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FUSE

Fungal Systematics and Evolution

VOLUME 8
DECEMBER 2021
PAGES 1–8

doi.org/10.3114/fuse.2021.08.01

Lentinula madagasikarensis sp. nov., a relative of shiitake mushrooms from Madagascar

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Key words:
Africa
biogeography
edible mushrooms
Omphalotaceae
systematics
1 new taxon

Abstract: We describe the first species of *Lentinula* from Africa, *Lentinula madagasikarensis sp. nov*. The new taxon, which was collected from central Madagascar, is strikingly similar to *L. edodes*, the shiitake mushroom. A BLAST search using ITS sequences from *L. madagasikarensis* as the query retrieves a mix of *Lentinula, Gymnopus*, *Marasmiellus*, and other members of *Omphalotaceae* as the top hits. A 28S phylogeny of the *Omphalotaceae* confirms placement of *L. madagasikarensis* within *Lentinula*. An ITS phylogeny places *L. madagasikarensis* as the sister group of *L. aciculospora*, which is a neotropical species. *Lentinula madagasikarensis* is characterized by robust basidiomata with vinaceous pilei, prominent floccose scales near the pileus margin, florets of sphaeropedunculate cheilocystidia, and subcylindrical basidiospores. This report constitutes a 4 000-mile, trans-oceanic range extension for *Lentinula*.

Citation: Looney BP, Buyck B, Menolli Jr N, Randrianjohany E, Hibbett D (2021). *Lentinula madagasikarensis sp. nov.*, a relative of shiitake mushrooms from Madagascar. *Fungal Systematics and Evolution* **8**: 1–8. doi: 10.3114/fuse.2021.08.01
Received: 14 April 2021; **Accepted:** 3 May 2021; **Effectively published online:** 1 June 2021
Corresponding editor: P.W. Crous

INTRODUCTION

Lentinula is a group of lignicolous agarics that includes the shiitake mushroom, *L. edodes*. Shiitake is a traditional food in East Asia and it has reportedly been cultivated in China for *ca*. 1 000 years (Chang & Miles 1987). However, the genus *Lentinula* has a broad distribution that spans much of South Asia, Australasia, and tropical and subtropical regions of the Americas (Pegler 1983). *Lentinula* has been resolved as a monophyletic group within the *Omphalotaceae* (*Agaricales*), which also contains *Gymnopus*, *Rhodocollybia*, and other collybioid mushrooms (Wilson & Desjardin 2005, Matheny *et al.* 2006, He *et al.* 2019, Oliviera *et al.* 2019).

Seven species have been formally described in *Lentinula*. Pegler (1983) included five species in his monograph of *Lentinula*: *L. edodes* in eastern Asia; *L. lateritia* in southeast Asia and Australasia; *L. novae-zelandiae* in New Zealand; *L. boryana* from the Gulf Coast of North America to South America; and *L. guarapiensis* from Paraguay. The latter is known only from the type collection (Spegazzini 1883). Using a combination of morphology, mating compatibility, and ITS sequences, Mata & Petersen (2000) described a new species from Costa Rica, *L. aciculospora*. Mata *et al.* (2001) then segregated a previous synonym, *Armillaria raphanica*, as *L. raphanica* from Pegler's broad concept of *L. boryana*. *Lentinula raphanica* is reported by Mata *et al.* (2001) from Florida, Louisiana, Puerto Rico, Costa Rica, Venezuela, and Brazil (*i.e.*, overlapping with *L. boryana s. str.*). Phylogenetic analyses of ITS sequences suggest that *L. lateritia* and *L. edodes* may each contain multiple species-level lineages, but these have not been formally described (Hibbett *et al.* 1998).

Most species of *Lentinula* grow on wood of *Fagales*, specifically *Fagaceae* or *Nothofagaceae* (Pegler 1983). The absence of *Lentinula* from Europe and most of North America (where oaks and their relatives abound, and outdoor log cultivation of shiitake is successful) is puzzling. Historical biogeographic analyses have suggested that the present distribution of *Lentinula* could reflect an ancient trans-Beringian distribution, which would require local extinction in North America (Hibbett 2001), but alternative dispersal routes via the southern hemisphere cannot be rejected.

During a study of the wild edible mushrooms of Madagascar, Buyck (2008) made two collections of a fungus that closely resembled *L. edodes*. Here, we present morphological and molecular analyses of the Malagasy material, which we describe as *L. madagasikarensis*.

MATERIALS AND METHODS

Sampling and phylogenetic inference

Two collections were made by Buyck (2008) in native forests of central Madagascar with endemic *Uapaca densifolia*

(*Phyllanthaceae*) and *Sarcolaenaceae*, and introduced *Eucalyptus robusta* in 2006 and 2008. Both collections have been deposited at the fungarium (PC) of the Muséum National d'Histoire Naturelle, Paris. Genomic DNA was extracted from both collections in 2020 using the Extraction Solution-based method of Looney *et al.* (2020).

