

Phylogenetics and evolution of nematode-trapping fungi (Orbiliiales) estimated from nuclear and protein coding genes

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Abstract: The systematic classification of nematode-trapping fungi is redefined based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA and β -tubulin genes. Molecular data were analyzed with maximum parsimony, maximum likelihood and Bayesian analysis. An emended generic concept of nematode-trapping fungi is provided. *Arthrobotrys* is characterized by adhesive networks, *Dactylellina* by adhesive knobs, and *Drechslerella* by constricting-rings. Phylogenetic placement of taxa characterized by stalked adhesive knobs and non-constricting rings also is confirmed in *Dactylellina*. Species that produce unstalked adhesive knobs that grow out to form loops are transferred from *Gamsylella* to *Dactylellina*, and those that produce unstalked adhesive knobs that grow out to form networks are transferred from *Gamsylella* to *Arthrobotrys*. *Gamsylella* as currently circumscribed cannot be treated as a valid genus. A hypothesis for the evolution of trapping-devices is presented based on multiple gene data and morphological studies. Predatory and nonpredatory fungi appear to have been derived from nonpredatory members of *Orbilialia*. The adhesive knob is considered to be the ancestral type of trapping device from which constricting rings and networks were derived via two pathways. In the first pathway adhesive knobs retained their adhesive material forming simple two-dimension networks, eventually forming complex three-dimension networks. In the second pathway adhesive knobs lost

their adhesive materials, with their ends meeting to form nonconstricting rings and they in turn formed constricting rings with three inflated-cells.

Key words: β -tubulin, evolution, fungi, phylogeny, predatory, systematics, rDNA

INTRODUCTION

Nematode-trapping fungi can produce various trapping devices to capture nematodes and other microscopic animals (Duddington 1951a, b; Barron 1977; Scholler et al 1999; Ahrén et al 2004) and are important in biological control (Dong et al 2004). The three basic types of trapping devices are adhesive knobs, constricting rings and adhesive networks and these can be subdivided further into seven types of trapping device (i.e., simple adhesive branches, unstalked adhesive knobs, stalked adhesive knobs, nonconstricting rings, constricting rings, two-dimensional networks and three-dimensional networks (Rubner 1996)). The majority of nematode-trapping fungi are hyphomycetes, placed within the Orbiliales (Orbiliomycetes) based on morphological and/or molecular studies. Other examples include *Nematotonus concurrens* Drechsler whose teleomorph belongs to the genus *Hohenbuehelia* (Basidiomycetes), which uses both adhesive traps and adhesive spores, while *Coprinus comatus* (O.F. Müll) Gray and *Pleurotus ostreatus* (Jacq.) Kumm. (Basidiomycetes) produce toxins from specialized hyphal stalks to immobilize and digest the nematode (Barron and Thorn 1987, Dong et al 2004).

Since the pioneering work by Drechsler (1937), nematode-trapping fungi have been classified in a number of genera based on morphology of conidia (shape, septa and size) and conidiophores (branching, modifications of the apex). Traditional taxonomic concepts relied heavily on conidia and conidiophore morphology without taking in account the importance of trapping devices. This has led to a situation where species with diverse types of trapping devices have been assigned to one genus, while others with similar trapping devices can be found in different genera (Glockling and Dick 1994; Liu and Zhang 1994, 2003; Zhang et al 1996).

With molecular technology, traditional generic classification, generally based on the morphology of conidial characters, was challenged. Rubner (1996) first used trapping structure to try to rationalize the

classification of the nematode-trapping fungi, which was justified by the following molecular data. Phylogenies based on rDNA sequences have indicated that trapping devices are more informative than other morphological characters in delimiting genera (Liou and Tzean 1997, Pfister 1997, Ahrén et al 1998, Scholler et al 1999, Kano et al 2004). Ahrén et al (1998) found that nematode-trapping fungi clustered into three lineages: species with constricting rings, species with various adhesive structures (net, hyphae, knobs and nonconstricting rings) and species have no trapping devices. Based on results obtained from morphological and molecular characters, Hagedorn and Scholler (1999) and Scholler et al (1999) classified nematode-trapping fungi into four genera: *Dactylellina* characterized by stalked adhesive knobs including species characterized by nonconstricting rings and stalked adhesive knobs; *Gamsylella* characterized by adhesive branches and unstalked knobs; *Arthrotrix* characterized by adhesive networks; and *Drechslerella* characterized by constricting rings.

In this study DNA sequences from 28S and 5.8S rDNA and β -tubulin were analyzed to understand the evolution of trapping devices and further examine the relationships among nematode-trapping fungi. Sequenced taxa were selected to provide evidence for: (i) the evolution of trapping devices and (ii) the importance of adhesive branches, unstalked knobs, stalked adhesive knobs and nonconstricting rings in the taxonomic placement of species.

