



New species of *Hohenbuehelia*, with comments on the *Hohenbuehelia atrocoerulea* – *Nematoctonus robustus* species complex

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Key words

28S
5 new taxa
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Abstract Four new species of *Hohenbuehelia* (Fungi: *Pleurotaceae*) are described in the group of *Hohenbuehelia atrocoerulea* and *Hohenbuehelia grisea*. *Hohenbuehelia algonquinensis*, found on *Pinus* in Ontario, Canada, may be distinguished macroscopically from bluish collections of *H. atrocoerulea* and watery grey-brown collections of *H. grisea* by its coal-black pileus. *Hohenbuehelia canadensis*, on or associated with *Pinus* in both Ontario and Alberta, Canada, and *Hohenbuehelia nimueae*, on *Salix* in Ontario and *Abies* in Wyoming, USA, have similarly dark fruiting bodies and were previously misidentified as *H. approximans* (which we treat as a synonym of *H. grisea*), *H. atrocoerulea*, *H. mustialensis* or *H. nigra*. The latter species is shown to be a member of *Resupinatus*, despite the presence of prominent metuloid cystidia in its hymenium. *Hohenbuehelia carlothornii* has been found in Costa Rica; collections of the sexual fruiting bodies were previously identified as *H. grisea* and isolates from soil nematodes were identified by the anamorph name *Nematoctonus robustus*. That name has been treated as a synonym of *H. atrocoerulea* but, given the genetic and geographic variation within this complex, we transfer it to *Hohenbuehelia* as a distinct species. Sequences of the nuclear ribosomal DNA internal transcribed spacer region (ITS), the D1/D2 variable region of the large subunit gene, and a portion of the translation elongation factor (TEF1) gene provide good separation and support for these new species. A key to the dimidiata species of *Hohenbuehelia* of North America and Europe is provided.

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INTRODUCTION

Hohenbuehelia is a genus of mushroom fungi (Basidiomycota: Agaricales) that forms a sister group to the much better known oyster mushrooms of the genus *Pleurotus* (Thorn et al. 2000). Both genera are nematophagous, with *Hohenbuehelia* attacking nematodes through production of adhesive knobs on its hyphae (the so-called predatory mode) or on germinated asexual conidia that are produced from denticles on its hyphae or on germinated basidiospores from the sexual fruiting body or mushroom (the parasitoid mode, Barron 1977). The asexual stages of *Hohenbuehelia* were formerly named separately in the anamorph genus *Nematoctonus* (Drechsler 1941, Thorn & Barron 1986), but under the recent one fungus – one name rules (Taylor 2011) adopted in Melbourne in 2011 (McNeill et al. 2012), all members of this monophyletic group should be known as *Hohenbuehelia* (Thorn 2013).

Within *Hohenbuehelia*, one species group that has caused considerable taxonomic debate centers on *H. atrocoerulea*, described from Sweden, and *H. grisea*, described from the USA. Both species (and several others that are macroscopically similar) have dimidiata, often imbricate fruiting bodies, ranging in colour from pale greyish tan in *H. grisea* to blue-black in *H. atrocoerulea*, with whitish to pale ochre-buff lamellae. However, the colour of the fruiting bodies varies, often with the maturity of the collection, and truly blue-black collections are rare, with the result that all variants are sometimes grouped under one species name (e.g., Elborne 1995) or treated sepa-

rately (e.g., Elborne 2008). The anamorphs of both species and of *H. approximans*, *H. cyphelliformis* and *H. nigra* have been reported to be morphologically indistinguishable and treated as *Nematoctonus robustus* (Thorn & Barron 1986), which was described from forest litter in Ghana (Jones 1964). Although molecular analyses showed several distinct clades within the *H. grisea* - *N. robustus* complex (Koziaik et al. 2007b), those authors chose not to address the taxonomic questions raised, and the name *N. robustus* was treated as a synonym of *H. atrocoerulea* by Thorn (2013).

Here we describe four new species based on collections from Canada, USA and Costa Rica, based on morphological distinctions and phylogenetic analyses of the nuclear ribosomal DNA internal transcribed spacer region (ITS) and the D1/D2 variable region of the large subunit gene. In addition, we discuss the differentiation of and correct names for several other species in this complex, and report that *Agaricus niger* has been misinterpreted by Thorn & Barron (1986) and others; it is a species of *Resupinatus* and does not produce conidia in culture formerly referable to as a *Nematoctonus robustus* anamorph.

MATERIALS AND METHODS

Macroscopic descriptions, from fresh material except where noted, follow Largent (1986) and Vellinga (1988), with colour codes from Kornerup & Wanscher (1978). Microscopic structures in both fresh and dried materials were examined in different mountants: water, L4 (7.2 g KOH, 160 mL glycerine, 840 mL dH₂O, 7.6 g NaCl and 5 mL Ciba-Geigy Invadin, Clémentçon 1972), Melzer's reagent, and ammoniacal Congo red (Kirk et al. 2008). Dried fragments were rehydrated in water and mounted in L4. All microscopic measurements were car-

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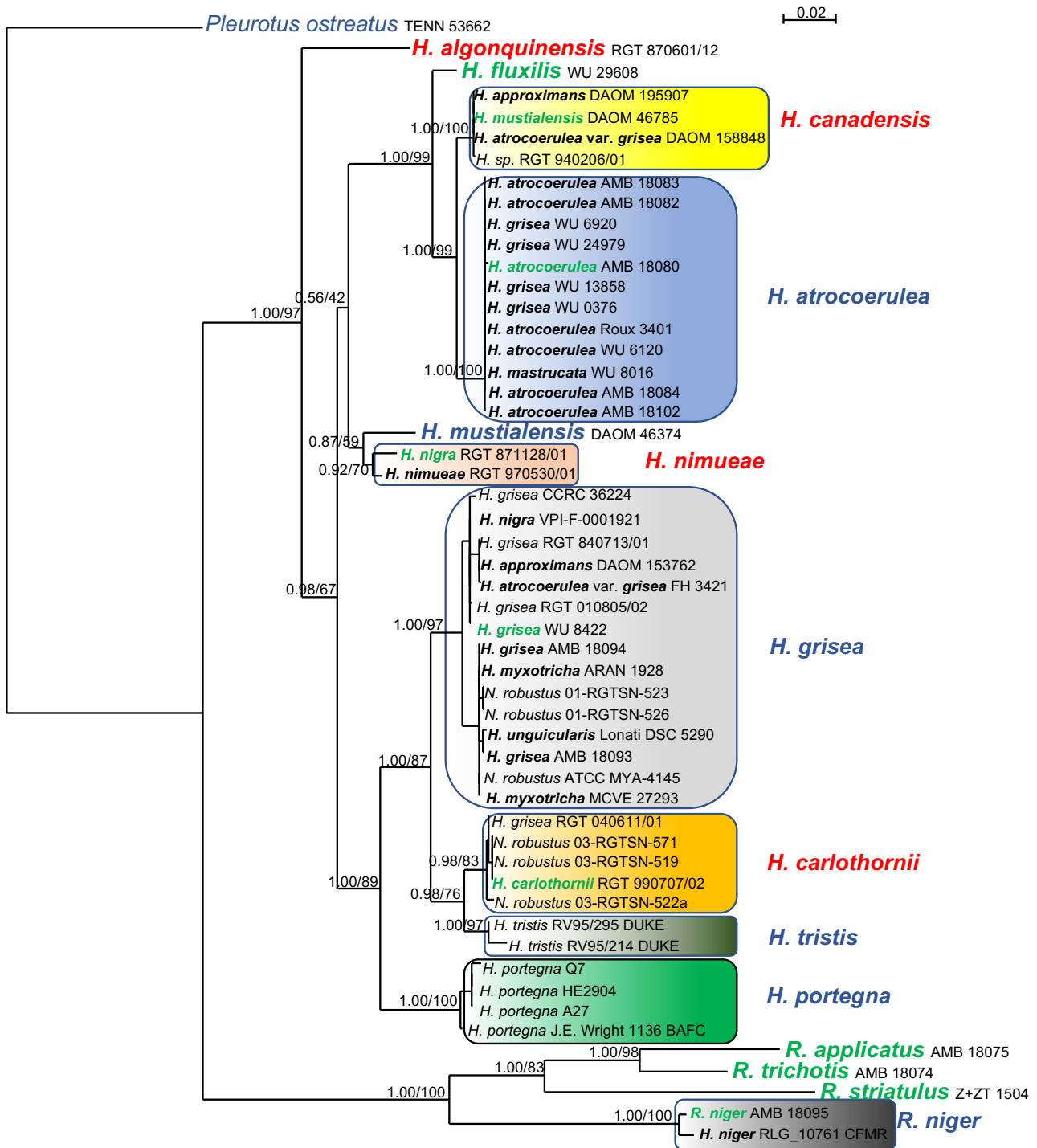


