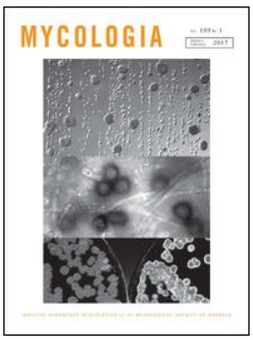

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Multilocus phylogenetic and coalescent-based methods reveal dilemma in generic limits, cryptic species, and a prevalent intercontinental disjunct distribution in *Geopyxis* (Pyronemataceae s. l., Pezizomycetes)

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Abstract: Species limits in the small genus *Geopyxis* are debatable because of problems with interpreting the few phenotypic features and poor documentation of types. To clarify species boundaries and diversity, we studied the morphology of 74 specimens of *Geopyxis* from the Northern Hemisphere, including five types, and sequenced four loci for 57 representatives: the nuc rDNA ITS1-5.8S-ITS2 (ITS), D1-D2 domains of nuc 28S rDNA (28S), translation elongation factor (*tef1*), and (or) part of the second largest subunit of the RNA polymerase II (*rpb2*) (5–7 region). Eight species are delimited. Six species are shown to be highly supported as reciprocally monophyletic: *G. aleurioides* sp. nov., *G. alpina* s. l., *G. carbonaria*, *G. delectans*, *G. korfii*, and *G. majalis*. In addition, coalescent-based Bayesian species delimitation shows *G. alpina* s. l. constitutes three cryptic species: *G. alpina* s. str., *G. deceptiva* sp. nov., and *G. rehmii*. ITS-28S sequences of type material show that *G. vulcanalis* and *G. foetida* are synonyms of *G. carbonaria*. A lectotype is designated for *Humaria delectans* and the name is combined in *Geopyxis*. Morphological characters that can be used to distinguish *Geopyxis* species are presence/absence of a long stipe, spore size and shape, and pigmented resinous exudates in medullary and ectal excipulum. *Geopyxis carbonaria* and *G. delectans* produce apothecia almost exclusively on burned ground. Bayesian analyses detected highly supported conflicts among different loci regarding generic delimitation and species relationships. Two hypogeous genera, *Stephensia* and *Hydnocystis*, are confirmed to nest within *Geopyxis*. The relationships between species of *Geopyxis* and *Tarzetta*, *Stephensia shanorii* and *Paurocotylis pila*, are unresolved. Six out of eight species of *Geopyxis* recognized in this study have intercontinental disjunct distributions.

Key words: gene conflicts, genealogical species, pyrophilous, taxonomy, type studies

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Introduction

Geopyxis (Pers.) Sacc. (Pyronemataceae s. l., Pezizales) is widely known for the charismatic post-fire species *G. carbonaria* (Alb. & Schwein.) Sacc. that produces apothecia abundantly on burned ground in boreal or montane coniferous forests. As currently recognized, *Geopyxis* is a small group of species with a cosmopolitan distribution, although 27 *Geopyxis* names are listed as current names in Index Fungorum. The species produces small to medium, 0.3–2.5 cm in diam, sessile or stipitate, cupulate to flattened apothecia on burned or unburned soil. *Geopyxis* is distinguished from other members of Pyronemataceae s. l. by the glabrous receptacle surface; yellow, orange to brown apothecial colors; smooth or very finely warted, ellipsoid to subfusiform spores without guttules when mature; and straight paraphyses with yellow to orange granules when fresh. Based on phylogenetic analyses of partial 28S sequences, *Geopyxis* has been shown to be paraphyletic by the nesting of *Stephensia bombycina* (Vittad.) Tul. & C. Tul., *Paurocotylis pila* Berk. (Læssøe and Hansen 2007, Perry et al. 2007), and *Hydnocystis piligera* Tul. (Alvarado et al. 2011, Tedersoo et al. 2013) within it. These are semi-hypogeous to hypogeous taxa with passive spore dispersal. Recently, *Geopyxis* was recommended to be removed from Pyronemataceae s. str. with *Tarzetta* and a small group of other genera based on a four-gene phylogeny (Hansen et al. 2013). Species of *Geopyxis* have been indicated to be saprobic, facultatively biotrophic, weakly parasitic, mutualistic under certain conditions (Egger 1986, Egger and Paden 1986, Vrålstad et al. 1998), endophytic and/or endolichenic (Tedersoo et al. 2013).

In Europe, species of *Geopyxis* have mostly been reported under the names *G. carbonaria*, *G. alpina* Höhn., *G. foetida* Velen., and *G. majalis* (Fr.) Sacc. and in North America under *G. carbonaria* and *G. vulcanalis* (Peck) Sacc. Over the last 35 y, three new species were described, i.e. *G. grandis* Thind & Kaushal (1981), *G. rehmii* Turnau (1984), *G. korfii* W.Y. Zhuang (in Zhuang and Liu 2006), from India, Poland, and China, respectively. Numerous questions were left unanswered in the

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literature on ambiguous species delimitations (see Garnweidner et al. 1991), and most species received contradictory taxonomic treatments. For example, *G. foetida* was regarded as a distinct species by Velenovský (1922, 1934), Svrček (1976), and Garnweidner et al. (1991), whereas it was synonymized with *G. vulcanalis* by Kotlaba (1969); *Geopyxis vulcanalis* was accepted as a species with short stipe on unburned ground (Seaver 1928, 1942, 1961; Larsen and Denison 1978; Thind and Kaushal 1981; Huhtinen 1984; Perry et al. 2007; Hansen et al. 2013), but Rifai (1968) considered the type of *G. vulcanalis*, with distinctly stipitate apothecia, as only a form of *G. carbonaria*. Morphological descriptions under the same name (e.g. *G. majalis*) in different literature appear to refer to different species (Grelet 1937, Eckblad 1968, Rifai 1968, Engel and Hanff 1984, Raymundo et al. 2012). Delimitations among the orange-colored species, such as *G. alpina*, *G. majalis*, and *G. rehmii* need to be clarified (Garnweidner et al. 1991). The number of species of *Geopyxis* is still unclear due to the unsolved taxonomic puzzles.

Using sampling from Asia, Europe, and North America, four-locus DNA data, morphology, and comparative species delimitation methods including genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al. 2000, Dettman et al. 2003) and a coalescent-based Bayesian species delimitation method (implemented in the program BPP) (Yang and Rannala 2010), we aim to: (i) uncover the genetic diversity within *Geopyxis*; (ii) solve the real identities of the species in *Geopyxis*, especially the debatable *G. foetida*, *G. majalis*, and *G. vulcanalis*; and (iii) settle the generic limits. To achieve this, several types or other original material were sequenced and/or morphologically studied. The results suggested a dilemma in the generic delimitation, unexpected but inevitable synonyms of currently used names, presence of cryptic species, and a geographically wide distribution pattern.

MATERIALS AND METHODS

Taxon and gene sampling.—A total of 74 collections of *Geopyxis* from 13 European, two North American, and four Asian countries were studied morphologically, including type or original material of *G. foetida*, *G. korfii*, *Humaria delectans* Starbäck, *G. majalis*, and *G. vulcanalis*. To obtain an estimate of the genetic diversity within *Geopyxis*, we generated 56 nuc rDNA ITS1-5.8S-ITS2 (ITS) and 48 nuc 28S rDNA (28S) sequences (the 28S of the remaining eight samples were obtained by us previously [Perry et al. 2007, Hansen et al. 2013]). The sampling aimed to cover the morphological variation and geographical distribution of *Geopyxis* and to include samples from both burned and unburned ground. In addition, we retrieved 25 ITS and 27 28S sequences of *Geopyxis* from GenBank and UNITE (total of 80 ITS and 75 28S sequences), including 29 sequences from 16 environmental, endophytic, and endolichenic samples. These samples were selected based on the following three criteria: (i) both ITS and 28S sequences were available; (ii) the sequences differed from the sequences obtained by us or; (iii) they covered additional ecological niches and/or biogeographical areas. One unpublished GenBank ITS sequence (HQ641122) was included in our dataset because of the Chinese origin that broadened our geographical sampling. Two protein-coding genes, translation elongation factor (*tef1*) and the second largest subunit of RNA polymerase II (*rpb2*), were sequenced for 23 of the 56 samples. These sequences, plus two sequences from Hansen et al. (2013), represent the full range of phylogenetic diversity displayed by ITS and 28S. In addition, ITS, 28S, *tef1*, and/or *rpb2* were sequenced for four semi-hypogeous to hypogeous taxa, *Hydnocystis* aff. *japonica* (Kobayasi) Trappe, *P. pila*, *S. bombycina*, and *S. shanorii* (Gilkey) Gilkey that have been suggested to be closely related to *Geopyxis* on the basis of phylogenetic analysis of 28S data (Læssøe and Hansen 2007, Perry et al. 2007, Alvarado et al. 2011). To further explore the generic boundaries of *Geopyxis*, we included four species of *Tarzetta* (Hansen et al. 2013).

For recognizing species based on genealogical concordance of ITS, 28S, *rpb2*, and *tef1* we included only our own samples, because none of the *Geopyxis* samples in GenBank had sequences of all four loci. To avoid noise caused by potential unverified sites in the sequences retrieved from GenBank, we also used only our own samples for the BPP analyses. The samples sequenced by us covered the range of generic variation within *Geopyxis*, except for the small variation represented by four environmental samples (AEA11305, AEA11277, AZ0755, AZ0439). For the BPP analyses, we used all samples of the core group of *Geopyxis* (i.e. exempting *G. delectans*) but excluded those of *Stephensia* and *Hydnocystis*. The three hypogeous species were excluded because our species delimitation model, a prior for BPP analysis (Yang and Rannala 2010), is based on the outcome of GCPSR (see farther under *Species delimitation analyses*), and the hypogeous species could not be delimited by GCPSR because too few samples were available with all four-gene regions.

Morphological methods.—Macroscopic descriptions are based on our own or other collectors' field observations. Microscopic measurements and descriptions are based on dried material unless otherwise stated. Color codes are from Kornerup and Wanscher (1961). For observation of spores, a small piece of an apothecium with well-developed hymenium was dipped into a drop of water on a slide to release mature spores. The slide was then kept in a moist Petri dish for 2–4 h. Twenty spores were measured in water from one apothecium of each collection. The measurements (and Q values) are given as MIN–*MEANmin*–*MEANmax*–MAX, in which MIN = the lowest value of all spores measured; *MEANmin* = the lowest mean value for the measured collections; *MEANmax* = the highest mean value for the measured collections; and MAX = the highest value of all spores measured. Q value = the ratio of length and width, and n refers to the total number of spores measured. Structural features of the excipulum were studied using vertical, median sections made by hand.

Sections were rehydrated in water for at least 4 h. Measurements and descriptions were made on material mounted in water. When cells did not fully recover, 5% or 10% KOH was added after water. Melzer's reagent (MLZ) and 10% KOH were added to water mounts to observe reactions of exudates or other pigmentation. Cotton Blue in lactic acid was used to observe spore ornamentation. Microscopical observations were made with a Nikon 80i microscope using both bright field and Nomarski Differential Interference Contrast (DIC). Photos were taken with a Nikon Digital Sight DS-Fi1 Camera. Unless otherwise indicated in the legends, the photographs presented in this paper were taken by X.H. Wang and K. Hansen. The exclamation point indicates that type or other original material was examined by us.

DNA extraction, amplification, and sequencing.—DNA was extracted from dried apothecia as outlined in Hansen et al. (1999), except that the material was not ground in liquid nitrogen but shaken in a cell disruptor (MINI-BEADBEATER™, BioSpec Products, Bartlesville, Oklahoma) for 20 s at 4600 rpm. For the five types or original specimens that were more than 90 y old, DNA was extracted following a protocol outlined by Stielow et al. (2013). A piece of dried apothecium was shaken in the cell disruptor for 20 s at 4200 rpm. After 24 h of homogenization and subsequent 48 h precipitation, the supernatant was processed from step 12 in the E.Z.N.A.® Forensic DNA Kit (D3591-01) (standard protocol). The last washing step was performed four times with 50 µl Elution Buffer. Primers ITS1 or ITS5 and ITS4, and LR0R and LR5 were used to amplify the ITS and 28S, respectively. For older material, when PCR failed or products were weak, internal primers 5.8S and ITS3 were used with ITS5 and ITS4, respectively, to amplify the ITS-1 and ITS-2 regions separately. The following program was used for PCR amplification of ITS and 28S: 5 min at 94 C, 35 cycles of 30 s at 94 C, 30 s at 60 C, and 90 s at 72 C and a final extension of 72 C for 7 min. The annealing temperature was decreased to 55 C when PCR failed or showed weak bands. For amplification of ITS-1 and ITS-2 in separate pieces, a slightly modified program was used: 5 min at 94 C, 35 cycles of 30 s at 94 C, 30 s at 55 C, and 50 s at 72 C, and a final extension of 72 C for 7 min. PCR primers and programs of *rpb2* and *tef1* followed Hansen et al. (2013), except that we only sequenced 5–7 regions of *rpb2* in this study. For old or problematic samples where the 6–7 regions did not successfully amplify, newly designed internal *Geopyxis* specific primers, Geopyxis-MR (5′-TCTCGGTCTCTGATGTC-3′) and Geopyxis-MF (5′-AGCCATGAAGTRAGTGTTATCC-3′), were used with the general *rpb2* primer 6F and fungal specific *rpb2* primer fRPB2-7CR (Liu et al. 1999), respectively, to amplify the 6–7 regions in two parts. PCR products (22 µl) were either purified using 5.5 µl mixture of FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (4:1) (Thermo Fisher Scientific, Waltham, Massachusetts), or QIAquick Gel Extraction Kit (Qiagen, Valencia, California) when there were multiple bands, by following the manufacturers' instructions. The sequencing reactions were performed with BigDye™ Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). The sequencing reactions were then purified with DyeEX 96 Kit (Qiagen, Valencia, California) and run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

Alignments and phylogenetic analyses.—Sequences were edited and assembled with Sequencher 4.10.1 (Gene Codes, Ann Arbor, Michigan) and are deposited in GenBank as accession Nos. KU932420–KU932581 (SUPPLEMENTARY TABLE I). One dataset with all 86 ITS and 79 28S sequences of *Geopyxis*, *Hydnocystis*, *Paurocotylis*, and *Stephensia* (except for the divergent *S. shanorii*) was analyzed. This dataset was used to aid in species identification and demonstrate the ecological diversity and geographical distributions of *Geopyxis*. The ITS region contained highly divergent regions, within which only certain subsets of the taxa or populations could be aligned. Therefore, parts of the final dataset were interleaved in alternating blocks of aligned complete sequences and aligned partial sequences. For an improved alignment, no outgroup was included in the analyses of this dataset. Four other datasets were prepared for single and/or combined analyses: a reduced ITS with 28 sequences (only our sequences of *Geopyxis*), 28S with 38 sequences, *rpb2* with 35 sequences (with *S. bombycina*, *H. piligera*, and *P. pila* missing compared with the 28S dataset), and *tef1* with 36 sequences (with *H. piligera* and *T. catinus* [Holmsk.] Korf & J.K. Rogers missing compared with the 28S dataset). These four datasets were used to delimit species, and the 28S, *rpb2*, and *tef1* datasets were used to construct the phylogeny of *Geopyxis* and/or investigate the generic limits. An additional hypogeous species, *S. shanorii*, and four species of *Tarzetta* were included in the 28S, *rpb2*, and *tef1* datasets due to their close/unresolved relationship with *Geopyxis* (Perry et al. 2007, Hansen et al. 2013). A set of three species, *Ascodesmis nigricans*, *Pseudombrophila theioleuca*, and *Pulvinula convexella*, that are the closest relatives of the *Geopyxis* lineage (Hansen et al. 2013), was used as an outgroup for the single and combined analyses of 28S, *rpb2*, and *tef1*. No outgroup was included in the analyses of the reduced ITS dataset, and the tree was midpoint rooted. The five datasets (ITS-28S, reduced ITS, 28S, *rpb2*, *tef1*) are available from TreeBASE under S18974.

