



# *Hygrophorus* sect. *Olivaceoumbrini*: new boundaries, extended biogeography and unexpected diversity unravelled by transatlantic studies

J.-M. Bellanger<sup>1,\*</sup>, R. Lebeuf<sup>2</sup>, E. Sesli<sup>3</sup>, M. Loizides<sup>4</sup>, C. Schwarz<sup>5</sup>, P.-A. Moreau<sup>6</sup>, K. Liimatainen<sup>7</sup>, E. Larsson<sup>8,9</sup>

## Key words

*Hygrophorus latitabundus*  
*Hygrophorus olivaceoalbus*  
*Hygrophorus persoonii*  
phylogeny  
taxonomy  
waxcap

**Abstract** As currently delineated, *Hygrophorus* sect. *Olivaceoumbrini* is a polyphyletic assembly within subg. *Colorati*, encompassing glutinous and pigmented taxa. According to available literature, between a dozen and twenty species may belong in the section, mostly represented in continental and boreal forests of Europe and North America. However, the limited phylogenetic and biogeographic coverage of the genus does not presently allow for a reliable assessment of its taxonomic boundaries, nor does it provide a complete picture of species diversity within sect. *Olivaceoumbrini*. In an ongoing effort to confer an evolutionary backbone to *Hygrophorus* systematics, we assembled and analysed a dataset comprising 268 intercontinental sequences, including holotypes of 7 taxa previously not positioned phylogenetically, and enriched with collections from largely unexplored Mediterranean and Anatolian ecosystems. Overall, 30 clades are identified within 5 distinct lineages, including 11 species putatively new to science. Seven of these are formally described here as *H. agathosmoides*, *H. albofloccosus*, *H. canadensis*, *H. limosus*, *H. marcocontui*, *H. pinophilus* and *H. pustulatoideus* spp. nov. This enriched coverage of section *Olivaceoumbrini* s.lat. calls for a re-evaluation of its natural boundaries into a core monophyletic clade, including *H. olivaceoalbus* and five closely related lookalikes, as well as the assignment of the section rank to the four remaining lineages: sect. *Fuscocinerei* sect. nov., sect. *Limacini* sect. nov., sect. *Nudolidi* sect. nov. and sect. *Tephroleuci*, respectively. We also stabilize the usage of six historical names, *H. glutinifer*, *H. hyacinthinus*, *H. mesotephurus*, *H. olivaceoalbus*, *H. pustulatus* and *H. tephroleucus*, through designation of two neotypes, three lectotypes and four epitypes.

**Citation:** Bellanger J-M, Lebeuf R, Sesli E, et al. 2021. *Hygrophorus* sect. *Olivaceoumbrini*: new boundaries, extended biogeography and unexpected diversity unravelled by transatlantic studies. *Persoonia* 46: 272–312. <https://doi.org/10.3767/persoonia.2021.46.10>.  
Effectively published online: 2 July 2021 [Received: 28 October 2020; Accepted: 17 December 2020].

## INTRODUCTION

Typified by *Hygrophorus eburneus*, the genus *Hygrophorus* (waxcaps) was introduced in 1836 by Fries (Fl. Scan.: 339), and its inter- and infra-generic arrangements have been in flux ever since. The genus was initially organized into three informal subgeneric ‘tribes’, named *Limacium*, *Camarophyllus* and *Hygrocybe* by Fries (1838), which were later elevated to generic rank by Kummer (1871). In those early taxonomic treatments, the genus *Limacium* more or less corresponded to what is nowadays recognised as *Hygrophorus* s.str. However, not all authors were in agreement with this arrangement and Karsten

(1876), for one, recognised the genera *Hygrophorus*, *Camarophyllus* and *Hygrocybe* (as ‘*Hydrocybe*’) instead. Singer’s (1951) treatment of *Hygrophorus* laid the foundations for the contemporary interpretations of the genus, by recognising the taxonomic importance of bilateral lamellar trama. In its current understanding, *Hygrophorus* includes biotrophic species with a tricholomatoid or omphaloid aspect, thick and waxy, adnate to decurrent lamellae, divergent hymenial trama, narrowly elongated basidia with prominent sterigmata, and white spore deposits of hyaline, ellipsoid to lacrymoid and guttulate spores (Singer 1951, 1986, Hesler & Smith 1963, Kühner 1980, Arnolds 1990).

Approximately 100 species of *Hygrophorus* have been described to date, almost exclusively from temperate and boreal forests of the northern hemisphere (Candusso 1997, Kirk et al. 2008). Few taxa are known from Australia (Young & Wood 1997, Young 2005) and Central America (Halling & Mueller 2005), but the genus is mostly absent from the tropics and, traditionally, *Hygrophorus* species have been strongly associated with colder, and to a lesser extent Mediterranean, climates (Singer 1986). The genus has been taxonomically treated in a number of monographic works in the last century (Hesler & Smith 1963, Arnolds 1990, Bon 1990, Breitenbach & Kränzlin 1991, Courtecuisse & Duhem 1994, Candusso 1997), though none of these treatments had employed molecular phylogenetic analyses and mostly dealt with the genus on a regional or continental scale.

<sup>1</sup> CEFÉ, CNRS, Univ. Montpellier, EPHE, IRD, INSERM, 1919 route de Mende, F-34293 Montpellier Cedex 5, France. ORCID: 0000-0001-9289-2349; corresponding author e-mail: [jean-michel.bellanger@cefe.cnrs.fr](mailto:jean-michel.bellanger@cefe.cnrs.fr).

<sup>2</sup> Saint-Casimir, Québec, Canada.

<sup>3</sup> Department of Biology, Fatih Faculty of Education, Trabzon University, Trabzon, Turkey.

<sup>4</sup> P.O. Box 58499, Limassol 3734, Cyprus.

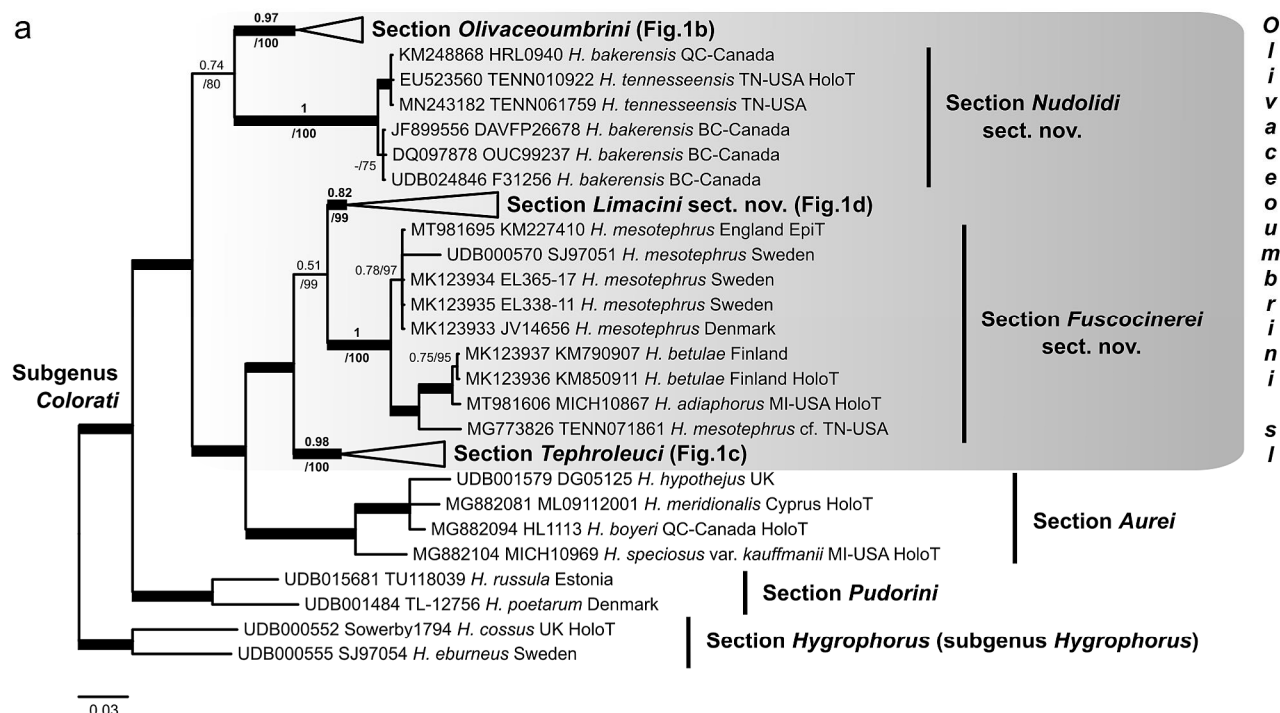
<sup>5</sup> Norris Center for Natural History, Environmental Studies Department, 1156 High Street, UC Santa Cruz, 95060, California, USA.

<sup>6</sup> Fac. Pharmacie de Lille, Univ. Lille, ULR 4515 - LGCgE, Laboratoire de Génie Civil et géo-Environnement, F-59000 Lille, France.

<sup>7</sup> Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, United Kingdom.

<sup>8</sup> Biological and Environmental Science, University of Gothenburg, Box 461, SE40530 Göteborg, Sweden.

<sup>9</sup> Gothenburg Global Biodiversity Centre, Box 461, SE40530 Göteborg, Sweden.



**Fig. 1** ITS phylogeny of *Hygrophorus* subgenus *Colorati*. Phylogenetic trees inferred from the combined Bayesian and Maximum likelihood analysis of 267 ITS sequences falling in sect. *Olivaceoumbrini* s.lat. plus, for rooting purposes, 8 sequences in sect. *Aurei*, *Pudorini* and *Hygrophorus*. Branches with strong statistical support (SH-aLRT > 0.8 and BPP ≥ 95 %) are highlighted as thick lines. a. Condensed phylogeny with sequences in sect. *Tephroleuci*, *Limacini* and *Olivaceoumbrini* s.str. collapsed; b. expanded sect. *Olivaceoumbrini* s.str.; c. expanded sect. *Tephroleuci*; d. expanded sect. *Limacini*. The short sequences (ITS2 only) of the isotype of *H. bakerensis* TENN014219, of the isotype of *H. agathosmus* f. *albus* LUG8454 and of collection ADP051014-1 fall, respectively, within the *I.H. bakerensis*, *I.H. agathosmoides* and *I.H. agathosmus* clades. Sequences from type material are highlighted in bold in panels b–d. Grey boxes highlight sect. *Olivaceoumbrini* s.lat. (Lodge et al. 2014) in panel a and the seven novel species described in the manuscript in panels b–d.

Macro-morphological characters of basidiomata have always been considered of critical importance in *Hygrophorus* systematics and species identification. Although colour can often vary due to the presence or absence of dark intracellular pigments and incrustations in the suprapellis and stipitipellis (Hesler & Smith 1963, Kovalenko 2012, Moreau et al. 2018), it is usually an important feature, as is the density of the lamellae and their attachment to the pileus. A number of species emanate strong and distinct odours, ranging from pleasant or aromatic in some species (bitter almonds, hyacinth, dried peaches), to disagreeable in others (raphanoid, mephitic, goat moth larvae). Several species may further display discolourations on the lamellae or the context upon drying, or marked reactions to chemical solutions (potassium hydroxide, ammonia, or iron sulfate) in various parts of the basidioma (Hesler & Smith 1963, Arnolds 1990). At the micro-anatomical level, the size and shape of the basidiospores is perhaps the most important feature, though the presence or absence of cystidioid elements, pigmentation, clamp connections and gleoplerous hyphae can sometimes provide valuable clues (Hesler & Smith 1963, Arnolds 1990, Candusso 1997, Larsson & Jacobsson 2004, Lodge et al. 2014, Moreau et al. 2018).

Species of *Hygrophorus* form ectomycorrhizal (EcM) associations with a wide spectrum of trees and shrubs, mostly members of the *Betulaceae*, *Corylaceae*, *Fagaceae*, *Pinaceae* and *Tiliaceae* (Gronbach & Agerer 1986, Hobbie & Agerer 2010, Tedersoo et al. 2010, Lodge et al. 2014). However, intercellular hyphae penetrating the root cortex cells of the host-tree have also been documented on some occasions (*H. olivaceoalbus*), suggesting that some species may form an aggressive type of mycorrhiza bordering on parasitism (Agerer 2012). Above-ground observations of co-occurring species, as in the case of *H. exiguus* and *Tricholoma inamoenum*, also suggest that cheating on other fungi might occur within the genus (Larsson et al. 2014). Host and substrate preferences are often highly

specific within different lineages, such as for instance in the yellowing species of sect. *Aurei* (Moreau et al. 2018), the foetid species of sect. *Hygrophorus* (Larsson & Jacobsson 2004), the species in sect. *Pudorini* (Jacobsson & Larsson 2007) or subsect. *Olivaceoumbrini* s.lat. (Larsson & Bendiksen 2020). In the Mediterranean region, a number of taxa appear to be broadly or strictly associated with shrubs of the *Cistaceae* family (Malençon & Bertault 1975, Lavorato 1991, Pérez-de-Gregorio et al. 2009, Loizides & Kyriakou 2011, Loizides 2016), though phylogenetic assessments from *Cistaceae* ecosystems are lacking and the taxonomic status of several of these species remains unclear.

At the turn of the millennium, the advent of molecular tools in mycology allowed for the delineation of phylogenetic boundaries defining a monophyletic *Hygrophorus* clade (Matheny et al. 2006, Larsson 2010). In an elaborate revision of *Hygrophoraceae* employing multigene phylogeny, morphology, ecology and pigment chemistry, Lodge et al. (2014) confirmed the monophyly of the genus, also recognising three evolutionarily supported subgenera in addition to multiple sections and subsections, not all of which were nonetheless monophyletic. Furthermore, several early-described binomials are yet to be phylogenetically positioned within the genus, resulting in ongoing taxonomic and nomenclatural instability. At the same time, several hotspots of biodiversity, particularly the Mediterranean basin and the Caucasus, remain largely unexplored, and a comprehensive revision of *Hygrophorus* integrating phylogenetic, morphological, biogeographical and ecological data on an intercontinental scale is still lacking (Lodge et al. 2014, Moreau et al. 2018).

Two recent studies (Larsson et al. 2018, Moreau et al. 2018) contributed to narrowing this gap, by revising sect. *Aurei* and subsect. *Tephroleuci* of subgenus *Colorati*. These studies unveiled cryptic diversity and taxonomic ambiguities in sect. *Olivaceoumbrini*, in particular within the species complexes of *H. agath-*

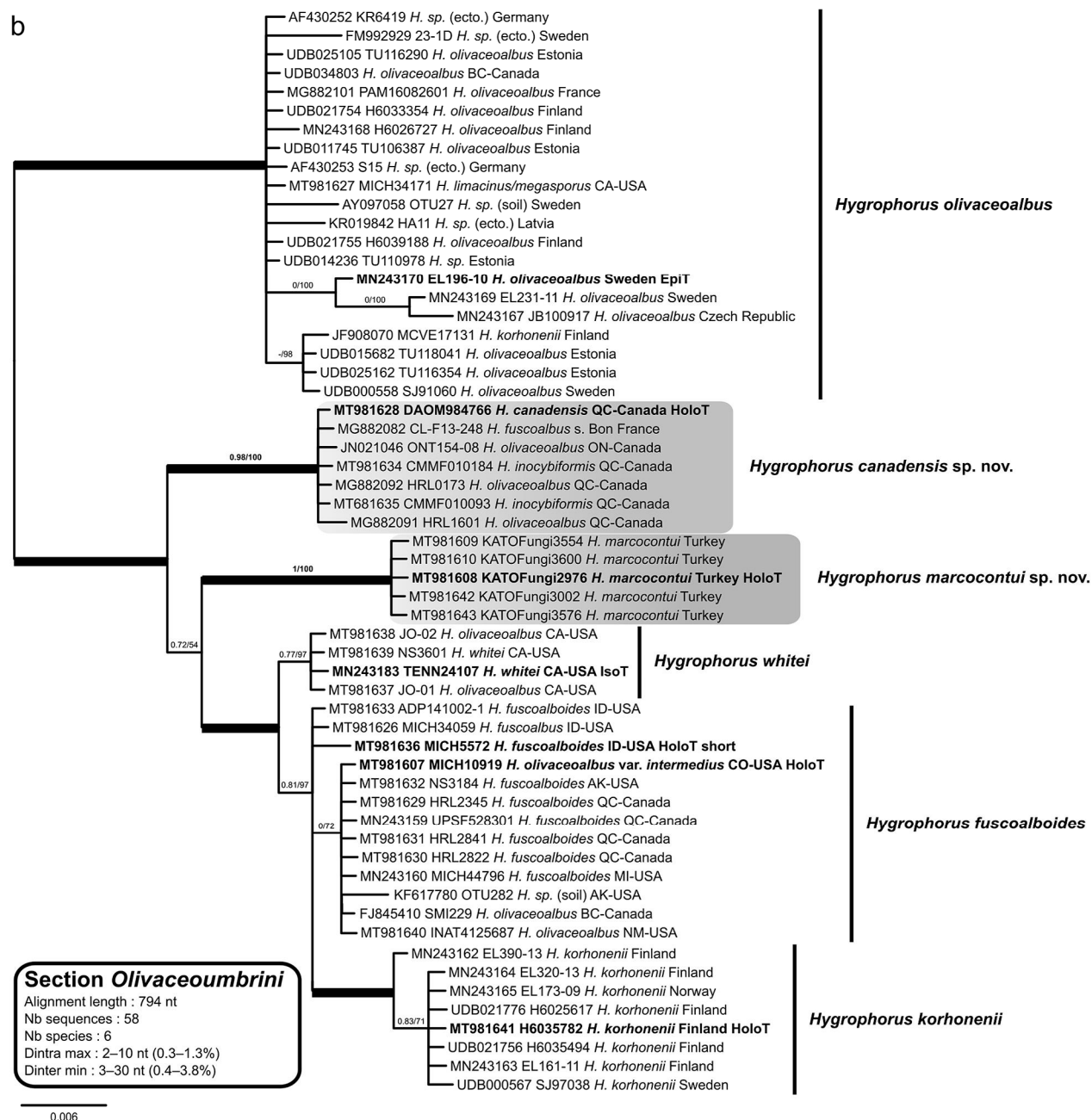


Fig. 1 (cont.)

*osmus* (subsect. *Tephroleuci*) and *H. olivaceoalbus* (subsect. *Olivaceoumbrini*). As currently defined, sect. *Olivaceoumbrini* is a polyphyletic assembly within subg. *Colorati* that includes a monophyletic subsect. *Tephroleuci* and, depending on taxon sampling and the loci analysed, a paraphyletic or polyphyletic subsect. *Olivaceoumbrini* (Lodge et al. 2014; Fig. 1a), resulting in a confusing and artificial taxonomy not accurately reflecting evolutionary history. At least two unnamed species have been detected in sect. *Olivaceoumbrini* by Moreau et al. (2018): i) a North American lineage misidentified as *H. olivaceoalbus*; and ii) a transatlantic lineage represented by two Canadian collections also assigned to *H. olivaceoalbus*, and French collections previously ascribed to *H. fuscoalbus* (Bon 1990), a highly controversial and variously interpreted taxon. Similarly, four distinct phylogenetic lineages were delineated by Larsson et al. (2018) among collections identified as *H. agathosmus*, a species previously believed to be easily recognised in the field by its strong scent of bitter almond. Although one of the newly detected lineages was described by these authors under the name *H. suaveolens*, and a neotype was designated for *H. aga-*

*thosmus*, the remaining two lineages detected by molecular analyses were left without formal binomials.

To better understand phylogenetic relationships and clarify species diversity within sect. *Olivaceoumbrini*, we analysed a large number of collections of intercontinental origin, encompassing the east and west coasts of North America, the continental, Atlantic and Mediterranean regions of Europe, and the Mediterranean and Black Sea regions of Turkey. Our primary goals were to:

- better define the evolutionary limits of sect. *Olivaceoumbrini* more accurately reflecting phylogenetic results;
- shed light on species diversity in the section and formally describe as yet unnamed phylogenetic lineages;
- elucidate species diversity in neglected hotspots of biodiversity, particularly the Mediterranean basin and Turkey;
- phylogenetically position in the section as many existing binomials as possible;
- taxonomically revise, on a phylogenetic basis, the limits of known species in the section; and
- stabilize the usage of old binomials by sequencing type material and designating lecto-, neo- and epitypes where needed.

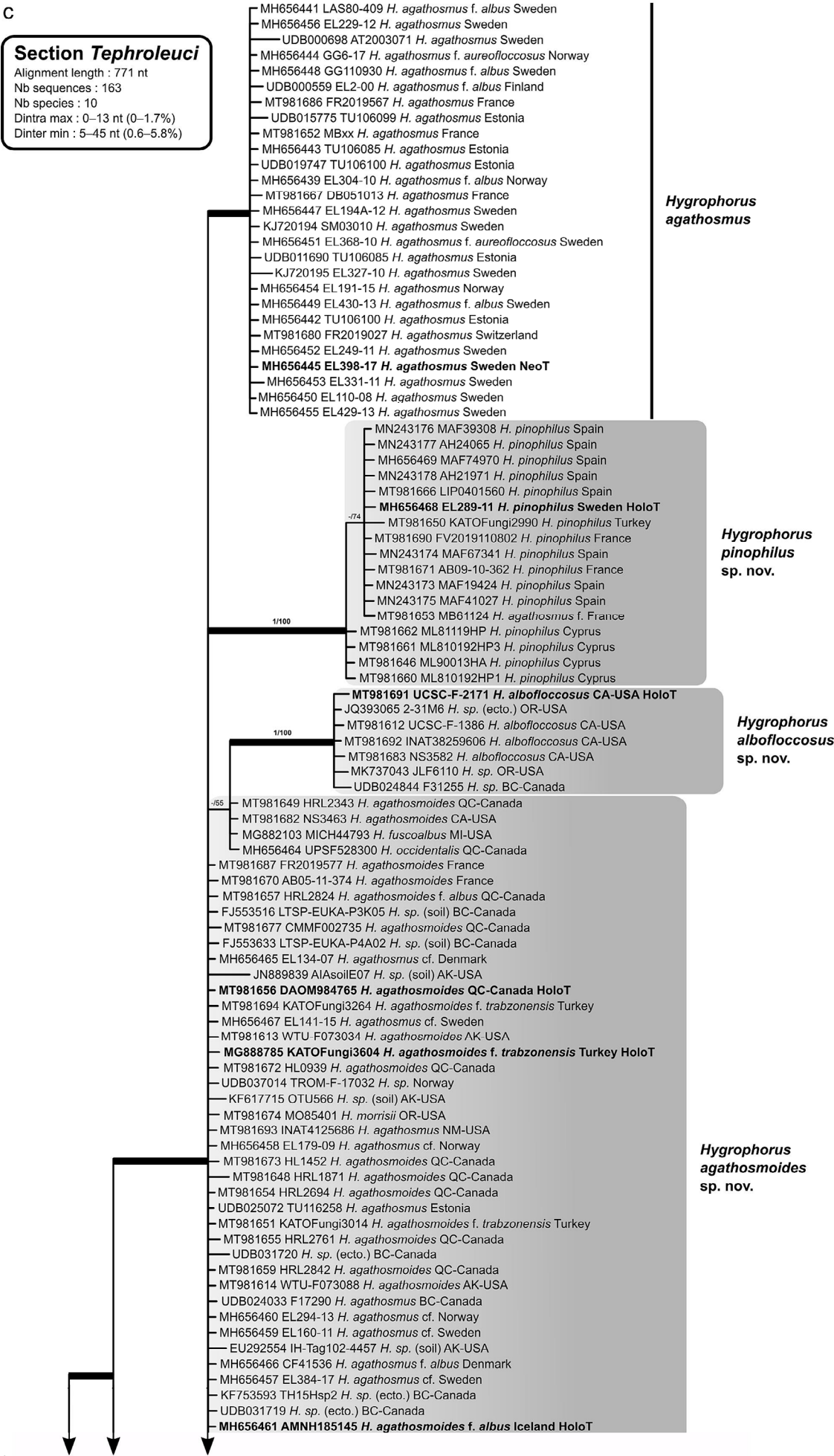


Fig. 1 (cont.)



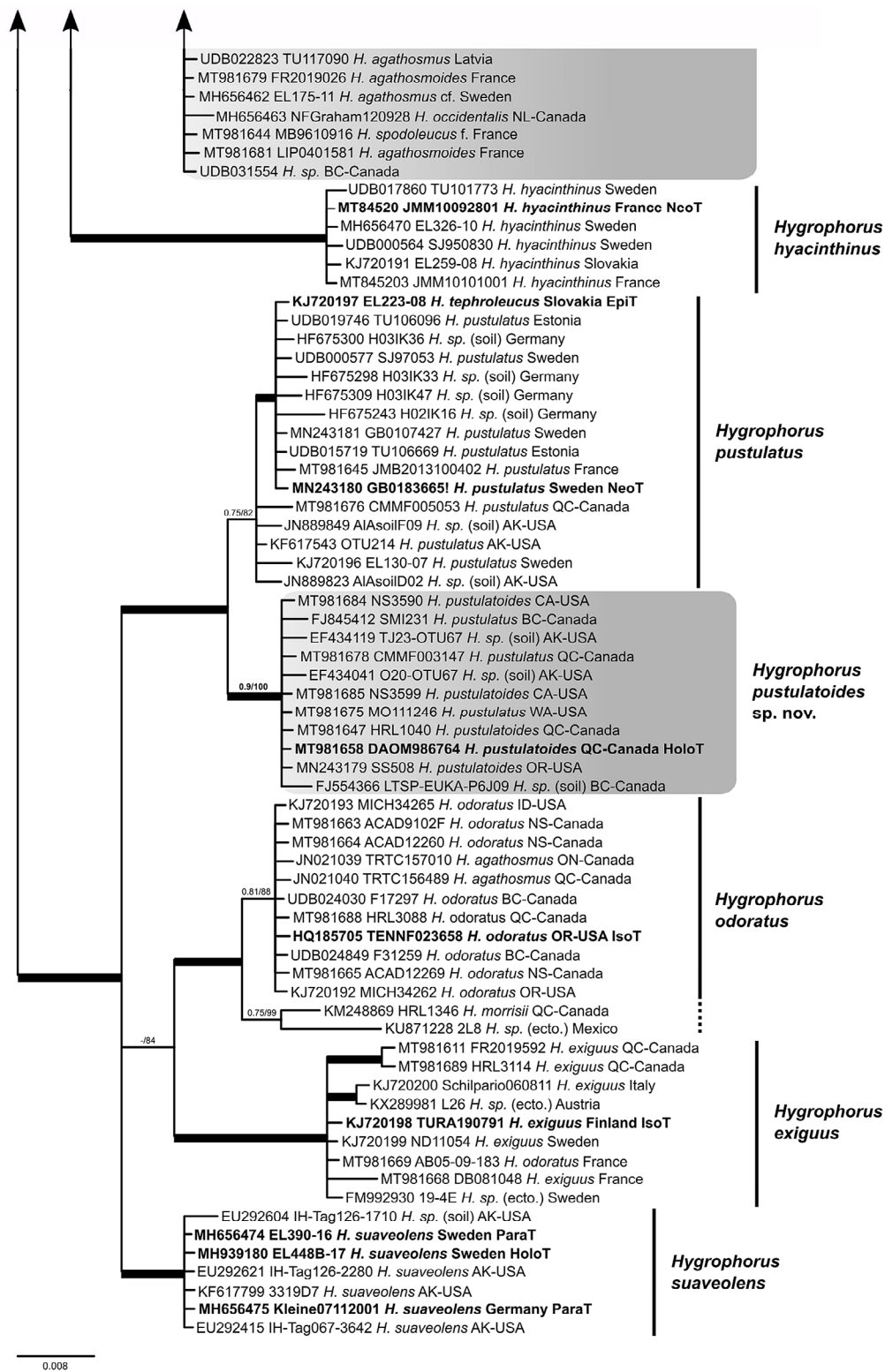


Fig. 1 (cont.)

The present work constitutes the first taxonomic revision of *Hygrophorus* sect. *Olivaceoumbriini* on a worldwide scale.

MATERIALS AND METHODS

Studied material

More than a hundred vouchers specimens from Canada, the Czech Republic, Cyprus, Finland, France, Greece, Norway, Slovakia, Spain, Sweden, Switzerland, Turkey and the USA were morphologically studied. Of these, 119 collections, including 7 *Typus* samples, were sequenced at the ITS locus (listed

in Table 1 and marked with an asterisk in the 'Other studied material' in the Taxonomy section). All collections are kept in the private herbaria of mycologists listed in the Taxonomy section and, when indicated, in official Herbaria abbreviated following Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>).

Morphological studies

Fresh basidiomata were photographed *in situ* and the habitat, altitude, soil characteristics and nearby trees and shrubs were noted. Detailed observations of macromorphological characters were made on fresh, dehydrated and rehydrated material, and compared to photographic material and field notes. Iron sulfate

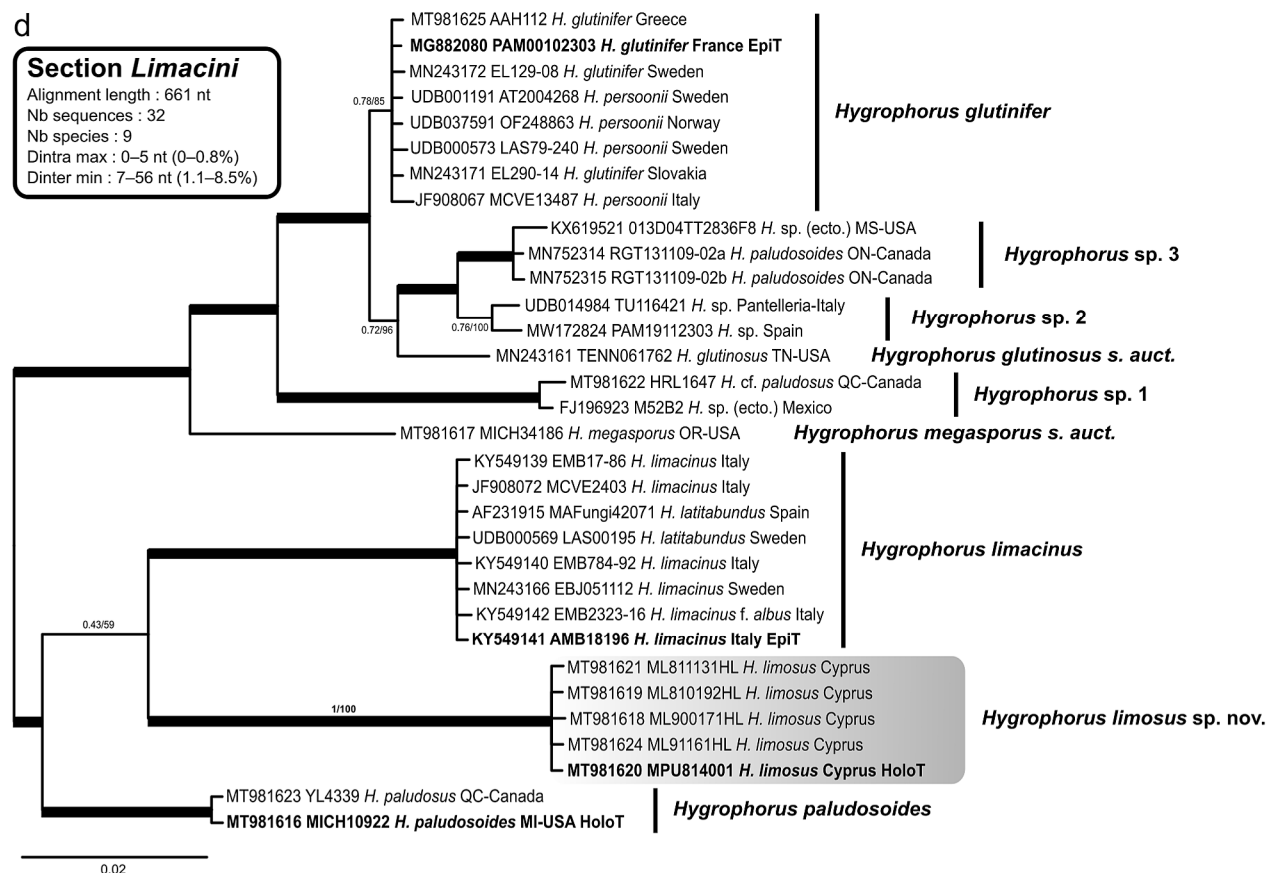


Fig. 1 (cont.)

(FeSO<sub>4</sub>), ammonia (NH<sub>4</sub>OH) and potassium hydroxide (KOH) were applied to test possible colour changes on the pileus, context and stipe of fresh basidiomata. Colours are described according to Kornerup & Wanscher (1978). Micro-anatomical studies were conducted on sections from the pileus, stipe and lamellae, rehydrated in 70 % ethanol or treated in concentrated ammonia solution and subsequently mounted in Congo red, 1 % SDS, or in 3–5 % KOH. Four optical microscopes were used: a Zeiss Axio Imager A2 trinocular research microscope, an AM trinocular Scope T360B, a Nikon Labophot and a Zeiss Axioskop 2 microscope. Basidiospores (hereafter referred to as spores), basidia, cystidioid elements and the other microstructures were measured with the aid of an optical micrometer or with Axio Imager software. Description of different cystidial forms follows Bas et al. (1988). However, the unexpectedly broad range of variability of these sterile cells among populations of some species and the difficulty to unambiguously assign them to previously defined terms such as cystidia, cystidioles, pseudocystidia or declinations of the latter, prompted us to collectively refer to these structures as ‘cystidioid elements’. Spore measurements were taken from natural deposits on the stipe surface obtained from fresh or dry basidiomata, mounted in water or in ammoniacal Congo red, or, if no naturally discharged spores were detected on the stipe, from dried fragments of lamellae. A minimum of 30 spores were measured from each basidioma and the Me (average length and width), Q (minimum and maximum length/width ratio) and Qm (average length/width ratio) were calculated. Spore measurements exclude apiculus. Basidial measurements exclude sterigmata, which are given separately.

