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Abstract: An abundant fruiting of a black morel was encountered in temperate northwestern New South Wales (NSW), Australia, during a mycological survey in Sep 2010. The site was west of the Great Dividing Range in a young, dry sclerophyll forest dominated by *Eucalyptus* and *Callitris* north of Coonabarabran in an area known as the Pilliga Scrub. Although the Pilliga Scrub is characterized by frequent and often large, intense wildfires, the site showed no sign of recent fire, which suggests this species is not a postfire morel. Caps of the *Morchella elata*-like morel were brown with blackish ridges supported by a pubescent stipe that became brown at maturity. Because no morel has been described as native to Australia, the collections were subjected to multilocus molecular phylogenetic and morphological analyses to assess its identity. Results of these analyses indicated that our collection, together with collections from NSW and Victoria, represented a novel, genealogically exclusive lineage, which is described and illustrated here as *Morchella australiana* T. F. Elliott, Bougher, O'Donnell & Trappe, sp. nov.

Key words: Ascomycota, *Callitris*, *Eucalyptus*, Morchellaceae, Morels, Pezizales, Western Australia

Introduction

True morels (*Morchella*) are hypothesized to have evolved in the early Cretaceous in the northern hemisphere where they currently exhibit high continental endemism (O'Donnell et al. 2011). The restricted distribution of most species suggests that long distance (LDD) dispersal via ascospores is not a very efficient mechanism for invading novel niches (Du et al. 2012a, b; Taşkın et al. 2010, 2012), especially ones in the southern hemisphere. Thus, the discovery of an unnamed, putatively Asian endemic *M. esculenta* Clade species, designated *Mes*-16 in New Zealand and the African countries of Kenya and Tanzania (Degreef et al. 2009), were interpreted as anthropogenic introductions into the southern hemisphere (Du et al. 2012a) rather than a result of LDD. However, based on multilocus phylogenetic data presented herein, a *M. elata*-like species collected in Victoria (940098) and New South Wales (950197) in 1994 and 1995 respectively by Helen Faris (Faris et al. 1996) and by two of us (Elliott and Trappe) in Sep 2010 in temperate northwestern NSW appears to represent a novel, phylogenetically distinct species currently known only from southeastern Australia. Our collection site was north of Coonabarabran in a biologically distinctive area known as the Pilliga Scrub (Rolls 1981), approximately 300 km northwest of Sydney in a young, dry sclerophyll forest dominated by *Eucalyptus* and *Callitris* spp. Because this morel fulfills the requirements of phylogenetic species recognition based on genealogical concordance (Taylor et al. 2000) and genealogical discordance was not detected (Dettman et al. 2003), it is described and illustrated here as *Morchella australiana* T.F. Elliott, Bougher, O'Donnell & Trappe., sp. nov.

Materials and methods

Specimens and cultures.—Specimens were photographed in situ, collected and macroscopic features were recorded the same day before drying on an electric, portable food dehydrator at 50 C. Microscopic characters were recorded by standard light microscopic procedures from mounts in water, 5% KOH and Melzer's reagent; all spore measurements were taken from water mounts. Colors reported followed the Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) system (Kelly and Judd 1976). Air-dried ascospores of *M. australiana* OSC 145974 were mounted on a stub, coated with gold-palladium and viewed and photographed with a JEOL 6400V scanning electron microscope (JEOL USA, Peabody, Massachusetts). Pure cultures were established by isolating ascospores that had germinated on 3% water agar (Difco, Detroit, Michigan) supplemented with approximately 40 μg streptomycin and

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TABLE I. Australian collections of *Morchella* studied

