

Circumscription of species of *Hodophilus* (Clavariaceae, Agaricales) in North America with naphthalene odours

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Abstract: Five North American *Hodophilus* species with naphthalene-like odours are now recognized based on sequence and (or) morphological data and molecular annotation of type collections. Two well-supported eastern North American species do not match any of the studied types and are described here as new: *Hodophilus hesleri* and *Hodophilus smithii*. The previously described *Hodophilus paupertinus* is found to represent an autonomous species and appears restricted to western North America. *Hodophilus subfuscus* is found to be an independent lineage in eastern North America. A morphological type study of *Hodophilus peckianus* shows that it is a distinct species and not represented among recent collections. Multilocus phylogenetic analyses of European and North American material of species with naphthalene odours reveal no species with transatlantic distributions. Overall, *Hodophilus* comprises two superclades (the *Hodophilus foetens* superclade and the *Hodophilus micaceus* superclade) and 16 terminal clades that correspond to phylogenetic species. This study introduces a new approach for morphological delimitation of agaricoid Clavariaceae combining shape and dimensions of particular elements in the pileipellis and caulocystidia. All previously described taxa included in this study, which were previously treated in the genera *Hygrophorus*, *Camarophylloopsis*, or *Hygrotrama*, are formally transferred to *Hodophilus*.

Key words: agaricoid, Clavariaceae, multilocus phylogeny, morphology, type studies.

Résumé : Cinq espèces de *Hodophilus* d'Amérique du Nord ayant une odeur qui s'apparente à celle du naphthalène sont maintenant reconnues sur la base de données de séquences et (ou) morphologiques et de l'annotation moléculaire de collections types. Deux espèces de l'est de l'Amérique du Nord bien appuyées ne correspondent à aucun des types étudiés et sont décrites ici comme étant nouvelles : *Hodophilus hesleri* et *Hodophilus smithii*. *Hodophilus paupertinus* précédemment décrit s'avère représenter une espèce autonome et semble restreint à l'ouest de l'Amérique du Nord. *Hodophilus subfuscus* s'avère constituer un lignage indépendant de l'est de l'Amérique du Nord. Une étude du type morphologique de *Hodophilus peckianus* montre qu'il constitue une espèce distincte et qu'il n'est pas représenté dans les collections récentes. Des analyses phylogénétiques multilocus de matériel provenant d'espèces d'Europe et d'Amérique du Nord possédant une odeur de naphthalène ne révèlent aucune distribution transatlantique des espèces. Globalement, *Hodophilus* comprend deux superclades (le superclade *Hodophilus foetens* et le superclade *Hodophilus micaceus*) et 16 clades terminaux qui correspondent aux espèces phylogénétiques. Cette étude introduit une nouvelle approche de délimitation morphologique des Clavariaceae agaricoïdes combinant la forme et les dimensions d'éléments particuliers du pileipellis et de la caulocystide. Tous les taxons précédemment décrits compris dans cette étude, qui étaient traités dans le genre *Hygrophorus*, *Camarophylloopsis* ou *Hygrotrama*, sont formellement transférés vers *Hodophilus*. [Traduit par la Rédaction]

Mots-clés : agaricoïdes, Clavariaceae, phylogénie multi-locus, morphologie, études de type.

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Introduction

Hodophilus foetens (W. Phillips) Birkebak & Adamčík is a well-known species characterized by its unpleasant camphor-like odour together with skatol and methylmercaptan-like components, which persists for some years in dried herbarium specimens (Arnolds 1990). Based on its phylogenetic position, it has been recently transferred from the genus *Camarophylloopsis* Herink (*Cam.*) to the genus *Hodophilus* R. Heim (*Ho.*) (Birkebak et al. 2016). Members of both genera had been previously treated within the genus *Hygrophorus* Fr. (*Hy.*) (Dennis 1953; Singer 1959; Hesler and Smith 1963). The genus *Hodophilus*, typified by *Hy. foetens* W. Phillips (Heim 1966), is characterized by a hymeniderm pileipellis composed of globose, obpyriform to sphaero-pendunculate terminal elements and absence of clamp connections (Birkebak et al. 2016).

In Europe, all *Camarophylloopsis* collections with such strong odours have been identified as a single species, *Cam. foetens* (W. Phillips) Arnolds (Boertman 2012; Kovalenko et al. 2012), originally described from Wales in the UK. (Phillips 1878). Hesler and Smith (1963) reported four *Hodophilus* taxa with strong odours from North America (treated in the genus *Hygrophorus*), including the European type species (Smith and Hesler 1954). They distinguished *Hy. foetens* from other North American taxa based on a darker brown colour of the pileus, a stipe covered by squamules, and a hymeniderm pileipellis composed of enlarged cells. *Hygrophorus subfuscenscens* var. *odora* A.H. Sm. & Hesler is the second North American taxon with strong odours. It was described with a hymeniderm pileipellis and distinguished from previous species based on a paler overall colour of the basidiomata. The remaining two North American taxa described with strong odours, *Hy. peckianus* Howe and *Hy. paupertinus* A.H. Sm. & Hesler, were both distinguished by their trichoderm pileipellis with less inflated and less dense terminal cells. They differ from each other by narrower lamellae of the latter.

In this study we taxonomically revise species with strong odours, because it is easy to sort them out among the 27 species described in the genus *Camarophylloopsis* (<http://www.mycobank.org/>). Some of these have been combined recently in the genus *Hodophilus* (Birkebak et al. 2016), but others are of uncertain systematic position. The odour of European collections of *Ho. foetens* is most frequently described as similar to *Tricholoma sulphureum* (Bull.) P. Kumm. or *Thelephora palmata* (Scop.) Fr. (e.g., Phillips 1878; Heim 1969; Moser 1978), but also as strong, fetid, gas-like (Boertman 2012) or naphthalene-like (Courtecuisse and Duhem 1994). Hesler and Smith (1963) described the odours of North American taxa as pungent (*Hy. foetens*); exceedingly strong, penetrating, disagreeable (*Hy. paupertinus*); strong, offensive (*Hy. peckianus*);

and very distinctly disagreeable when the flesh is bruised (*Hy. subfuscenscens* var. *odora*). Ghyselinck (2003) analysed odour descriptions of *Ho. foetens* by various authors and concluded that the best fitting term to describe them as naphthalene-like (similar to the odour of moth balls). We further refer to the strong odours among these taxa as of naphthalene.

Hesler and Smith's monograph (1963) is the most recent comprehensive North American taxonomic study of what are now recognized as *Hodophilus* species. No molecular studies have been published supporting species delimitation of species of *Hodophilus* with naphthalene or otherwise strong odours and testing the transatlantic distribution of *Ho. foetens*. A study by Birkebak et al. (2016) suggested that genetic diversity of both North American and European collections corresponds to more than one species with naphthalene odours. Here we seek to circumscribe species of North American *Hodophilus* taxa described with naphthalene odours based on morphological observations and a multilocus phylogenetic analysis using authentic (including type) material and recent well-documented collections. We seek to confirm whether the European species *Ho. foetens* occurs in North America, or if there are any other species with strongly disagreeable or naphthalene-like odours that have transatlantic distributions. We also seek to evaluate the taxonomic importance of the naphthalene odour for classification within the genus *Hodophilus*.

Materials and methods

Taxon sampling

Types of *Hy. foetens*, *Hy. peckianus*, *Hy. paupertinus*, and *Hy. subfuscenscens* var. *subfuscenscens* and *Hy. subfuscenscens* var. *odora* were included together with 14 other North American collections of *Hodophilus* (Supplementary data, Table S1¹). Sequences from GenBank related to North American *Hodophilus* species with naphthalene odours were also used. Twenty European collections identified as *Ho. foetens* were used to test the transatlantic distribution of species with naphthalene odours. Twenty other *Hodophilus* specimens without this smell were included in our phylogenetic taxon scheme to clarify relationships within the genus *Hodophilus* (Supplementary data, Table S1). Six other Clavariaceae species were added as outgroups, including *Cam. atrovolutina* (Romagn.) Arnaud, *Cam. schulzeri* (Bres.) Herink, *Cam. deceptiva* (A.H. Sm. & Hesler) Bon, *Clavaria fuscata* Oudem., *Clavaria pullei* Donk, and *Ramariopsis corniculata* (Schaeff.) R.H. Petersen based on Birkebak et al. (2016).

DNA extraction, PCR, and sequencing

Three gene regions (nLSU ribosomal RNA, ITS, and *rpb2*) were sequenced and analyzed. Protocols of

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2016-0091>.

Birkebak et al. (2013) were followed for DNA extraction, PCR, and sequencing. Primer pairs ITS1F–ITS4 (White et al. 1990; Gardes and Bruns 1993) 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 rLSU region. The primer pair b6F and b7.1R (Matheny 2005) was used to amplify and sequence the most variable region of the *rpb2* gene. Sequencing was performed at the UT Genomics Core facility (Knoxville, Tennessee, USA) and at the SEQme sequencing Company (Dobříš, Czech Republic).

Phylogenetic analyses

Alignments for individual regions were created in ClustalX (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 the RAxML GUI (Silvestro and Michalak 2012) with 1000 bootstrap replicates. Bayesian inference (BI) was performed in MrBayes version 3.2.2 (Ronquist et al. 2011) running 10 000 000 generations and sampling parameter states and trees every 10 000 generations. To ensure convergence had been reached, the mean standard deviation of split frequencies was monitored to ensure that it fell below 0.01, and trace files of the parameters were examined to ensure proper mixing. A burn-in of 25% was used. We consider bootstrap values >70% and posterior probabilities >0.95 as strong 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 USA and Canada are abbreviated, and country abbreviations follow the three-letter ISO code (International Organization for Standardization, Geneva, Switzerland). All sequences are deposited in GenBank. The concatenated final alignment has been deposited at TreeBASE (S19050).

Morphological analyses

Macromorphological descriptions were prepared from fresh material shortly after collection from the field. Colour nomenclature standards follow Kornerup and Wanscher (1967). All micromorphological characteristics 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 using 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. Microscopical

structures were examined on desiccated herbarium specimens in Congo red solution with ammonia after a short treatment in warm aqueous 10% KOH. The Q-value is the length:width ratio of basidiospores. Measurements exclude ornamentation. Statistics for measurements of microscopic characteristics are based on 30 measurements and given as the mean ± SD; values in parentheses are the measured minimum or maximum values. Basidiospores were tested in Melzer's reagent for amyloid or dextrinoid reactions (Moser 1978).

To find micro-morphological differences among studied taxa, the shape and size of the following elements were compared: basidiospores, basidia, caulocystidia, marginal cells on the lamellar edge, and terminal and subterminal cells of hyphae in the pileipellis. Pileipellis elements near the pileus margin and pileus centre were observed and evaluated separately, as several publications suggested that pileipellis structure may change depending on position from the pileus margin (e.g., Ronikier and Moreau 2007; Adamčík and Buyck 2011).

