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Phylogenetic diversity of true morels (*Morchella*), the main edible non-timber product from native Patagonian forests of Argentina

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ABSTRACT

Morchella species are edible fungi in high demand and therefore command high prices in world markets. Phenotypic-based identification at the species-level remains inadequate because of their complex life cycles, minor differences and plasticity of morphological characteristics between species, and the lack of agreement between scientific and common names. In Patagonia–Argentina, morels are associated with native forests of *Austrocedrus chilensis* (Cordilleran or Chilean cypress) and *Nothofagus antarctica* (ñire) and several exotic conifers that were introduced from western North America. Little is known about their taxonomy and phylogenetic relationships with other species in the genus. This work focused on the identification of collections of *Morchella* from Patagonia and their phylogenetic relationships with other species from the Northern Hemisphere. The comparison was made by analysis of DNA sequences obtained from four loci: the nuclear ribosomal internal transcribed spacer region (ITS) and the partial RNA polymerase I gene (*RPB1*) for the complete collection; and ITS, *RPB1*, RNA polymerase II gene (*RPB2*), and translation elongation factor (*EF1-α*) for the species-rich Elata Subclade. Analyses of individual and combined data sets revealed that Patagonian morels belong to the Elata Clade and comprised three strongly supported species-level lineages from both Patagonian native forest, and exotic trees introduced from western North America. One lineage was identified as *Morchella frustrata* phylogenetic species *Mel-2*, which is known from the USA and Canada. The second lineage, which appeared to be 'fire-adapted', was identified as *Morchella septimelata* phylogenetic species (*Mel-7*), which is also known from the USA. This species was collected from burned native forests mainly composed of *A. chilensis* and *N. antarctica* but also *Pseudotsuga menziesii* (Mirb.) Blanco, which is native to western North America. The phylogenetic analyses suggested that the third species from Patagonia was nested within the species-rich Elata Subclade and represents a new species-level lineage (informally designated *Mel-37*) within Elata Clade. The present collections from Patagonia constitute the southernmost latitude from which *Morchella* has been reported to date. The identification of two Argentine morels as North American taxa is therefore a remarkable biogeographic pattern. In view of the hypothesis that the Elata Clade originated in western North America, we speculate that at

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least two of the lineages colonized South America from North America *via* long distance dispersal, migration or, more likely, they were introduced with the exotic tree species that they were collected near.

Introduction

Little is known about wild edible mushrooms from native Patagonian forests. However, their novelty in the market offers great potential as a profitable non-timber forest product (De Michelis & Rajchenberg 2002). True morels (*Morchella* spp., Morchellaceae, Ascomycota) are recognized as one of the most economically important wild edible mushroom worldwide, given their demand as a gourmet product (Boa 2004).

Fructification of morels in Patagonia occurs during a few weeks every spring (late September–late November) mainly in *Austrocedrus chilensis* (Don) Flor. et Boutl. and *Nothofagus antarctica* (Forts f.) Oersted mixed native forests (de Michellis & Rajchenberg 2002). As in the Northern Hemisphere, prolific fructification after wildfires has been reported (Pilz *et al.* 2007; Greene *et al.* 2010; Keefer *et al.* 2010).

Morchella is a monophyletic and well defined genus within the Morchellaceae. It possesses a complex life cycle and is characterized by minor phenotypic differences and morphological plasticity between species, including variation in stalk and head colour, shape and texture, along with differences in ecological conditions for fructification (Pilz *et al.* 2007). Because of this complexity, combined with a lack of agreement on scientific and common names, morphological differentiation of species is problematic (Pilz *et al.* 2007; Kuo *et al.* 2012). DNA-based molecular techniques have been extensively used to infer relationships within *Morchella*. The techniques have included restriction fragment length polymorphisms (RFLPs) (Bunyard *et al.* 1994), amplified fragment length polymorphisms (AFLPs) (Pagliaccia *et al.* 2011), and multilocus sequence typing from the combined use of sequences from the internal transcribed spacer regions (ITS), RNA polymerase I (*RPB1*), RNA polymerase II (*RPB2*), translation elongation factor (*EF1-α*), and partial LSU rDNA gene sequences (Taşkin *et al.* 2010; Du *et al.* 2012). Even though the ITS rDNA region has been used in several studies for assessing *Morchella* genetic diversity (Wipf *et al.* 1996; Stefani *et al.* 2010; Pagliaccia *et al.* 2011), its value for identifying phylogenetic species previously inferred from multilocus DNA sequence data was only recently confirmed (Du *et al.* 2012).

Molecular systematic studies of *Morchella* identified the Esculenta clade (yellow morels) and Elata Clade (black morels) as monophyletic sister groups, with *Morchella rufobrunnea* as a basal monotypic sister clade. Among the Esculenta and Elata Clades, species reconstruction has been recently based on the phylogenetic species concept based on multilocus molecular analyses (Taşkin *et al.* 2010; O'Donnell *et al.* 2011; Du *et al.* 2012). At least 34 phylogenetic species have been recognized in the Northern Hemisphere, characterized by a high degree of continental endemism within the genus (O'Donnell *et al.* 2011; Du *et al.* 2012). Recently, Kuo *et al.* (2012) performed a taxonomic revision of *Morchella* from Canada and the United States and described 14 of the 19 phylogenetic species from that region, based on morphology, ecology and distribution. Unfortunately, in their phylogenetic analyses, only a few collections from South America were analysed. In Patagonia, southern South America, the taxonomy of *Morchella* is limited to the recognition of five morphological species, and virtually nothing is known about their geographical distribution or phylogenetic relationships with other species in the genus. Species described from southern South America include *Morchella patagonica* Speg., restricted to that region, and the European taxa *Morchella elata* Fr., *Morchella conica* Pers., *Morchella intermedia* Boud., and *Morchella esculenta* (L.) Pers. (Spegazzini 1909; Dominguez *et al.* 1987; Gamundi 1975, 2010). Given recent advances in understanding phylogenetic and biogeographic relationships within *Morchella* (O'Donnell *et al.* 2011; Du *et al.* 2012), the inclusion of molecular information about the unexplored Southern Hemisphere such as the Patagonian forest biome offers the opportunity to increase the knowledge of genetic diversity, evolution and global biodiversity patterns of this group of fungi.

