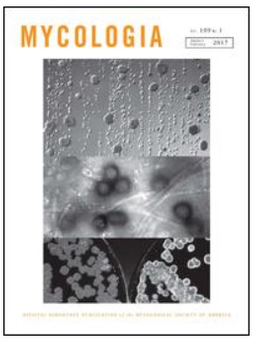

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Unraveling the *Inocybe praetervisa* group through type studies and ITS data: *Inocybe praetervisoides* sp. nov. from the Mediterranean region

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Abstract: Species in the *Inocybe praetervisa* group are characterized by producing nodulose to angular basidiospores and a bulbous, marginate, white stipe devoid of any pinkish to reddish tinge. Species delimitation problems and common misinterpretations in the *I. praetervisa* group have not yet been resolved through type studies and analysis of molecular data. This study seeks to clarify the taxonomy and nomenclature of species around *I. praetervisa*. Analyses of the nuc rDNA internal transcribed regions (ITS) recovered two major groups within the *I. praetervisa* group that can be separated on the basis of cystidial morphology. The study of three authentic and topotypic specimens in the Bresadola herbarium revealed that the name *I. praetervisa* has been misapplied often. The ITS region of one of the specimens was obtained, and this specimen is designated as epitype in support of a lectotype. *Inocybe rivularis* is demonstrated to be a later synonym of *I. praetervisa*, while *Inocybe phaeocystidiosa* is the correct name for the species most often misdetermined as *I. praetervisa*. *Inocybe salicis-herbaceae* and *I. praetervisa* var. *flavofulvida* are shown to be synonyms of *I. phaeocystidiosa* based on ITS sequence data from type collections. A new species sister to *I. phaeocystidiosa* with a Mediterranean distribution is described as *I. praetervisoides*. Cystidial morphology, distribution of caulocystidia, basidiospore morphology and ecology are shown to be the main diagnostic characters for separating the species. *Inocybe praetervisa* and *I. phaeocystidiosa* have a transoceanic distribution in Europe and North America, whereas *I. praetervisoides* so far is known only from the Mediterranean region.

Key words: Agaricales, barcoding, Inocybaceae, *Inocybe phaeocystidiosa*, molecular taxonomy, systematics

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Introduction

Inocybe praetervisa Quél. is the type species of the *I. praetervisa* group (subsection *Praetervisae* Bon 1998), a taxonomically intricate assemblage of taxa belonging to section *Marginatae* Kühner (1933). This section includes species characterized by nodulose to angular basidiospores and a more or less bulbous marginate stipe that never produces pinkish or reddish tinges. *Inocybe praetervisa* is a frequently used name, often described and/or cited in monographs and modern taxonomic literature mostly in Europe (see Materials and methods). *Inocybe praetervisa* is also the type species of the genus *Astrosporina* J. Schröt. (Horak 1968). This genus is not recognized phylogenetically (Matheny et al. 2002) and thus is considered a synonym of *Inocybe* (Fr.) Fr.

Several phylogenies have shown that *Inocybe* species with nodulose basidiospores do not form a monophyletic group (Matheny et al. 2002, Matheny 2005, Kropp et al. 2010, Ryberg et al. 2010). The species of the *I. praetervisa* group were included by Ryberg et al. (2010) in clade VII, characterized by a marginate bulbous stipe covered with caulocystidia. A distinct feature of many taxa in the *I. praetervisa* group is the more or less intense darkening in the flesh and on the stipe surface and a yellow-brown intracellular pigment in the trama and hymenial cells, especially in old or dried specimens. This character has proved to be useful for characterizing the *I. praetervisa* group but only on a qualitative basis in that it shows variable intensity (Kuyper 1990) and probably is influenced by several factors (especially the drying process) as well as intraspecific variation.

Sequences in GenBank show that many taxa in the *I. praetervisa* group have been misinterpreted or erroneously determined and many different names have been applied to some species. This reveals the need for a revision of the group through type studies using morphological and molecular species recognition approaches. Esteve-Raventós et al. (2015) have recently undertaken a revision of the *I. xanthomelas* Boursier & Kühner complex and have showed that the "xanthomelas clade" is not closely related phylogenetically to the *I. praetervisa* group. The species in the "xanthomelas clade" differ in a more pronounced darkening (even blackening) and elongate or slender sublageniform cystidia with a well defined and protruding neck.

In contrast darkening is absent or indistinct in species of the *Inocybe mixtilis* (Britzelm.) Sacc. clade, which seems not to be closely related phylogenetically to *I. praetervisa* or to *I. xanthomelas*. The recently proposed epitype of *I. mixtilis* (Marchetti et al. 2014) is an important step in disentangling the subsection *Praetervisae* sensu Bon (1998). Traditionally *I. praetervisa*, *I. mixtilis* and *I. xanthomelas* have been considered closely related on the basis of morphological characters.

The results presented here reveal that the concept of *I. praetervisa* adopted by many mycologists is different from the original concept. Most modern taxonomists have followed Kühner's (1933) interpretation, but we consider his concept of *I. praetervisa* heterogeneous. Type studies and ITS data reported here demonstrate that *I. praetervisa*, as described by Bresadola, is not uncommon in boreal and subalpine areas of Europe and North America (Canada). It is also cited by Kobayashi (2002) from Japan but, according to his description, these reports probably correspond to a different taxon.

MATERIALS AND METHODS

Specimens were collected in different European countries, and many of these collections are deposited in AH (Alcalá University Herbarium, Spain). Additional collections were loaned from the Bresadola herbarium (S; Swedish Museum of Natural History, Stockholm), Kühner's Herbarium (G; Conservatoire et Jardin botaniques de la Ville de Genève, Switzerland) and Jukka Vauras Herbarium (TUR; Herbarium of the University of Turku, Finland). Some collections are deposited in private herbaria: Erminio Ferrari (EF) in Italy, Fermín Pancorbo (FP) and Agustín Caballero (AC) in Madrid and La Rioja (Spain), respectively. Some of these collections have been deposited as duplicates in AH (with permission of the collectors). Specimens examined is provided as supplementary material.

