Mycological Progress (2021) 20:781–795
https://doi.org/10.1007/s11557-021-01703-x

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ORIGINAL ARTICLE

Two new species of *Morchella* from *Nothofagus* forests in Northwestern Patagonia (Chile)

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Received: 22 December 2020 / Revised: 17 April 2021 / Accepted: 20 April 2021

Abstract

In Chile, species of true morels have traditionally been identified on the basis of few morphological characteristics, but overall the genus *Morchella* has been poorly investigated and no studies combining morphological with molecular data exist. Here, *Morchella* collections from native forests of *Nothofagus* in Chilean Patagonia were characterized by combining morphological taxonomy with four-gene phylogenetic analysis. The phylogenetic relationships inferred from the concatenated dataset revealed that all collections belonged to the species-rich Elata clade and two new species were identified, which are formally described as *M. andinensis* and *M. aysenina*. *Morchella andinensis* was previously reported under the phylogenetic code *Mel*-37 from Argentinean Patagonia, while *M. aysenina*, which did not cluster with any other previously published groups of sequences, could be endemic to Chilean Patagonia. A third species, the transcontinental *M. tridentina*, is reported for the first time in Chile. It is hoped that these results will contribute to the limited knowledge of the genus *Morchella* in Chile and southern South America. *Morchella andinensis* and *M. tridentina* from Chilean and Argentinean Patagonia and *M. aysenina* constitute the southernmost collections of morels in the Southern Hemisphere.

Keywords Elata clade · Morchella andinensis · Morchella aysenina · Patagonia morels · Phylogeny

Introduction

Morchella is an emblematic widely distributed genus within Ascomycota, forming edible, highly appreciated ascomata. *Morchella* species have attracted the attention of mycologists and captured the interest of the broader public for a long time (Kuo 2005; Pilz et al. 2007). However, taxonomic arrangements in *Morchella*, especially at species level, have been historically problematic due to high plasticity of morphological features and the existence of cryptic species in the genus (Pilz et al. 2007; Masaphy et al. 2010). Hence, integrative methodological approaches are currently employed in the study of the genus, combining molecular with morphological, ecological, and distributional data (Loizides et al. 2015, 2016; Baroni et al. 2018). Contrary to previous concepts postulating the genus is composed of few cosmopolitan species, recent studies have demonstrated that most morel species are endemic, often confined to specific regions or

Section Editor: Zhu-Liang Yang

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continents. Three major groups, the Rufobrunnea clade (sect. *Rufobrunnea*), Esculenta clade (sect. *Morchella*), and Elata clade (sect. *Distantes*) currently accommodate all known morel species inferred from research mostly carried out in the Northern Hemisphere (Arora 1986; Guzmán and Tapia 1998; Kuo 2005; Pilz et al. 2007; Clowez 2012; Du et al. 2012a, b; Richard et al. 2015; Du et al. 2019). Apart from a critical revision of previously known species, mainly based on advanced molecular tools (O'Donnell et al. 2011; Du et al. 2012a, b; Kuo et al. 2012; Richard et al. 2015), documenting and describing morel species from poorly studied regions, especially in the Southern Hemisphere, pose an additional challenge.

Early mentions and descriptions of true morels from the Southern Hemisphere date from the late nineteenth and early twentieth centuries and were based on outdated taxonomic models (Armstrong 1880; Spegazzini 1909). Both groups, Esculenta and Elata, have been reported from Australia and New Zealand (Armstrong 1880; Gates and Ratkowsky 2016), but it wasn't until recently that collections were characterized using DNA analysis. Elliot et al. (2014) added three new taxa to the Australian inventory, one of which *M. australiana* T.F. Elliott, Bougher, O'Donnell and Trappe is putatively endemic to Australia. The first records of *Morchella* from southern South America were published by Spegazzini (1909) as "*M. conica*" Pers, based on collections from the eastern Andes between the Neuquen and Chubut regions in Argentina. Later, records of the same species from the Araucanía (Spegazzini 1918) and Valparaiso regions of Chile (Spegazzini 1921) were added. Espinosa (1929) reported *M. esculenta* (L.) Pers being sold on a market in Santiago de Chile. Those identifications, however, are highly doubtful since *M. esculenta* does not seem to occur in the Americas, while the name *M. conica* appears to be invalid, having been applied to different species (Richard et al. 2015).

Morchella patagonica Speg., the first "endemic" South American species described by Spegazzini (1909), has, to our knowledge, not been recorded since. *Morchella elata* Fr. was reported from Patagonia by Gamundí (1975) and already considered a species complex rather than a single taxon by that author. Gamundí (2010) also mentions records of *M. intermedia* Boud. and *M. semilibera* DC. based only on morphological characterization for Argentina. More recently and using molecular analysis, *M. tridentina* Bres. (as *M. frustrata* M. Kuo, *Mel*-2), *M. eximia* Boud. (as *M. septimelata* M. Kuo, *Mel*-7), and a new phylospecies informally labelled as *Mel*-37 belonging to the Elata clade were reported from Argentinean Patagonia (Pildain et al. 2014). Whereas *M. tridentina* and *M. eximia* have been described from several countries and continents (Kuo et al. 2012; Taşkın et al. 2010, 2012; Pildain et al. 2014; Richard et al. 2015; Loizides et al. 2015, 2016), *Mel*-37 would correspond to a new species whose presence has so far only been reported in Argentina. According to Pildain et al. (2014), none of the three species reported matches *M. patagonica*, as described by Spegazzini (1909). Recently, two new species of *Morchella* belonging to the Esculenta clade have been described from tropical zones: *M. gracilis* T.J. Baroni, Iturr. and Laessoe for Venezuela, Ecuador, and the Dominican Republic and *M. peruviana* Holgado, Aguilar, Quispe and T.J. Baroni for Peru, substantially expanding the known distribution range of the genus in South America (Baroni et al. 2018).

In southern Chile (northwestern Patagonia), commercial collection of *Morchella* spp. during spring (October–November) yields an important income for local collectors, mainly due to exports (INFOR 2016). As a result of a high demand for morels and an increasing concern for overharvesting and damage to natural morel habitats (e.g., by intentional forest fires in order to stimulate fructification), there is an increasing need to create national policies for sustainable management practices. Therefore, it is necessary to expand current knowledge on the diversity, distribution, and ecology of *Morchella* in Chile. In the present study, we focused our research on *Morchella* species from *Nothofagus* forests in northwestern Patagonia. Three species of the Elata clade are reported from Chilean Patagonia, one of them a first record for Chile and the other two proposed as new to science. One of those is present in Chilean and Argentinean Patagonia and was previously reported as *Mel*-37, while the other new taxon is currently only known from Chilean Patagonia. Our results are a further step forward toward an integrative taxonomic concept of *Morchella* in Patagonia, an area which constitutes the latitudinal distribution limit of morels in the Southern Hemisphere.

Material and Methods

Collection of ascocarps

During spring of 2013 and 2014, fresh ascocarps of *Morchella* were collected in native forests from two locations: Ñirehuao (45°12′S L) and Cochrane (47°2′S L) in the region of Aysen in northwestern Patagonia (Chile). Thirty-seven ascocarps (14 beige and 23 black) were collected and packed separately in paper bags for consecutive morphological characterization and molecular identification. Each ascocarp was imaged in situ by digital photography, and its habitat and surrounding vegetation were characterized. All the collections used in this study were deposited in the Fungal Herbarium of University of Concepción, Chile (see details in Table 1).