Collection no.[a]	Phylogenetic ID[b]	NRRL no.[c]	Date	Location
UWS940098	*Morchella australiana*	—	Spring 1994	Victoria
UWS950197	*Morchella australiana*	—	Spring 1995	Cowra, New South Wales
T35077	*Morchella australiana*	NRRL 54674	4 Aug 10	7 km N of Bugaldie
PERTH7572670	*Morchella rufobrunnea*	NRRL 26617	1 Jun 94	Murdoch University, Perth
PERTH7712979	*Morchella rufobrunnea*	NRRL 26620	16 Jul 85	University of Western Australia, Perth
PERTH5485452	*Morchella septimelata*	NRRL 26618	14 Aug 93	Chitelup Forest Block, Frankland River
PERTH7572131	*Morchella septimelata*	NRRL 26621	1 Aug 95	Boranup Forest Leeuwin Naturaliste National Park
PERTH7598394	*Morchella* sp.	NRRL 26619	5 Oct 96	Plavins Forest Block, Dwellingup

[a] UWS, University of Western Sydney; Trappe, Oregon State University, Oregon, USA; PERTH, Western Australian Herbarium, Kensington, Western Australia, PERTH5485452 *Morchella septimelata* was described as *M. elata* (Bougher and Syme 1998).

[b] Employing multilocus DNA sequence data (O'Donnell et al. 2011).

[c] NRRL, ARS Culture Collection, ARS-USDA, Peoria, Illinois, USA.

40 U penicillin/mL (P4333 Sigma-Aldrich, St Louis, Missouri). Pure cultures (TABLE I) are stored in the ARS Culture Collection (NRRL, http://nrrl.ncaur.usda.gov/) in liquid nitrogen vapor at −175 C in a cryogen composed of 1% DMSO and 10% skim milk.

Molecular biology and phylogenetics.—These procedures were identical to those reported by O'Donnell et al. (2011), except that the maximum likelihood bootstrap (ML-BS) analyses, which included 1000 pseudoreplicates of the data, were run with GARLI 1.0 (Zwickl 2006) on the CIPRES Science Gateway TeraGrid (Miller et al. 2010) employing the GTR + I + Γ model of evolution. Putative species lineages were considered to be genealogically exclusive if one or more of the individual partitions and the combined dataset supported species monophyly at ≥ 70% bootstrap support and none of the partitions contradicted species monophyly. Nucleotide sequence data were deposited in GenBank (accession numbers KC753466–KC753481), and the concatenated 52-taxon four-locus NEXUS file and the most parsimonious tree were deposited in TreeBASE (accession number S13998, tree number Tr61889).

RESULTS

Molecular phylogenetics.—Maximum likelihood (ML) and maximum parsimony (MP) bootstrap (BS) analyses of the ITS+LSU rDNA partition and the combined four-gene dataset strongly supported the genealogical exclusivity (Taylor et al. 2000) of these three collections from southeastern Australia (TABLE II, FIG. 1):

TABLE II. Tree statistics and summary of maximum parsimony phylogenetic analyses of *Morchella elata* Subclade datasets

Locus	No. characters[a]	No. MPTs[b]	MPT length	CI[c]	RI[d]	Syn[e]	Aut[f]	PIC/bp (%)[g]	MP bootstrap support (%) *M. australiensis*[h]
EF-1α	1599	1408	235	0.84	0.95	147	37	9.2	<50
RPB1	794	>5000	75	0.92	0.97	60	6	7.6	64
RPB2	906	>10 000	93	0.9	0.95	60	21	6.6	<50
rDNA	1319	432	159	0.84	0.95	96	22	7.3	100
Combined	4618	18	618	0.78	0.92	363	86	7.9	100

[a] Number of aligned nucleotide positions.

[b] MPTs, most-parsimonious trees.

[c] CI, consistency index.

[d] RI, retention index.

[e] Syn, synapomorphy or parsimony informative character.

[f] Aut, autapomorphy or parsimony uninformative character.

[g] PIC/bp, parsimony-informative characters/base pair.

[h] MP, maximum parsimony bootstrap support based on 1000 pseudoreplicates of the data.

