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Venturioscypha nigropila (*Hyphodiscaceae*, *Helotiales*) – a new genus and species from xeric *Pinus* bark

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Abstract

A new genus and species, *Venturioscypha nigropila*, is proposed for a minute inoperculate discomycete with long, cylindrical, partly flexuous, dark blackish-brown, smooth, finally thick-walled hairs. It has been collected repeatedly in Europe on dead, corticated branches of *Pinus* spp. attached to living or recently dead trees. At first glance the species resembles members of *Pirottaea* (*Pyrenopezizaceae*), but the relationship is shown by molecular phylogenetics to be close to *Hyphodiscus, Hyphopeziza*, *Fuscolachnum*, and *Venturiocistella* (*Hyphodiscaceae*). These genera differ in having hairs with more or less conspicuous warts, in *Hyphopeziza* also with glassy solidifications, and *Venturiocistella* in having in addition long, stiff, thick-walled, apically acute, dark brown hairs, which are warted in their lower part. The hair wall of *Venturioscypha* appears superficially smooth, but the surface is inconspicuously pitted as viewed under light microscopy. *Venturioscypha* is unique in *Hyphodiscaceae* by its peculiar hairs, inamyloid asci with a thin apical wall that ruptures irregularly by a terminal split at spore discharge, spores with a delicate sheath, and apothecial proliferation.

Introduction

A remarkable minute inoperculate discomycete with long cylindrical, blackish-brown hairs was collected and studied by the first author for the first time in 1978. It occurred on a corticated branch of *Pinus* sp., probably *P. mugo* ssp. *uncinata*, in the nature reserve Waldmoor-Torfstich (also known as Würzbacher Moor) in the northern part of Schwarzwald (Black Forest) in south-western Germany. Various collectors made further records of this species during 1990–2022 on different *Pinus* species in different European countries, always on bark and sometimes near but never on resin. Most of these collections were examined in the living state. In her study on nuclear DNA contents, Weber (1992: 37, 115) briefly mentioned the species under the name "*Pirottaea*" cf. *pini* Höhn. based on a sample from Vosges (H.B. 4139a). A triple (3×) DNA content was noted in the vegetative hyphae (in comparison to the lowest content of 1×). For morphological (inamyloid asci) and ecological reasons (drought-tolerant apothecia growing on bark), a placement in the *Encoelioideae* was considered in that study, with particular relationship to species of *Crumenulopsis* J.W. Groves (today *Cenangiaceae*). In a paper dealing mainly with *Venturiocistella* Raitv., Baral (1993) transferred *Pirottaea pini*, which has an hemiamyloid ascus apical ring and grows on resinous *Pinus* bark, to *Venturiocistella,* and briefly stated that the unidentified fungus with only one type of hairs has, at best, a marginal position in that genus. In the present paper, we describe it as a new genus and species, *Venturioscypha nigropila* that belongs to the *Hyphodiscaceae*, based on a unique combination of morphological features and a nine-gene phylogeny.

Materials and methods

Material and morphological studies

Material of *Venturioscypha* was received from various collectors or collected by the authors during the period of 1978–2022. The material was deposited in the fungaria of BRA, C, H and TUR, and in the private fungaria of B. Wergen (B.W.), E. Stöckli (E.S.), and H.O. Baral (H.B.). The taxa for molecular phylogenetic study were selected based on published and unpublished phylogenies of *Hyaloscyphaceae* s.l. and *Leotiomycetes* (Han et al. 2014, Johnston et al. 2019, Kosonen et al. in prep.).

Macroscopic characters were described from fresh or rehydrated apothecia. Microscopic study was predominantly based on living (*) elements following standards of vital taxonomy (Baral 1992), and for comparison also with dead (†) elements. Apothecia were rehydrated after different intervals for testing their drought tolerance. Tap water (H_2O) and Lugol's solution (IKI) were used as mounting media, and potassium hydroxide (KOH, ca. 5 %) for testing colour reactions or pigment solubility, resistance of oil drops (LBs), and for iodine tests with KOH pre-treatment. Ascus amyloidity prior to KOH was tested using high-concentrated IKI (1 % iodine (I_2) + 3 % KI (potassium iodide), and Melzer's reagent (MLZ) after treatment with KOH.

Macro- and microscopy was done using the equipment mentioned in Baral & Polhorský (2019) and Baral et al. (2020), whereas E. Stöckli used an Olympus CX41 microscope and a Nikon Coolpix E8400 camera (2015–2016), and a Zeiss AXIO Lab. A1 microscope with a AxioCam ERc 5c camera (2018–2020). WGS84 coordinates of the collection sites were copied to Microsoft© Excel and converted to kml-format using Earth Point (http://www.earthpoint.us/ExcelToKml.aspx). The kml file was opened in Google© Earth, from which **Fig. 11** was taken. A culture was produced from the sample TUR215407 following the procedure described in Kosonen et al. (2021). Colour codes for cultures refer to Cailleux (1981).

Additional abbreviations used: SCBs = KOH-soluble cytoplasmic bodies, VBs = refractive KOH-soluble vacuolar bodies, CRB = Brilliant Cresyl blue (aqueous), PVA = polyvinyl acetate, idem = the same, ibid. = from the same geographical region, vid. = examined, ø = unpreserved. Values in {} indicate the number of collections studied for the feature.

DNA extraction, sequencing and phylogenetic analyses

DNA was extracted and sequenced from two samples of *V. nigropila* in two separate laboratories using

Table 1. Species included in the molecular phylogenetic analyses, with voucher information and GenBank accession numbers. Some collections were re-identified/ re-named by us and the original names (in GenBank) are given in parenthesis. Sequences generated in this study are in bold. "n/a" refers to collection data or a sequence not available.

Species	Collection / culture number	Host / substrate	Country	Year	Collectors	GenBank accession number					
						ITS	LSU	TEF1	RPB2	RPB1	RPC2 et al.[1]
Amorphotheca resinae	Amore1; ATC22711	jet fuel	n/a		n/a	genome	genome	genome	genome	genome	genome
Bisporella sp.	Bissp1; PMI 857	soil	n/a	2016	n/a	genome	genome	genome	genome	genome	genome
Calycellina leucella	MP150937	(?)*Salix* leaves	Finland	2015	M. Pennanen	MT231682	MT231682	MT241672	MT228667	MT216612	n/a
Calycina marina	TROM:F26101	dead seaweed (*Ascophyllum nodosum*)	Norway	2014	T. Rämä	genome	genome	genome	genome	genome	genome
Hyphodiscus luxurians	CBS 647.75	decaying wood	Netherlands	1975	W. Gams	GU727560	GU727560	n/a	n/a	n/a	n/a
Cistella spicicola	TUR116388	*Diphasiastrum complanatum* dead sporophylls	Finland	1996	U. Söderholm	GU727553	GU727553	n/a	n/a	n/a	n/a
Fuscolachnum inopinatum	SBRH855	*Lycopodium*	Germany	2015	P. Püwert	OL752697	OL752697	n/a	n/a	n/a	n/a
Fuscolachnum misellum	SBRH799b	*Rubus fruticosus* leaves	Netherlands	2014	S. Helleman	KX501124	KX501129	n/a	n/a	n/a	n/a
Fuscolachnum pteridis (as *Scolecolachnum nigricans*)	MFLU 18-1817	dead stems	China	2016	A.H. Ekanayaka	MK584975	MK591973	n/a	n/a	n/a	n/a
Gamarada debralockiae	T6G9	hair roots of *Woollsia pungens*	Australia	2000	D. J. Midgley	genome	genome	genome	genome	genome	genome
Glutinomyces brunneus	ta2uD7	surface-sterilized root	Japan	2015	N. Nakamura	LC218306	LC315171	n/a	n/a	n/a	n/a
Glutinomyces inflatus	hig5dE3	surface-sterilized root	Japan	2015	N. Nakamura	LC315170	LC218289	n/a	n/a	n/a	n/a
Hyphodiscus brachyconius (as *Catenulifera brachyconius*)	CBS 700.73	decaying wood	Germany	1973	W. Gams	GU727557	GU727557	n/a	n/a	n/a	n/a
Hyphodiscus brevicollaris (as *Catenulifera brachyconius*)	CBS 126.74	*Phellinus* sp.	Germany	1975	W. Gams	GU727561	GU727561	n/a	n/a	n/a	n/a
Hyphodiscus hyaloscyphoides	TNS-F13588, NBRC 104869[2]	*Betula ermanii*	Japan	2006	n/a	AB546944	AB546945	n/a	JN086892	LC431668	n/a
Hyphodiscus hymeniophilus	TNS-F31801	decaying coniferous wood	Japan	1992	n/a	AB546948	AB546946	n/a	JN086901	n/a	n/a
Hyphodiscus hymeniophilus	TNS-F31802	n/a	Japan	1992	n/a	AB546951	AB546950	n/a	n/a	n/a	n/a
Hyphodiscus hymeniophilus	CBS 602.77	*Alnus viridis*	Switzerland	1970	P. Raschle	DQ227264	DQ227264	n/a	n/a	n/a	n/a
Hyphodiscus hymeniophilus	CBS 529.87	*Antrodia serialis*	Germany	1985	H. Schmid-Heckel	GU727555	GU727555	n/a	n/a	n/a	n/a
Hyphodiscus hymeniophilus	MUCL9042	n/a	n/a	n/a	n/a	DQ227259	DQ227259	n/a	n/a	n/a	n/a
Hyphodiscus hymeniophilus	CBS 490.67	*Piptoporus betulinus*	Germany	1965	W. Gams	DQ227261	DQ227261	n/a	n/a	n/a	n/a
Hyphodiscus otanii	TNS-F7099	unidentified wood	Japan	1995	n/a	AB546949	AB546947	n/a	JN086902	n/a	n/a