Morphological characterization

The collected specimens were transported to the laboratory where diagnostic macro- and microscopic morphological

Table 1 Representative *Morchella* collections from Northwestern Patagonia (Chile) used in this study

Species	Specimen voucher	Location/GPS coordinate	Collection date	Altitude (m)	Dominant vegetation	GenBank accession numbers			
						ITS	*TEF1*	*RPB1*	*RPB2*
Morchella tridentina	UDEC–LAF101-14	Ñirehuao, Aysén 45°12′15.9″S; 71°42′30.5″W	15 Nov 2013	750	*Nothofagus pumilio* *Dactylis glomerata* *Gaultheria mucronata* *Maytenus distichia*	MN355529	MN611981	MN602601	MN611968
Morchella tridentina	UDEC–LAF102-29	Ñirehuao, Aysén 45°12′15.9″S; 71°42′30.5″W	26 Oct 2014	750	*N. pumilio* *D. glomerata* *G. mucronata* *M. distichia*	MN355530	MN611982	MN602602	MN611969
Morchella tridentina	UDEC–LAF103-2	Ñirehuao, Aysén 45°12′15.1″S; 71°42′32.5″W	26 Oct 2014	750	*N. pumilio* *D. glomerata* *Anemone multifida* *M. distichia*	MN355531	MN611983	MN602603	MN611970
Morchella tridentina	UDEC–LAF104-1	Ñirehuao, Aysén 45°12′14.9″S; 71°42′31.5″W	26 Oct 2014	750	*N. pumilio* *D. glomerata* *A. multifida* *M. distichia*	MN355532	MN611984	MN602604	MN611971
Morchella andinensis sp. nov.	UDEC–LAF105-10	Ñirehuao, Aysén 45°12′12.6″S; 71°42′33.5″W	26 Oct 2014	743	*N. pumilio* *Taraxacum officinale* *Trifolium pratense* *Osmorhiza chilensis*	MN355520	MN611972	MN602592	MN611959
Morchella andinensis sp. nov.	UDEC–LAF106-8	Ñirehuao, Aysén 45°12′12.5″S; 71°42′29.2″W	26 Oct 2014	750	*N. pumilio* *T. officinale* *T. pratense* *O. chilensis*	MN35552	MN611977	MN602597	MN611964
Morchella andinensis sp. nov.	UDEC–LAF107-76	Cochrane, Aysén 47°21′11.5″S; 72°40′43.4″W	23 Oct 2014	234	*N. antarctica* *Holcus lanatus* *A. multifida* *Poa pratensis*	MN355522	MN611974	MN603594	MN611961
Morchella andinensis sp. nov.	UDEC–LAF108-77	Cochrane, Aysén 47°21′11.5″S; 72°40′43.4″W	23 Oct 2014	260	*N. antarctica* *H. lanatus* *A. multifida* *P. pratensis*	MN355524	MN611976	MN602596	MN611963
Morchella aysenina sp. nov.	UDEC–LAF111-78	Ñirehuao, Aysén 45°12′14.9″S; 71°42′31.5″W	26 Oct 2014	738	*N. pumilio* *D. glomerata* *A. multifida* *M. distichia*	MN355527	MN611979	MN602599	MN611966

Table 1 (continued)

Species	Specimen voucher	Location/GPS coordinate	Collection date	Altitude (m)	Dominant vegetation	GenBank accession numbers			
						ITS	*TEF1*	*RPB1*	*RPB2*
Morchella aysenina sp. nov.	UDEC–LAF112-33	Cochrane, Aysén 47°21'12.9"S; 72°40'44.2"W	23 Oct 2014	230	*N. antarctica* *A. multifida* *A. millefolium* *R. rubiginosa*	MN355528	MN611980	MN602600	MN611967
Morchella aysenina sp. nov.	UDEC–LAF113-35	Cochrane, Aysén 47°21'12.7"S; 72°40'43.5"W	23 Oct 2014	232	*N. antarctica* *A. multifida* *A. millefolium* *R. rubiginosa*	MN355526	MN611978	MN602598	MN611965

UDEC-LAF Universidad de Concepción-Los Angeles Fungal Herbarium. Specimens collected on undisturbed sandy loam soil, slightly acidic

features were studied from fresh and air-dried ascocarps, respectively. Microscopic observation of small pieces of dried hymenium, previously rehydrated and mounted in 4% KOH solution, was performed using a Zeiss Primo Star light microscope and documented with a Moticam 2000 camera; measurements were taken with Motic Images Plus 2.0 software. At least 50 ascospores were measured in water from each fresh mature specimen. For scanning electron microscopy, ascospores were mounted on a stub, coated with gold, and examined with a JEOL JSM 6380LV scanning electron microscope (SEM, JEOL USA, Peabody, Massachusetts). Finally, the ascocarps were air dried and deposited as voucher material in the Fungal Herbarium of the Department of Plant Science and Technology, University of Concepción (under code UDEC-LAF, Table 1).

Sample collection for DNA extraction

For the molecular studies, approximately 5 g of fresh pilei was aseptically removed from 37 ascocarps with a sterilized blade and dehydrated in sealed plastic bags containing silica gel. Approximately 25 mg of dried material were ground in liquid nitrogen in an autoclaved pestle and mortar. Pulverized samples were re-suspended in 500 μL of genomic lysis buffer. Total DNA was extracted using the Quick g-DNA Miniprep kit (Zymo Research, USA) following the manufacturer's instructions.

PCR amplification and sequencing

To investigate phylogenetic relationships between the 37 morel specimens, nuc rDNA ITS1-5.8S-ITS2 (ITS) was amplified using the primers ITS1 and ITS4 (White et al. 1990; Gardes and Bruns 1993). The PCR reaction mix was the following: 1-μl DNA; 0.2-μl GoTaq® (Promega, USA); 0.5-μl primer ITS1 (50 μM); 0.5-μl primer ITS4 (50 μM); 0.2-μl dNTPs (10 mM); 10-μl 5× buffer; 3 μl of $MgCl_2$ (25 mM); and 33.8 μl of PCR ultra-pure water. The cycling parameters were 5 min at 95°C followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s and a final hold at 72°C for 5 min. A subset of 11 samples (Table 1) chosen from each of the clusters of the phylogenetic tree developed with ITS was analyzed using a four-locus dataset comprising portions of the ITS, *RPB1*, *RPB2*, and *TEF1* genes (Taşkın et al. 2010). The primers used in this study are described in Supplementary Table S1. The cycling parameters for *RPB1*, *RPB2*, and *TEF1* genes were 90 s at 94°C followed by 40 cycles at 94°C for 30 s, 55°C for 90 s, and 72°C for 3 min and a final hold at 72°C for 5 min. The amplification of each region was verified by electrophoresis in 1.0 % (w/v) agarose gels prestained with 1:10,000 Gel Red DNA gel stain (Biotium, USA). The marker used was the Kapa^{TM} Universal Ladder. Electrophoresis was carried out in 1×TAE (40-mM Tris-

acetate, 1 mM EDTA, pH 8.0) at 75V for 2 h. Once gene amplification was verified, DNA samples were sequenced by Macrogen (Korea).

Phylogenetic analysis

The chromatograms were analyzed and edited using the Gene Tool Lite 1.0 software (Doubletwist, Inc., Oakland, CA, USA). Sequence alignments and phylogenetic analyses were conducted with MEGA X (Kumar et al. 2018). The reference sequences used for phylogenetic analyses were retrieved from GenBank (Supplementary Table S2). The alignments were made by ClustalX and edited manually. The phylogenetic tree was inferred by using the maximum likelihood method and GTR+I+G model with a bootstrap analysis based on 1,000 replicates to evaluate the confidence of the nodes. The BLAST search tool from the National Center for Biotechnology Information (NCBI) was used to find *Morchella* sequences. A concatenated matrix of the four loci was analyzed as a single partition, and that matrix is available on TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S25644).

