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Inocybe lemmi, a new species of section Marginatae from the alpine region of Sweden

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A new species, *Inocybe lemmi*, is described from alpine areas of Sweden. It is closely related to *I. candidipes,* associated with *Pinus ponderosa* and described from the southwestern United States. Both belong to the *I. praetervisa* group in section *Marginatae*, reserved for species characterized by a stipe with abundant caulocystidia in the upper half, which are sparse in lower half of the stipe. Other species in the *I. praetervisa* group have distinctly nodulose heterodiametric spores, while *I. lemmi* and *I. candidipes* have basally nodulose to angular spores with an elongated apex. A single collection from the alpine zone in Colorado was identified as representing a third lineage, closely related to *I. lemmi* and *I. candidipes*. The holotype of the recently described species, *I. tundrae,* was studied and is confirmed to be a later synonym of *I. rivularis*.

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Introduction

The name *Inocybe praetervisa* Quél. has been used in Fennoscandia for specimens of section *Marginatae* Kühner that mostly grow in parks and deciduous forests and have a rimose yellowish-brown cap lacking a velipellis. Micromorphologically it is characterized by having distinctly nodulose heterodiametric spores, utriform to broadly fusiform cheilo- and pleurocystidia with rather thick walls and crystals at the apex. Several collections have been deposited in Fennoscandian herbaria under this name, but the species concept used is very broad and can be applied to several taxa (Jacobsson & Larsson 2012).

Recently *I. praetervisa* was typified by Esteve-Raventós et al. (2016) and the ITS sequence from an epitype was generated. In the same paper the authors also synonymized *I. rivularis* Jacobsson & Vauras with *I. praetervisa*, referring to the similarity in morphology and ITS sequence data. However, only one ITS sequence of *I. rivularis* was included in the phylogenetic analyses. The results in Esteve-Raventós et al. (2016) initiated a larger study dealing with the *I. praetervisa* species complex. A broad sampling of specimens was selected and included, collected in different ecological habitats and from a wide geographic origin. The phylogenetic analyses based on ITS and LSU sequence data showed the *I. praetervisa* complex to be comprised of four closely relat-

ed species in Europe: *I praetervisa*, *I. rivularis*, *I. favrei* Bon, and a new species described as *I. arctica* E. Larss., Vauras & C.L. Cripps (Larsson et al. 2017).

In Esteve-Raventós et al. (2016) *I. praetervisa* was analysed with a broader sampling of taxa from section Marginatae, and detailed morphological studies were made to characterize the recovered major clades by morphology. The *I. praetervisa* clade was then defined to include species with a stipe that have abundant caulocystidia in the upper half but sparse to almost absent caulocystidia in lower half of the stipe. This result was confirmed in the study by Larsson et al. (2017).

Just recently Ludwig (2017) published "Pilzkompendium 4", dealing with Cortinariaceae in Europe, where he also included the genus *Inocybe*. He described 47 new species of *Inocybe*. One of them, *I. tundrae* E. Ludw., is in morphology very similar to *I. rivularis*. The type specimen originates from Abisko area in northern Sweden, associated with *Betula nana* L., *Salix* spp., and *Dryas octopetala* L.

In 2016, an inventory of macrofungi was undertaken by the Swedish Mycological Society (http://www.svampar.se) in alpine region of the Padjelanta National Park in Northern Sweden (https://www.sverigesnationalparker.se/en/choose-park---list/padjelanta--badjelannda-national-park/). Many collections of the *I. praetervisa* complex were taken. Two of them were characterized by having spores more angular in outline and less nodulose than the other species in the *I. praetervisa* complex.

Kropp & Matheny (2004) described *I. candidipes* Kropp & Matheny from montane environments in Arizona in the southwestern United States. It was associated with *Pinus ponderosa* Douglas ex C. Lawson. The species is characterized by having basally nodulose spores with an elongated apex, i.e. rocket-shaped in outline. In the phylogenetic analysis, the species came out in a separate clade together with a sequence named *I. glabrodisca* P.D. Orton, and not as expected with other species with similar spore morphology, such as *I. chelanensis* Stuntz, in the *Cortinatae* clade.