Species	Collection / culture number	Host / substrate	Country	Year	Collectors	GenBank accession number					
						ITS	LSU	TEF1	RPB2	RPB1	RPC2 et al.[1]
Hyphodiscus sp.	ICMP 21723	*Hypoxylon* sp.	New Zealand	1999	P.R.Johnston	genome	genome	genome	genome	genome	genome
Hyphodiscus sp.	KH.15.20 (S)	pyrenomycete	Estonia	2015	K. Hansen	**ON241826**	**ON241826**	**ON246252**	**ON228705**	**ON246257**	n/a
Hyphodiscus sp.	KH.15.26 (S)	wood	Sweden	2015	K. Hansen	**ON241824**	**ON241824**	**ON246251**	**ON228704**	**ON246256**	n/a
Hyphodiscus sp.	KUS-F52558	twigs	Korea	2009	n/a	JN033421	JN086724	n/a	JN086866	n/a	n/a
Hyphodiscus sp.	UBCF23770	*Phaeolus* sp. basidiomata	Canada	n/a	n/a	KC581301	KC581301	n/a	n/a	n/a	n/a
Hyphodiscus theiodeus	TNS-F32000	Unidentified decaying wood	Japan	2000	n/a	AB546953	AB546952	n/a	n/a	n/a	n/a
Hyphopeziza pygmaea	TNS-F17940	leaves	Japan	2006	n/a	JN033448	JN086748	n/a	JN086894	n/a	n/a
Leptodontidium irregulare	CBS 152.60	leaf litter	Japan	n/a	n/a	MH857936	MH869480	n/a	n/a	n/a	n/a
Leptodontidium obscurum	CBS 405.85	soil from *Pinus* forest	Netherlands	1985	W. Gams	MH861893	MH873582	n/a	n/a	n/a	n/a
Leptodontidium trabinellum	SH17/39 (S)	n/a	Sweden	2017	S. Huhtinen	**ON241825**	**ON241825**	**ON246250**	**ON228702**	**ON246254**	n/a
Leptodontidium trabinellum (as L. elatius var. elatius)	CBS 624.69	forest soil	USA	1962	G.L. Hennebert	MH859388	MH871159	n/a	n/a	n/a	n/a
Oidiodendron majus	Oidma1	n/a	n/a	n/a	n/a	genome	genome	genome	genome	genome	genome
Rutstroemia firma	Rutfi1	*Quercus robur* dead branches	Netherlands	1985	H.A. van der Aa	genome	genome	genome	genome	genome	genome
Sclerotinia sclerotiorum	UF70	*Phaseolus vulgaris*	USA	n/a	n/a	genome	genome	genome	genome	genome	genome
Scolecolachnum (as *Psilachnum*) *pteridii*	CPC_24666	fronds of *Pteridium arachnoideum*	Brazil	2011	R.W. Barreto	KU597797	KU597764	n/a	n/a	n/a	n/a
Scutoscypha fagina	TK7178 (S)	*Quercus robur* leaf	Sweden	2016	T. Kosonen	MT231710	MT231710	MT241710	MT228693	MT216636	n/a
Soosiella minima	PRM 922619	soil	Czech Republic	2007	M. Hujslova	JX124328[3]	MH877623	n/a	n/a	n/a	n/a
Venturiocistella japonica	TNS-F18030, NBRC 106633[2]	*Cercidiphyllum japonicum* leaf	Japan	2006	n/a	JN033447	AB546954	n/a	JN086893	LC431669	n/a
Venturiocistella sp.	KUS-F52028	leaves of *Acer pseudosieboldianum*	Korea	2008	n/a	JN033391	JN086694	n/a	not used[3]	n/a	n/a
Venturioscypha nigropila	TUR215407	*Pinus uncinata*	Switzerland	2016	E. Stöckli	**ON241823**	**ON241823**	**ON246253**	**ON228703**	**ON246255**	n/a
Venturioscypha nigropila	BRACR33227	*Pinus sylvestris*	Poland	2020	A. Polhorský	**MZ621146**	**MZ621145**	n/a	n/a	n/a	n/a

[1] Includes (partial) sequences from all of the following genes: RPC2, RPA1, RPA2, SF3B1 and TFB4.

[2] The RPB1 sequences of *Hyphodiscus hyaloscyphoides* and *Venturiocistella japonica* originate from the second collection listed, respectively NBRC 104869, Japan, 2006, T. Hosoya, and NBRC 106633, Japan, 2006, Y. Harada & K. Tanaka. The other gene regions originate from the first collection listed TNS-F18030 and TNS-F13588.

[3] The RPB2 sequence is available, but *Venturiocistella* sp. (KUS-F52028) was not included in the nine-gene dataset because of other missing genes, and therefore the RPB2 sequence was not used in our study. *Venturiocistella* sp. was included in the ITS-LSU phylogeny.

slightly different protocols: BRA CR33227 at the Natural History Museum in Bratislava and TUR215407 at the Swedish Museum of Natural History.

For BRA CR33227, total genomic DNA was extracted from fresh apothecia using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with prolonged incubation time of up to 3 h after addition of the RNA-lytic enzyme. PCR was performed using a C1000 Touch™ Thermal Cycler. The PCR reactions were conducted in 25 µL total volume using a GoTaq Flexi PCR kit (Promega), with the reaction mixture containing 20–25 ng DNA template, 1 µl of each primer (10 µM), 5 µl Buffer (5x), 2.5 µl dNTP (2 mM), 2 µl $MgCl_2$ (25 mM), 0.2 µl GoTaq Flexi polymerase, and ultra pure water added for the final volume. The ITS region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) was amplified with primers ITS5 and ITS4 (White et al. 1990) and the 5' end of the LSU rDNA (spanning domains D1 and D2) was amplified with the primers LR0R and LR5 (Vilgalys and Hester 1990). PCR reactions 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 sec per cycle) and 10 min final elongation at 72 °C. The PCR products were analysed on 2 % agarose gel with GelRed® Nucleic Acid Gel Stain, and the amplified products were purified using a Thermosensitive Alkaline Phosphatase (FastAP) and Exonuclease 1 (Exo 1) (Thermo Fisher Scientific Inc., USA) according to manufacturer's instructions. The ITS and LSU rDNA regions were sequenced in a commercial laboratory (Eurofins Genomics GmbH, Cologne, Germany) using the same primers as for PCR.

For TUR215407, total genomic DNA was isolated from fresh mycelia using the DNeasy Plant Mini Kit, following the standard protocol for fresh plant material. Five different gene regions, ITS, LSU, RPB1, RPB2, and TEF-1α, were amplified for TUR215407. The PCR cycle details and primers used are given in Kosonen et al. (2021). Purified PCR products were sequenced by Macrogen Inc. (the Netherlands), using the same primers as in the PCR.

The nucleotide sequences of RPC2, RPA1, RPA2, SF3B1 and TFB4 were obtained from whole genome sequence projects (WGS) available in GenBank. A trimmed nucleotide sequence of the corresponding partial gene region from the dataset of Johnston et al. (2019) was used as a template to BLAST against the WGS data. In the nine-gene dataset, these five genes are available only for the eight taxa with published genomes (**Table 1**).

Sequence alignment and phylogenetic analyses

Newly produced sequences were edited and assembled in Sequencher 5.4.6 (Gene Codes, Ann Arbor, Michigan) and deposited in GenBank (**Table 1**). Initial studies showed *Venturioscypha* ITS and LSU sequences having high similarity to *Hyphodiscus* sequences in GenBank. To assess the phylogeny of *Venturioscypha* and its related taxa, a nine-gene dataset was assembled. Another dataset, including only ITS and LSU sequences, was assembled to study the diversity around *Venturioscypha*, as multiple gene sequences were only available for a restricted number of taxa. Nucleotide sequences were aligned manually using AliView (Larsson 2014). The spliceosomal introns were excluded from the analyses. The protein coding regions (RPB1, RPB2, TEF-1α, RPC2, RPA1, RPA2, SF3B1, and TFB4) were analysed with two distinct partitions: 1) first and second codon positions; and 2) third codon positions. In the analyses of the combined nine-gene dataset, the LSU rDNA was specified as one partition. Thus, the concatenated nine-gene dataset was analysed with 17 partitions. *Sclerotinia sclerotiorum* (Lib.) de Bary was used as an outgroup in the nine-gene dataset, and was used for rooting purposes together with *Rutstroemia firma* (Pers.) P. Karst., based on their placement in the "sclerotinioid clade" that is outside (early diverging to) the "pezizelloid clade" including *Hyphodiscus* Kirschst. (Johnston et al. 2019). *Leptodontidium trabinellum* (P. Karst.) Baral, Platas & R. Galán was used as an outgroup in the ITS-LSU dataset based on results from the nine-gene dataset, and was used for rooting purposes together with *L. irregulare* (de Hoog) de Hoog.

Since the gene regions RPC2, RPA1, RPA2, SF3B1, and TFB4 were available only for eight of the 20 taxa, a separate four-gene (LSU, RPB1, RPB2, and TEF-1α) dataset was analysed for comparison to test the effect of missing data in the nine-gene dataset. The tree topologies from the two analyses were similar, and the support values of the backbone nodes were higher in the nine-gene phylogeny. None of the

nodes had lower support in the nine-gene phylogeny compared to the four-gene tree. Therefore, we proceeded with the nine-gene phylogeny.

All analyses were run on CIPRES Science Gateway (Miller et al. 2010) using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) in MrBayes v. 3.2.7a on XSEDE (Ronquist & Huelsenbeck 2003) and Maximum Likelihood-based inference (ML) in RAxML-HPC2 v. 8.2.10 (Stamatakis 2014). The Bayesian analyses were run in parallel using the "mixed model" option (Ronquist et al. 2012) with all parameter values, except branch length and tree topologies, unlinked. The analyses were initiated with random trees and consisted of four parallel searches, with four chains each. The single gene datasets were analysed separately using 5 M generations, and the combined nine-gene dataset using 10 M generations. The chains were sampled every 500 generations in the 5 M generation runs and every 10 K generations in the 10 M runs. The last 75 % of the posterior tree samples were used to assemble a consensus tree and to calculate the posterior probabilities (PP). Maximum likelihood bootstrap analyses (ML-BP) were performed using 1000 rapid bootstrap replicates from random starting trees, followed by a thorough ML search similarly using 1000 replicates. The analyses used a GTRGAMMA model for the rate heterogeneity allowing all free model parameters to be estimated by the program.

Prior to combining the datasets, data congruence was studied visually by comparing the single gene trees based on Bayesian and ML analyses of the LSU, RPB1, RPB2 and TEF-1α regions, available for most taxa in the nine-gene dataset. Also the ITS and LSU datasets were first analysed separately and the trees studied for conflicts. No supported conflicts (ML-BP ≥ 75 % or PP ≥ 0.95) were observed between any of the single gene trees. The ITS-LSU dataset were analysed unpartitioned.

Results

Nucleotide sequences, congruence and data partitions

Twenty-two sequences from five samples of four species of *Hyphodiscaceae* (two *Venturioscypha nigropila,* one of *Hyphodiscus hymeniophilus* (P. Karst.) Baral, *Hyphodiscus* sp., and *L. trabinellum*) were produced in this study as listed in **Table 1.** Sequences of *V. nigropila* comprise partial SSU (BRA CR33227), ITS and LSU D1–D4 (BRA CR33227 & TUR215407) and TEF-1α, RPB1, and RPB2 (TUR215407). A total of 142 sequences were retrieved from GenBank of which 80 originated from WGS projects. The nine-gene dataset contains 11046 characters and 20 taxa. Of the 20 taxa included in the nine-gene dataset, two taxa lack RPB1, six taxa lack TEF-1α, and 12 taxa lack RPC2, RPA1, RPA2, SF3B1, and TFB4. Sequences of LSU and RPB2 are available for all taxa. The ITS-LSU dataset contains 1143 characters, available for all 29 taxa.

