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Updated checklist of the *Culicoides* Latreille biting midges (*Diptera*: *Ceratopogonidae*) of Galicia (NW Spain): Diversity, distribution and medical-veterinary relevance

Alejandro Polina[1] | **Yasmina Martínez-Barciela**[1] | **José Manuel Pereira**[2] | **Fernando Cobo**[2] | **Elvira Íñiguez**[3] | **Javier Lucientes**[4] | **Rosa Estrada**[4] | **Josefina Garrido**[1]

[1]Departamento de Ecoloxía e Bioloxía Animal, Facultade de Bioloxía, Universidade de Vigo, Vigo, Spain

[2]Departamento de Zooloxía, Xenética e Antropoloxía Física, Facultade de Bioloxía, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[3]Consellería de Sanidade, Xunta de Galicia, Santiago de Compostela, Spain

[4]Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain

Correspondence
Alejandro Polina, Departamento de Ecoloxia e Bioloxia Animal, Facultade de Bioloxia, Universidade de Vigo, Vigo 36310, Spain.
Email: apolina@uvigo.es

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Abstract

Introduction: *Culicoides* Latreille biting midges are vectors of high concern as they can transmit serious veterinary diseases such as bluetongue virus or epizootic haemorrhagic disease virus, among others. Little is known about these vectors in Galicia, so a comprehensive literature review and an intensive monitoring were carried out in the region.

Material and methods: The Autonomous Community of Galicia was sampled through three different vector surveillance projects between 2004 and 2023. A total of 239 sampling points were deployed alongside the Galician territory. In addition, a literature review of *Culicoides* in Galicia related content was made by consulting several digital repositories.

Results: A total of 33 species of *Culicoides* belonging to 8 subgenera were identified. Among them, 15 are considered or suspected to be potential vectors of several pathogens of medical and/or veterinary interest. In addition, 20 of them are reported for the first time in the region. Updated distribution maps of the *Culicoides* biting midges of Galicia were provided, including several notes regarding their ecology and relevance for both public health and animal welfare.

Conclusions: The present work is one of the most complete works made at regional level in Spain to date. As Galicia's economy relies heavily on livestock farming, this work will provide a solid baseline in order to develop new research lines in the future regarding prevention to vector-borne diseases.

KEYWORDS
biting midges, *Culicoides*, Galicia, public health, vectors, veterinary health

1 | INTRODUCTION

Culicoides Latreille biting midges (*Diptera*: *Ceratopogonidae*) are one of the most important vectors in veterinary terms (González & Goldarazena, 2011) as they can transmit several diseases such as bluetongue virus (BTv), African horse sickness virus, epizootic haemorrhagic disease virus (EHDv), Schmallenberg virus (SBv) and many other pathogens like *Haemoproteus* sp. protozoa or *Onchocerca* sp. nematoda. *Culicoides* are holometabolous insects that can breed on a wide variety of habitats as long as they have abundant organic matter and humidity or water for egg development. Animals such as cows, sheep, goats or horses are their main hosts, but occasionally they can also feed on humans (González & Goldarazena, 2011).

The genus *Culicoides* includes 1347 valid species worldwide, after revision carried out by Borkent and Dominiak (2020). In Spain, 85 species belonging to 10 subgenera and one undefined group (*incertae sedis*) can be found (Alarcón-Elbal & Lucientes, 2012; González et al., 2023). This group is relatively well studied throughout the country, but irregularly considering the different regions.

There is an enormous lack of information in Galicia. First studies regarding *Culicoides* date back to 2008, when some results of the 'Programa Nacional de Vigilancia de la Lengua Azul' (PNVLA) referring to BTv vectors such as *Culicoides (Avaritia) imicola* Kieffer, *Culicoides (Avaritia) obsoletus* (Meigen, 1818) and *Culicoides (Culicoides) pulicaris* (Linnaeus) were published (Lucientes et al., 2008). Considering Galicia as a predominantly rural region where agriculture, and especially livestock farming, are key drivers of its economic development, the need to address animal welfare became increasingly urgent. Following the 'One Health' multidisciplinary approach, the regional government of Galicia (Xunta de Galicia), in collaboration with the University of Vigo (UVigo) and the University of Santiago de Compostela (USC), created the 'Rede Galega de Vixilancia de Vectores' (REGAVIVEC) in 2017. This network aims to monitor arthropod and update data regarding potential vectors of medical-veterinary relevance such as ticks, mosquitoes, sand-flies and biting midges. The Counselling of Health of the Xunta de Galicia is responsible for the annual publication of the results obtained by vector surveillance, making available the information on species collected during the sampling campaigns.

Further, in collaboration with REGAVIVEC, a PhD thesis was carried out by Silva-Torres (2021) with contributions on the monitoring network improvement. This work included several species not reported in the region previously, which contributed enormously to increase knowledge regarding the distribution and bioecology of *Culicoides* biting midges.

No more information related to this group have been provided to date. Therefore, the present study aims to create an updated checklist of the *Culicoides* genus for the Autonomous Community of Galicia based on a thorough review of existent literature and on unpublished data of several projects carried out in the field over the years. The information gathered on the distribution, diversity, ecology and sanitary-veterinary interest of these insects will represent a useful tool for vector surveillance and control programs.

2 | MATERIALS AND METHODS

2.1 | Study area

Galicia is the most northwestern region in Spain and represents 5.8% of the country with an extension of 29.575 km^2. It is divided in four provinces: A Coruña in the North West; Lugo in the North East; Ourense in the South East; and Pontevedra in the South West. The whole area has two predominant climate areas: oceanic temperate in the north and some western parts, catalogued as *Cfb* according to the Köppen climate classification (temperate with mild summers); and Oceanic Mediterranean in the middle and southern region, catalogued as *Csb* (dry with mild summers). There is a third climate area but much smaller, restricted to some margins of the Miño River, catalogued as *Csa* (Typical Mediterranean – dry with hot summers) (Consello da Cultura Galega, 2008; Agencia Estatal de Meteorología (AEMET), 2018). In *Cfb* climate area, the mean temperature for the hottest month never exceeds 22°C, but there are at least 4 months, in which mean temperature is above 10°C. It is the same in the *Csb* case, but its summers are way drier than in *Cfb*. In *Csa*, the precipitation rate in the driest month does not surpass 40 mm, and the mean temperature in the hottest month is above 22°C.

