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Tomentellopsis rosannae sp. nov. (Basidiomycota, Thelephorales), first species in the genus described from the Southern Hemisphere

Tomentellopsis rosannae sp. nov. (Basidiomycota, Thelephorales), primera especie del género descripta del hemisferio sur

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ABSTRACT

Patagonian collections of the corticioid genus *Tomentellopsis* have been treated in the past as *T. echinospora*, a common Northern Hemisphere species. New collections with DNA sequence data are distinct from the Northern Hemisphere taxon and must be considered a different species, endemic to the temperate subantarctic forests of Patagonia. We use molecular and morphological methods to study this new corticioid fungus and describe it as *Tomentellopsis rosannae* sp. nov.

Keywords — Andes; ectomycorrhizal; resupinate; Thelephoraceae.

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RESUMEN

Colecciones patagónicas del género corticioide *Tomentellopsis* han sido tratadas en el pasado como *T. echinospora*, especie común en el hemisferio norte. Nuevas colecciones con secuencias de ADN disponibles son distintas de las del hemisferio norte y deben ser tratadas como una especie diferente, endémica de los bosques templados subantárticos de Patagonia. Utilizando métodos moleculares y morfológicos para estudiar a este hongo, lo describimos aquí como *Tomentellopsis rosannae* sp. nov.

Keywords — Andes; ectomicorrícico; resupinado; Thelephoraceae.

INTRODUCTION

The small genus *Tomentellopsis* Hjortstam comprises a few corticioid thelephoraceous species with echinulate, hyaline to pale brown spores, simple septa and light colored basidiomes (Kõljalg, 1996). Although these are relatively easy to separate from species in the genus *Tomentella*, the limits between species within the genus are more difficult to establish. Based on morphological analyses, Kõljalg (1996) concluded that "All species in this genus are close to each other, but more material is needed in order to determine how many taxa are distinguishable". The inclusion of molecular data from ectomycorrhizal (ECM) roots later showed that the genus is actually more diverse than previously thought (Kõljalg *et al*., 2002, 2009).

Based on morphological criteria, fungal materials from unexplored areas have traditionally been identified by taxonomists as European species (Menolli & Capellari, 2014). A deeper knowledge on the specificity of certain mycorrhizal associations challenged this paradigm and most taxa or even lineages of ectomycorrhizal fungi in Southern Gondwana landmasses are now recognized as unique and separated from morphologically similar allies from the Northern Hemisphere (e.g. Truong *et al*., 2017). Molecular methods have confirmed this situation also for some Patagonian members of the Thelephoraceae (Kuhar *et al*., 2016) that had previously been interpreted as Northern Hemisphere species (e.g. Greslebin, 2002).

In this framework, Patagonian collections clearly belonging to the genus *Tomentellopsis* have until now been included in the concept of *Tomentellopsis echinospora* (Ellis) Hjortstam in a broad sense, with indications that some features deviate from the original diagnosis (Greslebin, 2002).

The aim of this communication is to reassess the situation of this taxon in the light of molecular data provided by new collections from the Southern Hemisphere.

MATERIALS AND METHODS

Descriptions

Descriptions of fresh basidiomes were made following Kõljalg (1996) under a light microscope (LM) and a Hitachi SU5000 Schottky Field-Emission scanning electron microscope (SEM) at the University of Florida Interdisciplinary Center for Bio-

technology Research (ICBR) Electron Microscopy Core Facility. Basidiospores were measured in 3% KOH in frontal and lateral view including ornamentation. At least 20 elements were measured in each estimation. Argentinean collections are identified with the acronym "AG" (Alina Greslebin) and deposited in the HFCF Herbarium (Chubut, Argentina), while Chilean collections are identified with the acronym "MES" (Matthew E. Smith) and deposited in the FLAS Herbarium (Florida, USA).

Molecular Methods

Fresh basidiomes were sampled in a field laboratory using sterile forceps and placed into Extraction Buffer from the Extract-N-Amp DNA extraction kit (Sigma-Aldrich, St. Louis, Missouri). Samples were transported to the University of Florida and extraction procedures were performed according to the manufacturer's instructions.

Polymerase chain reaction (PCR) of the ribosomal Internal Transcribed Spacer region (ITS) was made with primers ITS1F and ITS4 (White *et al*., 1990; Gardes & Bruns, 1993). The PCR conditions were 94° C for 5 min, followed by 35 cycles of 1 min at 94° C, 1 min at 55° C, and 2 min at 72° C, followed by 7 min at 72° C. Sanger sequencing was conducted at the ICBR at the University of Florida.