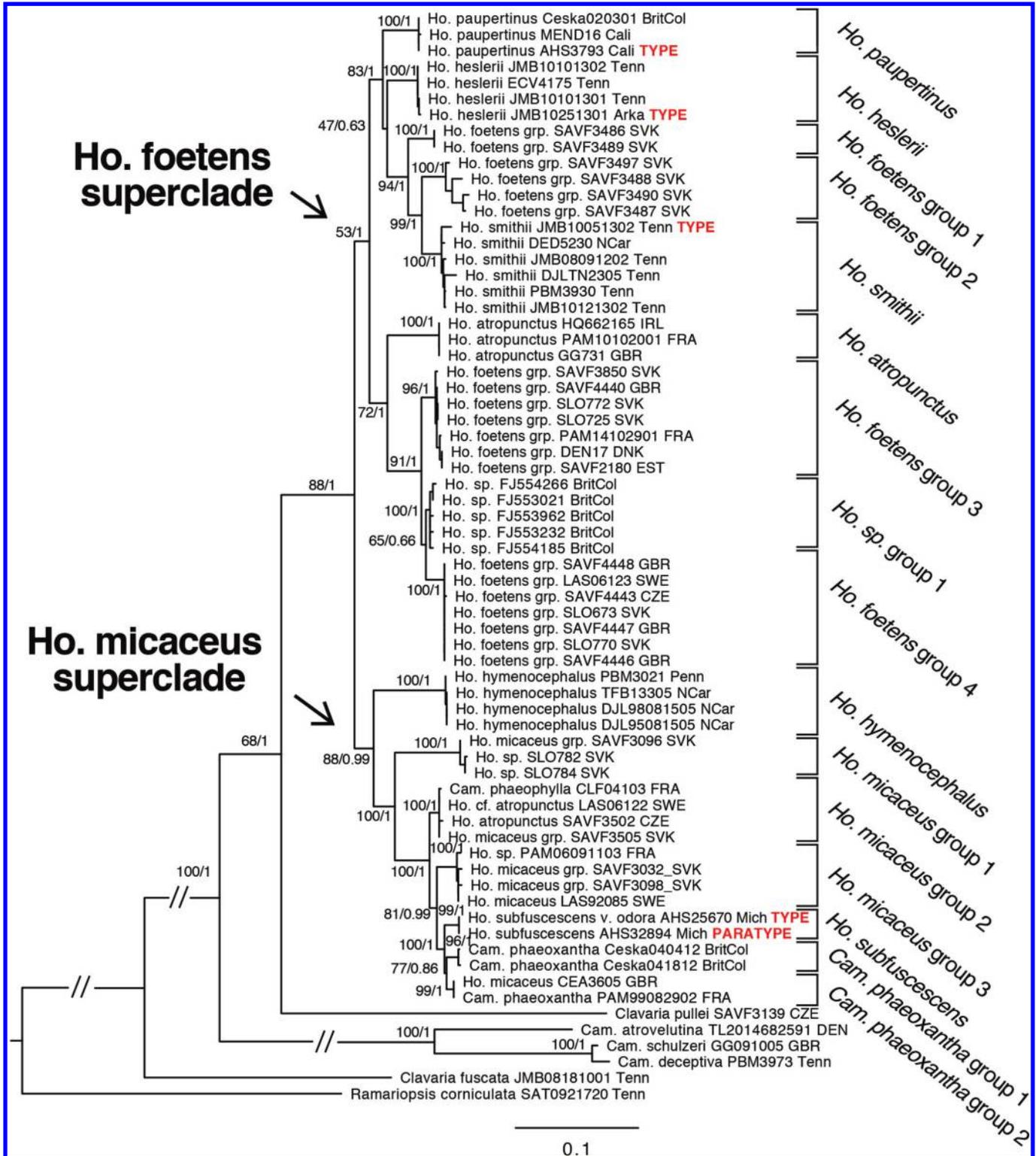
Results

Phylogenetic analyses

All nodes recovered from the ML inference were also recovered by the BI analysis. All recent collections of North American *Hodophilus* with naphthalene odours are grouped in three well-supported clades (Fig. 1). Only one of these clades is associated with a named species based on the sequence of the type specimen of *Hy. paupertinus*, and the other two are named as the new species *Ho. hesleri* and *Ho. smithii* below. In addition to these three clades, the type ITS sequence of *Hy. subfuscescens* var. *odora* type has an isolated position from the rest of *Hodophilus* collections with naphthalene odours and is identical with the ITS sequence of a paratype of *Hy. subfuscescens* var. *subfuscescens*.

European collections of *Hodophilus* with naphthalene odours (putatively identified as *Ho. foetens*) form four well-supported clades that correspond to different phylogenetic species. All collections with naphthalene odours, other than the type of *Hy. subfuscescens* var. *odora*, are part of one *Ho. foetens* superclade with moderate support. Within this, there is one clade with samples identified as *Ho. atropunctus* (Pers.: Fr.) Birkebak & Adamčík (an odourless species), and one clade of sequences originating from soil samples from Canada with an unknown odour. Sister to the *Ho. foetens* superclade is a second well-supported *Hodophilus* core clade of mostly odourless taxa, designated as the *Ho. micaceus* superclade. The one exception to being odourless in this clade is the *Hy. subfuscescens* var. *odora* type collection. Samples morphologically identified as *Cam. atropuncta*, *Cam. phaeoxantha*, and *Cam. micacea* all undoubtedly belong to the genus *Hodophilus*, but all contain multiple species under each name.

Fig. 1. Maximum Likelihood phylogeny inferred from three loci (ITS, LSU, and *rpb2*) with species-level clades 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. Also included are collection labels, country or state/province, and whether this represents a type collection. Bootstrap values followed by Bayesian posterior probabilities are indicated at nodes. [Colour online.]



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Table 1. Mean values of 30 measurements of selected micro-morphological characteristics observed on *Hodophilus* taxa with unpleasant odours.

Species	Herbarium No.	Spores			Caulocystidia		TC margin			TC centre		
		L	W	Q	L	W	L	W	Q	L	W	Q
<i>Ho. foetens</i>	K(M) 0009276*	5.9	4.3	1.39	31.6	8.7	27.2	14.3	2.10	23.4	11.4	2.03
<i>Ho. hesleri</i>	JMB10252013-01*	4.8	4.0	1.21	21.8	5.9	28.7	12.3	2.40	20.1	12.7	1.98
	JMB10102013-01	5.4	4.5	1.21	32.0	6.1	32.8	14.0	2.40	28.7	14.3	2.07
	JMB10102013-02	5.1	4.2	1.23	27.9	5.4	31.8	12.3	2.70	30.0	12.1	2.48
	ECV4175	5.5	4.4	1.26	26.1	6.4	30.0	14.6	2.10	26.3	15.1	1.76
<i>Ho. paupertinus</i>	MICH10923*	6.6	5.0	1.33	32.0	4.7	25.2	11.0	2.62	25.2	8.8	3.50
	Ceska020301	6.5	5.3	1.24	32.3	5.7	29.7	9.8	3.16	31.6	9.9	3.40
	MEND16	5.4	5.2	1.25	27.4	4.3	42.2	11.9	3.80	27.9	6.7	4.50
<i>Ho. peckianus</i>	NYS f3880*	6.1	4.8	1.27	40.1	8.6	26.0	16.3	1.67	23.3	14.7	1.71
<i>Ho. smithii</i>	JMB10052013-02*	6.0	4.5	1.33	39.5	9.0	30.7	15.2	2.05	29.2	9.9	3.10
	JMB10122013-02	6.0	4.7	1.29	25.4	8.4	22.8	10.0	2.29	28.6	10.8	2.77
	JMB08092012-02	5.7	4.4	1.28	31.8	10.2	32.3	13.1	2.57	37.4	12.7	3.07
	PBM3930	5.8	4.3	1.34	33.6	9.1	29.2	14.8	2.06	34.7	11.7	3.17
<i>Hy. subfuscescens</i> var. <i>subfuscescens</i>	MICH10952*	5.2	4.1	1.26	26.5	5.1	29.5	22.3	1.44	23.5	18.0	1.32
<i>Hy. subfuscescens</i> var. <i>odora</i>	MICH 10953*	5.2	4.1	1.28	33.7	5.8	26.4	17.8	1.52	28.9	20.8	1.42

Note: *, Type specimens; TC, margin/centre (terminal cells in pileipellis near the pileus margin/centre); L, length; W, width; Q, length:width ratio; The shaded boxes indicates important differences.

Morphological delimitation of genetically defined groups

Our morphological observations showed several differences among species defined by the molecular analyses. The most distinct differences represented by micro-morphological characteristics are labelled in Table 1. Mean basidiospore length of *Ho. hesleri* and *Ho. subfuscescens* do not exceed 5.5 μm . Mean caulocystidia width of *Ho. foetens*, *Ho. peckianus*, and *Ho. smithii* is broader than 8 μm . Terminal cells of hyphae in the pileipellis near the pileus margin are shorter with a length:width ratio up to 1.7 in *Ho. peckianus* and *Ho. subfuscescens*. In *Ho. paupertinus*, the presence of very narrow cylindrical terminal cells in the pileipellis near the pileus centre

contributes to its smallest mean width (up to 10 μm). This species and *Ho. smithii* have the greatest length:width ratio of the terminal cells near the pileipellis centre.

The shape of the terminal cells differ between the margin and the centre of the pileus in *Ho. hesleri* and *Ho. smithii*. The length:width ratio of the first is smaller near the pileus centre, whereas the second species shows an opposite pattern. Both species are similar in the shape of terminal cells near the pileus margin but demonstrate clear differences in terminal cell morphology at the pileus centre. Macro-morphological characteristics do not seem to exhibit much difference among the studied species other than the olive-buff tints of *Ho. subfuscescens* (Table 2).

Taxonomy

Artificial key to North American *Hodophilus* species with an unpleasant odours

- 1A. Caulocystidia on average wider than 8 μm 2
 1B. Caulocystidia on average narrower than 7 μm 3
 2A. Terminal cells of hyphae near the pileus centre sphaero-pedunculate, capitate, obpyriform or clavate, towards the base usually distinctly pedunculate ***Hodophilus smithii***
 2B. Terminal cells of hyphae near the pileus centre mostly globose, obpyriform and not distinctly pedunculate ***Hodophilus peckianus***
 3A. Pileipellis near the pileus centre with frequent non-inflated cylindrical or clavate terminal cells. . . . ***Hodophilus paupertinus***
 3B. Pileipellis near the pileus centre with obpyriform, globose, or sphaero-pedunculate terminal cells 4
 4A. Terminal cells of hyphae near the pileus centre obpyriform or globose with a ratio of length:width <1.5, subterminal cells often very short and small (up to 10 μm) ***Hodophilus subfuscescens***
 4B. Terminal cells of hyphae near the pileus centre obpyriform or sphaero-pedunculate with a ratio of length:width >1.7, subterminal cells rarely short and small (mostly >10 μm) ***Hodophilus hesleri***

Hodophilus hesleri Adamčík, Birkebak & Looney, sp. nov., Figs. 2C, 3A–3C, 4A, 5A, and 6A

MYCOBANK NO.: MB815983.