Results

The ITS amplicon obtained from all 37 collections was 700 bp long for the black morphotypes and 850 bp for the beige morphotypes. All collections nested in the Elata clade as defined by Taşkın et al. (2010) and O'Donnell et al. (2011) and fall into three different subgroups. Twenty black morels clustered with *Mel*-37 from Argentinean Patagonia (Pildain et al. 2014). Another three black specimens clustered on their own but close to *Mel*-17, *M. eohespera* (*Mel*-19), *M. purpurascens* (*Mel*-20), and *Mel*-34, while 14 beige specimens were conspecific with *M. tridentina* (*Mel*-2). Eleven specimens were selected for further sequencing and phylogenetic analysis, including four samples of *Mel*-37, four of *M. tridentina*, and the three black specimens which clustered separately (Table 1). In the ITS maximum likelihood phylogenetic tree (Fig. 1), specimens UDEC-LAF 105-10, 106-8, 107-76, and 108-77 (black morphotypes) showed identical ITS sequences and cluster with *Mel*-37 from Argentina. Specimens UDEC-LAF 111-78, 112-33, and 113-35 (black morphotypes) clustered together and were close to *Mel*-17 and *Mel*-34 from China and *M. eohespera* and *M. purpurascens* from Sweden. Finally, all the beige morphotypes (UDEC-LAF 101-14, 102-29, 103-2, and 104-1) clustered with *M. tridentina* in a well-supported monophyletic lineage (100% bootstrap support) (Fig. 1). A fragment of 770 bp of the *RPB1* gene was successfully amplified for the 11 specimens. The *RPB1* phylogeny (Supplementary Fig. S1) was generally consistent with the specimen clustering by the ITS rDNA. Specimens UDEC-LAF 111-78, 112-33, and 113-35 also showed identical *RPB1* sequences, but in the phylogenetic tree, these were closer to *M. hispaniolensis* (*Mel*-18) from the Dominican Republic and *M. conifericola* (*Mel*-32) from Turkey. In the phylogenetic tree generated from the concatenated genes (combining ITS-*RPB1*-*RPB2* and *TEF1* sequence data) (Fig. 2), the position of the specimens was similar to that in the ITS tree. Specimens UDEC-LAF 105-10, 106-8, 107-76, and 108-77 (black morphotypes) were included in the *Mel*-37 phylogenetic species and are formally described here as *M. andinensis* (see "Taxonomy" section). Again, the black morphotypes UDEC-LAF 111-78, 112-33, and 113-35 clustered together in a lineage that was close to *M. hispaniolensis* from the Dominican Republic. Therefore, these three specimens are described as *M. aysenina* (see "Taxonomy" section), a new species in the Elata clade. Finally, the beige morphotypes UDEC-LAF 101-14, 102-29, 103-2, and 104-1 consistently clustered with *M. tridentina* (Fig. 2).

Taxonomy

Morchella andinensis A. Machuca, M. Gerding and D. Chávez, sp. nov., Fig. 3a, b, c, d, e, and f

MycoBank MB 833803

Etymology: The epithet refers to the currently known area of distribution of the taxon along the Patagonian Andes.

Diagnosis: Ascomata 50–120 mm alta; capitulum sporogenum ovatum vel conicum, cavum; costae 12–22, nigrescentes vel umbrino griseae, regulariter ordinatae; alveoli colore ocraceo olivaceum vel umbrino olivaceum; stipes aequaliter crassus vel subbulbosus, cavus, eburneus, furfuraceus; asci cylindrici, 8 sporigeri, 207–303 × 14–19 µm, haud amyloidei; paraphysis cylindraceae, rariter bifurcatae, 50–129 × 6–8 µm, ad basim 2–3 septatae, apice capitatum vel clavatum; ascosporae longitudinaliter estriatae sub microscopium electronicum, 18–23 × 12–15 µm, Q = 1.58; acroparaphysis clavatae vel fusiformes; tegumento stipitis ex hyphis ramificatis, elementis apicalis catenulatis longis vel simplicis, cellulis ellipsoideis, oblongis vel clavatis; gregaria vel solitaria ad terram et humum in silvis patagonicis nothofagineis (*N. pumilio*, *N. antarctica*), vernalis; (**Typus** UDEC-LAF107-76), GenBank: ITS = MN355522; *TEF1* = MN611974; *RPB1* = MN602594; *RPB2* = MN611961.

Macromorphological description: Ascomata 5–12 cm high; pileus 3–4.7(6) × 2–3(4) cm, ovoid to conical, rarely acuminate, hollow, with 12–22 vertical ridges, more or less regularly aligned, sometimes sinuous, connected by sunken, transecting horizontal ridges, attached to the stipe with a sinus of variable depth, usually shallow; ridges beige to ochre when young, dark gray to pure black with age, sometimes with olive hues, finely tomentose; pits primarily elongated vertically,

