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Slovak record extends the knowledge of the distribution of *Hohenbuehelia josserandii*

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The rare species *Hohenbuehelia josserandii* was recorded in Slovakia for the first time. The locality in the Súľovské vrchy Hills is one of the easternmost in Europe. Basidiomata were produced on branches of freshly fallen silver fir (*Abies alba*). Field observations and morphological characteristics of the species are described in detail and compared with published data. Distribution data are visualised on a map. The ITS nrDNA sequence of the Slovak collection is in agreement with the sequence of the *H. josserandii* holotype.

Key words: *Agaricomycotina*, morphology, ITS nrDNA sequence, ecology, *Abies alba*, Europe.

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Vzácny druh *Hohenbuehelia josserandii* bol na Slovensku zaznamenaný po prvýkrát. Lokalita v Súľovských vrchoch patrí k najvýchodnejším v Európe. Plodnice sa tvorili na konároch čerstvo spadnutej jedle bielej (*Abies alba*). Pozorovania v teréne a morfologické znaky druhu sú podrobne opísané a porované s publikovanými údajmi. Údaje o rozšírení sú vyobrazené na mape. Sekvencia ITS nrDNA zo slovenského zberu sa zhoduje so sekvenciou z holotypu *H. josserandii*.

Introduction

The species *Hohenbuehelia josserandii* Consiglio et Setti (*Pleurotaceae*, *Agaricomycotina*) was introduced as new to science in 2017 (Consiglio et Setti 2017). It was named in honour of the French mycologist Marcel Josserand. Josserand (1933) was the first who described this species in detail, but under the misapplied name *Pleurotus silvanus* (Sacc.) Sacc. In the recent monograph on *Hohenbuehelia* Schulzer in Europe by Consiglio et Setti (2018), *H. josserandii* is one of the 24 recognised species. The monograph provides a comprehensive view of this group of fungi, and all species are indisputably defined by molecular characters.

Each new record of a species, including *H. josserandii*, may however modify previous knowledge of morphological characters and ecological preferences, as shown by e.g. Holec et Zehnálek (2020). The aim of this paper is to present the first record of *H. josserandii* from Slovakia and to compare its characteristics with published data.

MATERIAL AND METHODS

Morphology and ecology. Macromorphological characters of *Hohenbuehelia josserandii* were described from fresh material. References to colours followed Kornerup et Wanscher (1978). A dried specimen was used to examine micromorphological characters. Microscopic characters of the pilei were observed in a 3% aqueous solution of KOH, microscopic characters of the lamellae in ammoniacal Congo red after a short pre-treatment in a 3% aqueous solution of KOH. The slices were made manually with a razor blade. The structures were measured directly under an Olympus BX41 light microscope using an eyepiece micrometer. Magnifications of 100× and 400× were used for tissues, and an oil-immersion lens at a magnification of 1000× for cells. Drawings of microscopic structures were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of 2000×. Statistic calculations were based on 30 (spores, basidia, basidioles and cystidia) and 20 (other microscopic structures) measurements per specimen. Q = ratio of length and width of spores. The range of microscopic characters is given as minimum, maximum (in parentheses), average ± standard deviation, and average values. Exceptions are width of tramal hyphae and depth of tissue, for which the range of minimum and maximum sizes is given. Morphological terminology is adopted from Vellinga (1988). The specimen was deposited in the SLO herbarium (herbarium of the Comenius University Bratislava, Slovakia). Wood decay stages are indicated according to Heilmann-Clausen (2001). Degree of forest naturalness was assessed according to the Czech Natural Forests Databank (https://naturalforests.cz/databank-terminology-proposal-for-terminology). Nomenclature of fungal taxa mentioned in the text on the ecology of the species follows the MycoBank database (https://www.mycobank.org).

DNA extraction, PCR and sequencing. Total genomic DNA was extracted from dried tissue using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with a prolonged incubation time of up to 3 h after addition of the RNA-lytic enzyme. PCR reaction was performed using a C1000 Touch™ Thermal Cycler. The target region of the internal transcribed spacer regions of ribosomal DNA (ITS) was amplified using primers ITS5 and ITS4 (White et al. 1990). The large subunit of ribosomal DNA (LSU) was amplified using primers LR0R and LR5 (Vilgalys et Hester 1990). The PCR reactions were conducted in a total volume of 25 µl using a GoTaq Flexi PCR kit (Promega, Madison, USA), the reaction mixture containing 20–25 ng of total DNA template, 1 µl of both primers (10 µM), 5 µl of Buffer (5×), 2.5 µl of dNTP (2 mM), 2 µl of $MgCl_2$ (25 mM), 0.2 µl GoTaq Flexi polymerase and the final volume was supplemented with ultrapure water. The PCR reactions for the ITS and LSU regions were set up as follows: 3 min initial denaturation at 95 °C, 32 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min + increasing time 2 s per cycle), 10 min final elongation at 72 °C. The PCR products were visualised on 2% agarose gel, and purified using Thermosensitive Alkaline Phosphatase (FastAP) and Exonuclease 1 (Exo 1) (Thermo Fisher Scientific, Santa Clara, USA) according to the manufacturer's instructions. The partial gene was sequenced in a commercial laboratory (Eurofins Genomics, Cologne, Germany). Newly generated sequences were deposited in GenBank.

Phylogenetic analysis. Phylogenetic analysis was performed on the sequences of the ITS nrDNA region (LSU sequences were unavailable for the target species). The ITS dataset comprised 26 sequences of 11 taxa (Tab. 1). Our initial sampling included the species of *Hohenbuehelia josserandii* lineage as defined by Holec et Zehnálek (2020): *H. atrocoerulea* (Fr.) Singer, *H. canadensis* Consiglio, Setti et Thorn, *H. culmicola* Bon, *H. fluxilis* (Fr.) P.D. Orton, and *H. ilerdensis* Courtec., Vila et Rocabruna, and was further supplemented with morphologically similar species as defined by Consiglio et Setti (2018): *H. leightonii* (Berk.) Watling ex Courtec. et P. Roux, *H. mustialensis* (P. Karst.) Thorn, *H. pinacearum* Thorn, and *H. unguicularis* (Fr.) O.K. Mill. *Pleurotus ostreatus* (Jacq.) P. Kumm. served as an outgroup. Evolutionary analysis was conducted in MEGA X software (Kumar et al. 2018) by using the maximum likelihood and Tamura-Nei model (Tamura et Nei 1993) with 1000 bootstrap replications. All obtained phylogenetic trees were edited and visualised in MEGA X software. The tree with the highest lg likelihood (–2882.37) is presented. Bootstrap support values higher than 70% are shown at the nodes (lower values were discarded).