#### DNA extraction, amplification and sequencing

DNA extraction and PCR amplification was conducted with the REExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis,

MO, USA) or DNeasy Plant Mini kit (Qiagen, Hilden, Germany), PCR reactions using Illustra PuReTaq™ ReadyToGo PCR beads (GE Healthcare, Buckinghamshire, United Kingdom), following the manufacturer’s instructions. The internal transcribed spacers and 5.8S of nuc rDNA (ITS barcode) were amplified from each collection, with primers ITS-1F, ITS-4b, ITS-4, ITS-2 and ITS-3 (White et al. 1990, Gardes & Bruns 1993), as described in Moreau et al. (2018). Amplicons were purified with QIAquick PCR purification kit (Qiagen, Hilden, Germany) or by Eurofins Genomics, Ebersberg, Germany, prior to sequencing. Raw sequence data were edited and assembled with Codon Code Aligner v. 4.1.1 (CodonCode Corp., Centerville, MA, USA) or Sequencher v. 5.1 (Gene Codes, Ann Arbor, Michigan), and deposited in GenBank under the accession numbers indicated in Table 1.

#### Phylogenetic analyses

For the present work, we assembled a dataset consisting of 268 ITS sequences, including 119 newly generated sequences and 149 published sequences (GenBank and UNITE) belonging in sect. *Olivaceoumbrini sensu* Lodge et al. (2014). Phylogenetic analyses were all performed online at [www.phylogeny.lirmm.fr](http://www.phylogeny.lirmm.fr) (Dereeper et al. 2008) and on the CIPRES Science Gateway ([www.phylo.org/index.php/](http://www.phylo.org/index.php/)). Multiple sequence alignment was carried out with MUSCLE v. 3.7 (Edgar 2004) and edited first manually to adjust some homologous indels, then with Gblocks v. 0.91b, set to lowest stringency in the selection of conserved blocks (Castresana 2000, Talavera & Castresana 2007). Maximum likelihood (ML) phylogenetic analyses were performed with PhyML v. 3.0 (Guindon et al. 2010), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric, Shimodaira-Hasegawa, version of the approximate likelihood-ratio test (SH-aLRT), implemented in the latest release of PhyML and which ensures high accuracy

Table 1 Sequenced material for the present work.

Species	Herbarium	Herb./Voucher Id.	Collector	Putative ECM host	Collection date	Country	State/Province	GenBank ITS
<i>Hygrophorus adiaaphorus</i> <i>H. agathosmoides</i> f. <i>agathosmoides</i>	MICH	10867 (holotype)	A.H. Smith	<i>Picea</i> , <i>Abies</i>	15 Oct. 1960	USA	Michigan	MT981606
	DAOM	984765/HLR2823 (holotype)	R. Lebeuf	<i>Picea</i>	10 Oct. 2018	Canada	Quebec	MT981656
	CMMF	002735	Y. Lamoureux	<i>Picea</i>	10 Oct. 1995	Canada	Quebec	MT981677
	Coll. pers.	HLR2761	R. Lebeuf	<i>Picea</i> , <i>Alnus</i> , <i>Salix</i>	13 Sept. 2018	Canada	Quebec	MT981655
	Coll. pers.	HL0939	H. Lambert	<i>Picea</i>	15 Oct. 2010	Canada	Quebec	MT981672
	Coll. pers.	HL1452	H. Lambert	<i>Picea</i>	16 Oct. 2015	Canada	Quebec	MT981673
	Coll. pers.	HLR2842	R. Lebeuf	<i>Picea</i> , <i>Abies</i>	17 Oct. 2018	Canada	Quebec	MT981659
	Coll. pers.	HLR2343	R. Lebeuf	<i>Picea</i>	19 Oct. 2016	Canada	Quebec	MT981649
	Coll. pers.	HLR2694	R. Lebeuf	<i>Alnus</i> , <i>Salix</i> , <i>Picea</i>	2 Sept. 2018	Canada	Quebec	MT981654
	Coll. pers.	HLR1871	R. Lebeuf	<i>Picea</i>	26 Sept. 2014	Canada	Quebec	MT981648
<i>H. agathosmoides</i> f. <i>albus</i> <i>H. agathosmoides</i> f. <i>trabzonensis</i>	LIP	MB9610916 (as <i>H. spodiocleucus forma</i> )	M. Bon	<i>Picea</i> , <i>Abies</i>	Oct. 1996	France	Isère	MT981644
	Coll. pers.	FR2019577	J.-M. Bellanger	Conifers	18 Oct. 2019	France	Lozère	MT981687
	LIP	0401581	J.-M. Coquelle	<i>Picea</i>	18 Nov. 2016	France	Nord	MT981681
	Coll. pers.	FR2019026	L. Deparis	<i>Picea</i>	23 Aug. 2000	France	Savoie	MT981679
	Coll. pers.	AB05-11-374	A. Bidaud	<i>Picea</i>	Nov. 2005	France	Haute-Savoie	MT981670
	WTU-F	073088	S. Trudell	<i>Betula</i> , <i>Picea</i>	8 Sept. 2009	USA	Alaska	MT981614
	WTU-F	073034	S. Trudell	<i>Picea</i>	30 Sept. 2013	USA	Alaska	MT981613
	Coll. pers.	NS3463	N. Siegel	<i>Sequoia</i> , <i>Picea</i> , <i>Tsuga</i>	18 Dec. 2018	USA	California	MT981682
	Coll. pers.	INAT4125686	C. Schwarz	NA	26 Aug. 2016	USA	New Mexico	MT981693
	Coll. pers.	MO85401 (as <i>H. morrisii</i> )	S. Krstic	<i>Picea</i>	29 Dec. 2011	USA	Oregon	MT981674
<i>H. agathosmus</i> <i>H. alborfoccosus</i>	Coll. pers.	ADP051014-1	A.D. Parker	<i>Picea</i>	14 Oct. 2005	USA	Washington	MT981615
	Coll. pers.	HLR2824	R. Lebeuf	<i>Picea</i>	10 Oct. 2018	Canada	Quebec	MT981657
	KATO Fungi	3014	E. Sesli	NA	15 Oct. 2010	Turkey	Trabzon	MT981651
	KATO Fungi	3264	E. Sesli	<i>Alnus</i>	27 Sept. 2013	Turkey	Trabzon	MT981694
	Coll. pers.	FR2019567	J.-M. Bellanger	<i>Picea</i>	13 Oct. 2019	France	Gard	MT981686
	Coll. pers.	DB051013	D. Borgarino	<i>Picea</i> , <i>Abies</i>	13 Oct. 2005	France	Isère	MT981667
	LIP	MBxx	M. Bon	<i>Abies</i>	1 Oct. 1986	France	Pas-de-Calais	MT981652
	Coll. pers.	FR2019027	L. Deparis	Mixed forest	5 Nov. 2000	Switzerland	Genève	MT981680
	LUG	8454 (isotype)	NA	<i>Picea</i>	NA	Italy	NA	MT981605
	UCSC-F	2171 (holotype)	C. Schwarz	<i>Pseudotsuga</i> , <i>Sequoia</i> , <i>Arbutus</i>	18 Jan. 2020	USA	California	MT981691
<i>H. canadensis</i> <i>H. exiguus</i>	UCSC-F	1386	C. Schwarz	NA	8 Jan. 2016	USA	California	MT981612
	Coll. pers.	NS3582/HLR2888	N. Siegel	<i>Pseudotsuga</i> , <i>Notholithocarpus</i> , <i>Arctostaphylos</i> , <i>Quercus</i>	20 Jan. 2019	USA	California	MT981683
	Coll. pers.	INAT38259606	C. Schwarz	<i>Pseudotsuga</i>	29 Jan. 2020	USA	California	MT981692
	DAOM	984766/HLR2344 (holotype)	R. Lebeuf	<i>Picea</i>	14 Oct. 2016	Canada	Quebec	MT981628
	CMMF	10093 (as <i>H. inocybiformis</i> )	J. Labrecque	<i>Abies</i>	23 Sept. 2007	Canada	Quebec	MT981635
	CMMF	10184 (as <i>H. inocybiformis</i> )	J. Labrecque	<i>Picea</i> , <i>Populus</i> , <i>Betula</i>	19 Oct. 2007	Canada	Quebec	MT981634
	Coll. pers.	HLR3114	R. Lebeuf	<i>Picea</i> , <i>Abies</i> , <i>Betula</i> , <i>Populus</i>	24 Sept. 2019	Canada	Quebec	MT981689
	Coll. pers.	FR2019592	R. Lebeuf	<i>Picea</i>	6 Oct. 2019	Canada	Quebec	MT981611
	Coll. pers.	AB05-09-183 (as <i>H. odoratus</i> )	F. Armada, A. Bidaud & J. Cavet	<i>Abies</i>	21 Sept. 2005	France	Isère	MT981669
	Coll. pers.	DB081048	D. Borgarino	<i>Picea</i> , <i>Abies</i>	Oct. 2008	France	Isère	MT981668
<i>H. fuscoalboides</i>	MICH	5572/AHS46726 (holotype)	A.H. Smith & Bigelow	NA	25 Aug. 1954	USA	Idaho	MT981636
	UPS-F	528301	J.W. Groves	NA	18 Sept. 1963	Canada	Quebec	MN243159
	Coll. pers.	HLR2345	R. Lebeuf	<i>Picea</i> , <i>Betula</i>	19 Oct. 2016	Canada	Quebec	MT981629
	Coll. pers.	HLR2822	R. Lebeuf	<i>Picea</i>	10 Oct. 2018	Canada	Quebec	MT981630
	Coll. pers.	HLR2841	R. Lebeuf	<i>Pinus</i> , <i>Abies</i>	17 Oct. 2018	Canada	Quebec	MT981631
	Coll. pers.	NS3184	N. Siegel	<i>Picea</i>	29 Aug. 2018	USA	Alaska	MT981632
	MICH	10919/AHS52370 (holotype of <i>H. olivaceoalbus</i> var. <i>intermedius</i> )	A.H. Smith	<i>Picea</i>	17 Aug. 1956	USA	Colorado	MT981607
	MICH	34059/AHS47140 (as <i>H. fuscoalbus</i> )	A.H. Smith	NA	31 Aug. 1954	USA	Idaho	MT981626
	Coll. pers.	ADP141002-1	A.D. Parker	<i>Thuja</i> , <i>Tsuga</i>	2 Oct. 2014	USA	Idaho	MT981633
	MICH	44796/Bartelli3291	I. Bartelli	<i>Pinus</i>	11 Oct. 1968	USA	Michigan	MN243160

Table 1 (cont.)

Species	Herbarium	Herb./Voucher Id.	Collector	Putative ECM host	Collection date	Country	State/Province	GenBank ITS
<i>H. fuscoalboides</i> (cont.) <i>H. glutinifer</i>	Coll. pers.	INAT4125687	C. Schwarz	NA	26 Aug. 2016	USA	New Mexico	MT981640
	Coll. pers.	AAH112	A. Antonis	<i>Fagus</i>	24 Nov. 2019	Greece	Thessaloniki	MT981625
	GB	EL290-14	E. Larsson	<i>Quercus</i>	23 Oct. 2014	Slovakia	Nova Bosaca	MN243171
	GB	EL129-08	E. Larsson	<i>Quercus</i>	11 Sept. 2008	Sweden	Närke	MN243172
<i>H. glutinosus</i> s. auct.	TENN-F	061762	E.B. Lickey	<i>Quercus, Tsuga</i>	19 Oct. 2006	USA	Tennessee	MN243161
<i>H. hyacinthinus</i>	LIP	0401691/JMM10092801 (neotype)	J.-M. Moingeon	<i>Picea, Abies</i>	28 Sept. 2010	France	Doubs	MT845204
	Coll. pers.	JMM10101001	J.-M. Moingeon	<i>Picea, Abies</i>	10 Oct. 2010	France	Doubs	MT845203
	H	6035782 (holotype)	M. Korhonen & T. Ulvinen	<i>Picea</i>	29 Aug. 1977	Finland	Koillismaa	MT981641
	GB	EL320-13	E. Larsson	<i>Picea</i>	31 Aug. 2013	Finland	Muonio	MN243164
<i>H. korhonenii</i>	GB	EL390-13	E. Larsson	<i>Picea</i>	6 Sept. 2013	Finland	Rovaniemi	MN243162
	GB	EL173-09	E. Larsson	<i>Picea</i>	5 Sept. 2009	Norway	Steinkjer	MN243165
	GB	EL161-11	E. Larsson	<i>Picea</i>	31 Aug. 2011	Sweden	Jokkmokk	MN243163
	GB	68803	E. Bohus Jensen	<i>Pinus</i>	12 Nov. 2005	Sweden	Gotland	MN243166
<i>H. limacinus</i>	MPU	814001/ML81113HL2 (holotype)	D. Markides	<i>Pinus</i>	3 Nov. 2018	Cyprus	Prodromos	MT981620
<i>H. limosus</i>	Coll. pers.	ML811131HL	M. Loizides	<i>Pinus</i>	13 Nov. 2018	Cyprus	Karvounas	MT981621
	Coll. pers.	ML91161HL	M. Loizides	<i>Quercus, Pinus</i>	16 Jan. 2019	Cyprus	Pera Pedi	MT981624
	Coll. pers.	ML900171HL	M. Loizides	<i>Pinus</i>	17 Oct. 2009	Cyprus	Troodos	MT981618
	Coll. pers.	ML810192HL	M. Loizides	<i>Pinus</i>	29 Oct. 2018	Cyprus	Troodos	MT981619
<i>H. marcocontui</i>	KATO Fungi	2976 (holotype)	E. Sesli	<i>Picea</i>	8 Oct. 2010	Turkey	Giresun	MT981608
	KATO Fungi	3002	E. Sesli	<i>Picea</i>	15 Oct. 2010	Turkey	Trabzon	MT981642
	KATO Fungi	3554	E. Sesli	<i>Picea</i>	18 Oct. 2015	Turkey	Trabzon	MT981609
	KATO Fungi	3576	E. Sesli	<i>Picea</i>	18 Oct. 2015	Turkey	Trabzon	MT981643
<i>H. megasporus</i> s. auct.	KATO Fungi	3600	E. Sesli	<i>Picea</i>	26 Oct. 2015	Turkey	Trabzon	MT981610
	MICH	34186	A.H. Smith	<i>Pinus</i>	24 Oct. 1946	USA	Oregon	MT981617
<i>H. mesotephrius</i>	K(M)	227410 (epitype)	L. Goodwin	<i>Fagus, Quercus</i>	10 Oct. 2015	England	South Hampshire	MT981695
<i>H. odoratus</i>	Coll. pers.	HRL3088	R. Lebeuf	<i>Pinus</i>	24 Sept. 2019	Canada	Quebec	MT981688
	ACAD	9102F	K.A. Harrison	<i>Conifers</i>	11 Oct. 1968	Canada	Nova Scotia	MT981663
	ACAD	12260	K.A. Harrison	<i>Conifers</i>	14 Oct. 1968	Canada	Nova Scotia	MT981664
	ACAD	12269	K.A. Harrison	<i>Conifers</i>	14 Oct. 1968	Canada	Nova Scotia	MT981665
<i>H. olivaceoalbus</i>	GB	183666/EL196-10 (epitype)	E. Larsson	<i>Picea</i>	26 Aug. 2010	Sweden	Jämtland	MN243170
	GB	JB100917	J. Borovička	<i>Picea</i>	17 Sept. 2010	Czech Republic	North Bohemia	MN243167
	H	6026727	U. Salo & P. Salo	<i>Picea</i>	1 Oct. 2011	Finland	Uusimaa	MN243168
	GB	EL231-11	E. Larsson	<i>Picea</i>	13 Sept. 2011	Sweden	Västra Götaland	MN243169
<i>H. paludosoides</i>	MICH	34171 (as <i>H. limacinus/megasporus</i> )	A.H. Smith	NA	9 Dec. 1956	USA	California	MT981627
	MICH	10922 (holotype)	A.H. Smith	<i>Fagus</i>	16 Sept. 1961	USA	Michigan	MT981616
	Coll. pers.	YL4339 (as <i>H. paludosus</i> )	Y. Lamoureux	<i>Fagus, Populus</i>	1 Oct. 2015	Canada	Quebec	MT981623
	Coll. pers.	ML81119HP	M. Loizides	<i>Pinus</i>	9 Nov. 2018	Cyprus	Trooditissa	MT981662
<i>H. pinophilus</i>	Coll. pers.	ML90013HA	M. Loizides	<i>Pinus</i>	3 Oct. 2009	Cyprus	Troodos	MT981646
	Coll. pers.	ML810192HP1	M. Loizides	<i>Pinus</i>	29 Oct. 2018	Cyprus	Troodos	MT981660
	Coll. pers.	ML810192HP3	M. Loizides	<i>Pinus</i>	29 Oct. 2018	Cyprus	Troodos	MT981661
	Coll. pers.	AB09-10-362	A. Bidaud	<i>Pinus</i>	Oct. 2009	France	Drôme	MT981671
<i>H. paludosoides</i>	Coll. pers.	FV2019110802	F. Valade	<i>Pinus</i>	8 Oct. 2019	France	Essonne	MT981690
	LIP	MB61124 (as <i>H. agathosmus forma</i> )	M. Bon	<i>Quercus, Pinus</i>	Nov. 1966	France	Vendée	MT981653
	MA	F19424	J.A. Oria de Rueda	<i>Pinus</i>	1 Jan. 1987	Spain	Almería	MN243173
	MA	F41027	L. Romero de la Osa	<i>Pinus</i>	16 Dec. 1995	Spain	Andalusia	MN243175
<i>H. pinophilus</i>	LIP	0401560/AGI18121501	A. Gasch Illescas & P.-A. Moreau	<i>Pinus</i>	15 Dec. 2018	Spain	Andalusia	MT981666
	MA	F67341	F.D. Calonge	<i>Pinus</i>	15 Nov. 1983	Spain	Avila	MN243174
	MA	F39308	J. Rodriguez	<i>Pinus</i>	17 Dec. 1997	Spain	Galicia	MN243176
	AH	21971	C. Illiana & A. Altés	<i>Pinus, Arctostaphylos</i>	19 Nov. 1997	Spain	Madrid	MN243178
<i>H. pinophilus</i>	AH	24065	C. Illiana & A. Altés	<i>Pinus, Arctostaphylos</i>	3 Dec. 1997	Spain	Madrid	MN243177
	KATO Fungi	2990	E. Sesli	<i>Picea</i>	8 Oct. 2010	Turkey	Giresun	MT981650



Table 1 (cont.)

Species	Herbarium	Herb./Voucher Id.	Collector	Putative ECM host	Collection date	Country	State/Province	GenBank ITS
<i>H. pustulatus</i>	DAOM	984764/HRL2832 (holotype)	R. Lebeuf	<i>Picea</i>	15 Oct. 2018	Canada	Quebec	MT981658
	CMMF	003147 (as <i>H. pustulatus</i> )	Y. Lamoureux	<i>Picea</i>	25 Sept. 1997	Canada	Quebec	MT981678
	Coll. pers.	HRL1040	R. Lebeuf	<i>Picea</i>	24 Oct. 2011	Canada	Quebec	MT981647
	Coll. pers.	NS3590	N. Siegel	<i>Picea</i>	27 Jan. 2019	USA	California	MT981684
	Coll. pers.	NS3599/HRL2890	N. Siegel	<i>Picea</i>	28 Jan. 2019	USA	California	MT981685
	GB	SS508	S. Svantesson	<i>Alnus, Pinus, Tsuga</i>	1 Oct. 2018	USA	Oregon	MN243179
<i>H. pustulatus</i>	Coll. pers.	MO11246 (as <i>H. pustulatus</i> )	A.D. Parker	<i>Picea, Abies</i>	23 Sept. 2012	USA	Washington	MT981675
	GB	0183665/EL185-14 (neotype)	E. Larsson	<i>Picea</i>	21 Sept. 2014	Sweden	Västergötland	MN243180
	CMMF	005053	R. Boyer	<i>Abies, Picea</i>	3 Oct. 2001	Canada	Quebec	MT981676
	Coll. pers.	JMB2013100402	J.-M. Bellanger	<i>Picea</i>	04 Oct. 2013	France	Ardeche	MT981645
	GB	0107427	A. Aronsson	<i>Picea, Abies</i>	2011	Sweden	Västergötland	MN243181
<i>H. sp. 1</i>	Coll. pers.	HRL1647 (as <i>H. cf. paludosus</i> )	A. Paul	<i>Quercus, Carya</i>	11 Oct. 2013	Canada	Quebec	MT981622
<i>H. sp. 2</i>	Coll. pers.	PAM2019112303	A. Gasch Illescas & P.-A. Moreau	<i>Quercus</i>	23 Nov. 2019	Spain	Andalusia	MW172824
<i>H. tennesseensis</i>	TENN-F	061759	E.B. Lickey & R. Parker	Mixed hardwoods, <i>Tsuga, Pinus</i>	18 Oct. 2006	USA	Tennessee	MN243182
<i>H. whitei</i>	TENN-F	024107 (isotype)	A.H. Smith & White	<i>Picea</i>	15 Dec. 1956	USA	California	MN243183
	Coll. pers.	JO-02 (as <i>H. olivaceoalbus</i> )	J. Olson	Conifers ( <i>Picea, Abies, Tsuga, Sequoia</i> )	6 Jan. 2019	USA	California	MT981638
	Coll. pers.	JO-01 (as <i>H. olivaceoalbus</i> )/HRL2892	J. Olson	Conifers ( <i>Picea, Abies, Tsuga, Sequoia</i> )	13 Jan. 2019	USA	California	MT981637
	Coll. pers.	NS3601	N. Siegel	<i>Picea</i>	28 Jan. 2019	USA	California	MT981639

Coll. pers.: private herbarium of collector; NA: Not available

when SH-aLRT > 0.8 (Anisimova et al. 2011, Bellanger et al. 2015). Bayesian inference (BI) of phylogeny was performed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Two runs of four Monte Carlo Markov Chains each were performed for 1 000 000 generations, with stationarity convergence estimated by the Potential Scale Reduction Factor = 1 (Gelman & Rubin 1992). Tree and parameters were sampled every 1 000 generations (1 000 trees). The initial burn-in was set to 25 % (250 trees). Branch support was assessed by Bayesian posterior probabilities (BPP). Trees were built using FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited with Inkscape 0.91 (<https://inkscape.org/fr/>).

RESULTS

The phylogenetic analysis of our ITS dataset resolves 30 clades in sect. *Olivaceoumbrini* s.lat., distributed in 5 supported lineages (Fig. 1a; Table 2). The latter do not constitute a monophyletic group, but rather display paraphyletic or polyphyletic relationships to each other, confirming the artificial nature of the section as previously delineated by Lodge et al. (2014). In order to provide an evolutionarily-based scaffold for the infrageneric systematics of *Hygrophorus*, we propose here to restrict sect. *Olivaceoumbrini* to its core monophyletic clade and to assign section rank to each of the four other lineages (see Taxonomy).

In its narrow boundaries delineated here, sect. *Olivaceoumbrini* is a strongly supported monophyletic lineage (0.97/100 % branch support, as SH-aLRT/BPP, respectively and hereafter) that includes sequences of the type species *H. olivaceoalbus* and 5 additional morphologically very similar species (Fig. 1b)<sup>1</sup>. The second lineage (0.98/100 % support) of sect. *Olivaceoumbrini* s.lat. overlaps with the current subsect. *Tephroleuci* and includes 10 supported clades interpreted as species (Fig. 1c). The third lineage (0.82/99 % support) encompasses species akin to the well-known European species *H. limacinus* (= *H. latitabundus*) and *H. glutinifer* (= *H. persoonii*, see below), traditionally integrated within subsect. *Olivaceoumbrini*, as well as several North American binomials so far of unknown phylogenetic position, representing in total 9 supported species (Fig. 1d). We here introduce sect. *Limacini* sect. nov. to accommodate the latter, a taxonomic arrangement which further contributes to rendering sect. *Olivaceoumbrini* monophyletic (Taxonomy). The fourth lineage (1/100 % support) so far includes *H. mesotephrosus*, *H. adiaphorus* (incl. *H. betulae*, see below), and a third species represented by a single sequence from Tennessee (USA); we here emend and validate as sect. *Fuscocinerei* sect. nov. the old Friesian group '*Fuscocinerei l. lividi*' for this clade (Fig. 1a; Taxonomy). The last lineage (1/100 % support) includes sequences of *H. tennesseensis* and *H. bakerensis*, and is here formally introduced as sect. *Nudolidi* sect. nov. (Taxonomy).

Twenty-seven out of 30 (90 %) of these clades are currently represented by two or more sequences (Fig. 1; Table 2). Of these, only 6 (22 %) appear to naturally occur on both the North American and European continents, in concordance with the results of previous studies indicating considerable continental endemism among species of the genus. The dominant putative host trees of species in the emended section are *Pinus* and *Picea* spp., and the few clades encompassing species associated with broadleaved trees all belong to sect. *Limacini* and *Fuscocinerei* (Table 1).

Among the identified phylopecies, 10 lack Latin binomials that can be unambiguously assigned to them and thus represent species putatively new to science. Seven of these novelties are

<sup>1</sup> While this study was in press, Wang et al. (Mycoscience 2021) reported on a seventh species in sect. *Olivaceoumbrini*, *Hygrophorus annulatus*, so far restricted to China.

**Table 2** Phylogenetic overview of sect. *Olivaceoumbrini* s.lat. (Lodge et al. 2014).

Section (alignment length)	Species	Nb sequences	Support (ML/BI)	D <sub>intra</sub> max <sup>a</sup>	D <sub>inter</sub> min <sup>b</sup>	Closest neighbour
<i>Fuscocinerei</i> (632 nt)	<i>H. adiaphorus</i>	3	0.98/100	1	31	<i>H. cf. mesotephurus</i>
	<i>H. mesotephurus</i> <sup>c</sup>	5 (4)	0.78/97	14 (1)	34	<i>H. adiaphorus</i>
	<i>H. cf. mesotephurus</i>	1	na	na	31	<i>H. adiaphorus</i>
<i>Limacini</i> (661 nt)	<i>H. glutinifer</i>	8	0.78/85	1	8	<i>H. glutinosus</i> s. auct.
	<i>H. glutinosus</i> s. auct.	1	na	na	8	<i>H. glutinifer</i>
	<i>H. limacinus</i>	8	0.99/100	0	42	<i>H. paludosoides</i>
	<i>H. limosus</i>	5	1/100	2	56	<i>H. limacinus</i>
	<i>H. megasporus</i> s. auct.	1	na	na	36	<i>H. glutinifer</i>
	<i>H. paludosoides</i>	2	0.98/100	2	49	<i>H. glutinifer</i>
	<i>H. sp. 1</i>	2	1/100	5	32	<i>H. glutinifer</i>
	<i>H. sp. 2</i>	2	0.76/100	2	7	<i>H. sp. 3</i>
	<i>H. sp. 3</i>	3	0.89/100	2	8	<i>H. sp. 2</i>
<i>Nudolidi</i> (661 nt)	<i>H. bakerensis</i>	4	–/76	0	14	<i>H. tennesseensis</i>
	<i>H. tennesseensis</i>	3	0.98/100	2	14	<i>H. bakerensis</i>
<i>Olivaceoumbrini</i> (794 nt)	<i>H. canadensis</i>	7	0.98/100	3	17	<i>H. whitei</i>
	<i>H. fuscoalboides</i>	13	–/–	3	3	<i>H. whitei</i>
	<i>H. korhonenii</i>	8	0.95/100	4	6	<i>H. fuscoalboides</i>
	<i>H. marcocontui</i>	5	1/100	2	20	<i>H. whitei</i>
	<i>H. olivaceoalbus</i>	21	1/100	10	30	<i>H. whitei</i>
	<i>H. whitei</i>	4	0.77/97	3	3	<i>H. fuscoalboides</i>
<i>Tephroleuci</i> (771 nt)	<i>H. agathosmoides</i>	49	–/–	7	6	<i>H. agathosmus</i>
	<i>H. agathosmus</i>	28	0.94/99	3	6	<i>H. agathosmoides</i>
	<i>H. albofloccosus</i>	7	1/100	1	13	<i>H. agathosmoides</i>
	<i>H. exiguus</i>	9	0.99/100	13	26	<i>H. suaveolens</i>
	<i>H. hyacinthinus</i>	6	1/100	0	45	<i>H. agathosmoides</i>
	<i>H. odoratus</i> <sup>d</sup>	11 (13)	0.81 (0.85)/88 (100)	3 (13)	20	<i>H. suaveolens</i>
	<i>H. pinophilus</i>	17	1/100	1	22	<i>H. agathosmoides</i>
	<i>H. pustulatoides</i>	11	0.9/100	4	5	<i>H. pustulatus</i>
	<i>H. pustulatus</i>	16	0.75/82	6	5	<i>H. pustulatoides</i>
	<i>H. suaveolens</i>	7	0.92/100	6	17	<i>H. pustulatus</i>

<sup>a</sup> Maximal number of nucleotide differences between sequences within each clade, excluding those occurring in the first and last 30 positions, but including those occurring at heterozygotic sites. PolyN indels are counted as unique events.

<sup>b</sup> Minimal number of nucleotide differences between sequences belonging in distinct clades, excluding those occurring in the first and last 30 positions and those occurring at heterozygotic sites. PolyN indels are counted as unique events.

<sup>c</sup> The number in parentheses correspond to the clade excluding sequence UDB000570, which displays signs of poor quality.

<sup>d</sup> The numbers in parentheses correspond to the most inclusive clade (dotted line in Fig. 1c, see text for details).

na not applicable (only one sequence in the clade).

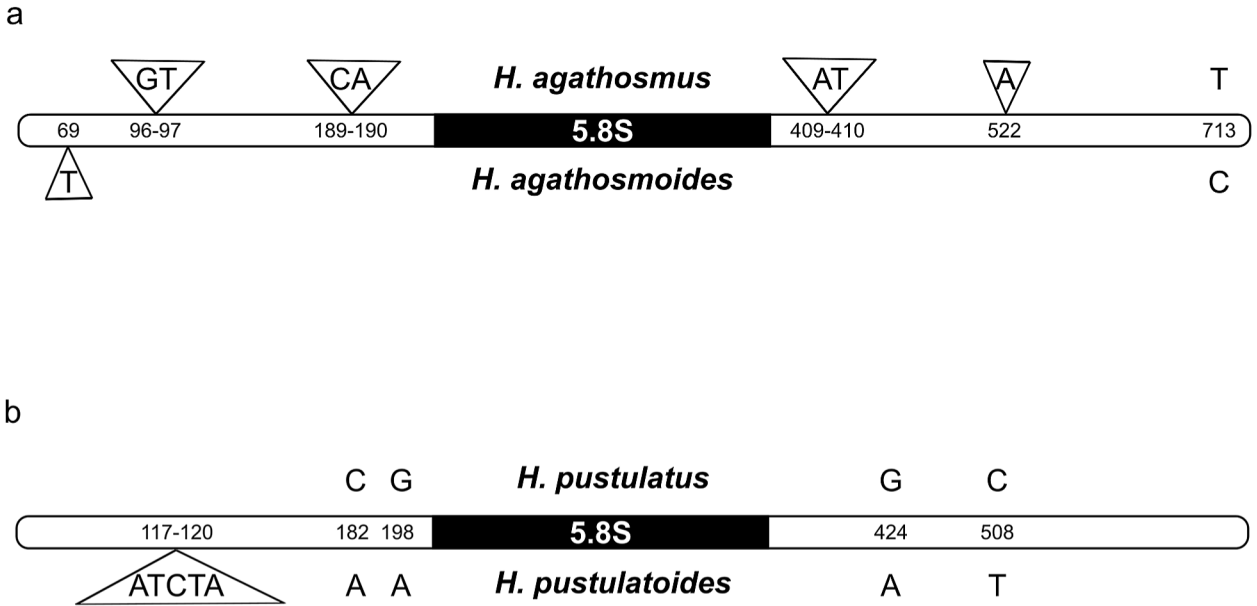
formally introduced below (Taxonomy) as *H. agathosmoides*, *H. albofloccosus*, *H. canadensis*, *H. limosus*, *H. marcocontui*, *H. pinophilus* and *H. pustulatoides* spp. nov., while the remaining 3 require more sampling to be formally described, and are here provisionally referred to as *H. sp. 1–3*. The biogeographical distribution of these new species is predominantly concentrated in the Mediterranean and Black Sea ecoregions, since four of them are either broadly represented in ( $n = 2$ ), or restricted to ( $n = 2$ ) southern European countries and Turkey.

