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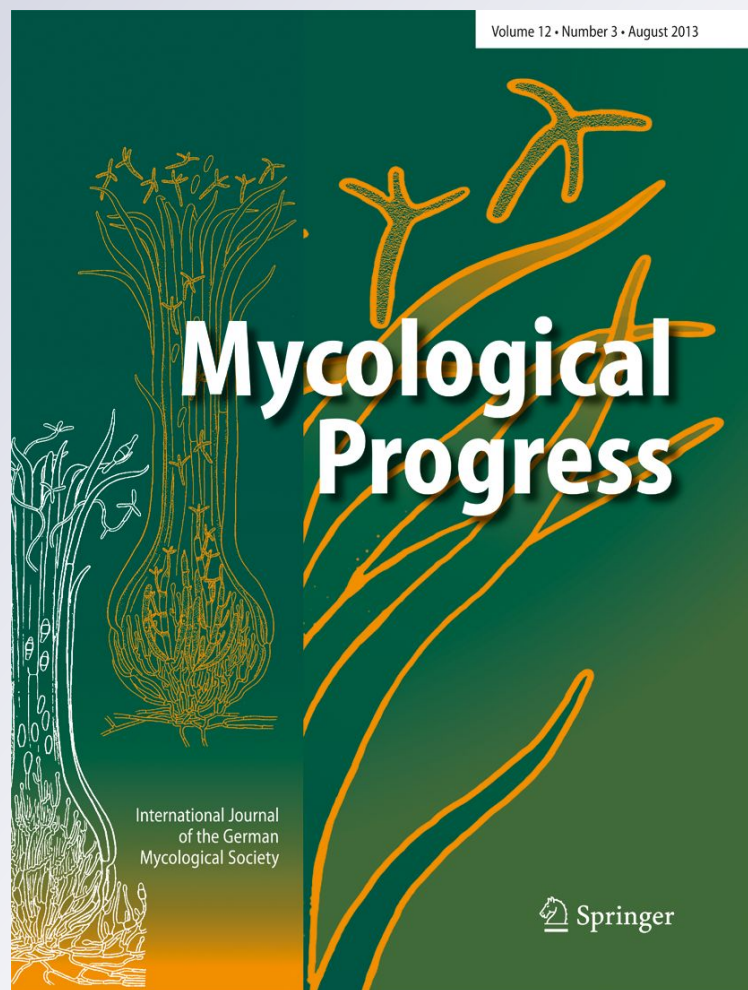
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# *Hodophilus* (Clavariaceae, Agaricales) species with dark dots on the stipe: more than one species in Europe

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**Abstract** *Hodophilus atropunctus* is traditionally defined as the only species of this genus with dark brown or black dots on the stipe. Multi-locus phylogenetic reconstruction recognised two distinct clades morphologically corresponding to this species concept. The limited morphological description in the protologue of *H. atropunctus* and absence of a type specimen were limitations in an assignment of this name to one of the recognised phylogenetic species. The emended species concept and the selection of a neotype are based on careful analyses of the colour of the basidiomata and how this changes during

maturation and drying. The name *H. atropunctus* is assigned to the paler of the two species which also shows colour change across the pileus and along the length of the stipe when dry. The second darker species is described here as new, *H. variabilipes*, but only seven out of 14 collections examined belonging to this taxon had distinct dark coloured dots on the stipe surface.

**Keywords** Agaricoid · *Camarophyllopsis* · Multi-locus phylogeny · Morphology · Type studies

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## Introduction

*Hodophilus atropunctus* (Pers.: Fr.) Birkebak & Adamčík is the only known species of the genus with distinct dark dots on the stipe surface (Bon 1977; Boertmann 2012; Kovalenko et al. 2012). It has traditionally been classified within the genus *Camarophylloopsis* Herink (Boertmann 2012; Kovalenko et al. 2012), but recent phylogenetic studies demonstrated polyphyly of the agaricoid species in the family *Clavariaceae* Chevall. and *H. atropunctus* was allocated to the genus *Hodophilus* R. Heim ex R. Heim together with other species with a hymeniform pileipellis (Birkebak et al. 2016). Further phylogenetic studies of the genus *Hodophilus* demonstrated the existence of several species within the traditional concept of *H. foetens* (W. Phillips) Birkebak & Adamčík, defined by an unpleasant naphthalene odour (Adamčík et al. 2016, 2017). These studies suggested that the strong, naphthalene-like odour defines not a single species but rather a group of species called the *H. foetens* superclade with several members in North America and Europe. Only a few collections with naphthalene-like odours belong to the second superclade of the genus (*H. micaceus* superclade) containing mainly odourless species. Adamčík et al. (2016, 2017) also showed that *H. atropunctus* collections identified based on darker dots on the stipe surface are placed within both superclades.

In this study, we aim to specify how widespread these distinct dark dots are among *Hodophilus* collections and whether this character is species-specific and useful for morphological delimitation. We also sought other morphological characters which might additionally distinguish the various species with dark dots on the stipe.

## Materials and methods

### Taxon sampling

Altogether, 14 *Hodophilus* collections with dark spots on the stipe surface are analysed, of which nine are newly sequenced in this study. Another nine collections with high sequence similarity were added, including six newly sequenced. All *Hodophilus* collections with dark spots or with high sequence similarity to them are listed in the Supplementary Table 1. For phylogenetic placement we used sequences previously published by Adamčík et al. (2017).

### DNA extraction, PCR, and sequencing

Three gene regions (nrLSU, nrITS and *rpb2*) were amplified, sequenced and analysed. Protocols of Birkebak et al. (2013) were followed for DNA extraction, PCR, and sequencing.

