

Exploring the European *Trichophaea*-like discomycetes (*Pezi-zales*) using morphological, ecological and molecular data. Part 2: Type revision of *Lasiobolidium spirale* and *L. mercantourense* sp. nov.

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Abstract: The revision of type material of *Lasiobolidium spirale*, type of the genus *Lasiobolidium*, is presented and illustrated with colour photographs for the first time. We also present the discovery of a new species, named *Lasiobolidium mercantourense*. Morphological and molecular data of this species are given, as well as a comparison with closest species. Finally, an updated key to *Lasiobolidium* is provided.

Keywords: Ascomycota, Pyronemataceae, rDNA, phylogeny, taxonomy, 1 new species.

Résumé : la révision du matériel type de *Lasiobolidium spirale*, type du genre *Lasiobolidium*, est présentée et illustrée pour la première fois avec des photographies en couleur. Nous présentons également la découverte d'une nouvelle espèce, nommée *Lasiobolidium mercantourense*. Les données morphologiques et moléculaires de cette espèce sont données, ainsi que la comparaison avec les espèces les plus proches. Enfin, une clé mise à jour des *Lasiobolidium* est fournie.

Mots-clés : Ascomycota, Pyronemataceae, ADN ribosomal, phylogénie, taxinomie, 1 nouvelle espèce.

Introduction

In the first part of our study on *Trichophaea*-like species (VAN VOOREN *et al.*, 2021), we amended the cleistothecial genus *Lasiobolidium* Malloch & Cain to include apothecial species thanks to new molecular data. Comparative analyses of these apothecial species were conducted to propose a new taxonomical frame of this genus. As the molecular results of a putative new species and those from type collections of *Lasiobolidium spirale* Malloch & Cain, the type of the genus, arrived lately in the editorial process of our article, we provide herein new phylogenetic analyses, as well as full descriptions and illustrations of these two taxa.

Material and methods

Morphological study. — Methods are identical to those defined in VAN VOOREN *et al.* (2021).

DNA extraction, amplification and sequencing. — Methods are identical to those defined in VAN VOOREN *et al.* (2021).

Phylogenetic analyses. — Sequences were first aligned in MEGA X (KUMAR *et al.*, 2011) software with its MUSCLE application (EDGAR, 2004) and then manually corrected. Evolutionary analyses were conducted in MEGA X with 24 nucleotide sequences for the LSU region (826 positions), and 13 nucleotide sequences for the combination of ITS and LSU (1652 positions). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible (GTR) model (NEI & KUMAR, 2000) with 1000 bootstrap iterations. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution (4 categories) was used to model evolutionary rate differences among sites.

The phylograms were graphically edited with the online software iTOL v6 (<https://itol.embl.de>, LETUNIC & BORK, 2019).

The sequences generated during this study were deposited in GenBank and are listed in Table 1.

Molecular results

Phylogenetic analyses were conducted to precisely evaluate the taxonomic position of the studied collections (including our putative new species), after the preliminary results given by the BLAST search (ALTSCHUL *et al.*, 1990). We first chose a phylogeny based on the LSU region to compare our results with the phylogeny published by PERRY *et al.* (2007, Fig. 1). We used a selection of taxa of *Pseudombrophilaceae* as outgroup because of the existence in this group of a sequence erroneously determined as *L. spirale* (MH872132).

We also proposed a phylogeny combining the ITS and the more conservative LSU region to compare the topologies of this two-gene analysis with the three-gene phylogeny obtained by VAN VOOREN *et al.* (2021, Fig. 1) where *Lasiobolidium* was supported as a monophyletic clade. As outgroup, two *Lasiobolidium*-like species belonging to *Pseudombrophilaceae* were chosen.

As shown by PERRY *et al.* (2007), HANSEN *et al.* (2013) and VAN VOOREN *et al.* (2021), *Lasiobolidium spirale* is strongly supported within *Pyronemataceae*, contrary to *L. orbiculoides* Malloch & Benny, nested in *Pseudombrophilaceae*, although its taxonomic position is still unclear (HANSEN *et al.*, 2005; LINDEMANN & ALVARADO, 2017). The strain CBS 866.71, erroneously determined as *L. spirale*, also belongs to *Pseudombrophilaceae*. The correct position of other cleistothecial species of *Lasiobolidium* is unknown because there are no molecular data available.

