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Crepidotus thermophilus comb. nov., a reassessment of *Melanomphalia thermophila*, a rarely collected tropical agaric

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Abstract: *Melanomphalia thermophila* (Sing.) Sing. is a rarely collected agaric previously known only from Florida and Brazil. This taxon was originally described as a species of *Tubaria* and much of Singer's rationale for placing *Tubaria* within the Crepidotaceae (Imai) Sing. was based on anatomical similarities between *T. thermophila* and *Crepidotus* (Fr.) Staude. In later works, *T. thermophila* was transferred to *Melanomphalia* M.P. Christ., again forming the basis upon which Singer placed *Melanomphalia* within the Crepidotaceae. Based on examination of newly collected specimens from Puerto Rico and Panama, type studies, and nuclear large subunit rDNA analysis, we conclude that this taxon is, in fact, a centrally stipitate *Crepidotus*. *Melanomphalia thermophila* is transferred to *Crepidotus*, fully described and illustrated.

Key Words: Crepidotaceae, nuclear large subunit rDNA sequences, *Tubaria*, type studies

INTRODUCTION

Tubaria thermophila Sing. was originally described from Florida (Singer 1948), where it held an isolated position within the genus as the only taxon with exosporial ornamentation. *Tubaria* Sect. *Thermophila* Sing. eventually came to include two species (Singer 1962), and it was largely based on similarities in spore ornamentation between this section and *Crepidotus* Sect. *Echinosporae* Pilát that led to Singer's transfer of *Tubaria* (W.G. Smith) Gill. from the Cortinariaceae R. Heim ex Pouzar to the Crepidotaceae (Imai) Sing. (Singer 1951, 1962).

Melanomphalia M.P. Christ. was a monotypic genus that Singer (1955) originally placed in the Cortinariaceae. Again, this decision was based on similarities in exosporial ornamentation, in this case between the type, *M. nigrescens* M.P. Christ., and *Inocybe platensis* Speg., which he transferred to *Melanomphalia* (Singer 1955). In later works, Singer reconsidered his treatment of these genera by transferring *Tubaria* Section *Thermophila* to *Melanomphalia* and placing *Melanomphalia* in the Crepidotaceae because of the stated anatomical similarities between *M. thermophila* and its allies and some *Crepidotus* species (Singer 1967, 1971). Thus the Crepidotaceae sensu Singer came to include, among others, the pleurotoid genus *Crepidotus* (including both smooth- and ornamented-spored taxa), and the stipitate genera *Melanomphalia* (all members with ornamented basidiospores), *Tubaria*, and *Simocybe* Karst. (the latter two containing only smooth-spored taxa) (Singer 1986).

We have made several collections of a dark reddish brown stipitate agaric from the Luquillo municipality of Puerto Rico in subtropical moist and wet forest between 70 and 110 m elevation. Anatomical features, including basidiospore ornamentation, are consistent with a diagnosis of *Melanomphalia thermophila*. In this paper we fully describe and illustrate *M. thermophila* and present the results of a phylogenetic analysis that lead us to propose a new combination, *Crepidotus thermophilus*.

MATERIALS AND METHODS

Morphology.—Color designations of basidiomata are given as general color terms, such as ochre, or in some cases color designations are from Kornerup and Wanscher (1978) and are indicated in the following manner, 7C5—Brownish Orange (where "7C5" designates a plate, column, and row, respectively). Methods used to prepare microscopic structures for data collection are those of Baroni (1981). All measurements of microscopic structures were made in mounts of 3% KOH or 10% NH_4OH. The measurement of basidiospores includes the hilar appendix or apiculus. The designations used for basidiospore measurements are those of Baroni and Horak (1994) with the exception that the symbol E (length/width of an individual spore) is herein designated as Q (for quotient). All measurements were made with an Olympus BHS light microscope under Hoff-

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TABLE I. Taxa selected for sequencing analysis

