Polyporus tubaeformis (Basidiomycota, Polyporaceae) – identity, ecology and distribution in the Czech Republic, Austria, Slovakia and Ukraine

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Holec J., Vampola P., Kout J., Beran M., Krisai-Greilhuber I., Hahn C. & Kolařík M. (2021): *Polyporus tubaeformis* (Basidiomycota, Polyporaceae) – identity, ecology and distribution in the Czech Republic, Austria, Slovakia and Ukraine. – Sydowia 73: 245–256.

Records of *Polyporus tubaeformis* are newly reported from the Czech Republic, Austria, Slovakia, and Ukraine. They originate from mountains at the elevation 750–1250 m a.s.l., namely Bohemian Forest, Northern Limestone Alps, Western and Eastern Carpathians. The habitats are a) steep rocky slopes above glacial lakes grown by natural montane Norway spruce forests with admixed *Betula* and *Sorbus* and b) valleys of montane brooks grown by shrubs or scattered mixed or spruce forests. Common features of the habitats are their near-natural to natural character, relatively cold climate and high air humidity and/or soil moisture. Basidiomata were found on wood or woody stems of broadleaved trees or shrubs: *Alnus incana*, *Rubus idaeus*, *Rubus* sp., *Salix* sp., *Sorbus aucuparia*, and *Sorbus* sp. Our data confirm *P. tubaeformis* as a species having predominantly boreal-montane distribution in Europe. Our collections belong to the European population of *P. tubaeformis,* which differs from the North American one in the ITS rDNA barcode. The separate species status of *P. tubaeformis* and *Picipes subtubaeformis* was confirmed by differences in ITS and LSU rDNA sequences. The revision of available molecular data showed that materials reported as *P. tubaeformis* from Japan represent other species. Consequently, records from temperate China and Russian Far East should be revised using molecular data.

Keywords: Fungi, DNA sequences, Bohemian Forest, the Alps, the Carpathians.

Polyporus tubaeformis (P. Karst.) Ryvarden & Gilb. is a stipitate polypore from the Melanopus group of *Polyporus* (Núñez & Ryvarden 1995a). Although described already in 19ᵗʰ century, it has long been included under *Polyporus badius* (Pers.) Schwein. or *Polyporus melanopus* (Pers.) Fr. and its full acceptance as a separate species started much later (Niemelä & Kotiranta 1991, Ryvarden & Gilbertson 1994). *Polyporus tubaeformis* is distinguished by infundibuliform, brown to reddish brown, rather thin and small pileus (up to 70 mm broad and 3 mm thick, Dai 1996), very narrow pores (5–7 per mm), central or slightly eccentric, slender, dark, velvety, finely wrinkled stipe, rather short spores (up to 9 µm) and generative hyphae with clamp connections (Ryvarden & Melo 2017). Further microscopic differences are summarized by Dai (1996). Based on molecular genetic data, the species has recently been transferred to a newly described genus *Picipes* Zmitr. & Kovalenko (Zmitrovich & Kovalenko 2016) as *Picipes tubaeformis* (P. Karst.) Zmitr. & Kovalenko. As long as the genus-level classification of fungi is still an ongoing process and also for practical reasons we use the traditional name *Polyporus*.

Polyporus tubaeformis is rather common in boreal habitats of Norway, Sweden and Finland but rare in Central Europe (Ryvarden & Melo 2017). There are also records from Scotland (Krueger 2002, Krüger et al. 2006), France (Rivoire 2020), Italy (Bernicchia & Gorjón 2020), Denmark (Anonymus 1), Estonia (Anonymus 2), Latvia (Anonymus 3), and Russia (e.g. Dai 1996, Volobuev 2013, Vlasen-

ko & Vlasenko 2015). Well-documented records exist from the USA (Krueger 2002, Krüger et al. 2006, Vlasák 2015) and internet reports from Canada (e.g. www.gbif.org). Older reports from China (Dai 1996, 1999, 2000) are not included in the newest work on diversity of Chinese polypores (Cui et al. 2019). *Polyporus tubaeformis* has been also reported from Japan (Nuñez & Ryvarden 1995b, Sotome et al. 2008), but as we are showing in this study, molecular data of Japanese collections do not fit the species.

Concerning the occurrence in Central Europe, *P. tubaeformis* has been reported only from Germany (Krueger 2002, Krüger et al. 2006, Anonymus 4) and briefly mentioned from Austria (Dämon & Krisai-Greilhuber 2017). Our aim is to evaluate the occurrence, ecology and taxonomy of *P. tubaeformis* based on new or hitherto unpublished data from the Czech Republic, Austria, Slovakia and Ukraine.

Material and methods

Morphology

Description of macromorphology is based on fresh basidiomata and their photographs. Micromorphological characters were studied on dried material using Melzer's reagent. The emphasis was on diagnostic characters, especially the size and shape of spores. At least 30 spores were measured for each collection.

DNA study

Genomic DNA was isolated from dried vouchers using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany). A ribosomal DNA (rDNA) fragment containing the internal transcribed spacers (ITS1 and ITS2), the 5.8S subunit and partial sequence of the 28S subunit, was amplified and sequenced as described in Holec et al. (2019). In addition, a reverse PCR primer LR7, and sequencing primer LR3R (Vilgalys & Hester 1990) were used to get longer part of the 28S rDNA. DNA extraction of the Austrian herbarium materials and amplification of ITS region of nuc rDNA containing ITS1-5.8S-ITS2 follow Hahn et al. (2019); the sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems). Unfortunately, sequencing of two relatively recent collections (PRM 890858, PRM: JH 109/2007) was unsuccessful. Consequently, two old collections (PRM 820896, PRM 497748) were not studied molecularly due to the anticipated difficulties.

Eight sequences (Tab. 1) were deposited in the EMBL database under the codes LR898979–LR898983 (ITS rDNA) and LR898976–LR898978 (28S rDNA). Sequences were compared with data from the NCBI GenBank using a BlastN similarity search and the taxon selection was focused on the identification of *P. tubaeformis* sequences. A matrix containing ITS sequences of *P. tubaeformis* and related species was aligned in MAFFT 6 using the G-INS-i strategy (Katoh et al. 2009). Final ITS dataset had 30 sequences and 610 positions with 168 variable characters and 12 singletons. LSU dataset had 18 sequences and 612 positions with 47 variable characters and 5 singletons. Maximum likelihood (ML) phylogenetic analyses were performed in PHYML (Guindon et al. 2010) using the default settings and 500 bootstrap replicates with the K2+G model for both datasets determined using MEGA X (Kumar et al. 2018). Bayesian phylogenetic analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). A metropolis coupled Markov chain Monte Carlo search algorithm with 5,000,000 generations was used. The resulting trees were sampled every 1000 generations. Chain convergence was assessed using Tracer 1.4 (http://tree.bio.ed. ac.uk/software/tracer), and the first 20% of trees were discarded as burn-in. The final tree was rooted by *Polyporus varius* (Pers.) Fr. Minimum Evolution phylogenetic tree was computed in MEGA X with 500 bootstrap replicates, using LogDet distances and pairwise deletion option.

