Phellinus caribaeo-quercicolus sp. nov., parasitic on Quercus cubana: taxonomy and preliminary phylogenetic relationships

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Abstract: Phellinus caribaeo-quercicolus sp. nov. is described from several collections made in western Cuba, so far exclusively on *Quercus cubana*. The species is characterized by a perennial, resupinate basidiomes, cushion-shaped to nodulose and multilayered when old, apically hooked to hamate hymenial setae, and ellipsoid to broadly ellipsoid, thinto thick-walled, hyaline to faintly yellowish basidiospores, $4.5-5.5 \times 3.5-4.5 \,\mu m$. The species is compared to other *Phellinus* species with hooked setae, especially *Phellinus undulatus*, also recorded in Cuba. The preliminary phylogenetic relationships of *Ph. caribaeo-quercicolus* within the poroid Hymenochaetales complex of genera is presented and discussed here.

Key words: neotropics, taxonomy, Phellinus undulates, phylogeny, xanthochroic polypores

INTRODUCTION

Poroid Hymenochaetales have been researched extensively in Cuba, mainly by Herrera Figueroa and Bondartseva, resulting in a series of studies dealing with the taxonomy and local biogeography of this complex group (Herrera Figueroa 1979; Herrera Figueroa and Bondartseva 1982, 1985; Bondartseva and Herrera Figueroa 1977, 1980a, b, 1981; Bondartseva et al 1992). Fifty-three species from seven genera

(*Phylloporia* Murrill, *Inonotus* P. Karst., *Coltricia* S.F. Gray, *Coltriciella* Murrill, *Onnia* P. Karst., *Cyclomyces* Kze and *Phellinus* Quél.) then were recorded (Herrera and Bondartseva 1985).

At the time however several collections could not be satisfactorily attributed to any existing species concept, and although they were thought to represent undescribed species this remained unpublished, mainly because of the paucity of available material (Herrera Figueroa 1979). Other collections were referred to species concepts that since have been challenged and are in need of revision.

As a continuation of this taxonomical and biogeographical survey, several new field trips yielded new materials and pure cultures of some of the poorly defined taxa, and a better characterization was obtained. Among them, samples of a Phellinus s.l. species left unnamed by Herrera Figueroa (1979) were collected several times and critically studied but could not be satisfactorily placed in any of the known taxa from the area (Ryvarden 2004), North America (Gilbertson and Ryvarden 1987), or elsewhere (Corner 1991, Dai 1999, Gilbertson and Ryvarden 1987, Larsen and Cobb-Poulle 1990, Ryvarden and Gilbertson 1994, Ryvarden and Johansen 1980). These collections were characterized by a resupinate, perennial basidiome; often thick with up to 12 tubes layers, then strongly cushion-shaped to nodulose and with the margin indurate (FIGS. 4, 5); hymenial setae apically hooked to very commonly hamate (Fig. 2); basidiospores ellipsoid to broadly ellipsoid, thin- to thick-walled, hyaline to faintly yellow, averaging $5.1 \times$ 3.8 µm (Fig. 1). From an ecological point of view this taxon so far has been found growing almost exclusively on living Quercus cubana A. Rich (Fagaceae) in the Western Province.

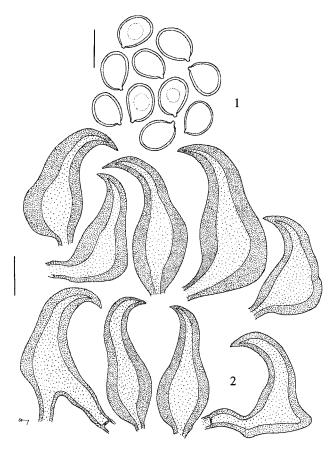
Herrera Figueroa (1979) compared this species to *Phellinus caryophilleus* (Cooke) Ryvarden, a rare South American species, known exclusively from the type specimen in Brazil (Lowe 1957, Larsen and Cobb-Poulle 1991, Ryvarden 2004) and with scattered apically hooked hymenial setae.

The macro- and micromorphology of the present taxon also prompted us to consider a relationship to the similar *Phellinus undulatus* (Murrill) Ryvarden (David and Rajchenberg 1985, Larsen and Cobb-Poulle 1990, Loguercio-Leite and Wright 1995, Ryvarden 2004, Ryvarden and Johansen 1980), a neotropical species also occurring in Cuba (Bondartseva

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FIGS. 1, 2. *Phellinus caribaeo-quercicolus*, MUCL 46004 (Holotype). 1. Basidiospores. 2. Hymenial setae. Bar = $5 \mu m$.

and Herrera Figueroa 1992, Herrera Figueroa 1979, Ryvarden 2004, pers obs).

