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Short communication

Maasoglossum, a basal genus in Geoglossomycetes

Vincent P. Hustad [a,b,*], *Andrew N. Miller* [b]

[a] *Department of Plant Biology, University of Illinois at Urbana-Champaign, 505 S. Goodwin Ave., Urbana, IL 61801, USA*

[b] *Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, IL 61820, USA*

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ABSTRACT

The genus *Maasoglossum* is examined using morphology, ecology, and molecular systematics of the internal transcribed spacer region and large subunit of the nuclear ribosomal RNA gene, all of which support the placement of *Maasoglossum* among the basal members of Geoglossomycetes. The morphology of the genus extends the range of ascocarp and ascospore development in Geoglossomycetes. The ecology and conservational significance of the genus is discussed, a nomenclatural transfer of *Geoglossum aseptatum* to *Maasoglossum* is made, and an emended description of *Maasoglossum* is provided.

The class Geoglossomycetes is a basal ascocarp-producing member of the 'Leotiomyceta' (Schoch et al. 2009). Kirk et al. (2008) suggest 48 species in four genera belong in the class, while Index Fungorum lists over 200 names for these same four genera. Recent research indicates eight genera belong in the class: *Geoglossum* Pers., *Glutinoglossum* Hustad et al., *Hemileucoglossum* Arauzo, *Leucoglossum* S. Imai, *Nothomitra* Maas Geest., *Sabuloglossum* Hustad et al., *Sarcoleotia* S. Ito & S. Imai, and *Trichoglossum* Boud. (Hustad et al. 2013; Arauzo and Iglesias 2014). The prevalence of rarely collected species has made an accurate estimation of the number of species in the class difficult.

The rare species, *Geoglossum aseptatum* Hakelier ex Nitare, is characterized by light brown, aseptate mature ascospores, a character not found in any other member of the genus *Geoglossum*. Preliminary molecular analysis (data not shown) suggested that this fungus belonged in a separate lineage from *Geoglossum* and a review of the literature suggested a possible link between *G. aseptatum* and the genus *Maasoglossum* Thind & R. Sharma.

The genus *Maasoglossum* was described by Thind and Sharma (1984) to accommodate a single species, *M. verrucisporum*, described from a collection made in 1981 from the temperate Eastern Himalayan forest of Bhutan. The authors noted the macroscopic similarity of the ascocarp to that of *Geoglossum*, but that it differed in the hymenium of *M. verrucisporum* contained small ridges and grooves that became more prominent upon drying, a character rarely encountered

* *Corresponding author*. Department of Plant Biology, University of Illinois at Urbana-Champaign, 505 S. Goodwin Ave., Urbana IL 61801, USA. Tel.: +01 618 843 0784; fax: +01 217 265 4678.
E-mail address: vhustad@illinois.edu (V.P. Hustad).
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in *Geoglossum*. Additionally, the one-celled, lightly-colored, warted ascospores precluded the placement of this fungus in *Geoglossum*. Since no other members of the Geoglossaceae *sensu lato* contain ornamented ascospores, they were unsure of the placement of this genus within Geoglossaceae *sensu lato* or Leotiomycetes (*Microglossum*). The genus is currently regarded as *incertae sedis* within Leotiomycetes (Lumbsch and Huhndorf 2010). In this paper we provide an in-depth assessment of the genus *Maasoglossum* based on morphology, ecology, and molecular systematics, and test the hypotheses that *Maasoglossum* is a basal member of Geoglossomycetes and that *G. aseptatum* belongs in the genus *Maasoglossum*.

For this study, the type specimens of both *Geoglossum aseptatum* and *Maasoglossum verrucisporum* were obtained and examined. Dried ascomata were hand-sectioned and squash mounted in 5% KOH and micromorphological characters were observed. Images of pertinent micromorphological characters were captured using a QImaging QColor 3 digital camera mounted on an Olympus BX51 compound microscope using differential interference microscopy. Images were processed with Adobe Photoshop v. 7.0 (Adobe Systems Inc., Mountain View, California). A minimum of 30 measurements was made for each micromorphological character using NIH Image v. 1.63 (National Institutes of Health, Bethesda, Maryland). Taxonomic novelties and associated data were deposited in MycoBank (Crous et al. 2004).

