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Authors: Lücking, Robert, Truong, Ba Vuong, Huong, Dang Thi Thu, Le, Ngoc Han, Nguyen, Quoc Dat, et al.

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ROBERT LÜCKING[1*], BA VUONG TRUONG[2], DANG THI THU HUONG[3], NGOC HAN LE[3], QUOC DAT NGUYEN[4], VAN DAT NGUYEN[5], ECKHARD VON RAAB-STRAUBE[1], SARAH BOLLENDORFF[1], KIM GOVERS[1] & VANESSA DI VINCENZO[1]

Caveats of fungal barcoding: a case study in *Trametes* s.lat. (*Basidiomycota*: *Polyporales*) in Vietnam reveals multiple issues with mislabelled reference sequences and calls for third-party annotations

Abstract: DNA barcoding using the nuclear internal transcribed spacer (ITS) has become prevalent in surveys of fungal diversity. This approach is, however, associated with numerous caveats, including the desire for speed, rather than accuracy, through the use of automated analytical pipelines, and the shortcomings of reference sequence repositories. Here we use the case of a specimen of the bracket fungus *Trametes* s.lat. (which includes the common and widespread turkey tail, *T. versicolor*) to illustrate these problems. The material was collected in Vietnam as part of a biodiversity inventory including DNA barcoding approaches for arthropods, plants and fungi. The ITS barcoding sequence of the query taxon was compared against reference sequences in GenBank and the curated fungal ITS database UNITE, using BLASTn and MegaBLAST, and was subsequently analysed in a multiple alignment-based phylogenetic context through a maximum likelihood tree including related sequences. Our results initially indicated issues with BLAST searches, including the use of pairwise local alignments and sorting through Total score and E value, rather than Percentage identity, as major shortcomings of the DNA barcoding approach. However, after thorough analysis of the results, we concluded that the single most important problem of this approach was incorrect sequence labelling, calling for the implementation of third-party annotations or analogous approaches in primary sequence repositories. In addition, this particular example revealed problems of improper fungal nomenclature, which required reinstatement of the genus name *Cubamyces* (= *Leiotrametes*), with three new combinations: *C. flavidus*, *C. lactineus* and *C. menziesii*. The latter was revealed as the correct identification of the query taxon, although the name did not appear among the best BLAST hits. While the best BLAST hits did correspond to the target taxon in terms of sequence data, their label names were misleading or unresolved, including [Fungal endophyte], [Uncultured fungus], *Basidiomycota*, *Trametes* cf. *cubensis*, *Lenzites elegans* and *Geotrichum candidum* (an unrelated ascomycetous contaminant). Our study demonstrates that accurate identification of fungi through molecular barcoding is currently not a fast-track approach that can be achieved through automated pipelines.

Key words: *Basidiomycota*, fungal barcoding, *Polyporales*, sequence contamination, *Trametes*, *Trametes cubensis*, *Trametes menziesii*, Vietnam

1 Botanischer Garten und Botanisches Museum, Freie Universität Berlin, Königin-Luise-Straße 6–8, 14195 Berlin, Germany; *e-mail: r.luecking@bgbm.org (author for correspondence).

2 Institute of Tropical Biology, Department of Biological Resources, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan St., District 3, Ho Chi Minh City, Vietnam.

3 Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

4 Southern Institute of Ecology, Vietnam Academy of Science and Technology, 01 Mac Dinh Chi, Ben Nghe, District 1, Ho Chi Minh City, Vietnam.

5 Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

Introduction

The true fungi (*Fungi*) represent the third largest kingdom in terms of known species and the second largest with respect to estimated richness, with between 2.2 and 3.8 million species (Hawksworth & Lücking 2018). Due to their simple body plan, fungi have few diagnostic characters compared to plants and animals (Nagy & al. 2017; Lücking 2019). This renders their accurate identification based on phenotype often difficult or even impossible (Lücking & al. 2020). Molecular barcoding has therefore become an important tool in the identification of fungi, as well as other organisms, and is even being implemented in citizen science projects (Geiger & al. 2016; Beenken & al. 2017; Bubner & al. 2019; Chiovitti & al. 2019).

For fungi, the mycological community has agreed on the nuclear internal transcribed spacer of the nuclear ribosomal DNA cistron (ITS) as universal barcoding marker (Schoch & al. 2012). While this marker works rather well in most fungal groups, in some lineages it does not provide sufficient resolution and secondary barcoding markers such as *TEF1* and *COX1* (*COI*) have been proposed (Chen & al. 2009; O'Donnell & al. 2015; Al-Hatmi & al. 2016; Raja & al. 2017; Tepkinar & Kalmer 2019). In some cases, phenomena such as hybridization, introgression or gene duplication also complicate the use of the ITS barcoding marker (Lindner & Banki 2013; Li & al. 2013, 2017; Lücking & al. 2020).

In addition to these technical difficulties, identification of fungi through molecular barcoding faces other issues. One is the quality and completeness of existing reference databases (Nilsson & al. 2006; Meier 2008; Begerow & al. 2010; Tedersoo & al. 2011; Kõljalg & al. 2013; Tanabe & Toju 2013; Hofstetter & al. 2019). For instance, GenBank currently includes ITS sequences for approximately 45 000 fungal species, which represent 30% of the currently accepted and formally described 140 000 to 150 000 species and just 1.5% of an estimated mean of 3 million species (Lachance 2006; Hawksworth & Lücking 2018; Species Fungorum 2020a). This means that the current probability that a randomly selected species will have a highest-scoring mismatch in GenBank is 70% based on the number of known species and 98.5% with respect to estimated global species richness.

The second problem is the often observed naivety when implementing molecular barcoding. Both manual approaches and automated pipelines typically rely on pairwise similarity assessments using a fixed threshold level. For instance, the curated ITS database UNITE uses 98.5% as default value, although thresholds can be set at other levels between 97% and 100% (Abarenkov & al. 2010; Kõljalg & al. 2013; Nilsson & al. 2019). The default threshold of 98.5% has also been recommended in other studies and is supported by empirical data for selected fungal groups (Jeewon & Hyde 2016; Vu & al. 2016). Strictly speaking, fixed thresholds for

species delimitation do not exist; the actual threshold is lineage-specific and depends on the phylogenetic context. In some groups, species can be distinguished by pairwise similarity thresholds as narrow as 99.5% (Vu & al. 2016; Lücking & al. 2017). Based on observed identity values alone, it is therefore not possible to decide whether the closest BLAST hit is actually the species in question or a closely related taxon.

Finally, a substantial proportion of ITS reference sequences deposited in GenBank and other databases are wrongly or incompletely labelled (Harris 2003; Vilgalys 2003; Nilsson & al. 2005, 2006, 2012, 2014; Meier 2008; Bidartondo & al. 2008; Lücking & al. 2012; Kõljalg & al. 2013; Hofstetter & al. 2019). As of 25 March 2020, GenBank returned 1 367 715 fungal ITS sequences using the structured query <Fungi[organism] AND (5.8S[title] OR ITS1[title] OR ITS2[title] OR ITS[title] OR "internal transcribed spacer"[title])>. Of these, only 443 645 (32%) were fully identified to species with a Latin binomial, though not necessarily correctly so. Consequently, the best BLAST matches may not have species-level and often not even genus-level identifications. Sequence labels are often incorrect, either due to misidentifications or outdated taxonomic concept. In some cases, taxon names are associated with sequences that represent unrelated contaminants (Lücking & Nelsen 2018). As a result, a species-level clade can contain numerous different names, and the same name can appear in various and even unrelated clades. Problems in sequence annotation can even perpetuate themselves when using erroneous annotations of BLAST hits to annotate newly generated sequences (e.g. Gilks & al. 2002). Using curated secondary databases and/or improved data standards have been proposed as possible solutions (Droege & al. 2016; Geiger & al. 2016). For fungal ITS, UNITE and RefSeq "Targeted Loci" are the most commonly consulted curated databases (Abarenkov & al. 2010; Kõljalg & al. 2013; Schoch & al. 2014; O'Leary & al. 2016; Nilsson & al. 2019).

All these issues complicate molecular barcoding and render this approach difficult, often resulting in errors comparable to or even greater than phenotype-based (morphological) identifications. Therefore, great care is required when implementing DNA barcoding (Nilsson & al. 2012; Hyde & al. 2013; Hofstetter & al. 2019; Lücking & al. 2020). In this study, we use the example of the widespread bracket fungus *Trametes* Fr. s.lat., a well-studied genus (Corner 1989; Ryvarden 1991; Ko & Jung 1999; Zhang & al. 2006; Justo & Hibbett 2011; Vlasák & Kout 2011; Welti & al. 2012; Zmitrovich & Malysheva 2013; Carlson & al. 2014; Cui & al. 2019). The study is part of a current project of molecular barcoding of Vietnamese fungi, to illustrate these problems and outline strategies to resolve them, in order to arrive at reliable results. We thereby place particular emphasis on the process of subsequent verification of the initial identification based on ITS barcoding.

Material and methods

Field work

Fresh material of a broad diversity of macrofungi was gathered during a joint excursion to Cúc Phương National Park in Vietnam in May 2019, with participation by E.R.S. and S.B. On the first field day, the group went to the western part of the park in Hòa Bình province, 400 m south of Xóm Khanh village near Bưởi river, where material was gathered in secondary rainforest. On the subsequent days, the group collected in primary forest or on trails close to visitor attractions, all in Ninh Bình province. Around the park centre (Bống), the group worked along the trail to "Silver cloudy peak" (Đinh Mây Bạc) and on the main road c. 2 km SE of the park centre, and along the trail "Bird watching" (dominated by *Dracontomelon duperreanum* Pierre). Other collecting sites were situated along the "Trail to the ancient tree" (dominated by *Terminalia myriocarpa* Van Heurck & Müll. Arg.), to the "Cave of the prehistoric man" (Động người xua) c. 6.7 km NW of the visitor centre, and along the trail to Kahnh village 2 km NW of the park centre. All fungi were collected on decaying wood, mainly on tree trunks or on bark of fallen trees. In total, 41 specimens of macrofungi were sampled during the excursion and subsequently processed at the BGBM, including full digitization. Voucher specimens are deposited in B and VNMN.

