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The genus *Hebeloma* in the alpine belt of the Carpathians including two new species

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Abstract: Between 2002 and 2012 regular visits to the Carpathians were made and a number of *Hebeloma* spp. were collected from the alpine area. In total 44 collections were made that represent 11 species, two of which, *Hebeloma grandisporum* and *H. oreophilum*, are described here as new. Of the 11 species, four (*H. alpinum, H. marginatulum* and the two species described as new) are known only from alpine or Arctic habitats. *Hebeloma dunense* and *H. mesophaeum* are commonly found in, but not restricted to, alpine habitats. The other five species (*H. aanenii, H. laterinum, H. naviculosporum, H. vaccinum, H. velutipes*) are usually found in lowland or boreal habitats. *Hebeloma naviculosporum* is reported for the first time from the alpine zone and *H. alpinum* for the first time as growing with *Helianthemum.* All but two species (*H. alpinum, H. marginatulum*) are reported for the first time from the Carpathian alpine zone. In this paper we discuss the habitat, the 11 recorded species and give detailed descriptions of the two new species, both morphologically and molecularly. A key for *Hebeloma* species from sect. *Hebeloma* occurring in Arctic-alpine habitats is provided.

Key words: Agaricales, basidiomycetes, ectomycorrhizal host, *RPB2, TEF1a*

INTRODUCTION

High-mountain areas and Arctic regions, with alpine grasslands and tundra formations developed under severe climatic conditions, belong to the most interesting natural model systems for ecological and biogeographical studies (Körner 2003). They harbor specific groups of plants, animals and fungi that evolved from adaptation to these harsh environmental conditions and further diversified through vicariance, ecological niche shifts and other processes. In circumpolar regions Arctic tundra extends over large, continuous areas, whereas in the mountains of lower latitudes the alpine zone is fragmented. The Carpathians are one of the main European mountain chains. Although the 209 000 km^2 total surface and about 1300 km length make them comparable in size to the Alps (Kondracki 1978), they are characterized by a considerably different physiography. They are much lower in altitude than the Alps and the alpine belt extends over a much smaller area (1000 km^2, which is 3.5% of the total surface; Ozenda 1985) and is highly fragmented (Pawłowski 1970) (FIG. 1).

Macrofungi are important elements of Arctic-alpine ecosystems; they are, for example, mycorrhizal symbionts of several key plant species of such areas, such as *Betula nana, Dryas octopetala, Kobresia* spp. and dwarf *Salix* spp. (Gardes and Dahlberg 1996). A systematic survey of Arctic-alpine fungal species in all important areas of Europe is indispensable for comparative studies dealing with such questions as biogeography and history of the formation of Arctic and alpine biota, taxonomic composition and degree of specificity of Arctic-alpine fungus flora.

Within Europe, relatively comprehensive data are available especially from the Alps and the Scandinavian mountains (e.g. Favre 1955; Bruchet 1970; Gulden and Lange 1971; Bon 1986; Kühner and Lamoure 1986; Vesterholt 1989, 2005). In addition to these traditional studies European Arctic-alpine fungal communities have been studied by high throughput sequencing (e.g. Bjorbækmo et al. 2010, Fujiyoshi et al. 2011, Lentendu et al. 2011, Geml et al. 2012, Botnen et al. 2014). Also the Arctic region of Greenland has been relatively well studied mycologically (Borgen et al. 2006).

Although the Carpathians are known as an important European diversity hot spot and center of endemism (Pawłowski 1970, Ronikier 2011, Bálint et al. 2011) and to harbor ancient, relic lineages of Arctic-alpine plants (e.g. Ronikier et al. 2012), their Arctic-alpine fungus flora remains almost unknown. Some data are available for the highest Carpathian massif,

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the Tatra Mountains (e.g. Pilát 1926, Nespiak 1960, Fellner and Landa 1993). Recently data on fungi also have been reported from the southern Carpathians where the largest surface of the alpine zone occurs (Ronikier 2008). However, no wide-ranging analysis has been conducted to date. Here we focus on the genus *Hebeloma* and report on results of 11 y observations conducted in the alpine zone of all high-mountain massifs of the Carpathians.

Hebeloma is one of the important genera in Arctic-alpine habitats (Gardes and Dahlberg 1996, Timling and Taylor 2012). *Hebeloma* spp. are ectomycorrhizal (Marmeisse et al. 1999). Many species are well known as alpine (and Arctic) specialists, occurring in such habitats with *Salix* spp. and *Dryas* spp. (Bruchet 1970, Vesterholt 2005, Eberhardt et al. 2015). Only two *Hebeloma* species (viz. *H. marginatulum, H. bruchetii*) were reported previously from the alpine belt of the Carpathians (Bruchet 1970, Fellner and Landa 1993), both of them from localities in the Tatra Mountains. No records of *Hebeloma* species from other parts of the alpine belt of the Carpathians are available. The aim of this study is to assess the diversity of *Hebeloma* species in the alpine belt of the Carpathians based on morphological and molecular analysis of specimens collected during the first long-term survey and to compare, as far as we can, the diversity of *Hebeloma* spp. in the highly fragmented alpine zone of the Carpathians with that in the more continuous alpine area of the Alps.

In this paper the distribution of species in other alpine and Arctic regions is discussed partially based on literature data but given the confusion that has existed with regard to species delimitation in the genus (e.g. Vesterholt 2005), we also provide information on species distribution based on our experience of collections examined morphologically and phylogenetically. We detail our collections and describe two new species collected in the Carpathians during this project, and also provide a key to the species of *H.* sect. *Hebeloma* from Arctic-alpine habitats in Europe.

MATERIALS AND METHODS

Description of study area and collection of material.—The Carpathians are one of the most important European mountain ranges, and lie east of the Alps and north of the mountains of the Balkan Peninsula. They are divided into three biogeographical sections: western, eastern and southern Carpathians, each composed of several well-delimited massifs bearing their own proper names (e.g. the Tatra Mountains, the highest Carpathian massif reaching an elevation of 2655 m). The Carpathians are built of crystaline schist, volcanic and metamorphic rocks with granite intrusions and limestone deposits (Kondracki 1978). Vegetation in the alpine belt of these mountains is diverse and shaped mainly by geological features and microclimatic conditions. On limestone, dolomite and mylonite (non-calcareous) bedrock various plant communities with *Dryas octopetala* and *Salix reticulata* as dominant ectomycorrhizal plant species develop on shallow, humus-enriched soils containing a large proportion of gravel. Another important ectomycorrhizal plant species, *S. herbacea*, occupies snow bed and snow field vegetation developed on silicate bedrock where snow persists for a long time, as well as the most exposed localities on silicate crests with shallow and acid soils. *Salix retusa* s.l., including *S. kitaibeliana*, is common in rock, scree and stony grassland habitats. *Helianthemum* spp. occur on neutral to basic soils in various plant communities, whereas *Kobresia myosuroides* and *Salix alpina* are important to a lesser extent in the Carpathian alpine belt (Kliment and Valachovič 2007, Ciocârlan 2009). Finally *Bistorta vivipara* is the most ubiquitous herbaceous ectomycorrhizal species occurring in various altitudinal belts, bedrocks and plant communities.

Material for this study was collected within the framework of a project carried out by one of us (AR) devoted to mycological inventory of the alpine belt of the Carpathians, during 11 vegetation seasons (2002–2012) at more than 350 localities in almost all Carpathian massifs with an alpine belt. Because of difficult access to most alpine areas of the Carpathians (lack of roads and other infrastructure) the localities were visited irregularly. Some sites were visited only once, while others, more easily accessible and with the best developed alpine area (e.g. Bucegi Mountains, parts of the Făgăraş Mountains, the Tatra Mountains) were visited several times. The Carpathian massifs where specimens of *Hebeloma* were collected are illustrated (FIG. 1). Those massifs visited during field work, within which no *Hebeloma* specimens were found (the Cindrel Mountains, Ceahlău Mountains, Iezer-Papuşa Mountains, Lotru Mountains, Latoriţei Mountains and Piatra Craiului Mountains in the southern Carpathians and Rodnei Mountains in the eastern Carpathians) are not highlighted in the map. At each site collections were photographed (in most cases) and placed in plastic boxes. Pieces of lamellae were placed in the CTAB buffer (Largent and Baroni 1988) for use in further molecular analysis. All important characters were noted the same day or at the latest the next day in daylight (for a comparable description of colors). Immediately after description the collections were put in plastic bags with silica gel for drying. Of the more than 1300 specimens of fungi collected during field work, the genus *Hebeloma* was represented by 44 collections found at 27 localities in these Carpathian massifs: the Tatra Mountains and Nízke Tatry Mountains in the western Carpathians, Bucegi Mountains, Făgăraş Mountains, Parâng Mountains, Builă-Vânturariţa Mountains and the Retezat Mountains in southern Carpathians (FIG. 1). In addition, a relic population of *Dryas octopetala*, a key Arctic-alpine mycorrhizal dwarf shrub, growing in a pine forest in a low massif of Slovenský Raj (almost 800 m) was studied. The collections are deposited at the Herbarium of the Polish Academy of Sciences (KRAM) in Krakow. Each collection also has a H.J. Beker (HJB) Database Record number; these are also cited in that we intend to publish this database and we think these numbers therefore will be useful for future researchers.

