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Hebeloma hiemale Bres. in Arctic/Alpine Habitats

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Abstract: *Hebeloma hiemale* Bres. is a frequently collected species and occurs with many hosts in a wide variety of habitats. It is an ectomycorrhizal species that has been recorded with a broad range of both deciduous and coniferous trees, including *Salix* and *Populus*. However, it is not generally regarded as an arctic/alpine fungus and we are not aware of any previous reports of *H. hiemale* in these habitats. During ISAM VIII 2008 this species was collected with *Salix reticulata* on the Beartooth Plateau in Montana above tree-line at 3000 m asl. We also report a number of other arctic or alpine collections of *H. hiemale* from Greenland, Svalbard, Iceland and the U.S.A. (Wyoming and Colorado). This is the first report of *H. hiemale* from the Rocky Mountain alpine zone.

Key words: Agaricales, Arctic-alpine, Basidiomycota, *Hebeloma hiemale*, ectomycorrhizal fungi

Introduction: *Hebeloma hiemale* Bres. is one of the most common *Hebeloma* species throughout Europe. It has been recorded with a variety of hosts including *Betula*, *Carpinus*, *Corylus*, *Fagus*, *Larix*, *Quercus*, *Picea*, *Pinus*, *Populus*, *Salix* and *Tilia*. Similarly it has been recorded in a wide variety of habitats including most types of woodland or scrub, calcareous and acid soils, and boggy and dry environments. It has been frequently recorded in sand dunes where it is associated with *Salix repens* L. For example Watling (2005) records *H. hiemale* with *Salix repens* on St. Kilda in the Hebridean Islands of Scotland. It also has been recorded with a number of other species of *Salix*. Milne et al (2007) record *H. hiemale* (although named as *Hebeloma* sp.) from a mycorrhizal root tip in a sub-arctic habitat with *Salix lapponicum* L. Record DQ414727 from this study is the only sequence of *H. hiemale* in GenBank recovered from soil or mycorrhiza samples, which is surprising considering how frequently basidiomes are recorded for this species.

A number of species of *Hebeloma* are known to occur in alpine and arctic habitats usually associated with various species of *Salix* but also regularly recorded with *Dryas* spp. However, we are not aware of *H. hiemale* included in lists of arctic or alpine fungi.

During ISAM VIII, *H. hiemale* was collected on the Beartooth Plateau in Montana above tree-line at 3000 m asl where it was growing with *Salix reticulata* L. It is certainly a new record for this site (Cripps & Horak 2008a, 2008b) and possibly for the whole Rocky Mountain alpine zone. In our *Hebeloma* database we currently have an additional 15 collections of *Hebeloma hiemale* recorded in arctic or alpine environments. The Beartooth Plateau collection adds further evidence that a consistent group of *Hebeloma* species occurs throughout the arctic/alpine zone. These additional collections are from Greenland, Svalbard, Iceland and the U.S.A. (Wyoming and Colorado). We are not yet aware of any

collections from the alpine zones of Central Europe (confirmed as this species) but we would expect to find it in these locations. The hosts for these other Arctic-alpine collections were recorded as *Salix arctica* Pall., *S. glauca* L., *S. herbacea* L., *S. polaris* Wahlenb., *S. reticulata*, and *Dryas octopetala* L.

In this paper we provide a full description of the Beartooth Plateau collection of *Hebeloma hiemale* as well as a complete description for the species worldwide. We reserve providing a full key to the arctic/alpine *Hebeloma* species for a future publication, but will indicate how *H. hiemale* may be distinguished from the other *Hebeloma* spp. known to occur in these habitat.

We also provide molecular evidence using ITS sequence data for collections of *H. hiemale* from arctic or alpine locations showing that this taxon represents the same one collected in dunes or other lowland areas (not arctic/alpine) with *Salix* or other ectomycorrhizal hosts. We also provide evidence that *H. hiemale* is a taxon distinct from the other non-cortinate *Hebeloma* species that occur in arctic or alpine environments.

Materials and Methods: ITS sequence data were obtained from all database collections (Beker et al., to be published online) of arctic and alpine *Hebeloma hiemale* as well as from a number of other collections of *H. hiemale* from different habitats. Sequences were produced by direct sequencing following the protocols detailed in Eberhardt et al. (2009) and references therein. Table 1 summarizes the sequences of *H. hiemale* considered in this paper. Sequences were aligned visually using Se-Al (version 2.0a11; Rambaut 2002). When raw data suggested the presence of two different ITS types within the same organism, the two sequences were included in both length variants in the alignment. This was done by manually comparing the trace results of the forward and reverse reads. If the traces could not be interpreted, i.e. because of multiple length-variant sites closely together, the

Table 1: GenBank accession no. of *Hebeloma hiemale* sequences, original location and putative hosts.