The BRA CR33227 partial SSU-ITS sequence includes SSU intron S1506 (see Baral et al. 2020: 124), while the ITS obtained from TUR215407 is incomplete in the beginning of the ITS1 (6 bp missing). The ITS sequences of the two samples differ by 3 bp in the ITS1 and 3 bp in the ITS2, and the LSU sequences differs by 2 bp in the D1 domain. In the ML analysis of the nine-gene dataset the single best scoring tree was recovered with –InL= 63244.701915. The Bayesian analysis reached an average standard deviation of split frequencies of 0.0041 after 10 M generations. The Potential Scale Reduction Factor stabilized at 1.000 (± 0.004) for all except nine of the 222 parameters, for which the value was within ±0.008. From the combined ITS-LSU analysis a single best scoring tree was recovered with –InL= 5693.501064. The Bayesian analysis reached an average standard deviation of split frequencies of 0.010 after 5M generations. The Potential Scale Reduction Factor stabilized at 1.000 (±0.001) for all parameters.

Phylogenetic placement of Venturioscypha based on a combined nine-gene phylogeny

Bayesian and ML analyses of the nine-gene dataset produced identical topologies, except for the unsupported placement of *Gamarada debralockiae*

and *Venturiocistella japonica* that are resolved as a monophyletic group in the ML (ML-BP 58 %) and as successive sisters in the Bayesian phylogeny (PP 0.78) (**Fig. 1**). *Venturioscypha* is nested within a highly supported family *Hyphodiscaceae* (ML-BP 98 %, PP 1.00, **Fig. 1**). *Leptodontidium* (*Leptodontidiaceae*) and *Amorphotheca-Oidiodendron* (*Amorphothecaceae-Myxotrichaceae*) form successive sister lineages to *Hyphodiscaceae* (ML-BP 89–98 %, PP 1.00). This clade, with representatives of *Hyphodiscaceae*, *Leptodontidiaceae*, *Amorphothecaceae* and *Myxotrichaceae,* forms a sister group to the *Pezizellaceae,* i.e. *Calycina* spp. (including "*Bisporella*"), *Calycellina leucella* and *Scutoscypha fagina* (ML-BP 75–100 %, PP 1.00). The five species of *Hyphodiscus* form a highly supported monophyletic group (ML-BP 100%, PP 1.00). *Hyphodiscus*, *Hyphopeziza pygmaea*, and *Venturioscypha nigrophila* form a highly supported clade (ML-BP 79 %, PP 1.00), but the exact relationships among these taxa are without support; *V. nigropila* and *H. pygmaea* resolve as a sister lineage to the *Hyphodiscus* clade (ML-BP 48 %, PP 0.61).

Diversity of taxa surrounding Venturioscypha based on an ITS-LSU phylogeny

There is no supported conflict between the phylogenies produced by ML and Bayesian analyses of the ITS-LSU dataset, but overall only few nodes have support in the ingroup. All species of *Hyphodiscus* form a highly supported monophyletic group (ML-BP 79 %, PP 1.00) (**Fig. 2**). *Venturioscypha* belongs to a supported clade of *Hyphodiscus*, *Hypho-*

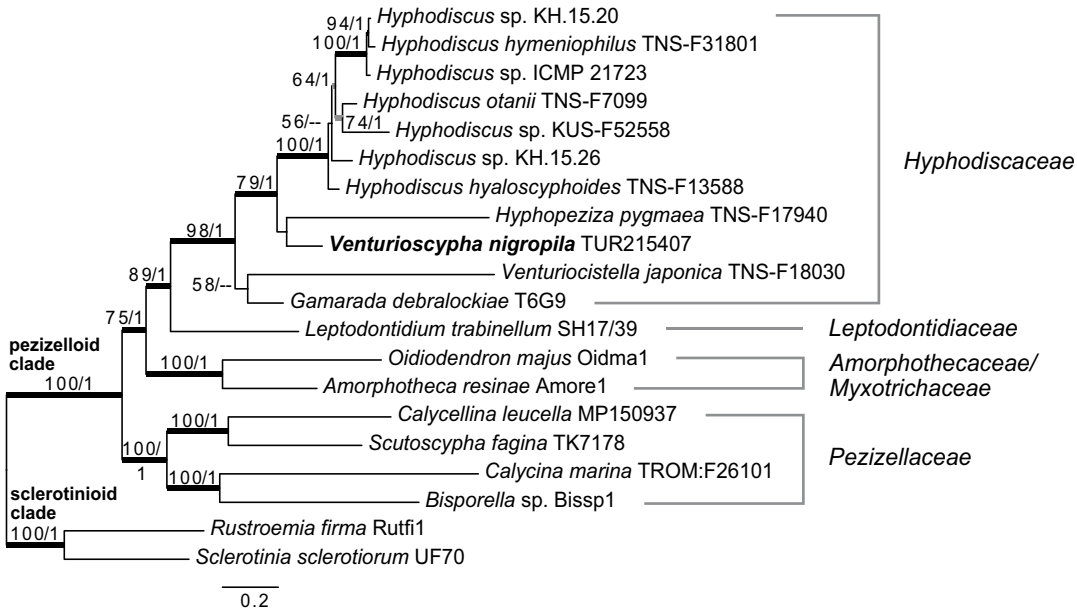

Fig. 1. A Maximum Likelihood phylogram of the nine-gene (RPB1, RPB2, TEF-1α, RPC2, RPA1, RPA2, SF3B1, TFB4 and LSU rDNA) dataset of *Hyphodiscaceae* and related families within the "pezizelloid clade." *Sclerotinia sclerotiorum* was used as the outgroup for the analyses and for rooting purposes together with *Rutstroemia firma*. Thick black branches received both Bayesian posterior probabilities (PP) ≥ 0.95 and maximum likelihood bootstrap values (ML-BP) ≥ 75 %. Exact support values are given at the branches as ML-BP ≥ 50 % / PP ≥ 0.95. Collection numbers are given after the taxon names. The new genus and species described in this paper are in **bold**.

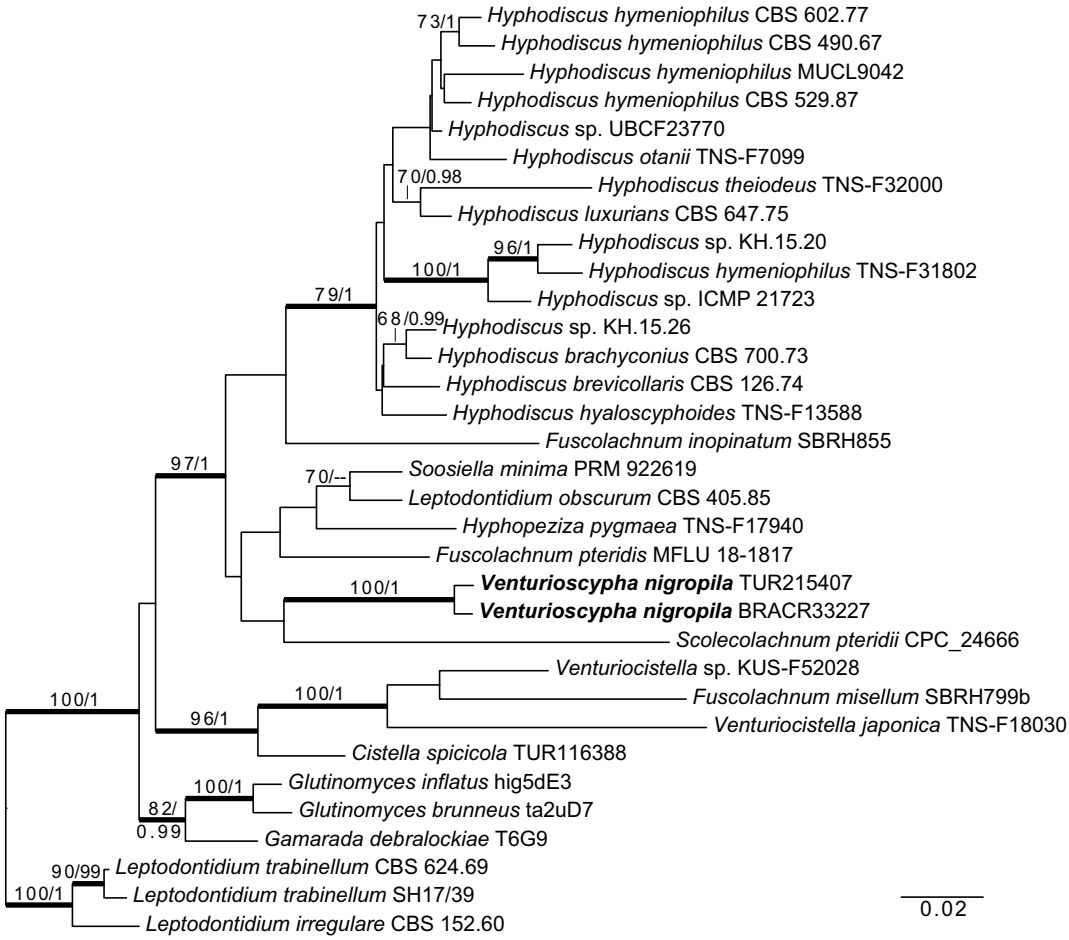

Fig. 2. A Maximum Likelihood phylogram of the ITS-LSU dataset of *Hyphodiscaceae*. *Leptodontidium trabinellum* was used as the outgroup for the analyses, and the tree was rooted using both *L. trabinellum* and *L. irregulare*. Thick black branches received both Bayesian posterior probabilities (PP) ≥ 0.95 and maximum likelihood bootstrap values (ML-BP) ≥ 75 %. Exact support values are given at the branches as ML-BP ≥ 50 % / PP ≥ 0.95. Collection numbers are given after the taxon names. The new genus and species described in this paper are in **bold**.

peziza, *Fuscolachnum* p.pt., *Leptodontidium obscurum*, *Scolecolachnum*, and *Soosiella minima* (ML-BP 97%, PP 1.00), but the relationship among these taxa are without support. Resolved outside this clade, is another highly supported clade of *Venturiocistella* sp., *V. japonica*, *"Cistella" spicicola*, and *Fuscolachnum misellum* (ML-BP 96%, PP 1.00). Within the clade, *"Cistella" spicicola* forms a sister lineage to a clade of *Venturiocistella* spp. and *F. misellum* (ML-BP 100 % and PP 1.00).

