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ORIGINAL ARTICLE

South American morels in the Elata group: mitosporic states, distributions, and commentary

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Abstract

The occurrence and distribution of morels in *Nothofagaceae* forests of South America are addressed based on our field studies in Southern Chile and Argentina. Both ascomata and mitosporic colonies were collected. In addition, ascomata were procured from commercial harvesters. A four-gene (ITS, *RPB1*, *RPB2*, *TEF1-α*) and ITS phylogeny assigned these *Morchella* ascomatal and mitosporic collections to four Elata clade lineages, *M. andinensis*, *M. aysenina*, *M. eximia*, and *M. tridentina,* which were each well supported by ML and Bayesian analyses. The placement of our collections of the two lineages unique to South America, *M. andinensis* (previously cited as Mel-37) and *M. aysenina*, expands their known distribution in South America. Most of the field-collected mitosporic colonies in our study belong to the *M. eximia* "fire adapted lineage." This is the first report of *M. eximia*, under this name, in Chile. Since the mitosporic colonies are frequent in the field, these collections help to expand the geographical range of currently described species.

Keywords Argentina · Chile · *Costantinella* · Mitosporic colonies · *Morchella* · *Nothofagaceae* forests

Introduction

Reports of *Morchella* species occurring in South America have historically been scarce and have almost universally applied names of European taxa to specimens. Recently, three publications have expanded the knowledge of the species of *Morchella* occurring in South America and the Caribbean using modern techniques. In the first of these contributions, Pildain et al. (2014) sequenced ascomata from Argentina and demonstrated the presence of *Morchella tridentina* (as *M. frustrata*) and *M. eximia* (as *M. septimelata*) as well as an unnamed clade of apparent South American endemics which they assigned the informal identifier *Mel-37*. Later, Baroni et al. (2018) described three new species from northern South America (Venezuela, Ecuador and Peru) and the Dominican Republic. More recently, two species were described from the *Nothofagaceae* forests of southern Chile (Machuca et al. 2021). One of the species described by Machuca et al. (2021), *M. andinensis*, is applicable to the species identified as *Mel-37* by Pildain et al. (2014). This species, along with *M. aysenina*, belongs in the *Morchella* Elata clade. *Morchella peruviana*, from Peru, and *M. gracilis* from the Dominican Republic, Ecuador, and Venezuela (Baroni et al. 2018) belong to the *Morchella* Esculenta clade and will not be further discussed here.

There have been no previous reports of mitosporic colonies of the morels in South America. These "spore mats" cover plant debris and soil in disturbed areas such as trail verges, road cuts or fire pits and following forest burns. They often produce prodigious numbers of spores and are commonly seen but regularly dismissed. Microscopically, they are composed of erect, arboriform verticillately branched systems, with recurved and crest-like cells which successively produce smooth, hyaline, globose mitospores. The conidiophores often branch forming verticillate arranged tiers. The conidiophores are interspersed with brownish, verrucose setae. Carris et al. (2015) demonstrated that these *Morchella* spore mats occur widely in the northwestern USA and used molecular phylogenetic techniques to connect these mitosporic colonies to described species of *Morchella*.

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Interestingly, Carris et al. (2015) reported that while morel ascomata are frequently found in the spring, they encountered spore mats largely in the autumn. Carris et al. (2015) also summarized the literary history of these mitosporic states, formerly referred to the form genus *Costantinella*.

The *Costantinella* state can be obtained in cultures of *Morchella* from ascospores and ascomatal tissues. The mitospores, however, do not germinate even under a range of laboratory manipulations (Carris et al. 2015) and their function in the life cycle of morels is uncertain. Previously, authors have suggested that the mitospores in *Pezizales* may serve as reproductive propagules or as spermatia (Healy et al. 2013). With the first possible function counter indicated by the failure to germinate, authors such as Du et al. (2017) suggest these spores may serve as spermatia and include the anamorphic state in their diagram of the proposed life cycle of *Morchella*. This function seems to be supported by studies of mating-type idiomorphs in several species of the Elata clade indicating that species studied are heterothallic (Du et al. 2017; Chai et al. 2017), but conclusive evidence is lacking.

Our purpose is to better understand the geographical and vegetational parameters in which morels occur in southern South America. As a starting point, we assembled all records of South American morels published and available in databases. Since modern species identification of morels depends in large part on analysis of DNA sequences (which is lacking in most reports) and the taxonomy of the genus *Morchella* has undergone upheaval in the past decade, the information which can be gleaned from these reports is inherently limited. Still, the data provide insight into the broad occurrence of these fungi across the continent.

To expand on this prior knowledge in a way that supports modern taxonomic techniques, we collected specimens of *Morchella* in Chile and Argentina, expending particular effort in searching for *Morchella* spore mats. The inclusion of spore mats helps to expand the geographical range of currently described species. Additionally, knowledge of spore mats contributes to an understanding of habitats and time of occurrence. An additional purpose of our study was to encourage the sustainable commercial harvest of these fungi in the *Nothofagaceae* forests. It is known that some species of *Morchella* produce ascomata in the years following large fire events, but this is not universally true for all species (Pilz et al. 2004). In an attempt to promote morel growth, some harvesters intentionally burn *Nothofagaceae* forests, a destructive and unsustainable practice we wish to discourage (Pfister 2016; Furci 2013, https://ffungi.org/eng/campaigns/). To begin to determine how important these burn-associated morels are to the Patagonian morel trade, samples were purchased from market collectors over a 2-year period. DNA was extracted from representative specimens from these collections, and ITS, *RPB1*, *RPB2*, and *TEF1-α* were sequenced for inclusion in phylogenetic analyses.

Materials and methods

Specimens

In the course of our field work in Southern Chile and Argentina, we collected *Morchella* ascomata and spore mats and also purchased ascomata in markets. As was reported in the Northern Hemisphere, we found ascomata in the spring but generally collected spore mats in the autumn (Carris et al. 2015).

The 15 South American *Morchella* ascomata included in this study were from several sources (Table 1). The collection numbers of ascomata collected by commercial harvesters in Chile are M1-M7 and G1, G3-G6. Giuliana Furci collected G2. All were harvested in the Nahuelbuta Mountain Range of Chile (exact locations unknown) except for M1 which was collected by a commercial gatherer in Punta Arenas, Chile. DT-CL-2017 was collected by Daniela Torres of the Fungi Foundation in 2017 from a pine plantation (Empedrado, Maule Region, northwest of the Nahuelbuta Mountain Range) where several devastating wildfires occurred the previous year. An additional ascoma was collected by Alija Mujic (AM-AR15-013, CORD) in Argentina in 2015. See Table 1 for the fungaria in which they are deposited.

Mitospore colonies were collected in *Nothofagus* forests of the Patagonian region of Argentina and Chile in April or May of 2015, 2016, and 2017. Cultures were not attempted since these historically have not been successful. Fifteen of the mitospore colonies were identified through morphological examination of field collections and DNA sequence BLAST analyses (Table 2).