In this study, we have selected for sequencing the two specimens from Sweden with angular spores, together with a previously identified specimen with very similar morphology, collected in the alpine zone in Colorado, United States, that was expected to represent the same species. Also, the ITS region of the holotype of *I. tundrae* was generated to be analysed and compared with sequence data of *I. rivularis* and the other species in the *I. praetervisa* clade. As the phylogenetic analyses suggest, the two specimens from Sweden represent an independent species, closely related to, but separate from both *I. candidipes* and the species represented by the single collection from Colorado. They are here described as new to science.

Material and methods

Morphological methods The majority of the sequenced specimens in this study were collected by the authors. The holotype of *Inocybe tundrae* was received as loan from herbarium M, and permission to extract DNA and sequence the ITS region was granted. Abbreviations of herbaria follow Index Herbariorum (http://sweetgum.nybg.org/science/ih/). Macro-morphological characters were noted and cross-sections photographed from some fresh basidiocarps. Micro-morphological characters were measured and drawn from dried material mounted in 10% NH_4OH solution following the methodology described in Vauras & Kokkonen (2009).

Molecular methods A subset of twenty-four sequences from Larsson et al. 2017, representing *Inocybe praetervisa*, *I. rivularis*, *I. favrei and I. arctica* were selected and included in the analyses. In addition, the LSU of *I. glabrodisca* (AY239022) and *I. candidipes* (AY239019) generated in Kropp & Matheny (2004) were taken from GenBank and included in the data set. Sequence data of *I. decemgibbosa* (Kühner) Vauras and *I. mixtilis* (Britzelm.) Sacc. were used as out-group taxa in the analysis. Sequences from the complete internal transcribed spacer (ITS) region and about 1400 base pairs (bp) of the 5′end of the large subunit (LSU) of the nuclear ribosomal DNA were generated. Protocols for DNA extraction, PCR and primers used follow Larsson et al. (2017). Sequences were edited and assembled using Sequencher 5.1 (Gene Codes, Ann Arbor, Michigan). Sequences generated for this study have been deposited in GenBank. Alignment was performed using the L-INS-i strategy as implemented in MAFFT v. 7.017 (Katoh & Standley 2013). The alignment was adjusted using Aliview 1.17.1 (Larsson 2014). For inferring phylogenetic relationships among species heuristic searches for the most parsimonious trees were performed using PAUP* (Swofford 2003). All transformations were considered unordered and equally weighted. Variable regions with ambiguous alignment were excluded and gaps were treated as missing data. Heuristic searches with 1,000 random-addition sequence replicates and TBR branch swapping were performed, by the bootstrap method using 1,000 heuristic search replicates with 10 random taxon addition sequence replicates

and TBR branch swapping, the latter saving at most 100 trees in each replicate.

In addition a Bayesian analysis was carried out in BEAST 2.4.7 (Bouckaert et al. 2014), with a best-fit model of nucleotide evolution and partitioning supplied by the automated partitioning analysis in PAUP 4.0a build 159 (Swofford 2003). As suggested by PAUP, the nucleotide evolution model HKY+G was used for the ITS1 and ITS2 spacers and K80+G was used for the 5.8S and LSU genes in the Bayesian analysis. The xml-file for the BEAST run was prepared using BEAUti 2.4.7 (Bouckaert et al. 2014). Trees were set as linked and clock models as unlinked for the four DNA regions, applying a lognormal, relaxed clock to each. The clock rate of each region was estimated, using a lognormal prior with mean in real space and the clock rate set to one. Markov Chain Monte Carlo (MCMCMC) chains were run for 10 million generations with tree and log files sampled every 1,000 generations. The MCMC analysis converged well in advance of the 10 % burn-in threshold, had ESS values above 200 for all parameters, and chain mixing was found to be satisfactory, as assessed using Tracer 1.6.0 (Rambaut et al. 2014). After discarding the trees prior to the burn-in threshold a maximum clade credibility tree was computed in TreeAnnotator 2.4.7 (Bouckaert et al. 2014) from the remaining trees.