Tab. 1. ITS nrDNA sequences used in the phylogenetic analysis. The newly generated sequence is marked in boldface.

Species	Country of origin	ITS GenBank accession no.	Specimen voucher / isolate / strain	Sequence origin / reference
Hohenbuehelia atrocoerulea	Austria	KT388029	WU 2364	Mentrida (2016)
Hohenbuehelia atrocoerulea	France	MF459674	LIP CPh2	Unpublished
Hohenbuehelia atrocoerulea	Denmark	MH137798	C 30448 (isolate)	Consiglio et Setti (2018)
Hohenbuehelia canadensis	Canada	KU355356	DAOM 158848	Consiglio et Setti (2018)
Hohenbuehelia canadensis	Canada	KY124253	DAOM 46785 holotype	Consiglio et Setti (2018)
Hohenbuehelia culmicola	France	KU355323	Roux 3488	Consiglio et Setti (2018)
Hohenbuehelia culmicola	France	NR154081	LIP 1034 neotype	Consiglio et Setti (2016)
Hohenbuehelia fluxilis	Austria	KU355326	WU 29608 neotype	Consiglio et Setti (2016)
Hohenbuehelia ilerdensis	France	MG553639	Roux 3924	Consiglio et Setti (2018)
Hohenbuehelia ilerdensis	Hungary	MH266015	FP20140111	Unpublished
Hohenbuehelia josserandii as *H. pinacearum*	Austria	KT388035	WU 16207	Mentrida (2016)
Hohenbuehelia josserandii	Czechia	MT525863	PRM 953246	Holec et Zehnálek (2020)
Hohenbuehelia josserandii	Czechia	MT525861	PRM 953225	Holec et Zehnálek (2020)
Hohenbuehelia josserandii	France	KU355354	Roux 3270 holotype	Consiglio et Setti (2018)
Hohenbuehelia josserandii	Germany	KU355353	Karasch EG10-812-T-F	Consiglio et Setti (2018)
Hohenbuehelia josserandii	**Slovakia**	**PP047587**	**SLO 2805**	**This study**
Hohenbuehelia leightonii	Austria	MG553640	WU 5846	Consiglio et Setti (2018)
Hohenbuehelia leightonii	Italy	MH137811	AMB 18107 (isolate) epitype	Consiglio et Setti (2018)
Hohenbuehelia mustialensis	Canada	KY124252	DAOM 46374	Consiglio et Setti (2018)
Hohenbuehelia mustialensis	France	OM368067	DA-14038	Unpublished
Hohenbuehelia pinacearum	Canada	MH137813	DAOM 80600 isotype	Consiglio et Setti (2018)
Hohenbuehelia pinacearum	Canada	MH137814	DAOM 84313	Consiglio et Setti (2018)
Hohenbuehelia unguicularis	Canada	MH861918	CBS 855.85 (strain)	Vu et al. (2019)
Hohenbuehelia unguicularis	France	MH873003	CBS 606.79 (strain)	Vu et al. (2019)
Pleurotus ostreatus	France	KX449514	Champ-110 (strain)	Pérez-Izquierdo et al. (2017)
Pleurotus ostreatus	Poland	OR613450	BWPH (strain)	Unpublished

RESULTS

Hohenbuehelia josserandii Consiglio et Setti, Riv. Micol. 60(1): 20, 2017 Figs 1–7

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

Description

Macroscopic characters. Basidiomata pileate, sessile (stipe absent), gregarious (in groups on six branches of one fallen tree, ca 300 basidiomata in total). Pileus 5–15 mm in diameter, rounded flabelliform or reniform (as seen from above), hemispherical or campanulate, later plano-convex (as seen from aside); hygrophanous; margin inflexed, later straight, undulate, translucently striate (up to a quarter to a half); surface brownish grey (5F2) or greyish brown (5F3), white strigose when young, these colours and covering disappearing with age (growth) except for area near the point of attachment, turning to yellowish brown (5E5, 5E4) and white velutinous towards the margin, yellowish brown (5D5) to yellowish (5D4) and almost glabrous on the margin. Lamellae up to 2 mm high, ventricose, only 3–6 reaching the point of attachment, other ones arranged in 3–4 lengths, rarely forked, coloured beige to greyish brown: orange white (5A2) when young, later orange grey (5B2) or greyish orange (5B3) to greyish brown (5D3); lamellae edges yellowish brown (5E4–5F4), entire to finely serrulate. Context very thin, brownish grey (5C2), smell indistinct, taste mealy.

Microscopic characters. Basidiospores (7)7.4–8.2–8.9(10) × (3.2)3.7–4.1–4.4(4.8) µm, Q = (1.63)1.84–2.01–2.18(2.3), oblong or subcylindrical in frontal view, phaseoliform in side view, with distinct hilar appendix, smooth, hyaline, thin-walled, inamyloid. Basidia 4-spored, (23)24.5–27.1–29.6(33) × (6.5)6.6–7–7.4(8) µm, clavate or narrowly clavate, hyaline to light brown, thin-walled. Basidioles (11)16.2–20.7–25.2(30) × (3.5)4.5–5.6–6.6(7.8) µm, clavate or narrowly clavate, hyaline to light brown, thin-walled. Cheilocystidia of two types: i) non-metuloid: (15)17.4–20.7–23.9(26) × (5.5)5.9–6.6–7.2(9) µm, mostly clavate, less frequently fusiform, at apex constricted into 1(–3) neck 1–2 µm broad, bearing a globose, ellipsoid or cylindrical head 1–2.5 µm wide, hyaline, thin-walled or thick-walled (wall up to 0.5 µm thick); ii) metuloid: (20)24.6–36.2–47.7(60) × (6)6.6–7.8–9(10) µm, mostly narrowly fusiform, less frequently fusiform or clavate, top obtuse or acute, mostly with incrustation, brown or light brown, with 1–3.5 µm thick wall. Pleurocystidia metuloid: similar to the metuloid cheilocystidia, but only narrowly fusiform, all with incrustation, light brown or hyaline, (37)43.8–54.7–65.6(82) × (7)7.5–9.2–11(13) µm. Lamellar trama of densely arranged, parallel to subparallel hyphae 3–6 µm broad, hyaline, thin-walled or thick-walled (wall up to 1 mm thick). Pileus trama of ± densely

Fig. 1. Basidiomata of *Hohenbuehelia josserandii*: above – surface, below – hymenophore. Súľovské vrchy, SLO 2805 (for details, see Collection studied). Photo F. Fuljer.