ETYMOLOGY: In honor of Dr. Lexemuel Ray Hesler.

HOLOTYPE: TENN 070842 (coll. No. JMB10251301).

Pileus (2.5–)4–11 mm broad, convex, weakly depressed near the centre when old, margin not striated, sometimes slightly crenulated, surface smooth, slightly rough

Table 2. Comparison of field characteristics observed on *Hodophitium* taxa with unpleasant odours as interpreted by Hesler and Smith (1963)^a, Howe (1874)^b, and according to our observations^c.

Species epithet	Pileus colour	Lamellar colour	Odour	Width of lamellae	Stipe surface
<i>foetens</i> ^a	Cinnamon brown	Wood brown	Pungent	Medium broad	First scabrous-dotted
<i>subfuscescens</i> var. <i>odorat</i> ^a	Isabella colour to pale olive buff	Pale olive buff, greyer at maturity	Disagreeable when flesh is bruised	Moderately broad	Glabrous
<i>paupertimus</i> ^a	Sordid Isabella, soon dark brownish grey	Pallid to pale drab	Strong disagreeable	Very narrow	Glabrous
<i>peckianus</i> ^b	Fulgurous paler buff-brown dry	Pallid, becoming darker with age	Strong, like lace-wing	Broad	Smooth
<i>hesleri</i> ^c	Pale grey brown, darker near the centre	Brownish grey to grey brown	Naphthalene also with other components	1–2 mm wide	Smooth, slightly pruinose near lamellae
<i>smithii</i> ^c	Pale grey brown	Milk-coffee to greyish brown	Naphthalene also with other components	1–1.5 mm wide	Smooth or with fine blackish punctuations

when young, becoming granulose and cracking when old, matt, hygrophanous, colour when moist near the margin dark blond (5D3–5D4), young with white outline, near the centre greyish brown to chocolate brown (6E4–6F4), eye brown (7F4) to black, when dry near the margin orange grey (5B2–5B3), birch grey (5C2), dry near the centre brownish orange (5C3). **Stipe** 9–24 mm × 0.75–2.00 mm, smooth and reflective except where slightly pruinose near lamellae, upper part bronze (5E5), brownish orange (5C3), or brown (6D3–6E4) when young, becoming darker with age dark blond (5D4) to chocolate brown (6F4), near the base first dark blond (5D4), later hair brown (5E4) or chocolate brown (6F4), flexuous, sometimes narrowed near base. **Lamellae** 1–2 mm wide, $L = 12–23$, $l = 0–1$, short decurrent, brownish orange to grey brown (5C3–6C3–6D3, 5B3–5D2). **Flesh** elastic, with a naphthalene odour but also mixed with other farinaceous or unpleasant components, dry orange grey (5B3), wet greyish brown (6E3).

Basidiospores (4.2–)4.8–6.0(–7.2) $\mu\text{m} \times$ (3.8–)4.0–4.8(–5.8) μm , mean = 5.4 $\mu\text{m} \times$ 4.4 μm , Q (length: width) = (1.04–)1.13–1.40(–1.55), mean $Q = 1.26$, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled, hilar appendage 0.4–0.6(–1.0) μm long. **Basidia** 4-spored, (31.0–)34.5–40.5(–43.0) $\mu\text{m} \times$ (4.5–)5.0–6.5(–7.0) μm , mean = 37.5 $\mu\text{m} \times$ 5.6 μm , hyaline, narrowly clavate, attenuated and flexuous toward base. **Basidioles** cylindrical to narrowly clavate, often flexuous, obtuse, (19.0–)26.0–38.0(–45.0) $\mu\text{m} \times$ 4.0–6.0(–6.5) μm , mean = 31.9 $\mu\text{m} \times$ 5.0 μm . **Pleurocystidia** absent. **Marginal cells** on the lamellae not well-differentiated, similar to basidiola on lamellar sides. **Lamellar trama** of strongly intricate, subparallel, scarcely branched and irregularly inflated, hyphae 4–10(–15) μm wide, composed of cells 20–100 μm long. **Subhymenium** pseudoparenchymatic, 20–25 μm deep, composed of branched, dense, hyphae 2–4 μm wide. **Pileipellis** near the pileus margin a hymeniderm, composed of sphaero-pedunculate or obpyriform cells arranged in one rank, terminal cells measuring (16.5–)22.5–37.5(–45.5) $\mu\text{m} \times$ (10.5–)11.0–18.0(–24.5) μm , mean = 30.0 $\mu\text{m} \times$ 14.6 μm , $Q = (1.19–)1.57–2.63(–2.92)$, mean $Q = 2.1$; subterminal cells rarely inflated, usually cylindrical or fusiform, short and small cells (shorter than 10 μm) occasional, usually not branched, often bearing incrustated pigments, measuring (3.5–)8.5–34.0(–63.0) $\mu\text{m} \times$ (3.0–)4.0–9.0(–12.0) μm , mean = 21.3 $\mu\text{m} \times$ 6.6 μm . Terminal cells of hyphae in pileipellis near the pileus centre comparatively shorter, mainly obpyriform, (15.5–)19.5–33.0(–44.0) $\mu\text{m} \times$ (10.5–)12.0–18.0(–22.0) μm , mean = 26.4 $\mu\text{m} \times$ 14.9 μm , $Q = (1.06–)1.39–2.21(–2.83)$, mean $Q = 1.8$. Pileus trama of interwoven hyphae, 3–10 μm wide, subparallel, irregularly inflated and often branched. **Caulocystidia** fascicled, thin-walled, repent or ascending, with terminal cells clavate or obpyriform, obtuse or rarely apically constricted, occasionally flexuous towards septum, measuring (10.0–)15.0–36.5(–58.0) $\mu\text{m} \times$ 4.5–8.5(–11.0) μm ,

Fig. 2. Basidiomata field aspect of (A) *Hodophilus smithii* sp. nov. (JMB10051302, holotypus) photo by B.P. Looney. (B) *Hodophilus smithii* (PBM3930) photo by P.B. Matheny. (C) *Hodophilus hesleri* sp. nov. (ECV4175) photo by S. Trudell. (D) *Hodophilus peckianus* (labelled as “*Hygrophorus Peckii* Howe”) drawing by C.H. Peck. Courtesy New York State Museum, Albany, New York, USA. (E) *Hodophilus paupertinus* (Ceska020704 from the same locality as Ceska020301) photo by O. and A. Ceska. (F) *Hodophilus subfuscescens* (AHS32894, holotypus) photo by A.H. Smith. This image is the property of the Regents of the University of Michigan. Scale bar = 1 cm. All photos and drawings reproduced with permission. [Colour online.]

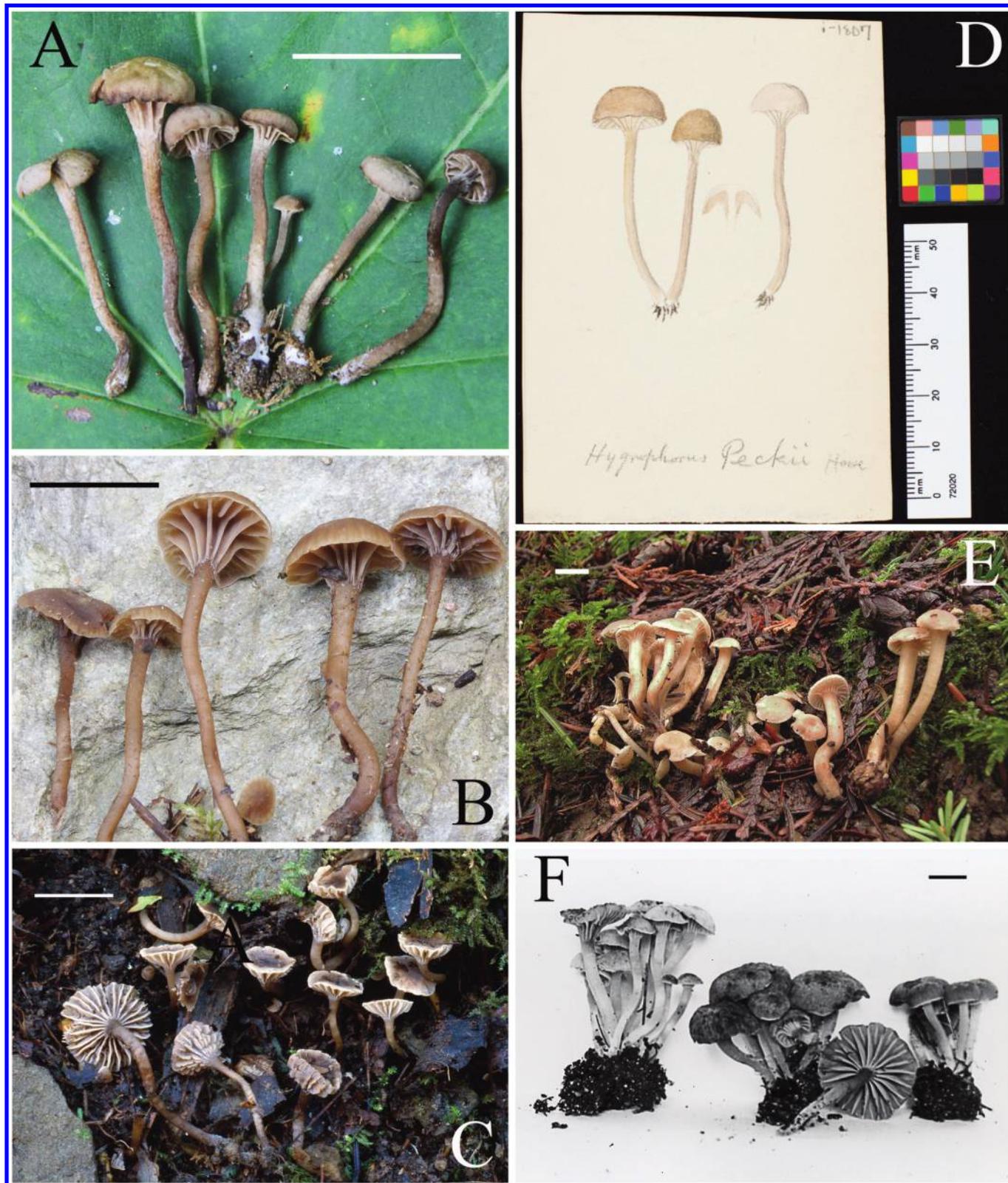
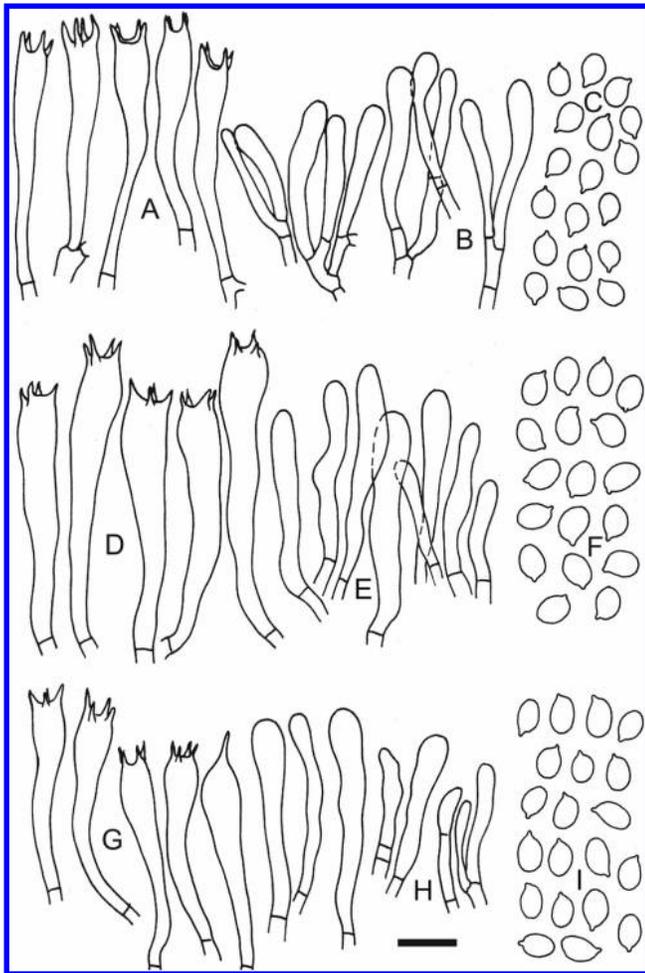


