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Geosiphon pyriforme, a Fungus Forming Endocytobiosis with *Nostoc* (Cyanobacteria), Is an Ancestral Member of the Glomales: Evidence by SSU rRNA Analysis

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Abstract. *Geosiphon pyriforme* inhabiting the surface of humid soils represents the only known example of endocytobiosis between a fungus (Zygomycotina; macrosymbiont) and cyanobacteria (*Nostoc;* endosymbiont). In order to elucidate the taxonomical and evolutionary relationship of *Geosiphon pyriforme* to fungi forming arbuscular mycorrhiza (AM fungi), the small-subunit (SSU) ribosomal RNA genes of *Geosiphon pyriforme* and *Glomus versiforme* (Glomales; a typical AM fungus) were analyzed and aligned with SSU rRNA sequences of several Basidiomycetes, Ascomycetes, Chytridiomycetes, and Zygomycetes, together with all AM-fungal (Glomales) sequences published yet.

The distinct group of the order Glomales, which includes *Geosiphon,* does not form a clade with any other group of Zygomycetes. Within the Glomales, two main lineages exist. One includes the families Gigasporaceae and Acaulosporaceae; the other one is represented by the genus *Glomus,* the members of which are very divergent. *Glomus etunicatum* and *Geosiphon pyriforme* both form independent lineages ancestral to the Glomales. The data provided by the present paper confirm clearly that *Geosiphon* represents a fungus belonging to the Glomales. The question remains still open as to whether or not *Geosiphon* is to be placed within or outside the genus *Glomus,* since this genus is probably polyphyletic and not well defined yet. *Geosiphon* shows the ability of a *Glomus*-like fungus to form a "primitive" symbiosis with a unicellular photoautotrophic organism, in this case a cyanobacterium, leading to the conclusion that a hypothetical association of a *Glomus*-like fungus with a green alga as a step during the evolution of the land plants appears probable.

Key words: *Geosiphon pyriforme* — *Glomus versiforme* — Glomales — Endocytobiosis — Molecular evolution — Small-subunit rRNA

Introduction

Geosiphon pyriforme is the only known case of endocyanosis of a fungus. The organism was first recognized by F. von Wettstein (1915), who described it as a siphonal algae but also suggested the presence of chitin. Knapp (1933) recognized *Geosiphon* as a fungus with endosymbiotic cyanobacteria and described it as an intracellular phycomycetal lichen. The endosymbiotic cyanobacterium usually is *Nostoc punctiforme.* The biology of *Geosiphon pyriforme* has recently been reviewed by Mollenhauer and Kluge (1994) and Kluge et al. (1994). Its ultrastructure was first investigated by Schnepf (1964). The question remained open as to which group of the Zygomycotina the fungal partner of the symbiotic *Geosiphon pyriforme* consortium belongs. The comparison of shape and ultrastucture of *Geosiphon* spores with that described for fungi belonging to the Glomales led to the conclusion that *Geosiphon* has strong affinities with the Glomales and could potentially be an arbuscular my-

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Table 1. SSU rRNA sequences used for the phylogenetic analyses

Organism	Accession numbers	Taxonomic position
Athelia bombacina	M55638	Basidiomycotina
Boletus satanas	M94337	Basidiomycotina
Coprinus cinereus	M92991	Basidiomycotina
Cronartium ribicola	M94338	Basidiomycotina
Filobasidiella neoformans	M55625	Basidiomycotina
Leucosporidium scottii	X53499	Basidiomycotina
Peridermium harknessii	M94339	Basidiomycotina
Schizophyllum commune	X54865	Basidiomycotina
Spongipelis unicolor	M59760	Basidiomycotina
Thanatephorus praticola	M92990	Basidiomycotina
Xerocomus chrysenteron	M94340	Basidiomycotina
Aspergillus fumigatus	M55626	Ascomycotina
Aureobasidium pullulans	M55639	Ascomycotina
Blastomyces dermatitdis	M55624	Ascomycotina
Candida albicans	X53497	Ascomycotina
Coccidioides immitis	M55627	Ascomycotina
Colletotrichum gloeosporioides	M55640	Ascomytocina
Kluyveromyces lactis	X51830	Ascomytocina
Leucostoma persoonii	M83259	Ascomycotina
Neurospora crassa	X04971	Ascomycotina
Penicillium notatum	M55628	Ascomycotina
Podospora anserina	X54864	Ascomycotina
Saccharomyces cerevisiae	M27607	Ascomycotina
Schizosaccharomyces pombe	X54866	Ascomycotina
Pneumocystis carinii	X12708	uncertain affiliation
Blastocladiella emersonii	M54937	Chytridiomycotina
Chytridium convervae	M59758	Chytridiomycotina
Neocallimastix sp.	M59761	Chytridiomycotina
Spizellomyces acuminatus	M59759	Chytridiomycotina
Acaulospora rugosa WV 935	Z14005	Zygomycotina
Acaulospora spinosa WV 860	Z14004	Zygomycotina
Entrophospora columbiana WV 877	Z14006	Zygomycotina
Entrophospora sp. WV 796	Z14011	Zygomycotina
Glomus etunicatum INVAM 147	Z14008	Zygomycotina
Glomus intraradices DAOM 197198	X58725	Zygomycotina
Glomus mosseae WV 156	Z14007	Zygomycotina
Glomus vesiculiferum	L20824	Zygomycotina
Gigaspora albida WV 1034	Z14009	Zygomycotina
Gigaspora gigantea WV 932	Z14010	Zygomycotina
Gigaspora margarita DAOM 194757	Z58726	Zygomycotina
Scutellospora dipapillosa WV 929	Z14013	Zygomycotina
Scutellospora pellucida WV 873	Z14012	Zygomycotina
Basidiobolus ranarum	D29946	Zygomycotina
Conidiobolus coronatus	D29947	Zygomycotina
Endogone pisiformis CRBF #0001	X58724	Zygomycotina
Entomophthora muscae	D29948	Zygomycotina
Mucor racemosus	X54863	Zygomycotina
Smittium culisetae	D29950	Zygomycotina
Zoophthora radicans	D29949	Zygomycotina
Micromucor ramannianus NRRL5844	X89435	Zygomycotina[a]
Mortierella polycephala NRRL22890	X89436	Zygomycotina[a]
Mucor mucedo NRRL3635	X89434	Zygomycotina[a]
Syncephalastrum racemosum NRRL2496	X89437	Zygomycotina[a]
Thraustochytrium kinnei	L34668	Chromista
Ulkenia profunda	L34054	Chromista
Zea mays	K02202	Plantae
Stylonychia pustulata	X03947	Protista