MATERIALS AND METHODS

Morphological examination.—Isolates were cultured on cornmeal agar (CMA) at 25 C for 1 wk to check the characters of conidia and conidiophores. To verify the morphology of trapping devices cultures were cut into small cubes and inoculated with the free-living nematode, *Paragrellus redivivus* (Linne) Goodey at 25 C for 3–5 d (Duddington 1955, Wyborn et al 1969). All morphological characters were recorded and photographed. The mean measurement is based on 50 counts.

DNA extraction.—Fungal cultures were grown 1 wk on PDA at 25 C before DNA extraction. The mycelia were scraped from the surface of the agar and used as starting material. DNA was extracted with a modified CTAB method as outlined by Jeewon et al (2002, 2004).

Amplification and sequence of genomic DNA.—DNA amplification was performed by polymerase chain reaction (PCR). Primer pairs LROR (Bunyard et al 1994) and LR5 (Vilgalys and Hester 1990), ITS5 and ITS4 (White et al 1990), Bt1ABt1B and Bt2ABt2B (Glass and Donaldson 1995) were used to amplify partial large subunit (28S), the complete ITS (including 5.8S) and partial β -tubulin, respectively.

The PCR reaction mixture consisted of 0.3 μ L of 1.5 units of Taq DNA polymerase, 5 μ L of 10 \times buffer, 1.5 μ L of 25 μ M MgCl₂, 4 μ L of 2.5 mM dNTPs, 1.5 μ L of 10 μ M primer, 3 μ L of DNA template and made up to a final volume of 50 μ L with double-distilled sterile water. PCR reaction were performed with these steps: 3 min at 95 C, 30 cycles of 1 min at 94 C, 50 s at 52 C, 1 min at 72 C and a final extension step of 10 min at 72 C. Amplified products were electrophoresed on 1% agarose gels to check for size and purity.

Purified PCR products were sequenced directly on both strands with the same primers that were used for amplification. Reactions were performed in an Applied Biosystem 3730 DNA Analyzer at the Genome Research Centre (University of Hong Kong). All the sequences have been deposited in GenBank (TABLE I).

Phylogenetic analysis.—DNA sequences were aligned with additional sequences obtained from GenBank (TABLE I) with BioEdit (Hall 1999) and Clustal X 1.83 (Thompson et al 1997). Manual gap adjustments were made to improve the alignment. Phylogenetic analyses were conducted with PAUP* version 4.0b10 (Swofford 2002) and MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). Alignment gaps were treated as missing data. The characters were weighted at different transition-transversion ratios (TTr) to determine the most appropriate parameters giving the best trees. Maximum parsimony trees were found with 1000 heuristic search and including parsimony-informative characters in stepwise (random) addition and tree bisection and reconstruction (TBR) as branch swapping algorithm. MAXTREES were set to 5000, branches of zero length were collapsed and all most parsimonious trees were saved. Branch support for all parsimony analyses was estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search consisting of 10 random-addition replicates for each bootstrap replicate. Partition homogeneity test (Farris et al 1995, Huelsenbeck et al 1996), as implemented in PAUP, was performed to check whether different genes could be combined.

To select the best-fit model of evolution for the maximum likelihood (ML) and Bayesian analyses, MrModeltest 2 (Posada and Crandall 1998, Nylander 2004) was used based on a tree calculated under neighbor joining criterion under the Jukes Cantor model (JC69) (Jukes and Cantor 1969). ML tree was built with a heuristic search, addition sequence set to "as", with TBR branch swapping algorithm. Bayesian analyses were conducted with MrBayes 3.0b4. Markov chains were run for 1 000 000 generations and trees were sampled every 100th generation resulting in 10 000 trees. The burn-in number was determined by graphically visualizing the likelihood scores. The first 1000 trees, which represented the burn-in phase of the analysis, were discarded, and the remaining 9000 trees were used for calculating posterior probabilities in the consensus tree.

Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI) and log likelihood (-Ln L) were calculated for all trees generated under parsimony and likelihood optimality criteria. Kishino-Hasegawa tests (Kishino and Hasegawa 1989) and Templeton tests (Tem-

