Decrypting the Hebeloma crustuliniforme complex: European species of Hebeloma section Denudata subsection Denudata (Agaricales)

U. Eberhardt1,2, H.J. Beker3, J. Vesterholt4

Key words
Hebeloma eburneum
Hebeloma helodes
Hebeloma lutescens
Hebeloma minus
Hebeloma pusillum
MCM7
mitochondrial SSU
Salix

Abstract
Hebeloma subsection Denudata includes the type of H. section Denudata, Hebeloma crustuliniforme, as well as the majority of the taxa commonly included in the Hebeloma crustuliniforme complex. Complementing the work of D.K. Aanen and co-workers, and using refined morphological and molecular methods we were able to recognize further individual taxa within the section. Fifteen species occurring in Europe are assigned to H. subsection Denudata. Of these, we describe eight species as new, namely H. aeneni, H. aurantiombrinum, H. geminatum, H. louiseae, H. luticystidiatum, H. paliddolabiatum, H. perexiguum and H. saliciola. Naucoria bellotiana, a species very similar to H. alpinum is recombined into Hebeloma. A key to Hebeloma subsection Denudata is provided. We demonstrate that within this subsection there is good overall consistency between morphological, phylogenetic and biological species concepts. In contrast to current opinion, in this group there is little species overlap, particularly when also considering species frequencies, between arctic and alpine floras on one hand and temperate on the other.

INTRODUCTION

Hebeloma is a genus of ectomycorrhizal fungi occurring in many different habitats in the northern hemisphere (Marmeisse et al. 1999) and indeed worldwide, with the possible exception of regions, where Fagales are not endogenous, such as Northern South America (Tedersoo et al. 2010) or Africa. However, as typical nursery fungi, Hebeloma spp. are likely to have been introduced through human activity (see Sulzbacher et al. 2013). Hebeloma crustuliniforme is one of the most often recorded (Vesterholt et al. 2014) species of the genus Hebeloma, but it is widely recognised that what has in the past been referred to as H. crustuliniforme is among the most notorious species complexes that have long defied recognition of individual taxon. In Vesterholt et al. (2014) we proposed an epitope for H. crustuliniforme so as to tie this name to a particular taxon within this complex, which we then suggested should be referred to as H. crustuliniforme (Bull.) Quél. emend. Vesterh., U. Eberh. & Beker to avoid confusion with earlier applied concepts of the taxon. As we stated at the time, this was the first step towards unravelling this complex of species within sect. Denudata.

More than 10 years ago, D.K. Aanen and others (Aanen 1999, Aanen et al. 2000, Aanen & Kuyper 2004) carried out a profound study of the H. crustuliniforme complex, using three different approaches, sporocarp morphology, molecular studies and intercompatibility tests, testing for dikaryotization between pairs of monokaryotic strains. They found 20 intercompatibility groups (ICGs) within the complex. A small number of strains were incompatible with some strains of other ICGs or could not be unambiguously assigned to a unique ICG.

1 Staatliches Museum für Naturkunde Stuttgart, Rosenstein 1, D-70191 Stuttgart, Germany; corresponding author e-mail: unula.eberhardt@smns-bw.de.
2 Ghent University, Dept. Biology, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.
3 Rue Père de Deken 19, B-1040 Bruxelles, Belgium.
4 Natural History Museum of Denmark, Gothersgade 130, DK-1123 Copenhagen K., Denmark. Deceased.

It is tempting to consider ICGs as biological species and they may well represent biological species, but as Aanen & Kuyper (1999) point out, the production of basidiospores in the dikaryotic mycelia generated in the intercompatibility tests could not be tested, therefore we do not know what the implications of the observed intercompatibility in nature are. In general, on the population level, partial intercompatibility may correspond to incomplete speciation (Aanen & Kuyper 1999). Almost all of a quite high number of strains stemming from 110 collections could be unambiguously assigned to a single ICG, thus supporting the notion that distinct biological taxa have already formed in this species aggregate.

Aanen & Kuyper (1999) linked this finding of partial compatibilty between some ICGs with the difficulty of morphologically separating ICGs into morpho-species, suggesting that partial intercompatibility might contribute to the failure to form distinct morphological and ecological syndromes. Accordingly, Aanen and co-workers (Aanen 1999, Aanen et al. 2000, Aanen & Kuyper 2004) adopted wide species concepts, i.e. H. crustuliniforme including H. alpinum with six ICGs and H. pusillum (four ICGs), partially overlapping with H. helodes (at least six ICGs). In the later work (Aanen & Kuyper 2004) H. helodes and H. pusillum were merged.

Molecular studies showed that three of the ICGs of the H. crustuliniforme complex were molecularly quite distinct from the other 17 ICGs. This supported a view published initially by Boekhout (1982), then molecularly supported (Aanen et al. 2000, Boyle et al. 2006), suggesting that there are indeed two distinct groups within this complex, one species complex around H. velutipes (syn. H. leucosarx acc. Grilli 2007) and another in sect. Denudata, around H. crustuliniforme. It has found its way into the classification system by Vesterholt (2005), which recognized two distinct sections Velutipes and Denudata, each including taxa that were formerly considered representatives of the H. crustuliniforme complex.
The focus of the present study is on _H_. sect. _Denudata_ and particularly on members of the _H. crustuliniforme_ complex and related taxa assigned to subsect. _Denudata_. _Denudata_ (described more fully below) of which _H. crustuliniforme_ is the type. The delimitation of _H_. subsect. _Denudata_ adopted in this paper is based on morphological characters. Within the scope of this paper we do not demonstrate the molecular delimitation of _H_. subsect. _Denudata_. A separate publication (Eberhardt et al. in prep.) will be concerned with the classification of subsections of _H_. sect. _Denudata_ and species from these other subsections.

With regard to species delimitation, we follow de Queiroz (2007) unified species concept that treats different criteria such as morphological distinctness, monophyly, evolutionary distance or intercompatibility as independent lines of evidence for recognizing separately evolving lineages, i.e. species. This implies that not all lines of evidence necessarily support a species or the assignment of a particular specimen to a species, particularly in young species or species with a recent history of range changes. Hybridization and deep coalescence are additional processes, which may prevent representatives from the same biological species from forming monophyletic clades in phylogenetic analyses. In practice, we recognize species that are morphologically distinct and/or which form a monophyletic group in phylogenetic analysis of one or several loci, which is not contradicted by supported monophyletic groupings in phylogenetic results of any other locus.

We obtained data from the ITS, two nuclear coding genes and two variable regions of the mitochondrial SSU to investigate species limits within _H_. subsect. _Denudata_. The goal was to obtain data from all DNA regions for all collections on which species descriptions were based. Taxonomic types were not only included in the descriptions, but as far as possible also in the sequence analyses in order to link molecular results to traditional taxonomy.

Morphological methods have been refined. A large number of morphological characters have been parameterised in order to allow a relatively complete description and easy comparison between collections. These morphological characters span both macroscopic and microscopic detail. We have made extensive use of the work of Vesterholt (2005) and studies cited therein who adopted a classification to describe spore characters and formalised chelciocystidia descriptions. For the chelciocystidia we have further refined their classification to better describe their shape.

Most of the material used by Aanen and co-workers for their ICG studies has kindly been made available by Th.W. Kuyper. Thus, we are now able not only to recognize taxon clusters, but also to relate them to existing species and, thanks to the previous work of Aanen and others, to relate them to ICGs. The rigorous statistical approach of analysing numerous collections and their characters has also helped in the recognition and understanding of ecological preferences.

Within _H_. subsect. _Denudata_ we include fifteen species occurring in Europe, eight of which are new to science. _Hebeloma crustuliniforme_ was described in a recent publication (Vesterholt et al. 2014); this paper provides descriptions of the remaining fourteen taxa encountered in Europe and a key to all fifteen species, allowing the reader to appreciate species distinctions. Eleven of the fifteen species are likely to correspond to eleven molecular and related taxa assigned to subsect. _Denudata_. Eleven of the fifteen species are likely to correspond to eleven ICGs identified by Aanen and Kuyper (Aanen 1999, Aanen & Kuyper 1999, 2004). As discussed above three ICGs relate to species outside _H_. sect. _Denudata_ and the remaining ICGs relate to species in other subsections of _H_. sect. _Denudata_ and will be addressed more fully in Eberhardt et al. and Grilli et al. (in prep.). We demonstrate that within this subsect – in spite of occasional problems in morphologically distinguishing between two species, rare mismatches between morphological and molecular evidence and gaps in the molecular data matrix when older specimen could not be sequenced for certain loci – that there is good overall consistency between morphological, molecular and seemingly also biological species circumscriptions. Implications of earlier taxonomic decisions (Aanen 1999, Aanen & Kuyper 2004) with regard to the _H. crustuliniforme_ complex and results based on environmental sequencing studies (reviewed by Timling & Taylor 2012) supported the view that agarics, including _Hebeloma_ spp., of the arctic or alpine regions are essentially the same as temperate taxa at the species level. According to the results presented here for the _H. crustuliniforme_ complex and _H_. subsect. _Denudata_, this assumption is debatable. Though taxa of both biomes are doubtlessly closely related, qualitative and quantitative differences can be observed between the arctic/alpine flora as opposed to floras of lower latitudes and altitudes.

**MATERIALS AND METHODS**

The majority of collections cited in species descriptions and used for molecular studies are included in the private herbarium of H.J. Beker. Some collections were obtained from other collectors and their private collections. Additional collections were obtained from public herbaria. The latter are referred to by their acronym, followed by a gap and a collection number. Private collection or collection numbers do not include gaps. Collections not contained in the herbarium of H.J. Beker are also referred to by their HUB database record number. This database contains all data pertaining to the collections and is intended for publication at a later stage.

**Molecular analyses**

Sequence data were obtained of five different DNA regions, ITS, _RPB2_, _MCM7_ (a DNA replication licensing factor) and the variable regions _V6_ and _V9_ of the mitochondrial SSU r-DNA. Not all data could be obtained for all collections; for some collections, mostly older collections, none or only partial ITS sequences could be obtained. Sequences were submitted to GenBank with the accession numbers KM390027–KM390104, KM390107–KM390759, KM390763–KM390775 (newly obtained for this study) and AY312982, JN943484–JN943481, KF309396–KF309406 and KF309426–KF309498. Details of DNA extraction, PCR and sequencing primers have been provided earlier (Eberhardt et al. 2009, 2013, Eberhardt & Beker 2010, Vesterholt et al. 2014). Raw sequence data were edited in Sequencher (v. 4.9, Gene Codes Corporation, Ann Arbor, MI, USA). Ambiguous base calls were regularly encountered in sequences from nuclear ribosomal and protein-coding loci. Length variant ITS copies within the same amplicon were treated as described in Eberhardt et al. (2013). In these cases the attempt was made to segregate the two constituent sequences, presumably representing different nuclei (Aanen et al. 2001), separately. Sequences with more than one indel were treated under the assumption that the two most likely constituent sequences were the two most similar ones, i.e. minimizing the number of assumed base exchanges. For analyses of concatenated alignments, the intragenomic consensus with the least number of ambiguous positions was used.

Sequence alignments were done in Mafft v. 7 (Katoh & Standley 2013) as implemented on http://mafft.cbrc.jp/alignment/software/, using the FFT-NS-i option for coding genes and the ITS and E-INS-i option for the variable mitochondrial SSU regions. Gap recoding following Simmons & Ochoterena (2000) was done using FastGap v. 1.2 (Borchsenius 2009) for the _V6_ and _V9_ sequence alignments. PartitionFinder (Lanfear et al. 2012)
These were measured while still on the lamella edge and width at the apex about 100 cheilocystidia were measured. To determine the average beloma, an important character in the separation of species within the genus Hebeloma, immediately becoming dark brick to dark reddish brown). The average width of the cheilocystidium apex appears to be strongly dextrinoid, excluding the apiculus. The maximum length to width is calculated. 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. Additional measurements (not included in any of our keys) included spore area, circumference and colour.

The assessment of spore characters follows Vesterholt (2005): spore ornamentation on a scale from 00 (smooth) to O4 (ornamentation fairly strong, and always visible without immersion), the looseness of the perispore P0 (not loosening) to P3 (strongly and constantly loosening) and the dextrinoidity of the spores in Melzer’s reagent from D0 (indextrinoid) to D4 (strongly dextrinoid, immediately becoming dark brick to dark reddish brown). 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. These were measured while still on the lamella edge and by measuring all cystidia where the apex could be properly focused and 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 (A), width at narrowest point in central region (M) and maximum width in lower half (B). The measurements are given in this order and an average value was calculated for each of these measurements. Further, the ratios A/M, A/B and B/M are calculated for each cystidium and then these too are averaged. The measurements were made in 5 % KOH.

All microscopic measurements are made using a Leica DM-RXA2 microscope system with a Leica DC300 camera connected to a computer running Leica IM1000 image capture software and Leica QWin image analysis software. Photographs of all relevant characters are taken and then all measurements and analysis is carried out on the computer system using the image analysis software, fed into spread sheets, where statistics are calculated, and then automatically transferred into the database. An advantage of this approach is that all measurements are repeatable and all information relating to these measurements, including all photographs, is stored with the rest of the collection information in the database.

Keys were built on the database as a set of complex queries. More than 1 250 collections of H. sect. Denudata and more than 4 000 collections of Hebeloma, including their collection details, ecology and, for more than half these collections, morphometric parameters and results have been entered into the database. By having all data fully parameterised, collections can be compared with ease and database queries can be used to isolate those collections with similar features. This in turn enables keys to sections and to species to be built and continually be tested across a large number of collections. Principal component analyses were done in R (R Core Team 2014, Vu 2011).

RESULTS

All collections cited in the species descriptions of this paper were included in the molecular studies. The minimum goal was to obtain ITS sequences. For known species, collections were selected from a wide variety of habitats throughout Europe and in some cases also from other continents. We were able to locate and obtain type material for each of these taxa, as well as type material for a number of species that we believed were related to these taxa and might belong to this subsection, and this was also included in our studies. In total, ITS sequence data were obtained for over 550 collections and 271 collections were subjected to further and more detailed analysis. Hebeloma mediorufum was used as outgroup. This taxon, up to now only known from New Zealand, is, according to all DNA regions included in the analyses and the study of Rees et al. (2013), the closest relative of all known European members of H. sect. Denudata, without being inside the clade representing the European species of H. sect. Denudata.

No molecular data could be obtained for the lectotype of H. he- lodes (G 00053920; database record HJB1000054) and the holotype of H. eburneum (MPU GM1122; database record HJB1000095). The same applies to one collection used in the description of H. aanenii (collected by G. Bresadola, S F14406, database record HJB13470); H. eburneum (collected by G. Malencou, MPU GM1415, database record HJB12229); and for H. pusillum (collected by D. Aanen WBS 9648, database record HJB12518), though for the latter a V6 sequence could be obtained. For H. pusillum GLM GL42941 (database record HJB10993) the ITS sequence published by Boyle et al. (2006), AY312982, was used. In two cases only partial ITS sequences could be obtained: for the lectotype collections of H. alpinum
sequences of the respective species, are not listed.

Ingroup excluding

H. luteicystidiatum

Clades supported by bootstrap support values higher than 70 % in single locus analyses. In bold are support values supporting clades incompa-
tible between some of the single locus ML results. n.a. – not applicable. Clades consisting of sequences assigned to a single species, but not containing all sequences of the respective species, are not listed.

Table 1

| Species               | No. | Min(inter) | Max(intra) |
|-----------------------|-----|------------|------------|
| H. auranitoumbrinum   | 35  | 0          | 1.1        |
| H. alpinum            | 34  | 0          | 0.2        |
| H. aurantioumbrinum   | 12  | 0          | 0.3        |
| H. crustuliniforme    | 20  | 0.2        | 0.2        |
| H. eburnum            | 35  | 0          | 1.2        |
| H. geminatum          | 26  | 0          | 0.3        |
| H. helodes            | 21  | 0          | 0.5        |
| H. lousiæ             | 3   | 0.5        | 0          |
| H. luteicystidiatum   | 4   | 0.2        | 0          |
| H. lutense            | 23  | 0.2        | 0          |
| H. mediorufum         | 4   | 3.4        | 0          |
| H. minus              | 10  | 0          | 1.0        |
| H. pallidolabiatum    | 2   | 0          | 0.2        |
| H. perexiguum         | 1   | 0.3        | n.a.       |
| H. pusillum           | 36  | 0.6        | 1.1        |
| H. salicicola         | 21  | 0          | 0.2        |

(G GK13674; database record HJB1000060) only the ITS1; for the isotype of H. lutense (L 0054088, database record HJB1000011) only the ITS2; the ITS sequence of the holotype of H. lutense (P 59.232, database record HJB1000253) is complete.

Table 2

| Clade                               | ITS  | V6 of mitSSU | V9 of mitSSU | RPB2 | MCM7 |
|-------------------------------------|------|--------------|--------------|------|------|
| H. aurantioumbrinum                 | 90   | 97           | 91           | 100  |      |
| H. helodes                          | 89   | 100          | 90           | 70   |      |
| H. aurantioumbrinum & H. helodes    | 93   | 100          | 99           | n.a. | 100  |
| H. luteicystidiatum                 | 73   |              |              |      |      |
| Ingroup excluding H. luteicystidiatum & H. perexiguum | 96   | 100          | 98           | 99   |      |
| H. lutense                          | 77   | 88           | 100          | 99   | 100  |
| H. perexiguum & H. pusillum         | 74   | 100          | 99           | 96   | 99   |
| H. crustuliniforme                  | 74   | 100          | 99           | 96   | 99   |
| H. crustuliniforme complex excluding H. crustuliniforme | 90   |              |              |      |      |
| H. lousiæ                           | 99   | 84           | 100          | 99   |      |
| H. minus                            | 77   |              |              |      |      |
| H. minus & H. lousiæ                | 82   |              |              |      |      |
| H. lousiæ & H. pallidolabiatum & H. minus | 78   |              |              |      |      |
| H. salicicola                       | 82   |              |              |      |      |
| H. salicicola excluding HJB13087 & HJB9072 | 78   |              |              |      |      |
| H. pallidolabiatum                  | 81   |              |              |      |      |
| H. pallidolabiatum & HJB13087 (H. salicicola) & HJB12204 (H. alpinum) | 81   |              |              |      |      |
| H. salicicola & H. pallidolabiatum  | 80   |              |              |      |      |
| HJB9072 (H. salicicola) & HJB11986 (H. alpinum) | 80   |              |              |      |      |
| HJB12806 (H. auranitii) & HJB10290, HJB12537, HJB12996 & HJB12804 (H. eburneum) | 78   |              |              |      |      |
| H. auranitii excluding HJB10450 & H. eburneum | 81   |              |              |      |      |
Fig. 1 Best of 100 ML results of European Hebeloma subsect. Denudata of single gene analyses with 1,000 bootstrap replicates and H. mediorum as outgroup. Only bootstrap values of ≥ 75% are given. Species are colour coded (see legend). 

- a. ITS; b. V6 region of the mitSSU; c. RPB2. For the ITS, length variant copies of the ITS from the same specimen were both included in the analysis and indicated by 'I' and 'II'.
to species clades mentioned before. *Hebeloma eburneum* is monophyletic without support. Only a single sequence could be obtained for *H. luteicystidiatum*, so that we do not know about the clade support. As in V6, the clade of the *H. crustuliniforme* complex receives a high bootstrap support. *Hebeloma aanenii*, *H. alpinum* and *H. geminatum* are paraphyletic.

Sequences for MCM7 were difficult to obtain, but 120 sequences of the same number of collections could be obtained; alignment length was 867 bp. The analysis supports eight species with bootstrap. *Hebeloma salicicola* forms an unsupported species clade. Sequence variation is rather high within some species of the *H. crustuliniforme* complex (i.e. *H. aanenii*, *H. minus*, *H. salicicola*) and, judging from SNPs, also intragenomically. We assume that the intragenomic variation is the result of heterokaryocity (Aanen et al. 2001). In high quality reads there are not more than two superposed peaks in a position.

Table 2 summarizes bootstrap results of single locus analyses, which were used for testing compatibility of the results of single gene analyses prior to concatenation. Results of single locus analyses are incompatible only with regard to the placement of type collections is indicated by the name of the respective species at the side of the clade; the colour of the font matches the current taxonomic placement of the species. a. V9 region of the mitSSU; b. MCM7.