[a] Unpublished sequences, kindly provided by Kerry O'Donnel (Peoria, USA)

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VANS1       GTCTAGTATAATCGTTATACAGG..............
GEO1a       ...TAGTATAATCGTTATACAGGTGA...........
GEO1b       ......TATAATCGTTATACAGGTGAAAC........
GEO1c       .........AATCGTTATACAGGTGAAACTGC.....
GEO1d       ............CGTTATACAGGTGAAACTGCGAA...
GEO1e       ..............TATACAGGTGAAACTGCGAATGG
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Fig. 1. Oligonucleotide sequences of the used 5′ side primers for PCR amplifications of the SSU rRNA gene of *Geosiphon pyriforme* and *Glomus versiforme*. *VANS1* after Simon et al. (1992, 1993b). *GEO1a–GEO1e* oligonucleotides constructed after the consensus sequences of known AM-fungi. The primer finally used, GEO1 (see Fig. 2), corresponds to GEO1b (*bold letters*).

corrhiza (AM) fungus (Schüßler et al. 1994; for the revised classification of AM fungi [Glomales] see Morton and Benny 1990). The main argument in favor of this view was the finding that the spores, which are the most important character for the taxonomy of the AM fungi, resemble spores of the genus *Glomus*, in particular spores described for *Glomus versiforme* (Bonfante et al. 1990), but are distinct from spores of other genera of the Glomales. In an attempt to understand the biology of *Geosiphon* and to place this organism in its phylogenetic position with the AM fungi and other fungal classes, we have employed gene amplification using polymerase chain reaction (PCR), cloning, and sequencing of the small-subunit (SSU) ribosomal RNA genes (Gray et al. 1984; Bruns et al. 1991, 1992). The data obtained were interpreted based on distance, parsimony, and maximum-likelihood approaches (Felsenstein 1989). Using 59 complete SSU rRNA sequences, we have generated a phylogenetic tree including the sequences of *Geosiphon pyriforme* and *G. versiforme*, 13 AM-fungi (Glomales), 14 Ascomycetes, 11 Basidiomycetes, 11 Zygomycetes, four Chytridiomycetes, and additionally one higher plant, two chromists, and one protist as outgroups (Table 1).

Materials and Methods

Sample Preparation. The DNAs of both *Geosiphon pyriforme* and *Glomus versiforme* were in each case isolated from one single spore. *G. versiforme* (strain NRRL5844) was kindly provided by Valeria Bianciotto and Paola Bonfante (Turin, Italy). *Geosiphon pyriforme* spores

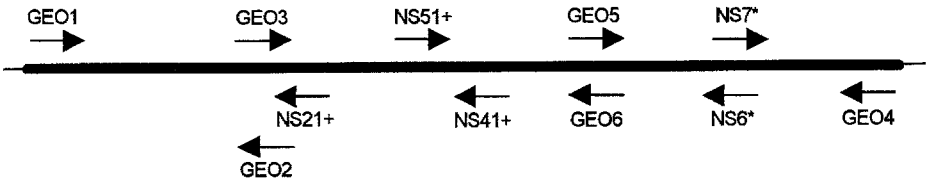


Fig. 2. Diagram of the relative location of the SSU primers used for amplification and sequencing reactions. *Arrows* indicate direction of primer extension. *Thin lines* represent flanking intergenetic spacers. + and * primer sequences described by Simon et al. (1992) and White et al. (1990). The remaining primer sequences are as follows: *GEO1:* TATAATCGTTATACAGGTGAAAC; *GEO2:* CAATTGTTCCTCGTTAAG; *GEO3:* CTTAACGAGGAACAATTGGA; *GEO4:* AAACCTTGTTACGACTTTTACTT; *GEO5:* GCAATAACAGGTCTGTT; *GEO6:* ACAGACCTGTTATTGC.

were harvested from 5–6-month-old laboratory cultures, free of fungal contamination. The spore surfaces were cleaned by 4 × 15 s sonication pulses followed by rinses with sterile water (Wyss and Bonfante 1993). Afterward the water was removed by placing the spores on sterile filter paper. Spores were collected with sterile toothpicks, placed in a microcentrifuge tube, and crushed with a miniature pestle in 20 µl sterile water. Highly purified DNA was prepared by combining the chelex method (Perkin Elmer, Weiterstadt, Germany) and the Gel Extraction Kit from Genomed (Bad Oeynhausen, Germany) (Hengen 1994). The 20-µl spore suspension was mixed with 40 µl of chelex resin (25% in sterile water), and sterile water was added to a final volume of 100 µl. After vortexing, the preparations were first sonicated once with a 10-s pulse, then frozen and thawed in liquid nitrogen four times, and heated finally at 95°C for 20 min. After short centrifugation, the supernatants were transferred to fresh 0.5-ml Eppendorf tubes, 15–30 µl of the Jetsorb suspension from Genomed (Bad Oeynhausen, Germany) was added, and DNA preparations were completed by following the protocol of the producer. DNA eluates were stored at −20°C and diluted tenfold before being used as templates for PCR.

PCR Conditions. First PCR amplification reactions with universal primers and the taxon-specific SSU primer VANS1 (Simon et al. 1992) gave only weak amplification products. Thus we have constructed five primers, GEO1a–e, beginning with the VANS1 sequence going downstream the consensus sequence of the known AM fungi in 3-bp steps (Fig. 1). From the five constructed primers only GEO1b–e gave strong amplification products with GEO2 as corresponding PCR primer. For sequence analysis we amplified overlapping fragments with the primer pairs GEO1-GEO2 and GEO3-GEO4 (Fig. 2). The reaction mix consisted of 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 µM of each dNTP, 15 pmol of two appropriate primers, and 1.5–2.5 U PrimZyme DNA Polymerase (Biometra, Göttingen, Germany) in 100-µl total reaction volume, overlaid with light paraffin oil. The amplification reaction was performed in a Biometra personal cycler programmed as follows: 1 × 5 min at 95°C; 35 cycles of 30 s at 95°C, 45 s at 52°C, 90 s at 72°C; 1 × 5 min at 72°C, and a cool-down step to 4°C.