Previous reports

Previous records of South American morels were also sought out. Many of these records were reported in scientific publications. The identifications of the reported specimens cannot usually be verified since sequences are lacking and many historically confused names for European species (e.g., *M. esculenta*, *M. conica*, *M. elata*) have been applied in South America. Where specimens could be identified at the species level this information was recorded. In addition to literature reports, herbarium and online reports were also sought using gbif.org, MyCoPortal (2021), and inaturalist.org. Care was taken not to double-count reports. The collections at the Farlow Herbarium (FH) were also manually searched. Online reports lacking specimens or photographic documentation were not considered since verification was impossible. Online reports associated with photographic documentation were reviewed by DHP and JKM on 21 July 2021 and were identified to section by DHP based on gross morphology, where possible.

Table 1 *Morchella* ascomata collected in Argentina and Chile during 2015–2017. GenBank accession numbers are of sequences generated in this study. Unavailable sequence marked with a dash (–)

Morchella collection no. and assigned species	Elata Clade "Mel" Code	Herbarium no.	Country of origin[a]	Collector	GenBank Accession Numbers			
					ITS	*TEF1-α*	*RPB1*	*RPB2*
M1, *M. aysenina*	NA	FH 00941464	Chile	Commercial Harvesters	MT552948	MT614279	MT568981	MT552962
M2, *M. tridentina*	Mel-2	FH 00941465	Chile	Commercial Harvesters	MT552949	MT614280	MT568982	MT552963
M3, *M. andinensis*	Mel-37	FH 00941466	Chile	Commercial Harvesters	MT552950	MT614281	MT568983	MT552964
M4, *M. tridentina*	Mel-2	FH 00941467	Chile	Commercial Harvesters	MT552951	MT614282	MT568984	MT552965
M5, *M. andinensis*	Mel-37	FH 00941468	Chile	Commercial Harvesters	MT552952	MT614283	MT568985	MT552966
M6, *M. tridentina*	Mel-2	FH 00941469	Chile	Commercial Harvesters	MT552953	MT614284	MT568986	MT552967
M7, *M. andinensis*	Mel-37	FH 00941470	Chile	Commercial Harvesters	MT552954	MT614285	MT568987	MT552968
G1, *M. tridentina*	Mel-2	FH 00941471	Chile	Commercial Harvesters	MT552955	MT614286	MT568988	MT552969
G2, *M. andinensis*	Mel-37	FH 00941480	Chile	Giuliana Furci	MT552956	MT614287	MT568989	MT552970
G3, *M. tridentina*	Mel-2	FH 00941472	Chile	Commercial Harvesters	MT552957	MT614288	MT568990	MT552971
G4, *M. tridentina*	Mel-2	FH 00941473	Chile	Commercial Harvesters	MT552958	MT614289	MT568991	MT552972
G5, *M. andinensis*	Mel-37	FH 00941474	Chile	Commercial Harvesters	MT552959	MT614290	MT568992	MT552973
G6, *M. tridentina*	Mel-2	FH 00941475	Chile	Commercial Harvesters	MT552960	MT614291	MT568993	MT552974
DT-CL-2017, *M. eximia*	Mel-7	FH 00941479	Chile	Daniela Torres	MT552961	MT614292	MT568994	MT552975
AM-AR15-013, *M. andinensis*	Mel-37	FLAS-F-63733	Argentina	Alija Mujic	KY462277	–	–	–

[a] M1 was collected by a commercial harvester in Punta Arenas, Chile

M2-M7 and G1-G6 collected in Nahuelbuta Mountain Range (exact locations unknown), Chile

DT-CL-2017 collected from a Pine Plantation, Empedrado, Maule Region, northwest of the Nahuelbuta Mountain Range, Chile

AM-AR15-013 collected from Río Negro, Road to Cerro Catedral, San Carlos de Bariloche, Argentina

Molecular techniques and phylogenetic analyses

DNA extractions of *Morchella* ascomata were performed using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland). Tissue was ground using a BIO 101 Thermo FastPrep FP120 Cell Disrupter (Qbiogene Carlsbad, California). DNA dilutions of either 1/10 or 1/100 were used for polymerase chain reaction (PCR) amplification of (i) the nuclear ribosomal internal transcribed spacer region (ITS) using ITS1F (Gardes and Bruns 1993) or ITS5 as the 5' primer and ITS4 (White et al. 1990) as the 3' primer; (ii) the translation elongation factor1 gene (*TEF1-α*) using primers EF1-df and EF1-1567R (Rehner and Buckley 2005); (iii) the RNA polymerase I gene (*RPB1*) using RPB1AFasc (Hofstetter et al. 2007) and RPB1Cr (Matheny et al. 2002); and the RNA polymerase II gene (*RPB2*) using primers RPB2-7CF (Liu et al. 1999) and RPB2-3053R (Reeb et al. 2004). Hyphae and spores from fresh mitospore colonies were isolated in extraction solution, aseptically crushed with a dissecting needle, and extracted at 95 degrees for 10 min, followed by a dilution of 3% BSA, following the protocol of Vandepol et al. (2020). Amplification was performed for ITS as described for ascomata. PCR amplifications were carried out in a BIO-Rad thermocycler. For PCR, 5 μl of 1/10 and 1/100 dilutions of the DNA extracts were used as templates in a reaction volume of 20 μl. The Bio-Rad Iproof High-Fidelity PCR Master Mix (Hercules, CA #1725310) was used for PCR amplification of the ITS and *TEF1-α* gene regions with thermal cycling parameters as described in Pfister et al. (2020). The *RPB1* and *RPB2* regions were most successful using the high-fidelity platinum Taq by Invitrogen (Waltham, MA #11304011) for PCR amplification. The PCR cycling parameters used for *RPB1* and *RPB2* are described respectively in Mitchell et al. (2021) and Pfister et al. (2020).

The PCR products of all genes were subsequently prepared for PCR purification and Sanger Sequencing using GeneWiz Inc. (Cambridge, MA) sequencing facilities. When PCR reactions resulted in multiple bands, the Qiagen Gel extract kit protocol (Germantown, MD, #28704) was used to purify the desired PCR product to prepare for Sanger sequencing. The forward and reverse sequences from each PCR product were edited using Sequencher 5.1 (GeneCodes, AnnArbor, Michigan). The 72 new DNA sequences are deposited in GenBank and listed in Tables 1 and 2. Alignments are included as Supplementary Files S1 and S2.

The phylogenetic trees presented in this study are based on two data sets both using *Morchella tomentosa* as the outgroup. The first data matrix included DNA sequences of the ITS,