Fig. 3. Hymenium elements and spores of three *Hodophilus* species with naphthalene odours. *Hodophilus hesleri* sp. nov. (holotypus): (A) Basidia. (B) Basidiola. (C) Spores. *Hodophilus paupertinus* (holotypus): (D) Basidia. (E) Basidiola. (F) Spores. *Hodophilus smithii* sp. nov. (holotypus): (G) Basidia. (H) Basidiola. (I) Spores. Scale bar = 10 μm .

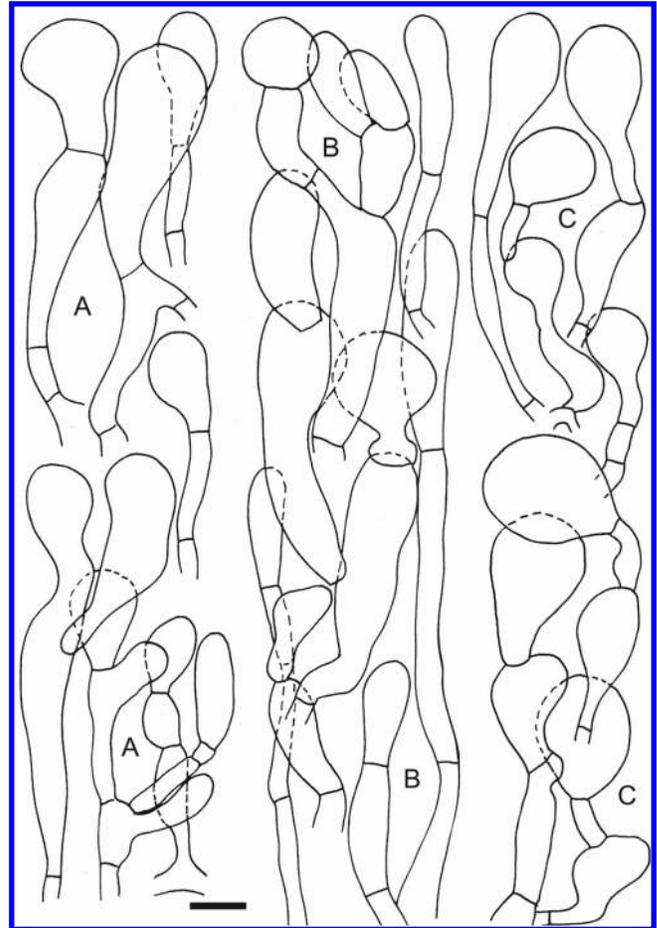


mean = 25.7 μm \times 6.5 μm , subterminal cells occasionally short and small (shorter than 10 μm). Stipe trama of hyphae 4–12(–15) μm wide, parallel, and composed of cells 15–90 μm long. **Clamp connections** absent in all tissues.

MATERIAL EXAMINED: USA. Arkansas. Stone County. Gunner pool recreation area, in deciduous forest on riverbank of North Sylamore Creek, 35°58'57.5"N; 92°28'33.5"W, on naked soil, 25 October 2013, J.M. Birkebak and S. Adamčík JMB10251301 (TENN 070842, holotype); North Carolina. Haywood County. Along Highway 32 near Big Creek, 9 October 2010, E.C. Vellinga ECV4175 (TENN 065670); Tennessee. Cambell County. Norris Dam State Park, Andrews Ridge Trail, 36°14'30.2"N; 84°07'30.6"W, 10 October 2013, J.M. Birkebak and S. Adamčík JMB10101301, JMB10101302 (TENN 070837, TENN 070838).

COMMENTARY: *Hodophilus hesleri* is similar in field aspect and in microscopy to *Ho. peckianus* from which it differs by narrower caulocystidia and by the absence of globose

Fig. 4. Hyphal terminations in pileipellis near the pileus margin of three *Hodophilus* species with naphthalene odours. (A) *Hodophilus hesleri* sp. nov. (holotypus). (B) *Hodophilus paupertinus* (holotypus). (C) *Hodophilus smithii* sp. nov. (holotypus). Scale bar = 10 μm .



terminal cells in the pileipellis. It is possible that there are some historical reports of *Ho. hesleri* misidentified as *Ho. peckianus*.

Hodophilus paupertinus (A.H. Sm. & Hesler) Adamčík, Birkebak & Looney, comb. nov., Figs. 2E, 3D–3F, 4B, 5B, and 6B

MYCOBANK NO.: MB815984.

≡ *Hygrophorus paupertinus* A.H. Sm. & Hesler, *Lloydia* 5(1): 13. 1942.

≡ *Armillariella paupertina* (A.H. Sm. & Hesler) Singer, *Lilloa* 22: 216. 1951.

≡ *Aeruginospora paupertina* (A.H. Sm. & Hesler) Singer, *Sydowia* 15(1–6): 46. 1962.

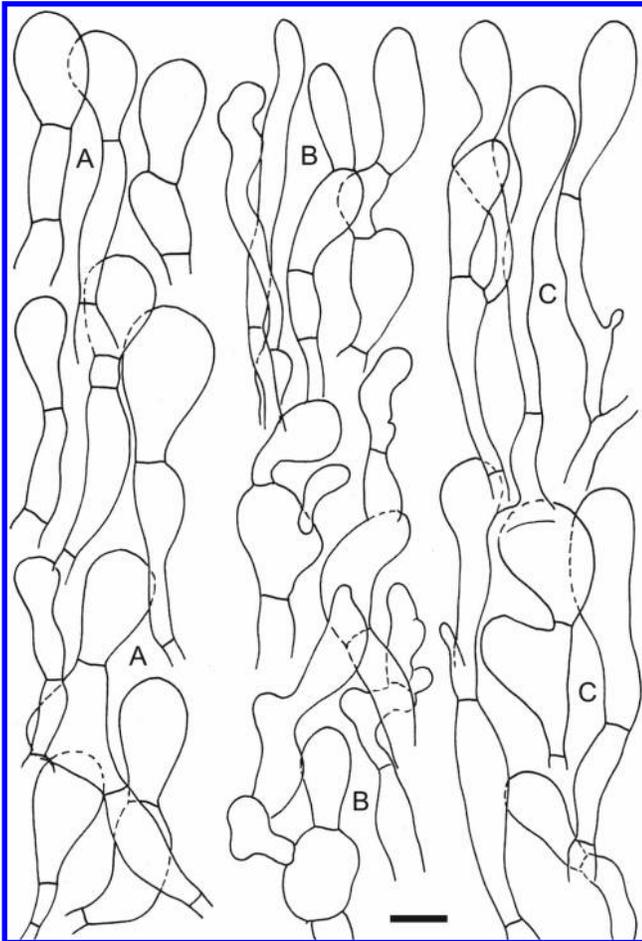
≡ *Hygrotrama paupertinum* (A.H. Sm. & Hesler) Singer, *Beih. Sydowia* 7: 4. 1973.

≡ *Camarophylloopsis paupertina* (A.H. Sm. & Hesler) Boertm., *Bibl. Mycol.* 192: 75. 2002.

HOLOTYPE: MICH 10923 (coll. No. A.H. Smith 3793).

