

Rhodocybe fusipes (Entolomataceae), a new species from Amazonian ‘terra-firme’ forest of Brazil

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Rhodocybe fusipes (Entolomataceae) is described as a new taxon belonging to sect. *Rufrobrunnea*. The specimens were found in the Amazon rain forest of Brazil, growing on soil, among litterfall. This new species is morphologically characterized by its clitocyboid habit, reddish orange pileus, close lamellae, contrasting white, fusiform stipe with radicate base, globose to short ellipsoid basidiospores, absence of cystidia and by a cutis-like pileipellis. Molecular data (nrITS) support the recognition of this new species.

Keywords: Agaricales, Basidiomycota, rDNA ITS, molecular phylogeny, Neotropical fungi, taxonomy. 1 new species.

Rhodocybe s.l. Maire included approximately 50 taxa (He et al. 2019) spread among temperate and tropical regions (Singer & Digilio 1951, Pegler 1977, Baroni 1981, Baroni & Halling 1992, Baroni & Gates 2006). Recent multigene phylogeny rearranged the *Rhodocybe-Clitopilus* clade in five monophyletic clades/genera (Kluting et al. 2014): *Clitopilus* (Rabenh.) P. Kumm; *Clitocella* Kluting, T.J. Baroni & Bergemann; *Clitopilopsis* Maire; *Rhodocybe* Maire s.str. and *Rhodophana* Kühner. Among them, *Rhodocybe* s.str. is now restricted to species with pleurotoid, collybioid, mycenoid, clitocyboid or tricholomatoid habit, variously colored; attachment of lamellae ranging from adnexed to subdecurrent; basidiospores angular in polar view with 6–12 facets of pronounced undulate-pustulate ornamentations; cystidia mostly present and clamp connections absent (Kluting et al. 2014).

In Brazil, studies on *Rhodocybe* are limited to six publications: Singer (1973, 1989) described *R. conica* Singer and *R. crepidotoides* Singer, from Amazonian and Atlantic rain forests of Amazonia and Paraíba States, respectively; Pegler (1997) re-

ported the occurrence of *R. crepidotoides* and *R. pseudonitellina* Dennis from Atlantic rain forest of São Paulo State; de Meijer (2006) listed *R. aff. albovelutina* (G. Stev.) E. Horak, *R. cf. caelata* (Fr.) Maire, *R. caelatoidea* Dennis, *R. aff. mellea* T.J. Baroni & Ovrebo, *R. aff. conchata* E. Horak, *R. cf. mycenoides* and *R. pseudonitellina* from Atlantic rain forest of Paraná state, but most require revision; later, de Meijer (2008) described *R. levispora* de Meijer, a name that probably corresponds to genus *Rhodophana*, due to presence of clamp connection. More recently, Silva-Filho et al. (2018) reported *R. caelatoidea* and *R. galerinoides* Singer also from Atlantic rain forest in Southern Brazil.

During field trips in order to study macrofungi in areas of Central Amazonia forest (Komura et al. 2016, 2017), an interesting species of *Rhodocybe* was collected, a priori, similar to *R. incarnata* T.J. Baroni & Halling, described from Venezuela (Baroni & Halling 1992). In this context, in which *Rhodocybe* is still a poorly studied genus in Brazil, we describe *Rhodocybe fusipes* as a new species based on morphological characters and phylogenetic hypoth-

esis by ITS, which also confirms its placement in *Rhodocybe* sect. *Rufrobrunnea* T.J. Baroni.

Materials and methods

Sampled areas

The specimens were collected from 'Estação Experimental de Manejo Florestal do INPA (ZF-2)', Manaus, Amazonas State (02° 37' and 02° 38' S; 60° 09' and 60° 11' W) in a "terra-firme" tropical rainforest of the Brazilian Amazon. This region presents a forest with acid and very clayey oxisol soil with a high concentration of minerals, due to wood residues left from selective logging (Ferreira et al. 2001). Regarding their flora, more than 300 plant species belonging to 173 genera and 57 families occur there (Jardim & Hosokawa 1986/1987).