Metropolis-coupled Markov chain Monte Carlo (MCMCMC) methods as implemented in MrBayes 3.2.1 (Ronquist et al. 2012) and maximum likelihood (ML) inference as implemented in RAxML 7.2.6 (Stamatakis 2006) were conducted as described in Hansen et al. (2013). The introns in the protein-coding genes (*rpb2*, *tef1*) were highly variable between the core group of *Geopyxis* (i.e. exempting *G. delectans*) and the rest of the taxa and could not be unambiguously aligned. Therefore, the introns in taxa outside the core group of *Geopyxis* were excluded from all analyses. All gene regions were analyzed using the nucleotides. The two protein-coding genes were partitioned as: (i) first and second codon positions; (ii) third codon position; and (iii) introns. In the combined 28S-*rpb2*-*tef1* analyses, the 28S was specified as a distinct partition. Thus, the concatenated three-gene datasets were analyzed with seven partitions. Incongruences among the equivalent datasets were examined by comparing the ML bootstrap proportions (ML-BP) and Bayesian inference posterior probabilities (BI-PP) for the same set of

taxa. A conflict was assumed to be significant when two different relationships (one monophyletic and the other non-monophyletic) for the same set of taxa were both supported with ML-BP ≥75% and BI-PP ≥0.95. Trees were viewed in FigTree 1.3.1 and exported to Adobe Illustrator CS5.

Species delimitation analyses.—GCPSR was employed to investigate species limits in *Geopyxis*. To identify independent evolutionary lineages under the GCPSR, we followed two criteria based on Dettman et al. (2003): (i) genealogical concordance, the clade was present in the majority (¾) of the single-locus genealogies; and (ii) genealogical nondiscordance, the clade was well supported in at least one single-locus genealogy, as judged by both ML-BP ≥75% and BI-PP ≥0.95 and was not contradicted in any other single-locus genealogy at the same level of support. The ITS, 28S, *rpb2*, and *tef1* genealogies were visually compared to find concordance. To decide which independent evolutionary lineages represented phylogenetic species one ranking criterion was used, i.e. "exhaustive subdivision: all individuals had to be placed within a phylogenetic species" (from Dettman et al. 2003). That is, if an individual was not included in one of the lineages, we traced down the nodes of the tree from that individual collapsing clades not subtended by thick branches until all individuals were included in a clade subtended by a thick branch and recognized such clades as phylogenetic species.

To explore potential hidden species diversity within the core clade of *Geopyxis* and test the performance of GCPSR, we applied a multilocus coalescent-based Bayesian species delimitation method as implemented in BPP 3.1 (for Bayesian Phylogenetics and Phylogeography; Yang & Rannala 2010, Yang 2015). The method uses reversible-jump MCMC to sample different species delimitation models and estimate the posterior probability of each model. To perform the BPP analyses, the samples were assigned to species according to the outcome of GCPSR, except *G. alpina* s. l. was split into three pre-defined species because it showed genetic structure in all four genealogies and a congruent pattern in both substitutions and INDELs. Accordingly a seven-species delimitation model was used as a prior for the BPP analyses. The three pre-defined species, *G. alpina* s. str., *G. deceptiva*, and *G. rehmii*, are not exclusively allopatric, and thus do not merely represent three geographically separated populations; the short branches seem to suggest recent speciation. The BPP program analyzes the data under a multispecies coalescent (MSC) model that is able to distinguish species that have only very recently diverged and whose reciprocal monophyly cannot be achieved due to incomplete lineage sorting (Yang and Rannala 2010, Zhang et al. 2011). We employed BUCKy 1.4.0 (Ané et al. 2007, Larget et al. 2010) to infer a species tree, to be used as a guide-tree in the BPP analyses. BUCKy considers biological processes such as hybridization and incomplete lineage sorting, which could result in different loci having different genealogies (Larget et al. 2010). To prepare for the BUCKy analyses, we first performed a BI analysis of each of the four loci, using four simplified datasets composed of the core group of *Geopyxis* (clade A in FIGS. 2, 3), with *G. delectans* as an outgroup. The settings in these BI analyses were the same as those given above for the full datasets. BUCKy uses the complete tree files generated by the BI analyses as input files, to estimate the Concordance Factor (CF) of each clade, i.e. the proportion of loci that have the clade, to infer the relationships between the species (the primary concordance tree built from clades with the highest estimated CFs). The BUCKy analyses consisted of 1 000 000 generations, four runs, and four chains for the MCMC sampling. A CF value >75% was regarded as significant.

In the BPP framework, two parameters need to be specified: $\theta = 4N_e\mu$ and τ. A larger value of θ means a large ancestral effective population size, and a relatively large value of τ implies an early divergence. A larger θ combined with a small τ favors a conservative species model (i.e. fewer species) (Leaché et al. 2010, Yang and Rannala 2010). Unlike some former studies using BPP that have host, ecological, or geographical prior information (i.e. Hambäck et al. 2013), our work used only the genetic pattern to set prior species delimitation models. Therefore, a trial including a more conservative setting was conducted to help detect potential over-splitting. We first used the A00 analysis (Yang 2015) to evaluate θ and τ from our own data. The estimated mean ancestral θ is 0.0007–0.0014, and τ is 0.0010–0.0013. On the basis of this estimation, we used three combinations of θ and τ, under a gamma distribution prior. The first combination θ (1,10) and τ (1,10) assumes a large ancestral population size and deep divergence. The second combination θ (1,10) and τ (2,2000) is used to test the performance with the most conservative setting (fewer species). The third combination θ (2,2000) and τ (2,2000) uses the estimated θ and τ, which assume a smaller ancestral population size and more recent divergence.

Due to PCR failure, more than two-thirds of the samples are missing in our *rpb2* and *tef1* datasets. Although BPP allowed some missing sequences in some of the loci, we tried to test the effect of the number of loci on the performance of the method. To do that, we first used ITS and 28S that included all samples, then sequentially added *rpb2* and *tef1*. Each of the analysis with a different number of loci and prior combinations was run two times independently using a random seed (−1). The congruence of the two runs was compared to arrive at a conclusive result. Each analysis consisted of 50 000 MCMC generations, sampling every fifth generation and discarding 10% as burn-in. Since the gaps are informative in the pre-delimitation of species, the analyses were run with "cleandata = 0", i.e. alignment gaps and ambiguous nucleotides were used in the likelihood calculation with gaps treated as missing data. A posterior probability ≥95% was accepted as significant support for a node to be split into two species.

RESULTS

Nucleotide variation and introns.—The ITS alignment including all terminals consisted of 1076 characters (18S: 1–41, ITS1: 42–687, 5.8S: 688–837, ITS2: 838–1032, 28S: 1033–1076). The ITS region (including 5.8S) varies considerably in length between the species, from 450 bp (*G. delectans*) to 712 bp (*P. pila*). The pattern of INDELs corresponds to the species delimited by BPP, except for one insertion of 26 bp

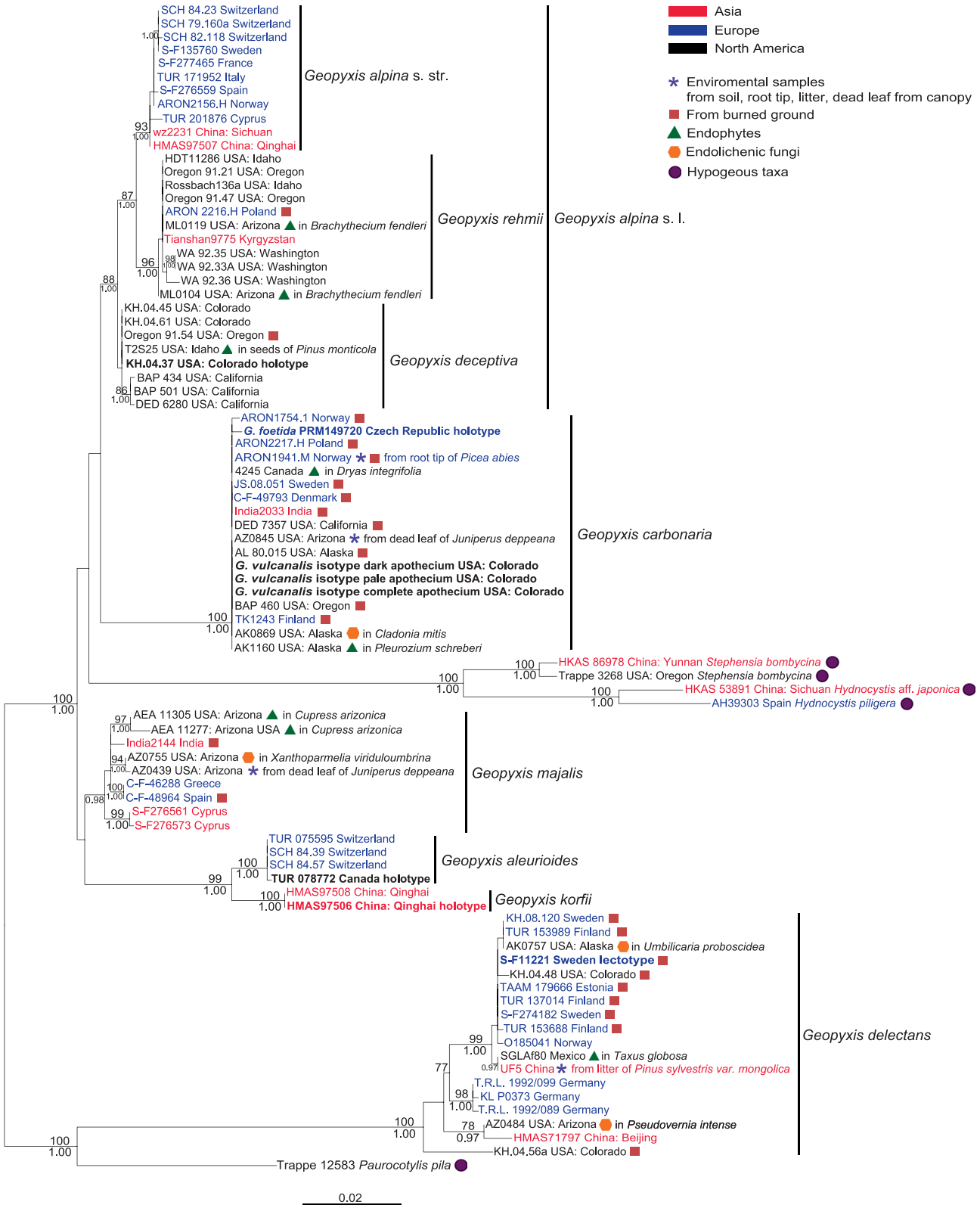

FIG. 1. Maximum likelihood phylogram of combined ITS-28S of *Geopyxis* and related taxa. Maximum likelihood bootstrap ≥70% and Bayesian posterior probabilities ≥0.95 are indicated above and below the internodes. The species names given are as delimited by a multilocus coalescent-based Bayesian species delimitation method. *Geopyxis alpina* s. l. consists of three cryptic species. All sequences are derived from ascomata, unless marked as environmental samples, endophytes, or endolichenic fungi. Type collections are shown in boldface.

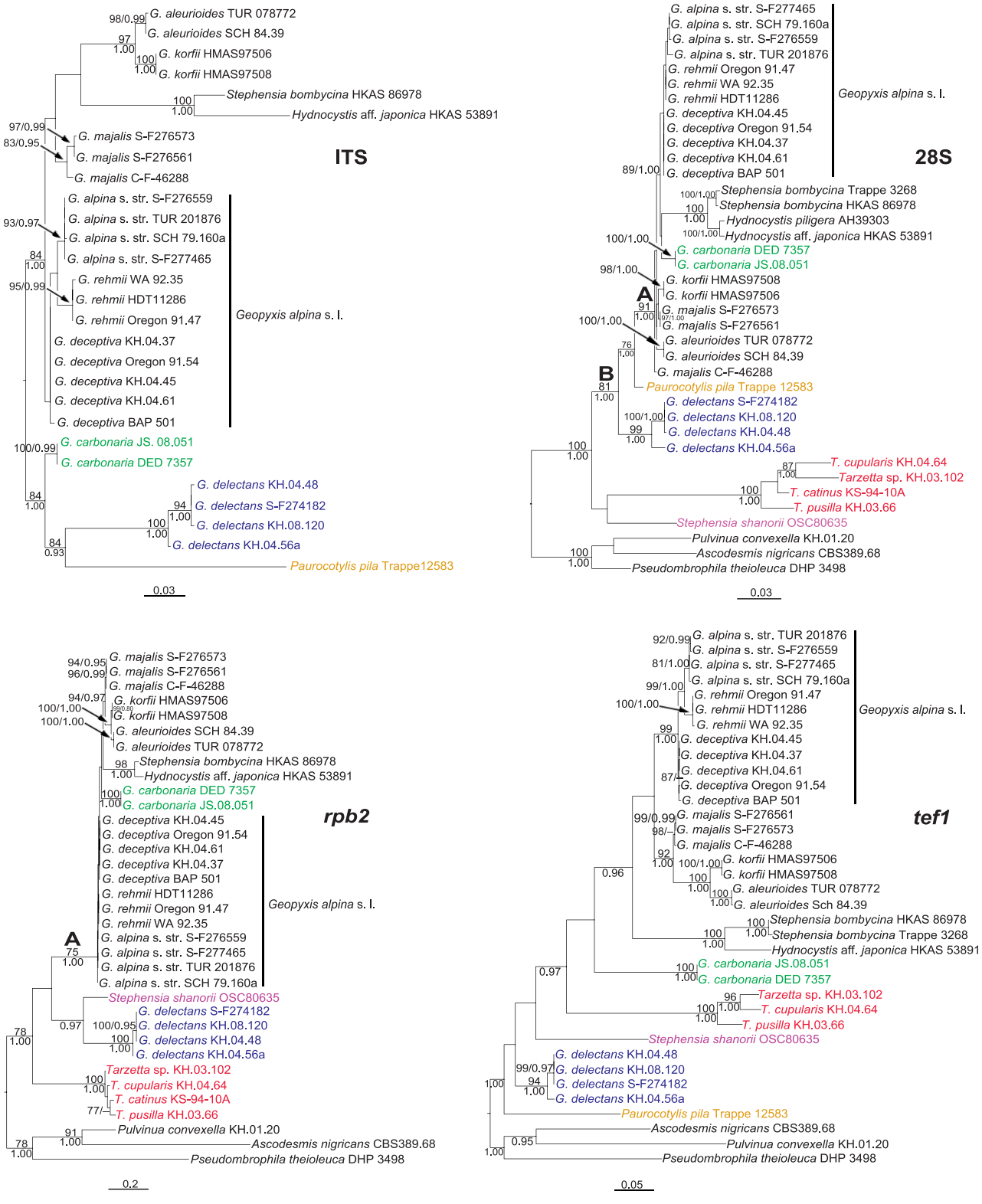

FIG. 2. Maximum likelihood phylograms of ITS, 28S, *rpb2*, and *tef1*, of *Geopyxis* and closely related taxa, with the ITS tree midpoint rooted, and the 28S, *rpb2*, and *tef1* trees rooted with *Ascodesmis nigricans*, *Pseudombrophila theioleuca*, and *Puvinula convexella*. Maximum likelihood bootstrap (ML-BP) ≥70% and Bayesian posterior probabilities (BI-PP) ≥0.95 are indicated above and below the internodes, or as ML-BP/BI-PP beside the nodes. In the online publication, taxa/clades showing supported conflict in placement between the single genealogies are highlighted in color.