ORIGINAL DESCRIPTION: Pileus (5) 10–20 mm, latus, subplanus, siccus, isabellinus demum umbrinus; caro cinerea, odore

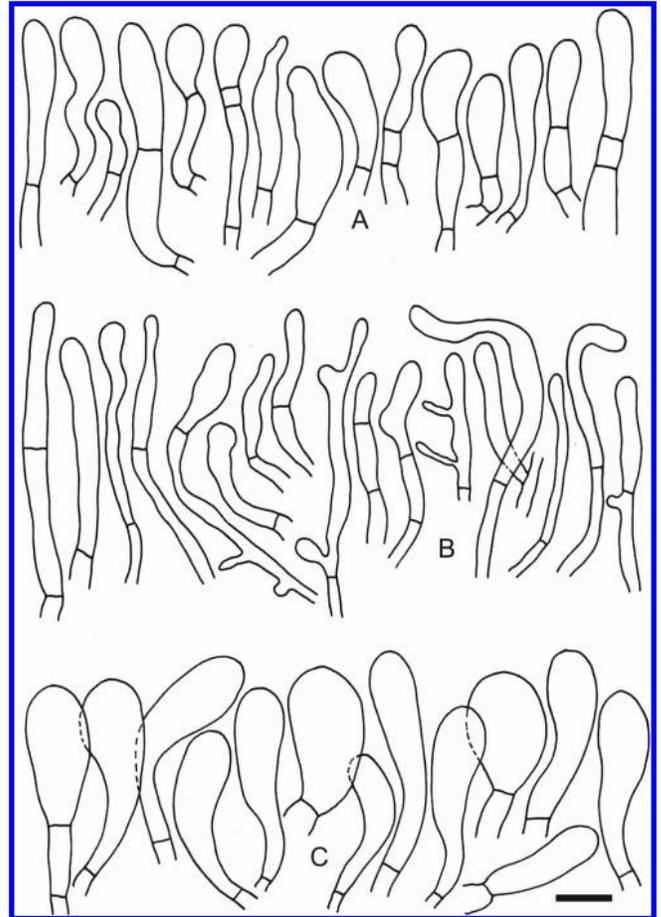
Fig. 5. Hyphal terminations in pileipellis near the pileus centre of three *Hodophilus* species with naphthalene odours. (A) *Hodophilus hesleri* sp. nov. (holotypus). (B) *Hodophilus paupertinus* (holotypus). (C) *Hodophilus smithii* sp. nov. (holotypus). Scale bar = 10 μ m.



subnauseosa; lamellae angustatae, distantes vel dibdistantes, decurrentes, subcinereae; stipes 1–2(3) cm. Longus, 3–6 mm. Crassus, fragilis, solidus demum cavus, isabellinus demum umbrinus, glaber; sporae 5–6 \times 4–5.5 μ m, subgloboosae.

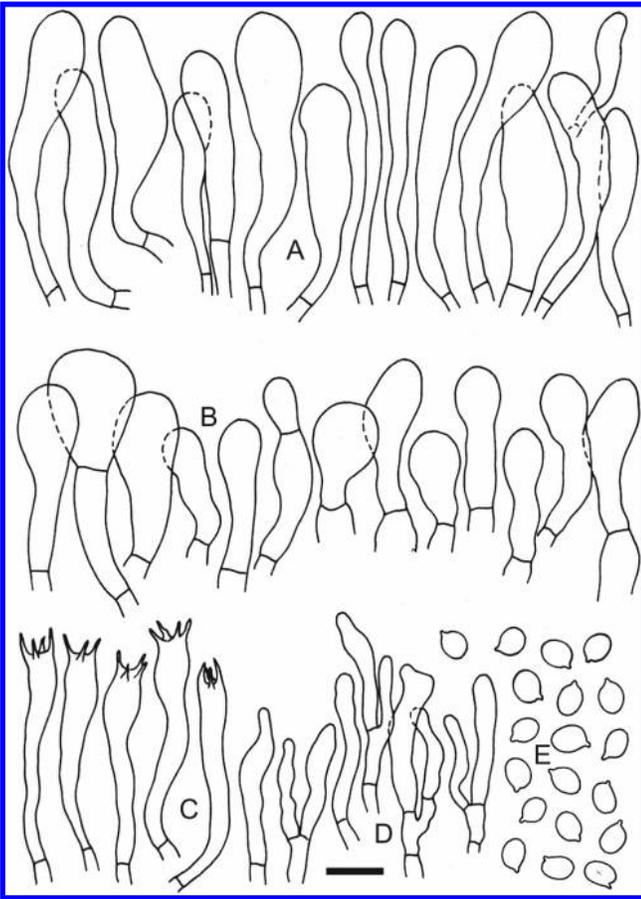
Basidiospores (4.6–)5.7–6.7(–7.5) μ m \times (4.3–)4.7–5.4(–6.0) μ m, mean = 6.4 μ m \times 5.0 μ m, $Q = (1.09)$ –1.16–1.36(–1.51), mean $Q = 1.26$, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled, hilar appendage up to 1 μ m long. **Basidia** 4-spored, 47.0–56.0 μ m \times 5.0–7.5 μ m, hyaline, narrowly clavate, attenuated and flexuous toward base. **Basidioles** cylindrical to narrowly clavate, obtuse, often flexuous, ca. 3.5–7.0 μ m wide. **Pleurocystidia** absent. **Marginal cells** on the lamellar edge not well-differentiated, similar to basidiola on lamellar sides. **Lamellar trama** composed of subparallel hyphae of very variable width, 3–17 μ m wide, septa usually more distant than 100 μ m. **Pileipellis** near the pileus margin transition from hymeniderm to trichoderm, composed of hyphal terminations very variable in shape, oriented mainly upward but some also repent, often with 1–3 irregularly inflated cells and some

Fig. 6. Caulocystidia of three *Hodophilus* species with naphthalene odours. (A) *Hodophilus hesleri* sp. nov. (holotypus). (B) *Hodophilus paupertinus* (holotypus). (C) *Hodophilus smithii* sp. nov. (holotypus). Scale bar = 10 μ m.



without inflated cells, terminal cells cylindrical, clavate, ellipsoid, globose, obpyriform, occasionally also lobate, measuring (12.0–)17.0–49.0(–108.0) μ m \times (5.0–)7.0–15.0(–23.5) μ m, mean = 32.1 μ m \times 10.9 μ m, $Q = (1.89)$ –1.72–4.55(–7.35), mean $Q = 3.13$; subterminal cells often narrower, cylindrical or fusiform, often also inflated, never short (i.e., shorter than 10 μ m), often branched, measuring (10.0–)18.5–51.0(–86.5) μ m \times (3.5–)5.5–13.0(–23.0) μ m, mean = 34.7 μ m \times 9.3 μ m. Pileipellis near the pileus centre showing more distinct contrast between cellular structure and narrow cylindrical hyphae, composed of often smaller and more irregular nodulous–flexuous–lobate elements, some terminal cells sphaero-pedunculate, obpyriform, and globose, others clavate or subcylindrical, terminal cells measuring (12.5–)16.5–41.5(–74.0) μ m \times (4.0–)5.0–13.0(–24.5) μ m, mean = 29.2 μ m \times 9.2 μ m, $Q = (0.74)$ –1.78–5.34(–11.4), mean $Q = 3.56$; subterminal cells sometimes inflated but sometimes very narrow, cylindrical, often also inflated, occasionally flexuous or nodulous, never short (shorter than 10 μ m), often branched, measuring (8.0–)12.0–34.0(–54.0) μ m \times (3.0–)4.5–11.0(–18.0) μ m, mean = 23.0 μ m \times 7.7 μ m. **Caulocystidia** dispersed or in small or larger fascicles, thin-walled, repent or ascending,

Fig. 7. *Hodophilus peckianus* (holotypus). (A) Caulocystidia. (B) Marginal cells on the lamellar edge. (C) Basidia. (D) Basidiola. (E) Spores. Scale bar = 10 μm .



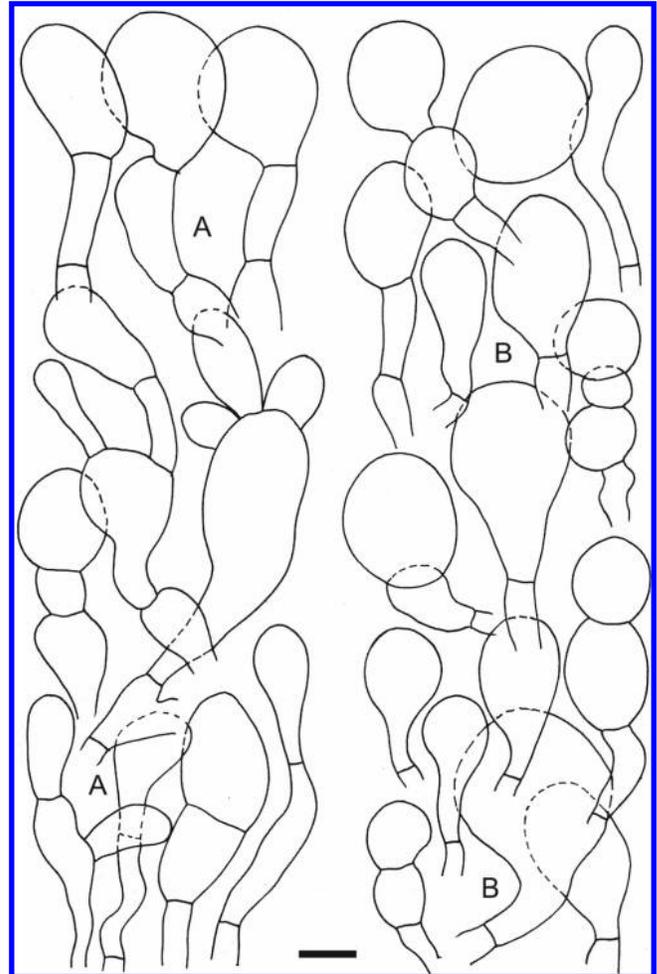
clavate or cylindrical, occasionally with lateral diverticulae, often flexuous at basal part, with terminal cells measuring (15.0–)20.5–40.5(–57.0) μm \times (2.0–)3.5–6.0(–7.5) μm , mean = 30.5 μm \times 4.7 μm . **Clamp connections** absent in all tissues.

MATERIAL EXAMINED: **Canada.** British Columbia. Capital Regional District. Colwood, Royal roads University property, 3 February 2001, A. and O. Ceska Ceska11032001 (TENN 070844); **USA.** California. Humboldt County, Orick, on soil, 5 December 1935, A.H. Smith 3793 (MICH 10923, holotype); Mendocino County. Jackson State Forest off Highway 20, 28 January 2012, D. Smith MEND16 (TENN 070840).

COMMENTARY: *Hygrophorus paupertinus* was described and reported originally from California (Smith and Hesler 1942; Hesler and Smith 1963) and is the only species of *Hodophilus* currently known from western North America. Molecular sequence data from the type and two other western collections are in full agreement. A recent collection from British Columbia confirms that *Ho. paupertinus* is likely widely distributed along the West Coast.

Hodophilus peckianus (Howe) Adamčík, Birkebak & Looney, comb. nov., Figs. 2D, 7, and 8

Fig. 8. *Hodophilus peckianus* (holotypus). (A) Hyphal terminations in pileipellis near the pileus centre. (B) Hyphal terminations in pileipellis near the pileus margin. Scale bar = 10 μm .



MYCOBANK NO.: MB815985.

\equiv *Hygrophorus peckianus* Howe, Bull. Torrey Bot. Club 5: 43. 1874.

\equiv *Camarophyllus peckianus* (Howe) Murrill, N. Amer. Fl. (New York) 9(6): 389. 1916.

\equiv *Hygrotrama peckianum* (Howe) Singer, Beih. Sydowia 7: 4. 1973.

\equiv *Camarophylloopsis peckiana* (Howe) Boertm., Bibl. Mycol. 192: 75. 2002.