Morphological analyses

Macroscopic characteristics were described based on fresh material. Color codes were based on Kornerup & Wanscher (1978). Dimensions of basidiospores are given as (<10 %, minimum length) average minimum length – average maximum length (<10 %, maximum length) × (<10 %, minimum width) average minimum width – average maximum width (<10 %, maximum width). For biometric measurements of the basidiospores, we follow the emended methodology of Wartchow & Gamboa-Trujillo (2012). All microstructures were analyzed using KOH 5 % and Congo red and described according to terminology used by Largent et al. (1977). Microscopic measurements and photographs were made under a Nikon Eclipse Ni (LM) with Nikon DS-Ril camera coupled using the NIS-Elements Ar v.4.51.00 software. Specimens were deposited in the Herbaria JPB, INPA (Thiers 2019) and in the Mycological collection at the 'Universidade Federal do Rio Grande do Norte' (UFRN-Fungos).

Molecular methods

The DNA was extracted from fresh basidioma using the FTA® card (Dentinger et al. 2010). The nuclear ribosomal internal transcribed spacer region (nrITS) was amplified and sequenced using primers ITS1-F and ITS4-B (White et al. 1990). Obtained sequences were assembled and edited with the software BioEdit (Hall 1999).

Phylogenetic analysis

For this study, a dataset composed of nrITS sequences of 28 specimens was constructed, using

25 sequences from NCBI (Genbank) and UNITE (Köljalg et al. 2013, unite.ut.ee/), and three new generated sequences from the new species and the isotype of *R. incarnata*. The sequences represent species of *Rhodocybe* s.str., *Clitocella mundula* (Lasch) Kluting, T.J. Baroni & Bergemann and *C. fallax* (Quél.). Kluting, T.J. Baroni & Bergemann were used as the outgroup. The dataset was aligned using MAFFT v.7 (Katoh & Standley 2013), under the Q-INS-i criteria. Seaview v.4 (Gouy et al. 2010) was used for manual alignment. For Maximum Likelihood RAxML v8.2.X (Stamatakis 2006) was used. The best nucleotide model was selected with AIC (Akaike Information Criterion) using jModelTest 2v.1.6 (Guindon & Gascuel 2003, Darriba et al. 2012). Bayesian inferences (BI) were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 were used in the CIPRES Science Gateway 3.1 (Miller et al. 2010). The final alignment and the resultant topologies were deposited in TreeBASE, ID: 25696.

Results and discussion

The final nrITS dataset included 28 taxa sequences, and the nrITS alignment resulted in 947 characters (including introduced gaps). The best evolutionary model estimated for the alignment was TPM2uf+G. The bootstrapping search for the ML analysis stopped after 156 replicates. In phylogenetic inferences based on nrITS (Fig. 1), the genus *Rhodocybe* and the sect. *Rufrobrunnea* form well-supported clades (100% BS / 1.0 BPP). Within section *Rufrobrunnea* the studied specimens clustered in an independent, well-supported lineage in a supported terminal branch (100% BS / 1.0 BPP) separated from *R. incarnata*.

Taxonomy

Rhodocybe fusipes Silva-Filho, D.L. Komura & Wartchow, **sp. nov.** – Figs. 2–13.
Mycobank no.: MB835472

Diagnosis. – Distinguished by clitocyboid basidiomata; orange to reddish and slightly umbonated pileus; close to crowded, white lamellae, white or pale sordid orange fusiform and radicate stipe; globose to subglobose basidiospores (4.5)5–5.5 × 4–5(5.5) µm; absence of cystidia; a cutis-like pileipellis and unique nrITS sequences.

Holotypus. – BRAZIL, Amazonas, Manaus, Estação Experimental de Manejo Florestal ZF-2, 21 May 2012, *leg.* D.L. Komura 587 (INPA259371).

Description. – Basidiomata, clitocyboid. – Pileus 13–47 mm diam., convex to plane convex

