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Short note

First record of *Phlebia tremelloidea* (Bres.) Parmasto in Italy

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Abstract

Phlebia tremelloidea is a rare, poorly known boreonemoral species producing resupinate basidiomata on fallen trunks. This species has been proposed to be synonymous with *Pseudophlebia lindtneri* (Pilát) C.L. Zhao, despite the long-standing nomenclatural history conflicts with the priority of Giacomo Bresadola (1920). *Phlebia tremelloidea* has been reported from several European Countries, but its distribution range appears shifted towards North-East Europe with very scattered or no records elsewhere. A specimen of *P. tremelloidea* was found in May 2023 (re-surveyed in 2024) in the State Strict Natural Reserve Bosco Siro Negri along the Ticino River. It represents the unique record in Italy (Pavia province) even including possible synonyms. The available exsiccata have been conserved in the University of Pavia and in Annarosa Bernicchia's private herbarium. Molecular barcode (ITS region) performed on the dried basidioma confirmed the macro- and micromorphological identification; besides, sequences from LSU, SSU and two sub-regions in RPB2 have been deposited as well in GenBank for the first time. This study increases the knowledge about distribution and ecology of *P. tremelloidea* and provides new molecular data in support of the taxonomic debate on this species and its relatives.

Keywords
Phlebia lindtneri sensu lato, deadwood, corticioid fungi, Bosco Siro Negri, morphological identification, molecular barcode, rarity

Introduction

Phlebia tremelloidea is a poorly known corticioid species in *Meruliaceae* Rea (Polyporales Gäum.) with resupinate basidiomata characterized by non-radial, horizontal folds, elastic consistency and top lamprocystidia (i.e., prominent encrusted cystidia). According to Bernicchia and Gorjón (2010), the putatively sister species *P. lindtneri* (Pilát) Parmasto is not discriminated based on morphology.

Phlebia tremelloidea was first described in 1920 as *Peniophora tremelloidea* Bres. by Bresadola (1920) based on Westfalian specimens and moved to *Phlebia* Fr. by Parmasto (1967); *Lilaceophlebia tremelloidea* (Bres.) Zmitr. has been proposed to be an obligate synonym (Zmitrovich and Ezhov, 2015) mainly based on ecological differences. Notwithstanding, *P. tremelloidea* is retained to be the current name according to Mycobank (www.mycobank.org), unlike Index

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Fungorum (www.indexfungorum.org). Both the sources agree in considering the species synonym with *Phlebia lindtneri sensu lato*, but Index Fungorum only redirects to the mentioned *L. tremelloidea* as the current name, whereas Mycobank indicates the current name of *P. lindtneri* is *Pseudophlebia lindtneri* (Pilát) C.L. Zhao. The latter proposal arises from Zhao et al. (2023) for which the proposed *Pseudophlebia* C.L. Zhao *genus novum* is represented by a few sequences of *P. lindtneri* and *P. setulosa* (Berk. & M.A. Curtis) C.L. Zhao. However, the latter shows inconsistent positions in different cladogram topologies as well as morphological description inconsistent with Hjortstam et al. (1988) and Bernicchia and Gorjón (2010).

As a whole, knowledge about *P. tremelloidea sensu lato* is quite poor and mostly referred to putative *P. lindtneri* specimens. The distribution range of this species is broadly Eurosiberian (Zmitrovich and Ezhov, 2015) with very scattered records in Central Europe and Balkans (Blaser S., Fuljer F., Greilhuber I., Larsson K.H., Mešić A., Logar R., Ryvarden L., Senn-Irlet B., Tortic M., Yurchenko E., Zíbarová L. personal communications; Karasiński et al., 2015; Holec et al., 2019; Bolshakov et al., 2022) and no records in Italy (Saitta et al., 2011) or in Western Alps. The aim of this work was to present the first record of *P. tremelloidea* in Italy based on both morphological and molecular identification.

