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Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status

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Abstract: Three species of pleurotoid, putatively lignicolous basidiomycetes previously described in the genus *Lactarius* sect *Panuoidei* were redescribed from fresh material collected in Guyana and Japan. In Guyana, *Lactarius panuoides* and *Lactarius campinensis* were restricted to forests dominated by ectomycorrhizal *Dicymbe* species (Caesalpiniaceae) and basidiomata of both pleurotoid species were subtended by ectomycorrhizal rootlets. This suggested an ectomycorrhizal nutritional mode for these fungi. Analysis of DNA sequences in the nuclear large subunit region taken from basidiomata and co-occurring ectomycorrhizae of *L. panuoides* and *L. campinensis* confirmed that these fungi are ectomycorrhizal and that their fruiting habit on organic deposits and well rotted wood at elevated positions is not due to saprotrophy, as previously assumed. *Lactarius uyedae* was collected in Japan from mature temperate forests dominated by Fagaceae, yet ectomycorrhizal rootlets were not found in association with the basidiomata. Morphological features and analysis of DNA sequences suggested that *L. panuoides* and *L. uyedae* have affinities with established sections of the genus *Lactarius,* regardless of the pleurotoid morphology of their basidiomata, and that *L. campinensis* should be transferred to the genus *Russula.* Discussion is provided for the genus *Pleurogala,* which was erected to accomodate putatively lignicolous, pleurotoid species of *Lactarius.*

Key Words: basidiomycetes, ectomycorrhizae, molecular phylogenetics, neotropics, saprotrophy

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INTRODUCTION

Lactarius section *Panuoidei* was described by Singer (1952) to accommodate *Lactarius panuoides* Singer from Trinidad. The outstanding characters of this section are the pleurotoid morphology of the small basidiomata, combined with poorly developed sphaerocytes, and a tendency to produce a subiculum of thick-walled hyphae (Singer 1984). Following description of *L. panuoides,* two other species were described in section *Panuoidei* from the Brazilian Amazon, *L. campinensis* Singer and *L. igapoensis* Singer, as well as a fourth extratropical species, *L. uyedae* Singer from Japan (Singer 1984). Singer (1952, 1984) and subsequent authors (Dennis 1970, Pegler and Fiard 1979, Redhead and Norvell 1993, Verbeken 1998) presumed that these pleurotoid *Lactarius* species were not ectomycorrhizal but instead were lignicolous, functioning either as deadwood decomposers or as parasites of living trees. This presumption was based on Singer's original observation that *L. panuoides* fruited gregariously from elevated positions on tree trunks and other woody substrates. Because of the pleurotoid morphology and highly unusual nature of the presumed saprotrophy for the genus *Lactarius,* Redhead and Norvell (1993) proposed a new genus, *Pleurogala,* to accommodate the species of *Lactarius* section *Panuoidei.* Recently, Buyck and Horak (1999) extended the knowledge of pleurotoid Russulaceae by describing two new species of pleurotoid *Russula.*

Recent mycological explorations in Guyana have discovered extensive areas of forest dominated by ectomycorrhizal species of *Dicymbe* Spruce ex Benth. (Caesalpiniaceae, tribe Amherstieae) (Henkel 1999). A diverse assemblage of Russulaceae, including pleurotoid species, has been collected in association with *Dicymbe* from this previously unexplored area, as well as numerous species from other ectomycorrhizal basidiomycete families including the Boletaceae, Amanitaceae, Cortinariaceae, and Cantharellaceae (Henkel 1999, unpubl).

This paper reports on three pleurotoid Russulaceae originally described in the genus *Lactarius*: two neotropical species from the Pakaraima Mountains of Guyana, *L. panuoides* and *L. campinensis,* and one temperate species, *L. uyedae,* from mountainous regions of central Japan. The purposes of this paper

are to: (i) provide new distribution records and descriptions for these species; (ii) assess their ectomycorrhizal status; (iii) explore the taxonomy and molecular systematics of pleurotoid Russulaceae, and (iv) propose a new combination for *L. campinensis* Singer.

MATERIALS AND METHODS

Collecting expeditions were made in May–Jun 1998 and May–Jun 1999 to the Upper Ireng River Basin along Guyana's western border with Brazil, in the south-central Pakaraima Mountains (general area: 5° 05′ N; 59° 58′ W). Fungi were collected from an area of approximately 5 km^2 surrounding a previously established base camp (Henkel 1999). In Japan, *L. uyedae* was collected during periods of heavy rain in Jul–Aug 1998 in temperate midlatitude areas of the island of Honshu from old-growth forests and other undisturbed areas surrounding local shrines. 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 were examined for fresh color characteristics. Macrochemical spot tests, when performed, were according to the methods of Singer (1986). Basidiomata were dried slowly over charcoal and subsequently placed in containers with silica gel to prevent spoilage in the excessively humid conditions; in addition, some basidiomata were immersed in FAA for preservation. Japanese collections were dried on a convection drier.

Microscopic anatomical details were determined from dried and pickled basidiomata and preserved ectomycorrhizae with a Leitz Ortholux microscope with bright-field and Nomarski interference optics. For basidiospores, basidia, and cystidia, at least 20 individuals were observed and measured per taxon. Scanning electron micrographs of spores and cystidia were obtained with a Phillips 501 microscope.

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.

For molecular analysis, ectomycorrhizal rootlets near both *L. panuoides* and *L. campinensis* were collected from underlying substrate along with adjacent basidiomata of each respective species (1–5 mm proximity). Additional rootlets were stored in FAA for morphological analysis. Ectomycorrhizae and fresh basidiome tissue were placed in 2× CTAB buffer for subsequent DNA extraction (Zolan and Pukkila 1986). Genomic DNA was extracted following a chloroform : isoamyl alcohol protocol (Zolan and Pukkila 1986). Portions of the nuclear gene coding for the large ribosomal subunit RNA (nLSU rDNA) were amplified with either the primer pairs 5.8SR/LR7 or LR0R/LR7 and sequenced with the primers LR0R, LR3R, LR5 and LR16 (Vilgalys and Hester 1990, Hopple and Vilgalys 1999). Amplified PCR products were purified using Qia-quick DNA columns (Bio 101, La Jolla, California) or Ultra-free-MC filters (Millipore) and sequenced with dye terminator cycle sequencing chemistries using 370A or 373A automated DNA sequencers (Applied Biosystems, Foster City, California). Sequence chromatograms for forward and reverse sequences were compared, edited, compiled and aligned against the sequences of other taxa in the Russulaceae (TABLE I) (alignments are available upon request from the authors). Phylogenetic analyses were performed using PAUP* v.4.0b (Swofford 1998). Regions with ambiguous alignment were removed from the analyses, all transformations were weighted equally, and gaps in alignment were either treated as missing data or excluded. Heuristic searches of an abbreviated Russulaceae dataset including several *Russula* and *Lactarius* species were conducted according to the method outlined by Olmstead and Palmer (1994). The relative robustness of individual branches was estimated by bootstrap resampling (Felsenstein 1985).

TAXONOMY

Lactarius panuoides Singer; Kew Bull. 7: 300. 1952. FIGS. 1–5

≡ *Pleurogala panuoides* (Singer) Redhead and Norvell; Mycotaxon 48:377. 1993.