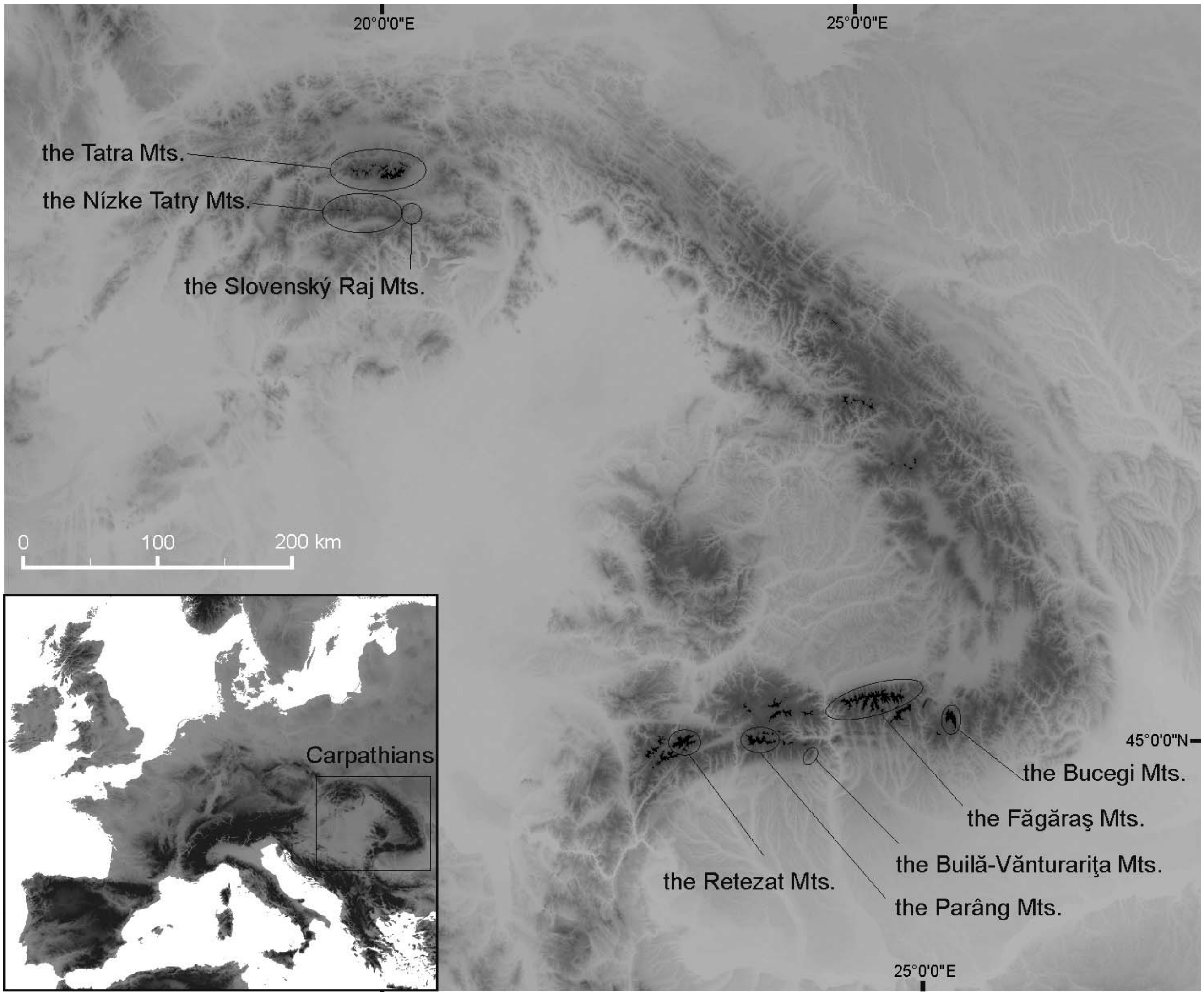

FIG. 1. The Carpathians with the localization of the visited Carpathian massifs. Black area = alpine belt.

Molecular analyses.—Sequence data of the ITS (ITS1-5.8S-ITS2, internal transcribed spacers of the nuc rDNA) were obtained for all collections. For a selection of collections DNA sequences of the partial *RPB2* (encoding the second largest subunit of RNA polymerase II) and *TEF1a* (translation elongation factor 1-α) genes also were obtained. In addition to sequences from the Carpathian collections sequences of the same or closely related taxa and *H. crustuliniforme* (in the sense of Vesterholt et al. 2014), *H. cylindrosporum, H. sinapizans,* and *H. theobrominum,* that is types or representatives of the main sections of *Hebeloma* according to Vesterholt (2005), were included in the analyses.

Details of DNA extraction, ITS and *RPB2* PCR and sequencing primers have been provided (Ronikier and Ronikier 2011, Eberhardt 2012, Eberhardt et al. 2013). PCR and sequencing primers for partial *TEF1a* sequences (forward elo31 m, 5′–TTC ATC AAG AAC ATG ATC AC–3′ and reverse a 1 : 1 mix of elo33R_R, 3′–GAC GTT GAA RCC RAC RTT GTC–5′ and elo33_W, 3′–GAC GTT GAA WCC RAC RTT GTC–5′) were constructed based on results of Stielow et al. (2014). PCR reactions were done with Bioline (London, UK) MyTaq and annealing temperatures of 48 C. Raw sequence data were edited in Sequencher 4.9, Gene Codes Corp., Ann Arbor, Michigan). Indels and ambiguous base calls in corresponding positions of forward and reverse traces were regularly encountered in sequences from nuclear ribosomal and protein-coding loci. For sequence reads where length deviant variants occurred, which was observed in all three loci on occasion, the consensus sequence with the lowest number of ambiguous positions was used.

Sequence alignments were done in Mafft 7 as implemented on http://mafft.cbrc.jp/alignment/software/, with E-INS-i option. Maximum likelihood analyses were done as heuristic searches in RAxML (8.1.2, Stamatakis 2014), including bootstrap analyses, with the number of replicates determined by the MRE option. Concatenation of alignments was done in SequenceMatrix (Vaidya et al. 2011). Before the concatenation of different datasets, their compatibility was tested following the principle of Kauff and Lutzoni (2002), which assumes a conflict to be significant if two relationships for the same set of taxa, one being monophyletic and the other nonmonophyletic, are supported by bootstrap greater than 70%.

Maximum likelihood searches for tree building were carried out locally with 100 (ITS) or 10 (*RPB2*, *TEF1a*, concatenated data) replicates with the GTRGAMMA model, selecting the best result for each analysis. Fast bootstrap searches were done locally or on the CIPRES server (Miller et al. 2010) using the MRE option. Trees were visualized with FigTree 1.4.0 (Rambaut 2012). The assignment of collections and sequences to species follows morphology. The taxonomic backbone for this study is provided by Vesterholt (2005), whom we follow with respect to the infrageneric classification.

Morphological analysis.—Morphological analysis was carried out with the help of a Biolomics-based database system (7, www.bio-aware.com). Whenever possible macroscopic information was collected in field notes and input as parametric data.

All microscopic measurements were made with a Leica DMRXA2 microscope system with a Leica DC300 camera (without color filters and with a halogen light source, usually using differential interference contrast) connected to a computer running Leica IM1000 image capture software and Leica QWin image analysis software. Photographs of all relevant features were taken, and all measurements and analysis were carried out with the image analysis software, fed into spreadsheets, where statistics were calculated, and 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 on the database.

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 along with the median, standard deviation and 5% and 95% percentiles. The assessment of spore characters follows Vesterholt (2005): spore ornamentation on a scale from O0 (smooth) to O4 (ornamentation fairly strong and always visible without immersion), the loosening of the perispore P0 (not loosening) to P3 (strongly and consistantly 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, approx. 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 in this order, including 5% and 95% percentiles and median (only for the apex), 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 are averaged. The measurements were made in 5% KOH.

The key to species of *Hebeloma* sect. *Hebeloma* occurring in the alpine belt was built on the database as a set of complex queries. At the time of writing we have analysed 118 alpine collections (and an additional 151 Arctic collections) belonging to *H.* sect. *Hebeloma* out of some 4500 collections of *Hebeloma*, including their collection details and ecology. For most collections, morphometric parameters were obtained. By having all data fully parameterized, 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 to be continually tested across a large number of collections.

RESULTS

We obtained ITS sequences for all collections cited below and *RPB2* and *TEF1a* sequences for Arctic or alpine members of *H.* sect. *Hebeloma*. Sequence data were submitted to GenBank (KT071011–KT071107, SUPPLEMENTARY TABLE I). Alignments were submitted to TreeBASE (S17371). Topologies (FIG. 2) are unrooted and are displayed according to results (Boyle et al. 2006 unpubl), using *Hymenogaster*, *Naucoria*, *Galerina* and *Gymnopilous* sequences as outgroup.

A total of 11 species of *Hebeloma* were collected in the Carpathians. (FIG. 2A), belonging to *H.* sects. *Denudata*, *Hebeloma*, *Myxocybe*, *Velutipes* following Vesterholt (2005). *Hebeloma naviculosporum* previously had not been classified to section.

Hebeloma alpinum is paraphyletic in relation to *H. aanenii* and *H. crustuliniforme*, the other included members of *H.* subsect. *Denudata*. The species *H. aanenii* is monophyletic but does not receive bootstrap support. *Hebeloma vaccinum*, another member of *H.* sect. *Denudata*, but not of *H.* subsect. *Denudata* sensu Eberhardt et al. (2015), forms a sister clade to *H.* subsect. *Denudata* clade. *Hebeloma laterinum* and *H. cylindrosporum*, both members of *H.* sect. *Myxocybe* form a joint clade and are well supported. *Hebeloma velutipes*, *H. sinapizans*, *H. theobrominum* and *H. naviculosporum* —the former three single representatives of their sections and the latter not included in Vesterholt (2005) —form well supported "species" clades, which are outside any of the indicated "section" clades (FIG. 2A). *Hebeloma* sect. *Hebeloma* receives high bootstrap support based on ITS, but the Arctic or alpine species included in this tree other than *H. nigellum* (not recorded from the Carpathians) do not. Because we have only a single collection of *H. grandisporum* we do not know whether this species is monophyletic and supported.

The remaining phylogenies (FIG. 2B–D) were inferred only for Arctic-alpine members of *H.* sect. *Hebeloma.* In the *RPB2* topology all species are monophyletic and all but *H. mesophaeum* are well supported (FIG. 2B). In the *TEF1a* result (FIG. 2C) *H. oreophilum* and *H. nigellum* are not resolved. In addition, *H. mesophaeum* is paraphyletic in relation to the highly supported clade of *H. dunense.* There is no conflict between the topologies of the three loci used with respect to species clades; potential conflicts pertaining to the relationship of the species among each other cannot be detected in unrooted phylogenies but are irrelevant for the question at hand. The results of the concatenated data are displayed (FIG. 2D). Here all species appear monophyletic but again *H. mesophaeum* does not receive support and *H. oreophilum* is monophyletic and supported (75%), but it does receive high support (FIG. 2B) (*RPB2*). As before we cannot know whether *H. grandisporum* would be monophyletic in this setting.