Results

The aligned complete dataset consisted of 34 sequences and 2104 characters. After exclusion of ambiguous regions mainly from the beginning and the end of the data set 1946 characters remained for the analysis. Of these, 1713 were constant, 35 were variable but parsimony uninformative, and 198 (10.2%) were parsimony informative. The maximum parsimony analysis yielded 23250 equally most parsimonious trees (length = 285 steps, CI = 0.9228, and RI = 0.9552). One of the trees is presented as a phylogram in Fig. 1. The bootstrap analysis recovered the *Inocybe praetervisa* species complex with 100% support. Five supported clades with medium to strong bootstrap support were found within the complex corresponding to *I. praetervisa* (95%), *I. favrei* (89%), *I. rivularis* (88%), *I. arctica* (96%), and a clade including the herein new described species *I. lemmi* (95%), and the two single branches representing *I.* cf. *candidipes* and *I. candidipes* (81%). The four last clades form a subclade within the complex with a support value of 62%. The Bayesian analysis recovered the same clades as the MP analysis, here with strong support, a Bayesian posterior probability (BPP) value of 1.0 (Fig. 1). The Bayesian tree topology was identical to the tree presented in Fig. 1.

The sequence of the ITS region of the type of *I. tundrae* was found to be identical to the type of *I. rivularis*. The LSU of *I. glabrodisca* AY239023 was found to be identical with that of *I. praetervisa*.

Taxonomy

Inocybe lemmi E. Larss., Vauras & C.L. Cripps **sp. nova** – Figs. 1–2, 4

MycoBank no.: MB823614

Diagnosis: Medium-sized species of section *Marginatae*, pileus with orange-brown to reddish brown colours, with white patches of velipellis. Stipe indistinctly pruinose to base. Spores oblong, indistinctly nodulose, measuring (11.0–)11.6–14.3(–15.2) × 6.4–8.3(–8.4) µm, pleurocystidia measuring (46–)54–72(–82) × (12–)14–19(–22) µm. Habitat alpine, on calcareous soil with dwarf willows and *Bistorta vivipara*.

Typus: **SWEDEN**. **Lule lappmark**. Jokkmokk, Padjelanta National Park, N side of the lake Vastenjaure, the fjeld Arranoaijvve. Alpine site on S slope, on fairly bare, calcareous soil near *Salix herbacea* coll., *S. reticulata*, *Dryas octopetala*, *Bistorta vivipara, Saxifraga oppositifolia* and *Silene acaulis*, alt. ca 860 m, 67°29.8′N, 16°36.8′E, 11.VIII.2016 *Vauras 31462F* (TUR-A 204243 – holotypus, GenBank Acc. No. MG574394; GB, MONT, Herb. Ditte Bandini – isotypi).

Etymology: *lemmi* (gen.) refers to the mammal Norwegian lemming, *Lemmus lemmus*, living in the same habitat and having similar colouration on part of its fur.

Pileus 1.5–3.7 cm in diam, when young hemisphaeric to conico-convex, later plano-convex to applanate, with or without umbo, umbo broad and round; orange-tinged brown to reddish brown, smooth to subtomentose around centre, outwards felty to radially fibrillose, often breaking up to some scales, some of them uplifted or recurved, often with abundant whitish velipellis; margin rarely splitting. *Lamellae* normally