Primer pairs ITS1F-ITS4 (Gardes and Bruns 1993; White et al. 1990) were used to amplify the ITS region. Combinations of LR0R-LR7, LR0R-LR5, or LR0R-LR16 (<http://sites.biology.duke.edu/fungi/mycolab/primers.htm>) were used to amplify and sequence the nLSU 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

Alignments for individual regions were created in CLUSTAL X (Larkin et al. 2007) and manually adjusted by eye in AliView (Larsson 2014). Individual alignments were concatenated in SeaView version 4 (Gouy et al. 2010). PartitionFinder (Lanfear et al. 2014) was used to identify the best partition scheme and molecular models under the AICc criterion. Maximum likelihood (ML) phylogenetic reconstruction was performed with RAxML version 7.4.2 (Stamatakis et al. 2008) implemented in RAxML GUI (Silvestro and Michalak 2012) with 1000 bootstrap replicates. Bayesian inference (BI) was performed in MrBayes v3.2.2 (Ronquist et al. 2011) running ten million generations and sampling parameter states and trees every ten thousand generations. In order to ensure convergence had been reached, the average standard deviation of split frequencies was monitored to ensure it fell below 0.01, and trace files of the parameters were examined to ensure proper mixing. A 25% burn-in was used. We consider bootstrap values >70% and posterior probabilities >0.95 as strong statistical support for clades. Bootstrap values between 50 and 70% and posterior probabilities between 0.80 and 0.95 can be considered as moderate support for clades. States and provinces for the United States and Canada are abbreviated and country abbreviations follow the three-letter ISO code (International Organisation for Standardisation, Geneva, Switzerland). All sequences are deposited in GenBank. The concatenated final alignment has been deposited at TreeBASE (20805).

### Morphological analyses

Macromorphological 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 “l” (Vellinga 1988). Colour nomenclature standards follow Kornerup 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 micromorphological

characters were observed as those in our previous study on European *Hodophilus* species with a naphthalene odour (Adamčík et al. 2017). Pileipellis elements near the pileus margin and the pileus centre were observed and evaluated separately. Features were observed under an Olympus CX-41 light 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. Q-value is the length/width

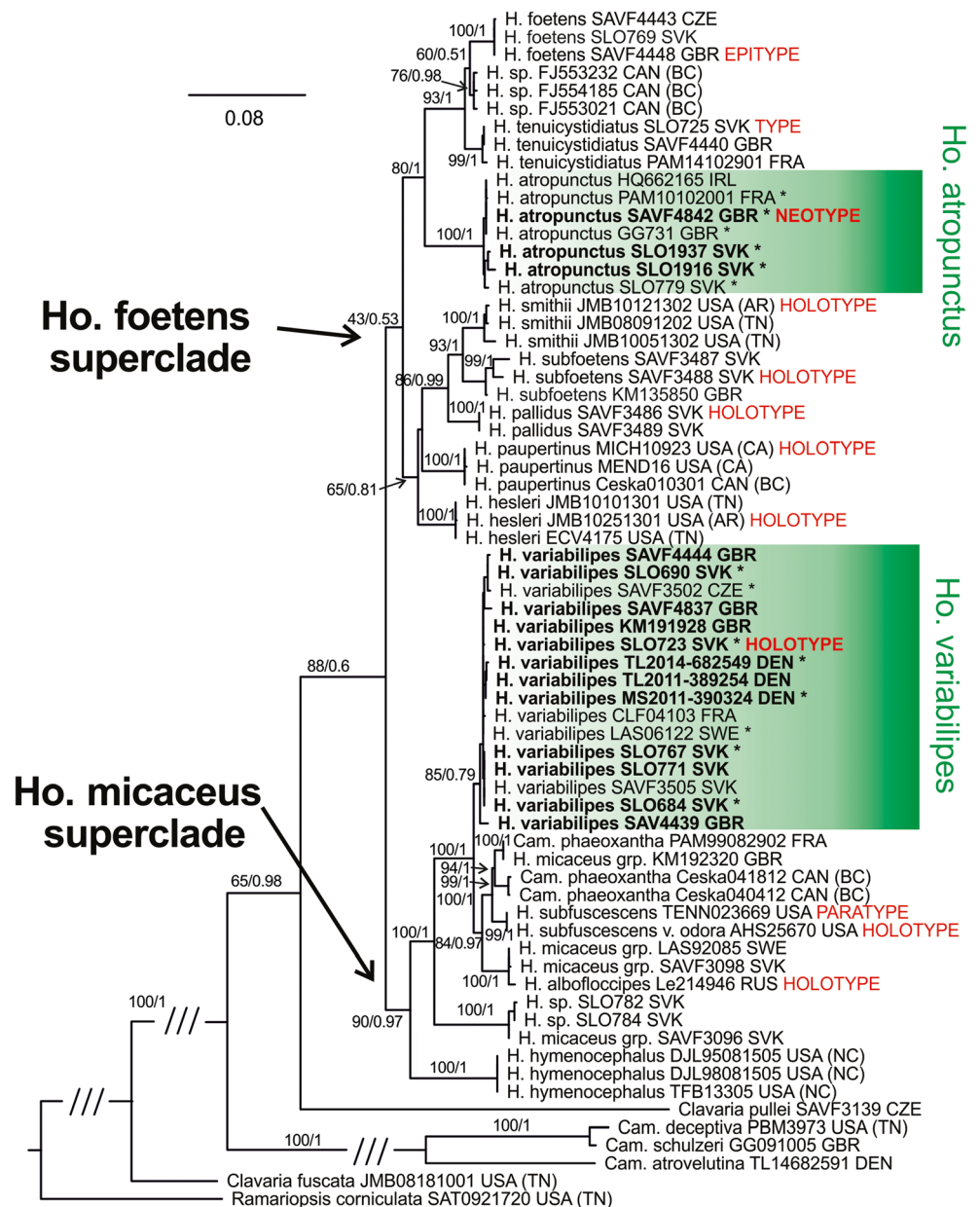
ratio of basidiospores. Statistics of microscopic dimensions are based on 30 measurements and given as a mean value plus/minus standard deviation; values in parentheses give measured minimum or maximum values. Basidiospores were tested in Melzer's reagent for amyloid or dextrinoid reactions (Moser 1978).

## Results

### Phylogenetic analyses

Results of ML inference show identical tree topology with BI analysis (Fig. 1) and with previous studies (Adamčík et al.