Morphological study.—Preparations of cystidia, basidia and basidiospores were mounted both in water and 5% ammonium hydroxide 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). Scanning electron micrographs (SEM) were obtained with a Zeiss DSM-950. For ultramicroscopic studies the material was rehydrated in concentrated ammonium hydroxide (28–30%) for 30 min, dehydrated in aqueous ethanol (70%) for 30 min, fixed for 2 h in pure ethylene glycol dimethylether (= 1,2-dimethoxymethane) and immersed in pure acetone at least 2 h. This was followed by critical point drying and sputtering with gold-palladium. This technique allows the use of little material (small portions of lamellae).

Basidiospore measurements are quoted according to Heinemann and Rammeloo (1985). Colors of basidiomata were compared with reference colors in Munsell® (1994). Terminology follows Kuyper (1986) and Vellinga (1988). Herbarium acronyms follow Thiers (2012). Specific bibliographies, monographs and keys on *Inocybe* from different continents have been used as a general tool for the study and recognition of collections: Kauffman (1924), Heim (1931), Malençon and Bertault (1970), Stuntz (1978), Alessio and Rebaudengo (1980), Kuyper (1986), Nishida (1989), Stangl (1989), Bon (1998), Kobayashi (2002), Zitzmann (2002), Ferrari (2006, 2010), Larsson et al. (2009), Kropp et al. (2010), Jacobsson and Larsson (2012), Kokkonen and Vauras (2012), Outen and Cullington (2012).

DNA extraction, PCR amplification and sequencing.—DNA was extracted from dried specimens. The extraction procedure followed Doyle and Doyle (1987). The nuc rDNA ITS1-5.8S-ITS2 region (ITS) was amplified, employing ITS1 and ITS4 primers for PCR and sequencing (White et al. 1990). The QIAGEN PCR purification kit was used for PCR-product cleaning. The amplicons were sequenced by Macrogen Europe, the Netherlands.

Novel sequences generated (SUPPLEMENTARY TABLE I) were subjected to BLAST to screen for contaminants. Sequences were assembled and edited with Sequencher 4.7 software (Gene Codes Corp., Ann Arbor, Michigan) and submitted to the EMBL/GenBank- /DDBJ databases (Cochrane et al. 2010). The alignment was done with MAFFT 6.864b under the anysymbol option (Katoh and Toh 2008) and was optimized manually in MacClade (Maddison and Maddison 2003).

Phylogenetic analyses.—Taxa were selected based on more inclusive analyses of rDNA ITS and partial 28S sequences of *Inocybe* with nodulose basidiospores (Fernando Esteve-Raventós unpubl). Specimens sequenced in this study are presented (SUPPLEMENTARY TABLE I). A sequence from *Inocybe mixtilis* (GenBank accession KJ938767) was selected as the outgroup. The whole alignment is available in TreeBase under accession number T17824. The alignment was analyzed with Bayesian (MB) and máximum-likelihood (ML) approaches. The Bayesian analysis was conducted in MrBayes 3.2.1. (Ronquist et al. 2012). The substitution model was sampled across the GTR space (Ronquist et al. 2012). Two parallel analyses of four MCMC chains were run for 10 million generations, starting from a random tree, and sampling one tree every 100th generation. To check whether the chains had converged, determine whether the mixing was adequate and to choose an appropriate burn-in, log-likelihood values were plotted against the time generation with Tracer 1.5 (Rambaut and Drummond 2007). Stationarity was assumed when the average standard deviation of split frequencies fell below 0.01. A burn-in sample of 25 000 trees was discarded from each run. To assess branch confidence, a 50% majority rule consensus tree was computed with the remaining 150 002 trees with the SUMT command of MrBayes. Bayesian PP values $\geq$ 95 % were considered to be significant. The ML analysis was implemented via CIPRES Science Gateway (Miller et al. 2010), employing the RAxML HPC2 on XSEDE tool (Stamatakis 2006), using mixed models of evolution, starting from a random tree and leaving the remaining options as default. For branch confidence 1000 ML bootstrap replicates were conducted with rapid bootstrapping. A 50%

majority rule consensus tree was made to obtain the bootstrap values in PAUP 4.0 Beta for Mac (Swofford 2002). ML bootstrap values were placed on the majority rule Bayesian phylogram.

RESULTS

Eighteen new ITS sequences were generated (SUPPLEMENTARY TABLE I) and were aligned with 35 sequences downloaded from GenBank. The complete alignment contained 53 taxa and 736 characters. The Bayesian analysis reached an average standard deviation of split frequencies of 0.01 after 445 000 generations. The 50% majority rule consensus tree obtained from the MB analysis is presented (FIG. 1). The ML analysis resulted in a single best ML tree of −lnL = 3360.963231. Two major supported clades were inferred by both analyses: i. Clade A, encompassing *I. flavobrunnescens* Esteve-Rav., G. Moreno & Bizio, *I. hirculus* Vauras, *I. praetervisoides* and *I. phaeocystidiosa* Esteve-Rav., G. Moreno & Bon (PP and ML 100%); and ii. Clade B, encompassing *I. decemgibbosa* (Kühner) Vauras *I. fibrosoides* Kühner, *I. margaritispora* (Berk.) Sacc., *I. phaeosticta* Furrer-Ziogas, *I. tabacina* Furrer-Ziogas and *I. praetervisa* s. str. (PP 98%; ML70%). Species in clade A and B differ in morphological patterns of cystidia, more protruding and elongate, sublageniform to fusiform in clade A and shorter, more ventricose and/or broader, subclavate, utriform in clade B. Sequences identified as *I. praetervisa* in GenBank nest either in the *I. phaeocystidiosa* clade (HQ604596) or in the *I. praetervisa* clade (HQ604401, HQ604397), which included a sequence derived from an authentic Bresadola specimen. Sequences obtained from the type specimens of *I. praetervisa* var. *flavofulvida*, *I. salicis-herbaceae* and *I. phaeocystidiosa* are in the *I. phaeocystidiosa* clade. The *I. phaeocystidiosa* clade and the *I. praetervisa* clade reveal a transoceanic distribution in temperate and boreal areas of the northern hemisphere but do not reveal a clear internal geographic structure. Three Mediterranean specimens identified as *I. praetervisoides* form a strongly supported clade (PP and ML 100%) sister to *I. phaeocystidiosa* but lack branch support.