Table 2 *Morchella* anamorphs from South America used in this study

Collection no.	FLAS/Herbarium no.	*Morchella* species	ITS GenBank No.	Country	Habitat	Substrate	Collector
MES-1185	FLAS-F-63157	*M. andinensis*, Mel-37	MT952464	Argentina	*Nothofagus dombeyi*	along bare soil of cut under forest	Rosanne Healy
MES-1225	FLAS-F-63186	*M. eximia*	KY462439	Argentina	*Nothofagus dombeyi*	on charcoal of fire pit	Donald H. Pfister
MES-1253	FLAS-F-63206	*M. eximia*	MT952465	Argentina	*Nothofagus dombeyi*	on burned wood (charcoal) and leaf	Matthew Smith
MES-1261	FLAS-F-63212	*M. eximia*	MT952466	Argentina	*Nothofagus dombeyi*	on charcoal in burn pile	Donald H. Pfister
MES-1286	FLAS-F-63232	*M. andinensis*, Mel-37	MT952467	Argentina	*Nothofagus dombeyi*	on soil along road cut	Rosanne Healy
MES-1329	FLAS-F-63257	*M. andinensis*, Mel-37	MT952468	Argentina	*Nothofagus dombeyi, N. pumilio, N. nervosa*	on soil	Rosanne Healy
MES-1330	FLAS-F-63258	*M. tridentina*	MT952469	Argentina	*Nothofagus dombeyi, N. pumilio, N. nervosa*	on base of Chusquaea	Rosanne Healy
MES-1716	FLAS-F-64494	*M. andinensis*, Mel-37	MT952470	Chile	*Nothofagus pumilio*	on soil in bank	Matthew E. Smith
MES-1849	FLAS-F-64600	*M. tridentina*	KY462590	Argentina	*Nothofagus dombeyi, N. pumilio*	on soil and woody debris at the edge of the trail	Rosanne Healy
MES-1912	FLAS-F-64621	*M. eximia*	MT952471	Argentina	*Nothofagus dombeyi*	in fire pits on charcoal	Rosanne Healy
MES-1914	FLAS-F-64623	*M. eximia*	MT952472	Argentina	*Nothofagus dombeyi*	in fire pits on charcoal	Rosanne Healy
MES-1915	FLAS-F-64624	*M. eximia*	MT952473	Argentina	*Nothofagus dombeyi*	in fire pits on charcoal	Rosanne Healy
MES-2107	FLAS-F-64738	*M. andinensis*, Mel-37	MT952474	Argentina	*Nothofagus antarctica, N. dombeyi*	on soil embankment	Rosanne Healy
MES-2112	FLAS-F-64742	*M. tridentina*	MT952475	Argentina	*Nothofagus antarctica, N. dombeyi*	on dead down leaves and twigs	Rosanne Healy
MES-2752	FLAS-F-65330	*M. aysenina*	MH930233	Chile	*Nothofagus pumilio*	on soil	Matthew E. Smith

RPB1, *RPB2*, and *TEF1-α* genes from 14 *Morchella* ascomata collected in Chile (Table 1) and DNA sequences of 78 *Morchella* taxa from GenBank (Table 3) representing species in the Elata clade of a worldwide distribution (Kuo 2008; O'Donnell et al. 2011; Clowez 2012; Kuo et al. 2012; Elliott et al. 2014; Richard et al. 2015; Taşkın et al. 2016; Baroni et al. 2018; Petrželová and Sochor 2019). Eleven species were identified from South America by Pildain et al. (2014) and Machuca et al. (2021) and three of the taxa were anamorphs of *M. brunnea*, *M. populiphila*, and *M. snyderi* collected and sequenced by Carris et al. (2015). The second dataset included DNA sequences of ITS rDNA for the above-mentioned taxa plus *M. brunnea* M698 and 5 additional taxa identified from South America by Pildain et al. (2014) and Machuca et al. (2021) (*M. eximia* CIEFAP 70 & 80, *M. tridentina* UDEC-1, UDEC-29, CIEFAP 1, Table 3), and the 15 mitosporic taxa plus an additional ascoma (AM-AR15-013) collected from Argentina and Chile (Tables 1 and 2) during 2015–2016.

DNA sequences were aligned using MAFFT through the CIPRES Science Gateway (Miller et al. 2010). Maximum likelihood (ML) and Bayesian analyses were performed through the CIPRES Science Gateway (Miller et al. 2010) for phylogenetic analyses of the ITS and four-gene datasets. Each locus of the four-gene data set was first analyzed separately. There was no significant incongruence in support values at the nodes among the single gene analyses, allowing the single-gene datasets for each taxon to be concatenated. The concatenated four-gene data matrix was analyzed as a single partition. Both phylogenetic methods used GTR+G+I, the most parameter-rich model of sequence evolution (Abadi et al. 2019). This model was also selected by Machuca et al. (2021) for their phylogenetic analyses of *Morchella* species from Chilean *Nothofagaceae* forests. The ML analyses used RAxML-HPC2 on XSEDE (v. 8.2.12) (Stamatakis 2014) using the default parameters, and branch support was determined by 1,000 bootstrap replicates. Bayesian inference used MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001) and consisted of four Markov chain Monte Carlo (MCMC) chains initiated from random trees for 10,000,000 generations and with tree sampling every 1,000 generations. The first 25% of trees were discarded as the burn-in phase in each analysis, and posterior probability (PP) values were determined from the remaining trees. The program Tracer v1.6.0 (http://beast.bio.ed.ac.uk/) determined the point at which a converged Bayesian analysis was achieved.

Table 3 *Morchella* species included in study. *Morchella* strains where species names or "Mel" code not yet assigned listed as NA. Sequences marked with 'Not Used' are available in GenBank but they were not used in the analyses. Unavailable sequence marked with a dash (–)