Taxon[a]	Collection No.	GenBank accession No.	Source
Agaricaceae			
Agaricus bisporus (Large) Singer	SAR 88/411	U11911	Chapela et al 1994
Leucocoprinus cepaestipes (Sow. : Fr.) Patouillard	EFM 548	U85286	Johnson 1997
Coprinaceae			
Coprinus atramentarius (Bull. : Fr.) Fries	C 114 = VT 1131	AF041484	Hopple 1994
Psathyrella candolleana (Fr.) Maire	J 181	AF041531	Hopple 1994
Bolbitiaceae			
Bolbitius vitellinus (Pers.) Fries	SAR 84/100	U11913	Chapela et al 1994
Conocybe rickenii (Schaeff.) Kühner	J 183	AF041546	Hopple 1994
Strophariaceae			
Pholiota squarrosoides Peck	JJ 7	AF042568	Moncalvo et al 2000
Stropharia rugosoannulata Farlow ex Murrill	D 258	AF041544	Hopple 1994
Cortinariaceae			
Cortinarius iodes Berkeley & Curtis	JM 96/23	AF042613	Moncalvo et al 2000
Dermocybe marylandensis Ammirati & Smith	JM 96/24	AF042615	Moncalvo et al 2000
Crepidotaceae			
Crepidotus amygdalosporus Kühner	MCA 258	AF205675	Aime 1999
Crepidotus aureus Horak	OKM 27300	AF205685	Aime 1999
Crepidotus cinnabarinus Peck	MCA 387	AF205686	Aime 1999
Crepidotus fraxinicola Murrill	OKM 26748	AF205697	Aime 1999
Crepidotus mollis (Fr.) Staude	OKM 26279	AF205677	Aime 1999
Crepidotus thermophilus (Sing.) comb. nov.[b]	TJB 8496	AF205691	Aime 1999
Crepidotus thermophilus[b]	OKM 27270	AF205669	Aime 1999
Crepidotus versutus (Peck) Saccardo	MCA 250	AF205695	Aime 1999
Simocybe serrulata (Murr.) Singer	OKM 27046	AF205688	Aime 1999
Simocybe sp.	MCA 294	AF205699	Aime 1999
Simocybe sp.	MCA 424	AF205687	Aime 1999
Outgroups			
Gymnopus dryophilus (Bull. : Fr.) Murrill[c]	RV 83/180	AF042595	Moncalvo et al 2000
Omphalotus nidiformis Berk.	VTCC 1946.8	AF042621	Moncalvo et al 2000
Crinipellis maxima Smith & Walker	DAOM 196019	AF042630	Moncalvo et al 2000

[a] Taxa listed following the classification of Singer (1986).
[b] Originally published as *Melanomphalia* sp.
[c] Accessioned as *Collybia dryophila.*

man interference optics using a semi-automated image analysis system (a GTCO digitizer pad and Metrics5 software written by Dr. David Malloch). Descriptive statistical analysis of the measurements was obtained using EXCEL97 and/or SigmaStat 1.0. Scanning electron micrographs (SEMs) were produced with an ISI Supra IIIA scanning electron microscope generally run at 10 Kev. Methods for preparation of samples for SEM are those of Baroni (1981). Where noted, coordinates of collecting sites were obtained with a hand held GPS device and were referenced to map datum WGS84.

Sequence analysis.—To ascertain the natural affinities of *M. thermophila,* DNA was extracted from two Puerto Rican collections made in consecutive years. Methods for extraction of DNA, amplification, and sequencing follow Aime (1999). Primers LR0R, LR3R, LR5, and LR7 (Moncalvo et al 2000) were used to sequence a portion from the 5′-end of the nuclear large subunit rDNA (nLSU). For sequence analysis, we assembled a data set of previously published sequences (TABLE 1), by selecting two generic exemplars from each family following the classification of Singer (1986) in order to approximate the range of diversity found within the dark-spored Agaricales. Three taxa of white-spored agarics were included for rooting purposes, with *Gymnopus dryophilus* chosen as the outgroup. Taxa within *Crepidotus* were selected to include a broad cross-section of the phenotypic diversity inherent in the genus.

