Pseudotricharina lanigera (*Pezizales*), a new species from the Patagonian region of Argentina

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Abstract: A species of *Pseudotricharina*, similar in sequence and morphology to the type species *P. intermedia*, is described from a soil bank in a *Nothofagus* forest of the Andes Mountains of Argentina. This is only the second species of *Pseudotricharina* to be described and the first known from the Southern Hemisphere.
Keywords: Ascomycota, cup fungus, phylogeny, *Pyronemataceae*, taxonomy.

Resumen: Una nueva especie de *Pseudotricharina* se descripta para la región Andina de Argentina creciendo en suelo expuesto en bosques de *Nothofagus*. Esta especie posee morfología y secuencia de ITS similares a la especie tipo *P. intermedia* y constituye la segunda especie de *Pseudotricharina* descrita y la primera para el hemisferio sur.
Palabras clave: Ascomycota, discomycete, filogenia, *Pyronemataceae*, taxonomía.

Introduction

During recent studies of the fungi in Nothofagaceae forests of South America our research group has been systematically collecting fungi and obtaining ITS rDNA barcodes. This effort has significantly increased available sequence data from Patagonia and also raised awareness of new species in the region (Truong *et al.*, 2017). One of these species was included in phylogenetic analyses of ITS and LSU during efforts to revise the genus *Tricharina* Eckblad (*Pyronemataceae*) (Van Vooren *et al*., 2017). Their analyses unequivocally placed this new species in the genus *Pseudotricharina* Van Vooren, Tello & M. Vega. The genus *Pseudotricharina* was described based on collections of the type species, *P. intermedia* Van Vooren, Tello & M. Vega, in the Mediterranean regions of Spain, Italy and Greece. Here we morphologically describe this new species, and distinguish it from the only other known species of *Pseudotricharina*.

Materials and methods

Apothecia were collected from the same site on two dates in May 2016. Fresh specimens were photographed on site, and cleaned of debris and preserved in DNA extraction buffer. They were then dried over low heat in a dehydrator, and stored in plastic bags as voucher specimens for further morphological examination. For molecular methods, DNA was extracted using an Extract-N-Amp Plant kit (SigmaAldrich). The ITS and LSU regions were PCR-amplified using primers ITS1f (Gardes & Bruns, 1993), ITS4 (White *et al.*, 1990), LROR, and LR5 (Vilgalys & Hester, 1990). Sanger sequencing was performed using the same primers at the Interdisciplinary Center for Biological Research at the University of Florida. Phylogenetic methods were those of Van Vooren *et al.* (2017). GenBank accession numbers are MES-2065 KY364034 (ITS) KY364071 (LSU), MES-2152 KY364033 (ITS) KY364072 (LSU).

For morphological analysis, dried specimens were soaked in water and cleaned of soil debris. Apothecia were then sectioned for photomicroscopy and measurements were obtained for relevant features (e.g. excipular hairs, excipulum, hymenium, and ascospores). All microscopic measurements were obtained from apothecial sections mounted in water. Spore ornaments were visualized and photographed from ascospores mounted in cotton blue in lactophenol. Reaction of material mounted in Melzer's solution, cotton blue in lactophenol, and 2% KOH were recorded. From each collection 30 ascospores were measured, excluding ornamentation, to obtain average length, width and Q (length/width) values. The range is given with extremes in parentheses.

Results

The ITS and LSU sequences of *Pseudotricharina lanigera* were included in the phylogenetic analyses of Van Vooren *et al.* (2017, Fig. 1 and 2). The topologies of the ITS and LSU RAxML trees clearly show the placement of this new species in the *Pseudotricharina* clade. The ITS phylogeny shows the new species as sister to an undescribed species known only from environmental sequences from *Pterygodium* (orchid) roots in South Africa, and both are depicted as a sister clade to *P. intermedia*. The LSU phylogeny shows *P. lanigera* and *P. intermedia* as sister species, but there were no LSU sequence available for the root tip from South Africa from which the closely related ITS sequence was derived.

Taxonomy

Pseudotricharina lanigera Healy, D. Torres, Pfister & M.E. Sm., *sp. nov.* — MB821469

Diagnosis: *Pseudotricharina lanigera* is distinguished from other genera by a combination of the following: inamyloid operculate asci; ellipsoid symmetrical ascospores with isolated, prominent warts; whitish to light orange ascomata that tend to develop beneath the soil surface; presence of copious brown, excipular hairs that are long, flexuous, often anastomosed and binding soil tightly; and distinguished from the most similar species, *P. intermedia*, by its wider ascospores (Q 1.4–1.9). Holotype MES-2152 (CORD).