Collection code & Herbarium no.*	GenBank acc. no.	Country of origin	Associated plants
L WAG-W9650	AF124703#	France	<i>Betula</i> sp.
L WAG-W9666	AF124707#	Netherlands	<i>Populus</i> sp.
HJB9479	GQ869481	Spain	<i>Pinus sylvestris</i>
HJB9384	GQ869482	England	<i>Betula</i> sp.; <i>Quercus</i> sp.
HJB9527	GQ869483	Belgium	<i>Salix</i> sp.
HJB8890	GQ869484	Belgium	<i>Carpinus betulus</i>
HJB8919	GQ869485	Belgium	Broadleaf
HJB9150	GQ869486	Belgium	<i>Picea</i> sp.
HJB8920	GQ869487, GQ869488	Belgium	Broadleaf
HJB10163	GQ869489, GQ869490	Belgium	<i>Picea</i> sp.
HJB10764	GQ869491	Norway	<i>Salix</i> sp.
C JV93-543, HJB10839	GQ869492, GQ869493	Iceland	<i>Betula</i> sp.
C JHC00-067, HJB10724	GQ869494	Norway	<i>Pinus</i> sp.
HJB11236	GQ869495, GQ869496	Belgium	<i>Populus</i> sp.
HJB11180	GQ869497	Scotland	<i>Salix repens</i>
HJB11030	GQ869498	Iceland	<i>Salix herbacea</i>
BR VJ03072, HJB11499	GQ869499, GQ869500	Belgium	<i>Alnus</i> sp.; <i>Betula</i> sp.
K (M)106237, HJB11606	GQ869501, GQ869502	England	unknown
HJB11695	GQ869503	Belgium	Broadleaf
HJB11699	GQ869504	France	<i>Fagus</i> sp.; <i>Picea</i> sp.
HJB11704	GQ869505	France	<i>Fagus</i> sp.; <i>Quercus</i> sp.
HJB11703	GQ869506, GQ869507	France	<i>Fagus</i> sp.; <i>Quercus</i> sp.
HJB11701	GQ869508	France	<i>Fagus</i> sp.; <i>Picea</i> sp.; <i>Pinus</i> sp.
HJB11702	GQ869509, GQ869510	France	<i>Fagus</i> sp.; <i>Picea</i> sp.; <i>Pinus</i> sp.
PDD PL10406, HJB 11570	GQ869511	New Zealand	<i>Salix cuprea</i>
HJB8455	GQ869512, GQ869513	England	<i>Betula pendula</i>
E 00244083, HJB11905	GQ869514	Scotland	<i>Picea sitchensis</i>
C TB99.258, HJB12195	GQ869515	Greenland	<i>Dryas octopetala</i> ; <i>Salix arctica</i>
C TB99.118, HJB12210	GQ869516	Greenland	<i>Dryas octopetala</i> ; <i>Salix arctica</i>
C TB06.067, HJB12193	GQ869517	Greenland	<i>Dryas octopetala</i> ; <i>Salix</i> sp.
C TB06.081, HJB12202	GQ869518	Greenland	<i>Dryas octopetala</i>
HJB11948	GQ869519	Svalbard & Jan Mayen Islands, Norway	<i>Salix polaris</i>
HJB11965	GQ869520	Svalbard & Jan Mayen Islands, Norway	<i>Salix polaris</i>
HJB11964	GQ869521, GQ869522	Svalbard & Jan Mayen Islands, Norway	<i>Salix polaris</i>
HJB12034	GQ869523	Svalbard & Jan Mayen Islands, Norway	<i>Salix polaris</i>
C TB99.146, HJB12200	GQ869524	Greenland	<i>Salix arctica</i>
HJB12443, NLF26.07.08k6	GQ869525	Canada	<i>Alnus viridis</i> ; <i>Betula pendula</i> ; <i>Larix laricina</i> ; <i>Picea glauca</i>
HJB12457\$	GQ869526	United States	<i>Salix reticulata</i>
ZT 8901, HJB12544	GQ869527	Greenland	<i>Salix glauca</i>
ZT 6417, HJB12571	GQ869528	United States	<i>Dryas octopetala</i> ; <i>Salix reticulata</i>
ZT 8072, HJB12574	GQ869529	United States	<i>Salix</i> sp.
ZT 9828, HJB12581	GQ869530	United States	<i>Dryas octopetala</i> ; <i>Salix reticulata</i>
C JV86-799, HJB12356	GQ869531	Denmark	<i>Picea</i> sp.
C 7490F, HJB12369	GQ869532	Finland	<i>Populus tremula</i>
C 7242F, HJB 12370	GQ869533	Finland	<i>Corylus avellana</i> ; <i>Fraxinus excelsior</i> ; <i>Ulmus</i> sp.

* Collection codes: C Copenhagen, Denmark; E Edinburgh, UK; K Kew, UK; L Leiden, Netherlands; PDD Auckland, New Zealand; HJB personal herbarium H.J. Beker.

Previously published as *H. helodes*.

