RESEARCH ARTICLE

Advances in the knowledge of the *Inocybe mixtilis* group (*Inocybaceae, Agaricomycetes*), through molecular and morphological studies

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Abstract *Inocybe mixtilis* constitutes a complex of species characterized by nodulose-angulose spores, absence of cortina and a more or less bulbous marginate stipe that is not darkening when desiccated. In order to elucidate species limits within the *I. mixtilis* complex, an ITS-RPB2 phylogeny was performed and interpreted using morphological and ecological characters. Six supported clades were obtained in our analyses that correspond to *I. mixtilis*, *I. subtrivialis*, and four new species to science: *I. ceskae*, *I. johannis-stanglii*, *I. nothomixtilis* and *I. occulta*. Species within this complex can be morphologically recognized through a unique combination of morphological characters, such as the spore shape, cystidial length and shape, presence and development of the velipellis and pileus colour and viscidity. Nevertheless, those characters overlap, especially among *I. mixtilis*, *I. ceskae* and *I. occulta*, and intermediate collections are therefore more reliably identified through ITS-sequencing. Two species, *I. ceskae* and *I. occulta* are present in both North America and Europe, while the rest are so far only known in Europe, or Europe and Asia (*I. mixtilis*). All species, except *I. johannis-stanglii*, seem to be able to establish ectomycorrhizal association both with conifers and angiosperms. Descriptions, colour illustrations and a key to all known species in the *I. mixtilis* group are provided.

INTRODUCTION

Inocybe (Agaricales, Basidiomycota) is one of the most diverse genera of fungi, with around 500 (Kirk et al. 2011) or even considerably more (Matheny 2009) species worldwide and is also one of the most common and abundant in ectomycorrhizal communities. For this reason, *Inocybe* is one of the most cited genera in mycological and ecological studies of ectomycorrhizal communities and also with a large number of unidentified or misidentified sequences in GenBank (Ryberg et al. 2008). This is due to serious taxonomic and nomenclatural problems that persist in *Inocybe,* mainly due to the existence of cryptic or semi-cryptic species that can be separated by molecular data, or subtle morphological, ecological or chorological differences. On the other hand, there are many species yet to be described in Europe, especially in the Mediterranean region (Esteve-Raventós et al. 2016).

Molecular data have importantly contributed to infer phylogenetic relationships and to disentangle targeted species complexes within *Inocybe*. Multigene phylogenetic analyses have shown that *Inocybe* nests in the Agaricoid clade and is classified in the family *Inocybaceae* by some authors (Matheny et al. 2006), or in *Crepidotaceae* when *Inocybaceae* is merged with

its sister family (Petersen et al. 2010). In a study assessing traditional taxonomic schemas through multilocus molecular data, Matheny (2009) discussed phylogenetic relationships within the whole genus. This author placed the genus *Inocybe* into the more restricted family concept of *Inocybaceae*. Other approaches for the study of the *Inocybaceae* were based on other genomic regions like RPB1 and RPB2 (Matheny et al. 2002, Matheny 2005), and even on analysing large sets of unidentified ITS sequences assigned to *Inocybe* and deposited in public databases (Ryberg et al. 2008). Further studies employing also molecular data have addressed related genera like *Auritella* (Matheny & Bougher 2006), subgenera like *Mallocybe* (Cripps et al. 2010) or sections like *Marginatae* (Kropp et al. 2010, Esteve-Raventós et al. 2016) or *Rimosae* (Larsson et al. 2009). At species level, several studies have integrated distributional and/or morphological data together with the ITS region to disentangle species complexes and to describe new species of *Inocybe* (e.g., Cripps et al. 2010, Larsson et al. 2014, 2017, Esteve-Raventós et al. 2016, La Rosa et al. 2017, Matheny & Bougher 2017). This integrative approach has proved useful to unravel species complexes.

This study targets a species complex that is in need of revision, the *I. mixtilis* group. *Inocybe mixtilis*, described by Britzelmayr (1885) and consensually placed in section *Marginatae* (Kühner 1933), is characterized by its nodulose to angular basidiospores, lacking a cortina, and a more or less bulbous marginate stipe which is not darkening when desiccated. The epitype material proposed for *I. mixtilis* by Marchetti et al. (2014) allows for a more accurate phylogenetic placement and a stable interpretation of *I. mixtilis*. Examination and sequencing of further material by us suggest that *I. mixtilis* is actually a species complex containing several undescribed species. Thus,

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Table 1 ITS sequences of the taxa employed in the study, indicating present and original identifications, geographical origin, ecology, voucher numbers, GenBank and UNITE accession numbers. **Bold** entries refer to newly generated sequences.

Identification (*I. = Inocybe*)	Original identification	Geographical origin	Ecology	Voucher specimen	GenBank/UNITE accession number	
					ITS	RPB2
Crepidotus calolepis	–	Hungary, Szabolcs-Szatmar-Bereg	Unknown	WU 28902/CR04A	KF879617	–
I. ceskae	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	TUR-A 203414 (holotype)	**KX290789**	**MH496023**
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0042321	**KY393432**	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F18984	HQ604481	–
	I. mixtilis	Canada, British Columbia	*Pseudotsuga menziesii*	UBC F19182	HQ604482	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F19107	HQ604478	–
	I. sindonia	Canada, British Columbia	Unknown	UBC F19221	HQ604484	–
	I. mixtilis	Canada, British Columbia	*Arbutus menziesii*, *Pseudotsuga menziesii*	UBC F19202	HQ604483	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F18974	HQ604478	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F19079	HQ604479	–
	I. umbratica	Canada, British Columbia	Unknown	UBC F18954	HQ604477	–
	I. praetervisa	Canada, British Columbia	Unknown	UBC F19237	HQ604486	–
	I. mixtilis	USA, Oregon	*Pseudotsuga menziesii*	OSC 1064046	EU525952	–
	I. mixtilis	Canada, British Columbia	In mixed forest with *Arbutus menziesii*	UBC F19412	HQ604487	–
	I. glabrodisca	Canada, British Columbia	Unknown	UBC F19553	HQ604480	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F18974	HQ604485	–
I. flavobrunnescens	–	Portugal, Estremadura	*Quercus faginea*	AH 29883 (holotype)	KJ938784	–
I. hirculus	–	Finland, Varsinais-Suomi	*Betula pubescens*, *Picea abies*, *Pinus sylvestris*, *Salix* sp.	TUR-A 2577/ K51 (holotype)	FJ531872	–
I. johannis-stanglii	*I. pallida*	Germany, Baden-Württemberg	*Betula pendula*, *Picea omorica*	KR-M-0038039 (holotype)	**KX290791**	**MH496018**
	–	Austria, Oberösterreich	*Abies alba*, *Larix decidua*, *Picea abies*	KR-M-0043320	**KX290792**	–
	–	Austria, Oberösterreich	*Abies alba*, *Larix decidua*, *Picea abies*	KR-M-0043321	**KX290793**	**MH496019**
	I. pallida	Germany, Bayern	*Betula* sp., *Picea omorika*	M-0021483	**KX290835**	–
I. krieglsteineri	–	Spain, Andalucia	*Pinus pinea*	AH 44479	KJ938770	–
I. mixtilis	–	Germany, Bavaria, Hörbach	*Picea abies*	M-0219661 (epitype)	KM873369	–
	I. viscossisima var. *bulbosovelata*	Italy, Piemonte	*Pinus strobus*	EF 45/2000 (paratype of *I. viscossisima* var. *bulbosovelata*)	**KX290790**	**MH496026**
	–	Finland, Etelä-Häme	*Betula* sp., *Picea abies*, *Pinus sylvestris*, *Salix* sp.	TUR-A 145111	**KX290839**	–
	–	Finland, Etelä-Häme	*Pinus sylvestris*, *Quercus robur*, *Tilia* sp.	TUR-A 182874	**KX290841**	–
	I. tabacina	Germany, Schleswig-Holstein	*Alnus* sp., *Betula* sp.	M-0022249	**KX290836**	–
	I. cf. *tabacina*	Germany, Baden-Württemberg	In forest	M-0022251	**KX290837**	–
	–	Finland, Varsinais-Suomi	*Picea abies*	TUR-A 199995	**KX290842**	–
	–	Germany, Sachsen-Anhalt	*Picea abies*	KR-M-0043295	**KX290794**	–
	–	Germany, Sachsen-Anhalt	*Picea abies*	KR-M-0043296	**KX290795**	–
	–	Spain, Canary Islands	*Pinus canariensis*	KR-M-0043297	**KX290796**	–
	–	Germany, Rheinland-Pfalz	*Quercus robur*	KR-M-0043298	**KX290797**	–
	–	Germany, Baden-Württemberg	*Pinus sylvestris*	KR-M-0043299	**KX290798**	–
	–	Germany, Baden-Württemberg	*Betula pendula*, *Pinus mugo*, *Salix caprea*	KR-M-0043300	**KX290799**	–
	–	Austria, Oberösterreich	*Abies alba*, *Larix decidua*, *Picea abies*	KR-M-0043301	**KX290800**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043302	**KX290801**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043303	**KX290802**	–
	–	Germany, Rheinland-Pfalz	*Alnus glutinosa*, *Corylus avellana*, *Pinus sylvestris*, *Quercus robur*	KR-M-0043304	**KX290803**	**MH496021**
	–	Germany, Rheinland-Pfalz	*Picea abies*	KR-M-0043305	**KX290804**	–
	–	Germany, Rheinland-Pfalz	*Picea abies*	KR-M-0043306	**KX290805**	–
	–	Austria, Salzburg, Tamsweg	*Alnus incana*, *Picea abies*	KR-M-0043307	**KX290806**	**MH496020**
	–	Germany, Bayern	*Picea abies*	KR-M-0043308	**KX290807**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043309	**KX290808**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043310	**KX290809**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043311	**KX290810**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043325	**KX290811**	–
	–	Germany, Hessen	*Pinus sylvestris*, *Quercus robur*	KR-M-0043326	**KX290812**	–
	–	Spain, Basque Country	*Picea abies*, *Fagus*	ARAN-Fungi 04711	MH500842	**MH496022**
	–	France, Nord, Cyzoing	*Pseudotsuga menziesii*	LIP PAM05103003	HQ586870	–
	–	Italy	Unknown	MCVE 21534	JF908218	–
	–	Sweden	Unknown	EL13104	AM882838	–
	–	Sweden	Unknown	EL5904	AM882839	–
	–	Sweden	Unknown	EL2604	AM882837	–
	–	Sweden	Unknown	EL9604	AM882835	–

Table 1   (cont.)

Identification (*I.* = *Inocybe*)	Original identification	Geographical origin	Ecology	Voucher specimen	GenBank/UNITE accession number	
					ITS	RPB2
I. mixtilis (cont.)	–	Denmark	Unknown	EL16107	FN550894	–
	–	Estonia, Põlva	Unknown	TU118169	UDB015355	–
	–	Estonia, Hiiu	Unknown	TU118144	UDB015338	–
	–	Italy	Unknown	MCVE 13860	JF908151	–
I. nothomixtilis	–	Spain, Basque Country	*Pinus radiata*, *Quercus robur*	AH 24510 (holotype)	**KX290780**	–
	–	Spain, Balearic Islands, Mallorca	*Cistus monspeliensis*, *Pinus halepensis*	AH 46558	**KX290786**	**MH496025**
	–	Spain, Cataluña	*Cistus monspeliensis*, *C. salviifolius*	AH 26921	**KX290784**	–
	I. tabacina var. *pseudovolvata* ad int.	France, Île-de-France	*Carpinus betulus*, *Fagus sylvatica*, *Quercus* sp.	PC 0738072	**KX290838**	–
	I. mixtilis var. *aurata* nom. inval.	Italy	Unknown	Herb. Alessio, without number	**KX290813**	–
	–	Italy, Toscana	*Quercus* sp.	KR-M-0043322	**KX290814**	–
	–	Germany, Baden-Württemberg	*Betula pendula*, *Fagus sylvatica*, *Quercus petraea*	KR-M-0038133	**KX290815**	–
	–	Germany, Hessen	*Pinus sylvestris*, *Quercus rubra*	KR-M-0043319	**KX290816**	–
I. obtusiuscula	–	France, Savoie	*Salix herbacea*	LIP PAM02081710	HQ586869	–
I. ochracea	–	Germany, Bavaria, Augsburg	*Alnus incana*, *Fagus sylvatica*, *Salix* sp.	M Stangl 88/K65 (holotype)	FJ755803	–
I. occulta	*I. mixtilis*	Spain, La Rioja	*Quercus ilex* subsp. *ballota*	AH 36443 (holotype)	**KX290787**	**MH496017**
	–	Spain, Castilla-León	*Pinus pinea*	AH 30697	**KX290785**	–
	–	Spain, Andalucía	*Quercus suber*	AH 22108	**KX290779**	–
	–	Spain, Castilla-La Mancha	*Cistus ladanifer*, *Pinus pinaster*	AH 40499	**KX290788**	–
	–	Spain, Castilla-León	*Quercus robur*	AH 24987	**KX290781**	**MH496016**
	I. mixtilis	Spain, Cataluña	*Salix pyrenaica*	AH 21576	**KX257359**	–
	–	Finland, Enontekiön Lappi	*Betula pubescens* subsp. *czerepanovii*	TUR-A 145165	**KX290840**	–
	–	Spain, Castilla-León	*Fagus sylvatica*	AH 24989	**KX290782**	–
	I. mixtilis	Spain, Cataluña	*Dryas octopetala*, *Salix retusa*	AH 26731	**KX290783**	**MH496015**
	–	Germany, Bayern	*Larix decidua*, *Picea abies*	KR-M-0038028	**KX290817**	–
	–	Germany, Rheinland-Pfalz	*Picea abies*	KR-M-0038125	**KX290818**	–
	–	Germany, Baden-Württemberg	*Helianthemum nummularium*, *Pinus sylvestris*, *Quercus robur*	KR-M-0043312	**KX290819**	–
	–	Austria, Tirol	*Picea abies*	KR-M-0043313	**KX290820**	–
	–	Germany, Bayern	*Larix decidua*, *Pinus mugo*	KR-M-0043314	**KX290821**	–
	–	Austria, Kärnten	*Picea abies*	KR-M-0043315	**KX290822**	–
	–	Austria, Salzburg, Tamsweg	*Picea abies*	KR-M-0043316	**KX290823**	–
	–	Germany, Baden-Württemberg	*Picea abies*	KR-M-0043323	**KX290824**	–
	–	Germany, Baden-Württemberg	*Fagus sylvatica*, *Picea abies*	KR-M-0043318	**KX290825**	–
	–	Germany, Baden-Württemberg	*Abies procera*	KR-M-0043317	**KX290826**	–
	–	Germany, Rheinland-Pfalz	*Betula pendula*, *Larix decidua*, *Quercus robur*	KR-M-0043230	**KX290827**	–
	–	Netherlands, Friesland	*Pinus sylvestris*	KR-M-0038029	**KX290828**	–
	–	Germany, Bayern	*Betula pendula*, *Larix decidua*, *Pinus sylvestris*	KR-M-0043324	**KX290829**	–
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0046554	**KX290830**	–
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0046555	**KX290831**	–
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0046558	**KX290832**	–
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0046557	**KX290833**	–
	–	Finland, Koillismaa	*Betula* sp., *Picea abies*, *Pinus sylvestris*	KR-M-0046556	**KX290834**	–
	I. mixtilis	Estonia, Saare	Unknown	TU120084	UDB023622	–
	I. praetervisa	Canada, British Columbia	Unknown	UBC F19174	HQ604492	–
	I. jacobi	Canada, British Columbia	Mixed forest with *Arbutus menziesii* and *Dodecatheon hendersonii*	UBC F19302	HQ604491	–
	I. mixtilis	Australia, Australian Capital Territory	*Pinus* sp., *Ulmus* sp.	CANB 574539	KP308781	–
	I. mixtilis	USA, Oregon	*Pseudotsuga menziesii*	OSC 1064111	EU525968	–
	I. mixtilis	USA, Oregon	*Pseudotsuga menziesii*	OSC 1064112	EU525941	–
	I. mixtilis	USA, Oregon	*Pseudotsuga menziesii*	OSC 1064115	EU525969	–
	I. mixtilis	Italy	Unknown	MCVE 5052	JF908121	–
	I. mixtilis	France, Pas-de-Calais	*Betula* sp., *Pinus pinaster*, *Populus tremula*	LIP PAM 07110104	HQ586872	–
	I. mixtilis	Sweden	Unknown	BJ920916	AM882840	–
	I. mixtilis	Canada, British Columbia	Unknown	UBC F19341	HQ604490	–

Table 1   (cont.)

Identification (*I.* = *Inocybe*)	Original identification	Geographical origin	Ecology	Voucher specimen	GenBank/UNITE accession number	
					ITS	RPB2
I. occulta (cont.)	*I. mixtilis*	Canada, British Columbia	Unknown	UBC F19030	HQ604488	–
	I. mixtilis	Italy	Unknown	MCVE 91	JF908089	–
	I. mixtilis	Mexico, Morelos	Unknown	GO-2010-169	KC152132	–
	I. xanthomelas	Italy	Unknown	MCVE 6549	JF908127	–
	I. mixtilis	Italy	Unknown	MCVE 9844	JF908141.2	–
I. phaeocystidiosa	–	Spain, Segovia	*Pinus sylvestis*	AH 9154 (holotype)	KT203789	–
I. praetervisa	–	Italy, Rabbi	In coniferous wood	SF229598 (epitype)	KT203792	–
I. praetervisoides	–	Spain, Madrid	*Quercus ilex* subsp. *ballota*	AH 29863 (holotype)	KT203794	–
I. saliceticola	–	Finland, Pohjois-Karjala	*Alnus incana*, *Betula* sp., *Salix* sp.	TUR-A 19738F/ K11 (holotype)	FJ755796	–
I. subtrivialis	–	Spain, Castilla-La Mancha	*Quercus ilex*, *Cistus*	AH 19225 (holotype)	MH496013 / MH496014	–
	–	Spain, Madrid	*Cistus ladanifer*, *Pinus pinaster*	AH 26789	**KX354977**	**MH496024**
I. tabacina	–	France, Wannehin	*Populus nigra*	LIP PAM05071302	HQ586865	–
I. urbana	–	Italy, Torino	*Tilia* sp.	AMB 17142 (holotype)	KT229613	–
I. xanthomelas	–	Spain, Madrid	*Betula pendula*	AH 29895	KJ938774	–

the aim of this work is to revise the *I. mixtilis* complex based on sequence data of two genetic markers (ITS and RPB2) and morphological data and to contribute to a better understanding of the genus *Inocybe*.

MATERIALS AND METHODS

Morphological study

Specimens studied were collected across Europe and were mostly deposited in KR (Karlsruhe, Germany), AH (Alcalá de Henares, Spain) and ARAN herbaria (Aranzadi Science Society). Additional collections were loaned from the Johann Stangl (M; Munich, Germany), Jukka Vauras (TUR-A; Turku, Finland) and Guillaume Eyssartier (PC; Paris, France) herbaria, while other collections were loaned from private herbaria: Erminio Ferrari (EF; Italy), Roberto Fernández-Sasia (RFS; Bizkaia, Spain), Jordi Vila García (JVG; Barcelona, Spain) and Agustín Caballero Moreno (AC; La Rioja, Spain).

Microscopical features were described from mountings in water and 3–5 % ammonium hydroxide solution and observed by light microscopy with the aid of an oil immersion objective. Photomicrographs were taken with a Nikon (Eclipse 80i) microscope and a digital camera Nikon (DS-5 M) and (in the case of D. Bandini) with a Leica DM-750-microscope and a Zeiss AxioCam ERc5s. Scanning electron micrographs were obtained using a Zeiss DSM-950. For ultramicroscopic studies, the samples were rehydrated in concentrated ammonium hydroxide (28–30 %) for 30 min, dehydrated in aqueous ethanol (70 %) for 30 min, fixed for 2 hours in pure ethylene glycol dimethylether (= 1.2-dimethoxymethane), and finally immersed in pure acetone for at least 2 hours. This was followed by critical point drying and sputtering with gold-palladium. This technique allows the use of very little material (small portions of lamellae).