DNA Sequencing. The amplification products were gel-purified on a 1.3% agarose gel, extracted with the Jetsorb gel extraction kit of Genomed, cloned into pUC 18 Vector, and transformed into *E. coli* DH5α competent cells. Plasmid DNA from positive clones were prepared with the QIAprep spin plasmid kit of Qagen. The resulting purified DNA was sequenced with a chain-termination sequencing kit (T7 sequencing kit, Pharmacia, Freilburg, Germany).

Data Analysis. The sequences determined in the present study were aligned with sequences representing all fungal classes, a higher plant, a ciliate, and two chromists. Additionally, four unpublished Zygomycotina sequences were obtained from Kerry O'Donnel (Peoria, IL, USA; see Table 1). In the complete dataset, long "noninformative" gaps occurring in single sequences were reduced to a length of one by replacing gaps by *N* since these gaps in most cases probably represent single deletion-events and would otherwise be overestimated in parsimony analysis. For the same reason, inserts in single sequences have been reduced to a length of one site. We have also replaced short parts of two Zygomycotina and one Chytridiomycetes sequences by *N,* where these were the only sequences uncertain to be in alignment. This allowed us to use the information of the other sequences at these sites for the analysis. For a discussion of this alignment method see Bruns et al. (1992). For the phylogenetic analyses the SSU rRNA coding regions of *Stylonychia pustulata, Zea mays, Ulkenia profunda,* and *Thraustochytrium kinnei* were used as outgroup taxa. Preliminary experiments with *Plasmodium falciparum* as outgroup gave the same results. Out of the whole aligned sequences, a dataset of 1,593 sites certain to be in alignment was taken for the construction of phylogenetic trees. Most analyses were run on a 486DX2/66 PC with the Microsoft Windows executables of the PHYLIP program package, version 3.55c (Felsenstein 1982, 1989). The analyses were bootstrapped up to 1,000 times to estimate robustness of tree structure, input order of species was randomized, and *Stylonychia* was taken as outgroup. After the construction of a phylogenetic tree including all 59 species (see Table 1) by the neighbor-joining method (Saitou and Nei 1987; Nei et al. 1995) with Kimura parameters (Kimura 1980) and the parsimony method (Felsenstein 1983), 17 species were excluded from the dataset before further analyses with the Fitch and Margoliash (1967) and maximum-likelihood (Felsenstein 1981) methods because of computer time constraints. Maximum-likelihood analysis was run with the program fastDNAml (Olsen et al. 1994) on UNIX computer. Phylogenetic reconstructions show only groupings with bootstrap values of more than 50%. Others were collapsed to polytomies in order to prevent misinterpretations of these low bootstrap supports.

Nucleotide Sequence Accession Numbers. Reported sequences are deposited in the EMBL database. Accession numbers are X86686 *Geosiphon pyriforme* and X86687 *Glomus versiforme* (strain HC/F-E01). The sequences of *Micromucor ramannianus* (strain NRRL5844), *Mortierella polycephala* (strain NRRL22890), *Mucor mucedo* (strain NRRL3635), and *Syncephalastrum racemosum* (strain NRRL2496) are not published and were kindly provided by Kerry O'Donnel (Peoria, IL, USA). All sequences used for the analysis are summarized in Table 1. Citation data are available in the EMBL database.

Results

SSU rRNA genes from *Geosiphon pyriforme* and *Glomus versiforme* were amplified by PCR. The two new SSU rRNA gene sequences span a length between the amplification primers of 1,712 (*Glomus versiforme*) and 1,715 (*Geosiphon pyriforme*) bases, excluding an estimated length of about 65 bp at the 5′ and 30 bp at the 3′ end of the SSU rRNA molecule. The sequences were

