

Five new species in *Piloderma* (Atheliales, Basidiomycota) and epitypification of *P. byssinum*

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ABSTRACT

Piloderma constitutes a small genus of soft, corticioid, ectomycorrhizal, widely distributed and mostly very common species. Microscopically, its members have traditionally been recognised by their simple-septate hyphae and by their small, thick-walled, colourless to pale yellow spores. We describe five new species from northern Europe based on molecular and morphological data: *P. frondosum* sp. nov., *P. fugax* sp. nov., *P. lamprolithum* sp. nov., *P. luminosum* sp. nov. and *P. mirabile* sp. nov. All the new species, except *P. luminosum* seem to be more or less rare. *Piloderma fugax* has a strong preference for old-growth forest – a lifestyle seemingly deviating from the rest of the genus. *Piloderma mirabile* is a sister species to *P. sphaerosporum* and the first known species with clamped hyphae. *Piloderma lamprolithum* is closely related to *P. exiguum* and distinguished by its large encrusting crystals. *Piloderma luminosum* is very closely related to, and morphologically semicryptic with, *P. byssinum*. To clarify its distinction from *P. byssinum*, an epitype is designated for the latter. *Piloderma frondosum* also belongs to the *P. byssinum* cluster and is distinguished by its association to broadleaved trees. An updated key to all *Piloderma* species is provided.

1. Introduction

The genus *Piloderma* (*Pilodermataceae*, *Atheliales*, *Basidiomycota*) currently comprises 13 species of corticioid, ectomycorrhizal fungi. Its members are distributed predominantly within the Northern Hemisphere and most of them are more or less commonly occurring (Larsson et al., 2024). Especially in boreal forests, *Piloderma* species are often frequently encountered in both specimen surveys and soil samples – for example, according to a nation-wide soil sequence dataset from Sweden, *Piloderma sphaerosporum* is present in 504 out of 1806 sample sites (Soil fungi in Swedish woodland, 2024), making it the most commonly sampled agaricomycete in the country.

Basidiomata of *Piloderma* are white, yellow or ochre and occur in two forms whose phylogenetic relationship is unclear: the first is soft but

adnate to the substrate, with a poorly developed subiculum and lacks hyphal cords, while the second is equally soft but loosely attached, pellicular, has a well-developed, byssoid subiculum and often hyphal cords visible on the substrate (Larsson et al., 2024). Microscopically, the two forms are similar and rather featureless: the hyphal system contains only generative hyphae without clamps, sterile elements are lacking from the hymenium, the basidia carry four, rarely two, sterigmata (varying within specimens as well as species) and the spores are small, smooth and colourless to pale yellow, in some species with a dextrinoid reaction (Eriksson et al., 1981). A more noticeable morphological feature for the genus is the consistently thick spore walls (l.c.). Another characteristic, present in most species, is the occurrence of crystalline encrustation on the surface of hyphae. Larsson et al. (2024) found the size and shape of such crystals to be one of the most important features

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for species identification within *Piloderma*. The most morphologically similar genus and also one of the most closely related is *Tretomyces*, which hitherto has been differentiated from *Piloderma* primarily by the presence of clamped hyphae (Sulistyo et al., 2021). However, see the results of the present study.

The comparatively small differences in morphological differentiation within *Piloderma* has historically made delimitation of species difficult and resulted in wide circumscriptions. Until very recently the genus comprised only six described species – *Piloderma byssinum*, *Piloderma fallax*, *Piloderma lanatum*, *Piloderma olivaceum*, *Piloderma reticulatum* and *P. sphaerosporum* – and three names with unclear synonymy: *Piloderma bicolor*, *Piloderma croceum* and *Piloderma lapillicola*. Larsson et al. (2024) concluded through type studies that *P. bicolor*, *P. croceum* and *P. lapillicola* are synonyms of *P. fallax* and proceeded to describe a further seven species from Scandinavia, all of which are morphologically distinguishable.

According to Soil fungi in Swedish woodland (2024), many *Piloderma* species are common, but a number of rarer species, with only one or a few occurrences, are also present in the data. In this study, the first aim is to target these rare species for taxonomic description, through systematic study of large basidiomata collections made by ourselves and others. The second aim is to resolve taxonomic issues in the species complex of *P. byssinum*.

2. Materials and methods

2.1. Specimen sampling

Specimens were collected by SS, RI and TH in the field in Finland, Lithuania, Norway and Sweden during the autumns of 2018–2023 and deposited in herbaria UPS, OULU and BILAS. For the *Piloderma byssinum* complex, this material was complemented with older specimens collected by KHL, deposited in herbaria GB and O. In addition, MK carefully examined a large part of the thousands of corticioid basidiomata that constitute the occurrence dataset of Nordén et al. (2017), deposited in herbaria O and OULU. Vouchers of all specimens originally deposited in other herbaria were loaned to herbarium UPS and studied by SS. Loans of specimens for comparative study from herbaria H, OSC and UC to UPS were also granted.

2.2. Morphological examination

Dried basidiomata were studied with a Leica DM2500 compound light microscope equipped with differential interference contrast lenses. Preparations were made in 3 % KOH and Melzer's solution. Measurements were made in KOH at 630x magnification, using the LAS X Life Science Microscope Software.

2.3. Applied species concept and recognition of species

We interpret sympatrically occurring individuals belonging to different phylogenetically supported clades that can be distinguished morphologically and/or ecologically as belonging to different species, since the formation of distinct ecological niches/morphologies coupled to certain genotypes under such circumstances are unlikely if recombination were occurring. For this purpose we deem the easily amplified ITS sequence sufficient. However, in cases where both ecology and morphology are vague/overlapping, additional DNA regions should be sequenced to prove genetic isolation – in this study Tef1 α .

2.4. DNA extraction, amplification, and sequencing

For the present study 17 ITS and 13 Tef1 α DNA sequences were generated (Table 1). The DNA encoding nLSU contain little variation in *Piloderma* and was hence not included in analyses. DNA was extracted from dried basidiomata. Extraction was either carried out with Quick

DNA Fungal/Bacterial Mini Prep Kit (Zymo Research) or using Chelex, following the protocol of Ferencova et al. (2017). Amplification was conducted using the primer pair ITS1F-ITS4 for the complete ITS sequence (White et al., 1990; Gardes and Bruns, 1993), or ITS1F-ITS2 for ITS1 and ITS3-ITS4B1 for ITS2 (White et al., 1990; Tedersoo et al., 2007), and EF983F-EF1567R for Tef1 α (Rehner and Buckley, 2005). The PCR conditions used for ITS were: initial denaturation at 95° for 3 min, 35 cycles of denaturation at 95° for 30 s, annealing at 50° for 45 s and elongation at 72° for 1 min, followed by a final incubation step at 72° for 10 min. PCR conditions for ITS1 and ITS2 were the same, except that an annealing temperature of 55° was used. PCR conditions for Tef1 α followed Sulistyo et al. (2021). Amplified products were purified with ExoSAP-IT (Applied Biosystems, Foster City, CA) and sent for sequencing to MacroGen Europe (Amsterdam, the Netherlands). For sequencing the same primers were used as for amplification, with the exception of the region ITS1, for which the primer pair ITS1-ITS2 were used, and for the region ITS2, for which ITS3-ITS4 were used (White et al., 1990). Sequences were assembled and manually corrected using Asseq (2019).

2.5. Phylogenetic analyses

The dataset was enriched with four ITS sequences from BOLD, 12 sequences from UNITE and 43 sequences from GenBank (Table 1). Sulistyo et al. (2021) recognised three genus-level clades in *Pilodermataceae*, viz. *Piloderma*, *Tretomyces*, and *Stereopsis vitellina*. *Tretomyces* differs from *Piloderma* by having consistently clamped hyphae and *Stereopsis vitellina* by producing stipitate basidiomata. Since *Tretomyces* and *Stereopsis vitellina* have ITS sequences that are rather deviating compared to *Piloderma*, we did not include them in our analyses in order to maximize sequence information within the genus. We followed Sulistyo et al. (2021) and used *Athelopsis subin conspicua* and a Genbank sequence identified as *Leptosporomyces raunkiaerii* as outgroup.

Alignments were made in AliView v. 1.18 (Larsson, 2014), utilising the L-INS-i strategy, as implemented in MAFFT v. 7.017 (Katoh and Standley, 2013). Introns and low-quality ends were manually trimmed from the sequences prior to analysis. Gblocks v. 0.91b (Castresana, 2000; Talavera and Castresana, 2007) was applied to trim the alignments of problematic character regions (e.g. missing data, saturated sites and sections with unclear homology), using the Less Stringent option on the web server at <http://phylogeny.lirmm.fr/> (Dereeper et al., 2008).