Sequences were manually aligned and analyzed in PAUP* 4.0b2 (Swofford 2001). Sequence alignments are deposited in TreeBASE S791. The data matrix included a total of 1193

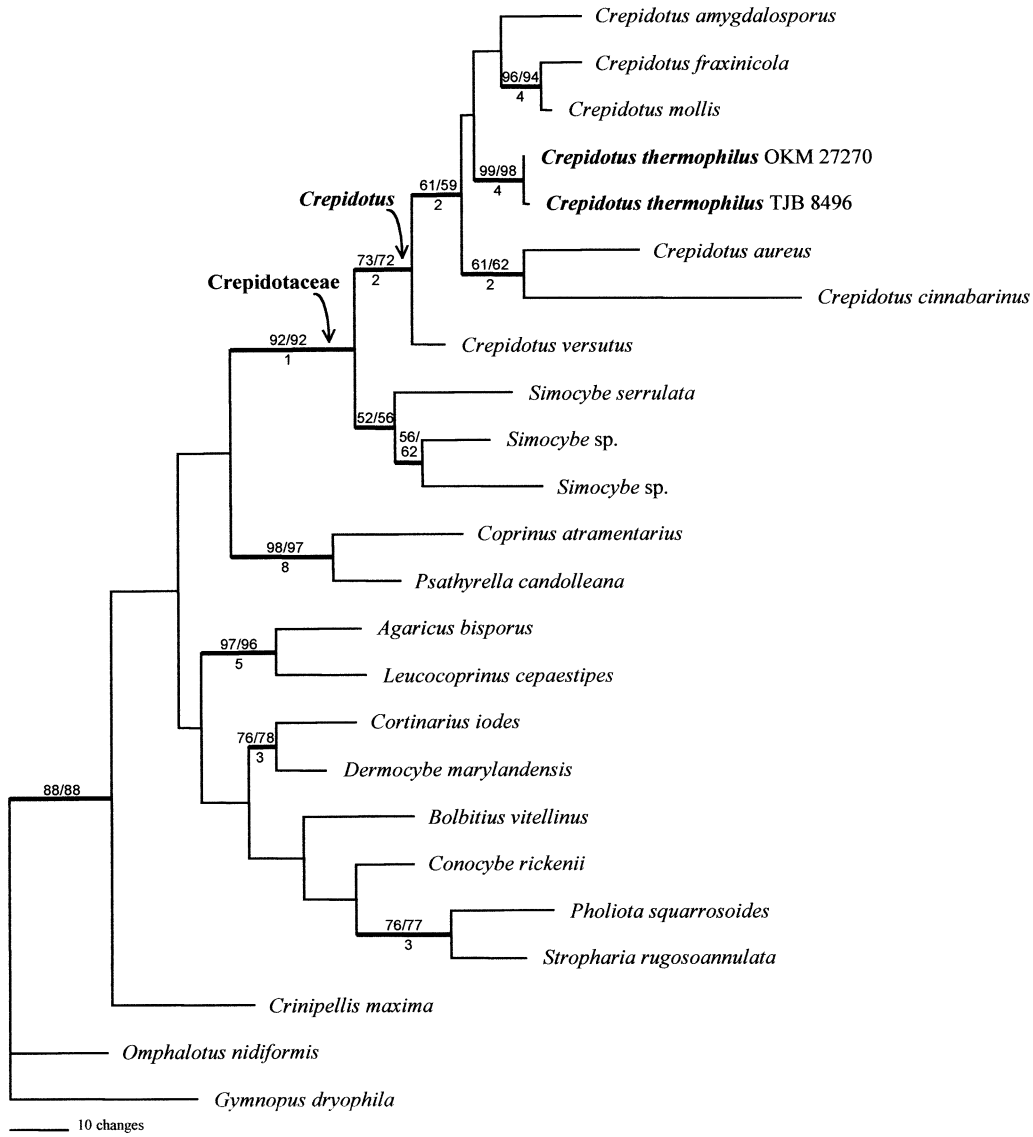


FIG. 1. Phylogenetic assignment of *Crepidotus thermophilus* within *Crepidotus* (Fr.) Staude. Analysis based on a portion from the 5′-end of the nuclear DNA encoding the large ribosomal subunit. The first of six most parsimonious trees is depicted (length = 587, RI = 0.54, CI = 0.50). Branches with strong statistical support are indicated by a bold line. Bootstrapping values (1000 replicates) are given as first number above supported branch; jackknifing values (1000 replicates) follow; decay values are indicated below branch.