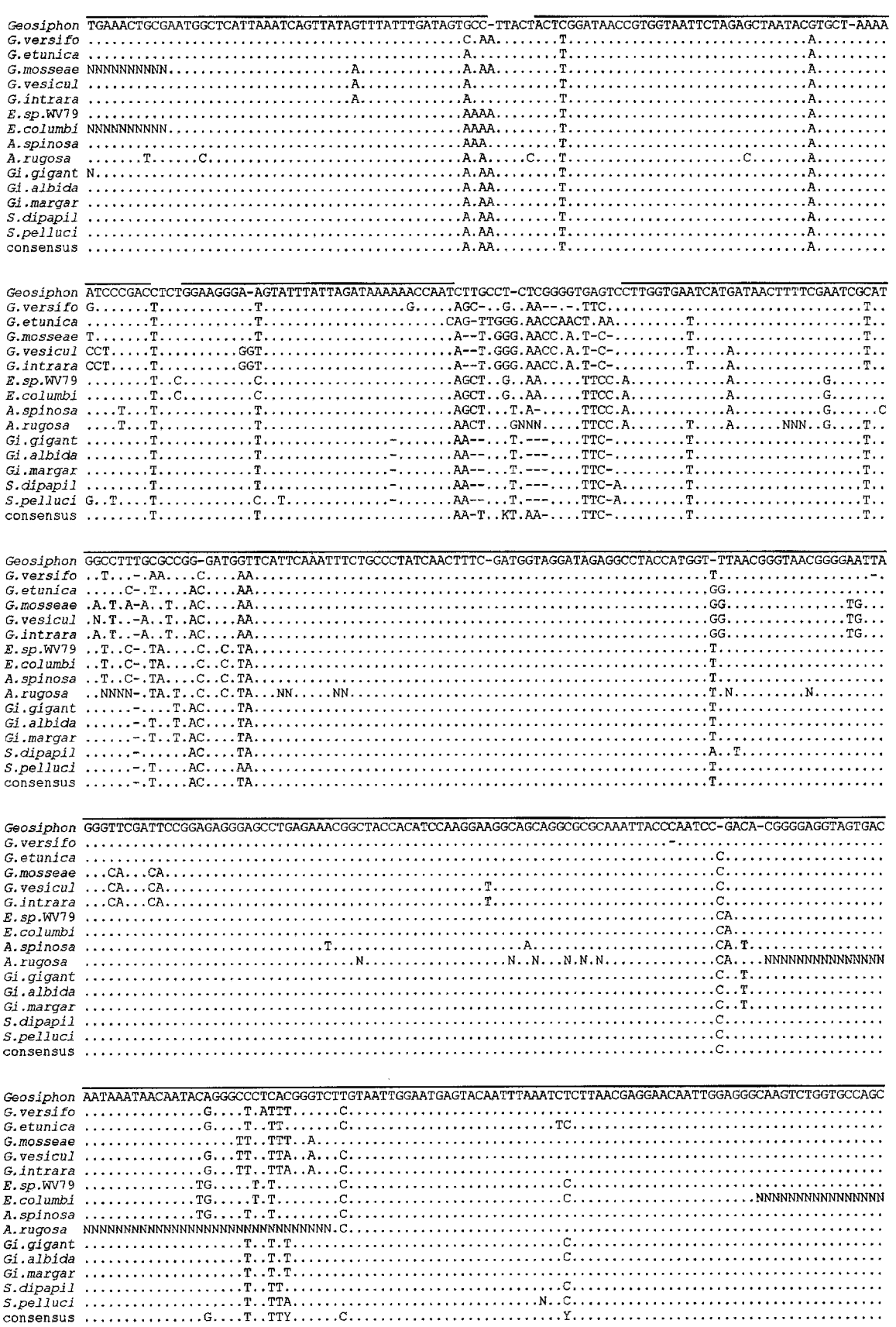


Fig. 3. Alignment of the SSU rRNA sequences of the Glomales. The sites used for the analyses are marked by a line. Each *N* denotes an unknown nucleotide, each *dash* a deletion or insertion. A *dot* indicates that the nucleotide is identical to that in the *Geosiphon* sequence. The consensus sequence of the Glomales (without *Geosiphon*) is also shown.

