



Generic circumscriptions in *Geoglossomycetes*

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Key words

Ascomycota
earth tongues
Geoglossum
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Abstract The class *Geoglossomycetes* is a recently created class of *Ascomycota*, currently comprised of one family (*Geoglossaceae*) and five genera (*Geoglossum*, *Nothomitra*, *Sarcoleotia*, *Thuemenidium* and *Trichoglossum*). These fungi, commonly known as earth tongues, have long been a subject of mycological research. However, the taxonomy within the group has historically been hindered by the lack of reliable morphological characters, uncertain ecological associations, and the inability to grow these fungi in culture. The phylogenetic relationships of *Geoglossomycetes* were investigated by conducting maximum likelihood and Bayesian analyses using a 4-gene dataset (ITS, LSU, MCM7, RPB1). Five well-supported monophyletic clades were found that did not correspond exactly with the currently recognised genera, necessitating a taxonomic revision of the group. Two new genera are proposed: *Glutinoglossum* to accommodate *G. glutinosum* and the newly described species *G. heptaseptatum*, and *Sabuloglossum* to accommodate *S. arenarium*. The type species of *Thuemenidium*, traditionally included within the *Geoglossaceae*, is confirmed as belonging to a separate lineage that is only distantly related to *Geoglossomycetes*.

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INTRODUCTION

Schoch et al. (2009b) created the class *Geoglossomycetes* and the order *Geoglossales* to contain three genera (*Geoglossum*, *Sarcoleotia* and *Trichoglossum*) in *Geoglossaceae*, which was previously placed in *Leotiomyces*. Fifty-three species in five genera (*Geoglossum* (22 species), *Nothomitra* (3 species), *Sarcoleotia* (4 species), *Thuemenidium* (5 species), and *Trichoglossum* (19 species)) are currently accepted in *Geoglossomycetes* (Kirk et al. 2008, Hustad et al. 2011); though many synonyms, dubious names and invalid names have been published in the group to date. Furthermore, several varieties and other infraspecific taxa have been recognised within the genera *Geoglossum* and *Trichoglossum*.

Geoglossomycetes are typically characterised by large, dark, club-shaped, terrestrial ascocarps with a fertile hymenium originating at the apex of the ascocarp, eventually intergrading with (*Geoglossum* and *Trichoglossum*) or abruptly terminating at (*Nothomitra* and *Sarcoleotia*) a sterile stipe. *Geoglossomycetes* ascospores range from dark brown to black, fusiform and multi-septate (*Geoglossum* and *Trichoglossum*), to light-coloured to hyaline, ellipsoid-fusiform and sparsely septate (*Nothomitra* and *Sarcoleotia*). Many morphological characters used to separate taxa are ambiguous within the group, as evidenced by more than 200 years of confusing classification at not only the species level, but also at higher taxonomic ranks. Identification of species is frequently compromised by a lack of appreciation that spore pigmentation and septation may not develop until a very late stage. *Geoglossomycetes* have been reported from every continent except Antarctica and are common components of many temperate and tropical mycobiota. Although previously identified in molecular environmental samples of soil hyphae and root endophytes (Bergemann & Garbelotto 2006, Wang et al.

2011), clear ecological connections between these fungi and plant hosts are lacking. Furthermore, the ascospores of these taxa do not germinate in culture, and their anamorphic states (if they exist) are unknown (Wang et al. 2006).

Despite being the focus of a number of morphological studies, modern molecular phylogenetic analyses of *Geoglossomycetes* are sparse. GenBank currently houses sequences from only 17 of 53 *Geoglossomycetes*. Preliminary molecular studies (Pfister & Kimbrough 2001, Wang et al. 2005) indicate that *Geoglossaceae* (as circumscribed at the time) does not form a monophyletic clade within *Leotiomyces* leading these authors to propose removal of several taxa from *Geoglossaceae*. These studies suggest that the inoperculate method of ascus dehiscence is not a sufficiently significant character to continue to group all earth tongues within *Leotiomyces* and that several taxa form a separate monophyletic clade basal to *Leotiomyces* deserving of a higher taxonomic rank. Sandnes (2006) examined nrDNA and found *Geoglossum*, *Sarcoleotia* and *Trichoglossum* to form a monophyletic clade. Using a 6-gene phylogeny with five ingroup species, Schoch et al. (2009b) found these genera to form a monophyletic group basal to *Leotiomyces* and proposed the class *Geoglossomycetes* and order *Geoglossales* to contain *Geoglossum*, *Sarcoleotia* and *Trichoglossum* within a single family, the *Geoglossaceae*. Ohenoja et al. (2010) recognised the wide separation between *Geoglossum* and the type species of *Thuemenidium*, although they did not complete the taxonomic work necessary to revise the latter genus. Recently, Hustad et al. (2011) included *Nothomitra* in *Geoglossomycetes* based on a 3-gene phylogeny.

Taxa assigned to *Geoglossomycetes* are considered to be of conservation significance in several European countries. There, many species are typical members of 'unimproved grassland' (non-intensively managed semi-natural grassland habitats that have not been treated with nitrogen fertiliser). The number of species present, along with those from three other fungal groups (the *Clavariaceae*, *Entolomataceae* and *Hygrophoraceae*) has been used as a proxy for grassland health, impacting on

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conservation value assessments (Newton et al. 2003, Genney et al. 2009). *Thuemenidium atropurpureum*, until recently assumed to be part of the *Geoglossomyces* clade, is listed under UK legislation (under its synonym *Geoglossum atropurpureum*) as a UK Priority species (<http://jncc.defra.gov.uk/speciespages/2290.pdf>) within the national Biodiversity Action Plan. Species of *Geoglossomyces* are included in the Norwegian (Kålås et al. 2010), Swedish (Gärdenfors 2010) and Swiss (Senn-Irlet et al. 2007) Red Data Lists.

The goal of this study was to examine the phylogenetic relationships within *Geoglossomyces* and its component genera using a robust 4-gene phylogeny with the largest sampling of species to date.

MATERIALS AND METHODS

Morphological analysis

Specimens were identified based on the morphology of ascomata and microscopic characters using the pertinent literature (e.g., Masee 1897, Durand 1908, Imai 1941, Nannfeldt 1942, Mains 1954, Maas Geesteranus 1964, Roobeek 2008, along with original species descriptions). Mature ascospores were obtained for measurement by tapping ascomata in a drop of water on a slide (Mains 1954). Ascomata were hand-sectioned and squash-mounted in water and images of micromorphological characters were captured with a QImaging QColor3 digital camera mounted on an Olympus BX51 compound microscope using differential interference microscopy. Images were processed using Adobe Photoshop v. 7.0 (Adobe Systems Inc., Mountain View, California). A minimum of 30 measurements was taken for all micromorphological structures when possible using NIH Image v. 1.63 (National Institutes of Health, Bethesda, Maryland). Taxonomic novelties and nomenclatural data were deposited in MycoBank (Crous et al. 2004).

Molecular procedures

Total genomic DNA was extracted from dried ascomata using a QIAGEN DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) and gene fragments were PCR amplified and sequenced following the methods outlined in Promputtha & Miller (2010) and Raja et al. (2011). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA), consisting of the ITS1, 5.8S and ITS2 regions, was amplified and sequenced using a combination of the primers ITS1F (Gardes & Bruns 1993), ITS5, ITS1, ITS4 (White et al. 1990) and ITS4A (Larena et al. 1999). A variety of primer combinations were used during amplification due to the frequent presence of introns in the 3' end of the adjacent 18S ribosomal small subunit in *Geoglossomyces*. The 28S large subunit (LSU) nrDNA region was amplified using JS1 (Landvik 1996) and LR6 (Vilgalys & Hester 1990) and sequenced with these primers in addition to the internal primers LR3 (Vilgalys & Hester 1990) and LR3R (Rehner & Samuels 1995).