Colours of basidiomata, either in fresh or dry condition, were standardized following Munsell® (1994). Most of the terminology used for microscopic features follows Kuyper (1986) and Vellinga (1988). L_m, W_m and Q_m refer to the mean spore length, width and ratio, respectively. Herbarium acronyms follow Thiers (2012). General bibliography, monographs or contributions to specific groups of *Inocybe* were used in the determination of the samples, i.e., Kauffman (1924), Heim (1931), Malençon & Bertault (1970), Stuntz (1978), Alessio & Rebaudengo (1980), Kuyper (1986), Nishida (1989), Stangl (1989), Bon (1998),

Kobayashi (2002), Ferrari (2006, 2010), Jacobsson & Larsson (2012), Matheny (2016) and Bandini (2017).

DNA extraction, amplification and sequencing

Total DNA from BAN (Bandini) samples was extracted from dry herbarium specimens employing a modified protocol based on Murray & Thompson (1980). A portion of each sample was blended with the aid of a micropestle in 600 µL CTAB buffer (CTAB 2 %, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65 °C. A similar volume of chloroform : isoamylalcohol (24 : 1) was added and carefully mixed with the samples until their emulsion. Samples were then centrifuged for 10 min at 13 000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70 %, centrifuged again for 2 min and dried. Genomic DNA was finally resuspended in 200 µL ddH2O.

For the rest of the specimens, genomic DNA was extracted from dried herbarium specimens using the Prepman Ultra Sample Preparation Reagent (Thermo Scientific, USA). One hundred µL of extraction solution were added to 1.5 ml Eppendorf tubes containing small portions of lamellae and context and vortexed for 1 min. These tubes were incubated for 10 min at 100 °C in a heated block and then centrifuged at 12 000 rpm for 2 min. Fifty µL of the supernatant were transferred to a new tube and stored at -20 °C. DNA concentration was determined using a NanoDrop ND-1.000 spectrophotometer (Thermo Scientific, USA).

We sequenced two regions: the nuclear ITS1-5.8S-ITS2 region using the primers ITS1F-ITS4 (White et al. 1990) and the RNA polymerase II subunit (RPB2) between primers RPB2-6F-bRPB2-7R for the RPB2 region (Matheny 2005). PCR reactions were performed in 25 µL per reaction under a program consisting of a hot start at 95 °C for 5 (6 in the case of Spanish isolates) min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s, respectively) and a final 72 °C step 10 min. PCR products were checked in 1 % agarose gels, and positive reactions sequenced in both directions. Sequencing primers were the same as employed for PCR amplification. Raw sequence data was manually edited using Chromas 2.5.1 software (Technelysium Pty Ltd., Southport, Queensland, Australia). The identity of obtained consensus sequences was verified through the BLASTn query algorithm in GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (https://unite.ut.ee/) databases.

Phylogenetic analyses

Two genomic regions were sequenced for phylogenetic reconstruction and two alignments were thus assembled. A first ITS-RPB2 alignment aimed to test species boundaries and the monophyly of the *Inocybe mixtilis* group using representative sequences of each species. This dataset comprised 1 410 characters divided into two partitions (ITS, 707 characters; RPB2, 703) and 22 taxa. *Inocybe calopedes* (KJ778839, KJ756415) and *I. subfibrosoides* (KP636879, KM656117) were selected as outgroup because sequences of both markers were available for it in GenBank and blast searches indicated similarity to sequences of the *I. mixtilis* group. Gene congruence was evaluated manually by comparing supported clades among

single-gene genealogies (Mason-Gamer & Kellogg 1996). Each locus was subjected to a Maximum Likelihood (ML) analysis as described below. A supported clade for one marker was considered to be in conflict when contradicted with significant support by another (bootstrap support > 70 %). As no conflict was detected, the ITS and RPB2 regions were concatenated. A second ITS alignment was constructed to place specimens sequenced only for the ITS region. This alignment comprised 771 characters and 120 sequences, of which 67 were generated by us, while the rest were downloaded from GenBank and UNITE databases (Table 1). Sequences were aligned in MUSCLE software (Edgar 2004) of MEGA6 (Tamura et al. 2013) and MAFFT (http://mafft.cbrc.jp/alignment/server/; Katoh et al.
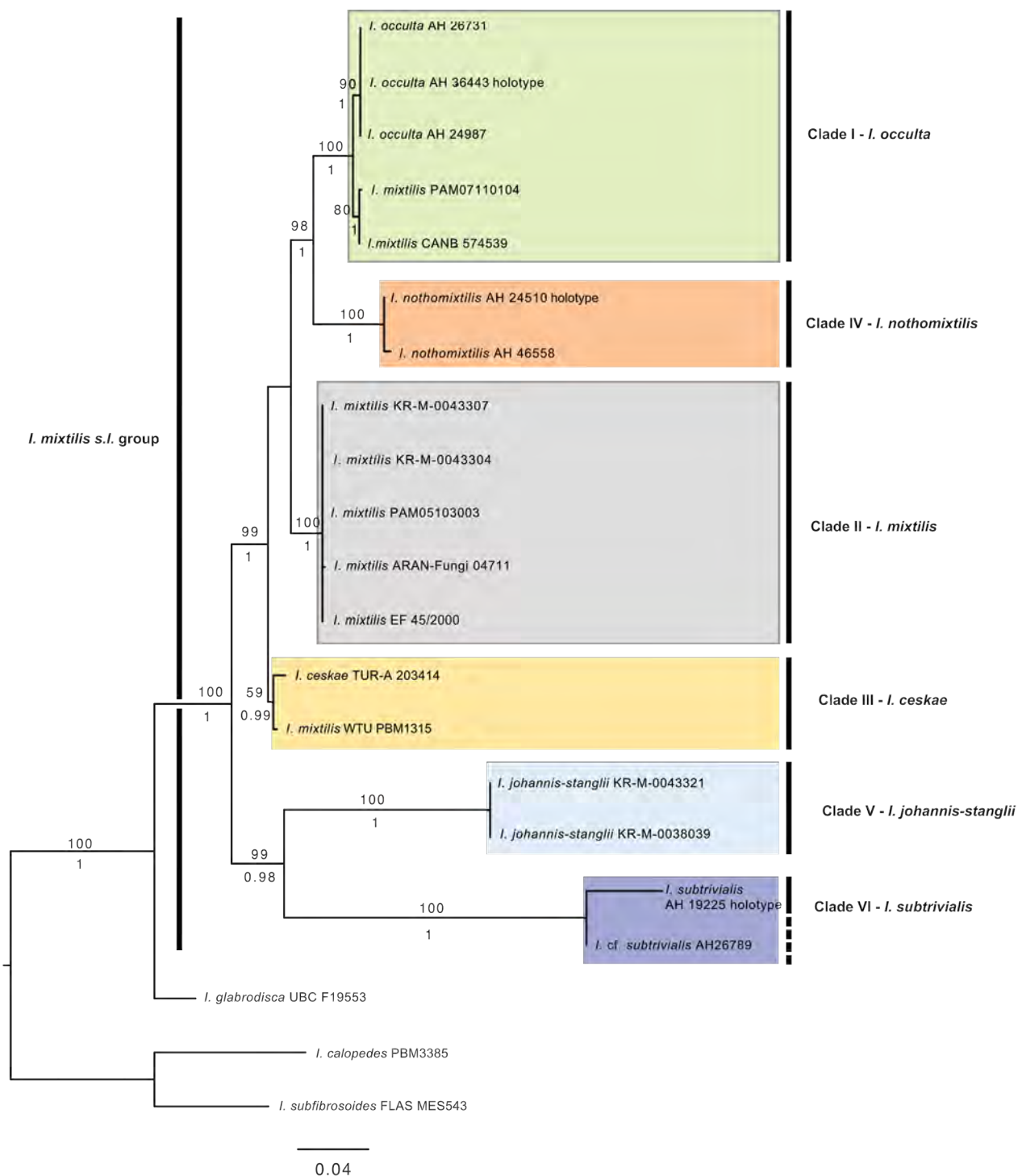
Fig. 1 The Bayesian 50 % majority-rule consensus tree inferred from ITS-RPB2 regions. Numbers above and below nodes represent Bayesian posterior probabilities and ML bootstrap values, respectively. Only nodes significantly supported in one of the analyses bear values. Sequences of *Inocybe stellata* were used as outgroup. Coloured boxes represent different taxa recognized for the *Inocybe mixtilis* species complex.

2005) and adjusted manually. An ITS sequence of *Crepidotus caulolepis* was used as outgroup. Ambiguously aligned positions and sequence ends were trimmed at both ends.

Bayesian inference of phylogenetic relationships was performed using MrBayes v. 3.2.3 (Ronquist et al. 2012), applying a Gamma+P-Imvar model of rate heterogeneity and a GTR substitution model. Markov Monte Carlo Chains (MCMC) were run for 2 M generations; two independent runs with four chains were performed for trees sampled every 1 000 steps, setting the heating temperature as 0.05. Burnin was set at 25 %. ML phylogenetic analysis was conducted using the dataset alignment with the E-INS-i strategy of MAFFT, employing default settings in RAxML v. 8.2.9 (Stamatakis 2006), via CIPRES platform

(https://www.phylo.org/; Miller et al. 2010). To assess branch confidence 1 000 non-parametric bootstraps were performed.

RESULTS

The 50 % majority-rule consensus phylogenetic tree obtained in the Bayesian analysis of the ITS-RPB2 alignment is shown in Fig. 1. The inferred tree was fully supported in both analyses of this alignment. The *I. mixtilis* group is monophyletic (PP 1; ML-BP 100 %), being *I. glabrodisca* sister to it. Five of the species attributed here to the *I. mixtilis* group received strong support, namely *I. johannis-stanglii* (PP 1; ML-BP 100 %), *I. mixtilis* (PP 1; ML-BP 100 %), *I. nothomixtilis* (PP 1; ML-BP 100 %),
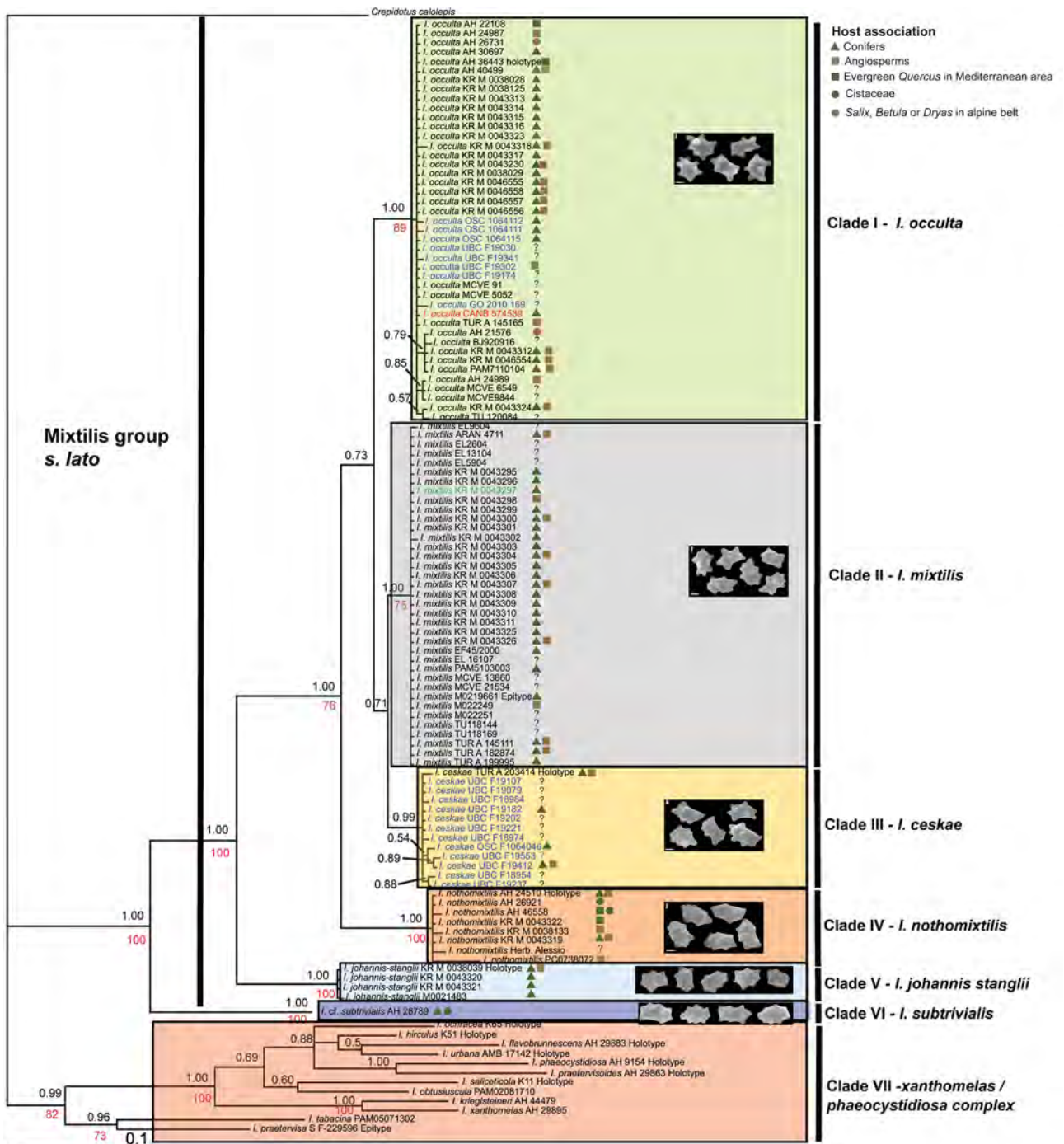
Fig. 2 The Bayesian 50 % majority-rule consensus tree inferred from ITS region. Numbers above and below nodes represent Bayesian posterior probabilities and ML bootstrap values, respectively. Only nodes significantly supported in one of the analyses bear values. Phylogram was rooted with an ITS sequence of *Crepidotus caulolepis*. Coloured boxes represent different taxa recognized for the *Inocybe mixtilis* species complex together with the *I. xanthomelas / phaeocystidiosa* group. Sequences are coloured according to their geographical origin: Europe (black), North America (blue), Australia (red), Canary Islands (green). Host associations are given for each collection when known.

I. occulta (PP 1; ML-BP 100 %) and *I. subtrivialis* (PP 1; ML-BP 100 %), while *I. ceskae* was only supported in the Bayesian analysis (PP 0.99; ML-BP 59 %), *I. johannis-stanglii* and *I. subtrivialis* form a strongly supported clade that is basal to the rest of the species of the *I. mixtilis* group (PP 0.98; ML-BP 99 %).

The 50 % majority-rule consensus tree of the Bayesian analysis of the ITS region shows a similar topology, but *I. subtrivialis* and *I. johannis-stanglii* do not form a monophyletic clade (Fig. 2). The six species of the *I. mixtilis* group receive support in at least the Bayesian analysis. Intraspecific sequence variability was extremely low for all treated taxa.

Each of the clades could be morphologically characterized based on a unique combination of several characters, such as the spore shape, the cystidial length, the pileus colour and viscidity, and the presence and development of the velipellis. Nevertheless, these characters were overlapping. Ecology and host specificity showed some patterns (Fig 2). While *I. ceskae* and *I. subtrivialis* are restricted to taiga forests and Mediterranean forests, respectively, other species like *I. mixtilis* exhibited a broader ecological amplitude and associates with both conifers and angiosperms. Likewise, a species seems to be restricted to Europe (*I. mixtilis*), while *I. occulta* and *I. ceskae* have a transoceanic distribution and were also present in North America.

TAXONOMY

Inocybe mixtilis (Britzelm.) Sacc., Syll. Fung. 5: 780. 1887. — Fig. 3, 4

≡ *Agaricus mixtilis* Britzelm., Ber. Naturhist. Vereins Augsburg 28: 152, 1885.

MycoBank number MB141839.

Lectotypus. Britzelmayr (1882), Dermini aus Südbayern: f. 21, *sub nomen Agaricus rimosus*, designated by Marchetti et al. (2014:149). Epitypus: Germany, Bayern, Hörbach, Haspelmoor, Landkreis Fürstenfeldbruck, Fichtenwald (*Picea* forest), auf Nadelstreuhumus wachsend, gesellig, 26 Sept. 1981, leg. J. Stangl, M-0219661, designated by Marchetti et al. (2014: 149; previously as neotype by Stangl 1984: 332). ITS barcode GenBank KM873369.

Selected descriptions and iconography — Stangl (1984), Marchetti et al. (2014: 149).

Basidiomes often gregarious, rarely solitary. *Pileus* 10−35(−45) mm diam, 5−20 mm high; shape when young subconical, paraboloid or campanulate, then hemispherical, broadly convex to expanded, often showing a broad distinct umbo since young, usually depressed around umbo in mature specimens and uplifted towards the margin; margin slightly inflexed when young, later somewhat deflexed to straight, initially even, often becoming somewhat undulate, irregular or torn in old specimens, not striate, rarely subhygrophanous (e.g., KR-M-0043299), occasionally crenate to appendiculate in young specimens by fugacious velipellis remnants; colour somewhat variable, but most often golden yellow or shiny yellow-ochre with orange, copper or brownish hue (Mu 10YR 8/8, 7.5YR 8/6, 7/6−7/8, 6/6−6/8), somewhat darker in centre (Mu 5YR 6/6; 7.5YR 6/6), but in some collections also uniformly brownish, mostly with faint orange or reddish tinge (Mu 5YR 6/4−6/6; 10YR 6/4−6/6), sometimes with darker umbo; surface entirely glabrous and smooth, very often with a lardy or greasy appearance, very finely radially fibrillose to slightly rimose, especially at the margin, where the paler flesh underneath is sometimes exposed; in a few collections (e.g., KR-M-0043325) the surface may also appear minutely verrucose or even cracked around the umbo (KR-M-0043306), or very finely felty (KR-M-0043310); velipellis apparently absent or, if present, fugacious and only visible in very young fruitbodies, leaving delicate appendiculate

whitish remnants on the pileus margin (KR-M-0043301), or very unusually (as commented by Ferrari 2006 for *I. viscosissima* var. *bulbosovelata*), covered by a thin velar patch ('biancogrigiognolo', greyish white); surface distinctly lubricous and sticky in wet conditions and hence agglutinating soil particles or humus remains. *Lamellae* subventricose, but almost linear in some collections (e.g., KR-M-0043302), 2−5 mm deep, almost free to adnexed, sometimes also adnate with a decurrent tooth, usually rather crowded, L = 50−60(−70), I = 1−2(−3); colour initially white or whitish, then greyish, quite often showing a faint violet hue, brownish grey, sometimes pale rusty-brown or pinkish rusty-brown when old; edge even to crenulate, whitish or paler than faces, but in old specimens sometimes concolorous or even somewhat darker. *Stipe* 15−50(−60) × (2−)3−5(−6) mm, solid, fibrose; shape cylindrical or tapering upwards, sometimes more or less curved near the base, which is always distinctly marginately bulbous; bulb 7–12 mm broad, edge mostly sharp or even volviform, occasionally showing a fibrillose rim from velipellis reminiscent of a 'pseudovolva'; colour pure white, whitish to beige or pale straw to pale ochraceous, more rarely lemon yellow (KR-M-0043301, Mu 10YR 8/6), never darkening; surface evenly and densely pruinose all over. *Cortina* not seen, even in young primordia. *Context* firm, fibrose, whitish in pileus, whitish or beige to very pale straw in stipe, whitish in the bulb; smell faint, acidulous or more or less spermatic, especially when cut; taste not recorded. *Colour of exsiccata*: pileus generally uniformly brownish (Mu 10YR 4/3−4/6 or 6/6−6/8), also reddish brown (Mu 7.5YR 4/4−4/6); stipe concolorous or somewhat lighter than pileus (Mu 10YR 5/6−5/8), never showing any distinct darkening (either browning or blackening) upon drying.

