



A new species of *Sabuloglossum* (Geoglossaceae, Ascomycota) from montane areas

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Abstract

The fungal genus *Sabuloglossum* (earth tongues) was originally described as a monotypic genus based on the species *Geoglossum* (*Microglossum*) *arenarium*. It typically occupies sandy coastal habitats and forms mycorrhizas with *Empetrum nigrum* (Ericaceae). While studying material from central Europe (Czech Republic, Slovakia, Austria, and Germany), ascomata morphologically very similar to *S. arenarium* were observed. They were ecologically limited to mid-altitudinal mountainous habitats and associated with *Calluna vulgaris* or *Vaccinium* sp. pl. (Ericaceae). The relationship to *S. arenarium* and related genera was evaluated using a combination of genetic (nrITS1–5.8S–ITS2 and the 28S nrLSU regions) and comparative morphological analysis. Macro- and micromorphological investigations revealed that mountain populations differ from typical *S. arenarium* in longer apical cells of paraphyses, more frequently septated ascospores, and the presence of inflated cells in the stipe squamules. In phylogenetic analyses, mountain populations appeared in sister position to *S. arenarium* in a strongly supported clade. According to these morphological and

genetic analyses, in addition to ecological and host-type specificities, the mountain populations from central Europe are proposed here as a distinct species, *S. monticola* sp. nov. Our study indicates that the speciation of *S. monticola* may have been driven predominantly by a shift in ecology and mycorrhizal association.

Keywords: biodiversity; central Europe; distribution; earth tongues; phylogeny

Introduction

The classical order Leotiales includes two morphologically similar families Geoglossaceae (eight genera) and Leotiaceae (three genera) that have been shown to be genetically distant (Frey 2016). The common feature of this order is the formation of cylindrical or club-shaped ascocarps. According to this taxonomical concept they are called "earth tongues" or "geoglossoid fungi". The application of molecular approaches revealed that the genera formerly considered as characteristic earth tongues (*Geoglossum* Pers., *Trichoglossum* Boud. and *Microglossum* Gillet) do not represent a single monophyletic group. Early molecular analyses indicated that *Microglossum* was most closely allied to Leotiomycetes, while *Geoglossum* and *Trichoglossum* formed a separate monophyletic clade within the Geoglossaceae (Platt 2000; Pfister and Kimbrough 2001; Spatafora et al. 2006; Wang et al. 2006a, b; Hibbett et al. 2007). Earth tongues, in general, are rare, often endangered, but widely distributed, growing in various biotopes from peat bogs to grasslands and sand habitats, distributed from lowlands to mountain sites. Eleven genera (Geoglossaceae: *Geoglossum*, *Glutinoglossum* Hustad, A.N. Mill., Dentinger & P.F. Cannon, *Hemileucoglossum* Arauzo, *Leucoglossum* S. Imai, *Nothomitra* Maas Geest., *Sabuloglossum* Hustad, A.N. Mill., Dentinger & P.F. Cannon, *Sarcoleotia* S. Ito & S. Imai, *Trichoglossum*; Leotiaceae: *Leotia* Pers., *Microglossum*, *Thuemenidium* Kuntze) are currently included in both families and, according to our previous studies (Kučera et al. 2012; 2014a,b; Fedosova et

al. 2017), about 20 species are expected to occur in central Europe. Index Fungorum (2021) lists over 250 species names in these genera. Identification of geoglossoid fungi is difficult and the only comprehensive identification key was published 21 years ago (Ohenoja 2000). Meanwhile, 31 new species have been described and several taxa were transferred, based on molecular data, to other genera (Hustad et al. 2013; Arauzo and Iglesias 2014; Kučera et al. 2014 a, b; 2017; Loizides et al. 2015; Crouz et al. 2017, 2020 a, b; Ekanayaka et al. 2017, 2019; Fedosova et al. 2017; Ohenoja 2010).

The genus *Sabuloglossum* was established by Hustad et al. in 2013, with a single species, *S. arenarium* (Rostr.) Hustad, A.N. Mill., Dentinger & P.F. Cannon. Apart from genetic and morphological traits this species can be easily distinguished according to its characteristic habitat type and ecology: it grows on sand or fine gravel in coastal areas and forms mycorrhizas with *Empetrum nigrum* (Nitare 1982). The history of the nomenclature and taxonomy of *S. arenarium* is convoluted. Rostrup (1892) described this fungus as *Microglossum arenarium* Rostr. from sand dunes in Denmark but did not designate a holotype. Masee (1897) placed this taxon into the genus *Mitrula* Fr. where he accommodated many of geoglossum-like fungi. Later, Durand (1908) studied material from Denmark (coll. Rostrup), Greenland (coll. Klartz ex herb. Rostrup), Labrador and Newfoundland (collected and described by Peck as *Leptoglossum latum* Peck in 1895) stated that all specimens are conspecific. He selected an epitype from material collected in Greenland and moved the taxon to the genus *Corynetes* Hazsl. Lloyd (1916) placed this taxon into the genus *Geoglossum* and in 1992 Korf and Petersen transferred the species to the genus *Thuemenidium*. Polyphyly of the genus *Thuemenidium* was for the first time shown by Ohenoja et al. (2010) and subsequently confirmed by Hustad et al. (2013).

The occurrence of *Sabuloglossum arenarium* was reported from numerous localities in Eurasia, North America, and Japan (Nitare 1982, Ohenoja et al. 2010, Hustad et al. 2013; Tejklová et al. 2015, Lüderitz et al. 2016, Fedosova 2019).

In 2004 an ascomata initially determined to be *S. arenarium* were collected in eastern Germany. It was found on the Großen Arber Mountain (elev. 1400 m) in sandy/gravelly acidic soil and closely associated with plants of the family Ericaceae. This population of *S. arenarium* was originally considered as relict from the Pleistocene era (Beenken and Horn 2008 as *Geoglossum arenarium*). Later, in 2014 a morphologically similar fungus was found in similar mountainous habitats in Hercynian massif, specifically in the Krkonoše Mts. (elev. 1025 m) and Hrubý Jeseník Mts. (elev. 1300 m) (Tejklová et al. 2015 and Zíbarová 2016). The localities lacked *Empetrum* shrubs which are primarily associated with the ascomata of *S. arenarium*. In contrast, the mountain populations seemed to be associated with *Calluna vulgaris* or *Vaccinium* sp. pl. (Ericaceae). These populations with different accompanying host species represent a separate taxon. . With extended sampling of the “mountain type” of *S. arenarium* based on the characteristic mountain distribution, micromorphology, and phylogenetic analyses we describe here a new, morphologically very similar species, *Sabuloglossum monticola*.

Materials and methods

Sampling and study design

We analysed four sequences of *Sabuloglossum* from montane habitats of Central Europe (Czechia, Austria and Slovakia, for details, see Table 1) and related members of earth tongues to uncover their taxonomic identity and phylogenetic relationships. These related species included (number of sequences/number of species): *Sabuloglossum arenarium* inhabiting sand habitats (three sequences), *Geoglossum* (22/16), *Glutinoglossum* (2/2), *Hemileucoglossum* (2/2), *Leucoglossum* (2/1), *Maasoglossum* K.S. Thind & R. Sharma (2/2), *Nothomitra* (1/1),

Sarcoleotia (2/1) and *Trichoglossum* (4/3). *Graddonina coracina* (Bres.) Dennis was used as an outgroup. Where available, we included type sequences of analysed taxa (Table 1).