2.2 | Data sources

In order to build a detailed and updated catalogue, three different sources of unpublished data were used in the present study:

- 'Rede Galega de Vixilancia de Vectores' project funded by the Xunta de Galicia through an agreement between the Counselling of Health, the Counselling of Rural Environment, the UVigo and the USC since 2017. Used data correspond to 2017–2023 period.
- 'Programa Nacional de Vigilancia de la Lengua Azul' project funded by the Spanish Government and carried out by the University of Zaragoza, collecting data from various points spread throughout the whole Spanish territory. Used data correspond to 2004–2023 period.
- Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation (CLIMOS) project funded by the European Union and developed by 29 different European research institutions. In Spain, the project is coordinated by the University of Murcia, being the UVigo one of the collaborating partners. Used data correspond to the year 2023.

As a result, 239 samplings were deployed alongside the Galician territory (Figure 1), allowing the entomological study of most of its municipalities.

In addition, a comprehensive review of the related bibliography of *Culicoides* in Galicia was made. All sources were consulted through ResearchGate, Google Scholar, Scopus and many other digital repositories as TESEO, DigitUM or Minerva using the following keywords

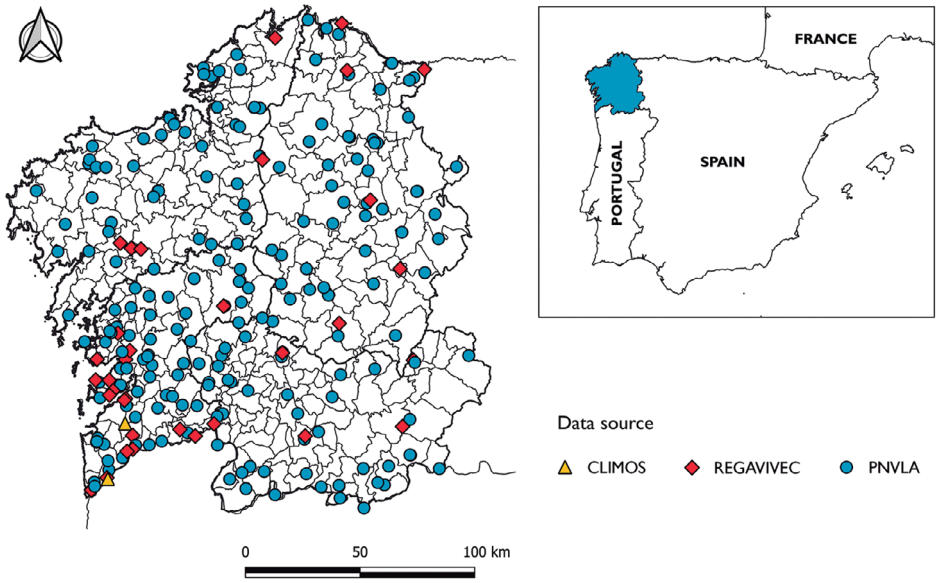

FIGURE 1 On the left, sampling points deployed throughout the Autonomous Community of Galicia. Municipalities (NUTS3) are also represented. On the right, situation of the region in the Iberian Peninsula.

in English, Spanish and Galician if possible: Ceratopogonidae (ceratopogónidos), *Culicoides*, biting midges (jejenes/xexenes), Galicia, Spain (España), bluetongue (lengua azul/lingua azul). All literature of scientific or official agency origin (articles, books, conference proceedings, government reports etc.) was considered valid, with no time range limit established.

2.3 | Data collection

Sampling points were selected depending on the type of vector monitored for the REGAVIVEC project. These points included ports, airports, big fuel stations, livestock farms, dog kennels, dwellings and wetlands. Different adult traps were placed alongside the Galician territory: BG-Sentinel 2 baited with BG-Lure (composed of lactic acid, ammoniac and fatty acids) (Biogents AG Company), BG-PRO equipped with a CO_2 generator (sugar, water and yeast grains) (Biogents AG Company) and CDC-miniature light traps fitted with incandescent 4-W white bulbs or 4-W UV lights (John W. Hock Company). Traps were placed weekly or fortnightly depending on the sampling point. With a few exceptions that were under surveillance throughout the year, monitoring started in April and ended in November, when climatic conditions were non-optimal. BG-Sentinel 2 and BG-PRO traps were settled on the floor, connected from a power supply or a 12-V battery. CDC-light traps were hung at a height of 1.5–1.8 m as well as connected right from an electrical source or a 6-V battery. All these adult traps were operated for 24 h. Samples were collected and preserved in ethanol 70% until processing.

PNVLA sampling points were selected looking for suitable areas for *Culicoides* occurrence. These points were sheep and cow farms mainly, but other goat and equine livestock farms were also studied. Collection procedure was only CDC-miniature light traps equipped with 4-W UV bulbs, as described above. Traps were placed weekly in sentinel places, but there were also some many points sampled irregularly. CDC-tools were hung at a height of 1.5–1.8 m inside the stables and kept operated for 48 h. Specimens were stored in ethanol 70% until identification.

The CLIMOS project sampling points were determined attending to potential areas of phlebotomine sand-flies appearance. In this way, two sites in Galicia were designated: a cattle farm and a dog kennel. The collection procedure consisted in two CDC-miniature light traps equipped with 4-W white bulbs per sampling point. These traps were hung at a height of 0.5 m and set-up every 28 days. Traps kept operating for 48 h, but collections were taken every 24 h in order to preserve the samples. These specimens were dry stored at −20°C until processing.

Biting midges were identified by following the morphological identification keys provided by Rawlings (1996), González and Goldarazena (2011) and Mathieu et al. (2012).