Basidiospores (6.7−)7.1−9.8(−10.5) × (5.1−)5.2−7.9(−8.1) µm, Q = (1−)1.1−1.4(−1.5), L_m = 8.2−9, W_m = 6.1−6.7, Q_m = 1.3−1.4 [n = 100, from 4 collections], mostly isodiametric to subisodiametric, more rarely subheterodiametric, with nodulose profile which is often 'margaritiform' or 'pseudoasteriform' (star-like arrangement), usually presenting quite distinct (7−)9−13(−16), obtuse to subconical knobs, (0.75−)1−1.5(−2) µm high, (1.2−)1.4−1.7(−2) µm broad at the base; apicula distinct, often curved; yellow in ammonia. *Basidia* 20−32 × 8−11.5 µm, clavate, (2−)4-spored, with sterigmata 3−5(−7) µm long. *Pleurocystidia* abundant, metuloid, (38−)40−48−66(−70) × (12−)14−18−25(−26) µm, Q = (1.8−)1.9−2.6−3.4(−3.5) [n = 40], variable in shape among collections, mostly sublageniform, subfusiform to subutriform, often ventricose and extending upwards into a defined neck (see Marchetti et al. 2014: 151),
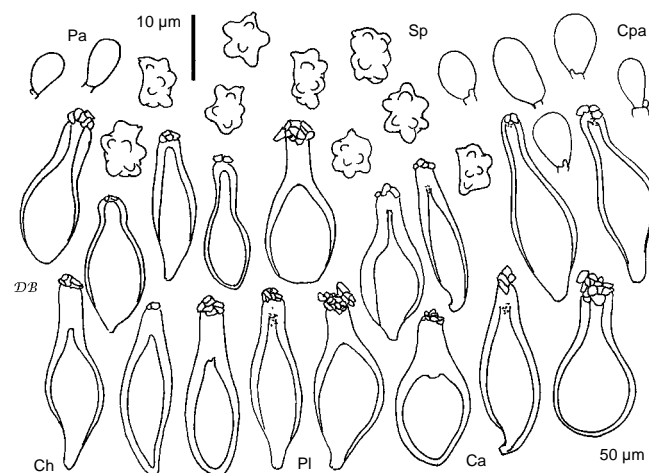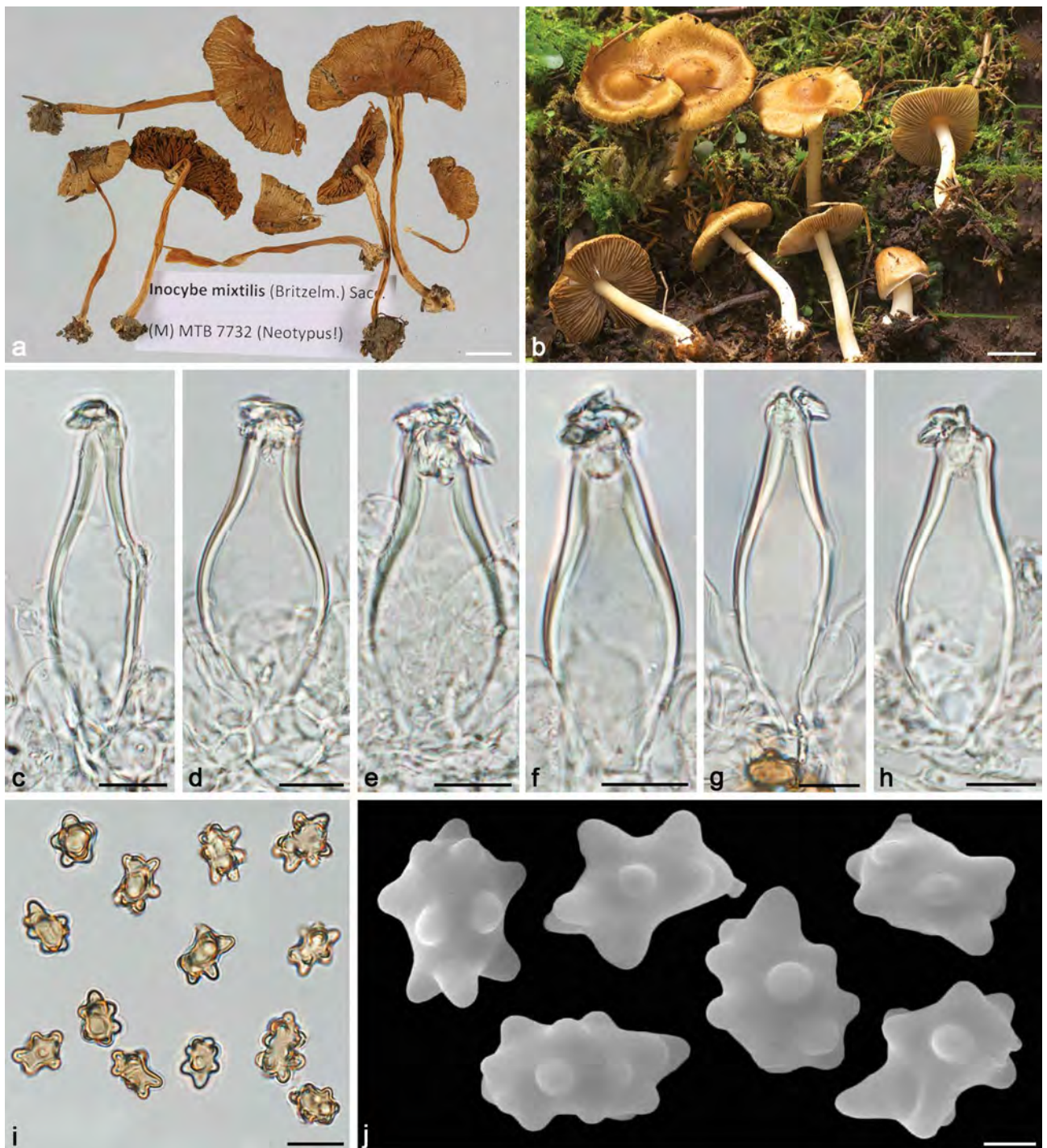

Fig. 3 *Inocybe mixtilis* KR-M-0043296: microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.

Fig. 4 *Inocybe mixtilis*. a. M-0219661 (epitype designated by Marchetti et al. 2014); b. KR-M-0043296, basidiomata *in situ*; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from KR-M-0043296). — Scale bars: a–b = 1 cm; c–i = 10 μm; j = 2 μm.

base rounded, not pedicellate or with short rounded pedicel; content hyaline or rarely with orange-yellowish, or yellow greenish amorphous matter; mostly crystalliferous at apex; walls (1–)1.5 μm thick (at the bulge), –3.5(–5) μm thick (at the apex), sometimes coalescing along the neck, yellowish in ammonia. *Lamellar edge* homogeneous and sterile, composed of abundant cheilocystidia and numerous clavate to subovoid paracystidia. *Cheilocystidia* abundant, similar in shape and size to pleurocystidia (35–)38–*50*–65(–67) × (12–)13–*17*–21(–26) μm [n = 40], with similar thick walls and crystalliferous apex. *Hymenophoral trama* regular to subregular, consisting of parallel to interwoven hyphae, 4–15(–20) μm wide, cylindrical to subfusiform in shape, often constricted at the septa, hyaline. *Subhymenium* poorly developed, consisting of 2–3 layers of small, subisodiametric, irregular cells, 4–6 μm diam. *Pileipel-*

lis constituted by an ixocutis, made up of parallel hyphae, 3–5 μm wide, with finely encrusting and parietal golden to orange yellowish pigment; subcutis with wider and paler elements, up to 20 μm broad; epicutis in young fruitbodies sometimes covered by thin hyaline hyphae, with scattered free ends (belonging to velipellis remains). *Stipitipellis* consisting of a cutis bearing numerous bundles of caulocystidia similar to hymenial cystidia, sometimes with tapering neck, (35–)42–*49*–64(–65) × (13–)14–*19*–27(–33) μm [n = 40], mostly sublageniform, subfusiform or subutriform, rarely narrowly clavate or nearly cylindrical, often crystalliferous at apex, intermixed with extremely abundant globose, ovoid to subclavate paracystidia; both elements forming the dense and conspicuous pruina that extends to the bulbous stipe base. *Clamp-connections* present in all tissues.

Habitat & Distribution — *Inocybe mixtilis* has a broad ecological range, but shows a marked preference for acid soils, sometimes with a sandy or gravelly texture, being also rather frequent in boggy or peaty soils in forests, where the epitype was encountered. It can be often found in nitrified locations, such as parks, roadsides, cemeteries or gardens in urban areas and also conifer reforestations (e.g., in northern France under *Pseudotsuga menziesii*, GenBank HQ586870, leg. & det. P.A. Moreau). Most collections are associated with pure stands of coniferous trees, especially in *Picea abies* forests, but also with *Pinus* (*P. mugo*, *P. pinea*, *P. sylvestris*). One collection from Germany grew under *Quercus robur*, and several included in the UNITE database (Kõljalg et al. 2013) are associated with broadleaf trees. All information available suggests that *I. mixtilis* shows a broad host range.

Although certainly mistaken for *I. occulta* and *I. ceskae*, *I. mixtilis* is widespread in Europe, but much less common in southern Europe. For instance, after extensive sampling in the Iberian Peninsula, only a specimen could be collected in an area of Eurosiberian affinity. Interestingly, the southernmost collection of *I. mixtilis* was made in La Palma (Canary Islands, Spain) under *Pinus canariensis* in laurisilva forest (Macaronesian biogeographical Region). Unlike *I. occulta*, *I. mixtilis* is unknown above the timberline in alpine areas, although its presence there is probable.

Up to date, *I. mixtilis* is unknown from North America. Current information indicates that deposited sequences from North American records of *I. mixtilis* correspond to *I. ceskae* and *I. occulta*, in spite that the name *I. mixtilis* appears commonly cited in the United States (Smith 1938, Grund & Stuntz 1980, 1983, Cripps 1997, Matheny 2005, Cripps & Horak 2008). Not recorded by Kobayashi (2002) from Asia, a sequence obtained from an ectomycorrhizal root, nearly identical to our sequences, suggests that *I. mixtilis* is present in Iran (UNITE UDB005301).

Additional specimens examined. Austria, Oberösterreich, Bezirk Braunau am Inn, Kobernausser Wald, near Schalchen, alt. 525 m, in acid boggy soil with *Sphagnum* in mixed forest with *Picea abies*, *Abies alba* and *Larix decidua*, 17 Aug. 2014, *D. Bandini, J. Christan & L. Quecke* (KR-M-0043301); Salzburg, Bezirk Tamsweg, Tamsweg, near Prebersee, alt. c. 1500 m, in boggy acid soil with *Picea abies* and *Alnus incana*, 14 Aug. 2013, *H. Prelicz & B. Haberl* (KR-M-0043307). – Finland, Etelä-Häme, Lammi, Biological Station, in park near *Quercus robur*, *Tilia* and *Pinus sylvestris*, 13 Aug. 2008, *J. Vauras* (TUR-A 182874; duplicate KR-M-0046560); Somero, Häntälä, W of the road to Kerkola, NW of Haapasaari farm house, former field planted with *Pinus sylvestris*, with scattered young trees of *Picea abies*, *Betula*, *Salix*, partly mossy and grassy, with abundant litter of *Pinus*, 11 Oct. 1995, *J. Vauras* (TUR-A 145111; duplicate KR-M-0046559); Varsinais-Suomi, Salo, Helisnummi, cemetery, S part, on lawn near *Picea abies*, 16 Sept. 2014, *J. Vauras* (TUR-A 199995; duplicate KR-M-0046561). – Germany, Baden-Württemberg, Alb-Donau-Kreis, Illerrieden, TK 7726, auf Wiese an Waldrand (grassy soil in forest), 10 Oct. 1978, *M. Enderle*, det. J. Stangl (as *I.* cf. *tabacina*) (M-0022251); Landkreis Calw, Neuweiler, Agenbach, TK 7217/4, alt. c. 950 m, under *Picea abies*, 19 July 2012, *D. Bandini & B. Oertel* (KR-M-0043302); Kreis, Freudenstadt, Baiersbronn, Nature Reserve 'Wilder See - Hornisgrinde', TK 7415/1, alt. 1031 m, acid soil of mixed forest with *Pinus mugo*, *Salix caprea* and *Betula pendula*, 12 Sept. 2014, *D. Bandini* (KR-M-0043300); Heidelberg, Königstuhl, TK 6618/1, alt. c. 460 m, acid soil with *Picea abies*, 25 Oct. 2014, *D. Bandini*, (KR-M-0043303); ibidem, alt. c. 460 m, in acid soil with *Picea abies*, 25 Sept. 2014, *D. Bandini* (KR-M-0043325); ibidem, at about 5 km distance from the former location, alt. c. 500 m, with *Picea abies*, 25 Oct. 2014, *D. Bandini* (KR-M-0043310); Rhein-Neckar-Kreis, Schönbrunn, TK 6519/4, alt. c. 360 m, boggy acid soil with *Picea abies*, 2 Nov. 2014, *D. Bandini* (KR-M-0043309); Rhein-Neckar-Kreis, Sandhausen, TK 6617/4, alt. c. 110 m, sandy soil with *Pinus sylvestris*, 21 Oct. 2012, *D. Bandini & B. Oertel* (KR-M-0043299); Bayern, Kreis Rottal-Inn, Julbach, TK 7743/2, alt. c. 360 m, with *Picea abies*, 15 Aug. 2014, leg. A. Hussong, KR-M-0043308; Schwarzwald-Baar-Kreis, Schonach im Schwarzwald, Rensberg, TK 7815/1, alt. c. 950 m, with *Picea abies*, 20 July 2012, *D. Bandini & B. Oertel* (KR-M-0043311); Hessen, Landkreis Bergstraße, Bürstadt, Waldfriedhof Lampertheim, TK 6317/3, alt. 95 m, in acid sandy soil under *Pinus sylvestris* and *Quercus rubra*, 26 Oct. 2014,

D. Bandini (KR-M-0043326); Rheinland-Pfalz, Landkreis Bad-Kreuznach, Bad Sobernheim, Meddersheim, Dornberg, TK 6211/2, alt. c. 400 m, acid ground with *Quercus robur*, 2 Nov. 2013, *D. Bandini & B. Oertel* (KR-M-0043298); Rhein-Pfalz-Kreis, Böhl-Iggelheim, TK 6615/1, alt. c. 110 m, with *Quercus robur*, *Pinus sylvestris*, *Corylus avellana* and *Alnus glutinosa*, 27 Sept. 2014, *D. Bandini* (KR-M-0043304); Landkreis Trier-Saarburg, Kell am See, TK 6306/4, alt. c. 360 m, boggy acid soil with *Sphagnum* and *Picea abies*, 2 Oct. 2011, *D. Bandini* (KR-M-0043306); ibidem, at about 5 km from the former location, alt. c. 400 m, with *Picea abies*, 6 Oct. 2011, *D. Bandini & B. Oertel* (KR-M-0043305); Sachsen-Anhalt, Landkreis Harz, Nature Reserve 'Kramershai', TK 4229/4, alt. c. 600 m, boggy acid soil with *Sphagnum* and *Picea abies*, 1 Oct. 2012, *D. Bandini & B. Oertel* (KR-M-0043295); ibidem, alt. 680 m, boggy acid soil under *Picea abies*, 2 Oct. 2012, *D. Bandini & B. Oertel* (KR-M-0043296); Schleswig-Holstein, Lübeck, TK 2130, Friedhof bei Weide, Erle (cemetery with willows and alders), 26 Sept. 1981, *H. Glowinski*, det. J. Stangl (as *I. tabacina*) (M-0022249). – Italy, Piemonte, Novara, Fara Novarese, alt. c. 210 m, in clay soil under *Pinus strobus*, 9 Nov. 2000, *E. Ferrari* (EF45/2000, paratypus of *I. viscosissima* var. *bulbosovelata*, Ferrari 2006). – Spain, Basque country, Gipuzkoa, Amezketa, Ondarreko langa, alt. 595 m, under planted *Picea abies* and *Fagus sylvatica* on calcareous ground, 20 Oct. 2016, *I. Olariaga* (ARAN-Fungi 04711); Islas Canarias, La Palma, Breña Baja, Parque Natural de Cumbre Nueva, Montaña de la Venta, alt. c. 1450 m, in *Pinus canariensis* forest, 15 Dec. 2013, *J. Christan* (KR-M-0043297). – Switzerland, Graubünden, Ramosch, alt. c. 1300 m, in *Picea abies* forest, 14 Sept. 1995, *F. Esteve-Raventós, E. Horak & G. Moreno* (AH 40432).

Notes — *Inocybe mixtilis* is undoubtedly one of the most cited nodulose-spored species in the genus and has always been considered as a common well known representative of section *Marginatae* Kühner (1933). Sequence data from collections deposited as *I. mixtilis* in GenBank and UNITE are very numerous and reflect this fact, as well as the extensive iconography and descriptions available in literature (see Materials and Methods).

Stangl (1984) neotypified *I. mixtilis* using a collection from the Bavarian location where Britzelmayr first collected and described it, but this choice is not correct as Britzelmayr's original plate (sub *A. rimosus*) was available for lectotypification, as Marchetti et al. (2014) proposed. These authors chose as epitype the 'neotype' selected by Stangl (M-0219661). ITS sequence data obtained from the epitype have been fundamental to start to unravel this taxon complex and to prove the existence of some other closely related species like *I. occulta* and *I. ceskae*.

The description of *I. mixtilis* given here is based on extensive material and complements Stangl's (1984). The holotype and further material show several distinct diagnostic characters:

1. the rather viscid pileus with a greasy or lardy appearance (mostly in wet conditions);
2. spores with protruding knobs arranged often in a 'margaritiform' appearance (hence isodiametric to subisodiametric); and
3. cystidial length of about 50 µm in average, their shape being normally ventricose and tapering upwards into a very often clearly differentiated neck (lageniform to sublageniform).

However, non-typical collections of *I. mixtilis* are difficult to morphologically distinguish from *I. ceskae* and *I. occulta*, which have more heterodiametric spores. All three species can be found in boreal taiga forests of the Northern Hemisphere.

Two examined collections determined by J. Stangl as *I. tabacina* from (M) had an identical ITS region compared to the epitype of *I. mixtilis*. This fact provides reliable information regarding the cromatic variation of the pileus in *I. mixtilis* (see description) and its final colour in dry specimens (exsiccata). Specimens of *I. mixtilis* with a more brownish orange pileus are mistaken for *I. tabacina*, especially in dry conditions. However, *I. tabacina* shows a tobacco brown to buff brown pileus, without any orange or yellow colour (in fresh condition), and microscopically both species show very different cystidia, being generally slender and fusiform in *I. tabacina* and ventricose to utriform in *I. mixtilis*.

A sequence obtained from a typical specimen of *I. tabacina* fell outside the *I. mixtilis* complex in our ITS analyses (Fig. 2), and also in previous published analyses (Fig. 1; clade B in Esteve-Raventós et al. (2016).

Our analyses (Fig. 1, 2) also confirm that the paratypes of *I. viscosissima* var. *bulbosovelata* (Ferrari 2006) belong to *I. mixtilis*, but a synonymy is not proposed here since the holotype was not studied (see Specimens examined).

Inocybe ceskae Bandini, Esteve-Rav. & B. Oertel, *sp. nov.* — MycoBank MB819790, ITS barcode GenBank KX290789; Fig. 5, 6

Etymology. Named after Oluna Ceska who has made numerous mushroom collections and contributed to the knowledge of fungal diversity in Canada (see notes).

Holotypus. FINLAND, Koillismaa, Kuusamo, Oulanka National Park, W of the Research Station, N of the river Oulanganjoki, Liikasenvaarantie 135, next to gravelled parking place and houses, alt. 166 m, ETRS89 N66°22'10.2" E29°18'37.2", on sandy and mossy soil, near *Pinus sylvestris*, *Betula* sp. and *Picea abies*, 21 Aug. 2015, *D. Bandini*, *J. Vauras & B. Oertel* (TUR-A 203414). Isotypes in KR-M-0046013 and AH 46568.

Diagnosis: *Inocybe ceskae* is characterised by a yellowish beige, straw-coloured to pale ochre, smooth and sticky pileus, a thin whitish evanescent velipellis in young basidiomata, shortly heterodiametrical nodulose spores with rather protruding knobs, and short cystidia (average ≤ 50 μm long). *Inocybe ceskae* has been found in one locality in boreal forest in Northern Europe (*Pinus*, *Betula*, *Picea*) and also occurs in humid and subtemperate coastal regions of the Pacific North (North America).