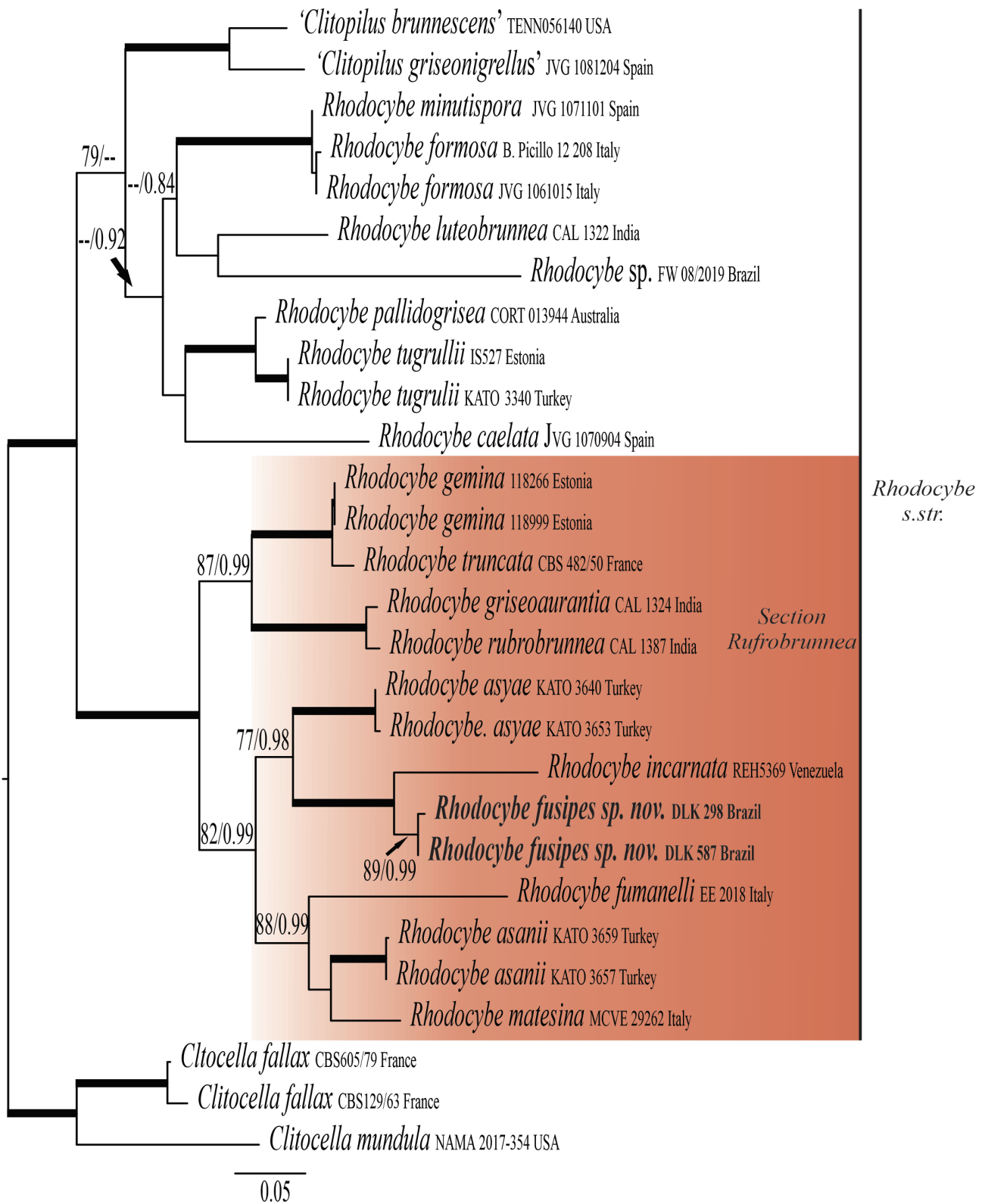
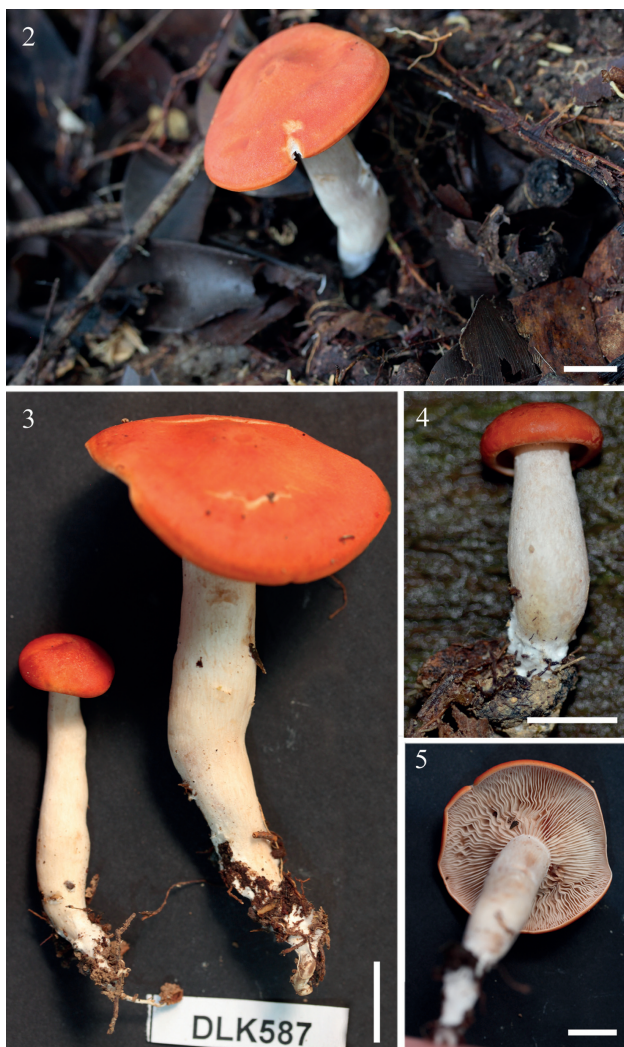