TABLE I. List of taxa and GenBank accession numbers used in this study

Species	Source of cultures	Geographic origin	Trapping devices	GenBank Accession Numbers			
				rDNA		β-tubulin	
				ITS	28S	β-1A1B	β-2A2B
<i>A. anchonia</i>	CBS109.37	USA	CR	AY965753	AY902799	AY965786	AY965815
<i>A. brochopaga</i>	CBS218.61	USA	CR	U72609	AY261176	AY965794	AY965823
<i>A. entomopaga</i>	CBS642.80	Australia	AK	AY965758	AY965774	AY965802	AY965831
<i>M. candidum</i>	YMF1.00036	China	AK&NCR	AY965749	AY902801	AY965778	AY965808
<i>M. cionopagum</i>	CBS585.91	Portugal	AK&NW	AY965756	AY965772	AY965800	AY965829
<i>M. drechsleri</i>	YMF1.00573	China	AK	AY695063	AY965765	AY965784	AY965813
<i>M. ellipsosporum</i>	CA-7	China	AK	AY965759	AY261157	AY965803	AY965832
<i>M. gephyrophagum</i>	CBS178.37	USA	AK&NW	U51974	AY261173	AY965792	AY965821
<i>M. haptotylum</i>	SQ-95-2	China	AK	AF106523	AY902791	AY965775	AY965805
<i>M. lysipagum</i>	YMF1.00535	China	AK&NCR	AY695067	AY261165	AY965782	AY965812
<i>M. mammillatum</i>	CBS229.54	UK	AK	AY902794	AY902802	AY965795	AY965824
<i>M. multiseptatum</i>	YMF1.00127	China	AK	AY965751	AY965764	AY965781	AY965811
<i>M. parvicolle</i>	YMF1.00029	China	AK&AB	AY965748	AY965761	AY965777	AY965807
<i>M. phymatopagum</i>	CBS325.72	Netherlands	AK&AB	U51970	AY261156	AY965798	AY965827
<i>M. robustum</i>	YMF1.01413	China	AK&AB	AY965755	AY965769	AY965789	AY965818
<i>M. sclerohyphum</i>	YMF1.00041	China	AK	AY902806	AY965762	AY965779	AY965834
<i>M. shuzhengense</i>	YMF1.00584	China	AK&AB	AY965752	AY965766	AY965785	AY965814
<i>M. sichuanense</i>	YMF1.00023	China	AK&UCR	AY902795	AY902803	AY965776	AY965806
<i>M. tentaculatum</i>	CBS206.64	USA	AK	AF106531	AY902792	AY965793	AY965822
<i>M. thaumasium</i>	CBS176.37	USA	NW	U51972	AY261137	AY965791	AY965819
<i>M. yunnanense</i>	CBS615.95	China	AK&UCR	AY965757	AY965773	AY965801	AY965830
<i>M. sp</i>	KG-55	China	AK&UCR	AY965747	AY965760	AY965804	AY965833
<i>M. sp</i>	YMF1.01405	China	AK&UCR	AY965754	AY965768	AY965788	AY965817
<i>D. arcuata</i>	CBS174.89	UK	AK&NW	AF106527	AY261129	AY965790	AY965820
<i>D. asthenopaga</i>	CBS262.83	Australia	AK	U51962	AY965770	AY965796	AY965825
<i>D. copepodii</i>	CBS487.90	New Zealand	AK	U51964	AY965771	AY965799	AY965828
<i>D. leptospora</i>	YMF1.00117	China	AK&UCR	AY965750	AY965763	AY965783	AY965809
<i>D. oxyspora</i>	YMF1.10000	China	None	AF106537	AY965767	AY965787	AY965816
<i>D. rhombospora</i>	CBS280.70	Germany	None	AY902793	AY902790	AY965797	AY965826
<i>D. varietas</i>	YMF1.00118	China	AK&UCR	AY902805	AY902800	AY965780	AY965810
<i>Neurospora crassa</i>	CBS709.71	USA		AY681193	AY681158	AY974799	AY681226
<i>Sordaria fimicola</i>	CBS723.96	Papua New Guinea		AY681188	AY681160	AY974800	AY681228

AK = adhesive knobs, AB = adhesive branches, CR = constricting rings, UCR = non-constricting rings, NW = networks.

pleton 1983) were performed for all trees to compare tree topologies. Trees were figured in Treeview (Page 1996).

RESULTS

rDNA data.—The 28S and 5.8S dataset included 32 taxa, each with 1046 characters. Eleven ambiguously aligned characters were excluded. The remaining 1035 characters included 808 constant characters, 28 variable parsimony-uninformative characters and 199 parsimony-informative characters. Unweighted analysis resulted in 15 parsimony trees. Three parsimony trees were obtained when transition was weighted 1.5 times over transversion. SYM+I+G were selected as the best-fit model for maximum likelihood and Bayesian analyses.

The Bayesian tree with bootstrap supported from 1000 replicated and Bayesian posterior probability (bpp) are provided (FIG. 1).

Taxa forming both nonconstricting rings and stalked adhesive knobs clustered with taxa forming only adhesive knobs within clade A (85% bpp). Two nonpredatory fungi, *Dactylella rhombospora* Grove and *D. oxyspora* (Sacc. & Marchal) Matsush., and two predatory fungi, *Monacrosporium multiseptatum* H.Y. Su & K.Q. Zhang and *D. varietas* Y. Li, K.D. Hyde & K.Q. Zhang, grouped together as defined in clade D with 99% bpp and 99% bootstrap support. Species with constricting rings (clade B) received high support as a monophyletic clade (99% bpp and 99% bootstrap support). Taxa characterized by unstalked adhesive knobs and adhesive branches did not cluster

