxa) and superpáramo (American I taxa) belts (Figure 1) (Stančik and Peterson, 2007). These species thrive in different environments and elevations, representing a wide range of biotic and abiotic conditions that may be associated with their natural adaptive genetic variation.

We used environmental niche modeling (ENM) of the four taxa to (i) build climatic envelopes of the species in their native range and identify contributing variables using ENMs based on current geographic data and present and past climate data, (ii) examine whether niche divergence accelerates evolution between the American I and American II groups and tests whether within-group speciation was associated with niche divergence or niche conservatism, (iii) compare niche overlap and niche breadth between the species to test for potential outperforming of American I or American II taxa; and (iv) testing for potential phylogenetic signal of ENM traits and correlation of niche overlap and phylogenetic distance.

Materials and methods

Distribution data

The selected sample set provided a large amount of occurrence data for American I (*F. chimborazensis*, *F. vaginalis*) and American II (*F. asplundii*, *F. subuliflora*) species (Figure 1). American I taxa are narrow endemics to Ecuador, distributed in the superpáramo belt (Figure 1a). American II taxa have broader geographic distribution ranges throughout the páramo and lower zones of the superpáramo belts of Colombia, Ecuador and Peru (Figure 1b), showing sympatric distributions with American I group species in some places. Occurrence data of the species under study were obtained from our own collections, herbarium vouchers (AAU, HUTPL, LOJA, MO, QCA, US), and verified records. The georeferenced data of each species was filtered and refined to remove duplicated data, uncertain geographic references, or data with null values for the climatic variables used, employing the options of the R package *dplyr* (Wickham et al., 2022). To improve model comparability and to avoid biasing models toward data from particular regions for more widespread or more endemic species, presence data was thinned to a single record within each grid cell at 0.92 x 0.92 km spatial resolution. We obtained a total of 217 georeferenced occurrences across the four taxa (26 for *F. chimborazensis*, 36 for *F. vaginalis*, 54 for *F. asplundii*, 101 for *F. subulifolia*) (Supplementary Table S1; Figure 1).

Environmental variables

Environmental variables for use in niche modeling must be taxon-specific (Syphard and Franklin, 2009). We selected variables associated with temperature and precipitation, which are known to constrain plant distribution across spatial scales (Grossman, 2021) and change slowly over time (Hu et al., 2015), thus having more predictive power at large temporal and spatial scales than other ecological variables, and being important for the ecology of *Festuca*. We used *getData raster* command (Etten et al., 2022) to automatically download data for 19 bioclimatic variables from the Worldclim database (https://www.worldclim.org/) for the current climate at 1 km resolution (30 arc seconds) and for the Last Glacial Maximum (LGM; ~20 kya) climate, according to the Community

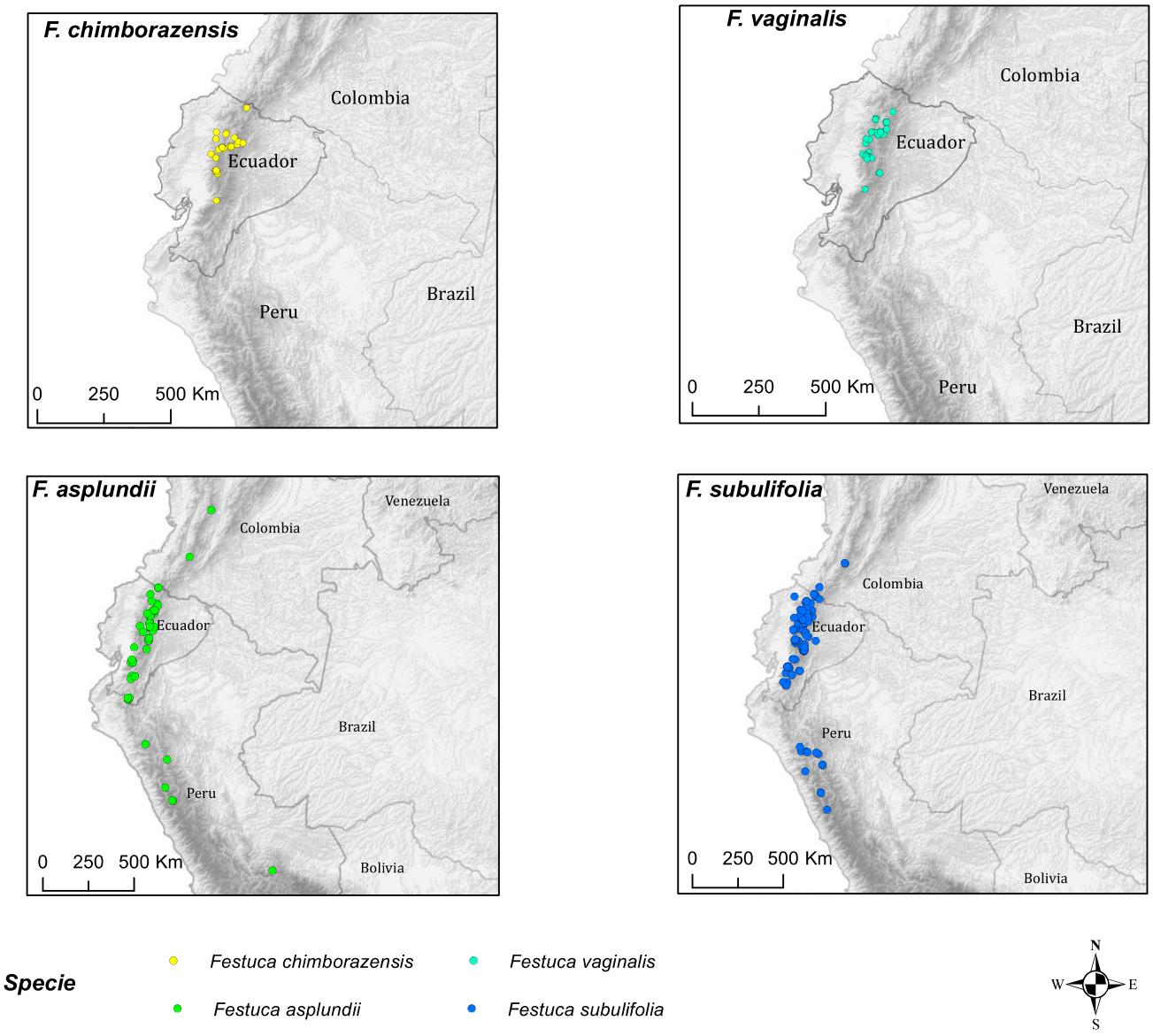


Figure 1. Distribution ranges and georeferenced presence data of the American I *Festuca chimborazensis* (yellow) and *F. vaginalis* (light blue) and American II *F. asplundii* (green) and *F. subulifolia* (dark blue) species under study.

Climate System Model version 4, CCSM4 (Gent et al., 2011), at 4.5 km resolution (2.5 seconds of arc). The bioclimatic variables of each taxon studied were refined and extracted for the northern Andean region using software packages (ArcGis, R). The variables were evaluated using the *VIF* function within the *car* package in R, discarding those with a correlation value ≥5 and selecting only the most suitable variables for the construction of reliable niche models. Climate data included five Bioclim variables (Temperature seasonality, Maximum temperature of warmest month, Temperature annual range, Annual precipitation, Precipitation seasonality) for climate modeling of all studied taxa (Supplementary Table S1). We restricted climate modeling to the same variables to build interpretable niche models that could be compared between taxa from different North-Andean regions. Occurrence data for taxa under current conditions were projected to the past LGM climatic envelopes to estimate potential niche gains or losses for each species by comparing range distributions and the effect of climate change on niche contractions or expansions. For this, we assumed that the biotic and abiotic conditions of each taxon in the LGM were the same as today (López-Alvarez et al., 2015; Leipold et al., 2017).

Niche modeling

Climate niche modeling was performed using the MaxEnt maximum entropy model (Phillips et al., 2004) with the *Maxnet* package in R (Phillips, 2021). Maxent is a presence-background technique that estimates suitability through a similarity index that resembles a heterogeneous point process or a logistic regression function (Hu et al., 2015). Maxent performs better than alternative modeling methods based solely on presence data (Elith et al., 2006). We used the *dismo* package (Hijmans et al., 2022) and the thinned presence data and associated climate data to build the Maxent niche models. We employed the *rgeos* package (Bivand and Rundel, 2021) to create a mask with an extension of 50 km around each of the occurrences and avoid spatially projected modeling in areas where the occurrence of the modeled species is unlikely. The mask was used to generate the background points, sampling at least 10% of the cells that were inside the mask. Background points with an assigned value of 0 were also associated with climate data, and both presence and background points were used to construct a prediction envelope showing the likelihood of the taxon occurring throughout the range (Grossman, 2021). To reduce sampling bias, we overlapped 30 independent bootstrap replicated

Maxnet models for each taxon. For each modelling, 75% of the records were used for model training and 25% for testing model performance. The discrimination performance of the model was assessed by measuring the area under the receiver operating curve (AUC), a threshold-independent statistical method that discriminates predictions of presences over absences better than expected by chance with values ranging from poor (0 – <0.7) to moderate (0.7 – 0.9) and good predictions (>0.9) (Elith et al., 2006; Peterson et al., 2008). Suitable species distributions of each taxon in each time window were mapped using ArcGIS. Species distribution models for each taxon at each time interval were exported in TIFF format and mapped with ArcGIS. The lowest 10% percentile threshold of the training presence values for each species was discarded for each of the models. The LGM and current time models were overlaid on the North Andes shapefile and exported.

To investigate differences in current climatic envelopes among taxa, we performed nonparametric Kruskal-Wallis tests and Dunn's pairwise tests for all species pairs for each of 19 bioclimatic variables using *multcompView* package in R (Spencer Graves, 2019). To discriminate the species in the environmental space and identify the variables that most contributed to their differentiation, we performed a Principal Component Analyses (PCA) of data extracted from the 19 bioclim variables using *ade4* (Jombart et al., 2022) and *ecospat* packages (Di Cola et al., 2017) in R. Furthermore, we evaluated niche divergences using the attributes of the ENMs.

Testing niche divergence and conservatism and estimations of niche breath

We evaluated the similarity or divergence in ecological niches between the studied species through sets of pairwise niche comparisons using *ecospat*. To remove the confounding effects of spatial autocorrelation in bioclimatic variables, we employed environmental niche model (ENM)-based equivalence and background similarity tests to quantify, respectively, niche identity and niche divergence-*vs*-conservatism (Warren et al., 2008; McCormack et al., 2010) among the *Festuca* taxa from the northern Andes. Pairwise niche overlap was assessed using Schoener's *D* metric (Schoener, 1968), which measures the proportional similarity of two distributions as an indicator of niche overlap. The niche equivalency test (or identity test) uses a bootstrap approach to assess whether the niches of two taxa are statistically equal. In this test, occurrence points of both taxa

are pooled together and resampled 100 times; for each resampling, new Maxent models are created and if the empirical D value falls outside of this distribution of simulated null D values, the niches are considered non-equivalent (McIntyre, 2012; Grossman, 2021). The niche similarity test (or background test) explores whether two niches are more or less similar than expected by chance. This test incorporates the environmental heterogeneity of the geographic ranges where the species occur and tests whether the niche overlap is more or less similar than expected based solely on regional environmental (background) differences (null model) or on differences in niche suitability. A null distribution of overlapping values is generated by comparing the niche model of species A with a niche model created from a set of random points drawn from the background of species B (with the same number of occurrences of A), and vice-versa. This process is repeated 1000 times for each reciprocal comparison, generating a null distribution of 2000 values for Schoener's *D* for each pair of species (Warren et al., 2008; McCormack et al., 2010). We performed the niche equivalency and niche similarity tests using the *ecospat.niche.equivalency.test* and *ecospat.niche.similarity.test* functions of the *ecospat* package. According to (McCormack et al., 2010), the niche similarity test could be used to test for niche divergence (D), when overlap values are smaller than the null distribution, or niche conservatism (C) when they are bigger. The niche similarity test is considered more relevant to the speciation process than the niche identity test (Smith and Donoghue, 2010).

We estimated the niche breadth of each species in each time window following (Warren et al., 2010) using the function *nicheBreadth* in *ENMtools* package (Cardillo and L. Warren, 2016). The breadth of a species' niche is directly related to the extent of its geographic distribution. We estimated averaged niche breadth values for *Festuca* taxa from the northern Andes under the current and LGM-CCSM4 climates. For this, we computed Levin's concentration metrics using suitability scores generated from each Maxent model, with values ranging from 0 (minimum niche breadth when only one grid cell in the geographic space has a suitability other than zero) and 1 (maximum niche breadth when all grid cells have non-zero suitability) (Cardillo and L. Warren, 2016).

Phylogenetic analyses of environmental niches

We assessed whether particular components of ENMs (mean values of climatic niche variables) and niche breath showed evidence of phylogenetic signal and whether climatic niche overlap was associated with phylogenetic distance. For this, we used a Loliinae phylogeny based on nuclear and plastid sequences (Moreno-Aguilar et al., 2020) and trimmed for the *Festuca* taxa studied, which was topologically congruent with that of the Loliinae species tree constructed from multiple nuclear single-copy-genes (Moreno-Aguilar et al., unpublished data). We tested the potential phylogenetic signal of climate niche traits using Blomberg's K (Blomberg et al., 2003) and Pagel's lambda (Pagel, 1999) with the *phylosig* function of the package *phytools* (Revell, 2012). For both tests, values >1 indicate that niche traits have more phylogenetic signal than expected, values = 1 that traits are consistent with the tree topology (phylogenetic signal), and values = 0 that there is no influence of shared ancestry on trait values (phylogenetic independence). Phyloheatmaps for the standardized values of these continuous characters were generated with *phytools*. We assessed whether niche overlap (empirical D) for each species pair from each lineage (American I, American II) was associated with phylogenetic distance (Elliott and Davies, 2017) using a Mantel test with the *mantel* function of the *vegan* package (Oksanen et al., 2019).

Results

Niche variation, influence of environmental variables and niche overlap

We found considerable variation in the environmental preferences of the American I and American II *Festuca* taxa (Table 1). Sixteen of the 19 climatic variables analyzed (all except Mean diurnal range, Minimum temperature of coldest month, and Precipitation of warmest quartet) detected significant differences among the four species, two between *F. chimborazensis* and *F. subulifolia* (Max. temperature of warmest month, Temperature annual range), two between *F. asplundii* and *F. subulifolia* (Isothermatily, Temperature seasonality), three between *F. chimborazensis* and *F. asplundii* (Isothermatily, Temperature seasonality, Precipitation of coldest quartet), six between *F. vaginalis* and *F. asplundii* (Max. temperature of warmest month, Annual precipitation, Precipitation of wettest month, Precipitation of driest

month, Precipitation of wettest quartet, Precipitation of wettest quartet), and eight between *F. vaginalis* and *F. subulifolia* (Min. temperature of coldest month, Mean temperature of wettest quarter, Mean temperature of driest quarter, Mean temperature of warmest quarter, Mean temperature of coldest quarter, Annual precipitation, Precipitation of wettest month, Precipitation of wettest quarter) (Table 1; Supplementary Table S2), indicating that each species has unique ecological preferences. *F. vaginalis* was associated with the lowest value for minimum temperature of coldest month (-1.1C) and lowest value for annual precipitation (837.50 mm), *F. chimborazensis* with the lowest value for temperature seasonality (38.29C) and highest value for precipitation of wettest quarter (403.29 mm), *F. asplundii* with the highest value for temperature seasonality (52.45C), *F. subulifolia* with the highest values for maximum temperature of warmest month (14.0C) and highest mean temperatures for all quartets (Table 1), and *F. subulifolia* and *F. asplundii* with the highest values for annual precipitation (1105.29C, 1103.92C) (Table 1).

The environmental space defined by the two first PC axes provided some additional support for different environments occupied by the studied fescues (Figure 2). Most of the American I samples clustered together in the central part of the bidimensional plot defined by the PC1 (48.9% of variance) and PC2 (22.7%) axes, while the American II samples clustered, respectively, at the positive and negative ends of PC2, and also overlapped with the American I samples (Figure 2). The variables that most contributed to the main PC axes were bio8, bio9, bio10, bio11 (PC1), and bio2, bio7, bio14, bio15, bio17 (PC2) (Table 1; Supplementary Table S3).

Table 1: Mean values of the 19 bioclimatic variables analyzed for climate niche optima of the American I *Festuca chimborazensis* (FC) and *F. vaginalis* (FV) and American II *F. asplundii* (FA) and *F. subulifolia* (FS) species in their native North Andean region. These variables were used for comparative interspecific statistical analysis (Kruskal-Wallis nonparametric test among the four species; Dunn's pairwise tests: significant differences for variables are indicated with color dots: FA vs FS (violet), FC vs FA (red), FC vs FS (pink), FV vs FA (green), FV vs FS (orange), see Supplementary Table S2; significance: p< 0.05*, p<0.01**, p<0.001***). Variables selected for environmental niche modeling are highlighted in bold.

Bioclimatic variable	Code	*F. chimborazensis*	*F. vaginalis*	*F. asplundii*	*F. subulifolia*	Kruskal-Wallis	p-value
Annual mean temperature	Bio1	7.31	5.07●	7.20	8.04●	14.1886**	0.0027
Mean diurnal range	Bio2	10.26	10.52	10.12	10.45	6.879	0.0759
Isothermality	Bio3	87.88●	87.45	84.77●●	86.53●	13.4643**	0.0037
Temperature seasonality	**Bio4**	**38.29●**	**40.48**	**52.45●●**	**43.54●**	**13.1177****	**0.0044**
Maximum temperature of warmest month	**Bio5**	**13.01●**	**10.94●**	**13.10●**	**14.00●**	**16.7732*****	**0.0008**
Min temperature of coldest month	Bio6	1.33	-1.10●	1.13	1.90●	7.3515	0.0615
Temperature annual range	**Bio7**	**11.68●**	**12.04**	**11.98**	**12.09●**	**10.0816***	**0.0179**
Mean temperature of wettest quarter	Bio8	7.57	5.33●	7.31	8.24●	14.5196**	0.0023
Mean temperature of driest quarter	Bio9	6.88	4.55●	6.92	7.69●	14.4140**	0.0024
Mean temperature of warmest quarter	Bio10	7.66	5.40●	7.64	8.43●	15.0830**	0.0017
Mean temperature of coldest quarter	Bio11	6.78	4.49●	6.45	7.43●	13.2837**	0.0041
Annual precipitation	**Bio12**	**1050.21**	**837.50●●**	**1103.92●**	**1105.29●**	**8.9401***	**0.0301**
Precipitation of wettest month	Bio13	141.89	104.83●●	144.61●	140.66●	13.4375**	0.0038
Precipitation of driest month	Bio14	35.68	33.29●	50.42●	46.41	10.6668*	0.0137
Precipitation seasonality	**Bio15**	**40.69**	**37.98**	**32.67**	**34.24**	**8.3650***	**0.039**
Precipitation of wettest quarter	Bio16	403.29	295.12●●	383.10●	382.76●	11.0022**	0.0117
Precipitation of driest quarter	Bio17	131.82	122.12●	182.69●	167.20	11.7406**	0.0083
Precipitation of warmest quarter	Bio18	350.43	234.79	297.85	316.54	3.6254	0.3049
Precipitation of coldest quarter	Bio19	162.25●	137.829	242.05●	205.06	14.5048**	0.0023

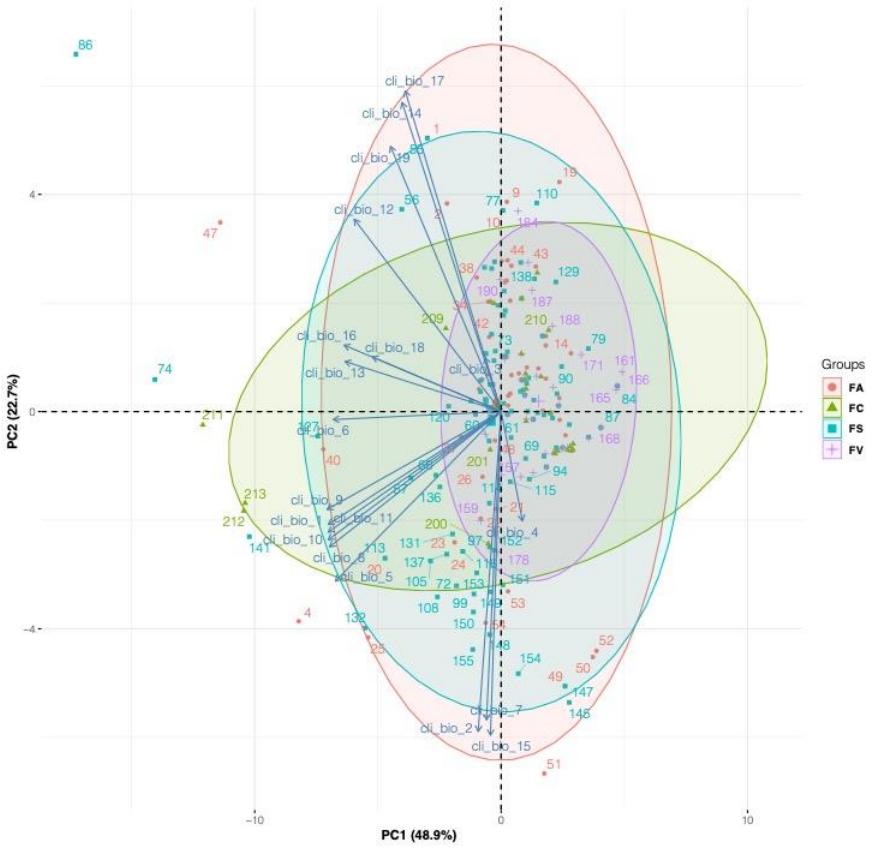
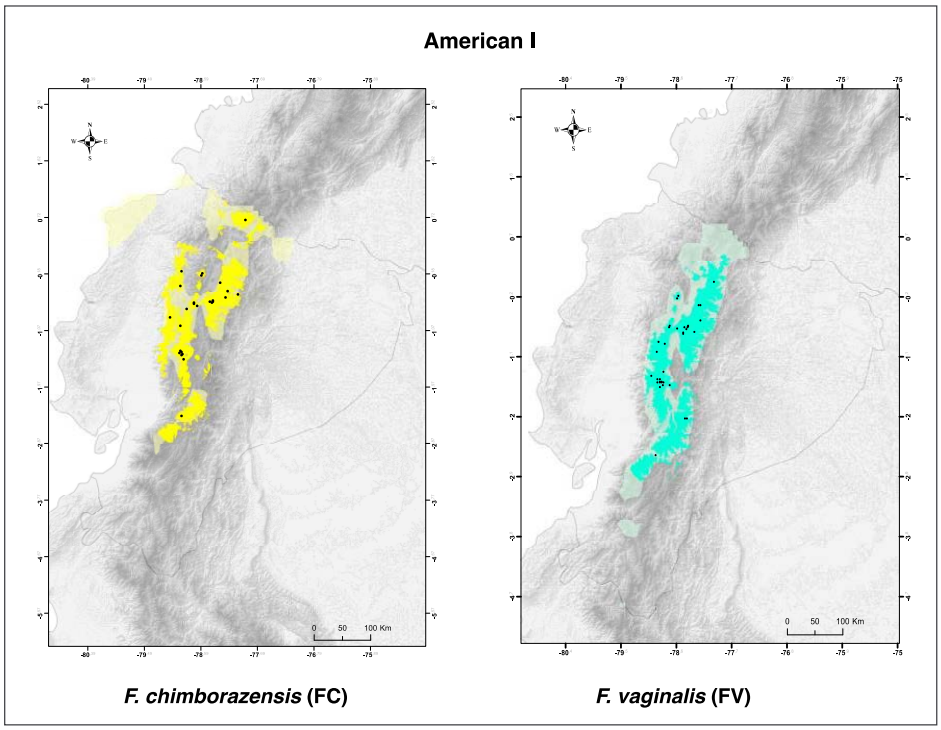


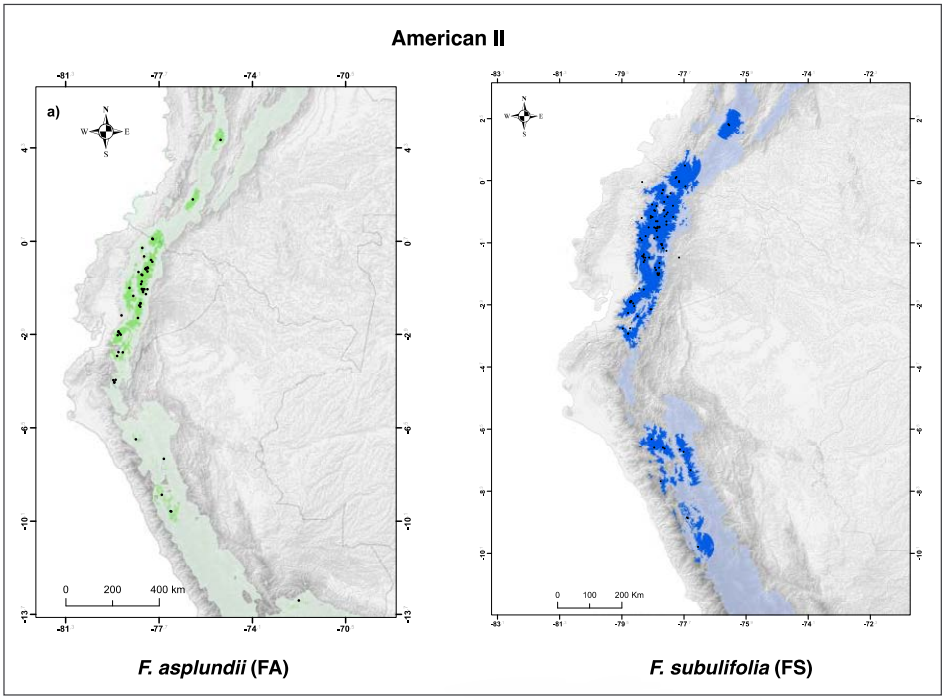
Figure 2. Bidimensional principal component analysis (PCA) plot of Northern Andean American I *Festuca chimborazensis* (green) and *F. vaginalis* (violet) and American II *F. asplundii* (salmon) and *F. subulifolia* (aquamarine) records based on data from 19 bioclimatic variables. PC1 and PC2 accounted for 48.9% and 22.7% of the variance, respectively.

The current models (Figure 3) were consistent with their known distributions (Figure 1) and exhibited good predictions for their respective ranges (AUC values 0.87 – 0.91; Figure 3). They revealed different reductions and shifts in the areas suitable for the occurrence of each of the four species from the LGM to the present (Figure 3). Projections from the predicted current niche model indicated that the American I species expand into the superpáramo belt of Ecuador (Figures 3a, 3b), with *F. vaginalis* showing a larger suitable range (Figure 3b) than that of *F. chimborazensis* (Figure 3a). ENMs of American II species revealed potential distributions in the páramo and lower superpáramo belts of the northern Andes (Figures 3c, 3d) and the two species exhibited striking differences in range suitability. *F. subulifolia* showed a potential distribution range (Figure 3d) almost double that of *F. asplundii* (Figure 3c). Predicted LGM niche model projections showed distribution ranges only slightly larger than those of current ENMs for American I taxa *F. chimborazensis* and *F. vaginalis* (Figures 3a, 3b); by contrast,

LGM-ENMs of American II taxa *F. asplundii* and *F. subulifolia* revealed considerable range expansions to suitable areas than current ENM distributions (Figures 3c, 3d).



***F. chimborazensis* (FC)** ***F. vaginalis* (FV)**



***F. asplundii* (FA)** ***F. subulifolia* (FS)**

Figure 3. Summarized maps of ecological niche models (ENMs) constructed with Maxent for the species American I *Festuca chimborazensis* (yellow) and *F. vaginalis* (light blue) and American II *F. asplundii* (green) and *F. subulifolia* (dark blue) in their respective Northern Andean native ranges under current climatic conditions (bright) and Last Glacial Maximum (LGM) (shaded). The georeferenced points correspond to occurrence data (Supplementary Table S1; Figure 1). AUC values for ENMs: FC-current 0.9133 ± 0.01, FV-current 0.9065 ± 0.01, FA-current 0.8745 ± 0.01, FS-current 0.8789 ± 0.01, FC-LGM 0.9116 ± 0.01, FV-LGM 0.9096 ± 0.01, FA-LGM 0.8736 ± 0.01, FS-LGMt-0.8813 ± 0.01. Maps were generated with ArcGis.

Comparative analysis of overlapping areas in the environmental niche distribution models of the American I and American II species detected ranges of shared potential occupancy under current conditions (Table 2, Figure 4). The current niches of American I taxa showed greater overlap [*F. chimborazensis* (FC) vs *F. vaginalis* (FV), D = 0.5429] than those of the American II taxa [*F. asplundii* (FA) vs *F. subulifolia* (FS), D = 0.2901] (Table 2; Figures 4a, 4b). Intergroup comparisons detected greater niche overlap of American I *F. chimborazensis* with American II taxa (FC vs FS, D = 0.3842; FC vs FA, D = 0.2828) (Table 2; Figures 4c, 4d than those of American I *F. vaginalis* with them (FV vs FS, D = 0.1823; FV vs FA, D = 0.0845) (Table 2; Figures 4e, 4f). Niche identity tests for current conditions found significant environmental differences in the pairwise comparisons of *F. chimborazensis* vs *F. vaginalis* and *F. asplundii vs F. subulifolia*, which indicated that niches were non-identical ($P < 0.01$), while the tests were not significant between *F. chimborazensis* vs *F. asplundii*, *F. chimborazensis vs F. subulifolia, F. vaginalis vs F. asplundii,* and *F. vaginalis vs F. subulifolia* ($P > 0.05$) (Table 2).

Table 2: Niche overlap values and results of tests of niche identity test and niche similarity test for different species-pair comparisons of American I and American II *Festuca* taxa in current climatic conditions. The observed and simulated overlap values are based on Schoener's *D* metric. In the background similarity test, the values correspond to the estimated niche overlap within a species pair when presence data from species A is projected onto the niche distribution of species B and viceversa. Significance: p-value < 0.01**, < 0.01***, n. s., non-significant.

Species pair (A-B)	Niche overlap	Identity	Similarity	
			A → B	B → A
F. chimborazensis-F. vaginalis	0.5429	0.6642**D	similar***	similar***
F. asplundii-F. subulifolia	0.2901	0.4653**D	similar***	similar***
F. chimborazensis-F. asplundii	0.2828	0.4047 n.s.	n.s.	n.s.
F. chimborazensis-F. subulifolia	0.3842	0.5286 n.s.	n.s.	n.s.
F. vaginalis-F. asplundii	0.0845	0.2625 n.s.	n.s.	n.s.
F. vaginalis-F. subulifolia	0.1823	0.3729 n.s.	n.s.	n.s.

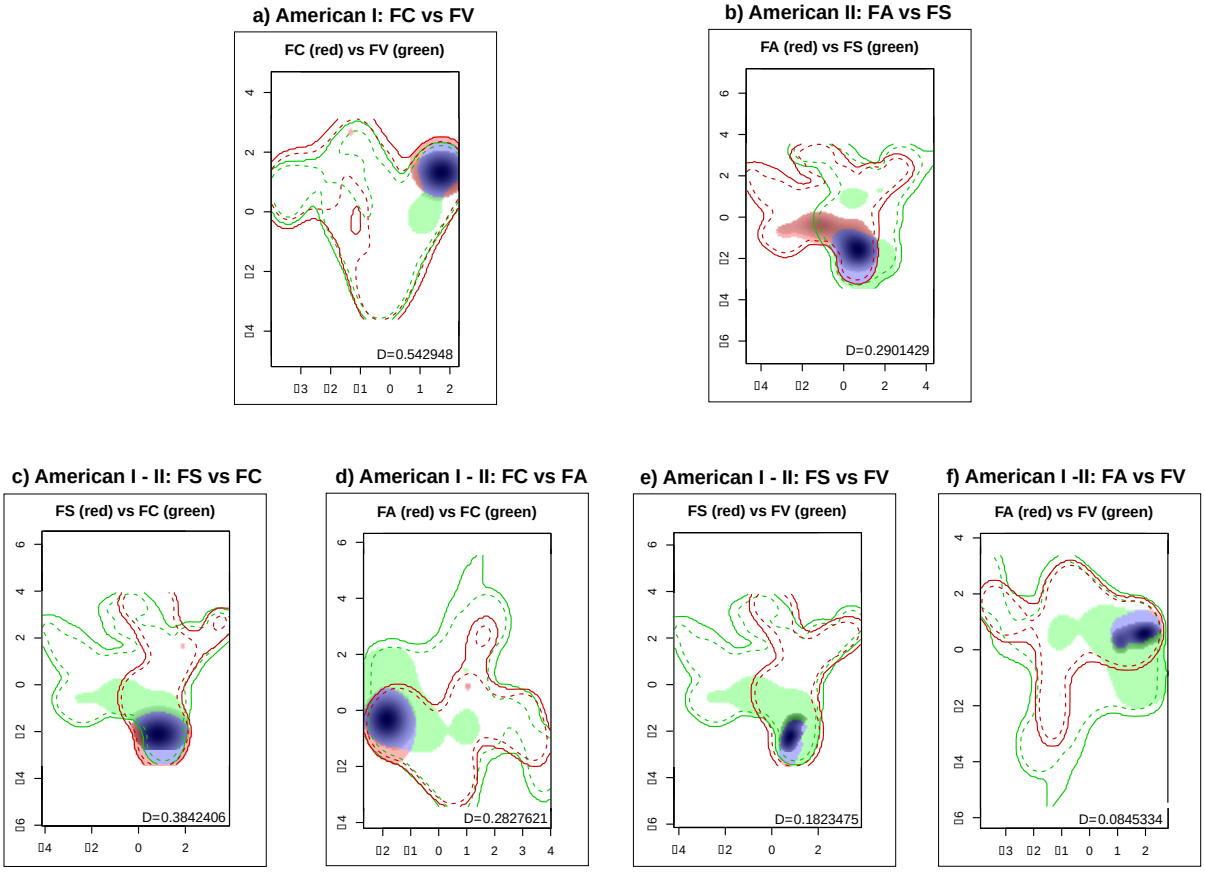


Figure 4. Niche overlap plots between species pairs of American I [*F. chimborazensis* (FC), *F. vaginalis* (FV)] and American II [*F. asplundii* (FA), *F. subulifolia* (FS)] *Festuca* species based on occurrence and background data from current climatic conditions (**a**) FC vs FV, (**b**) FA vs FS, (**c**) FC vs FS, (**d**) FA vs FC, (**e**) FS vs FV, (**f**) FA vs FV.

Niche divergence vs conservatism and niche breadth

Niche similarity tests for reciprocal comparisons of each *Festuca* taxa species-pair showed support for niche conservatism for intraclade taxa compared to null models of background divergence ($P > 0.05$; Table 2, Figure 5). Reciprocal comparisons of American I (*F. chimborazensis* vs *F. vaginalis*) and American II (*F. asplundii* vs F. *subulifolia*) taxa revealed significant evidence of niche conservatism (Table 2; Figures 5a, 5b), while the four interclade comparisons (*F. chimborazensis* vs *F. asplundii*; *F. chimborazensis* vs *F. subulifolia*, and *F. vaginalis* vs *F. asplundii*; and *F. vaginalis* vs *F. subulifolia*) did not deviate from null expectations (Figures 5c, 5d, 5e, 5f).

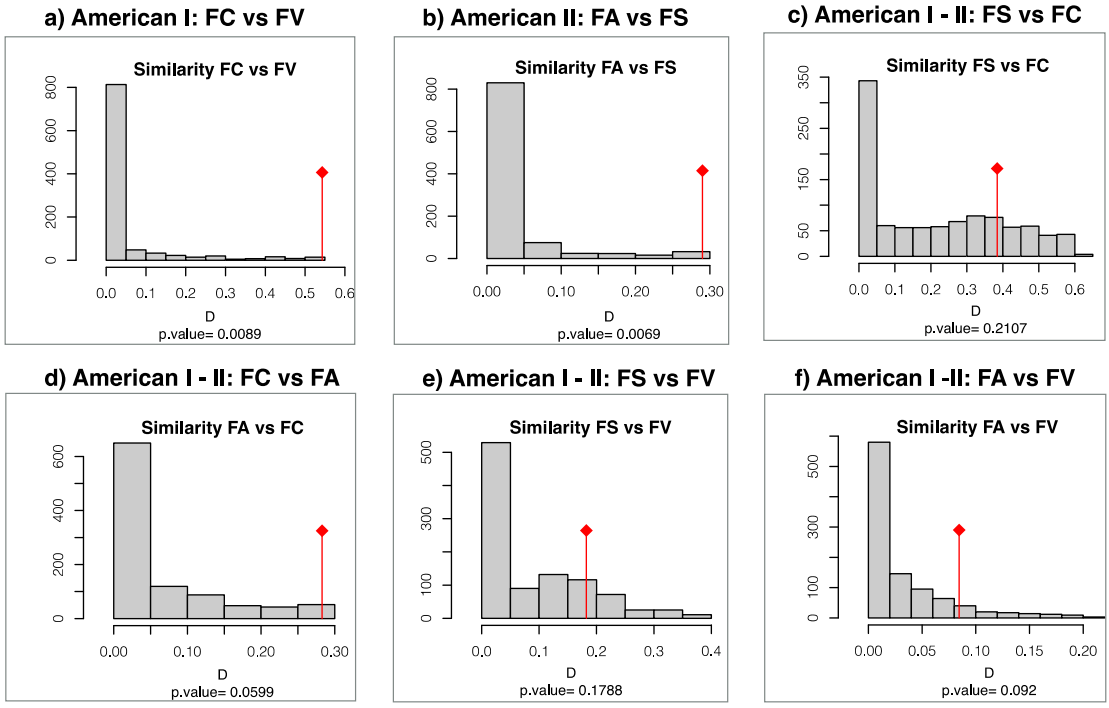


Figure 5. Plots of niche similary tests from environmental niche models of the *Festuca* taxa studied [*F. chimborazensis* (FC), *F. vaginalis* (FV), *F. asplundii* (FA), *F. subulifolia* (FS)]. Niche overlap values (red line) were compared with null distributions of background divergence for each species pair. Empirical values of niche overlap larger than the null distribution support

niche conservatism, and the results are inconclusive when the niche overlap value is similar to the null distribution background. (**a**) FC vs FV, (**b**) FA vs FS, (**c**) FS vs FC, (**d**) FC vs FA, (**e**) FV vs FS, (**f**) FV vs FA.

The estimated niche breadth values for the four species in their current native range were greater for *F. asplundii* (0.69445) than for *F. subulifolia* (0.67655), *F. chimborazensis* (0.6319) or *F. vaginalis* (0.6149), while those estimated for their LGM ranges were also greater for *F. asplundii* (0.60375) than for *F. subulifolia* (0.54525), *F. chimborazensis* (0.53415) or *F. vaginalis* (0.4947) (Table 3).

Table 3: Mean niche breadth estimates for American I and American II *Festuca* taxa under study in current and past (LGM) climatic envelopes.

Species	**Current time**	**LGM**
F. asplundii	0.69445	0.60375
F. subulifolia	0.67655	0.54525
F. chimborazensis	0.6319	0.53415
F. vaginalis	0.6149	0.4947

Phylogenetic signal of niche traits and phylogenetic distance and niche overlap

Blomberg's K and Pagel's lambda values were not significant ($p > 0.05$) for most ENM climatic traits and the niche breadth trait when tested on the *Festuca* phylogeny (Supplementary Table S4; Figure 6). However, the temperature annual range trait carried a phylogenetic signal for lambda values (Supplementary Table S4). American II taxa showed higher normalized values for this variable than American I taxa (Figure 6). The assessment of the relationship between phylogenetic distance and niche overlap through the Mantel correlation test indicated that both variables were not related ($r = -0.8109$; $p = 1$).

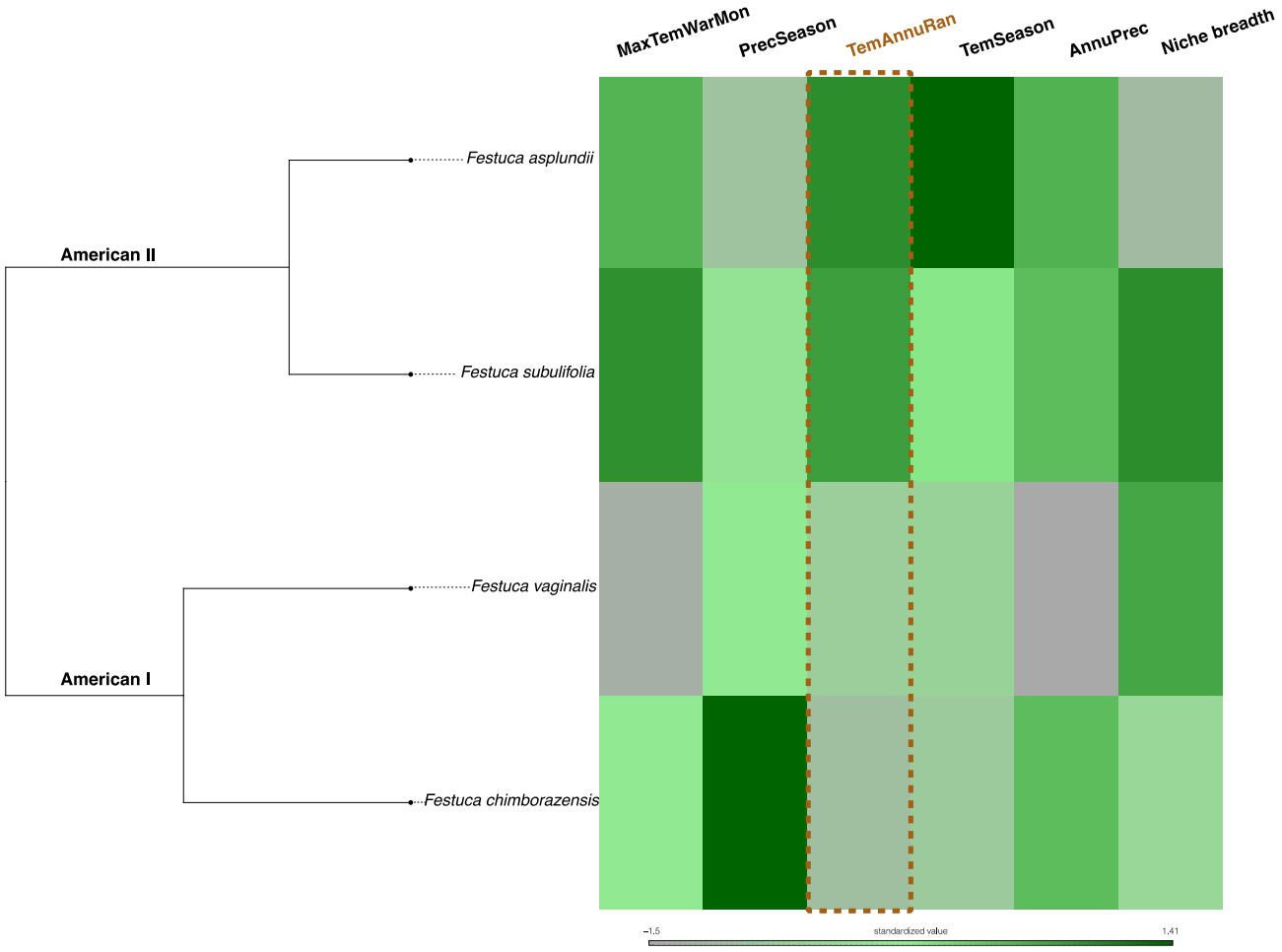


Figure 6. Maximum likelihood tree cladogram (combined nuclear and plastid loci) showing the relationships between the studied species of *Festuca* from the northern Andes and phyloheat maps of the normalized values of five niche climate variables (Temperature seasonality, Max. temperature of warmest month, Temperature Annual range, Annual precipitation, Precipitation seasonality) and niche breath. The variable showing a significant phylogenetic signal is highlighted in red.

Discussion

Intraclade American I and American II niche conservatism trends in North Andes Festuca species

The degree to which closely related, or partially sympatric, species display niche conservatism or niche divergence is relevant to understanding the role ecology plays in speciation (Wiens, 2004; Hu et al., 2015; Pyron et al., 2015). Niche differentiation may accelerate evolution, as predicted under ecological speciation (Nosil, 2012), while niche conservatism implies that

species divergence responds to evolutionary history rather than environmental conditions and that species tended to be ecologically similar (McCormack et al., 2010). Although our statistical tests showed differentiation in climatic variables for each of the four *Festuca* species studied (Table 1) and non-equivalent niches for each of the American I and American II species-pairs (Table 2), the niche similarity tests confirmed the existence of niche conservatism between the two American I species *F. chimborazensis* and *F. vaginalis* and the two American II species *F. asplundii* and *F. subulifolia* (Table 2; Figures 5a, 5b), which also showed the highest values of niche overlap (Table 2; Figure 4a, 4b). Our analyses were inconclusive regarding the niche similarities of American II *F. asplundii* and *F. subulifolia* and American I taxa, which had the lowest niche overlap values (Table 2; Figures 4c, 4d, 4e, 4f).

The niche conservatism between *F. chimborazensis* and *F. vaginalis* may have resulted from their recent split from the common ancestor (~1.5 Mya; (Moreno-Aguilar et al., 2020), supporting the hypothesis of ancestral niche inheritance and the tendency of niche to conserve through diversification (Wiens and Donoghue, 2004). The two species show large sympatric spatial distributions (Figures 1, 3) and across similar altitudinal ranges, growing between 3500 - 4000 masl (*F. chimborazensis*) and 3500 - 5000 masl (*F. vaginalis*), and sharing common climate features like low temperature variables (Supplementary Table S1). Although the two taxa differ in some preferred habitats, with *F. chimborazensis* thriving in humid pajonales and *F. vaginalis* in volcanic rocks and dry grasslands (Stancik and Peterson, 2007), their large niche overlap (Table 2; Figure 4a) suggest that the adaptations to different edaphic conditions could be recent, and could have evolved concomitantly with their phylogenetic divergence.

Likewise, our analyses supported the hypothesis that American II *F. asplundii* and *F. subulifolia* are distributed in identical or similar niches (Table 2; Figure 5b), and had relatively large niche overlap (Table 2; Figure 4b), despite the overall large size of their sympatric distributions (Figures 1, 3). *F. asplundii* grows at higher altitudes (3300–4300 masl) than *F. subulifolia* (2900–3700 masl); the former taxon is found in swampy patches, being a dominant species in flooded pajonales, while *F. subulifolia* is also a dominant species, spreading in various plant communities in moist to well-drained grasslands, often exposed to fire and other disturbances (Stančik and Peterson, 2007). *F. asplundii* and *F. subulifolia* probably also inherited an ancestral niche though they rapidly adapted to different environmental niches in a relatively short evolutionary time (Figure 5; (Minaya et al., 2017; Moreno-Aguilar et al., 2020). Niche parameters that are locally heterogeneous are expected to drive ecological speciation because

they capture variation in resources that are crucial for divergent selection (McCormack et al., 2010; Hu et al., 2015).

The low niche overlap between the species of the two divergent lineages of FL *Festuca* could be related to the potential influence of the distinct maternal (ancestral) progenitors of each allopolyploid group (Minaya et al., 2017), and the relatively older age of the American II (~1.7 Mya) than the American I ancestors (Moreno-Aguilar et al., 2020), which left more time for expansion and ecological diversification to the first group. Our data indicate a lack of widespread niche divergence among clades (Table 2; Figure 5). These results are consistent with the expectation that closely related clades will not be equivalent in their environmental niches, but will generally be more similar than expected given the suites of environments available to them (Hu et al., 2016). The strong environmental differences associated with distinct geographic ranges favor the divergence of the ecological niches of the species (Nakazato et al., 2010). Thus, the niche diversifications of the American I and American II taxa could also respond to their allopatric distributions (totally or partially) in, respectively, the most extreme environmental conditions of the superpáramo (American I taxa) and the less drastic conditions of the páramo (American II taxa) (Table 1; Supplementary Table S1).

Niche attributes, phylogenetic signal of niche traits, and recent niche contractions stronger in American II species than in American I species

The estimated niche breadth values of the North Andes *Festuca* species (Table 4) were not fully balanced with their niche distributional covers (Figure 3). Niche breadth analysis detected the highest levels of heterogeneous environmental variability for *F. asplundii* and *F. subulifolia* (Table 3). The high environmental heterogeneity of *F. subulifolia* may be related to its adaptation to the diverse climate and edaphic conditions of pajonal communities, installed on wet or dry soils, and its ability to proliferate as a 'ruderal' species, colonizing disturbed habitats in various páramo and lower areas of the superpáramo (Stančik and Peterson, 2007). Its ecological characteristics would also explain its higher niche overlap values with the other studied species in all pairwise comparisons (Table 3; Figure 4). By contrast, *F. asplundii* is adapted to constrained habitats of grassland wetlands, where the other species do not thrive in (Stancik and Peterson, 2007). *F. asplundii* is the most ecologically distinct species of the taxa studied, showing the lowest niche overlaps (Table 2; Figures 4b, 4c, 4e) with the remaining species. *F. chimborazensis* and *F. vaginalis* had

similar potential distribution ranges and niche breadths (Figures 3a, 3b; Table 3). *F. vaginalis* adapted not only to wet grasslands but also dry communities and volcanic outcrops throughout its greater altitudinal range that reaches the upper limit of plant life in the superpáramo (Stančik and Peterson, 2007).

We found no evidence of phylogenetic signal in the niche breadth optima nor in the means of the five bioclimatic variables used for niche modeling for Bloomers's K, and we only detected a significant signal for Pagel's lambda for Temperature annual range (Supplementary Table S4; Figure 6). This would imply that closely related American I and American II taxa were not more likely to be similar than expected by chance on most of these niche parameters. Consistent with these results, pairwise niche overlap was also not associated with phylogenetic distance. Although tropical mountain plants, such as those in the páramo and superpáramo belts of the North Andes, tolerate large diurnal temperature fluctuations from day to night (Salariato et al., 2022), the only niche trait that showed phylogenetic signal in our lamdba's tests (Temperature annual range; Supplementary Table S5) supported the main trends of sympatric speciation of the American I species through selective adaptation to the cooler climate of the superpáramo (Figures 1, 3), such as those proposed for other northern Andean angiosperms (Vargas et al., 2022).

Our LGM projections indicated that the areas suitable for the occurrence of these species in the Northern Andes were generally much wider in the past (Figure 3), probably as a consequence of the size and environmental suitability of the new ranges in lower altitudinal belts and the existence of altitude refugia (Antonelli et al., 2018; Pérez-Escobar et al., 2022). Past and present distributions evidenced that subsequent climate changes caused the loss of suitable habitats and the shifts of the species' ranges to their remaining suitable climatic conditions, currently restricted to the highest north-andine peaks (Figure 3). These predicted niche expansions and contractions of North Andean American I and American II fescues coincided with similar elevational range shifts of closely related *Festuca* afroalpine species during the time since the LGM occurred in the Tropical Africa "sky-islands" (Mairal et al., 2021). The higher proportions of range contractions predicted for American II species relative to American I species (Figure 3) could be due to postglacial aridification of the Andes in northern and central Peru (Latorre et al., 2003). The highest regressions of suitable niches for *F. subulifolia* and *F. asplundii* correspond to their Puna areas in Peru (Figures 3c, 3d). Paleoclimate studies identified dry phases during Holocene in the western slope of the

Andes and the Puna region and the establishment of the current hyperarid conditions in the low-lying belts of the Puna after 3.2 kya (Latorre et al., 2003) that likely created inhospitable habitats for these American II fescues that retreated to suitable habitat spaces in the present-day humid Puna and páramos (Figures 3c, 3d).

