MYCOLOGIA
2018, VOL. 110, NO. 6, 1205–1221
https://doi.org/10.1080/00275514.2018.1533772

Taylor & Francis
Taylor & Francis Group

Four new species of *Morchella* from the Americas

Timothy J. Baroni [a], Michael W. Beug [b], Sharon A. Cantrell[c], Teresa A. Clements [d], Teresa Iturriaga[e], Thomas Læssøe[f], María E. Holgado Rojas [g], Frank M. Aguilar[h], Miguel O. Quispe[h], D. Jean Lodge[i], and Kerry O'Donnell [j]

[a]Department of Biological Sciences, PO Box 2000, State University of New York–College at Cortland, Cortland, New York 13045; [b]The Evergreen State College, Olympia, Washington 98505; [c]Department of Biology, Universidad del Turabo, PO Box 3030, Gurabo, Puerto Rico 00778; [d]1430 W. Wagon Wheel Road, Cottonwood, Arizona 86326; [e]Illinois Natural History Survey, University of Illinois, 1816 South Oak Street, Champaign, Illinois 61820-6970; [f]Natural History Museum of Denmark/Department of Biology, University of Copenhagen, Copenhagen Ø, DK-2100, Denmark; [g]Centro de Investigación y Producción de Hongos Alimenticios y Medicinales (CIPHAM), Universidad Nacional de San Antonio Abad, Cusco, Perú; [h]Herbario Vargas Cuz, Facultad de Ciencias, Universidad Nacional de San Antonio Abad, Cusco, Perú; [i]Center for Forest Mycology Research, Northern Research Station, United States Forest Service, United States Department of Agriculture, Luquillo, Puerto Rico 00773-1377; [j]Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604-3020

ABSTRACT

Morphological and molecular phylogenetic studies of true morels (*Morchella*) in North America, the Dominican Republic, Venezuela, Ecuador, and Peru led to the discovery of four undescribed species of *Morchella*. Two new species in the Elata clade, one from the Dominican Republic, initially distinguished by the informal designation *Mel*-18, and a newly discovered sister species from northern Arizona, are now recognized. *Mel*-18 is described as a novel phylogenetically distinct species, *M. hispaniolensis*. Its sister species from Arizona is described as *M. kaibabensis*, also recovered as an endophyte of Rocky Mountain juniper. Two additional species in the Esculenta clade, *M. peruviana* discovered in Peru and *M. gracilis* (previously reported as *Mes*-14) from the Dominican Republic, Venezuela, and Ecuador, are described as new. We also demonstrate that scanning electron microscopy (SEM) imaging of ascospores using rehydration/dehydration/critical point drying preparation techniques provides for enhanced resolution of spore wall surfaces, thereby increasing the number of morphological traits available to assess differences among otherwise closely related species.

ARTICLE HISTORY
Received 16 February 2018
Accepted 5 October 2018

KEYWORDS
Arizona; Ascomycota; Dominican Republic; Ecuador; endophytes; molecular phylogenetics; Morchellaceae; Peru; Pezizales; Venezuela; 4 new taxa

INTRODUCTION

Recent multigene phylogenetic investigations on the genus *Morchella* have identified three main clades (Esculenta, Elata, and Rufobrunnea) and revealed a significant number of undescribed taxa on a global scale based on the recognition of cryptic phylogenetic species (Taşkın et al. 2010, 2012; O'Donnell et al. 2011; Du et al. 2012a, 2012b; Richard et al. 2015). In papers by O'Donnell et al. (2011) and Du et al. (2012a, 2012b), in addition to geographic information on known collections, they also assigned informal clade and species identifiers to phylogenetically distinct lineages either already named or unmatched to existing morphological species, e.g., *Mes*-1–27 for taxa in the Esculenta clade and *Mel*-1–36 for those in the Elata clade. *Mes*-1 of the Esculenta clade is *Morchella steppicola* Zerova, *Mes*-4 is the recently described *M. americana* Clowez & Matherly (Clowez 2012), and *Mel*-1 the recently described *M. tomentosa* Kuo (Kuo et al. 2012). Over 60 taxa have now been recognized globally (O'Donnell et al. 2011; Du et al. 2012a, 2012b), but additional informal designations still lack formal binomial names.

The process of determining and describing the known phylogenetic species of morels is ongoing (Elliott et al. 2014; Pildain et al. 2014; Taşkin et al. 2016; Voitk et al. 2016). Clowez (2012) published a monograph on European and American morels, describing more than 20 new species based on morphological features. Kuo et al. (2012) also published a paper describing over a dozen new species of American morels using morphological and molecular data. Unfortunately, in several cases, the same taxon was described under different names in these two publications. Richard et al. (2015) reexamined the taxonomic status of the European and North American morel taxa using a multilocus phylogenetic study based on designated types or original materials. In this work, accepted valid species names were assigned to about 50% of the known

CONTACT Timothy J. Baroni Tim.Baroni@cortland.edu

Published online 04 Dec 2018

phylogenetic taxa indicated by the *Mes* and *Mel* designators. Richard et al. (2015) also provided a summary of synonyms for each accepted taxon. In the case of *M. vulgaris* (*Mes*-17), a European morel with a designated epitype, at least eight different synonyms are listed.

Despite the progress made on North American morels, knowledge concerning their diversity and phylogenetic relationships in South America (Spegazzini 1909; Gamundi 1975, 2010; Domínguez de Toledo 1987; Pildain et al. 2014; Pinzón-Osorio and Pinzón-Osorio 2017), Mexico, and the Caribbean (Gómez 1971; Guzmán et al. 1985; Guzmán and Tapia 1998) is limited. Pildain et al. (2014) reported *M. frustrata* Kuo (now considered *M. tridentina* Bres. per Richard et al. 2015) and *M. septimelata* Kuo, two fire-adapted species recently described from North America, from Argentina. The latter two species may have been introduced into South America via tree plantations of nonnative plants (Pildain et al. 2014). However, they also listed one provisionally new taxon they labeled *Mel*-37, an addition to the informal classification, based on morphological and molecular data. These three taxa, the two fire-adpated species originally described from North America and *Mel*-37, were reported occurring with native *Austrocedrus* and *Nothofagus* tree species (Pildain et al. 2014). Speggazini (1909) had described *M. patagonica* Speg, from Patagonia as well, but this taxon was not reported by Pildain et al. (2014). Three new species have been described from Central America and Mexico, *M. herediana* Gómez from Costa Rica (Gómez 1971), *M. guatemalensis* Guzmán, M.F. Torres & Logem. from Guatemala (Guzmán et al. 1985), and *M. rufobrunnea* Guzmán & Tapia from southern Mexico (Guzmán and Tapia 1998). Several *Morchella* species previously reported from South America, Central America, and Mexico based on European morphological species, e.g., *M. esculenta, M. elata*, and *M. conica*, require reexamination using molecular systematic data to determine which morel species actually occur in these areas (Du et al. 2012b).

The following contribution provides complete descriptions and images for four new American species of morels recognized by a four-gene phylogenetic analysis. It also illustrates the usefulness of properly prepared ascospores for scanning electron microscopy (SEM) imaging and morel taxonomy. Two of the species, *Mel*-18 and *Mes*-14, were informally designated in a previous study (O'Donnell et al. 2011) but are here formally described from the neotropics. Two recently discovered new species not listed in the *Mel*/*Mes* informal designation scheme are also presented, one from Peru in the Esculenta clade and the second from Arizona in the Elata clade. Most of these new taxa are found at higher elevations. All except *M. gracilis* are not widespread geographically and do not seem to produce ascomata frequently nor in abundance when they do occur. These new discoveries raise the number of morel species known from the Americas to 27 (Gómez 1971; Guzmán et al. 1985; Richard et al. 2015).

MATERIALS AND METHODS

Specimens and cultures.—Two collections of *M. kaibabensis*, two of *M. hispaniolensis*, one of *M. peruviana*, and 11 of *M. gracilis* were analyzed in the present study (TABLE 1). Some species are represented by only one or two collections at this time, owing to the remoteness of the areas explored and/or the unsafe political or social difficulties in reentering these areas. In addition, the holotype of *M. palazonii* Clowez & L. Romero (Clowez et al. 2015) was borrowed and subjected to multilocus sequence analysis to infer its position in the Esculenta clade.

Collections were photographed in situ and/or in the laboratory. Macroscopic features were recorded from fresh specimens. Colors were noted using Ridgway (1912), with color names in quotation marks, e.g., "Warm Buff," or Kornerup and Wanscher (1978), with color names coded, e.g., yellow brown (5C5, Topaz). In some cases, general color terms, e.g., hazel, were also recorded and are indicated without quotation marks/parentheses. The term sulcus refers to the depressed groove between the cap and stipe connection when present.

Tissue sections were prepared from dried material rehydrated in 95% ethanol (ETOH) followed by soaking in distilled water or directly in distilled water, then mounted in Melzer's reagent, 14% clear ammonia, or 3% clear potassium hydroxide (KOH). Some preparations were made in 1% Congo red in ammonia to differentiate hyphal structures under transmission light microscope (LM) using bright-field and differential interference contrast (DIC) optics (Zeiss KF2 [Carl Zeiss Microscopes, Oberkochen, Germany] or Olympus BX50 [Olympus Corporation of the Americas, Waltham, Massachusetts]).

In the descriptions of microscopic structures, the following notations were used: n = number of spores measured, X = mean, ± standard deviation, Q = the length divided by the width of an individual spore and then given in a range, and Q^m = the mean of the range for Q values. Ascospores were also observed under a scanning electron microscope (SEM) from either air-dried samples or samples that had been rehydrated and critical point–dried in CO_2 (Kluting et al. 2014). These dried tissues were mounted on SEM stubs and coated with 300–350 Å of gold or gold-palladium prior to

Table 1. DNA sequences used in this study.