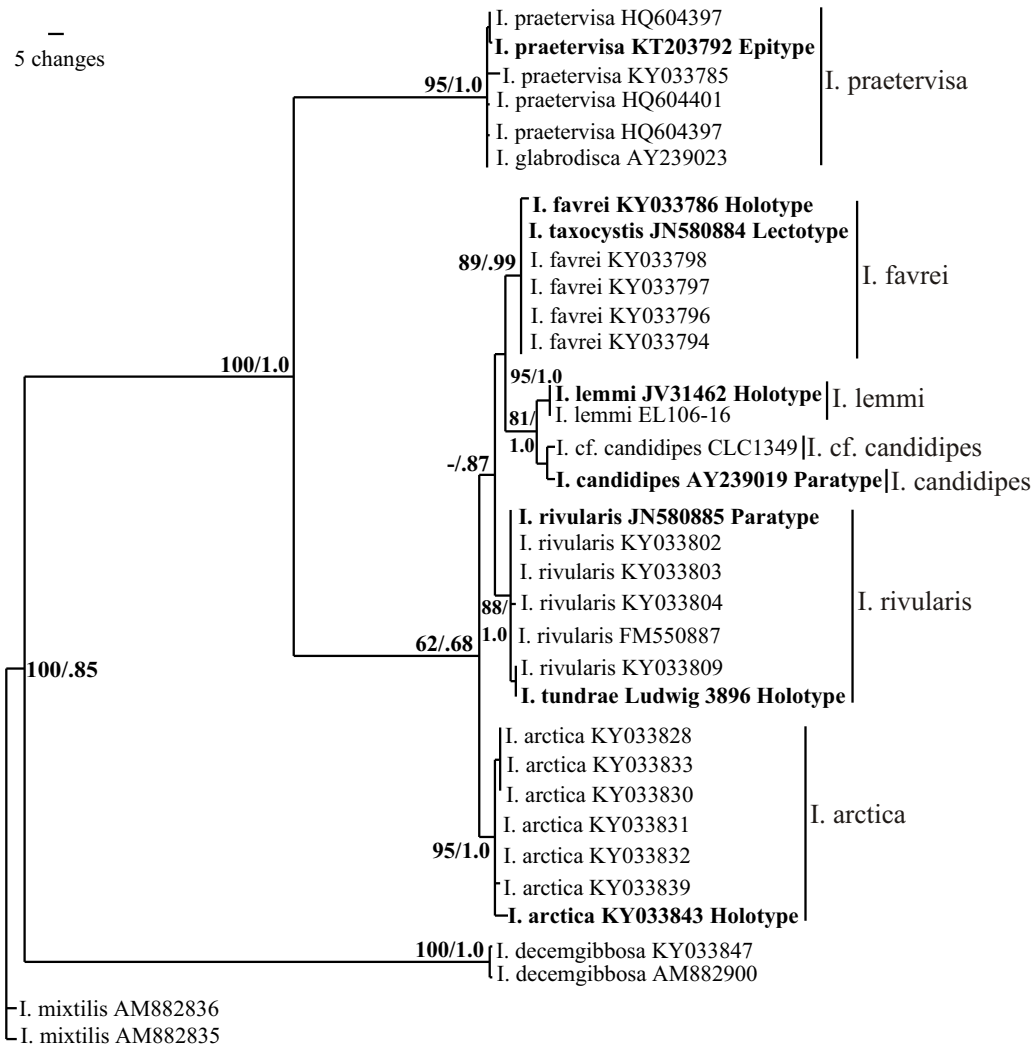

Fig. 1. One of the most parsimonious trees from the phylogenetic analysis based on nuclear rDNA ITS and partial LSU sequences. Parsimony bootstrap values and Bayesian posterior probabilities are indicated on branches. Clades discussed in the text are indicated with bars and species names. Sequences originating from type specimens are marked in bold.

crowded, up to 5 mm wide, subventricose, narrowly adnate, first whitish, then pale grey, greyish brown, yellowish brown, pale brown, edge concolorous. *Stipe* 1.2–2.0 × 0.3–1.0 cm, equal, even at base, or base subbulbous with slight bulb and some indistinctly submarginately bulbous; first whitish, later pale brown, orange-brown to reddish brown but base whitish, apex scarcely white-pruinose, indistinctly longitudinally fibrous, especially in lower part. *Cortina* not seen. *Context* quite soft, whitish in pileus and stipe when young, later partly yellow-brown to brown in stipe. *Smell* weak, somewhat acidulous.

Spores (11.0–)11.6–*12.9*–14.3(–15.2) × 6.4–*7.2*–8.3(–8.4) µm, range of mean values 12.4–13.2 × 7.1–7.2 µm, Q = (1.5–)1.6–*1.80*–2.1(–2.2), range of mean Q –values 1.75–1.84 (100 spores from 2 collections); oblong, with up to 5 indistinct nodules, fairly thick-walled, with granular mass inside, yellow-brown. *Basidia* (28–)30–*36*–40(–41) × (10–)11–*12*–13(–14) µm, clavate, 4-spored

Fig. 2. Inocybe lemmi, holotype, photographed in situ. – Photo: J. Vauras.

