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Review

The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, *Fungi**

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A data set with 1551 fungal sequences of the small subunit ribosomal RNA has been analysed phylogenetically. Four animal sequences were used to root the tree. The parsimony ratchet algorithm in combination with tree fusion was used to find most parsimonious trees and the parsimony jackknifing method was used to establish support frequencies. The full-length consensus tree, of the most parsimonious trees, is published and jackknife frequencies above 50 % are plotted on the consensus tree at supported nodes. Until recently attempts to find the most parsimonious trees for large data sets were impractical, given current computational limitations. The parsimony ratchet in combination with tree fusion was found to be a very efficient method of rapid parsimony analysis of this large data set. Parsimony jackknifing is a very fast and efficient method for establishing group support. The results show that the *Glomeromycota* are the sister group to a monophyletic *Dikaryomycota*. The majority of the species in the *Glomeromycota*/*Dikaryomycota* group have a symbiotic lifestyle – a possible synapomorphy for a group '*Symbiomycota*'. This would suggest that symbiosis between fungi and green plants evolved prior to the colonization of land by plants and not as a result of the colonization process. The *Basidiomycotina* and the *Ascomycotina* are both supported as monophyletic. The *Urediniomycetes* is the sister group to the rest of the *Basidiomycotina* successively followed in a grade by *Ustilaginomycetes*, *Tremellomycetes*, *Dacrymycetales*, *Ceratobasidiales* and *Homobasidiomycetes* each supported as monophyletic except the *Homobasidiomycetes* which are left unsupported. The ascomycete node begins with a polytomy consisting of the *Pneumocystidomycetes*, *Schizosaccharomycetes*, unsupported group with the *Taphrinomycetes* and *Neolectales*, and finally an unnamed, monophyletic and supported group including the *Saccharomycetes* and *Euascomycetes*. Within the *Euascomycetes* the inoperculate euascomycetes (*Inoperculata*) are supported as monophyletic excluding the *Orbiliomycetes* which are included in an unsupported operculate, pezizalean sister group together with *Helvellaceae*, *Morchellaceae*, *Tuberaceae* and others. *Geoglossum* is the sister group to the rest of the inoperculate euascomycetes. The *Sordariomycetes*, *Dothideomycetes*, *Chaetothyriomycetes* and *Eurotiomycetes* are each highly supported as monophyletic. The *Leotiomycetes* and the *Lecanoromycetes* both appear in the consensus of the most parsimonious trees but neither taxon receives any jackknife support.

INTRODUCTION

The polyphyletic origin of the various fungal-like taxa is generally acknowledged with all major groups, *Fungi*, *Oomycota* (included in the *Straminipila*), *Plasmodiophoromycota*, *Myxomycota*, *Dictyosteliomycota*, *Acrasiomycota* evolving in different lineages as related to major or well-known eukaryotes (Alexopoulos, Mims & Blackwell 1996). Fungal-like groups, such as '*Myxomycetes*', *Oomycetes*, and *Plasmodiophoromycetes*, are not included in the *Fungi* and believed to be more closely related to other eukaryotes (Hawksworth 1991, Tehler 1988, 1995, Tehler *et al*. 2000).

Evidence clearly indicates that the chitinous fungi, *Fungi*, constitutes a monophyletic group including *Chytridiomycetes*, *Zygomycetes*, *Ascomycotina* and *Basidiomycotina* (for references see Hawksworth *et al*. 1995). Their monophyly is supported by both morphological, chemical and other characters such as chitin in the cell walls, hyphal organization, heterotrophism by absorption of predigested food, a-aminoadafic (AAA) lysine synthetic pathway, and the flagellar apparatus,

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if present (Tehler 1988). Monophyly is also supported by sequences from the SSU rDNA (Bruns, White & Taylor 1991, Lipscomb *et al*. 1998, Tehler *et al*. 2000, Wainright *et al*. 1993).

Alexopoulos *et al*. (1996) and Hawksworth *et al*. (1995) use the kingdom name *Fungi* for this group, equalling to *Eumycota* as used by Tehler *et al*. (2000). In the following we will use *Fungi* or chitinous fungi for this particular group of organisms since nearly all of its members share cell walls with chitin (Prillinger 1982, Tehler 1988, 1995).

Various suggestions on the evolution of the *Fungi* have been put forward: on the basis of the lysine biosynthesis pathway, Vogel (1964) postulated that the nonflagellate fungi, *Amastigomycotina*, together with the nonlysine-producing protozoa and metazoa were derived from the euglenids. Cavalier-Smith (1987) suggested a choanoflagellate as a common ancestor of fungi and animals. None of these suggestions received support from parsimony jackknifing of SSU rDNA sequences (Lipscomb *et al*. 1998). Recently it was suggested that the *Microsporidia* constituted the sister group to the *Fungi* (Hirt *et al*. 1999) but that hypothesis has been contradicted by data from both ribosomal sequences (Lipscomb *et al*. 1998) and protein sequences (Tanabe, Watanabe & Sugiyama 2002). We conclude that the hypothesis describing a sister group relationship between chitinous fungi and animals is today the most well established and most widely adopted among phylogeneticists (Tehler *et al*. 2000).

The present study aims to: (1) find well supported fungal groups among the *Fungi*; and (2) find the most parsimonious trees for data sets of this magnitude in order to gain an indication of possible sister group relationships of groups that do not receive jackknife support.

We also wanted to publish the full length tree with all terminal sequences included to make it convenient for mycologists to view all details of the tree rather than the less informative summary trees.

MATERIALS AND METHODS

Taxon sampling

Nucleotide sequences of ribosomal RNA, in particular the small subunit of the rRNA, are now available from thousands of eumycete species. We downloaded the most recent data set of aligned small subunit RNA sequences from the European ribosomal RNA database with 1553 eumycete terminals (down-loaded Feb. 2002). A compilation of all complete, or nearly complete, small subunit ribosomal RNA sequences can be consulted via the World Wide Web at http://oberon.rug.ac.be:8080/rRNA/ssu/index.html. Sequences are provided in aligned format. The alignment takes into account the secondary structure information derived by comparative sequence analysis of thousands of sequences. Additional information such as literature references, accession numbers, taxonomy, secondary structure models and nucleotide variability maps, is also available (Wuyts *et al*. 2002).

Two sequences of *Calicium tricolor* (accession nos. L37534 and L37733) were considered spurious by the authors (Gargas & Taylor 1995). The problematic nature of those sequences was also confirmed by Tehler *et al*. (2000) and therefore they were excluded from the analyses. The number of species is approximately 19% lower than the actual number of terminals because some species are represented by more than one sequence. The names of all terminals strictly follow that of their respective GenBank entry[1]. We have found numerous obvious misidentifications, misspellings and outdated names in the data set. But in order to avoid any misunderstanding from the original GenBank entry no measures have been taken to correct names or interpret or evaluate the status of the taxa involved. This highlights the importance that depositers of sequences also deposit voucher specimens in public collections (Agerer *et al*. 2000).

Acanthocoepsis unguiculata (*Choanoflagellates*), *Mnemiopsis leidyi* (*Ctenophora*), *Trichoplax adhaerens* (*Placozoa*) and *Scypha ciliata* (*Porifera*) were chosen as outgroups on basis of the results from Lipscomb *et al*. (1998). All of these species arise from the basal part of the animal clade or from the basal polytomy in the fungal-animal clade.

The final data set consisted of 6561 aligned positions for 1555 terminals. Among the 1822 informative positions 23.91% of the cells were scored as insertions/deletions (treated as missing data by the analysis programs). 0.7% of the cells were scored as polymorphic due to ambiguities in the sequences.

Phylogenetic analyses

Parsimony ratchet

Due to the inherent difficulties in analysing large data sets several different search strategies were applied to this matrix. Preliminary, informal, testing of the various search strategies indicated that the parsimony ratchet (Nixon 1999) in combination with tree fusion (Goloboff 1999) was the most effective search method.