Another result of these analyses is that our putative new species is clearly nested into the *Lasiobolidium* clade and appears phylogenetically distinct from the other known species.

Table 1 – Collections sequenced for this study

Name	Coll. Ref.	Collector	Country	GenBank Accession numbers	
				ITS	LSU
<i>Lasiobolidium mercantourense</i>	LY NV 2020.10.38	N. Van Vooren	France	OL619531	OL619793
<i>Lasiobolidium parvisporum</i>	CVL060521(1)	F.J. Valencia	Spain	OL653171	OL653173

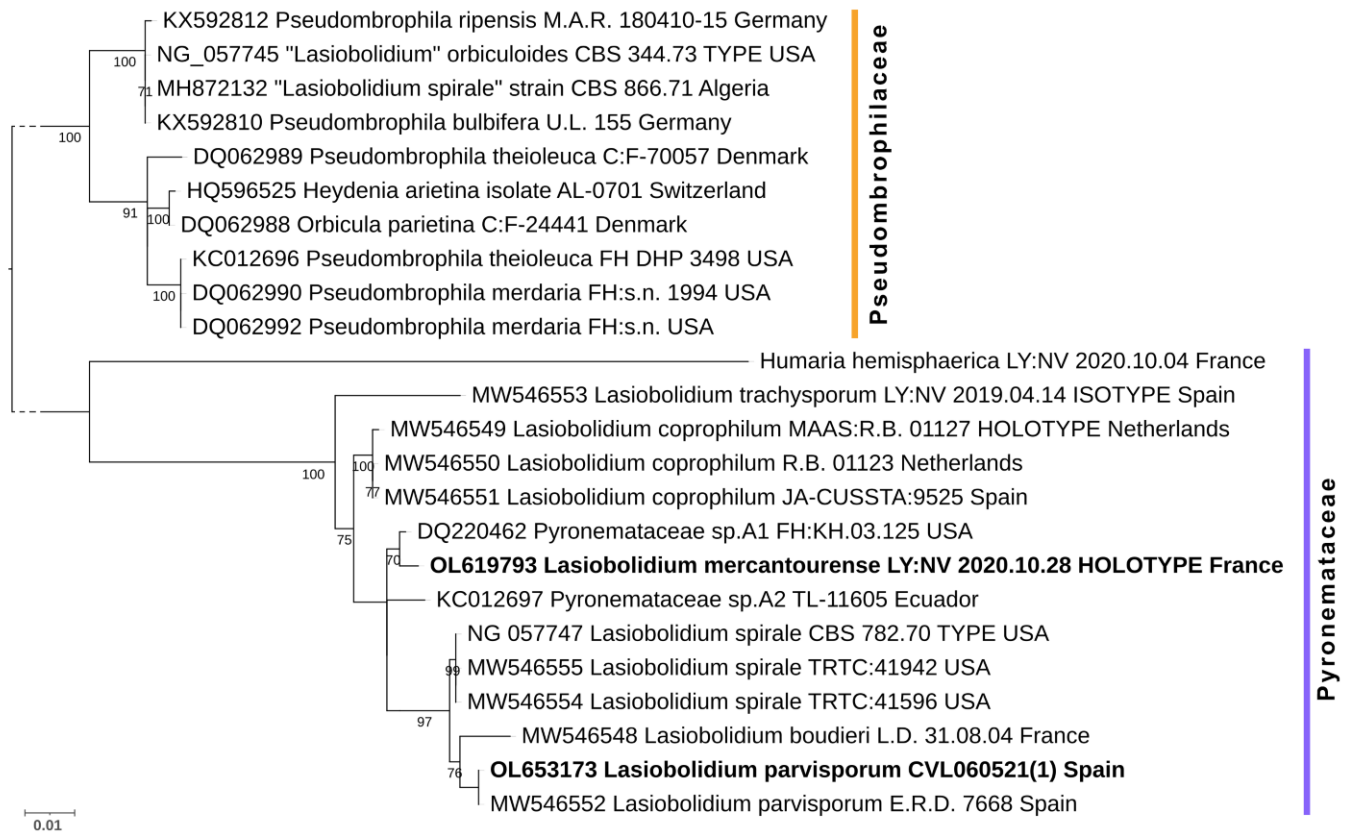


Fig. 1 – Phylogram of maximum likelihood (ML) of *Lasiobolidium* based on the LSU region, rooted with *Pseudombrophilaceae* spp. Nodes with $\geq 70\%$ ML bootstrap support are annotated with their support values. Terminals that represent sequences obtained during this study are in bold.