**Fig. 1** Maximum Likelihood phylogeny inferred from three loci (ITS, LSU, and *rpb2*) with species-level clades containing collections with dark coloured dots (\* – labelled by asterisk) on the stipe highlighted as well as the two known superclades composing the genus *Hodophilus*. Collection labels are updated with appropriate taxon labels except where collector identifications disagree. In bold are new samples added in this study. Also included are collection labels, country (and in some cases state/province), and whether this represents a type collection. Cam. – *Camarophylloopsis*. Bootstrap values followed by Bayesian posterior probabilities are indicated at nodes



2016, 2017). All collections with dark dots on the stipe are placed in two unrelated superclades (the clade nomenclature follows Adamčík et al. 2016). One strongly supported clade (100/1) placed in the *H. foetens* superclade contains exclusively collections with dark dots on the stipe and is morphologically assigned to *H. atropunctus*. The second moderately supported (85/0.79) clade with dark-dotted collections is placed in the *H. micaceus* superclade and contains also collections with pale white granulations or completely smooth stipe. This clade is described below as a new species, *H. variabilipes*.

### Morphological delimitation of genetically defined species

The black or dark brown dots on the stipe surface may be present and very similar in both phylogenetically defined species. We did not find significant differences in basidioma dimensions or shape, number of lamellae or surface structures. It seems that the only relevant field character is the colour of the basidiomata, but even this can be very similar (Table 1), partly because both species are hygrophane. They can be recognised only when maturity, moisture and different parts of the basidiomata are considered. *Hodophilus atropunctus* (represented by the collections in *H. foetens* superclade) has a brown colour of the pileus when fresh and young (Figs. 2, 3). It starts to discolour from the pileus margin with maturing and in dry condition (Fig. 4), and finally it becomes distinctly paler near the margin than near the pileus centre in dry conditions (Fig. 5). *Hodophilus variabilipes* has darker brown colours when young and fresh (Figs. 6, 8), starts to discolour near the pileus centre (Fig. 7), and is uniformly coloured in dry conditions (Fig. 9). The stipe display a similar colour pattern: *H. atropunctus* has usually a distinctly paler colour near the lamellae and *H. variabilipes* has a more or less uniform stipe colour all over the length. The lamella colour of both species are pale brownish to pale brown when young, and in mature conditions the lamellae of *H. atropunctus* are light brown to

yellowish brown (Figs. 4, 5) and those of *H. variabilipes* brown to dark brown (Figs. 8, 9).

The original description of *Agaricus atropunctus* Pers.: Fr. (Persoon 1801) is very brief and no herbarium specimen or illustration linked to protologue is available. We decided to assign this old name to the clade nested in the *H. foetens* superclade because it has a match with some parts of the protologue: it has contrasting dots on the stipe the pileus is described as “dilute cinereo” and of the lamella colour as “incarnato-cinereae s. pallidae” (incarnate-greyish, pale).

Contrary to the relatively large phylogenetic distance between the two species, they have relatively similar microscopic structures (Table 2). We were only able to identify differences in spore dimensions and in the pileipellis structure near the pileus centre. Spores of both species are of approximately the same length, but *H. atropunctus* has more elongated spores (average  $Q \geq 1.29$ ). The elements of the pileipellis are only different near the pileus centre, but not consistent among the studied material. In general, *H. atropunctus* has more elongated terminal cells (average length/width ratio  $> 1.8$ ) and subterminal cells usually in average longer than 25  $\mu\text{m}$  and with average width not exceeding 8  $\mu\text{m}$ .

### Taxonomy

*Hodophilus atropunctus* (Pers.: Fr.) Birkebak & Adamčík, Mycologia 108: 867. 2016.

Figs. 2–5, 10–15

$\equiv$  *Agaricus atropunctus* Pers.: Fr., 1801, Synopsis methodica fungorum: 353. 1801; Systema mycologicum 1: 195. 1821.

$\equiv$  *Omphalina atropuncta* (Pers.: Fr.) Quél., Bulletin de la Société Botanique de France 24: 319. 1877.

$\equiv$  *Omphalia atropuncta* (Pers.: Fr.) Sacc., Sylloge Fungorum 5: 320. 1887.

**Table 1** Comparison of selected field characters observed on European *Hodophilus* taxa with dark dots on the stipe

Morphological characters	<i>H. atropunctus</i>	<i>H. variabilipes</i>
Pileus colour	fresh or wet yellowish grey, olive brown, yellowish brown to brown, in dry conditions discolouring first at the margin to yellowish brown, light brown, brownish grey, orange grey to beige,	fresh or young basidiomata brown, dark brown to greyish brown, in dry conditions discolouring first at the centre to yellowish brown, greyish brown, orange grey to olive brown
Stipe colour	near the lamellae light brown to yellowish brown, towards the base dark brown	concolorous along entire length, greyish yellow, brownish orange, yellowish brown or dark brown
Stipe surface	covered by black dots or squamules, towards the base sometimes change to black fibrils	usually with very distinct dark brown floccules or granules and towards the base fibrils, some collections with completely smooth and shiny stipe surface, rarely with fine white granulations along entire length
Lamella colour	at first beige or orange grey, soon greyish to brownish orange, greyish brown, light brown to yellowish brown	when young light brown or greyish brown, when mature brown to dark brown

**Figs. 2–9** Basidiomata field aspect of *Hodophilus atropunctus* (left) and *H. variabilipes* (right). 2. Young basidiomata in fresh conditions (SLO1937) photo by S. Jančovičová. 3. Nearly mature basidiomata in wet conditions (SLO1916) photo by S. Jančovičová. 4. Mature basidiomata in wet conditions (SLO779) photo by S. Jančovičová. 5. Mature basidiomata in dry conditions (SAV F-4842, neotype of *H. atropunctus*) photo by M. Adamčík. 6. Young basidiomata in fresh conditions (SLO771) photo by S. Jančovičová. 7. Young basidiomata in dry conditions (SAV F-4837) photo by M. Adamčík. 8. Mature basidiomata in wet conditions [C (DMS-389254)] photo by T. Læssøe. 9. Mature basidiomata in dry conditions (SLO723, holotype of *H. variabilipes*) photo by S. Jančovičová. All collections except of SAV F-4837 and C (DMS-389254) (Figs. 7–8) had distinct dark coloured dots on the stipe surface. Scale bar = 1 cm



≡ *Camarophyllus atropunctus* (Pers.: Fr.) J.E. Lange, Dansk botanisk Arkiv 9 (6): 96. 1938.