Fig. 3. *Inocybe* cf. *candidipes*. *CLC 1349*. – Photo: C. Cripps.

(n = 40). *Pleurocystidia* (46–)52–*63*–78(–82) × (12–)13–*16*–20(–22) µm (n = 43), clavate to fusoid, some subcapitate, often with pedicel, with up to 3 µm thick, pale yellowish wall, usually with crystals at apex; rather scarce. *Cheilocystidia* fairly similar to pleurocystidia but on average shorter and more variable, (32–)42–*55*–64(–72) × 12–*16*–20(–21) µm (n = 42), some with yellow-brown contents, abundant; paracystidia abundant, mainly clavate, (18–)21–*27*–37(–41) × (8–)10–*12*–14(–16) µm (n = 36). *Caulocystidia* descending to base of stipe but scattered, mostly hyphal-like and thin-walled, rather cylindrical, but some with thicker walls to 2 µm thick, many with yellow-brown contents, at stipe apex (32–)38–*49*–61(–64) × (9–)11–*14*–18(–19) µm (n = 31); cauloparacystidia at stipe apex mainly clavate, 14–*25*–35 × 7–*10*–14 µm (n = 15), scarce. *Clamp connections* present in all tissues.

***Additional specimen examined*: SWEDEN. Lule lappmark.** Jokkmokk, Padjelanta National Park, Tuottar, 13.VIII.2016 *Larsson EL106-16* (GB, TUR-A, GenBank Acc. No. MG574395).

Inocybe lemmi is so far only known from two localities in the Padjelanta National Park in Northern Sweden. Both localities are on calcareous fjelds in the alpine zone. Potential ectomycorrhizal partners at both sites were *Salix reticulata* L. and *Bistorta vivipara* (L.) Gray. Also on the sites close by were *Dryas octopetala*, *Salix herbacea* L. and *S. polaris* Wahlenb. Other noted plants on the sites indicating rich alpine meadow areas were *Thalictrum alpinum* L., *Equisetum variegatum* Schleich., *Saxifraga aizoides* L. and *Pedicularis lapponica* L. *Inocybe lemmi* seems to be rare and to have a preference for calcareous soil. The two specimens were collected in mid-August.

Inocybe* cf. *candidipes – Figs. 1, 3

Pileus 10–20 mm in diam, conico-convex, yellow-brown to medium brown, scaly-fibrillose, some scales uplifted or recurved, with conspicuous whitish velipellis patches on each scale, sometimes striking in contrast; margin entire. *Lamellae* attached, sinuate, rather thick, white at first, then grey or grey brown; L=40 with lamellulae; edge white. *Stipe* 15-20 x 3-5 mm, equal, squared-off at base, or with slight bulb, or indistinctly submarginate, shining white, then watery brown, pruinose to base, also indistinctly longitudinally fibrous in lower part. *Cortina* not seen. *Context* white. *Smell* faint. *Spores* (10.8–)10.9–*11.8*–13.1(–13.4) × (6.2–)6.3–*6.9*–7.8(–8.6) µm, Q = 1.55–*1.70*–1.85(–1.9), (40 spores); oblong, indistinctly nodulose, with small apiculus, generally rectangular in outline. *Basidia* 33–*37*–42 × 12–*12*–13 µm clavate, 4-spored (16 basidia). *Pleurocystidia* (47–)56–*60*–66(–70) × 14–*17*–21 µm (20 pleurocystidia), clavate to short-fusoid, some tending towards subcapitate, with or without crystals at apex, wall mostly pale yellow; rather scarce. *Cheilocystidia* 38–*50*–60 × 16–*19*–24 µm (n = 12), similar to pleurocystidia, some with yellow contents, abundant, with abundant pyriform to clavate paracystidia interspersed, 21–*26*–31 × 9–*13*–15 µm (n = 10). *Caulocystidia* at stipe apex (32–)38–*49*–61(–63) × (11–)12–*16*–20(–22) µm (n = 31), a few, even to the base, mostly hyphal-like and thin-walled, cylindrical, but some with thicker walls to 1 µm thick; cauloparacystidia at stipe apex mainly clavate, 18–*28*–38 × 9–*11*–12 µm (n = 10), scarce. *Clamp connections* present in all tissues.

