Mycologia, 94(3), 2002, pp. 545–553.
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Russulaceae of the Pakaraima Mountains of Guyana. I. New species of pleurotoid *Lactarius*

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Abstract: Morphological and habitat descriptions, illustrations and taxonomic discussions are presented for two newly described species of pleurotoid *Lactarius*, *L. brunellus* and *L. multiceps*, from the Pakaraima Mountains of Guyana. A third species, *L. igapoensis*, is synonymized with *L. panuoides*.

Key Words: basidiomycetes, ectomycorrhiza, neotropics

INTRODUCTION

Recent efforts to document ectomycorrhizal fungi from Guyana have focussed on taxa associated with the ectotrophic legume genera *Dicymbe* Spruce ex Benth. and *Aldina* (Benth.) Endl. in the Pakaraima Mountains (Henkel 1999, Henkel et al 2000, Miller et al 2001, Simmons et al 2001). During the course of these studies it became apparent that many species are undescribed and that previously described but rarely collected species could benefit from additional detailed morphological and habitat descriptions and illustrations. Many of these fungi belong to the Russulaceae.

Lactarius section *Panuoidei* was originally erected by Singer (1952) to accommodate *Lactarius panuoides* Singer from Trinidad. Two additional species from the neotropics and one from Japan were subsequently described (Singer 1984). The outstanding characters of section *Panuoidei* are the pleurotoid morphology of the small basidiomata, poorly developed sphaerocytes, and a tendency to produce a subiculum of thick-walled hyphae, a character suite highly atypical for *Lactarius* (Singer 1984). Henkel et al (2000) redescribed three pleurotoid species of Russulaceae, transferring one from the genus *Lactarius* to the genus *Russula*, and confirming an ectomycorrhizal habit for two of the species. Recent expeditions to Guyana have uncovered two additional pleurotoid species associated with *Dicymbe corymbosa* Spruce ex Benth. that are described here. The present paper is the first in a series that will describe or redescribe and illustrate members of the Russulaceae from the Pakaraima Mountains of Guyana. It is hoped that this work will facilitate the accurate recognition and documentation of these and other members of the Russulaceae elsewhere in the neotropics.

Accepted for publication October 29, 2001.

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MATERIALS AND METHODS

Collecting expeditions were made in May–Jun 1998 and 1999 to the Upper Ireng River Basin and May–Ju1 2001 to the Upper Potaro River Basin along Guyana's western border with Brazil in the south-central Pakaraima Mountains (geographic coordinates: 5° 05′ N; 59° 58′ W). Basidiomata were examined in the field for fresh characteristics. Color characteristics were coded according to Kornerup and Wanscher (1981; code noted in parentheses) and described subjectively. Spore deposits on acetate sheets were examined for fresh color characteristics and spore dimensions. A large $FeSO_4$ crystal was rubbed directly on the stipe, lamellae and flesh and color changes noted. Basidiomata were dried using large-bead silica gel and later placed in resealable plastic bags with small-bead silica gel to prevent spoilage in the excessively humid conditions. Additional basidiomata in various stages of development were preserved in FAA. Ectomycorrhizal rootlets collected from the subicula of both species were also placed in FAA for subsequent anatomical observations.

Dried and preserved basidiomata and ectomycorrhizae were observed under an Olympus BH-2 microscope with bright-field optics and microscopical observations recorded. At least 20 individual basidiospores were observed and measured per collection.

Herbaria designations are from Holmgren et al (1990) and include: BRG—University of Guyana, Georgetown; DUKE—Duke University, Durham, North Carolina; VPI—Virginia Polytechnic Institute, Blacksburg, Virginia; INPA—Instituto Nacional de Pesquisas de Amazonia, Manaus, Brazil. Some collections are retained in Miller and Aime personal herbaria.

TAXONOMY

Lactarius brunellus S. L. Miller, M. C. Aime et T. W. Henkel sp. nov. FIGS. 1–3, 4–10

Etymology. Latin *brunellus*, referring to the diminutive brown basidiomata.

