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Contribution to European representatives of the genus *Hygrocybe*: Two new species and neotypification of *Hygrocybe mucronella*

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Abstract: *Hygrocybe alpina* and *Hygrocybe amara* are described here as new species closely related to *Hygrocybe mucronella*. *Hygrocybe alpina* is described from Slovakia and Sweden, but is probably more widespread, especially in alpine regions of Central and Northern Europe. *Hygrocybe amara*, a well delimited species based on molecular characters, is known only from Slovakia, but probably it is more widespread across Europe and misidentified as *H. mucronella* s.s. *Hygrocybe mucronella* is neotypified here, because no type material has been preserved. A fully amended description is given based on molecular evidence and morphology.

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INTRODUCTION

This study is a part of ongoing molecular phylogenetic and morphological revision of the Central European representatives of the genus *Hygrocybe*, conducted since 2020. Partial results of the research have been published by Fuljer *et al.* (2021, 2022).

The genus *Hygrocybe* as defined by Lodge *et al.* (2013) comprises terrestrial species with a pileus that is thin, tender and sometimes striate, with a moist, lubricous or viscid surface; stipe hollow or stuffed, splitting or fibrillose, generally smooth at the apex, with a moist or viscid surface. Basidiocarps are frequently brightly coloured, though grey, brown, white or even black species also occur (or combinations of these colours).. Lamellae are waxy and they are usually but not always thick. The lamellar trama structure is always, regular, subregular or interwoven. The basidiospores are always hyaline, inamyloid, thin-walled, and smooth (Boertman 2010). Morphological diversity of *Hygrocybe* species has been confirmed by molecular research in the last decades and new species has been described on regular basis worldwide (Læssøe & Boertmann 2008, Ainsworth *et al.* 2013, Lodge *et al.* 2013, Wang *et al.* 2018, Fuljer *et al.* 2021, Jordal & Larsson 2021, Fuljer *et al.* 2022). Here we present two new species of the *H. mucronella* group and we propose a neotype of *H. mucronella*. Further research in this field will reveal the phylogenetic position of the species described in this study.

MATERIAL AND METHODS

Collections and morphological analyses

Waxcaps were collected in Czechia, Norway, Poland, Slovakia, Spain and Sweden during 1990–2022, from July to December, by K. Bergelin, F. Fuljer, G. Gaarder, J. B. Jordal, V. Kautman, I. Kautmanová, J. Komár, J. Kuriplach, E. Larsson, P. Škubla, and M. Zajac. Most of the collections were from Slovakia. Soil type of Slovak sites has been determined based on the geological map of Slovakia (<https://app.geology.sk/pgm/>).

Descriptions of macro-morphological features were based on fresh material. Colours were coded according to the Pantone colour chart (Pantone Colour Finder 2021). Twenty basidiomata were studied and measured from the holotype collection.

The micromorphology of the studied specimens was investigated by F. Fuljer and I. Kautmanová using a Kapa Mic D117 with TouPCam EP 8.3 Mpx microscope camera, a Leica SM-Lux, a DIC microscope Nikon Eclipse Ni-U and microphotography were captured by a Nikon DS-Ri2 camera. Nis-Elements Basic Research and AmScope imaging software were used to measure and examine microscopic features. Tissues, spores and other micro-morphological structures were examined fresh or rehydrated in H₂O or in Congo Red ammonia solution.

Altogether 50 spores per each type collection were studied and measured; spores were measured mainly from spore deposits in H₂O. Thirty basidia, thirty sterigmata and thirty basidioles from five basidiomata were investigated from the rehydrated material in ammonial Congo Red solution. Other microscopic structures, such as gill trama, pileipellis and stipitipellis were observed in basidiomata from type materials. Q value refers to the division of length and width of microscopic structures. Q_{av} refers the average value of Q and av. refers the average length and width of microscopic features.

Type materials were deposited in the fungarium of the Slovak National Museum-Natural History Museum, Bratislava (BRA). Nomenclature follows Lodge *et al.* (2013) and Index Fungorum (indexfungorum.org).

DNA extraction and sequencing

Total genomic DNA was extracted from dried tissue using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with prolonged incubation time of up to 3 h after addition of the RNA-lytic enzyme. The PCR was performed using C1000 Touch™ Thermal Cycler. Target region of the internal transcribed spacer regions of ribosomal DNA (ITS) was amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White *et al.* 1990). The large ribosomal subunit of ribosomal DNA (LSU) was amplified using primers LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAACTTCG-3'; Vilgalys & Hester 1990). The amplification reactions were conducted in 25 µL total volume using a GoTaq Flexi PCR kit (Promega), the reaction mixture containing 20–25 ng total DNA template, 1 µL of both primers (10 µM), 5 µL of Buffer (5×), 2.5 µL of dNTP (2 mM), 2 µL of MgCl₂ (25 mM), 0.2 µL GoTaq Flexi polymerase and the final volume was added with ultrapure water. The amplification reaction for ITS and LSU regions was set up as follows: 3 min initial denaturation at 95 °C, 32 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min + increasing time 2 s per cycle) 10 min final elongation at 72 °C. The PCR products were analysed on 2 % agarose gel and then purified using Thermosensitive Alkaline Phosphatase (FastAP) and Exonuclease 1 (Exo 1) (Thermo Fisher Scientific Inc., USA) according to manufacturer's instructions. The amplicons were sequenced in a commercial laboratory (Eurofins Genomics GmbH, Cologne, Germany).

Our dataset is composed of 42 nrDNA ITS sequences, selected after an initial analysis using published and our unpublished ITS sequences. A combined dataset of 28 ITS and LSU sequences is also provided. Newly generated sequences were submitted to BOLD and GenBank databases. Sequence similarity searches were performed using GenBank BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST/>) and the BOLD Identification System (<https://www.boldsystems.org/>).

RESULTS AND DISCUSSION

Phylogeny

In total 71 *Hygrocybe* sequences were used for the analysis. Phylogenetic analyses were conducted in MEGA X (Kumar *et al.* 2018) by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei 1993). An ITS tree with the highest

log likelihood is shown in Fig. 1. The combined ITS and LSU tree of 28 selected “mucronella group” sequences is shown in Fig. 2. GenBank accession numbers are listed in Table 1. All of the species described as new to science or typified in this study received high statistical support. Altogether 28 LSU and 32 ITS barcode sequences were newly generated for this study.

Taxonomy

Hygrocybe alpina Fuljer, Zajac, Boertm. & Kautmanova, *sp. nov.* MycoBank MB 853535. Figs 3, 4, 5B.

Etymology: Name refers to supposed alpine distribution of the species.

Typus: Slovakia, Belianske Tatry Mts., Vysoké Tatry village, Zadné Jatky peak, 49.2388111°N, 20.2294611°E, alt. 1 940 m, alpine permanent grassland, without any management, 5 Sept. 2019, *F. Fuljer* (BRACR 38271 **holotype**; ITS GenBank OR524760, LSU GenBank OR524740).

