

Taxonomy of *Hohenbuehelia auriscalpium*, *H. abietina*, *H. josserandii*, and one record of *H. tremula*

JAN HOLEC^{1*}, PETR ZEHNÁLEK^{1,2}

¹National Museum, Mycological Department, Cirkusová 1740, CZ-193 00 Praha 9, Czech Republic

²Charles University, Faculty of Science, Department of Botany, Benátská 2, CZ-128 01 Praha 2, Czech Republic

*corresponding author: jan_holec@nm.cz

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The taxonomy of *Hohenbuehelia auriscalpium* (Fungi, Basidiomycota, *Pleurotaceae*) and its putative synonym *H. abietina* is evaluated based on material from natural forests in the Czech Republic and Ukraine. The rare species *H. josserandii* is described in detail based on rich collections from Boubínský prales virgin forest in the Czech Republic. All specimens were studied both morphologically and molecularly (ITS and 28S nrDNA, EF1- α). The recently published conclusion that *H. auriscalpium* and *H. abietina* are conspecific was confirmed. The species grows on wood of deciduous trees as well as conifers. The thickness of the gelatinous pileus layer and the presence or absence of pileocystidia proved to be taxonomically irrelevant. One collection from the studied dataset significantly deviates by its DNA sequences and may represent a separate taxon close to *H. auriscalpium*. Another collection originally identified as *H. auriscalpium* turned out to be *H. tremula*. A higher range of some morphological characters was found in *H. josserandii* as compared with published data. The species prefers wood of *Abies alba*, namely branches of trees which fell 2–3 years ago.

Key words: fungi, morphology, DNA study, ecology, distribution, Europe.

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Taxonomie druhu *Hohenbuehelia auriscalpium* (Fungi, Basidiomycota, *Pleurotaceae*) a jeho předpokládaného synonymního druhu *H. abietina* je vyhodnocena s využitím sběrů z přirozených lesů v České republice a na Ukrajině. Vzácný druh *H. josserandii* je detailně popsán na základě bohatých sběrů z Boubínského pralesa. Všechny sběry byly studovány jak morfologicky, tak molekulárně (ITS a 28S nrDNA, EF1- α). Nedávno publikované zjištění, že *H. auriscalpium* a *H. abietina* představují stejný druh, bylo potvrzeno. Tento druh roste na dřevě listnáčů i jehličnanů. Ukázalo se, že tloušťka gelatinózní vrstvy klobouku a přítomnost nebo nepřítomnost pileocystid jsou taxonomicky nepodstatné znaky. Jeden studovaný sběr se významně lišil svými sekvencemi a mohl by představovat samostatný taxon blízký druhu *H. auriscalpium*. Jiný sběr původně také určený jako *H. auriscalpium* představuje druh *H. tremula*. U druhu *H. josserandii* byla ve srovnání s publikovanými údaji zjištěna

větší šíře některých morfologických znaků. Tento druh preferuje dřevo jedle bělokoré, zejména větve stromů padlých před 2–3 lety.

INTRODUCTION

The genus *Hohenbuehelia* Schulzer comprises gilled agaricoid fungi with crepidotoid to pleurotoid, rarely collybioid-omphalinoid basidiomata, living as saprotrophs mostly on dead wood, but also on decaying grasses and mosses (Elborne 2012). *Hohenbuehelia* anamorphs formerly classified to the genus *Nematoctonus* Drechsler belong to nematode-trapping fungi. They capture nematodes by production of adhesive knobs (e.g. Thorn et al. 2000). *Hohenbuehelia* species are characteristic by the presence of a gelatinous layer between epicutis and pileus trama and by having two types of cystidia: a) thick-walled metuloid cystidia covered with calcium oxalate crystals, b) thin-walled cystidia with 1–3 rostra terminated by a capitulum, mostly covered with a glutinous drop (Elborne 2012). The recent European monograph (Consiglio & Setti 2018) recognises 24 species.

In the past three decades, the first author of this paper has devoted himself to fungi of Central European natural forests, mostly composed of *Fagus sylvatica*, *Picea abies* and *Abies alba*. He has collected rich material of *Hohenbuehelia* from dead wood of these tree species. Collections from *Abies alba* were identified as *H. abietina* Singer & Kuthan, those from *Fagus* as *H. auriscalpium* (Maire) Singer, based on Singer & Kuthan (1980) and Elborne (2012). However, Consiglio & Setti (2018) showed that *H. abietina* is conspecific with *H. auriscalpium*. Thus, the first aim of this paper is to verify which taxonomic concept is in better agreement with the morphological characters of our material and its DNA sequences. Secondly, we present rich material and DNA sequences of the rare fungus *H. josserandii* Consiglio & Setti, a species described recently (Consiglio & Setti 2017) based on *Pleurotus silvanus* (Sacc.) Sacc. sensu Josserand (1933).

MATERIAL AND METHODS

Morphological study. Descriptions of macrocharacters are based on fresh collections and their photographs. In *H. auriscalpium* and *H. abietina*, only diagnostic characters are discussed, as some data on the macrocharacters of studied collections is missing. Microscopic characters were observed in a 5% KOH solution on hand-made razor blade radial sections of pilei and pressurised mounts of lamellae, using magnifications of 200× and 400× for tissues and 1000× for cells. Conventional light microscopy and differential interference (Nomarski) contrast were applied. All structures were measured directly under an Olympus

BX43 microscope using an eyepiece micrometer. Spore size is presented as the main data range (10–90 percentile values), flanked by limit values in parentheses, of all spores measured (20 measurements per collection). Q = length/width quotient of all spores measured, Q_{av} = mean value of Q. Voucher specimens are kept in the Mycological Department of the National Museum, Prague (herbarium PRM). For codes of other herbaria, see Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>). Locality names in bold are used in collection lists. For segments of Boubínský prales, see Holec et al. (2015: 163).