Several other neotropical Phellinus s.l. species have similar curved or hooked hymenial setae and ellipsoid, hyaline to yellowish basidiospores and could be confused with the unidentified taxon. They are Ph. anchietanus Decock & Ryvarden, Ph. uncinatus Rajchenb., Ph. wahlbergii (Fr.) Reid (presently known as Fuscoporia wahlbergii (Fr.) T. Wagner & M. Fisch., Wagner and Fischer 2002), Ph. setulosus (Lloyd) Imazeki, Ph. uncisetus Robledo et al or still even Ph. semihispidus Ryvarden (Corner 1991, Cunningham 1965, Decock and Ryvarden 1997, Larsen and Cobb-Poulle 1990, Loguercio-Leite and Wright 1995, Rajchenberg 1987, Reid 1975, Robledo et al 2003, Ryvarden 2004, Ryvarden and Johansen 1980). However all these taxa differ significantly from our collections in several either macro- and/or microscopic features.

Phellinus caribaeo-quercicolus sp. nov. is described and compared to the above mentioned taxa. Its preliminary phylogenetic relationships with some other poroid Hymenochaetales with apically curved hymenial setae are inferred from a parsimony analysis based on partial nuclear ribosomal large subunit sequence data. The results are presented and discussed.



FIGS. 3–5. Basidiomes of *Phellinus caribaeo-quercicolus*. 3. Young basidiome (arrow) developing in wound of a living Q. cubana, MUCL 47108; 4, 5. Mature basidiomes on a living tree (No. 4, MUCL 47093) or dead stump (No. 5, MUCL 47110) of Q. cubana. Bars: 3 = 600 mm; 4-5 = 250 mm.

MATERIALS AND METHODS

Herbarium specimens are preserved at HAC, HAJB and MUCL (Holmgren et al 1990). Strains used in this study are preserved at BCCM/MUCL and in the Cuban Coleccion de Recursos Geneticos Fungicos (CRGF). Specimens were examined in Melzer's reagent, lactic acid cotton blue (Kirk et al 2001) and KOH 4%. Colors are described according to Kornerup and Wanscher (1981). All microscopic measurements were performed in Melzer's reagent. In presenting the size range of the microscopic elements 5% of the measurements were excluded at each end and are given in parentheses, when relevant. $\bar{\mathbf{x}}=$ arithmetic mean, $\mathbf{R}=$ the ratio of length/width of basidiospores and $\bar{\mathbf{x}}_{R}=$ arithmetic mean of the ratio R.

Sequencing.—DNA was extracted from freshly collected mycelium grown in malt broth at 25 C in the dark. Extractions were carried out with QIAGEN Dneasy Plant Mini Kit (QIAGEN Inc.), and purified with Geneclean® III kit (Q-Biogene), following the manufacturer's recommendations. The primer pair LROR-LR6 (White et al 1990) was used to amplify the 5' end of the nr LSU DNA regions. Successful PCR reactions resulted in a single band observed on a 0.8% agarose gel, corresponding to approximately 1200 bps. Polymerase chain reaction products were cleaned with the QIAquick® PCR purification kit (250) (QIAGEN Inc.), following the manufacturer's protocol. Sequencing reactions were performed with CEQ DTCS Quick Start Kit® (Beckman Coulter), according to the manufacturer's recommendations, with the primers LROR, LR3, LR3R, LR5 (biology.duke.edu/fungi/mycology/primers). Nucleotide sequences were determined with a CEQ 2000 XL capillary automated sequencer (Beckman Coulter). Initially nucleotide sequences were automatically aligned with Clustal X for Macintosh (version 1.5b), then manually adjusted as necessary with the editor in PAUP* (version 4.0b10).

The final dataset comprised 43 sequences (43 taxa) and 917 characters, including gaps. Three small inserts were present in both Coltricia species (5 and 3 nucleotides) and in the sequence of *Pseudoinonotus dryadeus* (5 nucleotides) and each were recoded as a single event. Phylogenetic analysis of the aligned sequences was performed with the maximum parsimony method of PAUP* version 4.0b10 (Swofford 2002) with gaps treated as fifth base. The most parsimonious trees were identified with heuristic searches with random addition sequence (1000), MAXTREES set to 100, and further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority rules in the bootstrap consensus tree. Analysis conditions were tree bisection addition branch swapping (TBR), starting tree obtained via stepwise addition, steepest descent not in effect, MULTREES effective.