Two nuclear loci were amplified and sequenced with standard primer sets: ITS1F-ITS4 (White *et al.* 1990) for ITS, and LROR-LR5 (Vilgalys & Hester 1990) for 28S. Both strands were sequenced using the Sanger method, by Psomagen, New York, NY, and sequences have been deposited into GenBank (accession Nos. MW810299–MW810302).

Two datasets were used to infer relationships at different scales, Dataset 1 was used to assess the placement within the *Omphalotaceae* and Dataset 2 was used to infer placement within *Lentinula*. Dataset 1 used the 28S *Omphalotaceae* matrix from Oliveira *et al.* (2019) as a starting alignment. Dataset 2 included 60 ITS sequences, representing all known lineages of *Lentinula*, which were downloaded using *emerencia* (Nilsson *et al*. 2005), and six sequences of *Gymnopus* spp. as the outgroup.

Sequences of the Madagascar material were manually aligned with the two datasets in AliView v. 1.27 (Larsson 2014). Phylogenetic inference under Maximum Likelihood was performed in RAxML-VI-HPC using raxmlGUI v. 2.0 with autoMRE bootstopping criteria (Stamatakis 2006, Edler *et al*. 2020). Bayesian analysis was performed in MrBayes v. 3.2.7a using Metropolis coupled MCMC under a GTR +G model through the CIPRES Science gateway (Ronquist *et al.* 2012). The analysis consisted of 3 runs of 50 M generations and four chains sampled every 5 000 generations. Finally, a distance matrix of nine representative sequences of different taxa was generated from the final ITS alignment in R using the dist.dna function of the 'ape' package. Alignments and resulting trees have been deposited in TreeBASE (https://treebase.org/) under study number 28094.

Morphological analysis

Morphological field notes were taken on fresh basidiomata using color standards given in parentheses (Kornerup & Wanscher 1978). Microscopic observations were made on a Nikon Eclipse e600 compound microscope with a mounted SPOT RT Slider digital camera. Dried specimens were rehydrated in 3 % KOH and observed in phase contrast or stained with phloxine and by bright field microscopy. Melzer's reagent was used for testing amyloidity. At least 20 observations were made for each feature. Microscopic feature measurements are given as lower limit–[arithmetic mean]–upper limit with *n* = total number of observations and Q-value for basidiospores calculated as arithmetic mean of the lengths divided by arithmetic mean of the widths for the lower limit–[arithmetic mean]–upper limit.

RESULTS

Molecular systematics

BLAST searches using ITS sequences from *L. madagasikarensis* as the queries retrieved sequences of *Lentinula, Gymnopus*, *Marasmiellus*, and other members of *Omphalotaceae* as the top hits (results not shown). Phylogenetic analyses of Dataset 1 recovered all of the major clades of *Omphalotaceae* identified by Oliveira *et al.* (2019), but often with weak bootstrap support (BS) (Fig. 1). *Lentinula* was strongly supported as monophyletic (BS 91 %). The sister group of *Lentinula* was not resolved with confidence, but a clade containing *Lentinula* and Clades A–J of Oliveira *et al.* (2019) was strongly supported (BS 99 %).

The *Lentinula*-focused ITS analyses of Dataset 2 placed *L. madagasikarensis* as sister to *L. aciculospora* with moderate BS (84 %) (Fig. 2). Two major groups were resolved in *Lentinula*, one weakly-supported group containing all Asian-Australasian species (BS 56 %) and one strongly-supported clade containing all American species, plus *L. madagasikarensis* (BS 98 %). There are three major groups in the Americas, *L. aciculospora* (BS 100 %), *L. boryana* (BS 86 %), and *L. raphanica* (BS 100 %).

The Asian-Australasian clade contains five lineages that have previously been termed Groups 1–5 based on ITS data (Hibbett *et al.* 1998). Each of the ITS-based groups are moderately to strongly supported as monophyletic, but relationships among them are not well resolved (Fig. 2). Groups 1 and 5 correspond to *L. edodes sensu lato*, including cultivated shiitake mushrooms. Based on sampling in the present study and that of Hibbett *et al.* (1998), Group 1 includes isolates from China, Japan, South Korea, Philippines, India, and the Russian Far East, whereas Group 5 includes isolates from China, Nepal and India. Group 2 and Group 4 both include collections from Papua New Guinea; Group 2 also includes collections from Australia. Group 3 is equivalent to *L. novae-zelandieae*, from New Zealand.

