



# *Hodophilus phaeophyllus* complex (Clavariaceae, Agaricales) is defined as new phylogenetic lineage in Europe

Slavomír Adamčík<sup>1</sup> · Bálint Dima<sup>2</sup> · Katarína Adamčíková<sup>3</sup> · Gilles Corriol<sup>4</sup> · Thomas Læssøe<sup>5</sup> · Pierre-Arthur Moreau<sup>6</sup> · Miroslav Caboň<sup>1</sup> · Soňa Jančovičová<sup>7</sup>

Received: 22 October 2019 / Revised: 12 November 2019 / Accepted: 18 November 2019  
© German Mycological Society and Springer-Verlag GmbH Germany, part of Springer Nature 2020

## Abstract

The study deals with the last unexplored morphological group of the genus *Hodophilus* defined by absence of distinct odours, absence of yellow colours and absence of darker dots on the stipe. The phylogenetic reconstruction of the whole genus based on nrITS, nrLSU and *RPB2* sequences placed all European members having these morphological characters in a monophyletic group defined here as a new section *H. sect. Phaeophylli*. The remaining European members of the genus are placed in two additional groups classified as section *H. sect. Hodophilus* and the new section *H. sect. Micacei*. Five species are recognised within section *Phaeophylli* which is typified by *H. phaeophyllus* that is lecto- and epitypified. Three new species belonging to this section are described: *H. carpathicus*, *H. decurrentior* and *H. stramineus*. *Hodophilus decurrentior* is the only species showing distinct morphological differences under the microscope. The identification of other species of the section depends mainly on the colour of basidiomata. An updated key to all European members of the genus is provided.

**Keywords** Agaricoid · *Camarophyllopsis* · Multi-locus phylogeny · Infrageneric classification

Section Editor: Zhu-Liang Yang

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11557-019-01544-9>) contains supplementary material, which is available to authorized users.

✉ Slavomír Adamčík  
slavomir.adamcik@savba.sk

Bálint Dima  
cortinarius1@gmail.com

Katarína Adamčíková  
katarina.adamcikova@ife.sk

Gilles Corriol  
gilles.corriol@cbnmpm.fr

Thomas Læssøe  
thomasl@bio.ku.dk

Pierre-Arthur Moreau  
pierre-arthur.moreau@univ-lille2.fr

Soňa Jančovičová  
sona.jancovicova@uniba.sk

- 1 Institute of Botany, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia
- 2 Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/c, Budapest H-1117, Hungary
- 3 Department of Plant Pathology and Mycology, Institute of Forest Ecology, Slovak Academy of Sciences Zvolen, Akademická 2, 949 01 Nitra, Slovakia
- 4 Conservatoire botanique national des Pyrénées et de Midi-Pyrénées, Vallon de Salut - BP70315, 65203 Bagnères-de-Bigorre Cedex, France
- 5 Natural History Museum of Denmark/Department of Biology, Universitetsparken 15, 2100 Copenhagen Ø, Denmark
- 6 Université de Lille, Fac. Pharma. Lille EA 4483 IMPECS, 59000 Lille, France
- 7 Department of Botany, Faculty of Natural Sciences, Comenius University in Bratislava, Révová 39, 811 02 Bratislava, Slovakia

## Introduction

The family Clavariaceae is known to include clavarioid fungi (Singer 1975), but recent phylogenetic studies have classified three agaricoid genera within this family (Birkebak et al. 2013, 2016). Most of the species in these three genera were previously placed in a single genus *Camarophylloopsis* Herink (Arnolds 1986), but the majority has recently been combined in *Hodophilus* R. Heim ex R. Heim based on molecular support and type studies (Birkebak et al. 2016, Adamčík et al. 2018). Our previous phylogenetic studies on *Hodophilus* recognized two major lineages within the genus (Adamčík et al. 2016, 2017a, 2017b, 2018); the first corresponding to species without a distinctive odours that grouped in the *H. micaceus* superclade and the second to species with mainly a strong naphthalene odours that grouped in the *H. foetens* superclade. Species with dark dots on stipe previously identified as *H. atropunctus* represent two different species, placed in these two lineages but each in different one. These studies were specifically directed at foetid species or to members of the *H. micaceus* superclade with yellow or yellow-brown stipes. Within the latter lineage, there are two well-supported residual clades, one represented by a single North American species, *H. hymenocephalus* (A.H. Sm. & Hesler) Birkebak & Adamčík, and the other by some unidentified collections from Europe. Basidiomata in both residual clades have brown stipes without yellow or with yellow-brown tints, no darker dots on stipe and no foetid odours. Such morphotypes have been recognised in the European literature as *C. hymenocephala* (A.H. Sm. & Hesler) Arnolds or *C. phaeophylla* (Romagn.) Arnolds (Boertmann, 2012, Kovalenko et al. 2012). Recently, Arauzo and Iglesias (2018) identified one European residual clade as *H. phaeophyllus* (Romagn.) Arauzo & P. Iglesias and described *H. hymenocystis* Arauzo & P. Iglesias as a new species that is morphologically similar to *H. phaeophylla* but not closely related according to their analysis of ITS nrDNA region.

We could not trace any type collection nor authentic material, except the published figure, of *C. phaeophylla*, and a correct epitypification of this name relies on a good knowledge of species diversity and variability within the genus, as well as knowledge of the type locality. In this study, we want to test the concept of this name as proposed by Arauzo and Iglesias (2018) and to specify species limits and relationships within this last underexplored group of *Hodophilus* with brown stipes and non-naphthalene odours. We apply a similar approach as in our previous studies, and we base our study on available collections from different areas of Europe, analyse them by multi-locus phylogeny, and support the species delimitation by statistically evaluated morphological observations.

## Materials and methods

### Taxon sampling

Altogether, 25 European *Hodophilus* collections with brown stipes without yellow tinges, darker dots on stipe and naphthalene odours were analysed. For the phylogenetic placement, we used sequences previously published by Adamčík et al. (2018) supported by ITS sequences retrieved from Arauzo and Iglesias (2018). All sequences are presented in the Supplementary Table 1.

### DNA extraction, PCR and sequencing

Three gene regions (nrITS, nrLSU and *RPB2*) were amplified, sequenced and analysed. Protocols of Birkebak et al. (2013) were followed for DNA extraction, PCR and sequencing. The primer pair ITS1F-ITS4 (Gardes and Bruns 1993, White et al. 1990) was used to amplify the ITS region. Combinations of LR0R-LR7, LR0R-LR5 or LR0R-LR16 (<https://sites.duke.edu/vilgalyslab/files/2017/08/rDNA-primers-for-fungi.pdf>) were used to amplify and sequence the nrLSU region. The primer pair b6F and b7.1R (Matheny 2005) was used to amplify and sequence the most variable region of the *RPB2* gene between conserved domains 6 and 7. Sequencing was performed at the SEQme sequencing Company (Dobříš, Czech Republic).

### Phylogenetic analyses

Sequences of individual gene regions were aligned using MAFFT online version 7 (<http://mafft.cbrc.jp/alignment/server>). The E-INS-I method (Katoh and Standley 2013) was selected for aligning the nrDNA ITS, the G-INS-I method (Katoh et al. 2005) for the nrDNA LSU, and the FFT-NS-I method (Katoh et al. 2002) for *RPB2* region, all under default settings. Manual adjustments and concatenation of the individual alignments were done in SeaView 4 (Gouy et al. 2010). FastGap 1.2 (Borchsenius 2009) was used to code the phylogenetically informative indels in the ITS region following the simple indel coding algorithm (Simmons et al. 2001). Adding indel characters to the nucleotide alignment of ITS sequences increases the robustness of phylogenetic analyses (Nagy et al. 2012). After concatenating the nucleotide and binary data, the partitioned alignment was subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses, which were performed in raxmlGUI (Silvestro and Michalak 2012) and MrBayes 3.1.2 (Ronquist et al. 2012), respectively. ML analysis was done using 1000 rapid ML bootstrap searches. The GTRGAMMA nucleotide substitution model was selected for the three nucleotide partitions (ITS, LSU, *RPB2*), and the default setting for binary data was used for the indel partition. BI was performed with the GTR +  $\Gamma$  + I

model of evolution for the nucleotide partitions, and the two-parameter Markov model was set for the indel partition. The BI settings were as follows: four Markov chain Monte Carlo (MCMC) over 10 million generations, sampling every 1000th generation, two independent runs, and burn-in of 35% (the first 3500 trees were discarded). Post burn-in trees were used to compute a 50% majority rule consensus phylogram. *Ramariopsis corniculata* was chosen as the outgroup.

Phylogenetic trees from both ML and BI analyses resulted in largely congruent topologies (Fig. 1). ML bootstrap values (BS) > 70% and Bayesian posterior probabilities (PP) > 0.95 were considered evidence for statistical branch support. All sequences are deposited in GenBank. The concatenated final alignment has been deposited in TreeBASE (TB2:S25267).

## Morphological analyses

Macro-morphological descriptions were prepared from fresh material shortly after collection from the field. The number of full-length lamellae is treated in the species descriptions as “L”. The number of short lamellulae between each pair of full length lamellae is labelled as “I” (Vellinga 1988). Colour nomenclature standards follow Korerup and Wanscher (1967).

Microscopic structures were examined on herbarium specimens in Congo red solution with ammonia after a short treatment in aqueous 10% KOH. The same micro-morphological characters were observed as those in our previous study on European *Hodophilus* species with naphthalene odours (Adamčík et al. 2017a). Pileipellis elements near the pileus margin and the pileus centre were observed and evaluated separately. Features were observed under an Olympus CX-41 microscope with an oil-immersion lens at a magnification of 1000×. All drawings of microscopic structures, with the exception of basidiospores, were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of 2000×. Basidiospores were scanned with an Artray Artcam 300MI camera and measured by Quick Micro Photo (version 2.1) software. Enlarged scanned pictures of spores were used for measuring with an accuracy of 0.1 µm and for making line drawings. All other elements are measured with accuracy of 0.5 µm. *Q* is the length/width ratio of basidiospores and pileipellis elements. Statistics of microscopic dimensions are based on 30 measurements and given as a mean value plus/minus standard deviation; values in parentheses give measured minimum or maximum values.