The excursion and the laboratory and analytical work were organized as part of the project "VIETBIO – Innovative approaches to biodiversity discovery and characterization" (BMBF, grant 01DP17052; https://www.internationales-buero.de/en/vietbio_innovative_approaches_to_biodiversity_discovery_and_characterization.php). VIETBIO is a bilateral German-Vietnamese research project supported by the German Federal Ministry for Education and Research (BMBF). The main objective of VIETBIO is the development and transfer of an integrated biodiversity discovery and monitoring system for Vietnam. Collaboration partners are the Museum für Naturkunde Berlin – Leibniz-Institut für Evolutions- und Biodiversitätsforschung (MfN), the Botanischer Garten und Botanisches Museum, Freie Universität Berlin (BGBM), and four Vietnamese institutions, which all belong to the Vietnam Academy of Science and Technology (VAST): the Vietnam National Museum of Nature (VNMN) and the Institute of Ecology and Biological Resources (IEBR) in Hanoi, as well as the Southern Institute of Ecology (SIE) and the Institute of Tropical Biology (ITB) in Ho Chi Minh City. Activities within VIETBIO include joint field sessions in Vietnam and training of Vietnamese researchers in state-of-the-art methods during working visits at MfN and BGBM. One of the four training modules of this project comprised molecular barcoding approaches, including DNA extraction, sequence generation and barcoding analysis. VIETBIO was therefore an ideal study ground to evaluate and document the caveats of molecular barcoding of fungi.

After initial assessment of fungal specimens for which data for the ITS barcoding marker were generated, we selected the genus *Trametes* s.lat. as a case study. *Trametes* is a well-known, cosmopolitan genus of bracket fungi (Corner 1989; Ryvarden 1991; Ko & Jung 1999; Zhang & al. 2006; Justo & Hibbett 2011; Vlasák & Kout 2011; Welti & al. 2012; Zmitrovich & Malysheva 2013; Carlson & al. 2014; Cui & al. 2019) and is also widely used in biotechnological studies (Ludwig & al. 2004; Nyanhongo & al. 2007). The ITS-barcoding marker has been shown to work reasonably well in this genus for species delimitation (Zhang & al. 2006; Justo & Hibbett 2011; Vlasák & Kout 2011; Welti & al. 2012; Zmitrovich & Malysheva 2013; Olusegun 2014; Carlson & al. 2014; Cui & al. 2019). For the analysis, we selected the following specimen (Fig. 1): Vietnam, Ninh Bình Province, Cúc Phương National Park, forest SW of main road, c. 2 km SE of park centre (Bống), 20°19'58.44"N, 105°36'28.44"E, 350 m, primary rainforest over limestone, on dead log and branches, 8 May 2019, *S. Bollendorff & al., VietBio Botany 920* (B 70 0107235, VNMN).

Laboratory work

New fungal ITS sequences for the target sample were generated in the laboratories of the BGBM, using a standardized approach to specimen documentation and laboratory work established by the GBOL project (Geiger & al. 2016). Genomic DNA was extracted from dried fungal tissue using a modified CTAB method after Doyle & Doyle (1987). The resulting DNA-stock solution (isolate DB42771) was diluted 1:10 with sterile water to create work solutions that were used for polymerase chain reaction (PCR). PCR was performed on a peqSTAR 96 HPL Thermocycler (PeqLab, Erlangen, Germany). The mixture for one reaction consisted of 10 µL of dNTPs 20 pm/µL, 5 µL of 10× Taq-buffer S, 2 µL of each primer with a concentration of 10 pm/µL (ITS1F: Gardes & Bruns 1993; ITS4: White & al. 1990), 0.3 µL of peqGOLD Hot Taq DNA Polymerase with 5 units/µL (PEQL01-8120, VWR International GmbH, Darmstadt, Germany), 5 µL of betaine (5M) and 4 µL of DNA template. Ultrapure H_2O was added to obtain a final volume of 50 µL. Temperature profiles for the PCR amplifications consisted of an initial denaturation step of 1:30 min at 95°C, followed by 35 cycles of 30 s denaturation at 95°C, 1 min of primer annealing at 50°C and 1 min of extension at 72°C, and a final elongation period of 10 min at 72°C. PCR products were electrophoresed for approximately 2.5 hours on a 1.5% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer (pH 8.0) and stained with SYBR-Gold (Life Technologies no. S11494, Carlsbad, California, U.S.A.). Bands were excised from the gel and cleaned using the GenepHlow Gel/PCR kit (Geneaid, New Taipei, Taiwan). Cycle sequencing was carried out by Macrogen Europe (Amsterdam, Netherlands), using the same primers as in

the PCR reactions. After quality check, the forward and reverse read (Suppl. Files S1, S2) were assembled into a single contig submitted to GenBank (MT928350).

Analytical approach

For the molecular barcoding approach, we implemented the following steps. First, we blasted the query sequence in GenBank using both MegaBLAST and BLASTn (Altschul & al. 1990, 1997; Tan & al. 2006). For both BLAST options, we used default settings as follows: [MegaBLAST] Expect threshold = 10, Word size = 28, Max matches in a query range = 0, Match/Mismatch Scores = 1,−2, Gap costs = linear; [BLASTn] Expect threshold = 10, Word size = 11, Max matches in a query range = 0, Match/Mismatch Scores = 2,−3, Gap costs = Existence: 5, Extension: 2. We also blasted the query sequence in UNITE, first using the default BLASTn option [https://unite.ut.ee/analysis.php] and then performing local BLAST in BioEdit 7.2.5 (Hall 1999, 2011), using the most recent General FASTA Release 8.2 for Fungi, with either singletons set as RefS or including global and 97% singletons (Abarenkov & al. 2020a, 2020b).

After confirmation that the query sequence belonged to *Trametes* s.lat., we downloaded all ITS sequences for this genus from GenBank (including recent segregates and related genera such as *Coriolopsis* Murrill, *Leiotrametes* Welti & Courtec., *Lenzites* Fr., *Polyporus* P. Micheli ex Adans.). This resulted in a total of 1518 ITS sequences (Suppl. Table S3). The sequences were aligned using MAFFT 7.164 (Katoh & Standley 2013), with the [--auto] and [--sort] function (Suppl. File S4). After assessing sequence patterns between groups of aligned sequences, we selected a subset of 89 sequences including the query sequence (Table 1). For this subset, we computed the best-scoring tree under maximum likelihood using RAxML 8 (Stamatakis 2014), with the universal GTR-Gamma model and 1000 bootstrap pseudoreplicates.

Results and Discussion

BLAST results

MegaBLAST returned as best matches for the query sequence numerous unidentified or incompletely identified reference sequences, including [Fungal endophyte], *Basidiomycota* sp., [Uncultured fungus], *Polyporales* sp., *Agaricales* sp. and *Trametes* sp. The only more specific, yet imprecise identification was *Trametes* cf. *cubensis*. Matches with unambiguous species identifications included (in sequence of decreasing Max Score) *Lenzites elegans*, *Geotrichum candidum*, *Leiotrametes lactinea*, *Leiotrametes flavida* and *Trametes cubensis* (Fig. 2). Note that we do not give authorities for these name citations because they merely represent sequence ID labels, and authorities would convey a false sense of accuracy. The following matches corresponded to a near-99.5%
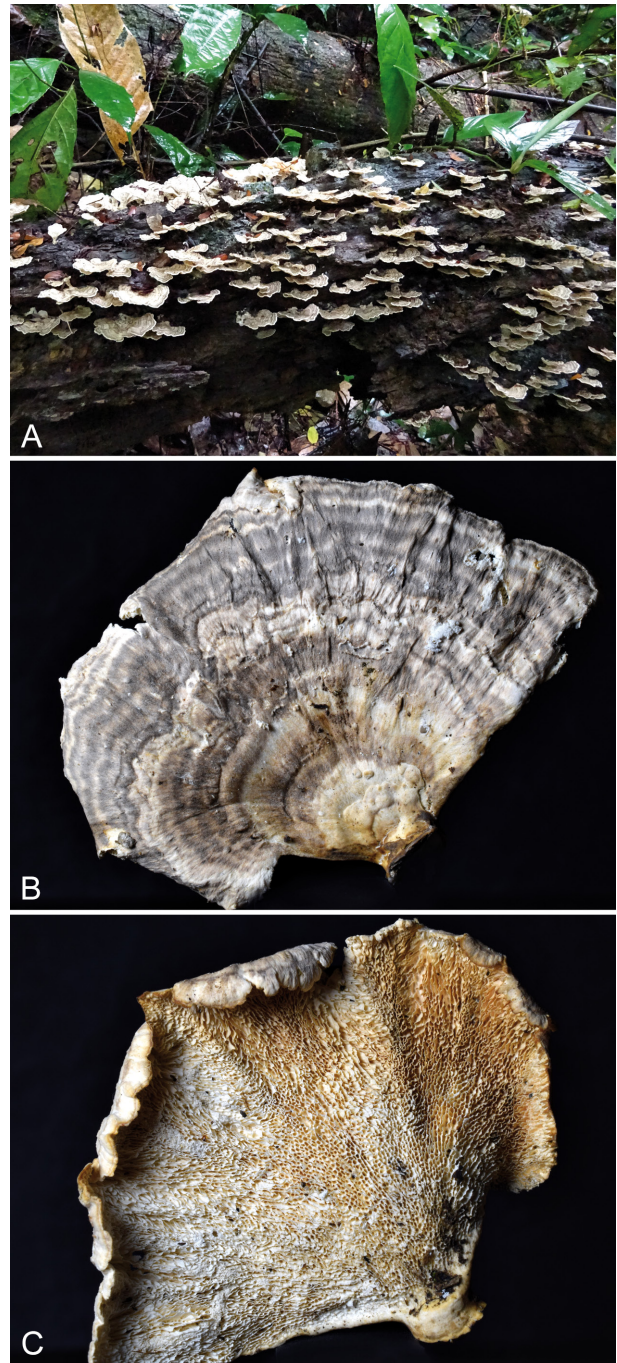
Fig. 1. Material of *Trametes* sp. from Vietnam (B 70 0107235) used for the fungal ITS barcoding exercise. – A: population in situ; B: basidioma from above showing colour zonation; C: basidioma from below, showing pored hymenophore.

similarity threshold: [Fungal endophyte], *Trametes* cf. *cubensis*, *Basidiomycota* sp. (99.48% each), *Lenzites elegans* (99.47%) and *Geotrichum candidum* (99.46%). Below the standard 98.5% threshold level, additional matches were *Trametes* cf. *cubensis* and [Uncultured fungus]. Results using BLASTn were largely congruent, with minor differences in the sorting of matches according to the Max Score (Fig. 3). Besides the substantial ambiguity and lack of definition of these BLAST results, obviously mislabelled reference sequences included the

Table 1. ITS GenBank accession numbers for sequences used in the phylogenetic analysis. The newly generated sequence is indicated in boldface (for detailed voucher information see Material and methods). Authorities are not given for name citations because they merely represent sequence ID labels, and authorities would convey a false sense of accuracy.