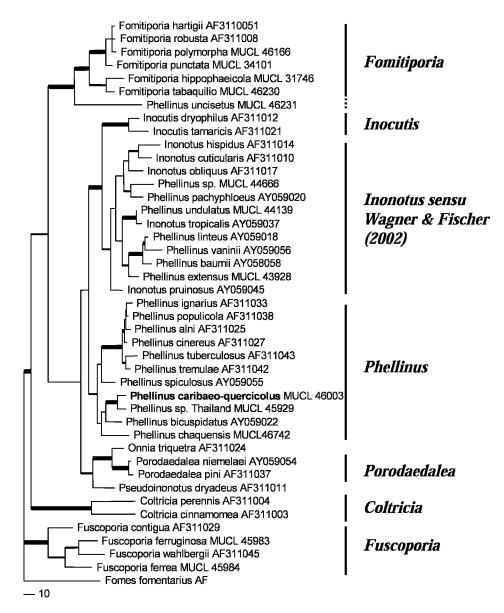
Sequences used.—Coltricia cinnamomea (Jacq.) Murrill, AF311003; C. perennis (L.) Murrill, AF311004; Fomes fomentarius (L.) J.J. Kickx, AF261538; F. hartigii (Allesch. & Schnabl.) Fiasson & Niemelä, AF3110051; F. hippophaeicola (H. Jahn) Fiasson & Niemelä, MUCL 31746 =

AY 618207; F. punctata (Fr.: P. Karst.) Murrill, MUCL 34101 = AY 618200; F. polymorpha M. Fisch., MUCL 46166 = DQ122393; F. robusta (P. Karst.) Fiasson & Niemelä, AF311008; Fuscoporia contigua (Pers.) G. Cunn., AF311029; F. ferrea (Pers.) G. Cunn, MUCL 45984 = DQ122398; F. ferruginosa (Schrad.) Murrill, MUCL 45983 = DQ122397; F. wahlbergii (Fries) T. Wagner & M. Fisch., AF311045; *Inocutis dryophila* (Berk.) Fiasson & Niemelä, AF311012; I. tamaricis (Pat.) Fiasson & Niemelä, AF311021; Inonotus cuticularis, AF311010; I. hispidus (Bolton) P. Karst., AF311014; I. obliquus (Fr.) Pilát, AF311017; I. pachyphloeus (Pat.) T. Wagner & M. Fisch., AY059020; I. pruinosus, AY059045; I. tropicalis (M. Lars. & Lomb.) T. Wagner & M. Fisch., AY059037; Onnia triquetra (Pers.) Imazeki, AF311024; Phellinus alni (Bond.) Parm., AF311025; Ph. baumii Pilát, AY058058; Ph. bicuspidatus Lombard & M.J. Larsen, AY059022; Ph. caribaeo-quercicolus Decock & S. Herrera, MUCL 46003 = DQ127279; MUCL 46004 = DQ127280; MUCL 46005 = DQ127281; MUCL 46011 = DQ127282; Ph. chaquensis (Iaconis & Wright) Wright & Desch. MUCL 46742 = DQ122396; Ph. cinereus (Niem.) M. Fisch., AF311027; Ph. extensus (Lev.) Pat. MUCL 43928 = DQ349099; Ph. ignarius (L.: Fr.) Quél., AF311033; Ph. linteus (Berk. & Curt.) Teng, AY059018; Ph. populicola Niemelä, AF311038; Ph. spiculosus (Campb. & Davids.) Niem., AY059055; Ph. uncisetus Robledo et al MUCL 46231 = DQ122395; Ph. undulatus (Murrill) Ryvarden MUCL = 44114 = DQ131562; MUCL 44139 = DQ131461; Phellinus sp., MUCL 44666 = DQ131563; Phellinus sp., MUCL 45929 = DQ127283; Phellinus vaninii Ljub., AY059056; Ph. tabaquilio Urcelay et al MUCL 46230 = DQ122394; Ph. tremulae (Bondartsev) Bondartsev & Borisov, AF311042; Ph. tuberculosus (Baumg.) Niemelä, AF311043; Porodaedalea niemelaei M. Fisch., AY059054; Po. pini AF311024; Pseudoinonotus dryadeus (Pers.: Fr.) T. Wagner & M. Fisch., AF311011.

RESULTS

The four recent collections of Ph. caribaeo-quercicolus all had identical sequences and just one sequence per species was maintained for the analysis, idem for the two collections of Ph. undulatus. A heuristic search with 1000 random additions produced 20 equally parsimonious trees, 1019 steps in length (CI = 0.489, RI = 0.324).

