

***Phellinus coronadensis*: a new species from southern Arizona, USA**

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Abstract: *Phellinus coronadensis* is characterized and described morphologically as a new species from southern Arizona, USA. This fungus was previously reported as *P. torulosus* based on morphological similarities of the basidiomes and type of wood decay. However, *P. coronadensis* is restricted to two mountain ranges in southern Arizona and found almost exclusively on living southwestern white pine (*Pinus strobiformis*). *Phellinus torulosus* is found primarily in Europe and parts of Asia and is primarily associated with hardwood hosts. Based on sequence analysis of small subunit mitochondrial ribosomal DNA (mt-SSU), we determined that *P. coronadensis* is in a different lineage from *P. torulosus* and apparently more closely related to the *P. Pini* complex. The taxon associated with southwestern white pine, being distinct and not yet having been validly named, is proposed as a new species here.

Key Words: *Phellinus*, wood decay fungi

INTRODUCTION

The use of gene trees has become increasingly important in identifying lineages and reproductively isolated populations of fungi (see reviews in Anderson and Kohn 1998, Harrington and Rizzo 1999). Such phylogenetic analyses can identify lineages where diagnosable phenotypic characters may be found that may help in species delimitation (Harrington and Rizzo 1999). This is particularly important when morphological characters are few or have apparently converged to the point where it is difficult to separate similar taxa. In addition, molecular phylogenetic analysis can be useful for testing whether species with disjunct ranges are within the same lineage even if

mating tests cannot be completed (Koufopanou et al 1997).

Such a case of a disjunct range has been reported for the wood-decay fungus, *Phellinus torulosus* (Pers.) Bourdot & Galzin (Phylum Basidiomycota, Family Hymenochaetaceae). This species is found in the warmer parts of Europe and northern Africa into the Caucasus and south of the Caspian Sea (Ryvarden and Johansen 1980, Parmasto 1985, Larsen and Cobb-Poulsen 1990, Fischer and Bresinsky 1992, Ryvarden and Gilbertson 1994). It also appears to range into Pakistan and northern India (Parmasto 1985). Reports of *P. torulosus* have also come from Japan and North America (Larsen and Cobb-Poulsen 1990). Gilbertson and Burdsall (1972) examined much of the material described from North America and determined that most collections were actually *Phellinus gilvus* (Schw.) Pat. However, they considered material from the Santa Catalina and Pinaleño Mountains of southern Arizona to be conspecific with European collections of *P. torulosus* (Gilbertson and Burdsall 1972). These Arizona collections were highly similar to the European material in morphological characters of the basidiomes and in the type of wood decay (white pocket rot), although some ecological differences were noted. In Europe, *P. torulosus* is primarily a root pathogen and saprobe on hardwood tree species and occasionally conifers (primarily *Cedrus* Trew or *Cupressus* L.). North American collections, on the other hand, were restricted to southwestern white pine (*Pinus strobiformis* Engl.), with one report from Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Gilbertson and Burdsall 1972).

As part of our ongoing molecular phylogenetic studies of the Hymenochaetaceae, we tested the hypothesis that *P. torulosus* from Europe and Arizona are conspecific. DNA sequences from the small subunit mitochondrial ribosomal locus (mt-SSU) were used to infer phylogenies across the family. Our results indicate that European and North American collections do not share a common ancestor and are, in fact, distantly related within the Hymenochaetaceae. Because of these findings and newly discovered morphological differences, we are describing the Arizona collections as a new species, *Phellinus coronadensis*.

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MATERIALS AND METHODS

Morphological studies.—Basidiomes of *P. torulosus* and *P. coronadensis* were hand-sectioned and examined microscopically in Melzer's solution, 2 % KOH (w/v) or water. Color descriptions are based on Kornerup and Wanscher (1981). All specimens are deposited in the Center for Forest Mycology Research (CFMR) Madison, Wisconsin. Additional specimens are deposited at the University of Arizona (ARIZ).