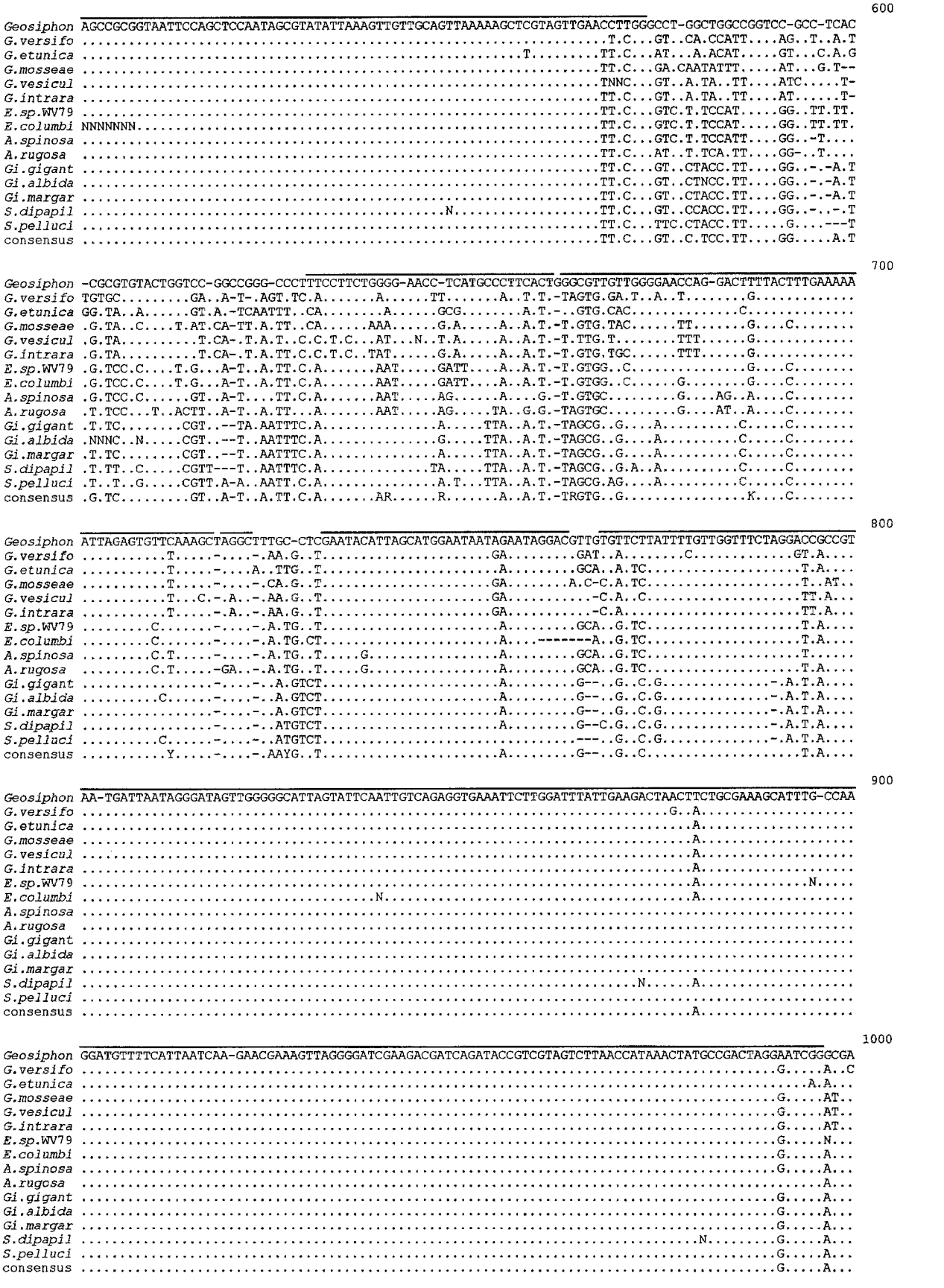
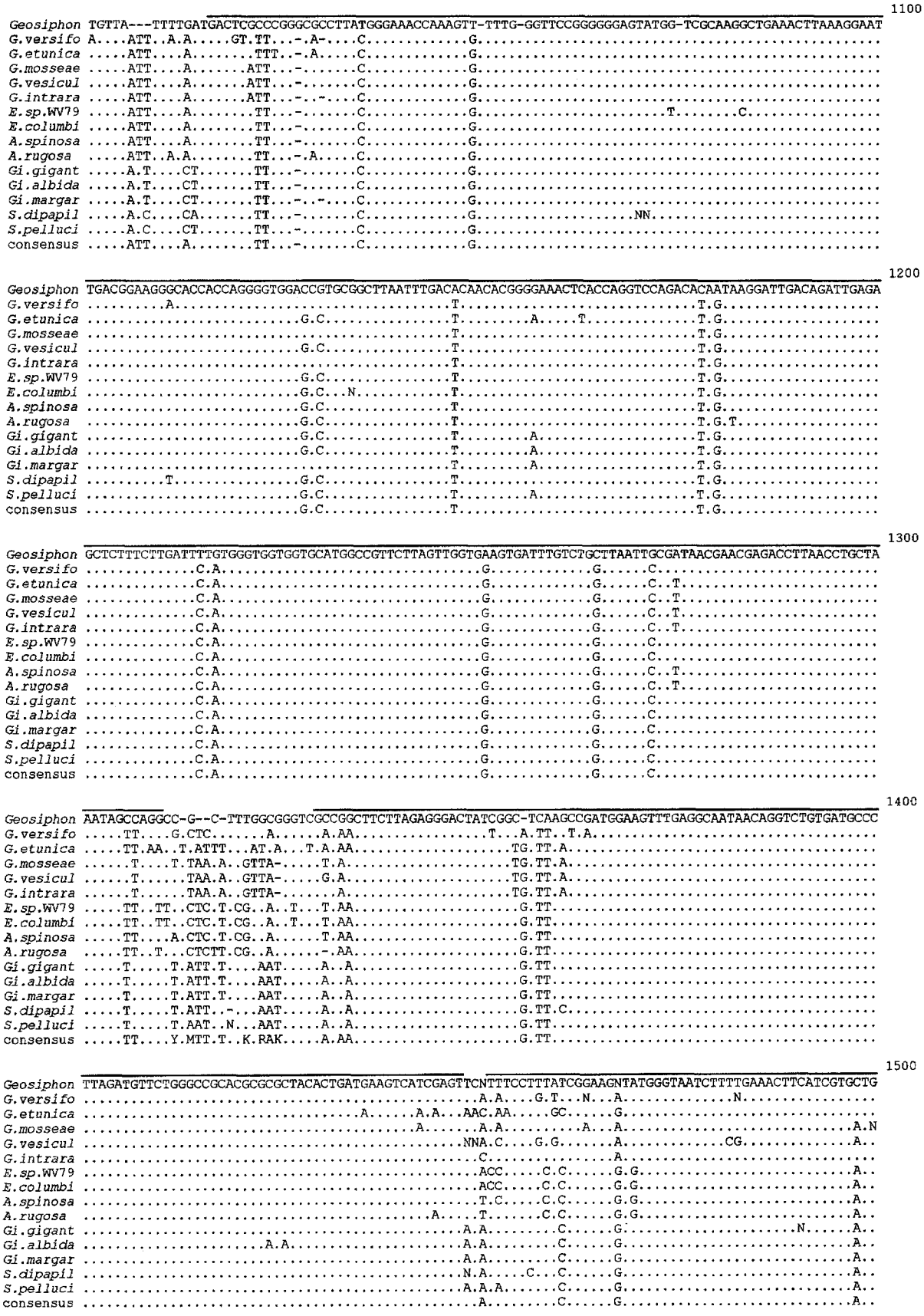


Fig. 3. Continued.



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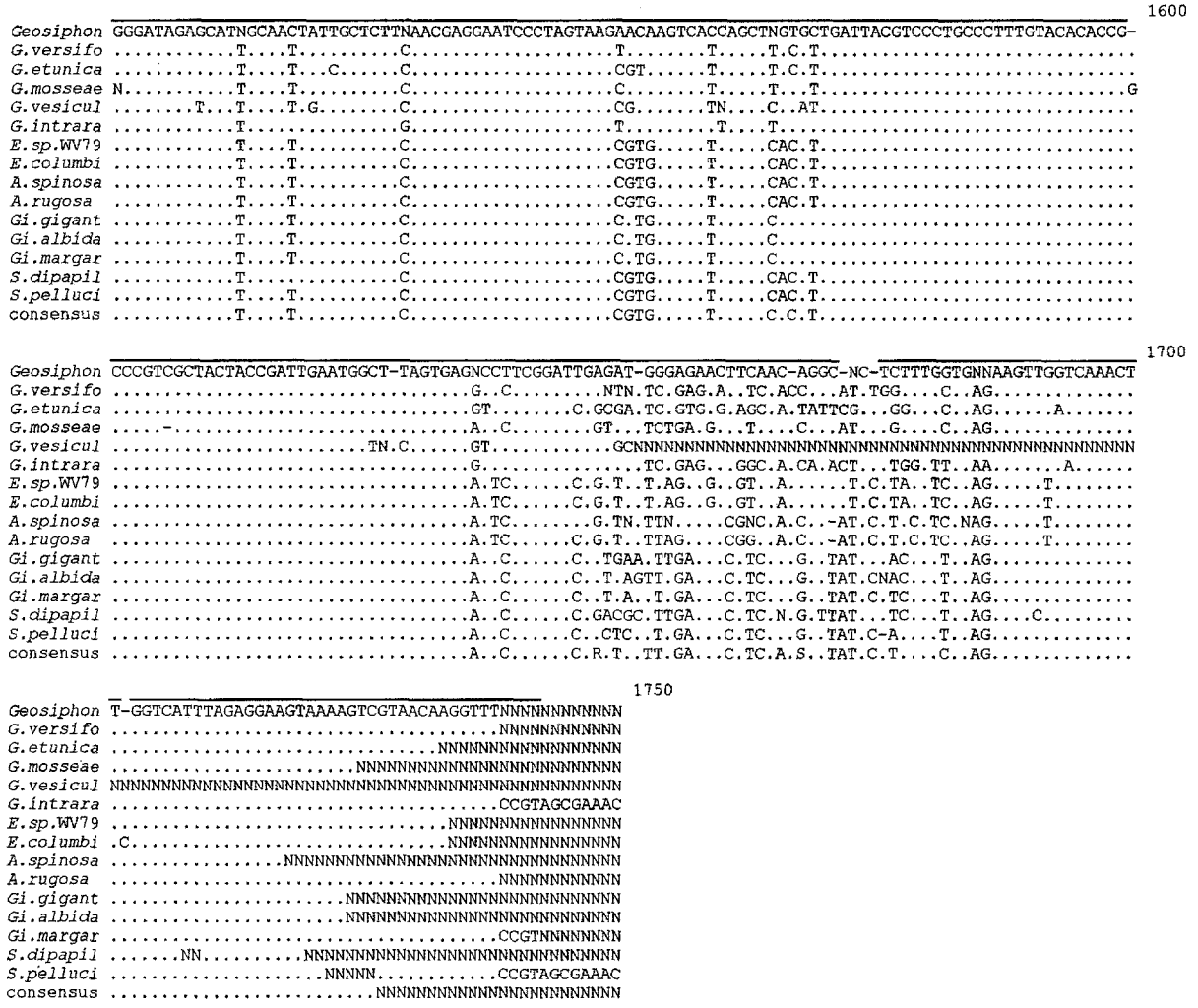