Collections studied

Data from herbarium labels were translated to English. Original spelling of some locality names is given in parentheses. Coordinates of localities not georeferenced directly in the field were derived additionally from maps as the approximate locality centre followed by the highest expected error in brackets. Their expected elevation range or approximate elevation have been added as the qualified estimation based on personal knowledge of the localities. Herbarium acronyms are given in accordance with Index Herbariorum (http://sweetgum. nybg.org/science/ih/). Collections marked with asterisk (*) were successfully sequenced (see also Tab. 1).

Polyporus tubaeformis

CZECH REPUBLIC. Bohemian Forest (= Šumava Mts.), NNW of the town of Železná Ruda, rocky wall above glacial lake **Černé jezero** („Jezerní stěna"), approximate coordinates 49.1754311 N, 13.1785497 E (error up to 1 km), expected ele-

Tab. 1. *Polyporus* collections (*P*.: *Polyporus*, *Pi*.: *Picipes*) used in the phylogenetic analysis. The sequences generated in this study are printed in bold. Type specimens are marked by "T".

Species	Voucher or strain number	Sequence accession no.	Country	Reference
P. americanus	JV 0509-149 (T)	KC572002 (ITS), KC572041 (LSU)	USA	Dai et al. (2014)
P. badius	Cui 10501	KC572015 (ITS), KC572053 (LSU)	China	Dai et al. (2014)
P. badius	Cui 2600	KX900107 (LSU)	China	Dai et al. (2014)
P. badius	JV 0809-4	KC572019 (ITS)	USA	Dai et al. (2014)
P. conifericola (deposited as *P. tubaeformis*)	WD1839	AB587634 (ITS), AB368101 (LSU)	Japan	Sotome et al. (2011)
P. conifericola	Dai 11114 (T)	JX473244 (ITS), KC572061 (LSU)	China	Zhou et al. (2016)
P. conifericola	Dai 10190	KX899981 (ITS), KC572060 (LSU)	China	Zhou et al. (2016)
P. melanopus	H 6029190	JQ964422 (ITS)	Finland	Xue & Zhou (2012)
P. melanopus	MJ 400-93	KC572027 (ITS)	Czech Republic	Zhou et al. (2016)
P. melanopus	H 6003449	JQ964422 (ITS), KC572064 (LSU)	Finland	Xue & Zhou (2012)
P. melanopus	TENN11465	AJ487951 (LSU)	Austria	Krüger et al. (2006)
P. melanopus	**BRNM 718734**	**LR898982 (ITS)**	**South Korea**	**this study**
P. subtubaeformis	Dai 11870 (T)	KU189752 (ITS), KU189784 (LSU)	China	Zhou et al. (2016)
P. subtubaeformis	Cui 10793	KU189753 (ITS)	China	Zhou et al. (2016)
P. subtubaeformis	Cui 10543	KX900044 (ITS), KX900160 (LSU)	China	unpublished
P. tubaeformis	TENN55689	AF511441 (ITS)	USA (CA)	Krüger et al. (2006)
Pi. tubaeformis	JV 0309-1	KC572034 (ITS), KC572072 (LSU)	USA	Dai et al. (2014)
Pi. tubaeformis	TENN55417	AF511442 (ITS)	USA (CA)	Krüger et al. (2006)
P. tubaeformis	TENN55689	AF511440 (ITS)	USA (CA)	Krüger et al. (2006)
P. tubaeformis	JV 0308-69A	KC572033 (ITS)	USA	Dai et al. (2014)
P. tubaeformis	O 63528	AF511443 (ITS)	Norway	Krüger et al. (2006)
P. tubaeformis	Niemelä 6855	KC572036 (ITS), KC572073 (LSU)	Finland	Dai et al. (2014)
P. tubaeformis	TENN55881	AF511439 (ITS)	Scotland	Krüger et al. (2006)
P. tubaeformis	**CB 21851**	**LR898979 (ITS), LR898977 (LSU)**	**Czech Republic**	**this study**
P. tubaeformis	**Kout 20Aug2019**	**LR898980 (ITS), LR898976 (LSU)**	**Slovakia**	**this study**
P. tubaeformis	**WU 40225**	**LR898981 (ITS),** submitted by M. Kolařík **MW343491 (ITS),** secondarily submitted by I. Krisai-Greilhuber	**Austria**	**this study**
P. tubaeformis (deposited as *P. leptocephalus*)	UBC:F19745	HQ604799 (ITS)	USA (OR)	unpublished
P. varius	CBS 454.50	MH856707 (ITS), MH868224 (LSU)	France	Vu et al. (2019)
P. varius	**MJ 392/89**	**LR898983 (ITS), LR898978 (LSU)**	**Czech Republic**	**this study**
Polyporus sp.	SWFC 006332	MK838843 (ITS)	China	unpublished
Polyporus sp.	SWFC 006329	MK838842 (ITS)	China	unpublished
Polyporus sp. (deposited as *P. tubaeformis*)	WD2353	AB368102 (LSU)	China	Sotome et al. (2008)