TAXONOMY

Inocybe praetervisa Quél. in Bres, Fung. Trident. 1: 35. 1881. FIG. 2

≡ *Astrosporina praetervisa* (Quél.) J. Schröt. in Cohn, *Krypt.-Fl. Schlesien* 3(1): 576. 1889.

Typification: Bresadola, Fung. Trident. 1: Tab. 38. 1881 (**lectotype**, designated here). ITALY. Rabbi, Jul 1909, in silvis coniferis, leg. *G. Bresadola*, S-F229598 (**epitype**, designated here).

MycoBank: MBT201675.

ITS barcode GenBank: KT203792.

= *Inocybe rivularis* Jacobsson & Vauras, *Windahlia* 18: 18. 1989 (holotype, FINLAND. Oulun Pohjanmaa, Hakala, 4 Aug 1986, J. Ruotsalainen & J. Vauras 2152F [TUR 98791]. Isotype in GB).

Notes: The French mycologist Lucien Quélet is the attributable authority of this species, which was published in Bresadola (1881); however, it was Giacomo Bresadola who described and provided an excellent watercolor painting in the original description in Fungi Tridentini (Bresadola 1881:35, Pl. XXXVIII). The original painting includes all the main diagnostic features that make this species recognizable, including habit, color, basidiospores and cystidia. This plate is the only original material of *I. praetervisa* known to us, and we therefore propose a lectotypification with it.

There are no known collections of *I. praetervisa*, which predate the original description in Fungi Tridentini (PC, TR, S). Of interest, three Bresadola specimens of *I. praetervisa* were found in S, all gathered after 1881; however, all of them were collected in the same localities mentioned in the protolog. Horak (1968) pointed out the lack of original material of *I. praetervisa* in Bresadola's collections (mostly located at TR) and suggested that such a specimen might be found in Quélet's Herbarium at PC. No Quélet specimen of *I. praetervisa* is deposited at PC (Patrice Lainé pers comm).

An examination of these collections confirmed that they are all conspecific and adhere to the original species concept. The most distinctive characters can be summarized as follows: i. robust habit (pileus 30–60 mm diam, stipe 40–70 × 4–10 mm); ii. pileus narrowly conical when young, smooth and shiny (lubricous when wet), often showing an asymmetric shape; pale fulvous-ochraceous to buff or light yellow brown when young (Mu 10YR 7/3-4, 6/3-6), becoming browner with age (Mu 10YR 5/3-6); surface distinctly rimose in the outer half; iii. lamellae pale, whitish at first, with a grayish reflection (Psathyrella-like) during the early maturing process; iv. stipe white in early stages, more or less marginate bulbous, pruinose in the upper half, sparsely or apparently not pruinose in the lower half or lower third, browning in dry specimens, not distinctly blackening upon drying or in exsiccatum; v. basidiospores 8.4–10.8–13.3(–13.5) × 6.4–7.35–8.3(–8.5) µm, Q_m 1.25–1.47–1.7 (n = 33), heterodiametric, provided with 8–11 distinct obtuse knobs, 1–3 µm high and up to 2(–2.7) µm broad at base; vi. cheilo- and pleurocystidia broad, (50–)55–65(–80) × (18–)20–26(–28) µm, utriform, broadly fusiform, subclaviform or rarely sublageniform, with short and obtuse neck, apex not very crystalliferous or without crystals, walls 2–3.5 thick at the apex, pale yellow in ammonia solutions; vii. yellowish intracellular pigment present in some hymenial cells (cystidia, basidia,