Pileus 6–13 mm, hemispherical, campanulate, margin crenate; surface smooth, slightly lubricous when young, later dry, with silky sheen, scarlet red to carmine red (Pantone 7625C to Pantone 7627C), sometimes with yellow orangish patches (Pantone 714C). *Stipe* 10–35 × 1–2 mm, slender, terete; contorted, rarely slightly compressed; hollow; surface smooth, dry to slightly viscid, with silky sheen, scarlet red (Pantone 7625C to Pantone 7627C), with paler base (Pantone 7570C). *Lamellae* broadly adnate, with a decurrent tooth, distant, brittle, scarlet red (Pantone 7625C to Pantone 7627C), with salmon tinge. *Context* concolourous with surface, thin; rather fragile. *Smell* indistinct. *Taste* bitter. *Spore deposit* white. *Basidiospores* irregularly ellipsoid, oblong, phaseoliform, often constricted, sometimes with widened or asymmetrical base, thin-walled, smooth, hyaline, non-amyloid, with one big vacuole, (7.4–)8.1–12.3(–13.5) × (4.3–)4.6–6.2(–6.8) µm, av. 9.7 × 5.4 µm, Q = (1.3–)1.4–2.1(–2.5), Q_{av} = 1.71–1.86. *Basidia* (37–)39–58(–63) × (7–)7.2–9.5(–11.4) µm, av. 49 × 8.4 µm, (1–) 2–4-spored, clavate to narrowly clavate, guttulate, sterigmata (3.2–)3.3–7.7(–7.8) µm, awl-shaped. *Basidioles* (28–)30–52(–54) × (3.9–)4.4–8.6(–8.8) µm, av. 44 × 6.6 µm, cylindrical to clavate. *Cystidia* absent. *Pileipellis* an ixocutis made up of repent, ascending, cylindrical, irregular hyphae, thickened at septa, size of hyphae (31–)37–138(–139) × (2.1–)2.6–5.2(–5.5) µm. *Gill trama* a subregular, made up of cylindrical or ±inflated elements, constricted at septa, (21–)33–130(–150) × (5–)6.4–18.5(–18.7) µm. *Clamps* relatively abundant in all tissues.

Habitat and distribution: Known from Slovakia and Sweden, probably more widespread, especially in alpine regions of Central and Northern Europe, but misidentified as *H. mucronella* or *H. salicis-herbaceae*. Growing solitary, rarely in small groups or groups. Occurs in alpine zone, in unmanaged grasslands, from July to September, on calcareous soils.

Additional materials examined: Slovakia, Belianske Tatry Mts., Vysoké Tatry village, Bujačí peak, alt. 1 900–1 950 m, alpine unmanaged grassland, 13 Jul. 2018, *M. Zajac*, BRACR38272; *ibid.*, under Predné Jatky peak, 49.2306639°N, 20.2561211°E, alt. 1 904 m, alpine unmanaged grassland, 24 Aug. 2021, *F. Fuljer*, BRACR38274; *ibid.*, under the Zadné Jatky peak, 49.238856°N, 20.228126°E, alt. 1 923 m, alpine unmanaged grassland, 19 Aug. 2021, *F. Fuljer*, BRACR38273. Sweden, Lule lappmark, Jokkmokk, Padjelanta NP, Svärtinjunjes, 67.5315100°N, 16.7734761°E,

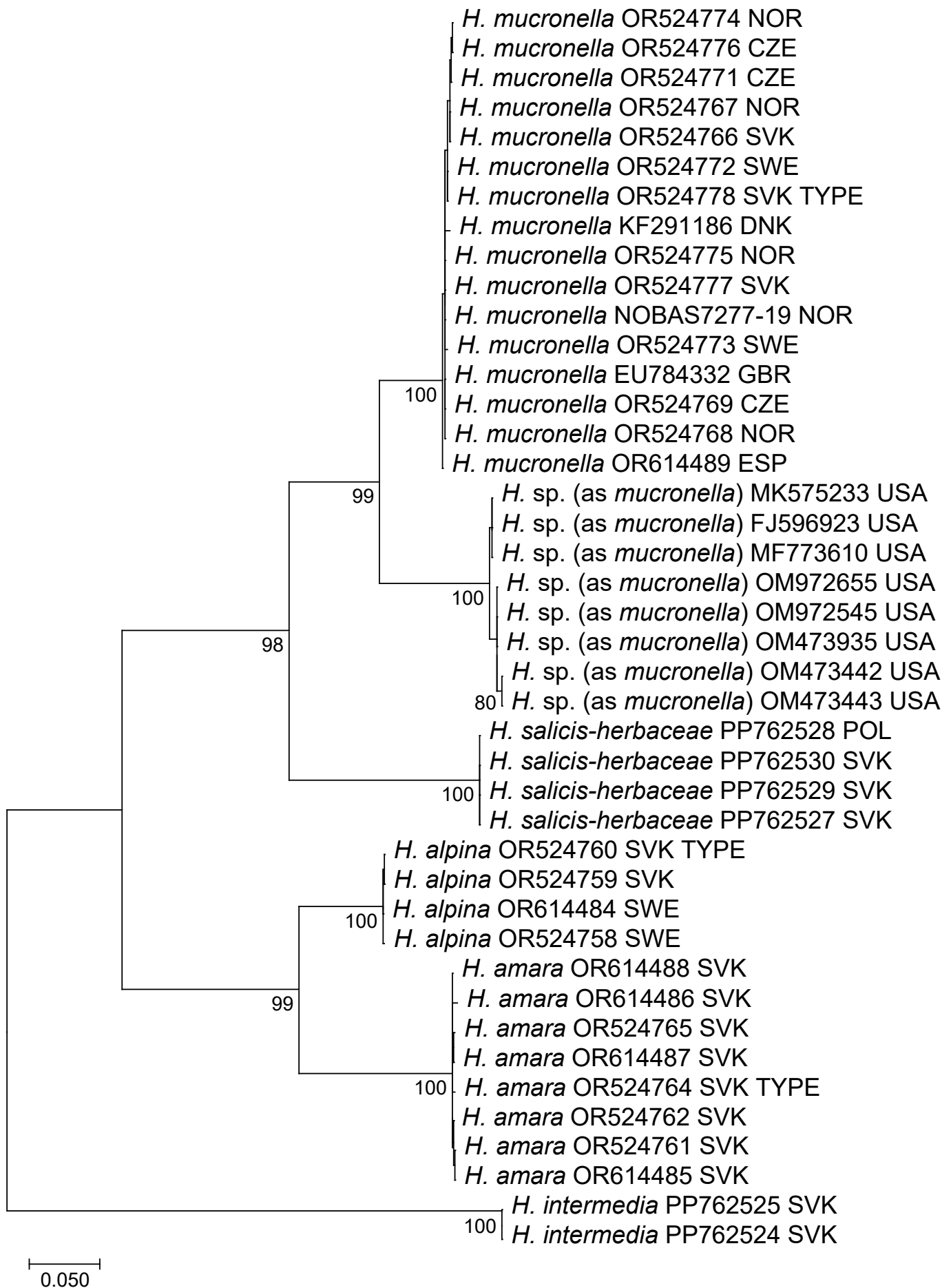


Fig. 1. Phylogenetic tree from the Maximum Likelihood analysis of the former *Hygrocybe mucronella* group, based on the ITS dataset. Bootstrap support values from the ML analysis ($BS \geq 70\%$) are shown at the nodes. *Hygrocybe intermedia* is used as outgroup.