Pileus 5–8 × 2–15 mm, subreniform to spathulate, convex to applanate, at base dull tannish-orange (5A4 KW) lightening to off-white distally, surface hispid to finely pubescent, scrobiculate-venose at maturity, base with dense white substrigose tomentum, astipitate or with minute lateral stipe 1–3 × 1–2 mm; context thin, white. *Odor* none; *taste* mild. *Lamellae* off-white to light cream, subdistant, thin, decurrent, tomentose near base, alternating with 1–3 lamellulae; latex copious, white, aqueous, drying orangish-brown in several minutes. *Basidiospores* white in fresh deposit, 7.5–9.5 × 7–8 μm, subglobose, hyaline with amyloid ornamentation 0.5–1 μm high, of numerous crowded verrucae joined by thin connectives forming an almost complete reticulum with small mesh; suprahilar plage with thickened amyloid central spot; hilar appendix 2–3 × 1.5–2 μm. *Basidia* clavate, 4-sterigmate, 45–60 × 10–12 μm, 4-spored; sterigmata 7–9 × 1–2 μm. *Pseudocystidia* infrequent to abundant, 3–8 μm wide, cylindrical or irregular contorted, rounded or blunt-pointed at the apex, frequently branching at or below the level of the hymenium, rarely emergent; walls usually thick, 1–1.5 μm wide, gelatinous in appearance; contents refractive, granulose to guttate, negative in sulfovanillan, connected deeply to the laticiferous hyphae in the trama and with similar contents. *Pleurocystidia* absent. *Hymenophoral trama* composed of interwoven, hyaline, thin-walled hyphae, 2–4 μm wide; lactiferous hyphae 8–10 μm wide, lightly to heavily encrusted, with refractive guttate contents; sphaerocytes rarely encoun-

TABLE I. Specimens used in the molecular phylogenetic analysis of the pleurotoid taxa[a]

Taxon	Collection	Origin	GenBank
Bondarzewia berkeleyi (Fr.) Singer	SLM 9507	USA, North Carolina	AF218563
L. fumosus var. *fumosus*	SLM 9546	USA, Virginia	AF218551
L. lignyotellus	SLM 9592	USA, West Virginia	AF218557
L. piperatus	SLM 9559	USA, Virginia	AF218556
L. deceptivus Peck	SLM 9535	Canada, Montreal	AF218550
L. panuoides Singer	DUKE-TH 6843	Guyana	AF218561
L. panuoides ectomycorrhizae	DUKE-TH 6843	Guyana	AF218566
L. peckii var. *peckii* Burlingham	SLM 9517	USA, Virginia	AF218554
L. scrobiculatus var. *pubescens* A. H. Smith	SLM 9544	USA, Virginia	AF218558
L. speciosus Burlingham	SLM 9509	USA, North Carolina	AF218555
L. subdulcis (Fries) S.F. Gray	SLM 9673	USA, North Carolina	AF218552
L. subpurpureus Peck	SLM 9572	USA, Virginia	AF218553
L. uyedae Singer	VPI-MCA 584	Japan	AF218562
R. adusta Fries	SLM 9530	Canada, Montreal	AF218554
R. brevipes var. *acrior* Shaffer	SLM 9555	USA, Vermont	AF218545
R. brunneola Burlingham	SLM 9524	Canada, Montreal	AF218548
R. campinensis (Sing.) Henkel, Aime and Miller	DUKE-TH 6844	Guyana	AF218564
R. campinensis ectomycorrhizae	DUKE-TH 6844	Guyana	AF218565
R. discopus Heim	PC-B 97218	Africa	AF218543
R. foetentula Peck	SLM 9501	USA, Virginia	AF218546
R. mairei Peck	DUKE-RV89/62	USA, North Carolina	RMU11926
R. radicans Heim	PC-B97109	Africa	AF218547
R. romagnesii Singer	DUKE-JJ60	USA, North Carolina	AF042572
R. silvicola Shaffer	SLM 9571	USA, Virginia	AF218549
R. violacea Quélet	PC-SM/BB 34	Europe	AF218559
R. virescens (Schaeffer) Fries	PC-SM/BB 8	Europe	AF041548
R. xerampelina (Schaeffer) Fries	VPI-JW 378	USA, Colorado	AF218542

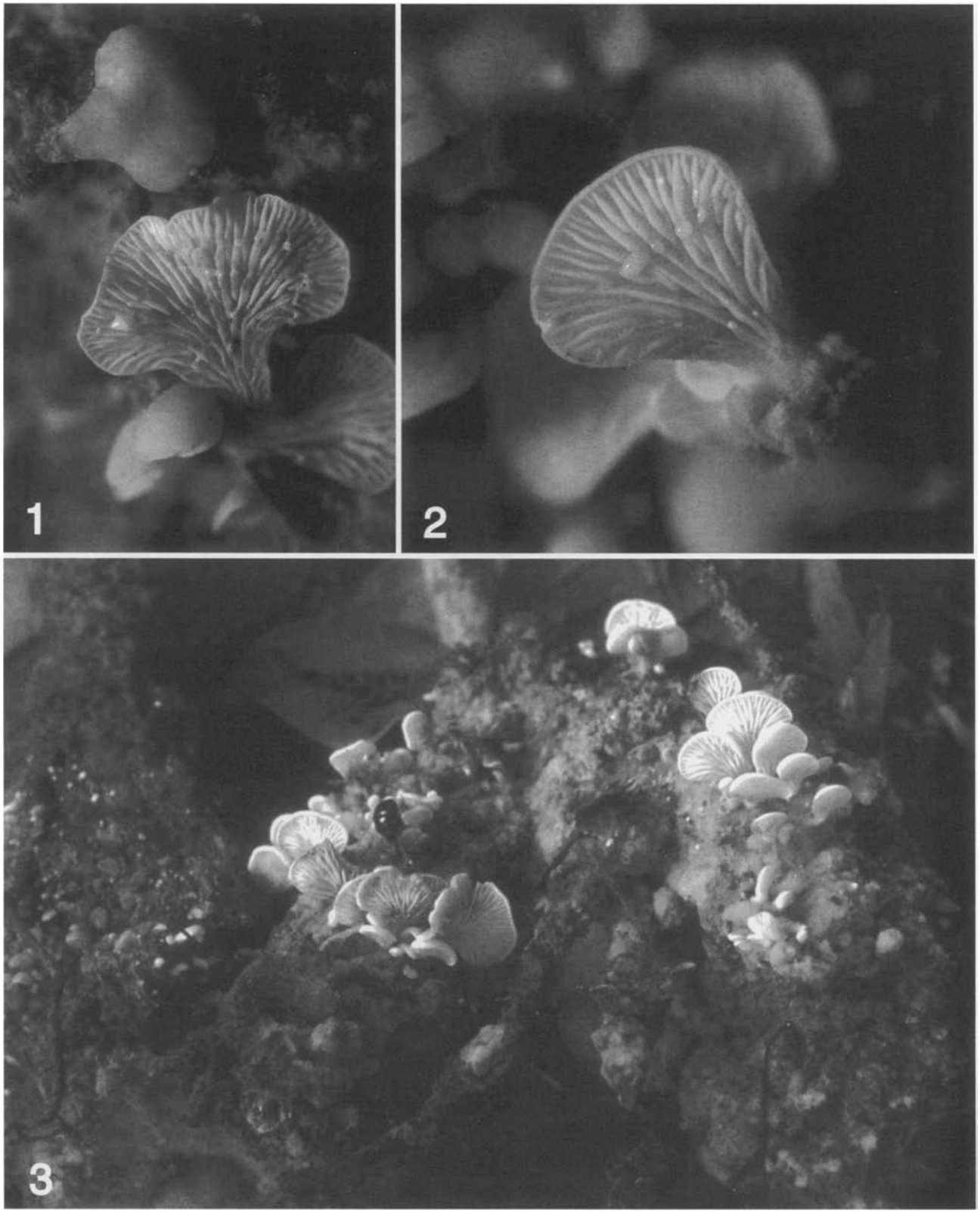
[a] *L.* = *Lactarius*, *R.* = *Russula*. Herbarium source is listed with each collection number: DUKE = Duke; PC = Paris Museum of Natural History; SLM = Steven L. Miller, personal collection; VPI = Virginia Tech.