vation range 1010–1300 m a.s.l., natural montane Norway spruce forests, on dead *Sorbus* trees, August 1926 *leg. & det*. A. Hilitzer as *Polyporus picipes* Fr., 17 January 2008 rev. Z. Pouzar as *Polyporus tubaeformis*, 19 July 2019 *rev*. P. Vampola as *Polyporus tubaeformis* (PRM 820896). – Bohemian Forest (= Šumava Mts.), 2 km S of the centre (church) of the town of Železná Ruda, valley called **Ferdinandovo údolí**, approximate coordinates 49.1190556 N, 13.2341386 E (error up to 30 m), elevation 750 m a.s.l., mixed scattered man-influenced stand below asphalt road through the valley (*Picea*, *Salix*, *Fagus*, *Betula* etc.), *Alnus incana*: twig lying on soil, 19 June 1997 *leg. & det*. J. Holec as *Polyporus* sp., 26 April 2019 *rev*. P. Vampola as *Polyporus tubaeformis* (PRM 890858). – Bohemian Forest (= Šumava Mts.), 0.8 km SEE of the centre (church) of the village of Srní, W of Vydra river (Povydří protected area), **valley of Hrádecký potok brook**: left bank, approximate coordinates 49.0834419 N, 13.4905431 E (error up to 50 m), elevation ca 790 m a.s.l., small forest meadow grown by grass and mosses, surrounded by high-grown *Picea* forest, on decaying woody stem of *Rubus idaeus* lying on soil, 28 August 2007 *leg*. J. Holec and M. Beran, *det*. Z. Pouzar, 20 September 2019 *rev*. P. Vampola as *Polyporus tubaeformis* (PRM: JH109/2007). – Bohemian Forest (= Šumava Mts.), SWW of the village of Nová Pec, E slope NW of the glacial lake **Plešné jezero**, 48.7777433 N, 13.8641761 E (error up to 50 m), elevation ca 1120 m a.s.l., close to marked tourist trail from the lake dam to the Stifter's monument, young *Picea* stand with admixed *Betula* and *Sorbus*, before 2008 surrounded by old montane Norway spruce forest which has been killed by bark beetle in about 2008–2015 and currently re-forest spontaneously, on thin fallen decaying trunk of *Sorbus aucuparia*, 29 June 2018 *leg. & det*. M. Beran, 16 November 2019 *rev*. P. Vampola as *Polyporus tubaeformis* (CB 21851*). – AUSTRIA. Upper Austria, Mühlviertel, Böhmerwald, district Rohrbach, community Ulrichsberg, close to the settlement of **Schöneben**, 48.7040833 N, 13.9487222 E, elevation ca 950 m a.s.l., *Picea* forest, on unidentified branch, 11 September 2017 *leg*. P. Kresitschnig s.n., *det*. I. Krisai-Greilhuber (WU 40225*). – Upper Austria, Mühlviertel, Böhmerwald, district Rohrbach, community Ulrichsberg, **Sulzberg hill**, 48.6949722 N, 13.9500556 E, mapping grid square 7349/2, elevation ca 1000 m a.s.l., *Picea* forest, on unidentified twig, 14 September 2017 *leg*. ÖMG (Austrian Mycological Society), *det*. I. Krisai-Greilhuber (WU 40188). – Upper Austria, Northern Limestone Alps, district Kirchdorf an der Krems, community Rosenau am Hengstpaß, **Hengstpass**, 47.7019444 N, 14.4611111 E, mapping grid square 8252/4, elevation 900 m a.s.l., on small branch of *Alnus*, 27 August 2018 *leg*. K. Oberhuber, *det*. Ch. Hahn & I. Krisai-Greilhuber (WU 40837). Styria, district and community Liezen, Machl, 47.588471 N, 14.280014 E, mapping grid square 8451/2, elevation 800 m a.s.l., brook side on moist lying small branch of *Salix*, 30 August 2018 *leg*. A. Gallé, G. Friebes et al., *det*. Ch. Hahn & I. Krisai-Greilhuber (fungarium Hahn). – SLOVAKIA. Západné Tatry Mts., 9.5 km NNE of the village of Podbanské, **Tichá dolina** valley, approximate coordinates 49.2221328 N, 19.9497936 E (error up to 50 m), elevation 1250 m a.s.l., shrubs dominated by *Salix* along brook and asphalt road passing the valley (outside closed Norway spruce stands which prevail in the vicinity), between the brook and asphalt road, moist site saturated by water from a small stream, covered by herbs, mosses, dead plants and pieces of fallen twigs, on dead woody stem of *Rubus*, 20 August 2019 *leg. & det*. J. Kout, 20 Nov 2019 *rev*. P. Vampola (herbarium of the University of West Bohemia, Plzeň*; duplicate: MJ). – UKRAINE. Zakarpatska Oblast Province, Eastern Carpathians, NNE of the

town of Tiachiv, in forests above the village of **Nimetska Mokra** („Německá Mokrá“), approximate coordinates 48.3726336 N, 23.8478006 E (error up to 3 km), elevation range given by Pilát 800–1300 m a.s.l., substrate: *Fagus sylvatica*, July 1932 *leg. & det*. A. Pilát as *Polyporellus melanopus*, 31 September 1992 *rev*. Z. Pouzar as *Polyporus tubaeformis*, 19 July 2019 *rev*. P. Vampola as *Polyporus tubaeformis* (PRM 497748).

Polyporus melanopus

REPUBLIC OF KOREA. Kyonggi-do prov., Guri, **Donggureung** (East Nine Tombs), 37.6175000 N, 127.1336111 E, mixed forest of *Quercus mongolica*, *Q. acutissima*, admixed *Pinus multiflora* and *Sorbus* sp., elevation 30 m a.s.l., on fallen twig of *Quercus mongolica*, 11 July 2009 *leg*. V. Antonín, *det*. P. Vampola as *P. tubaeformis* (BRNM 718734*).

Polyporus varius

CZECH REPUBLIC. Bohemian-Moravian Highlands (= Českomoravská vrchovina), N of Telč, close to the village of Doupě, **Roštejnská obora** nature reserve, approx. coordinates 49.2528761 N, 15.4307689 E (error up to 400 m), elevation ca 650 m a.s.l., near-natural mixed forest dominated by *Fagus sylvatica*, on fallen branch of *Fagus sylvatica*, 14 July 1989 *leg. & det*. P. Vampola as *P. tubaeformis* (MJ 392/89*).

Results and discussion

Polyporus tubaeformis (P. Karst.) Ryvarden & Gilb.

Basionym. – *Polyporellus varius* subsp. *tubaeformis* P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 9: 69 (1883). ≡ *Polyporellus tubaeformis* (P. Karst.) P. Karst., Acta Societatis Scientiarum Fennicae 16: 526 (1888). ≡ *Polyporus tubaeformis* (P. Karst.) Ryvarden & Gilb., Synopsis fungorum (Oslo) 7: 578 (1993). ≡ *Picipes tubaeformis* (P. Karst.) Zmitr. & Kovalenko, International Journal of Medicinal Mushrooms 18 (1): 36 (2016). – For further synonyms see www.mycobank.org.

Macromorphological characters. – Figs. 1–10.