Fig. 1 Phylogeny of selected members of the *Hohenbuehelia* clade based on a Bayesian and Maximum Likelihood (RAxML) inference analysis of a supermatrix of three nuclear gene regions (nrITS, nrLSU, TEF1). Bayesian posterior probability values and Maximum Likelihood bootstrap values are shown on the branches. For each taxon voucher number and original determination are given. The relevant GenBank accession numbers are shown in Table 1. Newly sequenced collections are in **bold**, new species names are in red and other species names are in blue, and the original determinations of type collections are in green.

ried out under oil immersion at 1000× with a Zeiss Axioscope 40. Spore measurements were made by photographing all the spores (taken from the hymenophore of mature specimens) occurring in the visual field of the microscope using Mycomètre software (Fannechère 2011). Spore dimensions with exclusion of the hilar appendix are given as: average minus standard deviation – average plus standard deviation of length × average minus standard deviation – average plus standard deviation of width; Q = average minus standard deviation – average plus standard deviation of ratio length/width; Qm = average of ratio length/width; V_m = average of the volume (in μm³). The approximate spore volume was calculated as that of an ellipsoid (Gross 1972, Meerts 1999). Photomicrographs were taken with

a Canon PowerShot A640 digital camera. Herbarium acronyms follow Thiers (2017). Author citations follow Index Fungorum (<http://www.indexfungorum.org/authorsoffungalnames.htm>).

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). A portion of each sample was ground with the aid of a micropestle in 600 μL CTAB buffer (hexadecyltrimethylammonium bromide 2 %, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform : isoamyl alcohol (24 : 1) was added carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13000 g, and the DNA in the supernatant was precipitated with an equal volume of

isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold 70 % ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL double distilled H₂O. PCR amplification was performed with the primers ITS1F and ITS4 for the ITS region (Gardes & Bruns 1993, White et al. 1990); primers LR0R and LR5 for amplify the 5' end of the LSU rDNA region (Vilgalys & Hester 1990), and primers EF1-983F and EF1-1567R for a portion of the TEF1 gene (Rehner & Buckley 2005). PCR reactions were performed using a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s, respectively) and a final 72 °C step 10 min. PCR products

were checked in 1 % agarose gels, and positive reactions were sequenced with primers ITS1F, ITS4, LR0R/LR5 or EF1-983F/EF1-1567R. Chromatograms were checked searching for putative reading errors, and these were corrected. Sequences in Fig. 1 with accession numbers starting with KU or KY, except KY679141–KY679146, obtained in the lab of RGT following Koziak et al. (2007b), were obtained by Dr. Pablo Alvarado (Oviedo, Spain), following the methods reported in Alvarado et al. (2010, 2012). The sequences involved in the present study, including those downloaded from GenBank, were checked and assembled with MEGA 6.0 (Tamura et al. 2013), and aligned by MUSCLE. The GTR+G model was chosen for each alignment.

Table 1 Species, specimens and their GenBank accession numbers of ITS, nLSU and TEF1 sequences used in this study.

Species	Specimen/culture	Locality	GenBank Accession Numbers		
			ITS	nLSU	TEF1
<i>Hohenbuehelia algonquinensis</i>	RGT 870601/12 UWO (culture T-434)	Canada: ON	KU355341	AF139950-AF139951	KU355456
<i>Hohenbuehelia atrocoerulea</i>	AMB 18080	Hungary	KU355304	KU355389	KU355439
	AMB 18083	Italy	KU355303	–	KU355438
	AMB 18084	Italy	KU355301	KU355388	KU355436
	AMB 18082	Italy	KU355302	–	KU355437
	AMB 18102	Hungary	KY698000	KY698001	–
	Roux 3401	France	KU355305	–	KU355440
	WU 0376, as <i>H. grisea</i>	Austria	KU355306	–	–
	WU 13858, as <i>H. grisea</i>	Austria	KU355310	–	–
	WU 24979, as <i>H. grisea</i>	Austria	KU355311	–	–
	WU 6120	Austria	KU355307	–	KU355441
	WU 6920, as <i>H. grisea</i>	Austria	KU355308	–	–
	WU 8016, as <i>H. mastrucata</i>	Austria	KU355309	KU355390	KU355442
<i>Hohenbuehelia canadensis</i>	DAOM 158848, as <i>H. atrocoerulea</i> var. <i>grisea</i>	Canada: ON	KU355356	–	–
	DAOM 195907, as <i>H. approximans</i>	Canada: ON	KY124255	–	–
	DAOM 46785, as <i>H. mustialensis</i> (holotype)	Canada: AB	KY124253	–	–
	RGT 940206/01 UWO, as <i>Hohenbuehelia</i> sp. (culture UAMH 5317)	Canada: AB	AF139960	AF139960	–
<i>Hohenbuehelia carlothornii</i>	RGT 990707/02 AMB 18106	Costa Rica	KY698012	KY698013	–
	03-RGTSN-519 UWO, as <i>Nematoctonus robustus</i>	Costa Rica	EF409757	EF409757	–
	03-RGTSN-522a UWO, as <i>N. robustus</i>	Costa Rica	EF409758	EF409758	–
	03-RGTSN-571 UWO, as <i>N. robustus</i>	Costa Rica	EF409759	EF409759	–
	RGT 040611/01 INBio (culture UWO), as <i>H. grisea</i>	Costa Rica	EF409756	EF409756	–
<i>Hohenbuehelia fluxilis</i>	WU 29608	Austria	KU355326	–	–
<i>Hohenbuehelia grisea</i>	01-RGTSN-523 UWO, as <i>N. robustus</i>	Costa Rica	EF409761	EF409761	–
	01-RGTSN-526 UWO, as <i>N. robustus</i>	Costa Rica	EF409762	EF409762	–
	AMB 18093	Italy	KY124249	–	–
	AMB 18094	Italy	KY124251	–	–
	ARAN 1928, as <i>H. myxotricha</i>	Spain	KU355327	–	–
	ATCC MYA-4145, as <i>N. robustus</i>	USA: CA	EF546657	EF546657	–
	CCRC 36224	Republic of Korea	AY265835	–	–
	DAOM 153762, as <i>H. approximans</i>	Canada: ON	KY124254	–	–
	FH 3421, as <i>H. atrocoerulea</i> var. <i>grisea</i>	USA: IN	KU355328	–	KU355446
	Lonati DSC 5290, as <i>H. unguicularis</i>	Italy	KY124250	–	–
	MCVE 27293, as <i>H. myxotricha</i>	Italy	KU355329	KU355394	KU355447
	RGT 010805/02 UWO	Costa Rica	EF409760	EF409760	–
	RGT 840713/01 DAOM	Canada: ON	AF139952	AF139952	–
	VPI-F-0001921 (culture VT 1324 = T-132), as <i>H. nigra</i>	USA: VA	KY679143	KY679143	–
WU 8422	Austria	KU355330	–	–	
<i>Hohenbuehelia mustialensis</i>	DAOM 46374	Canada: AB	KY124252	–	–
<i>Hohenbuehelia nimueae</i>	RGT 871128/01 UWO (culture T-489 = CBS 212.91), as <i>H. nigra</i>	Canada: ON	KY679144	KY679144	–
	RGT 970530/01 UWO	USA: WY	KY679145	KY679145	–
<i>Hohenbuehelia portegna</i>	A27	China?	KC414247	–	–
	HE2904	China?	KC505559	–	–
	Q7	China?	KC414261	–	–
	J.E. Wright 1136 BAFC	Argentina	AF139959	AF139959	–
<i>Hohenbuehelia tristis</i>	RV95/214 DUKE	Australia	–	AF042601	–
	RV95/295 DUKE	Australia	–	AF135171	–
<i>Pleurotus ostreatus</i>	TENN 53662 (= AFTOL-ID 564)	Austria	AY854077	AY645052	AY883432
<i>Resupinatus applicatus</i>	AMB 18075	Italy	KU355368	KU355411	–
<i>Resupinatus niger</i>	AMB 18095	Italy	KU355371	KU355413	–
	RLG 10761 CFMR (culture T-001 = VT 1520)	USA: AZ	KY679146	KY679146	–
<i>Resupinatus striatulus</i>	Z + ZT 1504	France	KU355374	–	–
<i>Resupinatus trichotis</i>	AMB 18074	Italy	KU355378	KU355416	–

The ITS, LSU and TEF alignments were performed separately. In the ITS alignment the 18S region was trimmed. A combined ITS/LSU/TEF1 dataset was constructed using *Pleurotus ostreatus* as outgroup. The combined data matrix (2 183 bp, 659 for ITS, 960 for LSU and 564 for TEF1) comprised 53 collections (including 18 from GenBank). Both Bayesian inference (BI, Ronquist et al. 2012) and maximum likelihood (ML, Stamatakis 2014) analyses produced the same topology; the consensus Bayesian inference+RAXML tree with both Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap (MLB) values is shown (Fig. 1).