tered, when present at junctions with lamellae, in nests of 3–6, each sphaerocyte 10–15 μm wide. Subhymenial layer not well developed. *Pileipellis* an irregular layer of chains or clusters of clavate to subglobose, thin-walled cells 15–40 μm wide, giving rise to scattered to abundant thick-walled, hairs 40–80 × 5–8 μm, cylindrical to contorted and irregular, walls 1–2 μm thick, hyaline to pale brown in KOH, negative in sulfovanillin. *Stipitipellis* a variably thick layer or discontinuous mounds of hyaline, subglobose, ovoid or clavate, mostly thick-walled elements, 15–35 μm wide, often giving rise to erect hairs, these 300 × 5–8 μm, thick-walled, straight or irregularly contorted, often septate. *Subiculum* composed entirely of hyaline, linear hyphae 6–7 μm wide, thick walls 2–2.5 μm wide, septate, occasionally curving apically, infrequently branching, often interspersed with filamentous green algae and cyanobacteria. *Clamp connections* not observed. *Macrochemical observations*: pileus surface: KOH browning slowly; NH_4OH slowly tan; $FeSO_4$ bluish gray slowly.

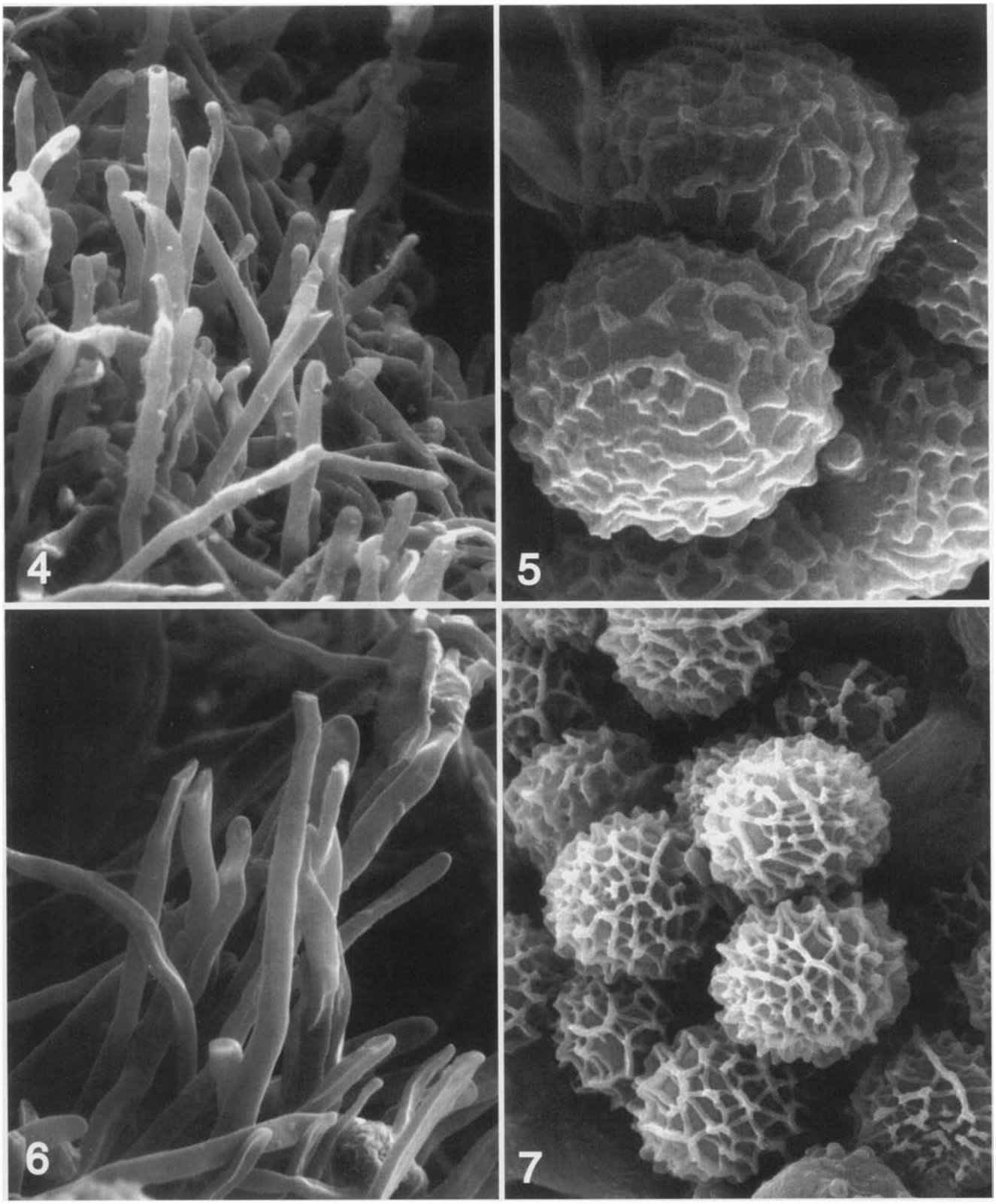
Ectomycorrhizae formed with *Dicymbe altsonii* Sandw. varying from irregularly to monopodially pinnate, mantle and extramatrical hyphae forming a white, densely tomentose to substrigose mass; in microscopic cross-section with abundant extramatrical hyphae; these hyphae linear, thick walled, septate, loosely interwoven but largely anticlinal to the root; occasionally similar hyphae forming capitate cystidia, capitulae globose; mantle of tightly interwoven hyphae; Hartig net well developed.

Habit, habitat, and distribution. Caespitose to imbricate in troops on lower trunks of saplings and larger trees, arising from a tough, persistent subiculum which enshrouds saplings and is often interspersed with bryophytes and algae, subtended by ectomycorrhizal rootlets; collected in May and Jun during very wet weather from forests dominated by *D. corymbosa* Spruce ex Benth. and *D. altsonii*; also known from mesic forest in Trinidad and Guadeloupe.

Specimens examined. GUYANA. Pakaraima Mountains, Upper Ireng River, 1 km downstream from confluence with Sukabi River, 700 m elev, in riverine forest on sapling of *D. altsonii*, 15 May 1998, *Henkel 6401* (BRG, DUKE); Upper Ireng River, west slope of Mt. Kukuinang, 3 km east of mouth of Sukabi River, 900 m elev, under *D. corymbosa*, 31



FIGS. 1–3. *Lactarius panuoides* habit (*Henkel 6401*). 1. Lamellae with exuding latex and brown discoloration following injury, pleurotoid habit with eccentric stipe, ×7. 2. Abundant latex from freshly cut lamellae, ×7. 3. Basidiome development from mat-like subiculum, ×2.



FIGS. 4–7. Scanning electron micrographs of pleurotoid *Lactarius* species. 4, 5. *Lactarius panuoides* (*Henkel 6401*). 4. Thick-walled pileus hairs, ×1200. 5. Basidiospores, ×11 000. 6, 7. *Lactarius uyedae* (*Aime 584*). 6. Thick-walled pileus hairs, ×2500. 7. Basidiospores, ×5000.

May 1998, *Henkel 6815* (BRG, DUKE); Upper Ireng River, lower Suruwabaru Creek, 700 m elev, in *Dicymbe*-dominated forest, 23 May 1999, *Henkel 7043* (BRG, DUKE). BRAZIL. AMAZONAS: *Lactarius igapoensis* Singer, Igarape do Tarumazihno, 16 Apr 1980, *Singer B12099* (HOLOTYPE, INPA).

