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Rhodocybe-Clitopilus clade (Entolomataceae, Basidiomycota) in the Dominican Republic: new taxa and first reports of *Clitocella*, *Clitopilus*, and *Rhodocybe* for Hispaniola

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Abstract

Based on morphological and phylogenetic investigations, four new species, *Clitocella termitophila*, *Clitopilus velutinus*, *Rhodocybe dominicana*, and *Rhodocybe pseudoalutacea*, one new variety, *Rhodocybe luteocinnamomea* var. *fulva*, and first reports of *Clitopilus prunulus*, *Rhodocybe mellea*, and *Rhodocybe roseiavellanea* are described and illustrated from the island of Hispaniola. Three protein coding genes, *RPB2*, *TEF1*, and *ATP6*, were used to compare taxa and make phylogenetic inferences about novel species and varieties. Where necessary, because of non-availability of these protein coding gene data for every taxon, ITS sequences were used to confirm uniqueness or species similarity for *C. velutinus* and *R. mellea*, respectively. This contribution is a result of and based on numerous expeditions in the Dominican Republic by two different groups over a 20-year period that were designed to gather data on macrofungal biodiversity of this region. The new agaric taxa documented here contribute to the limited but growing knowledge on biodiversity of the Entolomataceae (Agaricales) for the Dominican Republic and the island of Hispaniola.

Keywords Agaricales · *ATP6* · *RPB2* · *TEF1* · Termite nests · New taxa

Introduction

Rhodocybe Maire, *Clitopilus* (Fr. ex Rabehn.) P. Kumm., *Clitopilopsis* Maire, *Clitocella* Kluting, T. J. Baroni & Bergemann, and *Rhodophana* Kuhner are genera of Entolomataceae that represent a phylogenetic unit currently referred to as the Rhodocybe-Clitopilus clade (Moncalvo et al. 2002; Co-David et al. 2009; Kluting

et al. 2014). The Rhodocybe-Clitopilus clade, with approximately 300 species described so far worldwide, is the smaller of the two monophyletic groups that make up the Entolomataceae, i.e., the Entoloma clade and the Rhodocybe-Clitopilus clade. Members of the Rhodocybe-Clitopilus clade are mainly found as saprotrophs in tropical and/or temperate ecosystems in both hemispheres (Maire 1926; Singer 1947, 1969, 1973; Singer and

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Digilio 1952; Dennis 1953, 1961; Pegler 1977, 1983; Moser 1978; Horak 1979a, 1979b [1978]; Baroni 1981; Ovrebo and Baroni 1988, 2007; Noordeloos 1988, 2012; Baroni and Halling 1992; Baroni and Horak 1994; Baroni and Gates 2006; Yang 2007; Henkel et al. 2010; Raj and Manimohan 2018; Jian et al. 2020). Species of the Rhodocybe-Clitopilus clade are recognized by their attached lamellae, flesh pink or reddish-brown spore deposits, basidiospores ornamented with longitudinally ridged or bumpy-pustulate surface in face view, minutely angled in polar view with 6–12 facets, and with evenly cyanophilic spore walls (Kluting et al. 2014). The basidiospore surface ornamentation for these taxa is typically obvious in transmitted light microscopy, but for some species, it might be quite subtle and only clearly demonstrated under scanning electron microscopy (Baroni 1981; Kluting et al. 2014).

Only eighteen species of the Rhodocybe-Clitopilus clade have been documented so far from land masses around the Caribbean Basin (Dennis 1953, 1961; Baroni 1981; Pegler 1983; Baroni and Halling 1992; Ovrebo and Baroni 1988, 2007), an area encompassing the northern most portions of South America, all of Central America, and the Greater and Lesser Antilles. This number is not believed to be representative of the biodiversity of this group for such a large extent of land that supports a wide variety of habitats.

Over the past two decades, field research in the Dominican Republic by two different research teams was carried out to provide documentation on macrofungal biodiversity and a significant number of publications resulted from those efforts (Lodge et al. 2001; Armstrong and Baroni 2004; Cifuentes et al. 2005; Ortiz-Santana et al. 2007; Baroni et al. 2008; Justo et al. 2012, 2015a, b, c; Vizzini et al. 2012, 2016, 2017a, b, 2018; Angelini et al. 2014; Parra et al. 2018). However, even with this encouraging progress, the mycota for this diverse ecological region in the Caribbean, with ecosystems ranging from wet tropical seashore vegetation to the cold, nearly temperate regions of the high mountains of Armando Bermúdez National Park, is still mostly underexplored and unknown.

This report is the first on members of the Rhodocybe-Clitopilus clade for the Dominican Republic and the island of Hispaniola. We present here, based on morphological and molecular analyses (*RPB2*, *TEF1*, *ATP6*, and *ITS*), four new species, *Clitocella termitophila*, *Clitopilus velutinus*, *Rhodocybe dominicana*, and *Rhodocybe pseudoalutacea* and one new variety, *Rhodocybe luteocinnamomea* var. *fulva*, and report for the first time the presence of *Rhodocybe mellea* T. J. Baroni & Ovrebo, *Rhodocybe roseiavellanea* (Murrill) Singer, and *Clitopilus prunulus* (Scop.) P. Kumm. Species of *Clitopilopsis* and *Rhodophana* have yet to be documented from the island.

Materials and methods

Collecting and morphological descriptions

Collections were photographed in the field with either film or digital cameras. Notes on morphological characters of basidiomata were obtained before drying samples with a food dehydrator. Colors used in the descriptions are based on colors found in Kornerup and Wanscher (1978) or are general color terms. Additional color information was obtained by matching color chips in Kornerup and Wanscher (1981) to the color of the digital images in some cases.

Dried tissues were revived in 95% ETOH and then distilled H₂O before sectioning and mounting in 3% KOH or Congo Red in a 10% ammonia solution. Also, dry tissue samples of thin sections of the pileus and stipe surfaces and lamella edges were mounted directly in 3% KOH or Melzer's reagent to observe and record cellular structure or amyloid color reactions, respectively. Cyanophilic reactions were made on small dry lamella pieces placed in Cotton Blue in lactic acid, heated for 30 s over an alcohol lamp flame until the mountant produced vapor. The blue-stained tissue was then moved into a drop of clear lactic acid to destain for approximately 1 min. The tissue was finally mounted in a drop of clear lactic acid on a new clean slide and gently squashed under the coverslip to separate cells for observation. For basidiospore measurements, $n = 34/1$ means that 34 individual spores were measured from one basidioma, and $m = 5.3 \pm 0.4 \times 3.7 \pm 0.3 \mu\text{m}$ describes the mean with standard deviation for each of these measurements. Q^m indicates the mean for the range of Q values for individual basidiospores.

The SEM images of basidiospores were obtained from rehydrated, then dehydrated, and critical point dried samples of lamella pieces (Kluting et al. 2014) on a JEOL JSM 6010PLUS/LA scanning electron microscope run at 20 Kv with a working distance of 8–10 mm and a spot size of 30 to 50. Digital images of microscopic structures under the light microscope (LM) were obtained on a Spot Insight 3 shot color, 2 megapixel camera (Diagnostic Instruments, Inc.) using mainly DIC optics on an Olympus BX50 light microscope.

DNA extraction, PCR amplification, sequencing

All extractions, amplification, and sequencing for the new taxa were performed by ALVALAB (www.alvalab.es). Information about the collections sampled with detailed information on species, collector's identifier, herbarium, and accession number of the voucher specimens is provided in Table 1. Bold type indicates that the sequences were generated for this research. All other sequence data in normal type, used for comparison and phylogenetic analyses, come from GenBank. Molecular markers used to infer phylogenetic relationships for these fungi, as well as protocols and primers used for amplification, followed Kluting et al. (2014). The phylogenetic analysis shown here is

Table 1 DNA sequences produced for this study or obtained from GenBank for this analysis. All other sequences used in the phylogenetic analysis and Figs. 1–3 are cited in Kluting et al. (2014)

Species	Collection identifiers	Source	Locality	GenBank accession numbers			
				ITS	RPB2	TEF1	ATP6
<i>Clitocella termitophila</i>	ANGE 264	This paper	Dominican Republic	MN893319			
<i>Clitopilus albidus</i>	CAL 1319	GenBank—Raj and Manimohan (2018)	India	MF926596	MF946579		
<i>Clitopilus chrischonensis</i>	TO HG 1994	GenBank	Switzerland	HM623128			
<i>Clitopilus fusiformis</i>	SAAS1892—holotype	GenBank—Wang et al. (2017)	China	KU751777	KY385633		
<i>Clitopilus kamaka</i>	PDD 96106	GenBank—Cooper (2014)	New Zealand	KJ461903			
<i>Clitopilus scyphoides</i>	CBS 127-47	GenBank	France	MH856181			
<i>Clitopilus subscyphoides</i>	CAL 1326	GenBank—Raj and Manimohan (2018)	India	MF927542	MF946581		
<i>Clitopilus prunulus</i>	TJB 9425	This paper	Dominican Republic		MN893320	MN893330	MN893338
<i>Clitopilus velutinus</i>	ANGE 535	This paper	Dominican Republic	MN784991	MN893321	MN893331	MN893339
<i>Rhodocybe dominicana</i>	ANGE 464	This paper	Dominican Republic		MN893322	MN893332	MN893340
<i>Rhodocybe dominicana</i>	ANGE 473	This paper	Dominican Republic		MN893323	MN893333	MN893341
<i>Rhodocybe luteocinnamomea</i> var. <i>fulva</i>	ANGE 169	This paper	Dominican Republic		MN893324	MN893334	MN893342
<i>Rhodocybe mellea</i>	TJB 9805	This paper	Dominican Republic	MN784992	MN893325		MN893343
<i>Rhodocybe mellea</i>	ANGE 893	This paper	Dominican Republic	MN784993	MN893326		MN893344
<i>Rhodocybe mellea</i>	TJB 9823	This paper	Belize	MN784994			
<i>Rhodocybe mellea</i>	CO 2157—isotype	This paper	Costa Rica	MN785995			
<i>Rhodocybe pseudoalutacea</i>	TJB 9466	This paper	Dominican Republican		MN893327	MN893335	MN893345
<i>Rhodocybe pseudoalutacea</i>	TJB 9507	This paper	Dominican Republican		MN893328	MN893336	MN893346
<i>Rhodocybe pseudoalutacea</i>	ANGE 947	This paper	Dominican Republican		MN893329	MN893337	
<i>Lyophyllum decastes</i>	Sundberg 091007a	GenBank	Fennoscandia	HM572548			

Bold face type indicates that the sequences were generated for this research. Normal type face indicates existing ones from GenBank

based on partial sequences of the following three protein-coding genes that are commonly used in phylogenetic relationship studies in fungi: *RPB2*, *TEF1*, and *ATP6*. These three genetic markers were chosen because of the high support for internal and terminal nodes reported in the study by Kluting et al. (2014).