B a s i d i o m a t a pileate with central to excentric stipe. – P i l e u s 10–50 mm, orbicular to semiorbicular with even or slightly lobate margin, planoconvex with depressed centre when young, then slightly to deeply infundibuliform with inflexed margin, ochre-brown to reddish-brown when young, with darker centre, then dark red-brown, surface matt, velvety, then glabrous and finely radially wrinkled, context thin. – T u b e s 1–2 mm long, subdecurrent when juvenile, then deeply decurrent, pores narrow, 5–7 per mm, round to slightly angular, greyish-whitish to cream when young, later pale ochre. – S t i p e 10–30 × 1.5–6(7) mm, with shallow pores on the top, cylindrical or slightly widened towards base or narrower in medium part and widened towards both ends, tomentose to finely hairy when young, with yellowish apex and gradually becoming rusty brown, red-brown to dark brown to-
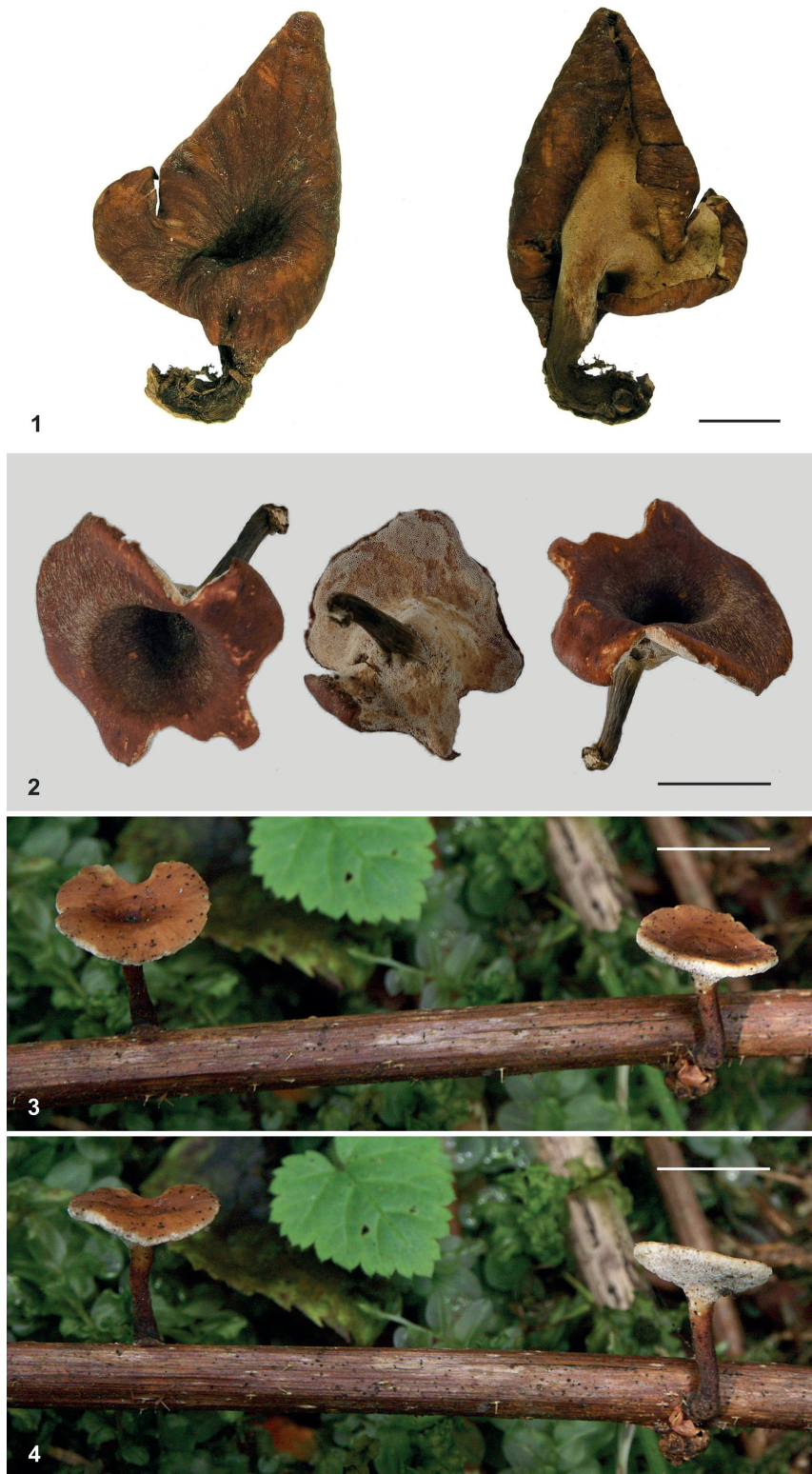
Figs. 1–4. *Polyporus tubaeformis*, Šumava Mts. (=Bohemian Forest), Czech Republic. **1.** Černé jezero (PRM 820896), photo P. Vampola: the same dried basidioma from different angles. **2.** Plešné jezero (CB 21851), photo P. Vampola: the same dried basidioma from different angles. **3–4.** Valley of Hrádecký potok brook (PRM: JH 109/2007), photo J. Holec. For details see Collections studied. Scale bars = 10 mm.
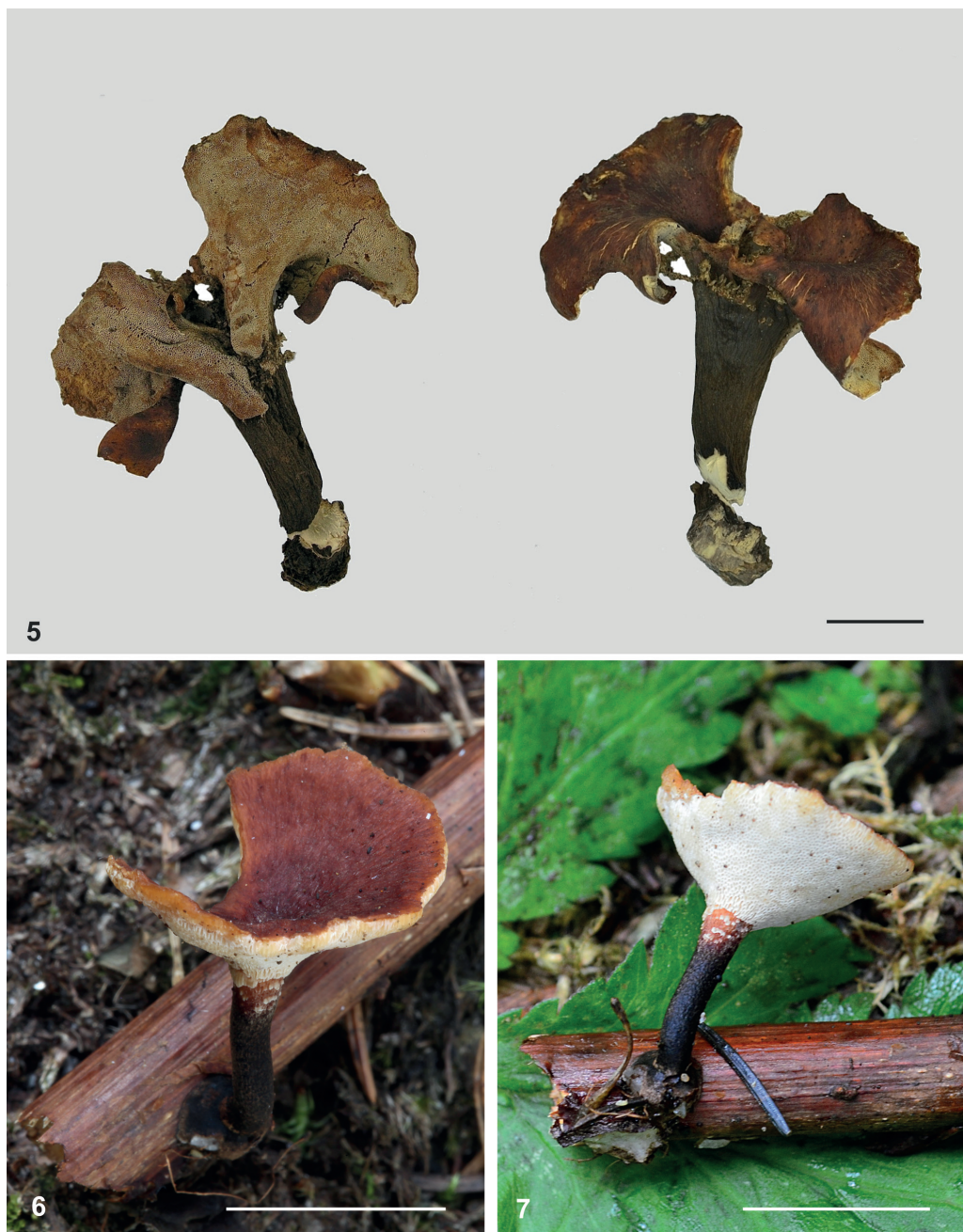
Figs. 5–7. *Polyporus tubaeformis*, The Carpathians. **5.** Nimetska Mokra, Ukraine (PRM 497748), photo P. Vampola: the same dried basidioma from different angles. **6–7.** Tichá dolina, Slovakia (herbarium of the University of West Bohemia, Plzeň), 6: photo M. Kříž, 7: photo J. Kout. For details see Collections studied. Scale bars = 10 mm.