Commentary. Lactarius panuoides is distinguished in the field by the off-white, subreniform to spathulate, imbricate, latex-bearing, mild-tasting basidiomata arising from the thick, persistent subiculum. The Guyana collections fit published descriptions of the holotype by Singer (1952), Pegler and Fiard (1979), and Verbeken (1998) exceedingly well. The infrequently encountered tramal sphaerocytes seen in our collections have not been reported previously. *Lactarius panuoides* differs from the phenotypically similar *L. igapoensis* in having paler basidiomata and higher spore ornamentation: 0.5–1 μm in *L. panuoides* and 0.4–0.5 μm in *L. igapoensis*. In the original collections Singer made no mention of co-occurring ectomycorrhizae with *L. panuoides* and, due to the fruiting of the fungus on woody substrates, a lignicolous habit was presumed. In the Guyana collections the presence of identical thick-walled, septate, hair-like elements in the subiculum, pileipellis, and subtending ectomycorrhizae was strongly suggestive of an ectomycorrhizal status for *L. panuoides*. In addition, *L. panuoides* was restricted to forest stands dominated by ectotrophic *Dicymbe* trees. Numerous conical subicula were repeatedly found at ground level, completely enshrouding the bases of adjacent tree saplings. The persistent subicula ranged up to 50 cm in height and served as sources of dozens of basidiomata during several months of the rainy season. While filamentous green algae and cyanobacteria were invariably present in the subicula, the possibility of lichenization remains to be explored.

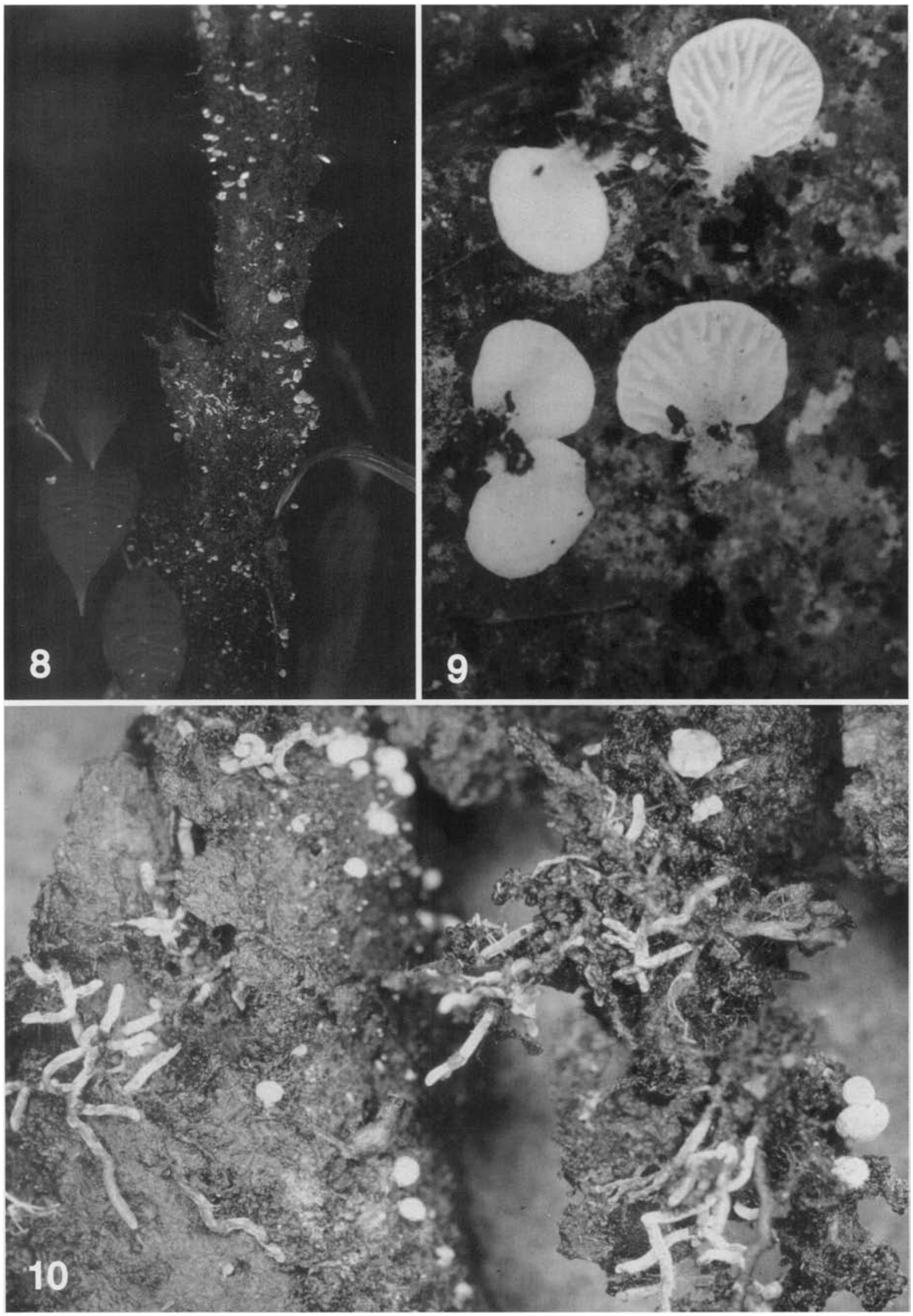
Russula campinensis Henkel, Aime et Miller comb. nov. FIGS. 8–18

≡ *Lactarius campinensis* Singer; Nova Hedwigia 40: 438. 1984.

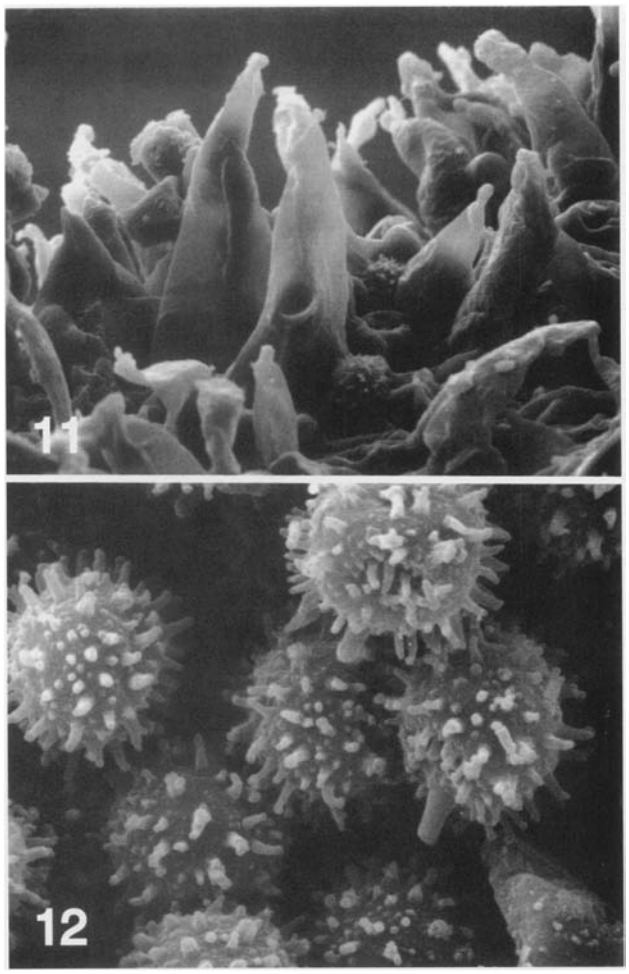
≡ *Pleurogala campinensis* (Singer) Redhead and Norvell; Mycotaxon 48:377. 1993.

