



## *Cellulariella warnieri* (Basidiomycota, Polyporales) and its doubles

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### Abstract

Species of *Polyporales* with a lamelliform hymenophore were traditionally classified in the genus *Lenzites*, until recent phylogenetic analyses revealed their affinities with various poroid lineages, leading to the re-classification of most of them. Nevertheless, the phylogenetic relationships of the lamellate *Lenzites warnieri* Durieu & Mont. have yet to be resolved. The recent erection of *Cellulariella* Zmitr. & V. Malysheva to encompass *L. warnieri* and *L. acuta* did little to disentangle the cross-synonymies between lamellate polypores of the *Trametes* group. To clarify the phylogenetic affinities of *L. warnieri*, we combined molecular data based on five markers (LSU, ITS, EF1- $\alpha$ , RPB1, RPB2) with morphological features of various collections belonging to the *Trametes* clade. In Bayesian phylogenetic reconstructions based on RPB1, RPB2 and EF1- $\alpha$ , *L. warnieri* has an unresolved position on globally poorly supported cladograms. Conversely, phylogenetic analyses of the combined (ITS+LSU) sequences support a monophyletic clade encompassing *L. warnieri* and *L. acuta*, which forms a sister group, within a broader clade encompassing *Leiotrametes*, *Pycnoporus* and the sub-clade *T. ljubarskyi* – *T. cingulata*. These phylogenetic results are also supported by micromorphological data. Here we redefine the genus *Cellulariella* based on the moderate amount of scarcely branched, not flagelliform binding hyphae in the context, the pointed and fusiform hyphal ends protruding through the hymenium and a strictly lamelliform (lenzitoid) hymenophore. A lectotype is designated for *Cellulariella warnieri*. The name *Lenzites acuta* has also been misapplied to a species of *Leiotrametes*, possibly identical to *Lenzites tenuis*. *Lenzites tenuis* is here recombined as *Leiotrametes tenuis* comb. nov.

**Keywords** – *Lenzites* – phylogeny – *Polyporaceae* – taxonomy – *Trametes*

### Introduction

In the order *Polyporales* Gäum. (Basidiomycota, Agaricomycetes), most species with a lamelliform hymenophore were traditionally classified in the genus *Lenzites* Fr. (Fries 1835), until their affinities with poroid species led to the re-classification of most of them. Currently, as confirmed by molecular data, the type species of *Lenzites*, *Lenzites betulinus* (L.) Fr. is included in

the mostly poroid genus *Trametes* Fr. (Justo & Hibbett 2011, Welti et al. 2012). Consequently, all species formerly classified in *Lenzites* because of their lamelliform (or, by extension, daedaleoid) hymenophore have already been assigned to a genus in conformity with phylogenetic data, but for a small minority. Amongst them, the Mediterranean species *Lenzites warnieri* Durieu & Mont., originally described from Algeria, is easy to identify but has not yet found a natural place in modern systematics despite available molecular sequences. Vellinga et al. (2015) reported *Lenzites warnieri* forming an unsupported branch within the *Trametes s. lat.* clade, which makes its generic attribution undecidable, especially if *Trametes* were split into several genera as proposed by Welti et al. (2012). Meanwhile, exposing their personal interpretation of still unsupported phylogenetic reconstructions of *Trametes s. lat.*, Zmitrovich & Malysheva (2013) created *Cellulariella* Zmitrovich & V. Malysheva, based on *Lenzites acuta* Berk. along with *L. warnieri*, without further justification.

Before being confirmed as an autonomous species by David (1967), *L. warnieri* was often interpreted as a lamellate form of *Daedalea quercina* (Kavina & Pilát 1936). Microscopic features led David (op. cit.) to consider it (under the posterior synonym *Lenzites reichardtii* Schulzer) as a closer relative of *Lenzites betulinus*, a conclusion supported by phylogenetic results and physiology (Nobles 1958, Welti et al. 2012).

During our fungal surveys in the Neotropics (French Guiana and the Lesser Antilles), we learned about another unnamed species within *Leiotrametes* Welti & Courtecuisse. This species is characterized by a highly variable hymenophore and was probably identified as «*Daedalea quercina*» by Patouillard (in Duss 1904) from Guadeloupe (Welti et al. 2012). This confusion extends to *D. tenuis* Berk., a tropical Asian species also characterized by a variable hymenophore that was considered by Berkeley (1842) to be close “in many respects” to *D. quercina*.

Compared to *D. tenuis*, the lamelliform hymenophore of *Lenzites warnieri* is a constant feature, although the primordial zone may be locally daedalean in some specimens (Rivoire 2020). Conversely, Ryvarden & Johansen (1980) described *L. acuta* with a highly variable hymenophore, from lamellate to poroid. Further bibliographic investigations and comparisons with herbarium collections suggested that the long list of synonyms enumerated, e.g. by Ryvarden & Johansen (1980), Zmitrovich & Malysheva (2013) or MycoBank ([www.mycobank.org](http://www.mycobank.org), continuously updated) hides important morphological differences. Heterogeneity of both hymenophoral and microscopical features within *L. acuta s. lat.* required additional investigations.

This study aims at clarify the phylogenetic position of *Lenzites warnieri*, to discuss the legitimacy of *Cellulariella* within the *Trametes*-clade (Welti et al. 2012) and to refine its circumscription through the study of the type collections of *L. acuta*, *L. warnieri* and an additional collection of the latter from the Algerian type locality. This study aims also at disentangle the cross-synonymies between *L. acuta* and *D. tenuis*.

## Materials & Methods

### Microscopical observations

Thin radial and transversal sections were made on various exsiccata of molecularly confirmed collections of *L. warnieri*, holotype specimens of *L. acuta* and *Daedalea tenuis* Berk., and various species of *Trametes s.str.*, *Artolenzites* Falck, *Leiotrametes* and *Pycnoporus* P. Karst. A total of 29 specimens were analyzed (Table 1). Three staining processes were used to highlight the hyphal constitution: 1) stained with Congo red, then examined, 2) stained with Congo red before examination with KOH 5%, 3) stained 10 min in Congo red, washed in KOH 5%, stained again 10 min in Cotton blue lactophenol, observed in 1% Ruthenium red in aqueous solution.

### DNA extraction and PCR amplifications

Genomic DNA was extracted from the context of 5 dry specimens and 2 dikaryotic isolates (Table 2). Each sample was incubated 1 h at 65°C in 500 µL extraction buffer (100 mM Tris/HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) CTAB, 0.2% (v/v) 2-mercaptoethanol and 0.1 mg

mL<sup>-1</sup> proteinase K). One volume of chloroform/isoamyl alcohol (24/1; v/v) was added after incubation. The mixture was gently mixed and centrifuged 10 min at 9500 g.

**Table 1** List of specimens studied for morphology.

| Genus                | Species                | Herbarium number          |
|----------------------|------------------------|---------------------------|
| <i>Artolenzites</i>  | <i>A. elegans</i>      | SW/Mart10-91 (LIP)        |
|                      | <i>A. elegans</i>      | SW/Mart11-07 (LIP)        |
|                      | <i>A. elegans</i>      | SW/Mart10-78 (LIP)        |
| <i>Cellulariella</i> | <i>C. warnieri</i>     | ND169 (LIP)               |
|                      | <i>C. warnieri</i>     | Epitype 0001798 (LIP)     |
|                      | <i>C. warnieri</i>     | Neotype PC 0723637        |
|                      | <i>Lenzites acuta</i>  | Holotype K(M) 168874      |
| <i>Leiotrametes</i>  | <i>L. lactinea</i>     | SW/Guad10-42 (LIP)        |
|                      | <i>L. lactinea</i>     | SW/Mart10-93 (LIP)        |
|                      | <i>L. lactinea</i>     | GUY/08-16 (LIP)           |
|                      | <i>L. lactinea</i>     | Guad/10-181 (LIP)         |
|                      | <i>L. menziesii</i>    | SW/Mart14-16 (LIP)        |
|                      | <i>L. menziesii</i>    | SW/Mart15-22 (LIP)        |
|                      | <i>L. sp.</i>          | SW/Guy12-78 (LIP)         |
|                      | <i>L. sp.</i>          | SW/Guy12-39 (LIP)         |
|                      | <i>Daedalea tenuis</i> | Holotype K(M) Cuming 2037 |
| <i>Pycnoporus</i>    | <i>P. sanguineus</i>   | SW/Mart08-02 (LIP)        |
|                      | <i>P. sanguineus</i>   | SW/Mart06-17 (LIP)        |
|                      | <i>P. sanguineus</i>   | SW/Mart06-18 (LIP)        |
| <i>Trametes</i>      | <i>T. versicolor</i>   | SW/F_NSR16-02 (LIP)       |
|                      | <i>T. betulina</i>     | SW/F_NSR16-01 (LIP)       |
|                      | <i>T. hirsuta</i>      | SW/F_NSR16-03 (LIP)       |
|                      | <i>T. maxima</i>       | RC/GUAD10-87 (LIP)        |
|                      | <i>T. maxima</i>       | SW/Mart10-14 (LIP)        |
|                      | <i>T. polyzona</i>     | RC/Bali13-005 (LIP)       |
|                      | <i>T. polyzona</i>     | SW/Mart15-56 (LIP)        |
|                      | <i>T. polyzona</i>     | SW/Mart11-05 (LIP)        |
|                      | <i>T. cingulata</i>    | unknown                   |
|                      | <i>T. ljubarskyi</i>   | MOU139/957 (LIP)          |

