

New species of *Tomentella* (Thelephorales) from the Patagonian Andes forests

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Abstract: The genus *Tomentella* forms abundant ectomycorrhizae in coniferous and deciduous forests worldwide. Molecular identification of root tips suggests undescribed species in the *Nothofagus* forests of Patagonia, Argentina. *Tomentella tenuissima*, *T. pulvinulata* and *T. patagonica* are described here as new to science based on morphological and molecular analyses. Their host range is addressed using available soil sequences. The identity of previous records of *T. galzinii* and *T. radiosa* are discussed with morphological and molecular evidence.

Key words: ectomycorrhiza, *Nothofagus*, Thelephoraceae

INTRODUCTION

Species of *Tomentella* Pers. ex Pat. (Thelephoraceae, Basidiomycota) produce resupinate basidiomes that form cottony or spider web-like layers on the surface of soil, twigs or other plant debris (Köljalg 1996) and were long regarded as saprotrophs (e.g. Larsen 1974). Since the first finding of an ectomycorrhiza (ECM) formed by a *Tomentella* species (Danielson and Pruden 1989), many works have confirmed the mycorrhizal status of this genus not only associated with ECM trees but also forming other mycorrhizal associations with Orchidaceae, Ericaceae, Monotropaceae and Pyrolaceae (Bidartondo et al. 2000, 2004; Selosse et al. 2002; Tedersoo et al. 2007). Although basidiomes are inconspicuous, *Tomentella* is an abundant and diverse genus, species of which are of great ecological importance as ectomycorrhiza formers in

temperate and tropical forests (Gardes and Bruns 1996, Trowbridge and Jumpponen 2004, Haug et al. 2005, Jakucs and Erős-Honti 2008, Jakucs et al. 2015).

In North America and temperate Eurasia *Tomentella* has been studied intensively and revised (Larsen 1974, Köljalg 1996), and new species and records have been reported from Africa (Yorou and Agerer 2008; Yorou et al. 2011, 2012), Asia (Thind and Rattan 1971, Jung 1994), Australia (Agerer and Bougher 2001) and tropical South America (Haug et al. 2005, Smith et al. 2011). Records of this genus based on the amplification of DNA sequences from environmental samples or root tips far outnumber those based on aboveground findings in many regions of the world. These records have provided valuable ecological and biogeographical information, but it is evident also that deeper studies in taxonomy and distribution of species are needed, as suggested by Jakucs and Erős-Honti (2008).

In Patagonia, Argentina, sequences obtained from root tips from pristine *Nothofagus* forests have been treated as unidentified OTUs nested within the genus *Tomentella* in that they did not match any known species (Nouhra et al. 2013). Although its importance in these forests seemed evident, reports of described species based on identifiable basidiome collections are scarce and the literature is limited. Greslebin (2002) recorded two species in Tierra del Fuego (southern Patagonia): *T. crinalis* (Fr.) M.J. Larsen, recently restored to its basionymic status in the non-mycorrhizal genus *Odontia* Pers. as *O. ferruginea* Pers. (Tedersoo et al. 2014), and *T. radiosa* (P. Karst.) Rick. Fernández et al. (2013) reported abundance of *Tomentella* from ectomycorrhizal root tips of *Nothofagus* spp. seedlings based on molecular analyses, but only one of them (on *N. nervosa*) had high similarity with a described species, *T. ellisii* (Sacc.) Jülich & Stalpers. To date, however, no basidiome of this species has been found in Patagonia. The UNITE database (Köljalg et al. 2005, <http://www.unite.ut.ee>) also reports *T. galzinii* Bourdot from Patagonia based on a specimen collected in *Nothofagus pumilio* forests in Tierra del Fuego.

The aims of this study was to describe new taxa of *Tomentella* found in the Patagonian *Nothofagus* forests of southern Argentina and to discuss the identity of records of the genus.

MATERIALS AND METHODS

Study sites and specimens studied.—Specimens of *Tomentella* were collected in *Nothofagus* forests of Chubut province, Argentina. Basidiomes were deposited at Herbarium BAFC,

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with duplicates at the Forest Pathology herbarium at Centro Forestal CIEFAP. Additional exemplars from other locations also were studied. Herbaria acronyms follow Thiers, B. (<http://sweetgum.nybg.org/science/ih/>).

Descriptions.—Description of dried basidiomes were made following Kõljalg (1996) under light microscope (LM) and a scanning electron microscope (SEM EDS, Inca Energy, Oxford Instruments, scanning electron microscope with field emission gun [FEG] Zeiss DSM 982 Gemini secondary electron in-lens detector). Basidiospores were measured in 3% KOH in frontal and lateral view without including ornamentation. At least 20 elements were measured in each estimation. Color codes of basidiomes are in parentheses according to Munsell Soil Color Charts (1990).

DNA extraction and PCR conditions.—DNA was extracted with the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich) according to the manufacturer's instructions. The rDNA ITS1-5.8S-ITS2 region (ITS) was amplified with primers ITS1F/ITS4 (White et al. 1990, Gardes and Bruns 1993), and the 28S region (28S) was amplified between approximate positions 24 and 990 with primers LR5 and LR0R (Viřgalys and Hester 1990). The PCR reaction was carried out with the REExtract-N-Amp PCR ReadyMix (Sigma-Aldrich) under the following conditions for both regions: an initial denaturing step at 94 C for 4 min, 35 cycles of 30 s at 94 C, 45 s at 55 C and 70 s at 72 C and a final extension for 10 min at 72 C. Amplified DNA fragments were purified and sequenced by Macrogen (Seoul, South Korea) with the primers ITS4 for ITS and LR0R for 28S.