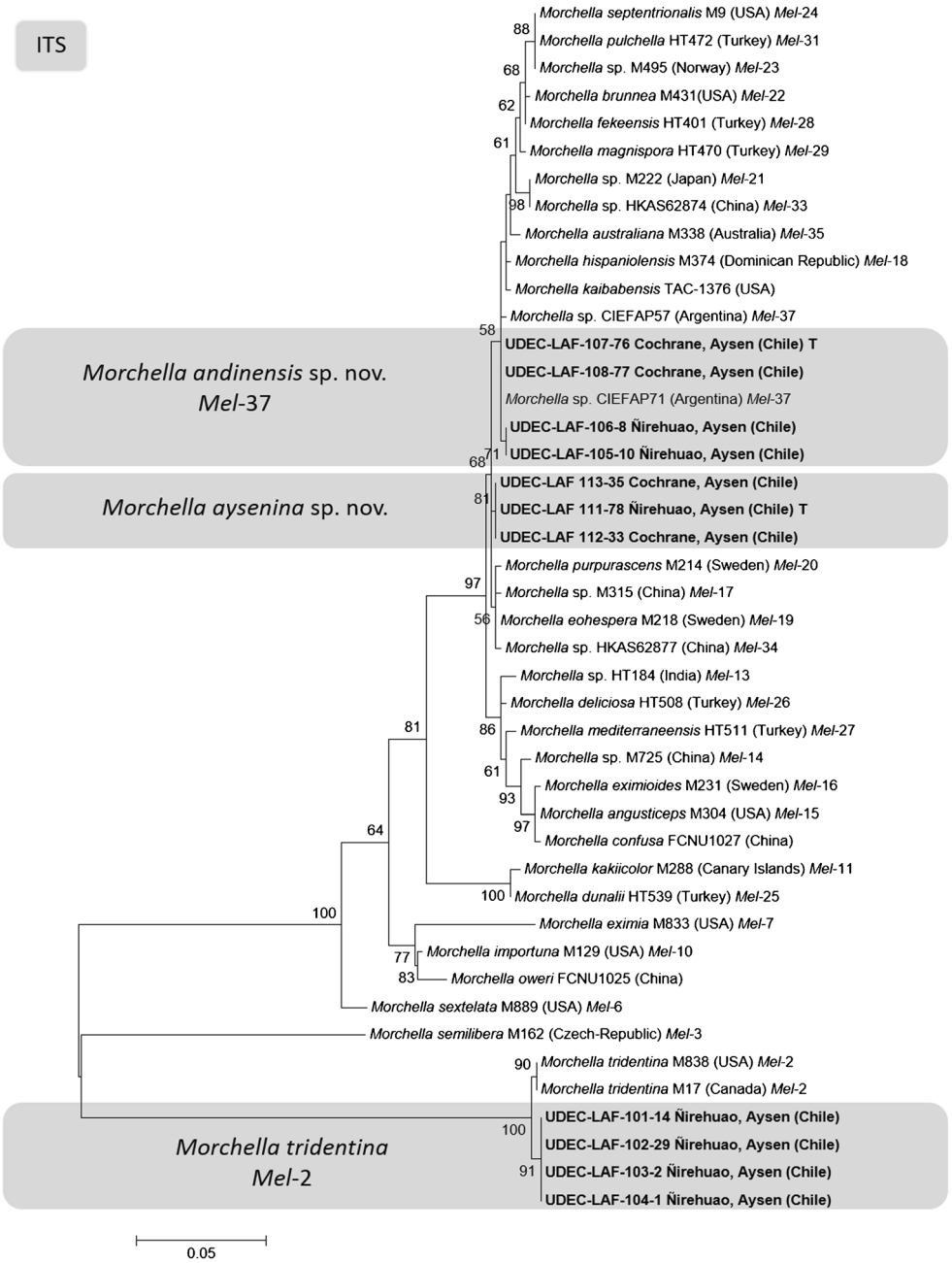

Fig. 1 Maximum likelihood phylogeny based on ITS genes of *Morchella* specimens within the Elata clade. Chilean Patagonian specimens are highlighted in gray boxes and taxonomically revised specimens in bold font. **T**, Type collections of *M. andinensis* and *M. aysenina*. Bootstrap values are indicated on branches only when higher than 65%. The other species sequences in the phylogram were obtained from GenBank (accession number in Table S2)

asymmetrical and irregular in outline, 4–7 mm deep, glabrous, ochre, initially concolorous with ridges, darkening with age but contrasting with ridges; stipe cylindrical to subbulbous, 2–3 (5.5) cm × 1–1.5 cm, hollow, ivory to cream, usually furfuraceous, with whitish granules, sometimes becoming longer than pileus with age; context fragile, cream to beige, inner surface finely granular.

Micromorphological description: Ascospores 18–23 (25) × (10.5) 12–15 (16) μm, Q = 1.58 in water, elliptical to ovoid, hyaline, appearing smooth under the light microscope, without oil droplets, under the SEM surface longitudinally striate or ridged, longitudinal ridges connected by numerous short anastomoses; asci 207–303 × 14–19 μm, cylindrical, inamyloid, eight-spored; paraphyses 50–129 × 6–8 μm, hyaline, inamyloid, cylindrical, uniseriate, occasionally bifurcate, 2–3 septate in the lower half and occasionally constricted at the septa, apices polymorphic, capitate to clavate or subclavate; acroparaphyses clavate to (sub)fusiform, 53–112 ×13–25 μm; terminal elements of stipe hairs elongate catenulate or simple, cells elliptical, cylindrical or clavate, 67–242 × 10–15 μm.

Ecology: Appearing in spring (October–early November), solitary or in scattered groups, in deciduous *Nothofagus* forest dominated by *N. pumilio* (at Ñirehuao

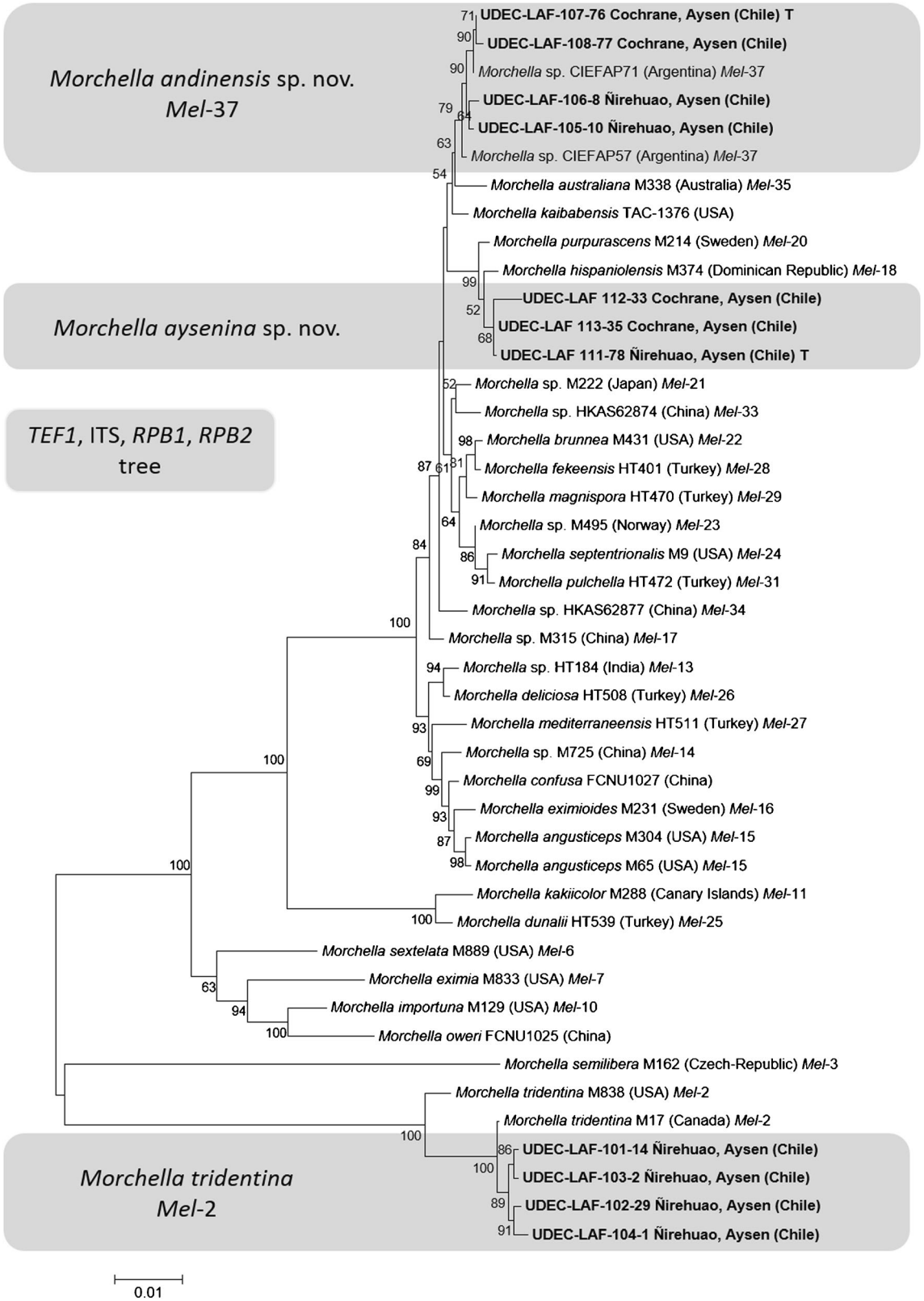

Fig. 2 Maximum likelihood tree based on *TEF1*, ITS, *RPB1*, and *RPB2* concatenated genes of *Morchella* specimens within the Elata clade. Chilean Patagonian specimens are highlighted in gray boxes and taxonomically revised specimens in bold font. **T**, Type collections of *M. andinensis* and *M. aysenina*. Bootstrap values are indicated on branches only when higher than 65%. The other species sequences in the phylogram were obtained from GenBank (accession number in Table S2)