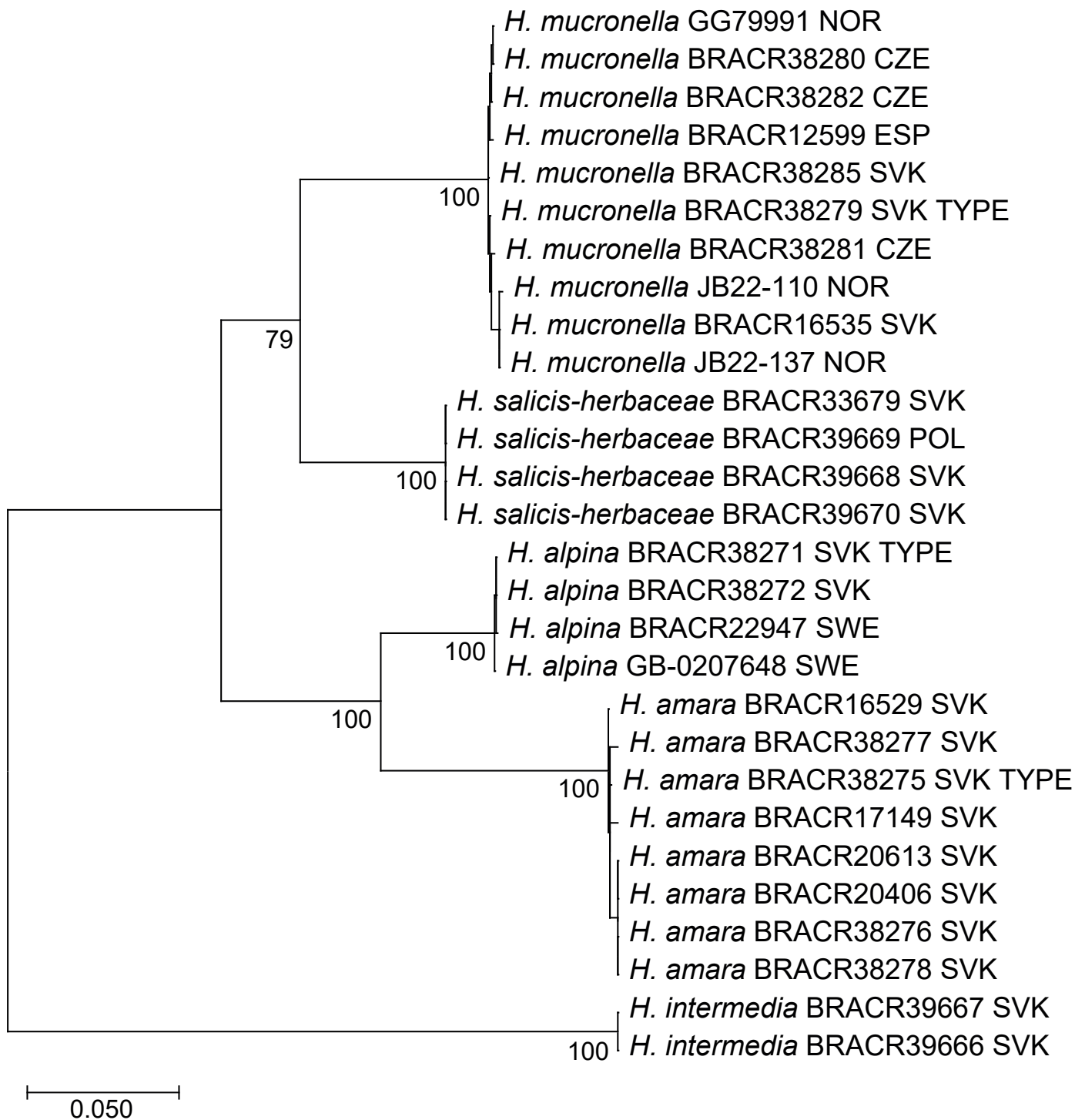


Fig. 2. Concatenated ITS-LSU tree derived from the Maximum Likelihood analysis with the positions of *Hygrocybe alpina*, *H. amara* and *H. mucronella*. Bootstrap support values from the ML analysis (BS \geq 90 %) are shown at the nodes. *Hygrocybe intermedia* is used as outgroup.

alt. 760 m, mosaic alpine grassland vegetation, 19 Aug. 2016, *E. Larsson*, GB-0207648; Hemavan, Gierevaartoe Mt., 65.7805962°N, 15.0641917°E, alt. 700 m, alpine heathland, 21 Aug. 2015, *E. Larsson*, BRACR22947.

Notes: *Hygrocybe alpina* forms a sister clade to *H. amara*. Morphologically and ecologically this species resembles *H. mucronella*, *H. salicis-herbaceae* and *H. amara*, differing from these three species by having larger and slightly differently shaped spores, scarlet to carmine-red pileus, and typical occurrence in alpine grasslands and heathlands. From *H. mucronella* and *H. amara* it differs also by strictly scarlet-red colour of basidiomata.

Hygrocybe salicis-herbaceae is more robust and though younger basidiomata can be scarlet-red, older ones are distinctly paler (rarely almost yellow-orange or even yellow). Taste of *H. salicis-herbaceae* is more rancid than bitter after prolonged chewing and gives a very unpleasant feeling in throat and on back of tongue, while *H. alpina* is immediately bitter. *Hygrocybe alpina* has been recorded at sites with calcareous bedrock, while *H. salicis-herbaceae* is acidophilic. Currently *H. alpina* is known from two localities (while one locality consists of three different microlocalities) in Slovakia, and two localities in Sweden, all of them located in alpine zones.



Fig. 3. *Hygrocybe alpina* basidiomes *in situ*. **A.** Collection BRACR38271 (Holotype). **B, C.** Collection BRACR38272. **D, E.** Collection BRACR38274. Scale bars = 10 mm.

Hygrocybe amara Fuljer, Zajac, Boertm. & Kautmanova, *sp. nov.*
Mycobank MB 853536. Figs 6–8, 5A.

Etymology: Name refers to a typical bitter taste of basidiomata.

