Four new species of *Morchella* from the Americas

Timothy J. Baroni, Michael W. Beug, Sharon A. Cantrell, Teresa A. Clements, Teresa Iturriaga, Thomas Læssøe, María E. Holgado Rojas, Frank M. Aguilar, Miguel O. Quispe, D. Jean Lodge, and Kerry O’Donnell

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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.

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 (Taşkin 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
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-Orsorio and Pinzón-Orsorio 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 non-native 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-adapted species originally described from North America and Mel-37, were reported occurring with native Austrocedrus and Nothofagus tree species (Pildain et al. 2014). Spegazzini (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 Körnerup 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 Qm = 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 CO2 (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.

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<thead>
<tr>
<th>Species</th>
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<th>Locality</th>
<th>GenBank accession numbers</th>
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*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 Taşkin 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. delicosa 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. peruviiana 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 Biotechnology Information (NCBI) GenBank as accesses 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. peruviiana CIPHAM-004 (= NRRL 66754) from the Peruvian Andes, and the holotype of M palazonii PhC149 from Spain (FIG. 2). Morchella gracilis and M. peruviiana 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.

MycoBank MB823939


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. kaibabensis.

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
young, not becoming thin or eroded at maturity; tran-
secting ridges not nearly as dark as vertical ones. Pits
primarily vertically elongated, although in some asco-
mata 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 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 = Hatra Taşķın; M = O’Donnell laboratory genomic DNA
code; NRRL = ARS Culture Collection; TAC = Terri A. Clements.
**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 Alimenticios 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, Qn = 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 capitulate and 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-brown in KOH. Sterile elements on ridges (acroparaphyses) dark fuscous-brown from intraparietal and plasmatic or vacular pigments, cells mostly swollen and clavate but also cylindrical, lanceolate, subcapitate, capitulate, 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 textula obtita, and finally a textula alata: outermost layer pale yellow in KOH, 400–480 µm thick, composed of mostly thin-walled, globose or greatly inflated ellipsoidial 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 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. 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; *RPB1*<sub>b</sub> = 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 pruinulate-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<sup>95</sup> = 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 separte 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 clamp 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*<sub>a</sub> = MH014736; *RPB2*<sub>b</sub> = 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
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. 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 gracle ascoma.

**Diagnosis:** Distinguished from other species in the Esclenta 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 × 12 ± 1.2 µm; Q = 1.2–2.0, Q95 = 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 separte below, sometimes branched below, and in rows or clusters, neither found in hymenium with ascii. 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 oblitata; 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-
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 occidentalis*, 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: Paschocha, in *Chusquea*-dominated mountain forest, on soil at cut trail edge with *Geocoryne variispora* Korf, 2850 m, 18 May 2002, T. Lessoe TL-9571 (collection split with part at Pontificia Universidad Catolica 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 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; Taşkin et al. 2016; Voitk et al. 2016) and phase-contrast LM (İsiloğlu et al. 2010). Ascospores of *M. gracilis*, *M. kaibabensis*, and *M. peruvianna* 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.
**Morchella peruviana** Holgado, Aguilar, Quispe & T.J. Baroni, sp. nov.


*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 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 sub-capitate, 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 oblitera; 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.

Ascospores (17–19)18–22 × 10–14 µm (n = 21, X = 19.5 ± 1.7 × 11.9 ± 1.3; Q = 1.3–1.9, Qm = 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 sub-capitate, 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 oblitera; 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.

*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.

**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.
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; Taşkin 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 ascomycete 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).

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**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⟩

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