Materials and Methods

Sampling area

A single specimen was found in the State Strict Natural Reserve (RNIS) "Bosco Siro Negri" (Zerbolò, Italy), 62 m a.s.l., on May 10 (2023), on a single decaying fallen trunk, presumably *Quercus robur* L.; the same growth station (probably the same individual) has been re-surveyed on May 24 (2024). The specimen was therefore treated as a single individual based on the guidelines in Girometta et al. (2023). The collection site coordinates were: 9.05639 E 45.20914 N based on Google Maps and the Geoportale Nazionale (http://www.pcn.minambiente.it/viewer/). The Reserve "Bosco Siro Negri" is managed by the Department of Earth and Environmental Sciences of the University of Pavia (DSTA-Unipv) and represents one of the last residuals of the typical lowland forest in Western Po Plain, with high amounts of deadwood (https://boscosironegri.unipv.it/; Girometta et al., 2020).

Morphological identification

Macro- and micromorphological identification was cross-checked in DSTA-Unipv and Annarosa Bernicchia's private laboratories. The following instrumentation was used in DSTA-Unipv: Zeiss 475052-9901 and Nikon Labophot II; slides were mounted in KOH 3%. The following instrumentation was used in Bernicchia's lab: Optech SZ-NT stereomicroscopes and Leitz Vialux 22FB optical microscopes equipped with Optika 4083.13 H camera; slides were mounted in diluted Congo Red (1%). Fresh, dried and re-hydrated samples were examined as well. It should be noticed that re-hydration aimed to break the cryptobiosis in the dormant specimen. Due to the lack of spores in the fresh sample collected in 2023, sporulation was thus forced by incubating a fragment of basidioma for 2 days at room temperature in humid chamber represented by a Petri plate with a water-soaked paper on the bottom. Instead, the sample collected in 2024 yielded abundant spores both in the fresh sample and after incubation in the humid chamber. Morphological data were analyzed by Microsoft Excel 2016 and SPSS26 (IBM).

Exsiccata

Fragments of the fresh resupinate basidiomata (15 cm^2 for the sample 2023 and > 100 cm^2 for the sample 2024) were dried at 30 °C, then preserved at -18 °C for 1 month and finally stored in a sealed box. About 1/3 of the specimen collected in 2023 was used for analyses. Exsiccata portions have been conserved in the University of Pavia and in Annarosa Bernicchia's private herbarium.

Molecular barcode and phylogeny

DNA extraction, amplification, purification and sequencing were performed as in Girometta et al. (2020). The following nuclear DNA regions were amplified (primers in brackets): ITS (ITS1-ITS4; ITS1F-ITS4B; ITS5-ITS4); LSU (LR0R-LR7); SSU (NS1-NS8); RPB2 (5F-7CR; 6F1-7R1). The primer sequences are available at the Hibbett's Lab (https://www2.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.pdf), which has in turn served as a reference for several authors, such as Liu et al. (2023) and Spirin et al. (2024) for Polyporales. Sequences were processed by Sequencher 5.0 and Maximum Likelihood phylogeny was inferred by MEGA7 with MUSCLE and/or ClustalW alignment; 1,000 bootstrap was set for both alignment and cladogram (Angeles Flores et al., 2023). The newly generated sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/); metadata and sequences used to generate the phylogenetic trees are reported in the Supplementary Tables S1 and S2.

The following species were introduced in phylogenetic reconstruction: *P. lindtneri*, *P. radiata* Fr., *P. rufa* (Pers.) M.P. Christ, *P. setulosa* (Berk. & M.A. Curtis) Nakasone [current name: *Pseudophlebia setulosa* (Berk. & M.A. Curtis) C.L. Zhao], *P. tremelloidea*, *Pseudophlebia semisupina* (C.L. Zhao, B.K. Cui & Y.C. Dai) C.L. Zhao [also known as *Ceriporiopsis semisupina* C.L. Zhao, B.K. Cui & Y.C. Dai, in Zhao & Cui]. *Aurantiporus mayaensis* (Ginns, D.L. Lindner & T.J. Baroni) Zmitr. was also introduced based on the phylogenetic affinity suggested by Chen et al. (2021) and Zhao et al. (2023).

Results and discussion

Morphological description

Basidioma patches resupinate and widely effused, about 2 mm thick when fresh and 20 cm (sample 2023) to about 50 cm (sample 2024) long, subhyaline, homogenously whitish to pearl grey along the whole section, elastic when fresh, hard but not brittle when dry. Margin distinct. Hymenophore folded-merulioid, alveoles < 2 mm wide; notably, folds in the present specimen showed mostly reclined folds instead of standing ones, that is reported to be the typical morphology (Zmitrovich and Ezhov, 2015; supplementary Fig. S1).