*Hygrophorus limosus*, so far known mostly from high elevation *Pinus nigra* subsp. *pallasiana* forests on the island of Cyprus, is morphologically similar to *H. limacinus* and phylogenetically resolves as its sister species despite the high (8.5 %, Table 2) nucleotide divergence at the ITS locus. *Hygrophorus marcocontui* and *H. canadensis* are part of the *H. olivaceoalbus* complex and are associated with, respectively, Turkish *Picea orientalis* forests and *Picea* spp. trees of eastern North American origin. The latter species has been previously referred to as ‘*H. fuscoalbus*’ by Bon (Bon 1990, Moreau et al. 2018). *Hygrophorus agathosmoides*, *H. albofloccosus* and *H. pinophilus* all belong to the *H. agathosmus* complex and emit a typical bitter-almond odour, much like *H. agathosmus* s.str. The former seems so far to be endemic to the west coast of North America, where it may be strictly associated with Douglas fir (*Pseudotsuga menziesii*), whereas the other two, previously referred to as ‘*Hygrophorus* sp.’ and ‘*H. aff. agathosmus*’ in Larsson et al. (2018), display much broader ecological ranges: xerophilic European pine forests for *H. pinophilus*, and spruce forests of Europe, North America and Turkey for *H. agathosmoides*. As previously reported (Larsson et al. 2018), *H. agathosmoides* and *H. agathosmus*

do not display reciprocal monophyly in probabilistic methods of phylogenetic inference (Fig. 1c). However, a close inspection of polymorphism distribution within this lineage in our enriched dataset indicates that the two species diverge from each other by one substitution and five indels (1 to 2 nucleotide-long) at the ITS locus, with no evidence of gene flow between the two genotypes, supporting their evolutionary autonomy (Fig. 2a; Suppl. Table 1). Three distinct forms of *H. agathosmoides* are further delineated and formally recognised, with f. *trabzonensis* displaying remarkable morpho-anatomical and ecological differences from f. *agathosmoides* (Taxonomy). Crypticism within the *H. pustulatus* lineage is also here unveiled and as a result, *H. pustulatoides*, so far restricted to North American spruce (*Picea* spp.) forests, is introduced as the sister species of the transcontinental *H. pustulatus*. The two species segregate by four single nucleotide polymorphisms and one indel on ITS (Fig. 2b; Suppl. Table 2).

Besides these novelties, our wide taxon sampling expands current knowledge of the natural distribution range of several species, incl. *H. agathosmus* s.str., previously restricted to Northern Europe and Russia (Larsson et al. 2018), but here also confirmed in France and Switzerland in sympatry with *H. agathosmoides*, and *H. exiguus*, confirmed in France and Quebec (Canada) for the first time (Fig. 1c).

Finally, our analyses phylogenetically confirm the placement of *H. fuscoalboides*, *H. korhonenii*, *H. whitei* and *H. olivaceoalbus* var. *intermedius* (as a heterotypic synonym of *H. fuscoalboides*) in the emended sect. *Olivaceoumbrini*, and reveals that *H. paludosoides* and *H. adiaphorus* are part of, respectively, sect. *Limacini* and sect. *Fuscocinerei* (Fig. 1b, d, a, respectively). The



**Fig. 2** Fixed ITS polymorphisms within the *H. agathosmus* and *H. pustulatus* complexes. Nature and position of fixed single nucleotide polymorphisms and indels between: a. *H. agathosmoides* and *H. agathosmus*; and b. *H. pustulatoides* and *H. pustulatus*. Inserted nucleotide(s) are indicated in triangles above or below the ITS locus, according to the relevant species in the pair. See Supplementary Table 1 and 2 for a more detailed distribution of polymorphisms within these two complexes.

latter species, so far known from a single locality and coniferous forest in Michigan (USA), is here revealed to be ITS-identical to *H. betulae* (one indel of difference, Table 2), a rare species recently described from a subalpine birch forest in Northern Finland (Larsson & Bendiksen 2020; Fig. 1a).

In order to build the present work on solid nomenclatural grounds, we also stabilise the usage of six historical names by designating three lectotypes, two sequenced neotypes and four sequenced epitypes for *H. glutinifer*, *H. hyacinthinus*, *H. mesotephros*, *H. olivaceoalbus*, *H. pustulatus* and *H. tephroleucus*, respectively (Nomenclature).

## NOMENCLATURE

***Hygrophorus glutinifer* Fr., Epicr. Syst. Mycol. (Upsaliae): 322. 1838 — MycoBank MB 169607; Fig. 3a–b, 15b**

**Basionym.** *Agaricus glutinosus* Bull., Herb. France 6: t. 258. 1786, nom. illeg. (non *A. glutinosus* Schaeff., 1774).

**Obligate synonyms.** *Agaricus albobrunneus* f. *glutinosus* Fr., Syst. Mycol. 1: 37. 1821, nom. sanct.

**Synonyms.** *Hygrophorus dichrous* Kühner & Romagn., Flore Analytique des Champignons Supérieurs: 60. 1953, nom. inval. (art. 41.5 and 41.6); *Hygrophorus persoonii* Arnolds, Persoonia 10 (3): 365. 1979.

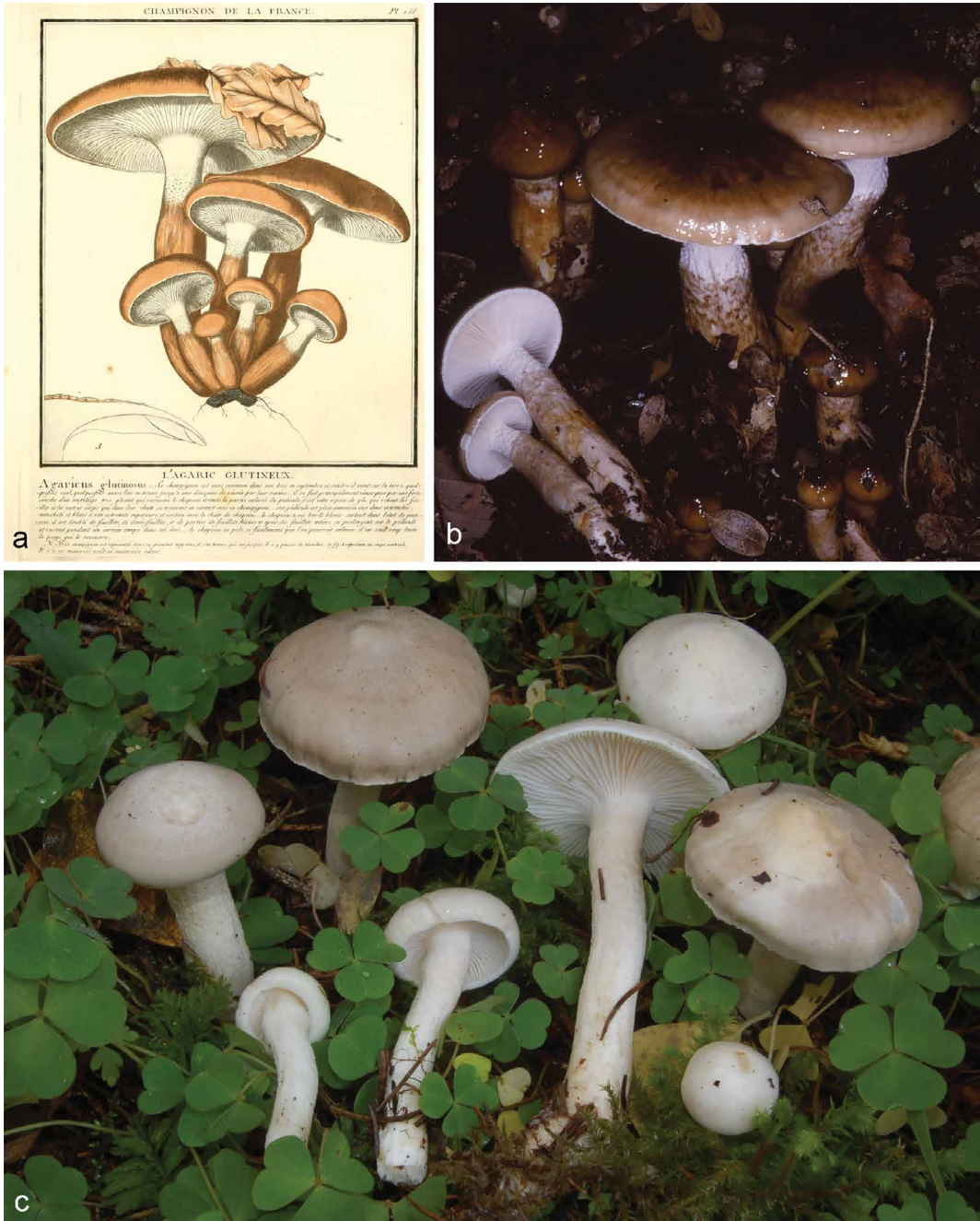
**Lectotype, here designated.** Bulliard, J.-B. P. (1786) *Herbier de la France*, vol. 6: t. 258 (as *Agaricus glutinosus*), MycoBank MBT 10001729.

**Epitype, here designated.** FRANCE, Savoie, Saint-Jean-de-Chevelu, in a *Quercus pubescens* woodland with *Buxus sempervirens* on calcareous soil, 23 Oct. 2000, P.-A. Moreau, LIP0401690\*, MycoBank MBT 10001730, GenBank ITS MG882080. In support of the lectotype designated above.

**Notes** (in collaboration with C. Papetti) — When describing *H. glutinifer*, Fries (1838) explicitly referred to two plates from Bulliard, pl. 258 (1786) and pl. 539 B (1792), both displayed under the name *Agaricus glutinosus*. Because the latter binomial had two older homonyms (*A. glutinosus* Schaeff. 1774, now *Gomphidius glutinosus* (Schaeff.) Fr., and *A. glutinosus* Curtis 1777, illeg., a synonym of *Protostropharia semiglobata* (Batsch) Redhead et al.), a nomenclatural conflict that would make Bulliard's name illegitimate according to the current Code of nomenclature (Turland et al. 2018, art. 53.1), Fries replaced the epithet *glutinosus* by *glutinifer*, in order to avoid any ambi-

guity in the future usage of his new name. While the description alone is not precise enough to diagnose with certainty what we used to name *H. persoonii* nowadays, combining it with the plate 258 from Bulliard leaves no doubt about the identity of this species, especially when considering the short accompanying text (in French), highlighting its main distinguishing features. Because Fries maintained *H. glutinifer* in the 'Hymenomycetes Europaei' (1874) he probably recognised the species as distinct once he compared it to its closest lookalikes. As evidenced by Gillet (1874), and by material found by us in PC (*Herbier Général*, coll. J.-B. Desmazières, '1863 n° 8' as *Agaricus glutinifer*, data not shown), the name was in use in the second half of the 19th century and applied to the species described here. However, the binomial disappeared from the literature, likely under the influence of Quélet (1888) who misapplied the name *H. olivaceoalbus* to this species, and Bataille (1910) who reported it as *H. limacinus*. *Hygrophorus glutinifer* seems to have been overlooked by Kühner & Romagnesi (1953) and Arnolds (1979).

From a morphological point of view, the species is intermediate between *H. limacinus* and *H. olivaceoalbus*, which consequently resulted in numerous misinterpretations by past authors (see Papetti 2016 for details). For instance, Ricken (1915) misinterpreted this taxon as the species now called *H. abieticola* Gröger & Bresinsky. The most reliable criterion to separate *H. glutinifer* from its lookalikes has been unveiled by Kühner & Romagnesi (1953), when they noticed the distinctive greenish blue reaction of the mucus on pileus and stipe to ammonia (unnoticed by Bataille) of their newly introduced taxon *H. dichrous*. This peculiar chemistry can also be observed under the microscope, with the coloration of hyphal extracellular deposits in KOH or NH<sub>4</sub>OH. Prior to this publication, the three species could be distinguished from each other by the size of basidiomata and ecology, but these criteria are not always reliable, especially for *H. limacinus*, which can be found in the same broadleaved tree woodlands as *H. glutinifer* (Papetti 2016). The taxonomy of *H. dichrous* has never been questioned, however, and the invalid publication of the latter (art. 41.5 and 41.6) prevented its use. It has not been possible to complement and validate the description of *H. dichrous*, because in 1958, Hongo validly published a homonym for a Japanese species (Hongo 1958). As



**Fig. 3** Typification of *Hygrophorus glutinifer* and *Hygrophorus hyacinthinus*. a. Lectotype of *H. glutinifer* P. Bulliard (1786); b. epitype of *H. glutinifer* P.-A. Moreau 00102303/LIP0401690; c. neotype of *H. hyacinthinus* J.-M. Moingeon 10092801/LIP0401691. — Photos by: b. P.-A. Moreau; c. J.-M. Moingeon.

a result, Arnolds (1979) introduced the new name *H. persoonii* to accommodate this species, explicitly citing *H. dichrous* as an invalid synonym.

The biogeographical distribution of *H. glutinifer* spans most of the European continent, including the southern regions of the Scandinavian Peninsula (<https://www.gbif.org/en/species/2538803>), therefore there is little doubt Fries encountered the species – possibly not in his early times at Femsjö and Lund since Bulliard’s taxon is cited as a variety of *Ag. albobrunneus* in the *Systema Mycologicum* (1821, 1832), but more likely when he started prospecting calcareous oak forests around Uppsala before the Epicrisis (1838). For these reasons, we conclude that *H. glutinifer* constitutes an earlier synonym of *H. persoonii*, and that this name should be used for the *H. limacinus*-like species fruiting under broadleaved trees (mostly *Quercus* spp.) with a positive reaction to ammonia solutions.

*Hygrophorus persoonii* var. *fuscovinosus* (Bon 1975, 1981) was described as a chromatic variant of *H. glutinifer* with occasional odour of bitter almond, found under *Quercus ilex*. Our unique personal collection of *H. sp. 2* (PAM2019112303, Fig. 1d) fits this diagnosis and is suspected to represent this taxon. However, as the type collection (LIP: Bon 3113) failed to yield DNA sequences, recent collections of this variety, ideally from its original area, are required before its taxonomic status can be resolved.

***Hygrophorus hyacinthinus*** Quél., *Enchiridion Fungorum*: 48. 1886 — MycoBank MB 164867; UNITE SH1177581.08FU; Fig. 3c, 10h

*Obligate synonyms.* *Hygrophorus agathosmus* var. *hyacinthinus* (Quél.) Quél., *Flore mycologique de la France et des pays limitrophes*: 265. 1888 (also (Quél.) Krieglst., *Die Großspilze Baden-Württembergs* 3: 110. 2001, comb. superfl.).

*Synonym.* *Hygrophorus agathosmus* f. *hyacinthinus* (Quél.) Pilát (‘1951’ 1952), Klic kurcování našich hub hřibovitých a bedlovitých: 75.



*Neotype, here designated.* FRANCE, Doubs, Frasne, under *Picea abies* and *Abies alba* on limestone, 28 Sept. 2010, J.-M. Moingeon, JMM10092801/ LIP0401691\* (isoneotype in GB), MycoBank MBT 10001731, GenBank ITS MT845204.

Notes — *Hygrophorus hyacinthinus* is a rare and rather poorly known European species associated with *Picea abies* forests, usually on rich and calcareous soils. It is reminiscent of *H. agathosmoides* and *H. agathosmus*, which are far more commonly occurring. *Hygrophorus hyacinthinus* was regarded by Quélet (1888) himself, and later by Krieglsteiner (2001), as a variety of *H. agathosmus*, and as a forma by Pilát (1951). The species concept may have been misinterpreted due to it being rare, and the fact that *H. agathosmus* shows a broad variation in macro-morphology. As presented in the study by Larsson et al. (2018), cryptic speciation within the latter species was revealed when comparing ITS sequences, and the com-

plex has been further explored and disentangled in this study. *Hygrophorus hyacinthinus* differs from its closest lookalikes by having less distinct floccules on the stipe apex, a more greyish tone of the pileus colour, and emits a pleasant sweet odour of *Hyacinthus orientalis* or *Narcissus papyraceus*, more delicate than the soap-like bitter almond odour of *H. agathosmoides* and *H. agathosmus*. The molecular phylogenetic analysis supports the recognition of *H. hyacinthinus* as a distinct species within sect. *Tephroleuci* (Fig. 1c).

The species was described from the eastern part of France and as no original material from Quélet’s collections has been preserved, we here designate a sequenced neotype from the same area. It must be quoted that Quélet (1886) described his species with a ‘pure white’ pileus, leading Ludwig (2012) to introduce a f. *murinus* for the most usual, mouse-grey aspect of the species.



Fig. 4 Typification of *Hygrophorus mesotephrus*. a–b. Isolectotypes Berkeley K(M)92925 (1853, A) & Broome K(M)92927 (1853, B); c. epitype L. Goodwin K(M)227410. — Photo by: c. L. Goodwin.

***Hygrophorus mesotephurus*** Berk. & Broome, Ann. Mag. Nat. Hist. 13: 402, t. 15: 2. 1854 — MycoBank MB 181367; UNITE SH1177587.08FU; Fig. 4, 17a

*Synonym.* *Hygrophorus olivaceoalbus* var. *gracilis* Maire, Treb. Mus. Ci. Nat. Barcelona, sér. bot. 15 (2): 53. 1933.

*Lectotype.* GREAT BRITAIN, England, Wiltshire, Bowood, 19 Oct. 1853, C.E. Broome, collection M.J. Berkeley, K(M)92925, collection C.E. Broome, K(M)92927 (isolectotype, designated by us, see Notes below), collectively designated by Orton as 'type material' (1960: 258, 426).

*Epitype, here designated.* GREAT BRITAIN, England, South Hampshire, New Forest, Eyeworth Wood, on steep woodland bank, on soil, amongst *Musci*, *Fagus sylvatica*, *Quercus* sp., 10 Oct. 2015, L. Goodwin, K(M)227410\*, MycoBank MBT 10001732, GenBank ITS MT981695. In support of the lectotype cited above.

**Notes** — Three original collections, perfectly preserved, matching Berkeley & Broome's protologue (i.e., collected by C.E. Broome at Bowood before 1854) are conserved at Kew, one dated 10 Oct. 1853 and two dated 19 Oct. 1853. No collection was designated as holotype by the authors, and thus a lectotype had to be designated among these three collections. Orton (1960) made this move, probably unintentionally, by explicitly citing the 19 Oct. collection as 'type material' in the legend of spore drawings (f. 388a p. 426) after having indicated that he had revised this material at Kew (p. 258). Because the two parts K(M)92925 and K(M)92927 represent the same original collection split between the coauthors and are conspecific as far as morphology can be interpreted, the former is here referred to as lectotype and the latter is distinguished as isolectotype. We did not attempt to sequence this old material, but instead we stabilize the binomial within this clade by epitypifying it with a recent sequenced collection, originating from a broadleaf woodland in England less than 100 km away from the type locality.

*Hygrophorus mesotephurus* is an uncommon European species found in beech (*Fagus sylvatica*) forests, but has been difficult to classify in the genus because of its unique combination of characters. For instance, Fries (1874) divided his *Hygrophorus* subg. *Limacium* into five 'groups' (invalid: Turland et al. 2018, art. 21.1) based on the colour of the pileus: *Albi l. albolutescentes*; *Rubentes*; *Fulventes l. flavi*; *Olivaceo-umbrini*; and *Fuscocinerei l. lividi*, and admitted *H. mesotephurus* in the last, together with *H. fuscoalbus*, *H. agathosmus*, *H. tephroleucus* and *H. pustulatus*. Bataille (1910) placed it in his subsect. *Discoidei* with *H. discoideus* and *H. arbustivus*. Candusso (1997) and Lodge et al. (2014) transferred it in sect. *Olivaceoumbrini* with, e.g., *H. olivaceoalbus*, *H. limacinus* (as *H. latitabundus*) and *H. glutinifer* (as *H. persoonii*), but available phylogenies indicate it does not form a monophyletic group with *H. olivaceoalbus* and allied taxa (Larsson 2010; Fig. 1a).

Our sampling effort shows that *H. mesotephurus*, together with *H. adiaphorus* and a morphologically similar species from Tennessee (USA), forms a strongly supported clade sister to sect. *Limacini* and shares evolutionary history with the latter and sect. *Tephroleuci* (Fig. 1a).

Bon (1977, 1990) was the only author who suspected *H. mesotephurus* represented an independent taxon within subg. *Limacium*, and intended to revive Fries' fifth group as 'subsect. *Fuscocinerei*' with *H. mesotephurus* as its type species, *H. fuscoalbus* (*sensu* Bon, here named *H. canadensis*), and the American interpretation of *H. pustulatus* (incl. *H. pustulatoides*) by Hesler & Smith (1963). Because Bon (1977: 39) did not provide any diagnosis nor direct citation to a previous diagnosis, the name, however, remained invalid.

We propose here to validate it following Bon's concept, i.e., based on *H. mesotephurus* and his North American relatives, distinct from all other European lineages.

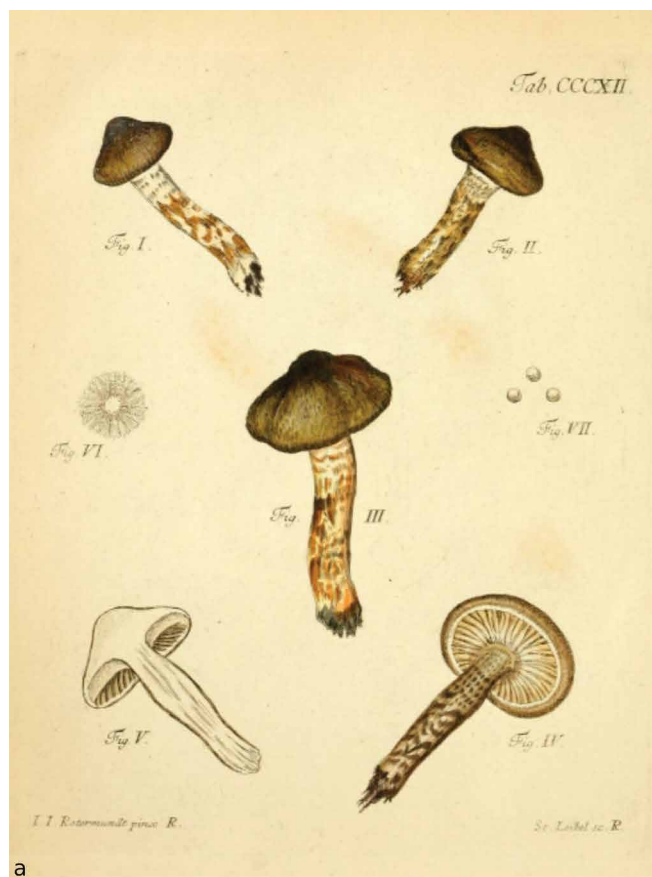
***Hygrophorus olivaceoalbus*** (Fr.) Fr., Epicr. Syst. Mycol. (Upsaliae): 324. 1838 — MycoBank MB 180673; UNITE SH1169761.08FU; Fig. 5, 7a

*Basionym.* *Agaricus olivaceoalbus* Fr., Observ. Mycol. 1: 5. 1815, nom. sanct.

*Obligate synonyms.* *Agaricus limacinus* subsp. *olivaceoalbus* (Fr.) Pers., Mycol. Eur. 3: 95. 1828; *Limacium olivaceoalbum* (Fr.) P. Kumm., Der Führer in die Pilzkunde: 119. 1871.

*Lectotype, here designated.* Schaeffer, J.C. (1774) Fungorum qui in Bavaria et Palatinatu circa Ratisbonam nascuntur icones, vol. 4, t. 312 (as *Agaricus limacellus*), MycoBank MBT 10001733.

*Epitype, here designated.* SWEDEN, Jämtland, Revsund, Ammerön, *Picea abies* dominated mixed coniferous forest, 26 Aug. 2010, E. Larsson, EL196-10/GB0183666!, MycoBank MBT 10001734, GenBank ITS MN243170. In support of the lectotype designated above.



a



b

**Fig. 5** Typification of *Hygrophorus olivaceoalbus*. a. Lectotype J.C. Schaeffer (1774); b. epitype E. Larsson 196-10/GB0183666!. — Photo by: b. E. Larsson.



Notes — When Fries described *Agaricus olivaceoalbus* (Fries 1815), he did not refer to any illustration or authentic material. He regarded *A. olivaceoalbus* as a common species in the coniferous forests dominated by *Picea abies* around Femsjö in Sweden. Later in *Systema Mycologicum* (Fries 1821), he emended the description and cited an illustration by Schaef-fer (1774) as representative for the species. This illustration is here selected as lectotype and fits the current concept of *H. olivaceoalbus*, contrary to the other iconographic material from Bulliard ('*Agaricus glutinosus*', pl. 258, 539 and 587 f. 1) considered by Fries as a variety 'b' of the former. Fries himself reinterpreted later (1838) Bulliard's plates 258, 539 and 587 as *H. glutinifer*, a legitimate but forgotten synonym of *H. persoonii* (see Papetti 2016 and above). In the Stockholm herbarium, there is a painting of *H. olivaceoalbus* from Uppsala signed by Fries (No. 617). The painting is not dated, but as Fries did not start illustrating fungi before 1845, it was not at hand at the time for the description (Strid 1994). The epitype selected here originates from *Picea abies* dominated mixed coniferous forest in Central Sweden, and fits Fries painted illustration of *H. olivaceoalbus* well. It also fits the protologue and sanctioning description.

As delimited here, the type species in the section is phylo-genetically variable (Fig. 1b; Table 2), possibly due to accel-erated evolution resulting from a trophic mode bordering on parasitism (Agerer 2012). Also, the present analysis indicates that collections identified as *H. olivaceoalbus* by past and con-temporary mycologists are scattered in at least 5 distinct clades:

1. a strictly North-European lineage here confirmed as *H. korhonenii*;
2. *H. canadensis*, here formally introduced;
3. & 4. two strictly North American lineages here confirmed as *H. fuscoalboides* and *H. whitei*, respectively; and
5. the current European concept of *H. olivaceoalbus*, also represented in the New World.

We designate a lectotype together with a sequenced epitype to stabilise the usage of the latter binomial.

***Hygrophorus pustulatus* (Pers.) Fr., Epicr. Syst. Mycol. (Upsaliae): 325. 1838 — MycoBank MB 187146; UNITE SH1411532.08FU; Fig. 6a, 10g**

*Basionym.* *Agaricus pustulatus* Pers., Synopsis Methodica Fungorum: 354. 1801, nom. sanct.

*Obligate synonym.* *Limacium pustulatum* (Pers.) P. Kumm., Der Führer in die Pilzkunde: 119. 1871.

*Synonyms.* *Hygrophorus tephroleucus* (Pers.) Fr., Epicr. Syst. Mycol. (Upsaliae): 325. 1838. See below.

*Neotype, here designated.* SWEDEN, Västergötland, Skeplanda, Rösena, Slereboäns NR, in *Picea abies* dominated mixed coniferous forest, 21 Sept. 2014, E. Larsson, EL185-14/GB01836651\*, MycoBank MBT 10001735, Gen-Bank ITS MN243180.

Notes — *Agaricus pustulatus* was originally described by Persoon (1801) as an ash grey species with ash grey squamu-lae on the stipe, but he did not refer to any painting, drawing or authentic material. In *Systema Mycologicum* (1821), Fries wrote that he found the species growing in coniferous forest and especially abundantly under *Picea abies*, with no refer-ence to any illustration or material either. In the Stockholm herbarium, there is a painting of *H. pustulatus* from Uppsala signed by Fries (No. 624), and dated 1845 (Strid 1994). In the study of the Persoon herbarium by Singer (1961), no material of *A. pustulatus* is cited. We conclude that there is no material available for lectotypification of this name, and that a neotype has to be chosen to stabilize its usage.

Confirming the presumptions of Melot (1981) and Bon (1990), the present work unveils cryptic species among collections

previously identified as *H. pustulatus*, with the detection of a morphologically very similar, strictly North American lineage here introduced as *H. pustulatoides*. To help distinguish the two species and fix the usage of the historical binomial, we desig-nate a neotype for *H. pustulatus*, based on a recent sequenced collection from the western part of Sweden.

***Hygrophorus tephroleucus* (Pers.) Fr., Epicr. Syst. Mycol. (Upsaliae): 325. 1838 — MycoBank MB 191619; UNITE SH1411532.08FU; Fig. 6b–c**

*Basionym.* *Agaricus tephroleucus* Pers., Synopsis Methodica Fungorum: 351. 1801, nom. sanct.

*Obligate synonym.* *Limacium tephroleucum* (Pers.) P. Kumm., Der Führer in die Pilzkunde: 119. 1871.

*Synonym.* *Hygrophorus pustulatus* (Pers.) Fr., Epicr. Syst. Mycol. (Up-saliae): 325. 1838.

*Lectotype, here designated.* Batsch, A.J.G.K. (1789) Elenchus Fungorum, continuatio secunda, plate 34, figure 196a (as *Agaricus discors*), MycoBank MBT 10001736.

*Epitype, here designated.* SLOVAKIA, Banskobystrický Kraj, Veporské vrchy Mts, Cierny Balog, Dobrotsky´ prales Nature Reserve, in *Picea* dominated mixed coniferous forest, 7 Oct. 2008, E. Larsson, EL223-08/GB0207586, MycoBank MBT 10001737, GenBank ITS KJ720197. In support of the lecto-type designated above.

Notes — *Agaricus tephroleucus* and *A. pustulatus* were both described by Persoon (1801: 351, 354). The original descrip-tions of the two species, both growing '*in pinetis ad terram*' (under conifers, on ground) are very similar: the distinctive feature is the presence of viscose papillae on the pileus of *A. pustulatus* ('*papillis viscosis obsito*'), while the pileus of *A. tephroleucus* is squamulose. Persoon mentions a stipe with black squamules in *A. tephroleucus* ('*squamulis nigris*'), white squamules in *A. pustulatus* ('*stipite squamuloso candidis*'); and ash-coloured squamules ('*squamulis cinereis*') in the variety  $\beta$  *consimilis*, frequent in Rhenan coniferous forests. The detailed description of *A. pustulatus*, mentioning a pileus '*saepe flex-uosus... nonnumquam subdepressus*' suggests that Persoon described rather mature specimens. A second variety of *A. pustulatus* ( $\gamma$  *A. brevis*, with short white stipe and glabrous darkening pileus) rather evoke *H. agathosmus*.

Based on Persoon's texts, one may consider that both descrip-tions concern different aspects of the same species. But Fries (1821: 34) and most later authors kept them separated, still considering the importance of the papillae of the pileus to dis-tinguish two species. For Fries, *A. pustulatus* is still defined with a white to ash-coloured squamulose stipe (and Fries includes his own *A. agathosmus* in this early concept of *A. pustulatus*, before separating them again in the Epicrisis; Fries 1838); there, *A. tephroleucus* was still mainly characterized by its stipe with black squamules. Batsch's (1789) illustration of his *A. discors*, cited without conviction by Persoon and Fries and representing the only original material available for a typification of *A. tephro-leucus*, does not provide any decisive diagnostic element. In the Epicrisis (1838: 325), probably based on personal collections, Fries emended his description of *H. tephroleucus*: the species is instead described as coarsely fibrillose on the stipe, squamulose on the pileus, uniformly dark-coloured (except the white lamel-lae) and finally compared to *H. olivaceoalbus*. Because Quélet's interpretation of *H. olivaceoalbus*, based on Bulliard's plate 258 cited by Fries (as *A. olivaceoalbus* b; 1821: 37), concerns another species common in European broadleaved calcareous forests (*H. glutinifer*), Quélet's followers such as Konrad (1936: 43) deduced from Fries' comparison that *H. tephroleucus* was the 'slender form' of *H. olivaceoalbus*, a Northern and mountain species unknown to most French mycologists of that period. Introducing *H. olivaceoalbus* var. *gracilis* (= *H. mesotephros*) from Catalanian beech forests, Maire (1933) also followed the French tradition of a robust *H. olivaceoalbus* associated with



**Fig. 6** Typification of *Hygrophorus pustulatus* and *Hygrophorus tephroleucus*. a. Neotype of *H. pustulatus* E. Larsson 185-14/GB01836651; b. lectotype of *H. tephroleucus* A.J.G.K. Batsch (1789); c. epitype of *H. tephroleucus* E. Larsson 223-08/GB0207586. — Photos by: a, c. E. Larsson.

broadleaved trees. This tradition ended with the recognition of *H. olivaceoalbus* in Fries' strict sense by Kühner & Romagnesi (1953: 60), who re-named the continental interpretation of Fries' species as *H. dichrous* (now *H. glutinifer*).

Throughout the 19th and 20th centuries, *H. pustulatus* has been univocally applied to a common species, fruiting late in the season under *Picea abies* throughout Europe, featuring a granulose stipe with white to dark grey squamules, and with a squamulose or granulose pileus surface. In contrast and as discussed by Melot (1981), the application of the name *H. tephroleucus* remained unstable. On one hand, authors who followed Persoon and Fries' *Systema* kept *A. pustulatus* and *A. tephroleucus* separated but hardly distinguishable; for instance Lange (1940: pl. 163A) illustrated the second as 'the smallest of all the *Limacium*s', with paler colours and less contrasting scales than *H. pustulatus* (pl. 162G). On the other hand, Kalchbrenner (1874: plate 17, f. 6) illustrated under this name a fungus with an umbonate, then depressed pileus with dark fibrillose squamules on both pileus and stipe, only slightly reminiscent of *H. pustulatus* but with squamose (not fibrillose) stipe. However, this plate was acknowledged by Fries in his concept of *H. tephroleucus* in *Hymenomycetes Europaei* (1874: 411).

From France, Métrod (1938: 70) and Bon (1974: 338; 1977: 30) seem to have found, and illustrated, a dark, entirely fibrillose fungus matching Fries' last description. Such a dark *H. tephroleucus* shifts towards another mythic species described with a black, umbonate and entirely fibrillose pileus: *H. fuscoalbus*, compared by Bon (1990) to *H. inocybiformis*. Should Métrod's *H. tephroleucus/fuscoalbus* exist as a separate species in continental Europe, it would match neither Persoon's protologue, nor Fries' sanctioning description.

