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Species delimitation in *Trametes*: a comparison of ITS, RPB1, RPB2 and TEF1 gene phylogenies

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Abstract: *Trametes* is a cosmopolitan genus of white rot polypores, including the ''turkey tail'' fungus, *T. versicolor*. Although *Trametes* is one of the most familiar genera of polypores, its species-level taxonomy is unsettled. The ITS region is the most commonly used molecular marker for species delimitation in fungi, but it has been shown to have a low molecular variation in *Trametes* resulting in poorly resolved phylogenies and unclear species boundaries, especially in the *T. versicolor* species complex (*T. versicolor* sensu stricto, *T. ochracea*, *T. pubescens*, *T. ectypa*). Here we evaluate the performance of three protein-coding genes (TEF1, RPB1, RPB2) for species delimitation and phylogenetic reconstruction in *Trametes*. We obtained 59 TEF1, 34 RPB1 and 55 RPB2 sequences from 69 individuals, focusing on the *T. versicolor* complex and performed phylogenetic analyses with maximum likelihood and parsimony methods. All three protein-coding genes outperformed ITS for separating species in the *T. versicolor* complex. The multigene phylogenetic analysis shows the highest amount of resolution and supported nodes separating *T. ectypa*, *T. ochracea*, *T. pubescens* and *T. versicolor* with strong support. In addition three slineages are resolved in the species complex of *T. elegans*. The *T. elegans* complex includes three species: *T. elegans* (based on material from Puerto Rico, Belize, the Philippines), *T. aesculi* (from North America) and *T. repanda* (from Papua New Guinea, the Philippines, Venezuela). The utility of gene markers varies, with TEF1 having the highest PCR and sequencing success rate and RPB1 offering the best backbone resolution for the genus.

Key words: gene phylogenies, PolyPEET, Polyporales, systematics, taxonomy

Introduction

The genus *Trametes* Fr. (Polyporales, Basidiomycota) is characterized by pileate sessile basidiocarps, trimitic hyphal systems, smooth non-dextrinoid and non-amyloid spores, absence of true hymenial cystidia and white rot wood decay (Ryvarden 1991). Species are present in almost all forest ecosystems and are found frequently on numerous genera of hardwoods throughout northern temperate forests (Gilbertson and Ryvarden 1987). They play an important role in natural ecosystems as wood decomposers and show enormous potential for bioremediation and biodegradation endeavors, making them both ecologically and economically important. The limits of the genus and its relations with closely related genera such as *Coriolopsis* Murrill., *Lenzites* Fr. and *Pycnoporus* P. Karst. have been studied using a five gene dataset by Justo and Hibbett (2011), who concluded that a broad generic concept for *Trametes* was the optimal taxonomic and nomenclatural option for this group in view of the phylogenetic results. Other authors (Welti et al. 2012) have proposed a different taxonomic arrangement, in which four genera are recognized within *Trametes* based on monophyly of groups inferred from ITS and RPB2 sequences, as well as differences in morphology: these include (i) a lineage corresponding to ''genuine'' *Trametes* species; (ii) *Pycnoporus* species; (iii) *Artolenzites* Falck., including the tropical ''*Lenzites*'' *elegans* (Spreng.) Pat. and (iv) *Leiotrametes* Welti & Courtec., including three tropical species, *Trametes menziesii* (Berk.) Ryv., *T. lactinea* (Berk.) Sacc. and ''*Leiotrametes* sp.'' (Welti et al. 2012). The study of Justo and Hibbett (2011) also presented a species phylogeny based on nuclear ribosomal internal transcribed spacer (nrITS) data, with 155 isolates representing 25 putative species-level entities, which illustrates the problems in the species taxonomy of *Trametes* that are the focus of the present paper. First, we address the incorporation of new ITS sequences from unsampled taxa to resolve taxonomic and nomenclatural controversies in the genus. Second, we take a closer look at the taxonomy and phylogeny of two problematic clades in the genus: the *T. versicolor* and *T. elegans* species complexes using a multilocus dataset.

Trametes versicolor, commonly known as the ''turkey tail'', is among the most common species within the genus and has been reported on 295 woody plant species including conifers and angiosperms (Grand and Vernia 2002; USDA database http://nt.arsgrin.gov/fungaldatabases/fungushost/fungushost.cfm). This species, together with *T. pubescens*, *T. ochracea*

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and *T. ectypa*, form a strongly supported clade in the ITS phylogeny of Justo and Hibbett (2011), but the internal topology of the clade is poorly resolved. These four species reveal high morphological similarity but are recognized as separate taxa by Gilbertson and Ryvarden (1987), who used the color and texture of the pileus as a pivotal character for species delimitation. Tomšovský and Homolka (2004) demonstrated that *T. versicolor*, *T. ochracea* and *T. pubescens* are not sexually compatible.

Trametes elegans, as it is recognized by Gilbertson and Ryvarden (1987), is widespread in tropical and subtropical environments and demonstrates extremely variable hymenophore morphology ranging from a lamellate to poroid hymenophore, sometimes in the same specimen (Ryvarden and Johansen 1980, Gilbertson and Ryvarden 1987, Quanten 1997). Gilbertson and Ryvarden (1987) cite the species as common in southeastern USA but occurring as far north as Wisconsin and west to Texas. The ITS phylogeny of Justo and Hibbett (2011) recovered three clades among collections identified as *T. elegans*, and some geographic structure was apparent in that collections from the continental USA grouped separately from Caribbean and southeastern Asian collections.

In the present study we examine the potential of three protein-coding genes, RPB1 (RNA polymerase II largest subunit), RPB2 (RNA polymerase II second largest subunit) and TEF1 (translation elongation factor 1-alpha), for resolving species delimitation in the *T. versicolor* and *T. elegans* species complexes.

