Studies in Perenniporia s.l. Perenniporiella tepeitensis comb. nov., an addition to Perenniporiella: evidence from morphological and molecular data

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Abstract – Perenniporia tepeitensis (basionym: Coriolus tepeitensis) is showed to belong to Perenniporiella, on the basis of both morphological and molecular data (DNA sequence data from the ribosomal LSU, ITS, and tef1). Hence, the new combination Perenniporiella tepeitensis is proposed. The species is redescribed on the basis of the type specimen and several original collections from eastern Mexico and USA. A key to Perenniporiella is proposed.

Neotropical polypores / phylogeny / taxonomy

Résumé – Perenniporia tepeitensis (basionyme : Coriolus tepeitensis) est transférée dans le genre Perenniporiella, sur base de critères morphologiques et d’analyse de séquences d’ADN (grande sous-unité ribosomale, zone ITS + 5.8S, et un fragment du gène codant pour la facteur d’élongation tef1). La nouvelle combinaison Perenniporiella tepeitensis est proposée, et l’espèce est redécrite. Une clé d’identification des espèces de Perenniporiella est proposée.

Polypores néotropicaux / phylogénie / taxonomie

INTRODUCTION

Decock & Ryvarden (2003) established Perenniporiella2 for 3 morphologically homogeneous polypores viz. P. neofulva (type species) and P. micropora, both previously placed in Perenniporia (Ryvarden 1987, 1990), and P. pendula. Robledo et al. (2009) added P. chaquenia, a species so far know only from the

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2 Authorships of scientific names listed in Table I are not repeated in the text.
Yungas forest in the Argentinean Andes. Robledo et al. (2009) also evidenced that *Perenniporiella*, as circumscribed then, is monophyletic when considered from a phylogenetic perspective.

In continuation of the revision of *Perenniporia* s.l. from Meso- and South America (Decock and Herrera Figueroa 2000, Decock and Ryvarden 1999a, b, 2000, 2003, Decock et al. 2001), we have reconsidered the taxonomic placement of *P. tepeitensis*. Ryvarden (1985) transferred the species, originally described as *Coriolus tepeitensis* Murrill (Murrill 1912), to *Perenniporia* on the basis of a dimitic hyphal system with dextrinoid skeletal hyphae and thick-walled basidiospores. Both features, in Ryvarden's classification scheme at that time, supported this placement. However, the main morphological characters of the species, especially the effused-reflexed basidiomata, the basidiospores morphology, and, to a lesser degree, the vegetative hyphae deviate from *Perenniporia* s.s. (Decock and Stalpers 2006) and point toward *Perenniporiella* (Decock and Ryvarden 2003). Phylogenetic inferences based on partial nuc ribosomal LSU, ITS-5.8S, and a 1200 bp fragment of the house keeping gene *tef1* also unequivocally placed this species in the latter genus.

The results are commented below, the new combination *Perenniporiella tepeitensis* is proposed, and the species re-described and illustrated.

**MATERIALS AND METHODS**

*Material and Collection localities.* — Specimens studied are preserved at CFMR, ENCB, MUCL, and NY (herbarium acronyms are from Thiers B. [continuously updated]). Original MUCL materials were collected in the eastern mountain range (*Sierra Madre oriental*) of Mexico, in the *El Cielo* Biosphere reserve (Prov. Tamaulipas, Gómez Farías, approximately 23°03′35.6″ N, 99°12′45″ W), at an elevation of about 1300 masl. The local ecosystem is mesophilous mountain forest, characterized by the botanical genera *Liquidambar, Quercus, Juglans, Dalbergia*, etc. (Rzedowski 2006). Additional original specimens and strains collected in southeastern USA were received from NY and CFMR.

MUCL original strains were isolated from basidiome tissues during fieldwork, on malt extract agar supplemented with 2 ppm benomyl (benlate) and 50 ppm chloramphenicol, and later, when necessary, purified in the laboratory. Living cultures are preserved at MUCL, and a representative strain at the CBS. Additional strains were received from CFMR (Madison, USA).