Pileus 6–19 mm in diametro, conchatus ad dimidiatus, pallide brunneus ad brunneus, velutinus ad hispidulus, siccus. Lamellae subdistantes, subdecurrentes, tenues, lamellulis singulis ad tribus alternantes, albae ad cremeae. Stipes nullus vel 1–3 × 2–3 mm, cylindricus, excentricus vel lateralis, albus, tomentosus ad fasciculatus. Sapor mitis. Contextus tenuis, cremeus. Latex aqueus ad lacteus, in aëre aliquot minutis tingens carnem lamellasque castaneus. Sporae albae in cumulo, 6.8–8.4 × (5.6) 6.0–7.2 μm (Q = 1.13–1.16), subglobosae ad breviter ellipsoideae, echinulatae spinulosae. Pseudocystidia infrequentia ad abundantia, 2.5–3.5 μm lata. Pileipellis epithelium crassitie 2–5 cellularum, cellulis obovatis ad obpyriformibus trichomata multa parietibus crassis producentibus. Fructificans e subiculo expanso hirto involvente truncos, stipites, radicesque arborum in silvis tropicalibus humidis *Dicymbes corymbosae* Spruce ex Benth. (Caesalpiniacearum) in montibus Pakaraima, Guyana, Maio et Julio 2000. HOLOTYPUS: Henkel 7641 (BRG, DUKE).

Pileus 6–15 (19) mm broad, ungulate, conchate, flabelliform or dimidiate to substipitate, young primordia white with brown disks, mature specimens brown (7E7) at disk fading to light brown (6D6–8 to 6E6–8) at margin, extreme margin white when young, brown with age, velutinous to hispidulous when young, smoother and occasionally shiny with age; margin entire, inrolled when young, occasionally crenate when older; context thin, fragile, cream. *Stipe* present or absent, if present short cylindrical, 1–2 mm broad, tomentose to fasciculate, white. *Odor* not distinctive. *Taste* mild to strongly fungal. *Lamellae* off-white to light cream, subdistant, thin, subdecurrent to decurrent, tomentose near base, multitiered with lamellulae irregularly interspersed. *Latex* scant except when young and fresh, watery to milky, discoloring flesh and lamellae reddish brown in several minutes.