Typus: Slovakia, Kysucká vrchovina Mts., Snežnica village, Brodenec, 49.2647581°N, 18.7793219°E, alt. 440 m, mesophilic mowed grassland, 15. Oct. 2021, *F. Fuljer* (BRACR 38275 **holotype**; ITS GenBank OR524764, LSU GenBank OR524744).

Pileus 3–20 mm, at first hemisphaerical, campanulate, later convex to almost applanate, margin crenate; surface smooth, lubricous when young, later dry, pale orange, yellowish orange to orange with reddish hue (Pantone 1235C, 1375C and 1505C),

very often with dark orangeish red central part (Pantone 173C and 1665 C), often translucently striate. **Stipe** 18–47 × 0,7 – 3,5 mm, slender, terete, occasionally compressed; hollow; surface smooth, firstly viscid, later dry, often uniformly coloured or under pileus paler and near base darker, yellowish orange, pale orange, orange, or rarely entirely dirty yellow (Pantone 124C, 138C, 716C, 7555C and 7751C). **Lamellae** broadly adnate, with a decurrent tooth, distant, brittle, pale yellow (Pantone 7499C). **Context** concolourous with surface, thin; rather fragile. **Smell** indistinct. **Taste** bitter. **Spore deposit** white. **Basidiospores** pear-shaped, irregularly ellipsoid, almost always with widened or asymmetrical base, rarely ellipsoid or oblong, but always medially constricted, thin-walled, smooth, hyaline, non-amyloid, in majority with one big vacuole, (7.0–)7.2–9.2(–9.5) ×

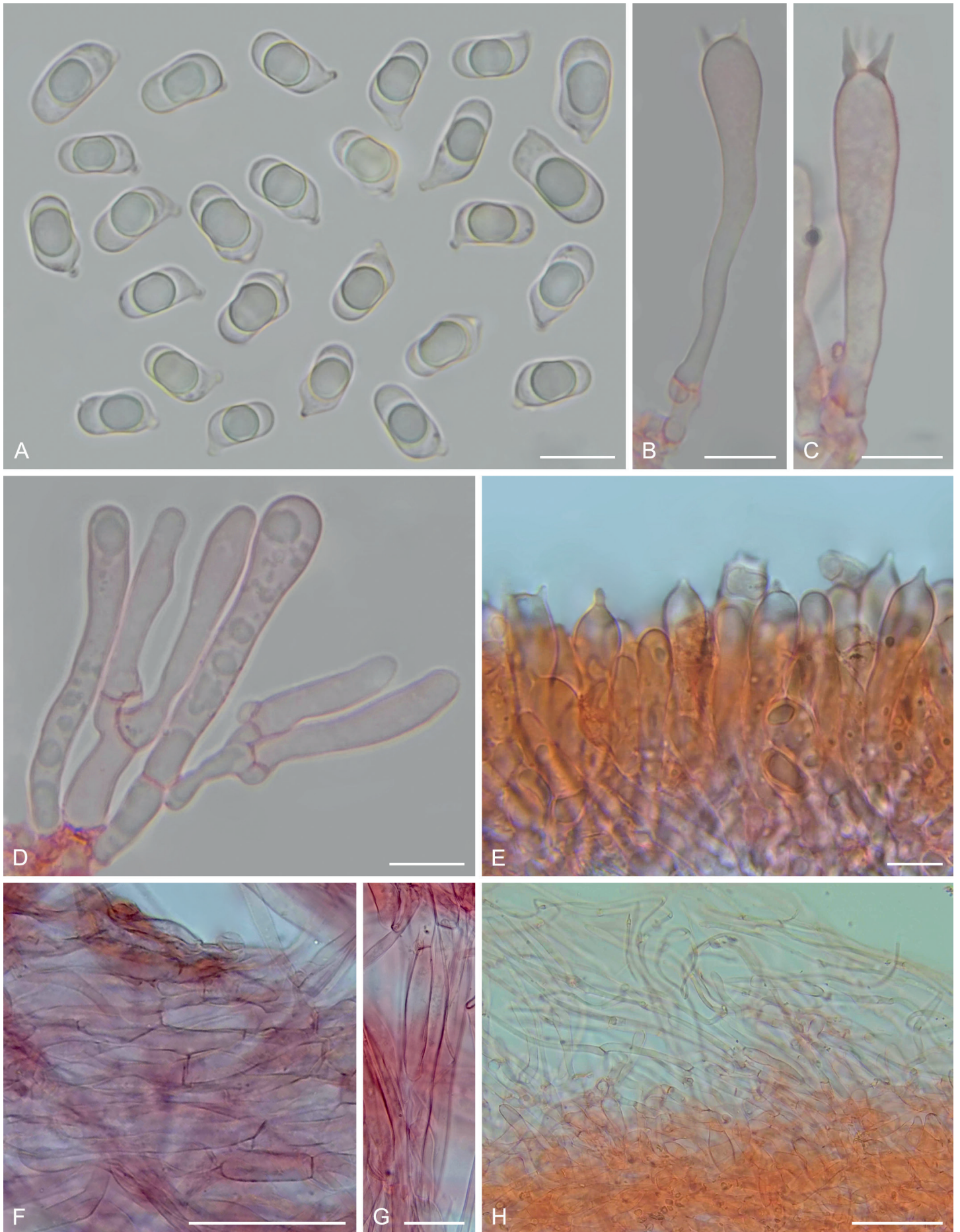


Fig. 4. *Hygrocybe alpina* micro-morphological characters [A, E. Collection GB-0207648; B, C, D, H. Collection BRACR38271 (Holotype); F, G. Collection BRACR22947]. **A.** Basidiospores. **B, C.** Basidia. **D.** Basidioles. **E.** Hymenium. **F, G.** Gill trama. **H.** Pileipellis. Scale bars: A–E = 10 µm; F, H = 50 µm; G = 20 µm.



Fig. 5. Habitats of type localities. A. *Hygrocybe amara*. B. *H. alpina*. C. *H. mucronella*.

(4.6–)4.9–6.8(–7.1) μm , av. $8.2 \times 6 \mu\text{m}$, $Q = (1.1\text{--})1.2\text{--}1.5(–1.7)$, $Q_{\text{av.}} = 1.32\text{--}1.39$. *Basidia* (30–)34–55 \times (6.6–)7.0–8.8(–9.1) μm , av. $42 \times 7.8 \mu\text{m}$, 4-spored, clavate to narrowly clavate, guttulate, sterigmata (4.6–)5.5–11.0(–11.8) μm , awl-shaped. *Basidioles* (29–)33–48(–49) \times (6.4–)6.6–8.3(–8.4) μm , av. $39 \times 7.4 \mu\text{m}$, cylindrical to clavate. *Cystidia* absent. *Pileipellis* an ixocutis made up of repent, ascending, cylindrical, irregular hyphae, slightly thickened at septa, gelatinised, size of hyphae (23–)24–128(–175) \times (2.3–)2.7–4.5 μm . *Gill trama* a subregular, cylindrical or \pm irregularly inflated elements, constricted at septa, (30–)37–156(–163) \times (10.4–)10.5–15.4(–16.6) μm . *Clamps* relatively abundant in all tissues.

