Biology and molecular ecology of subiculate *Lactarius* species from Guyana

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Miller, S. L. (Botany Department, University of Wyoming, Laramie, WY 82071, U.S.A) & T. W. Henkel (Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, U.S.A). Biology and molecular ecology of subiculate *Lactarius* species from Guyana. Memoirs of The New York Botanical Garden 89: 297–313, 2004.—To date four species of *Lactarius* that produce sporocarps from a well-developed subiculum have been collected from the Pakaraima Mountains of Guyana. Three of these, *L. panuoides, L. multiceps,* and *L. brunellus* are pleurotoid. An additional undescribed species, *L. subiculatus* nom. prov., is centrally stipitate from a short stipe. All these species belong to subgenus *Lactifluus,* which in sequence analysis of the ITS region appears basal in the *Lactarius* phylogeny. Depending on the species, the subiculum can vary in size from several square centimeters to several hundred square meters, and can cover rocks, soil, roots, saplings and tree trunks. Sequence analysis has shown that all these species form ectomycorrhizae with the leguminous host tree *Dicymbe corymbosa* (Caesalpiniaceae). The subiculum in *L. panuoides* and *L. brunellus* is extensive and thick, measuring to 15–20 cm deep. The inner portion of the subiculum appears to be well humified material and outer layers indicate a coexistence or competition with algae and/or bryophytes. A plaque-like slime has been found in close proximity to subicula from three of these fungi. The plaques appear to be detrimental to recruitment of *Dicymbe* saplings, with many dead or dying saplings observed covered by the plaque. Sequence analysis has shown that all except the plaque found associated with *L. brunellus* are of non-Russuloid fungal origin and that subiculate *Lactarius* species often share the boles of young saplings with the plaque fungi. Descriptive data on the biology, morphology and extent of the subiculum in these four species is provided, along with molecular documentation of their phylogeny and ectomycorrhizal status.

KEY WORDS: ectomycorrhiza, neotropics, subiculum, South America, *Lactarius,* Russulaceae, basidiomycetes, Guyana

Introduction

A subiculum is defined as "a net-, wool- or crust-like growth of mycelium under fruit-bodies" (Hawksworth et al., 1995). While not common in species of *Lactarius* from temperate regions, a few species such as *L. subserifluus* Longyear, *L. subdulcis* (Pers.:Fr.) Gray, and *L. citriolens* Pouzar have been described with a dense growth of white or colored hyphae present on the extreme base of the stipe (Heilmann-Clausen et al., 1998; Hesler & Smith, 1979). Verbeken (1996) reported a limited number of African *Lactarius* species with a similar subiculum restricted to the stipe base. However, much more extensive subicula have been described from species of pleurotoid *Lactarius* found in South America (Dennis, 1970; Singer, 1952, 1984; Singer et al., 1983). The extensive nature of these subicula and their habit of covering well-rotted tree stumps and logs has led most authors to conclude that the South American species of *Lactarius* are saprotrophic or parasitic (e.g., Singer, 1984). This has somewhat clouded the taxonomic picture for these fungi (Verbeken, 1998), and has resulted in various hypotheses regarding evolution in the Russulales (Redhead & Norvell, 1993).

In the last several years, four species of *Lactarius* that produce basidiomata from a well-developed subiculum have been encountered in the Pakaraima Mountains of Guyana (Henkel et al., 2000; Miller et al., 2002). Three

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of these—*L. panuoides* Singer, *L. multiceps* S. L. Mill., Aime & T. Henkel, and *L. brunellus* S. L. Mill., Aime & T. Henkel—are pleurotoid. An additional, as yet undescribed species, *L. subiculatus* nom. prov., is centrally stipitate from a short, ornamented stipe. These fungi are interesting and prominent components of the ectomycorrhizal mycota associated with trees of the leguminous species *Dicymbe corymbosa* Spruce ex Benth. in the mountains of western Guyana. This paper presents ecological information on these unusual fungi. Data on the biology, morphology, and extent of the subicula formed by these four species are provided, along with molecular documentation of their identity, phylogeny, and ectomycorrhizal status.

Materials and Methods

Collecting expeditions were made in May–June 2000 and 2001 to the Upper Potaro River along Guyana's western border with Brazil, in the south-central Pakaraima Mountains (general area: 5°05'N; 59°58'W). Basidiomata and subicula were examined in the field for fresh characteristics and photographed. Color characteristics were described subjectively. Most subicula were composed of numerous above-ground points that were easily visible. Measurements of subicula were based on observation of these visible points on trees, bases of saplings, horizontal roots, rocks, and soil in an unbroken or seemingly continuous well-defined area, with the assumption that all subicula with equivalent characteristics belonged to the same species and genet.

Tissue from fresh field-collected sporocarps, subicula, and plaque material was placed in 2× CTAB buffer and ground with a plastic pestle. Genomic DNA was extracted using CTAB buffers as previously described (Miller & Buyck, 2002) then purified using Geneclean III (Qbiogene, Carlsbad, CA, U.S.A.). Aqueous genomic preparations were frozen at −20°C until use.

PCR amplification was performed using Sigma *Taq* polymerase, tailed primer pair M13–ITS5/M13–ITS4 (White et al., 1990) in a programmable thermal cycler (Model PTC–100, MJ Research, Inc.). PCR products were electrophoresed through a 1% agarose gel stained with ethidium bromide, excised, and repurified using Geneclean III. The PCR products were further purified using Wizard PCR Preps (Promega Corp., Madison, WI, U.S.A.). DNA sequencing reactions were performed using primers M13–Forward and M13–Reverse labeled with IRD–700 and IRD–800, respectively, in preparation for simultaneous bidirectional sequencing (LI-COR Biotechnology Division, Lincoln, NE, U.S.A.). Amplified PCR products were sequenced with the SequiTherm Excel II DNA Sequencing Kit (Epicentre Technologies, Madison, WI, U.S.A.) and analyzed on a LI-COR Gene ReadIR 4200-2 automated sequencer. Base ImagIR (ver. 4.0, LI-COR) was used to process the data. Forward and reverse sequences were compiled, edited, and initially aligned using AlignIR (ver. 1.2, LI-COR) against a large dataset containing Russulales sequences obtained throughout the world. Final alignment was performed manually. The species examined in this study and their geographic origins are listed in Table 1.