			GenBank Accession Numbers			
Morchella Species and Strain	Elata Clade "Mel" Code	Country of Origin	ITS	*TEF1-α*	*RPB1*	*RPB2*
M. andinensis UDEC-8	Mel-37	Chile	MN355525	MN611977	MN602597	MN611964
M. andinensis UDEC-10	Mel-37	Chile	MN355520	MN611972	MN602592	MN611959
M. andinensis UDEC-76 Type	Mel-37	Chile	MN355522	MN611974	MN602594	MN611961
M. andinensis UDEC-77	Mel-37	Chile	MN355524	MN611976	MN602596	MN611963
M. andinensis CIEFAP 5	Mel-37	Argentina	KJ439678	KJ569626	KJ569594	KJ569620
M. andinensis CIEFAP 69	Mel-37	Argentina	KJ439677	KJ569629	KJ569595	KJ569623
M. angusticeps M37	Mel-15	USA	GU551412	GU551375	GU551449	GU551489
M. angusticeps M54	Mel-15	USA	GU551431	GU551394	GU551468	GU551513
M. australiana M337	Mel-35	Australia	KC753471	KC753467	KC753474	KC753479
M. australiana M338	Mel-35	Australia	KC753472	KC753468	KC753475	KC753480
M. australiana T35077	Mel-35	Australia	KC753470	KC753466	KC753477	KC753478
M. australiana M371	NA	Australia	KC753473	KC753469	KC753476	KC753481
M. aysenina UDEC-33	NA	Chile	MN355528	MN611980	MN602600	MN611967
M. aysenina UDEC-35	NA	Chile	MN355526	MN611978	MN602598	MN611965
M. aysenina UDEC-78 Type	NA	Chile	MN355527	MN611979	MN602599	MN611966
M. brunnea M36	Mel-22	Canada	GU551422	GU551385	GU551459	GU551500
M. brunnea M431	Mel-22	USA	GU551414	GU551377	GU551451	GU551491
M. brunnea M698	Mel-22	USA	–	GU551563	GU551661	GU551517
M. brunnea 11-29-Dung	Mel-22	USA	KM204687	KM204662	KM204728	KM204755
M. conifericola HT106	Mel-32	Turkey	JN085140	JN085084	JN085200	JN085256
M. conifericola HT479	Mel-32	Turkey	JN085127	JN085071	JN085187	JN085243
M. deliciosa S F27848	Mel-26	Sweden	JN085110	JN085054	JN085170	JN085226
M. deliciosa HT508	Mel-26	Turkey	JN085131	JN085075	JN085191	JN085247
M. dunalii HT436	Mel-25	Turkey	JN085117	JN085061	JN085177	JN085233
M. dunalii HT540	Mel-25	Turkey	JN085137	JN085081	JN085197	JN085253
M. eohespera M16	Mel-19	Netherlands	GU551409	GU551372	GU551446	GU551483
M. eohespera M510	Mel-19	Switzerland	GU551420	GU551383	GU551457	GU551497
M. eohespera M735	Mel-19	China	KT819368	GU551566	GU551664	GU551713
M. eximia M688	Mel-7	USA	JQ723041	GU551016	GU551099	GU551140
M. eximia M833	Mel-7	USA	JQ723042	GU550997	GU551080	GU551121
M. exima CIEFAP 80[a]	Mel-7	Argentina	KJ439665	–	Not Used	–
M. exima CIEFAP 70[a]	Mel-7	Argentina	KJ439667	–	Not Used	–
M. eximioides M231	Mel-16	Sweden	GU551428	GU551391	GU551465	GU551508
M. eximioides M489	Mel-16	Norway	GU551421	GU551384	GU551458	GU551499
M. eximioides M539	Mel-16	Denmark	GU551430.	GU551393	GU551467	GU551512
M. exuberans M103	Mel-9	USA	JQ723046	GU551549	GU551647	GU551696
M. fekeensis HT401	Mel-28	Turkey	JN085114	JN085058	JN085174	JN085230
M. fekeensis HT510	Mel-28	Turkey	JN085133	JN085077	JN085193	JN085249
M. hispaniolensis M374	Mel-18	Dominican Republic	MH014725	GU551554	GU551652	MH014741
M. hispaniolensis M375	Mel-18	Dominican Republic	JQ723059	MH014720	MH014731	MH014742
M. importuna HKAS62870	Mel-10	Germany	JQ321902	JQ321870	JQ321966	JQ321998
M. kaibabensis TAC1708	NA	USA	MH014728	MH014722	MH014733	MH014738
M. magnispora HT470	Mel-29	Turkey	JN085122	JN085066	JN085182	JN085238
M. magnispora HT471	Mel-29	Turkey	JN085123	JN085067	JN085183	JN085239
M. mediterraneensis HT448	Mel-27	Turkey	JN085118	JN085062	JN085178	JN085234
M. mediterraneensis HT520	Mel-27	Turkey	JN085135	JN085079	JN085195	JN085251

Table 3 (continued)

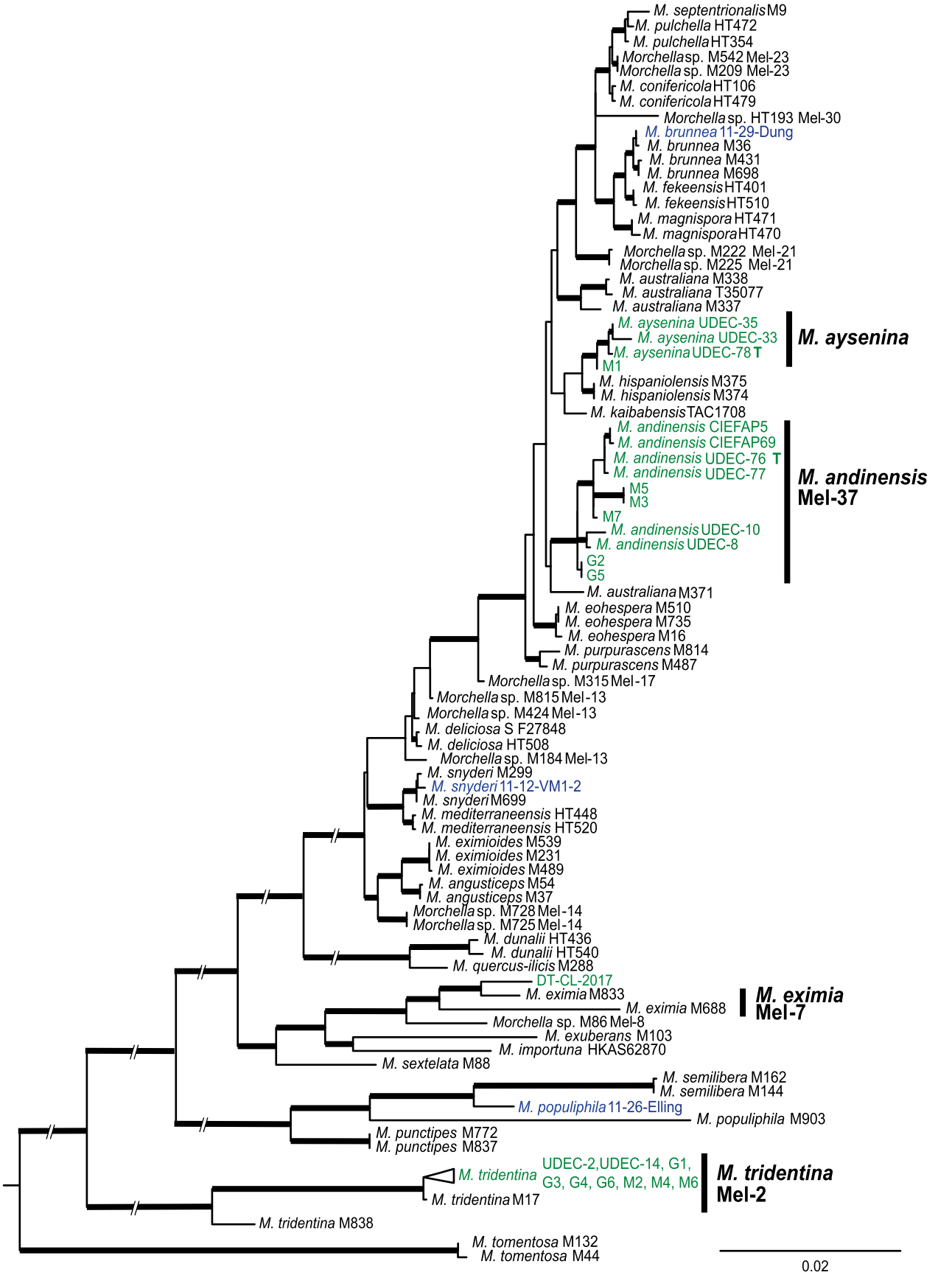
Morchella Species and Strain	Elata Clade "Mel" Code	Country of Origin	GenBank Accession Numbers			
			ITS	*TEF1-α*	*RPB1*	*RPB2*
M. populiphila M903	Mel-5	USA	JQ723033	GU551000	JQ670120	JQ670131
M. populiphila 11-26-Elling	Mel-5	USA	KM204689	KM204666	KM204718	KM204744
M. pulchella HT354	Mel-31	Turkey	JN085112	JN085056	JN085172	JN085228
M. pulchella HT472	Mel-31	Turkey	JN085124	JN085068	JN085184	JN085240
M. punctipes M837	Mel-4	USA	JQ723030	GU551008	GU551091	GU551132
M. punctipes M772	Mel-4	USA	JQ723029	GU550995	GU551078	GU551119
M. purpurascens M487	Mel-20	Norway	GU551419	GU551382	GU551456	GU551496
M. purpurascens M814	Mel-20	China	GU551438	GU551014	GU551097	GU551525
M. quercus-ilicis M288	Mel-11	Netherlands	JQ723065	GU551567	GU551665	JQ723065
M. semilibera M144	Mel-3	USA	JQ723024	GU550993	GU551075	GU551116
M. semilibera M162	Mel-3	Czech Republic	JQ723023	GU551552	GU551650	GU551699
M. septentrionalis M9	Mel-24	USA	JQ723064	GU551556	GU551654	GU551703
M. sextelata M88	Mel-6	USA	JQ723039	GU551546	GU551644	–
M. snyderi M299	Mel-12	USA	GU551413	GU551376	GU551450	GU551490
M. snyderi M699	Mel-12	USA	–	GU551564	GU551662	GU551518
M. snyderi 11-12-VM1-2	Mel-12	USA	KM204768	KM204764	KM204766	KM204767
M. tomentosa M132	Mel-1	USA	JQ723018	GU550994	GU551076	GU551117
M. tomentosa M44	Mel-1	USA	JQ723019	GU551553	GU551651	GU551700
M. tridentina M17	Mel-2	Canada	JQ723020	GU550989	GU551071	GU551112
M. tridentina M838	Mel-2	USA	JQ723021	GU550998	GU551081	GU551122
M. tridentina UDEC-1[a]	Mel-2	Chile	MN355532	–	–	–
M. tridentina UDEC-2	Mel-2	Chile	MN355531	MN611983	MN602603	MN611970
M. tridentina UDEC-14	Mel-2	Chile	MN355529	MN611981	MN602601	MN611968
M. tridentina UDEC-29	Mel-2	Chile	MN355530	Not used	Not used	Not used
M. tridentina CIEFAP 1[a]	Mel-2	Argentina	KJ439695	–	Not used	–
Morchella sp. M86	Mel-8	USA	JQ723045	GU551548	GU551646	GU551695
Morchella sp. M184	Mel-13	India	JQ723053	GU551555	GU551653	GU551485
Morchella sp. M424	Mel-13	India	GU551429	U551392	GU551466	GU551511
Morchella sp. M815	Mel-13	China	GU551439	GU551015	GU551098	GU551526
Morchella sp. M725	Mel-14	China	GU551435	GU551398	GU551472	GU551520
Morchella sp. M728	Mel-14	China	JQ723054	GU551565	GU551663	GU551522
Morchella sp. M209	Mel-23	Finland	GU551427	GU551390	GU551464	GU551506
Morchella sp. M542	Mel-23	Denmark	JQ723063	GU551562	GU551660	GU551514
Morchella sp. M222	Mel-21	Japan	GU551411	GU551374	GU551448	GU551488
Morchella sp. M225	Mel-21	Japan	JQ723062	GU551559	GU551657	GU551507
Morchella sp. HT193	Mel-30	Turkey	JN085141	JN085085	JN085201	JN085257
Morchella sp. M315	Mel-17	China	JQ723057	GU551561	GU551659	GU551510