Genus/higher taxon labels as used in GenBank	Species	ITS GenBank accession number	Genus/higher taxon labels as used in GenBank	Species	ITS GenBank accession number
Cubamyces	*menziesii*	**MT928350**	*Trametes*	*lactinea*	HM756192
Geotrichum	*candidum*	KU377517	*Trametes*	*lactinea*	HM756193
Trametes	*cubensis*	JN164905	*Trametes*	*lactinea*	JN645069
Trametes	*cubensis*	JN164922	*Trametes*	*lactinea*	JN645072
Trametes	*cubensis*	JN164923	*Trametes*	*lactinea*	JN645076
Trametes	*cubensis*	JN164989	*Trametes*	*lactinea*	JN645102
Trametes	*cubensis*	KP771708	*Trametes*	*lactinea*	JN645104
Trametes	*cubensis*	KU863059	*Trametes*	*lactinea*	JX082368
Trametes	*cubensis*	KY948714	*Trametes*	*lactinea*	JX082369
Trametes	*cubensis*	MF363158	*Trametes*	*lactinea*	KC848319
Trametes	*cubensis*	MG719297	*Trametes*	*lactinea*	KC848320
Trametes	*cubensis*	MH016940	*Trametes*	*manilaensis*	KC848314
Trametes	*cubensis*	MH212092	*Trametes*	cf. *manilaensis*	KC848321
Trametes	*cubensis*	MN068933	*Trametes*	*menziesii*	JN645071
Trametes	cf. *cubensis*	KC848315	*Trametes*	*menziesii*	JN645085
Trametes	cf. *cubensis*	KC848318	*Trametes*	*menziesii*	JN645103
Trametes	cf. *cubensis*	KC848323	*Trametes*	*menziesii*	KC848289
Trametes	cf. *cubensis*	KC848324	*Trametes*	*menziesii*	KC848326
Trametes	cf. *cubensis*	KJ654414	*Trametes*	*menziesii*	KC848328
Trametes	cf. *cubensis*	KJ654415	*Trametes*	*orientalis*	KX880643
Trametes	cf. *cubensis*	KJ654416	*Trametes*	*orientalis*	KX880644
Trametes	cf. *cubensis*	KJ654510	*Trametes*	*orientalis*	KX880645
Trametes	cf. *cubensis*	KJ654513	*Trametes*	*sanguinea*	KM596815
Trametes	cf. *cubensis*	KJ654514	*Pycnoporus*	*cinnabarinus*	AF363756
Trametes	cf. *cubensis*	KJ654515	*Pycnoporus*	*cinnabarinus*	AF363766
Lenzites	*elegans*	HQ248217	*Pycnoporus*	*cinnabarinus*	KX880629
Lenzites	*elegans*	JN182901	*Pycnoporus*	*cinnabarinus*	MH855575
Trametes	*elegans*	AY684178	*Pycnoporus*	*cinnabarinus*	MK795188
Trametes	*elegans*	EU661879	*Pycnoporus*	*coccineus*	HM595574
Trametes	*elegans*	KM438012	*Pycnoporus*	*coccineus*	JF308952
Trametes	*elegans*	KP262029	*Pycnoporus*	*coccineus*	KP255836
Trametes	*elegans*	KP780433	*Pycnoporus*	*coccineus*	KP255837
Trametes	*elegans*	KR080517	*Pycnoporus*	*coccineus*	KP255839
Trametes	*elegans*	KT763333	*Pycnoporus*	*sanguineus*	FJ234191
Trametes	*elegans*	LC120834	*Pycnoporus*	*sanguineus*	FJ234195
Trametes	*elegans*	LC176779	*Pycnoporus*	*sanguineus*	FJ234196
Trametes	*elegans*	MG270573	*Pycnoporus*	*sanguineus*	GQ982884
Leiotrametes	*flavida*	KC589130	*Pycnoporus*	*sanguineus*	GQ982886
Leiotrametes	*flavida*	KC589131	*Agaricales*	sp.	MK079616
Leiotrametes	*lactinea*	KC589126	*Agaricales*	sp.	MK079617
Leiotrametes	*lactinea*	KC589127	*Agaricales*	sp.	MK079618
Leiotrametes	*lactinea*	KC589128	*Polyporales*	sp.	MF621973
Leiotrametes	*lactinea*	KC589129	*Basidiomycota*	sp.	JX416577
Leiotrametes	*lactinea*	KP012950	*Basidiomycota*	sp.	JX416578
Leiotrametes	*lactinea*	KU982588	Fungal endophyte		KR016326
Leiotrametes	*lactinea*	MG712334	Uncultured fungus		GQ999222
Leiotrametes	*lactinea*	MH855702	Uncultured fungus		GQ999228
Leiotrametes	*lactinea*	MH862825	Uncultured fungus		GQ999291
Leiotrametes	*lactinea*	MH910526	Uncultured fungus		GQ999293
Leiotrametes	*lactinea*	MK564604	Uncultured fungus		GQ999305
Trametes	*lactinea*	GQ982888	Uncultured fungus		KF800601
Trametes	*lactinea*	HM756191	Uncultured fungus		KM104070

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download ▾ GenBank Graphics Distance tree of results ⚙

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Fungal endophyte isolate 622 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5	1050	1050	100%	0.0	99.48%	KR016326.1
Trametes cf. cubensis 11B-25 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S	1050	1050	100%	0.0	99.48%	KJ654514.1
Basidiomycota sp. FPF38b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl	1050	1050	100%	0.0	99.48%	JX416578.1
Basidiomycota sp. FPF38a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl	1050	1050	100%	0.0	99.48%	JX416577.1
Trametes cf. cubensis 11B-26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5	1044	1044	100%	0.0	99.31%	KJ654515.1
Trametes cf. cubensis 6-LS-3-B-34N(FB D)-A 18S ribosomal RNA gene, partial sequence; internal transcribe	1033	1033	100%	0.0	98.96%	KJ654513.1
Uncultured fungus clone LX042400-122-057-F05 internal transcribed spacer 1, partial sequence; 5.8S riboso	1033	1033	100%	0.0	98.96%	GQ999293.1
Uncultured fungus clone L042882-122-062-H07 internal transcribed spacer 1, partial sequence; 5.8S ribosom	1033	1033	100%	0.0	98.96%	GQ999222.1
Uncultured fungus clone LX042400-122-057-F03 internal transcribed spacer 1, partial sequence; 5.8S riboso	1027	1027	100%	0.0	98.79%	GQ999291.1
Lenzites elegans isolate PCT.22 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.	1024	1024	97%	0.0	99.47%	HQ248217.1
Trametes cf. cubensis 4-LS-2-A-55(FBA)A 18S ribosomal RNA gene, partial sequence; internal transcribed s	1014	1014	97%	0.0	99.12%	KJ654510.1
Uncultured fungus clone L042883-122-063-A01 internal transcribed spacer 1, partial sequence; 5.8S ribosom	1011	1011	97%	0.0	99.11%	GQ999228.1
Geotrichum candidum strain C234 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	1007	1007	96%	0.0	99.46%	KU377517.1
Leiotrametes lactinea voucher NAMA 2015-172 Mushroom Observer # 247141 small subunit ribosomal RNA	1005	1005	100%	0.0	98.10%	MH910526.1
Trametes cf. cubensis 11F-21b 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8	1005	1005	100%	0.0	98.10%	KJ654416.1
Trametes cf. cubensis 11F-21A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8	1005	1005	100%	0.0	98.10%	KJ654415.1
Leiotrametes lactinea voucher S.D. Russell MycoMap 6699 small subunit ribosomal RNA gene, partial seque	1002	1002	100%	0.0	97.93%	MK564604.1
Uncultured fungus clone LX042767-122-058-D10 internal transcribed spacer 1, partial sequence; 5.8S riboso	1002	1002	97%	0.0	98.76%	GQ999305.1
Polyporales sp. 2 TT-2017 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1000	1000	100%	0.0	97.93%	MF621973.1
Trametes sp. isolate Gv07 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1000	1000	100%	0.0	97.93%	MF101395.1
Leiotrametes flavida strain DMC813 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1000	1000	100%	0.0	97.92%	KC589132.1
Leiotrametes flavida strain DMC811 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	1000	1000	100%	0.0	97.92%	KC589130.1
Uncultured fungus clone rcw_134 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 a	1000	1000	100%	0.0	97.93%	KM104070.1
Uncultured fungus clone CMH510 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,	1000	1000	100%	0.0	97.93%	KF800601.1
Trametes cubensis voucher TJV93_213sp 18S ribosomal RNA gene, partial sequence; internal transcribed s	1000	1000	100%	0.0	97.93%	JN164923.1
Trametes cubensis voucher CR96 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,	1000	1000	100%	0.0	97.93%	JN164922.1
Leiotrametes lactinea strain DMC348 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	998	998	100%	0.0	97.76%	KC589127.1
Trametes cubensis voucher CRM90 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	998	998	99%	0.0	97.93%	JN164989.1
Agaricales sp. isolate 29-ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac	996	996	99%	0.0	97.92%	MK079617.1
Trametes cubensis voucher AJ866 small subunit ribosomal RNA gene, partial sequence; internal transcribed	996	996	99%	0.0	98.09%	KY948714.1
Agaricales sp. isolate 32ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed space	994	994	99%	0.0	97.92%	MK079618.1
Agaricales sp. isolate 26-ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac	994	994	99%	0.0	97.92%	MK079616.1
Leiotrametes lactinea strain DMC377 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	994	994	100%	0.0	97.76%	KC589128.1
Leiotrametes lactinea strain DMC346 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	994	994	100%	0.0	97.59%	KC589126.1
Leiotrametes lactinea strain CBS 362.35 small subunit ribosomal RNA gene, partial sequence; internal transc	992	992	99%	0.0	97.92%	MH855702.1
Trametes cubensis isolate UZ526_17 small subunit ribosomal RNA gene, partial sequence; internal transcribe	992	992	99%	0.0	97.92%	MF363158.1
Trametes cubensis voucher UOC-SIGWI-S26 18S ribosomal RNA gene, partial sequence; internal transcribe	992	992	99%	0.0	97.75%	KP771708.1

Fig. 2. MegaBLAST results for the query sequence of *Trametes* sp. from Vietnam.

ascomycetous yeast *G. candidum* (*Saccharomycetales*: *Dipodascaceae*) and *Agaricales* sp. (given that *Trametes* s.lat. is a member of *Polyporales*).