The topologies of the trees regarding the recovery and the relative position of different major generic entities within the poroid Hymenochaetales were in accordance with some works (Decock et al 2005; Wagner and Fischer 2001, 2002). In particular the Fomitiporia Murrill clade (bootstrap value 90%), the Fuscoporia Murrill clade (bootstrap value 88%), the Phellinus Quél. clade (bootstrap 60%), and the larger clade (bootstrap 84%) containing Inocutis Fiasson & Niemelä (bootstrap 100%) and Inonotus sensu Wagner and Fischer (bootstrap value 65%) were

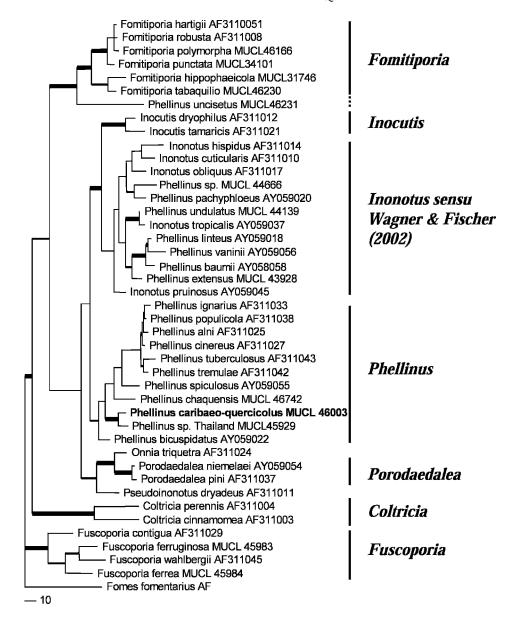


FIGS. 6, 7. Two EPTs (see text for explanation). Branches in bold are supported by bootstrap value greater than 80% (thick) or 60–79% (moderately thick). Bootstrap values smaller than 60 are not highlighted.

supported. Two of the 20 EPTs are represented (Figs. 6, 7).

The unidentified taxon phylogenetically is more closely related to the *Ph. ignarius* group than to any other alliances within the poroid Hymenochaetales, this group forming a moderately supported clade (bootstrap 65%). Within this clade, among the 20 EPTs, two main topologies concerning our species were recovered. In both topologies the Cuban species is closely related to an unidentified collection originating from Thailand (CHIANG MAI PROV.: Doi Suthep, Huai Kok Ma, 18°48′[62]N, 098°54′[60]E, on a large, dead, fallen trunk of an unidentified angiosperm, covering a large surface

below the trunk, etc., 30 Jul 2004, C. Decock and Th-04/121, MUCL 45929 [duplicata in herb. BIOTECH, Bangkok, Thailand]), both taxa forming a well supported clade (bootstrap 99%). In the less frequent topology (Fig. 6., five out of the 20 EPTs and also present in the bootstrap consensus tree) this two-species clade is included in a larger one containing also *Ph. bicuspidatus* Lombard & M.J. Larsen (Lombard and Larsen 1985) and *Ph. chaquensis* (Iaconis & Wright) Wright & Desch. (Iaconis and Wright 1953). However this four-species clade is not supported (bootstrap value 15%). In the second topology (Fig. 7, 12 out of the 20 EPTs), the *Ph. caribaeo-quercicolus* clade remains isolated.



Figs. 6, 7. Continued

TAXONOMY

Phellinus caribaeo-quercicolus Decock et S. Herrera, sp. nov. FIGS. 1–5

Fructificatio resupinata, effusa vel pulvinata usque noduliformis et ad maturitatem multistrata, tum pseudopileum denigricans adnexum; pororum facies umbrina plerumque griseo-fusca; pori rotundi, (5–)6–7/mm, (100–) 100–175 (–200) µm; contextus tenuissimus; tubis pluristratis, 8 usque 60 mm crassis; systema hypharum dimiticum, hyphae generatoriae hyalinae ad pallide luteae, afibulatae, hyphae skeletales flavo-brunneae, crassitunicatae, aseptatae, (2.5–)2.7–3.5(–3.8 µm) diam ($\bar{\mathbf{x}}=3.1~\mu\mathrm{m}$); setae hymeniales ferrugineo-fuscae, ventricosae, apice acutae, rectae aut saepe curvatae vel hamatae, (15.0–)15.0–28.0(–34.5) × (5.3–)6.0–11.0(–11.0) µm, ($\bar{\mathbf{x}}=21.5~\times~7.7~\mu\mathrm{m}$); basidiosporae ellipsoideae, leviter crassitunicatae, hyalinae vel

pallide luteae, (4.5–)4.5–5.7(–6.0) \times (3.5–)3.5–4.3(–4.7) μ m ($\bar{x}=5.2\times3.9~\mu$ m); cystidiola et chlamydosporae nullae.