Thus, the aim of the present study was to infer phylogenetic relationships of morels from the continental region of Patagonia–Argentina with species from other regions of the world, using DNA sequences from the nuclear ribosomal ITS and a portion of the *RPB1 gene*, as well as *RPB2* gene and *EF-1α*.

Material and methods

Fungal material

A total of 65 ascocarps of *Morchella* spp. collected and GPS referenced during the spring of 2010 and 2011 in the Patagonian provinces of Chubut, Río Negro and Neuquén (Argentina) were included in a preliminary species diversity analysis (Pildain *et al.* 2013). Based on this screening, 34 *Morchella* collections were chosen for the phylogenetic study. Details of the *Morchella* collections used in this study are listed in Supplementary Table S1. Each collection site was characterized by the surrounding dominating tree species and any signs of recent fire. Ascocarps were air-dried for subsequent analysis, and all collections were deposited in the Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP, Esquel, Argentina) herbarium.

Morphological studies

Morphology, colour, size, shape, and any significant features of the head and stalk were recorded. Pit and rib alignment and shape were also described and photographed, and shape

and size of ascospores, asci, and sterile elements were recorded.

DNA extraction and PCR protocol

Herbarium specimens were cleaned with a toothbrush and examined under a dissecting microscope before extraction of DNA. Approximately 10 mg of dried pileus tissue was ground using a micropestle in a 1.5-ml microcentrifuge tube, resuspended in 300 μL bead solution (UltraClean™ Microbial DNA Isolation Kit, MO BIO laboratories, Solana Beach, California) and incubated at 70 °C for 10 min, then following the manufacturer's instructions. When culture isolates were available (Supplementary Table S1), mycelia were grown in 2 mL medium consisting of malt peptone broth with 10 % (v/v) of malt extract (Merck) and 0.1 % (w/v) Bacto peptone (Difco), in 15 mL glass tubes. The cultures were incubated at 25 °C for 10 d in darkness. Total DNA was extracted as above and stored at −20 °C.

PCR amplification and sequencing of the isolates from Patagonia were performed for the two loci most often used in *Morchella* phylogenetics, the nuclear ribosomal ITS, and the partial *RPB1* gene; while for nine isolates within the species-rich Elata Subclade, *RPB2* and *EF1-α* gene sequences were generated to ascertain their identity. The ITS rDNA region was amplified using primers ITS1 and ITS4 (White *et al.* 1990), the *RPB1* fragment was amplified by the primers RPB-1A and RPB-1C (Stiller & Hall 1997), the *RPB2* fragment with the primers RPB2-7cf and RPB2-3053r (Liu and Hall, 1999; Reeb *et al.* 2004), and *EF1-α* was amplified using the primers EF-526F, EF-1567R, EF-2F, and EF-2218R (Rehner & Buckley 2005). The PCR reaction mix for all PCR reactions contained dNTPs (0.25 mM of each); 2.5 mM $MgCl_2$; 1× PCR buffer supplied with the Taq-polymerase enzyme; 0.1 μM of each primer; 3 μl of 50–100 ng μl^{-1} DNA was used as a template; and 1.25 U of GoTaq-polymerase (Promega, Madison, WI, USA). The final reaction volume was 50 μL. PCR reactions were performed in a thermal cycler (My Cycler™, BioRad) and the amplification program was according to Taşkin *et al.* (2010), with an initial denaturation step at 94 °C for 90 s, followed by 40 cycles of a denaturation step at 94 °C for 30 s, a primer annealing step at 55 °C for 30 s and an elongation step at 68 °C for 3 min, and a final elongation step at 68 °C for 5 min. PCR products were separated on a 1 % (w/v) agarose gel stained with GelRed™ Nucleic Acid Stain (Biotium Inc., Hayward, USA) and the bands were visualized under UV illumination. The amplified fragments were purified and sequenced on an ABI 3700 automated sequencer (Perkin–Elmer, Foster City, CA) at Macrogen Inc. (Seoul, South Korea). The resulting DNA sequences were trimmed to remove primer binding sites using Geneious® 6.1.5 software (Biomatters Ltd) and homology searches of NCBI (National Centre for Biotechnology Information – Bethesda, MD) were conducted using BLASTN (Altschul *et al.* 1997). Sequences generated in this study were submitted to GenBank and their accession numbers are presented in Supplementary Table S1.