3 | RESULTS

A total of 33 species of *Culicoides* belonging to eight subgenera (*Avaritia* Fox, 1955, *Beltranmyia* Vargas, 1953, *Culicoides* Latreille, 1809, *Monoculicoides* Khalaf, 1954, *Oecacta* Poey, 1853, *Sensiculicoides* Shevchenko, 1977, *Silvaticulicoides* Glukhova, 1972 and *Wirthomyia* Vargas, 1973) and one *incertae sedis* have been identified throughout the study. Among them, 15 are considered or suspected to be potential vectors of several pathogens of medical and/or veterinary interest (Table 1).

Twenty out of the 30 species collected during 2004 and 2023 samplings are new records for Galicia: *C. (Culicoides) fagineus* Edwards (1939), *C. (Culicoides) lupicaris* Downes and Kettle (1952), *C. (Monoculicoides) nubeculosus* (Meigen, 1830), *C. (Monoculicoides) parroti* Kieffer (1914), *C. (Oecacta) brunnicans* Edwards (1939), *C. (Sensiculicoides) comosioculatus* Tokunaga (1956), *C. (Sensiculicoides) gejgelensis*

TABLE 1 List of *Culicoides* biting midges recorded in Galicia (Spain) classified by subgenus.

Genus	Subgenus	Species	Province[a]	Source[b]	Sampling	Veterinary relevance[c]
Culicoides	*Avaritia* Fox, 1955	*dewulfi* Goethebuer, 1936	L	EC, LR	CDC UV	BTv
		imicola Kieffer, 1913	AC, P	EC, LR	CDC UV	AHSv, AKAv, BTv, EHDV, *Leishmania infantum*[d]
		obsoletus s.l. (Meigen, 1818)	AC, L, O, P	EC, LR	BG-PRO, BG-SEN, CDC UV, CDC WL	AHSv, BTv, EHDv[d], SBv
		obsoletus s.s. (Meigen, 1818)	AC, L, O, P	EC, LR	CDC WL, CDC UV	AHSv, BTv, EHDv[d], SBv
		scoticus Downes & Kettle, 1952	L, P	EC	CDC UV	AHSv, BTv, SBv
	Beltranmyia Vargas, 1953	*circumscriptus* Kieffer, 1918	AC, L, O, P	EC, LR	BG-PRO, CDC UV, CDC WL	*Haemoproteus* sp., *Leishmania infantum*[d]
	Culicoides Latreille, 1809	*fagineus* Edwards, 1939	L, O	EC	CDC UV	–
		impunctatus Goethebuer, 1920	L, O, P	EC, LR	BG-PRO, CDC UV	–
		lupicaris Downes & Kettle, 1952	P	EC	CDC WL, CDC UV	AHSv, BTv
		newsteadi Austen, 1921	AC, L, O, P	EC, LR	BG-PRO, CDC UV, CDC WL	BTv[d]
		pulicaris (Linnaeus, 1758)	AC, L, O, P	EC, LR	CDC WL, CDC UV	AHSv, BTv
		punctatus (Meigen, 1818)	AC, L, O, P	EC, LR	CDC WL, CDC UV	BTv[d], SBv[d]
	Monoculicoides Khalaf, 1954	*nubeculosus* (Meigen, 1830)	AC, L, O	EC	CDC UV	*Onchocerca cervicalis*, *Trypanosoma* sp.[d]
		parroti Kieffer, 1914	AC, L, O	EC	CDC UV	–
	oecacta Poey, 1853	*brunnicans* Edwards, 1939	AC, L, O, P	EC	CDC UV	–
	Sensiculicoides Shevchenko, 1977	*clastrieri* Callot, Kremer & Deduit, 1962	O	LR	–	–
		comosioculatus Tokunaga, 1956	P	EC	CDC UV	–
		festivipennis Kieffer, 1914	AC, L, O, P	EC, LR	CDC UV, CDC WL	SBv[d], *Leucocytozoon caulleryi*
		gejgelensis Dzhafarov, 1964	P	EC	BG-PRO, CDC UV, CDC WL	-
		heliophilus Edwards, 1921	L	EC	CDC UV	–
		heteroclitus Kremer & Callot, 1965	P	EC	CDC UV	–
		jumineri Callot & Kremer, 1969	AC, L	EC	CDC UV	–
		kibunensis Tokunaga, 1937	L, O	LR	-	*Haemoproteus* sp.[d]
		kurensis Dzhafarov, 1960	AC, L	EC	CDC UV	–
		maritimus Kieffer, 1924	L, O, P	EC	CDC UV, CDC WL	–
		odiatus Austen, 1921	L, O, P	EC	CDC UV	–
		pictipennis (Staeger, 1839)	P	EC	CDC UV	*Haemoproteus* sp.[d]
		univittatus Vimmer (1932)	AC, L, O, P	EC	CDC UV	–

(Continues)

TABLE 1 (Continued)

Genus	Subgenus	Species	Province[a]	Source[b]	Sampling	Veterinary relevance[c]
	Silvaticulicoides Glukhova, 1972	*achrayi* Kettle & Lawson, 1955	AC, L, O, P	EC	CDC UV, CDC WL	–
		fascipennis (Staeger, 1839)	AC, L, O, P	EC	CDC UV	–
	Wirthomyia Vargas, 1973	*minutissimus* (Zetterstedt, 1855)	P	EC	CDC UV	–
		segnis Campbell & Clinton, 1959	L, P	EC	CDC UV	*Haemoproteus* sp.[d]
	incertae sedis	*pallidicornis* Kieffer, 1919	P	EC	CDC UV	*Haemoproteus* sp.[d]

Note: Information regarding entomological collection is provided. Literature data were consulted through González and Cobo (2006), Lucientes et al. (2008) and Silva-Torres (2021). Veterinary relevance was consulted through Kitaoka and Morii (1963), Mellor (1974), Mellor et al. (1990), Caracappa et al. (2003), Meiswinkel (2007), Dijkstra et al. (2008), Stephan et al. (2009), Dik et al. (2012), Larska et al. (2013), Slama et al. (2014), Goffredo et al. (2015), Federici et al. (2016), Foxi et al. (2016), Svodová et al. (2017), Žiegytė et al. (2021, 2022) and Muz et al. (2023).

[a]Provinces: AC = A Coruña; L = Lugo; O = Ourense; P = Pontevedra.

[b]Source: EC = Entomological Collection; LR = Literature Review.