Basidiomes usually gregarious, rarely solitary. *Pileus* 15–30 mm diam; shape campanulate or obtusely conical to hemi-
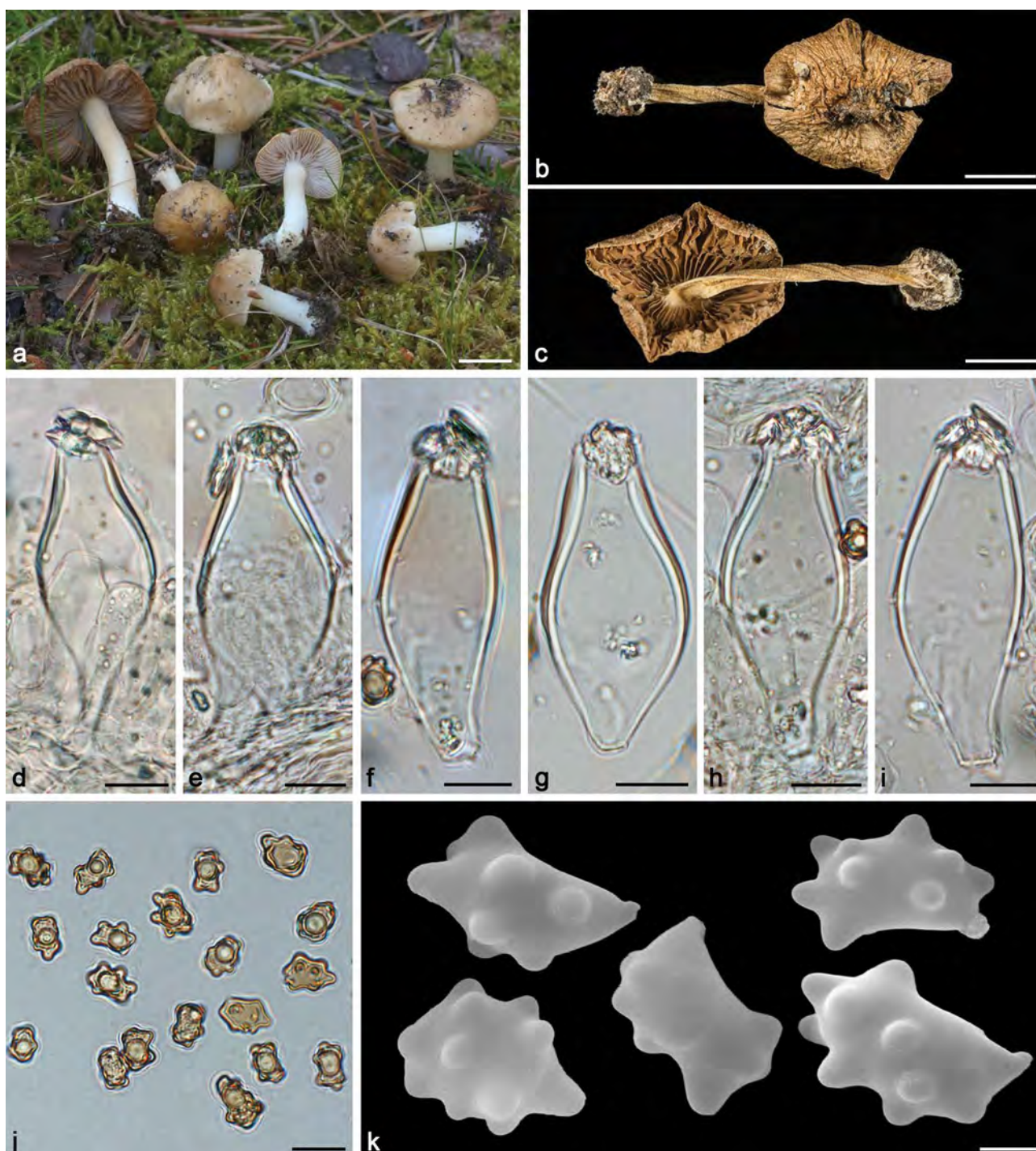
Fig. 5 *Inocybe ceskae* TUR-A 203414 (holotype). a–c. Basidiomata *in situ* and in dry condition; d–i. hymenial cystidia; j. spores under optical microscope; k. spores under SEM. — Scale bars: a–c = 1 cm; d–j = 10 μm; k = 2 μm.

spherical, then convex applanate when expanded, sometimes undulate when young, often showing a low and broad umbo, sometimes not or hardly umbonate; margin straight or deflexed, somewhat undulate in expanded specimens, not striate, not distinctly hygrophanous; colour beige-ochre, straw-yellow to pale ochre (Mu 10YR 8/2–8/6, 2.5Y 8/2–8/4), sometimes more intense in colour or with faint orange hue towards the centre, paler and whitish at the extreme margin because of the velipellis; surface radially appressed-fibrillose, smooth and glabrous when young, sometimes becoming finely felty fibrillose when old, lubricous and sticky in wet conditions, adhering soil and humus particles; velipellis present, distinct in young fruitbodies, as a very thin whitish coating especially at margin but also over the entire pileus, soon disappearing and nearly absent in washed or aged specimens. *Lamellae* ventricose to subventricose, 2–5 mm deep, nearly free to adnate or adnexed with decurrent tooth, moderately crowded to rather crowded, L = 40–60, l = 1–3; colour initially whitish, beige or greyish, showing a rather distinctive pinkish or purplish hue, especially towards the lamellar edge, then becoming brownish grey or even deep brown to orange or rusty brown when old; edge crenulate, whitish when young, sometimes concolorous with age. *Stipe* 20–45 × 2–5 mm, solid, fibrose; shape cylindrical to slightly tapering upwards, base most often abruptly marginately bulbous; bulb 6–9 mm broad, agglutinating abundant soil and humus material and sometimes hidden and difficult to perceive; colour whitish when young, then becoming yellowish with age (Mu 2.5Y 8/1–8/4) but not especially browning or darkening; surface evenly and densely pruinose all over, also discernible under the lens in the lower third of stipe and near the bulb. *Cortina* not seen, even in young primordia. *Context* fibrose, firm, whitish in pileus, stipe and bulb, concolorous to the surface in the stipe cortex; smell more or less spermatic when cut, otherwise sometimes indistinctive. *Colour of exsiccata*: pileus shiny brownish to brownish orange at the centre (10YR 7/4–7/6), pale brown, beige or straw-coloured towards the margin; stipe similar to pileus (10YR 8/4–8/6), not showing evident darkening (deep brown to blackish) upon drying; lamellae brown, deep brown to rusty brown.

Basidiospores (6.7–)7–11.1(–11.5) × (5–)5.2–7.6(–7.7) µm, Q (1.2–)1.3–1.6(–1.7), L_m = 8.6–8.9, W_m = 6.2–6.4, Q_m = 1.4 [n = 100, from 2 collections], heterodiametrical to subheterodiametrical, seldom subisodiametrical, often with a curved adaxial face in profile, presenting (8–)9–12(–14) distinct and rounded to conical-obtuse knobs, these often irregularly protruding, 0.7–1.4 µm high, 1.1–1.6(–2) µm broad at base; apicula distinct, curved; without germ pore and sometimes with a large central guttula. *Basidia* 18–30 × 7.5–11.5 µm,
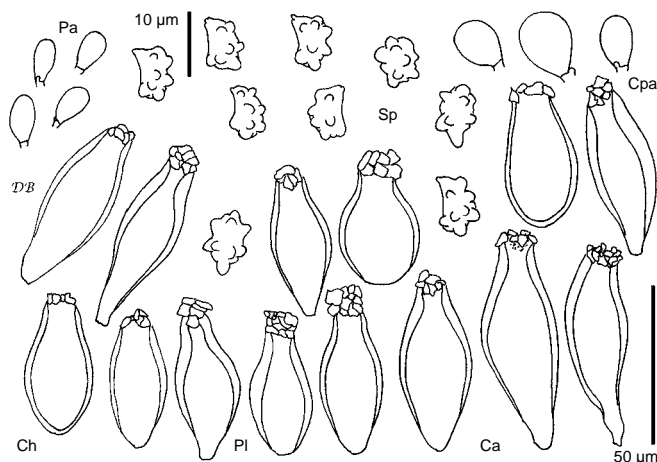
clavate, sometimes with small guttulae, (2–)4-spored, with sterigmata 2.5–5(–7) µm long. *Pleurocystidia* abundant, metuloid, (38–)40–48–60(–65) × (16.5–)18–22.7–30.5(–31) µm, Q = 2.1–2.3–3.3 [n = 40]; shape mostly rather ventricose and broadly subutriform with wide neck, but also broadly fusiform, generally not pedicellate or tapering into a short rounded base, rarely with a short pedicel; rather crystalliferous at apex; walls (1–)1.5–2 µm, often thickened towards the neck or apex, 2–4.5 µm thick, but not coalescing at the neck, pale yellowish in ammonia; content hyaline, occasionally pale yellowish. *Lamellar edge* nearly homogeneous and sterile, composed of numerous cheilocystidia and often rather small subclavate or subovoid paracystidia. *Cheilocystidia* abundant, similar in shape and size to pleurocystidia, (36–)37–46–54(–55) × (16–)18–22–25(–29) µm [n = 40], with similar thick walls, rather crystalliferous at apex and sometimes showing a pale yellowish content. *Hymenophoral trama* subregular, constituted by more or less interwoven hyphae, 4–26 µm wide, subcylindrical, subfusiform to almost ovoid in shape, mostly constricted at septa, hyaline. *Subhymenium* poorly developed, consisting of 2–3 layers of small subisodiametric or irregular cells, 4–6.5 µm diam, hyaline. *Pileipellis* consisting of an epicutis, composed of slightly gelatinized parallel hyphae, 4–12 µm wide, with incrusting and intracellular yellowish pigment; hyphae somewhat paler to almost hyaline in deeper layers (subcutis), up to 25 µm wide; epicutis sometimes covered by thin hyaline hyphae with scattered free ends, from remnants of velipellis. *Stipitipellis* consisting of a cutis of subcylindrical hyphae, constricted at septa, bearing numerous bundles of caulocystidia similar to hymenial cystidia, (29.5–)27.5–47.2–63.5(–67.5) × (17.5–)18.5–21.9–26.5 µm [n = 40], crystalliferous at apex, intermixed with abundant hyaline subclavate paracystidia, similar to those of lamellar edge; these elements constitute the dense and abundant pruina reaching the bulb. *Clamp connections* present in all tissues.

Habitat & Distribution — The holotype collection was found in Finland on sandy soil, next to a gravelled parking place in an anthropogenic area, but it is unknown to us whether the soil had calcareous profile. Several Canadian collections (British Columbia) were also gathered in human-influenced locations (see Notes), while our two European collections (holotype and paratype) were made in taiga forest, suggesting that *I. ceskae* is associated with conifers (*Pinus* or *Picea*) with the presence of *Betula* species. In the Pacific Coast of Canada (British Columbia) and north-western USA (Oregon), *I. ceskae* is associated with *Pseudotsuga menziesii* (Douglas-fir) and probably also with *Tsuga heterophylla* (western hemlock). *Inocybe ceskae* occurs further south along the Western Cordillera, and reaches Sierra Nevada Foothills (Northern California), where it is probably associated with *Arbutus menziesii* and *Quercus*. (Smith et al. 2002, Morris et al. 2008). Kennedy et al. (2012) have assessed the potential for the existence of common mycelial networks between *A. menziesii* and *P. menziesii*; *Arbutus menziesii* hosts a highly diverse mycorrhizal fungal community with similar composition to those found on other angiosperm and *Pinaceae* hosts (including *P. menziesii*). This fact could explain why *I. ceskae* has been found forming ectomycorrhiza with *A. menziesii* and some oaks in more southern and subtemperate areas of Northern California (Morris et al. 2008).

Due to its transoceanic distribution, it is very probable that *I. ceskae* extends throughout boreal to subboreal taiga forests in both continents and also Siberia.

Fig. 6 *Inocybe ceskae* TUR-A 203414 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.

Notes — Our description is based on the holotype and the paratype, both gathered in the same spot in consecutive years. These materials are conspecific with eleven collections at UBC and all form a strongly supported monophyletic clade (Fig. 1). The UBC materials were mostly determined as *I. mixtilis* from Canada (see Table 1) by Oluna Ceska, after whom we name this species. Photographs, drawings and notes of these Canadian vouchers are available on the internet (www.biodiversity.ubc.ca/museum/herbarium/). All of them were gathered in different localities in British Columbia, alt. 30−240 m, in the so-called Marine West Coast Forests, characterized by a temperate and moist climate. In all UBC material in which the ecology is noted the ectomycorrhizal host is *Pseudotsuga menziesii*, except for two specimens collected under *Tsuga heterophylla* (F19812) and *Arbutus menziesii* (F19202), respectively.

Seven of these collections at UBC were identified as *I. mixtilis* and actually belong to *I. ceskae*. The other four were originally determined as *I. glabrodisca*, *I. praetervisa*, *I. sindonia* and *I. umbratica*. These identifications suggest that the pileus colour can be pale and interestingly, the fruitbody named *I. glabrodisca* (F19553) is, as explicitly told, a very aged one, and apparently brownish.

Unfortunately, the examination of these UBC materials was not authorized while undertaking this study and this has refrained us from designating a North American collection as the holotype.

According to Oluna Ceska's drawings and annotations, there must be a certain range of variation in macro- and micromorphological features, but the resemblance with the holotype is obvious:

1. the range of pileus size in the Canadian vouchers is 25 (F23607) to 55 mm diam (F18984);
2. most exsiccata show a light yellowish to pale straw-coloured pileus, mostly broadly umbonate, and the surface is often shiny and glabrous, with adhered soil particles suggesting that they were lubricous or viscid;
3. fresh pileus colour indicated by Oluna Ceska ranges from ochre (F18954) to yellow-ochre or yellow-brown, or darker yellowish brown or even very pale (F18984). The centre of the pileus in one collection is described as 'with an ochre-orangy patch' (F18974);
4. an obtusely umbonate pileus is present in photos and sketches of most fruitbodies;
5. lamellar colour is whitish brown to greyish brown turning to dark olive (F18974);
6. the colour of the stipe is white, 'white silky' or whitish with a yellow tinge (F18984);
7. the stipe is always depicted pruinose over the entire length, and a marginate bulbous base is distinct in F19221 (photograph of fresh specimens) and also in Ceska's sketches (e.g., F18984); and
8. the stipe length is variable among these collections, up to 80 mm long (F18954) and −8 mm wide, with a bulb up to 11 mm (F18984). On the contrary, basidiomata in F23607 are very slender, with pileus up to 30 mm diam, with a prominent umbo and stipe up to 40 × 2.5−4 mm.

Unfortunately, microscopic features are roughly drawn. Spores show distinct, often prominent knobs, with measurements 8–10(−11) × 6–7 µm (F18954), 8–9(−10) × 6–7 µm (F18974), 7–9.5(−10) × 6.5–7(−8) µm (F18984) and 8–11 × 6–7 µm (F19553). Cystidia are ventricose with an attenuated obtuse neck and thick walls (−3 µm), but their base is never depicted. Measurements are only indicated for three collections: 45−55 × 12−13 µm (F18954), 45−50 × (10−)13−20 µm (F19553) and 45−50 × 15−22 µm (F19553), which reflects the small size and variable width of cystidia.

Although somewhat paler in colour, the habit of *I. ceskae* is similar to *I. mixtilis* and *I. occulta*. *Inocybe mixtilis* is shown to be sister to *I. ceskae* in our ITS-RPB2 phylogeny (Fig. 1), but it differs from the latter in showing a more intensely yellow to orange pileus, lubricous or sticky in wet conditions, and, microscopically, a large proportion of the spores have protruding obtuse knobs that give the spores a 'pseudoasteriform' or 'margaritiform' shape. Some collections of *I. ceskae* and *I. occulta* may share a thin whitish velipellis, which is mostly ephemeral and observed in young specimens not washed by rain, especially at pileus margin. Nevertheless, both species are sometimes difficult to recognize morphologically, but *I. ceskae* shows paler colours and shorter cystidia in average. *Inocybe ceskae* and *I. occulta* can occur in similar habitats, being *I. occulta* more widespread geographically, also extending to warmer and more temperate climates in Europe and North America. *Inocybe johannis-stanglii* and *I. nothomixtilis* show distinct heterodiametrical spores with a lower ornamentation. Furthermore, *I. johannis-stanglii* has a greyish velipellis and larger spores and cystidia, while *I. nothomixtilis* prefers instead southern, subtemperate to warm ecosystems where it is associated mainly with *Fagaceae* and *Cistaceae*.

Based on material from Kamchatka (Eastern Siberia, Russia), Peintner & Horak (2002) attributed to *I. mixtilis* a pale-coloured pileus, heterodiametric spores provided with distinct knobs and short cystidia, that suggest *I. ceskae* instead. This fact substantiates our hypothesis of *I. ceskae* being more widespread over circumpolar regions of the Northern Hemisphere.

Inocybe johannis-stanglii Bandini, Esteve-Rav. & G. Moreno, *sp. nov.* — MycoBank MB819791. ITS barcode GenBank: KX290791; Fig. 7, 8

Etymology. Named after Johann Stangl, for his great contribution to the knowledge of the genus *Inocybe*.

Holotypus. GERMANY, Baden-Württemberg, Heidelberg, TK 6518/3, alt. 120 m, churchyard of Johanneskirche, on lawn with *Picea omorika* and *Betula pendula*, 4 July 2012, *D. Bandini*, KR-M-0038039. Isotype AH 46580.

Misapplied names
– *Inocybe pallida* Velen. *sensu* Stangl, Hoppea 46: 308. 1989.

Selected descriptions and iconography: Stangl (1989: 308, Taf. 34/2); Ferrari et al. (2014: 106, 175)

Diagnosis: *Inocybe johannis-stanglii* is characterized by: a) the distinctly heterodiametric spores, measuring (7.6−)8.4−*10.1*−12(−13) × 6−*6.9*−7.8(−8.2) µm, Q = (1.2−)1.3−*1.5*−1.8(−1.9) µm, provided with numerous (8−16), low and rounded knobs; b) thin greyish velar patches on the pileus; c) abruptly bulbous stipe, not always distinctly marginate; d) intense lilac hues on lamellae when young; and e) cystidia often tapering into a distinct pedicel. Associated with conifers in central Europe in natural to semi-natural woodlands areas.

Basidiomes usually gregarious, rarely solitary. *Pileus* 10−30 mm diam; shape when young campanulate, subconical to hemispherical, then convex to expanded when old, sometimes somewhat undulate or irregular, sometimes with a broad low obtuse umbo; margin mostly straight, occasionally deflexed; colour when young pale ochre, then mostly dull ochre (Mu 10YR 7/6−7/8), brownish ochre or hazel brown (Mu 10YR 6/6−6/8 or 5/6−5/8), or, due to the presence of velipellis greyish brownish, entirely or only at margin and umbo; surface glabrous or finely felty to finely fibrillose with adpressed, not diverging fibres, in some collections slightly hygrophanous (e.g., KR-M-0043321); often lubricous in wet conditions and with adhering soil particles; velipellis distinct, especially in young fruitbodies, as a thin greyish layer covering the pileus centre or as scattered patches.

Lamellae ventricose to subventricose, 2−5 mm deep, almost free, adnexed, emarginate-adnexed or emarginate with decurrent tooth, moderately crowded to crowded, L = 40−60, l = 1−2; colour initially whitish, very often with a pale vinaceous grey, greyish lavender or light lilac tinge, finally greyish brownish, sometimes with a lilac tinge; edge fimbriate, whitish to even concolorous in old basidiomata. *Stipe* 20−40 × 2−4 mm, solid, fibrose; shape cylindrical or slightly tapering upwards, base bulbous or often faintly marginately bulbous; bulb up to 8 mm broad, mostly rounded marginate, in some collections hardly or not marginate and apparently clavate bulbous (e.g., in KR-M-0043320); colour whitish when young, then also wax-coloured or very pale brown at most (Mu 10YR 8/3−8/4), never showing a strong darkening when bruised or with age; surface evenly and densely pruinose all over, reaching the bulbous base.

Colour of exsiccata: pileus rather uniform light brown, greyish brown (Mu 10YR 5/4−5/6, 10YR 4/4), stipe beige to straw yellow (10YR 7/4−7/6) or yellowish brown (Mu 10YR 6/4), never showing any distinct darkening upon drying. *Cortina* not seen, even in young specimens. *Context* fibrose, firm, whitish in the pileus and also in the stipe when young, then with a brownish tinge, especially in the upper half and the cortex of the stipe; smell indistinct, weakly spermatic when cut.