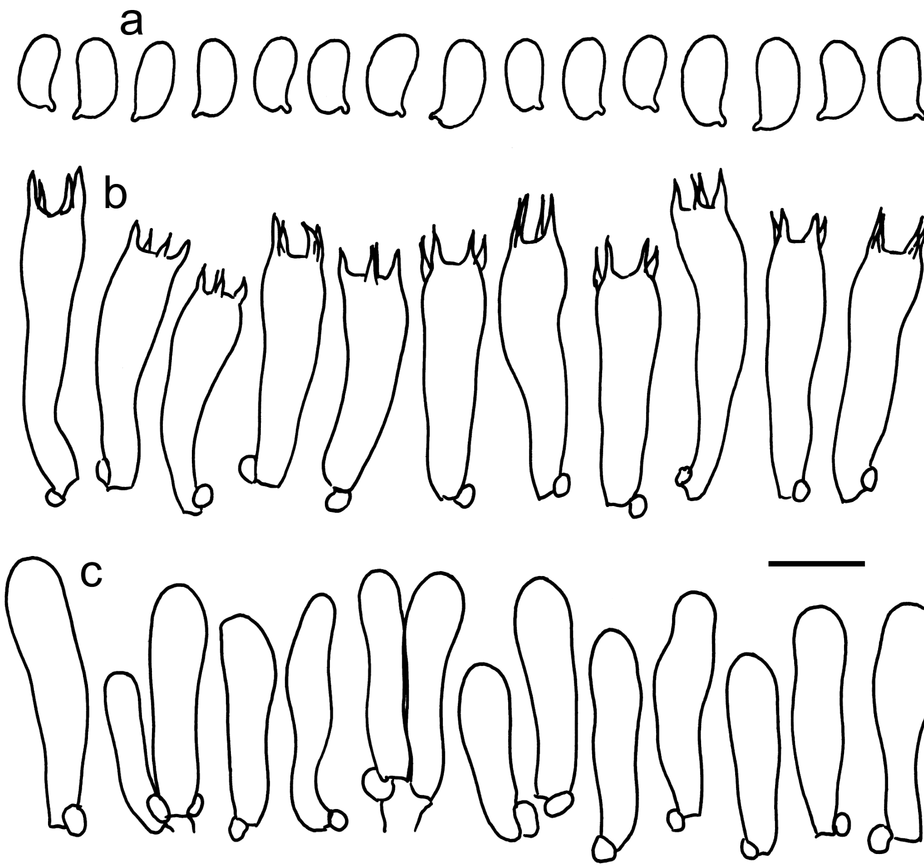

Fig. 2. *Hohenbuehelia josserandii*: **a** – basidiospores, **b** – basidia, **c** – basidioles. Súľovské vrchy, SLO 2805 (for details, see Collection studied). Scale bar = 10 µm. Drawing S. Jančovičová.

arranged, interwoven hyphae 3–7 µm broad, hyaline, thin-walled or thick-walled (wall up to 1 µm thick; pileus trama separated from the overlying gelatinous layer by a ca 70–120 µm deep, brown layer of densely arranged, subparallel hyphae 3–6 µm broad, brown, thin-walled or thick-walled (wall up to 1 µm thick), brown encrusted. Gelatinous layer ca 250–350 µm deep, of loosely arranged, interwoven (in various directions) hyphae 2–5 µm broad, hyaline or light brown, thin-walled, slightly brown encrusted or smooth, embedded in a hyaline gelatinous matter. Pileipellis of two layers: i) intricate trichoderm layer above the gelatinous layer ca 50–140 µm deep, consisting of ± densely arranged, interwoven to parallel, raised hyphae 2–6 µm broad, brown, with up to 1 µm thick wall, brown

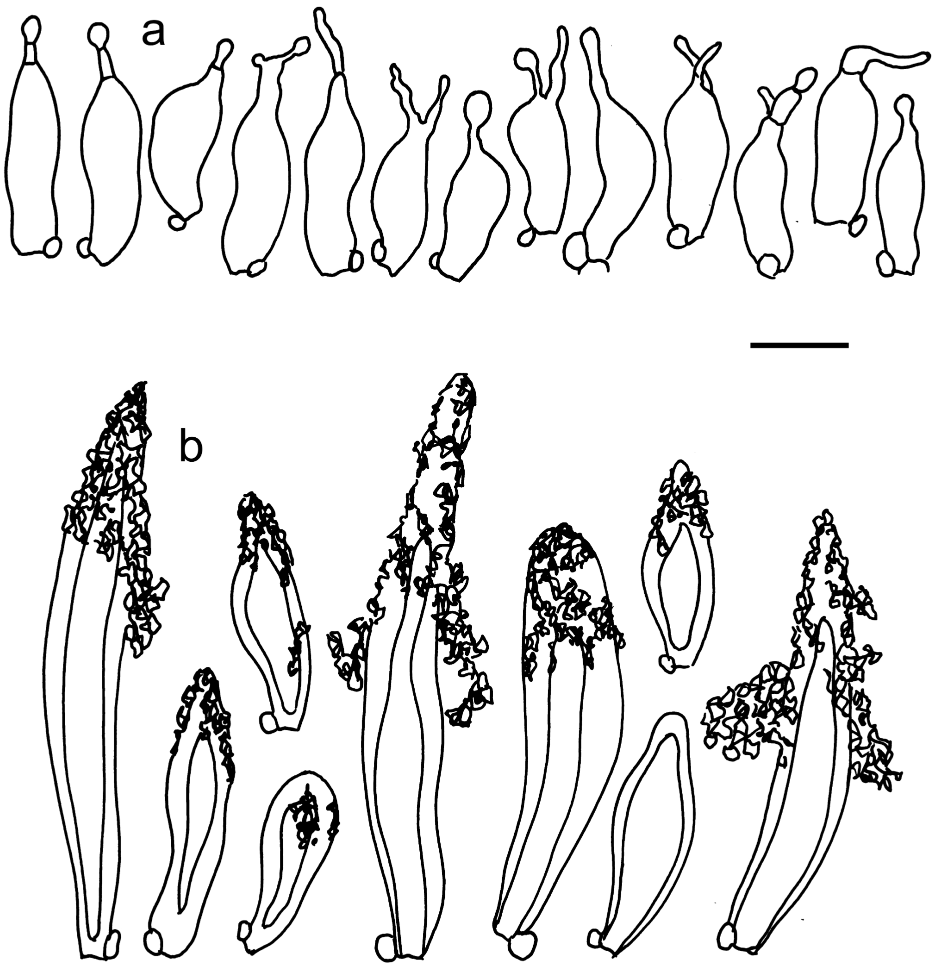

Fig. 3. *Hohenbuehelia josserandii*: **a** – non-metuloid cheilocystidia, **b** – metuloid cheilocystidia. Súľovské vrchy, SLO 2805 (for details, see Collection studied). Scale bar = 10 μm. Drawing S. Jančovičová.

encrusted or smooth; ii) a superficial trichoderm layer ca 100–200 μm deep, consisting of densely arranged, parallel, erect, cylindrical, straight or slightly flexuous hyphae 2–4(5) μm broad, hyaline, thin-walled or with up to 0.5 μm thick wall, smooth, at some places grouped in pyramidal fascicles; terminal cells 50 μm long, top obtuse. Pileocystidia not observed. Clamp connections present in all tissues.