[a] Taxa not included in four-gene DNA sequence analysis

Results

Phylogenetic analyses of the four-gene dataset including ITS, *RPB1*, *RPB2*, and *TEF1-α* DNA sequences (Fig. 1) determined that the 15 *Morchella* ascomata (Table 1) collected in Argentina and Chile during 2015–2016 all belong to the highly diverse Elata clade (Taşkın et al. 2010; O'Donnell et al.

Fig. 1 Phylogenetic relationships among *Morchella* anamorphs and teleomorphs collected in Chile in the Elata clade. The tree was determined from maximum likelihood (ML) analysis of combined ITS rDNA, *TEF1-α*, *RPB1*, and *RPB2* DNA sequence data. The morel specimens from Chile are highlighted in green. Anamorphs of *M. brunnea*, *M. populiphila*, and *M. snyderi* are highlighted in blue. Branches in bold indicate ML bootstrap support ≥ 75% and Bayesian posterior probabilities ≥ 95%. The tree was rooted with *Morchella tomentosa*


M. septentrionalis M9
M. pulchella HT472
M. pulchella HT354
Morchella sp. M542 Mel-23
Morchella sp. M209 Mel-23
M. conifericola HT106
M. conifericola HT479
Morchella sp. HT193 Mel-30
M. brunnea 11-29-Dung
M. brunnea M36
M. brunnea M431
M. brunnea M698
M. fekeensis HT401
M. fekeensis HT510
M. magnispora HT471
M. magnispora HT470
Morchella sp. M222 Mel-21
Morchella sp. M225 Mel-21
M. australiana M338
M. australiana T35077
M. australiana M337
M. aysenina UDEC-35
M. aysenina UDEC-33
M. aysenina UDEC-78 T
M. aysenina
M1
M. hispaniolensis M375
M. hispaniolensis M374
M. kaibabensis TAC1708
M. andinensis CIEFAP5
M. andinensis CIEFAP69
M. andinensis UDEC-76 T
M. andinensis UDEC-77
M5
M3
M7
M. andinensis UDEC-10
M. andinensis UDEC-8
G2
G5
M. andinensis Mel-37
M. australiana M371
M. eohespera M510
M. eohespera M735
M. eohespera M16
M. purpurascens M814
M. purpurascens M487
Morchella sp. M315 Mel-17
Morchella sp. M815 Mel-13
Morchella sp. M424 Mel-13
M. deliciosa S F27848
M. deliciosa HT508
Morchella sp. M184 Mel-13
M. snyderi M299
M. snyderi 11-12-VM1-2
M. snyderi M699
M. mediterraneensis HT448
M. mediterraneensis HT520
M. eximioides M539
M. eximioides M231
M. eximioides M489
M. angusticeps M54
M. angusticeps M37
Morchella sp. M728 Mel-14
Morchella sp. M725 Mel-14
M. dunalii HT436
M. dunalii HT540
M. quercus-ilicis M288
DT-CL-2017
M. eximia M833
M. eximia M688
M. eximia Mel-7
Morchella sp. M86 Mel-8
M. exuberans M103
M. importuna HKAS62870
M. sextelata M88
M. semilibera M162
M. semilibera M144
M. populiphila 11-26-Elling
M. populiphila M903
M. punctipes M772
M. punctipes M837
M. tridentina UDEC-2,UDEC-14, G1, G3, G4, G6, M2, M4, M6
M. tridentina M17
M. tridentina Mel-2
M. tridentina M838
M. tomentosa M132
M. tomentosa M44
0.02

2011). In the four-gene phylogeny (Fig. 1), these *Morchella* ascomata collections were assigned to four phylospecies, *M. tridentina*, *M. eximia*, *M. andinensis*, and *M. aysenina*, each well supported by ML and Bayesian analyses. Single-gene analysis of ITS, *RPB1*, *RPB2*, and *TEF1-α* DNA sequence data from the ascomata collected in Chile (Table 1) showed 99 to 100% sequence similarity for each single-gene analysis. There is some variation within the *M. andinensis* clade; up to 7% *RPB1* DNA sequence variation among isolates in this clade was observed.