The presence of free, top, obtuse lamprocystidia confirms the identification and clearly discriminates from white forms of *P. rufa*. Re-hydrated specimen in humid chamber produced only a few 4.6–6 (5.3) × 2.5 (2.5) µm ellipsoid to oblong-ellipsoid spores with average Q = 2.12 (N = 3). These values are within the species range, although smaller than reported by Zmitrovich and Ezhov (2015), possibly due to the harsh condition the specimen was forced to re-grow. The apparent lack of sporulation in fresh sample 2023 has been reported by Zíbarová too (personal communication) and it is possibly consistent with a prolonged lifetime span of basidiomata. Notwithstanding, sample 2024 was surveyed during massive sporulation time; spores resulted in 5–5.9 (5.4) × 3.0–4.0 (3.4) µm and average Q = 1.6 (N = 38). Based on the Levene's test for variance comparison and t-test for independent samples, this sample is quite similar to Zmitrovich and Ezhov (2015) one as concerns length (t = 23.5%), whereas width and Q are significantly different (t = 0% each). Images of the basidioma are shown in Fig. 1 and supplementary Figures S2–S7.

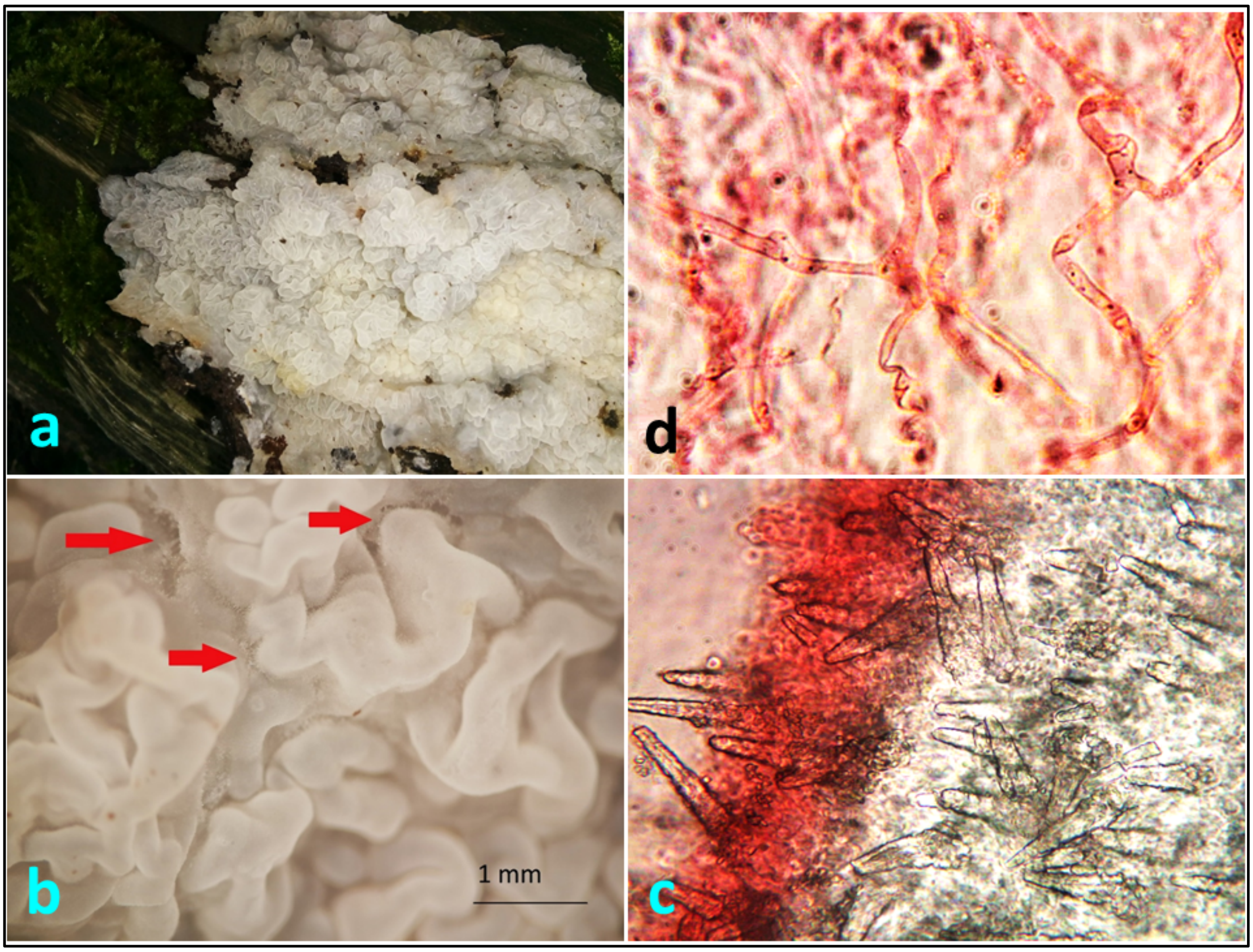