The upper phase was placed in a new tube and gently mixed with one volume of isopropanol before centrifugation. The resulting pellet was rinsed in 70% (v/v) ethanol and centrifuged. The ethanol was then removed and the pellet was air-dried in order to remove any remaining traces. The pellet was finally solubilized in TE buffer (10 mM Tris/HCl pH 8. 0.1 mM EDTA) at 4°C overnight. PCR reactions were performed in a final volume of 50 µL containing 1 µL of DNA, 80 mM Tris·HCl pH 9.4, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.5 mM MgCl<sub>2</sub>, 0.2% (w/v) Tween-20, 0.2 µM dNTPs, 0.2 µM of each primer and 1.25U Taq DNA Polymerase (Euromedex, Souffelweyersheim, France). Primers used for the ITS PCR amplification were ITS-1F (CTTGGTCATTTAGAGGAAGTAA) (Gardes & Bruns 1993) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). Primers used to amplify RPB2 were bRPB2-6F (TGGGGYATGGTNTGYCCYGC) and bRPB2-7.1R (CCCATRGCTGYTTMCCCATDGC) (Matheny et al. 2002). Primers used for the LSU region PCR amplification were LR0R (ACCCGCTGAACCTTAAGC) and LR5 (TCCTGAGGGAACTTCG) (Vilgalys & Hester 1990). Primers used to amplify RPB1 were RPB1-Af (GARTGYCCDGGDCAYTTYGG) and RPB1-Cr (CCNGCDATNTCRTRTCCATRTA) (Matheny et al. 2002). To amplify the elongation factor EF1-α the primers were EF1-983F (GCYCCYGGHCAYCGTGAYTTYAT) and EF1-2218R (ATGACACCRACRGCRACRGTYTG) (Rehner & Buckley 2005). PCR reactions for ITS and RPB2 were conducted as follows: an initial denaturation at 95°C for 2 minutes followed by 40 cycles of 30 s at 95°C; 30 s at 53°C for ITS or 50°C for RPB2; 1 min at 72°C followed by a final

step at 72°C during 5 min. 5 µL of PCR products were resolved at 8 V.cm<sup>-1</sup> on a 1.5% (w/v) agarose gel with 0.5 µg. mL<sup>-1</sup> ethidium bromide in TAE buffer (40 mM Tris/acetate pH 8.3, 1 mM EDTA). PCR reactions for LSU, RPB1 and EF1- $\alpha$  sequences were conducted as follows: an initial denaturation at 94°C for 3 min followed by 10 cycles of 30 s at 94°C, 60 s at 65 °C, 1 min 30 at 72°C decreasing the temperature by 1°C per cycle. Then 35 cycles of 30 s denaturation at 94°C, 60 s at 55°C and 1 min 30 at 72°C followed by a final step at 72°C during 10 min.

Regarding the strain BRFM 972, PCR reactions for LSU were conducted as follows: an initial denaturation step at 94°C for 2 min followed by 40 cycles of 15 s denaturation at 94°C, 30 s annealing at 51°C and 1 min elongation at 72°C. A final extension step at 72°C for 7 min was added at the end of the PCR. PCR products were then sequenced by the Sanger method with ITS-1F, RPB2-b6F, LR0R, RPB1-AF and EF1-983F as sequencing primers.

**Table 2** List of the new sequences generated.

| Species                                  | Strain/Herbarium number | Country    | ITS      | LSU      |
|--|-------------------------|------------|----------|----------|
| <i>Cellulariella (Lenzites) warnieri</i> | BRFM 972                | France     | —        | MW435560 |
| <i>Cellulariella (Lenzites) warnieri</i> | LIP 0001798             | Algeria    | MW435553 | —        |
| <i>Trametes</i> sp.                      | SWMart14_15             | Martinique | MW435554 | MW435561 |
| <i>Trametes membranacea</i>              | SWMart15_15             | Martinique | MW435555 | —        |
| <i>Trametes pavonia</i>                  | BRFM 1554               | Martinique | MW435556 | —        |
| <i>Trametes polyzona</i>                 | SWMart15_56             | Martinique | MW435557 | —        |
| <i>Trametes polyzona</i>                 | RC Bali 13005110        | Indonesia  | MW435558 | —        |

### Sequence alignments

Sequences generated for this study and those obtained from GenBank (Table 3) were aligned under Clustal W (Higgins et al. 1994) and carefully refined manually on the editor in Mega6 (Tamura et al. 2013). ITS, RPB2, RPB1, EF1- $\alpha$ , and LSU sequences have respectively an alignment of 560 bp with 173 variable regions and 121 parsimony characters, 700 bp with 310 variable regions and 261 parsimony characters, 705 bp with 298 variable regions and 240 parsimony characters, 456 bp with 155 variable regions and 121 parsimony characters and 821 bp with 112 variable regions and 80 parsimony characters. For the combined datasets, ITS and LSU sequences were aligned individually then concatenate. Combined sequences have an alignment of 1396 bp with 350 variable regions and 222 parsimony characters.

### Phylogenetic analyses

For each marker a Bayesian analysis was monitored under Mr Bayes v3.1 (Ronquist & Huelsenbeck 2003). According to the Bayesian Information Criterium (BIC) score, SIM+I+G were chosen for RPB1, ITS, LSU and the combined sequences (ITS+LSU), GTR+I+G for RPB2 and K80+I+G for EF1- $\alpha$  sequence analyses as the optimal substitution model defined by TOPALi v2.5 (Milne et al. 2004). Bayesian analyses were conducted using four Metropolis coupled Markov chain Monte Carlo (MCMC) with one sampled in every hundred trees. The first 5,000 trees were excluded from our analyses. For all Bayesian analyses, potential scale reduction factors (PSRF) were reasonably close to 1.0 for all parameters. Bayesian Posterior Probabilities (Bayesian PP) of each node were obtained with 50% majority rules with all compatible partitions. Each method gap was scored as missing and trees were rooted with *Lopharia cinerascens* (Schwein.) G. Cunn.

A Bayesian 50% majority rule consensus tree was shown for each gene. The alignments and phylogenetic trees were deposited in TreeBASE (Reviewer access URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S28879?x-access-code=ec9ba09104ff2683885b1c168fb72efc&format=html>).

**Table 3** Sequence list from GenBank.

| Species                        | Source  | No.             | GenBank Accession No. |          | Country of origin  |
|--------------------------------|---------|-----------------|-----------------------|----------|--------------------|
|                                |         |                 | LSU                   | ITS      |                    |
| <i>Cellulariella warnieri</i>  | voucher | MH72            | –                     | MN909986 | Pakistan           |
| <i>Corioloopsis polyzona</i>   | strain  | OH-184-sp       | AY333817              | –        | Taiwan             |
| <i>Daedalea microsticta</i>    | voucher | 18612           | –                     | FJ403209 | Costa Rica         |
| <i>Daedaleopsis flavida</i>    | strain  | 5A              | JF712849              | –        | India              |
| <i>Leiotrametes</i> sp.        | strain  | CIRM-BRFM 1056  | –                     | JN645059 | French Guiana      |
| <i>Leiotrametes</i> sp.        | strain  | CIRM-BRFM 1080  | –                     | JN645063 | French Guiana      |
| <i>Leiotrametes menziesii</i>  | strain  | CIRM-BRFM 1369  | –                     | JN645085 | French West Indies |
| <i>Leiotrametes menziesii</i>  | strain  | CIRM-BRFM 1281  | –                     | JN645071 | French West Indies |
| <i>Leiotrametes menziesii</i>  | strain  | CIRM-BRFM 1368  | –                     | JN645103 | French West Indies |
| <i>Leiotrametes lactinea</i>   | strain  | CIRM-BRFM 1282  | –                     | JN645072 | French West Indies |
| <i>Leiotrametes lactinea</i>   | strain  | CIRM-BRFM 1371  | –                     | JN645104 | French West Indies |
| <i>Leiotrametes lactinea</i>   | strain  | CIRM-BRFM 1251  | –                     | JN645069 | French Guiana      |
| <i>Lenzites acuta</i>          | voucher | Cui 10091       | KX900689              | KX900642 | China              |
| <i>Lenzites acuta</i>          | voucher | M0138338        | –                     | KF573028 | New Guinea         |
| <i>Lenzites acuta</i>          | voucher | Dai 13595       | KX900691              | KX900644 | China              |
| <i>Lenzites acuta</i>          | voucher | Dai 13103       | KX900690              | KX900643 | China              |
| <i>Lenzites acuta</i>          | voucher | Dai 11621       | –                     | KC848333 | China              |
| <i>Lenzites betulinus</i>      | strain  | DAOM180504      | AF261543              | –        | Canada             |
| <i>Lenzites betulinus</i>      | voucher | Cui 7234        | KC848389              | KC848304 | China              |
| <i>Lenzites vespacea</i>       | voucher | Cui 8758        | KC848339              | –        | China              |
| <i>Lenzites vespacea</i>       | voucher | M0138336        | –                     | KF573027 | New Guinea         |
| <i>Lenzites vespacea</i>       | voucher | Dai 13613       | KX900692              | KX900645 | China              |
| <i>Lenzites warnieri</i>       | strain  | CIRM-BRFM 972   | –                     | GU731567 | France             |
| <i>Lenzites warnieri</i>       | voucher | UOC_KAUNP _MK29 | –                     | KP794599 | Sri Lanka          |
| <i>Lenzites warnieri</i>       | strain  | JZ27            | –                     | MG719284 | India              |
| <i>Lenzites warnieri</i>       | voucher | UOC_KAUNP _K05  | –                     | KR867658 | Sri Lanka          |
| <i>Lopharia cinerascens</i>    | strain  | EL63_97         | AY586687              | –        | USA                |
| <i>Lopharia cinerascens</i>    | strain  | CBS 125884      | MH875543              | MH864085 | New Zealand        |
| <i>Lopharia cinerascens</i>    | voucher | PDD_995857      | –                     | JQ694103 | New Zealand        |
| <i>Pycnoporus cinnabarinus</i> | strain  | KHL8557         | AY586703              | –        | Sweden             |
| <i>Pycnoporus cinnabarinus</i> | strain  | MUCL-30555      | –                     | AF363764 | Belgium            |
| <i>Pycnoporus cinnabarinus</i> | strain  | DAOM72065       | AF393074              | –        | Canada             |
| <i>Pycnoporus coccineus</i>    | strain  | MUCL-38525      | –                     | JN645094 | Australia          |
| <i>Pycnoporus coccineus</i>    | voucher | Cui 7096        | KC848414              | KC848330 | China              |
| <i>Pycnoporus puniceus</i>     | strain  | MUCL-47087      | –                     | FJ234199 | Cuba               |
| <i>Pycnoporus puniceus</i>     | isolate | BCC27595        | FJ372708              | FJ372686 | Thailand           |
| <i>Pycnoporus sanguineus</i>   | isolate | M66             | HM595619              | HM595574 | China              |