Parsimony ratchet tree searches were performed using WinClada version 1.00.08 (Nixon 2003) and NONA version 1.6 (Goloboff 1993). As currently implemented, WinClada version 1.00.08 (Nixon 2003) controls NONA version 1.6 (Goloboff 1993) through the use of a procedure file. The ratchet algorithm, as modified from the original algorithm proposed by Nixon (1999), greatly increases the effectiveness of tree search (K. C. Nixon, pers. comm.). WinClada instructs NONA to do the following calculations:

(1) Using the original weights for each character (in this case 1 for all characters) calculate a Wagner

[1] A list with accession numbers is available from A.T.

tree using a single random terminal addition sequence. Holding up to two trees in memory, swap using Simple Pruning Regrafting (SPR) followed by Tree Bisection Reconnection (TBR). Save the resulting tree to a file.

(2) A set percentage of the nodes in the resulting tree are randomly selected and constrained to their current topology (in this case 10%).
(3) A set percentage of the characters are selected with replacement (in this case 15%). Each time a character is selected its weight is increased by 1.
(4) Holding one tree in memory swap using TBR.
(5) Randomly select characters for deactivation using the jackknife sampling procedure as described by Farris *et al.* (1996). This results in the deactivation of approximately 37% of the characters.
(6) Holding one tree in memory swap using TBR.
(7) Remove the constraints from all nodes, activate all characters, and reset all weights to their original values.
(8) Holding one tree in memory swap using TBR. Save the resulting tree to a file.
(9) Steps 2–8 are repeated a set number of times (in this case either 200 or 500).

Ratchet tree files, containing either 201 or 501 trees, were submitted to TNT version 0.2k (Goloboff, Farris & Nixon 2002) for tree fusion. Between 10 and 100 rounds of unidirectional tree fusion were conducted on each ratchet tree file (saving equal length trees). In addition multiple ratchet files (up to four) were concatenated and submitted for between 10 and 1000 rounds of tree fusion (saving equal length trees).

Parsimony jackknifing

Another way to address the problem of analysing large, ambiguous data sets is parsimony jackknifing (Farris *et al.* 1996), a technique that has been incorporated into the Xac computer program (Farris 1997). In contrast to searching for multiple most parsimonious trees and identifying supported groups by computing a consensus, jackknifing uses resampling to find well-supported groups. Resampling works by calculating a tree for each of a large number of subsamples (pseudoreplicates) of characters from the data, then finding a summary tree, which comprises the groups occurring in a majority of the trees for subsamples. This approach has proved to be very useful for rapid analyses of large data sets (Källersjö *et al.* 1998, Lipscomb *et al.* 1998, Tehler *et al.* 2000). In parsimony jackknifing the data are internally resampled with a jackknifing technique (Farris *et al.* 1996). In parsimony jackknifing the tree for each pseudoreplicate is found by parsimony analysis, and each pseudoreplicate is formed by randomly selecting characters from the data without replacement, each character having a fixed chance 1/e (about 37%) of being excluded. With this resampling technique the actual number of characters used may vary from replicate to replicate. The resampling procedure can be repeated up to 10 000 times. The program automatically discards groups found in less than 50% of the trees for pseudoreplicates, thus eliminating unjustified (poorly supported) resolution caused by ambiguous data sets.

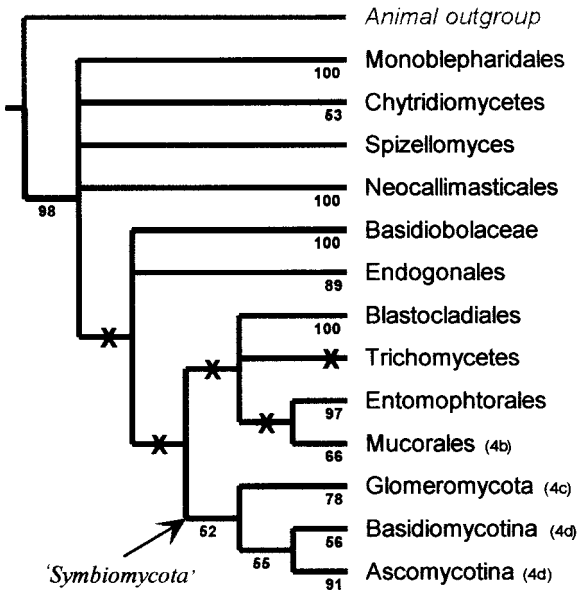

Fig. 1. The *Fungi*, summary of the consensus ratchet tree. Parsimony jackknife frequencies are given at supported nodes. A cross indicates unsupported node. (For detailed resolution see Fig. 4a or subfigure as indicated within parantheses.)

The Xac program (Farris 1997) provides branch swapping and random addition sequences. In this analysis we used SPR branch swapping with five random addition sequences per pseudoreplicate. The number of pseudoreplicates used in this analysis was 1000. The computer employed was a PowerBook G4/500.

RESULTS

The consensus tree from the most parsimonious trees as received from the parsimony ratchet analysis are shown as three summary trees (Figs 1–3) and the full length consensus (Fig. 4). Jackknife frequencies are plotted on supported nodes.

Presumed shortest trees, 40521 steps long, were found in five independent initiations (the actual number of independent hits on the shortest trees is likely much higher, but TNT does not report the information necessary to determine how many independent hits were made). The Consistency Index (CI) of these trees is 0.10 (uninformative characters excluded) and the Retention Index (RI) is 0.81. The strict consensus Fig. 4 has 241 collapsed nodes (of 1553 possible nodes). Although the parsimony ratchet was able to find near optimal trees (less than 5 steps longer than optimal) in all cases, only one 500 iteration ratchet was able to find the shortest trees. Tree fusion was successful in finding shortest trees only when the source trees were derived from multiple ratchet files.

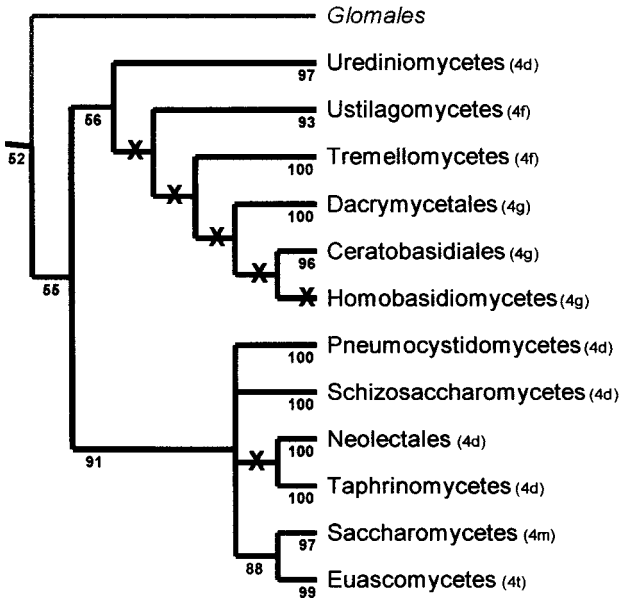

Fig. 2. The *Dikaryomycota* (*Ascomycotina* and *Basidimycotina*), summary of the consensus ratchet tree. Parsimony jackknife frequencies are given at supported nodes. A cross indicates an unsupported node. A number of species normally referred to as *Tremellomycetes* are not included in the node with 100% support. (For detailed resolution see Fig. 4 or subfigure as indicated within parantheses.)

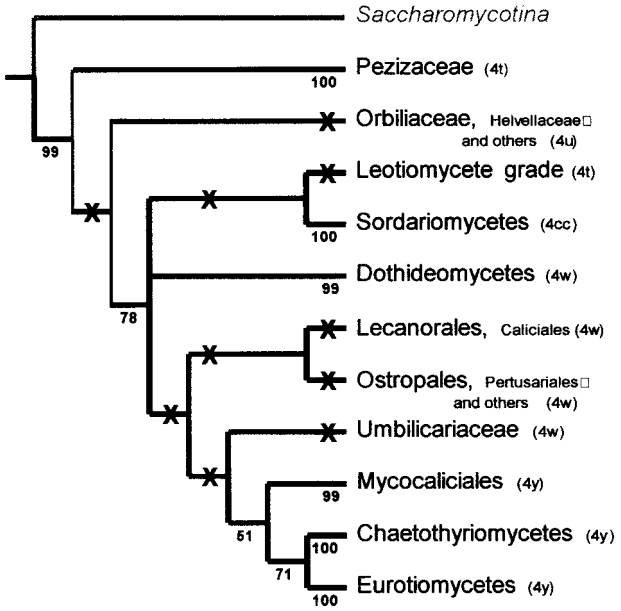

Fig. 3. The *Euascomycetes*, summary of the consensus ratchet tree. Parsimony jackknife frequencies are given at supported nodes. A cross indicates an unsupported node. A number of species normally referred to as *Dothideomycetes* are not included in the node with 99% support. (For detailed resolution see Fig. 4 or subfigure as indicated within parantheses.)