## Results

### Phylogenetic analyses

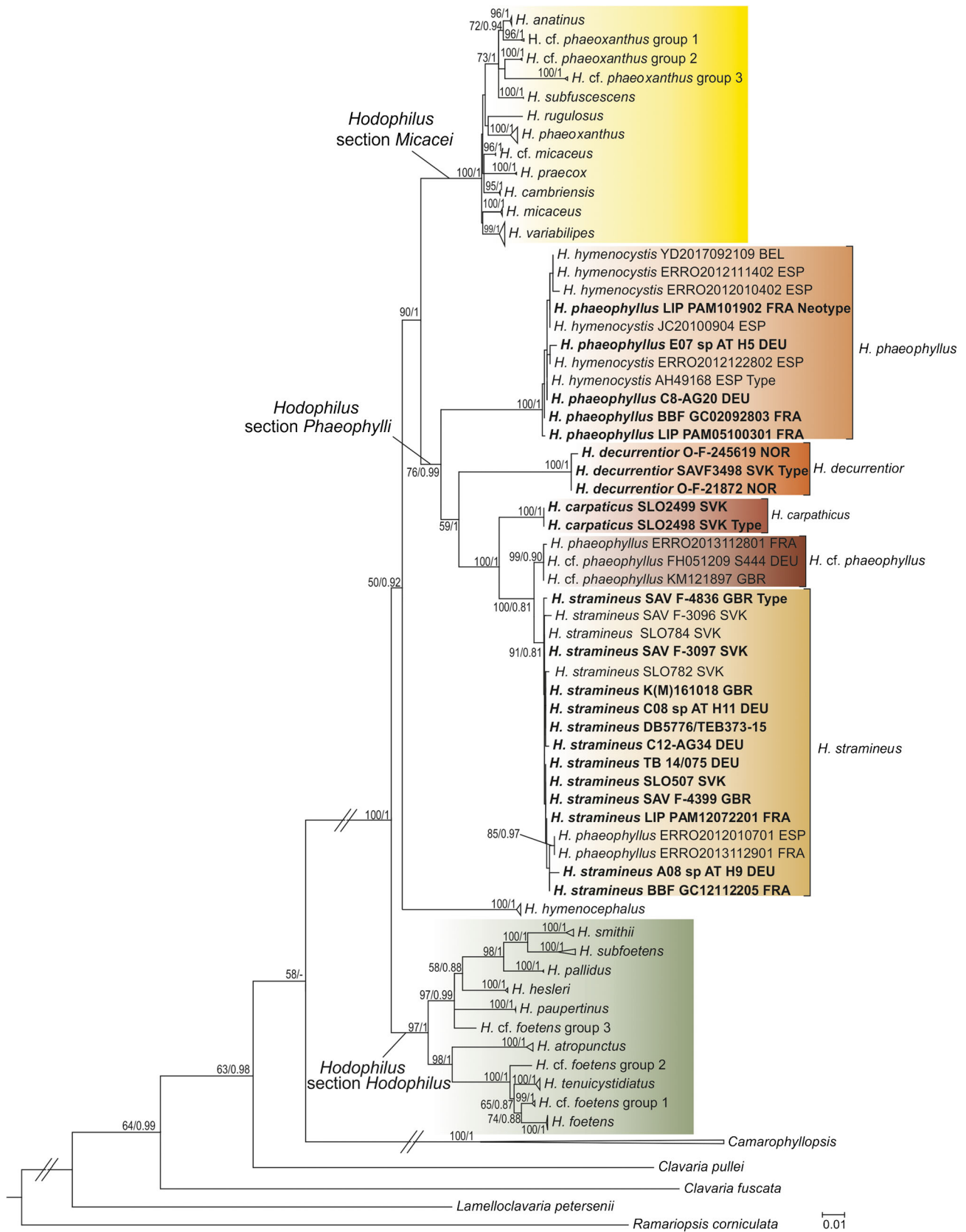
The final dataset consists of 172 samples represented by 168 ITS, 116 LSU and 87 *RPB2* sequences; 23 of them are

published for the first time in this study. European collections of the genus *Hodophilus* are grouped in three monophyletic groups supported by both ML and BI analyses. These groups are described as new sections and are morphologically defined below. The section *Hodophilus* contains European and North American members with naphthalene odours and *H. atropunctus* that has no distinct odour and has dark dots on the stipe. Section *Micacei* also contains *Hodophilus* members from both continents; these have distinct yellow colour on at least some parts of the stipe, with the exception of *H. variabilipes* with yellow-brown colour and sometimes also darker dots on the stipe. All collections without distinct odours and without yellow colour and darker dots on the stipe are placed in section *Phaeophylli*. The latter section contains only European collections clustered with strong support in five clades corresponding to phylogenetic species. Based on morphology explained below, we assigned one species clade to the previously described *H. phaeophyllus*, and this species is designated as the type of the section. Three other species of this section are described as new in this study. One species clade (labelled as *H. cf. phaeophyllus*) is composed of three collections and is not further described in this study because of *RPB2* amplification failure and absence of field descriptions. All sequences identified as *H. hymenocystis* by Arauzo and Iglesias (2018), including the type of the species, are placed in our *H. phaeophyllus* clade, suggesting synonymy of the two names. Sequences identified by these authors as *H. phaeophyllus* are placed in two different clades, one of them clustered within the *H. cf. phaeophyllus* clade and the other two within a new clade corresponding to *H. stramineus*.

### Morphological delimitation of genetically defined species

Our previous taxonomic studies on the genus *Hodophilus* often showed little differences in microscopic structure and proved the importance of field characters for species circumscription. Among the five species recognised by phylogenetic analysis, one lacks any field notes, and therefore, we did not include it in our morphological analysis. Available descriptions and photographs allow us to conclude that all species of the studied lineage have brown stipes without conspicuous yellow tints or darker dots, and they all share an absence of a strong naphthalene odour. All four described species here have caulocystidia of a very irregular shape, which are often twisted or lobate, but this character was observed also in *H. phaeoxanthus*, a member of section *Micacei* (Adamčík et al. 2018).

Our observations of macro-morphological characters on the four studied species recovered differences especially in colour. Table 1 compares colours of different parts of the basidiomata retrieved from our own observations with the original description of *H. phaeophyllus*. To analyse the pileus





◀ **Fig. 1** Maximum likelihood (RAxML) phylogeny inferred from three loci (nrITS, nrLSU, *RPB2*) and additional indel coding of the ITS region. Newly generated sequences are highlighted in boldface. Names of taxa are followed by collection labels, country, and whether this represents a type collection. ML bootstrap values followed by Bayesian posterior probabilities are indicated at nodes. Clades not in focus in this paper are compressed

colour, it is important to recognise if the basidiomata are in a fresh and moist condition or if they are dry, because pilei of all species are hygrophaneous. In fresh condition, *H. carpathicus*, *H. decurrentior* and *H. phaeophyllus* have dark brown pilei and *H. stramineus* light brown (Figs. 2–9). The most dramatic change from moist to dry condition we observed in *H. phaeophyllus* that discolours to reddish grey or whitish (Figs. 4–5). The stipe also changes its colour during maturing. Most species develop darker brown colours towards the base when mature, and only *H. carpathicus* has dark brown colours already in young stages. The lamellae of *H. stramineus* are light brown also at maturity; we observed consistently darker brown lamellae colours in *H. carpathicus* and *H. decurrentior*, and the lamellae of *H. phaeophyllus* are initially pale whitish but soon turn to dark brown.

The collections that are assigned to *H. phaeophyllus* have a perfect colour match with the original diagnosis of the species, i.e. dark brown pileus colours turning to pale greyish-whitish when dry; stipe becoming darker near the base with age and lamellae developing a dark brown colour with age. Our data indicate that *H. hymenocystis* is a later synonym of *H. phaeophyllus*. Arauzo and Iglesias (2018) applied the name *H. phaeophyllus* for collections that fell within two different species (*H. stramineus* and an undescribed species, see Fig. 1). The species concept adopted by these authors is discussed further below.

Under the microscope, *H. decurrentior* is the only species easily distinguishable from all other members of the genus by higher spore  $Q > 1.4$  (Table 2). The caulocystidia and terminal cells in the pileipellis near the pileus centre in *H. carpathicus* are very irregular in shape and size, with many small elements making their average length and width smaller than in other species of the studied lineage. In *H. phaeophyllus*, the broad marginal cells on the lamellae edges recall the terminal cells in the pileipellis. Sometimes, they are hard to find, disappear towards the stipe and are only present near the pileus

**Table 1** Comparison of basidiomata colourations observed in European *Hodophilus* taxa of the *H. phaeophyllus* lineage. The colour codes follow Komerup and Wanscher (1967)

Colours	<i>H. stramineus</i>	<i>H. carpathicus</i>	<i>H. decurrentior</i>	<i>H. phaeophyllus</i> this study	<i>H. phaeophyllus</i> protologue	
Pileus	Moist/fresh	Greyish brown (café-au-lait 6D3) or paler brown near the margin, darker brownish orange (golden blonde 5C4), brownish grey (6C2), olive brown (4F7–5F7) at the centre	Coffee brown (5F6) to chocolate (6F4) near the margin, coffee brown (5F7) at the centre	Greyish brown (7F3)	Brown (6E5) to greyish brown (café-au-lait 6D3) near the margin, dark brown (burnt amber 6F6) at the centre	Dark brown
	Dry	Greyish orange (5B3), yellowish brown (hair brown 5E4 to bronze 5E5), grey brown (5C3) or greyish yellow (champagne 4B4)	Brown (mustard 5E6), coffee brown (5F7) near the margin and greyish brown (nougat 5D3) at the centre	Hair brown (5E4)	Reddish grey (7B2) to whitish	Greyish brown to ash-blond or greyish-whitish, sometimes shaded brown, especially at the centre or slightly flushed by ochraceous cream
Stipe	Near lamellae	Orange grey (5B2), brownish orange (5C3), blonde (4C4), beige (4C3), greyish yellow (champagne 4B4)	When young unicoloured, dark brown (chestnut 6F7), when mature paler (coffee brown, 5F7)	Concolourous with pileus but paler near the lamellae and the base	Whitish to orange grey (5B2), light ochre, then flesh brown (6B3) to brownish orange (7C3)	First light brownish white (paler than pileus), then light brown
	Near base	Darker, first greyish brown/nougat (5D3), soot brown (5F5), then dark brown/chocolate (6F4), chestnut (6F6, 6F7)	Mustard brown (5E6)		Whitish, then reddish grey (7B2) to dark brown (7F4)	Often dark blackish brown
Lamellae	Young brownish orange (5C4), mature yellowish brown (5E4, 5E7) or brownish orange (6D3)	Tobacco brown (5F6) to chocolate brown (6F4)	Brown (6E4)	Whitish (5A2), then reddish grey (7B2), brownish orange (6C3) to dark brown (6F6)	When young brown to brownish grey, then brown and with slight purple or chocolate or grey brown	