In addition to the ribosomal genes, two protein coding genes were also used to infer taxonomic relationships at both lower and higher taxonomic levels. The minichromosome maintenance complex component 7 (MCM7) gene is a single-copy gene that codes for a DNA replication licensing factor required for DNA replication initiation and cell proliferation (Moir et al. 1982, Kearsey & Labib 1998). MCM7 has been found to produce highly accurate phylogenies in fungi (Aguileta et al. 2008, Schmitt et al. 2009), and it has been shown to be reliable across a wide range of ascomycete taxa, including *Geoglossomyces* (Raja et al. 2011). The primers 709F and 1348R (Schmitt et al. 2009) were used for PCR amplification and sequencing MCM7. The second protein-coding gene used

in this analysis was the RNA polymerase II subunit 1 (RPB1) gene. RPB1 codes the largest subunit of RNA polymerase II, the polymerase responsible for synthesising messenger RNA in eukaryotes. RPB1 was shown by Schoch et al. (2009a) to have the highest per-site informativeness (Townsend 2007) across six genes in the Ascomycota. The primers RPB1af and RPB1cr (Matheny et al. 2002) were used for amplification and sequencing of the RPB1 gene.

Phylogenetic analyses

Alignments of individual genes were created manually by eye in Sequencher 4.9 or by using Muscle v. 3.7 (Edgar 2004) in Seaview v. 4.2 (Galtier et al. 1996). Ambiguous regions were removed from the individual gene datasets using Gblocks v. 0.91b (Castresana 2000) under the following parameters: minimum number of sequences for both conserved and flanking regions = 22, maximum number of contiguous nonconserved positions = 8, minimum length of a block = 10, and allowed gap positions in 30 sequences. The Akaike Information Criterion (AIC) (Posada & Buckley 2004), implemented using jModelTest v. 0.1.1 (Posada 2008), determined GTR+I+G as the best-fit model of evolution for all four genes and this model was used in both maximum likelihood and Bayesian inference. Maximum likelihood (ML) analyses were performed using PhyML (Guindon & Gascuel 2003) under the GTR substitution model with six rate classes and invariable sites optimised. An unrooted BioNJ starting tree was constructed and the best of nearest neighbour interchange (NNI) and subtree pruning and regrafting (SPR) tree improvement was implemented during the heuristic search. Nonparametric bootstrap support (Felsenstein 1985) (BS) was determined with 100 replicates. Clades were considered significant and highly supported when BS \geq 70 % (Hillis & Bull 1993).

Bayesian inference employing a Markov Chain Monte Carlo (MCMC) algorithm was performed using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) on the CIPRES Science Gateway Teragrid (Miller et al. 2010) as an additional means of branch support. The GTR+I+G model with six rate classes was employed. Four independent chains of MCMC were run for 10 million generations to ensure that trees were not trapped in local optima. Clades with Bayesian posterior probability (BPP) \geq 95 % were considered significant and highly supported (Alfaro et al. 2003). Effective sample size (ESS) was estimated using Tracer v. 1.5 (Rambaut & Drummond 2009). Individual datasets of ITS, LSU, MCM7 and RPB1 were examined for potential conflict before concatenated into a single dataset for total evidence analysis (Kluge 1989, Eernisse & Kluge 1993). Individual gene phylogenies were considered to be incongruent if clades with significant ML BS and BPP (\geq 70 % BS and/or \geq 95 % BPP) were conflicting in the individual tree topologies (Wiens 1998, Alfaro et al. 2003, Lutzoni et al. 2004). Since there were no incongruencies found among the individual datasets, all genes were concatenated using Seaview v. 4.2 with the following gene order: ITS, LSU, MCM7, RPB1. Phylogenetic analyses were then performed on the concatenated dataset as above. Alignments and analyses were deposited in TreeBASE (<http://treebase.org>) under submission ID 13597.

Shimodaira-Hasegawa (S-H) tests (Shimodaira & Hasegawa 1999) were performed in PAUP v. 4.0b10 (Swofford 2003) to test generic hypotheses. Separate maximum likelihood analyses were conducted with: 1) all taxa in *Geoglossum* constrained to be monophyletic; and 2) *Thuemenidium* species constrained in a monophyletic genus. S-H tests using RELI approximation and 1 000 bootstrap replicates were then conducted to compare these constrained trees to the most-likely tree.

RESULTS

Fifty-nine sequences were newly generated in this study, including 13 ITS, 13 LSU, 12 MCM7 and 21 RPB1 sequences (Table 1). These were analysed together with 12 ITS, 12 LSU and 12 MCM7 sequences from our previous studies (Hustad & Miller 2011, Hustad et al. 2011) along with 35 sequences obtained from GenBank. Forty-three collections representing a total of 15 *Geoglossomycetes* and nine outgroup species were included in the analyses. Of the 43 taxa included in the final dataset, one taxon lacks ITS, 19 lack MCM7 and 22 lack RPB1 (Table 1). In the combined dataset, sequences for all four markers were available for 50 % of the taxa. For those taxa with missing data, at least two of the four DNA markers were available in 95 % (20/21) of the taxa.

No incongruencies were found among the individual datasets. The final combined data matrix had an aligned length of 2 887 base pairs, which was reduced to 2 393 after the removal of 494 ambiguous characters by Gblocks. Of the 2 393 characters used in these phylogenetic analyses, 125 were constant, 648 were parsimony-uninformative and 1 620 were parsimony-informative. A burn-in of 10 % was estimated using Tracer v. 1.5 to be sufficient to remove the pre-stationary posterior probability distribution, producing an ESS value of 520.912. The standard deviation of split frequencies was determined by MrBayes v. 3.1.2 to be 0.002488 at the end of the Bayesian analysis.

Fig. 1 represents the most likely tree produced by PhyML of the 4-gene dataset of *Geoglossomycetes* generated in this study.

Five well-supported clades of *Geoglossomycetes* were recovered. *Geoglossum* occurred as a distinct clade with high overall support (97 % BS, 100 % BPP). *Trichoglossum* was well supported as monophyletic with 95 % BS and 100 % BPP branch support. Five representatives of *Geoglossum glutinosum*, containing two individuals of a previously undescribed cryptic species, were recovered as a well-supported (85 % BS, 100 % BPP) clade distinct from the main *Geoglossum* clade. *Thuemenidium* was found to be paraphyletic with *T. arenarium* existing as a clade with strong support (100 % BS, 100 % BPP) separate from *T. atropurpureum*, which is most closely related to *Microglossum* in *Leotiomyces*. *Sarcoleotia* and *Nothomitra* were supported (74 % BS, 98 % BPP) as the most basal clade in *Geoglossomycetes*.

Two S-H tests were performed comparing the most-likely tree (Fig. 1) with the maximum likelihood trees from a search constrained to recover 1) all taxa in *Geoglossum* (i.e., including *G. glutinosum*) as monophyletic; and 2) *Thuemenidium* (i.e., *T. atropurpureum* and *T. arenarium*) as monophyletic. In both cases, this test rejected the hypothesis of a monophyletic *Geoglossum* (-ln L difference = 48.13503, P = 0.001) and monophyletic *Thuemenidium* (-ln L difference = 540.18521, P = 0.001).

Table 1 List of taxa, collection numbers, fungarium accession numbers and GenBank numbers for specimens used in this study.