Other search procedures, implemented in TNT, such as a driven search using a combination of drift (100 rounds), and tree fusion were at best able to find trees 1 step longer than most parsimonious (40522). Conducting tree fusion on trees derived by more conventional means (e.g. from multiple addition sequences with SPR and/or TBR swapping) was also unsuccessful.

The 1000 replicate parsimony jackknife calculation in Xac needed 154 h to finish (6.3 d).

DISCUSSION

The gross topology of the consensus ratchet tree accords very well with a previous study of a large sequence data set (Tehler *et al.* 2000) except for the position of *Blastocladiales* as a sister group to the *Trichomycetes*. However, only three *Blastocladiales* sequences are included in the data set and their trichomycete relationship needs to be corroborated by the inclusion of sequences from more species.

The backbone of the summary ratchet consensus tree of all major groups has only three nodes that receive any jackknife support (Fig. 1). The first node is the *Fungi* itself with a 100% support. The second node includes the *Glomeromycota* and the *Dikaryomycota* with a 52% support. Members of *Glomeromycota* (Schüssler, Schwarzott & Walker 2001) are characterized by the ability to form endomycorrhizas, the so-called vesicular-arbuscular mycorrhizas. One genus in *Glomeromycota*, *Geosiphon*, uses a lifestyle analogous to lichen-forming fungi since it has incorporated a *Nostoc* cyanobacterium in its cells, endocyanosis. That the sister group to the *Dikaryomycota*, the *Glomeromycota*, forms mycorrhizas and even includes a form of lichenization, is certainly interesting since that would indicate that the symbiotic lifestyle among the *Dikaryomycota* (*Ascomycotina* and *Basidiomycotina*) is a plesiomorphic feature (Tehler *et al.* 2000). Mycorrhiza-forming members are very common in the *Dikaryomycota* not only among the *Basidiomycotina*, but also among the *Ascomycotina*. It is also notable that approximately half of the ascomycete species are lichen-forming. The evolution of mycorrhizal fungi, lichenization, and also pathogenicity, might date back to a common ancestor that evolved a strategy with factors for recognizing green plants and structures for infecting green plants (Tehler *et al.* 2000). Such an event would make a synapomorphic feature for a group of chitinous fungi including all *Dikaryomycota* and the *Glomeromycota* (and possibly other zygomycete groups such as *Endogonales*), here tentatively called the '*Symbiomycota*' (Tehler 2002). The data does not contain enough information to resolve the position of the *Endogonales* of which many representatives form ectomycorrhizas (Fig. 1).

Fossil glomalean fungi have been reported from the Ordovician era, at least 450 Myr ago (Redecker, Kodner & Graham 2000). In geological time that coincides fairly well with the evolution of land plants. Given the fossil evidence, did the '*Symbiomycota*' evolve prior to or after plants moved onto the land? Or did the symbiotic lifestyles evolve as a result of this event enabling green plants to colonize the new environment as suggested by Heckman *et al.* (2001)? In

the material reported by Redecker *et al.* (2000), there was no evidence of association with green plants. They estimated that land colonization began 505 Myr ago, and that the *Dikaryomycota* diverged from *Glomeromycota* much earlier, 600–620 Myr ago. The date of divergence for the *Glomeromycota* and *Dikaryomycota* matches fairly well with that of Berbee & Taylor (2000), but Heckman *et al.* (2001) pushed the date of divergence back to 1200 Myr ago and the date of land colonization back to 700 Myr ago. The estimates of Heckman *et al.* (2001) and Redecker *et al.* (2000) indicate a considerable lag time between the divergence of the *Glomeromycota* and the *Dikaryomycota* and that of land colonization. It may very well have been that the colonization of land was facilitated by a symbiosis between green plants and fungi as suggested by Heckman *et al.* (2001) but symbiosis did probably not evolve as a result of that process. Our present and earlier results (Tehler *et al.* 2000) show that it is more plausible to believe that the predecessors of the '*Symbiomycota*' evolved the factors and structures to acquire a symbiotic lifestyle much earlier, in an aquatic environment. Otherwise, and less likely, those features would be polyphyletic evolving as separate events in the *Glomeromycota* and the *Dikaryomycota* (*Ascomycotina* and *Basidiomycotina*).

Finally, the third node including all *Dikaryomycota* (*Ascomycotina* and *Basidiomycotina*) has 55% support. A summary of major groups within the *Dikaryomycota* are shown in Fig. 2. The molecular support for the *Dikaryomycota* is low, but there is at least one morphological feature to support it: the dikaryon. Dikaryotic growth is the formation of continuously growing dikaryotic cells. It is a unique feature of paramount importance for the *Dikaryomycota* since it has never been convincingly reported from any other taxa. The *Basidiomycotina* are poorly supported whereas the *Ascomycotina* are highly supported. This is in contrast to earlier results and experience (Tehler 1988, Tehler *et al.* 2000) that indicate nodes that are highly supported by sequence data usually also have morphological data to support them as well. The *Basidiomycotina* in this study, including the *Urediniomycetes*, *Ustilagomycetes*, *Tremellomycetes*, *Dacrymycetales* and *Homobasidiomycetes*, are characterized by several morphological features such as, multilaminate cell wall, nuclear membrane broken down at mitosis, free-living dikaryotic mycelium, somatogamy, and meio-blastospores (Tehler 1988) but nevertheless receive a mere 56% support from sequence data (Fig. 2). The *Ascomycotina* on the contrary, composed of the *Schizosaccharomycetes*, *Pneumocystidomycetes*, *Taphrinomycetes*, *Neolectales*, *Saccharomycetes* and *Euascomycetes* have few obvious morphological features (Tehler 1988) in common although the jackknife frequency is high 91% (Fig. 2). Free cell formation is generally considered a specific feature of the *Ascomycotina* although it is evidently shared or partly shared with the *Basidiomycotina*. Early stages of basidiospore development follow the same general pattern as that of the free cell formation process in the *Ascomycotina*: the haploid nuclei become free in the cytoplasm and develop into individual cells together with part of the plasma from the mother cell (*cfr* McLaughlin 1982). The *Basidiomycotina* are specialized by way of their nuclei and part of the plasma, which are forced to migrate by a vacuolation process, through a sterigma into a special structure formed by the sporangium wall, which will be cut off from the basidium and in which the spore formation is completed. Thus, the basidiospores are actually internally produced (Bessey 1950), and only superficially exogenetical (Bresinsky 1983, Clemençon 1977). Within the *Ascomycotina* one sister group relationship receives some support, namely the *Saccharomycetes* and the *Euascomycetes*, and the *Euascomycetes* themselves receive nearly full support (99%; Fig. 2).

Internally supported, major euascomycete groups are (Fig. 3): (1) the *Pezizaceae* (100%; but not the *Pezizales*, *Pezizomycetes sensu*, Eriksson *et al.* (2003)); (2) a major group of inoperculate euascomycetes (83%; excluding the *Orbiliaceae*); (3) the *Sordariomycetes* (100%); (4) the *Eurotiomycetes* (100%) and the *Chaetothyriomycetes* (100%) both in a sister group relationship with 72% support; and (5) the two latter taxa are included in a poorly supported group together with the *Mycocaliciales* (52%).

The *Arthoniales*, a large and important group of predominantly lichen-forming fungi, were not represented by any species in the present analyses. Earlier analyses (e.g. Gargas *et al.* 1995, Kauff & Lutzoni 2002) have shown that representatives of the *Arthoniales* are joined into a well supported clade together with the *Sordariomycetes*.