characters (including gaps), 139 of which were parsimony-informative. Parsimony analyses were performed using heuristic search algorithms with multiple (10) random sequence additions to generate starting trees, and tree-bisection-reconnection (TBR) branch-swapping. Bootstrapping frequencies (Hillis and Bull 1993) were calculated using TBR branch swapping with 1000 replicates; support of greater than 50% was considered significant. Jackknifing frequencies (Lanyon 1985) were calculated using TBR branch swapping with 1000 replicates; support of greater than 50% was considered significant. Decay values (Bremer 1988) were calculated with AutoDecay 4.0.1 (Eriksson 1998) in PAUP 3.1.1 (Swofford 1993).

RESULTS

Type examinations show our Puerto Rico collections to be identical to *Melanomphalia thermophila* (Sing.) Sing. To infer the natural position of this taxon within the Crepidotaceae, DNA was isolated and sequenced from two Puerto Rican collections. Analysis of nLSU sequences, within both an extensive dataset of over 154 agaric taxa (Moncalvo et al 2000, dataset available at http://www.botany.duke.eeu/fungi/mycolab), and within the pruned dataset presented (FIG. 1) show this taxon to be a component of *Crepidotus*. This species

FIG. 2. *Crepidotus thermophilus* TJB 8496. Basidiomata.

is described and illustrated, and a new combination proposed as follows.

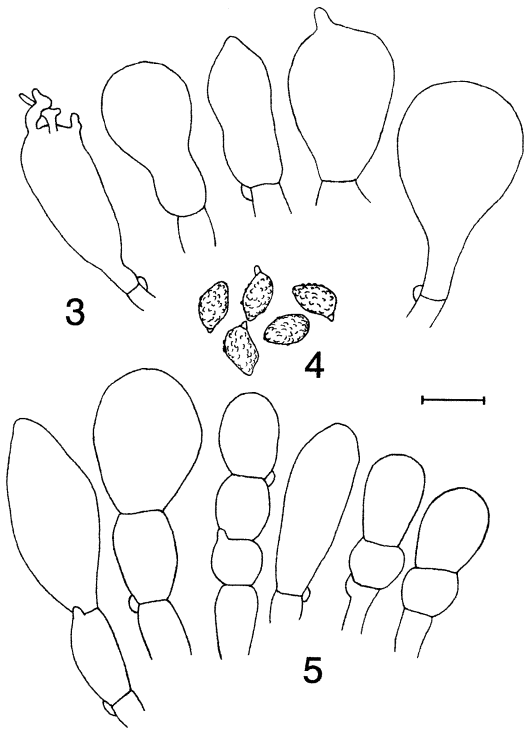
TAXONOMY

Crepidotus thermophilus (Sing.) Aime, Baroni, et O.K. Miller, comb. nov. FIGS. 2–13

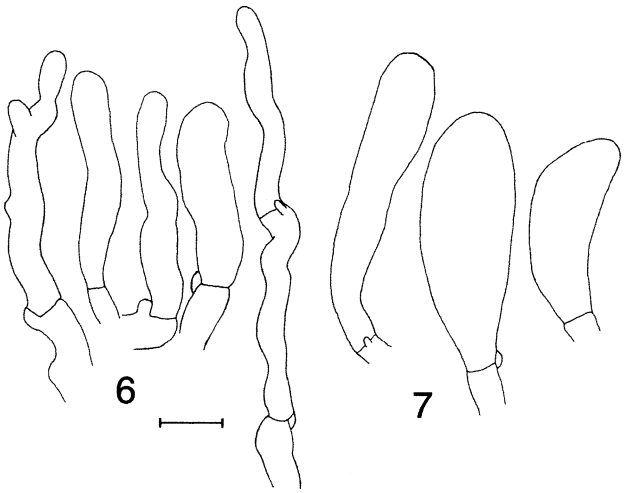
≡ *Tubaria thermophila* Sing., Papers Mich. Acad. Sci., Arts & Letters 32:145. 1948.