≡ *Hygrophorus atropunctus* (Pers.: Fr.) A.H. Sm. & Hesler, Lloydia 5(1): 15. 1942.

≡ *Aeruginospora atropuncta* (Pers.: Fr.) M.M. Moser, Kleine Kryptogamenflora von Mitteleuropa – Die Blätter- und Bauchpilze (Agaricales und Gastromycetes) IIb/2: 70. 1967.

≡ *Hygrocybe atropuncta* (Pers.: Fr.) P.D. Orton & Watling, Notes from the Royal Botanical Garden Edinburgh 29 (1): 134. 1969.

≡ *Hygrotrama atropuncta* (Pers.: Fr.) Singer, Beihefte zur Sydowia 7: 3. 1973.

≡ *Camarophyllopsis atropuncta* (Pers.: Fr.) Arnolds, Mycotaxon 25 (2): 642. 1986.

*Neotypus (designated here)*: United Kingdom. Wales, Pembrokeshire, Orierton field study centre, 8 October 2016, S Adamčík (SAV F-4842).

**Pileus** (Figs. 2–5) (4)7–14(25) mm broad, convex to plano-convex, sometimes weakly depressed near the centre; margin inflexed, later straight, sometimes slightly crenate, when wet weakly translucently striate up to half of the diameter; surface

**Table 2** Average values of 30 measurements of selected micromorphological characters observed on the studied *Hodophilus* specimens with dark dots on the stipe

species epithet	herbarium no.	spores			caulocystidia		TC margin			STC margin		TC centre			STC centre		marginal cells	
		L	W	Q	L	W	L	W	Q	L	W	L	W	Q	L	W	L	W
<i>atropunctus</i>	SLO779	5.0	3.9	1.30	26.9	7.3	27.3	17.6	1.66	22.4	8.1	29.3	12.3	2.48	30.9	7.5	–	–
	GG211144	5.0	3.8	1.32	28.9	11.9	28.3	13.4	2.46	25.7	6.2	32.9	15.8	2.21	27.2	7.8	–	–
	LIP (PAM10102001)	5.0	3.8	1.31	20.6	8.1	30.2	20.5	1.51	22.8	9.2	28.2	15.1	2.08	27.1	7.6	–	–
	SAV F-4842	5.1	4.0	1.29	–	–	36.3	24.3	1.57	16.9	7.7	39.0	21.8	1.84	23.6	7.5	–	–
	SLO1937	–	–	–	24.4	8.4	33.2	22.7	1.48	17.1	7.0	36.7	19.0	2.03	25.4	7.1	19.1	8.7
	SLO1916	5.0	3.8	1.33	26.6	7.3	27.8	19.5	1.45	12.3	6.5	30.4	20.2	1.56	22.5	7.6	–	–
<i>variabilipes</i>	SAV F-3502	5.1	4.2	1.21	29.3	8.8	31.9	22.3	1.48	27.0	12.6	37.3	22.9	1.80	21.1	8.1	–	–
	C (TL 2014-682549)	5.2	4.1	1.26	33.8	10.8	33.0	24.3	1.39	25.6	10.1	37.2	26.8	1.40	21.9	9.0	24.2	9
	SAV F-3505	5.0	4.1	1.23	24.2	8.7	26.6	19.2	1.41	12.2	7.3	29.2	18.0	1.66	19.8	8.8	27.0	11.3
	SAV F-4444	4.8	4.0	1.20	23.2	13.3	32.6	21.1	1.61	18.2	8.0	25.5	17.8	1.52	19.4	7.7	–	–
	C (MS 2011-390324)	5.2	4.1	1.26	30.9	10.2	35.9	29.1	1.27	22.5	12.7	35.5	27.9	1.29	23.5	12.9	29.4	13.6
	C (TL 2011-389254)	5.0	4.1	1.20	37.2	10.3	40.8	26.0	1.63	34.0	16.7	38.0	28.0	1.39	23.9	11.9	25.9	8.5
	SLO723	5.3	4.3	1.24	22.2	6.6	41.2	23.0	1.87	24.9	9.5	34.5	19.9	1.78	17.3	6.1	26.3	9.2
	SLO771	5.5	4.5	1.22	28.9	8.4	37.1	22.9	1.66	12.4	5.9	27.9	16.8	1.63	18.8	6.9	22.9	8.8
	SLO767	5.0	4.2	1.17	34.2	10.1	33.3	23.7	1.46	19.0	10.7	28.8	20.1	1.47	16.8	8.1	–	–
	K(M) 191928	5.1	4.4	1.18	33.5	13.6	28.8	21.1	1.48	17.0	6.6	32.1	22.7	1.80	18.4	8.1	–	–