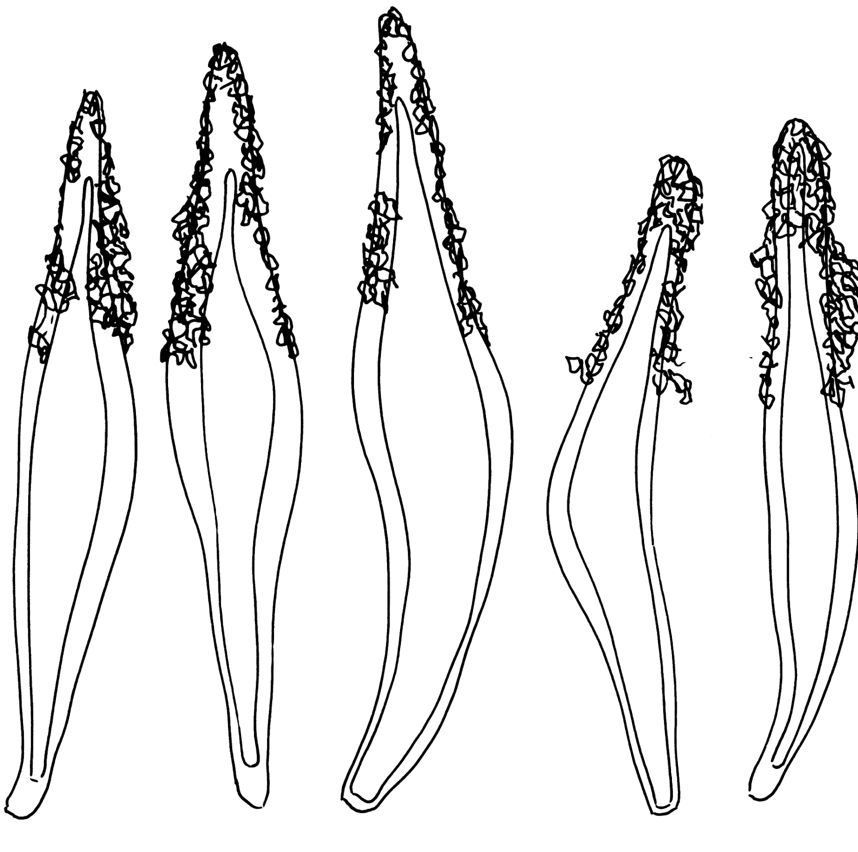

Fig. 4. *Hohenbuehelia josserandii*: metuloid pleurocystidia. Súľovské vrchy, SLO 2805 (for details, see Collection studied). Scale bar = 10 µm. Drawing S. Jančovičová.

Collection studied

Slovakia. Súľovské vrchy Hills, NE of the town of Považská Bystrica, north of Veľký Manín hill, 49°07'56.5" N, 18°29'56.4" E, 825 m a.s.l., ca 150-year old forest on marly limestone, dominated by *Fagus sylvatica*, with admixed *Abies alba* and *Picea abies*, on bark of 4–8 cm thick branches protruding from fallen *Abies alba* trunk, decay stage 2, 25 October 2022, leg. F. Fuljer (SLO 2805).

Ecology

Hohenbuehelia josserandii was found in the Súľovské vrchy Hills, north of Veľký Manín hill. The habitat was ca 150-year-old natural forest on marly limestone, dominated by *Fagus sylvatica*, with admixed *Abies alba* and *Picea abies*,