MATERIALS AND METHODS

DNA extraction and sequencing.—DNA for 69 isolates, from which ITS data had been studied in Justo and Hibbett (2011), was readily available. DNA from three isolates was obtained from specimens collected in the Virgin Islands National Park (St John, US Virgin Islands) 4 Feb–12 Feb 2012. Protocols for DNA extraction, PCR and sequencing are the same as those outlined in Justo and Hibbett (2011). PCR amplification and sequencing of the ITS region was performed with primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993). Primers EF1-983F and EF1-1567R were used to amplify approximately 500 bp of TEF1 (Rehner and Buckley 2005). Primers RPB1-Af and RPB1-Cr (Stiller and Hall 1997, Matheny et al. 2002) were used to amplify the conserved region between domains A and C of RPB1, approximately 1400 bp long. Additional sequencing primers include RPB1-Int2.2f (Binder et al. 2010) and RPB1-Int2.1r (Frøslev et al. 2005). The 6–7 region of RPB2, approximately 700–800 bp long, was amplified with primers RPB2-b6F and RPB2-b7.1R (Liu et al. 1999, Matheny 2005). Sequencing was done on an ABI 3130 DNA sequencer (Applied Biosystems). Raw sequence data were edited and assembled in Sequencher 4.7 (Gene Codes Corp.).

Sequence alignment and phylogenetic analyses.—Sequences were aligned in MAFFT 6 (Katoh and Toh 2008; http://mafft.cbrc.jp/alignment/server/) using the ''G-INS-I'' strategy. Aligned sequences were exported as a single nexus file, which was manually adjusted with MacClade 4.08 (Maddison and Maddison 2002). Two ITS datasets were assembled: (i) an extended dataset that includes all newly generated sequences plus publicly available sequences in GenBank since the publication of Justo and Hibbett (2011); and (ii) a core ITS dataset that includes only the 69 isolates of *Trametes* that were selected for the generation of new protein-coding gene data. We also assembled individual datasets for RPB1, RPB2 and TEF1 and one combined four-gene dataset (ITS, RPB1, RPB2, TEF1). Two representatives of the *Grifola frondosa* (Dicks.) Gray. complex were selected as outgroups for the extended ITS dataset, and *Lopharia cinerascens* (Shwein.) G. Cunn. was chosen as outgroup for all other datasets. Two phylogenetic analyses were performed on all datasets, a maximum likelihood analysis (ML) using RAxML 7.2.8 (Stamatakis et al. 2008) under a GTR model with 100 bootstrap replicates and an equally weighted parsimony analysis (MP) performed with PAUP*4.0.b10 (Swofford 2002) using 1000 bootstrap replicates. Parsimony analyses were performed with the same parameters described in Justo and Hibbett (2011). Nodes were considered strongly supported if they scored a bootstrap value greater than 70% in both analyses. A search for conflicts between the core ITS dataset and each of the protein-coding genes was performed by comparing the resulting trees from each dataset and looking for strongly supported positive conflict.

RESULTS

New sequences and alignments.—161 new sequences were generated: 13 ITS, 34 RPB1, 55 RPB2 and 59 TEF1. In addition, 14 unpublished ITS sequences generated by Dr Otto Miettinen (Clark University) were included in the extended ITS dataset. Amplification of protein-coding genes was attempted in 69 isolates. PCR and sequencing of TEF1 genes succeeded in 96% of the isolates, while for RPB2 and RPB1 the success rates were 91% and 65% respectively. GenBank numbers and collection information are provided (SUPPLEMENTARY TABLE I). GenBank numbers for sequences not generated in this study are provided in the corresponding figures. A comparative overview of the datasets analyzed here is presented (TABLE I), with the exception of the extended ITS dataset. All alignments were deposited in TreeBASE under study number S14650.

Extended ITS dataset.—This dataset includes 230 sequences of *Trametes*. A total of 504 most parsimonious trees were recovered in the MP analyses (consistency index = 0.45, retention index = 0.92). Out of the 679 total characters, 227 (33%) were

TABLE I. Overview of the alignment and analyses

Dataset	Number of ingroup sequences	Total characters	Parsimony informative characters	Most parsimonious trees	Consistency index/retention index	Strongly supported nod1es
ITS	81	685	129 (18%)	828	0.57/0.87	23 out of 42 (55%)
RPB1	53	1313	524 (40%)	263	0.49/0.78	27 out of 39 (70%)
RPB2	74	728	271 (37%)	288	0.40/0.78	28 out of 49 (57%)
TEF-1	78	528	172 (33%)	669	0.42/0.83	28 out of 42 (67%)
Combined	81	3524	1096 (31%)	650	0.45/0.80	36 out of 47 (77%)

parsimony informative. The best tree from the ML analysis is illustrated (FIG. 1).

A total 33 putative species are recognized in the analyses (FIG. 1). The *T. versicolor* and *T. elegans* complexes are discussed separately. Important differences and novelties with respect to the ITS phylogeny of Justo and Hibbett (2011) are: (i) *Lenzites acuta* Berk. and *L. vespacea* (Pers.) Ryv. both belong in *Trametes* and are not closely related to other lamellate species of *Trametes* (*T. betulina*, *T. elegans*, *L. warnieri* Mont. & Durieu, ''*Lenzites* sp.''); (ii) newly generated sequences of *T. villosa* auth. from the US Virgin Islands group with sequences obtained from GenBank under that name from Guadeloupe and Argentina; however these group separately with sequences from Tennessee and Mexico, suggesting the existence of cryptic species; (iii) sequences of *T. sanguinea* and ''*Pycnoporus*'' *coccineus* appear as separate in the analyses although without strong support; (iv) sequences under the name *T. ljubarskii* Pilát. from France and India form a paraphyletic group and represent two different species.