**Figs. 2–9** Basidiomata field aspect of species in the *H. phaeophyllus* lineage. **2** *H. carpathicus* (SLO2498, holotypus), photo S. Jančovičová. **3** *H. decurrentior* (SAV F-3498, holotypus), photo V. Stanová. **4** *H. phaeophyllus* in moist condition (LIP PAM00101902, epitype), photo P.-A. Moreau. **5** *H. phaeophyllus* in dry condition (GC02092803), photo G. Corriol. **6** *H. stramineus* in moist condition (SLO784), photo S. Jančovičová. **7** *H. stramineus* in dry condition (SAV F-4836), photo D. Harries. **8** *H. stramineus* in dry condition (PAM12072201), photo P.-A. Moreau. **9** Young basidiomata of *H. stramineus* (GC12112205), photo G. Corriol. Scale bar = 1 cm



margin, where the lamellae are very narrow. It is thus difficult to separate *H. phaeophyllus* from *H. stramineus*.

Below, we provide a preliminary key to European *Hodophilus* species described here and in our previous studies (Adamčík et al. 2017a, 2017b, 2018). We did not include *H. fuscofoetens* Arauzo, P. Iglesias & Fern.-Vic described recently by Arauzo and Iglesias (2018), because it is represented only by a single published ITS sequence that fall outside the genus *Hodophilus* in our analyses (not shown in the tree) and it

may represent another genus of the family *Clavariaceae*. Another species described by these authors, *H. praecox* Arauzo, is distinguished by a different phenology (in spring), but the authors did not recognise any morphological differences, possibly because their description lack sufficient detail. We have included putatively the species in the key as morphologically close to *H. anatinus* due to the similar stipe coloration and relatively narrow terminal cells in the pileipellis reported in the original description, but particularly the second character



**Table 2** Average values of 30 measurements of selected micro-morphological characters observed on the four species of the *H. phaeophyllus* lineage. Abbreviations: TC margin/centre and STC margin/centre, terminal and subterminal cells in pileipellis near the pileus margin or centre; *L*, length in  $\mu\text{m}$ ; *W*, width in  $\mu\text{m}$ , *Q*, length/width ratio.

Values in italics indicate important differences (with no or little overlap between at least one species pair). En dash indicates missing values due to absence of a structure or insufficient conditions of the studied herbarium specimen

Species epithet	Fungarium no.	Spores			Caulocystidia		TC margin			STC margin		TC centre			STC centre		Marginal cells	
		<i>L</i>	<i>W</i>	<i>Q</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>Q</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>	<i>Q</i>	<i>L</i>	<i>W</i>	<i>L</i>	<i>W</i>
<i>H. carpathicus</i>	SLO2498	4.7	3.8	1.21	28.1	<b>6.8</b>	26.9	17.2	1.59	20.5	9.15	<b>27.8</b>	<b>14.4</b>	2.12	21.3	8.7	–	–
	SLO2499	4.6	3.9	1.18	30.2	<b>7.1</b>	36.1	20.4	1.85	17.7	6.8	<b>27.9</b>	<b>16.7</b>	1.77	23.5	8.8	27.7	9.7
<i>H. decurrentior</i>	SAV3498	<b>5.6</b>	3.9	<b>1.43</b>	33.7	8.0	42.1	23.3	<b>1.88</b>	22.0	7.5	40.6	20.3	2.00	26.8	8.8	24.5	7.3
	NOBAS299916	<b>5.7</b>	3.8	<b>1.52</b>	–	–	28.6	21.3	1.35	17.7	6.3	32.4	22.1	1.55	20.6	8.9	–	–
	NOBAS287816	<b>6.3</b>	3.7	<b>1.72</b>	–	–	30.9	16.4	<b>1.92</b>	16.6	6.6	34.4	17.1	2.01	25.5	8.2	–	–
<i>H. phaeophyllus</i>	PAM05100301	4.9	3.8	1.29	29.7	9.8	32.6	22.8	1.42	19.4	7.3	31.7	22.0	1.44	20.7	7.8	–	–
	PAM00101902	4.5	3.6	1.25	32.3	11.6	32.7	25.0	1.30	15.5	6.4	35.2	24.8	1.42	19.8	6.9	27.7	9.7
<i>H. stramineus</i>	SAV4836	4.4	3.6	1.21	33.0	7.4	29.9	17.6	1.81	14.7	7.0	31.6	18.7	1.76	15.5	6.1	–	–
	SAV4836_SJ	4.6	4.1	1.13	30.5	8.4	26.2	19.0	1.41	10.4	6.5	31.9	19.4	1.73	19.1	8.2	–	–
	SLO782	4.5	3.6	1.24	–	–	33.1	24.3	1.41	10.7	5.6	36.7	22.5	1.67	14.3	6.8	–	–
	SAV4399	4.6	3.7	1.22	28.9	8.7	33.3	24.0	1.39	20.0	7.3	34.8	22.8	1.59	15.1	6.8	–	–
	SLO784	4.5	3.6	1.25	35.3	10.3	35.6	24.9	1.47	13.6	7.9	37.0	24.2	1.53	15.0	5.8	18.4	6.6
	SLO507	4.2	3.4	1.24	32.9	9.2	36.5	27.9	1.35	10.0	7.7	33.6	22.7	1.48	14.0	7.6	–	–
	SAV3097	4.7	3.8	1.24	38.7	13.3	28.7	22.1	1.32	15.3	8.0	29.7	22.5	1.32	13.5	6.9	–	–
	PAM12072201	4.2	3.3	1.27	40.5	8.6	37.0	25.6	1.46	13.6	6.5	34.7	24.8	1.40	11.4	6.2	–	–

should be investigated by observations from different parts of the pileipellis.

**Taxonomy**

Key to known European *Hodophilus* species

- 1 Basidiomata with distinct naphthalene odours and without conspicuous yellow colours on any part, subterminal cells in pileipellis rarely small (shorter than 5  $\mu\text{m}$ ), caulocystidia rarely lobate or twisted . . . . . 2
  - 1\* Basidiomata without strong and conspicuous naphthalene odours, sometimes with distinct yellow colours, subterminal cells in pileipellis frequently small, caulocystidia sometimes irregularly inflated, nodulose, lobate and twisted. . . . . 5
    - 2 Lamellae moderately close ( $L = 18\text{--}30$ ). . . . . 3
      - 2\* Lamellae distant [ $L = 10\text{--}18(20)$ ]. . . . . 4
        - 3 Basidiomata becoming dark brown to black, especially near the pileus margin upon maturation or drying; caulocystidia on average wider than 7.5  $\mu\text{m}$  . . . . . *H. foetens*
          - 3\* Mature or dry basidiomata not becoming darker; caulocystidia on average up to 7  $\mu\text{m}$  wide . . . . . *H. tenuicystidiatus*
            - 4 Pileus orange grey to greyish orange when fresh, drying pale dull orange; terminal cells of hyphae near the pileus centre with ratio of length/width mainly < 2.5. . . . . *H. pallidus*

- 4\* Pileus brown, grey brown, brownish grey, or when dry dark brown; terminal cells of hyphae near the pileus centre with ratio of length/width mainly > 2.5. . . . . *H. subfoetens*
  - 5 Stipe with distinct dark dots. . . . . 6
    - 5\* Stipe never with dark dots. . . . . 7
      - 6 Pileus becomes pale from the margin and darker at the centre when dry; stipe distinctly darker near the base. . . . .
        - . . . . . *H. atropunctus*
          - 6\* Pileus becomes pale from the centre outwards and is uniformly coloured when dry; stipe uniformly coloured, usually yellow-brown. . . . . *H. variabilipes*
            - 7 Stipe with distinct yellow colours at least on some parts. . . . . 8
              - 7\* Stipe without distinct yellow parts. . . . . 12
                - 8 Young basidiomata completely yellow; stipe vivid yellow and remaining so even when mature. . . . . *H. micaceus*
                  - 8\* Young basidiomata with yellowish brown pileus; stipe colour soon changing and becoming partly to almost completely brown with age. . . . . 9
                    - 9 Stipe starts to brown near the apex, terminal elements in pileipellis (at least near the centre) relatively narrow  $Q > 1.7$ . . . . . 10
                      - 9\* Stipe of young basidiomata near apex usually paler; terminal elements in pileipellis near pileus centre usually with  $Q < 1.6$ . . . . . 11
                        - 10 Stipe persistently vivid yellow, but when mature with olive and near apex also brownish tints, terminal elements in

pileipellis (near pileus margin?) very narrow ( $Q_{av} = 2.5$ ), fruiting in early season until July. . . . . *H. praecox*

10\* Stipe of young basidiomata near apex usually darker yellow brown to grey brown; terminal elements in pileipellis near pileus centre with  $Q_{av} = 1.7$ – $2.1$ , fruiting in August and later. . . . . *H. anatinus*

11 Stipe at first yellow to brownish yellow, in age gradually changing to dark grey brown to almost black near base; spores with  $Q_{av} \leq 1.2$ . . . . . *H. cambriensis*

11\* Stipe usually two-coloured with paler yellow, golden yellow, brownish orange near apex and light brown, yellowish brown or greyish brown colours near base, not becoming distinctly darker with age; spores usually with  $Q_{av} > 1.2$ . . . . . *H. phaeoxanthus*

12 Pileus and stipe usually uniformly yellow-brown; caulocystidia regular, not lobate or twisted. . . . . *H. variabilipes*

12\* Pileus and stipe without conspicuous yellow colours or stipe darker near the base; caulocystidia  $\pm$  lobate and twisted. . . . . 13

13 Spores narrowly ellipsoid,  $Q_{av} > 1.4$ . . . . . *H. decurrentior*

13\* Spores broadly ellipsoid,  $Q_{av} < 1.3$ . . . . . 14

14 All parts of basidiomata dark brown when young and in moist condition; pileus remains distinctly brown even when dry; caulocystidia and pileipellis at the pileus centre with elements of very different sizes. . . . . *H. carpathicus*

14\* Some parts of basidiomata, especially lamellae and adjacent surface of the stipe, pale brownish when young; pileus light brown or rapidly discolouring to light brown to almost whitish; caulocystidia and elements of pileipellis at the pileus centre of relatively similar size. . . . . 15

15 Pileus with prevailing light brown colours in moist and dry condition; lamellae light brown also at maturity. . . . . *H. stramineus*

15\* Pileus dark brown when young or moist, strongly changing colour to pale grey or almost whitish; lamellae at first pale brownish and greyish then dark brown. . . . . *H. phaeophyllus*

#### *Hodophilus* section *Hodophilus* R. Heim

*Type species:* *H. foetens* (W. Phillips) Birkebak & Adamčík, Mycologia 108(5): 866. 2016

*Diagnosis:* Basidiomata typically with distinct naphthalene odours, one species without a distinct odour but with distinct dark dots on the stipe.