Best-fitting substitution models and, for ITS, partitions for Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were estimated using ModelFinder, as implemented on the IQ-TREE server (Kalyaanamoorthy et al., 2017), assuming ITS1, 5.8S, ITS2 as minimal partitions (Chernomor et al., 2016). According to the program the best fit for ITS was achieved by the model HKY + G, while treating the entire alignment as a single partition, and for Tef1 α TN + G.

The ML analysis of the ITS alignment was conducted using IQ-TREE (Nguyen et al., 2015) using the online server at <http://iqtree.cibiv.univie.ac.at/> (Trifinopoulos et al., 2016). Branch support was estimated as SH-aLRT test (Guindon et al., 2010) and ultrafast bootstrap support (Hoang et al., 2018).

For the BI analysis of the ITS alignment we used BEAST 2.7.5 (Bouckaert et al., 2019). A lognormal, relaxed clock model was assumed, as a test run showed a coefficient of variation well above 0.1 (i.e. implying a relatively high rate variation among branches). The clock rate was estimated in the run, using a lognormal prior with a mean set to one in real space. We set the growth rate prior to lognormal, with a mean of 5 and a standard deviation of 2. These priors were set according to the STACEY package documentation (Jones, 2014). The Markov Chain Monte Carlo chain was run until the analysis converged well in advance of the 10 % burn-in threshold, had ESS values well above 200 for all parameters, and satisfactory chain mixing, as assessed in Tracer v. 1.7.2 (Rambaut et al., 2018). After discarding the burn-in trees, a maximum clade credibility tree was identified by TreeAnnotator v. 2.7.5

Table 1

List of sequences used in phylogenetic analyses with accession numbers from the databases GenBank, UNITE (starting with UDB) or BOLD (starting with FISAP, FUFUIW and NOCOR). Sequences produced in this study are indicated in bold face.

| Species | Voucher | Herbarium | Genbank/UNITE/BOLD ITS | Genbank/UNITE/BOLD Tef1 α | Country | Reference |
|--------------------------------|---------------------------|-----------|------------------------|----------------------------------|-----------------------|---------------------------|
| <i>Piloderma byssinum</i> | KHL8501 | GB | DQ469282 | | SE | Larsson et al. (2024) |
| | JS15688 | O | DQ469280 | | NO | Larsson et al. (2024) |
| | SS1137 | UPS | PQ791716 | PQ790256 | SE | This study |
| | SS631 | UPS | PQ791717 | PQ790257 | SE | This study |
| | KHL13488 | O | OQ803271 | | SE | Larsson et al. (2024) |
| | SS607* | UPS | PQ791718 | PQ790258 | SE | This study |
| | SS1093 | UPS | PQ791719 | PQ790255 | SE | This study |
| | KHL8456 | GB | DQ469283 | | SE | Larsson et al. (2024) |
| | GB-0121002 | GB | LR694206 | LR694222 | SE | Sulistyo et al. (2021) |
| | SS699 | UPS | PQ791720 | PQ790259 | SE | This study |
| | <i>Piloderma cinicola</i> | KHL18326 | GB | OQ803267 | | SE |
| SS1019 | | UPS | OQ803261 | | SE | Larsson et al. (2024) |
| <i>Piloderma craurum</i> | JS25853 | O | UDB001727 | | NO | Larsson et al. (2024) |
| | JS21891 | O | UDB001728 | | NO | Larsson et al. (2024) |
| <i>Piloderma egens</i> | KHL18343 | GB | OQ803275 | | SE | Larsson et al. (2024) |
| | JS24861 | O | UDB001733 | | NO | Larsson et al. (2024) |
| <i>Piloderma exiguum</i> | SS1142 | UPS | OQ803281 | | SE | Larsson et al. (2024) |
| | EL 323/12 | GB | OQ803280 | | SE | Larsson et al. (2024) |
| <i>Piloderma fallax</i> | MR00338 | UPS | LR694207 | LR694223 | SE | Sulistyo et al. (2021) |
| | BS1710030 | UPS | LR694205 | LR694221 | SE | Sulistyo et al. (2021) |
| <i>Piloderma frondosum</i> | szYM479 | / | LC547645 | | RU | Miyamoto et al. (2021) |
| | szYM184 | / | LC547646 | | RU | Miyamoto et al. (2021) |
| | SS1141* | UPS | PQ791715 | PQ790260 | SE | This study |
| | YO4-30I | / | AB831864 | | JP | Yagame et al. (2016) |
| | LM176 | / | KM576315 | | ES | Suz et al. (2014) |
| <i>Piloderma fugax</i> | SG037_A03 | / | KP889952 | | CA | Genbank, unpublished |
| | ECUBC35 | / | FJ236851 | | CA | Lim and Berbee (2013) |
| | JN6840* | O, OULU | PQ791721 | | SE | This study |
| | EST1423251 | / | UDB0404735 | | EE | Tedersoo et al. (2014) |
| <i>Piloderma humile</i> | SS380 | O | NOCOR377-18 | | NO | BOLD |
| | KHL14996 | O | UDB024825 | | NO | Larsson et al. (2024) |
| <i>Piloderma lamprolithum</i> | JS20687 | O | UDB001730 | | NO | Larsson et al. (2024) |
| | EST20_116213 | / | UDB0116442 | | EE | Tedersoo et al. (2014) |
| | GAVA.380 | OULU | FUFUIW2051-23 | | FI | BOLD |
| | isolate 9 | / | ON261497 | | CN | Genbank, unpublished |
| | szYM49 | / | LC547654 | | RU | Miyamoto et al. (2021) |
| | isolate 1 | / | ON261489 | | CN | Genbank, unpublished |
| | GAVA.236 | OULU | FUFUIW1094-21 | | FI | BOLD |
| | 2_207 | / | HQ271370 | | MX | Kennedy et al. (2011) |
| | JN9319* | O, OULU | FISAP1102-16 | | NO | BOLD |
| | KZ17 | / | EF372399 | | DE | Zimmer et al. (2007) |
| | TO10-OTU194 | / | EF434142 | | US | Taylor et al. (2007) |
| <i>Piloderma lanatum</i> | KHL13416 | GB | OQ803277 | | NO | Larsson et al. (2024) |
| | JS10446 | O | UDB001742 | | NO | Larsson et al. (2024) |
| <i>Piloderma luminosum</i> | AOE23-11 | UPS | PQ791708 | | SE | This study |
| | PEM220817 | UPS | PQ791707 | PQ790250 | SE | This study |
| | SS1008* | UPS | PQ791709 | PQ790254 | SE | This study |
| | SS615 | UPS | PQ791710 | | SE | This study |
| | O-F-253647 | O | UDB038226 | | NO | Larsson et al. (2024) |
| | JS20399 | O | DQ469281 | | NO | Larsson et al. (2024) |
| | SS1106 | UPS | PQ791711 | PQ790249 | SE | This study |
| | AOE23-05 | UPS | PQ791712 | PQ790252 | SE | This study |
| | LB 1165/08 | O | OQ803270 | | NO | Larsson et al. (2024) |
| | SS641 | UPS | PQ791713 | PQ790251 | SE | This study |
| | SS610 | UPS | PQ791714 | PQ790253 | SE | This study |
| <i>Piloderma mirabile</i> | KGN187/98 | GB | DQ469279 | | SE | Larsson et al. (2024) |
| | BILAS 51744* | BILAS | PQ791722 | | LT | This study |
| | clone 217 | / | KJ769284 | | RU | Malysheva et al. (2014) |
| | EST16_210528 | / | UDB0708777 | | EE | Tedersoo et al. (2014) |
| | MBN0213_23 | / | KC840632 | | CA | Nadeau and Khasa (2016) |
| JLP3362 | / | DQ377394 | | US | Parrent et al. (2006) | |
| <i>Piloderma ochraceum</i> | SG027-B01 | / | KP889405 | | US | Genbank, unpublished |
| | KHL13481 | GB | OQ803274 | | SE | Larsson et al. (2024) |
| <i>Piloderma olivaceum</i> | CFMR-S-12 | CFMR | GU187535 | GU187738 | FI | Binder et al. (2010) |
| | BS1710031 | UPS | LR694208 | LR694224 | SE | Sulistyo et al. (2021) |
| <i>Piloderma rallum</i> | KHL13431 | GB | OQ803282 | | NO | Larsson et al. (2024) |
| | SS729 | UPS | OQ803284 | | SE | Larsson et al. (2024) |
| <i>Piloderma sphaerosporum</i> | JS20011 | O | UDB001749 | | NO | Larsson et al. (2024) |
| | JS24262 | O | UDB001750 | | NO | Larsson et al. (2024) |
| <i>Piloderma sp.</i> | 8-46P1 | / | JQ393100 | | US | Kennedy et al. (2012) |
| | UVIC7 | / | KT272107 | | CA | Kranabetter et al. (2015) |
| | OSC63458 | OSC | DQ371931 | | US | Dunham et al. (2007) |

(continued on next page)

Table 1 (continued)

| Species | Voucher | Herbarium | Genbank/UNITE/BOLD ITS | Genbank/UNITE/BOLD Tef1 α | Country | Reference |
|------------------------------------|------------|-----------|------------------------|----------------------------------|---------|-------------------------|
| | UC2023203 | UC | KP814484 | | CA | Rosenthal et al. (2017) |
| | UC2023191 | UC | KP814486 | | CA | Rosenthal et al. (2017) |
| <i>Atheliopsis subinconspicua</i> | GB-0058732 | GB | LR694197 | LR738852 | SE | Larsson et al. (2024) |
| <i>Leptosporomyces raunkiaerii</i> | HHB-7628 | CFMR | GU187528 | | US | Binder et al. (2010) |

(Bouckaert et al., 2019).