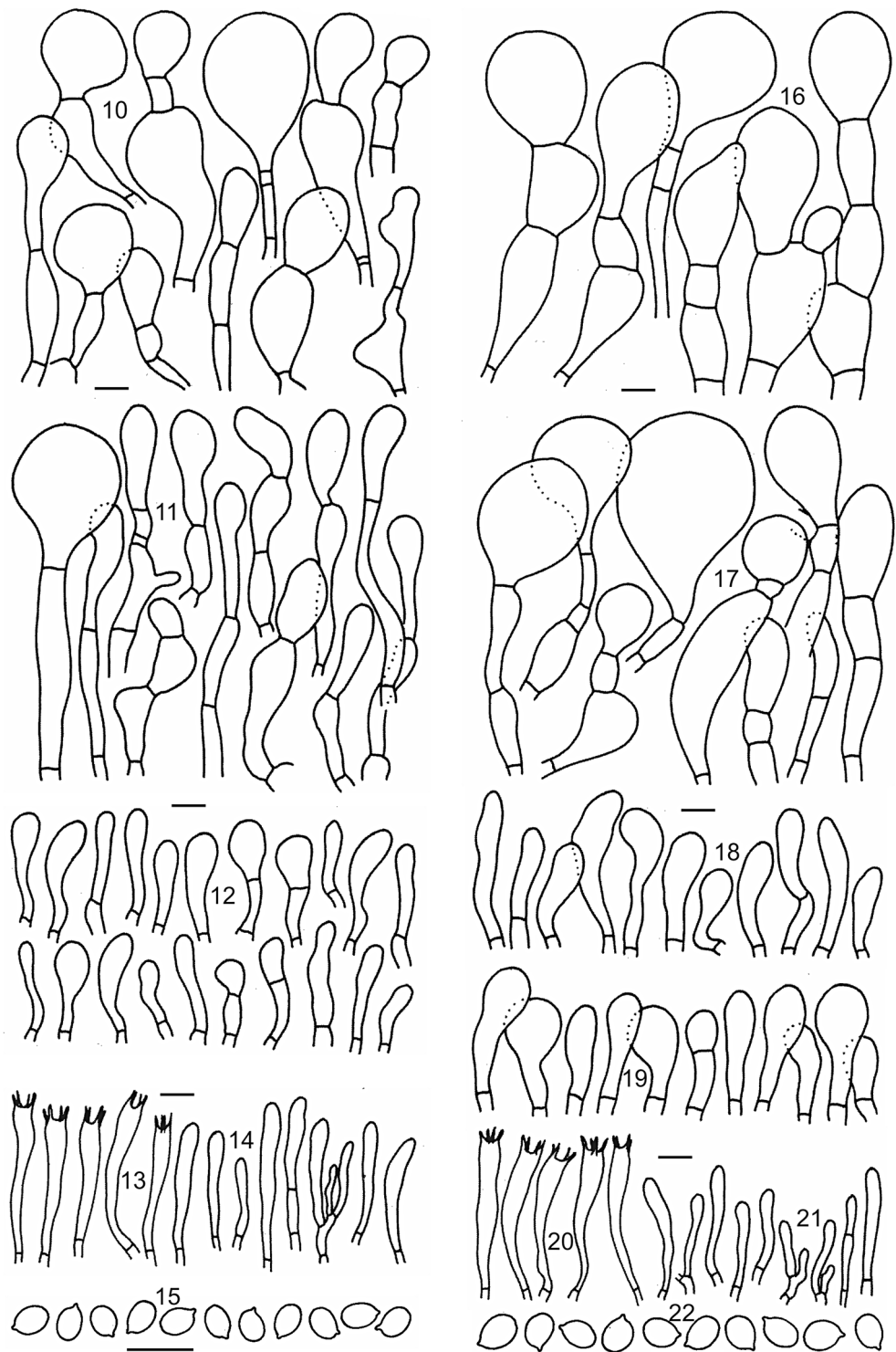
\* type specimens. TC and STC margin/centre – terminal and subterminal cells in pileipellis near the pileus margin/centre, L – length in  $\mu\text{m}$ , W – width in  $\mu\text{m}$ , Q – length/width ratio. The shaded boxes indicate important differences. Em dash indicates missing values due to absence of a structure or insufficient conditions of the studied herbarium specimen

mat, smooth, under lens velvety or finely pubescent, when old towards the centre slightly rugulose or veined, when dry cracked concentrically; hygrophanous; fresh basidiomata yellowish grey (putty 4B2), beige (4C3), yellowish brown (bronze 5E5 to hair brown 5E4) to brown (soot brown 5F5), towards the centre olive brown (khaki 4D5), yellowish brown (clay 5D5) to brown (soot brown 5F5), when dry or mature paler and typically with distinct contrast between the paler margin and the darker centre, near the margin beige (4C3), orange grey (birch bark 5B2), brownish grey (6C2), light brown (café-au-lait 6D3) to yellowish brown (hair brown 5E4), towards the centre usually light brown (café-au-lait 6D3), yellowish brown (clay 5D5, bronze 5E5) to brown (soot brown 5F5). **Stipe** 10–36(50)  $\times$  1–4 mm, cylindrical and narrowed towards the base, usually flexuous, sometimes compressed or longitudinally grooved; usually along the entire length covered by black dots or squamules, towards the base sometimes with black fibrils, at the base sometimes white tomentose; near the lamellae light brown (dark blonde 5D4) to yellowish brown (hair brown 5E4 to bronze 5E5), towards the base dark brown (negro 6F3 to 7F3 or chocolate 6F4). **Lamellae** 1–3.5 mm deep, L = 14–24(26), l = (0)1–3, short to long decurrent; at first beige (4C3) or orange grey (5B2), soon greyish orange (5B3), brownish orange (5C3), greyish brown (nougat 5D3), light brown (dark blond 5D4) to yellowish brown (bronze 5E5), often with distinct pink shade; edges concolorous, often near the stipe with darker, fine fibrils or dots, sometimes paler than the sides. **Flesh** 0.5–3 mm thick in the half pileus radius, elastic; odour indistinct but unpleasant with age.

**Basidiospores** (Fig. 15) (4.1)4.6–5.4(6.4)  $\times$  (3.1)3.5–4.2(4.9)  $\mu\text{m}$ , av. 5.0  $\times$  3.9  $\mu\text{m}$ , Q (length/width) = (1.13)1.21–1.39(1.62), av. Q = 1.29, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled; hilar appendage 0.3–0.6  $\mu\text{m}$  long. **Basidia** (Fig. 13) 4-spored, (33)36–42(46.5)  $\times$  (5)5.5–6.5  $\mu\text{m}$ , av. 38.9  $\times$  5.9  $\mu\text{m}$ , hyaline, narrowly clavate, attenuated and flexuous towards the base. **Basidioles** (Fig. 14) cylindrical to narrowly clavate, often flexuous, obtuse, ca. 15–43  $\times$  2–6  $\mu\text{m}$ . **Pleurocystidia** absent. **Marginal cells** on the lamellar edges usually not well differentiated, similar to basidioles on lamellar sides, but in the collection SAV F-1937 also with conspicuous, broadly clavate ones, measuring (7)13.5–25(30)  $\times$  (4)5.5–12(19)  $\mu\text{m}$ . **Lamellar trama** of intricate, subparallel, undulate, (3)5–10(12)  $\mu\text{m}$  wide hyphae, composed of 20–100  $\mu\text{m}$  long (often shorter than 50  $\mu\text{m}$ ) cells. **Subhymenium** pseudoparenchymatic, ca. 20  $\mu\text{m}$  deep, composed of 2.5–4  $\mu\text{m}$  wide hyphae. **Pileipellis** a hymeniderm, composed of cells arranged mainly in one rank; terminal cells near the pileus margin (Fig. 10) obpyriform or clavate, less frequently sphaeropedunculate, usually thin-walled, measuring (13.5)20.5–39.5(68)  $\times$  (5.5)13–26(37)  $\mu\text{m}$ , av. 30.2  $\times$  19.4  $\mu\text{m}$ , Q = 0.85–2.6(10), av. Q = 1.7; subterminal cells usually distinctly narrower, cylindrical or clavate, occasionally also inflated, short and small cells (shorter than 5  $\mu\text{m}$ ) rare, rarely nodulose or with lateral projections, unbranched, measuring (2)9.5–30(49)  $\times$  (2)3.5–11.5(20)  $\mu\text{m}$ , av. 19.7  $\times$  7.4  $\mu\text{m}$ . Terminal cells of hyphae near the pileus centre (Fig. 11) usually narrower, mainly broadly clavate, often sphaeropedunculate, obpyriform or ellipsoid, often