[c]Veterinary relevance: AHSv = African Horse Sickness virus; AKAv = Akabane virus; BTv = bluetongue virus; EHDv = epizootic haemorrhagic disease virus; SBv = Schmallenberg virus.

[d]Competence remains unclear.

Dzhafarov (1964), *C. (Sensiculicoides) heliophilus* Edwards (1921), *C. (Sensiculicoides) heteroclitus* Kremer and Callot (1965), *C. (Sensiculicoides) jumineri* Callot and Kremer (1969), *C. (Sensiculicoides) kurensis* Dzhafarov (1960), *C. (Sensiculicoides) maritimus* Kieffer (1924), *C. (Sensiculicoides) odiatus* Austen (1921), *C. (Sensiculicoides) pictipennis* (Staeger, 1839), *C. (Sensiculicoides) univittatus* Vimmer (1932) *C. (Silvaticulicoides) achrayi* Kettle and Lawson (1955) *C. (Silvaticulicoides) fascipennis* (Staeger, 1839), *C. (Wirthomyia) minutissimus* (Zetterstedt, 1855), *C. (Wirthomyia) segnis* Campbell and Clinton (1959) and *C. pallidicornis* Kieffer (1919) (*incertae sedis*).

Regarding entomological collection data, 175 out of 239 sampling points deployed in the three projects were positive for *Culicoides* presence (73.2%). Adding up the literature records, 137 out of the 317 Galician municipalities have been positive for biting midges' occurrence (43.2%). This can be considered a large area as just 173 municipalities have registered any kind of entomological data. The province of Pontevedra has been more extensively studied with data from 53 municipalities from a total of 62 (85.4%). It is followed by the province of Lugo with 37 municipalities studied out of 67 (55.2%); Ourense with 43 out of 92 (46.7%); and A Coruña with 36 out of 95 (37.9%). The latter shows a lack of information in almost 2/3 of the territory.

A table with abundances per species (Table 2) is provided, adding up all results for the three projects analysed in the present work.

Regarding literature review, only three references were found along with the periodical information updated by the European Centre for Disease Prevention and Control (ECDC) on its webpage. These references correspond to González and Cobo (2006), Lucientes et al. (2008) and Silva-Torres (2021).

Based on all of these data, several distribution maps have been made at municipality level (NUTS3) (Figures 2–4) using QGIS 3.26.0 Buenos Aires (QGIS Development Team, 2009). Distribution map could not be produced for *Culicoides (Avaritia) chiopterus* (Meigen, 1830) as related information were only found at provincial level.

Subgenus *Avaritia*

Note 1. *C. chiopterus*: Species reported by the ECDC on their periodical distribution maps (ECDC, 2023). According to their data, this species can be found in A Coruña and Lugo provinces.

Note 2. *Culicoides dewulfi*: Species also showed on the ECDC distribution maps, but just at provincial level. This work demonstrated its presence in O Valadouro (Lugo), whereas the ECDC increases occurrence range to Ourense and Pontevedra provinces (Figure 2a).

Note 3. *C. imicola*: Its presence in the region was already mentioned by Lucientes et al. (2008). Entomological data register its occurrence in seven municipalities in the provinces of A Coruña and Pontevedra, especially in the latter (Figure 2b).

Note 4. *C. obsoletus* s.l.: All females collected in the three projects belonging to the *C. obsoletus* group were classified as *C. obsoletus* sensu lato, as only morphological identification was made.

TABLE 2 Absolute abundance (*n*) for each species identified through the three compiled projects.

Species	*n*
Culicoides achrayi	5058
Culicoides brunnicans	1809
Culicoides circumscriptus	548
Culicoides comosioculatus	24
Culicoides dewulfi	1
Culicoides fagineus	4
Culicoides fascipennis	118
Culicoides festivipennis	479
Culicoides gejgelensis	43
Culicoides heliophilus	2
Culicoides heteroclitus	3
Culicoides imicola	8
Culicoides impunctatus	25
Culicoides jumineri	26
Culicoides kurensis	54
Culicoides lupicaris	12
Culicoides maritimus	66
Culicoides minutissimus	22
Culicoides newsteadi	847
Culicoides nubeculosus	2153
Culicoides obsoletus s.s.	23
Culicoides obsoletus s.l.	89,219
Culicoides odiatus	11
Culicoides pallidicornis	6
Culicoides parroti	4
Culicoides pictipennis	3
Culicoides pulicaris	7969
Culicoides punctatus	10,694
Culicoides segnis	33
Culicoides scoticus	11
Culicoides univittatus	1108

Note 5. *C. obsoletus* s.s.: Species were confirmed through morphological identification of the genitalia of the males. It was confirmed its presence in the four Galician provinces (Figure 2d).

Note 6. *Culicoides scoticus*: Silva-Torres (2021) mentioned that she found several males through her research. According to entomological records made in the present study, it can be found in Monforte de Lemos (Lugo) and Ponte Caldelas (Pontevedra) (Figure 2e).

Subgenus *Beltranmyia*

Note 7. *Culicoides circumscriptus*: Silva-Torres (2021) reported this species for the first time in A Rúa, in the province of Ourense. Results presented in this work confirm its distribution range in A Coruña, Ourense and Pontevedra (Figure 2f).

Subgenus *Culicoides*

Note 8. *C. fagineus*: Its presence is reported for the first time in Galicia, being collected in O Valadouro (Lugo) and A Gudiña (Ourense) (Figure 2g).

Note 9: *Culicoides impunctatus*: It was recently detected for the first time in Galicia by Silva-Torres (2021) in Quiroga and Sarria municipalities (Lugo). Entomological data presented in this work expand its known distribution to Ourense and Pontevedra (Figure 2h).

Note 10. *C. lupicaris*: Species reported for the first time in Galicia due to the results of the three projects along. It was collected in three municipalities, all of them in the province of Pontevedra (Figure 2i).

Subgenus *Monoculicoides*

Note 11. *C. nubeculosus*: This species is newly reported in Galicia. It was captured in A Coruña, Lugo and Ourense provinces (Figure 3a).