Species	Collection identifiers/cultures[a]	Source	Locality	GenBank accession numbers			
				ITS	*TEF1*	*RPB1*	*RPB2*
M. galilaea	DH-629, M308, NRRL 22924	This study; O'Donnell et al. 2011	Hawaii	MH014710	GU551154	GU551266	GU551322
M. galilaea	DED-7254, M685, NRRL 37041	This study; O'Donnell et al. 2011	Java, Indonesia	MH014709	GU551147	GU551259	GU551315
M. gracilis	TJB-9483, CORT13766, DR-2481	This study	Dominican Republic	MH014706	MH014703	MH014714	MH014717
M. gracilis	Cesari s.n., "USB," M324	O'Donnell et al. 2011; Du et al. 2012a	Aragua, Venezuela	JQ723087	GU551163	GU551275	GU551331
M. gracilis	Cesari s.n., "USB," M330	O'Donnell et al. 2011; Du et al. 2012a	Aragua, Venezuela	JQ723085	GU551530	GU55162	GU551677
M. gracilis	TL-9571, M684, C-F-58308	O'Donnell et al. 2011; Du et al. 2012a	Pichincha, Ecuador	JQ723086	GU551148	GU551260	GU551316
M. gracilis	J. Llovera & L. Villalles #8,"USB" M686, NRRL37053–37064	This study	Parque Nacional El Anda, Venzuela	MH014707	MH014704	MH014715	MH014718
M. hispaniolensis	Cantrell RD-9744, DR-298 M374, NY02861410	This study	Dominican Republic	MH014725	—	—	MH014741
M. hispaniolensis	Cantrell RD-9745, DR-326 M375, NY02861411	This study	Dominican Republic	MH014726	MH014720	MH014731	MH014736
M. kaibabensis	TAC-1376, KOD1438, NRRL 66752	This study	Arizona	MH014727	MH014721	MH014732	MH014737
M. kaibabensis	TAC-1708, KOD1793, ARIZ- AN043595	This study	Arizona	MH014728	MH014722	MH014733	MH014738
M. palazonii	PhC149	This study; Clowez et al. 2015	Spain	KT883899	MH781723	MH781725	MH781726
M. peruviana	CIPHAM-004, KOD1445–1447, NY02861412, NRRL 66754	This study	Peru	MH014708	MH014705	MH014716	MH014719
M. purpurascens	C-15146, M456	This study	Denmark	MH014730	MH014724	MH014735	MH014740
M. purpurascens	C-16014, M453	This study	Denmark	MH014729	MH014723	MH014734	MH014739

[a]Cultures: NRRL curated at ARS Culture Collection (nrrl.ncaur.usda.gov) and KOD in the laboratory of Kerry O'Donnell.

examination using a JEOL 6400V (xx, xx) described in Elliott et al. (2014) or a JEOL 6010PLUS/LA at 20 kV described in Matheny et al. (2017).

Pure cultures of the four species featured in the present study were obtained by germinating ascospores overnight on 3% water agar supplemented with antibiotics (Elliott et al. 2014). Once the identity of each pure culture was verified by sequencing portions of four marker loci (see below), it was suspended in a cryogen composed of 10% skim milk, to which 1% dimethyl sulfoxide (DMSO) was freshly added for long-term storage at −175 C in liquid nitrogen vapors in the United States Department of Agriculture Agricultural Research Service (ARS) Culture Collection (NRRL, http://nrrl.ncaur.usda.gov/) and at −80 C in the laboratory of Kerry O'Donnell, with KOD accession numbers. All voucher specimens, except those from Venezuela, are deposited in herbaria designated by internationally accepted codes following Thiers [continuously updated] (http://sweetgum.nybg.org/science/ih/). All collections cited from Venezuela are deposited in the herbarium of the Universidad Simon Bolivar, Sartenejas, Caracas, which currently lacks an internationally recognized code. Those collections are given the provisional designation "USB" in this paper. Also, all individual collections that yielded DNA for this analysis are labeled with a separate unique identifier, e.g., M324. These unique identifying M numbers are helpful in tracking the "USB" collections that lack herbarium accession numbers at this time or even collector field numbers. Unfortunately, the "USB" collections are currently not accessible due to political unrest, but they remain curated at Universidad Simon Bolivar for the foreseeable future. Therefore, these M numbers are necessary for tracking the original DNA extractions from those "USB" collections that are now being kept at −20 C in the O'Donnell laboratory. In addition, the M numbers are indispensable for searching for previously deposited *Morchella* sequences in GenBank. A summary of unique identifiers for each species can be found in TABLE 1.

DNA extraction, sequencing, and phylogenetics.—Protocols for extracting total genomic DNA from pure cultures and herbarium specimens, polymerase chain reaction (PCR) amplification, and DNA sequencing have been published previously (O'Donnell et al. 2011). Portions of the following four genetic marker loci were sampled: translation elongation factor 1-α (*TEF1*), RNA polymerase II largest (*RPB1*) and second largest (*RPB2*) subunits, and the nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region. See Taşkın et al. (2010) for PCR protocols and sequencing primers. ABI 3730 (Applied Biosystems,

Foster City, California) sequence chromatograms were edited with Sequencher 5.2.4 (Gene Codes, Ann Arbor, Michigan), exported as NEXUS files, and then aligned with MUSCLE software (Edgar 2004) in SeaView (Gouy et al. 2009).

Two four-gene data sets were assembled, one for the Esculenta clade and the second for the Elata clade. The best-fit model of molecular evolution for each of the four partitions (*TEF1, RPB1, RPB2*, ITS) was identified using ModelFinder (Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion scores. Maximum likelihood (ML) analyses were implemented in IQ-TREE (Nguyen et al. 2015; http://www.iqtree.org/), applying separate models for each partition (Chernomor et al. 2016). Based on results of more inclusive phylogenetic analyses (Voitk et al. 2016), and preliminary analyses that suggested that *M. hispaniolensis* and *M. kaibabensis* might be sister taxa in the Elata clade, we constructed and analyzed a four-locus data set that included ingroup sequences of the 10 closest relatives of the former two taxa. The ML phylogeny was rooted on sequences of *M. deliciosa* from Sweden and Turkey based on the analysis presented in Voitk et al. (2016), which indicated that it was an appropriate outgroup. Additionally, based on our preliminary analyses that indicated that *M. gracilis* and *M. peruviana* of the Esculenta clade might be nested with *M. galilaea* and three unnamed species from China (*Mes*-13, *Mes*-15, *Mes*-19), we constructed and analyzed a data set with seven ingroup taxa, in which the ML phylogeny was rooted on sequences of *M. americana* based on more inclusive analyses (fig. 1 in Clowez et al. 2015 and Loizides et al. 2016). The Esculenta and Elata four-gene data sets were combined and analyzed after ML bootstrapping (1000 pseudoreplicates) of the individual gene data sets did not reveal any conflict between strongly supported nodes. DNA sequences generated in the present study were deposited in the National Center for Biotecnology Information (NCBI) GenBank as accessions MH014703–MH014744 and MH781722–MH781727. NEXUS files and ML trees inferred for the combined Elata and Esculenta clade data sets were deposited, respectively, at TreeBASE as accession ID S22385, Tree ID numbers Tr113172 (Elata clade) and Tr113171 (Esculenta clade).

RESULTS

Molecular phylogenetics.—The four-gene Elata clade data set amounted to 4.39 kb and was used to investigate the species status and evolutionary relationships of two putatively novel true morels, *M. hispaniolensis* Cantrell RD-9744 (= NRRL 26636) and Cantrell RD-9745 (= NRRL 26632) from the Dominican Republic and *M. kaibabensis* TAC-1376 (= NRRL 66752) and TAC-1708 (= NRRL 66753) from the Kaibab Plateau in northern Arizona. *Morchella hispaniolensis* and *M. kaibabensis* both received 100% bootstrap support in the ML bootstrap analysis (FIG. 1); however, their reciprocal monophyly and evolutionary relationships with other species in the Elata clade were unresolved.

The Esculenta clade data set included 3.6 kb. Multilocus sequence data were generated in the present study for *M. gracilis* TJB-9483 from the Dominican Republic, *M. peruviana* CIPHAM-004 (= NRRL 66754) from the Peruvian Andes, and the holotype of *M palazonii* PhC149 from Spain (FIG. 2). *Morchella gracilis* and *M. peruviana* received 92% bootstrap support as reciprocally monophyletic sister groups in the partitioned ML bootstrap analysis (FIG. 2); however, their relationship with *M. palazonii* and other members of the ingroup were unresolved.

TAXONOMY

Morchella kaibabensis Beug, T.A. Clem. & T.J. Baroni, sp. nov. FIG. 3A–D

MycoBank MB823939

Typification: USA. ARIZONA: Coconino County, along Forest Road 293, Kaibab Plateau, North Rim, Grand Canyon National Park, 36.40806, −112.26692, 2700 m, with *Picea engelmannii* and *Picea pungens*, 21 May 2017, *T.A. Clements & D.M. Fulton TAC-1708* (*KOD1793*) (**holotype** ARIZ AN043595). Ex-type culture: NRRL 66753. GenBank: ITS = MH014728; *TEF1* = MH014722; *RPB1* = MH014733; *RPB2_a* = MH014738; *RPB2_b* = MH014744.

Etymology: kaibabensis (Latin), named after the Kaibab Plateau in northern Arizona where the specimens were collected.

Diagnosis: Distinguished from other members of the Elata clade by phylogenetic analysis of combined *TEF1*, ITS, *RPB1*, and *RPB2* sequences. Differing from the similar looking *M. brunnea* by a shorter, firm-fleshed stipe, and from *M. snyderi*, which it resembles and occurs in the same geographic location, but *M. snyderi* mostly produces ascomata in clusters, not singly as in *M. kaibabnesis*.