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Supplementary Tables

Supplementary Table S1: Presence data of the American I *Festuca chimborazensis* (FC) and *F. vaginalis* (FV) and American II *F. asplundii* (FA) and *F. subulifolia* (FS) taxa in the Northern Andes and associated data of 19 bioclimatic variables used for ecological niche modeling. Available at Github/Bioflora/ENM Festuca species.

Supplementary Table S2: Dunn's pairwise tests of mean values for 19 bioclimatic variables between the American I and American II species of *Festuca* under study (*F. chimborazensis* (Fchim), *F. vaginalis* (Fvag), *F. asplundii* (Fasp), *F. subulifolia* (Fsub)).

Annual mean temperature (Bio1)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.9590836	0.33752	1.0000
2	Fasp-Fsub	-1.4656969	0.14273	0.8564
3	Fchim-Fsub	-2.1643359	0.03044	0.1826
4	Fasp-Fvag	2.0243239	0.04294	0.2576
5	Fchim-Fvag	0.8124326	0.41654	1.0000
6	Fsub-Fvag	3.4970205	0.00047	0.0028
Mean diurnal range (Bio2)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.9215727	0.35675	1.0000
2	Fasp-Fsub	-1.3645513	0.17239	1.0000
3	Fchim-Fsub	-2.0460705	0.04075	0.2445
4	Fasp-Fvag	0.753771	0.45099	1.0000
5	Fchim-Fvag	-0.2178928	0.82751	1.0000
6	Fsub-Fvag	2.0061883	0.04484	0.2690
Isothermality (Bio3)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	-3.2217141	0.00127	0.0077
2	Fasp-Fsub	-2.95197533	0.00316	0.0189
3	Fchim-Fsub	1.22888472	0.21912	1.0000
4	Fasp-Fvag	-2.28360357	0.02239	0.1344
5	Fchim-Fvag	1.05633259	0.29082	1.0000
6	Fsub-Fvag	0.01510069	0.98795	1.0000
Temperature seasonality (Bio4)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	3.1414911	0.00168	0.0101
2	Fasp-Fsub	2.6437751	0.0082	0.0492
3	Fchim-Fsub	-1.3783237	0.1681	1.0000
4	Fasp-Fvag	2.6572048	0.00788	0.0473
5	Fchim-Fvag	-0.6692369	0.50334	1.0000
6	Fsub-Fvag	0.66271	0.50752	1.0000
Maximum temperature of warmest month (Bio5)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	1.4554296	0.14555	0.8733
2	Fasp-Fsub	-1.1587703	0.24655	1.0000
3	Fchim-Fsub	-2.46709	0.01362	0.0817
4	Fasp-Fvag	2.4785911	0.01319	0.0791
5	Fchim-Fvag	0.7355584	0.462	1.0000
6	Fsub-Fvag	3.7350342	0.00019	0.0011
Min temperature of coldest month (Bio6)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.5310125	0.59541	1.0000
2	Fasp-Fsub	-0.9312495	0.35172	1.0000
3	Fchim-Fsub	-1.2901773	0.19699	1.0000

4	Fasp-Fvag	1.6451162	0.09995	0.5997
5	Fchim-Fvag	0.8892701	0.37386	1.0000
6	Fsub-Fvag	2.6184729	0.00883	0.0530
Temperature annual range (Bio7)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	1.9782881	0.0479	0.2874
2	Fasp-Fsub	-0.6253474	0.53174	1.0000
3	Fchim-Fsub	-2.6248422	0.00867	0.0520
4	Fasp-Fvag	1.5632112	0.118	0.7080
5	Fchim-Fvag	-0.5137243	0.60744	1.0000
6	Fsub-Fvag	2.2649397	0.02352	0.1411
Mean temperature of wettest quarter (Bio8)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.8532475	0.39352	1.0000
2	Fasp-Fsub	-1.7199217	0.08545	0.5127
3	Fchim-Fsub	-2.2445959	0.02479	0.1488
4	Fasp-Fvag	1.8205822	0.06867	0.4120
5	Fchim-Fvag	0.739245	0.45976	1.0000
6	Fsub-Fvag	3.4905012	0.00048	0.0029
Mean temperature of driest quarter (Bio9)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	1.2459943	0.21277	1.0000
2	Fasp-Fsub	-1.059589	0.28933	1.0000
3	Fchim-Fsub	-2.163908	0.03047	0.1828
4	Fasp-Fvag	2.3617858	0.01819	0.1091
5	Fchim-Fvag	0.8307518	0.40611	1.0000
6	Fsub-Fvag	3.520691	0.00043	0.0026
Mean temperature of warmest quarter (Bio10)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	1.1375326	0.25532	1.0000
2	Fasp-Fsub	-1.3375415	0.18105	1.0000
3	Fchim-Fsub	-2.2595231	0.02385	0.1431
4	Fasp-Fvag	2.213005	0.0269	0.1614
5	Fchim-Fvag	0.8060502	0.42021	1.0000
6	Fsub-Fvag	3.5953018	0.00032	0.0019
Mean temperature of coldest quarter (Bio11)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.641698	0.52107	1.0000
2	Fasp-Fsub	-1.721428	0.08517	0.5110
3	Fchim-Fsub	-2.016361	0.04376	0.2626
4	Fasp-Fvag	1.722971	0.08489	0.5094
5	Fchim-Fvag	0.852475	0.39395	1.0000
6	Fsub-Fvag	3.383944	0.00071	0.0043
Annual precipitation (Bio12)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.7895493	0.42979	1.0000
2	Fasp-Fsub	0.2709722	0.78641	1.0000
3	Fchim-Fsub	-0.6482658	0.51681	1.0000
4	Fasp-Fvag	2.6960756	0.00702	0.0421
5	Fchim-Fvag	1.5317645	0.12558	0.7535
6	Fsub-Fvag	2.7459509	0.00603	0.0362
Precipitation of wettest month (Bio13)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	1.360057	0.17381	1.0000
2	Fasp-Fsub	0.463783	0.6428	1.0000
3	Fchim-Fsub	-1.118977	0.26315	1.0000
4	Fasp-Fvag	3.310402	0.00093	0.0056
5	Fchim-Fvag	1.520669	0.12834	0.7701
6	Fsub-Fvag	3.258942	0.00112	0.0067
Precipitation of driest month (Bio14)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	2.2964965	0.02165	0.1299
2	Fasp-Fsub	1.7129979	0.08671	0.5203
3	Fchim-Fsub	-1.1760882	0.23956	1.0000

4	Fasp-Fvag	2.9791569	0.00289	0.0173
5	Fchim-Fvag	0.3796667	0.70419	1.0000
6	Fsub-Fvag	1.818785	0.06894	0.41367
Precipitation seasonality (Bio15)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	-2.7472763	0.00601	0.0361
2	Fasp-Fsub	-1.7162097	0.08612	0.5167
3	Fchim-Fsub	1.6624199	0.09643	0.5786
4	Fasp-Fvag	-1.8133319	0.06978	0.4187
5	Fchim-Fvag	1.0130713	0.31103	1.0000
6	Fsub-Fvag	-0.5278787	0.59758	1.0000
Precipitation of wettest quarter (Bio16)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	0.782335	0.43402	1.0000
2	Fasp-Fsub	0.1326	0.89451	1.0000
3	Fchim-Fsub	-0.7465915	0.45531	1.0000
4	Fasp-Fvag	2.9170817	0.00353	0.0212
5	Fchim-Fvag	1.7236514	0.08477	0.5086
6	Fsub-Fvag	3.1091268	0.00188	0.0113
Precipitation of driest quarter (Bio17)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	2.4084777	0.01602	0.0961
2	Fasp-Fsub	1.638553	0.10131	0.6078
3	Fchim-Fsub	-1.3546215	0.17554	1.0000
4	Fasp-Fvag	3.1022173	0.00192	0.0115
5	Fchim-Fvag	0.3795658	0.70427	1.0000
6	Fsub-Fvag	2.0187696	0.04351	0.2611
Precipitation of warmest quarter (Bio18)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	-1.42808623	0.15327	0.9196
2	Fasp-Fsub	-1.39009151	0.1645	0.9870
3	Fchim-Fsub	0.48215074	0.6297	1.0000
4	Fasp-Fvag	-0.01891175	0.98491	1.0000
5	Fchim-Fvag	1.30080314	0.19333	1.0000
6	Fsub-Fvag	1.174396	0.24024	1.0000
Precipitation of coldest quarter (Bio19)	**Comparison**	**Z**	**P.unadj**	**P.adj**
1	Fasp-Fchim	2.6221001	0.00874	0.0524
2	Fasp-Fsub	1.8997001	0.05747	0.3448
3	Fchim-Fsub	-1.3859275	0.16577	0.9946
4	Fasp-Fvag	3.4930117	0.00048	0.0029
5	Fchim-Fvag	0.5101552	0.60994	1.0000
6	Fsub-Fvag	2.2260152	0.02601	0.1561

Supplementary Table S3: Contributions of each of the 19 bioclimatic variables to the main axes PC1 and PC2 of the Principal Component Analysis (PCA).

Bioclimatic variables	**Code**	**CS1**	**CS2**
Annual mean temperature	Bio 1	-0.3034153	-0.1407608
Mean diurnal range	Bio 2	-0.0401953	-0.373678
Isothermality	Bio 3	-0.0278012	0.06294243
Temperature seasonality	Bio 4	0.03742033	-0.1277108
Maximum temperature of warmest month	Bio 5	-0.2906131	-0.1980825
Min temperature of coldest month	Bio 6	-0.2943492	-0.009303
Temperature annual range	Bio 7	-0.0256513	-0.3601239
Mean temperature of wettest quarter	Bio 8	-0.3009714	-0.1579701
Mean temperature of driest quarter	Bio 9	-0.3052917	-0.1143673
Mean temperature of warmest quarter	Bio 10	-0.3032148	-0.1497358
Mean temperature of coldest quarter	Bio 11	-0.3031766	-0.1318199
Annual precipitation	Bio 12	-0.2576513	0.22466532
Precipitation of wettest month	Bio 13	-0.2736832	0.05853271
Precipitation of driest month	Bio 14	-0.1737997	0.36105702
Precipitation seasonality	Bio 15	-0.0186331	-0.3780368
Precipitation of wettest quarter	Bio 16	-0.2753268	0.07760378
Precipitation of driest quarter	Bio 17	-0.1683079	0.37449987
Precipitation of warmest quarter	Bio 18	-0.2260766	0.06393322
Precipitation of coldest quarter	Bio 19	-0.1940866	0.30967837

Supplementary Table S4: Phylogenetic signal of five environmental variables used to build the niche models under current climatic conditions and a sixth variable (niche breadth) assessed in the *Festuca* phylogenetic tree (Moreno-Aguilar et al. 2020) trimmed for the American I (*F. chimborazensis, F. vaginalis*) and American II (*F. asplundii, F. subulifolia*) taxa under study. Blomberg's K and Pagel's lambda values close to one indicate phylogenetic signal and values close to zero phylogenetic independence. K, p-values based on 1000 randomizations; lambda, p-values based on the Likelihood Ratio test. Significant test values are indicated in bold.

Bioclimatic variables	**Cluster_Name**	**Phylogenetic signal on American I and American II tree**			
		K	**p-value**	**lambda**	**P**
Maximum temperature of warmest month	Bio 5	1.2769	0.152	1.3011	0.253
Precipitation seasonality	Bio 15	0.9529	0.162	0.3346	0.832
Temperature annual range	**Bio 7**	1.8533	0.161	**1.3945**	**0.035**
Temperature seasonality	Bio 4	0.8187	0.244	0.3397	0.737
Annual precipitation	Bio 12	0.8898	0.372	0.0001	1.000
Niche_breadth	----	0.4386	1.000	0.0001	1.000

C.6. IAPT chromosome data 36/2 (Taxon 2022). Second-step lectotypifications of two names of *Festuca* subgenus *Erosiflorae* (Loliinae, Pooideae, Poaceae) (Correspondence Phytotaxa 2022)

C.6.1. IAPT chromosome data 36/2. *Festuca andicola, F. caldasii, F. chimborazensis* subsp. *micacochensis, F. subulifolia*

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IAPT CHROMOSOME DATA

IAPT chromosome data 36 – Extended version

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IAPT chromosome data 36/1

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Methods are described in Daviña (2001) and Barba-González & al. (2010).

* First chromosome count for the species.

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IAPT chromosome data 36/2

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POACEAE

Festuca andicola Kunth

$2n = 42$, $x = 6$, CHN. Ecuador: Loja, Saraguro, Cerro de Arcos, 3.563037°S, 79.463008°W, 3650 m; 21 May 2018, *M.F. Moreno & al. F90_i* (HUTPL 14037).

Festuca caldasii (Kunth) Kunth

$2n = 28$, $x = 4$, CHN. Ecuador: Loja, Catamayo, route Las Chinchas–Tambara, 3.967127°S, 79.515866°W, 2050 m; 26 May 2018, *M.F. Moreno & al. F98_i* (HUTPL 14054).

Festuca chimborazensis subsp. *micacochensis* Stančík

$2n = 42$, $x = 6$, CHN. Ecuador: Chimborazo, Riobamba, route Chimborazo–Guaranda, 1.44243°S, 78.93002°W, 4138 m; 30 Sep 2017, *M.F. Moreno & al. F58_i* (HUTPL 14065).

Festuca subulifolia Benth.

$2n = 42$, $x = 6$, CHN. Ecuador: Chimborazo, Riobamba, route Chimborazo–Guaranda, 2.17711°S, 78.51006°W, 3472 m, 1 Oct 2017, *M.F. Moreno & al. F60_i* (HUTPL 14099).

The Ecuadorian Andean paramos are considered hotspots of global biodiversity, hosting about 6.7% of the world's endemic plants (Myers & al., 2000). The most abundant plant communities of these paramunean ecosystems are the pajonales, extensive grazed communities dominated by cold seasonal grasses adapted to the high Andean climate (Sklenář & Ramsay, 2001). Among the most frequent pajonal grasses are species of the genus *Festuca*. This genus is the largest of the pooid subtribe Loliinae Dumort. and consists of more than 600 species distributed in temperate and mountainous regions of both hemispheres (Catalán, 2006). Approximately a quarter of the South American *Festuca* species are endemic to the North Andean region. Stančík (2004) recognized 23 species of *Festuca* in Ecuador and Stančík & Peterson (2007)

53 in the paramos of the northern Andes. *Festuca* species show a uniform chromosome base number of $x = 7$, and ploidy levels range from diploids to dodecaploids (Catalán, 2006). Phylogenetic studies have detected the existence of two main clades within *Festuca* and the Loliinae, the Broad-leaved (BL) and the Fine-leaved (FL) lineages, each containing different South American sublineages (Catalán & al., 2004, 2007; Catalán, 2006; Inda & al., 2008; Minaya & al., 2017; Moreno-Aguilar & al., 2020). Cytogenetic works have shown that all Southern Hemisphere *Festuca* species analyzed so far are polyploids (Dubcovsky & Martínez, 1992; Catalán, 2006; Šmarda & Stančík, 2006); however, a large number of *Festuca* species from South America have not been studied chromosomally yet.

Here we report new chromosome counts for four *Festuca* species from the Ecuadorian paramos: *Festuca caldasii* (Kunth) Kunth is a tetraploid with $2n = 28$ chromosomes, whereas *F. andicola* Kunth, *F. chimborazensis* subsp. *micacochensis* Stančík and *F. subulifolia* Benth. are hexaploids with $2n = 42$ chromosomes. The chromosomal study was performed following the protocol of Jenkins & Hasterok (2007). Chromosome counting was performed in two to five metaphasic cells per individual using DAPI-stained meristematic root cells; the staining was performed with the DAPI fluorescent marker (4′, 6-diamino-2-phenylindole), and counting was done using a Motic BA410 fluorescence microscope. Chromosome numbers of *F. caldasii* and *F. chimborazensis* subsp. *micacochensis* are given here for the first time. The chromosome-based hexaploid level detected in *F. chimborazensis* subsp. *micacochensis* agrees with its inferred ploidy from genetic analysis of nuclear genes (Díaz-Pérez & al., 2014). Šmarda & Stančík (2006) reported different chromosome numbers and ploidy levels for *F. andicola* and *F. subulifolia* (tetraploids with $2n = 28$); thus, our chromosome data provide new ploidy levels for these species.

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IAPT chromosome data 36/3

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* First chromosome count for the species.

FABACEAE

Tribe Dipterygeae

**Dipteryx lacunifera* Ducke

$2n = 16$, CHN. Brazil, Piauí, Bom Jesus, 09°04′57.2″S, 44°19′41.8″W, 298 m, 7 Dec 2021, *S.C. Silva & M. Lenara* (TEPB 205). [Fig. 4]

The genus *Dipteryx* Schreb. belongs to the tropical tribe Dipterygeae Polhill, family Leguminosae-Papilionoideae, and it comprises ~12 species dispersed through South and Central America (Ducke, 1948). The species commonly contains chemical compounds as coumarins, isoflavones, triterpenoids, fatty acids and furanocassan diterpenoids (Nakano & Suárez, 1970; Nakano & al., 1979; Godoy & al., 1989; Vieira Júnior & al., 2007), whose properties have aroused interest from food and cosmetic industry (Mendes & Silveira, 1994; Jang & al., 2003).

Dipteryx lacunifera Ducke (= *Coumarouna lacunifera* Ducke) is popularly known as Gurgueia's nut, donkey's nut or garampara (Fig. 4A). In Brazil, it can be found in the Amazon forest, Northeast and Central-North regions, mainly in Maranhão and Piauí states.

C.6.2. Second-step lectotypifications of two names of *Festuca* subgenus *Erosiflorae* (Loliinae, Pooideae, Poaceae)

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Festuca Linnaeus (1753: 73) is the largest genus of subtribe Loliinae. *Festuca* includes about 600 species distributed throughout the world (Catalán 2006). Nearly 80 species of the genus are found in South America (Ospina *et al.* 2015), and 56 of them in the Andean region (Stančík & Peterson 2007), where the Andes represent an important secondary center of *Festuca* diversity. The type species of *Festuca* subgen. *Erosiflorae* Alexeev (E.B. Alexeev 1986:11), *F. quadridentata* Kunth (Humboldt & al. 1815: 154), is distributed in Ecuador, and the other two species of the subgenus, *F. dichoclada* Pilg. (Pilger 1906: 514) and *F. horridula* Pilg. (Pilger 1906: 514), in the north of Peru. During our study of herbarium specimen and precise review of the type specimens of these names, as well as of the taxonomic literature and the protologues, we noted the need to typification of two names of *F.* subgen. *Erosiflorae*. Following article 9 of the ICN (Shenzhen Code, Turland *et al.* 2018, hereafter the ICN) second-step lectotypifications are designated here.

Regarding the typification of the name *F. quadridentata* Kunth, Alexeev (1986: 12) cited as "type" the *A.J.A. Bonpland & F.W.H.A Humboldt 3221* collection that is located in P. The term "type" is correctable to "lectotype" (Art. 9.10, Turland *et al.* 2018). There are two sheets of potential type material at P and it requires a second-step lectotypification (Art. 9.17 of the ICN). We chose the material from P 00669427 as the lectotype as it contains a better specimen than that from P 00625328, which is considered here an isolectotype. It must be added that in

the protologue of *F. quadridenta*, Kunth cited two localities: "prope Guamote et in radicibus montis altissimi Condorasto, alt. 1590 hexap." After careful revision of the manuscripts of A.J.A. Bonpland & F.W.H.A Humboldt (Humboldt & Bonpland 1773-1858, Lack 2004) on their journey thorough Ecuador from Quito to Alausi in July 1802 (Sandwith 1926), we find no mention of the locality "Condorasto" in the collection number 3221, and the syntypes from the locality of Condorasto have not been located in any herbarium. Thus, only the type material from Guamote could be used for typification.

For the name *Festuca dichoclada* Pilg., Soreng *et al.* (2003: 325) cited the "holotype" *A. Weberbauer 3230* housed at B, and this action must be accepted as the first-step of lectotypification (Art. 9.17of the ICN). The term "holotype" is also correctable to "lectotype" (Art. 9.10 of the ICN). As two herbarium sheets exists of the original material '*A. Weberbauer 3230*', it warrants a second-step lectotypification (Art. 9.17of the ICN) to narrow down it to a single sheet. We choose the specimen from B 100002570 as the lectotype because its sheet preserved with the original label.

Typification

Festuca quadridentata Kunth (Humboldt & al.1815: 154).

Lectotype (first-step designated as "typus" by Alexeev 1986: 12):—ECUADOR. Guamote, [without date] *A.J.A. Bonpland & F.W.H.A Humboldt 3221* (P, two sheets: P 00669427, P 00625328 images!): second-step designated here:—ECUADOR. Guamote, [without date], *A.J.A. Bonpland & F.W.H.A Humboldt 3221* (P 00669427 image!; isolectotypes (B-W 02072010 image!, B 100002572 image!, BAA 00002067 [fragment] image!, BAA 00002068 [fragment] image!, P 00625328 image!, US 01231924 [fragment?] image!). Image of second-step lectotype available at: https://science.mnhn.fr/institution/mnhn/collection/p/item/p00669427?listIndex=1&listCount=3&lang=en_US.

Festuca dichoclada Pilg. (Pilger 1906: 514).

Lectotype (first-step designated as holotype by Soreng et al. (2003: 325):—PERÚ. Cordillera Blanca, río Caraz, Dep. Ancachs, 3300-3600 m, 9 June 1903, *A. Weberbauer 3230* (B, two sheets: B 100002570, B 100002571 images!): second-step designated here:—PERÚ. Cordillera Blanca, río Caraz, Dep. Ancachs, 3300-3600 m, 9 June 1903, *A. Weberbauer 3230* (B 100002570 image!); isolectotypes (B 100002571 image!, BAA 00000856 [fragment] image!, US 00513487 [fragment] image!, MOL 00007849 image!). Image of second-step lectotype available at https://herbarium.bgbm.org/object/B100002570.

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APARTADO D

DISCUSIÓN GLOBAL Y CONCLUSIONES FINALES

D. DISCUSIÓN GLOBAL Y CONCLUSIONES FINALES

D.1. Discusión global

En esta tesis se ha abordado el mayor estudio de representantes de *Festuca* y otros subgéneros de Loliinae llevado a cabo hasta la fecha, incluyendo a representantes de todos sus linajes y grupos taxonómicos, e incorporando a especies pascícolas y forrajeras de gran interés ecológico y económico y de distribución mundial. Estas especies han sido analizadas genómicamente y filogenómicamente por primera vez a nivel de subtribu, empleando datos del plastoma completo y de genes nucleares copia simple, genes ribosomales 45S y 5S y elementos del repeteoma. Las filogenias obtenidas de las diversas fuentes genómicas han apoyado un marco evolutivo que muestra la divergencia de dos linajes principales de Loliinae de hojas anchas (BL) y de hojas finas (FL) y 24 sub-linajes repartidos entre ellos. Se ha podido determinar que las variaciones de los tamaños genómicos de las Loliinae dependen de las abundancias de sus elementos repetitivos, habiéndose detectándose indicios de paleo-poliploidización en linajes diploides BL y de pérdidas masivas de repeteoma en algunos linajes hibridógenos. Se estima que los elevados niveles de discordancia intragenómica nuclear observados en Loliinae han podido ser consecuencia del barajeo incompleto de linajes (ILS) y sus altas tasas de introgresión. Los resultados indican que la hibridación y la alopoliploidía han ocurrido frecuentemente en linajes tanto ancestrales como recientes de Loliinae habiendo contribuido a su diversificación. Estos estudios abren el camino a futuras investigaciones de los procesos y mecanismos de especiación en las Loliinae utilizando genomas completos de estas plantas.

Los trabajos desarrollados en esta tesis doctoral han permitido obtener nuevos resultados y de alto interés en relación a los objetivos propuestos en cada uno de sus seis capítulos, cuyos hitos más destacados se describen a continuación.

Capítulo 1. La museómica constituyó una herramienta crucial para desentrañar la desafiante historia evolutiva de *Megalachne* y *Podophorus* en Juan Fernandez en Chile. La utilización de métodos de secuenciación de siguiente generación (*Next Generation Sequencing*, NGS) ha permitido obtener resultados de secuenciación a baja cobertura de especímenes de herbario de estas especies que de otra forma hubiera sido imposible analizar y obtener datos fiables para reconstruir su filogenia y su evolución. Los datos genómicos del espécimen tipo de 164 años de antigüedad de *P. bromoides* permitió ubicar a esta especie extinta, y a las de su género hermano *Megalachne*, en el árbol evolutivo de las Loliinae, evidenciando que forman

parte de un linaje propio (Fernandeziano) dentro del linaje American-Vulpia-Pampas del clado FL de las Loliinae de hojas finas. El desarrollo de este estudio permitió la inclusión de ciertas especies del género *Festuca* que no habían sido analizadas molecularmente anteriormente (*F. caldasii, F. holubii, F. molokaiensis*). Con este estudio aumentó el número de géneros de Loliinae afines a *Festuca* a 14. Los análisis de los árboles filogenéticos del plastoma y del cistron nuclear ribosomal rDNA 35S mostraron incongruencias topológicas del clado Fernandeziano, lo que sugirió la posible naturaleza híbrida y poliploide de estas plantas. Las especies *F. caldasii* y *F. holubii* recolectadas en los páramos andinos ecuatorianos se emplazaron dentro de los linajes South American de hojas anchas (BL) y American II de hojas finas (FL), respectivamente. *F. molokaiensis* se emplazó dentro del linaje intermedio Subulatae-Hawaiian. Los análisis de datación y biogeográficos estimaron el origen del linaje Fernandeziano a partir de ancestros continentales de la región Pampa-Ventana en la transición del Mioceno-Plioceno, seguido de dispersión a larga distancia a la paleo-isla volcánica Santa Clara-Masatierra, emergida en esa época, y de posteriores divergencias y dispersiones de Masatierra a Masafuera que pudieron favorecer la reciente especiación *in-situ* de los taxones de *Megalachne* y *Podophorus* en estas islas en el Plioceno-Pleistoceno.

Capítulo 2. Los datos obtenidos sobre la composición y la abundancia de los elementos repetitivos confirmaron la decisiva contribución del repeteoma a la diversificación del tamaño genómico de las Loliinae. Las proporciones medias que mostraron los elementos repetitivos en las especies estudiadas fueron del 51.8% del tamaño genómico, y un rango que fluctuaba entre el 68.7% en algunos diploides, como *Lolium persicum* (2x), y el 30.7% en poliploides de alta ploidía, como *Festuca arundinacea letourneuxiana* (10x). Dentro de los elementos repetitivos identificados en todas las especies de Loliinae, los retrotransposones Ty1_Copia/Retand y Ty3_gypsy/Angela constituyeron las familias más comunes, con una presencia promedio de, aproximadamente el 7 y 6 %, respectivamente. Tres elementos repetitivos resultaron ser específicos de ciertas especies: Ty1_Copia/Reina (*L. saxatile*), Ty1_Copia/Ota (*F. letourneuxiana*) y Ty1_Copia/Tat (*F. simensis*). Mediante análisis de correlación y contribución, se demostró que 6 de los 25 elementos identificados en todas las Loliinae mostraron mayor contribución correlacionada con el tamaño del genoma, siendo los elementos Angela (19.5 %), TAR (0.642 %), Tekay (6.47 %), LTR (5.49 %) y Retand (10.7 %) los que mostraron mayor significación. Los conservados elementos Angela mostraron mayor correlación con la variación del tamaño genómico y una mayor señal filogenética, y los elementos Athila evidenciaron proliferaciones recientes fundamentalmente en *Lolium*. Las

redes filogenéticas de Loliinae basadas en el cómputo de árboles NJ del repeteoma presentaron congruencia topológica con el árbol nuclear 35S, detectando el aislamiento del linaje Schedonorus con respecto a las BL y FL Loliinae, y una estructura geográfica para los estrechamente emparentados linajes americanos American I, American II, American-Vulpia-Pampas, y American-Neozeylandic. La evolución del repeteoma de Loliinae sugirió un escenario de alopoliploidizaciones recurrentes seguidas de diploidizaciones que pudieron originar los grandes tamaños genómicos de las Loliinae BL diploides (1.5 veces mayores que los de las Loliinae FL diploides, y con familias 5S correspondientes a paleo-poliploides), y reorganizaciones genómicas en linajes altamente hibridógenos, que causaron pérdidas masivas del repeteoma y las consecuentes contracciones de los genomas, de especies poliploides de Schedonorus y Aulaxyper.

Capítulo 3. Las filogenias plastómicas y nucleares de las especies Meso y Suramericanas en estudio fueron congruentes en el emplazamiento evolutivo de *F*. subgen. *Mallopetalon* (de hojas anchas) en el linaje American II de las Loliinae de hojas finas, y del resto de las especies BL estudiadas en dos linajes de Loliinae de hojas anchas, Mexico-Central-South American I (MCSAI, que incluía a *F*. sect. *Glabricarpae, F*. subgen. *Asperifolia, F. superba*) y MCSAII (*F*. subgen. *Erosiflorae, F*. sect. *Ruprechtia, F. argentina*). La revisión sistemática apoyó las circunscripciones taxonómicas de Alexeev para estos rangos supraespecíficos y apoyó el cambio en la adscripción subgenérica de algunas especies que habían planteado otros autores. *F*. subgen. *Erosiflorae* resultó ser el único grupo monofilético en el árbol 5S. El árbol IGS (45S) recuperó los linajes BL Loliinae esperados. Sin embargo, a nivel general las discordancias topológicas plastoma-nucleo corroboraron el origen hibrido y alopoliploide de estos grupos, confirmado por la asimetría de algunos de sus cariotipos, e indicaron que las especies MCSAII derivaron probablemente de un ancestro paterno BL Leucopoa, las MCSAI y MCSAII de un ancestro materno BL Meso-Suramericano, y Mallopetalon de ancestros FL American I-II. Como consecuencia de nuestro estudio sistemático evolutivo, describimos un nuevo subgénero de *Festuca* (*F*. subgen. *Coironhuecu, subgen. nov.*) para acomodar a la especie *F. argentina*, que muestra rasgos fenotípicos y evolutivos exclusivos (dioecia, hojas plegadas, gluma inferior 3-nervada, hábito robusto, emplazamiento filogenético en el linaje MCSAII del clado BL).

Capítulo 4. Este estudió abarcó el máximo número de especies de Loliinae analizadas genómicamente hasta la fecha (136), de las cuales 81 fueron estudiadas por primera vez

molecularmente. De los 353 genes secuenciados mediante *target capture* para este estudio, se recuperaron 241 presentes en 133 especies. Cinco especies fueron excluidas al no poder obtenerse sus genes o por la carencia de una proporción considerable de ellos para el análisis. Adicionalmente, mediante la metodología *genome skimming* se estudiaron 130 especies de Loliinae, recuperando para todas ellas las secuencias del plastoma y del cistron nuclear ribosomal 35S rDNA. De los resultados obtenidos, el árbol máximo verosímil basado en la supermatriz de genes nucleares copia simple de Loliinae y el árbol de especie (Astral) mostraron unas topologías altamente concordantes entre sí y con respecto a las de otros componentes del genoma nuclear (genes ribosomales, repeteoma) y del plastoma para los linajes principales BL y FL y varios de sus sub-linajes, apoyando el marco evolutivo conceptual de la subtribu. Pese a ello, el nivel de discordancia intragenómica detectado por los genes copia simple fue en general elevado para la mayoría de los nodos del árbol de especie, probablemente como consecuencia de la ocurrencia de ILS, de la escasa resolución de algunos genes, y del impacto de la hibridación en las filogenias. Las pruebas Procrustes de hibridación confirmaron la existencia de niveles rampantes de introgresión en las Loliinae, tanto a niveles profundos como someros de la filogenia, y detectaron cuatro linajes BL (Subulatae-Hawaiian, Schenodorus, Tropical-South African, Mexico-Central-South American) y cinco FL (American II, Exaratae-Loretia, Aulaxyper, Afroalpine, American-Neozeylandic) altamente hibridógenos. Estos datos fueron corroborados por las altas tasas generales de hibridación estimadas para el grupo interno. Aunque los niveles de discordancia intragenómica pudieron haberse incrementado debido a sesgos en la selección de ortólogos, las filogenias recobradas fueron consistentes y revelaron aspectos claves en las relaciones de parentesco de estas gramíneas.

Capítulo 5. Los modelos de nicho ambiental construidos para las cuatro especies de festucas norteandinas (*F. asplundii, F. procera, F. chimborazensis, F. vaginalis*) con cinco variables bioclimáticas (temperatura estacional, temperatura máxima en el mes más cálido, rango de temperatura anual, precipitación anual, precipitación estacional) en el tiempo presente mostraron una distribución acorde con sus presencias. Análisis estadísticos de 19 variables ambientales con los datos de ocurrencia detectaron diferencias significativas para 16 de ellas entre distintos pares de especies, presentando cada especie caracteres únicos de nicho climático óptimo. Estos datos concuerdan con los hábitats y las características ambientales observadas para cada especie (*F. chimborazensis* (FC): pajonales húmedos del superpáramo; *F. vaginalis* (FV): pajonales secos-húmedos del superpáramo; *F. asplundii* (FV): pajonales encharcados del páramo; *F. subulifolia* (FS): pajonales diversos del páramo y zonas disturbadas). Análisis de

solapamiento de nicho mostraron mayores solapamientos en los nichos de las especies American I (FC, FV) que en los de las American II (FA, FS). Las pruebas de identidad de nicho indicaron que tanto las especies American I como las American II presentaban nichos no idénticos. Sin embargo, las pruebas recíprocas de similitud de nicho evidenciaron la existencia de conservatismo de nicho tanto entre las especies American I como entre las American II, sugiriendo que las especies de uno y otro linaje muestran una tendencia a mantener el nicho ancestral, y que sus diversificaciones se han producido principalmente por mecanismos evolutivos más que por adaptación ecológica. Las especies del páramo (FA, FS) mostraron mayores heterogeneidades (amplitudes) de nicho que las especies del superpáramo. Una de las cinco variables climáticas de nicho (rango anual de temperatura) mostró señal filogenética. Las proyecciones de nichos mostraron mayores disponibilidades de áreas adecuadas en el LGM que en el presente para las cuatro especies, detectando reducciones de nicho considerablemente mayores para las especies American II del páramo que las American I del superpáramo debidas probablemente a los intensos procesos de aridificación habidos en el cinturón vegetal de la puna-páramo en Perú durante el Holoceno.

Capítulo 6. En la primera nota breve los nuevos recuentos cromosómicos efectuados en cuatro especies de *Festuca* norteandinas detectaron números cromosómicos correspondientes a niveles de ploidía tetraploides en *F. caldasii* (2n=4x=28) y a niveles hexaploides (2n=6x=42) en *F. andicola*, *F. chimborazensis* subsp. *micacochensis* y *F. subulifolia*, confirmando la presencia exclusiva de taxones poliploides de Loliinae en el hemisferio sur. En la segunda nota breve se proporcionaron segundas lectotipificaciones para los nombres *Festuca quadridentata* Kunth, al haber diversos pliegos para la colección citada como tipo de esta especie depositada en P (*A.J.A. Bonpland & F.W.H.A Humboldt 3221*), y *F. dichoclada* Pilg., al existir dos pliegos de herbario del material tipo original de esta especie (‘*A. Weberbauer 3230*’).

D.2. Conclusiones

1. El uso de metodologías de secuenciación genómica *genome skimming* y de captura génica en las Loliinae ha proporcionado bases de datos de plastomas completos y de genes ribosomales 45S y 5S, elementos repetitivos, y 241 genes copia simple nucleares con los que se han desarrollado las primeras reconstrucciones filogenómicas de la subtribu. Estos árboles evolutivos han incorporado a más de un tercio de sus especies y han identificado 24 linajes en los clados principales de Loliinae de hojas anchas (BL) y hojas finas (FL). La alta congruencia topológica de las filogenias obtenidas a partir de las distintas bases de datos genómicas apoya el marco evolutivo propuesto para las Loliinae y su empleo en pruebas de procesos de especiación. Los análisis citogenéticos de Loliinae, en especial de las poco investigadas festucas suramericanas, han suministrado nuevos datos de números cromosómicos, tamaños genómicos y niveles de ploidía para estas especies, corroborando la ausencia de diploides en el hemisferio sur.
2. La aplicación de aproximaciones museómicas al estudio de las Loliinae, empleando muestras de herbario antiguas y recientes, y la obtención de datos de plastomas completos y del gen ribosomal nuclear 35S, permitieron inferir el origen evolutivo de las gramíneas endémicas del archipiélago de Juan Fernández (Chile) *Megalachne* y *Podophorus*. Las filogenias respaldan su posible ubicación filogenética en el linaje Fernandeziano, dentro del clado de hojas finas de la subtribu Loliinae. Los análisis de datación molecular y de reconstrucción de áreas ancestrales identificaron la región Pampeana-Ventana como lugar de origen probable de los ancestros de estas gramíneas en el Mioceno-Plioceno, y su posterior dispersión a larga distancia a las islas oceánicas.
3. El estudio del ADN repetitivo nuclear en las Loliinae corroboró la relación existente entre la abundancia de elementos repetitivos y las variaciones en los tamaños genómicos de estas gramíneas. Los elementos del repeteoma más frecuentes fueron los retrotransposones Retand y Angela, mientras que otras familias exhibieron proliferaciones sólo en algunos linajes. Los elementos repetitivos mostraron una elevada resolución filogenética para los principales linajes de Loliinae (BL, FL, Schedonorus). Las Loliinae BL diploides presentaron mayores contenidos de repeteoma (y mayor tamaño genómico) que las Loliinae FL diploides y patrones ribotípicos 5S concordantes con su posible origen paleo-poliploide. Los bajos contenidos de repeteoma de especies de Schedonorus y Aulaxyper con alto nivel de ploidía pudieron deberse a pérdidas

masivas de elementos repetitivos tras reorganizaciones genómicas en estas especies alopoliploides altamente hibridógenas.

4. Los estudios sistemáticos, evolutivos y nomenclaturales de las apenas estudiadas festucas de hojas anchas de México y Centro-Sur-América (MCSA) permitieron re-circunscribir taxonómicamente a los subgéneros *Asperifolia, Erosiflorae* y *Mallopetalon* y a las secciones *Glabricarpae* y *Ruprechtia* de *Festuca*,establecer sus orígenes y estabilizar la nomenclatura de dos nombres del subgénero *Erosiflorae*. Las filogenias basadas en datos del plastoma y de genes ribosomales (35S. IGS, 5S) y repeteoma nucleares recobraron tres linajes principales, uno perteneciente a las Loliinae de hojas finas (Mallopetalon) y dos a las Loliinae de hojas anchas (MCSA-I: Asperifolia, Glabricarpae, *F. superba*; MCSA-II: Erosiflorae, Ruprechtia, *F. argentina*). Las inferencias sugieren que las especies MCSAII derivaron probablemente de un ancestro paterno BL Leucopoa, las MCSAI y MCSAII de un ancestro materno BL Meso-Suramericano, y Mallopetalon de ancestros FL American I-II. Se describió un nuevo subgénero de *Festuca*, *F.* subgen. *Coironhuecu*, para clasificar en él a *F. argentina*, taxonómica y evolutivamente distinta del resto de los rangos supraespecíficos del género.
5. El primer estudio filogenómico de Loliinae basado en unos cientos de genes nucleares copia simple recobró una topología concatenada y un árbol de especie coalescente altamente congruentes entre si y consistentes con las topologías de otras fuentes genómicas. Los altos niveles de discordancia intragenómica nuclear detectados por los genes copia simple en las Loliinae pueden ser consecuencia del impacto de ILS y la hibridación en estos linajes recientemente evolucionados y el posible sesgo metodológico en la selección de ortólogos. Las pruebas Procrustes de hibridación confirmaron la existencia de niveles rampantes de introgresión en las Loliinae, tanto a niveles profundos como someros de la filogenia, y detectaron cuatro linajes BL (Subulatae-Hawaiian, Schenodorus, Tropical-South African, Mexico-Central-South American) y cinco FL (American II, Exaratae-Loretia, Aulaxyper, Afroalpine, American-Neozeylandic) altamente hibridógenos.

6. Los modelos de nicho ambiental de especies de *Festuca* norte-andinas evolutivamente próximas de los linajes de hojas finas American I (*F. chimborazensis, F. vaginalis*) y American II (*F. asplundii, F. subulifolia*) mostraron mayores solapamientos para las especies American I del superpáramo que para las American II del páramo, y nichos no

idénticos entre sí. Las pruebas de similitud recíproca de nicho indicaron que tanto las especies American I como American II presentaban conservatismo de nicho, sugiriendo que sus respectivas diversificaciones pudieron ser debidas a mecanismos evolutivos distintos de los de la especiación ecológica. Las proyecciones de nichos mostraron mayores disponibilidades de áreas adecuadas en el LGM que en el presente para las cuatro especies, detectando reducciones de nicho mayores para las especies American II del páramo que las American I del superpáramo causadas probablemente por los procesos de aridificación de la puna-páramo durante el Holoceno.

Conclusions

1. The use of *genome skimming* and gene capture methodologies in the Loliinae has provided databases of complete plastomes and 45S and 5S ribosomal genes, repetitive elements, and 241 nuclear single copy genes for phylogenomic reconstructions of the subtribe. These evolutionary trees have incorporated more than a third of the Loliinae species and have identified 24 lineages within its two main broad-leaved (BL) and fine-leaved (FL) clades. The high topological congruence of the trees obtained from the different genomic databases supports the proposed evolutionary network for Loliinae and its use for testing speciation processes. Cytogenetic analyses of Loliinae, especially of the poorly studied South American fescues, have proportionated new data on chromosome number, genome size and ploidy levels for these species, corroborating the absence of diploids in the southern hemisphere.
2. The application of museomic approaches to the study of Loliinae, using old and recent herbarium samples, and obtaining data from complete plastomes and the 35S nuclear ribosomal gene, allowed us to infer the evolutionary origin of the endemic grasses of the Juan Fernández archipelago (Chile) *Megalachne* and *Podophorus*. The phylogenies support their phylogenetic placement in the Fernandezian lineage, within the fine-leaved clade of Loliinae. Molecular dating and reconstruction analyses of ancestral areas identified the Pampean-Ventanian region as the probable place of origin of the Miocene-Pliocene ancestors of these grasses, and their subsequent long-distance dispersal to the oceanic islands.
3. The study of nuclear repetitive DNA in the Loliinae corroborated the relationship between the abundance of repetitive elements and the variations in the genomic sizes of these grasses. The most frequent repeatome elements were the Retand and Angela retrotransposons, while other families exhibited proliferations in only some lineages. The repetitive elements showed a high phylogenetic resolution for the main Loliinae lineages (BL, FL, Schedonorus). Diploid broad-leaved Loliinae presented higher repeatome contents (and larger genome size) then diploid fine-leaved Loliinae and 5S ribotypic patterns consistent with their possible paleo-polyploid origin. The low repeatome contents of highly polyploid Schedonorus and Aulaxyper species could be due to massive losses of repetitive elements following genomic rearrangements in these highly hybridogenic and allopolyploid species.

4. Systematic, evolutionary and nomenclatural studies of the poorly investigated broad-leaved fescues of Mexico and Central-South-America (MCSA) allowed taxonomically re-circumscribing the subgenera *Asperifolia, Erosiflorae* and *Mallopetalon* and the sections *Glabricarpae* y *Ruprechtia* of *Festuca*, establish their origins and stabilize the nomenclature of two names of the subgenus *Erosiflorae*. Phylogenies based on data from the plastome and ribosomal genes (35S, IGS, 5S) and nuclear repeatome recovered three main lineages, one belonging to FL Loliinae (*Mallopetalon*) and two to BL Loliinae (MCSA-I: *Asperifolia*, *Glabricarpae*, *F. superba*; MCSA-II: *Erosiflorae*, *Ruprechtia*, *F. argentina*). Inferences suggest that MCSAII species were probably derived from a BL Leucopoa paternal ancestor, MCSAI and MCSAII from a BL Meso-South American maternal ancestor, and *Mallopetalon* from FL American I-II ancestors. A new subgenus of *Festuca*, *F.* subgen. *Coironhuecu* was described to classify the taxonomically and evolutionarily unique *F. argentina*, within it.
5. The fist phylogenomic study of Loliinae based on a few hundred single copy nuclear genes recovered a concatenated topology and a coalescent species tree highly congruent with each other and consistent with topologies from other genomic sources. The high levels of intragenomic discordance detected by single copy genes in Loliinae may be a consequence of the impact of ILS and hybridization on these recently evolved lineages and potential methodological bias in ortholog selection. Procrustean co-phylogenetic hybridization tests confirmed rampant levels of introgression in the Loliinae, both at deep and shallow levels of the phylogeny, and detected four BL (Subulatae-Hawaiian, Schenodorus, Tropical-South African, Mexico-Central-South American) and five FL (American II, Exaratae-Loretia, Aulaxyper, Afroalpine, American-Neozeylandic) highly hybridogenic lineages.
6. Environmental niche models of evolutionarily close northern Andean *Festuca* species of the American I (*F. chimborazensis, F. vaginalis*) and American II (*F. asplundii, F. subulifolia*) fine-leaved lineages showed greater overlaps for the superpáramo American I species than for the páramo American II species, and non-identical niches. Reciprocal niche similarity tests indicated that both American I and American II species exhibited niche conservatism, suggesting that their respective diversifications resulted from evolutionary mechanisms other than ecological speciation. Niche projections showed greater suitability of areas in the LGM than in the present for the four species, detecting higher range shifts for the American II than for the American I species, probably caused by the aridification of the puna-páramo vegetation belt during the Holocene.

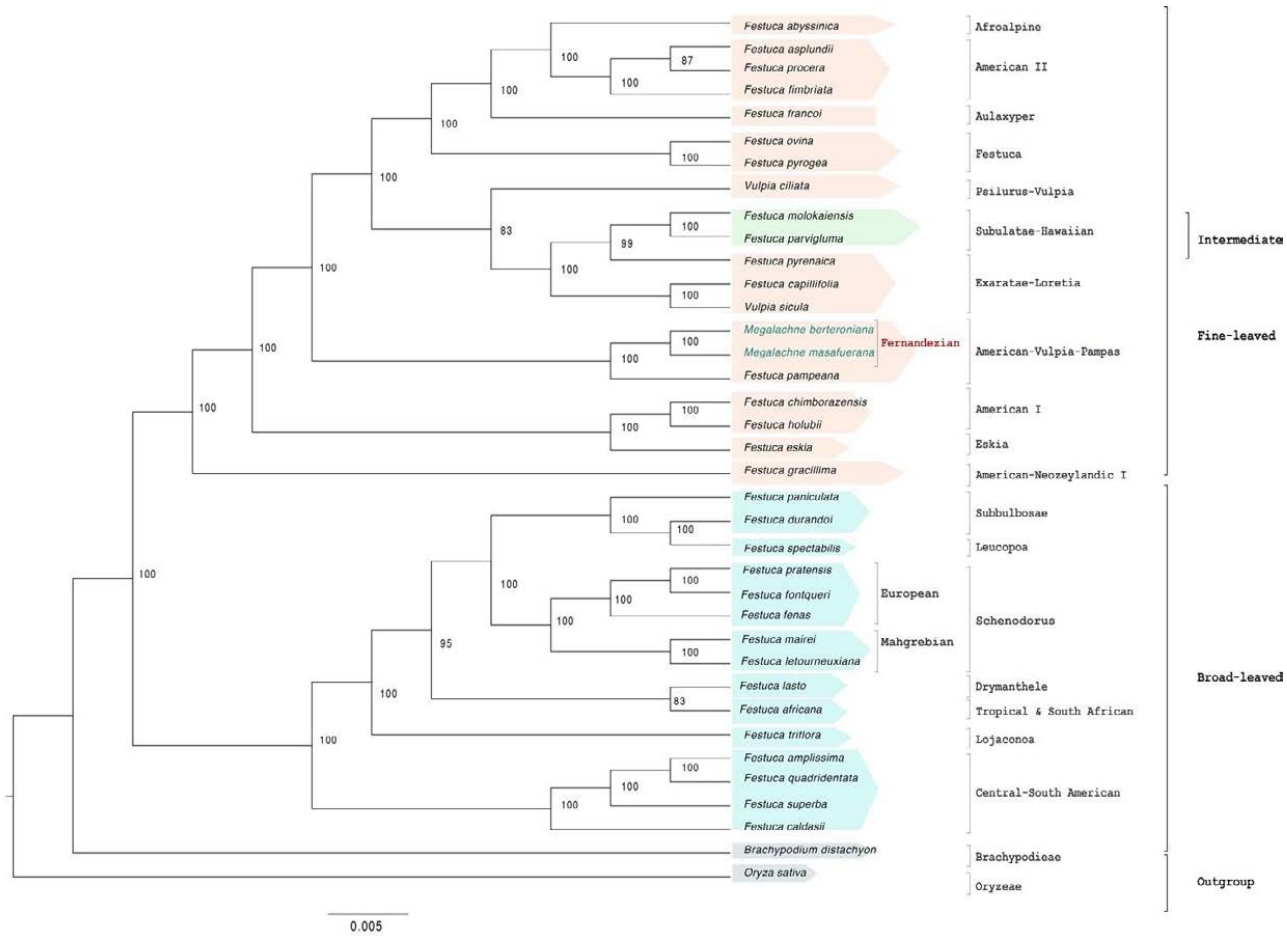
APARTADO E

APÉNDICES

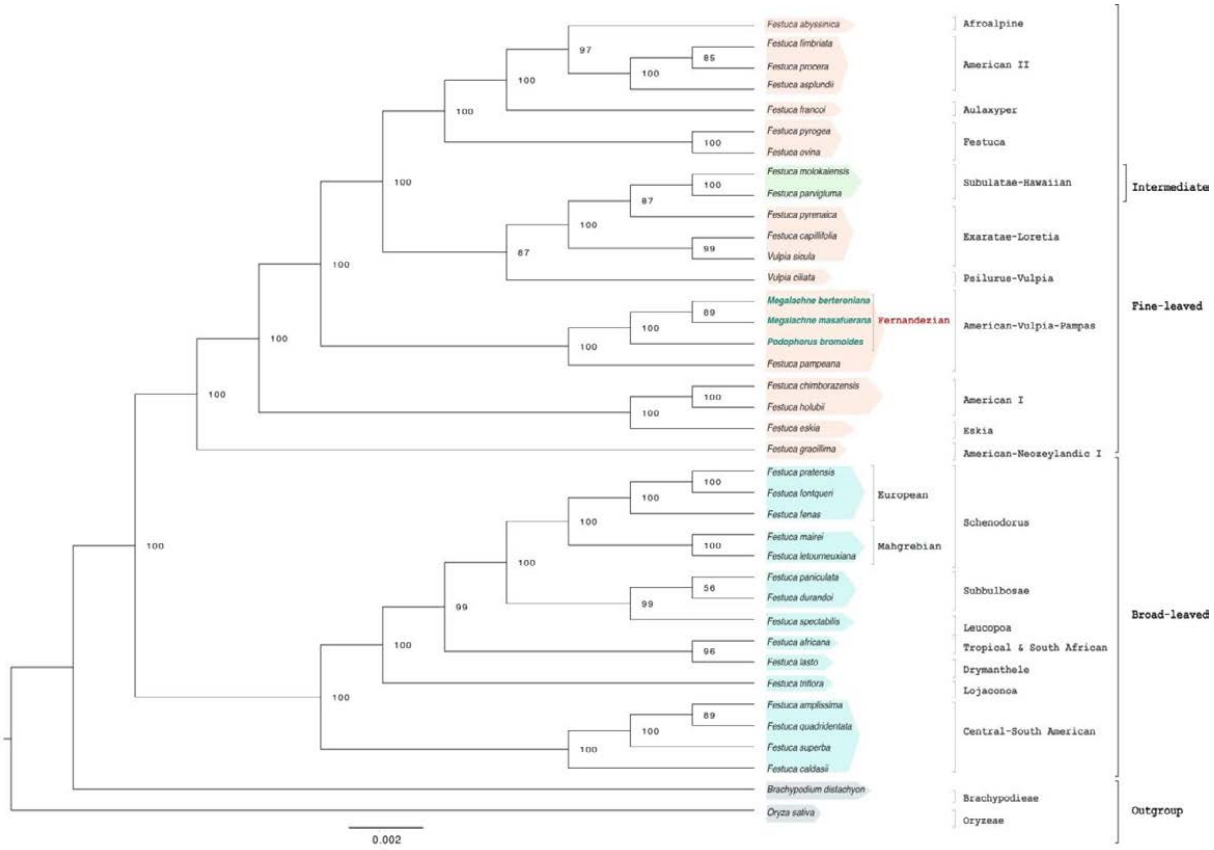
E. APÉNDICES

Apéndice 1. Información suplementaria del Capítulo C.1.