Etymology: From the Latin "*lana*", which means "wool", and "*gerere*" which means "to bear".

Apothecia up to 8 mm in diameter, and 3–5 mm high, round or somewhat irregular in shape due to compression by soil and pebbles, white to yellowish orange exterior, covered with light brown hairs that are matted together with soil. Apothecia begin their growth closed, at or below the soil surface, opening out at or just above the soil surface. Hymenium varies from cream white to light yellow to orange. Color is not indicative of maturity. Some apothecia were fruiting among moss, but more often they were embedded in bare soil. Mature apothecia are cup shaped, not flattened (Fig. 1 A-C). Those below the soil surface look like a cavity in the soil, but most have their apothecial margin above the soil surface. Elongated, flexuous, anastomosing hair-like hyphae that emanate from the cells below the margin of the excipulum hold the soil tightly obscuring

the excipulum, and anchor the apothecium to the soil. Thus these hyphae are here referred as "anchor cells".

Ectal excipulum 160–180 µm thick, composed of more or less isodiametric large cells measuring up to 40 × 40 µm. Outermost cells rounded to angular, walls 1–1.5 µm thick (Fig. 1 D). The tissue adjacent to the hymenium is composed of parallel hyphae that terminate as short, straight, smooth, hyaline elements 80–100 µm long with rounded tips, and variable widths 4–10 µm wide (Fig. 1 E). Next to these elements there are progressively longer hyphal "hairs" that form the excipular margin (Fig. 1 F). Below the hairs at the apex of the margin there is a layer of hairs that are longer than the depth of the cup, flexuous, smooth, hyaline to light brown, and short or up to 440 µm long, and 4 µm wide (Fig. 1 F lh). These are present around the entire margin and are visible with a hand lens. The tips of these

