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Article

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A new species of *Bjerkandera* (Polyporales, Basidiomycota) from Europe

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Abstract

Morphological and molecular revision of pore fungi from Czechia and France revealed a new species, *Bjerkandèra lecomtei, sp. nov.* This species is characterized by annual resupinate basidiomata, yellow ochre to deep brownish pore surface, a monomitic hyphal system with clamp connections on generative hyphae, ellipsoid to cylindrical basidiospores measuring 4.9–8.3 × 2.9–4.9 µm, and growing on hardwood (*Acer campestre* in floodplain forest, *Quercus* sp. beams, etc.). It differs from the most European *Bjerkandera* species by resupinate basidiome and spore sizes. Macroscopic and microscopic features and phylogenetic analyses of ITS and LSU rDNA markers are illustrated.

Key words: Phylogeny, Phanerochaetaceae, taxonomy, wood-decaying fungi

Introduction

Bjerkandera P. Karst. (Phanerochaetaceae, Polyporales), typified by *B. adusta* (Willd.) P. Karst. was established in 1879. Currently eleven species of *Bjerkandera* are recognized worldwide: *B. adusta, B. albocinerea* Motato-Vásq., Robledo & Gugliotta (in Motato-Vásquez *et al.* 2021: 234), *B. atroalba* (Rick 1935: 25) Westph. & Tomšovský (Westphalen *et al.* 2015), *B. carnegieae* (D. V. Baxter 1941:110) Robledo, Nakasone & B. Ortiz (in Robledo *et al.* 2021: 234); *B. centroamericana* Kout, Westph. & Tomšovský (Westphalen *et al.* 2015), *B. ecuadorensis* Y.C. Dai, Chao G. Wang & Vlasák (in Wang *et al.* 2021: 155), *B. fulgida* Y.C. Dai & Chao G. Wang (in Wang *et al.* 2021: 157), *B. fumosa* (Pers. 1801:530) P. Karst. (1879:38), *B. mikrofumosa* Ryvarden (2016:44), *B. minispora* Y.C. Dai & Chao G. Wang (in Wang *et al.* 2021:160) and *B. resupinata* Y.C. Dai & Chao G. Wang (in Wang *et al.* 2021:163). The recently published combination *B. xuchilensis* (Murrill 1912:143) O. Miettinen (in Miettinen *et al.* 2023) is likely an older synonym of one of the currently recognized American species, namely, *B. albocinerea, B. atroalba, B. centroamericana* and *B. mikrofumosa*. Basidiomata of *Bjerkandera* are resupinate, effused to pileate; hymenophore is poroid. The pileate species are characterized with a dark line separating the tube layer from the context. Hyphal system is monomitic, generative hyphae with clamp connections, cystidia are absent, basidiospores are smooth and negative in Melzer's reagent. All known species are wood-decaying fungi causing white rot, saprotrophs or facultative parasites on wounded or damaged trees with endophytic stage. Cultures produce arthroconidia (Robledo *et al.* 2021) and asexual morph of *B. adusta* was described as *Geotrichopsis mycoparasitica* Tzean & Estey (Tzean & Estey 1991, Vu *et al.* 2019, Stalpers *et al.* 2021). The genus is distributed worldwide in forest biomes. Only *B. carnegieae* is associated with woody Cactaceae in desert areas of southwestern USA (Arizona) and Mexico (Robledo *et al.* 2021). Phylogenetic relationships of *Bjerkandera* were studied by Westphalen *et al.* (2015), Motato-Vásquez *et al.* (2020), Robledo *et al.* (2021) and Wang *et al.* (2021).

The position of *Bjerkandera* in Phanerochaetaceae was confirmed by Justo *et al.* (2017). The molecular phylogenetic studies usually based on sequences of internal transcribed spacer (ITS) and large subunit (LSU) of ribosomal RNA gene confirmed the positions of *Bjerkandera* spp. within the genus (Motato-Vásquez *et al.* 2021, Robledo *et al.* 2021, Westphalen *et al.* 2015, Wang *et al.* 2021). Other genetic markers, namely the second largest subunit of RNA polymerase II and the translation elongation factor 1-alpha, are less frequently used in *Bjerkandera* phylogeny (Robledo *et al.* 2021, Westphalen *et al.* 2015).

In this study, one sample originally identified as "*Ceriporiopsis* s.l." from Czechia and recent collections from France are studied based on phylogeny of sequences of ribosomal RNA gene (ITS+LSU) and morphological examination. Based on these collections, the new species *Bjerkandera lecomtei* is described and illustrated.