wards base, later completely dark brown with velvety surface, longitudinally wrinkled. – S t i p e c o n t e x t whitish to cream-coloured.

Micromorphological characters

H y p h a l s y s t e m dimitic. – G e n e r a t i v e h y p h a e with clamp connections, hyaline, thin-walled, 2–5 µm wide. – S k e l e t a l - l i g a t i v e h y p h a e thick-walled, up to 8 µm wide. – B a s i d - i a tetrasporic, clavate, with basal clamp connection, 15–20 × 6–7 µm. – B a s i d i o s p o r e s narrowly ellipsoid to cylindrical, smooth, inamyloid, 5.5–9.0 × 2.5–3.5 µm.

Figs. 8–10. *Polyporus tubaeformis,* Austria. **8.** Schöneben, Böhmerwald (= Bohemian Forest) (WU 40225), photo I. Krisai-Greilhuber, the same basidioma from different angles. **9–10.** Machl, The Alps (fungarium Hahn), photo C. Hahn. For details see Collections studied. Scale bars = 10 mm.
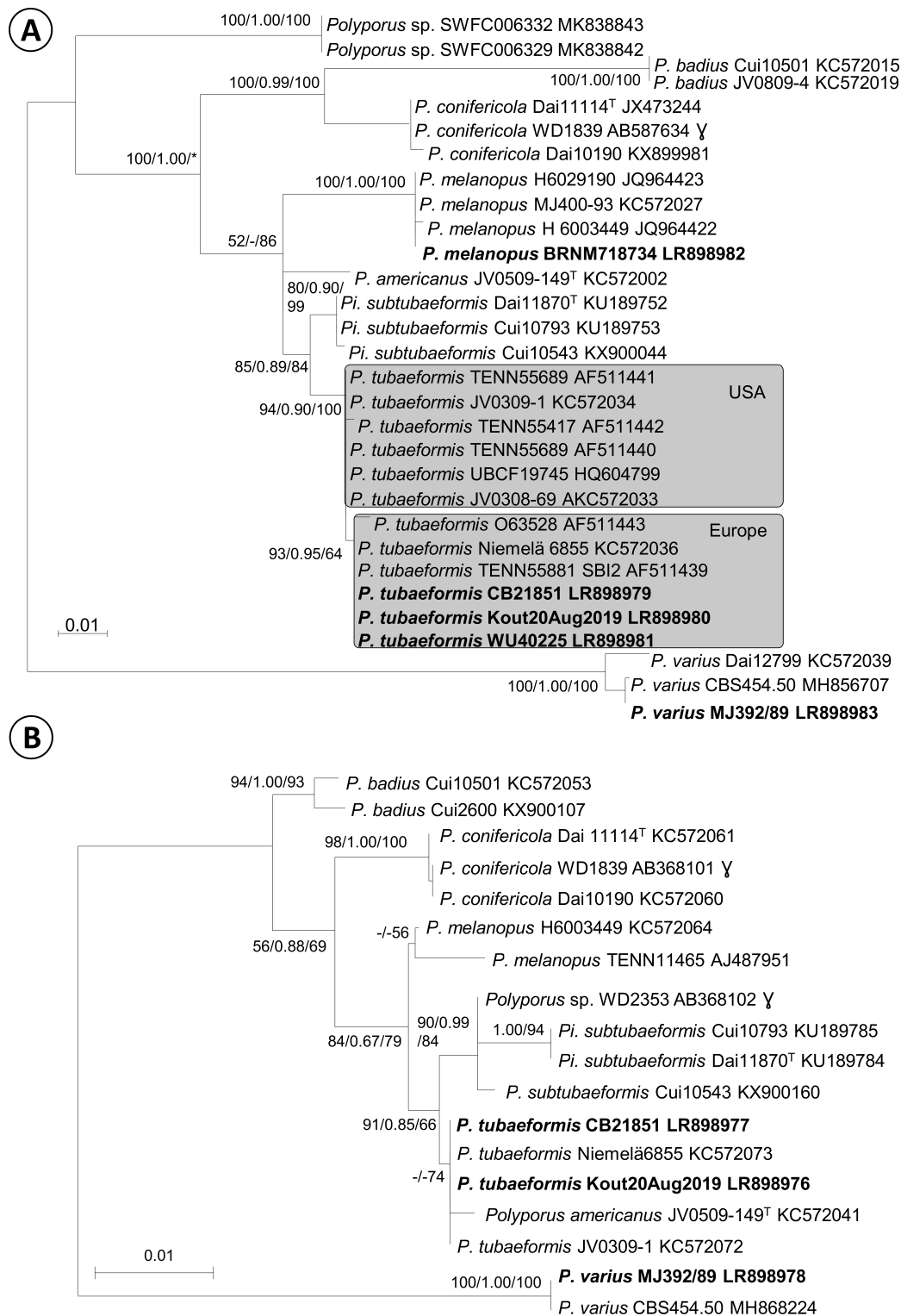
Fig. 11. Phylogeny of ITS rDNA (A) and LSU rDNA (B) sequence data for *Polyporus tubaeformis* and related species inferred from maximum–likelihood analysis (ML). Numbers beside internal nodes are ML bootstraps followed by Bayesian MCMC posterior probabilities and Minimum evolution tree bootstraps. Bootstrap support ≥ 50 % and posterior probabilities ≥ 0.90 % are shown. Asterisks mark nodes which were presented in the ML analysis only. Newly generated sequences are in bold. Ƴ This sign means sequences labelled as *P. tubaeformis* by Sotome et al. (2008) but in fact representing other species. *P*.: *Polyporus*, *Pi*: *Picipes*.