Molecular phylogenetic studies.—Species included in this study collection numbers, and accession numbers for mt-SSU sequences deposited in GenBank are listed below: *Trichaptum abietinum* (Dicks : Fr.) Ryv., SFC 960608–11, AF036632; *T. abietinum*, FPL 8973, U27078; *T. bifforme* (Fr.) Ryv., CBS 324.29, AF036635; *T. bifforme*, HHB-7316, AF036634; *Coltricia perennis* (Fr.) Murr., DSH 93-198, U27028; *Hydnochaete olivacea* (Schw. : Fr.) Banker, FP-102077, AF387552; *Hymenochaete arida* (P. Karst) Sacc., HHB-3683, AF387553; *H. badioferruginea* (Mont.) Lév., L-15559; AF387554; *H. Pinnatifida* Burt., FP-106761, AF387555; *H. rubiginosa* (Dicks : Fr.) Lév., HHB-17212, AF387557; *H. sprete* Peck, FP-104279, AF387556; *Hymenochaete* species, HHB-15827, AF387558; *Hymenochaete* species, PR-1480, AF387559; *Inonotus dryadeus* (Pers. : Fr.) Murr., JPL-544, AF387560; *I. Hispidus* (Bull. : Fr.) P. Karst, FPL 3597, U27044; *I. obliquus* (Pers. : Fr.) Pilát, RLG 3746, AF387561; *I. quercustris* Blackwell & Gilbn., RLG 14997, AF387562; *I. tomentosus* (Fr. : Fr.) S. Teng, (A allele) AF252892; *Phellinus coffeatorporus* Kotl. & Pouz., CRM 11, AF387563; *P. coronadensis*, AAB-1507, AF387564; *P. coronadensis*, AAB-1506, AF387565; *P. coronadensis*, RLC-9385, AF387566; *P. everhartii* (Ell. et Gall.) A. Ames, FP-71019, AF387567; *P. fastuosus* (Lév.) Ryv., L-13411, AF387568; *P. ferreus* (Pers.) Bourd. & Galz., HHB-12783, AF387569; *P. gilvus*, FPL 5528, U27060; *P. gilvus*, DAOM-94082, AF387570; *P. ipiarius* (L. : Fr.) Quél., FPL 5599, U27061; *P. ignarius*, TN-455, AF387571; *P. nigrolimitatus* (Rom.) Bourd. & Galz., FPL 135110, AF387572; *P. nigrolimitatus*, Colo-51–94, AF387573; *P. pini* (Thore : Fr.) A. Ames sensu lato, NM-6, AF387574; *P. pini sensu lato*, FP133110, AF387575; *P. pini sensu lato*, AZ-9, AF387576; *P. ralunensis* Adask., Gilbn., & Blanchette, JEA-1611, AF387577; *P. repandus* (Overh.) Gilbn., FPL 105605, AF387578; *P. robustus* (Karst.) Bourd. & Galz., FPL 106252, AF387579; *P. senex* (Nees et. Mont.) Imaz., Masuka 1029, AF387580; *P. senex*, US 1100791, AF387581; *P. senex*, Ryv. 27166, AF387582; *P. senex*, WD-842, AF387583; *P. senex*, RAB 97-5, AF387584; *P. senex*, HHB-15005, AF387585; *P. texanus* (Murr.) A. Ames, RLG 7775, AF387586; *P. torulosus*, DMR-IT1, AF387587; *P. torulosus*, RLG-14299, AF387588; *P. torulosus*, HHB-17211, AF387589; *P. torulosus*, US0348842, AF387590; *P. undulatus* (Murr.) Ryv., DMR 96–33, AF387591; *P. wahlbergii* (Fr.) D. Keid, PG-3, AF387592; *P. wahlbergii*, RAB 97–2, AF387593; *Phylloporia ribis* (Schum. : Fr.) Ryv., FPL 10677, U27065.

Except for those obtained directly from GenBank, sequences were determined from DNA extracted from living cultures or herbarium specimens that are on deposit in CFMR. Nucleic acids were isolated using a modification of

the method of Ceniz (1992). Cultured mycelia or fruiting body tissue was ground in extraction buffer (200 mM Tris, pH 8, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and 10% [v/v] glass beads. Cellular debris was precipitated with 3 M NaOAc, pH 5.2. Nucleic acids were precipitated from supernatant with an equal volume of isopropanol and then suspended in TE buffer.