Materials and methods

Morphological studies

The studied specimen is deposited in the Herbarium of Museum of Highlands Jihlava (Muzeum Vysočiny Jihlava in Czech) Czechia (MJ), within valuable polypore collection of Petr Vampola (Vampola and Charvátová 2021). Morphological descriptions are based on dried voucher specimen examined using an Olympus SZX12 stereomicroscope. Microscopic features are described from dried material mounted in KOH and Melzer's reagent using an Olympus BX51 light microscope (Japan) allowing for differential interference contrast with a magnification of 1000×. Basidiospores were measured from sections cut from the tubes using the Motic Images Plus software 3.0. To represent the variation in the size of spores, 5 % of measurements were excluded from each end of the range and are given in parentheses. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. Colour terms and abbreviations follow Kornerup and Wanscher (1983).

DNA extraction, amplification and sequencing

DNA from dried fungal material was isolated and the PCR of the Internal transcribed spacer (ITS) and Large subunit (LSU) of nuclear ribosomal RNA gene was performed according to Tomšovský *et al.* (2010). The PCR products were purified and sequenced by Elisabeth Pharmacon (Brno, Czechia) with the same primers as used in PCR. Newly generated sequences were deposited in the NCBI GenBank database. The ITS and LSU datasets were constructed using included best hits from NCBI GenBank obtained using BlastN search including all sequences of the reference species in *Bjerkandera*. All sequences analysed in this study are listed in **Table 1**.

TABLE 1. A list of species, specimens and GenBank accession numbers of sequences used in this study.

Species	Sample no	Country	GenBank Accession no.		Reference
			ITS no.	LSU no.	
Bjerkandera adusta	BRNM 771948	Czechia	KT305935	KT305935	Westphalen *et al.* 2015
B. adusta	BRNM 771946	Argentina	KT305936	KT305936	Westphalen *et al.* 2015
B. adusta	Dai 15665	China	MW507098	MW520205	Wang *et al.* 2021
B. adusta	X-41	USA	KC176334	KC176334	n. a.
B. "adusta"	CVP43	Spain	MT821136	-	n. a.
B. albocinerea	MV55917, holotype	Brazil	MH025419	MH025419	Motato-Vásquez *et al.* 2020
B. albocinerea	Dai 16411	USA	MW507102	MW520207	Wang *et al.* 2021
B. atroalba	SP 446205	Brazil	KT305930	KT305930	Westphalen *et al.* 2015
B. atroalba	SP 445672	Brazil	KT305931	KT305931	Westphalen *et al.* 2015
B. carnegieae	JV0407/27-J	USA	MW507122	-	Wang *et al.* 2021
B. carnegieae	RLG7277T	USA	KY948792	KY948854	Justo *et al.* 2017
B. centroamericana	BRNM 771949, isotype	Mexico	KT305933	KT305933	Westphalen *et al.* 2015
B. centroamericana	BRNM 771950	Mexico	KT305934	KT305934	Westphalen *et al.* 2015
B. ecuadorensis	JV1906/C16-J, isotype	Ecuador	MW507105	-	Wang *et al.* 2021

......*continued on the next page*

TABLE 1 (Continued)

Species	Sample no	Country	GenBank Accession no.		Reference
			ITS no.	LSU no.	
B. fulgida	Dai 16107, holotype	China	MW507106	MW520209	Wang *et al.* 2021
B. fulgida	Dai 12284	China	MW507107	-	Wang *et al.* 2021
B. fumosa	BRNM 771947	Slovakia	KT305937	KT305937	Westphalen *et al.* 2015
B. fumosa	Cui 10747	China	MW507111	MW520212	Wang *et al.* 2021
B. lecomtei	**MJ 2889, holotype**	**Czechia**	**PP104713**	**PP104712**	**This study**
B. lecomtei	**Sabet 2280**	**France**	**PV226174**	**PV226177**	**This study**
B. lecomtei	**Sabet 2464**	**France**	**PV226175**		**This study**
B. lecomtei	**Sabet 2686**	**France**	**PV226176**		**This study**
B. mikrofumosa	JV1707/10J-2	Costa Rica	MW507114	-	Wang *et al.* 2021
B. mikrofumosa	MV363	Brazil	MH023526	MH023526	Motato-Vásquez *et al.* 2020
B. minispora	Dai 15234, holotype	China	MW507115	MW520214	Wang *et al.* 2021
B. minispora	Cui 5376	China	MW507116	MW520215	Wang *et al.* 2021
B. resupinata	Dai 16642, holotype	Thailand	MW507117	MW520216	Wang *et al.* 2021
B. resupinata	Cui 8017	China	KU509526	-	Wang *et al.* 2021
Hapalopilus rutilans	Mushroom Observer 487451	USA	OP297813	OP297813	n. a.
Phlebiopsis gigantea	FBCC986		LN611134	LN611134	Kuuskeri *et al.* 2015
Terana caerulea	Dai 22632	China	OL457973	OL457442	n. a.

Bold = Newly generated sequences in this study. N. a. = No reference is available at the Genbank record.