Dataset selection.—ITS Sequences of the most similar taxa identified to species were queried in UNITE with BLASTN option and in GenBank NCBI (<http://www.ncbi.nlm.gov>) with the MEGABLAST option (Zhang et al. 2000). Additional sequences when available were included in the study to represent as many species of the genus as possible. UNITE and NCBI accession numbers as well as specimen id. and country of provenance of the sequences used are provided (TABLE I). Because the ITS sequences of *Thelephora* and *Tomentella* are too divergent to be aligned unambiguously with sequences of the sister genus *Odontia*, no outgroup was chosen, following Tedersoo et al. (2014). All the available 28S sequences corresponding to identified basidiomes were downloaded from UNITE and GenBank NCBI, and *Odontia fibrosa* UDB018683 was used as outgroup for 28S dataset analysis.

Sequence and phylogenetic analyses.—ITS sequences were aligned with L-INS-i strategy as implemented in MAFFT 7.0 (Katoh and Standley 2013). Ambiguously aligned regions were trimmed from the alignments with Gblocks 0.91b (Castresana 2000). The aligned matrix is available from TreeBASE (<http://purl.org/phylo/treebase/phylovs/study/TB2:S18619>). Nucleotide substitution models were selected with jModelTest 2.1 (Darriba et al. 2012), using the Akaike information criterion (AIC). Maximum likelihood (ML) analysis was performed in PHYML as implemented on the south of

France bioinformatics platform (<http://www.atgc-montpellier.fr/phyml/>) following Guindon et al. (2010) under the GTR nucleotide substitution model. Bootstrap values of the most likely tree were calculated with 1000 repetitions. To estimate the branch support of the studied taxa under different analysis methods maximum parsimony (MP) analyses were carried out in PAUP 4.0b10 (Swofford 2002). Bootstrap resampling method (BS) was calculated as implemented in PAUP (1000 replicates with 10 random addition sequences and TBR swapping) and B/MCMC analyses were conducted with MrBayes (Huelsenbeck and Ronquist 2001) with 10 000 000 generations starting with a random tree and employing four simultaneous chains. The first 100 000 generations (i.e. the first 1000 trees) were discarded as burn-in. TRACER1 (<http://evolve.zoo.ox.ac.uk/software.html/tracer/>) was used to ensure that stationarity was achieved after the first 100 000 generations.

RESULTS

Phylogenetic analysis.—ML analysis of the ITS dataset revealed low resolution in the deeper nodes of the topology but 99–100% bootstrap supported clades at the species level in the studied taxa, whose monophyly also was supported by high posterior probabilities resulting from the Bayesian analyses and the bootstrap support under MP (FIG. 1). ML analyses based on the 28S sequence alignments also suggested the monophyly of *T. patagonica*, but a more extensive taxon sampling and additional high quality 28S sequences of all the studied taxa are needed to infer phylogenetic relations of the Patagonian species within other representatives of the genus (SUPPLEMENTARY FIG. 1). The most likely tree is illustrated in supplementary materials. Three monophyletic entities were recognized and are described below as the new species *Tomentella tenuissima*, *T. patagonica* and *T. pulvinulata*.

A sequence available in the UNITE database of a Patagonian voucher collection identified as *T. galzinii* (UDB011106) revealed a high percentage identity and grouped together with other Patagonian sequences from root materials but unrelated to the northern hemisphere *T. galzinii* (here treated as *T. galzinii* Patagonia, FIG. 1).

TAXONOMY

Tomentella tenuissima Kuhar & Rajchenb., sp. nov.

FIGS. 2a; 3a, b

MycoBank MB812073

UNITE SH: SH177836.07FU

Typification: ARGENTINA. Chubut, Futaleufú, Cañadón Huemules, –42.767777, –71.45694, under a cushion of mosses in a pure *Nothofagus pumilio* forest, 14 Jun 2014, *F. Kuhar 14051* and *G. Romano* (**holotype** BAFC 52369). Isotype CIEFAP FK14051. GenBank accession Nos.: ITS = KT032082, 28S = KT032100.

TABLE I. GenBank/UNITE accession numbers and specimen ID of sequences used in the phylogenetic analyses