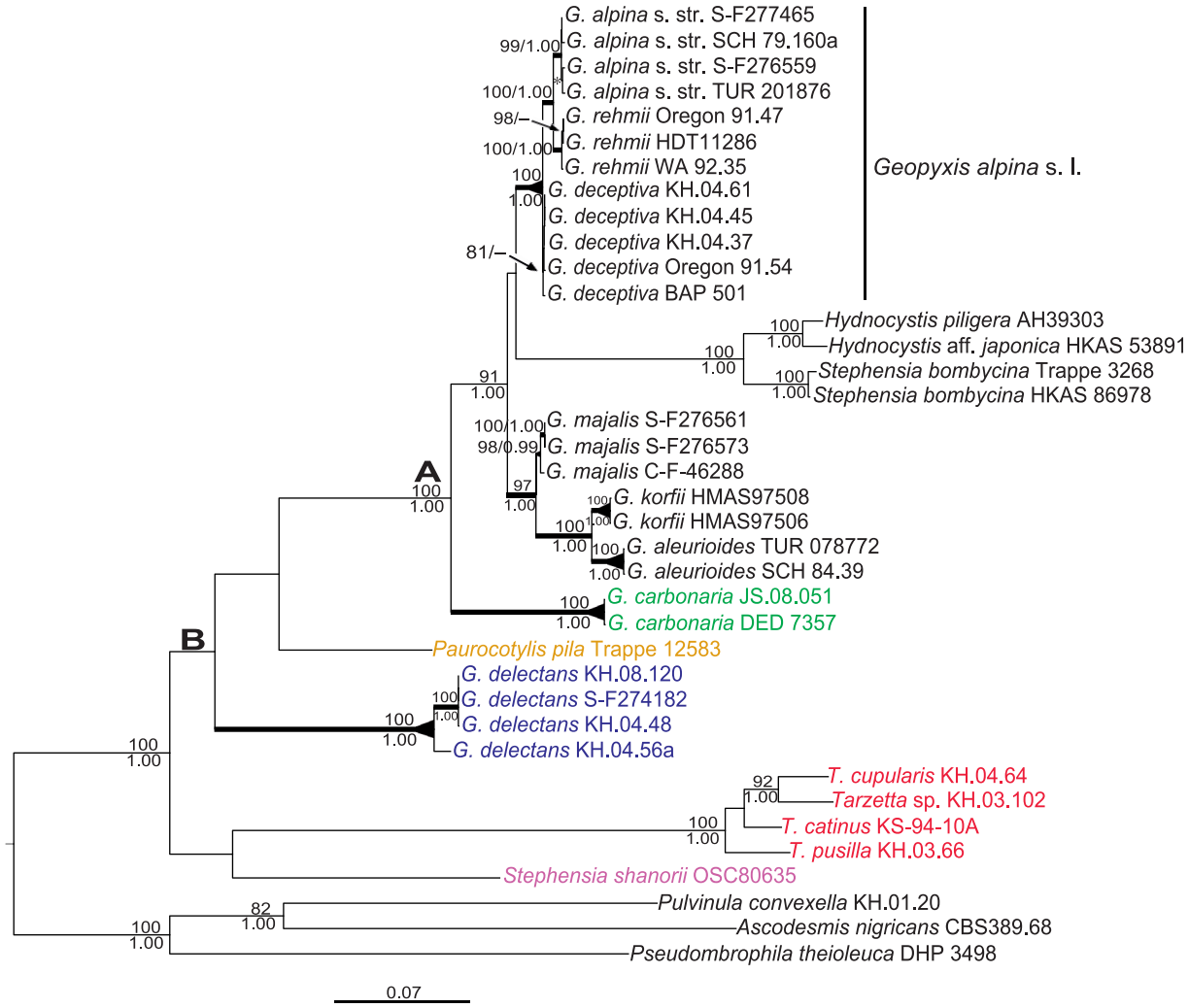

FIG. 3. Maximum likelihood phylogram of combined 28S-*rpb2*-*tef1* of *Geopyxis* and its relatives. The thick branches indicate thirteen independent lineages of *Geopyxis* (the fourteenth is shown with an asterisk) and the triangles at the nodes six species recognized by genealogical concordance phylogenetic species recognition. Support values for the nodes are as in FIG. 2. *Geopyxis alpina* s. l. comprises three cryptic species, on the basis of a coalescent-based Bayesian species delimitation method. In the online publication, taxa/clades shown in color refer to those whose placements have conflicts in different genealogies.

in three samples of *G. rehmii* (WA92.33, WA92.35, WA92.36), which is shared by *G. alpina* s. str. and *G. deceptiva* and five samples of *G. majalis* (C-F-46288, C-F-48964, S-F276561, S-F276573, India 2033). ITS-1 is more variable than ITS-2, in both length and substitutions. The 28S dataset included 833 characters, and the sequences are almost the same in length in all species.

In the datasets for combined analyses, the 28S alignment has 837 characters. The *rpb2* alignment (5–7 region) includes 1153 characters, with one 55 bp long spliceosomal intron. Among the 1098 bp exons of *rpb2,* 261 parsimony-informative characters are in the third codon position and 58 in the 1–2 codon positions. The *tef1* alignment contains 1438 characters and four spliceosomal introns, placed throughout the region and present in all samples. Their combined length is 227 bp. The first intron is located at the beginning of the sequences, 43 bp after the 3′ end of the primer 526F. Among the 1211 bp exons, 273 parsimony-informative characters are in the third codon position and 28 in the 1–2 codon positions.

Diversity and geographical distribution pattern of Geopyxis *species based on the ITS-28S phylogeny.*—Phylogenetic analyses of the most taxon-inclusive ITS-28S dataset resolved the eight species of *Geopyxis* delimited by BPP (see *Species estimation using a coalescent method* below) (FIG. 1). Six of these were highly supported by both ML-BP and BI-PP. *Geopyxis deceptiva* had no support, and *G. majalis* was highly supported only by BI-PP. *Geopyxis alpina* s. str., *G. deceptiva*, *G. delectans*,

G. majalis, and *G. rehmii* contain one or more small subclades that were strongly supported by ML-BP and/or BI-PP. Four samples of the hypogeous *Stephensia* and *Hydnocystis* formed a highly supported clade (ML-BP 100%, BI-PP 1.00), and *P. pila* constituted a separate branch as sister to *G. delectans*. Six of the eight *Geopyxis* species included samples from more than one continent, among which *G. carbonaria*, *G. delectans*, *G. majalis*, and *G. rehmii* included samples from Asia, North America, and Europe. *Geopyxis deceptiva* and *G. korfii* included samples only from western North America and northwestern China, respectively. Five of the eight *Geopyxis* species, i.e. *G. carbonaria*, *G. deceptiva*, *G. delectans*, *G. majalis*, and *G. rehmii* included sequences from both apothecia and cultures derived from living plants or lichens, i.e. endophytes or endolichenic fungi. The endophytic, endolichenic, and environmental sequences, obtained from GenBank, are here identified or re-identified to species (FIG. 1, SUPPLEMENTARY TABLE I; see farther under DISCUSSION).

Phylogeny of Geopyxis.—Supported conflict was detected between the individual gene phylogenies (28S, *rpb2*, and *tef1*) for combined analyses in terms of relationships among the species and in the generic limits of *Geopyxis*. In BI and ML analyses of the 28S and *rpb2* datasets, *G. carbonaria*, the type species of *Geopyxis*, formed a strongly supported monophyletic group with most other species of *Geopyxis* (exempting *G. delectans*) and species of *Hydnocystis* and *Stephensia* (28S: ML-BP 91%, BI-PP 1.00; *rpb2:* ML-BP 75%, BI-PP 1.00) (clade A in FIG. 2). *Geopyxis delectans* and *Tarzetta* were resolved (with or without *S. shanorii*) as successive sister groups to the rest of *Geopyxis* in these phylogenies, and strongly supported by 28S (ML-BP 81%, BI-PP 1.00). The placement of *S. shanorii* is in conflict in the 28S and *rpb2* phylogenies. It is strongly excluded from *Geopyxis* (clade B in FIG. 2) in the 28S phylogeny (ML-BI 81%, BI-PP 1.00), while forming a supported monophyletic group with *G. delectans* in the *rpb2* phylogeny (BI-PP 0.97). In contrast to the 28S and *rpb2* phylogenies, species of *Tarzetta* grouped with *Geopyxis* in Bayesian analyses of *tef1* (BI-PP 0.97); *Geopyxis delectans*, *S. shanorii*, and *P. pila* were resolved as a sister group to the rest of the ingroup, although without support (SUPPLEMENTARY FIG. 1). The relationships among *Tarzetta*, *G. carbonaria*, and the rest of the core group of *Geopyxis* and hypogeous taxa (clade B) were, however, without support in the *tef1* phylogeny. In the reduced ITS phylogeny (FIG. 2), *G. carbonaria* formed a strongly supported group with *G. delectans* and *P. pila* (ML-BP 84%, BI-PP 1.00), as sister to the rest of *Geopyxis*, *Stephensia*, and *Hydnocystis*.

Despite the conflict among the individual gene phylogenies, analyses were conducted on the combined three-gene dataset, 28S, *rpb2*, and *tef1*, to explore total evidence (e.g. reviewed by Huelsenbeck et al. 1996). Although Bayesian and ML analyses produced an identical, highly resolved phylogeny, critical deeper branches were without support (FIG. 3). A core group of *Geopyxis*, exempting *G. delectans*, is highly supported (ML-BP 100%, BI-PP 1.00), with species of *Hydnocystis* and *Stephensia* nested within it (clade A). *Paurocotylis pila*, *G. delectans*, and *Tarzetta-S. shanorii* formed three successive sister lineages, but their relationships are without support. The localized conflicts in the placement of *S. shanorii*, *G. delectans*, and *Tarzetta* appear to obstruct support for the deeper nodes in the phylogeny. Conversely, the three genes together contributed strong support for several subclades, i.e. *G. aleurioides-G. korfii* and *G. majalis*; the three cryptic species within *G. alpina* s. l.; and the two *Hydnocystis* species and *S. bombycina*. These three subclades formed a highly supported group (ML-BP 91%, BI-PP 1.00).

Species recognition using GCPSR.—Fourteen independent lineages were determined for the epigeous species in *Geopyxis* by GCPSR (thick branches in FIG. 3). Six of these are inferred as species following exhaustive subdivision (marked by a triangle on the node). In the analyses of the combined 28S, *rpb2*, and *tef1* datasets, all species were supported as monophyletic by 98–100% BI-PP and ML-BP (TABLE I). All species, except *G. alpina* s. l., were strongly supported as monophyletic by ML-BP (≥80%) and BI-PP (≥95%) in at least two of the individual genealogies (TABLE I). *Geopyxis alpina* s. l. and *G. majalis* were not resolved as monophyletic in the *rpb2* and 28S genealogies, respectively (FIG. 2), but their monophyly was not strongly contradicted in any of these trees. These two species contain samples that have different ITS lengths (561–587 bp in *G. alpina* s. l. and 552–578 bp in *G. majalis*), besides several substitutions. *Geopyxis alpina* s. l. had internal phylogenetic structure, i.e. included two independent evolutionary lineages (indicated by thick branches in FIG. 3). The ITS sequence of KH.04.56a differs in 21 base pairs from the other three samples of *G. delectans*, and formed a separate, independent branch. This sample was included in *G. delectans* when using exhaustive subdivision of GCPSR.

Species tree inference using BUCKy.—The guide species tree inferred from the analysis of the four-gene Bayesian concordance implemented in BUCKy is shown (FIG. 4). The relationships among all clades are highly supported (CF ≥ 75%). Within the clade of *G. alpina* s. l, the five samples of *G. deceptiva* did not form a monophyletic group. The CF factors for this unresolved relationship were with low support (CF ≤ 61%), and not to be oversplitting, considering the identical 28S

TABLE I. Support values from the individual gene partitions and three-gene combined dataset for each species of *Geopyxis* delimited using Geological Concordance Phylogenetic Species Recognition. Limits of these species correspond to the nodes with triangles (FIG. 3). Values are shown as ML-BP/BI-PP. Missing values apply to non-monophyly

Species recognized	ITS	28S	*rpb2*	*tef1*	Combined 28S-*rpb2*-*tef1*
G. aleurioides	98/0.99	100/1.00	100/1.00	100/1.00	100/1.00
G. alpina s. l.	58/—	71/0.89	—/—	99/1.00	100/1.00
G. carbonaria	100/0.99	100/1.00	100/1.00	100/1.00	100/1.00
G. delectans	100/1.00	99/1.00	100/1.00	94/1.00	100/1.00
G. korfii	100/1.00	98/1.00	99/0.80	100/1.00	100/1.00
G. majalis	83/0.95	—/—	96/0.99	98/0.81	98/1.00

sequences and only 1–2 bp differences in ITS, *rpb2*, and *tef1*, we assigned these samples to only one species. Instead, due to the moderate CF (75%) for the relationships among the three major clades of *G. alpina*-*G. deceptiva*-*G. rehmii*, *G. aleurioides*-*G. korfii*-*G. majalis*, and *G. carbonaria* (FIG. 4), we tested three alternative guide-tree topologies in the BPP analyses: 1. ((((*G. alpina*, *G. rehmii*), *G. deceptiva*), ((*G. aleurioides*, *G. korfii*), *G. majalis*)), *G. carbonaria*); 2. ((((*G. alpina*, *G. rehmii*), *G. deceptiva*), *G. carbonaria*), ((*G. aleurioides*, *G. korfii*), *G. majalis*)); 3. (((*G. alpina*, *G. rehmii*), *G. deceptiva*), (((*G. aleurioides*, *G. korfii*), *G. majalis*), *G. carbonaria*)) (trees shown in SUPPLEMENTARY FIG. 2).

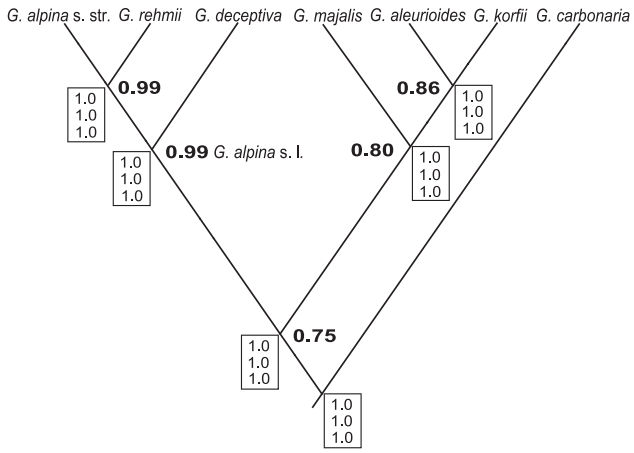

FIG. 4. Guide species tree of *Geopyxis* inferred from ITS, 28S, *tef1*, and *rpb2* sequences with BUCKy. The speciation (splitting of the node) probabilities, generated by coalescent-based Bayesian species delimitation analyses (using BPP), are provided for each node under each combination of priors for θ and τ: top, θ (1,10) and τ (1,10), assuming large ancestral population size and deep divergence; middle, θ (1,10) and τ (2,2000), testing the performance with the most conservative setting (fewer species); bottom, θ (2,2000) and τ (2,2000), which are close to the estimated θ and τ and assumes smaller ancestral population size and shallow divergence. The values outside the frame are the Concordance Factors obtained in BUCKy, standing for the support on the species relationships. *Geopyxis alpina* s. l., recognized as a single species by genealogical concordance phylogenetic species recognition, is supported as three cryptic species by BPP.

Species estimation using a coalescent method.—All BPP analyses produced congruent results. Independent of the three different guide-tree topologies used, the seven-species model received full posterior probability (FIG. 4). The three different settings on θ and τ did not affect the posterior probability for the seven-species model. The analyses of two loci (ITS, 28S) and three loci (ITS, 28S, *rpb2*) had the same outcome as using the four loci (ITS, 28S, *rpb2*, *tef1*). The pre-split of *G. alpina* s. l. as three species, *G. alpina* s. str., *G. rehmii*, and *G. deceptiva*, was fully supported. The samples of *G. alpina* s. str. are from Europe and Asia, *G. rehmii* from North America and Europe, and *G. deceptiva* from North America.

TAXONOMY

Geopyxis (Pers.) Saccardo, Syll. Fung. 8:63. 1889.

≡ *Peziza* (unranked) *Geopyxis* Pers., Mycol. Eur. 1:224. 1822.

≡ *Peziza* "tribus" *Geopyxis* (Pers.) Fr. Syst. Mycol. 2:42, 55. 1822.

Type species: Geopyxis carbonaria (Alb. & Schwein.) Sacc., selected by Boudier, Hist. Classific. Discomyc. Europe:53. 1907.

Apothecia discoid, cupulate to urnulate, 3–25 mm in diam, 2–18 mm high, fleshy, sessile to stipitate, stipe up to 14 mm long × 3 mm thick. Hymenium yellow, orange, orange-red, saffron, ochraceous buff, brownish yellow; receptacle surface concolorous with hymenium or darker, smooth or with brown warts; margin raised, crenulate, cream-colored to white. Asci cylindrical, operculate, 8-spored, hyaline, base with croziers. Spores uniseriate, ellipsoid to subfusiform, hyaline, smooth, sometimes ornamented with very fine warts or low ridges, eguttulate. Paraphyses of equal width or slightly enlarged at apices, mostly straight, when fresh with yellow to orange-red granules or guttules, when dried with dense or sparse hyaline granules. Ectal excipulum of textura angularis; cells thick-walled,

hyaline or with yellowish-brown walls. Medullary excipulum of textura intricata, hyphae hyaline or with amber-like resinous exudates, not dissolving or changing in KOH or MLZ.