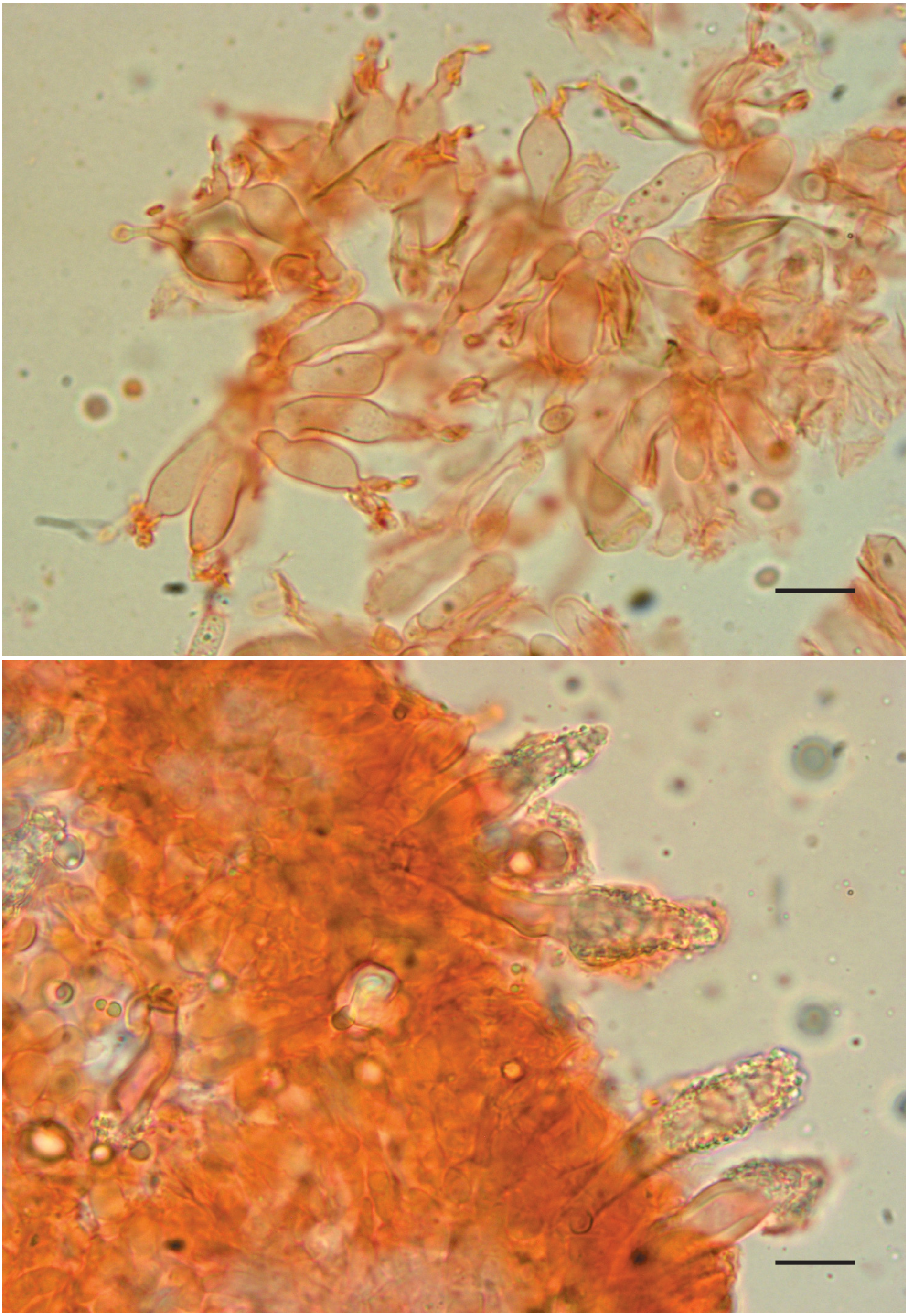

Fig. 5. *Hohenbuehelia josserandii*: above – non-metuloid cheilocystidia, below – metuloid cheilocystidia (stained in Congo red). Súľovské vrchy, SLO 2805 (for details, see Collection studied). Scale bars = 10 µm. Photo S. Jančovičová.

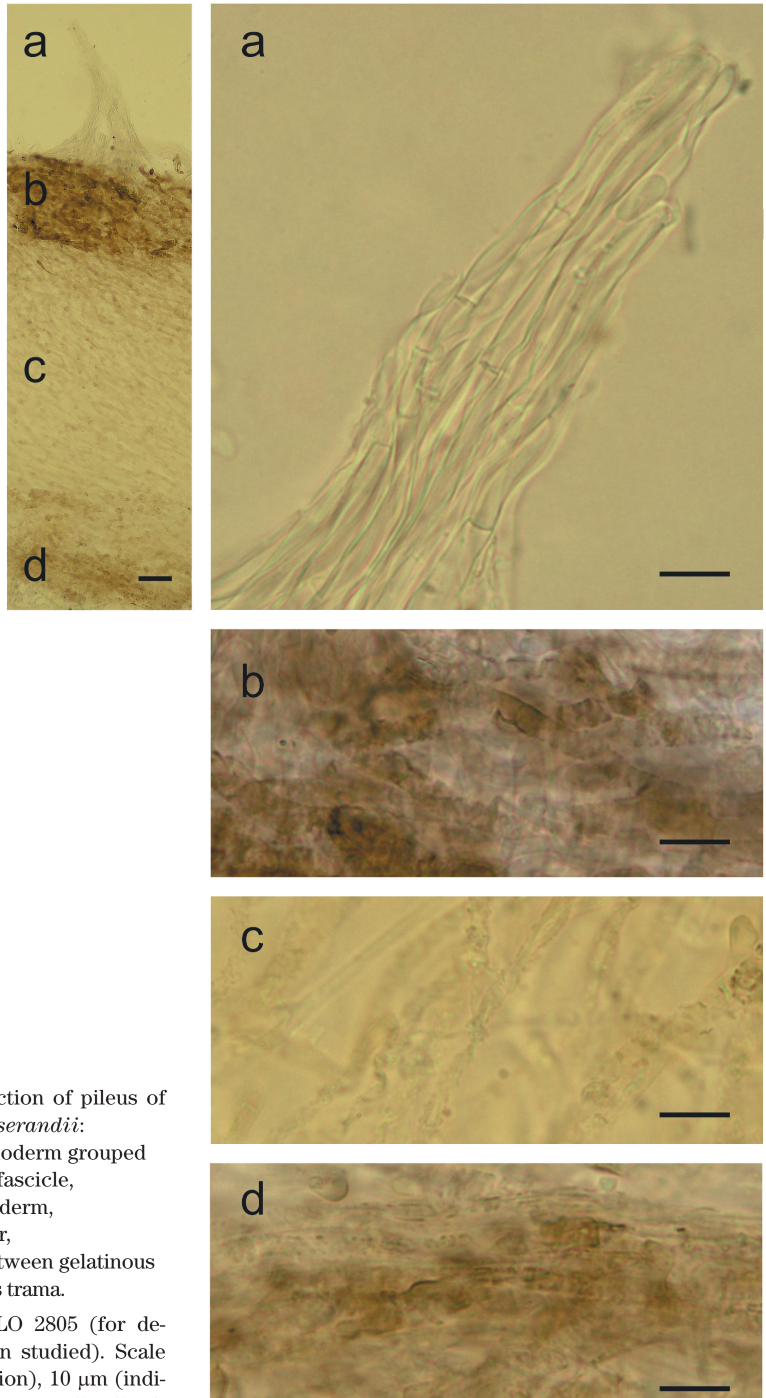

Fig. 6. Vertical section of pileus of *Hohenbuehelia josserandii*:
a – hyphae of trichoderm grouped in a pyramidal fascicle,
b – intricate trichoderm,
c – gelatinous layer,
d – brown layer between gelatinous layer and pileus trama.

Súľovské vrchy, SLO 2805 (for details, see Collection studied). Scale bars = 50 µm (section), 10 µm (individual layers). Photo S. Jančovičová.

Fig. 7. Habitat and substrate of *Hohenbuehelia josserandii*: above – fallen *Abies alba*, below – branches on which basidiomata were produced. Súľovské vrchy, SLO 2805 (for details, see Collection studied). Photo F. Fuljer.

rarely with *Acer pseudoplatanus* and *Tilia* sp. (ISLHP on-line). About three hundred basidiomata were growing on branches of an uprooted trunk of *Abies alba* of decay stage 1–2. The tree had been uprooted two to three years ago. The trunk diameter at breast height was 55 cm, the circumference 171 cm and the bark cover ca 80%. The branches on which the basidiomata were produced were attached to the trunk and protruding into the air (not lying on the soil), 4–8 cm thick, with a circumference of 13–25 cm and bark cover of 90–95%. Basidiomata grew on their sides and top (never directly on the trunk or twigs).

On the day of the find in October 2022, also other fungi were present on the same branches as *H. josserandii*: *Aleurodiscus amorphus* parasitised by *Phaeotremella mycetophiloides*, *Hyphodontia spathulata*, *Panellus mitis* (growing also on the trunk) and *Hirschioporus abietinus*.