Basidiomes perennial, separable to adnate, resupinate, either effused following the substrate, or cushion-shaped, becoming with age strongly cushion-shaped to nodulose, 70–150 mm long, 40–130 mm wide, from 8 mm up to 60 mm thick in the thickest part of strongly multilayered specimens; when growing on a vertical substrate, the upper edge develops in old multilayered specimens a pseudo pileus by the marginal accumulation of tubes layers, whose upper surface indurates and forms a hard crust, up to 500 μm thick, that turn dark grayish brown to grayish black to black (dark brown in section), slightly concentrically sulcate, and finally rimose, sometime greenish due to algal development;

consistency hard, woody; texture densely fibrous, basidiomes easily broken into pieces when dried, especially between tube layers; margin up to 0.5 mm wide, densely and minutely velutinous with short, densely packed erected hyphae, white to pale yellow at the very margin, soon turning rusty brown (6[D-E]6, 6D[6-8] [cinnamon, dead leaf, up to rust]); pore surface in shade of brown, commonly (grayish) chocolate brown (6E[5-6], leather, cocoa brown), slightly glancing with light, then light brown, grayish light brown (6D[5–6], sunburn to cinnamon), grayish chocolate brown (6E4), weathering to more grayish (6E[3-4] up to 7F3, grayish brown); pores round, ellipsoid when oblique, (5-)6-7/mm, (100-)100- $175(-200) \, \mu \text{m} \, \text{diam} \, (\bar{x} = 136 \, \mu \text{m}); \, \text{dissepiments}$ entire, thin, slightly wavy on elongated portion; subiculum thin to almost absent, negligible compared to the tube layers, concolorous with the older tube layer; tubes multilayered, with up to 12 individual, usually clearly distinct layers, each 3-5 mm thick, and totaling up to 60 mm thick, in some specimens, the individual layers easily separated when drying; tube layer in shade of brown, the older layers (grayish) light brown (6D[5-6], sunburn to camel, 7D8, burnt Sienna), variably mottled with white streaks made of hyphae filling the old tubes, and contrasting with the last, younger tube layer which is darker brown (6[E-F]6, cocoa brown to burnt amber); hyphal system dimitic, identical in the context and hymenophoral trama, the skeletal hyphae having a subparallel orientation in the latter; *generative hyphae* hyaline to faintly yellowish, thin-walled, slightly branched, 2–3 μm wide, skeletal hyphae densely packed, yellowish brown, thickwalled, but with the lumen open but narrow, (2.5-) $2.7-3.5(-3.8 \mu m)$ diam ($\bar{x} = 3.1 \mu m$); hymenial setae present, in variable abundance, both within and between specimens, commonly ventricose, occasionally with a hyphal-like base, or with a backward process, the apex pointed, acute, commonly curved, slightly to strongly hamate, rarely straight, (15.0-)15.0-28.0 $(-34.5) \times (5.3-)6.0-11.0(-11.0) \mu m, (\bar{x} = 21.5 \times 1.5)$ 7.7 µm); cystidioles absent; basidioles subglobose to barrel-shaped; basidia barrel-shaped, $7.5-8.5 \times 4.5-$ 6.5 µm, with four small sterigmata; basidiospores ellipsoid to broadly ellipsoid, thin-walled and hyaline when young, but thick-walled and hyaline to faintly yellowish when mature, with 0-1 gutta, negative in Melzer's reagent, $(4.5-)4.5-5.7(-6.0) \times (3.5-)3.5 4.3(-4.7) \mu \text{m} \ (\bar{x} = 5.2 \times 3.9 \mu \text{m}), R = (1.2-)1.2-$ 1.4(-1.6) ($\bar{x}r = 1.3$); basidiospores print pale yellowish (4A3); chlamydospore absent; substrate: usually known from living trunks or branches of Quercus cubana, identification of other host unknown; type of rot: a white rot; sexuality: unknown; geographic distribution: currently known exclusively in western Cuba.

HOLOTYPE: CUBA, PROVINCIA PINAR DEL RÍO: Municipio La Palma, in a mixed *Pinus caribaea/ Quercus cubana* forest, on a living trunk of *Quercus cubana*, about 2 m above soil, 9 Sep 2004, C. Decock, J. Ortiz and R. Carbonell Paneque, CU-04/120, MUCL 46004, HAC (culture ex-holotype MUCL 46004 = CRGF 347).