Morphological studies

The macro-morphological characters (shape, dimensions and colour of ascoma, stipe surface) of new collections were observed in fresh material. The micro-morphological structures (asci, ascospores, paraphyses, structures on the stipe) were observed both in dried and fresh material using a light microscope with an oil immersion lens. Fragments of ascomata were examined in tap water, 5% KOH, Melzer's reagent (MLZ), Lugol's solution (IKI) and a solution of Congo red in ammonia. Values of micro-morphological characters were estimated as an average of 30 measurements for each character (minimum/maximum values of the measurements are in parentheses) in each of the studied collection. Collecting sites are georeferenced and the coordinates are reported in the WGS 84 system. The voucher specimens are deposited in the fungarium of the Institute of Botany, Slovak Academy of Sciences in Bratislava (SAV), Museum of Eastern Bohemia in Hradec Králové (HR) and in Botanische Staatssammlung München (M).

DNA extraction, amplification and sequencing

DNA extraction and amplification of the internal transcribed spacer region of the nuclear ribosomal DNA (nrITS1–5.8S–ITS2, ITS) and the 28S nuclear ribosomal large subunit region (nrLSU, LSU) were performed by Alvalab (Spain). Total genomic DNA was extracted using a modified protocol based on Murray and Thompson (1980). Amplification of nrDNA was performed following Mullis and Faloona (1987) using a Taq polymerase (VWR, USA), and the primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993) and LR0R and LR5 (Vilgalys and Hester 1990, Cubeta et al. 1991). The PCR mix (40 µl)

included 1 × polymerase buffer, 2 mM MgCl₂, 0.2 mM total dNTPs, 0.5 μM of each primer and 1 μl of polymerase. The amplifications were carried out using the following cycle: 35 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min, final extension at 72 °C for 10 min and cooling to a constant of 4 °C. PCR product purification and Sanger sequencing was carried out at the commercial company STAB VIDA (Portugal). GenBank accession numbers of the sequenced DNA regions are provided in Table 1.

Phylogenetic analyses

To uncover the identity of mountain-dwelling *Sabuloglossum* accessions and reveal their phylogenetic position within closely allied taxa, we used concatenated ITS-nrLSU datasets (45 concatenated sequences × 1326 bp). Altogether we analysed 45 ITS and 38 nrLSU sequences. Missing LSU sequences or their parts were replaced by N, while indels were coded as a missing data. All analysed sequences were edited and aligned in Geneious Prime 2021.2.2 (www.geneious.com). Maximum likelihood analysis (ML) and Bayesian inference (BI) were performed on the concatenated dataset. Before phylogenetic analyses were performed, the best-fit partitioning schemes and models were estimated for the concatenated dataset, using the greedy search mode as implemented in the PartitionFinder v. 2.1.1 (Lanfear et al. 2016). The dataset was divided into four partitions accompanied by relevant nucleotide substitution models: nrLSU (GTR + Γ + I), ITS1 (GTR + Γ), 5.8S (GTR + Γ + I) and ITS2 (GTR + Γ). BI analysis was conducted in MrBayes v. 3.2.7a, using four independent chains, 10 million generations, and a sampling frequency of 1000 (Ronquist et al. 2012). Before the computation of the Bayesian majority consensus tree, the first 25% of sampled trees were discarded. Nodes with posterior probability (PP) values higher than 0.95 were considered as significant. ML was computed in RAxML v. 8.2.12 (Stamatakis 2014). Bootstrap support was computed from 1000 re-samplings. Only branches having the bootstrap support > 75% were

considered supported. Both analyses were performed using the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). Visualization and editing of the trees were done in FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>).

RESULTS

Molecular phylogenetic analysis

Both BI and ML analyses uncovered that the montane *Sabuloglossum* accessions formed a well supported (1.0 PP/100 BS) clade in a sister position to the *Sabuloglossum arenarium* clade (Fig 1). Both clades were strongly supported (1.0 PP/97 BS) suggesting their congeneric position within the genus *Sabuloglossum*. The final matrix was deposited in TreeBASE with accession no. S28128.

Taxonomy

Sabuloglossum monticola V. Kučera, Slovák, Janošík, sp. nov. MB 839263 (Figs 2, 3)

Etymology: Name reflects the mountain origin of collected material.

Diagnosis: Differs from *S. arenarium* by the following combination of characters: less robust ascomata, 0–7 septate ascospores, smaller asci (up to $140 \times 14 \mu\text{m}$), predominantly longer apical cells of paraphyses, and scales on the stipe that contain some inflated cells ($18\text{--}38 \times 10\text{--}19 \mu\text{m}$). Characteristic is occurrence in mountain areas at an elevation above 1000 m above sea level.

Holotype: SLOVAKIA, NP Malá Fatra, between peaks Žitné and Kraviarske, $49^{\circ}12'54.1''$ N, $19^{\circ}0'54.7''$ E, 1226 m asl., by the road, on soil, 29 Oct 2016 leg. Z. Egertová (SAV F-11291, ITS sequence GenBank MW471105, LSU sequence GenBank MW471102).

Description:—*Ascomata* usually scattered in small groups on bare soil, clavate, stipitate, 4–35 mm tall, 3–5 mm thick, black. *Ascigerous part* clavate to broadly clavate, usually takes up more than 1/2 of the total ascomata length, black, mace shaped, oval to lobed in cross section, distinctly delimited from the stipe, smooth. *Stipe* cylindrical, oval in cross section, 2–18 × 0.6–5 mm, usually robust, squamulose with scales arranged in horizontal lines. *Subterranean part* at the stem base a small pseudosclerotium (up to 3 × 5 mm) consisting of brown hyphae, Ericaceae roots and soil particles, 1(3) ascomata per pseudosclerotium. *Asci* thin-walled, club-shaped, (105)120–145(155) × 12–14(17) µm, Q = 7.3–11.6, [living (170)190–215(240) × (17)19–22 µm], inoperculate, 8-spored, with euamyloid apical ring and inamyloid wall in MLZ and IKI, arising from croziers. *Ascospores* elongate, fusiform, sometimes slightly curved, with rounded ends, (24)29–36(46) × (4)5–6 µm, Q = (4.6)5.8–7.2(8), [living (30)42–45(47) × (4.6)5–5.6(6.5) µm] hyaline, predominantly 1–7 septate, some of the ascospores aseptate (collections with only aseptate ascospores rarely seen). *Ascoconidia* not observed. *Paraphyses* about the same length, brownish in whole length, darker in apical part gradually lighter to the base, filiform, sparsely septate, curved or straight, sometimes branched; apical cells 25–40 × 3–4 µm when cylindrical to 23–50 × 4–8 (10) µm when inflated, not agglutinated. *Stipe surface* squamulose, scales made of tufts of short hyphae with cells 20–50 × 3–5 µm accompanied by hyphae with inflated apical cells (18–38 × 10–19 µm) and setose hairs resembling cells. For supplementary description and photo-documentation see *S. arenarium* in Tejklová et al. (2015) and Zíbarová (2016).