RESULTS

Phylogeny

Sequences for 30 collections of *Hohenbuehelia* and 5 collections of *Resupinatus* were acquired in this study (Table 1). A phylogenetic analysis yielded strong support for the *Hohenbuehelia atrocoerulea* – *H. grisea* group, and ten species within it (Fig. 1). The *H. grisea* clade contains two subclades with low support, both ranging from temperate zones to the neotropics; because of this and the small degree of variation, all of these representatives are considered conspecific. Sister to *H. grisea* are the new species *H. carlothornii*, with all sequences from Costa Rica, and *H. tristis* from Australia. *Hohenbuehelia portegna*, with one representative from Argentina and three sequences submitted from China, is a sister to *H. carlothornii*, *H. grisea* and *H. tristis*. Remarkably, *H. atrocoerulea*, of which *H. grisea* has frequently been treated as a form (Pilát 1935) or variety (Peck 1891, Kauffman 1918, Coker 1944, Thorn & Barron 1986), appears on a separate branch as a well-supported clade, with representatives from Europe and China. Its sister species is the new species *H. canadensis*, with sequences from Alberta and Ontario, and the two are subtended by a single sequence of *H. fluxilis* from Austria, *H. algonquinensis* from Ontario, and a branch with a sequence of *H. mustialensis* from Alberta and two sequences of the new species *H. nimueae* from Ontario and Wyoming. Two sequences of the taxon generally known as *H. nigra* are placed with strong support in the sister genus *Resupinatus*.

Within several species clades are sequences identified by different names in the herbarium or in GenBank: e.g., *H. approximans*, *H. myxotricha*, *H. nigra* and *H. unguicularis* within the *H. grisea* clade; *H. grisea* and *H. mastrucata* within the *H. atrocoerulea* clade; and *H. approximans*, *H. atrocoerulea* var. *grisea*, *H. mustialensis*, and *Hohenbuehelia* sp. within the *H. canadensis* clade. This reflects both the difficulty of species distinction within this group and the differing species concepts, or applications of names to taxa recognized, over time. The latter difficulty has been at least partly resolved by designation of lectotypes, neotypes and epitypes of species in this group (Consiglio 2016, 2017a, b, c). This has allowed us to treat *H. grisea* and *H. atrocoerulea* as distinct species, with both *H. approximans* and *H. myxotricha* as synonyms of *H. grisea*, and, on the basis of phylogenetic and morphological studies presented here, to recognize and describe below four new species. Two of these are from Canada (*H. algonquinensis* and *H. canadensis*), one from Canada and the United States (*H. nimueae*) and one is from Costa Rica (*H. carlothornii*).

Taxonomy

Hohenbuehelia algonquinensis Consiglio, Setti & Thorn, sp. nov. — MycoBank MB820331; Fig. 2a–e

Etymology. *algonquinensis* (Latin), of Algonquin, from the collection locality.

Holotype. CANADA, Ontario, Algonquin Provincial Park, Booth's Rock Trail, N45.52° W78.39°, on *Pinus strobus* in woods with *Tsuga canadensis*, 1 June 1987, R.G. Thorn #870601/12 (UWO), ex-type culture T-434 (UWO).

Diagnosis: A species of *Hohenbuehelia* distinguished from others with dimidiate fruiting bodies by its small, glossy black pileus and contrasting white or off-white lamellae, its broad basidiospores, and by its diagnostic ITS and LSU sequence data, accession numbers KU355341, MG519563, MG529564.

Basidiomata unguulate to dimidiate, laterally attached without pseudostipe, 0.5–2.0 cm broad × 0.5–1.2 cm deep, glossy and nearly black to watery 7E2 to 7AB1 towards margin, with fine pure white frosting towards the base of young fruiting bodies; margin decurved to acute and wavy-applanate, not translucent-striate when fresh. *Lamellae* pure white to pale grey at first (7AB1), slightly off-white in age (3A1½), moderately close, multiseriate, very finely frosted under a lens from metuloid cheilo- and pleurocystidia. *Basidiospores* hyaline, inamyloid, smooth, ellipsoid to elongate-ellipsoid to oblong, at times slightly amygdaliform in side view, 7.5–8.8 × 4.7–5.6 µm (on average 8.1 × 5.2) (n = 32), Q = 1.45–1.70 (on average 1.58), V_m = 116 µm³, with dull, granular content or with one or more oily drops. *Basidia* 4-spored, clavate, 23–27 × 7.3–7.8 µm; sterigmata up to 3 µm long. *Hymenophoral trama* irregular, made up of gelatinized hyphae up to 10 µm wide, hyaline in L4, yellow in Melzer's. *Cheilometuloids* isolated or in groups along the lamellar edge, subfusoid, generally with a narrow base, mostly with a lanceolate apical part and partly covered with refringent, yellow or whitish, crystalline granules, 34–42 × 6.8–8.7 µm. Mounted in Melzer's they react pale reddish (dextrinoid reaction), while in cresyl blue the wall stains vinaceous red (metachromatic reaction). *Cheilocystidia* (leptocystidia) of the gloeosphex type (Thorn & Barron 1986), 16–20 × 5.3–7.8 µm, in most cases with one or two swellings protruding from the apical portion, with a neck and capitulum of 5.8–8.7 × 1.5–2.4 µm, often capped with a tiny, vanishing drop of mucus to 8 µm diam. *Pleurometuloids* similar to cheilometuloids, 43–52 × 6.8–9.7 µm. *Pileipellis* consisting of an ixocutis of variously intertwined, gelatinized, filamentous, smooth hyphae, 2.5–5.0 µm wide, yellow in Melzer's, with a dark brown, encrusting epiparietal pigment; towards the point of attachment a trichoderm of hairs up to 12 µm wide, often tufted. *Gelatinous zone* c. 250 µm thick, hyphae 2–6 µm wide. *Pileitrama* marked by a narrow layer of cylindrical, horizontal to subparallel hyphae, with both brown intracellular and encrusting pigment, then pale below, 450–1000 µm thick, made up of hyphae up to 12 µm wide and with walls up to 1 µm thick. *Clamp connections* ubiquitous.

Cultural characteristics — In culture on water agar with nematodes. *Vegetative hyphae* (1.3–)1.7–2.8 µm diam, thin-walled, consistently clamped. *Assimilative hyphae* (in nematodes) (1.2–)1.5–3.0(–3.5) µm diam, thin-walled, clamped. *Predatory adhesive knobs* both intercalary (on short side-branches) and terminal, the adhesive mucoid balls 7–10 × 5.3–7.2 µm, and the hour-glass secretory cells 6.5–8.1 × 2.5–3.0 µm. *Conidia*, produced from slender, tapering denticles from hyphae or clamp connections, (9.3–)10.6–13.8(–14.1) × (2.7–)3.3–4.1(–4.8) µm, cylindrical or distally tapering, straight or curved. *Mating type* heterothallic, bipolar (unifactorial).

Habitat — Singly or occasionally in small imbricate clusters on fallen branches of *Pinus strobus* in mixed woods with *Tsuga canadensis*.