Maximum parsimony analyses were performed using PAUP* 4.0b5 (Swofford, 2001), and gaps in alignment were treated as missing data or excluded. Due to the large size of the dataset, heuristic analysis strategies designed by Maddison et al. (1992) and Olmstead and Palmer (1994) were used to find islands of parsimony. The relative robustness of individual branches and clade stability were estimated by bootstrap analysis (Felsenstein, 1985; Hillis & Bull, 1993) and decay indices (Bremer, 1988). Bootstrap values were generated using the settings 100 replicate searches on all parsimony-informative characters using 10 random sequence addition replications and TBR (tree-bisection-reconnection) branch swapping algorithms in PAUP*. Decay indices were calculated with AutoDecay 4.0 (Eriksson, 1998) with constraints tested in PAUP* and decay values output as a NEXUS tree file displayed with TreeView (Page, 1996). Discussion on choice of outgroups can be found in Miller and Buyck (2002).

Results

Subiculum Distribution

Subicula of the four species of *Lactarius* could be recognized as belonging to a given species despite the fact that basidiomata were not always present. Morphological characteristics of the subicula from these four species are presented in Table 2 and Figs. 1–23. Visible subiculum points of a given species were frequently uniform in the height to which they covered the boles of saplings in a given area (see Figs. 10, 13–16). In addition to actively growing and occasionally fruiting subicula, moribund subicula of *L. panuoides* and *L. brunellus* were observed, indicating that although the subicula of these two species are large and presumably long-lived, they are dynamic entities subject to competition and death.

The subiculum size ranged from 0.04 to 375 m^2 among the four species (Table 3). Likewise, subicula formed by individual species also varied greatly in size. *Lactarius panuoides* formed, by far, the largest subiculum

TABLE I
SPECIMENS USED FOR MOLECULAR STUDY OF THE SUBICULATE LACTARIUS SPECIES FROM GUYANA

Fungus	Source[a]	Geographic origin
Lactarius alnicola	NAMA 1 299	U.S.A., Colorado
L. argillaceifolius	JW 411, VPI	U.S.A., North Carolina
L. aspideoides	SLM 9581	U.S.A., Virginia
L. atroviridus	No coll.	U.S.A., New York
L. brunellus	SLM 10168	Guyana
L. brunellus ectomycorrhizae	SLM 10028	Guyana
L. brunellus plaque	SLM 10028	Guyana
L. brunellus subiculum	SLM 10028	Guyana
L. camphoratus	SLM 9656	U.S.A., West Virginia
L. chelidonium	SLM 9649	U.S.A., New York
L. chrysorrheus	LA 1538, SOL	U.S.A., Virginia
L. corrugis	LA 1539, SOL	U.S.A., Virginia
L. croceus	SLM 9551	U.S.A., New York
L. deceptivus	SLM 9535	Canada, Montreal
L. deliciosus	JW 381, VPI	U.S.A., Colorado
L. fumosus var. *fumosus*	SLM 9546	U.S.A., Virginia
L. glyciosmus	CLC 1217, SOL	U.S.A., Colorado
L. hygrophoroides	SLM 9608	U.S.A., West Virginia
L. hysginus	SLM 9627	U.S.A., Virginia
L. indigo	SLM 9579	U.S.A., Virginia
L. lignyotellus	SLM 9592	U.S.A., West Virginia
L. maculatipes	SLM 9546	U.S.A., Virginia
L. mucidus	JW 384, VPI	U.S.A., Colorado
L. multiceps	SLM 10136	Guyana
L. multiceps ectomycorrhizae	SLM 10146	Guyana
L. multiceps subiculum	SLM 10146	Guyana
L. occidentalis	SLM 9653	U.S.A., New York
L. panuoides	SLM 10104	Guyana
L. panuoides subiculum	SLM 10104	Guyana
L. peckii var. *peckii*	SLM 9563	U.S.A., Virginia
L. piperatus var.*glaucescens*	SLM 9559	U.S.A., Virginia
L. pseudoflexuosus	SLM 9610	U.S.A., West Virginia
L. repraesentaneus	LA 1352, SOL	U.S.A., Wyoming
L. rubrilacteus	SLM 9878	U.S.A., California
L. rufus	SLM 9650	U.S.A., New York
L. salmoneus	BK 14	U.S.A., Mississippi
L. scrobiculatus	SLM 9588	U.S.A., West Virginia
L. subdulcis	SLM 9673	U.S.A., North Carolina
L. subiculatus	SLM 10114	Guyana
L. subiculatus ectomycorrhizae	SLM 10047	Guyana
L. subiculatus subiculum	SLM 10047	Guyana
L. subiculatus subiculum 2	SLM 10153	Guyana
L. subpalustris	SLM 9550	U.S.A., Virginia
L. subvellereus	SLM 9584	U.S.A., West Virginia
L. thyinos	SLM 9648	U.S.A., New York
L. uvidus	NAMA 1-283	U.S.A., Colorado
L. veitus	SLM 9595	U.S.A., West Virginia
L. volemus	LA 1540, SOL	U.S.A., Virginia
Russula brevipes	LP 445, SOL	U.S.A., Wyoming

[a]Herbarium source is listed with each collection number: BK, Brad Kropp, personal collection; NAMA, North American Mycological Association, Chicago Natural History Museum; SLM, Steven L. Miller, personal collection; SOL, Solheim Mycological Herbarium; VPI, Virginia Tech.