Sequence alignment and phylogenetic analyses

Sequences generated in this study were manually edited using BioEdit 7.0.9.0 (Hall 1999). Sequences that were retrieved from the GenBank nucleotide database are presented in Supplementary Table S2. Sequence alignments were performed automatically using MAFFT (Katoh & Standley 2013) and are available from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S14401). Phylogenetic analyses were based on the terminal region of ITS1 section (154 bp), the complete 5.8S (155 bp), and the start and terminal regions of the ITS2 section (256 bp). Regions of sequence ambiguity within the alignment were identified by GBLOCK version 0.91b (Castresana 2000), and removed from subsequent phylogenetic analyses. The two concatenated data sets (ITS – *RPB1* and ITS – *RPB1*–*RPB2*–*EF1-α*) were assessed for congruence using the Partition homogeneity test in PAUP* 4.0b10 (Swofford 2002). Analyses were conducted *via* maximum parsimony (MP) in PAUP* 4.0b10 and Bayesian inference (BI) in MrBayes v.3.0B4 (Ronquist & Huelsenbeck 2003). Maximum Parsimony analysis of the combined data set was performed, gaps were alternately classified as a fifth state or as missing data, there was equal weighting of characters and transformations, with heuristic searches using random addition of sequences (1000 replicates), TBR (tree bisection reconnection) branch swapping, and MAXTREES was set to auto-increase. A bootstrap analysis was performed with 1000 pseudoreplicates with random addition of sequences. For Bayesian analyses, the nucleotide substitution models were selected statistically with the help of jModelTest (Posada 2008; program available at http://darwin.uvigo.es). Models were selected *via* the AIC criterion (AIC; Akaike 1974). The following models were used: (1) for ITS – *RPB1*, GTR + I + G, and TrN + I + G, respectively; (2) for ITS – *RPB1*–*RPB2*–*EF1-α*, the models selected were TrNeF + G, TrN + I, TrNEF + G, and TrN + I + G, respectively. The B/MCMC analyses were conducted with 10 000 000 generations starting with a random tree and employing four simultaneous chains. The first 100 000 generations (i.e., the first 1000 trees) were discarded as burn-in for the chain. TRACER1 (http://evolve.zoo.ox.ac.uk/software.html/tracer/) was used to ensure that stationarity was achieved after the first 100 000 generations. Trees inferred from the ITS–*RPB1* data set were rooted with sequences of *M. rufobrunnea* Guzmán & F. Tapia (ITS: DQ355922; *RPB1*: GU551666) and *M. esculenta* (ITS: AJ543737; *RPB1*: DQ471117), and the tree inferred from the concatenated data set for the species-rich Elata Subclade were rooted with a sequence of *Morchella* sp. *Mel-11* collection M288 (Supplementary Table S2).

Results

The phylogenetic analysis of *Morchella* that included collections from three different provinces of Patagonia–Argentina is presented in Fig 1. This analysis has provided the first estimation of phylogenetic relationship of morels from southern South America. Amplification of the ITS rRNA region with ITS1 and ITS4 primers resulted in amplicons of different size ranging from 700 bp to 950 bp corresponding to black morels (Elata Clade) according to Du *et al.* (2012). This was also confirmed using BLASTN, which revealed that the sequences of Argentinean morels belonged to the Elata Clade. MP analyses of the full data set from Patagonia were done for the ITS gene region in order to ascertain the affiliation of the specimens