DNA study. DNA was extracted from 14 dried specimens using a Zymo Research Fungal/Bacterial Kit (Zymo Research, Orange, CA, USA). Nuclear rDNA regions ITS and 28S were amplified with primer sets ITS1F, ITS4 and N1, N4 (White et al. 1990, O'Donnell 1993). Protein-coding marker elongation factor 1 α (EF-1 α) was amplified using primer pair 983F, 2218R (Rehner & Buckley 2005). For each marker, polymerase MyTaq HS Red DNA Polymerase (Bioline, Meridian Bioscience, Memphis, TN, USA) was successfully used. For PCR conditions for amplification of ITS and 28S nrDNA, see Koukol (2010); for EF-1 α , see Caboň et al. (2017, as cycling parameters for rpb2). The success of PCRs was confirmed by visualisation of PCR products with electrophoresis on 1% TAE agarose gel with ethidium bromide. The PCR products were purified with AMPure (Agencourt AMPure XP-PCR Purification Kit; Beckman Coulter, USA). The obtained PCR fragments were sequenced from both directions with identical primers as used for amplification at the Sequencing Laboratory of the OMICS Core Facility, BIOCEV (Vestec, Czech Republic). A total of 32 sequences were generated (Tab. 1), which were combined with 110 sequences of the three analysed markers taken from GenBank with respect to Consiglio & Setti (2018) to establish a final dataset for phylogenetic analysis. Datasets of each used marker were aligned separately using MAFFT algorithms contained in Geneious 7.1.9 (Biomatters, Auckland, New Zealand). Each alignment was analysed in jModelTest 2.1.5 (Darriba et al. 2012) to find the best substitution model using the Bayesian information criterion. The final concatenated alignment consisted of 69 sequences and 1636 characters. The species *Pleurotus calyptratus* was selected as outgroup. Phylogenetic analysis was carried out by Bayesian inference using MrBayes 3.2.2 (Ronquist et al. 2012) with the following conditions: two independent runs, 5 million generations, every 1000th generation sampled. The first 25% of samples were removed as burn-in and the remaining trees were used to compute a 50% majority rule consensus tree with posterior probabilities (PP) as branch support.

Abbreviations. BA: working code for *Abies alba* trunks monitored in detail (Holec & Kučera, submitted to Mycological Progress); BB: working code for *Picea abies* trunks monitored in detail (Holec et al. 2020); JH: collection by Jan Holec; ID: tree code in dendrometric database administered by the Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Brno, Czech Republic.

Tab. 1. Sequences generated during this study.

Species	Country	Locality	Sequence accession no.			Voucher
			ITS rDNA	28S rDNA	EF1- α	
<i>H. auriscalpium</i>	Czech Rep.	Jelení vrch	MT525853	MT534045	–	PRM 902143
<i>H. auriscalpium</i>	Czech Rep.	Velká Pleš	MT525856	MT534048	–	PRM 915239
<i>H. auriscalpium</i>	Czech Rep.	Kokořínský důl	MT525854	MT534046	–	PRM 909363
<i>H. auriscalpium</i>	Czech Rep.	Povydíří	MT525862	MT534054	–	PRM: JH 137/2007
<i>H. auriscalpium</i>	Czech Rep.	Pažení	MT525852	MT534044	–	PRM 897151
<i>H. auriscalpium</i>	Czech Rep.	Zátoňská hora	MT525857	MT534049	–	PRM 924531
<i>H. auriscalpium</i>	Czech Rep.	Zátoňská hora	MT525858	MT534050	MW054658	PRM 924536
<i>H. auriscalpium</i>	Czech Rep.	Coufava	MT525860	MT534052	MW054659	PRM: JH 71/2009
<i>H. auriscalpium</i>	Czech Rep.	Mionší	MT525851	MT534043	–	PRM 848861
<i>H. josserandii</i>	Czech Rep.	Boubínský prales	MT525861	MT534053	–	PRM 953225
<i>H. josserandii</i>	Czech Rep.	Boubínský prales	MT525863	MT534055	–	PRM 953246
<i>H. josserandii</i>	Czech Rep.	Boubínský prales	MT525864	MT534056	–	PRM 953248
<i>H. tremula</i>	Czech Rep.	Boubínský prales	MT525859	MT534051	MW054660	PRM 935569
<i>H. sp.</i>	Ukraine	Mt. Menchul	MT525855	MT534047	MW054657	PRM 909447

RESULTS AND DISCUSSION

Hohenbuehelia auriscalpium (Maire) Singer, Lilloa 22: 255, 1951 [“1949”]

Figs. 1–3, 6

Basionym: *Pleurotus auriscalpium* Maire, Bull. trimest. Soc. mycol. Fr. 46: 220, 1931 [“1930”]

= *Hohenbuehelia abietina*, Singer & Kuthan, Česká Mykol. 34(2): 61, 1980

Description. For original descriptions, see taxonomic citations listed after the names; for detailed recent description, see Consiglio & Setti (2018).

Diagnostic characters. Characters given as diagnostic for recognition of *H. auriscalpium* and *H. abietina* by Singer & Kuthan (1980), Elborne (2012) and Consiglio & Setti (2018) were compared in our material. The results are summarised in Tab. 2 and discussed below.

Thickness of gelatinous layer between epicutis and pileus trama. Singer & Kuthan (1980) considered a thickness of 20–50 μm and radial arrangement of hyphae the main diagnostic character of *H. abietina* against *H. auriscalpium* (40–105 μm , subparallel hyphae). Similarly, Elborne (2012) mentioned thicknesses of 36–60 μm versus 50–150 μm . In our material, the value varied between 25 and 115(125) μm . Collections from conifers as well as *Fagus* both had a low (up to 65 μm) and a higher thickness, respectively (Tab. 2). The arrangement of hyphae was variable depending on stage of ontogenetic development. Based on our material, the thickness of the gelatinous layer does not separate collections from conifers and deciduous trees.



Fig. 1. *Hohenbuehelia auriscalpium*, Zátoňská hora, fallen trunk of *Abies alba*, PRM 924536 (for details, see Collections studied). Photo J. Holec.



Fig. 2. *Hohenbuehelia auriscalpium*, Povydří, fallen trunk of conifer, PRM: JH 137/2007 (for details, see Collections studied). Photo J. Holec.

Tab. 2. Morphological characters of collections preliminary identified as *Hohenbuehelia auriscalpium* and *H. abietina*. For details, see Collections studied.