*Specimen description.* — Colors are described according to Kornerup and Wanscher (1981). Section were carefully dissected under a stereomicroscope in hot (40°C) NaOH 3% solution, and later examined in NaOH 3% solution at room temperature. Sections were also examined in Melzer’s reagent and lactic acid cotton blue to provide evidence of a staining reaction. All the microscopic measurements were carried out in Melzer’s reagent. In presenting the size range of several microscopic elements, 5% of the measurements at each end of the range are given in parentheses when relevant. In the text, the following abbreviations are used: Ave = arithmetic mean, R = the ratio of length/width of basidiospores, and AveR = arithmetic mean of the ratio R.
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Sequencing. — DNA extraction, amplification, and sequencing of the nuclear ribosomal 5′ end of the large ribosomal subunit; ITS = ITS1, 5.8S, ITS2; tef = fragment between exons 4 and 8 of the translation elongation factor 1 alpha

Phylogenetic analysis. — The species, strains / specimens, and sequences used in this study are listed in Table 1. Twelve specimens representing 5 species were included in the various phylogenetic analyses. Nucleotide sequences were automatically aligned with Clustal X (version 2.0.11) (Thompson et al. 1997), then manually adjusted as necessary with the text editor in PAUP* (version 4.0b10). Perenniporiella neofulva was chosen as the outgroup (Robledo et al. 2009).

Phylogenetic analysis of the aligned sequences was performed using the maximum parsimony method of PAUP* version 4.0b10 (Swofford 2002) with gaps treated as fifth bases.

Table 1. List of species / collections / sequences used in the phylogenetic analyzes, and their GenBank accession number. 28S = 5′ end of the large ribosomal subunit; ITS = ITS1, 5.8S, ITS2; tef = fragment between exons 4 and 8 of the translation elongation factor 1 alpha

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<th>Genus / Species names</th>
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T, PT = Type, Paratype.
The most parsimonious trees were identified using heuristic searches with random addition sequence (1000), and further evaluated by bootstrap analysis (bv), 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.

To detect topological conflicts among data partitions, the nodes between the majority-rule consensus trees obtained in the MP analysis from the individual data sets were compared. Paired trees were examined for conflicts only involving nodes with BS > 70%. A conflict was assumed to be significant if two different relationships for the same set of taxa (one being monophyletic and the other being non-monophyletic) were observed on the rival trees.

**RESULTS**

*Morphology.* — The hyphal system in *P. tepeitensis* is dimitic with slightly branched vegetative hyphae in the context and branched in the hymenophoral trama, then with a poorly differentiated arboriform branching pattern, with a short stalk and longs, skeletal-like branches (Figs. 5-6). The basidiospores are subglobose to globose, not truncate, thick-walled, apparently without any germ pore (Fig. 7), and variably dextrinoid. Both features, in addition to a resupinate to effused reflexed, light-weighted basidiomata, agree with the definition of *Perenniporiella* (Decock and Ryvarden 2003, Robledo et al. 2009). This definition reveals the difference with *Perenniporia* that has thick-walled, basidiospores, with a truncate apex, the latter resulting from the development of a large apical germ pore by wall dissolution (Decock & Stalpers 2006).

*DNA sequences data.* — The collections of *P. tepeitensis* from Mexico and the USA differ slightly in their sequences, the differences ranging from 1-5 divergent positions (max. 1 difference in LSU; 1-3 in ITS and tef1). These differences are not considered taxonomically significant at species level, especially when all the morphological features point toward conspecificity.

*Phylogenetic analysis.* — Heuristic MP searches for the LSU (870 positions, 25 variable positions parsimony-uninformative, 11 variable positions parsimony-informative), ITS (616 positions, 48 variable positions parsimony-uninformative, 37 variable positions parsimony-informative) and *tef1* datasets (1172 positions, 136 variable positions parsimony-uninformative, 95 variable positions parsimony-informative), considered individually, yielded respectively 1 (36 steps; CI = 1.0, RI = 1.0), 1 (104 steps; CI = 0.885, RI = 0.821), and 12 (293 steps; CI = 0.915, RI = 0.860) equally MPTs. These MPTs exhibited the same topology. By comparing the topologies obtained from MP bootstrap consensus tree for the individual datasets, no conflicts involving significantly supported nodes were found and the three datasets were combined.

*Combined dataset analysis.* — 2658 characters, gaps included, of which 143 variable characters were parsimony-uninformative and 209 characters were parsimony-informative. An exhaustive search produced 4 equally most parsimonious trees (438 steps; CI = 0.904, RI = 0.843), with a single topology (Fig. 1) concordant with the trees obtained by the individual data set.
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Fig. 1. One of the 4 most parsimonious trees obtained from an exhaustive search (TL: 438 steps; CI: 0.904, RI: 0.843). Ag = Argentina, Cu = Cuba, Mx = Mexico, USA = United States of America.
The phylogenetic inferences resolved all the morphologically based species as terminal branches, confirming their status of individual species. These branches are well supported for *P. pendula*, *P. chaquenia*, and *P. tepeitensis*, whereas *P. micropora* is represented by a single sequence.

The collections of *P. tepeitensis* form a monophyletic clade closely related to *P. chaquenia* and to a lesser degree, *P. pendula*, confirming the placement of this species in *Perenniporiella*; hence the proposed new combination.