The sequence mislabelled with the name *Geotrichum candidum* (KU377517) is a typical example of things that can go wrong in the process of generating, identifying and depositing sequence data that subsequently become reference sequences. The sequence was generated as part of a study of fungi causing cushion gall disease in *Theobroma cacao* L. in Venezuela (Castillo & al. 2016). The

Sequences producing significant alignments:

Select: All None   Selected:0

Alignments | Download ⌄ | GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Fungal endophyte isolate 622 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and	1026	1026	100%	0.0	99.48%	KR016326.1
Trametes cf. cubensis 11B-25 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S	1026	1026	100%	0.0	99.48%	KJ654514.1
Basidiomycota sp. FPF38b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl	1026	1026	100%	0.0	99.48%	JX416578.1
Basidiomycota sp. FPF38a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl	1026	1026	100%	0.0	99.48%	JX416577.1
Trametes cf. cubensis 11B-26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and	1018	1018	100%	0.0	99.31%	KJ654515.1
Uncultured fungus clone LX042400-122-057-F05 internal transcribed spacer 1, partial sequence; 5.8S riboso	1012	1012	100%	0.0	98.96%	GQ999293.1
Uncultured fungus clone L042882-122-062-H07 internal transcribed spacer 1, partial sequence; 5.8S ribosom	1012	1012	100%	0.0	98.96%	GQ999222.1
Trametes cf. cubensis 6-LS-3-B-34N(FB D)-A 18S ribosomal RNA gene, partial sequence; internal transcribe	1010	1010	100%	0.0	98.79%	KJ654513.1
Uncultured fungus clone LX042400-122-057-F03 internal transcribed spacer 1, partial sequence; 5.8S riboso	1008	1008	100%	0.0	98.79%	GQ999291.1
Lenzites elegans isolate PCT.22 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.	1001	1001	97%	0.0	99.47%	HQ248217.1
Trametes cf. cubensis 4-LS-2-A-55(FBA)A 18S ribosomal RNA gene, partial sequence; internal transcribed s	993	993	97%	0.0	99.12%	KJ654510.1
Uncultured fungus clone L042883-122-063-A01 internal transcribed spacer 1, partial sequence; 5.8S ribosom	990	990	97%	0.0	99.11%	GQ999228.1
Geotrichum candidum strain C234 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	985	985	96%	0.0	99.46%	KU377517.1
Uncultured fungus clone LX042767-122-058-D10 internal transcribed spacer 1, partial sequence; 5.8S riboso	983	983	97%	0.0	98.76%	GQ999305.1
Leiotrametes flavida strain DMC813 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	982	982	100%	0.0	97.92%	KC589132.1
Leiotrametes flavida strain DMC811 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	982	982	100%	0.0	97.92%	KC589130.1
Leiotrametes lactinea voucher NAMA 2015-172 Mushroom Observer # 247141 small subunit ribosomal RNA	980	980	100%	0.0	97.93%	MH910526.1
Trametes cf. cubensis 11F-21b 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8	980	980	100%	0.0	97.93%	KJ654416.1
Trametes cf. cubensis 11F-21A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8	980	980	100%	0.0	97.93%	KJ654415.1
Leiotrametes lactinea voucher S.D. Russell MycoMap 6699 small subunit ribosomal RNA gene, partial seque	977	977	100%	0.0	97.75%	MK564604.1
Polyporales sp. 2 TT-2017 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	976	976	100%	0.0	97.75%	MF621973.1
Trametes sp. isolate Gv07 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	976	976	100%	0.0	97.75%	MF101395.1
Uncultured fungus clone rcw_134 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 a	976	976	100%	0.0	97.75%	KM104070.1
Uncultured fungus clone CMH510 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,	976	976	100%	0.0	97.75%	KF800601.1
Trametes cubensis voucher TJV93_213sp 18S ribosomal RNA gene, partial sequence; internal transcribed s	976	976	100%	0.0	97.75%	JN164923.1
Trametes cubensis voucher CR96 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,	976	976	100%	0.0	97.75%	JN164922.1
Leiotrametes lactinea strain DMC348 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	975	975	100%	0.0	97.58%	KC589127.1
Trametes cubensis voucher CRM90 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	974	974	99%	0.0	97.75%	JN164989.1
Daedalea flavida strain CBS 158.35 small subunit ribosomal RNA gene, partial sequence; internal transcribed	973	973	99%	0.0	97.91%	MH855616.1
Agaricales sp. isolate 29-ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac	972	972	99%	0.0	97.75%	MK079617.1
Leiotrametes lactinea strain DMC346 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	972	972	100%	0.0	97.41%	KC589126.1
Trametes cubensis voucher AJ866 small subunit ribosomal RNA gene, partial sequence; internal transcribed	971	971	99%	0.0	97.91%	KY948714.1
Leiotrametes lactinea strain DMC377 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	971	971	100%	0.0	97.58%	KC589128.1
Agaricales sp. isolate 32ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac	970	970	99%	0.0	97.74%	MK079618.1
Agaricales sp. isolate 26-ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac	970	970	99%	0.0	97.74%	MK079616.1
Trametes cubensis voucher UOC-SIGWI-S26 18S ribosomal RNA gene, partial sequence; internal transcribe	969	969	99%	0.0	97.58%	KP771708.1
Leiotrametes lactinea strain CBS 362.35 small subunit ribosomal RNA gene, partial sequence; internal transc	968	968	99%	0.0	97.74%	MH855702.1
Trametes cubensis isolate UZ526_17 small subunit ribosomal RNA gene, partial sequence; internal transcribe	968	968	99%	0.0	97.74%	MF363158.1

Fig. 3. BLASTn results for the query sequence of *Trametes* sp. from Vietnam.

study did not implement a specific pipeline to identify the ITS sequences generated from the fungal cultured, but simply stated: "Similarity was inspected for each sequence against the non-redundant database maintained by the National Center for Biotechnology Information using the BLAST algorithm …" (Castillo & al. 2016: 134). The sequence in question was deposited under the name *G. candidum*, but in the published paper identified

as *Clonostachys rosea* f. *catenulata* (J. C. Gilman & E. V. Abbott) Schroers., a species in the *Bionectriaceae* (*Hypocreales*), quite unrelated to *G. candidum* Link (in a different subphylum of *Ascomycota*). Yet, both mega-BLAST and BLASTn place this sequence unambiguously in *Trametes* s.lat. (*Polyporales*). Not only is there a mismatch between the published identification and that deposited in GenBank (which ultimately serves as reference identification) in this case, but both identifications are also plain wrong. Unfortunately, such cases are not rare (Nilsson & al. 2012). Where clades have been amply sampled, such problems will eventually reveal themselves, but they can have substantial consequences for automated identification pipelines and instances where such sequences are the only ones available for a given name.

Blasting the query sequence in the UNITE curated ITS database (Abarenkov & al. 2010; Kõljalg & al. 2013; Nilsson & al. 2019), using the default analysis option [https://unite.ut.ee/analysis.php], did not markedly improve the result (Fig. 4). The best matches included the unresolved sequence labels [*Fungi*] and [*Polyporales*] (both matching the highest score), the genus names *Daedaleopsis*, *Leiotrametes*, *Lenzites* and *Trametes*, and the fully resolved species names *Dipodascus geotrichum*, *Trametes elegans* (both matching the highest score), *Leiotrametes lactinea*, *T. menziesii* (both matching the second highest score) and *T. flavida*. When using the UNITE General FASTA Release for local BLAST [https://unite.ut.ee/repository.php; Abarenkov & al. 2020a, b], we obtained *T. cubensis* (JN164989) unambiguously as best hit, both with singletons set as RefS and when including global and 97% singletons (Suppl. Files S5, S6).

The sequence mislabelled as *Geotrichum candidum* (KU377517) in GenBank returned the label *Dipodascus geotrichum* in the BLAST result from UNITE (Fig. 4). The label name in the corresponding UNITE record [https://unite.ut.ee/bl_forw.php?id=706809] was since updated based on our findings (R. Nilsson, pers. comm. July 2020). The name *D. geotrichum* (E. E. Butler & L. J. Petersen) Arx is the currently accepted name for *G. candidum* according to Species Fungorum [http://www.speciesfungorum.org/GSD/GSDspecies.asp?RecordID=313244]. This synonymy was automatically provided through UNITE when blasting query sequences before the name update, although the underlying sequence has nothing to do with either *Geotrichum* or *Dipodascus* but represents a basidiomycete in *Trametes* s.lat. (see above).

Phylogenetic analysis

Initial alignment and sorting of the 1518 ITS sequences encompassing *Trametes* s.lat. in MAFFT placed the query sequence from Vietnam in a well-defined group of reference sequences including the names *Leiotrametes flavida*, *L. lactinea*, *Lenzites elegans*, *Trametes cubensis*, *T. elegans*, *T. lactinea*, *T. manilaensis*, *T. menziesii* and *T.*

Sequences producing significant alignments:	Score (Bits)	E Value
MG270573 Trametes elegans	946	0.0
KU377517 Dipodascus geotrichum	946	0.0
KR016326 Fungi	946	0.0
KR016012 Fungi	946	0.0
KM265876 Fungi	946	0.0
KJ654514 Trametes	946	0.0
JX416578 Polyporales	946	0.0
JX416577 Trametes	946	0.0
HQ248217 Trametes elegans	946	0.0
KC848326 Trametes menziesii	942	0.0
HM756193 Leiotrametes lactinea	942	0.0
KJ654515 Trametes	939	0.0
KJ654510 Trametes	939	0.0
KC848289 Trametes menziesii	937	0.0
GQ999228 Polyporales	935	0.0
GQ999293 Polyporales	933	0.0
GQ999222 Polyporales	933	0.0
KJ654513 Trametes	930	0.0
GQ999305 Polyporales	928	0.0
GQ999291 Polyporales	928	0.0
KC848328 Trametes menziesii	919	0.0
KP262029 Trametes elegans	913	0.0
MK024175 Trametes	902	0.0
MH855616 Trametes flavida	902	0.0
KT210101 Lenzites	902	0.0
KT210099 Trametes flavida	902	0.0
KT210096 Trametes flavida	902	0.0
KT186190 Daedaleopsis	902	0.0
KR154994 Trametes	902	0.0
KP780433 Trametes elegans	902	0.0
KF425651 Trametes flavida	902	0.0
KC589132 Leiotrametes	902	0.0
KC589131 Leiotrametes	902	0.0
KC589130 Leiotrametes	902	0.0
JX082361 Lenzites	902	0.0
JF712851 Trametes flavida	902	0.0
HQ916734 Trametes	902	0.0

Fig. 4. Results of blasting the query sequence of *Trametes* sp. from Vietnam in the curated fungal ITS database UNITE.

orientalis (Suppl. File S4) To analyse this subset phylogenetically, we included an outgroup of sequences corresponding to three species of *Pycnoporus* P. Karst. (Justo & Hibbett 2011; Welti & al. 2012; Carlson & al. 2014), for a total of 89 terminals (75 ingroup terminals including the query sequence). The best-scoring maximum likelihood tree resolved the ingroup into four lineages (Fig. 5). The distribution of identified names among these lineages was thereby highly ambiguous. Lineages A and B (three terminals each) included two names each, lineage C included five names (two homotypic) plus the query sequence, and lineage D, comprising the bulk of ingroup sequences, encompassed seven names (two homotypic). The only names restricted to a single lineage were *Leiotrametes flavida* (lineage A), *T. menziesii* (lineage C), and *T. manilaensis* and *T. orientalis* (lineage D).