Of major interest with respect to lichen-forming fungi are the *Lecanoromycetes* since that taxon alone contains about 35% of all euascomycete species and about 75% of all lichenized species. Most authors agree that lichenized fungi do not form a monophyletic group but the question whether or not the *Lecanoromycetes* are monophyletic is not settled yet. The present analyses include lecanoromycete representatives from *Agyriales*, *Gyalectales*, *Lecanorales* (*Bacidiaceae*, *Caliciaceae*, *Cladoniaceae*, *Lecanoraceae*, *Lecideaceae*, *Parmeliaceae*, *Porpidiaceae*, *Rhizocarpaceae*, *Sphaerophoraceae*, *Stereocaulaceae*), *Medeolariales*, *Ostropales*, *Pertusariales*, *Peltigerales* (*Nephromataceae*, *Peltigeraceae*), *Teloschistales* and the families *Baeomycetaceae*, *Icmadophilaceae* and *Coccotremataceae*. Hafellner (1994) stated that the ascus features keep this otherwise heterogeneous lecanoralean group together. Molecular studies, although poorly supported and with limited taxon sampling, constantly point in the direction of monophyly for the *Lecanoromycetes* (DePriest 1994, Gargas *et al.* 1995, Lumbsch *et al.* 2002).

In the present analyses the *Lecanoromycetes* appear in the consensus ratchet tree (Figs 3–4) and thus sustain the hypothesis of a major monophyletic group of lichenized ascomycetes. However, the present data does

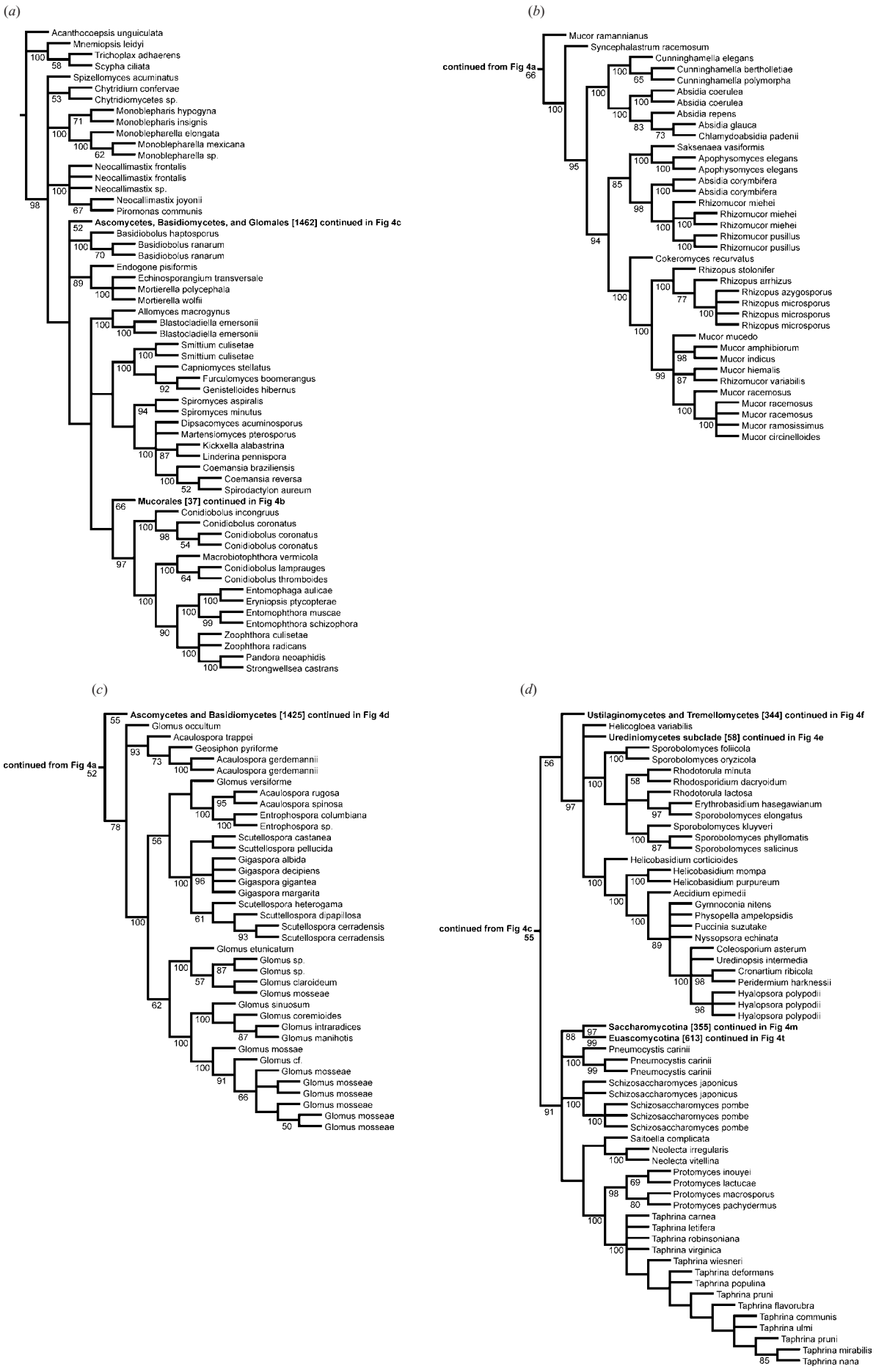
(a)
Acanthocoepsis unguiculata
Mnemiopsis leidyi
Trichoplax adhaerens
Scypha ciliata
Spizellomyces acuminatus
Chytridium confervae
Chytridiomycetes sp.
Monoblepharis hypogyna
Monoblepharis insignis
Monoblepharella elongata
Monoblepharella mexicana
Monoblepharella sp.
Neocallimastix frontalis
Neocallimastix frontalis
Neocallimastix sp.
Neocallimastix joyonii
Piromonas communis
Ascomycetes, Basidiomycetes, and Glomales [1462] continued in Fig 4c
Basidiobolus haptosporus
Basidiobolus ranarum
Basidiobolus ranarum
Endogone pisiformis
Echinosporangium transversale
Mortierella polycephala
Mortierella wolfii
Allomyces macrogynus
Blastocladiella emersonii
Blastocladiella emersonii
Smittium culisetae
Smittium culisetae
Capniomyces stellatus
Furculomyces boomerangus
Genistelloides hibernus
Spiromyces aspiralis
Spiromyces minutus
Dipsacomyces acuminosporus
Martensiomyces pterosporus
Kickxella alabastrina
Linderina pennispora
Coemansia braziliensis
Coemansia reversa
Spirodactylon aureum
Mucorales [37] continued in Fig 4b
Conidiobolus incongruus
Conidiobolus coronatus
Conidiobolus coronatus
Conidiobolus coronatus
Macrobiotophthora vermicola
Conidiobolus lamprauges
Conidiobolus thromboides
Entomophaga aulicae
Eryniopsis ptycopterae
Entomophthora muscae
Entomophthora schizophora
Zoophthora culisetae
Zoophthora radicans
Pandora neoaphidis
Strongwellsea castrans
(b)
Mucor ramannianus
Syncephalastrum racemosum
continued from Fig 4a
Cunninghamella elegans
Cunninghamella bertholletiae
Cunninghamella polymorpha
Absidia coerulea
Absidia coerulea
Absidia repens
Absidia glauca
Chlamydoabsidia padenii
Saksenaea vasiformis
Apophysomyces elegans
Apophysomyces elegans
Absidia corymbifera
Absidia corymbifera
Rhizomucor miehei
Rhizomucor miehei
Rhizomucor miehei
Rhizomucor pusillus
Rhizomucor pusillus
Cokeromyces recurvatus
Rhizopus stolonifer
Rhizopus arrhizus
Rhizopus azygosporus
Rhizopus microsporus
Rhizopus microsporus
Rhizopus microsporus
Mucor mucedo
Mucor amphibiorum
Mucor indicus
Mucor hiemalis
Rhizomucor variabilis
Mucor racemosus
Mucor racemosus
Mucor racemosus
Mucor ramosissimus
Mucor circinelloides
(c)
Ascomycetes and Basidiomycetes [1425] continued in Fig 4d
Glomus occultum
Acaulospora trappei
Geosiphon pyriforme
continued from Fig 4a
Acaulospora gerdemannii
Acaulospora gerdemannii
Glomus versiforme
Acaulospora rugosa
Acaulospora spinosa
Entrophospora columbiana
Entrophospora sp.
Scutellospora castanea
Scuttellospora pellucida
Gigaspora albida
Gigaspora decipiens
Gigaspora gigantea
Gigaspora margarita
Scutellospora heterogama
Scuttellospora dipapillosa
Scutellospora cerradensis
Scutellospora cerradensis
Glomus eturicatum
Glomus sp.
Glomus sp.
Glomus claroideum
Glomus mosseae
Glomus sinuosum
Glomus coremioides
Glomus intraradices
Glomus manihotis
Glomus mossae
Glomus cf.
Glomus mosseae
Glomus mosseae
Glomus mosseae
Glomus mosseae
Glomus mosseae
(d)
Ustilaginomycetes and Tremellomycetes [344] continued in Fig 4f
Helicogloea variabilis
Urediniomycetes subclade [58] continued in Fig 4e
Sporobolomyces foliicola
Sporobolomyces oryzicola
Rhodotorula minuta
Rhodosporidium dacryoidum
Rhodotorula lactosa
Erythrobasidium hasegawianum
Sporobolomyces elongatus
Sporobolomyces kluyveri
Sporobolomyces phyllomatis
Sporobolomyces salicinus
Helicobasidium corticioides
Helicobasidium mompa
Helicobasidium purpureum
Aecidium epimedii
Gymnoconia nitens
Physopella ampelopsidis
Puccinia suzutake
Nyssopsora echinata
continued from Fig 4c
Coleosporium asterum
Uredinopsis intermedia
Cronartium ribicola
Peridermium harknessii
Hyalopsora polypodii
Hyalopsora polypodii
Hyalopsora polypodii
Saccharomycotina [355] continued in Fig 4m
Euascomycotina [613] continued in Fig 4t
Pneumocystis carinii
Pneumocystis carinii
Pneumocystis carinii
Schizosaccharomyces japonicus
Schizosaccharomyces japonicus
Schizosaccharomyces pombe
Schizosaccharomyces pombe
Schizosaccharomyces pombe
Saitoella complicata
Neolecta irregularis
Neolecta vitellina
Protomyces inouyei
Protomyces lactucae
Protomyces macrosporus
Protomyces pachydermus
Taphrina carnea
Taphrina letifera
Taphrina robinsoniana
Taphrina virginica
Taphrina wiesneri
Taphrina deformans
Taphrina populina
Taphrina pruni
Taphrina flavorubra
Taphrina communis
Taphrina ulmi
Taphrina pruni
Taphrina mirabilis
Taphrina nana