\$ ISAM VIII Beartooth collections.

sequences were not included in the analyses. Ambiguous code was used for suspected base pair substitutions. Length variation among different copies of the ITS was found in 10 of the 43 analyzed collections.

To demonstrate the monophyly of *H. hiemale*, the newly obtained ITS sequences were added to the published dataset of Aanen et al. (2000), AF124665 – AF124716 which contains data of 49 lowland and alpine collections of *Hebeloma* from Europe, including three outgroup taxa. The alignment also was prepared in Se-Al by visual examination. The ML analysis was done in RAxML (Stamatakis 2006) with 100 replicates of heuristic search and 1000 replicates of bootstrap under the GTRMIX model FigTree (version 1.2.3, Rambaut 2009) was used to visualize the results.

Ambiguous base calls were (under specific conditions) considered as signal rather than noise and treated as indications of intragenomic variation (IGV). See Eberhardt et al. (2009) for details. Counts (bp) of IGV were done visually; intraspecific variation (ISV, i.e. sequence variation between different collections not attributable to intragenomic variation) was analyzed in PAUP (version 4.0b10, Swofford 2002) as absolute difference counts using the standard settings of PAUP* for DNA data. Sequences of the 43 *H. hiemale* collections and of intercompatibility group (icg) 21 (Aanen et al. 2000, GenBank acc. no. AF124703 and AF124707) were considered in the analyses.

For detecting and visualizing potential infraspecific substructure of *H. hiemale* and icg 21 ITS sequence variation, a NJ bootstrap analysis (1000 replicates) and a Neighbor-Net analysis were performed with Splitstree (version 4.10; Huson & Bryant 2006), using uncorrected-p distances and averaging over ambiguous states. For Neighbor-Net analysis ordinary least square distances were used with a threshold of 10^{-4} . Details of the morphological analyses are given in

Eberhardt et al. (2009). For each collection at least 50 spores were measured in Melzer's reagent, excluding the apiculus. The maximum length and width of each spore was measured and its Q value (ratio of length to width) calculated. Average length, width and Q value were calculated and recorded alongside the median, standard deviation and 5% and 95% percentiles. The assessment and coding of spore characters follows Vesterholt (2005). This includes 'O' for spore ornamentation types, 'P' for amount of loosening of the perispore and 'D' for the level of dextrinoid reaction. The average width of the cheilocystidium apex appears to be an important character in the separation of species within *Hebeloma* (Vesterholt 2005). It is also important, when determining the average apex width, not to be selective with regard to the cystidia chosen for measurement. To determine the average width at the apex, about 100 cheilocystidia were measured. For other measurements, at least 20 cheilocystidia, separated from the lamella edge, were measured from each collection. Because of the complex shapes of the cheilocystidia, four measurements were made: length, width at apex, width at narrowest point in central region and maximum width in lower half. The measurements are given in this order and an average value was calculated for each of these measurements. The measurements were made in 5% KOH. Unless otherwise mentioned, cited specimens are presently located in the personal collection of H.J. Beker (HJB), Belgium.

Taxonomy

Hebeloma hiemale Bres. (Fig. 1, Fig. 2)

Bresadola 1892: 52-53, pl. CLX 1, iconotype.

Beartooth Plateau Collection

Macroscopic features (Fig. 1): *Pileus* 21–32 mm in diam., broadly umbonate, dry, clay-buff to Isabella (sensu Vesterholt 2005), fairly uniform across much of the pileus but getting a little paler towards the margin; margin entire, not striate.

Lamellae emarginate, 5 mm deep at the widest point, moderately close with about 60 full length lamellae and usually between 1 and 3 lamellulae, brown following spore maturity; edge fimbriate, paler than lamella surface; droplets visible with the naked eye. *Stipe* central, cylindrical or clavate towards the base, 28–42 mm long, with central width 5–7 mm (up to 15 mm at the base), white or alutaceous (light tan to medium yellow), not significantly discoloring on handling, becoming hollow with age; surface dry, pruinose particularly towards the apex. *Cortina* not observed. *Flesh* cream to pale brown, not discoloring when bruised. *Odor* raphanoid, taste not recorded. Spore print brownish olive.

Microscopic features (Fig. 2): *spore color* under the microscope yellow brown; *spores* amygdaloid with small apiculus and many with a distinct but small papilla at the end opposite the apiculus, guttulate with one or occasionally more oily drops, almost smooth to weakly ornamented, with a slight loosening of the perispore visible at high magnification in some spores, many spores distinctly but weakly dextrinoid (O1/2, Po/1, D1/2); spore size based on $n = 58$ spores (5 % to 95 % percentile range) $9.6\text{--}11.3 \times 5.9\text{--}6.7 \mu\text{m}$, median $10.5 \times 6.3 \mu\text{m}$, on average $10.5 \times 6.3 \mu\text{m}$ (S. D. length $0.56 \mu\text{m}$ and width $0.25 \mu\text{m}$), Q value (for 5 % to 95 % percentile range) 1.54–1.78, median 1.65 and av. 1.66 (S. D. 0.08).