Pairwise distances of ITS sequences of *L. madagasikarensis* to other species of *Lentinula* ranged from 11 % *vs*. *L. edodes* (Group 5)*, L. lateritia* (Group 2), *L. novae-zelandiae*, and *L. aciculospora*, to 20 % *vs. L. raphanica* (Fig. 3). Pairwise sequence distances among three isolates from the Americas are 11–20 %, whereas those among five Asian-Australasian isolates are 1–4 % (Fig. 3).

Taxonomy

Lentinula madagasikarensis Buyck, Randrianjohany & Looney, ***sp. nov.*** MycoBank MB 839129. Fig. 4A–G.

Etymology: The specific epithet is derived from Madagasikara, which is the Malagasy name for Madagascar. To our knowledge, there is no Malagasy name for this species.

Diagnosis: Robust basidiomata with vinaceous pileus color and large, tufted scales near pileus margin merging into an appendiculate margin. Fibrous, ivory-colored stipe with squamules. Small and narrow basidiospores. Sphaeropedunculate cheilocystidia forming scattered florets.

Typus: **Madagascar**, Moramanga district, Alaotra-Mangoro region, Andasibe, 18°56'S 48°25'E, on *E. robusta* log, 20 Jan. 2006, *B. Buyck & V. Hofstetter* 06.007 (holotype PC0142531).

Description: *Pileus* convex to hemisphaerical when young, soon umbonate to applanate or broadly depressed, firm and fleshy, up to 6–7 cm diam.; surface smooth, sometimes scurfy or with minute scales or granules and almost greasy at the touch in the central portion, toward the margin rapidly becoming covered with a paler, hairy-fibrillose scurf, building more or less concentrically arranged, thick, floccose deposits with fibrillose extremities pointing downward; dark reddish brown (9EF6-8) to vinaceous or purplish brown (14EF5-8) at center when young, at maturity becoming paler, pale reddish brown to ochraceous brown (3A5-6, 4B5-8, 5C5-7) but retaining locally some darker