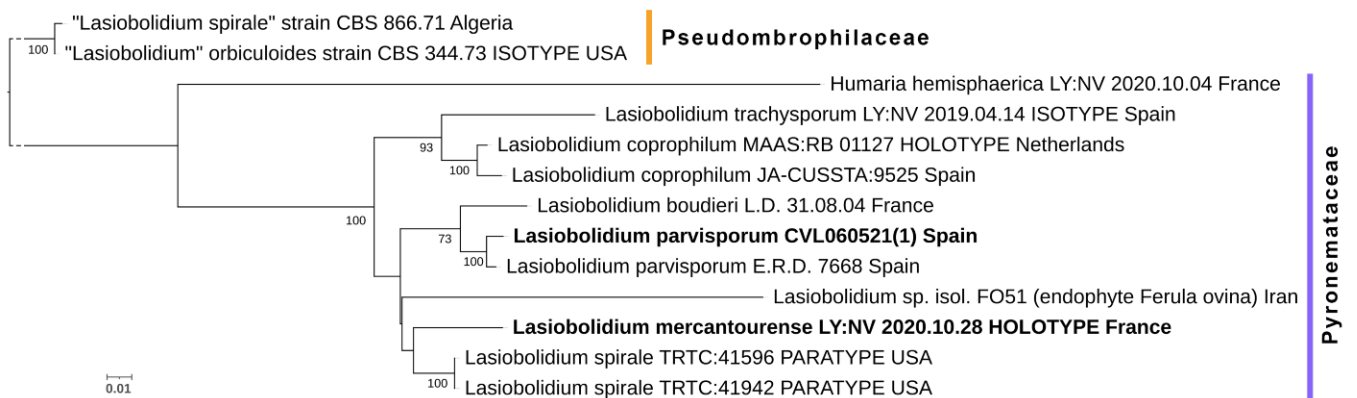


Fig. 2 – Phylogram of maximum likelihood (ML) of *Lasiobolidium* based on the combination of ITS and LSU regions, rooted with *Lasiobolidium*-like species belonging to *Pseudombrophilaceae*. Nodes with $\geq 70\%$ ML bootstrap support are annotated with their support values. Terminals that represent sequences obtained during this study are in bold.

Taxonomy

Circumscription of *Lasiobolidium*

An emended definition of *Lasiobolidium* was provided by VAN VOOREN *et al.* (2021). In this context, VAN VOOREN *et al.* (2021) expressed their doubt that the two fimicolous species, *L. helicoideum* Locq.-Lin. and *L. recurvatum* Locq.-Lin. (LOQUIN-LINARD, 1983) could belong to this genus, because of their type of hairs, i.e. thin-walled and not septate, as well as their coloured ascospores. *L. gracile* Yaguchi, Someya & Udagawa seems also excluded from this genus because of its hairs sparsely covered by brown-pigmented knots. *L. fallax*

Locq.-Lin. and *L. aegyptiacum* Moustafa & Ezz-Eldin share morphological affinities with *L. spirale* and may be accepted in this genus, waiting for molecular evidences. Finally, a full description of known apothecial species, i.e. *L. boudieri* (Grelet) Van Vooren & Valade, *L. parvisporum* (Benkert) Van Vooren, M. Carbone & Rubio, *L. coprophilum* Van Vooren, Bronckers & Valencia and *L. trachysporum* Valencia, M. Vega & Van Vooren, is given in VAN VOOREN *et al.* (2021). Note that *Paratrichophaea michiganensis* (Kanouse) Pfister is supposed to be a member of *Lasiobolidium* based on its description (KANOUSE, 1958; PFISTER, 1988) but we did not have the opportunity to study and sequence this species.