Phylogenetic analyses

Sequence alignment was done in MAFFT v. 7.222 (Katoh and Standley 2013) under the Q-INS-I strategy (considering rRNA secondary structure). The models for the two datasets selected by Mega7 (Kumar *et al.* 2016) were K2+G both for ITS and LSU dataset. Bayesian analysis (BA) were conducted with MrBayes 3.2.7a (Ronquist *et al.* 2012). Four independent runs of four Markov chains were performed for 1 M generations, with trees sampled every 100 generations. The first 50 % of the sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a majority rule consensus and calculate Bayesian Posterior Probabilities (BPP) of the clades. Maximum likelihood (ML) searches were conducted in Mega 7 (Kumar *et al.* 2016). Branch support for ML analysis was determined by 1 000 bootstrap replicates. Sequences of Phanerochaetaceae spp. *Hapalopilus rutilans* (Pers.) Murrill, *Phlebiopsis gigantea* (Fr.) Jülich and *Terana caerulea* (Lam.) Kuntze, obtained from GenBank, were used as the outgroups (Justo *et al.* 2017).

Results

Molecular phylogeny

The ITS sequences of the new species showed the highest, 99.58 % identity with sequence of *Bjerkandera adusta* P43 (MT821136) but only ≤ 92.5 % identity with sequences of other *Bjerkandera* spp. The LSU sequences showed the highest, 98.84% identity with sequence of *B. resupinata* (MW520216).

The final ITS alignment included 32 sequences with 670 nucleotide sites, from which 469 were constant and 114 parsimony-informative and 72 singleton sites. The final LSU alignment included 23 sequences with 1035 nucleotide sites, from which 935 were constant, 53 were parsimony-informative, and 47 were singleton sites. Bayesian analyses resulted in a nearly congruent topology to ML analyses, and thus only the ML trees are provided (Figs. 1, 2). The phylogenetic analyses confirmed the position of the specimen within *Bjerkandera* separated from all currently recognized species. Results of ITS analysis (Fig. 1) indicate the basal position of *B. lecomtei* within other *Bjerkandera* spp. On the contrary, the LSU phylogram revealed proximity of the new species to *B. fulgida, B. resupinata* and *B. carnegieae* (Fig. 2).