Pileus 2–7(–17) mm broad, applanate, conchate to flabelliform to dimidiate, white to cream (4A3 KW), aging tan, margin inrolled when young, smooth to finely pulvurulent under lens, finely sulcate over distal ⅔ due to underlying lamellae, context very thin, white. *Odor* none; *taste* strongly acrid when fresh. *Lamellae* concolorous with pileus, finely pulvurulent under lens, polydymous with 5–6 through-lamellae and 2–3 lamellulae, thick, distant, adnate to slightly decurrent, browning slightly upon pressure; latex absent. *Stipe* minute, lateral, subequal, concolorous with pileus, 0.7 × 0.5 mm, subtended by substrigose hairs; *subiculum* none. *Basidiospores* subglobose, 8.4–10.2 × 8.4–9.5 μm, hyaline, with amyloid echinulate-spinose ornamentation; ornamentation 0.8–2(–3.5) μm high, usually acute, rarely bifurcated at tip, lacking connectives and faint amyloid verrucae often present between the larger elements; hilar appendix prominent, 3–4 × 1.5–2 μm, hyaline; suprahilar plage small, weakly and inconsistently amyloid. *Basidia* clavate, 45–47 × 10–14 μm, hyaline, 4-sterigmate; sterigmata 6–7 × 1.5–2 μm. *Pleuro-* and *cheilocystidia* numerous, projecting slightly above hymenium, thin-walled, tapering upwards to acute, mucronate to subcapitate tip, 65–90 × 8–12 μm, with needle-like crystalline contents visible in KOH and sulfovanillin, under bright light dark bluish-purple in sulfovanillin, inserted in the subhymenium or often deeper in the trama, then connecting with sparse, sulfovanillin-positive conducting hyphae of the hymenophoral trama, these appearing pseudocystidia-like with septations visible. *Marginal cells* undifferentiated. *Hymenophoral trama* of irregular, interwoven, hyaline, partially gelatinized hyphae, 4–6 μm wide; sphaerocytes in nests of 2–10 scattered throughout the trama but most conspicuous at junctions of lamellae, each sphaerocyte 10–20(–30) μm wide; conducting hyphae infrequent, 3–5 μm wide, refractive with needle-like contents, sulfovanillin-positive, not guttate. *Pileus trama* of strongly interwoven, hyaline hyphae, 4–8 μm wide, septate and often sinuous; conductive hyphae infrequent. *Pileipellis* a dense trichodermium of fusoid, rounded, occasionally subcapitate dermatocystidia, 15–35 × 5–7 μm; interspersed with scattered larger fusoid-acuminate to mucronate dermatocystidia, 30–130 × 8–11 μm, these with granular to needle-like contents throughout, brownish to purple in sulfovanillin, rarely pale gray or blackish; hypoderm of irregular to subisodiametric hyaline elements, 5–10 μm wide. *Stipitipellis* composed of long, thick-walled hairs, 40–100 × 3–4 μm, irregularly twisted and contorted, often swollen at the base, occasionally septate, walls refractive; interspersed with clavate to cylindrical fusoid or mucronate thin-walled hyphae, 45–60 × 6–7 μm, hyaline in KOH and sulfovanillin; along with scattered cylindrical to fusiform hyphal end cells, 35–50 × 5–12 μm, guttate or with irregular granular contents, purplish-brown in sulfovanillin. *Clamp connections* not observed.

Ectomycorrhizae formed with *D. altsonii* monopodially pinnate with well developed mantle, light yellow; extramatrical hyphae inevident; microscopically with thick mantle, hyphae tightly packed, occasionally with bifurcating tips; under scalp section mantle



FIGS. 8–10. *Russula campinensis* habit (*Henkel 6844*). 8. Pleurotoid basidiomata on standing dead palm trunk, ×0.4. 9. Pleurotoid habit and lamellae, ×5. 10. Basidiomata fruiting from extensive mat of ectomycorrhizal rootlets, ×1.5.



FIGS. 11, 12. Scanning electron micrographs of *Russula campinensis* (*Henkel 6844*). 11. Partially collapsed, capitate dermatocystidia, ×1625. 12. Basidiospores, ×6000.

consisting of a coarse net of irregularly shaped, thick hyphae; Hartig net well developed.

Habit, habitat, and distribution. Gregarious but not imbricate or caespitose on trunks of saplings and larger trees, from base to 2 m in height, arising from accumulations of organic matter consistently permeated by ectomycorrhizal rootlets, true subiculum wanting; collected in May and June during very wet weather in forests dominated by *D. corymbosa* and *D. altsonii*; known also from type locality in the central Brazilian Amazon.

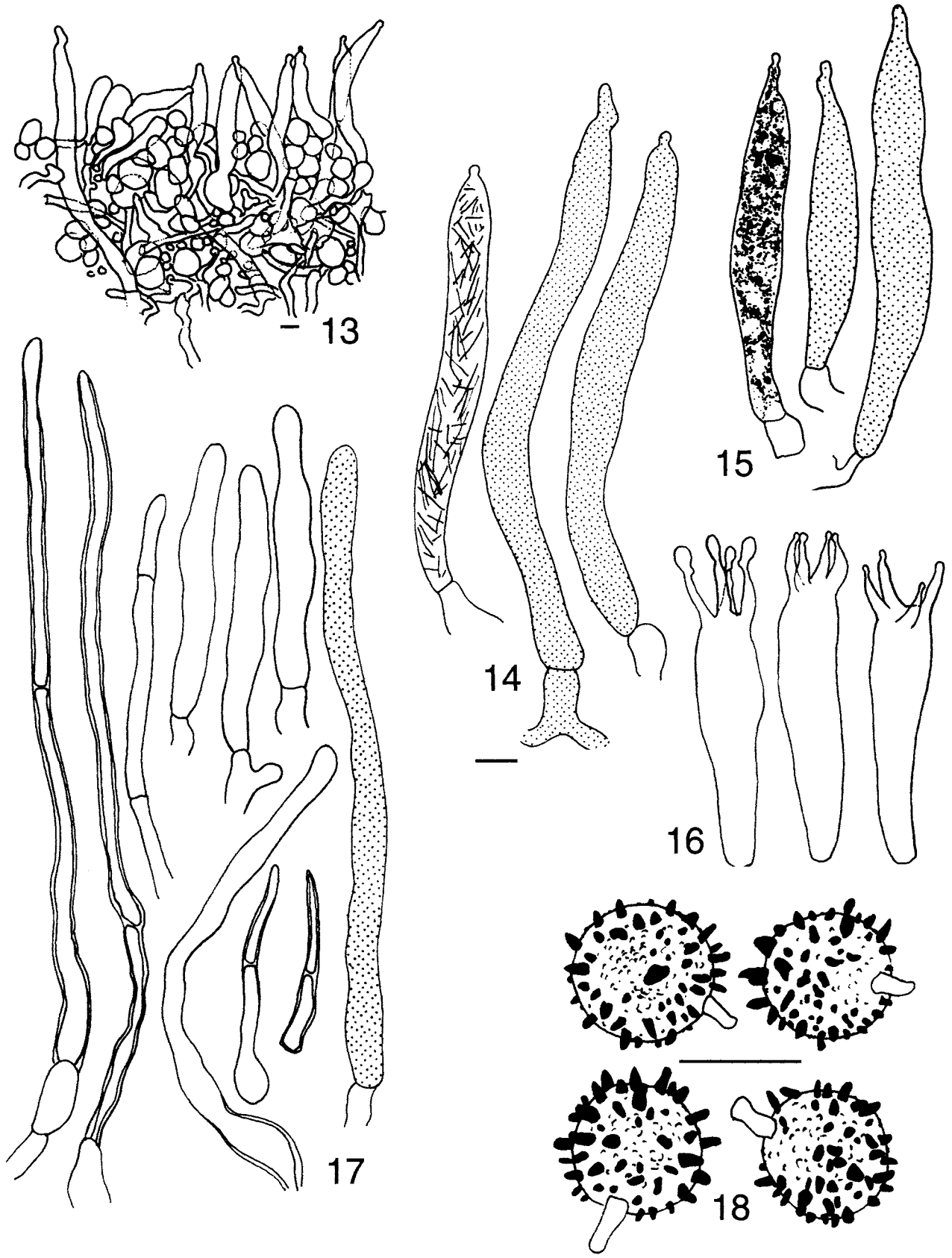
Specimens examined. GUYANA. Pakaraima Mountains, Upper Ireng River, 3 km west of confluence with Sukabi River, 800 m elev, on trunk of understory palm in upland forest dominated by *D. altsonii*, 1 Jun 1998, *Henkel 6844* (BRG, DUKE); Upper Ireng River, 2 km east of confluence with Sukabi River, 920 m elev, on trunk of large *D. corymbosa*, 6 Jun 1998, *Henkel 6893* (BRG, DUKE); Upper Ireng River, lower reaches of Suruwabaru Creek, on trunk of large *D. corymbosa*, 21 May 1999, *Henkel 7023* (BRG, DUKE).