Habitat and distribution: Known from Slovakia, probably more widespread across Europe, but misidentified as *H. mucronella* s.s. Growing gregarious, rarely in small groups and often hidden in higher grass and vegetation. Reported from foothill, semi-natural mesophilic grasslands, from late September to beginning of December, on calcareous or slightly calcareous soils.

Additional materials examined: Slovakia, Javorníky, Petrovice village, Sádky, 49.2561194°N, 18.5336111°E, alt. 361 m, mesophilic mowed grassland, 10 Nov. 2020, *F. Fuljer*, BRACR38276; Kysucká vrchovina, Snežnica village, Brodenec, 49.2647583°N, 18.7793222°E, alt. 440 m, mesophilic mowed grassland, 29 Oct. 2019, *F. Fuljer* & *M. Zajac*, BRACR38278; *ibid.*, 7 Nov. 2023, *M. Zajac*, BRACR39029; Malé Karpaty, Kočín village, Chrib, 48.6066528°N, 17.6502978°E, alt. 281 m, overgrown grassland by different shrubs, 17 Nov. 2010, *J. Komár*, BRACR17149; *ibid.*, Pezinok town, Stará hora (nad Kejdou), alt. 230 m, grassy place between old vineyards, 16 Sep. 2010, *V. Kautman* & *J. Kuriplach*, BRACR16529; *ibid.*, 14 Nov. 2013, *V. Kautman*, BRACR20406; Podtatranská kotlina, Liptovské Matiašovce village, Vyšné Matiašovce, 49.1676769°N, 19.5652169°E, alt. 675 m, mesophilic mowed grassland, 17 Oct. 2022, *F. Fuljer*, BRACR38277; Strážovské vrchy Mts., Trenčianske Teplice, semi-natural managed grassland, 14 Oct. 1990, *P. Škubla*, BRACR33938; Žilinská kotlina, Malé Lednice village, Cibul'ková, alt. 500 m, mowed grassland, 24 Oct. 2013, *I. Kautmanová*, BRACR20613.

Notes: *Hygrocybe amara* is closely related to *H. alpina* and *H. mucronella*. Generally, all three species differ in spore morphology, colour of basidiomata and ecology. *Hygrocybe alpina* is an alpine species, while *H. amara* and *H. mucronella* occur in coastal, foothills or lowland semi-natural grasslands. One of the most distinctive taxonomic characters is the Q value of spores. Based on the examined material the average Q value of *H. amara* is 1.32–1.39, while Q value of *H. mucronella* is 1.51–1.63 and Q value of *H. alpina* is 1.71–1.86. Spores of *H. alpina* are irregularly ellipsoid, oblong, phaseoliform, often slightly constricted and rarely with widened or asymmetrical base, while spores of *H. amara* and *H. mucronella* are predominately pear-shaped or irregularly ellipsoid, distinctly constricted and very often with widened or asymmetrical base. Spores of *H. amara* are also slightly broader (*H. mucronella* has spores broad 4.4–6.1 μm , while *H. amara* 4.9–6.8 μm). The pileus of *H. amara* is pale orange, yellowish orange to orange coloured, often with darker (orange-red) central part, while pileus of *H. alpina* is scarlet to carmine-red and pileus of *H. mucronella* is orange-red to reddish orange (rarely entirely orange).

Neotypification and emendation of *Hygrocybe mucronella*

Hygrocybe mucronella (Fr.) P. Karst., *Bidrag till Kännedom av*

Finlands Natur och Folk **32**: 235. 1879. MycoBank MB 438468. Figs 5C, 9–11.

Basionym: *Hygrophorus mucronellus* Fr., *Epicrisis Systematis Mycologici*: 331. 1838.

Synonyms: *Hygrocybe mucronella* var. *mucronella* (Fr.) P. Karst., *Bidrag till Kännedom av Finlands Natur och Folk* **32**: 235. 1879.

Agaricus puniceus β *acutus* Lasch, *Linnaea* **3**: 381. 1828.

Hygrocybe mucronella f. *siccipes* (Bon) Lécure, *Index Fungorum* **384**: 1. 2019.

Hygrocybe mucronella var. *mite* (Kühner) E. Ludw., *Pilzkompendium* (Eching) **3**: 322. 2012.

Typus: Slovakia, Javorníky Mts., Melocik, Veľké Rovné village, 49.3419169°N, 18.5087961°E, alt. 796 m, semi-natural cattle grazed mesophilic grassland, 2 Oct. 2022, *F. Fuljer* (BRACR 38279 **neotype** designated here, MBT 10019641; ITS GenBank OR524778, LSU GenBank OR524755).

Emended description: *Pileus* 3–16 mm, at first hemispherical, campanulate, later convex to almost applanate, margin crenate; surface smooth, lubricous when young, later dry, orange (Pantone 7409C to 7413C), orange-red and reddish orange (Pantone 7578C to 7579C), often with yellow or yellow-orange edge (Pantone 142C) and red centre (Pantone 7597C), often translucently striate and when dry with a characteristic matt surface. *Stipe* 13–42 \times 0.7–2.2 mm, slender, terete; hollow; surface smooth, firstly viscid, later dry, orange-yellow or yellowish orange (Pantone 124C, 130C, 137C), often with paler base (Pantone 128C) and darker upper part (Pantone 152C). *Lamellae* broadly adnate, with a decurrent tooth, distant, brittle, pale yellow, yellow-orange (Pantone 600C, 7401C, 7507C), sometimes with reddish or salmon hue. *Context* concolourous with surface, thin; rather fragile. *Smell* indistinct. *Taste* bitter and can usually be recorded just by putting tip of tongue on the pileus (note: some people cannot taste the bitterness). *Spore deposit* white. *Basidiospores* pear-shaped, irregularly ellipsoid, very often with widened or asymmetrical base, rarely obovoid, ellipsoid or oblong, often medially constricted, thin-walled, smooth, hyaline, non-amyloid, with one big vacuole, (6.5–)6.9–8.9(–9.7) \times (4.0–)4.4–6.1(–6.9) μm , av. $7.9 \times 5.2 \mu\text{m}$, $Q = (1.2\text{--})1.3\text{--}1.7(–1.8)$, $Q_{\text{av.}} = 1.51\text{--}1.63$. *Basidia* (33–)36–45(–47) \times (6.2–)6.4–8.0(–8.2) μm , av. $40 \times 7.2 \mu\text{m}$, 4-spored, rarely 2-spored, clavate to narrowly clavate, guttulate, sterigmata (3.9–)4.4–7.5(–8.3) μm , awl-shaped. *Basidioles* (26–)27–40(–47) \times (4.4–)4.8–7.3(–7.5) μm , av. $34 \times 5.8 \mu\text{m}$, cylindrical to clavate. *Cystidia* absent. *Pileipellis* an ixocutis made up of repent, ascending, cylindrical, irregular hyphae, slightly thickened at septa, 50–120 μm thick, gelatinised, size of hyphae (22–)27–123(–156) \times 2.9–4.7(–5) μm . *Gill trama* a subregular, cylindrical or \pm irregularly inflated elements, constricted at septa, (23–)37–123(–155) \times (3.5–)4.8–15.2(–19.9) μm . *Clamps* relatively abundant in all tissues.