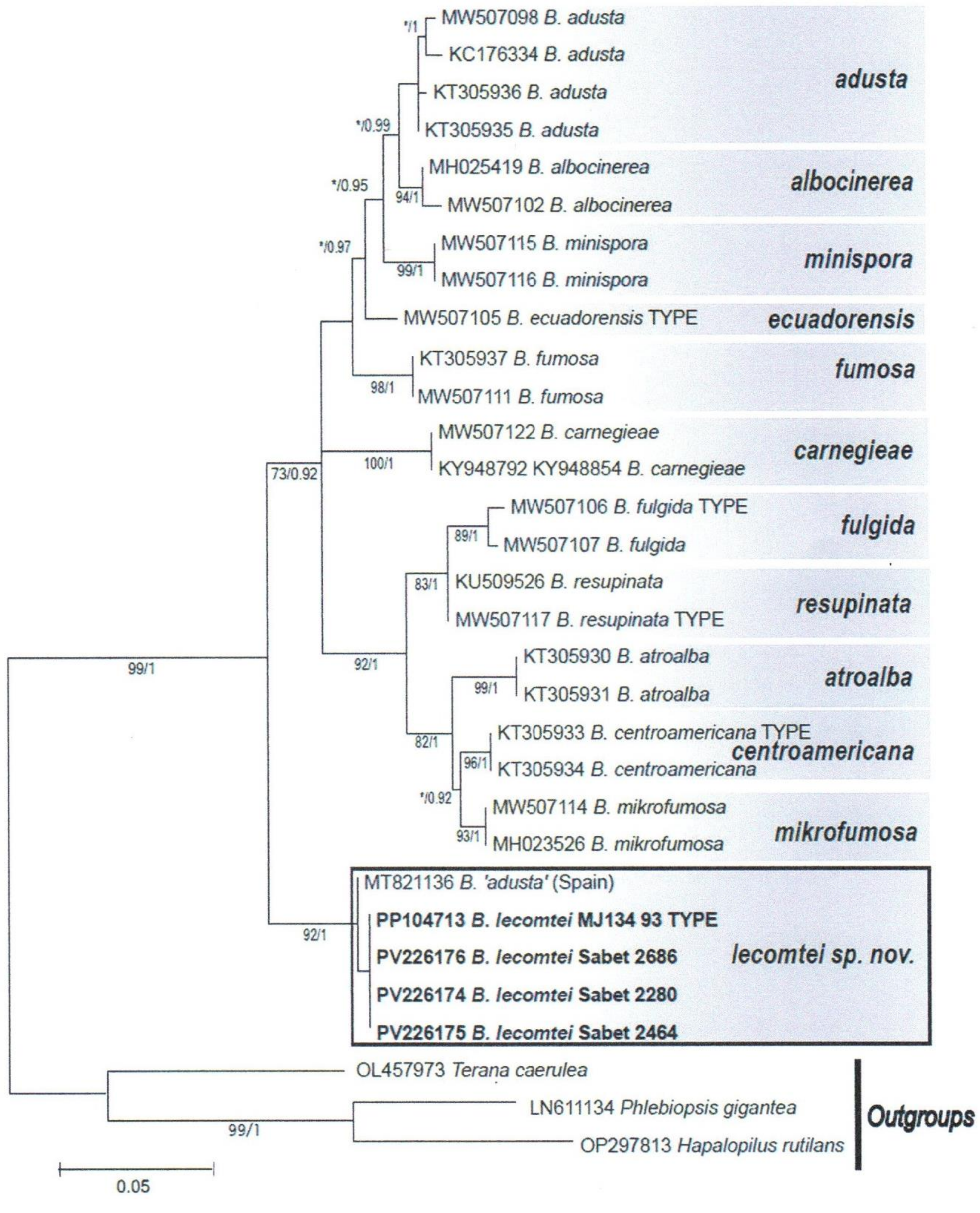

FIGURE 1. Phylogeny of *Bjerkandera* by ML analysis based on ITS dataset. Branches are labeled with maximum likelihood bootstrap values (ML) higher than 70% and Bayesian Posterior Probabilities (BPPs) more than 0.90. The asterisk (*) marks lower values of statistical support. The bar indicates the number of expected substitutions per position. New sequences are in bold.