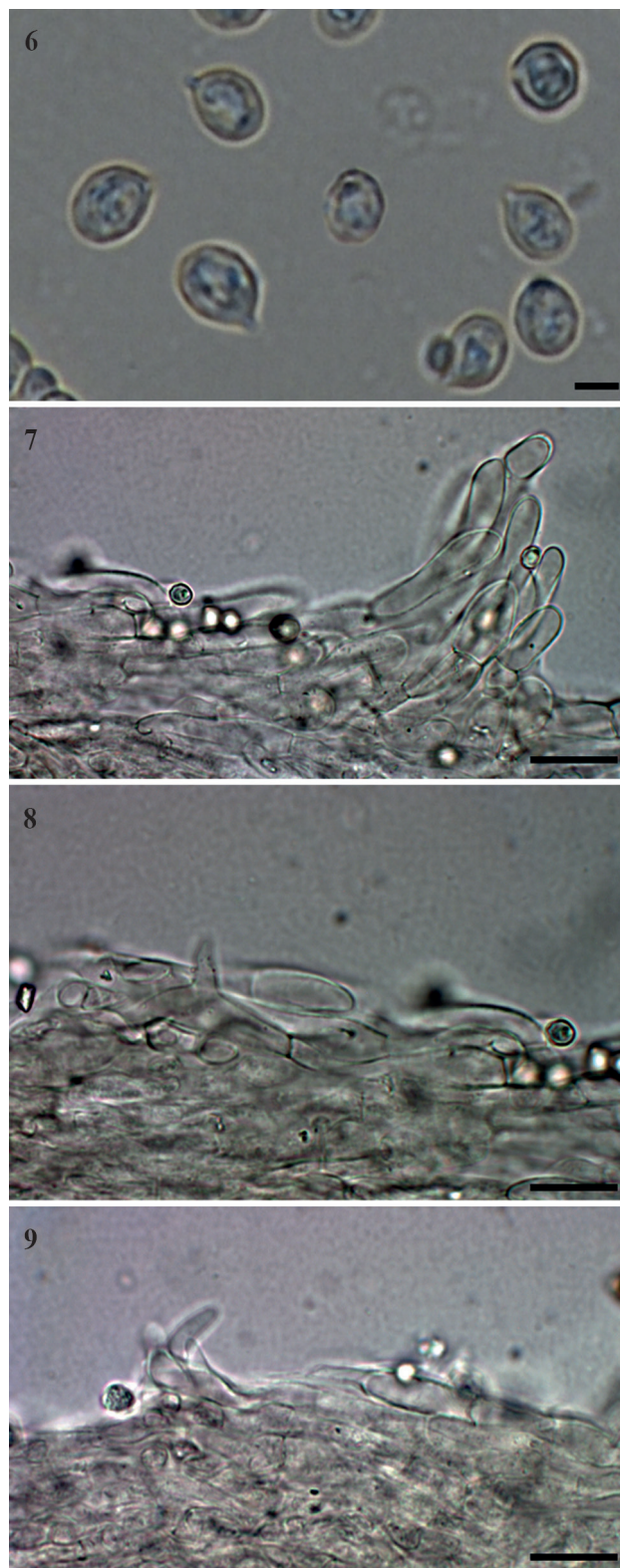