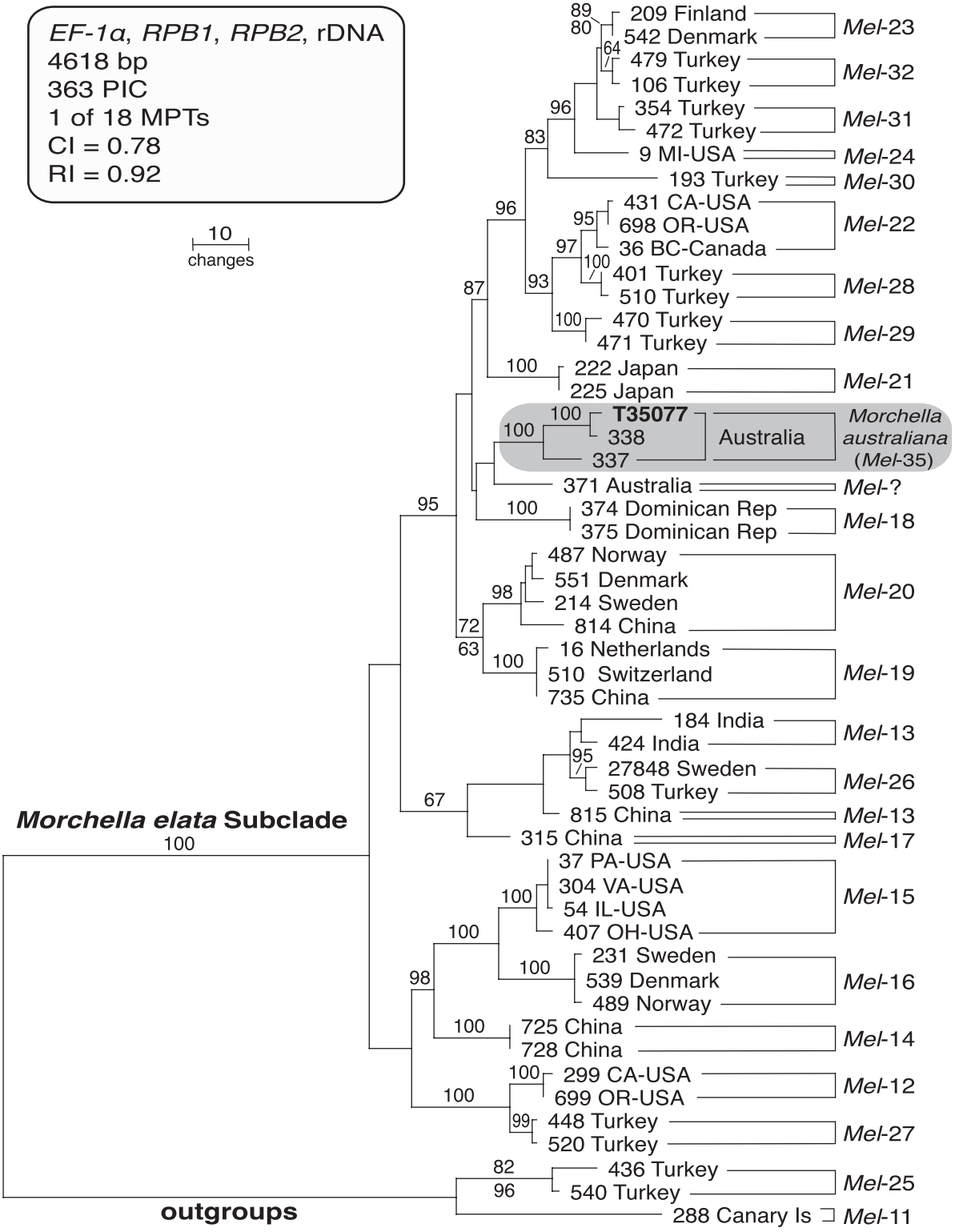

FIG. 1. One of 18 equally parsimonious trees inferred from a four-locus dataset (Swofford 2003) for the *Morchella elata* Subclade rooted on sequences of *Mel*-11 and *Mel*-25. The 52-taxon dataset comprised 4613 nucleotide positions of which 363 were parsimony informative (PIC). Numbers above nodes indicate ML-BS values based on 1000 pseudoreplicates of the data. MP-BS values are only indicated below nodes when they differed by more than 5% of the ML-BS value. Each phylogenetic species is identified by a unique Arabic number. The holotype of *M. australiana* (T35077) together with two other collections from New South Wales/Victoria (TABLE I, 337 = UWS940098, 338 = UWS950197) formed a strongly supported lineage (ML-BS/MP-BS = 100%). CI = consistency index, RI = retention index.