#### *Hodophilus* section *Micacei* Adamčík & Dima, sect. nov.

*Mycobank No.:* MB 833031.

*Etymology:* The name refers to epithet of the type species.

*Type species:* *H. micaceus* (W. Phillips) Birkebak & Adamčík, Mycologia 108(5): 867. 2016

*Diagnosis:* Basidiomata typically without distinct naphthalene odours and with yellow colour on the stipe, one species with yellow-brown stipe.

#### *Hodophilus* section *Phaeophylli* Adamčík & Dima, sect. nov.

*Mycobank No.:* MB 833032.

*Etymology:* The name refers to epithet of the type species.

*Type species:* *H. phaeophyllus* (Romagn.) Arauzo & P. Iglesias, Errotari 15: 330. 2018

*Diagnosis:* Basidiomata pale or dark brown, without distinct naphthalene odours, without yellow colour and dark dots on stipe.

#### *Hodophilus carpathicus* Jančovičová & Adamčík, sp. nov.

Figs. 2, 10–13, 29–30

*Mycobank No.:* MB 833033.

*Etymology:* The name refers to the Carpathian Mountains, the area of origin of the studied material.

*Holotypus:* Slovakia. Malá Fatra Mts, Kláštor pod Znievom, ca 1.5 km SE from the village center, 48° 57' 48.93" N, 18° 49' 14.14" E, alt. 481 m, old pasture with *Juniperus* shrubs, 13 Sep 2016, V. Kučera (SLO2498).

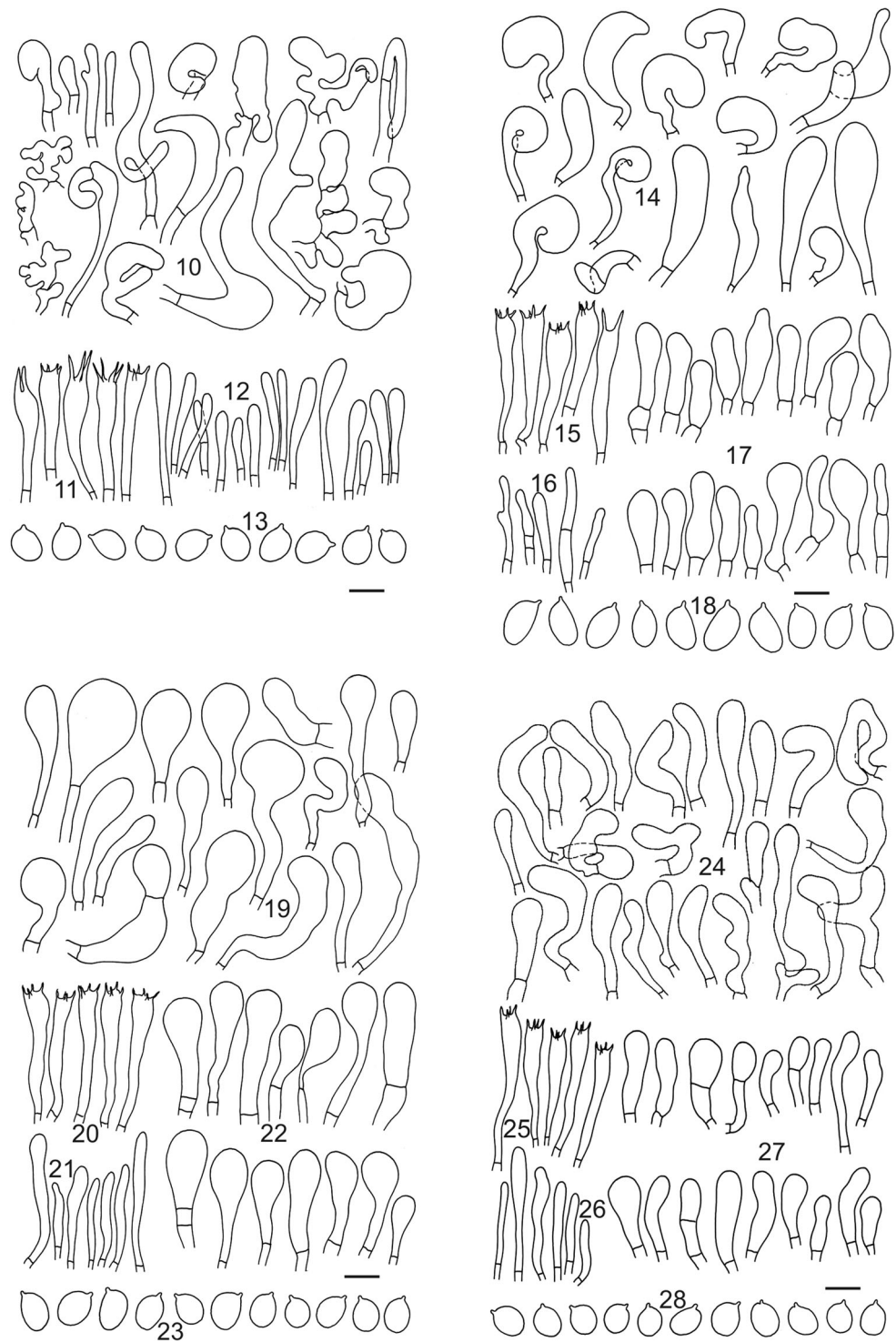
*Diagnosis:* All parts of basidiomata dark brown when young and in moist condition; pileus remains moderately brown also when dry; flesh without a strong odour. Spores subglobose to broadly ellipsoid, in average  $4.6 \times 3.9 \mu\text{m}$ ,  $Q_{av} = 1.2$ ; caulocystidia often strongly nodulose or lobate, flexuous or spirally coiled, sometimes coralloid, very irregular in size, in av.  $28.9 \times 6.9 \mu\text{m}$ .

**Pileus** 7–17 mm broad, hemispherical, convex to applanate, usually weakly depressed at the centre; margin often lobate, inflexed, even denticulate, when moist slightly translucently striate; surface matt, distinctly rugulose, weakly hygrophanous, when moist and fresh coffee brown (5F6) to chocolate (6F4) near the margin, coffee brown (5F7) at the centre; when dry mustard (5E6), coffee brown (5F7) near the margin and nougat (5D3) at the centre. **Stipe** 25–35  $\times$  2–4 mm, usually narrowed towards the base, often longitudinally compressed, flexuous especially towards the base, hollow; smooth, glabrous, matt; when young uniformly coloured, dark brown (chestnut 6F7); when mature paler near the lamellae, coffee brown (5F7), and near the base mustard brown (5E6). **Lamellae** L 22–24, 10–1, up to 2 mm wide, decurrent, tobacco brown (5F6) to chocolate brown (6F4); edge entire, concolorous. **Flesh** elastic, nougat (5D3) in pileus; odour indistinct, with weakly unpleasant component when drying (sweaty).

**Basidiospores** (4.2)4.4–4.9(5.5)  $\times$  (3.4)3.6–4.1(4.4)  $\mu\text{m}$ , av.  $4.6 \times 3.9 \mu\text{m}$ ,  $Q = (1.07)1.13$ – $1.26(1.42)$ ,  $Q_{av} = 1.2$ , subglobose to broadly ellipsoid, hyaline, smooth, thin-walled. **Basidia** 4-spored, narrowly clavate, (27)32–42(46)  $\times$  6–7(8)



**Figs. 10–28** Microscopic structure of *Hodophilus carpathicus* (SLO2498, holotypus). **10** Caulocystidia. **11** Basidia. **12** Basidioles. **13** Spores. *H. decurrentior* (SAV F-3498, holotypus). **14** Caulocystidia. **15** Basidia. **16** Basidioles. **17** Marginal cells. **18** Spores. *H. phaeophyllus* (LIP PAM00101902, epitype). **19** Caulocystidia. **20** Basidia. **21** Basidioles. **22** Marginal cells. **23** Spores. *H. stramineus* (marginal cells SLO784, other elements SAV F-4836, holotypus). **24** Caulocystidia. **25** Basidia. **26** Basidioles. **27** Marginal cells. **28** Spores. Drawings by S. Jančovičová. Scale bar = 5  $\mu\text{m}$  for spores, 10  $\mu\text{m}$  for all other elements



$\mu\text{m}$ , av.  $37.1 \times 6.6 \mu\text{m}$ . **Basidiola** cylindrical to narrowly clavate, obtuse, (13)19–33(40)  $\times$  (2.5)3–5(6)  $\mu\text{m}$ , av.  $26.1 \times 4.1 \mu\text{m}$ . **Pleurocystidia** absent. Well defined **marginal cells** not observed. **Pileipellis** a hymeniderm, rarely a transition to an epithelium; terminal cells near the pileus margin obpyriform, ellipsoid or broadly clavate, often with thickened

walls (up to 1  $\mu\text{m}$ ), often with dark brown parietal pigments and dark brown incrustations on subterminal cells, (15)20.5–43.5(78)  $\times$  (9)12–25.5(35)  $\mu\text{m}$ , av.  $32 \times 18.8 \mu\text{m}$ ,  $Q = (1.08)1.3\text{--}2.23(3.18)$ ,  $Q_{\text{av}} = 1.76$ ; subterminal cells usually distinctly narrower, rarely inflated, unbranched, (2)8–30(42)  $\times$  4–12(22.5)  $\mu\text{m}$ , av.  $19.1 \times 8 \mu\text{m}$ ; small cells (shorter than 5