The ITS phylogeny (Fig. 2) included ITS sequences from 15 *Morchella* mitospore colonies (Table 2) collected from Argentina and Chile. They were found to be phylogenetically related to the four previously mentioned lineages: i) the *M. tridentina* "Mountain blond Morel" lineage which includes the M2, M4, M6, G1, G3, G4, G6, and 3 mitospore collections (all collected on unburned soil or plant matter); ii) the *M. eximia,* "Fire adapted lineage" which includes DT-CL-2017 (collected from a post-burn pine forest) and six mitosporic isolates (all collected in burn pits); iii) the *M. andinensis* /Mel-37 lineage, originally recognized as a distinct, unnamed species by Pildain et al. (2014) and recently described by Machuca et al. (2021), includes M3, M5, M7, G2, G5, AM-AR15-013 and 5 mitospore colonies (all collected on unburned soil); and iv) the fourth and new lineage described by Machuca et al. (2021) as *M. aysenina* includes M1 and the mitospore colony collection MES-2752 (collected on unburned soil). The sequence variation observed among the mitospore collections and respective ascomata within each of the four phylospecies was less than 1%.

Our literature and on-line searches yielded over 140 reports of *Morchella* species from South America.

Comments on mitosporic features

Morchella species form *Costantinella*-type anamorphs (Matruchot 1892) which differ little among the species studied in South America. All form superficial patches on soil and debris. Initially, they may be white but soon become pale tan or light yellowish tan. The superficial appressed hyphae on the substrate give rise to upright conidiophores and setae (Fig. 3). Conidiophores may also arise from the base of the setae. The setae tend to be pale brown and are to a greater or lesser degree verruculose and can reach a length of almost a millimeter. Conidiophores are differentiated from the vegetative hyphae and are lightly colored and smooth. Conidiophores produce 3–5 verticillate branches of conidiogenous cells. The whorls are closely or distantly spaced, and each whorl consists of 3–5 lageniform or subcylindrical cells. Spores are produced successively forming denticles on one side of the curved crest of the lengthening fertile region of the conidiogenous cell. Spores are globose, 1-celled, hyaline, and smooth. Spores are copiously produced and when dry sometimes can be seen billowing from disturbed colonial mats. See Carris et al. (2015) for detailed descriptions of several species.

Discussion

Our collections and analyses supplement and broaden the knowledge and distribution of morels in South America. Based on four-gene and ITS phylogenies, the specimens collected in this study were assigned to Elata clade lineages that were first identified as occurring in South America by Pildain et al. (2014) and Machuca et al. (2021) (Figs. 1 and 2). Two of these lineages, *M. andinensis*/Mel-37 and *M. aysenina*, are unique to South America (Pildain et al. 2014; Machuca et al. 2021).

Morchella tridentina represents one of the most widely collected morel species in southern South America as reported by Pildain et al. (2014) for Argentina, and Machuca et al. (2021) and this study for Chile (Figs. 1 and 2). It was also found frequently in the studies of Taşkın et al. (2012) for Turkey. Loizides (2017) highlight that *M. tridentina* is geographically wide ranging and is found mostly in undisturbed areas, and unlike *M. rufobrunnea,* which is ruderal, *M. tridentina* has been collected with native vegetation and thus may not be introduced as Pildain et al. (2014) suggested.

The majority of the mitosporic colonies produced by a single species in our study belong to the *M. eximia* lineage (Fig. 2). All of these were collected from fire pits in the fall. The *M. eximia* clade also included Argentine collections from Pildain et al. (2014) reported as *M. septimelata*. Machuca et al. (2021) did not report *M. eximia* from among their *Morchella* collections from *Nothofagaceae* forests in Chile.

Our collections also represent two lineages unique to South America, *M. andinensis*/Mel-37 and *M. aysenina.* Analysis of ITS rDNA sequence data (Fig. 2) expanded the *M. aysenina* clade of Machuca et al. (2021) to include a new *Morchella* ascomatal collection, M1, and mitosporic mat (MES-2752) (Tables 1 and 2). Five of our mitosporic collections are represented in the *M. andinensis*/Mel-37 lineage along with six ascomatal collections thus expanding the geographic distribution of this species as well. An additional mitosporic mat collection (MES-3944) was recently made in Chile during a 2022

Fig. 2 Phylogenetic relationships of *Morchella* anamorphic taxa collected in Chile and Argentina to *Morchella* species in the Elata clade. The tree was determined from maximum likelihood (ML) analysis of ITS rDNA sequence data. *Morchella* specimens from Chile or Argentina are highlighted in green. The morel anamorphs are highlighted in blue. Branches in bold indicate ML bootstrap support ≥ 75% and/or Bayesian posterior probabilities ≥ 95%. The tree was rooted with *Morchella tomentosa*