We could not trace any formally proposed synonymy between *H. pustulatus* and *H. tephroleucus* before Melot (1981) and Candusso (1997), who both retained *H. pustulatus* as the preferred name (Turland et al. 2018, art 11.5). Nevertheless, our phylogenetic analysis of sect. *Tephroleuci*, based on a broad taxon sampling, fails to identify two distinct European *H. pustulatus*-like lineages, but instead supports a single morphologically variable species that includes elements fitting both Persoon's diagnoses. Therefore, we here close the debate by epitypifying *H. tephroleucus* with a collection from this clade displaying black squamules on the stipe, consistent with both the original species concept and the current one, which merges it within *H. pustulatus*.





**Fig. 7** Overview of sect. *Olivaceoumbrini*. a. *H. olivaceoalbus* PAM16082601; b. *H. canadensis* HRL2344/DAOM984766 (holotype); c. *H. fuscoalboides* HRL2345; d. *H. korhonenii* EL390-13/GB0183574; e. *H. marcocontui* KATO Fungi 3554; f. *H. whitei* JO-01/HRL2892. — Photos by: a. P.-A. Moreau; b–c. R. Lebeuf; d. E. Larsson; e. E. Sesli; f. J. Olson.

*Hygrophorus suzannae* Melot (Melot 1992) remains an enigmatic species, related to *H. pustulatus* but with white stipe with concolorous scales, possibly conforming to Persoon's original concept of *H. pustulatus*. No original material nor recent collection of this taxon was available to us.

## TAXONOMY

***Hygrophorus* sect. *Olivaceoumbrini*** (Bataille) Konrad & Maubl., *Icones Selectae Fungorum* 6: 137. 1937 emend. — MycoBank MB 804088; Fig. 7

*Basionym.* *Hygrophorus* (unranked) *Olivaceo-umbrini* ('*Olivaceo-umbrini*') Bataille, *Mém. Soc. Émul. Doubs*, sér. 8, 4: 163. 1910.

*Type species.* *Hygrophorus olivaceoalbus* (Fr.) Fr., *Epicr. Syst. Mycol. (Upsaliae)*: 324. 1838.

**Diagnosis** — Pileus glutinous, bistre, greyish brown, fuliginous or olivaceous at least in centre, sometimes fading or yellowing with age; lamellae sub-decurrent, distant, white; stipe glutinous, white with greyish olive-brown fibrils from veil remnants, sometimes with a partial veil forming an annulus, apex white, dry, floccose. Reaction to alkali negative in most species, or confined to the stipe base in others, according to the literature.

**Notes** — In order to define it in its natural monophyletic boundaries, the section is here restricted to *H. olivaceoalbus* and its closest relatives: *H. canadensis* sp. nov., *H. fuscoalboides*, *H. korhonenii*, *H. marcocontui* sp. nov. and *H. whitei* (Fig. 1b)<sup>1</sup>. Other sequenced species that were initially included in subsect. *Olivaceoumbrini* are now transferred to sect. *Fuscocinerei*

<sup>1</sup> While this study was in press, Wang et al. (Mycoscience 2021) reported on a seventh species in sect. *Olivaceoumbrini*, *Hygrophorus annulatus*, so far restricted to China.



sect. nov., sect. *Limacini* sect. nov. or sect. *Nudolidi* sect. nov. In its new limits, sect. *Olivaceoumbrini* is so far represented in coniferous forests of North America, Europe and Turkey.

***Hygrophorus canadensis*** Lebeuf & P.-A. Moreau, sp. nov. — MycoBank MB 836857; UNITE SH1239057.08FU; Fig. 7b, 8

*Etymology.* Refers to its Canadian origin.

*Holotype.* CANADA, Quebec, Saint-Casimir, rang du Rapide N, 4 km west of the village centre, under old *Picea glauca* on the edge of a grass field, N46°38'6.98" W72°10'15.28", 20 m asl, 14 Oct. 2016, R. Lebeuf, HRL2344/DAOM984766\* (isotype in LIP), GenBank ITS MT981628.

*Diagnosis* — Medium-sized agaric fruiting in mid- to late fall, resembling *Hygrophorus olivaceoalbus*, but differing macroscopically from it by its lack of olivaceous tints in the pileus and stipe, its slightly viscid but soon dry stipe, and microscopically by its smaller spores on average ( $11.3\text{--}11.8\text{--}12.3 \times 6.2\text{--}6.7\text{--}7.0 \mu\text{m}$ ), its pileipellis which is an ixocutis instead of an ixotrichoderm, as well as the presence on the stipitipellis hyphae of a gelatinous cuff covered with small specks of refractive material.

*Pileus* 15–55(–60) mm across, conico-convex to convex, with an incurved margin when young, becoming plano-convex with or without an acute papilla or small umbo, later depressed at the centre around the papilla or umbo, if present, with a rounded to deflexed margin, covered with innate dark brown fibrils which are typically more dense at the centre, viscid to glutinous in wet conditions, dark greyish brown to blackish at the centre, light brown at the margin. *Lamellae* adnate at first then arcuate-subdecurent, at times decurrent, L = 32–60, I = 1–3, subdistant, thickish, waxy, with finely eroded edge, pure white, sometimes with a greyish tint. *Stipe* 45–90  $\times$  3–9 mm, cylindrical, equal or slightly enlarged toward the base, often narrower at the apex, usually bent, bearing a dry white fibrillose partial veil leaving an annular zone on the stipe and fleeting remnants on the pileus margin; under the annular zone covered with pale brown (young specimens) to darker brown fibrils forming a ragged pattern over a whitish background; slightly viscid but soon dry, white and smooth or pruinose above the annular zone, solid. *Context* white, thin in the pileus; odour absent, taste mild. *Spore deposit* white.

*Spores* ( $n = 280$ )  $9.0\text{--}16.0$ (–18.5)  $\times$  (5.2–)6.0–9.0  $\mu\text{m}$ , ave.  $11.3\text{--}11.8\text{--}12.3 \times 6.2\text{--}6.7\text{--}7.0 \mu\text{m}$ ,  $Q = 1.62\text{--}1.76\text{--}1.99$  (excluding outlier large spores borne on 2-spored basidia), elliptical, more rarely sublacrymoid or obovoid, smooth, hyaline, thin-walled, inamyloid, with one to three small guttules with a wide and obtuse hilar appendage. *Basidia* 4-spored, rarely 2-spored, narrowly clavate, 50–73  $\times$  9–12  $\mu\text{m}$ , sterigmata 4–9(–11)  $\mu\text{m}$  long. *Hymenophoral trama* divergent, made up of cylindrical or inflated hyaline hyphae, in some collections 35–130  $\times$  4–26  $\mu\text{m}$ , in other shorter and narrower, 10–65  $\times$  2–9(–17)  $\mu\text{m}$ , with thin or thickened walls ( $\leq 0.5 \mu\text{m}$ ). *Subhymenium* ramose, made of short hyphae 3–5  $\mu\text{m}$  wide. *Hymenopodium* undifferentiated. *Cystidioid elements* absent. *Suprapellis* an ixocutis 20–150  $\mu\text{m}$  thick, made up of mostly repent or interwoven, gelatinized, branched, often curved hyphae, with thin or thickened wall, 1.5–7  $\mu\text{m}$  wide, with a brown intracellular and incrusting pigment; presence of refractive granular material in the gelatinous layer, specially on the outermost part. *Subpellis* made up of several rows of compact, parallel, hyaline, non-gelatinized hyphae 2–12  $\mu\text{m}$  wide with thin or thickened walls. *Pileitr trama* made up of subparallel, non-gelatinized, cylindrical to inflated, hyaline hyphae 3–24  $\mu\text{m}$  wide, with thin or thickened walls. *Stipitipellis* an ixocutis 10–175  $\mu\text{m}$  wide, consisting of cylindrical, thin-walled hyphae 2.5–8  $\mu\text{m}$  wide, repent or interwoven, often curved, with an intraparietal brown pigment, producing a gelatinous cuff covered with small specks of refractive material. *Stipititr trama* made up of parallel hyphae 3–12  $\mu\text{m}$  wide, with

thin or thickened wall. *Clamp connections* present in all tissues; medallion clamps frequent.

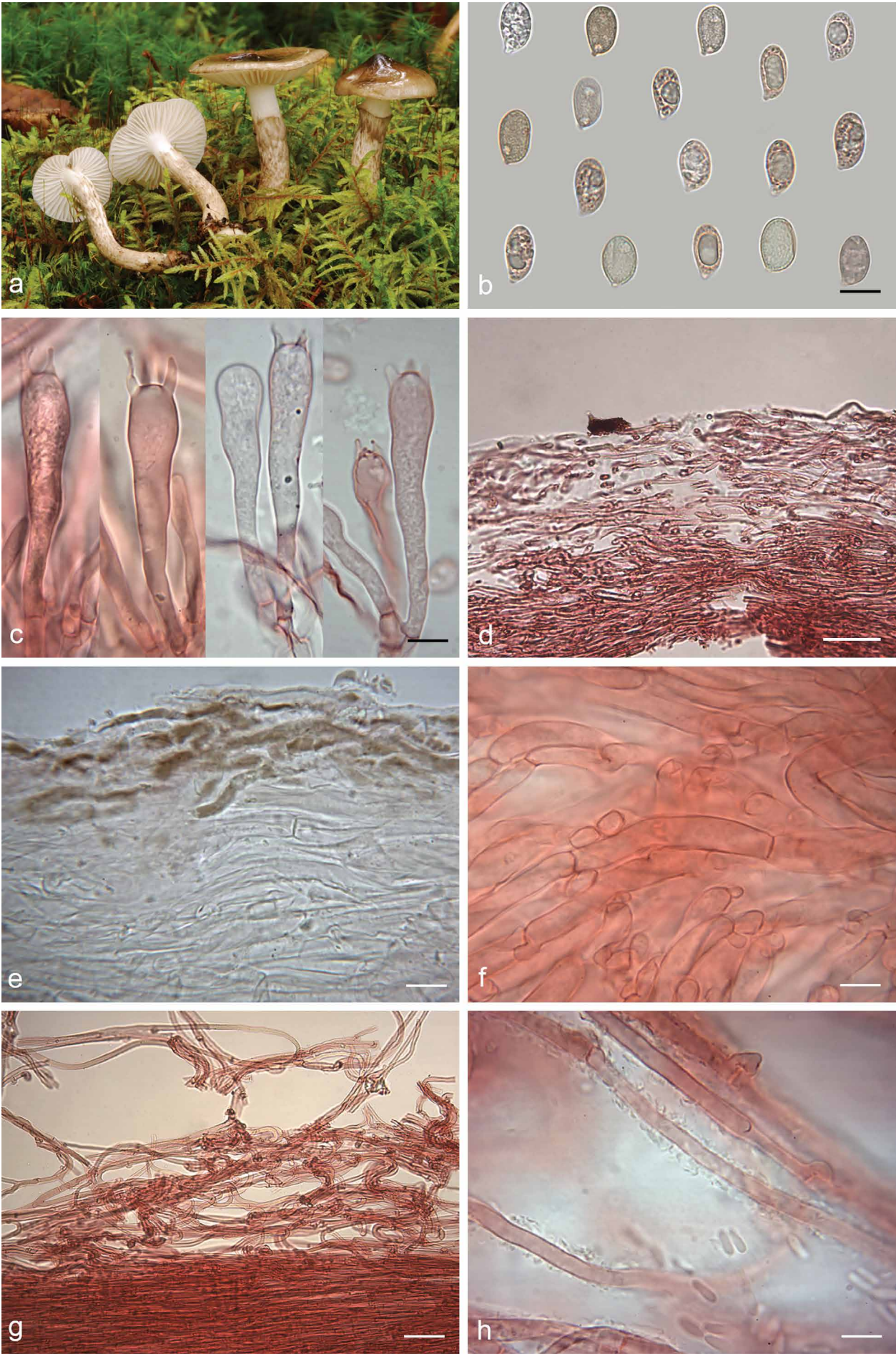
*Chemical reactions* — KOH,  $\text{NH}_4\text{OH}$  and  $\text{FeSO}_4$  all negative on pileus, context and stipe of fresh basidiomata.

*Ecology, Phenology & Distribution* — Appearing in small or large troops, sometimes caespitose, in coniferous forest floor under *Picea* spp., frequently in plantations, in mid- to late fall, often just before the first deep frosts. Confirmed so far from the provinces of Quebec and Ontario, in Canada, but also from France (as *H. fuscoalbus* s. Bon (e.g., Bon 1990, Gavériaux 1997)) in the Canadian war memorial of Beaumont-Hamel under *Picea* sp. imported from the Canadian province of Newfoundland.

*Edibility* — Eaten by some people without ill effects but said to be rather insipid. However, no toxicological studies have been carried out so far.

*Other collections studied.* CANADA, Quebec, Sainte-Anne-de-Bellevue, Arboretum Morgan, under indigenous *Picea* sp., N45°25'53.54" W73°56'41.87", 48 m asl, 25 Oct. 2008, R. Lebeuf, HRL0173\*; Rivière-à-Pierre, ZEC Rivière-Blanche, under *Picea glauca*, *Abies balsamea* and *Betula papyrifera*, in moss, N47°2'52.66" W72°6'55.09", 273 m asl, 13 Sept. 2013, R. Lebeuf, HRL1601\*; Lac-Beauport, chemin Tour du Lac, under *Picea glauca*, *Populus balsamifera* and *Betula papyrifera*, in lawn among needles and leaves, 9 Oct. 2007, J. Labrecque, CMMF010184\* (as *H. inocybiformis*); Lac-Beauport, pinède Rourke, in a conifer forest, under *Abies balsamea*, beside a path, on the soil, 23 Sept. 2007, J. Labrecque, CMMF010093\* (as *H. inocybiformis*). — FRANCE (Canadian territory), Somme, Beaumont-Hamel, Mémorial canadien, under *Picea* sp. imported from the Canadian province of Newfoundland, N50°4'22.39" E2°38'52.79", 1140 m asl, Nov. 2013, C. Lécure, CL/F13.248/LIP0301692\* (as *H. fuscoalbus*), GenBank MG882082.

*Notes* — This species has been long known in Eastern Canada, but was previously confused with *H. olivaceoalbus*. As previously reported (Moreau et al. 2018), the two species are genetically distinguishable by 24 substitutions (SNPs) and 15 insertions-deletions (indels), representing 5 % of sequence divergence at the ITS locus (Fig. 1b and data not shown). Phylogenetically, *H. canadensis* is actually closest to the Californian *H. whitei*, from which it differs by 17 evolutionary events (Table 2). From a morphological point of view, *H. canadensis* can be separated from *H. olivaceoalbus* by its pileus that is conical when young and lacks olivaceous tones, its stipe which is slightly viscid or dry instead of glutinous, its distribution which is restricted so far to eastern North America and its association with trees of North American origin and, microscopically, by its smaller spores (9–18  $\times$  6–9  $\mu\text{m}$  in *H. olivaceoalbus* (Kovalenko 2012, Arnolds 1990)). *Hygrophorus fuscoalboides* differs macroscopically from *H. canadensis* mostly by its stipe that is wider at the apex (8–25 mm), giving it a more robust habit. *Hygrophorus whitei* is morphologically very similar to *H. canadensis* in having a slender stipe, but it is restricted so far to Northern California. *Hygrophorus korhonenii*, so far restricted to Fennoscandia, is very similar to *H. canadensis* on account of its conical pileus when young, greyish colours, and weakly viscid to dry stipe, but it differs by its larger pileus (20–80 mm) and wider stipe (5–20 mm) (Harmaja 1985). Outside sect. *Olivaceoumbrini*, *H. adiaphorus*, a poorly known species so far only reported from two disjunct transcontinental localities and here emended in another lineage (see sect. *Fuscocinerei* below), differs from *H. canadensis* by the absence of brown inner fibrillose veil on the stipe, absent or rare clamp connections, and relatively smaller spores (8.5–11.7  $\times$  4.8–6.9  $\mu\text{m}$ ) from mostly bisporic basidia. *Hygrophorus megasporus*, presently known from western North America, has a viscid stipe lacking brown fibrils beneath the glutinous layer, a glabrous pileus and much larger spores measuring 10–20  $\times$  7–9  $\mu\text{m}$  (Hesler & Smith 1963).



**Fig. 8** *Hygrophorus canadensis* sp. nov. a. Collection HRL1601 *in situ*; b. spores; c. basidia; d–e. pileipellis, radial section; f. lamellar trama hyphae; g. stipitipellis hyphae; h. details of stipitipellis showing hyphae surrounded by a gelatinous cuff covered with small specks of refractive material. — Scale bars: b–c, e–f, h = 10  $\mu$ m; d, g = 50  $\mu$ m. — Photo by: a. R. Lebeuf.



***Hygrophorus fuscoalboides*** Hesler & A.H. Sm., North American Species of *Hygrophorus*: 384. 1963 — MycoBank MB 332239; UNITE SH1703880.08FU; Fig. 7c

*Synonym.* *Hygrophorus olivaceoalbus* var. *intermedius* Hesler & A.H. Sm., North American Species of *Hygrophorus*: 293. 1963.

*Holotype.* USA, Idaho, Custer Co., Cape Horn Summit, Boise National Forest, 44.3638–115.2678, 25 Aug. 1954, A.H. Smith & Bigelow, AHS46726/ MICH5572\*, ITS GenBank MT981636.

*Taxonomic description* — Hesler & Smith (1963), pp. 384–385 (*H. fuscoalboides*) and pp. 293–294 (*H. olivaceoalbus* var. *intermedius*).

*Emended micro-anatomy* — *Spores* ( $n = 196$ ), elliptical, more rarely amygdaliform or oblong, smooth, hyaline, thin-walled, in-amyloid,  $9.0\text{--}10.8\text{--}14.0 \times 5.5\text{--}6.5\text{--}8.0\ \mu\text{m}$ ,  $Q = 1.67$  in collections with 4-spored basidia,  $9.0\text{--}12.1\text{--}17.0 \times 6.0\text{--}7.4\text{--}10.0\ \mu\text{m}$ ,  $Q = 1.62$  in collection with mixed 2-, 3- and 4-spored basidia. *Basidia* 4-spored in most collections, in some collections 2-, 3- and 4-spored mixed, narrowly clavate,  $42\text{--}67 \times 8\text{--}11\ \mu\text{m}$ , sterigmata  $4\text{--}8\ \mu\text{m}$  long,  $7\text{--}15\ \mu\text{m}$  in collections with 2-, 3- and 4-spored basidia. *Hymenophoral trama* divergent, made up of hyphae  $5\text{--}20\ \mu\text{m}$  wide. *Subhymenium* ramose. *Hymenopodium* undifferentiated. *Cystidioid elements* not found. *Suprapellis* an ixocutis  $100\text{--}200\ \mu\text{m}$  thick, made up of strongly gelatinized hyphae  $2\text{--}7\ \mu\text{m}$  wide, with an intracellular and incrusting brown pigment. *Pileitrama* made of thin-walled subparallel hyphae  $5\text{--}18\ \mu\text{m}$  broad. *Stipitipellis* made of slightly gelatinized thin-walled hyphae  $3\text{--}9\ \mu\text{m}$  wide. *Clamp connections* present in all tissues, including large and smaller medallion clamps abundant in the pileipellis and stipitipellis.

*Other collections studied.* CANADA, Quebec, Saint-Casimir, rang du Rapide N, 4 km west of the village centre, under *Picea glauca*, 19 Oct. 2016, R. Lebeuf, HRL2345\*; Grondines, 3<sup>e</sup> rang, between Guilbault road and Dussault road, in *Picea glauca* plantation, in calcareous soil, 10 Oct. 2018, R. Lebeuf, HRL2822\*; Saint-Alban, Parc naturel régional de Portneuf, sentier à Ti-Mé, in *Picea glauca* plantation with some *Abies balsamea*, 17 Oct. 2018, R. Lebeuf, HRL2841\*; L'Islet Co., Saint-Aubert, 18 Sept. 1963, J.W. Groves, UPS-F528301\*. — USA, Alaska, Stetson Creek Trail, Cooper Landing, among mosses under *Picea*, 29 Aug. 2018, N. Siegel, NS3184\*; Colorado, San Miguel Co., Trout Lake, San Juan Mountains, under *Picea*, 17 Aug. 1956, A.H. Smith, AHS52370/ MICH10919\* (holotype of *H. olivaceoalbus* var. *intermedius*); Idaho, Bonner Co., under *Thuja* and *Tsuga*, 2 Oct. 2014, A.D. Parker, ADP141002-1\*; Valley Co., Upper Payette Lake, Payette National Forest 45.145–116.0267, 31 Aug. 1954, A.H. Smith, AHS47140/ MICH34059\* (as *H. fuscoalbus*); Michigan, Marquette Co., Huron Mountain Club, under *Pinus banksiana*, 11 Oct. 1968, I. Bartelli, 3291/ MICH44796\*; New Mexico, Taos Co., Wheeler Peak Wilderness, 26 Aug. 2016, C. Schwarz, INAT4125687\*.

*Notes* — As phylogenetically circumscribed here, this species constitutes a paraphyletic assembly close to *H. korhonenii* and *H. whitei*, from which it can be distinguished by 6 (3 SNPs + 3 indels) and 3 (2 SNPs + 1 indel) nucleotide differences, respectively (Fig. 1b; Table 2). Hesler & Smith (1963) compared this species with *H. fuscoalbus* and *H. limacinus* var. *intermedius*, from which it would mostly differ by a dry (not glutinous) stipe. It has already been demonstrated (Moreau et al. 2018) that the first was a doubtful name, interpreted by Hesler & Smith (1963) as '*H. agathosmus* s. lat.' (likely *H. agathosmoides*, Fig. 1c). The second is likely a mislabelling of *H. olivaceoalbus* var. *intermedius*, which is shown here to be conspecific with *H. fuscoalboides*. Taken together, these findings indicate that the extent of gluten on the stipe in this lineage has been overemphasized in the past and should be considered with caution when it comes to distinguishing the species from its closest relatives.

***Hygrophorus korhonenii*** Harmaja, Karstenia 25 (2): 42. 1985 — MycoBank MB 103452; UNITE SH1703879.08FU; Fig. 7d

*Holotype.* FINLAND, Koillismaa, Kuusamo, Liikasenvaara, Sirkkapuro, fairly rich spruce forest near Siikaupaja, 29 Aug. 1977, M. Korhonen & T. Ulvinen, H6035782\*, ITS GenBank MT981641.

*Taxonomic description* — Harmaja (1985), Kovalenko (2012: 290).

*Other collections studied.* FINLAND, Ostrobothnia ultima, Rovaniemi, Ounasjoki, Marrakoski, *Picea abies* dominated mixed forest, 6 Sept. 2013, E. Larsson, J. Vauras & S. Jacobsson, EL390-13/GB0183574\*; Lapponia kittilensis, Muonio, Pallas-Ounastunturi National Park, old moist *Picea abies* dominated mixed forest, 31 Aug. 2013, E. Larsson & J. Vauras, EL320-13/GB0183575\*. — NORWAY, Nord-Trøndelag, Steinkjer, Skratåsén, 5 Sept. 2009, mixed *Picea abies* dominated forest on calcareous soil, E. Larsson & M. Jeppson, EL173-09/GB0183575\*. — SWEDEN, Lule lappmark, Jokkmokk, Sitoåtno, moist *Picea abies* dominated mixed forest, 31 Aug. 2011, E. Larsson, EL161-11/GB0183575\*; Medelpad, Tuna, Ängömsåsen, mixed coniferous forest, 9 Oct. 1997, S. Muskos, SM97038/GB0124681.

*Notes* — This is the second European species in the *H. olivaceoalbus* group, so far restricted to Fennoscandia. Phylogenetically, the clade is strongly supported and closest to *H. fuscoalboides*, from which it differs by 3 SNPs and 3 indels (Fig. 1b; Table 2). In spruce forests of northern Europe, where the two species often co-occur, *H. korhonenii* can be distinguished from *H. olivaceoalbus* by a slightly stouter habit, a cap usually more broadly umbonate, with greyish brown rather than olivaceous tinges, stipe with greyish brown striped banding pattern, and the relatively smaller spores  $10\text{--}14 \times 5\text{--}7.5\ \mu\text{m}$  (in *H. olivaceoalbus*  $10\text{--}16 \times 7\text{--}8.5\ \mu\text{m}$ ). Harmaja (1985) gives additional micro-anatomical and ecological differences, but as he included two collections from Quebec in his studied material, his description may partly correspond to *H. canadensis* or *H. fuscoalboides* and must be treated with caution.

***Hygrophorus marcocontui*** Sesli, Bellanger & Liimat., *sp. nov.* — MycoBank MB 836858; Fig. 7e, 9

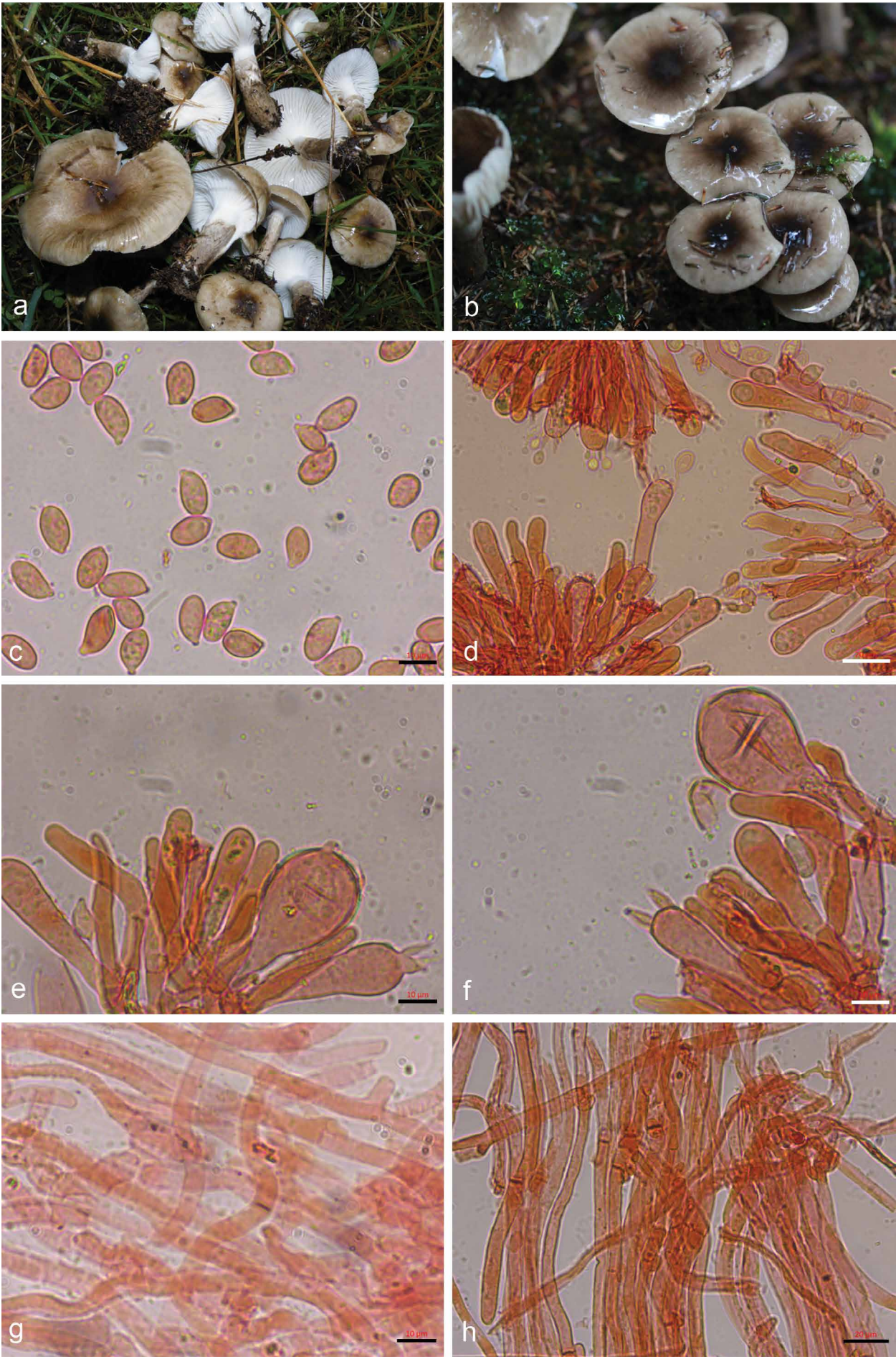
*Etymology.* Named in honour of the Italian mycologist, Marco Contu, who described many agarics new to science.

*Holotype.* TURKEY, Giresun-Kümbet plateau, under *Picea orientalis*, N40°33'30.71" E38°25'59.58", 1717 m asl, 8 Oct. 2010, E. Sesli, KATO Fungi 2976\*, GenBank ITS MT981608.

*Diagnosis* — Characterised by a pale brown to olive and convex to plane pileus. Over time, it may become ochre or slightly pinkish brown, pale or dark brown, and slightly umbonate or umbilicate. It also can be distinguished by its glutinous and generally splitting margin, decurrent, very distant and snow-white lamellae; a cylindrical, usually curved, lubricous to glutinous, mottled, pale grey, olive-brown and arachnoid universal-veiled stipe; typically a fungal odour and indistinct taste; ellipsoid, on average  $11.1 \times 6.9\ \mu\text{m}$  basidiospores;  $57.5 \times 13\ \mu\text{m}$ , clavate, 2–4-spored basidia;  $46\text{--}82 \times 18\text{--}30\ \mu\text{m}$ , clavate, pyriform or globose cystidioid elements on lamellar faces and strict association with *Picea orientalis*.

*Pileus* 25–75(–90) mm across, conical or convex when young, soon expanding to applanate, umbilicate, more rarely infundibuliform with an obtuse umbo, sometimes irregular, margin usually cracked and sometimes undulating; surface pale grey, olive, ochre or slightly pinkish brown or dark brown; typically, dark grey to blackish brown at the centre and lighter towards the margin; viscid to glutinous in wet conditions. *Lamellae* snow-white, very distant, rather thick, typically decurrent, L = 40–50, l = 1–3, waxy, anastomosed. *Context* solid, snow white, moderately thick and fleshy; odour and taste indistinct. *Stipe* 40–95  $\times$  5–15 mm, cylindrical to narrowly clavate, base at times somewhat inflated or tapering to even rooting, sometimes slightly flattened, usually more or less curved; longitudinally mottled; apex white, dry, pruinose, below lubricous to glutinous in moist conditions,





**Fig. 9** *Hygrophorus marcocontui* sp. nov. a–b. Collections KATO Fungi 2976 (holotype) & KATO Fungi 3600 *in situ*; c. spores; d. hymenium with basidia and basidioles; e–f. cystidioid elements found on the lamella surface of some collections; g. pileipellis hyphae; h. stipitipellis hyphae. — Scale bars: c, e–g = 10 µm; d, h = 20 µm. — Photos by: a–b. E. Sesli.

ochraceous to pale brown with olivaceous or greyish brown woolly scales on a whitish background, darker towards the base. The thin, membranous, glutinous and short-lived universal veil forms a more or less well-defined annular zone and scaly bands below. *Spore deposit* white.

*Spores* ( $n = 100$ ) ellipsoid to ovoid, smooth and guttulate, hyaline, thin-walled,  $(7-9.5-13.5(-15) \times (4.8-6-8(-8.7) \mu\text{m}$  (on average,  $11.1 \times 6.9 \mu\text{m}$ ),  $Q = 1.6-1.8$ , apiculus long. *Basidia* 2–4-spored (sometimes 1-spored) clavate to pyriform,  $46-68.7(-78.2) \times (11-12.9-15(-15.5) \mu\text{m}$ , on average  $57.5 \times 13 \mu\text{m}$  (sterigmata up to  $10 \mu\text{m}$  long), many with coarse vacuolar content, with basal clamp. Basidioles similar to the basidia or smaller,  $40-50 \times 9-12 \mu\text{m}$ . *Cystidioid elements* absent from lamellar edges but present in some collections on lamellar faces, subfusiform or fusiform, narrowly clavate, spathulate, or subcylindrical, often flexuous and narrowing at the apices, thin-walled, hyaline,  $(26.4-34-59.9 \times (6.2-7.1-9.9(-11.5) \mu\text{m}$ . *Subhymenium* ramose,  $14.6-24.7 \mu\text{m}$  thick, not gelatinized. *Hymenial trama* divergent, slightly gelatinized, made up of  $4.1-9.8 \mu\text{m}$ , cylindrical, colourless, thin-walled, scarcely branched hyphae (sometimes with short, thick-walled thromboplerous hyphae),  $48.3-181.4 \times 14.9-24.3 \mu\text{m}$ . *Hymenopodium* undifferentiated. *Pileipellis* an ixocutis ( $220-270 \mu\text{m}$  thick), made up of cylindrical,  $4.6-8 \mu\text{m}$  wide, thin- to slightly thick-walled, smooth to minutely punctate yellowish brown hyphae, rarely branched. *Subpellis* ( $60-75 \mu\text{m}$  thick) made up of cylindrical, weakly gelatinized hyphae with elements  $100-170 \times (8.6-13.3-29 \mu\text{m}$ . *Pileitrama* hyaline, made up of thin-walled, cylindrical,  $2.3-7.1 \mu\text{m}$  wide or sarcoid hyphae up to  $21.4 \mu\text{m}$  wide, smooth to locally weakly granular. *Stipitipellis* an ixocutis made up of slender, cylindrical,  $2.4-6.4 \mu\text{m}$  wide, smooth to slightly granular, hyaline and thin-walled, hyphae, not branched, with yellow-brown intracellular pigment. *Stipititrama* pale, made up of thin- to slightly thick-walled,  $6-15.2 \mu\text{m}$  wide, smooth to locally granular, sometimes uniformly yellowish hyphae. *Clamp connections* present at all septa.

Chemical reactions — KOH,  $\text{NH}_4\text{OH}$  and  $\text{FeSO}_4$  all negative on pileus, context and stipe of fresh basidiomata.