In UNITE, the sequences of all four lineages represented a single species hypothesis at the default threshold of

Table 2. Geographic origin of sequenced samples in the *menziesii* clade. The ocean air samples do not have specific locality information; the authors of that study gave the sampling area as follows: "The cruise covered regions between China, Australia, Antarctica, and Argentina, including the East China Sea, South China Sea, South Pacific Ocean, East Indian Ocean, South Atlantic Ocean, and Southern Ocean" (Fröhlich-Nowoisky & al. 2012: 1129).

ITS GenBank accession number	Geographic origin	Reference
JN645085	Martinique	Welti & al. 2012
JN645103	Martinique	Welti & al. 2012
HQ248217	Colombia	Navia & al. 2011
JX416577	Colombia (soil and litter sample)	López-Quintero & al. 2013
JX416578	Colombia (soil and litter sample)	López-Quintero & al. 2013
KU377517	Venezuela	unpublished
HM756193	Mauritius	Vlasák & Kout 2011
KC848289	China	unpublished
KC848326	China	unpublished
KC848328	China	unpublished
MT928350 (isolate DB42771)	Vietnam	this paper
KP262029	India	Lakshmi & al. 2017
MG270573	India	unpublished
KJ654510	Indonesia	Glen & al 2014
KJ654513	Indonesia	Glen & al 2014
KJ654514	Indonesia	Glen & al 2014
KJ654515	Indonesia	Glen & al 2014
KR016326	Papua New Guinea (fungal endophyte)	Vincent & al. 2016
JN645071	New Caledonia	Welti & al. 2012
GQ999222	ocean air sample (uncultured fungus)	Fröhlich-Nowoisky & al. 2012
GQ999228	ocean air sample (uncultured fungus)	Fröhlich-Nowoisky & al. 2012
GQ999291	ocean air sample (uncultured fungus)	Fröhlich-Nowoisky & al. 2012
GQ999293	ocean air sample (uncultured fungus)	Fröhlich-Nowoisky & al. 2012
GQ999305	ocean air sample (uncultured fungus)	Fröhlich-Nowoisky & al. 2012

98.5% (1.5% distance): *Trametes cubensis* (Mont.) Sacc. | SH1565941.08FU. Setting the threshold to 99.5% (0.5% distance) recovered the species hypotheses as follows: Lineage A (3 sequences; *Leiotrametes flavida*) returned *Polyporaceae* Fr. ex Corda | SH1954860.08FU (34 sequences, only few matching). Lineage B (3 sequences; unnamed) returned *T. elegans* (Spreng.) Fr. | SH1954901.08FU (3 sequences, exact match). Lineage C (15 sequences; *T. menziesii*) returned in *Dikarya* | SH1954863.08FU (24 sequences, mostly matching). Finally, lineage D (53 sequences; *T. cubensis*) returned *T. cubensis* (Mont.) Sacc. | SH1954850.08FU (109 sequences, mostly matching). Neither threshold thus exactly matched the lineages based on the phylogenetic analysis.

Generic placement of the query sequence

The exact identification of the query sequence not only depended on the identification of its most closely relat-ed sequences, but also on the taxonomic concept in the target group, both at genus and species level. Indeed, BLAST results did not allow to identify the correct genus from simple inspection. The generic classification in the trametoid clade, including widely used genera such as *Trametes* s.str., as well as *Lenzites* and *Pycnoporus*, is disputed and in flux (Corner 1989; Justo & Hibbett 2011; Welti & al. 2012). Based on a five-marker data set, Justo & Hibbett (2011) discussed alternative classification scenarios, including the distinction of up to five genera: *Trametes* s.str. (= *Coriolus* Quél.), *Lenzites* (= *Pseudotrametes* Bondartsev & Singer), *Coriolopsis*, *Artolenzites* Falck and *Pycnoporus* (incl. *Cubamyces* Murrill). In their topology, *Pycnoporus* s.lat. [incl. *T. cubensis* (Mont.) Sacc. and *T. ljubarskyi* Pilát] was resolved as supported sister to a clade containing the remaining genera, so a two-genus solution (*Pycnoporus* s.lat. vs. *Trametes* s.lat.) would have also been possible. The authors instead opted to recognize a single, large genus *Trametes*, subsuming

Pycnoporus and other names into synonymy. Their concept was still much narrower than that of Corner (1989), who also suggested to include *Daedaleopsis* J. Schröt., *Datronia* Donk and *Earliella* Murrill within *Trametes*, genera shown to fall outside the trametoid clade (Justo & Hibbett 2011; Justo & al. 2017). On the other hand, while recognizing the trametoid clade as a single genus, *Trametes*, Justo & Hibbett (2011) maintained a larger number of genera in the polyporoid sister clade, including the aforementioned three genera, plus *Amauroderma* Murrill, *Ganoderma* P. Karst., *Lentinus* Fr. and *Polyporus*. This was also reflected in a recent three-marker study focusing on family-level delimitations (Justo & al. 2017), where the core of *Polyporaceae* was divided into two strongly supported clades, one representing a single genus, *Trametes* s.lat., and the other including the bulk of the remaining genera. The main argument for this differential taxonomic approach was the apparent absence of clear phenotypical characters separating the variously proposed within the trametoid clade, with the exception of the orange-red pigmented *Pycnoporus*, which in a narrow sense was nested within a grade of lineages lacking or with inconspicuous pigments (Justo & Hibbett 2011).

Using three markers, Welti & al. (2012) obtained a topology similar to that of Justo & Hibbett (2011), although less well resolved, dividing the trametoid clade into three supported subclades plus one singleton. The first subclade containing *Pycnoporus*, the *Trametes lactinea* clade and the *T. ljubarskyi* clade, the second subclade corresponded to *Artolenzites* (*T. elegans*), and the third subclade to *Trametes* s.str. plus *Lenzites*. Welti & al. (2012) opted for a more fine-scaled concept, retaining *Artolenzites* and *Pycnoporus* as separate genera and, as a consequence, proposing the formal recognition of the *T. lactinea* clade as a new genus, *Leiotrametes* Welti & Courtec., overlooking that the name *Cubamyces* was already available for this clade (Justo & Hibbett 2011; Carlson & al. 2014; Kalichman & al. 2020). Besides the unique pigmentation found in *Pycnoporus*, the authors provided presumably diagnostic phenotype features for *Leiotrametes* (= *Cubamyces*), such as a glabrous upper surface, absence of a black line under the pileipellis and lack of parietal crystals. Gomes-Silva (2010) reported the absence of a black line not only for *T. cubensis* and *T. lactinea*, but for a number of unrelated species, including *T. pubescens* (Schumach.) Pilát (which belongs in *Trametes* s.str.), *T. pavonia* (Hook.) Ryvarden [representing a small, distinctive lineage within the trametoid clade, close to *T. gibbosa* (Pers.) Fr.], and *T. modesta* (Kunze ex Fr.) Ryvarden (which falls outside the trametoid clade). Therefore, the taxonomic usefulness of this feature at genus level is unclear. Nevertheless, we consider the four-genus solution (in phylogenetic order: *Trametes* s.str., *Artolenzites*, *Pycnoporus*, *Leiotrametes*) a workable compromise at present, given the frequent lack of a clear, straightforward correlation between phenotype and phylogenetic relationships in many fungal lineages (Lücking & al. 2020).

The reason for adopting this concept is in line with the guidelines laid out by Vellinga & al. (2015), including discussing alternative options. First, the *Pycnoporus* clade is both highly distinctive and monophyletic and it is one of the best-known tropical macrofungi; subsuming it within *Trametes* s.lat. would result in the nomenclatural loss of an enigmatic element of tropical fungal biota, recognized far beyond expert mycologists. Therefore, to fulfil the criterion of reciprocal monophyly, *Trametes* s.lat. is best split into more than one genus, requiring the recognition of *Cubamyces*. The taxonomic and geographic coverage of the trametoid clade is very broad compared to other polypores (Justo & al. 2017); for the ITS alone more than 1500 accessions are available (Suppl. File S4), and the underlying topology for the recognition of both *Pycnoporus* and *Cubamyces* is well supported by the combined use of three to five markers. An important guideline that Vellinga & al. (2015) were not including is that a classification should always reflect phylogenetic relationships, regardless of whether this is fully in line with phenotype features, particularly in organisms where the phenotype is known to exhibit high evolutionary plasticity. In this case, while *Cubamyces* is more similar to *Trametes* s.str., it is phylogenetically more closely related to *Pycnoporus*, and so the similarity with *Trametes* is plesiomorphic and, for some reason, *Cubamyces* evolved as a lineage separate from *Trametes* s.str. without much diverging from it phenotypically. This concept of (semi-)cryptic genera is analogous to (semi-)cryptic species, although rarely recognized as such.

The recently published *Compendium of generic names of agarics and Agaricales* (Kalichman & al. 2020) also accepted *Cubamyces* as a separate genus within the trametoid clade, along with *Artolenzites*, *Cellulariella* Zmitr. & Malysheva, *Coriolopsis*, *Cubamyces*, *Lenzites*, *Pilatotrama* Zmitr., *Pycnoporus*, *Sclerodepsis* Cooke and *Trametes* s.str. Our approach is conservative in comparison, but there seems to be a strong tendency to accept more than one genus in the trametoid clade.