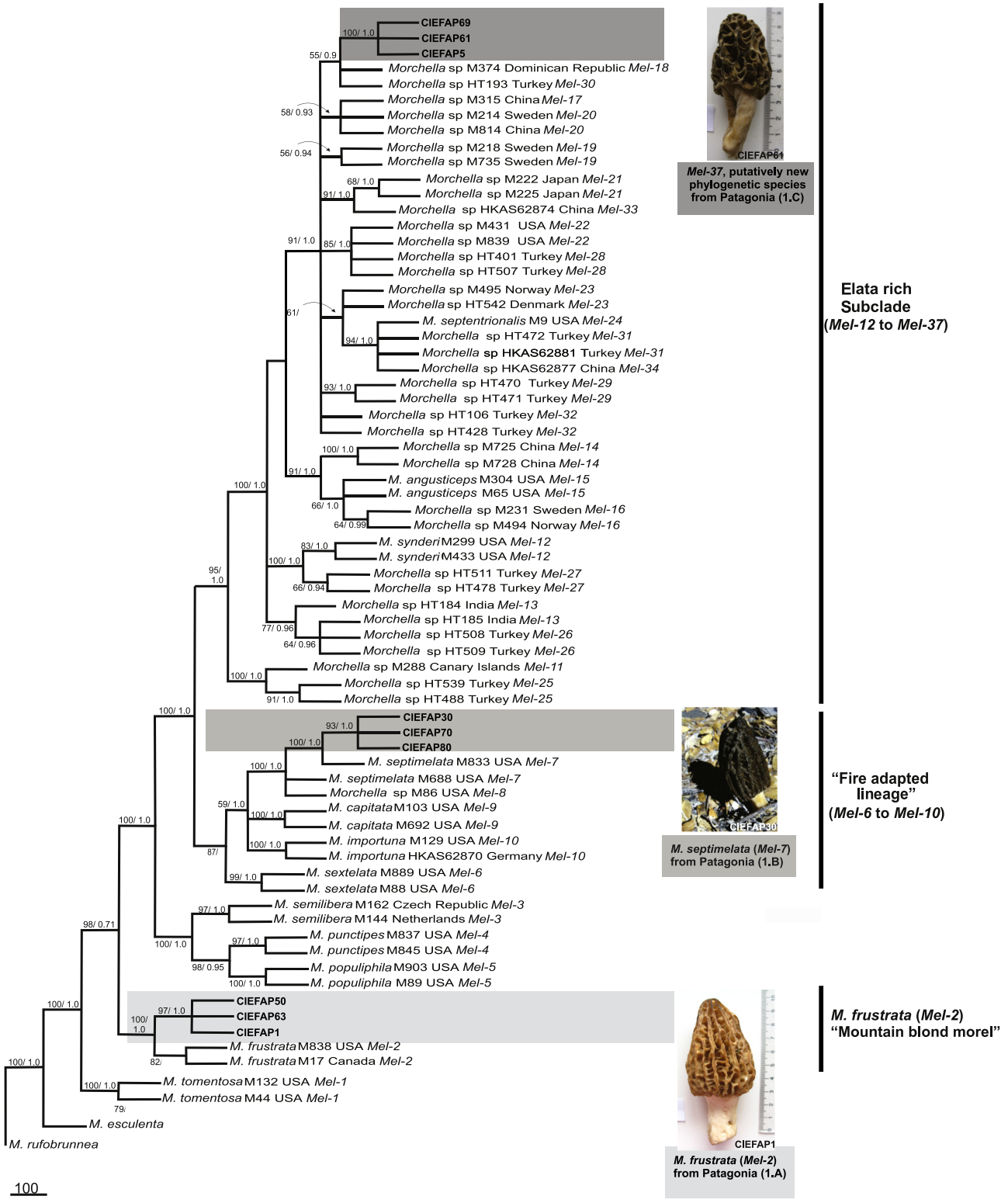

Fig 1 – Phylogenetic relationships of *Morchella* inferred from ITS and *RPB1* sequences *via* parsimony (MP) and Bayesian (BI) analysis. Bootstrap values ≥50 % and Bayesian posterior probabilities (≥0.9) for internal nodes are given on the branches (MP/BI). Phylogenetically distinct species within this clade are identified according to Taşkin *et al.* (2010) and Du *et al.* (2012). Latin binomials are applied to Mel-1 (*M. tomentosa*), *Mel*-3 (*M. semilibera*), *Mel*-4 (*M. punctipes*) and the species described by Kuo *et al.* (2012). GenBank numbers in boldface indicate specimens for which sequence data were generated in the present study.

collected (Supplementary Fig S1). The combined ITS and *RPB1* data set contained 72 sequences and included 1399 characters of which 833 were constant and 425 were parsimony-informative. The parsimony analysis found 60 most parsimonious trees (tree length 1095, consistency index 0.72 and retention index 0.91). The Bayesian analysis found two trees. The topologies inferred from the tree searches of the concatenated data sets did not differ when gaps were coded as missing data or as a fifth state (topologies not shown); the combined analysis is presented in Fig 1. Analyses of the individual and combined data sets revealed that the Patagonian morels belonged to the Elata Clade and that they were resolved as three strongly supported species-level lineages. The first lineage commonly known as the 'mountain blond morel' (Pilz *et al.* 2004 2007; Kuo *et al.* 2012) clustered with *Morchella frustrata* M. Kuo, corresponding to phylogenetic species *Mel-2* (O'Donnell *et al.* 2011) from the USA and Canada (Fig 1A), with strong monophyly bootstrap support; the ITS amplicon was 950 bp in length. Specimens of *M. frustrata* were collected from stands dominated by *Aristotelia chilensis* (D.Don.) Pic. Serm. & Bizarri, *Nothofagus antarctica* (Forst.) Oerst, *Nothofagus dombeyi* (Mirb.) Blume, *Lomatia hirsuta* (Lam.) Diels and from a mixed stand of *A. chilensis* – *N. dombeyi* – introduced *Pinus ponderosa* Dougl. Ex Laws., which had been affected by fire. However, within these areas, some isolates were collected from spots with abundant herbs and trees only marginally affected by fire. In general, they possessed conical pilei (a few were sub-globose) that were ochre-yellow to tan, becoming brown with maturity, (8) 6–3.5 (2.5) cm high and (5) 4–3 (1.5) cm wide at the widest point. Another characteristic of this species was the glabrous ribs, which were mainly longitudinal and straight, and concolor with pits that were elongated or irregular. Stalks were white, smooth or slightly grainy, sometimes furrowed and widening to the base, 2–3 (4) cm high and 1–2 cm wide. Ascospores were elliptical, smooth, (14) 20–24 (26) × (9) 14–19 (17) μm; asci were (115) 150–170 (230) × 17–19 μm. Elements on sterile ridges were septate, clavate or subclavate, 85–110 × 27–34 μm.