Taxonomy

Venturioscypha Baral, T. Kosonen & Polhorský gen. nov. – MycoBank MB846820

ETYMOLOGY: for the hairs resembling those of *Venturia*.

TYPE SPECIES: *Venturioscypha nigropila* Baral, T. Kosonen, Stöckli, Wergen & Polhorský

DIAGNOSIS: Differs from *Venturiocistella* in apothecia covered by only one type of dark brown, finally thick-walled hairs (**Fig. 3: 1g–h**), which are smooth or densely covered with fine, pore-like dots (**Fig. 3: 1i**), and in inamyloid asci without apical wall thickening (**Figs. 3: 1l–m, 2f–g**).

Venturioscypha nigropila Baral, T. Kosonen, Stöckli, Wergen & Polhorský sp. nov. – MycoBank MB846821 **Figs. 3–9.**

ETYMOLOGY: referring to the blackish hairs under incident light.

HOLOTYPE: Poland, **Lesser Poland**, Nowy Targ, Czarny Dunajec, Podczerwone, *Pinus sylvestris branch*, 15.II.2020, A. Polhorský (BRA CR33227).

APOTHECIA solitary to gregarious or sometimes densely crowded, 0.2–0.5(–0.7) mm diam. when hydrated {5} excluding hairs (0.2–0.9 mm including hairs), 0.12–0.2 mm tall, receptacle 85–120 µm thick at base, 70–90 µm at mid flanks, superficial on bark, stipe typically immersed in algal layer; disc pale to dark grey when fully hydrated, whitish when drying out, flat, exterior densely covered by projecting blackish hairs; stipe short cylindrical to strongly obconical, 0.04–0.12(–0.2) × 0.07–0.18 mm {6}; 2–4 generations may develop from the same stipe by proliferation {4}, remnants of ectal excipulum and hairs of old apothecia still present below the new generation (visible in external view and in median section), sometimes hairs proliferating in centre of disc {3}; when dry, disc of young apothecia completely covered by marginal hairs, mature apothecia only slightly contracted and with fully exposed hymenium. **ASCI** *(30–)38–55(–59) × (9.7–)10–12.5(–13.5) µm {8}, †(25–)30–48 × 7.5–9.5 µm {2}; spores 4–8-seriate in a fascicle {8} but often one spore somewhat out of the bundle, pars sporifera *20–25(–29) –> 17–23 µm long, †25–37 µm; apex (*) hemispherical to very broadly conico-truncate, (†) conical to medium truncate, apically thin-walled {7} (†0.2–0.5 µm thick), lateral wall †0.5–1.3 µm thick {2}, IKI– {9}, MLZ– (with or without KOH), opening by a terminal split, periascus absent in IKI but sometimes visible in KOH; base unstalked or with short stipe, arising from croziers {8}; asci not liberating spores in water mount {3} but ejecting when adding IKI, wall surface CRB–, immature asci with fusion nucleus 4 µm diam. (nucleolus 1.6 µm). **ASCOSPORES** *(10.5–)12–15.5(–17.5) × (2.2–)2.4–3(–3.4) µm {9}, †10–14(–18) × 1.8–2.7 µm {2}, nonseptate within living asci {7}, fusoid to naviculiform, straight, sometimes slightly heteropolar (towards base more tapering and at apex slightly to medium bent); with ~5–20 minute LBs (0.2–0.3 µm diam.) mostly grouped close to both ends but also scattered in each half, lipid content (0.5–)1–2 {9}, without glycogen regions, with a single nucleus 2–2.2 µm diam. (visible in IKI); with very inconspicuous polar gel sheaths being compressed and therefore refractive within the living asci but swelling after spore liberation {3}, spore wall surface and sheath CRB– {2}; overmature 1((–2))-septate {7}, not increasing in size, readily forming polar or lateral germ tubes in senescent apothecia. **PARAPHYSES** cylindrical, straight to ± flexuous or slightly bent at the non-inflated or often slightly narrowed apex, terminal cell *(9–)11–31(–35) {3} × 1.7–3.3 µm {4}, 12 µm shorter up to 10 µm longer than living asci {4}, 0–6 µm longer than dead asci {1}, rarely furcate (antler-like), smooth or often covered by a thin, irregular rough exudate (rough layer detaching from cell wall in KOH), lower cells *10–17 {1} × 1.8–2.7 µm {2}, partly dichotomously branched and with anastomoses towards base, eguttulate or sometimes with a few or many non- or slightly refractive, globose to angular or elongated vacuoles (unstained in IKI) and a few minute LBs (**Fig. 3: 1k**), rarely with strongly refractive SCBs in terminal and lower cells, staining pale red-brown in IKI (**Fig. 9: e–f**). **MEDULLARY EXCIPULUM** hyaline, 20–40 µm thick, in young apothecia little developed, at older stages two-layered: upper layer of ± vertically oriented, gelatinised textura porrecta, hyphae *1.3–2.5 µm wide, lower layer in receptacle of 10–15 µm thick, non-gelatinised t. porrecta or very loose textura intricata; in stipe of gelatinised dense t. (prismatica-)angularis, cells *2.5–4.5 µm wide, gel deep lilac in CRB, each cell containing 1–2 large LBs and a

Fig. 3. *Venturioscypha nigropila*. **1a**, **2a.** dry apothecia (1a young, in side view); **1b**, **2b.** hydrated apothecia; **1c**, **2c.** apothecia in median section (2c showing several generations of apothecia formed by proliferation from the same stipe); **1d.** idem, marginal region; **1e.** idem, ectal excipulum and hair bases at upper flanks; **1f**. same as **1e**, in surface view; **1g.** marginal hairs; **1h.** apex of marginal hairs; **1i–j.** detail of hairs (1i showing finely dotted surface); **2d.** detail of medullary excipulum in stipe (cells containing LBs); **1k**, **2e.** mature asci and paraphyses; **1l–m**, **2f–g.** ascus apices (immature, mature, and after ejection); **1n–p**, **2e.** croziers at ascus base; **1q**, **2h.** mature ascospores (with detaching polar sheaths); **1r.** overmature ascospores (one with germ tube). – Living state, except for 1i–j (in H_2O), 1l–m (in MLZ), 2f (in KOH), 2g (in IKI). – 1. H.B. 4897. Germany, Oberpfalz, Hirschau, Haarbühl, *Pinus strobus*; 2. H.B. 5284a. France, Vosges, Gérardmer, Tourbière de la Morte Femme, *P. mugo* ssp. *uncinata*. – Del. H.O. Baral.

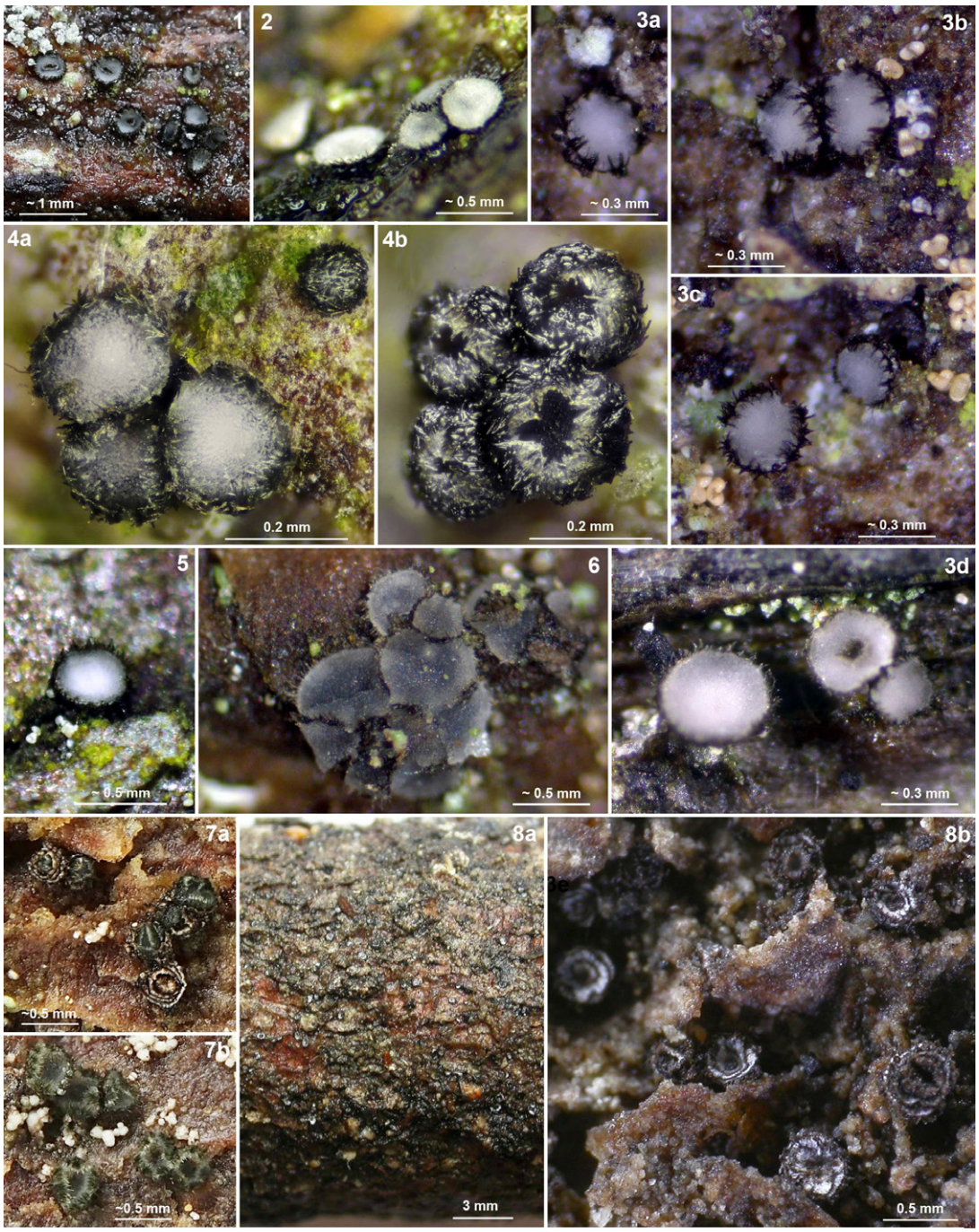

Fig. 4. *Venturioscypha nigropila*: (re)hydrated apothecia at different stage of development on xeric coniferous bark (1 & 3d with hymenial proliferations). – **1.** TUR215407. Switzerland, Jura, Tramelan, *Pinus mugo* ssp. *uncinata* (phot. E. Stöckli); **2.** E.S. 2015-83a. idem; **3a–d.** E.S. 2018-83b. idem (3d: phot. M. Hairaud); **4a–b.** 15.V.2018. Germany, Schwarzwald, Triberg, *P. sylvestris* (phot. B. Wergen); **5.** 30.VI.2020. Finland, Uusimaa, *P. sylvestris* (phot. J. Äikäs); **6.** E.S. 2020-130. France, Savoie, Méribel-Mottaret, *P. cembra* (phot. E. Stöckli); **7.** BRA CR33228. Slovakia, Žilina, High Tatras, *P. mugo* ssp. *mugo* (phot. M. Zajac); **8a–b.** H.B. 5284a. France, Vosges, Gérardmer, *P. mugo* ssp. *uncinata* (phot. H.O. Baral).