Under the genus concept accepted here, our phylogenetic analysis (Fig. 5) placed the query sequence from Vietnam into the genus *Leiotrametes* (Welti & al. 2012). These authors formally distinguished two species: *L. lactinea* (Berk.) Welti & Courtec. and *L. menziesii* (Berk.) Welti & Courtec. A third species, *L. flavida* (Lév.) S. Falah & al., was added later (Falah & al. 2018), but that combination was not validly published (Turland & al. 2018: Art. 41.1; May & al. 2019: Art. F.5.1). These three names correspond to lineages A, C and D in our ITS-based phylogeny, whereas lineage B remained unnamed (Fig. 5). Unfortunately, when establishing *Leiotrametes*, Welti & al. (2012) overlooked *Trametes cubensis*, which based on sequence data had been shown to be closely related to, and perhaps synonymous with, *T. lactinea* by Justo & Hibbett (2011). Because *Polyporus cubensis* Mont. antedates *P. lactineus* Berk. by six years (Montane 1837; Berkeley 1843), the epithet *cubensis* has
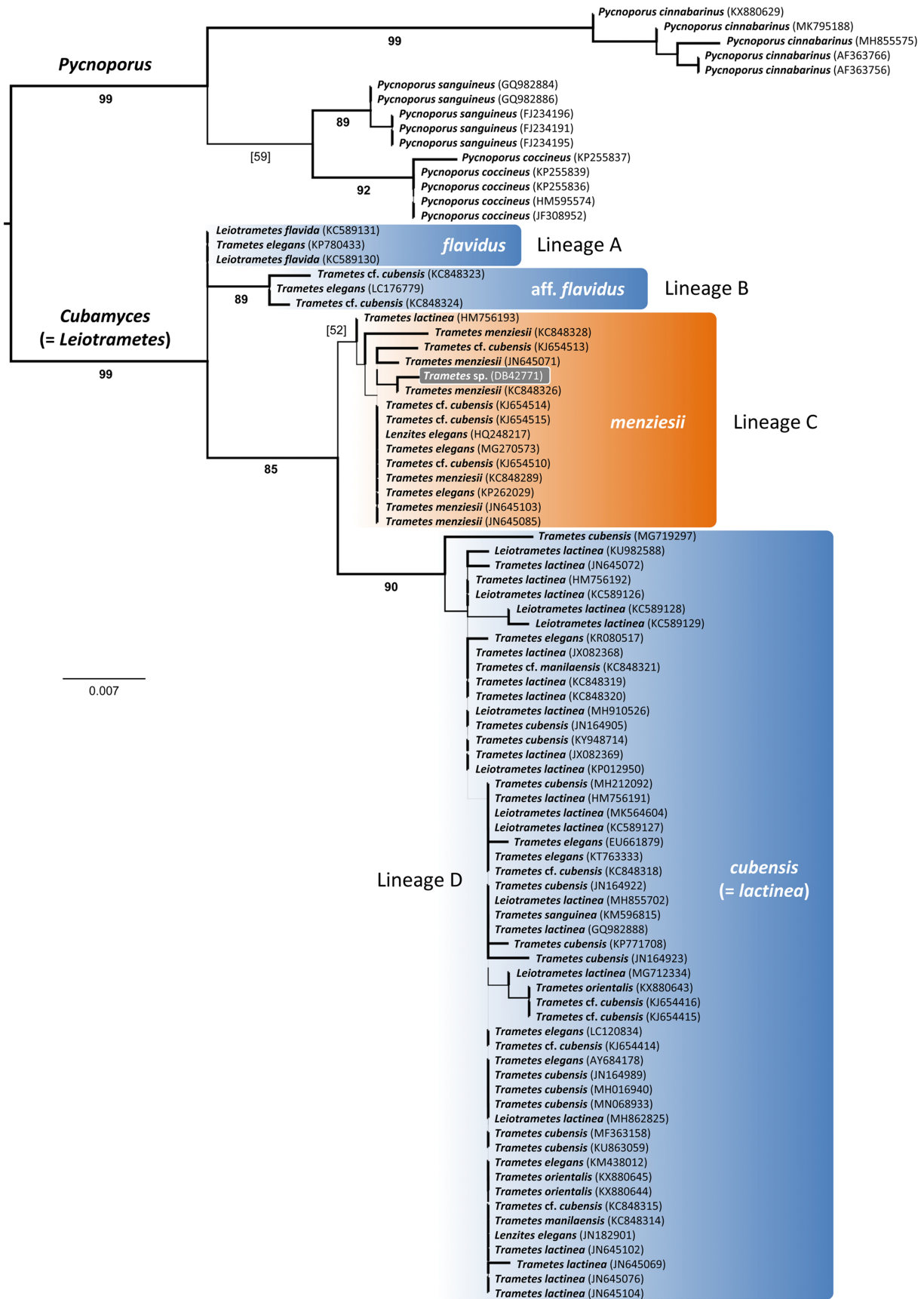
Fig. 5. Best-scoring maximum likelihood tree based on the ITS fungal barcoding marker of the clade containing the query sequence of *Trametes* sp. from Vietnam. Terminal labels indicate the original sequence labels, whereas stem branch names and boxes indicate the applicable genus and species names after a nomenclatural verification process.

priority when the two names are considered synonymous, but in either case the generic name *Cubamyces* antedates *Leiotrametes*.

Species delimitation in *Cubamyces*

Our analysis clearly separated *Leiotrametes flavida* (≡ *Cubamyces flavidus*, see below) and *L. menziesii* (≡ *C. menziesii*, see below), but placed most terminals labelled *Trametes cubensis* or *L. lactinea* into a single lineage (D), which besides *T. cubensis* (17 instances) and *L. lactinea* (23 instances) included also the names *T. elegans* (Spreng.) Fr. (seven instances), *T. manilaensis* (Lloyd) Teng (two instances) and *T. orientalis* (Yasuda) Imazeki (three instances). Of these, the use of the name *T. elegans* clearly represented misidentifications, as this species belongs in the *Artolenzites* clade (Justo & Hibbett 2011; Welti & al. 2020) and genuine samples are documented in GenBank by multiple ITS sequences (Suppl. File S4). Unfortunately, no type material has been sequenced for any of the other four names, and the question therefore arises to what extent the submitted identifications are genuine. In the case of *T. cubensis*, five of the 23 accessions stem from expert identifications, namely JN164905, JN164922, JN164923, JN164989 and KY948714 (Justo & Hibbett 2011; Justo & al. 2017); all others, including all labelled *T.* cf. *cubensis* in lineages B and C, resulted from DNA barcoding studies and hence represent secondary identifications. The five accessions based on expert identifications all belong to lineage D, the *cubensis/lactinea* clade. In the case of *L. lactinea*, 18 out of 24 accessions were based on expert identifications (Vlasák & Kout 2011; Berrin & al. 2012; Welti & al. 2012; Vu & al. 2019), including several unpublished sequences, all also clustering in lineage D. The five accessions bearing the names *T. manilaensis*, *T.* cf. *manilaensis* and *T. orientalis* were all apparently based on non-expert identifications and, as far as we can tell, these accessions have not been published other than in GenBank.

In the taxonomic literature, *Trametes cubensis* and *Leiotrametes lactinea* are generally distinguished by the reddish brown upper cuticle ("basal crust") in the former (Corner 1989; Gomes-Silva & al. 2010; Zmitrovich & al. 2012), a character not mentioned in the protologue (Montagne 1837). According to Zmitrovich & al. (2012), *T. cubensis* also has larger basidiospores than *L. lactinea* (7–9.5 × 3–3.5 μm vs. 5.5–7 × 2.5–3 μm). The two species do agree in some peculiar anatomical details, such as cystidiiform ends in the skeletal and binding hyphae, also characteristic for *Lenzites* within the trametoid clade (Corner 1989). Their ITS-based placement in a single clade, without resolution, therefore leaves three interpretations: (1) one of the two sets of accessions is entirely based on misidentifications; (2) the two taxa represent a single species and the reddish brown coloration beneath the tomentum is of no taxonomic value; (3) the two taxa represent separate species but ITS cannot resolve them.

Considering the expert identifications for both taxa in the clade, we can reject option (1). Also, the *cubensis/lactinea* clade encompasses accessions from all tropical regions, including the amphi-Caribbean region in Florida and northern South America (Venezuela, French Guiana), as well as India, Sri Lanka and Thailand (Fig. 6), i.e. the type regions for both taxa, so it would be extremely unlikely that either *T. cubensis* or *L. lactinea* existed in these regions as separate taxa that have not yet been sequenced.

Option (2) is a possibility, given the presumed phenotypic plasticity in these fungi at genus and species level. For instance, Corner (1989) found strong morphological resemblance of *Trametes cubensis* with *Earliella scabrosa* (Pers.) Gilb. & Ryvarden [as *Trametes scabrosa* (Pers.) G. Cunn.], a distantly related taxon in the polyporoid clade (Justo & Hibbett 2011; Justo & al. 2017), but at no point compared *T. cubensis* to *L. lactinea*, underlining the likelihood of phenotypic homoplasy in these fungi. Conspecificity of *T. cubensis* with *L. lactinea* would also be in line with the status of another name used in this clade, *T. orientalis*. Hattori & Ryvarden (1994) considered this taxon a possible variant of *T. lactinea*, although morphological differences were recognized by these authors and also by Zmitrovich & al. (2012), who distinguished *T. orientalis* from *T. lactinea* by the slightly broader basidiospores (5–6 × 3–3.5 μm vs. 5.5–7 × 2.5–3 μm) and the orange-brown vs. cream to tan colour of the pileus. Given the heterotypic synonymy already established for both *T. cubensis* and *L. lactinea*, with at least five synonyms (Species Fungorum 2020a), it would not be surprising to discover that *T. cubensis*, *L. lactinea*, plus *T. manilaensis*, and *T. orientalis*, all refer to the same species. However, because of the apparent morphological and anatomical differences between *T. cubensis* and *Leiotrametes lactinea* (Corner 1989; Gomes-Silva & al. 2010; Zmitrovich & al. 2012), we believe that option (3) is the most likely explanation. ITS has been shown to exhibit lack of resolution in recently evolving species complexes of fungi including lichens (Lücking & al. 2020) and, in some cases, approaches with microsatellite markers or RADseq demonstrated the presence of distinct lineages even when ITS was identical, such as in the lichenized genus *Usnea* Dill. ex Adans. (Lagostina & al. 2018; Grewe & al. 2018). We therefore consider *T. cubensis* and *L. lactinea* two closely related but separate species. The same potentially applies to *T. manilaensis* and *T. orientalis*, although in this case we cannot be certain that the accessions deposited under these names were correctly identified based on morphology and anatomy. As outlined above, *T. orientalis* has been considered a possible variant of *T. lactinea*; therefore, even if representing a distinct taxon, misidentifications by non-specialists are likely. The same applies to *T. manilaensis*, which is distinguished from *T. lactinea* largely by cylindric vs. ellipsoid basidiospores (Zmitrovich & al. 2012), a feature that would be difficult to assess by non-specialists, even if both were distinct species.
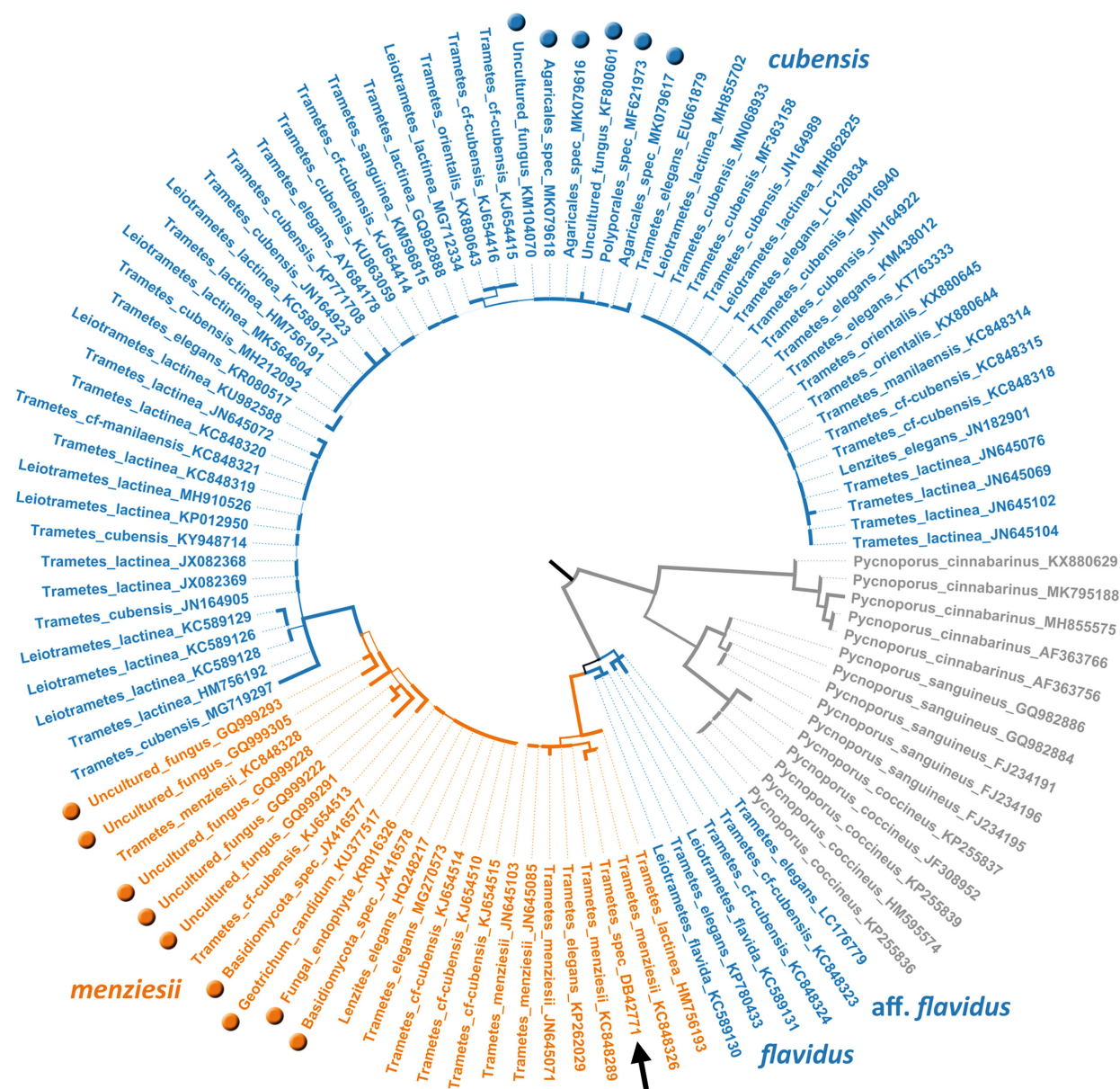
Fig. 6. Best-scoring maximum likelihood tree (circle tree) based on the ITS fungal barcoding marker of the clade containing the query sequence of *Trametes* sp. from Vietnam, with the 15 best-matching unnamed and incompletely labeled sequences from megablast and BLASTn results added and highlighted. The arrow indicates the query sequence.