FIG. 2. *Morchella australiana*. A–C. A. Habit photo of holotype collection in mixed *Eucalyptus* and *Callistris* forest 7 km N of Bugaldie in NSW. B. Capitate elements on sterile ridge. C. Ascospore with irregular longitudinal and interconnecting transverse ridges. Bars: A = 2 cm, B = 25 µm. C = 10 µm.

T35077 from Bugaldie, New South Wales (NSW) in 2010 by Elliott and Trappe (FIG. 2), UWS940098 (= 337, FIG. 1) and UWS950197 (= 338) from NSW/Victoria in 1994 and 1995 respectively by Helen Faris, who at the time was associated with Western Sydney University (Faris et et. 1996). Although three partitions (i.e. *EF-1α*, *RPB1*, *RPB2*) did not support monophyly of this Australian lineage, they did not contradict it. To better understand the diversity of *Morchella* in Australia, molecular phylogenetic identifications of five additional collections from Western Australia revealed that urban morel fruitings from the University of Western Australia, Perth, in Jul 1985 and Murdoch University, Perth, in Jun 1994 were *M. rufobrunnea*; those from the Jarrah Forest region in association with *Eucalyptus marginata*, Chitelup Forest Block,

Frankland River area, and a *E. diversicolor* burn site in Boranup Forest within Leeuwin-Naturaliste National Park, near Margaret River, were the postfire morel, *M. septimelata* (TABLE I; = *Mel*-7 sensu O'Donnell et al. 2011); and the phylogenetic identity of a morel (PERTH7598394, TABLE I) collected in Oct 1996 in a burned stand of *Eucalyptus marginata* in the Plavins Forest Block, Dwellingup, was unresolved (= 371, FIG. 1).

TAXONOMY

Morchella australiana T.F. Elliott, Bougher, O'Donnell & Trappe, sp. nov. FIG. 2
MycoBank MB803363

Macrocharacters.—Ascomata (60–)80–130 mm tall. Pileus subconical to ovate, (25–)40–60 × 30–50 mm at the widest point, attached to stipe with a sinus 1–2 mm deep and wide, pitted and ridged. Pileus ridges ± 2 mm broad, 10–14 primary vertical ridges with transecting horizontal ridges to meandering ridges showing only slight tendency to a vertical alignment, in youth the margins rounded, pubescent, and light orange-yellow (ISCC-NBS 70), with age the margin pubescence collapsing and the margins becoming dark grayish yellowish brown (ISCC-NBS 81) to silvery black. Pits vertically elongated to irregular, glabrous, 5–7 mm deep, in youth light orange-yellow (ISCC-NBS 70), with age darkening to moderate orange-yellow (ISCC-NBS 71). Stipe cylindrical or slightly downward tapered, shallowly fluted at the base, 30–75 × 15–30 mm, the surface beset with minute circumferential bands and aggregations that with a hand lens appear to be pubescent (but see microcharacters below), in youth yellowish white (ISCC-NBS 92), with age darkening to light orange-yellow (ISCC-NBS 7). Context and inner surface whitish in youth, with age becoming concolorous with the outer surface; inner surface with whitish pubescence.