Substrate: On burned or unburned ground.

Distribution: Northern Hemisphere, also reported from Australia (Rifai 1968).

Notes.—The genus was established by Saccardo (1889) to accommodate a large assemblage of species with fleshy, stipitate, cupulate, glabrous apothecia and ellipsoid, hyaline spores. Boudier (1907) delimited the genus to terricolous/pyrophilous species with large, sessile to stipitate, cupulate, glabrous apothecia; nonamyloid, operculate asci; slender paraphyses; and ellipsoid, smooth, eguttulate spores. The eight species that Boudier (1907) accepted were, however, still a mixture with only superficial resemblance. The selection of *G. carbonaria* as the type species (Boudier 1907) made it possible for later workers to apply the genus name to species surrounding the type species, following the concept of Boudier (e.g. Eckblad 1968, Rifai 1968, Svrček 1976, Dennis 1978, Thind and Kaushal 1981).

On the basis of GCPSR and Bayesian species delimitation, eight species were recognized within *Geopyxis*: *G. aleurioides*, *G. alpina* s. str., *G. carbonaria*, *G. deceptiva*, *G. delectans*, *G. korfii*, *G. majalis*, and *G. rehmii*. *Geopyxis aleurioides* and *G. deceptiva* are described as new species. *Geopyxis deceptiva* has traditionally been named as *G. vulcanalis* in North America. These taxa cover *Geopyxis* species documented in recent literature, except for the Indian *G. grandis*, which we were unable to get on loan. Below we provide a key to the species and give full descriptions for *G. aleurioides*, *G. carbonaria*, *G. deceptiva*, *G. delectans*, and *G. majalis*. *Geopyxis alpina*, *G. deceptiva*, and *G. rehmii* cannot be distinguished from each other morphologically and are keyed out as one entry. Notes are given for *G. alpina*, *G. rehmii*, and *G. korfii*.

KEY TO SPECIES OF *GEOPYXIS*

1. Apothecia almost exclusively on burned ground, clearly stipitate, often with long stipes, or sessile, broadly attached to the substrate; spore length mostly <15 µm (mean length ≤ 14.6 µm) 2
1. Apothecia on burned or unburned ground, short stipitate, often with stipe partly or fully buried in the substrate, or sessile, narrowly attached to the substrate; spore length mostly >15 µm (mean length ≥15.5 µm) 3
   2. Apothecia most often clearly stipitate (stipe 1–14 mm), cupulate or urnulate, ochraceous, brown, brownish orange, rarely light orange, receptacle surface smooth; spores mostly subfusiform to fusiform; medullary excipulum with abundant amber-like yellowish brown resinous exudates . . . . . *G. carbonaria*
   2. Apothecia sessile with base broadly attached to the substrate, often discoid, reddish orange, receptacle surface with small brown warts; spores ellipsoid or subcylindrical with rounded ends; medullary excipulum with only few scattered amber-like yellowish brown resinous exudates . . . . . *G. delectans*
3. Apothecia delicate, thin-fleshed; medullary excipulum rich in pigmented resinous exudates; spores ellipsoid to broadly subfusiform . . . . *G. alpina*, *G. deceptiva*, *G. rehmii*
3. Apothecia more robust, thick-fleshed; medullary excipulum with few pigmented resinous exudates; spores broadly subfusiform to fusiform 4
   4. Ectal excipulum often with golden resinous exudates on the hyphal walls . . . . . *G. majalis*
   4. Ectal excipulum lacking golden resinous exudates . . 5
5. Apothecia bright orange, orange-red; spores mostly fusiform, mean Q value = 1.99 . . . . . *G. aleurioides*
5. Apothecia yellow, pale orange; spores slightly broader subfusiform, mean Q value = 1.73 . . . . . *G. korfii*

Geopyxis aleurioides K. Hansen & Huhtinen, sp. nov. FIGS. 5f, j, 6a

MycoBank MB817840; ITS barcode GenBank: KU932478

Typification: CANADA. MANITOBA: Churchill, 100 m south of Northern Studies Center, on sandy-gravelly roadside, 24 Aug 1981, *S. Huhtinen 81/125* (**holotype** TUR, **isotype** S) !

Etymology: Named after the strong resemblance to species of the genus *Aleuria* in the apothecia shape and color.

Apothecia gregarious, densely clustered, urnulate to cupulate, sometimes becoming strongly flattened, discoid, partly deformed by mutual pressure, 4–17 mm in diam, up to 8 mm high when dried, thick-fleshed, sessile or short stipitate, narrowly attached to the substrate; stipe short, clearly defined, up to ca. 5 mm long and 2 mm thick, completely buried in substrate; margin distinctly raised, often cracking into lobes, crenulate, paler. *Hymenium* red-brown, apricot, or intensely reddish orange. Receptacle surface concolorous or slightly darker, below paler, smooth.

Asci cylindrical, 8-spored, 200–230 × 10–13 µm, hyaline, base with croziers. Spores 13.5–15.7–16.9–18.5 × 7.0–8.2–8.4–9.0 µm (Q = 1.68–1.92–2.01–2.34) (n = 80), uniseriate, subfusiform, more often fusiform, hyaline, smooth, without guttules. Paraphyses mostly straight, of equal width, some slightly enlarged toward apices, rarely deformed at apices, 1.5–3 µm broad at the base and middle part, 2.5–3.5(–4.0) µm broad at apices, hyaline, with small slightly or moderately refractive guttules and granules at the middle-upper parts. Ectal excipulum of textura angularis, mostly not clearly differentiated from the medullary excipulum, 50–90 µm thick, of 3–8 cells layers, cells 10–30 × 10–25 µm,

subglobose, ellipsoid, rarely pyriform, short cylindrical or irregularly swollen, more isodiametric near medullary excipulum, irregularly orientated or more or less perpendicular to surface, some adjacent to the medullary excipulum parallel with surface, walls 1–2 µm thick, more thick-walled and rounded toward outer surface, hyaline to pale yellowish brown, some outermost single cells projecting. Medullary excipulum of textura intricata, (200–)250–350(–380) µm thick, hyphae compactly interwoven, 3–6 µm in diam, cylindrical, some hyphae inflated up to 6–10 µm, sausage-shaped, more or less parallel with surface, mostly hyaline, some forming yellowish brown bundles, in KOH these hyphae separate, more yellowish brown underneath the subhymenium; amber-like yellowish brown resinous exudates very scattered to absent, when present often coating the septa, not changing in KOH or MLZ.

Substrate: On unburned ground.

Other specimens examined: SWITZERLAND. GRAUBÜNDEN: S-Charl, on sandy roadside, 29 Aug 1984, *E. Müller & T. Schumacher* (TUR 075595); S-Charl, toward Val Tavrü and Alp Tavrü, near the bridge, alt. 2000 m, on soil with mosses, 29 Aug 1984, *H. Dissing, SCH 84.39* (C-F-57373); S-Charl, Val Sesvanna, near Alp Sesvanna, near the bridge, alt. 2300–2400 m, under *Pinus,* along path, 31 Aug 1984, *H. Dissing, SCH 84.57* (C-F-57391).

Notes.—This new species was provisionally reported as *Geopyxis* cf. *vulcanalis* (Huhtinen 1984). Based on our multigene analyses, it is closely related to *G. korfii*, which is known only from northwestern China (Qinghai Province). Both species have rather fleshy, sessile, or very short stipitate, cupulate to discoid apothecia, often growing in dense clusters (Huhtinen 1984, Zhuang and Liu 2006), but *G. aleurioides* differs in the bright orange to reddish orange hymenium, compared to the yellow, pale orange to pinkish yellow hymenium in *G. korfii*. Both species have only very few amber-like resinous exudates in the medullary excipulum. Spores of *G. aleurioides* (Q mean value = 1.9–2.0) are slightly narrower than those of *G. korfii* (Q mean value = 1.73–1.74) (FIGS. 6a, i, 7). *Geopyxis aleurioides* is similar to *G. majalis* in the tapering spores, but *G. majalis* generally has a thinner medullary excipulum and golden yellowish brown resinous exudates in the ectal excipulum. *Geopyxis aleurioides* is recognized as a distinct species both by GCPSR and BPP analysis.

Geopyxis alpina Höhn., Ann. Mycol. 3:556. 1906 (1905). FIGS. 5a, i, 6b

= *Geopyxis flavidula* Velen., Monogr. Discom. Bohem. 1:338. 1934.

Typification: No type designated. AUSTRIA. LOWER AUSTRIA: at the mountain pass Krummbachsattel (1400 m) by the mountain Schneeberg, on naked, humus poor (limestone) soil (material not examined).

Typification: CZECH REPUBLIC. CENTRAL BOHEMIA: Prague, in the park Stromovka (= Královská obora), on moist ground, Jun 1924, *O. Zvěřinová* (**lectotype** PRM 149241, designated by Svrček [1976]).

Other specimens examined: CHINA. QINGHAI: Minhe, Xigou, alt. 2600 m, 18 Aug 2004, *W.Y. Zhuang & C. Y. Liu 5231* (HMAS 97507, as *G. korfii* in Zhuang and Liu [2006]); SICHUAN: border between Maerkang and Xiaojin, Mengbi Mountains, near Yakou, on grassy meadow on south side of ridge, 31°42′22″N, 102°18′50″E, alt. 3950–4150 m, 6 Sep 1997, *D. Hibbett & Z. Wang wz2211* (FH); border between Maerkang and Lixian, near Shanjiaoba, along ridge on west side of highway, 31°51′1″N, 102°40′13″E, alt. 4100–4350 m, in alpine meadows and *Rhododendron* thickets, 8 Sep 1997, *D. Hibbett & Z. Wang wz2231* (FH); Daofu, Geka, near Geka village, 30°51′1″N, 101°16′36″E, alt. 3490–3550 m, in mixed forest of *Picea*, *Abies*, and *Quercus* and adjacent grazed meadows, 30 Aug 1997, *D. Hibbett & Z. Wang wz2188* (FH). CYPRUS: west part of Troodos Range, Pafos Forest, Tripolis Nature Reserve, Cedar Valley, ca. 1.5 km southeast of Tripolis Hill, alt. 1000 m, in bank of a seasonally dry brook, in old forest of *Cedrus libanii* ssp. *brevifolia* and smaller deciduous bushes, 8 Apr 2014, *J. Issakainen et al.* (TUR 201876). FRANCE. FRANCHE-COMTÉ: Doubs, near the village Entre-les-Fourgs, Ruisseau de la Jougnena, 14 Jun 2009, *G. Moyne* (S-F277465, dupl. private herb. N. Van Vooren: NV 2009.06.24). ITALY. BELLUNO: Falcade, Le Buse, on litter of *Picea abies* needles, 16 Aug 2005, *E. Campo* (TUR 171952). SPAIN. BASQUE COUNTRY: Araba, San Zadornil, Nograro, on earthy calcareous slope under *Pinus sylvestris*, 27 Apr 2011, *P. Arrillaga et al.* (S-F276559, dupl. ARAN A8100350). SWEDEN. UPPLAND: Djursholm, kvart. Drott 5, under *Quercus*, greensward, 26 Sep 1948, *G. Berggren* (S-F135760). SWITZERLAND. GRAUBÜNDEN: Ramosch, 2 km southeast of Ramosch, east of the bridge near Resgia, alt. 1100 m, on moist soil among *Marchantia*, 6 Sep 1979, *H. Dissing, SCH 79.160A* (C-F-56936); same locality, 6 Sep 1979, *H. Dissing, SCH 79.167* (C-F-56944); 6 Sep 1979, *H. Dissing, SCH 79.175* (C-F-56952); Ramosch, south of the River Inn, east of the bridge near Resgia, alt. 1100 m, on silt among herbs (*Hepatica*), growing with *Smardaea purpurea*, 26 Aug 1984, *H. Dissing, SCH 84.23* (C-F-57357); Schmelboden, along road and river Landwasser to Brombänz, alt. 2263–2463 m, in soil along road, 4 Sep 1982, *I. Gamundi, SCH 82.118* (C-F-57094); S-charl, from parking area downriver from Clemgia, alt. 1750 m, on silt among grasses in moist area near the river, 8 Sep 1982, *H. Dissing, SCH 82.191* (C-F-57166).

Notes.—In the combined phylogeny of 28S, *rpb2*, and *tef1* (FIG. 3), and the species tree inferred from the three genes with BUCKy (FIG. 4), *G. alpina* s. str., *G. rehmii*, and *G. deceptiva* formed a highly supported clade. GCPSR was able to recognize only *G. alpina* s. str. and *G. rehmii* as independent evolutionary lineages, but the BPP analyses delimited three distinct species. *Geopyxis alpina* s. str. and *G. rehmii* are sister species (FIG. 4) and sympatric in

FIG. 5. Apothecia and excipulum characters of *Geopyxis* species. a–f. Apothecia. a. *G. alpina* s. str. (S-F276559). b. *G. carbonaria* (JS.08.051). c. *G. vulcanalis*, apothecium with long stipe (isotype, K). d. *G. deceptiva* (**holotype** FH). e. *G. majalis* (**isotype** K). f. *G. aleurioides*, dry apothecia (**holotype** TUR). g–l. Excipulum characters of dried material, in water. g. *G. majalis* (S-F276573), ectal excipulum showing intensely pigmented walls of the outermost cells. h. *G. carbonaria* (DED 7357, SFSU), almost colorless cells of ectal excipulum. i. medullary excipulum of *G. alpina* (SCH 82.118, C) showing yellowish amber-like exudates. j. *G. aleurioides*

Eurasia. All three species have orange apothecia that lack a well-developed stipe, broadly ellipsoid spores with more or less tapering ends, and a medullary excipulum with numerous amber-like resinous exudates (Höhnel 1905, Turnau 1984, pers obs). Morphological features of these species overlap, making them indistinguishable. The collections of *G. alpina* studied by us lacked a distinctive odor, except for the collection from Spain, which had a very unpleasant odor when bruised. This odor recalls the name *G. foetida*, originally described from Bohemia, Czech Republic (Velenovský 1922) but here synonymized with *G. carbonaria* based on our molecular data from the holotype.

We consider the species, including the three samples from the alpine regions in Switzerland (SCH 82.118, SCH 79.160a, SCH 84.23) to be *G. alpina*, because the type locality of *G. alpina* is in the Alps (Schneeberg, Austria). The species including the sample ARON 2216.H from Poland we regard as *G. rehmii*, because it was collected from the type locality of *G. rehmii* (Mount Turbacz). This sample was provided by the author of *G. rehmii*, K. Turnau (Vrålstad et al. 1998), and we assume that it represents authentic *G. rehmii*. The species, including only western North American samples, previously identified as *G. vulcanalis*, we describe as a new species *G. deceptiva*. *Geopyxis alpina* has also been reported from Slovakia (Mihál et al. 2011), Bulgaria (Dimitrova and Gyosheva 2009), Pakistan (Khalid et al. 2000), and India (Thind and Kaushal 1981). Due to the presence of the cryptic species, however, these reports need to be confirmed with molecular data.

Most of the samples examined have rather thin-fleshed apothecia. On this basis, we follow Svrćek (1976) in synonymizing yellow and thin-fleshed *G. flavidula* with *G. alpina*. Distinguishing *G. alpina* from *G. majalis* is a debatable taxonomic topic (Garnweidner et al. 1991). Careful comparison between the collections of *G. alpina* and *G. majalis* suggests four features that distinguish the two species: (i) the cells in the ectal excipulum are golden yellowish brown in *G. majalis* but almost colorless in *G. alpina*; (ii) yellowish brown amber-like resinous exudates are much more common in the medullary excipulum of *G. alpina* than in *G. majalis*; (iii) paraphyses of *G. alpina* have mostly sparse, non-refractive contents, whereas in *G. majalis* the contents are refractive and dense; and (iv) spores of *G. alpina* have more rounded poles than those of *G. majalis*. The sparse contents in the paraphyses, less pigmented cells in the ectal excipulum, and yellowish brown resinous exudates in the medullary excipulum of *G. alpina* give a less vividly colored species.