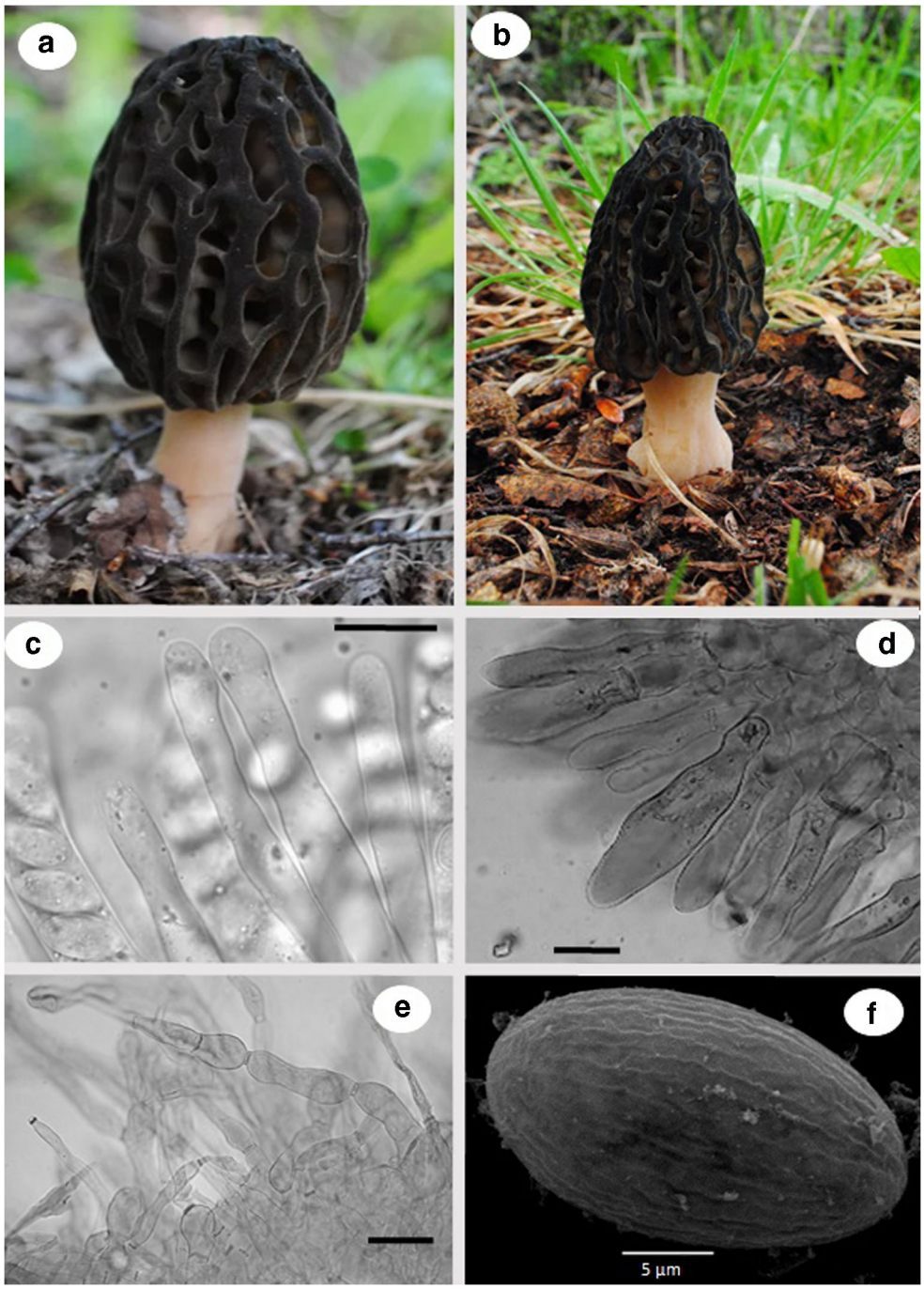

Fig. 3 Ascocarps of *Morchella andinensis* sp. nov. in situ (**a, b**) (images by F. Silva) and micromorphological features (**c, d, e**) (images by D. Chávez) and (**f**) (image by R. Oliva). **c** asci and paraphyses (bar = 25 µm); **d** acroparaphyses (bar = 20 µm); **e** stipe hairs (bar = 20 µm); **f** SEM image of an ascospore showing a regular pattern of longitudinal striations on wall surface

site) and *N. antarctica* (at Cochrane site), with abundant herbaceous understorey such as *Achillea millefolium*, *A. multifida*, and *Holcus lanatus*, at elevations ranging from 260 (Cochrane) to 750 m (Ñirehuao), on neutral or slightly acidic sandy loam with abundant leaf litter; the collection sites showed some grazing activity, but no sign of forest fire.

Distribution: Both slopes of the southern Andes in Chile (Aysen Region, this study) and Argentina (Chubut Province; see Pildain et al. 2014).

Comments: *Morchella andinensis*, according to our results, is identical with the phylogenetic species *Mel*-37 first documented in Argentina (Pildain et al. 2014). The Argentinian material had been collected from similar native forest habitats as our Chilean specimens, but also from mixed forests of *N. dombeyi* and under the introduced conifer *Pseudotsuga menziesii*. Ascocarps of *M. andinensis* showed less variability in shape than *M. tridentina* and were typically found inside shady forests, very close to *Nothofagus* trees, occasionally in groups located only on the sun-facing side of the stem and also

close to rotten tree trunks; less frequently, we recorded specimens from forest edges or open grassland. The collections from shady forest showed intense black pilei, whereas those in more open areas were pale black, always with ochre to brownish-colored pits. Old ascocarps can display a stipe equal or greater in width and height than the pileus. *Morchella andinensis* can be distinguished from *M. tridentina* mainly by its smaller ascocarps which have the tendency to turn black at maturity, but not rust-colored like *M. tridentina*, and also appear earlier in the season. At the microscopical level, the catenulate terminal stipe hair elements can be conspicually long, which may be a useful diagnostic feature. Also, the striation pattern of spores of *M. andinensis* as seen under SEM is similar to *M. aysenina* and more prominent than that of *M. tridentina*.

Specimens examined: see Table 1.

Morchella aysenina A. Machuca, M. Gerding & D. Chávez, sp. nov., Fig. 4a, b, c, and e

MycoBank MB 838257

Etymology: The epithet refers to the region of Aysén, Southern Chile, where the species was collected for the first time.

Diagnosis: Ascomata 60–80 mm alta; capitulum sporogenum subglobosum vel conicum, cavum; costae longitudinalae 16–20, griseobrunneae vel umbrinae, tomentosae, regulariter ordinatae, coniunctionibus transversalibus inferioribus; alveoli costis concolori, irregulari, longitudinaliter elongati, glabri; stipes aequaliter crassus vel subbulbosus, cavus, 20–25 × 10–15 mm, eburneus; asci cylindrici, 8 sporigeri, 215–300 × 15–25 μm, haud amyloidei, paraphysis cylindraceae, 73–112 × 6–10 μm, apice cylindraceae vel subclavatum; ascosporae striis longitudinalibus parallelis et anastomosantibus sub microscopium electronicum, 18–22 × 10–13 μm, Q = 1.63; acroparaphysis clavatae vel fusiformes; tegumento stipitis ex hyphis ramificatis, elementis apicalis catenulatis cortis vel simplicis, cellulis subglobosis, ellipsoideis, oblongis vel clavatis, saepe tortilibus; gregaria vel solitaria ad terram et humum in silvis patagonicis nothofagineis (*N. pumilio*, *N. antarctica*), vernalis; (**Typus** UDEC-LAF111-78), GenBank: ITS = MN355527; *TEF1* = MN611979; *RPB1* = MN602599; *RPB2* = MN611966.

Macromorphological description: Ascomata 6–8 cm high; pileus 4.5–5.5 × 2.8–3.5 cm, subglobose to conical, hollow, with 16–20 regular, sinuous vertical ridges, connected by sunken, transecting horizontal ridges, attached to the stipe with a shallow sinus; ridges grayish brown to dark brown, tomentose; pits primarily elongated vertically, markedly sinuous, 5–9 mm deep, glabrous, concolorous with ridges; stipe cylindrical to subbulbous, 2–2.5 cm × 1–1.5 cm, shorter than the pileus, hollow, ivory to cream; context firm.