TABLE II

MORPHOLOGICAL CHARACTERISTICS OF SUBICULA PRODUCED BY FOUR *LACTARIUS* SPECIES FROM GUYANA

	Color	Texture	Habit
Lactarius panuoides	White or pallid white, young, medium gray to dull gray-brown in age, frequently flushed and mottled with pallid white from new growth or reddish brown and light or dark green to blue-green from algae and/or bryophytes	Smooth to irregular, pitted or granular; tough, fleshy and spongelike when moist; of densely interwoven hyphae	Enshrouding lower trunks of living or dead saplings, conical to irregular, frequently covering bases of larger trees and exposed roots and nearby rocks and logs; subtended by ectomycorrhizal rootlets
L. brunellus	White to pallid white, young, light to medium gray or gray-brown in age; tufts and fascicles of new growth white against darker background of gray, and light or dark green to blue-green from interspersed algae and/or bryophytes	Shaggy, tufted, and fasciculate; new fascicles most abundant and conspicuous near sites of fruiting; of loosely arranged and protruding fascicles overlaying thin or thick layer of loosely interwoven hyphae	Enshrouding the bases of living or dead saplings, the bases of large tree boles, stumps, horizonal roots, and rocks; subtended by ectomycorrhizal rootlets
L. multiceps	White to pallid white young, light to medium gray or gray-brown in age; tufts and fascicles of new growth white against darker background of gray and light or dark green to blue-green from interspersed bryophytes	Shaggy, tufted, and fasciculate; new fascicles most abundant and conspicuous near sites of fruiting; of loosely arranged and protruding fascicles overlaying moderately thin layer of loosely interwoven hyphae	Enshrouding the bases of living or dead small-diameter saplings and fine pendant roots; subtended by ectomycorrhizal rootlets
L. subiculatus	White to pallid white young, flushed or mottled with tan to pale gray-brown in age; tufts and fascicles of new growth white	Shaggy and fasciculate; new fascicles small, most abundant and conspicuous near sites of fruiting; of loosely arranged and protruding fascicles overlaying very thin layer of loosely interwoven hyphae	Forming small to large patches on the bases of large tree boles, often in depressions and recesses between boles and/or enshrouding the bases of small-diameter saplings and fine pendant roots; subtended by ectomycorrhizal rootlets

observed, with one measuring 15 × 25 m (375 m^2) and another 12 × 7.5 m (90 m^2). Large subicula were also produced by *L. brunellus* with individual subicula measuring 6 × 4 m (24 m^2), 5 × 9 m (45 m^2) and 7 × 7 m (49 m^2).

The average number of observable points in a single subiculum ranged from 2 to 34. *Lactarius brunellus* exhibited the highest number of average visible points, due primarily to one subiculum with 196 visible points in a 375 m^2 area. Calculations of the number of visible points after removing the exceptionally large subicula from each species indicated for most species each subiculum contains approximately 20 visible points. *Lactarius subiculatus* consistently showed the lowest number of visible points, around 2 to 3 per subiculum.

The points within a single subiculum varied greatly in sporocarp production. *Lactarius panuoides* subicula averaged approximately the same number of sporocarp active points as sporocarp inactive points, whereas in *L. brunellus* and *L. multiceps,* approximately two to seven times as many subiculum points were inactive as were active. The subicula of *L. subiculatus* were much smaller, and those that were found on small saplings were often detected because of the presence of sporocarps, so it is not surprising that most subicula were sporocarp active.

In general, living saplings of <3 cm diam. were a preferred substratum for subiculate species of *Lactarius,* as virtually all species utilized that substratum class to a great extent. Fewer subicula were observed on moribund or visibly dying saplings of *Dicymbe* than on

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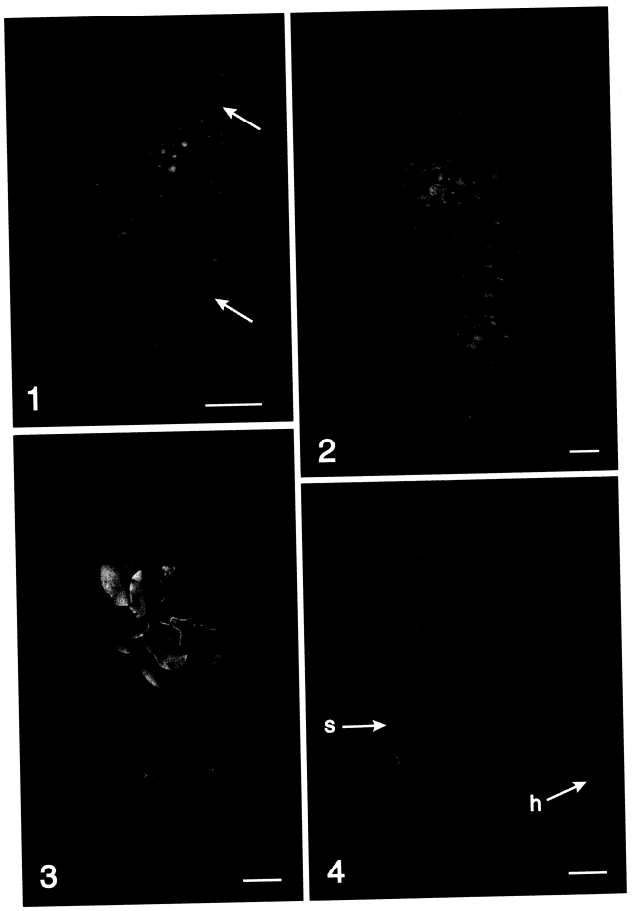
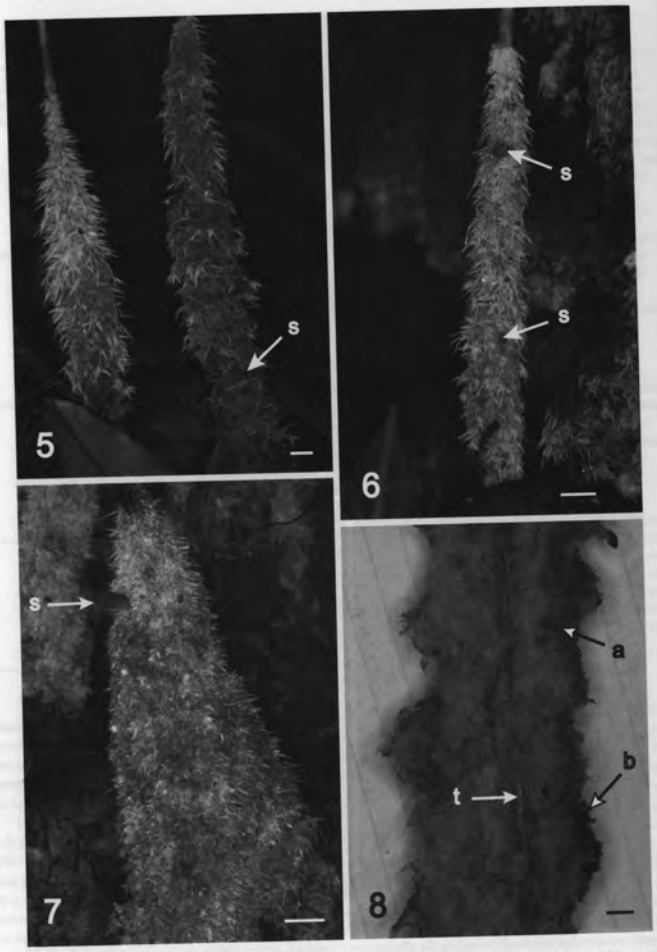