Basidia cylindrical to clavate, 4-spored, $31\text{--}36 \times 9.3\text{--}10.9 \mu\text{m}$, on average $33.5 \times 9.8 \mu\text{m}$.

Pleurocystidia not found. *Cheilocystidia* mostly hourglass-shaped, i.e. clavate in the upper part, the majority narrower in their central region and swollen again in the lower half, sometimes more cylindrical in their lower half (clavate-stipitate), often with apical or median thickening and occasionally with septa; based on 117 cheilocystidia, width of apex (5 % to 95 % percentile range) $5.9\text{--}8.6 \mu\text{m}$, median $7.4 \mu\text{m}$ and av. $7.4 \mu\text{m}$ (S.D. $0.81 \mu\text{m}$); based on 23 selected cheilocystidia (5% to 95% percentile range) the overall dimensions were $47\text{--}72 \times 6.9\text{--}8.5 \times 3.8\text{--}5.5 \times 4.6\text{--}7.5 \mu\text{m}$ and av. $58 \times 7.8 \times 4.6 \times 5.7 \mu\text{m}$

(see methods). *Caulocystidia* resemble cheilocystidia but tend to be larger. *Pileipellis* an ixocutis with an epicutis in the range $60\text{--}95 \mu\text{m}$ deep, with embedded hyphae up to $5 \mu\text{m}$ broad, often encrusted, hyaline or occasionally pigmented. Cutis orange brown, made up of cylindrical to isodiametric elements. Clamp connections present throughout basidiome. *Habitat*: Basidiomes in a small scattered group with *S. reticulata* in alpine scrub on acid soil.

Material studied: USA. Montana, Carbon County, Beartooth Plateau, site 1: Birch Site Quad Creek (N45.02456; W109.41079, alt. 3000m), Aug. 8 2008, leg. M. Nauta, HJB12457.

Figure 1. *Hebeloma hiemale*, collection HJB12457, Beartooth Plateau (MT, USA). Photograph H.J. Beker

***Hebeloma hiemale* Bres. (Fig. 3)**

Composite description based on 43 collections worldwide

Macroscopic features: *Pileus* up to 80 mm in diam., convex to broadly umbonate, dry or slightly viscid surface, occasionally with some spotting but rarely hygrophanous, with fairly uniform color across most of the pileus but sometimes darker in center or paler at margin, clay-buff to dark pinkish buff to Isabella or ochraceous, can approach clay-pink in the center and pale pinkish buff or cream near the margin; margin smooth, slightly involute in immature basidiomes, wavy (undulate) in older specimens.

Figure 2. Collection HJB12457 of *Hebeloma hiemale*: A. spores x 1600 in Melzer's Reagent, B. spore ornamentation x1600 in Melzer's Reagent, C. cheilocystidia x1000 in 5% KOH, showing basal swelling D. cheilocystidium with median thickening x1000 in 5% KOH, E. basidium x1000 in 5% KOH, F. epicutis x80 in 5% KOH. Bars = 10µm in A-E and = 100µm in F. Photographs H.J.Beker.

Lamellae adnate to emarginate, 3–6 mm deep at the widest point, sub-distant to close, with 35–70 full length lamellae and usually between 1 and 3 lamellulae, cream to alutaceous when young, later brown following spore maturity; edge fimbriate, paler than lamellae surface; droplets on lamellae usually visible with naked eye but occasionally only seen with 10x lens. *Stipe* central, cylindrical or clavate towards the base, up to 80 mm long, with central width of 4–14 mm (base up to 15 mm), white or alutaceous, sometimes discoloring from the base upwards particularly when handled, becoming hollow with age and often with a superior hanging string visible in stipe when cut in half; surface dry, pruinose particularly towards the apex. *Cortina* not observed. *Flesh* cream or pale brown, sometimes discolouring when bruised. *Odor* raphanoid occasionally with a cacao component but sometimes no smell recorded, taste

raphanoid. *Spore print* brownish olive to umber. *Microscopic features*: *Spore color* under the microscope yellow to yellow brown; *spores* amygdaloid, with small apiculus and many with a distinct but small papilla at the end opposite the apiculus, guttulate with one or occasionally more oily drops, most weakly ornamented, usually some almost smooth and occasionally some distinctly ornamented spores, with a slight loosening of the perispore visible at high magnification in some spores, occasionally with distinctly loosening perispore, most distinctly but weakly dextrinoid [O1,O2,(O3); Po,P1,(P2); D1,D2], (based on at least 50 spores of each of the 43 collections), spore range 9.0–14.3 × 5.2–7.9 µm (for 5 % to 95 % percentiles), on average 10.0–12.7 × 5.6–7.3 µm, median 10.0–12.7 × 5.6–6.3 µm (S. D. length 0.72 µm and width 0.30 µm), Q value (5% to 95% percentile range) 1.41–2.29, with av. 1.61–2.05, median 1.63–2.04 (S.D.