Descriptions

Lasiobolidium spirale Malloch & Cain, *Can. J. Bot.*, 49 (6): 853 (1971) – Pl. 1.

Original diagnosis: “*Coloniae in agaro “corn meal” secundum diametrem aetate 40 dierum 5 cm, ad appressas arachnoideae, hyalinae, azonatae; hyphae 2.0–5.0 µ crassae, hyalinae et laeves vel luteobrunneae et crustatae, irregulariter inflatae; ascogonia 10–17 µ crassa, septata, convoluta; ascocarpae 170–750 µ crassae, subglobosae vel globosae vel irregulares, luteo-brunneae, appendiculatae, granulosae, nonstiolatae; peridium ascocarporum bipartitum; appendices ascocarporum 5.0–10.5 µ crassae, in peridio aequaliter dispositae, aurantio-luteae, crassitunicatae, nonseptatae, helicoideae; asci 35–62 × 12–21 µ, irregulariter dispositi, clavati, octospori, evanescentes, nonstipitati vel brevi stipitati, nonamyloidei; ascosporeae 12–17 × 9.0–12 µ, ellipsoideae, hyalinae, laeves, sine porae; conidia nulla.*”

HOLOTYPE: *In fimo Bovis tauri*, Bosler, Albany Co., Wyoming, U.S.A., 1 Sept. 1964, Cain, TRTC 43808. In Cryptogamic Herbarium, University of Toronto.”

Type: Coll. TRTC 43808 (holotype).

The holotype contains only four apothecia. Therefore, for the type study, we used the attached slides, in order to not damage this scanty material. The ascocarps of the type seem in good condition. They are subglobose to globose, yellow-brown, 0.5–0.7 mm diam., densely covered by long curled light brown to hyaline hairs (2–3 mm in length). The fruitbodies are growing on the substrate, and the hairs obviously serve to anchor them (Plate 1A + C).

The label of the type is in conformity with the data indicated in the protologue, except the name of the determinator, J.C. Krug, is not indicated in the latter.

The observations made during the examination of the slides were supplemented by the study of three paratype collections (see data below).

Description (†) of microscopical characters:

The structure of the **excipulum** could not be observed. **Peridium** of *textura globulosa/subglobosa*, composed of brown, thick-walled cells up to 20 µm diam.; some cells on the outermost part of the peridium are elongated (ca. 30 × 10 µm). **Hairs** on all sides of the ascomata, hyaline to brown, thick-walled, not septate, not rooting, up to 3000 µm in length, 6.5–10 µm wide, widened at the base up to 20 µm. **Asci** 8-spored, without reaction in IKI; they are all collapsed, therefore a reasonable measurement is not possible. **Paraphyses** not seen. **Ascospores** biseriata, (13.2–)13.9–15.3 × 9.6–10.6 (–11.2) µm, Q = 1.4–1.6 [X = 14.6 × 10.1 µm, Qm = 1.5 (N = 20)], ellipsoid, thick-walled, hyaline to light brown, smooth.

Additional studied collections: USA. Nevada, Mineral Co., Montgomery Pass, on horse dung, 22 Aug. 1957, *leg.* and *det.* R.F. Cain, TRTC 41596 (paratype), ITS: MW546588, LSU: MW546554. Nevada, White Pine Co., McGill, on horse dung, 21 Aug. 1957, *leg.* R.F. Cain, *det.* P. Adilakshmi, TRTC 41942 (paratype), ITS: MW546589, LSU: MW546555. Nevada, White Pine Co., McGill, on cow dung, 21 Aug. 1957, *leg.* R.F. Cain, *det.* A. Punugu, TRTC 42029 (paratype).

Distribution: *Lasiobolidium spirale* is apparently only known from the USA. This is quite astonishing because, in the case of coprophilous species, it is not uncommon to find them distributed worldwide, not least due to the global livestock economy.

Comments: The observations made during the re-examination of the type material are mainly consistent with the original description by MALLOCH & CAIN (1971). The differences are so small that they do not require further comment. Being a cleistothecial species, it was astonishing that all genetically closely related species were *Trichophaea*-like discomycetes. However, this different morphology (cleistothecium vs. apothecium) hides a number of microscopical similarities we depicted in VAN VOOREN *et al.* (2021). A similar observation in the genus *Warcupia* Paden & J.V. Cameron — where the type is made of cleistothecial ascomata (PADEN & CAMERON, 1972) — was recently published by CARBONE *et al.* (2021) who described a new apothecial species.