Description: Ascomata 60–90 mm tall. Pileus conical to ovate, 30–60 × 20–55 mm at the widest point, attached to stipe with a sulcus 3–5 mm deep and 5–10 mm wide, small white radial ridges cross the sulcus; pileus pitted and ridged, ridges 2–6 mm broad, with 10–20 primary vertical ridges and some shorter, secondary, vertical ridges between irregular pits, with sunken transecting ridges often at some angle to the horizontal, ridges finely tomentose, “Pale Olive-Buff” to “Pinkish Buff” when young, aging “Fuscous Black,” dried ridges “Deep Mouse Grey,” flattened when

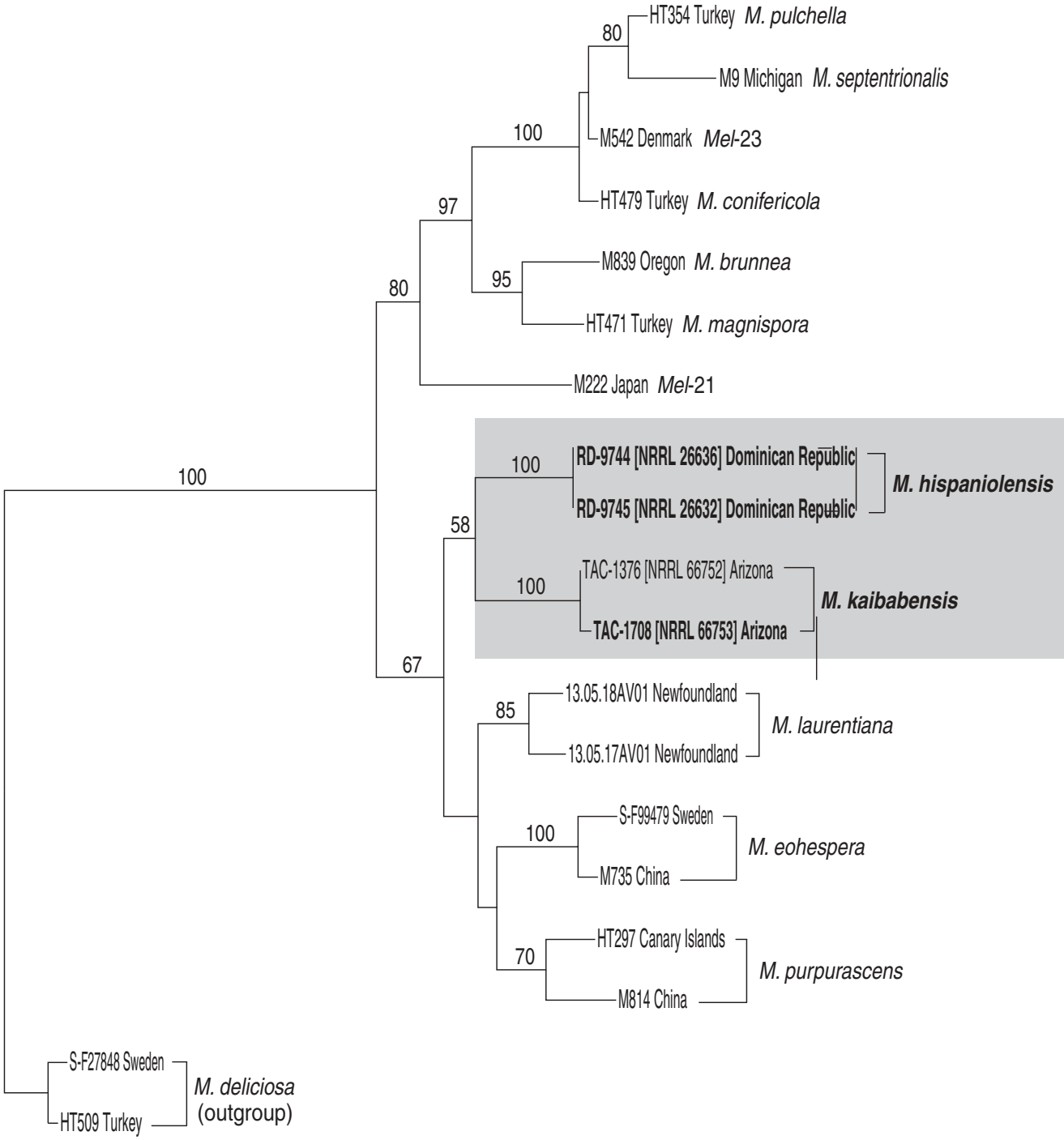

Figure 1. ML phylogeny inferred for 12 ingroup species in the Elata clade of *Morchella* based on analysis of a 4.39-kb four-gene data set including portions of *TEF1, RPB1, RPB2*, and ITS. Numbers above nodes represent ML bootstrap support values based on 1000 pseudoreplicates of the data. Ex-type strains are identified by bold font. HT = Hatıra Taşkın; M = O'Donnell laboratory genomic DNA code; NRRL = ARS Culture Collection; TAC = Terri A. Clements.

young, not becoming thin or eroded at maturity; transecting ridges not nearly as dark as vertical ones. Pits primarily vertically elongated, although in some ascomata wider than tall, finely tomentose; "Pale Olive-Buff " to "Honey Yellow" when young, aging "Dark Citrine," drying "Warm Buff" (FIG. 3A–B). Stipe cylindrical to clavate with chambers at base, 20–30 mm tall, 15–45 mm wide, with small ridges extending across sulcus to the pileus, stipe surface finely mealy with soft off white granules, overall colorless to "Pale Pinkish Buff." Context of stipe and pileus white to "Ivory Yellow," 2–3 mm thick, layered and chambered

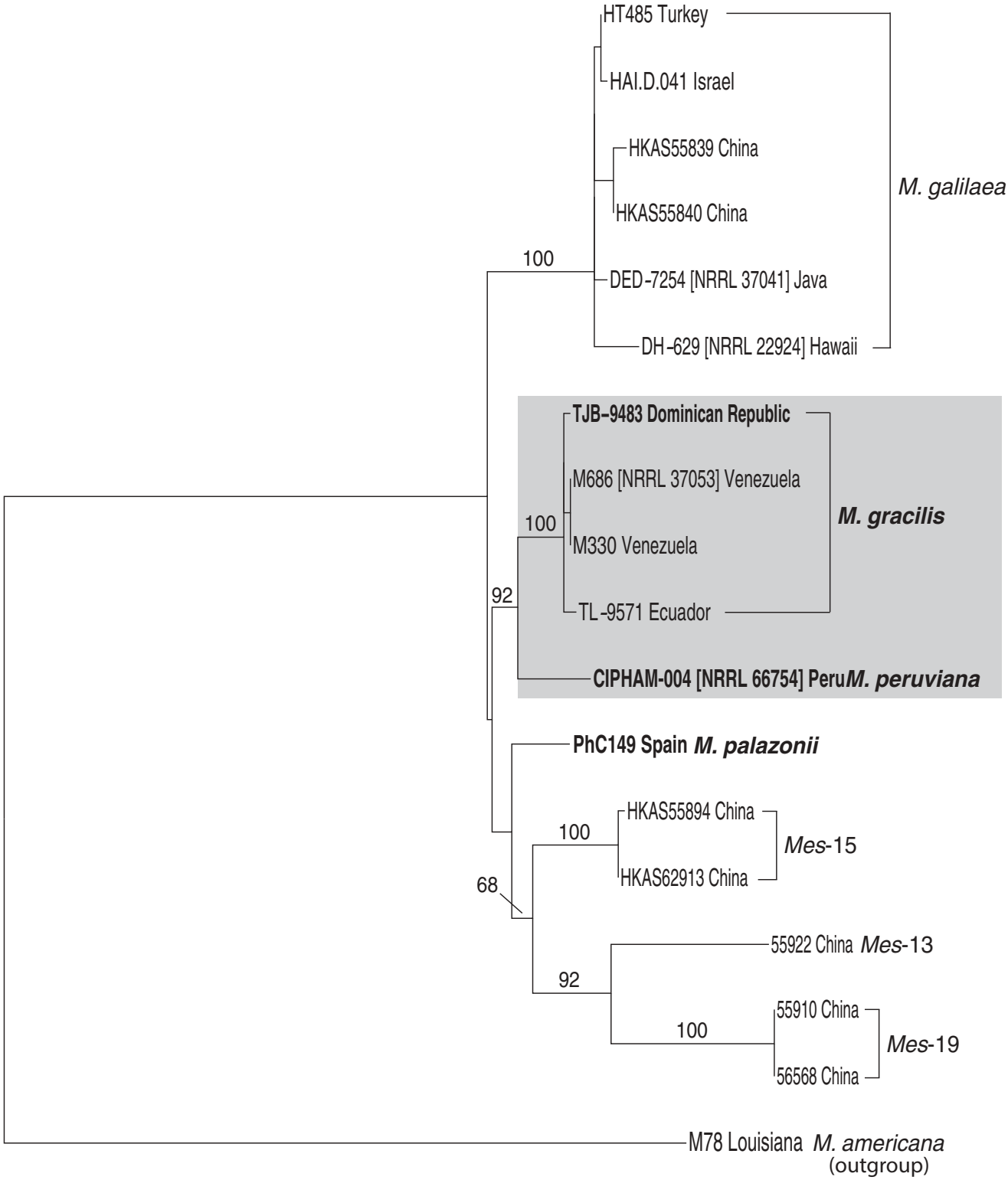

Figure 2. ML phylogeny of seven ingroup species in the Esculenta clade of *Morchella* based on analysis of a 3.6-kb data set including portions of *TEF1, RPB1, RPB2*, and ITS. ML branch support is based on 1000 pseudoreplicates of the data. Holotype collections of *M. gracilis* TJB-9483, *M. peruviana* CIPHAM-004, and *M. palazonii* PhC149 are identified by bold font. CIPHAM = Centro de Investigación y Producción de Hongos Alimenticiois y Medicinales; DED = Dennis E. Desjardin; DH = Don Hemmes; HKAS =Herbarium of Cryptogams; Kunming Institute of Botany; M = O'Donnell lab genomic DNA code; NRRL = ARS Culture Collection; PhC = Philippe Clowez; TJB = Timothy J. Baroni; TL = Thomas Læssøe.