Sequences of other representatives of the genus were retrieved from GenBank along with some Australian and one New Zealand sequence from the UNITE Database (https://unite.ut.ee/) with a high ITS similarity to the vouchers from Chile and Argentina. A sequence of the genus *Polyozellus* from GenBank was used as outgroup. Sequence data are shown in Table 1. The dataset was aligned with L-INS-i strategy as

Table 1. Sequences used for the phylogenetic analyses.
Tabla 1. Secuencias utilizadas para los análisis filogenéticos.

GenBank Accession N°	Species	Voucher	Origin
AJ410759	*Tomentellopsis zygodesmoides*	JS-27216	Norway
AJ410761	*Tomentellopsis zygodesmoides*	KHL-8653	Norway
ON832657	*Tomentellopsis rosannae*	MES-2740	Chile
MT366690	*Tomentellopsis rosannae*	MES-3338	Chile
HQ533015	*Tomentellopsis* sp.	PDD-95789	New Zealand
AJ410779	*Tomentellopsis bresadoliana*	RS-09494	Finland
AJ410778	*Tomentellopsis bresadoliana*	RS-04299	Finland
MK602779	*Tomentellopsis pulchella*	KHL16366	Norway
AM747527	*Tomentellopsis echinospora*	KHL110333	Norway
AY641459	*Tomentellopsis submollis*	AB15 8QH	UK
AJ410760	*Tomentellopsis zygodesmoides*	TAA-159775	Estonia
AJ410774	*Tomentellopsis submollis*	RS-22498	Finland
AM086447	*Tomentellopsis submollis*	P24-F	UK
UDB016681	*Tomentellopsis* sp.	TUF100489	Australia
MH310781	*Tomentellopsis echinospora*	KHL110333	Sweden
UDB016690	*Tomentellopsis* sp.	TUF100454	Australia
UDB016673	*Tomentellopsis* sp.	TUF100528	Australia
HQ533015	*Tomentellopsis* sp.	PDD 95789	New Zealand
MK290675	*Polyozellus humicola*	SS212	Sweden

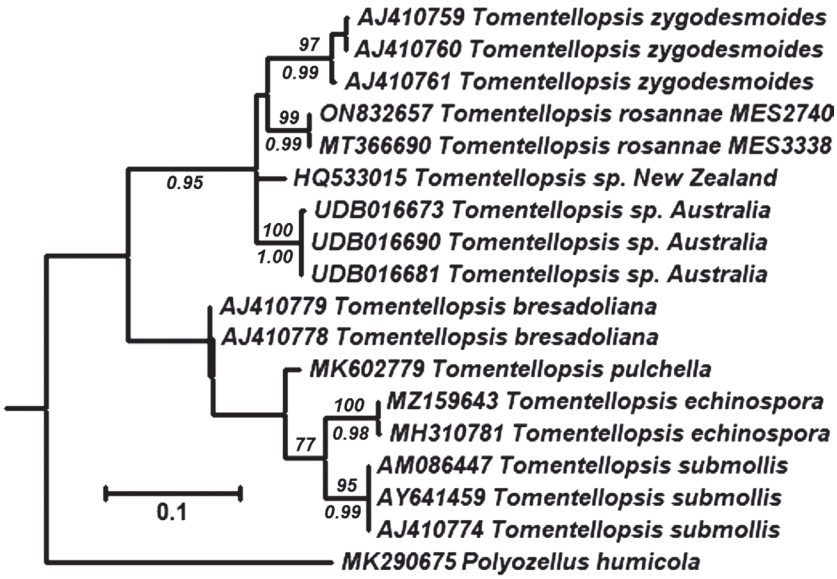

Fig. 1. Most likely tree showing the monophyly of the species described here in relation to other known species of the genus *Tomentellopsis*. Significant clade support is indicated over the branches (>75% ML bootstrap) and below them (>0.95 BPP).

Fig. 1. Árbol más probable que muestra la monofilia de la especie aquí descrita en relación con otras especies conocidas del género *Tomentellopsis*. Se indican los soportes significativos sobre las ramas (> 75% ML bootstrap) y debajo de ellas (> 0.95 BPP).

implemented in MAFFT 7.0 (Katoh & Standley, 2013) and analyzed after trimming the ends. Nucleotide substitution models were selected with jModelTest 2.1 (Darriba *et al*., 2012). Maximum likelihood (ML) analyses were made in PHYML. Bootstrap values of the highest likelihood tree were calculated with 1000 repetitions and B/MCMC analyses were conducted with MrBayes (Huelsenbeck & Ronquist, 2001) with 8 000 000 generations to calculate Bayesian Posterior Probabilities (BPP).