Updated key to the species of *Lasiobolidium*

- | | |
|--|---|
| 1. Ascomata cleistothecial, less than 1 mm diam. | 2 |
| 1. Ascomata apothecial, hairs upward-pointing | 4 |
| 2. Ascomata with helically coiled hairs; ascospores ± biseriata, 14–15.5 × 9.5–10.5 µm; on dung (horse, cow) | <i>L. spirale</i> |
| 2. Ascomata with wavy or irregularly coiled hairs | 3 |
| 3. Asci cylindrical; ascospores uniseriate, 11–12 × 8–9 µm; in soil | <i>L. aegyptiacum</i> |
| 3. Asci clavate; ascospores ± biseriata, 9–10 (12) × 5.5–7 (8) µm; on dung (camel) | <i>L. fallax</i> |
| 4. Ascospores smooth | 5 |
| 4. Ascospores ornamented by low and minute warts | <i>L. trachysporum</i> |
| 5. Apothecia growing on dung (horse, cow) | <i>L. coprophilum</i> |
| 5. Apothecia growing on other substrates | 6 |
| 6. Clavate macrocells present at the margin, up to 180 µm long, hairs often multifurcate; on wood | <i>L. macrocystis</i> |
| 6. Clavate macrocells absent or shorter | 7 |
| 7. Asci 8-spored | 8 |
| 7. Asci 4-spored | <i>Paratrichophaea michiganensis</i> |
| 8. Marginal hairs ≤ 500 µm | 9 |
| 8. Marginal hairs > 500 µm, reaching 850 µm long or sometimes more; ascospores 15–20 × 10–12 µm, containing bipolar small granules, disappearing at maturity | <i>L. boudieri</i> |
| 9. Ascospores 16.2–17.7 × 10.6–12.2 µm, containing persistent small granules; marginal hairs reaching 500 µm long .. | <i>L. parvisporum</i> |
| 9. Ascospores 19–21 × 12–13.5 µm, containing bipolar small granules, disappearing at maturity; marginal hairs up to 340 µm long | <i>L. mercantourense</i> |