TAXONOMY

Eleven species are recognized in the studied material of which three are Arctic-alpine specialists. Both of the two newly described taxa, *H. grandisporum* and *H. oreophilum,* were collected from alpine habitats. The list of species is arranged in alphabetical order.

Hebeloma aanenii Beker, Vesterh. & U. Eberh. FIGS. 3, 4A

Specimens examined: SLOVAKIA. ŽILINA: western Carpathians, Nízke Tatra Mountains, Mount Veľky Bok, summit area, N48.9402780, E19.8813800, 1720 m, in meadow, among moss, *Salix* sp. and *Bistorta vivipara,* 10 Sep 2006, *A. Ronikier, S. Adamčík, V. Kučera, V. Kautman* (KRAM F-47190, Database Record HJB13120).

Comment: This recently described species is known so far from Europe and New Zealand where it grows in various habitats and forms mycorrhizae with a wide range of trees or shrubs including *Dryas* (Eberhardt et al. 2015). The Carpathian alpine collection is typical albeit with basidiome size at the lower end of the range for the species. For a description see Eberhardt et al. (2015). *Hebeloma aanenii* normally can be distinguished from *H. alpinum, H. vaccinum* and related taxa of sect. *Denudata* based on ITS data (Eberhardt et al. 2015). A description of *H. aanenii* and a key to *H.* subsect. *Denudata* was studied by Eberhardt et al. (2015).

Hebeloma alpinum (J. Favre) Bruchet FIGS. 3, 4B

Specimens examined: ROMANIA. PRAHOVA: southern Carpathians, Bucegi Mountains, Munţele Caraiman, calcareous rocks near the marked trail to Mount Caraiman, N45.4141670, E25.4927700, 2400 m, in meadow among *Dryas octopetala,* 26 Jul 2004, *M. Ronikier, A. Ronikier, J. Cieślak* (KRAM F-46670, Database Record HJB13110); N45.4147220, E25.4927700, 2330 m, in meadow, among moss, *D. octopetala* and *B. vivipara,* 1 Aug 2006, *M. Ronikier, A. Ronikier, H. Knudsen* (KRAM F-46666, Database Record HJB13112). VÂLCEA: southern Carpathians, Builă-Vănturariţa Mountains, S crest of Munţele Builă, N45.2405560, E24.0927780, 1815 m, among *Helianthemum* sp., 28 Aug 2009, *A. Ronikier, M. Ronikier* (KRAM F-57432, Database Record HJB14244). SLOVAKIA. PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, Hlúpe Sedlo pass, N49.2388890, E20.2277780, 2000 m, in meadow among *D. octopetala,* 19 Aug 2006, *A. Ronikier, M. Ronikier* (KRAM F-46669, Database Record HJB13118); N-E slopes of Mount Ždiarska Vidla (Plačlivá Skála), N49.2447220, E20.2094400, 2070 m, in meadow among *D. octopetala,* 23 Jul 2007, *A. Ronikier, S. Adamčík* (KRAM F-47192, Database Record HJB13123); between Vyšné Kopské Sedlo pass and Kopské Sedlo pass, N49.2327780, E20.2191670, 1850 m, among *D. octopetala,* 17 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57424, Database Record HJB14236); Jatky crest, N49.2372220, E20.2327780, 2025 m, among *D. octopetala* and *S. reticulata,* 3 Aug 2010, *A. Ronikier, P. Mleczko* (KRAM F-57434, database record HJB14247).

Comment: This Arctic-alpine species is associated mainly with *Salix,* known from European mountains and Arctic regions also outside Europe (Eberhardt et al. 2015). The Carpathian alpine collections are typical but include one collection with *Helianthemum,* an associate that we have not seen recorded elsewhere. For a description and a key to *H.* subsect. *Denudata* see Eberhardt et al. (2015). Eberhardt et al. (2015) were not able to find a single molecular locus that always distinguishes *H. alpinum* from related taxa, but when using several loci all other species can be distinguished from *H. alpinum.*

Hebeloma dunense L. Corb. & R. Heim FIGS. 3, 4C.

Specimens examined: POLAND. MAŁOPOLSKA: western Carpathians, Tara Mountains, western Tatra Mountains, Kasprowy Wierch Massif, Mount Beskid, summit area, N49.2302780, E19.9894400, 1900 m, in meadow, 29 Jul 2002, *H. Knudsen, A. Ronikier* (KRAM F-53052, database record HJB13107; KRAM F-53053, Database Record HJB13108); N slopes of Mount Ciemniak, summit area, N49.2311111, E19.9033330, 2088 m, among *S. reticulata,* 2 Aug 2010, *A. Ronikier, P. Mleczko* (KRAM F-57434, database record HJB14246). ROMANIA. SIBIU: southern Carpathians, Fagaraş Mountains,

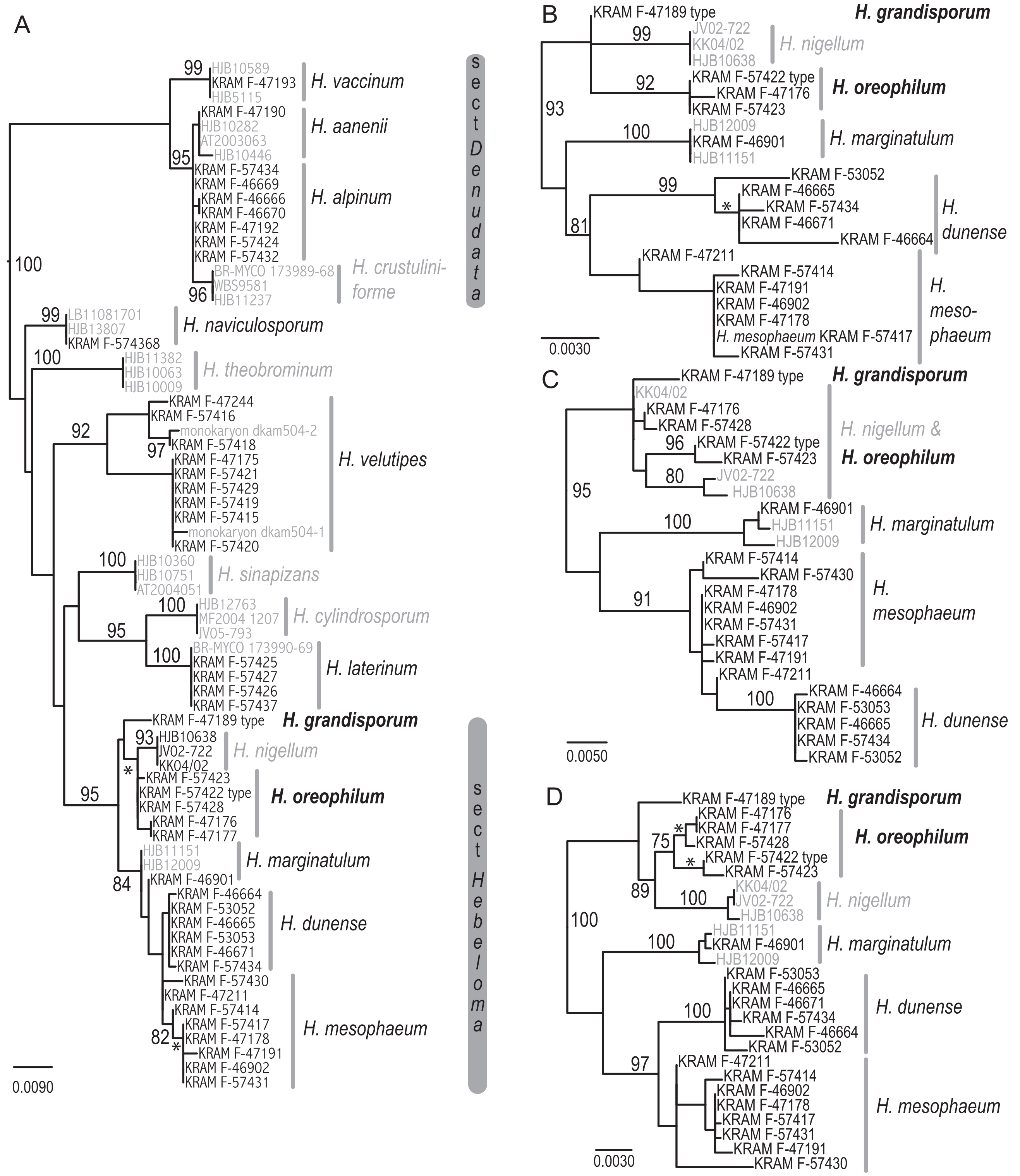

FIG. 2. Maximum likelihood results. *H.* – *Hebeloma*. The infrageneric classification where indicated follows Vesterholt (2005). Collections and species names in gray have not been collected in the study area. Bootstrap values ≥75% are shown. Asterisk indicates bootstrap support of ≥75% within species clades. A. Unrooted ITS topology, with support values of 550 bootstrap replicates. For *H. velutipes*, sequences of two monokaryotic strains produced from a single-spore deposit (Aanen and Kuyper 2001) were included. B. Unrooted *RPB2* topology of *H.* sect. *Hebeloma*, with support values of 1000 bootstrap replicates. C. Unrooted *TEF1a* topology of *H.* sect. *Hebeloma*, with support values of 1000 bootstrap replicates. D. ITS, *RPB2* and *TEF1a* concatenated; unrooted topology with 700 bootstrap replicates.