PCR amplifications used 0.5–1 µL of unquantified DNA in 30 µL reactions with *Taq* DNA polymerase (2.5 U, Promega) and *Taq* Extender Additive (2.5U, Stratagene) or cloned *Pfu* DNA polymerase (0.25 U, Stratagene), 0.2 mM each dNTP, and 5–10 pmol of each primer in the 1× reaction buffer (20 mM Tris-HCl, pH 8.8, 10 mM KCl, 10 mM (NH₄)₂ SO₄, 2 mM MgSO₄, 0.1% Triton X-100, 0.1 mg/mL BSA) supplied with the *Taq* Extender Additive and supplemented with 2.5 mM MgSO₄.

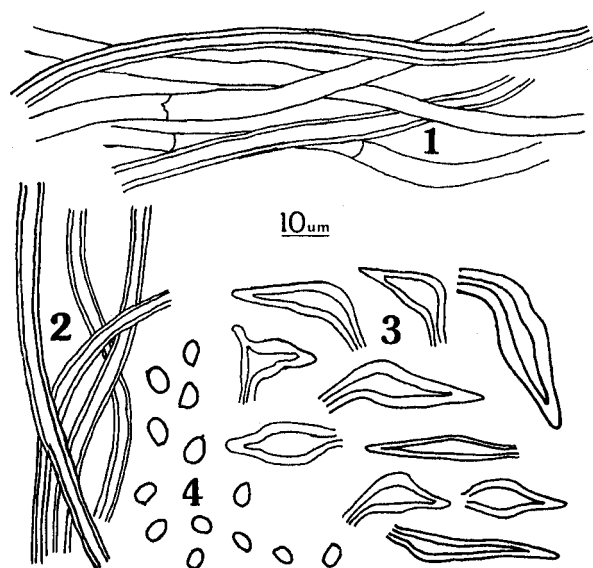
Previously published primers MS1 and MS2 (White et al 1990) were used for amplification of mt-SSU products using the following thermal profile: 95 C (3 min) followed by 35 cycles of 95 C (40 s), 58 C (1 min), 72 C (1 min), with one final cycle at 72 C (10 min). Templates for cycle sequencing were prepared using QIA PCR-preps (QIAGEN) to purify products, or treated directly in PCR reactions with Exonuclease I/Shrimp Alkaline Phosphatase enzymes (USB PCR product Pre-sequencing kit). DNA sequences were generated from both strands using the same primers, MS1 and MS2, and done by either the Advanced Plant Genetics or DRS Sequencing Facilities at University of California, Davis. Results were proofed, edited, and merged into individual contigs for each taxon using SEQUENCHER 3.0 (Gene Codes) software.

Multiple sequence alignments of taxon sequences were made initially with CLUSTALW 1.4 (Thompson et al 1994), then manually adjusted using the multiple-alignment sequence editor, SEQPUP/PPC 0.6f (Gilbert 1996). Further taxa were added to initial alignments using SEQPUP. The final alignment is available as NCBI PopSci Number 19070853 (National Institutes of Health, Bethesda, MD). Phylogenetic analyses (parsimony, distance and maximum likelihood) were performed using PAUP*4.0 (Swofford 2000) with all character changes unordered and unweighted. Small insertion/deletions (indels) were encoded as additional characters. Uninformative characters and those missing or in difficult to align (hypervariable) regions were excluded from analyses. Parsimony analyses used random addition in 1000 replicates for heuristic searches to find the most parsimonious trees for each data set. Neighbor Joining distance analyses (Saitou and Nei 1987) were performed using a ML measure of genetic distance. To assess relative support for monophyletic groups, bootstrap analyses were conducted using 10 000 replicates with resampling (Felsenstein 1985).