The second species, informally called 'fire-adapted' (Fig 1B) was identified as *Morchella septimelata* M. Kuo (Kuo *et al.* 2012), phylogenetic species *Mel-7* (O'Donnell *et al.* 2011). *Morchella* species in the fire-adapted clade also included *Morchella capitata* M. Kuo & M.C. Carter (*Mel-9*), *M* importuna* M. Kuo, O'Donnell & T.J. Volk (*Mel-10*), and *Morchella sextelata* M. Kuo (*Mel-6*) (Fig 1B). Patagonian collections were collected from burned native forest composed mainly of *A. chilensis* and *Nothofagus antarctica,* and burned plantations of introduced *Pinus ponderosa* and *Pinus radiata* D. Don. mixed with native *A. chilensis*, *N. antarctica*, *Aristotelia maqui* (Mol.) Stunt, *Schinus patagonicus* (Phil.) I.M. Johnst. ex Cabrera and *L. hirsuta*. This species formed a strongly supported post-fire adapted lineage, with conical or sub-conical brown pilei and smaller spores (18–25 × 10–15 μm). Patagonian collections of *Morchella septimelata* yielded ITS amplicons of 626–628 bp. They were characterized by conical pilei, with an acute or flat apex that were brown or greyish brown, 6.5–3.5 cm high and 2–3 cm wide at the widest point, glabrous or finely tomentose ribs, mainly longitudinal and straight, with occasional secondary ribs, and concolor with pits and darkening with age, pits elongated, white stalks, finely verrucose, cylindrical or widening to the base, sometimes with a subterranean portion, 3–4 (5) cm high and (0.5) 1–1.5 cm wide. Ascospores were elliptical, smooth, 17–22 × (9) 10–15 μm; asci were 160–210 × 15–16 (20) μm, elements on sterile ridges capitate, 85–110 μm high × 17–19 μm at apex and 13–4 μm at base, septate, with no colour change or slightly yellowish when treated with KOH.

Phylogenetic analyses of the ITS – *RPB1* data set also identified a species-level lineage from Patagonia within the species-rich Elata Subclade, with ITS amplicons of 652 bp (Fig 1D). The concatenated four-gene analysis performed for the species-rich Elata Subclade revealed a novel phylogenetic species that was designated *Mel-37*, which appears to be endemic to Patagonia (Fig 2). The four-gene data set for 44 taxa included 3224 characters, of which 297 were parsimony-informative. A heuristic search using unweighted characters yielded 50 most parsimonious trees 451 steps in length (CI = 0.830 and RI = 0.922).

Patagonian collections of the phylogenetic species *Mel-37* were associated with a mixed stand of *N. dombeyi*, introduced *Pseudotsuga menziesii* (Mirb.) Blanco, *Lomatia hirsuta* or *A. chilensis* with *L. hirsuta* and *Aristotelia maqui*, with no evidence of fire. Morphologically they were characterized by conical pilei, with an acute or flat apex that was light ochre turning to dark brown with maturity, (5.5) 5–3.5 cm high and 2–3 (3.5) cm wide at the widest point. Ribs were glabrous, straight or sinuous, with occasional secondary ribs that were concolor with pits and darkening with age, pits were irregular in shape. Stalks were white, turning yellowish at maturity, finely verrucose, cylindrical or widening to the base; 3–3.5 (8) cm high and 1–1.5 (3.2) cm wide. Ascospores were elliptical, smooth, 17–20 × (9) 12–13 (14.5) μm; asci 115–190 × 15–17 (20) μm. Elements on sterile ridges were septate and capitate, 90–150 (200) μm high × 15–20 μm at the base and 27–30 μm at the apex, with no colour change when treated with KOH.

Discussion

The three Elata Clade species of *Morchella* from Argentina were identified as *Morchella frustrata* (*Mel-2*), *Morchella septimelata* (*Mel-7*) and a putatively Patagonian novel phylogenetic species *Mel-37* within the species-rich Elata Subclade (O'Donnell *et al.* 2011). These results were supported using MP and BI analyses (Fig 1). The Elata Clade is typically found in temperate deciduous and coniferous forest biomes, mountain habitats and high elevations, and include several species that are fire-adapted. These niche characteristics are present in the southern Patagonian Andes, which possess a temperate forest biome that extends from around 33°S to 55°S latitude over much of southern Chile and the eastern slopes of the Patagonian Andes in Argentina. Morels in Patagonia are present in mixed *Nothofagus* spp. and *Austrocedrus chilensis* forests. Since *Morchella esculenta* has been reported in Cordoba province in Argentina (Dominguez de Toledo 1987), but specimens were unavailable for this study, we cannot conclude that only the Elata Clade is represented in southern South America. Thus, there is a need for more exhaustive sampling in that region.

Morchella conica, *Morchella elata*, *Morchella esculenta*, and *Morchella intermedia* were reported in Argentina in the early 1920s, but it remains to be determined whether these