Fig. 3. Continued.

aligned with 57 eukaryotic SSU rRNAs and 1,593 sites certain to be in alignment were taken for the construction of phylogenetic trees.

Figure 3 shows an alignment of all known SSU rRNA gene sequences of AM fungi, representative for the three families recognized so far, with the two new sequences of *Geosiphon pyriforme* and *Glomus versiforme* (a full alignment of all 59 species is available from the authors).

The phylogenetic analysis by all methods used strongly indicates that *Geosiphon* and the other Glomales are forming a monophyletic group. In the neighbor-joining (1,000 replicates; Fig. 4) and parsimony analysis (200 replicates) the monophyly is supported by 90%. The percentage in the neighbor-joining analysis rises to 96% if the *G. etunicatum* sequence is excluded from the data set (Fig. 4) or if a reduced dataset not including some species which do not form groups with others is used (Fig. 5). The Fitch-Margoliash analysis (100 replicates) of the reduced dataset also gives strong bootstrap support of 96% for this group (Fig. 5). Maximum-likelihood analysis leads to the same monophyletic clade, including *Geosiphon,* and 80% bootstrap support (50 replicates; Fig. 5).

Within the genus *Glomus* greater divergence is seen, such as between the families Acaulosporaceae and Gigasporaceae. The monophyletic origin of the group comprising *G. intraradices, G. vesiculiferum,* and *G. mosseae* is strongly supported, whereas the relationship of *G. versiforme* to this branch is less significant. Within the Glomales *G. etunicatum* and *Geosiphon* form very early branches. Despite these poorly defined early branches within the Glomales, these form a distinct group with high bootstrap support in all analyses and seem to be more closely related to the Basidiomycetes and Ascomycetes than to the Zygomycetes and Chytridiomycetes.

Discussion

SSU rRNAs are relatively large molecules containing highly conserved regions as well as small, partially conserved elements, and sequence data permit the inference of phylogenetic investigations on different taxonomic levels (Bruns et al. 1991). To find further support for the assumption by Schüßler et al. (1994) that *Geosiphon*