Geopyxis carbonaria (Alb. & Schwein.) Sacc. Syll. Fung. 8:71. 1889. FIGS. 5h, k, l, 6c–e

Basionym: Peziza carbonaria Alb. & Schwein., Consp. Fung.:314. 1805, tab. IV. fig. 2: Fr., Syst. Mycol. 2:62. 1822.

Typification: No type designated. GERMANY. FREE STATE OF SAXONY: seen at Quizdorf, "verlornes Wasser", Tränke, Polsbruch "ingens" (by Niesky), "uncialis", abundant on burned places in forest, on charcoal, in fall and spring (material not examined).

? = *Peziza pulchra* W.R. Gerard, Bull. Torrey Bot. Club 4:64. 1873.

= *Peziza vulcanalis* Peck in Hayden, Ann. Rep. U.S. Geol. Survey 6:792. 1873.

≡ *Geopyxis vulcanalis* (Peck) Sacc., Syll. Fung. 8:65. 1889.

Typification: USA: COLORADO. "Snake River?", in the crater of an extinct volcano, on soil, 16 Jul 1872, *J.M. Coulter*, collection communicated by C.H. Peck, ex herb. Cooke (**isotype** K[M] 181130) !

= *Geopyxis foetida* Velen., České Houby 4–5:858. 1922.

Typification: CZECH REPUBLIC: Bohemia australis, Třeboň, ad terram humidam in societate Marchantiae, Aug 1918, *R. Weinzettl* (**holotype** PRM149720) !

Apothecia gregarious to densely gregarious, urnulate, funnel-shaped, subglobose with margin incurved, cupulate, sometimes later opening with margin cracking downward into the cup in several places, shallowly cupulate, 3–25 mm in diam, 2–18 mm high, regular, sometimes becoming slightly irregular due to mutual compression, fleshy, mostly clearly stipitate, stipe up to 14 mm long, 4 mm broad, expanding gradually or abruptly into the apothecium, cylindrical, solid, sometimes totally buried in the substrate, concolourous with or paler than the receptacle surface; margin distinct, crenulate, cream-colored to whitish. Flesh pale yellow. Hymenium ochraceous, brown (6D8–6E8), brownish orange, rarely pale orange, darker when drying. Receptacle surface concolourous, smooth, slightly darker or lighter than the hymenium. Odor none or fetid.

Asci 190–230(–250) × 9–13 µm. Spores 12.0–13.5–16.5–18.0 × 6.5–7.4–9.6–11.0 µm (Q = 1.51–1.71–1.98–2.26) (n = 400), subfusiform to fusiform, rarely ellipsoid with round ends, smooth, rarely finely warted (seen on surface using ×1000 bright field and DIC),

←

(**holotype** TUR), colorless cells of ectal and medullary excipulum lacking resinous exudates. k. *G. carbonaria* (BAP 460), showing rich resinous exudates in medullary excipulum. l. *G. carbonaria* (JS.08.051), colorless cells of ectal excipulum and moderately rich resinous exudates in medullary excipulum. Bars: g–l = 20 µm. Photos: a. Ibai Olariaga. b. Juan Santos. c, e, f. Jennifer Kearey.

with aggregated granules, slightly to moderately refractive. Paraphyses of equal width or slightly enlarged toward apices, some subclavate, 1.5–3 µm in diam at the basal and middle portions, 2.5–5(–6) µm in diam at apices, straight, rarely slightly bending, rarely with a small knob near the apex, with small guttules and granules in the whole length or middle-upper portion, mostly strongly refractive, rarely slightly refractive with sparse contents, when fresh with scattered yellowish brown granules and droplets, dissolving or becoming hyaline to pale yellow in MLZ. Ectal excipulum of textura angularis, mostly (20–)30–50 µm thick, rarely up to 60–80 µm thick, (2–)3–4(–5) cells thick, regular, even in thickness, with very few cells projecting, clearly delimited from the medullary excipulum, very rarely poorly defined; outermost 1–2 cells (7–)15–30 × (7–)10–20 µm, often more thick-walled, round, and smaller than the inner cells, ellipsoid to pyriform, with wall 1–2.5 µm thick, more or less with the long axes parallel with the surface, hyaline or pale yellowish brown; inner 2–3 cell layers, cells 12–30(–50) × 10–30 µm, subglobose, elongate ellipsoid, polygonal, remaining so, or more or less hyphoid toward the medullary excipulum, more or less perpendicular to the surface, some cells with yellowish brown resinous exudates. Medullary excipulum of textura intricata, variable in thickness, (50–)120–200(–250) µm thick; hyphae loosely interwoven, rarely compactly interwoven, mostly (3–)4–7 µm in diam, cylindrical, more or less parallel with the surface, hyaline, often more yellowish brown near the subhymenium, intermixed with fusiform, pyriform or even subglobose hyphae inflating up to 10–20 µm wide, these hyphae uncommon in inner medullary excipulum, more common near ectal excipulum; amber-like resinous exudates common to numerous, as a refractive yellowish brown substance coating the hyphae, more often coating the hyphal septa, more common toward the ectal excipulum and/or subhymenium, 1–2 µm thick.

Substrate: On burned ground.

Other specimens examined: CANADA. QUÉBEC: La Verendrye Park, near Lac Roand, on soil among burned wood and moss, 11 Sep 1963, *M. Pantidou & M. E. Elliott 63-54* (C, dupl. DAOM93255). CZECH REPUBLIC. PRAGUE: Karlštejn, on burned ground, with *Peziza praetervisa* and *Flammula carbonaria*, 29 May 1965, *F. Kotlaba, Z. Pouzar, M. Svrček*, and *H. Dissing* (C-F-48912). DENMARK. EAST JUTLAND: Vester Vanned Plantage, mixed forest on sandy soil, with *Aleuria*, 13 Nov 1982, *T. Læssøe* (C-F-49793). FINLAND. PERÄPOHJANMAA: Rovaniemi, Povarivaara, on ground burned 2 y ago in *Picea* forest, 66.417N 25.828E, 25 May 2014, *T. Kekki 1243* (S, dupl. TUR). INDIA. HAMACHAL PRADESH: Narkanda, Mahasu, on burned soil in open place in coniferous forest, 14 Mar 1965, *K.S. Thind 2033* (C); Soja, Kulu, on burned soil and buried pieces of decorticated wood in coniferous forest, 19 Sep 1965, *K.S. Thind 2055* (C). JAPAN: without locality (apothecia with small pieces of charcoal at the base) (K[M] 179958, *C. Wright 149*, US North Pacific, E.E., ex herb. Berkeley, isotype of *Peziza lepida* Berk. & M.A. Curtis). NORWAY. NORDLAND: Rana, Far moen, 25 km southeast of Mo i Rana, on burned ground, 4 Sep 1973, *H. Dissing, Rana 73.038* (C-F-53897); Rana, Hammernes, Langvatnet, 15 km northeast of Mo i Rana, on calcareous burned soil, 8 Sep 1976, *T. Schumacher, Rana 76.038A* (C-F-61399). POLAND: Bialowieza, southeast of Bialystok, ca. 2 km north of Stara Bialowieza, among needles on burned place, 8 Oct 1984, *H.F. Gøtzsche, HFG 84.104* (C). SWEDEN. UPPLAND: Uppsala, Norra Lunsen Nature Reserve, burned area near Lunsentorpet, 28 Aug 2008, *J. Santos, JS.08.051* (S); Uppsala, Västland, Sätra Nature Reserve, growing on lime-rich soil in a burned clearcut coniferous forest, 23 Sep 2008, *K. Hansen & J. Santos, KH.08.136* (S). USA. ALASKA: between Anchorage and Denali National Park, on burned place, 14 Aug 1980, *P.M. Petersen & H. Dissing, AL 80.015* (C-F-56094); Fairbanks, on route to Toolik Lake, near Dalton Highway, on burned soil on a slope, growing with *Peziza* sp., 11 Aug 1996, *H. Dissing, AL 96.007* (C-F-56259); Fairbanks, Dietrick Campground, south of Brooks Range, 65°33′N, 148°53′W, on exposed roadside, 20 Aug 1996, *H. Dissing, AL 96.031* (C-F-56282); CALIFORNIA: Calaveras County, near Big Trees State Park, Darby burned area, on burned soil under conifers, alt. ca. 1200 m, 20 Apr 2002, *D.E. Desjardin, DED 7357* (SFSU); Humboldt County, Trinidad, Spruce Cove, terrestrial, on burned over soil and woody debris, *H.E. Parks 6922* (S-F136337); COLORADO: Big Elk Meadows, on large burned area (3 y old), 13 Sep 2004, *K. Hansen, KH.04.50* (FH); Roosevelt Park, on small fireplace at campground, 13 Sep 2004, *K. Hansen & V. Evenson, KH.04.51* (FH); OREGON: Deschutes County, Mount Cache, junction of NFS Roads 2068 and 2067, on soil of burn in *Abies* forest, 17 May 2003, *B.A. Perry, BAP 460* (FH).

Notes.—The identities and delimitations of *G. carbonaria* and *G. vulcanalis* have been argued for a long time. Peck (1873) originally described *G. vulcanalis* with cup "funnel-form, stipitate" and "hymenium pale orange", based on a collection from an extinct volcano. Later, Peck (1879) reported the species from burned ground and emphasized that the color "externally, is brown or ochraceous-brown, and the disc is orange or yellow-orange" and always with "a distinct stem". The funnel-shaped apothecia with a distinct stipe and the later record on burned ground suggest that *G. vulcanalis* is highly similar to *G. carbonaria*. The concept of *G. vulcanalis* changed with Seaver (1928, 1942, 1961), who applied the name to a species with stipe "up to 5 mm., but often so short that the apothecia appear sessile" and from unburned ground. Most subsequent authors have followed this interpretation (Larsen and Denison 1978, Thind and Kaushal 1981, Huhtinen 1984, probably Tylutki 1993, Perry et al. 2007, Hansen et al. 2013, Kaya et al. 2016). Groves and Hoare (1954) also separated *G. vulcanalis* from *G. carbonaria* (*G. cupularis* sensu auct.) but based on bigger spores and habitat. They did not

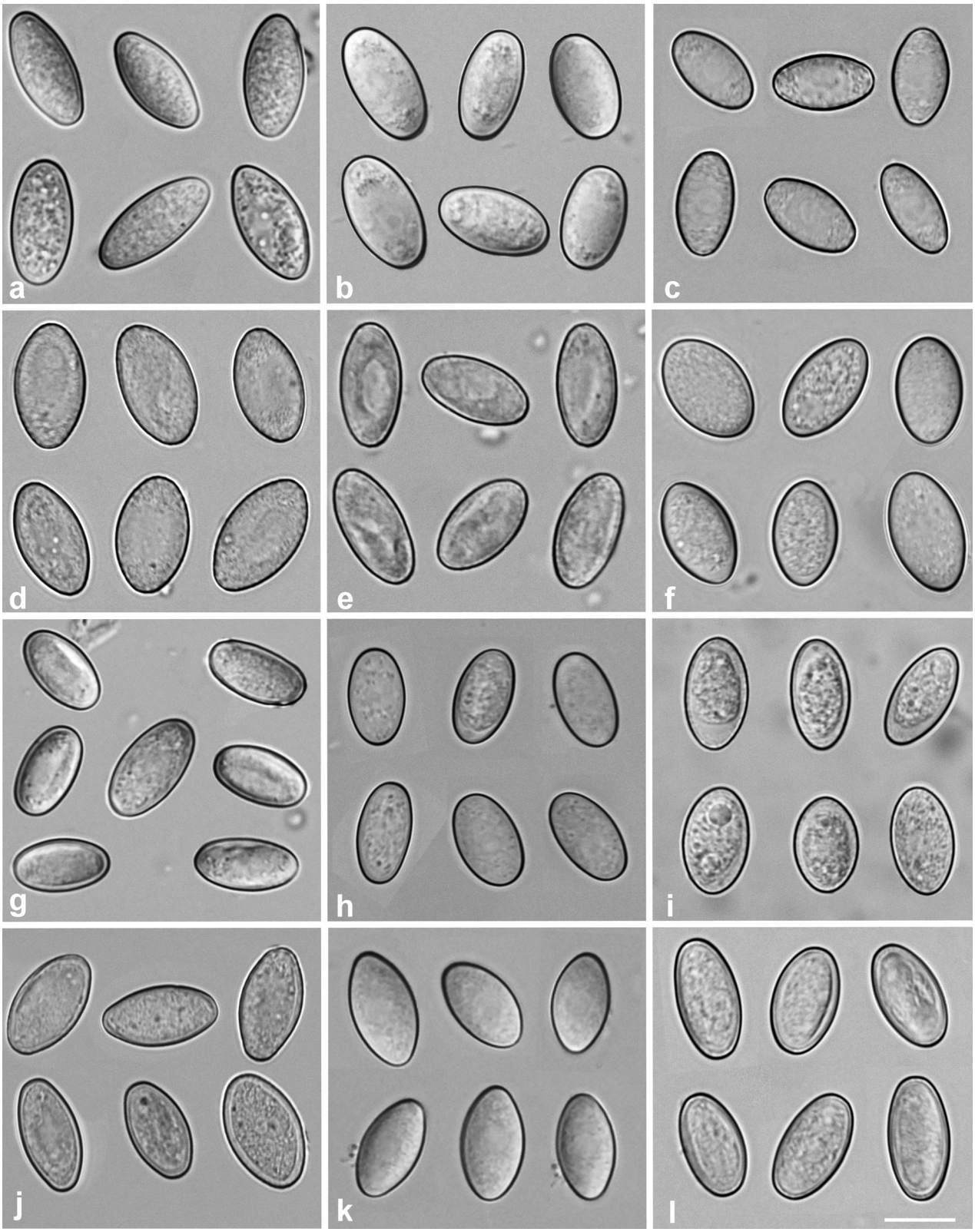

FIG. 6. Spores of *Geopyxis* species. a. *G. aleurioides* (**holotype** TUR). b. *G. alpina* s. str. (S-F276559). c. *G. carbonaria* (JS.08.051). d, e. *G. vulcanalis* (**isotype** K). d. From complete apothecium with long stipe. e. From darker apothecium. f. *G. deceptiva* (**holotype** FH). g. *G. delectans* (**lectotype** S). h. *G. delectans* (KH.04.48). i. *G. korfii* (**holotype** HMAS). j. *G. majalis* (**isotype** K). k. *G. majalis* (S-F276573). l. *G. rehmii* (HDT 11286). All from dried material, in water. Bar: a–l = 10 µm.