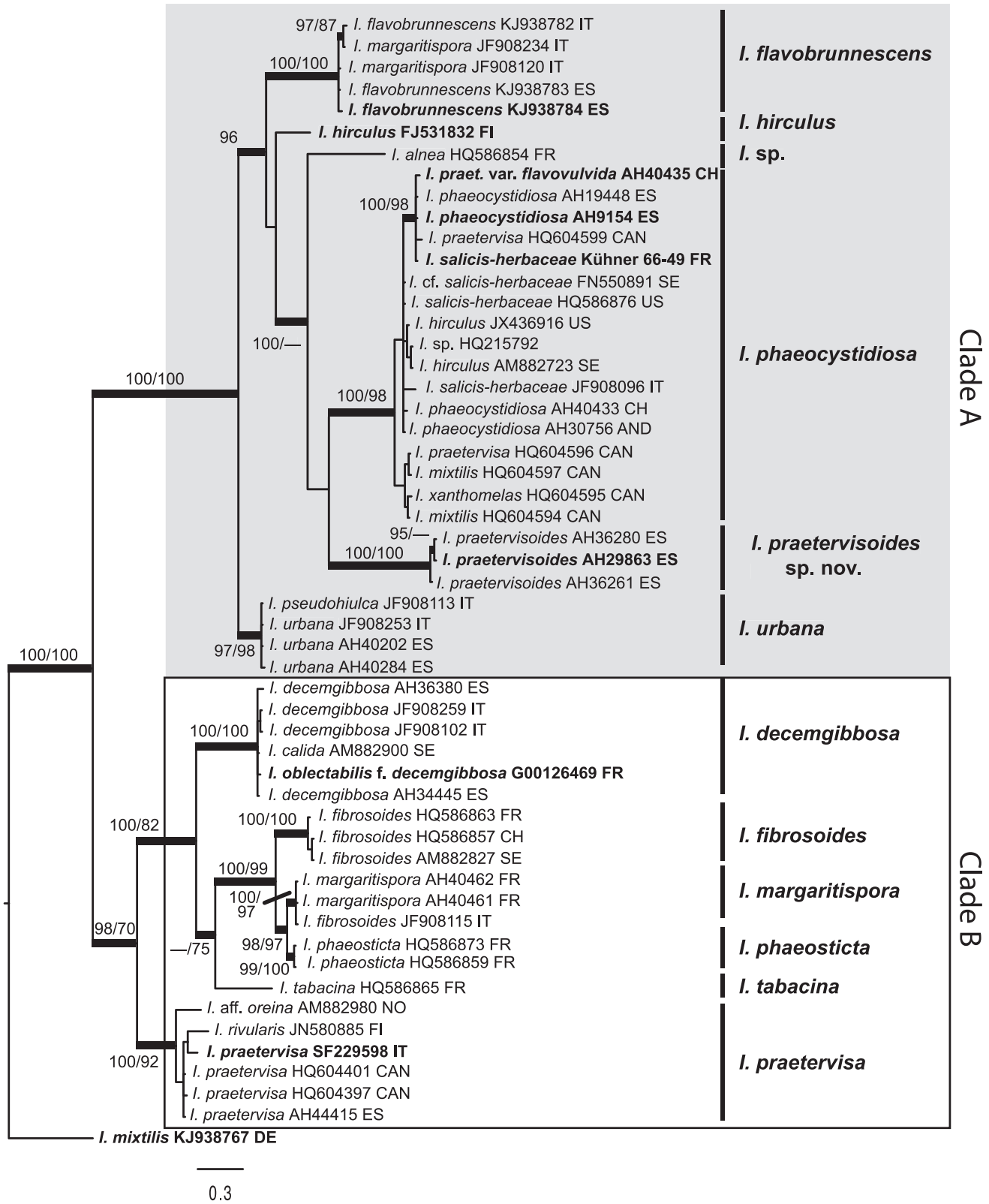

FIG. 1. Bayesian inference 50% majority-rule consensus phylogram of *Inocybe pratervisa* group from ITS sequence data. Bayesian posterior probabilities (≥ 95%)/maximum likelihood bootstrap values (≥ 70 %) are next to nodes. Thick lines indicate branches that are supported either in the Bayesian or maximum-likelihood analysis. Type collections are highlighted in boldface. Country of origin for each collection is provided with ISO country codes. Names of species recognized are indicated by vertical bars.

FIG. 2. *Inocybe praetervisa* Quél. A. Authentic material from Stockholm Herbarium (S), designated as epitype (S-F229598). B. Basidiomata in situ (AH 44438). C–H. Hymenial cystidia. I. Basidiospores under optical microscope. J. Basidiospores under SEM. (C–J from the epitype). Bars: A, B = 1 cm; C–I = 10 µm; J = 2 µm.

trama) but never brown or dark brown; and vii. caulocystidia numerous on the upper half of the stipe, becoming sparse and rare on the lower half or third; in some collections apparently absent and only some clavate paracystidia present, either thin- or thick-walled, mostly not crystalliferous.

An ITS sequence was obtained from one of Bresadola's collections (S-F229598) and so we designate this collection as the epitype supporting the lectotype proposed here.

The sequence obtained from a paratype of *I. rivularis* (3610F, JN580885), collected in the type locality, nests in the same clade as the epitype specimen of *I. praetervisa*. This supports the synonymy of *Inocybe rivularis* Jacobsson & Vauras and *I. praetervisa*. *Inocybe rivularis* originally was described from the boreal zone in northern Europe (Finland and Sweden), where it is a frequent species that grows in humid mixed forests with conifers (*Picea*), *Betula* and other riparian trees such as *Alnus* and *Salix* and is often found in *Sphagnum*

FIG. 3. *Inocybe phaeocystidiosa* Esteve-Rav., G. Moreno & Bon. A, B. Holotype (AH 9154). C. Paratype (AH 3953). D. Basidiomata in situ (AH 19448). E, I. Hymenial cystidia under optical microscope. J. Hymenial cystidium by SEM. K. Basidiospores under optical microscope. L. Basidiospores by SEM. (E–L, from holotype). Bars: A–D = 1 cm, E–K = 10 µm, L = 2 µm.

bogs in slightly acidic soils (Jacobsson and Vauras 1989). These authors assumed that *I. rivularis* is probably associated with *Betula*, which appeared to be the constant ectomycorrhizal host in every collection they studied. The morphological features of *I. rivularis* also fit *I. praetervisa* in every respect. *Inocybe rivularis* originally was considered by its authors an unusual species within section *Marginatae* Kühner owing to the lack of caulocystidia on the lower half of the stipe and the presence of an ephemeral velum at the pileus margin. In some collections we studied caulocystidia seemed to be sparse or apparently absent on the lower