Note 12. *C. parroti*: Its presence is reported for the first time in Galicia. It is an unusual species in the Galician region, and its distribution was constated in only four municipalities from A Coruña, Lugo and Ourense (Figure 3b).

Subgenus *Oecacta*

Note 13. *C. brunnicans*: This species is registered for the first time in Galicia. It was collected in the four provinces, being more disperse in Lugo as it was detected in five different municipalities (Figure 3c).

Subgenus *Sensiculicoides*

Note 14. *Culicoides clastrieri*: Silva-Torres (2021) noted its presence in only one municipality of the province of Ourense, in A Rúa (Figure 3d).

Note 15. *C. comosioculatus*: This species is newly reported in Galicia. It was collected in Ponte Caldelas (Pontevedra) in 2018, but it was not detected again to date (Figure 3e).

Note 16. *Culicoides festivipennis*: Species reported for the first time by Silva-Torres (2021) in a cattle farm in Monforte de Lemos (Lugo). Data presented in this work complement this record as it was also recorded in A Coruña, Ourense and Pontevedra provinces (Figure 3f).

Note 17. *C. gejgelensis*: It is reported for the first time in Galicia as it was detected in three municipalities of Pontevedra (Figure 3g), during 2022 and 2023.

Note 18. *C. heliophilus*: It is reported here for the first time in Galicia. It was detected twice in a cattle farm in O Valadouro (Lugo) in 2019 (Figure 3h). It is considered a rare and unusual species.

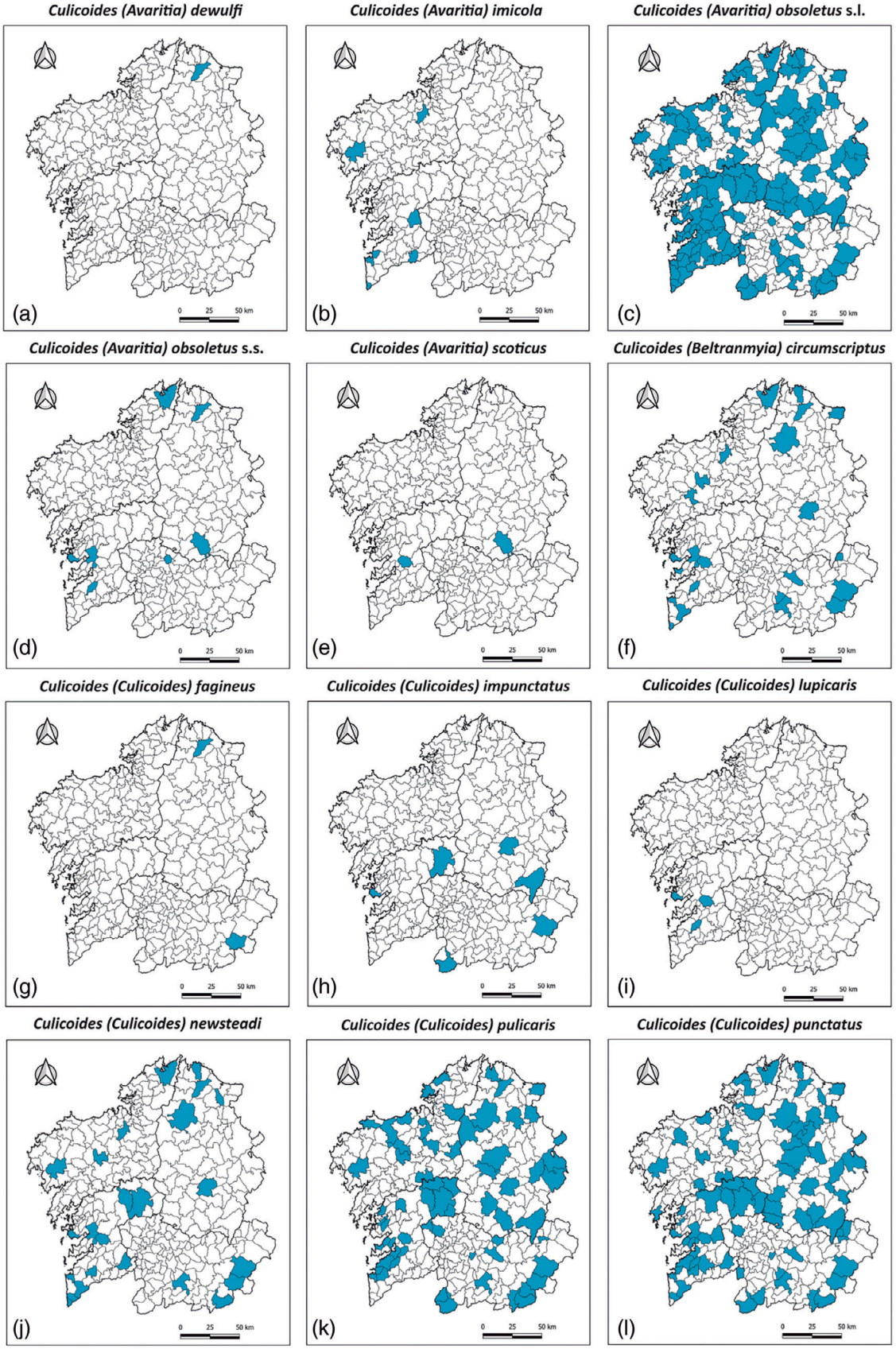

FIGURE 2 Distribution maps for biting midges of subgenera *Avaritia* (a–e), *Beltranmyia* (f) and *Culicoides* (g–l) in Galicia. Blue: present. White: absent or never observed.