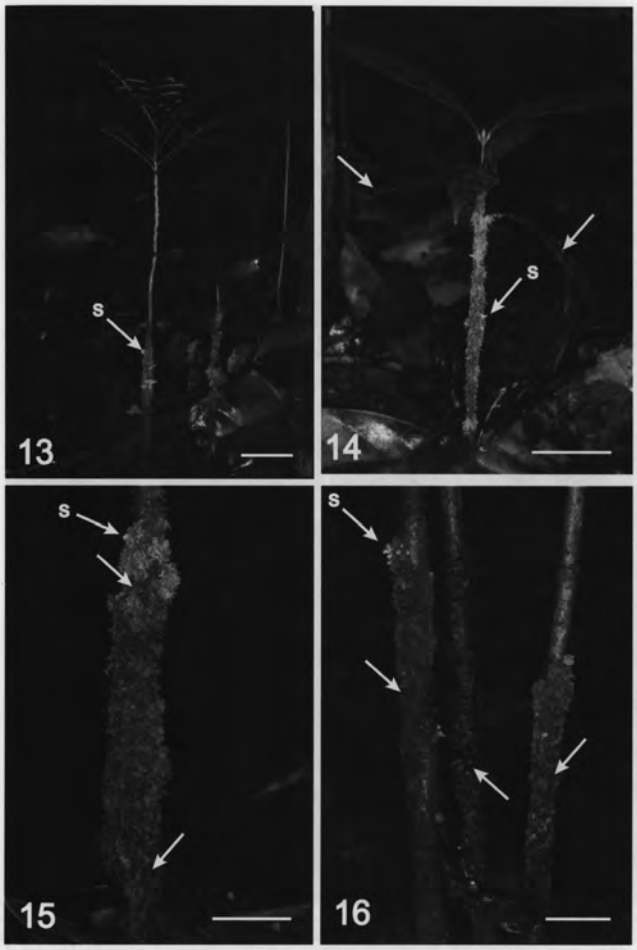
FIGS. 1–4. Habits of *Lactarius panuoides* subicula. **1, 2.** Conical subicula on the base of small sapling with young sporocarp initials or mature sporocarps. Arrows indicate patches of cyanobacteria or algae. **3.** Irregular subiculum on soil and well-decomposed log producing mature sporocarps. **4.** Longitudinal section of subiculum showing the layered appearance. Arrow at **s** indicates the outer surface of the subiculum; arrow at **h** indicates well-lumified material in the interior of the subiculum. Scale bars: 1: 5 cm; 2–4: 1 cm.



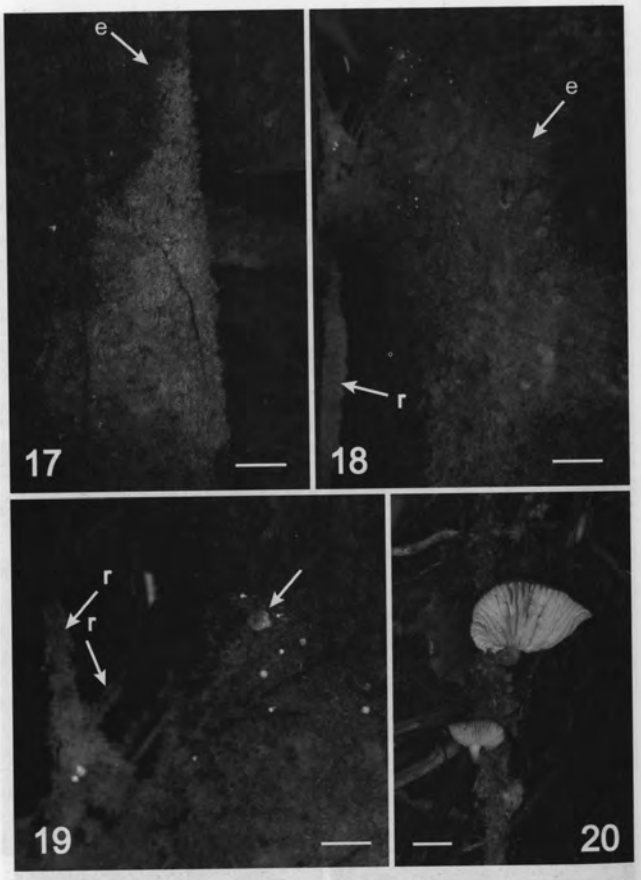
FIGS. 5–8. Habits of *Lactarius brunellus* subicula. 5–7. Conical subicula on the bases of small saplings with young sporocarp initials or mature sporocarps (arrows at **s**). **8.** Longitudinal section of subiculum enshrouding small-diameter *Dicymbe* sapling. Arrow at **t** indicates trunk of sapling. Arrows at **a** and **b** indicate patches of cyanobacteria, algae, and bryophytes near the outer surface of the subiculum. Scale bars: 1 cm.

FIGS. 9, 10. Habits of *Lactarius brunellus* subicula. **9.** Large bole of *Dicymbe corymbosa* covered by extensive subiculum. **10.** Cluster of visible subiculum points approximately 8 m from the tree shown in Fig. 9. Scale bars: 9: 0.5 m; 10: 10 cm.