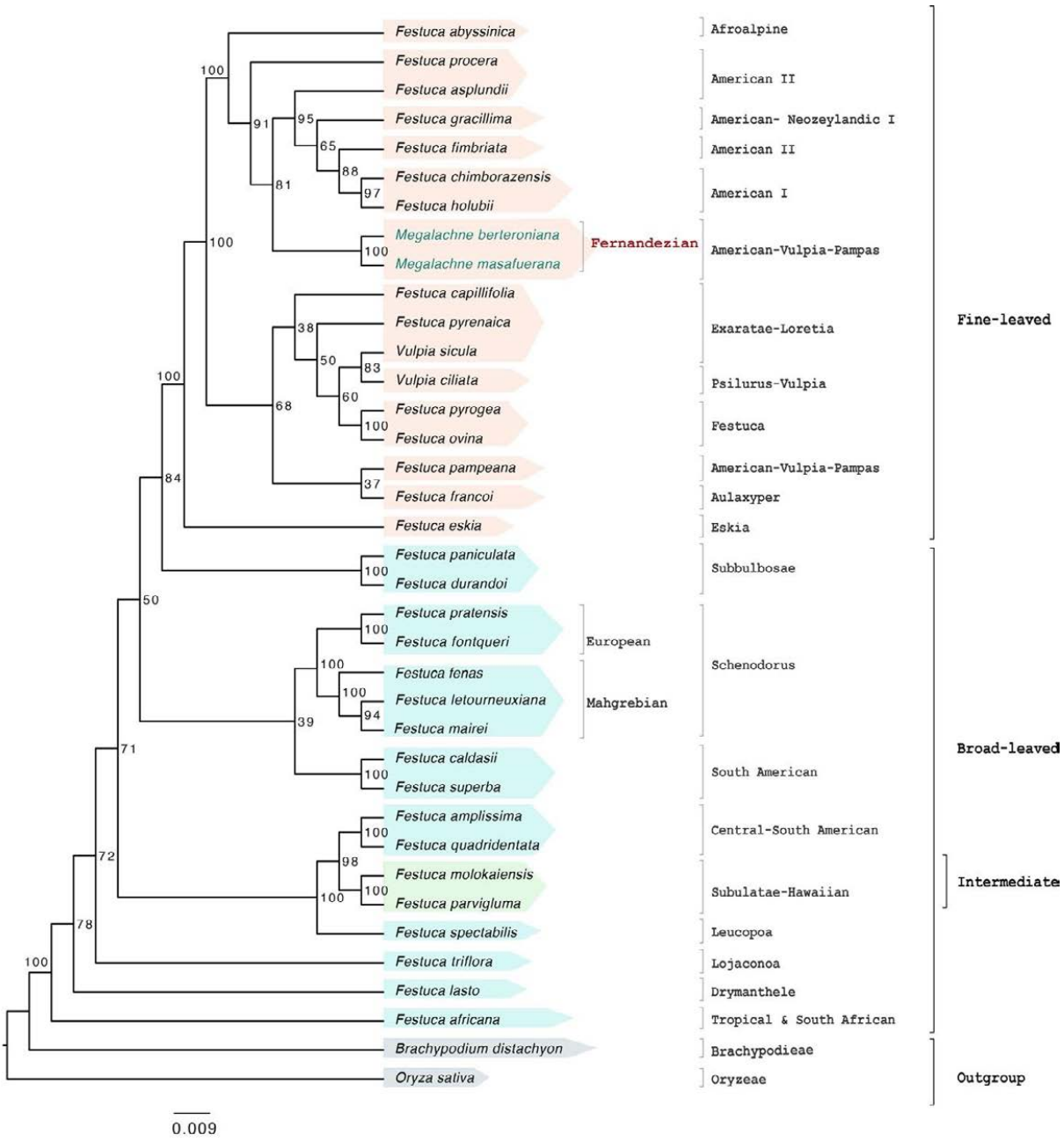
Supplementary Figures



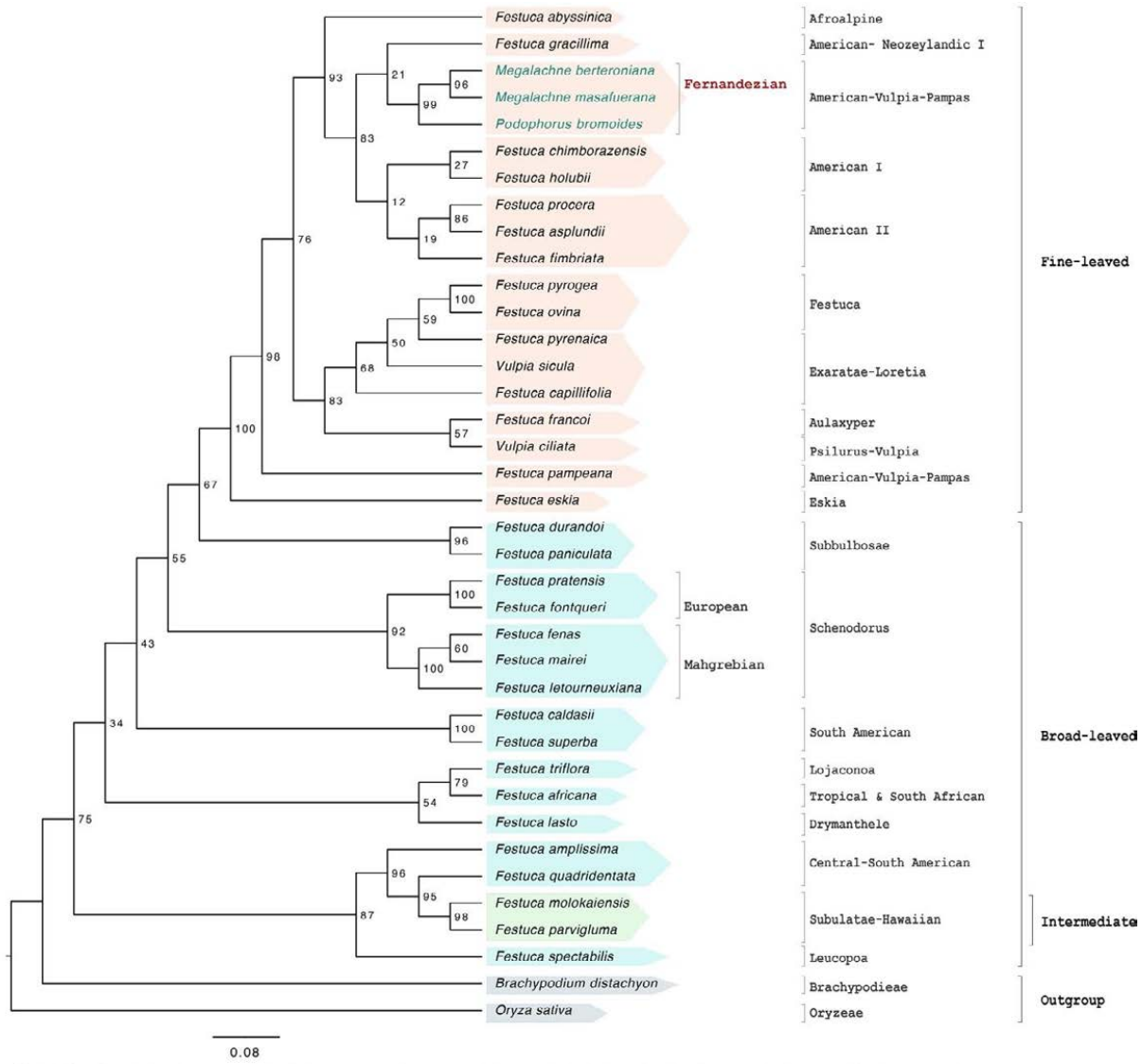
Supplementary Figure 1A. Maximum likelihood full plastome cladogram (35 Loliinae taxa, Podophorus excluded) constructed with IQTREE showing the relationships among the studied Fernandez and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).



Supplementary Figure 1B. Maximum likelihood reduce plastome cladogram (36 Loliinae taxa, Podophorus included) constructed with IQTREE showing the relationships among the studied Fernandez and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).

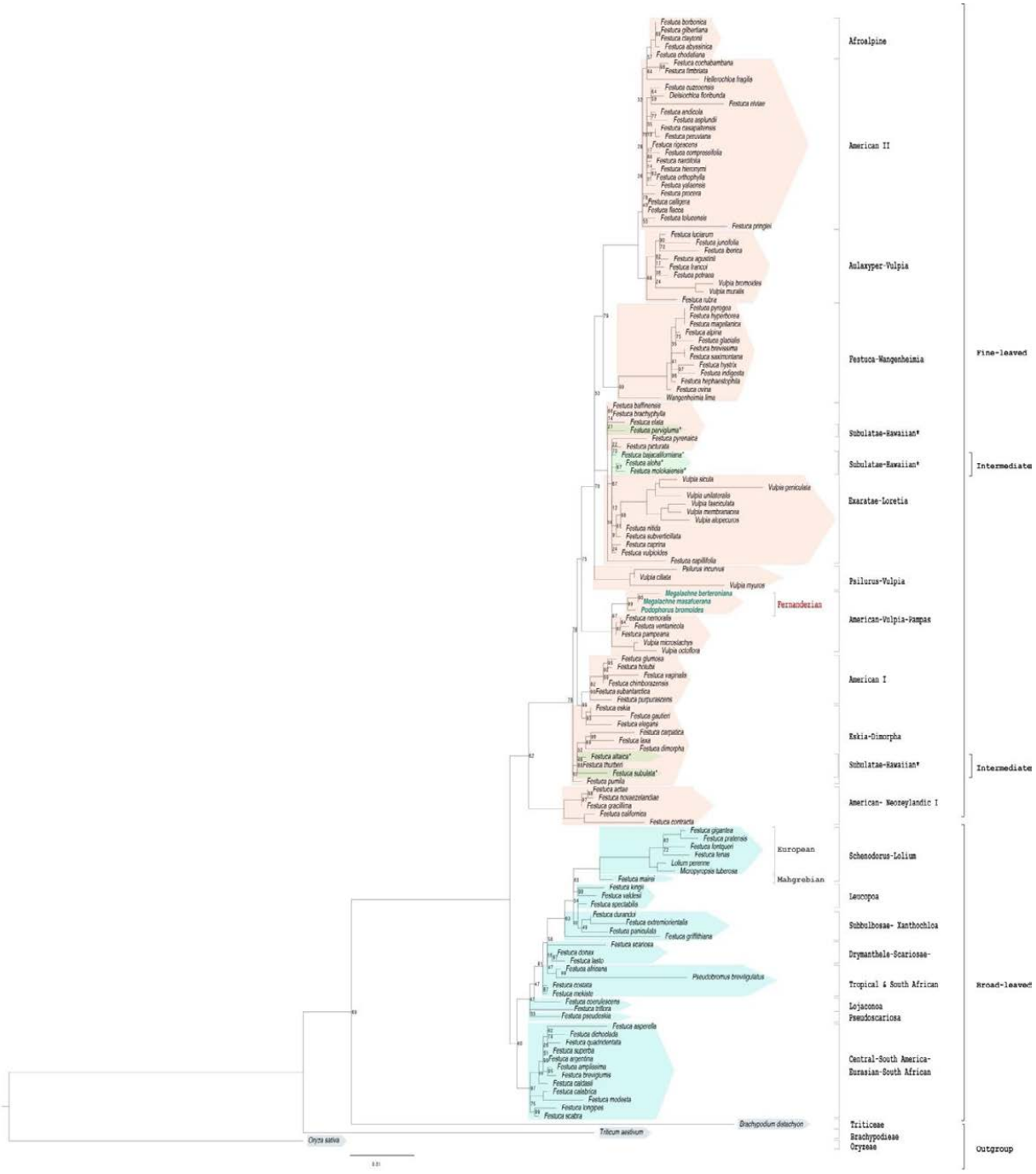


Supplementary Figure 1C. Maximum likelihood rDNA cistron cladogram (35 Loliinae taxa, Podophorus excluded) constructed with IQTREE showing the relationships among the studied Fernandez and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).

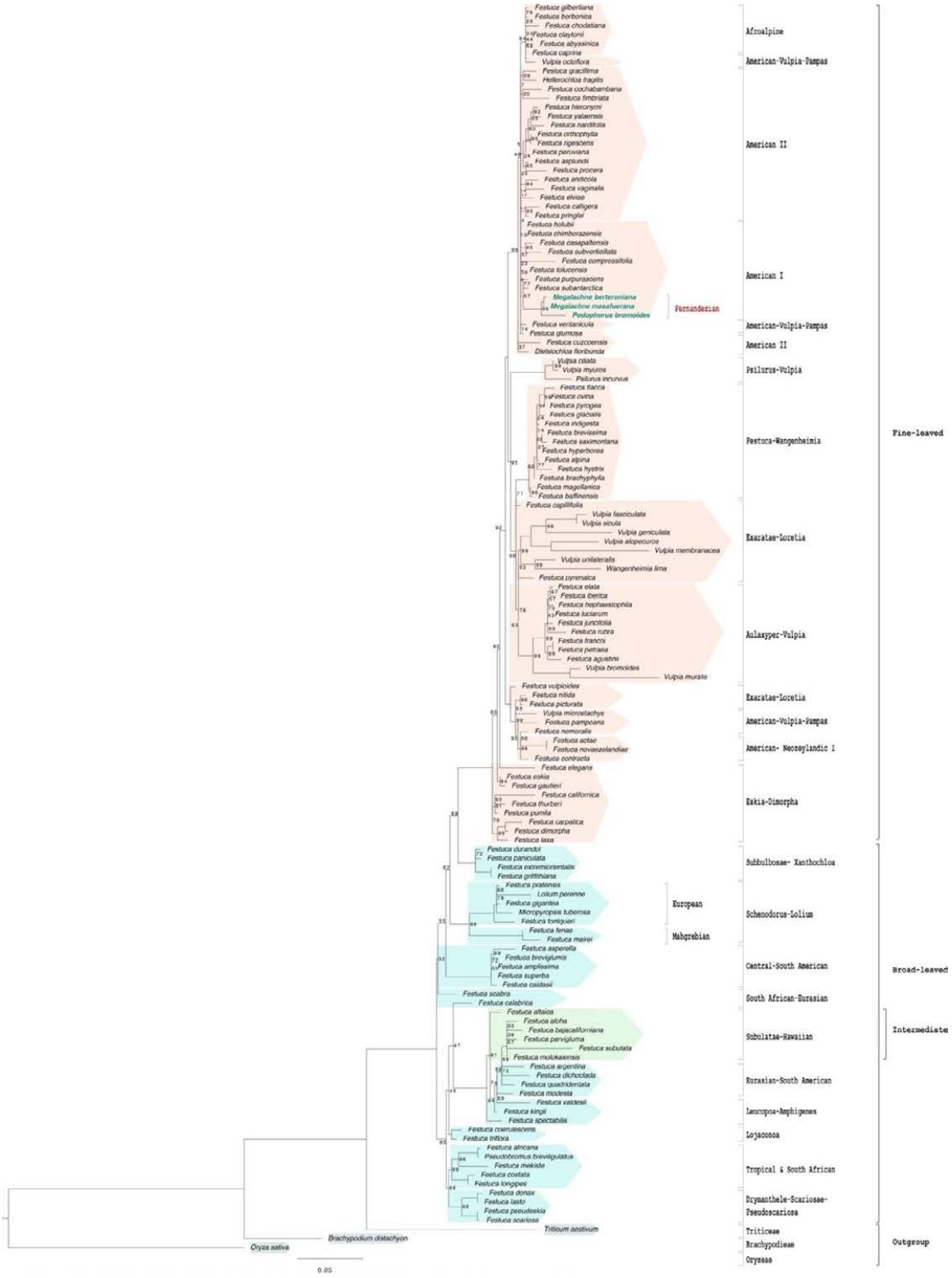


Suppl. Fig. 1D. Maximum likelihood nuclear ITS cladogram (36 Loliinae taxa, *Podophorus* included) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses.
Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).

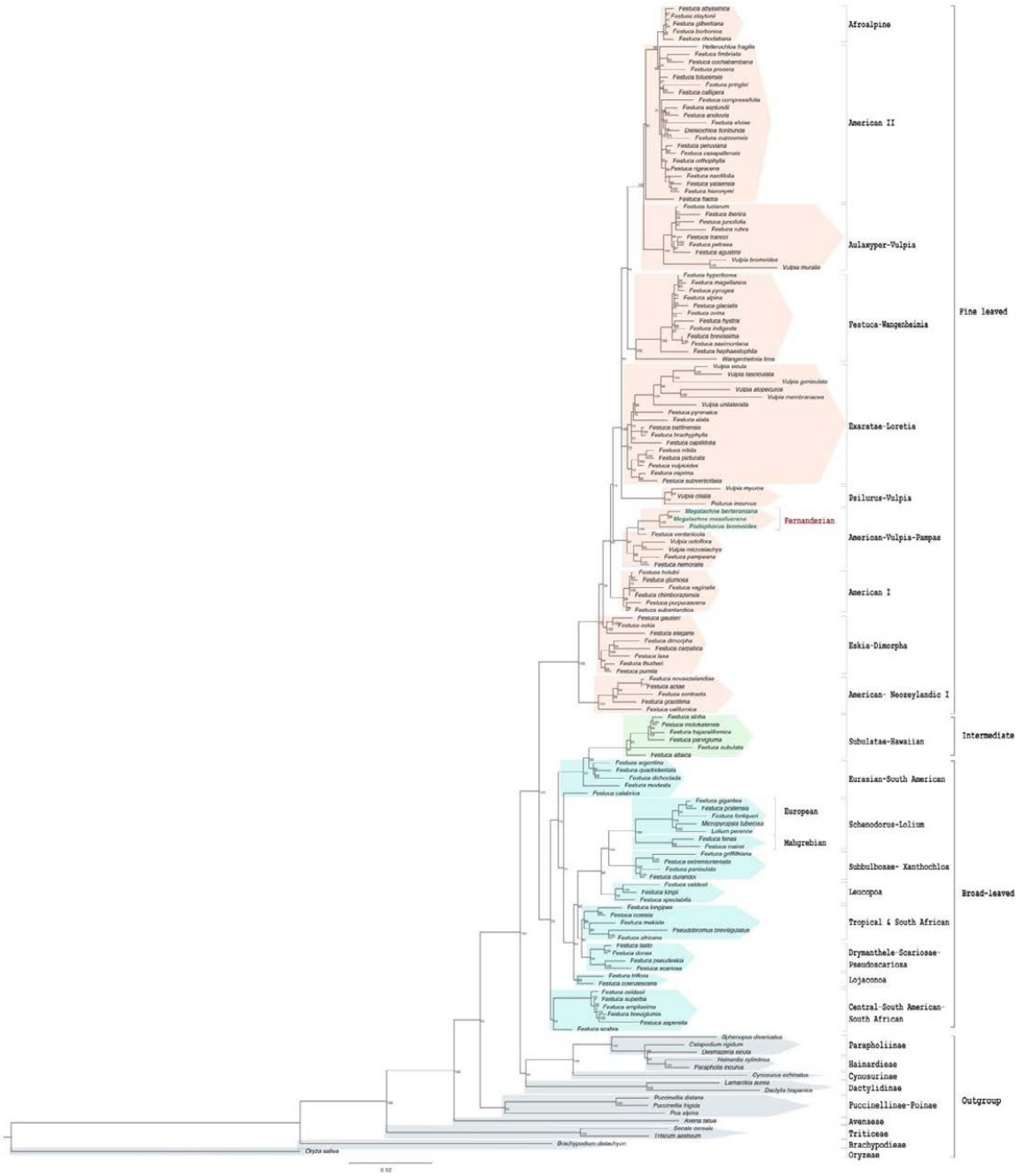
Supplementary Figure 1D. Maximum likelihood nuclear ITS cladogram (36 Loliinae taxa, Podophorus included) constructed with IQTREE showing the relationships among the studied Fernandez and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS).



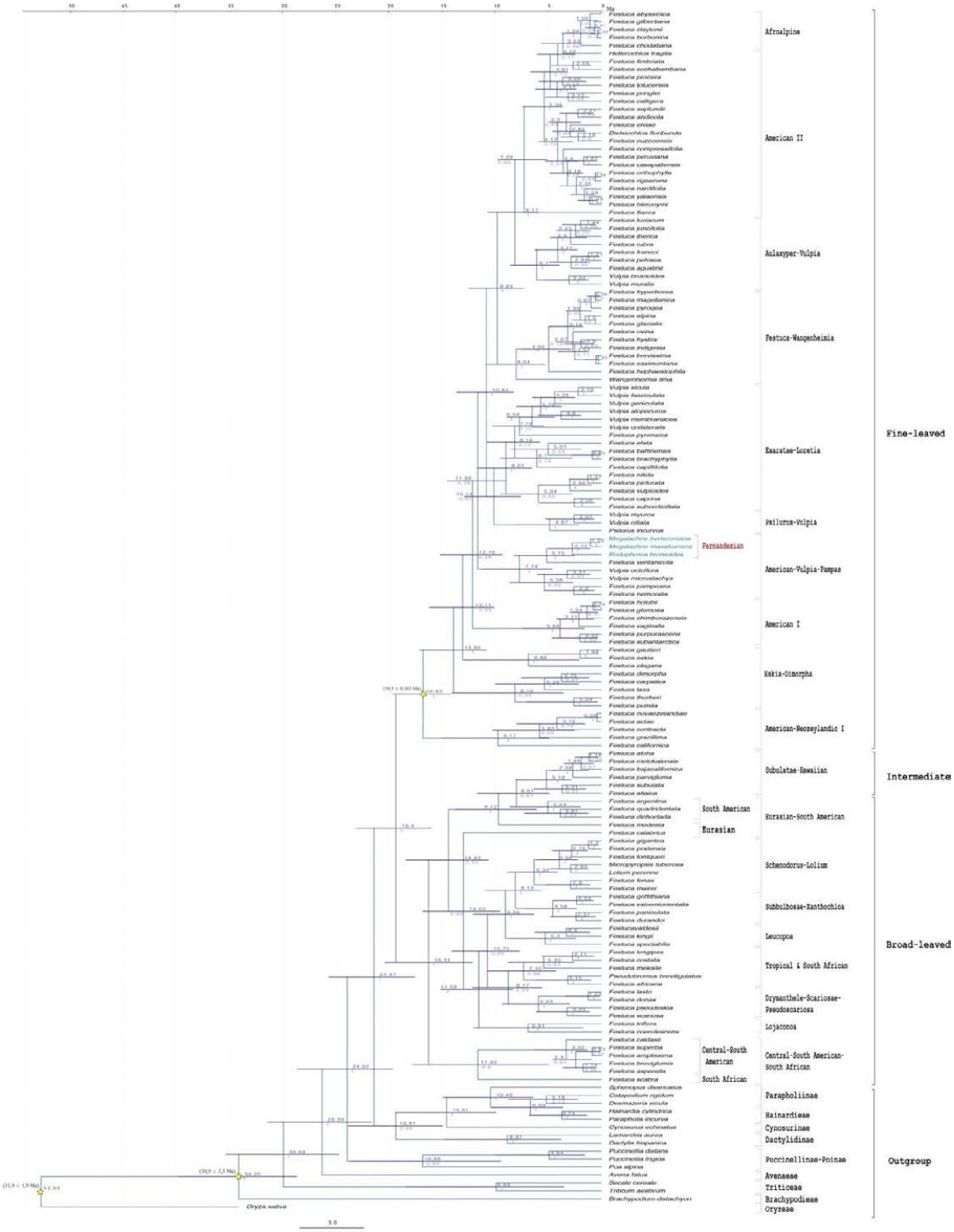
Supplementary Figure 2A. Maximum likelihood plastid TLF tree (135 taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) < 100%; the remaining branches have 100% BS values.



Supplementary Figure 2B. Maximum likelihood nuclear ITS tree (135 taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) < 100%; the remaining branches have 100% BS values.



Supplementary Figure 2C. Maximum likelihood combined ITS-TLF tree (135 taxa) constructed with IQTREE showing the relationships among the studied Fernandezian and Loliinae grasses. Oryza sativa was used to root the trees. Numbers indicate branches with UltraFast Bootstrap supports (BS) < 100%; the remaining branches have 100% BS values.



Supplementary Figure 3. Fully expanded Bayesian maximum clade credibility dated chronogram of 135 Loliinae taxa constructed with Beast2 using nuclear ITS and plastid TLF loci showing estimated nodal divergence times (medians, in Ma) and 95% highest posterior density (HPD) intervals (bars) above branches and Posterior Probability Support (PPT) values below branches. Stars indicate secondary nodal calibration priors (means +- SD, in Mya) for the crown nodes of the BOP, Brachypodium+ core pooids, and fine-leaved Loliinae clades..

Suppl. Table 1. List of taxa included in the phylogenetic study of the Fernandezian and other Loliinae grasses. Taxon, source, ploidy level, nuclear ITS, plastid *trn*TL and *trn*LF, plastome and rDNA cistron Genbank codes, average alignment insert size, total number of pair-end reads, number of plastome assembled reads, and number of rDNA cistron assembled reads are indicated for the corresponding samples.

Taxon	Source	Ploidy	GenBank Accession code			GenBank/Phytozome Accession code			Average alignment insert size	Total number of pair-end reads	Number of plastome assembled reads	Number of rDNA cistron assembled reads
			ITS	trnTLF	trnTL	Plastome		rDNA cistron				
Dielsiochloa floribunda (Pilg.) Pilg.	Bolivia: La Paz; Cumbre near La Paz ; Muller J.;MA 721312	?	DQ539563	DQ631428	DQ631494	------	------	------				
Festuca abyssinica Hochst. ex A. Rich.	Tanzania: Kilimanjaro; Afroalp PopSpecNo TZ-0095-5; Afroalp CatNoFull O-DP-42737	4x	------	------	------	**SAMN14647043**	**SRR11576633**	**MT145276**	166	12041	62836	68734
Festuca actae Connor	New Zealand: Canterbury; Banks peninsula; Lake Forsyth; Lloyd K.M. 57589	6x	AY524829	AY528949	EF584998	------	------	------				
Festuca africana (Hack.) Clayton	Uganda: Gahinga; hypericum forest; Namaganda 190Vg; MHU1603; 3170m	10x	KY368800	KY368852	KY368903	**SAMN14647044**	**SRR11576632**	**MT145277**	195	13549	193396	23500
Festuca agustinii Linding.	Spain: Canarias; Tenerife; Anaga Bailadero; A.Santos	2x	AY099005	AY099003	EF584999	------	------	------				
Festuca aloha Catalán, Soreng & P.M.Peterson	USA: Hawaii; Kauai; PTBG 17678; R. Wood 1704	?	GQ162206	GQ162208	-	------	------	------				
Festuca alpina Suter	Spain: Hawaii Kauai; PTBG 17678; R. Wood 1704	2x	AF303415	AF478522	EF585001	------	------	------				
Festuca altaica Trin.	Canada: Yukon Territory; Teslin Lake; Soreng R.J. 5996; US	4x	AF532952	AF533055	EF585002	------	------	------				
Festuca amplissima Rupr.	Mexico: Chihuahua; Barranca del Cobre; Peterson P.M.; Catalan P. 17573	6x	------	------	------	**SAMN14647045**	**SRR11576621**	**MT145278**	220	12058	104462	27272
Festuca andicola Kunth	Ecuador: Azuay; Cuenca; Parque Nacional Cajas; HUTPL14032	4x	**EF584922**	**EF592955**	**EF585009**	------	------	------				
Festuca argentina (Speg.) Parodi	Argentina: Santa Cruz; Lago Argentino; Peterson P.M. 17158	4x	EF584923	EF592957	EF585012	------	------	------				
Festuca arundinacea Schreb. subsp. *arundinacea* var. *letourneuxiana* (St.-Yves) Torrecilla & Catalán	Morocco: Atlas Mountains, ABY BN400	10x	------	------	------	**SAMN14647059**	**SRR11576625**	**MT145292**	250	16839	97892	145790
Festuca asperella E.B. Alexeev	Mexico: Mexico DF; Morelos boundary; Trott et al. 142; MO 2744225	?	KY368796	KY368848	-	------	------	------				
Festuca asplundii E.B. Alexeev	Ecuador: Loja; Saraguro; Vía Urdaneta-Yacuambi	6x	------	------	------	**SAMN14647046**	**SRR11576610**	**MT145279**	300	25088	169540	24566
Festuca baffinensis Polunin	Canada: Northwest Masik River Valley; Gillespie L.J. & al.7116	4x	EF584925	EF592958	EF585013	------	------	------				
Festuca bajacalifornica Gonz.-Led. & S.D. Koch	Mexico : Peterson; 5287; US	?	KY368814	KY368865	KY368914	------	------	------				
Festuca borbonica Spreng.	Mascarenes: Reunion Island; Besnard GB03-2013	?	KY368840	KY368892	KY368939	------	------	------				
Festuca brachyphylla Schult. & Schult. f.	USA: Alaska; North Slope Borough Prudhoe Bay; Soreng R.J. 6243 US	6x	EF584927	KY368876	EF585016							
Festuca breviglumis Swallen	Mexico: Jalisco; Ciudad Guzman; Peterson P.M.; Rosales O.16078	?	AY367952	EF592960	EF585017	------	------	------				
Festuca brevissima Jurtzev	USA: Alaska; Denali Borough Alaska Range; Soreng R.J.6021 US	2x	EF584928	EF592961	EF585018	------	------	------				
Festuca calabrica Huter, Porta & Rigo	Italy: Calabria; Cosenza; Muller J. 10838	?	KY368798	KY368850	KY368901	------	------	------				

Festuca caldasii (Kunth) Kunth	Ecuador: Catamayo; route Las Chinchas -Tambara	?	------	------	------	**SAMN14647047**	**SRR11576604**	**MT145280**	248	9863	158092	17516
Festuca californica Vasey	USA: Oregon; Benton County; Wilson B. 7014	8x	AF532956	AF533054	EF585020	------	------	------				
Festuca calligera (Piper) Rydb.	USA: New Mexico; Sangre Cristo Mts; Allred K. 8262	4x	EF584929	EF592962	EF585021	------	------	------				
Festuca capillifolia Dufour ex Roem. & Schult.	Morocco : Middle Atlas; Ifrane National Park; PC 77.17	2x	------	------	------	**SAMN14647048**	**SRR11576603**	**MT145281**	228	13430	183114	150400
Festuca caprina Nees	South Africa: Eastern Cape; Catalan & Pimentel; UZ-SA026	4x	KY368825	KY368877	KY368923	------	------	------				
Festuca carpatica F. Dietr.	Slovak Republic: VysokeL; Tatry Mts. Tisovnice; Marhold K. s.n.	4x	AY099006	AY099001	EF585023	------	------	------				
Festuca casapaltensis Ball	Peru: Lima; Canta Cordillera Viuda; Peterson 20289; Soreng; and Romashchenko. US	?	KY368832	KY368884	KY368930	------	------	------				
Festuca chimborazensis E.B. Alexeev	Ecuador: Riobamba; route Chimborazo-Cotopaxi; HUTPL14066	4x	------	------	------	**SAMN14647049**	**SRR11576602**	**MT145282**	254	10913	311132	41408
Festuca chodatiana (St.-Yves) E.B. Alexeev	Uganda: Elgon; Mnt. Namaganda 322E	4x	KY368842	KY368894	KY368941	------	------	------				
Festuca claytonii E.B. Alexeev	Uganda: Elgon; Mnt. Namaganda 271E	4x	KY368843	KY368895	KY368942	------	------	------				
Festuca cochabambana E.B.Alexeev	Bolivia: Cochabamba; Chapare; Muller J. 9277	?	EF584931	EF592964	EF585026	------	------	------				
Festuca coerulescens Desf.	Spain: Cadiz; Jerez de la Frontera; Catalan P. & al.; UZ-91.2000	2x	AF538363	AF533051	EF585027	------	------	------				
Festuca compressifolia J. Presl	Peru: Huancavelica; Tayacaja; Peterson P. M. 14144 & O. Tovar; MO 5750428	?	KY368833	KY368885	KY368931	------	------	------				
Festuca contracta Kirk	South Georgia Islands: HU Peter s/n; JE.	6x	KY368819	KY368870	-	------	------	------				
Festuca costata Nees	South Africa: Eastern Cape; Amathole Mnts.; Stuttenheim 2; Catalan & Pimentel; UZ-SA024	4x	KY368801	KY368853	KY368904	------	------	------				
Festuca cuzcoensis Stančík & P.M. Peterson	Peru: Cuzco; Calca; Peterson P.M. & Refulgio-Rodriguez 16582	?	EF584932	EF592966	EF585029	------	------	------				
Festuca dichoclada Pilg.	Peru: Junin; Tarma Maraynioc; Peterson P.M.; Tovar O. 14056; US-3421417	?	EF584933	EF592967	EF585031	------	------	------				
Festuca dimorpha Guss.	France: Alpes de Haute-Provence Col des Champs; Korneck D. s.n; Herb. Muller J. 10969	2x,4x	AF519982	AF519987	EF585032	------	------	------				
Festuca donax Lowe	Portugal: Madeira: Porto Moniz; Catalan P. & Sequeira M., UZ-MS4515	2x	EF584935	EF592968	EF585033	------	------	------				
Festuca durandoi Clauson	Portugal: Serra Arga; Alto do Espinheiro	2x	------	------	------	**SAMN14647050**	**SRR11576601**	**MT145283**	217	12688	42884	153004
Festuca elata Keng ex E.B. Alexeev	China: Yunnan; Lushui; Gaoligong Shan; Soreng R.J.& al. 5268; US-3420890	?	EF584937	EF592970	EF585037	------	------	------				
Festuca elegans Boiss.	Spain. Granada; Baza; Cebolla & Rivas-Martinez s.n.;UAM	2x,4x	AF303406	AF478509	EF585038	------	------	------				
Festuca elviae Briceño	Venezuela: Mérida; Laguna de Coromoto; Catalán P. s.n. MERC	4x		AF543517	EF585039	------	------	------				
Festuca eskia Ramond ex DC.	Spain: León; Picos de Europa: Colladines 3; FE321	2x	------	------	------	**SAMN14647051**	**SRR11576600**	**MT145284**	300	24041	215594	304740
Festuca extremiorientalis Ohwi	Russia: Krasnoyarsk; Pen'kovskaya & N Sozinova; LE	4x	KY368812	KY368863	KY368912	------	------	------				
Festuca fenas Lag.	Spain: W Mediterranean; PI289654	4x	------	------	------	**SAMN14647052**	**SRR11576599**	**MT145285**	271	16112	221450	61950
Festuca fimbriata Nees	Argentina: Misiones; Dpto. Apóstoles; Marcelo Kostling 44; UZ 498.08	6x	------	------	------	**SAMN14647053**	**SRR11576631**	**MT145286**	173	15741	64234	41572

Festuca flacca Hack. ex E.B. Alexeev	Ecuador: Pichincha; Amaguana; Volcano Pasochoa; Stancik D.; US-3428939	4x	EF584938	EF592971	EF585041	------	------	------				
Festuca fontqueri St.-Yves	Morocco: Rif Mountains; Talassemtane National Park; Outa-El-Kadir to Dj. Lakraa	2x	------	------	------	**SAMN14647054**	**SRR11576630**	**MT145287**	300	22187	721238	214938
Festuca francoi Fern. Prieto, C. Aguiar, E. Días & M.I. Gut.	Portugal: Açores: Terceira, Caldeira de St. Bárbara, nas cristas expostas e taludes do último 1 km até ao topo, Sequeira, MS4403	2x	------	------	------	**SAMN14647057**	**SRR11576627**	**MT145290**	186	17592	213218	77606
Festuca gautieri (Hack.) K. Richt.	Spain: Girona Pyrenees; Nuria; Catalan P.; Mirones V. UZ	2x	AF303414	AF478507	EF585044	------	------	------				
Festuca gigantea (L.) Vill.	Spain: Navarra; Arce; Aizpuru & Catalan; UZ s. n.	6x	AF303416	AF533043	EF379003	------	------	------				
Festuca gilbertiana E.B. Alexeev ex S.M. Phillips	Ethiopia: Simen; Namaganda	?	KY368844	KY368896	-	------	------	------				
Festuca glacialis Miégev. ex Bureau	Spain: Huesca Pyrenees; Cotiella; Catalan P. 2002	2x	AF303428	AF478523	EF585045	------	------	------				
Festuca glumosa Hack. ex E.B. Alexeev	Ecuador: Imbabura; Cayambe Volcano Cayambe; Stancik.; US-3428930	4x	EF584940	EF592973	EF585046	------	------	------				
Festuca gracillima Hook. f.	Argentina: Tierra de Fuego; Catalan. UZ- 482.08	6x	------	------	------	**SAMN14647055**	**SRR11576629**	**MT145288**	224	13888	167296	93250
Festuca griffithiana (St.-Yves) Krivot.	Afghanistan: Hajigak pass. Furse 8489; LE	?	KY368813	KY368864	KY368913	------	------	------				
Festuca hephaestophila Nees (Nees)	Mexico: Nuevo Leon; Sierra Madre Oriental; Peterson P.M.;Knowless M.B. 13347; US	?	EF584943	EF592976	EF585048	------	------	------				
Festuca hieronymi Hack.	Argentina: Córdoba; Yacanto de Calamuchita. Catalan P. UZ-383.08	6x	KY368835	KY368887	KY368933	------	------	------				
Festuca holubii Stančík	Ecuador: Saraguro; route to Cerro de Arcos; HUTPL14071	?	------	------	------	**SAMN14647056**	**SRR11576628**	**MT145289**	249	10264	253806	56342
Festuca hyperborea Holmen ex Fred.	Canada: Northwest Territories; Prince Patrick Island; Gillespie L.J.; Consaul L.L. 6893	4x	EF584946	EF592978	EF585050	------	------	------				
Festuca hystrix Boiss.	Spain: Almeria; Sierra de Gador; Catalan P. & al.; UZ-31.2000	2x	AF478480	AF478520	EF585051	------	------	------				
Festuca iberica (Hack.) K. Richt.	Spain: Granada; Sierra Nevada; Borreguiles de S. Juan; Catalan P. & al.; UZ- 77.2000	6x	AY118087	AF478516	EF585052	------	------	------				
Festuca indigesta Boiss.	Spain: Granada; Sierra Nevada; Catalan P. & al.; UZ-43.2000	6x	AF303426	AF478519	EF585054	------	------	------				
Festuca juncifolia Chaub.	Spain: Lugo Viveiro; Brieiro; Arenales de Area; López-Rodríguez J.A. 1366; UZ	8x	AF478478	AF478515	EF585057	------	------	------				
Festuca kingii (S. Watson) Cassidy	USA: Colorado; Boulder Co; Flat Irons; Catalan P. UZ-1.93	8x	AF303410	AY099004	EF585058	------	------	------				
Festuca lasto Boiss.	Spain: Cadiz; Jerez de la Frontera: Los Alcornocales	2x	------	------	------	**SAMN14647058**	**SRR11576626**	**MT145291**	300	21581	224446	56486
Festuca laxa Host	Slovak Republic: Veliki Stador; Gutermann et al. 38483	4x	KY368816	KY368867	KY368917	------	------	------				
Festuca longipes Stapf	South Africa: Cape Province; Encobo district. Catalan & Pimentel. UZ- SA031	?	**KY368804**	**KY368856**	**KY368907**	------	------	------				
Festuca luciarum Connor	New Zealand: Maungaharuru Range; Hawkes Bay; LloydK.M. 57621	8x	AY524828	AY528939	EF585064	------	------	------				
Festuca magellanica Lam.	Argentina: Tierra de Fuego; Ctra. Haberton; Catalan & A. Sarria UZ-461.08	6x,8x	KY368829	KY368881	KY368927	------	------	------				
Festuca mairei St.-Yves	Morocco: Mahgrebian; PI-610941	4x	------	------	------	**SAMN14647060**	**SRR11576624**	**MT145293**	254	19134	302524	91040
Festuca mekiste Clayton	Kenya: Mt. Elgon National Park; Carvalho 4521	?	KY368805	KY368857	KY368908	------	------	------				
Festuca modesta Nees ex Steud.	China: Yunnan Fugong (Bijiang); Soreng R.J. & al. 5227 US-3420887	2x	EF584953	EF592985	EF585068	------	------	------				

Festuca molokaiensis Soreng, P.M. Peterson & Catalán	USA: Hawai: Molokai; BISH 728771	?	------	------	------	**SAMN14647061**	**SRR11576623**	**MT145294**	219	12188	46122	43038
Festuca nardifolia Griseb.	Argentina: Salta Abra; El Acay; Peterson P.M. 10379	?	EF584954	EF592986	EF585070	------	------	------				
Festuca nemoralis Türpe	Argentina: Jujuy; Yala; Laguna Rodeo; Catalan 358.08 & J. Müller.	8x	KY368821	KY368872	KY368920	------	------	------				
Festuca nitida Kit. ex Schult.	Montenegro: Prutas; Hangmulde nordöstlich unter dem Gipfe; Gutermann et al. 38891; UZ	2x	KY368826	KY368878	KY368924	------	------	------				
Festuca novae-zelandiae (Hack.) Cockayne	New Zealand: Fiorland; Lloyd K.M. 57940	6x	AY524832	AY528941	EF585073	------	------	------				
Festuca orthophylla Pilg.	Bolivia: Tarija; Aviles; Copacabana; Muller J. 9245	8x	EF584957	EF592989	EF585075	------	------	------				
Festuca ovina L.	Russia: Leningradskaya Oblast'; Gatchinskii Raion; № 4365; coll. N.S. Probatova & A.P. Sokolovskaya; PC 54	2x	------	------	------	**SAMN14647062**	**SRR11576622**	**MT145295**	215	11364	69246	79642
Festuca pampeana Speg.	Argentina: Buenos Aires; Sierra de la Ventana; sendero al Cerro Ventana; P. Catalan 428.08	8x	------	------	------	**SAMN14647063**	**SRR11576620**	**MT145296**	223	14862	72914	235404
Festuca paniculata (L.) Schinz & Thell	Spain: Caceres; Puerto de los Castaños; UZ 40.07	2x	------	------	------	**SAMN14647064**	**SRR11576619**	**MT145297**	300	35808	473682	403042
Festuca parvigluma Steud.	China: Henan: Neixiang Xian; Baotianman Nature Reserve; dowstream from Pingfang. MO 4922557	4x	------	------	------	**SAMN14647065**	**SRR11576618**	**MT145298**	190	15872	243310	105592
Festuca peruviana Infantes	Peru: Lima; Canta; Cordillera Viuda; Peterson P.M. 20293	?	KY368836	KY368888	KY368934	------	------	------				
Festuca petraea Guthn. ex Seub	Portugal: Azores; St. Maria; Maia; Sequeira M. 4393	2x	EF584962	EF592994	EF585081	------	------	------				
Festuca picturata Pils	Austria: Tirol; Zillertaler Alpen Plauener Hütte; Schonswetter & Katharina; UZ 11.08	2x	KY368827	KY368879	KY368925	------	------	------				
Festuca pratensis Huds.	England: USDA/283306	2x	------	------	------	**SAMN14647066**	**SRR11576617**	**MT145299**	271	30021	245576	112544
Festuca pringlei St.-Yves	Mexico: Zacatecas; Peterson P.M. 21440; US	?	AF303421	AF478503	EF379007	------	------	------				
Festuca procera Kunth	Ecuador: Bolivar; P.M. Peterson 9297 & E.J. Judziewicz; MO 3853384	4x	------	------	------	**SAMN14647067**	**SRR11576616**	**MT145300**	263	12189	675414	278236
Festuca pseudeskia Boiss.	Spain: Granada; Sierra Nevada; Collado del Diablo; Catalan P. & al.; UZ-73.2000	2x	AF519979	AY099000	EF585084	------	------	------				
Festuca pumila Chaix	Slovak Republic: Veliki Stador. Gutermann 38499, UZ 4.08	2x	KY368817	KY368868	KY368918	------	------	------				
Festuca purpurascens Banks & Sol. ex Hook. f.	Argentina: Santa Cruz; Lago Argentino; Peterson P.M. 17147	6x	EF584964	EF592996	EF585087	------	------	------				
Festuca pyrenaica Reut.	Spain: Huesca; P. N. Ordesa; Tobacor	4x	------	------	------	**SAMN14647068**	**SRR11576615**	**MT145301**	300	40669	1104654	133298
Festuca pyrogea Speg.	Argentina: Tierra de fuego; Cabo San Pablo; 494.08; 30/12/2008	?	------	------	------	**SAMN14647069**	**SRR11576614**	**MT145302**	193	16835	31716	90354
Festuca quadridentata Kunth	Ecuador: Chimborazo; Alao; 3200 msnm; Coll. M. Acosta Solis; 01/03/ 1944; NMNH-SI 1911313	?	------	------	------	**SAMN14647070**	**SRR11576613**	**MT145303**	130	15091	61858	14906
Festuca rigescens (J. Presl) Kunth	Peru: Ancash; Recuay; Cordillera Blanca; Peterson;Refulio Rodriguez; US-3423058	?	EF584966	EF592999	KY368936	------	------	------				
Festuca rubra L.	Finland: A.Kosonen (Hb.Univ.Oulu) JACA JA-474496	6x	EF584968	EF593001	EF585097	------	------	------				
Festuca saximontana Rydb.	USA: Oregon; Soreng R. J. 6021-1	6x	EF584969	EF593002	EF585098	------	------	------				
Festuca scabra Vahl	South Africa; KwaZulu Natal; Underberg; Sani Pass; Catalan & Pimentel; UZ-SA047	4x,6x	KY368797	KY368849	KY368900	------	------	------				
Festuca scariosa Lag. ex Willk.	Spain: Almeria; Sierra Filabres; Las Menas; Catalan P.& al.; UZ- 62.2000	2x	AF519978	AY098999	EF585100	------	------	------				

Festuca spectabilis Jan	Italy: Lombardia; Bergamo; Passo della Presolana; Muller J.8229	6x	------	------	------	**SAMN14647071**	**SRR11576612**	**MT145304**	221	12960	42352	90950
Festuca subantarctica Parodi	Argentina: Santa Cruz; Peterson P.M. 17163	?	EF584973	EF593004	EF585103	------	------	------				
Festuca subulata Trin.	USA: Oregon; Clatsop County; Saddle Mnts; Wilson B. 10512	2x,4x	AF532953	AF533056	EF585104	------	------	------				
Festuca subverticillata (Pers.) E.B. Alexeev	USA: West Virginia; Grant Co.; Peterson P.M.; Saarela J.M. 15784; US	6x	EF584974	EF593006	EF585106	------	------	------				
Festuca superba Parodi ex Türpe	Argentina: Jujuy; Yala; Laguna Rodeo; arroyo al W de la laguna; P. Catalan 356.08 & J. Müller.	8x	------	------	------	**SAMN14647072**	**SRR11576611**	**MT145305**	221	12193	22702	54668
Festuca thurberi Vasey	USA: New Mexico; Sangre Cristo; Mts; Allred K. 8257	4x,6x	EF584975	EF593007	EF585107	------	------	------				
Festuca tolucensis Kunth	Venezuela: Mérida; Páramo de Piedras Blancas; Catalan P.; UZ-99.2000; MERC	4x	EF584976	EF593038	EF585108	------	------	------				
Festuca triflora J.F. Gmel.	Morocco: Rif Mountains; Bab Barret-Ketama; P.Catalan & Mario Mairal; PC 39.17	2x	------	------	------	**SAMN14647073**	**SRR11576609**	**MT145306**	300	24472	543924	145104
Festuca vaginalis (Benth.) Laegaard	Ecuador: Chimborazo; Riobamba; Vía Chimborazo-Guaranda; HUTPL14121	4x	**EF584977**	**EF593010**	**EF585111**	------	------	------				
Festuca valdesii Gonz.-Led. & S.D. Koch	México: Cohauila P.M. Peterson 21456 US	?	**MT022521**	**MT040974**	**MT040975**	------	------	------				
Festuca ventanicola Speg.	Argentina: Buenos Aires; Sierra de la Ventana; Catalan UZ- 418.08	6x	KY368823	KY368874	KY368922	------	------	------				
Festuca vulpioides Steud.	South Africa: Eastern Cape; Amathole Mnts. Catalan & Pimentel; UZ- SA032	?	KY368828	KY368880	KY368926	------	------	------				
Festuca yalaensis Joch. Müll. & Catalán	Argentina: Jujuy; Yala; Laguna Rodeo; Catalan & Müller; UZ-358.08	?	GQ849279	GQ849280	GQ849281	------	------	------				
Hellerochloa fragilis (Luces) Rauschert	Venezuela: Mérida; Páramo de Piedras Blancas; Catalán P. s.n. MERC	?	AF303401	AF478504	EF379024	------	------	------				
Lolium perenne L.	UK: Wales (cutivated seeds PI 619001 USDA-Pullman)	2x	AF303401	AF478504	EF379024	------	------	------				
Megalachne berteroniana Steud.	Chile: Juan Fernandez archipelago; Masatierra; Cordón; Salsipuedes. Leg. T. Stuessy; D. Crawford; P. López; J. Soto. 11751 (0S)	?	------	------	------	**SAMN14647074**	**SRR11576608**	**MT145307**	184	5288	34688	46952
Megalachne masafuerana (Skottsb. & Pilg. ex. Pilg.) Matthei	Chile: Juan Fernandez archipelago; Masafuera; Cuchillo del Iman. Leg. A; Landero; L. Gaete. 9150 (0S)	?	------	------	------	**SAMN14647075**	**SRR11576607**	**MT145308**	184	6134	81518	48050
Podophorus bromoides Phil.	Chile: Juan Fernandez archipielago; Masatierra	?	**MT022522**	**Github**	**Github**	**SAMN14668162**	**SRR11586977**	------	197	6694	228488	-----
Micropyropsis tuberosa Romero-Zarco & Cabezudo	Spain: Huelva; Almonte; Catalan P. & al.; UZ-89.07	2x	JQ972944	AF533037.1	EF379013	------	------	------				
Pseudobromus breviligulatus Stapf ex A. Camus	Madagascar: Andringitra National Park; Vorontsova F1221	?	KY368806	KY368858	KY368909	------	------	------				
Psilurus incurvus (Gouan) Schinz & Thell.	Spain: Malpartida de Plasencia. UZ 31.07	4x	JQ972945	AF478533	JQ973013	------	------	------				
Vulpia alopecuros (Schousb.) Dumort.	Portugal: Algave; Meia Praia Lagos; Stace C.A.; LEI	2x	AF478491	AF487617	EF585117	------	------	------				
Vulpia bromoides (L.) Gray	Spain: Lugo; Lancara; Lopez Rodrıguez J.A. 01080; UZ	2x	AF478485	AF487616	EF585119	------	------	------				
Vulpia ciliata Dumort.	Spain: Toledo; Mar de Ontígola; UZ 109.07	4x	------	------	------	**SAMN14647076**	**SRR11576606**	**MT145309**	220	11801	83344	104788
Vulpia fasciculata (Forsk.) Samp.	Spain: Barcelona; Vilanova; Pyke S. 15.2000; UZ	4x	AF478487	AF478528	EF585121	------	------	------				
Vulpia geniculata (L.) Link	Spain: Sevilla; Constantina; JACA J29397	2x	AF478490	AF478531	EF585123	------	------	------				
Vulpia membranacea (L.) Dumort.	Spain: Cadiz; Sanlucar de Barrameda; La Algaida; Catalan P.; UZ-8.2002;	2x	AY118090	AY118101	EF585124	------	------	------				