HOLOTYPE: NYS f3880.

ORIGINAL DESCRIPTION: Odorous, rather firm, gregarious or subcaespitose; pileus fleshy, convex or slightly depressed in the centre, smooth, hygrophanous, fuliginous when moist, paler buff-brown when dry, the margin curved and sometimes wavy; lamellae subdistant, broad, thick, arcuate, decurrent, pallid when young, becoming darker with age; stem smooth, stuffed or hollow, subflexuous, often compressed and attenuated below, coloured like the pileus; spores subglobose, rough, .0002 in. in diameter.

Plant 1–2 inches high (1 inch = 2.54 cm), pileus 5–10 lines broad; stem about 1 line thick.

Ground under *Pteris aquilina*. Lake Pleasant, August.

Odor quite strong, resembling that emitted by some species of Golden-eyed lace-wing flies (*Chrysopa*). The colour of the moist plant is almost exactly like that of *Lactarius fuliginosus*, Fries. Related to *Hygrophorus Cantharellus*, Schw.

Basidiospores (5.4–)5.6–6.5(–7.0) $\mu\text{m} \times$ (4.0–)4.4–5.1(–5.6) μm , mean = 6.1 $\mu\text{m} \times$ 5.8 μm , $Q = (1.13\text{--})1.21\text{--}1.34\text{--}(1.40)$, mean $Q = 1.28$, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled, hilar appendage 0.6–0.9(–1.0) μm long. **Basidia** 4-spored, (30.0–)36.5–45.0(–50.0) $\mu\text{m} \times$ 5.0–6.5(–7.5) μm , mean = 40.8 $\mu\text{m} \times$ 5.9 μm , hyaline, narrowly clavate, attenuated and flexuous toward base. **Basidioles** cylindrical to narrowly clavate, often flexuous, and apically constricted, ca. 2.5–6.0 μm wide. **Pleurocystidia** absent. **Marginal cells** on the lamellar edge well differentiated, clavate to obpyriform, pedunculate or not, (10.0–)15.0–28.0(–35.0) $\mu\text{m} \times$ (5.5–)6.5–12.5(–15.5) μm , mean = 21.5 $\mu\text{m} \times$ 9.3 μm . **Lamellar trama** composed of strongly undulate and intricate but parallel, scarcely branched, and irregularly inflated hyphae 2–8(–10) μm wide, some hyphae with yellow pigments. **Pileipellis** near the pileus margin transitions from a hymeniderm to an epithelium, composed of globose, sphaero-pedunculate or obpyriform cells arranged in 1–2 (rarely 3) ranks, terminal cells measuring (12.0–)16.5–35.0(–45.0) $\mu\text{m} \times$ (7.5–)11.5–21.0(–30.0) μm , mean = 26.0 $\mu\text{m} \times$ 16.3 μm , $Q = 0.94\text{--}2.42\text{--}(4.50)$, mean $Q = 1.67$; subterminal cells often inflated, some narrow cylindrical, short and small cells (shorter than 10 μm) rare, usually not branched, often bearing incrusting pigments, measuring (23.0–)30.0–50.5(–70.0) $\mu\text{m} \times$ (4.5–)6.5–11.0(–13.0) μm , mean = 40.1 $\mu\text{m} \times$ 8.6 μm . Pileipellis elements near the pileus centre very similar to those near the margin, terminal cells of hyphae measuring (9.0–)15.0–31.5(–42.0) $\mu\text{m} \times$ (6.0–)8.0–21.0(–31.0) μm , mean = 23.3 $\mu\text{m} \times$ 14.7 μm , $Q = (0.82\text{--})1.05\text{--}2.36\text{--}(3.50)$, mean $Q = 1.71$. **Caulocystidia** fascicled, thin-walled, repent or ascending, clavate, obtuse, usually pedunculate and flexuous towards septum, with terminal cells measuring (9.0–)12.5–31.5(–50.0) $\mu\text{m} \times$ 4.5–14.0(–23.0) μm , mean = 22.2 $\mu\text{m} \times$ 9.2 μm . **Clamp connections** absent in all tissues.

MATERIAL EXAMINED: USA. New York. Hamilton County. Lake Pleasant, ground, under *Pteris aquilina*, August (NYS f3880, holotype).

COMMENTARY: *Hygrophorus peckianus* is the oldest name of agaricoid Clavariaceae species described and reported from North America (Howe 1874). Coker (1948) suggested a close relationship with the European species *Ho. foetens*, but *Ho. peckianus* was not widely accepted because of the European bias for accepting a single species (*Ho. foetens*) with a strong disagreeable odour. Smith and Hesler (1954) reported *Ho. foetens* from Michigan, and later from Idaho (in Hesler and Smith 1963), and discussed its dif-

ferences from *Ho. peckianus*. They also recognized two more species with unpleasant odours (in Hesler and Smith 1963). Comparing the descriptions of *Ho. peckianus* by Coker (1948) and Smith and Hesler (1954) with the original diagnosis suggests that their interpretation of the species was correct. Hesler and Smith (1963) place the species in the section *Camarophylloopsis* because the hyphae are not oriented perpendicularly to form a hymeniderm. In fig. 8 of their publication (Hesler and Smith 1963) they illustrated and described end-cells in the pileipellis (terminal cells) as pyriform, clavate ovoid, or subglobose, similar to our observation on the type. Because of the poor condition of the type with mostly collapsed microscopic elements, we were not able to judge and confirm the perpendicular orientation of hyphae. However, it is possibly a useful characteristic to distinguish the species from other similar species.

Hodophilus peckianus has been reported from New York (Howe 1874), Massachusetts (Murrill 1916), North Carolina (Coker 1948), Michigan (Smith and Hesler 1942), and Tennessee (Hesler and Smith 1963). The drawing included in Fig. 2 was sketched by C.H. Peck and is labelled as “*Hygrophorus Peckii* Howe”, which is a combination that Howe did not make. Although not linked to a particular collection, it is likely that this represents *Hy. peckianus* rather than *Hy. peckii* G.F. Atk., based on the colour of stipe, lack of glutinous coating of stipe, and a lack of striations on the pileus margin.

Hodophilus smithii Adamčík, Birkebak & Looney, sp. nov., Figs. 2A–2B, 3G–3I, 4C, 5C, and 6C

MYCOBANK NO.: MB815986.

ETYMOLOGY: in honor of Dr. Alexander Hanchett Smith.

HOLOTYPE: TENN 070839 (coll. No. JMB10051302).

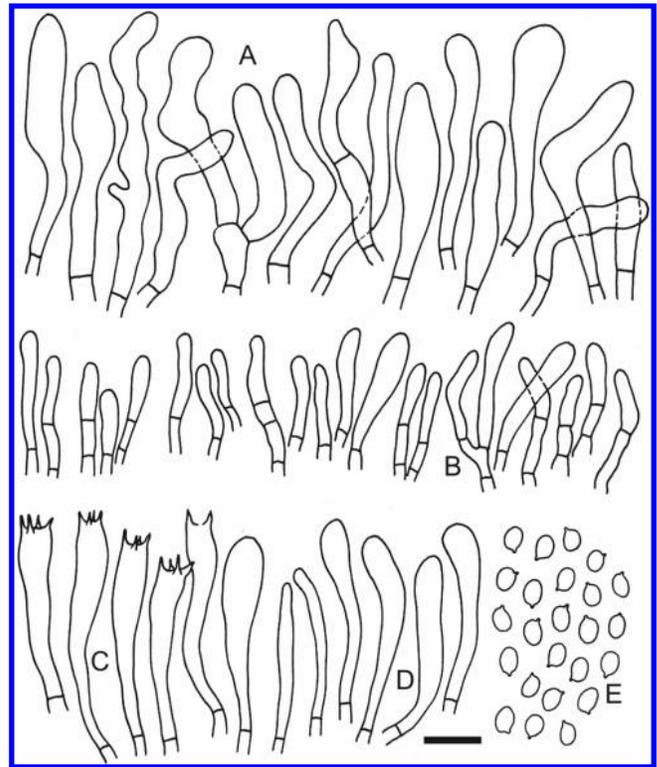
Pileus 2–10 mm broad, convex, margin not striate, sometimes slightly crenulated, surface smooth, matt, hygrophanous; colour when moist near the margin brownish orange (5C3), nougat to grey-brown (5D3–6D3), fawn brown (7F4), near the centre concoloured or nutria brown (5F3), when dry near the margin orange-grey (5B2), birch grey (5C2), greyish brown (6E3–6E4), dry near the centre concoloured or golden (5C4). **Stipe** (6–)9–30(–37) mm \times 0.75–1.50 mm, smooth or with fine blackish punctuations in zones oriented horizontally, near the lamellae camel-brown (6D4), greyish brown (6E3), chocolate brown (6F4), towards base sometimes darker, sometimes black or almost so all over, sometimes with white tomentum on base, flexuous, slightly narrowed near base. **Lamellae** 1.0–1.5 mm wide, $L = 12\text{--}18$, $l = 0\text{--}1$, deeply decurrent, milk-coffee to greyish brown (6D3–6E3), towards edge paler golden (5C4). **Flesh** elastic, with very strong naphthalene odour but also with farinaceous or other unpleasant components, in pileus orange white (5A2), greyish brown (6C2–6D3).