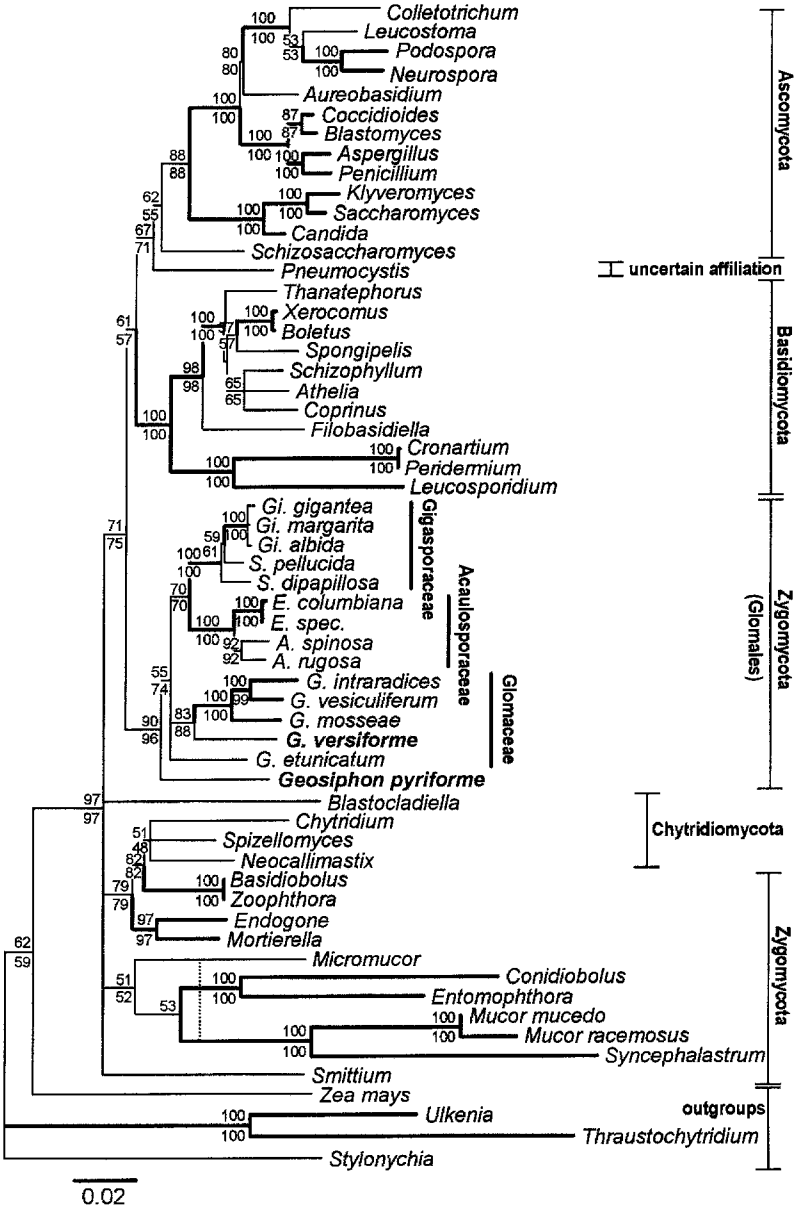

Fig. 4. Phylogenetic analyses of 55 fungal SSU rRNA sequences with a ciliate, a plant, and two chromists as outgroups. Neighbor-joining consensus tree. *Numbers* on internal branches are percent bootstrap values of 1,000 replicates. *Thick lines* delineate the topology that is supported by 95% or more of the bootstraps. Branches with bootstrap values below 50% are reduced to polytomies. The *upper numbers* and branch lengths correspond to the computation of the complete dataset of 59 sequences. *Lower numbers* denote the values obtained if *Glomus etunicatum* was excluded from the analysis. This causes a single change in the branching order leading to an occurrence of *Micromucor* ancestral to the Mucorales with low bootstrap support of 49% (indicated by *broken line*). A parsimony consensus tree (not shown) obtained by 200 bootstraps shows only two changes in the tree topology: (1) *Micromucor* is indicated ancestral to the Mucorales with bootstrap support of 65% (same topology like obtained by the neighbor-joining analysis without *G. etunicatum*), (2) *Filobasidiella* is placed at the *Peridermium I Cronatium I Leucosporidium* branch with bootstrap support of 60%. The Glomales clade is supported by 90% in the parsimony analysis.

pyriforme belongs to the order Glomales, we analyzed the SSU rRNA genes of *Geosiphon* and *Glomus versiforme.*

The phylogenetic relationships found indeed show *Geosiphon* as a member of the Glomales, but representing a very early branch within this group. The same can be said for *Glomus etunicatum.* This is consistent with the results of Simon et al. (1993a), who found unexpected large differences among the genus *Glomus* and suggested that *G. etunicatum* might be placed in a separate family (Simon et al. 1993a,b). The differences of SSU sequences within the genus *Glomus* are larger then between the phylogenetic younger families Acaulosporaceae and Gigasporaceae. The suggested subdivision of the genus *Glomus* is supported by our results, adding a further independent phylogenetic branch, represented by *Geosiphon pyriforme,* which does not cluster with any of the Glomales species sequenced so far. The affiliation of *G. versiforme* to the monophyletic clade of *G. vesiculiferum, G. intraradices,* and *G. mosseae* also shows only low-to-medium bootstrap support. The sequence analyses of Simon et al. (1993a) indicate that *Glomus* represents the oldest genus of the Glomales. *Glomus*-like fungi probably were the ancestral endomycorrhizal fungi and originated in the Paleozoic era at the time when the first land plants appeared (415 Myr; Simon et al. 1993a). This is in agreement with the exclusivity of *Glomus*-like fossil records (Stubblefield et al. 1987) and the discovery of arbuscules in 400-Myr-old *Aglaophyton* fossils (Remy et al. 1994). Walker (1992) pointed out that even by classical criteria the genus *Glomus* is already certainly phylogenetic and that the taxonomic position of some *Glomus* species within the order Glomales is not clear. By classical criteria, there are at least three distinct groups within the genus *Glomus* (Walker 1992), and until more SSU rRNA gene sequences of species belonging