A multigene analysis was conducted for ITS and Tef1 α , using STACEY (Jones, 2017), as implemented in BEAST 2.7.5 (Bouckaert et al., 2019). The settings applied were the same as for the ITS BI analysis, with the addition that clock rates were estimated independently and the prior PopPriorScale was set to a mean of -7 and a standard deviation of 2, in accordance with the STACEY package documentation (Jones, 2014).

For all species treated in the Taxonomy section, links to UNITE SHs (version 10) are provided (Abarenkov et al., 2023). All SH datasets have permanent digital object identifiers (DOIs).

3. Results

The final alignments for ITS and Tef1 α , after processing in Gblocks, were 481 and 546 nucleotides long, respectively. ML and BI analyses produced topologically similar trees. Figure 1 shows the ITS ML

phylogram with values considered significant received from the SH-aLRT test (≥ 80), ultrafast bootstrap (≥ 95), and BI tree (≥ 0.95) indicated on branches. Figure 2 shows the multigene analysis (STACEY), for the *Piloderma byssinum* group, for which both ITS and Tef1 α sequences were successfully generated.

Five species are described as new. *Piloderma fugax*, *Piloderma lamprolithum* and *Piloderma mirabile* correspond to supported clades in the ITS phylogeny (Fig. 1) and are morphologically distinct. Generation of Tef1 α sequences for these species failed, despite repeated attempts, probably due to age and limited size of the specimens. *Piloderma mirabile* is closely related to *Piloderma sphaerosporum*, while *Piloderma fugax* has an unclear placement within the genus. *Piloderma lamprolithum* is sister to *Piloderma exiguum*, according to both the ML and the BI analyses but the clade only has support in the latter. It is delimited to encompass two clades, one of which is poorly supported, separated by nine nucleotides across seven loci. Within *Piloderma* this constitutes a relatively large difference,

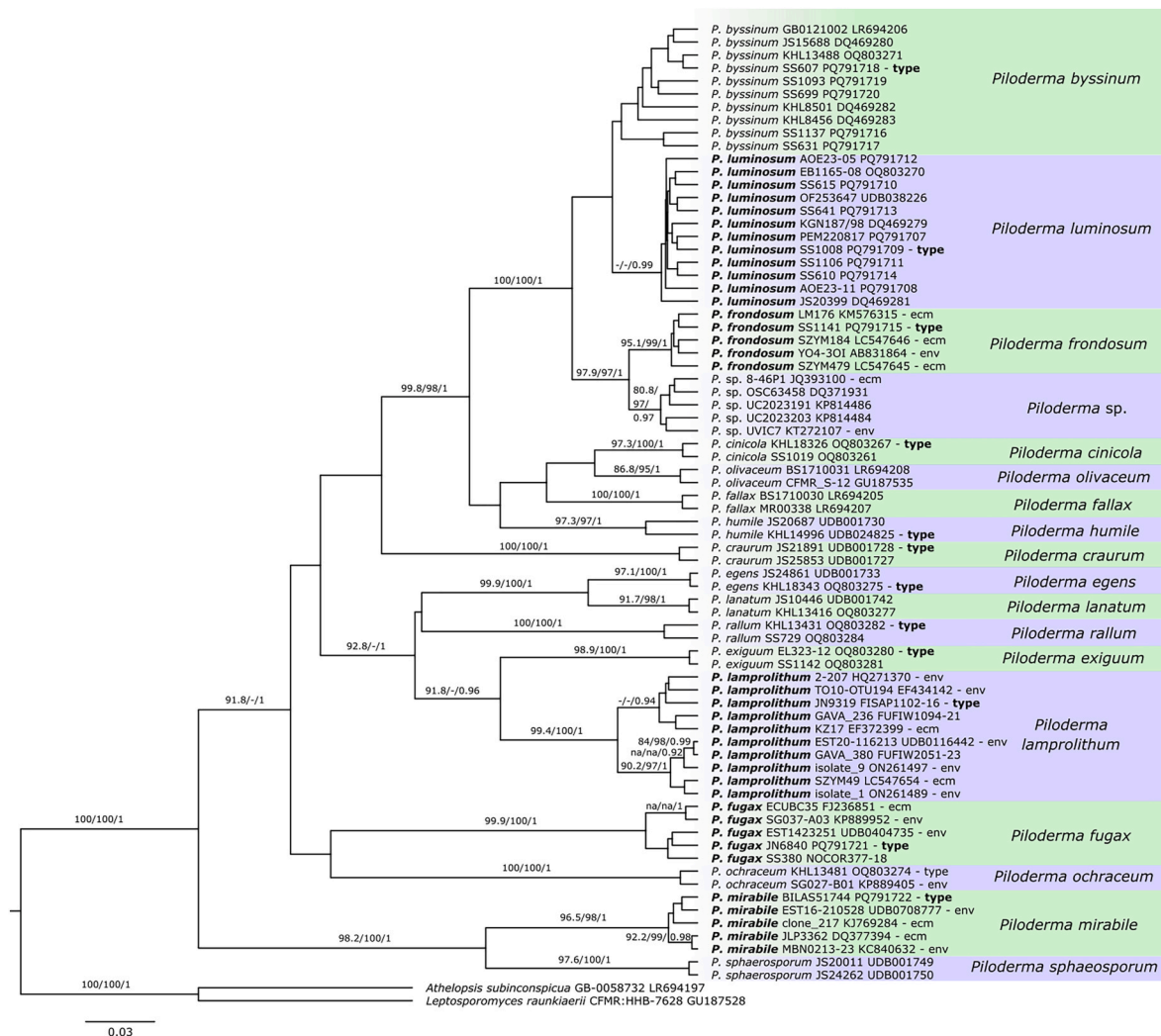


Fig. 1. Maximum Likelihood tree of *Piloderma*, based on ITS sequences. Support values considered significant received from SH-aLRT test (≥ 80), ultrafast bootstrap (≥ 95), and Bayesian posterior probabilities (≥ 0.95) are indicated on branches. New species are indicated in bold face.

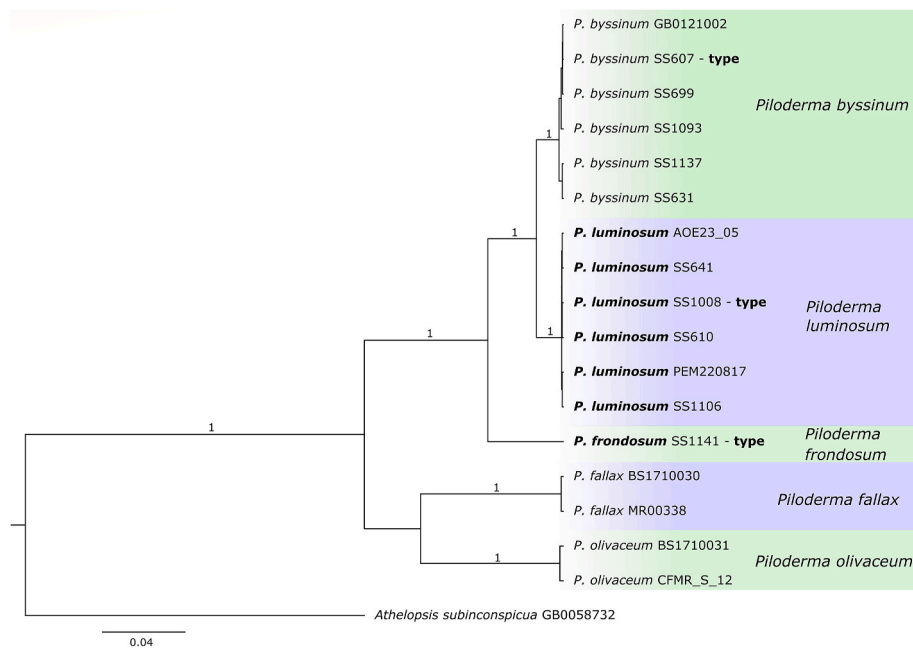


Fig. 2. STACEY species tree of the *Piloderma byssinum* group, based on ITS and Tef1 α sequences. Support values, in the form of Bayesian posterior probabilities, considered significant (≥ 0.95) are indicated on branches. New species are indicated in bold face.

which might equate to a separation of species, but in the absence of more basidiomatal specimens (two from one clade and one from the other) from which other genetic regions could be sequenced or a well-founded morphological difference inferred, we cannot reject that all specimens belong to the same species and therefore currently treat them as one. The description of *Piloderma mirabile* is based on a single specimen only, but this specimen has consistently clamped hyphae – a feature which makes it unique within *Piloderma*.