PARATYPE SPECIMENS: CUBA, PROVINCIA PI-NAR DEL RÍO: Municipio La Palma, Encinar del Caimito, el Burén, in a mixed Pinus caribaea/Quercus cubana forest, on a living trunk of Q. cubana, about 2.5 m high, 9 Sep 2004, C. Decock, J. Ortiz and R. Carbonell Paneque, CU-04/119, MUCL 46003 (culture ex-specimen MUCL 46003 = CRGF 346); in a hole at the base of a living trunk of Q. cubana, 9 Sep 2004, C. Decock, J. Ortiz and R. Carbonell Paneque, CU-04/121, MUCL 46005, HAC (culture ex-specimen MUCL 46005 = CRGF 348); on a large living trunk of Q. cubana, 1 Oct. 2005, C. Decock and R. Carbonell Paneque, CU-05/242, MUCL 47093 (culture exspecimen MUCL 47093 = CRGF 561); in a hole of dead stump of Q. cubana, 1 Oct 2005, C. Decock and R. Carbonell Paneque, CU-05/243, MUCL 47094 (culture ex-specimen MUCL 47094 = CRGF 562); Encinar La Jagua, on a living branch of Q. cubana, 2 Oct 2005, H. Maraite, CU-05/250, MUCL 47100 (culture ex-specimen MUCL 47100 = CRGF 563); 8 km from Consolación del Norte, in a dead fallen trunk, unidentified angiosperm, 28 Jun 1973, S. Herrera, J.L. Ortiz, G. González, HAC-Herrera 1387; west from La Palma, Consolación del Norte, in a Q. cubana forest, 6 Jun 1970, H. Kreisel, HAJB- Kreisel 2166; Municipio Viñales, Encinar de la Finca Lele, at the base of a living trunk of Q. cubana, 4 Oct 2005, C. Decock, CU-05/262, MUCL 47108 (culture ex-specimen MUCL 47108 = CRGF 564); on a dead stump of Q. cubana, 4 Oct 2005, C. Decock, CU-05/267, MUCL 47110 (culture ex-specimen MUCL 47110 = CRGF 565); CU-05/268, same data, MUCL 47111 (culture ex-specimen MUCL 47111 = CRGF 566); Municipio San Andrés, in a Q. cubana forest, in a hole at the base of a living trunk of Q. cubana, 10 Sep 2004, C. Decock, J. Ortiz and R. Carbonell Paneque, CU-04/ 132, MUCL 46011 (culture ex-specimen, MUCL 46011 = CRGF 350); south from Viñales, on living trunk of Q. cubana,16 Jun 1969, H. Kreisel, HAJB-Kreisel 1139.

Remarks. Phellinus caribaeo-quercicolus is characterized by perennial, resupinate, effused to cushion-shaped or nodulose basidiomes, thick to very thick and then distinctly multilayered when old and well developed, and with the marginal area becoming indurate, black and eventually, rimose (FIGS. 5–6), 6–7 pores/mm, commonly apically hooked to slightly or strongly hamate hymenial setae (FIG. 2),

and ellipsoid to broadly ellipsoid, thin- to thick-walled, hyaline to faintly yellowish basidiospores (Fig. 1), 4.5–5.5) \times 3.5– $4.0~\mu m$, averaging $5.1~\times$ $3.8~\mu m$. Apart from one specimen collected from an unidentified host, the species has been found growing only on living or dead *Quercus cubana* in either pure or mixed stands (then in association with *Pinus caribaea* Morelet) (Capote and Berazaín 1984) in western Cuba in an ecosystem characterized by severe drought in winter. The species grows preferably on trunk or branches of old trees or on dead stump.

The perennial basidiomes, the dimitic hyphal system, and the hyaline to faintly yellow, thin- to slightly thick-walled basidiospores justified the placement in the *Ph. ignarius* (L.:Fr.) Quél. alliance (Dai 1999, Wagner and Fischer 2002), a fact supported by our preliminary phylogenetic inference, in which the species nested within the moderately supported (bootstrap 60%) *Phellinus s.s.* clade, as Wagner and Fischer (2002) circumscribed it.

More specifically in our phylogenetic analysis (FIGS. 6, 7) Ph. caribaeo-quercicolus's closest relatives appears to be an, as yet, unidentified collection originating from northern Thailand (MUCL 45929), both taxa forming a well supported clade (bootstrap 99%). The Thai collection shares with Ph. caribaeoquercicolus a combination of macro- (perennial, resupinate basidiomes) and microscopic characteristics (apically hooked hymenial setae, and ellipsoid to broadly ellipsoid, slightly thick-walled, hyaline to faintly yellowish basidiospores), that also makes the two taxa morphologically closely related. The Thai collection differs in having a thinner and more widely effused basidiomes, more than 1 m long, thick-walled and uni- to more commonly bi- or trirooted hymenial setae, and slightly smaller basidiospores. Furthermore it occurs in humid, tropical mountainous forest in northern Thailand.