Fig. 4.

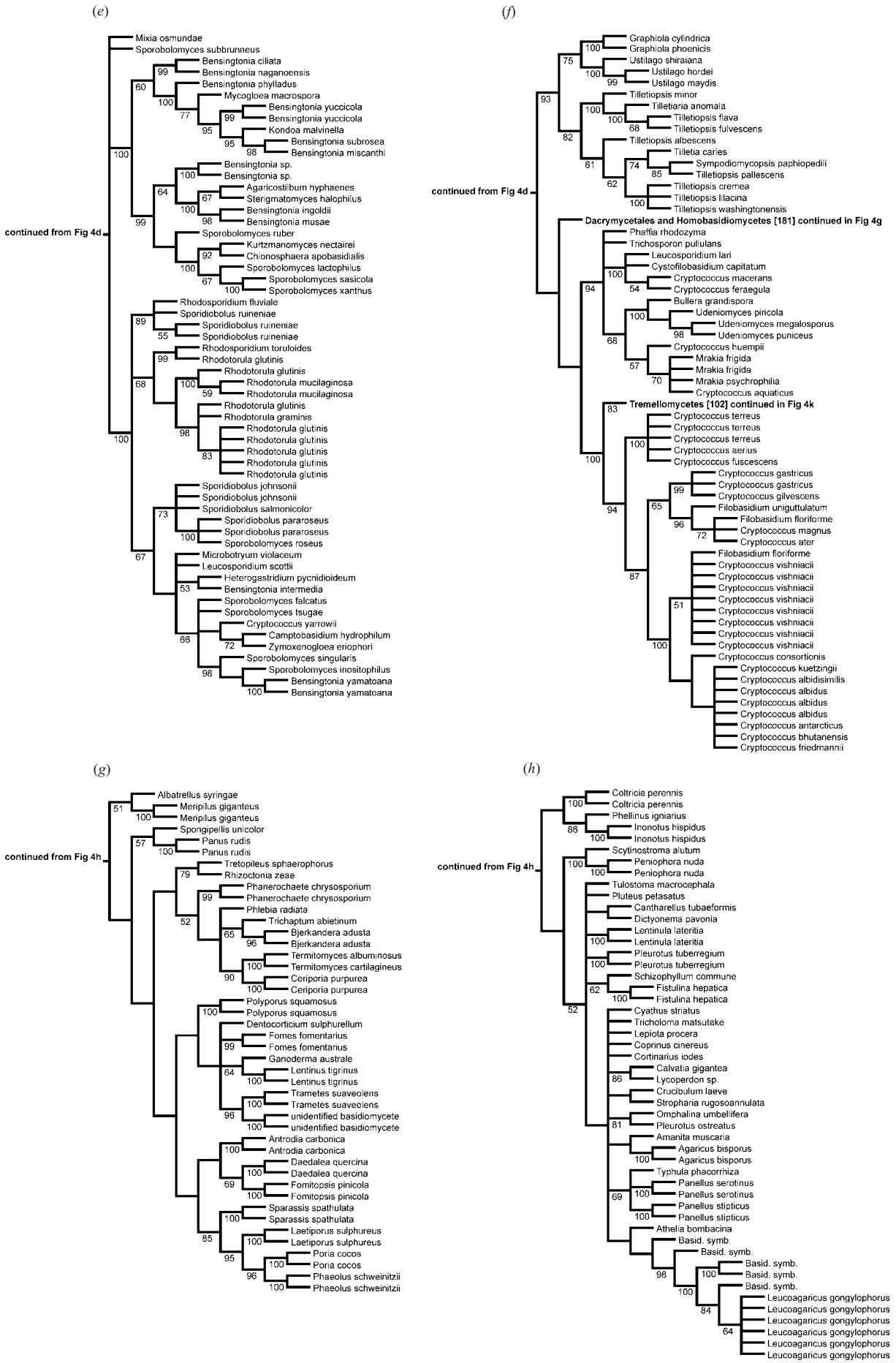

Fig. 4. Continued.