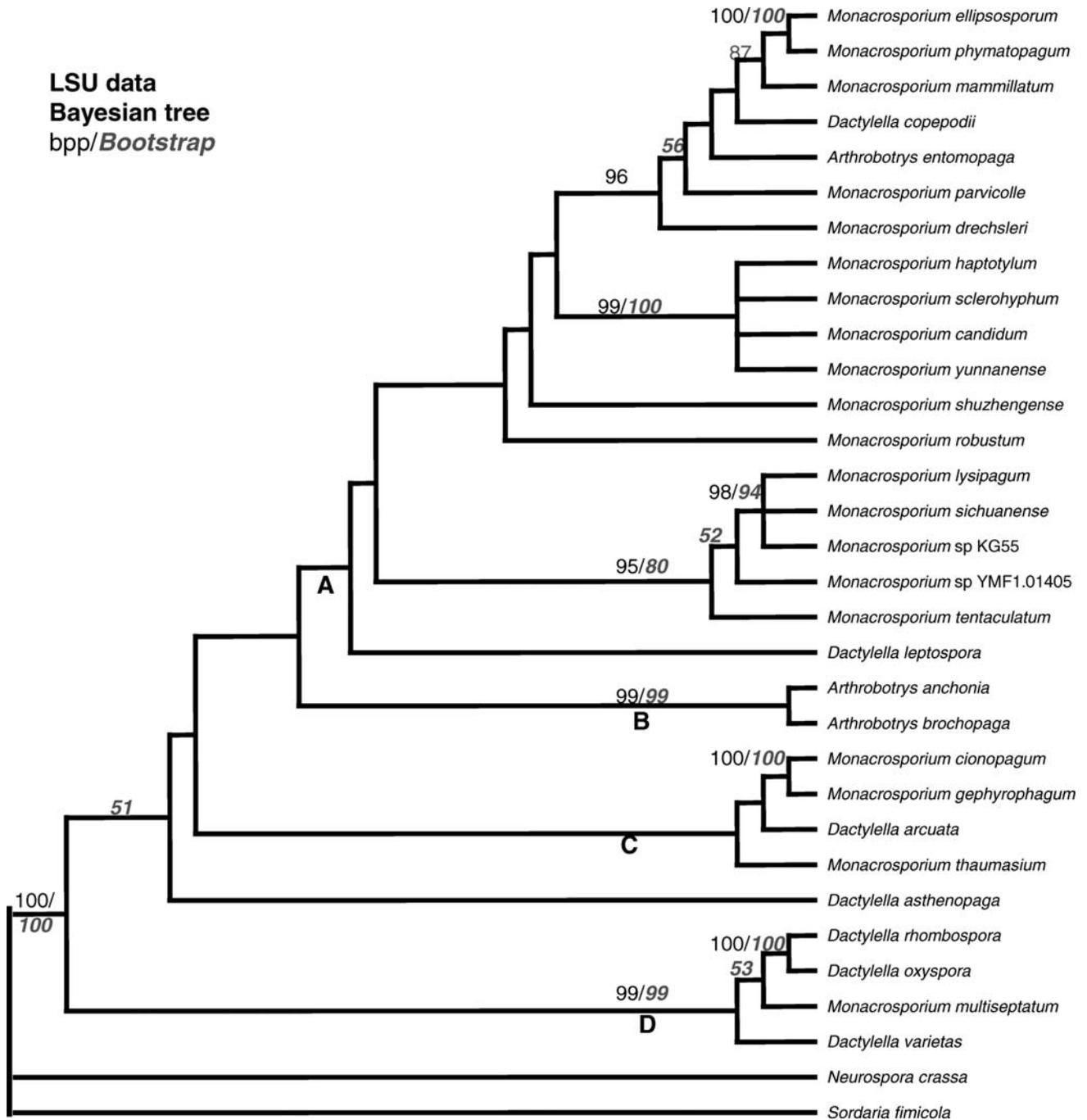


FIG. 1. Bayesian tree based on rDNA sequences. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates and Bayesian posterior probability. *Neurospora crassa* and *Sordaria fimicola* are used as outgroups.

together. *Monacrosporium parvicolle* (Drechsler) R.C. Cooke & C.H. Dickinson, *M. phymatopagum* (Drechsler) Subram., *M. robustum* J.S. McCulloch and *M. shuzhengense* X.F. Liu & K.Q. Zhang characterized by unstalked adhesive knobs with adhesive hyphae growing out to form loops clustered in clade A, while

M. cionopagum (Drechsler) Subram., *M. gephyrophagum* (Drechsler) Subram. and *D. arcuata* Scheuer & J. Webster, characterized by unstalked adhesive knobs with adhesive hyphae that grow out to form networks, grouped with *M. thaumasium* (Drechsler) de Hoog & Oorschot (clade C).