Fig. 1. The ML phylogeny based on ITS sequences. *Rhodocybe fusipes* sp. nov. in bold. Bootstrap values and posterior probabilities indicated if they exceed 60 % and 0.80, respectively. The thicker branches represent those with maximum bootstrap and posterior probability values (100 % BS / 1.0 BPM). Scale bar represents the expected number of nucleotide changes per site.

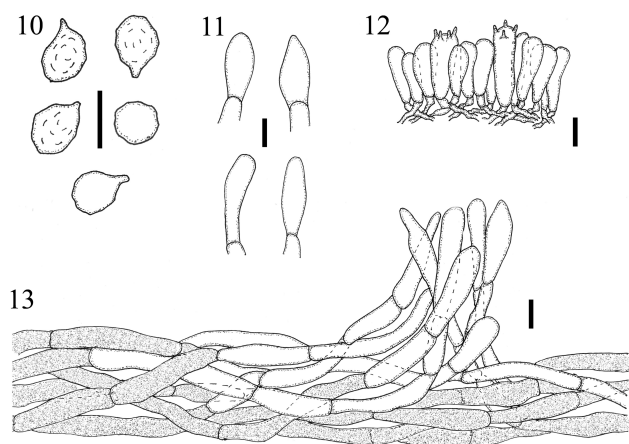


Figs. 2–5. Basidiomata of *Rhodocybe fusipes*: 2. DLK 587 in situ. 3. DLK 587 in situ. 4. DLK 298 immature basidiomata. 5. DLK 587 in situ. All photos by D.L. Komura. Bars 10 mm.



Figs. 6–9. Photographs of *Rhodocybe fusipes* microstructures, in KOH: 6. Basidiospores. 7. Pileipellis. 8. Pileipellis. 9. Stipitipellis. Bars 10 μ m.

with low broad umbo, deep orange (6A8), orange (6B8), reddish orange (7A8) (7B8), high red (10A8); surface smooth to slightly pruinose, not hygrophalous; margin smooth, decurved to incurved, slightly lobed (Figs. 2–4); context 1–3 mm thick, fleshy, white (6A5), unchanging. – Lamellae short decurrent to uncinat, close to crowded, sometimes forked, membranous, white (1A1) becoming reddish (8A2) with the age; margin smooth becoming eroded, concolorous; lamellulae of three lengths (Fig. 5). – Stipe 43–63 \times 5–10 (apex), 8–13 (center), 5–8 mm (base), central, fusiform, white (1A1), with brownish orange (7C8), greyish orange (5B4) fibrils and stains; surface longitudinally fibrillose; consistency fleshy; radican, up to 12 mm long; basal mycelium strigose,



Figs. 10–13. Drawings of *Rhodocybe fusipes* microstructures: **10.** Basidiospores. **11.** Terminal hyphae of pileipellis. **12.** Side of Hymenium. **13.** Pileipellis. Bars 10 μm .

white (1A1) (Figs. 2 - 5); context solid, white (1A1). – Spore print not observed.

Basidiospores $(4.5)5\text{--}5.5 \times 4\text{--}5(5.5) \mu\text{m}$; $n = 40/2/2$; $L = 5.13 \mu\text{m}$, $W = 4.5 \mu\text{m}$; $Q = 1.00\text{--}1.25$, $Q_m = 1.12$; globose to short ellipsoid in profile view, angular in polar view with 8–10 angles, undulate/pustulate in all views; thin-walled, inamyloid, hyaline; hilar appendix evident, up to $1.8 \mu\text{m}$ long (Figs. 6, 10). – Basidia $27\text{--}31.5 \times 5\text{--}7 \mu\text{m}$, cylindrical-clavate to clavate, tetrasporic, thin-walled, hyaline (Fig. 7); sterigmata up to $4 \mu\text{m}$ high. – Pleurocystidia and cheilocystidia absent. – Lamellar edge fertile, composed of basidioles and scattered basidia. – Lamellar trama subregular, composed of cylindrical hyphae, $3\text{--}6 \mu\text{m}$ diam., smooth, thin-walled, hyaline. – Pileipellis a cutis with mostly radially oriented hyphae, hyphae $4.5\text{--}8 \mu\text{m}$

Tab. 1. Species, voucher collection, origin and GenBank and UNITE accession numbers of nrITS sequences used in the molecular analyses.