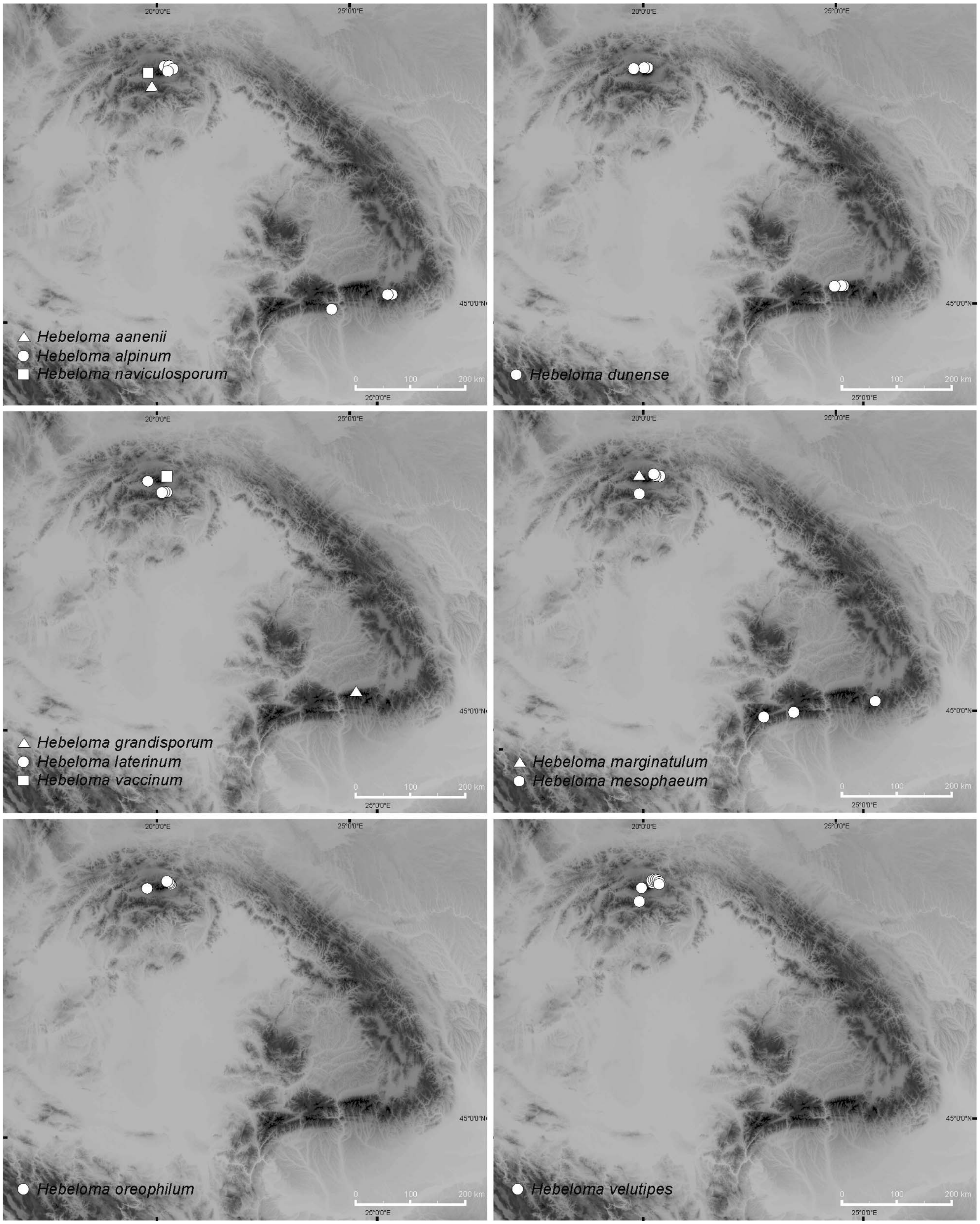

FIG. 3. Distribution maps of *Hebeloma* species found in the alpine belt of the Carpathians. Black area = alpine belt.

FIG 4. *Hebeloma* collections from the alpine belt of the Carpathians. A. *H. aanenii* (KRAM F-47190). B. *H. alpinum* (KRAM F-57432). C. *H. dunense* (KRAM F-46664). D. *H. laterinum* (right, cut basidiome; KRAM F-57427) and *H. naviculosporum* (left; KRAM F-57436). E. *H. marginatulum* (KRAM F-46901). F. *H. mesophaeum* (KRAM F-57417). G. *H. vaccinum* (KRAM F-47193). H. *H. velutipes* (KRAM F-57421).

upper part of Valea Podragului valley (toward Şaua Podragului Pass), N45.6047220, E24.6883300, 2270 m, among *S. retusa*, 5 Aug 2004, *M. Ronikier, A. Ronikier* (KRAM F-46665, database record HJB13111); the pass in the N-NW ridge of the Arpaşu Mare Massif, E facing slopes (blue hiking trail), N45.6061110, E24.6725000, 2100 m, among moss, *S. reticulata* and *S. retusa*, 28 Jul 2006, *M. Ronikier, A. Ronikier* (KRAM F-46664, database record HJB13115); Mount Paltinului, summit area, N45.5986110, E24.6066600, 2410 m, in meadow, among *S. reticulata*, 4 Aug 2006, *H. Knudsen, A. Ronikier, M. Ronikier* (KRAM F-46671, Database Record HJB13116).

Comment: The species occurs with *Salix* in bogs and sand dunes (Vesterholt 1989, 2005). While we know this species to be common in alpine and Arctic habitats, as far as we are aware, this is the first published report of the fungus from the alpine belt. The Carpathian alpine collections are variable in size, color and presence of veil remnants (from almost absent to conspicuous). For a description see Vesterholt (2005) where it is described under the name *Hebeloma collariatum* Bruchet. *Hebeloma dunense* is a member of *H.* sect. *Hebleoma* and is monophyletic in all topologies (Fig. 2).

Hebeloma grandisporum Beker, U. Eberh. & A. Ronikier, sp. nov. Figs. 3, 5, 6
MycoBank: MB812871

Typification: ROMANIA. SIBIU: southern Carpathians, Fagaraş Mountains, upper part of Valea Podragului Valley (toward Şaua Podragului Pass), N45.6047220, E24.6883300, 2270 m, among *Salix retusa*, 25 Jul 2006, *M. Ronikier, A. Ronikier* (holotype KRAM F-47189). Database record HJB13114).

Etymology: From Grandis (Latin) = large and sporum (Latin) = spore.

Diagnosis: Hebeloma grandisporum belongs to *H.* sect. *Hebeloma* based on the presence of a cortina and the lageniform or ventricose cheilocystidia. It has consistently two-spored basidia and large amygdaloid and limoniform spores (ave. 15.4 × 8.9 µm), which are strongly dextrinoid. The ITS of the species is distinct from ITS sequences of all known species of *Hebeloma* from Europe.

Basidiomes in scattered group. Pileus small, up to 20 mm diam, convex to umbonate, sometimes broadly; surface slightly viscid, tacky when moist occasionally spotting never hygrophanous; cuticle cream or warm buff to Isabella or yellowish brown or cinnamon, paler toward the margin which is cream; pileus margin usually straight becoming upturned with age. Lamellae emarginate to adnate, distant to subdistant (L = 24); cream, alutaceous or brown when young, later umber to sepia following spore maturity; edge fimbriate, paler than lamella surface; droplets on the lamella edge are not present; lamellulae frequent. Stipe central, cylindrical to clavate; white or alutaceous, rarely discoloring from the base; surface dry, usually fibrillose, occasionally more pruinose or velute; interior stuffed when young but sometimes becoming hollow with age. Cortina present. Flesh thin, cream or pale brown. Odor raphanoid. Flavor not recorded. Spore deposit not recorded. Exsiccata dark and firm.

Spores amygdaloid, sometimes limoniform, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall and a distinct papilla; some spores guttulate with one or more oily drops, weakly ornamented with no sign of a loosening perispore and strongly dextrinoid becoming orange-brown (O2; P0; D3); spore under the microscope pale brown; spores based on 51 specimens 14.0–16.5 × 8.5–9.5 µm, with median 15.5 × 9.0 µm and ave. 15.4 × 8.9 µm with S. D. length 0.83 µm and width 0.42 µm, Q value 1.56–1.84, with median 1.73 and ave. 1.72 with S. D. 0.09. Basidia cylindrical to clavate and two-spored, 31–44 × 7.0–9.5 µm, with ave. 36 × 8.4 µm. Pleurocystidia not found. Cheilocystidia lageniform or ventricose and occasionally septate or knee-shaped; width of apex range 5.0–7.0 µm, with median 6.0 µm and ave. 5.8 µm with S.D. 0.66 µm; based on 23 cheilocystidia the ranges are 34–53 × 5.0–7.0 × 4.5–6.5 × 6.0–10.5 µm while the averages are 42 × 5.8 × 5.6 × 8.2. The ave. cheilocystidia ratios were: A/M = 1.04; A/B = 0.71; B/M = 1.49. Caulocystidia resemble cheilocystidia but up to 100 µm long. Pileipellis is an ixocutis with epicutis 130 µm thick, embedded hyphae up to 5 µm broad, smooth or occasionally encrusted, colorless or occasionally pigmented; cutis: brown and made up of cylindrical to isodiametric elements. Trama below the subcutis contains isodiametric elements. Clamp connections present throughout the basidiome.

Habitat: Our single collection of *H. grandisporum* is from the alpine zone of the southern Carpathians in Romania and grew with *Salix*.