Basidiospores (7.6−)8.4−12(−13) × 6−7.8(−8.2) µm, Q = (1.2−)1.3−1.8(−1.9), L_m = 8.3−9.1, W_m = 6.6−7.1, Q_m = 1.4−1.5 [n = 100, from 4 collections], heterodiametric, with nodulose profile, presenting (8−)10−16 low, rounded to broadly conical knobs, 0.4−1.2(−1.4) µm high, 1−2(−2.2) µm broad at base; yellow in ammonia, without germ pore and sometimes with a large central guttule. *Basidia* 22.5−32 × 8.5−12 µm, clavate,
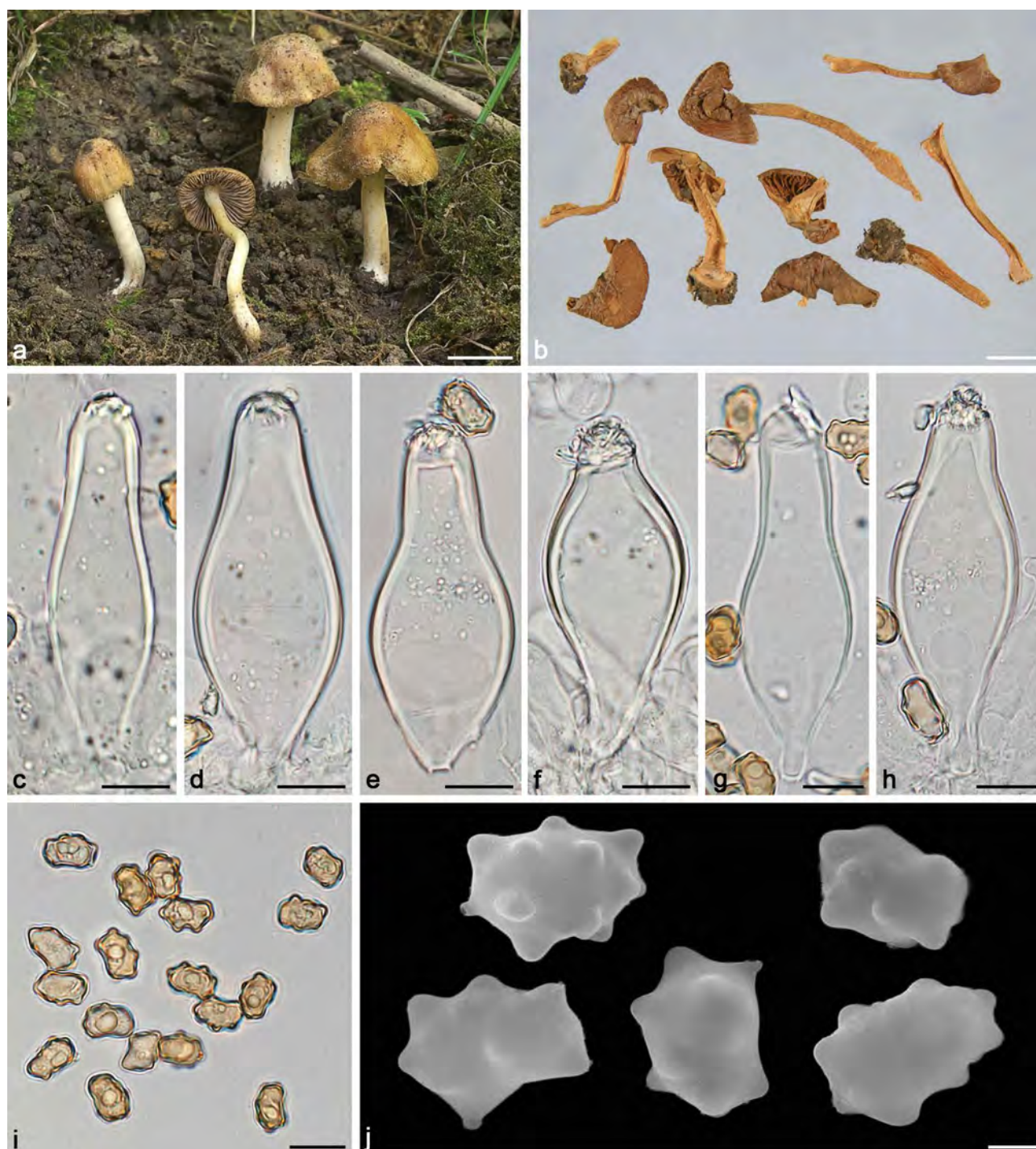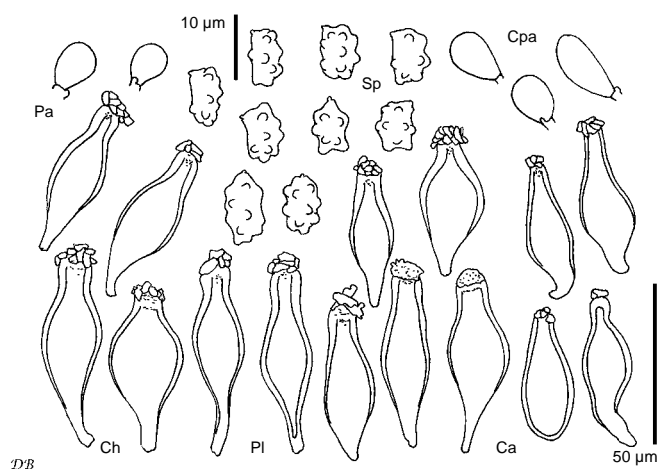

Fig. 7   *Inocybe johannis-stanglii*. a. KR-M-0038039 (holotype) *in situ*; b. *Inocybe pallida* sensu Stangl M-0021484; c−h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from KR-M-0038039, holotype). — Scale bars: a−b = 1 cm; c−i = 10 µm; j = 2 µm.

4-spored, in some collections mixed with 2-spored, with sterigmata 2.5–6 µm long. *Pleurocystidia* abundant, metuloid, (35–)45–*55*–63(–65) × (11–)13–*17.5*–21(–24) µm, Q = 1.9–*3*–4.4 [n = 40], subutriform, sublageniform, also subfusiform, occasionally subcylindrical or subclavate, often rather ventricose, mostly with rather short obtuse neck, base often tapering into a more or less distinct, sometimes curved pedicel; apex usually rather crystalliferous; walls 1–2.5 µm, often thickened towards the neck, up to 3.5 µm thick, yellowish in ammonia; content generally hyaline, but sometimes showing a pale yellow to glaucous amorphous content in ammonia solution. *Lamellar edge* almost homogenous and sterile, consisting of numerous hyaline cheilocystidia intermixed with subclavate paracystidia. *Cheilocystidia* abundant, similar in shape and size to pleurocystidia, (31–)33–*53*–63(–70) × (11.5–)12–*16*–21(–25) µm [n = 40], with similar walls and often crystalliferous. *Hymenophoral trama* regular to subregular, consisting of parallel-arranged to slightly interwoven hyphae, 4–26(–30) µm wide, subcylindrical to subfusiform in shape, mostly more or less constricted at septa, hyaline. *Subhymenium* poorly developed, consisting of 1–2 layers of small subisodiametric cells, 3.5–6.5 µm diam. *Pileipellis* consisting of an epicutis, often slightly gelified, constituted by parallel-arranged hyphae 5–12 µm wide, with encrusting and intracellular brownish orange to yellowish pigment, somewhat wider and paler and almost whitish in the subcutis, up to 20(–25) µm wide, mostly covered by thin hyaline hyphae, with scattered free ends (velipellis remains). *Stipitipellis* consisting of a cutis of subcylindrical hyphae, slightly constricted at septa, bearing numerous bundles of caulocystidia, (37–)39–*46*–55(–64) × (12–)13–*15.5*–20(–21) µm [n = 40], crystalliferous at apex, intermixed with numerous paracystidia, similar to those of the lamellar edge; both elements forming a dense and conspicuous pruina, clearly reaching the bulbous base. *Clamp connections* present in all tissues.

Habitat & Distribution — *Inocybe johannis-stanglii* is associated with conifers (*Abies*, *Picea*, *Larix*) in temperate continental forests of Europe, and is also found in urban and man-influenced areas like gardens, parks, churchyards and roadsides, where it thrives near planted or introduced firs or spruces like *Picea omorika* (= *Abies omorika*, Serbian spruce), which grows spontaneously in mountainous areas of Serbia, between 800–1400 m alt., and is planted in Germany and Switzerland. *Inocybe johannis-stanglii* appears to prefer neutral to basic soils, judging from Austrian material and the holotype, but collecting further material is necessary to confirm this assumption.

Inocybe johannis-stanglii is known with certainty from Austria and Germany, but considering its habitat, it could be widespread in continental conifer forests. The collection reported from Switzerland by Breitenbach & Kränzlin (2000, as *I. pallida*) seems to correspond to *I. johannis-stanglii* as well. *Inocybe pallida* has been reported by Solak et al. (2009) from Turkey, but this find cannot be confirmed to be conspecific with *I. johannis-stanglii* or *I. subtrivialis* with available information.

Additional specimens examined. Austria, Oberösterreich, Bezirk Braunau am Inn, Kobernausser Wald, near Schalchen, alt. c. 525 m, mixed forest with *Picea abies*, *Abies alba*, *Larix decidua*, 17 Aug. 2014, *D. Bandini, J. Christan & L. Quecke* (KR-M-0043320); in about 2 km distance from the former location, alt. c. 550 m, mixed forest with *Picea abies*, *Abies alba*, *Larix decidua*, 17 Aug. 2014, *D. Bandini, J. Christan & L. Quecke* (KR-M-0043321). – Germany, Bayern, Augsburg-Bergheim, TK 7631, garden of A. Sedlmeir, with *Betula* and *Picea omorika*, 28 Oct. 1987, *J. Stangl* (M-0021484, as *I. pallida sensu* Kuyper); 9 Nov. 1987, *J. Stangl* (M-0021483, as *I. pallida sensu* Kuyper); Baden-Württemberg, Heidelberg, TK 6518/3, alt. 120 m, churchyard of Johanneskirche, on lawn with *Picea omorika* and *Betula pendula*, 18 July 2012, *D. Bandini* (KR-M-0046122); 19 July 2012, *M. Rave* (KR-M-0038082); 31 July 2014, *D. Bandini* (KR-M-0042290, duplicate in TUR-A 203094).

Notes — *Inocybe johannis-stanglii* is a well-delimited species within the Mixtilis group, as revealed by our analyses (Fig. 1, 2). Morphologically, the presence of a thin greyish velipellis and the heterodiametric spores, the largest known in this group (often longer than 10 µm), are diagnostic. Spores of *I. johannis-stanglii* recall those of *I. nothomixtilis* and *I. subtrivialis* in shape and ornamentation, with low but distinct numerous knobs. The hymenial cystidia, in average longer than 50 µm, are also very particular because of having a base tapered into a pedicel. Caulocystidia are also abundant near the bulb of the stipe, which is not distinctly marginate in some specimens.

The presence of a distinct velipellis has also been depicted by Stangl (1989, Taf. 34/2) from two collections deposited at M that were seen by T.W. Kuyper and determined as *I. pallida* by the latter. Both collections have identical ITS sequences compared to the holotype of *I. johannis-stanglii*. Stangl (1989) named both collections as *I. pallida* following Kuyper (1985), who revised Velenovský's *Inocybe* collections at PR and PRC. Velenovský (1920) originally described and depicted *I. pallida* with a narrow stipe (1–2 mm), progressively enlarged towards the base, but never really bulbous, and angulose spores in outline. The material was gathered in a xerophilous open grassland habitat, with presence of *Helianthemum*, *Thymus*, *Phleum* and *Trifolium*. This particular habitat suggests a possible relationship with the ectomycorrhiza-forming *Helianthemum* (*Cistaceae*), common in heliophilous and warm habitats in Mediterranean and continental European areas. Some years later, Velenovský (1947) gave a Latin diagnosis of *I. pallida* and added some microscopical data, like spores '12-14 µm, cuneatae, angulato-aculeatae', cystidia measuring 50–60 × 25–40 µm, and habitat 'in gramine collium, in fruticibus Ruborum non raro'. Kuyper's revision (1985: 394 and f. 33) of the holotype of *I. pallida* confirmed the angulose outline of the spores ('with indistinct nodulae'), the very wide cystidia × 20–24(–26) µm, with walls up to 1.5 µm thick and caulocystidia descending over halfway of stipe. Kuyper also suggested that *I. pallida* is related to *I. decipiens*, but differed in the 'smaller, more angular spores'.

The original concept of *I. pallida* remains difficult to interpret with certainty and sequencing of type material is necessary to infer its position among the nodulose/angulose-spored *Inocybe* species. Nevertheless, this is probably impossible to achieve, since the holotype is preserved in a liquid solution (Kuyper 1985), which is a mixture of formaldehyde, acetic acid and water (Antonín 1986, 1990), and its DNA is presumably highly degraded. Thus, an epitype for *I. pallida* should be selected, preferably using material from the type locality, in order to establish a consistent interpretation.

Fig. 8 *Inocybe johannis-stanglii* KR-M-0038039 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.

In the meantime, morphological and ecological features of the holotype of *I. pallida* strongly suggest that this taxon could be a member of the *I. decipiens*/*dunensis* complex due to the angular spores (with hardly protruding knobs, if any), large ventricose utriform cystidia without tapering base, an indistinctly bulbous stipe, caulocystidia only descending halfway of the stipe and a xerophilous habitat. All of this suggests a closer relationship with the *I. decipiens*/*dunensis* complex, as earlier suggested by Esteve-Raventós & Caballero Moreno (2009: 112). Sequences identified as *I. pallida* in GenBank show that this name has been consistently employed for species of *Inocybe* of the *I. decipiens*/*dunensis* group.

Inocybe nothomixtilis Esteve-Rav., Bandini & V. González, *sp. nov.* — MycoBank MB819792; ITS barcode GenBank KX290780; Fig. 9, 10

Etymology. From Latin *nothus*, which means false, and *mixtilis* (*Inocybe*), which represents a different but very close species.

Holotypus. SPAIN, Basque Country, Araba (Alava), Zuia, Markina, in acid, peaty soil, among *Sphagnum* spp. and other mosses near *Erica tetralix* in a *Quercus robur* stand, with some reforested pines (*Pinus radiata*) and *Sorbus aria* nearby, 29 Aug. 1996, *R. Fernández-Sasia*, RFS no. 960829-01 (AH 24510). Isotypes KR-M-0046551 and in CEME-EMI (Centro de Estudios Micológicos de Euskadi-Euskadiko Mikologia Ikastegia).

= *Inocybe tabacina* var. *pseudovolvata* Eyssart. *ad int.*, Bull. Soc. Mycol. France 122: 177. 2006 [not validly published].
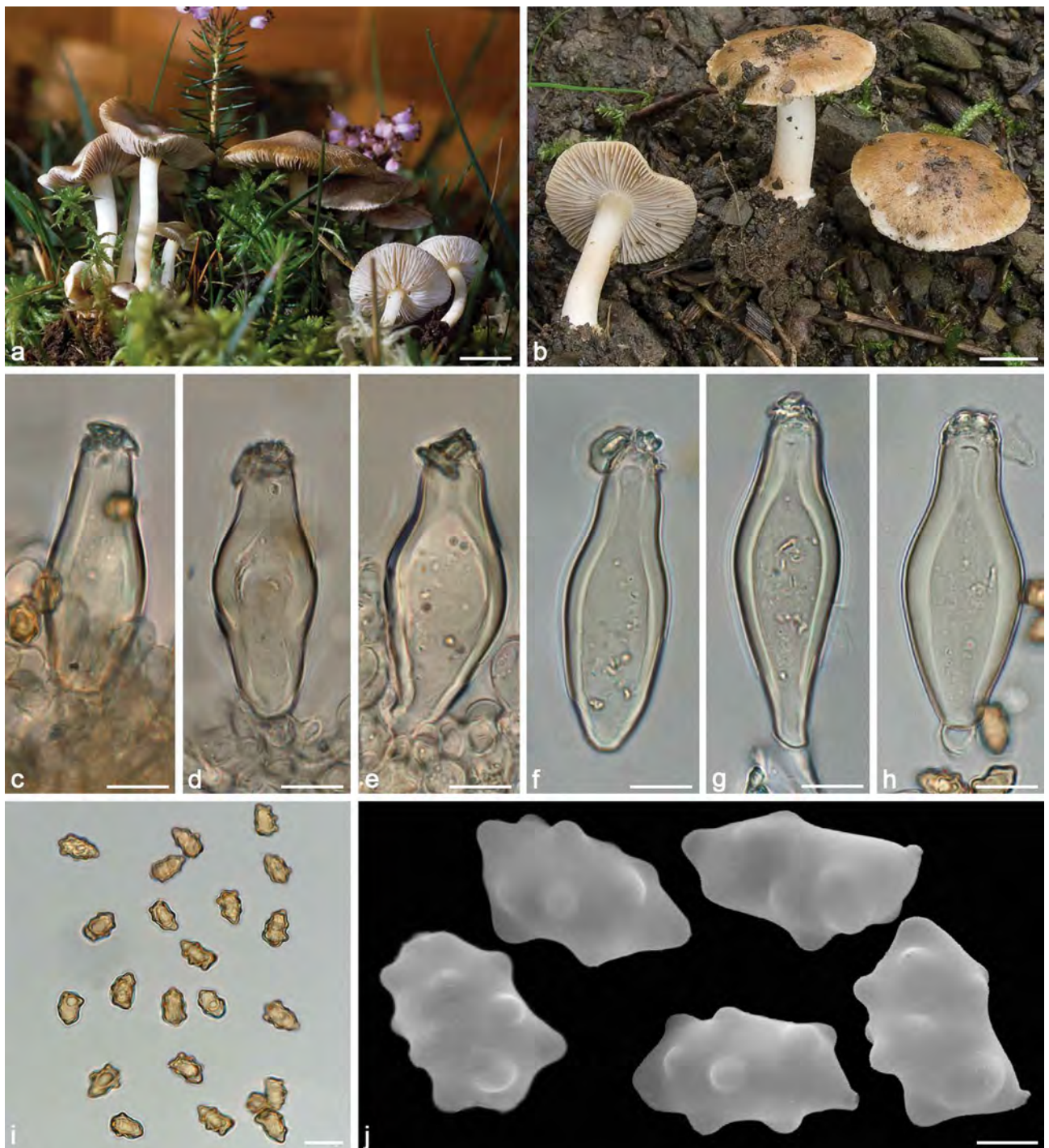
Fig. 9 *Inocybe nothomixtilis*. a. AH 24510 (holotype) *in situ*; b. KR-M-0043322 *in situ*; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from AH 24510, holotype). — Scale bars: a–b = 1 cm; c–i = 10 μm; j = 2 μm.

Diagnosis: Macroscopically *I. nothomixtilis* resembles *I. mixtilis* in habit, size and colours, but it is distinguished by: a) a developed whitish velipellis, easily observed on the pileus and the edge of the bulb in primordia or young specimens; b) the heterodiametric basidiospores 7–*8.5*–10.1(–11) × 5–*5.9*–6.8(–6.9) μm, Q = 1.2–*1.45*–1.7(–1.8) [n = 100, from 4 collections], with 10–13 low, obtuse and well-delimited knobs; c) rather short cystidia of average length ≤ 50 μm; d) pileipellis not viscid or slimy, but sticky to lubricous in wet conditions, or even dry. Distributed in temperate continental and Mediterranean regions of Europe.