Habitat: on bare soil or between bryophytes *Ditrichum heteromallum*, *Oligotrichum hercynicum*, *Nardia scalaris*, *Polytrichum* sp. pl., *Dicranella* sp. pl., in vicinity of plants of Ericaceae family and grasses.

Distribution: Austria, Czech Republic (Tejklová et al. 2015 as *Sabuloglossum arenarium*), Germany (Beenken and Horn 2008 as *Geoglossum arenarium* (Rostr.) Lloyd), Slovakia (Fig 4).

Specimens examined: AUSTRIA, Styria, Schladminger Tauern, Sankt Nikolai im Sölktal, Etrachböden, 47°16'27.98" N, 14°3'37.44" E, 2055 m asl., trampled edge of tourist trail with co-occurring bryophyte taxa (*Ditrichum heteromallum*, *Oligotrichum hercynicum*), 10 Sept 2015 leg. L. Janošík (SAV F-11256, ITS sequence GenBank MW471104); Styria, Wölzer Tauern, Irdning-Donnersbachtal, Ilgenberg, Mölbegg, 47°27'7.22" N, 14°10'54.69" E, 1955 m asl., trampled edge of tourist trail, with co-occurring bryophyte taxa (*Ditrichum heteromallum*, *Oligotrichum hercynicum*, *Nardia scalaris*) and Ericaceae shrubs (*Vaccinium vitis-idaea* and *Rhododendron ferrugineum*), 9 Sept. 2015 leg. L. Janošík (SAV F-11252); Salzburg, Hohe Tauern, Sportgastein, 1 km E of Riffelscharte, 47°3'36.22" N, 13°1'27.72" E, 1950 m asl., trampled edge of tourist trail, with co-occurring bryophyte taxa (*Ditrichum heteromallum*, *Nardia scalaris*) and Ericaceae shrubs (*Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Rhododendron ferrugineum*), 25 Sept. 2016 leg. L. Janošík (PRC 4685).

CZECH REPUBLIC, Bohemia, Krkonoše Mts., Špindleruv Mlýn, Labský důl valley, 700 m SE of Pančava waterfall, 50°45'34" N, 15°33'13" E, 1025 m asl., trampled edge of tourist trail, with co-occurring bryophyte taxa (*Dicranella heteromalla*, *Oligotrichum hercynicum* and *Polytrichum* cf. *formosum*), close to *Festuca* sp. and *Vaccinium* sp. pl., granite bedrock, 19 Sept 2014, leg. T. Tejklová, V. Samková, J. Doležal (HR 94300, ITS sequence GenBank, MW471103, LSU sequence GenBank MW471101); Moravia, Hrubý Jeseník Mts., small meadow between Polom peak and Hášova cottage, 50°08'59.5" N, 17°05'14.8" E, 1112 m asl., trampled surface of old tourist trail, with co-occurring bryophyte taxa (*Ditrichum*

heteromallum, *Polytrichum* sp., *Oligotrichum hercynicum*), *Clavaria* cf. *argillacea*, 11 Oct 2014 leg. V. Halasů (V.H. 37, SAV F-11600).

GERMANY, Bavaria, Bayerisch Eisenstein, northern upper slope of the Grosser Arber Mt., close to the summit area, 49°07' N, 13°08' E, 1400 m asl., 26 Sept 2004 leg. L. Beenken & K. Horn (Herb. L. Beenken No. 4950 in M, habitat and accompanying vegetation described in Beenken and Horn 2008).

SLOVAKIA, Martin district, Malá Fatra Mts., Martinské hole recreation area, Veľká lúka meadow, 49°5'23.03" N, 18°48'57.72" E, 1437 m asl., trampled edge of tourist trail, covered with small bryophytes (*Ditrichum heteromallum*, *Oligotrichum hercynicum*, *Nardia scalaris*), and Ericaceae shrubs (*Vaccinium myrtillus*), near temporal water bodies, 10 Sept 2016 leg. L. Janošík (SAV F-11522); Martin district, Malá Fatra Mts., Martinské hole recreation area, Veľká lúka meadow, 49°5'30.48" N, 18°48'56.16" E, 1447 m asl., trampled edge of tourist trail, with co-occurring bryophyte taxa (*Polytrichum* sp., *Oligotrichum hercynicum* etc.), 19 Sept 2017 leg. A. Polhorský (SAV F-11594); ibidem 9 Nov 2018 leg. M. Krivuš (SAV F-11596); Martin district, Malá Fatra Mts., Šútovo village, Generál, 49°10'20.21" N, 19°3'15.34" E, 1138 m asl., edge of the road, 12 Nov 2020 leg. M. Krivuš (SAV F-11661); Martin district, Malá Fatra Mts., southern part, Šútovo village, Chleb Mt., trail Čierťaz - Generál - Snílovské saddleback, 49°10'13.15" N, 19°3'17.50" E, 1095–1450 m asl., edge of the touristic trail, 9 Nov 2020 leg. M. Krivuš (from several micro-localities SAV F-11662, SAV F-11663, SAV F-11664).

Discussion

The genus *Sabuloglossum* as a member of class Geoglossomycetes was described by Hustad et al. (2013). In the light of our study, it is apparent that at least two species, *S. arenarium* and *S. monticola*, are present in the genus. These two species clearly differ genetically, however,

morphological differences are less obvious. Both species form pseudosclerotia composed of hyphae, Ericaceae roots and soil particles. The subterranean pseudosclerotia of *S. arenarium* are larger than the above-ground ascomata and up to 10 or more ascomata often arise from a single pseudosclerotium. (Lüderitz et al. 2016, Nitare 1982). The pseudosclerotia of *S. monticola* are, in contrast, much smaller and only one or rarely a few ascomata develop on them, though this characteristic may also be influenced by different soil textures.

Taxonomic determination of *S. monticola* is based on a combination of several crucial diagnostic characters including inflated cells of hyphae forming scales on the stipe surface (up to $18\text{--}38 \times 10\text{--}19 \mu\text{m}$), length of apical cells of paraphyses ($23\text{--}50 \mu\text{m}$), weak coloration of whole paraphyses which increase to the apex, and frequently septate ascospores (1–7, usually at least some of the ascospores are septate, although ascomata with aseptate ascospores may occur). Furthermore, compared to *S. arenarium*, ascomata of *S. monticola* seem to be less robust with the fertile part usually club-shaped. Additionally, *S. monticola* inhabiting inland mountain ranges at the altitude over 1000 m a.s.l. Probably the first collection of *S. monticola* was presented by Beenken and Horn (2008) under the name *Geoglossum arenarium* (Herb. L. Beenken No. 4950!). Even though it was not possible to amplify DNA from this sample, the morphology and habitat clearly indicate that it belongs to *S. monticola*. The paraphyses are brown in colour throughout with long apical cells and the cells in scales on the stipe surface are distinctly inflated. Moreover, occurrence in elevation about 1400 m, lack of *Empetrum* sp. pl. and distinctly presence of other plants of family *Ericaceae* support this identification. All collections of *S. arenarium* mentioned by Tejklová et al. (2015) and Zíbarová (2016) according to our observations also represent *S. monticola*.