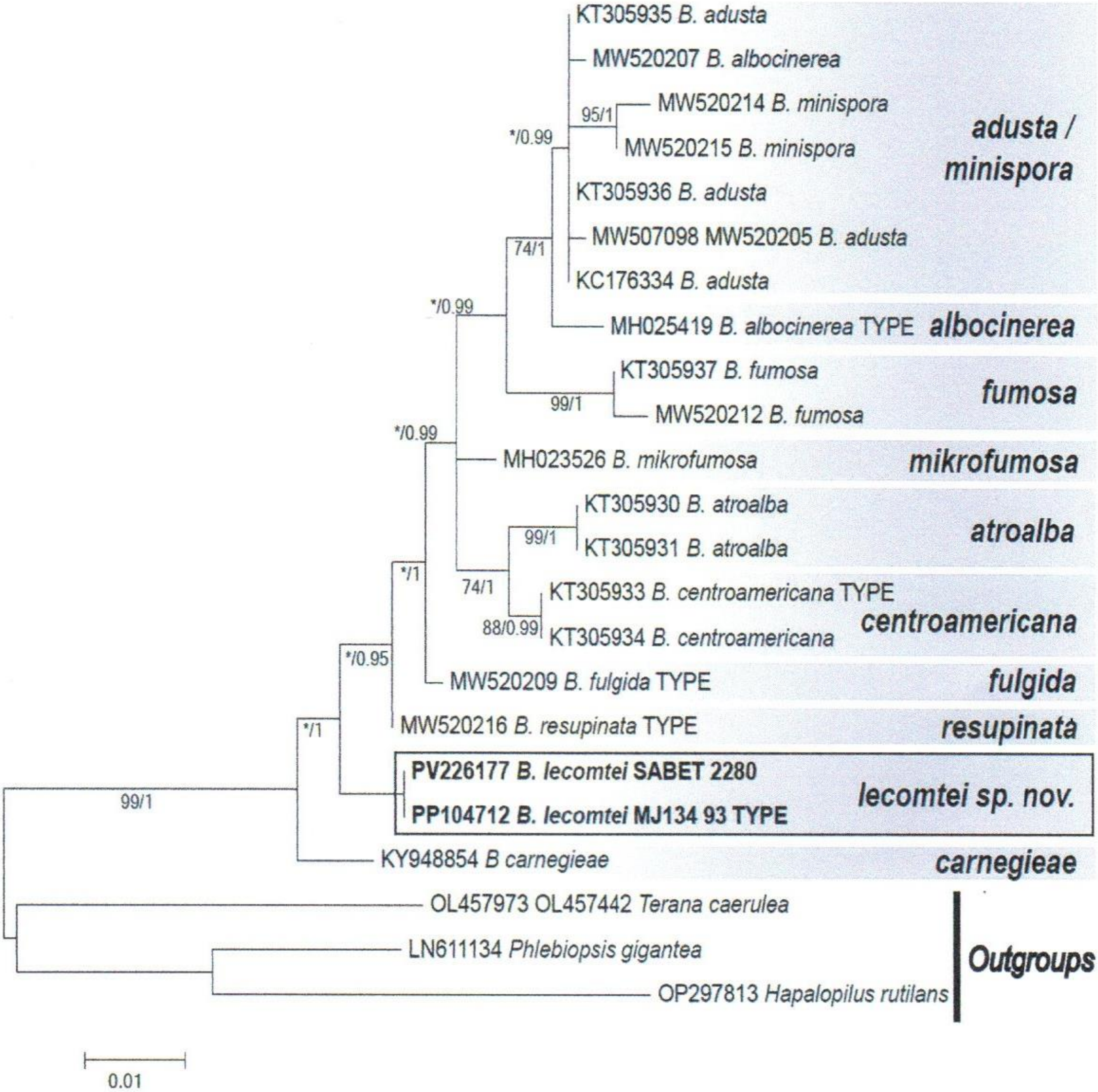

FIGURE 2. Phylogeny of *Bjerkandera* by ML analysis based on LSU dataset. Branches are labeled with maximum likelihood bootstrap values (ML) higher than 70% and Bayesian Posterior Probabilities (BPPs) more than 0.90. The asterisk (*) marks lower values of statistical support. The bar indicates the number of expected substitutions per position. New species is in bold.

Taxonomy

Bjerkandera lecomtei Tomšovský, V. Sabet, Welti, P.-A. Moreau & Dumez, ***sp. nov.***
MycoBank:—857869, Index Fungorum number:—IF901612; Figs. 3, 4, 5

Etymology:—*lecomtei,* dedicated to the Belgian mycologist Marcel Lecomte for generously shearing his broad experience in microscopy and field mycology .

Diagnosis:—Differs from temperate *Bjerkandera* spp. by resupinate basidiomes and absence of differentiated hypophyllum ("black line"). Similar *Bjerkandera carnegieae* and *B. resupinata* have different basidiospores and DNA sequences of ITS and LSU regions of ribosomal RNA gene.

Holotype:—Czechia, Bulhary, dead arm of river, alluvial forest NE of the village, 12 km BW of Břeclav, *Acer campestre*—on fallen trunk, coordinates approximately 48.8348564N, 16.7571736E, elevation 170 m, 04 May 1993, Petr Vampola (P. Vampola 134/93), holotype MJ 2889, isotype BRNM 844459.

Basidiomata annual, resupinate, adnate, corky, occasionally up to 1 m broad, with a width between 30 and 50 cm., 0.5 mm thick at base. *Pore surface* whitish when fresh then yellow ochre to yellowish brown or brownish (5C6, 5C7, 5E8), deep brownish when dry, sterile margin thin pale white to cream (4A3), slightly fimbriate (or tomentose); pores mostly angular, sometimes rounded, 1–4 (5) per mm; dissepiments thin, entire to lacerated. *Subiculum* pale cream (4A3); up to 0.2 mm thick with a distinct hymenopodial zone between subiculum and tubes in the type collection, not distinct in French (indoor) collections. Tubes up to 3 mm long, concolorous with the pore surface (5C6, 5C7), becoming dark in KOH.

