Generic circumscriptions in Geoglossomycetes

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Key words
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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, RP81). 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. heptasepatum, 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 Geollasales to contain three genera (Geoglossum, Sarcoleotia and Trichoglossum) in Geoglossaceae, which was previously placed in Leotiomycetes. 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 Leotiomycetes 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 Leotiomycetes and that several taxa form a separate monophyletic clade basal to Leotiomycetes 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 Leotiomycetes and proposed the class Geoglossomycetes and order Geollasales to contain Geoglossum, Sarcoleotia and Trichoglossum within a single family, the Geoglossaceae. Ohenjoa 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...
conservation value assessments (Newton et al. 2003, Gen-
ney et al. 2009). *Thuemenidium atropurpureum*, until recently
assumed to be part of the Geoglossomycetes clade, is listed
under UK legislation (under its synonym *Geoglossum atropur-
pureum*) as a UK Priority species (http://jncc.defra.gov.
uk/_speciespages/2290.pdf) within the national Biodiversity
Action Plan. Species of Geoglossomycetes are included in the
Norwegian (Kålås et al. 2010), Swedish (Gårdenfors 2010) and
Swiss (Senn-Irlet et al. 2007) Red Data Lists.

The goal of this study was to examine the phylogenetic rela-
tionships within Geoglossomycetes 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 asco-
 mata and microscopic characters using the pertinent literature
(e.g., Massée 1897, Durand 1908, Imal 1941, Nannfeldt 1942,
Main 1954, Maas Geesteanus 1964, Roodbeek 2008, along
with original species descriptions). Mature ascospores were
obtained for measurement by tapping ascomata in a drop of
water on a slide (Main 1954). Ascomata were hand-sectioned
and squash-mounted in water and images of micromorphologi-
cal characters were captured with a QImaging QCcolor3 digital
camera mounted on an Olympus BX51 compound microscope
using differential interference microscopy. Images were pro-
cessed using Adobe Photoshop v. 7.0 (Adobe Systems Inc.,
Mountain View, California). A minimum of 30 measurements
was taken for all micromorphological structures when pos-
sible 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, Cali-
ifornia) 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 Geo-
glossomycetes. The 28S large subunit (LSU) nrDNA region
was amplified using JS1 (Landvik 1996) and LR6 (Vilgalys
& Hester 1999) and sequenced with these primers in addition to
the internal primers LR3 (Vilgalys & Hester 1999) and LRR3
(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 mainte-
nance 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 al.
1982, Kearsey & Labib 1998). MCM7 has been found to
produce highly accurate phylogenies in fungi (Aguiet et al.
2008, Schmitt et al. 2009), and it has been shown to be
reliable across a wide range of ascomycete taxa, including
Geoglossomycetes (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 RPB1f 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 Sea-
view v. 4.2 (Galtier et al. 1996). Ambiguous regions were removed
from the individual gene datasets using Gblocks v. 0.91b (Cas-
tresana 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 ≥ 70 % (Hillis & Bull 1993).

Bayesian inference employing a Markov Chain Monte Carlo
(MCMC) algorithm was performed using MrBayes v. 3.1.2 (Huel-
senbeck & 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 em-
ployed. 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)
≥ 95 % were considered significant and highly supported (Al-
far 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 (Klug 1989, Eernisse & Kluge 1993).
Individual gene phylogenies were considered to be incongruent
if clades with significant ML BS and BPP (≥ 70 % BS and/or
≥ 95 %) 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 Geoglossom constrained
to be monophyletic; and 2) *Thuemenidium* species constrained
in a monophyletic genus. S-H tests using RELL 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 95 % 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 characters, 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.

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.