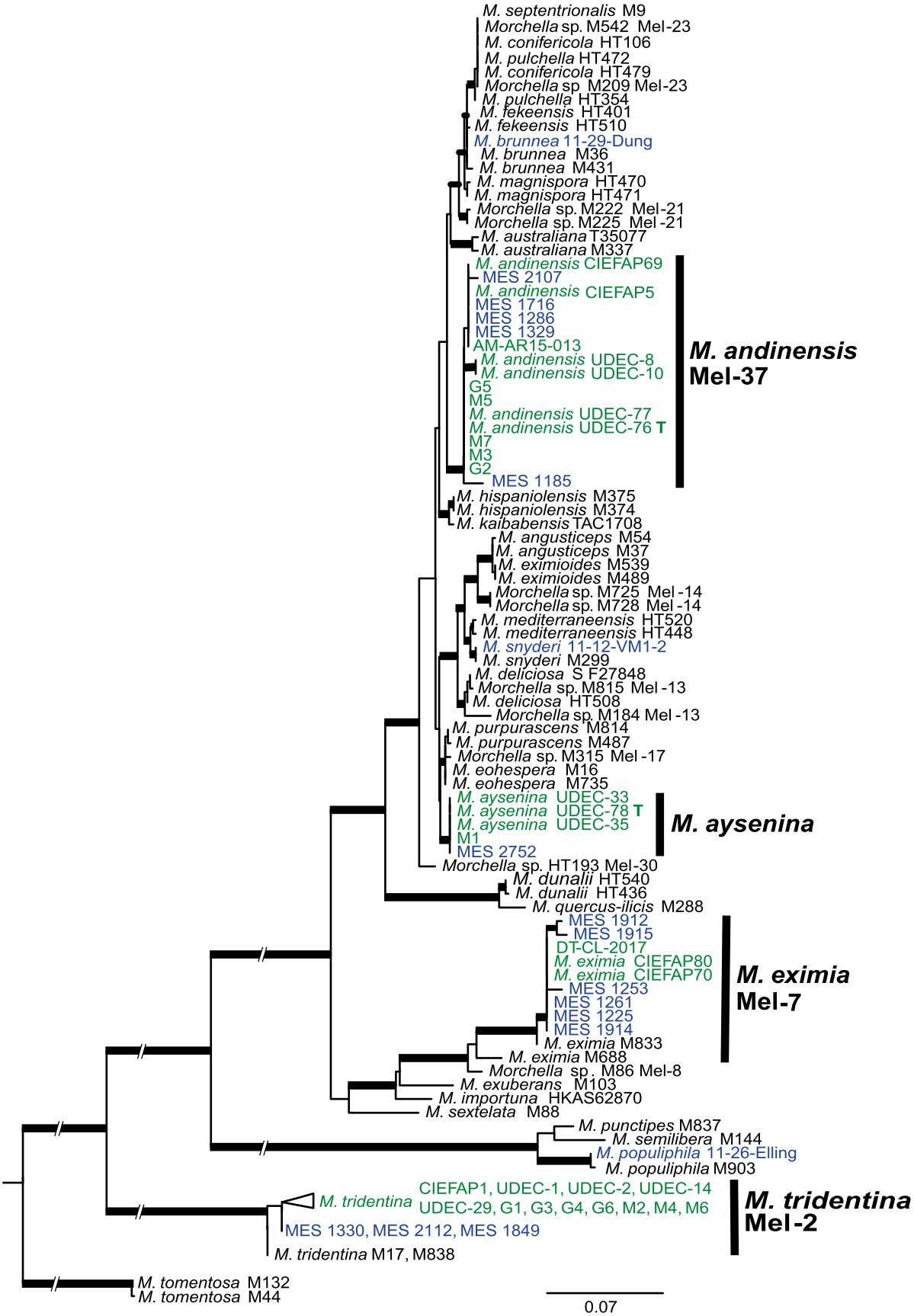

M. septentrionalis M9
Morchella sp. M542 Mel-23
M. conifericola HT106
M. pulchella HT472
M. conifericola HT479
Morchella sp. M209 Mel-23
M. pulchella HT354
M. fekeensis HT401
M. fekeensis HT510
M. brunnea 11-29-Dung
M. brunnea M36
M. brunnea M431
M. magnispora HT470
M. magnispora HT471
Morchella sp. M222 Mel-21
Morchella sp. M225 Mel-21
M. australiana T35077
M. australiana M337
M. andinensis CIEFAP69
MES 2107
M. andinensis CIEFAP5
MES 1716
MES 1286
MES 1329
AM-AR15-013
M. andinensis UDEC-8
M. andinensis UDEC-10
G5
M5
M. andinensis UDEC-77
M. andinensis UDEC-76 T
M7
M3
G2
MES 1185
M. andinensis Mel-37
M. hispaniolensis M375
M. hispaniolensis M374
M. kaibabensis TAC1708
M. angusticeps M54
M. angusticeps M37
M. eximioides M539
M. eximioides M489
Morchella sp. M725 Mel-14
Morchella sp. M728 Mel-14
M. mediterraneensis HT520
M. mediterraneensis HT448
M. snyderi 11-12-VM1-2
M. snyderi M299
M. deliciosa S F27848
Morchella sp. M815 Mel-13
M. deliciosa HT508
Morchella sp. M184 Mel-13
M. purpurascens M814
M. purpurascens M487
Morchella sp. M315 Mel-17
M. eohespera M16
M. eohespera M735
M. aysenina UDEC-33
M. aysenina UDEC-78 T
M. aysenina UDEC-35
M1
MES 2752
M. aysenina
Morchella sp. HT193 Mel-30
M. dunalii HT540
M. dunalii HT436
M. quercus-ilicis M288
MES 1912
MES 1915
DT-CL-2017
M. eximia CIEFAP80
M. eximia CIEFAP70
MES 1253
MES 1261
MES 1225
MES 1914
M. eximia Mel-7
M. eximia M833
M. eximia M688
Morchella sp. M86 Mel-8
M. exuberans M103
M. importuna HKAS62870
M. sextelata M88
M. punctipes M837
M. semilibera M144
M. populiphila 11-26-Elling
M. populiphila M903
M. tridentina
CIEFAP1, UDEC-1, UDEC-2, UDEC-14
UDEC-29, G1, G3, G4, G6, M2, M4, M6
MES 1330, MES 2112, MES 1849
M. tridentina M17, M838
M. tridentina Mel-2
M. tomentosa M132
M. tomentosa M44
0.07

Fig. 3 *Morchella andinensis* (a–g). **a**, **b** ascomata (MES-3585, bar=2cm), **c** mitosporic mat (MES-2107, bar=1cm), **d** mitosporic mat (MES-1716, bar=5mm), **e–f** conidiophores with mitospores (MES-1716, bar = 20μm), **g** seta from mitosporic mat (MES-1716, bar = 20μm). *Morchella aysenina* (h-k). **h** dried ascomata (M1, bar = 3 cm), **i** mitosporic mat (MES-2752, bar=5mm), **j–k** conidiophores with mitospores (MES-2752, bar = 20μm)

collecting trip. An incomplete sequence of this collection was not included in our analysis but it appears to be part of the *M. andinensis* clade based on initial ML analysis. All of these were collected from soil or vegetation near or on soil in the fall.

The incorporation of field collections of the *Costantinella* mitosporic mats from southern South America builds on the work of Healy et al. (2013) and Carris et al. (2015). Carris et al. (2015) specifically correlated mitospore colonies and ascomata of *Morchella* species from collections in the Pacific Northwestern USA. The mitospore mats collected in this study were morphologically similar to those reported by Carris et al. (2015); these all belong to the Elata clade of *Morchella* (Richard et al. 2015). The distinctive verrucose setae observed among the conidiophores by Carris et al. (2015) were also found in our specimens. It remains to be seen if mitospore colonies in the Esculenta clade also produce setae. The mature length of sporogenous tips of the four *Morchella* species studied here are relatively short as compared to the *Gyromitra* and *Disciotis* anamorphs reported in the Carris et al. (2015) study, and most are recurved, but there were examples in *M. aysenina and M. andinensis* where some of the conidiogenous cells remained straight at maturity.

Judging from our specimens collected from nature and those described from culture by Carris et al. (2015), there are few characters that can be used to distinguish among the species based on the mitosporic state. In culture the colonies are fawn color, and the hyphae are pale to medium brown. This corresponds to the appearance of the field collected specimens. Setae attain a length of up to 70 μm with a great deal of variation. The setae are pigmented and verrucose below and tend to be smooth and hyaline near the apex. Similar variations are seen in conidiophore length and pigmentation. There are some structural differences in the arrangement and number of verticels (from 3 to 6 per locus), the amount of secondary branching, and the degree of crowding along the length of the

conidiophore. These characters may all be malleable and affected by the conditions under which they develop. We did not attempt to culture our collections. The uniformity of morphology among the species studied so far suggests that identification will require DNA sequence acquisition.

Several *Morchella* species have been previously reported in South America. Patouillard and Lagerheim (1891) list *M. conica* (though unverified, this is part of the Elata clade) from Ecuador and state that their report is the first report of the species from South America. Gamundí (1975) described a single *Morchella* collection as *M. elata* from Port Harberton in Argentine Tierra del Fuego. It is dark brown, almost black, similar in color to those in Europe. She noted the variations in ascospore size and shape of the pileus, stating that her collection agrees with the description given by Rifai (1968) for material from Australia. She pointed out that Rifai considered *Morchella* species in general to represent "aggregate species" as has been borne out in molecular phylogenetic studies. Gamundí's collection has somewhat swollen but not capitate acroparaphyses. At about 54.87820°S, 67.32861°W, this specimen from Tierra del Fuego represents the southernmost occurrence of a species of *Morchella* in the world. An unverified record of *M. semilibera* from the Falkland Islands is discussed by Pegler et al. (1980), but at a lower latitude. The species identification for both remains problematic; as members of the highly diverse Elata clade, sequence data is needed to give precise identifications. There are a number of additional unverified internet records of *Morchella* species (mostly *M. conica* and *M. elata*) in South America. These reports are largely without voucher specimens. Ultimately, DNA sequencing will determine whether additional species are present, as the names applied cannot be easily mapped onto modern concepts.