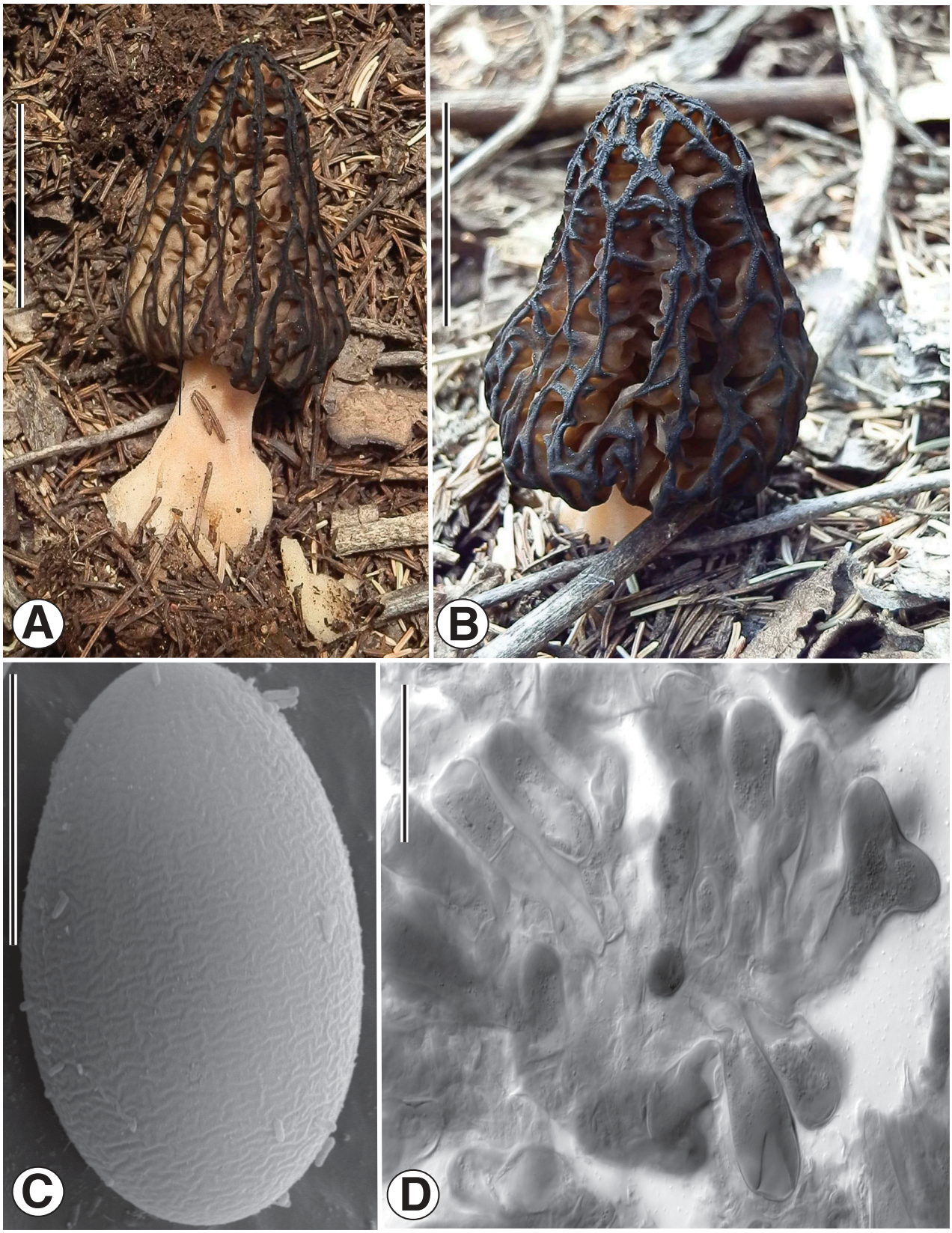

Figure 3. *Morchella kaibabensis*. All images from the holotype TAC-1708. A–B. Ascomata (images by T. A. Clements). C. SEM image of ascospore. D. Acroparaphyses. Bars: A–B = 35 mm; C = 10 µm; D = 50 µm.

near the stipe base, upper portion of the stipe becoming ridged in most specimens. Sterile inner surface white and pubescent. Odor and taste unknown.

Ascospores (17.5–)21–26(–27) × (10–)13–18 µm (n = 20, X = 23 ± 1.5 × 15 ± 1.4; Q = 1.3–1.8, Q^m = 1.6 ± 0.1), ellipsoid, hyaline, smooth or finely stippled at 1000× under DIC, finely rugulose under SEM, longitudinal striae absent or obscure (FIG. 3C); spore wall ±1 µm thick. Asci cylindrical, 200–300 × 18–23 µm, 8-spored, hyaline, inamyloid, operculate, thin-walled. Paraphyses scattered, versiform, terminal cell typically cylindrical to subclavate but some capitate or lageniform; 200–300 µm long, 4–6 µm wide at the base, expanding up to 32 µm at the apex; septate with terminal cell 65–130 × 18–32 µm, hyaline or pale yellowish-tan in KOH. Sterile elements on ridges (acroparaphyses) dark fuscous-brown from intraparietal and plasmatic or vacuolar pigments, cells mostly swollen and clavate but also cylindrical, lanceolate, subcapitate, capitate, or dichotomously branched (FIG. 3D), 120–170 × 10–25(–40) µm, septate with terminal cell 56–96 µm long and filled with dark fuscous-brown, often coagulated pigments. Stipe tissues composed of three distinct layers, from outermost inward, a textura globosa, then a textura oblita, and finally a textura globosa: outermost layer pale yellow in KOH, 400–480 µm thick, composed of mostly thin-walled, globose or greatly inflated ellipsoidal cells, often with collapsing accordion-like walls, 22–70 × 20–40 µm, producing scattered mounds of clustered, suberect, inflated, thin-walled, mostly fusoid or conical, some very short conical, some clavate terminal cells on top of short chains of inflated cells (granulate ornamentation), individual terminal cells 20–32(–52) × 12–20(–42) µm; middle layer 240–320 µm thick, composed of tightly interwoven, mostly thick-walled cylindrical hyphae, 5–16 um wide, with very thick-walled (up to 2 µm), darkly golden refractive septa that appear donut-like when viewed directly down a hypha through the septum; innermost layer 320–400 µm thick, resembling the outermost layer, composed of large, inflated, thin-walled cells, 30–52 × 26–44 µm.

Ecology and distribution: On moist forest soils at 2700–2800 m under spruce and pine (*Picea, Pinus*) and aspen (*Populus*), also isolated as an environmental sample from living tissue of *Juniperus scopulorum* (GQ153010); Arizona (type) and New Mexico (GQ153010). May.

Other specimens examined: USA. ARIZONA: Coconino County, north of Forest Road 222, on the North Rim of the Grand Canyon, in the Kaibab National Forest, 36.40295, −112.23186, 2700 m, on soil under *Picea engelmannii* and *Picea pungens* with *Pinus ponderosa* and *Populus tremuloides* nearby, 19 May 2016, *T.A. Clements & D.M. Fulton TAC-1376* (KOD1438 and NRRL 66752), GenBank: ITS = MH014727; *TEF1* = MH014721; *RPB1* = MH014732; *RPB2_a* = MH014737; *RPB2_b* = MH014743. NEW MEXICO, San Juan County, Navajo Nation, isolated from photosynthetic tissue of *Juniperus scopulorum*, culture by A. E. Arnold DC2069 (ARIZ; ITS and 28s rRNA, GenBank GQ153010).

Comments: Ascomata of *M. kaibabensis* were collected in mid- to late May in the Kaibab National Forest. Also occurring in the same area at that time was *M. brunnea* M. Kuo (*Mel*-22), which differs from *M. kaibabensis* by the longer, more fragile stipes, although it can only reliably be distinguished from *M. kaibabensis* by DNA analysis. *Morchella brunnea* is widespread in the Pacific Northwest, California, Montana, and Arizona, fruiting from late Mar through mid-May depending on the elevation. *Morchella snyderi* M. Kuo & Methven (*Mel*-12) also is known from the same area at the same time of season but sometimes can be distinguished by the tendency to grow in clusters. When occurring singly, *M. snyderi* can only be reliably distinguished from *M. kaibabensis* by DNA data. We have studied collections of *M. snyderi* from the Pacific Northwest, California, Montana, and Arizona where it occurs from mid-Apr through May. *Morchella eohespera* Beug, A. Voitk & O'Donnell (*Mel*-19), like *M. brunnea*, typically has a longer and more fragile stipe. Although often associated with spruce, *M. eohespera* is so far known only from more northern locations in North America, Europe, and Asia.

In a BLASTn search of the NCBI database, a 100% match to *M. kaibabensis* was discovered. The matching sequence, GQ153010, was obtained from an endophyte culture taken from living photosynthetic tissue of *Juniperus scopulorum* obtained by A. E. Arnold (University of Arizona) from a station east of the Grand Canyon National Park in the Chuska Mountains in the Navajo Nation. Two other species of *Morchella, M. sextelata* M. Kuo (*Mel*-6) and *M. snyderi*, have also been documented as endophytes (Baynes et al. 2012). These morels isolated from cheatgrass (*Bromus tectorum*) were considered important partners for cheatgrass survival in the fire-dominated ecology of the plant, since they increased seed survival in high heat conditions produced by such fires. In the present case with *Juniperus*, it is not obvious what the ecological role may be for evolution of this association. Morels, at least those in the Elata clade, may be more common as endophytes than we currently realize.

The ascospores of *M. kaibabensis*, as viewed under SEM, reveal a subtle rugulose ornamented surface (FIG. 3C). This type of ascospore surface ornamentation is

similar to that found in *M. gracilis* but less pronounced. All similarities end there, since these two species possess different ascomata morphologies and occur in different clades. The acroparaphyses of *M. kaibabensis* are also distinctive due to the inflated and variable nature of the terminal cells and the dense dark internal and often coagulated pigmentation. Additional color images of *M. kaibabensis* taken by T. A. Clements may be found on MushroomObserver.org (MO277102, MO239180).

Morchella hispaniolensis S.A. Cantrell, Lodge, T.J. Baroni & O'Donnell, sp. nov. FIG. 4A–D
MycoBank MB823940

Typification: DOMINICAN REPUBLIC. LA VEGA PROVINCE: South of Constanza off Route 41, in the Cordillera Central, south of Parque Nacional Valle Nuevo, terrestrial in pine needle litter in old growth *Pinus occidentalis* forest, 18.774, –70.6272, 2200 m, 8 Jan 1997, *S. A. Cantrell RD9744* (*DR-298, M374*) (**holotype** NY02861410). Ex-type cultures: NRRL 26636–26637. GenBank: ITS = MH014725; *RPB2_b* = MH014741.

Etymology: *hispaniolensis* (Latin), from the island of Hispaniola.

Diagnosis: Distinguished from other members of the Elata clade by phylogenetic analysis of combined *TEF1*, ITS, *RPB1*, and *RPB2* sequences, by the dark honey- to hazel-colored rounded conical pileus, and by the strongly ridged ornamented ascospores under the SEM.

Description: Ascomata 6.5–12.5 cm tall. Pileus conical, 30–65 mm high, 20 mm broad at base, 28 mm broad at widest point just below middle, tapering to a rounded conical apex, longitudinally pitted with 4–6 elongated pits; pits 5–20 mm long, 3–5 mm broad, mostly wrinkled and undulate inside each pore but otherwise glabrous; ridges finely hoary-pubescent, honey-colored when young, hazel at maturity. Stipe cylindrical or enlarged at apex and base, subtly ridged from apex to base, 35–60 mm tall, 12 mm wide at apex and up to 17 mm wide at base with narrowest width 10 mm, pale cream color, densely pruinate-granular overall. Odor and taste unknown.