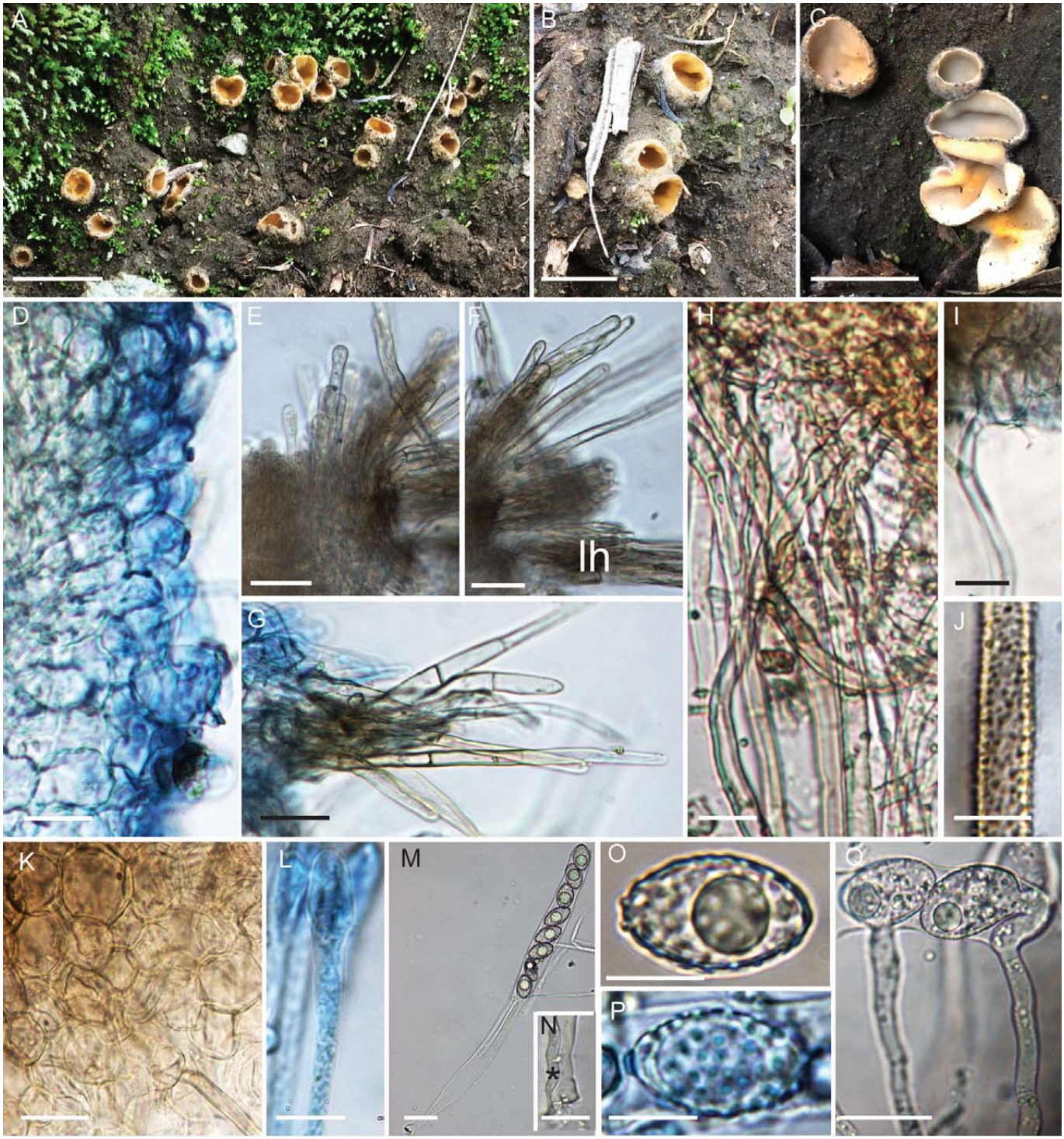

Plate 1 – *Pseudotricharina lanigera*

A: Apothecia *in situ*, bar = 20 mm. B: Apothecia *in situ*, bar = 10 mm. C: Apothecia *in situ*, bar = 10 mm. D: Excipular cells on side of apothecium, bar = 20 µm. E: Hyphal elements next to the hymenium, bar = 20 µm. F: Marginal hairs, long hyphal hairs (lh), bar = 20 µm. G: Younger group of the long marginal hairs, showing the base to tip structure, bar = 20 µm. H: Anchor hairs, bar = 10 µm. I: Anchor hair from bulbous cell in excipulum, bar = 10 µm. J: Rough walls of anchor cell hair, bar = 10 µm. K: Excipular cells near base of apothecium, bar = 20 µm. L: Paraphyses bar = 10 µm. M: Ascus with ascospores, bar = 20 µm. N: Base of ascus with crozier remnants and paraphysis (*), bar = 20 µm. O: Ascospore, bar = 10 µm. P: Ascospore, bar = 10 µm. Q: Ascospores germinated in ascus, bar= 20 µm. D-Q from ascomata redhydrated in water and unstained, except for D, G, I, L and P which were stained in cotton blue in lactophenol.

long hair-like hyphae are rounded to tapered, as seen in a younger tuft in Fig. 1 G. Like the hyphal elements adjacent to the hymenium, the marginal hyphal hairs are produced from more or less parallel hyphae .The anchor hairs (Fig. 1 H) below the margin originate from inflated, lobate to somewhat angular cells at the surface, or subsurface of the excipulum (Fig. 1 I). Many, if not all, excipular cells at the surface below the margin produce one or more long hyphae. These hyphae are difficult to measure because they are bound tightly to the soil and they break when the soil is removed. However, after careful dissection some hairs measured as long as 3 mm. They are of variable width (up to 8 µm wide near the base), but more or less equal in width further from the base (about 4 µm wide). They are flexuous, hyaline to light brown, anastomosing, and occasionally branching. Most are smooth, but some hairs have rough walls (Fig. 1 J). The cell walls of the hairs are 1.5 µm thick. The outer excipular cells below the margin, gradually become larger and more angular (Fig. 1 K). **Medullary excipulum** intergraded with the ectal excipulum, with hyaline, smaller cells. Paraphyses with light yellow granular cytoplasm, the same length or slightly longer than asci, 3–4 µm wide, gradually inflated to 5–8 µm wide at the tips, septate, with branching not observed (Fig. 1 L). **Asci** 184–250 × 13–16.5 µm, inamyloid, containing eight uniseriate spores, operculate, cylindrical (Fig. 1 M), with inconspicuous crozier at base (Fig. 1 N). **Ascospores** 18–20.5 (23) × 11–13 µm, 20 × 12.3 µm on average, with an average Q value of 1.6, ranging from (1.4) 1.6–1.7 (1.9), and ornamented with small warts, and usually with one large oil drop when rehydrated in water. Spore ornaments are usually isolated (not attached to each other), and coarse when mature, up to 1 µm high (Fig. 1 O), of varying shape and length, dissolving and disappearing in 2% KOH, cyanophilic in cotton blue in lactophenol (Fig. 1 P).