| Species | Voucher collection | Origin | ITS accession n° |
|---------------------------------------|-------------------------|---------------|------------------|
| <i>Clitocella fallax</i> | CBS605/79 | France | AF357018 |
| <i>Clitocella fallax</i> | CBS129/63 | France | AF357017 |
| <i>Clitocella mundula</i> | NAMA 2017-354 | USA | MH979252 |
| ' <i>Clitopilus brunnescens</i> ' | TENN056140 | USA | HQ222033 |
| ' <i>Clitopilus griseonigrellus</i> ' | LIP JVG 1081204 | Spain | KU862859 |
| <i>Rhodocybe asanii</i> | KATO 3657 | Turkey | KX834265 |
| <i>Rhodocybe asanii</i> | KATO 3659 | Turkey | KX834263 |
| <i>Rhodocybe asyae</i> | KATO 3640 | Turkey | KX834266 |
| <i>Rhodocybe asyae</i> | KATO 3653 | Turkey | KX834268 |
| <i>Rhodocybe caelata</i> | JVG 1070904-2 | Spain | KU862855 |
| <i>Rhodocybe formosa</i> | JVG 1061015 | Italy | KU862857 |
| <i>Rhodocybe formosa</i> | Herb. B. Picillo 12/208 | Italy | KU862858 |
| <i>Rhodocybe fumanelli</i> | EE-2018 | Italy | MH399225 |
| <i>Rhodocybe fusipes</i> | DLK 587 Paratype | Brazil | MN306210 |
| <i>Rhodocybe fusipes</i> | DLK 298 Holotype | Brazil | MN306209 |
| <i>Rhodocybe gemina</i> | 118999 | Estonia | UDB023556 |
| <i>Rhodocybe gemina</i> | 118266 | Estonia | UDB015436 |
| <i>Rhodocybe griseoaurantia</i> | CAL 1324 | India | KX083571 |
| <i>Rhodocybe incarnata</i> | REH5369 Isotype | Venezuela | MT254071 |
| <i>Rhodocybe luteobrunnea</i> | CAL 1322 | India | KX083570 |
| <i>Rhodocybe matesina</i> | MCVE:29261 | Italy | KY629961 |
| <i>Rhodocybe minutispora</i> | LIP JVG 1071101 | Spain | KU862860 |
| <i>Rhodocybe pallidogrisea</i> | CORT 013944 | Australia | KX271752 |
| <i>Rhodocybe rubrobrunnea</i> | CAL 1387 | India | KX951452 |
| <i>Rhodocybe</i> sp. | FW 08/2019 | Brazil | MT408946 |
| <i>Rhodocybe truncata</i> | CBS 482/50 | France | EF421110 |
| <i>Rhodocybe tugrulii</i> | IS527 | Estonia | UDB011605 |
| <i>Rhodocybe tugrulii</i> | KATO 3340 | Turkey | NR154436 |

diam., smooth to slightly encrusted, thin-walled, grayish in KOH, sometimes colorless, with scattered clusters of erect terminal cells; terminal elements $21\text{--}43 \times 6\text{--}8.5 \mu\text{m}$, cylindrical to slightly clavate, sometimes with mucronate apex smooth, thin-walled, hyaline; terminal elements becoming scarce towards the margin (Figs. 7, 8, 13). – *Pileus trama* composed of irregular hyphae $6\text{--}8 \mu\text{m}$ diam., periclinally oriented, smooth, thin-walled, hyaline, sometimes grayish pigmented. – *Stipitipellis* a cutis composed of cylindrical hyphae $4.5\text{--}8.5 \mu\text{m}$ diam., smooth, thin-walled, hyaline; terminal cells $20\text{--}30.5 \times 4.5\text{--}8.5 \mu\text{m}$, cylindrical to cylindrical-clavate, thin-walled smooth to finely encrusted, hyaline (Fig. 9). – *Caulocystidia* absent. – *Stipititrama* subregular, composed of hyphae $3\text{--}6 \mu\text{m}$ diam., smooth, thin-walled, hyaline. – *Oleiferous* hyphae (thrombopleurous) scattered, present in pileus trama and stipititrama. – *Clamp connections* absent in all tissue.

Etymology. – Latin ‘fusi’ = spindle; and ‘pes’ = foot. In reference to the fusiform stipe shape.