Figure 3. Collections of *Hebeloma hiemale*: A. HJB11965, arctic collection from Svalbard, B. 7490F (HJB12369) non-arctic collection from Finland, C. HJB11704 non-arctic/alpine collection from France; D. HJB 11948 arctic collection from Svalbard. Photographs (B) J. Vauras; (A), (C) & (D) H.J. Beker.

0.11). *Basidia* cylindrical to clavate, 4-spored, $27.3\text{--}39.1 \times 8.3\text{--}9.8 \mu\text{m}$. *Pleurocystidia* not found. *Cheilocystidia* mostly hourglass-shaped, i.e. clavate in the upper part, the majority narrower in their central region and swollen again in the lower half, sometimes more cylindrical in their lower half (clavate-stipitate) or more cylindrical in their upper half (ventricose or lageniform), often with apical or median thickening, occasionally with septa, sometimes bent in center; based on about 100 cheilocystidia from each of 43 collections (for 5 % to 95 % percentile range), apex width $4.3\text{--}10.8 \mu\text{m}$, on average $5.6\text{--}8.5 \mu\text{m}$, median $5.7\text{--}8.5 \mu\text{m}$ (S.D. $0.96 \mu\text{m}$); based on all 43 collections, overall average for *Cheilocystidia* $35\text{--}62 \times 5.6\text{--}8.5 \times 3.6\text{--}4.9 \times 4.9\text{--}7.4 \mu\text{m}$. *Caulocystidia* resemble cheilocystidia but tend to be larger. *Pileipellis* an ixocutis with an epicutis in the range $40\text{--}150 \mu\text{m}$ thick; embedded hyphae up to $5.5 \mu\text{m}$ broad, often encrusted, hyaline or occasionally pigmented. Cutis buff to orange brown and made up of cylindrical to isodiametric elements. *Clamp connections* present throughout the fruiting body.

Habitat and distribution: *Basidiomes* usually in small scattered groups, but occasionally solitary and rarely caespitose. Hosts include: *Betula*, *Carpinus*, *Corylus*, *Fagus*, *Larix*, *Quercus*, *Picea*, *Pinus*, *Populus*, *Salix* and *Tilia*. Soil conditions have been recorded as wet, dry, acid and calcareous. Habitats include woodland, roadside and dunes as well as arctic and alpine. Geographically the 43 collections cover Belgium, Canada, Denmark, England, Finland, France, Greenland, Iceland, New Zealand, Norway, Scotland, Spain, Svalbard and the United States. Fig. 4 shows the distribution of all 43 collections while Fig. 5 shows the distribution of the arctic/alpine collections.

Material examined: BELGIUM: Foret de Soignes, (N50.79; E04.38; alt. 53m) on rotten litter in a mixed woodland under *Carpinus betulus*, 16.10.2003, H. Beker, HJB8890;

Barvaux, (N50.34; E05.49; alt. 185m) on rotten litter in a mixed woodland under deciduous trees, 18.10.2003, H. Beker, HJB8919; Barvaux, (N50.34; E5.49; alt. 185m) on rotten litter in a mixed woodland under deciduous trees, 18.10.2003, H. Beker, HJB8920; Barvaux, (N50.34; E05.49; alt. 185m) on wet soil in a mixed woodland under *Salix* sp., 18.10.2003, H. Beker, HJB9527; Tiewinkel, (N50.9666667; E05.2333333; alt. 25m) on wet soil in a woodland including *Alnus* sp. and *Betula* sp., 16.10.2003, J. Volders, VJ03072, double HJB11499; Namur, (N50.46; E04.86; alt. 120m) on rotten litter in a conifer woodland under *Picea* sp., 21.11.2003, M. Ghyselinck, HJB9150; Goutelle, (N50.0150000; E05.2291667; alt. 401m) on grassy soil in a mixed woodland under *Picea* sp., 3.10.2004, M. Lenne, HJB10163. Brussels: (N50.79; E04.38; alt. 110m) on bare soil under *Populus* sp., 7.10.2005, H. Beker, HJB11236; Andenne, (N50.4755333; E05.1154833; alt. 150m) on bare soil in a mixed woodland under deciduous trees, 10.9.2006, H. Beker, HJB11695. CANADA: North West of Konrad Lake, (N56.2276180; W62.7917520; alt. 147m) in a mixed woodland including *Alnus viridis*, *Betula pendula*, *Larix laricina*, *Picea glauca*, 26.7.2008, E. Ohenoja, NLF26.07.08k6, double HJB12443. DENMARK: Løvenholm Skov, (N56.43; E10.47; alt. 30m) on mossy soil on a woodland roadside under *Picea* sp., 2.10.1986, J. Vesterholt & S. Svane, JV86-799, C, double HJB12356; ENGLAND, Swallowcliffe, nr Salisbury, (N51.04; W02.05; alt. 127m) on soil, 29.11.1964, J B Hindley, K(M)106237, (HJB11606); Sherrards Wood, Welwyn Garden City, (N51.83; W00.21; alt. 73m) on wet soil under *Betula* sp. and *Quercus* sp., ?10.2002, A. Burnham, HJB9384; La Baraka, Virginia Water (N51.4093800; W00.5650113; alt. 70m) on grassy soil in an urban garden under *Betula pendula*, 5.11.2002, H. Beker, HJB8455. FINLAND: Jomala comune, Ramsholmen NR, (N60.09; E19.86; alt. 10m) on grassy soil in a grazed woodland including *Corylus avellana*, *Fraxinus excelsior* and *Ulmus* sp., 7.9.1992, J. Vauras, 7242F, split as HJB12370; Mariehamn,