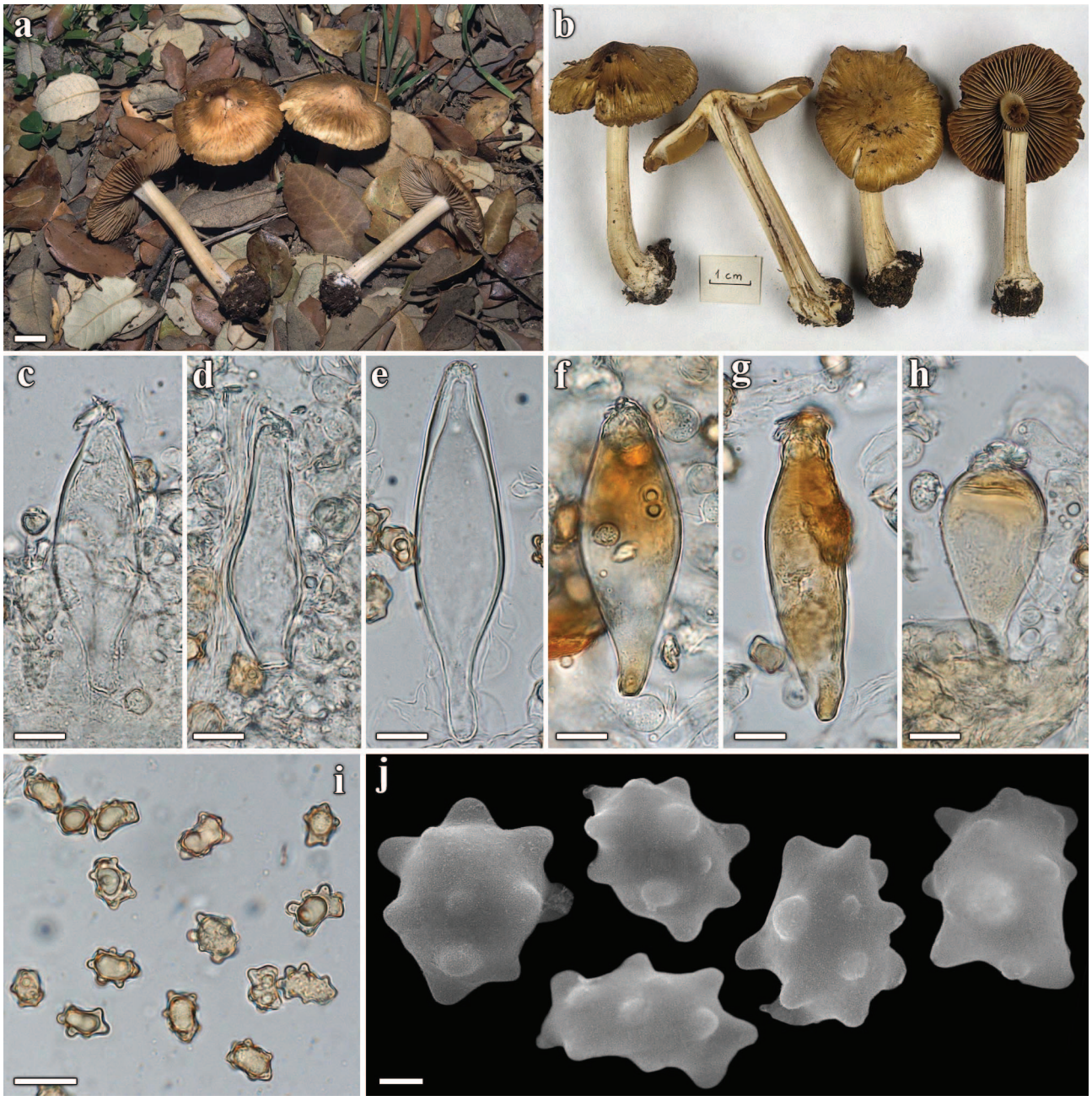

FIG. 4. *Inocybe praetervisoides* Esteve-Rav., G. Moreno & Olariaga, sp. nov. (Holotype AH 29863). A, B. Basidiomata. C–H. Hymenial cystidia (some with brownish content). I. Basidiospores under optical microscope. J. Basidiospores by SEM. Bars: A, B = 1 cm; C–I = 10 µm; J = 2 µm.

half (smooth under the lens) and thus difficult to observe. With reference to the habitat, which was not described in detail by Bresadola, the presence of *Betula* is possible in the Rabbi area because *Betula* is common in the Italian Alps. The Spanish collections were gathered in *Pinus sylvestris* forests, but *Betula* occurs near the streams and brooklets, in granitic soils. The progressive browning of the basidiomata (golden brown to bronze) during development is also a diagnostic feature that is more intense in old specimens and dry material or exsiccata but never develops blackish coloration.

As inferred from sequences in GenBank (FIG. 1), *I. praetervisa* is also represented in Norway (AM882980, as *I.* aff. *oreina*, Ryberg et al. 2008) and Canada (HQ604397, HQ604401 as *I. praetervisa*, Berbee et al. unpubl). It has a summer fruiting period and a boreal distribution but occurs in montane and subalpine stages of mountain ranges of central and southern Europe.

Inocybe phaeocystidiosa Esteve-Rav., G. Moreno & Bon in Esteve-Raventós & Moreno, Doc. Mycol. 17(67):18. 1987 (holotype, SPAIN: Segovia, San Rafael, near the Alto del León, 1500 m, 29 May 1985, in *Pinus sylvestris* forest on granitic soil, *G. Moreno & F. Esteve-Raventós*, AH 9154).

ITS barcode GenBank: KT203789 FIG. 3

= *Inocybe salicis-herbaceae* Kühner, *Doc. Mycol.* 19(74): 24. 1988 (holotype, SWITZERLAND: Graubünden, S-charl, Schombrina, 2100 m, 15 Aug 1966, in *Salix herbacea* shrubs, *R. Kühner*, GR 66-49 [G00110923]). ITS barcode GenBank: KM226889

= *Inocybe praetervisa* var. *flavofulvida* E. Ferrari & Brignoli, *Boll. Gruppo Micol. G. Bresadola* 43(1): 17. 2000 (holotype, SWITZERLAND: Vallese, Briga, south side of Passo del Sempione, 2400 m, 5 Sep 1999, in *Salix herbacea* shrubs, *E. Brignoli & E. Ferrari*, EF 53/99. Isotype AH40435). ITS barcode GenBank: KT203787