**Table 3** Continued.

| Species                           | Source  | No.             | GenBank Accession No. |          | Country of origin  |
|-----------------------------------|---------|-----------------|-----------------------|----------|--------------------|
|                                   |         |                 | LSU                   | ITS      |                    |
| <i>Pycnoporus sanguineus</i>      | strain  | CIRM-BRFM 896   |                       | FJ234188 | French Guina       |
| <i>Pycnoporus sp</i>              | isolate | ZW02-30         | AY684160              | –        | –                  |
| <i>Trametes cingulata</i>         | strain  | MUCL-40167      | –                     | JN645075 | Zimbabwe           |
| <i>Trametes cingulata</i>         | strain  | DMC814          | KC589159              | KC589133 | Cameroon           |
| <i>Trametes cinnabarina</i>       | strain  | CBS 375.34      | MH867081              | MH855576 | Belgium            |
| <i>Trametes cinnabarina</i>       | strain  | DAOM72065       | AF261536              | –        | Canada             |
| <i>Trametes conchifer</i>         | voucher | Dai 8367        | –                     | KC848276 | China              |
| <i>Trametes conchifer</i>         | voucher | FP106793sp      | JN164797              | JN164924 | USA                |
| <i>Trametes cubensis</i>          | voucher | AJ177           | JN164787              | JN164905 | USA                |
| <i>Trametes cubensis</i>          | voucher | TJV93           | –                     | JN164923 | USA                |
| <i>Trametes drummondii</i>        | voucher | BJFC12708       | KC848391              | –        | China              |
| <i>Trametes duplexa</i>           | voucher | Dai 12039       | KC848348              | KC848262 | China              |
| <i>Trametes ectypa</i>            | voucher | FP106037T       | JN164803              | JN164929 | USA                |
| <i>Trametes ectypa</i>            | voucher | FP103976sp      | –                     | JN164961 | USA                |
| <i>Trametes elegans</i>           | strain  | CIRM-BRFM 1280  | –                     | JN645070 | New Caledonia      |
| <i>Trametes elegans</i>           | strain  | CIRM-BRFM 1122  | –                     | JN645066 | French Guiana      |
| <i>Trametes elegans</i>           | strain  | CIRM-BRFM 1378  | –                     | JN645105 | French West Indies |
| <i>Trametes elegans</i>           | voucher | FP105679sp      | JN164799              | JN164944 | USA                |
| <i>Trametes elegans</i>           | voucher | Dai 10748       | JN048785              | JN048766 | China              |
| <i>Trametes ellipsoidea</i>       | voucher | Yuan 3453       | KC848345              | KC848259 | China              |
| <i>Trametes ellipsospora</i>      | voucher | Cui 8384        | KC848337              | –        | China              |
| <i>Trametes ellipsospora</i>      | voucher | Cui 6259        | KC848335              | JN048767 | China              |
| <i>Trametes gibbosa</i>           | strain  | Wu 9411-7       | AY351924              | –        | Taiwan             |
| <i>Trametes gibbosa</i>           | voucher | Cui 7390        | KC848387              | KC848302 | China              |
| <i>Trametes hirsuta</i>           | strain  | CIRM-BRFM 994   | –                     | GU731578 | France             |
| <i>Trametes hirsuta</i>           | voucher | Dai 12319       | KC848383              | KC848298 | China              |
| <i>Trametes hirsuta</i>           | strain  | Wu 9410-39      | AY351922              | –        | Taiwan             |
| <i>Trametes junipericola</i>      | voucher | HUBO 6916       | –                     | AY684171 | Italy              |
| <i>Trametes junipericola</i>      | voucher | 145295 (O)      | KC017763              | KC017758 | China              |
| <i>Trametes aff. junipericola</i> | strain  | BRFM 25         | –                     | JN645088 | China              |
| <i>Trametes ljubarskyi</i>        | voucher | Li 286          | KC848415              | KC848331 | China              |
| <i>Trametes ljubarskyi</i>        | strain  | CIRM-BRFM 957   | –                     | JN645097 | France             |
| <i>Trametes ljubarskyi</i>        | voucher | PRM_622107      | –                     | AY684174 | France             |
| <i>Trametes ljubarskyi</i>        | voucher | Wei 1653        | KC848416              | KC848332 | China              |
| <i>Trametes manilaensis</i>       | voucher | Dai 10747       | KC848398              | KC848314 | China              |
| <i>Trametes cf manilaensis</i>    | voucher | Cui 6240        | –                     | KC848321 | China              |
| <i>Trametes marianna</i>          | voucher | BJFC12714       | KC848418              | KC848334 | China              |
| <i>Trametes maxima</i>            | voucher | Dai 12274       | KC848394              | KC848310 | China              |
| <i>Trametes maxima</i>            | strain  | CIRM-BRFM 1367  | –                     | JN645084 | French West Indies |
| <i>Trametes maxima</i>            | voucher | UOC_KAUNP_MK78b | –                     | KR907875 | Sri Lanka          |
| <i>Trametes maxima</i>            | voucher | Zhou 147        | KC848393              | –        | China              |
| <i>Trametes membranacea</i>       | voucher | PRSC82          | JN164805              | JN164945 | Puerto Rico        |

**Table 3** Continued.

| Species                       | Source  | No.            | GenBank Accession No. |          | Country of origin  |
|-------------------------------|---------|----------------|-----------------------|----------|--------------------|
|                               |         |                | LSU                   | ITS      |                    |
| <i>Trametes menziesii</i>     | Voucher | Yuan 3555      | KC848410              | KC848326 | China              |
| <i>Trametes meyenii</i>       | strain  | CBS 453.76     | MH872762              | MH860991 | India              |
| <i>Trametes aff. meyenii</i>  | strain  | CIRM-BRFM 1121 | –                     | JN645065 | French Guiana      |
| <i>Trametes aff. meyenii</i>  | strain  | CIRM-BRFM 1361 | –                     | JN645083 | French Guiana      |
| <i>Trametes ochracea</i>      | voucher | HHB13445sp     | JN164812              | JN164954 | USA                |
| <i>Trametes ochracea</i>      | voucher | Dai 2005       | KC848357              | –        | China              |
| <i>Trametes ochracea</i>      | voucher | Yuan 2477      | KC848356              | –        | China              |
| <i>Trametes ochracea</i>      | strain  | CBS 257.74     | –                     | JN645077 | Netherland         |
| <i>Trametes orientalis</i>    | strain  | Wu 9708-190    | AY351920              | –        | Taiwan             |
| <i>Trametes pavonia</i>       | voucher | FP103050sp     | JN164806              | JN164958 | USA                |
| <i>Trametes pocas</i>         | voucher | Dai 11577      | KC848340              | KC848253 | China              |
| <i>Trametes pocas</i>         | voucher | Dai 11577      | –                     | KC848253 | China              |
| <i>Trametes pocas</i>         | strain  | Wu 9901-18     | AY351919              | –        | Taiwan             |
| <i>Trametes polyzona</i>      | voucher | OAB0195        | MK736961              | MK736986 | Benin              |
| <i>Trametes polyzona</i>      | strain  | CBS 319.36     | –                     | JN645078 | Zimbabwe           |
| <i>Trametes polyzona</i>      | strain  | WR710-1        | –                     | JN848329 | Thailand           |
| <i>Trametes polyzona</i>      | strain  | RYNF13         | –                     | KT281117 | Thailand           |
| <i>Trametes pubescens</i>     | voucher | FP101414sp     | JN164811              | JN164963 | USA                |
| <i>Trametes pubescens</i>     | voucher | Cui 7569       | KC848377              | –        | China              |
| <i>Trametes pubescens</i>     | voucher | Cui 5904       | KC848376              | –        | China              |
| <i>Trametes pubescens</i>     | voucher | Cui 7571       | KC848375              | –        | China              |
| <i>Trametes sanguinea</i>     | voucher | PRSC95         | JN164795              | JN164982 | Puerto Rico        |
| <i>Trametes socotrana</i>     | voucher | OAB0162        | MK736963              | MK736988 | Benin              |
| <i>Trametes socotrana</i>     | voucher | OAB0131        | MK736962              | MK736987 | Benin              |
| <i>Trametes socotrana</i>     | strain  | MUCL-38649     | –                     | JN645073 | Zimbabwe           |
| <i>Trametes socotrana</i>     | voucher | BJFC12724      | KC848397              | KC848313 | China              |
| <i>Trametes sp</i>            | voucher | Yuan 6455      | KC848386              | –        | China              |
| <i>Trametes sp</i>            | voucher | Zhou 223       | KC848346              | –        | China              |
| <i>Trametes stipitata</i>     | voucher | Yuan 3273      | KC848360              | KC848275 | China              |
| <i>Trametes suaveolens</i>    | strain  | CBS 296.33     | MH866480              | MH855012 | Germany            |
| <i>Trametes suaveolens</i>    | strain  | CIRM-BRFM 578  | –                     | JN645090 | France             |
| <i>Trametes suaveolens</i>    | strain  | DAOM196328     | AF261537              | –        | Canada             |
| <i>Trametes subsuaveolens</i> | voucher | Cui 269        | KC907404              | –        | China              |
| <i>Trametes tephroleuca</i>   | voucher | Wei 1518       | KC848379              | –        | China              |
| <i>Trametes tephroleuca</i>   | voucher | Cui 7977       | KC848381              | KC848296 | China              |
| <i>Trametes thujae</i>        | Voucher | Dai 4953       | KC848373              | KC848288 | China              |
| <i>Trametes thujae</i>        | voucher | Dai 5055       | KC848371              | –        | China              |
| <i>Trametes velutina</i>      | voucher | Dai 10149      | KC848358              | –        | China              |
| <i>Trametes versicolor</i>    | strain  | IUM00100       | DQ208414              | –        | Korea              |
| <i>Trametes versicolor</i>    | strain  | CBS 296.33     | MH866900              | MH855444 | Netherlands        |
| <i>Trametes versicolor</i>    | strain  | CIRM-BRFM 1219 | –                     | JN645113 | France             |
| <i>Trametes villosa</i>       | voucher | FP71974R       | JN164810              | –        | USA                |
| <i>Trametes villosa</i>       | strain  | CBS 334.49     | MH868069              | MH868069 | Argentina          |
| <i>Trametes_villosa</i>       | strain  | CIRM-BRFM 1375 | –                     | JN645101 | French West Indies |