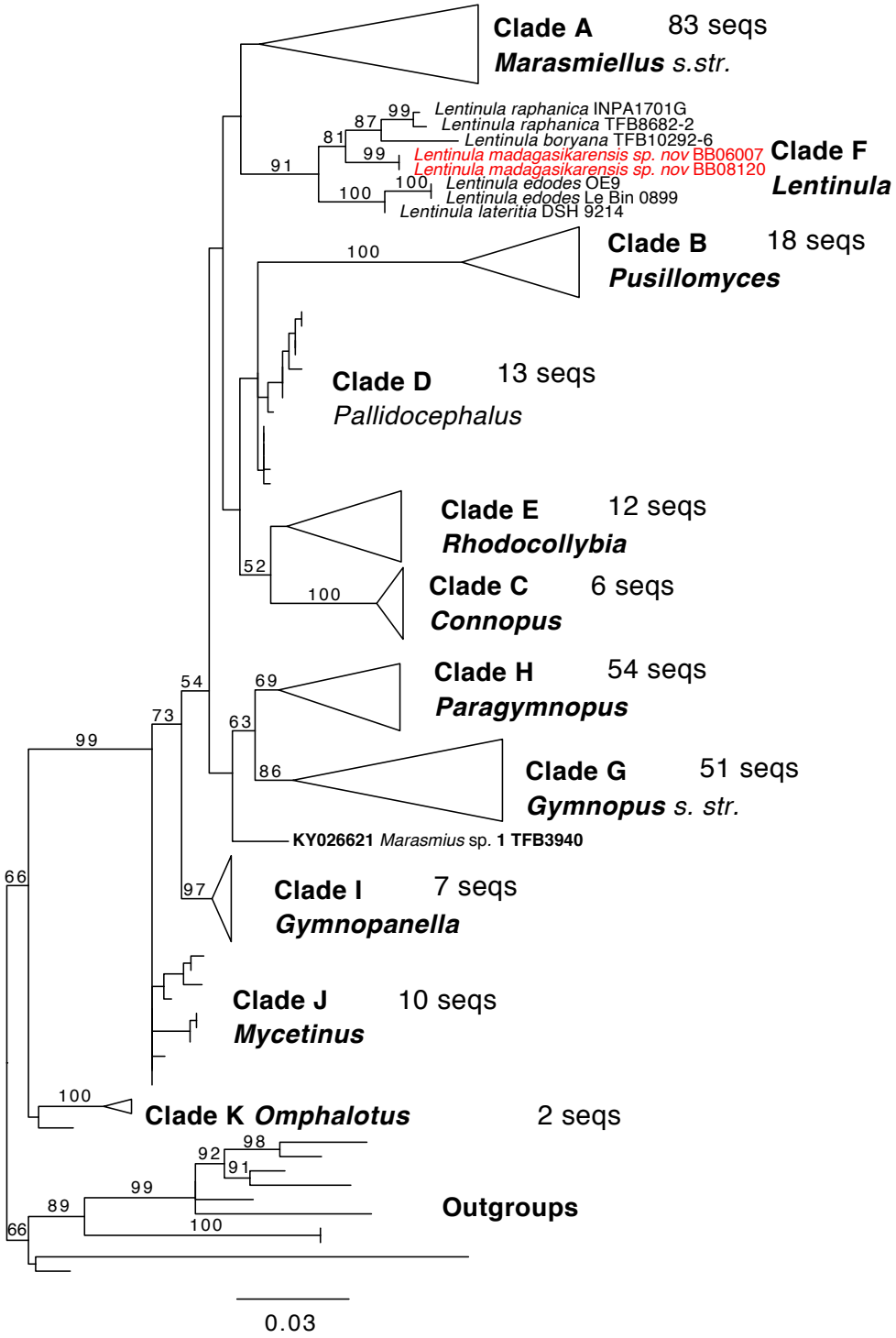

Fig. 1. Maximum-likelihood phylogeny of *Omphalotaceae* based on the 28S sequence dataset of Oliveira *et al.* (2019) with 788 bootstrap replicates according to autoMRE bootstopping criteria. Major clades from the original study are labeled and collapsed with number of samples included in each clade. BS values ≥ 50 % are included with the branches.

shades. *Lamellae* adnato- or adnexo-sinuate with a decurrent tooth, later frequently subfree, unequal with up to 4–5 series of lamellulae of different lengths, whitish to ivory, discolouring brownish after injury, crowded; edge concolorous, entire or irregularly serrulate. *Stipe* up to 4 × 2.5 cm; stout and firm, always shorter than the pileus diameter, central to mostly eccentric, cylindrical or a little widening to almost bulbous near the base, strongly fibrillose-squamose over the entire surface from thick fibrils or squamae pointing out- or upward, solid. *Partial veil* a thick cottony tissue, strongly fibrillose-squamose on the lower surface from fibrils pointing downward, fugacious, soon breaking up and limited to remnants on the pileal margin or sometimes leaving a (partial) ring on upper part of the stipe. *Context* firm-fleshy, pale coloured, not discolouring but often colored yellowish in the stipe near the attachment to the woody host surface. *Spore print* white. *Basidiospores* oblong to subcylindrical, 5.0–[6.0]–7.0 × 2.0–[3.0]–3.5 µm; Q = 1.7–[2.1]–2.4 (*n* = 30), hyaline, inamyloid, thin-walled, smooth; hilar appendage conspicuous; contents heterogenous or homogeneous. *Hymenium* composed mostly of basidia and basidioles. *Basidia* 11.0–[19.0]–26 ×