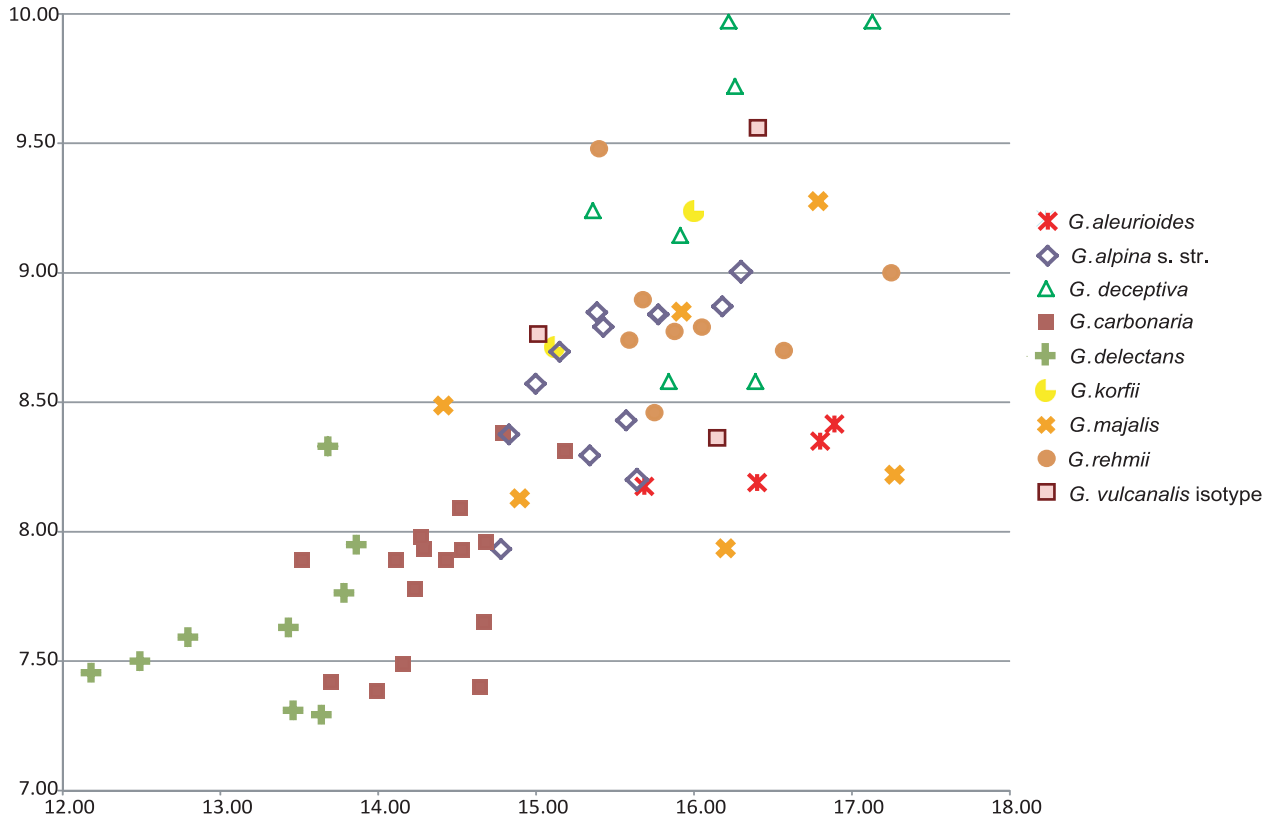

FIG. 7. Spore dimensions of eight *Geopyxis* species. Each symbol represents the average length and width based on 20 spores from one apothecium of one collection, except the spores of the isotype of *Geopyxis vulcanalis* (synonym of *G. carbonaria*) are based on 20 spores from three apothecia, respectively.

weigh color and shape of apothecia. The isotype of *G. vulcanalis* in K was already studied by Rifai (1968), who suggested that it is only a "luxuriant form" of *G. carbonaria*, differing in slightly larger spores and thicker ectal excipulum. Nevertheless, because of uncertainty in habitat and morphological variability within the species, Rifai postponed listing *G. vulcanalis* as a synonym of *G. carbonaria*, therefore, his suggestion was never followed.

The holotype in NY of *Peziza vulcanalis* is missing (L. Leonardi pers comm). The isotype (K[M] 181130) includes a complete apothecium (FIG. 5c), an almost complete cup and corresponding stipe, and broken pieces of two additional apothecia. All apothecia have a clear stipe, as also noted by Rifai (1968), from the same type. The complete apothecium is of typical appearance of *G. carbonaria*. Burned debris was found from the substrate of this specimen. Three apothecia were re-examined (all but the smallest one). The spore sizes vary among these apothecia and are larger than the other *G. carbonaria* specimens examined (FIGS. 6c–e, 7): 14.5–16.1–17.5 × 8.0–8.4–9.0 µm in the palest apothecium; 13.5–15.0–16.0 × 8.0–8.8–9.9 µm in the darker, broken apothecium; 15.0–16.5–18.0 × 8.5–9.6–11.0 µm in the complete apothecium with long stipe (FIG. 5c). The ectal excipulum of the darker and complete apothecia (70–80 µm thick) are thicker than the paler one. These observations agree with Rifai's (1968). The ITS and 28S sequences of the three apothecia are identical, except the ITS of the darker apothecium might differ in two base pairs that are degenerated. In the ITS-28S phylogeny, these three apothecia are nested within *G. carbonaria*. Therefore, we consider the morphological variations to be intraspecific and synonymize *G. vulcanalis* with *G. carbonaria*. *Geopyxis vulcanalis* sensu Seaver likely refers to *G. deceptiva* or *G. rehmii*.

Geopyxis foetida was described as a species closely related to *G. carbonaria* but certainly different in the repulsive smell (Velenovský 1922, 1934) and much larger apothecia (Velenovský 1934). Svrček (1974) restudied the holotype and observed that the spore sizes are very variable and some small spores are finely warted. He agreed with Velenovský that *G. foetida* is different from *G. alpina* and *G. carbonaria*. This was followed by Garnweidner et al. (1991), who used the name for a German *Geopyxis* collection with fetid odor and long stipe. Different from the researchers above, Kotlaba (1969) synonymized *G. foetida* with *G. vulcanalis*, probably emphasizing the stipitate apothecia. One apothecium of the holotype was loaned

from herbarium PRM for us to study. It is funnel-shaped with a distinct stipe. ITS and 28S sequences of this apothecium are identical with those of *G. carbonaria*, and it has comparable spore sizes and excipulum structure with *G. carbonaria*. A tiny piece of charcoal was found at the base of the apothecium. Synonymizing *G. foetida* with *G. carbonaria* is inevitable. We found that the isotype of *Peziza lepida* Berk. & Curtis is identical to *G. carbonaria*, in agreement with Rifai (1968). It is, however, a later homonym of *P. lepida* Pers.

Geopyxis carbonaria is variable in morphology. The color, typically yellowish brown, varies considerably between different collections and may change with environmental conditions. Pale yellow, yellowish orange, pale orange, orange, ochraceous orange, and golden-yellow hymenia have been continuously recorded for different collections that we have studied. The size of apothecia may vary and we have observed a population (KH.04.50) with very small mature apothecia (cup up to 3 mm in diam, 2.5 mm high, stipe 0.5 mm). An unpleasant odor, described for *G. foetida* (= *G. carbonaria*), has also been confirmed in three other species, i.e. *G. alpina*, *G. rehmii*, and *G. majalis*. In conclusion, three features remain available/reliable for recognizing *G. carbonaria*: clearly stipitate apothecia, an average spore length that in general does not exceed 15 μm (FIG. 7), and burned ground as the habitat. *Geopyxis carbonaria* has been reported to occur 16 to 139 wk after burning (Petersen 1970). The burned substrate may be overlooked if it burned long before the production of apothecia and if mixed with non-charred debris, as happened with the types of *G. vulcanalis* and *G. foetida*. *Peziza pulchra* could also be the same as *G. carbonaria* if any remnant of burned substrate is found with the type. *Geopyxis carbonaria* has been reported from unburned ground where the natural conditions had been disturbed and on a clay riverbank (with raised pH-values to 7.3–8.6 and $CaCO_3$ content in the surface layer; Petersen 1970, Huhtinen 1985), and one of the collections we studied (C-F-49793) was from unburned ground. Recently *G.* cf. *carbonaria* was suggested to secondarily colonize ectomycorrhiza on *Alnus* spp. formed by species of Basidiomycota (Tedersoo et al. 2009, 2010). Our analysis on their ITS-28S sequence (UDB002879) and additional *G.* cf. *carbonaria* sequences deposited by the authors in UNITE (https://unite.ut.ee/) (UDB002884, UDB02883, UD002882, UDB002881, UDB002880) found that these actually represent a species of *Tarzetta*.

Geopyxis deceptiva X.H. Wang & K. Hansen sp. nov. FIGS. 5d, 6f

MycoBank MB817841; ITS barcode GenBank: KU932466

Typification: USA. COLORADO: Boulder County, Caribou Forest Road 505, northwest of Nederland, along stream, 10 Sep 2004, *K. Hansen & V. Evenson, KH.04.37* (**holotype** FH) !

Etymology: For being deceptive in comparison with *G. alpina* and *G. rehmii*, and because mycologists were deceived to believe it represented *G. vulcanalis*.

Apothecia scattered or gregarious, deeply cupulate, cupulate to urnulate, 3–11 mm in diam, 2–9 mm high, sometimes expanding to strongly flattened, discoid, narrowly attached to the substrate, partly deformed by mutual pressure, thin-fleshed, sessile or short stipitate, stipe often buried in substrate, rarely clearly stipitate, sometimes base tapering downward, forming a tapering stipe, stipe (when present) 0.5–3 mm long, 0.5–1 mm broad, glabrous or covered with white tomentum; margin distinctly raised, often cracked, paler, cream to white, crenulate. Hymenium pale orange (5A4–5), orange to brownish yellow (5B8–5C7), grayish to brownish orange (6B–C5–6), ochraceous yellow to brownish yellow (5B–C7–8), upon drying orange to brownish orange (6B–C7). Receptacle surface concolorous or paler, smooth. Odor indistinctive, rarely unpleasant.

Asci cylindrical, 8-spored, 230–270(–300) × 10–15 (–17) μm, hyaline, base with croziers. Spores 13.5–16.3–17.1–18.5 × 7.5–8.4–10.0–11.0 μm (Q = 1.41–1.63–1.93–2.09) (n = 200), uniseriate, ellipsoid to subfusiform, rarely typically fusiform, hyaline, smooth, without guttules, moderately to strongly refractive with small granules and amorphous content. Paraphyses mostly straight, of equal width, some slightly enlarged toward apices, rarely with deformed or forking apices, 1.5–3 μm in diam at the basal and middle portions, 2–4 μm in diam at the apices, hyaline, with sparse slightly refractive granules and amorphous content in the middle-upper portions, when fresh with small hyaline vacuoles. Ectal excipulum of textura angularis, clearly differentiated from the medullary excipulum, rarely poorly defined, 30–70 μm thick, of (1–)2–5(–7) cells thick; outermost 1–2 cells 6–25 × 15–20 μm, more thick-walled and rounded than the inner cells, subglobose, ellipsoid, rarely pyriform, with walls 1–2.5 μm thick, irregularly oriented or more or less parallel with the surface, mostly hyaline, rarely pale yellowish brown, some single cells projecting, forming an uneven surface; inner 3–5 cells 10–30(–40) × 5–20 (–35) μm, irregularly swollen, subglobose, pyriform or short cylindrical, more isodiametric near medullary excipulum, more or less perpendicular to the surface, hyaline or pale yellowish brown, walls 0.5–1.5 μm thick. Medullary excipulum of textura intricata, variable in thickness between individuals, (70–)100–200(–250) μm thick, unevenly colored; hyphae compactly interwoven, 3–6 μm in diam, cylindrical, those close to ectal

excipulum more swollen, inflating up to 10–15(–20) µm, sausage-shaped, elongate ellipsoid, rarely globose, more or less parallel with the surface, mostly hyaline, some places yellowish brown with resinous exudates; amber-like resinous exudates scattered to numerous, unevenly distributed, often coating the septa, not changing in KOH or MLZ.

Substrate: Often on burned ground, rarely from unburned ground.

Other specimens examined: USA. CALIFORNIA: Sierra County, Green Acres area, Weber Lake Road, near Bassett's Station, alt. 1615 m, scattered in soil in montane coniferous woods, 7 Jun 1995, *N. Wilson, DED 6280* (SFSU); Sierra County, Highway 49, Chapman Creek Campground, gregarious in mossy soil of *Abies-Pinus* forest, 11 Jun 2002, *B.A. Perry, BAP 434* (FH); Sierra County, Wild Plum Campground, off Highway 49, near Sierra City, gregarious on soil with mosses, 5 Jun 2003, *A. Wilson, BAP 501* (FH); COLORADO: Boulder County, Caribou Forest Road 505, along brooklet, 9 Sep 2004, *K. Hansen & V. Evenson, KH.04.45* (FH); Gilpin County, Northern Gample Gulch, Perigo, 15 Sep 2004, *K. Hansen, KH.04.61* (FH); OREGON: Sahari Falls, Hood River Meadows, road down from Cloud Cap, on soil along roadside, on burned ground, 9 Jul 1991, *H. Dissing, Oregon* 91.54 (C-F-56514).

Notes.—The BPP analyses of 28S, *rpb2*, and *tef1* recognize *G. deceptiva* as a distinct species (FIG. 4). Based on our study, it is impossible to distinguish it from *G. alpina* and *G. rehmii* morphologically. The morphological description given here is based on the examined collections, all of which have been shown by molecular data to be *G. deceptiva*. Most previous reports of this species were given under the name *G. vulcanalis* sensu Seaver (1928, 1942), considered to be a North American *Geopyxis* occurring on unburned ground. The name *G. vulcanalis* has, however, been misapplied; the isotype of *G. vulcanalis* is actually *G. carbonaria* (FIG. 1). *Geopyxis deceptiva* is still reported only from North America on mostly unburned ground; a single collection was on burned ground. For additional notes, see under *G. alpina* and *G. carbonaria*.

Geopyxis delectans (Starbäck) K. Hansen & X.H. Wang comb. nov. FIGS. 6g, h, 8
MycoBank MB817843; ITS barcode GenBank: KU932498

Basionym. *Humaria delectans* Starbäck, Bot. Notiser:211. 1898.

≡ *Aleuria delectans* (Starbäck) Boud., Hist. Class. Discom. Eur. (Paris):45. 1907.

≡ *Kotlabaea delectans* (Starbäck) Svrček, Česká Mykol. 28 (3):131. 1974.

Typification: SWEDEN. UPPLAND: Knifsta, among mosses on burned ground, Aug 1895, *K. Starbäck* (S-F11221, **lectotype** designated here) !

Apothecia shallowly cupulate to expanded, constricted in the center, 8–17 mm in diam, up to 5 mm high, sessile, broadly attached to the substrate, or with stipe ca. 1.5 mm long, 1 mm broad, stipe completely buried; margin distinctly raised, incurved at very upmost rim, white, crenulate, often cracking when cup is expanding, brown when drying or in age. Hymenium bright orange (5A8, close to 6B8, 6B8), brown-orange (6C8). Receptacle surface concolourous or slightly darker (5C8, 5B7), with small brown pustules, sometimes with white mycelia at the middle and lower parts, rarely with white mycelia toward the base. Odor none.

Asci cylindrical, (165–)180–200 × 10–12 µm. Spores 11.00–12.2–13.9–15.0 × 6.0–7.3–8.3–9.5 µm (Q = 1.39–1.64–1.88–2.31) (n = 200), ellipsoid with round ends, sometimes subcylindrical, rarely with slightly narrowing ends, smooth, slightly or moderately refractive with sparse or dense granular and amorphous content. Paraphyses more or less swollen toward apices, rarely clavate or capitate, some with deformed or knobbed apices or narrowing again at upmost tips, mostly straight, rarely slightly bending, some branching, 1.5–3 µm in diam at the base and middle part, 2.5–4(–5) µm in diam at the apices, hyaline to pale yellowish, with strongly refractive small guttules and granules at the middle-upper portions, when fresh with refractive, bright yellowish orange or orange granules, turning green or olivaceous in MLZ. Ectal excipulum of textura angularis, mostly clearly differentiated from the medullary excipulum, 40–75 µm thick, of (2–)3–7 (–8) cells; outermost 1–2 cells 5–15 × 5–13 µm, more thick-walled, rounded and smaller than the inner cells, globose, subglobose, irregularly ellipsoid, walls (1–)1.5–2.5(–3) µm thick, yellowish brown, intensely colored in some parts, often with some (3–10) cells aggregated, forming pustules up to 20–40 µm high and 30–40 µm wide, those cells darker and more thick-walled than the others; inner 3–5 cells 10–30(–40) × 8–20(–30) µm, subglobose, more often elongate, polygonal, pyriform, more or less cylindrical or hyphoid near the medullary excipulum, mostly perpendicular to the surface, in some places more or less of textura prismatica, but those adjacent to the medullary excipulum more often parallel to the surface, hyaline to pale yellowish brown, not changing in MLZ. Medullary excipulum of textura intricata, (70–)100–250 µm thick; hyphae compactly interwoven, 3–5 µm in diam, cylindrical, rarely inflating up to 8–15 µm, more or less parallel with the surface, hyaline, compactly interwoven and more yellowish brown underneath the subhymenium; amber-like yellowish brown resinous exudates very scattered, more common near the ectal excipulum, often coating the septa.

Substrate: On burned ground.