Phylogeny of the Trametes versicolor *complex.*—30 isolates from the core 69-taxa dataset belonging to the *T. versicolor* complex were selected for phylogenetic analysis. The individual ITS (core dataset), RPB1, RPB2 and TEF1 phylogenies for this group are illustrated (FIG. 2). The full individual phylogenies are provided (SUPPLEMENTARY INFORMATION). The results from the concatenated four-gene dataset are illustrated (FIG. 3). No conflicts were detected among the datasets analyzed in the present study.

The resulting phylogeny of the core ITS (FIG. 2) dataset is similar to the extended ITS dataset and the phylogeny of Justo and Hibbett (2011). Both *T. versicolor* and *T. ectypa* isolates cluster as separate groups in the ML and MP analyses but with no bootstrap support. One of the isolates of *T. ochracea* (HHB12282sp) groups with *T. versicolor* and the Argentinean isolate of *T. versicolor* (BAFC285) is not nested with the rest of *T. versicolor* isolates, so neither species forms a clade. *Trametes ochracea* and *T. pubescens* are recovered as monophyletic but with poor support in the ML analysis, and their placement collapses in the strict-consensus MP tree.

In the individual analyses all three protein-coding genes give better separation of the taxa in this complex (FIG. 2). The Argentinean isolate BAFC285 appears nested within *versicolor* isolates (RPB1), nested within *ectypa* isolates (RPB2) or separate from all other taxa (TEF1), but none of these positions receives strong bootstrap support. The isolate HHB12282sp groups with *T. ochracea* isolates in the RPB1, RPB2 and TEF1 phylogenies. *Trametes conchifer* appears outside the *T. versicolor* complex in the ITS and RPB1 phylogenies but nested within in the RPB2 and TEF1 phylogenies, although in both cases there is no strong support for this placement.

In the combined four-gene dataset (FIG. 3), *T. conchifer* is the sister taxon of the *T. versicolor* complex, in which five strongly supported lineages are recovered: *T. pubescens*, *T. ochracea*, *T. ectypa*, *T.* cf. *versicolor* (BAFC285) and *T. versicolor*.

Phylogeny of the Trametes elegans *complex.*—Twelve isolates belonging to the *T. elegans* complex were analyzed. The ITS (core dataset), RPB1, RPB2 and TEF1 phylogenies for this group are depicted (FIG. 4). Three strongly supported clades are recovered in the four-gene dataset (FIG. 3) and for convenience are named here *Trametes elegans* I, II and III. *Trametes elegans* I is composed of isolates from continental USA and is recovered in all four individual datasets with strong support except in the RPB2 dataset (FIG. 4). *Trametes elegans* II contains predominantly isolates from the Caribbean region, although one isolate from the Philippines (FPR10) is also included here. This clade is not recovered in the core ITS or RPB2 dataset (FIG. 4), but it is recovered (with weak support) in the extended ITS dataset (FIG. 1a) and with strong support in the TEF1 (FIG. 4) and four-gene dataset (FIG. 3). Only one RPB1 sequence is available from this group of samples. *Trametes elegans* III contains predominantly isolates from southeastern Asia, although one isolate from Venezuela (OH271sp) also is included here. This clade is recovered with strong support in all analyses