***Specimen examined*: USA. Colorado.** Pitkin/Lake County, sawatch Range, Independence Pass, 12.VIII.1999 *Cripps CLC 1349* (MONT, TUR-A, GenBank Acc. No. MG574396).

Inocybe cf. *candidipes* was collected in an alpine locality in the mountains of Colorado, where both dwarf and shrubby willows occur. It was originally labeled *I. favrei* (*I. taxocystis*). In Arizona, *I. candidipes* was associated with *Pinus ponderosa* in montane environments (Kropp & Matheny 2004). Microscopically the collection from Colorado has wider spores than *I. candidipes* (5.0–*5.6*–6.0(–6.5) µm), and the spore outline is not similar to *I. chelanensis* as was written for *I. candidipes* in Kropp & Matheny 2004. However, having only one collection, we prefer to call the Colorado collection *I.* cf. *candidipes*.

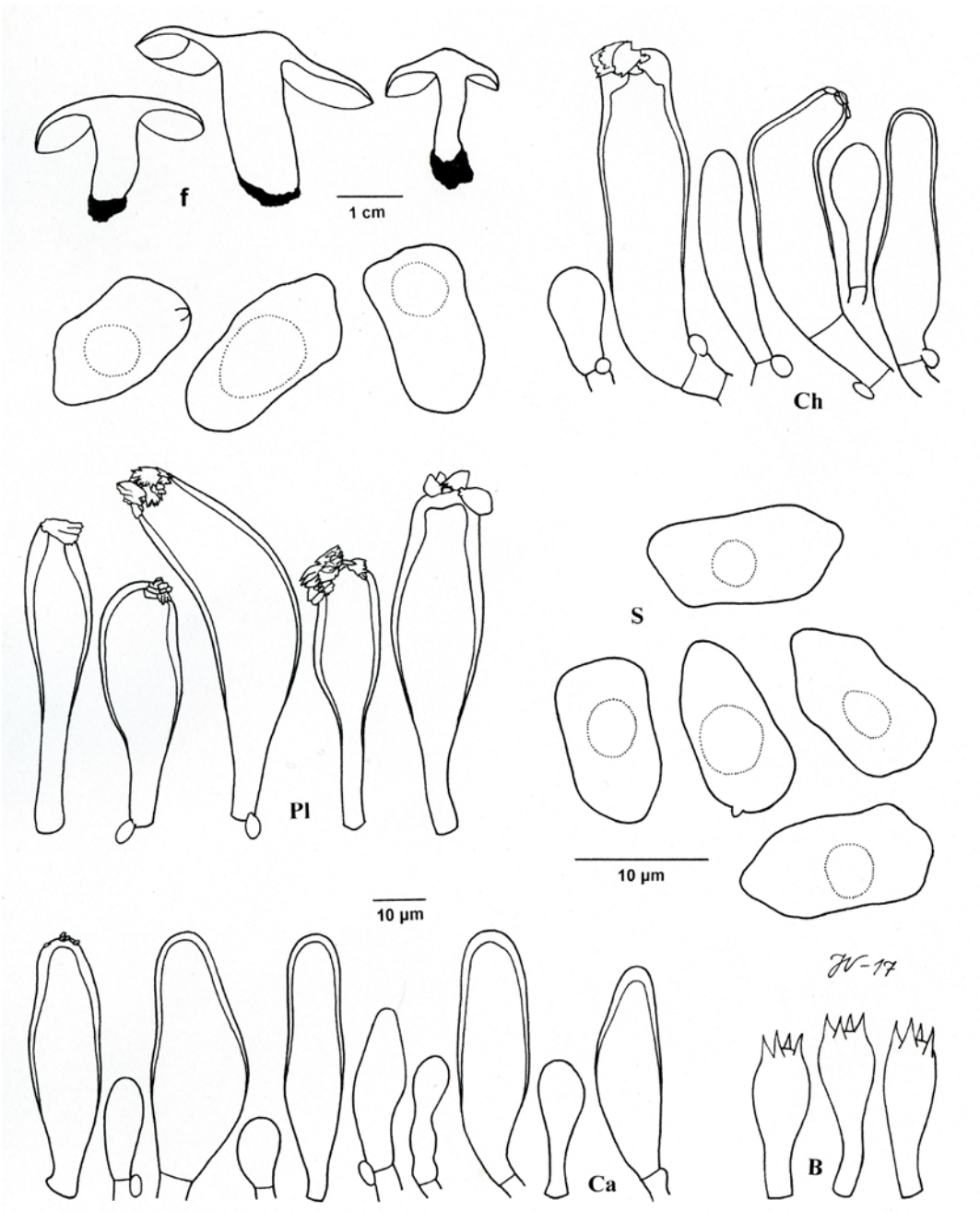

Fig. 4. Microscopical characters and cross-sections of fruiting bodies (f) of *Inocybe lemmi* (holotype). B = basidia, Ca = caulocystidia and paracystidia at stipe apex, Ch = cheilocystidia and paracystidia, Pl = pleurocystidia, S = spores.

Discussion

In this paper we describe *Inocybe lemmi*, based on two collections originating from two separate localities in the middle alpine zone in Sweden. In morphology it resembles *I. candidipes* and the single collection herein named *I.* cf. *candidipes,* by having spores with an angular outline and only scattered cystidia on the stipe. At first we thought they could represent one and the same species, but it is hard to evaluate when only few collections are available originating from three rather disparate geographical areas. All these lineages are closely related (Fig. 1) and may represent rather rare species.

Inocybe lemmi and *I.* cf. *candidipes* both originate from the alpine zone. In the ITS sequence, they differ by five substitutions and three insertion/deletion events, in the LSU region by four substitutions, two ambiguous sites, and one insertion/deletion event. And as the Swedish collections also differ in the micro-morphology by having larger spores than those measured in the collection *I.* cf. *candidipes,* we decided to recognize *I. lemmi* as a distinct species.