In our analysis this two-species clade appears either isolated (Fig. 7) or forming a larger one together with Ph. bicuspidatus and Ph. chaquensis (Fig. 6). However this latter clade is not supported by the analysis (bootstrap 15%). Phellinus bicuspidatus is a North American species with perennial resupinate basidiomes, hymenial setae, ellipsoid to broadly ellipsoid basidiospores, and a preference for living on Quercus sp. as substrate. It is morphologically distinguished by its hyaline basidiospores and nonhooked but commonly bicuspidate setae (Lombard and Larsen 1985). Phellinus chaquensis is a South American taxon, currently known only from xerophilous forests of the Chaco domain in northern Argentina (Cabrera 1976), where it seems to be endemic, and reported on living Schinopsis (Anacardiaceae) and Caesalpinia

(Fabaceae) (Iaconis and Wright 1953). The species is easily differentiated from our taxon by pileate basidiomes, with a thick crust becoming strongly rimose with age, hymenial setae with a straight, not hooked or curved apex, and more distinctly colored basidiospores (Iaconis and Wright 1953).

The affinities of Ph. caribaeo-quercicolus and of the Thai collection might be sought in the Ph. setulosus (Lloyd) Imazeki group (Corner 1991, Robledo et al 2003). The setal and basidiospores morphology of the Thai collection especially could be related to that group, to which as a consequence Ph. caribaeoquercicolus also might belong. Several authors have provided modern descriptions of Ph. setulosus (Corner 1991, Cunningham 1965, Quanten 1997, Robledo et al 2003, Ryvarden 2004, Ryvarden and Johansen 1980). The main features of the species are a pileate basidiome, a dimitic hyphal system, ventricose, thickwalled, apically curved to hamate hymenial setae, and subglobose, thin- to thick-walled, hyaline to faintly vellow basidiospores. Robledo et al (2003) on the basis of a revision of the type specimen emphasized a peculiarity not mentioned previously, which is the multi-, commonly bi- or trirooted setae. This peculiarity is identical in the Thai's collection, which could indicate a close relationship between the latter and Ph. setulosus. Both species then would differ in the basidiome habit (viz. respectively completely resupinate, without any sign of marginal pileus development, and pileate, and some additional biometric details). Phellinus caribaeo-quercicolus also differs from Ph. setulosus in having a resupinate habit, a slightly different setal morphology, mono- to occasionally birooted, and slightly larger basidiospores. However no sequence of Ph. setulosus is available at the moment thus impeding any phylogenetic (DNA based) inference of its relationships with these other taxa.

Some other neotropical *Phellinus s.l.* species characterized by curved to hooked hymenial setae also could be compared to *Ph. caribaeo-quercicolus*, although from a phylogenetic point of view they might belong to other alliances. They are *Ph. anchietanus* (Decock and Ryvarden 1997), *Ph. uncinatus* (Rajchenberg 1987) and *Ph. undulatus* (David and Rajchenberg 1985, Ryvarden 2004, Loguercio-Leite and Wright 1995), all three with resupinate basidiomes; *Ph. caryophilleus*, *F. wahlbergii* (Ryvarden 2004), *Ph. semihispidus* (Ryvarden 2004), having pileate basidiomes.

Herrera Figueroa (1979) tentatively referred to her collections of *Ph. caribaeo-quercicolus* as *Ph. caryophil-leus*. The latter species is known only from the type specimen that originated from Brazil (Ryvarden 2004). Although it shares apically hooked setae with

TABLE I. Basidiospores and hymenial setae size in various collections of Ph. caribaeo-quercicolus

	Basidiospores range	Average	Hymenial setae range	Average
MUCL 46003	Sterile	sterile	$17.0 - 35.0 \times 6.0 - 11.0$	24.4×8.4
MUCL 46004*	$(4.5-)4.8-5.5(-5.8) \times (3.5-)3.7-4.35(-4.3)$	5.3×3.9	$17.5 - 23.5 \times 6.0 - 8.2$	20.3×7.3
MUCL 46005	$(4.5-)4.7-5.8(-6.0) \times (3.5-)3.5-4.7(-4.7)$	5.4×4.1	$15.5 - 25.0 \times 5.5 - 11.0$	21.3×7.4
MUCL 46011*	$(4.5-)4.5-5.3(-5.3) \times (3.5-)3.5-4.1(-4.1)$	4.9×3.8	$15.0 - 23.0 \times 5.3 - 9.0$	18.1×7.2

^{*}Measurement taken from basidiospores print collected in nature.

Ph. caribaeo-quercicolus, Ph. caryophilleus is distinguished by having a pileate basidiome (applanate to conchate) and more globose, distinctly colored (yellow to rusty brown) basidiospores (Lowe 1957, Ryvarden 2004).