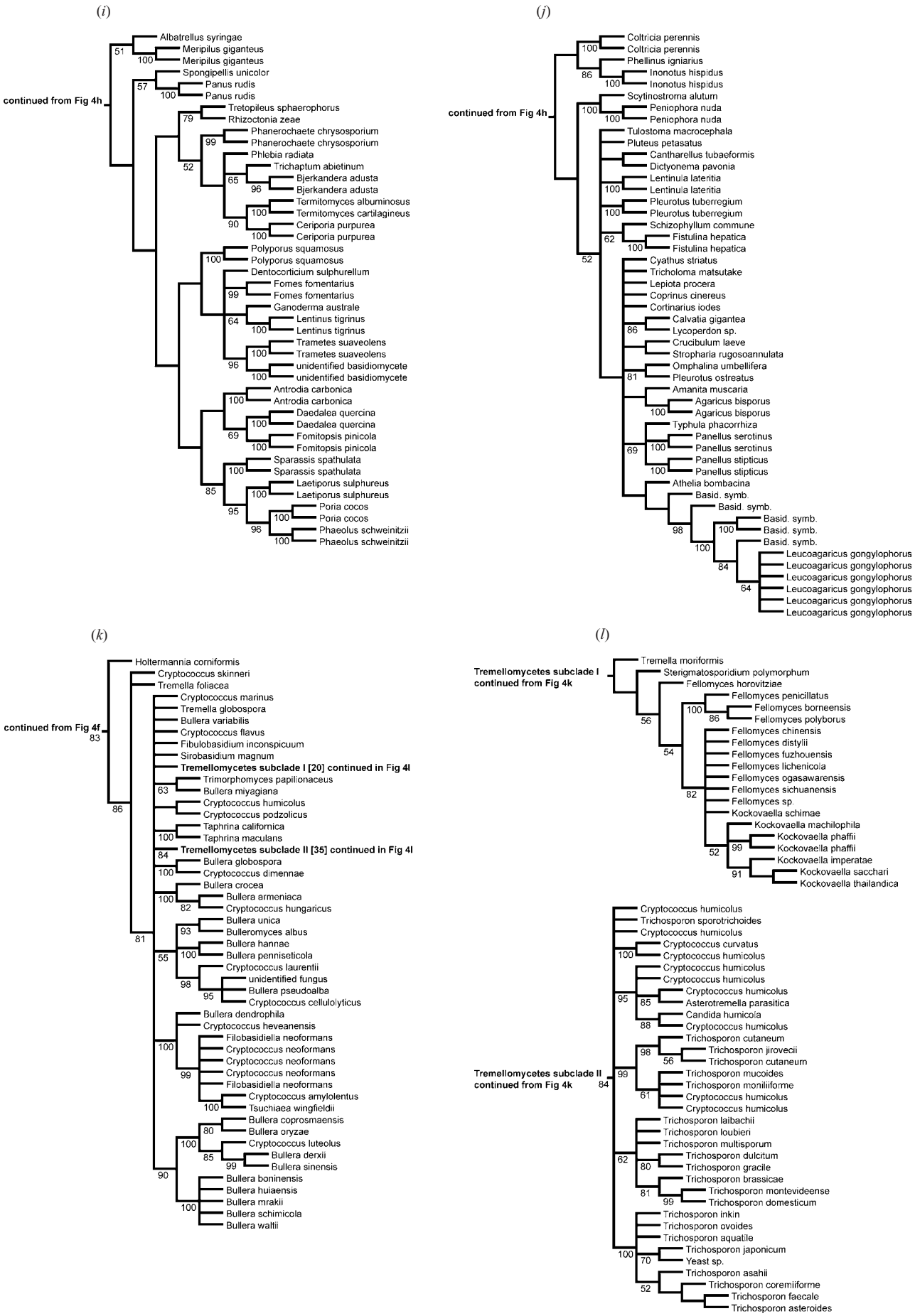

Fig. 4. Continued.

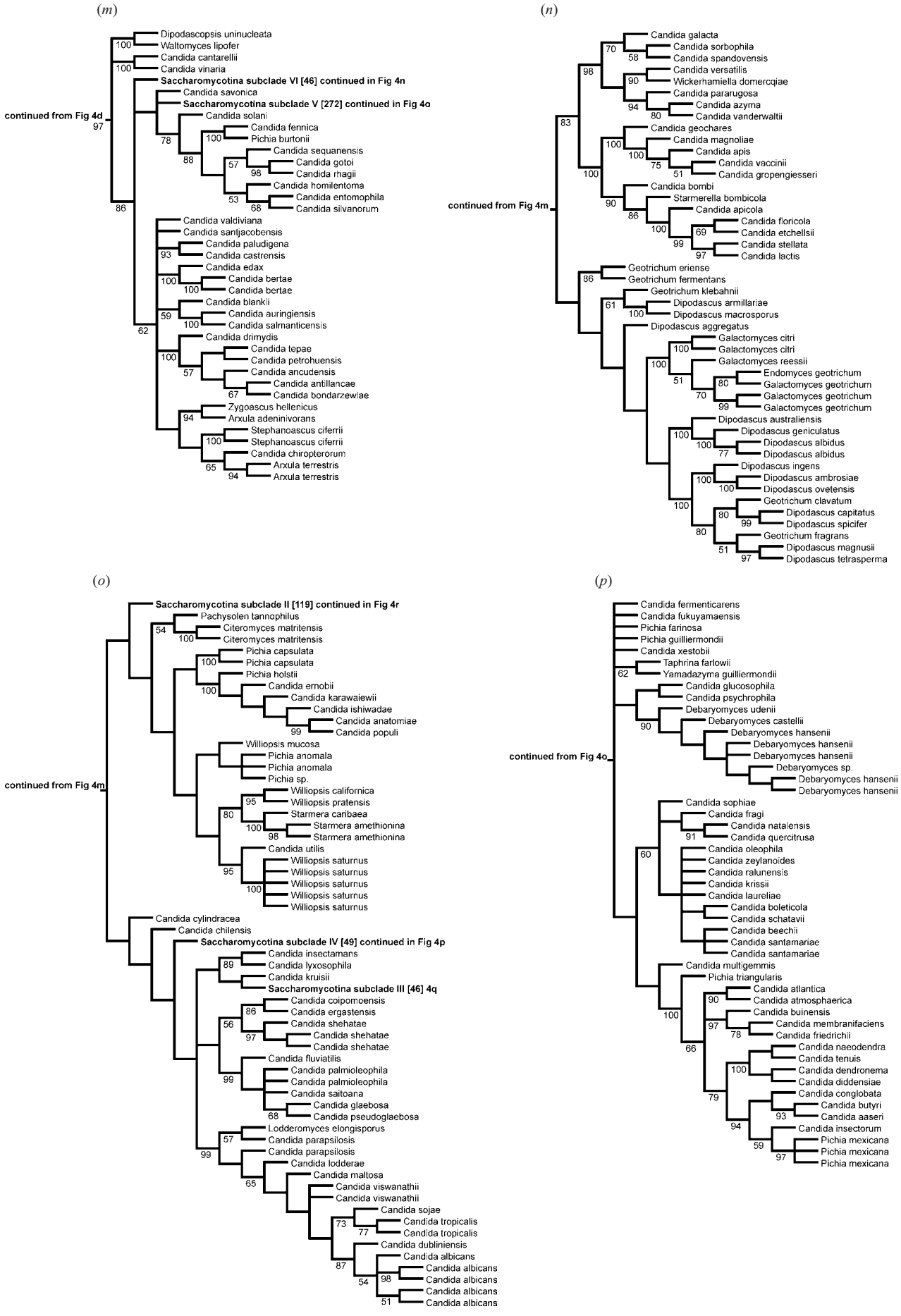

Fig. 4. Continued.