Habitat. – In ‘terra firme’, an Amazon ecosystem that houses a high diversity of Lecythidaceae, Sapotaceae, Burseraceae and Fabaceae species (Oliveira 2000), on soil, among litterfall, probably saprotrophic.

Distribution. – Known only from type locality.

Material examined. – BRAZIL. Amazonas, Manaus, Estação Experimental de Manejo Florestal ZF-2, 21 May 2012, leg. D.L. Komura 587 (INPA 259371, Holotype, JPB, Isotype); *ibid.*, April 2012, leg. D.L. Komura 298 (UFRN 3288 Paratype).

Discussion

Rhodocybe fusipes is characterized by its orange to reddish pileus with a broadly umbonate disc; adnate to short decurrent, close to crowded and sometimes furcate white lamellae; white or pale sordid orange stipe with distinctly tapered fusiform base; subglobose to broadly ellipsoid basidiospores; absence of cheilocystidia and a cutis-like pileipellis. Central stipe, reddish pileus and absence of hymenial pseudocystidia and clamp connections indicate that *R. fusipes* belongs to *R. sect. Rufrobrunnea* (Baroni 1981).

Rhodocybe incarnata is closely related to *R. fusipes* in its phylogenetic relationships and morphological similarities, such as: convex to plane-convex and reddish orange pileus ($20\text{--}55 \text{ mm}$ diam.), adnate to sinuate-adnate, close to crowded lamellae and contrasting white or pale sordid orange fusiform-

like stipe (Baroni & Halling 1992). However, *R. incarnata* produces cylindrical, ventricose-rostrate cheilocystidia, cylindrical caulocystidia, shorter basidia ($20.3\text{--}24 \times 5.6\text{--}6.4 \mu\text{m}$) with siderophilous and cyanophilous bodies, and a pileipellis with short ($10.5\text{--}25 \mu\text{m}$ long) terminal elements (Baroni & Halling 1992). *Rhodocybe asyae* from Turkey also is a phylogenetically close taxon. *Rhodocybe asyae* was found near to coniferous trees, absent in the Amazonian forest, and it is morphologically differentiated by salmon pink to reddish brown pileus, smaller stipe ($25\text{--}30 \times 2\text{--}5 \text{ mm}$), broadly ellipsoid to ellipsoid basidiospores and presence of versiform cheilocystidia (Sesli & Vizzini 2017).

In the key for South American *Rhodocybe* s.l. species presented by Baroni & Halling (1992), the absence of both pleuro- and cheilocystidia, leads to *R. pseudonitellina*, originally described from Venezuela (Dennis 1953). This is the only species of *R. sect. Rufrobrunnea* with subglobose to broadly ellipsoid basidiospores that does not produce hymenial cystidia. *Rhodocybe pseudonitellina* has basidiospore size, arrangement of lamellar trama and pileipellis (Baroni 1981) similar to *R. fusipes*, but differs mainly in the macromorphology: *R. pseudonitellina* has brownish orange and campanulate to convex pileus, adnate and subdistant lamellae and reddish brown, non-radicant and hollow stipe (Pegler 1977, Baroni 1981), but in contrast, *R. fusipes* has reddish orange and convex to plane-convex pileus, close to crowded and short decurrent to uncinat lamellae and whitish, radican and solid stipe.

Another five *Rhodocybe* species belonging to *sect. Rufrobrunnea* were originally described from the Neotropical region: *R. hygrophoroides* T.J. Baroni & Halling, *R. laeta* Singer, *R. luteocinnamomea* T.J. Baroni & Ovrebo, *R. testacea* Dennis and *R. rickii* Singer. All mentioned species contain greyish orange, brownish orange or reddish-brown pileus, broadly ellipsoid to ellipsoid basidiospores and, except for *R. luteocinnamomea* and *R. testacea*, all of them produce some kind of hymenial cystidia (Baroni 1981, Baroni & Halling 1992).

The phylogenetic relationship between the Neotropical species of *Rhodocybe* remains uncertain due to absence of ITS sequences of described species. It seems, the rain forests of the Neotropical region reveal a great diversity of *Rhodocybe*, but are still underexplored. Thus, the discovery of new species and the inclusion of new sequences are expected to provide a better understanding of the taxonomic delimitations of the *Rhodocybe* species.

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