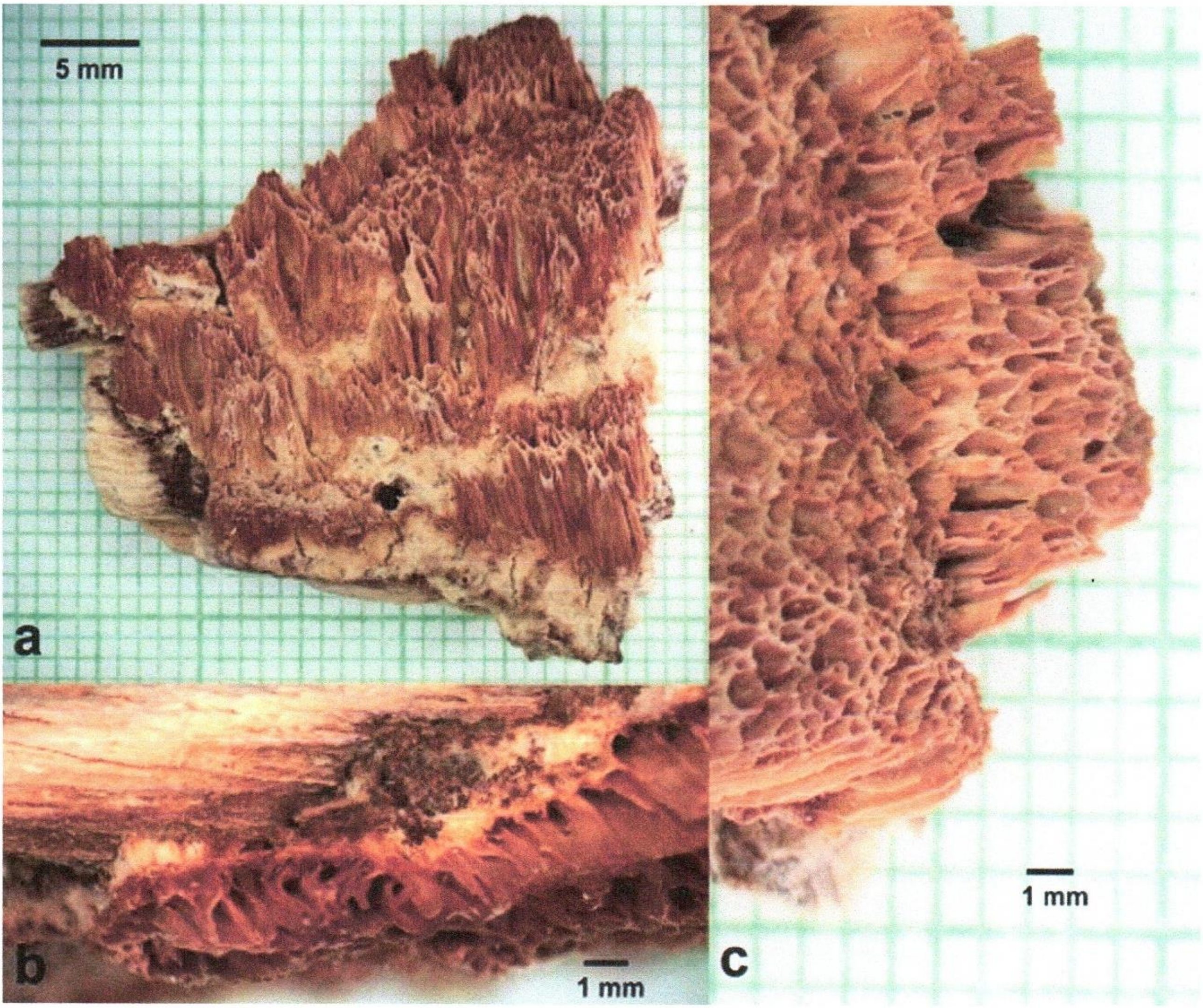

FIGURE 3. Basidiomata of *Bjerkandera lecomtei* BRNM 844459. a General view of basidiome. b Subiculum and tubes. c Tubes.

Basidiospores on holotype, n = 35 (5.5–)5.7–6.8–8(–8.3) × (3.4–)3.5–3.8–4.2(–4.9) µm, Q = (1.49–)1.56–1.81–2.03(–2.14); on V. Sabet 2464, n =35) (4.9–)5.5–6.1–6.7(–7.2) × (2.9–)3.2–3.5–3.9(–4.0) µm, Q = (1.4–) 1.6–1.7–2.0(–2.1); average values of both specimens L = 6.46 µm, W = 3.66 µm, Q = 1.76; ellipsoid or cylindro-ellipsoid, some slightly allantoid, thin-walled; negative in Melzer's reagent. *Basidia* 14.4–21.5 × 4.6–6.5µm, clavate, 4-spored; basidioles similar, 3.3–5.1 µm wide. *Cystidia and cystidioles* absent. *Hyphal system* monomitic, generative hyphae with clamp connections, smooth, colourless; hyphae of subiculum thick-walled with a wide lumen, rarely branched, 3.5–4.9 µm in diam; hyphae of tubes thin- to slightly thick-walled, frequently branched, 2.3–3.6 µm in diam.

The French material is somewhat distinct from the description of the Czech collection in its subiculum, which is approximately 300 µm thick and characterized by an alternation of compact zones composed of fascicles of unbranched, either horizontally or vertically oriented hyphae (3–8 µm wide) with moderately thickened walls (0.3–1 µm thick, locally yellowish in KOH), and looser zones predominantly composed of thin- to thick-walled, frequently branched and lobate, interwoven hyphae (2–4 µm wide). The tramal hyphal system is mainly composed of fascicles of unbranched, vertically oriented hyphae thinner (<4 µm) and shorter hyphae, thin or thick-walled, often highly and shortly branched, together forming a compact and cohesive structure overall.

Material examined: CZECHIA. South Moravian Region, Bulhary, dead arm of river, alluvial forest NE of the village, 10 km BW of Břeclav, *Acer campestre*—on fallen trunk, coordinates approximately 48.8348564N, 16.7571736E, 170 m a.s.l., 04 May 1993, Petr Vampola (P. Vampola 134/93), holotype MJ 2889, isotype BRNM 844459; PP104713

(ITS), PP104712 (LSU). FRANCE. Loiret, Jargeau, on a house beam (*Quercus* sp), Nov 2024, Pierre-Yves Sachot (V. Sabet, 2280, LIP); GenBank: PV226174 (ITS), PV226177 (LSU). Same locality, 12 Dec 2024, Pierre-Yves Sachot (V. Sabet, 2464, LIP); Genbank: PV226175 (ITS). Meurthe-et-Moselle, Jarville-la-Malgrange, on a house beam, 24 January 2025 (V. Sabet, 2686, LIP); Genbank: PV226176 (ITS).