≡ *Melanomphalia thermophila* (Sing.) Sing., Atas Instituto de Micologia 5:481. 1967.

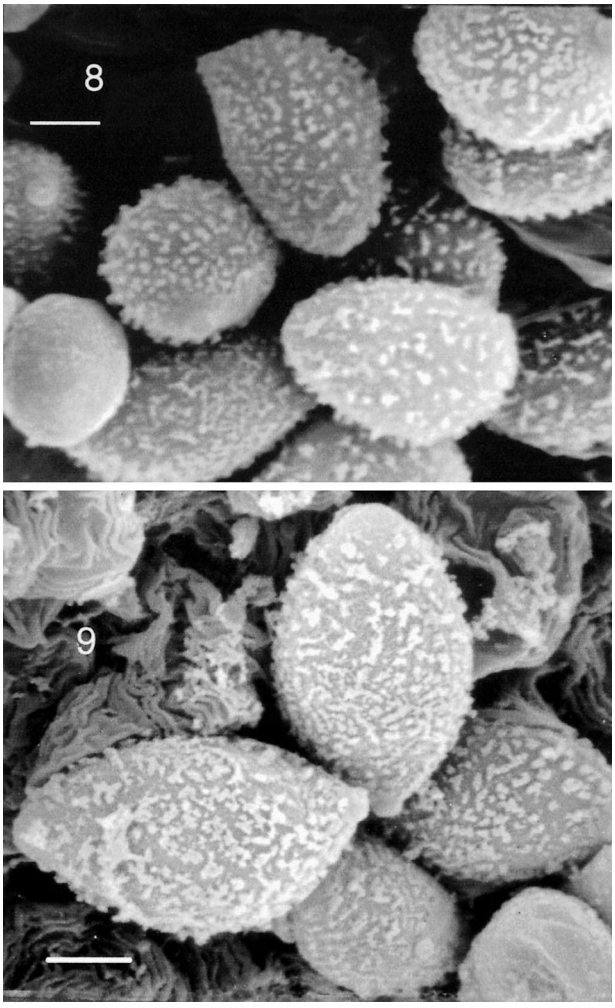
Pileus (FIG. 2) a deep rich reddish brown (6D5-7 to 7D8—Sienna, Brick Red or Terra Cotta) slightly fading with age and expansion to Cinnamon Brown (6D6), 20–50 mm broad, convex becoming broadly convex, occasionally broadly umbonate, then plane, eventually uplifted, undulate and incised around the margin with age, moist or dry, becoming appressed fibrillose squamulose with age. *Lamellae* pale tan (5A2 to 5B3—Orange White or Greyish Orange), short decurrent, close to crowded (2–3 tiers of lamellulae), narrow (up to 2 mm), edges concolorous or slightly paler and fimbriate. *Stipe* pale creamy white (4A2–3—Yellowish White or Cream), 1.5–4.0 mm wide at apex, 20–35 mm long, equal, terete, often flexuous, central or very slightly eccentric, glabrous except for white fibrillose-pruinose apex, white mycelioid or strigose covering at base, solid and white context. *Odor* and *Taste* not distinctive. *Spore deposit* light reddish-brown. *Basidiospores* (FIGS. 4, 8–9, 13) 7.0–10.7 × (4–)4.8–5.9(–6.3) μm (n/6 = 136, L^m = 8.7 ± 0.83, W^m = 5.4 ± 0.38, Q = 1.29–2.12, Q^m = 1.62 ± 0.17; HOLOTYPE n = 21, 7.9–10.7 × 4.5–6, L^m = 9.6 ± 0.69, W^m = 5.3 ± 0.37, Q = 1.56–2.05, Q^m = 1.82 ± 0.14), amygdaliform in profile view, broadly fusiform-elliptical in face view, round in polar view, verrucose, yellow brown in 3% KOH. *Basidia* mostly 2-sterigmate, some 1 or 4 sterigmate, clavate, 17.8–24.3 × 6.4–8.0 μm. *Cheilocystidia* (FIGS. 3, 10) abundant, hyaline, versiform, but mostly inflated, clavate, obpyriform, sphaeropedunculate, or broadly fusiform, some cells with apical digiform projections, 22–44 × 11–23 μm. *Pleurocystidia* absent. *Lamella trama* composed of a ± parallel, hyaline, cylindric or mostly inflated hyphae, (3.2) 8–17 μm in diam. *Pileus context* a hyaline layer of loosely interwoven, cylindric or slightly inflated, frequently branched hyphae, 4–