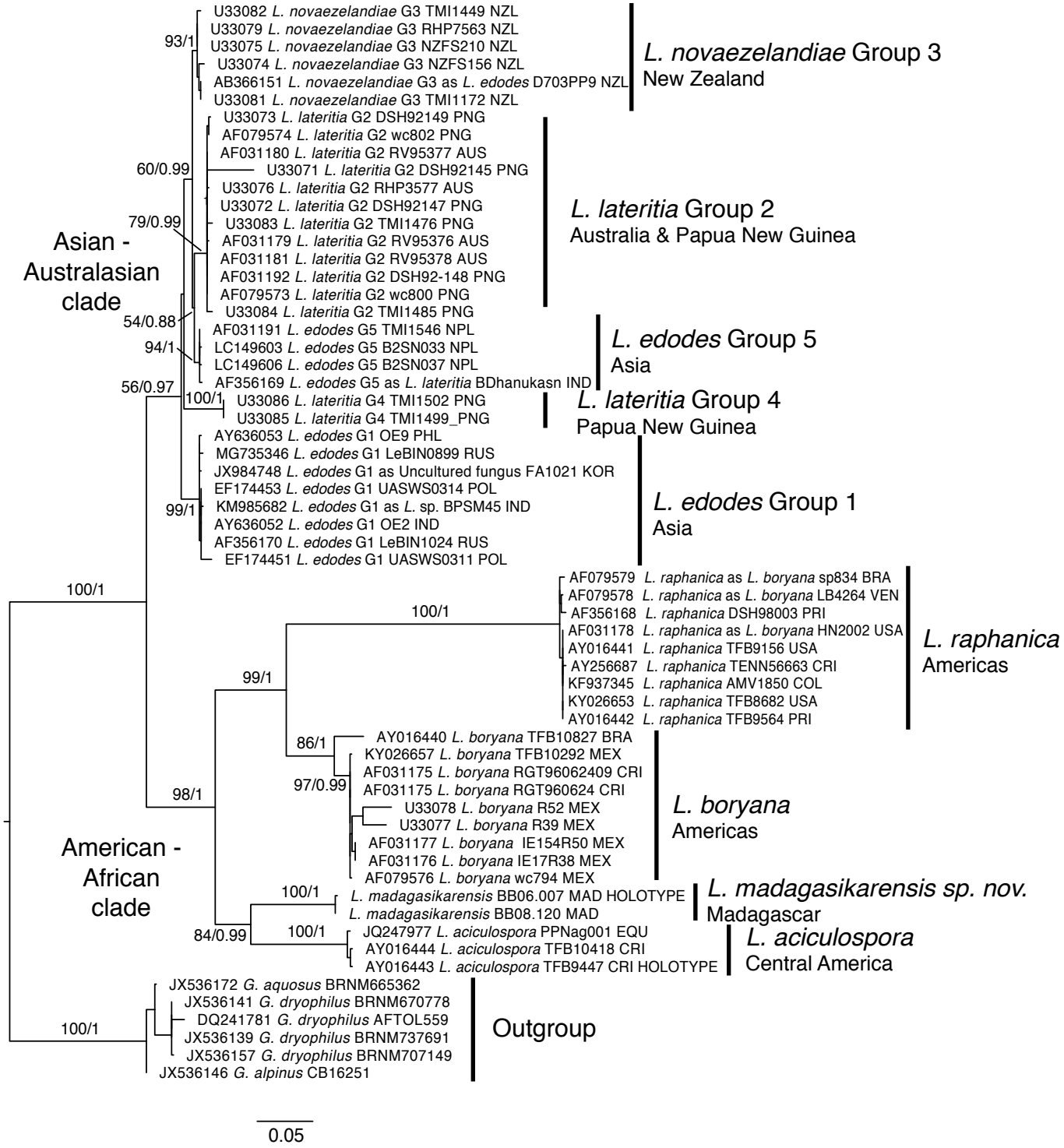

Fig. 2. Maximum-likelihood phylogeny of *Lentinula* based on Dataset 2 of ITS. BS values ≥ 50 % / Bayesian PP values ≥ 0.80 are included with the branches. Sequence labels include GenBank accession numbers and collection numbers. G1–G5 in the Asian-Australasian clade correspond to ITS-based groups from Hibbett *et al.* (1998). Country codes: AUS = Australia, BRA = Brazil, COL = Colombia, CRI = Costa Rica, IND = India, KOR = South Korea, MEX = Mexico, NPL = Nepal, NZL = New Zealand, PHL = Philippines, PNG = Papua New Guinea, POL = Poland (possible cultivar), PRI = Puerto Rico, RUS = Russia, USA = continental USA, VEN = Venezuela.

4.0–[5.0]–5.5 µm (*n* = 40), oblong to cylindrical, clavate with a median constriction, with a clamp connection at the base, four-sterigmate; sterigmata slender, up to 3 µm long. *Pleurocystidia* absent. *Cheilocystidia* 15.0–[28]–46 × 7.0–[11.0]–16.0 µm (*n* = 20), clavate to sphaeropedunculate, inflated apically without lobes, thin-walled, smooth, forming dense clusters or florets, infrequent. *Lamellar trama* regular to subregular; hyphae 4.0–15.0 µm diam., thin- to thick-walled. Subhymenium rudimentary. *Epicutis* 30–50 µm thick; hyphae 3.0–7.0 µm diam, brown in mass, hyaline singly, repent, interwoven and subregular cutis; hyphae in scales erumpent, ending in eroded hyphal fragments. *Subpellis* composed of frequently branching, irregular hyphae 5.0–11.0 µm diam, hyaline, thick-walled. *Stipitipellis* composed of interwoven hyphae 3.0–7.0 µm diam, branching, with floccons formed of obtuse terminal end cells. *Caulocystidia* absent.