Habitat and distribution: Known and reported from most European countries. Growing in semi-natural grasslands, herb rich woodlands and road verges, from September to November, on calcareous or slightly calcareous soils.

Additional materials examined: Czechia, Moravskoslezské Beskydy Mts., Bíla village, close to Bíla Ostravice river, 49.437583°N, 18.440063°E, alt. 555 m, edge of mesophilic mowed and sheep grazed grassland, close to road, on place where calcareous gravel was ploughed, between gravel, 11 Oct. 2022, *F. Fuljer*, BRACR38280;



Fig. 6. *Hygrocybe amara* basidiomes *in situ*. A, B, F. Collection BRACR38275 (Holotype). C, D. Collection BRACR39029. E. Collection BRACR38278. Scale bars = 10 mm.



Fig. 7. *Hygrocybe amara* details of macro-morphological characters (A, B. Collection BRACR38275; C–G. Collection BRACR39029). A, C, E. Pileus. B, D, F. Lamellae. G. Stipe. Scale bars = 10 mm.

Table 1. Phylogenetically analysed collections with countries of origin, herbarium numbers and GenBank Accession numbers.

| Species | Origin | Fungarium number | GenBank Accession No. | |
|--------------------------------------|----------------------------------|------------------------|------------------------|----------|
| | | | ITS | LSU |
| <i>Hygrocybe mucronella</i> | Czechia | BRACR38281* | OR524769 | OR524749 |
| | | BRACR38282* | OR524771 | OR524750 |
| | | BRACR38280* | OR524776 | OR524753 |
| | Denmark | D. Boertmann 2006/73 | KF291186 | — |
| | Norway | JB22-110* | OR524768 | OR524748 |
| | | JB22-213* | OR524767 | — |
| | | JB22-137* | OR524775 | OR524752 |
| | | GG79991* | OR524774 | OR524751 |
| | | O-F-245865 | NOBAS7277-19** | — |
| | Slovakia | BRACR38279* (neotype) | OR524778 | OR524755 |
| | | BRACR16535* | OR524777 | OR524754 |
| | | BRACR38285* | OR524766 | OR524746 |
| | Spain | BRACR12599* | OR614489 | OR614495 |
| | Sweden | KBE-07.164* | OR524772 | — |
| | | KBE-05.192* | OR524773 | — |
| | UK | RBG Kew K(M)127308 | EU784332 | — |
| | <i>Hygrocybe alpina sp. nov.</i> | Slovakia | BRACR38271* (holotype) | OR524760 |
| BRACR38272* | | | OR524759 | OR524739 |
| Sweden | | GB-0207648* | OR524758 | OR524738 |
| | | BRACR22947* | OR614484 | OR614491 |
| <i>Hygrocybe amara sp. nov.</i> | Slovakia | BRACR38275* (holotype) | OR524764 | OR524744 |
| | | BRACR38277* | OR524765 | OR524745 |
| | | BRACR38278* | OR524761 | OR524741 |
| | | BRACR38276* | — | OR524743 |
| | | BRACR20613* | OR524762 | OR524742 |
| | | BRACR33938* | OR614488 | — |
| | | BRACR17149* | OR614486 | OR614493 |
| | | BRACR16529* | OR614487 | OR614494 |
| | | BRACR20406* | OR614485 | OR614492 |
| <i>Hygrocybe sp. (as mucronella)</i> | USA | — | MK575233 | — |
| | | TENN CLB010 | MF773610 | — |
| | | DJL06TN64 | FJ596923 | — |
| | | — | OM972545 | — |
| | | — | OM972655 | — |
| | | — | OM473935 | — |
| | | — | OM473442 | — |
| | | — | OM473443 | — |
| <i>Hygrocybe salicis-herbaceae</i> | Poland | BRACR39669 | PP762528 | PP762516 |
| | Slovakia | BRACR33679 | PP762527 | PP762515 |
| | | BRACR39668 | PP762529 | PP762517 |
| | | BRACR39670 | PP762530 | PP762518 |
| <i>Hygrocybe intermedia</i> | Slovakia | BRACR39666 | PP762525 | PP762513 |
| | | BRACR39667 | PP762524 | PP762512 |