Sequence alignment and phylogenetic analysis

Sequences were assembled and edited using CODONCODE ALIGNER 4.2.7 or SEQUENCHER 4.2.2. Alignment was performed manually on MESQUITE 3.2 using the previous master alignment for the Rhodocybe/Clitopilus clade produced by Kluting et al. (2014). Introns were removed from *RPB2* and

TEF1 sequences before analysis was performed by using AUGUSTUS (GENE PREDICTION—<http://bioinf.uni-greifswald.de/augustus/>) and by comparison to previously published sequences for each gene region. A concatenated matrix of the three loci for the data was analyzed as a single partition, and that matrix is available on TREEBASE (<http://purl.org/phylow/treebase/phylows/study/TB2:S24817>).

Maximum likelihood (ML) analyses were conducted using RAxML ver. 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway 3.3 (Miller et al. 2010) generating 1000 bootstrap (MLBS) replications that provided a consensus tree with bootstrap values generated for all branches using a GTRCAT nucleic acid model for the bootstrapping phase. The tree was

then visualized in FigTree v1.4.3 (Rambaut 2012) and prepared for presentation in Adobe Illustrator (Fig. 1).

In order to confirm the phylogenetic uniqueness of *Clitopilus velutinus*, it was necessary to compare morphologically similar taxa represented in GenBank, and unfortunately, only ITS sequences were available for these taxa. Also, to confirm the presence of *Rhodocybe mellea* in the Dominican Republic, we were allowed to extract DNA from an ISOTYPE specimen, but only ITS sequences could be obtained. Therefore, these ITS sequences for selected taxa of *Clitopilus* and *Rhodocybe* were aligned using MUSCLE (Edgar 2004), and maximum likelihood phylogenetic analyses were obtained on MEGA7 (Kumar et al. 2016) and prepared in ADOBE ILLUSTRATOR (Figs. 2–3).

Results

Phylogenetic analysis

The ML tree for the concatenated dataset is presented with MLBS values for branches (Fig. 1). The

multigene analysis shows strong backbone support for the genera (*Rhodophana*, *Clitopilopsis*, *Clitocella*, *Clitopilus*, and *Rhodocybe*). Four new species and one new variety are highlighted by the phylogenetic results, and three previously described species known from the greater Caribbean area and Central America or from North America and Europe are also confirmed for the first time for the Dominican Republic with this analysis.

All sequences generated during this study are deposited in GenBank under the accession numbers (MN893319–MN893346 and MN784991–MN784995).

Morphological and taxonomic results

The morphological data mostly agree with the phylogenetic analyses, but with some interesting caveats. For more details, see the discussions under *R. pseudoalutacea* and *R. luteocinnamomea* var. *fulva*.

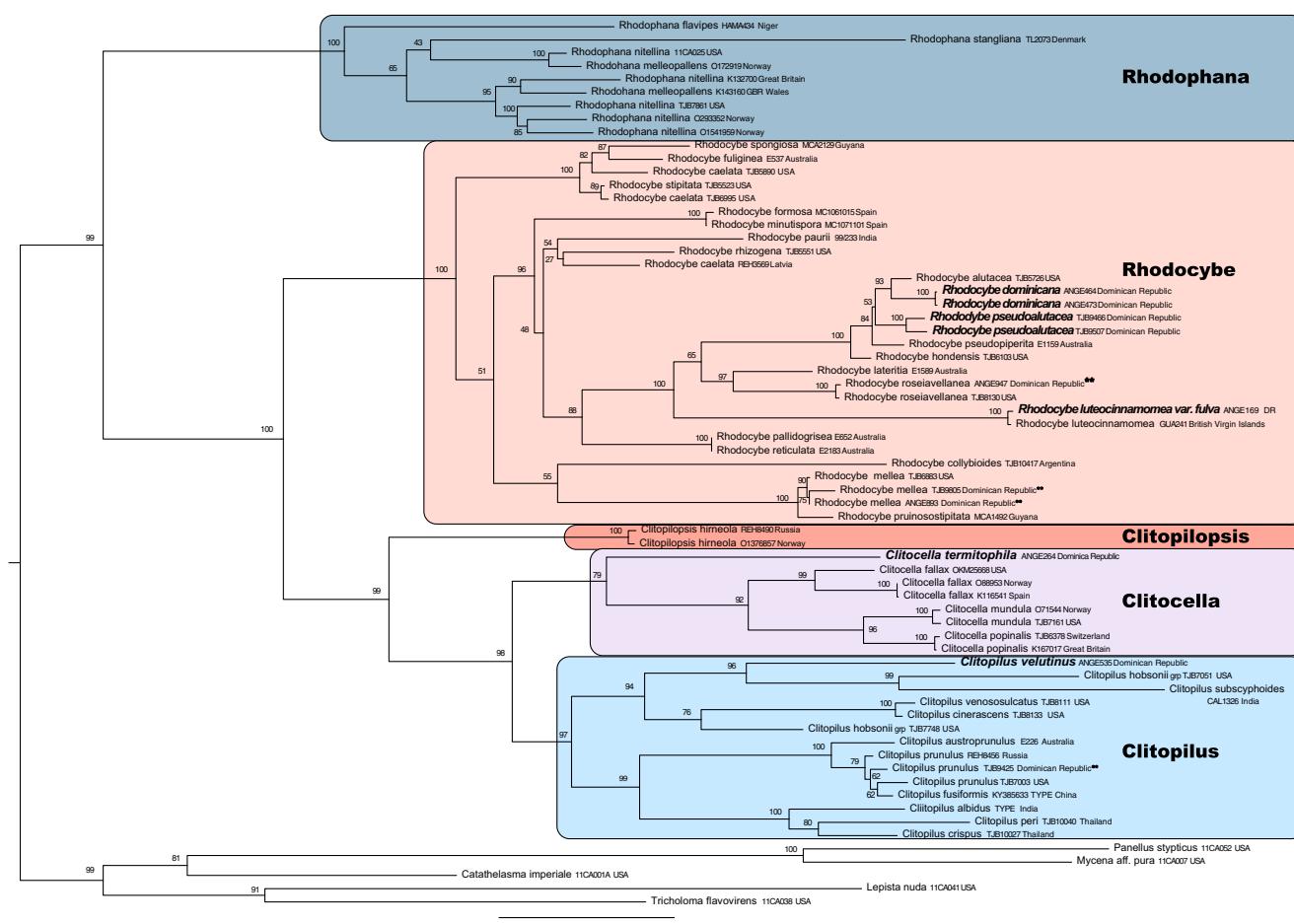


Fig. 1 Three-gene (*RPB2*, *TEF1*, and *ATP6*) ML phylogenetic analysis of Rhodocybe-Clitopilus clade highlighting collections found in the Dominican Republic in comparison to a global sampling of known taxa.

Species reported for the first time from the Dominican Republic are marked with double asterisks. All new taxa are in bold italic

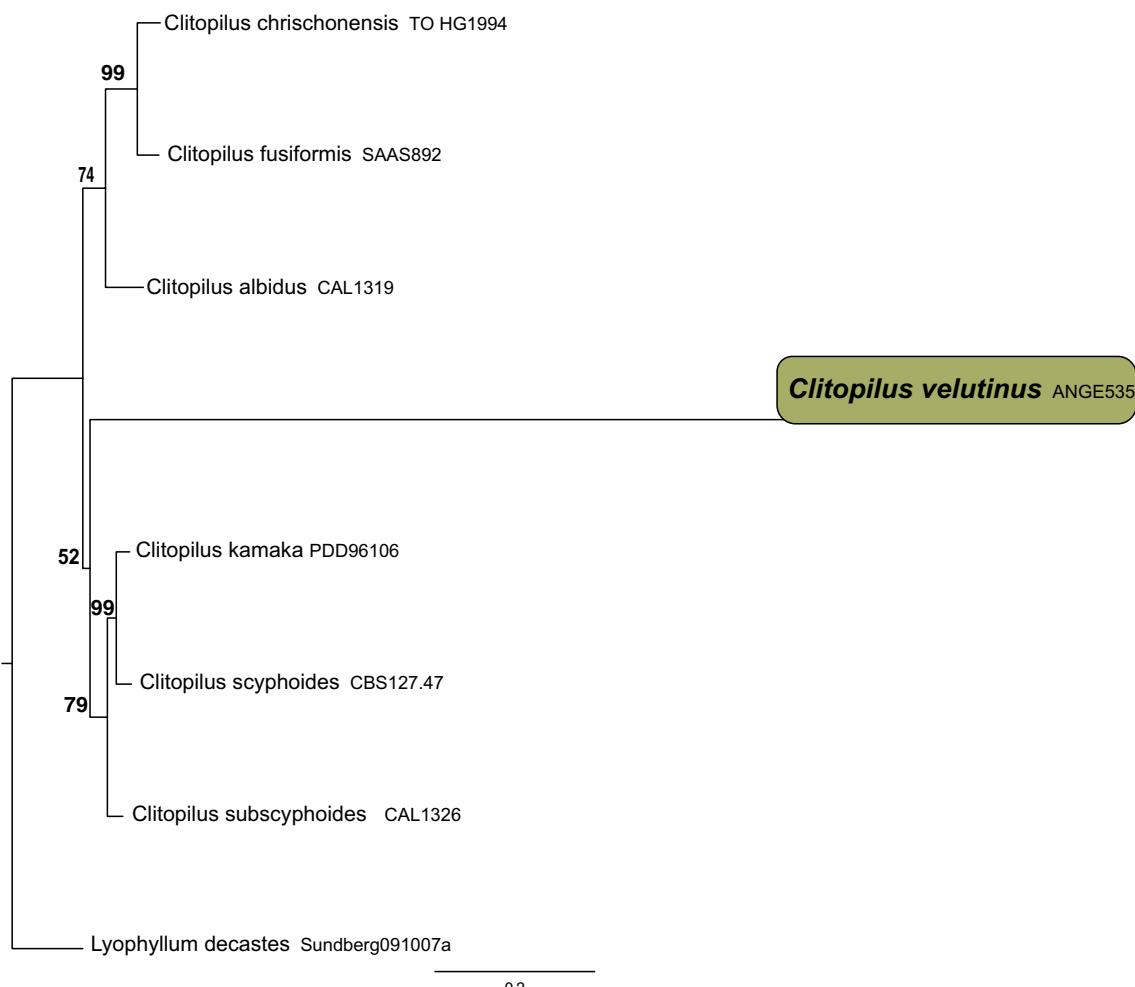


Fig. 2 ITS ML phylogenetic analysis of *Clitopilus velutinus* in comparison to other small white *Clitopilus scyphoides*-like taxa available in GenBank

New taxa

Clitocella termitophila T.J. Baroni & Angelini, sp. nov.