Ecology, Phenology & Distribution — Appearing in large, sometimes caespitose groups, in wet, mossy *Picea orientalis* forests, on calcareous soil. So far only known from Turkey, mostly found at elevations between 1000–1700 m asl where it appears to be common in October, but may be present at other elevations.

Edibility — Probably edible but no toxicological studies have been carried out so far.

*Other collections studied.* TURKEY, Trabzon-Mačka-Sevinç area, on grass alongside *Picea orientalis* forest, 18 Oct. 2015, E. Sesli, KATO Fungi 3576\*; under *Picea orientalis*, 26 Oct. 2015, E. Sesli, KATO Fungi 3600\*; on grass alongside *Picea orientalis* forest, 18 Oct. 2015, E. Sesli, KATO Fungi 3554\*; Trabzon-Akçaabat-Hidirnebi, on needle litter among mosses under *Picea orientalis*, 15 Oct. 2010, E. Sesli, KATO Fungi 3002\*.

Notes — This new species is well supported phylogenetically and differs from its closest relative, *H. whitei*, by 11 SNPs and 9 indels, representing 2.5 % sequence divergence (Fig. 1b; Table 2). Morphologically, *H. marcocontui* may be compared with *H. olivaceoalbus*, *H. korhonenii*, as well as with *H. limacinus* and *H. glutinifer*, in the new sect. *Limacini*, although its narrow distribution does not appear to overlap with some of these taxa. *Hygrophorus olivaceoalbus* differs by its smaller and lighter-coloured pileus (20–60 mm), larger basidiospores ( $(9-10-16(-18) \times (6-7-8.5(-9) \mu\text{m}$  acc. to Kovalenko (2012)) and by its lack of cystidioid elements. The other morphologically similar taxon, *H. korhonenii*, is a non-cystidioid species with a longer (70–120 mm) stipe, different gill attachment (broadly adnate to shortly decurrent), oblong basidiospores of similar size ( $10-14 \times 5-7.5 \mu\text{m}$  on average) and slightly smaller ( $65 \times 10 \mu\text{m}$ ) 4-spored basidia. *Hygrophorus limacinus* is also mor-

phologically similar, but has smaller basidiospores ( $9.8 \times 6.2 \mu\text{m}$  on average), white to pale pinkish cream and broadly adnate to subdecurrent lamellae, a robust, 15–40 mm wide clavate to ventricose stipe and slenderly clavate basidia. Another non-cystidioid species, *H. glutinifer*, has smaller basidiospores ( $8.1-10.8(-11.3) \times (5.3-5.5-6.5(-6.7) \mu\text{m}$  according to Arnolds (1979)), broadly adnate to subdecurrent lamellae, and yields a dark blue-green pileal reaction with KOH. *Hygrophorus glutinifer* is also found in different habitats, fruiting in association with broadleaved trees (mostly *Quercus*) and appears to be limited to western Europe (Kovalenko 2012).

***Hygrophorus whitei*** Hesler & A.H. Sm., North American Species of *Hygrophorus*: 280. 1963 — MycoBank MB 547470; Fig. 7f

*Holotype.* USA, California, Humboldt Co., Trinidad, Patrick's Point State Park, 41.13611-124.15139, on soil under *Picea*, 15 Dec. 1956, A.H. Smith & White, AHS56693/MICH10968 (isotype TENN-F-024107)\*, ITS GenBank MN243183.

Emended taxonomic description — *Pileus* 30–60 mm across, viscid, grey-brown with innate virgate fibrils, darker in the centre. *Lamellae* white, distant, typically adnate to decurrent, waxy. *Stipe* 60–100  $\times$  5–10 mm, enlarged at the base, bearing a cortinoid white partial veil leaving an annular zone, narrower above the annular zone, viscid but soon dry, covered with grey-brown appressed fibrils below veil, white above the annular zone. *Context* soft, white; odour indistinct, taste mild to slightly bitter.

*Spores* ( $n = 99$ ), elliptical, subamygdaliform, smooth, hyaline, thin-walled, inamyloid,  $9.0-10.9-15.0 \times 5.0-6.4-8.0 \mu\text{m}$  ( $9-11 \times 5-6(-7) \mu\text{m}$  in Hesler & Smith 1963),  $Q = 1.59-1.70-1.79$ . *Basidia* 4-spored, clavate,  $46-67 \times 9-12 \mu\text{m}$ , sterigmata  $4-10 \mu\text{m}$  long. *Hymenophoral trama* divergent, made up of hyphae  $3-26 \mu\text{m}$  wide ( $9-12 \mu\text{m}$  in Hesler & Smith 1963), gelatinized near apex. *Subhymenium* ramose. *Cystidioid elements* not found. *Suprapellis* an ixocutis  $100-600 \mu\text{m}$  thick, with some ascending hyphae, made up of strongly gelatinized hyphae  $3-6 \mu\text{m}$  wide, with an intracellular and incrusting brown pigment. *Pileitrama* made of subparallel hyphae  $5-24 \mu\text{m}$  broad with thickened wall; rare thromboplerous hyphae about  $6 \mu\text{m}$  wide present. *Clamp connections* present in all tissues, including large medallion clamps abundant in the pileipellis.

All specimens examined were collected in Northern California in mixed conifer forests (*Picea*, *Abies*, *Tsuga*, *Sequoia*) or under *Picea sitchensis*.

*Other collections studied.* USA, California, Humboldt Co., Eureka, Freshwater Rd, HSU Forest, under mixed conifers (*Picea*, *Abies*, *Tsuga*, *Sequoia*), 13 Jan. 2019, J. Olson, JO-01\*; Davison Rd, PC Redwood State Park, under mixed conifers (*Picea*, *Abies*, *Tsuga*, *Sequoia*), 6 Jan. 2019, J. Olson, JO-02\*; Trinidad Beach SP, Elk Head, under *Picea sitchensis*, 28 Jan. 2019, N. Siegel, NS3601\*.

Notes — In the protologue, *H. whitei* (misspelled as *H. whiteii*) is compared with *H. eburneus* and said to be closest to *H. flavodiscus*, a common species in sect. *Aurei* found under white pine in eastern North America (Hesler & Smith 1963). The species is described as having a warm buff pileus and a pallid stipe, features which are not directly evoking those in sect. *Olivaceoumbri*. Three recent sequenced collections are sufficiently different from this original description to justify the emended description of this species provided above.

This species is phylogenetically very close to *H. fuscoalboides*, but the two clades differ from each other by 3 fixed nucleotide differences (2 substitutions + 1 indel) at the ITS locus and are strongly supported as distinct in the Bayesian analysis (Fig. 1b; Table 2). The three recent collections here studied display anatomic features compatible with those of *H. whitei* and are morphologically very similar to *H. canadensis* and *H. fuscoalboides*.





**Fig. 10** Overview of sect. *Tephroleuci*. a. *H. agathosmus* DB051013; b. *H. agathosmoides* f. *albus* AMNH-185145/GB0183713 (holotype); c. *H. albofloccosus* INAT37760508 (not sequenced but same mycelium as INAT38259606); d. *H. exiguus* HRL3114; e. *H. pinophilus* AB09-10-362; f. *H. pustulatoides* NS3599/ HRL2890; g. *H. pustulatus* CMMF005053; h. *H. hyacinthinus* EL326-10; i. *H. odoratus* HRL3088; j. *H. suaveolens* Kleine 07110801. — Photos by: a. D. Bor-garino; b. G.G. Eyjólfssdóttir; c. C. Schwarz; d, i. R. Lebeuf; e. A. Bidaud; f. N. Siegel; g. R. Boyer; h. E. Larsson; j. J. Kleine.



They differ from the former by their growth restricted to the west coast of North America and from the latter by their slenderer habit. Thus, although the type material of the species seems to deviate quite substantially from the recent sequenced material, *H. whitei* fits well in the section. Because all collections of the species, including the type, originate from Northern California, it is possible that *H. whitei* is endemic to this ecoregion.

***Hygrophorus* sect. *Tephroleuci*** (Bataille) Candusso, *Hygrophorus*. Fungi Europaei 6: 97. 1997 — MycoBank MB 538776; Fig. 10

*Basionym.* *Hygrophorus* (unranked) *Tephroleuci* Bataille, Mém. Soc. Émul. Doubs, sér. 8, 4: 164. 1910.

*Type species.* *Hygrophorus tephroleucus* (Pers.) Fr., Epicr. Syst. Mycol. (Upsaliae): 325. 1838.

**Diagnosis** — Pileus viscid to completely dry, white, grey, cinereous, bistre or greyish brown; lamellae distant or subdistant, adnate to subdecurrent, white; stipe usually dry or subviscid, white, basally with greyish tinges, sometimes with dark greyish brown fibrils or granules from veil remnants; often with strong and distinct odours of bitter almonds, hyacinth, acetylaldehyde, or amyl acetate. So far represented in coniferous forests of North America, Europe and Anatolia.

**Notes** — In its currently revised circumscription, sect. *Tephroleuci* is a strongly supported monophyletic clade and encompasses 10 species (Fig. 1c; Table 2): *H. agathosmoides* sp. nov., *H. agathosmus*, *H. albofloccosus* sp. nov., *H. exiguus*, *H. hyacinthinus*, *H. odoratus*, *H. pinophilus* sp. nov., *H. pustuloides* sp. nov., *H. pustulatus* and *H. suaveolens*.

***Hygrophorus agathosmoides*** Lebeuf, E. Larss. & Bellanger sp. nov. — MycoBank MB 836859; UNITE SH1411531.08FU; Fig. 10b, 11

*Etymology.* Named after its similarity to *H. agathosmus*.

*Holotype.* CANADA, Quebec, Grondines, 3<sup>e</sup> rang, between Guilbault road and Dussault road, in *Picea glauca* plantation, in calcareous soil, N46°38'29.59" W72°3'14.90", 38 m asl, 10 Oct. 2018, R. Lebeuf, HRL2823/DAOM984765\* (isotype in LIP), ITS GenBank MT981656.

**Diagnosis** — Differs from *H. agathosmus* by having less inflated thin-walled hyphae in the lamellar trama 5–12(–18) µm vs 7–20(–24) µm in *H. agathosmus*, somewhat more robust basidiomata, and also by fruiting in man-modified environments, such as parks and plantations, whereas *H. agathosmus* is mainly found in old-growth moist *Picea* forests on somewhat richer soils. Has a broad intercontinental distribution range while *H. agathosmus* is so far only known from Europe.

**Pileus** (15–)20–60(–80) mm across, hemispherical or convex when young, expanding to flat or depressed, often with a low obtuse umbo, viscid to glutinous in wet conditions, in North American collections occasionally entirely dark brown when young (6F6) but typically orange brown (5B2, 5C2, 5C3, 5C4) at all stages of development but darker at the centre (5C3, 5D3, 5D4, 5F4) due to the presence of innate brown fibrils, in European collections rather grey to brownish grey (5B1, 5B2), margin at first incurved, becoming straight, with age uplifted to revolute, sometimes undulating, bearing white fibrillose remnants of a narrow fleeting partial veil, the remnants disappearing in mature specimens. **Lamellae** broadly adnate then subdecurrent to decurrent, arcuate, distant, thick, waxy, white, occasionally interveined, 2–6 mm wide, L = 40–68, l = 1–3, with smooth edge. **Stipe** (20–)30–65 × 3–10(–15) mm, cylindrical, equal or slightly enlarging towards the base, dry to slightly viscid, whitish at first then pale greyish brown, covered from the apex to the half or lower third with white floccules turning greyish brown to brown with age, smooth below, with age becoming finely fibrillose. **Context** white, thick; odour of bitter almond, often difficult to detect in cold weather, taste mild. **Spore deposit** white.

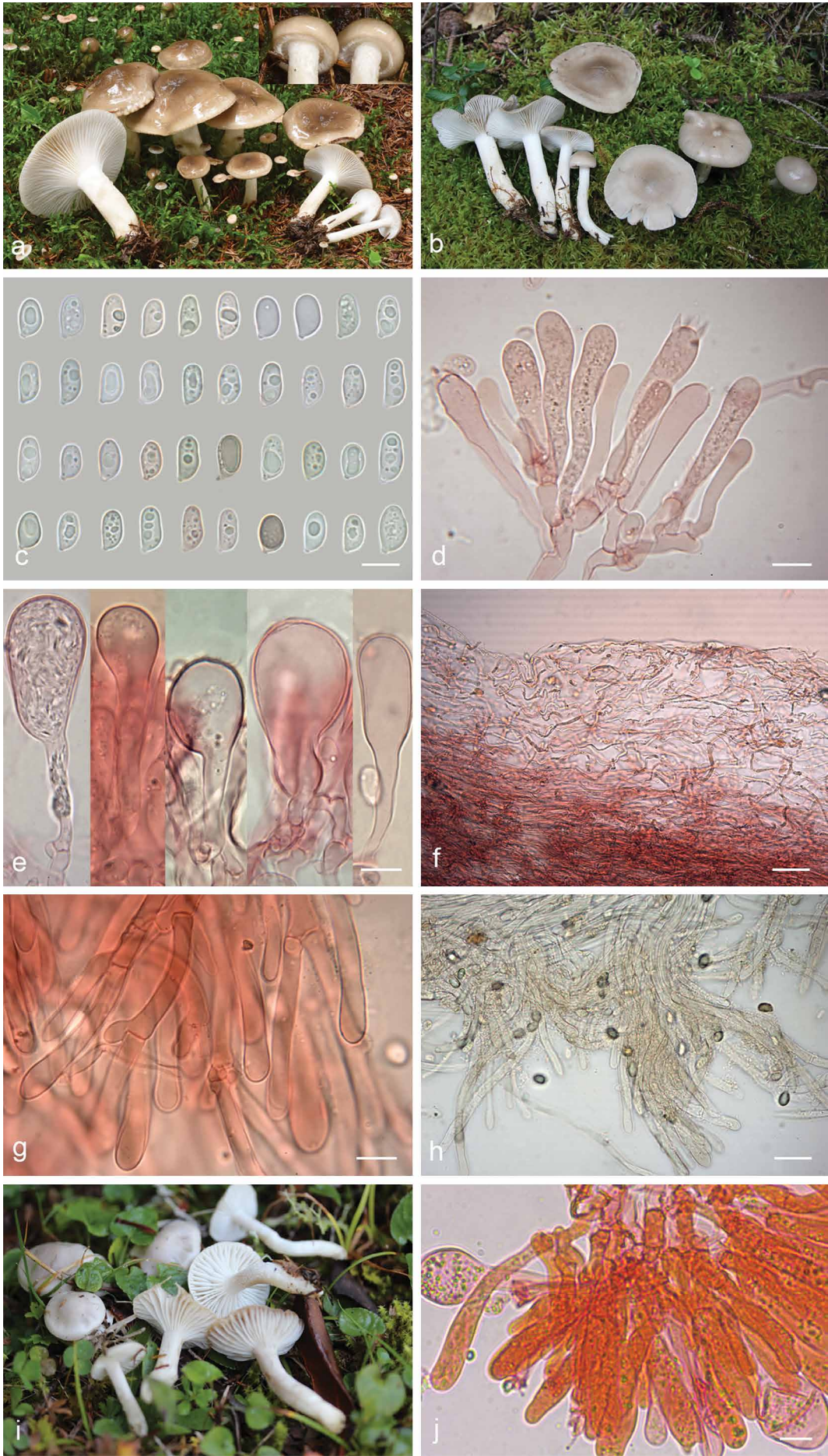
**Spores** (*n* = 230, Canadian collections), elliptical to ovoid-oblong, rarely subamygdaliform, smooth, hyaline, thin-walled, inamyloid, (7.0–)8.8–9.0–9.5(–11.5) × (4.1–)5.0–5.3–5.7(–6.5) µm; Q = 1.53–1.69–1.81. **Basidia** mostly 4-spored, some 2-spored, narrowly clavate, 44–68 × 7–9 µm, sterigmata 5–9 µm long. **Hymenophoral trama** divergent, made up of 15–60 × 4–8 µm, cylindrical or inflated, rarely branched, thin-walled, hyaline hyphae. **Subhymenium** ramose, made of short hyphae 4–6 µm wide. **Hymenopodium** undifferentiated. **Mediostratum** of subparallel 30–100 × 5–12(–18) µm thin-walled hyphae; occasional thromboplerous hyphae up to 350 µm long and 3–6 µm wide, rarely branched, may be observed. **Cystidioid elements** generally absent, but observed in most collections from the west coast of North America, 18–61 × 13.5–25 µm, mostly clavate, thick-walled, often filled with refractive granulose content, present on lamellar edge and/or faces. **Suprapellis** an ixotrichoderm 200–500 µm thick, made up of distant, strongly gelatinized, yellowish or hyaline, granulose, branched, thin-walled, interwoven hyphae 2–4 µm wide; occasional narrow thromboplerous hyphae may be observed. **Subpellis** 100–250 µm thick, not gelatinized, made up of cylindrical to slightly inflated branched hyphae 4–7 µm wide, densely arranged in a subregular to interwoven pattern, with a thickened wall and brown intracellular pigment, rarely incrustated; inconsistently, occasional long thromboplerous hyphae present. **Pileitrama** made up of interwoven cylindrical to mostly inflated, branched, hyaline hyphae 4–15 µm broad, with thin or thickened wall. **Stipitipellis** a cutis of cylindrical thin-walled hyphae, 3–5 µm wide. **Stipititrama** made up of straight to contorted, branched, thin-walled hyphae 5–11 µm wide. **Floccules** at apex made up of clumps of erect, branched hyphae with intracellular brown pigment; terminal cells cylindrical or clavate, 25–45 × 5–8 µm. **Clamp connections** present in all tissues.

**Chemical reactions** — KOH, NH<sub>4</sub>OH and FeSO<sub>4</sub> all negative on pileus, context and stipe of fresh basidiomata.

**Ecology, Phenology & Distribution** — Gregarious to caespitose under *Picea* in basic (calcareous) or acidic soil, in needle litter or in grass, frequent in young *Picea* plantations, but also growing with old trees in open areas and in young, natural forests, in parks and along roadsides. Fruiting in the fall until the first deep frosts, preferring cold temperatures. So far known from British Columbia, Newfoundland and Quebec in Canada, from Alaska, California, Michigan, New Mexico, Oregon and Washington States in the USA, and from Denmark, Estonia, Finland, France, Latvia, Norway and Sweden in Europe.

**Edibility** — Eaten by some people without ill effects and said to be quite good. However, no toxicological studies have been carried out so far.

*Other collections studied.* CANADA, Quebec, l'Islet Co., Saint-Aubert, 20 Sept. 1963, J.W. Groves, UPS F-528300 (MH656464, as *H. occidentalis*); Saint-Bruno-de-Montarville, 10 Oct. 1995, Y. Lamoureux, CMMF002735\*; Anse-de-Roche (Sacré-Coeur-sur-le-Fjord), under *Picea* and *Abies*, at forest edge, in sandy soil (sand dune), N48°12'29.0" W69°52'09.5", 150 m asl, 15 Oct. 2010, H. Lambert, HL0939\*; Grondines, Guilbault road, near highway 40, in *Picea glauca* plantation, N46°37'22.43" W72°3'38.56", 40 m asl, 26 Sept. 2014, R. Lebeuf, HRL1871\*; Amos, under *Picea* and *Larix* in grass in residential area (imported soil and sand), N48°33'52.7" W78°06'15.9", 320 m asl, 16 Oct. 2015, H. Lambert, HL1452\*; Saint-Casimir, rang du Rapide N, 4 km west of the village centre, under *Picea glauca* in rich neutral or basic soil, N46°38'6.98" W72°10'15.28", 20 m asl, 19 Oct. 2016, R. Lebeuf, HRL2343\*; Radisson, city park, mixed forest of *Alnus*, *Salix* and *Picea*, in acidic sandy soil, N53°47'53.84" W77°36'56.32", 198 m asl, 2 Sept. 2018, R. Lebeuf, HRL2694\*; Chibougamau, chemin de La Baie Queylus, in leaves under *Picea*, *Alnus* and *Salix*, in acidic sandy soil, N49°44'7.78" W74°26'1.20", 379 m asl, 13 Sept. 2018, R. Lebeuf, HRL2761\*; Saint-Alban, Parc naturel régional de Portneuf, sentier à Ti-Mé, in *Picea glauca* plantation with a few *Abies balsamea* close by, in mossy calcareous soil, N46°42'16.66" W72°5'9.31", 42 m asl, 17 Oct. 2018, R. Lebeuf, HRL2842\*. — FRANCE, Haute Savoie, Chêne-en-Semine, in a plain plantation of *Picea abies*, in chalky-clay soil, Nov. 2005, A. Bidaud, AB05-11-374\*; Isère, Gresse en Vercors, likely



**Fig. 11** *Hygrophorus agathosmoides* sp. nov. a. Collection HRL2823/DAOM984765 *in situ* (holotype); b. collection EL294-13 *in situ*; c. spores; d. hymenium; e. cystidioid elements found in the hymenium of some western North American collections; f. pileipellis, radial section; g–h. floccules on stipe; i–j. *H. agathosmoides* f. *trabzonensis*, *in situ* (i, collection KATO Fungi 3264) and cystidioid elements on lamellar faces (j, collection KATO Fungi 3604, holotype). — Scale bars: c–e, g, j = 10  $\mu$ m; f = 50  $\mu$ m; h = 25  $\mu$ m. — Photos by: a. R. Lebeuf; b. E. Larsson; i. E. Sesli.



under *Picea* and *Abies*, Oct. 1996, *M. Bon*, MB9610916\* (as *H. spodoleucus* forma); Lozère, Le Pont-de-Montvert, mixed conifers, acidic soil, 18 Oct. 2019, *J.-M. Bellanger*, FR2019577\*; Nord, Le Villiers-au-Tertre, Le Polygone, under planted *Picea abies*, 18 Nov. 2018, *J-M Coquelle*, LIP0401581\*; Savoie, Landry, Barmont, in grasses under *Picea abies*, 23 Aug. 2000, *L. Deparis*, FR2019026\*. — NORWAY, Trøndelag, Steinkjer, Skratåsén, mixed forest with *Picea abies*, 5 Sept. 2009, *E. Larsson* & *M. Jeppson*, EL179-09 (MH656458); Troms, Storjford, Lulledalen, close to Mullejokka, mixed coniferous forest on calcareous soil, under *Picea abies*, 30 Aug. 2013, *E. Larsson*, EL294-13 (MH656460). — SWEDEN, Lule Lappmark, Jokkmokk, Sitoätno, mixed *Picea abies* dominated forest, 31 Aug. 2011, *E. Larsson*, EL160-11 (MH656459); *ibid.*, Kassavare, moist mixed coniferous forest with *Picea abies*, *Betula pendula*, *Pinus sylvestris*, 1 Sept. 2011, *E. Larsson*, EL175-11 (MH656462); Lycksele Lappmark, Tärna, Voitaŧjaure, mixed coniferous forest, 21 Aug. 2015, *E. Larsson*, EL141-15 (MH656467); Öland, Böda, Kesnäsudden, grazed meadow area with *Pinus sylvestris* and *Picea abies*, 5 Oct. 2017, *E. Larsson*, EL384-17 (MH656457). — USA, Alaska, Glacier Ranger Station (Chugach National Forest), 145 Forest Station Rd., Girdwood, clustered in lawn near *Betula* and *Picea*, 8 Sept. 2009, *S. Trudell*, WTU-F-073088\*; *ibid.*, clustered in lawn under *Picea*, 30 Sept. 2013, *S. Trudell*, WTU-F-073034\*; California, Redwood National Park, Davison Rd, mp. 3.4, scattered under *Sequoia*, *Picea* and *Tsuga*, 18 Dec. 2018, *N. Siegel*, NS3463\*; New Mexico, Taos Co., Wheeler Peak Wilderness, 26 Aug. 2016, *C. Schwarz*, INAT4125686\*; Oregon, Multnomah Co., Northwestern Portland, in a grass strip along sidewalk (suburban area), under *Picea*, 29 Dec. 2011, *S. Krstic*, Mushroom Observer 85401\* (as *H. morrisii*); Washington, Pend Oreille Co., under *Picea*, 14 Oct. 2005, *A.D. Parker*, ADPO51014-1\*.

**Notes** — Phylogenetically, this species is quite polymorphic (Suppl. Table 1) and lacks synapomorphies at the ITS locus, resulting in the basal and paraphyletic assemblage of representative sequences, from which emerges the well supported and monophyletic clades of *H. agathosmus*, *H. pinophilus* and *H. albofloccosus* (Fig. 1c; Table 2). It is closest to *H. agathosmus*, from which it differs by only 1 substitution and 5 indels (1–2 nt-long) but with no evidence of gene flow between the two lineages (Fig. 2a; Suppl. Table 1). *Hygrophorus agathosmoides* is the most frequently encountered species of the *H. agathosmus* complex in North America, and is also present in Europe, where it seems to produce slightly larger spores ((8.8–)9.5–9.9–10.1(–11.2) × (4.9–)5.7–5.9–6.1(–6.8) µm,  $Q = 1.62–1.68–1.72$ ,  $n = 174$ ). It grows under *Picea* on both calcareous and acidic soils, in young forests, particularly young plantations, but also in open or grassy areas with young or old trees. It is very similar to *H. agathosmus*, which, according to the sequences available, does not seem to occur in North America and is restricted to old-growth rich forests. The newly described *H. albofloccosus*, so far restricted to the west coast of North America, may be confused with *H. agathosmoides* in this wide ecoregion where the two species co-occur. However, the former seems associated with *Pseudotsuga menziesii*, bears stipe floccules that do not turn brown with age, is usually more robust, and produces shorter spores (average  $8.2 \times 5.2$  µm, compared to  $9.0 \times 5.3$  µm). *Hygrophorus suaveolens*, another species with a bitter-almond scent recently described from Europe (Larsson et al. 2018), grows under *Pinus*, usually on acidic soil and has a glutinous stipe, remaining distinctly viscid when old; it is also known from soil samples collected in Alaska. Another relatively close species with the same odour, *H. exiguus*, described from old-growth *Picea abies* dominated forest in Finland, and also confirmed from Sweden, South Europe and Canada (Larsson et al. 2014 and this study), has a much smaller fibrillose pileus (8–25 mm), a slender stipe (3–6 mm) and lamellae often showing a pinkish tint. It grows in association with *Tricholoma inamoenum*. Another species with a similar odour, *H. odoratus*, described by Smith & Hesler (1954) from Oregon, USA, is so far confirmed from both coasts of North America. That species, however, produces more slender basidiomata (pileus 5–40 mm) with a glabrous to minutely scabrous stipe, stains lemon yellow when bruised or with age (Hesler & Smith 1963, Bird & Grund 1979) and has

larger spores (11–14 × 6.5–8 µm acc. to Hesler & Smith 1963; or 9.5–12.5(–14) × 6–8(–8.5) µm acc. to Bird & Grund 1979). The poorly known *H. morrisii*, described by Peck (1899) and so far restricted to eastern North America, may also be confused with *H. agathosmoides*, but it is still unclear whether it is distinct or not from *H. odoratus* (Fig. 1c, dashed line; Table 2). Among the other new species described herein with a bitter-almond odour, *H. pinophilus* has spores similar to those of *H. agathosmoides*, measuring 8.5–9.7–11.3 × 4.8–5.9–6.6 µm, but grows under *Pinus* and is so far restricted to Europe. *Hygrophorus hyacinthinus*, known from Europe and growing in calcareous soils, has a different odour of *Narcissus tazetta* (Larsson et al. 2018), acetylaldehyde (Arnolds 1990) or amyl acetate (English candies, Bon 1990). In cold temperatures, when its almond odour cannot be perceived, *H. agathosmoides* could also be confused with *H. pustulatus* and *H. pustulatoides*, but these two species are generally less robust, lack a partial veil, and their stipes develop distinct, darker floccules at apex and quickly stains yellow to yellow-orange with KOH at the base. The name *Hygrophorus occidentalis* has been applied to some North American collections of *H. agathosmoides*, but it was shown previously (Larsson et al. 2018), and is confirmed here (holotype sequenced, not shown) that this binomial belongs in sect. *Hygrophorus*, close to *H. eburneus*.

***Hygrophorus agathosmoides* f. *albus* E. Larss. & Lebeuf, f. nov.** — MycoBank MB 836860; Fig. 10b

**Etymology.** Relates to the atypical white colours of basidiomata.

**Holotype.** ICELAND, Vaglaskógur Fnjóskadal, Arnþórslunur, in a plantation of *Picea sitchensis* and *Betula pubescens*, 13 Sept. 2009, *G.G. Eyjólfssdóttir*, AMNH-185145 (isotype GB0183713), GenBank ITS MH656461.

**Diagnosis** — Differs from the type of *H. agathosmoides* by the lack of pileus colour, being off-white, sometimes with a pale cream buff colour or greyish tone in the disc zone, and having white floccules at stipe apex.

**Other collections studied.** CANADA, Quebec, Grondines, 3<sup>e</sup> rang, between Guilbault road and Dussault road, in *Picea glauca* plantation, in basic soil, N46°38'29.59" W72°3'14.90", 38 m asl, 10 Oct. 2018, *R. Lebeuf*, HRL2824\*. — DENMARK, Lolland, Fuglsang Storskov, under *Picea abies*, 3 Oct. 2007, *E. Larsson*, EL134-07 (MH656465); Sjælland, Store Dyrehave S of Hillerød, under *Picea abies*, 10 Nov. 1999, *B.T. Olsen*, C-F41536 (MH656466).

**Notes** — Albinic and pale forms of *H. agathosmus* are rather often encountered and have been described as *H. agathosmus* f. *albus* by Candusso (1997). Similarly, white and pale forms of *H. agathosmoides* are also encountered and accordingly, we here introduce a forma *albus* for these specimens. Sequencing the holotype of the former (LUG8454) revealed that these two white forms are not conspecific. Many white *Hygrophorus* specimens collected in *Picea abies* forests deposited in public Herbaria, at least in Northern Europe, under the name *H. piceae* have later been shown to represent the white forma of *H. agathosmoides* or *H. agathosmus*. Actually, many species in sect. *Tephroleuci* are known to form albinic basidiomata, such as *H. hyacinthinus* (Quélet's 1886 original description) or *H. pustulatus* (f. *niphoides* Cugnot 2004).

***Hygrophorus agathosmoides* f. *trabzonensis* Sesli, f. nov.** — MycoBank MB 836861; Fig. 11i–j

**Etymology.** Named after the Trabzon massif in northern Turkey, where this phenotype was first observed.

**Holotype.** TURKEY, Trabzon, Maçka-Sevinç area, on a lawn of a spruce forest, 1141 m asl, 26 Oct. 2015, *E. Sesli*, KATO Fungi 3604, GenBank ITS MG888785.

**Diagnosis** — Differs from *H. agathosmoides* by its smaller size, stickier and differently coloured pileus; hyacinth-like odour; slightly burning taste; larger and more elongated basidiospores; larger basidia and frequent presence of cystidioid elements on lamellar faces.

*Pileus* (10–)20–50(–60) mm across, smooth, hemispherical or convex when young, soon expanding to applanate or becoming depressed in the centre with an indistinct, obtuse umbo; margin sometimes cracked and undulating; surface silver-grey or pale grey to whitish at first, becoming ash coloured or smoke grey within an hour after collection, slightly darker toward the centre; viscid to strongly glutinous in wet conditions. *Lamellae* broadly adnate to subdecurrent, distant, thick, waxy, sometimes forked, white to cream or silver ash coloured;  $L = 30\text{--}45$ ,  $I = 1\text{--}3\text{--}(4)$ ; lamellar edge smooth. *Context* solid, whitish, thick in the centre and thin toward the margin; *odour* similar to hyacinths and taste mild to slightly hot. *Stipe* (30–)40–60 × 5–10 mm, cylindrical, usually curved, sometimes somewhat enlarged toward the base; whitish to pale grey, silver-grey or ash coloured, typically pruinose, whitish fibrillose to finely floccose, solid. *Spore deposit* white.