Phellinus anchietanus and Ph. undulatus both form resupinate basidiome. Phellinus anchietanus presently known from a single collection originating from southern Brazil has a thinner basidiome, additional extrahymenial (marginal or tramal) setae and smaller $(3.7-4.2 \times 3.0-3.8 \,\mu\text{m})$, subglobose basidiospores (Decock and Ryvarden 1997); these features deviate from Ph. caribaeo-quercicolus. Phellinus undulatus, also present in western Cuba (Bondartseva et al 1992, Ryvarden 2004, pers obs), has a thinner basidiome (<5 mm thick), larger pores, 4-6/mm, and faintly brown basidiospores that do not exceed 4.5 μm long and 3.5 μm wide (Loguercio-Leite and Wright 1995, Lowe 1966, Ryvarden 2004, Ryvarden and Johansen 1980, pers obs). Phellinus undulatus also has different ecological requirements and occurs in western Cuba in a wetter forest ecosystem and consequently on different hosts. So far the species has not been reported from Q. cubana or from the Q. cubana forest. Furthermore our phylogenetic studies demonstrate that its affinities lie in Inonotus sensu Wagner and Fischer (2002) and not with Phellinus s.s. This result supports a previous phylogenetic analysis based on the mit SSU sequence data and in which Ph. undulatus also was linked directly to Inonotus (more particularly I. obliquus [Fr.] Pilát, I. hispidus [Bolton] P. Karst., and I. quercustris M. Blackwell & Gilbn.) (Rizzo et al 2003).

Phellinus uncinatus Rajchenb. has subglobose to globose, thick-walled, and dextrinoid basidiospores (Rajchenberg 1987), which clearly would indicate affinities with a different alliance within the *Phellinus* complex of genera. These affinities should be sought, in all probability, in *Fomitiporia* Murrill. The latter genus is typically characterized by the same dextrinoid basidiospores (Decock et al 2005, Fischer 1996, Wagner and Fischer 2002, but see below).

Phellinus semihispidus and F. wahlbergii both have pileate basidiomes and thin-walled, hyaline basidiospores (Corner 1991, Cunningham 1965, Larsen and Cobb-Poulle 1990, Loguercio-Leite and Wright 1995, Reid 1975, Ryvarden and Johansen 1980, Ryvarden 2004), in which features they differ from our species. Furthermore *F. wahlbergii*, previously placed in *Phellinus*, was shown to belong to the *Fuscoporia* alliance. *Phellinus semihispidus* also might belong there.

Phellinus uncisetus also has a pileate basidiome, longer setae, of a different morphology, with a bulbous base and elongated apical part, and larger, more distinctly globose/subglobose basidiospores (6.0–7.0 \times 5.5–6.0 µm, Robledo et al 2003). Furthermore in our phylogenetic analysis *Ph. uncisetus*' closest relatives appear to be species of the *Fomitiporia* clade.

DISCUSSION

Phellinus caribaeo-quercicolus is described based on morphological features and molecular data. Its placement in *Phellinus s.s.* is supported by both morphological (e.g. dimitic hyphal system, thickwalled, hyaline to faintly yellow basidiospores) and molecular data, the species appearing in a phylogenetic analysis based on nuclear LSU, closer to the *Ph. ignarius* group than to any other generic alliance.

In Cuba *Ph. caribaeo-quercicolus* was found on living *Quercus cubana*, in pure and mixed forest. The species should be sought in other regions of the Caribbean where *Quercus* occurs.

The species so far is related to an unidentified collection (representing probably an undescribed taxon) from northern Thailand. Both taxa might be related to the *Ph. setulosus* group. However this hypothesis should be tested by inclusion of the latter species in a more complete phylogenetic analysis.

Phellinus undulatus, although having morphological similarities with Ph. caribaeo-quercicolus, nested within the Inonotus sensu Wagner and Fischer (2002) clade, where its closest relative so far appears to be I. tropicalis. Our studies thus support previous results that, although based on two independent genes, respectively nuc rDNA LSU (Wagner and Fischer 2002) and mit rDNA SSU (Rizzo et al 2003), affirmed the close relationships of I. tropicalis (Wagner and Fischer 2002) and Ph. undulatus (Rizzo et al 2003)

with *Inonotus sensu* Wagner and Fischer (2002). Hence this would support the transfer of *Ph. undulatus* into *Inonotus sensu* Wagner and Fischer (2002), but for the time being we think this transfer would be premature. This will be discussed separately.

Hooked to hamate hymenial setae are widespread over the poroid Hymenochaetales and their presence does not indicate any kind of phylogenetic relationships apart from those with closely related species in the species complex. Hooked to hamate hymenial setae are found for instance in *Inonotus sensu* Wagner and Fischer, *Inonotus* P. Karst. s.s. (Ryvarden and Gilbertson 1994), *Mensularia* Laz. (Gilbertson and Ryvarden 1987), *Phellinus* Quél. s.s., Fuscoporia Murrill and, in all probability, Fomitiporia Murrill. This morphological feature has arisen independently on several occasions.

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