Basidiospores (5.0–)5.4–6.3(–7.2) $\mu\text{m} \times$ (3.8–)4.1–4.8(–5.2) μm , mean = 5.9 $\mu\text{m} \times$ 4.5 μm , $Q = (1.08\text{--})1.22\text{--}$

1.40(–1.55), mean $Q = 1.31$, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled, hilar appendage 0.45–0.80 μm long. **Basidia** 4-spored, (28.0–)33.0–42.5(–49.0) $\mu\text{m} \times$ (5.0–)6.0–7.5(–8.0) μm , mean = 37.7 $\mu\text{m} \times$ 6.8 μm , hyaline, narrowly clavate, attenuated and flexuous toward base. **Basidioles** cylindrical to narrowly clavate, often flexuous, obtuse, (17.0–)24.5–37.5(–49.0) $\mu\text{m} \times$ (3.0–)4.0–6.5(–8.0) μm , mean = 31.0 $\mu\text{m} \times$ 5.2 μm . **Pleurocystidia** absent. **Marginal cells** on the lamellae not well-differentiated, similar to basidiola on lamellar sides. **Lamellar trama** of strongly undulate and intricate, subparallel, scarcely branched and irregularly inflated, 3–10(–15) μm wide hyphae, composed of cells 10–90 μm long. **Subhymenium** pseudoparenchymatic, 20–25 μm deep, composed of branched, dense, hyphae 3–5 μm wide. **Pileipellis** near the pileus margin a hymeniderm, mainly composed of sphaero-pedunculate, occasionally also obpyriform cells arranged in one rank, terminal cells measuring (10.0–)20.0–39.5(–58.0) $\mu\text{m} \times$ (7.0–)10.0–17.5(–24.0) μm , mean = 29.8 $\mu\text{m} \times$ 13.7 μm , $Q = (1.00\text{--})1.47\text{--}3.01(–4.00)$, mean $Q = 2.24$; subterminal cells rarely inflated, usually cylindrical, short and small cells (shorter than 10 μm) very rare, usually unbranched, measuring (5.0–)13.5–35.0(–53.0) $\mu\text{m} \times$ (3.0–)3.5–13.0(–30.0) μm , mean = 21.2 $\mu\text{m} \times$ 8.1 μm . Terminal cells of hyphae in pileipellis near the pileus centre usually somewhat longer, mainly clavate-pedunculate or sphaero-pedunculate, rarely obpyriform, (15.0–)23.0–42.0(–66.0) $\mu\text{m} \times$ (6.0–)8.0–14.5(–21.0) μm , mean = 32.5 $\mu\text{m} \times$ 11.3 μm , $Q = (1.18\text{--})2.06\text{--}3.99(–5.67)$, mean $Q = 3.03$. Pileus trama of interwoven, 3–10 μm wide, subparallel, irregularly inflated hyphae, composed of cells 10–80 μm long. **Caulocystidia** repent and dispersed or fascicled and ascending, thin-walled, often inflated, obpyriform or sphaero-pedunculate, others broadly clavate, usually not flexuous, with terminal cells measuring (13.0–)21.0–44.0(–78.0) $\mu\text{m} \times$ (4.0–)7.0–11.5(–17.0) μm , mean = 32.6 $\mu\text{m} \times$ 9.1 μm , with darker parietal and sometimes also dark incrusted pigments. Stipe trama hyphae 4–10 μm wide, parallel, often anastomosed, composed of cells 10–120 μm long. **Clamp connections** absent in all tissues.

MATERIAL EXAMINED: USA. North Carolina. Big Creek, at very beginning of Baxter Creek trail, 9 August 2012, E.C. Vellinga JMB08091202 (TENN 067461); Macon County. Highlands, Horse Cave Road, Rusty Falls area, 15 July 1991, D.E. Desjardin DED5230 (TENN 050035, as *Hygrophorus subfuscescens* var. *odora*); Tennessee, Blount County. Cades Cove, Primitive Baptist Church, 35°36'08"N; 83°48'48"W, 9 August 2005, E.B. Lickey DJL04TN23 (TENN); 1.1 miles (1 mile = 1.6 km) up Schoolhouse Gap trail, 19 July 2013, P.B. Matheny PMB3930 (TENN 070843); Monroe County. Cherokee National Forest, Walnut Grove Picnic Area, 35°19'36"N; 84°09'41"W, lawn on a strip between parking place and the road, under deciduous trees, 5 October 2013, J.M. Birkebak and S. Adamčík JMB10051302 (TENN 070839, holotype); Anderson County.

Fig. 9. *Hodophilus subfuscescens* var. *odora* (holotypus). (A) Caulocystidia. (B) Marginal cells on the lamellar edge. (C) Basidia. (D) Basidiola. (E) Spores. Scale bar = 10 μm .



Norris Dam State Park, Grist Mill trail, naked soil on margin of the trail in deciduous forest, 12 October 2013, J.M. Birkebak and S. Adamčík JMB10121302 (TENN 070841). **COMMENTARY:** In determining the identity of North American collections of *Ho. foetens* (Smith and Hesler 1954, Hesler and Smith 1963), *Ho. smithii* presents the best candidate for their original concept. Smith and Hesler (1954) described incrusted pigment on the hyphae of the pileipellis and a scabrous-dotted stipe of *Ho. foetens* that may somewhat correspond to caulocystidia incrusted by dark pigments and arranged in fascicles (thus appearing in the field as darker dots on the stipe surface). However, all of our collections of *Ho. smithii* have comparatively smaller basidiomata with pilei not exceeding 10 mm, whereas pilei of *Ho. foetens* are described as 10–40 mm wide. The identity of North American collections of *Ho. foetens* has to be revealed by the study of more recent and authentic material.

Hodophilus subfuscescens (A.H. Sm. & Hesler) Adamčík, Birkebak & Looney, comb. nov., Figs. 2F, 9, and 10

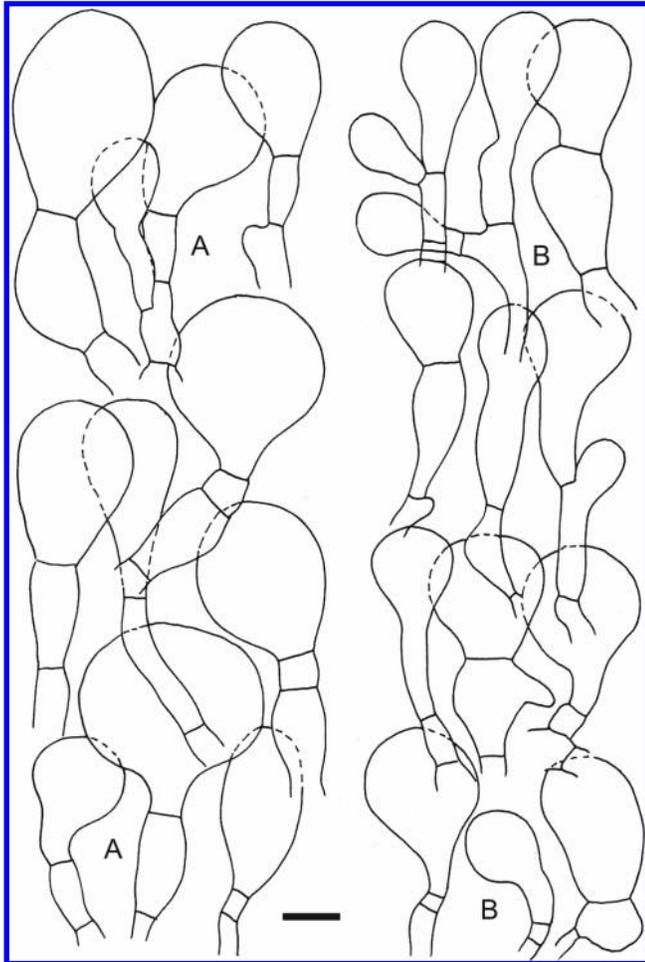
MYCOBANK NO.: MB815987.

≡ *Hygrophorus subfuscescens* A.H. Sm. & Hesler, Sydowia 8: 318. 1954.

≡ *Camarophylloopsis subfuscescens* (A.H. Sm. & Hesler) Arnolds, Mycotaxon 25(2): 643. 1986.

≡ *Hygrotrama subfuscescens* (A.H. Sm. & Hesler) Singer, Beih. Sydowia 7: 3. 1973.

Fig. 10. *Hodophilus subfuscescens* var. *odora* (holotypus). (A) Hyphal terminations in pileipellis near the pileus centre. (B) Hyphal terminations in pileipellis near the pileus margin. Scale bar = 10 μm .



= *Camarophyllopsis subfuscescens* var. *odora* (A.H. Sm. & Hesler) Arnolds, Mycotaxon 25(2): 643. 1986.

= *Hygrophorus subfuscescens* var. *odora* A.H. Sm. & Hesler, Sydowia 8: 318. 1954.

HOLOTYPE: MICH 10952 (coll. No. A.H. Smith 32894).

ORIGINAL DESCRIPTION: Pileus 6–25 mm latus, connexus, glaber, udus, olivaceo-luteus, demum olivaceo-brunneus; lamellae distantes, decurrentes, subluteae, demum sordide avellaneae; stipes 2–4 cm. longus, 1.5–3 mm. crassus, glaber, pallide luteus, dein cinereus; sporae 5–6 \times 4–5 μ . Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Mackinaw City, Mich. Aug. 6, 1949, Smith n. 32894.

Basidiospores (4.3–)4.9–5.6(–6.1) $\mu\text{m} \times$ (3.5–)3.9–4.3(–4.6) μm , mean = 5.2 $\mu\text{m} \times$ 4.1 μm , $Q = (1.16)$ –1.20–1.34(–1.49), mean $Q = 1.27$, broadly ellipsoid to ellipsoid, hyaline, smooth, inamyloid, not dextrinoid, thin-walled, hilar appendage 0.7–1.0 μm long. **Basidia** (2–)4-spored, (31.0–)34.5–43.0(–49.0) $\mu\text{m} \times$ (5.0–)5.5–7.5(–9.0) μm , mean = 38.6 $\mu\text{m} \times$ 6.6 μm , hyaline, narrowly clavate, attenuated and flexuous toward base. **Basidioles** cylindrical to narrowly clavate, often slightly flexuous to-

wards the base, ca. 2–7 μm wide. **Pleurocystidia** absent. **Marginal cells** on the lamellar edge not well-differentiated, similar to basidiola but smaller, cylindrical to clavate, (10.0–)10.5–19.0(–24.0) $\mu\text{m} \times$ 2.5–4.0(–5.5) μm , mean = 14.9 $\mu\text{m} \times$ 3.1 μm , mixed with dispersed basidia. **Lamellar trama** composed of undulate and intricate but parallel, scarcely branched and irregularly inflated hyphae 3–9 μm wide. **Subhymenium** pseudoparenchymatic, 15–25 μm deep, composed of branched, dense hyphae 2–4 μm wide. **Pileipellis** near the pileus margin hymeniderm, composed of sphaero-pedunculate or obpyriform cells arranged usually in 1 rank, terminal cells measuring (15.0–)20.0–35.5(–53.0) $\mu\text{m} \times$ (8.5–)14.0–26.0(–36.5) μm , mean = 28.0 $\mu\text{m} \times$ 20.1 μm , $Q = (0.86)$ –0.92–2.04(–4.50), mean $Q = 1.48$; subterminal cells mainly narrow cylindrical, short and small cells (shorter than 10 μm) occasional, branched or not, measuring (2.0–)7.5–31.0(–58.5) $\mu\text{m} \times$ (3.5–)4.0–19.0(–24.0) μm , mean = 19.4 $\mu\text{m} \times$ 8.4 μm . Pileipellis elements near the pileus centre very similar to those near the margin, terminal cells of hyphae measuring (15.0–)19.0–33.5(–47.0) $\mu\text{m} \times$ (12.0–)14.0–24.5(–35.0) μm , mean = 26.0 $\mu\text{m} \times$ 19.4 μm , $Q = (0.83)$ –1.04–1.70(–2.70), mean $Q = 1.37$. Pileus trama of interwoven hyphae 3–15 μm wide, subparallel, irregularly inflated and often branched. **Caulocystidia** in dense fascicles, thin-walled, usually ascending or erect, clavate or cylindrical, obtuse, flexuous and often also moniliform, with terminal cells measuring (10.0–)20.0–40.5(–61.5) $\mu\text{m} \times$ (3.5–)4.0–7.0(–9.5) μm , mean = 33.3 $\mu\text{m} \times$ 5.4 μm . Trama of stipe parallel, unbranched hyphae 5–10 μm wide, composed of cells often shorter than 30 μm and rarely longer than 100 μm . Stipe trama hyphae 2–11 μm wide, parallel, composed of cells 20–100 μm long. **Clamp connections** absent in all tissues.