In spite of these incompatibilities we decided to concatenate the single locus alignments to see whether species not receiving support or being paraphyletic in single locus analyses would form (supported) monophyla in concatenated analyses. All loci were included in the concatenation, as there was no indication that mitochondrial DNA data show a completely different evolutionary history from nuclear data. Fig. 3 shows the ML result including five loci and all collections (158) for which data were available for at least three of the five concatenated loci. If only collections were included with a full dataset of five sequenced loci, the resulting topology is very similar, but the bootstrap support is better. Only *H. alpinum* (paraphyletic) and *H. minus* do not receive support ≥ 75 % and all other species, apart from *H. geminatum* and *H. aanenii* receive bootstrap support of 90 % or more. With four genes out of five, the result differs from the depicted phylogram by supporting the *H. geminatum* clade with 78 % bootstrap and the *H. aanenii* clade with 77 % bootstrap. With a minimum of two genes out of five, *H. aanenii*, *H. eburneum*, *H. geminatum*, and *H. minus*, in addition to *H. alpinum* that is paraphyletic in all analyses, also become paraphyletic.

In the ML result based on a minimum of three loci, one collection is ‘misplaced’ with regard to its morphological species

![Fig. 2](image-url)
In this case, the V9 sequence of the collection is ≥ 75% are shown. Type collections are in ICG – Intercompatibility group (see Aanen & Kuyper 1999).

Fig. 3 thus implicitly in the paraphyletic part of the tree constituting H. alpinum, H. geminatum, H. alpinum assignment, namely H. alpinum HJB11051. DNA extraction, PCR and sequencing were repeated, but several markers place the collections in H. geminatum. One collection, H. geminatum, HJB11545, is not included in the H. geminatum clade and thus implicitly in the paraphyletic part of the tree constituting H. alpinum. In this case, the V9 sequence of the collection is odd and differs from the rest of the H. geminatum V9 sequences (and all other taxa with long V9 sequences in the alignment) in a number of positions. It differs very clearly from H. alpinum V9 sequences, which are considerably shorter than H. geminatum V9 sequences.
In Fig. 3 the intercompatibility group (ICG) assignment of (Aanen & Kuyper 1999) is stated for all collections for which this information was available. The figure shows that there is full agreement between the ICG, taxon names based on morphology and the molecular results. Collection WBS 9605, compatible with two ICGs, belongs to *H. alpinum* morphologically and also clusters with other collections of this taxon.

We have been able to construct queries on the database, based purely on morphological characters, which can select the collections of a single clade (or ICG) as demonstrated in the keys provided below, with the single exception of *H. aanenii* and *H. geminatum* where there is clear evidence for two distinct species but we have been unable, thus far, to find a morphological character or set of characters on which such separation can consistently be unambiguous. Table 3 summarizes the most important morphological properties and ICG assignment (following Aanen & Kuyper 1999) of the recognized species.

Principal component analyses with three parameters (average spore area, cheilocystidia apex width and number of complete lamellae, Fig. 4a), and with two parameters (average spore area and width of complete lamellae, Fig. 4b).

![Fig. 4](image)
area and cheilocystidia apex width, Fig. 4b) that are important for species identification were run for the four taxa that are most difficult to distinguish from each other morphologically (H. aenennii, H. crustuliniforme, H. eburneum and H. geminatum) and H. alpinum which is most difficult to distinguish from these four in molecular terms. This analysis shows that these parameters alone are sufficient to group many collections correctly to species.

**TAXONOMY**

**Hebeloma section Denudata** (Fr.) Sacc.

*Type. Hebeloma crustuliniforme (Bull.) Quél. emend. Vesterh., U. Eberh. & Beker.*

**Hebeloma subsection Denudata**

Within subsect. *Denudata* we recognise the following fifteen taxa:

- H. aenennii, H. alpinum, H. aurantioumbrinum, H. crustuliniforme, H. eburneum, H. geminatum, H. helodes, H. luisaeae, H. luteicystidiatum, H. lutense, H. minus, H. pallidolabiatum, H. perexiguum, H. pusillum and H. salicicola

Cortina absent; universal veil not observed; smell usually more or less radish-like but sometimes absent; the lamellae usually with clear droplets but occasionally absent, particularly in dry weather, but then often leaving brown or rusty stains on the lamellae. Spores amygdaloid, sometimes with a papilla, O1-3; P0-2; D0-2, occasionally with some spores up to D3, av size 9.1–13.7 × 4.9–7.7 μm, Qav 1.60–2.17. **Cheilocystidia** with a majority capitately-, clavate- or spathulate-stipitate; i.e. swollen at the apex but fairly cylindrical below this apical swollen area but sometimes with a few cheilocystidia a little swollen near the base (capitate-, clavate- or spathulate-lageniform), particularly in smaller, possibly less mature cheilocystidia; average length between 40 μm and 75 μm; av width dimensions (μm): 6.8 < base < 5.4; 9.1 < median < 4.9; 3.4 < apex < 5.4; 3.2 < base < 6.4. Ratios: A/M > 1.6; A/B > 1.45; B/M < 1.35.

Notes — The main morphological feature that distinguishes species of *H*. subsect. *Denudata* is the shape of the cheilocystidium, significantly swollen at the apex, constricted in the median part and little swollen, if at all, in the bottom half, except in smaller, possibly less mature cheilocystidia.

Below we give descriptions of all species of *H*. subsect. *Denudata* discussed in this paper, with the exception of *H. crustuliniforme*, which was described in Vesterholt et al. (2014). Although a number of these species have been treated extensively in the literature, our diagnosis may be narrower (based wholly on the collections cited in this paper) and hence we feel a full description is necessary in order to properly define the morphological species concepts.

**Key to Hebeloma subsection Denudata**

We provide a separate key for arctic/alpine collections. While this inevitably means some repetition between the two keys we believe the practical advantages make it worthwhile.
Fig. 5 Photo of: a. *Hebeloma aeneii* BR-MYCO 173987-66 (holotype); b. *H. aurantioumbrinum* BR-MYCO 173985-64 (holotype); c. *H. geminatum* C-F-90152 (JV96-341) (holotype); d. *H. louiseae* BR-MYCO 173982-61 (holotype); e. *H. luteicystidiatum* BR-MYCO 166233-72 (holotype); f. *H. pallidolabiatum* BR-MYCO 174908-17 (holotype); g. *H. perexiguum* BR-MYCO 173979-58 (holotype); h. *H. salicicola* BR-MYCO 173977-56 (holotype). — Photos: a, b, d, f–h. H. Beker; c. J. Vesterholt; e. P. Derboven.
Key to Hebeloma subsection Denudata

1. Alpine or arctic collection with Salix or Dryas... Key 1
1. Not alpine or arctic, whatever the association... Key 2

Key 1 – Hebeloma subsection Denudata

1. Av L ≥ 60 and av spore length < 11 μm... Denudata Key 2 – 11
1. Av L < 60 or av spore length ≥ 11 μm... 2
2. Av L ≥ 40, spore length ≥ 11 μm with distinct papilla... H. alpinum
2. Any of the above conditions not satisfied... 3
3. Spores O3 and D2 and not D1... H. salicicola
3. Spores not O3 or not D2 or D1... 4
4. Av width of cheilocystidia apex ≥ 8.5 μm... H. aurantiombrinum
4. Av width of cheilocystidia apex ≥ 8 μm or cheilocystidia not... 5
5. Cheilocystidium ratio A/B > 1.8... 6
5. Cheilocystidium ratio A/B ≤ 1.8... 7
6. Spores O1 or O2, few if any spores O3... H. louiseae
6. A large number of spores O3... H. minus
7. Majority of spores O1 and av spore length ≤ 12 μm... H. perexiguum
7. At least some spores at least O2 and av spore length > 12 μm... H. pallidolabiatum

Key 2 – Hebeloma subsection Denudata

1. L ≥ 60... 2
1. L ≤ 60... 9
2. Av width of cheilocystidia apex < 8 μm and many cheilocystidia sinuate... H. lutense
2. Av width of cheilocystidia apex ≥ 8 μm or cheilocystidia not sinuate... 3
3. Av spore length < 11 μm... 4
3. Av spore length ≥ 11 μm... 5
4. Cheilocystidia without consistent and distinct apical thickening... H. aurantiombrinum
4. Cheilocystidia with consistent and distinct apical thickening... H. helodes
5. Pileus almost uniformly coloured and pale (usually 3A2 or 4A2)... H. minus
5. Pileus distinctly 2-coloured (with brown centre) and av stipe width in centre ≥ 6 mm... H. eburneum
5. Pileus distinctly 2-coloured (with brown centre) and av stipe width in centre ≤ 7 mm... 6
6. L ≤ 50 and cheilocystidia have a thick apical wall often appearing yellow under the microscope... H. minutissimum
6. L > 30 and cheilocystidia have a thick apical wall often appearing yellow under the microscope... H. luteolystidiatum
7. Av stipe Q ≥ 12, spore dextrinoidity at least D5... H. salicicola
7. Av stipe Q ≤ 12 or many spores at most D1... 8
8. Av spore Q < 1.9... H. minus
8. Av spore Q ≥ 1.9... H. pusillum
9. Av spore width is ≥ 6.4 μm or av, spore length > 10.75 μm and av spore width > 6 μm... 10
9. Av spore width < 6.4 μm and av, spore length ≤ 10.75 μm or av spore width ≤ 6 μm... 11
10. Av width of cheilocystidia apex ≥ 8 μm... H. eburneum
10. Av width of cheilocystidia apex < 8 μm... H. crustuliniforme
11. Av width of cheilocystidia apex > 9 μm... H. geminatum
11. Av width of cheilocystidia apex ≥ 8 μm and ± 9 μm... H. aanenii/H. geminatum
11. Av width of cheilocystidia apex < 8 μm... H. aanenii
Fig. 6  *Hebeloma aanenii* (BR-MYCO 173987-66, holotype). a, b. Spores and spore ornamentation ×1 600 in 5 % KOH; c, d. spores and spore ornamentation ×1 600 in 5 % Melzer’s reagent; e. cheilocystidia ×1 000 in 5 % KOH; f. caulocystidia ×500 in 5 % KOH; g. basidia ×1 000 in 5 % KOH; h. epicutis hyphae ×500 in 5 % KOH. — Scale bars: 10 µm.
width of apex holotype 5–95 % percentile range 5.5–9.0 µm, with median 7.3 µm and av 7.2 µm with SD 1.01 µm; across 36 collections median 6.2–9.0 µm and av 6.2–9.0 µm (of the 36 collections almost 80 % have the cheilocystidium apex at 8 µm); with n ≥ 20 selected cheilocystidia of 36 collections the apex width of apex at the apex. Pileipellis resembles cheilocystidia but are often more clavate-lageniform shaped, up to 100 µm long and 11 µm wide at the apex. Pileipellis is an ixocutis with a very thick epicutis 100–400 µm, embedded hyphae up to 5–7 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented.

Cutis yellowish and made up of cylindrical to isodiametric elements. Subcutis contains isodiametric elements and the trama below the subcutis contains cylindrical, ellipsoid and thick sausage shaped elements up to 20 µm broad. Clamp connections present throughout the basidiome.

Habitat & Distribution — Hebeloma aanenii appears to be widespread across Europe, although we do not have confirmed records from south-west Europe. It appears to grow in a variety of habitats, on both acid and calcareous soils, often on woodland pathways and also in alpine areas. It appears to form mycorrhiza with a variety of trees. Records include Abies, Betula, Carpinus, Dryas, Eucalyptus, Fagus, Helianthemum, Picea, Pinus, Populus, Quercus, Salix and Tilia. We have a confirmed collection of H. aanenii with Salix sp. in New Zealand.

Additional specimens examined. BELGIUM, prov. Luxembourg, Barvaux (c. N50.34 E5.49, alt. 200 m) on wet soil in mixed woodland under Salix sp., 18 Oct. 2003, E. Emmett, HJB8896; prov. Luxembourg, Wilbauroche (N49.81335 E5.27330, alt. 312 m) on rotten litter in broadleaf woodland under Corylus av. (= Betula) sp., 17 Sept. 2004, H. Beker HJB10607; prov. Luxembourg, Gouette (N50.01500 E5.229167, alt. 401 m) on grassy soil in mixed woodland under Picea sp., 3 Oct. 2004, M. Chyżelinski HJB10164; prov. Namur, Biron (c. N50.30 E5.11, alt. 250 m) on rotten litter in conifer woodland under various broadleaf trees, 7 Oct. 2004, M. Lene HJB10282; prov. Brussels, Scheutbos (N50.8531667 E4.2950500, alt. 50 m) on grassy soil in broadleaf woodland under Betula sp., Salix sp., 28 Oct. 2007, H. Beker HJB12164. – CZECH REPUBLIC, Moravia, LPA Moravian Karst, NNR Vyvre Punktý (N49.37250 E16.72623, alt. 487 m) on soil in mixed woodland under Fagus sylvatica, 10 Oct. 2008, V. Antonin HJB12717; Moravia, LPA Moravian Karst, NNR Vyvre Punktý (N49.37250 E16.72623, alt. 487 m) on soil in mixed woodland under Populus sp., 10 Oct. 2008, S. Kelly

Notes — The dominant cheilocystidial shape, clavate- to spathulate-stipitate clearly defines H. aanenii as belonging to H. subsection Deduntata. Denudata. Hebeloma aanenii is a constituent of the H. crustuliniforme complex and most likely corresponds to ICG2 of Aaen & Kyuper (1999). It is likely that many collections of this species have been recorded under the name H. crustuliniforme and exist worldwide in herbaria under this name. It is morphologically most similar to H. crustuliniforme, H. eburneum, H. alpinum and H. geminatum. But its spores, normally < 11 µm long and < 6 µm wide distinguish it from the first three species. It can be distinguished from other members of this subsection by the number of complete lamellae, which is always ≥ 60. Until now we have found no consistent morpho-
Fig. 8  Hebeloma alpinum (G GK13674, lectotype). a, b. Spores and spore ornamentation ×1 600 in 5 % Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e, f. cheilocystidia ×1 000 in 5 % KOH; g. basidium ×1 000 in 5 % KOH; h. cheilocystidia ×500 in 5 % KOH; i. epicutis hyphae ×500 in 5 % KOH. — Scale bars: 10 µm.
logical character to unambiguously separate *H. aanenii* and *H. geminatum*. However, we can often separate these two taxa. The cheilocystidium average apex width for *H. aanenii* is usually smaller than that for *H. geminatum*. From our records, if the average apex width of the apex of the cheilocystidium is < 8 μm then the collection is almost certainly *H. aanenii*. However, the average apex width can reach 9 μm. Average apex widths in this interval between 8–9 μm can be found in either taxon. This is responsible for the overlap of the ellipses of *H. aanenii* and *H. geminatum* in the PCA diagram in Fig. 4b.

In spite of the rather large intraspecific ITS variation it should normally be possible to recognize *H. aanenii* based on this locus. Only a single collection (WBS 9620, database record HJB12508) out of 33 is not included in the *H. aanenii* clade. Based on three or five loci, *H. aanenii* is monophyletic and weakly supported (Fig. 3), including WBS 9620. In none of the single locus phylogenies do all *H. aanenii* sequences form a monophyly. The combination of V6 and V9 also unambiguously identifies *H. aanenii*, though neither locus on its own suffices.

*Hebeloma alpinum* (J. Favre) Bruchet, Bull. Mens. Soc. Linn. Lyon 39, 6 (Suppl.); 68. 1970. — MycoBank MB314944; Fig. 6, 9

Type. SWITZERLAND, Val dal Botsch (c. N46.65 E10.10, alt. c. 2600 m) alpine scrub, on calcareous soil with Dryas octopetala and Salix herbacea, 27 Aug. 1949, J. Favre, lectotype G GIK13674; database record HJB1000060, selected by Vesterholt in Symb. Bot. Upsal. 30 (no. 3): 134, 1995.

**Basidiomes** usually in scattered groups, sometimes solitary, rarely caespitose. *Pileus* 12–70 mm diam, convex often umbo-nate, tacky when moist but never hygrophanous; **cuticle colour** quite variable from cream through pinkish buff to Isabella and yellow brown, to clay buff, cinnamon or even sepia, often unicoloured but sometimes paler towards the margin which may be pinkish buff, clay buff or greyish buff through to cream or white; *pileus margin* often involute particularly in young basidiomes; *Lamellae* emarginate, usually moderately crowded (*L* = 40–72); maximum depth of 3–9 mm; *colour* of the holotype, 5–95% percentile range 11.2–13.3 × 6.6–8.0 μm, with median 12.3 × 7.3 μm and av 12.3 × 7.3 μm with SD length 0.65 μm and width 0.45 μm, Q value 5–95% percentile range 1.50–1.86, with median 1.69 and av 1.69 with SD 0.11; *spore size* based on 34 collections medians 10.9–13.7 × 6.1–7.6 μm and av 11.0–13.7 × 6.1–7.7 μm with SD length 0.53–1.16 μm and width 0.25–0.57 μm, Qav 1.60–1.97. **Basidium** cylindrical to clavate and 4-spored, 25–45 × 7.1–11.7 μm, with av 28–40 × 8.0–11.1 μm and avidium Qav in the range 3.0–4.3. **Pleurocystidia** found, cheilocystidia clavatistipitate or spathulate-stipitate, occasionally swollen towards the base (clavate-lageniform), sometimes with thickening of the apex or thickening of the median, sometimes sepatate, rarely bífurcate, sinuate or rostrate; **width of apex** holotype 5–95% percentile range 6.4–9.7 μm, with median 7.8 μm and av 7.9 μm with SD 1.10 μm; across 34 collections median 6.8–9.8 μm and av 6.8–9.8 μm; with n ≥ 20 selected cheilocystidia of 34 collections the 5–95% percentile ranges are 30–92 × 4.8–13.1 × 2.7–6.3 × 2.3–8.1 μm while the averages are 40–71 × 6.8–9.8 × 3.6–5.1 × 3.2–6.1 and 58 × 7.9 × 4.4 × 4.3 μm av for the holotype. The av cheilocystidia ratios for the 34 collections were: A/M = 1.61–2.74; A/B = 1.52–2.71; B/M = 0.87–1.24. **Caulocystidia** resemble cheilocystidia, up to 120 × 11 μm wide at the apex. *Pileipellis* is an ixocutis with an epicutis from relatively thin to medium thick, 60–160 μm, embedded hyphae up to 5–6 μm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. *Subcutis* yellow to orange and made up of cylindrical to isodiametric elements. **Trama below the subcutis** contains angular, ellipsoid, cylindrical, spherical and sausage-shaped elements up to 18 μm broad. **Clamp connections** present throughout the basidiome.

**Habitat & Distribution** — *Hebeloma alpinum* has been recorded with a number of species of Salix in both arctic and alpine environments. It has also been recorded with *Dryas octopetala* when Salix was not recorded as present. We strongly suspect that it can be mycorrhizally associated with *Dryas*. We have no records of this taxon outside of such habitats. Our database records of *H. alpinum* have the following Saliceae associations: *Salix herbacea*, *S. polaris*, *S. reticulata* and *S. retusa*. We have records in both acid and calcareous soils, bare, grassy, snowy and sandy. As well as the 34 collections upon which our description has been based, we have a number of other confirmed records of *H. alpinum* including a number from outside of Europe. So far we have no records on our database from alpine areas outside Europe but we suspect it may well be present in these regions too.