$\mu\text{m}$ ) rare or occasional. Terminal cells near the pileus centre often narrower than those near the pileus margin,  $(8.5)18\text{--}37.5(53) \times (6.5)10\text{--}21.5(31) \mu\text{m}$ , av.  $27.8 \times 15.5 \mu\text{m}$ ,  $Q = (0.83)1.07\text{--}2.81(6.46)$ ,  $Q_{\text{av.}} = 1.94$ , variable in shape and size, some obpyriform or ellipsoid and very variable in size, others narrower and clavate; subterminal cells similar to those near the pileus margin,  $(2)9.5\text{--}35(54) \times (2.5)4.5\text{--}13.5(21.5) \mu\text{m}$ , av.  $22.4 \times 8.8 \mu\text{m}$ . **Caulocystidia** without dark pigments, thin-walled, repent, usually densely clustered; often strongly nodulose or lobate, flexuous or spirally coiled, often ventricose, sometimes coralloid, occasionally clavate and regular, obtuse, very variable in size  $(10)14\text{--}44(82) \times (3)4\text{--}10(16.5) \mu\text{m}$ , av.  $28.9 \times 6.9 \mu\text{m}$ . **Clamp connections** absent in all parts.

*Additional material examined: Slovakia.* Malá Fatra Mts, Kláštor pod Znievom, ca 1.5 km SE from the village centre,  $48^\circ 57' 48.93'' \text{N}$ ,  $18^\circ 49' 14.14'' \text{E}$ , old pasture with *Juniperus* shrubs, alt. 481 m, 13 Sep 2016, V. Kučera (SLO2499).

***Hodophilus decurrentior*** Adamčík, Jančovičová, Læssøe & Dima, sp. nov.

Figs. 3, 14–18, 31–32

Mycobank No.: MB 833034.

*Etymology:* The name refers to the deeply decurrent lamellae.

*Holotypus:* Slovakia. Záhorská nížina Lowland, Závod vilage, Abrod National Nature Reserve,  $48^\circ 32' 0'' \text{N}$ ,  $17^\circ 0' 25'' \text{E}$ , on soil in low repent scrubs of *Salix* sp. and tall vegetation of *Molinia caerulea*, 30 Sep 2002, S. Adamčík (SAV F-3498).

*Diagnosis:* Pileus greyish brown (7F3), when dry hair brown (5E4); stipe concolorous but paler near the lamellae and at the base; lamellae usually deeply decurrent, brown (6E4); flesh without a strong odour. Spores narrowly ellipsoid to oblong, av.  $5.9 \times 3.8 \mu\text{m}$ ,  $Q = 1.37\text{--}1.74$ ,  $Q_{\text{av.}} = 1.56$ ; caulocystidia clavate, frequently flexuous, occasionally curved, av.  $33.7 \times 8 \mu\text{m}$ ; pileipellis mainly a hymeniderm, terminal cells near the pileus margin with average  $Q > 1.76$ .

**Pileus** 5–13 mm broad, hemispherical to convex, not or weakly depressed at the centre; margin inflexed also at maturity, crenate, when moist weakly translucently striate up to 3 mm; surface matt, smooth, rugulose at the centre, hygrophanous, when moist and fresh greyish brown (7F3), when dry hair brown (5E4). **Stipe** 18–25  $\times$  1–3 mm, narrowed towards the base, flexuous; smooth, matt; concolorous with pileus but near apex and at the base paler. **Lamellae** L = 11–19, l = 0–1, deeply decurrent, brown (6E4); edge entire, concolorous. **Flesh** fragile; grey-brown, unchanging; odour indistinct.

**Basidiospores**  $(4.9)6.3\text{--}6.5(7.5) \times (3.3)3.5\text{--}4.1(4.5) \mu\text{m}$ , av.  $5.9 \times 3.8 \mu\text{m}$ ,  $Q = (1.27)1.37\text{--}1.74(2.21)$ ,  $Q_{\text{av.}} = 1.56$ , ellipsoid to oblong, hyaline, smooth, thin-walled. **Basidia** 4-spored, rarely 2-spored, narrowly clavate,  $(30)32.5\text{--}41(47) \times (5)5.5\text{--}7(7.5) \mu\text{m}$ , av.  $36.5 \times 6.2 \mu\text{m}$ . **Basidiola** cylindrical to

narrowly clavate, obtuse,  $(11)21\text{--}33.5(38) \times (3)3.5\text{--}5.5(7) \mu\text{m}$ , av.  $27 \times 4.5 \mu\text{m}$ . **Pleurocystidia** absent. Well-differentiated **marginal cells** on the lamellar edges observed only near the pileus margin of SAV F-3498, narrowly or broadly clavate, occasionally subcylindrical or lageniform, rarely sphaeropedunculate, apically obtuse,  $(15.5)19.5\text{--}29.5(41) \times (3.5)5.5\text{--}9(11.5) \mu\text{m}$ , av.  $24.5 \times 7.3 \mu\text{m}$ . **Pileipellis** a hymeniderm, rarely a transition to an epithelium, with intraparietal pigments especially at subterminal cells; terminal cells near the pileus margin obpyriform, sphaeropedunculate, subglobose or broadly clavate, walls not distinctly thickened (up to  $0.5 \mu\text{m}$  thick),  $(20)24\text{--}45.5(63) \times (10.5)14.5\text{--}26(38) \mu\text{m}$ , av.  $34.7 \times 20.3 \mu\text{m}$ ,  $Q = (1)1.27\text{--}2.26(3.76)$ ,  $Q_{\text{av.}} = 1.76$ ; subterminal cells usually distinctly narrower, cylindrical, rarely inflated, not branched,  $(2.5)5.5\text{--}31(65) \times (2)3.5\text{--}10(19) \mu\text{m}$ , av.  $18.3 \times 6.7 \mu\text{m}$ ; small cells (shorter than  $5 \mu\text{m}$ ) rare or occasional. Terminal cells near the pileus centre similar in size and shape,  $(13)25\text{--}47(59) \times (9)13.5\text{--}25.5(33) \mu\text{m}$ , av.  $35.9 \times 19.5 \mu\text{m}$ ,  $Q = (1)1.33\text{--}2.54(3.46)$ ,  $Q_{\text{av.}} = 1.93$ , occasionally nodulose near the bases; subterminal cells more frequently inflated,  $(2)11\text{--}38(59) \times (3)4\text{--}13.5(25) \mu\text{m}$ , av.  $24.6 \times 8.6 \mu\text{m}$ . **Caulocystidia** without dark pigments, thin-walled, ascending or repent, usually clustered in patches; terminal cells mostly narrowly clavate to clavate, rarely ventricose, often pedunculate, frequently flexuous, occasionally curved to twisted, obtuse,  $(18)24\text{--}43.5(51) \times (6)6.5\text{--}9.5(12) \mu\text{m}$ , av.  $33.7 \times 8 \mu\text{m}$ . **Clamp connections** absent in all parts.

*Additional material examined: Norway.* Oslo, Gressholmen,  $59^\circ 53' 02.04'' \text{N}$ ,  $10^\circ 43' 07.32'' \text{E}$ , in grass turf on calcareous rocks, 7 Oct 2013, T. Læssøe & A. Molia NOBAS2878-16 (O-F-21872); *ibid.*, 16 Oct 2012, T. Læssøe & A. Molia NOBAS2999-16 (O-F-245610).

***Hodophilus phaeophyllus*** (Romagn.) Arauzo & P. Iglesias, *Errotari* 15: 330. 2018

Figs. 4–5, 19–23, 33–34

$\equiv$  *Hygrophorus rugulosus* var. *phaeophyllum* Romagn., *Bull. trimest. Soc. mycol. Fr.* 86: 874. 1971

$\equiv$  *Camarophyllopsis phaeophylla* (Romagn.) Arnolds, *Mycotaxon* 25(2): 643. 1986

$\equiv$  *Hygrotrama phaeophylla* (Romagn.) Arnolds, *Persoonia* 12(4): 477. 1985

$\equiv$  *Hygrotrama rugulosa* var. *phaeophylla* (Romagn.) [as “*rugulosus* var. *phaeophyllus*”] Bon, *Doc. Mycol.* 7(27-28): 46. 1977

*Holotypus:* France. Forêt de Coye, le Caillou Blanc, à Chaumontel (V. d’O.), 12 Sep 1951, *Romagnesi* 51.267 [no specimen located in Romagnesi’s herbarium in PC].

*Lectotypus (designated here):* *Bull. trimest. Soc. mycol. Fr.* 86: 872, Fig. 3. 1971. MycoBank typification number MBT389233.

*Epitypus* (designated here): France. Pas-de-Calais. Terril de Pinchonvalles, thickets of *Crataegus* and *Betula*, on soil, 19 Oct 2000, P-A Moreau (LIP PAM00101902). MycoBank typification number MBT389232.

= *Hodophilus hymenocystis* Arauzo & P. Iglesias, *Errotari* 15: 317. 2018

**Original diagnosis:** A typo differ colore magis e fusco murino ac lamellis brunneis.

**Emended diagnosis:** Pileus near the margin pale brown to brown (6D3–6E5), at the centre dark brown (6F6), when dry reddish grey (7B2) to whitish; stipe concolorous but when young paler near the apex and at the base; lamellae first whitish when mature dark brown (6F6); flesh without a strong odour. Spores broadly ellipsoid to ellipsoid, av.  $4.7 \times 3.7 \mu\text{m}$ ,  $Q = 1.17\text{--}1.37$ ,  $Q_{av} = 1.27$ ; caulocystidia mainly clavate to broadly clavate, av.  $31.1 \times 10.7 \mu\text{m}$ ; pileipellis mainly a hymeniderm, terminal cells near the pileus margin with  $Q_{av} = 1.36$ .