Other specimens examined: FINLAND. ETELÄ-HÄME: Somero, Palikainen, Grid 27°E 67303:3192, on coal and ashes in messy loam, 7 Jul 2000, *M.L. Heinonen & P. Heinonen 578-2000* (TUR 137014); Tammela, Riihivalkama, Grid 27°E 674078:32241, on top of the ashy/sandy ground layer in burned coniferous forest, 25 Aug 1998, *M.L. Heinonen & P. Heinonen 695-1998F* (TUR 153688); Tammela, Saari, Grid 27°E 674049:32335, on rocky heath soil in burned coniferous forest, 10 Aug 1998, *M.L. Heinonen & P. Heinonen 585-1998F* (TUR 153989). NORWAY. OSLO: Bygdøy Sjöbad, Kongeskogen, on burned ground, 13 Jul 1957, *F.E. Eckblad* (O-F185041, as "*G. Majalis*" in Eckblad [1968]). SWEDEN. NORRBOTTEN: Boden, Ekopark Storklinten, Gruvberget, on burned soil, *Pinus* and *Picea* herb-rich forest with some *Betula pubescens* and *Populus tremula*, 25 Jun 2013, *S. Westerberg* (S-F274182); SÖDERMANLAND: Sorunda, Stora Vika, by limestone quarry, on burned ground, 18 Sep 2008, *K. Hansen & J. Santos, KH.08.120* (S); same locality, *JS.08.085* (S); UPPLAND: Knifsta, among mosses and grasses on burned ground, 11 Jun and 13 Jul 1895, *K. Starbäck* (Vestergren, Micromycetes Rariores Selecti No. 51, S-F66460). USA. COLORADO: Big Elk Meadows, among mosses on 3 y old burned ground, 13 Sep 2004, *K. Hansen & V. Evenson, KH.04.48* (FH); same locality, 13 Sep 2004, *K. Hansen & V. Evenson, KH.04.49* (FH); 13 Sep 2004, *K. Hansen & V. Evenson, KH.04.56a* (FH).

Notes.—When Starbäck (1898) published this species, he cited material "Legi 11.6 et 13.7.1895". This material was distributed in Vestergren, Micromycetes rariores selecti 51. Two collections from this exsiccate, in S and UPS, are both with these two dates and thus include two gatherings (with no indication of how to separate these). No part of this material can therefore be used for typification (Art. 8.2 and 9.2, ICN; McNeill et al. 2011). In S there is another collection from the type locality: "Uppsala: Knifsta", made by Starbäck, "August 1895" (S-F11221). This collection is not mentioned in the original description, but it can be regarded as original material because it was collected before the description of *H. delectans*, and it is therefore likely to be part of the material upon which Starbäck based his description (Art. 9.3 ICN). We therefore designate this collection as lectotype of *Humaria delectans*.

The morphology of the exsiccate collection S-F66460 and the lectotype are identical, except that more substrate is left with the lectotype and thus, the apothecia clearly shows the broadly attached base. The spores of the lectotype (12.0–13.2–15.0 × 6.5–7.6–8.5 μm) are smaller than those given by Starbäck (1898: 14–16 × 7.5–9 μm). The other specimens examined by us match well the original material. ITS and 28S sequences from the lectotype are identical to those from the newly collected material. *Geopyxis delectans* can be easily distinguished from most *Geopyxis* species by the broadly attached apothecial bases, brown warts on the outer surface of the receptacle (FIG. 8c, f) and smaller spores with rounded or even truncate ends (FIGS. 6h, 8e). The paraphyses have refractive, bright yellowish orange or orange granules when fresh, and in the dry material these are seen as highly refractive dense granules and droplets. Hyaline flexuous hyphae were observed in the lower part of the apothecia of four collections (JS.08.085, KH.08.120, S-F11221, S-F66460). Only in one of them (JS.08.085) could we see the hyphae originating from the outermost cells of ectal excipulum. This feature has to be studied further. *Geopyxis delectans* share the vividly orange, sessile to expanded, fleshy apothecia, and a medullary excipulum with very few amber-like resinous exudates with *G. aleurioides*, but *G. aleurioides* has much larger fusiform spores and more thick-fleshed apothecia. *Geopyxis delectans* has recently been reported from North America as *Geopyxis* sp. (Perry et al. 2007, Hansen et al. 2013) and from China as *G. majalis* (GenBank DQ458804 in Liu and Zhuang 2006). The sequence in UNITE (accession No. UDB000091) of an Estonian collection, labeled as *G. alpina*, is *G. delectans*. All of the collections studied are from burned ground, suggesting a pyrophilous species. The invalidly published *G. carbonaria* var. *sessilis* Grelet (1937) (lacking a Latin description or diagnosis) is most likely conspecific with *G. delectans* due to the sessile apothecia, smaller spores, and burned ground as its habitat.

In the phylogenetic trees (FIGS. 1, 2), *G. delectans* contains a clade of three collections from Sweden (KH.08.120, S-F274182) and USA (KH.04.48) and a sister lineage of a single collection from USA (KH.04.56a). This singleton is from the same locality as the two samples of *G. delectans*, KH.04.48 and KH.04.49, but it shows genetic divergence from the other samples of *G. delectans*, which are nearly identical. It has larger spores with somewhat tapering ends, which are reminiscent of *G. majalis* and *G. korfii*. Nevertheless, following exhaustive subdivision in GCPSR, we recognized this collection as *G. delectans*. To determine its identity fully, further sampling is needed.

Geopyxis delectans has most recently been treated in *Kotlabaea* (Svrček 1974, Benkert 2008). Phylogenetic studies in *Kotlabaea* using ITS and 28S sequences showed, however, that the genus is polyphyletic, and *K. delectans* is more closely related to species of *Geopyxis* and *Tarzetta* (Lindemann et al. 2015). In our ITS-28S phylogeny, these "*K. delectans*" sequences are nested within the clade of *G. delectans* but show several base pair differences from our other sequences (FIG. 1). *Humaria delectans* is here combined in *Geopyxis*, because it shares most morphological features with species in the core group of the genus, especially *G. aleurioides*

and *G. korfii*. See "Generic limit of *Geopyxis*" under DISCUSSION for detailed explanation.

Geopyxis korfii W.Y. Zhuang, Nova Hedwigia 83:180. 2006. FIG. 6i

Typification: CHINA. QINGHAI: Qilian, Zhamashi, alt. 2800 m, on mossy soil, 21 Aug 2004, *W.Y. Zhuang & C.Y. Liu 5468* (HMAS 97506 **holotype**) !

Other specimens examined: CHINA. QINGHAI: Qilian, Babaoxiang, alt. 2800 m, on mossy soil, 21 Aug 2004, *W.Y. Zhuang & C.Y. Liu 5439* (HMAS 97508).

Notes.—This species is still known only from the localities in Qinghai, China, given in the original description. Zhuang and Liu (2006) compared it to *G. carbonaria* and *G. rehmii*. Our four-locus data show that *G. korfii* is a well-delimited species, more closely related to *G. aleurioides* and *G. majalis*. Our examination of two specimens of *G. korfii* shows that it shares spores with tapering ends and a medullary excipulum with extremely few resinous exudates with *G. aleurioides* and *G. majalis* but in general has shorter and broader spores (13.5–15.1–16.0–17.5 × 8.0–8.7–9.2–10.0, Q = 1.49–1.73–1.74–1.94) (FIG. 7). See farther under *G. aleurioides*. The spores of *G. korfii* were shown by SEM to have an ornamentation of very fine warts and ridges (FIG. 7 in Zhuang and Liu 2006). Using DIC we saw a very fine ornamentation on the spore surface in HMAS 97508 in water but not in the holotype.

Geopyxis majalis (Fr.) Sacc., Syll. Fung. 8:72. 1889. FIGS. 5e, g, 6j, k

≡ *Peziza majalis* Fr., Nova Acta reg. Soc. Sci. Uppsal. ser. 3. 1:120. 1851 (preprint). [1855]

Typification: SWEDEN. UPPLAND: Uppsala?, *Th. M. Fries* (K[M]179960, ex herb. E. M. Fries [ex herb. Berkeley], **isotype**) !

Apothecia in groups to densely gregarious, first subglobose to cupulate, with base narrowly attached to the substrate, later expanded, 7.5–25 mm in diam, up to 15 mm high, fleshy, sessile, rarely short stipitate, stipe up to 2.5 mm long × 1.5 mm broad, expanding abruptly above into the cup; margin constricted, crenulate, pruinose, white. Hymenium pallid yellow, pale orange. Outside of receptacle pallid yellow, concolorous with the hymenium, pruinose or smooth. Odor indistinct or unpleasant.

Asci 220–260 × 10–13 (–15) µm. Spores 13.0–14.4–17.3–18.5 × 7.0–7.9–9.3–10.5 µm (Q = 1.46–1.70–2.10–2.38) (n = 120), subfusiform to fusiform, smooth, slightly refractive with sparse granular amorphous content. Paraphyses of equal width or slightly enlarged at apices, rarely with strongly deformed or knobbed apices, mostly straight, rarely slightly bending, 1.5–3 µm in diam at the base and middle portions, 2.5–4 µm in diam at the apices, hyaline, with sparse granules at the middle-upper portions and apices. Ectal excipulum of irregular textura angularis-globulosa, variable in thickness between different individuals, (30–)40–80(–100) µm thick, of (2–)3–7(–11) cells, clearly or vaguely delimited from the medullary excipulum; outermost 1–3 cells (7–)10–30(–40) × 7–25 µm, more rounded than the inner cells, globose, subglobose, irregularly ellipsoid, with wall 1–2.5(–3) µm thick, irregularly orientated, yellowish brown, some cells intensely colored with golden yellowish brown resinous exudates, in some places a few cells projecting; inner cells 10–25(–30) × 5–20(–30) µm, polygonal, rectangular, pyriform, cylindrical to hyphoid, mostly with the long axes perpendicular to the surface, in some places more of textura prismatica, hyaline to pale yellowish brown. Medullary excipulum of textura intricata, (120–)150–250(–300) µm thick; hyphae compactly interwoven, 3–5 µm in diam, cylindrical, those next to the ectal excipulum more often inflated up to 15 µm wide, in the inner medullary excipulum rarely inflating up to 7–10 µm wide, hyaline, underneath the subhymenium yellowish brown and more compactly interwoven, forming a colored zone, amber-like yellowish brown resinous exudates very scattered, more common under the subhymenium.

Substrate: On burned or unburned ground.

Other specimens examined: CYPRUS. LIMASSOL: Sfalantziotissa, under cypress trees by path side, 15 Feb 2012, *M. Loizides* (S-F276561); Pissouri, in open *Pinus brutia* forest with grassy pastures, 16 Mar 2011, *M. Loizides* (S-F276573). GREECE. Rhodos, 6 km west of Laerma, among mosses in *Pinus* forest, 12 Apr 1967, *L. Døssing* (C-F-46288); INDIA. HAMACHAL PRADESH: Chamba, Sara, on burned ground in coniferous forest, 27 Aug 1966, *K.S. Thind 2144* (C). SPAIN. Madrid, Ciudad Universitaria, on burned ground, 19 Apr 1974, *M. de la Torre & F. D. Calonge* (C-F-48964).

Notes.—Fries (1851) defined *P. majalis* as having "urceolato-campanulata" apothecia with "stipite brevi albo, margine prominulo flocculoso-crenato, disco aurantiaco". The macromorphology is followed in the illustration by Cooke (1875) based on specimens communicated by E.M. Fries. Most workers followed this concept (Grelet 1937, Garnweidner et al. 1991, Raymundo et al. 2012), except that Engel and Hanff (1984) reported a "*G. majalis*" from Germany with pale ochre hymenium and very unpleasant odor in age. The original description gave naked, moist soil (among streams) as the habitat, and the species has generally been recognized as nonpyrophilous (Grelet 1937; Huhtinen 1984; Garnweidner et al. 1991), except that Eckblad (1968) reported a collection from burned ground. We sequenced the voucher by Eckblad (O-F185041) and found that it is *G. delectans* (FIG. 1).

The isotype of *G. majalis* in K is in poor condition. The single, flattened apothecium (possible to examine;

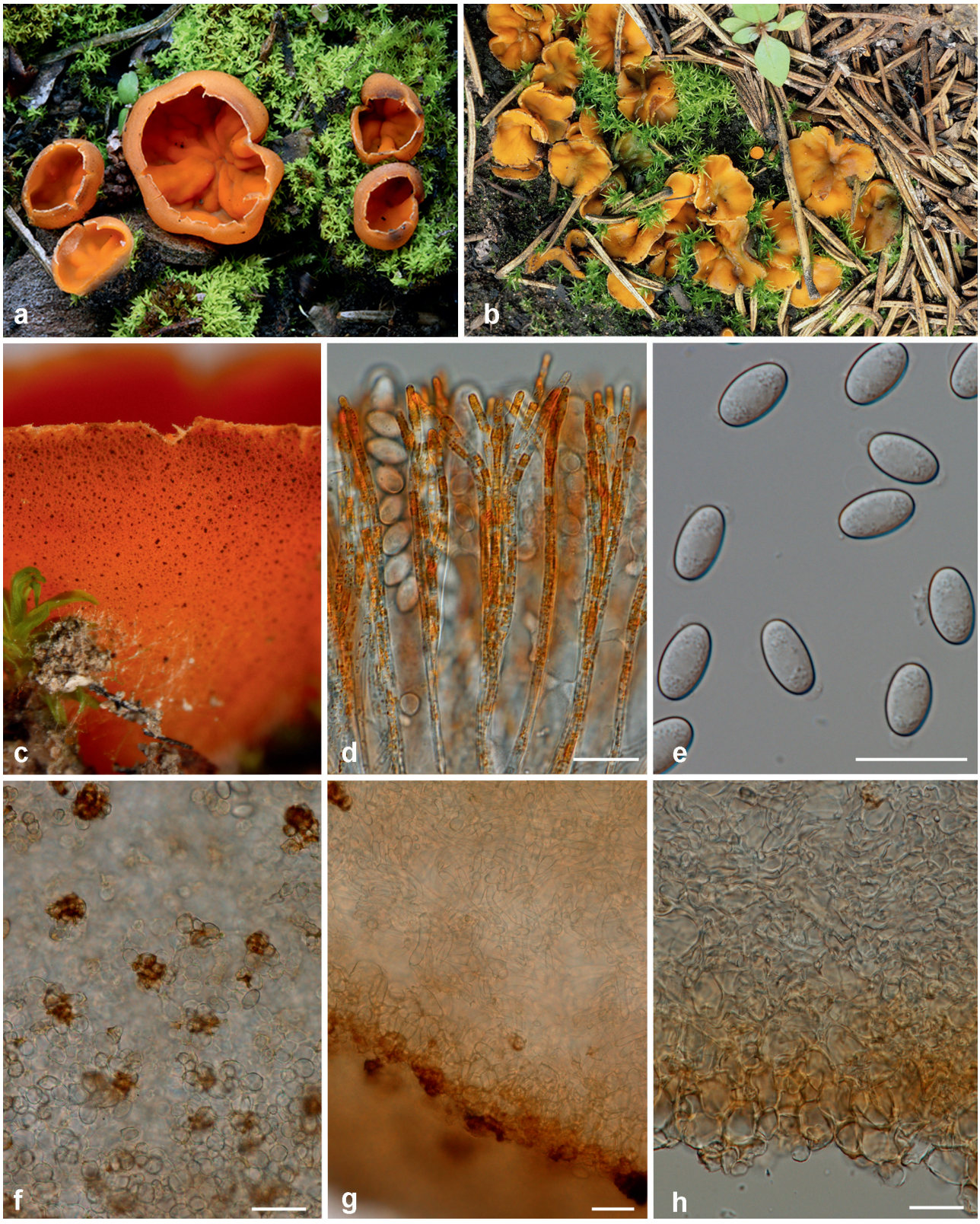

FIG. 8. *Geopyxis delectans*. a. Apothecia (JS.08.085). b. Apothecia (TUR 153989). c–h. (KH.08.120). c. Receptacle surface showing small brown pustules. d–g. Living material in water. d. Paraphyses filled with refractive, bright orange granules. e. Spores with granular content, especially toward the poles. f. Close-up of receptacle surface pustules, compiled from globose cells. g. Medullary and ectal excipulum, and outer pustules. h. Intensely pigmented cells of ectal excipulum and medullary with only scattered encrustations, dried material in water. Bars: d–h = 20 µm. Photos: a. Juan Santos. b. Pekka Heinonen.

the two additional remnants are covered in glue) is cup-shaped with a short stipe (FIG. 5e; dried cup 2.5 mm wide × 2 mm high, stipe 1 mm long × 0.5 mm broad) that is more visible than illustrated by Cooke (1875). The spores are 14.0–15.9–17.0 × 7.5–8.6–10.0 µm, mostly with tapering ends (FIG. 6j), and the medullary excipulum are without amber-like resinous exudates. From these features, we refer here collections from Europe (Cyprus, Greece, and Spain) and Asia (India) to *G. majalis*. *Geopyxis aleurioides*, newly described in this study, also has spores with narrowing ends (FIG. 6a) and an excipulum without incrustations (FIG. 5j). It has, however, a much thicker excipulum than what we observed in the type of *G. majalis*. To our knowledge *G. majalis* has not been recorded in Sweden since it was described.