## Results

### Hyphal features and stains

Twenty-nine specimens were used to study representing 15 species of *Trametes s. str.*, *Artolenzites*, *Leiotrametes*, and *Pycnoporus* (Table 1). Different staining reactions were tested (data not shown) on thin sections of various orientations across the basidiome. The retained techniques (see Materials and Methods) were those providing the best resolution of hyphal systems, by differentially staining generative, skeletal, and binding hyphae.

Generative hyphae are always thin-walled and colorless in both water and KOH. When stained with method 3 (see Materials and Methods), their content strongly fixes Cotton blue (especially in clamps and around septa) while Congo red stains the wall surfaces.

Skeletal hyphae remain colorless in both Congo red and Cotton blue while binding hyphae strongly fix Congo red (wall and content) and sometimes show a red color gradient with KOH, lumen being more colorful. Ruthenium red and Cotton blue (CB) provide a global view of hyphal organization and reveal mucoid deposits.

### Morphological inferences in the *Trametes*-clade

As shown in Table 4, the combination of four relevant micro-morphological characteristics allowed a clear-cut discrimination between the studied taxa.

### Upper side pileus structure

Welti et al. (2012) described 7 types of abhymenial pileus structures within the *Trametes* clade. Among them, types “e” (illustrated by *Leiotrametes* spp., *Lenzites warnieri*, and *L. acuta*) and “g” (illustrated by *Trametes cingulata* Berk. and *Trametes ljubarskyi* Pilát) are characterized by a surface structures composed of superficial skeletal hyphae filled with brown resinous content, responsible for a resinoid matrix on mature specimens in type g. Observations of a specimen of *L. warnieri* (LIP 0001798), recently collected in Algeria, improved our understanding of the development of the upper side structure of this species, which evolves, either with aging or for still unknown reasons, from type “e” to type “g”. Such an intermixed structure with brown intracellular pigment is also found in species of the genus *Leiotrametes* and in “*Daedalea*” *tenuis*. However, in these species, it does not evolve into a resinoid matrix. Species in the genera *Trametes*, *Pycnoporus*, and *Artolenzites* do not produce resiniferous hyphae.

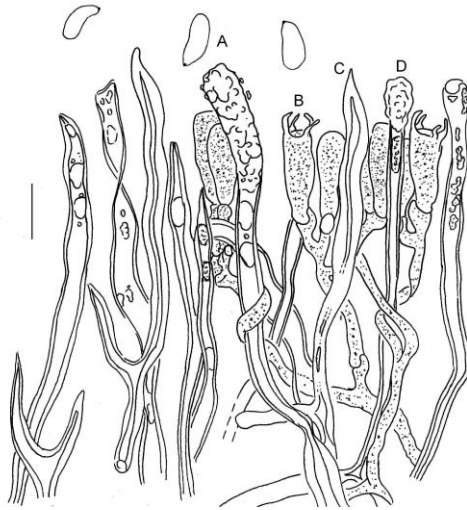
### Hymenial hyphal ends

They arise either from binding hyphae (Ryvarden & Gilbertson 1993) or from subhymenial skeletal hyphae (Zmitrovich 2018). This feature associated with a lamelliform hymenophore distinguished *Lenzites sensu* Ryvarden & Gilbertson (1993) from the genus *Trametes s. str.* Only *T. betulina* (L.) Pilát and *L. acuta* show pointed fusiform hyphal ends, coming from the binding hyphae and protruding through the hymenium, comparable with those of *L. warnieri* (Figs 1-2). Other hymenial hyphal ends were also found in *Artolenzites*, *Leiotrametes*, and *D. tenuis*, but they showed morphological differences: they were either funnel-shaped, obtuse or rounded, coming from the binding hyphae and sometimes protruding through the hymenium such as in *Leiotrametes* and *D. tenuis* (Fig. 3), or full of gelled substances and clavate, arising from skeletal hyphae but not protruding or barely through the hymenium, such as in *Artolenzites*.

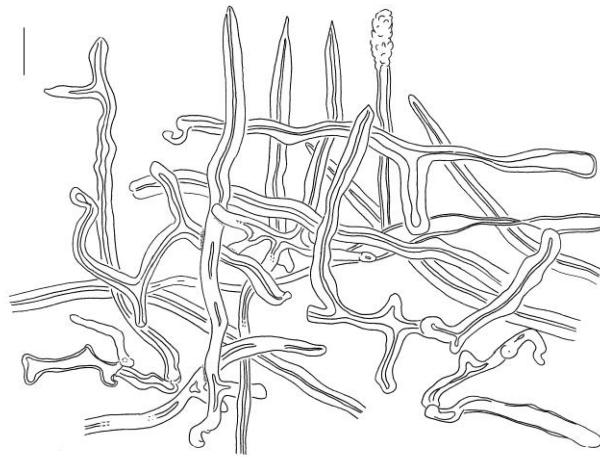
### Binding hyphae of context – abundance and description

The hyphal system in the context is duplex (upper part dimitic with skeletal hyphae and lower part trimitic) for *L. warnieri*, and *T. ljubarskyi*, while it is uniformly trimitic in the other genera of the *Trametes* clade as well as in *T. cingulata*, *L. acuta*, and *D. tenuis*. Only *Leiotrametes* and *Trametes s. str.* show a significant amount of entangled binding hyphae, intricately branched with flagelliform ends. The moderate amount of scarcely branched binding hyphae without flagelliform ends makes the context of *Artolenzites* and *L. acuta* easy to cut, and even easier for *L. warnieri*.

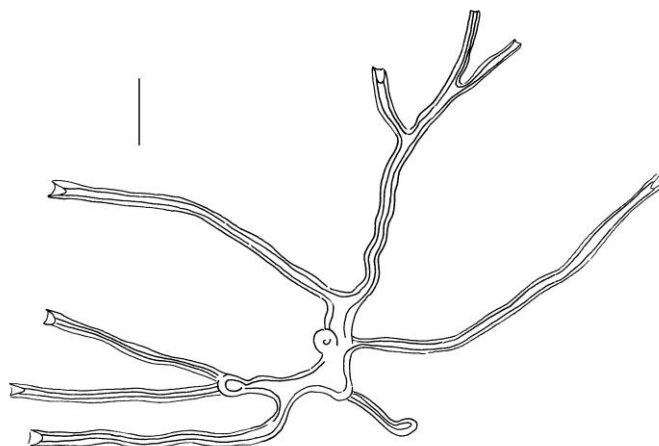




**Fig. 1** – Hymenium of *Cellulariella warnieri* (neotypus and epitypus). A Basidiospores. B Basidia. C Hymenial pointed hyphal ends protruding from the hymenium. D Non-septate terminal segments with crystalline encrustation. Scale bar: 10  $\mu$ m.