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        |
|--------------------------|------------------|---------------|-------------|--------------|-------------|-------------|
| Geoglossum barlae        | Moingeon s.n.    | ILLS 61034    | JQ256416    | JQ256433     | JQ256444    | KC222160    |
| Geoglossum cookeanum     | ANM 2257         | ILLS 61035    | JQ256417    | JQ256434     | JQ256445    | KC222161    |
| Geoglossum difforme      | J. Gaitsler s.n. | ILLS 67347    | KC222122    | KC222135     | N/A         | N/A         |
| Geoglossum glabrum       | ANM 10499        | ILLS 67349    | KC222124    | KC222137     | KC222149    | KC222163    |
| Geoglossum nigritum      | OSC 60610        | GenBank       | AY789316    | AY789317     | N/A         | N/A         |
| Geoglossum nitrigum      | AFTOL-ID 56      | GenBank       | DQ491490    | AY544650     | N/A         | N/A         |
| Geoglossum similare      | ANM 2171         | ILLS 61039    | JQ256421    | JQ256437     | JQ256448    | KC222165    |
| Geoglossum sphagnophilum | ASM 10528        | ILLS 67350    | KC222126    | KC222138     | KC222151    | KC222166    |
| Geoglossum umbratilis    | Pournara s.n.    | ILLS 67351    | KC222126    | KC222139     | KC222151    | KC222167    |
| Glutinoglossum glutinosum| ANM 2231         | ILLS 67352    | KC222126    | KC222141     | KC222153    | KC222170    |
| Glutinoglossum heptaseptatum| J. Gaitsler s.n.| ILLS 67354    | KC222129    | KC222142     | KC222154    | KC222171    |
| Glutinoglossum reptile    | J. Gaitsler s.n. | ILLS 67354    | KC222130    | KC222143     | KC222155    | KC222172    |
| Gradonia coracina        | ANM 2018         | ILLS 60491    | JQ256423    | JN102009     | JN672993    | KC222173    |
| Microglorium olearcum     | HH-DHSH7-103     | GenBank       | AY789308    | AY789309     | N/A         | N/A         |
| Microglorium rufum        | Ingo-Clark-Geo 163| GenBank       | DQ57360     | DQ470981     | N/A         | N/A         |
| Neolecota vitellina      | OSC 119159       | GenBank       | FJ171854    | FJ171881     | N/A         | N/A         |
| Nothomitra cinnamomea    | Moingeon s.n.    | ILLS 61042    | JQ256424    | JQ256439     | JQ256450    | KC222174    |
| Orbilia auricula         | AFTOL-ID 306     | GenBank       | DQ491512    | DQ470953     | N/A         | N/A         |
| Orbilia delicata         | DHP 108          | GenBank       | UT2595      | AY26178      | N/A         | N/A         |
| Sabuloglossum arenarium  | CFR 181007       | ILLS 61043    | JQ256426    | JQ256440     | JQ256452    | KC222175    |
| Sarcoleotia globosa      | OSC 63633        | GenBank       | AY789410    | AY789409     | N/A         | N/A         |
| Sarcoleotia turricula    | MBH 52476        | GenBank       | AY789429    | AY789428     | N/A         | N/A         |
| Spathularia flavida      | H253397          | GenBank       | AY789278    | AY789277     | N/A         | N/A         |
| Thuemendium atropurpureum| ASM 4931         | ILLS 67350    | JQ256427    | JQ256441     | JQ256453    | KC222176    |
| Trichoglossum hirsutum    | ANM 2233         | ILLS 67355    | KC222132    | KC222145     | KC222157    | KC222177    |
| Trichoglossum octopartium| J. Gaitsler s.n. | ILLS 61045    | JQ256428    | JQ256442     | JQ256454    | KC222178    |
| Trichoglossum octopartium| OSC 61726        | GenBank       | AY789314    | AY789314     | N/A         | N/A         |
|                        | AFTOL-ID 64      | GenBank       | DQ491494    | AY41853      | N/A         | N/A         |
|                        | 81362           | HAKS 55133    | KC222133    | KC222146     | KC222158    | KC222179    |
|                        | JPP 10191       | ILLS 61046    | JQ256429    | JQ256443     | JQ256455    | KC222180    |
|                        | ANM 2227        | ILLS 67356    | KC222134    | KC222147     | KC222159    | KC222181    |

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 Microglorium in Leotiomycetes. 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 Ge. 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 Geoglossomycetes (-ln L difference = 48.13503, P = 0.001) and monophyletic Thuemenidium (-ln L difference = 540.18521, P = 0.001).
Geoglossum

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

**Type species.** *Glutinoglossum glutinosum* (Pers.) E.J.Durand, **comb. nov.**

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

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

Asccarp 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. Asc 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 Holotype]).