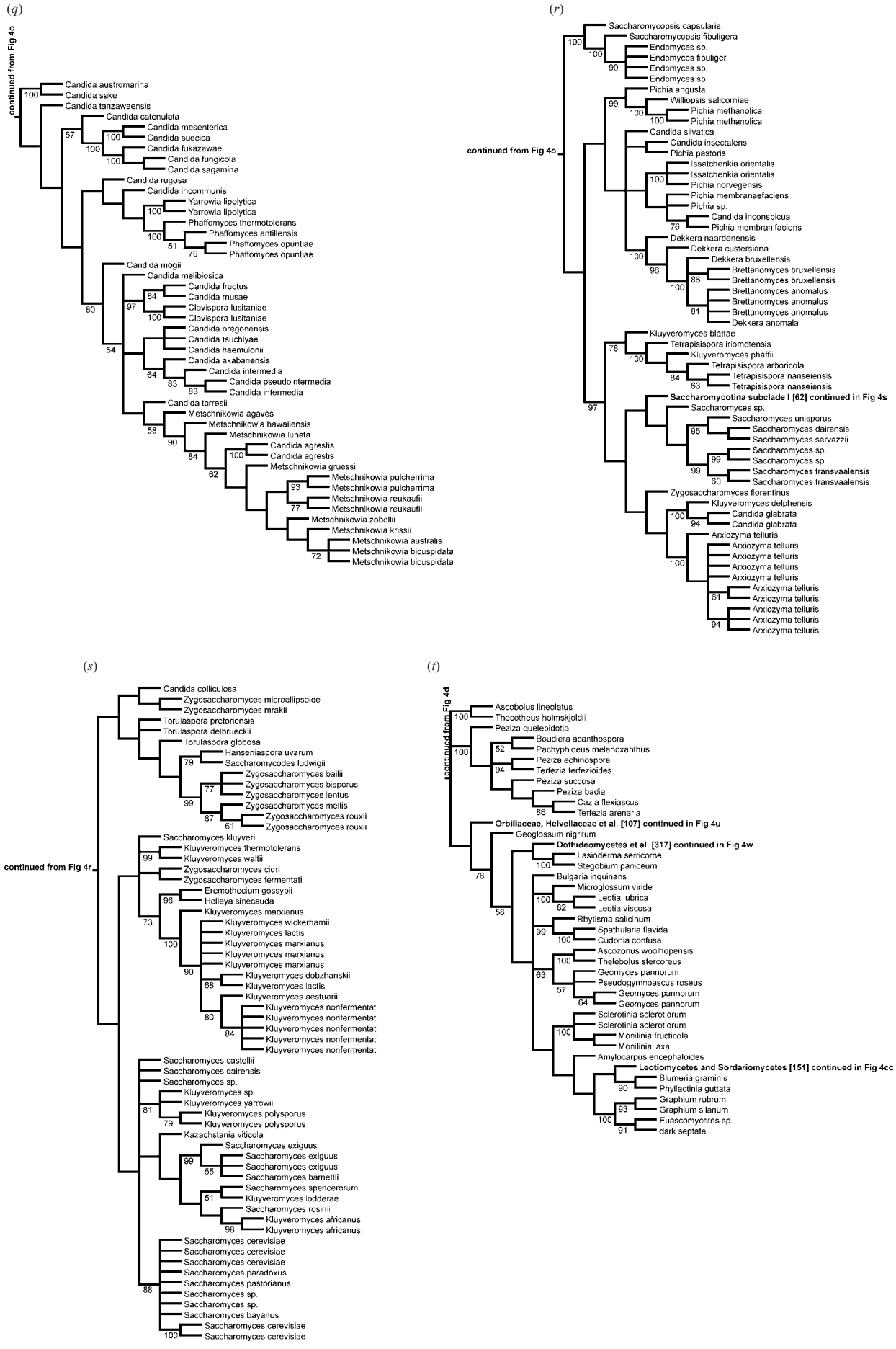

Fig. 4. Continued.

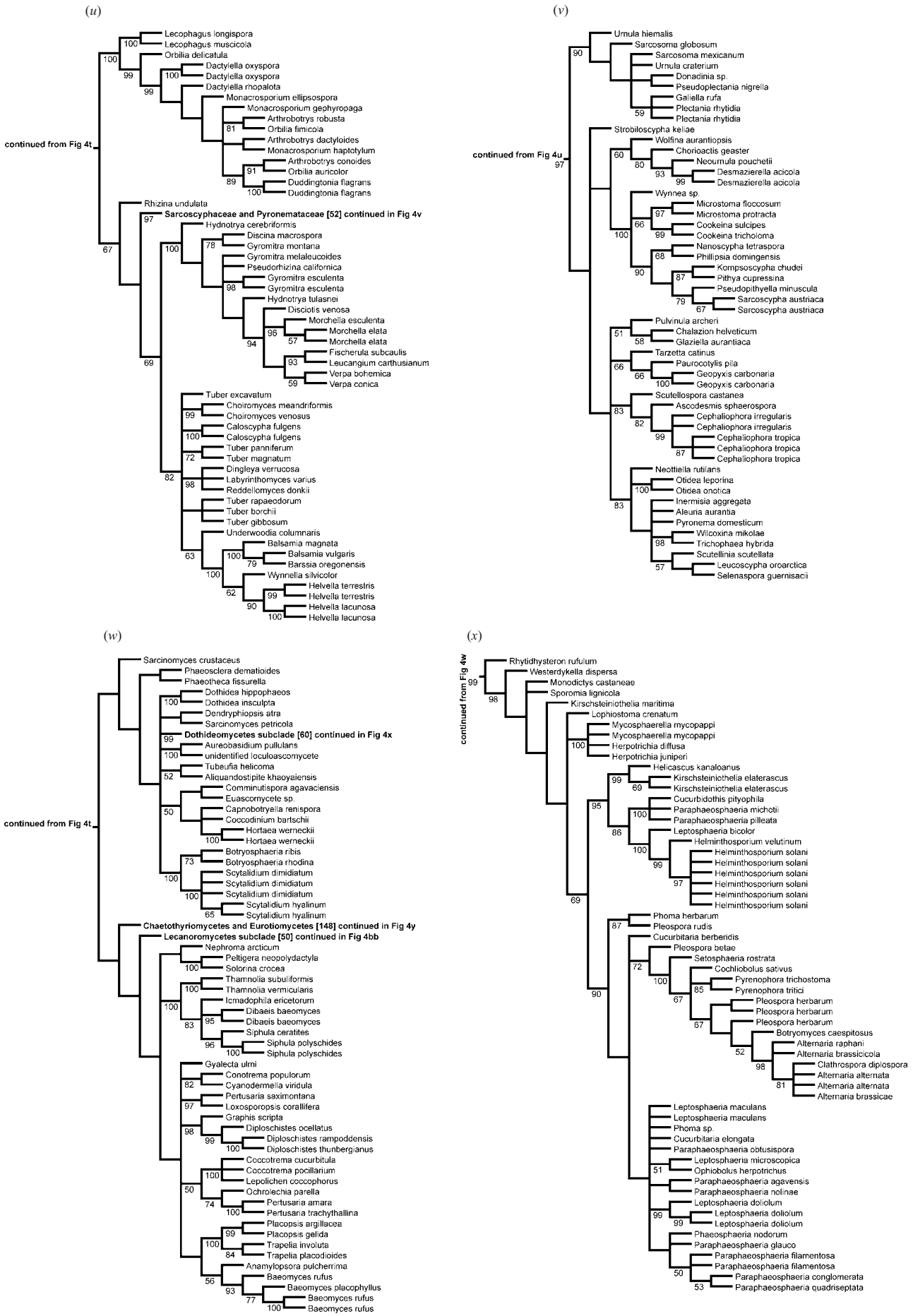

Fig. 4. Continued.

Fig. 4. Continued.