Notes: Most records of *I. praetervisa* have been misinterpreted in Europe (Kühner 1933, Favre 1955, Favre 1960, Alessio and Rebaudengo 1980, Horak 1987, Huhtinen 1987, Bizio 1995, Jamoni 2008, Consiglio et al. 2014), North America (Matheny 2008) and Asia (Kobayashi 2002). These records are mainly referred to *I. phaeocystidiosa* Esteve-Rav., G. Moreno & Bon, characterized by its large fusiform cystidia occurring on the stipe. Both *I. praetervisa* and *I. phaeocystidiosa* share a similar biogeographical distribution and probably ecology, but their morphological differences are distinct. These differences were overlooked by most mycologists because Bresadola's material had not been previously studied (Takahito Kobayashi revised the collections deposited at S, but his data remains unpublished). Some of the records of *I. praetervisa* from temperate frondose or mixed forests in central Europe might be other species of the *I. praetervisa* group (Stangl 1989, Breitenbach and Kränzlin 2000, Eyssartier and Roux 2011). In fact Kühner's (1933) own interpretation of *I. praetervisa* is heterogeneous, including *I. phaeocystidiosa* (Savoie subalpine collections) and other taxa from warmer French localities. The same has been proven for Kühner's interpretation of *I. xanthomelas* (Esteve-Raventós et al. 2015).

Esteve-Raventós et al. (1987) described *I. phaeocystidiosa* from pine forests in high mountain areas of the Spanish Central Massif (Sierra de Guadarrama). When collected both the holotype and paratype showed striking brownish caulocystidia (seen easily under the lens), and hence the species was so named. However, as pointed out by Kühner (1933) and Kühner and Romagnesi (1953), the context and flesh in the *I. praetervisa* group darken progressively and even turn blackish in the *I. xanthomelas-I. krieglsteineri* clade (Esteve-Raventós et al. 2015). This darkening also can be noticed in microscopic examination, in that elements of the trama and hymenium have a distinct yellow to dark brown intracellular pigment (cystidia, basidia), which become conspicuous in old specimens or after rapid desiccation. According to our own observations, and as remarked by Kuyper (1990), this character should be used with caution because it can be influenced quantitatively by external factors, such as the basidioma age, humidity or drying method.

Some macro- and microscopic features of *I. phaeocystidiosa* are different from those of *I. praetervisa* and allow a clear separation without molecular tools, and ITS analyses reveal that both species cluster in different clades (FIG. 1). The main diagnostic features of *I. phaeocystidiosa* include: i. pileus not distinctly rimose in the outer half, but radially fibrillose, occasionally cracked, conical convex in young stages, brown-yellowish in young specimens (Mu 10YR 6/6–8, 7.5YR 6/6–8), not turning especially darker with age or upon drying; ii. stipe distinctly and densely pruinose throughout, pruina apparent even near the marginate bulbous base (see young specimens); iii. pleurocystidia large to very large, 65–90(–100) × 15–23(–25) µm, markedly protruding, fusiform, progressively attenuated toward the apex, crystalliferous; walls thick (2–3.5 µm), pale yellowish in ammonia solutions; iv. basidiospores heterodiametric to subheterodiametric 9–11–12.9 × 6.6–8.2–10 µm. Q_m 1.1–1.33–1.56, (n = 30), ornamented with 11–14(–16) distinct obtuse knobs, 0.8–1.4 µm high and 1.3–2 µm broad at base; and v. flesh and stipe surface turning ash gray to dark gray on aged specimens or after drying, often unevenly. Like *I. praetervisa*, some young collections may have a thin, fugacious, white veil coating at the pileus margin, but the stipe always has abundant and dense caulocystidia reaching the marginate bulb.

Inocybe phaeocystidiosa is widespread in the alpine and boreo-alpine regions of Europe and North America. Kühner (1988) described this species from the French Alps as *Inocybe salicis-herbaceae* (see also Favre 1955 as *I. praetervisa*) in association with dwarf willows (mainly *Salix herbacea* but also with other small species such as *S. foetida*). Such boreo-alpine collections or ecological forms, such as the one described by Kühner (1988), are characterized by macroscopically smaller basidiomes, but microscopically they are similar to the montane or subalpine collections. Of interest, Ryberg et al. (2011) demonstrated that there is a weak specificity in ectomycorrhizal mushrooms associated with *Salix herbacea* and *S. polaris* in the tundra, and this statement could be applied to *I. phaeocystidiosa*, which also grows under coniferous trees in subalpine areas. Examination of the *I. salicis-herbaceae* holotype yielded the following microscopic data: i. basidiospores heterodiametric to (sub-)isodiametric, 9–10.3–11.5 (–11.7) × 6.9–7.9–8.8, Q_m = 1.2–1.3–1.4 (n = 21),

ornamented with obtuse distinct knobs (similar in outline to *I. phaeocystidiosa*); ii. pleurocystidia fusiform, large and protruding, 60–75(–90) × 14–22(–25) µm, walls thick (2–3.5 µm at apex), pale yellow in ammonia solutions, often with a yellow intracellular pigment; iii. cheilocystidia similar or slightly shorter, mixed with numerous clavate paracystidia; iv. caulocystidia similar to hymenial cystidia, abundant, mixed with numerous paracystidia forming bundles, even near the bulbous base. Measurements of microscopic characters correspond well to the range of variation observed in collections of *I. phaeocystidiosa*, and the ITS analyses show that the sequence of the holotype of *I. salicis-herbaceae* is almost identical to that from the holotype of *I. phaeocystidiosa* (one deletion in the holotype of *I. salicis-herbaceae*), which supports the synonymy of both taxa (FIG. 1).