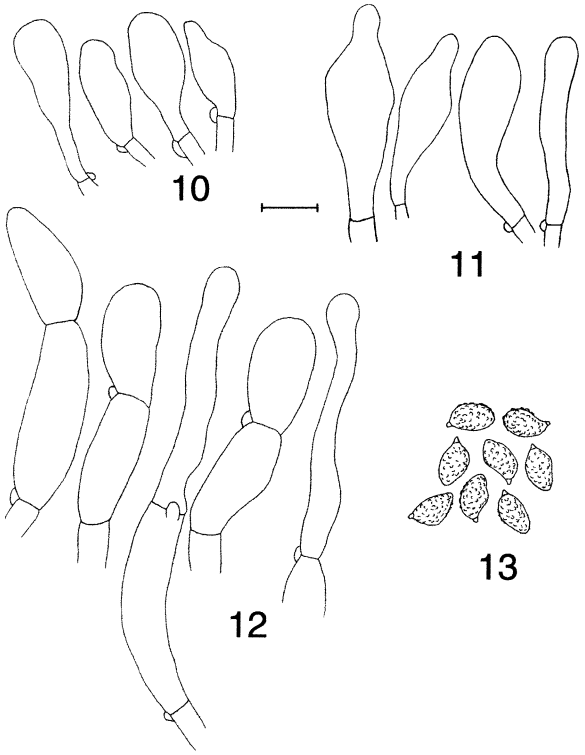
FIGS. 3–5. *Crepidotus thermophilus* TJB 8496. 3. Cheilocystidia. 4. Basidiospores. 5. Caulocystidia. Scale bar = 10 μm.



FIGS. 6–7. *Crepidotus thermophilus* Ovrebo 3808. 6. Caulocystidia from young basidiomata. 7. Caulocystidia from mature basidiomata. Scale bar = 10 μm.



FIGS. 8–9. *Crepidotus thermophilus* scanning electron micrographs of basidiospores. 8. Basidiospores of TJB 8496. 9. Basidiospores of Singer F20 (Syntype). Scale bars equal 2.5 μm.



FIGS. 10–13. *Tubaria thermophila*. 10. Cheilocystidia Singer F20 (Syntype). 11. Caulocystidia Singer F20/III. 12. Pilocystidia Singer F20 (Syntype). 13. Basidiospores Singer F20 (Syntype). Scale bar = 10 μm.

14 μm in diam. *Pileipellis* a rich yellow brown layer of loosely entangled, cylindric or slightly inflated hyphae, 6–18 μm in diam, producing ascendant versiform pilocystidiate end cells (FIG. 12), cylindrical or slightly inflated or some tapered fusoid, 30–120 × 6–18 μm, all cells with dense dark yellow brown vacuolar pigments. *Stipitipellis* a dingy yellowish brown layer of repent, cylindric hyphae, 2.4–5.6 μm in diam, with abundant versiform *caulocystidia* (FIGS. 5–7, 11) at apex, quite variable, on young specimens mostly cylindrical-contorted, narrowly fusoid or narrowly clavate, on mature specimens mostly composed of clusters of inflated clavate or spherical end cells, 14.6–46 × 9.7–18 μm, sometimes with these spherical end cells sitting atop chains of 2–4 swollen spherical subtending cells. *Clamp connections* present in all tissues.