RESULTS

The sequences corresponding to Patagonian *Tomentellopsis* constituted a well-supported monophyletic clade which we describe here as a new species, *T. rosannae* sp. nov. Australian and New Zealand sequences included due to their high similarity appeared close to the Patagonian vouchers. *Tomentellopsis zygodesmoides* (Ellis) Hjortstam was phylogenetically more similar to *T. rosannae* than the other previously described species, despite clear differences in spore shape and a distant geographical distribution.

Taxonomy

***Tomentellopsis rosannae* sp. nov.** Kuhar & Greslebin, Figs. 2 & 3
MycoBank #844581

Diagnosis.— Monomitic basidiomes with subiculum consisting on hyphae with thin to thickened walls hyaline to chestnut. Basidia up to 60 μm, tetrasporate, bearing globose echinulate spores.

Etymology.— to honor our friend and colleague, the mycologist Rosanne Healy, who collected the type specimen.

Holotype.— CHILE, Los Lagos, Puyehue National Park, Antillanca ski area. *Nothofagus pumillio* forest close to timberline, on embankment under overhang MES-2740, (FLAS-F-65322 (holotype), 12-IV-2017, Rosanne Healy, ITS: ON832657.

Basidiome resupinate, detachable from the substrate, loosely felt-like and hypochnoid; hymenial surface smooth, pallid yellow, ochre yellow or chestnut in age; margin thinning, fibrillar and arachnoid. Rhizomorphs absent. Hyphal system monomitic, composed of straight, simple-septate generative hyphae with infrequent ramifications. Subhymenial hyphae 3–5 μm in diameter, thin to slightly thick-walled, hyaline, yellowish to chestnut. Subicular hyphae yellowish to brownish, with thickened cell walls. Cystidia absent. Basidia clavate, not stalked, somewhat sinuous, hyaline, 35–60 × 7–8 μm, with 4 sterigmata and a simple septum at the base. Basidioles abundant, with the same morphological configuration as the basidia. Basidiospores globose in frontal view, 6–7.5 μm in diameter, ellipsoid to subglobose in lateral view, 7–8 × 6–7 μm, spiny, with isolated spines up to 1 μm long, slightly thick-walled, hyaline to yellowish in KOH.

Substrates.— fruiting on litter of *Drymis winteri*, *Nothofagus antarctica*, *N. betuloides*, and *N. pumilio* in autumn and spring. Putatively ectomycorrhizal with Nothofagaceae species.

Distribution.— Thus far known only from Nothofagaceae forests in the Andean Patagonia, Chile and Argentina.

Observations.— Morphologically, this species resembles *T. echinospora*, especially in the globose echinulate spores. However, *T. rosannae* can be clearly distinguished by the brownish subicular hyphae with thicker cell walls and somewhat larger basidiospores. *Tomentellopsis zygodesmoides* resembles closely *T. rosannae* in the subicular hyphae, but the basidia reaching 60 μm are a notable characteristic of *T. rosannae* that separate it from this species and all other in this genus. All basidia reported until now in the other species measure up to 35–40 μm long. Also, the disjunct biogeography associated with Patagonian *Nothofagus* forests is an unequivocal character differentiating this species from all the others known in the genus.