Basidiomes usually gregarious, rarely solitary. *Pileus* 15–30(–35) mm diam, 5–17 mm high, when young broadly conical to hemispherical campanulate, then expanded to broadly convex, convex or more rarely applanate, often with a broad and low umbo; margin slightly inflexed when young, then more straight upon expansion, initially entire or regular, then somewhat wavy to irregular in old specimens, not striate, not or very slightly hygrophanous in old or very soaked fruitbodies; colour somewhat variable, but always showing a range of yellow colours, from golden yellow to brownish yellow or orange-yellow in young specimens (Mu 10YR 6/8; 7.5YR 7/6–7/8), often more brownish to reddish yellow or brownish with a reddish orange hue in older specimens (Mu 7.5YR 6/8), sometimes pale yellowish to ochraceous, especially towards the margin (Mu 10YR 8/6 or 7/8); surface radially fibrillose, most often felty to even woolly-fibrillose with bunches of adpressed fibres, sometimes becoming glabrous towards the centre or in some collections even practically smooth in its entire surface (e.g., in *I. tabacina* var. *pseudovolvata*), lubricous and sticky but not viscid in wet conditions, with adhered soil and humus particles; velipellis clearly visible in young fruitbodies as a thin to somewhat thick whitish coating on a yellowish background, rather persistent, sometimes lanose-fibrillose and still visible at centre and/or margin in old specimens. *Lamellae* ventricose to subventricose, 2–4(–5) mm deep, nearly free or adnexed, moderately crowded, L = 40–48(–50), I = 1–2(–3); colour initially white or whitish, soon becoming beige or pale grey, brownish grey and finally ochre-brown, in some collections showing a typical lilacinous or pinkish lilac reflection (e.g., AH 24510, AH 26925, PC 0738072), never olivaceous at maturity; edge crenulate, whitish or paler than faces, sometimes concolorous by places. *Stipe* (10–)15–50 × (2–)3–6 mm, solid, fibrose, cylindrical or slightly tapering upwards, base bulbous (AH 24510), most often abruptly and marginately bulbous, in some specimens curved or sinuous (AH 24510); bulb 7–9 mm broad, in young specimens with well-delimited edge covered by a whitish fibrillose coating

reminiscent of a 'pseudovolva'; colour initially whitish to cream (Mu 10YR 8/3), sometimes showing light yellow tinges in adult specimens (Mu 10YR 8/6), never darkening; surface evenly pruinose all over, reaching the bulb, with pruina thin and dense, sometimes pruinose-fibrillose at the lower region, close to the bulb. *Colour of exsiccata*: pileus brownish (Mu 7.5YR 5/4); stipe beige to brownish yellow (Mu 10YR 6/4), never showing any distinct darkening (either browning or blackening) upon drying. *Cortina* not seen, even in young primordia. *Context* fibrose, firm, whitish in pileus centre, white to concolorous to the surface in the stipe, white in the bulb; smell weakly to strongly spermatic, especially when cut; taste mild.

Basidiospores 7–10.1(–11) × 5–6.8(–6.9) μm, Q = 1.2–1.7(–1.8), L_m = 8.3–9.1, W_m = 6.1–6.2, Q_m = 1.4–1.5 [n = 100, from 4 collections], heterodiametric, rarely subheterodiametric, with nodulose profile, presenting low but distinctive, 10–13 rounded to conical obtuse knobs, 0.5–0.7 μm high, (0.7–)0.9–1.1 μm broad at base, mostly distributed in abaxial position, sometimes presenting an apical knob up to 1.2 μm high and hence the spore outline showing an attenuated apex; yellow in ammonia, without germ pore. *Basidia* 22–30 × 8–11(–12) μm, clavate, 4-spored, with sterigmata 2–4(–5) μm long. *Pleurocystidia* moderately abundant to scattered, metuloid, (35–)38–*46.5*–55(–60) × 10–*15.5*–22(–28) μm, Q = 2.1–*3*–4 [n = 40], variable in shape depending on collections, often ventricose and subutriform with broad and short neck, also subfusiform, occasionally subcylindrical to subovoid, base attenuate-truncate (rather frequent in the holotype AH 24510) or tapering in a more or less distinct pedicel (e.g., in *I. tabacina* var. *pseudovolvata* voucher); generally rather crystalliferous at apex (hardly or not crystalliferous in Alessio's collection of *I. mixtilis* var. *aurata*); walls 1.5–2.5 μm, more or less uniformly thickened, in some collections (e.g., in the holotype) up to 3–4 μm at the neck and the extreme apex, and then sometimes walls coalescing (like a 'plug'), hyaline to very pale yellow in ammonia; content hyaline, never showing dark (brown to dark brown) pigments, but sometimes filled with a hyaline to pale yellowish amorphous content. *Lamellae edge* homogeneous and sterile, composed of numerous to scattered cheilocystidia and very abundant clavate to ovoid paracystidia, mixed with intermediate elements in shape, hyaline, occasionally pale yellow mostly in aged specimens. *Cheilocystidia* similar in shape and size to pleurocystidia, (26–)30–*44*–57(–60) × (10–)12–*16*–21(–24) μm [n = 40], with similar thick walls and crystalliferous apex. *Hymenophoral trama* regular to subregular, constituted of more or less parallel hyphae, 4–20(–25) μm wide, cylindrical to fusiform in shape, often constricted at the septa, hyaline or with pale yellow intracellular pigment. *Subhymenium* poorly developed, consisting of 1(–2) layers of small, subisodiametric cells, 4–6 μm diam. *Pileipellis* consisting of an epicutis, somewhat gelatinized (but not a distinct ixocutis), constituted by parallel hyphae up to 4 μm wide, with finely encrusting and intracellular parietal yellowish pigment; hyphae somewhat wider and paler in the subcutis, up to 20(–25) μm wide; the epicutis is sometimes covered by thin hyaline hyphae, with scattered free ends (velipellis remains). *Stipitipellis* consisting of a cutis bearing numerous bundles of caulocystidia similar to hymenial cystidia, (26–)30–*41.5*–53(–60) × (12–)13–*15.5*–19(–20) μm [n = 40], crystalliferous at apex, and intermixed with numerous paracystidia, similar to those of the lamellar edge (both elements forming a dense and conspicuous pruina), and clearly reaching the bulbous stipe base. *Clamp connections* present in all tissues.

Habitat & Distribution — In humus of warm or temperate natural forests of *Fagaceae* (*Fagus sylvatica*, *Quercus* spp.) and *Cistaceae* stands (i.e., *Cistus monspeliensis*, *C. salviifolius*), sometimes mixed with *Pinus* spp. *Inocybe nothomixtilis* is also
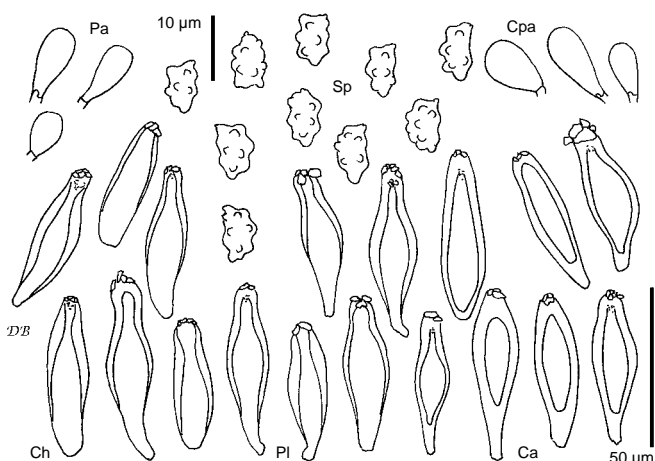
Fig. 10 *Inocybe nothomixtilis* AH 24510 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.

found in parks or urban areas and then in mixed forests with broad-leaved trees (e.g., *Betula*, *Carpinus*, *Tilia*) or conifers (e.g., *Pinus*, *Abies*). It prefers acid to decalcified soils with sandy texture. Its current known distribution includes France, Germany, Italy and Spain, but considering its habitat preferences, it is probably more widespread in Mediterranean and sub-Mediterranean European areas, and also in warm and temperate locations in continental Europe.

Additional specimens examined. France, Île-de-France, Seine-Saint-Denis, Parc du Sausset, under mixed broadleaved trees (*Fagus sylvatica*, *Carpinus betulus*, *Quercus* spp.), 25 Oct. 2006, *G. Eyssartier*, GE 06-110 (PC 0738072, as *I. tabacina* var. *pseudovolvata*). – Germany, Baden-Württemberg, Karlsruhe, TK 6916/3, alt. 118 m, in a lawn with *Quercus petraea*, *Fagus sylvatica* and *Betula pendula*, 1 June 2013, *D. Bandini & B. Oertel* (KR-M-0038133); Hessen, Landkreis Bergstraße, Bürstadt, Waldfriedhof Lampertheim, TK 6317/3, alt. 95 m, in acid sandy soil under *Pinus sylvestris* and *Quercus rubra*, 26 Oct. 2014, *D. Bandini* (KR-M-0043319, duplicate in TUR-A 203452). – Italy, Toscana, Castiglion Fiorentino, St. Stefano, alt. approx. 500 m, in Mediterranean forest near *Quercus* spp. (possibly *Q. pubescens* or *Q. cerris*), in presumably decalcified soils, 18 Oct. 2010, *M. Dondl* (KR-M-0043322). Locality unknown (probably Piemonte, Pamparato according to Alessio & Rebaudengo (1980)), under *Abies alba* (?), 24 June 1986, *C.L. Alessio*, (private herbarium of C.L. Alessio, as *I. mixtilis* var. *aurata*). – Spain, Cataluña, Barcelona, Collserola, Can Ferrer, in *Cistus* bushes stand (*C. monspeliensis* and *C. salviifolius*), in acid soil, 23 Oct. 2000, *J. Vila & X. Llimona* (AH 26921, duplicate in JVG 1001023-3); 31 Oct. 2000 (AH 26924, duplicate in JVG 1001031-3); Islas Baleares, Mallorca, Escorca, Predio Marjanor, alt. 500–700 m, among mosses in Mediterranean mixed forest with *Pinus halepensis*, *Cistus monspeliensis* and *Pistacia lentiscus*, in decalcified washed soils, 26 April 1994, *J.L. Siquier*, JLS 715B (AH 46558).

Notes — Habit and size of *I. nothomixtilis* fruitbodies are very similar to other taxa in the Mixtilis group, but *I. nothomixtilis* is characterized by its well-developed and rather persistent veil, at least in primordia and young specimens, as well as by its regularly felty to rather woolly-fibrillose pileus in most collections. This whitish veil, when not washed out by heavy rainfall, is easily observed on the pileus and on the bulb edge, forming here a 'pseudovolva'. In wet conditions, this velipellis covers a sticky but not really viscid pileus, which has often adhering soil particles and humus remains. In some collections the cuticle becomes cracked radially, forming adpressed-fibrillose scales, when withered or upon ageing. All specimens assigned here to *I. nothomixtilis* form a supported monophyletic clade in all analyses (Fig. 1, 2).

Furthermore, microscopical characters of *I. nothomixtilis* distinguish reliably from the other species. The spores in *I. nothomixtilis* are rather small, clearly heterodiametric ($Q_m = 1.45$), and show numerous (usually 10–13) knobs. These protuberances are small, low and rounded (defined as 'patatiform' by Bon (1998: 4), referring to *I. umbrina*). Spore shape and ornamentation remind of those of *I. johannis-stanglii* or *I. subtrivialis*, but these species have larger (often exceeding 10 µm) and more rectangular spores. A distinct thin velipellis is also present in *I. johannis-stanglii*, but this species shows larger cystidia mostly tapering into a distinct attenuate base. *Inocybe nothomixtilis* is characterized by its rather short cystidia, in average ≤ 50 µm, variable and ranging from truncate to shortly pedicellate at the base. Both taxa also differ by a different distribution and habitat preferences (see comment on *I. johannis-stanglii*).

Our analyses support that both *I. tabacina* var. *pseudovolvata* and a collection of *I. mixtilis* var. *aurata* are contaxic with *I. nothomixtilis*. Regarding *I. tabacina* var. *pseudovolvata*, Eyssartier (2006: 177) based his description on a single collection with tobacco-brown pilei ('paille brunâtre terne à beige-brun terne ou un peu roussâtre ou encore beige ochracé'), and practically devoid of any yellow tone. The pileus surface was described as nearly smooth, somewhat sticky and agglutinating some soil and humus particles (Eyssartier 2006: Planche V). Eyssartier named this taxon *I. tabacina* var. *pseudovolvata* because of a

well-developed velipellis forming a 'pseudovolva' around the bulb edge. As mentioned above, this character is very helpful to recognise *I. nothomixtilis*, especially when basidiomata are young and in good condition. Eyssartier also provided some interesting macrochemical reactions not tested by us: KOH 5 % yellow on pileus flesh and orange brown on pileus surface; silver nitrate chocolate brown after 10–15 min on pileus flesh.

Inocybe tabacina var. *pseudovolvata* was never validly published, but a detailed description was provided. We confirm here by examining the collection kept at PC that it matches *I. nothomixtilis* in most characters, especially in the heterodiametric and small spores ($Lxl_m = 8.4 \times 6$; Qm = 1.4, n = 21) and in having short cystidia ($L_m < 50$ µm). Eyssartier also pointed out some similarities between his variety and *I. mixtilis* but stated that *I. tabacina* var. *pseudovolvata* was distinguished by a well-developed veil and a darker pileus colour. However, no comparison of microscopic characters was made by him.

Inocybe mixtilis var. *aurata* was introduced by Alessio & Rebaudengo (1980: 302) to include some collections with a more yellow golden colour and a drier pileus ('pilei cuticulam aridiorem …'). Alessio described the pileus as practically dry, hence devoid of the characteristic slimy cuticle which is evident in wet conditions in *I. mixtilis*. This var. *aurata* was not validly published because two different gatherings were designated as the holotype (Art. 40.1). Moreover, no original material (Pamparato, Cabanet in 1972 and Roburentello in 1976) is located in E. Rebaudengo's private herbarium (Ceva, Italy), where it was said to be deposited.

Although most Alessio's *Inocybe* collections used for his monograph are lost (M. Candusso and E. Bizio, pers. comm.), we were able to study three authentic (but not original) collections of var. *aurata* kept in Alessio's private herbarium, sent to us by M. Candusso. The material is poorly preserved, mouldy and lacks accurate collection data. Two collections could be ITS-sequenced, one of them belonging to *I. nothomixtilis* (24 June 1986) and the other one conspecific with *I. mixtilis* (15 Sept. 1984). DNA extraction was successful and the ITS sequence obtained and analysed. The specimen dated 24 June 1986, 'prato nostro sotto abete bianco' is conspecific with *I. nothomixtilis*, while the one dated 15 Sept. 1984, belongs to *I. mixtilis*. A third collection collected also on 24 June 1986 is a leiosporeous species. From these facts, we conclude that Alessio's concept of var. *aurata* was heterogeneous.

Inocybe occulta Esteve-Rav., Bandini, B. Oertel & G. Moreno, *sp. nov.* — MycoBank MB819793; ITS barcode GenBank KX290787; Fig. 11, 12

Etymology. From Latin *occultus*, which means hidden or concealed, due to the likely confusion with similar taxa of the *Inocybe mixtilis* group.

Holotypus. Spain, La Rioja, Villarroya, 800 m, 30T WM76, on washed and decalcified sandy soil, in a *Quercus ilex* subsp. *ballota* forest, 17 May 2008, *A. Caballero*, AC3805 (AH 36443). Isotypus KR-M-0046550 and A. Caballero's private herbarium.

Selected description and iconography: Esteve-Raventós & Caballero Moreno (2009: 37, f. 11, photos 27–31 – as *I. mixtilis*).

Diagnosis: *Inocybe occulta* is morphologically rather close to *I. ceskae*, differing in the more intense yellow-orangish to orange-brown pileus colour and the slightly longer cystidia in average. *Inocybe occulta* is sister to *I. nothomixtilis* in our ITS-RPB2 analyses (Fig. 1), but it differs primarily from the latter in having more isodiametric spores with lower knobs. *Inocybe ceskae* and *I. mixtilis*, both with spores of similar shape ($Q_m = 1.3–1.4$) to *I. occulta*, cluster in a different clade and are phylogenetically distinct from *I. occulta* (Fig. 1).

Basidiomes usually gregarious, rarely solitary. *Pileus* (12–)15–30(–45) mm diam, shape initially campanulate or obtusely conical to hemispherical, then convex applanate when expanded, often with a low and broad umbo, but occasionally not or hardly umbonate; margin straight to slightly deflexed, sometimes wavy in expanded specimens, not striate, only slightly appendiculate by fugacious velar remnants when present; colour variable, often yellow to orange, dull to more or less bright, e.g., beige, yellowish ochre, buff yellow to golden yellow, orange-yellow to orange-brown (Mu 10YR 8/4–8/8, 7/6–7/8, 7.5YR 7/6–7/8 or 6/6–6/8), in some collections also light brown (Mu 10YR 5/6–5/8), at least towards the margin (e.g., KR-M-0046554 or KR-M-0046555), uniform or very often slightly darker at the centre or the umbo; surface radially appressed-fibrillose, smooth and glabrous, sometimes with a micaceous and/or a falsely hygrophanous appearance (e.g., KR-M-0046556), even not clearly rimose towards the margin, with lubricous aspect in wet conditions, somewhat sticky and hence often with adhering soil and humus particles; velipellis absent or scarcely developed, in this case fugacious but still observable in young specimens of some collections, especially towards the periphery and margin, forming small fibrillose patches or squamules, but mostly absent in many collections when washed by rainfall or aged. *Lamellae* ventricose to subventricose, 2–6 mm deep, practically free or hardly adnexed, often crowded, sometimes moderately crowded, L = 45–55, l = 1–2; colour initially whitish to beige or greyish, often showing a lilac to violet reflection ('Psathyrella-like'), when old becoming ochraceous to brownish grey and finally yellow-brown to orange-brown; edge finely fimbriate to crenulate, whitish or paler than the sides. *Stipe*

Fig. 11   *Inocybe occulta*. a. AH 36443 (holotype) *in situ*; b. KR-M-0046556 *in situ*; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from AH 36443, holotype). — Scale bars: a–b = 1 cm; c–i = 10 µm; j = 2 µm.

20–50(–55) × (2–)3–6(–8) mm, solid, fibrose; shape cylindrical to tapering upwards, base distinctly marginately bulbous, often agglutinating soil or humus debris; bulb 7–10(–12) mm broad, edge often clear-cut and even volviform, but velar rests absent or ephemeral in young stages; colour white or dirty white when young, often turning to ochraceous, ochraceous yellow or even bright yellow in some collections (e.g., AH 36148), or fawn with age (Mu 2.5Y 8/1–8/4), never browning nor blackening; surface evenly and densely pruinose all over, up to the bulb. *Cortina* not seen, even in young primordia. *Context* fibrose, firm, whitish, concolorous towards the cortex in pileus and stipe, hardly becoming yellowish with age or when drying; smell generally faint in fresh specimens, often indistinct, in some collections more or less spermatic when cut, exceptionally aromatical or even honey-like (AH 36443); taste not recorded. *Colour of exsiccata*: pileus brownish to orange brown (Mu 7.5YR 6/6–6/8, 5/6–5/8) to brown (7.5YR 4/6); stipe dirty whitish to ochraceous brown, paler than pileus (10YR 7/3–7/4 or 6/4–6/6), not darkening; lamellae deep brown orange.