Fig. 4a–d

Mycobank: MB 831958

Typification: Dominican Republic, Puerto Plata Province, near the Town of Sosúa in the vicinity of Puerto Chiquito, approximate location 19.750833, –70.526111, on downed, decaying, abandoned arboreal termite nest (*Nasutitermes corniger* Motschulsky) in a secondary strongly disturbed deciduous forest, 12 December 2013, leg. C. Angelini ANGE264 consisting of ten basidiomata (**holotype** JBSD 127398; **isotype** CORT 014751—small piece of pileus).

Diagnosis: Distinguished from other *Clitocella* species and all other taxa in the Rhodocybe-/Clitopilus clade by the pleurotoid stature combined with yellowish cream pileus and stipe colors, the decurrent lamellae, the eccentric stipe, the lack of hymenial cystidia, small elliptical, obscurely bumpy basidiospores, 4.8–5.6 × 3.2–4 µm, and absence of clamp connections. Also distinctive by growth on decaying arboreal termite nests and the *RPB2* molecular marker.

Etymology: The epithet refers to the unique substrate of decaying arboreal termite nests.

Basidiomata pleurotoid in stature. Pileus sordid yellowish cream (4B3—sand to near 4B4—champagne) or light ochre (5B2–3—alabaster or grayish orange), not hygrophanous, (5)10–25 (30) mm broad, convex with a depressed disc, becoming plano-convex to somewhat infundibuliform, moist, opaque, glabrous but appearing finely textured, margin inrolled. Context off white, 1.5–2.5 mm thick. Lamellae white or off white, with pale pinkish buff hues with age, decurrent, close, moderately broad (1.5–2.5 mm deep), edge even, lamellulae in 3–4 tiers of varying lengths. Stipe pale cream (4A2—yellowish white), (6)8–20(25) mm long, 2–4 mm broad, central at first, but soon mostly eccentric, solid, cylindrical, usually curved, glabrous but with fine white mycelioid base and white rhizoids. Odor and taste not determined.

Basidiospores 4.8–5.6(6.4) × (3)3.2–4 µm ($n = 34/1$, $m = 5.3 \pm 0.4 \times 3.7 \pm 0.3$, $Q = 1.2–1.6(2)$, $Q^m = 1.4 \pm 0.2$), ellipsoid or ± pip-shaped in profile view, ellipsoid in face view, very obscurely, and minutely angled in polar view (6–8 angles), and some appearing round in light microscopy,

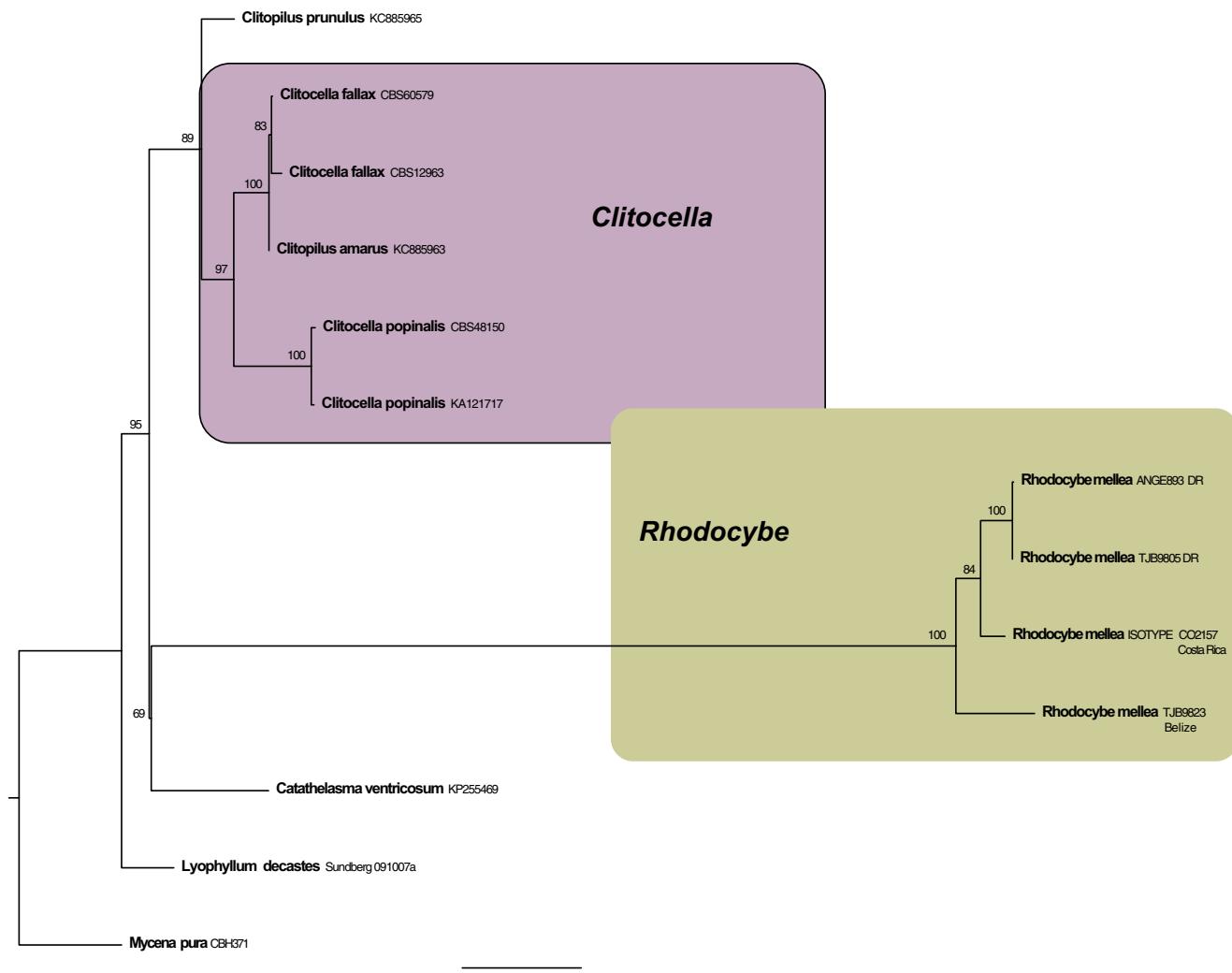


Fig. 3 ITS ML phylogenetic analysis of *Rhodocybe mellea* collections from Belize, Costa Rica, and the Dominican Republic

obscurely bumpy undulate in light microscopy, clearly undulate bumpy in SEM, walls evenly cyanophilic, not amyloid nor dextrinoid, \pm hyaline in 3% KOH. Basidia 4—sterigmate, clavate, lacking cyanophilic bodies, (17)20–26 \times 5.6–8 μm . Hymenial cystidia absent. Lamella trama hyaline, parallel, cylindrical hyphae, 4–7 μm in diam, hyphae of subhymenium narrowly cylindrical, 2–3.5 μm in diam. Pileus context hyaline or pale straw yellow, composed of loosely interwoven but radially arranged non-encrusted cylindrical hyphae, 4.8–9(11.3) μm in diam. Pileipellis, a brown repent and compact layer in 3% KOH, composed of cylindrical hyphae, 2.4–6 μm in diam, heavily or moderately encrusted with brown pigment. Stipitipellis hyaline or pale yellow, with repent, non-encrusted, cylindrical hyphae, 2–4 μm in diam. Clamp connections absent.

Habit and habitat: Small pleurotoid basidiomata, gregarious on decaying, abandoned nest of *Nasutitermes corniger* Motschulsky (= *N. costalis* Holmgren) in secondary tropical rain forest similar to the habitat described for

R. luteocinnamomea var. *fulva*. Fruiting in winter (December).

Discussion: Morphologically, basidiospores with rounded obscure angularity in polar view and obscure pustulate-bumpy surface ornamentation of the spores are typical features of species placed in the genus *Clitocella*. However, the ellipsoid basidiospores, the small stature and eccentric stipe, and the habit of growing on decaying woody materials of an arboreal termite nest set this new species apart from all other known species of *Clitocella*. Species of *Clitocella* typically produce subglobose or short ellipsoid or amygdaliform-shaped basidiospores, the basidiomata are mostly moderate sized or some species produce larger fruit bodies, all have central stipes, and none are lignicolous as they are found growing on decaying leaves, humus, or soil in various temperate habitats.