Fig. 1 – *Phlebia tremelloidea* in the“Bosco Siro Negri”: part of specimen 2024 in field (A); specimen 2024 at about 200× and lamprocystidia indicated by red arrows (B); lamprocystidia protruding and embedded from the hymenium in specimen 2023 (C).

Molecular barcode and phylogeny

All the ITS sequences of the two specimens confirmed the morphological identification with similarities > 98% with accessions of *P. lindtneri* and *P. tremelloidea* from both Mycobank and NCBI. Only the ITS1-ITS4 primer pair resulted in successful amplification, whereas ITS1F-ITS4B and ITS5-ITS4 always failed to amplify DNA extracts. Only four sequences of *P. lindtneri* (from Norway, Poland, Sweden and an undefined locality, maybe Japan) and one of *P. tremelloidea* (from Russia) are available in public repositories [last accessed on November 3, 2024]. Despite the molecular data seem currently insufficient to explore robust phylogenies and possible cryptic species, a preliminary maximum likelihood – ML phylogeny based on the ITS region only is shown in Fig. 2 (the non-condensed cladogram is shown in the Supplementary Fig. S8).

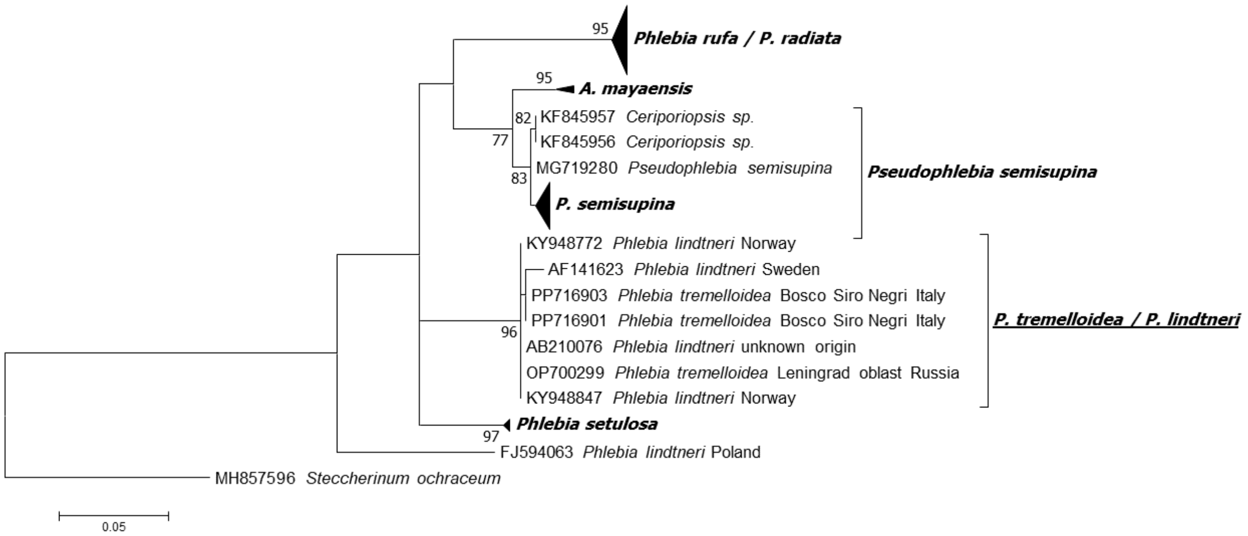

Fig. 2 – ML phylogeny resulting from the ITS region (1,000 bootstraps). Alignment by Muscle – MEGA7. Bootstrap cut-off is set at 70%.