In April 2023, we observed the substrate after overwintering. The trunk bark cover had decreased by 20% and the following fungi were growing on the branches: *Dacrymyces stillatus* s. l., *Durandiella gallica*, *Exidia pithya*, *Grovesiella* aff. *abieticola*, *Hymenochaete cruenta*, *Lachnellula subtilissima*, *Orbilia aprilis*, *Rutstroemia elatina*, and *Schizophyllum commune*.

In November 2023, in an attempt to re-find *H. josserandii* on the same substrate, we could not confirm its occurrence here or elsewhere in the area. The trunk bark cover had decreased by 60–70% (the trunk was almost barkless) and the branches, although still covered with bark, were softer and more water-soaked. We found the following fungi on the branches: *Aleurodiscus amorphus*, *Crepidotus kubickae*, *Dacrymyces stillatus* s. l., *Exidia pithya*, *Lachnellula subtilissima*, *Panellus violaceofulvus*, *Pleurotus* sp. and *Hirschioporus abietinus*.

DNA sequences and phylogenetic analysis

Two nrDNA regions (ITS and LSU) of the Slovak *Hohenbuehelia josserandii* collection were amplified. The ITS sequence can be accessed in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under number PP047587, the LSU sequence under number PP047586. Based on megablast search (https://blast.ncbi.nlm.nih.gov/Blast.cgi), our ITS sequence had the highest similarity (100%) to *H. josserandii* holotype from France (KU355354), also to *H. josserandii* from Czechia (MT525861, MT525863) and Germany (KU355353), and to *H. josserandii* (as *H. pinacearum*) from Austria (KT388035); the LSU sequence matched 100% identity to *H. josserandii* from Germany (KU355403). The phylogenetic analysis of the ITS nrDNA region showed that our Slovak collection belonged to a strongly supported clade composed of European representatives of *H. josserandii*. Related and morphologically similar species were shown to be autonomous (Fig. 8).

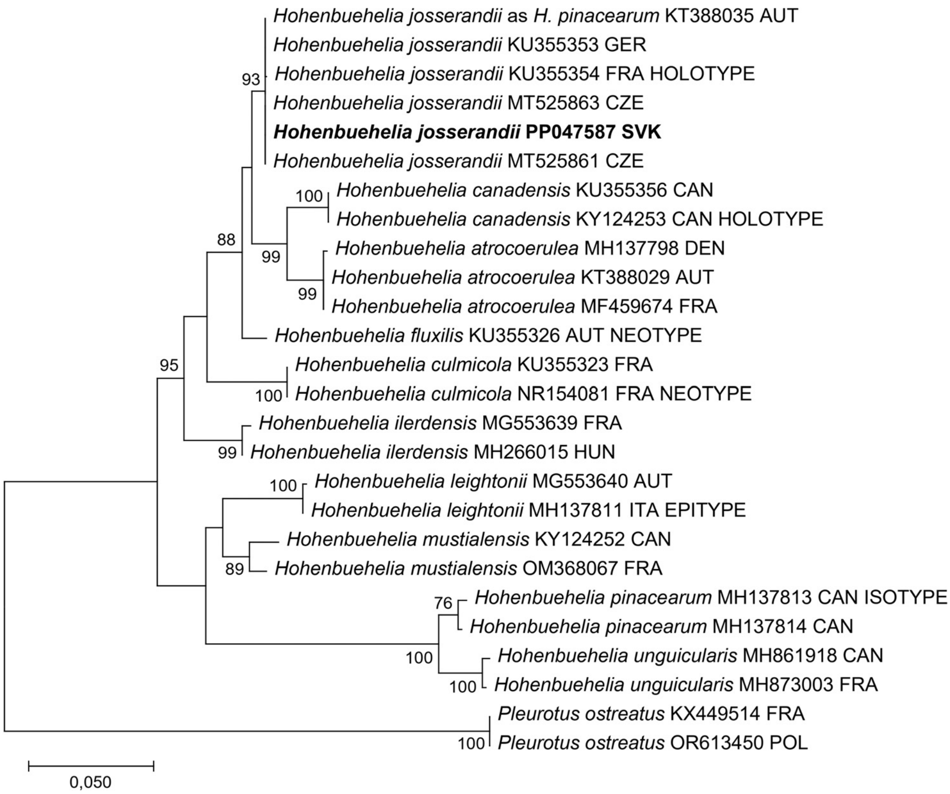

Fig. 8. Maximum likelihood tree based on phylogenetic analyses of the ITS nrDNA region with the position of *Hohenbuehelia josserandii* sequence generated in this study (in boldface). Sequences of *Pleurotus ostreatus* were used as an outgroup. Bootstrap support values higher than 70 (BS ≥ 70) are visualised at the nodes.

DISCUSSION

Identification

Following the key to the European species of the genus *Hohenbuehelia* by Consiglio et Setti (2018), *H. josserandii* belongs together with *H. mustialensis* and *H. unguicularis* to the group of species with sessile basidiomata, pileus < 2 cm broad, lamellae at first dark, basidia 4-spored. Since our collection of *H. josserandii* did not have dark lamellae, we also compared it with other species possessing the above characters, but with initially white or whitish lamellae: *H. atrocoerulea*, *H. cyphelliformis* (Berk.) O.K. Mill., *H. leightonii*. We also included the morphologically indistinguishable species *H. pinacearum* described from Canada, but with different DNA (Tab. 2). The name *H. pinacearum* has been used by some European authors for collections representing *H. josserandii* (e.g. Krieglsteiner 2001, Roux 2006).

Tab. 2. Comparison of *Hohenbuehelia josserandii* with morphologically similar *Hohenbuehelia* species (species sessile, pileus < 2 cm broad, 4-spored basidia) according to Consiglio et Setti (2018). Average values are underlined. Essential characters are boldfaced.