Piloderma frondosum and *Piloderma luminosum* are split from *P. byssinum* based on their support in the multi-gene analysis (Fig. 2). Both species produce basidiomata that seem to be microscopically identical to *P. byssinum* and are macroscopically different only under certain circumstances (see further under *P. byssinum* in the Taxonomy section). *Piloderma frondosum*, however, is also ecologically separated from the other two species by its association with broadleaved – in contrast to coniferous – trees. *Piloderma byssinum* and *P. luminosum* are separated by 14 nucleotides across ten loci in ITS, and three nucleotides in Tef1 α . Moreover, nine of the nucleotide differences across seven loci in ITS are insertions/deletions and are therefore excluded from analysis by the ML and BI algorithms. A likely fourth species within the group, *Piloderma* sp., is present in North America, as evidenced by ITS sequences from both basidiomatal specimens, root tips of coniferous trees and soil. It is morphologically indistinguishable from *P. luminosum* and *P. frondosum* and separated from these and *P. byssinum* by ca nine nucleotides. Available specimens studied (OSC63458, UC2023191 and UC2023203) were too old/sparse to allow sequencing of Tef1 α , and it was thus not described – see further reasoning in the Discussion.

The frequency of new and previously described *Piloderma* species present in the dataset of Soil fungi in Swedish woodland (2024) are shown in Fig. 3. It encompasses all species, except *P. mirabile*, *Piloderma ochraceum* and *Piloderma reticulatum* (only known from the type), and does not distinguish *P. luminosum* from *P. byssinum*. A trend can be observed, whereby, with a few exceptions, the most common species were described first, less common species were covered by Larsson et al. (2024), and three of the rarest species in Sweden are described in the current study.

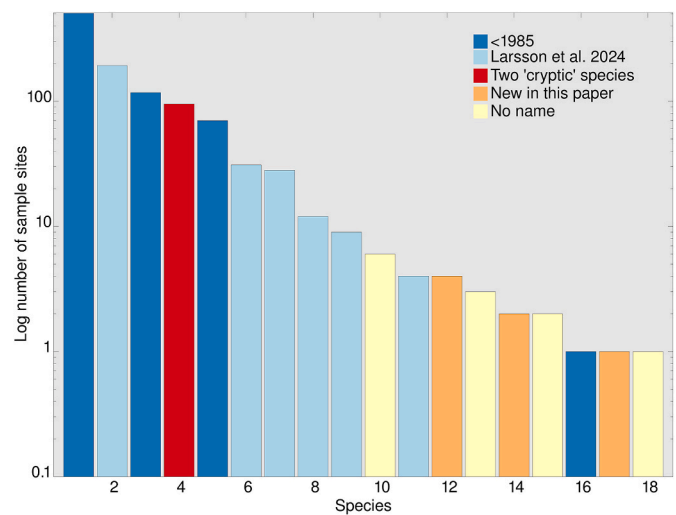


Fig. 3. Occurrence numbers of species and undescribed OTUs attributed to *Piloderma* in the dataset of Soil fungi in Swedish woodland (2024), colour coded according to date of formal description. *Piloderma byssinum* and *Piloderma luminosum* (red bar) were not treated as separate species by the study in question. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Taxonomy

***Piloderma byssinum* (P. Karst.) Jülich, Ber. Deutsch. Bot. Ges. 81: 418, 1969. Fig. 4.**

Basionym: *Lyomyces byssinus* P. Karst., Meddeland. Soc. Fauna Fl. Fenn. 11: 137, 1884.

Type: **Finland:** Mjölö. Oct. 1883, E. Wainio, ex herb. Karsten 681 (holotype: H6010754!). **Sweden:** Västerbotten: Umeå, Grössjön Nature Reserve, close to *Picea abies*, *Pinus sylvestris*, *Betula pubescens* and *Populus tremula* on acidic soil, on bark of *Betula pubescens*, 63.78174/20.34279, 4 Sep. 2022, S. Svantesson 607 (epitype: UPS, here designated), MycoBank typification no.: 10023931.



Fig. 4. *Piloderma byssinum* (epitype). Fresh basidiome in situ.

UNITE SH (0.5 %): SH1954086.10FU.

Selected illustrations (micromorphology): Larsson et al. (2024: Fig. 3).

Description: basidiomata resupinate, effused, loosely attached to the substrate by an extensive, byssoid subiculum, hymenium smooth or with small and sparse blisters, continuous, more or less pellicular, hymenium white to very pale yellow (cream), subiculum white, margin byssoid, hyphal cords usually present in subiculum and substrate. Hyphal system monomitic, hyphae 2.5–3.5 μm wide, without clamps, in the subiculum with thin to thickened walls, usually with short, baculiform, rod-like or elongated pyramidal crystals, lacking yellow encrustation in Melzer, in the subhymenium thin-walled, richly branched, often with encrustation that is coarser than in the subiculum. Basidia clavate to clavopedunculate, (10–)12–15(–25) \times 3.5–5 μm , with (2–)4 sterigmata. In yellowish parts of the hymenium, basidia and subbasidial cells often have pale yellow contents; subicular and lower subhymenial cells remain hyaline. Basidiospores subglobose to elliptic, 3–4(–4.5) \times 2.5–3 μm , smooth, with thickened walls, hyaline to slightly yellowish, cyanophilous, weakly dextrinoid.

Habitat: on decayed wood and litter of all kinds. Ectomycorrhizal with conifers. Known hosts are: *Picea abies*, *Pinus sylvestris* and *Pinus taeda* (Abarenkov et al., 2023).

Distribution: known from: Estonia, Finland, France, Italy, Lithuania, Sweden and United States (Abarenkov et al., 2023).

Remarks on identification: *Piloderma byssinum* is very similar to *Piloderma frondosum* and *Piloderma luminosum*. Basidiomata of *P. byssinum* remain white to cream-coloured upon maturity, while those of *P. luminosum* and *P. frondosum* range from white to, more commonly, bright yellow or orange, at least in parts. This colour difference appears to have two different causes: the cream colour observed upon maturity in *P. byssinum* and sometimes in *P. luminosum* and *P. frondosum* is likely the result mostly of increased spore-load in the otherwise white/hyaline basidiomata (the more spores present the stronger the colour), while the bright yellow to orange colour often observed in the latter two seems to be caused by a reaction to insect predation/damage. It appears to initially only be present around minute holes in the basidiomata, possibly caused by insect damage, and then spreads from there, colouring hyphae and basidia in the process. Interestingly, such holes are present also in *P. byssinum*, but they are much fewer and in this species the colour reaction spreads less and is limited to basidia and cells in the upper subhymenium. Microscopically the three species seem to be indistinguishable. While basidiomata of *P. frondosum* and *P. luminosum* with yellow to orange colours can sometimes be identified to species level through their association to host species – *P. frondosum* is associated with broadleaved trees and *P. luminosum* with conifers – white to

cream-coloured basidiomata in the vicinity of coniferous trees always have to be DNA-sequenced to be correctly identified. *Piloderma craurum* can also appear similar but differs by larger basidia and distinctly dextrinoid basidiospores. *Piloderma cinicola* has rounder and slightly smaller basidiospores and acicular instead of rod-like crystals on subicular hyphae. *Piloderma egens* and *Piloderma humile* differ by a tendency for elliptic basidiospores and by acicular crystals.

Remarks on epitypification: *Piloderma byssinum* has previously been more widely circumscribed and is here separated from *P. luminosum* based on consistent differences in the ITS and Tef1 α DNA regions, as well as the partly deviating basidiome colour. We have not sequenced the holotype of *Lyomyces byssinus*, due to its age and scarcity, but it is entirely white and the species is in its protologue described as “niveus”, i.e. white as snow (Karsten, 1884). Even though this description could also apply to basidiomata of *P. luminosum* and presumably *P. frondosum*, these species are commonly more strongly coloured, and therefore, given the choice, it is our opinion that the current selection provides the best fit.