Micromorphological description: Ascospores (16)18–22(26) × (9.5)10–13(16) μm, Q = 1.63 in water, elliptical to ovoid, hyaline, appearing smooth under the light microscope, without oil droplets, under SEM surface longitudinally striate or ridged, longitudinal ridges connected by numerous, short anastomoses; asci 215–300 × 15–25 μm, cylindrical, inamyloid, uniseriate, eight-spored; paraphyses 73–112 × 6–10 μm, hyaline, inamyloid, cylindrical, terminal cells typically cylindrical to subclavate, sometimes capitate or lageniform, 1–2 septate in the lower half; hymenium with accumulations of dark brown pigment; acroparaphyses clavate to subfusiform or fusiform, 45–92 × 10–25 μm; terminal elements of stipe hairs shortly catenulate or simple, cells subglobose, elliptical, cylindrical or clavate, often tortuous, 37–75 × 8–32 μm.

Ecology: Appearing in spring (October–early November), solitary or in scattered groups, in deciduous *Nothofagus* forest dominated by *N. pumilio* (at Ñirehuao site) and *N. antarctica* (at Cochrane site), with abundant herbaceous understorey such as *Achillea millefolium*, *A. multifida*, and *Holcus lanatus*, at elevations ranging from 260 (Cochrane) to 750 m (Ñirehuao), on neutral or slightly acidic sandy loam with abundant leaf litter; the collection sites showed some grazing activity, but no sign of forest fire.

Distribution: Southern Andes in Chilean Patagonia (Aysén Region).

Comments: *Morchella aysenina* is morphologically very close to *M. andinensis* and was found at the same collection sites; however, its position in the molecular phylogenetic tree is clearly different from *M. andinensis* and shows phylogenetic proximity to taxa from other biogeographical regions (see "Discussion" section). Although *M. aysenina* shows some differential microscopic traits from *M. andinensis*, further collections of *M. aysenina* are necessary to establish consistent morphological differences between the two species.

Specimens examined: see Table 1.

Morchella tridentina Bres., Fungi Tridentini 2 (11-13): 65 (1898), Fig. 5a, b, c, d, e, and f

= *Morchella frustrata* M. Kuo, in Kuo et al., Mycologia 104(5): 1167 (2012)

= *Morchella quercus-ilicis* Clowez, Ballester & L. Romero, Bull. Soc. Mycol. Fr. 126(3-4): 318 (2012) Synonymy established by Loizides et al., Mycol. Prog. 14:13 (2015)

Macromorphological description: Ascomata 5–19 cm high; pileus 3–7(10) × 2–4.5(6) cm, conical to subglobose or acuminate, hollow, with 15–18 vertical ridges, more or less regularly aligned, with numerous transverse ridges, attached to the stipe with a shallow sinus when young, without sinus in mature specimens; pale yellowish when young, pale ochraceous, buff or beige at maturity, usually with rust-colored or reddish stains, glabrous; pits vertically elongated,

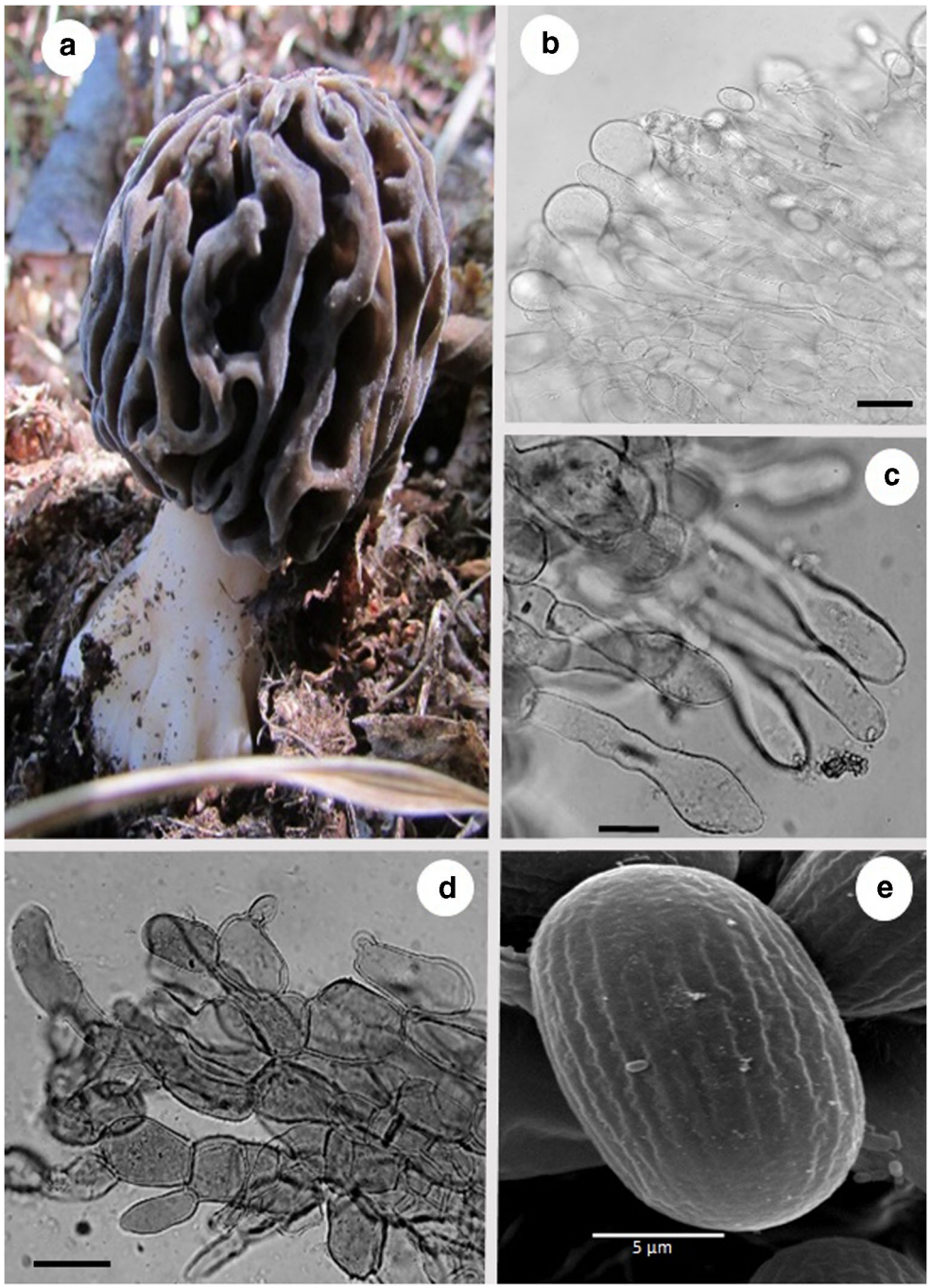

Fig. 4 Ascocarp of *Morchella aysenina* sp. nov. in situ (**a**) (image by D. Chávez) and micromorphological features (**b, c, d**) (images by D. Chávez) and (**e**) (image by R. Oliva). **b** asci and paraphyses (bar = 25 μm); **c** acroparaphyses (bar = 20 μm); **d** stipe hairs (bar = 20 μm); **e** SEM image of an ascospore showing longitudinal striations on wall surface

sinuous, in some ascomata asymmetrical and irregular in outline and size, shallow, 2–4 mm deep, more or less concolorous with ridges; stipe rarely cylindrical, usually clavate to subbulbous, 2–5(9) × 1.3–3 cm, twisted, hollow, whitish or ochraceous-white, with stains at the base, usually smooth, sometimes covered by whitish granules; context firm, whitish to cream.