Since we can exclude entirely erroneous identifications as the source for *Trametes cubensis* and *Leiotrametes lactinea* clustering in a single clade (lineage D), by extension both taxa must be congeneric, independent of their interpretation as a single or two separate species. It follows that the introduction of the genus *Leiotrametes* in the concept elaborated by Welti & al. (2012) was superfluous, because the older name *Cubamyces* Murrill (Murrill 1905) is available for this clade (Justo & Hibbett 2011; Carlson & al. 2014). Welti & al. (2012) entirely overlooked *T. cubensis* and so their name *Leiotrametes* is legitimate, but must nevertheless be replaced with *Cubamyces* if that lineage is recognized in a separate genus, as we do here. As a result, formal synonymization of *Leiotrametes* with *Cubamyces* is required, as well as

the combination of at least three names into *Cubamyces*, two of which represent distinctive lineages in the ITS-based phylogeny, namely *T. flavida* and *T. menziesii*, and *L. lactinea* if maintained as a separate species for the time being.

Cubamyces Murrill in Bull. Torrey Bot. Club 32: 480. 1905 [MycoBank MB 17418]. – Type: *Cubamyces cubensis* (Mont.) Murrill.
= *Leiotrametes* Welti & Courtec. in Fungal Diversity 55: 60. 2012 [MycoBank MB 563399]. – Type: *Leiotrametes lactinea* (Berk.) Welti & Courtec.

Cubamyces cubensis (Mont.) Murrill in Bull. Torrey Bot. Club 32: 480. 1905 [MycoBank MB 468969] ≡

Polyporus cubensis Mont. in Ann. Sci. Nat., Bot., sér. 2, 8: 364. 1837 ≡ *Trametes cubensis* (Mont.) Sacc., Syll. Fung. 9: 198. 1891 ≡ *Ungulina cubensis* (Mont.) Pat., Essai Tax. Hyménomyc.: 102. 1900 ≡ *Daedalea cubensis* (Mont.) A. Roy in Canad. J. Bot. 60: 1015. 1982 ≡ *Lenzites cubamyces* Teixeira in Revista Bras. Bot. 15: 126. 1992 [not *Lenzites cubensis* Berk. & M. A. Curtis in J. Linn. Soc., Bot. 10: 303. 1869].

Cubamyces flavidus (Lév.) Lücking, **comb. nov.** [MycoBank MB 836819] ≡ *Daedalea flavida* Lév. in Ann. Sci. Nat., Bot., sér. 3, 2: 198. 1844 ≡ *Striglia flavida* (Lév.) Kuntze, Revis. Gen. Pl. 2: 871. 1891 ≡ *Daedaleopsis flavida* (Lév.) A. Roy & A. Mitra in Canad. J. Bot. 61: 2979. 1984 ≡ *Trametes flavida* (Lév.) Zmitr. & al. in Int. J. Med. Mushr. 14: 310. 2012 ≡ *Leiotrametes flavida* (Lév.) S. Falah & al. in Biodiversitas 19: 634. 2018.

Cubamyces lactineus (Berk.) Lücking, **comb. nov.** [MycoBank MB 836820] ≡ *Polyporus lactineus* Berk. in Ann. Mag. Nat. Hist. 10: 373. 1843 ≡ *Trametes lactinea* (Berk.) Sacc., Syll. Fung. 6: 343. 1888 ≡ *Coriolus lactineus* (Berk.) G. Cunn. in Proc. Linn. Soc. New South Wales 75: 229. 1950 ≡ *Leiotrametes lactinea* (Berk.) Welti & Courtec. in Fungal Diversity 55: 60. 2012.

Cubamyces menziesii (Berk.) Lücking, **comb. nov.** [MycoBank MB 836821] ≡ *Polyporus menziesii* Berk. in Ann. Mag. Nat. Hist. 10: 378. 1843 ≡ *Polystictus menziesii* (Berk.) Fr. in Nova Acta Regiae Soc. Sci. Upsal., ser. 3, 1: 74. 1851 ≡ *Microporus menziesii* (Berk.) Kuntze, Revis. Gen. Pl. 3(3): 496. 1898 ≡ *Trametes menziesii* (Berk.) Ryvarden in Norweg. J. Bot. 19: 236. 1972 ≡ *Leiotrametes menziesii* (Berk.) Welti & Courtec. in Fungal Diversity 55: 60. 2012.

Accurate identification of the query sequence

As a result of this extensive verification process, the accurate identification of the query sequence from Vietnam at genus and species level was *Cubamyces menziesii*. This is in line with the morphological and anatomical features of the material (Fig. 1), including the comparatively small, zoned pileus in which the brownish zones become paler grey in the dried stage, the short basal stipe and the rather narrow basidiospores, 5–7 × 2–2.5 μm in size (Corner 1989; Zmitrovich & al. 2012). Given the initial BLAST results, this outcome was unexpected, as the epithet *menziesii* did not appear among the highest BLAST matches (Fig. 2, 3) and also did not appear when using the UNITE General FASTA Release with local BLAST. Surprisingly, the observed mismatch was entirely an artefact of mislabelled reference sequences (Fig. 7). The best matches from both megaBLAST and BLASTn, labelled *Trametes* cf. *cubensis* and *Lenzites elegans*, do not represent these species but belong in the *menziesii* clade. Also, analysis of the 15 unnamed best BLAST matches showed

that nine of them fall within the *menziesii* clade, whereas the remaining six fall within the *cubensis*/*lactinea* clade (Fig. 6). These and other top-scoring hits stem from non-expert barcoding and metabarcoding studies (Fröhlich-Nowoisky & al. 2012; López-Quintero & al. 2013; Glen & al. 2014; Castillo & al. 2016) and thus their identifications, if given at all, represent secondary identifications based on comparison with previously deposited sequences. A single original error can therefore perpetuate itself multiple times, in the process becoming inflated (Gilks & al. 2002). Indeed, after simply relabelling the BLAST results and highlighting their percentage identity values, the best hits for the query sequence were invariably *C. menziesii* (Fig. 7).

Our results also showed that BLAST results may be misleading by revealing wrong relationships. Based on the phylogenetic analysis, the reference sequence closest to the query sequence was *Trametes menziesii* (KC848326). This sequence did not appear among the 35 best BLAST hits (Fig. 2, 3), yet was phylogenetically the most closely related (Fig. 5). Automated identification pipelines using similarity threshold approaches may therefore produce erroneous identifications, and only verification using an alignment-based phylogenetic analysis is able to detect such issues. Overall, the problems of DNA barcoding associated with sequence labelling and similarity-based inference of phylogenetic relationships are well known (Nilsson & al. 2006; Kang & al. 2010; Ovaskainen & al. 2010; Hofstetter & al. 2019) but mostly ignored by automated identification pipelines and other BLAST-based approaches. This exemplifies the necessity to define standards for labelling of reference sequences and the importance of multiple alignment-based phylogenetic identifications (Nilsson & al. 2012, 2017; Schoch & al. 2014, 2017; Geiger & al. 2016; Lücking & al. 2020).

Overall, this study revealed numerous problems with similarity-based molecular barcoding in fungi, particularly if done automatically without critical check and verification, as is often the case in broad fungal biodiversity studies using metabarcoding approaches (Tedersoo & al. 2018; Ruppert & al. 2019). One might consider this example an outlier, but *Trametes* s.lat. is a group of conspicuous and well-known macrofungi that has been studied phylogenetically in much detail (Corner 1989; Ryvarden 1991; Ko & Jung 1999; Zhang & al. 2006; Justo & Hibbett 2011; Vlasák & Kout 2011; Welti & al. 2012; Zmitrovich & Malysheva 2013; Olusegun 2014; Carlson & al. 2014; Cui & al. 2019). Similar problems have been documented in other fungal DNA barcoding studies focusing on macrofungi (e.g. Hofstetter & al. 2019). These findings are troublesome, as the situation is likely worse in less well-known groups of microfungi (Lücking & al. 2020).