DNA sequences. – Fig. 11.

Identity of two specimens originally determined as *P. tubaeformis* but in fact representing *P. varius* (MJ 392/89) and *P. melanopus* (BRNM 718734) was confirmed by sequencing of their ITS-LSU gene (Fig. 11). Specimens of *P. tubaeformis* sequenced by us (from the Czech Republic, Austria, Slovakia) had identical ITS and LSU rDNA sequences. Their ITS rDNA barcode sequence was identical with a specimen TENN55881 collected in Scotland and differed in a single indel position from the sequence of the voucher Niemelä 6855 collected in Finland. That indel position is on the very beginning of the ITS1 regions, and from the context of other analysed sequences in Fig. 11 it looks rather as a sequencing error. Phylogenetic analysis placed our *P. tubaeformis* sequences in a highly supported clade (94/0.90/100) with other *P. tubaeformis* sequences. Within this clade, a North American and moderately supported European lineage can be distinguished (93/0.95/64) demonstrating the existence of two vicariant populations, which evidently result from an ongoing allopatric speciation. Also Krüger et al. (2006) found that European and Californian isolates of *P. tubaeformis* were sorted out according to geographic origin. Krüger et al. (2006) also showed that both populations were intercompatible, thus representing the same biological species. It is clear from our phylogenetic analysis (Fig. 11) that Japanese specimens identified as *P. tubaeformis* by Sotome et al. (2008) belong to the clades of *P. conifericola* H.J. Xue & L.W. Zhou (ITS from strain WD 1839) and of *Picipes subtubaeformis* J.L. Zhou & B.K. Cui (LSU from strain WD 2353). The *P. tubaeformis* clade is sister to the *Pi. subtubaeformis* clade. *Picipes subtubaeformis*, a species morphologically similar to *P. tubaeformis* and distributed in East Asia (China), has been described recently based on multigene analyses (Zhou et al. 2016, Cui et al. 2019). In our study, the separate analysis of ITS and LSU rDNA showed that both markers itself distinguish the two species well.

Taxonomy and similar species

In its typical form, *P. tubaeformis* is a rather characteristic species (see diagnostic characters in introduction). However, there may be confusion with other *Polyporus* species due to its considerable variability in shape and colour. Already Niemelä & Kotiranta (1991) stated that its pilei can be larger than in *P. melanopus*, a species generally considered more robust (see e.g. Ryvarden & Melo 2017). *Polyporus melanopus* also differs by a (usually) shorter stipe, larger pores (3–4 per mm) and the mostly flat, not infundibuliform pileus (see e.g. Ryvarden & Melo 2017). *Polyporus tubaeformis* sometimes forms very small („dwarfish") basidiomata. This happens when they develop on a spatially limited, low-nutrient substrate, which is the case in our records from *Rubus* twigs. Dwarfism is also known in other *Polyporus* species, e.g. *P. varius*. Young specimens of *P. varius*, sometimes having more or less funnel-shaped pileus, may also be mistaken for *P. tubaeformis*. Old specimens differ from *P. tubaeformis* by the lighter cap colour.

The range of spore size was broader in our material of *P. tubaeformis* (5.5–9.0 × 2.5–3.5 µm) in comparison with the range given by Ryvarden & Melo (2017: 7–9 × 3–3.5 µm). Certain problems can occur when basidiospores are measured, e.g. from dried specimens lacking macroscopic description. Even if the similar species *P. melanopus* and *P. varius* normally have longer spores (reaching the size of up to 10 µm in the former and 11 µm in the latter), in some cases, e.g. when immature, they produce smaller spores comparable with those of *P. tubaeformis*.

The study of some other characters can also be challenging under certain conditions. The macroscopically similar *P. badius* differs by simple-septate generative hyphae. For clear identification of *P. tubaeformis*, clamp connections have to be found on its generative hyphae. This is difficult especially when older collections with collapsed cells are studied. Older specimens of the potentially similar *P. badius* differ macroscopically due to the less funnel-shaped and bigger pileus (up to 15 cm broad) and the ± laterally inserted stipe. Also young specimens of *P. badius* may be difficult to identify macroscopically. In that case, absence of clamp connections on generative hyphae has to be verified.