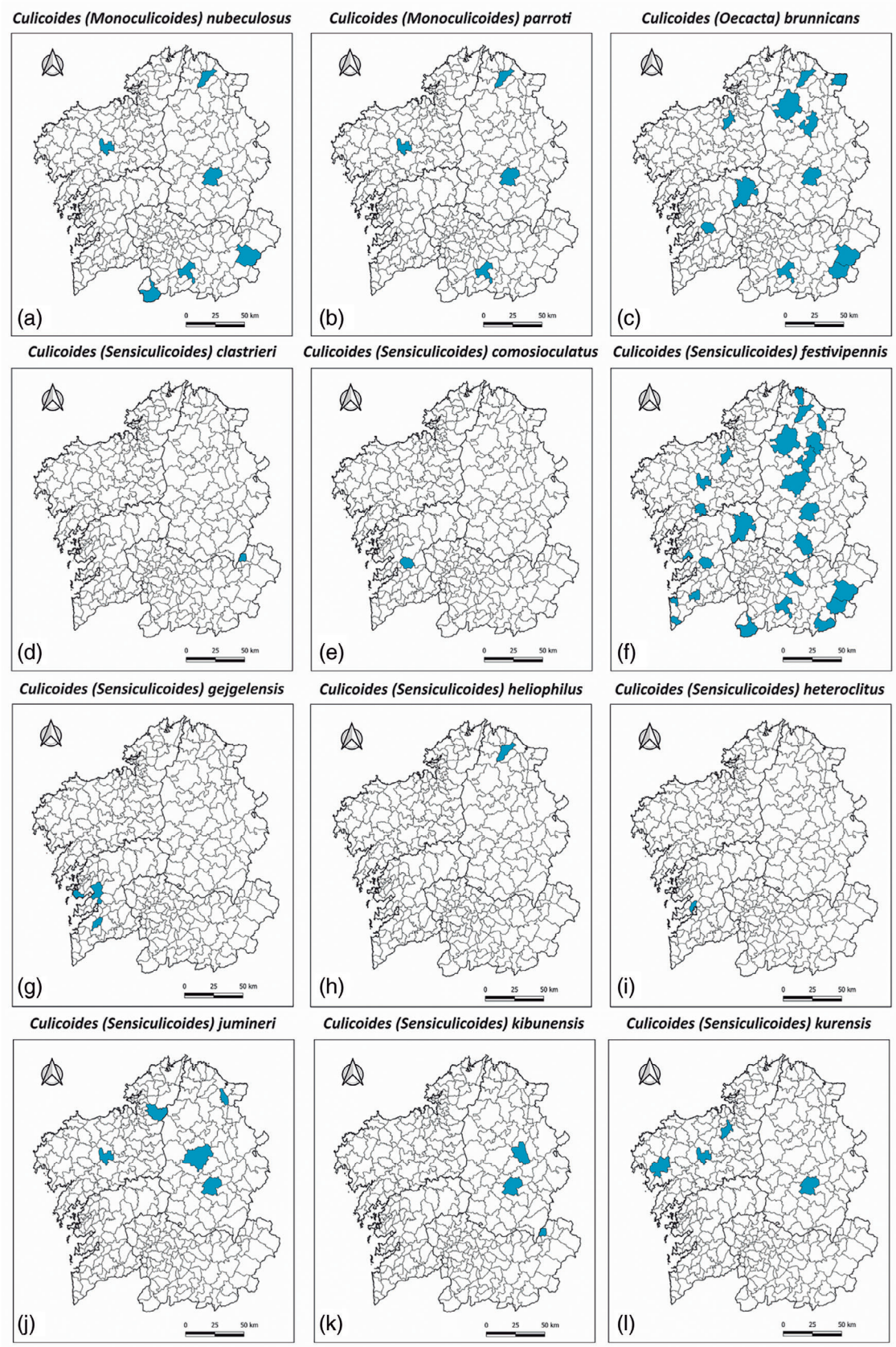

FIGURE 3 Distribution maps for biting midges of subgenera *Monoculicoides* (a–b), *Oecacta* (c) and *Sensiculicoides* (d–l) in Galicia. Blue: present. White: absent or never observed.