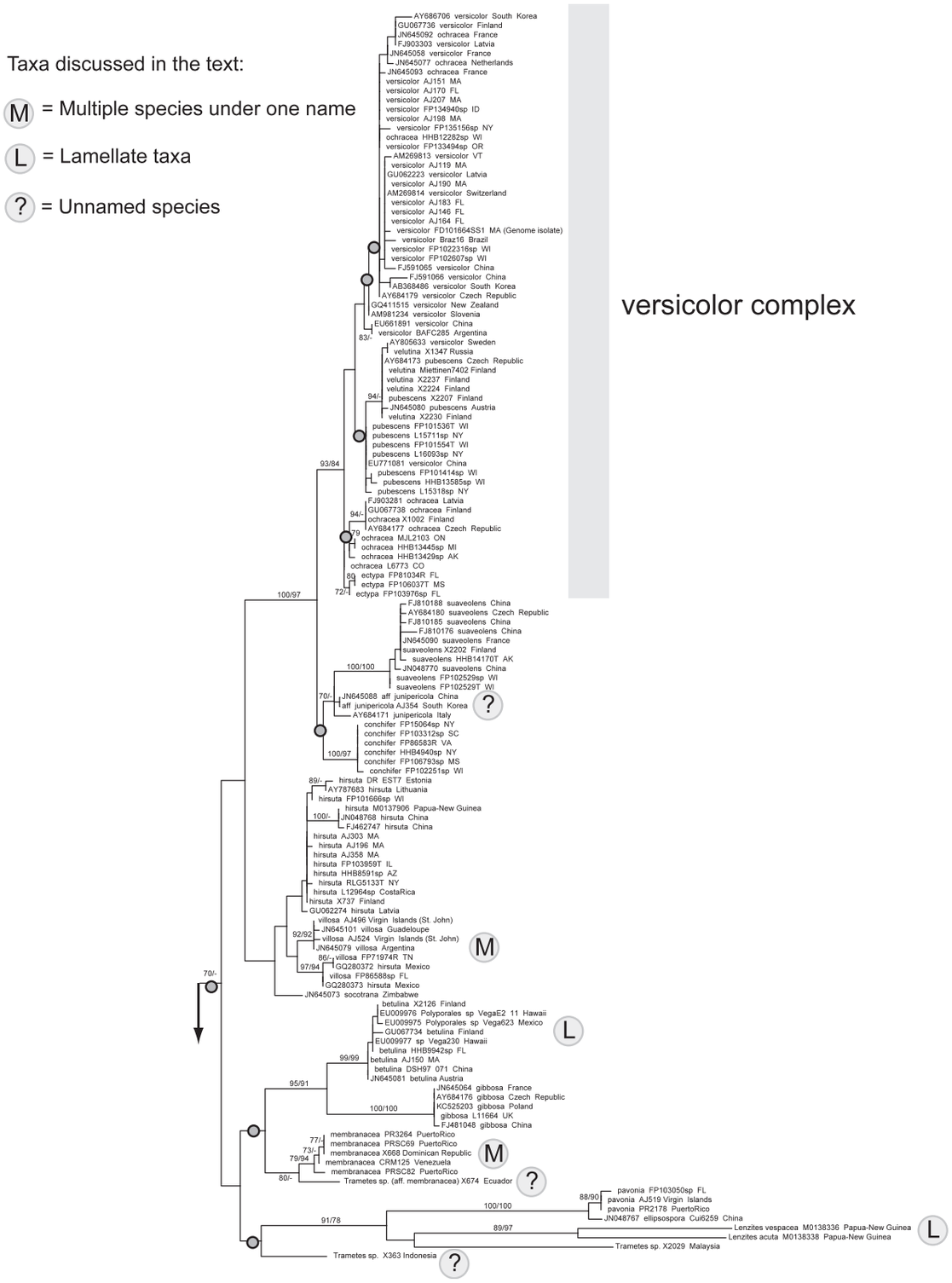

FIG. 1. Best tree from the ML analysis of the extended ITS dataset. Bootstrap values on or below branches (ML/MP). Gray circles outlined in black represent nodes that collapse in the strict consensus tree from PAUP.

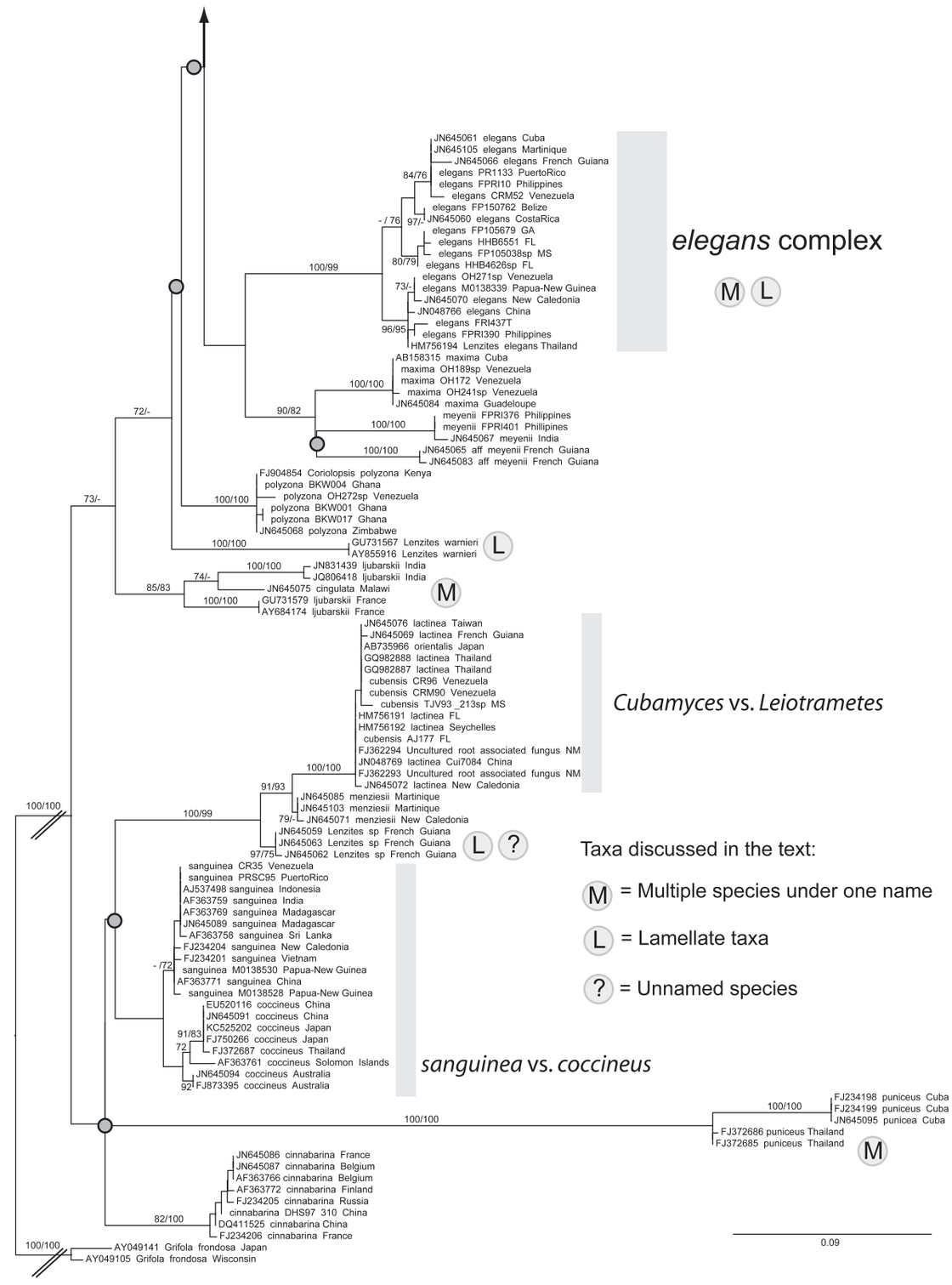

FIG. 1. Continued.