A well-supported (96% bootstrap) clade including *P. tremelloidea* and *P. lindtneri* segregates from all the other clades: *P. radiata/P. rufa*, *P. setulosa* and *Pseudophlebia* (Zhao et al., 2023). The topology of the cladogram based on the ITS region suggests that *P. tremelloidea* and *P. lindtneri* could be the same species but the number of available sequences is largely insufficient to solve the taxonomy of this taxa. Noteworthy, the Polish sequence FJ594063 is labelled as *P. lindtneri*, but a cross-check by NCBI BLAST and Mycobank Molecular ID revealed it belongs to *Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn ex Ryvarden, a species in *Incrustoporiaceae* Jülich. Besides, it should be noticed that the ITS-based tree results in typical polytomic topology lacking a true basal root and hampering the reconstruction of vertical phylogeny. Consequently, the present analysis doesn't support the segregation of *Pseudophlebia* and suggests the latter is paraphyletic.

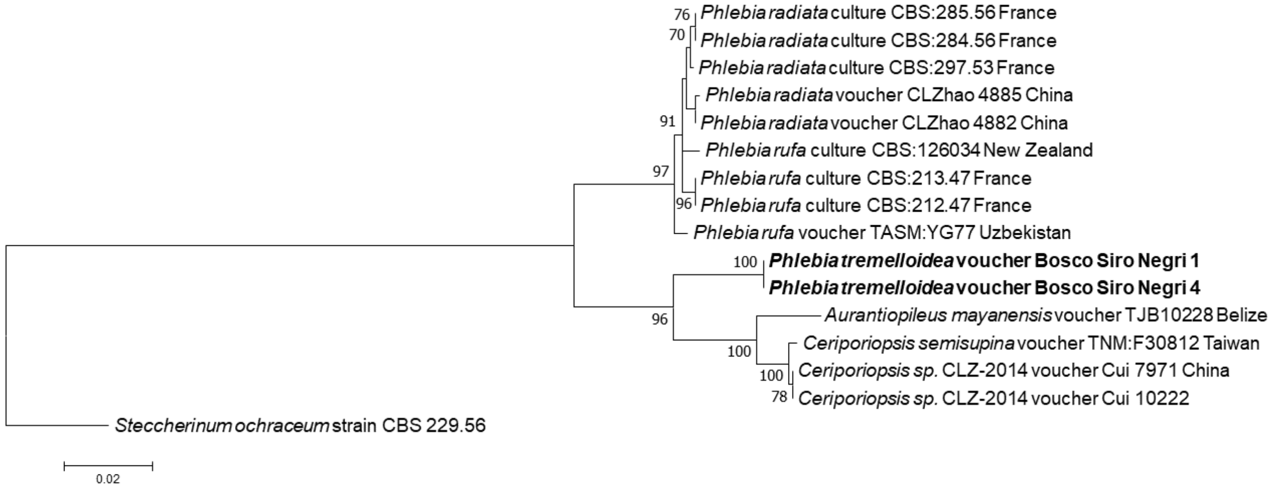

Fig. 3 – ML phylogeny resulting from the ITS+LSU concatenation (1,000 bootstraps). Alignment by Clustal W- Mega7. Bootstrap cut-off is set at 70%.

Based on the few available sequences in GenBank for ITS+LSU concatenation, the ML phylogeny consistently segregates the *Phlebia sensu stricto* clade from a macro-clade distinctly including the proposed *Pseudophlebia/Aurantiporus* clade and a *P. tremelloidea/P. lindtneri* one (Fig. 3). Non-condensed trees only based on LSU are shown in Supplementary Fig. S9.

In conclusion, the Italian specimen from Bosco Siro Negri has been confirmed to belong to *P. tremelloidea*; surveys in field are ongoing to detect new basidiomata in the only known Italian site. Molecular data don't support the segregation of *P. tremelloidea* from *P. lindtneri* and the former has nomenclatural priority based on chronology. Moreover, polytomic topology (particularly in the ITS-based phylogeny) suggests that *Phlebia sensu lato* is a paraphyletic tank still far from being clarified. Further investigation is therefore needed to support the segregation of genus *Pseudophlebia* with respect to close genera and the *Phlebia sensu stricto* core clade. The present study also resulted in newly generated ITS, SSU, LSU and RPB2 sequences for future studies.

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