**Additional specimens examined.** GREENLAND, Zackenberg, just south of Teldzamaden (c. N74.30 W21.00, alt. c. 700 m) in dry soil under *Salix herbacea*, 20 July 1999, T. Borgen TB99 022, duplicate HJB12194; Zacken-berg, 100 m west of Zackenberg River (c. N74.50 W21.00, alt. c. 700 m) on dry soil in dry soil under *Dryas octopetala*, 3 Aug. 1999, T. Borgen TB99 199, duplicate HJB12204. — ICELAND, Valavatn, (N64.86653 E23.5597167, alt. c. 301 m) on bare soil in *Salix herbacea*, 29 July 2005, H. Beker; H. Moller HJB11051. — ITALY, Lac Verney (N45.68183 E6.682441, alt. c. 2090 m) under *Salix reticulata*, 24 Aug. 2009, G. Coriole HJB13096. — NORWAY, Mare & Romsdal, Beiren kommune: Sukommfjellet (N66.87940 E14.28040, alt. c. 800 m) under *Dryas octopetala*, *Salix polaris*, *Salix reticulata*, 4 Aug. 2008. — P. Larsen Larsen 56-2008, HJB13008. — SWEDEN, Endalen (N78.197333 E15.7891167, alt. c. 29 m) on soil in grazed scrub under *Salix polaris*, 13 Aug. 2007, M. L. Beker HJB11988; Ekmanfjorden (N78.61705 E14.837183, alt. c. 9 m) on soil in maritime coastal scrub under *Salix herbacea*, 15 Aug. 2007, H. Beker, M. L. Beker HJB11997; Ekmanfjorden (N78.617033 E14.837883, alt. c. 12 m) on soil in maritime coastal scrub under *Salix polaris*, 15 Aug. 2007, H. Beker, M. L. Beker HJB12000; Ekmanfjorden (N78.617033 E14.837883, alt. c. 12 m) on soil in maritime coastal scrub under *Salix polaris*, 15 Aug. 2007, J. Sandmo HJB12002; Dicksonfjorden (N78.6207667 E14.8823000, alt. c. 24 m) on soil in maritime coastal scrub under *Salix polaris*, 15 Aug. 2007, H. Beker, M. L. Beker HJB12004; Dicksonfjorden (N78.6203333 E14.8845000, alt. c. 25 m) on soil in maritime coastal scrub under *Salix polaris*, 15 Aug. 2007, H. Beker, M. L. Beker HJB12005. — SWITZERLAND, Graubünden Samnaun (c. N46.940 E10.360, alt. c. 1900 m) under *Salix reticulata*, *Salix retusa*, 28 Aug. 1984. — H. Knudsen HC36-84, HJB10642; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under *Salix retusa*, 5 Sept. 1996, D. Aanen WBS 9605, database record HJB12498; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under *Salix retusa*, 5 Sept. 1996.
D. Aanen WBS 9607, database record HJB12499; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix retusa, 5 Sept. 1996. D. Aanen WBS 9609, database record HJB12500; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix retusa, 5 Sept. 1996. D. Aanen WBS 9613, database record HJB12502; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix retusa, 5 Sept. 1996. D. Aanen WBS 9606, database record HJB12504; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix sp., 5 Sept. 1996. D. Aanen WBS 9614, database record HJB12505; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix retusa, 5 Sept. 1996. D. Aanen WBS 9616, database record HJB12507; Adelboden, Bern (c. N46.500 E7.550, alt. c. 1900 m) on calcareous soil under Dryas octopetala, Salix sp., 5 Sept. 1996. D. Aanen WBS 9615, database record HJB12998; Spittelmatte (N46.440 E7.640, alt. c. 2000 m) on rotten litter in scrub under Dryas octopetala, 9 Aug. 2005. H. Beker, M.L. Beker HJB11087; Spittelmatte (N46.4195 E7.62375, alt. c. 2220 m) on herbaceous litter in scrub under Dryas octopetala, 9 Aug. 2005. H. Beker, M.L. Beker HJB11088; Spittelmatte (N46.41050 E7.62220, alt. c. 2000 m) on bare soil in scrub under Salix sp., 9 Aug. 2005. H. Beker, M.L. Beker HJB11094; Spittelmatte (N46.43953 E7.63781, alt. c. 2000 m) on rotten litter in scrub under Dryas octopetala, Salix sp., 9 Aug. 2005. H. Beker, M.L. Beker HJB11100; Corno Gries (N47.480 E8.500, alt. c. 2500 m) on grassy soil in scrub under Salix sp., 9 Aug. 2005. H. Beker, M.L. Beker HJB11104; Spittelmatte (N46.465833 E8.4069500, alt. c. 2500 m) on litter in scrub under Salix herbecae, 11 Aug. 2005. H. Beker, M.L. Beker HJB11123; Albulapass (N46.58200 E9.84300, alt. c. 2300 m) on bare soil in scrub under Salix sp., 12 Aug. 2005. H. Beker, M.L. Beker HJB11132; Morartersh (N46.43340 E9.93623, alt. c. 2002 m) on sandy soil in scrub under Salix sp., 13 Aug. 2005. H. Beker, M.L. Beker HJB11133.

Notes — Given the shape of its cheilocystidia, Hebeloma alpinum clearly belongs to H. subsect. Denudata. Hebeloma alpinum most likely corresponds to ICG4 of Aanen & Kuyper (1999). As discussed in the Discussion in more detail, some members of this species might be incompatible with H. eburneum (ICG3) or even with H. aenennii (ICG2) (Aanen & Kuyper 1999, Aanen et al. 2000). Hebeloma alpinum certainly appears to be confined to alpine or arctic habitats and can be readily separated from other alpine/arctic species in this section based on the number of lamellae, usually between 40 and 60 on average, and the size of the spores, on average ≥ 11 μm long and > 6 μm wide. It also has quite a robust stature. Microscopically it is closest to H. minus but it can normally be separated on macroscopic characters, since H. minus is smaller with darker-coloured pileus and with fewer lamellae than H. alpinum. Hebeloma alpinum is the most common Hebeloma species we have collected in alpine/arctic areas and can usually be determined, with reasonably high confidence, in the field.

Among the loci applied in this study, not a single one can distinguish H. alpinum on its own. Even when combining a minimum of three out of five loci (Fig. 3), H. alpinum is not monophyletic, though, apart from HJB11051 and HJB11154 (discussed in detail above and under H. geminatum), H. alpinum does not form mixed clades with other taxa. Again, the combination of V6 and V9 is probably the most reliable combination of fewer loci for identifying H. alpinum. Hebeloma alpinum V6 sequences cluster with H. geminatum and V9 sequences with H. eburneum, whereas H. geminatum V9 sequences are much longer and the great majority forms a monophylet, except for the two deviant collections, and H. eburneum V6 sequences cluster with H. aenennii.

We have examined material of Naucoria bellotiana (K K(M) 165365). This collection from Bellot Island in Canada was collected by Capt. Feilden on 14 August 1876 and described by M.J. Berkeley as Agaricus (Naucoria) bellotianus in the Journal of the Linnean Society, vol. 17, 1878, p. 14. This species certainly belongs to the genus Hebeloma and we make the new combination here:

Hebeloma bellotianum (Berk.) Beker & U. Eberh. comb. nov. — MycoBank MB809913

Hebeloma bellotianum has cheilocystidia that clearly place it in H. sect. Denudata subsect. Denudata. Given the habitat at N81.68 it falls into the arctic/alpine group from this subsection. The number of lamellae (estimated from the exsiccat) and spore size would mean that it would key out, among known species from this subsection, as H. alpinum to which it is certainly similar and were it not for the very large spores we would be confident this was the same species. However the spores of H. bellotiana measured, on average, 14.7 × 7.1 μm and the largest average spore size we have measured for H. alpinum (across 71 collections) is 13.7 μm long (and 7.7 μm wide). We have not been able to obtain any molecular data from this collection and cannot rule out that this may be a species from this subsection that we have not yet encountered. Thus at this point we hesitate to synonymise these species and await further evidence one way or the other.

Hebeloma aurantioumbrinum Beker, Vesterh. & U. Eberh., sp. nov. — MycoBank MB809906; Fig. 5b, 10, 11

Etymology. From auranto - orange and umbrinum - umber.

Type. Svalbard, Krudusenheia (N78.937333 E11.842533, alt. c. 9 m) on grazed scrub with Salix polaris, 10 Aug. 2007. M.L. Beker, H. Beker, holotype BR BR-MYCO 173985-64; isotypes C C-F-90148, HJB12058.

Diagnosis — Hebeloma aurantioumbrinum possesses the cheilocystidia typical of H. subsect. Denudata. It is a species typically occurring in alpine or arctic habitats where it can be recognized by the combination of an average spore width of < 7 μm and an average cheilocystidium apex width of < 8.5 μm. Outside these habitats it can be differentiated from other small species of the subsection (H. luteicystidiatum, H. helodes and H. pusillum) by its uniformly brownish orange pileus and by its cheilocystidium apex without abnormal wall thickening.

Basidiomes usually in scattered groups. Pileus up to 21 mm diam, umbonate, slightly tomentose at high magnification;
Fig. 10  *Hebeloma aurantioumbrinum* (BR-MYCO 173985-64, holotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c. cheilocystidia ×1 000 in 5 % KOH; d. cheilocystidia ×500 in 5 % KOH; e. basidium ×1 000 in 5 % KOH; f. epicutis hyphae ×1 000 in 5 % KOH; g. caulocystidia ×1 000 in 5 % KOH; h. caulocystidia ×500 in 5 % KOH. — Scale bars: 10 µm.
surface slightly viscid, tacky when moist rarely hygrophanous; cuticle colour from yellow brown to cinnamon to umbel with some orange, sometimes with a thin paler pinkish buff to clay buff margin; pileus margin usually straight, sometimes slightly scalloped. Lamellae emarginate, quite widely spaced (L = 26–39) with a maximum depth of 2.5 mm; colour cream, alutaceous or brown when young, later umberto sepia following spore maturity; edge fibrinate, paler than lamella surface; droplets on the lamella edge are usually present and visible to the naked eye, however in dry conditions they may not be seen; lamellules sparse. Stipe central, cylindrical occasionally clavate, stuffed, (14–)15–28 × 2.5–3.1(–3.5) mm and up to 6 mm at the base; white or alutaceous, with no visible discoloring when handled; surface dry, pruinose particularly towards the apex. Cortina not observed. Flesh rather thin, cream or pale brown. Smell raphanoid, sometimes weakly. Taste not recorded. Spore deposit clay-buff.

Spores amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore surface slightly viscid, tacky when moist rarely hygrophanous; cuticle colour from yellow brown to cinnamon to umbel with some orange, sometimes with a thin paler pinkish buff to clay buff margin; pileus margin usually straight, sometimes slightly scalloped. Lamellae emarginate, quite widely spaced (L = 26–39) with a maximum depth of 2.5 mm; colour cream, alutaceous or brown when young, later umberto sepia following spore maturity; edge fibrinate, paler than lamella surface; droplets on the lamella edge are usually present and visible to the naked eye, however in dry conditions they may not be seen; lamellules sparse. Stipe central, cylindrical occasionally clavate, stuffed, (14–)15–28 × 2.5–3.1(–3.5) mm and up to 6 mm at the base; white or alutaceous, with no visible discoloring when handled; surface dry, pruinose particularly towards the apex. Cortina not observed. Flesh rather thin, cream or pale brown. Smell raphanoid, sometimes weakly. Taste not recorded. Spore deposit clay-buff.

Spores amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore surface slightly viscid, tacky when moist rarely hygrophanous; cuticle colour from yellow brown to cinnamon to umbel with some orange, sometimes with a thin paler pinkish buff to clay buff margin; pileus margin usually straight, sometimes slightly scalloped. Lamellae emarginate, quite widely spaced (L = 26–39) with a maximum depth of 2.5 mm; colour cream, alutaceous or brown when young, later umberto sepia following spore maturity; edge fibrinate, paler than lamella surface; droplets on the lamella edge are usually present and visible to the naked eye, however in dry conditions they may not be seen; lamellules sparse. Stipe central, cylindrical occasionally clavate, stuffed, (14–)15–28 × 2.5–3.1(–3.5) mm and up to 6 mm at the base; white or alutaceous, with no visible discoloring when handled; surface dry, pruinose particularly towards the apex. Cortina not observed. Flesh rather thin, cream or pale brown. Smell raphanoid, sometimes weakly. Taste not recorded. Spore deposit clay-buff.

Fig. 11 Hebeloma aurantioumbrinum (BR-MYCO 173985-64, holotype). a. Basidium; b. Spores; c. Cheilocystidia. — Scale bars: 5 μm.
case when it might be collected in a boreal rather than alpine or arctic habitat (we have only seen one such collection), then the small size of mushroom, the apex of the cheilocystidia that does not show any abnormal thickening and the more or less unicoloured brownish orange pileus separates this species from the other small species of H. subsect. *Denudata*, namely *H. luteicystidiatum*, *H. helodes* and *H. pusillum*.

Molecularly, *H. aurantioumbrinum* is sister species to *H. helodes* from which it can only be separated based on molecular data.

*Hebeloma aurantioumbrinum* (M.M. Moser, Z. Pilzk. 36, 1: 72. 1970. = *Hebeloma cheilocystidiatum*, Z. Mykol. 53, 2: 294. 1987.

Type. *Morocco*, Azrou (c. N33.417 W5.217, alt. c. 1500 m) with *Cedrus libani* ssp. atlantica, 8 Nov. 1941, G. Malençon, MPU GM1122, database record HJB1000095.

*Basiocystidia* usually in scattered groups, sometimes solitary, sometimes caespitose. *Pileus* (8)–20–133 mm diam, convex often conical, surface often viscid, tacky when moist but never hygrophanous; *cuticle colour* always pale from pale cream or buff or brown when young, later umber to sepia following spore deposition; *margin* which is always pale cream or slightly yellowish; *upper surface* often involute particularly in young basidiomes but then straightening in older basidiomes, rarely serrate. *Lamella edge* always pale from pale cream to buff or occasionally involute, especially towards the apex. *Stipe* dry, pruinose to distinctly floccose, surface; droplets normally very visible on the lamella edge; *Flesh* white to pale buff.

*Smell* present throughout the basidiome.

**Habitat & Distribution** — *Hebeloma aurantioumbrinum* has been recorded on a wide variety of trees, primarily broadleaf but occasionally also with conifers. In Malençon’s original description he claims it is uniquely associated with *Cedrus* but we have numerous collections where no *Cedrus* was present. Trees recorded as associates include: *Betula ssp.*, *Carpinus betulus*, *Cedrus ssp.*, *Fagus ssp.*, *Larix ssp.*, *Picea ssp.*, *Pinus ssp.*, *Populus ssp.*, *Quercus ssp.*, *Salix ssp.*, *Tilia ssp.*. It has been recorded in various types of woodland, both broadleaf and conifer, parklands, churchyards, urban areas, dunes, Salix thickets, often close to paths. Habitats include grassy places as well as exposed and overgrazed alvar.

We have no records of this species from arctic or alpine habitats. The species occurs in calcareous, chalky, sandy and clayey, both wet and dry soil conditions.

*Additional specimens examined.* AUSTRIA, Tirol, Sitz, Forstgarten (c. N48.38 W16.72, alt. c. 1500 m) in plantation with *Picea sp.* and *Pinus sylvestris*, 18 Oct. 1962, F. Gobit (IB19620098, database record HJB1000071; this is the holotype of *H. perpallidum*). — BELGIUM, Brussels, Forêt de Soignes (N50.795333 E4.4279333, alt. c. 100 m) in broadleaf woodland on calcareous soil with *Fagus sylvatica*, 8 Sept. 2006, H. Beker-HJB11187; Forêt de Soignes (N50.795333 E4.4279333, alt. c. 100 m) in broadleaf woodland on calcareous soil with *Fagus sylvatica*, 8 Sept. 2006, H. Beker-HJB11189; Zaventem (c. N50.89 E4.46, alt. c. 40 m) in grassland under *Betula sp.*, 8 Oct. 2005, D. Deschatyter HJB111235; prov. Luxembourg, Reine (N50.31085 E4.4318333, alt. c. 180 m) in mixed woodland with *Picea sp.*, 8 Oct. 2004, M. Lenne HJB10290. — CZECH REPUBLIC, LPA Moravian Karst, NNR Vyvyr Punky (N49.37245 E16.73003, alt. c. 490 m) in mixed woodland on calcareous grassy soil under *Populus sp.*, 10 Oct. 2008, S. Kelly HJB12171; LPA Moravian Karst, NNR Vyvyr Punky (N49.40500 E16.73003, alt. c. 489 m) on calcareous grassy soil under *Populus sp.*, 1 Oct. 2008, S. Kelly HJB12172. — DENMARK, Brønderslev W of Århus, Egebjergvej (c. N56.15 E10.10, alt. c. 30 m) on grassy soil with *Salix sp.*, 7 Sept. 2004, H. Beker-HJB12720. — ESTONIA, Saaremaa, Salme commune, Kaugatoma-Löo (N58.09321 E22.17693, alt. c. 5 m) on calcareous chalky soil in urban broadleaf scrub under *Betula pubescens*, 18 Sept. 2008, J. Vesterholt Jv06-251, HJB13237; Hiiumaa, Pühala commune, Saare peninsula, Soonepla (N58.85049 E23.03069, alt. c. 10 m) in mixed woodland, on grassy calcareous soil with *Betula pubescens*, *Picea abies* and *Pinus sylvestris*, 25 Sept. 2008, J. Vesterholt Jv08-408; duplicate HJB12338;
Fig. 12  *Hebeloma eburneum* (MPU GM1122, holotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH. — *H. eburneum* (HJB12772), e, f. Cheilocystidia ×1 000 in 5 % KOH; g. basidia at gill edge ×500 in 5 % KOH; h. caulocystidia ×500 in 5 % KOH. — Scale bars: 10 µm.
Hiiumaa, Kõrgessare Commune, Reigi cemetery (N58.93808 E22.51068, alt. c. 10 m) in churchyard lawn on dry calcareous soil under Betula pubescens and Larix sp., 25 Sept. 2008, J. Vesterholte C JV08-405, duplicate HJB12343. — FINLAND, Turku town, Mäntymäki, near the cross of Vähä-Hilkäläntie and Luolavuorenkatu (c. N60.64055 E22.27442, alt. c. 40 m) on lawn under Tilia sp., 22 Sept. 2005, J. Vauras C TURA JV23655, duplicate HJB12339. — FRANCE, Vienne, Saint-Berme (N46.4542667 W0.0171467, alt. c. 17 m) in parkland on gravelly soil under Populus sp., 4 Nov. 2004, H. Beker HJB10525; Provence, Le Verdiere (c. N43.94 E4.877, alt. c. 30 m) with Pinus halepensis and Quercus ilex, 9 Nov. 1999, P.-A. Moreau C JV99-720, duplicate HJB10899. — GERMANY, Hemhut, Herrschaftsgarten (c. N51.010430 E14.758030, alt. c. 330 m) under Carpinus betulus, 29 Oct. 1999, G. Zschieschang GLM GL04795, database record HJB10979; Loassa (c. N40.3220 E11.410, alt. c. 310 m) under Betula sp., Populus sp., Salix sp., 24. Oct. 2004, D. Penke GLM HB079138, duplicate HJB12243; Parkplatz, Hilpoltstein, Bayern (c. N49.189 E11.184, alt. c. 380 m) with Tilia sp., 24 Sept. 1995, D. Aaen WBS 9511, database record HJB12501; Regensburg, Universitätsgelände (c. N49.00 E12.09, alt. c. 370 m) with Tilia sp., 4 Oct. 1978, J. A. Bresinsky M5340, database record HJB13014. — HUNGARY, Fót Com. Pest (c. N47.50 E19.40, alt. c. 220 m) in broadleaf woodland on sandy soil under Populus sp., 20 Oct. 1971, J. Schumeth BP 48.427, database record HJB1000052, this is the holotype of H. ochroalbidum. — MACEDONIA, Ezernari near Asamati village (N40.99359 E21.03931, alt. c. 855 m) in thicket under Populus sp., 10 Nov. 2008, S. amplexicaulis and on calcareous soil with Quercus alnus, c. 520 m) in parkland on grassy soil under Populus sp., 23 Sept. 2008, I. Katucka, H. Beker HJB12667; Mt Kamiensk (the outer damping ground of the Belchatow Lignite Mine), forest distr. 300b (near the cross, plot Z-5d, 12 year old plantation) HJB12670; Jelonka reserve (undisturbed), plot 16 HJB12671; Chotow, forest distr. 310a (c. N52.596 E23.363, alt. c. 180 m) in naturally regenerated conifer plantation with Pinus sylvestris and Alnus sp., 23 Sept. 2008, H. ochroalbidum, which were all described as large and fleshy with a very pale coloured cap. Moser saw the primary differences as the presence or absence of tears on the lamellae and this species from H. albobolossum, H. ochroalbidum and H. perpallidum, which were all described as large and fleshy with a very pale coloured cap. Moser saw the primary differences as the presence or absence of tears on the lamellae and the discolouration of the stipe. He regarded H. eburneum as a Mediterranean species and the others as more northern. He also believed spore size and the presence of a papilla on the spores, leading to a more limoniform shape, as significant (Moser 1970, 1985). The presence or absence of tears can be affected by the weather and while it is a good character it is not a dependable character. The discolouration of the stipe is also an important character but again can be affected by local ecology; for this species the stipe does not usually discolour very much with age, however, when conditions are very damp the stipe can exhibit some discolorising which starts from the base of the stipe. This species is morphologically close to H. aenanii and to H. geminatum but usually has rather larger spores; we have had one collection of H. eburneum that has smaller spores than usual and if the spores are both short and narrow it may become impossible to separate these species purely on the basis of the spore size. Hebeloma eburneum is also morphologically close to H. crustuliniforme but can be distinguished through its cheilocystidia, which have on average a larger apex. Hebeloma crustuliniforme also tends to have a rather more coloured cap.