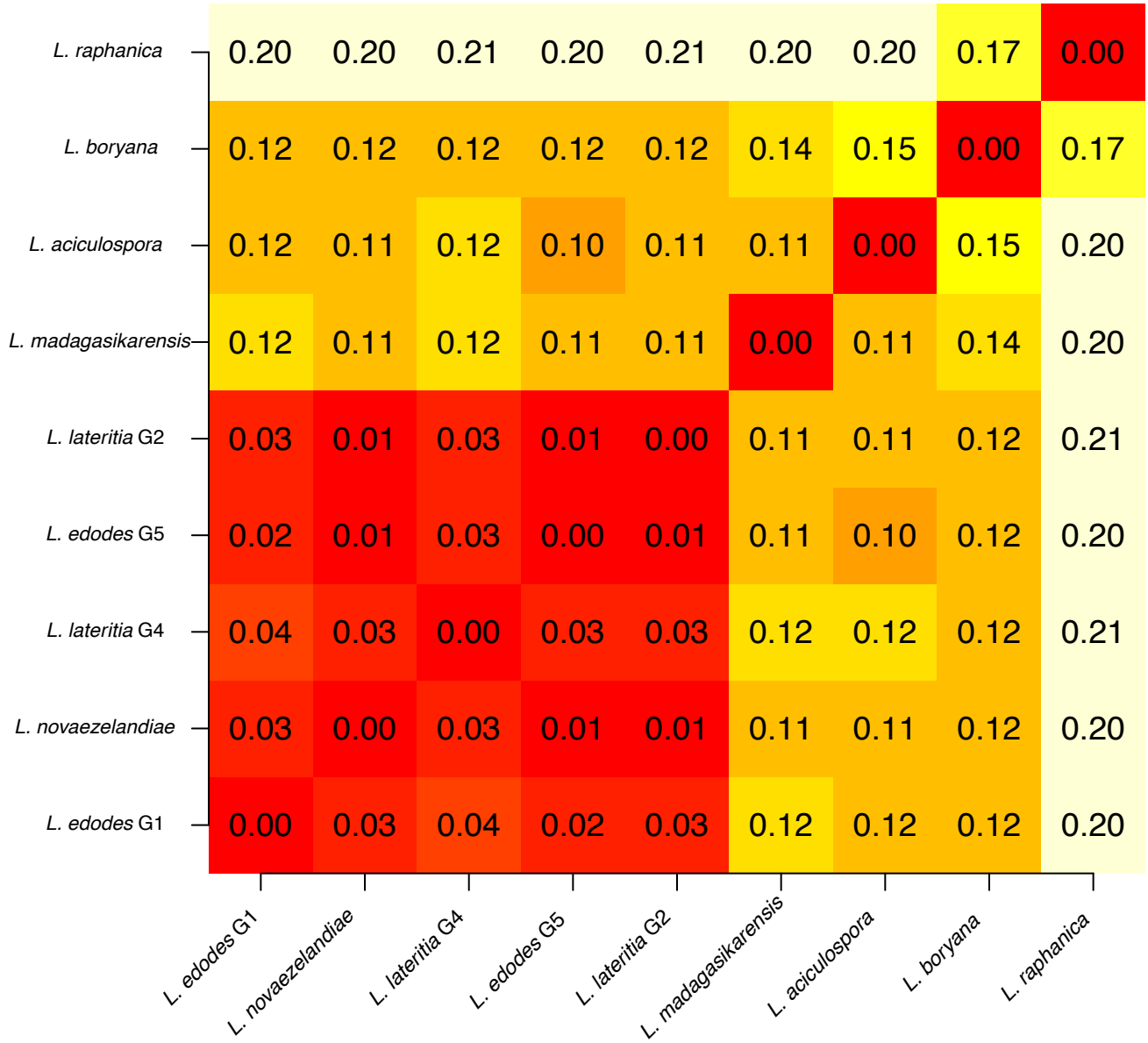

Fig. 3. Distance-matrix heat map of representative sequences of species of *Lentinula* based on pairwise distances from the ITS multi-sequence alignment given as proportion of divergence.

Habit and habitat: Gregarious to scattered on corticate logs of *E. robusta* and unidentified hardwood in humid, mixed mountain forests at higher elevations of the central plateau and eastern escarpment of Madagascar.

Additional specimen examined: **Madagascar**, Ankazobe district, Analamanga region, Ambohitantely Special Reserve, 18.161°S 47.302°E, in primary forest dominated by *U. densifolia* and *Sarcolaenaceae*, 22 Jan. 2008, *B. Buyck & V. Hofstetter* no. 08.120 (paratype PC0142532).