Voucher	Identification (with original identification in brackets)	Locality	Substrate	Basidioma length (mm, dry state*)	Basidioma width (mm, dry state*)	Basidioma shape	Pileus surface	Gelatinous layer thickness (µm)	Metuloid pileocystidia	Thin-walled pileocystidia
PRM 902143	<i>H. auriscalpium</i>	Jelení vrch	<i>Fagus sylvatica</i>	12	4	spathuliform	glabrous	75–115	–	–
PRM 915239	<i>H. auriscalpium</i>	Velká Pleš	<i>Fagus sylvatica</i>	16	7	spathuliform	finely white tomentose	38–50 (70)	–	–
PRM 909863	<i>H. auriscalpium</i>	Kokořínský důl	<i>Fagus sylvatica</i>	30	20	spathuliform	glabrous	37–63 (75)	–	–
PRM: JH 137/2007	<i>H. auriscalpium</i>	Povydí	conifer (<i>Abies?</i> <i>Picea?</i>)	20–65	15–45	spathuliform to flabelliform with lobate margin	white tomentose	50–75 (85)	–	frequent
PRM 897151	<i>H. auriscalpium</i> (as <i>H. abietina</i>)	Pažení	<i>Abies alba</i>	12	8	spathuliform	white tomentose	50–63	–	–
PRM 924531	<i>H. auriscalpium</i>	Zátoňská hora	<i>Abies alba</i>	30	17	spathuliform	white tomentose-squamulose	38–50	–	scarce
PRM 924536	<i>H. auriscalpium</i>	Zátoňská hora	<i>Abies alba</i>	25	35	flabelliform	white tomentose-squamulose	30–60	–	rare
PRM: JH 71/2009	<i>H. auriscalpium</i> (as <i>H. abietina</i>)	Couřavá	<i>Abies alba</i>	25	16	spathuliform	finely white tomentose	85–100 (120)	–	–
PRM 848861	<i>H. auriscalpium</i>	Mionší	<i>Abies alba</i>	40	18	spathuliform	finely white tomentose	50–65	–	–
PRM 935569	<i>H. tremula</i> (as <i>H. auriscalpium</i>)	Boubínský prales	<i>Picea abies</i>	15	7	spathuliform	glabrous	25–40 (50)	–	frequent
PRM 909447	<i>Hohenbuehelia</i> sp. (as <i>H. auriscalpium</i>)	Mt. Menchul	<i>Fagus sylvatica</i>	18	16	flabelliform	sparsely white tomentose-squamulose	63–100 (125)	–	frequent

*size of dried basidiomata (the size of fresh ones was not recorded for all collections)

Presence of pileocystidia. This character was stressed by Elborne (2012). Thin-walled capitate pileocystidia should be present and pileometuloids absent in *H. auriscalpium*, whereas pileocystidia absent and metuloid ones absent or rare in *H. abietina*. No pileocystidia were reported by Singer & Kuthan (1980) in their detailed original description of *H. abietina*. No pileometuloids were observed in any collection studied by us. The thin-walled capitate pileocystidia were not found in most collections from conifers and *Fagus* (Tab. 2). However, they were scarce to rare in two collections from *Abies* (found after 15 minutes of careful searching) and very distinct and frequent (at first sight) in two collections from conifers (Povydrří, Boubínský prales) and one from *Fagus* (Mt. Menchul), see Tab. 2 and Fig. 5. As shown below (DNA sequences), the collection from Boubínský prales (PRM 935569, Fig. 5a) represents *H. tremula* (Schaeff.: Fr.) Thorn & G.L. Barron, which is a species bearing pileocystidia (Consiglio & Setti 2018). However, the pattern of cystidia presence described above does not allow for their use to distinguish *H. auriscalpium* and *H. abietina* as proposed by Elborne (2012). Pileocystidia are frequently present in the conifer collection from Povydrří (Fig. 2), which is a typical *H. auriscalpium* molecularly (Fig. 6, difference in 1/626 positions). In collections with scarce to rare pileocystidia they grew out of hyphae forming bundles in the trichodermium. Macroscopically, the bundles form fine squamules on the pileus surface. As they are present in mature and old basidiomata only, the absence or rarity of pileocystidia could be a matter of ontogeny rather than phylogeny. Generally, the presence or absence of pileocystidia is taxonomically irrelevant in *H. auriscalpium*.

Macrocharacters. There is no difference in basidiomata size and shape between collections from conifers and *Fagus* (Tab. 2). In both cases the basidiomata can be both pale, small, slender, spathuliform and, on the other hand, darker, larger, thicker, and flabelliform with a lobate margin. Principally, the basidiomata change during ontogeny, being pale and glabrous to finely white tomentose when young and becoming darker and more tomentose to white tomentose-squamulose at maturity (Figs. 1–3).

DNA sequences. The results clearly show absence of any phylogenetic barriers between samples from conifers and deciduous trees. Our data are in agreement with the conclusion published by Consiglio & Setti (2018) that *H. auriscalpium* and *H. abietina* are conspecific. However, collections PRM 935569 (*Picea*, Boubínský prales, Czech Republic) and PRM 909447 (*Fagus*, Mt. Menchul, Ukraine) originally identified as *H. auriscalpium* clearly differ molecularly (Fig. 6). In the ITS nrDNA barcode region they differ as follows: PRM 935569: 50/626 positions ~ 7.9%; PRM 909447: 21/626 positions ~ 3.3%. Collection PRM 909447 (Figs. 4, 5b) could represent an undescribed species close to *H. auriscalpium*. Additional collections will be needed to resolve this issue. On the other hand,

specimen PRM 935569 evidently represents *H. tremula*. Surprisingly, our collection is closer to the Canadian sample, erroneously designed as neotype (for comments on typification, see Consiglio & Setti 2018), than to the European one (epitype correctly designated by Consiglio & Setti 2018). The topology of our phylogram indicates a paraphyletic status of *H. tremula*, as its clade includes another species, *H. longipes* (Boud.) M.M. Moser. This is in contrast to the results by Consiglio & Setti (2018), where the two species are separated.

Ecology. Specimens representing *H. auriscalpium* (see previous paragraph) originate from dead wood of *Abies alba* (5), *Fagus sylvatica* (3) and an unidentified conifer (*Abies* or *Picea*). Most of them are from natural forests located both at lower and higher elevations (from highlands to the mountains). This also applies to collections from *Abies*. It is thus not so that the fungus growing on conifers (putative *H. abietina*) is a montane species. For *H. auriscalpium*, Elborne (2012) also reports *Betula* and Consiglio & Setti (2018) mention *Quercus*.