TAXONOMY

Phellinus coronadensis Rizzo, Gieser & Burdsall, sp. nov. FIGS. 1–4

Pileus sessile, dimidiatus, crassus, imbricatus; superficies superior horizontalis, brunneus ad nigrum;



FIGS. 1–4. Line drawings of the microscopic characters of *Phellinus coronadensis*, RLG 9396. FIG. 1. Context hyphae. FIG. 2. Hyphae of pore trama. FIG. 3. Setae. FIG. 4. Basidiospores. Scale = 10 μ m.

superficies pororum in angulis 45 ad substratum, luteo-brunneus; pori rotundi, 5–7 per mm; contextus rufo-brunneus, proximo 11 mm crassus; hyphae septatae, 2.5–5.0 μ m diam, rufo-brunneus; setae raris, ventricosae, 15–23 $\times$ 7.5–9.0 μ m vel subulatus, 26–35 $\times$ 6–9 μ m; basidiosporae ovoidae, laeves, hyalinae, nonamyloidae, 4–6 $\times$ 3–4 μ m.

Basidiomes perennial, sessile, pileate, triangular in vertical section with the upper surface horizontal and the pore surface forming a 45-degree angle with that surface, thick, up to 46 cm wide, 11 cm thick, and 28 cm from the margin to the host; margin obtuse, rounded, finely velvety, up to 2 cm thick, greyish yellow (4B6) to light brown (6D7); upper surface in older areas smooth and crustose, sulcate, dark brown (7F4–5, 8F4), lighter toward the margin to light or yellowish brown (6D7, 6E6); pore surface smooth, brownish orange (5C6) to light brown (6D7) or yellowish brown (6E8), pores 5–7 per mm, circular with thick entire dissepiments; context up to 11 mm thick, hard and woody, of fibrous appearance, faintly zonate with fine black lines in some areas separating growth periods, brownish orange (5C6) to yellowish brown

(6E8), becoming black in 2% KOH, slightly zonate; tubes up to 5 mm long, stratified in 5–6 mm layers in older specimens, concolorous with or slightly paler than the context.

Hyphal system dimitic but difficult to distinguish two hyphal types because of intergrading wall thickness; *contextual hyphae* 2.5–5.0 μ m diam, skeletal hyphae thick walled (walls 1–1.5 μ m thick), darkly pigmented and rarely branched; generative hyphae 2.5–4.0 μ m diam, reddish brown to yellowish brown or only slightly thickened and paler, branching uncommon, septa rare; hyphae of pore trama mostly narrower (2.0–4.0 μ m diam) and of similar description; *setae* infrequent, of two types: 1) short, ventricose, 15–23 $\times$ 7.5–9 μ m, often rather abruptly constricted midway to apex; 2) subulate, 26–35 $\times$ 6–9 μ m, tapering gradually to apex, the ventricose protruding about 10 μ m, the subulate projecting about 20 μ m; *basidia* broadly clavate, 9–11 $\times$ 5–6 μ m, 4 sterigmate, lacking basal clamps; *basidiospores* hyaline, ovoid, obovoid or ellipsoid 4–6 $\times$ 3–4 μ m, not reacting with Melzer's reagent.

Habitat. At the base of living *Pinus strobiformis* and occasionally *Pseudotsuga menziesii* above 2500 m elevation in the Coronado National Forest in the state of Arizona, USA. Less frequently reported on dead standing trees.

HOLOTYPE. USA. ARIZONA: Graham County, Coronado National Forest, Pinaleno Mts., Riggs Flat Lake, on living *Pinus strobiformis*, 6-V-1970, RLG-9396, in CFMR conservatus; *isotypus* in ARIZ conservatus.