Vulpia microstachys (Nutt.) Munro	USA: California; W of San Luis Obispo; Soreng R.J. 7406	?	EF584981	EF593015	EF585125	------	------	------				
Vulpia muralis (Kunth) Nees	Spain: Zaragoza; Actur; Pyke & Catalan UZ-11.2000	2x	AY118091	AY118102	EF585126	------	------	------				
Vulpia myuros (L.) C.C. Gmel.	USA: Washington; King Co Seattle Lake Forest Park; Catalan P. UZ-54.2001	6x	AY118092	AY118103	EF585127	------	------	------				
Vulpia octoflora (Walter) Rydb.	USA: Washington; Okanogan; Peterson P.M. 3263	2x	EF584982.1	EF593016	EF585128	------	------	------				
Vulpia sicula (C. Presl) Link	Italia: Sicilia; Piano Battaglia - Battagliete; Madone	2x	------	------	------	**SAMN14647077**	**SRR11576605**	**MT145310**	223	11327	82474	148794
Vulpia unilateralis (L.) Stace	Spain: Zaragoza; Vedado de Peñaflor; Catalan P.; UZ-18.2002	2x	AY118095	AY118106	EF585130	------	------	------				
Wangenheimia lima (L.) Trin.	Spain: Zaragoza; Vedado de Peñaflor; Catalan P. & al.; UZ-17.2000	2x	AF478498	AF478536	EF585131	------	------	------				
Outgroups												
Avena fatua L.	Russia	6x	FJ794719.1	JF904749.1	JF904749.1	------	------	------				
Brachypodium distachyon (L.) P.Beauv.	Spain: Caceres; UZ 28.07	2x	AF303399	AF478500	DQ336855	NC_011032.1	NC_011032.1	Phytozome Bd21 v.3.1				
Catapodium rigidum (L.) C.E. Hubb.	Spain: Segovia; Sepúlveda; López Rodríguez J.A. s.n.; UZ	2x	AF532940	AF533034	EF584986	------	------	------				
Cynosurus echinatus L.	Spain: Soria; Monte Valonsadero; López Rodríguez J.A. s.n.; UZ	2x	AF532937	AF533031	EF584993	------	------	------				
Dactylis glomerata subsp. *hispanica* (Roth) Nyman	Spain: Zaragoza; Peñaflor; Catalán P. & al. s.n.; UZ	2x	AF393014	AF533027	EF584994	------	------	------				
Desmazeria sicula (Jacq.) Dumort.	Seed Bank; Royal Botanic Gardens Kew;17332; Soreng R.J. s.n. (BH)	2x	EF584917	EF592948	EF584989	------	------	------				
Hainardia cylindrica (Willd.) Greuter	Spain: Zaragoza; Parque Tio Jorge; Pyke S. s.n.; UZ	4x	AF532941	AF533035	EF584990	------	------	------				
Lamarckia aurea (L.) Moench	Spain: Zaragoza; Puenete de la Almozara; PC 14.2000; UZ	2x	AF532935	AF533029	EF584995	------	------	------				
Oryza sativa L.	China: National Hybrid Rice Research and Development Center (NHRRDC); China	2x	EF221613.1	EF137577.1	DQ415935	AY522331.1	AY522331.1	AP008215				
Parapholis incurva (L.) C.E. Hubb.	Spain: Zaragoza; Vedado de Pen˜aflor; Catalán P. & al. 23;2000; UZ	4x	AF532942	AF533036	EF584991	------	------	------				
Poa alpina L.	Andorra: Ordino; Llamas; Acedo & Alonso; 62; LEB	2x, 4x, 6x	EU792390	AY504635	DQ353986	------	------	------				
Puccinellia distans (L.) Parl.	Spain: Navarra; Lazagurría; JACA207897	2x, 4x, 6x	AF532934	AF533024	EF584985	------	------	------				
Puccinellia frigida (Phil.) I.M. Johnst.	Genbank	?	KF020797	JF904754	JF904753	------	------	------				
Secale cereale L.	USDA	2x	AF303400	AF478501	DQ336856	------	------	------				
Sphenopus divaricatus (Gouan) Rchb.	Spain; Zaragoza; Vedado de Penñaflor; Catalán P. & al. s.n.;UZ	2x, 4x	AF532939	AF533033	EF584992	------	------	------				
Triticum aestivum L.	K. Tanno RIB-15296S	6x	DQ981410	AB732940	JQ973001	------	------	------				

Suppl. Table 2a. Operational areas used in the stratified Loliinae DEC Lagrange analysis

Taxon	**Operational areas**												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Dielsiochloa floribunda (Pilg.) Pilg.	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca abyssinica Hochst. ex A. Rich.	0	0	1	0	0	0	0	0	0	0	0	0	0
Festuca actae Connor	0	0	0	0	0	0	1	0	0	0	0	0	0
Festuca africana (Hack.) Clayton	0	1	0	1	0	0	0	0	0	0	0	0	0
Festuca agustinii Linding.	0	0	0	0	0	0	0	0	0	0	1	0	0
Festuca aloha Catalán, Soreng & P.M.Peterson	0	0	0	0	0	0	0	0	0	0	0	1	0
Festuca alpina Suter	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca altaica Trin.	0	0	0	0	0	1	0	1	0	0	0	0	0
Festuca amplissima Rupr.	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca andicola Kunth	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca argentina (Speg.) Parodi	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca asperella E.B. Alexeev	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca asplundii E.B. Alexeev	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca baffinensis Polunin	0	0	0	0	0	1	0	1	0	0	0	0	0
Festuca bajacalifornіana Gonz.-Led. & S.D. Koch	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca borbonica Spreng.	0	1	0	0	0	0	0	0	0	0	0	0	0
Festuca brachyphylla Schult. & Schult. f.	0	0	0	0	0	1	0	1	0	0	0	0	0
Festuca breviglumis Swallen	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca brevissima Jurtzev	0	0	0	0	0	1	0	1	0	0	0	0	0
Festuca calabrica Huter, Porta & Rigo	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca caldasii (Kunth) Kunth	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca californica Vasey	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca calligera (Piper) Rydb.	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca capillifolia Dufour ex Roem. & Schult.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca caprina Nees	1	0	1	0	0	0	0	0	0	0	0	0	0
Festuca carpatica F. Dietr.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca casapaltensis Ball	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca chimborazensis E.B. Alexeev	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca chodatiana (St.-Yves) E.B.Alexeev	0	0	1	0	0	0	0	0	0	0	0	0	0
Festuca claytonii E.B.Alexeev	0	0	1	0	0	0	0	0	0	0	0	0	0
Festuca cochabambana E.B. Alexeev	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca coerulescens Desf.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca compressifolia J. Presl	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca contracta Kirk	0	0	0	0	0	0	1	0	0	1	0	0	0
Festuca costata Nees	1	0	1	0	0	0	0	0	0	0	0	0	0
Festuca cuzcoensis Stančík & P.M. Peterson	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca dichoclada Pilg.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca dimorpha Guss.	0	0	0	1	0	0	0	0	0	0	0	0	0

Festuca donax Lowe	0	0	0	0	0	0	0	0	0	0	1	0	0
Festuca durandoi Clauson	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca elata Keng ex E.B. Alexeev	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca elegans Boiss.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca elviae Briceño	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca eskia Ramond ex DC.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca extremiorientalis Ohwi	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca fenas Lag.	0	0	0	0	1	0	0	0	0	0	0	0	0
Festuca fimbriata Nees	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca flacca Hack. ex E.B. Alexeev	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca fontqueri St.-Yves	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca francoi Fern. Prieto, C. Aguiar, E. Días & M.I. Gut.	0	0	0	0	0	0	0	0	0	0	0	1	0
Festuca gautieri (Hack.) K. Richt.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca gigantea (L.) Vill.	0	0	0	0	1	1	0	0	0	0	0	0	0
Festuca gilbertiana E.B. Alexeev ex S.M. Phillips	0	0	1	0	0	0	0	0	0	0	0	0	0
Festuca glacialis Miégev. ex Bureau	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca glumosa Hack. ex E.B. Alexeev	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca gracillima Hook. f.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca griffithiana (St.-Yves) Krivot.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca hephaestophila Nees (Nees)	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca hieronymi Hack.	0	0	0	0	0	0	0	0	0	0	1	0	0
Festuca holubii Stančík	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca hyperborea Holmen ex Fred.	0	0	0	0	0	1	0	1	0	0	0	0	0
Festuca hystrix Boiss.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca iberica (Hack.) K. Richt.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca indigesta Boiss.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca juncifolia Chaub.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca kingii (S. Watson) Cassidy	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca lasto Boiss.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca laxa Host	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca longipes Stapf	1	0	0	0	0	0	0	0	0	0	0	0	0
Festuca luciarum Connor	0	0	0	0	0	0	1	0	0	0	0	0	0
Festuca magellanica Lam.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca mairei St.-Yves	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca mekiste Clayton	0	0	1	0	0	0	0	0	0	0	0	0	0
Festuca modesta Nees ex Steud.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca molokaiensis Soreng, P.M. Peterson & Catalán	0	0	0	0	0	0	0	0	0	0	0	1	0
Festuca nardifolia Griseb.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca nemoralis Türpe	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca nitida Kit. ex Schult.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca novae-zelandiae (Hack.) Cockayne	0	0	0	0	0	0	1	0	0	0	0	0	0
Festuca orthophylla Pilg.	0	0	0	0	0	0	0	0	0	0	1	0	0

Festuca ovina L.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca pampeana Speg.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca paniculata (L.) Schinz & Thell	0	0	0	1	0	1	0	0	0	0	0	0	0
Festuca parvigluma Steud.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca peruviana Infantes	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca petraea Guthn. ex Seub	0	0	0	0	0	0	0	0	0	0	1	0	0
Festuca picturata Pils	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca pratensis Huds.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca pringlei St.-Yves	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca procera Kunth	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca pseudeskia Boiss.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca pumila Chaix	0	0	1	0	0	1	0	0	0	0	0	0	0
Festuca purpurascens Banks & Sol. ex Hook. f.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca pyrenaica Reut.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca pyrogea Speg.	0	0	0	0	0	0	0	0	0	0	1	0	0
Festuca quadridentata Kunth	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca rigescens (J. Presl) Kunth	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca rubra L.	0	0	0	0	0	1	0	0	0	0	0	0	0
Festuca saximontana Rydb.	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca scabra Vahl	1	0	0	0	0	0	0	0	0	0	0	0	0
Festuca scariosa Lag. ex Willk.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca spectabilis Jan	0	0	0	1	0	1	0	0	0	0	0	0	0
Festuca subantarctica Parodi	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca subulata Trin.	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca subverticillata (Pers.) E.B. Alexeev	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca superba Parodi ex Türpe	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca thurberi Vasey	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca tolucensis Kunth	0	0	0	0	0	0	0	1	1	0	0	0	0
Festuca triflora J.F. Gmel.	0	0	0	1	0	0	0	0	0	0	0	0	0
Festuca vaginalis (Benth.) Laegaard	0	0	0	0	0	0	0	0	1	0	0	0	0
Festuca valdesii Gonz.-Led. & S.D. Koch	0	0	0	0	0	0	0	1	0	0	0	0	0
Festuca ventanicola Speg.	0	0	0	0	0	0	0	0	0	1	0	0	0
Festuca vulpioides Steud.	1	0	0	0	0	0	0	0	0	0	0	0	0
Festuca yalaensis Joch. Müll. & Catalán	0	0	0	0	0	0	0	0	0	1	0	0	0
Hellerochloa fragilis (Luces) Rauschert	0	0	0	0	0	0	0	0	1	0	0	0	0
Lolium perenne L.	0	0	0	0	1	1	0	0	0	0	0	0	0
Megalachne berteroniana Steud.	0	0	0	0	0	0	0	0	0	0	0	0	1
Megalachne masafuerana (Skottsb. & Pilg. ex. Pilg.) Matthei	0	0	0	0	0	0	0	0	0	0	0	0	1
Podophorus bromoides Phil.	0	0	0	1	0	0	0	0	0	0	0	0	0
Micropyropsis tuberosa Romero-Zarco & Cabezudo	0	0	0	0	0	0	0	0	0	0	0	0	1
Pseudobromus breviligulatus Stapf ex A. Camus	0	1	1	0	0	0	0	0	0	0	0	0	0

Psilurus incurvus (Gouan) Schinz & Thell.	0	0	0	1	0	1	0	0	0	0	0	0	0
Vulpia alopecuros (Schousb.) Dumort.	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia bromoides (L.) Gray	0	0	0	1	0	1	0	0	0	0	0	0	0
Vulpia ciliata Dumort.	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia fasciculata (Forsk.) Samp.	0	0	0	1	0	1	0	0	0	0	0	0	0
Vulpia geniculata (L.) Link	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia membranacea (L.) Dumort.	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia microstachys (Nutt.) Munro	0	0	0	0	0	0	0	1	0	0	0	0	0
Vulpia muralis (Kunth) Nees	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia myuros (L.) C.C. Gmel.	0	0	0	1	0	1	0	0	0	0	0	0	0
Vulpia octoflora (Walter) Rydb.	0	0	0	0	0	0	0	1	0	0	0	0	0
Vulpia sicula (C. Presl) Link	0	0	0	1	0	0	0	0	0	0	0	0	0
Vulpia unilateralis (L.) Stace	0	0	0	1	0	0	0	0	0	0	0	0	0
Wangenheimia lima (L.) Trin.	0	0	0	1	0	0	0	0	0	0	0	0	0

Suppl. Table 2b Dispersal rate matrices reflecting the palaeogeographic connectivity among the study areas in each historical scenario (time slices TSI, TSII, TSIII, TIV).

Time slice I (23 to 16 Ma)

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	--	0.5	0.75	0.01	0.25	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
B		--	0.75	0.01	0.01	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C			--	0.01	0.25	0.75	0.25	0.01	0.01	0.01	0.01	0.01	0.01
D				--	0.5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
E					--	0.75	1.0	0.01	0.01	0.01	0.01	0.01	0.01
F						--	1.0	0.5	0.01	0.01	0.01	0.01	0.01
G							--	0.5	0.25	0.75	0.01	0.01	0.01
H								--	0.01	0.01	0.01	0.01	0.01
I									--	0.25	0.01	0.01	0.01
J										--	0.5	0.25	0.01
K											--	0.75	0.01
L												--	0.01
M													--

Time slice II (16 to 7.2 Ma)

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	--	0.5	0.75	0.25	0.25	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
B		--	0.5	0.01	0.01	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C			--	0.25	0.5	0.75	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D				--	0.75	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
E					--	0.75	1.0	0.01	0.01	0.01	0.01	0.01	0.01
F						--	1.0	0.25	0.01	0.5	0.01	0.01	0.01
G							--	0.25	0.25	0.75	0.01	0.01	0.01
H								--	0.01	0.01	0.01	0.01	0.01
I									--	0.25	0.01	0.01	0.01
J										--	0.75	0.25	0.01
K											--	1.0	0.01
L												--	0.01
M													--

Time slice III (7.2 to 2.6 Ma)

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	--	0.5	0.75	0.01	0.25	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
B		--	0.5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C			--	0.01	0.5	0.75	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D				--	0.75	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
E					--	1.0	1.0	0.01	0.01	0.01	0.01	0.01	0.01
F						--	1.0	0.25	0.01	0.01	0.01	0.01	0.01
G							--	0.5	0.25	0.5	0.01	0.01	0.01

H								--	0.01	0.01	0.01	0.01	0.01
I									--	0.25	0.25	0.01	0.01
J										--	1.0	0.5	1.0
K											--	1.0	0.01
L												--	0.01
M													--
Time slice IV (2.6 Ma to present)													
	A	B	C	D	E	F	G	H	I	J	K	L	M
A	--	0.5	0.75	0.01	0.25	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
B		--	0.5	0.01	0.01	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C			--	0.01	0.25	0.75	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D				--	0.75	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01
E					--	0.75	1.0	0.01	0.01	0.01	0.01	0.01	0.01
F						--	1.0	0.5	0.01	0.5	0.01	0.01	0.01
G							--	0.75	0.25	1.0	0.01	0.01	0.01
H								--	0.01	0.01	0.01	0.01	0.01
I									--	0.25	0.25	0.01	0.01
J										--	1.0	0.75	1.0
K											--	1.0	0.01
L												--	0.01
M													--

Suppl. Table 2c Operational areas used in the stratified American-Vulpia-Pampas DEC Lagrange analysis.

Taxon	**Operational areas**				
	H	N	O	P	Q
Festuca nemoralis Türpe	0	0	1	0	0
Festuca pampeana Speg.	0	1	0	0	0
Festuca ventanicola Speg.	0	1	0	0	0
Megalachne berteroniana Steud.	0	0	0	1	0
Megalachne masafuerana (Skottsb. & Pilg. ex. Pilg.) Matthei	0	0	0	0	1
Podophorus bromoides Phil.	0	0	0	1	0
Vulpia microstachys (Nutt.) Munro	1	0	0	0	0
Vulpia octoflora (Walter) Rydb.	1	0	0	0	0

Suppl. Table 2d Dispersal rate matrices reflecting the palaeogeographic connectivity among the study areas in each historical scenario (time slices TSIII, TIV).

Time slice III (9 to 2.5 Ma)					
	H	N	O	P	Q
H	--	0.01	0.5	0.25	0.01
N		--	1.0	0.75	0.01
O			--	1.0	0.01
P				--	0.01
Q					--
Time slice IV (2.5 Ma to present)					
	H	N	O	P	Q
H	--	0.01	0.5	0.25	0.25
N		--	0.01	0.75	0.5
O			--	1.0	0.75
P				--	1.0
Q					--

Apéndice 2. Información suplementaria del Capítulo C.2.

Suppl. Table S1. Taxa included in the repeatome analysis of Loliinae. Taxonomic rank, taxon authorship, detailed localities and vouchers, and source of cytogenetic and genomic data. Group: BL, broad-leaved Loliinae; FL, fine-leaved Loliinae; Sch, Schedonorus. Chromosome number (2n), Ploidy, Genome size (2C, pg), Monoploid genome size (1Cx, pg; 1Cx, Mbp) and Genbank accession codes for plastome and nuclear ribosomal 35S and 5S genes are given for each sample. Values in bold correspond to new data generated in this study. Outgroups used in the phylogenomic analyses: *Oryza sativa, Brachypodium distachyon*.

Taxon	Code	Group	Locality	2n	Ploidy	2C (pg)	1Cx (pg)	1Cx (Mbp)	Genbank accession No.			Data source
									Plastome	35S rDNA	5S rDNA	
Festuca africana (Hack.) Clayton	FF	Broad-Leaved	Uganda: Gahinga; Namaganda 190Vg; MHU1603	70	10x	---	---	---	SAMN14647044	MT145277	**ON248974**	2n: Namaganda et al., 2006; genome data: this study
Festuca amplissima Rupr.	GG	Broad-Leaved	Mexico: Chihuahua; Barranca del Cobre; PC. 17573	42	6x	---	---	---	SAMN14647045	MT145278	**ON248975**	2n: Bolkhouskikh et al. 1969; genome data: this study
Festuca caldasii (Kunth) Kunth	NN	Broad-Leaved	Ecuador: Catamayo; Chinchas-Tambara; HUTPL14055	28	**4x**	**20.36**	5.09	4978.02	SAMN14647047	MT145280	**ON248977**	2n, 2C, genome data: this study
Festuca durandoi Clauson	PP	Broad-Leaved	Portugal: Serra Arga; Alto do Espinheiro;	14	2x	14.66 (4x)	3.66	3584.86	SAMN14647050	MT145283	**ON248980**	2n: Devesa & Martinez-Segarra 2020; 2C: Šmarda et al., 2008; genome data: this study
Festuca lasto Boiss.	HH	Broad-Leaved	Spain:Cadiz: Jerez de la Frontera; Los Alcornocales; UZ 29.08	14	2x	---	---	---	SAMN14647058	MT145291	**ON248989**	2n: Harper et al. 2004; genome data: this study
Festuca mekiste Clayton	AM	Broad-Leaved	Kenya: Mt. Elgon National Park, Kambi Mtamaiwa; Carvalho 4521	---	---	---	---	---	**SAMN27777779**	**ON243855**	**ON248992**	genome data: this study
Festuca molokaiensis Soreng, P.M. Peterson & Catalán	II	Broad-Leaved	USA: Hawai: Molokai, BISH 728771	---	---	---	---	---	SAMN14647061	MT145294	**ON248993**	genome data: this study
Festuca paniculata (L.) Schinz & Thell	QQ	Broad-Leaved	Spain: Caceres, Puerto de los Castaños, UZ 40.07	14	2x	7.65	3.83	3740.85	SAMN14647064	MT145297	**ON248996**	2n, 2C: Šmarda et al., 2008; genome data: this study
Festuca parvigluma Steud.	JJ	Broad-Leaved	China: Baotianman; Henan; Neixiang Xian; MO 4922557	28	4x	---	---	---	SAMN14647065	MT145298	**ON248997**	2n: Tateoka 1954; genome data: this study
Festuca scabra Vahl	AP	Broad-Leaved	South Africa: KwaZulu Natal; Cathedral Peak; UZ-SA047	28	4x	---	---	---	**SAMN27777781**	**ON243857**	**ON249003**	2n: Spies and Plessis 1986; genome data: this study
Festuca spectabilis Jan	LL	Broad-Leaved	Bosnia-Hercegovina: Troglav; Sajkovacko zdrlo	42	6x	---	---	---	SAMN14647071	MT145304	**ON249004**	2n: Markgraf-Dannenberg 1980; genome data: this study
Festuca superba Parodi ex Türpe	RR	Broad-Leaved	Argentina: Jujuy; Yala; Laguna Rodeo; PC 356.08	56	8x	---	---	---	SAMN14647072	MT145305	**ON249005**	2n: Dubcovsky and Martínez, 1992; genome data: this study
Festuca triflora J.F. Gmel.	MM	Broad-Leaved	Morocco: Rif Mountains, Bab Barret-Ketama; PC 39.17	14	2x	**7.84**	3.92	3833.76	SAMN14647073	MT145306	**ON249006**	2n, 2C, genome data: this study
Festuca abyssinica Hochst. ex A. Rich.	AB	Fine-Leaved	Tanzania: Kilimanjaro; Afroalp O-DP-42737	28	4x	---	---	---	SAMN14647043	MT145276	**ON248973**	2n: Namaganda et al. 2006; genome data: this study
Festuca asplundii E.B. Alexeev	AC	Fine-Leaved	Ecuador: Loja; Saraguro; HUTPL14046	---	6x	**21.23**	3.54	3460.49	SAMN14647046	MT145279	**ON248976**	2n: Smarda & Stancik 2006; 2C, genome data: this study
Festuca capillifolia Dufour ex Roem. & Schult.	SS	Fine-Leaved	Morocco: Middle Atlas; Ifrane National Park; PC 77.17	14	2x	---	---	---	SAMN14647048	MT145281	**ON248978**	2n: Devesa & Martinez-Segarra 2020; genome data: this study
Festuca chimborazensis E.B. Alexeev subsp. *micacochensis* Stančík	AD	Fine-Leaved	Ecuador: Riobamba; Chimborazo; HUTPL14066	42	6x	**13.48**	2.25	2197.24	SAMN14647049	MT145282	**ON248979**	2n, 2C, genome data: this study
Festuca eskia Ramond ex DC.	OO	Fine-Leaved	Spain: León, Picos de Europa, Colladines, FE321	14	2x	5.7	2.85	2787.3	SAMN14647051	MT145284	**ON248981**	2n: Kerguelen 1975; 2C: Marques et al. 2016; genome data: this study
Festuca fimbriata Nees	AE	Fine-Leaved	Argentina: Misiones; Dpto. Apóstoles; UZ 498.08	42	6x	---	---	---	SAMN14647053	MT145286	**ON248983**	2n: Dubcovsky & Martinez 1992; genome data: this study

Festuca francoi Fern. Prieto, C. Aguiar, E. Días & M.I. Gut	TT	Fine-Leaved	Portugal: Açores; Terceira; MS4403	12	2x	---	---	---	SAMN14647057	MT145290	**ON248984**	2n: Sequeira et al. 2009; genome data: this study
Festuca gracillima Hook. F.	AF	Fine-Leaved	Argentina: Tierra de Fuego; Estancia San Pablo; UZ482.08	42	6x	---	---	---	SAMN14647055	MT145288	**ON248986**	2n: Dollenz 1978; genome data: this study
Festuca holubii Stančík	AG	Fine-Leaved	Ecuador: Saraguro; route to Cerro de Arcos; HUTPL14071	---	---	---	---	---	SAMN14647056	MT145289	**ON248988**	genome data: this study
Festuca ovina L.	UU	Fine-Leaved	Russia: Leningradskaya Oblast'; Gatchinskii Raion; PC 54	14	**2x**	4.82	2.41	2356.98	SAMN14647062	MT145295	**ON248994**	2n, 2C: Smarda & al. 2008; genome data: this study
Festuca pampeana Speg.	VV	Fine-Leaved	Argentina: Buenos Aires; Sierra de la Ventana; PC 428.08	56	8x	---	---	---	SAMN14647063	MT145296	**ON248995**	2n: Dubcovsky & Martinez 1988; genome data: this study
Festuca procera Kunth	AH	Fine-Leaved	Ecuador:Chimborazo; Riobamba; HUTPL14079	28	4x	**14.88**	3.72	3638.16	SAMN14647067	MT145299	**ON248999**	2n: Smarda & Stancik 2006; 2C, genome data: this study
Festuca pyrenaica Reut.	WW	Fine-Leaved	Spain: Huesca; Pyrenees, Tobacor	28	4x	---	---	---	SAMN14647068	MT145300	**ON249000**	2n: Kerguelen 1975; genome data: this study
Festuca pyrogea Speg.	XX	Fine-Leaved	Argentina: Tierra de fuego, Cabo San Pablo; PC 494.08	---	---	---	---	---	SAMN14647069	MT145302	**ON249001**	genome data: this study
Festuca rubra L.	AN	Fine-Leaved	Argentina: Tierra de fuego; Cabo Annicolta; UZ 03.09	42	**6x**	13.68	2.28	2229.84	**SAMN27777780**	**ON243856**	**ON249002**	2n, 2C: Smarda et al. 2008; genome data: this study
Megalachne masafuerana (Skottsb. & Pilg. ex. Pilg.) Matthei	AJ	Fine-Leaved	Chile: Juan Fernandez islands; Masafuera. L. Gaete. 9150 (0S)	---	---	---	---	---	SAMN14647075	MT145308	**ON249018**	genome data: this study
Vulpia ciliata Dumort.	YY	Fine-Leaved	Spain: Toledo; Mar de Ontígola; UZ 109.07	28	4x	8.28	2.07	2024.46	SAMN14647076	MT145309	**ON249009**	2n: Stace & Cotton 1980; 2C: Smarda et al. 2008; genome data: this study
Festuca arundinacea Schreb. subsp. *arundinacea*	AX	Schedonorus	Spain: Coruña: Ferrol	42	6x	17.46	2.91	2845.98	**SAMN27777774**	**ON243850**	**ON249007**	2n, 2C: Kopecký et al., 2010; genome data: this study
Festuca arundinacea subsp. *atlantigena* (St.-Yves) Auquier	AY	Schedonorus	Morocco: Atlas mountains, ABY BN 807	56	8x	16.22	2.03	1982.895	**SAMN27777775**	**ON243851**	**ON248990**	2n, 2C: Ezquerro-López et al., 2017; genome data: this study
Festuca arundinacea Schreb. subsp. *arundinacea* var. *letourneuxiana* (St.-Yves) Torrecilla & Catalán	CC	Schedonorus	Morocco: Atlas Mountains, ABY BN 400	70	10x	19.7	1.97	1926.66	SAMN14647059	MT145292	**ON249010**	2n, 2C: Ezquerro-López et al., 2017; genome data: this study
Festuca dracomontana H.P. Linder	AQ	Schedonorus	South Africa: TVL; Haernertsburg; PRE 66429	---	---	---	---	---	**SAMN27777776**	**ON243852**	**ON249011**	genome data: this study
Festuca fenas Lag.	AA	Schedonorus	Spain:W Mediterranean; PI289654	28	4x	10.48	2.62	2562.36	SAMN14647052	MT145285	**ON248982**	2n, 2C: Ezquerro-López et al. 2017; genome data: this study
Festuca fontqueri St.-Yves	BB	Schedonorus	Morocco: Rif Mountains; Talassemtane National Park, PC 59.17	14	2x	**5.54**	2.77	2709.06	SAMN14647054	MT145287	**ON249008**	2n: Favarger et al. 1980; 2C, genome data: this study
Festuca gigantea (L.) Vill.	AO	Schedonorus	Norway: cultivated; 12/P2007	42	6x	20.75	3.46	3382.25	**SAMN27777777**	**ON243853**	**ON248985**	2n, 2C: Smarda et al. 2008; genome data: this study
Festuca gudoschnikovii Stepanov	AR	Schedonorus	Russia:Krasnoyarskii Krai; Yermakovskii Raion; PC 87	28	4x	---	---	---	**SAMN27777778**	**ON243854**	**ON248987**	2n: Probatova et al. 2017; genome data: this study
Festuca mairei St.-Yves	DD	Schedonorus	Morocco: Atlas Mountains; PI-610941	28	4x	10.04	2.51	2454.78	SAMN14647060	MT145293	**ON248991**	2n, 2C: Ezquerro-López et al. 2017; genome data: this study
Festuca pratensis Huds.	EE	Schedonorus	UK: England, USDA PI 283306	14	2x	6.5	3.25	3178.5	SAMN14647066	MT145301	**ON248998**	2n, 2C: Kopecky et al. 2010; genome data: this study
Festuca simensis Hochst. ex A. Rich.	AS	Schedonorus	Kenya: Mt. Kenya, Meteorological station; Namaganda 1750	28	4x	---	---	---	**SAMN27777782**	**ON243858**	**ON249012**	2n: Namaganda et al. 2006; genome data: this study
Lolium canariense Steud	AT	Schedonorus	Spain: Canary Islands; USDA PI 320544	14	2x	4.3	2.15	2102.7	**SAMN27777783**	**ON243859**	**ON249013**	2n: Inda & Wolny 2013; 2C: Huchinson et al. 1979; genome data: this study
Lolium perenne L	AL	Schedonorus	UK: Wales; USDA PI 619001	14	2x	5.51	2.76	2694.39	**SAMN27777784**	**ON243860**	**ON249014**	2n: Inda & Wolny 2013; 2C: Smarda et al. 2008; genome data: this study
Lolium persicum Boiss. & Hohen	AK	Schedonorus	Georgia; USDA PI 314446	14	2x	6.4	3.2	3129.6	**SAMN27777785**	**ON243861**	**ON249015**	2n: Inda & Wolny 2013; 2C: Huchinson et al. 1979; genome data: this study
Lolium rigidum Gaudin	AV	Schedonorus	Turkey; USDA PI 545604	14	2x	5.49	2.75	2684.61	**SAMN27777786**	**ON243862**	**ON249017**	2n: Inda and Wolny, 2013; 2C: Smarda et al. 2008; genome data: this study
Lolium saxatile H. Scholz & S. Scholz	AW	Schedonorus	Spain: Canary islands; AS	---	---	---	---	---	**SAMN27777787**	**ON243863**	**ON249016**	genome data: this study
Micropyropsis tuberosa Romero-Zarco & Cabezudo	AU	Schedonorus	Spain: Huelva: Almonte; UZ89.07	14	2x	---	---	---	**SAMN27777788**	**ON243864**	**ON249019**	2n: Romero-Zarco 1988; genome data: this study

Brachypodium distachyon (L.) P.Beauv.	---	---	Spain: Caceres; UZ 28.07	10	2x	---	---	---	NC_011032.1	Phytozome Bd21 v.3.1	CM000883.3	----
Oryza sativa L.	---	---	China: National Hybrid Rice Research and Development Center (NHRRDC); China		2x	---	---	---	AY522331.1	AP008215	----	----
Oryza eichingeri Peter	---	---	Philippines: International Rice Reserch Institute (IRRI)	---	2x	---	---	---	----	----	EF197127.1	----

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Table S2. Loliinae samples used in the repetitive DNA analysis. Genome skimming paired-end (PE) reads per sample and PE reads selected by Repeat Explorer2 per sample in each of the comparative analyses of the four Loliinae groups: Loliinae, BL (broad-leaved Loliinae), FL (fine-leaved Loliinae), Schedonorus.

Taxon	Code	Group	*Genome skimming* reads	Insert size	Loliinae		Schenodorus		Broad_leaved		Fine_leaved	
					Repeat Explorer reads	Repeat Explorer Genome coverage	Repeat Explorer reads	Repeat Explorer Genome coverage	Repeat Explorer reads	Repeat Explorer Genome coverage	Repeat Explorer reads	Repeat Explorer Genome coverage
Festuca africana (Hack.) Clayton	FF	Broad-Leaved	13,549,000	195	93,068	---	---	---	231,344	---	---	---
Festuca amplissima Rupr.	GG	Broad-Leaved	12,058,000	220	83,358	---	---	---	204,494	---	---	---
Festuca caldasii (Kunth) Kunth	NN	Broad-Leaved	9,863,000	248	68,088	0.094452353	---	---	168,162	0.08957966		---
Festuca durandoi Clauson	PP	Broad-Leaved	12,688,000	217	87,702	0.131176665	---	---	215,648	---	---	---
Festuca lasto Boiss.	HH	Broad-Leaved	21,581,000	300	148,516	---	---	---	367,034	---	---	---
Festuca mekiste Clayton	AM	Broad-Leaved	16,245,000	201	111,228	---	---	---	276,810	---	---	---
Festuca molokaiensis Soreng, P.M. Peterson & Catalán	II	Broad-Leaved	12,188,000	219	83,800	---	---	---	206,804	---	---	---
Festuca paniculata (L.) Schinz & Thell	QQ	Broad-Leaved	35,808,000	300	246,446	0.125689537	---	---	609,220	0.11920535	---	---
Festuca parvigluma Steud.	JJ	Broad-Leaved	15,872,000	190	108,708	---	---	---	270,022	---	---	---
Festuca scabra Vahl	AP	Broad-Leaved	21,174,000	211	145,346	---	---	---	359,062	---	---	---
Festuca spectabilis Jan	LL	Broad-Leaved	12,960,000	221	89,008	---	---	---	220,088	---	---	---
Festuca superba Parodi ex Türpe	RR	Broad-Leaved	12,193,000	221	83,988	---	---	---	207,694	---	---	---
Festuca triflora J.F. Gmel.	MM	Broad-Leaved	24,472,000	300	168,142	0.122643489	---	---	416,196	0.11631645	---	---
Festuca abyssinica Hochst. ex A. Rich.	AB	Fine-Leaved	12,041,000	166	82,794	---	---	---	---	---	158,960	---
Festuca asplundii E.B. Alexeev	AC	Fine-Leaved	25,088,000	300	172,022	0.13587258	---	---	---	---	331,124	0.114396833
Festuca capillifolia Dufour ex Roem. & Schult.	SS	Fine-Leaved	13,430,000	228	92,674	---	---	---	---	---	177,206	---
Festuca chimborazensis E.B. Alexeev subsp. *micacochensis* Stančík	AD	Fine-Leaved	10,913,000	254	75,418	0.213989234	---	---	---	---	143,276	0.180166525
Festuca eskia Ramond ex DC.	OO	Fine-Leaved	24,041,000	300	164,798	0.168688589	---	---	---	---	317,762	0.142026009
Festuca fimbriata Nees	AE	Fine-Leaved	15,741,000	173	107,956	---	---	---	---	---	207,162	---
Festuca francoi Fern. Prieto, C. Aguiar, E. Días & M.I. Gut	TT	Fine-Leaved	17,592,000	186	120,860	---	---	---	---	---	231,806	---
Festuca gracillima Hook. F.	AF	Fine-Leaved	13,888,000	224	95,164	---	---	---	---	---	183,168	---
Festuca holubii Stančík	AG	Fine-Leaved	10,264,000	249	70,992	---	---	---	---	---	135,198	---
Festuca ovina L.	UU	Fine-Leaved	11,364,000	215	78,084	0.199486506	---	---	---	---	149,724	0.167956069
Festuca pampeana Speg.	VV	Fine-Leaved	14,862,000	223	102,358	---	---	---	---	---	196,018	---

Festuca procera Kunth	AH	Fine-Leaved	40,669,000	263	280,040	0.129237225	---	---	---	---	536,736	0.108810249
Festuca pyrenaica Reut.	WW	Fine-Leaved	30,021,000	300	205,996	---	---	---	---	---	395,608	---
Festuca pyrogea Speg.	XX	Fine-Leaved	16,835,000	193	115,054	---	---	---	---	---	221,512	---
Festuca rubra L.	AN	Fine-Leaved	25,260,000	220	174,248	0.210860736	---	---	---	---	333,992	0.177532512
Megalachne masafuerana (Skottsb. & Pilg. ex. Pilg.) Matthei	AJ	Fine-Leaved	6,134,000	184	43,824	---	---	---	---	---	82,838	---
Vulpia ciliata Dumort.	YY	Fine-Leaved	11,801,000	220	81,058	0.348378608	---	---	---	---	155,632	0.293314585
Festuca arundinacea Schreb. subsp. *arundinacea*	AX	Schedonorus	15,556,000	206	---	---	136,784	0.09663341	---	---	---	---
Festuca arundinacea Schreb. subsp. *arundinacea* var. *letourneuxiana* (St.-Yves) Torrecilla & Catalán	CC	Schedonorus	16,839,000	250	116,320	0.244041867	151,754	0.14274275	---	---	---	---
Festuca arundinacea subsp. *atlantigena* (St.-Yves) Auquier	AY	Schedonorus	15,091,000	224	---	---	140,518	0.13869455	---	---	---	---
Festuca dracomontana H.P. Linder	AQ	Schedonorus	15,835,000	139	---	---	142,276	---	---	---	---	---
Festuca fenas Lag.	AA	Schedonorus	16,112,000	271	109,944	0.183497129	145,146	0.10732947	---	---	---	---
Festuca fontqueri St.-Yves	BB	Schedonorus	22,187,000	300	152,486	0.173560462	200,066	0.1015174	---	---	---	---
Festuca gigantea (L.) Vill.	AO	Schedonorus	20,914,000	223	143,564	0.139015656	188,600	0.08131177	---	---	---	---
Festuca gudoschnikovii Stepanov	AR	Schedonorus	13,994,000	208.75	---	---	126,240	---	---	---	---	---
Festuca mairei St.-Yves	DD	Schedonorus	19,134,000	254	131,436	0.191538836	172,776	0.11203315	---	---	---	---
Festuca pratensis Huds.	EE	Schedonorus	12,189,000	271	83,958	0.147926916	109,432	0.08652406	207.212	0.14029553	---	---
Festuca simensis Hochst. ex A. Rich.	AS	Schedonorus	14,159,000	192.5	---	---	127,318	---	---	---	---	---
Lolium canariense Steud	AT	Schedonorus	16,359,000	231.5	---	---	147,858	0.13079219	---	---	---	---
Lolium perenne L	AL	Schedonorus	28,103,000	244	192,992	0.174505437	253,800	0.10207013	478.612	0.16550289	---	---
Lolium persicum Boiss. & Hohen	AK	Schedonorus	25,523,000	241	175,334	0.150238275	229,658	0.087876	---	---	---	---
Lolium rigidum Gaudin	AV	Schedonorus	16,730,000	201.5	---	---	150,880	0.10244197	---	---	---	---
Lolium saxatile H. Scholz & S. Scholz	AW	Schedonorus	16,001,000	236	---	---	144,232	---	---	---	---	---
Micropyropsis tuberosa Romero-Zarco & Cabezudo	AU	Schedonorus	19,803,000	NA	---	---	178,422	---	---	---	---	---

Supplementary Table S5. Phylogenetic signal based on Blomberg's K values of repeat cluster contents obtained from the comparative RE2 analysis of Loliinae samples assessed in each of the four Loliinae groups, (**A**) Loliinae (38 samples, 39 clusters), (**B**) Broad-leaved (BL) Loliinae (13 samples, 96 clusters), (**C**) Fine-leaved (FL) Loliinae (17 samples, 122 clusters), (**D**) Schedonorus (16 samples, 167 clusters), using the *phylosig* option of the *phytools* R package. Cluster abundance values (number of PE reads) are indicated in Supplementary Table S3B. K values close to one indicate phylogenetic signal, values close to zero phylogenetic independence, and values >1 more phylogenetic signal than expected. p-values based on 1000 randomizations. Significant values are highlighted in bold.

(A) Loliinae

N. Cluster	Cluster_Name	Phylogenetic signal on combined tree	
		K	p-value
35	LTR_1	0.373703	0.2050
39	**LTR_2**	**0.583327**	**0.0060**
86	LTR_3	0.267521	0.6150
6	Angela_1	0.441390	0.0960
7	**Angela_2**	**0.847919**	**0.0020**
12	Angela_3	0.465467	0.0310
13	Angela_4	0.520696	0.0210
14	Angela_5	0.513860	0.0200
17	Angela_6	0.415452	0.1400
30	Angela_7	0.415190	0.1060
34	Angela_8	0.399333	0.1240
53	**Angela_9**	**0.649828**	**0.0030**
71	Angela_10	0.448955	0.0910
94	**Angela_11**	**0.636886**	**0.0040**
107	**Angela_12**	**0.743470**	**0.0010**
3	SIRE_1	0.411074	0.1290
9	SIRE_2	0.313160	0.4690
22	SIRE_3	0.361960	0.2340
29	SIRE_4	0.380547	0.1730
58	SIRE_5	0.321802	0.4230
67	SIRE_6	0.329360	0.4050
84	SIRE_7	0.330350	0.3800
85	SIRE_8	0.355221	0.3070
103	SIRE_9	0.333838	0.4040
126	**SIRE_10**	**0.463612**	**0.0490**
137	SIRE_11	0.339207	0.3850
50	TAR_1	0.358543	0.2190
139	TAR_2	0.279301	0.5300
28	Athila_1	0.386600	0.0910
41	EnSpm_CACTA_1	0.375853	0.1800
59	**EnSpm_CACTA_2**	**0.475749**	**0.0530**
75	EnSpm_CACTA_3	0.456950	0.1710
87	**EnSpm_CACTA_4**	**0.555016**	**0.0520**
108	**EnSpm_CACTA_5**	**0.503111**	**0.0510**
180	EnSpm_CACTA_6	0.374766	0.1810
106	.45S_rDNA_1	0.209001	0.8380
115	45S_rDNA_2	0.190001	0.8970
203	5S_rDNA_1	0.177649	0.9110

(B) Broad-leaved

N. Cluster	Cluster Name	Phylogenetic signal on combined tree		N. Cluster	Cluster Name	Phylogenetic signal on combined tree	
		K	p-value			K	p-value
40	Repeat_1	0.3435	0.5850	1	Tekay_1	0.5706	0.1220
57	Repeat_2	0.4259	0.3090	4	**Tekay_2**	**1.0530**	**0.0080**
58	Repeat_3	0.3954	0.4410	7	**Tekay_3**	**0.6307**	**0.0730**
70	Repeat_4	0.4548	0.2880	8	Tekay_4	0.3789	0.4640
84	Repeat_5	0.3713	0.4890	10	**Tekay_5**	**0.7060**	**0.0780**
108	Repeat_6	0.2951	0.7690	19	**Tekay_6**	**0.9883**	**0.0230**
110	Repeat_7	0.3704	0.5180	27	Tekay_7	0.6976	0.0370
179	Repeat_8	0.4154	0.3740	43	Tekay_8	1.1727	0.0040
189	Repeat_9	0.3301	0.6560	46	Tekay_9	0.3280	0.6190
218	Repeat_10	0.2661	0.8800	49	**Tekay_10**	**0.9624**	**0.0080**
100	LINE_1	0.3406	0.6240	62	**Tekay_11**	**1.0418**	**0.0080**
12	**LTR_1**	**1.0796**	**0.0050**	65	**Tekay_12**	**1.0957**	**0.0020**
256	Ale_1	0.2603	0.7670	114	**Tekay_13**	**0.8371**	**0.0230**
2	**Angela_1**	**0.6062**	**0.0830**	118	Tekay_14	0.5686	0.1650
3	Angela_2	0.3894	0.4440	129	Tekay_15	0.6237	0.1210
5	Angela_3	0.3994	0.3840	132	Tekay_16	0.3177	0.6310
13	Angela_4	0.5182	0.1780	24	Athila_1	0.2142	0.9100
21	**Angela_5**	**0.6538**	**0.0540**	42	**Athila_2**	**0.6794**	**0.0550**
22	Angela_6	1.2911	0.0020	48	Athila_3	0.5150	0.1750
31	Angela_7	0.5303	0.1320	55	Athila_4	0.4772	0.2190
32	Angela_8	0.5690	0.0950	60	**Athila_5**	**0.6296**	**0.0800**
34	Angela_9	0.5083	0.1890	80	Athila_6	0.2239	0.8610
39	Angela_10	0.5176	0.1500	97	**Athila_7**	**0.7035**	**0.0540**
53	Angela_11	0.3974	0.3990	104	**Athila_8**	**0.6905**	**0.0380**
64	Angela_12	0.4498	0.2500	185	Athila_9	0.5642	0.1200
72	Angela_13	0.4298	0.3210	11	Retand_1	0.5333	0.1620
79	Angela_14	0.4175	0.3480	45	EnSpm_CACTA_1	0.4333	0.4040
81	**Angela_15**	**0.7050**	**0.0450**	47	EnSpm_CACTA_2	0.5681	0.1320
95	Angela_16	0.3509	0.5720	59	EnSpm_CACTA_3	0.4286	0.3510
162	Angela_17	0.4655	0.2330	127	EnSpm_CACTA_4	0.3997	0.4200
198	Ikeros_1	0.4370	0.2990	140	EnSpm_CACTA_5	0.5053	0.2650
18	SIRE_1	0.4566	0.2890	144	EnSpm_CACTA_6	0.4883	0.2060
26	SIRE_2	0.4068	0.3970	150	EnSpm_CACTA_7	0.4796	0.2840
35	SIRE_2.1	0.5107	0.1750	176	EnSpm_CACTA_8	0.2484	0.8980
68	SIRE_4	0.3873	0.4810	199	EnSpm_CACTA_9	0.5272	0.1890
99	SIRE_5	0.4136	0.4120	86	**MuDR_Mutator_1**	**0.9483**	**0.0020**
192	SIRE_6	0.3916	0.4380	219	MuDR_Mutator_2	0.5809	0.1410
28	TAR_1	0.3529	0.5740	190	PIF_Harbinger_1	0.2860	0.7130
194	Tork_1	0.3740	0.4450	98	45S_rDNA_1	0.2531	0.8490
16	CRM_1	0.3987	0.4300	133	45S_rDNA_2	0.2497	0.8550
82	CRM_2	0.6587	0.0990	236	5S_rDNA_1	0.3941	0.4240
116	CRM_3	0.5217	0.1830	107	Satellite_1	0.5113	0.2440
223	CRM_4	0.3788	0.4460				