Basidiospores (7.2–)7.5–10(–10.5) × (5.2–)5.3–7.5 μm, Q = (1.1–)1.15–1.57(–1.63), L_m = 8–9.6, W_m = 5.9–7, Q_m = 1.3–1.5 [n = 100, from 4 collections], variable in shape (even within a specimen), from heterodiametrical to shortly heterodiametrical and less frequently subisodiametrical; spore ornamentation somewhat variable, mostly presenting 8–13 conical obtuse knobs, (0.9–)1.1–1.4(–1.7) μm high, 1.2–1.6 μm broad at base (but in KR-M-0043312 many spores show low knobs or even a polyhedrical angulose outline, 'sub-entolomatoid'); apicula distinct; without germ pore and sometimes with a central oil guttule. *Basidia* 22–28(–30) × 8–10 μm, clavate, 4-spored, with sterigmata 3–5 μm long. *Pleurocystidia* abundant, metuloid, (37–)39–*51.5*–64(–67) × (11.2–)11.5–*17*–22(–24) μm, Q = 2–*3*–4.1 [n = 40]; of variable shape among collections, normally ventricose fusiform to subutriform, occasionally ventricose sublageniform with obtuse apex and short neck, but also not distinctly ventricose in some collections; base normally not pedicellate, tapering into an obtuse base, seldom truncate; rather crystalliferous at apex in most collections; walls (1–)1.5–2.5 μm, often thickened towards the apex up to 3–4 μm, not coalescing at the neck, pale yellow in ammonia; content hyaline or pale yellowish, not showing dark pigments. *Lamellar edge* homogeneous and sterile, composed of numerous cheilocystidia and rather abundant small clavate to subovoid paracystidia, 7–12 μm broad. *Cheilocystidia* abundant, similar in shape and size to pleurocystidia, 33–*47*–61(–65) × 11.5–*17.5*–23 μm [n = 40], with similar thick walls, rather crystalliferous at apex, hyaline or showing a pale yellowish content. *Hymenophoral*
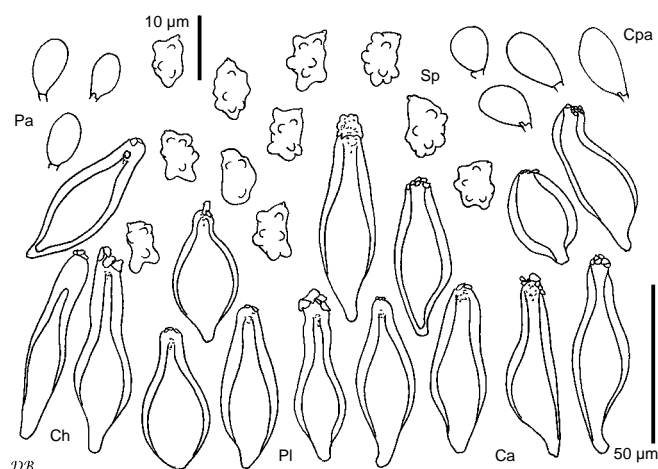
trama subregular, consisting of more or less parallel to interwoven hyphae, 3–12 μm wide, subcylindrical to subfusiform, hyaline. *Subhymenium* poorly developed, consisting of 2–3 layers of small branched to subisodiametric or irregular cells, 3.5–5 μm diam, hyaline. *Pileipellis* consisting of an epicutis of slightly gelified parallel hyphae, 3.5–15 μm wide, with incrusting and intracellular yellowish pigment; hyphae somewhat paler to almost hyaline in deeper layers (subcutis), up to 20(–25) μm wide. *Stipitipellis* consisting of a cutis of subcylindrical hyphae, often constricted at septa, bearing numerous bundles of caulocystidia similar to hymenial cystidia, (27–)30–*48.5*–66.5(–69) × (11–)11.5–*17*–22.5 μm [n = 40], crystalliferous at apex, intermixed with abundant hyaline, ovoid to subclavate paracystidia (× 8.5–18 μm), similar to those of lamellar edge; these elements constitute the dense and abundant pruina covering the whole stipe. *Clamp connections* present in all tissues.

Habitat & Distribution — *Inocybe occulta* occurs in a broad range of habitats and is distributed from cold boreal areas to warm Mediterranean regions of the Northern Hemisphere. Many collections were made in boreal to subboreal taiga forests in Europe (mixed forests including *Picea, Larix, Betula, Pinus*, etc.), but also in warmer and temperate habitats in central and southern Europe in association with conifers (*Picea, Larix, Pinus*) or broad-leaved trees like *Fagaceae* (*Fagus sylvatica, Quercus robur*, etc.). *Inocybe occulta* also occurs in more xeric Mediterranean habitats, in association with *Pinaceae* (*Pinus pinaster, P. pinea*, etc.), *Fagaceae* (evergreen oaks like *Quercus ilex, Q. suber*, etc.) and also *Cistaceae* (*Cistus* spp.). Interestingly, *I. occulta* is also present at the alpine stage in the Pyrenees, in *Dryas octopetala* and dwarf *Salix* communities on slightly calcareous soils. These data suggest that, unlike *I. ceskae* and *I. mixtilis*, *I. occulta* has probably a more extensive distribution and broader range of hosts. The known distribution of *I. occulta* in North America overlaps that of *I. ceskae*, being known in subtemperate coastal *Pseudotsuga* forests from the Northern Pacific (British Columbia, Canada) to Oregon (USA), here in young *P. menziesii* forests (Smith et al. 2002). According to sequences available in GenBank, *I. occulta* is found in Mexico (Morelos, Volcán El Pelado), presumably in native *Pinus* forests (KC152132) and also in Australia (Canberra), introduced with *Pinus* (KP308781).

Inocybe occulta does not show specific soil pH requirements. Many of the collections from montane areas in northern and continental Europe were gathered in calcareous soils, but it also occurs in decalcified, neutral to slightly acid soils with sandy texture in Mediterranean areas, (e.g., in evergreen-oak or Mediterranean pine forests, like *Pinus pinea* in Italy, ref. GenBank JF908141, Bizio pers. comm.), often with presence of *Cistus* bushes.

Additional specimens examined. AUSTRIA, Kärnten, Bezirk Spittal an der Drau, Reißeck, Penk, alt. 1100 m, under *Picea abies*, 16 Aug. 2013, *D. Bandini & B. Oertel* (KR-M-0043315); Salzburg, Bezirk Tamsweg, Tamsweg, near Prebersee, alt. 1500 m, in *Picea abies* forest, 15 Aug. 2013, *D. Bandini & B. Oertel* (KR-M-0043316); Tirol, Bezirk Landeck, Fließ, Pillerhöhe, alt. 1540 m, in *Picea abies* forest, 28 Aug. 2014, *B. Oertel & G. Schmidt-Stohn* (KR-M-0043313). – FINLAND, Enontekiön Lappi, Enontekiö, Kilpisjärvi, along the small river Tšahkaljohka, in rather moist forest of *Betula pubescens* subsp. *czerepanovii*, 15 Aug. 1990, *J. Vauras* (TUR-A 145165, duplicate in KR-M-0046553); Koillismaa, Kuusamo, Oulanka National Park, near the Research Station, mixed forest with *Pinus sylvestris*, *Picea abies* and *Betula* sp., 17 Aug. 2015, *J. Vauras, D. Bandini & B. Oertel* (KR-M-0046554); Kuusamo, Oulanka National Park, Ampumavaara, roadside with *Pinus sylvestris*, *Picea abies* and *Betula* sp., 20 Aug. 2015, *J. Vauras, D. Bandini & B. Oertel* (KR-M-0046555); Kuusamo, Oulanka National Park, Pähkänä, wayside with *Pinus sylvestris*, *Picea abies* and *Betula* sp., 21 Aug. 2015, *J. Vauras, D. Bandini & B. Oertel* (KR-M-0046556); Kuusamo, Oulanka National Park, foot of Ampumavaara, roadside with *Pinus sylvestris*, *Picea abies* and *Betula* sp., 22 Aug. 2015, *J. Vauras, D. Bandini & B. Oertel* (KR-M-0046557); Kuusamo, Oulanka National Park, Liikasenvaara, lawn of a house of Iso Sirkkalampi, in mixed forest

Fig. 12 *Inocybe occulta* AH 36443 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.

with *Pinus sylvestris*, *Picea abies* and *Betula* sp., 25 Aug. 2015, *J. Vauras, D. Bandini & B. Oertel* (KR-M-0046558). – GERMANY, Baden-Württemberg, Landkreis Breisgau-Hochschwarzwald, Titisee-Neustadt, Schwärzenbach, Black Forest, TK 8015/3, alt. 930 m, wayside near *Picea abies*, 18 Aug. 2014, *D. Bandini* (KR-M-0043323); Rhein-Neckar-Kreis, Sandhausen, TK 6617/4, alt. 100 m, Nature Reserve 'Sandhausener Düne, Pflege Schönau-Galgenbuckel', in calcareous sand dune with *Pinus sylvestris*, *Quercus robur* and *Helianthemum nummularium*, 13 Oct. 2012, *D. Bandini, B. Oertel & W. Winterhoff* (KR-M-0043312); Rhein-Neckar-Kreis, Wiesenbach, TK 6618/2, alt. 190 m, mixed forest with *Fagus sylvatica* and *Picea abies*, 17 Oct. 2012, *D. Bandini* (KR-M-0043318); at about 4 km distance from the former location, alt. 170 m, under *Abies procera*, 20 Oct. 2012, *D. Bandini* (KR-M-0043317); Bayern, Landkreis Berchtesgadener Land, Schönau am Königssee, Gotzenalm, TK 8443/4, alt. 1680 m, in calcareous soil with *Picea abies*, *Larix decidua* and *Juniperus communis*, 14 Aug. 2011, *D. Bandini & B. Oertel* (KR-M-0038028); Landkreis Garmisch-Partenkirchen, Garmisch-Partenkirchen, Riffelriss, TK 8531/4, alt. 1600 m, in calcareous soil with *Larix decidua*, *Pinus sylvestris* and *Betula pendula*, 1 Aug. 2012, *B. Oertel* (KR-M-0043324); ibidem, alt. 1660 m, in calcareous soil with *Larix decidua* and *Pinus mugo*, 20 Aug. 2012, *D. Bandini & B. Oertel* (KR-M-0043314, duplicate in TUR-A 203441); Rheinland-Pfalz, Landkreis Mainz-Bingen, Bingen, Stadtwald, TK 6013/1, alt. 300 m, mixed forest with *Quercus robur*, *Larix decidua* and *Betula pendula*, 10 Sept. 2011, *D. Bandini & B. Oertel* (KR-M-0043230); Landkreis Trier-Saarburg, Kell am See, TK 6306/4, alt. 460 m, under *Picea abies*, 1 Oct. 2011, *D. Bandini* (KR-M-0038125). – NETHERLANDS, Friesland, Ameland, Hollum, alt. 5 m, in calcareous sand dune, wood with *Pinus sylvestris*, 19 Sept. 2011, *D. Bandini* (KR-M-0038029). – SPAIN, Andalucía, Cádiz, Los Barrios, road from Los Barrios to Facinas (km. 5), Parque Natural de los Alcornocales, alt. 180 m, in acid soil under *Quercus suber*, 14 Dec. 1990, *F. Esteve-Raventós & A. Ortega* (AH 22108); Castilla-La Mancha, Guadalajara, Tamajón, El Chortal, alt. 1000 m, in sandy slightly acid soil in a reforested *Pinus pinaster* forest with *Cistus ladanifer*, 31 Oct. 2014, *G. Moreno* (AH 40499); Castilla-León, Burgos, Nava de Mena, Embalse de Ordunte, alt. 300 m, under *Quercus robur* in slightly acid and sandy soils, 14 Oct. 1998, *J.M. Barrasa & F. Esteve-Raventós* (AH 24987); Castilla-León, Burgos, Valle de Mena, Vivanco, Santuario de Cantonad, alt. 480 m, in *Fagus sylvatica* forest, 14 Oct. 1998, *J.M. Barrasa & F. Esteve-Raventós* (AH 24989); Valladolid, Tudela de Duero, in slightly acid and sandy soil in *Pinus pinea* forest, 23 Nov. 2002, *P. Juste* (AH 30697); Cataluña, Gerona, Queralbs, Val de Núria, Coma de l'Embut, alt. 2220 m, in calcareous soil among *Dryas octopetala* and *Salix retusa*, 8 Aug. 1996, *J. Vila* (AH 21573, duplicate in JVG 960808-26); 12 Aug. 1997, *J. Vila & X. Llimona* (AH 23481, duplicate in JVG 970812-8); 23 July 1999, *J. Vila* (AH 26731, duplicate in JVG 990723-15; published by Esteve-Raventós & Vila (1997), as *I. mixtilis*); Gerona, Setcases, Refugio de Ulldeter, alt. 2300 m, among *Salix retusa* in slightly calcareous soils, 2 Aug. 1997, *F. Esteve-Raventós & J. Vila* (AH 23480, duplicate in JVG 970802-4); Lérida, Espot, Estany de Sant Maurici, La Roca de l'Estany, alt. 2150 m, under *Salix pyrenaica* in calcareous soil, 12 Sept. 1996, *J. Vila & X. Llimona* (AH 21576, duplicate in JVG 960912-25; published by Esteve-Raventós & Vila (1997), as *I. mixtilis*); Extremadura, Cáceres, Jarandilla de la Vera, in acid soil under *Quercus pyrenaica*, 14 Nov. 2014, *J. Muñoz* (AH 44531); La Rioja, Santa Lucía, Valle de Ocón, alt. 800 m, in decalcified sandy soil in *Quercus ilex* subsp. *ballota* forest, 3 June 1997, *A. Caballero* (AH 36110, duplicate in AC 2008); 6 June 2009 (AH 40279, duplicate in AC 4102); Villarroya, alt. 800 m, in decalcified sandy soils of *Quercus ilex* subsp. *ballota* forest, 30 April 2006, *A. Caballero* (AH 36148, duplicate in AC 3189; published by Esteve-Raventós & Caballero Moreno (2009), as *I. mixtilis*).

Notes — This study suggests that *I. occulta* is the species with the broadest, transoceanic distribution within the Mixtilis group, and probably the most common one. Its epithet refers to the fact that this taxon has been overlooked and has probably been misinterpreted for *I. mixtilis* or other species of this group.

Inocybe occulta forms a well-supported clade in our analyses (Fig. 1) but it is sometimes difficult to recognize morphologically. Typical collections are characterized by the heterodiametrical spores, pleurocystidia 34 – 69 μm long and a pileus of intense colour. Nevertheless, those characters are somewhat overlapping in *I. mixtilis*, *I. ceskae* and *I. occulta*.

The spore shape in *I. occulta* and *I. ceskae* shows a clear tendency to be heterodiametric to shortly heterodiametric, while spores are generally isodiametric to subisodiametric in *I. mixtilis*. The three species are characterized by producing spores with a prominent ornamentation constituted by high knobs, in contrast

to those of *I. nothomixtilis*, *I. johannis-stanglii* and *I. subtrivialis*, which have considerably lower knobs.

Inocybe subtrivialis Esteve-Rav., M. Villarreal & Heykoop, Rev. Catalana Micol. 20: 157. 1997 — Fig. 13, 14

Holotypus. SPAIN, Castilla-La Mancha, Toledo, La Iglesuela, El Bonal, 30TUK525575, alt. 450 m, in acid sandy soil of *Quercus ilex* subsp. *ballota* forest with *Cistus ladanifer* and *Lavandula pedunculata* bushes, 16 Jan. 1996, *J.L. Aguirre, M. Heykoop & M. Villarreal* (AH 19225).

Selected descriptions and iconography: Esteve-Raventós et al. (1997: 157, f. 5). ITS barcode GenBank MH496013, MH496014.

Habitat & Distribution — *Inocybe subtrivialis* is currently known only in the Iberian Peninsula, but its ecology suggests it is more widespread and has been overlooked in Mediterranean Europe. Some records of *I. pallida* from Mediterranean environments might actually belong to *I. subtrivialis*, as that by Solak et al. (2009) in pine forests in southern Turkey. The typical habitat of *I. subtrivialis* are thermophilous Mediterranean evergreen oak forests (*Quercus ilex* subsp. *ballota*) or pine forests (*Pinus pinea*, *P. pinaster*), with presence of *Cistus.*

Specimens examined. SPAIN, Toledo, La Iglesuela, El Bonal, 30TUK525575, alt. 450 m, in acid sandy soil of *Quercus ilex* subsp. *ballota* forest with *Cistus ladanifer* and *Lavandula pedunculata* bushes, 16 Jan. 1996, *J.L. Aguirre, M. Heykoop & M. Villarreal* (AH 19225, holotype); 5 April 1996, *M. Villarreal & M.A. Jiménez* (AH 20435); 24 April 2000, *F. Esteve-Raventós & M. Villarreal* (AH 26783); AH 26784. — *Inocybe* cf. *subtrivialis*. SPAIN, Madrid, Rozas de Puerto Real, Embalse de los Morales, alt. 780 m, in acid sandy soil in a mixed forest with *Pinus pinaster* and *Cistus ladanifer* bushes, 14 May 2000, *F. Esteve-Raventós & M. Villarreal* (AH 26789).

Notes — *Inocybe subtrivialis* was described in detail by Esteve-Raventós & Caballero Moreno (2009), who commented on the similarities with *I. pallida* in the sense of Stangl (1989) (see comments under *I. johannis-stanglii*). Our ITS-RPB2 analyses (Fig. 1) revealed that both are close but independent species. Furthermore, both show a different distribution, being *I. subtrivialis* a characteristic thermophilous species associated with Mediterranean evergreen oaks (*Quercus* spp.), *Cistus* spp. and *Pinus* spp., in acid sandy soil (see also notes on *I. johannis-stanglii* for comparison).

Inocybe subtrivialis shows the following diagnostic characteristics: 1) pileus covered by whitish veil in young specimens, usually sticky to viscid in wet conditions, with a warm brownish yellow (Mu 10YR 5/6 – 5/8, 6/6 – 6/8), orange (Mu 7.5YR 6/6 – 6/8) to dark yellow-orange brown or 'bronze' colour (Mu 7.5YR 5/6 – 5/8, 4/6), smooth or often broken into appressed
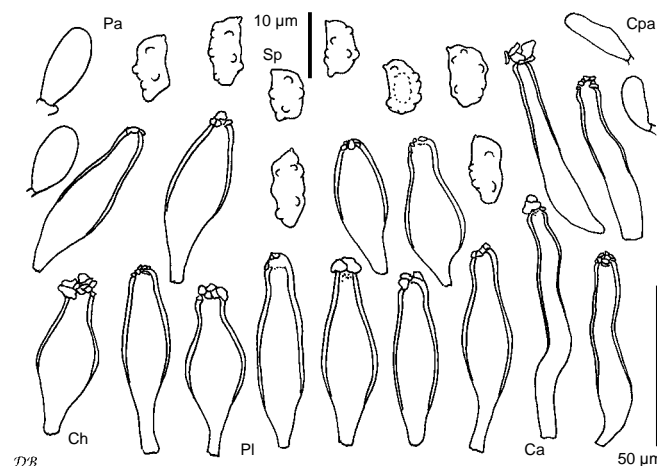
Fig. 13 *Inocybe subtrivialis* AH 19225 (holotype): microscopic features. Sp = spores, Pa = paracystidia, Cpa = cauloparacystidia, Ch = cheilocystidia, Pl = pleurocystidia, Ca = caulocystidia. — Drawn by Ditte Bandini.
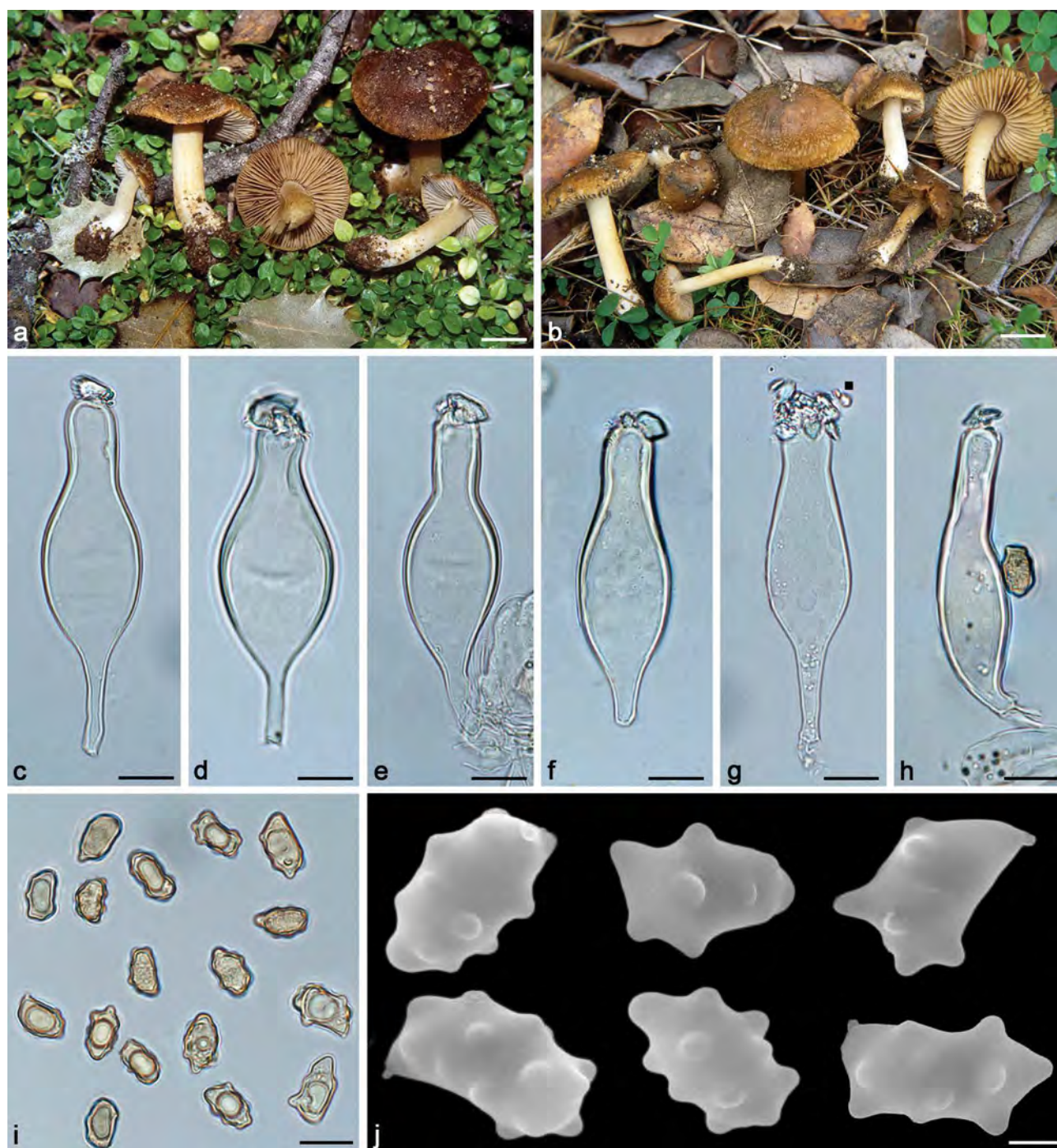
Fig. 14 *Inocybe subtrivialis.* a. AH 19225 (holotype) *in situ*; b. AH 26783 *in situ*; c–h. hymenial cystidia; i. spores under optical microscope; j. spores under SEM (all microphotos from AH 19225, holotype). — Scale bars: a–b = 1 cm; c–i = 10 μm; j = 2 μm.

scales; 2) stipe in most cases with a napiform or subbulbous base, in some collections with a slightly marginate bulb, white and typically becoming fuscous yellow with age (especially evident in exsiccata), densely pruinose all over but sometimes sparsely pruinose in the lower third; 3) lamellae without distinct violet or lilac shades when young; 4) spores heterodiametric, (8–)8.2–*10.5*–12.7(–12.8) × (5.2–)5.5–*6.4*–7.3(–7.5) μm, Q = (1.25–)1.3–*1.65*–2(–2.2) [n = 100, from 4 collections], provided with numerous (10–)12–16(17) low and obtuse knobs (0.35–0.75 μm high, 0.7–2 μm broad at base), but in some occasional collections without distinct knobs and hence subangulose in outline; 5) pleurocystidia mostly narrowly lageniform to subfusiform, also sublageniform provided with a differentiated obtuse neck and base often tapering into a pedicel, 46–*55.5*–65 × 9.5–*16*–22 μm [n = 40], walls (1–)1.2–2(–2.5) μm thick, yellowish (not very intense) in ammonia solutions, con-

tent often showing a pale yellow-fuscous intracellular pigment; and 6) caulocystidia similar to hymenial cystidia, often more slender or even hair-like near the base, mixed with clavate to sphaeropedunculate or subovoid paracystidia.