If species identification was attempted with a single locus only, then RB2 was probably the most reliable among the loci tested. The majority of H. eburneum ITS sequences form a H. eburneum clade (Fig. 1a). Most of the H. eburneum sequences placed outside this clade are phased copies from collections with length differences within their ITS. These sequences are in the unresolved part of the tree, together with H. alpinum and H. geminatum sequences. Typically, only one of the two phased alleles of a collection falls outside the H. eburneum clade. The only exception to this is the type of H. albobolossum where both alleles, if phased (results not shown as there are only ambiguities, no length difference in the direct ITS reads), are in the unresolved part of the tree. We did not obtain many RB2 sequences, but the results obtained so far argue against hybridization and suggest that the presence of unspecific ITS alleles in H. eburneum is more likely a consequence of incomplete lineage sorting than of frequent hybridization events. As in H. alpinum, the combination of V6 and V9 is another option for separating H. eburneum from other members of the H. crustuliniforme complex, because H. eburneum V6 sequences form mixed clades with H. aenanii and V9 sequences with H. alpinum, and the H. alpinum V6 sequences or the H. aenanii V9 sequences, respectively, are clearly different from H. eburneum based on presence/absence patterns of indels. In deciding on the priority of the name H. eburneum a number of factors have been taken into account:

---

Notes — Given the shape of its cheilocystidia, H. eburneum clearly belongs to H. subsect. Denudata. The species most likely corresponds to ICG3 of Aaen & Kuyper (1999). It appears to have a wide range both geographically and ecologically. There is no morphological or molecular evidence to separate this species from H. albobolossum, H. ochroalbidum and H. perpallidum, which were all described as large and fleshy with a very pale coloured cap. Moser saw the primary differences as being the presence or absence of tears on the lamellae and the discolouration of the stipe. He regarded H. eburneum as a Mediterranean species and the others as more northern. He also believed spore size and the presence of a papilla on the spores, leading to a more limoniform shape, as significant (Moser 1970, 1985). The presence or absence of tears can be

Fig. 13 Hebeloma eburneum (MPU GM1122, holotype). a. Basidia; b. spores; c. cheilocystidia. — Scale bars: 5 µm.
Hebeloma eburneum and H. perpallidum both have publication dates of 1970 (Maleçon & Bertault 1970, Moser 1970). Hebeloma ochroalbidum has an effective publication date of 1972 (Bouis 1972) and H. albocolossum has an effective publication date of 1986 (Moser 1985).

Hebeloma perpallidum was published in Z. Pilzk. vol. 36, Parts 1 & 2. We have been informed by the publishers that this was published in December 1970. Hebeloma eburneum was published in the Flore des Champignons Superieurs du Maroc Vol. 1 which is Volume 32 of Trave. Inst. Sci. Cherifien de la Fac. Sci. Rabat. We have not been able to establish a precise date of publication for this volume but: Hebeloma eburneum was listed in Index of Fungi 4, 3: 65 (early 1972); but perpallidum was not listed until a year later, in Index of Fungi 4, 5: 133 (early 1973).

The book by Maleçon & Bertault was reviewed in Bull. Soc. Mycol. France 86, 3: 793 on 7 May 1971 and most other publications reviewed in that issue of the journal have a publication date of 1969, or even 1968. This suggests that the book was published relatively early in 1970 and almost certainly not as late as December 1970.

The holotype of H. crustuliniforme var. tiliae is a mixed collection. The part of the collection that corresponds best with the description is designated here as lectotype of the species (M 0-151680, database record HJB13014, MycoBank MBT198154).

**Hebeloma geminatum** Beker, Vesterh. & U. Eberh., sp. nov. — MycoBank MB809907; Fig. 5c, 14, 15

Etymology. From the base geminus meaning a double or twin and to emphasise that this is one of two taxa that can be differentiated molecularly and biologically but for which we have found no unambiguous morphological character to separate them.

Type. **Demark**, Nystrom Kilplantage, S of Kridtstien (N57.00 E8.52, alt. c. 42 m) with Abies sp., 10 Oct. 1996, J. Vesterholt holotype C JV96-341; isotype BR BR-MYCO 173983-62, database record HJB10833.

**Diagnosis — Hebeloma geminatum with primarily capitately-clavate or spathulate-stipitate cheilocystidia belongs to H. subect. Denuata. It can be distinguished from other taxa of the section by its average number of lamellae, which is ≥ 60, its sect. Cortina color and neutral soils, often sandy or mossy and in rotten litter, in a range of woodlands and plantations, often on roadsides or pathsides, as well as in gardens and parklands and also on slagheaps.

**Additional specimens examined. Belgium**: prov. Brussels, Foret de Soignes (N50.790 E4.430, alt. c. 114 m) on rotten litter in broadleaf woodland under Fagus sp., 16 Sept. 2003, H. Beker HJB8633; prov. Antwerp, Geel, Deksheovevijver IBFL: C6.21.34 (c. N51.1667 E5.000, alt. c. 20 m) under Populus sp., 19 Oct. 2003, J. Volders VJ03073, duplicate HJB10969; prov. Luxembourg, Ste-Cecile (N49.7632 E5.269050, alt. c. 257 m) on rotten litter in broadleaf woodland, 17 Sept. 2004, L. Renne HJB10803; prov. Hainaut, Maubray (N50.5302833 E3.4948667, alt. c. 52 m) on rotten litter in broadleaf woodland on slagheap under Betula sp., Populus sp., Salix sp., 10 Nov. 2006, P.-A. Moreau HJB11736. — **Demark**, region NEZ, Tisivilde Hegn, East end (c. N56.020 E12.030, alt. c. 15 m) under Betula sp., Picea sp., Pinus sp., Populus sp., Quercus sp., Salix sp., on various acid and neutral soils, often sandy or mossy and in rotten litter, in a range of woodlands and plantations, often on roadsides or pathsides, as well as in gardens and parklands and also on slagheaps.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall but rarely with any papilla, guttulate with one or more oily drops, weakly ornamented to distinctly ornamented, sometimes with some sign of loosening perispore in a few spores and weakly dextrinoid (O2:03, (P0:01)P2; D0:01); spore colour under the microscope yellow- or grey-brown to brown; spore size based on n = 63 spores of the holotype, 5–95% percentile range 9.2–11.7 × 5.4–6.9 µm, with median 10.3 × 6.0 µm and av 10.4 × 6.0 µm with SD length 0.85 µm and width 0.51 µm, Q value 5–95% percentile range 1.58–1.89, with median 1.74 and av 1.74 with SD 0.1; spore shape based on 25 collections medians 9.8–10.8 (–11.6) × 5.4–6.4 µm and av 9.8–10.8 (–11.6) × 5.4–6.3 µm with SD length 0.38–1.02 µm and width 0.19–0.51 µm, Qav 1.65–1.93. Basidia cylindrical to clavate and 4-spored, 22–36 × 6.6–9.9 µm, with av 26–35 × 7.1–9.0 µm. Pleurocystidia not found. Cheilocystidia capitate-, clavate- and sometimes staphylate-siptitate, occasionally slightly swollen towards the base (clavate-lageniform) and occasionally with some median thickening, sinuate, septate or bifurcate; width of apex holotype 5–95% percentile range 6.8–12.2 µm, with median 9.4 µm and av 9.4 µm with SD 1.74 µm; across 25 collections median 8.0–10.4 µm and av 8.0–10.4 µm (of the 25 collections 10 have the cheilocystidium apex ≥ 9 µm); with ≥ 20 selected cheilocystidia of 25 collections the 5–95% percentile ranges are 36–91 × 6.2–13.2 × 2.6–6.2 × 2.4–7.2 µm while the averages are 50–72 × 8.0–10.4 × 4.0–4.7 × 3.7–5.0 and 55 × 9.4 × 4.2 × 5.0 µm av for the holotype. The av cheilocystidium ratios for the 19 collections were: A/M = 1.76–2.57, A/B = 1.68–2.65, B/M = 0.81–1.19. Caulocystidia resemble cheilocystidia, up to 70 µm long and 12 µm wide at the apex. Pleiopelis is an ixocutis with a medium thick epiixocutis 110–200 µm, embedded hyphae up to 5–8 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. Cutis yellowish and made up of cylindrical to isodiametric elements. Trama below the subcutis contains cylindrical, ellipsoidal, spherical and thick-sausage shaped elements up to 16 µm broad. Clamp connections present throughout the basidiome.

**Habitat & Distribution — Hebeloma geminatum** has been recorded with a variety of trees including: Abies, Betula, Corylus, Fagus, Picea, Pinus, Populus, Quercus, Salix, Tilia, on various acid and neutral soils, often sandy or mossy and in rotten litter, in a range of woodlands and plantations, often on roadsides or pathsides, as well as in gardens and parklands and also on slagheaps.

Spore deposit raphanoid, usually mild. Smell raphanoid. Tast usually mild. 

Fig. 14 Hebeloma geminatum (C-F-90152 (JV96-341, holotype)). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e. cheilocystidia ×500 in 5 % KOH; f. cheilocystidia ×1 000 in 5 % KOH; g. basidia ×1 000 in 5 % KOH; h. caulocystidia ×500 in 5 % KOH; i. caulocystidia ×1 000 in 5 % KOH. — Scale bars: 10 µm.
Baraka, Gorse Hill Road (N51.4093800 W0.5650113, alt. c. 70 m) on rotten litter in urban garden under Betula sp., 31 Oct. 2004, H. Beker HUB10384; West Kent, Tudeley Wood (c. N51.1660700 E0.3099706, alt. c. 88 m) on sandy soil in broadleaf woodland under Betula sp., Quercus sp., 22 Oct. 2005, A. Andrews HJB11545; Surrey, La Baraka, Gorse Hill Road (N51.4088400 E0.5650200, alt. c. 51 m) on mossy soil in urban garden under Betula pendula, 28 Oct. 2009, H. Beker HUB13309. — ESTONIA, Hiiumaa district, Puhtahale commune, Sarve (c. N58.180 E22.060, alt. c. 0 m) on alvar soil in woodland path under Betula sp., Corylus avellana, Juniperus sp., Pinus sylvestris, 16 Sept. 2001, J. Vauras 17298F, HJB10961. — FRANCE, Yvelines, St-Leger-en-Yvelines, Foret domaniale de Rambouillet (c. N48.6703 E1.7797, alt. c. 160 m) on sandy soil in conifer woodland plantation under Pinus sp., 3 Nov. 1998, G. Coriol GC88 11 03 02, HJB12834; Nord Pas de Calais, Auberchicourt (N50.3475667 E3.2351667, alt. c. 23 m) on grassy soil in broadleaf woodland slaghheap under Betula sp., Salix sp., 13 Nov. 2006, C. Lecuru HJB11801. — NETHERLANDS, Groningen, Eems haven (c. N53.455E E6.806, alt. c. 0 m) under Salix alba, S. caprea, S. repens, 3 Oct. 2006, D. Aaen WBS 9675, database record HJB12469. — NORWAY, Skibotndalen fieldstation Tromsø (c. N69.260 E20.510, alt. c. 260 m) under Betula pubescens, 6 Aug. 1995, M. Moser (as H. albocolossum) IB 19950057, HJB11629. — POLAND, Mt Kamiensk (N51.2245833 E19.403611, alt. c. 220 m) on calcareous, clayey, sandy soil in conifer woodland plantation and quarry site under Pinus sylvestris, Populus sp., Salix sp., 2 Nov. 2004, I. Kalucka LOD IK-H0004, HJB13188; Wolkawa Lekawksa, forest distr. 8C k (plot no. P-2), 9 year old pine plantation (75 % pine, 25 % birch) on former arable land (N51.3001500 E19.3972700, alt. c. 88 m) on sandy soil in broadleaf woodland under Betula pendula sp., 22 Oct. 2005, A. Andrews HJB11545; Surrey, La Baraka, Gorse Hill Road (N51.4093800 E0.5650113, alt. c. 700 m) in wet soil with Filipendula ulmaria, 24 June 2004, A. Taylor AT2004061, HJB10770; Uppsala, Uppsala Hospital (c. N59.600 E16.540, alt. c. 49 m) on grassy soil in mixed parkland under Picea sp., Pinus sp., Tilia sp., 5 July 2004, A. Taylor AT2004061, HJB10776.

Notes — Given the shape of its cheilocystidia, H. geminatum clearly belongs to H. subsect. Denudata. H. helodes geminatum most likely corresponds to ICG1 of Aaen & Kuyp (1999) and is a constituent of the ‘Hebeloma crustuliniforme complex’. It is likely that many collections of this species have been recorded under the name H. crustuliniforme and exist worldwide in herbaria under this name. It is morphologically similar to H. aenennii, H. alpinum, H. crustuliniforme and H. eburneum. But its spores, normally < 11 µm long and < 6 µm wide (rarely longer or wider) distinguish it from the first three species. It can be distinguished from other members of this subsection by the number of complete lamellae, which is always ≥ 60.

Until now we have found no consistent morphological character to separate H. aenennii and H. geminatum. However, we can often separate these two taxa through the use of a mixture of characters, although none appears fool proof. The best character appears to be the cheilocystidium apex width; for H. geminatum this is usually larger than that for H. aenennii. From our records, if the average width of the apex of the cheilocystidium is more than 9 µm then the collection is almost certainly H. geminatum. However, the average apex width can be as small as 8 µm and widths in this interval between 8–9 µm can be from either taxon. This is illustrated in the scatter diagram (Fig. 4b).

Of course one can combine characters, so, for instance a collection with large basidioles exhibiting a thick epicutis in excess of 200 µm and a cheilocystidium apex width of < 8.5 µm will almost certainly be H. aenennii. Similarly, a collection with small basidioles, a relatively thin epicutis and cheilocystidium apex width of > 8.5 µm will almost certainly be H. geminatum. A synoptic key will be far more powerful as a tool based on morphological characters; but we do not yet feel we have examined sufficient collections to build such a key with confidence.

It is worth noting that we do not have any confirmed records from southern Europe. Other than two Swiss alpine collections we have not seen (and therefore are not cited in the species description, WBS 9618, WBS 9621, corresponding to ICG1, Aaen & Kuyp 1999), our most southerly collection is from France at a latitude of more than N48.67.

Molecularly, H. geminatum and H. alpinum are very close and sequences of both species are paraphyletic in four out of the five loci tested, though, in MCM7 H. geminatum forms clades not including H. alpinum (apart from collection HJB11051 discussed elsewhere). The only locus that normally clearly separates H. geminatum from H. alpinum is V9. Hebeloma alpinum lacks an indel present in H. geminatum and some other members of H. subsect. Denudata. In fact, there is only a single bp difference between some H. aenennii and H. geminatum V9 sequences, but all other loci, including the ITS, can separate those two taxa.

Collection HJB11051 (see also in the Discussion) is genetically closer to H. geminatum in all loci tested than to H. alpinum, but corresponds morphologically with H. alpinum and, being an alpine collection, is also ecologically more typical for the latter species. One could argue that the genetic assignment is more meaningful in terms of ancestry and call the collection H. geminatum. However, as it is morphologically clearly different from typical H. geminatum, we decided not to include it here.

The V9 sequence of H. geminatum HJB11545, in spite of possessing the indel, differs quite strikingly in a number of positions from the ‘normal’ V9 of H. geminatum and in fact from all other V9 sequences obtained from members of its subsection. The placement of HJB11545 in the concatenated analyses has to be interpreted as being outside the H. geminatum clade as opposed to being a member of H. alpinum. Morphologically and ecologically, HJB11545 fits well into H. geminatum, only the spore width with an average of 5.4 µm is an outlier within this taxon.

Hebeloma helodes J. Favre, Beitr. Kryptogamenfl. Schweiz 10, 3: 214. 1948. — MycoBank MB286871; Fig. 16, 17

Type. SWITZERLAND, Vaud, Jura. Tourbiere du sentier, Vallée de Joux (c. N48.6122 E6.2358, alt. c. 1000 m) in wet soil with Filipedula ulmaria in a bog with Betula sp. and Pinus sp., 30 Aug. 1939, G. Favre 9139, lectotype G 00003920, database record HJB1000054, selected by F. Gröger in Myk. Mittbl. 30, 2: 46, 1987.

Basidiomes usually in scattered groups or sometimes solitary. Pileus 13–38 mm diam, convex, sometimes weakly umboinate,
Fig. 16 Hebeloma helodes (G 00053920, lectotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e. cheilocystidia ×1 000 in 5 % KOH; f. cheilocystidia ×500 in 5 % KOH; g. basidium ×1 000 in 5 % KOH; h. caulocystidium ×500 in 5 % KOH; i. epicuts hyphae ×500 in 5 % KOH. — Scale bars: 10 µm.
sometimes slightly depressed in the centre especially when older, often viscid, tacky when moist but never hygrophanous; cuticle colour quite pale and cream or white towards the margin of the centre sometimes darker from light ochraceous to dark beige or yellowish brown; pileus margin usually involute although this feature sometimes disappears in older basidiomes. Lamellae emarginate, moderately spaced (L = 37–54) with a maximum depth of 3–5 mm; colour cream, alutaceous or brown when young, later umber to sepia following spore maturity; edge fimbriate, significantly paler than lamella surface; droplets normally visible on the lamella edge even with the naked eye; lamellules frequent. Stipe central, usually cylindrical but sometimes slightly to distinctly clavate, becoming hollow with age, 15–60 × 3–4.5 mm and up to 7 mm at the base; white or alutaceous, occasionally with some slight brown discolouration at the base of the stipe; surface dry, fuscose particularly towards the apex. Cortina not observed. Flesh rather thin, whitish but more slightly coloured in the base of the stipe. The whole basidiome has a very slender appearance with the length of the stipe normally at least twice the width of the pileus. Smell not observed. Taste raphanoid, rarely absent.

**Spores** amygdaloid, with small apiculus and rounded at the end, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, rarely with any papilla, gullettate with one or more oily drops, from weakly to distinctly ornamented, sometimes slightly to distinctly clavate, becoming hollow with age, 15–60 × 3–4.5 mm and up to 7 mm at the base; white or alutaceous, occasionally with some slight brown discolouration at the base of the stipe; surface dry, fuscose particularly towards the apex. Cortina not observed. Flesh rather thin, whitish but more slightly coloured in the base of the stipe. The whole basidiome has a very slender appearance with the length of the stipe normally at least twice the width of the pileus. Smell raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, rarely with any papilla, gullettate with one or more oily drops, from weakly to distinctly ornamented, usually with some sign opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a definite thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.

**Spores** amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, raphanoid, rarely absent. Taste raphanoid, not bitter. Spore deposit Isabella to brownish olive.
Bialowiecki Park Narodowy (N52.7100 W23.84479, alt. c. 150 m) on grassy wet acid soil in broadleaf woodland under Fagus sylvatica, 5 Oct, 2002, H. Beker HJB98115.

Notes — Given the shape of its cheilocystidia, *H. helodes* clearly belongs to *H. subsect.* *Denudata*. The species most likely corresponds to ICG12 of Aanen & Kuyper (1999). We have no confirmed records of this taxon in arctic or alpine habitats although it does exist in these habitats. It can be readily distinguished from the other members of this subsection that grow in lowland areas based on the number of lamellae, 30–60, the small spore length, < 11 µm, the average width of the cheilocystidium apex, > 8 µm, and the regular apical thickening of the cheilocystidium. It has probably often been confused with *H. pusillum*, which is also a small *Hebeloma* sp., but *H. pusillum* is confined to *Salix*, often has < 30 full length lamellae, has a more fragile stature and usually a rather darker centre to the pileus. It also has significantly longer spores, on average. *Hebeloma luteicystidiatum* also has fewer lamellae and longer spores and is usually somewhat smaller. *Hebeloma aurantiombrinum* is rarely found in non-alpine/arctic habitats, but in any case can be distinguished microscopically because its cheilocystidia very rarely have any sign of apical thickening. It also usually has a much more brightly coloured pileus.

As shown in Fig. 1 and 2, all loci tested are suitable for distinguishing *H. helodes* from species other than *H. aurantiombrinum*. *Hebeloma helodes* cannot be distinguished molecularly from *H. aurantiombrinum* based on ITS, V6 or V9, but either of the coding genes RPB2 or MCM7 will be suitable for the identification of *H. helodes*.