Additional specimens examined. None. Only the holotype collection is known at present. RGT has studied *Hohenbuehelia* collections in major herbaria covering the collecting area of this species (Thorn & Barron 1986) and GC and LS have extensively sampled European taxa, and no other representatives of this species were found. Although blue-black collections have been reported as *H. atrocaerulea* (e.g., Christiansen 1959, with basidiospores 8–10 × 4–5 µm from 4-spored basidia), the micromorphology of each

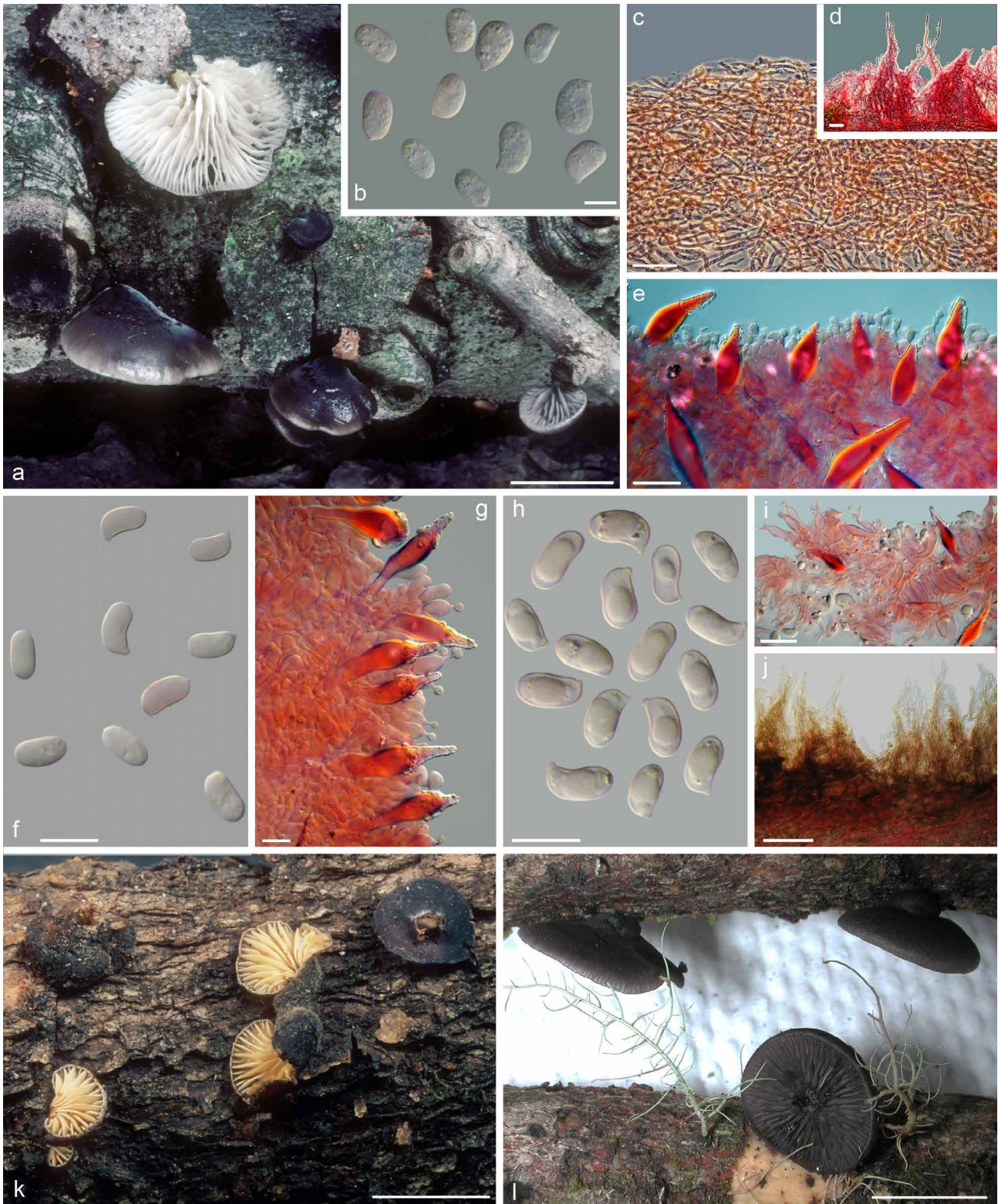


Fig. 2 Macro- and micromorphology of *Hohenbuehelia* species. — a–e. *Hohenbuehelia algonquinensis* RGT 870601/12 (holotype). a. Fruiting bodies, on *Pinus strobus* in Algonquin Park, Ontario; b. basidiospores; c. pileipellis; d. tufts of pileipellis hairs; e. cheilocystidia and cheilometuloids. — f–g. *Hohenbuehelia canadensis*. f. Basidiospores, DAOM 46785 (holotype); g. cystidia from the gill edge, including lanceolate, thick-walled cheilometuloids with apical incrustation and lecythiform, thin-walled cheilogloeosporic cystidia with apical capitulum surrounded by mucoid droplet, DAOM 195907. — h–k. *Hohenbuehelia nimueae*. h. Basidiospores of RGT 871128/01; i. cheilocystidia and cheilometuloids of RGT 871128/01; j. vertical section of pileipellis of RGT 871128/01; k. fruiting bodies of RGT 970530/01 on *Abies lasiocarpa* in Wyoming. — l. *Resopinatus niger*, fruiting bodies of RGT 010805/01, on unidentified hardwood, San Gerardo de Dota, Costa Rica. — Scale bars: a, k–l = 1 cm; b = 5 μ m; c–e, i = 20 μ m; f–h = 20 μ m; j = 50 μ m.

is different from this species. The very broad basidiospores are distinctive among dark, dimidiate species.

Hohenbuehelia canadensis Consiglio, Setti & Thorn, *sp. nov.* — MycoBank MB820332; Fig. 2f–g

Etymology. *canadensis* (Latin), of Canada, from the broad range of collection localities.

Holotype. CANADA, Alberta, Strachan, (approx.) N52.26° W115.14°, on *Pinus contorta* slash, 21 Oct. 1954, V.J. Nordin DAOM 46785 (= DAVFP 1224; as *Pleurotus atrocaeruleus* var. *griseus*, det. S.C. Hoare; cited as *Hohenbuehelia mustaliensis* [sic] by Thorn & Barron 1986).

Diagnosis: A species of *Hohenbuehelia* distinguished from others with dimidiate fruiting bodies by its small blackish fruiting bodies, less than 2 cm broad, cream to pale greyish yellow lamellae, and diagnostic ITS sequence, accession KY124253.

Description from dried herbarium specimens; no field collections seen fresh. *Basidiomata* unguulate to dimidiate, laterally attached by a broad pseudostipe, 0.5–1.5 cm broad × 0.5–1.2 cm deep, black, glabrous, but frosted with grey tomentum towards the base; margin decurved, very finely frosted or nearly glabrous. *Lamellae* cream to pale greyish yellow (dry; 4A2–4B3), moderately close, multiseriate, very finely frosted under a lens from metuloid cheilo- and pleurocystidia. *Basidiospores* hyaline, inamyloid, smooth, long ellipsoid to cylindrical, at times slightly amygdaliform, 6.9–8 × 3.6–4.2 µm (on average 7.5 × 3.9 µm) ($n = 96$); $Q = 1.76–2.06$ (on average 1.91), $V_m = 61 \mu\text{m}^3$, with dull, granular content or with one or more oil drops. *Basidia* 4-spored, clavate, 23–24 × 6.4–7.5 µm; sterigmata up to 5 µm long. *Hymenophoral trama* irregular, composed of gelatinized hyphae up to 7 µm wide, hyaline in L4, yellow in Melzer's. *Cheilometuloids* isolated or in groups along the lamellar edge, subfusoid, generally with a narrow base, dextrinoid and metachromatic, 34–38 × 9.1–11 µm, mostly with a lanceolate apical part and partly covered with refringent, whitish, crystalline granules. *Cheilocystidia* (gloeosphex) clavate, 13–19 × 5.6–7.1 µm, in most cases with one or two swellings protruding from the apical portion, with a neck and capitulum of 4.8–5.7 × 2.0–4.1 µm, often capped with a tiny, vanishing drop of mucus to 8 µm diam. *Pleurometuloids* similar to cheilometuloids, 57–62 × 10–12 µm. *Pileipellis* a cutis of variously intertwined, slightly gelatinized, filamentous hyphae, 3–8.5 µm wide, with brown encrusting pigment; in parts overlain by a trichoderm of hyaline, smooth, often tufted hairs up to 7 µm wide. *Gelatinous zone* 300–1000 µm thick, consisting of gelatinized hyphae, 2–5 µm wide, with an encrusting epiparietal pigment in the area close to the pileitrama. *Pileitrama* marked by a narrow layer of cylindrical, horizontal to subparallel hyphae with a brown intracellular pigment; below this a hyaline zone 50–250 µm deep, made up of hyphae up to 8 µm wide and with walls up to 1 µm thick. *Clamp connections* ubiquitous. *Mating type* unknown.

Habitat — Singly or occasionally in small imbricate clusters on branches of *Pinus contorta*, or unidentified twigs.

Additional specimens examined. CANADA, Alberta, Edson, (approx.) N53.56° W116.58°, isolated as a contaminant from basidiocarp of ?*Cortinarius* in *Pinus contorta* woods, 24 Aug. 1985, RGT 940206/01 (fruiting in culture, culture number T-454 = UAMH 5317; dup. at CFMR); Ontario, Mallorytown Landing, St. Lawrence Is. National Park, (approx.) N44.45° W75.86°, 22 June 1976, A. Carter (DAOM 158848, as *H. approximans*); Mazinaw Lake, Ontario, (approx.) N44.90° W77.20°, on ?*Populus* twig on ground, 31 July 1986, S.A. Redhead (DAOM 195907, as *H. approximans*).