Comment: The presence of a cortina and the lageniform or ventricose cheilocystidia clearly places this taxon in *H.* section *Hebeloma*. Within this section it can be differentiated on the basis of the large amygdaloid and limoniform spores that are distinctly to strongly dextrinoid. It is the only *Hebeloma* collection we have ever encountered where all basidia appear to be two-spored, which no doubt contributes to the large spore size. Because we have only a single collection we do not know whether the two-spored basidia are an individual aberration or constant character of the species, as for example, in *Lactarius acerrimus*, which happens to have the largest spores within its genus in Europe (Heilmann-Clausen et al. 1998). However, because the collection here described as *H.*

grandisporum is molecularly distinct in all loci tested and therefore presumably also biologically clearly distinct from all other taxa within *H.* sect. *Hebeloma*, regardless of the number of spores per basidium, we have decided to report it at this time. Molecular data support the inclusion of this species in *H.* sect. *Hebeloma.*

Hebeloma laterinum (Batsch) Vesterh. FIGS. 3, 4D

Specimens examined: SLOVAKIA. PREŠOV: western Carpathians, Slovenský Raj Mountains, S-E from Vernárska Tiesňava gorge, N48.9305560, E20.2897220, 790 m, in conifer woodland, among *D. octopetala* and *Pinus sylvestris*, 11 Sep 2009, *A. Ronikier, M. Ronikier* (KRAM F-57425, database record HJB14237, KRAM F-57426, database record HJB14238); N 48.9305280, E20.2896670, 785 m, among *D. octopetala* and *P. sylvestris*, 13 Oct 2010, *A. Ronikier, P. Mleczko* (KRAM F-57437, database record HJB14248). ŽILINA: western Carpathians, Tatra Mountains, western Tatra Mountains, S-E ridge of Mount Baranec, N49.1583330, E19.7569440, 1945 m, among *S. retusa*, 27 Sep 2009, *A. Ronikier, M. Ronikier* (KRAM F-57427, database record HJB14239).

Comment: With its short cylindrical cheilocystidia the species belongs in *H.* sect. *Myxocybe* and a morphological key to that section is provided in Vesterholt (2005). For a description see Vesterholt (2005). This is a woodland species occurring on calcareous soils with both coniferous and deciduous trees (Vesterholt 2005). Bruchet writes (1970) that "Il peut atteindre la zone alpine sous une forme un peu plus petite et trapue". We have examined Bruchet's specimens and Favre's material (1955) and also find that this has a small squat form. Carpathian collections from a relic *Dryas octopetala* locality from the Slovenský Raj Mountains (forest belt) are typical, while the alpine collection is smaller and squat.

Although macroscopically slightly different from lowland specimens, the alpine collections appear microscopically and molecularly identical to other collections and it therefore is likely that the macroscopic difference is the result of occurrence in the high mountain environment. ITS supports the inclusion of the Carpathian alpine collection into *H. laterinum* (FIG. 2A).

Hebeloma marginatulum (J. Favre) Bruchet FIGS. 3, 4E

Specimens examined: POLAND. MAŁOPOLSKA: western Carpathians, Tatra Mountains, western Tatra Mountains, Czerwone Wierchy Massif, Małołącka Przełęcz Pass, E slopes, N49.2355560, E19.9233300, 1930 m, in meadow, among *S. herbacea*, 24 Aug 2006, *A. Ronikier* (KRAM F-46901, database record HJB13119).

Comment: This is an Arctic-alpine species associated with *Salix* (Vesterholt 1989, 2005). The Carpathian alpine collection is typical. For a description see Vesterholt (2005). Whereas this taxon is not monophyletic in the ITS topology, *RPB2, TEF1a* and the results of the analysis of the concatenated dataset support *H. marginatulum* as monophyletic (FIG. 2).

Hebeloma mesophaeum (Pers.) Quél. FIGS. 3, 4F

Specimens examined: ROMANIA. DÎMBOVIŢA: southern Carpathians, Bucegi Mountains, at the Şaua Şugărilor Pass, N45.4316670, E25.4594400, 2400 m, in meadow, among moss and *D. octopetala*, 27 Jul 2004, *M. Ronikier, A. Ronikier* (KRAM F-47211, database record HJB13109), 2 Aug 2006, *M. Ronikier, A. Ronikier, H. Knudsen* (KRAM F-46902, database record HJB13113). HUNDEOARA: southern Carpathians, Parâng Mountains, E slopes of Mount Parângul Mare, below the summit, N45.3416670, E23.5413890, 2412 m, among *S. herbacea*, 25 Aug 2009, *A. Ronikier, M. Ronikier* (KRAM F-57430, database record HJB14242); southern Carpathians, Retezat Mountains, E slopes of Piatra Iorgovanului, N45.2831944, E22.8500000, 1985 m, among *D. octopetala* and *S. retusa*, 22 Aug 2009, *A. Ronikier, M. Ronikier* (KRAM F-57431, database record HJB14243). SLOVAKIA. ŽILINA: western Carpathians, Nízke Tatra Mountains, Mount Veľky Bok, summit area, N48.9402780, E19.8813800, 1720 m, in meadow, among moss, *Salix* sp. and *B. vivipara*, 10 Sep 2006, *A. Ronikier, S. Adamčík, V. Kučera, V. Kautman* (KRAM F-47191, database record HJB13121). PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, Jatky crest, N49.2366670, E20.2358330, 2000 m, in meadow, among moss, *D. octopetala* and *S. reticulata*, 24 Jul 2007, *A. Ronikier, M. Ronikier, S. Adamčák, W. Paul* (KRAM F-47178, database record HJB13124); N49.2344440, E20.2469440, 1970 m, among *D. octopetala* and *S. reticulata*, 15 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57417, database record HJB14229); W slopes of Mount Bujačí Vrh, N49.2307220, E20.2580560, 1875 m, among *D. octopetala* and *S. reticulata*, 15 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57414, database record HJB14226).

Comment: This is one of the most common species of the genus in Europe, occurring in various habitats including Arctic and alpine regions with various hosts (Vesterholt 1989, 2005). Some Carpathian collections exhibit a typically bicolored pileus, while others have more uniform colors. For a description see Vesterholt (2005). We have not found any single locus yet that supports *H. mesophaeum* as monophyletic, even though it does receive high support in the analysis of the concatenated dataset (FIG. 2D). We have not been able to find any supported subgroups within this taxon either. *Hebeloma mesophaeum* is supported as a member of *H.* sect. *Hebeloma* (FIG. 2A).

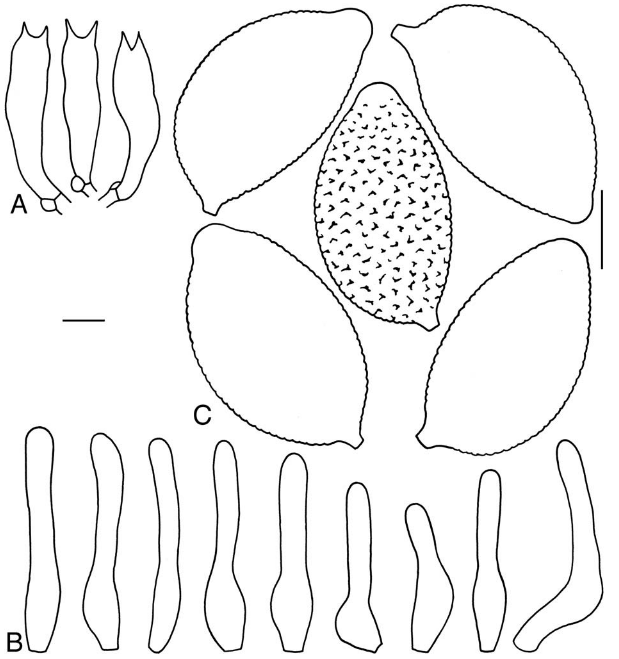

FIG. 5. Line drawing of *Hebeloma grandisporum* collection KRAM F-47189 (HJB13114) (holotype): A. Basidia × 1000. B. Cheilocystidia × 1000, bar = 10 µm. C. Spores × 1600, scale bar 5 µm.

Hebeloma naviculosporum Heykoop, G. Moreno & Esteve-Rav. FIGS. 3, 4D

Specimens examined: SLOVAKIA. ŽILINA: western Carpathians, Tatra Mountains, Western Tatra Mountains, S-E ridge of Mouny Baranec, N49.1583330, E19.7569440, 1945 m, among *S. retusa,* 27 Sep 2009, *A. Ronikier, M. Ronikier* (KRAM F-57436, database record HJB14568).

Comment: This is a rare species known from the type locality with *Pinus sylvestris* in Spain and all collections of which we are aware have been recorded with conifer trees. For a description see Heykoop et al. (1992). Typically the pileus has strong orange colors. The pileus surface of the Carpathian collection was cracked into small squamules with some orange pigment but far paler than other collections we have examined. This pale color makes it macroscopically similar to *H. laterinum* with which it co-occurred at the same locality. *Hebeloma naviculosporum* has cylindrical cheilocystidia and its navicular spores with high length/width ratio distinguish it from all other known European *Hebeloma* spp. including *H. laterinum.* These differences in morphology of an alpine collection might have been caused by exposure to more extreme conditions than typical for the species, but ITS data support its assignement to *H. naviculosporum.*

Hebeloma oreophilum Beker & U. Eberh., sp. nov. FIGS. 3, 7–9

MycoBank: MB812872

Typification: SLOVAKIA. PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, N slopes of Mount Hlúpy, vicinity of the summit, N49.2376390, E20.2222200, 1970 m, on mossy soil among *S. reticulata* and *S. retusa,* 17 Sep 2009, *A. Ronikier, S. Adamčík* (holotype KRAM F-57422). Isotype BR 5020184124533. Database record HJB14234.

Etymology: From ορεοφιλος (Greek) = mountain loving, to emphasize its presence in alpine habitats.

Diagnosis: Hebeloma oreophilum has the persisting cortina and the lageniform or ventricose cheilocystidia of *H.* section *Hebeloma.* The species is similar to *H. clavulipes* but has wider (ave. 6.8–7.2 µm) and more ovoid spores and occurs in the alpine habitat.

Basidiomes usually in scattered groups. Pileus up to 40 mm diam, convex; surface slightly viscid, tacky when moist occasionally appearing hygrophanous; cuticle clay-buff to Isabella to brownish olive or cinnamon, paler toward the margin which is cream to pinkish buff often slightly pruinose or tomentose; pileus margin often involute when young, then straight. Lamellae emarginate, subdistant (L = 44–48) with a maximum depth of 6 mm; cream, alutaceous or brown when young, later clay-buff following spore maturity; edge fimbriate, paler than lamella surface; droplets on the lamella edge are absent; lamellulae frequent. Stipe central, cylindrical, occasionally slightly clavate, 35–70 × 3–6 mm; clay-buff, discoloring from the base, which becomes brownish olive, sometimes strongly so, when handled or with age; surface dry, fibrillose, pruinose at apex above cortina; interior stuffed when young but becoming hollow with age. Cortina visible and reasonably persistent. Flesh thick, cream or pale brown. Odor raphanoid. Flavor slightly raphanoid, slightly bitter. Spore deposit color not recorded.