**Pileus** 5–15 mm broad, hemispherical to convex, slightly depressed at the centre; margin inflexed, crenate, when moist weakly translucently striate; surface matt, rugulose, veined, hygrophanous; when moist and fresh brown (6E5) to greyish brown/café-au-lait (6D3) near the margin, dark brown/burnt amber (6F6) at the centre, when dry reddish grey (7B2) to whitish. **Stipe** 20–30  $\times$  1.5–2.5 mm, sometimes enlarged near lamellae up to 3.5 mm, narrowed towards the base, flexuous, tortuous; pruinose, white powdery to white fibrillose, the base covered by sparse white mycelium; near the apex at first whitish, orange grey (5B2), to light ochre, then flesh brown (6B3) brownish orange (7C3), towards the base whitish, then reddish grey (7B2) to dark brown (7F4). **Lamellae**  $L = 12\text{--}16$ ,  $l = 0\text{--}1$ , up to 2 mm wide, deeply decurrent, whitish (5A2), then reddish grey (7B2), brownish orange (6C3) to dark brown (6F6). **Flesh** fragile; odour faint or herbaceous earthy.

**Basidiospores**  $(4.1)4.3\text{--}5(6) \times (3.1)3.4\text{--}3.9(4.2) \mu\text{m}$ , av.  $4.7 \times 3.7 \mu\text{m}$ ,  $Q = (1.1)1.17\text{--}1.37(1.58)$ ,  $Q_{av} = 1.27$ , broadly ellipsoid to ellipsoid, hyaline, smooth, thin-walled. **Basidia** 4-spored, hyaline, narrowly clavate and slightly flexuous toward the base,  $(26)29.5\text{--}38.5(47) \times (4.5)5\text{--}6.5(7) \mu\text{m}$ , av.  $34.1 \times 5.9 \mu\text{m}$ . **Basidiola** cylindrical to narrowly clavate, often flexuous, obtuse,  $(15)24\text{--}35.5(40) \times (2.5)3.5\text{--}5.5(7) \mu\text{m}$ , av.  $29.5 \times 4.4 \mu\text{m}$ . **Pleurocystidia** absent. **Marginal cells** observed in one collection only (PAM05100301), well differentiated, clavate to sphaeropedunculate, sometimes flexuous towards the base, rarely nodulose, obtuse,  $(16)20\text{--}35.5(45) \times (7)8\text{--}11(12.5) \mu\text{m}$ , av.  $27.7 \times 9.7 \mu\text{m}$ . **Pileipellis** a transition between a hymeniderm and an epithelium; terminal cells near the pileus margin subglobose, obpyriform, thin-walled or only with indistinctly thickened walls,  $(15)21.5\text{--}43.5(68) \times (10)18.5\text{--}29.5(40) \mu\text{m}$ , av.  $32.7 \times 23.9 \mu\text{m}$ ,  $Q = (1)1.07\text{--}1.65(2.43)$ ,  $Q_{av} = 1.36$ ; subterminal cells usually distinctly narrower, cylindrical, rarely inflated, occasionally branched,  $(3)4\text{--}30.5(94) \times 3\text{--}11(29) \mu\text{m}$ , av.  $17.4 \times 6.8 \mu\text{m}$ ; small cells (shorter than 5  $\mu\text{m}$ ) occasional. Terminal cells near the pileus

centre similar in size and shape to those near the pileus margin,  $(17)23.5\text{--}43(55) \times (8)17.5\text{--}29.5(37) \mu\text{m}$ , av.  $33.5 \times 23.4 \mu\text{m}$ ,  $Q = 0.97\text{--}2.04(5.25)$ ,  $Q_{av} = 1.48$ ; subterminal cells also similar to those near the pileus margin,  $(3)9.5\text{--}31(46) \times (3)3.5\text{--}11(22) \mu\text{m}$ , av.  $20.2 \times 7.4 \mu\text{m}$ . **Caulocystidia** without dark pigments, thin-walled, repent or ascending; terminal cells mainly clavate to broadly clavate, rarely obpyriform, occasionally pedunculate or flexuous towards the base, apically obtuse,  $(11)21\text{--}41.5(65) \times (6)7.5\text{--}14(21.5) \mu\text{m}$ , av.  $31.1 \times 10.7 \mu\text{m}$ . **Clamp connections** absent in all parts.

**Additional material examined:** **France.** Doubs. Boujailles, maison forestière de Chevreuille, among needles under *Thuja* sp., 3 Nov 2005, J-M. Moingeon PAM05100301 (LIP 0401638); Hautes-Pyrénées, ravin de la Tapère, *Buxus* litter, on calcareous soil, 28 Sep 2002, G. Corriol (BFF GC02092803). **Germany.** Thüringen. Jena, Jenaer Forst, on soil, 27 Sep 2014, T. Böhning C8-AG20; Thüringen, Jena, Nordfriedhof, 28 Jul 2009, A. Gminder E07 sp AT H5.

*Hodophilus stramineus* Jančovičová, Dima & Adamčík, sp. nov.

Figs. 6–9, 24–28, 35–36

MycoBank No.: MB 833035.

**Etymology:** The name refers to the pale straw colour of dry pilei.

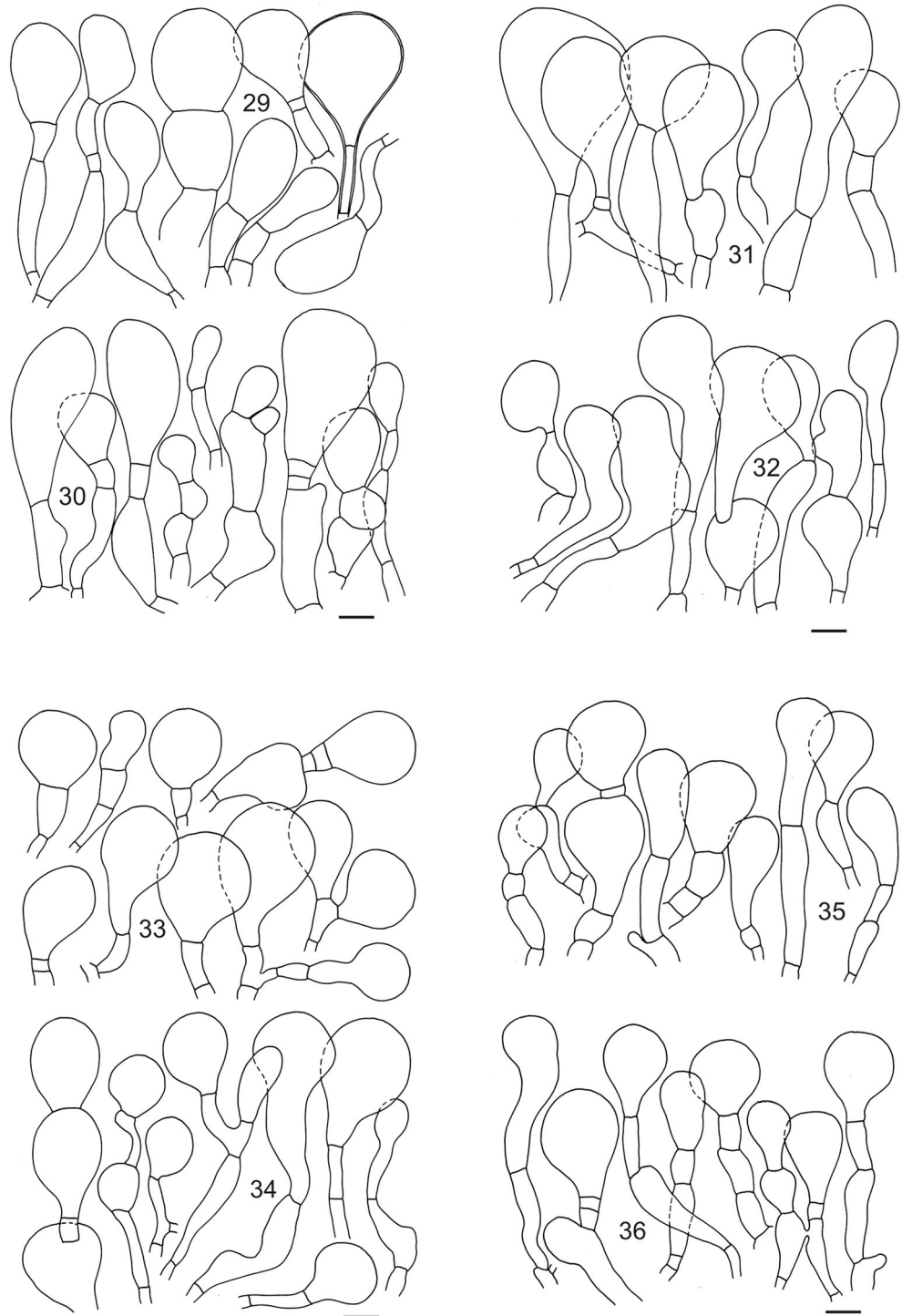
**Holotypus:** United Kingdom. Wales. Pembrokeshire, Orierton Wood, Orierton Field Study Centre,  $51^{\circ} 39' 10.55''$  N,  $4^{\circ} 57' 03.25''$  W, on ground at woodland edge associated with *Fraxinus*, *Acer*, *Quercus*, *Corylus*, *Hedera helix* and *Rubus*, 8 Oct 2016, D. Harries (SAV F-4836).

**Diagnosis:** Pileus light brown to moderately brown (6D3 to 5C4, 6C2, 4–5F7), fading to even paler yellowish brown or grey-brown colours when dry (5B3–B4); stipe with pale brownish, yellowish and greyish tints near the lamellae, darker grey-brown towards the base, where it becomes even darker when old; flesh without a strong odour. Spores in average  $4.4 \times 3.6 \mu\text{m}$ ,  $Q_{av} = 1.24$ ; pileipellis mainly a hymeniderm, terminal cells of the hyphae near the pileus centre mainly obpyriform, broadly clavate or sphaeropedunculate, with  $Q_{av} < 1.46$ .