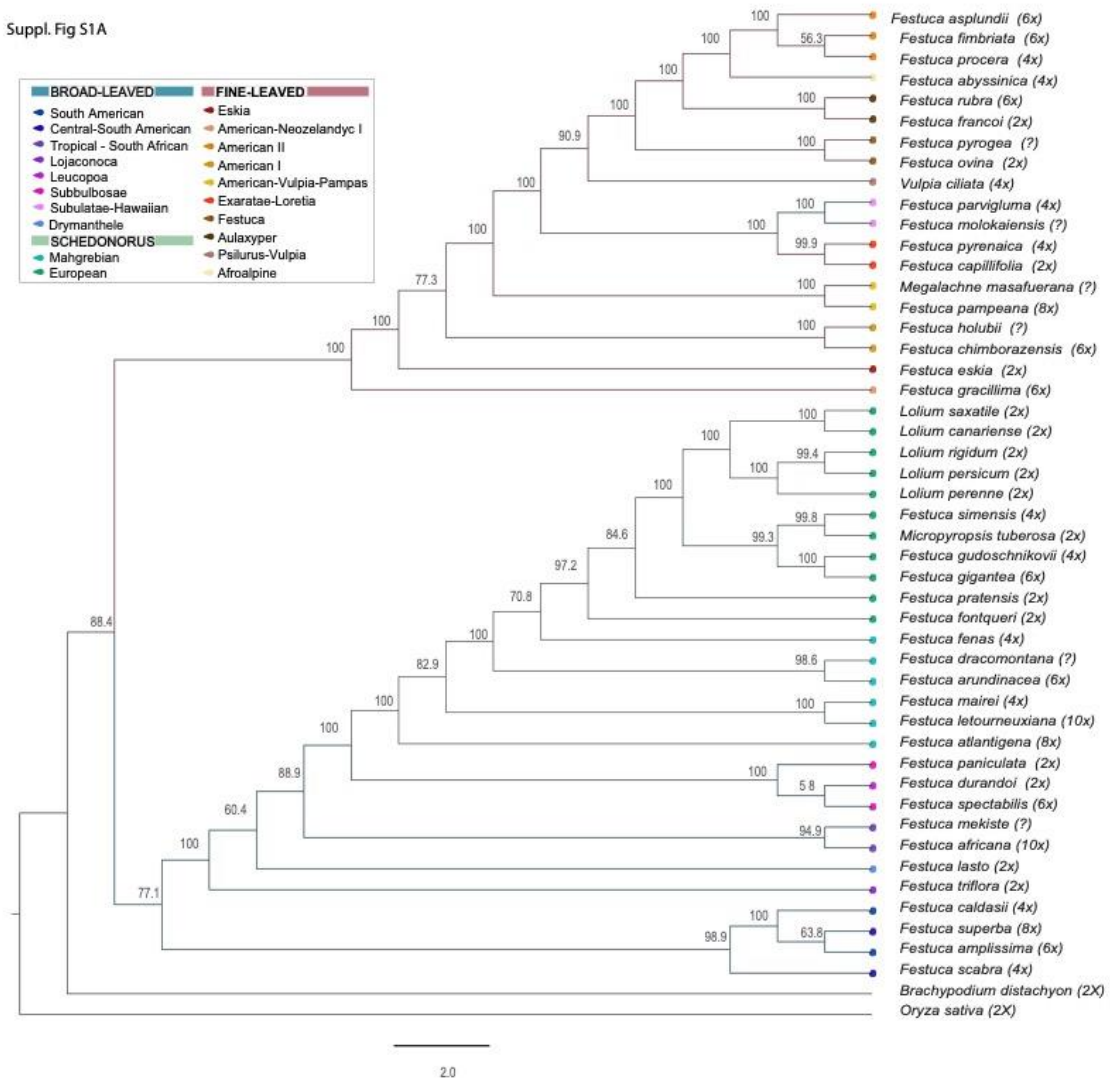
(C) Fine-leaved

N. Cluster	Cluster Name	Phylogenetic signal on combined tree		N. Cluster	Cluster Name	Phylogenetic signal on combined tree	
		K	p-value			K	p-value
18	LTR_1	0.5850	0.3040	178	Ivana	0.6122	0.2780
124	LTR_10	0.4946	0.5210	1	SIRE_1	0.4255	0.6880
36	LTR_2	0.3961	0.7580	10	SIRE_2	0.4795	0.5640
52	LTR_3	0.4874	0.4790	17	SIRE_3	0.4726	0.5560
61	LTR_4	0.3825	0.7960	20	SIRE_4	0.5103	0.4530
79	LTR_5	0.6444	0.1800	22	SIRE_5	0.4442	0.6720
82	LTR_6	0.5286	0.4160	33	SIRE_6	0.6636	0.1920
92	LTR_7	0.5928	0.2790	43	SIRE_7	0.4188	0.6890
107	LTR_8	0.6859	0.1720	58	SIRE_8	0.4299	0.6800
121	LTR_9	0.4593	0.6240	66	SIRE_9	0.4203	0.7200
51	45S_rDNA_1	0.5162	0.4370	68	SIRE_10	0.6319	0.2110
112	45S_rDNA_2	0.6238	0.2660	71	SIRE_11	0.5350	0.3940
183	5S_rDNA	0.5924	0.2420	86	SIRE_12	0.5048	0.4990
16	**Repeat_1**	**1.0080**	**0.0250**	114	SIRE_13	0.4121	0.7350
24	Repeat_2	0.4221	0.7190	15	TAR	0.5817	0.3120
67	Repeat_3	0.3501	0.8790	145	Tork	0.6339	0.1990
140	Repeat_4	0.4277	0.7000	25	Athila_1	0.3757	0.8400
34	Satellite_1	0.5174	0.4740	27	Athila_2	0.4745	0.5670
96	Satellite_2	0.3895	0.7970	32	Athila_3	0.4377	0.6350
6	EnSpm_CACTA_1	0.4334	0.6900	39	Athila_4	0.3759	0.8270
9	EnSpm_CACTA_2	0.4903	0.4900	40	Athila_5	0.5311	0.3540
46	EnSpm_CACTA_3	0.4561	0.6060	47	Athila_6	0.4955	0.4820
49	EnSpm_CACTA_4	0.4501	0.5980	54	Athila_7	0.4510	0.5860
55	EnSpm_CACTA_5	0.5194	0.4530	81	Athila_8	0.4363	0.6530
60	EnSpm_CACTA_6	0.4620	0.6040	131	Athila_9	0.4843	0.4760
64	EnSpm_CACTA_7	0.6836	0.2210	135	Athila_10	0.4966	0.4560
73	EnSpm_CACTA_8	0.4144	0.7190	138	Athila_11	0.6256	0.1840
80	EnSpm_CACTA_9	0.4872	0.5390	149	Athila_12	0.3980	0.7520
105	EnSpm_CACTA_10	0.5294	0.4210	187	Athila_13	0.4041	0.7130
125	EnSpm_CACTA_11	0.4066	0.7510	42	CRM_1	0.6362	0.2460
144	EnSpm_CACTA_12	0.5082	0.4910	59	CRM_2	0.3588	0.8750
157	EnSpm_CACTA_13	0.5125	0.4800	100	CRM_3	0.3510	0.8600
45	MuDR_Mutator_1	0.5426	0.3880	103	CRM_4	0.3466	0.8890
101	MuDR_Mutator_2	0.5144	0.4370	116	CRM_5	0.6577	0.2860
219	MuDR_Mutator_3	0.7445	0.2030	126	CRM_6	0.3726	0.8240
229	MuDR_Mutator_4	0.6158	0.3420	136	CRM_7	0.6476	0.2630
197	PIF_Harbinger	0.5061	0.4370	170	CRM_8	0.4988	0.4740
3	Angela_1	0.7349	0.1480	171	CRM_9	0.7605	0.0920
7	Angela_2	0.6601	0.2330	152	Ogre_1	0.6782	0.2880
11	Angela_3	0.7251	0.1630	184	Ogre_2	0.4720	0.5490
21	Angela_4	0.5692	0.3330	2	Retand_1	0.3831	0.7980
26	Angela_5	0.5260	0.4440	4	Retand_2	0.4944	0.5290
30	Angela_6	0.5605	0.3710	5	Retand_3	0.5164	0.4030
35	Angela_7	0.5329	0.3950	14	Retand_4	0.4230	0.7090
41	Angela_8	0.7546	0.1210	19	Retand_5	0.6771	0.1810
48	Angela_9	0.7401	0.1350	37	Retand_6	0.3956	0.7640
75	**Angela_10**	**0.8110**	**0.0830**	89	Retand_7	0.5288	0.4010
76	Angela_11	0.4887	0.5130	193	Retand_8	0.7818	0.1380
78	Angela_12	0.5210	0.3690	23	Tekay_1	0.5026	0.5260
117	Angela_13	0.5121	0.4270	85	**Tekay_2**	**1.1647**	**0.0900**
120	Angela_14	0.4530	0.6090	97	**Tekay_3**	**1.2022**	**0.0780**
153	Angela_15	0.4987	0.4770				

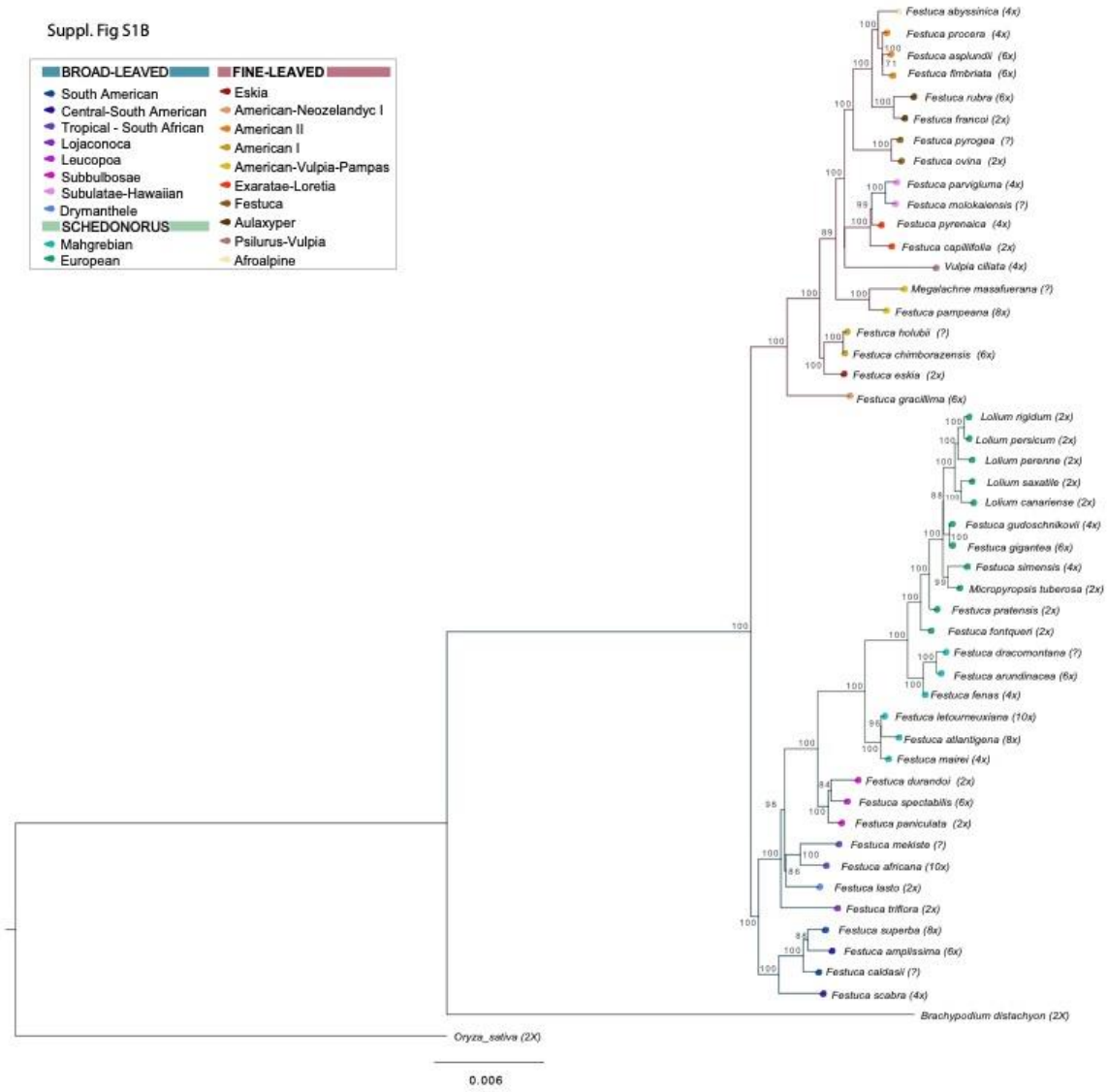
(D) Schedonorus

N. Cluster	Cluster Name	Phylogenetic signal on combined tree		N. Cluster	Cluster Name	Phylogenetic signal on combined tree	
		K	p-value			K	p-value
57	LINE_1	0.3705	0.3440	139	SIRE_10	0.1483	0.9960
157	LINE_2	0.2186	0.8270	67	TAR	0.1890	0.9090
2	**LTR_1**	**1.5842**	**0.0020**	183	Tork	0.2287	0.8030
12	LTR_2	0.3209	0.5250	11	Athila_1	0.5269	0.1640
27	**LTR_3**	**1.5812**	**0.0010**	26	Athila_2	0.4200	0.3390
56	**LTR_4**	**1.4556**	**0.0010**	29	Athila_3	0.3408	0.5330
64	**LTR_5**	**0.8215**	**0.0210**	31	Athila_4	0.2637	0.7040
71	**LTR_6**	**1.2780**	**0.0010**	33	Athila_5	0.4679	0.2820
79	**LTR_7**	**1.6481**	**0.0010**	42	Athila_6	0.6943	0.0380
113	**LTR_8**	**1.5144**	**0.0010**	44	Athila_7	0.3594	0.4870
149	**LTR_9**	**1.3891**	**0.0020**	45	Athila_8	0.1765	0.9040
158	**LTR_10**	**1.4747**	**0.0020**	47	Athila_9	1.5761	0.0010
187	LTR_11	0.2718	0.6820	48	Athila_10	0.2335	0.7880
82	45S_rDNAS1	0.6840	0.0570	54	Athila_11	0.3823	0.4270
93	45S_rDNA_2	0.5688	0.1450	59	**Athila_12**	**1.5937**	**0.0010**
127	5S_rDNA	0.3046	0.5960	60	Athila_13	0.2908	0.6600
83	**Repeat_1**	**0.8499**	**0.0050**	65	Athila_14	0.7097	0.0500
99	Repeat_2	0.5225	0.1030	76	Athila_15	0.1966	0.8560
105	**Repeat_3**	**1.1762**	**0.0010**	78	Athila_16	0.2506	0.7410
116	Repeat_4	0.4963	0.2720	80	Athila_17	0.2559	0.7610
130	**Repeat_5**	**1.1488**	**0.0010**	124	Athila_18	0.5208	0.1130
1	Satellite_1	0.7178	0.0290	190	Athila_19	0.2890	0.6350
9	Satellite_2	0.3350	0.5370	7	CRM_1	0.4637	0.1690
37	Satellite_3	0.5394	0.1320	30	CRM_2	0.2489	0.7650
39	Satellite_4	0.3527	0.4060	41	CRM_3	0.2486	0.7790
46	Satellite_5	0.4842	0.1040	61	CRM_4	0.4670	0.1910
97	Satellite_6	0.3450	0.5240	69	CRM_5	0.2767	0.6630
106	Satellite_7	0.3020	0.6540	123	CRM_6	0.2620	0.7110
109	Satellite_8	0.6066	0.0950	150	CRM_7	0.2515	0.7470
111	Satellite_9	0.5399	0.2640	154	**CRM_8**	**0.8502**	**0.0050**
126	Satellite_10	0.6675	0.0420	175	CRM_9	0.5515	0.0930
133	Satellite_11	0.4684	0.2770	4	Retand_1	0.2663	0.7290
171	Satellite_12	0.4450	0.2800	8	Retand_2	0.3144	0.5930
179	Satellite_13	0.3971	0.4150	10	Retand_3	0.3599	0.4780
185	Satellite_14	0.6319	0.0940	17	Retand_4	0.3321	0.5640
186	Satellite_15	0.5587	0.0530	18	Retand_5	0.2748	0.6990
22	EnSpm_CACTA_1	0.1688	0.9490	19	Retand_6	0.2932	0.6330
32	EnSpm_CACTA_2	0.2353	0.8030	21	Retand_7	0.3357	0.5110
87	EnSpm_CACTA_3	0.4075	0.2460	24	Retand_8	0.2946	0.6320
135	EnSpm_CACTA_4	0.3635	0.3550	38	Retand_9	0.2642	0.7020
136	EnSpm_CACTA_5	0.4831	0.1960	49	Retand_10	0.3449	0.5200
137	EnSpm_CACTA_6	0.1642	0.9480	53	Retand_11	0.2818	0.6370
203	EnSpm_CACTA_7	0.2418	0.7860	58	Retand_12	0.3672	0.4280
148	MuDR_Mutator_1	0.3375	0.4670	72	Retand_13	0.3412	0.5230
169	**MuDR_Mutator_2**	**0.8000**	**0.0050**	74	Retand_14	0.2868	0.6490
194	**MuDR_Mutator_3**	**1.7396**	**0.0010**	77	Retand_15	0.3579	0.3890
20	PIF_Harbinger_1	0.7504	0.0140	84	Retand_16	0.3713	0.3760
141	PIF_Harbinger_2	0.6238	0.0500	89	Retand_17	0.2949	0.6100
3	Angela_1	0.3177	0.5200	101	Retand_18	0.2817	0.6850
5	Angela_2	0.2702	0.6510	103	Retand_19	0.3941	0.3370

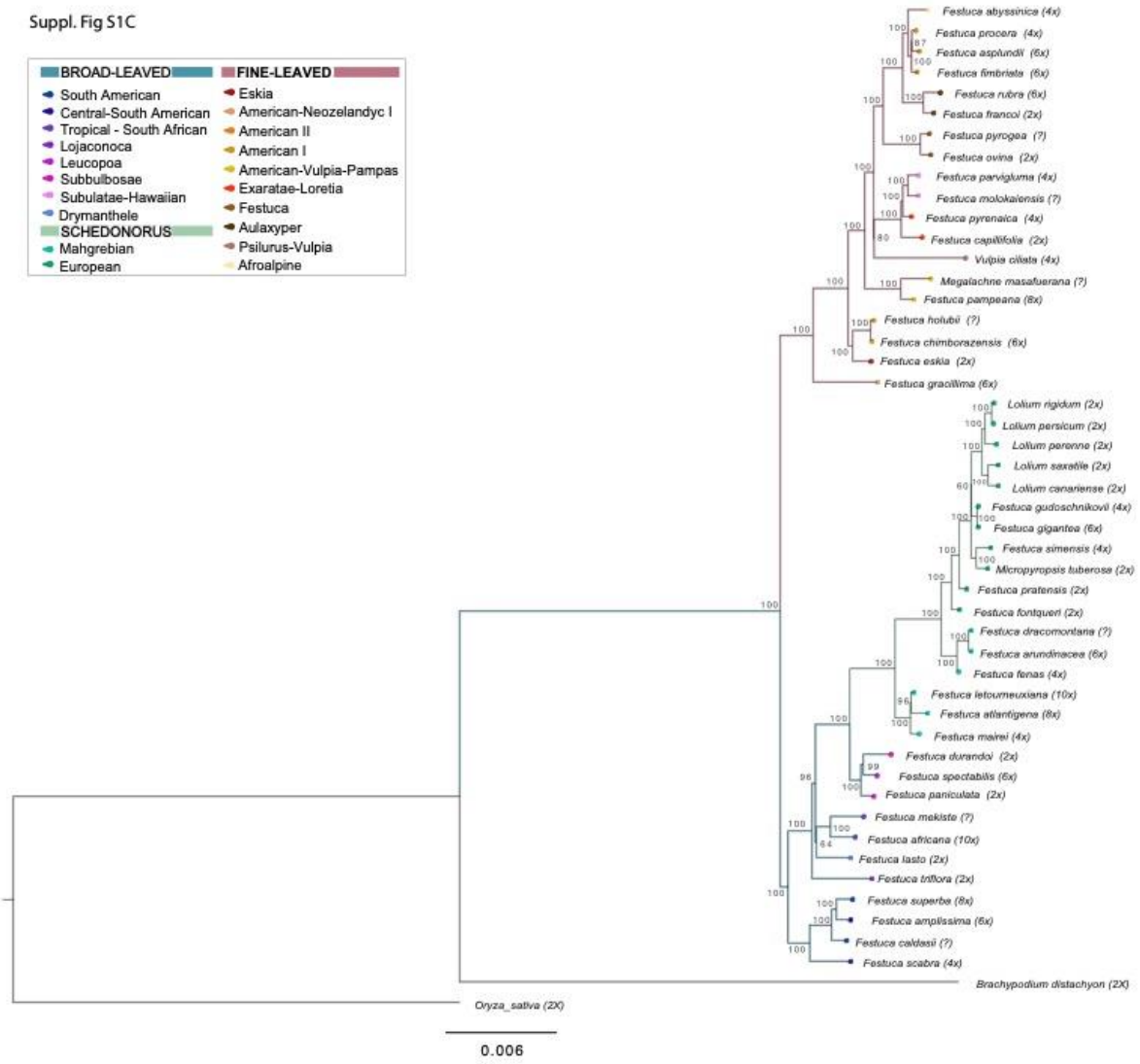
14	Angela_3	0.5073	0.1180	112	Retand_20	0.4008	0.3000
15	Angela_4	0.2170	0.8160	117	Retand_21	0.3912	0.3590
55	Angela_5	0.2384	0.7530	132	Retand_22	0.3261	0.4750
81	Angela_6	0.2473	0.7380	160	Retand_23	0.4118	0.3610
86	Angela_7	0.2889	0.6420	178	Retand_24	0.5538	0.0470
92	Angela_8	0.2993	0.6190	180	Retand_25	0.4416	0.1580
115	Angela_9	0.2473	0.7680	223	Retand_26	0.4754	0.1160
167	Ikeros	0.4735	0.1360	23	Tekay_1	0.3267	0.4720
13	SIRE_1	0.2846	0.6170	25	Tekay_2	0.4040	0.3530
16	SIRE_2	0.3148	0.5330	50	Tekay_3	0.2894	0.5810
40	SIRE_3	0.3128	0.5040	63	**Tekay_4**	**1.3463**	**0.0040**
43	SIRE_4	0.3197	0.5320	90	Tekay_5	0.1691	0.9050
52	SIRE_5	0.2477	0.7700	108	Tekay_6	0.4487	0.2940
68	SIRE_6	0.2165	0.8290	118	Tekay_7	0.4218	0.3150
95	SIRE_7	0.3357	0.4620	129	Tekay_8	0.2556	0.7410
98	SIRE_8	0.3081	0.5600	220	Tekay_9	0.5207	0.1700
122	SIRE_9	0.3181	0.5200				



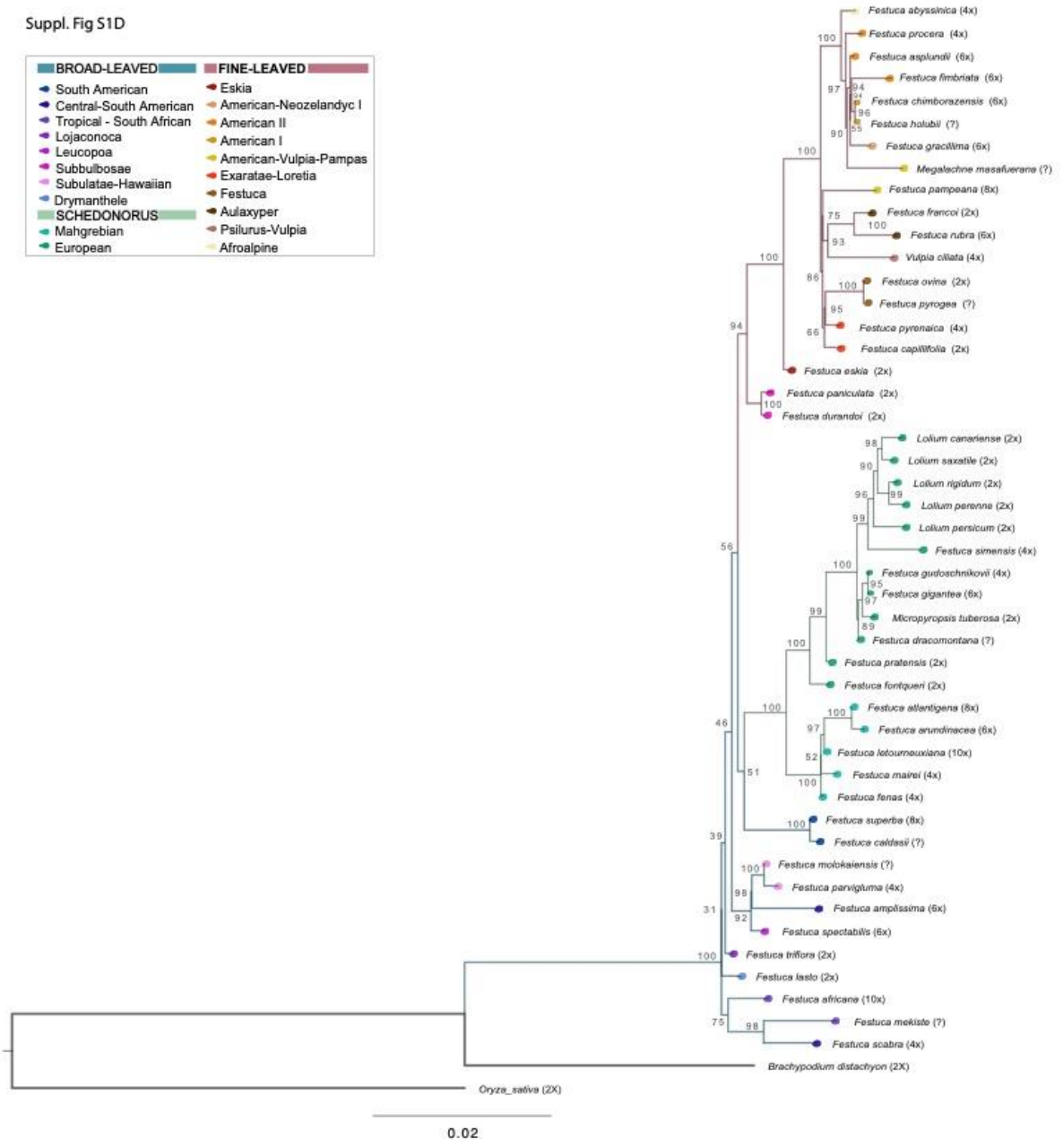
Supplementary Figure 1A. Combined (plastome + 35S rDNA) Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.



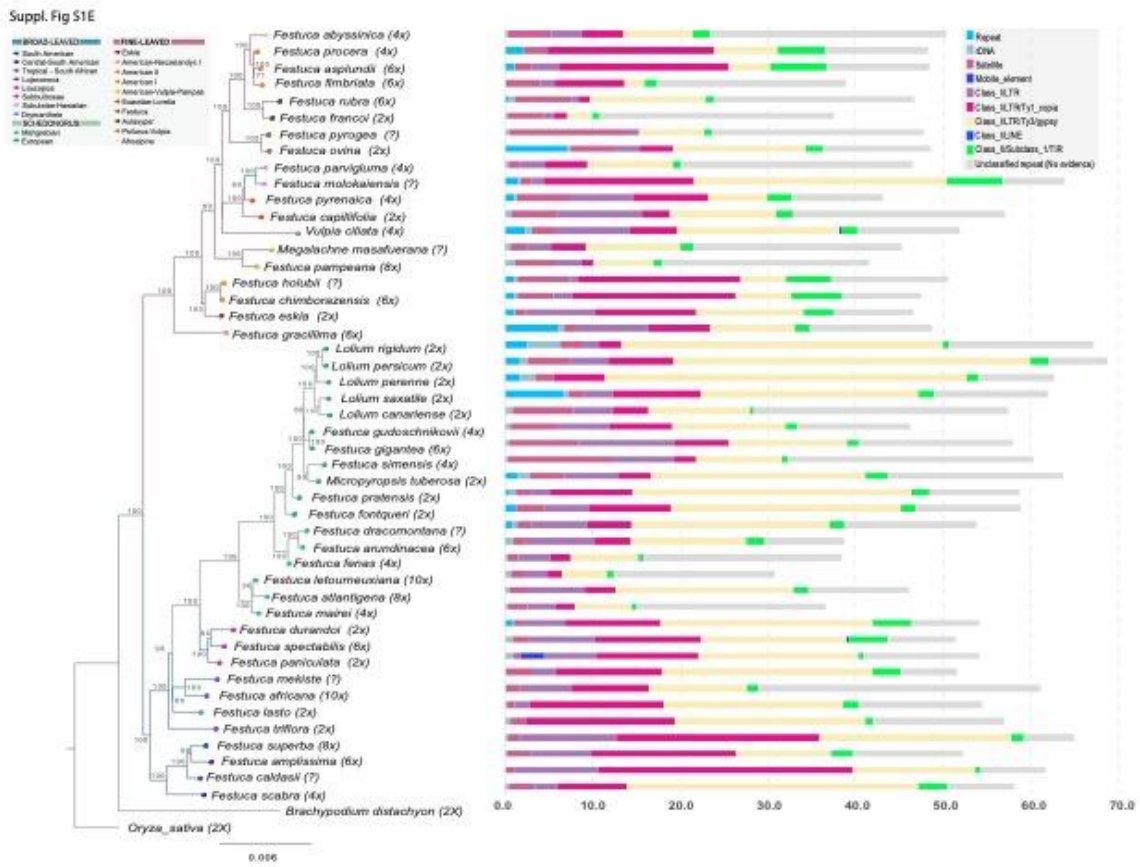
Supplementary Figure 1B. Maximum Likelihood phylogenomic trees of 47 Loliinae samples based Combined (plastome + 35S rDNA) data. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.



Supplementary Figure 1C. Maximum Likelihood phylogenomic trees of 47 Loliinae samples based on plastome data. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.

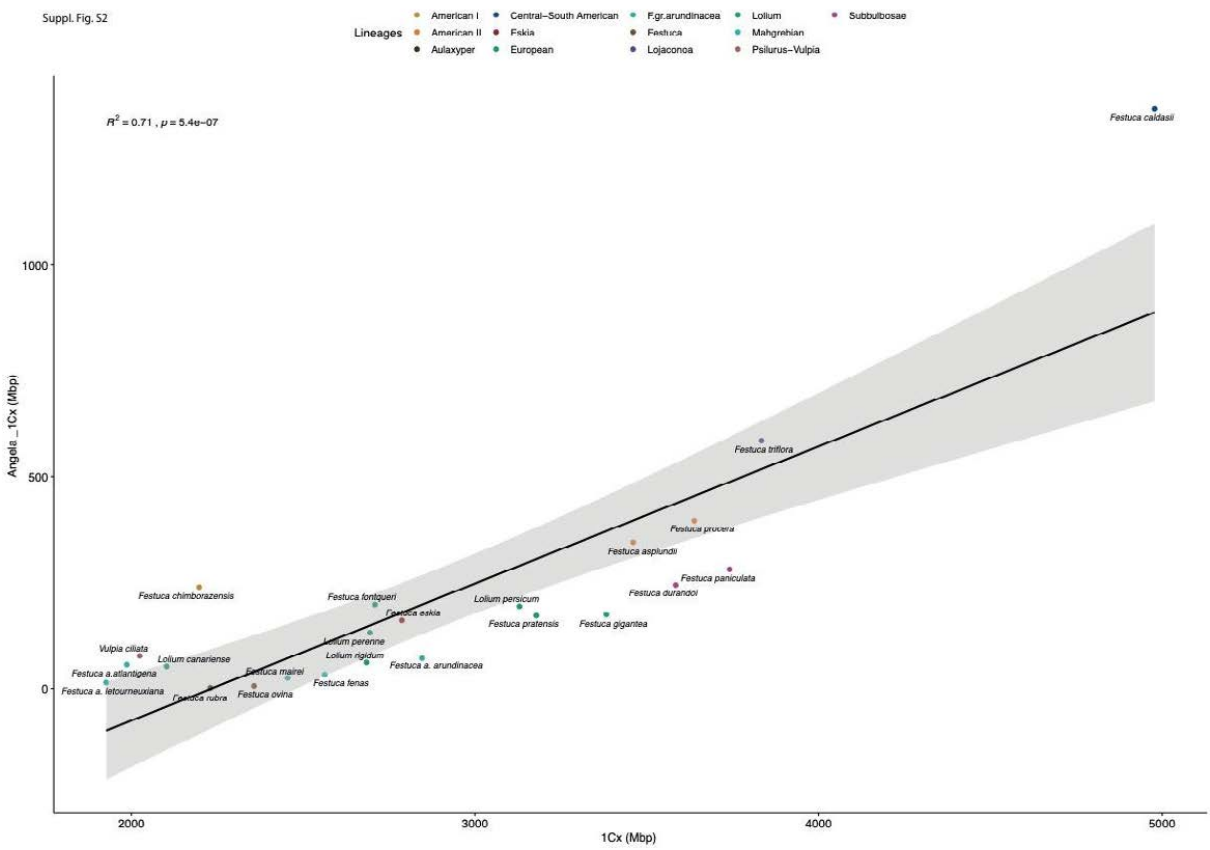


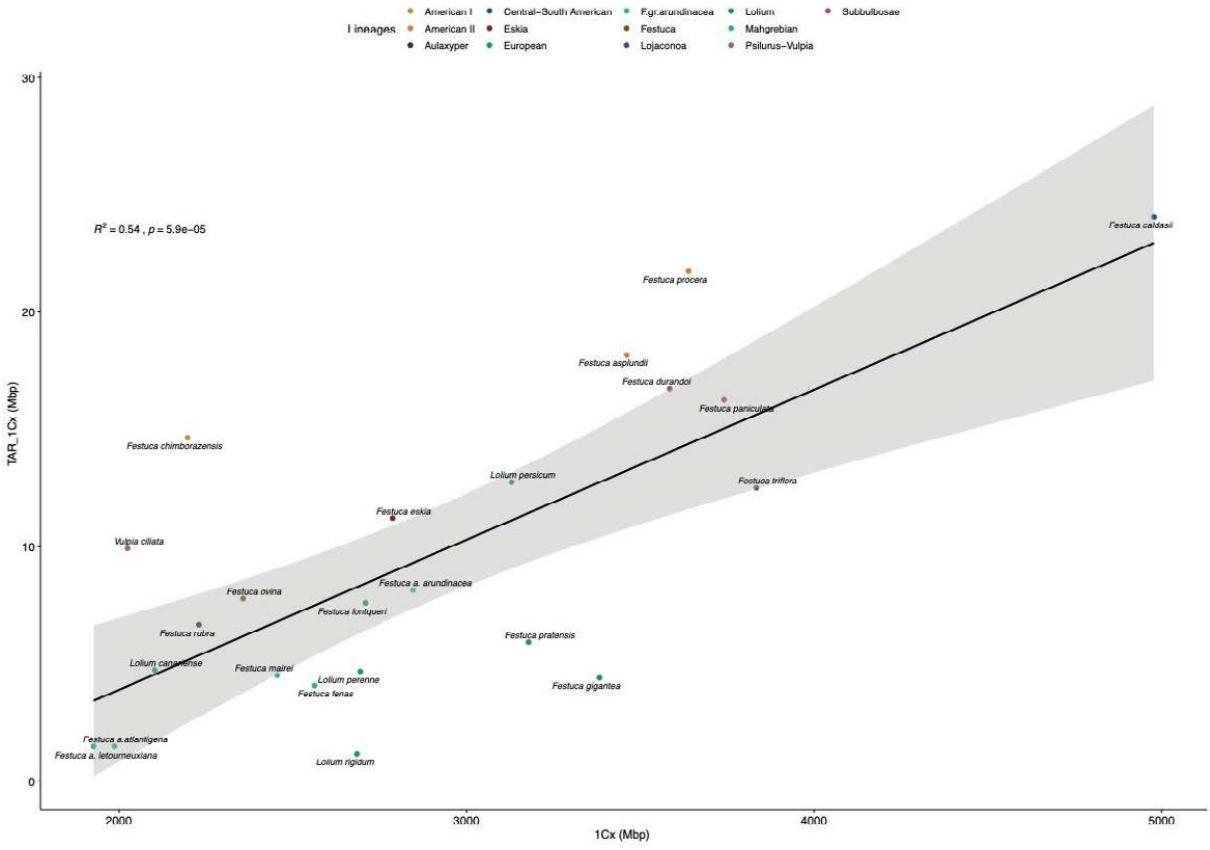
Supplementary Figure 1D. Maximum Likelihood phylogenomic trees of 47 Loliinae samples based on nuclear 35S rDNA data. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.

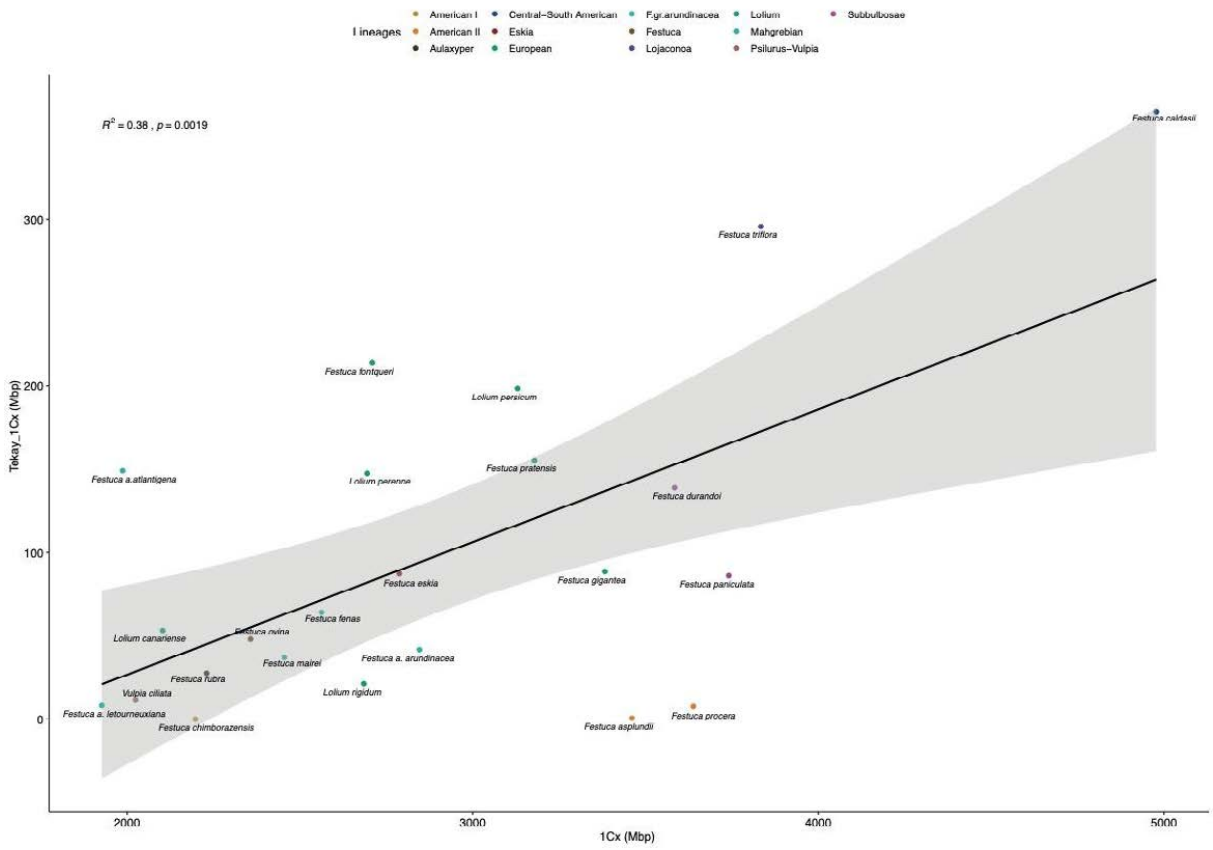


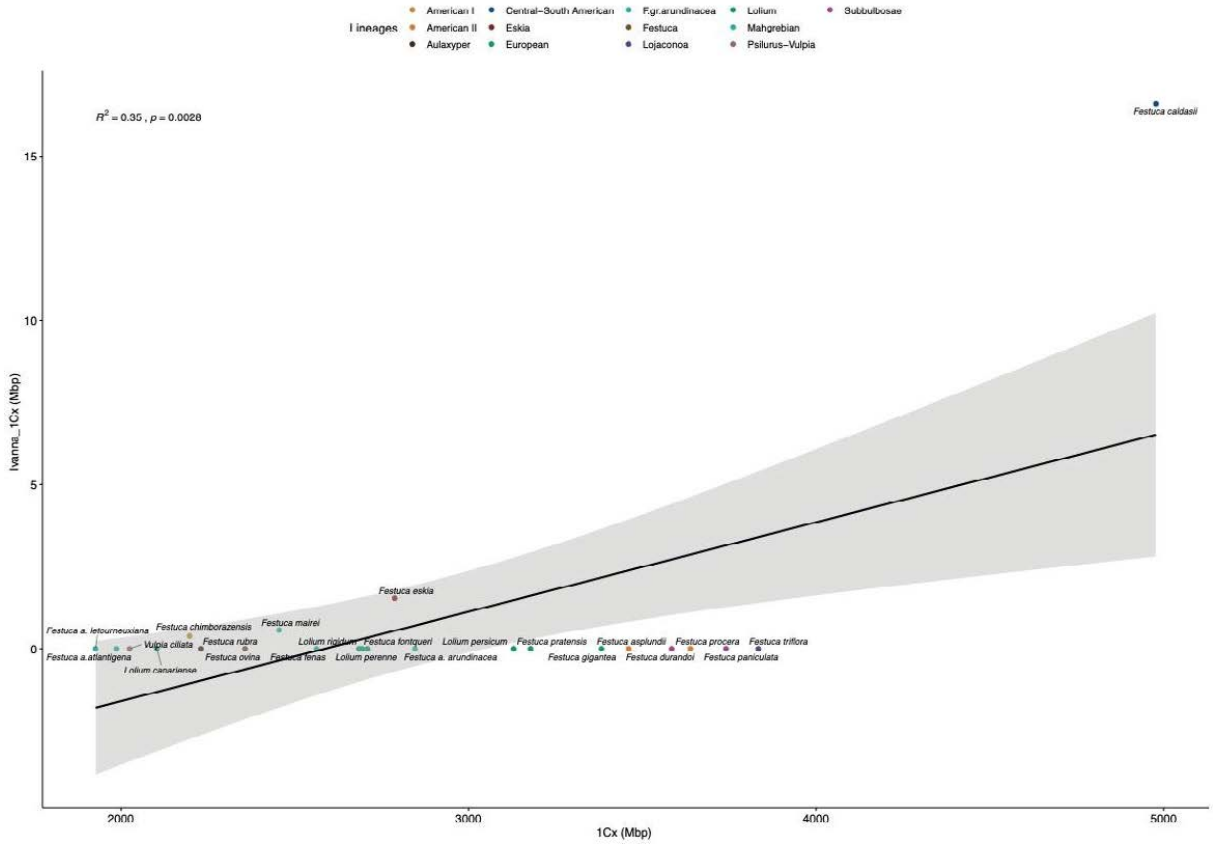
Supplementary Figure 1E. Histograms of repeat contents per holoploid genome (1C) retrieved from the individual Repeat Explorer 2 analyses of the studied Loliinae samples mapped onto the Maximum Likelihood combined phylogenomic tree (plastome + nuclear 35S rDNA cistron) of Loliinae. Ultrafast bootstrap support values are indicated on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root the trees. Color codes of Loliinae lineages are indicated in the charts. Scale bar: number of mutations per site.

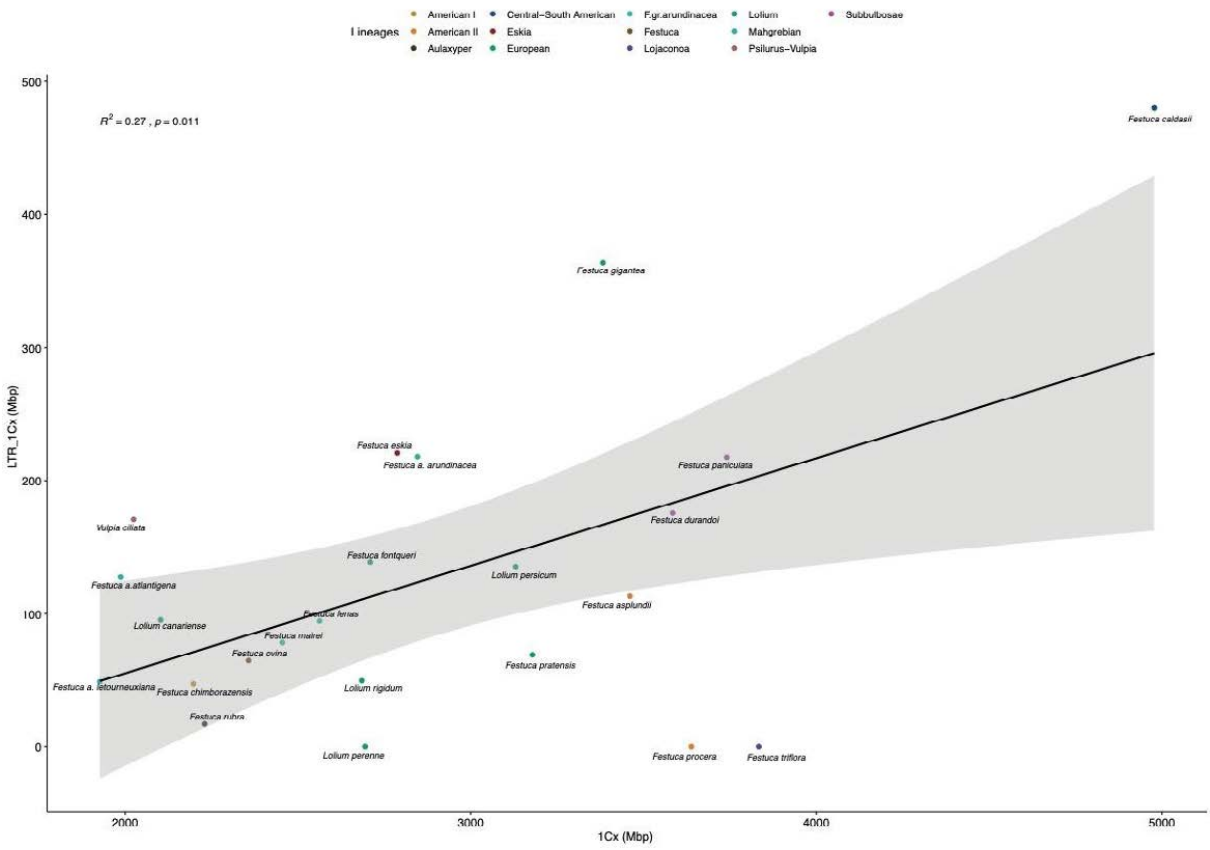
Suppl. Fig. S2

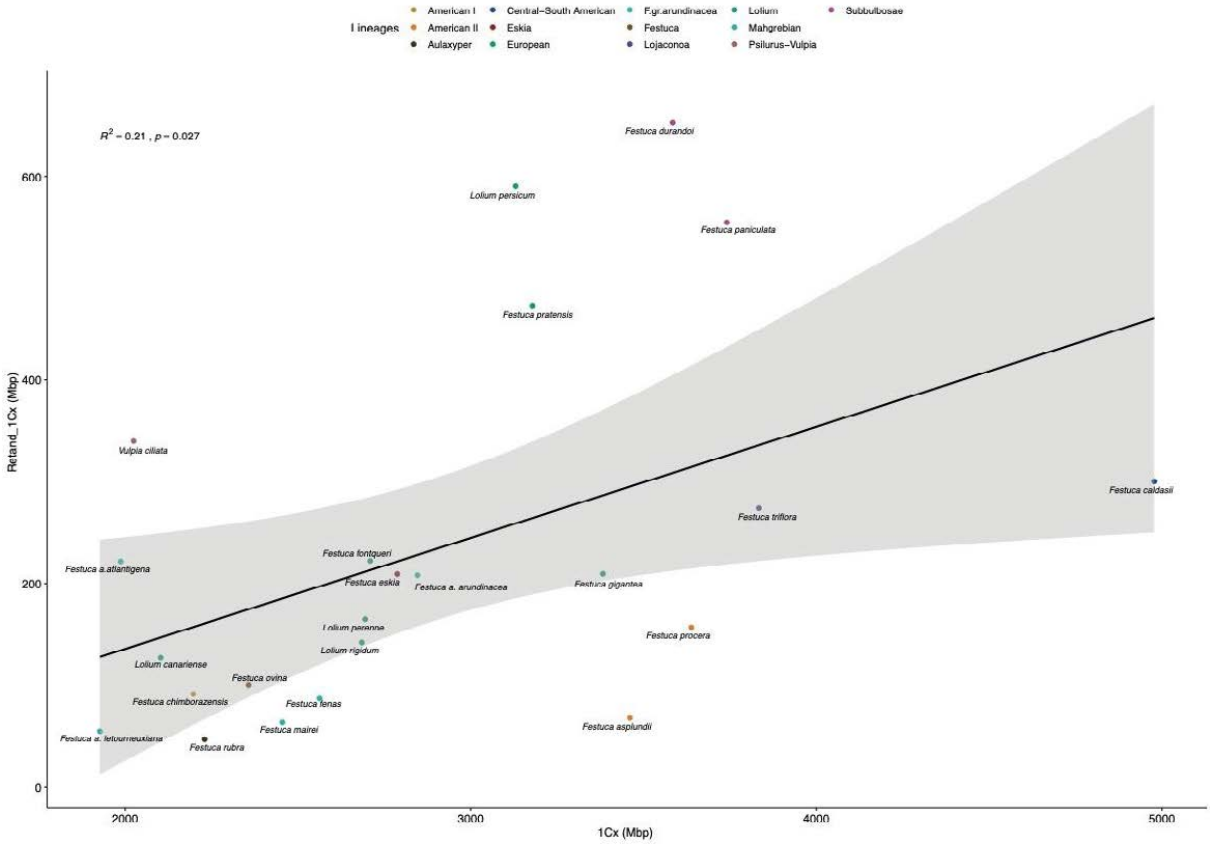

Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.71 , p = 5.4e−07
Angela _ 1Cx (Mbp)
1Cx (Mbp)
Festuca caldasii
Festuca triflora
Festuca procera
Festuca asplundii
Festuca paniculata
Festuca durandoi
Festuca chimborazensis
Festuca fontqueri
Festuca eskia
Lolium persicum
Festuca pratensis
Festuca gigantea
Vulpia ciliata
Lolium canariense
Festuca a.atlantigena
Festuca a. letourneuxiana
Festuca rubra
Festuca ovina
Festuca mairei
Festuca fenas
Lolium perenne
Lolium rigidum
Festuca a. arundinacea

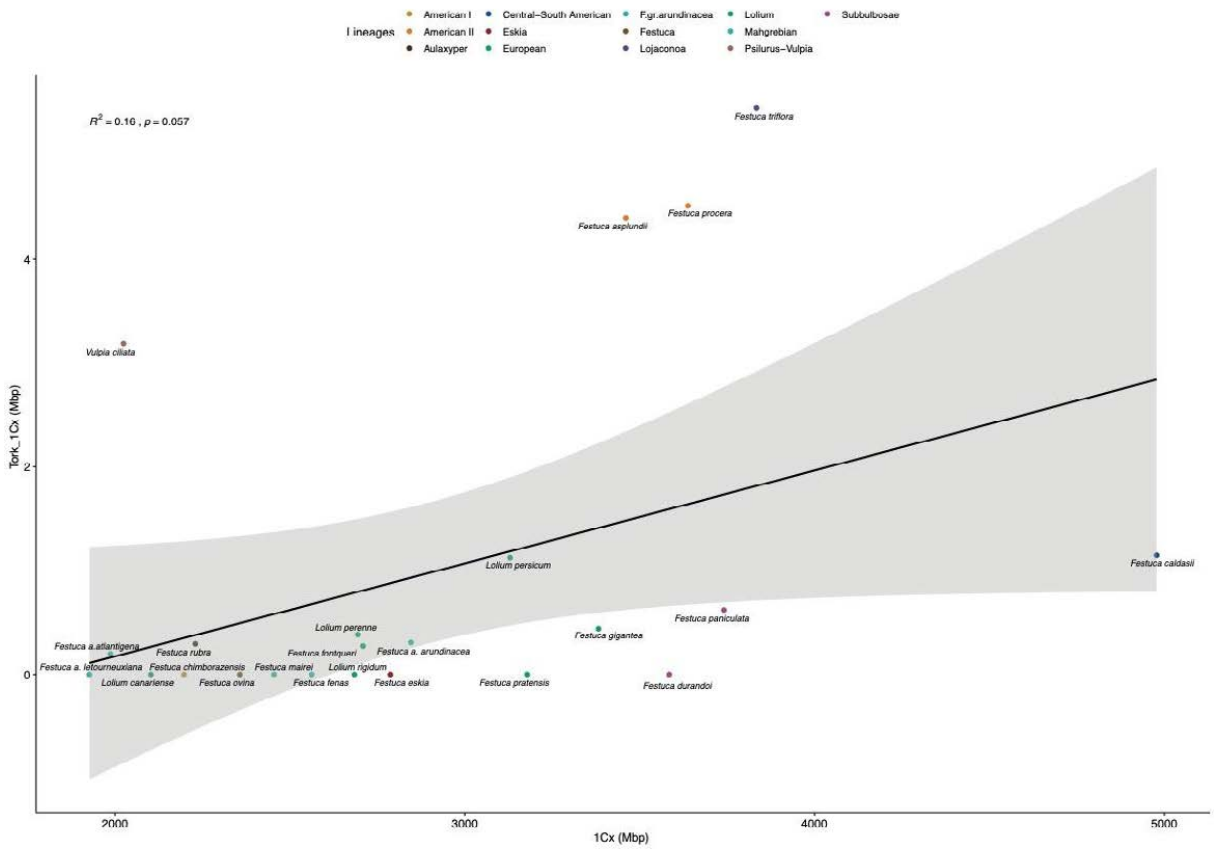

Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.54 , p = 5.9e−05
TAR_1Cx (Mbp)
1Cx (Mbp)
Festuca caldasii
Festuca procera
Festuca asplundii
Festuca durandoi
Festuca paniculata
Festuca chimborazensis
Lolium persicum
Festuca triflora
Festuca eskia
Vulpia ciliata
Festuca ovina
Festuca a. arundinacea
Festuca fontqueri
Festuca rubra
Festuca pratensis
Lolium canariense
Festuca mairei
Lolium perenne
Festuca fenas
Festuca gigantea
Festuca a.atlantigena
Festuca a. letourneuxiana
Lolium rigidum