**Figs. 10–22** Microscopic structure of *Hodophilus atropunctus* (left, SLO779) and *H. variabilipes* (right, SAV F-3502, marginal cells SAV F-3505). 10. Hyphal terminations in pileipellis near the pileus margin. 11. Hyphal terminations in pileipellis near the pileus centre. 12. Caulocystidia. 13. Basidia. 14. Basidioles. 15. Spores. 16. Hyphal terminations in pileipellis near the pileus margin. 17. Hyphal terminations in pileipellis near the pileus centre. 18. Caulocystidia. 19. Marginal cells on the lamellar edges. 20. Basidia. 21. Basidioles. 22. Spores. Drawings by S. Jančovičová. Scale bar = 10  $\mu$ m



pedicellate, measuring (12.5)22.5–42(60)  $\times$  (4.5)11–22.5(34)  $\mu$ m, av. 32.2  $\times$  16.7  $\mu$ m, Q = (0.63)1.28–2.88(5.33), av. Q = 2.08; subterminal cells usually not inflated and cylindrical, occasionally nodulose, measuring (2)13–40(63)  $\times$  (3)4.5–10.5(18)  $\mu$ m, av. 26.6  $\times$  7.5  $\mu$ m; pileus trama of intricate, irregularly oriented, 2.5–4  $\mu$ m wide hyphae. **Caulocystidia** (Fig. 12) dark brown

(pigments parietal and sometimes incrustated), usually in dense fascicles or patches, thin- or occasionally slightly thick-walled, repent or ascending; with terminal cells mainly clavate, occasionally subcapitate or obpyriform, obtuse, often pedicellate and flexuous, measuring (11)18–33.5(54.5)  $\times$  (4)5.5–11.5(20)  $\mu$ m, av. 25.6  $\times$  8.5  $\mu$ m. **Clamp connections** absent in all parts.

**Material examined:** **France.** Pas-de-Calais, Guînes, forêt domaniale, 20 October 2010, P Pirot *PAM10102001* (LIP); **Slovakia.** Podunajská nížina Lowland, Banka village, near the Koliba pod Ahojom, 26 September 2014, S Jančovičová (SLO779); Nízke Beskydy Mts., Pčoliné village, 6 October 2016, S Jančovičová (SLO1916); Nízke Tatry Mts., Hybe village, 8 October 2016, S Jančovičová (SLO1937); **United Kingdom.** Wales, Pembrokeshire, Orierton field study centre, 8 October 2016, S Adamčík (SAV F-4842, neotypus); Wales, Powys, Welshpool, Powis Castle, 22 November 2004, GW Griffith *GG731* (TENN 063729).

***Hodophilus variabilipes*** Jančovičová, Adamčík & Looney, sp. nov.

Figs. 6–9, 16–22

*Mycobank No.:* MB821290.

**Etymology:** The epithet refers to the variable presence of dark dots on the stipe surface.

**Holotypus:** Slovakia. Malé Karpaty Mts., Sološnica village, 9 October 2014, S Jančovičová (SLO723).

**Diagnosis:** Pileus dark brown when fresh or wet, hygrophanous, in dry condition becoming paler near the centre, when dry uniformly pale brown; stipe apex usually with dark brown floccules or granules, but sometimes with white or absent covering, background more or less uniformly brown; lamellae brown to dark brown when mature; flesh without a strong odour; spores in average  $5.1 \times 4.2 \mu\text{m}$ , av.  $Q = 1.22$ ; pileipellis mainly a hymeniderm, terminal cells of hyphae near the pileus centre mainly subglobose, obpyriform, with average length/width ratio  $< 2$ , subterminal cells of hyphae near the pileus centre often inflated or short, in average  $< 25 \mu\text{m}$  long and  $> 8 \mu\text{m}$  wide.

**Pileus** (Figs. 6–9) 4)5–22 mm broad, hemispherical, convex to plano-convex, rarely depressed near the centre; margin first slightly inflexed, soon straight, when mature slightly crenate, not or indistinctly translucently striate up to one third when wet; surface matt, velvety and later with fine, darker granules or pruina, at first smooth, but when mature becoming rugose or rough towards the centre, in dry conditions concentrically cracking; hygrophanous; the colour first becoming paler near the cap centre, fresh or young basidiomata brown (hair brown 5E4), dark brown (chocolate 6F4) to greyish brown (negro 6F3), when dry yellowish brown (clay 5D5), greyish brown (nougat 5D3, drab 5E3 to nutria 5F3), orange grey (5B2) to olive brown (4D4). **Stipe** (9) 15–42  $\times$  1–4 mm, usually flexuous, cylindrical and narrowed towards the base, sometimes compressed; usually with very distinct dark brown floccules or granules and towards the base fibrils, some collections with a completely smooth and shiny stipe surface, one collection with fine white granulations along the entire length, at the base white tomentose at times, usually concolorous along the entire length, greyish yellow (blonde 4C4), brownish orange (5C4), yellowish brown (bronze 5E5 to mustard

brown 5E6), dark brown (negro 6F3, chocolate 6F4, burnt umber 6F6 to chestnut 6F7). **Lamellae** (1.5) 2.5–5 (8) mm deep, distant to moderately close,  $L = (9) 14\text{--}24 (28)$ ,  $l = 1\text{--}3 (5)$ , sometimes interveined, short to deeply decurrent, when young light brown (dark blonde 5D4 to camel 6D4), greyish brown (nougat 5D3 to negro 6F3), when mature brown (6E4 to 6E5) to dark brown (teak 6F5 to chocolate 6F4); edge entire, concolorous or slightly paler than the sides. **Flesh** 0.5–2 mm thick in half radius of the pileus, elastic, pale beige to light brown; odour none or when old with indistinct unpleasant components.