Notes — Collections of this species have been misidentified as *H. approximans* or *H. atrocaerulea* var. *grisea* (Thorn & Barron 1986; both now *H. grisea*) or *H. mustaliensis* (Thorn & Barron 1986), but differ from *H. grisea* in their small, dark fruiting bodies, usually ≤ 2 cm broad and occurrence on conifer wood, from *H. mustaliensis* by their pale lamellae (greyish brown to black

in *H. mustaliensis*), and from both in their phylogenetic position. A black and white photograph of fresh fruiting bodies of UAMH 5317, produced in culture, was published by Thorn et al. (2000: f. 4), along with illustrations of cultures on water agar with nematodes, producing predatory adhesive knobs as well as toxin droplets similar to those of *Pleurotus* (Barron & Thorn 1987), and cylindrical, usually straight conidia (Thorn et al. 2000; Fig. 1–3).

Hohenbuehelia nimueae Consiglio, Setti & Thorn, *sp. nov.* — MycoBank MB820333; Fig. 2h–k

Etymology. *nimueae* (Latin), of Nimue, the Lady of the Lake from King Arthur's tales, in reference to the collecting habitat at the boggy margin of a shallow lake near Arthur, Ontario.

Holotype. CANADA, Ontario, Luther Bog, near Arthur, N43.905° W80.405°, on *Salix* near stream through *Sphagnum* bog with *Larix* and *Chamaedaphne*, 28 Nov. 1987, R.G. Thorn, T.A. Dickinson, et al. RGT #871128/01 (UWO), ex-type culture, T-489 (UWO; CBS 212.91, as *Hohenbuehelia nigra*).

Diagnosis: A species of *Hohenbuehelia* distinguished from others with dimidiate fruiting bodies by its dark, greyish brown pileus, finely frosted (not tomentose, as in *H. canadensis*) towards the base, and pale cream to brownish grey lamellae, and its diagnostic ITS-LSU sequence, accession KY679144.

Basidiomata cupulate-pendent to dimidiate, laterally attached without pseudostipe, 1.5–2.0 cm broad × 0.7–0.8 cm deep, greyish brown (6F4, 6½F3), with fine white frosting tomentum towards the base; margin undulating, at first incurved, opaque. *Lamellae* radiating from point of attachment, moderately close, 2–3-seriate, margins entire, becoming frosted, pale cream at first (4½A2–5A2), becoming greyish orange to brownish grey (6B2–6F3). *Context* gelatinous, tough, black, to 1 mm thick. *Basidiospores* hyaline, inamyloid, smooth, ellipsoid to ellipsoid-phaseoliform in profile, 7.7–8.8 × 4.1–4.8 µm (on average 8.2 × 4.4), $Q = 1.73–2.0$ (on average 1.86), with dull granular content or one or more oily drops. *Basidia* 4-spored, clavate, 21–26 × 6.8–7.8 µm. *Hymenophoral trama* irregular, gelatinized, hyaline; hyphae 2.5–5.2 µm diam. *Cheilometuloids* isolated or in groups along the lamellar edge, lanceolate, dextrinoid and metachromatic, partly covered with refringent, yellow or whitish, crystalline granules, 27–37 × 6.8–9.7 µm. *Cheilocystidia* (gloeosphex) clavate-leythiform, the base 13–17 × 4.8–6.3 µm, with one or two hour-glass secretory processes 4.4–5.0 × 1.9–2.4 µm thinly surrounded by hyaline mucus to 8 µm diam. *Pleurometuloids* mostly or entirely submerged in hymenium, clavate to lanceolate, 31–51(–66) × 7.8–8.7 µm, with hyaline apices but trilayered below with darkened median layer. *Pileipellis* a thin ixocutis, approximately 10–12 µm deep, of filamentous hyphae, 2–4 µm wide, with a brown, spiral-encrusting pigment, with hairs 4.6–7.7 µm broad with walls 1.2–2.5 µm thick, singly or in fascicles 8–12 µm thick, up to 100 µm tall. *Gelatinous zone* c. 210–440 µm thick as revived in 2% KOH; hyphae 1.5–3.2(–4.5) µm wide. *Pileitrama* a compact layer of cylindrical, horizontal brown hyphae 3–5 µm wide. *Clamp connections* ubiquitous.

Cultural characteristics — In culture on water agar with nematodes. *Vegetative hyphae* 2–3 µm diam, thin-walled, consistently clamped. *Predatory adhesive knobs* intercalary (on short side-branches), the adhesive mucoid balls 7–8 × 6.0–6.5 µm, and the hour-glass secretory cells 6.0–7.5 × 2.0–2.6 µm. *Conidia*, produced from slender, tapering denticles from hyphae or clamp connections, 12–14(–15) × 3.3–3.8 µm, $Q = 3.3–4.0$ (on average 3.6), cylindrical, curved, representing 90–180° of an arc. *Mating type* unknown.

Habitat — Fruiting in a small group on branches of dead, standing *Salix*; a second collection on *Abies lasiocarpa*.

Additional specimens examined. USA, Wyoming, Upper South Lodgepole Creek, below Tie City parking lot, elev. 2578 m, N41.25° W105.43°, on twigs and bark of fallen *Abies lasiocarpa* in woods with *Picea engelmannii*, 30 May 1997, R.G. Thorn & L.J. Hutchison, RGT #970530/01 (UWO; culture = T-971).

Notes — This species was initially misidentified by RGT as *H. nigra* sensu Thorn & Barron (1986), in which the descriptions included teleomorphic elements from the true *Resupinatus niger* (see below) and anamorphic elements from a culture derived from a collection identified by O.K. Miller Jr. as *Hohenbuehelia niger* that has proven to be *H. grisea* (KY679143). It resembles a small *H. grisea* or *H. canadensis* but differs in having lamellae becoming greyish orange to dark brownish grey and in its phylogenetic position, as sister to *H. mustialensis*. *Hohenbuehelia algonquinensis* is very similar but has broader basidiospores and is phylogenetically distant.

Hohenbuehelia robusta (F.R. Jones) Consiglio, Setti & Thorn, *comb. nov.* — Mycobank MB820334

Basionym (anamorph). *Nematoctonus robustus* F.R. Jones, Trans. Brit. Mycol. Soc. 47 (1): 57. 1964.

Holotype. IMI 102541 (Royal Botanic Gardens, Kew), cultured Dec. 1958 from leaf litter collected near Kumasi, Ghana (Jones 1964).

Notes — Because of its geographic origin, we consider it prudent to recognize this species as distinct from those north-temperate species with which it has been considered

synonymous (e.g., *H. atrocoerulea*, *H. grisea*) (Thorn & Barron 1986, Thorn 2013) and from the neotropical species described below as *H. carlothornii*. Despite the fact that the teleomorph of this species is unknown, the current Code (McNeill et al. 2012) dictates that the correct name for this species is in the teleomorphic genus (which has priority), together with all morphologically similar anamorphs for which molecular data are available. However, collections named *H. atrocoerulea* and *H. aurantiocystis* have been described from Kenya, Uganda and Tanzania (Pegler 1977), and these and other *Hohenbuehelia* collections from West Africa deserve molecular study. Unfortunately, molecular data on the holotype from Ghana are lacking, and no living strains are known.

Hohenbuehelia carlothornii Consiglio, Setti & Thorn, *sp. nov.*
— Mycobank MB820335; Fig. 3a–e

Etymology. *carlothornii* (Latin), in appreciation of Carlo Thorn, for his enthusiastic assistance – at three years of age – in collecting soils and other samples, including the paratype specimen (Fig. 3a–b), for a survey of the nematode-destroying fungi of Costa Rica.

Holotype. COSTA RICA, Puntarenas Province, Wilson Botanical Garden near San Vito, West Bank Trail at top of stairs past Rio Jaba, approx. N8.788° W82.964°, on dead log and adjacent fallen branches in wet primary Premontane Rain Forest (Holdridge 1971) at approx. 1080 m, 7 July 1999, R.G. Thorn #990707/02 (UWO, isotype AMB n. 18106), ex-type culture at UWO and INBio.