Spores amygdaloid, occasionally ovoid, with small apiculus and rounded at the end opposite the apiculus, with a distinct thinning of the spore wall and a weak papilla, some spores guttulate with one or more oily drops, weakly ornamented to distinctly ornamented, sometimes with some sign of loosening perispore in a few spores and strongly dextrinoid becoming orange-brown (O2,O3; P1; D3); spore under the microscope pale brown to brown; spore size based on 50 spores of the holotype, 10.5–12.0 × 6.5–7.5 µm, with median 11.0 × 7.0 µm and ave. 11.3 × 6.9 µm with S. D. length 0.78 µm and width 0.36 µm, Q value 1.47–1.78, with median 1.64 and ave. 1.65 with S. D. 0.14; dimensions based on five collections, medians 11.2–12.6 × 6.7–7.2 µm and ave. 11.3–12.7 × 6.8–7.2 µm with S. D. length 0.61–1.25 µm and width 0.26–0.59 µm, ave. Q 1.65–1.82. Basidia cylindrical to clavate and four-spored, 28–35 × 6.0–8.5 µm, with ave. 29.5–32.5 × 6.1–8.2 µm. Pleurocystidia not found.

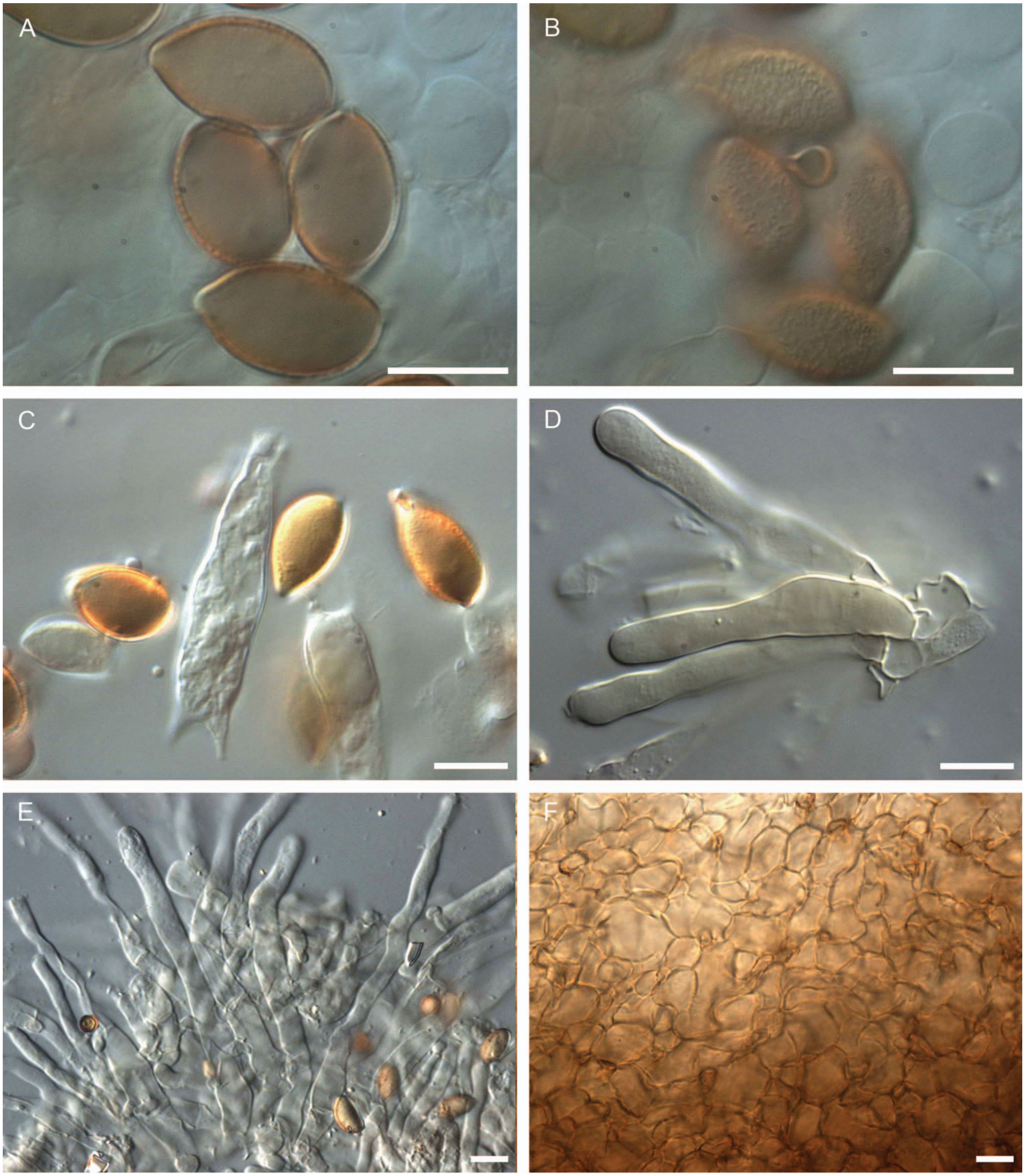

FIG. 6. *Hebeloma grandisporum* KRAM F-47189 (HJB13114) (holotype): A–B. Spores × 1600 in Melzer's reagent. B. Spore ornamentation. C. Basidium × 1000. D. Cheilocystidia × 1000. E. Caulocystidia × 500. F. Cutis × 500. Bars = 10 μm. Microographs H.J. Beker.

Cheilocystidia lageniform ventricose, occasionally cylindrical and sometimes with apical thickening, basal thickening, knee-shaped, median thickening, septate; width of apex holotype, 4.0–6.0 μm, with median 4.5 μm and ave. 4.8 μm with S. D. 0.62 μm; across five collections median 4.7–5.7 μm and ave. 4.8–5.7 μm, with at least 20 selected cheilocystidia of five collections 42–66 × 4.0–6.5 × 4.0–6.0 × 7.0–11.0 μm while the averages are 49–55 × 4.8–5.7 × 4.5–5.3 × 8.2–9.0 and 54 × 4.8 × 4.5 × 8.8 μm ave. for the holotype. The ave. cheilocystidia ratios for the five collections were: A/M = 1.03–1.24, A/B = 0.57–0.67, B/M = 1.57–1.94. Caulocystidia resemble cheilocystidia but are less ventricose and more cylindrical, often multiseptate, up to 120 μm long. Pileipellis is an ixocutis with a relatively thin epicutis 50–70 μm, embedded hyphae up to 5–7 μm broad, smooth, colorless or occasionally pigmented; cutis reddish brown and made up of cylindrical to isodiametric elements. Trama below the subcutis contains cylindrical to sausage shaped elements up to 18 μm broad. Clamp connections present throughout the basidiome.

Habitat: The five collections of *H. oreophilum* from the western Carpathians of Slovakia were collected within 40 km of each other. They were recorded from calcareous soil with either *Salix reticulata, S. retusa* or *Dryas octopetala.* The altitude was 1940–2120 m.

Additional specimens examined: SLOVAKIA. PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, Hlúpe Sedlo Pass, N49.2388890, E20. 2277780, 2000 m, in meadow, among moss and *S. reticulata*, 19 Aug 2006, *A. Ronikier, M. Ronikier* (KRAM F-47177, database record HJB13117); N49.2388890, E20.2275000, 1940 m, in meadow, among *S. reticulata*, 2 Sep 2009, *A. Ronikier* (KRAM F-47176, database record HJB13126); N slopes of the Mount Hlúpy, vicinity of a summit, N49.2376390, E20.2222200, 1970 m, among *S. reticulata* and *S. retusa*, 17 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57423, database record HJB14235). ŽILINA: western Carpathians, Tatra Mountains, western Tatra Mountains, E slopes of S-E ridge of Mount Baranec, N49.1708330, E19.7472220, 2114 m, on soil among *D. octopetala*, 27 Sep 2009, *A. Ronikier, M. Ronikier* (KRAM F-57428, database record HJB14240).

Comment: The persistent presence of a cortina and the lageniform or ventricose cheilocystidia clearly place this taxon in *H.* section *Hebeloma.* Within this section it can be differentiated on the basis of the amygdaloid and ovoid spores distinctly to strongly dextrinoid, the number of lamellae, which is always at least 40, and its occurrence in alpine habitats. It is closely related to *H. clavulipes* but can be differentiated from that species not only by the habitat but also by the wider spores (6.8–7.2 μm vs. 5.8–6.6 μm) which are more ovoid and rarely limoniform (with at most a weak papilla). Molecularly *H. oreophilum* clusters with other members of *H.* sect. *Hebeloma.* We have not found a single locus that always separates *H. oreophilum* from all other taxa, but within the context of alpine taxa, *H. oreophilum* is monophyletic with *RPB2* (support 92%) and, with lower support, in the result of the concatenated dataset (FIG. 2B, D).

Hebeloma vaccinum Romagn. FIGS. 3, 4G

Specimens examined: SLOVAKIA. PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, N slopes of Mount Hlúpy, N49.2369440, E20.2202780, 2050 m, in meadow, among *D. octopetala* and *S. reticulata*, 25 Jul 2007, *A. Ronikier, S. Adamčík* (KRAM F-47193, database record HJB13125).

Comment: This fairly common species occurs in bogs and sand dunes and occasionally in Arctic or alpine habitats under *Salix* and *Populus* (Vesterholt 2005). The Carpathian collection is typical in regard to all characters. For a description see Vesterholt (2005). From a morphological viewpoint *H. aanenii* and *H. alpinum* have primarily clavate-stiptate cheilocystidia, which separate them from *H. vaccinum,* which has predominantly clavate-ventricose cheilocystidia. The ITS distinguishes *H. vaccinum* and *H. cavipes* from all other *Hebeloma* spp.; these two taxa are best separated by *RPB2* sequences.