Species	Coll./Strain no.	Fungarium no.	ITS	LSU	MCM7	RPB1
<i>Geoglossum barlae</i>	Moingeon s.n.	ILLS 61034	JQ256416	JQ256433	JQ256444	KC222160
<i>Geoglossum cookeanum</i>	ANM 2257	ILLS 61035	JQ256417	JQ256434	JQ256445	KC222161
	J. Gaisler s.n.	ILLS 67347	KC222122	KC222135	N/A	N/A
<i>Geoglossum difforme</i>	ANM2169	ILLS 61036	JQ256418	JN673044	JN672990	KC222162
	VPH s.n.	ILLS 67348	KC222123	KC222136	KC222148	N/A
	ASM 10498	ILLS 67349	KC222124	KC222137	KC222149	KC222163
<i>Geoglossum glabrum</i>	ANM 2267	ILLS 61038	JQ256420	JQ256436	JQ256447	KC222164
	OSC 60610	GenBank	AY789318	AY789317	N/A	N/A
<i>Geoglossum nigratum</i>	AFTOL-ID 56	GenBank	DQ491490	AY544650	N/A	N/A
<i>Geoglossum simile</i>	ANM 2171	ILLS 61039	JQ256421	JQ256437	JQ256448	KC222165
	ASM 10528	ILLS 67350	KC222125	KC222138	KC222150	KC222166
<i>Geoglossum sphagnophilum</i>	Poumarat s.n.	ILLS 67351	KC222126	KC222139	KC222151	KC222167
<i>Geoglossum umbratile</i>	CFR 251108	ILLS 61040	JQ256422	JQ256438	JQ256449	KC222168
	Medardi s.n.	K(M): 169625	KC222127	KC222140	KC222152	KC222169
	Mycorec 1840	GenBank	AY789304	AY789303	N/A	N/A
<i>Glutinoglossum glutinosum</i>	ANM 2231	ILLS 67352	KC222128	KC222141	KC222153	KC222170
	J. Gaisler s.n.	ILLS 67353	KC222129	KC222142	KC222154	KC222171
	1100649	GenBank	N/A	AY789310	N/A	N/A
<i>Glutinoglossum heptaseptatum</i>	J. Gaisler s.n.	ILLS 63754	KC222130	KC222143	KC222155	KC222172
	J. Gaisler s.n.	K(M): 165359	KC222131	KC222144	KC222156	N/A
<i>Graddonia coracina</i>	ANM 2018	ILLS 60491	JQ256423	JN012009	JN672993	KC222173
<i>Microglossum olivaceum</i>	FH-DSH97-103	GenBank	AY789398	AY789397	N/A	N/A
<i>Microglossum rufum</i>	Ingo-Clark-Geo 163	GenBank	DQ257360	DQ470981	N/A	N/A
<i>Neolecta vitellina</i>	OSC 119159	GenBank	FJ171854	FJ171881	N/A	N/A
<i>Nothomitra cinnamomea</i>	Moingeon s.n.	ILLS 61042	JQ256424	JQ256439	JQ256450	KC222174
<i>Orbilina auricolor</i>	AFTOL-ID 906	GenBank	DQ491512	DQ470953	N/A	N/A
<i>Orbilina delicatula</i>	DHP 108	GenBank	U72595	AY261178	N/A	N/A
<i>Sabuloglossum arenarium</i>	CFR 181007	ILLS 61043	JQ256426	JQ256440	JQ256452	KC222175
	OULU-F077201	GenBank	GU324765	GU324764	N/A	N/A
	A. Voitk 9335	GenBank	GU324767	GU324766	N/A	N/A
<i>Sarcoleotia globosa</i>	OSC 63633	GenBank	AY789410	AY789409	N/A	N/A
	MBH 52476	GenBank	AY789429	AY789428	N/A	N/A
<i>Sarcoleotia turficola</i>	H253397	GenBank	AY789278	AY789277	N/A	N/A
<i>Spathularia flavida</i>	wz95	GenBank	AF433155	AF433144	N/A	N/A
<i>Thuemenidium atropurpureum</i>	ASM 4931	ILLS 61044	JQ256427	JQ256441	JQ256453	KC222176
	s.n.	K(M):135612	EU784253	AY789307	N/A	N/A
<i>Trichoglossum hirsutum</i>	ANM 2233	ILLS 67355	KC222132	KC222145	KC222157	KC222177
	J. Gaisler s.n.	ILLS 61045	JQ256428	JQ256442	JQ256454	KC222178
	OSC 61726	GenBank	AY789314	AY789313	N/A	N/A
	AFTOL-ID 64	GenBank	DQ491494	AY544653	N/A	N/A
	81362	HKAS 55133	KC222133	KC222146	KC222158	KC222179
<i>Trichoglossum octopartitum</i>	JPP 10191	ILLS 61046	JQ256429	JQ256443	JQ256455	KC222180
	ANM 2227	ILLS 67356	KC222134	KC222147	KC222159	KC222181

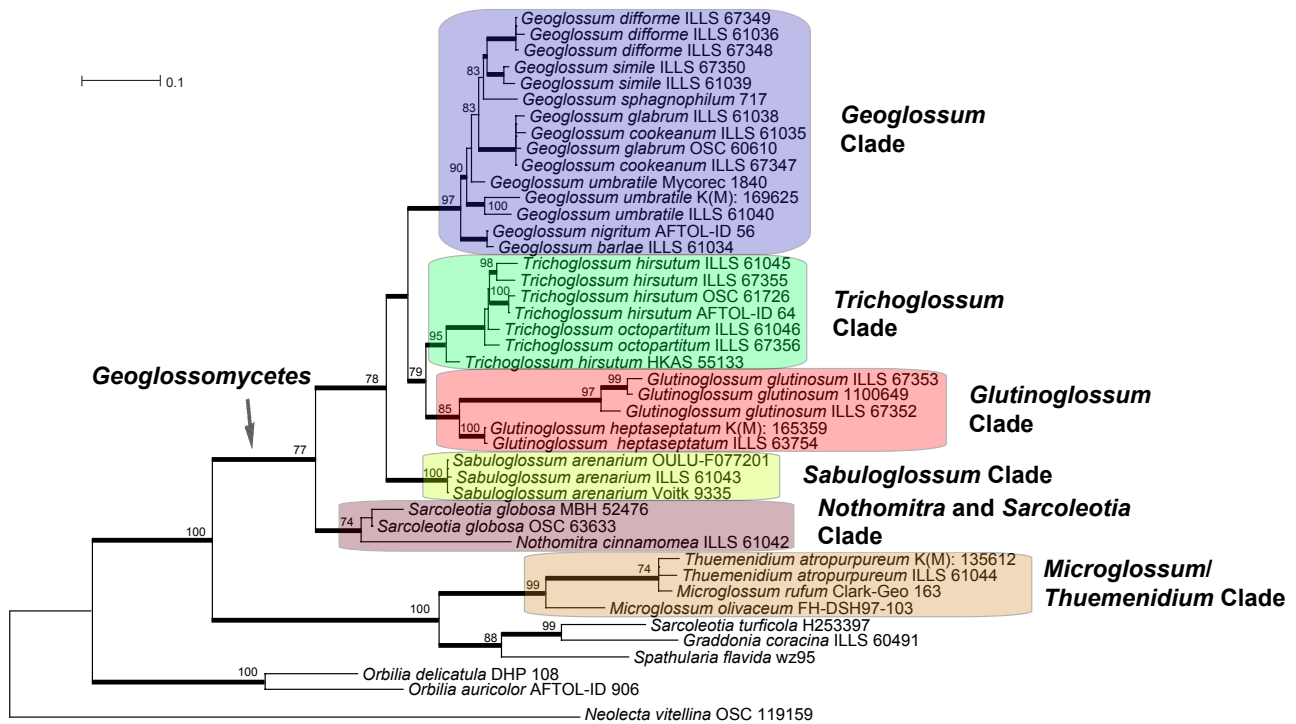


Fig. 1 PhyML maximum likelihood phylogeny of *Geoglossomycetes*, based on a combined dataset (2393 bp) of ITS, LSU, MCM7 and RPB1 DNA sequences representing 24 species (–lnL score = 18379.50385). Thickened branches indicate significant Bayesian posterior probabilities ($\geq 95\%$); numbers at nodes indicate significant PhyML bootstrap support values ($\geq 70\%$) based on 100 replicates. *Neolecta vitellina*, *Orbiliomyces* and *Leotiomyces* were used as outgroup taxa. Numbers associated with taxon names are fungarium accession numbers or strain numbers obtained from GenBank.

Taxonomy

Glutinoglossum Hustad, A.N. Mill., Dentinger & P.F. Cannon, *gen. nov.* — MycoBank MB801343

Type species. *Glutinoglossum glutinosum* (Pers.) Hustad, A.N. Mill., Dentinger & P.F. Cannon, *comb. nov.*

= ?*Geoglossum* subgen. *Cibalocoryne* Hazsl., Magyar Tud. Akad. Értes., A Termés-tud Kőr. 11, 19: 8. 1881.

Etymology. From Latin *glutinosus*, referring to the viscid character of the ascocarp.