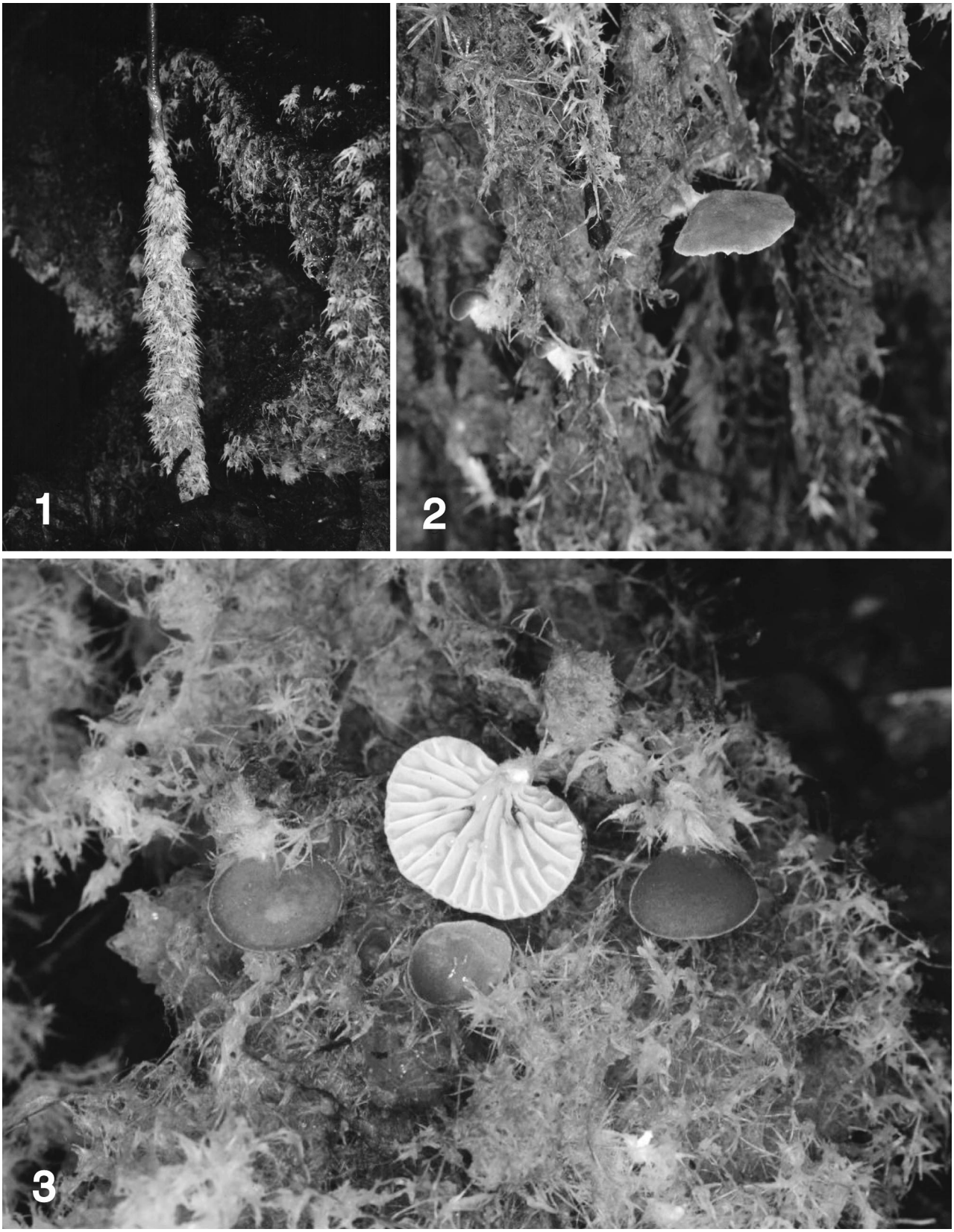
Basidiospores white in fresh deposit, 6.8–8.4 × (5.6)6.0–7.2 μm (Q = 1.13–1.16), subglobose to short ellipsoid, hyaline with amyloid echinulate-spinuose ornamentation; ornamentation 0.5–1.5(–2) μm high, usually acute with rare connectives; hilar appendix prominent 3–4 × 1.5–2 μm, hyaline; suprahilar plage large, nonamyloid. *Basidia* clavate, 43–53 × 9–10.5 μm, 4-spored; sterigmata 7–9 × 1–2 μm. *Pseudocystidia* infrequent to abundant, 2.5–3.5 μm wide, cylindrical or irregularly contorted, rounded or blunt-pointed at the apex, frequently branching at or below the level of the hymenium, not emergent, connected deeply to laticiferous hyphae in the trama; walls usually thin; contents similar to laticifer contents, refractive, negative in sulfovanillin. *Pleurocystidia* absent. *Hymenophoral trama* composed of interwoven, hyaline, thin-walled hyphae, 2–4 μm wide with scattered inflated cells 8–10 μm wide; lactiferous hyphae scattered, 8–11 μm wide with refractive guttate contents; sphaerocytes rare or absent. Subhymenial layer not well developed. *Pileipellis* a 2–5 cell thick epithelium composed of obovate, napiform to obpyriform or clavate thin-walled cells 10–20 μm wide with brownish contents in KOH, giving rise to scattered to abundant thick-walled hairs, the hairs 40–120 × 4–7 μm, cylindrical to slightly contorted, frequently with swollen bases, walls 1–2 μm thick, hyaline in KOH, negative in sulfovanillin. *Stipitipellis* a dense covering of erect hairs that are often tightly fasciculate, 500–1000 × 2–6 μm, and with 2–2.5 μm thick walls, straight or irregularly contorted, occasionally with irregular branches or knob-like termini, often irregularly septate, pseudocystidia 8–12 μm wide projecting above surface, irregularly contorted, pointed to irregular at the apex; walls usually thin; contents refractive, negative in sulfovanillin, connected deeply to laticiferous hyphae. *Subiculum* usually extensive, shaggy, off-white to greyish cream, composed of fascicles of hyaline, linear hyphae and hairs, 2–8 μm wide, thick walls 2–2.5 μm. *Clamp connections* not observed.

Macrochemical observations. $FeSO_4$—lamellae and flesh quickly dull green.