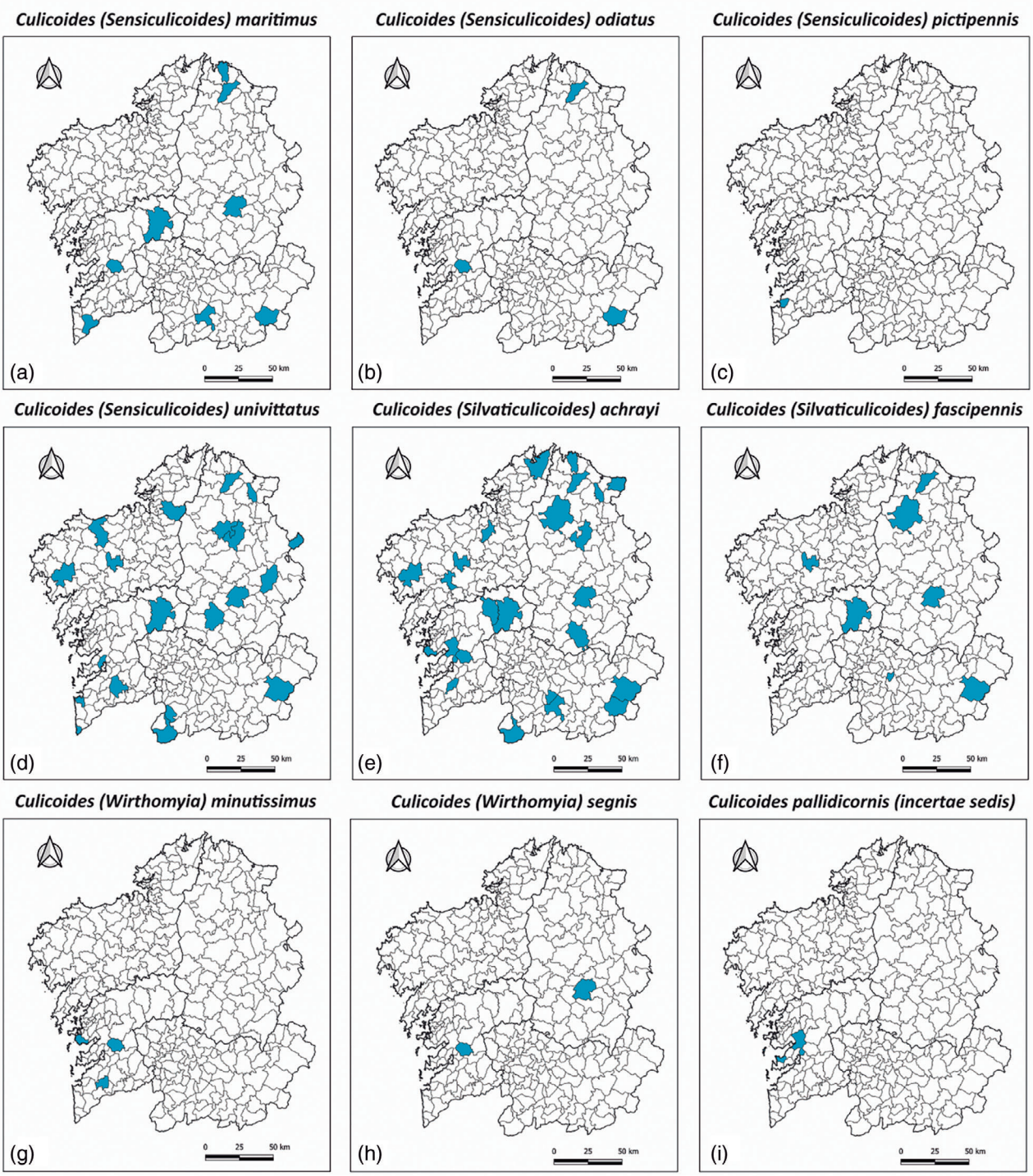

FIGURE 4 Distribution maps for biting midges of subgenera *Sensiculicoides* (a–d), *Silvaticulicoides* (e–f) and *Wirthomyia* (g–h); and unclassified (i) in Galicia. Blue: present. White: absent or never observed.

Note 19. *C. heteroclitus*: This species is a new report for the region. It was collected in 2006 and 2007 in two cattle farms from two municipalities of Pontevedra (Figure 3i).

Note 20. *C. jumineri*: This species is registered for the first time in Galicia. This species shows a distribution restricted to the northern half of Galicia, dispersed through several municipalities of A Coruña and Lugo (Figure 3j).

Note 21. *Culicoides kibunensis*: It was recently detected in Galicia by Silva-Torres (2021), and she noted its distribution in two municipalities of Lugo and in one of Ourense (Figure 3k).

Note 22. *C. kurensis*: This species is reported here for the first time in Galicia. It was collected relatively often during samplings carried out in 2008 in three municipalities of A Coruña and one in Lugo (Figure 3l).

Note 23. *C. maritimus*: It is newly reported for the Galician territory. Its distribution range includes the provinces of Lugo, Ourense and Pontevedra (Figure 4a).

Note 24. *C. odiatus*: It is newly reported for the region. This species was collected uninterruptedly from 2014 to 2017 with a range distribution restricted to three municipalities from Lugo, Ourense and Pontevedra (Figure 4b).

Note 25. *C. pictipennis*: It is a new species reported in Galicia, collected in Nigrán (Pontevedra) in 2006 (Figure 4c).

Note 26. *C. univittatus*: This species is registered here for the first time in Galicia. It was collected frequently in all four provinces (Figure 4d).

Subgenus *Silvaticulicoides*

Note 27. *C. achrayi*: It is reported for the first time in Galicia as it was detected consistently in all four provinces (Figure 4e). It was frequently collected during the ongoing surveillance.

Note 28. *C. fascipennis*: This species is a new record for Galicia. It was collected in all four provinces (Figure 4f) between 2007 and 2008 and, again, in 2019.

Subgenus *Wirthomyia*

Note 29. *C. minutissimus*: This species is newly recorded in Galicia as it was detected in three municipalities; all of them in the province of Pontevedra (Figure 4g). Specimens were collected interruptedly between 2018 and 2022.

Note 30. *C. segnis*: This species is reported for the first time in Galicia. It was collected in two cattle farms from two municipalities of Lugo and Pontevedra in 2011 (Figure 4h).

Unclassified (*incertae sedis*)

Note 31. *C. pallidicornis*: It is newly reported in the region. It was detected in two municipalities of Pontevedra during samplings carried out in 2022 and 2023 (Figure 4i).

4 | DISCUSSION

A great part of the Galician territory was studied thanks to the effort put into the three different projects carried out in the field and the reviewed literature. In fact, most of the Galician territory (54.6% of municipalities) have registered any kind of entomological data. As a result, 33 species of biting midges have been counted for this updated checklist, with notes on their vector competence according to specialized and current literature.

Eight species and one complex related or suspected as BTv vectors were identified. Among them, *C. obsoletus* s.l. was undoubtedly the most widespread taxa in Galicia, present in enormous abundances ($n = 89{,}219$) in 130 out of 173 municipalities with entomological data (75.1%). It was also collected in diverse areas: livestock farms, farmyards, dog kennels, dwellings, campsites, wetlands and meadows. The role of its members as BTv vector was repeatedly demonstrated in several studies (Meiswinkel et al., 2007; Dijkstra et al., 2008; Stephan et al., 2009; Foxi et al., 2016), and it was also suspected as the responsible of the serotype-1 outbreaks happened in Galicia between 2008 and 2009. After 14 years of eradication, four new serotype-4 outbreaks were registered in southern livestock farms (MAPA, 2023), consolidating this sickness as one of the most worrying vector-borne diseases in the region. It is not considered that *C. imicola*, the main BTv vector in Europe, could play any role as its populations were very small ($n = 8$). It was detected during the ongoing surveillance but its distribution range is restricted just to seven municipalities from only two provinces (A Coruña and Pontevedra). Among the confirmed potential BTv vectors, *C. pulicaris* was a widespread species as it was collected in 65 municipalities and in great numbers ($n = 7969$). *C. lupicaris*, which also belongs to the Pulicaris complex, was recorded for the first time in Galicia, but its populations were way smaller ($n = 12$) and restricted to the southwestern area of the province of Pontevedra. Both *C. pulicaris* and *C. lupicaris* were found in cattle, equine and sheep farms, but the first was also captured in a zoo stable.

Culicoides punctatus was collected in 79 municipalities and in large populations ($n = 10{,}694$). Another found species suggested as a BTv vector is *C. newsteadi*, even though it was detected in lesser councils (24) and in lesser numbers ($n = 847$). Goffredo et al. (2015) detected BTv genome in wild specimens, suggesting them as potential transmitters of the disease.