*Spores* ( $n = 160$ ) cylindrical-ellipsoid to ellipsoid, smooth and guttulate, hyaline, thin-walled,  $(7.5\text{--})9\text{--}12\text{--}(13) \times (5\text{--})6\text{--}8\text{--}(9.5) \mu\text{m}$ ; on average  $9.8 \times 6.6 \mu\text{m}$ ;  $Q = 1.2\text{--}1.8 \mu\text{m}$ . *Basidia* (1–)2–4-spored, clavate to pyriform, generally granulated, clamped,  $(41.5\text{--})50\text{--}60\text{--}(78) \times (7.5\text{--})8.5\text{--}11.5\text{--}(13) \mu\text{m}$ ; on average  $56.5 \times 9.8 \mu\text{m}$ . Sterigmata occasionally very long (up to 20  $\mu\text{m}$ ). Basidioles similar to the basidia or smaller,  $40\text{--}50\text{--}(55) \times (6.3\text{--})7\text{--}8.5\text{--}(9.5) \mu\text{m}$ . *Cystidioid elements* absent from lamellar edges but present on lamellar faces in some collections, clavate, pyriform or globose,  $(22\text{--})50\text{--}70\text{--}(94) \times (10.4\text{--})15\text{--}25\text{--}(26.9) \mu\text{m}$ . *Cystidioid elements* fusiform, narrowly clavate, cylindrical or flexuous, sometimes with narrowing or bulging ends, thin-walled, hyaline,  $(20.6\text{--})25\text{--}45\text{--}(53.5) \mu\text{m}$ . *Hymenial trama* not or only slightly gelatinized, made up of  $4.7\text{--}17.1 \mu\text{m}$ , cylindrical, hyaline, thin-walled and rarely branched hyphae (short, thick-walled thromboplerous hyphae sometimes present). *Subhymenium* sometimes ramose, comprised of a mixture of fusiform, clavate, cylindrical, ellipsoid, ovoid, subovoid, pyriform, clavate or lageniform cells,  $(36.2\text{--})45\text{--}75\text{--}(113) \times (3.7\text{--})5\text{--}25\text{--}(61.3) \mu\text{m}$ ; not gelatinized. *Hymenopodium* undifferentiated. *Pileipellis* an ixocutis ( $210\text{--}280 \mu\text{m}$  thick), made up of cylindrical, strongly gelatinized,  $(1.5\text{--})2.5\text{--}3.9\text{--}(4.7) \mu\text{m}$  thick, rarely branched, thin- to slightly thick-walled and smooth to minutely punctate hyphae, with greyish intracellular pigment. *Subpellis* ( $50\text{--}80 \mu\text{m}$  thick) slightly gelatinized, made up of cylindrical, pyriform, ellipsoid, clavate, or rarely lageniform,  $(28.3\text{--})40\text{--}85\text{--}(100) \times (12.4\text{--})14\text{--}22.1\text{--}(33) \mu\text{m}$  hyphae. *Pileitrama* hyaline, made up of thin-walled, cylindrical or rarely lageniform,  $4\text{--}7\text{--}(15.7) \mu\text{m}$  thick, smooth to locally slightly granular hyphae. *Stipitipellis* an ixocutis made up of cylindrical,  $(3.2\text{--})5\text{--}7\text{--}(8.8) \mu\text{m}$  thick, smooth to slightly granular, hyaline and thin-walled hyphae, sometimes branching. *Stipititrama* pale, made up of thin- to slightly thick-walled, smooth to locally granular hyphae  $11.1\text{--}21.2 \mu\text{m}$  diam, with occasionally enlarged, cylindrical, pyriform or eggplant-shaped hyphae,  $65.2\text{--}84.2\text{--}(101.3) \times 8.1\text{--}22 \mu\text{m}$ . *Clamp connections* present in all tissues.

**Ecology, Phenology & Distribution** — Gregarious to caespitose among grasses and mosses, in meadows and pastures along *Picea* forest clearings. So far restricted to elevations between 1000–1500 m asl in the Trabzon massif in Turkey, where it fruits in the fall.

**Edibility** — Probably not edible, there is no evidence of consumption by local people and no toxicological studies have been carried out so far.

**Other collections studied.** TURKEY, Trabzon, Akçaabat, Hidirnebi, on lawn with *Alnus glutinosa* and *Picea orientalis*, 27 Sept. 2013, E. Sesli, KATO Fungi 3264\*; in meadows and pastures among grasses and mosses, 15 Oct. 2010, E. Sesli, KATO Fungi 3014\*.

**Notes** — This local phenotype of *H. agathosmoides* is phylogenetically indistinguishable from the type, but displays a striking

combination of morphological, anatomical and organoleptic features that make it unique in sect. *Tephroleuci*. *Hygrophorus agathosmus* has a larger, less sticky, grey or yellowish pileus; longer ( $50\text{--}100 \times 6\text{--}15 \text{ mm}$ ), dry to moist stipe; almond-like odour; mild taste; ellipsoid and smaller ( $8\text{--}11 \times 5\text{--}6 \mu\text{m}$ ) basidiospores; smaller ( $48\text{--}58 \times 7\text{--}9 \mu\text{m}$ ) and 4-spored basidia; and lacks hymenial cystidioid elements. With the same odour, *H. hyacinthinus* differs by having a larger ( $20\text{--}100 \text{ mm}$ ) and less lubricous pileus; longer ( $50\text{--}100 \times 6\text{--}15 \text{ mm}$ ) stipe; ellipsoid and smaller ( $8.6\text{--}11.3 \times 4.8\text{--}6.2 \mu\text{m}$ ) basidiospores; and smaller ( $35\text{--}45 \times 7.5\text{--}9 \mu\text{m}$ ) 4-spored basidia (Bon 1990, Breitenbach & Kränzlin 1991, Kovalenko 2012). Another close species, *H. pinophilus*, lacks hymenial cystidioid elements and differs by having a beige-grey or grey to brownish grey pileus often with white patches; yellowish to pale brown (when injured) stipe; a strong almond odour; smaller basidiospores ( $7.5\text{--}9.5 \times 5\text{--}6.5 \mu\text{m}$ ); as well as shorter and narrower basidia ( $40\text{--}50 \times 7\text{--}9 \mu\text{m}$ ). Another relatively close species, *H. exiguus* described from Finland (Larsson et al. 2014), has a much smaller pileus ( $8\text{--}20 \text{ mm}$ ); shorter and thinner stipe ( $20\text{--}40 \times 3\text{--}4 \text{ mm}$ ); an indistinct taste and different odour in addition to ovoid to broadly ellipsoid, smaller basidiospores ( $9.2\text{--}11.4 \times 5.3\text{--}6.8 \mu\text{m}$ ).

***Hygrophorus albofloccosus*** C.F. Schwarz, Lebeuf & Bellanger, sp. nov. — MycoBank MB 836862; UNITE SH1552735.08FU; Fig. 10c, 12

**Etymology.** Refers to the white flocculence of the stipe, the most useful field character to separate it from similar-looking greyish to greyish brown almond-scented species of *Hygrophorus*.

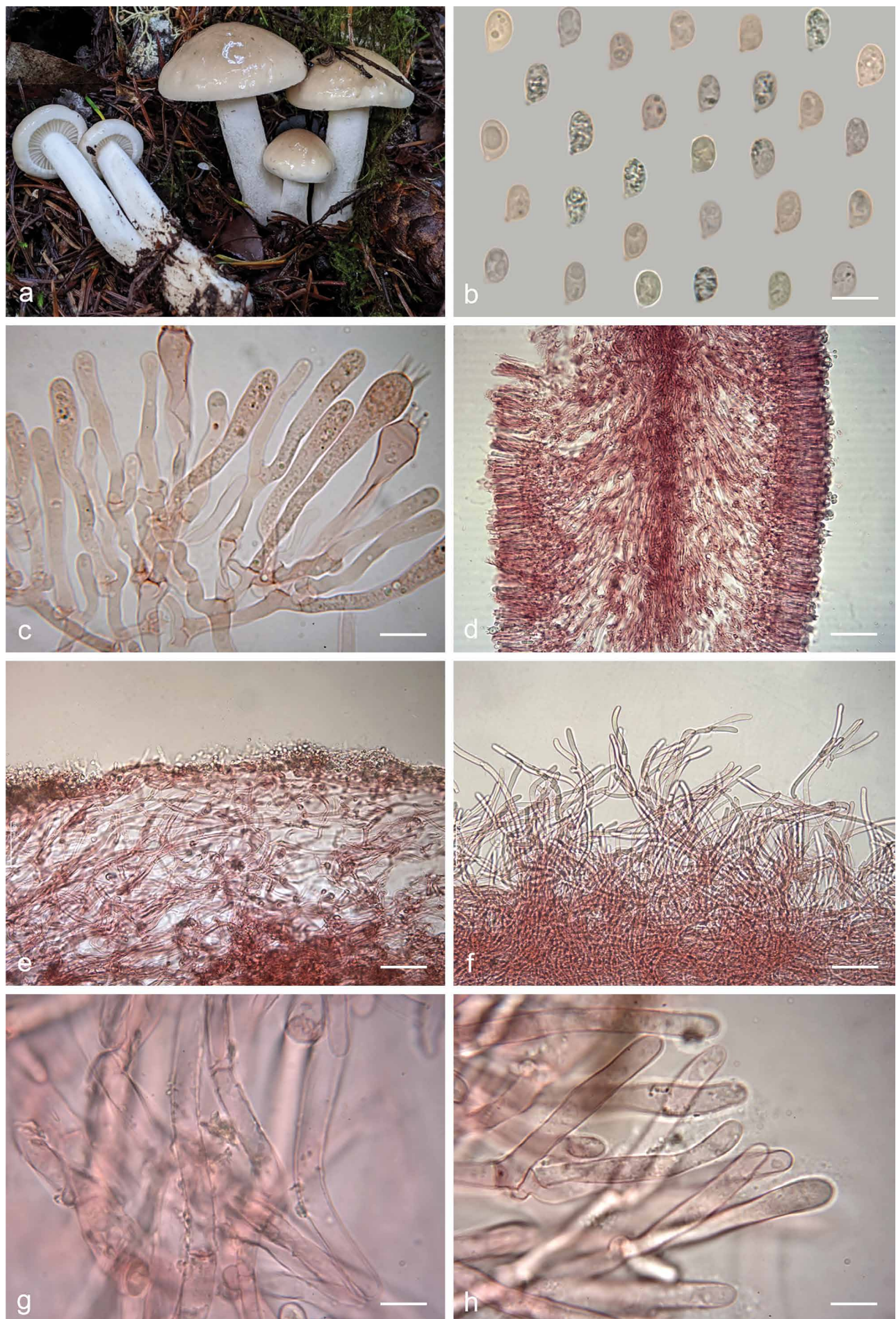
**Holotype.** USA, California, San Mateo County, Portola Redwoods State Park, along Iverson Trail in soil and duff under *Pseudotsuga menziesii*, *Sequoia sempervirens*, *Arbutus menziesii*, with understory of *Vaccinium ovatum*, N37°15'11.2068" W122°13'15.6864", 121 m asl, 18 Jan. 2020, C. Schwarz, UCSC-F-2171\*. GenBank ITS MT981691.

**Diagnosis** — Distinguished from *H. agathosmoides* and *H. agathosmus* by the lack of contrasting brown punctate flocculence on the upper stipe surface, the more robust habit, and its apparent association with *Pseudotsuga menziesii*. Occurs along the Pacific Coast of the United States and in British Columbia, Canada.

*Pileus*  $40\text{--}120\text{--}(150) \text{ mm}$  across, hemispherical when young, expanding to convex or plane, sometimes slightly uplifted and weakly wavy in age, smooth with barely-visible innate or tightly appressed radially-arranged fibrils, viscid and shiny when wet, becoming duller when dry, overall cool grey to greyish buff to greyish tan, sometimes with dark brown, typically much darker at centre, rather pallid towards margin; margin distinctly incurved at first, typically crenate or ribbed, extreme margin finely ornamented with white scurfy teeth appearing as velar remnants (but apparently never leaving a noticeable zone on the stipe). *Lamellae* usually broadly attached or slightly sinuate, subdistant to distant, with few lamellulae in 2–3 series, relatively broad, waxy-feeling, bright white when young, staying white or becoming ivory or pale greyish white. *Stipe*  $50\text{--}150 \times 8\text{--}20\text{--}(25) \text{ mm}$ , equal or tapering towards base, sometimes significantly, white to off-white, extensively finely scurfy or white-pruinose, especially near apex, smoother in age. *Context* thin in cap, thick in stipe, soft, fibrous, white to pale grey; odour mildly to strongly like bitter almond, taste not distinctive. *Spore deposit* white.

*Spores* ( $n = 192$ )  $7.0\text{--}8.2\text{--}9.5\text{--}(10) \times (4.5\text{--})5.0\text{--}5.2\text{--}6.0\text{--}(6.5) \mu\text{m}$ ,  $Q = 1.25\text{--}1.59\text{--}1.89$ , elliptical to oblong, smooth, hyaline, thin-walled, inamyloid. *Basidia*  $40\text{--}67 \times 6\text{--}9 \mu\text{m}$ , 4-spored, narrowly clavate, sterigmata  $4\text{--}7 \mu\text{m}$  long. *Hymenophoral trama* divergent, made up of cylindrical to inflated, thin-walled, hyaline hyphae measuring  $30\text{--}105 \times 3\text{--}18 \mu\text{m}$ . *Subhymenium* ramose, made of short hyphae  $2\text{--}4 \mu\text{m}$  wide. *Hymenopodium* undifferentiated. *Cystidioid elements* absent. *Pileipellis* an ixotrichoderm





**Fig. 12** *Hygrophorus albofloccosus* sp. nov. a. Collection UCSC-F-2171 *in situ* (holotype); b. spores; c. hymenium; d. lamella, radial section; e. pileipellis, radial section; f. stipitipellis; g. stipitipellis hyphae covered with refractive material; h. stipitipellis terminal hyphae. — Scale bars: b–c, g–h = 10  $\mu$ m; d–f = 50  $\mu$ m. — Photo by: a. C. Schwarz.



150–500 µm thick, made up of distant, strongly gelatinized, branched, interwoven hyphae 2–4 µm wide, with thin to thickened wall ( $\leq 0.5$  µm) and brownish intracellular and incrusting pigment. *Pileitrama* of densely interwoven, cylindrical to slightly inflated, branched, hyaline, thin-walled hyphae 3–20 µm broad. *Stipitipellis* made of repent, cylindrical, thin-walled hyphae 2–6 µm wide covered by a continuous or discontinuous layer of erect, septate, branched, cylindrical, hyaline to pale yellow hyphae 3–10 µm wide and up to 200 µm long, some covered by granular refractive crystals in some collections; terminal cells 27–140 × 3–9 µm, cylindrical with rounded apex. *Stipititrama* made up of straight, thin-walled hyphae 4–16 µm wide. No thromboplerous hyphae observed. *Medallion clamp connections* present in all tissues.

Chemical reactions — KOH negative on pileus, lamellae and stipe.

Ecology, Phenology & Distribution — Basidiomata scattered or solitary, occasionally in small clusters or sometimes large troops on ground in thick duff of coniferous forest, almost always near *Pseudotsuga menziesii*; usually in darker, wetter closed-canopy microhabitats in mixed evergreen forest. Commonly-associated overstory plants include *Sequoia sempervirens*, *Arbutus menziesii* and *Notholithocarpus densiflorus*. Commonly-associated understory plants include *Vaccinium ovatum* and various mosses. Fairly common to common from Santa Cruz County in central California northwards to British Columbia, Canada. In California fruiting from late fall through winter, typically late November into late January; earlier in more northerly parts of distribution.

Edibility — Edible, but the almond odour does not persist when cooked, and is infrequently collected by mushroom foragers along the Pacific coast.

*Other collections studied.* USA, California, Santa Cruz County, Whitehouse Canyon Rd, 8 Jan. 2016, *M. Benson*, UCSC-F-1386\*; Sonoma County, Monte Rio, Bohemian Grove, under *Pseudotsuga menziesii*, *Notholithocarpus densiflorus*, *Arctostaphylos* sp. and *Quercus lobata*, 20 Jan. 2019, *N. Siegel*, NS3582/HRL2888\*; Santa Cruz County, UC Santa Cruz Upper Campus, near Fuel Break road, in soil of nearly-pure stand of young *Pseudotsuga menziesii*, N37°0'22.2228" W122°3'35.9388", approximately 220 m asl, 29 Jan. 2020, *C. Schwarz*, INAT38259606\*.

Notes — This novel and well-supported species in the *H. agathosmus* complex is closest to *H. agathosmoides*, from which it differs by 17 evolutionary events (Fig. 1c; Table 2). Morphologically, the viscid grey pileus, white lamellae, dry stipe with fine white flocculence (not brownish), robust habit and strong bitter-almond odour render this species quite distinct in the field. *Hygrophorus agathosmoides*, present in the same range in far northern California, is quite similar but it is more delicate, has more extensive brown tones in the cap and develops brownish floccose punctations on the upper stipe. Its spores are also larger than those of *H. albofloccosus* (average  $9.0 \times 5.3$  µm, compared to  $8.2 \times 5.2$  µm), which might help distinguish the two. *Hygrophorus pustuloides* is similar, but is slenderer, has brownish dots on the stipe, and lacks any odour; it is common under *Picea sitchensis* in far northern California, whereas *H. albofloccosus* extends much further southwards. *Hygrophorus bakerensis* shares the strong sweet bitter-almond odour, but has a yellowish brown cap. *Hygrophorus occidentalis* is superficially similar but has a darker grey pileus, a viscid layer on the stipe, and lacks an odour.

***Hygrophorus exiguus*** E. Larss., E. Campo & M. Carbone in Larsson et al., *Karstenia* 54: 42. 2014 — MycoBank MB 808838; Fig. 10d

*Holotype.* FINLAND, Koillismaa, Kuusamo, Oulanka National Park, Am-pumavaara. In moist *Picea abies* dominated mixed forest, growing among fruiting bodies of *Tricholoma inamoenum*, 18 Aug. 2009, *Calledda*, *Pini*, *Boerio* & *Carbone*, TUR-A 190791. GenBank ITS KJ720198.

Taxonomic description — Larsson et al. (2014).

*Other collections studied.* CANADA, Quebec, Trécesson, in a young mixed boreal forest of *Picea*, *Abies*, *Populus* and *Betula*, among basidiomata of *Tricholoma inamoenum*, 24 Sept. 2019, *R. Lebeuf* HRL3114\*; Saint-Jean-des-Piles, Parc national de la Mauricie, under *Picea*, among basidiomata of *T. inamoenum*, 6 Oct. 2019, *R. Lebeuf*, FR2019592\* (not conserved). — FRANCE, Isère, Villard-de-Lans, in *Sphagnum* under *Abies alba*, among basidiomata of *T. inamoenum*, 1200 m asl, 21 Sept. 2005, *F. Armada*, *A. Bidaud* & *J. Cavet* AB05-09-183\*; Isère, Gresse-en-Vercors, under *Picea abies* and *Abies alba*, Oct. 2008, *D. Borgarino*, DB081048\*.

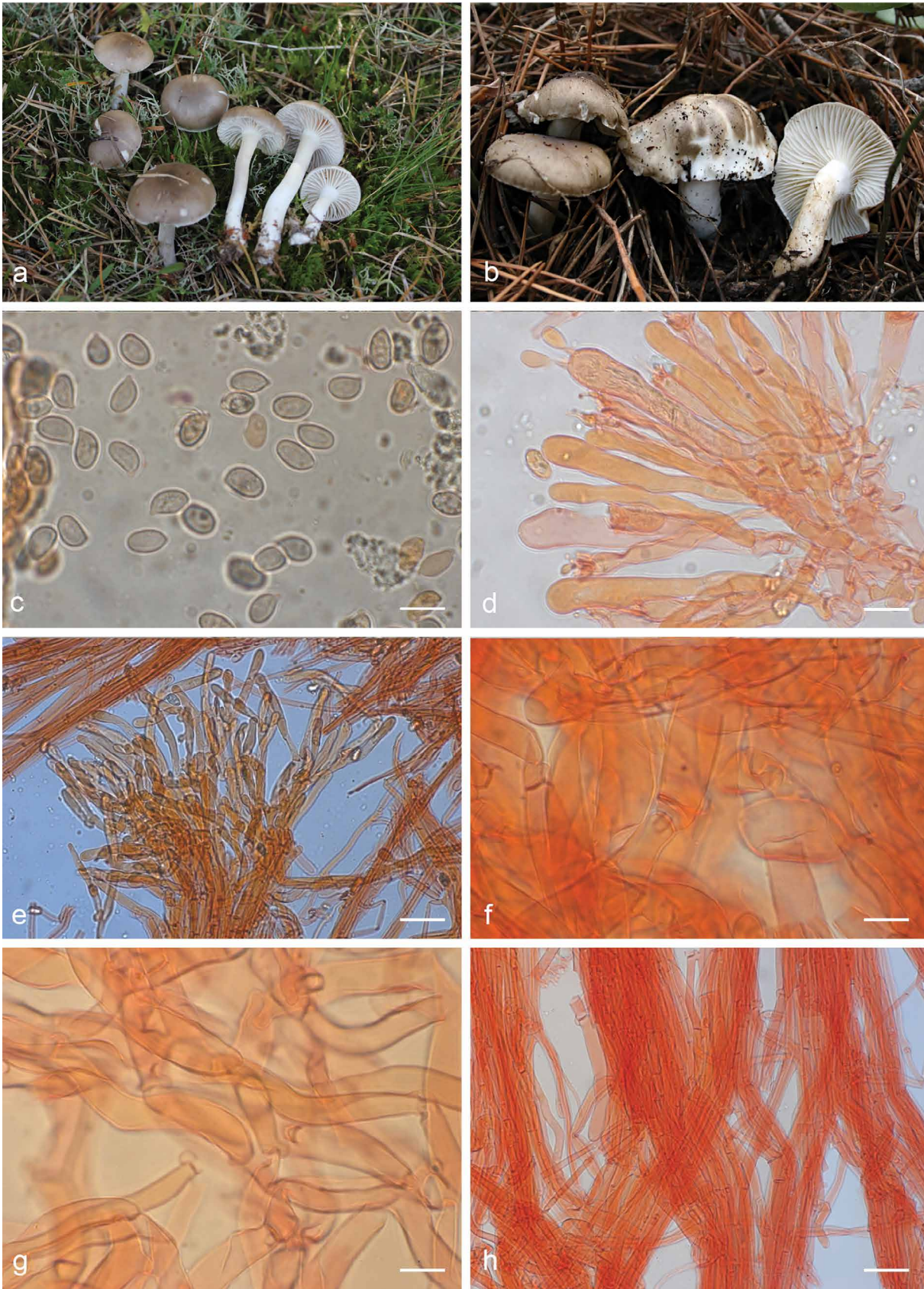
Notes — This species deviates from its closest relatives in many respects, including its small size and trophic mode, unique within the genus. As such, it can hardly be mistaken in the field, even in the absence of molecular information. Prior to our work, *H. exiguus* was confirmed in Fennoscandia, Austria and Italy, but the wider geographical distribution of its host *Tricholoma inamoenum* suggests that the species may be more overlooked than rare. In this context, our French and Quebec collections, all originating from typical mountainous or boreal *Picea/Abies* forests, among *T. inamoenum* basidiomata for most of them, did not come as a surprise. Less expected were the levels of variability of the ITS locus between sequences (Table 2), which topologically translate into the bushy clade depicted in Fig. 1c. Such internal phylogenetic structure, especially the long and supported branch of the two Canadian sequences, may support the taxonomic autonomy of some of these collections. However, a careful inspection of the number and nature of polymorphisms distinguishing sequences in the inclusive clade, coupled with the biogeographical origin of each collection, is more supportive of unusually high levels of sequence variability between populations of a single species rather than cryptic speciation. For instance, the two sequences from Quebec differ from their closest European counterparts by three SNPs and five 1 to 4 nt-long indels, which together constitute 8 evolutionary events (data not shown). However, the two French collections, originating from the same forest, differ from each other by two SNPs and six 1 to 6 nt-long indels, representing the same number of evolutionary events (data not shown). Thus, in contrast to what Fig. 1c may suggest at first sight, there is no clear phylogenetic support for two transatlantic species, but instead, evidence for high ITS polymorphism between sympatric populations in the French Alps. The only conserved Canadian collection (HRL3114) displays some anatomical differences when compared to the holotype, such as slightly smaller spores, a thinner gelatinous pileipellis, narrower hyphae of the lamellar trama and less inflated terminal cells of the stipitipellis floccules. However, the number of collections from both sides of the ocean that we could analyse was not sufficient to estimate the levels of variability of these micro-morphological features among populations. Considering the very similar macro-morphology and ecology of all sequenced material, we refrain, at least provisionally, from splitting the clade into two distinct species and we interpret the high levels of polymorphism of *H. exiguus* as the consequence of its presumed parasitism, often associated with accelerated evolution rates.

***Hygrophorus pinophilus*** E. Larss., Sesli & Loizides, sp. nov. — MycoBank MB 836863; Fig. 10e, 13

*Etymology.* Refers to the ecology and its association with *Pinus* spp.

*Holotype.* SWEDEN, Gotland, Gammelgarn, Danbo, Sjausrå, sandy heathland with *Pinus sylvestris*, 27 Sept. 2011, *E. Larsson*, EL289-11 (isotype GB0183712), GenBank ITS MH656468.

Diagnosis — Resembles *H. agathosmus*, but differs by producing basidiomata with more uniformly and somewhat darker greyish brown coloured pileus. Mainly distributed in southern Europe and associated with *Pinus*, while *H. agathosmus* is more restricted to Northern Europe and associated with *Picea abies*.



**Fig. 13** *Hygrophorus pinophilus* sp. nov. a. Collection EL289-11 *in situ* (holotype); b. collection ML411162HA *in situ*; c. spores; d. hymenium; e. floccule at stipe apex; f. subcuticular hyphae; g. inflated hyphae in lamella trama; h. pileipellis, cluster of parallel hyphae. — Scale bars: c–d, f, h = 10  $\mu$ m; e, g = 25  $\mu$ m. — Photos by: a. E. Larsson; b. M. Loizides.

*Pileus* 30–80(–12) mm across, subglobose to campanulate or conical with involute margin when young, convex to applanate when mature, with or without a slight broad umbo, greyish brown (5C2, 5D2) to somewhat darker greyish brownish (5E3, 5E4) in the centre, becoming more uniformly brown upon drying (5F6, 5F7), often with white fibrillose veil remnants on pileus

margin or sometimes with extensive white patches, occasionally somewhat roughened to subsquamulose in the middle, dry, when moist viscid but never glutinous. *Lamellae* adnate to arcuate-subdecurrent, L = 36–50, distant to subdistant, thick, waxy, with lamellulae, white, sometimes with a pale incarnate tint. *Stipe* up to 5–8  $\times$  0.5–1 cm, cylindrical, often bent, slightly



tapering towards the base, dry, slightly viscid when moist, at first off-white with minute floccules at the top, later with a pale greyish brown tone. *Context* white, with a mild taste. Odour sometimes indistinct but usually strong and sweet, reminiscent of bitter almonds and similar to *H. agathosmus*. *Spore deposit* white.

*Spores* ( $n = 208$ ) elliptical to ovoid-oblong, more rarely sub-amygdaliform, with a distinct, obtuse hilar appendage, smooth, hyaline, inamyloid,  $(8.5\text{--}9.5\text{--}9.8\text{--}10.0\text{--}11.3) \times (4.8\text{--}5.8\text{--}5.9\text{--}6.1\text{--}6.6) \mu\text{m}$ , average  $Q = 1.63\text{--}1.67\text{--}1.75$ . *Basidia* mainly 4-spored, narrowly clavate,  $(35\text{--}40\text{--}50\text{--}60) \times (5\text{--}7\text{--}9\text{--}10) \mu\text{m}$ , sterigmata  $6\text{--}8 \mu\text{m}$  long. Basidioles similar to the basidia or smaller,  $32.8\text{--}48.5 \times 6.2\text{--}7.2 \mu\text{m}$ . *Cystidioid elements* not found. *Subhymenium* ramose,  $9.1\text{--}16.7 \mu\text{m}$  thick, slightly gelatinized. *Hymenophoral trama* bilateral to divergent, composed of interwoven cylindrical hyphae  $4\text{--}8 \mu\text{m}$  wide and thin-walled inflated hyphae with terminal end cells up to  $18\text{--}20 \mu\text{m}$  wide. *Pileipellis* an ixotrichoderm up to  $400 \mu\text{m}$  thick; made up of compact interwoven, branched hyphae  $1.5\text{--}5 \mu\text{m}$  wide, in matrix smooth, hyaline or intracellular pigmented, upper layer with incrustated pigmented hyphae. *Subpellis* composed of densely arranged, sub-parallel interwoven hyphae up to  $15 \mu\text{m}$  broad. *Stipitipellis* a cutis, up to  $65 \mu\text{m}$  thick, made up of  $4\text{--}5 \mu\text{m}$  wide interwoven branched hyphae, with scattered terminal elements, smooth, hyaline, or with intracellular pigments. *Stipititrampa* of hyaline more or less parallel interwoven hyphae  $5\text{--}8 \mu\text{m}$  wide. *Floccules* at the apex made up of compact erect branched hyphae,  $3.5\text{--}5 \mu\text{m}$  wide, loosely scattered free ends, cylindrical or slightly enlarged, up to  $8\text{--}10 \mu\text{m}$  wide. *Clamp connections* present in all tissues.

*Chemical reactions* — KOH negative on pileus, lamellae and stipe.

*Ecology, Phenology & Distribution* — Growing solitary or in groups, sometimes caespitose, primarily under *Pinus sylvestris*, *P. nigra* and *P. pinaster* on serpentine, neutral to calcareous soil in southern Europe, but also under *P. sylvestris* on calcareous soil in Fennoscandia (Sweden and Norway), reported once under *Picea orientalis* in the Trabzon massif of Turkey. Fruiting from September to January.

*Edibility* — Probably edible, but not choice. No toxicological studies have been carried out so far.

*Other collections studied.* CYPRUS, Troodos, in *Pinus nigra* subsp. *pallasiana* forest on serpentine soil, 30 Oct. 2008, *M. Loizides*, ML800103HA; *ibid.*, 1 Nov. 2008, *M. Loizides*, ML8002111HA; *ibid.*, 3 Oct. 2009, *M. Loizides*, ML90013HA\*; *ibid.*, 28 Oct. 2009, *M. Loizides*, ML90020182HA; *ibid.*, 18 Nov. 2011, *M. Loizides*, ML11021181HA; *ibid.*, 19 Nov. 2014, *M. Loizides*, ML41021191HA; *ibid.*, 26 Nov. 2014, *M. Loizides*, ML41021162HA; *ibid.*, 29 Oct. 2018, *M. Loizides*, ML810192HP1\* & ML810192HP3\*; Trooditis, in *Pinus nigra* subsp. *pallasiana* and *Quercus alnifolia* forest, on serpentine soil, 9 Nov. 2008, *M. Loizides*, ML81119HP\*. — FRANCE, Drôme, Eygalayes, under *Pinus sylvestris* on calcareous soil, 850 m asl, Oct. 2009, *A. Bidaud*, AB09-10-362\*; Essonne, under *Pinus* sp., on calcareous soil, 8 Oct. 2019, *F. Valade*, FV2019110802\*; Vendée, under *Quercus* sp. and *Pinus* sp., Nov. 1966, *M. Bon*, MB61124/LIP0101693\* (as *H. agathosmus* forma). — NORWAY, Oppland, Gudbrandsdalen, under *Pinus sylvestris*, 13 Sept. 2018, *E. Bendiksen* (O). — SPAIN, Andalusia, Huelva, Alájar, Puerto de Linares, under *P. pinaster*, 16 Dec. 1995, *L. Romero de la Osa*, MA-F41027\*; Huelva, Almonaster La Real, under *P. pinaster*, 15 Dec. 2018, *A. Gasch Illescas*, AGI18121501/LIP0401560\*; Galicia, Pontevedra, Cangas, Barra, coastal *Pinus* woodland, 5 Dec. 1992, *M. Castro*, MA-F33947; *ibid.*, 17 Dec. 1997, *J. Rodríguez*, MA-F39308\*; Madrid, Cuenca, Fuente la Teja, Puente Sun Podro, in the litter of *P. nigra*, *Q. faginea* and *Arctostaphylos uva-ursi*, 19 Nov. 1997, *C. Illiana & A. Altés*, AH-21971\*; Madrid, Guadalajara, Molina de Aragón y Torremocha de Pinar, in the litter of *P. sylvestris* and *Arctostaphylos uva-ursi*, 3 Dec. 1997, *C. Illiana & A. Altés*, AH-24065\*; Madrid, Zarzalejo, Puerto de la Cruz Verde, in the litter of *P. sylvestris* and *P. pinaster*, 11 Dec. 2004, *F. Prieto*, MA-F74970 (MH656469); Almería, Sierra de Lúcar, *P. nigra*, 1 Jan. 1987, *J.A. Oria de Rueda*, MA-F19424\*; Avila, La Adria, Pinar de Casilla del Collado, 15 Nov. 1983, *F.D. Calonge*, MA-F9598; Navarra, Bigüezal, under *P. sylvestris*, 16 Dec. 1989, *L.M. García Bona*, MA-F67341\*. — TURKEY, Giresun-Kümbet plateau, under *Picea orientalis*, 8 Oct. 2010, *E. Sesli*, KATO Fungi 2990\*.

*Notes* — This newly described and phylogenetically well delimited species (Fig. 1c; Table 2) has been formerly identified as a distinct lineage and referred to as *H. sp.* in Larsson et al. (2018). It is morphologically similar to *H. agathosmoides*, *H. agathosmus*, *H. hyacinthinus* and *H. pustulatus*, from which it differs by at least 11 substitutions and 11 indels (relative to *H. agathosmoides*, Table 2). *Hygrophorus agathosmus* displays a greyer pileus, usually with a darker greyish brown disc zone, white lamellae without pinkish tint and is associated with *Picea abies*. *Hygrophorus agathosmoides* has more robust basidiomata, whitish lamellae usually with a pinkish tint in young and fresh specimens, and is also probably exclusively associated with *Picea* or *Abies* spp., in contrast to *H. pinophilus* that is so far confirmed to be associated with *Pinus*. *Hygrophorus hyacinthinus* differs by its striking hyacinth-like odour, slightly longer basidiospores, rare distribution and association with *Picea* spp. *Hygrophorus pustulatus* is odourless and has a grey-brown to black-brown, fibrillose-scaly pileus; dry, silky and typically punctate-floccose stipe; slightly longer ( $9\text{--}11 \mu\text{m}$ ) basidiospores and is associated with *Picea* spp.

***Hygrophorus pustulatoides*** Lebeuf, E. Larss. & Bellanger, *sp. nov.* — MycoBank MB 836864; UNITE SH1411539.08FU; Fig. 10f, 14

*Etymology.* Refers to its similarity to *Hygrophorus pustulatus*.