Species	Origin	Specimen ID	Accession No. ITS	NCBI/UNITE S28
<i>Odontia fibrosa</i>	India	TU115714		UDB018683
<i>Thelephora albomarginata</i>	Sweden	GBKHL8457		UDB018707
<i>Thelephora americana</i>	United States	JMT17465	TAU83487	
<i>Thelephora anthocephala</i>	Estonia	TAAM165304		UDB018693
<i>Thelephora anthocephala</i>	United States	src614	DQ974771	
<i>Thelephora caryophyllea</i>	Estonia	TAAM172626		UDB018694
<i>Thelephora caryophyllea</i>	Mexico	GO-2010-163	KC152242	
<i>Thelephora caryophyllea</i>	Poland	ID PAN 684	KM085427	
<i>Thelephora penicillata</i>	Estonia	TAAM169453		UDB018695
<i>Thelephora terrestris</i>	Estonia	TAAM162083		UDB018696
<i>Thelephora terrestris</i>	Poland	Hilsz. 1-IBL	FJ532478	
<i>Thelephora terrestris</i>	Poland	P17 M2 772	KM409440	
<i>Thelephora terrestris</i>	United States	JMP0086	EU819444	
<i>Tomentella albomarginata</i>	Finland	TU115350	UDB011599	
<i>Tomentella albomarginata</i>	Finland	TU115353	UDB011601	
<i>Tomentella albomarginata</i>	Sweden	KHL8457		UDB018707
<i>Tomentella asperula</i>	Estonia	TU108147		UDB018469
<i>Tomentella atramentaria</i>	Estonia	TU108866	UDB000955	
<i>Tomentella atramentaria</i>	Germany	4-A.Heller	UDB000236	
<i>Tomentella atramentaria</i>	Russia	FarEast149211		UDB018697
<i>Tomentella atroarenicolor</i>	Estonia	TU115438	UDB016303	
<i>Tomentella atroarenicolor</i>	Russia	TAAM149946	UDB000243	
<i>Tomentella badia</i>	China	TU115038		UDB018714
<i>Tomentella badia</i>	Estonia	TU108893	UDB000952	
<i>Tomentella badia</i>	Russia	TAAM159022	UDB000238	
<i>Tomentella botryoides</i>	Russia	TAAM149614	UDB000257	
<i>Tomentella botryoides</i>	Sweden	KHL8453		AY586717
<i>Tomentella bresadolae</i>	Estonia	TU115447	UDB016311	
<i>Tomentella brunneorufa</i>	Australia	TAAM159857	UDB000274	
<i>Tomentella bryophila</i>	Denmark	MC01-548	UDB000035	
<i>Tomentella bryophila</i>	United Kingdom	TAA187311	UDB001655	
<i>Tomentella bryophila</i>	India	TU115716		UDB018684
<i>Tomentella castanea</i>	Denmark	TL-6886	UDB000120	
<i>Tomentella cinerascens</i>	Estonia	TAAM159555	UDB000232	UDB018699
<i>Tomentella cinerascens</i>	Italy	019AS	UDB016498	
<i>Tomentella cinereoumbrina</i>	Iran	TU108230	UDB003298	
<i>Tomentella cinereoumbrina</i>	Italy	002AS	UDB016481	
<i>Tomentella coerulea</i>	Estonia	TAAM153804	UDB000266	
<i>Tomentella coerulea</i>	Estonia	TU115602	UDB016469	
<i>Tomentella coerulea</i>	China	TU115044		UDB018457
<i>Tomentella ellisii</i>	Estonia	TU114483	UDB003355	
<i>Tomentella ellisii</i>	Estonia	TU114484	UDB003356	
<i>Tomentella ferruginea</i>	Estonia	TAAM166877	UDB000256	
<i>Tomentella ferruginea</i>	Estonia	TU115605	UDB016471	
<i>Tomentella fuscocinerea</i>	Italy	004AS	UDB016483	
<i>Tomentella fuscocinerea</i>	Sweden	KHL11906	UDB000960	
<i>Tomentella fuscocinerea</i>	Estonia	TAAM149918		UDB018703

TABLE I. Continued

Species	Origin	Specimen ID	Accession No. ITS	NCBI/UNITE S28
<i>Tomentella galzinii</i>	Argentina	TU115232	UDB011106	
<i>Tomentella galzinii</i>	Estonia	TAAM166821	UDB000260	
<i>Tomentella galzinii</i>	Estonia	TU100674	UDB003276	
<i>Tomentella galzinii</i>	Finland	RS27093	UDB000264	
<i>Tomentella galzinii</i>	Russia	TAAM149734	UDB000263	
<i>Tomentella lapida</i>	Estonia	TU115491	UDB016346	
<i>Tomentella lapida</i>	United Kingdom	TAA187315	UDB001657	
<i>Tomentella lateritia</i>	Estonia	TU108551	UDB000954	
<i>Tomentella lateritia</i>	Norway	NF.S033	UDB000962	
<i>Tomentella lateritia</i>	Norway	NF.S045	UDB000963	
<i>Tomentella lateritia</i>	China	TU115020		UDB018446
<i>Tomentella lilacinogrisea</i>	France	TU100869	UDB003317	
<i>Tomentella lilacinogrisea</i>	Slovenia	TU115636	UDB020322	
<i>Tomentella lilacinogrisea</i>	Estonia	TU108189		UDB018468
<i>Tomentella pileocystidiata</i>	Seychelles	TU105068	UDB015029	
<i>Tomentella pileocystidiata</i>	Seychelles	TU105090	UDB017786	
<i>Tomentella pileocystidiata</i>	Seychelles	TU105054	UDB017789	
<i>Tomentella pilosa</i>	Estonia	TAAM152428	UDB000241	UDB018704
<i>Tomentella pilosa</i>	Estonia	TU100825	UDB003324	
<i>Tomentella punicea</i>	Estonia	TAAM158081		UDB018705
<i>Tomentella punicea</i>	Lithuania	TU100125	UDB000948	
<i>Tomentella punicea</i>	Lithuania	TU100126	UDB000950	
<i>Tomentella punicea</i>	Sweden	TU108925	UDB000949	
<i>Tomentella radiosa</i>	Ecuador	TU110022	UDB014068	
<i>Tomentella radiosa</i>	Estonia	TAAM108027	UDB000224	
<i>Tomentella radiosa</i>	Madagascar	TU108395	UDB016789	
<i>Tomentella radiosa</i>	Madagascar	TU108404	UDB016790	
<i>Tomentella radiosa</i>	Norway	NF.S010	UDB000964	
<i>Tomentella</i> sp.	Australia	TU110383		UDB013385
<i>Tomentella</i> sp.	China	TU115037		UDB018453
<i>Tomentella</i> sp.	India	TU115704		UDB018677
<i>Tomentella</i> sp.	India	TU115708		UDB018680
<i>Tomentella</i> sp.	Argentina	Env. sample		UDB007145
<i>Tomentella</i> sp.	Argentina	Env. sample		UDB007109
<i>Tomentella</i> sp.	Argentina	Env. sample		UDB007004
<i>Tomentella</i> sp.	Argentina	Env. sample		UDB007024
<i>Tomentella stuposa</i>	Estonia	TAAM159498	UDB000244	UDB018706
<i>Tomentella stuposa</i>	Finland	TAAM159816	UDB000246	
<i>Tomentella stuposa</i>	France	TU100887	UDB003314	
<i>Tomentella stuposa</i>	Gabon	TU108321	UDB016783	
<i>Tomentella stuposa</i>	Gabon	TU108342	UDB016788	
<i>Tomentella stuposa</i>	Madagascar	TU108415		UDB011876
<i>Tomentella subclavigera</i>	Estonia	TU100771	UDB003303	
<i>Tomentella subclavigera</i>	Estonia	TU115532	UDB016389	
<i>Tomentella subclavigera</i>	Finland	TU100729	UDB003310	
<i>Tomentella subclavigera</i>	France	TU100877	UDB003320	
<i>Tomentella subclavigera</i>	Norway	TU115207	UDB000259	