**Pileus** 5–21 mm broad, convex to plano-convex, usually weakly depressed at the centre; margin inflexed, slightly crenate, when moist translucently striate up to half the radius; surface matt, smooth, sometimes rough or rugulose at the centre, when dry locally cracking and forming a fine granulose structure, hygrophanous; when moist and fresh greyish brown/café-au-lait (6D3) or paler brown near the margin, darker, brownish orange/golden blonde (5C4), brownish grey (6C2), olive brown (4F7–5F7) at the centre, when dry greyish orange (5B3), yellowish brown/ hair brown (5E4) to bronze (5E5), grey brown (5C3) or greyish yellow/champagne (4B4). **Stipe** 12–35  $\times$  1–4 mm, cylindrical, usually narrowed towards the base, sometimes flexuous; smooth, shiny, sometimes near lamellae pruinose or granulose; near the lamellae orange grey



**Figs. 29–36** Microscopic structure of *Hodophilus carpathicus* (SLO2498, holotypus). **29** Hyphal terminations in pileipellis near the pileus margin. **30** Hyphal terminations in pileipellis near the pileus centre. *H. decurrentior* (SAV F-3498, holotypus). **31** Hyphal terminations in pileipellis near the pileus margin. **32** Hyphal terminations in pileipellis near the pileus centre. *H. phaeophyllus* (PAM00101902, neotype). **33** Hyphal terminations in pileipellis near the pileus margin. **34** Hyphal terminations in pileipellis near the pileus centre. *H. stramineus* (SAV F-4836, holotypus). **35** Hyphal terminations in pileipellis near the pileus margin. **36** Hyphal terminations in pileipellis near the pileus centre. Drawings by S. Jančovičová. Scale bar = 10  $\mu\text{m}$



(5B2), brownish orange (5C3), blonde (4C4), beige (4C3), greyish yellow/champagne (4B4); towards the base darker, at first greyish brown/nougat (5D3), soot brown (5F5), then dark brown/chocolate (6F4) or chestnut (6F6, 6F7). **Lamellae** L = 12–31, l = 0–1, up to 3 mm wide, decurrent to deeply decurrent, usually darker than pileus surface, young brownish orange (5C4), mature yellowish brown (5E4, 5E7) or brownish orange (6D3); edge entire, when young pale whitish, when

old concolourous. **Flesh** elastic, beige; odour indistinct, with a faint unpleasant component.

**Basidiospores** (3.9)4.1–4.7(5.4)  $\times$  (2.9)3.3–3.8(4.3)  $\mu\text{m}$ , av. 4.4  $\times$  3.6  $\mu\text{m}$ ,  $Q = (1.08)1.18\text{--}1.3(1.5)$ ,  $Q_{\text{av}} = 1.24$ , broadly ellipsoid, hyaline, smooth, thin-walled. **Basidia** 4-spored, narrowly clavate, (25)30.5–38(44)  $\times$  (4.5)5–6(7)  $\mu\text{m}$ , av. 34.2  $\times$  5.7  $\mu\text{m}$ . **Basidiola** cylindrical to narrowly clavate, obtuse, (14)21.5–33(42)  $\times$  (3)3.5–5(6)  $\mu\text{m}$ , av. 27.2  $\times$  4.4  $\mu\text{m}$ .

**Pleurocystidia** absent. Well defined **marginal cells** on the lamellae edges observed only in one collection (SLO784), mainly clavate, occasionally ellipsoid or obpyriform, obtuse, (10)12.5–24.5(34) × (4.5)5–8(10.5) μm, av. 18.4 × 6.6 μm. **Pileipellis** a hymeniderm, rarely a transition to an epithelium; terminal cells near the pileus margin obpyriform, broadly clavate or sphaeropedunculate, often with thickened walls (0.5–1 μm), (14)23.5–43.5(65) × (9)16.5–31(41) μm, av. 33.4 × 23.8 μm,  $Q = (0.7)1.04–1.88(4.21)$ ,  $Q_{av.} = 1.46$ ; subterminal cells usually distinctly narrower, thin-walled, cylindrical, rarely inflated and branched, (2)4.5–23.5(50) × (2)4–10.5(33) μm, av. 14 × 7.1 μm; small cells (shorter than 5 μm) rare or occasional. Terminal cells near the pileus centre similar to those near the pileus margin, (10)25–43(65) × (10)16–29(49) μm, av. 34 × 22.6 μm,  $Q = (0.79)1.09–2.07(4.7)$ ,  $Q_{av.} = 1.58$ ; subterminal cells also similar, (2)4–24(54) × (2)3.5–10(22) μm, av. 14.1 × 6.6 μm. **Caulocystidia** without dark pigments, thin-walled, ascending or repent; terminal cells mostly narrowly clavate to clavate, frequently flexuous or twisted, occasionally nodulose, apically obtuse, (10)23.5–47(69) × (5)6–13.5(26) μm, av. 35.3 × 9.7 μm. **Clamp connections** absent in all parts.

**Additional material examined:** **France.** Pas-de-Calais, Eperlecques, wet plantation of *Alnus incana* with *Molinia caerulea*, 22 Jul 2012, P.-A. Moreau PAM12072201 (LIP0401639); Hautes-Pyrénées: Castet de Gerde, broadleaf forest, on clay soil, 12 Nov 2012, G. Corriol (BFF GC12112205). **Norway.** Oslo, Bygdøy, Reinsdyrlia, on soil in *Tilia-Corylus* forest, 16 Sep 2015, T.E. Brandrud, B. Dima, DB5776 / TEB373-15 (O). **United Kingdom. England.** S Somerset, Swell Wood, near Fivehead, on bare, damp soil, under *Corylus avellana*, 18 Sep 2008, N.W. Legon (KM161018); **Wales.** Powys, Gregynog grounds, 24 Oct 2014, pasture on edge of *Quercus* forest, on soil, R. Foster (SAV F-4399). **Slovakia.** Podunajská nížina Lowland, Banka village, near the Koliba pod Ahojom, scrubs on the forest margin, on soil, 26 Sep 2014, S. Jančovičová (SLO782, SLO784); Malé Karpaty Mts, Plavecké Podhradie village, margin of meadow, near deciduous forest, 19 Oct 2014, S. Jančovičová (SLO507); Poľana Mts, Zvolen city, Arborétum Borová hora, soil on a stream bank, 30 Sep 2009, S. Adamčík (SAV F-3096; SAV F-3097). **Germany.** Thüringen, Jena, Rautal, on soil, 28 Sep 2014, T. Böhning TB14/075; Thüringen, NP Hainich, on soil, 15 Sep 2014, T. Böhning C12-AG34; Germany, Thüringen, Craula, NP Hainich, 31 Oct 2013, A. Gminder A08 sp AT H9; Thüringen, Hütscheroda, NP Hainich, 12 Nov 2013, A. Gminder C08 sp AT H11.

## Discussion

### How much can we trust morphology?

When discussing the situation and recent changes in species circumscriptions within the genus *Hodophilus* with other

mycologists, we received feedbacks like “yesterday, species identification was easy and tomorrow it will be difficult”. Indeed, previous species concepts were easy: all collections with naphthalene odours were identified as *H. foetens*, all with dark dots as *H. atropunctus*, with yellow stipes as *H. micaceus* and those collections without these marked characters were assigned to *H. hymenocephalus* or *H. phaeophyllus* (e.g. Boertmann 2012). Surprisingly, this easy concept of four species was supported also by earlier phylogenetic studies (Kovalenko et al. 2012). Studies of Adamčík et al. (2017a, 2017b, 2018) confirmed one after another that naphthalene odour, dark dots on stipes and yellow colours on stipes are characters associated with a range of phylogenetically defined species. Moreover, the study on species with dark dots demonstrated that odour and dark dots on the stipe are plesiomorphic characters present in unrelated lineages of the genus.

Our current study is dealing with the last unexplored group, representing the residual morphotypes not displaying any of above mentioned striking characters. We demonstrated that this group is represented by at least five species in Europe, four of which we describe here. Surprisingly, the residual European morphotypes are grouped in a single monophyletic lineage. However, our initial field identifications taught us that the morphological delimitation of this particular group is highly intriguing. Several members of the *H. micaceus* lineage may lose the yellow colour on the stipe with age and thus become very similar to any of the species in the *H. phaeophyllus* lineage. To avoid mistakes, identification should be based on collections with a least some young basidiomata. Some collections of *H. variabilipes* without dots may be similar especially to *H. stramineus* since both have yellow-brown colours, but the colours of the latter are duller and much paler when fresh or young and the stipe becomes darker towards the base.

We are aware that our current proposal for species circumscription is based on relatively few well annotated collections and may not cover the variability of characters sufficiently. Moreover, potential undiscovered species of the genus may reshuffle the distinguishing characters and the key. One undescribed species is present in our phylogeny represented by three ITS sequences. One sequence was obtained from a collection sent by Felix Hampe, who provided also a photograph showing very dark brown basidiomata similar to *H. carpathicus*. Also, a sequence with accession number MK139804 of collection ERRO-2013112801 identified by Arauzo and Iglesias (2018) as *H. phaeophyllus* belongs to this undescribed species and Fig. 23 in their study made by Patrice Tanchaud probably corresponds to this collection and shows also very dark brown basidiomata. Further phylogenetic and morphological delimitation of this species will require new, well annotated collections,

further work behind the microscope and more sequence work.

### Brown odourless *Hodophilus* species in European literature

The oldest name used for the residual brown morphotypes corresponding to the *H. phaeophyllus* lineage is *Hygrophorus hymenocephalus* A.H. Sm. & Hesler described from Tennessee, USA (Smith and Hesler 1940), recently combined in *Hodophilus* (Birkebak et al. 2016). A decade later, the species was reported from Denmark (Lange and Hansen 1950) and soon from England (Hora and Orton 1955). At that time, it was believed that different species of the genus (as *Hygrophorus*) may have a transoceanic distribution in Europe and North America and as a consequence, Romagnesi (1971) described *H. rugulosus* var. *phaeophyllus* Romagn. as a colour variety of the North American species *H. rugulosus* A.H. Sm. & Hesler also recently combined in *Hodophilus* (Adamčík et al. 2018). Arnolds (1986, 1990), who raised Romagnesi's variety to species rank (in the genus *Camarophylloopsis*) believed that *H. hymenocephalus* occurred in Europe but was distinct from *H. phaeophyllus*. Boertmann (2012) treated the names as synonyms. Nevertheless, there is no evidence of transoceanic distributions within the genus (e.g. Birkebak et al. 2016); *H. hymenocephalus* is placed apart from the *H. phaeophyllus* lineage in our tree and the use of this name should be abandoned in Europe.