The typical *S. arenarium* occupying coastal dunes has in general more robust ascomata with paraphyses distinctly coloured only in the most apical 2–3 cells, which are usually up to $20 \mu\text{m}$ long, lack inflated cells in stipe scales and with ascospores often aseptate

(or with 1–3 septa). Durand (1908) in his study described ascospores as "for a long time continuous, finally becoming at least 4-septate (probably 10 or more)" and Ohenoja et al. (2010) reported rarely septate ascospores, as well. The presence of spore septation alone is thus probably not sufficient to unambiguously separate these two taxa. In general, it seems that spore septation is more frequent in *S. monticola*, especially in mature ascomata, but this character alone is not sufficient to exclude *S. arenarium*.

Sabuloglossum arenarium occurs on sand dunes and in coastal areas stabilised by vegetation. Nitare (1982) suggested a boreal-maritime distribution, although two collections are known also from the mountains in Nordic countries – in Swedish Lapland. This unusual occurrence could be possibly explained by the maritime influence which reaches the sites from the west. The global distribution of this species is summarised in Hustad et al. (2013).

Sabuloglossum arenarium (Fig 5) appears to be a highly variable taxon and the reported ranges of several micromorphological characters are very broad. Ascus length varies from 100 μm (Rostrup 1892 as *Microglossum arenarium*, Durand 1908 as *Corynetes arenarius*) to 180 μm (Ohenoja et al. 2010 as *Thuemenidium arenarium*) and width from 12 μm (Rostrup 1892, Durand 1908) to 35 μm (Hustad et al. 2013). Ascospore dimensions differ in reports, ranging from 25–30 \times 4–6 μm (Rostrup 1892) to 27–37 \times 3.5–5 μm (Hustad et al. 2013). Material from Estonia (SAV F-10729), Sweden (SAV F-11665) and Finland (SAV F-10402) which we have studied (data not shown) fits this wide range very well.

Sabuloglossum monticola is well-supported as a new and well defined species of earth tongue, although it might be, at first sight misidentified with members of some related genera. Indeed, some mountainous species of the genus *Geoglossum*, particularly *G. dunense* Loizides, M. Carbone & P. Alvarado, *G. heuflerianum* Bail, *G. montanum* Nannf. and *G. alpinum* Eckblad, have similar black or blackish color, small ascocarps (up to 2 cm) and structures (warts or scales) on the stipe.

The phylogenetically unrelated *Geoglossum dunense*, which macroscopically strongly resembles *S. arenarium* and *S. monticola* also shares a similar habitat (sandy or gravelly soil). However *G. dunense* can be distinguished by its larger ($31\text{--}44 \times 8\text{--}10\text{ }\mu\text{m}$) coloured and 2–3 septate ascospores and thick-walled multiseptate, moniliform and sometimes forked paraphyses up to $7\text{ }\mu\text{m}$ wide at the apex (Loizides et al. 2015).

Geoglossum heuflierianum, was first reported by Bail in 1860 from Tyrol (Austrian Alps) without description. Saccardo validated the species in 1889 and provided this name with a short and scanty description. Nannfeldt (1942) wrote that this description is too vague to make it identifiable and he was unable to locate the voucher specimen in any of requested museums. When Fluri et al. (2017) reported the rediscovery of this taxon in the Swiss Alps, they presented collections which are possibly the first in more than 150 years. They designated a lectotype and emended description. This taxon differs from *S. monticola* in longer ($38.7\text{--}52.7 \times 8.3\text{--}11.3\text{ }\mu\text{m}$) brown ascospores with 2–4 septa and longer ($175\text{--}237 \times 22\text{--}31\text{ }\mu\text{m}$) asci but inhabits similar habitats on gravelly soil next to hiking trails.

Geoglossum montanum was described from trails in subalpine areas in Sweden (Nannfeldt 1942). Its asci are thicker ($120\text{--}150 \times 14\text{--}20\text{ }\mu\text{m}$) usually with only 4–6 longer ($50\text{--}65 \times 5\text{--}6\text{ }\mu\text{m}$) fuliginous-brown ascospores with 7 septa, but its macromorphology and habitat is similar to *S. monticola*. It was rarely collected from northern Europe, predominantly from Scandinavia (Nannfeldt 1942; Eckblad 1963; Olsen 1986; Nitare 1988; Ohenoja 1995, 2000; Elurikkus 2021). Unfortunately there is no sequence of this taxon in the GenBank database to reveal their evolutionary relationships.

Geoglossum alpinum was described by Eckblad in 1963 on the basis of specimens collected in Norway. It has wider ($130\text{--}150 \times 18\text{--}22\text{ }\mu\text{m}$) asci, fuliginous brown 7-septate and longer ($55\text{--}70 \times 5.5\text{--}7.5\text{ }\mu\text{m}$) ascospores and paraphyses strongly agglutinated by a dark brown amorphous matter. Also for this taxon there is no single sequence in the GenBank

database, so far it was rarely collected and noted in Iceland (Hallgrímsson 1987; Ohenoja 2000) and Norway (Eckblad 1963, Olsen 1986, Ohenoja 2000).

According to other sources summarised in Fluri et al. (2017) also *G. cookeanum* Nannf. ex Minter & P.F. Cannon, *G. fallax* E.J. Durand, *G. nigrum* (Pers.) Cooke, *G. staerbeckii* Nannf. and *G. umbratile* Sacc. occur in mountain areas. All of them can be easily distinguished from *S. monticola* by their ascospore length (more than 60 µm) and ascospore septation (seven or more).

Other similar species are *Glutinoglossum peregrinans* Fedosova & V. Kučera and *G. proliferatum* V. Kučera (Fedosova et al. 2017). Both were reported from mountain areas, but they clearly differ by their viscid ascocarps. According to our observations (SAV F-11393, SAV F-11666) also some taxa from the genera *Trichoglossum* and *Hemileucoglossum* may grow in mountain areas in Europe, but these are only mentioned here because in their young states they can macromorphologically resemble *S. monticola*.

Based on microscopy there are four other very similar taxa that should be mentioned.

Corynetes geoglossoides Eckblad was described in 1963 as a distinct taxon from *S. arenarium* (listed as *Corynetes arenarius*) even though it occurs in similar habitats and resembles *S. arenarium* in its appearance. The only difference is that *C. geoglossoides* contains some asci with pale brown ascospores which may be 1-septate. Hustad et al. (2013) treated this taxon to be conspecific with *S. arenarium* due to observation that ascospore pigmentation may occur here very late in the ontogenesis. They do not consider this character as sufficient for distinguishing a separate species. We noted that according to the original description this taxon should have a smooth stipe. Other microstructures seem to be almost identical with *S. arenarium* and overlap also with *S. monticola*. There is no sequence of this taxon in the GenBank database.

Maasoglossum asaseptatum (Hakelien ex Nitschke) Hustad & A.N. Mill., a member of the basal genus of the family Geoglossaceae, was for the first time collected and observed by Nils Hakelien in 1977. After seven years Nitschke (1984) described this unusual taxon as *Geoglossum asaseptatum*. Based on molecular data Hustad and Miller (2015) placed this taxon into genus *Maasoglossum* K.S. Thind et R. Sharma emend. Hustad et A.N. Mill. Until now of the four known localities of *M. asaseptatum* only the type locality in Sweden and the locality in Finland have been preserved (Hustad et Miller 2015). The information presented in ArtDatabanken (2019) suggest that the fungus may form ericoid mycorrhizas with shrubs and trees. The light brown, aseptate ascospores are only slightly smaller ($23\text{--}31 \times 4.8\text{--}6.8 \mu\text{m}$) than in *S. monticola*, asci are of comparable dimensions ($80\text{--}140 \times 10\text{--}16 \mu\text{m}$) but its paraphyses are agglutinated with an amorphous brown material and it has a smooth stipe and bigger ascomata (up to 7 cm).