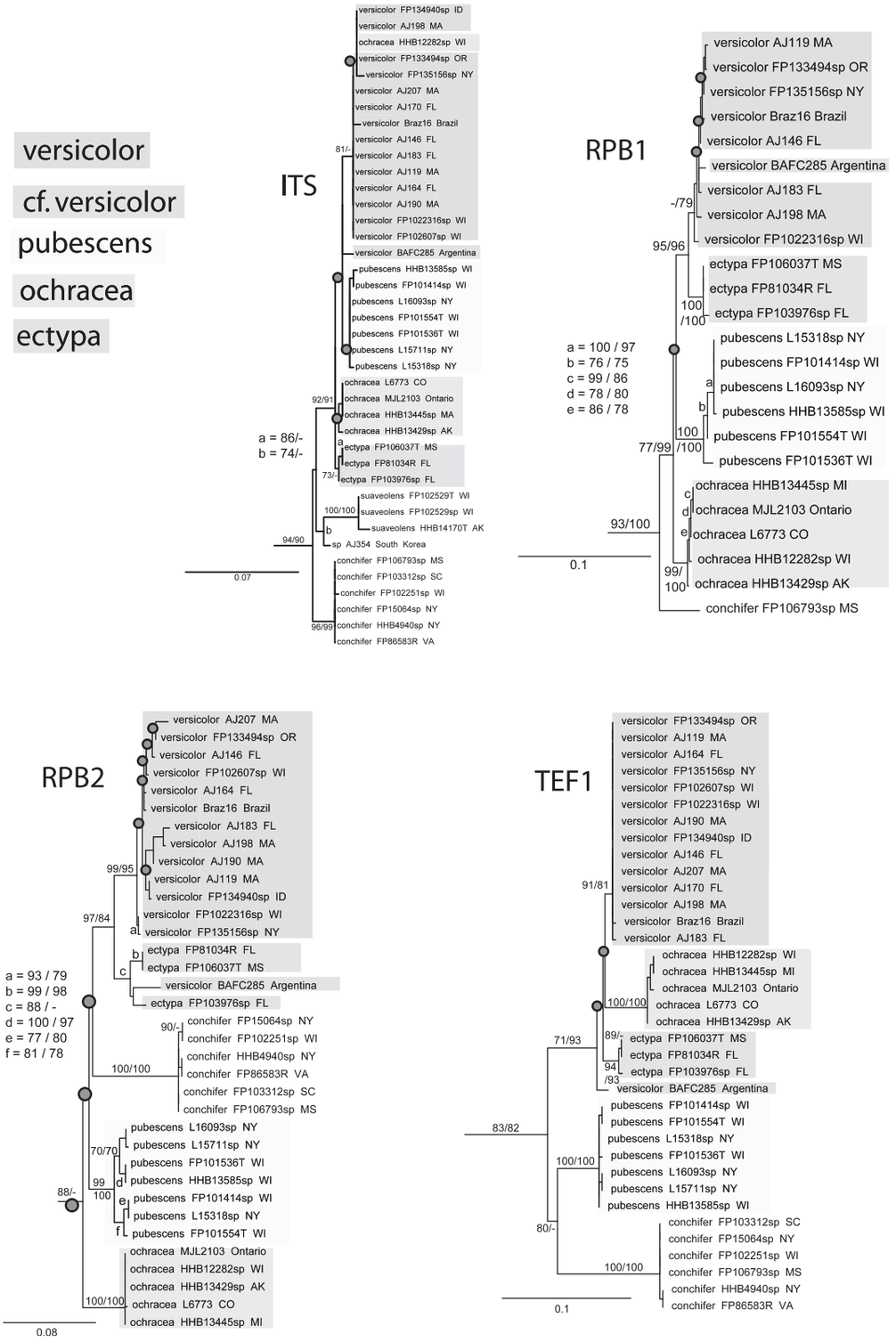

FIG. 2. *T. versicolor* complex as recovered in the best trees from the ML analyses of the individual gene datasets. Bootstrap values on or below branches (ML/MP). Gray circles outlined in black represent nodes that collapse in the strict consensus tree from PAUP.