*Hebeloma louiseae* Beker, Vesterh. & U. Eberh., sp. nov. — Myco-Bank MB809908; Fig. 5d, 18, 19

Etymology. To mark the support Louise Beker has provided to this entire project not only with her time but also travelling to remote places in search of *Hebeloma* spp.

Type. SVALBARV, Ossian near Ny Ålesund (N78.9260500 E12.4542667, alt. c. 3 m) on mossy soil with *Salix polaris*, 17 Aug. 2007, M.L. Beker, H. Beker holotype BR BR-MYCO 173982-61; isotypes C C-F-90149, HJB12019.

Diagnosis — *Hebeloma louiseae* was found in arctic conditions in Svalbard and is most similar to *H. minus*, from which it differs by the less prominent spore ornamentation - O1 or O2 and at most a few spores O3. It differs from other taxa of *Hebeloma* subsect. *Denudata* occurring in arctic conditions by its small number of complete lamellae (< 40) in combination with an average cheilocystidium apex width of ≥ 9 µm.

*Basidiomes* usually in scattered groups. *Pileus* up to 15 mm diam, convex or plano-convex to broadly umbovate; *surface* dry or slightly viscid, neither hygrophanous nor striate, sometimes with a slight pruinose layer; *cuticle colour* clay buff to Isabella sometimes with a thin paler margin; *pileus margin* straight, sometimes crenulate and slightly involute even in fully grown basidiomes. *Lamellae* emarginate, quite widely spaced (L = 30–38); *colour* cream, alutaceous or brown when young, later umber to sepia following spore maturity; *edge* fimbriate, paler than lamella surface; droplets on the lamellae edge were not seen; *lamellules* sparse. *Stipe* central, cylindrical sometimes slightly clavate or tapering towards the base, (8–)9.5–21.5(–25) × 2–3(–3.5) mm; white or alutaceous, sometimes discoloring brown near the base when handled; *surface* dry, with fine fibrils and pruina over the length of the stipe. *Flesh* not observed. * Flesh* rather thin, cream or pale brown, discoloring slightly when bruised. *Smell* raphanoid or absent. * Taste* not recorded. * Spore deposit* greyish brown.

*Spores* amygdaloid to limoniform, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall and often with a pronounced papilla, guttulate with one or more oily drops, almost smooth to very weakly ornamented, with no sign of loosening perispore and dextrinoidity ranging from completely indextrinoid to weakly dextrinoid (O1, O2; P0, D0, D1); *spore colour* under the microscope yellow brown to brown; *spore size* based on n = 69 spores of the holotype, 5–95 % percentile range 11.6–14.4 × 6.9–8.6 µm, with median 12.7 × 7.7 µm and av 12.8 × 7.7 µm with SD length 0.82 µm and width 0.53 µm. O value 5–95 % percentile range 1.50–1.80, with median 1.67 and av 1.66 with SD 0.1; *spore size* based on 3 collections medians 12.2–12.7 × 7.5–7.7 µm and av 12.3–12.8 × 7.5–7.7 µm with SD length 0.53–0.82 µm and width 0.36–0.53 µm, Qav 1.63–1.66. *Basidia* cylindrical to clavate and 4-spored, 28–56 × 8.6–11.6 µm, with av 33–48 × 9.5–10.5 µm. *Pleurocystidia* not found. *Cheilocystidia* clavate-stipitate to subcapitate-stipitate, occasionally slightly swollen towards the base and occasionally separte, some apical thickening, sinuate or bifurcate; *width of apex* holotype 5–95 % percentile range 7.9–12.3 µm, with median 9.7 µm and av 9.9 µm with SD 1.48 µm; across 3 collections median 9.0–9.8 µm and av 9.0–9.9 µm; with n ≥ 20 selected chelio-

cystidia of 3 collections the 5–95 % percentile ranges are 37–76 × 7.1–12.7 × 3.5–6.5 × 4.0–6.8 µm while the averages are 49–59 × 9.0–9.9 × 4.4–5.4 × 4.8–5.6 and 57 × 9.9 × 5.4 × 4.9 µm av for the holotype. The av cheilocystidia ratios for the 3 collections were: A/M = 2.01–2.42; A/B = 1.99–2.25; B/M = 0.92–1.24. *Caulocystidia* resemble cheilocystidia, up to 75 µm long and 12 µm wide at the apex. *Pleipellis* is an ixocutis with a medium thick epicutis up to a maximum of 100 µm and embedded hyphae up to 6 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. *Cutis* orange brown and made up of cylindrical to isodiametric elements. *Trama below subcutis* contains larger thick sausage shaped elements up to 15 µm broad. *Clamp connections* present throughout the basidiome.
Fig. 19 Hebeloma louiseae (BR-MYCO 173982-61, holotype). a, b. Spores ×1 600 in Melzer’s reagent or 5 % KOH, respectively; c, d. spore ornamentation ×1 600 in Melzer’s reagent or 5 % KOH, respectively; e, f. cheilocystidia ×1 000 in 5 % KOH; g. cheilocystidia ×500 in 5 % KOH; h. basidium ×1 000 in 5 % KOH; i. caulocystidia ×500 in 5 % KOH; j. cutis ×80 in 5 % KOH. — Scale bars: 10 µm, in j. 100 µm.
Habitat & Distribution — All 3 collections of *H. louiseae* have been collected in Svalbard in association with *Salix polaris*. Two of the collections have been on coastal shingle, not far from the water’s edge but the third collection was from a grazed scrub area 4–5 km from the sea.

Additional specimens examined. *Svalbard*, Endalen near Longyearbyen (N78.1940667 E15.7892500, alt. c. 28 m) on soil in grazed valley with *Salix polaris*, 13 Aug. 2007. *J. Sandmo* HJB11894; Ossian near Ny Ålesund (N78.9259500 E12.4541167, alt. c. 0 m) on grazed scrub with *Salix polaris*, 17 Aug. 2007, M.L. Beker, H. Beker HJB12023.

Notes — *Hebeloma louiseae* has most likely not been included in the intercompatibility tests of (Aanen & Kuyper 1999) and is currently only known from Svalbard, but it has been collected in two sites some 110 km apart. It is likely that it is *Salix*-specific. The chelioecystidia place this species within *H. subsect. Denudata*. Given its arctic habitat, small size and very weakly ornamented spores this species is easily distinguished from other members of *H. subsect. Denudata*. While it is possible that this species only exists on Svalbard it is likely that it could be found in other arctic habitats, but its sizeier and camouflaged appearance may mean that it is often overlooked. Also it may in the past have been confused with other small arctic/alpine *Hebeloma* spp.

*Hebeloma louiseae* is not only monophyletic, but also receives (high) bootstrap support in four out of five tested loci. Only V9 cannot distinguish it from *H. minus*. Although the name *H. crustuliniforme* has traditionally been applied to collections of medium and large sporocarps (Vesterholt et al. 2014), typically growing outside the arctic and alpine habitats, in evolutionary terms, the *H. crustuliniforme* complex also includes small, arctic/alpine taxa. The analyses of V6, V9 and RP2 suggest that *H. louiseae* belongs to the *H. crustuliniforme* complex. This is contradicted by its placement in the ITS phylogram.

*Hebeloma luteicystidiatum* Beker, Vesterh. & U. Eberh., *sp. nov.* — MycoBank MB809909; Fig. 5e, 20, 21

*Etymology.* From *luteus* - yellow and *cystidiatus* – having cystidia, to emphasise the thick apical wall that sometimes looks yellow under the microscope.

*Type. Belgium,* prov. Limburg, Houthalen (N51.0154833 E5.3518667, alt. c. 45 m) in wet boggy ground next to sludgeheap with *Salix sp.*, 22 Oct. 2006, *J. Volders* holotype BR-BR-MYCO 166233-72 (VJ06095); isotypes C C-F-90150, HJB11837.

Diagnosis — Based on its chelioecystidium shape, *H. luteicystidiatum* is a member of *H. subsect. Denudata*. The most distinctive and constant character of the species are the thick walls of the chelioecystidium apices. With its small stature and 2-coloured pileus, *H. luteicystidiatum* is similar to *H. pusillum*. From the latter it can be separated based on the pileus colour, which may be dark, but not dark brick, and the cystidia of *H. pusillum* do not have chelioecystidia with wall thickening at the apex. Macroscopically, *H. helodes* is similar in terms of coloration, but is larger, has more crowded lamellae and shorter spores.

*Basidiomes* solitary or in scattered groups. *Pileus* 6–15 mm diam, convex, slightly tacky when moist but never hygrophanous, at high magnification the pileus has a slightly feltly look; *cuticle colour* in the central region ochre to honey or rusty-coloured to dark brown but becoming paler towards the margin which may be anywhere from buff to cream to white; *pileus margin* usually straight, occasionally involute or eroded with age. *Lamellae* emargined, almost free, distant (L = 21–26) with a maximum depth of 2.5 mm; *colour* cream, alutaceous or brown when young, later umberto sepal following spore maturity; *edge* fimbriate, significantly paler than lamella surface; droplets normally visible on the lamella edge usually by the naked eye but certainly with a ×10 lens; *lamellules* occasional. *Stipe* central, cylindrical usually studded, rarely hollow, 12–30 × 1.0–2.5 mm usually with strong brown discoloration towards the base of the stipe; *surface* dry, pruinose to fuscose. *Cortina* not observed. *Flesh* rather thin, whitish but slightly more coloured in the base of the stipe. The whole basidiome has a very slender and fragile appearance with the stipe Q (ratio of stipe length to stipe width) in excess of 12. *Scent* raphanoid, sometimes absent. *Taste* not recorded. *Spore* deposit not recorded. *Exsiccata* fragile and brittle often dark, sometimes blackening.

Spores amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, sometimes with a papilla, sometimes guttulate with one or more oily drops, from almost smooth to normally weakly ornamented, sometimes with slightly loosening perispore in a few spores and weakly but sometimes distinctly dextrinoid (O1;O2, P0;P1(P2); D1;D2). *Spore colour* under the microscope yellow brown to brown; *spore size* based on n = 53 spores of the holotype, 5–95 % percentile range 10.3–13.0 × 5.8–6.9 µm, with median 11.6 × 6.4 µm and av 11.6 × 6.4 µm with SD length 0.80 µm and width 0.32 µm, Q value 5–95 % percentile range 1.63–2.01, with median 1.85 and av 1.84 with SD 0.12; *spore size* based on 4 collections medians 11.6–11.9 × 6.1–6.4 µm and av 11.6–11.9 × 6.1–6.5 µm with SD length 0.59–0.80 µm and width 0.31–0.36 µm, Qav 1.80–1.95. *Basidia* cylindrically to clavate and 4-spored, 25–39 × 6.4–10.1 µm, with av 28–34 × 7.1–9.4 µm and basidium Q av in the range 3.3–4.7. *Pleurocystidia* not found. *Chelioecystidia*, clavate-stipitate or spathulate-stipitate, occasionally subcapitate-stipitate or slightly swollen towards the base (subcapitate-lageniform or clavate-lageniform), always with distinctive thickening of the apex, so much so that the apex sometimes appears yellow under the microscope, occasionally separtate and rarely with median thickening; *width of apex* holotype 5–95 % percentile range 7.2–11.8 µm, with median 9.1 µm and av 9.3 µm with SD 1.45 µm; across 4 collections median 8.6–9.7 µm and av 8.8–9.9 µm; with n at least 20 selected chelioecystidia of 4 collections the 5–95 % percentile ranges are 37–77 × 6.7–12.6 × 3.0–5.7 × 2.7–6.1 µm while the averages are 50–62 × 8.8–9.9 × 4.0–4.3 × 4.0–4.9 and 53 × 9.3 × 4.0–4.9 µm av for the holotype. The av chelioecystidia ratios for the 4 collections were: A/B = 2.27–2.77; A/B = 2.21–2.68; B/M = 0.98–1.22. *Caulocystidia* resemble chelioecystidia, up to 90 × 10 µm wide at the apex. *Pileipellis* is an ixocutis with a thin epicutis 50–60 µm, embedded hyphae up to 5 µm broad, smooth or sometimes encrusted, hyaline.
Fig. 21  *Hebeloma luteicystidiatum* (BR-MYCO 166233-72, holotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c. spore with loosening perispore ×1 600 in 5 % KOH; d, e. spores and spore ornamentation ×1 600 in 5 % KOH; f. cheilocystidia ×1 600 in Melzer’s reagent; g. cheilocystidia ×1 000 in Melzer’s reagent; h. basidium ×1 000 in 5 % KOH; i. cheilocystidia at gill edge ×500 in 5 % KOH; j. trama below cutis ×500 in 5 % KOH. — Scale bars: 10 µm.
or occasionally pigmented. *Cutis* orange brown and made up of cylindrical to isodiametric elements. *Trama below subcutis* contains ellipsoid, cylindrical and angular elements up to 20 µm broad. *Clamp connections* present throughout the basidiome.

**Habitat & Distribution** — All records of *H. lutense* are in association with *Salix* with which it clearly has a mycorrhizal relationship. Furthermore, all collections are from wet boggy areas, usually in *Salix* thickets in woodland. Additional specimens examined. *Belgium,* prov. Limburg, Houthalen (N51.0154833 E5.3518667, alt. c. 45 m) in wet boggy ground next to sphagnum with *Salix* sp., 31 Oct. 2007, H. Beker HJB121740. — *France,* Yvelines, St-Rémy-des-Cherouze, Bois de Champfleury (N45.72402 E2.06501, alt. c. 100 m) on boggy wet ground under *Alnus* sp. and *Salix* sp., 12 Sept. 1999, G. Corrivel GC99081202, duplicate HJB12936. — *Netherlands,* Amsterdam (c. N52.22 E4.53, alt. c. 30 m) collected with *Salix* sp., 9 Sept. 1995, D. Aaenen WBS 9901, database record HJB12462.

Notes — Given the shape of its cheilocystidia, *H. lutense* clearly belongs to *H.* subset *Denuidata.* The species most likely corresponds to ICG6 of Aanen & Kuyper (1999). It certainly appears to be restricted to *Salix* and occurs on wet soil, usually in *Salix* thickets. The basidiomes are very small and we suspect it has either tended to be overlooked or confused with *H. pusillum* on account of its small slender appearance and 2-coloured cap. However, it can easily be separated from *H. pusillum,* macroscopically because of the centre which while quite dark is not the distinctive dark brick colour of *H. pusillum* and microscopically because *H. pusillum* never has a consistently thickening apex to its cheilocystidia. *Hebeloma helodes* has pileus colours more like *H. lutense* but is a larger species and has pileus orange brown and made up of cylindrical to isodiametric elements. *Cutis* orange brown and made up of cylindrical to isodiametric elements. *Trama below subcutis* of *H. helodes* also has rarer more crowded lamellae.

*Hebeloma lutense* Romagn., Bull. Trimestriel Soc. Mycol. France 81: 342. 1965. — MycoBank MB331750; Fig. 22, 23

*Caulocystidia* usually in scattered groups or sometimes caespitose. *Pileus* 15–58 mm diam, convex, sometimes umbo nate, slightly viscid, tacky when moist but never hygrophanous; *cuticle colour* often almost zonate with the centre from yellow brown to cinnamon to chestnut or even dark brick, sometimes then with a paler but still strongly coloured zone and finally pinkish buff to cream to almost white near the margin; *pileus margin* usually straight, sometimes involute and occasionally slightly scalloped. *Lamellae* emarginate, moderately spaced (L = 32–58) with a maximum depth of 5–10 mm; *colour cream,* alutaceous or brown when young, later umber to sepia following spore maturity; *edge fimbriate,* paler than lamella surface; *droplets normally visible* on the lamella edge even with the naked eye; *lamellules* frequent. *Stipe* central, sometimes cylindrical but more often clavate and occasionally even subbulbous, (15–)22.5–90 × 3–11 mm and up to 18 mm at the base, studded when young but later hollow and sometimes with a superior wick; white or alutaceous, often with some brown discoloration in older basidiomes; *surface dry,* pruinose to floccose particularly towards the apex. *Cortina* not observed. * Flesh* rather thick, whitish but slightly more coloured in the base of the stipe. *Smell* raphanoid, sometimes with hint of cocoa, rarely detected. Taste raphanoid to bitter. *Spore deposit* brownish olive toumber.

*Spores* amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall, occasionally with a weak papilla, guttleate with one or more oily drops, from almost smooth to weakly ornamented but more usually distinctly ornamented, usually with some sign of loosening perispore in a few spores and often in many spores and weakly but distinctly dextrinoid (Q10)O2.03, (P0)P1.22, D1.20; *spore colour* under the microscope yellow brown to brown; *spore size* based on n = 57 spores of the holotype, 5–95 % percentile range 9.2–11.7 × 5.5–6.6 µm, with median 10.6 × 6.0 µm and av 10.6 × 6.1 µm with SD length 0.73 µm and width 0.34 µm, Q value 5–95 % percentile range 1.62–1.88; with median 1.74 and av 1.74 with SD 0.9; *spore size* based on 22 collections: medians 9.4–11.7 × 5.2–6.3 µm and av 9.4–11.7 × 5.4–6.4 µm with SD length 0.46–1.08 µm and width 0.24–0.57 µm, Qav 1.63–1.91. *Basidia* cylindrical to clavate and 4-spored, 27–40 × 5.3–8.9 µm, with av 29–38 × 6.5–8.0 µm and basidium Qav unusually high in the range 4.0–5.7. *Pleurocystidia* not found. *Cheilocystidia* clavate or clavate-stipitate, occasionally slightly swollen towards the base (clavate-lageniform) and occasionally with septa, sometimes clamped, or median thickening and almost always with a large number of sinuate cheilocystidia; *width of apex* holotype 5–95 % percentile range 5.8–9.6 µm, with median 7.7 µm and av 7.7 µm with SD 1.22 µm; across 22 collections median 6.6–7.8 µm and av 6.7–7.9 µm; with n = 20 selected cheilocystidia of 22 collections the 5–95 % percentile ranges are 28–71 × 5.2–10.4 × 2.7–5.7 × 2.4–7.4 µm while the averages are 42–56 × 6.7–7.9 × 3.4–4.5 × 3.5–4.8 and 47 × 7.7 × 4.2 × 4.3 µm av for the holotype. The av cheilocystidia ratios for the 22 collections were: A/M = 1.63–2.39; A/B = 1.58–2.34; B/M = 0.95–1.22. *Caulocystidia* resemble cheilocystidia, up to 95 µm long and 9 µm wide at the apex. *Pileiellus* is an ixocuttis with a relatively thick epicutis 100–180 µm, embedded hypae up to 5 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. *Cutis* orange brown and made up of cylindrical to isodiametric elements. *Trama below subcutis* contains ellipsoid or thick sausage shaped elements up to 15 µm broad. *Clamp connections* present throughout the basidiome.

**Habitat & Distribution** — All our records of *H. lutense* have been collected in the presence of *Salix* spp. and it would appear likely that this species is confined to *Salix.* Many of the collections have been from wet or damp environments on acid soil. It is interesting to note that while our description is based on 22 collections of *H. lutense,* on our database we have 53 records. All of these are from Western Europe (highest longitude is < E11°).