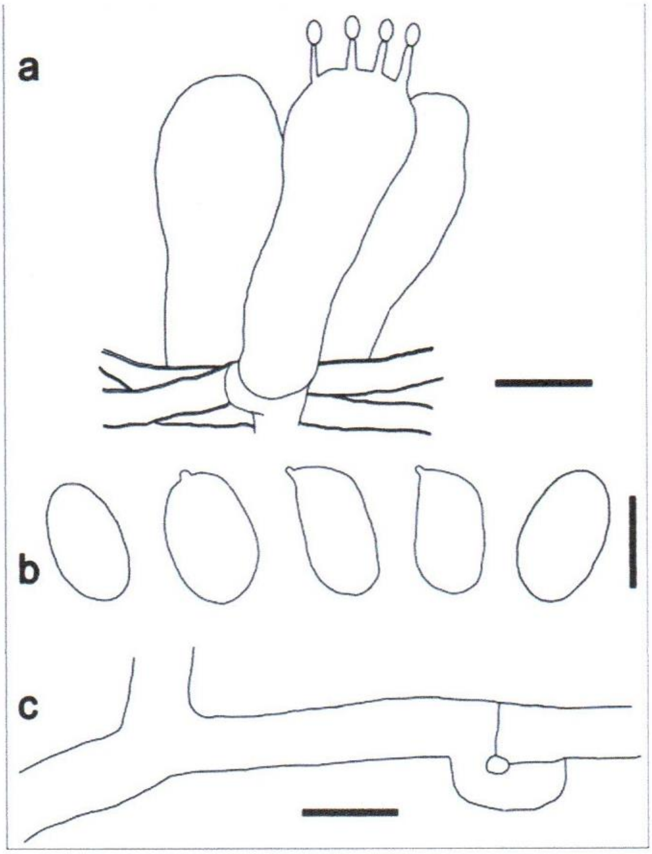

FIGURE 4. Microscopic structures of *Bjerkandera lecomtei* (MJ 2889, holotype). a Hymenium. b Basidiospores. c Generative hyphae. Scale bars: a–c = 5 µm.

Notes: *Bjerkandera lecomtei* is similar to *Raduliporus aneirinus (Ceriporiopsis aneirina)*. The two species differ in size of basidia and DNA sequences, while the dimensions of basidiospores and pores overlap. Other similar polypore occurring in the same habitat is *Pouzaroporia subrufa* having distinctly dimitic hyphal system. The color of *B. lecomtei* basidiomes is close to that of *B. carnegieae* but the species differ in ecology and basidiospore dimensions. The description of *B. lecomtei* is based one old herbarium specimen (collected in 1993 in nature) and three recent indoor collections in France. No fresh basidiome of the species was found recently in the type locality.

Discussion

Bjerkandera lecomtei was first discovered in the southernmost part of Czechia, in a floodplain forest with warm climate. A wide distribution of *B. lecomtei* in Europe and a broader spectrum of woody hosts was confirmed by the ITS sequence GenBank no. MT821136, identified as *B. adusta* (see in Table 1 and Fig. 1) from roots of *Olea europea* (cultivar Picual) in Spain. The ITS1 sequence of *B. lecomtei* matchs some records of the GlobalFungi database (Větrovský *et al.* 2020), the species was revealed among airborne mycobiota sequences of cropland (rice field) in North Italy (Franco Ortega *et al.* 2020). Our recent collections from beams (houses investigated for sanitary expertises) in France may confirm a xerophilic or thermophilic tendency, and suggests a natural Mediterranean distribution, with a special ability of growth in dry or confined conditions on hardwoods. The detailed revision of similar specimens deposited in south European fungaria is desirable to reveal distribution and ecological preferences of the species.

Phylogenetic analysis of the ITS marker (Fig. 1) suggests that *B. lecomtei* is clearly positioned basally within the *Bjerkandera* lineage, a position it shares with two other resupinate species, *B. carnegieae* and *B. resupinata*, according to the analysis of the LSU marker (Fig. 2)