TABLE I. Continued

Species	Origin	Specimen ID	Accession No. ITS	NCBI/UNITE S28
<i>Tomentella subvilacina</i>	Argentina	TU100932	UDB002972	
<i>Tomentella subvilacina</i>	Ecuador	TU110032	UDB014076	
<i>Tomentella subvilacina</i>	Estonia	TAAM167139	UDB000228	
<i>Tomentella subvilacina</i>	Norway	NF.S052	UDB000968	
<i>Tomentella subtestacea</i>	Denmark	MC01-546	UDB000034	
<i>Tomentella subtestacea</i>	Estonia	TU115449	UDB016312	
<i>Tomentella tedersooi</i>	Seychelles	TU103657	UDB017779	
<i>Tomentella tedersooi</i>	Seychelles	TU103668	UDB017780	
<i>Tomentella terrestris</i>	United States	EL9897		UDB018708
<i>Tomentella umbrinospora</i>	Estonia	TAAM149462	UDB000233	UDB018709
<i>Tomentella umbrinospora</i>	Italy	021AS	UDB016499	
<i>Tomentella viridula</i>	Estonia	TU108038		UDB016192
Uncultured fungus	Argentina	Env. sample	JX316237	
Uncultured fungus	Argentina	Env. sample	JX316247	
Uncultured fungus	Argentina	Env. sample	JX316316	
Uncultured fungus	Argentina	Env. sample	JX316401	
Uncultured fungus	Argentina	Env. sample	JX316488	
Uncultured fungus	Argentina	Env. sample	UDB008426	
Uncultured fungus	Argentina	Env. sample	UDB014402	
Uncultured Thelephoraceae	Argentina	Env. sample	KJ701296	
Uncultured Thelephoraceae	Argentina	Env. sample	KJ701297	
Uncultured Thelephoraceae	Australia	Env. sample	JF960840	
Uncultured Thelephoraceae	Australia	Env. sample	JF960841	
Uncultured Thelephoraceae	Australia	Env. sample	AY955494	
Uncultured Thelephoraceae	New Zealand	Env. sample	JQ282698	
Uncultured Thelephoraceae	New Zealand	Env. sample	JQ282699	
Uncultured Thelephoraceae	New Zealand	Env. sample	JQ282700	
<i>Tomentella tenuissima</i>	Argentina	BAFC 52369	KT032082	KT032100
<i>Tomentella tenuissima</i>	Argentina	CIEFAP FK15011	KT032083	
<i>Tomentella tenuissima</i>	Argentina	CIEFAP FK15010	KT032084	
<i>Tomentella tenuissima</i>	Argentina	BAFC 52374	KT032085	
<i>Tomentella tenuissima</i>	Argentina	CIEFAP FK15001	KT032086	
<i>Tomentella tenuissima</i>	Argentina	CIEFAP FK14070	KT032087	
<i>Tomentella pulvinulata</i>	Argentina	BAFC 52370	KT032088	KT032101
<i>Tomentella pulvinulata</i>	Argentina	BAFC 52371	KT032089	
<i>Tomentella patagonica</i>	Argentina	BAFC 52372	KT032090	KT032102
<i>Tomentella patagonica</i>	Argentina	BAFC 52373	KT032091	KT032103
<i>Tomentella patagonica</i>	Argentina	CIEFAP 2QN	KT032092	
<i>Tomentella patagonica</i>	Argentina	CIEFAP FK15002	KT032093	

Etymology: superlative form of the Latin *tenuis*: thin, fine, weak. In reference to the hymenophore consistency.

Diagnosis: Basidiome arachnoid, even, dark gray to blackish, with a violet iridescence, not separable from the substrate. Subhymenium consisting of clamped, smooth or seldom encrusted hyaline hyphae. Subiculum

formed by clamped, heavily encrusted dark hyphae. Hyphal cords and cystidia absent, basidiospores globose to tri-lobed with grouped echinulae.