The ITS sequence obtained from the holotype of *I. praetervisa* var. *flavofulvida* also confirms that it is conspecific with *I. phaeocystidiosa* (FIG. 1). An isotype of *I. praetervisa* var. *flavofulvida* also reveals similar morphological characters to *I. phaeocystidiosa*. It originally was described from the Swiss Alps, close to the Italian border, in alpine *Salix herbacea* beds (Ferrari and Brignoli 2000). Observations and measurements from the isotype are: i. basidiospores heterodiametric to subisodiametric, 9.1–10.6–12 × 7.5–8.6–9.7(–10) µm, with numerous obtuse knobs (similar in number and height to those of *I. phaeocystidiosa*), Q_m = 1.06–1.22–1.38(–1.46) (n = 30); ii. pleurocystidia fusiform, large and protruding, (60–)65–80(–90) × 17.5–21(–23) µm, often crystalliferous but sometimes devoid of crystals at the apex, with thick walls (2–3[–4] µm at the apex), pale yellowish to hyaline in ammonia solutions and usually with yellow-brown intracellular pigment; iii. cheilocystidia slightly smaller but also protruding and similar to pleurocystidia, 60–70(–75) × (14–)17–20 µm; and iv. caulocystidia similar to hymenial cystidia or somewhat shorter, abundant over the stipe surface, forming bundles with numerous paracystidia, even near the bulb.

Abundant brown-yellow pigment makes *I. praetervisa* var. *flavofulvida* basidiomes darker than usual and hence distinct, but this character seems environmentally influenced and merely quantitative. Before studying the isotype a close relationship was suspected with *Inocybe humilis* (Favre 1960) gathered originally from Swiss subalpine areas and, according to Favre, characterized by distinctive yellow. However, *Inocybe humilis* has much shorter (< 60 µm) and not protruding, fusoid ventricose cystidia with short, obtuse necks. Furthermore, the basidiospores in *I. humilis* are subisodiametric to isodiametric (substellate), and caulocystidia are rare and replaced by paracystidia and hair-like elements on the lower third of the stipe (Esteve-Raventós unpubl, according to type revision). Unlike *I. phaeocystidiosa*, the lower part of the stipe is fibrillose and not truly pruinose under the lens. No molecular data have been obtained from the type material or any other collection of *I. humilis*. Its morphological features suggest that it is a different taxon, perhaps related to *I. substellata* or *I. obtusiuscula* (Kühner 1988), owing to a similar stipe covering and cystidial morphology.

Inocybe praetervisoides Esteve-Rav., G. Moreno et Olariaga, sp. nov. FIG. 4
MycoBank MB812917

Typification: SPAIN. MADRID: Cenicientos, 3 May 2002, in humus of *Quercus ilex* subsp. *ballota* forest, on sandy granitic soil, leg. *J.C. Campos* (**holotype** AH 29863). ITS barcode GenBank: KT203794

Etymology: praetervisa (*L.*), *Inocybe-*, due to apparent confusion with this taxon.

Illustrations: Esteve-Raventós and Caballero Moreno (2009: photos 62–64, FIG. 22, as *I. xanthomelas*)

Pileus (15–)20–40(–45) mm diam, 15–25 mm high when young, at first broadly conical to campanulate, then expanded to convex or applanate at maturity, distinctly and broadly umbonate; margin deflexed to inflexed or straight at maturity, sometimes irregular, undulate to lobate, neither hygrophanous nor striate; color uniform or slightly darker toward the center, revealing a wide range of yellow tones, alutaceous, buff-yellow, ocher-yellow or yellow-brown (Mu 10YR 8/6, 7/6-8 or 6/6-8); surface radially fibrillose, silky and smooth, exceptionally radially or irregularly cracked toward the margin but never squamose, not rimose, shiny and sticky in wet condition but not viscid (some soil particles may adhere to pileus); velipellis fugacious and apparently absent, visible only as a whitish patch in primordia or on young specimens. Lamellae close (L = 40–48, l = 1–2), ventricose to subventricose, adnexed; initially dirty white to cream, then grayish to argillaceous, often with a lilac reflection (*Psathyrella*-like), then brown-ochraceous to grayish-brown, without olivaceous tints at maturity; edge paler to concolorous at maturity, crenulate. Stipe 25–50(–60) × 3–7(–8) mm, initially solid and fibrillose, becoming hollow in age, cylindrical or slightly tapering upward; base abruptly bulbous (often napiform) or even marginately bulbous in some specimens; bulb up to 9–10(–12) mm diam; initially white to argillaceous, then white-yellowish (Mu 2.5Y 8/1–4, 5Y 8/1) or straw-ocher (Mu 10YR 7/4–6), often darkening in age or during the drying process, either partially or completely, becoming grayish to grayish brown or grayish-black (Mu 10YR 6/2–4, 5/2–3); surface evenly and densely pruinose, less so in the lower one-third, but also dense near the bulb, longitudinally striate to sulcate in some specimens; cortina absent. Context firm, white in the pileus, yellowish to concolorous with the stipe surface; odor weak or practically absent, sometimes