Specimen AH 26789 is morphologically similar to *I. subtrivialis* but its ITS sequence deviates from the one obtained from the holotype specimen (11 changes and several insertions/deletions). Our material needs to be examined to more appropriately assess its identity. In addition to its habitat and distribution, *I. subtrivialis* differs from *I. johannis-stanglii* in the darker, brownish orange pileal colour, a strong tendency of the fruitbodies to become yellow fuscous to bronze on ageing or upon drying, slightly narrower spores and slenderer cystidia.

PROVISIONAL KEY FOR THE RECOGNITION OF THE KNOWN SPECIES OF THE INOCYBE MIXTILIS CLADE

1. Spores with Q_m = 1.4–1.65, provided with low knobs (0.5–1 µm high) 2
1. Spores with Q_m = 1.3–1.4, provided with clear and distinct protruding knobs (1–1.5 µm high) 4
2. Spore width W_m ≥ 6.5 µm; velipellis present, buff to greyish; lamellae with a violet to lilac reflection when young; cystidia bulgy and mostly pedicellate. — In temperate Europe . . . . *I. johannis-stanglii*
2. Spore width W_m < 6.5 µm 3
3. Spores with Q_m = 1.6, more or less rectangular heterodiametric; cystidia not very short (L_m = 55 µm), often pedicellate and with thin walls (1–2 µm); velipellis whitish to buff, often ephemeral; basidiomata generally becoming yellow-copperish to orange or brown copperish with age; lamellae without lilac or violet reflection when young. — In Mediterranean sandy forests, mainly under evergreen oaks (*Quercus* spp.), sometimes with the presence of *Cistus* and/or *Pinus* . . . . *I. subtrivialis*
3. Spores with Q_m = 1.4–1.5, polygonal heterodiametric, often tapering to an apical knob; cystidia short (L_m = 45–50 µm), not pedicellate, often with thick walls 2–3(–4) µm; velipellis rather developed and persistent, sometimes leaving a rim around the bulb margin; pileus sometimes fibrillose-shaggy, dry to weakly sticky in humid conditions. — In temperate to warm areas of Europe . . . . *I. nothomixtilis*
4. Spores mostly subisodiametric to isodiametric, with Q_m = 1.3–1.4, often with a 'margaritiform' shape; pileus in wet conditions remarkably lubricous and sticky, with a lardy aspect; hymenial cystidia often showing a differentiated neck (lageniform to sublageniform). — Only known with certainty from Europe, especially in north temperate to subboreal cold areas, rare in southern countries . . . . *I. mixtilis*
4. Spores with Q_m = 1.4–1.5, generally more heterodiametrical; pileus without a lardy aspect, slightly sticky in humid conditions, sometimes subhygrophanous 5
5. Pileus pale straw-coloured, yellowish beige, pale ochre, slightly sticky in wet conditions; cystidia ≤ 50 µm, fusiform, with attenuate but not pedicellate base. — Known only from cold subboreal areas of Europe and certainly also from temperate coastal forests of Pacific Canada extending to Oregon (USA) . . . . *I. ceskae*
5. Pileus darker, buff yellow, golden-yellow, orange-yellow or light brown, often with a subhygrophanous appearance; cystidia often with a sublageniform shape, often extending into a distinct neck, though sometimes variable, often > 50 µm long. — Widespread all over continental Europe, and also known from North America and Australia. . . . . *I. occulta*

DISCUSSION

Inocybe is a species-rich genus of ectomycorrhizal fungi. Due to lack of reference sequences and lack of comparative taxonomic studies, identification of *Inocybe* species is often challenging and many sequences and material remain unidentified or misidentified (Ryberg et al. 2008). Previous ITS-based phylogenies integrating comparative morphological studies and distributional data have proven to be a good approach to resolve several species complexes (e.g., Larsson et al. 2009, 2017, Cripps et al. 2010, Esteve-Raventós et al. 2015, 2016)

and six species are recognized here within the *I. mixtilis* group. The addition of RPB2 sequences to the ITS data yielded a more robust phylogeny, especially concerning deeper nodes. Our analyses confirmed also that a considerable amount of sequences remain being erroneously identified in GenBank (Ryberg et al. 2008). Thus, sequences of at least *I. ceskae* and *I. occulta* have been named as *I. mixtilis* in GenBank (Table 1).

The six species recognized in this study are rather well delimited using morphological, ecological and distributional characters. Among morphological characters, spore shape and cystidial length/shape, pileus colour and viscidity and the presence of velipellis were useful in species identification, but some characters overlap between close species. Thus, *I. mixtilis*, *I. ceskae* and *I. occulta*, distinct species according to our phylogenetic analyses (Fig. 1), cannot always be reliably identified through morphological species recognition and we consider them 'semicryptic' species. From a molecular point of view, these taxa are regarded as distinct phylospecies (Wilkins 2009), constituting three diagnosable, monophyletic entities that represent unique lineages in the complex. The term semicryptic has been applied to species subsumed under a collective epithet and having overlapping morphological characters (Bickford et al. 2006), as in the *I. mixtilis* group and also used in similar cases concerning some 'telamonioid' *Cortinarius* groups by Brandrud et al. (2015).

Interestingly, our phylogenetic analyses (Fig. 2) show certain patterns in host-specificity and distribution, but conclusions should be taken cautiously as our sampling includes a small representation of the potential diversity of the *I. mixtilis* group. Two species, *I. ceskae* and *I. occulta* have a transoceanic distribution, the former being apparently restricted to subboreal and boreal forests. On the contrary, *I. mixtilis* is so far only known in Europe and Asia, but examination of further specimens is needed to confirm this. In general, species of the *I. mixtilis* group seem to have a broad host-specificity since all species but *I. johannis-stanglii* seem to establish ectomycorrhizal association with both conifers and angiosperms, as earlier observed for other groups of *Inocybe* (Ryberg et al. 2011, Esteve-Raventós et al. 2016).

This study represents one more example of cryptic speciation in *Inocybe.* The use of the ITS region and comparative morphological studies were useful to disentangle the *I. mixtilis* complex also in this case and allowed us to make a step further towards a better understanding of this ecologically important and diverse genus of ectomycorrhizal fungi.

REFERENCES

Alessio CL, Rebaudengo E. 1980. Inocybe. Iconographia mycologica, Vol. 29, suppl. 3. Museo Tridentino di Scienze Naturali, Trento.

Antonín V. 1986. Studies in annulate species of Armillaria I. Study of type specimens of Armillaria cepaestipes Velenovský. Česká Mykologie 40: 38–40.

Antonín V. 1990. Studies in annulate species of Armillaria III. Species described by Josef Velenovský. Acta Musei Moraviae, Scientiae Biologicae 75: 129–132.

Bandini D. 2017. Startseite – Inocybe. www.inocybe.org. Accessed 25 Jan. 2017.

Bickford D, Lohman DJ, Sohdi NS, et al. 2006. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22: 148–155.

Bon M. 1998. Clé monographique du genre Inocybe (Fr.) Fr. (3ème partie: espèces gibbosporées = Sous-genre Clypeus Britz. = Genre Astrosporina Schroet.). Documents Mycologiques 28 (111): 1–45.

Brandrud TE, Bendiksen E, Dima B. 2015. Some new and little known telamonioid Cortinarius species from Norway. Agarica 36: 11–42.

Breitenbach J, Kränzlin F. 2000. Champignons de Suisse. Vol. 5. Mykologia, Luzern.

Britzelmayr M. 1882. Dermini aus Südbayern. Friedlaender & Sohn, Berlin.

Britzelmayr M. 1885. Hymenomyceten aus Südbayern, Cortinarii. Bericht des Naturwissenschaftlichen Vereins für Schwaben und Neuburg in Augsburg 28: 1–160.

Cripps CL. 1997. The genus Inocybe in Montana aspen stands. Mycologia 89: 670–688.

Cripps CL, Horak E. 2008. Checklist and ecology of the Agaricales, Russulales and Boletales in the alpine zone of the Rocky Mountains (Colorado, Montana, Wyoming) at 3000–4000 m a.s.l. Sommerfeltia 31: 101–119.

Cripps CL, Larsson E, Horak E. 2010. Subgenus Mallocybe in the Rocky Mountains alpine zone with molecular reference to European arctic-alpine material. North American Fungi 5: 97–126.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797.

Esteve-Raventós F, Caballero Moreno A. 2009. Especies nuevas e interesantes del género Inocybe (Inocybaceae, Basidiomycetes) en la Comunidad de La Rioja (España Peninsular). Taxones con esporas nodulosas I. Fungi non Delineati, vol. XLVII. Edizione Candusso, Alassio.

Esteve-Raventós F, Moreno G, Alvarado P, et al. 2016. Unravelling the Inocybe praetervisa group through type studies and ITS data: Inocybe praetervisoides sp. nov. from the Mediterranean region. Mycologia 108: 123–134.

Esteve-Raventós F, Moreno G, Bizio E, et al. 2015. Inocybe flavobrunnescens, a new species in section Marginatae. Mycological Progress 14: 14. doi: https://doi.org/10.1007/s11557-015-1036-0.

Esteve-Raventós F, Vila J. 1997. Algunos Inocybe de la zona alpina de los Pirineos de Catalunya, I. Revista Catalana de Micologia 20: 177–186.

Esteve-Raventós F, Villarreal M, Heykoop M. 1997. Estudios sobre el género Inocybe (Fr.) Fr. en la Península Ibérica e Islas Baleares. III. Especies recolectadas en el Valle del Tiétar (Avila y Toledo). Revista Catalana de Micologia 20: 153–162.

Eyssartier G. 2006. La Flore Mycologique du Parc du Sausset (Seine-Saint-Denis, France). Bulletin Trimestriel de la Société Mycologique de France 122: 161–183.

Ferrari E. 2006. Inocybe alpine e subalpine. Fungi non Delineati, vol. XXXIV–XXXVI. Edizioni Candusso, Alassio.

Ferrari E. 2010. Inocybe dai litorali alla zona alpine. Fungi non Delineati, vol. LIV–LV. Edizioni Candusso, Alassio.

Ferrari E, Bandini D, Boccardo F. 2014. Inocybe (Fr.) Fr., terzo contributo. Fungi non Delineati, vol. LXXIII–LXXIV. Edizioni Candusso, Alassio.

Grund DW, Stuntz DE. 1980. Nova Scotian Inocybes.V. Mycologia 72: 670–688.

Grund DW, Stuntz DE. 1983. Nova Scotian Inocybes.VII. Mycologia 75: 257–270.

Heim R. 1931. Le genre Inocybe précédé d'une introduction general à l'étude des Agarics Ochrosporés. Lechevalier & Fils, Paris.

Jacobsson S, Larsson E. 2012. Inocybe (Fr.) Fr. In: Knudsen H, Vesterholt J (eds), Funga Nordica: 981–1021. Nordsvamp, Copenhagen.

Katoh K, Kuma KI, Toh H, et al. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.

Kauffman CH. 1924. Inocybe. North American Flora 10: 227–260.

Kennedy PG, Smith DP, Horton TR, et al. 2012. Arbutus menziesii (Ericaceae) facilitates regeneration dynamics in mixed evergreen forests by promoting mycorrhizal fungal diversity and host connectivity. American Journal of Botany 99: 1691–1701.

Kirk PM, Cannon P, Minter DW, et al. 2011. Ainsworth & Bisby's Dictionary of the Fungi. 10th edition. CAB International, UK.

Kobayashi T. 2002. The taxonomic studies on the genus Inocybe. Nova Hedwigia, Beihefte 124: 1–246.

Kõljalg U, Nilsson RH, Abarenkov K, et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology. doi: https://doi.org/10.1111/mec.12481.

Kropp BR, Matheny PB, Nanagyulyan SG. 2010. Phylogenetic taxonomy of the Inocybe splendens group and evolution of supersection 'Marginatae'. Mycologia 102: 560–573.

Kühner R. 1933. Notes sur le genre Inocybe. Bulletin Trimestriel de la Société Mycologique de France 49: 81–121.

Kuyper TW. 1985. Studies in Inocybe – I. Revision of the new taxa of Inocybe described by Velenovský. Persoonia 12: 375–400.

Kuyper TW. 1986. A revision of the genus Inocybe in Europe I. Subgenus Inosperma and the smooth-spored species of subgenus Inocybe. Persoonia, Suppl. 3: 1–247.

La Rosa A, Bizio E, Saitta A, et al. 2017. Inocybe castaneicolor (Agaricales, Basidiomycota), a new species in section Splendentes. Phytotaxa 316 (1): 79–87.

Larsson E, Ryberg M, Moreau PA, et al. 2009. Taxonomy and evolutionary relationships within species of section Rimosae (Inocybe) based on ITS, LSU and mtSSU sequence data. Persoonia 23: 86–98.

Larsson E, Vauras J, Cripps CL. 2014. Inocybe leiocephala, a species with an intercontinental distribution range – disentangling the I. leiocephala – subbrunnea – catalaunica morphological species complex. Karstenia 54: 15–39.

Larsson E, Vauras J, Cripps CL. 2017. Inocybe pratervisa group – A clade of four closely related species with partly different geographical distribution ranges in Europe. Mycoscience: 1–11.

Malençon G, Bertault R. 1970. Flore des Champignons supérieurs du Maroc, Vol 1. Institut Scientifique Chérifien et Faculté des Sciences, Rabat.

Marchetti M, Franchi P, Consiglio G. 2014. Tipificazione di alcune Inocybe di Britzelmayr. Rivista di Micologia 57: 127–178.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Graminaeae). Systematics and Biology 45: 524–545.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Molecular Phylogenetics and Evolution 35: 1–20.

Matheny PB. 2009. A phylogenetic classification of the Inocybaceae. McIlvainea 18: 11–21.

Matheny PB. 2016. Key to species of Inocybe from eastern North America – v02 (20 Dec. 2016). http://mathenylab.utk.edu/Site/The_Inocybaceae_Post_files/Key_Inocybe_easternNA_v02.pdf. Accessed 26 Jan. 2017.

Matheny PB, Bougher NL. 2006. The new genus Auritella from Africa and Australia (Inocybaceae, Agaricales): molecular systematics, taxonomy and historical biogeography. Mycological Progress 5: 2–17.

Matheny PB, Bougher NL. 2017. Fungi of Australia: Inocybaceae. CSIRO Publishing.

Matheny PB, Hofstetter V, Aime MC, et al. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98 (6): 982–995.

Matheny PB, Liu YJ, Ammirati JF, et al. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). American Journal of Botany 89: 688–698.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computer Environments Workshop (GCE), 14 Nov. 2010, New Orleans: 1–8.

Morris MH, Smith ME, Rizzo DM, et al. 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (Quercus spp.) in a California woodland. New Phytologist 178: 167–176.

Munsell®. 1994. Soil color charts (revised edition). Macbeth Division of Kollmorgen Instruments Corp, New York.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8: 4321–4325.

Nishida F. 1989. Key to the species of Inocybe in California. Mycotaxon 34: 181–196.

Peintner U, Horak E. 2002. Inocybe (Basidiomycota, Agaricales) from Kamchatka (Siberia, Russia): taxonomy and ecology. Sydowia 54: 198–241.

Petersen G, Knudsen H, Seberg O. 2010. Alignment, clade robustness and fungal phylogenetics – Crepidotaceae and sister families revisited. Cladistics 26: 62–71.

Ronquist F, Teslenko M, Van der Mark P, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: https://doi.org/10.1093/sysbio/sys029.

Ryberg M, Andreasen M, Björk R. 2011. Weak habitat specificity in ectomycorrhizal communities associated with Salix herbacea and Salix polaris in alpine tundra. Mycorrhiza 21 (4): 289–296.

Ryberg M, Nilsson RH, Kristiansson E, et al. 2008. Mining metadata from unidentified ITS sequences in GenBank: a case study in Inocybe (Basidiomycota). BMC Evolutionary Biology 8: 50.

Smith AH. 1938. Certain species of Inocybe in the Herbarium of the University of Michigan. Papers of the Michigan Academy of Science, Arts and Letters 24: 93–105.

Smith JE, Molina R, Huso MMP, et al. 2002. Species richness, abundance, and composition of hypogenous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (Pseudotsuga menziesii) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 80: 186–204.

Solak MH, Isiloglu M, Kalmis E. 2009. Some new records of Inocybe (Fr.) Fr. from Turkey. Turkish Journal of Botany 33: 65–69.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics Applications Note 22: 2688–2690.

Stangl J. 1984. Inocybe mixtilis (Britz.) Sacc. International Journal of Mycology and Lichenology 1: 327–334.

Stangl J. 1989. Die Gattung Inocybe in Bayern. Hoppea 46: 5–388.

Stuntz D. 1978. Interim 'skeleton key' to some common species of Inocybe in the Pacific Northwest. Pacific Northwest Key Council (rev. ed 2004). www.svims.ca/council/Inocyb.htm [accessed 15 Jan. 2017].

Tamura K, Stecher G, Peterson D, et al. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Thiers B. 2012. Index Herbariorum. A global directory of public Herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [accessed 15 Jan. 2017].

Velenovský J. 1920. Ĉeské Houby, vols 1–2. Praha.

Velenovský J. 1947. Novitates Mycologicae Novissimae. Opera Botanica Cechica 4: 1–158.

Vellinga EC. 1988. Glossary. In: Bas C, Kuyper TW, Noordeloos ME, et al. (eds), Flora Agaricina Neerlandica: 54–64. Balkema, Rotterdam.

White TJ, Bruns TD, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand H, Sninsky JS, et al. (eds), PCR protocols: a guide to methods and applications: 315–322. Academic, San Diego.

Wilkins JS. 2009. Species: The history of an idea. University of California Press, Berkeley.