Micromorphological description: Ascospores (20)22–27 × (13)14–17(20) μm, Q = 1.53 in water, elliptical to ovoid, hyaline, smooth under light microscope, slightly wrinkled under SEM, without oil droplets; asci 250–337 × 17–22 μm, cylindrical to clavate, uniseriate, eight-spored, inamyloid; paraphyses 100–225 × 8–15 μm, hyaline, mostly clavate, with rounded apices, 3–4 septate in the lower half; acroparaphyses clavate to subfusiform or fusiform, 75–117 × 8–17 μm; terminal elements of stipe hairs simple, cells subglobose, elliptical, cylindrical or clavate, 50–105 × 9–25 μm.

Ecology: Appearing in spring (late October–November), typically growing in groups of four or more individuals, often fused at the base, in deciduous *Nothofagus* forest dominated

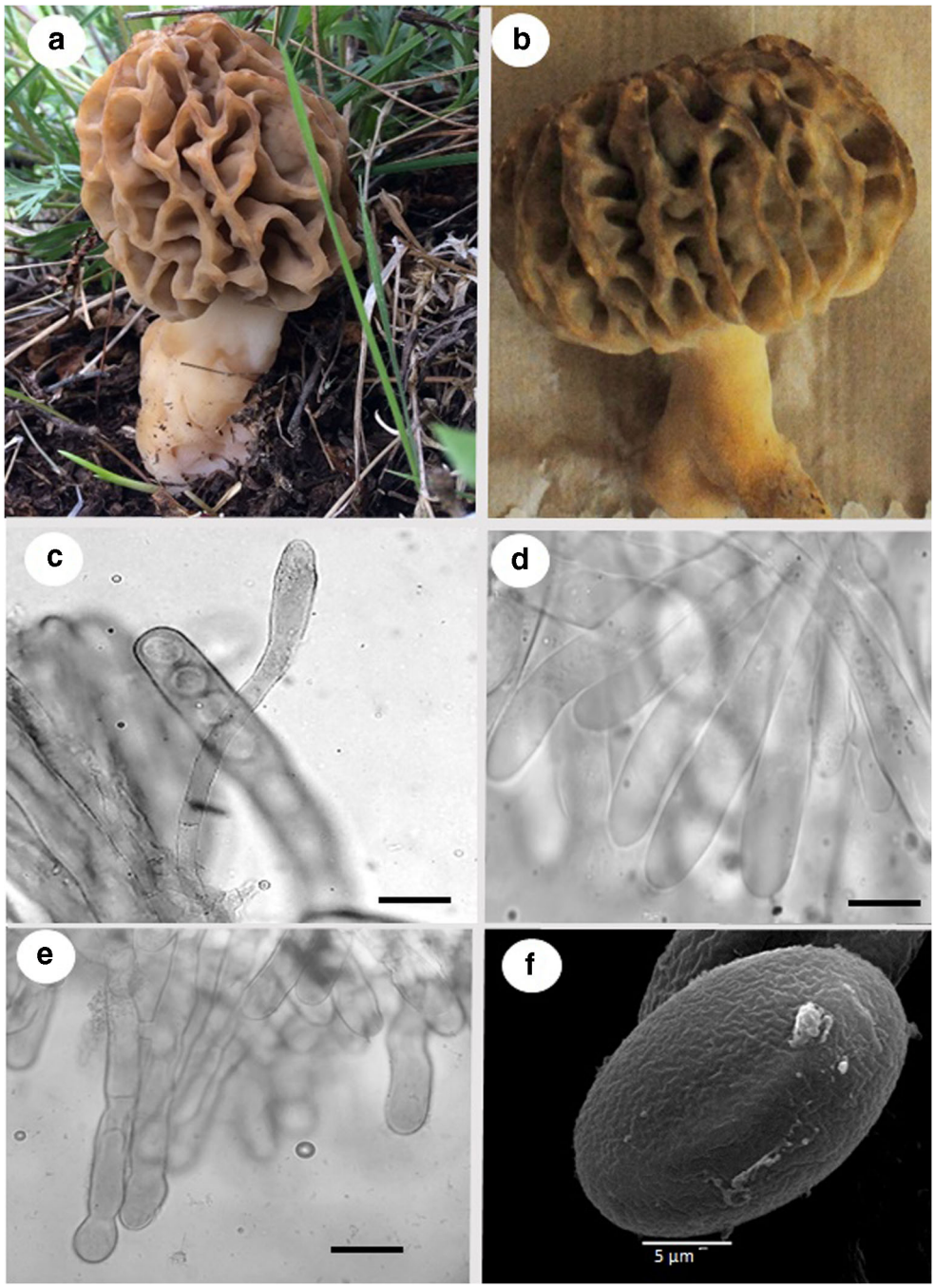

Fig. 5 Ascocarps of *Morchella tridentina* in situ (**a**) (image by A. Machuca) and at laboratory (**b**) (image by F. Silva) and micromorphological features (**c, d, e, f**) (images by D. Chávez) and (**f**) (image by R. Oliva). **c** asci and paraphyses (bar = 25 µm); **d** acroparaphyses (bar = 20 µm); **e** stipe hairs (bar = 20 µm); **f** SEM image of an ascospore showing wrinkles on wall surface

by *N. pumilio*, with abundant understorey dominated by shrubs like *Gaultheria mucronata* and *Maytenus distichia* and herbs such as *Dactylis glomerata* and *Anemone multifida*, at elevations above 700 m, preferring neutral or slightly acidic sandy loam soils; collection sites were characterized by little or no anthropogenic disturbance.

Distribution: widely distributed in the Northern Hemisphere and in South America at both slopes of the southern Andes in Chile (Aysen Region, this study) and Argentina (Chubut Province; see Pildain et al. 2014).

Comments: In most of the collections, the stipe of young specimens was shorter than the pileus, but like *M. andinensis* in some older specimens, the stipe was equal to or longer than the pileus. Typically, the specimens displayed an absence of sinus between pileus and stipe at maturity, and some showed a hole in the apex of the pileus. In the field, those specimens more exposed to sunlight showed a bright ochraceous-yellow pileus unlike those developed under the bushes, which were pale yellow and faded. Some specimens had a darkened apex due to dehydration or exposure to frost in the field.

Consistently, all young specimens from our study sites in Patagonia were pale yellowish in color, unlike that reported for collections from Eurasia. Occasionally the ascocarps of *M. tridentina* emerged in clusters of four or more fused by the stipe base. Due to its larger size, *M. tridentina* is usually preferred by mushroom collectors in Chilean Patagonia and also because, after harvest, *M. andinensis* tends to decompose more easily, acquiring an unpleasant and penetrant odor. We observed that both species usually do not mix but grow separately at specific, well-defined sites.

Specimens examined: see Table 1.

Table 2 shows a comparative summary of the main morphological, macroscopic, and microscopic traits of the three *Morchella* species found in Chilean Patagonia.

Discussion

Whereas taxonomy for morels in the Northern Hemisphere has been extensively revised and unified (Richard et al. 2015), state-of-the-art identification and comparison of species from both sides of the Southern Pacific are still incomplete, despite some important advances in recent years (Elliot et al. 2014; Pildain et al. 2014). Of the three taxa identified by Elliot et al. (2014) for the Australian Pacific area, viz., *M. australiana*, *M. rufobrunnea*, and *M. eximia* (as *M. septimelata*), only the latter has been reported from South America so far (Pildain et al. 2014). However, *M. australiana* which, according to the authors, may be endemic in Australia and *M. andinensis* appear closely related, and a possible relationship between these taxa should be further investigated.