**Fig. 2** – Trama of *Cellulariella warnieri* (neotypus). Scale bar: 10  $\mu$ m.



**Fig. 3** – Binding hyphae from trama of “*Daedalea*” *tenuis* (holotypus). Scale bars: 10  $\mu$ m.

**Table 4** Morphologic comparative studied between the lineage belonging to the *Trametes* clade.

| <b>Taxa of the <i>Trametes</i> clade</b>              | <i>Trametes s. str.</i>   | <i>Artolenzites elegans</i>   | <i>Cellulariella warnieri</i>  | <i>Cellulariella acuta</i> (typus)                         | <i>T. ljubarskyi</i>   | <i>T. cingulata</i>   | <i>Pycnoporus</i>  | <i>Leiotrametes</i> and <i>D. tenuis</i> (typus)   |
|---|---|---|--|--|--|---|--|--|
| <b>Upper side pileus structure</b>                    | trichoderm with differentiated subpellis, with incrustations                                  | intermixed structure without incrustations  | intermixed structure with brown intracellular pigment with resinoid matrix | intermixed structure with brown intracellular pigment      | intermixed structure with brown intracellular pigment and resinoid matrix  | intermixed structure with brown intracellular pigment and resinoid matrix | intermixed structure with incrustations at hyphal apex               | intermixed structure with brown pigment in skeletal hyphae   |
| <b>Hymenial hyphal ends</b>                           | not found (pointed fusiform coming from binding hyphae and protruding in <i>T. betulina</i> ) | clavate and full of gelled substances coming from skeletal hyphae and barely protruding | pointed fusiform coming from binding hyphae and protruding                 | pointed fusiform coming from binding hyphae and protruding | not found  | not found   | not found  | obtuse, rounded to funnel-shaped coming from binding hyphae and not protruding (but for <i>Leiotrametes</i> sp.) |
| <b>Abundance of the binding hyphae of the context</b> | significant and tangled   | moderate and isolate  | moderate and mostly isolate only in the lower part of the context (duplex) | moderate and mostly isolate                                | moderate and mostly isolate only in the lower part of the context (duplex) | moderate and isolate  | moderate and isolate   | significant and tangled  |
| <b>Description of the main context binding hyphae</b> | intricately branched with flagelliform ends   | scarcely branched, with obtuse tips, sometimes antler-shaped or bifid                   | scarcely branched, sometimes with apex shaped sword                        | scarcely branched, sometimes with apex shaped sword        | scarcely branched sometimes with flagelliform ends                         | frequently branched with flagelliform ends                                | frequently branched with flagelliform ends, sometimes hairpin-shaped | intricately branched, with flagelliform ends, sometimes hairpin-shaped   |

### Phylogenetic results and position of *Lenzites warnieri*

On reconstructions based on RPB1, RPB2, and EF1- $\alpha$ , *L. warnieri* had an unresolved position on globally low-supported cladograms (data not shown). A multigene analysis was also intended (not shown) without conclusive result. To resolve the phylogenetic relationships of *L. warnieri*, a combined (ITS+LSU) data set provided more robust results.

In the phylogenetic analysis based on the ITS region alone (Fig. 4) or the combined (ITS+LSU) data set (Fig. 5), sequences identified as *L. warnieri* and some identified as *L. acuta* (vouchers Cui 10091 and Dai 11621) are clustered in a strongly supported monophyletic clade (Figs 4-5). The phylogenetic analysis based on the combined (ITS + LSU) data set resolve significantly this clade as sister to a broader lineage which encompasses three other clades: *Leiotrametes*, *Pycnoporus*, and the *T. ljubarskyi* - *T. cingulata* - *Trametes marianna* (Pers.) Ryvarden clade. Within this broad clade, the *T. ljubarskyi* - *T. cingulata* - *T. marianna* clade appears as sister to the *Leiotrametes*-*Pycnoporus* clade (Fig. 5). Other sequences from specimens identified as “*Lenzites acuta*” in GenBank are clustered in 2 other clades: 1) Dai 13103 and Dai 13595, closely related to *Leiotrametes* sp. (ITS, Fig. 4), belong to the *Leiotrametes* clade; 2) MO138336 clustered with *Lenzites vespacea* (ITS data only, Fig. 4) belongs to the sub-lineage of the genus *Trametes*.

### The *Cellulariella* clade

To refine the taxonomy into this clade, pairwise comparisons were processed between available ITS sequences used in Fig. 4: *C. warnieri* from France (strain CIRM-BRFM 972) and Algeria (LIP0001798), Asian *C. warnieri* from India (strain JZ27), Sri-Lanka (vouchers UOC\_KAUNP\_MK29 and UOC\_KAUNP\_K05), and Pakistan (voucher MH-72) and *C. acuta* from China (vouchers Dai11621 and Cui 10091). The ITS-based *Cellulariella* clade (Fig. 4) showed weakly supported internal differences, leading us to question the autonomy between *C. acuta* and *C. warnieri*, which required deeper investigations.

On sequences of approx. 523 bp, for each pairwise alignment, internal variabilities (% identity) between collections of *C. warnieri* from France and Algeria, and from *L. acuta* from China are 99.4%. Among other Asian collections (India, Pakistan, Sri Lanka), the variability ranges between 99.4-99.8% (the sequence KAUNP\_K05, from Sri Lanka, was discarded with the value of 99.2 % due to 7 suspect substitutions at the end of ITS2 region).

Similarities between *C. warnieri* (France, Algeria) and Chinese *C. acuta* range from 97.5 to 97.9%. Similarities between the first and other Asian collections range from 97.9 to 98.2%, whereas the similarities between Chinese and other Asian collections are from 99.2 to 99.8%.

With a significant ITS barcode gap of 0.8% between European-Algerian and Asian sequences, two species can be characterized, *i.e.* 1) *C. acuta* encompassing all sequences originating from Asia, and 2) *C. warnieri* from Europe and North Africa.

### Taxonomy

***Cellulariella warnieri*** (Durieu & Mont.) Zmitr. & Malysheva, Index Fungorum 180: 1 (2014)  
[validation of *C. warnieri* (Durieu & Mont.) Zmitr. & Malysheva, Mikol. Fitopatol. 47(6): 376 (2013), inval., McNeill et al. 2013, art. 42.1 – no repository identifier indicated]

≡ *Lenzites warnieri* Durieu & Mont., Annls Sci. Nat., Bot., sér. 4 14: 182 (1860) (basionym)

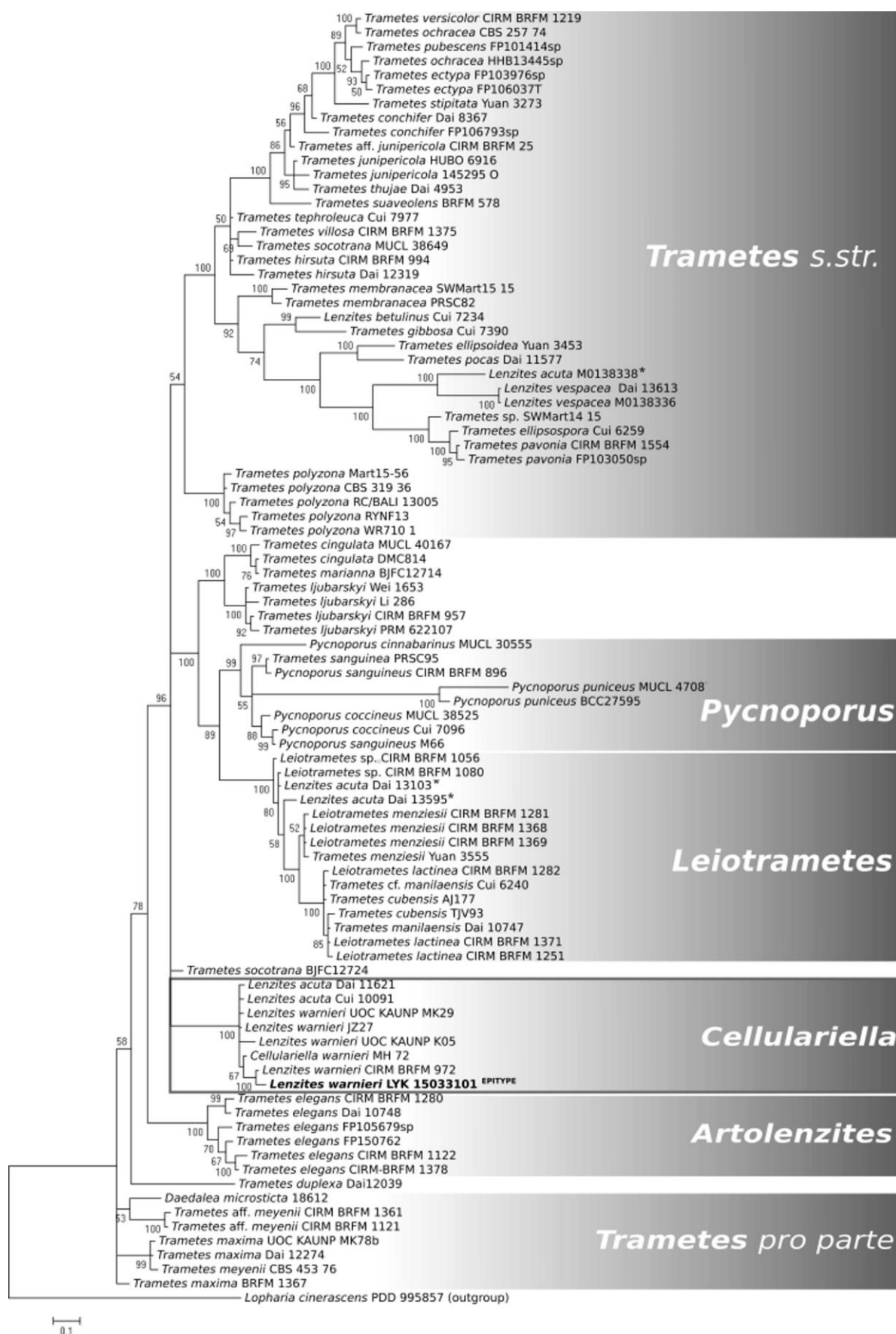
≡ *Cellularia warnieri* (Durieu & Mont.) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 452 (1898)