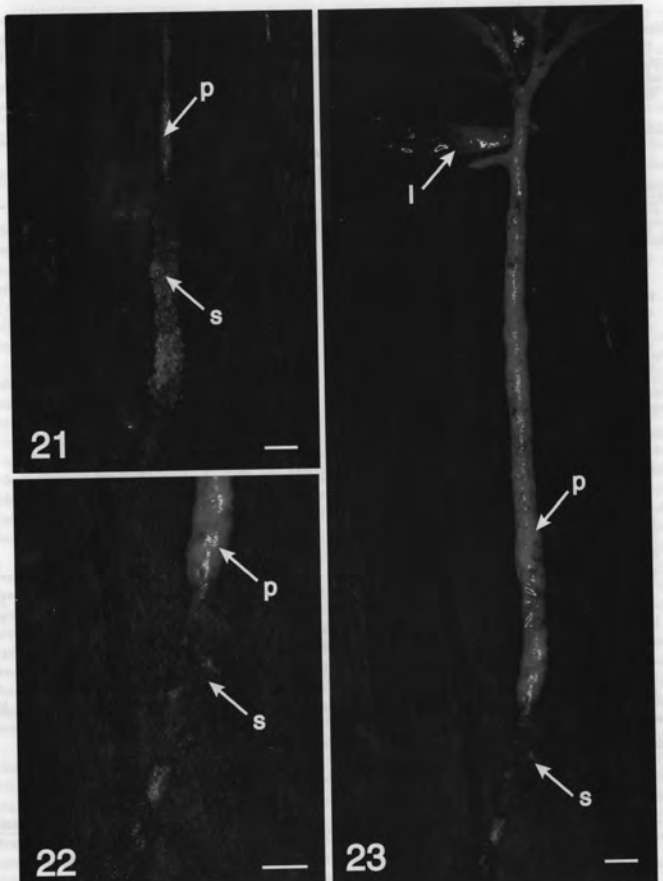
FIGS. 11, 12. Habits of *Lactarius brunellus* subicula. 11. Large bole of *Dicymbe corymbosa* covered by extensive subiculum. 12. Living horizontal root of the tree shown in Fig. 11. Scale bars: 11: 0.5 m; 12: 10 cm.



FIGS. 13–16. Habits of *Lactarius multiceps* subicula. All subicula are enshrouding small-diameter *Dicymbe corymbosa* saplings. 13, 14. Unlabeled arrows indicate sparse patches of subiculum covering leaves. Arrows at s indicate young sporocarp initials. 15, 16. Arrow at s indicates cluster of young sporocarp initials. Unlabeled arrow s indicates the presence of algae and/or cryptogams associated with the subiculum. Scale bars: 13, 14: 10 cm; 15, 16: 5 cm.



FIGS. 17–20. Habits of *Lactarius subiculatus* subicula. 17–19. Subicula on boles of large-diameter *Dicymbe corymbosa* trees. Arrows at **e** indicate the plaquelike leading edge of the subiculum. Arrows at **r** indicate subiculum enshrouding fine pendant roots. **19.** Detail of Fig. 18. Arrows at **r** indicate fine pendant roots. Unlabeled arrow indicates young sporocarp initials. **20.** Small-diameter sapling enshrouded by subiculum producing sporocarps Scale bars: 17, 18: 10 cm; 19, 20: 1 cm.



FIGS. 21–23. Plaques associated with *Lactarius subiculatus* subicula. Arrows at **s** indicate subicula. Arrows at **p** indicate plaques. The arrow at **l** indicates the plaque covering the leaves causing significant necrosis. Scale bars: 1 cm.

TABLE III

DESCRIPTIVE ECOLOGICAL DATA FOR FOUR SPECIES OF SUBICULATE *LACTARIUS* FROM GUYANA. THE NUMBER OF POINTS REFERS TO THE NUMBER OF INDIVIDUAL POINTS OF SUBICULUM VISIBLE ABOVE GROUND IN A WELL-DEFINED AREA OF OBSERVABLE DIMENSIONS. SUBICULA WERE CONSIDERED SPOROCARP ACTIVE (SA) IF SPOROCARPS WERE BEING PRODUCED ANYWHERE ON AN INDIVIDUAL POINT; OTHERWISE THEY WERE CONSIDERED SPOROCARP INACTIVE (SI). SAPLING CONDITION REFERS TO SAPLINGS THAT WERE ALIVE (LIV.), MORIBUND (MOR.) OR DEAD. SUBSTRATUM REFERS TO THE ENTITY PROVIDING SUPPORT FOR THE SUBICULUM AND DOES NOT IMPLY UTILIZATION OF THE SUBSTRATE BY THE FUNGUS FOR ENERGY.

Species Dimensions	No. points	Sporocarps (%) SA	SI	Sapling condition (%) Liv.	Mor.	Dead	Substratum (%) Sapling diam. <3 cm	3–10 cm	Other substrates (N)	
Lactarius panuoides										
1. 15 × 25 m (375 m²)	30	73	27	55	21	24	66	34	-Horizontal root	3
									-Tree 1 m diam.	4
2. 12 × 7.5 m (90 m²)	51	16	84	91	3	6	69	31	-Horizonal roots	14
									-Soil	14
3. 6 × 6 m (36 m²)	34	71	29	97	0	3	68	32		
4. 1 × 1.5 m (1.5 m²)	4	75	25	75	0	25	100	0		
5. 7 × 4 m (28 m²)	18	94	6	83	0	17	67	33		
6. 7 × 3.5 m (24.5 m²)	23	35	65	50	0	50	100	0	-Tree 2 m diam.	4
7. 0.25 × 0.25 m (0.06 m²)	1	100	0	100	0	0	100	0		
Average 79 m²	23	66	34	79	3	18	81	19		
Lactarius brunellus										
									-Litter branch	4
1. 5 × 9 m (45 m²)	196	30	70	83	3	14	99	1	-Well-rotted logs	2
									-Horizontal roots	9
									-Tree 0.6 m diam.	1
2. 1 × 1.5 m (1.5 m²)	7	86	14	86	0	14	100	0		
3. 2 × 1.5 m (3 m²)	4	100	0	100	0	0	100	0		
4. 4 × 3 m (12 m²)	24	8	92	96	0	4	96	4	-Tree 0.3 m	4
5. 4 × 1.5 m (6 m²)	15	7	93	100	0	0	93	7		
6. 1.5 × 1.5 m (2.25 m²)	4	0	100	100	0	0	100	0	-Tree 1 m	25
									-Tree 1 m	16
									-Tree 0.5 m	4
7. 6 × 4 m (24 m²)	24	58	42	100	0	0	98	2	-Aroid stems	13
									-Vine	4
									-Dead tree roots	8
8. 7 × 7 m (49 m²)	4	50	50	99	0	0	0	0	-Tree 2 m	33
Average 17.8 m²	39	42	58	96	0	4	86	2		
Lactarius multiceps										
1. ?	50	10	90	100	0	0	100	0		
2. ?	23	13	87	100	0	0	100	0		
3. 4 × 3 m (12 m²)	28	11	89	93	0	7	100	0		
4. 0.2 × .0.2 m (0.04 m²)	1	100	0	0	0	0	0	0	-Fine pendant root	100
Average 6.2 m²	26	33	67	73	0	2	75	0		
Lactarius subiculatus										
									-Patch on 0.6 m diam. tree	33
1. 0.5 × 0.5 m (0.25 m²)	3	67	33	0	0	0	0	0	-Vine pendant root	66
									-Patch, 0.5 cm diam. tree	100
2. 0.5 × 0.5 m (0.25 m²)	1	100	0	0	0	0	0	0		
3. 3 × 3 m (9 m²)	2	100	0	100	0	0	100	0		
Average 3.1 m²	2	89	11	33	0	0	33	0		

healthy or dead saplings for all subiculate Lactarius species, suggesting that saplings died quickly. Subicula also occurred frequently on 3–10 cm diam. trees colonized by *L. panuoides* and *L. brunellus,* but not by *L. multiceps* or *L. subiculatus.* However, the boles of large *Dicymbe* trees 0.3–2 m diam. were used as support by all species except *Lactarius multiceps.*