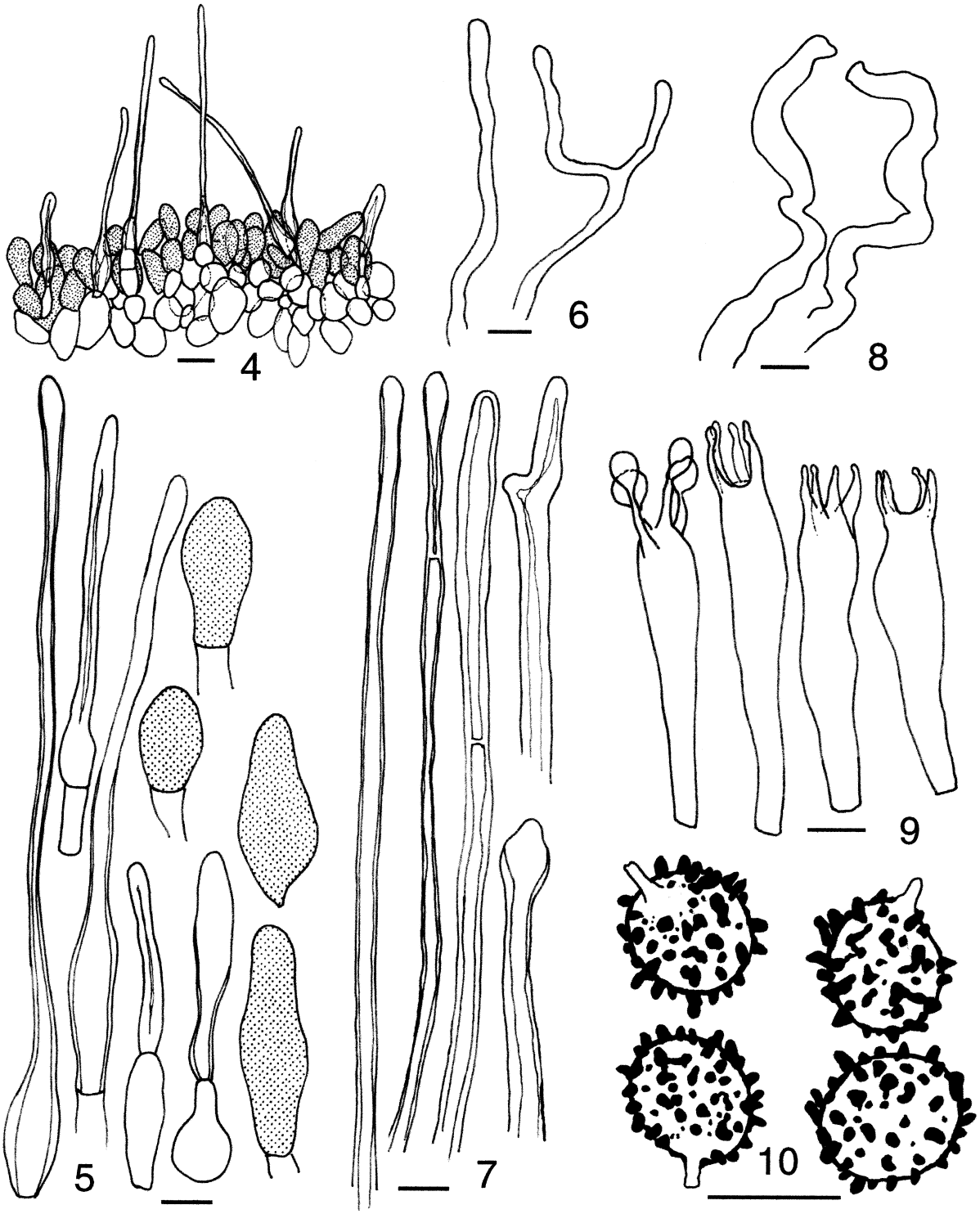
Habit, habitat, and distribution. Solitary to gregarious on lower trunks of saplings, larger trees, stumps and other elevated positions, arising from a shaggy, often deep and extensive persistent subiculum which enshrouds living and dead objects above the surface of the ground and which is often interspersed with or overriding bryophyte growth, subtended by ectomycorrhizal rootlets; found May through early July during wet weather in forests dominated by *D. corymbosa.* Collected near the Upper Potaro River, and observed but not collected by Henkel in the adjacent upper Ireng River Basin.

Specimens examined. GUYANA. Pakaraima Mountains, Upper Potaro River, 19 May 2000, *Miller 10028* (Miller personal herbarium), 24 May, *Aime 1016* (Aime personal herbarium), 30 May 2000, *Miller 10076* (Miller personal herbarium), 24 Jun 2000, *Miller 10168* (Miller personal herbarium); 18 Jul 2000, *Henkel 7641* (HOLOTYPE, BRG; DUKE ISOTYPE).

Commentary. Lactarius brunellus is one of at least three small pleurotoid subiculate *Lactarius* species found western Guyana forests. *Lactarius brunellus* is distinguished in the field by the ungulate, brown basidiomata with white margins, which arise from a shaggy fasciculate subiculum. The subiculum enshrouds bases of adjacent living or dead tree saplings, bases



FIGS. 1–3. *Lactarius brunellus* habit (*Miller 10028*). 1. Basidiomata arising from shaggy subiculum enshrouding base of *Dicymbe* sapling, × 0.6. 2. Three developmental stages of basidiomata on subiculum, ×2.1. 3. Pleurotoid habit with eccentric stipe. Note the lamellae exuding latex from fresh cut, ×2.0.