As shown above, the situation is further complicated by unresolved taxonomies or incomplete treatments of published names. In the present case, *Cubamyces menziesii* (lineage C) was strongly supported as sister to *C. cubensis* (lineage D), but was not supported as a separate

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	
☑	Fungal endophyte isolate 622 18S ribosomal RNA gene, partial s	1062	1062	100%	0.0	99.49%	KR016326.1	*menziesii*
☑	Trametes cf. cubensis 11B-25 18S ribosomal RNA gene, partial s	1062	1062	100%	0.0	99.49%	KJ654514.1	*menziesii*
☑	Basidiomycota sp. FPF38b internal transcribed spacer 1, partial	1062	1062	100%	0.0	99.49%	JX416578.1	*menziesii*
☑	Basidiomycota sp. FPF38a internal transcribed spacer 1, partial	1062	1062	100%	0.0	99.49%	JX416577.1	*menziesii*
☑	Trametes cf. cubensis 11B-26 18S ribosomal RNA gene, partial s	1057	1057	100%	0.0	99.32%	KJ654515.1	*menziesii*
☑	Trametes cf. cubensis 6-LS-3-B-34N(FB D)-A 18S ribosomal RN	1046	1046	100%	0.0	98.97%	KJ654513.1	*menziesii*
☑	Uncultured fungus clone LX042400-122-057-F05 internal transcr	1046	1046	100%	0.0	98.97%	GQ999293.1	*menziesii*
☑	Uncultured fungus clone L042882-122-062-H07 internal transcril	1046	1046	100%	0.0	98.97%	GQ999222.1	*menziesii*
☑	Uncultured fungus clone LX042400-122-057-F03 internal transcr	1040	1040	100%	0.0	98.80%	GQ999291.1	*menziesii*
☑	Lenzites elegans isolate PCT.22 18S ribosomal RNA gene, partia	1026	1026	96%	0.0	99.47%	HQ248217.1	*menziesii*
☑	Leiotrametes lactinea voucher NAMA 2015-172 Mushroom Obse	1018	1018	100%	0.0	98.13%	MH910526.1	*cubensis*
☑	Trametes cf. cubensis 11F-21b 18S ribosomal RNA gene, partial	1018	1018	100%	0.0	98.13%	KJ654416.1	*cubensis*
☑	Trametes cf. cubensis 11F-21A 18S ribosomal RNA gene, partia	1018	1018	100%	0.0	98.13%	KJ654415.1	*cubensis*
☑	Trametes cf. cubensis 4-LS-2-A-55(FBA)A 18S ribosomal RNA g	1016	1016	96%	0.0	99.12%	KJ654510.1	*menziesii*
☑	Leiotrametes lactinea voucher S.D. Russell MycoMap 6699 sma	1014	1014	100%	0.0	97.96%	MK564604.1	*cubensis*
☑	Trametes sp. isolate Gv07 small subunit ribosomal RNA gene, p	1013	1013	100%	0.0	97.96%	MF101395.1	*cubensis*
☑	Leiotrametes flavida strain DMC811 18S ribosomal RNA gene, p	1013	1013	100%	0.0	97.95%	KC589130.1	*flavida*
☑	Uncultured fungus clone CMH510 18S ribosomal RNA gene, par	1013	1013	100%	0.0	97.96%	KF800601.1	*cubensis*
☑	Trametes cubensis voucher TJV93_213sp 18S ribosomal RNA g	1013	1013	100%	0.0	97.96%	JN164923.1	*cubensis*
☑	Trametes cubensis voucher CR96 18S ribosomal RNA gene, par	1013	1013	100%	0.0	97.96%	JN164922.1	*cubensis*
☑	Leiotrametes lactinea strain DMC348 18S ribosomal RNA gene,	1011	1011	100%	0.0	97.79%	KC589127.1	*cubensis*
☑	Uncultured fungus clone L042883-122-063-A01 internal transcrit	1011	1011	96%	0.0	99.11%	GQ999228.1	*menziesii*
☑	Geotrichum candidum strain C234 internal transcribed spacer 1,	1009	1009	95%	0.0	99.46%	KU377517.1	*menziesii*
☑	Uncultured fungus clone rcw_134 18S ribosomal RNA gene, par	1009	1009	99%	0.0	97.95%	KM104070.1	*cubensis*
☑	Polyporales sp. 2 TT-2017 small subunit ribosomal RNA gene, p	1007	1007	100%	0.0	97.79%	MF621973.1	*cubensis*

Fig. 7. BLASTn result for the query sequence under a scenario of corrected reference sequence labels. For this graph, the BLAST was repeated, and therefore the individual scores are slightly different from those depicted in Fig. 3.

species (Fig. 5). When comparing the ITS between the two clades, there were eight consistent substitutions and two indels across a total of 560 sites (Suppl. File S4), resulting in 98.2% similarity, just below the broadly employed standard threshold of 98.5% (Abarenkov & al. 2010; Kõljalg & al. 2013; Jeewon & Hyde 2016; Vu & al. 2016; Nilsson & al. 2019). Notably, almost all differences were found in the ITS1 region, indicating a higher level of resolution in that region for this group of fungi. We therefore consider *C. menziesii* a good species, though closely related to *C. cubensis*, and the lack of support is likely an artefact of the taxon set used for the analysis. When reducing the taxon set to these two species, support for *C. menziesii* increased to 59% (not shown). The sequence labelled *Trametes* cf. *cubensis* (MG719297) appeared to be of lower quality, with several ambiguous or aberrant base calls, and removing this sequence increased support for both *C. menziesii* and *C. cubensis* to 100% each (not shown). Low-quality sequences may therefore be another potential source of erroneous results. Improper terminal trimming has been identified as one problem of sequence quality (Nilsson & al. 2017), because only a few aberrant terminal base calls greatly affect pairwise identity values, although it is not rare for sequences to exhibit low quality or odd base calls across the entire read.

Cubamyces menziesii is considered a subcosmopolitan, although largely Asian-Australasian species with numerous heterotypic synonyms, including, among others, *Trametes blumei* (Lév.) G. Cunn., *T. grisea* Pat., *T. meleagris* (Berk.) Imazeki, *T. murina* (Cooke) Ryvarden and *T. vittata* (Berk.) Bres. (Kiet 1988; Corner 1989; Buchanan & Rywarden 2000; Zmitrovich & al. 2012; Species Fungorum 2020b). None of these taxa appears to have been sequenced from original material or epitypes, and so their synonymy status is unclear. For instance, *T. murina* was treated as a separate species by Ryvarden (1972, 1978). Therefore, even if our exercise resulted in a phylogenetically accurate identification of the query sequence as *C. menziesii*, it still remains unclear whether this identification is ultimately correct. Given that the species was

originally described from Sumatra, Indonesia (Berkeley 1843), the identification of Vietnamese material with that name is likely. As in the *cubensis/lactinea* clade, the *menziesii* clade encompassed accessions from all tropical regions (Table 2), including several from the type region, Indonesia, and one expert accession from not too far away, New Caledonia (Welti & al. 2012). However, if *C. menziesii* turned out to be a complex of more than one species, some of its heterotypic synonyms originating from continental Southeast Asia would be alternative candidate names for the query sequence, such as *Polyporus nepalensis* Berk. (Nepal), *P. corium* Berk. (India), *P. gaudichaudii* Lév. (Singapore), and *P. thwaitesii* Berk. and *P. vittatus* Berk. (both Sri Lanka). However, because the currently accepted heterotypic synonymy for *C. menziesii* is in line with the geographic data of the sequenced material and with agreement in phenotypic characters (Kiet 1988; Corner 1989; Buchanan & Rywarden 2000; Zmitrovich & al. 2012), we consider *C. menziesii* as the valid identification in this case.

Conclusions

Our study demonstrates that accurate identification of fungi through molecular barcoding is currently not a fast-track approach that can be achieved through automated pipelines. Following up on an initial BLAST approach, the preferred method of automated barcoding, we had to go through numerous steps, including alignment-based phylogenetic analysis and a time-consuming verification process, including a thorough revision of the underlying classification and nomenclature, to arrive at an accurate identification. Numerous issues were revealed along the way, including (in part grossly) mislabelled reference sequences, mismatches between published and deposited sequence identifications, sequence quality, lack of important sequence metadata such as geographic origin, and even genus and species concepts and nomenclature.

Surprisingly, most of these problems could be easily remedied through two steps. The first would be options for third-party annotations of reference sequences directly in GenBank and other primary repositories. Curated secondary repositories such as UNITE provide an example of how sequence annotations can be implemented, but it is crucial that annotations become directly visible in primary repositories as well. Most workers download sequences for research from primary repositories, such as GenBank, and use the taxonomy given in the sequence label. They will therefore not be aware of separately published annotations not visible in the primary sequence label. The second would be the ongoing attempt to complete ITS reference sequences for as many fungi as possible.

Already the first option would make a substantial difference: if all ITS sequences tested and verified in this study could now be annotated by the authors of this paper directly in GenBank, further molecular barcoding approaches would immediately give the correct results already with BLAST approaches. Currently only the original submitters can update primary sequence data, and unfortunately, there seems to be low motivation to do so, even if there is awareness of issues. There is work involved in updating records, with no reward in terms of publications. However, as a community, we all depend on the quality of reference data, so we should not seek reward in housekeeping work but consider it a necessary obligation concerning our own data. Still, a much better solution would be enabling third-party annotations, as for instance standard in natural history collections. Imagine if only the original describer of a new species was allowed to annotate the type material! We therefore strongly advocate for the possibility of direct third-party annotations in GenBank and other primary sequence repositories, following established mechanisms in curated secondary databases such as UNITE.

In lieu of such a possibility, the use of third-party updates in flat table format is a possible alternative, listing the sequence accession number, the original and the corrected identification (where available with Index Fungorum or MycoBank registration number), and the study which provided the alternative identification, with its DOI (Suppl. Table S7).

Acknowledgements

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Supplmentary content

The following content is published electronically under Supplemental content online (see https://doi.org/10.3372/wi.50.50302).

Supplementary File S1. Forward chromatogram of the ITS query sequence (from isolate DB42771).

Supplementary File S2. Reverse chromatogram of the ITS query sequence (from isolate DB42771).

Supplementary Table S3. ITS GenBank accessions of the sequences used for the global analysis in this study.

Supplementary File S4. Global ITS alignment for the genus *Trametes* s.lat.

Supplementary File S5. Results of the search with the UNITE General FASTA Release for local BLAST, with singletons set as RefS.

Supplementary File S6. Results of the search with the UNITE General FASTA Release for local BLAST, including global and 97% singletons.

Supplementary Table S7. Example of an annotation table for published sequence accessions, using unique identifiers for accessions, proposed re-identifications (MycoBank registration numbers) and authority (DOI of corresponding study).