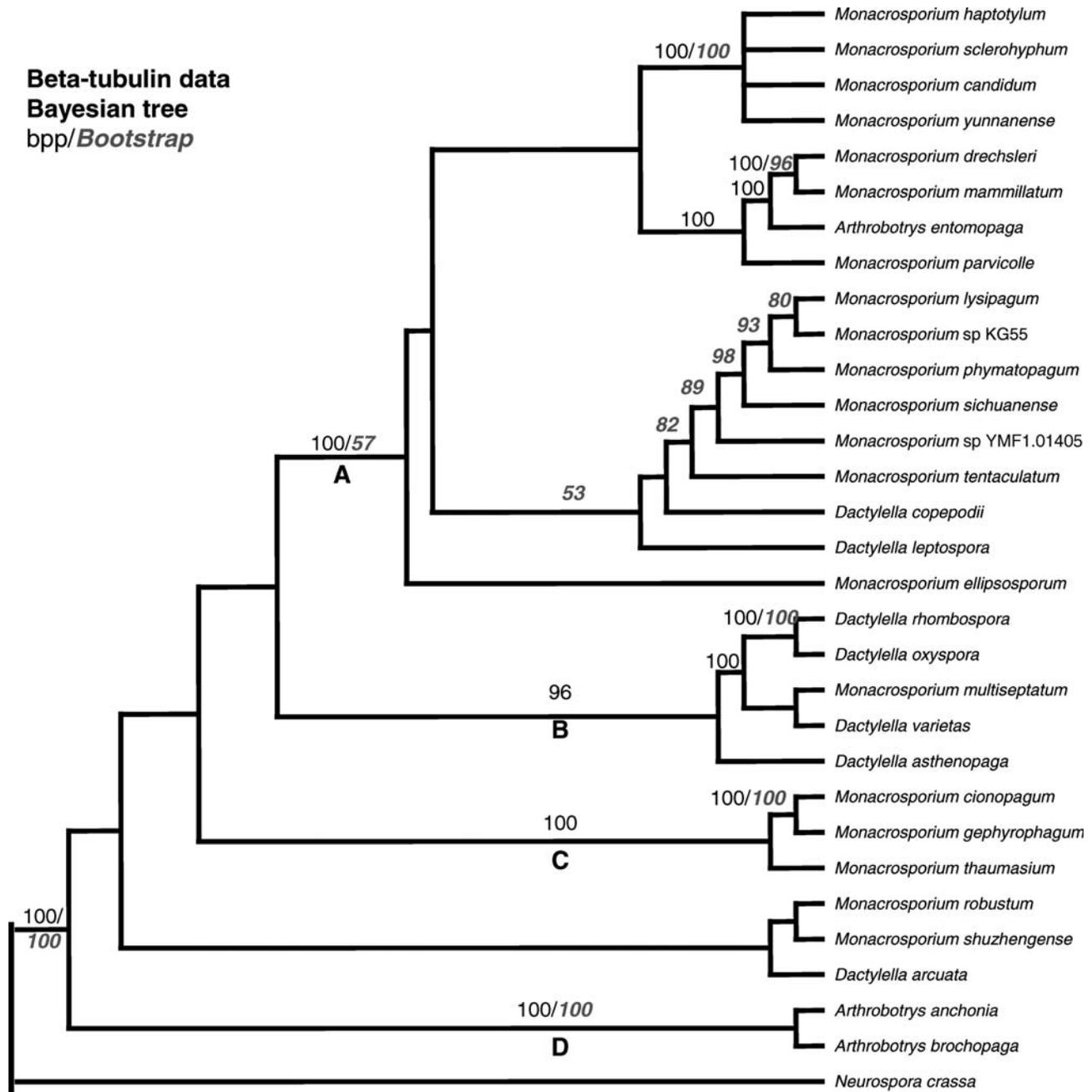


FIG. 2. Bayesian tree generated from β -tubulin sequences. Bootstrap values less than 50% and Bayesian posterior probability less than 95% are not shown. *Neurospora crassa* and *Sordaria fimicola* are used as outgroups.

β -tubulin.—The final alignment of β -tubulin-1 amplified with Bt1A and Bt1B and β -tubulin-2 amplified with Bt2A and Bt2B included 32 taxa each with 1203 characters; 546 ambiguously aligned characters were excluded. The resulting dataset consisted of 219 parsimony informative characters. Unweighted heuristic searches resulted in 12 trees, while one tree was obtained when a transition-transversion ratio of 1.5:1 was used. There were no significant differences between

trees generated from weighted and unweighted parsimony. The best-fit model selected for likelihood and Bayesian analyses by MrModeltest was GTR+G.