Microcharacters.—Ascospores 19–25 (–29) × (12–)13–15 μm in water, Q = 1.33–1.64(–1.83), ellipsoid, hyaline, with light microscopy in water or cotton blue mounts appearing smooth when intact but sometimes faintly and minutely longitudinally ridged when broken, in Melzer's reagent the ridges faintly evident on most spores, in SEM 32–46 longitudinal to slightly spiraling, straight to sinuous ridges 0.25–1.5 μm broad and often connected with low, crowded, transverse ridges; contents homogeneous; spore wall ± 1 μm thick; tips sometimes with minute bubbles clustered at the ends. Asci crowded, cylindrical, straight to slightly sinuous, 140–165 × 17–19 μm at maturity, eight-spored, hyaline, thin-walled. Paraphyses generally not exceeding the asci, scattered, cylindrical to clavate, obtuse, 5–10 μm broad, when clavate the apices expanded up to 12 μm, hyaline, thin-walled, septate. Elements on sterile ridges straight to slightly sinuous, obtuse to tapered, subcapitate, capitate or irregular, 75–175 × (7.5–)10–18 μm, but when subcapitate to capitate the apices up to 30(–47) μm broad, hyaline to brownish or brown, thin-walled, septate. Stipe context of loosely interwoven, hyaline, thin-walled hyphae 4–7 μm broad at the septa, some cells inflated up to 12–20 μm; minute ridges on stipe outer surface composed of aggregations of hyaline, thin-walled hyphae and inflated cells up to 10–60 μm broad, the ridges and intervening furrows with scattered to crowded, emergent, obtuse, septate, hyaline, thin-walled hyphal tips 12–20 μm broad and 30–100 μm long; inner stipe surface similar to outer but less prominently developed.

Holotype: Trappe 35077, a dried specimen deposited in MEL. AUSTRALIA, NEW SOUTH WALES, West of Coonabarabran on west side of Coonabarabran Road, 7 km N of Bugaldie at Bugaldie Creek, 31°3′41″S, 149°5′45″E, 350 m, in a young forest mixture of *Eucalyptus* spp. and *Callitris* sp., 4 Sep 2010, Todd Elliott and Jim Trappe.

Isotypes: CANB and OSC 145974.

Ex holotype culture: NRRL 54674.

Etymology.—Latin for "Australian," an epithet especially appropriate for the first morel apparently endemic to Australia.

Comments: We have designated *Morchella australiana* phylogenetic species *Mel*-35 because application of validly published binomials for most morels is problematic (Clowez 2012, Kuo et al. 2012) and molecular phylogenetic data are the most reliable means by which most species can be identified (Taşkın et al. 2010, O'Donnell et al. 2011, Du et al. 2012a). Although *Morchella australiana* is morphologically similar to several species within the *M. elata* Clade (i.e. *M. elata*, *M. angusticeps*, *M. capitata*, *M. septentrionalis*), its current known distribution (New South Wales and Victoria in southeastern Australia) and ecology (in association with *Eucalyptus* and *Callitris*) distinguish it from these European and North American species. Like *M. capitata*, *M. australiana* produces distinctive capitate elements on the sterile ridges; however, unlike the former, *M. australiana* apparently does not fruit on burn sites. With the present dataset, the phylogenetic position of PERTH7598394 *Morchella* sp. (= 371) from an *E. marginata* forest in the Jarrah Forest region in Dwellingup, Western Australia, (TABLE I) is unresolved. Our justification for treating *M. australiana* and PERTH7598394 *Morchella* sp. (= 371) as distinct lineages is based on the fact they did not form a genealogical exclusive lineage coupled with the fact

that the latter morel was collected in Western Australia on a burn site whereas *M. australiana* has been collected only in southeastern Australia on sites that were not burned. Thus, the available data suggest they have distinctive geographic distributions and ecologies. Additional collections of PERTH7598394 *Morchella* sp. (= 371) are needed however to assess whether it represents a genealogically exclusive sister lineage of *M. australiana*. Similarly, additional surveys of Australia are needed to assess which morels have been introduced (i.e. *M. rufobrunnea* and *M. septimelata* = *Mel*-7) and which appear to be native (i.e. *M. australiana*).

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