Distribution. Collections representing *H. auriscalpium* are from central Europe (Czech Republic), a region from which *H. abietina* has been described, namely from Salajka Nature Reserve in the Beskydy Mts. (type locality). We did not study the holotype (C 5626 in herbarium F), but a very similar collection from Mionší Nature Reserve located only 25 km away in the same mountain range. This collection, also from wood of *Abies alba*, replaced the holotype in our study, especially for the reason that no DNA data of the holotype was published by Consiglio & Setti (2018). We found out that neither its morphological characters nor DNA sequences deviate from material collected on *Fagus*. Generally, *H. auriscalpium* in its current sense, including *H. abietina* and *Hohenbuehelia spatulina* Huijsman as synonyms, is widespread in Europe and grows on wood of both deciduous and coniferous trees (Consiglio & Setti 2018).

Conclusions. The analysis mentioned above shows that the overlap and very similar morphological characters between collections from conifers and deciduous trees do not support distinction of *H. auriscalpium* and *H. abietina* as two separate species. The same applies to their DNA sequences. Consequently, we consider them as one species, namely *H. auriscalpium*, with *H. abietina* as a synonym. The same conclusion has already been published by Consiglio & Setti (2018), also based on morphological and molecular data. The mentioned authors studied several collections from conifer wood (*Picea abies*, *Abies alba*, *Pinus pinea*) originally identified as *H. abietina*, and sequenced three of them (from *Abies* and *Picea*). They matched morphologically and molecularly (ITS, RPB2) with collections from wood of deciduous trees.



Fig. 3. *Hohenbuehelia auriscalpium* ("*H. abietina*"), Coufava, fallen trunk of *Abies alba*, PRM: JH 71/2009 (for details, see Collections studied). Photo J. Holec.



Fig. 4. *Hohenbuehelia* sp., Mt. Menchul, fallen trunk of *Fagus sylvatica*, PRM 909447 (for details, see Collections studied). Photo J. Holec.

Collections studied***Hohenbuehelia auriscalpium***

Czech Republic. 10 km E of Klatovy, 1.5 km SEE of Habartice near Plánice, **Jelení vrch** Nature Reserve, N and NE slope, 620 m a.s.l., near-natural herb-rich *Fagus*/ravine forest, with *Picea*, *Abies*, *Acer pseudoplatanus*, *A. platanoides*, *Tilia* and *Ulmus*, on fallen decaying trunk of *Fagus sylvatica*, 18 Sep 2004 leg. J. Holec, det. J. Holec, JH 176/2004 (PRM 902143). – Křivoklátsko Protected Landscape Area, 2.7–3.2 km NE of the village of Týřovice S of Rakovník, **Velká Pleš** National Nature Reserve: S–N running stream valley between Malá Pleš and Prachoviště hills, 300 m a.s.l., seminatural mixed forest with ravine character: *Fagus*, *Fraxinus*, *Ulmus*, *Tilia*, *Acer*, on fallen decaying trunk of *Fagus sylvatica*, 29 Aug 2008 leg. L. Edrová, det. J. Holec, JH 94/2008 (PRM 915239). – Kokořínsko Protected Landscape Area, 0.8 km NEE of the village of Kokořín near Mšeno, **Kokořínský důl** Nature Reserve, steep E slope among sandstone rocks, 300 m a.s.l., mixed forest: *Pinus*, *Fagus*, *Quercus*, on fallen decaying trunk of *Fagus sylvatica*, 19 Oct 2007 leg. J. Holec, det. J. Holec, JH 397/2007 (PRM 909363, photographed). – Šumava Mts., **Povydrří** (valley of Vydra river), right bank, 0.1–0.5 km S of the settlement of Čeňkova Pila, Zone 1 of Šumava National Park, steep W slope, 670 m a.s.l., mixed near-natural forest (*Picea*, *Abies*, *Fagus*, *Alnus*, *Salix*, *Acer*, *Corylus*, *Betula*, *Pinus*), on fallen decaying trunk of a conifer covered with mosses, 29 Aug 2007, leg. M. Beran, det. J. Holec (PRM: JH 137/2007, photographed). – Šumava Mts., 2.5 km NNE of the village of Zátoň, Mt. Boubín: part named **Pažení**, Boubínský prales National Nature Reserve, vicinity of Lukenská cesta forest road, close to its junction with blue-marked tourist trail, SE slope, 960 m a.s.l., young near-natural *Fagus* forest with huge fallen trunks of *Abies*, on fallen trunk of *Abies alba* covered with mosses, 5 Aug 1998 leg. J. Holec, det. J. Holec, JH 414/1998 (PRM 897151, as *H. abietina*, photographed). – Šumava Mts., near Lenora, Mt. Zátoňská hora: 0.2–0.4 km SSW of the summit, **Zátoňská hora** Nature Reserve, S slope, stony to rocky ridge, 970 m a.s.l., natural ravine forest: *Fagus*, *Abies*, *Acer pseudoplatanus*, *A. platanoides*, *Picea*, *Ulmus glabra*, on branch of *Abies alba* lying on soil, 29 Aug 2014 leg. J. Holec, det. J. Holec, JH 106/2014 (PRM 924531); *ibid.*, 0.2–0.5 km SW of the summit, SW slope with boulders, 960 m a.s.l., natural montane forest: *Fagus*, *Picea*, *Abies*, *Acer pseudoplatanus*, *A. platanoides*, on fallen decayed trunk of *Abies alba*, 29 Aug 2014 leg. J. Holec, det. J. Holec, JH 112/2014 (PRM 924536, as *H. auriscalpium*, photographed). – Moravský kras (Moravian Karst), N of Brno, NNE of the village of Útěchov, **Coufava** Nature Reserve: W part, S-oriented side valley of the Coufava stream valley, 440 m a.s.l., N 49°17.691', E 16°38.263', multi-aged near-natural *Fagus* forest with admixed *Carpinus*, *Picea* and rarely *Abies*, on fallen decaying trunk of *Abies alba*, 26 Aug 2009 leg. A. Vágner & J. Holec, det. J. Holec (PRM: JH 71/2009, as *H. abietina*, photographed). – Beskydy Mts., near the village of Horní Lomná close to Jablunkov, **Mionší** National Nature Reserve, on fallen decaying trunk of *Abies alba*, 7 Sep 1969 leg. & det. Z. Pouzar (PRM 848861, as *H. auriscalpium*).