Ascocarp viscid-gelatinous, black, stipitate, with fertile hymenium usually restricted to the upper portion. *Paraphyses* prominent, continuing beyond the hymenium and forming a distinct gelatinous layer, mostly straight, pale brown, apical cell enlarged. *Asci* clavate to cylindrical with J+ apical pore. *Ascospores* slow-maturing, initially hyaline and aseptate, becoming septate and coloured in maturity.

Glutinoglossum glutinosum (Pers.) Hustad, A.N. Mill., Dentinger & P.F. Cannon, *comb. nov.* — MycoBank MB802301; Fig. 2

Holotype. In L, Herb. Lugd. Bat. no. 910.261-767 (L 0110938 [Persoon Herb.]), assumed to be collected in Europe.

Basionym. *Geoglossum glutinosum* Pers., *Observ. Mycol.* 1: 11. 1796.
 ≡ *Gloeoglossum glutinosum* (Pers.) E.J. Durand, *Ann. Mycol.* 6: 419. 1908.
 ≡ *Cibalocoryne* ('*Cibarocoryne*') *glutinosa* (Pers.) S. Imai, *Bot. Mag. (Tokyo)* 56: 525. 1942, *nom. inval.* (Art. 43.1).

= *Geoglossum viscosum* Pers., *Comment. Fung. Clav.*: 39. 1797.
 = *Geoglossum glutinosum* β *lubricum* Pers., *Mycol. Eur.* 1: 197. 1822.
 = ?*Geoglossum* (*Cibalocoryne*) *viscosulum* Hazsl., Magyar Tud. Akad. Értes., A Termés-tud Kőr. 11: 8. 1881.

Ascocarps scattered to caespitose, very viscid, becoming gelatinous when wet, clavate, 15–55 mm in height; hymenium black, 1/3 to 1/2 the length of the ascocarp, bilaterally compressed, clavate, cylindrical or ellipsoidal, 3–6 mm wide,

sometimes with a vertical median groove; stipe dark brown to black, terete, glabrous, viscid, 10–40 × 2–3 mm. *Paraphyses* hyaline below, light to dark brown above, 2–4 μm diam at base, 4–11 μm diam at apex, sparsely septate with the terminal cell enlarged and globose, broadly obovoid, or pyriform, continuing down the stipe in a thick gelatinous layer. *Asci* slender, clavate, (175–)220–265(–290) × (10–)12–16 μm, 8-spored, apical pore J+ in Melzer's reagent. *Ascospores* clavate, straight to slightly curved, (55–)70–90(–100) × 4–5.5 μm, often aseptate when young, usually 3- or 5-septate when fully mature, occasionally becoming 7-septate, initially hyaline, eventually becoming brown.

Habitat — On soil in wet places and in unfertilised grassland. Found associated with hardwoods in North America and commonly encountered in pastures and dune slacks in Europe. Reported from Africa: Macronesia (Spooner 1987); Asia: China (Tai 1944), India (Batra & Batra 1963, Maas Geesteranus 1965, Prasher & Sharma 1997), Japan (Imai 1941), Philippines (Baker 1914); Australasia: Australia (Spooner 1987), New Zealand (<http://hiddenforest.co.nz/>); Europe: Austria (von Keissler 1916), Bulgaria (Hinkova & Stoichev 1983), Czech Republic (this paper), Denmark (Lind 1913), Finland (Karsten 1871), France (Bigeard 1898), Germany (Rabenhorst 1857), Hungary (Hazslinszky 1881), Ireland (<http://www.gbif.org>), Netherlands (Oudemans 1873, this paper), Norway (Eckblad 1963), Sweden (Nannfeldt 1942), United Kingdom (Dennis 1978, this paper); North America: Bermuda (Waterson et al. 1945), Canada (Durand 1908), USA (Durand 1908, Mains 1954). These records probably encompass several species as defined using modern phylogenetic methods.

Conservation — Not formally assessed on a global scale but would probably be listed as of Least Concern, though in Europe its grassland habitat is widely threatened due to agricultural 'improvement'. It is listed as Critically Endangered in the Red Data Book of Bulgaria (Peev 2011).

Specimens examined. CZECH REPUBLIC, Mada Boleslav, Baba u Kosmonos, deciduous forest, south slope, 30 Oct. 2010, *J Gaisler s.n.* (ILL 64443);

Liberec, Hamrstejn, deciduous forest, south slope, 25 Aug. 2010, *J Gaisler s.n.* (ILLS 64451); Rasovka, mowed meadow, southeast slope, 4 Oct. 2010, *J Gaisler s.n.* (ILLS 67353); Jablonne v Podjestadi, in grass and moss, 20 Oct. 2010, *Z Egertova s.n.* (ILLS 64453). — THE NETHERLANDS, North Holland, Bergen, on Slaperdijk, N52°43', E4°39', 24 Nov. 2008, *CF Roobeek*, CFR-241108-D (ILLS 64449). — UNITED KINGDOM, Clitheroe, Billington, Whalley Old Road, Moonside Cottage, on acid, mossy soil, N53°48', W2°25', 12 Oct. 1996, *I Ridge s.n.* (ILLS 64450); Wales, Trefor, on short grass and moss, N52°59', W4°26', 9 Oct. 2011, *VP Hustad, PF Cannon, BTM Dentinger & AM Ainsworth*, ANM2456 (ILLS 64445); Snowdonia National Park, in sheep-grazed grass and moss, N53°4', W4°4', 13 Oct. 2011, *VP Hustad, PF Cannon & BTM Dentinger*, ANM2476 (ILLS 64446); Scotland, Skye, Sleat, Tokavaig, in short grass along roadway N57°7', W5°58', 16 Oct. 2011, *VP Hustad & PF Cannon*, ANM2485 (ILLS 64447); Talisker Beach, in sheep-grazed grass, N57°16', W6°27', 21 Oct. 2011, *VP Hustad, PF Cannon, DR Genney & AJ Silverside*, ANM 2529 (ILLS 64448). — UNITED STATES, North Carolina, Haywood Co., Great Smoky Mountains National Park, Cataloochee, Caldwell Fork Trail, mixed deciduous forest soil, N35°37', W83°6', 762 m elev., 14 Aug. 2009, *VP Hustad & AS Methven*, ANM2177 (ILLS 64360); Swain Co., Great Smoky Mountains National Park, Smokemont, mixed deciduous forest soil, N35°33', W83°18', 640 m elev., 16 Aug. 2009, *VP Hustad & AS Methven*, ANM2247 (ILLS 64444); Tennessee, Sevier County, Great Smoky Mountains National Park, Greenbrier, soil among *Thuidium* moss, N35°42', W83°22', 549 m elev., 15 Aug. 2009, *VP Hustad & AS Methven*, ANM2231 (ILLS 64443).



Fig. 2 *Glutinoglossum glutinosum*. a. *In situ* photograph of fresh ascocarps (© Jan Vesterholt/Mycokey); b. ascus from dried material (total magnification = 200 \times); c. ascospores from dried material (total magnification = 480 \times); d. paraphyses from dried material (total magnification = 400 \times). Micrographs from specimen ILLS 67353, used in this study. — Scale bars: b = 20 μ m; c, d = 10 μ m.

Glutinoglossum heptaseptatum Hustad, A.N. Mill., Dentinger & P.F. Cannon, *sp. nov.* — MycoBank MB802302; Fig. 3

Holotype. CZECH REPUBLIC, Hradec Králové, Betlem, moist pasture with moss, 20 Oct. 2010, *J Gaisler s.n.* (ILLS 63754).

Etymology. Refers to the predominantly 7-septate ascospores.

Macroscopically indistinguishable from *Glutinoglossum glutinosum*. Characterised by wider asci (170–205 \times 18–22 μ m) and predominantly 7-septate ascospores (55–60–80(–90) \times 4–6.5 μ m).

Habitat — On soil in wet places. At present, known only from a single locality in the Czech Republic. According to literature the species may also be present in Asia (Imai 1941), Australia (Spooner 1987), and North America (Mains 1954), but this very wide potential distribution may indicate that more than one taxon is involved.

Conservation — Not formally assessed. Its only definitely known locality, the Grassland Research Station Liberec, is a protected experimental pasture, subjected to extensive grazing since 1998, that was previously an abandoned meadow. The site is property of the Crop Research Institute Prague – Ruzyně and is not threatened by agriculture or urban sprawl.