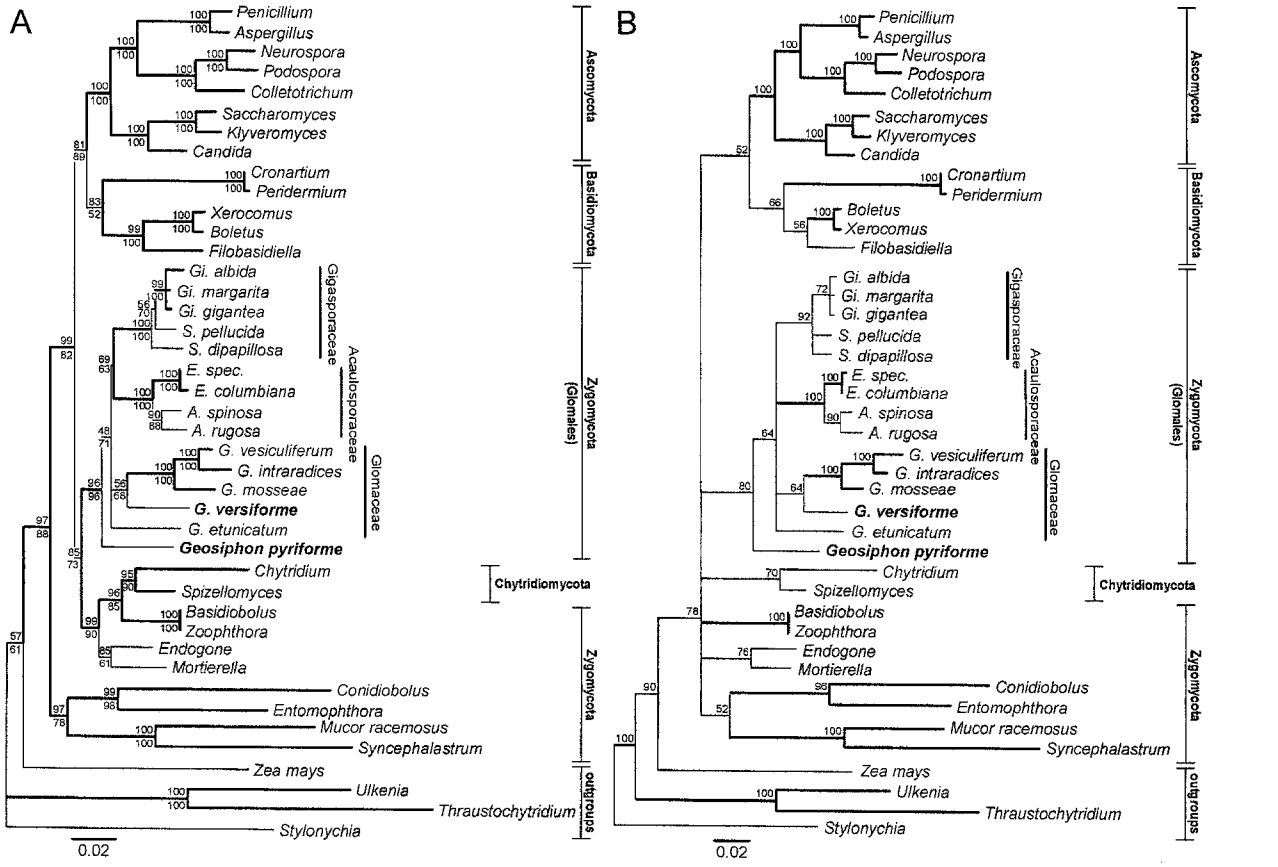

Fig. 5. Phylogenetic analyses of the reduced dataset (42 sequences) comprising 38 fungal SSU rRNA sequences. *Thick lines* delineate the topology that is supported by 95% or more of the bootstraps. Branches with bootstrap values below 50% are reduced to polytomies. **A** Neighbor-joining consensus tree obtained by 1,000 bootstrap replicates. Bootstrap values are given as *numbers above* branches. *Numbers below* the branches are the percentages of a Fitch-Margoliash analysis (100 replicates), showing an identical tree topology. **B** Consensus tree of a maximum likelihood computation (50 replicates).

to this group are known, a defined classification or subdivision of the genus *Glomus* will probably not be possible. At the moment it should be considered as the phylogenetically oldest group of the Glomales.

Thus, *Geosiphon* can be assumed to be a member of the genus *Glomus* if this is seen as a polyphyletic group of phylogenetically old branches inside the order Glomales, and the light and electron microscopic results (Schüßler et al. 1994) are taken as taxonomic criteria. Considered from a SSU rRNA analysis point of view, *Geosiphon* could belong to a clade comprising *Glomus* spp. not sequenced so far, or to an ancestral family within the Glomales. In our opinion it makes little sense to speculate about the definitive classification of *Geosiphon* until more Glomales sequences (in particular of the genus *Glomus*) are available. However, doubtless *Geosiphon* represents an old branch within the Glomales sequenced so far, and thus probably represents a very ancient kind of symbiosis between a member of the Glomales and a photoautotrophic organism. About 80% of all vascular plant species form arbuscular endomycorrhizas (Bonfante and Perotto 1995; Malloch et al. 1980) including ferns (Peterson et al. 1981) and Lycopodiaceae (Schmid and Oberwinkler 1993). Also, all three classes of Bryophytes show members with AM-like associations (Parke and Linderman 1980, Ligrone 1988; Ligrone and Lopes 1989; Stahl 1949). Probably the evolution of the land plants became highly promoted by the establishment of associations between plants and *Glomus*-like fungi, efficiently supplying the plants with water and nutrients from the soil (Malloch et al. 1980; Pirozynski 1981; Simon et al. 1993a). Pirozynski and Malloch (1975) postulated a partnership between two basically aquatic protists, a green alga and a "phycomycetous" fungus, as the initial step of land plant evolution, a hypothesis which became widely accepted (e.g., Price 1991; Lewis 1991; Kendrick 1991) but which until now has lacked direct evidence. *Geosiphon pyriforme* now indirectly supports this hypothesis by the formation of a symbiosis between a fungus belonging to the Glomales and a photoautotrophic prokaryote. Thus all other possible earlier associations between fungi and photoautotrophic organisms are less speculative but appear probable. At this point it should also be noted that, on the basis of their ultrastructural features and cytochemical properties, the symbiotic interfaces established between fungus/cyanobacterium in the *Geosiphon* symbiosis and arbuscule/root-cell in the AM symbiosis share many fea-

tures (Schüßler et al. 1996) and therefore have been suggested to be homologous.

Another interesting result of the present study is the grouping of *Endogone* together with *Mortierella* with high bootstrap support in all analyses. In the study of Bruns et al. (1992) a grouping of *Endogone* with the Chytridiomycetes, except *Blastocladiella,* with low bootstrap support has unexpectedly been shown. In our study the Chytridiomycetes still seem to form a monophyletic branch together with *Basidiobolus, Zoophthora, Endogone,* and *Mortierella* in the neighbor-joining and Fitch-Margoliash analysis. It is in particular the case with the reduced dataset, where some species which do not group with others (e.g., *Blastocladiella, Smittium, Micromucor*) are excluded from the analysis. In that case the noted clade is supported by bootstrap values of 99 and 90% in neighbor-joining and Fitch-Margoliash analysis, respectively. In contrast, in the parsimony analysis of the complete dataset and the maximum-likelihood analysis of the reduced dataset, there is no good support for this clade, leading to the conclusion that no statement should be made on the phylogeny of this group based on the present state of knowledge. This also shows the dependence of the obtained results on the method of analysis used. In this context one should remember that the grouping of *Geosiphon* within the Glomales—the topic of this study—is well supported by all analyses used. The old taxonomy with the order Endogonales including the AM fungi and the genus *Endogone* (Benjamin 1979), which is still used in many textbooks, should be avoided. The revised classification of Morton and Benny (1990) with the order Glomales represents a more "natural" taxonomy, although the placing of the Glomales inside the Zygomycotina is questionable.

Within the Zygomycotina and Chytridiomycetes, more sequences have to be provided in order to allow proper interpretation of their phylogenetic relationships. However, one may speculate that Zygomycotina are polyphyletic and share relationships to some Chytridiomycetes. In any case, contrary to the Ascomycotina and Basidiomycotina, these groups are taxonomically poorly defined to date.

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