***Hohenbuehelia* sp.**

Ukraine. Eastern Carpathians, NNE of Rachiv, NNE of the village of Kvasy, **Mt. Menchul** (1305 m), ca. 2.5 km W of the summit, slopes facing the Tisa river valley, NWW slope, 720 m a.s.l., N 48°09.570', E 24°17.070', near-natural multi-aged *Fagus* forest with admixed *Picea*, on fallen decaying trunk of *Fagus sylvatica* covered with mosses, 27 Jun 2007 leg. J. Holec, det. J. Holec, JH 45/2007 (PRM 909447, as *H. auriscalpium*, photographed).

Hohenbuehelia tremula (Schaeff.: Fr.) Thorn & G.L. Barron Figs. 5a, 6

= *Hohenbuehelia rickenii* (Kühner) Kühner, nom. inval. (see Consiglio & Setti 2018: 251)

Notes. This species was not the subject of our study. However, as shown in paragraphs above (Presence of pileocystidia, DNA sequences), one collection originally identified as *H. auriscalpium* turned out to be *H. tremula*. This identi-

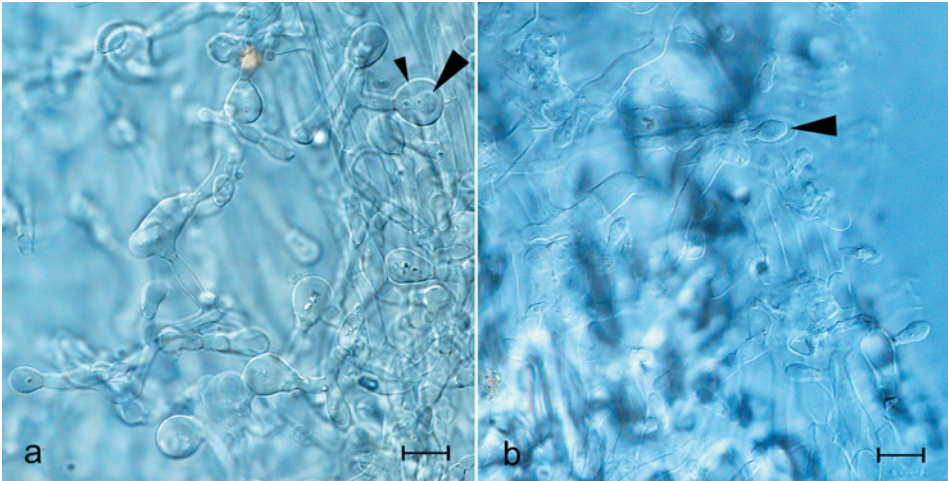


Fig. 5. Thin-walled pileocystidia: **a** – *Hohenbuehelia tremula*, Boubínský prales, PRM 935569; **b** – *Hohenbuehelia* sp., Mt. Menchul, PRM 909447. Large arrows: pileocystidium (only the best visible cell is labelled, others look similar). Small arrow: mucous body embedding cystidium head (only the best visible body is labelled, others look similar). Scale bars = 10 µm. Photo J. Holec.

fication was first demonstrated molecularly and subsequently also morphologically. Thin-walled capitate pileocystidia were frequent (Fig. 5a), which is a character of *H. tremula*. Spores measured $6.5\text{--}7.5 \times 4\text{--}4.5(5)$ µm, which is more than in *H. auriscalpium* ($5\text{--}6.3 \times 3.6\text{--}4.5$ µm; Consiglio & Setti 2018) and in agreement with the range given for *H. tremula* ($6.1\text{--}8.3 \times 4.3\text{--}5.1$ µm; Consiglio & Setti 2018). Unfortunately, the material is represented by just one small basidioma (Tab. 2) and we neither have a description of macrocharacters nor a photograph, which makes it impossible to treat this species in a detailed way. Concerning ecology, the record is from the lateral side of a fallen, moderately decayed trunk of *Picea abies*, in a place covered with bark. Such occurrence is somewhat unusual but fits the overall ecology of the species, which is reported from “the ground or needle litter in coniferous woods, or on sawdust and wood chips” (Consiglio & Setti 2018).

Collection studied

Czech Republic. Šumava Mts., near the village of Zátoň, **Boubínský prales** National Nature Reserve, fenced core area: segment BP1d (Czech Mycol. 67: 163), 970 m a.s.l., N 48°58.547', E 13°48.902', montane virgin forest: *Picea abies*, *Fagus sylvatica*, *Abies alba*, on fallen trunk of *Picea abies* (BB22 = ID 105433), decay stage 3: trunk exterior distinctly decayed, soft, 28 Sep 2015 leg. J. Holec, det. J. Holec, JH 384/2015 (PRM 935569). The collection is included in Holec et al. (2020: Electronic supplement C) under the name *H. auriscalpium*.

Fig. 6. Phylogeny of studied collections and related taxa derived from Bayesian inference. Branches are labelled with Bayesian posterior probabilities (values lower than 0.95 were discarded). Sequences in bold were obtained during this study.



Hohenbuehelia josserandii Consiglio & Setti, Riv. Micol. 60(1): 20, 2017

Figs. 6–12

= *Pleurotus silvanus* (Sacc.) Sacc. sensu Josserand, Bull. Soc. mycol. France 49(3–4): 360–364, 1933

Description. Fungus producing groups of several to dozens of basidiomata (Figs. 7–11), pileate, without stipe, shell-shaped with inflexed margin when young, then flabelliform to reniform, with undulate margin, attached laterally or by lateral part of upper side, elastic, 4–20 mm broad, almost black when young, then dark brown-grey, paler towards margin: beige-grey to olivaceous beige, inner half with fine, dense, tomentose to hairy, white covering, other parts slightly rugulose. Lamellae sparse, only 4–6 touching the point of attachment but intermixed with lamellae arranged in 3–5 levels, not intervenose, ventricose, pale grey-beige when young, then darker, grey-beige with olive tinge to grey-brown, darker towards edge, the very edge paler, silvery, finely serrulate. Flesh very thin, brownish. Smell indistinct. Taste cucumber-mealy.