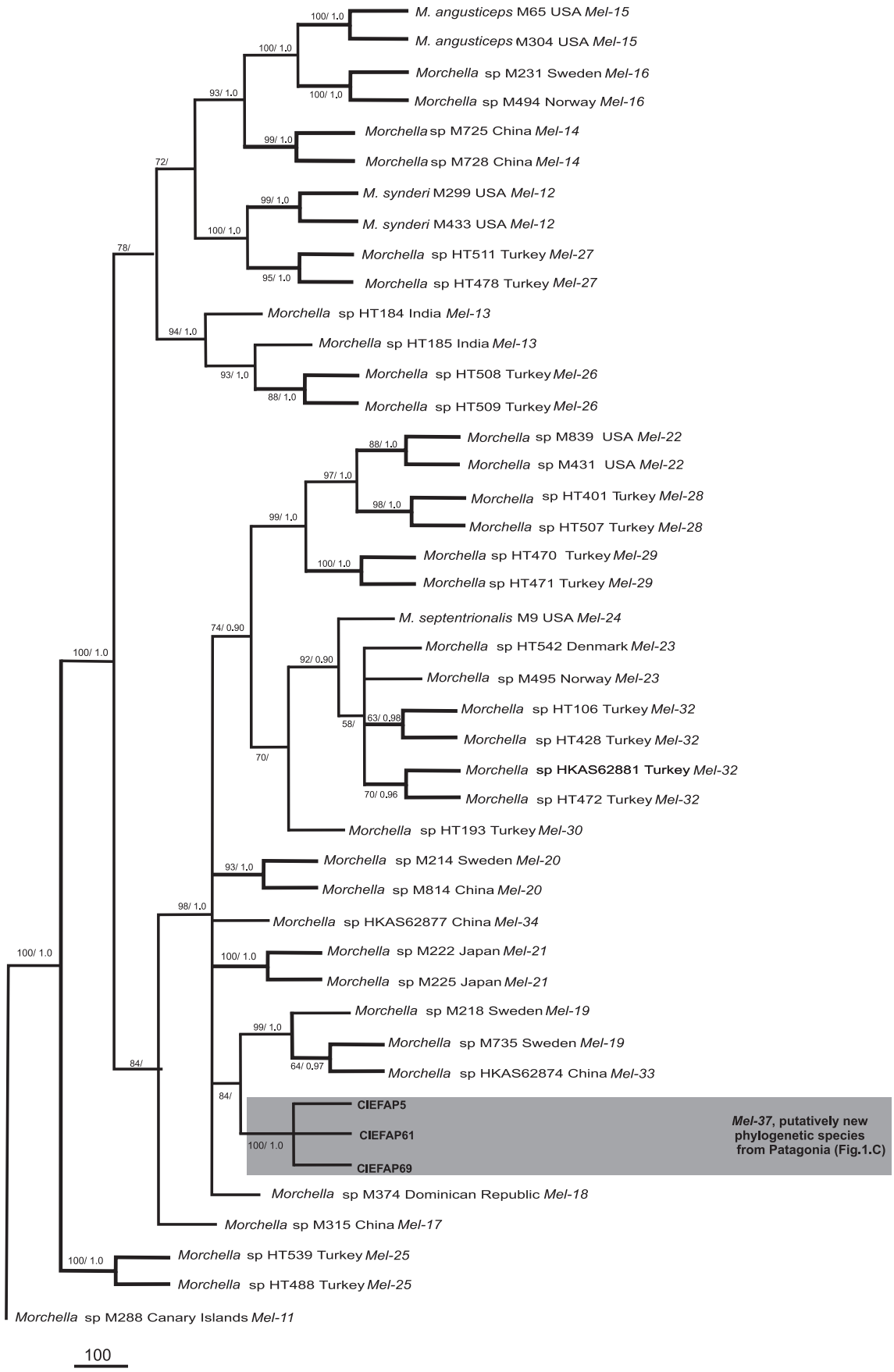

Fig 2 – One of sixty nine most parsimonious phylograms generated from the ITS – *RPB1*–*RPB2*–*EF1-α* concatenated data set for the species-rich Elata Subclade *via* parsimony (MP) and Bayesian (BI) analysis. Bootstrap values ≥50 % and Bayesian

European species are present in South America (Spegazini 1909; Dominguez de Toledo 1987; Gamundi 2010). O'Donnell *et al.* (2011) concluded that those names could not be applied to North American morels. *Morchella patagonica* which has only been reported from Patagonia, is a light brown species with ovoid, small pilei (2.5–4 cm tall × 1–1.5 cm wide) and ascospores that are 15–17 × 10–11 μm (Spegazzini 1909). The three species we collected do not match the description of *M. patagonica*. Therefore, further sampling of the region will be necessary in order to recollect species or obtain DNA data from the type specimen. This could be challenging, because the type is over 100 years old. The type was collected in Blest Harbour inside the Nahuel Huapi National Park (Spegazzini 1909), an area that was not accessible at the time of our survey.

The Patagonian collections of *M. frustrata (Mel-2)* were from mixed forests dominated by *A. chilensis*, *N. antarctica*, *N. dombeyi*, and *Lomatia hirsuta* and possessed pale pilei, similar to samples in collections of *M. frustrata* from the USA (Pilz *et al.* 2004, 2007; Kuo *et al.* 2012); however, the Patagonian collections possessed smaller asci and ascospores. As previously reported by Taşkin *et al.* (2012) for Turkey, *M. frustrata* represented the most widely collected morel in Patagonia in this study (Supplementary Table S1; Fig 1A). The Patagonian morel within the fire-adapted lineage (*Mel-6*, *Mel-7*, *Mel- 8*, *Mel-9*, and *Mel-10*) represents *M. septimelata* = *Mel-7* (Fig 1B). This fungus was described from the USA (Kuo *et al.* 2012) and is commonly referred to by commercial collectors as the 'pickle', 'pink' or 'green morel' (Pilz *et al.* 2007). *Morchella* spp. from post-fire sites in Patagonia were previously reported by Gamundi (1975), Gamundi & Horak (1993), and La Manna & Barroetaveña (2011). In the present study, specimens of *M. septimelata* were collected from native *A. chilensis* and *Nothofagus antarctica* forests or in a mixed stand with exotic *Pinus* spp. subjected to a recent fire (Supplementary Table S1). In contrast to Kuo *et al.* (2012)'s description, pilei of Patagonian specimens were smaller, no olive nor pinkish colours were detected, and ascospores and asci were slightly shorter. The species-rich Elata Subclade was also represented in Argentina, and a new phylogenetic species (*Mel-37*), currently known only from southern south America, is proposed (Fig 1C).