Figure 4. Distribution of 43 collections of *H. hiemale* cited.

Figure 5. Distribution of 16 arctic/alpine collections of *H. hiemale* cited.

- Ytternäs camping place, (N60.07; E19.94; alt. 10m) on gravelly soil in a lawn under *Populus tremula*, 11.10.1992, J. Vauras, 7490F, split as HJB12369. FRANCE: Foret d'Ecouvès, (N48.5474000; W00.0348167; alt. 409m) on bare soil in a mixed woodland including *Fagus* sp. and *Picea* sp., 6.10.2006, H. Beker, HJB11699; Foret Andaines, (N48.5807; W00.4582; alt. 170m) on rotten litter in a mixed woodland including *Fagus* sp., *Picea* sp. and *Pinus* sp., 7.10.2006, H. Beker, HJB11701; Foret Andaines, (N48.5807; W00.4582; alt. 170m) on rotten litter in a mixed woodland including *Fagus* sp., *Picea* sp. and *Pinus* sp., 7.10.2006, H. Beker, HJB11702; Foret Andaines, (N48.5807; W00.4582; alt. 170m) on rotten litter in a mixed woodland including *Fagus* sp. and *Quercus* sp., 7.10.2006, H. Beker, HJB11703; Foret Andaines, (N48.5807; W00.4582; alt. 160m) on wet litter in a mixed woodland including *Fagus* sp. and *Quercus* sp., 7.10.2006, H. Beker, HJB11704. GREENLAND: Zackenberg, (N74.5; W21.0; alt. 704m) on mossy soil in a scrub with *Salix arctica*, 29.7.1999, T. Borgen, TB99.146, double HJB12200; Zackenberg, (N74.5; W21.0; alt. 150m) in scrub with *Dryas octopetala* and *Salix arctica*, 27.7.1999, T. Borgen, TB99.118, double HJB12210; Zackenberg, (N74.5; W21.0; alt. 704m) on mossy soil in scrub with *Dryas octopetala* and *Salix arctica*, 9.8.1999, T. Borgen, TB99.258, double HJB12195; Zackenberg, (N74.5; W21.0; alt. 704m) on mossy soil in scrub with *Dryas octopetala* and *Salix* sp., 2.8.2006, T. Borgen, TB06.067, double HJB12193; Zackenberg, (N74.5; W21.0; alt. 704m) in scrub with *Dryas octopetala*, 3.8.2006, T. Borgen, TB06.081, double HJB12202; NW below Nasaasaaq, E-valley, E of Sisimiut (N66.925; W53.611; alt. 50m) with *Salix glauca*, 18.8.2000, E. Horak, ZT8901, ZT, double HJB12544. ICELAND: Egilsstadarskog, Sudur-Múlasysla: Egilsstadaskogur, (N65.25; W14.37; alt. approx. 250m) with *Betula* sp., 7.8.1993, J. Vesterholt, JV93-543, C, double HJB10839; Kinnarhryna, (N64.8370333; W23.4583000; alt. 242m) on bare soil in scrub with *Salix herbacea*, 27.7.2005, M.L.Beker, HJB11030 NEW ZEALAND: Kaituna Track, Golden Bay, (S40.66; E172.55; alt. 50m) on soil under a farmed hedge under *Salix cuprea*, 10.4.2006, P. Leonard, PL10406, PDD, double HJB11570. NORWAY: Ringerike, Ringen at Samsjoen, N of Hønefoss, (N63.09; E10.67; alt. 214m) under *Pinus* sp., 12.9.2000, J. Heilmann-Clausen, JHC00-067, C, double HJB10724; Tislet, (N61.08; W06.50; alt. 242m) on a pathside under *Salix* sp., 5.8.2004, A. Andrews, HJB10764. SCOTLAND UK: Ladmore, (N56.48; W03.53; alt. 85m) under *Picea sitchensis*, 2000, R. Watling, E00244083, E, (HJB11905); CorrieCas, (N57.782720; W04.864242; alt. 835m) on skeletal soil under *Salix repens*, 24.8.2005, G. Walker, HJB11180. SPAIN: Asno, (N40.845; W04.026; alt. 1261m) on rotten litter in a conifer woodland under *Pinus sylvestris*, 10.11.2003, E. Emmett, HJB9479. SVALBARD NO: Colesdalen, (N78.1104667; E15.0537167; alt. 48m) on soil in a grazed grassland with *Salix polaris*, 10.8.2007, M.L.Beker & H. Beker, HJB11948; Adventdalen, (N78.1733500; E16.0139667; alt. 25m) on grazed scrub with *Salix polaris*, 12.8.2007, M.L.Beker & H. Beker, HJB11964; Adventdalen, (N78.1742333; E16.0040167; alt. 28m) on soil in grazed scrub with *Salix polaris*, 12.8.2007, M.L.Beker & H. Beker, HJB11965; Ossian, (N78.9475667; E12.4355333; alt. 10m) on soil in coastal scrub with *Salix polaris*, 17.8.2007, M.L.Beker & H. Beker, HJB12034. UNITED STATES: Wyoming, Park County, Beartooth Pass, N of Mt. Wymont, Highline Trail, (N45.0046; W109.4070; alt. 3200m) on soil in mountain scrub with *Dryas octopetala* and *Salix reticulata*, 8.8.1998, E. Horak, ZT6417, ZT, double HJB12571; Montana, Carbon County, Beartooth Site 1: Birch Site Quad Creek, (N45.02456; W109.41079; alt. 3004m) on soil in scrub with *Salix reticulata*, 8.8.2008, M. Nauta, HJB12457. Colorado, Summit County, Loveland Pass, West-7, (N39.664; W105.864; alt. 3750m) in mountain scrub with *Salix* sp., 7.8.1999, E. Horak, ZT8072, ZT, double