Ascospores 16–22.5 × 11.5–16 µm (n = 34, X = 20.2 ± 1.3 × 13.5 ± 1.2 µm; Q = 1.3–1.9, Q^m = 1.5 ± 0.1), ovoid-ellipsoid or ellipsoid, appearing smooth under LM, under SEM strongly ridged from pole to pole (FIG. 4B) and with moderately developed, lower-in-height secondary lateral branching over most of spore surface, but also on some spores at both poles somewhat maze-like from equal-height ridges; wall slightly thickened, acyanophilic, inamyloid, uniseriate in ascus. Asci cylindrical or subclavate, 180–280 × 16–28 µm, 8-spored, inamyloid, operculate. Paraphyses hyaline in KOH, sparsely distributed, obscure, apices well below ascus tips in mature tissues; narrowly cylindrical, subclavate, or narrowly ventricose, septate 1 or 2 times, 100–165 × 5–14 µm. Sterile elements (acroparaphyses; FIG. 4D) on ridges and upper sides of pits subclavate, broadly ventricose, subcapitate, sometimes with broad or narrow, finger-like projections, sterile elements larger on ridges of pits, 94–140 × 24–46 µm, compared with those on sides of pits, 50–94 × 12–22 µm, often septate below, sometimes branched below, and in rows or clusters. Stipe tissues complex, composed of four distinct layers, from outermost inward, a textura porrecta, then a mixture of textura porrecta and textura globosa, then entirely textura porrecta, with innermost layer a mixture of textura porrecta and textura globosa: thin outermost layer pale yellow in KOH and a combination of repent, cylindrical, thin-walled hyphae, 4–8 µm wide, producing scattered mounds of clustered, erect, inflated, thin-walled, mostly clavate terminal cells on top of chains of inflated cells (granulate ornamentation); individual terminal cells 44–80 × 18–38 µm, sometimes penultimate cell branched with two terminal cells on a large, inflated, penultimate cell, all cells with golden refractive internal pigments; directly below the thin surface layer is a distinct layer, 160–240 µm thick, composed of large, thin-walled, inflated, isodiametric cells, 30–40 µm wide, in chains and surrounded by interwoven cylindrical hyphae, 4–8 µm wide; central layer 400–640 µm thick, composed of only loosely interwoven, thin-walled, cylindrical hyphae, 4–10 µm wide; innermost layer 160–240 µm thick, resembling the subsurface layer, composed of large, inflated, thin-walled cells in chains, 24–74 µm wide, interlaced with cylindrical hyphae 4–10 µm wide.

Ecology and distribution: Solitary or scattered on soil in needle litter under *Pinus occidentalis*, 2200 m; Dominican Republic, Hispaniola (type). Jan.

Other specimen examined: DOMINICAN REPUBLIC. LA VEGA PROVINCE: Same locality as holotype, under *P. occidentalis*, 8 Jan 1997, *S.A. Cantrell RD9745* (alternate ledger *DR-326* [= M375]; cultures: NRRL 26632–26634; NY02861411). GenBank: ITS = MH014726; *TEF1* = MH014720; *RPB1* = MH014731; *RPB2_a* = MH014736; *RPB2_b* = MN014742.

Comments: Morchella hispaniolensis (*Mel*-18; O'Donnell et al. 2011) is associated with *Pinus occidentalis*, an endemic pine of Hispaniola found at higher altitudes in the Central Mountains of the Dominican Republic. It occurs in Jan like *M. gracilis*. *Morchella hispaniolensis* is the first species of morel documented for the island that belongs in the Elata clade (FIG. 1). It appears to be rare known thus far only from two collections.

A second species of morel, *M. gracilis*, is described below in this report as also from Hispaniola, but this taxon occurs in South America as well. *Morchella gracilis* is a member of the Esculenta clade, differing from *M. hispaniolensis* by the

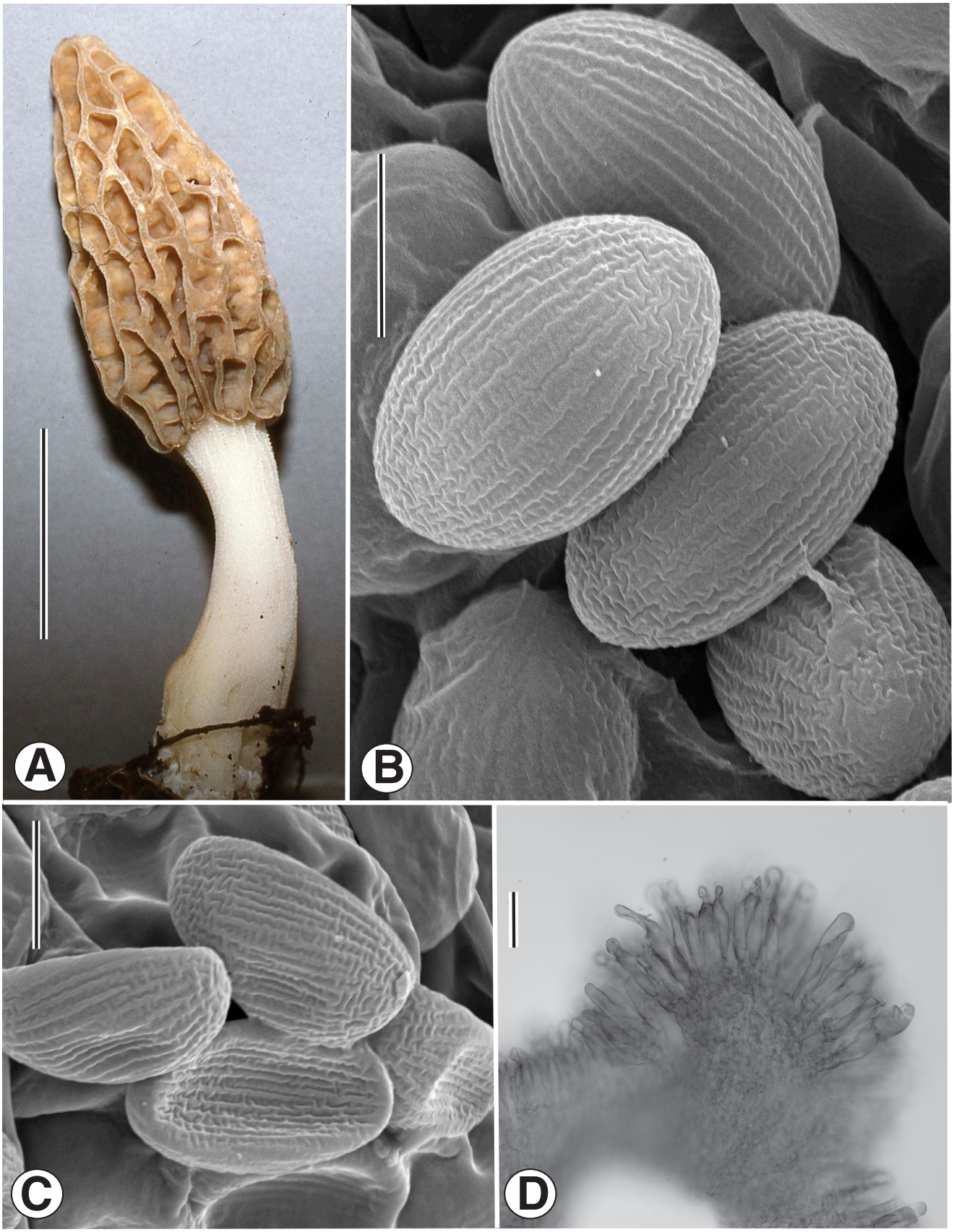

Figure 4. *Morchella hispaniolensis*. A. Ascoma, S.A. Cantrell RD-9745 (DR-326) (image by S. A. Cantrell). B. SEM image of ascospores after rehydration and critical point drying, S.A. Cantrell RD-9744 (= DR-298, holotype). C. SEM image of air-dried ascospores S.A. Cantrell RD-9744 (= DR-298, holotype). D. Acroparaphyses on ridge of pileus, S.A. Cantrell RD-9744 (= DR-298, holotype). Bars: A = 35 mm; B–C = 10 µm; D = 100 µm.

yellow colors at some phase of development. Additionally, as its name implies, *M. gracilis* has a slender, typically curving pileus that differs from the erect, broad, conical pileus of *M. hispaniolensis*. Microscopically, the two species are similar in ascospore morphology and distribution of sterile cells (acroparaphyses) on ridges of the pileus. One significant morphological difference between these two species is the strongly developed ridging along the length of the ascospores for *M. hispaniolensis*. These ridges were observed under SEM (FIG. 4B–C). *Morchella gracilis* ascospores lack the pole-to-pole ridges or striae observed in *M. hispaniolensis*, but they do show a more elaborate pattern of secondary short, lateral, maze-like, low-branching ridges of mostly equal height under SEM (FIG. 5C). This maze-like pattern of ascospore ornamentation is distinctive.

Morchella hispaniolensis is a phylogenetic sister species to *M. kaibabensis* in the Elata clade (FIG. 1). *Morchella kaibabensis* has been found so far only in Arizona and New Mexico. *Morchella hispaniolensis* is paler colored than *M. kaibabensis*. The pileus of *M. hispaniolensis* is honey-colored, turning a darker hazel color in some with age, with the ridges and concave pits of similar color. Although the pileus of *M. kaibabensis* starts out buff, it soon turns mouse gray or fuscous black, and the vertical ridges of the pits are noticeably darker than the rest of the pileus (FIG. 3A–B). In addition, the ascospores of *M. kaibabensis* are generally longer (21–26 × 13–18 µm) than those of *M. hispaniolensis* (16–22.5 ×11.5–16 µm), and the ascospores of *M. kaibabensis* are not obviously striate under the SEM.

Morchella gracilis T.J. Baroni, Iturr. & Læssøe, sp. nov. FIG. 5A–D
MycoBank MB823941

Typification: DOMINICAN REPUBLIC. SANTIAGO PROVINCE: Near town of Antonsape, inside Parque Bermudez, in vicinity of Antonsape Bueno Creek, about 0.4 km into the park from entrance trail head, on soil with mosses and lichens on trail bank near shoulder level with *Pinus occidentalis*, 19.200816, −70.999705, 1010 m, 11 Jan 2003, *TJB-9483* (*DR-2481*) (**holotype** CORT013766). GenBank: ITS = MH014706; *TEF1* = MH014703; *RPB1* = MH014714; *RPB2* = MH014717; 28S = MH014711.