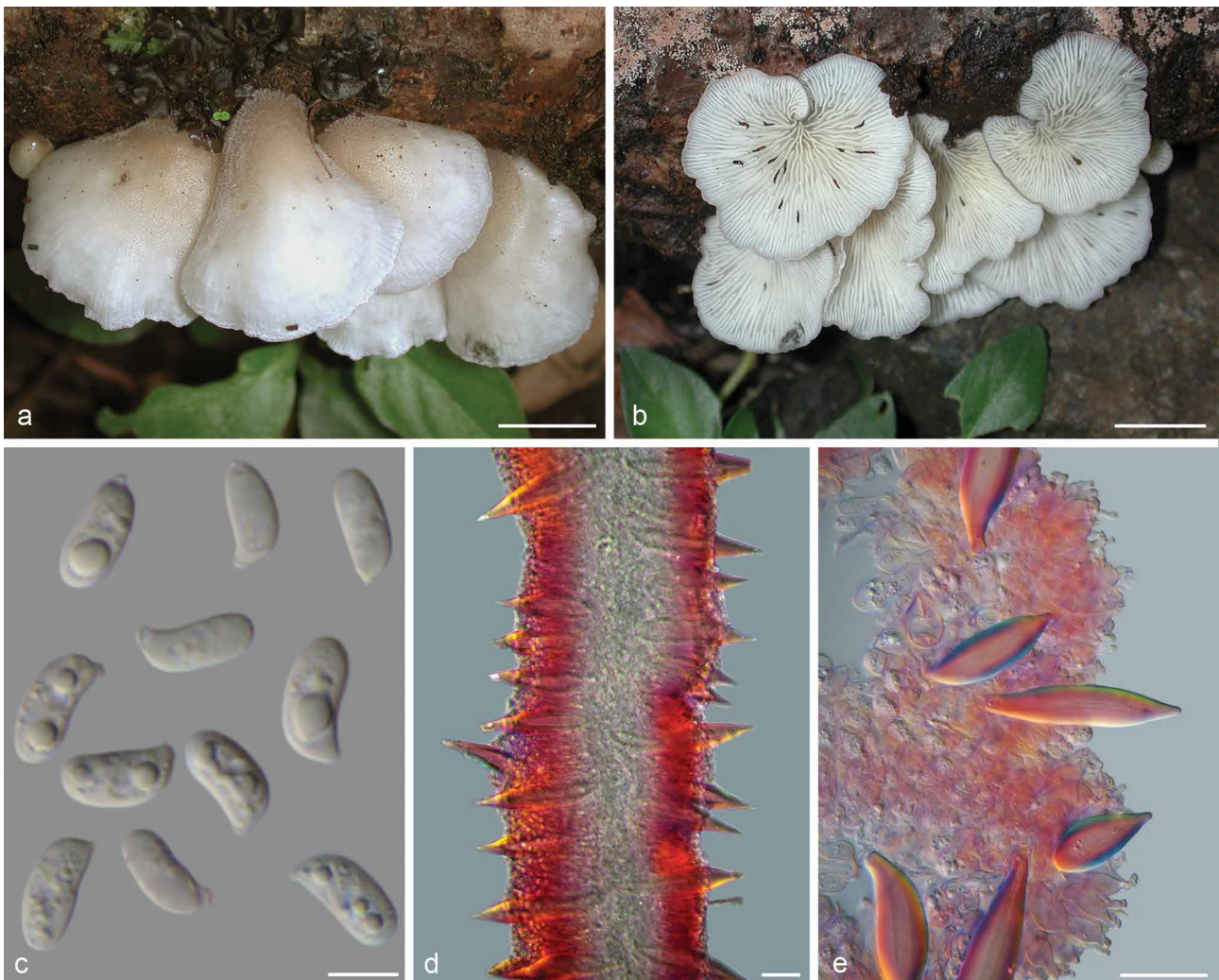


Fig. 3 a–e. *Hohenbuehelia carlothornii*. a–b. Under- and upper-side of fruiting bodies of RGT 040611/01 (paratype, INBio); c–e microscopic features of RGT 990707/02 (holotype, UWO); c. basidiospores; d. vertical section of lamella showing origin of metuloid pseudocystidia in lamellar trama; e. cheilocystidia and cheilometuloids. — Scale bars: a–b = 1 cm; c = 5 µm; d–e = 20 µm.

Diagnosis: A species of *Hohenbuehelia* with dimidiate fruiting bodies, distinguished from similar species by its glabrous, lubricous pileus, pallid colour, and its diagnostic ITS-LSU sequences, KY698012 and KY698013.

Basidiomata gregarious to imbricate, dimidiate to orbicular, laterally or dorsally attached without stipe or pseudostipe, 2.0–3.2 cm wide × 1.4–2.5 cm deep; margins acute, plane, very faintly translucent-striate; upper surface appearing moist, shiny, off-white (4½A1½), finely white-tomentose towards base. *Lamellae* radiating from point of attachment, narrow, close, white to off-white (4AB1½). *Basidiospores* hyaline, inamyloid, smooth, cylindrical in front view, allantoid in side view, 7–8.2 × 3.1–3.7 µm (on average 7.6 × 3.4 µm) (n = 32), Q = 2.08–2.42 (on average 2.25), V_m = 47 µm³, with dull, granular content or with one or more oil drops. *Basidia* 4-spored or sometimes 2-spored, clavate to subcylindrical, 19–24 × 5.2–6.8 µm; sterigmata up to 7 µm long. *Hymenophoral trama* irregular, made up of gelatinized hyphae up to 9.5 µm wide, hyaline in L4, yellow in Melzer's. *Cheilometuloids* isolated or in groups along the lamellar edge, subfusoid, dextrinoid and metachromatic, 30–46 × 11–14 µm, generally with a narrow base, mostly with a lanceolate apical part and partly covered with refringent, yellow or whitish, crystalline granules. *Cheilocystidia* (gloeosphex) clavate, 13–17 × 4.9–9.4 µm, in most cases with one or two swellings protruding from the apical portion, with a neck and capitulum of 3.9–7.8 × 1.9–2.1 µm, rarely capped with a tiny, vanishing drop of mucus. *Pleurometuloids* similar to cheilometuloids, 55–63 × 14–18 µm. *Pileipellis* an ixocutis of variously intertwined, slightly gelatinized, filamentous, smooth hyphae, 3–9.5 µm wide, yellow in Melzer's, with a light brown intracellular pigment, with undifferentiated terminal elements. *Gelatinous zone* c. 400 µm thick, hyphae 1.5–4.5 µm wide. *Pileus trama* 50–450 µm deep, made up of hyphae up to 9 µm wide and with walls up to 1 µm thick. *Clamp connections* ubiquitous.

Cultural characteristics — On water agar with nematodes, with assimilative and fertile hyphae hyaline, clamped, 1.6–2.4 µm diam; fertile hyphae producing predominantly terminal predatory adhesive knobs, and conidia holoblastically from tapering denticles up to 2.5 µm tall; adhesive knobs occasionally intercalary from short cylindrical branches, hour-glass secretory processes 3.2–9.6 µm long, base 1.6–3.2 µm wide, tip 0.8–3.2 µm wide, surrounded by a mucoid drop 4–7.2 × 2.4–5.6 µm; conidia cylindrical to allantoid, aseptate, (5.6–)7.2–13.6(–15.6) × (1.6–)2.4–4.0(–6.4) µm, Q = (2.1–)2.4–3.5(–4.0); conidia germinating with a tapering germ tube 5–25 µm long, with an hour-glass secretory process 3.2–5.6 µm long by 1.6–3.2 µm, surrounded by a subglobose mucoid droplet 3–8 × 3–6.5 µm. Previously described and illustrated by Koziak et al. (2007a) as *Nematoctonus robustus* (in part; their f. 15–17), who noted that isolates that proved to be this species were unusual in producing conidia that germinated on water agar with nematodes, a feature not seen in other isolates nor previously described for *N. robustus*. *Mating system* unknown.

Habitat — Isolated from soil and litter and fruiting on dead angiosperm wood in Tropical Wet Forest and Premontane Rain Forest in Costa Rica.

Additional specimen examined (paratype). COSTA RICA, Cartago Province, Guayabo National Monument, N9.97° W83.69°, on fallen branches in Premontane Rain Forest at 1130 m, 11 June 2004, R.G. Thorn, C.S. Thorn & A.T. Koziak, RGT #040611/01 (INBio; ITS-LSU sequence EF409756).

Conspecific cultures (paratypes). COSTA RICA, Puntarenas Prov., Area de Conservación Osa, Parque Nacional Corcovado, Sirena Biological Station, in Tropical Wet Forest at 5–100 m, N8.48° W83.60°, cultured from infected nematodes recovered from soil and litter collected 5–7 Nov. 2003, R.G. Thorn, 03-RGTSN-519, 03-RGTSN-552a and 03-RGTSN-571 (UWO, INBio; Koziak et al. 2007a).