HJB12574; Lake County, Sawatch Range, Twin Lakes, Independence Pass S, (N39.1115; W106.5668; alt. 3750m) on mountain scrub with *Dryas octopetala* and *Salix reticulata*, 13.8.2001, E. Horak, ZT9828, ZT, double HJB12581.

Results and Discussion: The sequences of *Hebeloma hiemale* form a distinct clade with 89 % bootstrap support in the context of a dataset of *Hebeloma* sequences representing all seven sections of *Hebeloma* considered in the classification of Vesterholt (2005) (Fig. 6). The *H. hiemale* clade also includes two sequences of icg (intercompatibility group) 21 of Aanen et al. (2000), suggesting that our concept of *H. hiemale* corresponds to this intercompatibility group. This is also in agreement with Aanen's (1999) description of the morphology of icg 21. Whereas Aanen et al. (2000) assumed that icg 21 would not associate with *Salix*, our observations suggest the opposite.

Figure 6. Maximum Likelihood analysis 52 *Hebeloma hiemale* sequences obtained for this study and 49 *Hebeloma* spp. and three outgroup sequences obtained by Aanen et al. (2000). Bootstrap analysis was calculated in 1000 replicates. The clade names follow the section level classification of Vesterholt (2005).

The clades recovered in Fig. 6 correspond with the results of the analyses done by Aanen et al. (2000). According to our analyses, *H. hiemale* is a sister clade to *H. crustuliniforme* s.l., which also includes a number of alpine taxa, e.g. *H. alpinum* and *H. minus*.

Taking a closer look at the intragenomic and intraspecific variation of the sequences of the 43 considered *H. hiemale* collections, the species appears rather variable at first glance with 44 variable positions in 465 aligned positions of the ITS1 and ITS2. Many of the single bp substitutions and all of the indels of 1-3 bp are attributable to intragenomic variation (Min(IGV) = 0 bp, Max(IGV) = 10 bp, Median (IGV) = 3 bp; the typical length of the two spacer regions together is 457bp. Intraspecific variation involves only 5 positions of the aligned ITS1 and ITS2 all of which are single bp substitutions (Min(ISV) = 0, Max(ISV) = 3, Median (IGV) = 0). Counting the number of sequences with at least one unique ambiguous position suggests a minimum of 17 unique alleles of the ITS. Not all of these ITS types are necessarily functional (not known) and it cannot be excluded that some ambiguities do not reflect sequence variation or errors. However, the coding regions (3' portion of the SSU and 5' portion of the LSU) are entirely free of ambiguities. The generally high number of ambiguities in the spacer regions and their lack of obvious patterns in the alignment suggest that the number of different ITS alleles in *H. hiemale* is rather high.

The high number of potential ITS types and the probably even higher number of different alleles, the relatively low number of collections per geographic area and the fact that the sequences were gained from dikaryotic samples do not allow the application of proper population genetic analyses. Neighbor joining bootstrap analyses gave no indication of population substructure. Only one sequence pair received support greater than 75 % (86 %; AF124707 and HJB8919). This also corresponds to the ML bootstrap results.

The Neighbor-Net analysis of *H. hiemale* sequences (Fig. 7) suggests that within the current dataset there is no obvious infraspecific structure related to geographical origin, altitude or mycorrhizal host species.