FIGS. 4–10. Microscopic features of *Lactarius brunellus* (*Miller 10028*). Scale bars = 10 μm. 4. Pileipellis epithelium composed of thick-walled hairs and brownish polymorphic cells. 5. Thick-walled hairs (left) and brownish thin-walled polymorphic cells (right) from the pileipellis. 6. Pseudocystidia. 7–8. Elements of the stipitipellis. 7. Thick-walled hairs. 8. Irregularly shaped pseudocystidia. 9. 4-sterigmate basidia. 10. Basidiospores with amyloid non-reticulate ornamentation and hyaline suprahilar plage.

of large tree boles, stumps, horizontal roots and rocks. Several individual subicula observed were extensive, with aboveground contiguous portions measuring over 15 m in diameter. Alternating layers of bryophyte and subiculum growth observed suggested competition for space by the subicula with leafy liverworts and other bryophytes. Such layering may indicate phenological differences for the two organisms or may reflect an ongoing struggle for position. *Lactarius multiceps* (described below) has a similar subiculum, which also may occupy large areas; it however has only been observed to enshroud smaller diameter saplings and produces yellow basidiomes. *Lactarius panuoides*, also present in this area, has off-white to dull tannish-orange basidiomes that often form large imbricate troops on lower trunks of saplings and larger trees. The subiculum of *L. panuoides* is much more densely interwoven and more sponge-like in texture.

Lactarius multiceps S. L. Miller, M. C. Aime et T. W. Henkel sp. nov. FIGS. 11–14, 15–20

Etymology. Latin *multiceps*, referring to the numerous basidiomata arising from a single subiculum.

Pileus 5–25 mm in diametro, ungulatus ad subreniformis, applanatis ad irregularis et sursum flexus, aureus ad fuscato-aurantiacus, juventute velutinus ad hispidulus, senectute praeter marginem glaber. Lamellae subdistantes ad distantes, subdecurrentes, tenues, lamellulis singulis ad tribus alternantes, ex aurantiaco albae. Stipes 1–5 × 2–3 mm, cylindricus, excentricus ad lateralis, arcuatus, pallide flavus sub tomento albo. Sapor acerrimus. Contextus tenuis, fragilis, pallide flavus. Latex parcus, aqueus. Sporae albae in cumulo, 7.2–9.2 × 6.4–8.0 μm (Q=1.12–1.15), subglobosae ad breviter ellipsoideae, ornamentatione verrucarum magnarum connexarum a reticulo completo e rugis et tenuibus connectivis composito. Pseudocystidia abundantia, 4.8–6.5 μm lata. Pileipellis epithelium crassitie 2–5 cellularum, cellulis obovatis ad obpyriformibus trichomata sparsa ad abundantia parietibus crassis producentibus. Fructificans e subiculo tenui hirto involvente truncos arborum parvarum et radices in silvis tropicalibus humidis *Dicymbes corymbosae* Spruce ex Benth. (Caesalpiniacearum) in montibus Pakaraima, Guyana, Junio ad Julio 2000. HOLOTYPUS: Henkel 7656 (BRG, DUKE).

Pileus 5–25 mm broad, ungulate, conchate, dimidiate or subreniform, convex to applanate to upturned with age, golden yellow, orange or brownish orange (5B7–8 to 6C–8) fading to yellowish white to light yellow (4A2–4) at margin, pale yellow to light yellow (4A3–4) with age, velutinous to hispidulous overall when young, hispidulous at margin in age; margin entire, inrolled when young, irregularly and obscurely sulcate due to underlying lamellae when older; context thin, fragile, pale yellow. *Stipe* eccentric, 1–5 mm long × 2–3 × mm wide, tomentose to fasciculate, pale yellow beneath white tomentum. *Odor* not distinctive. *Taste* strongly acrid. *Lamellae* orange white, subdistant to distant, thin, subdecurrent to decurrent, tomentose near base, multitiered with irregularly interspersed lamellulae. *Latex* scant, watery.