There were no contradictions in topologies among the Bayesian, parsimony and likelihood trees. The Bayesian tree is provided (FIG. 2). The tree based on β -tubulin data is similar to the trees based on rDNA data in topology, but higher support was received for clade A (FIG. 2). Species forming both stalked

adhesive knobs and nonconstricting rings group with species forming only stalked adhesive knobs in clade A with 100% bpp and 57% bootstrap support. Nonpredatory fungi, *Dactylella rhombospora* and *D. oxyspora*, appeared to be related phylogenetically to *Monacrosporium multiseptatum* and *D. varietas*, taxa with stalked adhesive knobs, within clade B (96% bpp). Species with constricting rings (clade D) and species with adhesive networks (clade C) were supported as monophyletic clades with 100% bpp, while species that produce both unstalked adhesive knobs and adhesive branches did not group together as a monophyletic clade. Phylogenies obtained from β -tubulin sequences are generally concordant with those based on rDNA sequences. The only topological difference is the phylogenetic placement of *M. robustum* and *M. shuzhengense*, which group within clade A in rDNA data as compared to outside clade A in β -tubulin phylogenies. Affinities of *M. phymatopagum* and *M. parvicolle* are well supported in clade A. However the phylogenetic affinities of *M. robustum* and *M. shuzhengense* cannot be established due to the difference observed in their phylogenetic placement. They were sister taxa to *Dactylella arcuata* (unstalked adhesive knobs and networks) in β -tubulin analyses (FIG. 2), however in rDNA analyses they were basal to clade A (FIG. 1).

Combined dataset.—A partition homogeneity test of the full dataset, combining rDNA (28S and 5.8S rDNA) and β -tubulin (1A1B and 2A2B), indicated that the different datasets could be combined ($P > 0.05$). The combined dataset consisted of 32 taxa each with 2249 characters. A total of 548 ambiguously aligned characters were excluded. Of the remaining 1701 characters, 1115 were constant and 431 were parsimony-informative.

Equally weighted maximum parsimony analysis generated two trees and weighted parsimony with a transition transversion ratio of 1.5:1 resulted in three trees, which were not significantly different from each other. For ML analysis, the best-fit model determined by MrModeltest 2 was the GTR+I+G model.

While minor topological differences were obtained under different optimality criteria (maximum parsimony, maximum likelihood and Bayesian), the topology among and within the well-supported clades (clades A, B and C) remained the same. Some of the major clades that collapsed and were not supported statistically in the rDNA and β -tubulin datasets were resolved and received reasonable bpp and bootstrap support in the combined dataset (FIG. 3).

Clade A received 100% bpp and 90% bootstrap support based on combined datasets. Taxa with both

nonconstricting rings and stalked adhesive knobs are not phylogenetically unrelated from taxa with only stalked adhesive knobs (subclades A2 and A3, FIG. 3). Clade B comprised two nonpredatory taxa (no trapping devices) and three predatory taxa with high support (100% bpp and 91% bootstrap support). This is consistent with results obtained from sequences analyses of individual genes (FIGS. 1, 2). Species with constricting rings (clade C) and *Monacrosporium thaumasium* with networks received 100% bpp. Phylogenetically *M. gephyrophagum*, *M. cionopagum* and *Dactylella arcuata* appear to be closely related to *M. thaumasium*. Another major topological difference observed in the combined dataset is the phylogenetic placement of taxa with unstalked adhesive knobs and adhesive branches. *Monacrosporium robustum* and *M. shuzhengense* formed a monophyletic clade (98% bpp and 60% bootstrap support) and sister to clade A (FIG. 3) as compared to sister to *Dactylella arcuata* (FIG. 2). Phylogenetic results obtained from the combined dataset are used to infer relationship among nematode-trapping fungi because most clades received good support.

DISCUSSION

Phylogeny of nematode-trapping fungi.—Nematode-trapping fungi previously were classified based on morphological characters of the conidia and comprised three genera, *Arthrobotrys*, *Dactylella* and *Monacrosporium subramanian* (1963). Recent studies with ITS and 18S rDNA sequences indicated that trapping devices are more informative than other morphological structures in delimiting genera (Liou and Tzean 1997, Pfister 1997, Ahrén et al 1998, Scholler et al 1999), and based on these studies a new classification scheme with four genera (*Arthrobotrys*, *Dactylellina*, *Gamsylella* and *Drechslerella*) was proposed by Scholler et al (1999).

Dactylellina.—*Dactylellina* M. Morelet was recognized by Scholler et al (1999) for species characterized by stalked adhesive knobs and included those species producing nonconstricting rings. Species producing both nonconstricting rings and stalked adhesive knobs could not be separated from the group producing only stalked adhesive knobs based on sequence analysis of the 18S and ITS regions of rDNA. Scholler et al (1999) however only included one taxon, *Monacrosporium haptotylum* (Drechsler) X.Z. Liu & K.Q. Zhang, which produced both nonconstricting rings and stalked adhesive knobs in their analyses. Furthermore nonconstricting rings were not produced by