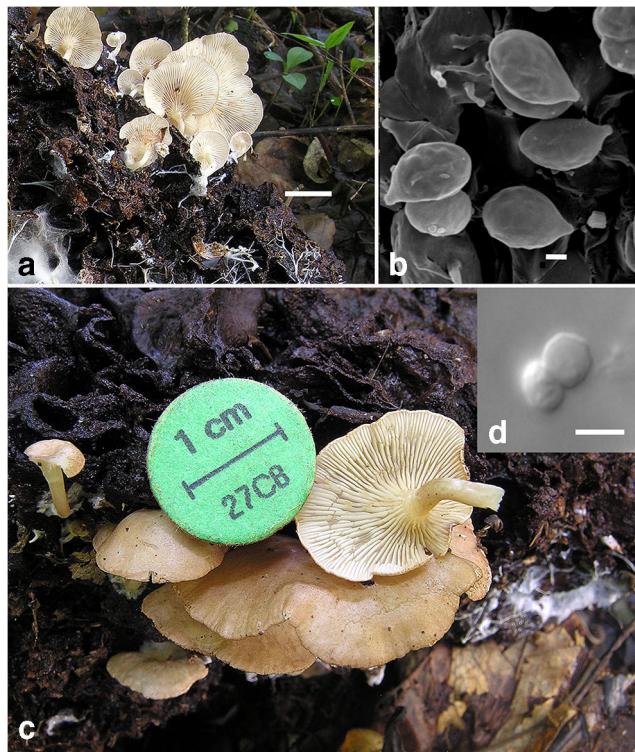


Fig. 4 *Clitocella termitophila*, ANGE264, type. **a** Basidiomata on abandoned arboreal termite nest. **b** SEM of basidiospores. **c** Basidiomata showing pileus surface. **d** Transmitted light micrograph (LM) of basidiospores in polar view showing subtle rounded angularity. Scale bars, a and c = 1 cm, b = 1 μ m, d = 4 μ m

***Clitopilus velutinus* T. J. Baroni & Angelini, sp. nov.**

Fig. 5a–f

Mycobank: MB 831959

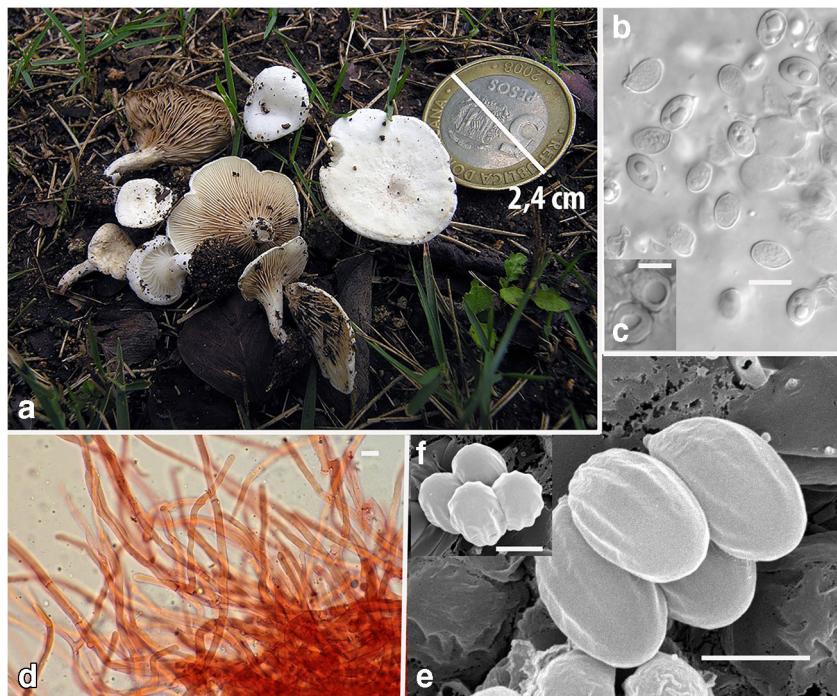
Typification: Dominican Republic, Santo Domingo, the National Garden (Jardin Botanico Nacional Dr. Rafael), approximate location 18.495833, –69.953056, terrestrial under deciduous trees, 11 December 2015, leg. C. Angelini ANGE535 consisting of eight basidiomata, (**holotype** JBSD 127422; **isotype** CORT 014618—small piece of pileus).

Diagnosis: Differing from other small white *Clitopilus* species with eccentric stipes, e.g., *C. scyphoides*, by a robust stature with stipe longer than pileus diameter, by the velvety pileus surface, by basidiospores with 10–14 ridges, and by ITS sequences.

Etymology: Referring to the velvety pileus surface.

Basidiomata medium to small clitocyboid in stature, resembling a small *Clitopilus prunulus*. Pileus pure white, sometimes faintly crème yellow in age, 10–25 mm, convex, then plane, sometimes with depressed center, not umbonate, the margin involute for a long time often irregularly lobed, dry, opaque, not hygrophanous, with the surface typically velvety. Context whitish, 2–3 mm thick, moderately fleshy, not cartilaginous. Lamellae white at first, then slowly rosy with the maturation of the spores, eventually pinkish ocher, arcuate-decurrent at first, then long decurrent on the stipe with age, subdistant, thick, broad, edges concolorous, lamellulae in mostly 3 tiers. Stipe white as the pileus, 20–40 mm long, 4–7 mm broad, some cylindrical, but often tapered towards the base, mostly eccentric, glabrous but surface may be bumpy,

Fig. 5 *Clitopilus velutinus*, ANGE535, type. **a** Basidiomata. **b** Light microscopy image of basidiospores. **c** Basidiospores in polar view. **d** Pileipellis. **e** SEM image of basidiospores. **f** SEM image of polar view of basidiospores. Scale bars, a = 2.4 cm, b = 10 μ m, c = 5 μ m, d = 10 μ m, e and f = 5 μ m



solid but somewhat fragile, white inside. Odor and Taste slight farinaceous. Spore print dark pinkish ocher.

Basidiospores $7\text{--}9 \times (4.5)5\text{--}6 \mu\text{m}$, ($n = 38/1$, $m = 8 \pm 0.5 \times 5 \pm 0.4$, $Q = 1.27\text{--}1.8$, $Q^m = 1.5 \pm 0.1$), ellipsoid in profile and face view, minutely angled in polar view (10–14 facets), faintly longitudinally striate with ridges running pole to pole, walls evenly cyanophilic, inamyloid, \pm hyaline in 3% KOH. Basidia (2)4 sterigmate, broadly clavate, lacking cyanophilic bodies, $23\text{--}38 \times 7\text{--}12 \mu\text{m}$, with sterigmata up to $5 \mu\text{m}$ long, with scattered thick-walled basidia strongly staining in Congo Red. Hymenial cystidia absent. Pileipellis hyaline, loosely erect entangled trichodermial arrangement of cylindrical hyphae, $(2)3\text{--}5 \mu\text{m}$ in diam. Stipitipellis hyaline compact layer of repent, parallel, non-encrusted, cylindrical, thin-walled hyphae, $5\text{--}7 \mu\text{m}$ in diam. Clamp connections absent.

Habit and habitat: Medium to small *Clitopilus prunulus* like basidiomata, gregarious, on soil in tropical forest garden. Fruiting in December.

Discussion: The basidiomata remind one of a more robust version of *Clitopilus scypoides* (Fr.) Singer known from Europe (Singer 1946, p. 554–557), mainly because of the small white basidiomata with slightly depressed pileus, slightly eccentric stipe and decurrent lamellae that eventually turn dark fleshy pink when the basidiospores mature. But the robust stature of *C. velutinus* immediately separates this taxon from *C. scypoides*. In addition, the erect interwoven trichodermial pileipellis and the shorter and slightly broader basidiospores with 10–14 angles/ridges in polar view separate *C. velutinus* from *C. scypoides* that produces basidiospores ($7.5\text{--}11.5 \times 4\text{--}5.5 \mu\text{m}$) with only 6–8 angles/ridges in polar view and a pileipellis composed of repent cylindrical hyphae (a cutis) (Singer 1946).

Singer (1946) also discussed two other varieties of *C. scypoides*, var. *floridanus* (Murrill) Singer and var. *submicropus* (Rick) Singer, both of which he found in Florida. The typical variety of *C. scypoides* was cited as only occurring in Europe (Singer 1946). The typical form of *C. scypoides* has a slender stipe (only up to 2 mm wide) that is shorter than the diameter of the pileus (from Singer 1946 as pileus dimension 5–25 mm broad, and stipe dimensions as 4–10 mm long—see <https://www.flickr.com/photos/biodivlibrary/8723366380> for an image of this taxon there labeled as *Clitopilus cretatus* Berk. & Broome, a synonym of *C. scypoides* sensu Index Fungorum: <http://www.indexfungorum.org/Names/Names.asp>). The varieties *floridanus* and *submicropus* described by Singer (1946) have stipes as long as the pileus is wide and basidiospores that are different in dimensions when compared to the typical *C. scypoides*. These two varieties also have a pileipellis composed of repent, cylindrical, interwoven, hyaline hyphae, much like the type variety, *scypoides* (Singer 1946). Neither of these varieties are morphologically comparable to *C. velutinus*. *Clitopilus scypoides* var. *intermedius*

(Romagn.) Noordel., another European taxon, also differs from *C. velutinus* by possessing a fruity odor, basidiospores with 6–8 angles in polar view and a repent cutis for a pileipellis (Noordeloos 1984).

Clitopilus subscyphoides WQ Deng, TH Li & YH Shen a recently described species from China (Deng et al. 2013), also reported from India (Raj and Manimohan 2018), is characterized by its entirely white omphalinoid basidiomata with umbilicate, slightly velutinus or finely pruinose pileus surface making it somewhat similar to *C. velutinus*. However, *C. subscyphoides* differs from *C. velutinus* by its small size (pileus under 10 mm broad), smaller basidiospores ($5\text{--}7 \times 3.5\text{--}4.5 \mu\text{m}$) with 8–10 prominent longitudinal rides and angles in polar view, and a repent cutis for a pileipellis (Raj and Manimohan 2018).

Unfortunately, most species of *Clitopilus* listed in GenBank only have an ITS sequence, and a very few have one of the three protein coding genes currently used to compare species of the Rhodocybe-Clitopilus clade. Therefore, the ITS sequence of *C. velutinus* was compared with ITS sequences of morphologically similar *Clitopilus* species retrieved from GenBank. Based on this single ITS marker, *C. velutinus* is phylogenetically distinct from *C. scypoides* and related taxa (Fig. 2).



Fig. 6 *Rhodocybe dominicana*, ANGE473, type. **a** Basidiomata. **b** SEM of basidiospores. **c** LM of basidiospores. **d** LM of pileipellis showing encrusted hyphae and inflated subpellis cells. Scale bars, **a** = 1 cm, **b** = $5 \mu\text{m}$, **c** = $5 \mu\text{m}$, **d** = $10 \mu\text{m}$

Rhodocybe dominicana T. J. Baroni & Angelini, sp. nov.