Basidiome resupinate, thin, arachnoid, even, pulverulent when fresh, loosely to slightly densely arranged, dark gray when fresh (10YR3/1) with a violet iridescence that vanishes soon after collecting, blackish

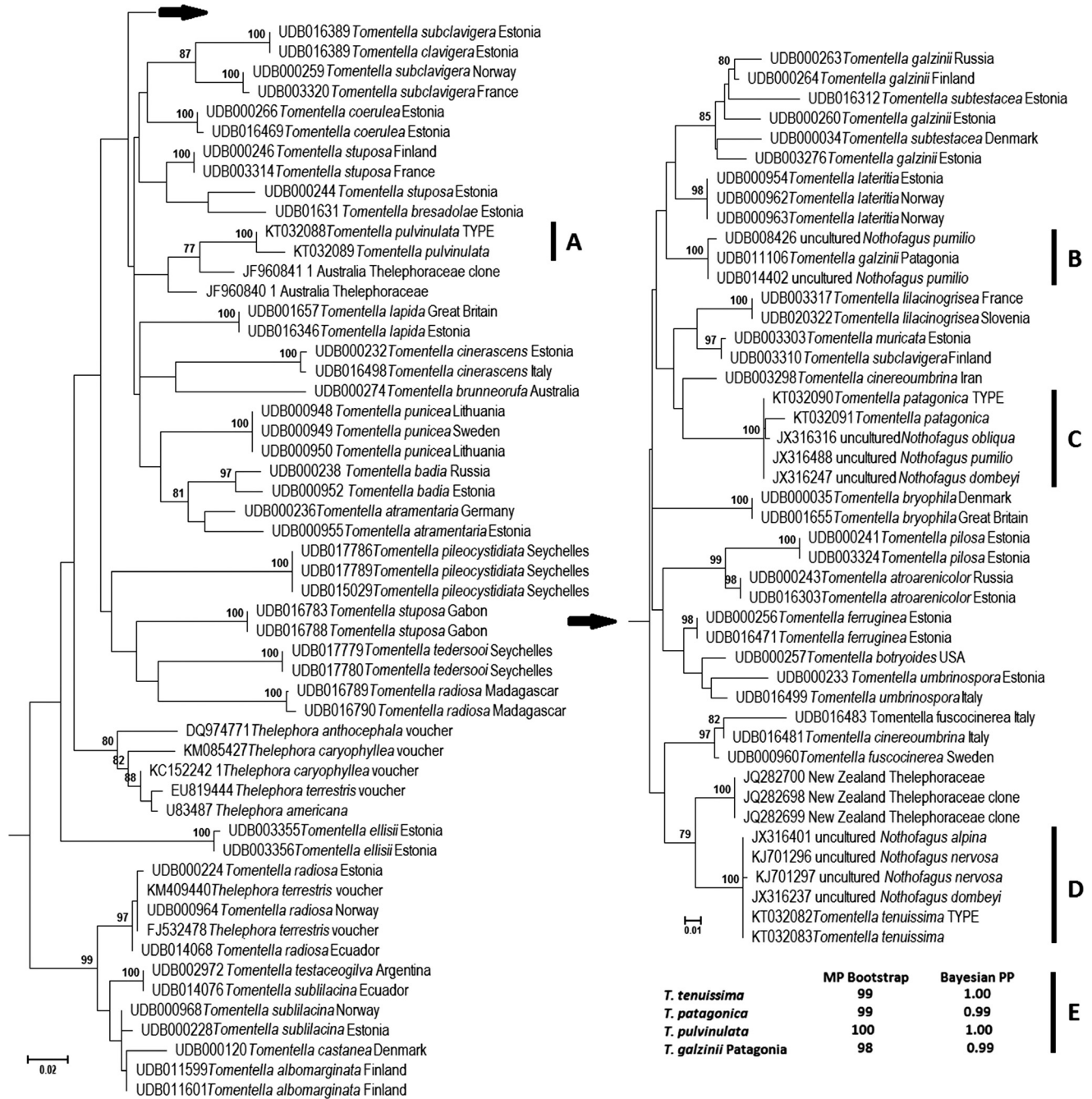


FIG. 1. Most likely unrooted tree showing the correspondence between basidiomes and environmental sequences of the studied taxa based on ITS sequences. *Tomentella pulvinulata* (A), Patagonian *T. galzinii* (B), *T. patagonica* (C), *T. tenuissima* (D). Significant bootstrap support of the ML analysis (BS > 75) are indicated above and below the branches. Bootstrap support under MP and Posterior probability of B/MCMC analyses of the branches corresponding to the studied taxa are shown separately (E).

upon drying (10HR2/1). Hymenophore consisting of irregularly distributed patches of loose fertile areas, not separable from the substrate. Subiculum blackish (10HR2/1), darker than the hymenophore. Hyphal cords absent. Sterile margin cobweb-like, indeterminate, concolorous with the subiculum.

Hyphal system monomitic. Generative hyphae clamped, of two types: subhymenial hyphae thin- to slightly thick-walled, smooth or, seldom with slightly irregular incrustations, 3–5 µm diam; subicular hyphae thick walled 3–10 µm diam, dark brown, typically branched in right angles, heavily encrusted