Geoglossum hakelienii Nitschke was described as *G. fumosum* by Hakelien (1967) from central Sweden. However, the name was found to be a later homonym of *G. fumosum* (Peck) Lloyd and therefore illegitimate (Art. 53.1 ICN, Turland et al. 2018) and the new name *G. hakelienii* was proposed by Nitschke (1983). The main difference from *S. monticola* seems to be the length of the ascomata (up to 4.5 cm), colour of the ascomata which is described as brown, as if impregnated by smoke, shorter ($100\text{--}125 \times 12\text{--}17 \mu\text{m}$) asci and occurrence in pasture-land. It is distributed both in Sweden and Norway (Olsen 1986). There is no sequence of this taxon in the GenBank database.

Geoglossum lineare Hakelien, another taxon described from pasture-land of Sweden (Hakelien 1967), has very similar characteristics of paraphyses and asci as *S. monticola*, but with significantly longer ($45\text{--}65 \times 4.5\text{--}5.5 \mu\text{m}$) ascospores and a smooth viscid stipe. It is reported from Norway (Olsen 1986), Sweden (Hakelien 1967; Ohenoja 2000), Germany, and Estonia (GBIF 2021). Also for this taxon there is no sequence in the GenBank database.

Both *Sabuloglossum* species are probably in close relationship with the plants of the family Ericaceae, *S. arenarium* is usually found with *Empetrum* sp. pl. and *Calluna* sp., and *S. monticola* in the vicinity of *Vaccinium* and *Calluna* species. *Sabuloglossum monticola* has a likely affinity with disturbed or synanthropised habitats such as tourist or forest animals trails, since the ascomata generally grow on the banks of such trails on fine gravel or fine stony soil. We hypothesize that disturbance caused by trampling may likewise create suitable habitats for survival and formation of ascomata in mountain areas.

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Declarations

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Conflicts of interest/Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of data and material

All used material and data are accessible.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Viktor Kučera, Lukáš Janošík, Marek Slovák and Ludwig Beenken. The first draft of the manuscript was written by Viktor Kučera and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

- Arauzo S, Iglesias P (2014) La familia Geoglossaceae ss. str. en la península Ibérica y la Macaronesia. *Errotari* 11:166–259
- ArtDatabanken (2019) *Geoglossum aseptatum*, slat jordtunga (in Swedish); [accessed 2021 Mar 9]. <https://artfakta.se/artbestamning/taxon/geoglossum-aseptatum-700>.
- Bail T (1860) Das Pilztypenherbar, im Auftrage des kaiserlich-österreichischen Ministeriums für Cultus und Unterricht. *Österr bot Z* 10:101–114
- Beenken L, Horn K (2008) First record of *Geoglossum arenarium* on Mount Großer Arber in the Bavarian Forest *Z Mykol* 74:119–126
- Crous PW, Wingfield MJ, Burgess TI et al (2017) Fungal Planet description sheets: 625–715. *Persoonia* 39:270–467. <https://doi.org/10.3767/persoonia.2017.39.11>
- Crous PW, Wingfield MJ, Chooi Y-H et al (2020a) Fungal Planet description sheets: 1042–1111. *Persoonia* 44:301–459. <https://doi.org/10.3767/persoonia.2020.44.11>
- Crous PW, Cowan DA, Maggs-Kölling G (2020b) Fungal Planet description sheets: 1112–1181. *Persoonia* 45:251–409. <https://doi.org/10.3767/persoonia.2020.45.10>

- Cubeta MA, Echandi E, Abernethy T, Vilgalys R (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* 81:1395–1400
- Durand EJ (1908) The Geoglossaceae of North America. *Annales Mycologici* 6:387–477
- Eckblad F–E (1963) Contributions to the Geoglossaceae of Norway. *Nytt Magasin for Botanikk* 10:137–158
- Ekanayaka AH, Hyde KD, Gareth J et al (2017) A new species of *Trichoglossum* (Geoglossales, Ascomycota) from Thailand. *Phytotaxa* 316:161–170.
<https://doi.org/10.11646/phytotaxa.316.2.5> 161
- Ekanayaka AH, Hyde KD, Gentekaki E et al (2019) Preliminary classification of Leotiomycetes. *Mycosphere* 10:310–489. <https://doi.org/10.5943/mycosphere/10/1/7>
- Elurikkus (2021) *Geoglossum montanum* Nannf. Elurikkus Occurrence; [accessed 2021 Mar 9]. https://elurikkus.ee/generic-hub/occurrences/search?taxa=Geoglossum+montanum&sort=occurrence_date&dir=desc&pageSize=#records.
- Fedosova AG, Popov ES, Lizoň P, Kučera V (2017). Towards an understanding of the genus *Glutinoglossum* with emphasis on the *Glutinoglossum glutinosum* species complex (Geoglossaceae, Ascomycota). *Persoonia* 41:18–38.
<https://doi.org/10.3767/persoonia.2018.41.02>
- Fedosova AG (2019): Семейство Geoglossaceae (Ascomycota) в России [The family Geoglossaceae (Ascomycota) in Russia]. Dissertation, Institute of Botany RAS, Sankt Petersburg
- Fluri H, Seen-Irlet B, Graf U, Beenken L (2017) *Geoglossum heuflerianum* – Wiederentdeckung einer alpinen Art in der Schweiz. *Österr Z f Pilzkunde* 26:87–97

- Frey W ed (2016) Syllabus of Plant Families. A. Engler's Syllabus der Pflanzenfamilien. 13th ed. 1/2 Ascomycota. Gebrüder Borntraeger Verlagsbuchhandlung, Stuttgart
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes — application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2:113–118.
- GBIF (2021) *Geoglossum lineare* Hakelier. GBIF Occurrence; [accessed 9 March 2021], https://www.gbif.org/occurrence/search?taxon_key=3484553.
- Hakelier N (1967) Three new Swedish species of *Geoglossum*. *Sven Bot Tidskr* 61:419–424
- Hallgrimsson H (1987) The family Geoglossaceae in Iceland. *Acta Bot Islandica* 9:61–67
- Hibbett DS, Binder M, Bischoff JF et al (2007) A higher-level phylogenetic classification of the Fungi. *Mycol Res* 111:509–547. <https://doi.org/10.1016/j.mycres.2007.03.004>
- Hustad VP, Miller AN (2015) *Maasoglossum*, a basal genus in Geoglossomycetes. *Mycoscience* 56:572–579
- Hustad VP, Miller AN, Dentinger BTM, Cannon PF (2013) Generic circumscriptions in Geoglossomycetes. *Persoonia* 31:101–111.
- Index Fungorum (2021); [accessed 2021 Mar 21]. <http://www.indexfungorum.org/names/Names.asp>.
- Kučera V, Lizoň, P (2012) Geoglossaceous fungi in Slovakia III. The genus *Geoglossum*. *Biologia* 67: 654–658. <https://doi.org/10.2478/s11756-012-0053-6>
- Kučera V, Lizoň P, Tomšovský M (2014a) A new green earthtongue *Microglossum parvisporum* sp. nov. *Sydowia* 66: 335–343
- Kučera V, Lizoň P, Tomšovský M et al (2014b) Reevaluation of the morphological variability of *Microglossum viride* and *M. griseoviride* sp. nov. *Mycologia* 106:282–290. <https://doi.org/10.3852/106.2.282>