Hebeloma velutipes Bruchet FIGS. 3, 4H

Specimens examined: SLOVAKIA. ŽILINA: western Carpathians, Nízke Tatra Mountains, Mount Veľky Bok, summit area, N48.9402780, E19.8813800, 1720 m, in meadow, among moss, *Salix* sp. and *B. vivipara*, 10 Sep 2006, *A. Ronikier, S. Adamčík, V. Kučera, V. Kautman* (KRAM F-47244, database record HJB13122). PREŠOV: western Carpathians, Tatra Mountains, Belanské Tatra Mountains, W slopes of Mount Bujačí Vrh, N49.2307220, E20.2580560, 1875 m, among *D. octopetala* and *S. reticulata*, 15 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57415, database record HJB14227, KRAM F-57416, database record HJB14228); Jatky crest, N49.2377780, E20.2319440, 2008 m, among *D. octopetala*, 2 Sep 2008, *A. Ronikier* (KRAM F-47175, Database Record HJB13127); N49.2344440, E20.2469440, 1970 m, among *D. octopetala* and *S. reticulata*, 15 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57418, database record HJB14230, KRAM F-57419, database record HJB14231); Hlúpe Sedlo Pass, N49.2388890, E20. 2263890, 1920 m, on mossy soil, among *S. retusa*, 17 Sep 2009, *A. Ronikier, S. Adamčík* (KRAM F-57420, database record HJB14232, KRAM F-57421, database record HJB14233); High Tatra Mountains, upper part of Mengusovská Dolina Valley, S from Veľké Hincovo Pleso lake, N49.1719440, E20.0627780, 1935 m, among *D. octopetala* and *S. retusa*, 28 Sep. 2009, *A. Ronikier, M. Ronikier* (KRAM F-57429, database record HJB14241).

Comment: This is one of the most common species in Europe, which can be found in many different habitats (Vesterholt 2005). In the alpine belt of the Carpathians the fungus is also common and all collections are typical. For a description and keys to *H.* sect. *Hebeloma* see Vesterholt (2005). Already Aanen and

FIG. 7. *Hebeloma oreophilum* KRAM F-57422 (HJB14234) (holotype). Photograph by A. Ronikier.

co-workers (2001) described that many *H. velutipes* collections (genotypes) show an extraordinary polymorphism in the ITS. Representatives of both genotypes are present in the Carpathian population as demonstrated by the inclusion of ITS sequences (FIG. 2A) of monokaryotic strains gained from single-spore cultures of the spore deposit of a single specimen (Aanen et al. 2001). Intragenomic variation also is common in coding genes (Eberhardt et al. 2013, 2015). As a result *H. velutipes* is difficult to delimit molecularly, at least until a better overview of the full extent of the intragenomic variation is known. From a morphological viewpoint it has primarily gently clavate cheilocystidia that are cheilocystidia that are not clearly constricted in the median part.

KEY TO SPECIES OF *HEBELOMA* SECTION *HEBELOMA* FROM ARCTIC-ALPINE HABITATS

We provide below a key to the species of *Hebeloma* section *Hebeloma* that can be found in Arctic and alpine habitats in Europe. This includes all five species collected in the Carpathians plus *H. nigellum*, which has not yet been recorded in this region.

1\. Spores amygdaloid with dextrinoidity D2 or D3 4
1\. Spores amygdaloid or not, with dextrinoidity D0 or D1 2
2\. Ave. spore length at most 10 µm or ave. spore width less than 6 µm and spores elliptical, only rarely amygdaloid, associated with various trees and shrubs . . . . *H. mesophaeum*
2\. Ave. spore length greater than 10 µm and ave. spore width at least 6 µm or if spores smaller then many spores amygdaloid, always with Saliceaceae. 3

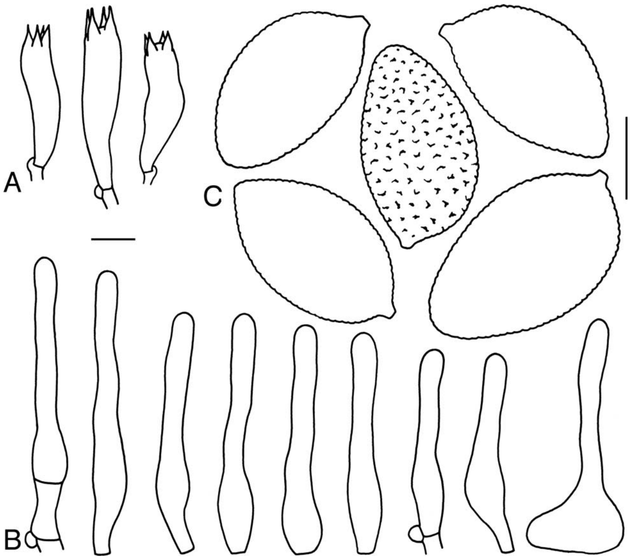

FIG. 8. Line drawing of *Hebeloma oreophilum* KRAM F-57422 (HJB 14234) (holotype). A. Basidia × 1600. B. Cheilocystidia × 1000; bar = 10 µm. C. Spores × 1600; bar 5 µm.

3\. Spores with some clear ornamentation (O1,O2) and an indistinct but clear dextrinoid reaction (D1). . . . . *H. dunense*
3\. Spores showing almost no ornamentation, even under immersion (O1) or completely indextrinoid (D0) . . . . *H. marginatulum*
4\. Number of complete lamellae (L) at least 40 . . . . *H. oreophilum*
4\. Number of complete lamellae (L) less than 40 5
5\. Ave. spore length at least 15 µm . . . . *H. grandisporum*
5\. Ave. spore length less than 15 µm. . . . . *H. nigellum*

DISCUSSION

We begin this discussion by again stressing the difficulty that mycologists have had with regard to morphological species delimitation in the genus *Hebeloma* and hence the confusion over species determination. Vesterholt (2005) stressed that literature references of *H. alpinum* should be treated with caution; as an example he referred to misinterpretation of this species by Bruchet (1970).

Many *Hebeloma* species have been exclusively reported from alpine and Arctic habitats (e.g. Bruchet 1970, Vesterholt 2005, Eberhardt et al. 2015). It is also interesting that a high number of otherwise typically lowland species can be found in Arctic and alpine environments, so it is possible that the potential to grow in cold environments is characteristic of many of the *Hebeloma* species. In the Carpathians we found 11 species. Three of them belong to the Arctic-alpine geographical element. They are *Hebeloma alpinum*, *H. marginatulum* and *H. oreophilum*. The first two species are common in Arctic and alpine regions. They have been reported from numerous localities in Greenland, Iceland, Russian Arctic, Svalbard, Scandinavian mountains, the Alps and the Pyrenees (e.g. Favre 1955, Bruchet 1970, Gulden and Lange 1971, Ohenoja 1971, Kühner and Lamoure 1986, Senn-Irlet et al. 1990, Heinrich and Olech 1999, Karatygin et al. 1999, Borgen et al. 2006, Corriol 2008). *Hebeloma alpinum* also was reported from Alaska and the Rocky Mountains (Miller 1998, Cripps and Horak 2008).

In the Carpathians *H. alpinum* appears common, recorded at seven localities in three massifs (FIG. 3). Based on the material we have studied we can confirm the occurrence of this species in Greenland, Iceland, Svalbard, Scandinavia, the Alps and the Pyrenees. In addition, we have records from Canada. *Hebeloma alpinum* occurs with *Salix* spp. and *Dryas* spp. and it has been reported to form mycorrhizae with both (e.g. Debaud et al. 1981, Eberhardt et al. 2015). All Carpathian records of this fungus are from calcareous areas where it grew among *Dryas octopetala* (sometimes together with *Salix reticulata*), but of note in the Builă-Vănturarița Mountains the species was growing with *Helianthemum* sp. (FIG. 4B). Although *Dryas octopetala* is also present in this area, it was not recorded in near the fungus, so it is likely that *H. alpinum* forms mycorrhizae also with *Helianthemum* sp. As far as we are aware this is the first time it has been recorded with this association.

Hebeloma marginatulum was recorded by us only once in the Tatra Mountains (the highest Carpathian massif) (FIG. 3) on siliceous bedrock and close to *Salix herbacea*, but Bruchet (1970) and Fellner and Landa (1993) report the species at three calcereous localities in this part of the Carpathians. Again we would suggest caution in that G. Bruchet, for instance, appears not to have been aware that the morphologically similar *H. dunense* also occurs in these habitats. We suspect his concept of *H. marginatulum* embraced both of these species. We found *H. marginatulum* on siliceous bedrock and close to *Salix herbacea*, but other Carpathian records are from siliceous and calcareous areas. According to Gulden and Lange (1971) *H. marginatulum* also probably was reported from the Caucasus Mountains and also has been reported from the Scottish Highlands (e.g. Watling 1987). However, we have examined this collection from Scotland both molecularly and morphologically and find it to be conspecific with *H. mesophaeum*. For *H. marginatulum* we have studied 65 collections. They are all from alpine or Arctic habitats, confirming species occurrence known from the literature in Greenland, Iceland, Svalbard, the Alps and the Norwegian mountains and also from subArctic area of Finland, Canadian Arctic and the Rocky Mountains of USA. *Salix* is recorded as a potential host for all these collections.

The newly described *H. oreophilum* seems to prefer base rich or neutral soils. All our Carpathian records of this species are from calcareous bedrock, where it was growing among *Salix reticulata*, *S. retusa* or *Dryas octopetala*.

Hebeloma dunense, a species originally described from sand dunes associated with *Salix repens* (Corbière and Heim 1929) occurs in a range of habitats. One Arctic report of the fungus is from Greenland (Borgen et al. 2006, as *H. collariatum*). As explained above we think it has been confused with *H. marginatulum*. However, out of 185 collections of *Hebeloma dunense* we have studied, 77 are alpine or Arctic, from Canada, France, Greenland, Italy, Russian Federation, Svalbard, Sweden, Switzerland and USA. All Carpathian records of the species are from non-calcareous locations with *Salix retusa* or *S. reticulata*.