≡ *Trametes warnieri* (Durieu & Mont.) Zmitr., Wasser & Ezhov in Zmitrovich et al., Int. J. Medic. Mushr. 14(3): 182 (2012)

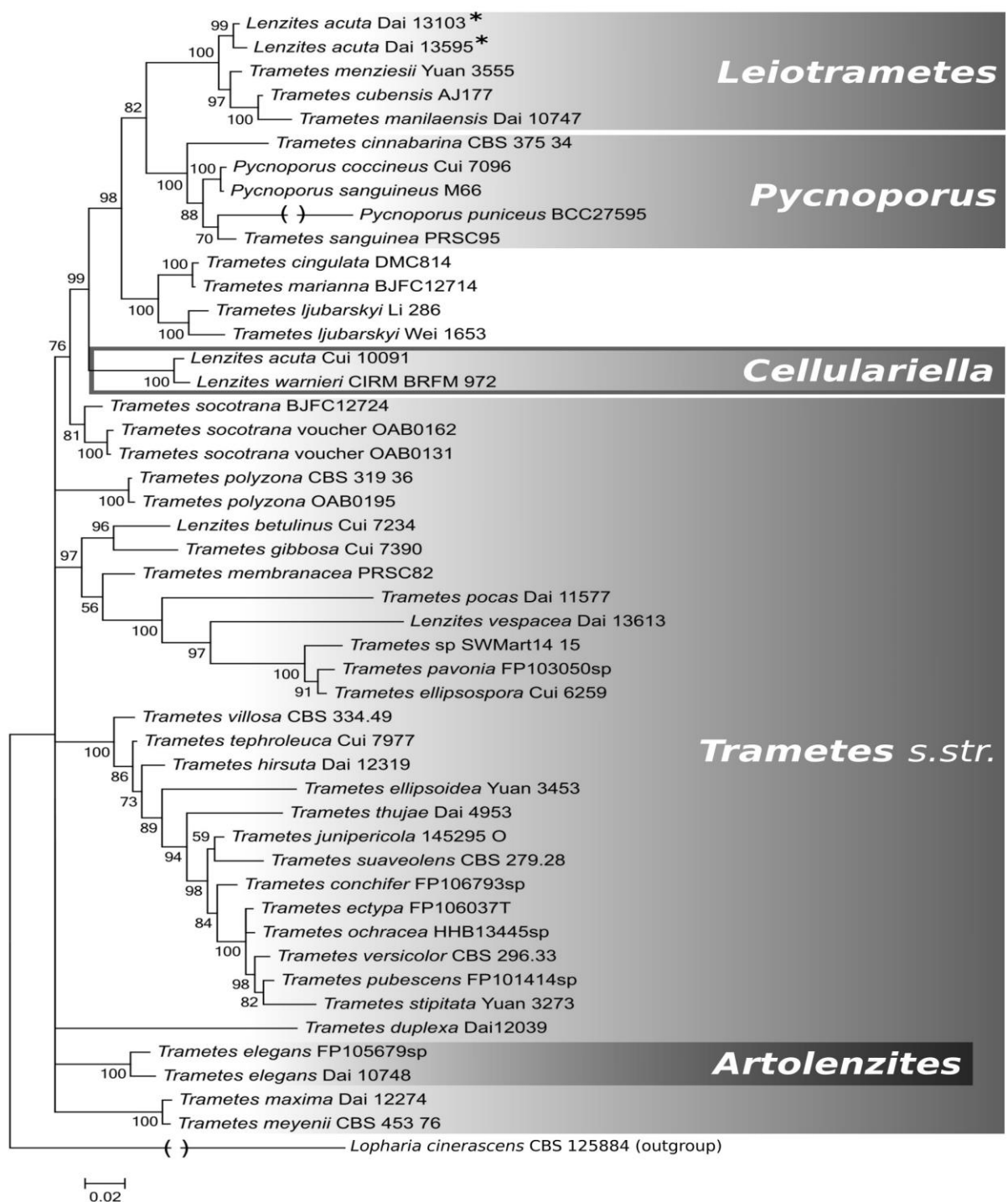
Typification:

Neotype (designated here): Algeria, Tipaza-Blideen Atlas, (1844); PC 0723637. MycoBank MBT 395712

Epitype (designated here): Algeria, Béjaia-Darguina, (2015-03-31); LIP 0001798, as support to the neotype designated above. MycoBank MBT 395713



**Fig. 4** – Phylogenetic relationships of *Cellulariella acuta* and *C. warnieri* within the *Trametes*-clade inferred from Bayesian analysis of the ITS rDNA dataset (50% majority rule consensus tree). Sequences of collections identified as “*Lenzites acuta*” in GenBank not belonging to *Cellulariella* clade are marked with a star (\*).



**Fig. 5** – Phylogenetic relationships of *Cellulariella acuta* and *C. warnieri* within the *Trametes*-clade inferred from Bayesian analysis of the combined (LSU+ITS) rDNA dataset (50% majority rule consensus tree). Sequences of collections identified as “*Lenzites acuta*” in GenBank not belonging to *Cellulariella* clade are marked with a star (\*).

= *Lenzites faventina* Caldesi, Nuovo G. bot. ital. 1: 133 (1869)

≡ *Cellularia faventina* (Caldesi) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 452 (1898)

≡ *Daedalea quercina* f. *lenzitoidea* Bres., Ann. Mycol. (Berlin) 14(3-4): 221 (1916) [as new name for *Lenzites faventina* Caldesi] ≡ *Trametes quercina* f. *lenzitoidea* (Bres.) Pilát in Kavina & Pilát, Atlas Champ. d'Europe, III, Polyporaceae (Praha) 1: 329 (1936).

= *Lenzites reichardtii* Schulzer, in Thümen, Mycoth. Univ., cent. 16: no. 1501 (1880)

≡ *Cellularia reichardtii* (Schulzer) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 452 (1898)

### Description (based on neotype and epitype collections)

Basidiomata annual, pileate, solitary, sessile and broadly attached, thick and corky becoming tougher with age; pileus slightly convex, subtriquetrous, flabelliform, semi-circular to reniform, anterior part flattened along with the substrate insertion level, circularly gibbose at mid-radius, slightly projecting up to 130 mm, 200 mm wide and 30 mm thick at base; pileus surface initially pubescent, glabrous with age, first black grey sometimes with silvery bloom, then progressively becoming beige grey in concentric grey brown pigmentation zones, progressively indurated and wrinkled, anterior part radially veined and slightly concentrically sulcate, posterior part rather granulose, cracked, warty to nodular, sometimes with acute nodules; margin glabrous, cinnamon-brown, regular to slightly undulate and obtuse; Hymenophore lamelliform, lamellae yellow cream, gradually grey-brown towards edge, without sterile marginal zone, sometimes dichotomously branching, crowded (10 per cm), lamellae thick, regular and shallow (2 mm) at start, becoming sinuous, broader (10-13 mm), more spaced and thinner (4-7 per cm), then undulating towards the base and finally locally daedalean towards the insertion point to the substrate. Lamellulae variable in length, short and conspicuous at the margin, blending to lamellae from a certain length, edge yellowish cream, regular, faintly rounded in some parts; context corky, particularly soft towards surface, easily sectioned with a razor blade, thin, pale brown and fluffy under surface, corky towards hymenophore; trama cream to pale cream, in continuity with context; hyphal system duplex in the context, dimitic with skeletal hyphae in upper part, trimitic in lower part, trimitic in hymenophoral trama; generative hyphae 2-3 µm wide, hyaline, clamped, sparingly branched and anastomosing, isolated or inter-twisted, thin-walled; vegetative hyphae of two types: skeletal hyphae 3-6 (rarely up to 10) µm wide, longitudinal, straight or flexuous, sometimes bifurcate, never ramose, thin- to thick-walled or almost solid, congophobic; binding hyphae (2)3-4(5) µm wide, hyaline in water, often strongly congophilic, always distinctly swelling in KOH 5%, variably branched, either dichotomously branched or more frequently with alternate lateral branches, never flagelliform, tortuous but sometimes easily mingled with true skeletal hyphae, all thick-walled to almost solid; context made of basically tri-directional fibres, mostly radially oriented, others more or less vertically crossed in X, mainly composed of inter-twisted thin mediate hyphae, thick-walled skeletal hyphae, and barely branched generative hyphae, contextual binding hyphae scarcely branched, conspicuous only towards dissepiment; pileus surface made of dense adpressed fibers, radially inter-twined, crossed by short, 15 µm-wide vertically oriented fascicules arising towards margin through pileipellis and connected to others by horizontal skeletal hyphae, all covered by mucoid deposits soluble in KOH; generative hyphae also branched and abundant. In older parts a secondary pileipellis occurs as an intermixed structure with brown intracellular pigment in a resinoid matrix; trama made of a majority of binding hyphae, strongly polymorphic, all sparsely branched with diverticules and short or long branches, entangled, sometimes with apex shaped sword or fusiform, mixed with typical skeletal and generative hyphae (Fig. 2); basidia cylindro-clavate, 15-18 x 4-6 µm, 4-spored with sterigmata 3-4 µm long, clamped; basidioles cylindrical to shortly clavate, clavate at maturity (Fig. 1); leptocystidia absent, skeletocystidia abundant forming catahymenium, straight or fusiform, often bearing apical colorless amorphous secretions or crystals not dissolving in KOH or Melzer if originating from non-septate terminal segments that resemble thin-walled skeletal, bifurcate or sword shaped at apex (matching the hymenial pointed hyphal ends *sensu* Ryvar den & Gilbertson (1993) when originating from binding hyphae (Fig. 1); basidiospores 7-9 x 3-4 µm, cylindrical, slightly incurved, hyaline, inamyloid (Fig. 1).