Etymology: *gracilis* (Latin), in reference to the slender gracile ascomata.

Diagnosis: Distinguished from other species in the Esculenta clade by the slender conical pileus, typically curved at apex (FIG. 5A–B), which is longitudinally elongate pitted, with the pileus dull yellow brown or honey-colored or pale grayish, with paler ridges almost white and hoary-pubescent around the pits. Ascomata occurring in disturbed areas, often on soil, with ascospores finely corrugate under SEM (FIG. 5C); also distinct by the phylogenetic analysis of combined *TEF1*, ITS, *RPB1*, and *RPB2* sequences.

Description: Ascomata 7.5–12.5 cm tall. Pileus slender, conical or narrowly conical, pointed and curved at apex, 30–65 mm high, 12–15 mm broad at base, 5.5 mm broad just below apex, with deep, longitudinally elongate alveolate pits, 5–15 mm long, 1–7.5 mm broad, with ridges finely hoary-pubescent, glabrous, but undulate or wrinkled elsewhere; dull yellow brown (5C5, Topaz), honey-colored, or pale grayish, with paler ridges (near 5A2, Orange White), some turning hazel color with age. Stipe equal, longitudinally ridged and wrinkled, especially over base, 40–60 mm tall, 11 mm wide, surface densely white pruinate-pubescent or soft mealy granulate overall, white or pale cream (nearly white) or becoming pale cream. Stipe and pileus hollow, densely pruinate-pubescent or mealy granulate covering on all surfaces inside stipe and pileus. Odor rich, mushroom-like when cut. Taste unknown.

Ascospores (15.5–)16–22.5 × 10.5–15.5 µm (n = 20, X = 19 ± 1.8 x 12 ± 1.2 µm; Q = 1.2–2.0, Q^m = 1.6 ± 0.2), ovoid-ellipsoid, appearing smooth under LM, rugulose or corrugated or maze-like under SEM (FIG. 5C); wall 0.5 µm thick, acyanophilic, inamyloid, uniseriate in ascus, pale yellow in deposit. Asci cylindrical or narrowly clavate, 160–380 × 16–30 µm, 8–spored, inamyloid, operculate. Paraphyses hyaline, cylindrical or narrowly clavate, septate 2–5 times over lower portion, 130–156 × 8–10 µm, some branched below first or second septum, hyaline in KOH. Sterile elements (acroparaphyses; FIG. 5D) on ridges and sides of pits subclavate, sterile elements longer and narrower on the ridges of pits, 60–114 × 10–12 µm, those on sides of pits are shorter and broader, 30–66 × 18–34 µm, both often septate below, sometimes branched below, and in rows or clusters, neither found in hymenium with asci. Stipe composed of three distinct layers: a thin outer layer of textura porrecta, an internal thicker layer of textura globosa, and an innermost layer of textura oblita; surface cells pale yellow in KOH; outermost layer a combination of repent, cylindrical, thin-walled hyphae, 4–10 µm wide, producing scattered mounds of clustered, erect, inflated, thin-walled cells in chains (granulate ornamentation), individual cells 20–66 × 18–34 µm; directly below this surface layer is a distinct layer, 200–400 µm thick, composed of large, thin-walled, inflated cells, 46–120 × 25–60 µm, stacked 3–4 cells or more deep with walls so thin some collapsing or folding in accordion-like fashion on anticlinal walls; innermost layer 200–400 µm thick, composed of interwoven, thick-

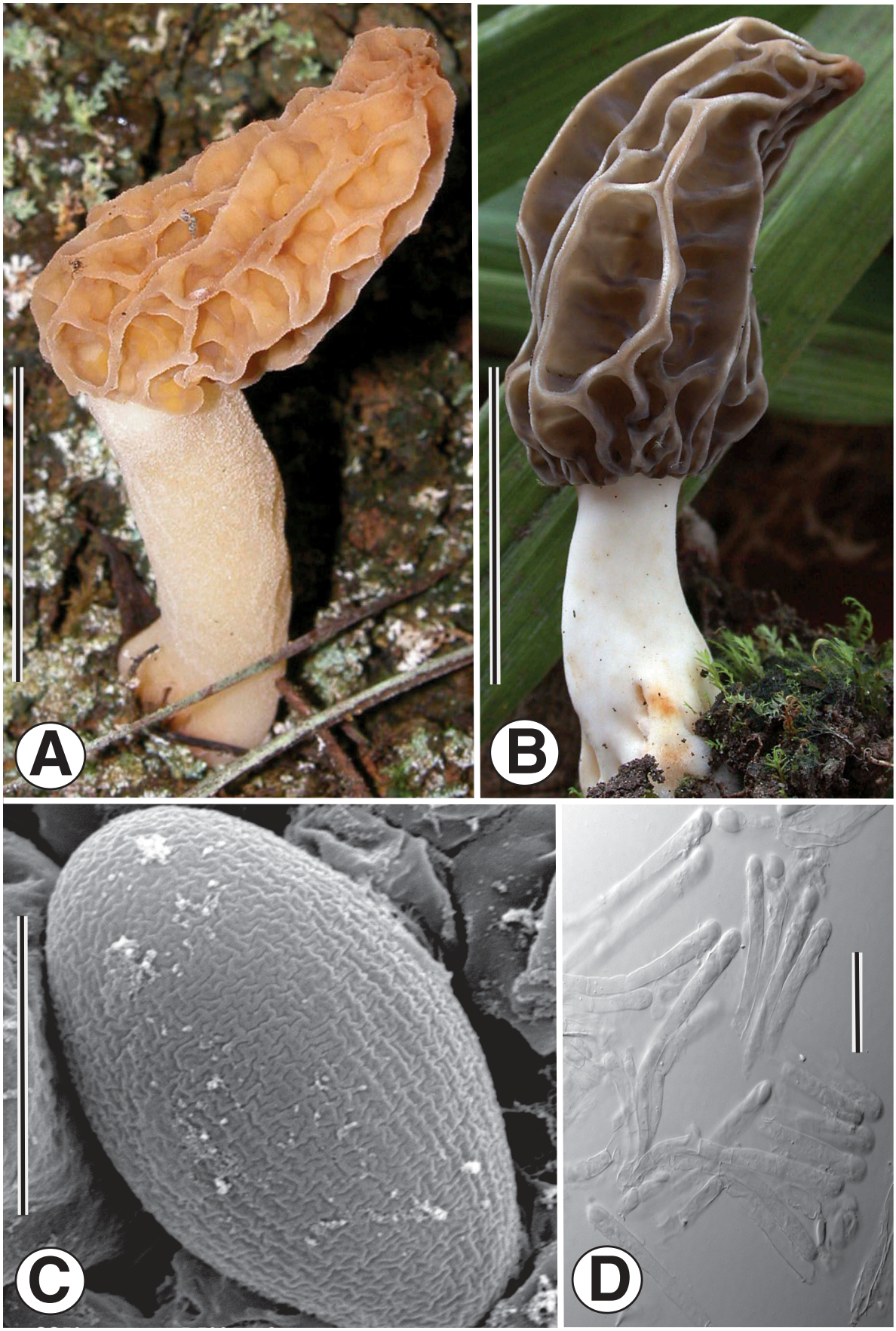

Figure 5. *Morchella gracilis*. A. Ascoma, TJB-9483, holotype (image by T. J. Baroni). B. Ascoma, TL-9571 (image by T. Læssøe). C. SEM image of ascospore, TJB-9483, holotype. D. Acroparaphyses, TJB-9483, holotype. Bars: A–B = 25 mm; C = 10 µm; D = 50 µm.

walled, cylindrical hyphae, 4–12 µm wide, with shiny refractive septa thicker than the side walls of the hyphae and appearing donut-like when viewed down a hyphal cell through a septum.

Ecology and distribution: Solitary or scattered on sandy-clay soil with *Pinus occidentialis*, on clay or rocky soils lacking much vegetation, with grasses, or in *Chusquea*-dominated forest, 1010–2850 m; Dominican Republic, Ecuador, and Venezuela. Jan, May–Jun, Oct.

Other collections examined: ECUADOR. PICHINCHA PROVINCE: Pasochoa, in *Chusquea*-dominated mountain forest, on soil at cut trail edge with *Geocoryne variispora* Korf, 2850 m, 18 May 2002, *T. Læssøe TL-9571* (collection split with part at Pontificia Universidad Católica del Ecuador as QCA(M)02155 and the other part at the Natural History Museum of Denmark as C-F-58308). VENEZUELA. ARAGUA STATE: Colonia Tovar, Sector La Capilla, 1700 m, among grass, no date, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M324); same location, 1800 m, terrestrial without vegetation, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M325); same general location in Sector Liceo Viejo, 1730 m, on shale rock, 23 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M326); same locality, 300 m from the old aqueduct, 1800 m, on slope lacking vegetation, 27 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M327); same general location, in Sector La Cara, 100 m from intersection of La Cava and entrance to Colinas Stubinger, 1800 m, on clay slope, 29 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M328); same locality, 100 m from crossroads of La Cava and the entrance to Colinas Stubinger, 1800 m, on clay slope, 29 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M329); same locality, same area and elevation as previous, on clay slope, 29 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M330 and M331); same general locality in Sector Capilla Abajo, 1850 m, on edge of trail, 30 Jun 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M332); same general locality in Sector Collin, 1625 m, on ground near wall, 22 Oct 1992, *C. Cesari s.n.* ("USB"; O'Donnell laboratory M333); Rancho Grande, Maracay, Parque Nacional El Avila, on earth, 28 Oct 1990, *R. Muradian s.n.* ("USB"; O'Donnell laboratory M334); same general locality in Parque Nacional El Avila, on road to Papelón, on soil of slope in disturbed area in cloud forest region, 30 Jul 2002, *J. Llovera & L. Villalba #8* ("USB"; O'Donnell laboratory M686, and ex-paratype cultures NRRL 37053–37064).

Comments: Morchella gracilis (*Mes*-14) may be adapted to disturbed and often bare soil habitats in cooler tropical cloud forests where it is distributed over a wide area in South America and in the Greater Antilles. As encountered for other members of the Esculenta clade, the pileus of *M. gracilis* can be grayish or yellowish (FIG. 5A–B). Although these color differences have not been documented as related to age of the ascoma for *M. gracilis*, such a color shift is documented for *M. americana* Clowez & C. Matherly of the Esculenta clade, the most common yellow morel in North America. The latter species typically starts out grayish then changes to yellowish with age as ascomata mature (Beug et al. 2014).