few smaller ones (large LBs near base 1–2.5(–4) µm diam., smaller in upper part), KOH-inert, also present in cells of inner ectal excipulum. **ECTAL EXCIPULUM** 15–50 µm thick at lower flanks {5}, irregularly oriented towards surface at an angle of 30–50° {2} up to 70–80° {2}; cells somewhat thick-walled, towards base thick-walled, inner cells prismatic, outermost cells globose to angular, *(4.5–)6–10(–13) × (3.5–)5–9 µm {3}; exudate among cells and on outer surface at first granular, yellowish-ochraceous-olive, gradually turning blackish-olivaceous-brown, more cloddy to continuous, unstained in CRB; at mid flanks and margin 10–25 µm thick, of t. prismatica oriented at 10–20°. **HAIRS** (28–)50–100(–120) × (2.7–)3.3–4(–4.5) µm {5}, densely covering the entire flanks and margin (sometimes hairs on lower flanks very short), emerging from outermost cells, cylindrical, slightly narrower towards apices to (1.5–)2–3(–3.5) µm {6}, ± straight to somewhat flexuous, mid to dark greyish-olive to blackish-brown, concolorous or subhyaline near apices; somewhat thick to finally thick-walled, wall in lower part of hairs 0.3–1 µm thick at margin {3} and 1–1.3(–1.5) µm thick at flanks {3}, thick wall 3-layered, usually wall thinner towards hair apices, wall not swelling in dead state; (0–)2–3(–4)-septate {4}, cells of equal length or apically longer, septa usually thick-walled; surface smooth {8} but overall densely finely dotted {3} (like pits, visible especially in PVA, perhaps only or mainly when thick-walled), CRB–. **ANCHORING HYPHAE** hyaline, smooth, *2–3.5 µm wide, forming a strongly gelatinised textura intricata {1}. **KOH** not releasing a pigment into the medium {2}, provoking no colour change of hairs and excipulum {H.B. 5284a} or a change from olivaceous-brown to orange-reddish-brown {BRA CR33227} (**Fig. 9: k-l**). **CHARACTERISTICS IN PURE CULTURE:** Radial growth moderate on malt-agar, ca. 15 mm in 30 days (room temperature, no daylight). Basic colour very dark brown (T51), margin abrupt and lighter, leather brown (N79) in colour. Occasionally zonate. No hyphal strands, but surface hyphae present. Yeast-like growth lacking, no anamorphs observed.

ECOLOGY: In thermoboreal and supra- to orotemperate regions of Europe, from planar to subalpine altitudes, e.g., in colline *Pinus sylvestris* and *P. strobus* forests and plantations, in mountainous peat bogs with *Pinus mugo* ssp. *uncinata*, once in a subalpine *Pinus cembra* forest, shady to sun-exposed, on dead twigs and branches still attached to living or recently dead trees 0.3–1.5 m above the ground, also on branches recently fallen to the ground but protruding into the airspace, corticated, 2–12 {5} up to 20–25 mm thick {6}, of cf. *Picea abies* {1}, *Pinus cembra* {1}, *P. mugo* ssp. *mugo* {1}, *P. mugo* ssp. *uncinata* {9/1}, *P. strobus* {3}, *P. sylvestris* {7}, on little to medium decayed bark {14} (on periderm, sometimes in deep cracks of bark), partly near resinous wounds, sometimes growing ± close to but never on resin, branches sometimes with still attached dead needles, often intermixed with green algae but also on naked bark devoid of algae, often very close to crustose or foliose *Lecanorales*. **TAXA IN CLOSE VICINITY** *Ciliolarina pinicola* {3}, *Chrysodisca peziculoides* {3}, *Crumenulopsis pinicola* {2}, *C. sororia* {1}, *Hypogymnia physodes* {1}, *Lachnellula calyciformis* {1}, *L. pseudofarinacea* {1}, *Lecanora* sp. {1}, cf. *Melanohalea* sp. {1}, *Micarea* cf. *peliocarpa* {1}, *Pezicula eucrita* {2}, *Resinomyces kirschsteinianus* {2}, *Sarea difformis* {1}, *S. resinae* {2}, *Therrya pini* {1}, *T. fuckelii* {2}, *Tympanis* sp. {1}, indet. crustose lichen {1}. **DROUGHT TOLERANCE:** After 1 month in the herbarium, mature asci and paraphyses still alive. **PHENOLOGY:** II–IX (probably throughout the year). **ALTITUDE:** 3–110 m (Fennoscandia), 445–1720 m (Central Europe). **GEOLOGY:** granite {3}, mica schist {2}, Upper Buntsandstein {1}, Keuper {1}, alluvial peat over calcareous Jurassic rock {1}.

SPECIMENS EXAMINED (all on xeric bark of gymnosperm branches): **Denmark**: **Sjaelland**, W of Helsinge city, NNE of Asserbo, Asserbo Plantage, 10 m, 56° 1' 55" N, 12° 1' 5" E, *Pinus sylvestris*, 9.III.2020, O. Martin, vid. T. Læssøe (DMS-10086342, C). — **Finland**: **Uusimaa**, SSE of Porvoo, NE of Storgård, border of Kråkö sandpit, 3 m, 60° 20' 44" N, 25° 41' 12" E, *P. sylvestris*, 30.VI.2020, J. Äikäs (ø). – **Etelä-Häme**, Pirkanmaa, NW of Lempäälä, NNE of Portaankorva, 110 m, 61° 21' 0" N, 23° 38' 0" E, *P. sylvestris*, 10.VIII.1995, U. Söderholm (ex U.S. 2360, TUR, dupl. H.B. 5337a). – 16 km SW of Somero, E of Halkjärvi lake, 60° 30' 30" N, 23° 40' 34.5" E, 91 m, *P. sylvestris*, 7.V.2022, S. Jakobsson (S.J. 5156, H). — **France**: **Lorraine**, **Vosges**, W of Gérardmer, E of Le Costet Beillard, Tourbière de la Morte Femme, 643 m, 48° 4' 23" N, 6° 49' 15" E, *P. mugo* ssp. *uncinata*, 22.VI.1990, G. Marson & J. Deny (H.B. 4139a). – idem, 18.V.1995, J. Deny (H.B. 5284a). – idem, 2.IX.1996, H.O. Baral (ø). – NW of Gérardmer, NNE of le Petit Liézey, la Haute Pinasse, la Goutte Loiselot, 850 m, 48° 6' 3" N, 6° 51' 0" E, cf. *Picea abies*, 6.IX.1996, H.O. Baral (ø). – **Rhône-Alpes**, **Savoie**, Parque de la Vanoise, SSE of

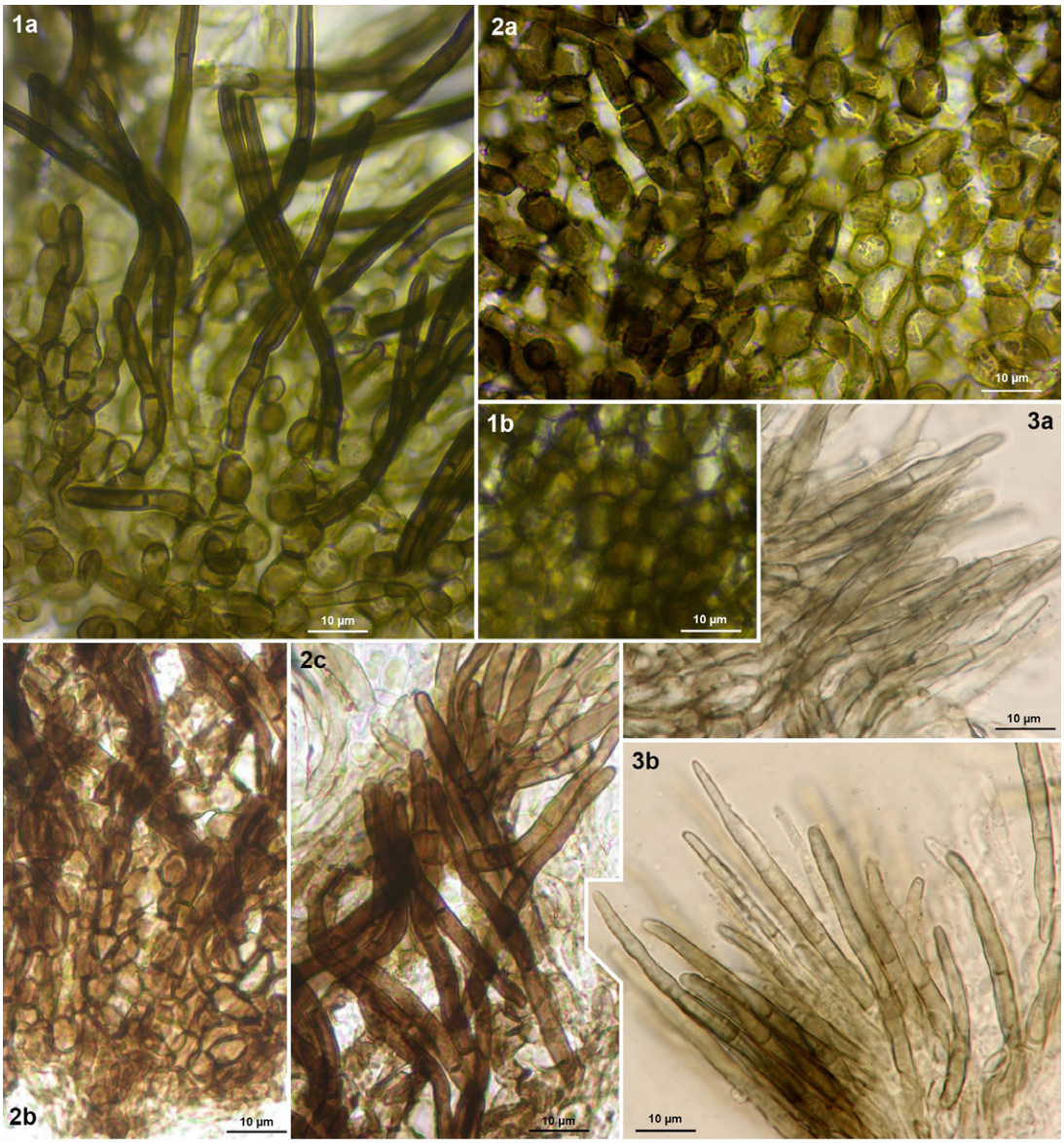

Fig. 5. *Venturioscypha nigropila*. **1–3.** Ectal excipulum and hairs in surface view or suqash mount (living state in water, colour differences due to different cameras and white balance). – **1.** E.S. 2020-130. France, Savoie, Méribel-Mottaret, *Pinus cembra*; **2.** E.S. 2018-83b. Switzerland, Jura, Tramelan, *P. mugo* ssp. *uncinata*; **3.** 15.V.2018. Germany, Schwarzwald, Triberg, *P. sylvestris*. – Phot. 1a–b, 2a: E. Stöckli; 2b–c: H.O. Baral, 3a–b: B. Wergen.