One remarkable biogeographical pattern from our study concerns the presence of putatively North America taxa in Argentina. Based on the low diversity of *Morchella* in Patagonia, and the fact that the Elata Clade putatively first evolved in western North America (O'Donnell *et al.* 2011), we suggest that *M. frustrata* and *M. septimelata* may have colonized South America from North America, and that there are three possible hypotheses to account for such apparent disjunction. The first, Long Distance Dispersal (LDD) and niche conservation, was proposed as a possible force to explain the disjunct distribution of *M. septimelata* in western North America, Europe and Asia (O'Donnell *et al.* 2011; Du *et al.* 2012). The second is a migration at the end of the Tertiary period. The amphi-boreal distribution from North America to Southern South America has been noted for a number of fungi and other taxa, such as the plant genus *Berberis* and the biotrophic fungi that cause powdery mildew disease; it is explained by the connection of South America to North America produced at the end of the Tertiary period through the closure of the Isthmus of Panama (Coates & Obando 1996; Burnham & Graham 1999; Niinomi *et al.* 2008). This connection coincided with the formation of the Andean corridor with cold, elevated areas that allowed the migration of cold-adapted taxa from the Northern Hemisphere into southern South America (Simpson 1983). The third hypothesis is a recent anthropogenic introduction of *Morchella*, as was proposed for *M. esculenta Mes-16* in New Zealand and Africa (Du *et al.* 2012a) and diverse other edible, pathogenic and ectomycorrhizal fungi (Coetzee *et al.* 2001; Dickie & Johnston 2008; Bonito *et al.* 2010). The existence of *M. frustrata* and *M. septimelata* collections from pure and mixed stands of exotic pine species and native trees could be explained with a co-introduction *Pinus* spp. and *Pseudotsuga menziesii* from western North America (Supplementary Table S1). The facultative mycorrhizal-like and/or endophytic nature demonstrated for *M. septimelata* (Baynes *et al.* 2012) may have also contributed to their current occurrence *via* human introduction and migration. However, this last hypothesis deals with the first report of *Morchella* in Patagonia pre-dating the exotic pine plantations in the region (Spegazzini 1909; Andenmatten *et al.* 2002). Further support for any of these hypotheses about the origins and evolutionary relationships of South American and North American morels need a more comprehensive biogeographical context, including additional sampling and DNA extraction of herbarium material.

In conclusion, we have established a comprehensive phylogeny that shows for the first time the relationships among morels from the Andean Forests in Patagonia, Argentina with other *Morchella* species found around the world. Future research will focus on sampling from presently underrepresented areas, e.g. Chile and the center of Argentina, to clarify the biogeographical patterns of *Morchella* in South America further, and to provide a more complete framework of *Morchella* evolution.

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posterior probabilities (≥0.9) for internal nodes are given on the branches (MP/BI). Phylogenetically distinct species within this subclade are identified according to Taşkin *et al.* (2010) and Du *et al.* (2012). Latin binomials are applied to the *Morchella* species described by Kuo *et al.* (2012). Highlight identifies the putatively novel distinct phylogenetic species from Patagonian forests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2014.03.008.

REFERENCES

Akaike H, 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389–3402.

Andenmatten E, Gonda HE, Letorneau F, Cortez GO, Bava JO, 2002. *Proyección de la distribución diamétrica en plantaciones de pino ponderosa en la provincia de Neuquén* Informe final, Proyecto PIA 15/58. SAGPyA, Argentina.

Baynes M, Newcombe G, Dixon L, Castlebury L, O'Donnell K, 2012. A novel plant-fungal mutualism associated with fire. *Fungal Biology* **116**: 133–144.

Boa E, 2004. *Wild edible fungi a global overview of their use and importance to people*. FAO/Forestry Department, Rome, Italy, Online at. www.fao.org/docrep/007/y5489e/y5489e00.htm#TopOfPage [accessed Jan. 2010].

Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R, 2010. A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host association and long-distance dispersal. *Molecular Ecology* **19**: 4994–5008.

Bunyard BA, Nicholson MS, Royse DJ, 1994. A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia* **86**: 762–772.

Burnham RJ, Graham A, 1999. The history of neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Garden* **86**: 546–589.

Castresana J, 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.

Coates, Obando JA, 1996. The geologic evolution of the Central American isthmus. In: Jackson JBC, Budd AF, Coates AG (eds), *Evolution and Environment in Tropical America*. Univ Chicago Press, Chicago, pp. 21–56.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ, 2001. The root fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* **10**: 387–396.

De Michelis A, Rajchenberg M, 2002. *Hongos comestibles: teoría y práctica para la recolección, elaboración y conservación*, 1st edn. INTA, Bariloche.

Dickie IA, Johnston P, 2008. *Invasive Fungi Research Priorities, With a Focus on* Amanita muscaria Landcare Research Contract Report: LC0809/027. Landcare Research New Zealand Ltd.

Domínguez de Toledo L, 1987. Sobre la presencia de *Morchella esculenta* PERS. en Córdoba (Morchellaceae – Pezizales). *Boletín de la Sociedad Argentina de Botánica* **25**: 79–84.

Du Xi-Hui, Zhao Qi, O'Donnell K, Rooney AP, Yang Zhu L, 2012. Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. *Fungal Genetics and Biology* **49**: 455–469.

Gamundi IJ, 1975. Fungi Ascomycetes Pezizales. In: Guarrera SA, Gamundí de Amos I, Rabinovich deHalperin D (eds), *Flora Criptogámica de Tierra del Fuego FECIC*, pp. 14–22 Buenos Aires.