Commentary. Russula campinensis is recognized in the field by the small, white, usually dimidiate basidiomata that are non-lactescent and acrid-tasting when fresh. In Guyana, *R. campinensis* fruited most commonly on the trunks of large, living *Dicymbe* trees up to two meters high, in bark depressions that had accumulated organic debris. These organic deposits were invariably permeated with ectomycorrhizal rootlets and served as persistent sources of basidiomata, with repeated fruiting observed over an entire rainy season from the same deposits.

Although the type specimen for *L. campinensis* was unavailable for examination (I. Araujo pers comm), the Guyana collections of *R. campinensis* were consistent with the habit, macro-, and micro-morphological features reported for *L. campinensis* by Singer (1984), including the distinguishing basidiospore and pileipellis features. Exceptions included: (i) the short, ventricose, multiseptate cheilocystidia noted by Singer in the type were inevident in the Guyana collections, and (ii) the lack of acrid taste in the type. The lack of acrid taste in Singer's material could be explained by the age of the basidiomata he collected. In the Guyana material, the sharp acrid taste was present only in the freshest basidiomata, and faded quickly with age.

Phylogenetic analysis indicated an affinity of *R. campinensis* for the genus *Russula* (FIG. 13). In addition, true sulfovanillin-positive dermatocystidia such as those present in *R. campinensis* are very common in *Russula,* and absent from *Lactarius* except for the African subgenus *Russulopsis* Verbeken, considered closely related to *Russula* (Heilman-Clausen et al 1998). Additionally, latex was not observed and was not reported by Singer (1984). This information provided the basis for the generic transfer.

The presence of hymenial cystidia arising as extensions of the sparse tramal conducting system (i.e., pseudocystidia sensu Singer 1986) in *R. campinensis* was difficult to interpret. These structures were consistent with Singer's original description of the taxon in *Lactarius*; yet pseudocystidia are normally considered to be absent from *Russula* (Buyck 1991). Such discrepancies may be expected, however. As noted by Buyck and Horak (1999), an African species of Russulaceae recently described as a *Lactarius* was variable in the presence or absence of conducting hyphae and pseudocystidia, making it difficult to assign the taxon to either *Russula* or *Lactarius* based on these criteria. *Russula campinensis* appears to be a neotropical taxon which is morphologically intermediate between the traditionally-defined genera *Russula* and *Lactarius,* a fact not inconsistent with the ongoing decay of generic boundaries in the Russulaceae as tropical taxa are more fully explored (Buyck 1995).



FIGS. 13–18. Microscopic features of *Russula campinensis* (*Henkel 7023*). 13. Extremities of the pileipellis. 14. Dermatocystidia. 15. Pleuromacrocystidia. 16. Basidia. 17. Elements of the stipitipellis. 18. Basidiospores. Bars = 10 μm.

Lactarius uyedae Singer; Nova Hedwigia 40: 436. 1984. FIGS. 6, 7

≡ *Pleurogala uyedae* (Singer) Redhead and Norvell; Mycotaxon 48:377. 1993.

Pileus 3–6 mm broad, flabelliform to spathulate, convex to applanate to upturned with age, pure white, some developing brownish-yellow stains with age, densely hispid when young, smoother with age; margin entire, inrolled when young, becoming wavy; context thin, fragile, white. *Odor* none; *taste* not determined. *Lamellae* thick, subdistant, adnate to slightly decurrent, white, densely hispid including interlamellar spaces, 10–13 per basidiome with 10–15 lamellulae; latex not observed. Stipe 1–3 mm × 0.5–1.5 mm, equal, eccentric, lateral or rarely absent, recurved, tomentose, with white strigose hairs basally. *Subiculum* none. *Basidiospores* subglobose to short ellipsoid, (7.5) 8.4–10.2 μm × (6) 7.5–8.4 μm, hyaline; ornamentation strongly amyloid, consisting of irregularly thickened ridges 0.5 μm high, connected by finer reticulum throughout; hilar appendix prominent, 2–2.5 μm × 1–1.5 μm; suprahilar plage inamyloid to faintly amyloid. *Basidia* clavate, 45–50 μm × 8–11 μm, hyaline, 4-sterigmate; sterigmata 7–9 × 1.5–2 μm. *Pleuromacrocystidia* and *cheilocystidia* abundant, cylindrical or irregular contorted, 50–120 μm × 7–9 μm, rounded or blunt-pointed at the apex, contents fine, granular or needle-like, grayish near the base in sulfovanillin. *Pseudocystidia* infrequent, occasionally barely emergent, slender, 2–4 μm wide, contorted, occasionally thick-walled near apex, pale brown in sulfovanillin, contents refractive, arising from tramal conducting hyphae. *Marginal cystidia* frequent. *Basidioles* palisadic. *Hymenophoral trama* irregular, interwoven, hyphae 1.5–5 μm wide; conducting hyphae 5–7 μm wide; sphaerocytes few to lacking. *Pileipellis* composed of thin-walled globose elements, 10–30 μm wide, giving rise to abundant, cylindrical to fusoid thick-walled hairs, 20–180 μm × 7–10 μm, acute, wall 1–3 μm thick, forming an erect pile, light golden in KOH; pileus trama essentially a deep extension of epithelium, conducting hyphae present, 6–8 μm wide, with fine granulose contents.

Habit, habitat, and distribution. Solitary to scattered among bryophyte mats on soil, rock surfaces, and on twigs in old-growth forests dominated by *Castanopsis* Spach. and *Fagus* L. Known only from the island of Honshu, Japan.

Specimens examined. JAPAN. SHIGA PREFECTURE: Otsu City, Sekinotsu Cho, Shinmochi Shrine, moss bed in 1–2 mm soil covering bare rock face with no major trees or tree roots in the vicinity, 9 Jul 1998, *M. C. Aime 527* (VPI). KYOTO PREFECTURE: Mount Kaikichi, Uji City, scattered but abundant on rock face along side of mountain path, in moss, bare soil and pieces of gravel with *Castanopsis* growing nearby, 19 Jul 1998, *M. C. Aime 584* (VPI). IBARAKI PREFECTURE: Ogawa Forest near Ogawa City, scattered in moss near creek bank in old growth forest predominated by *Fagus japonica,* 31 Jul 1998, *M. C. Aime 634* (VPI).