Fig. 6a–d

Mycobank: MB 831964

Typification: Dominican Republic, Puerto Plata Province, Town of Sosúa, on litter in a disturbed secondary deciduous forest proximal to the area of Sosúa Beach, approximate location 19.757315–70.516439, 4 December 2014, leg. C. Angelini ANGE473 consisting of five basidiomata (**holotype** JBSD 127399; **isotype** CORT 014746—small piece of pileus). **Diagnosis:** Differing from the somewhat similar *Rhodocybe roseavellanea* and *R. luteocinnamomea* by the two-layered pileipellis with inflated nearly globose cells making up the hyphae in the subpellis, from *R. roseavellanea* by the lack of hymenial cystidia and the habitat of secondary deciduous forest, and from all other taxa by the combined *RPB2*, *TEF1*, and *ATP6* sequences.

Etymology: The epithet refers to the country of origin, La República Dominicana.

Basidiomata clitocyboid in stature. Pileus buff-ocher (5B3—grayish orange) at first with a white inrolled margin, hygrophanous, and developing sordid cream hues (slightly darker than 4A2—yellowish white) over the center, but more highly colored (5B4–3) from mid-pileus to the margin with expansion, (15)25–50(65) mm broad, convex, some mature specimens becoming broadly depressed over the disc, often with margin undulate or undulate-pleated, moist, smooth, appearing glabrous but moderately covered with fine white canescence as moisture evaporates, this canescence obvious on inrolled margins in young stages. Context off white, 2–3.5 mm thick. Lamellae pale at first but becoming flesh pinkish (6B3—flesh), adnate becoming short decurrent or arcuate-decurrent with expansion, close, broad (2.7–3.8 mm deep), edges even or becoming irregularly undulate, lamellulae in 1–4 irregular tiers of varying lengths. Stipe buff with cream color hues (4A2–3—cream, light yellow), 30–45(55) mm long, 4–8(10) mm broad, cylindrical, central, cylindrical, solid, often curved, with fine white pubescence over the apex and covered by irregular patches of a white mycelial mat over the lower portions of the base, also with numerous white rhizoids. Odor and taste aromatic, floury like bread crust.

Basidiospores (4.8)5–7.2 × 3.5–4.8 µm ($n = 30/1$, $m = 6 \pm 0.5 \times 4.2 \pm 0.3$, $Q = 1.2\text{--}1.6$, $Q^m = 1.4 \pm 0.1$), ellipsoid or subamygdaliform in profile, ellipsoid in face view, minutely angular in polar view (6–9 angles), undulate-pustulate in all views, hyaline, walls evenly cyanophilic, inamyloid. Basidia 16–25 × 5–7 µm, 4-sterigmate, clavate, hyaline, lacking cyanophilic bodies. Hymenial cystidia absent. Lamella trama hyaline, hyphae parallel, cylindrical or slightly inflated, 4–16 µm in diam, hyphae of subhymenium narrowly cylindrical, 2–3.5 µm in diam. Pileipellis cream yellow or yellow-brown in 3% KOH, two-layered and 100–140 µm deep, suprapellis a thin layer, 10–30 µm deep, of repent cylindrical hyphae, 6–10 µm in diam, thin-walled, with pale yellow brown finely

encrusting pigments in 3% KOH, subpellis 90–110 µm deep, of inflated nearly globose, short-celled hyphae, mostly 18–30 µm wide by 26–46 µm long, also finely encrusted with brown pigments, but also with some interwoven cylindrical hyphae similar to suprapellis present. Pileus context hyaline, composed of radially arranged, cylindrical hyphae, 6–10 µm in diam, not encrusted with pigments. Stipitipellis hyaline or pale yellow, composed of repent, cylindrical hyphae, 6–8 µm in diam with occasional upturned cylindrical or subcapitate end cells (caulocystidia) scattered over the surface, 30–50 × 6–7 µm. Stipe context hyaline, parallel hyphae, not differentiated from the stipitipellis. Clamp connections absent.

Habit and habitat: Medium sized, clitocyboid, gregarious basidiomata occurring on decaying leaf litter substrates and in the same secondary deciduous forest area as *R. luteocinnamomea* var. *fulva*. Fruiting in winter (December).

Other specimens examined: Dominican Republic, Puerto Plata Province, Town of Sosúa, on litter in a secondary deciduous forest proximal to the area of Sosúa Beach, 4 December 2014, approximate location 19.757222, – 70.516611, near sea level, leg. C. Angelini ANGE464 consists of two basidiomata (JBSD 127400).

Discussion: *Rhodocybe dominicana* belongs in Section *Rufobrunnea* and is morphologically similar to *Rhodocybe roseavellanea* because of the moderately bulky size, pinkish colors of the pileus, adnate or short decurrent, close lamellae, and the amygdaliform or subamygdaliform-shaped basidiospores. *Rhodocybe dominicana* differs by the lack of cheilocystidia, by having a two layered pileipellis, by the habitat of growing in secondary deciduous maritime tropical forests that lack oak and pine, and by the combined molecular data of *RPB2*, *TEF1*, and *ATP6*.

Another species that might be confused with *R. dominicana* is *R. luteocinnamomea* var. *luteocinnamomea* described from Panama and the British Virgin Islands (Ovrebo and Baroni 2007) due to the somewhat similar colors of the basidiomata: i.e. pale buff-ochre colors of the pileus for both species, but for *R. luteocinnamomea* also with light cinnamon brown hues over the disc; the pinkish colored, close and at least short decurrent lamellae for each species, and the yellowish or cream-buff colors of the stipes. However, *R. luteocinnamomea* is a thinner fleshed species with the pileus context only 1.5–2 mm thick at the disc versus 2–3.5 mm for *R. dominicana*. Also, the basidiospores of *R. luteocinnamomea* are shorter and differently shaped (4.5–5.5(6) × (3.5)4–4.5(5) µm, with a Q^m of 1.23 (Ovrebo and Baroni 2007) versus (4.8)5–7.2 × 3.5–4.8 µm with a Q^m value of 1.4 for *R. dominicana*). The basidiospores are distinctly subglobose or broadly elliptical and angular or subangular in profile views with 5–7 irregular facets for *R. luteocinnamomea*. The basidiospores of *R. dominicana* are ellipsoid or subamygdaliform (Fig. 5b–c) and not

obviously angular in profile views. Another main difference separating the two is that the pileipellis of *R. dominicana* is two-layered with many large swollen nearly spherical cells in the subpellis, reminding one of the inflated cells of the hypodermium of *Mycena* species. This layer of inflated cells is missing in *R. luteocinnamomea*.

Rhodocybe piperita (Stevenson) Horak described from New Zealand and only known from that region so far, also has a pale colored pileus and stipe (pinkish buff), and pinkish decurrent lamellae, but the basidiomata are large (up to 80 mm broad) and have an odor and taste of pepper. In addition, the spores are much larger, $6.5\text{--}10.5 \times 5\text{--}7(8.5)$ μm (Baroni 1981) than those of *R. dominicana*.

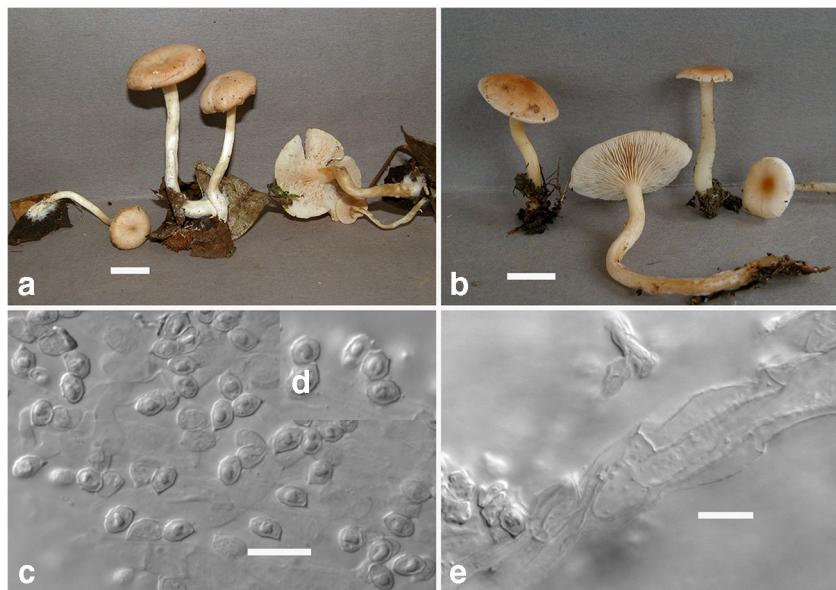
Based on our molecular studies, *R. dominicana* is phylogenetically related to *Rhodocybe alutacea*, a slender more petite North American taxon. However, these two taxa are morphologically separated by significant differences in basidiomata size (pileus 25–35 mm broad and stipe 23–35 mm long by 2.5–5.5 mm wide at apex for *R. alutacea*), by odor and taste which is farinaceous for *R. alutacea*, and by the pileipellis morphology of a simple compact repent layer in *R. alutacea*.

***Rhodocybe pseudoalutacea* T. J. Baroni, Lodge & Lacey sp. nov. Fig. 7a–d**

Mycobank: MB 831965

Typification: Dominican Republic, Santiago Province, near Las Placetas, off of Carretera Las Placetas, close to the bridge over the River Bao on the uphill side of the river on the left just off of the road, four fruiting bodies on leaf humus of mixed broad leaf trees, approx. location 19.242576–70.898551, 13 January 2003, leg. T. J. Baroni

Fig. 7 *Rhodocybe pseudoalutacea*. **a** Basidiomata, TJB9507, type. **b** Basidiomata TJB9502. **c** LM basidiospores TJB9502. **d** LM basidiospores in polar view TJB9502. **e** Pileipellis, showing encrusted hyphae TJB9502. Scale bars, a and b = 1 cm, c = 10 μm , d = 5 μm , e = 10 μm



TJB9507 with *E. Grand* (alt. ledger DR-2502), four fruit bodies (**holotype** CORT 013781).

Diagnosis: Distinguished from *Rhodocybe alutacea* by the brown pileus color, lack of cheilocystidia, and by the combined *RPB2*, *TEF1*, and *ATP6* sequences.