Total genomic DNA was extracted from approximately 0.5 cm^2 of hymenium tissue from a single dried ascoma with the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) for recently-collected specimens (collected within 20 years). For older specimens, DNA was extracted from approximately 0.2 cm^2 of hymenium tissue using the EZNA Forensic DNA kit (Omega Bio-Tek, Norcross, Georgia). Gene fragments were PCR amplified following the methods outlined in Promputtha and Miller (2010) and purified using a Wizard SV Gel and PCR Clean-Up System (Promega Corp., Fitchburg, Wisconsin). Sequences were generated on an ABI Applied Biosystems 3730XL high-throughput DNA capillary sequencer at the UIUC Keck Center for Comparative and Functional Genomics. Two regions of the nuclear rRNA gene were used for molecular phylogenetic analysis: the ca. 570 bp internal transcribed spacer (ITS) region, consisting of the ITS1, 5.8S, and ITS2 regions, was amplified and sequenced using a combination of the primers ITS5, ITS1, ITS2, ITS3, and ITS4 (White et al. 1990); and a ca. 630 bp fragment of the 28S large subunit (LSU) region was amplified and sequenced with the primers JS1 (Landvik 1996) and LR3 (Vilgalys and Hester 1990). Due to the age of the material, attempts to PCR amplify single-copy protein coding genes (i.e. *RPB1*, *RPB2*, *MCM7*) were unsuccessful. Outgroup species were chosen from closely related taxa based on previous studies (Wang et al. 2006; Hustad et al. 2013).

Sequence alignments were created by eye in Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan) and optimized if necessary using Muscle v. 3.8.31 (Edgar 2004) in SeaView v. 4.4.2 (Gouy et al. 2010). Ambiguous regions were removed from each dataset using Gblocks 0.91b (Castresana 2000) under the following parameters: for ITS: minimum number of sequences for both conserved and flanking regions = 15, maximum number of contiguous, nonconserved regions = 2, minimum length of a block = 2, and allowed gap positions in 50% of sequences; for LSU: same as ITS except minimum number of sequences for both conserved and flanking regions = 14.

The GTR + I + G model was determined to be the best-fit model of evolution for both ribosomal regions using the Akaike Information Criterion (AIC) (Posada and Buckley 2004) in jModelTest 2.0 v. 0.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012) on the XSEDE platform of the CIPRES Science Gateway Teragrid (Miller et al. 2010) and was used in phylogenetic analyses of each individual dataset. Maximum likelihood (ML) analyses were performed using PhyML 3.0 (Guindon et al. 2010) on the ATGC server (http://www.atgc-montpellier.fr/phyml/). The best of subtree pruning and regrafting (SPR) and nearest neighbor interchange (NNI) was implemented during the heuristic search. Nonparametric bootstrap support (Felsenstein 1985) (BS) was determined with 1000 replicates. Clades with BS values of ≥70% were considered significant (Hillis and Bull 1993).

Bayesian inference employing a Markov Chain Monte Carlo (MCMC) algorithm was performed using MrBayes v. 3.2.2 (Ronquist et al. 2012) on the XSEDE platform. Four independent chains of MCMC were run for 10 million generations. Clades with Bayesian posterior probability (BPP) values of ≥95% were considered significant (Alfaro et al. 2003). Tracer v. 1.5 (Rambaut and Drummond 2009) was used to estimate effective sample size (ESS) using the standard deviation of split frequencies produced by Bayesian analysis.

Individual ITS and LSU datasets were examined for potential conflict before concatenation into a single dataset for total evidence analysis (Kluge 1989; Eernisse and Kluge 1993). Individual gene phylogenies were considered to be incongruent if clades with significant ML BS and BPP support were conflicting in individual tree topologies (Wiens 1998; Alfaro et al. 2003; Lutzoni et al. 2004). Since no incongruencies were found among individual phylogenies, the ITS and LSU datasets were concatenated and final ML and Bayesian analyses were performed on the combined dataset. Alignments and trees are deposited in TreeBASE (http://treebase.org) under submission ID 16591.

Ten new sequences were generated in this study, five ITS and five LSU sequences (Table 1). These were analyzed together with 23 ITS and 21 LSU sequences from our previous studies (Hustad and Miller 2011; Hustad et al. 2011, 2013, 2014) along with sequences of Geoglossomycetes from previously published research (Pfister 1997; Zhao et al. 2001; Wang et al. 2002, 2005, 2006, 2011; Peterson 2008; Brock et al. 2009; Ohenoja et al. 2010). Twenty-eight collections, representing a total of 15 Geoglossomycetes species (including sequences from type species of each genus in Geoglossomycetes except *Sarcoleotia*) and eleven outgroup species, were included in the analyses. Of the 28 collections included in the final dataset, none lack ITS and only two lack LSU, with sequences for both markers available for 93% of the collections (Table 1).

The final combined data matrix had an aligned length of 2712 bp, which was reduced to 1110 bp after the removal of 1602 bp of ambiguously aligned regions by Gblocks. Of the 1110 characters used in the final phylogenetic analyses, 554 were constant and 556 were variable. A burn-in of 10% was estimated using Tracer v. 1.5 to be sufficient to remove the