Specimens examined. CZECH REPUBLIC, Hradec Králové, Betlem, 12 km north of Liberec, Protected Landscape Area Jizerske hory (Jizera Mountains), Grassland Research Station Liberec, moist pasture with *Festuca rubra*, *Agrostis capillaris*, *Cirsium palustre*, and moss, N50°50', W15°5', Oct. 2009, *J. Gaisler s.n.*, K(M): 165359; 20 Oct. 2010, *J. Gaisler s.n.* (ILLS 63754).

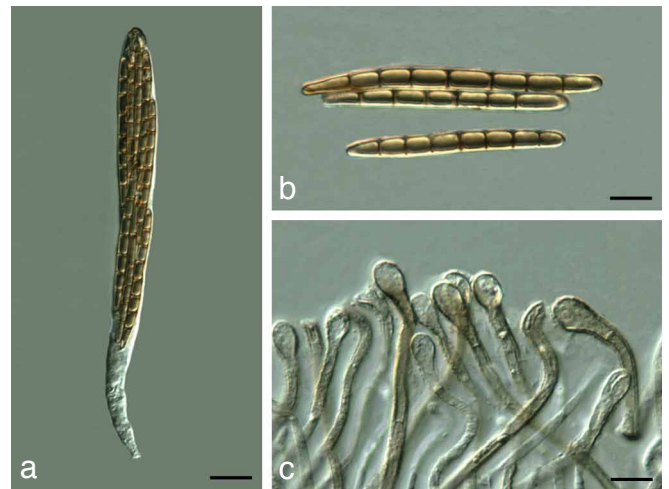


Fig. 3 *Glutinoglossum heptaseptatum*. a. Ascus from dried material (total magnification = 200 \times); b. ascospores from dried material (total magnification = 400 \times); c. paraphyses from dried material (total magnification = 400 \times). Micrographs from specimen ILLS 63754, used in this study. — Scale bars: a = 20 μ m; b, c = 10 μ m.

Sabuloglossum Hustad, A.N. Mill., Dentinger & P.F. Cannon, *gen. nov.* — MycoBank MB802197

Type species. *Sabuloglossum arenarium* (Rostr.) Hustad, A.N. Mill., Dentinger & P.F. Cannon, *comb. nov.*

Etymology. The genus name is derived from the Latin *sabulum*, referring to the ecology of its only known species.

Ascocarps brownish black to black with fertile head slightly darker than, though not distinct from, the stipe. *Stipe* often squamulose, terete. *Paraphyses* longer than asci, light to dark brown and somewhat inflated at the apex. *Ascospores* hyaline and smooth, often 1-celled though occasionally becoming septate at maturity, straight or slightly curved with rounded ends, often multiguttulate.

Sabuloglossum arenarium (Rostr.) Hustad, A.N. Mill., Dentinger & P.F. Cannon, *comb. nov.* – MycoBank MB802198; Fig. 4

Holotype. In C, no. C-F-70804 (ex. herb. Rostrup), collected in East Greenland, 17 Aug. 1890.

Basionym. *Microglossum arenarium* Rostr., Bot. Tidsskr. 18: 76. 1892.
 ≡ *Mitrla arenaria* (Rostr.) Masee, Ann. Bot. (Oxford) 11, 42: 283. 1897.
 ≡ *Corynetes arenarius* (Rostr.) E.J. Durand, Ann. Mycol. 6: 417. 1908.
 ≡ *Geoglossum arenarium* (Rostr.) Lloyd, Mycol. Notes 5: 8. 1916.
 ≡ *Thuemenidium arenarium* (Rostr.) Korf in Petersen & Korf, Nordic J. Bot. 2: 152. 1982.
 = *Leptoglossum latum* Peck, Bull. Torrey Bot. Club 22: 210. 1895.
 = *Corynetes geoglossoides* Eckblad, Nytt Mag. Bot. 10: 141. 1963.

Ascocarps brownish black, fertile head slightly darker but not distinct from stalk, caespitose, broadly and irregularly clavate, 20–40 mm in height, 5–20 cm thick at apex. Paraphyses dark brown, becoming nearly opaque above, filiform, strongly curved above, occasionally straight, 3–4 µm thick at apex, not agglutinated. Asci narrowly clavate, 130–160 × 18–35 µm, 8-spored, apical pore J+ in Melzer's reagent. Ascospores nearly cylindrical

with rounded ends or slightly clavate, hyaline, becoming yellowish to light brown with age, aseptate, 27–37 × 3.5–5 µm.

Habitat — On sand dunes and dune slacks, also in sandy soil alongside rivers and lakes. Reported from Asia: Japan (Imai 1941); Europe: Denmark (Rostrup 1892b, Lind 1913), Germany (Schade 1939), Greenland (Rostrup 1892a), Iceland (Hallgrímsson 1987), Netherlands (van Luyk 1919, Roobeek 2008), Norway (Rostrup 1904, Imai 1940, Eckblad 1963), Sweden (Andersson 1950, Granquist 1950), United Kingdom (Ramsbottom 1926) and North America: Canada (Labrador and Newfoundland; Durand 1908), USA (Mains 1955). This very wide distribution might indicate that the species as currently circumscribed is a composite.

Conservation — Not formally assessed on a global scale. *Sabuloglossum arenarium* was assessed as Endangered in the provisional Red Data List of British Fungi (Ing 1992). *Sabuloglossum arenarium* was mistakenly synonymised with *Thuemenidium atropurpureum* by Cannon et al. (1985). This may have confused some of the European conservation assessments of the latter species, which is listed on the Red Data List of nine European countries and was proposed for inclusion in the Appendices of the Bern Convention (Dahlberg & Croneborg 2003). *Sabuloglossum arenarium* has a much more restricted distribution in the UK than has *T. atropurpureum*, but may not face the same conservation threats due to its different ecological requirements.

Leptoglossum latum was described from sandy soil in Labrador by Peck (1895). We have not seen authentic material, but the description is very similar to that of *S. arenarium* so we follow Durand (1908) in placing the two species in synonymy. *Corynetes geoglossoides* was described by Eckblad (1963) as distinct from *S. arenarium* (treated by him as *Corynetes arenarius*), but the two taxa occur in identical habitats and have similar distributions. The only difference cited by Eckblad was that *C. geoglossoides* possessed some asci that eventually formed pigmented ascospores while all of those of *C. arenarius* remained hyaline. However, we have observed that ascospore pigmentation may occur very late in the developmental cycle and do not consider this as sufficient justification for maintaining the taxa as separate species in the absence of molecular data.

Specimens examined. SWEDEN, Västerbotten, Sävar parish, Långvikskatan, in sandy heath amongst *Empetrum*, 22 Oct. 1980, J. Nitare, Fungi Exsiccati Suecici 3301 [UPS(F-005445) 61577]. — THE NETHERLANDS, North Holland, near Bergen aan Zee, N52°41', E4°38', 18 Oct. 2007, C.F. Roobeek, CFR181007 (ILLS 61043).

DISCUSSION

The molecular phylogeny of *Geoglossomycetes* presented here is the most robust and taxonomically diverse sampling of the group to date. Our results concur with previous authors (Schoch et al. 2009b) that *Geoglossomycetes* forms a separate and well-supported clade within *Pezizomycotina* (77 % BS, 98 % BPP). Five well-supported clades representing six genera were shown to occur within *Geoglossomycetes* in our analyses (Fig. 1). In addition, our analyses confirmed the polyphyletic nature of *Thuemenidium* as claimed by Ohenoja et al. (2010), with one clade within the *Geoglossomycetes* and the other within the *Helotiales*.