Etymology: somewhat similar morphologically to *Rhodocybe alutacea* Singer.

Basidiomata clitocyboid in stature. Pileus brown (7E6; 6D7—raw sienna) or brownish orange (5C5–6—topaz, Pompeian yellow) over disc, light brown (6D5–6—sunburn, cinnamon brown) over margin or entire cap cinnamon brown (6D5–6) becoming paler and grayish orange (5B4) to pale orange/orange white (5A2–3) to pale cream (4A2—yellowish white) over the margin with moisture loss, hygrophanous, 10–35 mm broad, broadly convex, some with low broad umbo, becoming plano-convex then plane, some with shallow depressed disc, innately radiate fibrillose or matted with radiate silvery fibrils overall, glabrescent with expansion, margin incurved becoming plane, opaque. Context watery buff or white, 1–5 mm thick at disc. Lamellae pale orange pinkish (5A2–3—orange white, pale orange) or grayish orange (5B3) with slight flesh hues, adnate to subdecurrent or short decurrent, close to crowded, moderately broad (1–2 mm). Stipe buffy cream or dull cream color (between 4A2—yellowish white and 5A2 orange white), 15–50 mm long, 2–6 mm broad, central, equal or enlarged downwards, terete, fine white pruinose over apex, glabrous elsewhere or with sparse white mycelioid covering over base, also with fine white rhizoids radiating out from base, solid, becoming stuffed then hollow. Odor not distinctive or slightly of sulfur when first cut open. Taste mild or slightly sweet. Spore print pale pinkish in light deposit.

Basidiospores (5)5.5–7(8) \times (3.5)4–5(5.5) μm ($n = 77/3$, $m = 6.2 \pm 0.5 \times 4.27 \pm 0.4$, $Q = (1.1)1.2\text{--}1.6(1.8)$, $Q^m = 1.5 \pm 0.1$), mostly subamygdaliform, also ellipsoid in profile view,

ellipsoid in face view, minutely but obviously angular in polar view (7–10 angles), undulate-pustulate in all views, walls evenly cyanophilic, inamyloid. Basidia 4-sterigmate, clavate, lacking cyanophilic bodies, (17)20–26(29) × 5.6–8 µm. Hymenial cystidia absent. Lamella trama hyaline, parallel, cylindrical, 4–7 µm in diam, or with short inflated hyphae, 24–58 × 8–20 µm at top of lamella trama near pileus context. Pileus context hyaline or pale straw yellow, composed of loosely interwoven and radially arranged cylindrical or inflated hyphae, 4.8–14.5 µm in diam, with scattered undulating cylindrical refractive, septate, hyphae, 4–5 µm in diam, occasionally present. Pileipellis a pale yellowish or yellowish ochre repent, compact layer in 3% KOH, composed of moderately to finely encrusted cylindrical hyphae, 2.4–6.4 µm in diam, with obscure recumbent mostly clavate inflated end cells, 8–10 × 28–40 µm, over the disc on some pilei. Stipitipellis pale golden yellow, composed of repent, non-encrusted, cylindrical hyphae, 1.5–5 µm in diam., with hyaline, cylindrical, clavate, cylindrical-contorted, or versiform caulocystidia, 22–48 × 3.2–8 µm, singly or in clusters scattered over the apex. Clamp connections absent.

Habit and habitat: Slender clitocyboid basidiomata occurring scattered or gregarious on decaying leaf vegetation or well-decayed woody debris and small branches in moist creek bed area, also on leaf humus or even soil in drier areas, under broad leaved trees and shrubs or with *Pinus occidentalis* also present in the central areas of the Dominican Republic. Fruiting in winter (January).

Other specimens examined: Dominican Republic, Santiago Province, near Las Placetas, off of Carretera Las Placetas, close to the bridge over the River Bao, 13 January 2003, approximate location 19.242187, –70.897242, approx. 700 m, leg. T. J. Baroni TJB9502, two basidiomata (CORT 013780); same general area but further along road towards Las Placetas, 8 January 2003, approximate location 19.240578–70.898056, leg. T. J. Baroni TJB9466, with D. J. Lodge, L. Lacey, seven basidiomata (CORT 013777).

Discussion: *Rhodocybe pseudoalutacea* is similar morphologically to *Rhodocybe alutacea* Singer in stature and somewhat in colors of the basidiomata. The *R. pseudoalutacea* is a slender, clitocyboid species, like *R. alutacea*. The *R. pseudoalutacea* produces darker colors on the pileus, brown or cinnamon brown, than has been recorded for *R. alutacea* of the southeastern United States. *Rhodocybe alutacea* produces pinkish cinnamon or cinnamon buff hues when fresh. Both species have an hygrophanous pileus surface that becomes paler over the margin at first, then progressively losing color intensity towards the disc and becoming very pale grayish orange or pale cream in *R. pseudoalutacea* and even paler approaching white or pale pinkish buff for *R. alutacea*. Both species also have a pileus surface that is described as somewhat sericeous for *R. alutacea* and innately radiate fibrillose or matted fibrillose for *R. pseudoalutacea*. In both

cases, the pileus surface eventually becomes glabrous. In both cases, the stipe is pale colored, buffy, or dull cream colored in *R. pseudoalutacea* and light pinkish cinnamon or white in *R. alutacea*.

One distinctive morphological difference separating these two species is the presence of septate, cylindrical cheilocystidia scattered on the lamella edges of *R. alutacea*, but such septate cheilocystidia are lacking in *R. pseudoalutacea*. Two of the three collections of *R. pseudoalutacea* did have very widely scattered and infrequent more or less cylindrical cells barely projecting from some of the lamella edges, but these structures were not consistent in a basidiome, nor were they septate. However, based on the three gene molecular comparison, the Dominican Republic collections of *R. pseudoalutacea* are phylogenetically distinct from the North American *R. alutacea*.

***Rhodocybe luteocinnamomea* T. J. Baroni & Ovrebo var. *fulva* Angelini & T. J. Baroni var. nov.**

Fig. 8a–d

Mycobank: MB 831966

Typification: Dominican Republic, Puerto Plata Province, Town of Sosúa, on litter in a disturbed secondary deciduous forest just behind Sosúa Beach, approximate location 19.757315–70.516439, 10 December 2013, leg. C. Angelini ANGE169 consisting of two basidiomata (**holotype** JBSD 127397; **isotype** CORT 014745—small piece of pileus).



Fig. 8 *Rhodocybe luteocinnamomea* var. *fulva*, ANGE169, type. **a** Basidiomata. **b** LM of basidiospores. **c** LM of basidiospores at higher magnification. **d** LM of single basidiospore in polar view. Scale bars, a = 1 cm, b and c = 5 µm

Diagnosis: Phylogenetically identical to *Rhodocybe luteocinnamomea* with a three-gene analysis (*RPB2*, *TEF1*, and *ATP6*), but strikingly different from *Rhodocybe luteocinnamomea* by the fulvous colored pileus surface and the lack of encrusting pigments on the hyphae of the pileipellis.

Etymology: varietal name for the tawny orange color of the pileus surface.

Basidiomata clitocyboid in stature. Pileus tawny orange (5B4–5 grayish-orange, nearly 5B6 apricot yellow), 15–40 mm broad, convex with a low broad umbo in young specimens, becoming plano-convex, surface moist, smooth, hygrophanous, becoming pale cream (4A3—cream) over the center but with margin remaining tawny orange and incurved. Context 1.5–2 mm thick, off white. Lamellae off white, buff or pinkish buff (5A2—orange white) with maturity, adnate with short decurrent tooth, close, broad (2.5–3.5 mm deep) edge irregular and sinuous, lamellulae (1–)3 tiers. Stipe buff, with fine white fibrils over the apex, with a thick white fibrillose coating over the lower half in the smaller basidiome, 25–35 mm long, 3–5 mm broad, equal or slightly enlarged downwards, cylindrical, central, solid, somewhat curved, base covered by white mycelial mat. Odor of mushrooms. Taste not distinctive.

Basidiospores $4.5\text{--}5.6 \times (3.8)4\text{--}4.8 \mu\text{m}$, ($n = 21/1$, $m = 5.0 \pm 0.4 \times 4.1 \pm 0.3 \mu\text{m}$, $Q = 1.1\text{--}1.4$, $Q^m = 1.2 \pm 0.1$), subglobose or short broad ellipsoid in profile and face views, minutely angular in polar view (7–10 angles), finely bumpy-pustulate in all views, hyaline, walls evenly cyanophilic, inamyloid. Basidia $18\text{--}24(30) \times 6\text{--}7(8) \mu\text{m}$, 4-sterigmate, clavate, hyaline, lacking cyanophilic bodies. Hymenial cystidia absent. Lamella trama hyaline, hyphae parallel, cylindrical or slightly inflated, $3\text{--}7(12) \mu\text{m}$ in diam; hyphae of subhymenium cylindrical, $2\text{--}4 \mu\text{m}$ in diam. Pileipellis sordid cream or pale yellow-brown in 3% KOH, hyphae repent forming a cutis of cylindrical hyphae, $2.4\text{--}4(6) \mu\text{m}$ in diam, thin-walled, smooth, with pale yellow brown parietal pigments. Pileus context hyaline, composed of loosely interwoven, cylindrical or slightly inflated hyphae, $4\text{--}10 \mu\text{m}$ in diam. Stipitipellis hyaline, pale yellow or pale yellowish brown, similar to the pileipellis with repent, cylindrical hyphae $2.4\text{--}5 \mu\text{m}$ in diam, with scattered erect or ascending cystidiole end cells, variable in form but mostly cylindrical or subclavate, some septate, $13\text{--}20 \times 4\text{--}5.6 \mu\text{m}$. Stipe context hyaline, parallel hyphae, $6\text{--}8 \mu\text{m}$ diam. Repository hyphae present in lamella trama and pileus context, with pale yellow contents in 3% KOH, $6\text{--}8 \mu\text{m}$ in diam, shiny and undulating, sparsely septate. Clamp connections absent.