faintly spermatic or herbaceous-raphanoid; flavor not recorded. Color of exsiccata brown-yellow to dark brown or gray-brown (Mu 10YR 5/4–8, 4/2–6), more intense on the stipe surface. Basidiospores 9.4–10.8–12.3(–12.5) × 7–7.9–9 µm, Q_m 1.2–1.37–1.5(–1.6) [n = 30], heterodiametrical to subisodiametrical, with a distinctly nodulose profile, ornamented with (9–)13–17 obtuse knobs, 0.9–1.5(–2) µm high, 1–1.7(–2.5) µm broad at base, yellow in ammonia solutions, apiculus distinct. Basidia 25–36 × 9–14 µm, distinctly clavate, 4–(2)-spored, with sterigmata up to 5 µm long. *Pleurocystidia* numerous and protruding from the hymenium, metuloid, (50–)60–80(–100) × (11–)13–20(–25) µm, in general slender and narrowly to broadly fusiform to subcylindrical, occasionally subclavate, mostly devoid of a well delimited neck (and hence not lageniform), crystalliferous at apex; walls 1–2 µm thick, not or slightly thickened at the apex (–3 µm), yellow in ammonia solutions but variable in intensity; content often with a yellow-brown intracellular pigment, more or less evident depending on collections, normally developing upon drying or in aged specimens. Lamella edge mostly composed of numerous cheilocystidia and clavate paracystidia, with some non-crystalliferous intermediate elements, often with brown-yellow intracellular pigment, more or less evident depending on collections. Cheilocystidia abundant, similar in shape to pleurocystidia, often smaller, walls dull yellow in ammonia, apex crystalliferous. Hymenophoral trama regular, constituted of parallel hyphae, 5–18 µm wide, cylindrical to fusiform, often constricted at septa, with pale yellow intracellular pigment. Subhymenium consisting of 1–3 layers of small and subisodiametrical cells, 1–5 µm diam. Pileipellis an ixocutis or repent, parallel, densely packed cylindrical hyphae, 5–10 µm wide, with encrusting, slightly zebroid yellowish pigment; subcutis hardly differentiated, with slightly wider elements. Stipitipellis consisting of a cutis bearing numerous bundles of caulocystidia, intermixed with abundant paracystidia, similar in shape and size to hymenial cystidia, still abundant at the lower third and reaching the bulbous base. Clamp connections present in all tissues.

Habitat and distribution. Fruiting during spring (May and Jun) on humus in wet evergreen oak forests (*Quercus ilex* subsp. *ballota*) on sandy, acidic to decalcified soils. All known collections have been gathered in Spain; however, it is expected to be widespread in the Mediterranean region in similar habitats (see comments). All collections from La Rioja were published as *I. xanthomelas* by Esteve-Raventós and Caballero Moreno (2009).

Notes: Inocybe praetervisoides and *I. phaeocystidiosa* are sister species of similar morphology but exhibit different distributional patterns with *I. praetervisoides*, which has only been found in Mediterranean evergreen forests (*Quercus ilex*). The macro- and micromorphological characters of *I. praetervisoides* are similar to *I. phaeocystidiosa*, both with abundant large caulocystidia on the stipe and a distinct marginate bulb. In addition to different distributional patterns, the cystidia are more thin-walled in *I. praetervisoides* (FIG. 4c–h), and basidiospores have a greater density of knobs (FIG. 4i–j). Some of the *I. praetervisa* records from mesophilous and temperate European frondose or mixed forest or parks in central Europe (Stangl 1989, Breitenbach and Kränzlin 2000, Eyssartier and Roux 2011) do not represent *I. praetervisoides*. Some other taxa belonging to the *I. xanthomelas* clade (i.e. *I. hirculus*, *I. ochracea* [Stangl] Stangl or *I. urbana* Alessio [Esteve-Raventós et al. 2015]) could be confused with *I. praetervisoides*, but their cystidia or basidiospores are different.

When considering the existing records of Mediterranean *Inocybe* spp. with nodulose spores, the interpretation of *I. xanthomelaena* Kühner & Boursier by Malençon and Bertault (1970: 407–408) from Morocco is interesting and probably can be attributed to *I. praetervisoides*. During a revision of the material deposited in MPU under *I. xanthomelaena*, Bizio (2009) noticed that collection No. 1937 and No. 2551 are not conspecific; the former belongs to the *I. praetervisa* group, whereas No. 2551 is a different species, probably *I. glabrodisca* P.D. Orton. The original description of coll. No. 1937 conforms to *I. praetervisoides*: "… sous *Quercus suber*, en sol sableux, … cystides fusoïdes et brunnissantes (70 × 20 µm), … celles des faces fusoïdes-ventrues (65 × 23 µm)" and "… spores noduleuses, 9.4–11 × 7 µm, montrant d'ordinaire 9–10 bosses arrondies bien nettes dans un profil rectangulaire …". Although Malençon indicated that caulocystidia were present only on the stipe apex, Bizio (2009) observed that No. 1937 had caulocystidia along its length.

An apparently closely related species growing in similar habitats is *I. flavobrunnescens* (Esteve-Raventós et al. 2015), which can be separated from *I. praetervisoides* by sublageniform cystidia with a well differentiated neck and more subisodiametric basidiospores with somewhat less pronounced ornamentation with shorter knobs. Furthermore, the universal veil in *I. praetervisoides* is fugacious and often apparently absent whereas in *I. flavobrunnescens* it is abundant and usually persistent. Molecular studies show that they are not sister phylogenetic species; *I. flavobrunnescens* belongs in the *I. xanthomelas* clade, characterized by a medium-small habit and strongly darkening flesh.

DISCUSSION

This study aimed to resolve species limits and to elucidate the correct names for several species within the *I. praetervisa* group. ITS provides good resolution for

species delimitation in the *I. praetervisa* group, as observed in other species complexes within *Inocybe* (Ryberg et al. 2008, Larsson et al. 2009). Obtaining ITS sequence data from type specimens is a useful approach to enable the correct interpretation and application of *Inocybe* names. Several morphological characters are also useful for species delimitation and are correlated with the phylogenetic signal obtained from the ITS region. Basidiospore and cystidium morphology, the distribution of caulocystidia on the stipe and ecological niche characterize each of the phylogenetic species obtained in our ITS analyses. Nevertheless morphological characters separating the sister species *I. phaeocystidiosa* and *I. praetervisoides* are subtle and not clear-cut; both species have a different distribution and ecological niche. The distribution of *I. praetervisa*, *I. phaeocystidiosa* and *I. praetervisoides* have a clear biogeographic or bioclimatic pattern, but most probably they are not associated with a single ectomycorrhizal host (Ryberg et al. 2011). Whereas *I. praetervisa* and *I. phaeocystidiosa* are widely distributed in boreal and alpine areas in Europe and North America, *I. praetervisoides* seems to be confined to the Mediterranean region. This suggests that their distribution is conditioned by bioclimatic factors, rather than by the presence of a specific ectomycorrhizal host.

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