Although some support seems to exist for circumscribing groups of genera into higher-level hierarchies, we consider any creations of new orders and families to be premature at this stage. Nevertheless, *Sarcoleotia* and *Nothomitra* form a morphologically distinct and phylogenetically well-supported clade (74 % BS, 98 % BPP) within *Geoglossomycetes*. This separation suggests a higher-level differentiation from the remainder of *Geoglossomycetes*, possibly indicative of a separate order and

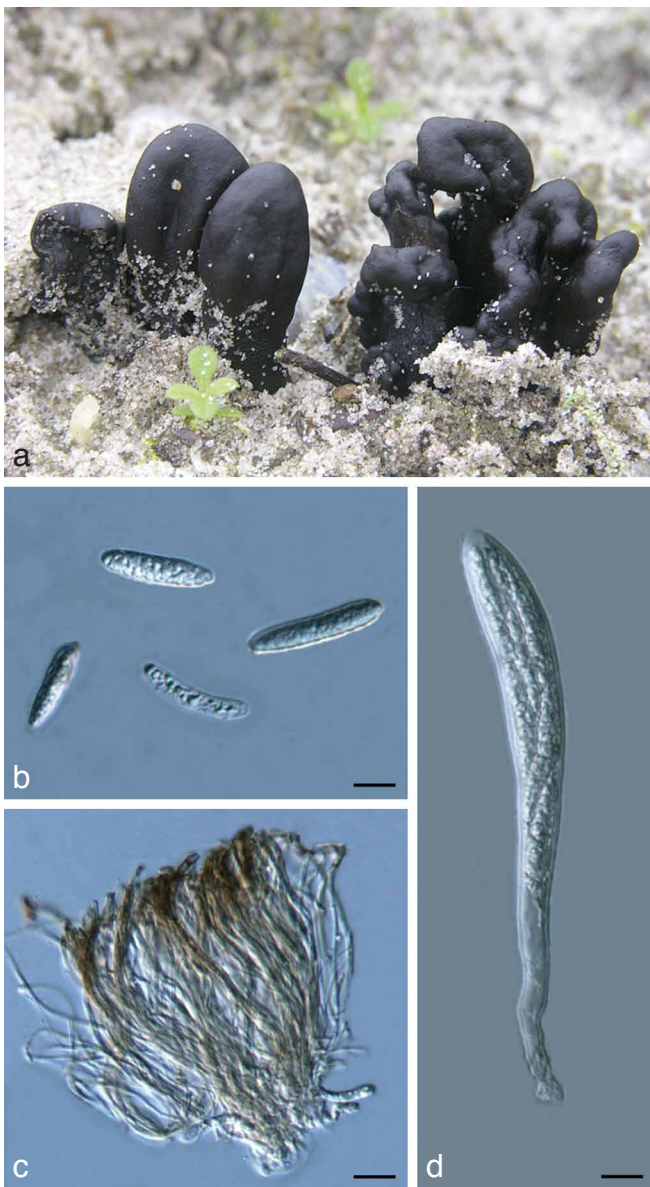


Fig. 4 *Sabuloglossum arenarium*. a. *In situ* photograph of fresh ascocarps (© Jan Vesterholt/Mycokey); b. ascospores from dried material (total magnification = 400×); c. paraphyses from dried material (total magnification = 200×); d. ascus from dried material (total magnification = 400×). Micrographs from specimen ILLS 61043, used in this study. — Scale bars: b, d = 10 µm; c = 20 µm.

family within the class. *Sabuloglossum* is highly distinct within *Geoglossomycetes* and may represent a separate family within the class. *Glutinoglossum* and *Trichoglossum* are also present on a well-supported distinct clade (79 % BS, 99 % BPP), and future research will determine if changes in higher-level taxonomy are warranted for each of these discrete groups.

Geoglossum clade

The genus *Geoglossum* occupies a well-supported clade (97 % BS, 100 % BPP). Sequences of two specimens identified previously as *G. glabrum*, the type species of the genus (see discussion below), occur in a well-supported clade with two representatives of *G. cookeanum*. As circumscribed by earlier authors (e.g., Durand 1908, Nannfeldt 1942), these taxa are separated by slight morphological differences and specimens used in this study were found to be indistinguishable based on molecular analyses. Comparison of the ITS locus (i.e., ITS1, 5.8S and ITS2 rDNA) of several collections of both taxa (data not shown) reveals less than 2 % variation in sequence across the entire gene region, providing a preliminary indication that only a single species is present (Hughes et al. 2009). Additional research is needed before definitive taxonomic changes can be made in this species complex.

Geoglossum difforme and *G. simile* are strongly supported as monophyletic species within *Geoglossum*. Collections identified as *G. umbratile* are polyphyletic with the single GenBank representative (only ITS and LSU sequences) differing from the two sequences generated in this project, suggesting the GenBank representative may be misidentified. Species delimitations within the *G. barlae*/*G. nigritum*/*G. umbratile* complex are ambiguous, and these taxa have occasionally (e.g., Masee 1897, Nannfeldt 1942) been considered synonymous. This species complex warrants additional study and will doubtlessly be a subject of future investigation.

Some controversy has surrounded the choice of *G. glabrum* Pers. as the type species of *Geoglossum* (Spooner 1987). Persoon first described the genus *Geoglossum* in 1794, including an abbreviated diagnosis and listing four species, *G. glabrum* (as a replacement name for *Clavaria ophioglossoides* L., a species for which no original material exists beyond a simple illustration; Vaillant 1727), *G. hirsutum* (a replacement name for *Clavaria atra* Batsch and now treated as *Trichoglossum hirsutum* (Pers.) Boud.), *G. lilacinum* (based on *Clavaria atropurpurea* Batsch, now *Thuemenidium atropurpureum* (Batsch) Kuntze) and *G. viride* (Schrad.) Pers. (based on *Clavaria viridis* Schrad., now *Microglossum viride* (Pers.) Gillet).

Persoon treated the genus in several subsequent publications (1796, 1797, 1799, 1801, 1822) and the name *Geoglossum* was sanctioned by Fries in *Systema Mycologicum* I (1821). The source of the nomenclatural debate centres around the admittedly minimal description of the genus in Persoon's 1794 publication and the suggestion that *G. glutinosum*, described in detail by Persoon a short time later (1796), represents the first complete description of a species in *Geoglossum* and thus represents the type of the genus. However, the description of the genus in both publications is largely identical and very similar in detail. *Observationes Mycologicae* I (Persoon 1796) contains a series of descriptions of four new taxa, and the most logical interpretation is that these (apart from *G. viride* Pers., which was subsequently transferred to *Microglossum*) were intended by Persoon as additional taxa rather than a circumscription of a new, distinct concept for the genus. This view is reinforced by the fact that two of the four species included are separated from the others by nearly 30 pages (*Geoglossum* species descriptions are found at numbers 17 and 18 on page 11, and also at numbers 83 and 84 on pages 39 and 40, respectively). Lastly, all eight of these species (with *G. glabrum* presented

first) were included in Persoon's subsequent and more detailed account of the genus (Persoon 1797), further evidence that he was not rejecting his 1794 account. Durand (1908) assumed *G. glabrum* to be the type of the genus due to this being the most prominent species discussed by Persoon in 1797 and subsequent publications (1799, 1801, 1822), and proposed Persoon's collection of *G. glabrum* (presumably collection no. 910.262-109 as this collection had been examined by Durand; van Luyk 1919) as the lectotype.

Van Luyk (1919) and Maas Geesteranus (1965) found five different species present in collections labelled *G. glabrum* in the Persoon fungarium at Leiden. These findings and the lack of material designated by Persoon himself led Maas Geesteranus (1965) to formally reject the epithet *G. glabrum*, while Spooner (1987) regarded *G. glabrum* as a *nomen ambiguum*. However, we consider that *G. glabrum* Pers., sanctioned by Fries (1821) and lectotypified by Durand (van Luyk 1919) may be confirmed as a species within modern concepts of *Geoglossum* and agree with Durand's (1908) view that *G. glabrum* rather than *G. glutinosum* should be taken as the type species of that genus. Of the five fungarium sheets within the *G. glabrum* cover in Persoon's collections (Maas Geesteranus 1965), one (910.261-770) was identified as *G. glabrum* with doubt by Persoon and in fact contains a depauperate, immature *Xylaria*. The other four all contain fungi referable to the *Geoglossaceae*, and three of those contain species now classified in *Geoglossum*. Sheet 910.261-768 contains seven ascomata of *G. fallax* and two of *G. cookeanum*, sheet 910.261-773 contains two ascomata of *G. fallax*. Sheet 910.262-109 (the material examined by Durand) includes two ascomata identified as *G. glabrum*, probably initially by Mougeot rather than Persoon (Maas Geesteranus 1965).