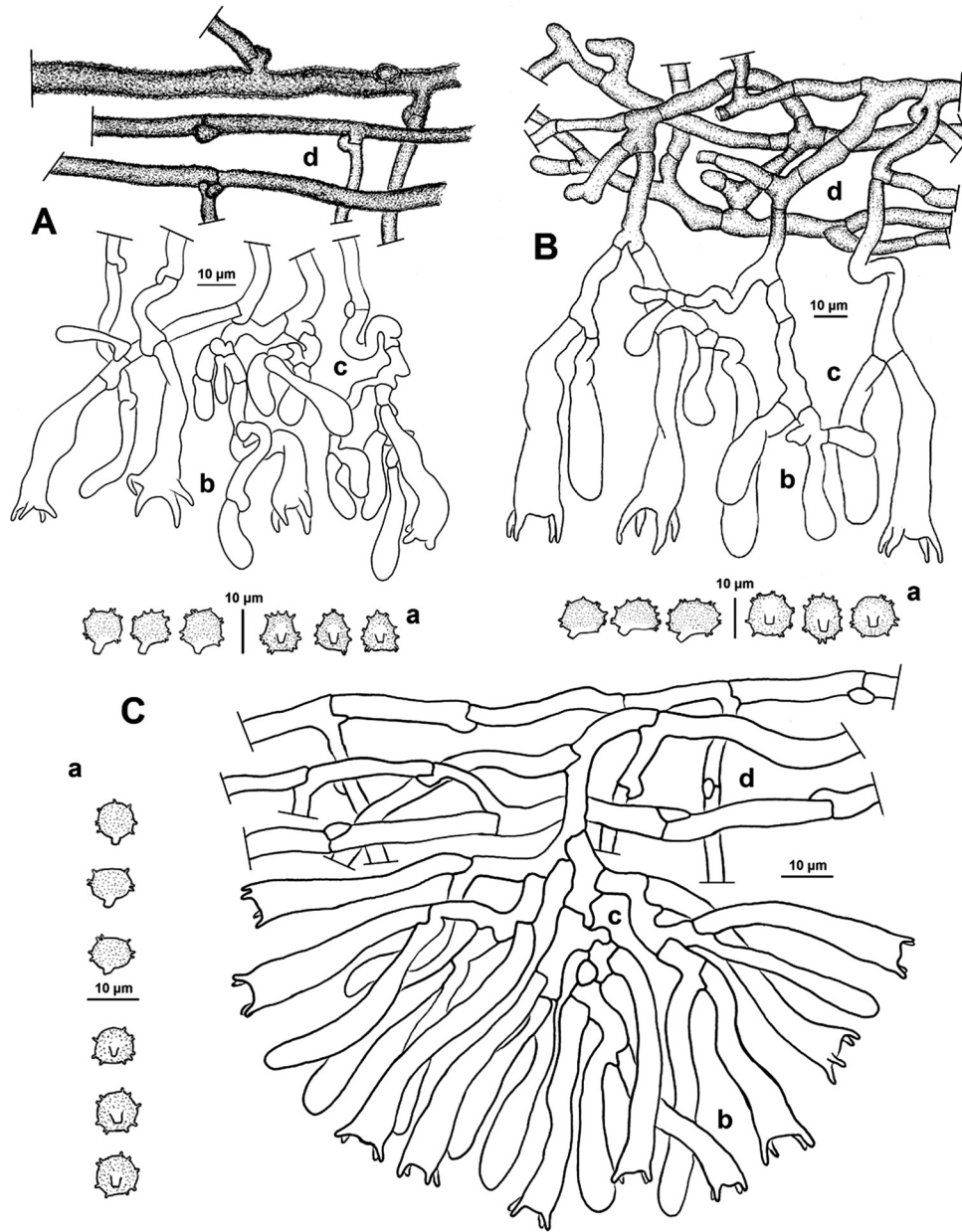


FIG. 2. *Tomentella tenuissima* (A), *T. patagonica* (B), *T. pulvinulata* (C), a. Basidiospores. b. Hymenium. c. Subhymenium. d. Subiculum. Bars = 10 μ m.

with granules of varying sizes. Intermediate hyphae showing a transition in the ornamentation, wall thickness and coloration. No eluates produced in 3% KOH. Basidia clavate to suburniform, cylindrical when young, 38–54 \times 8–10 μ m, four-spored, sterigmata up to 6 μ m long. Basidiospores highly variable, subglobose to elliptical in lateral view, 8–10 \times 6.5–8 μ m, globose to tri-lobed in frontal view, 7.5–9.5 μ m, with short echinulae that are grouped in 2–3; hilar appendix present and of variable length, without reaction in 3% KOH, IKI–. Cystidia absent.

Ecology and hosts: On rotten wood or moss cushions under *Nothofagus pumilio*. The species also is also present in pure *Nothofagus dombeyi* and *N. nervosa* forests, according to available soil sequences.

Other specimens examined: ARGENTINA. Chubut, Languiño, Lago Guacho, –43.8106, –71.4786 22. Apr 2015, F. Kuhar 15011 and V. Silva, on rotten wood under *N. pumilio* (CIEFAP FK15011). GenBank accession No.: ITS = KT032083.

Comments: *Tomentella lapida* (Pers.) Stalpers is morphologically similar but differs in the lighter basidiome and by the globose basidiospores bearing larger and