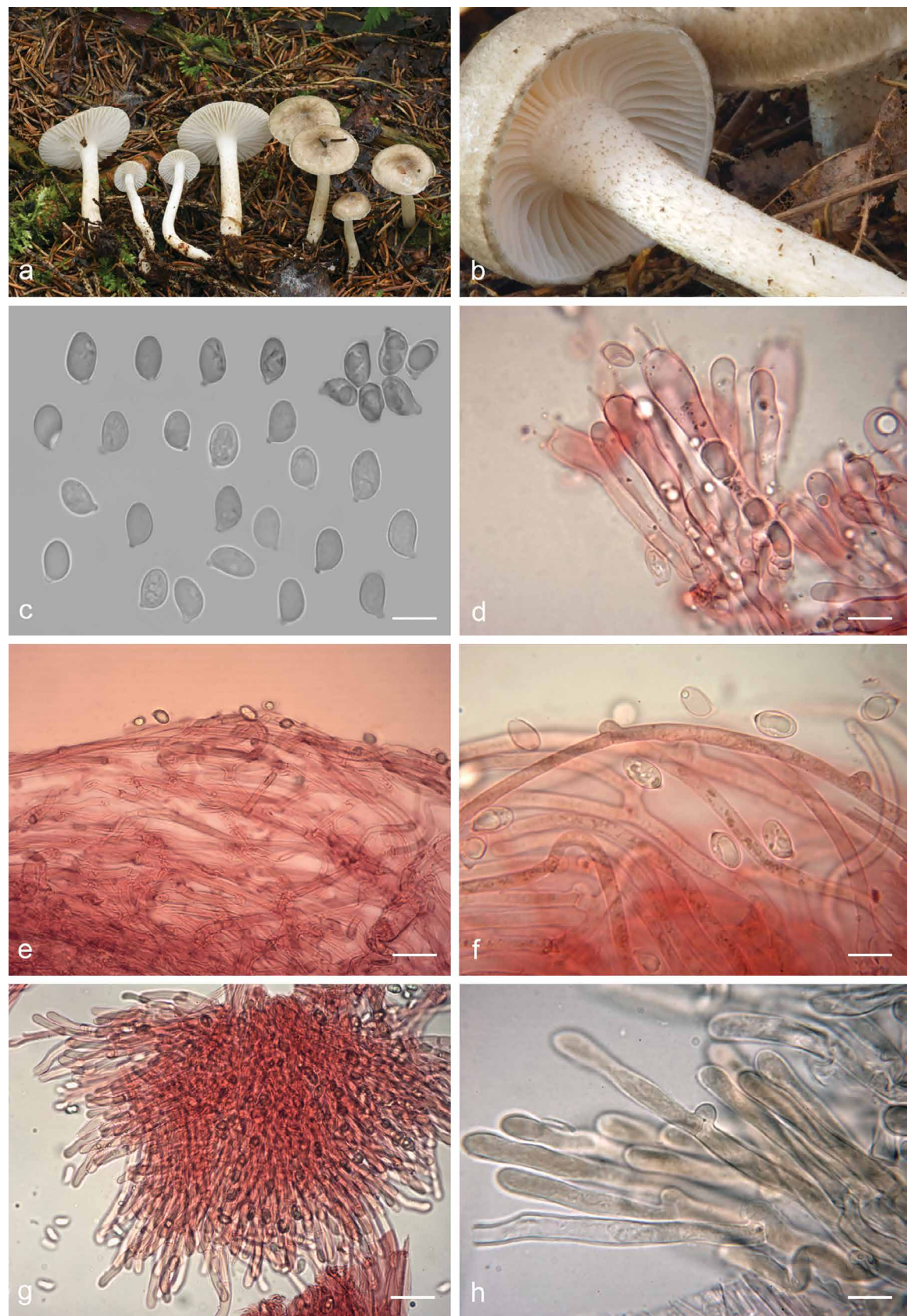
*Holotype.* CANADA, Quebec, Saint-Casimir, route du Rang Saint-Jérôme, in young *Picea glauca* plantation, in calcareous soil, in litter, N46°39'21.28" W72°10'41.16", 39 m asl, 15 Oct. 2018, *R. Lebeuf*, HRL2832/DAOM984764\* (isotype LIP), ITS GenBank MT981658.

*Diagnosis* — Medium-sized agaric fruiting in mid- to late fall under *Picea* spp. resembling *Hygrophorus pustulatus*, but differing from it by its non-squamulose pileus centre, microscopically by its less inflated thin-walled hyphae in the lamellar trama and by range restricted to North America.

*Pileus*  $15\text{--}50 \text{ mm}$  across, convex becoming applanate, at times with a conical umbo, depressed at centre with age, viscid, glutinous in wet conditions, pale greyish brown (5D3), darker at the disk, with appressed darker fibrils radiating under the gluten, at times appearing fibrillose in dry conditions; margin decurved when young, then straight, at first bearing a white tomentum. *Lamellae* broadly adnate to shortly decurrent, arcuate, distant, rather thick, waxy, off-white, intervened,  $2\text{--}5 \text{ mm}$  wide,  $L = 25\text{--}34$ ,  $I = 1\text{--}2$ , with smooth edge. *Stipe*  $25\text{--}60 \times 3\text{--}11 \text{ mm}$ , cylindrical, equal or tapering downwards, dry, white, solid, at least in the upper half covered with white floccules soon turning brown. *Context* white, thin, thicker toward the disk; odour indistinct, taste mild. *Spore deposit* white.

*Spores* ( $n = 300$ ) ellipsoid, oblong, ovoid, or amygdaliform, smooth, hyaline, thin-walled, monoguttulate or aguttulate in Congo Red, inamyloid,  $(7.0\text{--}8.0\text{--}9.4\text{--}11.0\text{--}12.0) \times 4.5\text{--}5.5\text{--}6.0\text{--}7.0) \mu\text{m}$ ,  $Q = 1.60\text{--}1.71\text{--}1.85$ , with a wide and obtuse hilar appendage. *Basidia*  $37\text{--}60 \times 8\text{--}11 \mu\text{m}$ , 4-spored, narrowly clavate; sterigmata  $4\text{--}7 \mu\text{m}$  long. *Hymenophoral trama* divergent, not gelatinized, made up of  $25\text{--}120 \times 3\text{--}15 \mu\text{m}$ , cylindrical to inflated, rarely branched and thin-walled hyaline hyphae. *Subhymenium* ramose, not gelatinized, made of short hyphae  $3\text{--}4 \mu\text{m}$  wide. *Hymenopodium* undifferentiated. *Cystidioid elements* not found. *Pileipellis* an ixotrichoderm or in places an ixocutis  $50\text{--}200 \mu\text{m}$  thick, made up of gelatinized, branched and thin-walled  $2\text{--}7 \mu\text{m}$  wide hyphae, either hyaline or with brown intracellular pigment; outermost hyphae often repent. *Pileitrampa* made up of subparallel, non-gelatinized, cylindrical to inflated, branched, hyaline, thin-walled hyphae  $5\text{--}17 \mu\text{m}$  wide. *Stipitipellis* a cutis of cylindrical, thin-walled, non-gelatinized hyphae  $2\text{--}6 \mu\text{m}$  wide. *Stipititrampa* made up of parallel, thin-walled hyphae  $3\text{--}14 \mu\text{m}$  wide. *Floccules* on stipe made of large tufts





**Fig. 14** *Hygrophorus pustulatoides* sp. nov. a. Collection HRL2832/DAOM984764 *in situ* (holotype); b. details of stipe; c. spores; d. hymenium; e–f. pileipellis, radial section; g–h. floccules on stipe. — Scale bars: c–d, f, h = 10 µm; e, g = 25 µm. — Photos by: a–b. R. Lebeuf.

of erect, multiseptate hyphae with brown intracellular pigment; terminal cells mostly cylindrical, less commonly subclavate at the apex, 18–52(–98) × 4–6 µm, or when subclavate 7–8 µm wide at the apex. *Clamp connections* present in all tissues, variously shaped; medallion clamps frequent, particularly in the pileipellis and stitipipellis.

**Chemical reactions** — KOH, NH<sub>4</sub>OH and FeSO<sub>4</sub> all negative on pileus, context and apex or mid-stipe of fresh basidiomata. Base of the stipe in exsiccata staining pale yellow to yellow-orange with KOH.

**Ecology, Phenology & Distribution** — Gregarious under *Picea* in needle litter, in either plantations or natural forests, in

acidic or basic soil. Fruiting in the fall until the first deep frosts, preferring cold temperatures. So far known from British Columbia and Quebec in Canada and from Oregon, Washington State and Alaska in the USA.

**Edibility** — Probably edible, but not choice. No toxicological studies have been carried out so far.

**Other collections studied.** CANADA, Quebec, Sainte-Anne-de-Bellevue, Arboretum Morgan, under *Picea* sp., N45°25'35.38" W73°56'34.22", 61 m asl, 24 Oct. 2011, R. Lebeuf, HRL1040\*; Montreal, 25 Sept. 1997, Y. Lamoureux, CMMF003147\* (as *H. pustulatus*). — USA, California, Humboldt Co., Arcata, 2269 Fickle Hill Road, scattered under *Picea sitchensis*, 27 Jan. 2019, N. Siegel, NS3590\*; Trinidad Beach SP, Elk Head, scattered under *Picea sitchensis*, 28 Jan. 2019, N. Siegel, NS3599/HRL2890\*; Oregon, Sherman Co., Wasco, under *Alnus* sp., *Pinus* sp., *Tsuga* sp., 1 Oct. 2018, S. Svantesson, SS508\* (GB); Washington, Pend Oreille Co., Gypsy Meadows FS 2220, in area with *Picea* and *Abies* predominating, 1280 m asl, 23 Sept. 2012, A.D. Parker, Mushroom Observer 111246\* (as *H. pustulatus*).

**Notes** — This species was totally cryptic prior to the present study and was likely confused with its sister species *H. pustulatus*. Only Melot (1981) and Bon (1990) suggested, without personal experience of the North American mushroom, that the basal viscosity of Hesler & Smith's *H. pustulatus* (1963) could make it distinct from the European species. Phylogenetically analysing a biogeographically broad ITS sequence dataset, however, including 10 vouchered specimens, unveiled a well-supported North American clade, sister to a weakly supported clade including sequences originating from both continents (Fig. 1c). The two species differ by 4 substitutions and 1 indel (Fig. 2b; Suppl. Table 2). Based on available sequences, *H. pustulatoides* appears to be more common than *H. pustulatus* in North America, and is confirmed from both coasts of the continent. Morphologically, *H. pustulatoides* differs from its sister species by a non-squamulose pileus at the centre and microscopically by narrower hyphae in the lamellar trama. It may also be mistaken for *H. agathosmoides* in its natural range, especially in cold temperatures when the typical bitter-almond odour is absent, but the latter species is generally more robust, bears a partial veil visible on the pileus margin on young specimens, and has a stipe developing paler brown floccules with age, also with a negative KOH reaction at the stipe base. *Hygrophorus odoratus* (including the possibly conspecific *H. morrisii*), another species with normally a bitter-almond odour, so far confirmed from both coasts of North America, has larger spores (11–14 × 6.5–8 µm acc. to Hesler & Smith 1963; or 9.5–12.5(–14) × 6.8(–8.5) µm acc. to Bird & Grund 1979), has a glabrous to minutely scabrous stipe, and stains lemon yellow when bruised or with age (Hesler & Smith 1963, Bird & Grund 1979). A few other species, such as *Hygrophorus agathosmus*, *H. hyacinthinus*, *H. pinophilus*, *H. exiguus* or *H. suaveolens*, may also resemble *H. pustulatoides*, but all emanate strong odours in normal conditions and only the last two are so far confirmed in North America (Arnolds 1990, Bon 1990, Larsson et al. 2014, 2018).

**Hygrophorus sect. Limacini** P.-A. Moreau, Bellanger, Loizides & E. Larss. sect. nov. — MycoBank MB 836867; Fig. 15

**Type species.** *Hygrophorus limacinus* (Scop.) Fr., Epicr. Syst. Mycol. (Upsaliae): 324. 1838.

**Etymology.** From the type species *Hygrophorus limacinus*.

**Diagnosis** — Species similar to those in sect. *Olivaceoumbriini* due to their dark olive brown pileus colours but producing usually larger or stouter basidiomata, often in fascicles, very glutinous and slippery in wet conditions. From literature and tested species, the reaction to alkali is usually strong to intense, while species in sect. *Olivaceoumbriini* yield a negative or weak KOH reaction, mostly confined to the stipe base. Most species are associated with broadleaved trees, especially in the Mediterranean region.

**Notes** — In its current context, this newly erected section forms a moderately supported clade and encompasses 9 lineages represented in both North America and Europe: *H. glutinosus* s. auct., *H. limacinus* (Fig. 15a), *H. limosus* sp. nov., *H. megasporus* s. auct., *H. paludosoides* (Fig. 15d), *H. glutinifer*, and three yet-to-be-named phylopecies referred to here as *H. sp. 1–3* (Fig. 1d, 15e–f). The type species of this new section is often referred to as *H. latitabundus*, following a late interpretation of the Britzelmayer binomial by Arnolds (1979). However, Papetti (2016) showed that the earlier name *H. limacinus* is the correct one for this species and stabilized it by epitypifying it with a sequenced Italian collection included in Fig. 1d. *Hygrophorus limacinus*, together with *H. glutinifer*, were until now treated together with *H. olivaceoalbus*, *H. mesotephros* and *H. bakerensis* in subsect. *Olivaceoumbriini* (Lodge et al. 2014). However, all available molecular phylogenies, including ours, indicate that such a grouping is polyphyletic and thus artificial (Larsson 2010, Lodge et al. 2014, this work Fig. 1a). Conversely, although *H. limacinus* and *H. glutinifer* are quite distant phylogenetically, they are consistently retrieved in a moderately to strongly supported clade. The sampling effort undertaken for the present work identified 7 lineages evolutionarily related to either one of these two species, while maintaining the sister-clade relationships of the two groups (Fig. 1d). As introduced here, sect. *Limacini* documents the previously unsuspected taxonomic vicinity of a few North American species to *H. limacinus* or *H. glutinifer*, respectively, phylogenetically positions a species new to science, *H. limosus*, as well as *H. sp. 1–3* in this section, and contributes to the monophyly of sect. *Olivaceoumbriini*. The green reaction of the mucus in alkali is not exclusive to *H. glutinifer* and is also present in '*H. sp. 2*'; data are lacking on the other yet unnamed species, but it could represent a synapomorphy for this subclade if confirmed.

**Hygrophorus limosus** Loizides & Bellanger, sp. nov. — MycoBank MB 836865; Fig. 15c, 16

**Etymology.** From '*limosus*' adj. (Latin), meaning glutinous or muddy, referring to the heavily glutinous pileus of the species.

**Holotype.** CYPRUS, Prodromos, in *P. nigra* subsp. *pallasiana* and *Sorbus aria* forest on serpentine soil, 3 Nov. 2018, D. Markides, ML81113HL2/MPU814001\*, ITS GenBank MT981620.

**Diagnosis** — Medium- to large-sized waxcap, very similar to *Hygrophorus limacinus* but producing darker and heavily glutinous basidiomata with a negative pileal reaction to alkali, a slender rooting stipe with prominent greyish brown bands, slightly larger spores (7.5–)9–12.5(–16) × (5.5–)7–8.5(–9.5) µm, and a marked preference for serpentine substrates. Found from late October to late December in high altitude *Pinus nigra* subsp. *pallasiana* forests, on the island of Cyprus.

**Pileus** 40–70(–110) mm, subglobose to campanulate with a deeply involute margin when young, gradually expanding to conico-convex or plano-convex and typically maintaining a broad dark umbo in the middle; very young basidiomata buff or pale brown in colour, soon becoming dark brown and greyish brown to black towards the centre, heavily glutinous and sticky, innately fibrillose under the mucus, typically with needles and debris firmly stuck onto the cuticle when dried. **Lamellae** adnate to subdecurrent at first, subdecurrent to decurrent in full maturity, thick and waxy, sometimes anastomosing and intercepted by lamellulae, moderately crowded, L = 60–70 (excluding lamellulae), white, usually with a faint salmon-pink reflection, drying cream. **Stipe** 6–12 × 1.5–3 cm, cylindro-clavate to ventricose, often flexuous or bent and tapering-rooting towards the base, with a well-defined annular zone below the apex, smooth or finely pruinose and pure white above the annular zone, covered by successive bands of thickly glutinous, dark brown or greyish brown flocci below, drying grey-black; partial veil glutinous, translucent, upon drying becoming membra-





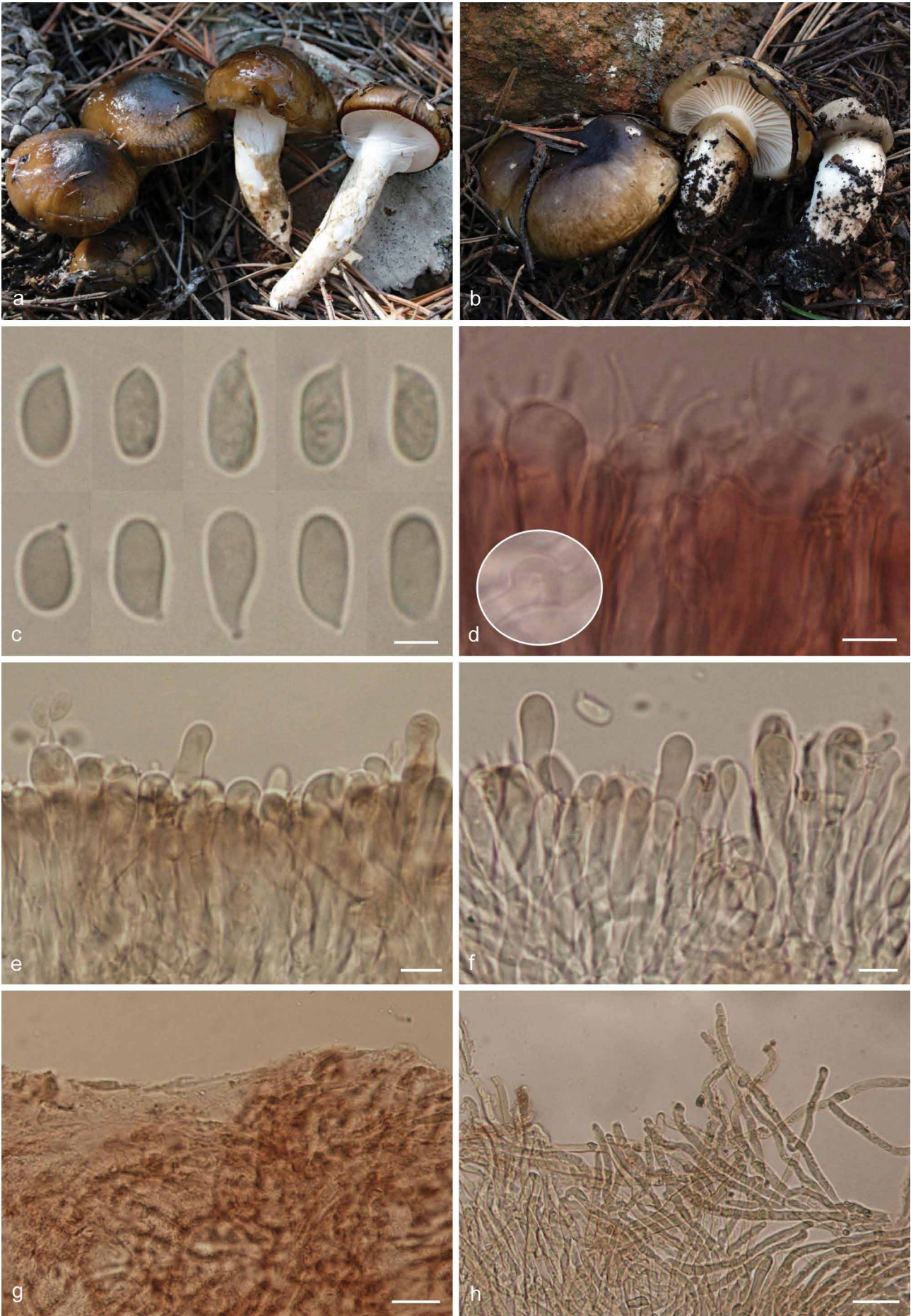
**Fig. 15** Overview of sect. *Limacini*. a. *H. limacinus* EL452-17 (not sequenced); b. *H. glutinifer* EL214-14; c. *H. limosus* ML80111HL (not sequenced); d. *H. paludosoides* YL4339; e. *H. sp.* 1 HRL1647; f. *H. sp.* 3 RGT131109-02. — Scale bar: d = 5 cm. — Photos by: a–b. E. Larsson; c. M. Loizides; d. Y. Lamoureux; e. A. Paul; f. R.G. Thorn.

nous, wafer-thin and opaque white. *Context* thick, somewhat cartilaginous, pure white, more or less odourless, or with a weak, faintly herbaceous odour when cut; taste mild, indistinct. *Spore deposit* white.

*Spores* ( $n = 100$ ) variable in size and shape, ellipsoid to broadly ellipsoid or somewhat ovoid, measuring  $(7.5\text{--})9\text{--}12.5\text{--}(16) \times (5.5\text{--})7\text{--}8.5\text{--}(9.5) \mu\text{m}$ ;  $\text{Me} = 10.6 \times 6.8 \mu\text{m}$ ; average  $Q = 1.14\text{--}1.5\text{--}2$ , with an oblique hilar appendage  $1\text{--}2 \mu\text{m}$  long; inamyloid, hyaline and smooth or microguttulate in water, appearing monoguttulate in KOH. *Basidia* 2–4-spored, clavate,  $43\text{--}59 \times 8\text{--}12 \mu\text{m}$ , some with oily refractive content in KOH; sterigmata  $4\text{--}7 \mu\text{m}$  long. *Cystidioid elements*  $29\text{--}69 \times 7\text{--}13 \mu\text{m}$ , basidiomorphous to polymorphic, long cylindrical, somewhat flexuous or subcapitate, rarely also capitate, thick-walled, often filled with refractive granulose content. *Hymenophoral trama* divergent, comprised of cylindrical, interwoven, frequently branched and thick-walled hyphae  $3.5\text{--}8\text{--}(10) \mu\text{m}$  wide, terminating in

inflated, clavate end-cells up to  $11 \mu\text{m}$  across. *Pileipellis* an ixotrichoderm composed of thick-walled, hyaline or brown-pigmented interwoven hyphae  $2\text{--}4.5 \mu\text{m}$  wide, sparsely branching and embedded in a gelatinous matrix; incrustations or extracellular pigment not seen. *Subpellis* composed of more or less parallel hyphae up to  $7 \mu\text{m}$  wide. *Stipitipellis* an ixotrichoderm, made up of extensively incrustated, loosely interwoven and occasionally branched hyphae  $4\text{--}5 \mu\text{m}$  wide, with yellowish brown intracellular pigment; terminal elements rounded to attenuated, not enlarged. *Stipititrama* composed of densely packed, more or less parallel arranged, subhyaline to yellowish brown and extensively incrustated hyphae  $3.5\text{--}9 \mu\text{m}$  wide. *Floccules* at the apex made up of compact tufts of tortuous, interwoven, branched and thick-walled hyphae  $2.5\text{--}5 \mu\text{m}$  wide, with attenuated terminal elements; incrustations not seen. *Clamp connections* abundant in all tissues, somewhat inconspicuous at the bases of basidia and basidioles, more distinct in the





**Fig. 16** *Hygrophorus limosus* sp. nov. a–b. Collections ML80111HL (not sequenced) & ML810192HL *in situ*; c. spores; d. basidia (insert: basal clamp); e–f. hymenium with basidia, basidioles and cystidioid elements; g. pileipellis, hyphae embedded in gelatinous matrix; h. floccules on stipe. — Scale bars: c–d = 5 µm; e–f = 10 µm; g–h = 20 µm. — Photos by: a–b. M. Loizides.

pileal and stipe hyphae where they are frequently present as prominent medallions.

**Chemical reactions** — KOH reaction negative on the pileus cuticle, but weakly to moderately yellow at the stipe apex and strongly ochraceous-yellow to briefly orange at the stipe base, slowly fading after several minutes.  $\text{NH}_4\text{OH}$  reaction negative on all parts of the basidioma.

**Ecology & Distribution** — Growing in small, sometimes caespitose groups, typically in high altitude (> 1200 m asl) *Pinus nigra* subsp. *pallasiana* forests. So far known only from the island of Cyprus, where it fruits from late October to late December, predominantly on serpentine substrates.

**Edibility** — Probably edible and sometimes collected for the table by the locals, though no toxicological studies have been carried out.

**Other collections studied.** CYPRUS, Karvounas, in *P. nigra* subsp. *pallasiana*, *Quercus alnifolia* and *Arbutus andrachne* forest on serpentine soil, 13 Nov. 2018, M. Loizides, ML811131HL\*; Pera pedi, in *Quercus coccifera* subsp. *calliprinos* and *P. brutia* forest on calcareous soil, 16 Jan. 2019, M. Loizides, ML91161HL\*; *ibid.*, M. Loizides, ML81113HL3; *ibid.*, in *P. nigra* subsp. *pallasiana* forest on serpentine soil, 1 Dec. 2018, M. Loizides, ML81211HL; Trooditissa, in *P. nigra* subsp. *pallasiana*, *Quercus alnifolia* and *Arbutus andrachne* forest on serpentine soil, 9 Nov. 2018, M. Loizides, ML81119HL; Troodos, in *Pinus nigra* subsp. *pallasiana* and *Sorbus aria* forest on serpentine soil, 30 Oct. 2008, M. Loizides, ML800103HL; *ibid.*, 1 Nov. 2008, ML80111HL; *ibid.*, in *P. nigra* subsp. *pallasiana* forest on serpentine soil, 17 Oct. 2009, M. Loizides, ML900171HL\*; *ibid.*, 19 Nov. 2014, ML411191HL; *ibid.*, 29 Oct. 2018, ML810192HL\*; *ibid.*, 3 Nov. 2018, ML81113HL1 & ML81113HL-2; *ibid.*, 20 Nov. 2018, M. Loizides, ML811102HL1, ML811102HL2, ML811102HL3 & ML811102HL4; *ibid.*, 2 Dec. 2018, M. Loizides, ML81212HL; *ibid.*, 7 Dec. 2018, ML81217HL.

**Notes** — Prior to this work, *H. limosus* had been overlooked and erroneously reported as '*H. latitabundus*' (= *H. limacinus*) in Loizides et al. (2011), due to its close morphological resemblance to the latter. However, the autonomy of *H. limosus* is strongly supported, as is its sister clade relationship to *H. limacinus*, even though the two species are quite distant from an evolutionary point of view, with no less than 56 substitutions and indels distinguishing them at the ITS locus (Fig. 1d; Table 2). Besides these phylogenetic differences, *H. limacinus* has a markedly calciphilous ecology and produces more robust and usually paler basidiomata with a stout, clavate to broadly ventricose stipe (Britzelmayer 1899, Arnolds 1979). Moreover, the pileus of *H. limacinus* stains orange with KOH, whereas none of our numerous collections of *H. limosus* showed a positive pileal reaction to alkali, but only an ochraceous-yellow reaction at the apex and an ochraceous-yellow or sometimes orange reaction at the stipe base. Microscopically, *H. limosus* appears to have larger spores on average than *H. limacinus*, albeit with high inter-individual variability and a very broad spore quotient of 1.14–2. Arnolds (1979, 1990), Breitenbach & Kränzlin (1991) and Candusso (1997) reported spores ranging from (7.8–)8.1–11.5(–13.4)  $\times$  4.7–7.7(–8.1)  $\mu\text{m}$  to 9.5–11.3(–14)  $\times$  6–7.5(–8)  $\mu\text{m}$  for *H. limacinus*, averaging 9.8  $\times$  6.2  $\mu\text{m}$ , as opposed to a spore average of 10.6  $\times$  6.8  $\mu\text{m}$  observed in *H. limosus*. So far, the known distribution range of the two sister-species does not appear to overlap, with *H. limosus* molecularly verified only from the island of Cyprus. *Hygrophorus glutinifer* is the second European taxon in the new section introduced here. This species, however, is exclusively associated with broadleaved trees (*Quercus* in particular), has a chestnut or honey-brown to ochraceous-brown pileus usually yielding a blue-green reaction with  $\text{NH}_4\text{OH}$ , and a stipe with paler, ochraceous-brown to olivaceous-brown bands, turning reddish orange at the base with KOH. Microscopically, it has narrower spores than *H. limosus*, measuring 8.1–10.8(–11.3)  $\times$  (5.3–)5.5–6.5(6.7)  $\mu\text{m}$  (Arnolds 1979). In sect. *Olivaceoumbrini*, *H. olivaceoalbus* has similar greyish

brown pileal colours, as well as olivaceous-brown bands on the stipe and spores similar to those of *H. limosus*, but produces distinctly smaller and slender basidiomata (< 6 cm across). The former also appears to be exclusively associated with *Picea* in northern localities (particularly Fennoscandia) and crystalline mountains of western and central Europe, and is yet to be molecularly confirmed in the Mediterranean region. In sect. *Aurei*, the recently described *H. meridionalis* Loizides et al. (Moreau et al. 2018) bears some superficial resemblance to *H. limosus*, mainly because of its dark pileal colours and glutinous bands on the stipe darkening with age. The two species co-occur in Cyprus, but are nonetheless easily distinguished in the field: *H. meridionalis* has an olivaceous-grey to almost black pileus usually lacking brown tinges, more distant arcuate-decurrent lamellae becoming yellowish in mature basidiomata and a different ecology, found in calcareous woodland and matorral in association with *Pinus brutia* and *P. halepensis*. *Hygrophorus meridionalis* also has a negative reaction to KOH.

### *Hygrophorus* sect. *Fuscocinerei* Bon ex Bellanger, P.-A.

Moreau & E. Larss., sect. nov. — MycoBank MB 836868; Fig. 17a–c

**Basionym.** Based on '*Hygrophorus* (sect. *Olivaceoumbrini*) subsect. *Fuscocinerei* (Fr.) Bon' in Bon, Doc. Mycol. 27–28: 39. 1977, inval. (see Notes below).

**Type species.** *Hygrophorus mesotephurus* Berk. & Broome, Ann. Mag. Nat. Hist. 13: 402, t. 15: 2. 1854.

**Diagnosis** — Small-sized and slender taxa, characterized by glutinous basidiomata, without or with ephemeral partial veil. Pileus with pale greyish to fuscous to olive-grey colours, but not pure white and strongly contrasted radially, with the centre distinctly darker than the margin. Stipe covered by a glutinous coating, up to a more or less well-delimited annular zone, but with no, or small traces of inner fibrils underneath.

**Notes** — Currently, this section encompasses *H. adiaphorus* (incl. *H. betulae*, see below and Fig. 17b), *H. mesotephurus* and an unnamed lineage represented by a single collection from Tennessee (USA) (Fig. 1a, Fig. 17c).

***Hygrophorus adiaphorus*** Hesler & A.H. Sm., North American Species of *Hygrophorus*: 295. 1963 — MycoBank MB 332217; Fig. 17b

? **synonym.** *Hygrophorus betulae* K. Bendiksen & E. Larss. in Larsson & Bendiksen, Karstenia 58: 4. 2020.

**Emended taxonomic description** — *Pileus* 15–35 mm diam, convex, with slight umbo when young, then plano-convex to flattened, later somewhat depressed in the centre, grey or pale whitish with an olive-grey tint, and with a distinct darker olivaceous-grey or fuscous-blackish centre, rough to finely scaly or with fine dark fibrils, strongly glutinous when moist. *Lamellae* adnate to shortly decurrent, L = 26–32, distant to subdistant, thick, waxy, with lamellulae, richly veined, white to cream coloured, when dried cream pallid or the edge yellowish. *Stipe* 35–75  $\times$  4–7 mm, cylindrical or slenderly subfusiform, tapering towards the base, at apex white, dry, first pruinose with minute floccules, with age smooth, below glutinous with light greyish velum, in the holotype no fibrillose velum present, base glutinous pale grey to yellowish brown tone, context whitish. Taste and smell indistinct. Spore deposit white.

**Spores** ( $n = 125$ ) elliptical, subelliptical to ovoid, with an obtuse hilar appendage, smooth, hyaline, inamyloid, (8.5–)10.1–10.2–10.3(–11.7)  $\times$  (4.8–)6.0–6.1–6.2(–6.9)  $\mu\text{m}$ , average  $Q = 1.65$ –1.68–1.70. *Basidia* mainly 4-spored, a few 1- or 2-spored, or, in the holotype, mainly 2-spored with about 10 % 1-spored and exceptional 4-spored, narrowly clavate, (33–)42–47(–54)  $\times$  5–8(–9)  $\mu\text{m}$ , sterigmata 6–8  $\mu\text{m}$  long, in KOH with oil drops,





**Fig. 17** Overview of sect. *Fuscocinerei* and sect. *Nudolidi*. a. *H. mesotephurus* EL338-11; b. *H. betulae* (? = *H. adiaphorus*); c. *H. cf. mesotephurus* TENN071861; d. *H. tennesseensis* HRL0940 (published as *H. bakerensis*); e. *H. bakerensis* (not sequenced but from Mt Baker, WA-USA). — Photos by: a. E. Larsson; b. K. Bendiksen; c. R.A. Swenie; d–e. R. Lebeuf.

in the holotype a reddish reaction with Melzer's reagent. *Cystidioid elements* not found. *Lamellar trama* bilateral to divergent, composed of interwoven thin-walled cylindrical inflated hyphae 10–12  $\mu\text{m}$  wide with terminal end cells up to 25  $\mu\text{m}$  wide. *Pileipellis* an ixotrichoderm up to 300  $\mu\text{m}$  thick; made up of loosely arranged interwoven, branched hyphae 1.5–5.5  $\mu\text{m}$  wide, in matrix smooth, hyaline or with intracellular pigments, upper layer very loosely interwoven, hyaline and pigmented hyphae, with extracellular granules in matrix. *Subpellis* composed of densely arranged, sub-parallel interwoven inflated hyphae up to 25  $\mu\text{m}$  broad. *Stipitipellis* an ixotrichoderm, up to 200  $\mu\text{m}$  thick, made up of 3.5–5.5  $\mu\text{m}$  wide interwoven branched hyphae, with scattered free end cells, smooth, hyaline, or with intracellular pigments. *Stipititrampa* of hyaline more or less parallel interwoven hyphae 5.5–8  $\mu\text{m}$  wide. *Floccules* at apex made up of compact erect interwoven cylindrical branched hyphae, 3–5  $\mu\text{m}$  wide, with slightly enlarged end cells up to 6.5  $\mu\text{m}$  wide. *Clamp connections* few but distinct in all tissues, regularly present in hymenial tissues and on epicuticular hyphae, or, as

in the holotype, absent from the hymenium but observed at the base of terminal elements of the pileipellis.