Notes: *Lentinula madagasikarensis* can readily be distinguished from its apparent sister species, *L. aciculospora*, by gross morphological characters including a darker, vinaceous pileus color in *L. madagasikarensis* and larger tufted scales concentrated near the pileus margin forming a distinctive appendiculate margin as well as microscopic differences in basidiospore size, which is smaller in *L. aciculospora* (5.6–8.8 × 1.6–2.8 µm), and shape of cheilocystidia that are gnarled and bluntly lobed in *L. aciculospora* (Mata & Petersen 2000). *Lentinula madagasikarensis* closely resembles *L. boryana* (Mata *et al.* 2001) but differs microscopically because the first never has appendages on cheilocystidia and caulocystidia are absent. Macroscopically, *L. madagasikarensis* differs from *L. boryana* in size, color, and robustness of the pilei, which are smaller (1.8–2.5 cm) and paler (light brown to golden brown or grayish orange) in *L. boryana* (Mata *et al.* 2001). Morphologically, *L. madagasikarensis* closely resembles *L. edodes* in pileus color and the robust aspect of the basidiomata, which also has a dark vinaceous brown and > 5 cm diam pileus (Pegler 1983). Microscopic characters are similar between the two species with similar size of the basidiospores, 5–6.5(–7) × 3–3.7 µm in *L. edodes* and general shape of cheilocystidia, also clavate without any apical appendages in *L. edodes* (Pegler 1983). However, basidiospores of *L. edodes* are slightly more ovoid (Q = 1.78) as reported by Pegler (1983) and cheilocystidia are not reported as sphaeropedunculate or forming florets as is typically found in *L. madagasikarensis*. The geographic distribution also separates *L. edodes* from *L. madagasikarensis* with the first broadly distributed in Asia and the latter known only from Madagascar.

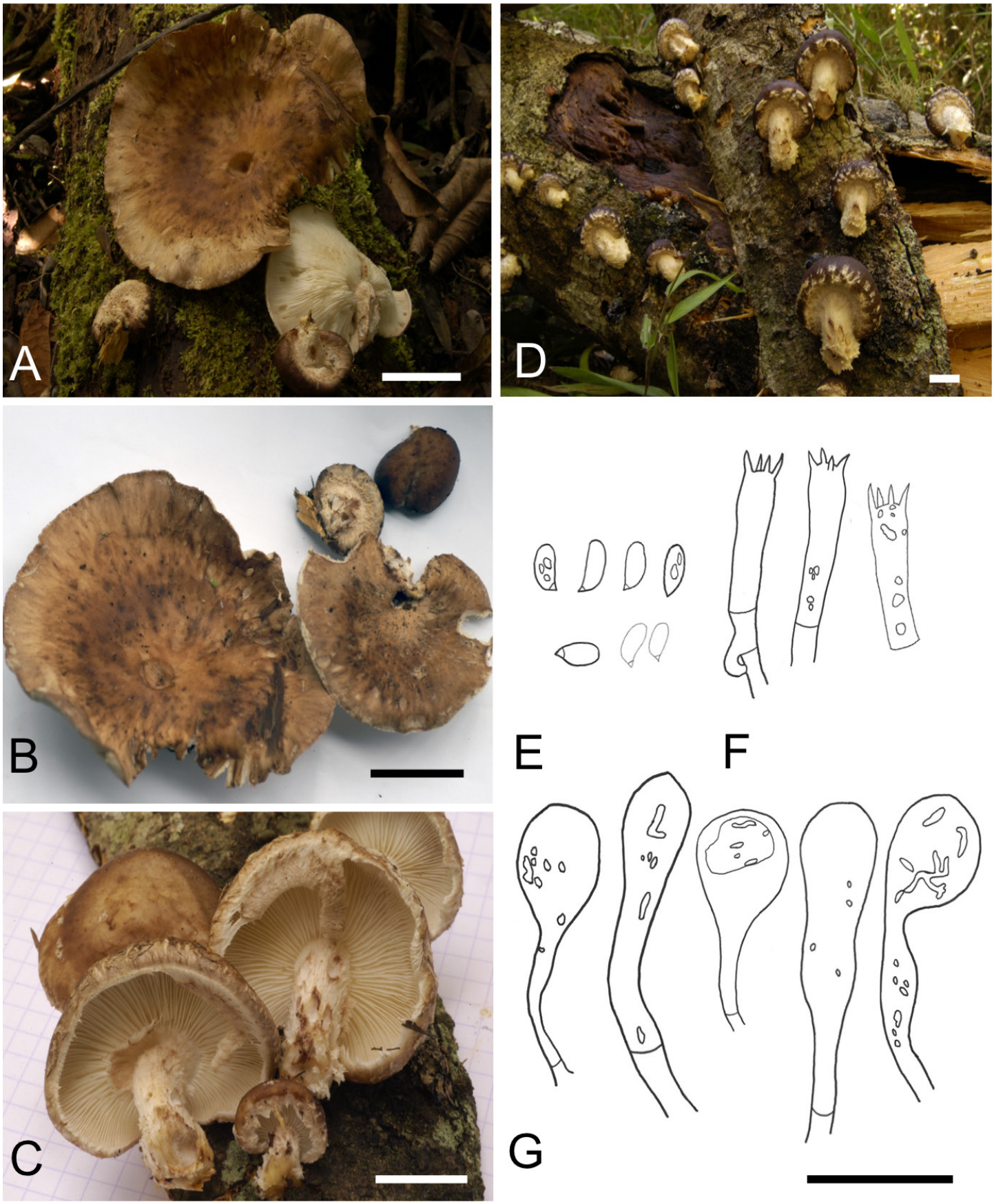

Fig. 4. A, B. Field photograph of *Lentinula madagasikarensis* (type specimen, PC coll. BB06.007). **C, D.** Field photographs of *Lentinula madagasikarensis* (paratype specimen, PC coll. BB08.120). **E.** Basidiospores. **F.** Basidia. **G.** Cheilocystidia. Scale bars: A–D = 2 cm, E–G = 20 µm.