**Basidiospores** (Fig. 22)  $(4.1)4.8\text{--}5.4(6) \times (3.3)3.9\text{--}4.5(4.9) \mu\text{m}$ , av.  $5.1 \times 4.2 \mu\text{m}$ ,  $Q = (1.07)1.15\text{--}1.28(1.42)$ , av.  $Q = 1.22$ , broadly ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled; hilar appendage up to 0.4–0.8  $\mu\text{m}$  long. **Basidia** (Fig. 20) 4-spored,  $(28)34.5\text{--}45.5(52) \times (5)6\text{--}7.5(8) \mu\text{m}$ , av.  $40.0 \times 6.7 \mu\text{m}$ , hyaline, narrowly clavate, attenuated, and flexuous towards the base. **Basidioles** (Fig. 21) cylindrical to narrowly clavate, obtuse, often flexuous, ca.  $9\text{--}35 \times 2\text{--}5.5 \mu\text{m}$ . **Pleurocystidia** absent. **Marginal cells** on the lamellar edges (Fig. 19) usually well differentiated, broadly clavate, obpyriform or sphaeropedunculate, measuring  $(13)20\text{--}32(55.5) \times (5.5)7\text{--}13.5(19) \mu\text{m}$ , av.  $25.9 \times 10.2 \mu\text{m}$ , but sometimes absent or similar to basidioles on lamellar sides, thin walled or rarely with slightly thickened walls. **Lamellar trama** composed of 2–8(10)  $\mu\text{m}$  wide, subparallel or occasionally interwoven and irregularly inflated hyphae, composed of cells usually shorter than 100  $\mu\text{m}$  (often  $< 50 \mu\text{m}$ ). **Subhymenium** 40–50  $\mu\text{m}$  deep, composed of irregularly oriented, intricate, 2–4  $\mu\text{m}$  wide hyphae, gradually passing to underlying hyphae of the trama. **Pileipellis** near the pileus margin (Fig. 16) a transition from hymeniderm to epithelium, hyphal terminations composed of 1–3 inflated cells; terminal cells obpyriform, subglobose or ellipsoid, rarely sphaeropedunculate or broadly clavate, measuring  $(12)22.5\text{--}45(87.5) \times (8)15.5\text{--}31(55) \mu\text{m}$ , av.  $33.9 \times 23.2 \mu\text{m}$ ,  $Q = (0.70)1.09\text{--}1.95(3.16)$ , av.  $Q = 1.52$ ; subterminal cells mainly inflated ellipsoid to broadly clavate, occasionally small (shorter and narrower than 5  $\mu\text{m}$ ), less frequently cylindrical and longer than 10  $\mu\text{m}$ , rarely nodulose, not branched, measuring  $(1.5)7\text{--}36(74) \times (2)3\text{--}17(40) \mu\text{m}$ , av.  $21.5 \times 10.1 \mu\text{m}$ . Pileipellis near the pileus centre (Fig. 17) a hymeniderm, hyphal terminations usually inflated only at terminal cells that are subglobose or obpyriform, occasionally ellipsoid or broadly clavate, measuring  $(11)21\text{--}44.5(99.5) \times (8)14\text{--}30(55) \mu\text{m}$ , av.  $32.8 \times 22.1 \mu\text{m}$ ,  $Q = (0.75)1.07\text{--}2.03(3.98)$ , av.  $Q = 1.55$ ; subterminal cells usually not inflated and short cylindrical, not branched, measuring  $(2)7.5\text{--}33(74) \times (2)2.5\text{--}15(44) \mu\text{m}$ , av.  $20.1 \times 8.8 \mu\text{m}$ ; pileus trama of intricate, subparallel, 2–6  $\mu\text{m}$  wide hyphae, composed of usually short, up to 50  $\mu\text{m}$  long cells, but often even shorter than 50  $\mu\text{m}$ . **Caulocystidia** (Fig. 18) crowded in large patches, mainly broadly clavate, rarely ellipsoid, fusiform or sphaeropedunculate, usually thin-

walled, sometimes very flexuous, rarely nodulous, usually with dark intracellular or parietal pigments, measuring (12)20.5–38.5(55) × (4)6.5–13.5(29) μm, av. 29.7 × 10.1 μm. Stipe trama of 3–12 μm wide, parallel hyphae, composed of cells often <50 μm long (rarely >100 μm long). **Clamp connections** absent in all parts.

**Material examined:** **Czech Republic.** Kokořínsko, Hradsko settlement, 20 October 2007, S Adamčík (SAV F-3502); **Denmark.** Jylland, Lysbro Skov, 24 September 2014, T Læssøe [C (DMS-682549), (photo JHP-14.212)]; Sjælland, Lergravene (Nivå), 24 September 2011, T Læssøe [C (DMS-389254)]; Jylland, Lysbro Skov, 27 September 2011, M Strandberg [C (DMS-390324)]; **France.** Nord, Douai, Canal de la Sensée, 15 November 2004, C Lécuru *CL/F04.103* (LIP); **Slovakia.** Biele Karpaty Mts., Nová Bošáca village, Grúň Natural Monument, 14 October 2002, K Devánová (SAV F-3505); Strážovské vrchy Mts., Bojnice city, Predné Štefankovo, 4 September 2014, V Kautman (SLO684); Považský Inovec Mts., Kálnica village, 17 September 2014, J Herman (SLO690); Biele Karpaty Mts., Krivoklát village, Krivoklátske lúky Natural Monument, 23 September 2014, S Jančovičová (SLO767); Podunajská nížina Lowland, Banka village, near the Koliba pod Ahojom, 26 September 2014, S Jančovičová (SLO771); Slovakia. Malé Karpaty Mts., Sološnica village, 9 October 2014, S Jančovičová (SLO723, holotypus); **Sweden.** Bohuslän, Tanum, Svenneby, 27 August 1992, L & A Stridvall *GB0060390* (LAS92/085); **United Kingdom.** Wales, Pembrokeshire, Somerton Farm, 4 October 2010, D Harries (SAV F-4439); England, West Yorkshire, Mirfield, Community of the Resurrection, 3 November 2012, J Blinkhorn [K(M)191,928]; Wales, Monmouthshire, Garn Ddyrys, the Bloreng, 2 November 2014, S Adamčík (SAV F-4444); Wales, Pembrokeshire, Orielton field study centre, 8 October 2016, S Adamčík (SAV F-4837).