MOLECULAR ANALYSES

Figure 24 shows the best most-parsimonious tree (likelihood score of −ln L = 5211.15240) based on weighted parsimony (transitions two times transversions) with a consistency index (CI) of 0.483, retention index (RI) of 0.673, and rescaled consistency index (RC) of 0.325. Other equally parsimonious trees in this island differed only in minor rearrangements within terminal clades.

Topology of the tree indicates that the subiculate habit has arisen in at least four species of South American *Lactarius* (Fig. 24); however, all four have been taxonomically placed in the subgenus *Lactifluus* (Burl.) Hesler & A. H. Sm. The subgenus *Lactifluus* contains a number of both temperate and tropical species (Verbeken, 1996) and in all of our molecular analyses this subgenus appears near the base of the topology in the *Lactarius* phylogeny, suggesting that these species and their subiculate habit may be quite ancient.

The molecular data also confirmed the ectomycorrhizal habit of *Lactarius subiculatus, L. multiceps,* and *L. brunellus* (Fig. 24), undoubtedly in association with *Dicymbe corymbosa* and possibly *D. altsonii* Sandw. Mycorrhizae of *Lactarius panuoides* were described in an earlier paper (Henkel et al., 2000) and were excluded from this analysis. In addition, the molecular data also confirmed that the subiculum material thought to be from each subiculate species did indeed correspond to those species.

Thin, moist, and opaque plaques were observed in close proximity with several of the subicula (Figs. 21–23). These were found primarily on small-diameter living and moribund saplings, and were especially noticeable near the leading edge of *Lactarius panuoides, L. brunellus,* and *L. subiculatus* subicula on large boles of trees. The plaques on the small-diameter saplings often developed apically to patches of subiculum recognizable as belonging to *L. panuoides, L. brunellus,* or *L. subiculatus,* and extended for substantial distances up the trunk, occasionally partially or completely covering the leaves (Figs. 21–23). Because of the morphological similarity of the plaques on the small-diameter saplings with the leading edge of massive subicula on the boles of large trees, these were initially interpreted as a young stage in the development of subicula. However, sequence analysis indicated that *L. brunellus* was the only member of the subiculate *Lactarius* group that was confirmed to form this plaque on small-diameter saplings, and a non-Russuloid origin was determined for the remainder of these plaques. Following GenBank BLAST searches, some of the non-Russuloid plaques were found to belong to members of the Sebacinaceae, jelly fungi that have been reported to form ectomycorrhizae with saprotrophic orchids (D. L. Taylor, unpubl. sequence submitted to GenBank, AY052375; Lim & Berbee, unpubl. *Tremellodendron* sequence submitted to GenBank, AF384862).

Discussion

The subicula of several species of South American *Lactarius* are impressive features of the forest landscape, covering the bases of small saplings and large-diameter trees, downed limbs and logs, rocks, horizontal roots, and soil. The subiculate habit has arisen in several species of South American *Lactarius* species all belonging to the subgenus *Lactifluus.* As far as we know, no other Russuloid species with extensive subicula have been described outside of the Neotropics. Verbeken (1993, 1996) recorded a basal tomentum, which she termed a subiculum, on several African species of *Lactarius* belonging to subgen. *Lactifluus* including *L. gymnocarpus* R. Heim ex Singer, *L. pseudogymnocarpus* Verbeken, and *L. caperatus* R. Heim & Gooss.-Font. However, none of the paleotropical or temperate species in this subgenus forms the extensive, above-ground subicula found in these neotropical species. Consistent with this observation, *L. uyedae* Singer, a closely related pleurotoid member of subgen. *Lactifluus* from Japan, lacks a subiculum (Henkel et al., 2000). Because subgen. *Lactifluus* appears as a well-defined, basal clade in our previous molecular analyses of the n-LSU (Henkel et al., 2000; Miller et al., 2001) as well as the present analysis of ITS data, we can safely hypothesize that subgen. *Lactifluus* is one of the most ancient groups in the genus and that the subiculate habit is plesiomorphic in *Lactarius.*

FUNCTIONAL BIOLOGY

The functional biology of the subiculum, aside from acting as a support for sporocarp production, is not fully known. Certainly, the larger the number of visible above-ground points with spore-producing basidiomata, the greater the sexual reproductive prominence the species will have in the local landscape. A subiculum