All *Morchella* species from the study sites in Chilean Patagonia (45°12′S–47°21′S latitude) were found between 260 and 750 masl in *Nothofagus* forests dominated by the deciduous species *N. pumilio* and *N. antarctica* on sites relatively undisturbed and not affected by wildfire. In these latitudes, morels fruit in spring (October–November) under conditions of strong wind, frost, rain, and occasional snowfall. All specimens recorded at these sites were part of the highly diverse Elata clade (*Mel*-), as defined by Taşkın et al. (2010) and O'Donnell et al. (2011). Within the Elata clade, three main phylogenetic lineages were recognized in Chilean Patagonia through a four-gene phylogenetic analysis: *M. tridentina* (phylospecies *Mel*-2, with ascomata that fade to beige at maturity), *M. andinensis* (phylospecies *Mel*-37, with dark gray ascomata that become black at maturity), and *M. aysenina* (with grayish brown to dark brown ascomata at maturity). *Morchella tridentina*, well-known from the Northern Hemisphere, is a new record for Chile. There is only one previous mention of this species in Chile being sold on a market, based on a sequence uploaded on a public database (Loizides et al. 2015), but no taxonomic details or geographic data were provided. *Morchella andinensis*, previously reported as *Mel*-37 from Argentina (Pildain et al. 2014), is a new species formally described for the first time in this work. The known area of distribution of *M. andinensis* is considerably extended by our results, and it appears that the species might be endemic in southern South America. The third taxon is represented by three collections of black morphotypes which are nested together in our phylogenetic trees, well-separated from *M. andinensis*. Because it represents a phylogenetically distinct species within the Elata clade, we have described it here as a new species with the name *M. aysenina*. Nevertheless, additional fresh material is necessary for establishing consistent morphological differences between *M. aysenina* and *M. andinensis*, and further surveys are required to determine if *M. aysenina* is also present outside Chilean Patagonia.

Morphologically, the collections of *M. tridentina* from Chilean Patagonia were similar to *M. tridentina* described by Kuo et al. (2012, as "*M. frustrata*") in the USA and also similar to *M. tridentina* described by Loizides et al. (2015) from Cyprus and Spain. A frequent feature in this species is the rusty or orange staining on the edge of the ridges that appears at maturity, also described by Loizides et al. (2015). However, the shape of the pileus appeared to be much more variable (conical to globose or subglobose) for collections from Chilean Patagonia than those described in the literature. On the other hand, collections of *M. andinensis* from Patagonia display some morphological characteristics which match other Elata clade species, e.g., the blackening of the hymenial ridges with age, as in *M. arbutiphila*, *M. disparilis*, and *M. dunalii* from Cyprus (Loizides et al. 2016), *M. eohespera* and *M. laurentiana* from Canada (Voitk et al. 2016), and *M. australiana* from Australia (Elliot et al. 2014). However, combined morphological traits of *M. andinensis* like the lack of pinkish-purple, pinkish buff, orange citrine, or grayish shades at any stage of development and the size and shape of stipe hairs ends are helpful in distinguishing it from similar species.

The Andean-Patagonian forests of southern South America are known for their highly endemic biota which also includes fungi (Palfner 2001; Palfner and Casanova-Katny 2019). On the other hand, many subcosmopolitan species have been reported from the region as well, but due to the lack of extensive inventories prior to the second half of the twentieth century, it is difficult to determine whether they form part of the native fungal communities since prehistoric times or if their presence is the consequence of introduction by human activities during the more recent past. *Morchella tridentina* is a typical case: although many *Morchella* species appear to be geographically restricted to specific regions or continents (Taşkın et al. 2012; Du et al. 2015; Richard et al. 2015), *M. tridentina* has been reported from Europe, North America, Asia (Taşkın et al. 2010; Du et al. 2015; Loizides et al. 2015; Richard et al. 2015), and South America, (Pildain et al. 2014 as

Table 2 Main macroscopic and microscopic morphological features of the three *Morchella* species from Northwestern Patagonia (Chile)

		M. andinensis sp. nov.	*M. aysenina* sp. nov.	*M. tridentina*
Macromorphology	Ascoma size	50–120 mm	60–80 mm	50–190 mm
	Pileus color	Dark gray to pure black at maturity	Grayish brown to dark brown	Pale yellowish to pale ochraceous, buff or beige with rust-colored stains at maturity
	Pileus shape	Ovoid to conical	Subglobose to conical	Conical to subglobose or acuminate
Micromorphology	Spore size	18–23 (25) × (10.5) 12–15 (16) μm	(16)18–22(26) × (9.5)10–13(16) μm	(20)22–27 × (13)14–17(20) μm
	Spore wall (SEM)	Longitudinally striate	Deeply longitudinally striate	Slightly wrinkled
	Ascus length/width	207–303 × 14–19 μm	215–300 × 15–25 μm	250–337 × 17–22 μm
	Paraphyses length/width	50–129 × 6–8 μm	73–112 × 6–10 μm	100-225 × 8-15 μm
	Paraphyses apex shape	Cylindrical	Cylindrical to subclavate to clavate	Mostly clavate, with rounded apices
	Paraphyses septa	2–3 septa in lower half	1–2 septa in lower half	3–4 septa in lower half
	Acroparaphyses length/width	53–112 × 13–25 μm	45–92 × 10–25 μm	75–117 × 8–17 μm
	Capitate elements	Absence	Presence	Absence
	Acroparaphyses shape	Clavate to (sub)fusiform	Clavate to (sub)fusiform	Clavate
	Stipe hairs length/width	67–242 × 10–15 μm	37–75 × 8–32 μm	50–105 × 9–25 μm
	Stipe hairs shape	Elongate catenulate or simple, cells elliptical, cylindrical, or clavate	Simple or shortly catenulate, cells subglobose, elliptical, cylindrical or clavate, sometimes tortuous	Simple, cells subglobose, elliptical, cylindrical, or clavate

"*M. frustrata*"; and this study). Its presence in South America could be the effect of anthropogenic activities, especially international traffic of widely used timber species and forestry machinery and materials, but non-anthropogenic dispersion by high altitude winds or migrating birds as spore vectors could also be possible (O'Donnell et al. 2011; Pildain et al. 2014; Du et al. 2015). Based on these assumptions, it has been suggested that *M. tridentina* could have colonized South America from North America through introduced forest trees (Pildain et al. 2014). However, Loizides et al. (2015, 2016) have questioned these hypotheses to explain the transcontinental distribution of some *Morchella* species. For this reason, studies are necessary before being able to establish with certainty the mechanism than explains the presence of the cosmopolitan *M. tridentina* in southern South America.

Although we could add to a better understanding of diversity and distribution of *Morchella* in temperate and subantarctic South America, there are still gaps to fill. A recent study using analysis of conserved genes demonstrated the existence of *M. tridentina* and *M. andinensis*, but not *M. aysenina*, outside Patagonia in the south-central Chile, associated always with native forests (in the case of *M. andinensis*), but also with plantations of *Eucalyptus* sp. (in the case of *M. tridentina*) (Sandoval 2017). Our results confirm that *M. andinensis* and *M. tridentina* are found in Chilean and Argentinean Patagonia, while *M. aysenina* has hitherto been found only in Chilean Patagonia. These results will hopefully contribute to expanding knowledge of the species of *Morchella* in Chile and in temperate South America.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-021-01703-x.

Acknowledgements We particularly wish to thank the collectors of the Aysén Region of Chilean Patagonia for their valuable collaboration with the location of the morel fruiting sites and information about the growing habit of the fungus in the field. We are also grateful for the collaboration of C. Gómez, F. Silva, and D. Montenegro in the field work and R. Oliva (UdeC) for the SEM images. We appreciate the valuable contributions of the reviewers of the first version of this manuscript.

Author contribution All authors contributed to the study conception and design. AM, GP, MG, PO, DCh, and YG did sample collection, material preparation, and data analysis. AM, MG, CC, and DCh wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

Funding This work was supported by the Research Fund for the Native Forest of the *Corporación Nacional Forestal* (CONAF-Chile) (project FIBN 077/2013).

Data Availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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