Additional specimens examined. *Belgium,* prov. Hainaut, Maubray (N50.5306167 E3.4941000, alt. c. 30 m) slough, on mossy soil, collected with *Salix* sp. and *Betula* sp., 10 Nov. 2006, P.-A. Moreau HJB117117; *Prov. Limbourg,* Tielenwick (c. N50.967 E5.233, alt. c. 25 m) collected on wet soil under *Betula* sp., 12 Sept. 2004, A. de Haan ADM04059, duplicate HJB10523; *prov. Hainaut,* Maubray (N50.5306167 E3.4941000, alt. c. 29 m) on rotten litter in broadleaf woodland on slagheap under *Betula* sp., *Salix* sp., 10 Nov. 2006, P.-A. Moreau HJB11726. — *Denmark,* WJ, Marbaek plantation north of Esbjerg UTM MG5757 TBU 27 (c. N55.550 E5.310, alt. c. 0 m) on wet soil under *Salix* sp., 17 Sept. 1995, J. Vesterholt JV95-357, duplicate HJB10876; *prov. Limbourg,* N56.651 E9.585, alt. c. 40 m) scattered at lake side with *Salix* sp., 24 Oct. 1996, T. Letasse TL4413, duplicate HJB10905. — *England,* Surrey, Bookham Common (c. N51.27 W0.37, alt. c. 100 m) in mixed woodland under *Betula* sp. and *Salix* sp., 15 Oct. 1995, P.D. Orton E 00076300, database record HJB121917: this forms part of a collection of *H. leucosarx* which was mixed and the part that does not represent *H. leucosarx* as recently selected by Grilli (2007); Surrey, Boldermere (N51.3144100 W0.4517446, alt. c. 30 m) on wet soil in mixed woodland under *Salix* sp., 28 Sept. 2002, H. Beker HJB6098. — *France,* Landes, Mios (N44.5936667 W0.407333, alt. c. 0 m) in damp litter in
Fig. 22  *Hebeloma lutense* (P 59.232, holotype). a, b. Spores and spore ornamentation ×1600 in 5% KOH; c, d. spores and spore ornamentation ×1600 in Melzer’s reagent; e. cheilocystidia ×1000 in Melzer’s reagent; f. cheilocystidia ×500 in Melzer’s reagent; g–i. basidia ×1000 in Melzer’s reagent; i. subcutis ×500 in 5% KOH; j. epicutis hyphae ×500 in 5% KOH. — Scale bars: 10 µm.
a broadleaf woodland with Salix sp., 17 Oct. 2005, H. Beker HJB11328; Alpes-Maritimes, Les Espagnols (N43.5090667 E6.7947833, alt. c. 186 m) on lakeside amongst wet litter in mixed woodland, under Populus sp. and Salix sp., 16 Oct. 2007, H. Beker HJB12122; Nord Pas de Calais, Saint Amand (N50.3976000 E3.4439833, alt. c. 20 m) on wet soil in broadleaf woodland scrub under Salix sp., 10 Nov. 2006, P.-A. Moreau HJB11755; Alpes-Maritimes, Les Espagnols (N43.5090333 E6.7947667, alt. c. 185 m) on litter in mixed woodland under Populus sp., Salix sp., 16 Oct. 2007, P. Cavanagh HJB12126. – **Netherlands**, Drente, Beilen, Wijster, Usbaantje (c. N52.52 E6.52, alt. c. 10 m) collected in wet *Sphagnum* with *Salix aurita*, *Salix cinerea* and *Salix repens*, 1 Oct. 1996, D. Aanen WBS 9662, database record HJB12522; Drente, Eemsterveld, langs de Drentse Aa (c. N53.15 E6.60, alt. c. 2 m) under *Salix* sp., 10 Oct. 1995, D. Aanen WBS 9671, database record HJB12808; Drente, Beilen, Wijster jabaantje (c. N52.82 E6.52, alt. c. 10 m) collected in acid wet soil with *Sphagnum* and *Salix repens*, 1 Oct. 1996, D. Aanen WBS 9663, database record HJB12523. – **Scotland**, Orkney (c. N59.64959 W3.0094460, alt. c. 0 m) in dune in sandy soil with *Salix repens*, 21 Aug. 2002, A. Andrews HJB9819; Loch Loya (c. N57.58 W3.85, alt. c. 10 m) in wet mixed woodland under *Betula* sp. and *Salix* sp., 25 Sept. 1995, P.D. Orton K(M)52712, database record HJB1000001. This forms part of the holotype collection of *H. leucosarx* which was mixed and the part not selected as typus for *H. leucosarx* by Grilli (2007); Easterness, Bogoch (N57.1659300 W3.8482160, alt. c. 235 m) in bog on pathside with *Salix* sp., 22 Aug. 2005, S. Huthinnen SH05/44, duplicate HJB11168. – **Spain**, Castilla y Leon, Rio Cerneja (N43.1177667 W3.4608833, alt. c. 835 m) in acid litter from the other members of this subsection. Sinuate cheilocystidia. — Scale bars: 5 µm.

Notes — Given the shape of its cheilocystidia, *H. lutense* clearly belongs to *H.* subsect. *Denuata*. The species most likely corresponds to ICG9 of Aanen & Kuyper (1999). Its sinuate cheilocystidia and its consistently long thin basidia distinguish it from the other members of this subsection. Sinuate cheilocystidia do occur in other species, for example *H. eburneum*, but in our experience there is no other species where sinuate cheilocystidia occur so frequently and is such a consistent character. Similarly other species in this subsection can have quite long narrow basidia but again this taxon has them consistently. The basidium Q for this taxon ranges from 4.0–5.7. The combination of these sinuate cheilocystidia and the high average Q of the basidia unambiguously define this taxon. Additionally, the number of complete lamellae < 60 and the average cheilocystidium apex width of < 8 µm, means it is highly unlikely to miss-determine this taxon.

The records on our database for *H. lutense* are confined to Salicaceae and to Western Europe, i.e. the most easterly collection is from Denmark. We suspect that an exclusive mycorrhizal association with Salix is correct. With regard to the geographical distribution we must await more confirmed records. While we do not have sufficient data to suggest that this species is restricted to Western Europe, it certainly appears more common in this area.

*Hebeloma lutense* is monophyletic and receives high bootstrap support in all tested loci apart from the ITS. The placement of the incomplete sequence of the isotype of *H. lutense* outside the *H. lutense* clade of the ITS result can be explained by missing data. The placement of a single *H. alpinum* collection (HJB11997; its ITS sequence has been confirmed by repetition and its V6 sequence is clearly not *H. lutense*) in the clade that otherwise only contains sequences from *H. lutense* collections only shows how similar the ITS is in this subsection. However, normally an ITS2 sequence of *H. lutense* should be sufficient to identify this species, too.

**Hebeloma minus** Bruchet, Bull. Mens. Soc. Linn. Lyon 39, 6 (Suppl.): 126. 1970. — MycoBank MB314960; Fig. 24, 25

**Type. France**, Savoie, Lac des Assiettes, Col de la Vanoise (c. N45.3839 E6.792, alt. c. 2500 m) on alpine scrub, in calcareous dry lake bed with *Salix herbacea*, 11 Sept. 1969, G. Bruchet, LY BR69-15, database record HJB1000065.

**Basidiomes** usually in scattered groups. *Pileus* 9–31 mm diam, convex to umboinate, slightly tacky when moist and sometimes hygrophanous; *cuticle colour* from dark pinkish buff or Isabella to brownish olive, greyish brown or umber sometimes unicoloured but sometimes paler towards the margin which may be pinkish buff, occasionally pruinose (givré) especially after frosting; *pileus margin* usually straight, sometimes involute particularly in young basidiomes. *Lamellae* emarginate to adnate, relatively distant (*L* = 30–34) with a maximum depth of 4–5 mm; *colour* cream, aluatous or brown when young, later umber to sepia following spore maturity; *edge* fimbriate, significantly paler than lamella surface; droplets normally visible on the lamella edge usually by naked eye but certainly with a ×10 lens, rarely absent; *lamellules* occasional. *Stipe* central, cylindrical or slightly clavate, rarely bulbous, becoming hollow with age, 10–40 × 1–8 mm and up to 10 mm at the base rarely with some weak discoloration towards the base of the stipe; *surface* dry, pruinose to floccose, especially towards the apex. *Cortina* not observed. *Flesh* medium thick, pale buff. *Smell* raphanoid, sometimes weak. *Taste* mild, occasionally weakly bitter or weakly raphanoid. *Spore deposit* brownish olive. *Exsiccate* fragile and brittle.

Spores amygdaloid or limoniform, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, and usually with a papilla, sometimes guttulate with one or more oily drops, from weakly to distinctly ornamented, sometimes with slightly loosening perispore in a few to many spores and weakly but sometimes distinctly dextrinoid (O2,O3; P0,P1,P2; D1(D2)); *spore colour* under the microscope yellow to pale brown; *spore size* based on *n* = 64 spores of the holotype, 5–95% percentile range 11.0–13.9 × 6.1–7.3 µm, with median 12.4 × 6.7 µm and av 12.4 × 6.7 µm with SD length 0.96 µm and width 0.40 µm, *Q* value 5–95% percentile range 1.68–2.07, with median 1.84 and av 1.86 with SD 0.12; *spore size* based on 10 collections medians 11.2–13.1 × 6.3–7.6 µm and av 11.2–13.1 × 6.2–7.7 µm with SD length 0.62–1.12 µm and width 0.32–0.54 µm, Qav 1.61–1.87. *Basidia* cylindrical to clavate and 4-spored, 27–39 × 8.2–11.5 µm, with av 27–35 × 8.9–10.9 µm and basidium Qav in the range 2.8–3.8. *Pleurocystidia* not found. *Cheilocystidia* capitulate-stipitate, clavate-stipitate or...
Fig. 24  *Hebeloma minus* (LY BR69-15, holotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e–h. cheilocystidia ×500 in 5 % KOH; i, j. basidia ×500 in 5 % KOH; k. trama below cutis ×500 in 5 % KOH. — Scale bars: 10 µm.
Hebeloma minus (LY BR69-15, holotype). a. Basidia; b. spores; c. cheilocystidia. — Scale bars: 5 µm.

Fig. 25

Additional specimens examined. FRANCE, Jura, Lac des Rouges Truites (c. N46.36252 E5.59531, alt. c. 940 m) under Salix caprea, 22 Sept. 1996, D. Aanen WBS 9630, database record HJB12512. — ICELAND, Nordur-Mulasysla, Hamborg, road to Jokladalur (c. N65.050 W14.917, alt. c. 400 m) in mountain heathland under Salix herbacea, 6 Aug. 1993, J. Vesterholt JV93-503, duplicate HJB10865; Nordur-Mulasysla, Hamborg, road to Jokladalur (c. N65.05 W14.917, alt. c. 400 m) in mountain heathland under Salix herbacea, 6 Aug. 1993, J. Vesterholt JV93-506, duplicate HJB10866; Valavatn (N84.8655500 W23.5597167, alt. c. 301 m) on rotten wood in coastal sand under Salix herbacea, 28 Aug. 1998, E. Horak ZT 4113, duplicate HJB12568; Oberaar (N46.5485667 E27.683333, alt. c. 2312 m) on soil in wasteland under Salix sp., 10 Aug. 2005, H. Beker, M.L. Beker HJB11107.

Notes — Given the shape of its cheilocystidia, H. minus clearly belongs to H. subsect. Denudata. The species most likely corresponds to ICG7 of Aanen & Kuyper (1999). It certainly appears to be restricted to Salix (and possibly Dryas) and while it appears to be predominantly an arctic/alpine species it can occur in subalpine habitats and presumably subarctic habitats. While at present we only have 11 recorded collections of H. minus we would suspect that it is widely distributed throughout the arctic and alpine regions, appearing occasionally in the boreal zone. When we look at the distribution of our collections they are clustered in Iceland, Svalbard and the French/Swiss Alps, plus we have one collection from Canada (not included in our description). Hebeloma minus appears morphologically close to H. alpinum and H. pallidolabiatum. It can be difficult to separate from H. alpinum microscopically but can be separated on macroscopic characters, since H. minus is smaller with a darker coloured pileus and with fewer lamellae than H. alpinum. Hebeloma minus is macroscopically very similar to H. pallidolabiatum but can be separated microscopically using the cheilocystidium ratio A/B which is always > 1.8 for H. minus but < 1.8 for H. pallidolabiatum. With regard to the other alpine/arctic species, it can be distinguished from H. aurantioumbrinum through the average width of the cheilocystidium apex which ≤ 8.5 µm for H. aurantioumbrinum while the average cheilocystidium apex for H. minus is always > 8.5 µm. It can be separated from H. louseae which has spores O1 or O2 and very rarely O3, while H. minus has many spores O3. In subalpine areas it is most similar to H. sicolorum and H. pusillum. But H. pusillum has av spore Q > 1.9 while H. minus av spore Q ≤ 1.9 and H. sicolorum has spores O2 or even D3 while H. minus spores are usually D1 and rarely even D2.

As H. alpinum, H. minus is a rather diverse species molecularly. The only single locus in which all available H. minus sequences form a supported monophyletic clade is RPB2. In other single locus analyses, H. minus forms mixed clades with either H. louseae or H. pallidolabiatum or some of the H. minus sequences are included in the unresolved H. crustuliniforme complex part of the tree.

Type: SBULBAR, Skansbukta, (N78.5156167 E16.0139500, alt. c. 34 m) on soil in coastal scrub under Salix herbacea, 14 Aug. 2007, M.L. Beker, H. Beker holotype BR-Myco 174908-17; isotypes C-C-92312, HJB11992.

Diagnosis — Hebeloma pallidolabiatum belongs, based on its cheilocystidium shape to H. subsect. Denudata. It can be separated from the other small arctic species of this section by the cheilocystidium ratio apex : base (A/B) which is always > 1.8 and the spores, most of which are always quite distinctly ornamented and > 12 µm in length.

Basidiomes in a scattered group. Pileus 12–21 mm diam, convex to broadly umbonate, slightly tacky when moist; cuticle colour 2-coloured, sepia to dark brick in the centre with a thin paler margin; pileus margin straight. Lamellae emarginate, quite
Fig. 26 Hebeloma pallidolabiatum (BR-MYCO 174908-17, holotype). a, b. Spores and spore ornamentation ×1 600 in 5 % KOH; c, d. spores and spore ornamentation ×1 600 in Melzer’s reagent; e, f. basidia ×1 000 in 5 % KOH; g, h. cheilocystidia ×1 000 in 5 % KOH; i. caulocystidia ×1 000 in 5 % KOH; j. trama below cutis ×500 in 5 % KOH. — Scale bars: 10 µm.
distant (L = 30–33); colour cream, alutaceous or brown when young, later umber to sepia following spore maturity; edge fimbriate, significantly paler than lamella surface droplets usually visible by naked eye; lamellules occasionally. Stipe central, cylindrical, 14–24 × 2.0–3.5 mm with some brown discoloration towards the base of the stipe; surface dry, pruinose. Corna not observed. Flesh rather thin, whitish but slightly more coloured in the base of the stipe. Smell raphanoid. Taste not recorded. Spore deposit isabellina to brownish olive.

Spores amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, at least under immersion, with no sign of loosening perispore and at most weakly dextrinoid (O2; P0; (D)0(D1,D2); spore colour under the microscope brownish yellow; spore size based on n = 58 spores of the holotype, 5–95% percentile range 12.0–14.1 × 7.0–8.5 µm, with median 12.8 × 7.5 µm and av 12.8 × 7.6 µm with SD length 0.65 µm and width 0.44 µm, Q value 5–95% percentile range 1.57–1.80, with median 1.69 and av 1.69 with SD 0.07; spore size based on 2 collections medians 12.8–13.5 × 7.2–7.5 µm and av 12.8–13.5 × 7.2–7.6 µm with SD length 0.65–0.89 µm and width 0.33–0.44 µm, Qav 1.69–1.88. Basidia cylindrical to clavate and 4-spored, 26–40 × 6.6–10.6 µm, with av 27–37 × 7.9–9.8 µm and basidium Qav 3.6–3.9. Pleurocystidia not found. Cheilocystidia clavate-stipitate sometimes slightly swollen towards the base giving an hourglass appearance clavate-lageniform, occasionally with septa (sometimes clamped); width of apex holotype 5–95% percentile range 6.8–11.5 µm, with median 8.8 µm and av 9.0 µm with SD 1.66 µm; across 2 collections median 8.5–8.8 µm and av 8.6–9.0 µm; with n ≥ 20 selected cheilocystidia of 2 collections the 5–95% percentile ranges are 48–73 × 6.8–11.5 × 4.1–6.4 × 3.6–7.9 µm while the averages are 58–59 × 8.6–9.0 × 5.1–5.4 × 5.9–6.3 and 59 × 9.0 × 5.1–6.3 µm av for the holotype. The av cheilocystidia ratios across the 2 collections were: A/M = 1.61–2.05; A/B = 1.57–1.69; B/M = 1.09–1.24. Caulocystidia resemble cheilocystidia, up to 90 µm long and 11 µm wide at the apex. Pileipellis is an ixocutis with a thin to medium thickness epicutis up to 80 µm, embedded hyphae up to 5.5 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. Cutis orange brown and made up of cylindrical to isodiametric elements. Trama below subcutis contains angular elements, sometimes shaped like thick sausages up to 20 µm broad. Clamp connections present throughout the basidiome.

Habitat & Distribution — We only have two collections of this species, both from Svalbard in arctic conditions with dwarf Salix but over 100 km apart. Consequently its habitat is not yet well understood.

Additional specimens examined. SVALBARD, Knudsenheia (N78.9375333 E11.8438500, alt. c. 8 m) on soil in grazed scrubland under Salix polaris, 18 Aug. 2007, M.L. Beker, H. Beker HJB12038.

Notes — Given the shape of its cheilocystidia, H. pallidolabiatum clearly belongs to H. subsect. Denudata. The species has most likely not been included in the intercompatibility tests of Aanen & Kuypers (1999). Of the members of H. subsect. Denudata it is most likely to be confused with the other small arctic/alpine species: H. louiseae, H. minus and H. perexiguum. It can be readily separated from the first two of these on the basis of the cheilocystidium ratio A/B which for this taxon is always < 1.8 whereas it is > 1.8 for both H. louiseae and H. minus. The separation from H. perexiguum is straightforward as the spores for H. pallidolabiatum are more ornamented and longer. Given the small number of collections on which our description is based it is possible that our description is too narrow but until more collections of this taxon are recorded we cannot be sure. Hebeloma pallidolabiatum is monophyletic in four out of five loci and supported by bootstrap in three of them. Two ITS sequences were obtained from the type collection, of which one is included in a (the) H. pallidolabiatum clade and the other is part of the unresolved H. crustuliniforme complex part of the tree. This provides further evidence that the ITS is less suited for species identification than any of the other loci.

Hebeloma perexiguum Beker, Vesterh. & U. Eberh., sp. nov. — MycoBank MB809911; Fig. 5g, 28, 29

Etymology. From per — very and exigus — small, to emphasise the small size of this mushroom.

Type: SVALBARD, London near Ny Alesund (N78.9631 E12.05035, alt. c. 5 m) in scrub on site of deserted settlement with Salix polaris, 18 Aug. 2007, M.L. Beker, H. Beker holotype BR-BR-MYCO 173799-58; isotype HJB12038.

Diagnosis — Hebeloma perexiguum belongs based on its cheilocystidium shape to H. subsect. Denudata. It can be recognized by the swollen basal part of the cheilocystidia and its small size. It can be recognized from other species of the subsect that are known to occur in arctic/alpine habitats and form small basidiomes, such as H. salicicola by its small number of full length lamellae, which is < 30, from H. minus and H. pallidolabiatum by its low spore ornamentation (O1), from H. aurantiombrinum by its spore width being > 7 µm and from H. louiseae by its average cheilocystidium apex width of < 9 µm. Basidiomes in a scattered group. Pileus 7–12 mm diam, convex, slightly tacky when moist; cuticle colour almost unicolour, greyish brown with a thin paler margin; pileus margin straight. Lamellae emarginate, quite distant (L = 24–26); colour cream, alutaceous or brown when young, later umber to sepia following spore maturity; edge fimbriate, significantly paler than lamella surface droplets not seen on the lamella edge; lamellules occasional. Stipe central, cylindrical, 10–15 × 2.0 mm with some weak brown discoloration towards the base of the stipe; surface dry, pruinose. Cornina not observed. Flesh rather thin, whitish but slightly more coloured in the base of the stipe. Smell absent. Taste not recorded. Spore deposit not recorded. Exsiccata fragile and brittle and pileus blackening.