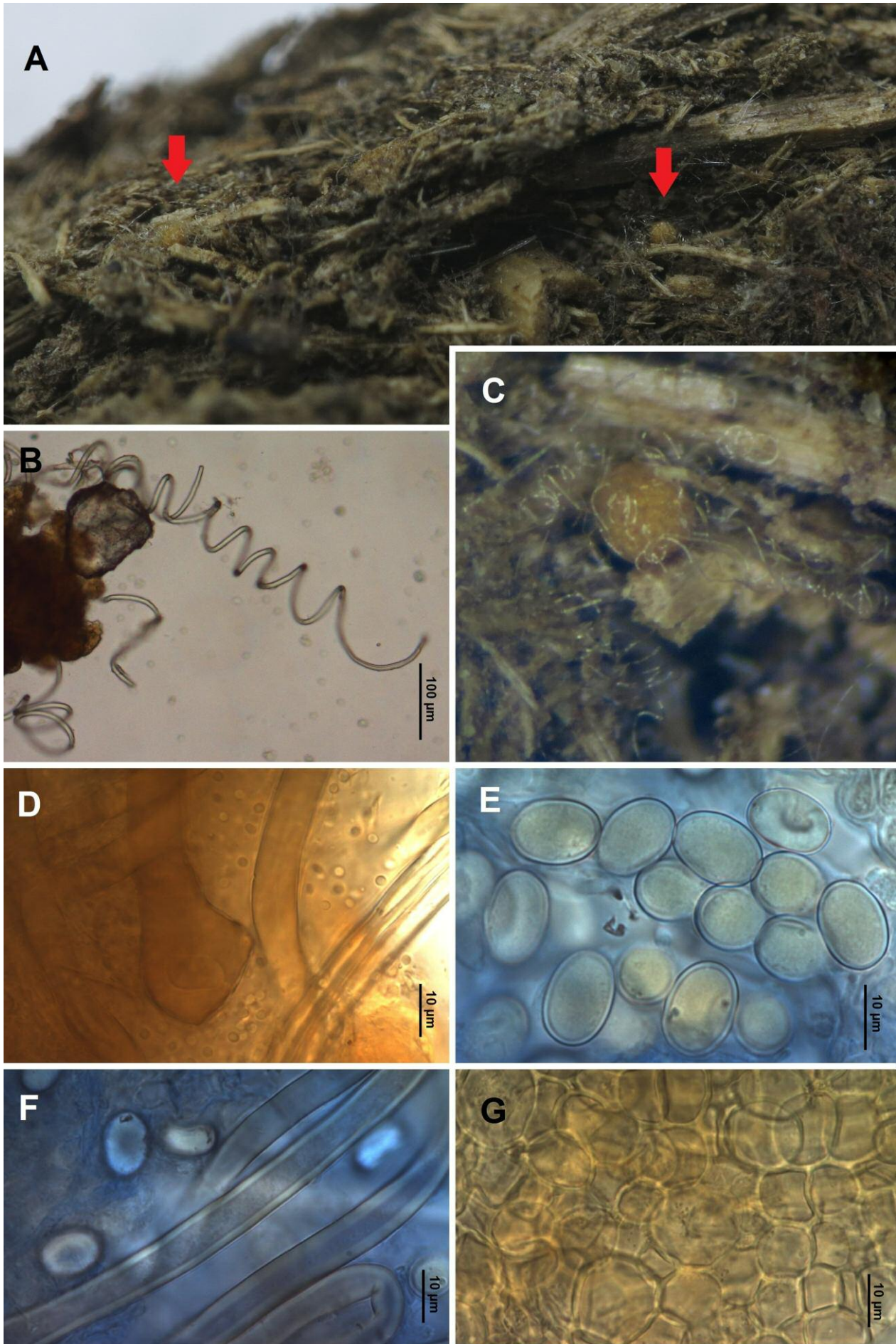


Plate 1 – *Lasiobolidium spirale*. A, C: Ascomata on substrate; B: Curled hair. D: Hair base; E: Ascospores, in cotton blue. F: Thick-walled hairs; G: Cells of the peridium. A, C, E + F from the holotype (TRTC 43808), B + D from the paratype TRTC 042029, G from the paratype TRTC 041596. All photos by U. Lindemann.

Lasiobolidium mercantourense Van Vooren, *sp. nov.* – MB 841999
– Pl. 2 & fig. 3

Diagnosis: Differs from *L. boudieri* by its wider ascospores, its shorter hairs and its mountainous habitat.

Type: coll. LY NV 2020.10.28 (holotype).

Etymology: From Mercantour, the name of the French national park where the species was found.

Apothecia 0.5–0.7 mm diam., sessile, ± obconical, hymenium flat, whitish grey; external surface concolorous, covered with short brown hairs, upward-pointing. **Marginal** hairy with brown hairs.

Subhymenium and **medullary excipulum** undifferentiated, of *textura globulosa*, made of small hyaline cells, 6–20 µm diam., mixed with more elongated to clavate cells. **Ectal excipulum** thin, of *textura globulosa/subangularis*, with hyaline cells, 6–27 µm wide. **Marginal cells** made of subglobose cells ± organised in *textura prismatica*. **Marginal hairs** 190–340 × 9–20 µm, superficial, septate, straight with a simple radicate base, sharp at the top, 2–3 µm thick-walled. **Excipular hairs** very similar, slightly shorter, sometimes emerging from a bulbous base. **Anchoring hyphae** present, rather short, septate, hyaline, ~3 µm wide. **Ascospores** uniseriate, ellipsoid, (18.2) 19–21 × (11) 12–13.5 µm, Q 1.5–1.7 (1.8) [X = 19.9 × 12.2 µm, Qm = 1.6, N = 30], hyaline, smooth, without lipid drops but containing bipolar small granules, disappearing at maturity. **Asci** cylindrical, 230–250 × 13–15 µm, arising from croziers, operculate, 8-spored. **Paraphyses** filiform, hyaline, not widened at the top, 2–3 µm diam.