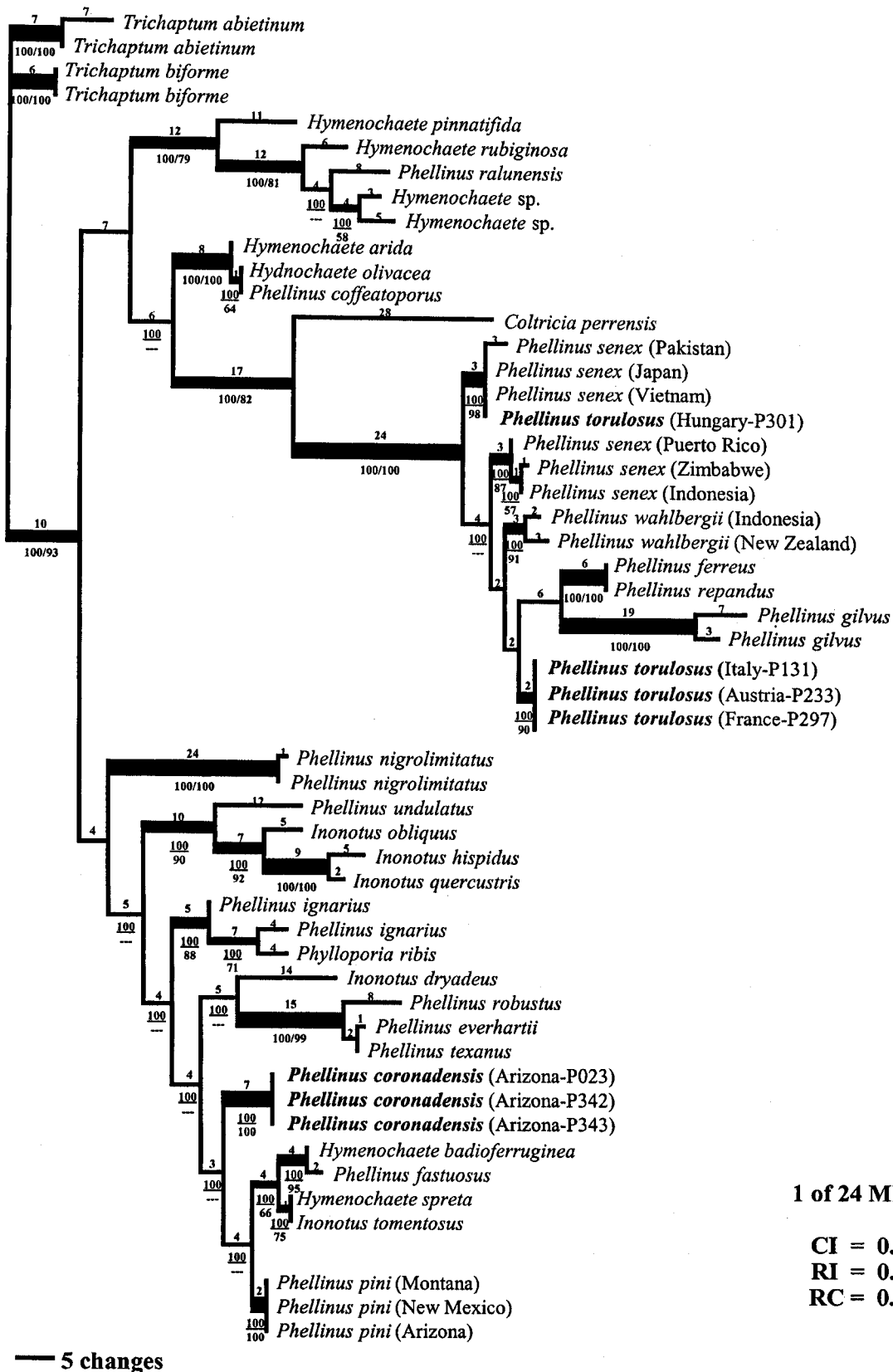
Etymology. From the Coronado National Forest, where all of the specimens of *P. coronadensis* have been found.

Specimens examined. *Phellinus coronadensis*: USA. ARIZONA: Coronado Nat. Forest, Summerhaven, Santa Catalina Mts., Pima County, all on base of *P. strobiformis*, RLG 7887, 9385, 9387, 9396 ABB1506*, 1507*, 1508, no number, 1995, VI.28; Webb Peak Area, Pinalino Mts., Graham County, on base of living *Pseudotsuga menziesii*, HHB 1504*. All specimens in CFMR, ARIZ. (*indicates cultures also available from CFMR)

Phellinus torulosus: ITALY. Popolunia at base of living *Quercus ilex* L., 20-IV-1994, DMR 94-IT1. FRANCE. Forêt domaniale de Montech, 10 km SW of Montauban, on *Quercus rubra* L, stump, HHB-17211. AUSTRIA. Burgenland, on *Morus* sp., RLG 14299. All specimens in CFMR, ARIZ.