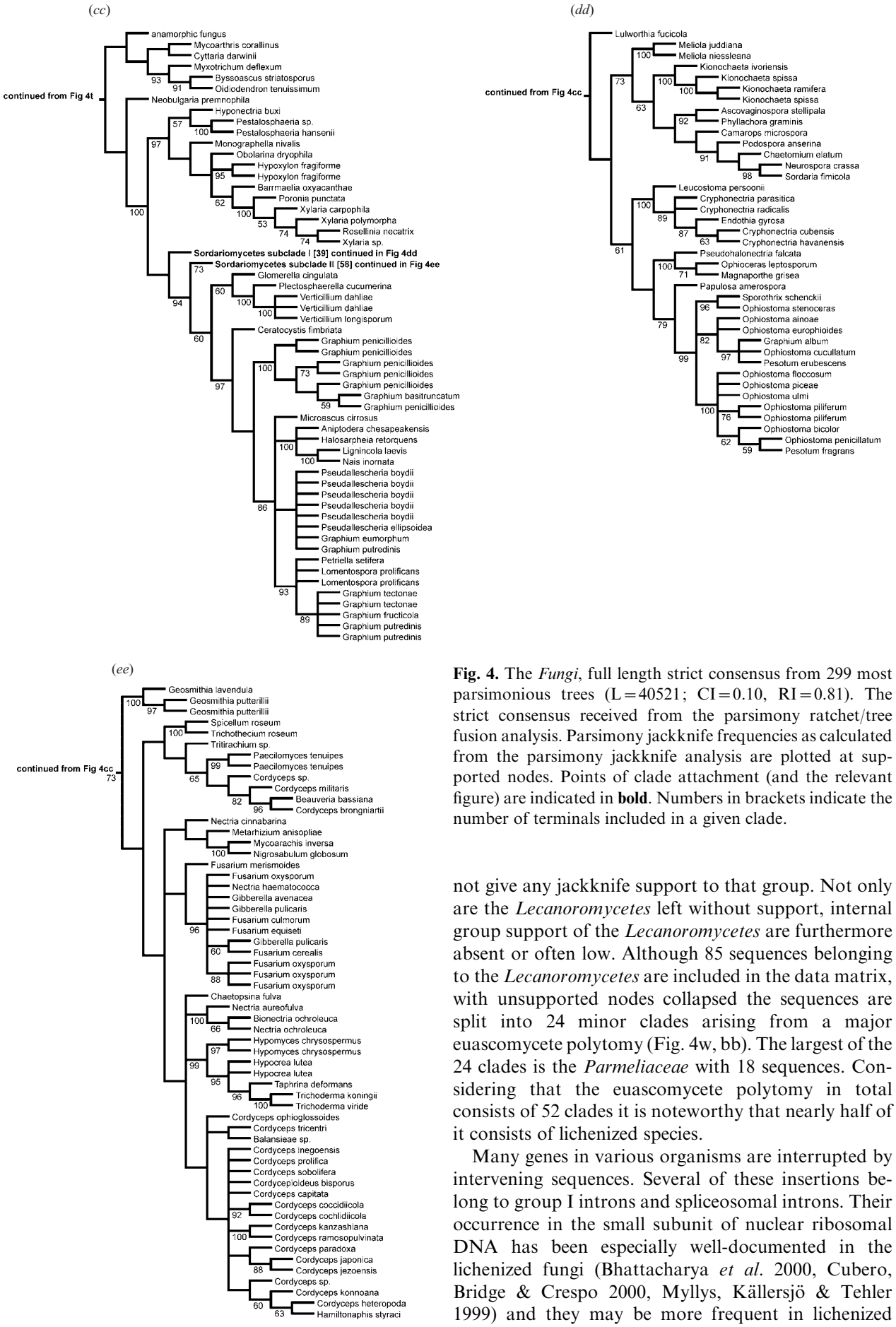

Fig. 4. The *Fungi*, full length strict consensus from 299 most parsimonious trees (L = 40521; CI = 0.10, RI = 0.81). The strict consensus received from the parsimony ratchet/tree fusion analysis. Parsimony jackknife frequencies as calculated from the parsimony jackknife analysis are plotted at supported nodes. Points of clade attachment (and the relevant figure) are indicated in **bold**. Numbers in brackets indicate the number of terminals included in a given clade.

not give any jackknife support to that group. Not only are the *Lecanoromycetes* left without support, internal group support of the *Lecanoromycetes* are furthermore absent or often low. Although 85 sequences belonging to the *Lecanoromycetes* are included in the data matrix, with unsupported nodes collapsed the sequences are split into 24 minor clades arising from a major euascomycete polytomy (Fig. 4w, bb). The largest of the 24 clades is the *Parmeliaceae* with 18 sequences. Considering that the euascomycete polytomy in total consists of 52 clades it is noteworthy that nearly half of it consists of lichenized species.

Many genes in various organisms are interrupted by intervening sequences. Several of these insertions belong to group I introns and spliceosomal introns. Their occurrence in the small subunit of nuclear ribosomal DNA has been especially well-documented in the lichenized fungi (Bhattacharya *et al.* 2000, Cubero, Bridge & Crespo 2000, Myllys, Källersjö & Tehler 1999) and they may be more frequent in lichenized

groups than in other fungi or in other organisms. Introns are usually difficult to align and their variable distribution suggest that they are mobile genetic elements capable of insertion and deletion (DePriest 1993, Myllys *et al*. 1999). This makes it even more difficult to make hypotheses of homology. Intron regions may be useful for studies at population level (Myllys, Tehler & Lohtander 2001) but they do not seem to contain sufficient phylogenetic structure for studies across higher taxonomic ranks such as family, order, or class. Most of the normal size Group I introns (200–400 bp) were probably removed for the present data prior to alignment at the European ribosomal RNA database (van de Peer, pers. comm.). However, in addition to the large Group I introns other much smaller insertions have commonly been found in lichenized fungi, so called short introns or degenerative group I introns (Grube, Gargas & DePriest 1996, Myllys *et al*. 1999) and short spliceosomal introns (Bhattacharya *et al*. 2000, Cubero *et al*. 2000). Short introns are only about 60 bp long and have probably not been removed in the alignment process. Bhattacharya *et al*. (2000) reports that a recent spliceosomal intron invasion likely occurred soon after the origin of the Euascomycetes and that the introns spread within many lineages of the tree, and particularly the lineages dominated by lichen symbionts. Thus, if ribosomal DNA in lichenized fungi actually have a higher tendency than other fungi to accumulate introns this could obscure the original phylogenetic structure of the data and serve as an explanation for the low resolution in the *Lecanoromycetes*. The question of algal origin of introns in lichens by way of the intimate, obligate, symbiotic lifestyle between the algae and the fungi (DePriest 1995) has been rejected by Bhattacharya *et al*. (1996, 2002).

Resampling methods are widely employed in systematics, and their use as such has seldom been considered objectionable. Jackknifing, however, has sometimes been misunderstood by authors who are more familiar with other resampling procedures. According to Felsenstein (2002, in main.html), for example:

'**RnA** [is] J. S. Farris's very fast program which uses parsimony to carry out jackknifing resampling of DNA sequence data. This would be nearly equivalent in properties to bootstrapping if the jackknifing were sampling random halves of the data, but Farris prefers to have each jackknife sample delete a fraction 1/e of the data, which will give most groups too much support (he would disagree with this statement).'

Actually the RNA program (for Rapid Nucleotide Analysis) – which is *not* the parsimony jackknifer, Xac – uses bootstrap resampling, not jackknifing. Felsenstein was right, though, about the 1/e deletion frequency employed when parsimony jackknifing *is* used. The question is why he supposed that jackknifing would give groups 'too much support,' by which he meant higher resampling frequencies than would be obtained by bootstrapping. It is a curious complaint, considering that Farris *et al*. (1996) chose the 1/e deletion frequency precisely in order to make the group resampling frequencies from parsimony jackknifing similar to those expected from bootstrapping. As they showed, a group supported by k uncontradicted characters would have expected frequency

$$1-p^k$$

with jackknife resampling using deletion frequency p. Felsenstein (1985), similarly, had found that such a group would have expected frequency

$$1-\mathrm{e}^{-k}$$

with bootstrap resampling, provided the total number of characters is large enough. As Farris *et al*. (1996) pointed out, it is immediately seen from these two expressions that jackknifing and bootstrapping can give the same expected frequencies only if $p=1/\mathrm{e}$.

Felsenstein (2002) did not explain what he thought was wrong with that observation; he simply did not mention it. Further, he did not make the grounds for his criticism entirely explicit. His only further comment on this point was (Felsenstein 2002, in seqboot.html):

'Delete-half-jackknifing … involves sampling a random half of the characters, and including them in the data but dropping the others. The resulting data sets are half the size of the original, and no characters are duplicated. The random variation from doing this should be very similar to that obtained from the bootstrap. The method is advocated by Wu (1986). It was mentioned by me in my bootstrapping paper (Felsenstein [1985]), and has been available for many years in this program as an option. Jackknifing is advocated by Farris *et al*. (1996) but as deleting a fraction 1/e (1/2.71828). This retains too many characters and will lead to overconfidence in the resulting groups.'

Aside from simply repeating the complaint, this adds only a reference to Wu (1986). Wu did conclude that delete-half jackknifing should give about the same results as bootstrapping, and so it would appear that Felsenstein's objection to parsimony jackknifing is based on Wu's results. In that event, however, Felsenstein's conclusion rests only on a faulty analogy. Wu's study concerned sampling variation in estimates of regression coefficients for continuous variables. Delete-half jackknifing may give the same results as bootstrapping in that case, but even if so this certainly does not mean that those two methods give the same group frequencies in tree calculations. Indeed, the simple algebraic result given above makes it obvious that delete-half jackknifing cannot generally give the same group frequencies as bootstrapping. Felsenstein's objection to parsimony jackknifing thus seems to lack any legitimate basis.

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