Studied collection: FRANCE. Alpes-de-Haute-Provence, Allos, Cluite forest, near “Fontaine de Valplane”, 44.247505° N 6.668315° E, 1800 m a.s.l., on soil, among small mosses, along a path, under conifers (*Abies alba*, *Picea abies*), 14 Oct. 2020, leg. N. Van Vooren, herb. LY NV 2020.10.28 (holotype); GenBank ITS OL619531, LSU OL619793.

Distribution: Only known from original locality. Its ecology (see below) suggests it could be found in other mountainous area of southern Europe.

Comments: This species was discovered during an inventory made in the mountains of the National Park of Mercantour (Southern France). It was found in an ecologically interesting forest because the latter is dominated by *Abies alba* and *Picea abies* rather than larches (*Larix decidua*), that dominate other subalpine forests

in this area. The first examination of these tiny ascomata immediately pointed to the genus *Lasiobolidium* as recently amended by us (VAN VOOREN *et al.*, 2021) because of the upward-pointing hairs and eguttulate ascospores. It was first compared to *L. boudieri* (Grelet) Van Vooren & Valade, but the latter possesses longer hairs and narrower ascospores. The ecological context is also different, since *L. boudieri* has to our knowledge not been reported from such a high elevation and is often reported from wet places with broadleaf trees and scrubs (see studied collections in VAN VOOREN *et al.*, 2021: 28). *L. parvisporum* (Benkert) Van Vooren, M. Carbone & Rubio is also similar to *L. mercantourense* but has shorter ascospores containing persistent granules.

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Plate 2 – *Lasiobolidium mercantourense* in situ.

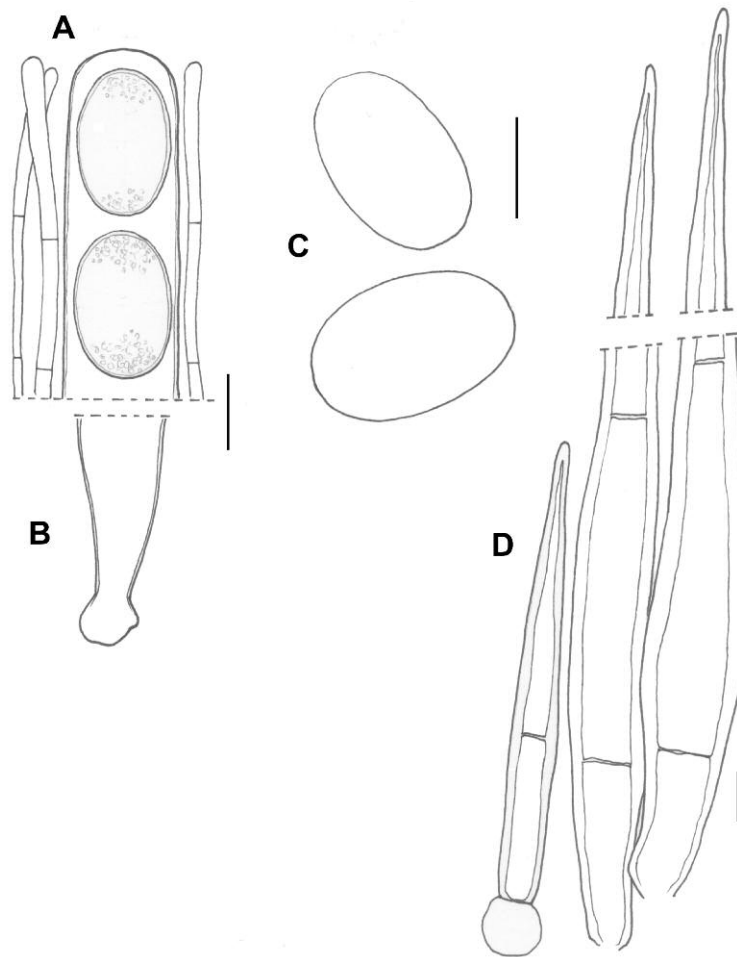


Fig. 3 – *Lasiobolidium mercantourense*, microscopical characters.

A. Top of ascus and paraphyses. B. Base of ascus. C. Ascospores. D. Hairs. All elements in water. Scale bars = 10 µm. Drawings: N. Van Vooren

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