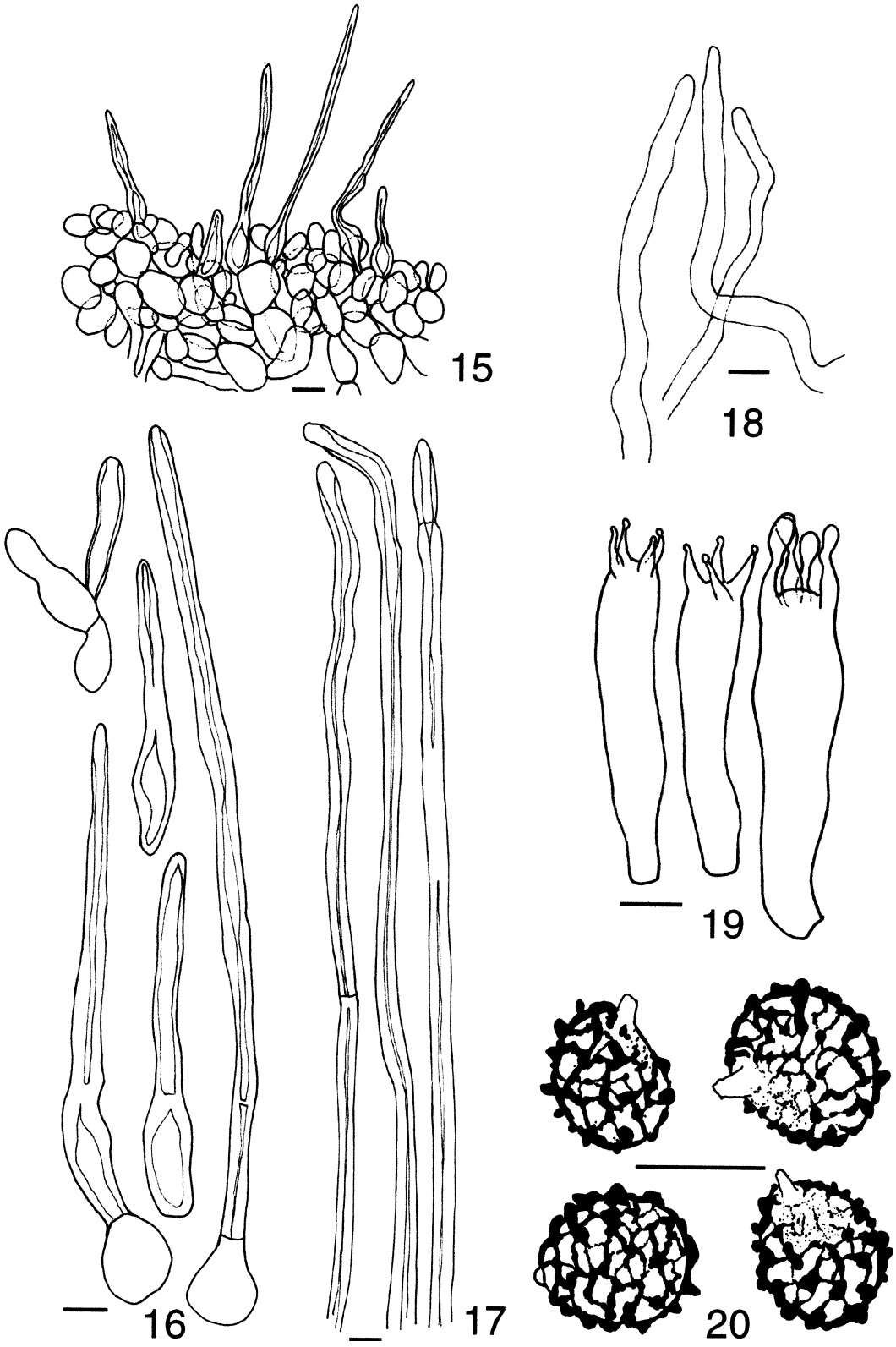
Basidiospores white in fresh deposit, 7.2–9.2 × 6.4–8.0 μm (Q = 1.12–1.15), subglobose to short ellipsoid, hyaline with amyloid ornamentation; ornamentation 0.5–1.0(1.5) μm high, large verrucae connected by complete reticulum of ridges and fine connectives; hilar appendix prominent 2–3 × 1.5–2 μm, hyaline; suprahilar plage large, with minute verrucae forming a faintly amyloid subreticulate pattern. *Basidia* clavate 44–48 × 8–13 μm, 4-spored; sterigmata 6.5–10 × 1–2 μm. *Pseudocystidia* abundant, 4.8–6.5 μm wide, cylindrical or irregularly contorted, rounded or blunt-pointed at the apex, occasionally emergent, connected deeply to laticiferous hyphae in the trama; walls usually thin; contents similar to laticifer contents, refractive, negative in sulfovanillin. *Pleurocystidia* absent. *Hymenophoral trama* composed of interwoven, hyaline, thin-walled hyphae, 2–4 μm diam; lactiferous hyphae 4–6 μm wide, contents refractive and guttate; sphaerocytes rare, when present in nests at interface of pileus trama and lamellae, each sphaerocyte 8–20 μm diam. Subhymenial layer not well developed. *Pileipellis* a 2–5 cell thick epithelium composed of globose, mostly thin-walled cells that are 10–25 μm diam and hyaline in KOH that gives rise to scattered to abundant thick-walled hairs, 40–220 × 4–7 μm, cylindrical to slightly contorted, frequently with swollen bases, walls 1–3.5 μm thick, hyaline in KOH, negative in sulfovanillin. *Stipitipellis* a dense covering of erect hairs, these 500–1000 × 4–5 μm with irregularly thickened 2–3.5 μm thick walls, straight or irregularly contorted, irregularly deeply branched and irregularly septate. *Subiculum* shaggy, off-white to greyish cream, composed of fascicles of hyaline, straight hyphae and hairs, 4–8 μm diam, walls 2–3.5 μm thick. *Clamp connections* not observed.