The slender gracile pileus of *M. gracilis* is characteristic for the species, along with the deep, longitudinally elongate pits and pileus that tends to curve or bend off at an angle, sometimes just at the apex. The habitat preference of bare soil or disturbed sites, not associated with any one plant or group of plants, also appears to be a consistent trait. *Morchella gracilis* shares some morphological features with *M. peruviana*, also described as new in this study (see below), and is known to be its sister species phylogenetically. For more detailed comparisons, see comments under *M. peruviana*. Also, another poorly known Central American species, *M. herediana*, shares some similarities with *M. gracilis* by ascomata size and form, including the sharply conical pileus with elongate pits. However, *M. herediana* differs from *M. gracilis* by the ivory white or beige-colored pileus with olive-citrine hues and the shorter and narrower ascospores (16.8–18 x 8–9.9 µm per Gómez 1971). *Morchella herediana* has not been the subject of molecular investigations and is only known from the original description (Gómez 1971).

An important feature that will be helpful in identification of *M. gracilis* is the type of ornamentation found on the surface of the ascospores. Under SEM, the ascospores of *M. gracilis* possess a uniform rugulose or corrugate-like surface ornamentation, lacking any well-developed ridges or striations. Strongly or obviously ridged ascospores have been reported for other morel taxa examined via SEM (Malloch 1973; Elliott et al. 2014; Loizides et al. 2016; Taşkın et al. 2016; Voitk et al. 2016) and phase-contrast LM (Işiloğlu et al. 2010). Ascospores of *M. gracilis, M. kaibabensis*, and *M. peruviana* are not obviously ridged or striate, but those of *M. hispaniolensis* possess striate or ridged ornamentations (FIG. 4B–C). The ridged or striate type of ornamentation as visualized under SEM appears to be the more common state for species of *Morchella*; however, ascospores of only a few taxa have been studied critically under the SEM to date.

A recent report documenting the first morel species in Colombia as *Morchella* sp., in the *M. elata* group (Pinzón-Osorio and Pinzón-Osorio 2017), appears to be of a collection of a single specimen of *M. gracilis* based on images of the ascomata, size of the ascospores, and the habitat of disturbed soil.

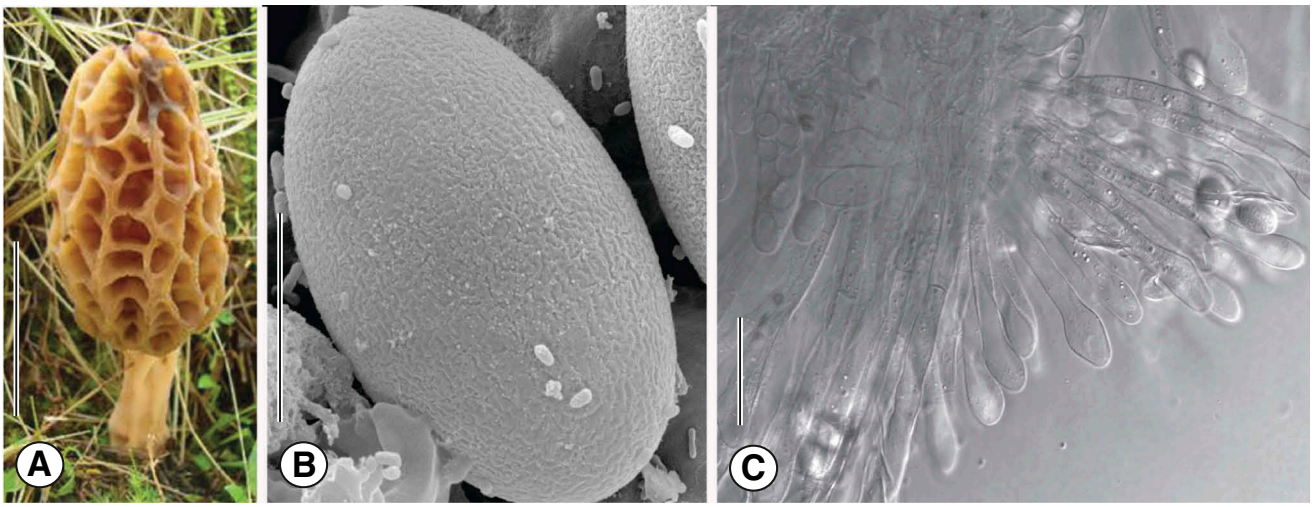

Figure 6. *Morchella peruviana*. All images from the holotype, F. Aguilar, M. Holgado & M. Quispe s.n. (CIPHAM-004). A. Ascoma (image by M. E. Holgado). B. SEM image of ascospore. C. Acroparaphyses. Bars: A = 25 mm; B = 7 µm; C = 40 µm.

Morchella peruviana Holgado, Aguilar, Quispe & T.J. Baroni, sp. nov. FIG. 6A–C
MycoBank MB823942

Typification: PERU. CUSCO REGION, ANTA PROVINCE: Chinchaypujio District, near Ocjra, 3600–3800 m, under *Escallonia resinosa* and *E. myrtilloides*, Mar 2016, *F. Aguilar, M. Holgado & M. Quispe s.n.* (*CIPHAM-004, KOD1445–1447*) (**holotype** NY02861412). Ex-type culture: NRRL 66754. GenBank: ITS = MH014708; *TEF1* = MH014705; *RPB1* = MN014716; *RPB2* = MH014719; 28S = MH014713.

Etymology: *peruviana* (Latin), in reference to the country of Peru where it is found.

Diagnosis: Morphologically similar to *M. gracilis* by the elongate, somewhat slender, pale-colored pileus, by the similar ascospore dimensions and with similar rugulose not striate ascospore surface ornamentation under the SEM, but differing from *M. gracilis* by variable acroparaphysis shapes, and by phylogenetic analysis of combined *TEF1*, ITS, *RPB1*, and *RPB2* sequences.

Description: Ascomata 50–70 mm tall. Pileus ovoid or ovoid elongate when young, conical with age or remaining ovoid, 39–54 mm high, 15–25 mm broad at widest point, when young with dominant vertical, more or less parallel ribs, forming narrow, elongate alveolate pits, with age pits expand revealing both primary and deeper secondary horizontal ridges inside pits, beige at first, turning brownish or sordid ochre-orange. Stipe cylindrical or wider at the base, with longitudinal ridges becoming more noticeable with age, surface white and furfuraceous overall, hollow and beige inside of stipe and pileus. Odor and taste unknown.

Ascospores (17–)18–22 × 10–14 µm (n = 21, X = 19.5 ± 1.7 × 11.9 ± 1.3; Q = 1.3–1.9, Q^m = 1.6 ± 0.1), ovoid-ellipsoid, appearing smooth under LM, not striate or ridged but rugulose or corrugated under SEM (FIG. 6B); wall appearing somewhat thickened, acyanophilic, inamyloid, uniseriate in ascus. Asci cylindrical or narrowly clavate, 150–250 × 15–24 µm, 8-spored, inamyloid, operculate. Paraphyses scattered, cylindrical or narrowly clavate or with slightly tapered apex, some septate, 103–154 × 10.5–16 µm. Sterile elements (acroparaphyses; FIG. 6C) on ridges of pits variously shaped from cylindrical or subcapitate, some with a distinctly swollen apex, with some resembling the head of a pit viper snake, also clavate, narrowly lanceolate, sometimes bifurcate, 80–144 µm long, 16–20 µm broad over widest area, 8–10 µm broad over narrowest basal area, often septate below, walls with pale brown intraparietal pigments. Stipe composed of three distinct layers: the outermost and innermost layers are textura globosa, the middle layer textura oblita; outermost layer 240–400 µm thick, mostly composed of hyaline, inflated, thin-walled hyphae, 26–60 × 22–42 µm, producing scattered pale golden yellow mounds in KOH of clustered, erect, inflated, thin-walled, cylindrical, or clavate end cells, 40–70 ×16–20 µm, some erect cells with a single septum, on short chains of two to three inflated cells (granulate ornamentation); the internal middle layer 300–480 µm thick, composed of interwoven, thick-walled (mostly 2 µm thick), refractive, cylindrical hyphae, 8–16 µm wide, septa between cells often more highly thickened and appearing donut-like when viewed down a hyphal cell through a septum; innermost layer 480–560 µm thick, composed of thin-walled, isodiametric, or elongate inflated cells 28–80 × 20–66 µm.

Ecology and distribution: Solitary on soil covered by moss and small herbaceous plants in relic forests with dominant vegetation of *Escallonia resinosa* and *E. myrtilloides*, 3600–3800 m; Peru. Mar.

Comments: Morchella peruviana, a member of the Esculenta clade, appears to be associated with woody *Escallonia* species in the high Andean native forests that line the stream beds in that region. It is a small and slender morel resembling *M. gracilis*, to which it is a sister species phylogenetically (FIG. 2). *Morchella gracilis* is currently known from Ecuador, Dominican Republic, Venezuela, and most likely Colombia (Pinzón-Osorio and Pinzón-Osorio 2017). These two species also share a similar distinctive ascospore surface ornamentation that is rugulose or corrugated and not striate from pole to pole as seen under SEM (FIG. 6B). The subtle rugulose ornamentation of *M. peruviana* is not as well developed as that of *M. gracilis* (FIG. 5C). In addition, the acroparaphyses on the ridges of the apothecium of *M. peruviana* (FIG. 6C) are more variable in form than those found in *M. gracilis*.

O'Donnell et al. (2011) previously hypothesized that *M. gracilis* (as *Mes*-14) was an anthropogenic introduction to the Americas from Asia because it was found in a clade dominated by taxa from China and Japan (*Mes*-12, *Mes*-13, *Mes*-15). However, with the discovery of *M. peruviana* in native Andean forests and confirmation of its phylogenetic connection to *M. gracilis*, the more likely scenario for these two evolutionarily linked taxa is that they share a most recent common ancestor whose evolutionary origin was in a region yet to be determined.