Basidiospores (7) 7.5–9 × 3.5–4 μm (n = 60, 3 collections), in one spore exceptionally 10 × 4.5 μm, on average 8.1 × 3.8 μm, Q = 1.75–2.57, Qav = 2.13, cylindrical to ellipsoid in face view, more or less phaseoliform in side view, some with slight median constriction, with distinct hilar appendix, smooth, hyaline, thin-walled, with granular content, without large oil droplets, inamyloid. **Basidia** 4-spored, 26–38 × 6–9 μm, cylindrical to narrowly clavate, brownish by sparse granular intracellular pigmentation, sterigmata 3–5 μm. **Metuloid cystidia** 35–75 × 7–11 μm, frequent on lamella edge, there smaller and sometimes clavate with acute rostrum (Fig. 12b), moderately frequent and larger on lamella surface, brown, fusiform to setiform with obtuse or acute apex, with apical incrustations, wall thick, 1–3(4) μm. **Thin-walled cystidia** present only on lamella edge, frequent, 16–30 × 5–10 μm, with swollen lower part: fusiform, clavate to barrel-shaped, upper part formed by 1–3 outgrowths having a 1–2 μm broad neck and 1–3 constrictions, terminated by a cylindrical to ellipsoid head 2–3 μm broad, sometimes embedded in a mucous body up to 5 μm broad. **Hymenium** overall brown, cells with brown wall and brown granular intracellular pigmentation. **Subhymenium** made up of densely arranged interwoven hyphae, brownish. **Lamellar trama** regular to subregular, pale brown, consisting of densely arranged parallel to subparallel hyphae 3–7 μm broad, slightly thick-walled, with pale yellow-brown, up to 1 μm thick wall and sparse granular intracellular pigmentation. **Pileus trama** consisting of interwoven hyphae 4–7 μm broad, hyaline, with up to 1 μm thick wall, separated from overlying gelatinised tissue by a narrow brown layer of densely arranged, subparallel, brown encrusted hyphae. **Gelatinous layer** between pileus trama and epicutis 125–200 μm thick, of loosely arranged upturned to interwoven flexuose hyphae embedded in a hyaline

gelatinous matter, 2–4(5) μm broad, slightly dark brown encrusted. Pileus surface composed of superficial trichodermium and underlying epicutis. Epicutis thin, $\pm 25 \mu\text{m}$, brown, made up of densely arranged parallel to slightly interwoven hyphae 2–4(5) μm broad, most of them with strong brown encrusting pigmentation. Trichodermium of loosely arranged erect to perpendicular hyphae 2–4(5) μm broad and up to 100 μm long, straight or slightly flexuose, at places arranged into pyramidal fascicles, cylindrical or repeatedly slightly constricted, hyaline or brownish by membranal and intracellular pigment, some with slightly thickened wall. Pileocystidia not observed. Clamp connections present in all tissues.

Diagnostic characters. Basidiomata in groups, small, 4–20 mm broad, shell-shaped, flabelliform to reniform, attached laterally or by lateral part of upper side, dark, black when young, then dark brown-grey with paler margin, inner half white tomentose-hairy, lamellae quite dark, grey-beige to grey-brown, basidiospores usually $7.5\text{--}9 \times 3.5\text{--}4 \mu\text{m}$, cylindrical to ellipsoid in face view, distinctly phaseoliform in side view. Growing on dead wood of *Abies*.

DNA sequences. The identity of our three collections was unambiguously confirmed by their agreement with the ITS nrDNA sequence of the *H. josserandii* holotype (Consiglio & Setti 2017). The *H. josserandi* lineage is strongly supported in our phylogenetic analysis (Fig. 6). Its position in a lineage containing *H. atrocoerulea*, *H. canadensis*, *H. fluxilis*, *H. culmicola* and *H. ilderdensis* is in accordance with Consiglio & Setti (2018).

Ecology. All records in this paper come from Boubínský prales, a virgin forest in the Šumava Mts. (Bohemian Forest), Czech Republic. The locality represents one of the best preserved mixed montane forests of Central Europe, composed of *Picea abies*, *Fagus sylvatica* and *Abies alba* (Vrška et al. 2012). It is well-known for its unique and rich mycobiota (Holec 2019, Holec et al. 2015, 2020). *Hohenbuehelia josserandii* was found here for the first time in 2019. The records come from a detailed survey of huge fallen trunks of *Abies alba* carried out in the same way as the previous study focused on *Picea abies* (Holec et al. 2020). The species was found on three of four monitored trunks in decay stage 1, i.e. freshly fallen trunks with hard wood, complete bark cover and branches with green needles (at the time of fall). Basidiomata grew in the upper trunk part on branches with a diameter of 3–7 cm (Figs. 7, 11), still attached to the trunk and waving in the air (not lying on the soil). Two trunks (BA10, 32) broke in October 2017 during windstorm Herwart, one trunk (BA30) was uprooted in autumn 2016. Our records come from November 2019, which means that the basidiomata had appeared 2–3 years after the fall of the trees and in a cold humid part of the year (rainy, with temperatures of $-3 \text{ }^{\circ}\text{C}$ to $5 \text{ }^{\circ}\text{C}$). However, it is also possible that some



Fig. 7. *Hohenbuehelia josserandii*, young basidiomata, Boubínský prales, PRM 953246 (for details, see Collections studied). Photo J. Holec.



Fig. 8. *Hohenbuehelia josserandii*, old basidiomata, Boubínský prales, PRM 953246 (for details, see Collections studied). Photo J. Holec.

basidiomata arose here earlier, namely between September 2018 and November 2019 when the trunks were not inspected. No basidiomata were observed during inspections in June 2017 (trunk BA30 only), July 2018, and September 2018. These data suggest that *H. josserandii* is either an early coloniser of branches of freshly fallen firs or an endophyte or weak parasite, the fructification of which is induced by tree fall. The endophytic way of life and subsequent fructification on branches of fallen firs is known in e.g. the ascomycete *Durandiella gallica* (Kowalski & Kehr 1992). Its fruitbodies (both anamorphic and teleomorphic)

were observed on branches of one of the *H. josserandii* trunks (BA30) already in spring 2017 (Holec et al. 2018). They were not longer present in November 2019.

The following fungal species were found on branches of *Abies alba* at the same time as *H. josserandii* (the number after the name indicates their frequency on three trunks hosting *H. josserandii*): *Aleurodiscus amorphus* 2, *Ascocoryne sarcoides* 1, *Basidioradulum radula* 1, *Crepidotus kubickae* 2, *Dacrymyces stillatus* 1, *Hypholoma capnoides* 1, *Hypochnicium erikssonii* 1, *Lachnellula calyciformis* 1, *Lachnellula subtilissima* 1, *Oligoporus caesius* 1, *Panellus mitis* 3, *Panellus violaceofulvus* 2, *Phlebia radiata* 1, *Scutellinia crinita* 1, *Stereum sanguinolentum* 3, *Trichaptum abietinum* 1. Those with a frequency of 2 and 3 can almost be considered as ‘obligate neighbours’. During field research, their presence could indicate a possible occurrence of *H. josserandii*.