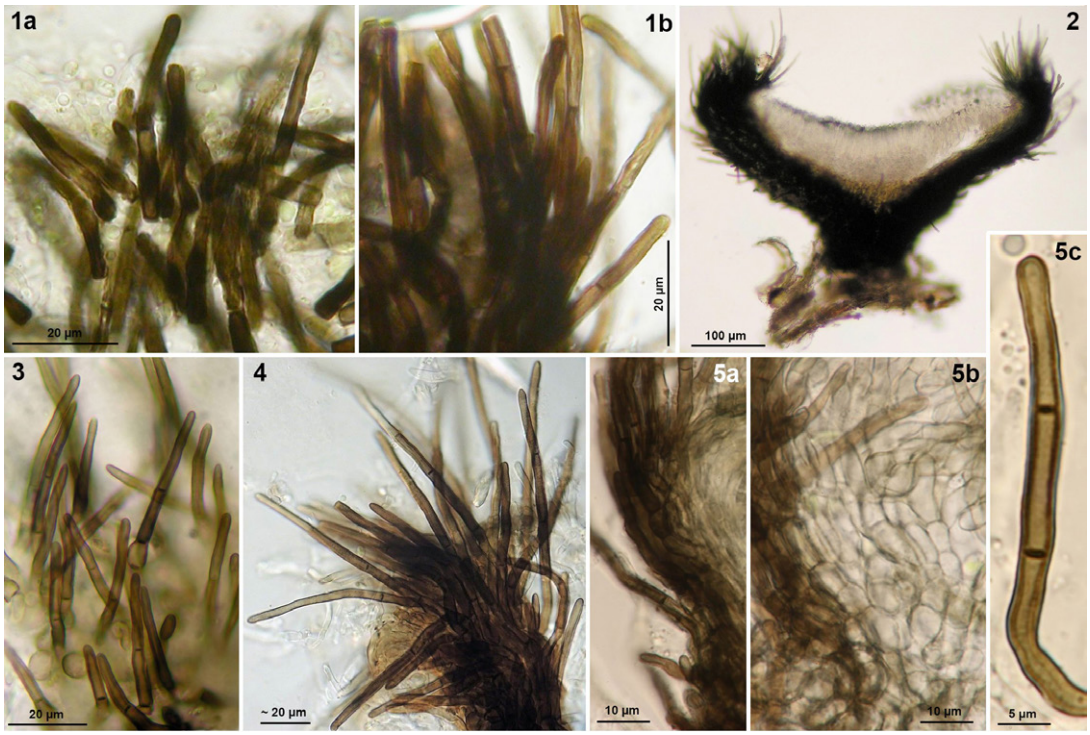

Fig. 6. *Venturioscypha nigropila*. **2.** apothecium in median section; **1a–b, 3–4.** hairs near margin, in external view or section (squash mount); **5a.** median section of marginal ectal excipulum with hairs; **5b.** idem, at base; **5c.** hair with indistinct surface dotting. – **1a–b.** in KOH, rest living state in water. – 1a–b. H.B. 5284a. France, Vosges, Gérardmer, *Pinus mugo* ssp. *uncinata* (phot. H.O. Baral); 2. TUR215407: Switzerland, Jura, Tramelan, *P. mugo* ssp. *uncinata* (phot. E. Stöckli); 3. 23.IX.2015. idem; 4. 9.III.2020. Denmark, Sjaelland, Asserbo, *P. sylvestris* (phot. T. Læssøe); 5a-c. 15.V.2018. Germany, Schwarzwald, Triberg, *P. sylvestris* (phot. B. Wergen).

Les-Allues, SSE of Méribel-Mottaret, N of Lac de Tuleda, 1720 m, 45° 21' 53" N, 6° 35' 8" E, *Pinus cembra*, 28.VIII.2020, E. Stöckli (E.S. 2020-130). — **Germany**: **Baden-Württemberg**, Schwarzwald, WNW of Calw, WSW of Oberreichenbach, Waldmoor-Torfstich, 680 m, 48° 43' 50" N, 8° 38' 40" E, *P.* cf. *mugo* ssp. *uncinata*, 28.V.1978, H.O. Baral (H.B. 2718). – SSE of Triberg, Geutsche, 920 m, 48° 6' 55" N, 8° 14' 35" E, *P. sylvestris*, 15.V.2018, B. Wergen (B.W.). – **Bayern, Oberpfalz**, NNE of Amberg, W of Hirschau, Haarbühl, 445 m, 49° 32' 45" N, 11° 55' 7" E, *P. strobus*, 14.IV.1991, E. Weber & H.O. Baral (H.B. 4380). – ibid., *P. strobus* & *P. sylvestris*, 12.VIII.1992, H.O. Baral & E. Weber (H.B. 4731, *P. strobus*). – ibid., *P. strobus*, 30.V.1993, H.O. Baral & E. Weber (H.B. 4897a). — **Poland**: **Lesser Poland**, WSW of Nowy Targ, SW of Czarny Dunajec, W of Podczerwone, close to Slovakian border, 717 m, 49° 24' 39" N, 19° 47' 22" E, *P. sylvestris*, 15.II.2020, A. Polhorský (BRA CR33227, **holotype**, GenBank: ITS MZ621146, LSU MZ621145). — **Slovakia**: **Žilina**, High Tatras, WNW of Poprad, NNW of Štrbské pleso, Mlynická dolina, 1480 m, 49° 8' 15" N, 20° 3' 11" E, *P. mugo* ssp. *mugo*, 13.VI.2020, M. Zajac (BRA CR33228). — **Switzerland**: **Jura**, N of Tramelan, WSW of Les Genevez, La Tourbière du Pâturage du Bas, 1015 m, ~ 47° 14' 28" N, 7° 5' 35" E, *P. mugo* ssp. *uncinata*, 2.VIII.2015, E. Stöckli (E.S. 2015-83a). – idem, 23.IX.2015 (ø). – idem, 28.V.2016 (TUR215407 , GenBank: ITS

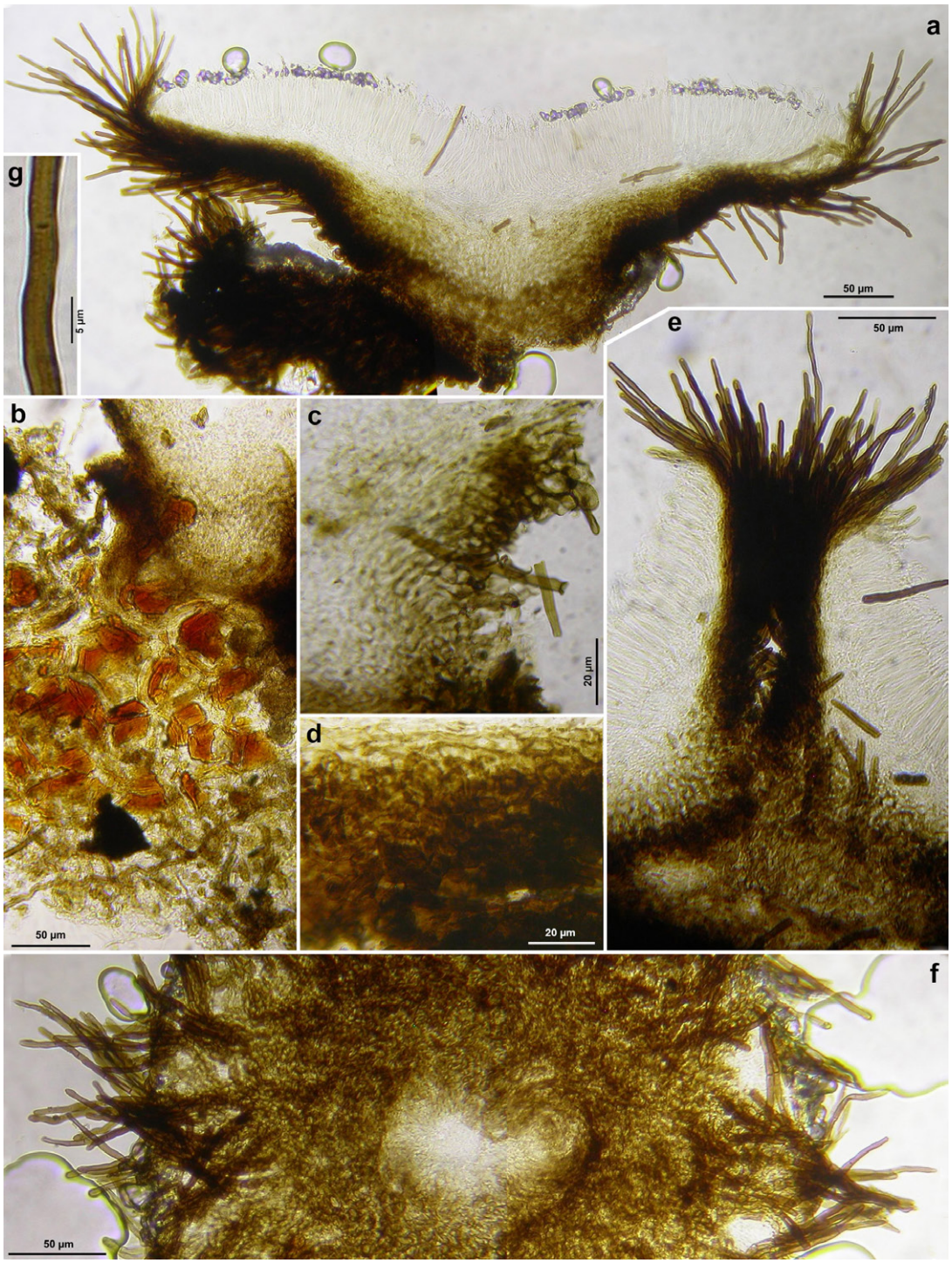

Fig. 7. *Venturioscypha nigropila* (H.B. 4139a: France, Vosges, Gérardmer, Tourbière de la Morte Femme, *Pinus mugo* ssp. *uncinata*). **a.** apothecium in median section; **b.** stipe base in median section, with bark cells below and fungal hyphae above and below; **c.** stipe in median section; **d.** idem, ectal excipulum at lower flanks; **e.** idem, two apothecia in mutual association; **f.** apothecium viewed from below; **g.** part of hair showing surface dotting. – Dead state (permanent slide, embedded in PVA). – Phot. H.O. Baral.