Species **Spore size (μm)** **Q (length and width ratio) of spores**	**Pileus colour (p)** **Lamella colour (l)** **Notes (n)**	**Hosts; substrates**
H. josserandii this study 7.4–8.2–8.9 × 3.7–4.1–4.4 Q = 1.84–**2.01**–2.18	p: brownish grey or greyish brown, then yellowish brown, yellowish on margin l: beige to greyish brown (see Description)	***Abies alba***; branches
Consiglio et Setti (2018) 7.0–7.5–8.0 × 3.3–3.6–3.9 Q = 1.93–**2.11**–2.30	p: greyish black or bluish black l: silvery grey, then dark olivaceous brown to black or blue-black	coniferous trees, ***Abies alba***; branches, trunks
Holec et Zehnálek (2020) 7.5–8.1–9 × 3.5–3.8–4.0 Q = 1.75–**2.13**–2.57	p: almost black, then dark brown-grey, margin paler l: grey-beige to grey-brown	***Abies alba***; branches
H. mustialensis 7.4–8.3–9.1 × 4.0–**4.6**–5.2 Q = 1.55–**1.82**–2.10	p: ash grey, then dark grey to black l: greyish white, then grey or greyish yellow, soon brown-black to black	pine trees, deciduous trees, *Pinus sylvestris*, *Salix cinerea*, *Tilia tomentosa*; branches, trunks
H. unguicularis 6.4–7.1–7.8 × 3.5–4.0–4.5 Q = 1.55–**1.79**–2.04	p: brownish to blackish l: silvery grey, brown-grey to black on drying	shrubs, **deciduous trees**, *Populus*, *Quercus* or *Castanea*, *Ulmus*; branches, trunks
H. pinacearum 6.7–8.1–9.5 × 3.2–3.7–4.2 Q = 2.00–2.20–2.39	p: black, bluish black l: silvery, then blue-black n: described from Canada, **an American sibling of the European *H. josserandii***	coniferous trees, *Abies balsamea*; branches, trunks
H. atrocoerulea 6.4–7.2–7.9 × 3.7–4.1–4.5 Q = 1.55–**1.75**–1.95	p: dark blue, bluish grey, brown-grey, grey, brownish ochraceous, dark brown, olive-brown, buff, cream or dingy white in dry weather l: whitish or cream, then yellow to orange-yellow n: pileus 1–6 cm broad	**deciduous trees**, herbs, *Fagus*, *Populus*, *Quercus*, *Q. ilex*, *Q. pubescens*, *Sambucus*, *Ulmus americana*; branches, trunks, stems
H. cyphelliformis 7.1–8.2–9.3 × 3.1–3.6–4.2 Q = 1.92–**2.30**–2.68	p: light grey to brown-grey, blackish on drying l: white to light ochraceous cream	deciduous trees, shrubs, coniferous trees, herbs, *Morus*, *Penstemon ovatum*, *Phytolaca americana*, *Pinus*, *Rubus idaeus*, *Sambucus nigra*, *Wisteria sinensis*; branches, trunks, stems
H. leightonii 8.7–10.1–11.5 × 4.2–**4.8**–5.4 Q = 1.82–2.11–2.41	p: black with a bluish or blackish brown shade, then brown-grey l: white to ash-grey, yellowish cream on drying	deciduous trees, coniferous trees, herbs, *Cornus sanguinea*, *Pinus pinaster*, *Populus*?, *Vitis vinifera*; branches, stems

We think that *H. josserandii* may not always have blackish grey colours on both pileus and lamellae (cf. Consiglio et Setti 2018). For example, the photograph of the collection which was later designated as the holotype (named *H. pinacearum* in Roux 2006) show basidiomata with distinctly light-coloured lamellae. Holec et Zehnálek (2020) described the lamellae of *H. josserandii* as pale grey-beige when young, then darker, grey-beige with olive tinge to grey-brown, darker towards edges, the very edges paler, silvery. On our Slovak collection, we observed beige to greyish brown lamellae (see Description) with yellowish brown edges. Considering that the lamella colour of *H. josserandii* changes (darkens) with age, it is likely that we found young basidiomata. Therefore, it is important to consider basidiomata of all ontogenetic stages.

Our microscopic observations showed that the presence and essential characteristics of all micromorphological structures are most consistent with the data published by Holec et Zehnálek (2020). We observed a similar structure of the pileipellis, with a stratification into distinct layers from the surface to the pileus trama (Fig. 6). While the trichoderm macroscopically causes a strigose pileus covering, the intricate trichoderm [as cutis, e.g. by Elborne (2012), or epicutis, e.g. by Holec et Zehnálek (2020)] determines the texture, consistency and colouration of the pileus. The thickness of some pileipellis layers varies between publications, e.g. gelatinous layer ca 250–350 μm deep in our case versus 125–200 μm according to Holec et Zehnálek (2020), or 90 μm according to Consiglio et Setti (2018); intricate trichoderm ca 50–140 μm deep in our case versus 25 μm according to Holec et Zehnálek (2020). We agree with Holec et Zehnálek (2020) that these differences are likely to be related to ontogenetic and intraspecific variation, as taxonomically stable characters of DNA, spores and hymenial structures are in good agreement.

Ecology

In their recent European monograph, Consiglio et Setti (2018) presented *H. josserandii* as a species growing on bark, dead standing trunks, fallen branches and dead branches of living conifers. The collections examined were from *Abies alba*, from the months of January, May, September and October.

A search of species occurrence data in Europe revealed *Abies alba* as the most common host tree. Some authors also mentioned other host trees, such as *Picea*, *Pinus* (Elborne 2012), *Pinus* (Watling et Gregory 1989), or *Corylus avellana* and *Fraxinus excelsior* (Krieglsteiner 2001). However, the identity of these hosts should be verified. In the cited works, *H. josserandii* basidiomata were mentioned to have been found also in November, December and February (see list in the caption to Fig. 9). Therefore, apart from March, April, June, July, and August, fructification of the species was recorded in all other months of the year.

Detailed ecological data on *H. josserandii* came from the neighbouring Czech Republic. Holec et Kučera (2020) and Holec et Zehnálek (2020) reported the species from Boubínský prales National Nature Reserve in the Šumava Mts, a virgin forest composed of *Picea abies*, *Fagus sylvatica* and *Abies alba*. The species was found on fallen *Abies alba* trees in November 2019 (2–3 years after fall), having trunks in decay stage 1 with hard wood and complete bark cover. The basidiomata grew on 3–7 cm thick branches still attached to the standing trunks and sticking out in the air. The authors also recorded other fungal species on these branches, some of which could indicate possible occurrence of *H. josserandii*: *Aleurodiscus amorphus*, *Crepidotus kubickae*, *Panellus mitis*, *P. violaceofulvus* and *Stereum sanguinolentum*. Holec et Zehnálek (2020) suggested that *H. josserandii* was either an early coloniser of branches of freshly fallen trees, or an endophyte or weak parasite, its fructification being induced by tree fall. The other known locality of *H. josserandii* in Czechia is Zámecký les Forest south of the town of Železná Ruda (Šumava Mts). Found in November 2021, the basidiomata were produced on branches and twigs sticking out of fallen *Abies alba* trees (1–3 years after fall). *Hohenbuehelia josserandii* was considered an almost exclusive fir specialist (Holec 2021).