Habit and habitat: Medium sized basidiomata with clitocyboid habit, fruiting on mixed decaying vegetation proximal to Playa Sosúa, in the general locality of 19.757222, –70.516611, in a secondary tropical forest of *Bucida buceras* L., *Guazuma tomentosa* H.B.K., *Roystonea hispaniolana*

(H.B.K.) O.F. Cook, *Hura crepitans* L., *Samanea saman* (Jacq.) Merrill, *Swietenia mahagoni* (L.) Jacq., *Terminalia catappa* L., *Calophyllum calaba* L., *Coccoloba diversifolia* Jacq., *Ocotea coriacea* (Sw.) Britton, *Acacia macracantha* Willd., *Celtis trinervia* Lam., *Bursera simaruba* (L.) Sarg., and with *Petivera alliacea* L. dominating the understory vegetation. Only known to fruit in the winter months (December) so far.

Discussion: We originally considered this collection an undescribed species reminiscent of *Rhodocybe incarnata* T. J. Baroni & Halling, known only from Venezuela. The tawny orange pileus colors and pale colored stipe were somewhat similar to aged basidiomata of *R. incarnata*. However, *Rhodocybe incarnata* differs by its tricholomatoid robust basidiomata with sinuate lamella attachment, a subtomentose pileus surface, and very different microscopic features such as the presence of cheilocystidia, and a trichodermial pileipellis of erect entangled cylindrical, septate hyphae. These features are lacking in our collection.

We were quite surprised when the collection matched the pale colored *R. luteocinnamomea* phylogenetically in our three-gene analysis (Fig. 1). The pileus colors cited in the original description of *R. luteocinnamomea* are at most light ochre to dull cinnamon brown and the pileus is hygrophanous, thus becoming even paler as the moisture leaves the cap. Either the original collections, six of them cited in the protolog (Ovrebo and Baroni 2007), were all over mature fruit bodies, or the Dominican Republic collection represents a distinctive color morph of this Caribbean species. The latter hypothesis is most likely the case. It would be unusual to find so many collections of a species from Panama and the British Virgin Islands that all were pale in color because they were collected in their late developmental cycles. We therefore recognize this taxon as a distinct variety.

When comparing the micromorphological data, the Dominican Republic collection matches the structures found for *R. luteocinnamomea*: Basidiospore size, shape, and surface ornamentation; lack of hymenial cystidia; structure and pigmentation of pileipellis hyphae, except for fine encrustations on the hyphae of the Panamanian and British Virgin Island collections, but only intraparietal pigments for the Dominican Republic collection.

Rhodocybe luteocinnamomea var. *fulva* belongs in Section *Rufobrunnea* (Baroni 1981) due to the lack of pseudocystidia, lack of clamp connections, and the brightly colored pileus surface.

New reports

Rhodocybe roseiavellanea (Murrill) Singer, Lilloa 22:609. 1951 [1949]

Fig. 9a–d

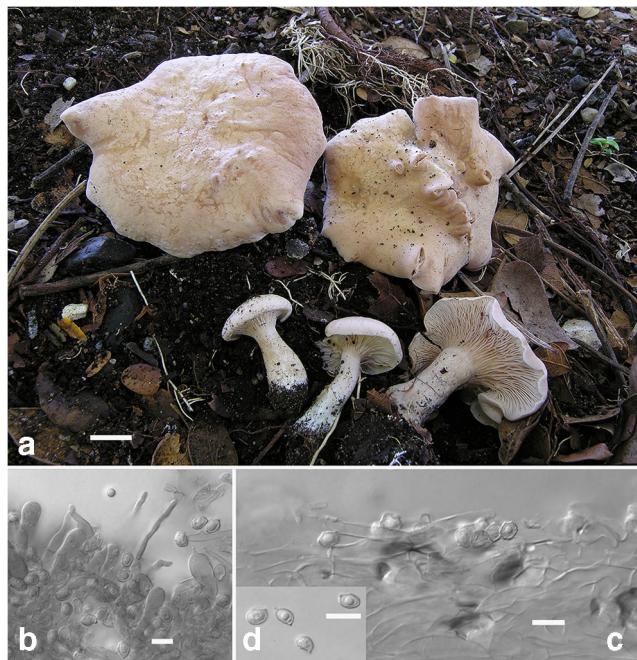


Fig. 9 *Rhodocybe roseavellanea*. **a** Basidiomata, ANGE956. **b** Cheilocystidia, ANGE947. **c** Pileipellis, ANGE947. **d** Basidiospores, ANGE947. Scale bars, a = 1 cm, b–d = 10 μm

A brief description is provided of the collections found in Puerto Plata Province along the northern coast of the Dominican Republic.

Basidiomata clitocyboid, fleshy. Pileus pinkish or rosy tinted over avellaneous ground color when fresh, fading to sordid with age, 15–80 mm broad, convex becoming plano-convex, broadly depressed, becoming somewhat uplifted with margin becoming wavy-undulate with age, margin inrolled or incurved consistently, dry, glabrous, but may become concentrically cracked with age. Context white, up to 10 mm thick. Lamellae pale alabaster white or pinkish buff at first, becoming darker pinkish flesh with age and eventually turning flesh brown, adnate or mostly decurrent, close, narrow (3–5 mm deep). Stipe concolorous with pileus, 20–40 mm long, 6–18 mm wide at apex, equal but some with a swollen base, terete, solid and white inside, sometimes becoming cavernous hollow. Odor and Taste not sampled.

Basidiospores (5.6)6.4–8 \times 4.5–5.6 μm ($n = 40/2$, $m = 7 \pm 0.6 \times 5 \pm 0.4$, $Q = 1.14\text{--}1.6$) $Q^{\text{m}} = 1.4 \pm 0.1$), subamygdaliform or ellipsoid in profile view, sometimes slightly angular at apical polar end, ellipsoid in face view, minutely and unevenly angled in polar view (6–8 angles), bumpy pustulate overall, walls evenly cyanophilic, inamyloid, pale golden yellow in mass in 3% KOH. Basidia 4-sterigmate. Cheilocystidia scattered, barely projecting, cylindrical. Pileipellis a narrow, yellow brown pigmented layer of compact, repent and/or entangled cylindrical hyphae, with scattered vertical, erect end cells, but not typically projecting above the surface, 3.2–7.2 μm in diam. Clamp connections absent.

Habit and habitat: Scattered or gregarious on decomposing leaves and humus. December.

Specimens examined: Dominican Republic, Puerto Plata Province, near the Town of Sosúa in Puerto Chiquito, next to a tree-lined driveway of the parking area of an old abandoned hotel on decomposing leaves of *Albizia (Samanea) saman* F. Muell., 2 December 2017, approximate location 19.750864–70.526111, leg. C. Angelini ANGE 947 consists of seven basidiomata (JBSD127424; CORT014741); same general location, 17 December 2017, leg. C. Angelini ANGE 956 consists of six basidiomata (JBSD127425; CORT014747).

Discussion: *Rhodocybe roseavellanea*, originally described from Florida, is a large fleshy species with stipe thickness reaching 10–20 mm, that occurs widely spread along the gulf coastal United States and the eastern seaboard as far north as Massachusetts (Baroni 1981). The pileus with delicate pinkish flush mixed in with muted pale brown (avellaneous) or pale grayish brown colors when the basidiomata are young and fresh, is characteristic for this species. The pileus colors fade to pale leather brown or even paler to eventually sordid and develop a concentric cracked pattern over the margin. Murrill also noted this loss of color with age for one of his Florida collections (Baroni 1981). The amygdaliform-shaped basidiospores, the pileipellis that is composed of interwoven or repent cylindrical hyphae that occasionally produce erect end cells, the lack of encrusting pigments on the pileipellis hyphae, and the often widely scattered cylindrical cheilocystidia are also distinctive features of this species.

Even though *R. dominicana* and *R. roseavellanea* can be found in the same general area, they should not be confused because *R. dominicana* produces cream colors that develop in the pileus and stipe with age. Also *R. dominicana* lacks cheilocystidia, and more importantly produces a distinctive two-layered pileipellis.

Phylogenetically, the collections of *R. roseavellanea* from the Dominican Republic are indistinguishable from those of North America (Fig. 1). Not only does the three-gene analysis confirm the connection, but a 99% ITS match between the Dominican Republic collections and one in GenBank from Tennessee (MF686525—PB Matheny PBM4056 = TENN070822!) also confirms the identification of the Dominican Republic collections.

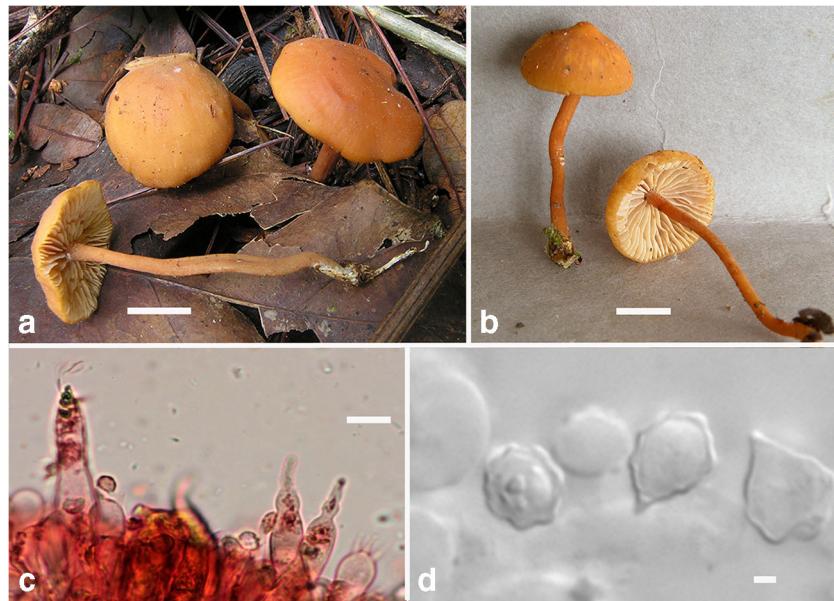
Rhodocybe mellea TJ Baroni & Ovrebo, Mycologia 80:513. 1988

Fig. 10a–d

= *Clitopilus melleus* (TJ Baroni & Ovrebo) Noordel. & Co-David

Basidiomata collybioid in stature. Pileus orange-brown (6 C-D 8 Brownish Orange, Light Brown or 7D 7–8 Terra Cotta, Burnt Sienna), slowly and slightly hygrophanous or strongly hygrophanous and becoming paler orange (5C5 topaz or 5B–

Fig. 10 *Rhodocybe mellea*. **a** Basidiomata, ANGE893. **b** Basidiomata, TJB9808. **c** Hymenial pseudocystidia, stained in Congo Red, ANGE893. **d** Basidiospores TJB9805. Scale bars, a and b = 1 cm, c and d = 10 μm



C7–8 golden yellow, oxide yellow, brownish yellow), 13–35 mm broad, conical or convex with conical or papillate or mamillate umbo at first or umbo lacking, becoming broadly convex to plane or somewhat uplifted and with strongly undulate margin, dry or slightly lubricous, glabrous, smooth, opaque or with faintly translucent-striate margin. Context concolorous with surface or pale buff, thin (1 mm), tough. Lamellae flesh brown (5C4 golden blonde) or pinkish orange (6B3 flesh to 6C4 red haired), adnexed or adnate, some with a short decurrent tooth, close, lamellae = 30, lamellulae = 3–4 tiers, seceding, irregularly forked, edge even, faces appearing waxy coated, broad (3–4 mm deep), typically turning yellow when bruised. Stipe more or less concolorous with pileus, 25–70 mm long, 1–5 mm broad, equal or gradually enlarged downwards on some, terete or mostly compressed with age, flexuous, white pruinose over apex or in some pruina lacking, glabrous elsewhere, smooth, pale ochre-buff fibrillose stuffed, tough and pliant. Odor not distinctive Taste astringent or slightly peppery.