Spores amygdaloid, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, sometimes with a papilla, not guttulate, almost smooth, with
Fig. 28  *Hebeloma perexiguum* (BR-MYCO 173979-58, holotype).  

a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e. basidium ×1 000 in 5 % KOH; f. cheilocystidia ×1 000 in 5 % KOH; g. cheilocystidia ×500 in 5 % KOH; h. epicutis hyphae ×1 000 in 5 % KOH; i. caulocystidia ×1 000 in 5 % KOH; j. caulocystidia ×500 in 5 % KOH. — Scale bars: 10 µm.
ornamentation hardly visible, with no sign of loosening perispore and weakly dextrinoid (O1; P0; D1); spore colour under the microscope brownish yellow; spore size based on n = 56 spores of the holotype, 5–95 % percentile range 9.9–13.4 × 6.4–8.1 µm, with median 11.7 × 7.2 µm and av 11.7 × 7.2 µm with SD length 1.15 µm and width 0.56 µm, Q value 5–95 % percentile range 1.43–1.86, with median 1.64 and av 1.63 with SD 0.12. Basidia cylindrical to clavate and 4-spored, 31–43 × 8.9–10.1 µm, with av 38 × 9.6 µm and basidium Qav 3.9. Pleurocystidia not found. Cheliocystidia spathulate-stipitate or clavate sometimes slightly swollen towards the base giving an hourglass appearance or even appearing lageniform, with some thickening of the apex, occasionally with septa; width of apex holotype 5–95 % percentile range 6.7–10.6 µm, with median 8.2 µm and av 8.5 µm with SD 1.56 µm and overall av dimensions 54 × 8.5 × 4.8 × 6.4 µm av for the holotype. The av cheliocystidia ratios were: A/M = 1.75; A/B = 1.45; B/M = 1.33. Caulocystidia resemble cheliocystidia, up to 75 µm long and 9 µm wide at the apex. Pileipellis is an ixocutis with a very thin epicutis up to 30 µm, embedded hyphae up to 6 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. Cutis orange brown and made up of cylindrical to isodiamic elements. Trama below subcutis isodiametric elements. Notes — Salix species, growing in arctic conditions with dwarf pigmented. broad, smooth or sometimes encrusted, hyaline or occasionally B/M = 1.33. long and 9 µm wide at the apex. Hebeloma pusillum J.E. Lange, Fl. Agaric Danic. 5 (Taxon. Conspr.): iv. 1940. — MycoBank MB275806; Fig. 30, 31 = Hebeloma pusillum var. longisporum Bruchet, Bull. Mens. Soc. Linn. Lyon 39, 6 (Suppl.): 126. 1970. = Hebeloma vaccinum var. cephalotum Enderle & Vesterh. in Die Pflanzen des Ulmer Raumes (Ulm): 379. 2004.

Type. DENMARK, Fyn, Langesæskovene S. of Morud (c. N55.45 E10.19, alt. c. 30 m) with Salix sp., Danske Agaricacéer, pl. 460 (aquarelle, C), reproduced in Lange 1938 as pl. 120C; same locality, 16 Oct. 1991, J. Vesterholt, epitypus C JKV1–685, database record HUB1000128, selected by J. Vesterholt in Fungi of Northern Europe 3: 82. 2005.

Basidiomes usually in scattered groups. Pileus 5–40 mm diam, convex or convoluted, sometimes with a small umbo, often viscid, slightly tacky when moist but never hygrophanous; cuticle colour cinnamon to sepia to dark brick in the central region and becoming paler towards the margin which may be anywhere from cream to clay coloured, distinctly 2-coloured in older specimens; pileus margin usually straight, occasionally involute when young and sometimes upturned with age. Lamellae emarginate, quite widely spaced (L = 20–38) with a maximum depth of 3–4 mm; colour cream, alutaceous or brown when young, later umber to sepia following spore maturity; edge fimbriate, significantly paler than lamella surface; droplets normally visible on the lamella edge even with the naked eye; lamellules occasional. Stipe central, cylindrical usually stuffed, rarely hollow, 14–58 × 1.5–2.5(–3.5) mm usually with some brown discolouration towards the base of the stipe; surface dry, pruinose. Cortina not observed. Flesh rather thin, whitish but slightly more coloured in the base of the stipe. The whole basidiome has a very slender and fragile appearance with the av stipe Q (ratio of stipe length to stipe width) usually well in excess of 11. Smell raphanoid, sometimes absent. Taste raphanoid. Spore deposit brownish olive to umber.

Sporae amygdaloid or fusoid, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, rarely with any papilla, sometimes guttulate with one or more oily drops, from weakly to distinctly ornamented, usually with some sign of loosening perispore in at least a few spores and often in many spores and weakly but sometimes distinctly dextrinoid ((O1)Q2; O3; (P0)P1,P2; (DO)D1,D2); spore colour under the microscope yellow brown to brown; spore size based on n = 55 spores of the epitope, 5–95 % percentile range 11.1–13.7 × 5.8–7.0 µm, with median 12.3 × 6.4 µm and av 12.3 × 6.4 µm with SD length 0.87 µm and width 0.48 µm, Q value 5–95 % percentile range 1.79–2.11, with median 1.92 and av 1.93 with SD 0.13; spore size based on 20 collections medians 11.3–13.6 × 5.6–6.7 µm and av 11.4–13.6 × 5.6–6.7 µm with SD length 0.64–1.04 µm and width 0.22–0.55 µm, Qav 1.91–2.22. Basidia cylindrical to clavate and 4-spored, 23–40 × 5.7–10.1 µm, with av 25–35 × 6.8–9.6 µm and basidium Qav in the range 3.0–4.4. Pleurocystidia not found. Cheliocystidia capitate-stipitate, clavate-stipitate or spathulate-stipitate, occasionally slightly swollen towards the base (capitate-lageniform or clavate-lageniform), occasionally with septa and rarely with median thickening, sometimes the cheliocystidia appear con-
Fig. 30 Hebeloma pusillum (C JV91-685, epitype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c. cheilocystidia x 630 in Melzer’s reagent; d. cheilocystidia ×500 in Melzer’s reagent; e, f. basidia ×1 000 in Melzer’s reagent; g. caulocystidia ×500 in 5 % KOH; h. epicutis hyphae ×500 in 5 % KOH; i. trama below cutis ×500 in 5 % KOH. — Scale bars: 10 µm.
glutinate and tend to break rather than separate; width of apex epitype 5–95 % percentile range 6.1–12.4 μm, with median 8.5 μm and av 9.0 μm; with SD 1.96 μm; across 20 collections median 7.9–9.9 μm and av 8.0–10.0 μm; with n ≥ 20 selected cheilocystidia of 20 collections the 5–95 % percentile ranges are 32–97 × 5.6–12.8 × 2.7–6.1 × 2.7–6.8 μm while the averages are 41–70 × 8.0–10.0 × 3.8–4.8 × 3.6–4.9 and 70 × 9.0 × 4.3 × 4.4 μm av for the epitype. The av cheilocystidia ratios for the 15 collections were: A/M = 1.71–2.71; A/B = 1.68–2.52; B/M = 0.94–1.20. Caulo cystidia resemble cheilocystidia, up to 75 × 12 μm wide at the apex. Pileipellis is an ixocutis with a thin epicutis 40–80 μm, embedded hyphae up to 6 μm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. Cutis red brown and made up of cylindrical to isodiametric elements. Trama below subcutis, but we suspect that it is widespread across all of Europe. 

**Notes** — Given the shape of its cheilocystidia, *H. pusillum* clearly belongs to *H. sect. Denudata*. The species most likely corresponds to ICG8 of Aanen & Kuyper (1999). The strongly 2-coloured cap, somewhat reminiscent of *H. mesophaeum* but with much more slender stature and without cortina leaves very few possibilities. Other small *Hebeloma* spp. have not such strongly 2-coloured caps, nor are they so slender. We have no confirmed records of this taxon in arctic or alpine habitats. It can be readily distinguished from the other members of this subsection that grow in lowland areas based on the distinctly 2-coloured cap, the number of lamellae 20–40, the slender basidiomes with stipe width ≤ 3.5 mm and stipe Q normally > 10, the spore length, > 11 μm, the large average width of the cheilocystidium apex ≥ 8 μm, and with no regular apical thickening of the cheilocystidium. It has probably often been confused with *H. helodes*, which is also a small *Hebeloma* sp., but *H. pusillum* is confined to *Salix*, has a more fragile stature and usually a rather darker centre to the pileus and significantly longer spores, on average. *Hebeloma lutecystidiatum* also has long spores but the very distinct apical thickening of the cheilocystidium distinguishes this taxon from *H. pusillum*, which rarely has any apical thickening of the cheilocystidium. *Hebeloma aurantiombrinum*, is rare in non-arctic/alpine environments but also has shorter spores on average. *Hebeloma minus* is also rare in non-arctic/alpine environments but in any case this taxon has a smaller spore Q from that of *H. pusillum*. *Hebeloma salicicola* could be confused with *H. pusillum* but normally it has a more robust stature with a wider stipe, a smaller stipe Q and the spores of *H. salicicola* are on average more dextrinoid.

*Hebeloma pusillum* forms species clades in all of the loci tested, and receives bootstrap support in all single locus results apart from ITS.

Having studied the description and holotype material of *H. vaccinum* var. cephalotum we are convinced this was a mixed collection of *H. salicicola*, *H. vaccinum* and *H. pusillum*. We have extracted DNA several times and every sequence we have generated is of *H. pusillum*, similarly the material we have examined is *H. pusillum*. However, the macroscopic description given for this taxon very much more resembles *H. salicicola*. We have also examined the isotype of this material, but that is *H. vaccinum*. So we conclude that we should synonymise it with *H. pusillum* but point out that the macroscopic description does not really match *H. pusillum*. See also the discussion following the description of *H. salicicola*.
Fig. 32 Hebeloma salicicola (BR-MYCO 173977-56, holotype). a, b. Spores and spore ornamentation ×1 600 in Melzer’s reagent; c, d. spores and spore ornamentation ×1 600 in 5 % KOH; e, f. cheilocystidia ×1 000 in 5 % KOH; g. cheilocystidia ×500 in 5 % KOH; h. basidium ×1 000 in 5 % KOH; i. caulocystidia ×1 000 in 5 % KOH; j. cutis ×125 in 5 % KOH. — Scale bars: 10 μm, in j. 100 μm.
larly and have concluded that there are no grounds for separating this variety. This is in agreement with Gröger (1987). The molecular sequences we have generated are identical to *H. pustillum* and our measurements of the spore length show it to be in the middle of the range for this species; we have included Bruchet’s collection within the set of collections upon which our overall species description is based.

The original iconotype of Lange is an excellent representation of this taxon. The epitype selected by Vesterholt is also representative.

**Hebeloma salicicola** Beker, Vesterh. & U. Eberh., sp. nov. — MycoBank MB809912; Fig. 5h, 32, 33

**Etymology.** Meaning lover of *Salix*, being exclusively mycorrhizal with *Salicaceae*.

**Type.** BELGIUM, De Panne, Westhoek, West Flanders (N51.08793 E2.57568, alt. c. 3 m) on sand in calcareous dune slack with *Salix repens*, 12 Oct. 2009, *H. Beker*, L. Davies holotype BR BR-MYCO 173977-56; isotype C C-F-90151, HJB13302.

**Diagnosis.** *Hebeloma salicicola* is a member of *H.* subsect. *Denudata* based on cheilocystidium shape, though the cystidia can be rather broad at the base. Macroscopically it is similar to *H. vaccinum*, but in contrast to *H. vaccinum*, mature specimens nearly always have a 2-coloured pileus with a darker centre. Microscopically *H. salicicola* differs from other members of its subsection by a combination of characters, i.e. its small stature, the low number of full length lamellae (<50), the rather strong dextrinoidity (D2,D3) and ornamentation of the spores (O2,O3), which are on average >11 µm.

**Basidiomes** usually in scattered groups, rarely solitary, sometimes gregarious. **Pileus** 10–48 mm diam, convex often umbonate, surface often viscid, tacky when moist but never hygrophanous; **cuticle colour** mature basidiomes are almost always 2-coloured with the centre from ochre to a rich red brown, dark brick or sepia and the margin from cream to clay pink or buff or Isabella; **pileus margin** usually straight but sometimes scalloped. **Lamellae** adnate or emarginated, sometimes almost free, usually quite distant with L = 30–49; maximum depth 2–9 mm; **spore colour** (O2,O3; (P0)P1,P2; D2,D3); under the microscope **becoming orange brown or light brick in Melzer’s reagent** spores and distinctly to rather strongly dextrinoid with spores sometimes with slightly loosening perispore in a few to many weakly ornamented but usually more distinctly ornamented, sometimes guttulate with one or more oily drops, sometimes with some thinning of the spore wall, and often with a papilla.

**Spores** amygdaloid or limoniform, sometimes fusoid, with small apiculus and rounded at the end opposite the apiculus, with some thinning of the spore wall, and often with a papilla, sometimes guttulate with one or more oily drops, sometimes weakly ornamented but usually more distinctly ornamented, sometimes with slightly loosening perispore in a few to many spores and distinctly to rather strongly dextrinoid with spores becoming orange brown or light brick in Melzer’s reagent (O2,O3; P0)P1,P2; D2,D3); **spore colour** under the microscope from yellow through yellow brown to brown; **spore size** based on n = 54 spores of the holotype, 5–95 % percentile range 11.6–13.9 × 6.4–7.8 µm, with median 12.7 × 7.2 µm and av 12.7 × 7.1 µm with SD length 0.67 µm and width 0.46 µm. Q value 5–95 % percentile range 1.67–1.92, with median 1.78 and av 1.78 with SD 0.08; **spore size** based on 21 collections medians 11.2–13.3 × 6.0–7.6 µm and av 11.2–13.3 × 6.1–7.5 µm with SD length 0.50–1.24 µm and width 0.25–0.51 µm, Qav 1.64–2.00. **Basidia** cylindrical to clavate and 4-spored, 22–38 × 7.2–10.2 µm, with av 24–37 × 8.0–9.9 µm and basidium Qav in the range 3.0–4.1. **Pleurocystidia** not found. **Cheilocystidia** capitate-stipitate or clavate-stipitate, sometimes swollen towards the base (capitate-lageniform or clavate-lageniform), usually with some thickening of the apex, occasionally with septa and occasionally bifurcate; **width of apex** holotype 5–95 % percentile range 7.5–10.8 µm, with median 8.9 µm and av 8.9 µm with SD 1.01 µm; across 21 collections median 7.8–10.6 µm and av 7.9–10.7 µm; with n ≥ 20 selected cheilocystidia of 21 collections the 5–95 % percentile ranges are 37–77 × 6.1–15.5 × 3.0–5.7 × 2.5–8.9 µm while the averages are 46–63 × 7.9–10.7 × 3.8–5.0 × 3.6–5.9 while the av for the holotype was 54 × 8.9 × 4.3 × 4.9. The av cheilocystidia ratios for the 17 collections were: A/M = 1.83–2.63; A/B = 1.60–2.82; B/M = 0.96–1.27. **Caulocystidia** resemble cheilocystidia but with a more swollen base, up to 85 × 13 µm wide at the apex. **Pileipellis** is an ixocutis with an epicutis medium thick, 80–150 µm, embedded hyphae up to 10 µm broad, smooth or sometimes encrusted, hyaline or occasionally pigmented. **Cutis** orange brown and made up of cylindrical to isodiametric elements. **Trama below subcutis** contains cylindrical, ellipsoid, ovate and sausage-shaped elements up to 16 µm broad. **Clamp connections** present throughout the basidiome. **Habitat & Distribution.** *Hebeloma salicicola* appears to be confined in its mycorrhizal association to *Salix* and *Populus*. It is common and often gregarious in calcareous dune slacks growing with *Salix repens*, where it appears to have two fruiting periods per year, in the spring and in the autumn. We also have one alpine record of *H. salicicola* with *Salix herbacea* and one arctic record where it was growing in association with *Salix polaris*. Other records (not from dunes or alpine or arctic) are from grassy or mossy, often sandy ground with *Populus* × *alba* or *Salix* sp. in scrub, gardens, grassland or woodland plantations, on both acid and calcareous soils.

**Additional specimens examined.** BELGIUM, prov. West Flanders, Westhoek (c. N51.04 E3.56, alt. c. 0 m) in dune on sandy, calcareous soil with *Salix repens*, 26 Apr. 2004, *H. Beker* HJB9911; prov. West Flanders, Westhoek
those of many other species of cystidia are perhaps more swollen in their lower half than
Sept. 2004, Salix herbacea, 23 Aug. 2009, J. Vauras C TURA JV08-278, duplicate HJB12323. – FINLAND, Uusimaa, Hanko, Tyvärminne, Tyvärminneby (c. N59.84 E23.2, alt. c. 10 m) on calcareous soil under Salix sp., 30 June 1998, J. Vauras C TURA JV13610, duplicate HJB10930. – FRANCE, Haute Savoie, Lac des aissiettes (N45.39001 E6.78529, alt. c. 2478 m) on mossy soil in mountain scrub under Salix herbacea, 23 Aug. 2009, L. Davies HJB13087. – NETHERLANDS, Groningen, Eemshaven (c. N53.448 E6.831, alt. c. 0 m) with Salix repens, 3 Oct. 1996, D. Aanen WBS 9670, database record HJB12473; Groningen, Eemshaven (c. N53.45 E6.83, alt. c. 0 m) with Salix repens, 3 Oct. 1996, D. Aanen WBS 9678, database record HJB12479; Lelystad, Oostvaardersplassen (Flevoland) (c. N52.45 E5.37, alt. c. 0 m) in dune with Salix repens, 11 Oct. 1995, D. Aanen WBS 9567, database record HJB12533. – POLAND, Kmiętnik (the outer damped ground of the Bei- chatow Lignite Mine) forest distr. 297 (by the pond) (N51.22136 E19.43988, alt. c. 340 m) conifer woodland plantation with Salix sp., 23 Sept. 2008, I. Kalucka, H. Beker HJB12677. – SPAIN, Madrid, Colmenarrejo (c. N40.56 W4.01, alt. c. 890 m) in broadleaf plantation with Populus × alba, 17 Apr. 2001, F. Prieto HJB9072. – SWEDEN, Osyria (Ossian) (N78.9257000 E12.4542167, alt. c. 2 m) on grassy soil in maritime coastal scrub under Salix polaris, 17 Aug. 2007, H. Beker, M.L. Beker HJB12020. – WALES, Anglesey, Newborough (N53.1483800 W4.0425270, alt. c. 0 m) on sand in dune under Salix sp., 24 Sept. 2001, H. Beker HJB5311.

Notes — Given the shape of its cheilocystidia, H. salicicola clearly belongs to H. sect. Denudata and, although its cheilocystidia are perhaps more swollen in their lower half than those of many other species of H. subsect. Denudata, this taxon still falls within the subsection parameters. The species most likely corresponds to ICG14 of Aanen & Kuyper (1999). It appears restricted to Salix and Populus and can be common, even very regular, on calcareous and sandy soil. Where it is often found alongside H. vaccinum with which, macroscopically, it can be confused particularly when young and still appearing almost unicoloured. In the key to this subsection we key it out in both subkeyes. It can be distinguished from other arctic/alpine species of this subsection through the number of lamellae, < 60, and the spores more ornamented or more dextrinoid than H. auran- tioumbrinum, H. alpinum, H. louliseae, H. minus, H. pallidola- biatium and H. perexiguum and usually without a very strong distinct papilla, unlike H. alpinum. It can be separated from other lowland species in this section through the number of full length lamellae 30–60, the spore length > 11 µm, the smallish stature and the dextrinoidity of the spores. In the past this species has probably been confused with H. vaccinum and perhaps H. pusillum in dunes and lowland areas and with H. minus in alpine and arctic habitats. Both ITS and RPB2 distinguish this species.

Originally, we referred to this taxon as ‘Hebeloma cephalotum’ as it matched closely with the description of H. vaccinum var. cephalotum Enderle & Vesterh. in Enderle (2004). However, as mentioned above, we have examined the holotype of this species (M M-0155166) and it appears to be a mixed collection. While some of the fragments we have examined may belong to H. salicicola, all fragments we have sequenced are of H. pusillum, which is certainly present, from a morphological perspective, in the material deposited. Similarly the microscopic morphological analysis we have carried out also corresponds to H. pusillum. Trying to resolve this, we also examined an isolate (C C43996) but this collection, based on both molecular and morphological study is H. vaccinum. We do believe that the material of H. vaccinum var. cephalotum studied by J. Vester- holt corresponded to the taxon we have called H. salicicola, however, given the mixed nature of both the holotype and the isolate of H. vaccinum var. cephalotum we conclude that it is safer to describe this taxon as new.