Macrochemical observations. $FeSO_4$—flesh dull green.

Habit, habitat, and distribution. Gregarious on lower trunks of saplings and fine pendant roots, arising from a shaggy, persistent subiculum; found June through July during wet weather in forests dominated by *D. corymbosa* Spruce ex Benth.

Specimens examined. GUYANA. Pakaraima Mountains, Upper Ireng River, 15 Jun 1999, *Henkel 7213* (BRG, DUKE); Upper Potaro River—4 km upstream from Ayanganna airstrip near base camp, 14 Jun 2000, *Miller 10136* (Miller personal herbarium); Blackwater Creek, *Dicymbe corymbosa* research site "Paluway #3",19 Jun 2000, *Miller 10146* (Miller personal herbarium), 20 Jul 2000, *Henkel*

FIGS. 11–14. *Lactarius multiceps*. 11. Basidiomata arising from shaggy subiculum enshrouding *Dicymbe* saplings (*Henkel 7656*). Note extensive development of the subiculum on leaf bases in the image at left, ×0.5. 12. Older basidiomata on subiculum (*Henkel 7656*), ×0.8. 13–14. Older basidiomata on subiculum. Note primordia and immature basidiomata at left, fully mature basidiomata on right. 13. Henkel 7213, ×1.0. 14. Henkel 8343, ×2.0.



FIGS. 15–20. Microscopic features of *Lactarius multiceps* (*Miller 10146*). Scale bars = 10 μm. 15. Pileipellis epithelium composed of thick-walled hairs and polymorphic cells. 16. Thick-walled hairs in pileipellis. 17. Thick-walled hairs in the stipitipellis. 18. Irregularly shaped pseudocystidia. 19. 4-sterigmate basidia. 20. Basidiospores with amyloid reticulate ornamentation and lightly amyloid and ornamented suprahilar plage.

7656 (HOLOTYPE BRG; ISOTYPE DUKE), 21 Jun 2001, *Henkel 8343* (BRG, DUKE).

Commentary. Lactarius multiceps is distinguished in the field by the numerous golden yellow to brownish orange basidiomes developing from a shaggy fasciculate subiculum. The subiculum is similar in appearance to that of *L. brunellus* although not as extensive in the collected representatives of *L. multiceps*, and was restricted to covering the bases of small diameter saplings and fine pendant roots. The stipe of *L. multiceps* is often well developed, and the mature basidiome may be eccentric rather than truly pleurotoid.

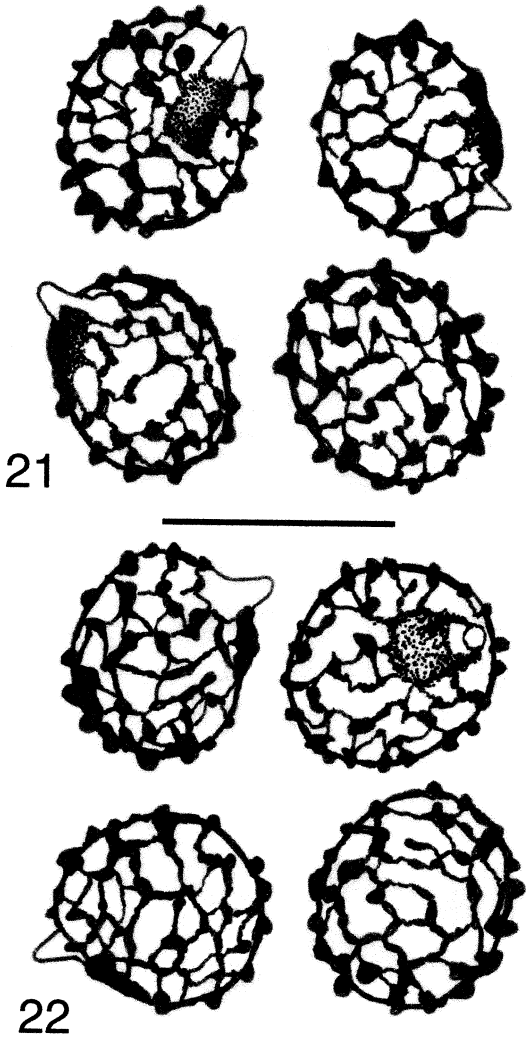
Lactarius multiceps most closely resembles *L. epitheliosus* Buyck and Courtecuisse (in Courtecuisse and Buyck 1991) which was collected in humid forests of French Guyana. Both species share diminutive size, yellow to yellow brown coloration, total lack of hymenial cystidia, and spore size and ornamentation. Unlike *L. multiceps*, *L. epitheliosus* exhibits a pronounced pleurotoid to eccentric habit, and the pileipellis consists of an epithelium from which abundant thick-walled hairs arise. No mention is made of a subiculum in the original description of *L. epitheliosus* and the taste, which in *L. multiceps* is strongly acrid, was unfortunately not tested in *L. epitheliosus*.