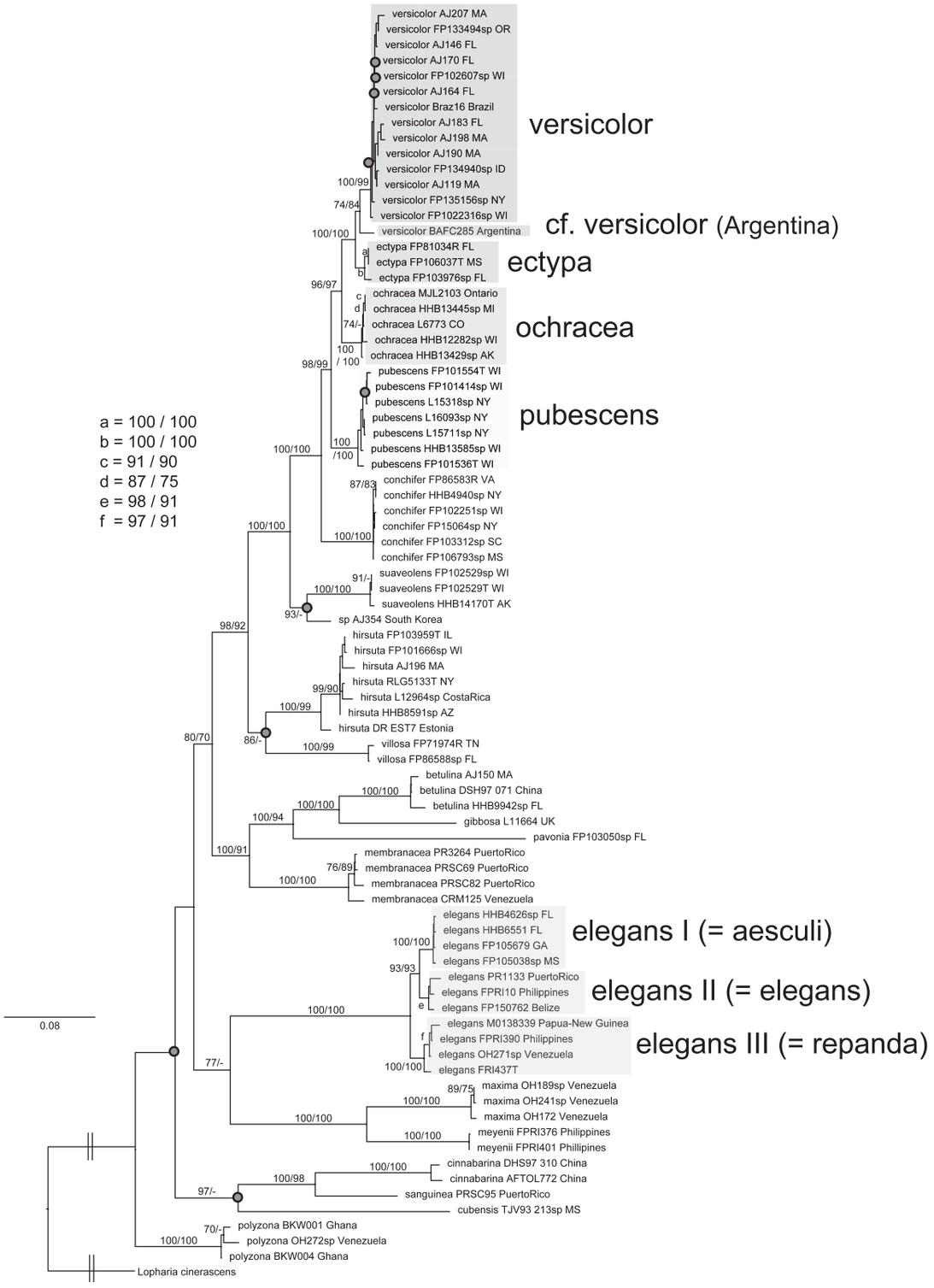

FIG. 3. Best tree from the ML analysis of four-gene dataset. Bootstrap values on or below branches (ML/MP). Gray circles outlined in black represent nodes that collapse in the strict consensus tree from PAUP.

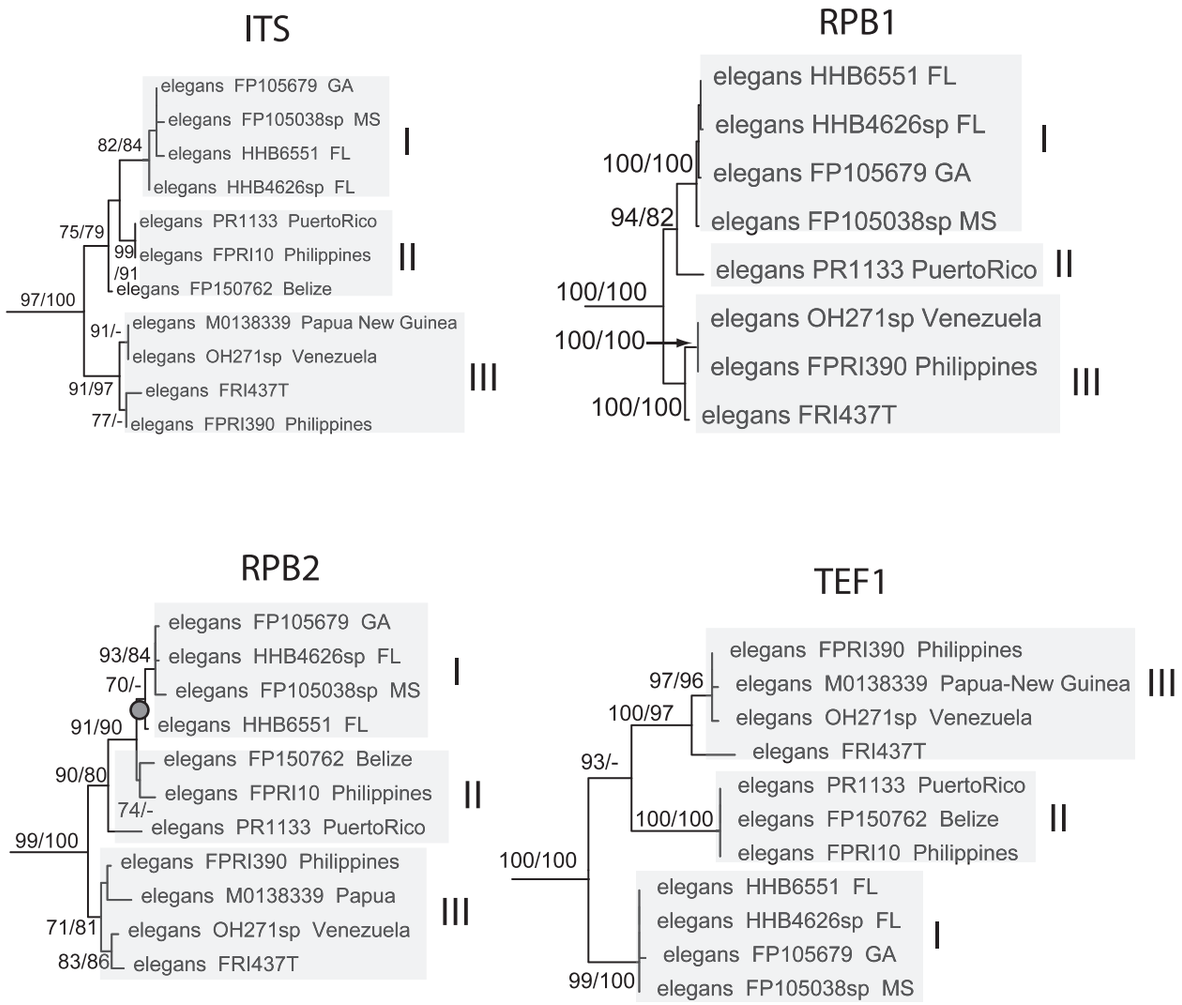

FIG. 4. *T. elegans* complex as recovered in the best trees from the ML analyses of the individual gene datasets. Bootstrap values on or below branches (ML/MP). Gray circles outlined in black represent nodes that collapse in the strict consensus tree from PAUP.