Concerning the members of the Obsoletus complex, four species were confirmed throughout the Galician region. *C. dewulfi*, *C. obsoletus* s.s. and *C. scoticus* were detected during the conducted surveillance by morphological identification of the male genitalia, whereas *C. chiopterus* were registered due to literature review. *C. obsoletus* s.s. turned out to be the best distributed and most abundant species ($n = 23$) over *C. scoticus* ($n = 11$) and *C. dewulfi* ($n = 1$), respectively. These results are consistent with other studies where the most abundant members of the Obsoletus complex were *C. obsoletus* s.s. and *C. scoticus*, respectively (Ramilo et al., 2012). *C. obsoletus/C. scoticus* are also demonstrated vectors of SBv, emerging disease suggested endemic in Spain at this moment (Larska et al., 2013; Jiménez-Ruiz et al., 2020).

Another emerging disease of concern is the EHDv, detected in several regions of Portugal and Spain, including Galicia. In the last year 2023, 29 veterinary districts reported EHDv cases on sentinel livestock farms (Xunta de Galicia, 2023), but it is expected that this number could be higher. The only known vector in Spain is *C. imicola*, but its role in Galicia is again discarded. Therefore, it is logical to consider *C. obsoletus* s.l. as the main vector, but studies could not demonstrate its competence yet (Dik et al., 2012; Federici et al., 2016).

Five species are suspected to be vectors of avian *Haemoproteus* sp. parasites, but its susceptibility was not confirmed to date (Žiegytė et al., 2021, 2022). *C. circumscriptus* is the best distributed one, being present in 23 municipalities. It could be particularly found close to areas with dogs such as dog kennels or even dwellings with many pets. *C. pallidicornis*, *C. pictipennis* and *C. segnis* are reported for the first time in

Galicia, but they are considered minoritarian species as they occurred in small numbers. The first one was collected in a dwelling with animals as well as in an organic-rich wetland, whereas the second and the third one were captured in cattle farms in 2006 and 2011, respectively. *C. kibunensis* was reported in low numbers by Silva-Torres (2021).

Some works also suggest the potential competence of *C. circumscriptus* along with *C. imicola* as vectors of *Leishmania infantum*. Slama et al. (2014) found individuals of both species naturally infected with *L. infantum*, but their role as vectors could not be confirmed to date and remains unclear.

Two found species were recently postulated as potential vectors of avian *Trypanosoma* sp.: *C. impunctatus* and *C. nubeculosus* (Svobodová et al., 2017), being the latter a new record for the Autonomous Community of Galicia. *C. nubeculosus* is also a confirmed vector of *Onchocerca cervicalis*, a very aggressive nematode parasite in equine animals (Mellor, 1974). On its part, *C. impunctatus* is a well-studied species in the United Kingdom as it is the main responsible of the 90% of the biting attacks on man and animals there (Blackwell, 2001). *C. nubeculosus* was found in relatively high numbers ($n = 2153$) with some peaks of abundance, whereas *C. impunctatus* has been collected in minor quantities ($n = 25$). Both species were collected in cattle and sheep farms; nevertheless, Silva-Torres (2021) also found some *C. impunctatus* individuals in dog kennels and even in a dwelling.

C. festivipennis is a generalist species considered a potential vector of *Leucocytozoon* sp. protozoa (Kitaoka & Morii, 1963). Its role as vector of many other sicknesses such as Schmallenberg disease is not excluded (Muz et al., 2023). It was mainly detected in cattle farms but also in organic-rich wetlands.

C. achrayi stands out as it was collected in high numbers ($n = 5808$), being the fourth most abundant specimen in the study. In any case, this species has no clear connection to disease transmission. Same applies to the rest of the species included on the catalogue. However, further studies regarding vector competence are needed in order to shed light on its actual relevance.

As mentioned, most of the territory of Galicia have registered some kind of entomological data. Considering the rugged orography of the territory, with many changes in altitude throughout it, these results are extremely positive. Nevertheless, it would be interesting if future surveillance could fill the existing gaps, as some of them are also livestock regions where biting midges could be found.

5 | CONCLUSIONS

Up to now, this study has reported 33 different species of *Culicoides* from 8 subgenera and 1 unclassified, including several of them considered of relevance for both public health and animal welfare. Twenty species have been reported for the first time in the Autonomous Community of Galicia, considered all of them autochthonous. Further, valuable information regarding distribution, incidence and veterinary-medical importance has been provided.

The displayed catalogue is one of the most complete works made at regional level in Spain to date. Now, this work is the building blocks of creating a baseline map of *Culicoides* in Galicia, and that future surveillance and research could be directed to understand disease dynamics, changes in the vector abundance over time and factors contributing to the presence or absence of these vectors. Moreover, this will be a solid baseline in order to develop new research lines in the future, as prevention associated to vector-borne diseases will be needed. Health problems at the human–animal–environment interface caused by zoonotic diseases still have significant repercussions on health and economy, so the multidisciplinary approach 'One Health' turns out to be more important than ever. Given that biting midges could adopt an important role as consequence of the globalisation and climate change effects, the study on their composition at local level and on their geographical distribution is key to provide an effective response.

AUTHOR CONTRIBUTIONS

All signatories contributed on the design and realisation of the study. Alejandro Polina, Yasmina Martínez-Barciela, José Manuel Pereira and Javier Lucientes participated in data collection. Alejandro Polina, Yasmina Martínez-Barciela and José Manuel Pereira wrote the first version of the text. Fernando Cobo, Javier Lucientes, Rosa Estrada and Josefina Garrido interpreted the results, made corrections with important intellectual contributions and developed the second version of the article. Fernando Cobo, Elvira Íñiguez, Javier Lucientes, Rosa Estrada and Josefina Garrido validated the methodology, supervised the correct execution of the study, managed the project resources and drafted the final version of the article. All the signatories have approved the final version of the article and are happy with its publication in its current form.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

None.

ORCID

Alejandro Polina https://orcid.org/0000-0002-9576-8308
Yasmina Martínez-Barciela https://orcid.org/0000-0002-9056-0153

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