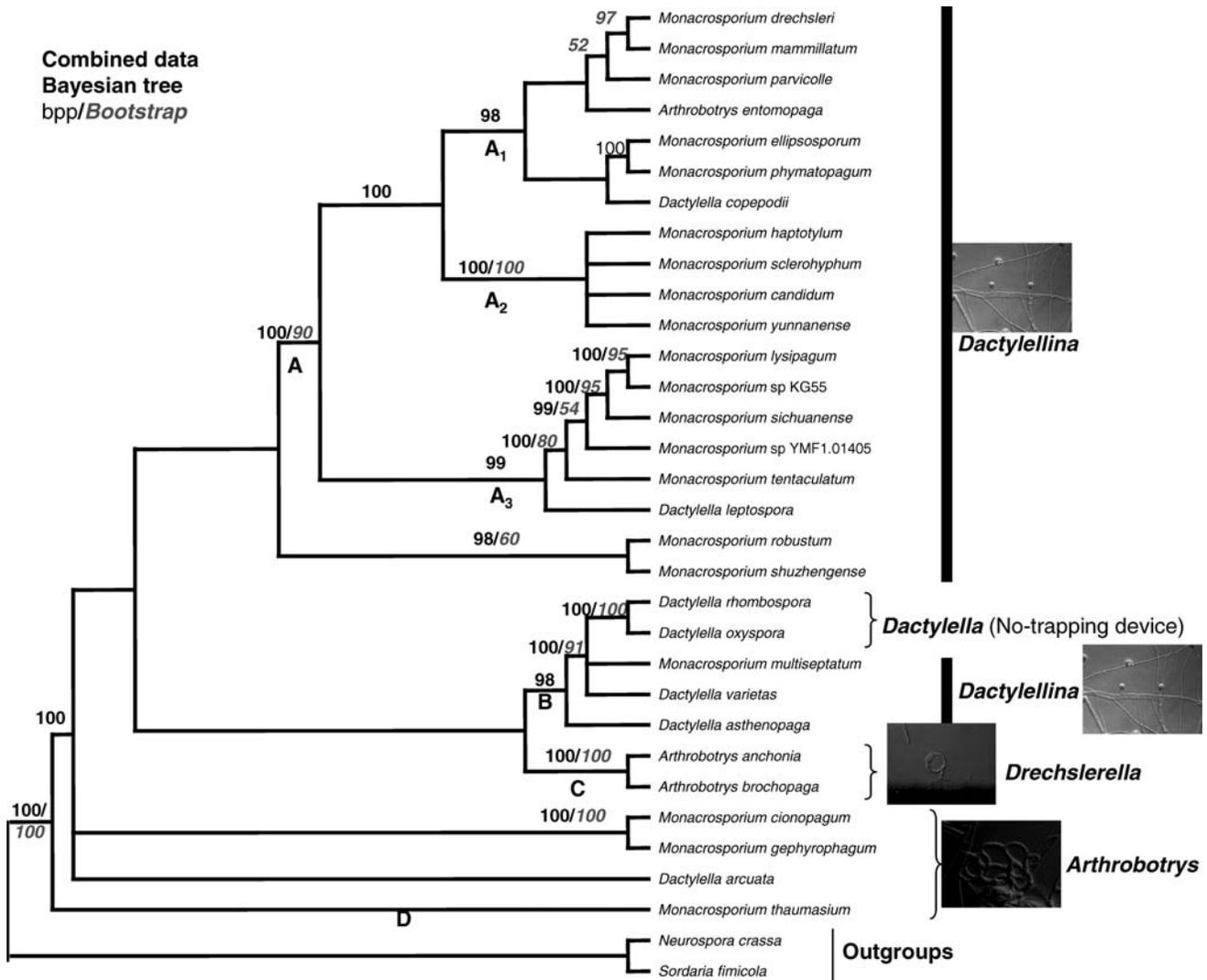


FIG. 3. Phylogenetic tree based on combined dataset of rDNA and β -tubulin. *Neurospora crassa* and *Sordaria fimicola* are used as outgroups. Bootstrap values obtained from 1000 replications and Bayesian posterior probability are shown above respective nodes.

this strain in culture. The inclusion of taxa producing nonconstricting rings in *Dactylellina* remained questionable given the limited taxon sampling.

In our study we included eight taxa producing both nonconstricting rings and stalked adhesive knobs. Phylogenies derived from combined gene datasets (rDNA and β -tubulin) do not support the monophyly of species with both nonconstricting rings and stalked adhesive knobs (FIG. 3, clade A) which group with species producing stalked adhesive knobs only. Within clade A, subclades A₂ and A₃ comprise taxa forming both nonconstricting rings and stalked adhesive knobs and taxa forming only stalked adhesive knobs with 100% bpp and 99% bootstrap support. Our data therefore support the conclusions

of Scholler et al (1999) in defining *Dactylellina* as including species that produce stalked adhesive knobs, with some species also producing nonconstricting rings.

The nonpredatory species *Dactylella oxyspora* and *D. rhopalota* Drechsler have been shown to be closely related to species producing constricting rings and species producing adhesive knobs (Ahrén et al 1998, Liou and Tzean 1997), with low statistical support. In our study however we used the nonpredatory taxa *D. rhombospora* and *D. oxyspora* and found them to have close affinities to species with adhesive knobs. It is possible that taxa with adhesive knobs have evolved from nonpredatory species, having gained the capability of capturing nematodes with these knobs, as concluded by Ahrén et al (1998). On the other hand

these taxa might have produced adhesive knobs at one time, but this trapping device might have been lost in some species in their subsequent development, thus losing their ability to trap nematodes. We have observed repeatedly that several strains characterized by adhesive knobs fail to produce any kind of trapping devices after successive subculturing.