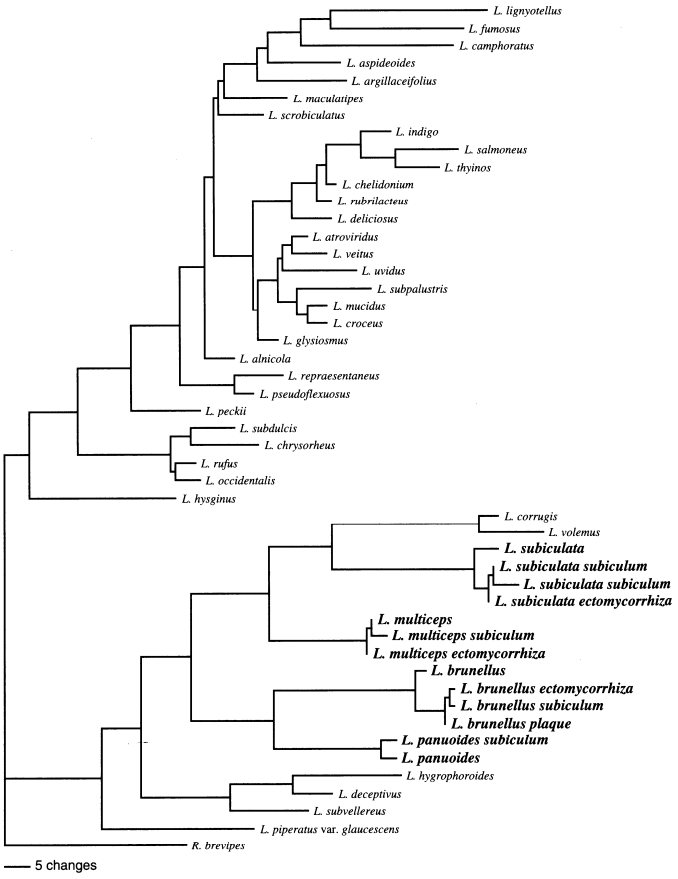


FIG. 24. Best (−ln likelihood) from an island of 11 equally most-parsimonious trees of 814 steps inferred from parsimony analysis of ITS1, 5.8S, and ITS2 nrDNA sequences depicted as a phylogram. In this analysis, transitions equal two times transversions. CI = 0.483, RI 0.673, RC 0.325. Sequences from subiculate *Lactarius* spp. sporocarps, ectomycorrhizae, and subicula are in boldface type.

with multiple above-ground points may simply be a way to increase surface area of the mycelium, basidiomata, and overall fecundity.

Other possibilities for functionality of the subiculum may lie in nutrient resource capabilities. Material from within individual points of the thick subicula of *Lactarius panuoides* and *L. brunellus* was composed of well-humified material with numerous ectomycorrhizae. Dennis (1970), Pegler (1979), and Singer (1984) presumed that many of the pleurotoid taxa ascribed to *Lactarius* were saprotrophic, parasitic, or facultatively ectomycorrhizal in the wettest parts of the tropics. This observation was largely due to the fact that many of these fungi fruit from a subiculum on a sapling or decomposed log well above ground level where most other species of *Lactarius* produce basidiomata. Lindeberg (1948, 1949) and Giltrap (1982) hypothesized that many ectomycorrhizal fungi are physiologically more similar to white rot fungi than to brown rot fungi, since many of them contain polyphenol oxidases used in lignin depolymerization. The genus *Lactarius* contains many species that have been shown to produce highly effective phenol-oxidases (Giltrap, 1982). In addition, many macrochemical color reactions, long found to be indispensable in the identification of certain *Russula* and *Lactarius* species, use phenolic substrates such as guaiac, guaiacol, phenol, formol, aniline, and phenol-aniline as their basis for colorogeny (Singer, 1986). These and other phenolic substrates are used extensively in the detection of phenol oxidase enzymes during lignin degradation (Marr, 1979; Marr et al., 1986). It may be that formation of numerous above-ground points in subiculate species is an effective resource-capture device, and that the fungi slowly decompose captured material and reclaim nutrients from dead subiculum hyphae. Future studies of the subiculate *Lactarius* from Guyana should be aimed at the ecophysiology of saprotrophic activity.

Along these lines, another possibility that was explored in our first manuscript on these fungi (Henkel et al., 2000) was that because most of the individual points of *Lactarius panuoides* and *L. brunellus* subicula are associated with cyanobacteria and/or green algae, in addition to cryptogams such as leafy liverworts, mosses, and ferns, the subiculum/ectomycorrhizal unit may be a device to obtain nitrogen from free-living nitrogen fixers or even possibly from a lichenized association.

One factor that may influence these putative symbiotic associations is the water-holding capacity of the subiculum. While perhaps counterintuitive given the wet tropical forests of the Pakaraima Mountains (>3000 mm/yr, Henkel, 2002), the spongelike texture of the subicula may have evolved as a mechanism to prevent desiccation. Free water could be produced by squeezing subicula of *Lactarius panuoides,* even after several days without precipitation. Short-term desiccation may be a reality in these forests even in the wet season; water-holding capacity in the subicula could also extend the period of biological activity and possibly fruiting in the subicula into the dry season. While speculative, it seems reasonable to assume that a water-holding mechanism that supports fruiting, algal/cyanobacterial growth and ectomycorrhizal function could be advantageous to these fungi.

The size and extent of individual subicula was estimated subjectively based on the assumption that all visible points of a subiculum from a given species in a well-defined area belonged to the same species and genet. This assumption may be justified as the range of diameters for subicula reported here is roughly equivalent to those described for genets of many temperate ectomycorrhizal fungi (~10–18 m: Bonello et al., 1998; Dahlberg, 1997; Dahlberg & Stenlid, 1994; de la Bastide et al., 1994; Gryta et al., 1997). The population structure of subiculate *Lactarius* species should be fully considered to address the assumption that these subicula are indeed a single genet.

During the study of substrate preferences for the four subiculate *Lactarius* species, it was observed that the subicula frequently occupied the bases of small-diameter saplings, including many saplings that were dead or moribund. The large number of dead or dying *Dicymbe* saplings indicated that some factor was responsible for the mortality of these saplings. Closer examination indicated that many of the moribund saplings were covered to a lesser or greater extent by a thin, opaque slimelike plaque. This plaque frequently enveloped the base of the sapling, was continuous with and/or slightly above the level of a recognizable patch of *Lactarius* subiculum near the base, and occasionally enveloped the leaves. Interpretation of these plaques in the field was that they were the initial stage of a subiculum and that older portions near the base developed into an active sporocarp-producing subiculum. This is, in fact, the scenario that develops on the boles of large *Dicymbe* trees where the leading edge of *L. panuoides, L. brunellus,* and *L. subiculatus* have a wet, plaquelike appearance, while older portions develop into recognizable subicula for each species. The fact that ectomycorrhizal fungi putatively associated with the roots were causing the death of these saplings was somewhat disturbing but not without precedent. The broad-host ranging ectomycorrhizal fungus *Thelephora terrestris* Ehr. ex. Fr. has been frequently reported to cause "smothering disease" of pines and other conifers in nurseries and a variety of