**Notes** — *Hygrophorus adiaphorus* and *H. betulae* are basically identical in the ITS sequence data as they only differ by a unique 5 nt-long indel in the ITS2 region (Table 2). In morphology, the measurements of pileus, stipe and lamellae, and of spores and basidia are more or less the same. However, there are also differences in the two descriptions. The pileus colour in *H. adiaphorus* is described as much darker (grey to cinereous with fuscous to blackish disc) than in *H. betulae*, where it is rather nearly white with an olive-grey disc zone. In *H. adiaphorus*, clamp connections are restricted to terminal elements of the pileipellis whereas in *H. betulae*, clamps are rather few but regularly occurring in the hymenium and on the epicuticular hyphae, and present in all tissue. In *H. adiaphorus*, basidia are mainly 2-spored whereas in *H. betulae*, 4-spored basidia dominate. The microscopic differences between *H. adiaphorus* and *H. betulae* seem then to be gradual, with the frequency



of 4-spored basidia and of clamp connections apparently correlated. In *H. adiaphorus*, the oil drops of basidia turn reddish in Melzer's reagent, but this reaction was not observed in *H. betulae*. The two also differ in their ecology, as the holotype of *H. adiaphorus* was collected under *Picea* and *Abies* in Wilderness State Park in Michigan (USA), whereas *H. betulae* is so far only found associated with *Betula pubescens* in the subalpine zone of Finland.

The species is obviously rare and no additional ITS sequence data are available in GenBank or UNITE databases to better evaluate its morphological, micro-anatomical, ecological and biogeographical plasticity. In an evolutionary point of view, *H. adiaphorus* is almost equidistant from the European *H. mesotephros* and the unique North American collection TENN071861 (Fig. 1a; Table 2).

***Hygrophorus* sect. *Nudolidi* Bellanger & Lebeuf, sect. nov. —**  
MycoBank MB 836869; Fig. 17d–e

*Type species. Hygrophorus tennesseensis* A.H. Sm. & Hesler, Lloydia 2: 40. 1939.

*Etymology.* Contraction of *Nuda*, i.e., naked, without any veil, and *Olida*, i.e., smelling, the two currently known species in the section combining these two features.

*Diagnosis* — Medium to large-sized fleshy basidiomata with a glutinous brown, tawny to ochraceous pileus, cream to white at the margin, stipe whitish to pale pinkish buff and dry, without any trace of glutinous veil or partial veil, and odour of bitter almonds or raw potatoes; taste mild or bitter. So far restricted to conifers of North America.

*Notes* — Currently includes *H. tennesseensis* (Fig. 17d) and *H. bakerensis* (Fig. 17e). *Hygrophorus bakerensis* was included in *Olivaceoumbrini* s.lat. but available phylogenies, including ours, indicate it lies in an isolated position within subg. *Colorati*, somehow intermediate between sect. *Aurei* and the other lineages of subsect. *Olivaceoumbrini* s.lat. (Larsson 2010, Lodge et al. 2014, this work Fig. 1a). Together with *H. tennesseensis*, this species forms a strongly supported clade that we introduce here at the section rank to yield a monophyletic sect. *Olivaceoumbrini* and, more generally, confer to the infra-generic taxonomy of *Hygrophorus* an evolutionary backbone. From a morphoanatomical point of view, *H. tennesseensis* and *H. bakerensis* are very similar but, according to Hesler & Smith (1963), they can easily be distinguished by their odour, taste and biogeography: raw potatoes, bitter and east coast for the former vs almond, mild and west coast for the latter. However, the American authors reported two Californian collections of *H. tennesseensis* in their monograph (Hesler & Smith 1963) and, conversely, a recent sequenced collection from Quebec (HRL0940) displays almond odour and mild taste, as would be expected from *H. bakerensis*. These two species have been merged and referred to as *H. bakerensis* in Moreau et al. (2018), but closer inspection of sequences in the two clades reveals 14 segregating evolutionary events (4 SNPs and 10 indels, Table 2). The two species are thus phylogenetically supported as distinct taxa but more samples originating from both coasts will be necessary in this lineage to identify reliable diagnostic features for each of them.

## DISCUSSION

### *From monophyly of sect. Olivaceoumbrini to an evolutionary scaffold for Hygrophorus*

In its original (Friesian) concept, the *Olivaceo-umbrini* (un-ranked) grouped together hygrophori with similar dark olivaceous cap tinges. Although the taxonomic status of this group was later validated at the section rank by Konrad & Maublanc

(1937), its natural boundaries remained unresolved throughout the 20th century. The phylogenetic revision of the genus from Lodge et al. (2014) revealed the artificial nature of sect. *Olivaceoumbrini* by demonstrating that, from an evolutionary point of view, the section is polyphyletic. To provide an updated and informative arrangement of sect. *Olivaceoumbrini* and related taxa, we here propose, for the first time, a unified taxonomy for this cluster of taxa at a transcontinental scale. The novel systematics introduced in the present work reduces the original section to a core content including the type *H. olivaceoalbus* and the five closely related species *H. canadensis*, *H. fuscoalboides*, *H. korhonenii*, *H. marcocontui* and *H. whitei*<sup>1</sup>. In the revised delimitation, sect. *Olivaceoumbrini* is so far restricted to coniferous forests of North America, Europe and Turkey. Other sequenced species initially included in the section are now shown to belong in one of the following monophyletic sections: *Fuscocinerei*, *Limacini*, *Nudolidi* or *Tephroleuci*.

Beyond improving taxonomy of species historically classified in *Olivaceo-umbrini*, our work thus contributes to defining a natural arrangement in the genus congruent with its evolutionary history, and in line with other recent studies following this approach (Lodge et al. 2014, Larsson et al. 2018, Moreau et al. 2018). Still, this challenging task is far from complete, and necessitates two complementary future research axes. First, classical monographs still include old names in need of phylogenetic assessment and, in all likelihood, further revision. This is for example the case with *H. morrisii* and *H. paludosus* from Hesler & Smith (1963), whose identity and autonomy relative to confirmed species are still pending. Historical collection mining and next generation sequencing will certainly help fill some gaps in the years to come, but it might be necessary to designate sequenced neo- or epitypes in some cases to fix usage of these old binomials. Second, as our work clearly demonstrates, taxon sampling, especially in poorly explored geographical regions, is key to uncover overlooked biodiversity, even in well-known and widely studied genera like *Hygrophorus*. Therefore, both human efforts and funding should be invested to support forays, sampling and dedicated taxonomic studies, with a focus on hot-spots of biodiversity and neglected ecoregions. In this respect, the recent work of Wang et al. (2020) on subsect. *Hygrophorus* in China is viewed as a positive response to the global concern of the academic community to the threatened fungal biodiversity at a worldwide scale.

### *Evolution and taxonomic significance of morphoanatomical features in Hygrophorus*

The systematics in *Hygrophorus* has long been based on morpho-anatomical, ecological and organoleptic features, whose taxonomic value can now be objectively evaluated in light of DNA phylogenies. Not surprisingly perhaps, the colour of basidiomata does not constitute a consistent taxonomic criterion at the genus scale, as species displaying dark olivaceous hues on the cap belong in three phylogenetically supported sections here, with the exceptions of sect. *Tephroleuci* and *Nudolidi*. The presence and extent of a glutinous veil on the stipe has also been used to distinguish species within the historical *Olivaceoumbrini*, especially by Hesler & Smith (1963) for North American taxa. This criterion has been overemphasized at least within *H. fuscoalboides*, and probably depends on microclimatic and edaphic conditions of the collection site. The strong and typical odour of bitter almond of *H. agathosmus* is here shown to be a feature of most species of sect. *Tephroleuci*. However, this organoleptic taxonomic criterion cannot be viewed as a

<sup>1</sup> While this study was in press, Wang et al. (Mycoscience 2021) reported on a seventh species in sect. *Olivaceoumbrini*, *Hygrophorus annulatus*, so far restricted to China.

synapomorphy of the latter lineage because: i) it is absent (probably lost) in *H. pustulatus* and *H. pustulatoides*, or different in *H. hyacinthinus*; and ii) it is also present outside sect. *Tephroleuci*, in sect. *Nudolidi*. Ecology has also been used to discriminate between similar-looking species in the genus. With the notable exception of *H. limacinus* (Papetti 2016) and possibly *H. adiaphorus* (Larsson & Bendiksen 2020), all species dealt with in the present work are associated either with coniferous trees or with broadleaved trees, but much more inclined to form associations with the former host type. It is worth noting, in this respect, that all known broadleaved-associated taxa are restricted to sect. *Limacini* and sect. *Fuscocinerei*, suggesting that host trees have been, and probably still are, major drivers of the evolution in hygrophori. Similarly, substrate conditions may have also contributed to speciation processes, such as in the case of *H. limosus*, which is almost exclusively found in high elevation ultramafic and serpentinized substrates on the island of Cyprus. Allopatric speciation (including insularity) seems to have occurred in all five lineages identified here, as most species are either continentalized or regionalized, with at least *H. alboflocosus*, *H. limosus*, *H. marcocontui* and *H. whitei* appearing as narrowly endemic to their respective ecoregions. In marked contrast, five species have been successful in colonizing transcontinental ecosystems with a probably Holarctic distribution: *H. agathosmoides*, *H. exiguus*, *H. olivaceoalbus*, *H. pustulatus* and *H. suaveolens*.

#### **Fine tuning species limits in *Hygrophorus* – difficult choices for difficult problems**

In the course of this study, we have been faced with challenging issues in defining species limits for some lineages, as a result of the difficult task of translating DNA phylogenies into a meaningful taxonomy. In the case of *H. exiguus*, unusually high variability of ITS sequences between populations from both sides of the Atlantic could have led us to recognize a cryptic Canadian species. However, considering the levels of genetic polymorphism between sympatric populations on the one hand, and the lack of significant morpho-anatomical and ecological differences between all collections on the other hand, we adopted the conservative taxonomic option of a single transcontinental species. In this case, we could interpret the unusual sequence variability of the ITS locus as indicative of a putatively accelerated evolution rate due to the parasitic trophic mode of *H. exiguus*. The pair *H. adiaphorus*/*H. betulae* displayed the opposite profile of two taxa with sub-identical ITS sequences, but displaying a number of morpho-anatomical, ecological and biogeographical differences that may justify keeping the two species separate. However, considering the very limited sampling of the clade, the possible presence of overlooked birches in the conifer forest from which the holotype of *H. adiaphorus* has been collected, and the high-resolution power of the ITS locus in *Hygrophorus*, further confirmed in the present work, we treated these two published names as synonyms, at least until more definitive data become available. The distinction of *H. whitei* from *H. fuscoalboideus* was not straightforward either, because recent collections falling in the two clades were not easy to distinguish taxonomically or ecologically. However, as a biogeographic signal appeared to support their phylogenetic sister-clade relationship, we tentatively maintained them as distinct species.

The most problematic case to deal with has certainly been that of *H. agathosmoides*. This plesiomorphic lineage relative to *H. agathosmus*, *H. alboflocosus* and *H. pinophilus* had been entirely cryptic prior to the work of Larsson et al. (2018), and furthermore does not constitute a monophyletic clade. Detailed inspection of ITS polymorphisms across a broad sampling of sequences falling in this group and in *H. agathos-*

*mus*, showed that despite the limited number of differences between the two groups of sequences, there is no evidence of gene flow, supporting two distinct species. Whilst this refined the 'outer boundaries' of *H. agathosmoides*, however, we still had to deal with the taxonomic status of some populations with astonishingly deviating phenotypes. In the Trabzon massif of Turkey, several collections displayed a combination of morpho-anatomical and organoleptic features that would easily prompt any field mycologist to describe them as a distinct species, in particular unusually inflated cystidioid elements on lamellar faces and a hyacinth-like odour. However, a posteriori careful observation of all the material phylogenetically assigned to *H. agathosmoides* revealed that: i) not all Turkish collections harboured such hymenial sterile structures; and ii) most of the western North American collections also featured similar inflated structures, although not exactly of identical shape and size. The presence of cystidioid elements on lamellae of *H. marcocontui* (this work) and of *H. yadigarii* (Sesli et al. 2018), both from the same mountainous region of Turkey, in addition to the presence of intermediate protruding or subcapitate structures in collections of *H. limosus* from Cyprus, suggests that some local or ecological cues may favour these structures. Beyond, these observations obviously recall old questions on the origin, significance and definition of *cystidia* (Léveillé 1837), which have been variously interpreted and subdivided over the years, an issue recently revisited by Loizides et al. (2020). The hyacinth-like odour itself is quite different from that of bitter almond, but the sister-clade relationship of *H. hyacinthinus* with the *H. agathosmoides*/*H. agathosmus*/*H. alboflocosus*/*H. pinophilus* complex suggests that the two underlying biosynthetic pathways may not be so distant, after all. These, in turn, might easily switch from one to the other, perhaps triggered by a limited number of genetic, epigenetic or environmental cues. Taking all this into consideration, we have concluded that *H. agathosmoides* is indeed a 'good species', having a remarkably broad biogeographical range and exhibiting extraordinary phenotypic plasticity, and hence introduced f. *trabzonensis* as a taxon to designate the strongly deviating Turkish collections.

Central to the taxonomic treatment of all critical cases described above has always been human choice, educated and guided by careful consideration of presently available taxon and biogeographical sampling. As such, our integrative analyses of both molecular and non-molecular data might ultimately be viewed as either a strength or a weakness of the present work, depending on one's vision of taxonomy in the DNA era. However, such multi-source approaches have successfully disentangled diversity within several critical genera in recent years, in an increasing effort to shed light on 'grey areas' of speciation and translate molecular phylogenies into meaningful taxonomic arrangements (Dayrat 2005, Will et al. 2005, Schlick-Steiner et al. 2009, Padial et al. 2010, Stadler et al. 2014, Zervakis et al. 2014, Zamora et al. 2015, Loizides et al. 2016, Wei et al. 2016, Zamora & Ekman 2020). This challenging task has in this case been made possible by complementing molecular data with rigorous morpho-anatomical, chemotaxonomical, developmental and behavioural analyses, as well as consideration of distributional patterns, ecological niches, substrate specificities and plant associations. Whilst this integrative approach might be neither perfect nor error-free, no automated barcoding method or other species-delimitation algorithm could have otherwise fixed such difficult issues in the *Olivaceoumbrini* complex. In essence, our efforts constitute an attempt to document, delimit, and describe biodiversity by utilising the broadest available range of information, and ultimately providing a practical systematic framework for both the academic and field mycologist communities alike.

**Acknowledgements** The authors are grateful to the following individual contributors for kindly providing us with material analysed in the present work: A. Antonis, K. Bendiksen, A. Bidaud, E. Bohus Jensen, D. Borgarino, R. Boyer<sup>†</sup>, F.D. Calonge, J.-M. Coquelle, L. Deparis, G.G. Eyjólfssdóttir, A. Gasch Illescas, L. Goodwin, B. Henne, J. Kleine, S. Krstic, H. Lambert, Y. Lamoureux, C. Lécure, B. Lefebvre<sup>†</sup>, D. Markides, J.-M. Moingeon, J. Olson, J.A. Oria de Rueda, A.D. Parker, A. Paul, J. Rodriguez, L. Romero de la Osa, N. Siegel, S. Svantesson, R.A. Swenie, K. Timvios, R.G. Thorn, S. Trudell and F. Valade. We also warmly thank the curators of the following herbaria, who sent us precious material, including types, for study and sequencing: ACAD, AH, AMNH, C, CMMF, H, K, LIP, LUG, MA, MICH, MPU, O, PC, TENN and UPS. The typification of *H. glutinifer* has been kindly provided by C. Papetti, who is here sincerely acknowledged. Samples sequenced by the first author were prepared at the Service of Genetic Markers in the Functional and Evolutionary Ecology Centre (GEMEX-CEFE) of Montpellier (Head: Marie-Pierre Dubois). Financial support was received from The Swedish Taxonomy Initiative, SLU Artdatabanken, Uppsala (EL) and the Karadeniz Technical University (BAP: FAT-2017-7044, ES).

## REFERENCES

- Agerer R. 2012. Asexual reproduction of *Hygrophorus olivaceoalbus* by intracellular microsclerotia in root cells of *Picea abies* – a winner of ozone stress? *Mycological Progress* 11: 425–434.
- Anisimova M, Gil M, Dufayard J-F, et al. 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology* 60: 685–699.
- Arnolds E. 1979. Notes on *Hygrophorus* III. The group of *Hygrophorus olivaceoalbus* (*Hygrophorus* subsect. *Olivaceoumbrini* Bat.) in North-Western Europe. *Persoonia* 10: 357–382.
- Arnolds E. 1990. Genus *Hygrophorus*. In: Bas C, Kuyper TW, Noordeloos ME, et al. (eds), *Flora Agaricina Neerlandica* 2: 115–133. Balkema, Lisse.
- Bas C, Kuyper TW, Noordeloos ME, et al. (eds). 1988. *Flora Agaricina Neerlandica: Critical monographs on families of Agarics and Boleti occurring in the Netherlands*. Vol. 1 A. General part. Balkema, Rotterdam-Brookfield.
- Bataille F. 1910. Flore monographique des *Hygrophores*. Mémoires de la Société d'émulation du Doubs, sér 8 (4): 132–189.
- Batsch, AJGK. 1789. *Elenchus fungorum*. Continuatio secunda. Halle.
- Bellanger J-M, Moreau P-A, Corriol G, et al. 2015. Plunging hands into the mushroom jar: a phylogenetic framework for *Lyophyllaceae* (Agaricales, Basidiomycota). *Genetica* 143: 169–194.
- Bird CJ, Grund DW. 1979. Nova Scotian species of *Hygrophorus*, Proceedings of the Nova Scotian Institute of Science 29: 1–131.
- Bon M. 1974. *Hygrophores du Centre-Est de la France étudiés au salon du museum 1971*. Bulletin mensuel de la Société linnéenne de Lyon 43 (9): 333–343.
- Bon M. 1975. Agaricales de la côte atlantique française. Documents Mycologiques 5 (17): 1–40.
- Bon M. 1977. Clé analytique des *Hygrophoraceae* Roze. 2. Les genres *Hygrophorus* et *Hygrotrama*. Documents Mycologiques 7 (27–28): 25–53.
- Bon M. 1981. Novitates. Combinaisons et noms nouveaux. Documents Mycologiques 11 (44): 28–31.
- Bon M. 1990. Les *Hygrophores*. *Hygrophoraceae* Lotsy. Documents Mycologiques Mémoire Hors Série n° 1.
- Breitenbach J, Kränzlin F. 1991. *Fungi of Switzerland*, Volume 3: Boletes and Agarics. Verlag Mykologia, Luzern, Switzerland.
- Britzelmayr M. 1899. Revision der Diagnosen zu den von M. Britzelmayr aufgestellten Hymenomyceten-Arten, Schluss. Botanisches Zentralblatt 80: 116–126.
- Bulliard JBFP. 1786. *Herbier de la France; ou Collection complète des plantes indigènes de ce royaume; avec leurs propriétés, et leurs usages en médecine*. Edited by the author, Paris, France. Tome 6 Fasc. 65–72: pl. 256–288.
- Bulliard JBFP. 1792. *Herbier de la France; ou Collection complète des plantes indigènes de ce royaume; avec leurs propriétés, et leurs usages en médecine*. Edited by the author, Paris, France. Tome 12 Fasc. 133–144: pl. 529–576.
- Candusso M. 1997. *Fungi Europaei* 6: *Hygrophorus* s.l. Edizioni Candusso, Alasio, Italia.
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552.
- Courtecuisse R, Duhem B. 1994. Guide des champignons de France et d'Europe. Delachaux et Niestlé, Paris, France.
- Cugnot J-M. 2004. *Hygrophorus pustulatus* f. *niphoides*, f. nov. Bulletin Mycologique et Botanique Dauphiné-Savoie 173: 5–9.
- Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407–415.
- Dereeper A, Guignon V, Blanc G, et al. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36: W465–W469.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Fries EM. 1815. *Observationes Mycologicae*. Gerhardt Bonnier, Copenhagen.
- Fries EM. 1821. *Systema Mycologicum* 1. Lund.
- Fries EM. 1832. *Index Alphabeticus Generum, Specierum et Synonymorum in Eliae Fries Systemate Mycologico ejusque Supplemento 'Elencho Fungorum' Enumeratorum*. Lundae.
- Fries EM. 1836. *Corpus Florarum provincialium suecicae* 1. Floram Scanicam, Uppsala.
- Fries EM. 1838. *Epicrisis systematis mycologici seu synopsis Hymenomycetum*. Uppsala.
- Fries EM. 1874. *Hymenomycetes Europaei sive Epicriseos systematis mycologici*. Uppsala.
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Gavériaux J-P. 1997. Deux contributions à l'inventaire mycologique. Bulletin de la Société Mycologique du Nord de la France 31 (62): 61–64.
- Gelman A, Rubin DB. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–511.
- Gillet CC. 1874. Les Hyménomycètes, ou Description de tous les champignons qui croissent en France avec l'indication de leurs propriétés utiles ou vénéneuses. Ch. Thomas, Alençon.
- Gronbach E, Agerer R. 1986. Charakterisierung und Inventur der Fichten-Mykorrhizen im Höglwald und deren Reaktion auf saure Beregnung. Forstwissenschaftliches Zentralblatt 105: 329–335.
- Guindon S, Lethiec F, Duroux P, et al. 2010. PHYML Online - a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* 33 (Web Server issue): W557–W559.
- Halling RE, Mueller GM. 2005. Common mushrooms of the Talamasca mountains, Costa Rica. *Memoirs of the New York Botanical Garden* 90: 1–195.
- Harmaja H. 1985. Studies on white-spored agarics. *Karstenia* 25: 41–46.
- Hesler LR, Smith AH. 1963. *North American species of Hygrophorus*. University of Tennessee Press, Knoxville.
- Hobbie EA, Agerer R. 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant and Soil* 327: 71–83.
- Hongo T. 1958. Studies on the Agaricales of Japan 1. The genus *Hygrophorus* in Shiga-Prefecture (1). *Journal of Japanese Botany* 33: 97–103.
- Jacobsson S, Larsson E. 2007. *Hygrophorus penarioides*, a new species identified using morphology and ITS sequence data. *Mycotaxon* 99: 337–343.
- Kalchbrenner K. 1874. *Icones selectae Hymenomycetum Hungariae* II: 21–36, pl. 11–20.
- Karsten P. 1876. *Mycologica Fennica* III, Basidiomycetes in Bidrag till Kännedom af Finlands. Natur och Folk, in Petter Adolf Karsten (1834–1917) *Collected Mycological Papers*, vol 1. Asher & Co. reprint, Amsterdam.
- Kirk P, Cannon P, Minter D, et al. 2008. *Dictionary of the Fungi*. 10th edition. CBS, The Netherlands.
- Konrad P. 1936. Notes critiques sur quelques Champignons du Jura. Bulletin de la Société Mycologique de France 52 (1): 35–53.
- Konrad P, Maublanc A. 1937. *Icones Selectae Fungorum*. 6: 1–558.
- Kornerup A, Wanscher JH. 1978. *Methuen handbook of colour*. Third edition, revised, Methuen London, London.
- Kovalenko A. 2012. *Hygrophorus* Fr. In: Knudsen H, Vesterholt J (eds), *Funga Nordica*, Nordsvamp, Copenhagen, Denmark: 282–293.
- Krieglsteiner GJ. 2001. *Die Großpilze Baden-Württembergs* 3: 1–634. Verlag Eugen Ulmer, Germany.
- Kühner R. 1980. Les hyménomycètes agaricoïdes (Agaricales, Tricholomatales, Pluteales, Russulales). Étude général et classification. Bulletin de la Société Linnéenne de Lyon, Numéro Spécial 49: 1–1027.
- Kühner R, Romagnesi H. 1953. *Flore analytique des champignons supérieurs*. Masson, Paris.
- Kummer P. 1871. *Der Führer in die Pilzkunde*. Luppe, Zerbst.
- Lange JE. 1940. *Flora Agaricina Danica* V: 1–105.
- Larsson E. 2010. *Hygrophorus*, a monophyletic genus with species showing strong host preferences. International Mycological Congress (IMC9), Edinburgh, Scotland. Poster Abstract P4: 111.
- Larsson E, Bendiksen K. 2020. *Hygrophorus betulae*, a new species described from subalpine birch forest in Finland. *Karstenia* 58: 1–9.
- Larsson E, Campo E, Carbone M. 2014. *Hygrophorus exiguus*, a new species in subgenus *Colorati* section *Olivaceoumbrini*, subsection *Tephroleuci*. *Karstenia* 54: 41–48.
- Larsson E, Jacobsson S. 2004. Controversy over *Hygrophorus cossus* settled using ITS sequence data from 200 year-old type material. *Mycological Research* 108: 781–786.



- Larsson E, Kleine J, Jacobsson S, et al. 2018. Diversity within the *Hygrophorus* *agathosmus* group (Basidiomycota, Agaricales) in Northern Europe. *Mycological Progress* 17 (12): 1293–1304.
- Lavorato C. 1991. Chiave analitica e note bibliografiche della micoflora del cisto. *Bollettino dell'Associazione Micologica e Ecologica Romana* 24: 16–45.
- Léveillé JH. 1837. Recherches sur l'hymenium des champignons. *Annales des Sciences Naturelles, Sér. 2*: 321–338.
- Lodge DJ, Padamsee M, Matheny PB, et al. 2014. Molecular phylogeny, morphology, pigment chemistry and ecology in *Hygrophoraceae* (Agaricales). *Fungal Diversity* 64: 1–99.
- Loizides M. 2016. Macromycetes within Cistaceae-dominated ecosystems in Cyprus. *Mycotaxon* 131 (1): 255–256.
- Loizides M, Alvarado P, Polemis E, et al. 2020. Multiple evolutionary origins of sequestrate species in the agaricoid genus *Chlorophyllum*. *Mycologia* 112 (2): 400–422.
- Loizides M, Bellanger J-M, Clowez P, et al. 2016. Combined phylogenetic and morphological studies of true morels (Pezizales, Ascomycota) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. *Mycological Progress* 15: 39.
- Loizides M, Kyriakou T. 2011. Fungi of the Cistus Maquis. *Field Mycology* 12 (1): 14–22.
- Loizides M, Kyriakou T, Tziakouris A. 2011. Edible & toxic fungi of Cyprus. Published by the authors.
- Ludwig E. 2012. Pilzkompendium, Band 3. Beschreibungen. Fungicon-Verlag, Berlin.
- Maire R. 1933. Fungi Catalaunici, series altera. Contributions à l'étude de la Flore Mycologique de la Catalogne. Publications de l'Institut Botànica de Barcelona III (4): 1–128.
- Malençon G, Bertault R. 1975. Flore des Champignons Supérieurs du Maroc. II. Travaux de l'Institut chérifien et de la Faculté des Sciences de Rabat, série Botanique et Biologie végétale 33: 1–541.
- Matheny PB, Curtis JM, Hofstetter V, et al. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia* 98: 982–995.
- Melot J. 1981. Éléments de la flore mycologique du Baar, II. Bulletin de la Société Mycologique de France 97 (2): 76–77.
- Melot J. 1992. Novitates - Combinations et taxa nova. *Documents Mycologiques* 22 (85): 20.
- Métrod G. 1938. *Hygrophorus tephroleucus* (Fries et Persoon) sensu Bresadola. *Bulletin de la Société Mycologique de France* 54: 70–72.
- Moreau P-A, Bellanger J-M, Lebeuf R, et al. 2018. Hidden diversity uncovered in *Hygrophorus* sect. *Aurei* (*Hygrophoraceae*), including the Mediterranean *H. meridionalis* and the North American *H. boyeri*, spp. nov. *Fungal Biology* 122: 817–836.
- Orton PD. 1960. New check-list of British Agarics and Boleti. Part III. Notes on genera and species in the list. *Transactions of the British Mycological Society* 43 (2): 159–439.
- Padial JM, Miralles A, De la Riva I, et al. 2010. The integrative future of taxonomy. *Frontiers in Zoology* 7: 16.
- Papetti C. 2016. Contributo alla conoscenza delle *Hygrophoraceae* – III. *Hygrophorus limacinus* o *H. latitabundus*? *Hygrophorus persoonii* o *H. dichrous*? *Rivista di Micologia* 59(4): 291–320.
- Peck CH. 1899. New species of fungi. *Bulletin of the Torrey Botanical Club* 26: 63–71.
- Pérez-de-Gregorio MÂ, Roqué C, Macau N. 2009. Apuntes sobre un *Hygrophorus* Fr. común en las comunidades cisticolas mediterráneas. *Errotari* 6: 22–28.
- Persoon CH. 1801. *Synopsis Methodica Fungorum*. Dietrich, Gottingen.
- Pilát A. 1951 '1952'. *Mushrooms*. Spring books, London.
- Quélet L. 1886. *Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium*. Doin, Paris.
- Quélet L. 1888. *Flore mycologique de la France et des pays limitrophes*. Octave Doin, Paris.
- Ricken A. 1915. Die Blätterpilze (Agaricaceae) Deutschlands und der angrenzenden Länder, besonders Oesterreichs und der Schweiz. Lief. 13–14: 385–448. Weigel. Leipzig.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Schaeffer JC. 1774. *Fungorum qui in Bavaria et Palatinatu circa Ratisbonam nascuntur, Icones* 4: 1–136.
- Schlick-Steiner BC, Steiner FM, Seifert B, et al. 2009. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421–438.
- Sesli E, Antonin V, Contu M. 2018. A new species of *Hygrophorus*, *H. yadigarii* sp. nov. (*Hygrophoraceae*), with an isolated systematic position within the genus from the Colchic part of Turkey. *Turkish Journal of Botany* 42: 224–232.
- Singer R. 1949 '1951'. The Agaricales in modern taxonomy. *Lilloa* 22: 1–832.
- Singer R. 1961. Type studies on Basidiomycetes X. *Persoonia* 2 (1): 1–62.
- Singer R. 1986. The Agaricales in modern taxonomy. 4th edn. Koeltz Scientific Books, Koenigstein.
- Smith AH, Hesler LR. 1954. Additional North American *Hygrophori*. *Sydowia* 8 (1-6): 304–333.
- Stadler M, Læssøe T, Fournier J, et al. 2014. A polyphasic taxonomy of *Daldinia* (*Xylariaceae*). *Studies in Mycology* 15: 1–143.
- Strid Å. 1994. A catalogue of fungus plates painted under the supervision of Elias Fries. Swedish Museum of Natural History, Stockholm.
- Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577.
- Tedersoo L, May TW, Smith ME. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20: 217–263.
- Turland NJ, Wiersema JH, Barrie FR, et al. (eds). 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten, Koeltz Botanical Books. <https://doi.org/10.12705/Code.2018>.
- Wang C-Q, Li T-H, Zhang M, et al. 2020. *Hygrophorus* subsection *Hygrophorus* (*Hygrophoraceae*, *Agaricales*) in China. *MycKeys* 68: 49–73.
- Wei X, McCune B, Lumbsch HT, et al. 2016. Limitations of species delimitation based on phylogenetic analyses: A case study in the *Hypogymnia* *hypotrypa* group (*Parmeliaceae*, *Ascomycota*). *PloS One* 11: e0163664.
- White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al. (eds), *PCR Protocols: a guide to methods and applications*: 315–322. Academic Press, New York.
- Will KW, Mishler BD, Wheeler QD. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* 54: 844–851.
- Young AM. 2005. *Fungi of Australia: Hygrophoraceae*. CSIRO Publishing, Australian Biological Resources Study, Canberra.
- Young AM, Wood AE. 1997. Studies on the *Hygrophoraceae* (Fungi, Homobasidiomycetes, Agaricales) of Australia. *Australian Systematic Botany* 10: 911–1030.
- Zamora JC, Calonge FD, Martín MP. 2015. Integrative taxonomy reveals an unexpected diversity in *Geastrum* section *Geastrum* (*Geastrales*, *Basidiomycota*). *Persoonia* 34: 130–165.
- Zamora JC, Ekman S. 2020. Phylogeny and character evolution in the *Dacrymycetes*, and systematics of *Unilacrymaceae* and *Dacryonaemataceae* fam. nov. *Persoonia* 44: 161–205.
- Zervakis GI, Ntougias S, Gargano ML, et al. 2014. A reappraisal of the *Pleurotus eryngii* complex – new species and taxonomic combinations based on the application of a polyphasic approach, and an identification key to *Pleurotus* taxa associated with *Apiaceae* plants. *Fungal Biology* 118: 814–834.

## Supplementary material

**Suppl. Table 1** Distribution of polymorphisms between *H. agathosmus* and *H. agathosmoides*.

**Suppl. Table 2** Distribution of polymorphisms between *H. pustulatus* and *H. pustulatoides*.