Generally, *H. josserandii* is reported from dead wood of conifers by Consiglio & Setti (2018). However, no vouchers from other conifer species than *Abies alba* are given by them. Our data also support the fact that *H. josserandii* prefers fir wood. The species has never been found on wood of *Picea abies* at Boubínský prales (Holec et al. 2015, 2020) although *Picea* is about ten times more abundant than *Abies* at the site (Vrška et al. 2012).

Distribution. The recent *Hohenbuehelia* monograph (Consiglio & Setti 2018) reports *H. josserandii* from 8 countries, all of them in western, northern and central Europe (west–east: from Great Britain to the Czech Republic, north–south: from Sweden to Austria). Our records from the Šumava Mts. (Bohemian Forest) have its ‘counterpart’ on the German side of the same mountain range: Waldhäuser Wald in Bavarian Forest National Park (leg. P. Karasch, see Consiglio & Setti 2018). Interestingly, there are no records from other parts of the natural distribution of *Abies alba* comprising also southern, eastern, and south-eastern Europe. We suppose that *H. josserandii* lives there but is overlooked or not recognised by local mycologists. On the other hand, it may prefer regions with an oceanic character of climate, i.e. those with milder temperatures and regular rains. Further records will help to answer this question.

Notes. The characters of our records essentially agree with the description by Consiglio & Setti (2018). However, there are some deviations, probably connected with the fact that we had the possibility to observe both young, mature and old basidiomata. Consiglio & Setti (2018) present photos and descriptions of young and mature ones, reaching the size of up to 15 mm. Our basidiomata reach 20 mm in size and are paler when old, especially on their outer part (Figs. 8–10). Their larger stature is probably reflected by a thicker gelatinous pileus layer (125–200 µm in our case versus ca. 90 µm given by Consiglio & Setti 2018). Thin-walled cheilocystidia in our material are larger (16–30 × 5–10 µm versus 15–18 × 5–7 µm; all values rounded to 1 µm), and the same applies to the



Fig. 9. *Hohenbuehelia josserandii*, young and mature basidiomata, Boubínský prales, PRM 953248 (for details, see Collections studied). Photo J. Holec.



Fig. 10. *Hohenbuehelia josserandii*, young basidiomata, Boubínský prales, PRM 953246 (for details, see Collections studied). Photo J. Holec.

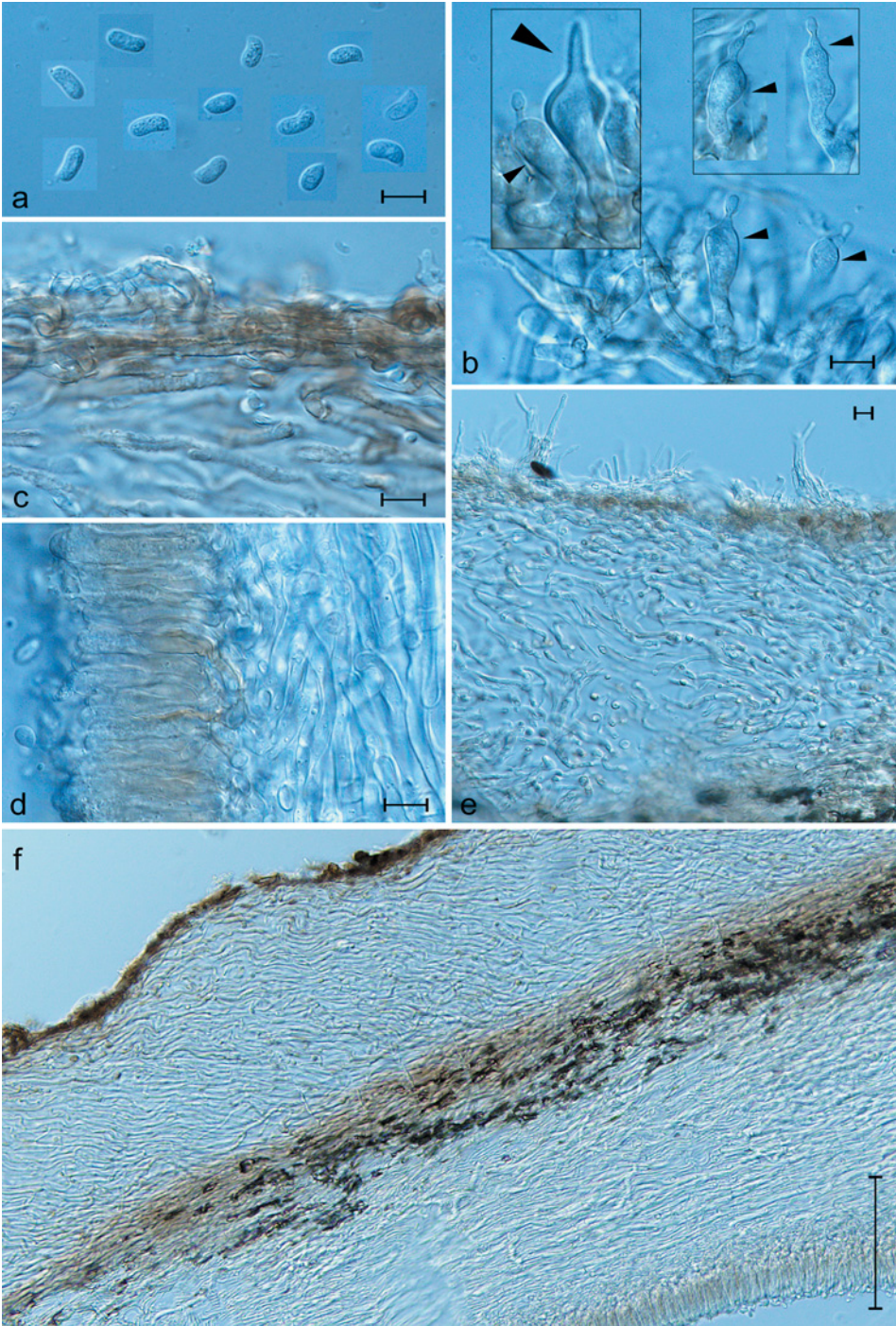
basidia ($26\text{--}38 \times 6\text{--}9 \mu\text{m}$ versus $23\text{--}26 \times 7\text{--}8$). Consiglio & Setti (2018) report an irregular and gelatinised lamellar trama, gelatinous epicutis (called suprapellis in