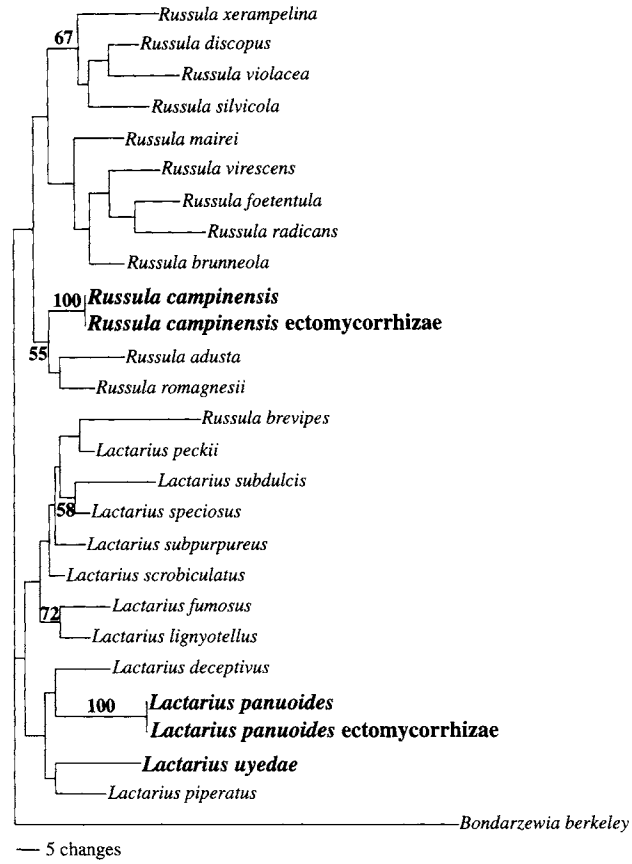


FIG. 19. Phylogeny of *Russula* and *Lactarius* including pleurotoid species based on nuclear large subunit rDNA sequences. One of 8 equally most parsimonius trees is depicted as a phylogram (tree length = 409, CI excluding uninformative characters = 0.4688). *Bondarzewia berkeleyi* was used as the outgroup taxon. Bootstrap values greater than 50% are noted above the respective branches.

Commentary. Morphologically *L. uyedae* is similar to *L. panuoides* and *L. igapoensis* in having a pileus surface composed of thin-walled globose elements intermixed with slender, thick-walled hairs. Verbeken (1998) reported the pileus hairs in *L. uyedae* to be shorter and less acute than those in *L. panuoides* and *L. igapoensis.* In the collections described here this was not the case; however, the morphology of the hairs was somewhat variable depending on the stage of basidiome development. Verbeken (1998) provided detailed drawings of elements in the pileus and hymenium. Singer (1984) reported a white, unchanging, and acrid latex for *L. uyedae,* though no latex could be demonstrated in the present collections.

Phylogenetic analysis.—FIGURE 19 shows one of eight most parsimonious trees that were obtained using 28

ingroup taxa and one outgroup taxon (length = 409, CI = 0.469, excluding uninformative characters). The tree was presented as a phylogram to show the number of character state changes per branch. The other trees differed only in minor rearrangements among terminal clades.

There was a relatively low overall level of divergence among the nLSU sequences. Bootstrap support values for branches were generally low, although several of the short branches were highly stable (FIG. 19). The analysis indicated that the nLSU sequences obtained from the basidiomata and co-occurring ectomycorrhizae in both *L. panuoides* and *R. campinensis*, were, respectively, identical.

The phylogenetic relationships among the pleurotoid taxa were less clear due to the low bootstrap support. *Russula campinensis* was positioned within a larger *Russula* lineage, with moderate bootstrap support for its inclusion in a clade also containing *Russula adusta* and *R. romagnesii*. *Lactarius panuoides* and *L. uyedae* appeared derived from a larger, monophyletic *Lactarius* lineage, although bootstrap support was lacking for their position within this lineage.

Lactarius appeared monophyletic within the Russulaceae. *Russula* appeared polyphyletic; while most species fell into one large clade, *R. brevipes* was positioned within the *Lactarius* clade.

DISCUSSION

Given the consistency with which species of *Russula* and *Lactarius* are considered to be ectomycorrhizal in other parts of the world, it was not surprising to find that basidiomata of *L. panuoides* and *R. campinensis* could be matched with co-occurring ectomycorrhizae using DNA sequence data. Additional circumstantial evidence supporting an ectomycorrhizal condition for these species was provided by transect data in which basidiomata of both *L. panuoides* and *R. campinensis* were found to be strictly spatially associated with *D. corymbosa* and *D. altsonii* (Henkel unpubl). Both *D. corymbosa* and *D. altsonii* heavily dominated areas of forest which were sharply juxtaposed with mixed rainforest containing no ectomycorrhizal trees and both species consistently exhibited ectomycorrhizal rootlets in the field. If saprotrophic, these fungi might be expected to have a wider local distribution, occurring in both mixed and *Dicymbe* stands, which was not the case.

In Japan, basidiomata of *L. uyedae* appeared restricted to forests dominated by mature *Castanopsis* and *Fagus* trees which are members of the Fagaceae, an angiosperm family considered to be obligately ectomycorrhizal. This relationship is suggestive of an ectomycorrhizal habit for the fungus. *Lactarius uyedae* also shares morphological and molecular similarities with the ectomycorrhizal *L. panuoides* which argued against great differences between the species in nutritional mode. Yet, for the collections reported here, careful excavations revealed no ectomycorrhizal roots in the vicinity of the basidiomata and putative ectomycorrhizal hosts, while present, were often some distance away. More intensive sampling of ectomycorrhizal rootlets from forests bearing *L. uyedae*, with DNA-based identification of the mycobionts, and possibly in vitro mycorrhizal synthesis, are necessary to determine the mycorrhizal status of this fungus.

The production of basidiomata in elevated positions on living trees or rotting wood is not necessarily indicative of saprotrophic nutrition (Rayner et al 1985). Elevated fruiting from woody support structures has been recorded in a variety of ectomycorrhizal basidiomycetes such as the boletes *Boletellus ananas* (Curt.) Murrill in the southeastern USA (Singer 1945), southeast Asia (Corner 1972), and Guyana (Henkel pers obs); *Boletellus ananiceps* (Berk.) Singer in Australia (Rayner et al 1985); and in *Tylopilus potamogeton* (Singer) var. *irengensis* Henkel and *Tylopilus exiguus* Henkel in Guyana (Henkel 1999). Heim (1970) noted such fruiting of *Russula annulata* ssp. *parasitica* Heim in Africa, as did Buyck and Horak (1999) with pleurotoid *Russula* species recently described from New Guinea and New Zealand. In the Pacific Northwest of the USA, *Russula bicolor* Burl. commonly fruits at heights of 1–2 m on well rotted stumps of large coniferous trees (S. L. Miller pers obs). The reason for production of fruiting bodies in elevated positions is unclear; possibly, there are requirements of the fungus for more air turbulence or less moisture than is present at ground level, thereby facilitating spore dispersal. Alternatively, woody substrates in temperate forests are often completely perfused with ectomycorrhizal root systems which may exploit nitrogen derived from free-living N-fixers common in decomposing wood (Weber and Sundman 1986, Jurgensen et al 1987) and basidiomata may arise from these substrates due to the proximity of their parent mycorrhizae. Similar evidence from the tropics is limited, but well rotted logs in Guyanese *Dicymbe* forests are usually permeated by ectomycorrhizal rootlets (Henkel pers obs).