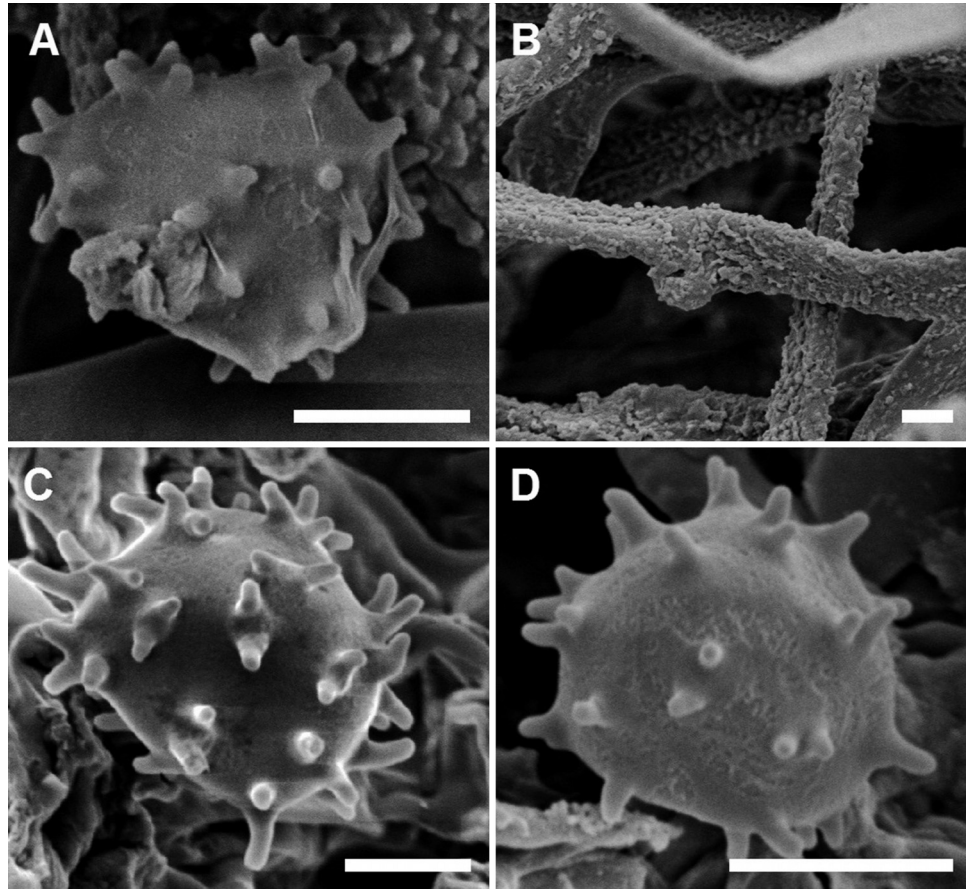


FIG. 3. SEM images of the basidiospores: *Tomentella tenuissima* (A), detail of the heavily encrusted, clamped subicular hyphae (B), *T. patagonica* (C), *T. pulvinulata* (D). Bars = 5 μ m.

narrower echinulae that are not grouped or conrescent. *Tomentella lilacinogrisea* Wakef. also resembles *T. tenuissima* but produces much smaller basidiospores (6–7 μ m, [Koljalg 1996]).

***Tomentella pulvinulata* Kuhar & Rajchenb., sp. nov.**

FIGS. 2c, 3d

Mycobank MB812074

Typification: ARGENTINA. Chubut, Futaleufú, Los Alerces National Park, Puerto Chucao, –42.729.505, –71.750.483, on rotten wood under *Nothogafus dombeyi*. 14 Oct 2014, F. Kuhar 14054 and G. Romano (**holotype** BAFC 52370)/ **isotype** CIEFAP FK14054). GenBank accession Nos.: ITS = KT032087, 28S = KT032101.

Etymology: from the Latin *pulvinulus*, diminutive form of *pulvinus* (cushion, pillow), which describes the hymenophore arrangement.

Diagnosis: Basidiome granulose, brick red to olive brown when dry, not separable from the substrate. Subhymenium consisting of clamped, hyaline hyphae arranged in cushion-shaped granules. Subiculum formed by clamped, hyaline or slightly pigmented hyphae. Hyphal cords and cystidia absent, basidiospores globose, echinulate.

Basidiome resupinate, thin, brick red when fresh, turning dark olive brown (2.5Y3/3) to grayish brown (10YR4/2) upon drying; not separable from the substrate. Hymenophore discontinuous, consisting of cushion-shaped, hemispherical and densely arranged granules with bluish eluates in KOH 3%. Sterile margin lacking. Subiculum concolorous, significantly thinner than the hymenophore. Hyphal cords absent. Hyphal system monomitic consisting of clamped, thin-walled hyphae. Subhymenial hyphae 3.5–6.0 μ m diam, densely arranged, hyaline, smooth, repetitively branching at the clamp connections so that each whole granulum or cushion is originated by a single or a few hyphae. Subicular hyphae 3.5–6 μ m diam, thin-walled, not encrusted, hyaline or with greenish yellow contents, not forming a dense layer but a sparse net connecting the granules. Basidia cylindrical to slightly clavate, narrow, 35–60 \times 3–7 μ m diam, hyaline, clamped at the base, four-spored, sterigmata up to 4 μ m long.

Basidiospores globose, 6–8.5 μ m, rarely slightly triangular in frontal view, ornamented with isolate or paired echinulae, hyaline to yellowish, greenish in 3% KOH, IKI–. Cystidia lacking.

Ecology and hosts: On well decayed wood under *N. dombeyi*.

Other specimens examined: ARGENTINA. Chubut, Futaleufú, Los Alerces National Park, Puerto Chucao, -42.729.599, -71.753.208, 14 Oct 2014, *F. Kuhar 14055* and *G. Romano*, on rotten wood under *N. dombeyi* (BAFC 52371). GenBank accession No.: ITS = KT032089.

Comments: Although clearly separated by the phylogenetic analyses, *Tomentella pulvinulata* has morphological similarity to *T. coerulea* (Bres.) Höhn. & Litsch and *T. lateritia* Pat. Both species have narrower subhymenial and subicular hyphae than *T. pulvinulata* and continuous hymenophores.

***Tomentella patagonica* Kuhar & Rajchenb., sp. nov.**
FIGS. 2b, 3c

Mycobank MB812075

UNITE SH: SH184540.07FU

Typification: ARGENTINA. Neuquén, San Martín de los Andes, Traful, between Lago Traful and Lago Villarino, -42.722777, -71.735000, on rotten wood of *Lomatia hirsuta* under *Nothofagus dombeyi*, 27 Sep 2010, *S. Pérez Gorjón 2682* (**holotype** BAFC 52372). GenBank accession Nos.: ITS = KT032090, 28S = KT032102.

Etymology: from the Latinized form *patagonicus*, native of Patagonia.

Diagnosis: Basidiome even, plumbeous gray, separable from the substrate. Subhymenium consisting of simple septate, hyaline hyphae arranged in cushion-shaped granules. Subiculum formed by simple-septate, smooth, thin- to slightly thick-walled brownish hyphae. Hyphal cords and cystidia absent, basidiospores ellipsoid, with bifurcate verrucae.