Additional sequenced specimens studied: **Norway:** Buskerud: Nore og Uvdal, Smådøldalen, on *Betula* sp. 2 Sep. 1997, J.N. Stokland et al., 15688 (O). **Sweden:** Uppland: Ingarö, Karlsdal, old forest with *Picea abies*, *Pinus sylvestris*, *Populus tremula*, *Corylus avellana* and *Betula pendula*, 59.284162/18.356395, 25 Oct. 2022, S. Svantesson 1137 (UPS); Nora, Östa SO, middle-aged forest with *Picea abies*, *Pinus sylvestris* and *Betula* spp., 601094/6670054 (SWEREF99), 24 Oct. 2022, S. Svantesson 1093 (UPS). Västerbotten: Nordmaling, Sörheden Nature Reserve, old growth forest, pure *Picea abies* stand, 728241/7070234 (SWEREF99), 6 Sep. 2022, S. Svantesson 631 (UPS). Västergötland: Alingsås, Rödene, Kampeå, conifer forest, 13 Oct. 1996, K.H. Larsson 8456 (GB); Horla, S of Melltorp, brook–valley on N-facing slope, 18 Nov 1997, K.H. Larsson 8501 (GB); Västra Tunhem, Hallesnipen-Ovandalen, on strongly decayed wood, 5 Oct. 2006, K.H. Larsson 13488 (GB). Östergötland: Ringarum, Forsum NO, old forest with *Pinus sylvestris*, *Picea abies*, *Populus tremula*, *Quercus petraea* and *Betula pubescens*, 587886/6468578 (SWEREF99), 18 Sep. 2022, S. Svantesson 699 (UPS).

***Piloderma frondosum* Svantesson, sp. nov., Fig. 5.**

Mycobank: 857053

Etym.: *Frondosus* (Lat.) = leafy. Referring to the ectomycorrhizal association with broadleaved trees.

Diagnosis: *Piloderma frondosum* is differentiated from other species in the *P. byssinum* complex based on its host association with broadleaved trees, as well as nucleotide differences in ITS and Tef1 α .

Type: **Sweden:** Uppland: Ingarö, Karlsdal, old forest with *Picea abies*,



Fig. 5. *Piloderma frondosum* (holotype). Dried basidiome.

Pinus sylvestris, *Populus tremula*, *Corylus avellana* and *Betula pendula*, on wood, 59.28416/18.35640, 25 Oct. 2022, S. Svantesson 1141 (holotype: UPS).

UNITE SH (1 %): SH1411941.10FU.

Selected illustrations (micromorphology): Larsson et al. (2024: Fig. 3 – same micromorphology as *P. byssinum*).

Description: basidiome resupinate, effused, loosely attached to the substrate by an extensive, byssoid subiculum, hymenium smooth or with small and sparse blisters, continuous, more or less pellicular, from the beginning white, then developing yellow to orange spots that may probably extend to the whole basidiome, subhymenium and subiculum remaining white, margin byssoid, white hyphal cords present in subiculum and substrate. Hyphal system monomitic, hyphae 2.0–3.5 µm wide, without clamps, thin-walled, with no reaction in Melzer's reagent, in the subhymenium, richly branched. Encrustation initially granular, then short-rod like, eventually present as large, bipyramidal crystals, found on all hyphae but most well-developed in the upper subhymenium. Basidia clavate to clavo-pedunculate, 12–16 × 5.5–7 µm, with (2–)4 sterigmata up to 4.5 µm long. In yellow or orange parts of the hymenium, scattered basidia and underlying hyphae of all types frequently have yellow to orange contents; elsewhere, hyaline. Basidiospores subellipsoid, 3.2–3.7(–3.9) × 2.7–3.0, Q = 1.2–1.3 µm, smooth, with slightly thickened walls, hyaline to slightly yellow, sometimes strongly yellow (in yellow/orange parts of hymenium), cyanophilous, not dextrinoid.

Habitat: on decayed wood and litter. Forming ectomycorrhiza with at least *Betula* sp., *Populus* sp., *Quercus petraea* and unspecified subtropical broadleaved trees (Abarenkov et al., 2023). Once recorded from the roots of *Tsuga diversifolia* in Japan and as the host of *Neottia* sp. (l.c.). It is thus clear that this species, in contrast to *P. byssinum* and *P. luminosum* forms ectomycorrhiza with broadleaved trees, but it remains unknown to what extent it can also associate with coniferous trees.

Distribution: basidiome encountered in: Sweden. Present in environmental and root tip samples also from China, Estonia, Japan, Russia and Spain (Abarenkov et al., 2023).

Remarks on identification: see under *P. byssinum*.

***Piloderma fugax* Svantesson & M. Kulju, sp. nov., Fig. 6.**

MycoBank: 857054

Etym.: *Fugax* (Lat.) = coy, elusive, fleeting, fugitive – in our view a fitting description of this hard-to-find little species, whose quickly passing appearances seem like those of a fugitive, hidden away in the scarce remnants of old-growth forest that can still harbour it.

Diagnosis: *Piloderma fugax* is differentiated from other species in *Piloderma* by the combination of cordless basidiomata, frequently

pedunculate basidia, persistent sterigmata, rather sparse encrustation and globose to subglobose spores.

Type: Sweden: Lule Lappmark: Gällivare, Blåkölen Woodland Key Habitat, [old growth forest] on coniferous wood, 66.86103/21.35781, 21 Sep. 2010, J. Nordén 6840 (holotype: O; isotype: OULU GAJ.17972).

UNITE SH (3 %): SH0195980.10FU.

Description: basidiomata resupinate, effused, thin, soft, adnate, hymenium smooth, pale yellowish white to pale brownish, sometimes with a pinkish tinge ("café au lait"), somewhat farinaceous when young, white, margin not differentiated. Hyphal cords absent. Hyphal system monomitic, hyphae thin-walled, without clamps. Subiculum very sparse, hyphae 2.5–5.5(–6.0) µm wide. Subhymenial hyphae of equal width to subicular hyphae, sometimes encrusted by granular to rod-like crystals. Basidia mostly clavo-pedunculate, sometimes clavate, 20–35 × 5.0–8.5 µm, with four slightly to markedly curved sterigmata up to 7 µm long that usually do not collapse after spore discharge. Basidiospores globose to subglobose, (3.7–)3.9–5.5 × (3.3–)3.5–5.0 µm, Q = 1.0–1.2, smooth, thick-walled, without oil-drops, hyaline, cyanophilous, not dextrinoid.

Habitat: on litter and dead wood. The only known host is *Tsuga heterophylla*, but records from forests dominated by other coniferous trees in Europe and Japan likely indicate a range of such species (Abarenkov et al., 2023). Out of seven localities of DNA sequences and specimens for which information is available, all constitute forests that are described either as virgin, old-growth or has a recorded tree age above 120 years (l.c.; Soil fungi in Swedish woodland, 2024).

Distribution: a widely distributed but seemingly rare species, with occurrences in Canada, Finland, Japan, Norway and Sweden (Abarenkov et al., 2023).

Remarks on identification: *Piloderma fugax* can be recognised by its lack of hyphal cords, frequently pedunculate basidia, sterigmata that persist after spore release, rather sparse encrustation and globose to subglobose spores. In all these features, apart from the encrustation (which is not always present), it is reminiscent of a larger version of *Piloderma sphaerosporum* or possibly *Piloderma ochraceum* – whose basidiomatal colour it may approach. Apart from having smaller microscopical features in general, these species always completely lack crystal encrustation. *Piloderma ochraceum* further differs by having partly gelatinised hyphae in the subiculum, while *P. sphaerosporum* has basidia and hyphae with drop-like inclusions. Other species that may appear similar are *Piloderma rallum* and *Piloderma reticulatum*. The former differs from *P. fugax* by its subellipsoid, rather than subglobose spores (slight difference in width), and the latter, though only known from its type, has smaller spores.