tree seedlings in nature (Ginns, 1986; Shaw, 1973). The name of this disease is appropriate because the mycelium of *T. terrestris* grows over the entire seedling from the base upward, including the leaves, essentially smothering the tree from lack of light. Coupled with the reports that South American pleurotoid *Lactarius* species are saprotrophic or parasitic and that *Lactarius* produces saprotrophic enzymes, it was not difficult to reach the tentative conclusion, at least in the field, that these subiculate species of *Lactarius* might be killing *Dicymbe* saplings. However, molecular identification of several of the plaque fungi indicated a non-Russuloid origin for most of these fungi. Only *Lactarius brunellus* was shown conclusively to form plaques on small saplings. Both *L. brunellus* and *L. multiceps* subicula were observed to extend to form thin patches of subiculum over the leaves of shorter *Dicymbe* seedlings, but no necrosis was recorded for these observations. Because of the uniformity in height of most subiculum points within a given species in a particular area, it is possible that only the smallest saplings and seedlings are adversely affected by the *Lactarius* species. The plaque fungi were identified as members of the Sebacinaceae, a group of Heterobasidiomycetes commonly known as "jelly fungi." Several of these fungi have also been reported from ectomycorrhizal roots of saprophytic orchids (*Neottia nidus-avis* (L.) Rich.) and salal (*Galtheria shallon* Pursh), and bear further study. The association of these fungi with patches of subiculum at the bases of small-diameter *Dicymbe* saplings also requires additional study. Several of the subicula near or covered by plaques looked distinctly unhealthy, and competition with the subiculate *Lactarius* by the plaque fungi is a distinct possibility.

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Literature Cited

Bonello P. T. D. Bruns & M. Gardes. 1998. Generic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens.* New Phytol. 38: 533–542.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

Dahlberg A. 1997. Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. Mycol. Res. 101: 47–54.

——— & J. Stenlid. 1994. Size, distribution and biomass of genets in populations of *Suillus bovinus* (L.: Fr.) Roussel revealed by somatic incompatibility. New Phytol. 127: 225–234.

de la Bastide P. Y., B. R. Kropp & Y. Piché. 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. New Phytol. 127: 547–556.

Dennis, R. W. G. 1970. Fungus flora of Venezuela and adjacent countries. Kew Bull., Addit. Ser. 3: 1–531.

Eriksson, T. 1998. AutoDecay version 4.0. Program distributed by the author, Stockholm University–Department of Botany, Stockholm.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Giltrap, N. J. 1982. Production of polyphenol oxidases by ectomycorrhizal fungi with special reference to *Lactarius* spp. Trans. Brit. Mycol. Soc. 78: 75–81.

Ginns, J. H. 1986. Compendium of plant disease and decay fungi in Canada 1960–1980. Agric. Canada Publ. 1813: 1–416.

Gryta H., J.-C. Debaud, A. Effosse, G. Gay & R. Marmeisse. 1997. Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. Molec. Ecol. 6: 353–364.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton & D. N. Pegler. 1995. Ainsworth & Bisby's dictionary of the Fungi. Ed. 8. CAB International, Wallingford, UK.

Heilmann-Clausen, J., A. Verbeken & J. Vesterholt. 1998. The genus *Lactarius.* Fungi of northern Europe. Vol. 2. Danish Mycological Society, Greve.

Henkel, T. W. 2002. Monodominance in the ectomycorrhizal *Dicymbe corymbosa* (Caesalpiniaceae) from Guyana. J. Trop. Ecol. 19: 417–437.

———, M. C. Aime & S. L. Miller. 2000. Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status. Mycologia 92: 1119–1132.

Hesler, L. R. & A. H. Smith. 1979. North American species of *Lactarius.* University of Michigan Press, Ann Arbor.

Hillis, D. M. & J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42: 182–192.

Lindeberg, G. 1948. On the occurrence of polyphenol oxidases in soil-inhabiting Basidiomycetes. Physiol. Pl. (Copenhagen) 1: 196–205.

———. 1949. Influence of enzymatically oxidized gallic acid on the growth of some hymenomycetes. Svensk Bot. Tidskr. 43: 348–447.

Maddison, D. R., M. Ruvolo & D. L. Swofford. 1992. Geographic origins of human mitochondrial DNA: phylogenetic evidence from control region sequences. Syst. Biol. 41: 111–124.

Marr, C. D. 1979. Laccase and tyrosinase oxidation of spot test reagents. Mycotaxon 9: 244–276.

Miller, S. L. & B. Buyck. 2002. Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. Mycol. Res. 106: 259–276.

———, D. W. Grund & K. A. Harrison. 1986. The taxonomic potential of laccase and tyrosinase spot tests. Mycologia 78: 169–184.

———, T. M. McClean, J. F. Walker & B. Buyck. 2001. A molecular phylogeny of the Russulales including agaricoid, gasteroid and pleurotoid taxa. Mycologia 93: 344–354.

———, M. C. Aime & T. W. Henkel. 2002. Russulaceae of the Pakaraima Mountains of Guyana. I. New species of pleurotoid *Lactarius.* Mycologia 94: 545–553.

Olmstead, R. G. & J. D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. Amer. J. Bot. 81: 1205–1224.

Page, R. D. M. 1996. Treeview: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357–358.

Pegler, D. N. 1979. Taxonomy and ecology of *Lactarius* (Agaricales) in the Lesser Antilles. Kew Bull. 33: 601–628.

Redhead, S. A. & L. Norvell. 1993. Notes on *Bondarzewia, Heterobasidion,* and *Pleurogala.* Mycotaxon 48: 371–380.

Shaw, C. G. 1973. Host fungus index for the Pacific Northwest—II. Fungi. Wash. State Agric. Exp. Sta. Circ. 336: 1–162.

Singer R. 1952. Russulaceae of Trinidad and Venezuela. Kew Bull. 7: 295–301.

———. 1984. Tropical Russulaceae II. *Lactarius* section *Panuoidei.* Nova Hedwigia 40: 435–447.

———. 1986. The Agaricales in modern taxonomy. Ed. 4. Koeltz Scientific, Koenigstein.

———, I. Araujo & M. H. Ivory. 1983. The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially central Amazonia. Beih. Nov. Hedwigia 77: 1–352.

Swofford, D.L. 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, MA

Verbeken, A. 1995. Studies in tropical African *Lactarius* species. 1. *Lactarius gymnocarpus* Singer ex R. Heim and allied species. Mycotaxon 55: 515–542.

———. 1996. Biodiversity of the genus *Lactarius* Pers. in tropical Africa. Ph.D. thesis, University of Gent, Belgium.

———. 1998. Studies in tropical African *Lactarius* species. 6. A synopsis of the subgenus *Lactariopsis* (Henn.) R. Heim emend. Mycotaxon 66: 387–418.

White, T. J., T. Bruns, S. Lee & J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322. *In:* M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (editors). PCR protocols: a guide to methods and applications. Academic Press, San Diego.