**Basionym.** Geoglossum glutinosum Pers., **Observ. Mycol.** 1: 11. 1796.

= Glueglossum glutinosum (Pers.) E.J.Durand, *Ann. Mycol.* 6: 1906.

= 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 lubicum Pers., *Mycol. Eur.* 1: 197. 1822.

= ?Geoglossum (Cibalocoryne) viscosulum Hazsl., *Magyar Tud. Akad. Értes., A Termés-tud Kör. 11: 8. 1881.

Asccarps 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 asccarp, 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 ovoid, or pyniform, continuing down the stipe in a thick gelatinous layer. Asc 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); South America: Brazil (von Keissler 1916), Chile (Wildhaber 1941), United Kingdom (Dennis 1978, this paper); North America: California (Hinkova & Stoichev 1983), Czech Republic (this paper), Denmark (Lind 1913), Finland (Karsten 1871), France (Bigeard 1898), Germany (Rabenhorst 1857), Hungary (Hazslinšzky 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 (Waterston 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. (ILLS 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 Podjestedi, 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, Trefo, on short grass and moss, N52°59', W4°27', 9 Oct. 2011, VP Hustad, PF Cannon, BTM Dentinger & AM Ainsworth, ANM2476 (ILLS 64446); Scotland, Skye, Sleat, Tokavaig, in short grass along roadway N57°7', W5°58', 16 Oct. 2011, VP Hustad & PF Cannon, ANM2247 (ILLS 64444); Great Smoky Mountains National Park, Smokemont, mixed deciduous forest soil, N35°37', W83°6', 762 m elev., 14 Aug. 2009, VP Hustad & AS Methven, ANM2231 (ILLS 64443). – United States, Tennessee, Sevier County, Great Smoky Mountains National Park, Cataloochee, Caldwell Fork Trail, mixed deciduous forest soil, N35°33', W83°18', 460 m elev., 10 Aug. 2009, VP Hustad & AS Methven, ANM2227 (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).

**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 × 18–22 µm) and predominantly 7-septate ascospores (55–)60–80(–90) × 4–6.5 µm.

**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 Ježerské hory (Užer 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).

**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.

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

**Fig. 3** *Glutinoglossum heptaseptatum.* a. Ascus from dried material (total magnification = 200×); b. ascospores from dried material (total magnification = 400×); c. paraphyses from dried material (total magnification = 400×). Micrographs from specimen ILS 63754, used in this study. — Scale bars: a = 20 µm; b, c = 10 µm.
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.
= Mitrula arenarium (Rostr.) Masseee, 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. 1962.
≡ 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 (Hallgrimsson 1987), Netherlands (van Luyk 1919, Roobeek 2008), Norway (Rostrup 1904, Imai 1940, Eckblad 1963), Sweden (Andersson 1950, Granquist 1950), United Kingdom (Ramshaw 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, sandy heath amongst Empetrum, 22 Oct. 1980, J. Nite, 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 Oehnora 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. similis* 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., Massue 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. illinicum* (based on *Clavaria atropurpurea* Batsch, now *Thueummium 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 that *G. glutinum* 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. glutinum* (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. glutinum* 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. glutinum*, while Spooner (1987) regarded *G. glutinum* as a nomen ambiguum. However, we consider that *G. glutinum* 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. glutinum* rather than *G. glutinosum* should be taken as the type species of that genus. Of the five fungarium sheets within the *G. glutinum* cover in Persoon's collections (Maas Geesteranus 1965), one (910.261-770) was identified as *G. glutinum* 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. glutinum*, 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. glutinum*, and should not therefore have been chosen as the 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. glutinum* 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. glutinum* 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. glutinum* 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. glutinum*.