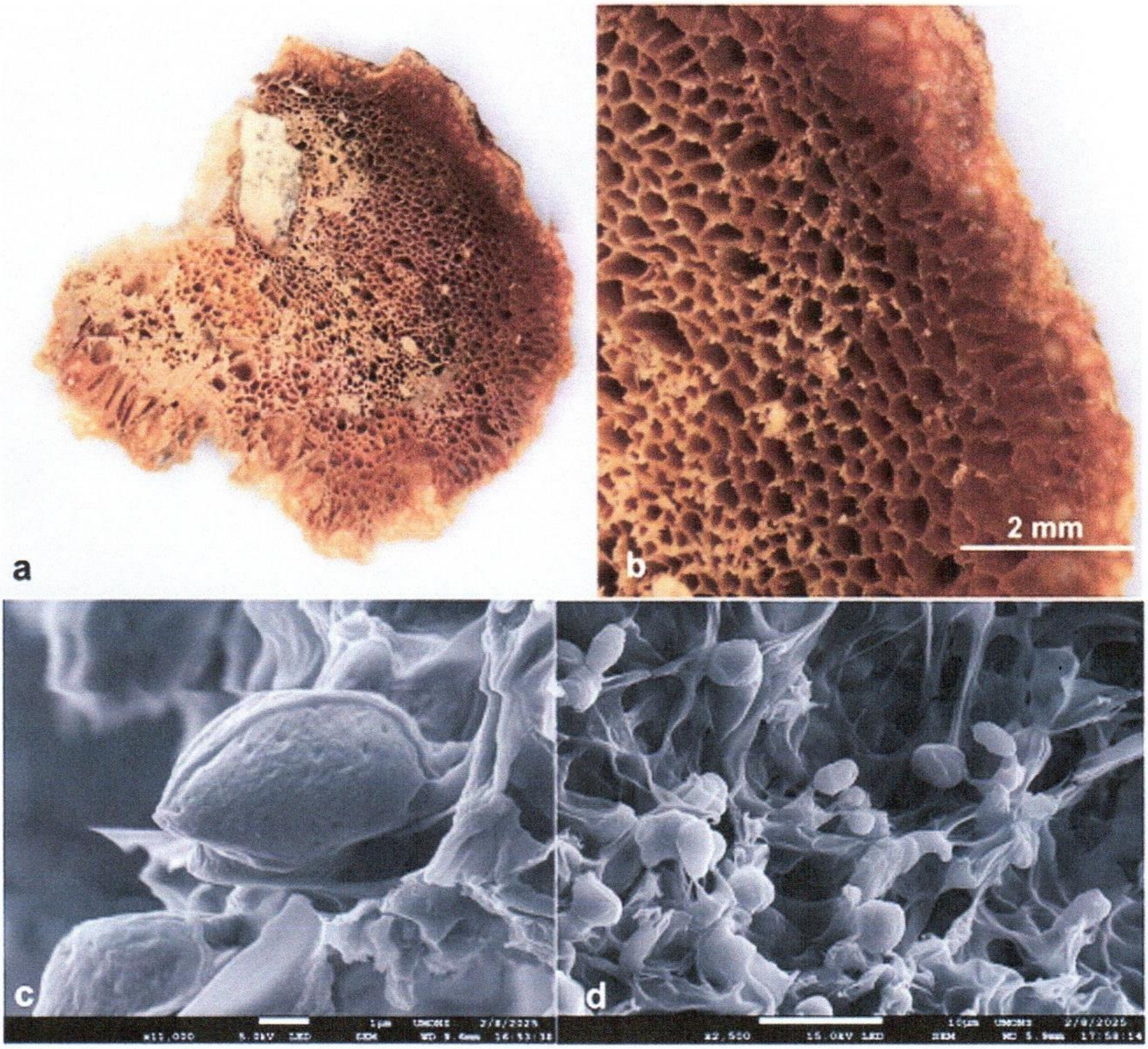

FIGURE 5. Microscopic structures of *Bjerkandera lecomtei* (V. Sabet 2464). a–b Hymenium; c Basidiospore under scanning electron microscope, bar = 1 µm; d Hymenium under scanning electron microscope, bar = 10 µm.

Updated key of *Bjerkandera* worldwide (based on Motato-Vásquez *et al.* 2020 and Wang *et al.* 2011)

1.	Basidiomata resupinate	2
-	Basidiomata effused-reflexed to pileate	5
2.	Growing on *Carnegiea gigantea* (saguaro cactus), North America	*B. carnegieae*
-	Growing on hardwoods, temperate Eurasia	3
3.	Hymenial surface ash grey, white towards margin, not browning nor reddening when drying	*B. adusta* f. *resupinata*
-	Hymenial surface whitish to yellowish ochre, brownish when dry, margin whitish	4
4.	Spores narrowly ellipsoid to cylindrical, Q = 1.55-2.03; Europe	*B. lecomtei*
-	Spores ellipsoid to broadly ellipsoid, Q = 1.40-1.42; China	*B. resupinata*
5.	Pores < 5 per mm	6
-	Pores > 5 per mm	7
6.	Pileal surface white to cream; basidiospores broadly ellipsoid	*B. atroalba*
-	Pileal surface buff to woody-coloured; basidiospores shortly cylindrical	*B. fumosa*
7.	Pileal surface white to cream when fresh	8
-	Pileal surface buff to grey when fresh	9
8.	Basidiospores subglobose to broadly ellipsoid	*B. centroamericana*
-	Basidiospores oblong-ellipsoid to ellipsoid	*B. albocinerea*
9.	Crystals present among hymenium	10
-	Crystals absent among hymenium	11
10	Pileal margin dark brown when dry	*B. mikrofumosa*

\- Pileal margin buff when dry .... *B. fulgida*
11 Basidiospores > 4.5 µm in length .... *B. adusta*
\- Basidiospores < 4.5 µm in length 12
12\. Basidiospores 3.1–4.2 × 2–2.8 µm, Q = 1.49–1.53 .... *B. minispora*
\- Basidiospores 3.9–4.5 × 2.7–3 µm, Q = 1.43 .... *B. ecuadorensis*

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