Our publication raises the number of known species in the *H. phaeophyllus* lineage from one or two previously accepted (*H. hymenocephalus* and *H. phaeophyllus*) to four described here and one undescribed species. We are fairly sure, that none of the previous publications included *H. decurrentior*, due to the deviating spore shape. Hora and Orton (1955) and Printz and Læssøe (1986) described the colours of *H. hymenocephalus* as dark brown without mentioning distinct discolourations that may correspond to *H. carpathicus* or the dark undescribed species recovered in our phylogeny. Surprisingly, three different publications described *H. phaeophyllus* (Moser 1978, Horak 2005) or *H. hymenocephalus* (Lange and Hansen 1950) with darker colours during maturation. This does not correspond to any species described in our study. We are not sure, whether this darkening species corresponds to the undescribed species in our phylogeny, or to another species, or if these descriptions are based on mixed collections, because species of *Hodophilus* often co-occur in small hotspots. Arnolds (1990) gave his description of *H. phaeophyllus* with colour codes according to Komerup and Wanscher (1967), thus we were able to compare colours with our descriptions. The pileus colours of the Arnolds' description cover both *H. phaeophyllus* and *H. stramineus* in our observations (Table 1). It is apparent, that without sorting of collections to phylogenetic species, sorting out the colour variation and dissimilarities were impossible. Kovalenko et al. (2012), who published the first limited phylogeny of *Hodophilus* (as *Camarophylloopsis*

*sensu lato*) based on *tefl* $\alpha$ , provided the only description of *H. phaeophyllus* that fits well to the concept of the species presented and typified in this study.

Recently, Arauzo and Iglesias (2018) recognised two European species that morphologically correspond to the *H. phaeophyllus* lineage, one as new, *H. hymenocystis*, and *H. phaeophyllus*. They distinguish the former by the presence of marginal cells at the lamellae edges and the paler colour of lamellae and pileus. Our colour comparison of the four species described in this study with the original description of *H. phaeophyllus* showed that its best match is within the species clade that contains an ex-type sequence of *H. hymenocystis*. There are two reasons why Arauzo and Iglesias (2018) applied their new name for *H. phaeophyllus*. Firstly, sequences identified by these authors as *H. phaeophyllus* belong to two different species according to our phylogeny: to the pale-coloured *H. stramineus* and to a dark coloured undescribed species. Secondly, the presence of marginal cells is not a constant feature within the species according to our earlier studies (Adamčík et al. 2018) and our current observations confirmed it (Table 1.). Arauzo and Iglesias (2018) argued that the original description of *H. phaeophyllus* reported an absence of marginal cells. Romagnesi (1971) described together with *Hygrophorus rugulosus* var. *phaeophyllus* also *H. phaeoxanthus* in the same paper, and for both he did not mention any defined elements of the lamellae edges. The type study of *H. phaeoxanthus* (Adamčík et al. 2018), however, revealed presence of marginal cells. To our experience, these elements are difficult to observe and are easily overlooked due to often very limited material (small basidiomata).

### Ecology and distribution

According to our observations, members of the *H. phaeophyllus* lineage are the rarest in Europe compared to the other two phylogenetic groups of the genus. *Hodophilus phaeophyllus* and *H. stramineus*, both known from France, Germany and Spain, the latter also from Great Britain and Slovakia, maybe the most common and widespread within the lineage. Collections of the undescribed species in our phylogeny originate from Spain, Great Britain and Germany. *Hodophilus decurrentior* is only known from Norway and Slovakia, but this is unlikely to reflect the true distribution. *Hodophilus carpathicus* is only known from two collections from the same site, but some descriptions of dark basidiomata from other sites may correspond to this species. The ecology of our collections is similar to other groups of the genus: grasslands, scrubs, forest margins or non-ectomycorrhizal woods, often on heavy clay soils with high pH. They may occur in mass fruitings with several species involved. At one small site in Denmark covering about 50 × 50 m all the classic four “species” co-occurred and produced thousands of fruitbodies. They were associated with *Camarophylloopsis schulzeri*, many clavarioid species and a long list of Geoglossaceae and



*Entoloma* species. The known occurrences of the genus suggest that its members prefer a humid climate with high annual rainfall or relatively stable humid conditions but known sites vary a lot in terms of climatic conditions and amount of exposure from completely open dry grassland to very wet closed forests such as alder carrs with lime deposits.

**Acknowledgements** The authors would like to thank Tanja Böhning, Tor Erik Brandrud, Rob Foster, David Harries, Andreas Gminder, Viktor Kučera, Jean-Marc Moingeon, Anna Molia and Erik Amfred Thomsen for their contribution of specimens, photographs and/or assistance with field work. We also thank the staff and curators of the herbarium at K, LIP and O (including NorBOL staff) for loans of the herbarium material.

**Funding information** The research of SA, MC, KA and SJ was granted by the national grant Vega 02/0018/18 and a grant from Slovak-American Foundation to SA. The research of BD was partly supported by the ELTE Institutional Excellence Program (1783-3/2018/FEKUTSRAT) of the Hungarian Ministry of Human Capacities.

## References

- Adamčík S, Looney BP, Birkebak JM, Jančovičová S, Adamčíková K, Marhold K, Matheny PB (2016) Circumscription of species of *Hodophilus* (Clavariaceae, Agaricales) in North America with naphthalene odour. *Botany* 94:941–956. <https://doi.org/10.1139/cjb-2016-0091>
- Adamčík S, Jančovičová S, Looney BP, Adamčíková K, Birkebak JM, Moreau PA, Vizzini A, Matheny PB (2017a) Circumscription of species in the *Hodophilus foetens* complex (Clavariaceae, Agaricales) in Europe. *Mycol Prog* 16:47–62. <https://doi.org/10.1007/s11557-016-1249-x>
- Adamčík S, Jančovičová S, Looney BP, Adamčíková K, Griffith GW, Læssøe T, Moreau PA, Vizzini A, Matheny PB (2017b) *Hodophilus* (Clavariaceae, Agaricales) species with dark dots on the stipe: more than one species in Europe. *Mycol Prog* 16:811–821. <https://doi.org/10.1007/s11557-017-1318-9>
- Adamčík S, Dima B, Adamčíková K, Harries D, Læssøe T, Moreau P-A, Jančovičová S (2018) European *Hodophilus* (Clavariaceae, Agaricales) species with yellow stipe. *Mycol Prog* 17:1097–1111. <https://doi.org/10.1007/s11557-018-1418-1>
- Arauzo S, Iglesias P (2018) Estudios en el género *Hodophilus* (Clavariaceae). *Errotari* 15:314–340
- Arnolds E (1986) Notes on Hygrophoraceae IX. *Camarophylloopsis* Herink, an older name for *Hygrotrama* Sing. *Mycotaxon* 25:639–644
- Arnolds E (1990) *Camarophylloopsis*. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds) *Flora Agaricina Neerlandica*, vol 2. A.A. Balkema, Rotterdam, Brookfield, pp 111–115
- Birkebak JM, Mayor JR, Ryberg M, Matheny PB (2013) A systematic, morphological, and ecological overview of the Clavariaceae (Agaricales). *Mycologia* 105:896–911. <https://doi.org/10.3852/12-070>
- Birkebak JM, Adamčík S, Matheny PB (2016) Multilocus phylogenetic reconstruction of the Clavariaceae (Agaricales) reveals polyphyly of the agaricoid members. *Mycologia* 108:860–868. <https://doi.org/10.3852/15-370>
- Boertmann D (2012) *Camarophylloopsis* Herink. In: Knudsen H, Vesterholt J (eds) *Funga Nordica*, 2nd edn. Nordsvamp, Copenhagen, pp 242–244
- Borchsenius F (2009) FastGap 1.2. Department of Bio-sciences, Aarhus University, Denmark. [http://www.aubot.dk/FastGap\\_home.htm](http://www.aubot.dk/FastGap_home.htm)
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224. <https://doi.org/10.1093/molbev/msp259>
- Hora FB, Orton PD (1955) Three new british agaric records. *Trans Br Mycol Soc* 38:400–404
- Horak E (2005) Röhrlinge und Blätterpilze in Europa. Spektrum Akademischer Verlag, München
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780. <https://doi.org/10.1093/molbev/mst010>
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511–518. <https://doi.org/10.1093/nar/gki198>
- Kornerup A, Wanscher JH (1967) *Methuen Handbook of Colour*, 2nd edn. Methuen Co., London
- Kovalenko AE, Malysheva EF, Morozova OV (2012) The genus *Camarophylloopsis* in Russia: new records and new species *C. albofoccepipes*. *Mikol Fitopatol* 46:54–66 [In Russian]
- Lange M, Hansen EB (1950) Notes on Danish Fungi. *Friesia* 4:61–65
- Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). *Mol Phylogenetics Evol* 35:1–20. <https://doi.org/10.1016/j.ympev.2004.11.014>
- Moser M (1978) *Keys to agarics and boleti*. Roger Phillips, London
- Nagy LG, Kocsubé S, Csanádi Z, Kovács GM, Petkovits T, Cs V, Papp T (2012) Re-mind the gap! Insertion – deletion data reveal neglected phylogenetic potential of the nuclear ribosomal internal transcribed spacer (ITS) of fungi. *PLoS ONE* 7:e49794. <https://doi.org/10.1371/journal.pone.0049794>
- Printz P, Læssøe T (1986) *Vokshatteslægten Camarophylloopsis* i Danmark. *Svampe* 14:83–92
- Romagnesi H (1971) Sur quelques espèces omphalioides d'agarics leucosporés. *Bull trimest Soc mycol Fr* 86:865–874
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Syst Biol* 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAXML. *Org Divers Evol* 12:335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Simmons MP, Ochoterena H, Carr TG (2001) Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. *Syst Biol* 50:454–462. <https://doi.org/10.1080/10635150120427>
- Singer R (1975) *The Agaricales in modern taxonomy*, 3rd edn. J. Cramer, Vaduz
- Vellinga EC (1988) Glossary. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds) *Flora Agaricina Neerlandica*, vol 1. A.A. Balkema, Rotterdam, Brookfield, pp 54–69
- White TJ, Bruns T, Taylor LS (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and application*. Academic Press, Inc., San Diego, pp 322–315

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.