and appears as sister of the clade containing *T. elegans* I and II except in the TEF1 dataset. In the TEF1 dataset *T. elegans* II and III appear as sister clades, however, this conflict is not strongly supported by MP analysis. In addition, a single isolate (PR1133) from Puerto Rico appears to cluster within *T. elegans* II in all analyses except in the RPB2 dataset, where it appears as sister of *T. elegans* I and II.

DISCUSSION

Taxonomic overview of Trametes.—The results of the extended ITS analyses (FIG. 1) illustrate the current problems in the species-level taxonomy of *Trametes*. Five of the taxa sampled here lack names, although molecular data suggests they are, in fact, unique species: aff. *junipericola* Manjón, G. Moreno & Ryv. (AJ354, JN645088), aff. *membranacea* auth (isolate X674), *Trametes* sp. (isolate X2029), aff. *meyenii* auth (JN645065, JN645083) and ''*Lenzites* sp.'' (JN645059, JN645063, JN645062). Isolates labeled *Trametes ljubarskii*, *T. punicea* and *T. villosa* sampled here all represent more than one species. In the cases of *T. punicea* (originally described from southeastern Asia) and *T. villosa* (originally described from Jamaica), geographically close isolates may help decide on the application of those names, but in other cases like *T. ljubarskii* (originally described from the Russian Far East), no isolates have been sampled from the areas near the type locality. Other unresolved problems include the relatively wide ITS variation in some species like *T. hirsuta* and *T. membranacea* and the unclear separation of *T. sanguinea* and ''*Pycnoporus*'' *coccineus*. The sparse sampling, the often difficult

separation of species in *Trametes* based on morphological characters and the convoluted nomenclatural history of tropical and subtropical species of *Trametes* make it difficult to resolve species-level taxonomy in the genus.

Taxonomic uncertainty at the species level often will complicate taxonomic issues at higher levels. For example, Welti et al. (2012) proposed the genus *Leiotrametes* to accommodate *Trametes lactinea* (as the type species) and *T. menziesii*. However, ITS data from *T. lactinea* is identical to that of *T. cubensis*, which is the type species of *Cubamyces*, a genus erected by Murrill more than a hundred years ago (Murrill 1905a). Thus we consider *Leiotrametes* a synonym of *Cubamyces* because it was discussed in Justo and Hibbett (2011).

Lamellate species of Trametes.—Based on our results, there are eight species of *Trametes* with a lamellate or lamellate-poroid hymenophore (FIG. 1): *Trametes betulina*, *Lenzites acuta* Berk., *Lenzites vespacea*, *Lenzites warnieri*, *Lenzites* sp. and the three species in the *T. elegans* complex discussed below. Ryvarden and Johansen (1980) highlighted the morphological similarities among *Lenzites acuta*, *L. vespacea* and *L. warnieri*, casting some doubt as to whether they represent different taxa. Our sequences of *L. acuta* and *L. vespacea* confirm that both species are separate from each other and from *L. warnieri* (FIG. 1), implying that there might have been multiple transitions from a poroid hymenophore to a lamellate one.

Combinations in *Trametes* for *L. vespacea* and *L. warnieri* have been prosposed by Zmitrovich et al. (2012). The taxon referred to as *Lenzites acuta* by Nuñez and Ryvarden (2001), Quanten (1997) and Ryvarden and Johansen (1980) is in need of a new name because the name *Trametes acuta* Lév., probably a synomyn of *Coriolopsis strumosa* (Fr.) Ryv., is preoccupied. The oldest name available for this taxon is *Daedalea tenuis* Berk. Although the combination *Trametes tenuis* (Hook.) Corner, based on *Boletus tenuis* Hook., already exists it was invalidly published (Corner 1989) under Art. 41.5 of the International Code of Nomenclature for Algae, Fungi and Plants (no reference to a basionym was made) (McNeill et al. 2012). Therefore the new combination is proposed here: **Trametes tenuis** (Berk.) Justo, comb. nov.; MycoBank: 805416; *Basionym: Daedalea tenuis* Berk., London J. Bot. 1:151 (1842).

Trametes versicolor *complex.*—Analysis of the individual (FIG. 2) and combined (FIG. 3) datasets confirm that, despite the lack of resolution in the ITS phylogenies, *T. versicolor*, *T. ochracea*, *T. pubescens* and *T. ectypa* all are separate species. All genes except RPB1 placed the Argentinean isolate BAFC285 separately from other *versicolor* collections although in different positions. This isolate apparently represents a separate lineage, but further sampling in South America is necessary to clarify its status. The Brazilian isolate of *T. versicolor* sampled here (''Braz16'') showed no significant molecular differences with respect to the northern hemisphere samples.

The grouping of the *T. ochracea* isolate HHB12282sp with *T. versicolor* in the ITS dataset (FIG. 2) is probably caused by the high similarity in ITS sequences of *versicolor* and *ochracea* (98–99%). The possibility that this anomalous placement was the consequence of hybridization between *T. ochracea* and *T. versicolor* is not supported because all protein-coding gene sequences from this isolate grouped with the other *T. ochracea* isolates and none of these sequences had hybrid *versicolor*/*ochracea* characteristics. Moreover, Tomšovský and Homolka (2004) found complete intersterility between their isolates of *T. ochracea* and *T. versicolor*. The ITS region of this isolate was resequenced to rule out human error.