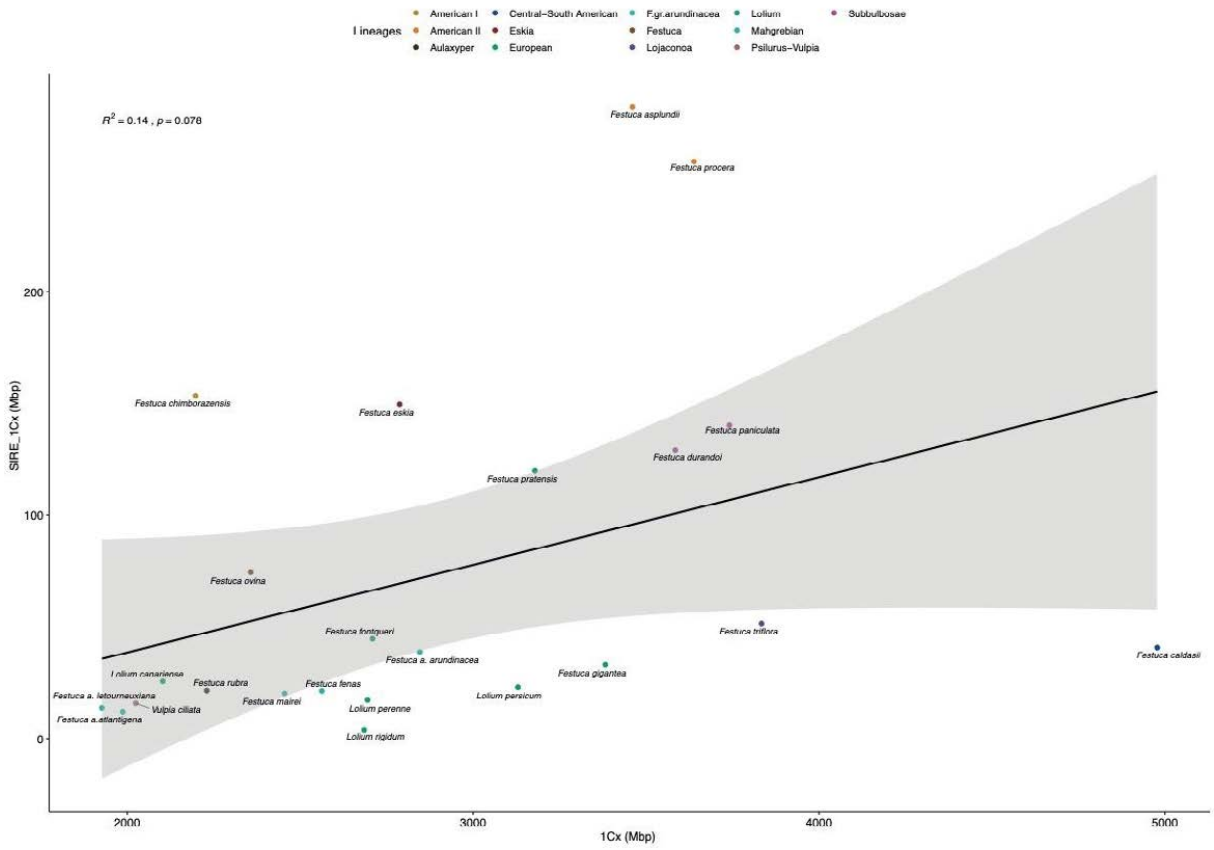

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.38 , p = 0.0019
Tekay_1Cx (Mbp)
1Cx (Mbp)
Festuca caldasii
Festuca triflora
Festuca fontqueri
Lolium persicum
Festuca a.atlantigena
Festuca pratensis
Festuca durandoi
Festuca eskia
Festuca gigantea
Festuca paniculata
Festuca fenas
Festuca ovina
Lolium canariense
Festuca a. arundinacea
Festuca mairei
Festuca rubra
Vulpia ciliata
Lolium rigidum
Festuca a. letourneuxiana
Festuca chimborazensis
Festuca asplundii
Festuca procera

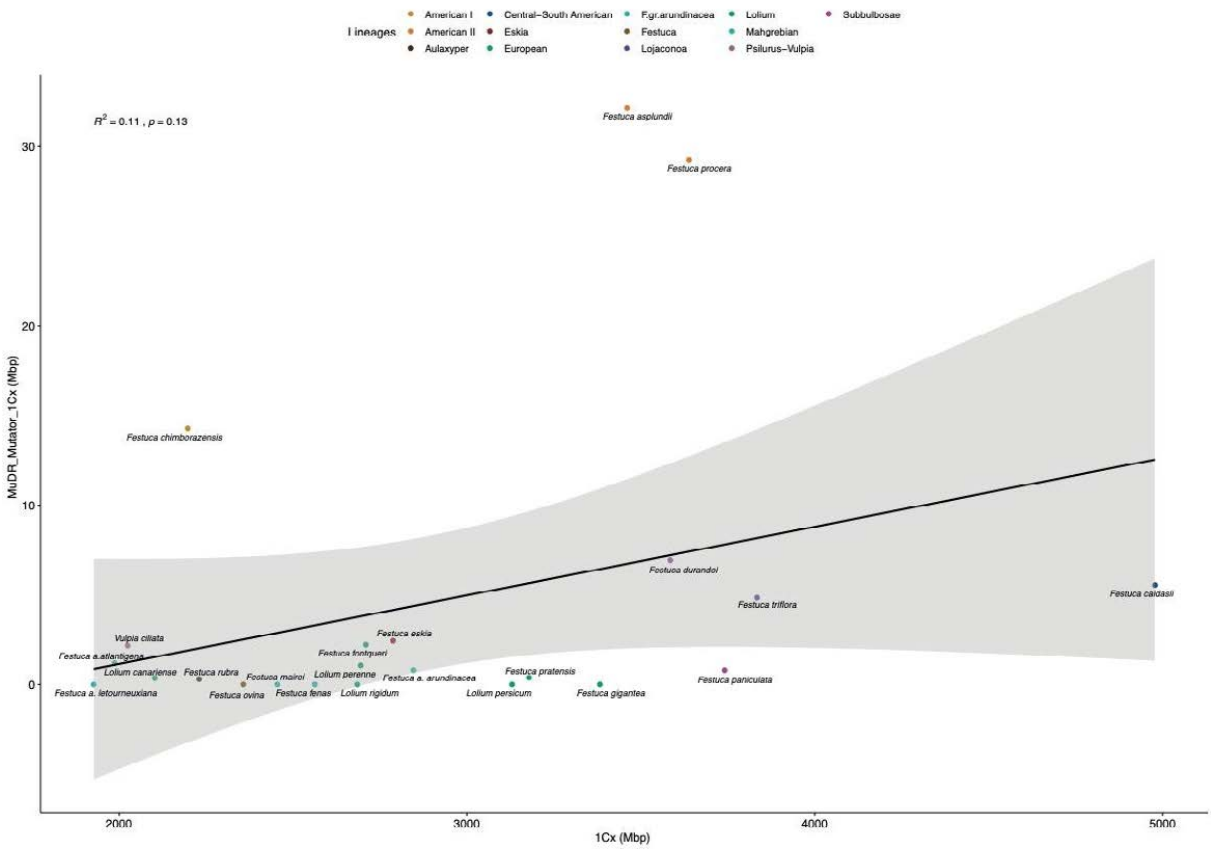

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.35 , p = 0.0026
Ivanna_1Cx (Mbp)
1Cx (Mbp)
Festuca caldasii
Festuca eskia
Festuca a. letourneuxiana
Festuca chimborazensis
Festuca mairei
Festuca a.atlantigena
Vulpia ciliata
Festuca rubra
Lolium rigidum
Festuca fontqueri
Lolium persicum
Festuca pratensis
Festuca asplundii
Festuca procera
Festuca triflora
Festuca ovina
Festuca fenas
Lolium perenne
Festuca a. arundinacea
Festuca gigantea
Festuca durandoi
Festuca paniculata
Lolium canariense

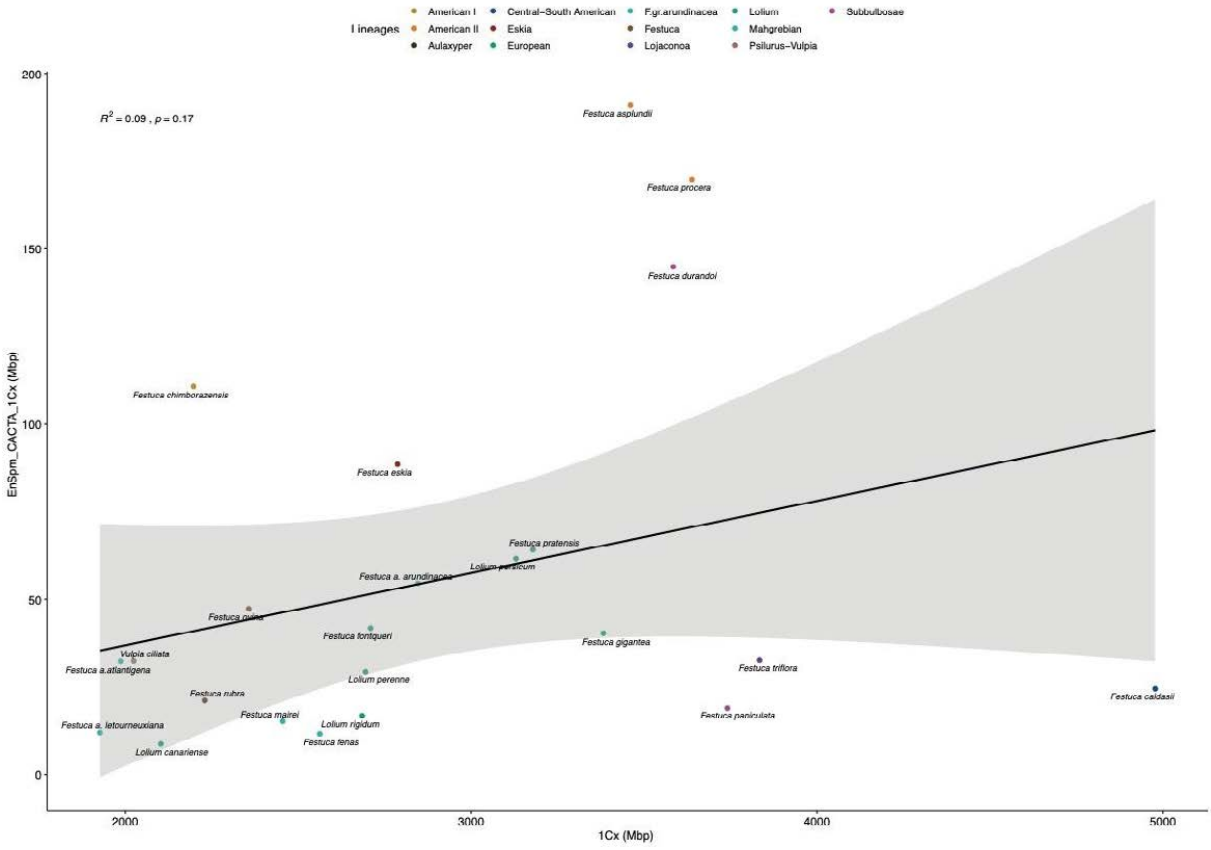

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.27 , p = 0.011
LTR_1Cx (Mbp)
1Cx (Mbp)
Festuca caldasii
Festuca gigantea
Festuca eskia
Festuca a. arundinacea
Festuca paniculata
Festuca durandoi
Vulpia ciliata
Festuca fontqueri
Festuca a.atlantigena
Lolium persicum
Festuca asplundii
Festuca lenas
Lolium canariense
Festuca mairei
Festuca ovina
Festuca pratensis
Festuca a. letourneuxiana
Festuca chimborazensis
Lolium rigidum
Festuca rubra
Lolium perenne
Festuca procera
Festuca triflora

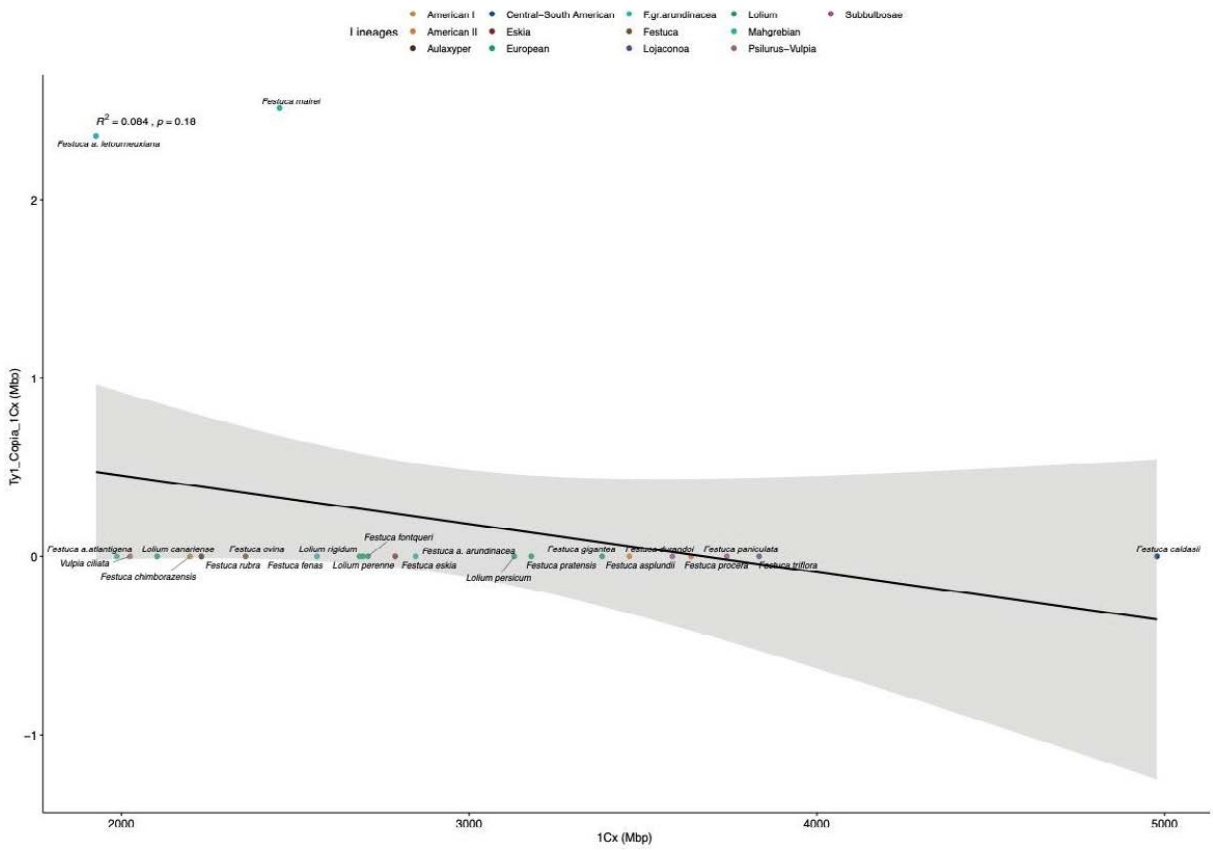

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.21 , p = 0.027
Retand_1Cx (Mbp)
1Cx (Mbp)
Festuca durandoi
Lolium persicum
Festuca paniculata
Festuca pratensis
Vulpia ciliata
Festuca caldasii
Festuca triflora
Festuca fontqueri
Festuca a.atlantigena
Festuca eskia
Festuca a. arundinacea
Festuca gigantea
Lolium perenne
Festuca procera
Lolium rigidum
Lolium canariense
Festuca ovina
Festuca chimborazensis
Festuca lenas
Festuca asplundii
Festuca mairei
Festuca a. letourneuxiana
Festuca rubra

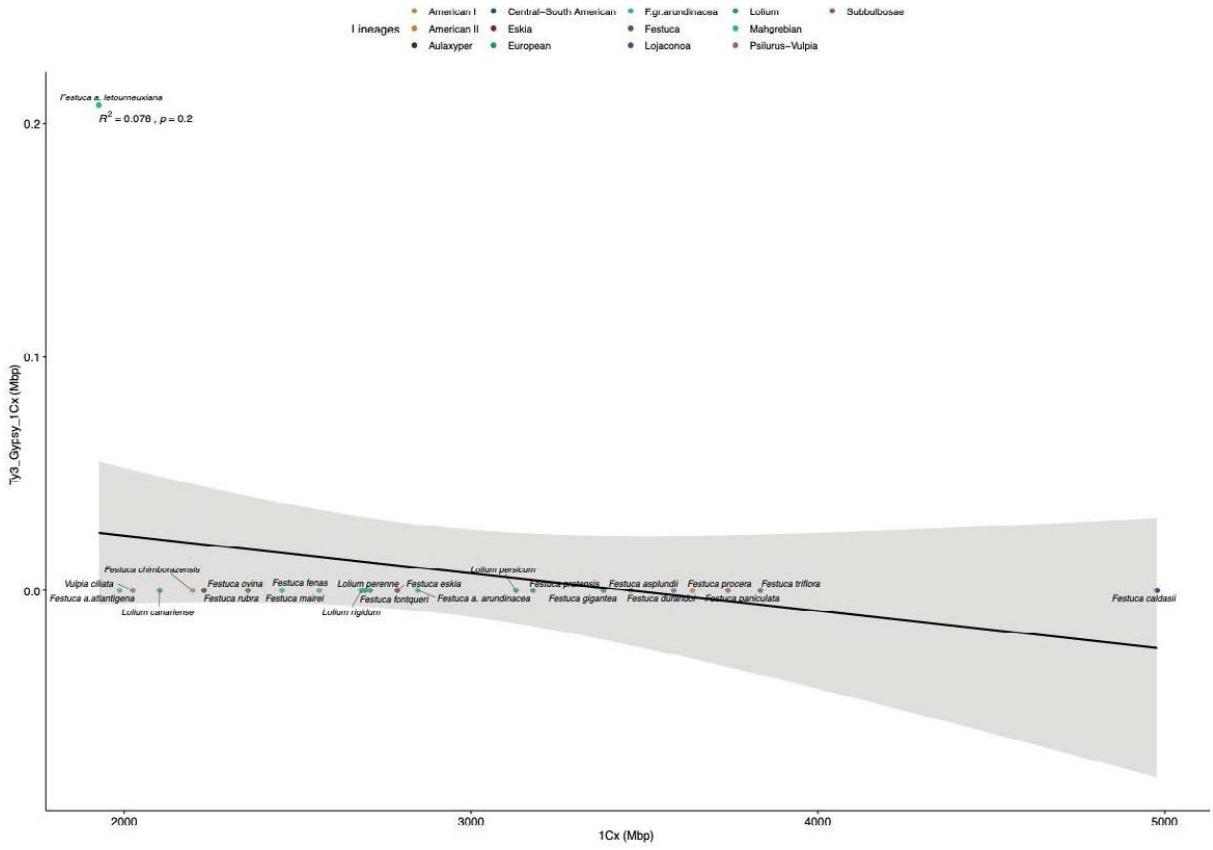

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.16 , p = 0.057
Tork_1Cx (Mbp)
1Cx (Mbp)
Festuca triflora
Festuca procera
Festuca asplundii
Vulpia ciliata
Lolium persicum
Festuca caldasii
Festuca paniculata
Festuca gigantea
Lolium perenne
Festuca a.atlantigena
Festuca rubra
Festuca fontquari
Festuca a. arundinacea
Festuca a. letourneuxiana
Festuca chimborazensis
Festuca mairei
Lolium rigidum
Lolium canariense
Festuca ovina
Festuca fenas
Festuca eskia
Festuca pratensis
Festuca durandoi

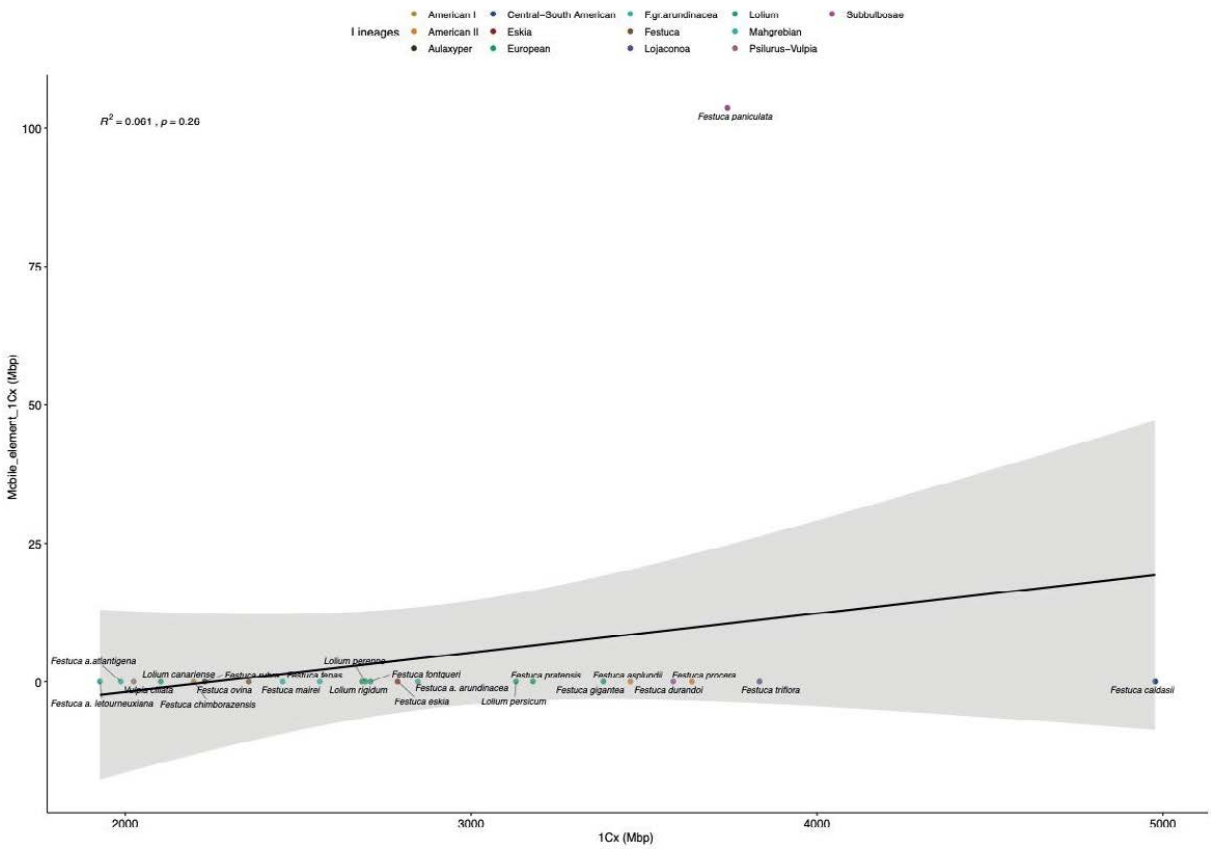

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.14 , p = 0.078
SIRE_1Cx (Mbp)
1Cx (Mbp)
Festuca asplundii
Festuca procera
Festuca chimborazensis
Festuca eskia
Festuca paniculata
Festuca durandoi
Festuca pratensis
Festuca ovina
Festuca fontquari
Festuca triflora
Festuca a. arundinacea
Festuca caldasii
Festuca gigantea
Lolium canariense
Festuca rubra
Festuca fenas
Festuca a. letourneuxiana
Lolium persicum
Festuca mairei
Vulpia ciliata
Lolium perenne
Festuca a.atlantigena
Lolium rigidum

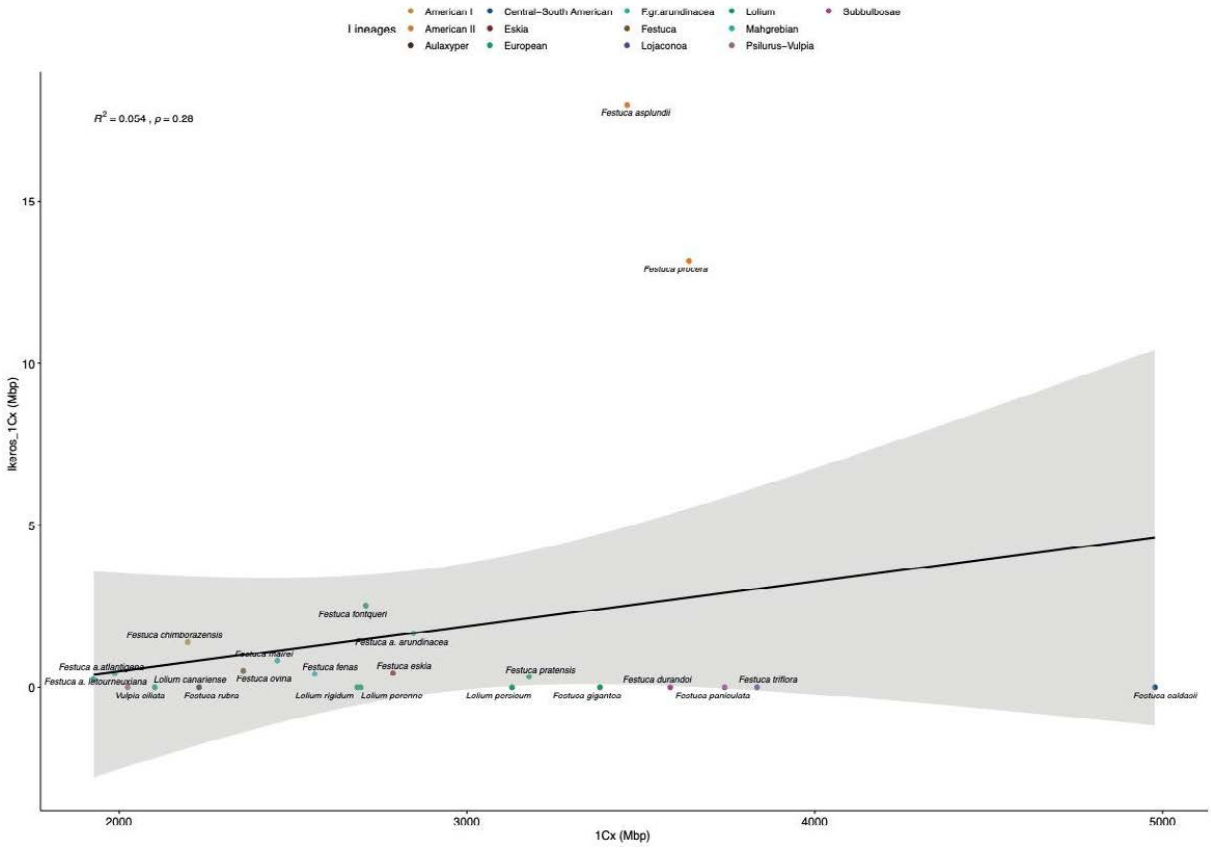

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.11 , p = 0.13
MuDR_Mutator_1Cx (Mbp)
1Cx (Mbp)
Festuca asplundii
Festuca procera
Festuca chimborazensis
Festuca durandoi
Festuca triflora
Festuca caldasii
Vulpia ciliata
Festuca eskia
Festuca fontqueri
Festuca a.atlantigena
Festuca rubra
Festuca mairei
Lolium perenne
Festuca a. arundinacea
Festuca pratensis
Festuca panniculata
Lolium canariense
Festuca a. letourneuxiana
Festuca ovina
Festuca fenas
Lolium rigidum
Lolium persicum
Festuca gigantea

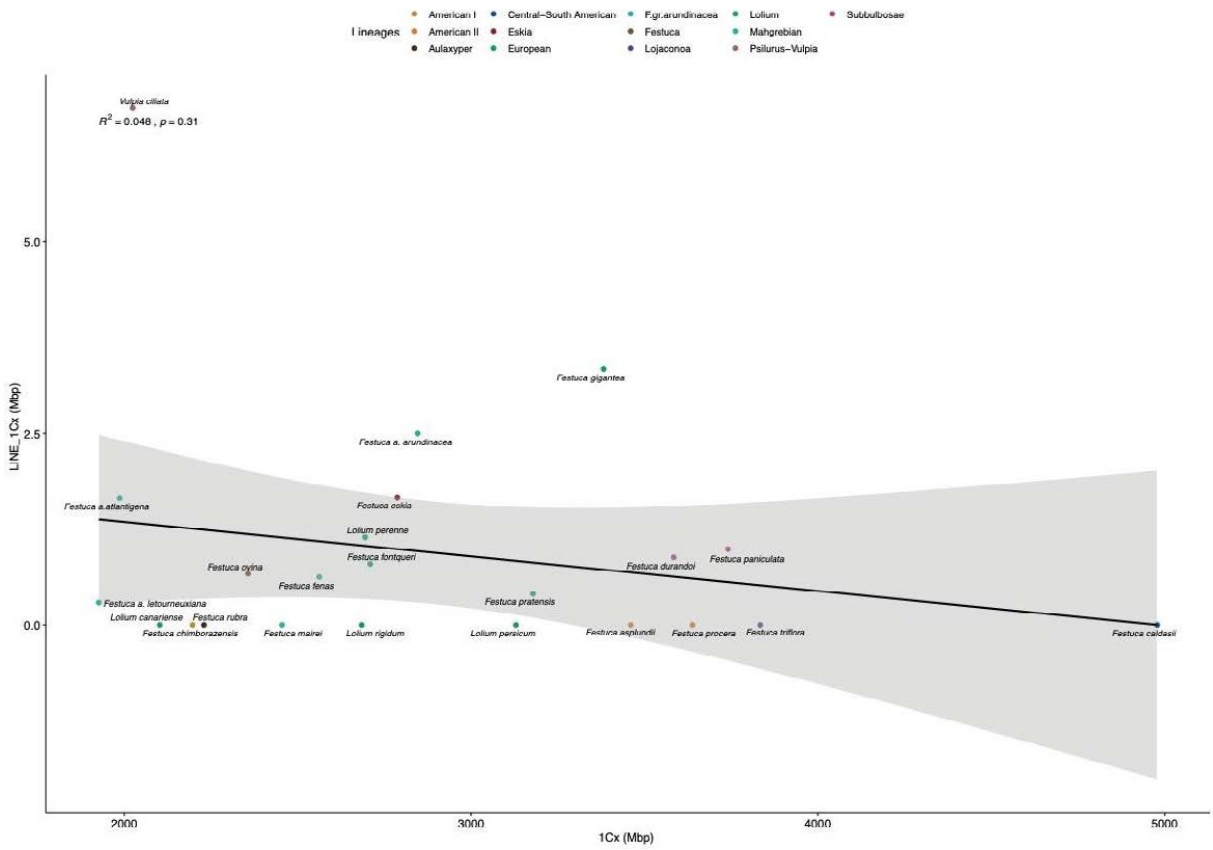

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.09 , p = 0.17
EnSpm_CACTA_1Cx (Mbp)
1Cx (Mbp)
Festuca asplundii
Festuca procera
Festuca durandoi
Festuca chimborazensis
Festuca eskia
Festuca pratensis
Festuca a. arundinacea
Lolium persicum
Festuca ovina
Festuca fontqueri
Festuca gigantea
Vulpia ciliata
Festuca a.atlantigena
Festuca triflora
Lolium perenne
Festuca caldasii
Festuca rubra
Festuca mairei
Festuca panniculata
Festuca a. letourneuxiana
Lolium rigidum
Festuca fenas
Lolium canariense

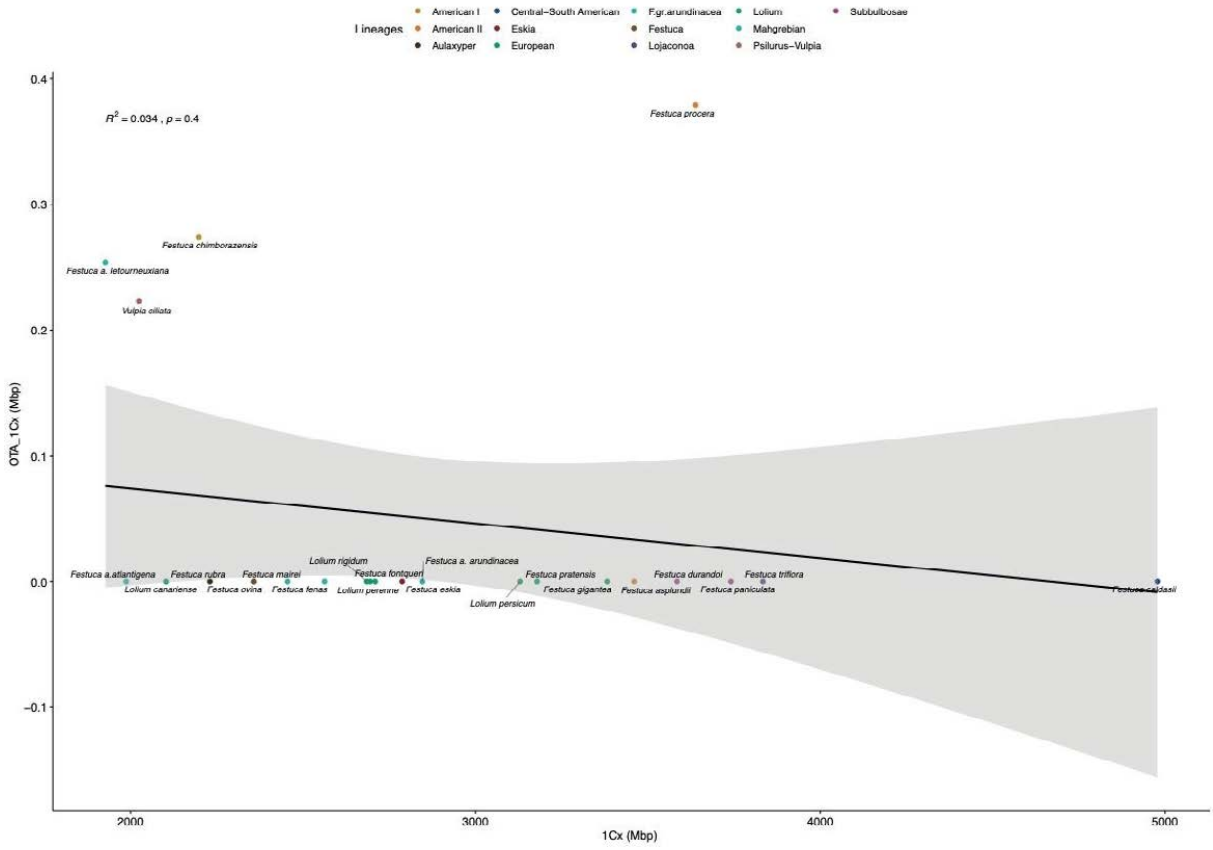

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
E.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
$R^2 = 0.084$, $p = 0.16$
Festuca a. letourneuxiana
Festuca mairei
Festuca fontqueri
Festuca caldasii
Ty1_Copia_1Cx (Mbp)
1Cx (Mbp)

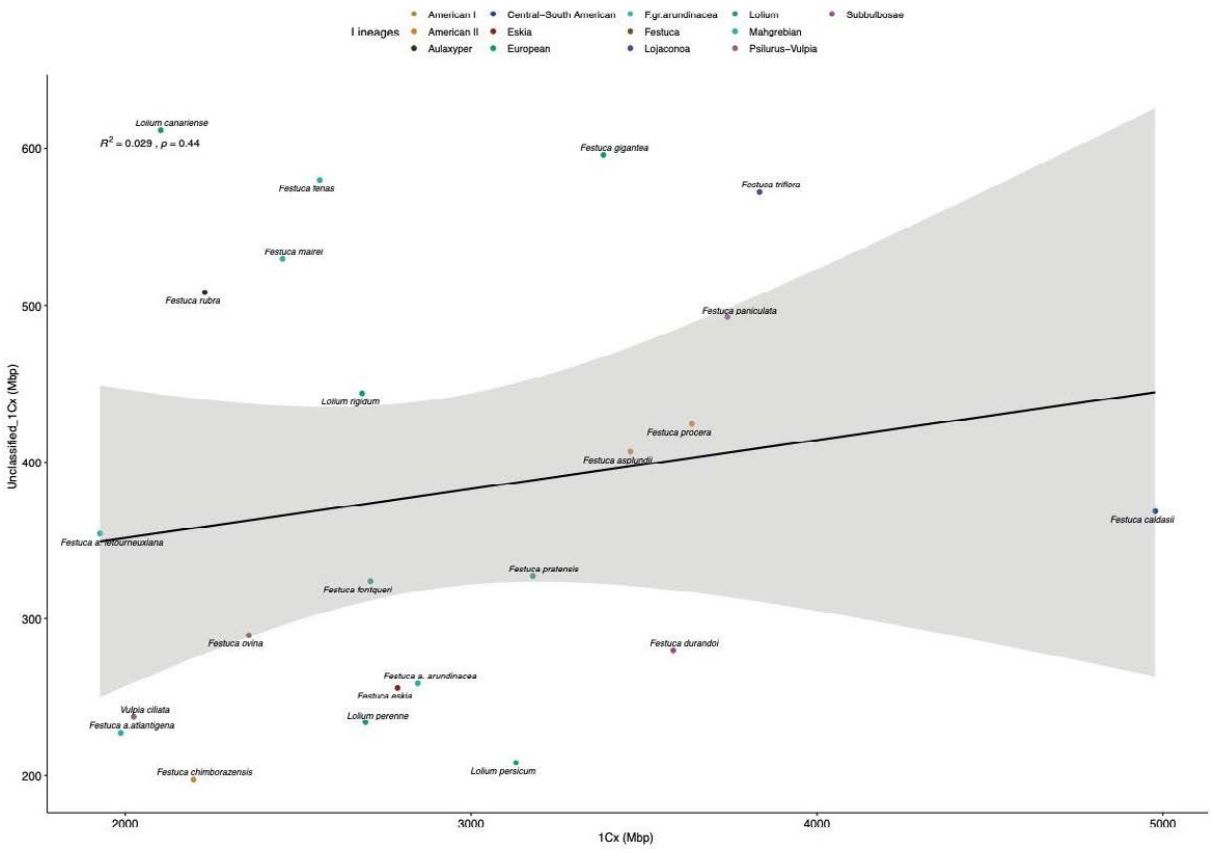

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
E.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
$R^2 = 0.078$, $p = 0.2$
Festuca a. letourneuxiana
Festuca caldasii
Ty3_Gypsy_1Cx (Mbp)
1Cx (Mbp)

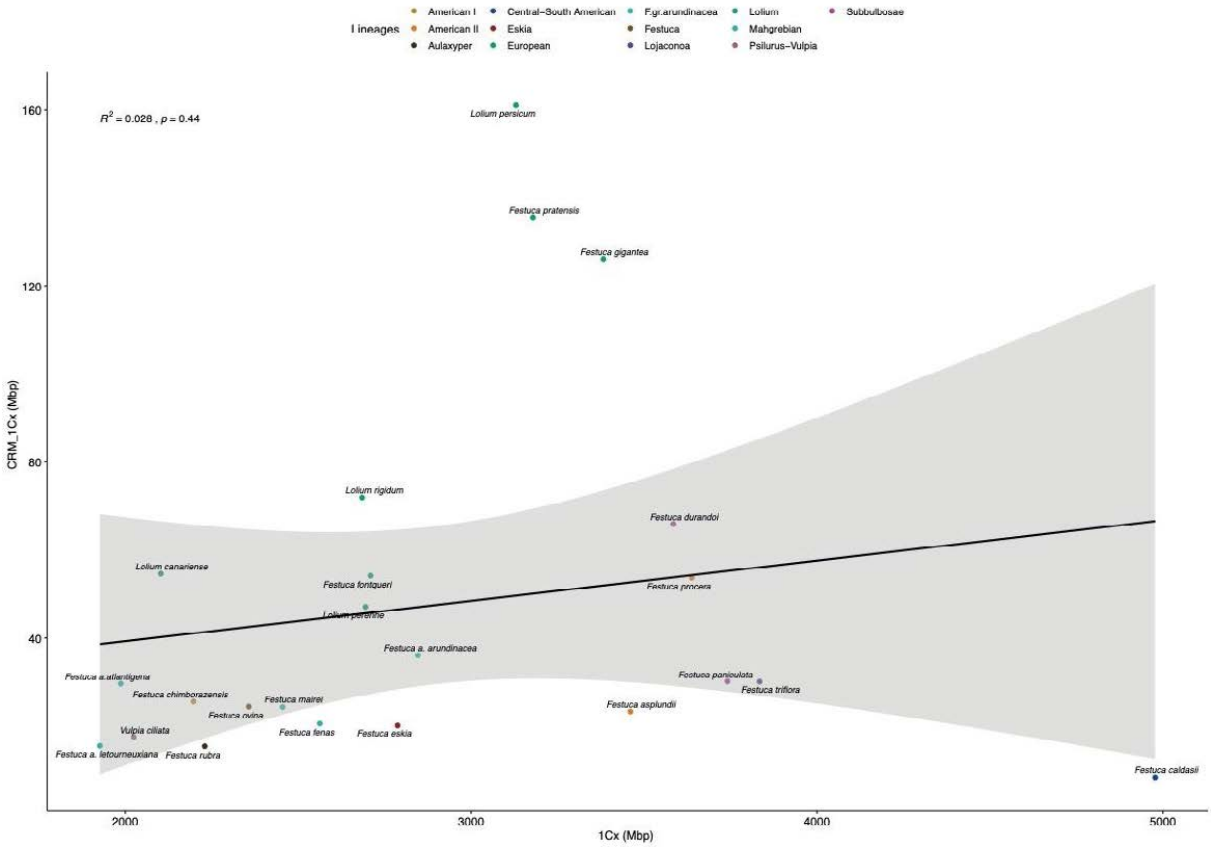

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.061 , p = 0.26
Festuca paniculata
Mobile_element_1Cx (Mbp)
1Cx (Mbp)
Festuca a.atlantigena
Lolium canariense
Festuca rubra
Vulpia ciliata
Festuca ovina
Festuca fenas
Festuca mairei
Lolium perenne
Lolium rigidum
Festuca fontqueri
Festuca a. arundinacea
Festuca eskia
Festuca pratensis
Lolium persicum
Festuca asplundii
Festuca gigantea
Festuca durandoi
Festuca triflora
Festuca caldasii
Festuca a. letourneuxiana
Festuca chimborazensis

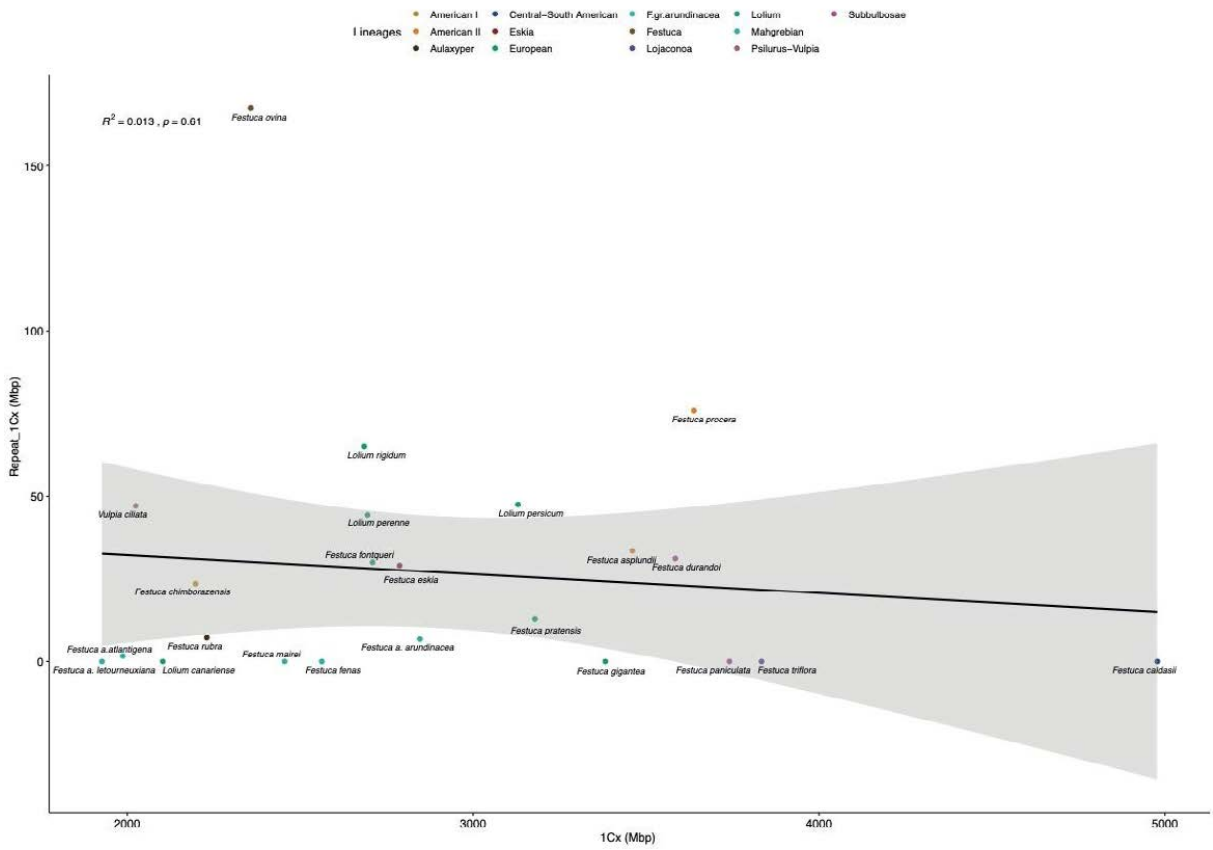

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.054 , p = 0.28
Festuca asplundii
Festuca procera
Ikaros_1Cx (Mbp)
1Cx (Mbp)
Festuca fontqueri
Festuca chimborazensis
Festuca a. arundinacea
Festuca mairei
Festuca a.atlantigena
Festuca a. letourneuxiana
Lolium canariense
Festuca ovina
Festuca fenas
Festuca eskia
Festuca pratensis
Festuca durandoi
Festuca triflora
Vulpia ciliata
Festuca rubra
Lolium rigidum
Lolium perenne
Lolium persicum
Festuca gigantea
Festuca paniculata
Festuca caldasii

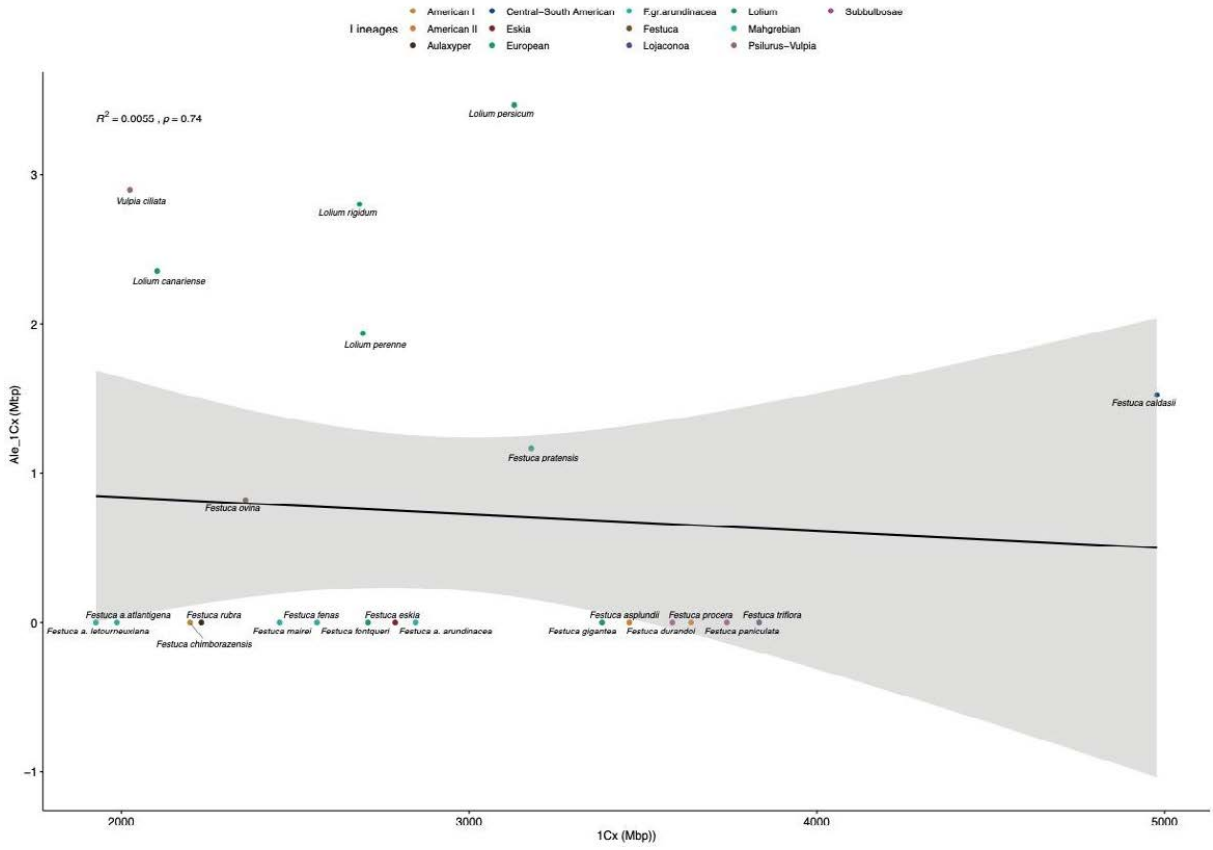

Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
Egr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
$R^2 = 0.046$, $p = 0.31$
LINE_1Cx (Mbp)
1Cx (Mbp)
Vulpia ciliata
Festuca gigantea
Festuca a. arundinacea
Festuca a.atlantigena
Festuca eskia
Lolium perenne
Festuca fontqueri
Festuca ovina
Festuca durandoi
Festuca paniculata
Festuca fenas
Festuca pratensis
Festuca a. letourneuxiana
Lolium canariense
Festuca rubra
Festuca chimborazensis
Festuca mairei
Lolium rigidum
Lolium persicum
Festuca asplundii
Festuca procera
Festuca triflora
Festuca caldasii