Notes — This species corresponds to the subgroup of *Hohenbuehelia grisea* – *Nematoctonus robustus* noted by Koziak et al. (2007b) with ITS1 of 251 bases and ITS2 of 212 bases, whereas the subgroup with ITS1 of 249 bases and ITS2 of 214 bases corresponds to *H. grisea* s.str. The anamorph of this species is morphologically indistinguishable from the anamorphs of *H. atrocoerulea*, *H. grisea* (including its synonym *H. approximans*), *H. cyphelliformis* (Thorn & Barron 1986), as well as *H. algonquinensis*. However, molecular data (Koziak et al. 2007b; this study) point to a well-defined clade of neotropical isolates, which we describe here as *H. carlothornii*, that is separate from each of these species.

Resupinatus niger (Schwein.: Fr.) Murrill, N. Amer. Fl. (New York) 9 (4): 242. 1915. — Fig. 21

Basionym. *Agaricus niger* Schwein., Schr. Naturf. Ges. Leipzig 1: 90. 1822.

= *Agaricus niger* Schwein.: Fr., Elench. Fung. 1: 26 (1828).

= *Pleurotus niger* (Schwein.: Fr.) Sacc., Syll. Fung. (Abellini) 5: 380 (1887).

= *Hohenbuehelia nigra* (Schwein.: Fr.) Singer, Lilloa 22: 256 (1951).

= *Hohenbuehelia latialis* Angeli & Contu, Micol. Veg. Medit. 22 (2): 120 (2008).

Misapplication: *Hohenbuehelia horakii* Courtec. *sensu* P. Roux, Mille et un champignons: 312 (2006); Eyssartier & Roux (2011: 962).

Basidiomata cupulate and dorsally attached to unguulate with dorsolateral pseudostipe, 0.6–1.5 cm broad × 0.5–1.2 cm deep, coal-black to paler (to 8F3) and somewhat translucent-striate towards margin, surface matte and glabrous to appressed fibrillose towards attachment; margin acute, not incurved. *Lamellae* radiating from point of attachment, blunt, thick, moderately close, multiseriate, grey brown (9E2½) with blackish margins. *Basidiospores* hyaline, inamyloid, ellipsoid to elongate-ellipsoid or phaseoliform, smooth, 6.2–7.6 × 3.7–4.5 µm (on average 6.9 × 4.1 µm); Q = 1.49–1.89 (on average 1.69); V_m = 63 µm³. *Basidia* 23–36 × 6.0–8.0 µm, 4-spored, clavate, with sterigmata up to 5 µm long. *Hymenophoral trama* hyaline, subregular to irregular, composed of hyphae dispersed in a gel matrix, up to 8 µm wide; subhymenium with intracellular, brownish yellow pigment. *Cheilocystidia* (leptocystidia) cylindrical to clavate, 24–30 × 7.8–8.8 µm, with one or more apical prongs 6.5–9 µm tall tipped by a tiny ball 2.4–3.6 µm diam. *Cheilometuloids* isolated or in clusters along the lamellar edge, subfusoid with narrow base, mostly apically lanceolate and partly capped with whitish, crystalline granules, with brownish cytoplasm, 28–45 × 7.4–10 µm. *Pleurometuloids* similar, 46–99 × 9.7–15 µm. *Pileipellis* a brownish cutis of interwoven hyphae, 2.5–6 µm wide, smooth or with brown encrusting pigment. *Gelatinous zone* up to 750 µm thick, with ascendant, hyaline hyphae 1.5–3.0 µm broad. Parallel orientation, a yellowish brown intracellular pigment and a brown epiparietal pigment. *Pileus trama* of horizontal, cylindrical, brownish hyphae, 1.3–3.5(–8) µm wide, is up to 550 µm thick. *Clamp connections* ubiquitous.

Cultural characteristics — RLG 10761 is non-nematophagous in culture on water agar with nematodes, and produces only clamped cylindrical vegetative hyphae and no form of conidia (Thorn & Barron 1984, 1986). Attribution of a conidial state formerly known as *Nematoctonus robustus* (Thorn & Barron 1986) was based on a culture (VT 1324) that proved to be *Hohenbuehelia grisea*.

Specimens examined. CANADA, Yukon Territory, Dawson, (approx.) N64.06° W139.43°, on *Alnus sinuata*, 6 July 1961, J. Holms, DAOM 89048 (= DAVP 12920) and DAOM 89049 (= DAVP 12924, det. J.W. Groves as *Pleurotus atrocaeruleus*). — COSTA RICA, San Jose Province, San Gerardo de Dota, along Río Savegre, 200 m above Las Cataratas, (approx.) N9.546° W83.81°, on unidentified hardwood branches, 5 Aug. 2001, R.G. Thorn 010805/01 (UWO). — FRANCE, Côte d'Azur, Port-Cros, on dead, rotten

hardwood (probably *Quercus ilex*), 28 Oct. 2001, M. Meyer & P. Hervé, Roux 3740 (det. P. Roux as *Hohenbuehelia horakii* Courtec.). – ITALY, Lazio, Castelporziano Presidential Estate, on rotten wood of *Quercus ilex*, 7 Nov. 2007, P. Angeli et al., MCVE n. 10781 (as *H. latialis*, holotype) and Galli (*H. latialis*, isotype) (MCVE); Lazio, Acquachiarra (Viterbo), 27 Oct. 2009, Gelsomini, AMB 18095 (neotype of *Agaricus niger*); Calcata (Viterbo), 11 Jan. 2013, Gelsomini, AMB 18097. – NORWAY, Nordland, Nordrana par., St. Alteren, rich *Alnus-Ulmus* wood, on decayed *Ulmus* wood, 10 Sept. 1976, N. Lundqvist, UPS n. F-131691 (as *H. reniformis*). – USA, Arizona, Turkey Creek, Chiricahua Mountains, Coronado National Forest, (approx.) N31.86° W109.34°, on *Quercus hypoleucoides*, 7 Sept. 1972, R.L. Gilbertson, RLG 10761 (CFMR, as *Resupinatus applicatus*; det. O.K. Miller Jr. as *Hohenbuehelia niger* [sic]); North Carolina, Salem, Syn.# 162 (possible holotype of *Agaricus niger*, PH).

Notes — The packet containing the Schweinitz specimen in PH is labelled '*Agaricus niger* L. v. Schw., Epic. 527, Salem, Syn. # 162'. This packet was created after the Schweinitz herbarium was bequeathed to the Philadelphia Academy of Science upon Schweinitz's death in 1834. The numbered references are to the species number in the *Epicrisis Systematis Mycologici* (Fries 1838) and in 'Synopsis Fungorum in America Boreali Media Degentium' (Schweinitz 1834) and are thus not helpful in determining the status of the specimen in the packet. However, the collecting locality 'Salem' provides a clue that the specimen predates the move by Schweinitz from Salem (now Old Salem), North Carolina to Bethlehem, Pennsylvania in 1821, shortly before the species *Agaricus niger* was published (Schweinitz 1822), based on his collections made prior to 1817 (Shear & Stevens 1917). Coker (1944) studied this specimen and one in the Michener collection at BPI and regarded them as 'authentic' but did not comment on their status as types. Albertó et al. (1998) cited these as holotype and isotype, respectively. However, given the uncertain status of the holotype, Consiglio (2017b) designated a modern specimen (AMB 18095) as neotype to better secure the usage of the name; if it can be proven that any Schweinitz collections (in PH, or perhaps FH, K or UPS) are truly original, these should be designated as holotype, isotype, lectotype or syntype, as appropriate, and the neotype designated as an epitype. The species is very distinctive and, despite its metuloid cystidia, is reminiscent of *Resupinatus applicatus*, as was pointed out by Berkeley & Curtis (1856) and Coker (1944) – more so than any species of *Hohenbuehelia*.

DISCUSSION

Each of the species described here as new had gone undetected, through misapplication or overly broad application of the names of previously described species of *Hohenbuehelia*. A part of the problem has been uncertain application of names for which there are no holotype specimens, including those for which the only original material is a figure depicting macroscopic features. Another part of the problem rests on old monographs (e.g., Pilát 1935, Coker 1944) in which the authors had what now appear to be overly broad species concepts with species in synonymy whose types had not been adequately studied. To bring some clarity and stability to these older names, Consiglio (2016, 2017a–c) has designated lectotypes, neotypes and epitypes for species names that lack modern, molecularly characterized holotypes. This, together with molecular studies (Koziaik et al. 2007b, this study, Consiglio & Setti in prep.), has enabled the untangling of controversies over the application and synonymy of names such as *Hohenbuehelia atrocoerulea* and *H. grisea*.