Morphological separation of the species in the *T. versicolor* complex relies heavily on the colors, zonation and texture of the pileus surface and to a lesser extent on pore and spore size. Therefore, old, weathered and/or sterile specimens can be challenging to identify. For full morphological descriptions and additional comments, readers should refer to Gilbertson and Ryvarden (1987) and Bernicchia (2005).

Trametes versicolor, *T. ochracea* and *T. pubescens* are common and widespread in boreal and temperate northern hemisphere, with *T. versicolor* being the most common of the three (Gilbertson and Ryvarden 1987, Ryvarden and Gilbertson 1994, Nuñez and Ryvarden 2001). *Trametes versicolor* and *T. pubescens* also occur in tropical areas of the northern hemisphere and in tropical and temperate forest of the southern hemisphere (Ryvarden and Johansen 1980, Rajchenberg 1982, Quanten 1997). *Trametes ectypa* seems restricted to the Gulf Coast of the southeastern USA and in the Caribbean islands (Gilbertson and Ryvarden 1987).

Trametes elegans *complex.*—The three lineages recovered in the combined dataset (FIG. 3) are thought to represent three separate species. No clear segregation of morphological characters among the three species was observed in the specimens sampled here, and individually each species would fit the morphological descriptions of *T. elegans* by Gilbertson and Ryvarden (1987), Quanten (1997) or Nuñez and Ryvarden (2001).

Geographical distributions are correlated with phylogenetic relationships (FIGS. 1a, 4); *T. elegans* I occurs exclusively in continental USA (Georgia, Mississippi, Tennessee), based on material sampled here; *T. elegans* II is widely distributed in Central and South America and the Caribbean region (Belize, Costa Rica, Cuba, French Guiana, Martinique, Venezuela) with only one isolate from southeastern Asia (Philippines); *T. elegans* III is predominant in southeastern Asia and Oceania (China, New Caledonia, Papua New Guinea, Philippines, Thailand) with only one isolate from South America (Venezuela). *Trametes elegans* originally was described from Guadeloupe (Fries 1821), therefore the clade named in this study, *T. elegans* II, is considered to represent the true *Trametes elegans*. The application of the name to samples outside tropical and subtropical America should be subject to further scrutiny and tested with molecular data.

The oldest name available for a southeastern Asian representative of the *T. elegans* complex is *Daedalea repanda* Pers. described from Rawak Island (Western Papua, Indonesia), therefore this name is adopted for ''elegans III'': **Trametes repanda** (Pers.) Justo, comb. nov. MycoBank 805417. Basionym: *Daedalea repanda* Pers. in Gaudichaud-Beaupré, Voy. Uranie 5:168 (1827).

The presence of *T. elegans* in the Philippines and *T. repanda* in Venezuela could be due to long-distance dispersal, either natural or anthropogenic, but additional sampling is required to answer this question.

The oldest name available for a member of the *T. elegans* complex described from continental USA is *Polyporus aesculi* Fr. (Fries 1828), a sanctioned nom. nov. for *Boletus aesculi-flavae* Schwein. described from North Carolina (Schweinitz 1828). Murrill transferred this species to the genus *Agaricus* (Murrill 1905b), which he used in a similar sense to the modern *Daedalea* and later to *Daedalea* (Murrill 1908). Murrill attributed to *D. aesculi* (Fr.) Murill. a reniform, rigid and azonate pileus and distribution confined to southern USA and recognized a second species, alternatively named *Agaricus deplanatus* (Link ex Fr.) Murrill. and *Daedalea amanitoides* P. Beauv., with a variously shaped, flexible and zonate pileus and purely tropical distribution (Murrill 1905b, 1908). This second species as described by Murrill contains elements of *T. elegans* and *T. repanda* as accepted here, and the morphological characters used to separate *aesculi* from *deplanatus*/*amanitoides* are far too variable to be reliable. However, Murrill's observation that the species in this group (in southern USA) is different than its tropical counterpart(s) is supported by the molecular data presented here (FIGS. 3, 4). The epithet *aesculi* is adopted here for this taxon: **Trametes aesculi** (Fr.) Justo, comb. nov. MycoBank 805418. Basionym: *Polyporus aesculi* Fr. In this case Schweinitz's name ''*aesculi-flavae*'' cannot be used and is not the correct basionym because Fries used the description when he named *P. aesculi*. These should be treated as synonyms, however, because *P. aesculi* is a sanctioned name, the combination *Trametes aesculi* (Fr.) Justo must be used.

Although the name *T. elegans* is widely used for collections made in USA its presence (in the strict sense as detected here) in North America has yet to be demonstrated. North American *T. elegans*, we predict, should be referred to as *T. aesculi*.

Taxonomic use of protein-coding genes.—When analyzed individually all three protein-coding genes tested here (RPB1, RPB2, TEF1) outperformed ITS in separating the species in the *T. versicolor* complex (FIG. 2). In the *T. elegans* complex RPB1 and TEF1 better resolved the species boundaries while RPB2 gave similar results to ITS (FIG. 4). In both cases TEF1 was the only gene that separated the five species in the *T. versicolor* complex and the three species in the *T. elegans* complex as they appear in the four-gene dataset (FIG. 3), although topological relations between the species were not resolved and differ within the *T. elegans* complex with respect to the other genes. RPB1 recovered the topological relations that more closely resemble the results of the four-gene dataset for both species complexes. Considering the high PCR/sequencing success rate of TEF1 we recommend the use of this gene for resolving species boundaries in other problematic complexes in *Trametes* but caution that this gene has limited power to resolve relationships among the species and deeper nodes in the phylogeny. To address deeper relationships, RPB1 seems to be the best suited of the three genes studied.

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