The pleurotoid habit is a rarity for members of the Russulaceae in temperate areas where most of the work on the family has been conducted; however, this habit appears to be more common in the tropics than previously believed, with new evidence from Guyana (here) and New Guinea (Buyck and Horak 1999). As Buyck (1995) pointed out, the taxonomic and systematic basis for studying the Russulaceae was established in the relatively depauperate flora of the

Northern Hemisphere. A wider degree of morphological diversity exists for both *Lactarius* and *Russula* in the tropics, not only in basidiome shape, but in features such as spore-print coloration, pileipellis structure, cystidial elements, basidiospore ornamentation, and presence or absence of a veil. A fully integrated taxonomy for the Russulaceae must take into account the great deal of variation present in the tropics.

The phenotypic distinction between *Russula* and *Lactarius,* although fairly definitive in the Northern Hemisphere, also becomes less obvious in the tropics (Buyck 1995). *Russula* and *Lactarius* are currently distinguished primarily on differing degrees and combinations of two characters: *Lactarius* contains abundant latex and relatively few sphaerocytes, while *Russula* generally contains no latex and has abundant sphaerocytes (Singer 1986). In addition, *Lactarius* possesses secretion hyphae that end in pseudocystidia in the hymenium, whereas pseudocystidia of this type are not considered to be present in *Russula* (Buyck 1991). Other characters, although not exclusive, help to distinguish these genera. In *Russula,* the caps are usually brighter in color, pigments are almost always vacuolar, fluorescence under UV light has been observed in all species tested, true dermatocystidia and encrusted primordial hyphae are present in many species, virtually all species have intervenose lamellae, and, except in section *Compactae* Fries, decurrent gills are not common. In *Lactarius,* bright colors are usually not seen except in subgenus *Lactarius* Hesler & Smith, pigments generally occur extracellularly as encrustations, fluorescence under UV light has yet to be reported, decurrent lamellae are common, few species have true dermatocystidia, primordial hyphae have never been observed, and many species lack intervenose gills (Hesler and Smith 1979, Romagnesi 1985, Heilman-Clausen et al 1998).

Such observations are particularly relevant in attempts to identify and classify taxa of pleurotoid Russulaceae. Critical examination of our fresh collections illustrated the difficulty in distinguishing between *Russula* and *Lactarius* and assigning these species to one or the other genus. Only in the larger *L. panuoides* was latex evident in fresh collections. Absence of a latex is not uncommon in diminutive species of *Lactarius* when slightly dessicated. On the other hand, Buyck and Horak (1999) reported a watery latex that was exuded from the lamellae of a pleurotoid fungus from New Guinea which they assigned to the genus *Russula.* The moderately acrid taste observed for *R. campinensis* commonly occurs in both *Russula* and *Lactarius,* as does the mild taste in *L. panuoides.* Microscopically the echinulate basidiospores of *R. campinensis* appear *Russula*-like, as do the sulfovanillin-positive hymenial cystidia, but these features also occur in *Lactarius.* The trichodermial pileipellis with true, sulfovanillin-positive dermatocystidia seen in *R. campinensis* rarely occurs in *Lactarius,* although it is common in *Russula* (Heilman-Clausen et al 1998). Hesler and Smith (1979) reported pileocystidia in the pileipellis of *Lactarius* species in subgenus *Russularia* (Fr.) Kauffman section *Thejogali* Hesler and Smith, however, these are quite different in appearance from the dermatocystidia in *R. campinensis* and do not stain in sulfovanillin. Sphaerocytes, while observed in the trama of both *L. panuoides* and *R. campinensis,* were not abundant, and were conspicuous only at the junctions of the lamella and pileus. Pseudocystidia, a common feature of *Lactarius* considered to be absent in *Russula,* were abundant in *L. panuoides* and *L. uyedae.* The presence of pseudocystidia-like elements in *R. campinensis* necessitates a reconsideration of the generic boundaries for *Russula.*

Although bootstrap support was generally weak, the topology of the nLSU tree provided some clues regarding the separation of *Lactarius* and *Russula,* and the possible disposition of the pleurotoid taxa described here. *Lactarius* appeared to be monophyletic. *Russula* appeared to be largely monophyletic, except for *R. brevipes.* Maire (1910) recognized the similarity between members of the genus *Russula,* such as *R. brevipes,* which are mostly white with dense tissues, and some species of *Lactarius,* by erecting the section *Lactarioidae* (= *Plorantes* Bat. ex Singer) of *Russula.* These observations provide some circumstantial support for the positioning of *R. brevipes* with *Lactarius* suggested by this study.

Analysis of nLSU sequence data suggested that *L. panuoides* and *L. uyedae* can be accomodated within the genus *Lactarius,* though low support values and the low number of taxa sampled limited any assessment of their subgeneric affinities. The shared features of pileipellis structure with a layer of globose elements giving rise to a turf of thick-walled hairs, reticulate spore ornamentation, and brown discoloration suggest a relatively close phenotypic relationship between *L. panuoides* and *L. uyedae.* This suite of characters is consistent with *Lactarius* subgenus *Lactifluus* (Burl.) Hesler & Smith section *Lactifluus* Hesler & Smith near *L. volemus* (Lalli and Pacioni 1992), where phylogenetic analysis of an internal transcribed spacer (ITS) sequence dataset with more complete taxon sampling indicated an affinity (S. L. Miller unpubl). Additional molecular analyses are needed to the assess the unity of Singer's section *Panuoidei.* It may become necessary to describe more sections of *Lactarius* in order to accomodate the pleurotoid taxa or pleurotoid taxa may be best placed in

existing sections of *Lactarius* regardless of their pleurotoid habit.

Sequence analysis of *R. campinensis* indicated an affinity of the taxon for the genus *Russula*. This generic placement is supported morphologically by the presence of sulfovanillin-positive dermatocystidia in *R. campinensis*. *Russula campinensis* was sister to the larger *Russula* clade within a modestly supported, smaller clade containing *R. adusta* and *R. romagnesii*, though no morphological synapomorphies are currently apparent which unite these taxa. Buyck and Horak (1999) recently described the first known species of pleurotoid *Russula*. Their discoveries, in addition to the combination of *R. campinensis* proposed here, suggest that other species of tropical Russulaceae might be critically re-evaluated using both morphological and molecular tools. Molecular studies with more comprehensive taxon sampling will help elucidate the evolutionary relationship between *Russula* and *Lactarius*.

Redhead and Norvell (1993) erected a new genus, *Pleurogala*, to accommodate the species of *Lactarius* sect *Panuoidei* Singer. They did so because of the putative lignicolous nutritional mode, pleurotoid habit and the presence of a subiculum with thick-walled hyphae in some of the species. The authors hypothesized that the saprotrophy of *Lactarius* sect *Panuoidei* and *Bondarzewia* was a plesiomorphic nutritional condition in the russuloid lineage from which the ectomycorrhizal mode was derived. An ectomycorrhizal relationship between *L. panuoides* and *R. campinensis* and tree hosts in the genus *Dicymbe* has now been confirmed. Molecular data suggested that species in sect. *Panuoidei* do not form a monophyletic clade within the Russulaceae but have affinities with either *Russula* or *Lactarius*. They do not occur in a sister position to the family as a whole. Additionally, the pleurotoid habit has been shown to be a feature of a number of tropical Russulaceae in both *Russula* and *Lactarius* and is not necessarily a critical morphological character at the generic level (Buyck and Horak 1999). The relationship between *Bondarzewia* and *Lactarius* sect. *Panuoidei* does not appear to be a close one although these fungi certainly belong in the increasingly well defined russuloid lineage. Therefore it is most appropriate to maintain *L. panuoides* and *L. uyedae* in the genus *Lactarius*, and to transfer *L. campinensis* into *Russula*.

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