DISCUSSION

The evolutionary distance, on the whole small, between all members of H. subsect. Denudata and particularly between some members of the H. crustuliniforme complex, suggest that, in spite of the demonstrated differentiation between clades and morphotypes, their presumably short evolutionary history may not have been sufficient for species to be fully differentiated in all characters. The analyses presented here give an impression of how well the characters distinguished above are supported by different analyses. With a full dataset of five genes, all species, apart from H. alpinum, are monophyletic and all, apart from H. minus, receive bootstrap support. Missing data introduce uncertainty in the results, which could not be avoided, as we were aiming for the best possible geographical, morphological and ecological representation for every species on the basis of the available material. Confronted with probable coalescence and possible hybridization, leading to an increased probability of recombination, phylogenetic analysis methods are not the most appropriate analysis methods. Both coalescence-based species tree methods or population genetic methods might be more appropriate. These kinds of analyses are geared towards haploid and unlinked markers and call for knowledge of population parameters; requirements that the available data do not readily fulfil. While we are still in the process of exploring the applicability of these methods, we do not expect the results will be very different from the results presented here with regard to species delimitation. The data presented above show that the DNA barcode (ITS) is, among the loci tested, perhaps the least well suited for discriminating between taxa of H. subsect. Denudata. A cut-off value of 3 % allows the separation of some H. pusillum and some H. eburneum sequences from the rest of the sequences, but the overwhelming majority of ITS sequences are more similar than 97 % and defined species identification with a 3 % cut-off. It is obvious from Table 1 that a blast search based identification based on any cut-off values of ITS similarity is bound to fail, if re-quiring all matches below a certain cut-off value to be assigned to the same species. In spite of this, complete ITS sequences can usually be correctly assigned to a species, because normally the great majority of very good matches belong to a single taxon and the great majority of sequences belonging to other species have noticeably worse scores. Furthermore, once the ITS data will be entered into the species hypotheses clustering of UNITE (Köjalg et al. 2013), we will be able to annotate the sequences in a way, so that the users of this database will be aware of the identification caveats within this group of species. The ML results suggest that the RPB2 is the most powerful single locus for recognizing species, but H. aeneni, H. alpinum and H. geminatum do not form monophyly. Obviously, this result may be biased due to the smaller number of sequences in the RPB2 dataset as opposed to some others, namely ITS. In our analyses, we found eight new species within H. subsect. Denudata, for which old names were not available. They are
described above as Hebeloma aenanii, H. aurantioumbrinum, H. geminatum, H. louiseae, H. luteicystidiatum, H. pallidolabiatum, H. perexiguum and H. salicicola, alongside descriptions of our concepts of known species (H. alpinum, H. eburneum, H. helodes, H. lutense, H. minus and H. pusillum). For all of these taxa, type material was studied. For the concept adopted for H. crustuliniforme (H. crustuliniforme (Bull.) Quél. emend. Vesterh., U. Eberh. & Beker) see Vesterholt et al. (2014). Molecular results suggest that the H. crustuliniforme complex includes nine taxa in Europe (H. aenanii, H. alpinum, H. crustuliniforme, H. eburneum, H. geminatum, H. louiseae, H. minus, H. pallidolabiatum and H. salicicola), of which H. alpinum is the least distinctive in molecular terms (followed by H. minus and H. geminatum), and H. aenanii and H. geminatum in morphological terms. Above, in the species comments of the taxonomy part of the paper, we discuss the lines of evidence leading to the acceptance of the respective species and how the species can be recognized.

The species classification adopted here is superficially in stark contrast to the taxonomical conclusions of Aanen & Kuyper (2004) who, also with regard to the determination of species by morphology alone, included H. aenanii, H. alpinum, H. crustuliniforme, H. eburneum, H. geminatum and H. salicicola as adopted here in H. crustuliniforme. Hebeloma helodes, H. luteicystidiatum, H. lutense, H. minus, H. pusillum and some other taxa outside of H. subsect. Denudata as defined here were merged in H. helodes. The latter species (H. helodes sensu Aanen & Kuyper 2004) was entirely based on morphology, not on intercompatibility test results, adopting a wide morphological concept. This concept is also not supported by the results of molecular analyses presented here, clearly showing a number of well-supported taxon clades, matching with morphological and ICG data.

Another important reason for the different morphological conclusions between this work and that of Aanen & Kuyper (2004) is that they used cheilocystidia length and width where here width ratios between different parts of the cheilocystidia were used to better capture cheilocystidia shapes. This does also explain why their concept of H. helodes does not fit into our concept of H. subsect. Denudata, which is strongly based on cheilocystidium shape. In addition, Aanen & Kuyper (2004) appear to have used size classes for spore measure means rather than the means of the measures themselves.

In the case of H. crustuliniforme sensu Aanen & Kuyper the species delimitation of Aanen & Kuyper (2004) was also related to intercompatibility between some members of different ICGs and the observation of a strain of ambiguous ICG membership. Their (Aanen & Kuyper 2004) reluctance to split H. crustuliniforme and H. helodes sensu auct. was further fed by molecular results based on ITS and IGS (intergenic spacer of the nuclear ribosomal RNA genes) data that – analogous to some results presented here – show that neither all ICGs nor all morphospecies readily form monophyletic clades in gene trees.

However, taking a different view on the intercompatibility test results, one could also argue as we are doing here: If indeed the great majority of basidiomes can unambiguously be assigned to a single ICG (Aanen & Kuyper 1999), it stands to reason that at least some ICGs do represent biological species, which are then likely to have evolved a distinct morphology. We did not use ICG membership as a criterion for species delimitation. In spite of occasional intercompatibility between different ICGs (Aanen & Kuyper 1999) in the core of the H. crustuliniforme complex (H. aenanii, H. alpinum, H. eburneum, H. geminatum), there is good correspondence between ICG data and the species limits used here, in that all collections belonging to the same ICG are assigned to the same species, none of the species includes more than one ICG. (The special case of collection WBS 9605, database record HJB12498, compatible ICG3 and ICG4 and included in the same clade as other ICG4 members, is discussed below in more detail.) Eleven of the species recognized by us here appear to correspond to ICGs of Aanen & Kuyper (1999) and should accordingly be biological species. Apart from H. aenanii and H. geminatum all of these species can also be unambiguously separated based on morphology. This could be taken as an indication that hybridization, i.e. crosses between different ICGs, does not play a major role in nature. A contributing factor could well be that the two species corresponding to the most promiscuous ICGs according to Aanen & Kuyper’s (1999) results, H. alpinum and H. eburneum, though widespread and common, are separated by their respective ecologies, in that the first is restricted to arctic and alpine areas where the latter does not occur. This is rather suggestive of speciation by isolation in progress, with residual intercompatibility in some genotypes (Aanen et al. 2000). The intercompatibility results (Aanen & Kuyper 1999) indicated possible, but less clear, intercompatibility of representatives of H. geminatum and H. aenanii and H. alpinum and H. aenanii. Both hybridisation and coalescence might be responsible for the lack of monophyly in some taxa and species clades in the shape of badly supported short-stemmed monophyly. The example of H. alpinum WBS 9605 (ICG 3/4) shows that on the basis of the molecular and morphological data we obtained, genotypes with exceptional biological intercompatibility cannot be detected. We do not know whether WBS 9605 could have produced viable offspring with a member of ICG3 and how the progeny might look. However, if viable offspring resulted, one would expect contradictions between morphology and genotype as in HJB11051, which is morphologically H. alpinum (ICG4) and genetically, based on five markers, H. geminatum (ICG1). Further, if HUB11051 was the direct product of hybridization, one would expect phased nuclear markers to belong to different taxon clades. This is not the case (results not shown). It is the only collection of H. alpinum we have from Iceland, and, given the relative vicinity of Iceland with Greenland, and given that we know that H. alpinum as delimited in Europe exists in Greenland, this might be an indication that the species delimitation between H. geminatum and H. alpinum as it works in Europe, might not do so with American material. In all of the 273 collections we examined molecularly, HUB11051 was the only one where a clear contradiction between genotype and morphology occurred.

It is tempting to see a key property of the H. crustuliniforme complex, actually of all species of H. subsect. Denudata, in their association to Salix ssp. While all the taxa of this subsection have been recorded with Salix, some of the taxa seem to only associate with this host genus (or with Salicaceae), see Table 3. Though the results reported by Tedersoo et al. (2013) implicitly caution against assuming that ectomycorrhizal taxa associating with one species of Salix would automatically form associations easily with other species of Salix, association with Salix could be considered an advantage to thrive during the climatic changes that took place in the northern hemisphere in the Pleistocene, and also to acquiring a Palearctic distribution. Hebeloma aurantioumbrinum, one of the less common species in Europe, has been found in North America. Hebeloma alpinum is known from Greenland. It is likely that at least the cold-adapted taxa like H. alpinum, H. minus and possibly also H. aenanii, H. geminatum and H. salicicola are circumpolar species. Sequence data from the US and Canada is publicly available, but as the results presented here show, ITS diversity is not a reliable predictor for species diversity in this group of fungi. Reviewing a number of environmental sequencing studies of ectomycorrhizal fungi from arctic northern hemisphere
environments, Timling & Taylor (2012) reported that *Hebeloma* spp. are among the fungal lineages that were recovered in all studies with at least moderate frequencies. Based on a 97% cut-off value they concluded, that almost 3/4 of the arctic phylootypes of ectomycorrhizal fungi can also be found outside the arctic. The results presented here for *H.* subsect. Denudata cast serious doubt on the appropriateness of such a statement, firstly with view to methodology – a 97% cut-off value is far too crude to capture spatial genetic structure in the genus *Hebeloma*; and secondly with regard to conclusions. There is a species overlap between arctic and alpine places, but the species overlap between arctic and temperate or boreal areas is small, if frequencies are also considered. Taxa that allegedly occur in both biomes (i.e., *H.* aanenii, *H.* aurantioumbrinum, *H.* geminatum, *H.* minus, *H.* salicicola), have a strong preference for the arctic/alpine areas or lesser latitudes and altitudes, and must, according to our database, be considered rare in their less favoured habitats. It is of course possible that the taxa that were so far only collected in Svalbard (*H.* *louiseae*, *H.* *pallidolabiatum* and *H.* *perexiguum*) are North American taxa and perhaps not restricted to arctic or alpine habitats. Given the low level of interspecific variation in *H.* subsect. Denudata, and the probability of intercompatibility of at least some members of some species to mate with members of other taxa, it appears unlikely, that putative North American taxa have persisted unchanged in Svalbard since the islands’ isolation, given the geological and vegetation history of the islands (summarized by Gemi et al. 2012). Long-distance dispersal may or may not be frequent from boreal or temperate regions to northern arctic environments (Gemi et al. 2012), but one could argue that not dispersal but climatic selection does play a key role in the arctic *Hebeloma* flora.

Dispersal in the other direction, from the arctic to boreal and temperate zones, might be a lot more successful, when looking at the example of *H.* *alpinum*. This strictly arctic/alpine taxon is a rather common species (around 80 records on the database) and associates with *Salix* or *Dryas*. It is most difficult to distinguish molecularly and could be confused with *H.* *aanenii*, *H.* *eburneum* and *H.* *geminatum*, which have wide host spectra and do not (typically) occur under arctic/alpine conditions. The situation is characterised by shared alleles and there is little evidence of shared divergence within *H.* *alpinum* compared to its temperate corticial congeners, which all show strong specific differentiation in a subset of the markers used. Incidentally, *H.* *alpinum* very likely corresponds to the most promiscuous ICG (Aanen & Kuyper 1999). Compatibility is a driver of speciation (Aanen et al. 2000). According to the results of Aanen and co-workers (Aanen & Kuyper 1999, Aanen et al. 2000) it is a quantitative rather than purely qualitative trait. This is true for pairings within the same ICG as well as between members of different ICGs. Loss of compatibil-ity may be precluded or followed by differentiation in morphology and genetic divergence in parts of the genome not directly involved in intercompatibility. While speciation and divergence are still in progress, every individual is likely to be a mosaic of genes and traits with different evolutionary histories. It appears likely that in the ancestral population of *H.* subsect. Denudata and in particular the ancestor of the *H.* crustuliniforme complex (with the possible exception of *H.* crustuliniforme itself) loss of compatibility and divergence in other characters did not always follow the same pattern. Possibly *H.* *alpinum* has maintained most of the traits of this ancestral population, retaining more ancestral variation, staying faithful to arctic/alpine habitats and associating with *Dryas* and *Salix*, but not with conifers or other broadleaves (non-arctic/alpine *Salix*) and preserving the highest level of intercompatibility (Aanen & Kuyper 1999). Based on the data we have it is impossible to unambiguously trace

the evolutionary history of the species of *H.* subsect. Denudata. The climatic oscillations during the Quaternary might even have arrested the potential of *H.* *alpinum* and intercompatible taxa, potentially bringing intercompatible members of different taxa in touch that normally would live in separate habitats or geographically distant areas, i.e. Europe and America. On a side note, it is remarkable that the closest relative of *H.* sect. Denudata, *H.* *mediorufum* (Rees et al. 2013) associates with *Nothofagus* and seems restricted to New Zealand, where *Salix* is not endogenous.

On an evolutionary timescale, species are transient. In cases like the *H.* crustuliniforme complex and related taxa it is to an extent subjective whether to consider divergence and non-compatibility as sufficiently advanced to separate groups of organisms into species. One might argue that it does not make sense to recognize or even describe species that are likely to be unidentifiable without huge effort and using identification tools that may not be widely used today. It may be argued that a consequence is that misnamed sequences and collections might crowd databases and be misleading rather than elucidating. Indeed, it is also possible that what we describe here as species may turn out to be somewhat differentiated sub-populations of to-date unstudied larger entities that are better suited to represent species. However, we believe that the dataset that forms the basis of this study is sufficiently extensive and sufficiently consistent to recognize entities, here described as species, that have diverged in the context of the climatic, geological and biological history of Europe.

Acknowledgements We are very much obliged to G. Walther for providing microscopic drawings. The help of A. Bogaerts from the herbarium in Meise (BR) and of K. Knudsen (C) for handling loans and managing deposits is greatly appreciated. Great thanks to Th. W. Kuyper for kindly making available to us material used by D.K. Aanen from WBS, now L. For help in translating various papers and descriptions we thank K. Kleine and M. Noodloeso. The authors would like to thank the herbaria in C, E, G, GLM, IB, K, L, LOD, LY, M, MKHN, MPU, O, OULU, P, PDD and TURA for the loan of collections for study and sequencing, BR for supplying material for morphological and for molecular analyses. Furthermore, we very much appreciated the help of A. Andrews, P. Antibus, V. Antonin, R. Androsoff, H. Baker, E. Benndiksen, P. Boisen Hansen, T. Borgen, A. Brand, P. Cavanagh, G. Corioli, C. Cripo, L. Davies, D. Deschuyter, E. Emmett, M. Enderlie, D. Ghyselink, M. Ghyselink, A. de Haan, H. Hallgrimsson, C. Hobart, E. Horak, S. Huhtinen, I. Kaluca, D. Karasinski, S. Kelly, T. Kirk, H. Knudsen, T. Lassae, P. Larsen, C. Lecuru, M. Lenne, P. Leonard, P-A. Moreau, E. Oehenoja, I. Oliana, A. Ibburg, J. Petersen, F. Preito, J-P. Proux, M. Rotheroe, P. Roux, J. Sandmo, D. Schafer, M. Storey, M. Tölö, M. Szczelesviski, A. Taylor, J. Vauras, J. Volders and any other collectors we may have forgotten by accident, for supplying us with interesting and exciting *Hebeloma* collections. We thank P. Derboven for the permission to use his photograph. Numerous people have helped in the lab to generate *Hebeloma* sequence data that were used directly or indirectly in this study. We would like to thank K. Dukik (CBS-KNAW Fungal Biodiversity Centre Utrecht), U. Furst, S. Garnica and J. Schade (Universiteit Tübingen), and R. Gadieeva, M. Jonsson, C. Lundström, J. Petterson and D. Öncü (SLU Uppsala) as well as the Uppsala University Genome Center and the Hubrecht Institute sequence facility.

REFERENCES

Aanen DK, Kuyper TW. 1999. Intercompatibility tests in the *Hebeloma crustuliniforme* complex. PhD thesis, Wageningen University, Netherlands.

Aanen DK, Kuyper TW. 1999. Intercompatibility tests in the *Hebeloma crustuliniforme* complex in northwestern Europe. Mycologia 91: 783–795.

Aanen DK, Kuyper TW. 2004. A comparison of a biological and phenetic species concept in the *Hebeloma crustuliniforme* complex within a phylogenetic framework. Persoonia 18: 285–316.

Aanen DK, Kuyper TW, Boekhout T, et al. 2000. Phylogenetic relationships in the genus *Hebeloma* based on ITS1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. Mycologia 92: 267–281.

Aanen DK, Kuyper TW, Hoekstra RF. 2001. A widely distributed ITS polymorphism within a biological species of the ectomycorrhizal fungus Hebeloma velutipes. Mycological Research 105: 284–290.

DENUDATA – Volume 35, 2015

146

Personio – Volume 35, 2015

146
Boekhout T. 1982. De secties Hebeloma (Fr.) Saccardo en Anthraccumphiela Boekhout nom. prov. van het geslacht Hebeloma (Fr.) Kummer in Nederland en aangrenzende gebieden. PhD thesis, Leiden University, Netherlands.

Buhos G. 1972. Hebeloma studies I. Annales Historico-Naturales Musei Nationalis Hungarici 64: 71–78.

Borchsenius F. 2009. FastGap version 1.2. Department of Biosciences, Aarhus University. http://www.aubot.dk/FastGap_home.htm.

Boyle H, Zimbras B, Renker C, et al. 2006. A molecular phylogeny of Hebe­loma species from Europe. Mycological Research 110: 369–380.

Eberhardt U, Beker HJ. 2010. Hebeloma vesterholtii, a new species in section Theobromina. Mycological Progress 9: 215–223.

Eberhardt U, Beker HJ, Vesterholt J, et al. 2013. European species of He­beloma section Theobromina. Fungal Diversity 58: 103–126.

Eberhardt U, Beker HJ, Vila J, et al. 2009. Hebeloma species associated with Cistus. Mycological Research 113: 153–162.

Enderle M. 2004. Die Pilzflora des Ulmer Raumes. Verein für Naturwissen­schaft und Mathematik in Ulm e.V., Ulm, Germany.

Geml J, Timling I, Robinson CH, et al. 2012. An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. Journal of Biogeography 39: 74–88.

Grill E. 2007. Type studies in Hebeloma unravelling a taxonomic-nomen­clatural tangle: What is Hebeloma leucosarx? Micologia e Vegetazione Mediterranea 22: 133–176.

Gröger F. 1987. Der Formenkreis des winzigen Fälblings, Hebeloma pusillum. Series Botanique et biologie végétale 32: 440–465.

Grilli E. 2007. Type studies in Hebeloma unravelling a taxonomic-nomen­clatural tangle: What is Hebeloma leucosarx? Micologia e Vegetazione Mediterranea 22: 133–176.

Kõljalg U, Nilsson RH, Abarenkov K, et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22: 5271–5277.

Lange JE. 1938. Studies in the Agarics of Denmark. Part XII. Hebeloma, Nauocoria, Tubaria, Galera, Bobitius, Phuteolus, Crepidotus, Pseudopaxillus, Paxillus. Dansk botanisk Arkv 9: 1–104.

Maddison WP, Maddison DR. 2011. Mesquite: A modular system for evolu­tionary analysis version 2.75, http://mesquiteproject.org.

Marteilisse R, Gryta H, Jargeat P, et al. 1999. Hebeloma. In: Caimey JWG, Chambers SM (eds), Ectomycorrhizal fungi: Key genera in profile: 89–127. Springer, Berlin, Germany.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 2010: 1–8.

Moser M. 1970. Beiträge zur Kenntnis der Gattung Hebeloma. Zeitschrift für Pilzkunde 36: 61–75.

Moser M. 1985. Beiträge zur Kenntnis der Gattung Hebeloma II. Sydowia. Annales Mycologici editi in Notitiam Scientiae Mycologicae Universalis, Series II, 38: 171–177.

Queiroz K de. 2007. Species concepts and species delimitation. Systematic Biology 56: 879 – 886.

Rees BJ, Midgley DJ, Marchant A, et al. 2013. Morphological and molecular data for Australian Hebeloma species do not support the generic status of Anamika. Mycologia 105: 1043–1058.

Sulzbacher MA, Grebenc T, Jacques RJS, et al. 2013. Ectomycorrhizal fungi from southern Brazil – a literature-based review, their origin and potential hosts. Mycosphere 4: 61–95.

Sulzbacher MA, Grebenc T, Jacques RJS, et al. 2013. Ectomycorrhizal fungi from southern Brazil – a literature-based review, their origin and potential hosts. Mycosphere 4: 61–95.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369–381.

Tedersoo L. 2011. ggbiplot: A ggplot2 based biplot. R package version 0.55. http://github.com/vqv/ggbiplot.

Vu VQ. 2011. ggbiplot: A ggplot2 based biplot. R package version 0.55. http://github.com/vqv/ggbiplot.