- Kučera V, Lizoň P, Tomšovský M (2017) Taxonomic divergence of the green naked-stipe members of the genus *Microglossum* (Helotiales). *Mycologia* 109: 46–54.
<https://dx.doi.org/10.1080/00275514.2016.1274620>
- Lanfear R, Frandsen PB, Wright AM, et al (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 4:772–773
- Lloyd CG (1916) *Mycological writings 5: The Geoglossaceae*. Cincinnati
- Loizides M, Carbone M, Alvarado P (2015) *Geoglossum dunense* (Ascomycota, Geoglossales): a new species from Mediterranean islands of Cyprus and Malta. *Mycological Progress* 14: Art. 41, 1–8
- Lüderitz M, Kamke M, Specht P et al (2016) Ergebnisse des 4. und 5. Dünepilzworkshops. *Zeitschrift für Mykologie* 82:355–448
- Massee G (1897) A monograph of the Geoglossaceae. *Annals of Botany* 11:225–306
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees, In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, LA: 1–8
- Mullis K, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155:335–350
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8:4321–4325
- Nannfeldt JA (1942) The Geoglossaceae of Sweden (with regard also to the surrounding countries). *Arkiv för botanik* 30A: 1–67
- Nitare J (1982) *Geoglossum arenarium*, sandjordtunga – ekologi och utbredning i Sverige. *Sven Bot Tidskr* 76:349–357

- Nitare J (1983) *Geoglossum hakelieri*, ett nytt namn för *G. fumosum* Hakelier. Windahlia 12:81–88 [1982]
- Nitare J (1984) *Geoglossum asaepatum* n. sp., a new earthtongue with brown continuous spores. Windahlia 14:37–42
- Nitare J (1988) Jordtungor, en svampgrupp på tillbakagång i naturliga fodermarker. Sven Bot Tidskr 82:341–368
- Ohenoja E (1995) Occurrence of *Geoglossum*, *Trichoglossum*, and *Microglossum* (Ascomycota, Leotiales) in Finland. Documents Mycologiques 25:285–294
- Ohenoja E (2000) Geoglossaceae Corda. In: Hansen L and Knudsen H (eds) Nordic Macromycetes Vol. 1: Ascomycetes, Copenhagen, pp 177–183
- Ohenoja E, Wang Z, Townsend JP et al (2010) Northern species of earth tongue genus *Thuemenidium* revisited, considering morphology, ecology and molecular phylogeny. Mycologia 102:1089–1095
- Olsen S (1986) Jordtunger i norge. Agarica 7:120–168
- Peck CH (1895) New species of fungi. Bulletin of the Torrey Botanical Club 22:198–211
- Petersen PM, Korf RP (1982) Some inoperculate Discomycetes and Plectomycetes from West Greenland. Nord J Bot 2:151–154
- Pfister DH, Kimbrough JW (2001) Discomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota VII. Part A. Systematics and evolution. Springer Verlag.
- Platt JL (2000) Lichens, earth tongues, and endophytes: evolutionary patterns inferred from phylogenetic analyses of multiple loci. Dissertation, Oregon State University
- Rambaut, A 2014. FigTree v1.4.2; [accessed 9 March 2021].
<http://tree.bio.ed.ac.uk/software/figtree/>

- Ronquist F, Teslenko M, Van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–542
- Rostrup E (1892) Mykologiske Meddelelser. Spredte lagttagelser fra Aaren 1889–1891. *Botanisk Tidsskrift* 18:65–78
- Saccardo PA (1889) Discomyceteae FR. *Sylloge Fungorum* 8:3–842
- Spatafora JW, Sung G-H, Johnson D et al (2006) A fivegene phylogeny of Pezizomycotina. *Mycologia* 98:1018–1028. <https://doi.org/10.3852/mycologia.98.6.1018>
- Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics* 30:1312–1313
- Tejklová T, Deckerová H, Gaisler J (2015) *Sabuloglossum arenarium* (Geoglossaceae) in the Czech Republic. *Czech Mycol* 67:85–94
- Turland NJ, Wiersema JH, Barrie FR et al eds (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books. DOI <https://doi.org/10.12705/Code.2018>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238–4246
- Wang Z, Binder M, Schoch CL et al (2006a) Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): a nuclear rDNA phylogeny. *Mol Phylogenet Evol* 41:295–312. <https://doi.org/10.1016/j.ympev.2006.05.031>
- Wang Z, Johnston PR, Takamatsu S et al (2006b) Toward a phylogenetic classification of the Leotiomycetes. based on rDNA data. *Mycologia* 98:1065–1075. <https://doi.org/10.3852/mycologia.98.6.1065>

White TJ, Bruns TD, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego

Zíbarová L 2016. *Geoglossum arenarium* (Pazoubek písečný); [accessed 2021 Mar 21]. <http://www.mykologie.net/index.php/houby/podle-morfologie/apothecia/item/2154-geoglossum-arenarium>.

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Table 1. Specimens and NCBI GenBank accession numbers of DNA sequences used in this study

Species	Voucher No.	GenBank accession No.		Notes
		ITS	LSU	
<i>Geoglossum brunneipes</i>	AH 44217	KP144090	–	holotype
<i>G. chamaecyparinum</i>	AH 44219	KP144098	–	holotype
<i>G. cookeanum</i>	ILLS 67347	KC222122	KC222135	
<i>G. cookeanum</i>	ILLS 61035	JQ256417	JQ256434	
<i>G. difforme</i>	ILLS 67348	KC222123	KC222136	
<i>G. difforme</i>	ILLS 67349	KC222124	KC222137	
<i>G. dunense</i>	TUR-A 199830	KP744516	KP744517	holotype
<i>G. fallax</i>	ILLS 61037	JQ256419	JQ256435	
<i>G. fallax</i>	Lueck 11	KP965776	KP965793	
<i>G. geesterani</i>	AH44218	KP144083	–	holotype
<i>G. glabrum</i>	ILLS 72358	KP657559	KP657564	
<i>G. glabrum</i>	ILLS 61038	JQ256420	JQ256436	
<i>G. heuflerianum</i>	Ueli Graf 25.08.2013/1	KP742955	–	
<i>G. nigrum</i>	OSC 100009	DQ491490	AY544650	
<i>G. raitviirii</i>	LE 303983	KT936308	KU986891	holotype
<i>G. scabripes</i>	AH 44220	KP144094	–	holotype
<i>G. simile</i>	ILLS61039	JQ256421	JQ256437	
<i>G. simile</i>	ILLS 71160	KF944381	KF944383	isoeptyp e