Additional specimens studied: Norway: Sør-Trøndelag: Tydal, Hilmo,

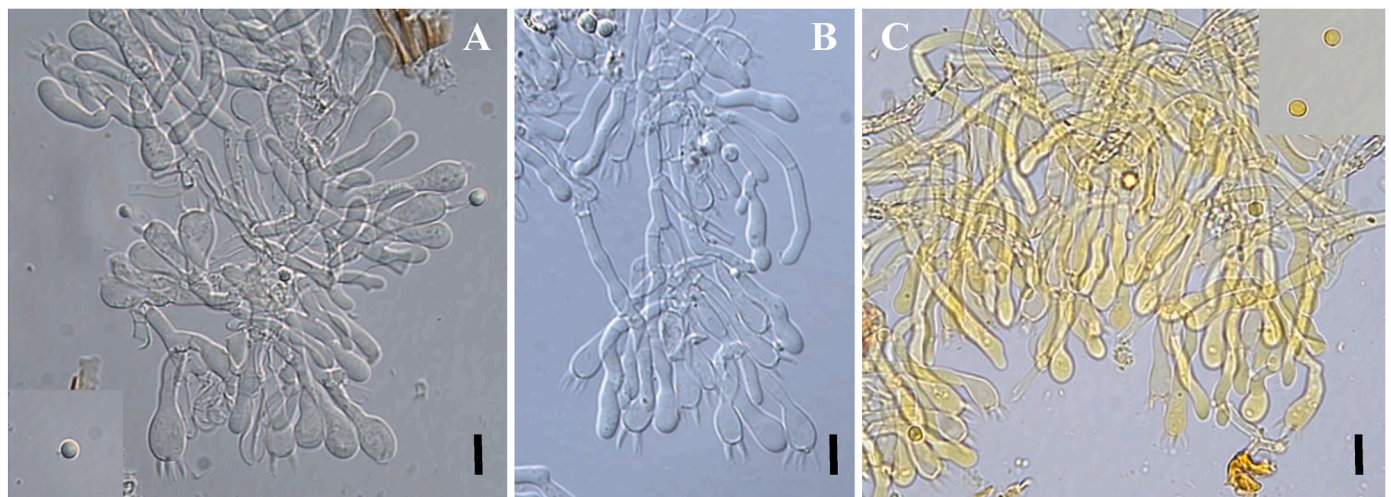


Fig. 6. *Piloderma fugax*. A (SS380): hymenium and inset spore in KOH. B (holotype): hymenium and subhymenium in KOH. C (holotype): hymenium, subhymenium and inset spores in Melzer's reagent. Scale bar = 10 µm.

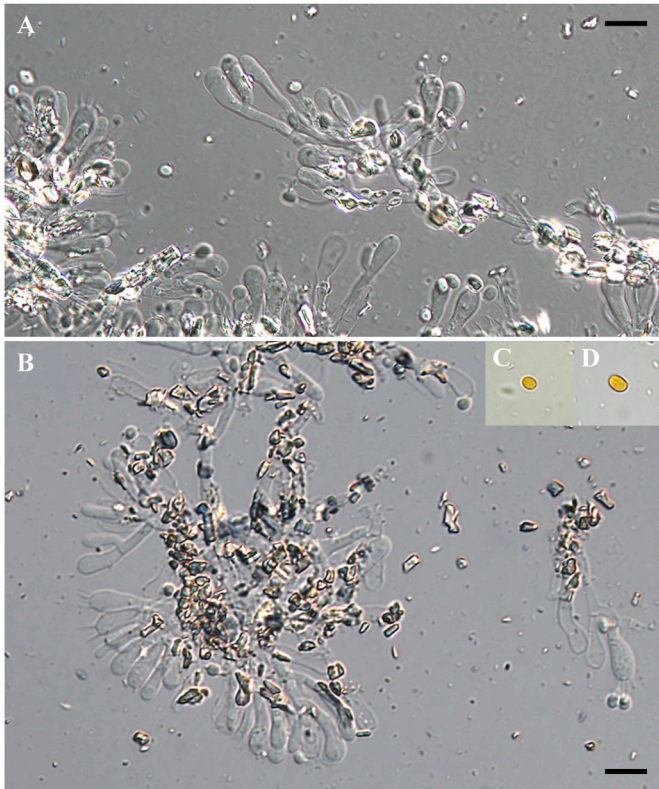


Fig. 7. *Piloderma lamprolithum*. A, B: hymenium and subhymenium in KOH. C: spore from a 4-spored basidium; D: spore from a 2-spored basidium, both in Melzer's reagent. A–C: holotype; D: OULU GAVA.236. Scale bar = 10 µm.

old, spruce-dominated [old-growth] forest, on *Picea abies*, 63,0432°N/11,39°E, 22 Sep. 2011, S. Svantesson 380 (O F-253693).

Piloderma lamprolithum Svantesson, M. Kulju & Helo, **sp. nov.**, Fig. 7.

MycoBank: 857055

Etym.: *Lampros* (Gr.) = shining; *Lithos* (Gr.) = stone. A name alluding to the abundant, coarse crystals present upon the subhymenial hyphae of this species.

Diagnosis: *Piloderma lamprolithum* is differentiated from other species in *Piloderma* by the combination of cordless basidiomata and very coarsely encrusted subhymenial hyphae.

Type: **Norway:** Sør-Trøndelag: Selbu, Råndalen Nature Reserve, [old growth forest] on *Picea abies*, 63.12559/11.23623, 20 Sep. 2011, J. Nordén 9319 (holotype: O; isotype: OULU GAM.20).

UNITE SH (1.5 %): SH1024082.10FU.

Description: basidiomata resupinate, effused, thin, soft, adnate, hymenium smooth, somewhat farinaceous, white to yellowish white (cream-coloured), whitish when young, margin loosely byssoid or arachnoid. Hyphal cords absent. Hyphal system monomitic, hyphae thin-walled, without clamps. Subiculum very sparse, hyphae 2.0–3.5 µm wide, smooth to completely enveloped by fine, crystalline encrustation. Subhymenial hyphae (2.4–)2.8–3.5(–3.9) µm wide, smooth but encrusted by coarse, rectangular to quadratic, rhomboid or angular-irregular, hyaline crystals of equal width to or several times as wide as hyphae. Basidia clavate, 10–25 × 5.0–7.0 µm, with two or four sterigmata up to 6 µm long. Basidiospores from two-spored basidia broadly ellipsoid or ovoid, (5.0–)5.5–6.1 × (3.9–)4.0–4.6(–4.8) µm and from four spored basidia subellipsoid (3.7–)3.8–4.4(–4.7) × 3.1–3.4(–3.7), Q = 1.2–1.4, smooth, thick-walled, with one oil-drop or granular contents, hyaline, cyanophilous, not dextrinoid.

Habitat: on litter and dead wood. Ectomycorrhizal with both conifer and broadleaved hosts. It has so far been found in the roots of *Abies*

alba, *Betula nana*, *Fagus sylvatica*, *Larix gmelinii*, *Picea abies*, *Pinus ponderosa*, *Quercus pubescens* and *Tsuga diversifolia*, as well as in forests consisting solely of tropical tree species (Abarenkov et al., 2023). Both of the Finnish localities – for which soil and vegetation data are available – are calcareous and herb-rich.

Distribution: soil and root tip DNA indicate that *Piloderma lamprolithum* is a species with a very wide distribution in the Northern Hemisphere. It is present in Bulgaria, Canada, China, Czechia, Estonia, Finland, Georgia, Germany, Hungary, India, Italy, Japan, Latvia, Malaysia, Mexico, Morocco, Norway, Romania, Russia, Sweden, Switzerland, Turkey and United States (Abarenkov et al., 2023).

Remarks on identification: *Piloderma lamprolithum* can be recognised by its lack of hyphal cords in combination with the abundant presence of coarse crystals in the subhymenium, which frequently are as wide as they are long and often wider than the hyphae. Among the species without hyphal cords the most similar are *P. fugax* and *P. rallum*, but both species differ by having finer encrustation, with crystals being granular to rod-shaped and narrower than hyphae.

Remarks on delimitation: this species contains two clades with a difference of nine nucleotide bases, across seven loci in the ITS region. Sequencing of Tef1α was attempted but failed. The single specimen belonging to one of the clades has two-spored basidia, while the two specimens belonging to the other clade include both a two-spored specimen and a four-spored specimen. There is a small size-difference between the two two-spored specimens, but without further collections it is impossible to know whether these measurements are representative for the two clades. A clear ecological difference does not seem to exist for the scarce material available. Although *T. lamprolithum* may represent two closely related species with a very similar ecology and morphology, we believe that there is not enough data to conclude this without the addition of further collections to support a morphological distinction or separation by several genetic regions. We therefore currently chose to regard them as one.

Additional specimens studied: **Finland:** Kainuu: Paltamo, Ellukka, 71412, 5327 (etrs-tm35fin), old *Picea abies*-dominated calcareous herb-rich forest, on a 3 cm diam. trunk of *Juniperus communis*, 1 Oct. 2022, T. Helo 20220798 (OULU GAVA.380); Sotkamo, Lauttolampi, 71188/35434 (YKJ), old *Picea abies*-dominated, calcareous, herb-rich forest, on a 2 cm diam. branch of *Picea abies*, 22 Aug. 2019, T. Helo 2019026 (OULU GAVA.236).



Fig. 8. *Piloderma luminosum* (holotype). Fresh basidiome in situ.