Basidiome resupinate, even, moderately thick, whitish to plumbeous gray (5YR7/1), not separable from substrate, continuous, crustose. Sterile margin lacking. Subiculum darker than the hymenophore (5YR3/1). Hyphal cords lacking. Hyphal system monomitic. Generative hyphae simple-septate, with intense bluish eluates in KOH 3%. Subhymenial hyphae 3.5–7 µm diam, hyaline, smooth, relatively tortuous, profusely branched. Subicular hyphae 3.5–7.5 µm diam, thin- to slightly thick-walled, irregularly thickened, not encrusted and formed by short hyphal segments, brownish at or near the branches. Basidia 7–11 × 40–55 µm, cylindrical to clavate, hyaline, bearing four sterigmata up to 7 µm long, bluish in KOH 3%. Basidiospores broadly ellipsoid in lateral view, 9–12 × 7–10 µm, ellipsoid in frontal view, 9–12 µm, ornamented with bifurcate verrucae, somewhat tuberculate-globose when immature, hyaline in distilled water, bluish in KOH 3%, IKI–.

Ecology and hosts: On rotten wood under *Nothofagus dombeyi*. The species also is present in pure *Nothofagus pumilio* and *N. obliqua* forests, according to environmental sequences.

Other specimens examined: ARGENTINA. Neuquén, San Martín de los Andes, Traful, between Lago Traful and Lago Villarino, -42.722776, -71.580277, on rotten wood under *Nothofagus dombeyi*, 17 May 2010, *M. Rajchenberg, A. de Errasti, S. Pérez Gorjón 2817* (BAFC 52373). GenBank accession Nos.: ITS = KT032091, 28S = KT032103.

Comments: *Tomentella patagonica* is similar to *T. fuscocinerea* (Pers.) Donk and *T. cinereoumbrina* (Bres.) Stalpers, but the latter differs by triangular or lobed, echinulated basidiospores and thick-walled subicular hyphae. Another similar species, *T. badia* (Link) Stalpers, with a frequently mealy granulose hymenophore, does not present bifurcate ornamentations on the spore wall.

DISCUSSION

Species of other genera within the Thelephorales have been scantily recorded in Patagonia. Singer (1969) recorded *Thelephora terrestris* as a common species “apparently forming ectotrophic mycorrhizae with *Nothofagus antarctica* and also with planted trees like *Cedrus* etc.” and also cited materials determined by Maas Geesteranus as *Phellodon melaleucus* (Fr.) Karst. Greslebin (2002) reported *Tomentellopsis echinospora* (Ellis) Hjortstam and *Odontia ferruginea* (as *Tomentella crinalis*). In a survey based on ITS sequences obtained from ECM root tips Nuhra et al. (2013) revealed that the genus *Tomentella* was better represented than previously suspected by the basidiome report of Greslebin (2002).

In an ongoing survey of the ectotrophic mycobiota associated with *Nothofagus pumilio* seedlings and on which this study is based *Tomentella* species appeared to form highly abundant associations with young plants (data not shown); this along with the cited work underlines the importance of this genus in Patagonia. The affinity among available environmental/uncultured Patagonian sequences with our specimens confirms that different *Nothofagus* species share most of the tomentelloid taxa, as Nuhra et al. (2013) suggest as a general result concerning all studied mycorrhizal groups. Environmental ITS sequences deposited at GenBank and UNITE databases that show high similarity with our specimens suggest that *T. tenuissima* and *T. patagonica* have no specificity for a single-host species within South American *Nothofagus*, all of them being found under at least three different *Nothofagus* species. The wide host range of *Tomentella* already has been demonstrated (Selosse 2006). The absence of closer environmental sequences or root tips matching *Tomentella pulvinulata* may be due to the relatively poor sampling history of this group in Patagonian Andes forests.

ML and Bayesian inference analyses based on ITS suggested possible relationships of *T. pulvinulata*,

T. tenuissima and *T. galzinii* Patagonia with other southern hemisphere (Australia, New Zealand) undescribed *Tomentella* species recovered from environmental samples. However, a more complete knowledge of southern hemisphere Thelephorales and its phylogeny is required to confirm these relationships.

The presence of other *Tomentella* species in Patagonia shown by Nouhra et al. (2013) also is corroborated by the present study. Greslebin (2002) reported *T. radiosa* from Tierra del Fuego; however, her description does not agree with the species as described by Koljalg (1996) but suggests, together with other features, a notable similarity to *T. tenuissima*. This collection is no longer available for study at BAFC. Therefore, further findings of exemplars matching the cited description are needed to confirm its identity and thus the presence of this species in Tierra del Fuego.

An available ITS sequence from an Argentinean *T. galzinii* collection TU115232 revealed high similarity with Patagonian soil sequences. This material was identified as *Tomentella* aff. *galzinii* by Greslebin in her doctoral dissertation (2001). Our analysis (FIG. 1) showed that this material corresponds to a new species from Patagonia (UNITE SH: SH189408.07FU) that is not described here because only one collection is available.

Peay (2014) suggests that a deep understanding of fungal communities based on metagenomics must be preceded by a species-centric knowledge of the autecology of the involved taxa (i.e. spore dispersal structures) to correctly interpret the meaning of environmental datasets because those studies are unable to discriminate sequences originated from spore banks, colonizing mycelium or other structures. The integration of morphological information and different aspects of the natural history of the Patagonian species of *Tomentella*, together with molecular data, will contribute to future studies based on extensive data generated through environmental sequencing.

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