<i>G. uliginosum</i>	SAV 10162	KJ152695	KJ152696	
<i>G. umbratile</i>	ILLS 61040	JQ256422	JQ256438	
<i>G. umbratile</i>	K(M) 169625	KC222127	KC222140	
<i>G. variabilisporum</i>	AH 44216	KP144101	–	holotype
<i>Glutinoglossum peregrinans</i>	LE 303988	KX694170	KX694207	holotype
<i>Gl. pseudoglutinosum</i>	SAV F-10903	KX694178	KX694215	holotype
<i>Graddonina coracina</i>	ILLS 60491	JQ256423	JN012009	outgroup
<i>Hemileucoglossum alveolatum</i>	MICH s.n.	KP657560	KP657565	
<i>H. pusillum</i>	SAV F-11293	MF353090	MF353093	holotype
<i>Leucoglossum leucosporum</i>	LE 291874	KP272114	KP272115	
<i>L. leucosporum</i>	LE 291891	KP272112	KP272113	
<i>Maasoglossum asaetatum</i>	UPS F-118883	KP657562	KP657567	
<i>M. verrucisporum</i>	CUP-IN-000606	KP657563	KP657568	isotype
<i>S. arenarium</i> as <i>Thuemenidium arenarium</i>	ILLS 61043	JQ256426	JQ256440	
<i>S. arenarium</i> as <i>Thuemenidium arenarium</i>	OULU-F077201	GU324765	GU324764	
<i>S. arenarium</i>	Andrus Voitik 9335	GU324767	GU324766	
<i>Sabuloglossum monticola</i>	SAV F-11291	MW471105 *	MW471102 *	holotype
<i>S. monticola</i>	SAV F-11594	MW471106 *	–	

<i>S. monticola</i>	SAV F-11256	MW471104 *	—	
<i>S. monticola</i>	HR 94300	MW471103 *	MW471101 *	
<i>Sarcoleotia cinnamomea</i> as <i>Nothomitra cinnamomea</i>	ILLS 61042	JQ256424	JQ256439	
<i>Sarcoleotia globosa</i>	OSC63633	AY789410	AY789409	
<i>S. globosa</i>	MBH52476	AY789429	AY789428	
<i>Trichoglossum hirsutum</i>	ILLS 67355	KC222132	KC222145	
<i>T. hirsutum</i>	ILLS 61045	JQ256428	JQ256442	
<i>T. octopartitum</i>	ILLS 67356	KC222134	KC222147	
<i>T. variable</i>	ERRO 2011012206	KP144106	—	

* – sequences obtained in this study.

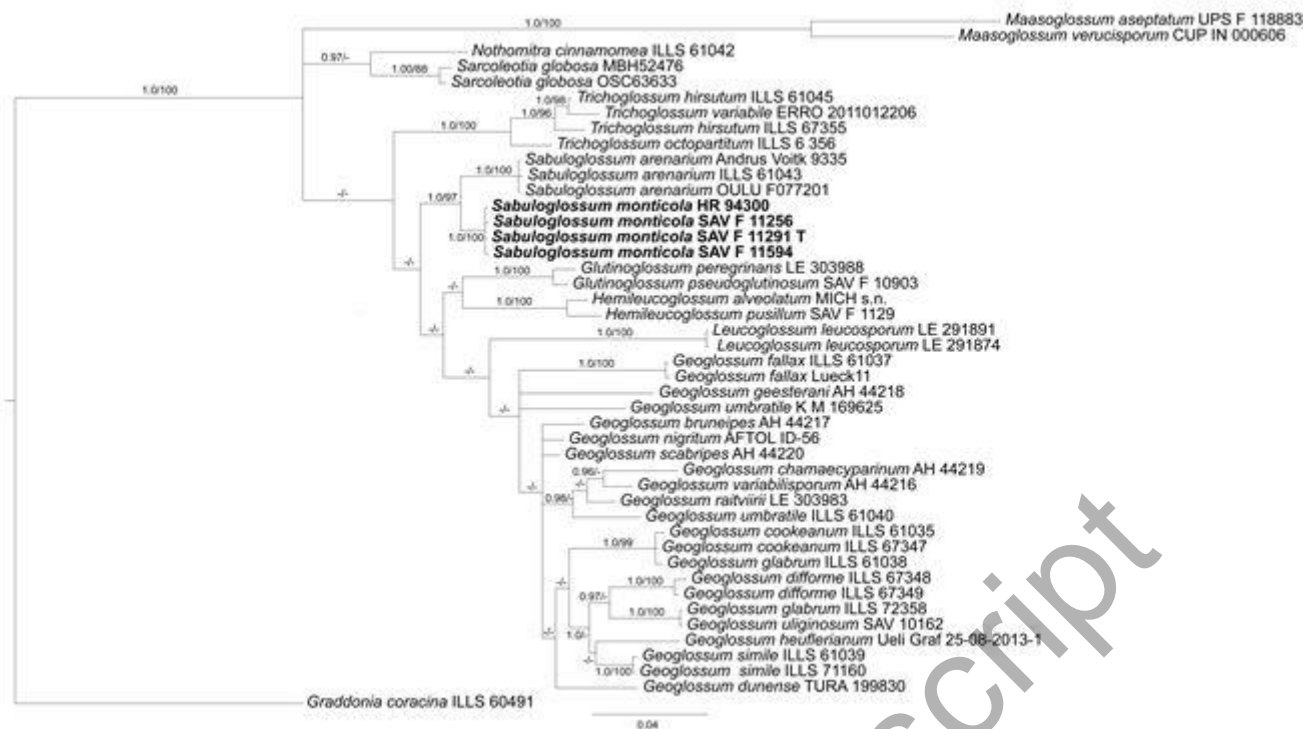


Fig. 1 Bayesian majority-rule consensus trees inferred from concatenated ITS and nrLSU dataset. Numbers above branches refer to posterior probability values of Bayesian inference (values above 0.95 are shown)/the bootstrap support as inferred for maximum likelihood analyses (values $\geq 75\%$ are shown). *Sabuloglossum monticola* is in bold



Fig. 2 *Sabuloglossum monticola* – ascomata and habitat. Pictures: **a, d–e, g** SAV F-11522, L. Janošík; **b, h** SAV F-11252, L. Janošík; **c** SAV F-11291 holotype, Z. Sochorová; **f** PRC 4685, L. Janošík



Fig. 3 *Sabuloglossum monticola* – microscopic characters. **a** free ascospores (water, SAV F-11594); **b** asci (water, SAV F-11522); **c** hymenium, septa in ascospores are highlighted by arrowheads (KOH, SAV F-11596); **d** hymenium (water, SAV F-11522), **e** euamyloid reaction of apical rings (IKI, SAV F-11594); **f–g** stipe scales (KOH, SAV F-11596); **h–i** paraphyses (water, SAV F-11594). Scale bars: **a, h–i** = 10 μ m, **b–g** = 20 μ m. Pictures: **a, e, h–i** A.