Unfortunately, we lack an ITS sequence of *I. candidipes* and only the LSU sequence data can be compared. *Inocybe* cf. *candidipes* differ from *I. candidipes* by four substitutions and one insertion/deletion event and its status will have to wait until the ITS sequence of *I. candidipes* is available. But in this case there is also a difference in the spore measurements between *I.* cf. *candidipes* and *I. candidipes*. *Inocybe lemmi* differs from *I. candidipes* by five substitutions and two insertion/deletion events in the LSU region.

The three lineages all belong to the *I. praetervisa* clade where the other species, *I. praetervisa, I. rivularis*, *I. favrei* (syn. *I. taxocystis*) and *I. arctica* have distinctly nodulose heterodiametric spores and abundant caulocystidia in the upper half of the stipe. Despite having fewer caulocystidia on the stipe, *I. lemmi* and *I.* cf. *candidipes* have the same distribution of caulocystidia, so the *I. praeterevisa* clade can still be characterized and defined as suggested by Estevo-Raventos et al. (2016).

Inocybe rivularis was described by Jacobsson & Vauras (1990) from moist forest habitats in the boreal zones of Fennoscandia. The species was found to have a rather wide distribution and to be common in suitable habitats, usually close to small spring brooks and associated with *Betula* spp. In the study by Larsson et al. (2017) it was confirmed as a distinct species, clearly separated from *I. praetervisa*, *I. favrei* and *I. arctica*, and also to have an intercontinental distribution and to occur in boreal forest ecosystems up to the lower alpine zone and associated with *Betula* spp. The recently described *I. tundrae* (Ludwig 2017) was found to be very similar in morphology and to have an ecology similar to *I. rivularis*. Here we show that the sequence of the ITS region from the holotype of *I. tundrae* is identical with that generated from a paratype of *I. rivularis,* collected from the same locality as the holotype. We therefore consider it as a later synonym of *I. rivularis* (Fig. 1).

The LSU sequence named *I. glabrodisca* in Kropp & Matheny (2004) included in our analyses is here identified as *I. praetervisa* (Fig. 1). The position in their study with *I. candidipes* close to the sequence named *I. glabrodisca* is here confirmed and it is now set that *I. candidipes* belongs in section *Marginatae* and the *I. praetervisa* clade.

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