Dried and then frozen one-year-old apothecia that were hydrated in tap water over night contained ascospores that germinated within the ascus, with one or two germ tubes (Fig. 1 Q).

Studied collections: Argentina. Bariloche, Nahuel Huapi National Park, 1 km before Lago Hess, on or in ground along cut bank of roadside, in forest dominated by *Nothofagus antarctica* and *N. dombeyi*, 16-May-2016, coll. Daniella Torres and Matthew Smith, MES-2065 Paratype (CORD MES-2065; FLAS-F-60662). Same place, 18-May-2016, coll. Rosanne Healy and Matthew Smith, CORD MES-2152 Holotype (Isotype FLAS-F-60663).

Discussion

Pseudotricharina lanigera is the second species of *Pseudotricharina* to be described and the first species known from the Southern Hemisphere. The type species of the genus, *P. intermedia*, was described from three Mediterranean areas, in Spain, Italy and Greece. *Pseudotricharina lanigera* looks very similar to *P. intermedia* in color, size, and habit. The margin hair-like hyphae are well depicted in Van Vooren *et al.* (2015, p. 343, Plate 1 O), and look the same as in *P. lanigera*. However, *P. lanigera* differs from *P. intermedia* in its habitat in a temperate climate, and in its wider ascospores (Q value of 1.4–1.9 for *P. lanigera* vs 1.7–2.3 for *P. intermedia*) with more pronounced warts. A third species has been detected based on environmental sequences of *Pterygodium* orchid roots from South Africa (Waterman *et al.*, 2011), which may give a clue to the ecology of other species of *Pseudotricharina*.

To our knowledge, this is the first report of anything resembling this species from Patagonia. We searched through descriptions of cup fungi from southern South America by Gamundí and others (Cash, 1957; Gamundí, 1964; Gamundí, 1971; Gamundí, 1973; Gamundí & Giaiotti, 1998; Gamundí *et al.*, 2004; Gamundí, 2010; Speggazini, 1887) but did not find any described taxa that matched the characters of this new species. The most similar described species was *Tricharina gilva* (Boud.) Eckblad (Gamundí, 1973, as *Trichophaea gilva*; Gamundí *et al.*, 2004; Gamundí, 2010) but *T. gilva* has smooth ascospores and acute, stiff hairs rather than the flexuous hairs with rounded tips observed in *Pseudotricharina*, and *T. gilva* fruits above rather than below the soil surface and lacks an orange hymenium. It differs from *Humaria* species in its fruiting habit, its flexuous rather than course, stiff hairs, and its color. The fruiting habit of *Pseudotricharina* species is similar to those of the genus *Sepultaria* (Cooke) Boud., although the ascomata are often only semi-immersed in soil. When they are completely immersed like *Sepultaria*, the apothecium of *P. lanigera* does not generally split as it opens at the soil surface, and the ascospores are warted rather than smooth (Burdsall, 1968). Additional differences between *Pseudotricharina* and other genera are discussed in Van Vooren *et al.* (2015). Phylogenetic evidence in Van Vooren *et al.* (2017) clearly shows that *Pseudotricharina* is genetically different from other genera of *Pezizales*, but shares a well-supported clade with *Anthracobia*, *Ascorhizoctonia*, *Cupulina*, *Geopora*, *Hoffmanoscypha*, *Paratricharina*, *Sepultariella*, *Tricharina*, and *Trichophaea*. Phylogenetic analyses using only ITS and LSU did not resolve which of these genera is most closely related to *Pseudotricharina*.

Pseudotricharina lanigera has only been collected at one site in Argentina, despite searching for fruiting bodies in similar areas in both Argentina and Chile between 2015-2017. The observed fruiting was extensive, with at least a hundred apothecia along several meters of the soil bank. If extensive gregarious fruiting is typical of this species, it should have been noticed by us or by other mycologists who explored Patagonia for Pezizalean fungi. We surmise that this species is either rare, or rarely fruits.

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