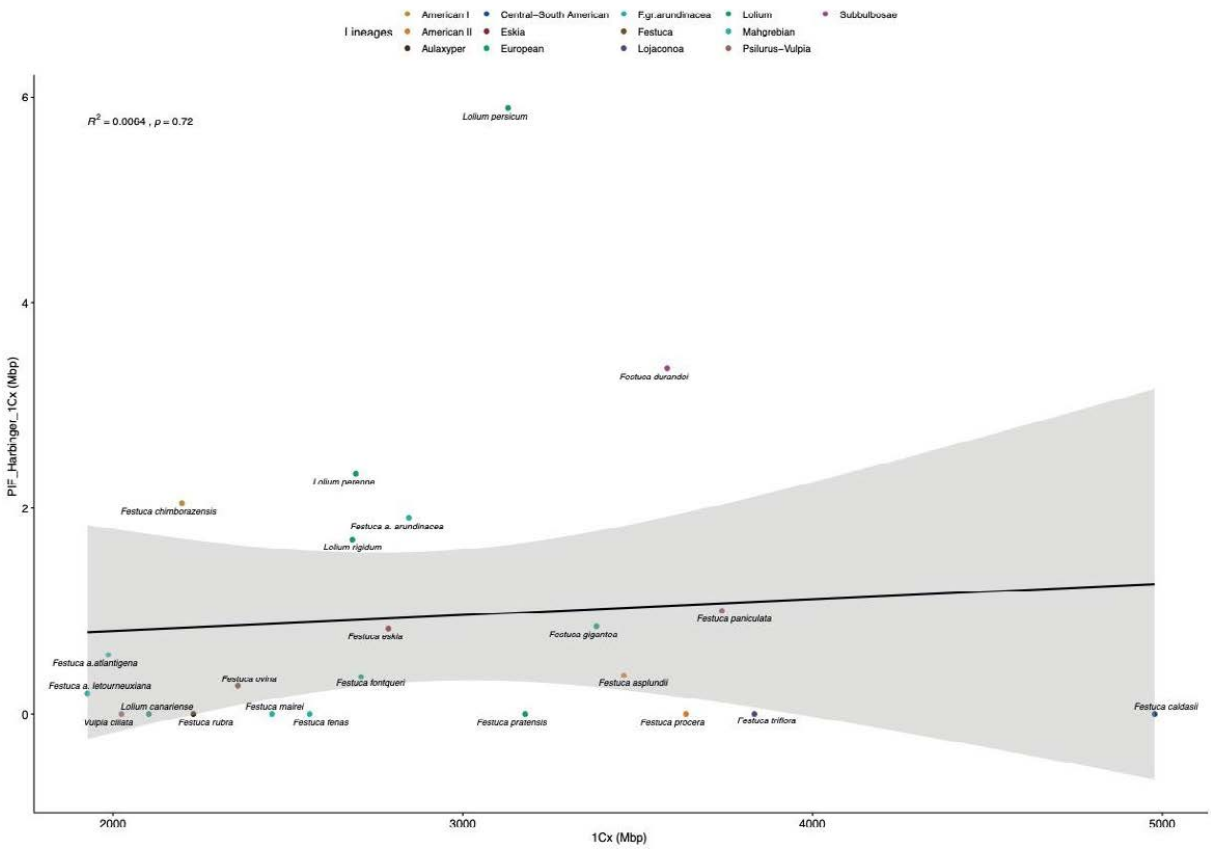

Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
Egr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
$R^2 = 0.034$, $p = 0.4$
OTA_1Cx (Mbp)
1Cx (Mbp)
Festuca procera
Festuca chimborazensis
Festuca a. letourneuxiana
Vulpia ciliata
Lolium rigidum
Festuca a. arundinacea
Festuca a.atlantigena
Festuca rubra
Festuca mairei
Festuca fontqueri
Festuca pratensis
Festuca durandoi
Festuca triflora
Lolium canariense
Festuca ovina
Festuca fenas
Lolium perenne
Festuca eskia
Festuca gigantea
Festuca asplundii
Festuca paniculata
Lolium persicum
Festuca caldasii

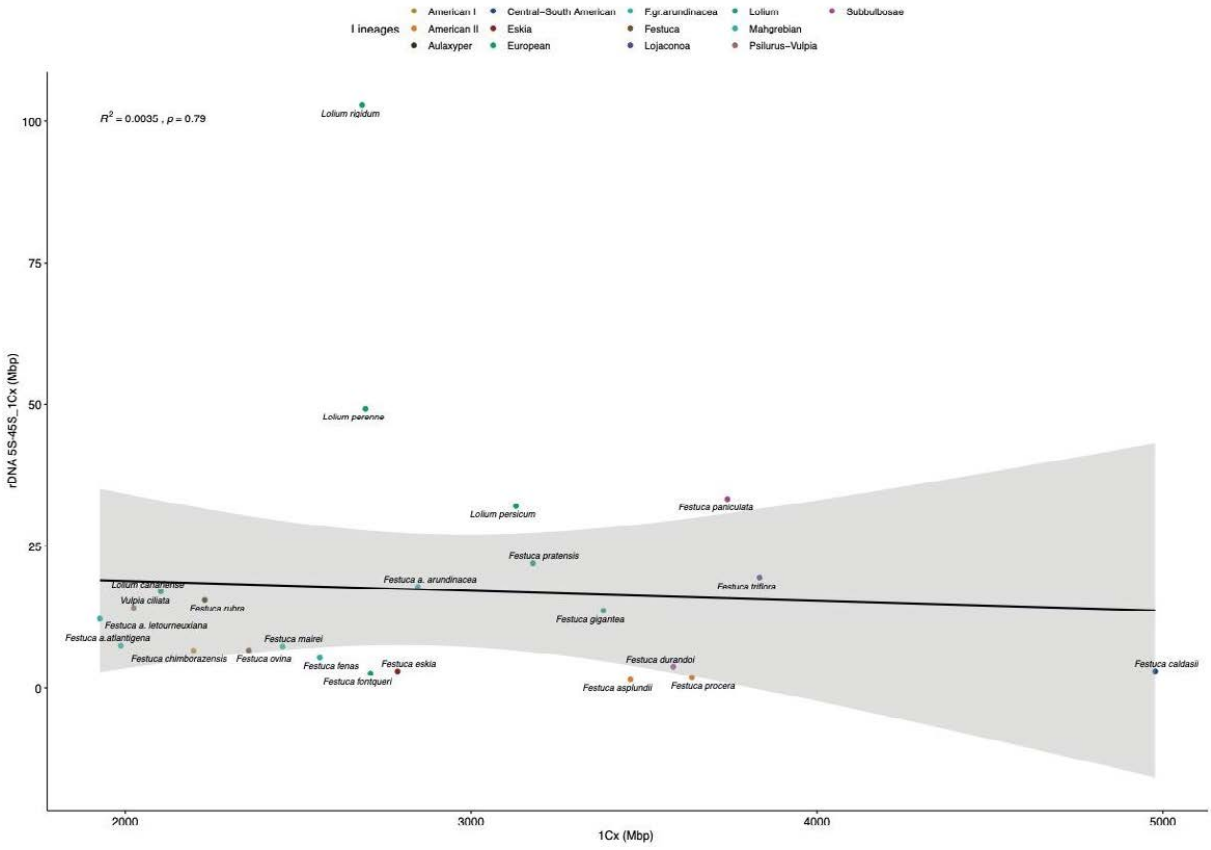

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.029 , p = 0.44
Lolium canariense
Festuca gigantea
Festuca tenas
Festuca trifiora
Festuca mairei
Festuca rubra
Festuca paniculata
Lolium rigidum
Festuca procera
Festuca asplundii
Festuca caldasii
Festuca a. letourneuxiana
Festuca pratensis
Festuca fontqueri
Festuca ovina
Festuca durandoi
Festuca a. arundinacea
Festuca eskia
Vulpia ciliata
Festuca a.atlantigena
Lolium perenne
Festuca chimborazensis
Lolium persicum
Unclassified_1Cx (Mbp)
1Cx (Mbp)
200
300
400
500
600
2000
3000
4000
5000

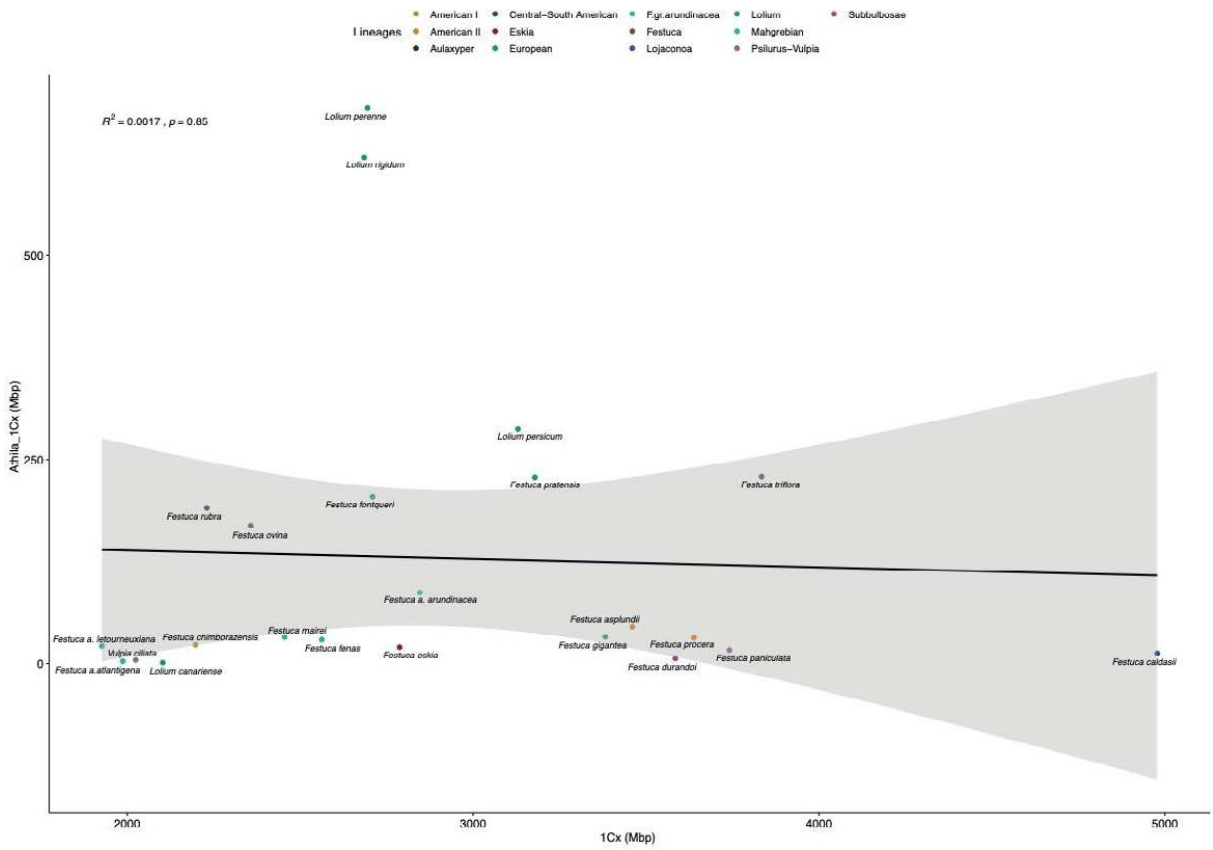

Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
F.gr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.028 , p = 0.44
Lolium persicum
Festuca pratensis
Festuca gigantea
Lolium rigidum
Festuca durandoi
Lolium canariense
Festuca fontqueri
Festuca procera
Lolium perenne
Festuca a. arundinacea
Festuca a.atlantigena
Festuca chimborazensis
Festuca mairei
Festuca ovina
Festuca paniculata
Festuca trifiora
Festuca asplundii
Vulpia ciliata
Festuca tenas
Festuca eskia
Festuca a. letourneuxiana
Festuca rubra
Festuca caldasii
CRM_1Cx (Mbp)
1Cx (Mbp)
40
80
120
160
2000
3000
4000
5000

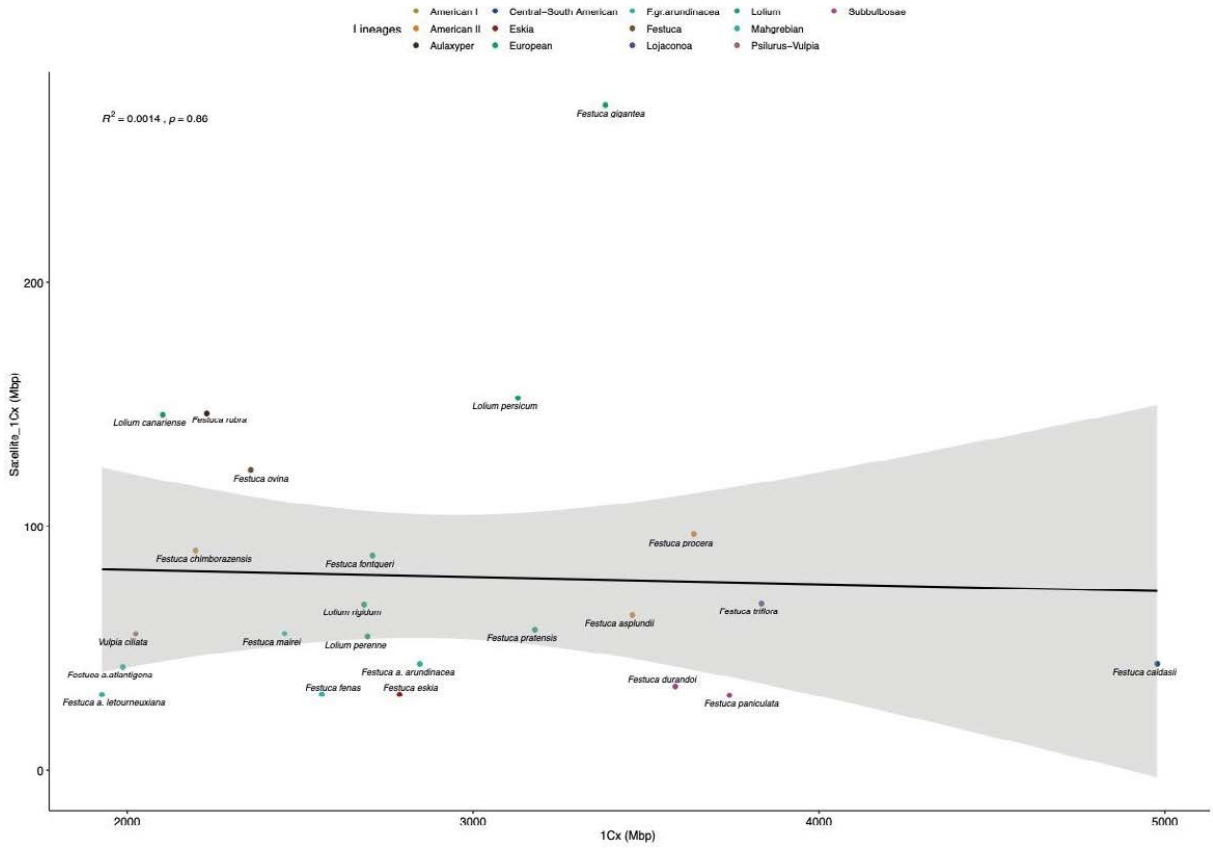

Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
Egr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.013 , p = 0.61
Repeat_1Cx (Mbp)
1Cx (Mbp)
Festuca ovina
Festuca procera
Lolium rigidum
Vulpia ciliata
Lolium perenne
Lolium persicum
Festuca fontqueri
Festuca asplundii
Festuca durandoi
Festuca eskia
Festuca chimborazensis
Festuca pratensis
Festuca a.atlantigena
Festuca rubra
Festuca mairei
Festuca a. arundinacea
Festuca a. letourneuxiana
Lolium canariense
Festuca fenas
Festuca gigantea
Festuca paniculata
Festuca triflora
Festuca caldasii


Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
Egr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.0055 , p = 0.74
Ale_1Cx (Mbp)
1Cx (Mbp))
Lolium persicum
Vulpia ciliata
Lolium rigidum
Lolium canariense
Lolium perenne
Festuca caldasii
Festuca pratensis
Festuca ovina
Festuca a.atlantigena
Festuca rubra
Festuca fenas
Festuca eskia
Festuca asplundii
Festuca procera
Festuca triflora
Festuca a. letourneuxiana
Festuca chimborazensis
Festuca mairei
Festuca fontqueri
Festuca a. arundinacea
Festuca gigantea
Festuca durandoi
Festuca paniculata


Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.0064 , p = 0.72
PIF_Harbinger_1Cx (Mbp)
1Cx (Mbp)
Lolium persicum
Festuca durandoi
Lolium perenne
Festuca chimborazensis
Festuca a. arundinacea
Lolium rigidum
Festuca paniculata
Festuca eskia
Festuca gigantea
Festuca a.atlantigena
Festuca ovina
Festuca a. letourneuxiana
Festuca fontqueri
Festuca asplundii
Lolium canariense
Festuca mairei
Festuca caldasii
Vulpia ciliata
Festuca rubra
Festuca tenas
Festuca pratensis
Festuca procera
Festuca trifiora


Lineages
American I
American II
Aulaxyper
Central−South American
Eskia
European
Fgr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus−Vulpia
Subbulbosae
R^2 = 0.0035 , p = 0.79
rDNA.5S-45S_1Cx (Mbp)
1Cx (Mbp)
Lolium rigidum
Lolium perenne
Lolium persicum
Festuca paniculata
Festuca pratensis
Festuca a. arundinacea
Festuca trifiora
Lolium canariense
Vulpia ciliata
Festuca rubra
Festuca gigantea
Festuca a. letourneuxiana
Festuca a.atlantigena
Festuca mairei
Festuca chimborazensis
Festuca ovina
Festuca tenas
Festuca eskia
Festuca durandoi
Festuca caldasii
Festuca fontqueri
Festuca asplundii
Festuca procera


Lineages
American I
American II
Aulaxyper
Central–South American
Eskia
European
Eyr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.0017 , p = 0.85
Athila_1Cx (Mbp)
1Cx (Mbp)
Lolium perenne
Lolium rigidum
Lolium persicum
Festuca pratensis
Festuca triflora
Festuca fontqueri
Festuca rubra
Festuca ovina
Festuca a. arundinacea
Festuca asplundii
Festuca a. letourneuxiana
Festuca chimborazensis
Festuca mairei
Festuca gigantea
Festuca procera
Festuca fenas
Festuca eskia
Festuca paniculata
Vulpia ciliata
Festuca durandoi
Festuca caldasii
Festuca a.atlantigena
Lolium canariense


Lineages
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American II
Aulaxyper
Central–South American
Eskia
European
Eyr.arundinacea
Festuca
Lojaconoa
Lolium
Mahgrebian
Psilurus–Vulpia
Subbulbosae
R^2 = 0.0014 , p = 0.86
Sarelita_1Cx (Mbp)
1Cx (Mbp)
Festuca gigantea
Lolium persicum
Lolium canariense
Festuca rubra
Festuca ovina
Festuca procera
Festuca chimborazensis
Festuca fontqueri
Lolium rigidum
Festuca triflora
Festuca asplundii
Vulpia ciliata
Festuca mairei
Lolium perenne
Festuca pratensis
Festuca a. arundinacea
Festuca durandoi
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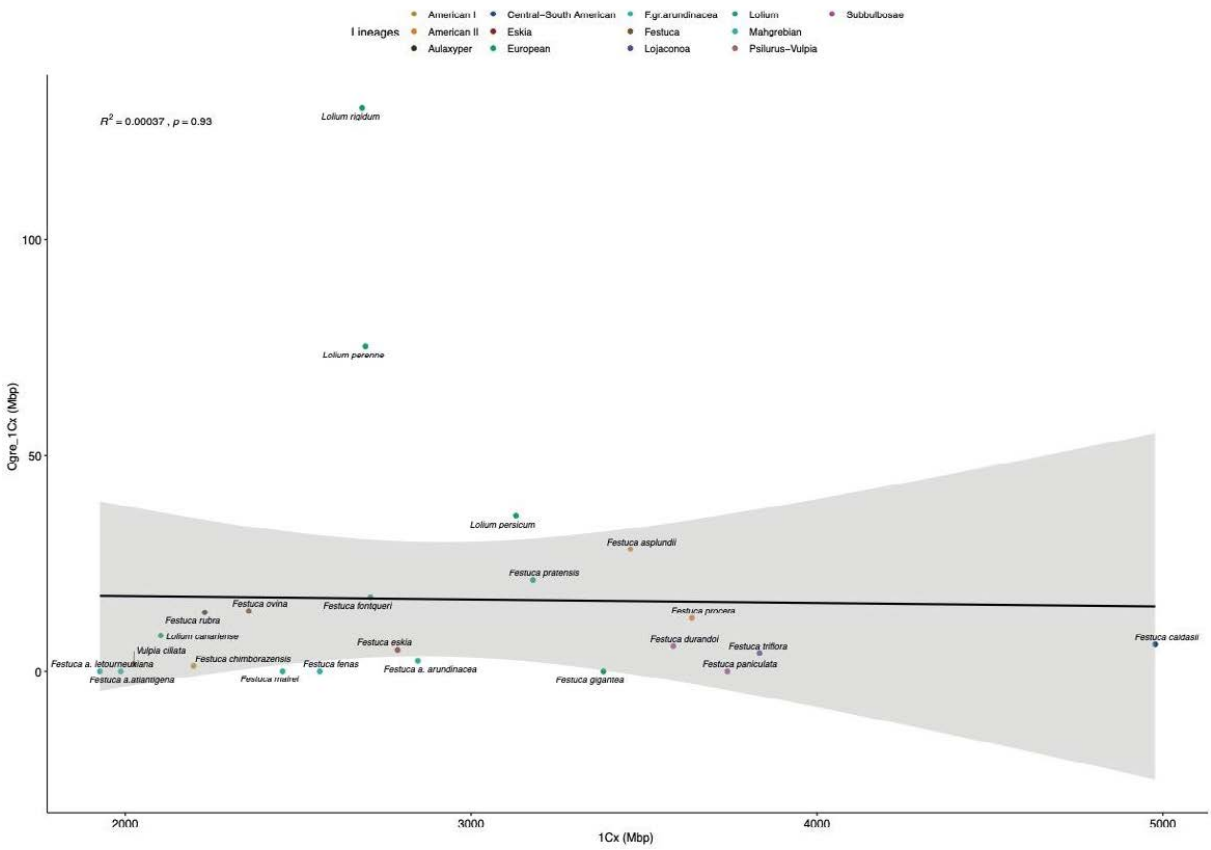
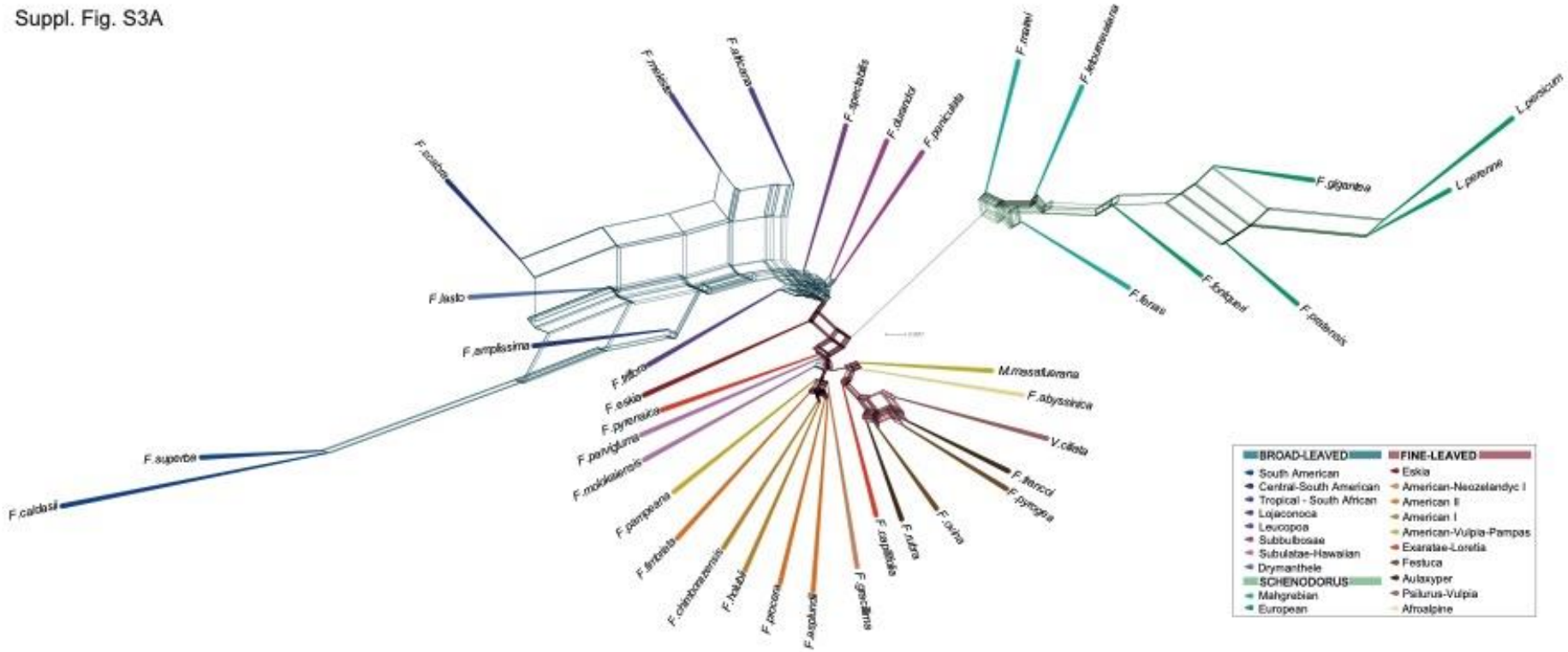
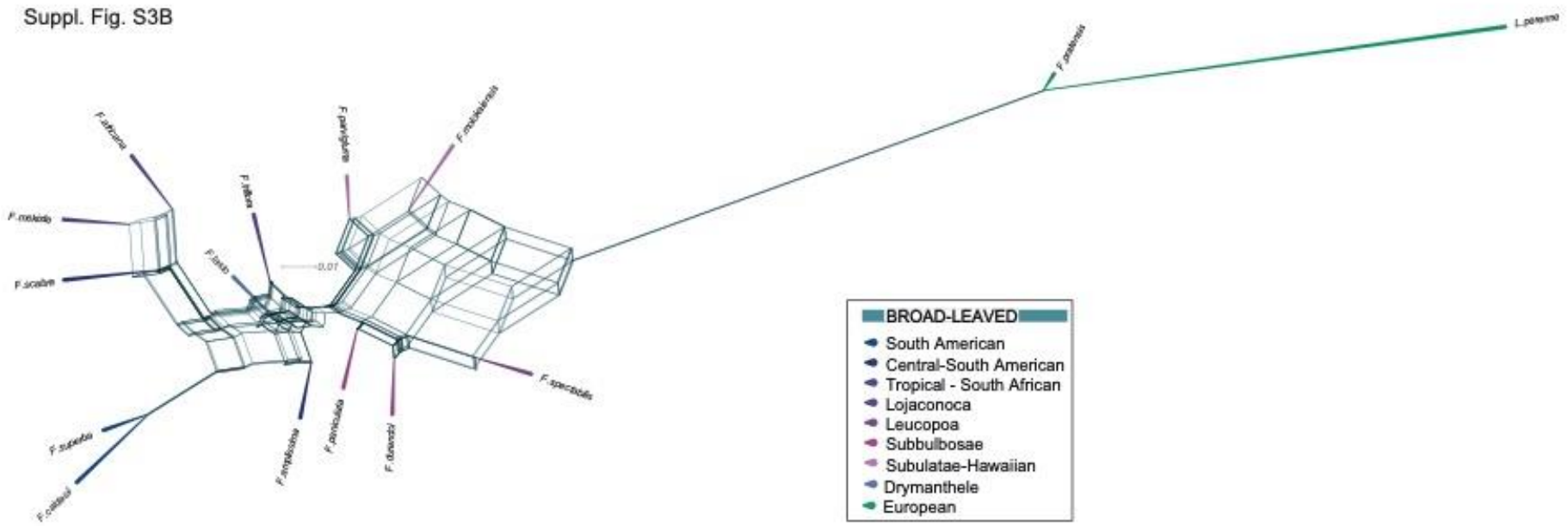
Festuca a.atlantigena
Festuca fenas
Festuca eskia
Festuca paniculata
Festuca a. letourneuxiana



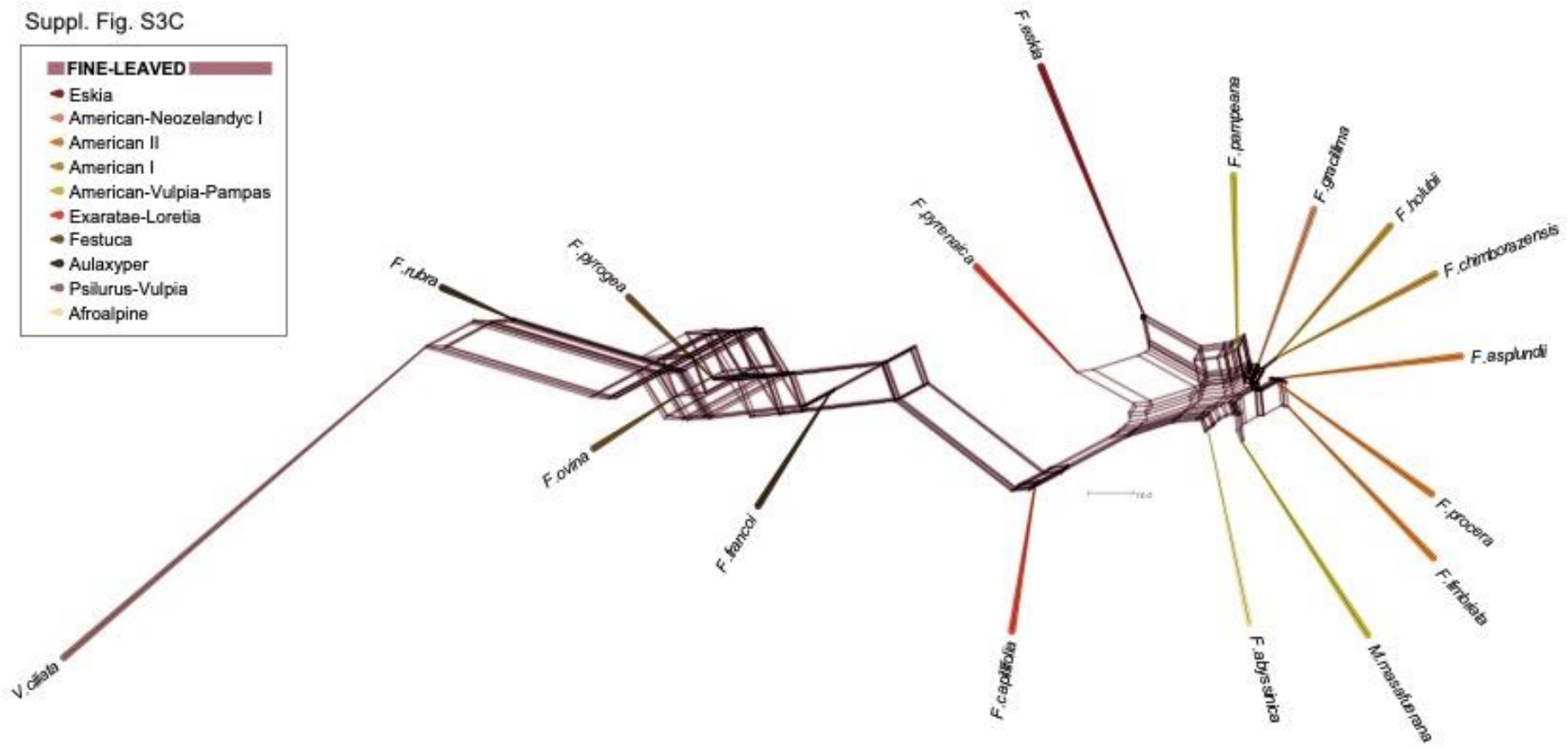
Supplementary Figure 2. Correlation plots of repeat content and genome size variation (1Cx) for the 23 Loliinae taxa with known genome sizes. Individual plots for the most represented repeat types found across the 23 Loliinae taxa with known genome size data (see **Table 2** and **Figure 2**). Color codes of Loliinae lineages correspond to those indicated in **Figure 1**.



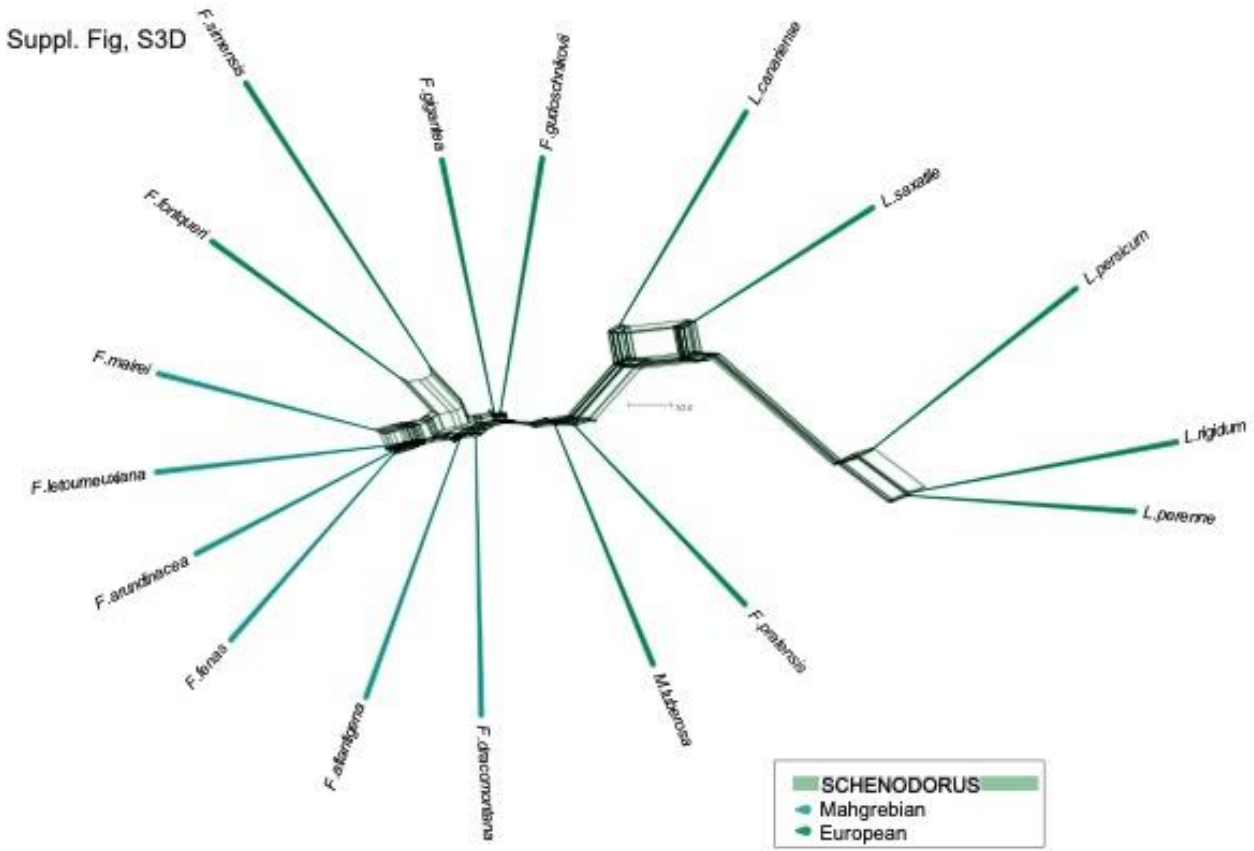
Supplementary Figure 3A. Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the Loliinae evolutionary group Loliinae. The networks were constructed from distance-based NJ trees computed with pairwise inverse distances between samples (see text). Color codes of Loliinae lineages are indicated in the respective charts. Scale bar: number of mutations per site.



Supplementary Figure 3B. Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the Loliinae evolutionary group broad-leaved (BL) Loliinae. The networks were constructed from distance-based NJ trees computed with pairwise inverse distances between samples (see text). Color codes of Loliinae lineages are indicated in the respective charts. Scale bar: number of mutations per site.



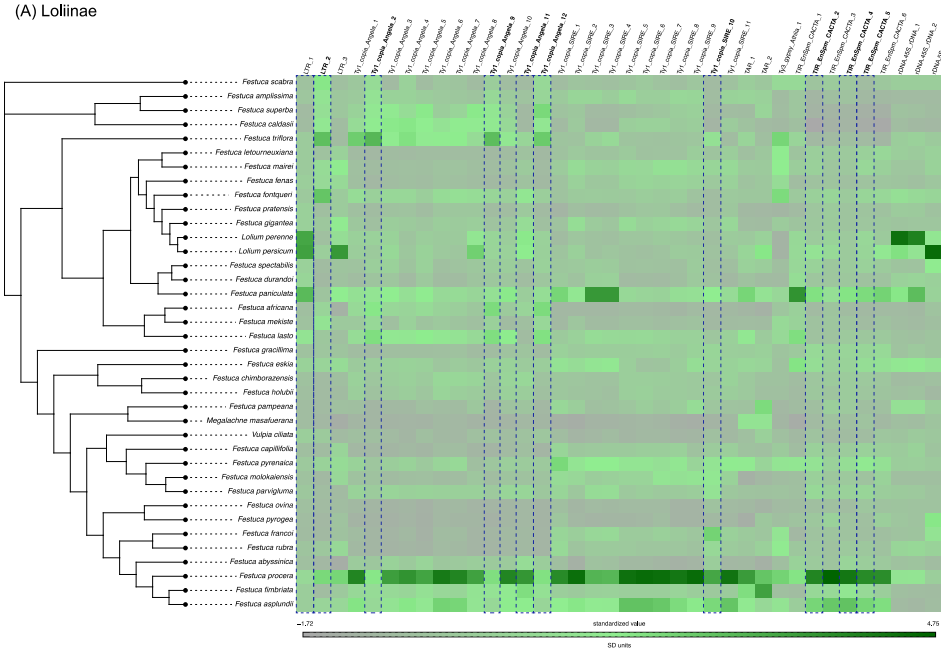
Supplementary Figure 3C. Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the Loliinae evolutionary group fine-leaved (FL) Loliinae. The networks were constructed from distance-based NJ trees computed with pairwise inverse distances between samples (see text). Color codes of Loliinae lineages areindicated in the respective charts. Scale bar: number of mutations per site.



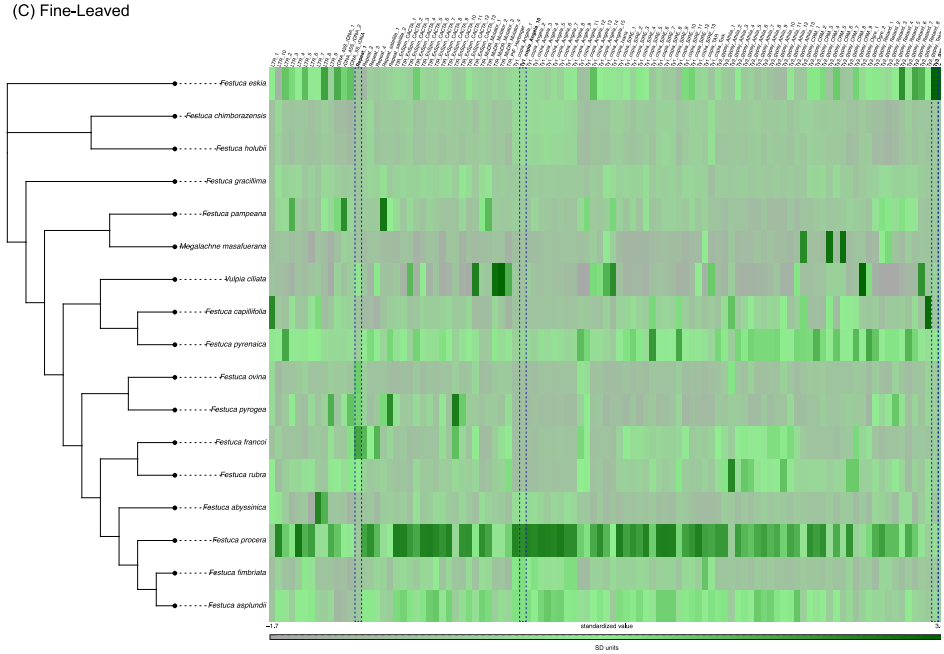
Supplementary Figure 3D. Evolutionary networks based on standardized repeat data sets obtained from the comparative RE2 analysis of the Loliinae evolutionary group Schedonorus Loliinae. The networks were constructed from distance-based NJ trees computed with pairwise inverse distances between samples (see text). Color codes of Loliinae lineages areindicated in the respective charts. Scale bar: number of mutations per site.

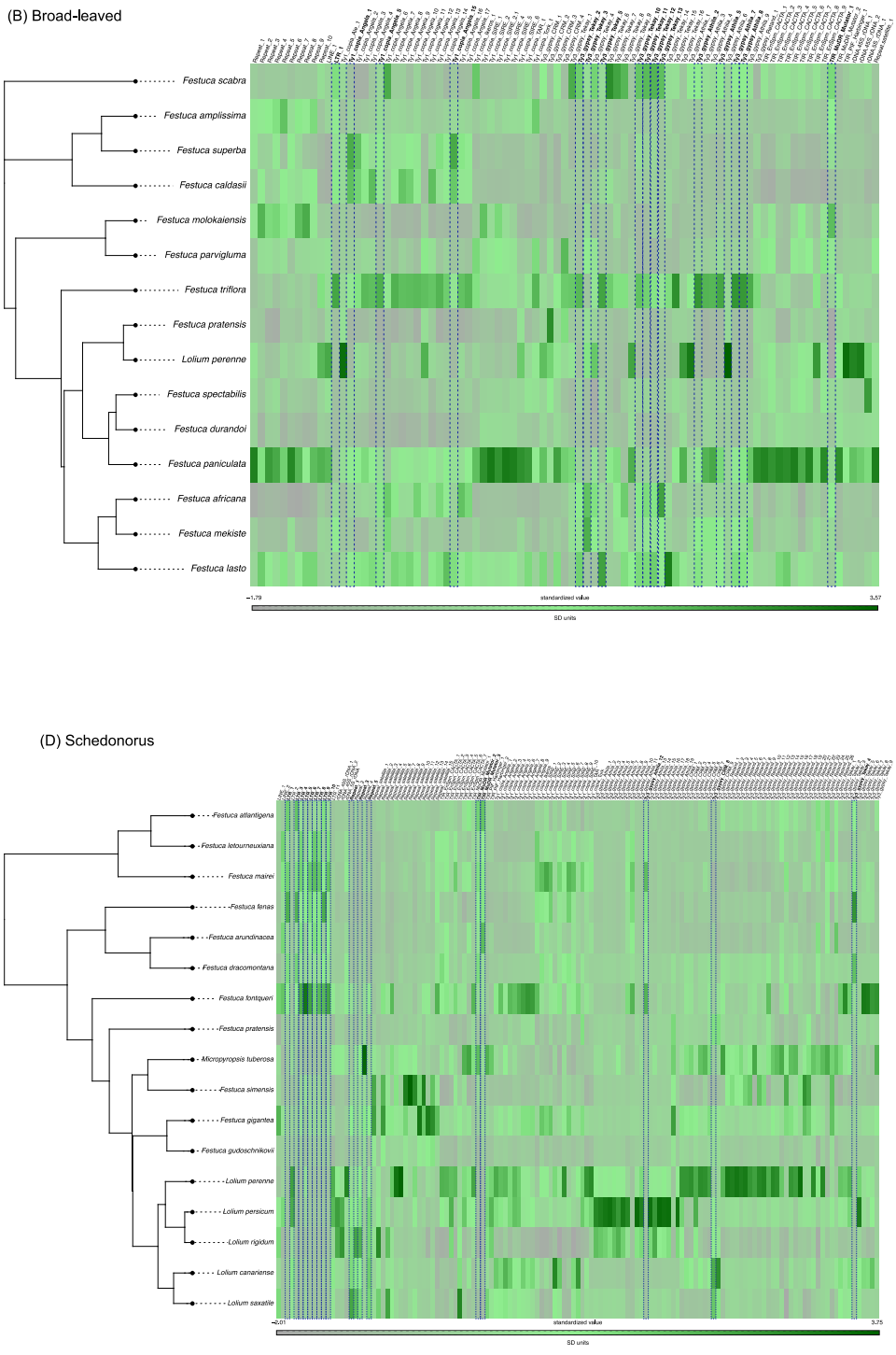
Suppl. Fig. S4

(A) Loliinae



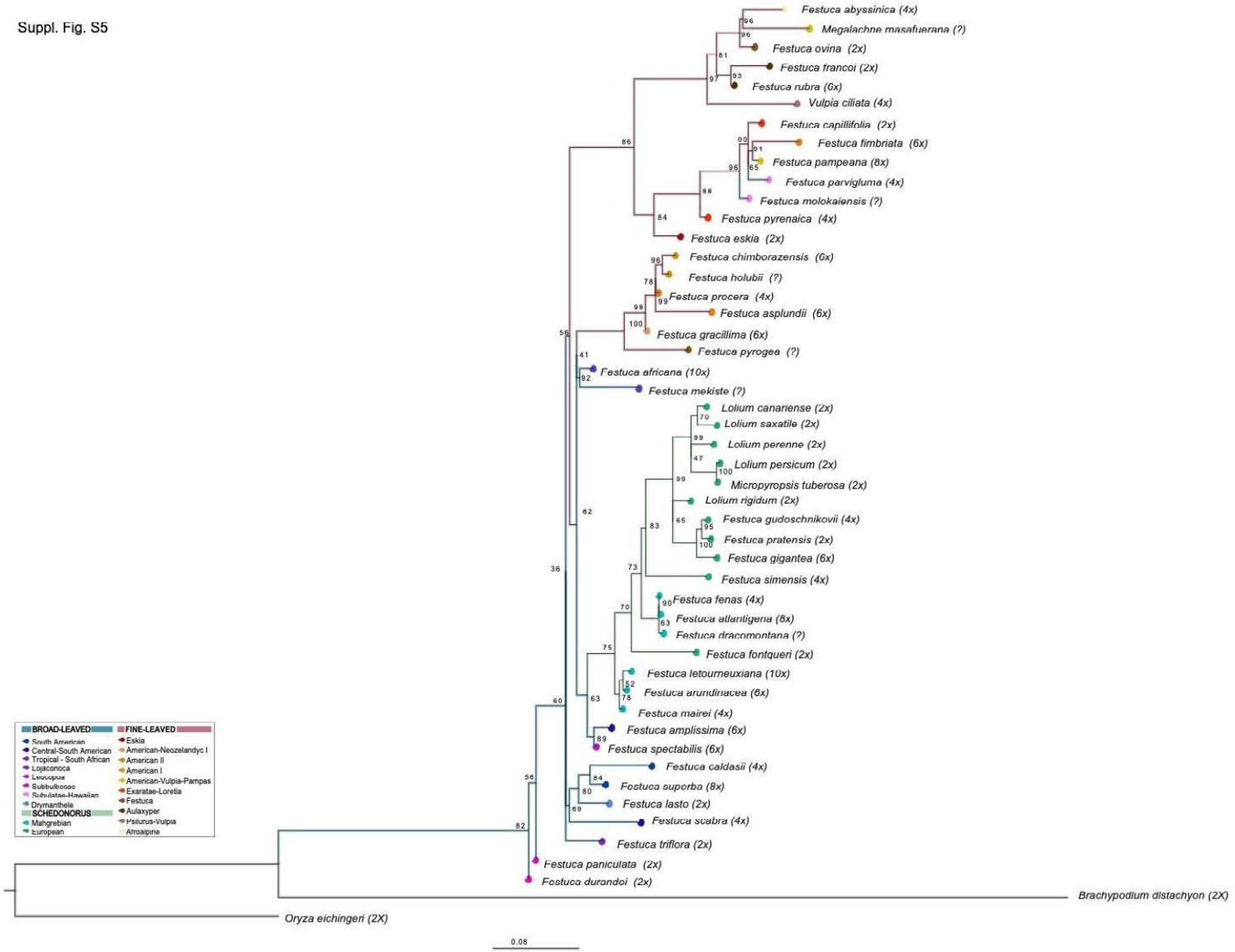
(C) Fine-Leaved





Supplementary Figure 4. Maximum Likelihood Loliinae tree cladograms (combined plastome + nuclear 35S rDNA cistron) showing the relationships among the studied samples in each of the four evolutionary groups of Loliinae and phyloheatmaps of normalized values for different sets of repeat clusters retrieved by RE2 from the comparative analysis of each group: **(A)** Loliinae (38 samples, 38 clusters), **(B)** broad-leaved (BL) Loliinae (15 samples, 96 clusters), **(C)** fine-leaved (FL) Loliinae (17 samples, 122 clusters), **(D)** Schedonorus (16 samples, 167 clusters). Repeat clusters showing significant phylogenetic signal are highlighted with dotted lines.

Suppl. Fig. S5



Supplementary Figure 5. Maximum Likelihood nuclear 5S rDNA cistron tree showing the relationships among the 47 studied Loliinae samples. Ultrafast bootstrap support values are indicated on branches. *Oryza eichingeri* and *Brachypodium distachyon* outgroups were used to root the tree. Color codes of Loliinae lineages are indicated in the chart. Scale bar: number of mutations per site.

Apéndice 3. Información suplementaria del Capítulo C.3.