Piloderma luminosum Svantesson, *sp. nov.*, Fig. 8.

Mycobank: 857056

Etym.: *Luminosus* (Lat.) = bright, shining. Referring to the vividly orange colour often seen in well-developed basidiomata.

Diagnosis: *Piloderma luminosum* is differentiated from other species in the *P. byssinum* complex based on the colour of its basidiomata, its host association with coniferous trees and nucleotide differences in ITS and Tef1 α .

Type: **Sweden:** Södermanland: Botkyrka, Riksten O, old forest with *Picea abies*, *Pinus sylvestris*, *Populus tremula* and *Betula pendula*, on wood, 59.18580/17.92012, 22 Oct. 2022, S. Svantesson 1008 (holotype: UPS).

UNITE SH (0.5 %): SH1954547.10FU.

Selected illustrations (micromorphology): Larsson et al. (2024): Fig. 3 – same micromorphology as *P. byssinum*).

Description: basidiomata resupinate, effused, loosely attached to the substrate by an extensive, byssoid subiculum, hymenium continuous, more or less pellicular, from the beginning smooth, white, soon after formation often developing yellow to bright orange spots and blisters that may extend to entire basidiome; subiculum remaining white, margin byssoid. Hyphal cords white, usually present in subiculum and substrate. Hyphal system monomitic, hyphae 2.5–3.5 μ m wide, without clamps; in the subiculum with thin to thickened walls, usually with short, baculiform, rod-like or elongated pyramidal crystals, lacking yellow encrustation in Melzer, in the subhymenium thin-walled, richly branched, often with encrustation that is coarser than in the subiculum. Basidia clavate to clavo-pedunculate, (10–)12–15(–25) \times 3.5–5 μ m, with (2–)4 sterigmata. In yellow or orange parts of the hymenium, scattered basidia and underlying hyphae of all types frequently have yellow to orange contents; elsewhere, hyaline. Basidiospores subglobose to elliptic, 3–4(–4.5) \times 2.5–3 μ m, hyaline to slightly yellowish, smooth, with thickened walls, cyanophilous, weakly dextrinoid.

Habitat: on decayed wood and litter of all kinds. Frequently recorded as an ectomycorrhizal associate of conifers. Known hosts are: *Larix gmelinii*, *Picea abies*, *Picea glauca*, *Pinus sylvestris*, *Pseudotsuga menziesii* and *Tsuga heterophylla* (Abarenkov et al., 2023). Once recorded from the roots of *Populus* and as ericoid mycorrhiza in *Vaccinium* (l.c.).

Distribution: widely distributed across the Northern Hemisphere. Known from Canada, Estonia, Finland, India, Italy, Latvia, Malaysia, Mexico, Norway, Pakistan, Russia, Sweden, Switzerland and United States (Abarenkov et al., 2023).

Remarks on identification: The yellow to orange discoloration sometimes seen in the hymenium may cause specimens to be identified as *Piloderma fallax* or *Piloderma olivaceum*. However, these two species have a bright yellow subiculum and yellow hyphal cords when fresh –

features never seen in *P. luminosum*. For further notes on identification: see under *P. byssinum*.

Additional sequenced specimens studied: **Norway:** Akershus: Lørenskog, Østmarka, Tretjernhøla nat. res., on *Picea abies* log, 25 Nov. 2008, E. Bendiksen 1165/08 (O). Buskerud: Modum, Dritardalen on *Picea abies*, 23 Sep. 1997, J.N. Stokland et al., 20399 (O). **Sweden:** Dalarna: Särna, Fulufjället, on *Picea abies*, 20 Sep. 2023, A. Öster 23-11 (UPS); Transtrand, Öjskogsfjället, on *Picea abies*, 17 Aug. 2023, A. Öster 23-05 (UPS). Norrbotten: Råneå, Blåkölens Naturreservat, 17 Aug. 2022 P.-E. Mukka (UPS). Närke: Lerbäck, Åsbrohammar, on *Picea abies*, 25 Apr. 1998, K.G. Nilsson 187 (GB). Uppland: Nora, Östa SO, middle-aged forest with *Picea abies*, *Pinus sylvestris* and *Betula* spp., 24 Oct. 2022, S. Svantesson 1106 (UPS). Västerbotten: Umeå, Grössjön Nature Reserve, old forest, close to *Picea abies* and *Populus tremula*, 63.781737/20.342791, 4 Sep. 2022, S. Svantesson 615 (UPS); Umeå, Stadsliden, old growth forest, close to *Picea abies*, *Pinus sylvestris* and *Betula pubescens*, 4 Sep. 2022, S. Svantesson 610 (UPS); Nordmaling, Sörheden Nature Reserve, old growth forest, pure *Picea abies* stand, 728241/7070234 (SWEREF99), 6 Sep. 2022, S. Svantesson 641 (UPS).

Piloderma mirabile Svantesson & Iršénaitė, *sp. nov.*, Fig. 9.

Mycobank: 857057

Etym.: *Mirabilis* (Lat.) = remarkable, extraordinary. A name alluding to the unexpected discovery of clamps in this species.

Diagnosis: *Piloderma mirabile* is differentiated from other species in *Piloderma* and from those in *Tretomyces* by its consistently clamped hyphae.

Type: **Lithuania:** Zarasų district: near lake Samanis, *Pinus sylvestris* forest with *Betula* and *Picea* (pines ca 180 years old), on mosses, 55.65396/26.05498, 18 Sep. 2018, R. Iršénaitė (holotype: BILAS 51744). UNITE SH (3 %): SH0195969.10FU.

Description: basidiome resupinate, effused, thin, soft, adnate, hymenium finely porulose, white to yellowish white (cream-coloured), margin arachnoid, white. Hyphal cords absent. Hyphal system monomitic, hyphae thin-walled to slightly thick-walled, with clamps at all septa, hyaline, not encrusted. Subiculum very sparse, hyphae 2.5–3.5 μ m wide. Subhymenial hyphae (2.5–)2.7–3.5(–3.8) μ m wide, on average slightly wider than subicular hyphae. Basidia clavate to clavo-pedunculate, 16–22 \times 4.7–5.7 μ m, with four sterigmata up to 4 μ m long. Basidiospores subglobose to subellipsoid, 3.2–3.5 \times 2.6–3.0, Q = 1.1–1.3, smooth, thick-walled, hyaline, cyanophilous, not dextrinoid.

Habitat: ectomycorrhizal with coniferous and ericaceous hosts. It has so far been found in the roots of *Kalmia angustifolia*, *Picea glauca*, *Pinus koraiensis*, *Pinus taeda* and *Tsuga canadensis* but is evidently forming ectomycorrhiza with European tree species as well (Abarenkov et al., 2023).

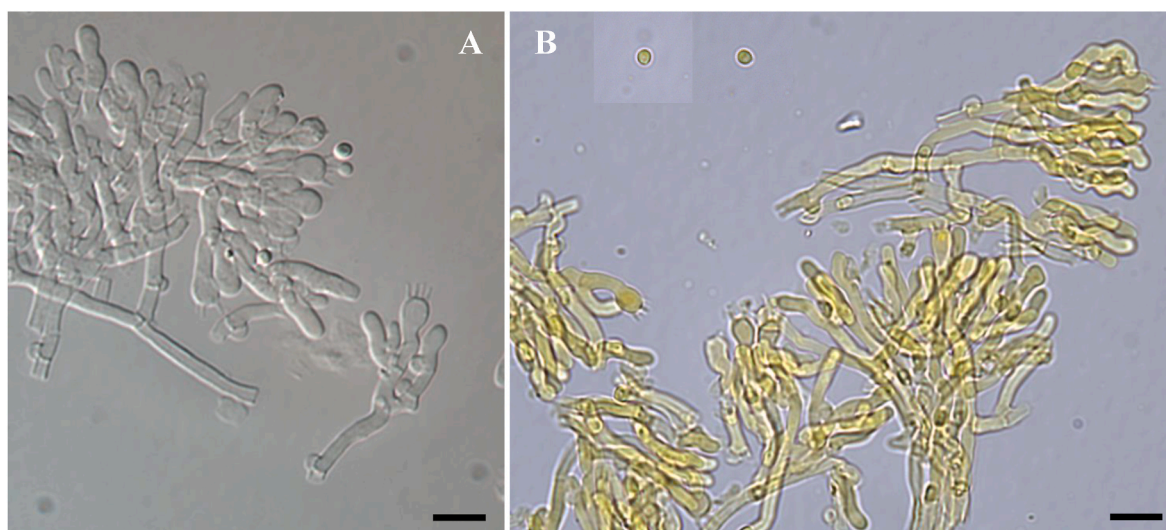


Fig. 9. *Piloderma mirabile* (holotype). A: hymenium and subhymenium in KOH. B: hymenium, subhymenium and inset spores in Melzer's reagent. Scale bar = 10 μ m.