Fig. 11. *Hohenbuehelia josserandii*, young and mature basidiomata, Boubínský prales, PRM 953285 (for details, see Collections studied). Photo J. Holec.

their work) and presence of pileocystidia (p. 165). We observed a regular to sub-regular and non-gelatinised trama (Figs. 12d, f), non-gelatinised epicutis (Fig. 12c) and no pileocystidia. Concerning spore size, we found a wider range than Consiglio & Setti (2018), namely $(7)7.5\text{--}9 \times 3.5\text{--}4 \mu\text{m}$ with $Q = 1.75\text{--}2.57$ versus $7\text{--}8 \times 3.3\text{--}3.9$ and $Q = 1.93\text{--}2.30$. However, the average Q value expressing the usual spore proportion is almost the same (2.13 versus 2.11). All these discrepancies most probably represent intraspecific and ontogenetic variation, as the most stable and taxonomically relevant characters like DNA sequences and appearance of spores and cystidia are in good agreement.

Fig. 12. *Hohenbuehelia josserandii*, microcharacters (PRM 953246: a, b, e; PRM 953285: c, d, f). **a** – basidiospores; **b** – metuloid cheilocystidium (large arrow), thin-walled cheilocystidia (small arrows); **c** – epicutis with overlying trichodermium and underlying gelatinous layer; **d** – hymenium (brown), subhymenium and lamellar trama; **e** – gelatinous layer in pileus covered with epicutis (brown) and trichodermium; **f** – radial section of pileus, from top to bottom: epicutis (brown), gelatinous layer, brown separating layer, pileus trama (interwoven hyphae), regular lamellar trama, subhymenium, hymenium (pale brown). Scale bars = 10 μm (a–e), 100 μm (f). Photo J. Holec. ►



According to Consiglio & Setti (2018), *Pleurotus silvanus* (Sacc.) Sacc. sensu Jossierand (1933) is conspecific with *H. jossierandii*, a name published in honour of M. Jossierand who first characterised the species in detail. Pilát's concept of *P. silvanus* (Pilát 1935) is hard to interpret, as he failed to mention some diagnostic characters (e.g. cystidia including the metuloid ones) and gave a very wide range of tree species as the substrate (both broadleaved and conifers). PRM, a herbarium keeping Pilát's collections, keeps two specimens labelled by him as *Pleurotus silvanus* (PRM 733531: Libert, Fung. Arduen. No. 19, on trunks and branches of *Ribes grussularia* [= *Ribes uva-crispa*]; PRM 733530: microscopic mount from the same collection). The material is old, scanty, in poor condition, unusable for a detailed revision. It was not studied by us for these reasons.

Similar species. As shown by Consiglio & Setti (2018), the morphologically almost identical *Hohenbuehelia pinacearum* Thorn described from Canada (Thorn 1986) differs by its DNA sequences and, thus, represents an American sibling of the European *H. jossierandii*. In the last three decades, the name *H. pinacearum* has been used by some European authors for collections representing *H. jossierandii*.

Hohenbuehelia mustialensis (P. Karst.) Thorn has similar basidiomata and also grows on wood of conifers (Consiglio & Setti 2018). It differs mainly by oblong (not phaseoliform) and wider spores, measuring $7.4\text{--}9.1 \times 4.0\text{--}5.2 \mu\text{m}$, on average $8.3 \times 4.6 \mu\text{m}$ (Consiglio & Setti 2018). Moreover, its basidiomata are larger (30–50 mm).

Hohenbuehelia unguicularis (Fr.) O.K. Mill. has small and dark basidiomata like *H. jossierandii*, but grows on wood of broadleaved trees and shrubs, the basidiomata are cupulate to conchate, spores shorter and slightly wider ($6.4\text{--}7.8 \times 3.5\text{--}4.5 \mu\text{m}$, on average $7.1 \times 4.0 \mu\text{m}$), ellipsoid to cylindrical, in side view only slightly phaseoliform (Consiglio & Setti 2018).

Hohenbuehelia jossierandii can also be mistaken for *H. leightonii* (Berk.) Watling ex Courtec. & P. Roux, which has basidiomata of similar size and colour and grows on conifer branches exceptionally (but mostly on deciduous ones plus rotting herbaceous stems). The first author of this paper originally identified the collections from Boubínský prales as *H. leightonii*. However, *H. leightonii* has larger spores ($8.7\text{--}11.5 \times 4.2\text{--}5.4 \mu\text{m}$), dorsally attached, cupulate or conchate basidiomata and paler lamellae: white, ash-grey to yellowish cream (Consiglio & Setti 2018).

Collections studied

Czech Republic. Šumava Mts., near the village of Zátoň, **Boubínský prales** National Nature Reserve, fenced core area: segment BP1d, montane virgin forest (*Picea abies*, *Fagus sylvatica*, *Abies alba*), 985 m a.s.l., N 48°58.393', E 13°48.866', *Abies alba*: fallen trunk **BA10** = ID 100738, decay stage 1: recently fallen trunk with bark, wood still hard, diam. (DBH) 96 cm, on twigs, 18 Nov 2019 leg. J. Holec, det. J. Holec & P. Zehnálek, JH 125/2019 (PRM 953225, photographed); *ibid.*, segment BP1e,

1020 m a.s.l., N 48°58.553', E 13°48.752', *Abies alba*: fallen trunk **BA32** = ID 106467, decay stage 1: recently fallen trunk, with bark, wood still hard, diam. (DBH) 87 cm, on twigs, 19 Nov 2019 leg. J. Holec, det. J. Holec & P. Zehnálek, JH 185/2019 (PRM 953246, photographed), JH 187/2019 (PRM 953248, photographed); *ibid.*, segment BP1h, 1100 m a.s.l., N 48°59.014', E 13°48.540', *Abies alba*: fallen trunk **BA30** = ID 114109, decay stage 1: recently fallen trunk with bark, wood still hard, diam. (DBH) 102 cm, on twig, 20 Nov 2019 leg. J. Holec, det. J. Holec, JH 256/2019 (PRM 953285, photographed).

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