Morphologically the *H. hiemale* collections also form a consistent group. Based on the morphological and molecular evidence all these collections do appear to represent the same species. There is no evidence for the delineation

Figure 7. Neighbor-Net analysis of the ITS sequences of 43 collections of *H. hiemale* and two sequences from GenBank also attributed to *H. hiemale*. Arctic alpine collections are indicated by bigger squares and bold print as opposed to lowland collections indicated by smaller squares and regular print. Split squares are used where two or more collections from different geographical areas coincided in the same node. Collections from *Salix* spp. are underlined. The letter preceding the collection no. or GenBank acc. no. indicates the country of origin (see legend). HJB12457 (see bottom) is the ISAM VIII Beartooth collection.

of alpine and arctic collections as a separate taxon. Thus, it appears that *H. hiemale* is a circumpolar species that has the ability to grow under arctic and alpine conditions. Based on this study, the existence of population substructure at the allelic level cannot be excluded, but so far there is no indication of any biogeographic or ecological infraspecific population substructure.

The above description of *H. hiemale* appears to conform well to the original species description given by Bresadola (1898). His illustration and description do indicate more of a contrast between the color at the centre of the pileus and that at the margin than we normally see but a number of the collections included in the above description do exhibit such a character. Bresadola gives the spore size as 12–13 x 6–7 µm which although slightly high is well within the above description.

Grilli (1997) provides a description based on four collections (collected between 1912 and 1924) determined by Bresadola. His composite description gives a spore size range of 10.7–12.6 x 5.5–6.6 µm and a Q range of 1.78–2.08. For the cheilocystidia he gives length as 42–57 µm, apex as 5.9–7.8 µm and basal width as 5.4–7.0 µm. Again this fits well with the above description. When looking at the average measurements of spores for each of the four collections we find they range from 11.4–12.4 x 5.8–6.5 µm and Q from 1.86–2.0. For the cheilocystidia the ranges for his averages are: 47.3–51.1 x 6.4–6.8 x 3.2–3.7 x 6.1–6.5 µm. Here his spore Q values are close to the top of our range and perhaps more importantly his range for the narrowest point in the top half of the cheilocystidium is surprisingly low. While this may lead us to some concern over confusion with *H. ingratum* Bruchet, the latter species has an average spore length of at most 11 µm, so Bresadola's collections are unlikely to represent this species. No holotype material exists for *H. hiemale* (there is an iconotype, plate CLX in Bresadola (1898)) and epitypification would be helpful. If it were possible to sequence

one of the collections described by Grilli, above, such a collection might be a good candidate for an epitype, hence we defer epitypification until this has been explored.

There are two collections of *H. hiemale* in the Swedish Museum of Natural History both collected by Bresadola in Gocciadoro, Trentino which is the location cited in the original 1898 description. Collection F14406 was collected in November 1914. A microscopic analysis reveals spores and cheilocystidia typical of the group of species *H. crustuliniforme* s.l. and the presence of a large number of full length lamellae would indicate this as being one of the larger species within this group. Further, the spores are quite short, averaging 10.6 µm, and so this collection would be highly unlikely to resemble Bresadola's icon or original description. Collection F28516 was collected in November 1900. This collection is mixed, as noted by E. Grilli. Part of the collection likely represents *H. cavipes* Huijsman, a species close to *H. hiemale* but with more ornamented and more dextrinoid spores, usually on average larger than those of *H. hiemale*. The spores of this collection have an average length of 13.2 µm and a Q over 2. The second part of the collection is more difficult to determine. Grilli suggested that it might be *H. truncatum* var. *pruinsum* M. M. Moser (which is synonymous with *H. erumpens* Contu). We were unable to find any cheilocystidia in the fragment we examined but the spores (av. 11.1 x 5.9 µm) were certainly too large for *H. erumpens*; although with spores at the shorter end of the range, this material could represent *H. hiemale*.

Hebeloma hiemale can be separated from other non-cortinate arctic or alpine species using the following characters:

- The majority of the spores are weakly ornamented (O2) albeit some may be more strongly ornamented or almost smooth, the perispore is not consistently strongly loosening (not P3) and the

majority of spores are weakly but distinctly dextrinoid (D2).

- The average spore length is between 10 and 12.7 μm .
- The average width of the apex of the cheilocystidium is less than 8.6 μm .
- If we denote the average width of the apex of the cheilocystidium by A, the av. width of the narrowest point in the top half of the cheilocystidium by M and the av. width of the widest point in the lower half by B, then A/M is at least 1.5, B/M is at least 1.2 and A/B is at most 1.65.

Using these characters it would appear, based on a number of collections in our database, that the only other arctic or alpine species that might be confused with *H. hiemale* is *H. ingratum*. However, based on our observations, the latter species usually has strongly ornamented spores of maximum av. length at most 11 μm and narrower cheilocystidia in the median with M at most 4.2.

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