Distribution: apart from the Lithuanian type, soil and root tip DNA indicate that *Piloderma mirabile* is a species with a scattered but very wide distribution in the Northern Hemisphere – it is present also in Canada, Estonia, Russia and United States (Abarenkov et al., 2023).

Remarks on identification: *Piloderma mirabile* is closely related to *P. sphaerosporum* but is unmistakable as the only *Piloderma* species with

clamps. It may appear similar to the species of *Tretomyces* but unlike them, whose hyphae have septa both with clamps and without, it has consistently clamped hyphae. Further, it has larger spores than *Tretomyces microsporus* and very slightly larger spores than *Tretomyces lutescens* (reference specimens might be needed to observe difference).

Key to the formally described species in *Piloderma* (adapted from Larsson et al., 2024)

| | |
|--|----------------------|
| 1. Basidiomata pellicular, subiculum well-developed, byssoid, visible hyphal cords often present on the substrate..... | 2 |
| Basidiomata more or less adnate, subiculum poorly developed, visible hyphal cords lacking..... | 11 |
| 2(1). Subiculum and hyphal cords yellow, after long storage pale yellow to pale pink..... | 3 |
| Subiculum and hyphal cords white..... | 4 |
| 3(2). Hymenium white, subiculum yellow..... | fallax |
| Hymenium and subiculum yellow to brown or olivaceous..... | olivaceum |
| 4(2). Basidiospores strongly dextrinoid..... | craurum |
| Basidiospores not or only weakly dextrinoid..... | 5 |
| 5(4). Basidiospores 2–2.5 µm wide..... | humile |
| Basidiospores 2.5–4.5 µm wide..... | 6 |
| 6(5). Basal hyphae with rod-like to elongated bipyramidal crystals..... | 7 |
| Basal hyphae with mainly acicular crystals..... | 9 |
| 7(5). Under broadleaved trees (in Japan documented from <i>Tsuga</i>)..... | frondosum |
| Under coniferous trees (white to cream basidiomata require molecular identification)..... | 8 |
| 8(7). Basidiomata white to yellow or orange, often more strongly coloured in spots.... | luminosum |
| Basidiomata white to cream..... | byssinum |
| 9(6). Basidiospores subglobose..... | cinicola |
| Basidiospores elliptic..... | 10 |
| 10(9). Basidiospores 3.5–4(–5.5) µm long..... | egens |
| Basidiospores 5–5.5(–6.5) µm long..... | lanatum |
| 11(1). Basidiomata ochraceous to pale brown..... | 12 |
| Basidiomata white to pale ochraceous..... | 13 |
| 12(11). Subicular hyphae partly gelatinised, subhymenial hyphae not encrusted, basidiospores 3.5–4 × 3–3.2..... | ochraceum |
| Subicular hyphae not gelatinised, subhymenial hyphae sometimes encrusted, basidiospores 3.9–5.5 × 3.5–5.0..... | fugax |
| 13(11). Hyphae with clamps..... | mirabile |
| Hyphae without clamps..... | 14 |
| 14(13). Basidia and subhymenial hyphae with drop-like inclusions..... | sphaerosporum |
| Basidia and hyphae lacking drop-like inclusions..... | 15 |
| 15(14). Basidiospores less than 3.5 µm long..... | exiguum |
| Basidiospores 3.5 µm long or longer..... | 16 |
| 16(15). Encrustation abundant, angular, coarse – crystals on subhymenial hyphae often wider than hyphae..... | lamprolithum |
| Encrustation lacking or sparse, when present granular, mostly fine – crystals narrower than hyphae..... | 17 |
| 17(16). Basidiospores 3.5–4 µm long..... | reticulatum |
| Basidiospores 4–5.5 µm long..... | 18 |
| 18(17). Basidiospores subglobose to elliptic 2.5–3.7 µm wide, basidia clavate..... | rallum |
| Basidiospores globose to subglobose 3.5–5.0 µm wide, basidia often pedunculate..... | fugax |

5. Discussion

Environmental DNA of fungi from sequencing of soil samples regularly display molecular variation far transgressing currently applied names and has thus in recent years brought into focus the communicative need for new species to be described (e.g. Nilsson et al., 2023). Within the genus *Piloderma*, Larsson et al. (2024) clarified the usage of existing names through type studies and described seven new species to science, most of which seem to be less common than previously known species but still not rare (Abarenkov et al., 2023; Soil fungi in Swedish woodland, 2024). In this study we have described a further five new species from northern Europe, three of which seem to be rare (l.c.). As a result of these two studies, the number of accepted *Piloderma* species has now tripled.

Rare species, by nature, take longer to find and even more so if they have inconspicuous basidiomata that are difficult to separate from previously described species in the field. In this study enough material to facilitate their description was achieved through extensive field sampling, barcoding, and a wide research network. However, it is clear that there are more species out there awaiting formal description.

The existence of a rare species seemingly limited to old-growth forest, *Piloderma fugax*, in a genus of species hitherto perceived as very common and ecologically indiscriminate is intriguing. The mechanisms behind this are hard to speculate upon, but it raises the question of whether other rare species in the genus – particularly those yet to be described – are associated with similar habitats. This, in turn, highlights the importance of studying them, from both taxonomical, conservation and ecological points of view.

The presence of clamps in *Piloderma mirabile* – a feature not previously observed in *Piloderma* – challenges the distinction between *Piloderma* and *Tretomyces*, at least morphologically. A narrow dataset was used in this study in order to maximise resolution for species delimitation, why generic delimitation is not certain. However, the close relationship between *Piloderma sphaerosporum* and *P. mirabile* indicates that in the current delimitation of genera, *P. mirabile* belongs in *Piloderma*. Even so, generic delimitation within *Pilodermataceae* is not well supported (Sulistyo et al., 2021), and should be studied with both more genetic regions and more species sampled.

Most new species described in this study are based on rather small and few collections. In general, one could argue that this is bad practice and should be avoided. However, in cases where rare species are targeted that few mycologists work with taxonomically (see e.g. Svantesson et al., 2021), it is our opinion that the benefits associated with a formal description outweighs the negative aspects of such endeavours. A species named and described can be identified in collection efforts by professionals and amateurs alike, targeted by conservational ventures and laws and is probably more likely to be the focus of ecological studies, while a taxonomic description that lacks the full morphological breadth of the species concerned can be updated when new specimens are encountered – likely at an accelerated rate as a result of its description.

However, we left *Piloderma* sp., a clade in the *P. byssinum* complex that likely constitutes a new species, undescribed, since we do not think the currently available data conclusively support its formal description at this stage – it is not morphologically distinct and we do not have more than one genetic region for it. The ITS sequences available are derived from soil samples and mycorrhizal root tips from USA and Canada, found on *Pseudotsuga menziesii* or in “pinaceous forest”. Although this seemingly sets it apart ecologically from *P. frondosum* – its closest relative according to the ITS phylogeny (Fig. 1) – *P. frondosum* has not been documented outside Eurasia, and a North American subpopulation with an intercontinental niche shift hence cannot be ruled out. We hope that publication of the current study can inspire the collection of fresh material and sequencing of additional genetic regions (at least Tef1 α) to let the matter be settled.

For two of the new species important parts of their descriptions were

elucidated from ecological metadata available in international gene databases; *Piloderma frondosum* is part of the semicryptic *Piloderma byssinum* group, but is separated from the other species therein due to its deviating association with broadleaved hosts, while *P. fugax* seems to be restricted to old growth forest. The basidiomata upon which description of the latter were made, as well as one of two paratypes of *Piloderma lamprolithum* were also part of a large collection of basidiomata made for a study on ecology (Nordén J. – to be published), which was carefully examined by one of us (MK). This testifies to the important interconnection between ecological studies of both basidiomata and ectomycorrhiza, international gene databases and taxonomic studies; the former two can play a vital role in the delimitation and ecological knowledge of species – especially in cases like this, where morphological characteristics are scarce – while taxonomic studies, in turn, can demonstrably pay back by providing more accurate delimitation for and names to facilitate communication of the units employed.

CRedit authorship contribution statement

Sten Svantesson: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lowie Tondeleir:** Writing – review & editing, Visualization, Investigation. **Matti Kulju:** Writing – review & editing, Investigation. **Reda Iršenaite:** Writing – review & editing, Investigation. **Björn D. Lindahl:** Writing – review & editing, Investigation. **Teppo Helo:** Investigation. **Karl-Henrik Larsson:** Writing – review & editing, Investigation, Conceptualization. **Martin Ryberg:** Writing – review & editing, Visualization, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Conflict of interest

The authors declare no conflict of interest.

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