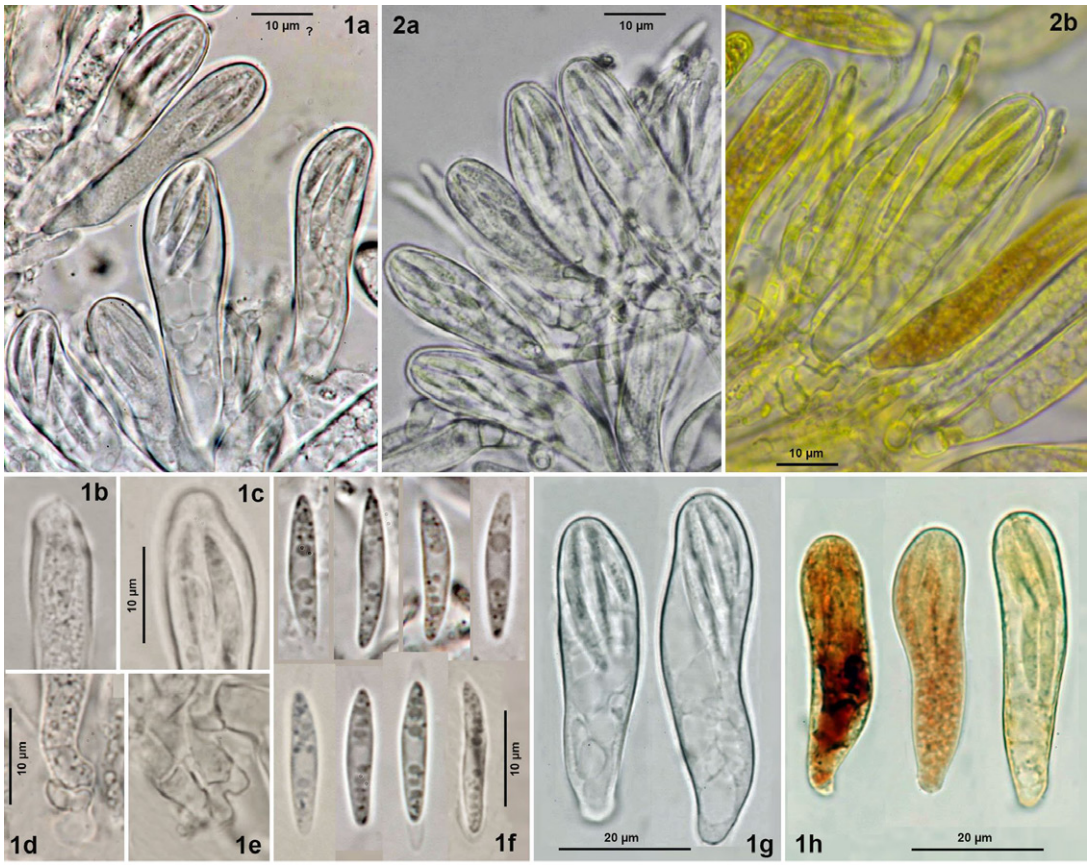

Fig. 8. *Venturioscypha nigropila*. **1a, 2a–b.** asci and paraphyses in squash mount; **1b–c.** ascus apices (immature and mature); **1d–e.** croziers at ascus base; **1f.** ascospores; **1g–h.** detached asci at different stages of maturity. – Living state, except for 1b–c & 1e; all in H_2O, except for 1f & 2b (in IKI). – 1. 15.V.2018: Germany, Schwarzwald, Triberg, *Pinus sylvestris* (phot. B. Wergen); 2. E.S. 2018-83b: Switzerland, Jura, Tramelan, *P. mugo* ssp. *uncinata* (phot. E. Stöckli).

ON241823, LSU ON241823, TEF1 ON246253, RPB2 ON228703, RPB1 ON246255.). – idem, 1.VI.2016 (E.S. 2016-83b). – idem, 9.VII.2017 (E.S. 2017-83c, not documented). – idem, 12.VI.2018 (E.S. 2018-83b).

Discussion

Phylogenetic position of *Venturioscypha*

The nine-gene phylogeny firmly places *Venturioscypha* in *Hyphodiscaceae*. Considering morphology, this seems a natural placement for a genus with gelatinised medullary and obliquely orientated ectal excipular cells, and prominent hairs. *Venturioscypha* represents a distinct branch in the phylogenies of related taxa, derived from analyses of both the nine-gene and the ITS-LSU datasets, and it is suggested being most closely related to *Fuscolachnum* p.pt., *Hyphodiscus*, and *Hyphopeziza*. Nevertheless, morphologically *Venturioscypha* resembles considerably *Venturiocistella* sharing short-celled, pigmented excipular cells and long, thick-walled, black-brown hairs. However, *Venturiocistella* differs in having hairs of two kinds: long, thick-walled, dark brown spiny hairs (often referred to as "setae" in the literature) that are warted in the basal part and gradually attenuated towards the pointed apices; and short, thin-walled, light brown cylindrical hairs that are entirely warted.

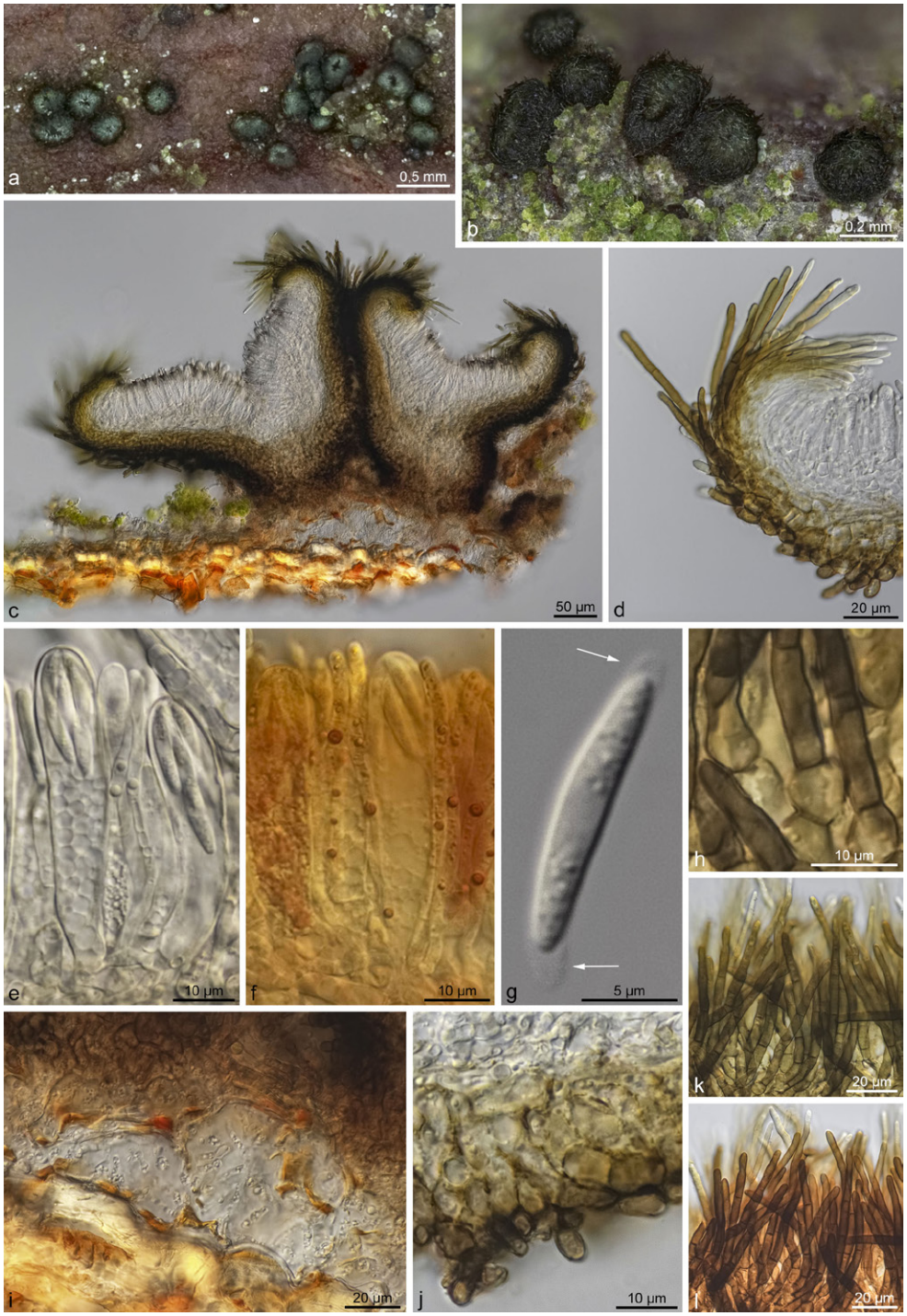

Fig. 9. *Venturioscypha nigropila* (holotype). **a–b.** hydrated, rather young apothecia on bark; **c–d.** median section through apothecia; **e–f.** hymenium in median section (paraphyses containing SCBs) **g.** ejected ascospore, arrows pointing to polar sheats; **h.** base of hairs; **i.** base of stipe with strongly gelatinised anchoring hyphae; **j.** ectal excipulum in median section; **k–l.** hairs in external view. – Living state (c–e, g–k in H_2O, f in IKI), except for l (in KOH). – a–l. BRA CR33227: Lesser Poland, Czarny Dunajec, Podczerwone, *Pinus sylvestris* (phot. A. Polhorský).

Fig. 10. Montane peat bog La Tourbière du Pâturage du Bas near Tramelan, Swiss Jura, with *Pinus mugo* ssp. *uncinata*. Phot. E. Stöckli (20.IX.2015, no collection of *Venturioscypha* made on this day).