Maas Geesteranus observed that the material on sheet 910.262-109 was almost certainly collected after publication of *G. glabrum*, and should not therefore have been chosen as lectotype by Durand. Typification of these early names is not an exact science as we cannot be confident that any of the material labelled as *G. glabrum* in Persoon's collections was collected prior to 1794. However, we can use it to gain some insight as to Persoon's concept of the taxa concerned, and we can be reasonably confident that *G. glabrum* falls within the modern concept of *Geoglossum* s.str. This is important for nomenclatural stability as *G. glutinosum* is now known to fall within a different clade of the *Geoglossaceae* and we no longer consider them to be congeneric. Bearing in mind the differing opinions as to the identity (or lack of identity) of *G. glabrum* over the years, we consider it premature to replace the names *G. fallax* (the predominant species in Persoon's fungarium collections), *G. cookeanum* (also present in Persoon's material) or *G. sphagnophilum* with *G. glabrum*.

Trichoglossum clade

Trichoglossum is recovered as a highly supported clade (95 % BS, 100 % BPP), though with only two species included in these analyses. A more intensive sampling of *Geoglossomycetes* including nine species of *Trichoglossum* but using only nrDNA also supports the monophyly of *Trichoglossum* (data not shown). *Trichoglossum hirsutum* is probably the most widespread and widely collected species of *Geoglossomycetes* and our analyses contain representatives from China, Europe, and North America. Our analyses indicate that this species is very diverse with European and North American material grouped together while the Chinese specimen occurs on a separate basal branch. A preliminary phylogeographic analysis using ITS nrDNA indicates that *T. hirsutum* is not monophyletic and cryptic speciation is likely to have occurred in this morphological species complex (data not shown).

Trichoglossum octopartitum did not form a monophyletic clade within *Trichoglossum* but was included in the highly supported

genus clade. The specimens of *T. octopartitum* used in this study were from European and North American material, suggesting distinct North American and European species. ITS sequences of both specimens differed by more than 10 %, further supporting the interpretation that separate species are present. Boudier (1885) separated the genus *Trichoglossum* from *Geoglossum* based on its prominent setae. *Geoglossum hirsutum* was transferred to *Trichoglossum* and designated as the type of the genus. The genus has been examined multiple times since its creation (Durand 1908, Sinden & Fitzpatrick 1930, Imai 1941, Mains 1954, Rifai 1965) with many new species and varieties described. Index Fungorum (<http://indexfungorum.org>) currently lists 47 names, including forms and varieties, and Kirk et al. (2008) acknowledge 19 species. Published molecular phylogenetic research also supports the genus as a well-supported clade (Sandnes 2006, Schoch et al. 2009b, Hustad & Miller 2011).

Glutinoglossum clade

Glutinoglossum was strongly supported as a distinct clade (85 % BS, 100 % BPP) comprised of at least two well-supported species, *G. glutinosum* and *G. heptaseptatum*. The most obvious morphological character of this genus is the conspicuous viscosity of the ascocarp, which is easily distinguished in the field (Fig. 2a). Ascocarp viscosity is not a character exclusive to *Glutinoglossum*, as several species, including *Geoglossum difforme*, also produce viscid ascocarps but have been shown to belong in the *Geoglossum* clade. Tardily septate ascospores of up to seven septa are also shared by both species of *Glutinoglossum*. Previous authors (Durand 1908, Imai 1941, Mains 1954, Spooner 1987) have noted the occurrence of a predominantly 7-septate form of *G. glutinosum*, though each was reticent to create a new species or form. Our phylogenetic analysis supports recognition of two distinct species of *G. glutinosum* and comparison of the ITS sequences reveals that the species differ by 8–10 % sequence dissimilarity (data not shown).

Further molecular analysis may lead to the inclusion of other species in *Glutinoglossum* in the future. *Geoglossum affine* E.J.Durand is similar to *G. heptaseptatum* with predominantly 7-septate ascospores but is differentiated by smaller ascospores (43–65 × 5–6 µm) and is presently known only from North America. According to Nannfeldt (1942) several other species of *Geoglossum* (*G. cohaerens*, *G. heufferianum* and *G. littorale*) are morphologically similar to *G. glutinosum* with viscid ascocarps and tardily-septate ascospores. Furthermore, Nitare (1983) considered *G. littorale* to be an immature form of *G. glutinosum*. Fresh material of these species is currently not available for molecular analysis so their taxonomic positions cannot be assessed here.

Viscid species of *Geoglossaceae* have been separated from the main group before. *Geoglossum glutinosum* was included in *Gloeoglossum* by Durand (1908), this genus being described as containing species of 'viscid-gelatinous consistency when fresh' and with paraphyses that are 'not confined to the hymenium but continue with unchanged form down the stem to its base'. According to Durand, *Gloeoglossum* contained two further species, *G. affine* and *G. difforme* (syn. *G. peckianum*). However, Durand chose *G. difforme* as type of *Gloeoglossum*, and our research places this species within the main *Geoglossum* clade.

Some authors (e.g., Imai 1941, Nannfeldt 1942, Holm in Farr et al. 1979) have treated *Cibalocoryne* (Hazslinszky 1881) at generic rank, and if that is correct it could constitute an earlier name for *Glutinoglossum*. However, while Hazslinszky's work is ambiguous in the rank at which *Cibalocoryne* is accepted, it is clearly subordinate to *Geoglossum* and as Mains (1954) and Maas Geesteranus (1965) stated, Hazslinszky himself referred to the taxon as a subgenus of *Geoglossum* at subgeneric rank

at one point in his work. Saccardo (1884) treated the only species included by Hazslinszky in his subgenus as a species of *Geoglossum*, and Imai (1941) attempted to use *Cibalocoryne* at generic rank but failed to make the necessary new combination. *Geoglossum (Cibalocoryne) viscosulum*, the only subordinate taxon belonging to *Cibalocoryne* in Hazslinszky (1881), was placed into synonymy with *Geoglossum glutinosum* by Nannfeldt (1942), but since Hazslinszky placed *G. glutinosum* into a different subgenus from *Cibalocoryne* we have doubts as to the acceptability of this action. We have not seen any material identified as *G. (Cibalocoryne) viscosulum*, but even if the synonymy were confirmed *Cibalocoryne* as a subgenus would not threaten the legitimacy of our newly erected genus *Glutinoglossum*.

Sabuloglossum clade

The genus *Sabuloglossum* is proposed to accommodate the fungus most recently known as *Thuemenidium arenarium*. Due to the low resolution of their LSU nrDNA phylogeny of *Geoglossomycetes*, Ohenoja et al. (2010) did not transfer *T. arenarium* into a new genus, retaining a paraphyletic concept of *Thuemenidium*. Our sample size is greater than that of Ohenoja et al. (2010) and the inclusion of three additional genes provides a well-resolved and strongly supported phylogeny confirming the recognition of *T. arenarium* as distinct from *T. atropurpureum* (100 % BS, 100 % BPP).

Microglossum arenarium was described by Rostrup (1892a) from material collected in Denmark on moist sand dunes. The species was transferred to the now obsolete genus *Corynetes* by Durand (1908) and then to the genus *Geoglossum* by Lloyd (1916). As Maas Geesteranus (1964) observed, *Corynetes* was originally described as a subgenus of *Geoglossum* by Hazslinszky (1881) rather than at generic rank as assumed by others (e.g., Nannfeldt 1942, Seaver 1951), and did not achieve generic status until Durand (1908) made the necessary rank change. It is therefore a junior synonym of *Thuemenidium* since this genus was described in 1891. The type of *Geoglossum* subg. *Corynetes* appears to be synonymous with *T. atropurpureum*, but authentic material has not been traced. *Microglossum arenarium* was finally transferred to *Thuemenidium* by Korf (Petersen & Korf 1982). Nitare (1981, 1982, 1984) studied several collections of *T. arenarium* and determined that it belonged in the genus *Geoglossum*. Cannon et al. (1985) considered *T. arenarium* to be a synonym of *T. atropurpureum*, however, the synonymy of these species is only found in this checklist (based most probably on a mis-reading of the text in Maas Geesteranus 1964). Both species have hyaline ascospores with slowly-appearing septation, however, they are markedly different in ascocarp morphology. *Thuemenidium atropurpureum* has a distinct purplish tinge when fresh, whereas *T. arenarium* is black in colour. *Thuemenidium atropurpureum* is found in humus and grassy soil, whereas *T. arenarium* is ecologically separated by its growth habit in sand and river gravel.