In addition, other species have been described in the *Polyporus melanopus* group recently, mostly based on molecular genetic data. This may complicate or even prevent classical morphological identification. *Picipes subtubaeformis* J.L. Zhou & B.K. Cui described from the temperate zone of China (Zhou et al. 2016, Cui et al. 2019) is morphologically very similar but differs by DNA sequences, slender stipe (Zhou et al. 2016: 17), slightly wider pores, and not infundibuliform but irregularly semicircular or elliptical pileus (Zhou et al. 2016: 20). *Picipes conifericola* (H.J. Xue & L.W. Zhou) J.L. Zhou & B.K. Cui, also desribed from China (Dai et al. 2014), has narrower pores (7–10 per mm) than *P. tubaeformis*, and grows exclusively on wood of conifers (Cui et al.

2019). Other recently described species from this group are *P. submelanopus* H.J. Xue & L.W. Zhou (Xue & Zhou 2012), found in northwest China, and *P. americanus* Vlasák & Y.C. Dai, from the USA (Dai et al. 2014). According to Dai et al. (2014) *P. americanus* and *P. tubaeformis* share a brown to fuscous upper surface, gelatinized tramal hyphae, similarly sized pores and basidiospores, presence of cystidioles, and growth on angiosperm wood, but *P. tubaeformis* has a deeply funnel-shaped pileus, entire dissepiments, less abundant cystidioles and differs clearly in ITS sequence.

Distribution and habitats. – Fig. 12.

All records from Austria, the Czech Republic, Slovakia and Ukraine originate from mountains (Fig. 12). Their elevation range is 750–1250 m a.s.l. which represents the submontane and montane belts. Czech and two Austrian records are from Bohemian Forest (belonging to Hercynian mountains) situated on the Austria/Germany/Czech Republic border, the third Austrian record originates from the mountain range Reichraminger Hintergebirge belonging to the Northern Limestone Alps. The Slovakian and Ukrainian collections are from the Carpathians (Western and Eastern Carpathians).

Two records originate from very unique sites – steep rocky slopes above glacial lakes (Bohemian Forest: Plešné jezero, Černé jezero) formed by montane glaciers of the former Pleistocene glaciation. This ecosystem, common e.g. in the Alps (including active glaciers), is extremely rare in the Czech Republic where it is present just as relict only in the Bohemian Forest. The mostly east-exposed, shaded and moist slopes, formerly covered with glaciers or situated close to them, now carry natural montane Norway spruce forests with admixed *Betula* and *Sorbus*. Three records are from valleys of montane brooks covered by shrubs or scattered mixed or spruce forests. One of these valleys was also shaped by the former montane glacier (Tichá dolina in Západné Tatry Mts.), two of them by water erosion. A common feature of all reported localities of *P. tubaeformis* is relatively cold climate (a consequence of high elevation), high air humidity and/or soil moisture. All records are from near-natural to natural habitats. Three of them represent relic sites of the highest nature conservation value (Černé jezero, Plešné jezero, Tichá dolina).

In France, *P. tubaeformis* is also known from montane regions (The Vosges, Alps, Massif Central), namely from the elevations 776–1600 m (Rivoire 2020). In Italy, a revised collection exists from the

Fig. 12. Localities of *Polyporus tubaeformis* in studied countries (Austria, Czech Republic, Slovakia, Ukraine; data from this paper) and adjacent region of Germany (Bavarian Forest, a part of the Bohemian Forest; data from Anonymus 4). Note the clear preference of the species for montane areas and accumulation of localities in Bohemian Forest (in all its parts: the Czech, German and Austrian one).

Alps of Devero (1650 m a.s.l., Val Ossola, Verbania-Ossola Province, Piemonte Region, very close to the border with Switzerland, in an *Alnus viridis/Salix* wood nearby a rivulet, leg. Marco Cartabia; pers. comm. A. Bernicchia 24 July 2020). This record, documenting *P. tubaeformis* from Italy, does not originate from mediterranean but alpine vegetation so the fungus cannot be considered a mediterranean species (compare Bernicchia & Gorjón 2020). In Germany, the species is known (Anonymus 4) from northern lowland areas close to Denmark (a country of frequent occurrence of *P. tubaeformis*), hilly areas of west central Germany (Wuppertal, Eifel National Park), and a mountain area (Bavarian Forest which is part of the Bohemian Forest). The Bavarian localities situated close to the villages of Seebachschleife, Altschönau and Mitterfirmiansreut (Anonymus 4) lie in the immediate vicinity of all Czech and two Austrian records from the Bohemian Forest. It is obvious that this mountain range is the richest area of *P. tubaeformis* occurrence in Central Europe (Fig. 12). Across Europe, *P. tubaeformis* is a good example of a species having predominantly boreal-montane distribution.

Substrates

Our records are from wood or woody stems of broadleaved trees or shrubs: *Sorbus aucuparia*, *Sorbus* sp., *Alnus incana*, *Rubus idaeus*, *Rubus* sp., *Salix* sp. and unidentified wood of deciduous tree. Basidiomata grew up from dead trees (Černé jezero; however, the tree part and position was not indicated), decaying fallen trunks, branches and twigs and woody stems of *Rubus*, mostly in medium stages of decay and on moist sites. In five of seven cases the decaying remnants occurred in habitats dominated by *Picea abies*. However, we have no records from spruce wood even if spruce is reported as substrate of *P. tubaeformis* by Niemelä & Kotiranta (1991) and Dai (1996). Pilát gives *Fagus sylvatica* as substrate of his collection from Ukraine. We are of the opinion that this information is problematic as Pilát worked in *Fagus*-dominated area and rather arbitrarily assigned beech as substrate for most of his records, even if other tree species were present. Unfortunately, there are no wood remnants in his specimen so that the substrate identity cannot be verified.

Generally, *Polyporus tubaeformis* is reported from dead wood of broadleaved trees (mostly *Alnus*, *Betula* and *Salix*, more rarely *Corylus*, *Fraxinus*, *Populus*, *Pyrus*, *Sorbus*), especially decaying wood debris on the ground, but also from conifers (*Abies*, *Picea*, *Pinus*, *Taxus*) and woody stems of *Rubus*

(Niemelä & Kotiranta 1991, Dai 1996, Ryvarden & Melo 2017, Rivoire 2020).

References

Anonymus 1 (n. d.) *Trompet-stilkporesvamp*; https://svampe.databasen.org/taxon/63823 (accessed 16 Jul 2020).

Anonymus 2 (n. d.) *Polyporus tubaeformis (P. Karst.) Ryvarden & Gilb., 1993*; https://elurikkus.ee/bie-hub/species/168465 (accessed 16 Jul 2020).