DISCUSSION

Two previously recognized phylogenetic species of *Morchella* (O'Donnell et al. 2011) are now described, *Mel*-18 (*M. hispaniolensis*) and *Mes*-14 (*M. gracilis*). However, in the process of sorting through new collections of American taxa using molecular systematic data, two additional species, *M. kaibabensis* and *M. peruviana*, were also discovered. With continued exploration of areas not examined critically in the past, new species of morels are still being discovered (Pildain et al. 2014). The process of continued biodiversity studies on morels and other macrofungi in the Americas provides future benefits for clarifying global diversity. In the case of morels, which appear to be morphologically variable, even within a species to some extent, molecular studies help with initial identification of new taxa. However, once a species is recognized, a careful and critical examination of morphological traits usually supports the uniqueness of the taxon.

We agree with the assessment by Loizides (2017) that careful and critical observations of morphological features of the ascomata and the ecology of a species offer valuable traits that can and will prove helpful with species recognition, even though currently many morel taxa can only be distinguished using molecular data (Richard et al. 2015). In this study, variation in acroparaphysis morphology and ascospore surface ornamentation has proven to be highly useful in discriminating individual taxa, supporting the hypotheses presented by Loizides (2017).

The majority of previously published SEM images of ascospores were not obtained from rehydrated and then critical point–dried (CPD) material. Rehydration and CPD treatment of reproductive tissue to provide accurate representation of the ornamentation on the surface of the spores is critical for determining fine structural detail of spore surface ornamentation in many groups of macrofungi. Using air-dried ascospore samples of morels to obtain SEM images has been the standard technique employed to date, as described in all the materials and methods sections of previous papers (Malloch 1973; Elliott et al. 2014; Taşkın et al. 2016). Use of air-dried untreated samples may obscure the fine detail of the ornamentation of the ascospore surface and in some cases appears to accentuate ridges or striations on the surface of the ascospores (Loizides 2017; see *M. hispaniolensis* in FIG. 4B–C). These partially collapsed untreated ascospores with strongly developed striations may obscure the secondary transverse or lateral branching patterns or other types of ascospore surface ornamentation. For example, in this study using rehydration and CPD preparation of ascocarp tissues, we found at least three different morphological types of ornamentation for morel ascospores: (i) primarily ridged or longitudinally striate with lower and shorter secondary lateral branching (FIG. 4B), (ii) a nonstriate rugulose surface ornamentation (FIGS. 5C, 6B), and (iii) a finely striate and minutely pebbled or textured surface ornamentation produced by erect rod-like agglutinated tips (unidentified taxon from Washington State not included here). In the future, we suggest that SEM documentation of ascospore surface ornamentation for morel species should employ rehydration and CPD preparations to ensure accurate evaluation of these morphological features. Such research will provide additional character states that have been shown here as useful in morel taxonomy.

Three different species of *Morchella* have now been documented forming endophytic symbioses with vascular plants. An earlier study (Baynes et al. 2012) confirmed an endophyte connection between *M. sextelata* (*Mel*-6), *M. snyderi* (*Mel*-12), and cheatgrass (*Bromus tectorum*). In this current study, while exploring GenBank for sequences that might match the new

species being described, our sequences for *M. kaibabensis* of the Elata clade were found to be identical to that of an endophyte culture that was taken from living photosynthetic tissue of *Juniperus scopulorum*. This collection came from the same general area (Arizona and New Mexico) where *M. kaibabensis* was discovered. So far, all known endophyte morel species are members of the Elata clade and endemic to western North America (Richard et al. 2015).

ACKNOWLEDGMENTS

We thank Robert H. Proctor for calling our attention to the IQ-TREE software package that was used to conduct phylogenetic analyses and Gail Doehring and Nathane Orwig for skilled technical assistance in various aspects of this study. We thank the Geology Department of SUNY Cortland for providing T.J.B. access to their scanning electron microscope and technical support. We also thank the curators and collection managers of the following herbaria for their assistance: A. Elizabeth Arnold and Joseph Aaron Myers, Robert L. Gilbertson Mycological Herbarium (ARIZ); David Giblin, University of Washington Herbarium (WTU); and Régis Courtecuisse and Christophe Lécuru, Université de Lille, France (LIP). Donald H. Pfister and Pierre-Arthur Moreau provided valuable presubmission comments of the manuscript and greatly improved the presentation.

FUNDING

T.J.B., D.J.L., and S.A.C. were funded by grants from the National Science Foundation Biodiversity Surveys and Inventories Program to the State University of New York College at Cortland (DEB-9525902 and DEB-0103621) for field work in the Dominican Republic. The USDA Forest Service Center for Forest Mycology Research Forest Products Laboratory provided additional logistical support for Caribbean work via a Joint Venture Agreement with SUNY Cortland. Field work in Ecuador by T. Læssøe was supported by a Danish government RUF grant.

ORCID

Timothy J. Baroni http://orcid.org/0000-0002-3411-4175
Michael W. Beug http://orcid.org/0000-0001-6767-6134
Teresa A. Clements http://orcid.org/0000-0003-4427-6802
María E. Holgado Rojas http://orcid.org/0000-0002-2285-8679
Kerry O'Donnell http://orcid.org/0000-0001-6507-691X

LITERATURE CITED

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

Beug M, Bessette AE, Bessette AR. 2014. Ascomycete fungi of North America: a mushroom reference guide. Austin, Texas: University of Texas Press. 488 p.

Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65:997–1008.

Clowez P. 2012 (2010). Les morilles une nouvelle approche mondiale du genre *Morchella*. Bulletin de la Société Mycologique de France 126:199–376.

Clowez P, Bellanger J-M, Romero De La Osa L, Moreau P-A. 2015. *Morchella palazonii* sp. nov. (Ascomycota, Pezizales): une nouvelle morille méditerranéen. Clé des *Morchella* sect. *Morchella* en Europe. Documents Mycologiques 36:71–84.

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 X-H, Zhao Q, O'Donnell K, Rooney AP, Yang ZL. 2012a. Multigene molecular phylogenetics reveal true morels (*Morchella*) are especially species-rich in China. Fungal Genetics and Biology 49:455–469.

Du X-H, Zhao Q, Yang ZL, Hansen K, Taşkın H, Büyükalaca S, Dewsbury D, Moncalvo J-M, Douhan GW, Robert VARG, Crous PW, Rehner SA, Rooney AP, Sink S, O'Donnell K. 2012b. How well do ITS rDNA sequences differentiate species of true morels (*Morchella*)? Mycologia 104:1351–1368.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Elliott TF, Bougher NL, O'Donnell K, Trappe JM. 2014. *Morchella australiana* sp. nov., an apparent Australian endemic from New South Wales and Victoria. Mycologia 106:113–118.

Gamundi IJ. 1975. Fungi Ascomycetes Pezizales. In: Guarrera SA, Gamundí de Amos I, Rabinovich de Halperin D, eds. Flora criptogámica de Tierra del Fuego. Buenos Aires, Argentina: FECIC. p. 14–22.

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

Gómez LD. 1971. Un Nuevo discomycete operculado de American Central: *Morchella herediana*, nov. sp. Darwinia16:417–426.

Gouy M, Guindon S, Gascuel O. 2009. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27:221–224.

Guzmán G, Tapia F. 1998. The known morels in Mexico, a description of a new blushing species, *Morchella rufobrunnea*, and new data on *M. guatemalensis*. Mycologia 90:705–714.

Guzmán G, Torres MF, Logemann H. 1985. Fungi from Guatemala I. A new species of *Morchella*. Mycologia Helvetica 1:451–459.

Işiloğlu M, Alli H, Spooner BM, Solak MH. 2010. *Morchella anatolica* (Ascomycota), a new species from southwestern Anatolia, Turkey. Mycologia 102:455–458.

Kalyaanamoorthy K, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kluting KL, Baroni TJ, Bergemann SE. 2014. Toward a stable classification of genera within the Entolomataceae: a phylogenetic re-evaluation of the *Rhodocybe-Clitopilus* clade. Mycologia 106:1127–1142.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Methuen & Co. 252 p., 30 pl.

Loizides M. 2017. Morels: the story so far. Field Mycology 182:42–53.

Loizides M, Bellanger J-M, Clowez P, Richard P, Moreau P-A. 2016. Combined phylogenetic and morphological studies of true morels (Pezizales, Ascomycota) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. Mycological Progress 15:1-28.

Malloch D. 1973. Ascospore sculpturing in *Morchella* (Ascomycetes: Pezizales). Canadian Journal of Botany 51:1519–1520.

Matheny PB, Baroni TJ, Simoni A, Holgado R, Sánchez-Garc ía M, Gates G. 2017. The wild edible mushroom *Pleurocollybia cibaria* from Peru is a species of *Gerhardtia* in the Lyophyllaceae (Agaricales). Cryptogamie Mycologie 382:205–212.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32:268–274.

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.

Pildain MB, Visnovsky SB, Barroetavena C. 2014. Phylogenetic diversity of true morels (*Morchella)*, the main edible non-timber product from native Patagonia forests of Argentina. Fungal Biology 118:755–765.

Pinzón-Osario CA, Pinzón-Osario J. 2017. Primer registro de la familia Morchellaceae (Ascomycota: Pezizales) para Colombia. Revista Peruana de Biología 24:105–110.

Richard F, Sauve M, Bellanger J-M, Clowez P, Hansen K, O'Donnell K, Urban A, Courtecuisse R, Moreau P-A. 2015. True morels (*Morchella*, Pezizales) of Europe and North America: evolutionary relationships inferred from multilocus data and a unified taxonomy. Mycologia 107:359–382.

Ridgway R. 1912. Color standards and color nomenclature. Washington, DC: Published by the author. 43 p., 53 pl.

Speggazini CL. 1909. Mycetes Argentinenses. Series 4. Anales del Museo Nacional de Historia natural de Buenos Aires 19:257–458.

Taşkın H, Büyükalac S, Doĝan 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. DOI: 10.3852/11-180

Taşkın H, Doğan H, Büyükalaca S, Clowez P, Moreau P-A, O'Donnell K. 2016. Four new morel (*Morchella*) species in the elata subclade (*M.* sect. *Distantes*) from Turkey. Mycotaxon 131:467–482.

Thiers B. [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. [cited 8 Feb 2018]. Available from: http://sweetgum.nybg.org/science/ih/

Voitk A, Beug MW, O'Donnell K, Burzynski M. 2016. Two new species of true morels from Newfoundland and Labrador: cosmopolitan *Morchella eohespera* and parochial *M. laurentiana*. Mycologia 108:31–37.