Gamundi IJ, 2010. Genera of Pezizales of Argentina 1. An updating of selected genera. *Mycotaxon* **113**: 1–60.

Gamundí IJ, Horak E, 1993. *Hongos de los Bosques Andino-patagónicos*, 1st edn. Vazquez Mazzini, Buenos Aires.

Greene DF, Hesket M, Pounden E, 2010. Emergence of morel (*Morchella*) and pixie cup (*Geopyxis carbonaria*) ascocarps in response to the intensity of forest floor combustion during a wildfire. *Mycologia* **102**: 766–773.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Katoh K, Standley DM, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Keefer ME, Winder R, Hobby T, 2010. Commercial development of non-timber forest resources: A case study of morels in the East Kootenay, British Columbia. *BC Journal of Ecosystems and Management* **11**: 39–51.

Kuo M, Dewsbury DR, O'Donnell K, Carter MC, Rehner SA, Moore JD, Moncalvo JM, Canfield SA, Stephenson SL, Methven AS, Volk TJ, 2012. Taxonomic revision of true morels (*Morchella*) in Canada and the United States. *Mycologia* **104**: 1159–1177.

La Manna L, Barroetaveña C, 2011. Propiedades químicas del suelo en bosques de *Nothofagus antarctica* y *Austrocedrus chilensis* afectados por fuego. *Revista de la Facultad de Ciencias Agrarias de la Universidad Nacional de Cuyo* **43**: 41–55.

Liu YL, Hall BD, 1999. Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. *Proceedings of the National Academy of Sciences, USA* **101**: 4507–4512.

Niinomi S, Takamatsu S, Havrylenko M, 2008. Molecular data do not support a southern hemisphere base of *Nothofagus* powdery mildews. *Mycologia* **100**: 716–726.

O'Donnell K, Rooney AP, Mills GL, Kuo M, Weber NS, Rehner SA, 2011. Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early cretaceous origin and high continental endemism and provincialism in the Holarctic. *Fungal Genetics and Biology* **48**: 252–265.

Pagliaccia D, Douhan GW, Douhan L, Peever TL, Carris LM, Kerrigan JL, 2011. Development of molecular markers and preliminary investigation of the population structure and mating system in one lineage of black morels (*Morchella elata*) in the Pacific Northwestern USA. *Mycologia* **103**: 969–982.

Pildain MB, Visnovsky S, Barroetaveña C, 2013. *Phylogenetic Diversity of True Morels (*Morchella*) in Native Patagonian Forests of Argentina* Proceedings of the 7th International Workshop on Edible and Mycorrhizal Mushrooms, IWEMM 7.

Pilz D, Weber NS, Carter MC, Parks CG, Molina R, 2004. Productivity and diversity of morel mushrooms in healthy, burned and insect-damaged forests of northeastern Oregon. *Forest Ecology and Management* **198**: 367–386.

Pilz D, McLain R, Alexander S, Villarreal-Ruiz L, Berch S, Wurtz TL, Parks CG, McFarlane E, Baker B, Molina R, Smith JE, 2007. *Ecology and Management of Morels Harvested from the Forests of Western North America. General Technical Report PNW-GTR-710.* US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland.

Posada D, 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**: 1253–1256.

Reeb V, Lutzoni F, Roux C, 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.

Rehner SA, Buckley E, 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. *Mycologia* **97**: 84–98.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Simpson BB, 1983. An historical phytogeography of the high Andean flora. *Revista Chilena de Historia Natural* **56**: 109–122.
Spegazzini CL, 1909. Mycetes Argentinenses. Series 4. Anales del Museo Nacional de Historia Natural de Buenos Aires/19: 257-458Spegazzini CL, 1918. Tercera contribución a la micología Chilena. Revista Chilena de Historia Natural 22: 42-43.Spegazzini CL, 1921. Mycetes Chilenses. *Boletín de la cademia Nacional de Ciencias, Córdoba* **25**: 1–124.
Stefani FOP, Sokolski S, Wurtz TL, Piche Y, Hamelin RC, Fortin JA, Berube JA, 2010. *Morchella tomentosa*: a unique belowground structure and a new clade of morels. *Mycologia* **102**: 1082–1088.
Stiller JW, Hall BD, 1997. The origin of red algae: implications for plastid evolution. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 4520–4525.
Swofford DL, 2002. *PAUP* 4.0: phylogenetic analysis using parsimony (* and other methods)*, 10th edn. Sinauer Associates, Sunderland Massachusetts.
Taşkın H, Büyükalaca S, Dogan HH, Rehner SA, O'Donnell K, 2010. A multigene molecular phylogenetic assessment of true morels (*Morchella*) in Turkey. *Fungal Genetics and Biology* **47**: 672–682.
Taşkın H, Büyükalaca S, Hansen K, O'Donnell K, 2012. Multilocus phylogenetic analysis of true morels (*Morchella*) reveals high levels of endemics in Turkey relative to other regions of Europe. *Mycologia* **104**: 446–461.
White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic, San Diego, pp. 315–322.
Wipf D, Munch JC, Botton B, Buscot F, 1996. DNA polymorphism in morels: complete sequences of the internal transcribed spacer of genes coding for rRNA in *Morchella esculenta* (yellow morel) and *Morchella conica* (black morel). *Applied and Environmental Microbiology* **62**: 3541–3543.