Geopyxis majalis displays some genetic structure in the ITS-28S phylogeny, correlated with the geographical sampling (FIG. 1). Morphological variability between different populations of this species is also striking. Four of the six collections studied by us have sessile cupulate apothecia, but those in the Indian collection (*K.S. Thind 2144*, C) are sessile to clearly stipitate (when fresh stipe up to 2.5 × 1.5 mm) as in the isotype. This Indian collection was reported previously as *G. carbonaria* (Thind and Kaushal 1981). The Spanish collection (C-F-48964) has the longest spores (mean: 17.3 × 8.2 µm) in the genus, while spores of one specimen from Cyprus are short (mean: 14.4 × 8.5 µm). The ectal excipulum can be very thin and clearly delimited from the medullary excipulum, as those in the Greek (C-F-46288) and Indian (*K.S. Thind 2144*, C) collections, and one apothecium of the collection from Pissouri, Cyprus (S-F276573); or moderately delimited, as in another apothecium of the same collection from Pissouri (S-F276573); or not clearly delimited, as in the collections from Limassol, Cyprus (S-F276561) and Spain (C-F-48964). The thickness of both ectal and medullary excipulum varies significantly between different individuals, with the ranges 30–100 µm and 120–300 µm, respectively.

Geopyxis rehmii Turnau, Nova Hedwigia 40:166. 1984. FIG. 6l

Typification: POLAND. MALOPOLSKA: Western Carpathian, the Gorce mountain range, Stare Wierchy (near Mount Turbacz), 960 m s.m., on burn in coniferous forest, 5 Sep 1979, *K. Turnau*, s.n. (**holotype** KRA).

Specimens examined: KYRGYZSTAN. TIANSCHAN INTERIOR: Montes Terskey Ala-Too, Tschon Koezod-su, alt. 2500 m, 7 Aug 1971, *K. Kalamees & A. Kollom 9775* (C-F-60839). USA. IDAHO: Latah County, Moscow Mountain, alt. 1200 m, in hard-packed soil on wood road, 24 Jul 1936, *George & R. Rossbach 136a* (FH); Priest River, Priest River Experimental Forest, Canyon Creek Road, gregarious in moss bed in coniferous forest, 5 Jul 1964, *H.D. Thiers, HDT11286* (SFSU); OREGON: Deschutes National Forest, Bear Valley, growing with *Funaria* on burned ground, 26 Jun 1991, *H. Dissing, Oregon 91.21* (C-F-56486); Horseshoe Creek, along roadside under *Pseudotsuga menziesii* and *Tsuga heterophylla*, 8 Jul 1991, *H. Dissing, Oregon 91.47* (C-F-56509); WASHINGTON: Clallam County, Olympic National Park, on silt in *Alnus* forest, 14 Sep 1992, *H. Dissing, WA 92.33A* (C-F-56562); same locality, 14 Sep 1992, *H. Dissing, WA 92.35* (C-F-56565); Clallam County, Olympic National Park, along road to Soleduck, 15 Sep 1992, *H. Dissing, WA 92.36* (C-F-56566).

Notes.—Geopyxis rehmii was described as a pyrophilous species different from *G. carbonaria* by the orange-red, subsessile apothecia and larger, roughened spores (as viewed by SEM). It was also recognized to have a lower tolerance to burn conditions; apothecia developed later (in second year) and toward the edge of the burns, until pH values had lowered in the center (in third year). Our data from broad sampling show that this species occurs on both burned and unburned places. It has a wide range of spore sizes (FIG. 7), and although it was described as a species with roughened spores (Turnau 1984), most of the collections studied by us have smooth spores. Morphologically it cannot be separated from *G. alpina* and *G. deceptiva* (see under *G. alpina*). In Poland it is restricted to mountain areas (Turnau 1984), which may also be the case in North America (based on the localities in the material examined by us). We found that the GenBank sequence (GQ223459), obtained from cultures from roots of the orchid *Gymnadenia conopsea* and identified as *G. rehmii* in Stark et al. (2009; using the closest BLAST hit [91%]) is in fact *S. bombycina*.

DISCUSSION

Species recognition and scarcity in distinguishing morphological features in Geopyxis.—Using GCPSR, we recognized six species within *Geopyxis*. One of these was shown to comprise three cryptic species by the BPP method. Among the six species recognized by genealogical concordance (GCPSR), only *G. carbonaria* and *G. delectans* can be readily identified by morphological characters and a pyrophilous habit. The morphological circumscriptions of *G. aleurioides*, *G. korfii*, and *G. majalis* need to be tested further with additional samples.

By fully comparing the macro- and microscopical characters of the samples, we conclude that in general the phenotypic characters that can be used to distinguish *Geopyxis* species are few and subtle. The species have apothecia in yellow, ochraceous, orange, orange-red, to brownish orange tones that overlap between species and may be influenced by environmental conditions.

The spore sizes overlap between many of the species (FIG. 7), and the spore shape varies within and between species, from ellipsoid (*G. alpina*, *G. delectans*, *G. deceptiva*, *G. rehmii*), ellipsoid with more or less tapering ends (*G. alpina*, *G. deceptiva*, *G. majalis*, *G. rehmii*) to fusiform (*G. aleurioides*, *G. carbonaria*, *G. korfii*) (FIG. 6). The excipulum structure is uniform within the genus. The amount of resinous exudates, which is reported in this study as a new character to aid in identification, also varies within the species. Our study shows that some characters that were previously thought to be of taxonomic importance, i.e. odor, presence/absence of a stipe, and burned/unburned habitat, sometimes are misleading. For instance, an unpleasant odor was used to distinguish *G. foetida* from *G. carbonaria* (Velenovský 1922, 1934; Garnweidner et al. 1991), but on the basis of ITS-28S sequences they are conspecific. In fact several species, i.e. *G. alpina*, *G. majalis*, and *G. rehmii*, produce both smelly and unsmelly apothecia. Burned versus unburned ground has been used to separate the non-pyrophilous *G. majalis* from *G. carbonaria* (Rifai 1968, Huhtinen 1984), and the moss-growing *G. korfii* from pyrophilous *G. rehmii* (Zhuang and Liu 2006). Our data show, however, that *G. majalis* and *G. rehmii* have broad niches, and *G. carbonaria* may exceptionally occur on unburned ground. Due to the scarcity in distinguishing morphological characters in *Geopyxis*, for now we have to rely strongly on molecular data to identify *Geopyxis* species correctly. Using BPP, we have demonstrated that two loci, ITS and 28S, are powerful enough to delimit all the species. Having a high rate of PCR success, the ITS and 28S regions together are recommended as good markers for identifying *Geopyxis* species.

Generic delimitation of Geopyxis.—Using congruence/nondiscordance between four gene regions (ITS, 28S, *rpb2*, *tef1*) and combined analyses of 28S, *rpb2*, and *tef1* (FIGS. 2, 3), we confirm that the two semi-hypogeous to hypogeous genera *Hydnocystis* (type species *H. piligera*) and *Stephensia* (type species *S. bombycina*) are deeply nested within *Geopyxis* (e.g. Perry et al. 2007, Alvarado et al. 2011; based on 28S sequences). We therefore consider these three genera to be congeneric. Unlike most hypogeous taxa, *Stephensia* and *Hydnocystis* s. str. are probably not ectomycorrhizal (Tedersoo and Smith 2013). This does not contradict the putative ecology of *Geopyxis*. *Hydnocystis* (Tul. & C. Tul. 1844) is the earliest generic name and thus has nomenclatural priority over *Geopyxis* and *Stephensia*. We postpone combining these genera until the species diversity and the usage of the names are more fully evaluated with a larger sampling.

In a comprehensive study of Pyronemataceae based on *rpb1*, *rpb2*, *tef1*, and 28S (Hansen et al. 2013), *Tarzetta* was resolved within *Geopyxis*, supported by parsimony bootstrap (81%), but without support in ML and BI analyses. The two *Geopyxis* (KH.04.37 as *G. vulcanalis* and KH.04.48 as *Geopyxis* sp.) and four *Tarzetta* samples from Hansen et al. (2013) are included in this study. With a much larger sampling of *Geopyxis* and several semi-hypogeous to hypogeous species, our combined three-gene phylogeny still leaves us with uncertainties in the generic limits of *Geopyxis* (FIG. 3). As such, at least three taxonomic treatments are possible: (i) the five lineages resolved (i.e. the core of *Geopyxis* [clade A], *P. pila*, *G. delectans* (= *Humaria delectans*), *Tarzetta*, and *S. shanorii*), are treated in a single genus; (ii) each of the five lineages are treated as separate genera; or (iii) *G. delectans* and *P. pila* are treated in *Geopyxis*, while *Tarzetta* and *S. shanorii* are kept as separate genera. To recognize both the shared morphological and ecological features and distinctions within the group, we advocate the last one. *Humaria delectans* share all morphological characters with species in *Geopyxis* (especially *G. aleurioides* and *G. korfii*), except for the brown warts on the outer surface of the receptacle (FIG. 8). The taxon produces apothecia exclusively on burned ground, as does the type species of *Geopyxis*. Therefore, we do not consider *H. delectans* to be sufficiently distinct to warrant a separate genus.

Paurocotylis pila produces unusual subglobose or irregular ascomata that at maturity are epigeous, bright orange-red, hollow and bladder-like, and without an organized hymenium and paraphyses. The cavity is lined with a powdery spore mass entangled with hyphae; asci disintegrate early (Dennis 1975). It has been suggested as "an aleurioid fungus gone underground" (Trappe 1979). As such, it fits nicely in *Geopyxis*. In contrast to *Geopyxis*, it is considered to occur naturally in the Southern Hemisphere (New Zealand) and as an introduction to the United Kingdom (Dennis 1975). *Paurocotylis* is not considered to be ectomycorrhizal (Dennis 1975, Castellano et al. 2004) but may be facultatively biotrophic, as is *Geopyxis*. *Tarzetta* constitute a coherent group based on molecular, morphological, and ecological data. The four *Tarzetta* species sampled here form a monophyletic group that has the longest branch in the phylogenies (FIGS. 2, 3). Although *Tarzetta* and *Geopyxis* are similar in apothecial shape (i.e. sessile to distinctly stipitate, deeply cupulate, sometimes with the margin/upper part cracking into lobes and expanding) *Tarzetta* differs in lacking bright yellow, orange to red organic pigments (it is without carotenoids, Arpin 1968). The exact nature of the pigments in *Geopyxis* still needs to be determined; Arpin (1968) studied two species (named

as *G. carbonaria* and *G. majalis*), but the content of carotenoids were so low that the type could not be determined. *Tarzetta* also differs in having an outer ectal excipulum of globose cells or fascicles of hyphal cells forming conical warts (Harmaja 1974) that give the outer surface a delicate furfuraceous to verrucose appearance, and spores with 2–3 large guttules. Furthermore, species of *Tarzetta* are ectomycorrhizal (e.g. Tedersoo et al. 2006) and occur on unburned ground almost exclusively under broad-leaved trees.

Wide geographical distributions and trophic strategies.—Multilocus species delimitation with sampling in different continents as presented here, provides insight into the geographical distribution patterns of species in *Geopyxis*. Based on the outcome of the finest species delimitation method BPP, among the eight species recognized by us, six are represented by samples from more than one continent: *G. alpina* s. str. has a Eurasian distribution; *G. aleurioides* a European-North America disjunct distribution; and the other four species, *G. carbonaria*, *G. rehmii*, *G. majalis*, and *G. delectans* a Eurasian-North American distribution (FIG. 1). Except for *G. majalis*, ITS sequences of the conspecific samples from different continents are identical or highly similar, with the same length and only 1–2 base-pair difference. ITS divergence is present in *G. delectans* but shows no geographical correlation. Only *G. deceptiva* and *G. korfii* are known from single continents, North America and Asia, respectively. The suggested endemism of *G. korfii* in Asia may be due to limited sampling.

DNA-based species-level investigations in ectomycorrhizal, saprobic, and pathogenic fungi have demonstrated both continental endemic (provincialism) and intercontinental disjunct species within a single genus or species complex (e.g. Hansen et al. 1999, 2002; Dettman et al. 2003; Bonito et al. 2010; Du et al. 2012; Feng et al. 2012; Hansen and Olariaga 2015; Harrower et al. 2015). Compared with those groups, *Geopyxis* is striking due to the low number of continental endemics. The cases of *G. carbonaria* and *G. delectans* are highly similar to the fire-adapted lineage of the Morchella Elata Clade, in which four of the five species have an intercontinental disjunct distribution pattern (Taskin et al. 2010, 2012; O'Donnell et al. 2011; Du et al. 2012). By analyzing the source of the *Geopyxis* sequences used in our study, we intend to link this distribution pattern with the pyrophilous trait of *G. carbonaria* and *G. delectans*, and the diverse survival/trophic strategies of *Geopyxis* species. *Geopyxis carbonaria* produces large quantities of apothecia soon after fire (Petersen 1970, Turnau 1984). It has been identified from ectomycorrhizal root tips of severely burned, wind-fallen *Picea abies*, on the basis of identical ITS sequences from culture isolates originating from these root tips and from spores of *G. carbonaria* and was suggested as a biotrophic (mycorrhizal) root associate (Vrålstad et al. 1998). In pure culture syntheses, *G. carbonaria* infected epidermal and cortical root cells (but not the vascular cylinder) of *Pinus contorta* that showed signs of moderate pathogenicity (chlorosis and reduced vigor), but it also formed a mantle and rudimentary Hartig net (Egger and Paden 1986). Looking at substrate hydrolysis patterns of post-fire ascomycetes in vitro, *G. carbonaria* showed capacities for being both saprobic and biotrophic, being able to degrade cellulose and lignin and produce phenol oxidase enzymes (Egger 1986, Egger and Paden 1986). Unlike ectomycorrhizal pezizalean species, spores of *G. carbonaria* germinate easily in axenic culture and produce a conidial stage (Vrålstad et al. 1998). By including Pezizomycetes or fungal sp. endophytic or endolichenic sequences from GenBank (Ganley and Newcombe 2006; Higgins et al. 2007; Hoffman and Arnold 2010; U'Ren et al. 2010, 2012, 2014; Soca-Chafre et al. 2011; Wijeratne et al. 2012), in our ITS-28S analyses (FIG. 1), we identify five of the eight species of *Geopyxis* as endophytic and/or endolichenic fungi in diverse hosts, in high latitudes or altitudes. While *G. carbonaria* and *G. delectans* produce apothecia almost exclusively on burned ground, *G. rehmii*, *G. deceptiva*, and *G. majalis* produce apothecia on both burned and unburned ground (FIG. 1). The number of endophytic/endolichenic and environmental sequences of *Geopyxis* in GenBank appear to be vast, and only a limited number were included in our analyses. Taken together, this evidence shows that *Geopyxis* species are saprobic/endophytic/endolichenic and possibly facultatively biotrophic/parasitic under certain conditions.

The diverse, seemingly nonspecialized, trophic strategies of *Geopyxis* species likely make it easier for these species to establish in new areas (or survive long periods of time), and may be an important part of the explanation for their disjunct distributions. In the studies of true morels, where geographical endemism prevails, intercontinental disjunct species within the fire-adapted Elata Clade were attributed to relatively recent human-mediated dispersal events (Taskin et al. 2010; O'Donnell et al. 2011) but with wider sampling were better explained by relatively recent long distance dispersal (LDD) (Du et al. 2012). Long-distance dispersal was the most likely explanation for the high similarity of ectomycorrhizal basidiomycetes amond Svalbard, an isolated high-arctic archipelago, boreal North America, and Europe (Geml et al. 2012). Similarly Harrower et al. (2015) found LDD to be the most likely means by which species in the ectomycorrhizal *Cortinarius violaceous* group dispersed and diversified from Australasia to the Americas. Future

molecular studies, including a larger sampling of *Geopyxis* species in distant but similar habitats (i.e. burns or forest litter) and reconstruction of historical ranges using molecular clock analyses could give insights into the origin and dispersal routes of these widely distributed *Geopyxis* species.

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