Basidiospores $5–7.2 \times (4.5)4.8–5.6 \mu\text{m}$ ($n = 20/1$, $m = 6.17 \pm 0.6 \times 5 \pm 0.4$, $Q = (1)1.07–1.4$, $Q^m = 1.2 \pm 0.2$), subglobose or broadly ellipsoid and somewhat angular on some in profile view, subglobose or broadly ellipsoid in face view, minutely angled in polar view (7–9 angles), strongly bumpy undulate-pustulate, even at 400x in light microscopy, walls evenly cyanophilic, inamyloid, \pm hyaline in 3% KOH. Basidia $22–31.6 \times 6.4–7.2 \mu\text{m}$, 4-sterigmate, clavate, lacking cyanophilic bodies, some with shiny refractive bodies in 3% KOH. Hymenial cystidia as pseudocystidia, mostly originating in lamella trama and filled with dark ochre-orange, coagulated pigments in distilled H_2O and 3% KOH, mostly fusoid, some lageniform, projecting beyond the hymenium, $40.5–101 \times 5.6–10.5 \mu\text{m}$. Lamella trama hyaline, but also with

pigment filled hyphae connected to pseudocystidia, parallel, cylindrical hyphae, $2.4–4.8 \mu\text{m}$ in diam. Pileus context hyaline, radially arranged non-encrusted hyphae, cylindrical or slightly inflated, $4.8–8 \mu\text{m}$ in diam. Pileipellis dark golden ochre in 3% KOH, repent, cylindrical hyphae, $2.4–6.4 \mu\text{m}$ in diam. Stipitipellis dark golden ochre as pileipellis, composed of repent, non-encrusted, cylindrical hyphae, $2.4–4 \mu\text{m}$ in diam., at the apex producing cylindrical, contorted, erect, projecting “caulocystidia,” $16–32 \times 2.4–3.2 \mu\text{m}$, these sometimes filled with ochre-orange, coagulated pigments similar to the pseudocystidia of the hymenium. Clamp connections absent.

Habit and habitat: Scattered on soil or well-decayed humus, under or near *Pinus occidentalis* or under mixed hardwoods. November.

Specimens examined: Dominican Republic, Santiago Province, near Pedregal, Plan Sierra Los Montones Convention Center, on well-decayed humus, 17 November 2003, approximate location 19.288524–70.925180, collected by Mycology Class, leg. T. J. Baroni TJB9805, consisting of 5 basidiomata (CORT 013885); similar location, collected by Mycology Class, leg. T. J. Baroni TJB9808, consisting of 2 basidiomata (CORT 013886); La Vega Province, Jarabacoa, near “Jardines del Montaña,” on well-decayed humus in mixed woods with dominant *Pinus occidentalis* present, 7 December 2016, approximate location 19.187068–70.590431, leg. C. Angelini ANGE893, consisting of 3 basidiomata (JBSD 127402); La Vega Province, Jarabacoa area, on the Mogote de Jarabacoa, on soil/leaf litter, 21 November 1999, 19.075404–70.668746, leg. T. R. Armstrong Curtis TRA258, 1 basidiome (CORT 014737); La Vega Province, near Manabao, on the property of Señor Cruz, approximately 19.066485–70.785131, on duff under

Pinus occidentalis, 21 November 1999, collected by Orson and Hope Miller, leg. T. J. Baroni TJB8974 three basidiomata (CORT 014471).

Discussion: *Rhodocybe mellea* was originally described from Costa Rica, but seems to be distributed widely in Central America, Mexico, and in a number of the Caribbean Islands such as Puerto Rico and now documented for Hispaniola and the Dominican Republic. The colors of the cap and stipe can be somewhat variable, ranging from orange or ochre-brown to light brownish, and then when losing moisture by hygrophany becoming quite pale and more yellowish or honey colored, hence the name. Also, the lamellae typically turn dull to bright yellow when bruised. The small subglobose or broadly ellipsoid basidiospores that are often somewhat angular in profile views and strongly pustulate-bumpy even under high dry magnifications, along with the striking darkly ochre-orange colored fusoid or lageniform hymenial pseudocystidia make this species distinct from all others.

In order to confirm similarity of all taxa cited from the Dominican Republic with *Rhodocybe mellea*, originally described from Costa Rica, we attempted sequencing of the ISOTYPE of *Rhodocybe mellea* (C. Ovrebo 2157). Unfortunately, only ITS sequences were obtained for this ISOTYPE collection of *R. mellea*. The ITS analyses (Fig. 3) of our collections compared with the ISOTYPE and collections from Belize when compared to a small subset of taxa in the Rhodocybe–Clitopilus clade that also have ITS sequences available in GenBank, show that the collections from the Dominican Republic, along with the Belize collection, are nested in a well-supported clade with the ISOTYPE collection from Costa Rica. These collections from the Dominican Republic, Costa Rica, and Belize appear to be all one species

that is not infrequently found in Central America and islands of the Greater Antilles in the Caribbean Basin. Unfortunately, all duplicate materials of this species originally deposited at JBSD have been lost to mold infections (personal communication with the current curatorial staff).

Clitopilus prunulus (Scop.) P. Kumm. Führ. Pilzk. 96. 1871

Fig. 11a–c

We provide a brief description of our collections of this species from the central mountain region of the Dominican Republic that compare morphologically and phylogenetically (Fig. 1) to this commonly encountered species in North America and Europe. This report is the first account of *C. prunulus* for Hispaniola and the Dominican Republic.

Basidiomata clitocyboid in stature, fleshy. Pileus white or sordid white with pale grayish buff cast and often with pale grayish tan or orange-gray (5B2—alabaster) over disc area, 13–40 mm broad, convex becoming plano-convex then plane, with inrolled margin, finely matted fibrillose overall under a lens or glabrous, smooth, soft to the touch. Context white, unchanging, solid, up to 10 mm thick. Lamellae white with cream hues at first, soon pale flesh pink (5A2 Orange White), decurrent, close or crowded, sometimes forked, sometimes intervenose, narrow (3 mm deep). Stipe white or concolorous with pileus, 13–30 mm long, 2–7(13) mm broad, ± equal, terete, finely matted white fibrillose especially over apex, or glabrescent downwards, white mycelium at base, flesh white, unchanging, solid. Odor farinaceous, cucumber-farinaceous. Taste strong farinaceous or rancid-farinaceous.

Basidiospores $8.8\text{--}11(16)\times 4.5\text{--}6.4 \mu\text{m}$ ($n = 32/2$, $m = 10.4 \pm 1.3 \times 5.2 \pm 0.5$), $Q = (1.57)1.76\text{--}2.35(2.89)$. $Q^m = 2.0 \pm 0.3$), amygdaliform or subfusiform in profile and face views, angled in polar view [(5)6(7) angles], with mostly 6 longitudinal ribs running pole to pole, walls evenly cyanophilic, not amyloid nor dextrinoid, pale straw yellow in 3% KOH. Basidia 4—sterigmate. Hymenial cystidia absent. Pileipellis a hyaline or pale brown layer of repent, cylindrical hyphae, 2.5–7 μm in diam, some hyphae with fine sandy-granular encrustations over disc area. Clamp connections absent.

Habit and habitat: Scattered, gregarious, or subcaespitose on soil or humus under *Pinus occidentalis*. November.

Specimens examined: Dominican Republic, San Juan Province, Parque Nacional José Del Carmen Ramírez, trail from Vallecito to Valle del Tetero with original departure from La Cienaga, on soil, 24 November 2002, approximate location 19.007186–70.891118, collected by Lance Lacey, leg. T. J. Baroni TJB9411, 3 basidiomata, (CORT 013671); same general location in Valle del Tetero, trail to Las Ballenas, 25 November 2002, approximate location 18.987281–70.930901, collected by D. J. Lodge, leg. T. J. Baroni TJB9414, 3 basidiomata, (CORT 013672); same general



Fig. 11 *Clitopilus prunulus*. TJB9425. **a** Basidiomata. **b** Basidiospores showing longitudinal ridges. **c** Basidiospores in polar view showing the typical 6 angles and ridges. Scale bars, a = 1 cm, b and c = 5 μm

location, entrance to first small valley leading to Casetta de Valle del Tetero, 26 November 2002, approximate 18.994639–70.922158, T. J. Baroni TJB9425, 10 basidiomata (CORT 013673).

Discussion: As is typical for the east coast North American version of *Clitopilus prunulus* some basidiospores are occasionally quite large and some of the hyphae of the pileus surface are finely granular encrusted, especially over the disc area. One unusual feature about these collections from the Dominican Republic is the rancid-farinaceous taste when fresh. The odor and taste were more intense than what are normally encountered. The duplicate splits of these collections originally deposited at JBSD were lost to mold infections of the dried basidiomata (personal communication with current curatorial staff).

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