Another species that is frequent in Arctic-alpine habitats as well as in lowland habitats is *H. mesophaeum*. It has been reported from many Arctic and alpine localities including Greenland, Svalbard, Russian Arctic, Scandinavian mountains, the Alps, the Pyrenees, the Rocky Mountains and the Bargusin Mountains in Russia (e.g. Favre 1955, Gulden and Lange 1971, Nezdoiminogo 1971, Ohenoja 1971, Karatygin et al. 1999, Borgen et al. 2006, Corriol 2008, Cripps and Horak 2008). Out of 286 collections we have analyzed, 67 are from alpine or Arctic locations in Austria, Canada, Faroe Islands, France, Greenland, Iceland, Italy, Norway, Scotland, Svalbard, Sweden, Switzerland and USA. The species also belongs to the set of the most common *Hebeloma* sp. in the alpine zone of the Carpathians; it was reported from eight ecologically diverse (calcareous and siliceous) localities dispersed throughout the range (FIG. 3).

The remaining species are typical lowland fungi but can be encountered in high-mountain ecosystems. The recently described *H. aanenii* is known to occur in both alpine and Arctic habitats but is uncommon in such environments (Eberhardt et al. 2015). Out of 94 collections of this taxon we have studied, only three are from alpine/Arctic habitats, namely from France and Iceland with *Salix* and from Switzerland with *Dryas*. The present record of *H. aanenii* from the Carpathians originates from a slightly lowered alpine belt (1720 m) in the Nízke Tatry Mountains in vegetation including ectomycorrhizal hosts as *Salix* sp. and *Bistorta vivipara*.

Hebeloma vaccinum was reported from Greenland and the polar part of the Ural Mountains (Karatygin et al. 1999, Borgen et al. 2006). Out of 76 collections we have analyzed seven from alpine or Arctic locations, two each from Greenland and Iceland and one each from each of France, Sweden and Switzerland. All

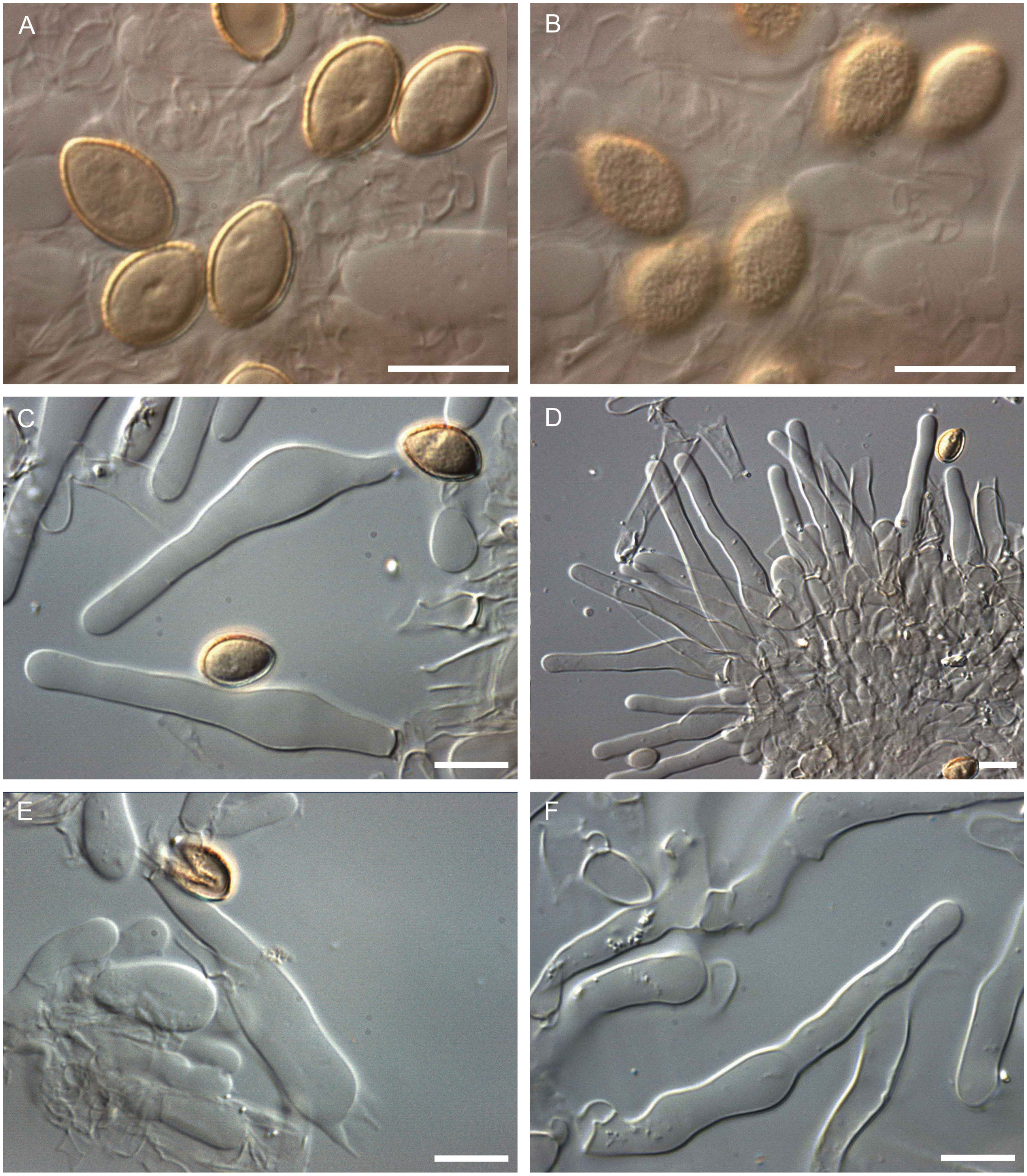

FIG. 9. *Hebeloma oreophilum* KRAM F-57422 (HJB14234) (holotype): A–B. Spores × 1600 in 5% KOH. B. Spore ornamentation; C–D. Cheiloystidia × 1000 and × 500, respectively. E. Basidium × 1000. F. Caulocystidia × 1000. Bars = 10 μm. Photographs by H.J. Beker.

records note the presence of *Salix*. The eighth, the Carpathian, alpine locality of the species is from a calcareous massif where *Salix reticulata* grows together with *Dryas octopetala*.

Within our 44 collections from the Carpathians there are four collections of *H. laterinum*. Three of these collections are not from true alpine habitats but from relic localities of *Dryas octopetala* at 790 m.

However, the fourth collection, recorded with *Salix retusa*, was collected at 1945 m on Mount Baranec in the Tatra Mountains and arguably could be regarded as being alpine. *Hebeloma laterinum* has been reported (as *H. edurum*) from the alpine belt of the Alps and the Pyrenees (Favre 1955, Bruchet 1970, Vila et al. 1998, Corriol 2008). Out of 87 collections of this taxon that we have studied in detail this is only the third from an alpine location; the others being the collections of Favre in 1946 at 2350 m with *Dryas* and according to Favre (1955) well above the tree line of the pines and that of Bruchet in 1966 (as *H. edurum* cited by Bruchet 1970) in Graubünden in the Swiss Alps at an altitude of 2400 m also with *Dryas* and with *Pinus mugo* present. *Hebeloma laterinum* is usually recorded with *Fagaceae* or *Pinaceae* in both low-altitude and subalpine habitats and also occasionally with *Helianthemum*. At our alpine locality we have not recorded the presence of *Pinus mugo* but the site is situated at 1950 m, and thus the presence of scattered plants or seedlings of *Pinus mugo* in the vicinity of fungus cannot be excluded. The relic site of *Dryas octopetala* is located on a calcareous crest inside a pine forest (*Pinus sylvestris*), so pine is the most likely mycorrhizal symbiont of the fungus there.

Hebeloma naviculosporum is uncommon, and all of the other 12 collections we have studied list *Picea* or *Pinus* as the most likely mycorrhizal partner. As mentioned above the presence of *Pinus mugo* at the locality cannot be excluded and although the fungus was growing among *Salix retusa* it might have been associated with a dwarf pine there. However, we cannot exclude the possibility that *Salix retusa* among which the basidiomes were found is also a mycorrhizal partner of the fungus at that locality. The Carpathian record is the first alpine locality of *H. naviculosporum*.

Hebeloma velutipes is common and as we mentioned above it appears to be ecologically unspecific and has been recorded with a multitude of mycorrhizal associates. We are aware of one Arctic literature record of this fungus in Iceland (Heinrich and Olech 1999, as *H. longicaudum* ss. Lange). We have analyzed almost 350 records of this taxon and apart from these records from the Carpathians there were 15 other collections from Arctic or alpine localities, from Finland, Greenland, Spain, Svalbard and Switzerland with *Dryas* and *Salix* recorded roughly in equal proportions as associate. We have nine collections of this fungus from the Carpathians, and all of them pertain to the western part of the range.

Eleven species of *Hebeloma* including six typical Arctic-alpine ones seems like a small number given that there are 17 European Arctic-alpine specialists from the genus. However, the alpine belt of the Carpathians is relatively small in comparison to, for example, the Alps (Ozenda 1985) or Arctic regions in the north and its fragmentation may be a reason for impoverishment of the fungal biota represented by *Hebeloma* species. In comparison we studied 146 collections from the Alps, across Austria, France, Italy and Switzerland; these represent 15 different species of which eight are typical Arctic-alpine specialists.

The taxonomy of fungi, also and especially to species has changed so much in recent years that observations from premolecular times or data from metagenomic ITS studies from bulk samples will not give a reliable picture of the *Hebeloma* species of a given region, particularly not of an alpine region (Eberhardt et al. 2015). East of the Carpathians is still terra incognita in view of modern treatments of *Hebeloma*; with this study we have made a start to shed light on the *Hebeloma* species diversity of the Carpathians.

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