Hohenbuehelia algonquinensis was originally identified in the herbarium as a 'large-spored variant of *H. atrocoerulea*', as

reported by Christiansen (1959) and Thorn & Barron (1986). Specimens of *H. canadensis* had been identified in the herbarium as *H. approximans*, *H. grisea* or *H. mustialensis*. The name *H. approximans* was also interpreted in different ways, with some (e.g., Singer 1951) suggesting it was close to the taxon with large spores and dusky lamellae known in Europe as *H. reniformis* and others (e.g., Coker 1944) emphasizing the snow-white lamellae of the protologue. The former concept is indeed close to *H. canadensis*, whereas specimens fitting the latter concept are conspecific with *H. grisea* based on ITS-LSU sequence data (Fig. 1). As a result, we consider *H. approximans* to be a synonym of *H. grisea*. The name *H. reniformis* is itself based on a misapplication by Fries (1874) of the name of a species with thick, red, lateral stipe and sulcate-crenate, milk-white pileus, described from the Essequibo River area of Guyana by Meyer (1818), to a quite different fungus from the Femsjo region of Sweden. This concept was carried on by Pilát (1935), but this European taxon should be known as *Hohenbuehelia leightonii* (Thorn 1986, Courtecuisse 2008). Finally, *H. carlothornii* was included within the *H. grisea* – *Nematoctonus robustus* complex by Koziaik et al. (2007a, b), despite the molecular distinctions and some morphological features that caused RGT to file specimens of the teleomorph in the herbarium as '*Hohenbuehelia* sp.' or '*Hohenbuehelia* cf. *grisea*'.

Resupinatus niger and its typonyms have long been variously misapplied, with some collections proving to be *H. grisea* (VT 1324; KY679143) or *H. nimueae* (RGT 871128/01, above). In addition, since the culture of *H. nigra* (RLG 10761) proved not to have a conidial anamorph referable (in the 1980s) to *Nematoctonus*, Thorn & Barron (1984, 1986) identified this as '*Resupinatus* sp.'. The sequence of this culture (AF042599, Moncalvo et al. 2002) proved to be a member of *Resupinatus*, but the confusion over what species name to apply to it remained until identical sequences were obtained from collections identified as *R. niger* (by RGT and GC) and were recognized (by GC) as conspecific with *Agaricus niger* as described by Schweinitz (1822). All of these collections have distinct metuloid cystidia, typical of *Hohenbuehelia* (Singer 1986), except that their walls are multilayered, with a median layer that is dark brown in optical section (Thorn & Barron 1986, as *H. nigra*). This and other species of *Resupinatus* such as *R. alboniger* have elliptical to allantoid basidiospores, similar to those of some *Hohenbuehelia* and quite different from the classic globose to subglobose spores of *R. applicatus*, the type species of *Resupinatus*. Thus, several of the prominent micromorphological distinctions between these two genera (Singer 1986, Thorn & Barron 1986) have been eliminated, leaving only the presence of a nematophagous conidial anamorph in *Hohenbuehelia* (lacking in *Resupinatus*) and the presence in *Resupinatus* of tapering spines, sometimes tipped with a small droplet, on cuticular and assimilative hyphae (absent in *Hohenbuehelia*). Hyphae of the pileipellis in *Hohenbuehelia* may also be distinguished by spiral bands of encrusting pigment (globular or amorphous in *Resupinatus*) and, when these are present, by hour-glass-shaped secretory structures surrounded by a mucoid ball, similar to the adhesive knobs of the nematophagous anamorph (Thorn & Barron 1986). Although the morphological distinctions of these two genera are beginning to blur, the DNA sequence data available to date shows they are quite distinct (Fig. 1). Much more work remains to be done to assess the status and generic placement of many older species names in this group and large areas of the world, including western North America and most of Asia and the tropics, have been poorly sampled for these taxa.

Key to species of *Hohenbuehelia* with dimidiate fruiting bodies from western Europe and north-eastern North America

1. Metuloid cystidia lacking in hymenium; pileipellis hyphae with amorphous-globular incrustation and sometimes with fine tapering spines each tipped with a droplet; spores globose or ellipsoid . . . *Resupinatus* (not included here)
1. Hymenial metuloid cystidia present, either projecting and visible as a frosting under a hand lens or immersed and only visible in microscopic section 2
2. Pileus reaching 6 cm, dimidiate-orbicular, pearl grey and covered with a deep tomentum that forms a reticulate pattern of spines. — Ontario and Quebec, south to Costa Rica, Europe *H. mastrucata*
2. Pileus smaller, or tomentum not forming a reticulum. 3
3. Pileus usually 2 cm or more broad 4
3. Pileus usually less than 1.5 cm broad 6
4. Pileipellis with pileogloeosphexes; pileus whitish to grey, pale beige, honey brown, dark brown, sometimes olive brown; spores 6.4–8.0 × 3.7–4.5 µm. — Europe, Asia *H. atrocoerulea*
4. Pileipellis without pileogloeosphexes; pileus off-white, pale beige to honey brown 5
5. Pileus pale beige to honey brown with paler margins, with pale to buffy tomentum toward base; spores 6.6–8.1 × 3.4–4.4 µm. — North America, south to Costa Rica, Europe *H. grisea*
5. Pileus off-white, with at most fine white tomentum towards base; spores 7.0–8.2 × 3.1–3.7 µm. — Costa Rica *H. carlothornii*
6. Lamellae white to cream or off-white when mature 7
6. Lamellae darker initially or in development 12
7. Pileus cupulate, grey-brown to black; lamellae few and distant, pure white; spores allantoid, 7.2–9.3 × 3.1–4.3 µm. — Ontario, Michigan and New York, Europe *H. cyphelliformis*
7. Pileus dimidiate; lamellae moderately close 8
8. Pileus pale, straw-coloured to pearl grey. 9
8. Pileus darker, blue-grey to black 10
9. Pileus straw-coloured or pale beige; basidia usually 4-spored; spores 6.6–8.1 × 3.4–4.4 µm; small forms previously known as *H. approximans* *H. grisea*
9. Pileus pearly grey with deep, translucent gelatinous zone; basidia 2-spored, with clamps; spores 8.1–9.9 × 3.8–4.8 µm. — Newfoundland (Thorn 2014) and Europe. *H. fluxilis**
10. Pileus blue-grey, with whitish to concolourous tomentum toward base; on wood of deciduous trees; spores 6.4–8.0 × 3.7–4.5 µm. — Europe, Asia *H. atrocoerulea*
10. Pileus deep brown to black; on wood of conifers (rarely deciduous) trees 11
11. Pileus glossy black, with fine white tomentum towards base; spores 7.5–8.8 × 4.7–5.6 µm. — Ontario *H. algonquinensis*
11. Pileus brown to black, finely frosted or with greyish tomentum towards base; spores 6.9–8.0 × 3.6–4.2 µm. — Alberta and Ontario *H. canadensis*

12. Metuloids immersed; pileus cupulate and remaining so 13
12. Metuloids projecting; pileus cupulate to dimidiate 14
13. Pileus grey-brown to blackish with silvery tomentum; usually on hardwood branches; spores 6.3–7.9 × 3.4–4.5 µm. — Boreal North America and Europe . . . *H. unguicularis*
13. Pileus jet black, glossy, with at most scattered fine white wisps; on branches of conifers; spores 6.8–8.6 × 3.1–3.9 µm. — Boreal North America and Europe *H. pinacearum*
14. On wood of conifers; pileus blackish with pebbly surface and coarse yellowish to black tomentum towards base; spores 7.4–9.1 × 4.0–5.2 µm. — Alberta and Finland *H. mustialensis*
14. On wood of deciduous trees; pileus less coarsely tomentose 15
15. Both pileus and lamellae black; metuloid cystidia trilayered, with dark brown middle layer as seen in the microscope; spores 6.2–7.7 × 3.7–4.4 µm. — Yukon to Costa Rica, Europe *Resupinatus niger*
15. Pileus dark greyish brown, finely white-tomentose towards base; lamellae at first creamy white then greyish brown; spores 6–8 × 3.2–4.4 µm. — Ontario and Wyoming *H. nimueae*

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* Mycoportal (mycoportal.org) shows collections under this name from Nova Scotia and Ontario in Canada and Florida and South Carolina in USA, but several appear conspecific with 2-spored forms of *H. grisea* such as RGT 840713/01 (Fig. 1; Thorn & Barron 1986).

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