DISCUSSION

Buyck (2008: 516) described the Malagasy *Lentinula* as a "shiitake look-alike." Our analyses confirm that his collections, from two different localities in Madagascar, represent a new species of *Lentinula*. *Lentinula madagasikarensis* was discovered 4 000 miles from the nearest well-documented wild populations of *Lentinula*, which are those of *L. edodes* Group 5 in India and Nepal. Its apparent sister group, *L. aciculospora*, occurs 9 000 miles away, in Costa Rica (Mata & Petersen 2000), Ecuador (Andrade *et al.* 2012), Panama (Piepenbring 2008), and Nicaragua (Gómez 2018). However, several collections identified as *L. edodes* or "*L. edodes*-like" have been reported from the Democratic Republic of the Congo (DRC), which is less than 2 000 miles from Madagascar (Global Biodiversity Information Facility [www.gbif.org] occurrence records 1836956264, 1840736410, 1840736444). As of this writing, we have not been able to study the material from the DRC.

At its narrowest point, the Mozambique Channel separates Madagascar and mainland Africa by less than 300 miles. Based on their geographic proximity, one would expect a *Lentinula* species from the DRC and *L. madagasikarensis* to be closely related. Even if they are, the presence of *Lentinula* in Madagascar is probably due to long-distance dispersal; Madagascar began to separate from continental Africa during the Late Jurassic Period, about 150-M-yr ago (de Wit 2003), whereas *Lentinula* is about 50-M-yr-old, according to a molecular clock analysis by Varga *et al.* (2019).

Both collections of *L. madagasikarensis* were found in similar habitats in the highlands of the Central Plateau at 1 500–2 000 m alt. One collection was found growing on wood of *E. robusta* and the other was on a fallen log of an "unidentified native tree" (Buyck 2008: 516), but both were in dense forests largely dominated by *U. densifolia* and various species of *Sarcolaenaceae*. All other species or species complexes of *Lentinula* are reported to occur on *Fagales*, particularly *Fagaceae* or *Nothofagaceae*, but some are also capable of growing on other hardwood substrates (Pegler 1983, Mata & Petersen 2000, McKenzie *et al.* 2000, Mata *et al.* 2001, Piepenbring 2008). There are no native *Fagaceae* or *Nothofagaceae* in Madagascar or Sub-Saharan Africa (Manos & Stanford 2001, Knapp *et al.* 2005). A recent discovery of 52-M-yr-old *Castanopsis* fossils from Patagonia shows that *Fagaceae* was present in the southern hemisphere in the early Eocene (Wilf *et al.* 2019), but that was late in the breakup of Gondwana, long after the opening of the South Atlantic Ocean. *Nothofagus* has an 80-M-yr fossil record, but there is no evidence of this group having existed in Africa or Madagascar (Knapp *et al.* 2005).

The phylogenetic placement of *L. madagasikarensis* is important for understanding host shifts and historical biogeography of *Lentinula*. The distribution of host ranges in *Lentinula* suggests that the ancestor of the genus decayed *Fagales*. If so, then *L. madagasikarensis* would represent an expansion onto hosts that are indigenous to Madagascar, as well as introduced eucalypts. Based on the ITS phylogeny, *L. madagasikarensis* is closely related to *L. aciculospora* within the American-African clade (Fig. 2), suggesting that it (and possibly the entire genus) is derived from an ancestor in the neotropics. However, many branches in the ITS phylogeny of *Lentinula* are not strongly resolved (Fig. 2). We defer formal analyses of historical biogeography and evolution of substrate ranges until we have a genome sequence for *L. madagasikarensis*, which we are currently pursuing.

ACKNOWLEDGEMENTS

BB expresses sincere thanks to the Committee for Research and Exploration of the National Geographic Society for funding part of the fieldwork through grant #7921-05. Colleagues of the CNRE at Antananarivo are acknowledged for excellent field assistance. Jadson J. S. Oliveira kindly provided the starting alignment for Dataset 1. Research at Clark University was supported by National Science Foundation award DEB-1456588 (DSH, PI).

Conflict of interest: The authors declare that there is no conflict of interest.

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