One particularly enigmatic species name, *Morchella patagonica* Spegazzini (1909), was based on material from Puerto Blest, Nahuel Huapi, Argentina, collected in December 1904. Of note in the original description are Spegazzini's (1909) comments that the asci become blue in iodine, the ascospores have two small guttules and the hymenium lacks paraphyses. These characters are anomalous in the genus *Morchella*. Spegazzini (1912) collected the species once again from Las Juntas, Catamarca, Argentina, in April 1910. For this specimen, he again noted the bluing of the asci in iodine, but this time described the spores as eguttulate. Gamundí (1964) reexamined the type specimen held at LPS in Spegazzini's herbarium. While she did note the guttules in the spores and the apparent lack of paraphyses, her examination did not reveal bluing in the hymenium, only the development of a reddish-purple shade. Unusually, she also describes that the ascospores have pointed ends. Both of Spegazzini's specimens were apparently examined by Dominguez de Toledo (1987), but with very few morphological observations given; she does not, however, mention the asci bluing in iodine. There also seem to be no reliable modern collections, with only the type collection being listed by Gamundí et al. (2004). Given the inconsistency in the descriptions and that the original material is not available for loan from LPS, we feel this name is better treated as a *nomen ambiguum*. We note, however, that we have an anamorph collection of *M. andinensis*/Mel-37 (MES-1185) from Puerto Blest, the locality indicated by Spegazzini (1909) for *M. patagonica*. This provides circumstantial evidence that *M. andinensis* might be a later name for *M. patagonica*, but without critical study the question remains unresolved.

The name *Morchella intermedia* Boud. was first applied in South America by Gamundí and Horak (1995). Their illustrations make it clear this name was used for a member (or members) of the Elata clade. This is broadly in keeping with Boudier's concept of the species, which was based on an illustration of *M. conica* by Krombholz. This name has been used by some other authors more recently (Gamundí et al. 2004; Wright and Albertó 2006; Molares et al. 2020), but given that it has not appeared even in the recent taxonomic literature on European morels, it should likely be replaced in the South American Funga with one or more of the Elata clade morels that were detected in this and other molecular studies.

Another set of intriguing reports of South American morels is a series of reports of apparent members of the Esculenta clade from Chile, northern Argentina, and southern Brazil. Not much can be made of a 1929 report from Chile (Espinosa Bustos 1929), but more recent reports give better evidence that the Esculenta clade members are present in southern South America. Dominguez de Toledo (1987) described collections she referred to *M. esculenta* from around the Villa Allende and Villa Warcalde, near Córdoba, Argentina. Two ascocarps were from grasses around fruit trees and two others were on soil near a grove of cultivated and native trees; all are preserved in CORD. Dominguez de Toledo (1987) thought her collections were closest to *M. patagonica*, the type specimen of which she had studied. She pointed out, however, that the anomalous characters of *M. patagonica* reported by Spegazzini (1909)—guttulate ascospore and the lack of paraphyses—were absent in her collections. Dominguez de Toledo (1987) also provides a key which includes *M. conica* (from Río Negro and Chubut), *M. elata* (from Neuquén and Tierra del Fuego), *M. esculenta* (from Córdoba), and *M. patagonica* (from Catamarca and Río Negro). Mahú (1987) reported an immature collection he referred to *M. vulgaris* from the coast near Santiago, Chile. The ascoma was collected among herbaceous plants near the edge of native scrub, Mahú provided illustrations and some description. Spegazzini (1918) had also reported *M. conica* from Chile which he found to be no different than those from Argentina. Nozari Susin and de Souza Campos (1995) reported three ascomata they referred to *M. esculenta* from Rio Grande do Sul, Brazil, from a residential garden, and provided a description; the specimens are preserved in

HURG. Cortez et al. (2004) also reported a collection of *M. esculenta* from Rio Grande do Sul, Brazil (preserved in SMDB), this collection in a forest among mosses under an orange tree; they provide illustrations and a description. Sobestiansky (2005) reported a collection of *M. esculenta* from the same state from mixed soil. The specimen is preserved at MBM. *Morchella esculenta* is also reported from Argentina by Daniele and Becerra (2013) and Dominguez et al. (2021). Their collections come from Tucumán, where they were collected under alders. These collections are preserved in CORD. Vignale et al. (2022) provide a description and several specimens from the Province of Misiones in the Paranaense Forest ecoregion of the Atlantic Forest. They provide morphological comparisons with the species previously reported in southern South America. Furci (2013) also reports *M. esculenta* from central Chile.

Molares et al. (2020) studied the wild edible fungi as used and understood by the Mapuche people of Patagonia. They observe that *M. tridentina*, *M. eximia* (as *M. septimelata*), and an unidentified morel (presumably *M. andinensis*) have great cultural value. These three species had the highest relative frequency of mention and were among the main market species along with *Suillus luteus* and *Cyttaria espinosae*. The morels were among the fungi favored for consumption within the community and commercial harvest and are a relatively new seasonal but significant source of income (Valenzuela-Flores 2003). In an earlier study in Peru (Franquemont et al. 1990), three species of *Morchella*, *M. deliciosa*, *M. elata*, and *M. esculenta* were reportedly consumed by the Chinchero community in Andean southern Peru.

Our data indicate that the two species most frequently found in the marketplace were *M. tridentina* and *M. andinensis*, which do not occur with burns. This agrees with the findings of Gerding et al. (2021). The fire adapted species *Morchella eximia* was not found in our market samples at all; the one ascomatal collection of *M. eximia* we include was from a pine plantation after a fire. Intentionally, set fires have been used to promote *Morchella* fruiting (Pfister 2016; Furci 2013, https://ffungi.org/eng/campaigns/). That our samples did not contain the fire induced species may be a positive indication that intentional burning of *Nothofagus* forests used to trigger harvesting of morels is futile, given the non-fire dependent nature of the native morels found in Chile and Argentina. It may also indicate that the sustainable harvest of *M. andinensis* and *M. aysenina* is sufficient for the Argentine and Chilean morel trade, since supplementation of harvest by burning forests is not supported by our results. Guidelines for sustainable and responsible collecting of morels are given by de Michelis and Rajchenberg (2006) and Pavez Andrades and Machuca (2021).

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Author contribution Donald H. Pfister conceived and organized the project and wrote the paper; Rosanne Healy did field collecting, photography, reviewed the manuscript, and performed sequencing and analysis; Kathrine LoBuglio prepared sequences, performed phylogenetic analyses, and reviewed the manuscript; Giuliana Furci provided field collections and assistance in the field logistics, and reviewed the manuscript; James Mitchell did literature searches and reviewed the manuscript; Matthew Smith provided funding for field work, collected samples, and reviewed the manuscript.

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Data availability All sequences are available on GenBank.

Declarations

Ethics approval and consent Not applicable

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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