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FIG. 5. One most parsimonious (MP) phylogram for mitochondrial small subunit ribosomal DNA. CI, consistency index; RI, retention index; RC, rescaled consistency index. Numbers above branches represent the number of steps (changes) between taxa; below branches represent 50% majority rule consensus values (for 24 trees)/bootstrap values based on 10 000 replicates. Wider branch widths represent relative bootstrap support. Information in parentheses indicates: lab collection numbers and/or locations for *P. coronadensis*, *P. torulosus* and related taxa.



DISCUSSION

Based on phylogenetic analysis of mt-SSU sequences, collections from Arizona originally named *P. torulosus* do not share a direct common ancestor with European collections of *P. torulosus* (FIG. 5). While the overall relationships across the family are not well resolved in this analysis, *P. torulosus* and *P. coronadensis* are clearly placed in distantly related terminal lineages and supported by high bootstrap values. All European collections of *Phellinus torulosus* grouped in a lineage that consisted of *P. senex* and *P. wahlbergii* (FIG. 5). These species all have similar basidiome, setal and spore characteristics with *P. torulosus*, and cause a white pocket rot (Larsen and Cobb-Pouille 1990). Other very closely related taxa include *P. gilvus*, *P. ferreus*, and *P. repandus* (FIG. 5).

The position of *P. coronadensis* within the *Hymenochaetaceae* is not clear, although it shared a most recent common ancestor with *Phellinus pini sensu lato*, *Inonotus tomentosus* and several *Hymenochaete* species (FIG. 5). From an ecological point of view, the grouping of *P. coronadensis* with the *P. pini* and *I. tomentosus* is not completely unexpected. The *P. pini* complex is made up of a number of pathogens on conifers (e.g., *P. pini*, *P. chrysoloma* (Fr.) Donk, *P. cancriformans* M. J. Larsen, Lombard & Aho), all of which cause white pocket rots (Larsen and Cobb-Pouille 1990).

While basidiome morphology of *P. torulosus* and *P. coronadensis* is highly similar, our re-examination of collections from Europe and Arizona has revealed several morphological differences. The setae of *P. coronadensis* are infrequent while in *P. torulosus* they are of frequent occurrence. The setae in *P. coronadensis* are of two shapes (FIG. 3): ventricose and measuring 15–23 × 7.5–9 µm, and subulate, measuring 26–35 × 6–9 µm, while in *P. torulosus* the setae are all subulate with measurements similar to those in *P. coronadensis*. The basidiospores in specimens of *P. coronadensis* are in general about 0.5 µm broader and longer than those of *P. torulosus*. The combination of morphological, ecological, and molecular genealogical characters, in association with the distinct geographic ranges, clearly indicate the delimitation of two species.

The generic placement of *P. coronadensis* is not completely straightforward. The perennial basidiomes place it within *Phellinus sensu lato*. Based on a variety of morphological and biochemical characters, a number of segregate genera have been described from within *Phellinus* Qué1, *sensu lato* (Fiasson and Niemelä 1984). For example, the association of *P. coronadensis* with the *Phellinus pini* lineage (FIG. 5) could potentially put this new species in the genus

Porodaedalea Murrill (Fiasson and Niemelä 1984). However, these segregate genera were based solely on studies of *Phellinus* and *Inonotus* P. Karst., rather than the *Hymenochaetaceae* as a whole (Fiasson and Niemelä 1984). From our molecular phylogenetic studies of the *Hymenochaetaceae*, it is clear that *Phellinus* as commonly perceived is not monophyletic (FIG. 5). While these preliminary data support to some extent the segregate genera described by Fiasson and Niemelä (1984), inclusion of *Hymenochaete* Lév., *Hydnochaete* Bres. and other genera in such studies complicates the results (FIG. 5). Therefore, until the complete phylogeny of the family is resolved, including possible linkages between the various lineages, we prefer to place *P. coronadensis* in the genus *Phellinus*.

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