The Slovak collection of *H. josserandii* is ecologically similar to the Czech records. With increasing ecological knowledge, there is a higher chance to find new localities of the species. As recommended by Holec (2021), we should focus on localities with known occurrences of other fir specialists with which *H. josserandii* co-occupies the substrate, e.g. *Cyphella digitalis* (Holec et al. 2022) or *Panellus violaceofulvus* (Jančovičová et al. 2016).

Distribution

Following the published data and the GBIF database (https://www.gbif.org/occurrence/search), *Hohenbuehelia josserandii* has been recorded in the following European countries: Austria (Consiglio et Setti 2018), Belgium (Elborne 1995), the Czech Republic (Holec et Kučera 2020, Holec et Zehnálek 2020, Holec 2021), France (Roux 2006, Consiglio et Setti 2017, 2018), Germany (Krieglsteiner 1979, 2001, Consiglio et Setti 2018), Great Britain (Watling et Gregory 1989, Henrici 2009), Greece (Læssøe et al. 2021), Poland (Gierczyk 2016), and Sweden (Elborne 2012). Our find in the Súľovské vrchy Hills is the first one for Slovakia. Although some authors (e.g. Consiglio et Setti 2018) mentioned the Netherlands among the countries of occurrence, Elborne (1995) stated that *H. josserandii* (as *H. pinacearum*) has not been found there.

At the time that Holec et Zehnálek (2020) published their paper, the country with the easternmost occurrence of *H. josserandii* was considered the Czech Republic and the southernmost Austria. The new record from Slovakia, together with online reports from Poland and Greece, have shifted the knowledge of the distribution range of the species to the east and south. It is very likely that dots on the map will expand in all directions, especially in areas where *Abies alba* occurs naturally (Fig. 9).

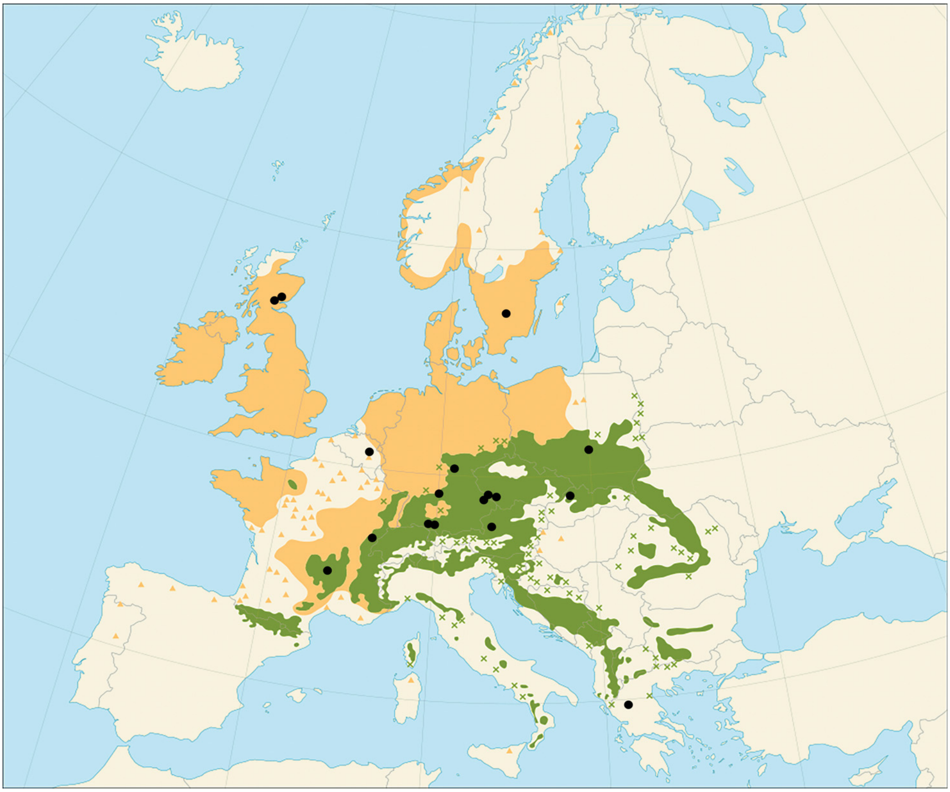

Fig. 9. Distribution of *Hohenbuehelia josserandii* in Europe.
Black dots (●) = distribution of *H. josserandii*; locality names are not edited. Green area and crosses (×) = native continuous range and isolated populations of *Abies alba*. Yellow area and triangles (▲) = introduced and naturalised (synanthropic) continuous range and isolated populations of *Abies alba*. Distribution map of *Abies alba* adopted from Caudullo et al. (2017).

Austria: Fuschl am See, Eibenseebach-Wildmoos, Salzburg-Umgebung, October (Consiglio et Setti 2018).
Belgium: Belgium (Elborne 1995, as *H. pinacearum*).
Czech Republic: Šumava Mts, Boubínský prales National Nature Reserve, on *Abies alba*, November (Holec et Kučera 2020, Holec et Zehnálek 2020); Zámecký les near Železná Ruda, on *Abies alba*, November (Holec 2021).
France: Bonneval, on *Abies alba*, September (Roux 2006, as *H. pinacearum*; Consiglio et Setti 2017, holotype of *H. josserandii*); Doubs, Goux-les-Usiers, on *Abies alba*, January (Consiglio et Setti 2018).
Germany: Bavaria, Freyung-Grafenau, Waldhäuser Wald, Bayerischer Wald National Park, on *Abies alba*, May (Consiglio et Setti 2018); Ostwürttemberg, Schwäbisch-Fränkischen Wald, on *Abies alba*, December to February; Coburger Land/Nordbayern, on *Abies alba*, January; Schwäbischen Allgäu, on *Abies alba*, November (Krieglsteiner 1979, as *Resupinatus silvanus*); Westallgäuer Hügelland, Sigger Wald, on *Abies alba*, May (Krieglsteiner 2001, as *H. pinacearum*).
Great Britain: Scotland, Perthshire/Perths, on *Pinus* (Watling et Gregory 1989, as *H. unguicularis*; Henrici 2009, as *H. pinacearum*); Scotland, Glamis, Angus, on *Abies* (Henrici 2009, as *H. pinacearum*).
Greece: Thessaly, Amárantos, on *Abies*, in October (Læssøe et al. 2021).
Poland: Radomsko, in February (Gierczyk 2016).
Slovakia: Súľovské vrchy, north of Veľký Manín hill, on *Abies alba*, October (this study).
Sweden: Sweden (Elborne 2012, as *H. pinacearum*).

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