Fuscolachnum, *Hyphodiscus*, *Hyphopeziza*, and *Venturiocistella* each have distinct hair shapes, but share the feature of more or less prominent warts on the hairs. *Venturioscypha* is clearly distinct by its peculiar one kind of hairs that are smooth or inconspicuously pitted (as viewed by the light microscope), non-amyloid asci with a thin apical wall that ruptures irregularly at spore discharge, spores with a delicate sheath, and apothecial proliferation. The exact relationship among *Venturioscypha*, *Hyphodiscus*, and *Hyphopeziza* is not resolved with support in the nine-gene phylogeny (**Fig. 1**) and *Fuscolachnum* is only represented in the ITS-LSU phylogeny due to missing sequence data. Also the branches leading to *Hyphopeziza* and *Venturiocistella* are very long. The difference in species/genus representation between the ITS-LSU and nine-gene tree shows one clear goal for future studies, i.e., a robust phylogenetic hypothesis for the *Hyphodiscaceae* demands for the acquisition of multiple genes from several taxa, which are presently only available with barcoding (ITS) sequences, or even taxa not yet sampled.

Morphological "look-alikes"

Based on morphological similarities, *Venturioscypha* could be considered related to *Pirottaea* Sacc., but that genus is closely related to *Pyrenopeziza* Fuckel or might even be a synonym of it based on the similar apothecial morphology. *Pirottaea* and *Pyrenopeziza* were included in "*Ploettnerulaceae*" by Baral (in Jaklitsch et al. 2016) because of the synonymy of *Ploettnerula* Kirschst. with *Pirottaea*. Based on a 3156-gene phylogeny (Johnston et al. 2019), "*Ploettnerulaceae*" was placed in the "mollisioid clade", whereas *Hyphodiscaceae* clustered in the "pezizelloid clade". Phutthacharoen et al. (2021) adopted the family "*Ploettnerulaceae*" in their molecular phylogenetic analysis, but used the anamorphic name *Rhexocercosporidium* U. Braun instead of *Pirottaea* and *Pyrenopeziza*, and misapplied *Pirottaea* to taxa now assigned to *Chaetoscypha* Syd. (*Helotiaceae*). Doweld, A.B. 2022: (2865–2866) Proposals to conserve the name *Pyrenopezizaceae* against *Excipulaceae* and *Pyrenopeziza*, nom. prot., with a conserved type (Fungi: Ascomycota: *Leotiomycetes*) Taxon 71:461–462. *Pirottaea* in the correct sense differs from *Venturioscypha* in having desiccation-intolerant, sessile apothecia without a stipe-like base,

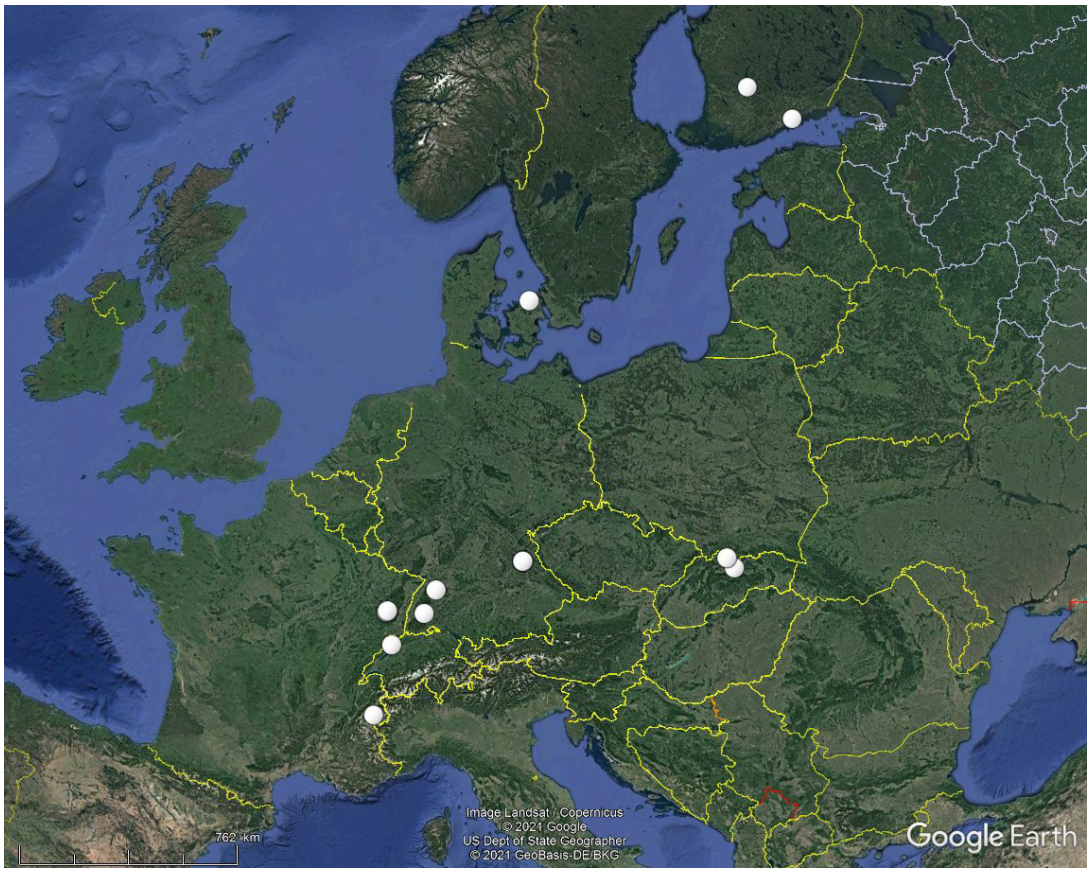

Fig. 11. Known distribution of *Venturioscypha nigropila*.

which generally develop beneath the epidermis of herbaceous plants, their erumpent growth being sometimes recognizable by the lifted epidermis around the mature apothecia. It further differs in an ectal excipulum of overall thin-walled textura angularis-globulosa being vertically oriented at the flanks and marginally often protruding beyond the disc, asci with euamyloid apical ring, and ascospores lacking a sheath.

The lichenicolous genera *Echinodiscus* Etayo & Diederich, *Diplolaeviopsis* Giralt & D. Hawksw., and *Macroskyttea* Etayo et al. somewhat resemble *Venturioscypha* in their smooth hairs and inamyloid asci, but their hairs are hyaline. *Echinodiscus* also differs in a violet ectal excipulum and the other two genera in a purplish colour change of the excipular pigments in KOH. Based on molecular phylogenetics, *Diplolaeviopsis* and *Macroskyttea* are closely related to *Unguiculariopsis* Rehm in the *Cordieritidaceae* (Suija et al. 2015 as "*Encoelioideae*", Etayo et al. 2015 as "encoelioid-clade", Pärtel et al. 2016). For *Echinodiscus* no molecular data was available.

To gain a better understanding of the surface microstructure of the hairs of *Venturioscypha* (see **Fig. 3: 1i, 7g**), they should be viewed by scanning electron microscopy (SEM). The structure is likely similar or identical to what has vaguely been seen on the smooth upper part of the spiny hairs of *Venturiocistella* (Baral in prep.).

Apothecial proliferation

The formation of new apothecia by proliferation from excipular shells of old apothecia, in which the hymenium has disappeared, was observed in four collections (H.B. 2718, 4139a, 5284a, BRA CR33228 – **Figs. 3: 2c, 4: 7, 8b**). This peculiarity illustrates the longevity

of the species by forming several generations from one apothecial stalk. Sometimes, hair proliferation in the middle of the disc was observed (**Fig. 4: 1, 3d**), as in *Proliferodiscus* J.H. Haines & Dumont (*Lachnaceae*). The repeated formation of new generations of apothecia was reported by Raitviir (2002) for the superficially similar *Involucroscypha involucrata* (B. Erikss.) Raitv., based on the documentation by Eriksson (1970: pl. 2), who misinterpreted the "involucre" as a feature of young apothecia, and on Raitviir's personal studies of more recent samples of the species. The monotypic genus *Involucroscypha* Raitv. was placed in *Hyaloscyphaceae* s.l., but Raitviir (2002) saw also similarities with *Coronellaria* (P. Karst.) P. Karst., a genus of the *Hysteropezizella*-complex. An ITS sequence of *I. involucrata* in UNITE (TAAM 165831) suggests in fact affinities to *Mollisiaceae*, which is related to the *Hysteropezizella*-complex.

Ecology

Venturioscypha nigropila seems to be restricted to xeric bark of *Pinus*. So far it has been found only on *Pinus mugo* ssp. *mugo* and ssp. *uncinata*, *P. sylvestris*, and *P. strobus*. The substrate of the first sample from Black Forest (Schwarzwald) collected in 1978 was thought at first to be *Abies alba*, but re-examination of the wood anatomy revealed it to be *Pinus*, based on the pits forming large trapezoid apertures. The apothecia occur generally on only slightly decayed bark often in close vicinity of lichens. The species has not been found on decorticated wood or on resinous bark, although the apothecia often occurred not too distant from resinicolous ascomycetes and sometimes close to a wound in the branch.

Apothecia of *Venturioscypha nigropila* occur on substrate distant to the ground, i.e., bark of dead, corticated twigs and branches attached to a usually living tree, and are tolerant to desiccation. They are easiest to spot when fully hydrated, but soon dry up during periods of drought. The dry apothecia remain fully alive for a considerable time period. The desiccation tolerance was confirmed by rehydrating apothecia one month after depositing them in the herbarium and finding the cells vital. Various other groups of *Helotiales* include drought-tolerant members. Within the *Hyphodiscaceae*, members of *Hyphodiscus* are usually found on more or less exposed substrate, in particular *H. theiodeus*. Other examples of drought-tolerant hairy *Helotiales* are *Hyaloscypha quercicola* and *H. minuta* (*Hyaloscyphaceae*), or *Perrotia flammea* and *Proliferodiscus tricolor* (*Lachnaceae*).

Venturioscypha nigropila was observed in different vegetation types. Collections from Vosges, Jura, and High Tatras were from the border or centre of mountainous peat bogs with *P. mugo* ssp. *mugo* and ssp. *uncinata*, *Abies alba*, *Picea abies*, *Vaccinium*, *Calluna* etc. (See **Fig. 10** depicting one typical collection site). The collections from Oberpfalz derive from a site with a young to mid-adult colline monoculture of *Pinus strobus*, the forest around this plantation being partially paludified. The sample from Savoie originates from a subalpine *Pinus cembra* forest. The collection from Poland was from the edge of *Picea abies* and *Pinus sylvestris* relict boggy forest with *Rhododendron tomentosum* and *Vaccinum myrtillus*, bordering a peat bog. No information on the vegetation was available for planar to mountainous collections on *Pinus sylvestris*.

The geology at the sites in Finland, Poland, Slovakia, Vosges, and Oberpfalz was acidic (granite, mica schist, Buntsandstein, Keuper), whereas in the Swiss Jura it was alluvial peat over calcareous Jurassic rock. The climatic preferences of the fungus so far include a subcontinental distribution within Central Europe with colline to mountainous areas and subalpine altitudes as well as occurrence in the thermoboreal belt of Northern Europe.

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