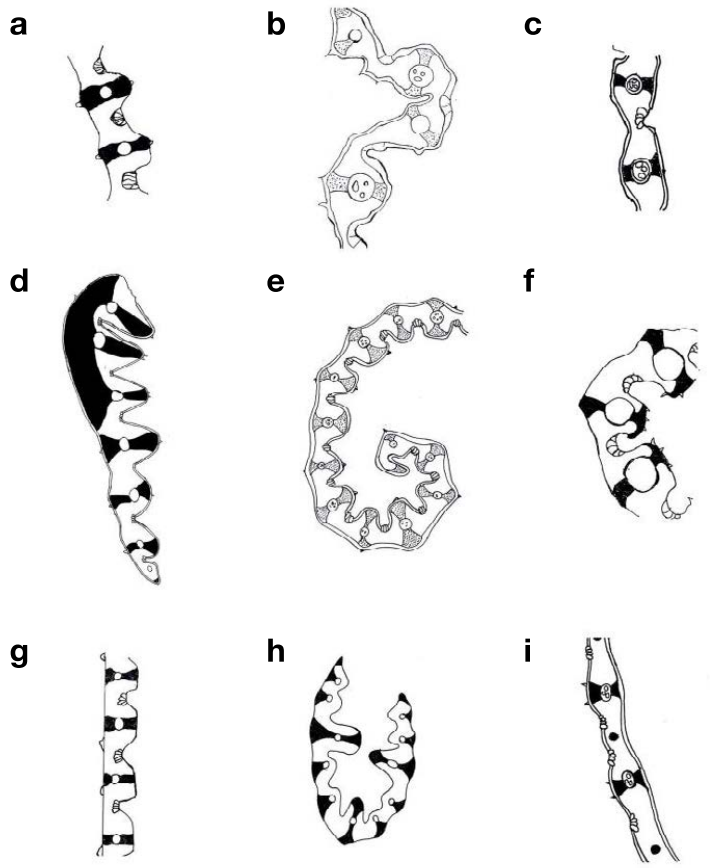


Figure S1: Anatomical leaf blade section of representative species of Mesoamerican and South-American broad-leaved *Festuca* taxa analyzed morphologically in this study. *F*. subgen. *Subulatae* sect. *Glabricarpae*: *F. venezuelana* (**a**); *F*. subgen. *Drymanthele s. l.*: *F. superba* (**b**); *F*. subgen. *Subulatae* sect. *Glabricarpae*: *F. breviglumis* (**c**); *F*. subgen. *Asperifolia*: *F. asperella* (**d**); *F*. subgen. *Erosiflorae*: *F. quadridentata* (**e**), *F. dichloclada* **(f)**; *F*. subgen. *Drymanthele* sect. *Ruprechtia*: *F. amplissima* (**g**); *F.* subgen. *Coironhuecu* (*subgen. nov*.): *F. argentina* (**h**); *F*. subgen. *Mallopetalon*: *F. fimbriata* (**i**). Drawings by José Alfredo Hidalgo-Salazar (**a**–**h**) and María Fernanda Moreno-Aguilar (**i**). [**a**: modified from Stancˇik & Peterson [31]; **b**: modified from Türpe [49]; c: Peterson PM. & Rosales O. 16117, US- 3524155; **d**: modified from Alexeev [38]; **e**: modified from St. Yves [46]; **f**: Smith et al. 10782, AAU; **g**: modified from Stancˇik & Peterson [31]; **h**: modified from Catalán & Muller [32]; **i**: Kostling M. 44, UZ 498.08].

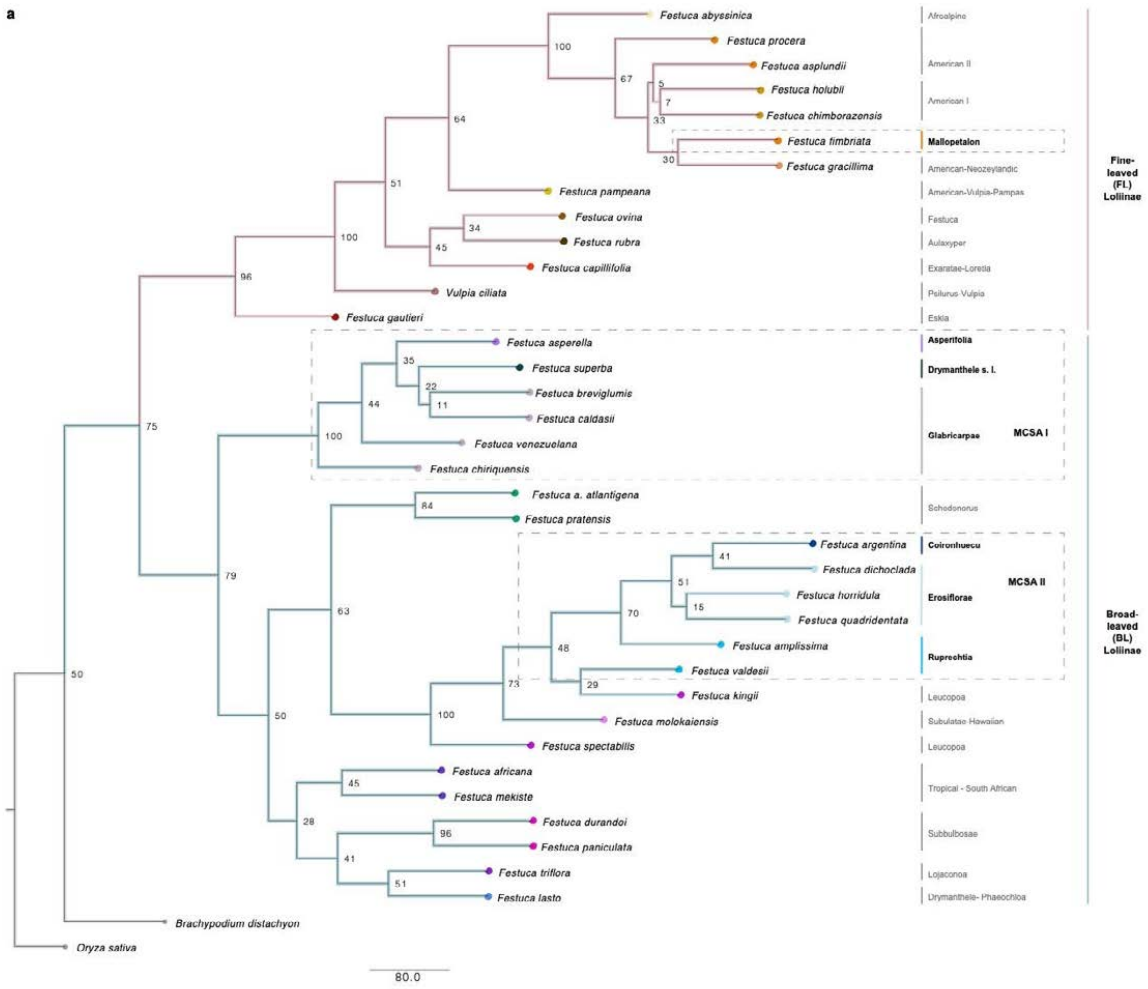


Figure S2a: Nuclear rDNA 35S Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root some trees.

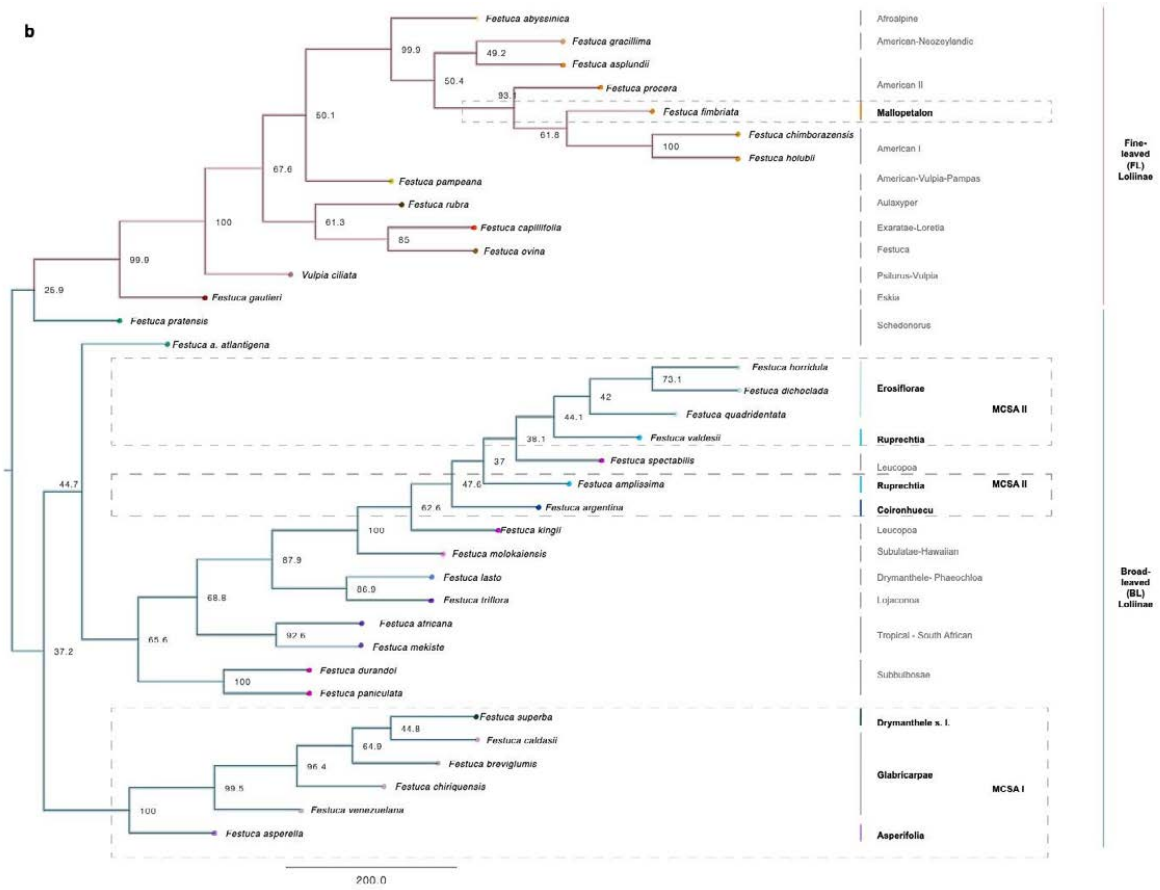


Figure S2b: Nuclear rDNA (45S) IGS Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root some trees.

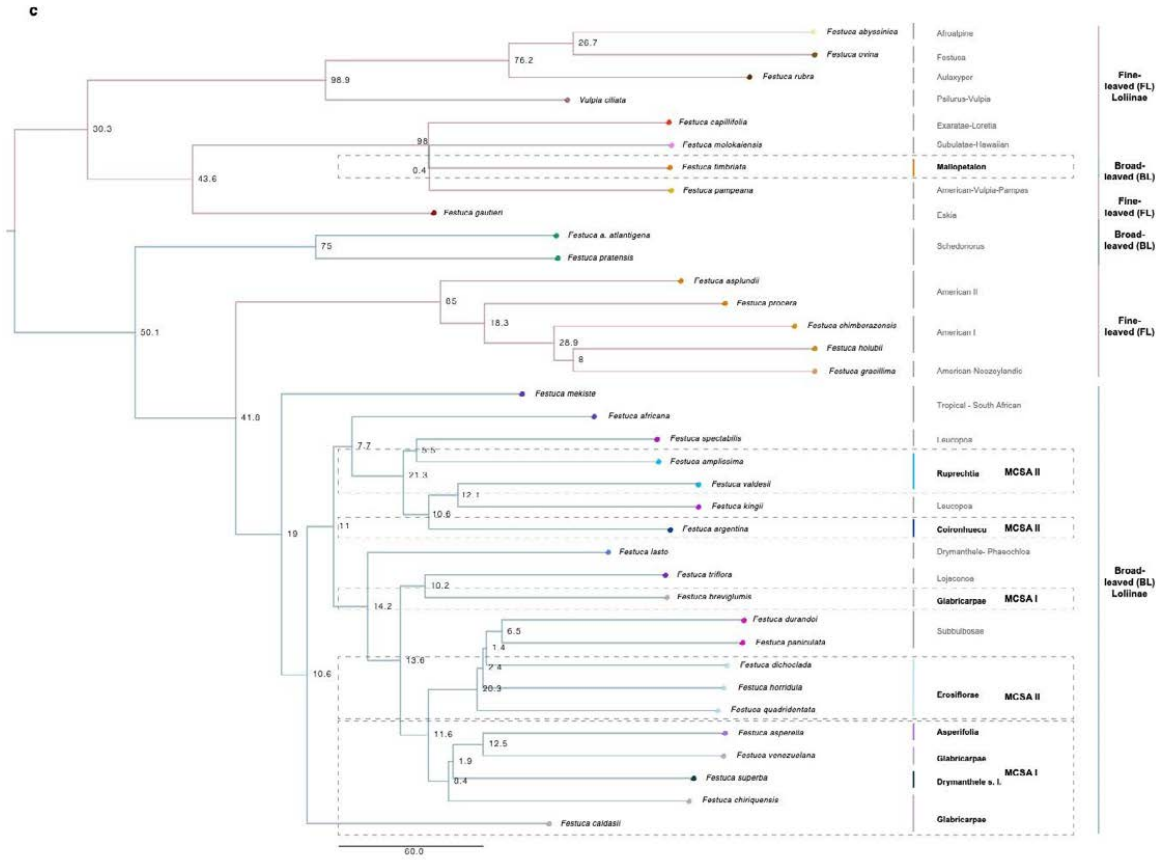


Figure S2c: Nuclear rDNA 5S Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root some trees.

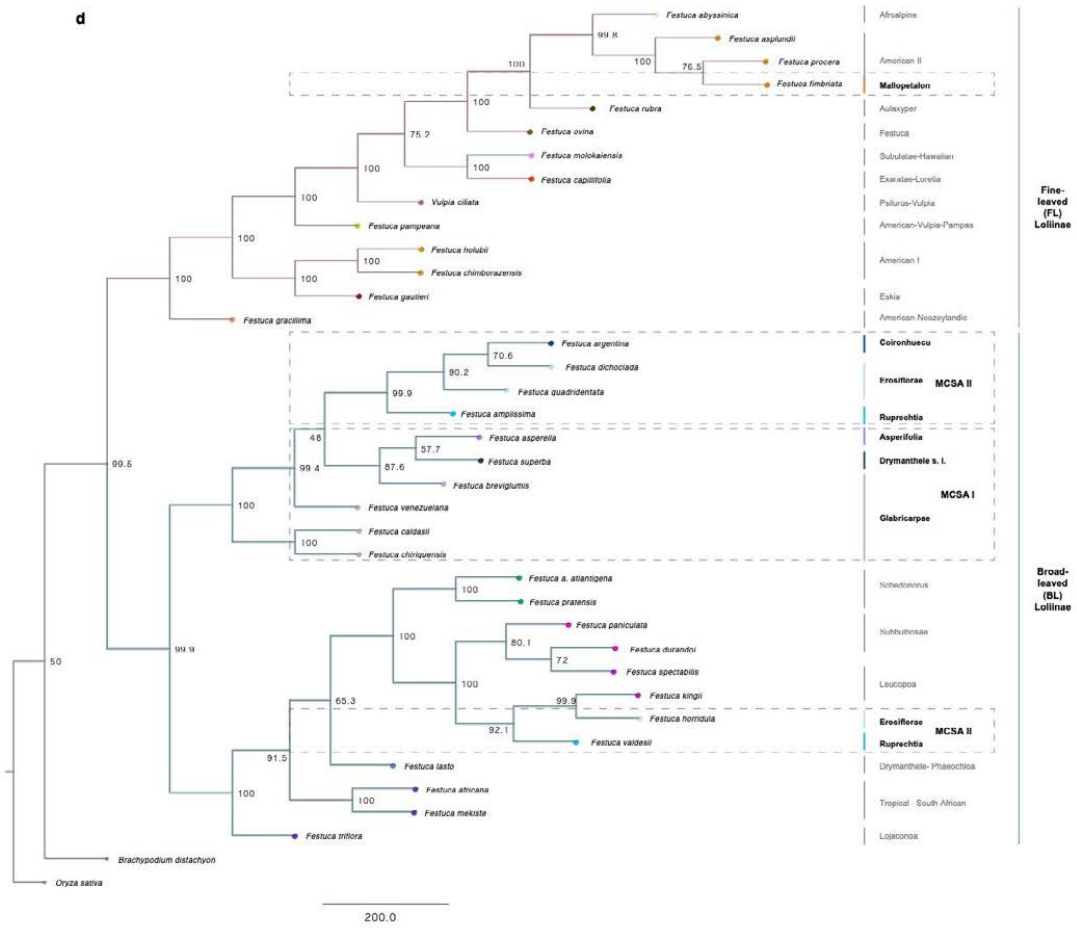


Figure S2d: Palstome Loliinae coalescent species tree computed through Singular Value Decomposition quartets (SVDq) analysis showing bootstrap support values on branches. *Oryza sativa* and *Brachypodium distachyon* outgroups were used to root some trees.

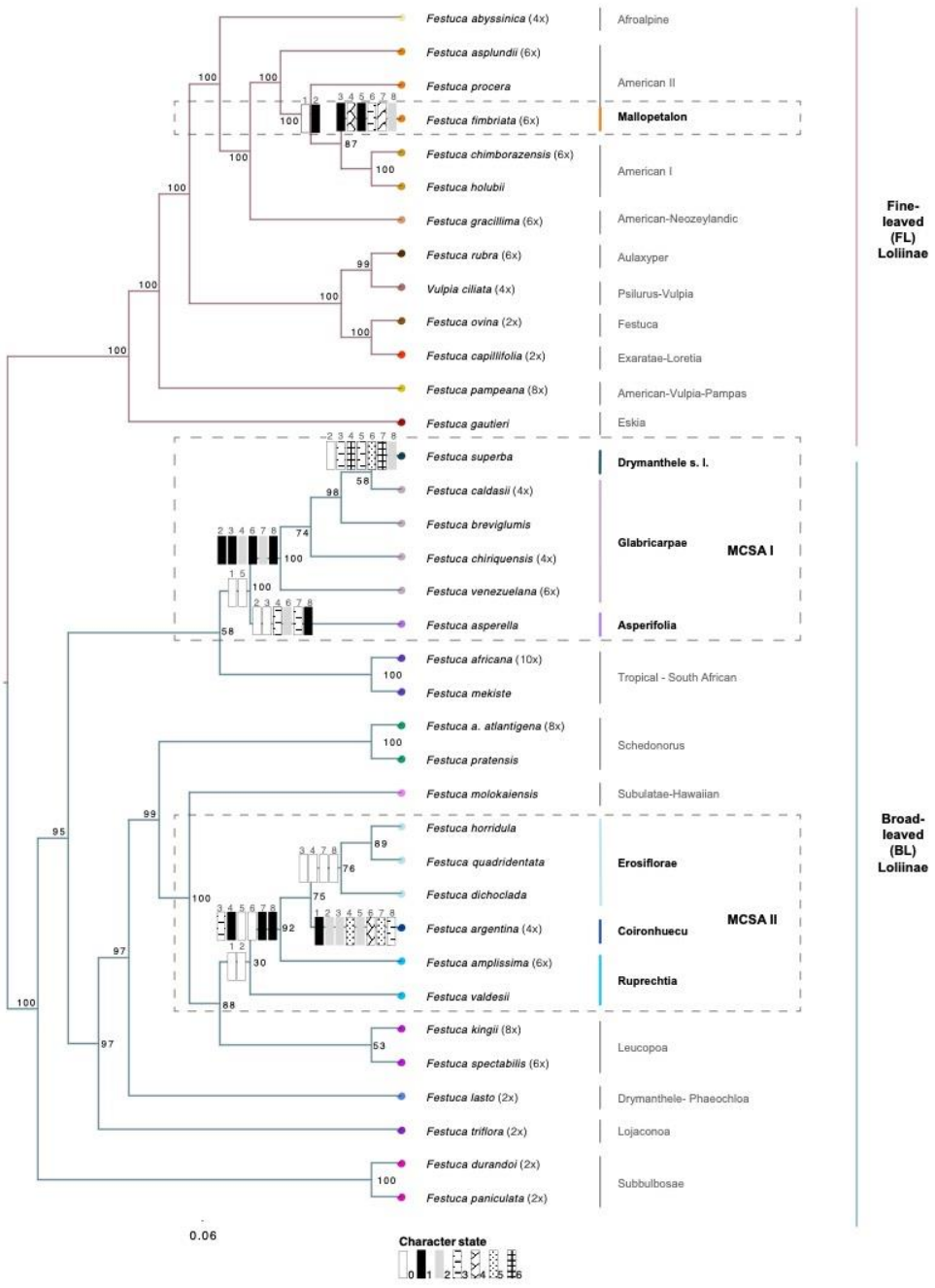


Figure S3: Morphological diagnostic traits mapped onto a Maximum Likelihood IGS cladogram tree of the Mesoamerican and South-American broad-leaved Festuca taxa studied and other representative species of the broad-leaved (BL) and fine-leaved (FL) Loliinae lineages. Traits codes: 1. Reproduction: monoecious (0), dioecious (1); 2. Habit: rhizomatous or caespitose or mixed (0), rhizomatose (1), caespitose (2); 3. Innovations: Extravaginal or intravaginal (0), intravaginal (2), extravaginal or/and intravaginal (3); 4. Ligule: membranaceous, apex acute, erose or lacerate, long (0), non- membranaceous, apex truncate shortly ciliate, or short membranaceous, apex truncate and ciliate, short (1), membranceous or hyaline, apex truncate or rounded, lacerate or dentate, or shortly ciliate, medium (2); membranaceous, apex truncate or slightly rounded and lacerate or dentate, medium-long (3); membranaceous, apex truncate, erose and ciliate, short (4); membranaceous, apex truncate and densely ciliate, short (5); 5. Leaf-blade: Flat, involute in the middle and subconvolute at the apex (0), largely flat (1), plicate, junciform (2), largely flat, subconvolute (3); 6. Inflorescence: erect (0), nutant or erect with nutant branches (1), erect or scarcely nutant (2), erect, laxe (3), erect, contracted (4), erect, branches flexuous (5); 7. Lemma apex: dentate or entire, unawned (0), entire, unawned (1), entire or bifid, awned (2), bifid, shortly awned or unawned (3), entire, scariose, rolled and fimbriate, unawned, muticous (4), entire, unawned, muticous or mucronulate (5), entire, unawned, muticous (6); 8. Ovary tip: glabrescent (0), glabrous or hispid (1), densely hairy (2), sparsely hispid (3)

Supplementary Table S1. List of 65 specimens examined taxonomically of the species under study [*Festuca* subgen. *Erosiflorae*, *F*. subgen. *Drymanthele* sect. *Ruprechtia*, *F*. subgen. *Subulatae* sect. *Glabricarpae*, *F*. subgen. *Asperifolia* and *F*. subgen. *Mallopetalon* sensu Alexeev, plus the newly described *F*. subgen. *Coironhuecu subgen. nov*. (*F. argentina*) and *F.* subgen. *Drymanthele sensu lato* (*F. superba*)], ranked in alphabetical order.

***Festuca amplissima* Rupr.** MEXICO. México: 3.1 mi SE Plan de Vegas [Vigas], 11 October 2001, Peterson P.M. 16150 & Herrera-Arrieda Y. (US). ***Festuca argentina* (Speg.) Parodi**. ARGENTINA. Chubut: Escalante, Canadon Pilar, Estancia Los Manantiales, 60 km N of Comodoro Rivadavia; 24 December 1938, Eyerdam W. J. s.n., Beetle A.A., Grondona E. (SI). Neuquén: Ruta 237, next to Estancia La Lonja, 28 November 1963, Vallerini J. 376 (SI). Río Negro: Bariloche, río Lemay, 19 February 2010, Catalán P. s.n. (UZ); Pilcaniyeu, 20 km al S de Paso Flores, 10 December 1981, Cabrera A.L. 33017 with Botta S., Ezcurra C. & Kiesling R. (SI). Santa Cruz: Lago Argentino, south shore near Calafate, 10 January 1939, Eyerdam W. J. s.n. with Beetle A.A. & Grondona E. (SI). San Julian and [unreadable], 1915, Carette E. 15 (SI). Catalán, P., no Lemey, 19 febr 2010, (UZ)**.** ***Festuca asperella* E.B. Alexeev.** MEXICO. México: Along Hwy. 95, south of Mexico City, just S of El Gordo, 21 August 1972, Dziekanowski C. T, Dunn D. B. & Bolingbroke 2022 (MO, isotype). ***Festuca breviglumis* Swallen.** MEXICO. Jalisco: 11.7 mi SW of Ciudad Guzman, 5 October 2001, Peterson P.M. & Rosales O. 16078 (US); 6.3 mi SW of Mazamitla along Hwy 110 towards Colima, 7 October 2001, Peterson P. M. & Rosales O. 16117 (US)**.** ***Festuca caldasii* (Kunth) Kunth.** ECUADOR. Chimborazo: Sipambe - Huigra ca km 10, 16 June 1999, Laegaard S. 20405 (AAU). Loja: Catacocha - La Toma km 28, 29 April 1992, Laegaard S. 102535 (AAU); Km 6 above Jimbura along Zumba road, 27 March 1994, Laegaard S. 105256 (AAU); Catamayo, Chinchas-Tambara, fecha, colectores Moreno-Aguilar M. F. F98_iii, Medina I. & Cango G. (HUTPL). ***Festuca carrascana* Stančík & Renvoize.** BOLIVIA. Cochabamba: José Carrasco Torrico, 10-15 km south of Totora along the road to Aiquile, 1 March 2005, Wood J. R. I. 21684 & Haigh A. (LPB, isotype). ***Festuca chiriquensis* Swallen.** PANAMA. Chiriqui: Chiriqui volcano, 29-30 September 1911, Hitchcock A. S. 8197 (US, holotype). ***Festuca chuquisacae* Stančík & Renvoize.** BOLIVIA. Chuquisaca: Tipoyo, cerro Obispo-San Juan, 24 April 1994, Wood J. R. I. 8337 (LPB, isotype). ***Festuca dentiflora* E.B. Alexeev ex Stančík & P.M. Peterson.** PERU. Huanuco: Baños, 1838, Wilkes Explor. Exped. 5 (US, holotype). ***Festuca dichoclada* Pilg.** PERU. Ancash: Huascarán National park, Quebrada Llaca, 24 May 1985, Smith N. 10782 (AAU). Cajamarca: Sais, José Carlos Mariátegui, km 20-40 on Suchubamba-San Juan road, 5 June 1984, Smith D. N. 7534 & Sánchez-Vega I. (MO). ***Festuca fimbriata* Nees.** ARGENTINA. Corrientes: Santo Tome, Arroyo Chimiray and Ruta 40, 8 October 1976, Quarin 3409 (CTES); Garruchos, 22 October 1954, Buikart A. 19659 (BAA, SI); estancia "Garruchos", 1 November 1950 Pedersen T. M. 819 (C, K, MO); Ruta 37, 5 km E of Governador Virasoro, 14 November 1974, Schini A. & Carnevali R. C. 10523 (SI, US); Timbo, costa Río Uruguay, 26 km SE of Colina Garabi, 16 September 1980, Ahumado & Schinini 4085 (CTES). Misiones: Apóstoles, Est. Agrotécnica, October 1977, Cabrera A. 28766, Botta S. M., Kiesling R., Rotman A. D., Tur N. & Zuloaga F. O. (SI); Ruta Prov. 40 and Ayo. Chimiray, 16 October 2000, Zuloaga F. O. & Morrone O. 7155 (SI); Capital, Ruta provinc. no. 1, 12 km S of Posadas, 16 November 1974, Schinini & Carnevali 10687 (CTES). ***Festuca horridula* Pilg.** PERU. Huancavelica: Huillca-orocon, 5 km NW of Conayca, 16 April 1955, Tovar O. 2431 (US). Lima: Salta Cuna, March 1943, Soukup J. 1944 (US); Huarochiri, 107 km W of Lima on Hwy 20 towards La Oroyo [Oroya] at Chicla, 5 April 1997, Peterson P. M. & Tovar O. 14019 (MO). ***Festuca jaliscana* E.B. Alexeev.** MEXICO. Jalisco: NE slopes of the Nevado de Colima, below Canoa de Leoncito, Barranca de la Rosa, 10 October 1952, McVaugh R. 13409 (US, holotype). ***Festuca lugens* (E. Fourn.) Hitchc. ex Hern.-Xol.** MEXICO. Oaxaca: Cumbre de Estepa, September 1842, Liebmann F. M. 6247, 502 (C, type). ***Festuca quadridentata* Kunth.**

ECUADOR. Cañar: S of El Tambo ca 1.5 - 3 km along road to Carshao, 10 June 1999, Laegaard S. 20287 & Sklenář P. (AAU). Chimborazo: Near Alao, along Río Alao, 21 September 1985, Laegaard S. 55290 (AAU); *idem*, Llactapamba, 1 March 1944, Acosta-Solis M. 7581 (US). Chimborazo/Morona Santiago: Hda. Huargualla - Hda. San Eduardo, way to NP Sangay, 20 July 1999 (AAU). ***Festuca steinbachii* E.B. Alexeev.** BOLIVIA. Cochabamba, Cejawald bei "La Aduana", 7 March 1929, Steinbach J. 9533 (LIL, isotype). ***Festuca superba* Parodi ex Türpe.** ARGENTINA. Jujuy: Lagunas de Yala, 30 January 1975, Zuloaga F. O. 383 & Deginani N. B. (SI); *idem*,16 February 1987, Nicora E. 8740, Gómez-Sosa E., Palasi C., Pensiero J. & Rúgolo Z. (SI); *idem*, 16 January 1988, Zuloaga F. O. 3545 & N. B. Deginani (SI); slope of Yala, 29 March 1992, Cabrera A. L. 34886, Deginani A. M., Tur N. & Ulibarri E. (SI); quebrada de Yala, 19 January 1976, Cabrera A. L. 27380, S. Arroyo, N. Bacigalupo, E. Nicora, E. Ulibarri (SI); from San Salvador de Jujuy to Termas de Reyes, 05 March 1987, Zuloaga F. O. 3008, Morrone O. (SI); Termas de Reyes, 5 February 1943, Parodi L. R. 14552 (BAA); *idem*, 07 January 1968, Cabrera A. L. 18879, Deferrari A. M., Frangi J., Marrone M. T., Petriella B. (SI); *idem*, entrada al Balneario, 25 November 2008, Catalan P. 360.08 & Müller J. (UZ); Yala, Laguna Rodeo, 24 November 2008, Catalan P. 356.08 & Müller J. (UZ). ***Festuca tancitaroensis* Gonz.-Led. & S.D. Koch.** MEXICO. Michoacan: Mt. Tancitaro, 25 July 1941, Leavenworth W.C. & Leavenworth Mrs. W.C. 1213 (US, holotype). ***Festuca urubambana* Stančík.** PERU. Cuzco: Urubamba, lower end of Quebrada Pumahuanca, a deep side-valley of R. Urubamba ca. 2-4km NW of Urubamba, 31 December 1962, Iltis H. H.-C. M. 853 & Ugent D. – V. (K, holotype). ***Festuca valdesii* Gonz.-Led. & S.D. Koch.** MEXICO. Coahuila de Zaragoza: Arteaga, Sierra Zapalinamé, 19 May 1990, Hinton G.S. 20278 & al. (IEB, isotype). ***Festuca woodii* Stančík.** COLOMBIA. Boyaca: Sierra Nevada del Cocuy, hacienda La Esperanza, 29 December 1985, Wood J. 5254 (COL, holotype). ***Festuca venezuelana* Stančík.** VENEZUELA. Tachira: La Grita, La Negra, cross of the two roads to La grita and Pogonero, 10-11 November 2000, Stančík D. 4262 (AAU, isotype).

Supplementary Table 2. Genome proportion of repeats estimated by Repeat Explorer2 for individual Loliinae samples (estimated percentages per holoploid genome, 1C). Values in bold correspond to new data generated in this study.

	Class I/LTR/Ty1_copia							Class I/LTR/Ty3_gypsy								Class II/TIR															
Loliinae taxon	**Ale**	**Angela**	**Ikeros**	**Ivana**	**SIRE**	**TAR**	**Tork**	**OTA**	**Athila**	**Tat**	**Ogre**	**Tekay**	**Retand**	**Reina**	**CRM**	**chromovirus**	**MuDR_ Mutator**	**EnSpm_ CACTA**	**hAT**	**PIF_ Harbinger**	**Ty1_ copia**	**Ty3_ gypsy**	**LTR**	**LINE**	**satellite**	**rDNA (5S-45S)**	**Mobile element**	**Repeat (conflicting evidences)**	**Unclassified (No evidence)**	**Total**
Broad Leaved (BL) Loliinae																															
Festuca asperella	**0.00**	**9.47**	**0.00**	**0.21**	**1.01**	**0.42**	**0.01**	**0.00**	**0.06**	**0.00**	**0.00**	**3.93**	**6.14**	**0.00**	**0.21**	**0.02**	**0.24**	**0.49**	**0.00**	**0.00**	**0.79**	**0.00**	**8.16**	**0.00**	**0.27**	**0.19**	**0.00**	**0.00**	**20.38**	**52.000**
Festuca breviglumis	**0.00**	**16.98**	**0.00**	**0.71**	**0.41**	**0.34**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**2.68**	**18.65**	**0.00**	**0.02**	**0.00**	**0.20**	**0.65**	**0.00**	**0.00**	**0.00**	**0.00**	**5.59**	**0.00**	**1.37**	**0.02**	**0.00**	**1.51**	**6.47**	**55.610**
Festuca chiriquensis	**0.00**	**38.63**	**0.00**	**0.30**	**0.86**	**0.27**	**0.09**	**0.00**	**0.53**	**0.00**	**0.00**	**6.36**	**6.76**	**0.00**	**0.22**	**0.00**	**0.32**	**0.58**	**0.02**	**0.00**	**0.00**	**0.40**	**6.82**	**0.00**	**0.64**	**0.15**	**0.00**	**0.02**	**4.53**	**67.500**
Festuca caldasii	0.03	27.45	0.00	0.33	0.82	0.48	0.02	0.00	0.26	0.00	0.13	7.32	6.03	0.00	0.17	0.00	0.11	0.49	0.00	0.00	0.00	0.00	9.64	0.00	0.88	0.06	0.00	0.00	7.40	61.597
Festuca superba	0.03	21.07	0.00	0.90	0.68	0.49	0.01	0.00	0.00	0.00	0.01	1.54	20.31	0.00	0.05	0.00	0.51	0.92	0.00	0.00	0.00	0.00	11.01	0.00	1.40	0.26	0.00	0.00	5.71	64.879
Festuca venezuelana	**0.00**	**15.44**	**0.00**	**0.14**	**0.38**	**0.15**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**4.94**	**4.85**	**0.00**	**0.06**	**0.00**	**0.05**	**0.09**	**0.00**	**0.00**	**0.31**	**0.00**	**10.50**	**0.00**	**0.90**	**0.25**	**0.00**	**0.00**	**26.22**	**64.280**
Festuca dichoclada	**0.00**	**7.89**	**0.00**	**0.59**	**1.62**	**0.71**	**0.03**	**0.00**	**0.04**	**0.00**	**0.00**	**1.30**	**13.81**	**0.00**	**2.01**	**0.00**	**1.73**	**2.70**	**0.05**	**0.00**	**0.00**	**0.00**	**7.12**	**0.00**	**1.61**	**0.15**	**0.00**	**1.16**	**8.75**	**51.270**
Festuca horridula	**0.00**	**8.97**	**0.01**	**0.80**	**1.06**	**0.19**	**0.00**	**0.00**	**0.33**	**0.00**	**0.28**	**2.89**	**13.92**	**0.00**	**1.83**	**0.00**	**1.27**	**0.72**	**0.02**	**0.05**	**0.05**	**0.00**	**8.02**	**0.05**	**3.51**	**0.35**	**0.00**	**0.02**	**6.83**	**51.170**
Festuca quadridentata	0.00	8.56	0.01	0.35	1.64	0.25	0.00	0.00	0.43	0.00	0.10	1.75	5.83	0.00	1.88	0.00	0.10	0.69	0.00	0.00	0.00	0.00	2.37	0.09	2.41	0.27	0.00	0.00	22.24	48.970
Festuca amplissima	0.00	12.43	0.05	0.11	3.04	0.71	0.23	0.00	0.26	0.00	0.02	2.05	7.87	0.00	0.67	0.00	0.79	1.54	0.00	0.00	0.00	0.00	6.89	0.16	2.72	0.12	0.00	0.00	12.58	52.254
Festuca valdesii	**0.00**	**11.38**	**0.00**	**0.75**	**1.82**	**0.66**	**0.06**	**0.00**	**0.22**	**0.00**	**0.00**	**1.39**	**9.65**	**0.00**	**3.40**	**0.00**	**0.24**	**0.68**	**0.00**	**0.00**	**0.00**	**0.00**	**11.07**	**0.04**	**2.94**	**0.41**	**0.00**	**1.04**	**10.17**	**55.920**
Festuca argentina	**0.00**	**6.77**	**0.04**	**0.41**	**8.84**	**0.70**	**0.05**	**0.00**	**0.43**	**0.00**	**0.20**	**3.23**	**16.33**	**0.00**	**1.41**	**0.00**	**0.75**	**2.33**	**0.00**	**0.00**	**0.00**	**0.00**	**4.67**	**0.10**	**2.66**	**0.29**	**0.00**	**1.45**	**6.04**	**56.700**
Festuca kingii	**0.11**	**6.72**	**0.01**	**0.37**	**1.42**	**0.26**	**0.00**	**0.00**	**0.04**	**0.00**	**0.00**	**0.65**	**12.77**	**0.00**	**0.27**	**0.00**	**0.27**	**0.63**	**0.00**	**0.00**	**0.00**	**0.00**	**6.37**	**0.01**	**4.72**	**0.45**	**0.00**	**0.00**	**18.67**	**53.630**
Festuca spectabilis	0.00	8.94	0.00	0.00	2.47	0.73	0.10	0.00	0.07	0.00	0.12	3.54	10.48	0.00	2.38	0.00	0.35	4.13	0.00	0.05	0.00	0.00	7.32	0.20	2.04	0.77	0.00	0.00	7.71	51.406
Festuca africana	0.00	8.42	0.00	0.00	0.46	0.06	0.00	0.00	0.30	0.00	4.63	4.75	1.32	0.00	0.14	0.00	0.13	1.14	0.00	0.00	0.00	0.00	5.74	0.00	1.70	0.04	0.00	0.00	32.26	61.096
Festuca mekiste	0.00	8.79	0.00	0.01	3.08	0.24	0.00	0.00	2.77	0.00	7.92	11.14	1.91	0.00	0.35	0.00	0.27	2.86	0.00	0.00	0.00	0.00	2.68	0.00	3.01	0.03	0.00	0.00	6.50	51.573
Festuca durandoi	0.00	6.81	0.00	0.00	3.60	0.47	0.00	0.00	0.17	0.00	0.16	3.87	18.20	0.00	1.84	0.00	0.19	4.04	0.00	0.09	0.00	0.00	4.90	0.02	0.96	0.10	0.00	0.87	7.81	54.121
Festuca paniculata	0.00	7.51	0.00	0.00	3.75	0.43	0.02	0.00	0.45	0.00	0.00	2.30	14.83	0.00	0.81	0.00	0.02	0.51	0.00	0.03	0.00	0.00	5.82	0.03	0.82	0.89	2.77	0.00	13.16	54.140
Festuca lasto	0.00	11.83	0.09	0.00	2.73	0.59	0.02	0.00	3.42	0.00	0.00	8.51	6.85	0.00	1.76	0.00	0.03	1.72	0.00	0.00	0.00	0.00	1.76	0.01	0.84	0.17	0.00	0.00	14.11	54.461
Festuca triflora	0.00	15.24	0.00	0.00	1.34	0.33	0.14	0.00	5.98	0.00	0.11	7.71	7.15	0.00	0.78	0.00	0.13	0.85	0.00	0.00	0.00	0.00	0.00	0.00	1.78	0.51	0.00	0.00	14.93	56.981
Festuca pratensis	0.04	5.41	0.01	0.00	3.77	0.19	0.00	0.00	7.18	0.00	0.66	4.89	14.88	0.00	4.26	0.00	0.01	2.02	0.00	0.00	0.00	0.00	2.17	0.01	1.81	0.69	0.00	0.40	10.29	58.684
Festuca atlantigena	0.00	2.84	0.02	0.00	0.60	0.08	0.01	0.00	0.14	0.00	0.00	7.50	11.16	0.00	1.49	0.00	0.06	1.62	0.00	0.03	0.00	0.00	6.42	0.08	2.13	0.37	0.00	0.09	11.43	46.088
Festuca molokaiensis	0.00	5.94	0.03	1.26	9.96	0.03	0.00	0.00	0.01	0.00	1.55	5.71	21.35	0.00	0.26	0.00	1.37	4.95	0.00	0.00	0.00	0.00	1.49	0.02	1.12	0.25	0.00	1.49	7.09	63.853
Fine leaved (FL) Loliinae																														
Festuca fimbriata	0.01	4.47	0.15	0.04	1.60	1.18	0.12	0.00	0.04	0.00	0.34	0.21	1.66	0.00	0.09	0.00	0.20	1.18	0.00	0.00	0.43	0.00	1.62	0.02	3.91	0.05	0.00	0.00	21.54	38.855
Festuca asplundii	0.00	9.97	0.52	0.00	8.17	0.53	0.13	0.00	1.31	0.00	0.82	0.02	1.96	0.00	0.67	0.00	0.93	5.52	0.00	0.01	0.00	0.00	3.27	0.00	1.83	0.04	0.00	0.97	11.75	48.412
Festuca procera	0.00	10.88	0.36	0.00	7.10	0.60	0.12	0.01	0.87	0.00	0.34	0.21	4.32	0.00	1.48	0.00	0.80	4.67	0.00	0.00	0.00	0.00	0.00	0.00	2.66	0.05	0.00	2.09	11.66	48.212
Festuca chimborazensis	0.00	10.89	0.06	0.02	6.98	0.67	0.00	0.01	1.06	0.00	0.06	0.00	4.17	0.00	1.16	0.00	0.65	5.04	0.00	0.09	0.00	0.00	2.15	0.00	4.09	0.30	0.00	1.07	8.99	47.449
Festuca holubii	0.00	10.87	0.07	0.00	6.86	0.76	0.00	0.00	0.61	0.00	0.02	0.01	3.52	0.00	1.10	0.00	0.54	4.54	0.00	0.01	0.00	0.00	0.90	0.01	5.96	0.42	0.00	0.99	13.32	50.518
Festuca pampeana	0.00	0.52	0.00	0.06	0.63	0.10	0.06	0.00	0.00	0.00	0.33	0.00	6.47	0.00	0.03	0.00	0.19	0.85	0.00	0.00	0.00	0.00	2.85	0.00	4.77	1.02	0.00	0.00	23.59	41.481
Festuca gracillima	0.00	4.50	0.15	0.02	1.74	0.61	0.00	0.00	0.87	0.00	1.22	0.01	6.05	0.00	1.54	0.00	0.92	0.76	0.00	0.00	0.00	0.00	8.23	0.00	1.45	0.61	0.00	6.04	13.95	48.684
Festuca abyssinica	0.00	3.65	0.00	0.07	0.96	0.08	0.00	0.00	0.15	0.00	1.85	1.78	3.83	0.00	0.34	0.00	0.41	1.56	0.00	0.00	0.00	0.00	3.45	0.00	4.93	0.31	0.00	0.00	26.92	50.267
Festuca rubra	0.00	0.08	0.00	0.00	0.96	0.30	0.01	0.00	8.55	0.00	0.61	1.24	2.11	0.00	0.69	0.00	0.01	0.95	0.00	0.00	0.00	0.00	0.76	0.00	6.56	0.70	0.00	0.32	22.79	46.646
Festuca ovina	0.03	0.26	0.02	0.00	3.16	0.33	0.00	0.00	7.18	0.00	0.59	2.04	4.26	0.00	1.04	0.00	0.00	2.01	0.00	0.01	0.00	0.00	2.75	0.03	5.22	0.28	0.00	7.10	12.28	48.550
Festuca capillifolia	0.00	0.75	0.00	0.00	2.32	0.14	0.01	0.00	1.58	0.00	0.22	4.87	4.03	0.00	1.41	0.00	0.00	1.86	0.00	0.00	0.00	0.00	9.77	0.00	5.16	0.65	0.00	0.00	24.23	57.019
Vulpia ciliata	0.14	3.81	0.00	0.00	0.78	0.49	0.16	0.01	0.22	0.00	0.08	0.56	16.79	0.00	0.86	0.00	0.11	1.60	0.00	0.00	0.00	0.00	8.45	0.33	2.76	0.70	0.00	2.33	11.74	51.766
Festuca gautieri	**0.00**	**4.85**	**0.04**	**0.11**	**4.41**	**0.41**	**0.00**	**0.00**	**0.14**	**0.00**	**0.21**	**1.25**	**13.15**	**0.00**	**1.09**	**0.00**	**0.21**	**2.14**	**0.00**	**0.04**	**0.00**	**0.00**	**7.35**	**0.02**	**1.35**	**0.86**	**0.00**	**0.00**	**10.71**	**48.340**

Apéndice 4. Factor de impacto y área temática de la revista

Revista	Año	Cuartil/Decil	Factor de impacto	Área WoS
Frontiers in Plants Sciences	2020	Q1/D1	4.402	Plant Sciences
Frontiers in Plants Sciences	2022	Q1/D1	6.627	Plant Sciences
Plants	2022	Q1	4.670	Plant Sciences
Taxon	2022	Q2	2.586	Plant Sciences

Apéndice 5. Justificación de la contribución de la doctoranda en los trabajos en coautoría

- **Moreno-Aguilar MF**, Arnelas I, Sánchez A, Viruel J, Catalán P. 2020. Museomics unveil the phylogeny and biogeography of the neglected Juan Fernandez archipelago *Megalachne* and *Podophorus* endemic grasses and their connection with relict Pampean-Ventanian fescues**.** *Frontiers in Plant Sciences* 11: 819. IF: 4.402, WOS, Plant Sciences (24/223), Q1, D1. doi 10.3389/fpls.2020.00819.

  La doctoranda participó en las salidas de campo en Ecuador, recolectando material vegetal de las especies de *Festuca*. Realizó la identificación en el herbario UTPL de las muestras colectadas. Llevó a cabo el procesamiento y trabajo experimental de laboratorio, extracción de ADN de muestras frescas, almacenadas en silica gel y de herbario, el procesamiento, la depuración y el ensamblaje de secuencias del genoma del plastoma, el ensamblaje por mapeo del gen nuclear ribosomal 35S y extracción de secuencias de loci nucleares (ITS) y plastídicos (*trn*TL-*trn*LF), y los análisis de reconstrucción filogenética, de datación molecular y de biogeografía. Participó en la redacción del manuscrito.

- **Moreno-Aguilar MF**, Inda LA, Sánchez-Rodríguez A, Arnelas I, Catalán P. 2022. Evolutionary dynamics of the repeatome explains contrasting differences in genome sizes and hybrid and polyploid origins of grass Loliinae lineages. *Frontiers in Plant Sciences* 13:901733. IF: 6.627, Q1, D1. doi 10.3389/fpls.2022.901733.

  La doctoranda participó en los estudios de estimación de tamaño genómico, desarrolló trabajo experimental de laboratorio, con la extracción de ADN de muestras seleccionadas. Llevó a cabo el procesamiento, la depuración y el ensamblaje de secuencias del genoma del plastoma, mapeo del gen rDNA 35S y los análisis de reconstrucción filogenética con estos datos. Desarrolló los análisis de identificación, caracterización y composición del repeteoma individual y comparado de las especies seleccionadas, realizó la extracción, alineamiento de secuencias y reconstrucción filogenética con datos del gen rDNA 5S, y los análisis estadísticos. Participó en la redacción del manuscrito.

- **Moreno-Aguilar MF,** Inda LA, Sánchez-Rodríguez A, Catalán P, Arnelas I. 2022. Phylogenomics and systematics of overlooked Mesoamerican and South American polyploid broad-leaved *Festuca* grasses differentiate *F.* sects. *Glabricarpae* and

Ruprechtia and *F.* subgen. *Asperifolia, Erosiflorae, Mallopetalon* and *Coironhuecu* (*subgen. nov*.). *Plants* 11:2303. IF: 4.67, Q1. doi 10.3390/plants11172303.

La doctoranda llevó a cabo el trabajo experimental de laboratorio y la extracción de ADN de muestras seleccionadas. Desarrolló el procesamiento, la depuración y el ensamblaje de secuencias del plastoma, el mapeo del gen rDNA 35S, y el análisis del ADN repetitivo de muestras individuales y comparadas, analizó los datos de abundancias del repeteoma y del gen rDNA 5S, y la obtención de las filogenias de cada base de datos. Participó en la redacción del manuscrito.

- **Moreno-Aguilar MF**, Inda LA, Arnelas I, Catalán P. 2022. IAPTchromosome data 36/2. *Festuca andicola*, *F. caldasii*, *F. chimborazensis* subsp. *micacochensis*, *F. subulifolia*. *Taxon* 71:1132-1134. IF:2.586.

  La doctoranda llevó a cabo los estudios de recuentos cromosómicos y de estimación de los niveles de ploidía de las muestras estudiadas. Participó en la redacción del artículo.

- Arnelas I, **Moreno-Aguilar MF**, Catalán P. 2022. Second-step lectotypifications of two names of *Festuca* subgenus *Erosiflorae* (Loliinae, Pooideae, Poaceae). *Correspondence. Phytotaxa (under review).*

  La doctoranda proporcionó los datos genómicos que permitieron conocer el emplazamiento filogenético de las especies del subgénero y participó en la revisión de materiales de herbario.

- **Moreno-Aguilar MF**, Viruel J, Sánchez-Rodríguez A, Probatova N, Ospina JC, Martínez-Segarra G, Devesa JA, Stewart A, Arnelas I, Catalán P. 2022. A nuclear single-copy-gene phylogeny of Loliinae (Poaceae): unraveling hybridization episodes. *(in prep.).*

  La doctoranda llevó a cabo el trabajo experimental de laboratorio, la extracción de ADN de 90 muestras adicionales seleccionadas y su posterior procesamiento, depuración y el ensamblaje de secuencias del plastoma, el mapeo del gen rDNA 35S y las reconstrucciones filogenéticas con estas bases de datos. Completó la extracción de ADN de 144 especies para la secuenciación mediante captura génica. Analizó los datos obtenidos para las reconstrucciones filogenéticas y las pruebas de hibridación mediante programas bioinformáticos, y participó en la redacción del artículo.

- **Moreno-Aguilar MF**, Benito B, Vicioso A, Sánchez-Rodríguez A, Arnelas I, Catalán P. 2022. Climatic niche conservatism of American I and American II fescue grasses from the North-Andean páramos (*Festuca*, Poaceae) (*in prep.).*

  La doctoranda participó en la obtención de datos de localidades de presencia de las especies endémicas de Ecuador y validó las ocurrencias de las especies norteandinas. Desarrolló los análisis de modelización de nicho ecológico de las especies seleccionadas en distintos escenarios temporales, y llevó a cabo los análisis estadísticos de variables climáticas y las pruebas de conservatismo de nicho y participó en la redacción del artículo.

Contribución de la doctoranda a otras publicaciones relacionadas con el tema de la tesis durante el periodo de desarrollo de su tesis (*Contribution of the doctorate student to other publications related to the topic of the thesis during the period of development of her thesis*):

- Kergunteuil A, Humair L, Maire AL, **Moreno-Aguilar MF**, Godschalx A, Catalán P, Rasmann S. 2020. Tritrophic interactions follow phylogenetic escalation and climatic adaptation. *Scientific Reports* 10: 2074.

  La doctoranda contribuyó con la extracción de ADN de parte de las especies en estudio la obtención de secuencias de loci nucleares y plastídicos, y el desarrollo de los análisis filogenéticos del conjunto de las especies en estudio.

- Grass Phylogeny Working Group III (several authors, incl. **Moreno-Aguilar MF**). 2022. Grass nuclear phylogenomics (*in prep*.).

  La doctoranda aportó datos de secuencias de genes nucleares copia simple y de plastomas de las especies de Loliinae incluidas en el estudio.