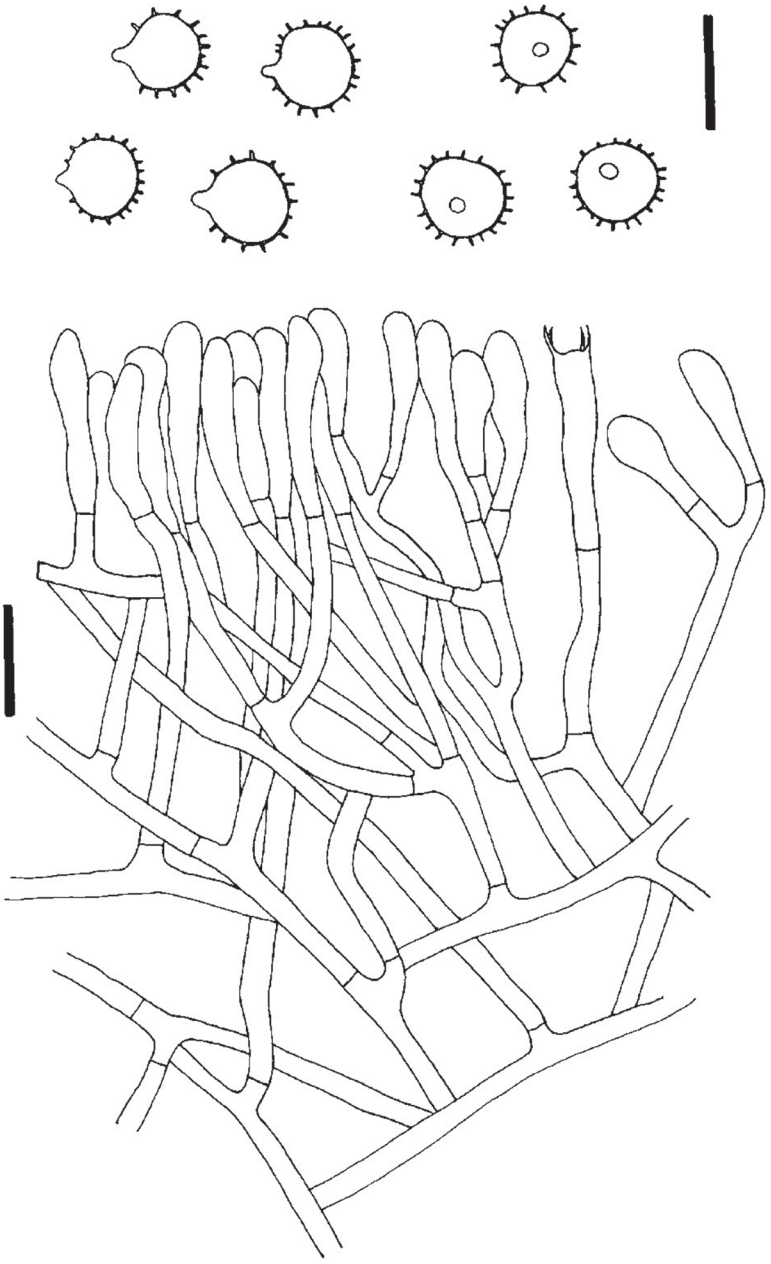

Fig. 2.*Tomentellopsis rosannae* sp. nov. A) Basidiospores, scale bar = 10 μm. B) Hymenium (Basidia). C) Subhymenium. D) Subiculum, scale bar = 25 μm.

Fig. 2.*Tomentellopsis rosannae* sp. nov. A) Basidiosporas, escala = 10 μm. B) Himenio (Basidios). C) Subhimenio. D) Subículo, escala = 25 μm.

Other studied material: ARGENTINA, Tierra del Fuego, Parque Nacional Tierra del Fuego, Ensenada, AG1331, 20-III-1998; AG1824, 9-XI-1998. Río Pipo, AG1758, 7-XI-98. Road to El Mirador AG1913, 25-IV-1999. Turbal y Castorera AG1927 25-III-1999. Dpto. Ushuaia, Estancia Moat, 1-2 Km before Río Chico AG1888, 24-III-1999. Lago Escondido, AG1965, 26-III-1999. CHILE, Los Ríos, Villarrica National Park, Coñaripe access MES-3338, 13 May 2019 (Collection destroyed, but image and ITS sequence available).

Fig. 3. A) Young basidiomes of *T. rosannae* on *Nothofagus* leaf litter. B and C) Basidiospores viewed under SEM.

Fig. 3. A) Basidiomas jóvenes de *T. rosannae* sobre la hojarasca de *Nothofagus*. B y C) Basidiosporas vistas bajo MEB.

DISCUSSION

Species of *Tomentellopsis* have generally been considered as ectomycorrhizal fungi (Kõljalg *et al*., 2009). However, previous studies assessing the ECM diversity using root tips from Patagonian *Nothofagus* forests (e.g., Nouhra *et al*., 2013) did not report species of this genus. Further explorations of the "pink ectomycorrhizae" characteristic of *Tomentellopsis* spp. (Kõljalg *et al*., 2002) will likely confirm the ECM nutritional mode for *T. rosannae.* Given that *T. rosannae* is always found in Nothofagaceae forests and that ECM fungi rarely transition to other trophic modes (Tedersoo *et al*., 2010; Tedersoo & Smith, 2013), it is logical to assume that this species is an ECM fungus.

The high similarity in ITS sequences between the Patagonian vouchers and other Southern Hemisphere materials (e.g. from Tasmania and New Zealand) suggests a common biogeographical history among closely related taxa with Nothofagaceae hosts. For the confirmation of these links, more robust multi-locus phylogenies will be needed. In our experience, the most complex issue when working with corticioid Thelephoraceae is the performance of the DNA extraction and amplification processes. This, together with the difficulties to obtain pure cultures, is the reason why most studies have been based solely on ribosomal ITS or LSU (e.g. Tedersoo *et al.*, 2014).

Finally, forthcoming research is being conducted in Patagonia to elucidate the fructification ecology of corticioid basidiomycetes on debris of *Nothofagus* species. These results will help us understand the value of these corticioid fungi as indicators of disturbance, but maybe also the role they play in the complex southern temperate forests.

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