## Discussion

### How do phylogenetically defined groups coincide with morphology?

Previous phylogenetic reconstructions of the genus *Hodophilus* (Adamčík et al. 2016, 2017) recovered two major clades: the *H. foetens* superclade dominated by species with naphthalene odour and rare small subterminal cells in the pileipellis and *H. micaceus* superclade dominated by species without strong odour and with frequent small subterminal cells (shorter than 5 μm) in the pileipellis (Figs. 16–17). *Hodophilus atropunctus* is so far the only known species nested in the *H. foetens* superclade that has a faint odour. In general, this species shows the pattern of pileipellis typical for the *H. foetens* superclade: terminal cells of hyphae near the

pileus centre are more elongated (average length/width ratio > 1.8) and the small subterminal cells are rare (Figs. 10–11). However, some collections of both species with dark dots on the stipe show overlapping values of these characters in the pileipellis (Table 2). Therefore, we think that for a quick and efficient initial (“field”) identification, we would propose to use naphthalene odour and dark dots on the stipe as the first step prior to subsequent microscopic examination. In this study, we did not provide a key, because we discovered that *H. variabilipes* often lacks darker dots on the stipe and such collections might be confused with some other species of the *H. micaceus* superclade with currently unresolved taxonomic delimitation and nomenclature (Adamčík et al. 2017). We think that field characters offer relatively high probability to recognise *H. atropunctus* and *H. variabilipes*, if the maturity and the humidity of basidiomata are considered (Table 1). Micro-morphological characters might be used to support the identification and although the characters discussed above show some overlap or very similar values (Table 2), in most cases they are convincing.

### Ecology and distribution

More than half of the *Hodophilus* collections with darker dots on the stipe originate from Slovakia and the United Kingdom and both species are represented in both countries. We did not observe any difference in ecological preferences, both species occur frequently near edges between forested areas and meadows, and have preference for either scrub or grassland habitats. Recent French collections of *H. atropunctus* come from deciduous forests with *Fagus* and *Carpinus* rich in with a well-developed shrub layer on clay soil, in agreement with the protologue (Persoon 1801: 353). At Orielton research centre in South Wales (UK), both species were found only a few meters apart. Our data suggest that both species grow in temperate areas of Europe and may share common habitat types. It is possible that the distribution of species with darker dots on the stipe is limited to Europe or Eurasia because they were not reported in the North American monograph on *Hygrophorus* sensu lato by Hesler and Smith (1963).

### *Hodophilus* with dark dots on the stipe in the European literature

The treatment of *Hodophilus* species with darker dots on the stipe in the current European literature (Printz and Læssøe 1986; Boertmann 2012; Kovalenko et al. 2012) is analogous to species with strong naphthalene odour (Adamčík et al. 2017); both species complexes were morphologically defined as a single species (*Camarophylloopsis atropuncta* and *C. foetens*) based on one striking morphological character easily recognisable in the field. However, the history of *H. atropunctus* does not relate straightforwardly to its current

concept. Persoon (1801) mentioned in the protologue of *Agaricus atropunctus* incarnate (flesh-coloured) lamellae of the species. Based on the presence of the pinkish tint, Fries placed it in the tribus “*Clitopilus*” (Fries 1821) and then in “*Eccilia*” (Fries 1874), both names of currently accepted genera and subgenera in the family Entolomataceae Kotl. & Pouzar. This concept was soon adopted by British mycologists and they described *A. atropunctus* as having pink angular spores (Smith 1875; Phillips 1878; Cooke 1884). The French mycologist L. Quélet attributed the name to a species with “foetid” odour (Quélet 1877) and later he considered it a possible synonym of *H. foetens* (Quélet 1898), following criticism by the British authors (Phillips 1878; Cooke 1884). Although influenced by Quélet, Bresadola (1928) introduced the description of *Omphalia atropuncta* (Pers.: Fr.) Sacc. that corresponds to the current concept of *H. atropunctus* (dark dots on the stipe and without naphthalene odour).

From the beginning of twentieth century, all European mycologists clearly applied the current concept for various combinations based on the name *A. atropunctus*, but in most cases their descriptions are either insufficient to recognise *H. atropunctus* from *H. variabilipes* or are ambiguous (Lange 1938; Bon 1977; Arnolds 1990; Boertmann 2012). Printz and Læssøe (1986) noted that the Danish collections fell in two colour groups. They illustrated what is here considered to be *H. atropunctus* sensu stricto. In our dataset, *H. variabilipes* is represented more than twice as often as *H. atropunctus* and this corresponds to the higher number of publications that might be assigned to the first species. Bresadola (1928) and Horak (2005) described *H. atropunctus* as having a dark brown colour on all parts, clearly corresponding to our concept of *H. variabilipes*. Moser (1978) and Kovalenko et al. (2012), in addition to the dark brown colour, described the pileus as becoming paler (beige) first near the centre when drying and the latter publication in addition reports an average length/width ratio of the spores as 1.23, both characters are very typical for *H. variabilipes*. The only clue that may correspond unambiguously to the concept of *H. atropunctus* adopted here are brown-pink or pinkish lamellae in descriptions or keys of some French authors (Heim 1969; Bon 1977).

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