Anonymus 3 (n. d.) *Kātiņpiepe Polyporus tubaeformis*; https://dabasdati.lv/lv/observation/guif9jc4qsibnt5otmro4gn4g3/ (accessed 16 Jul 2020).

Anonymus 4 (n. d.) *Pilze Deutschlands, Polyporus tubaeformis (P. Karst.) Ryvarden & Gilb. 1994*; http://www.pilze-deutschland.de/organismen/polyporus-tubaeformis-p-karst-ryvarden-gilb-1994-1 (accessed 29 Dec 2020).

Bernicchia A., Gorjón S.P. (2020) *Polypores of the Mediterranean Region*. Romar, Segrate.

Cui B.-K., Li H.-J., Li H.-J., Ji X., Zhou J.-L., Song J., Si J., Dai Y.-C. (2019) Species diversity, taxonomy and phylogeny of Polyporaceae (Basidiomycota) in China. *Fungal Diversity* **97**: 137–302.

Dai Y.-C. (1996) Changbai wood-rotting fungi 5. Study on *Polyporus mongolicus* and *P. tubaeformis*. *Annales Botanici Fennici* **33**: 153–163.

Dai Y.-C. (1999) Changbai wood-rotting fungi 11. *Polyporus* sensu stricto. *Fungal Science* **14**: 67–77.

Dai Y.-C. (2000) A checklist of polypores from Northeast China. *Karstenia* **40**: 23–29.

Dai Y. -C., Xue H.J., Vlasak J., Rajchenberg M., Wang B., Zhou L.W. (2014) Phylogeny and global diversity of *Polyporus* group Melanopus (Polyporales, Basidiomycota). *Fungal Diversity* **64**: 133–144.

Dämon W., Krisai-Greilhuber I. (2017) *Die Pilze Österreichs. Verzeichnis und Rote Liste 2016. Teil: Makromyzeten*. Österreichische Mykologische Gesellschaft, Wien.

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. (2010) New algorithms and methods to estimate maximum likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Hahn C., Friebes G., Krisai-Greilhuber I. (2019) *Sarcodon fennicus*, a boreo-montane stipitate hydnoid fungus with a remarkable smell. *Österreichische Zeitschrift für Pilzkunde* **27**: 43–52.

Holec J., Kunca V., Kolařík, M. (2019) *Tricholomopsis badinensis* sp. nov. and *T. sulphureoides* – two rare fungi of Euro-

pean old-growth forests. *Mycological Progress* **18**: 321–334.

Katoh K., Asimenos G., Toh H. (2009) Multiple alignment of DNA sequences with MAFFT. In: Bioinformatics for DNA sequence analysis, Vol. 537, *Methods in molecular biology* (ed. Posada D.), Humana Press Inc., Totowa: 39–64.

Krueger D. (2002) *Monographic studies in the genus Polyporus (Basidiomycotina)*; https://trace.tennessee.edu/utk_graddiss/2135 (accessed 30 December 2020).

Krüger D., Petersen R.H., Hughes K.W. (2006) Molecular phylogenies and mating study data in *Polyporus* with special emphasis on group "Melanopus" (Basidiomycota). *Mycological Progress* **5**: 185–206.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547–1549.

Li H.J., Cui B.K., Dai Y.C. (2014) Taxonomy and multi-gene phylogeny of *Datronia* (Polyporales, Basidiomycota). *Persoonia* **32**: 170–182.

Niemelä T., Kotiranta H. (1991) Polypore survey of Finland 5. The genus *Polyporus*. *Karstenia* **31**: 55–68.

Núñez M., Ryvarden L. (1995a) *Polyporus (Basidiomycotina) and related genera*. In: *Synopsis Fungorum*, Vol. 10, Fungiflora, Oslo: 1–85.

Nuñez M., Ryvarden L. (1995b) Polypores new to Japan 1. Species of *Polyporus*, with a note on *P. hartmanni*. *Mycoscience* **36**: 61–65.

Rivoire B. (2020) *Polypores de France et d'Europe*. Mycopolydev, Orliénas.

Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Ryvarden L., Gilbertson R.L. (1994) *European polypores. Part 2. Meripilus – Tyromyces*. In: *Synopsis Fungorum*, Vol. 7, Fungiflora, Oslo: 388–743.

Ryvarden L., Melo I. (2017) *Poroid fungi of Europe, 2nd Edition*. In: *Synopsis Fungorum*, Vol. 37, Fungiflora, Oslo: 1–430.

Sotome K., Hattori T., Ota Y., To-anun Ch., Salleh B., Kakishima M. (2008) Phylogenetic relationships of *Polyporus* and morphologically allied genera. *Mycologia* **100**: 603–615.

Sotome K., Hattori T., Ota Y. (2011) Taxonomic study on a threatened polypore, *Polyporus pseudobetulinus*, and a morphologically similar species, *P. subvarius*. *Mycoscience* **52**: 319–326.

Vilgalys R., Hester M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Vlasák J. (2015) *Polypores, Collection of Dr. Josef Vlasák, Hluboká nad Vltavou, Czech Republic, edition 18. II. 2015*; http://mykoweb.prf.jcu.cz/polypores/ (accessed 7 Nov 2019).

Vlasenko V.A., Vlasenko A.V. (2015) Diversity, distribution and ecology of the genus *Polyporus* south of Western Siberia (north Asia). *Current Research in Environmental & Applied Mycology* **5**: 82–91.

Volobuev S. (2013). Aphyllophoroid fungi of the Naryshkinskij Natural Park, Orel Region, Russia. *Folia Cryptogamica Estonica* **50**: 81–88.

Vu D., Groenewald M., de Vries M., Gehrmann T., Stielow B., Eberhardt U., Al-Hatmi A., Groenewald J.Z., Cardinali G., Houbraken J., Boekhout T., Crous P.W., Robert V., Verkley G.J.M. (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals threshold for fungal species and higher taxon delimitation. *Studies in Mycology* **92**: 135–154.

Xue H.-J., Zhou L.-W. (2012) *Polyporus submelanopus* sp. nov. (Polyporales, Basidiomycota) from Northwest China. *Mycotaxon* **122**: 433–441.

Zhou J.-L., Zhu L., Chen H., Cui B.-K. (2016) Taxonomy and phylogeny of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota) from China. *PLoS ONE* **11(8)**: e0159495.

Zmitrovich I.V., Kovalenko A.E. (2016) Lentinoid and polyporoid fungi, two generic conglomerates containing important medicinal mushrooms in molecular perspective. – *International Journal of Medicinal Mushrooms* **18**: 23–38.