Lactarius panuoides Singer Kew Bull. 7:300. 1952. FIGS. 21–22

≡*Lactarius igapoensis* Singer Nova Hedwigia 40:441. 1984.

In an earlier paper redescribing several pleurotoid species from Guyana (Henkel et al 2000), we recognized the possibility raised by Verbeken (1998) that *L. panuoides* and *L. igapoensis*, similar in many respects, might be synonymous. After additional collecting in the field and careful examination of type material of *L. igapoensis* (Singer B 10299, INPA), we have concluded that *L. panuoides* and *L. igapoensis* are in fact, synonymous.

The original descriptions of *L. igapoensis* (Singer 1984) and *L. panuoides* (Singer 1952) differ in three characters: habitat, color of the basidiomata, and height of spore ornamentation. The specific epithet "*igapoensis*" refers to the occurrence of this species in frequently flooded lowland habitats or "igapo." During a recent expedition to Guyana (May–Jul of 2000), large fruitings of *L. panuoides* near the banks of the Potaro River were observed to be frequently submerged following extensive rainstorms upriver. While submersion appeared to have little effect on fruiting of this fungus, basidiomata present before the flood and observed after the waters had receded were uniformly darker in color. The color of *L. panuoides* basidiomata is highly variable; young fresh basidiomata are pale while older ones stain variably or uniformly reddish brown upon injury, in age, or after flooding. Large fruitings of *L. panuoides* upon individual subicula can contain uniformly young, uniformly old, or more typically, a mixture of young and senescent basidiomata. It is likely that collections that Singer described as *L. igapoensis* were composed of young basidiomata that had recently been inundated by floodwater.



FIGS. 21–22. Microscopic features of *Lactarius panuoides* (≡*L. igapoensis*) basidiospores. 21. Basidiospores of *Lactarius igapoensis* Singer (HOLOTYPE, *Singer B12099*). 22. Basidiospores of *Lactarius panuoides* (*Miller 10099*).

The type material of *L. igapoensis* is in a poor state of preservation and direct comparison of all important microscopic morphological characters is not possible. Verbeken (1998) correctly noted that the type specimens of *L. igapoensis* were immature. Most spores we found were amorphous or broken and stained only slightly in Melzer's reagent, suggestive of premature or interrupted development. After numerous attempts, we found a few mature basidiospores in one basidiome from the type collection.

From these we find that mature basidiospores of both *L. panuoides* and *L. igapoensis* are subglobose, 7.5–9.5 × 7–8 μm, hyaline with amyloid ornamentation composed of numerous crowded verrucae joined by an almost complete reticulum (FIGS. 21–22). The ornamentation height (0.5–1 μm high for both species) is at the higher end of the range in the few *L. igapoensis* spores observed and the reticulum mesh is slightly more coarse than in *L. panuoides*. However, both of these characters are within the range exhibited by mature *L. panuoides* spores examined from spore prints taken from several collections. In addition, the basidiospores of both species are characterized by a suprahilar plage with thickened, darkly amyloid, central spot.

Other phenotypic similarities exist between *L. panuoides* and *L. igapoensis*. Both produce their basidiomata on an extensive subiculum described by Singer (1984) as ''tough-fleshy and thick-membranous-crustaceous, dry, white or pallid but at least in parts blue-green from algal cells; subglabrous.'' Basidiomata of both species have an epithelium giving rise to abundant thick-walled hairs, both have white latex, and both have abundant and similarly sized pseudocystidia. For these reasons, we conclude that *L. igapoensis* is synonymous with *L. panuoides*.

ACKNOWLEDGMENTS

This work was made possible by grants from the National Geographic Society's Committee for Research and Exploration to Henkel, NSF DEB-9974018 and USDA competitive grant 2000–02861 to Miller, and an Explorers Club of Virginia grant to Aime. Field assistance was provided in Guyana by Mimi Chin, Leonard Williams, Christopher Andrew, and Benny Cheong. This paper is number 62 in the Smithsonian Institution's Biological Diversity of the Guianas Program publication series.

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