### Remarks

Montagne (1860) did not designate a holotype, and no original material matching the indications of the protologue could be located at PC (National museum of natural history of Paris). A contemporary collected specimen from Algeria (1844, Atlas, behind Blidah) exists in Montagne's

herbarium (PC), without indication of the collector. This specimen is here designated as neotype, as it was likely accepted by Montagne to represent his original species. An epitype (MBT 395713) originating from the locality, collected recently and for which molecular data were available, is also designated. Both types locally show a daedalean hymenophore at the point of insertion to the substrate.

*Cellulariella warnieri* is usually fully lamellate with the exception of rare specimens (Rivoire 2020). These exceptions are due to a physical constraint related to the mode of insertion on the substrate, also observed on specimens *Trametes betulina* (L.) Pilát [for instance LIP SW/F\_NSR16-01, see table 1] in a more attenuated form.

***Cellulariella acuta*** (Berk.) Zmitr. & Malysheva, Index Fungorum 180: 1 (2014)

[validation of *Cellulariella acuta* (Berk.) Zmitr. & Malysheva, Mikol. Fitopatol. 47(6): 376 (2013), inval., McNeill et al. 2013, art. 42.1 – no repository identifier indicated]

≡ *Lenzites acuta* Berk., London J. Bot. 1(3): 146 (1842) (basionym)

≡ *Cellularia acuta* (Berk.) Kuntze, Revis. Gen. pl. (Leipzig) 3(2): 451 (1898)

≡ *Trametes acuta* (Berk.) Imazeki, Bull. Tokyo Sci. Mus. 6: 73 (1943)

≡ *Artolenzites acuta* (Berk.) Mossebo & Ambit in Ambit & Mossebo, Index Fungorum 268: 1 (2015) [validation of *Artolenzites acuta* (Berk.) Mossebo & Ambit in Ambit & Mossebo, Mycosphere 6(3): 282 (2015), inval., McNeill et al. 2013, art. 41.5 – basionym not fully indicated]

Typification: Philippine Islands, Holotype K(M)168874; MBT 380721 (Berkeley, 1842: 146)

### Holotype description

Basidiomata annual, pileate, solitary or laterally confluent, sessile to dimidiate, straightened from below by a discoid, sterile and oblique attachment, in some cases laterally substipitate when young, semi-circular to flabelliform, projecting up to 90 mm, 75 mm wide, flat to slightly convex, slightly depressed near the attachment zone; pileus surface “*of a beautiful grey umber or cinnamon, inclining toward the margin to tawny*” (Berkeley 1842), glabrous, sparingly granulose, scarcely radially striate, concentrically zonate by spaced concolorous furrows, less marked towards margin, more crowded around insertion; zones smooth, bumped, faintly marked by secondary furrows; margin acute, slightly undulate, flexuous; hymenophore strictly lamelliform, lamellae shallow if compared to *C. warnieri*, dark brown at the edge and zonally lighter until mid-length, radiating from base without daedaloid or poroid transient forms, approximatively 10 per cm at margin, ‘*forked, truncate-dentate at the points of division, edge very acute, lacerato-dentate*’ (Berkeley 1842), faintly bifid towards margin; lamellulae occasional, inconspicuous due to the acute margin; context relatively thin but corky, easily sectioned with a razor blade; hyphal system trimitic in context and trama; binding hyphae rare and scarcely branched in the context, never flagelliform, sometimes with acute terminal hyphal ends, abundant, branched and entangled in the trama; hymenial crystal-bearing hyphal ends abundant; generative hyphae, basidia and basidiospores not observed. The pileipellis in radial section shows a vertically oriented structure more conspicuous toward margin, as well as a brown intracellular pigment but without a resinoid matrix.

### Remarks

Most observed features, especially the vegetative hyphae and the hymenophoral structure, are similar to those of *L. warnieri*. Trimitic context (vs duplex in *C. warnieri*) thinner carpophore, acute margin, lacero-dentate edge of the lamellae as well as the lack of a resinoid matrix at the upper side level distinguish *C. acuta* from *C. warnieri*. Due to the variability of the ‘resinoid matrix’ character (see result part), analysis of the single isotype specimen did not allow us to fully evaluate this feature.

***Leiotrametes tenuis*** (Berk.) Welts & P.-A. Moreau, comb. nov.

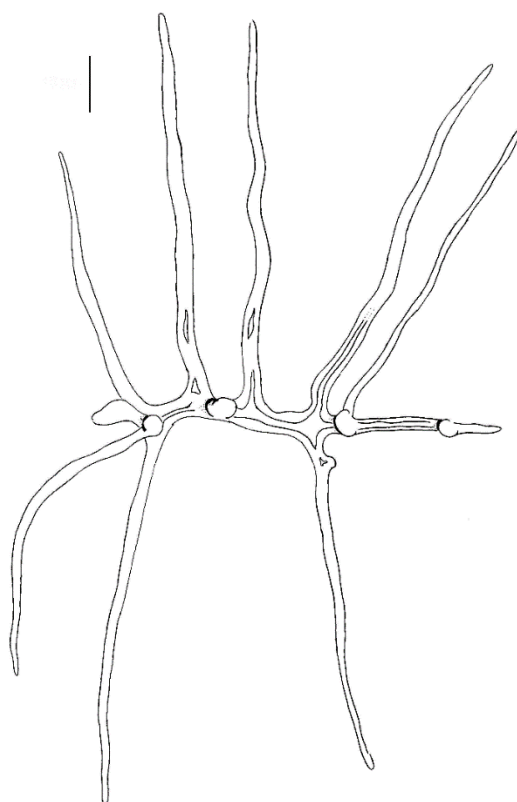
Mycobank number: MB 838542

≡ *Daedalea tenuis* Berk., London J. Bot. 1(3): 151 (1842), (basionym).

≡ *Lenzites tenuis* (Berk.) G. Cunn., Proc. Linn. Soc. N.S.W. 75(3-4): 244 (1950)  
≡ *Striglia tenuis* (Berk.) Kuntze, Revis. Gen. pl. (Leipzig) 2: 871 (1891)  
≡ *Trametes tenuis* (Berk.) Justo, in Carlson et al., Mycologia 106(4): 743 (2014)  
Holotype – Philippine Island, K(M), Cuming 2037; MBT 37440 (Berkeley 1842: 151)

### Holotype description

Basidiomata annual, pileate, solitary; pileus dimidiate, broadly and laterally attached, flat and slightly convex towards the margin, hemi-circular with a reniform trend, projecting up to 101 mm, 64 mm wide, corky, thin and acute at margin level, gradually thickening towards the attachment; Pileus surface “wood coloured inclining to umber, especially towards the expanded, very acute margin” (Berkeley 1842), glabrous, initially flat, slightly depressed and partly reddened or blackened by resin, roughened by radial, irregular and discontinuous furrows, folds and small bump especially at margin, with 3 main concentric and narrow bumpy zones, delimited by deep furrows, and with discontinuous and not always apparent, concentric colored or slightly furrowed zones; margin narrow, acute, slightly wavy; hymenophore originally poroid and remaining so in places and at margin, lamellae developing radially as they furcate to somewhat irpicoid, deeper towards base and mostly interrupted by wedges; hyphal system trimitic in context and trama; context made of a majority of binding hyphae frequently hairpin-shaped (Fig. 6), branched with flagelliform ends, abundant and mostly tangled; pileipellis of hardly interpretable, intermixed filamentous structure with brown pigment in skeletal hyphae; basidia, basidiospores and hymenial cystidia not found; hymenial surface mainly made of vegetative hyphae usually funnel-shaped at apex (Fig. 3) arising from subhymenial binding hyphae.



**Fig. 6** – Binding hyphae from the context of “*Daedalea*”*tenuis* (holotypus). Scale bars: 10 µm.

### Remarks

The branched, entangled binding hyphae with flagelliform ends within the context differs from the hyphal system of *Cellulariella* species, and refers to species of *Leiotrametes* Welte & Courtecuisse (Welte et al. 2012). The poroid hymenophore at margin, which evolves to a lamelloid



hymenophore with age and may vary from one to another specimen, is reminiscent of a species collected by Welte et al. (2012) from French Guiana and referred as “*Leiotrametes* sp.” (SW/Guy12-39, SW/Guy12-78; Table 1). Perpendicular at lamellae or less frequently at lamellulae, the conspicuous and irregular anastomoses at the margin level distinguish *Leiotrametes tenuis*, as well as the *Leiotrametes* sp. (SW/Guy12-39, SW/Guy12-78) from French Guiana, from species of genus *Cellulariella*. We do not propose here to consider the East Asian and Neotropical collections as conspecific, but we propose to interpret the Asian *D. tenuis* as a *Leiotrametes* species and we introduce above the new combination.