Nothomitra and Sarcoleotia clade

The most basal clade of *Geoglossomycetes* based on our sampling was found to contain the genera *Nothomitra* and *Sarcoleotia* (74 % BS, 98 % BPP). Hustad et al. (2011) recently placed *Nothomitra* within *Geoglossomycetes* based on a 3-gene phylogeny. Members of this clade are characterised by ascocarps with a distinct capitate hymenium that is clearly separated from the stipe when mature. This clade is most readily distinguishable from the remainder of *Geoglossomycetes* in that both genera produce hyaline to lightly-coloured ascospores that only occasionally become 3–5-septate when mature, whereas ascospores in all other genera of *Geoglossomycetes* are generally multiseptate and brown to dark brown in colour.

Sarcoleotia was found to be polyphyletic in our analyses, with *S. globosa* allied to *Geoglossomycetes* and *S. turficola* in *Leotiomyces*, in agreement with Wang et al. (2006) but contradicting Schumacher & Sivertsen (1987), who reduced the genus *Sarcoleotia* to a single species, *Sarcoleotia* (= *Ascocoryne*) *turficola*. Molecular data from the type species is needed before the taxonomic status of this genus can be finally addressed.

Sarcoleotia was described by Imai (1934), who separated the genus from *Leotia* based on the fleshy, non-gelatinous ascocarps and subcylindrical ascospores. *Sarcoleotia nigra* was designated the type species of the genus and described in the same publication from collections made in Hokkaido, Japan. Maas Geesteranus (1966) transferred *Helvella platypus* to the genus, creating *S. platypus* and considered *S. nigra* a synonym of *S. platypus*. Korf (1971a) transferred *Mitrua globosa* to *Sarcoleotia*, while Dennis (1971) transferred *Coryne turficola* to the genus. Subsequently, Korf (1971b) transferred *S. turficola* to *Ascocoryne turficola* based on the gelatinous tissue characteristic of *Ascocoryne*. Rahm (1975) reported *Sarcoleotia clandestina* from material collected in Switzerland, however, this name is a *nomen nudum* as no legal description of the species was ever made. Schumacher & Sivertsen (1987) examined *Sarcoleotia* and recognised only one species, *S. globosa*, concurring with the findings of Maas Geesteranus (1966) that *S. nigra* is a later synonym of *S. platypus*. Kirk et al. (2008) list only a single species. Wang et al. (2006) included both *S. globosa* and *Ascocoryne turficola* in their phylogeny based on 3-gene nrDNA analyses (LSU, SSU and 5.8S) with *A. turficola* shown to be most closely allied with the *Ascocoryne* clade. Bunyard et al. (2008) examined collections of *Ascocoryne turficola* from North America using nrDNA and found North American and European collections to be conspecific.

The genus *Nothomitra* was described by Maas Geesteranus (1964) to distinguish certain *Microglossum* species that possess a glabrous wavy hymenium that is not flattened and intergraded with the stipe, and parallel internal stipe hyphae. At present, three species are found in the genus. *Nothomitra cinnamomea* was designated as the type species from material collected in Upper Austria. *Nothomitra kovalii* was described from specimens collected in the Kuril Islands (Raitviir 1971) and *Nothomitra sinensis* was described from Northern China (Zhuang & Wang 1997). Hustad et al. (2011), using a 3-gene phylogeny of both nuclear ribosomal and protein-coding DNA, found support for including *Nothomitra* within *Geoglossomycetes*.

***Thuemenidium*/*Microglossum* clade**

Our findings concur with the results of previous authors (Wang et al. 2006, Schoch et al. 2009b, Ohenoja et al. 2010), in that *Thuemenidium atropurpureum* does not belong in *Geoglossomycetes* and is most closely aligned with the genus *Microglossum* in *Leotiomyces*.

Complexity surrounds *Thuemenidium* and the identity of its type species. The genus was erected by Kuntze (1891) as a replacement name for *Microglossum* Sacc. (1884), the author being under the impression that the name *Microglossa*, described for a genus of *Asteraceae* (de Candolle 1836), took precedence. The current rules of orthography indicate that the two names are not homonyms, but coincidentally Saccardo seems to have been unaware of the publication five years earlier of *Microglossum* Gillet (1879), subsequently lectotypified by Clements & Shear (1931) with *M. viride* and with this choice being confirmed by Maas Geesteranus (1964). Therefore, Saccardo's genus cannot be taken up.

Although Kuntze included two other species in his publication of *Thuemenidium* (based on *Microglossum multiforme* and *M. atropurpureum*), the only included species in Saccardo's

genus was *M. hookeri* (basionym *Geoglossum hookeri*), and this must be taken as type of the replacement generic name.

Microglossum hookeri is a later synonym of *Geoglossum hookeri*, a species first described by Cooke (1875) from a single specimen sent to him by M.J. Berkeley from an unknown locality. We agree with several subsequent authors (Massee 1897, Durand 1908, Imai 1941) and consider *Thuemenidium hookeri* to be a synonym of *T. atropurpureum*. We have not been able to establish the identity of *T. multiforme* (basionym *Geoglossum multiforme*) with certainty, but Nannfeldt (1942) and Eckblad (1963) treated that species as *Mitrua multiforme*. *Thuemenidium berteroi*, a component of the earth tongue mycobiota of the temperate Southern Hemisphere, was added to the genus via transfer from *Mitrua berteroi* by Gamundi (1977); we cannot at present confirm placement of this species.

Throughout the 20th century, most authors assumed a close relationship between *Thuemenidium* (in many papers listed as *Corynetes*) and *Geoglossum*, with both genera referred to the *Geoglossaceae*, and the heterogeneity of the former genus was not questioned. *Microglossum* was also assumed to belong to the *Geoglossaceae* by most authors. Initial molecular data led Wang et al. (2006) to suggest that *Thuemenidium* was more closely aligned with *Helotiales* than *Geoglossaceae*, and Schoch et al. (2009b) and Hustad et al. (2011) confirmed this positioning, placing the genus close to *Microglossum* and *Leotia* within the *Leotiomyces*. Ohenoja et al. (2010) found that *Thuemenidium* was a polyphyletic genus with *T. atropurpureum* closely related to *Microglossum* as a member of *Leotiomyces*, whereas *T. arenarium* occurred in *Geoglossomycetes* based on LSU nrDNA analyses. However, since the backbones of these phylogenies were unresolved, the authors chose not to revise the taxonomy of these *Thuemenidium* species.

The close association of *T. atropurpureum* with species of *Microglossum* in our phylogenetic tree tends to reinforce the view that this species should be formally reassigned to *Microglossum* (Huhndorf & Lumbsch note 270 <http://www8.umu.se/myconet/asco/litt/newNotes.html>). However, the type species of *Microglossum* Gillet, *M. viride* (Pers.) Gillet, has not been included in our study. As the synonymy of *Thuemenidium* with *Microglossum* would have legislative complications for the fungal conservation community, we prefer to keep the genera separate for the time being. Judging from phylogenetic analysis of the ITS sequences submitted to GenBank of fungi assigned to *Thuemenidium* and *Microglossum* (data not shown) and also our own field observations, several of the species within these genera are polyphyletic, and the complex is in need of revision.

CONCLUSIONS

Further molecular systematics research is needed in this group in order to determine strongly supported phylogenetic relationships that will ultimately lead to a robust taxonomical classification in this class. Our study reveals that several cryptic taxa occur within *Geoglossomycetes* and can only be discovered through detailed molecular analyses due to the simple morphology and incompletely known life histories of these fungi. Increased sampling of species and varieties is also necessary to determine the phylogenetic placement for the large number of currently available species names that still remain within *Geoglossomycetes*. Undoubtedly, new taxonomic novelties will be discovered that will lead to the proposal of additional taxa and further synonymy within this group.

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