MATERIAL EXAMINED: USA. Michigan. Cheboygan County. Mackinaw City, 45°47'02"N; 84°43'40"W, in hardwoods, 13 July 1947, A.H. Smith 32894 (MICH 10952, type of *Hodophilus subfuscescens* var. *subfuscescens*; TENN 023669, paratype); Emmet County. Maple River, Brutus, 13 July 1947, A.H. Smith 25670 (MICH 10953, type of *Hygrophorus subfuscescens* var. *odora*).

COMMENTARY: This study gives clear molecular and morphological arguments for the placement of *Hy. subfuscescens* in the *Ho. micaceus* superclade of the genus *Hodophilus* but fails to confirm odour as an appropriate criterion to recognize it as a distinct taxon from the typical inodorous variety. After the original description of *Ho. subfuscescens* (Smith and Hesler 1954), the species was adopted by later authors (e.g., Romagnesi 1971; Singer 1975) but also treated as a synonym of *Ho. micaceus* (Printz and Læssøe 1986). Based on morphological observation of the type specimens of both *Ho. subfuscescens* varieties, Kovalenko et al. (2012) considered the species as very similar to *Ho. micaceus* in all characteristics except for the more globose basidiospores of the former. Our phylogenetic analyses suggest *Ho. subfuscescens* is different from European *Ho.*

micaceus collections and also from other North American recognized species within the *Ho. micaceus* superclade. The morphological distinction from *Ho. foetens* superclade members, i.e., other species with unpleasant odours, is discussed above (different shape of terminal cells and presence of small cells in the pileipellis).

Discussion

Pileipellis structure and infragenetic classification

Hesler and Smith (1963), in the last North American monograph of the genus *Hygrophorus* (including all known North American *Hodophilus* species), used pileipellis structure (that is, the anatomical makeup of the pileus cuticle) as the basic characteristic for species classification. *Hygrophorus peckianus* and *Hy. paupertinus*, both having a trichoderm type of pileipellis, were classified in *Hygrophorus* sect. *Camarophylloopsis* Hesler & Smith and *Hy. foetens* and *Hy. subfuscens* var. *odora* with a hymeniderm (or in some places of the book interpreted as epithelium) type of pileipellis in *Hygrophorus* sect. *Hygrotrama* (Singer) Hesler & Smith. Among our studied material of collections with naphthalene odours, which includes types of the above mentioned taxa, all collections have inflated broadly clavate, sphaero-pedunculate, obpyriform, or globose elements usually arranged in one rank and oriented perpendicularly. These structures correspond more or less to a hymeniderm, sometimes with transitions to an epithelium if the subterminal cells or cells in lower ranks are inflated. According to our observations, the types of *Hy. foetens*, *Hy. peckianus* and *Hy. subfuscens* var. *odora* show a more distinctive transition to an epithelium (with more inflated subterminal cells and subsequent cells) than the *Hy. paupertinus* type and representatives of the other two species clades of North American *Hodophilus* with naphthalene odours. This does not agree with Hesler and Smith's concept of sections.

In addition to differences in the overall structure of the pileipellis, we observed differences in the shape and dimensions of its terminal elements. The size of terminal cells near the pileus margin and the pileus centre, together with basidiospore and caulocystidia dimensions, are presented for sequenced North American *Hodophilus* collections with naphthalene odours and types of *Hy. peckianus* and *Hy. foetens* (Table 1). Representatives of the *Ho. paupertinus*, *Ho. hesleri*, and *Ho. smithii* clades, together with the type of *Ho. foetens*, have terminal cells in the pileipellis near the pileus margin that are typically clavate, sphaero-pedunculate, or obpyriform, having a length:width ratio (Q) > 2. In contrast, the types of *Hy. peckianus* and *Hy. subfuscens* var. *subfuscens* and var. *odora* have a length:width ratio of the terminal cells near the pileus margin < 2 (1.67, 1.44, and 1.52 respectively). According to our morphological observations of European representatives of the *Ho. micaceus* superclade, and according to our type studies of related North American taxa (unpublished data), representatives of this group

typically have shorter and broader ($Q \leq 2$) terminal cells in the pileipellis. According to our observations, odourless members of the *Ho. micaceus* superclade typically have occasional to frequent small (length and width up to 5 μm) subterminal cells or cells at lower ranks of hyphae in pileipellis (Fig. 10). Such small subterminal cells are absent or rare in the *Ho. foetens* superclade and might represent an additional and even more stable feature for morphological recognition of two major clades within the genus *Hodophilus*.

Species nomenclature and morphological delimitation

Hygrophorus paupertinus is not only clearly identified by the position of the type sequence in the phylogenetic tree (Fig. 1), but it is probably the most distinctive species studied here. All three studied collections (type and two more recent ones) show a pileipellis composed of typical inflated terminal elements mixed with numerous narrower, clavate, or cylindrical elements. The species is re-described above, and combined in the genus *Hodophilus*.

The phylogenetic tree also clearly demonstrates that *Hy. subfuscens* var. *odora* is very different from other North American *Hodophilus* samples with naphthalene odours. Because of the close sequence identity with the typical variety, the variety 'odora' is not accepted here as a distinct infraspecific taxon, and the species is re-described based on the types of both varieties and combined in the genus *Hodophilus*.

The phylogenetic position of the type of *Hy. peckianus* is unknown, owing to unsuccessful attempts at sequencing, and this species represents a nomenclatural challenge for two unidentified North American clades of *Hodophilus* with naphthalene odours. Our morphological studies demonstrate two differences observed on the *Hy. peckianus* type specimen that distinguish it from both unidentified clades but also from the type of *Hy. foetens*; length:width ratios of terminal cells in the pileipellis near the pileus margin and the pileus centre are less than 2 (Table 1; Fig. 8) and subterminal cells are often inflated. Based on the pileipellis structure of the type specimen, *Hy. peckianus* is re-described here as a distinct species of the genus *Hodophilus* in addition to *Ho. paupertinus* and two other new *Hodophilus* species with naphthalene odours. Because of the lack of small subterminal cells (Fig. 8) we expect that *Hy. peckianus* is member of the *Ho. foetens* superclade.

In our *Hodophilus* phylogeny, all European collections of *Hodophilus* with naphthalene odours are grouped in well-supported species clades with no overlap with any North American clade. This suggests that the European species *Ho. foetens* probably does not occur in North America. Because there are no alternative published names for two so far unidentified clades, they are described here as two new species *Ho. hesleri* and *Ho. smithii*. The first is distinguished from other studied members of the *Ho. foetens* superclade by smaller spores (up to 5.5 μm long on average), narrow caulocystidia (up

to 6.4 μm wide on average), and a smaller length:width ratio for terminal cells in pileipellis near the pileus centre. The second species is defined by a combination of wide caulocystidia (at least 8.4 μm wide on average) and a greater length:width ratio for terminal cells in pileipellis near the pileus centre (on average between 2.77 and 3.17) (Table 1).

Importance of field characteristics

Hesler and Smith (1963) used several field characteristics to recognize four North American taxa with strong unpleasant odours: pileus and lamellar colour, width of lamellae, surface texture of the stipe and the pileus, and the odour of basidiomata. All of these characteristics are compared in Table 2. Concerning the colour of the pileus, both species described here as new are very similar. They have brown and grey tints of variable intensity because of a hygrophanous surface. We did not observe on our material for the two new species any cinnamon colour such as reported for *Hy. foetens* by Hesler and Smith (1963) nor Isabella or olive-buff colours such as reported by them for *Hy. paupertinus* and *Hy. subfuscescens* var. *odora*. The lamellae of both new species are grey brown, which probably corresponds to the pallid lamellae of *Hy. peckianus* and *Hy. paupertinus*. The odour of both new species is somewhat variable, naphthalene mixed with a farinaceous, methyl mercaptan or an unpleasant component. Hesler and Smith (1963) distinguished various odour types that seem to us subjective and difficult to recognize. The odour for *Ho. subfuscescens* var. *odora* seems to be useful, which is described as distinctly disagreeable when bruised. In our experience with both new species, their odours were so strong that they could be located among leaves even before having seen them. Hesler and Smith defined the lamellar width as specifically “very narrow” in *Hy. paupertinus*, which may correspond to the width of the lamellae of *Ho. hesleri* and *Ho. smithii* (up to 1.5–2) mm). However, since no specific size of the lamellae is given, the importance of this characteristic needs to be verified. Possibly a good field characteristic might be darker dots on the upper part of the stipe of *Ho. smithii*, which are probably caused by dark incrustated pigments on the caulocystidia. Hesler and Smith also reported the presence of a scabrous-dotted stipe surface (with no mention of darker colour), but only for American collections of *Hy. foetens*.

Ecology

In the North American literature (e.g., Hesler and Smith 1963), the ecology of *Hodophilus* taxa with unpleasant odours is defined very generally, most frequently in woods and on bare soil, often under bracken ferns. The only mention of grassland is associated with *Hy. peckianus*, reported also from lawns in addition to other forest habitats. Accordingly, we collected only one among seven of our North American collections of *Hodophilus* with naphthalene odours in a lawn on a strip between a

parking lot and the road in the Cherokee National Forest (JMB10052013-02), but this was surrounded by a forest in close proximity. In Europe, the reports of *Ho. foetens* more frequently originate from grasslands, e.g., pastures or meadows (e.g., Arnolds 1990; Boertman 2012). In our experience, *Hodophilus* species with naphthalene odours grow at locally humid places but not on damp soil. The suitable microclimate is probably supported by the shelter provided by various woody or herbaceous vegetation (forest, scrubs, high grass) in combination with either proximity to a water source or humid climate with frequent rains. In both continents, *Hodophilus* with naphthalene odours occur frequently on naked soil, e.g., steep slopes on riverbanks, footprints or paths of large animals, roadsites, etc. They are often associated with other clavarioid Clavariaceae and with *Hygrocybe* species. Most of our North American collections are from riverbanks, roadsides, and embankments next to trails, whereas our European collections are mainly from scrubs and high grass on forest/pasture margins.

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