## Discussion

### Phylogenetic affinities of “*Lenzites*” *warnieri*

In a previous phylogenetic analysis based on combined (ITS+RPB2) sequences, Welte et al. (2012) showed that the *Trametes*-lineage was composed of four highly supported clades, representing *Trametes* and *Artolenzites*, *Lenzites warnieri*, and a group of specimens with glabrous upper surface encompassing *Leiotrametes*, *Pycnoporus* and the sub-group *T. cingulata*-*T. ljubarskyi*. The phylogenetic relationships between these four clades remained unsolved.

Phylogenetic results based on RPB2 and EF1- $\alpha$  genes lack deep resolution in the *Trametes*-clade. According to the selected marker, *L. warnieri* is either supported as an isolated clade, or clustered without support within other genera of the *Trametes*-clade. Regarding the RPB1 gene, there is currently no publicly available sequence of *Trametes ljubarskyi* and *T. cingulata* at Genbank. We believe that the absence of these two species is a bias to the phylogenetic results. Conversely, the combined (ITS+LSU) analysis significantly placed *L. warnieri* as sister to the previously described large clade encompassing *Leiotrametes*, *Pycnoporus*, and the sub-clade *T. cingulata*-*T. ljubarskyi*. Within it, the subclade *T. ljubarskyi*-*T. cingulata* together with *T. marianna*, are placed as sister to the clade encompassing *Pycnoporus* and *Leiotrametes*. In many aspects, this topology of the Bayesian tree reflects the morphological characteristics (Table 4):

- the glabrous upper surface level is shared by *Lenzites warnieri* [(ND169 (LIP) and LIP0001798] as well as all species of the *Leiotrametes*-*Pycnoporus* clade including *Trametes ljubarskyi* and *T. cingulata* (Welte et al. 2012). Such a character is therefore synapomorphic for these two clades
- the brown intracellular pigment is symplesiomorphic since it characterizes *L. warnieri* and all the species belonging to the *Leiotrametes*-*Pycnoporus* clade with the exception of *Pycnoporus*
- the resinoid matrix is only shared by *L. warnieri* as well as *T. ljubarskyi* and *T. cingulata*; since all of these species are glabrous, these two sublineages form a paraphyletic group
- with the exception of the resinoid matrix, *L. warnieri* as well as *L. acuta* share most of the morphological characters studied (see Table 4 and taxonomic part); these two species are consequently monophyletic.

Zmitrovich & Malysheva (2013) proposed to include *L. warnieri* into *Cellulariella*, typified by *L. acuta*, which is a paleotropical species. Some ITS and LSU sequences available in GenBank as well as micromorphological features of the isotype of *L. acuta* (Table 4) tend to confirm a close phylogenetic affinity between both species (Figs 4-5).

### An emendation of the genus *Cellulariella*

With regard to the monophyly of the *L. acuta* - *L. warnieri* clade, we questioned the circumscription of *Cellulariella* provided by Zmitrovich & Malysheva (2013). *Cellulariella* is typified by *L. acuta*, and its delimitation is based on the following elements: “hymenophore daedaloid to lamellate; sclerohyphae hyaline, sympodially and rarely branched, inamyloid, without deposits; in some cases, with crystalline encrustation; context cream. Basidia clavate, basidiospores cylindrical”.

We agree with these authors regarding “the sympodially and rarely branched sclerohyphae”, which are interpretable as scarcely branched binding hyphae. However, we emend and refine here the other elements of the definition as follows:

“Daedaloid to lamellate hymenophore”: our specimens (ND169, LIP0001798, lectotype of *L. warnieri* and isotype of *L. acuta*) showed a homogeneous lamelliform hymenophore with *numerous* and clearly distinct lamellulae. Lamellae are dichotomously branched and dividing points are truncate-dentate. Sometimes, this feature is so emphasized that the lamellulae appear free from lamellae. Such structured and homogeneous lamelliform hymenophore is mostly present in *L. warnieri* and frequent in *L. acuta*. It is also found in *Trametes betulina* (type of the genus *Lenzites*) and was defined by Ryvarden & Gilbertson (1993) as ‘lenzitoid’. We never observed any specimen identified as *L. acuta* or *L. warnieri* not strictly lenzitoid as defined above, i.e. without easily identifiable lamellulae and with frequent and disorganized forks as well as perpendicular anastomoses between lamellae. All specimens identified as “*L. acuta*” with heterogeneous hymenophore or with perpendicularly oriented anastomose are closely related or attributed to *Leiotrametes tenuis* (see Taxonomy part).

“Sclerohyphae without deposits and in some cases with crystalline encrustation”: we assume that the authors refer to skeletal hyphae of the context. In our previous observations (Table 4; Welti et al. 2012), we observed a parietal crystalloid pigment in *Pycnoporus* or *T. cingulata* for instance, but we did not find this characteristic in *L. warnieri* or *L. acuta*.

Although pointed hyphal ends protruding through the hymenium were not mentioned by Zmitrovich & Malysheva (2013), Zmitrovich (2018) adds them in a second description of *Cellulariella*. Previously described in *L. warnieri* by both Ryvarden & Gilbertson (1993) and Bernicchia (2005), this feature is rare in polypores and therefore is an interesting character to be included in the definition of *Cellulariella*. Many authors, including ourselves (Welti et al. 2012), pointed out the inconsistency of hymenophoral structure for a definition at generic level in the *Trametes*-clade. The type of *Lenzites*, *L. betulinus*, shows the rarely combined features “lamelliform hymenophore” and “hymenial pointed hyphal ends” (Ryvarden 1991) and has been shown to be phylogenetically nested into the *Trametes* *ss. str.* clade (Tomšovský et al. 2006, Justo & Hibbett 2011, Welti et al. 2012). In *Cellulariella*, the uniqueness of the association of lenzitoid hymenophore, pointed fusiform binding hyphal ends protruding through the hymenium, and scarcely branched contextual binding hyphae without flagelliform ends (intricately branched with flagelliform ends in *T. betulina*) characterizes both *L. acuta* and *L. warnieri* within the *Trametes*-clade and supports the recognition of *Cellulariella* in the trametoid complex of genera.

We therefore emendate the definition of *Cellulariella* to include hyphal system of the context trimitic with scarcely branched binding hyphae, never intricate and without flagelliform ends, pointed fusiform binding hyphal ends protruding through the hymenium, and strictly homogeneous lenzitoid hymenophore with, in some cases, numerous lamellulae mostly free from lamellae, instead of “daedaleoid to lamellate”.

### ***Cellulariella* versus *Leiotrametes***

*Cellulariella* in its original definition (Zmitrovich & Malysheva 2013) could match species belonging to other clades of the *Trametes*-clade, especially *Leiotrametes*.

Such a broadly formulated description could lead to an erroneous placement of various daedaloid polypores in this genus. The imprecision in hymenophoral structure might also affect species concepts, for instance too broad a morphological concept of *Lenzites acuta*.

In the combined (ITS+LSU) and ITS phylogenetic datasets (Figs 4-5), vouchers identified as “*L. acuta*” are spread within three distinct lineages, respectively close to *L. vespacea* (clade of the genus *Trametes*), *Leiotrametes* sp., and *L. warnieri*. *Daedalea tenuis* is a usual synonym of *Lenzites acuta* (Ryvarden 1976), likely confused by an approximatively similar morphology. Microscopical features clearly confirm the phylogenetic affinities of these taxa.

The study of the isotype of *L. acuta* revealed a strictly lenzitoid hymenophore, a trimitic context with scarcely branched binding hyphae, lacking flagelliform ends, and hymenial fusiform

pointed hyphal ends, that agree with the definition of *Cellulariella* (see description in the taxonomy part and Table 4). The examination of the holotype of *D. tenuis*, synonymized to *L. acuta* by Ryvarden (1976) for instance, revealed a poroid to daedaleoid hymenophore, locally lamelliform but never strictly lenzitoid, intricately branched binding hyphae with tapering flagelliform ends, sometimes hairpin-shaped, both within the context and hymenophoral trama (Fig. 6), and usually funnel-shaped (not pointed) hymenial ends originating from binding hyphae, that agree with the definition of *Leiotrametes* (see taxonomy part and Table 4). The same result was achieved with *Leiotrametes* sp. from French Guiana.

Given that these morphological observations are congruent with phylogenetic results, it is concluded that *D. tenuis* belongs to the *Leiotrametes* whereas *L. acuta* belongs to *Cellulariella*. Consequently, the synonymy between both species is rejected and each of them is transferred into its corresponding genus: *Leiotrametes tenuis* (Berk.) Welti & P.-A. Moreau, comb. nov. (see taxonomy part) and *Cellulariella acuta* (Berk.) Zmitr. & Malysheva. The species from French Guiana described as “*Leiotrametes* sp.” in a previous work (Welti et al. 2012), phylogenetically close to the vouchers “*L. acuta*” Dai 13103 and Dai 13595 from China, is comparable to *L. tenuis* in many aspects. Recent and reliably identified material from the original locality of *L. tenuis* (Philippines) is urgently needed, to confirm the molecular position of the species and compare more reliably Asian and neotropical collections.

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