**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 comprehensive 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 6–10 % 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 µm) and is presently known only from North America. According to Nannfeldt (1942) several other species of Geoglossum (G. cohaerens, G. heuffelianum 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. difforne (syn. G. peckianum). However, Durand chose G. difforne 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 (Hazslinsky 1881) at generic rank, and if that is correct it could constitute an earlier name for Glutinoglossum. However, while Hazslinsky’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, Hazslinsky 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 Hazslinsky 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 subdomain belonging to Cibalocoryne in Hazslinsky (1881), was placed into synonymy with Geoglossum glutinosum by Nannfeldt (1942), but since Hazslinsky 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, Olenoja 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 Olenoja 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 Hazslinsky (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 Sarcoleota clade**

The most basal clade of Geoglossomycetes based on our sampling was found to contain the genera Nothomitra and Sarcoleota (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 capitulate 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 Leotiomycetes, in agreement with Wang et al. (2006) but contradicting Schumacher & Sivertsen (1987), who reduced the genus Sarcoleotia to a single species, Sarcoleotia (= Asccoryne) 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 ascosporas. 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 Helvetia platypus to the genus, creating S. platypus and considered S. nigra a synonym of S. platypus. Korf (1971a) transferred Mitrula globosa to Sarcoleotia, while Dennis (1971) transferred Coryne turficola to the genus. Subsequently, Korf (1971b) transferred S. turficola to Asccoryne turficola based on the gelatinous tissue characteristic of Asccoryne. Rahm (1975) reported Sarcoleoteca clandestina from material collected in Switzerland, however, this name is a nomen nudum.

Our findings concur with the results of previous authors (Wang et al. 2006, Schoch et al. 2009a, Ohenoja et al. 2010), in that our own field observations, several of the species within these collections to be conspecific.

The genus Nothismitra was described by Maas Geesteranus (1964) to distinguish certain Microglossum species that possess a glabrous waxy hymenium that is not flattened and intergraded with the stipe, and parallel internal stipe hyphae. At present, three species are found in the genus. Nothismitra cinna­momea was designated as the type species from material collected in Upper Austria. Nothismitra kovalii was described from specimens collected in the Kuril Islands (Raith­vi­1971) and Nothismitra 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 Nothismitra within Geoglossomycetes.

**Thuemenia 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 Thuemenia atropurpureum does not belong in Geoglossomycetes and is most closely aligned with the genus Microglossum in Leotiomycetes.

Complexity surrounds Thuemenia 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 Saccaro­di­ 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 Thuemenia (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 (Masséé 1897, Durand 1908, Imai 1941) and consider Thuemenia hookeri to be a synonym of T. atropurpureum. We have not been able to establish the identity of T. multiforme (basionym Microglossum multiforme) with certainty, but Mannfeldt (1942) and Eckblad (1963) treated that species as Mitrula multiforme.

Thuemenia berterei, a component of the earth tongue mycobiota of the temperate Southern Hemisphere, was added to the genus via transfer from Mitrula berterei by Gamundi (1977); we cannot at present confirm placement of this species.

Throughout the 20th century, most authors assumed a close relationship between Thuemenia (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 Thuemenia was more closely aligned with Heliotiales 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 Leotiomycetes. Ohenoja et al. (2010) found that Thuemienia was a polyphyletic genus with T. atropurpureum closely related to Microglossum as a member of Leotiomycetes, 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 Thuemienia 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 reallocated to Microglossum (Huhndorf & Lumbsch note 270 http://www8.umu.se/myconet/asc/o/t/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 Thuemenia 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 analyses of the ITS sequences submitted to GenBank of fungi assigned to Thuemenia 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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