* – sequences generated in this study, ** – BOLD accession number

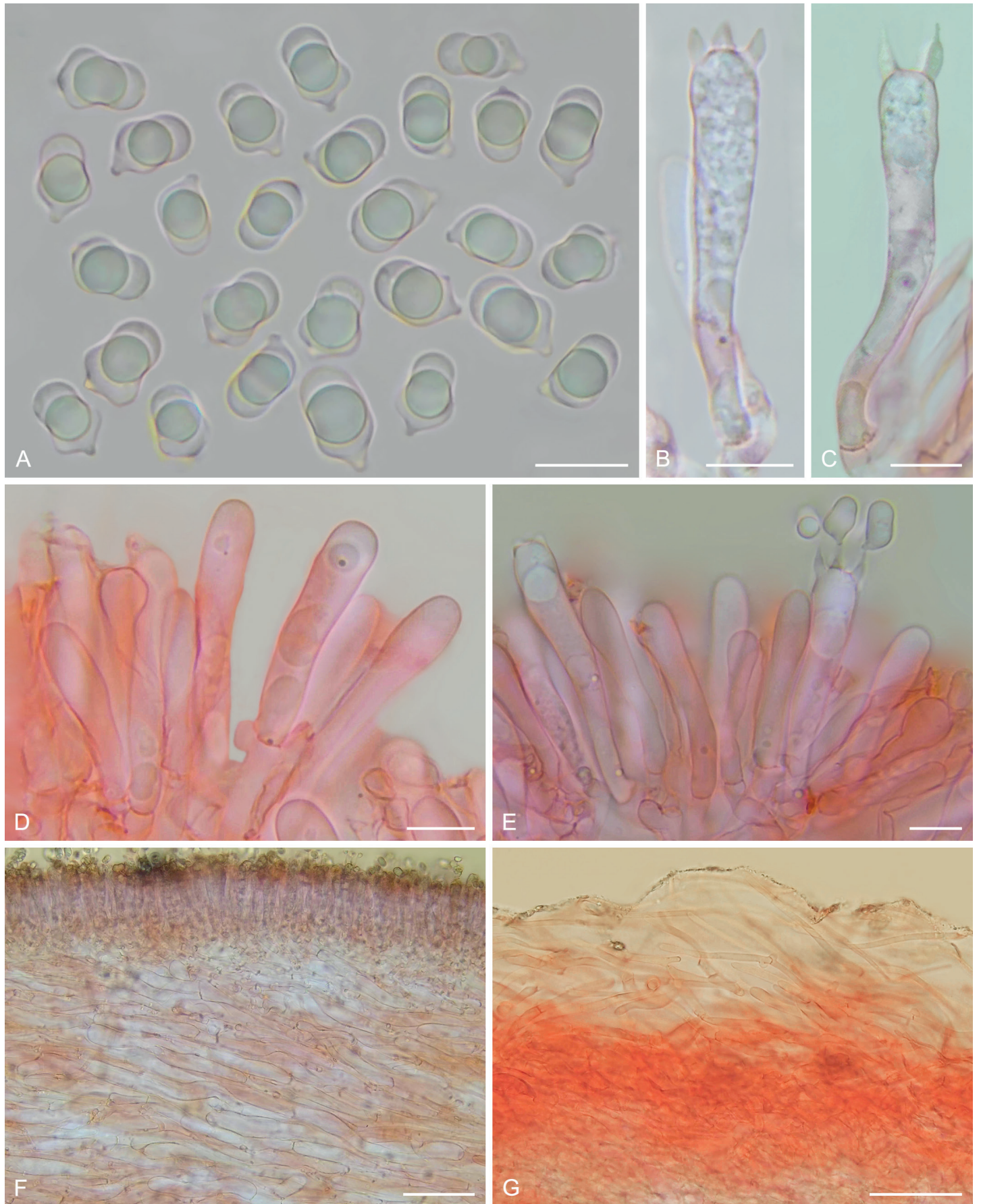


Fig. 8. *Hygrocybe amara* micro-morphological characters [A–E, G. Collection BRACR38275 (Holotype); F. Collection BRACR39029]. **A.** Basidiospores. **B, C.** Basidia. **D.** Basidioles. **E.** Hymenium. **F.** Gill trama. **G.** Pileipellis. Scale bars: A–E = 10 µm; F, G = 50 µm.



Fig. 9. *Hygrocybe mucronella* basidiomes in situ. A. Collection BRACR38279 (Neotype). B. Collection BRACR38286. C. Collection BRACR39028. D. Collection BRACR38284. E. Collection JB22-137. F. Collection BRACR38281. Scale bars = 10 mm.



Fig. 10. *Hygrocybe mucronella* details of macro-morphological characters (A–C, F, G. Collection BRACR39028; D, E. Collection BRACR38281). A, C, E. Pileus. B, D, F. Lamellae. G. Stipe. Scale bars = 10 mm.

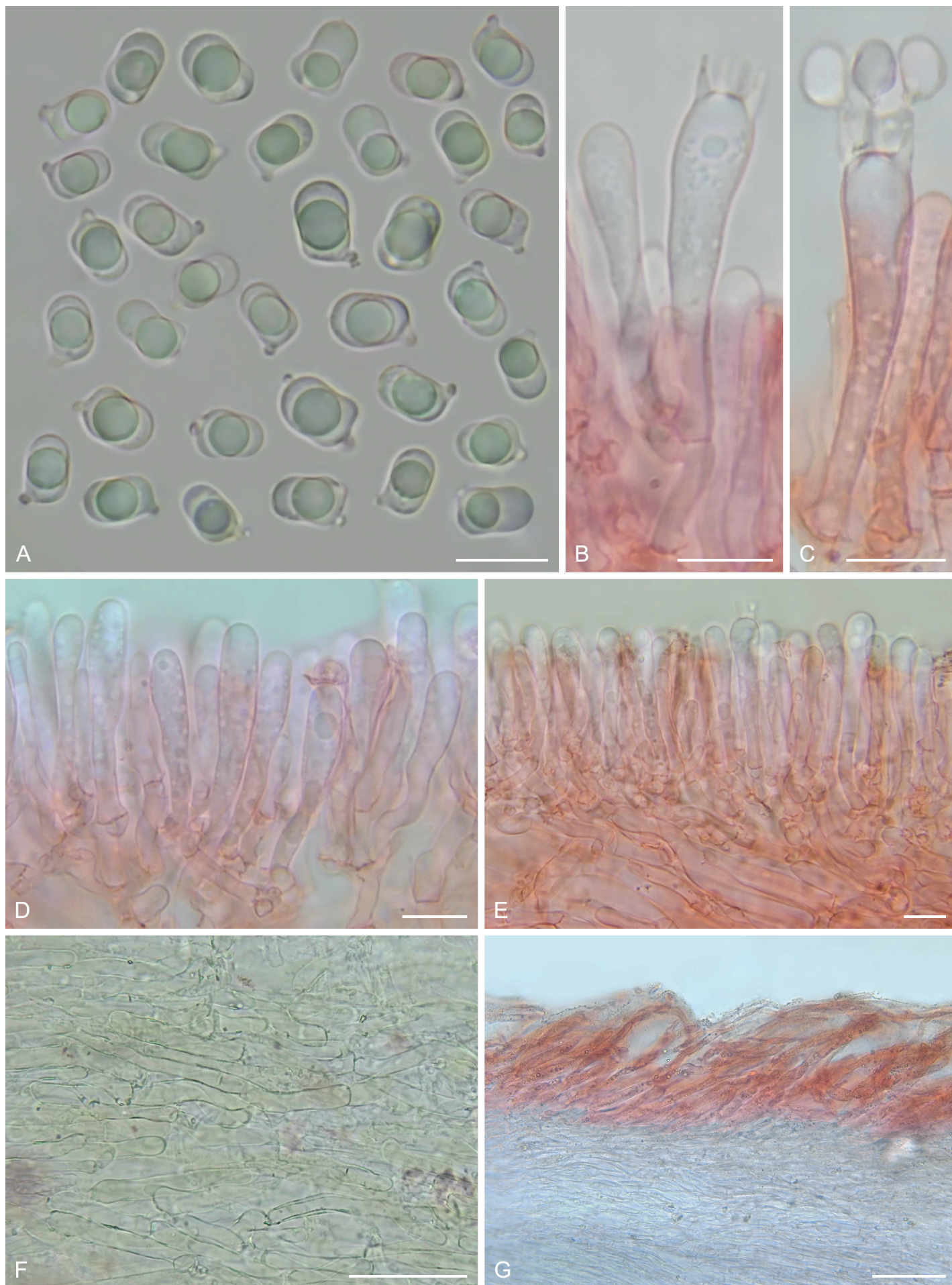


Fig. 11. *Hygrocybe mucronella* micro-morphological characters (A. Collection JB22-137; B–E. Collection BRACR38279; F, G. Collection BRACR39028). A. Basidiospores. B, C. Basidia. D. Basidioles. E. Hymenium. F. Gill trama. G. Pileipellis. Scale bars: A–E = 10 μ m; F, G = 50 μ m.