**TAXONOMY**

*Perenniporiella tepeitensis* (Murrill) Decock & Valenzuela comb. nov. Figs 2-7

Mycobank: MB 518040

**Basidiome** seasonal to reviviscient, resupinate, in small delimited, circular patches, up to 7 × 5 mm, becoming effused or fusing laterally, extending up to 20-55 × 20-30 mm, 1.5-3 mm thick, margin free, detached from the substrate; or effused-reflexed, the pileus portion 15-30 wide, projecting up to 7-15 mm, up to 3 mm thick at the base; or more rarely only pileate, then attached by the vertex; pileus/pileate part applanate in section, semicircular to broadly attached, rarely attached by the vertex, solitary or laterally fused, with a soft, flexible consistency; pileus surface glabrous, concentrically sulcate, whitish cream to pale grayish orange when fresh and young (4B4-5B4), zoned, with several pale to dark reddish or reddish brown (8C8-9C8, brownish red) bands, thin next to the margin, larger near the base, with age greenish overall due to algae; margin even, white when fresh, drying whitish to pale cream; pore surface white, whitish when fresh, pale grayish cream to pale corky on drying; pores even, round, 6-7(-8)/mm, (85-)95-140(-150) µm diam, (ave = 113 µm); dissepiments smooth, entire, (25-)30-55(-80) µm thick (ave = 44 µm); context/subiculum homogeneous, reduced to a thin layer up to 1 mm thick at the base in the pileate part, whitish and with a soft corky consistency when fresh, drying greyish orange, pale corky; tubes layer single or layered, up to 2 distinct layers, concolorous with the context, white or whitish when fresh, drying pale grayish orange, pale corky, with a corky consistency and a fibrous texture when fresh, up to 2.5 mm thick.

**Hyphal system** dimitic both in the context and the trama of the tubes; generative hyphae with clamps (clamps present at all septa on hyphae in culture), hyaline, thin-walled, 2-3.5 µm thick; vegetative hyphae hyaline, cyanophilous, weakly dextrinoid in the context, dextrinoid in the trama of the tubes, which is especially obvious in the dissepiments; context mainly composed of non-branched to sparingly branched vegetative hyphae, straight to sinuous, thick-walled, 2.5-3.5 µm (ave = 2.9 µm) in their main part; hymenophoral trama mainly composed of branched vegetative hyphae having a loose arboriform branching pattern, with an unbranched, scarcely differentiated, thick-walled (with the lumen open) basal stalk, (12-)25-45(-50) µm long, (ave = 37.5 µm), 2.5-4.0(-4.5) µm wide (ave = 2.9 µm), straight to geniculated, then occasionally with lateral aborted processes
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and slightly widened at the apical branching point, branching of 1-3 levels, the branches long (measured (50-)95-250 µm, ave = 160 µm), 2-2.5 µm wide (ave = 2.1 µm) in their main part, thick-walled, straight to sinuous.

Hymenium: basidia clavate, with a basal clamp, hyaline, thin-walled, 14-18×8-9 µm, with four small sterigmata; basidiospores broadly ellipsoid to subglobose, up to broadly obovoid, thick-walled, smooth, with a small apiculus, hyaline, non- to faintly dextrinoid, cyanophilous, (5.0-)5.5-6.5(-7.0) × (4.5-)4.5-5.5(-6.0) µm (ave = 5.8×5.0 µm), R = (1.0-)1.1-1.3(-1.4) (aveR = 1.16); chlamydospores absent.

Substratum: dead fallen branches of unidentified angiosperm; dead wood of Robinia pseudoacacia (black locust, Fabaceae). Also reported in lit. (Lowe 1952) on Sassafras (Lauraceae) and Sumach (Rhus glabra, Anacardiaceae).

Type of rot: white rot (production of laccase positive, tested with syringaldazine [Harkin and Obst 1974]).

Confirmed distribution: Mexico, Southeastern United States.

Holotype: MEXICO [PROV. Morelos]: Tepeite Valley, near Cuernavaca [approx. 19°00'38" N – 99°13'26" W], elev. 7000 ft asl (2134 m), on dead fallen branches, 28 Dec. 1909, W.A. & E.L. Murrill, 550, NY.