Habitat, habit, fruiting period. Scattered in leaf litter, soil, sandy soil, or on well decomposed woody debris in subtropical moist and borderline wet forests at low elevations. May through August and January. Known from Puerto Rico, Florida, Brazil, and Panama.

Specimens examined. PANAMA. PROVINCE OF PANAMA: Barro Colorado Island, Gatun Lake, W.M. Wheeler Trail, 14 May 2000, *C.L. Ovrebo 3808* (CSU). PUERTO RICO. MUNICIPIO LUQUILLO: between Luquillo and Sabana, off of Rt. 991, above Rio Sabana and a private chicken farm, N 18° 21′ 3.4″, W 65° 43′ 50″, approx. 70 m elev., 7 June 1997, *T.J. Baroni 8496* (CORT); same local, 14 Nov. 1996, *T.J. Baroni 8309* (CORT); same local, 15 January 1998, *O.K. Miller, Jr. 27270* (VPI); Luquillo Mts., CNF, near Sabana in borderline subtropical wet forest, 18° 19′ 34″, W 65° 43′ 22″, 14 July 1998, collected by J. Mercado, comm. *S. A. Cantrell PR4887* (NY). UNITED STATES. FLORIDA: Highlands Co., near Seabring, Highlands Hammock State Park, 2 August 1942, *R. Singer F 20* (SYNTYPE, FH); same local, August 1942, *R. Singer F 20/III* (''authentic'', FH).

DISCUSSION

Crepidotus thermophilus is recognized in the field by the combination of its reddish brown subtomentose

to fibrillose-punctate pileus surface, decurrent tan lamellae, pale creamy-white central stipe, and light reddish-brown spore deposit. Although the vast majority of described *Crepidotus* species are pleurotoid in form, the generic definition does not exclude taxa with a well-developed stipe. One other species of *Crepidotus* possesses a prominent stipe, *C. nyssicola* (Murr.) Sing. (Hesler and Smith 1965, Bigelow 1980). *Crepidotus nyssicola* lacks pigmentation in the pileus, has globose, echinulate spores and is known from temperate North America. *Crepidotus thermophilus* is differentiated by its amygdaliform, verrucose basidiospores and a Neotropical distribution. The inflated cheilocystidia and 2-sterigmate basidia of *C. thermophilus* are also distinctive for this species. Singer (1971) previously had reported this species from Florida in the United States and from Pernambuco in Brazil. We now have documented further collections from Puerto Rico and Barro Colorado Island in Panama. We suspect this rarely collected and apparently saprotrophic agaric will eventually be found widely distributed in subtropical and tropical habitats throughout the Caribbean basin and in the northern areas of South America.

Much of Singer's evolving concept of the Crepidotaceae was based on similarities in exosporial ornamentation between *Tubaria* (later *Melanomphalia*) *thermophila* and *Crepidotus* Sect. *Echinosporae*. Not surprisingly, basidiospore ornamentation in *C. thermophilus* as revealed by SEM (FIGS. 8–9) falls well within the range of variation for *Crepidotus*, consisting of low, distinct, verruculae, intermediate between that found in *C. variabilis* (Fr.) Kumm. (in Pegler and Young 1972) and *C. subverrucisporus* (Pilát) (in Senn-Irlet 1993). Previous work has suggested that the nature of exosporial ornamentation combined with the shape of basidiospores may be the best phenotypic indicator of phylogenetic relationships in the Crepidotaceae (Aime 1999). Significantly, neither basidiospore ornamentation nor shape in *C. thermophilus* share similarities with those published for the generic type of *Tubaria*, *T. furfuracea* (Pers. ex Fr.) Gill. (in Clémençon 1977), or with *M. nigrescens* (in Horak 1968, Montag 1996), the type species of *Melanomphalia*. Fresh material of *M. nigrescens* was not available for DNA analysis.

This study has raised questions regarding the natural phylogenetic affinities of both *Tubaria* and *Melanomphalia* that we are currently working to address. In addition, we are re-examining other species currently placed in *Melanomphalia* to determine whether they also belong in *Crepidotus*. If and when these taxa are transferred it would increase the number of species with well-developed central stipes currently placed in *Crepidotus*.

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