Table 2. Comparison of selected characters and ecological requirements of *H. alpina*, *H. amara* and *H. mucronella*.

| | <i>Hygrocybe alpina</i> | <i>Hygrocybe amara</i> | <i>Hygrocybe mucronella</i> |
|--|---|--|---|
| Pileus colour | scarlet red to carmine red, sometimes with yellow orangeish patches | pale orange, yellowish orange to orange, very often with dark orangeish red central part | orangeish red and reddish orange, rarely entirely orange, often with yellow or yellow-orange edge |
| Spores shape | irregularly ellipsoid, oblong, phaseoliform, often constricted, sometimes with widened or asymmetrical base | pear-shaped, irregularly ellipsoid, almost always with widened or asymmetrical base, rarely ellipsoid or oblong, but always medially constricted | pear-shaped, irregularly ellipsoid, very often with widened or asymmetrical base, rarely obovoid, ellipsoid or oblong, often medially constricted |
| Spores size [μm] | (7.4–)8.1–12.3(–13.5) × (4.3–)4.6–6.2(–6.8) | (7.0–)7.2–9.2(–9.5) × (4.6–)4.9–6.8(–7.1) | (6.5–)6.9–8.9(–9.7) × (4.0–)4.4–6.1(–6.9) |
| Spores av. [μm] | 9.7 × 5.4 | 8.2 × 6 | 7.9 × 5.2 |
| Spores Q | (1.3–)1.4–2.1(–2.5) | (1.1–)1.2–1.5(–1.7) | (1.2–)1.3–1.7(–1.8) |
| Spores Qav. | 1.71–1.86 | 1.32–1.39 | 1.51–1.63 |
| Basidia size [μm] | (37–)39–58(–63) × (7.0–)7.2–9.5(–11.4) | (30–)34–55 × (6.6–)7.0–8.8(–9.1) | (33–)36–45(–47) × (6.2–)6.4–8.0(–8.2) |
| Basidia av. [μm] | 49 × 8.4 | 42 × 7.8 | 40 × 7.2 |
| Basidia type | (1–)2- to 4-spored | 4-spored | 4-spored, rarely 2-spored |
| Habitat | Alpine grasslands | Foothill semi-natural mesophilic grasslands | Semi-natural grasslands, herb rich woodlands and road verges |

ibid., Dolní Lomná village, Kantořanka, 49.547067°N, 18.672406°E, alt. 495 m, edge of mesophilic mowed and sheep grazed grassland, close to road, on place where calcareous gravel was ploughed, 31 Oct. 2022, *F. Fuljer*, BRACR38281; *ibid.*, Staré Hamry village, Černá, 49.455920°N, 18.467477°E, alt. 530 m, edge of mesophilic mowed grassland, close to road, on place where calcareous gravel was ploughed, 6 Oct. 2022, *F. Fuljer* & *M. Zajac*, BRACR38282. **Norway**, Hordaland county, Bømlo, Lykling, Blyttarstemma, 59.7119°N, 5.1710°E, alt. 10 m, calcareous pasture, 7 Oct. 2022, *J.B. Jordal*, JB22-213; Innlandet county, Østre Toten municipality, Skreia: Hågåbakken, 60.6574722°N, 10.9392500°E, alt. 138 m, calcareous pasture, 9 Oct. 2022, *G. Gaarder*, GG79991; Møre og Romsdal county, Herøy municipality, Nerlandsøy village, Mulevika bay, 62.3533°N, 5.5258°E, alt. 7 m, calcareous pasture, 26 Sep. 2022, *J.B. Jordal*, JB22-110; *ibid.*, Giske municipality, Vigra village, Molnesfjellet, 62.5876°N, 6.1004°E, alt. 8 m, calcareous sandy pasture, 27 Sept. 2022, *J.B. Jordal*, JB22-137. **Slovakia**, Javorníky, Veľké Rovné village, Melocík, 49.3419169°N, 18.5087961°E, alt. 796 m, cattle grazed mesophilic grassland, 17 Sep. 2022, *F. Fuljer*, BRACR38283; *ibid.*, 24 Sep. 2023, BRACR39028; Kysucká vrchovina Mts., Snežnica village, under Vreťň hill, 49.267831°N, 18.787606°E, alt. 460 m, overgrown pasture, 18 Oct. 2019, *F. Fuljer* & *M. Zajac*, BRACR38284; Malé Karpaty Mts., Chtelnica village, Plešivá Hora, alt. 350 m, overgrown pasture, 12 Sep. 2010, *I. Kautmanová* & *V. Kautman*, BRACR16535; *ibid.*, Plavecké Podhradie village, Strelnica, 48.483572°N, 17.263375°E, alt. 259 m, overgrown edge of relatively dry grassland (shady place), 23. Oct. 2020, *F. Fuljer*, BRACR38285; Nízke Tatry Mts., Liptovská Teplička village, alt. 890 m, in grass in pasture, 5. Oct. 2001, *I. Kautmanová*, BRACR33936; Slovenský raj Mts., Telgárt village, Besník, 48.8604319N, 20.2322439E, alt. 1040 m, cattle grazed pasture on calcareous bedrock, 24 Sep. 2022, *F. Fuljer*, BRACR39001; Strážovské vrchy Mts., Trenčianske Teplice town, alt. 300 m, in city park, 10 Oct. 1990, *P. Škubla*, BRACR33937; Zvolenská kotlina, Banská Bystrica town, Jakub, 48.764216°N, 19.144047°E, alt. 460 m, cattle grazed pasture, 6. Oct. 2010, *M. Zajac*, BRACR38286. **Spain**, Izurtza, Larrinagatxu, alt. 50 m, edge of road, 15. Nov. 2008, *I. Kautmanová*, BRACR12599. **Sweden**, Brunnby, Kullaberg, Ransvik, 56.2925189°N, 12.4751239°E, alt. 50 m, deciduous forest on rich soil, 24 Sep. 2007, *K. Bergelin*, KBE-07.164; *ibid.*, Lerhamns fälad, 56.2536000°N, 12.5222739°E, alt. 3 m, grazed coastal heath, 13 Nov. 2005, *K. Bergelin*, KBE-05.192.

Notes: No type of *Hygrocybe mucronella* has been preserved and the diagnosis was partly based on a description by Lasch (1828) from Brandenburg in Germany. Later it has been described also by Fries (1838), and Karsten (1879) reported it from Skåne in Sweden, again with no specimen preserved. Specimens KBE-05.192 and KBE-07.164 collected by K. Bergelin in Brunnby are probably the closest records to Karsten's collection, but as no photos of these collections were available, we decided not to select one of them as neotype. North American ITS sequences downloaded from NCBI labelled as *H. mucronella* form a distinctive clade in our alignment and should be described as a new species in future.

Morphologically the closest species to *H. mucronella* are *H. alpina* and *H. amara*. For the differences between these species, see the "Notes" under the *H. amara* and *H. alpina* or Table 2.

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