Polhorský; **b, d** L. Janošík; **c, f–g** V. Kučera

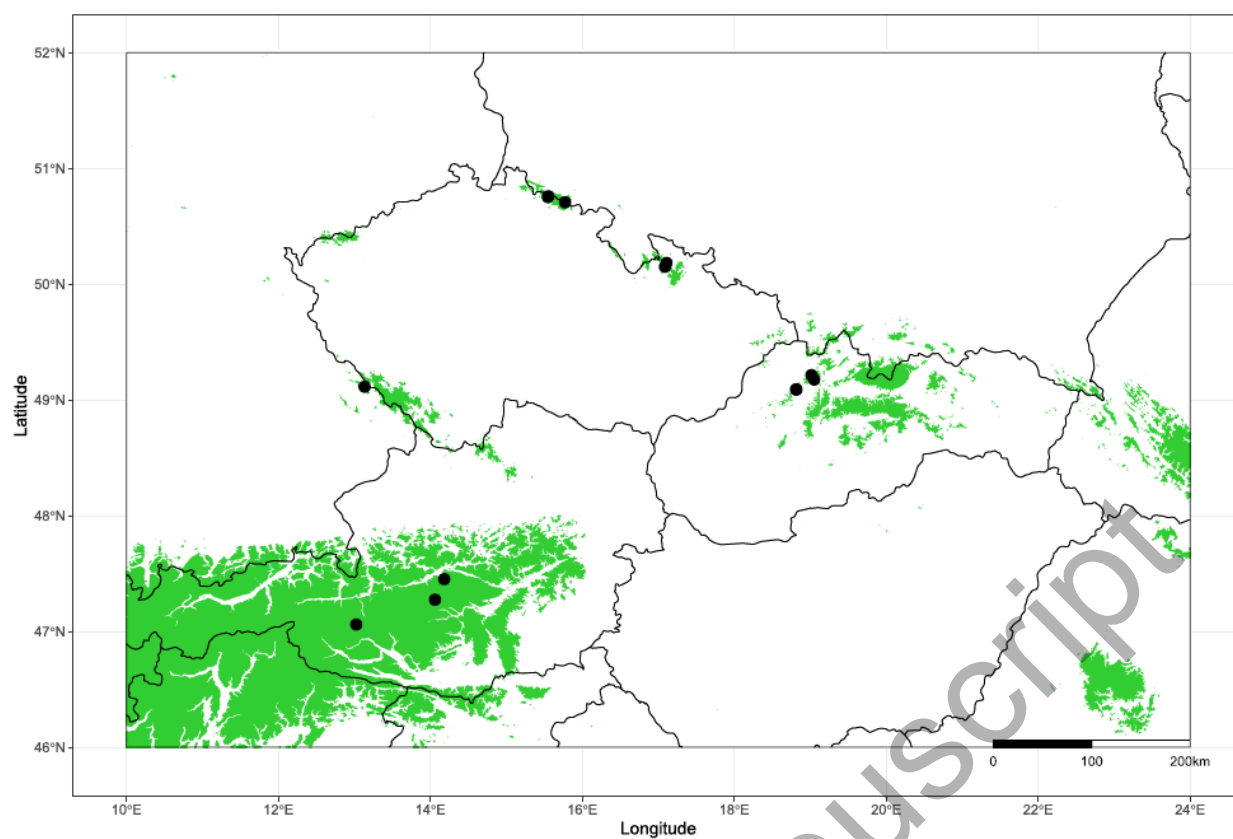


Fig. 4 Distribution of *Sabuloglossum monticola*. Mountain areas with altitude above 900 m are highlighted in green

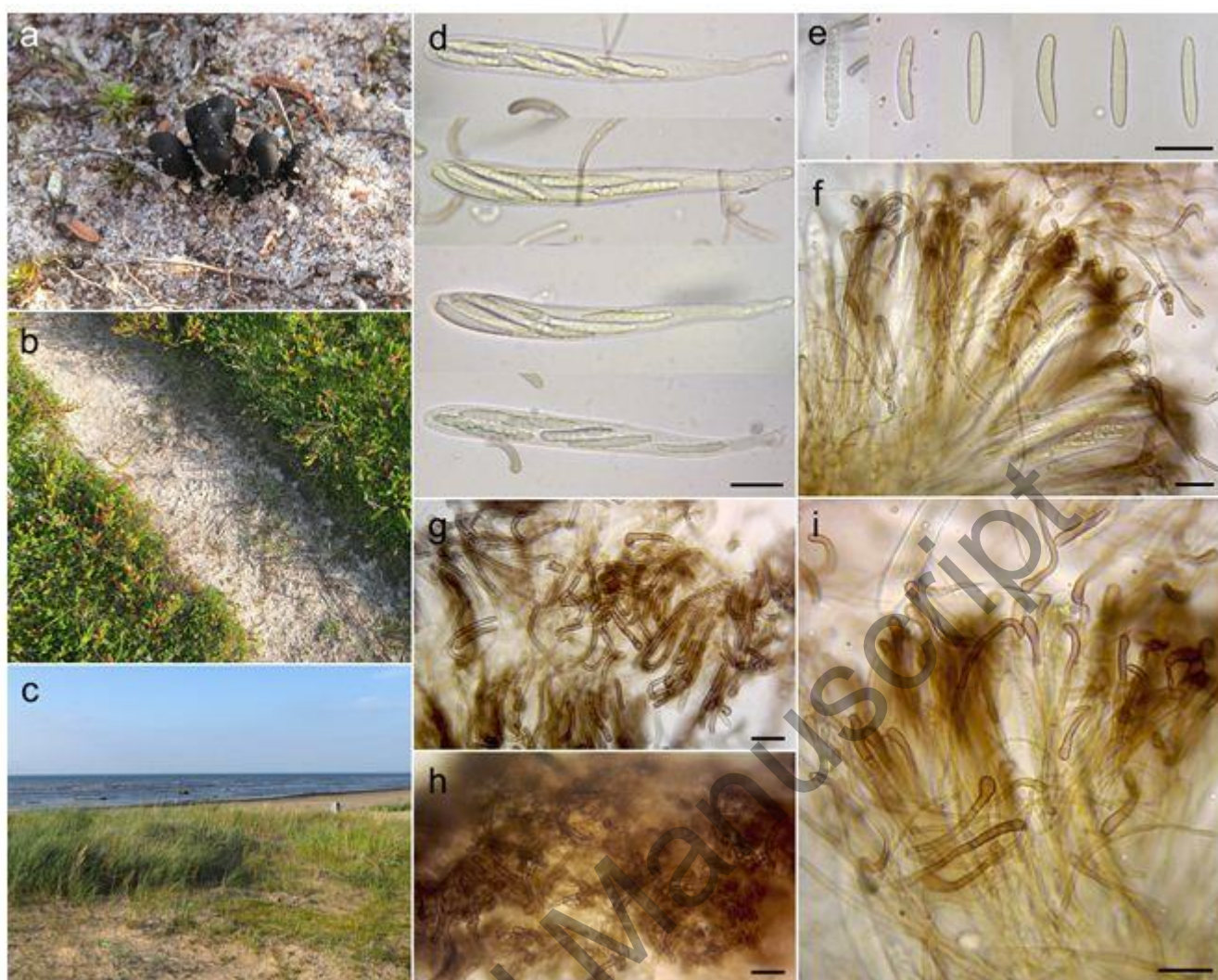


Fig. 5 *Sabuloglossum arenarium*. **a** ascomata (SAV F-11665); **b–c** habitat (SAV F-11665); **d** asci (SAV F-10729 & SAV F-11665); **e** free ascospores (SAV F-10729 & SAV F-11665); **f** hymenium (SAV F-10729); **g–h** stipe scales (SAV F-10729); **i** paraphyses (SAV F-10729 all in KOH). Scale bars: **d–i** = 20 µm. Pictures: a–i V. Kučera