Figs 2-4. Macroscopical features of basidiomes of Perenniporiella tepeitensis. 2-3. Basidiome, general view (MUCL 52300): 2, scale bar = 1 cm; 3, scale bar = 2 cm; 4. pore surface, scale bar = 1 cm.
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Additional specimens examined: MEXICO [PROV. Morelos:] Tepeite Valley, near Cuernavaca [approx. 19°00'38" N – 99°13'26" W], elev. 7000 ft asl (2134 m), on dead fallen branches, 28 Dec. 1909, W.A. & E.L. Murrill, 490 & 533, NY (paratypes); PROV. Tamaulipas: Reserva de la Biosfera El Cielo, Municipio de Gómes Farías, on the way to Altacima, 23°03'35.6" N – 99°02'45" W, elev. 1200-1400 masl, on dead fallen branches lying in the ground, unidentified angiosperm, 27-28 Jul. 2009, C. Decock and R. Valenzuela, MX-09-75, MX-09-86, MX-09-87, respectively MUCL 52296, 52300, 52301 (cultures ex- MUCL 52296 (= CBS), 52300, 52301); USA, NORTH CAROLINA: Asheville, Bent Creek Exp. Forest, on black locust (Robinia pseudoacacia L.), 16 Sep 1959, J. Lowe and R.L. Gilbertson, Lowe n° 11319 (NY; culture ex- CFMR L-11319-Sp); Asheville, 11 Aug 1962, J. Lowe, Lowe n° 12485 (NY; culture ex- CFMR L-12485-Sp); TENNESSEE: Great Smoky Mountains National Park, near Gatlinburg, Cherokee Orchid, on black locust (Robinia pseudoacacia L.) 26 Aug 1950, J. Lowe N° 4531 (FP 99690).

DISCUSSION

Perenniporiella tepeitensis has all the typical morphological features of Perenniporiella, including the hyphal system (Figs 5-6) and the broadly ellipsoid to subglobose, apically rounded, and thick-walled basidiospores (Fig. 7). The species is characterized by a resupinate to effused reflexed or more rarely pileate basidiome (Figs 2-4), 6-7 pores/mm, and basidiospores averaging 5.7 × 4.9 µm (Fig. 7).

Our Mexican collections differ slightly from the description of Gilbertson and Ryvarden (1987) in the pileus color and number of pores/mm. Gilbertson and Ryvarden (1987) reported a white to cream pileus, without any mention of brownish red tint, and 4-5/mm. Pores were measured as 6(-7)/mm in the type, reported 5-6/mm by Murrill (1912). In Lowe specimen (see list of specimens), the pileus is either absent or poorly developed, and then cork colored.

Perenniporiella tepeitensis is comparable to P. chaquenia and P. pendula: they share similar basidiomes, basidiospores, and vegetative hyphae. Perenniporiella chaquenia and P. tepeitensis differs in the number of pores/mm, 4-6 versus 6-7(-8)/mm, respectively. The pores have about the same diameter in both taxa, but the dissepiments are thicker in P. chaquenia compared to P. tepeitensis (on average, respectively 70 µm versus 40 µm). Furthermore, the pileus of P. tepeitensis is smooth while it is finely velutinate in P. chaquenia. Perenniporiella pendula is characterized by mostly pileate basidiomes, pendant to broadly attached, occasionally with a decurrent part, and (slightly) smaller pores (7-9/mm).

Perenniporiella micropora has related basidiome and basidiospores, but a duplex context and much smaller pores, 8-10/mm. Perenniporiella neofulva differs in having much thicker, more rigid basidiomes, and distinctly smaller basidiospores, on average ≤ 4.0 µm.

Perenniporiella tepeitensis, P. chaquenia, P. pendula, and P. micropora are also, in a phylogenetic perspective (Fig. 1), very closely related to each other, forming a well supported sub-clade, while P. neofulva is more distant (Robledo et al. 2009).
KEY TO SPECIES OF *PERENNIPORIELLA*

1a Basidiospores on average ≤ 4 µm long (3.5-4.5 × 3-4 µm) ........................................... *Perenniporiella neofulva*

1b Basidiospores on average ≥ 5 µm .................................................................................. 2

2a Context heterogeneous (duplex), with a loose upper layer and a denser lower layer; pores 8-10(-12)/mm .......................................................................................... *Perenniporiella micropora*

2b Context homogeneous; pores larger .................................................................................. 3

3a Pores 4-6/mm; dissepiments thick .............................................................. *Perenniporiella chaquenia*

This species is so far only known from the Chaquean mountain forests of Central Argentina

3b Pores 6-9/mm; dissepiments thin .................................................................................. 4

4a Pores 7-9/mm; basidiospores subglobose to globose, 4.5-6.0 × 4.0-4.8 µm, averaging 5.2 × 4.5 µm .............................................................................................. *Perenniporiella pendula*

4b Pores 6-7/mm; basidiospores subglobose to globose, 5.5-6.5(-6.5) × (4.5-) 4.5-5.5(-6.0) µm (ave = 5.7 × 4.9 µm) ...................................................................... *Perenniporiella tepeitensis*

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REFERENCES


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