Gamsylella.—The new genus *Gamsylella* was proposed for species characterized by producing unstalked adhesive knobs and adhesive branches Scholler et al (1999). Six species were placed in this genus including *Dactylella arcuata*, *D. lobata* Dudd., *Monacrosporium gephyrophagum*, *M. robustum*, *M. parvicolle* and *M. phymatopagum*. In this study we included these six taxa plus a new species *M. shuzhengense*. Morphological observations from culture revealed that none of these taxa only formed unstalked adhesive knobs, which was just one temporary structure formed at the first stage of life. These unstalked knobs could then grow out to form branches, which will further change in different species. In *D. arcuata*, *M. gephyrophagum* and *M. cionopagum*, the branches then fuse to form two- or three-dimension adhesive networks, whereas in *M. parvicolle*, *M. shuzhengense*, *D. lobata* and *M. phymatopagum* the branches fuse to form loops or helices. Therefore, based on our observation, unstalked adhesive knobs should not be treated as a unique type of trapping-device and this character should not be given taxonomic importance.

Scholler et al (1999) showed *Gamsylella* to be monophyletic when using *Dactylella arcuata*, *D. lobata*, *M. gephyrophagum* and *M. phymatopagum* in their analyses.

In this study we used more representatives of *Gamsylella sensu* Scholler et al (i.e. *Dactylella arcuata*, *Monacrosporium cionopagum*, *M. gephyrophagum*, *M. parvicolle*, *M. phymatopagum*, *M. robustum* and *M. shuzhengense*) and a combination of rDNA and β -tubulin to provide a better phylogenetic interpretation. *Monacrosporium parvicolle* and *M. phymatopagum* grouped with *Dactylellina* species (FIG. 3, clade A). *Monacrosporium shuzhengense* and *M. robustum* were sister taxa to *Dactylellina*. *Dactylella arcuata*, *Monacrosporium gephyrophagum* and *M. cionopagum*, which form unstalked adhesive knobs that grow into networks, are close to *M. thaumasium*, which Scholler et al (1999) included in *Arthrobotrys*. Kano et al (2004) found that 14 species with networks grouped as a monophyletic clade supported by ITS data and *M. gephyrophagum* and *M. cionopagum* also were related closely to this clade.

Based on findings reported here, we think it is necessary to emend the genetic concepts of nema-

tode-trapping fungi. *Dactylella arcuata*, *Monacrosporium gephyrophagum* and *M. cionopagum* are transferred from *Gamsylella* to *Arthrobotrys*, a genus where all species form unstalked adhesive knobs that develop into adhesive networks. The other taxa in *Gamsylella* (i.e., *M. parvicolle*, *M. phymatopagum*, *M. shuzhengense* and *M. robustum*) are transferred to an emended *Dactylellina*, a genus in which species can form stalked adhesive knobs, with some species producing nonconstricting rings or unstalked adhesive knobs that develop into loops; *Gamsylella* does not merit generic status.

Dactylellina candidum.—Rubner (1996) treated *Monacrosporium candidum* (Nees) X.Z. Liu & K.Q. Zhang, *M. haptotylum* (Drechsler) X.Z. Liu & K.Q. Zhang and *M. sclerohyphum* (Drechsler) X.Z. Liu & K.Q. Zhang as conspecific under the name *M. haptotylum*. *Monacrosporium yunnanense* K.Q. Zhang, X.Z. Liu & L. Cao (CBS 615.95) is similar to *M. candidum* in having spindle-shaped, mostly 4-septate conidia, and branched conidiophores, bearing 3–10 conidia in a loose capitulate arrangement. The only difference being that *M. candidum* and *M. yunnanense* form both stalked adhesive knobs and nonconstricting rings, while *M. haptotylum* and *M. sclerohyphum* form only stalked adhesive knobs. These four taxa (FIG. 3, subclade A2) are phylogenetically closely related receiving high bootstrap support and bbp (FIGS. 1–3), based on the rDNA, β -tubulin and combined datasets, and this grouping agrees well with the morphological characters of the taxa. These four taxa therefore can be considered as conspecific and should be named *Dactylellina candidum* based on our results.

Arthrobotrys gephyropaga.—In this study *Monacrosporium cionopagum* is closely related to *M. gephyrophagum* with 100% bpp and 100% bootstrap support (FIGS. 1, 2, 3) based on molecular data. This is in agreement with the morphological based classification scheme of Rubner (1996) in which *M. cionopagum* is considered a synonym of *M. gephyrophagum*. *Monacrosporium gephyrophagum* is transferred to *Arthrobotrys* based on the molecular results of this study.

Accepted genera of nematode trapping fungi.—Based on multigene data analyses, three genera, *Arthrobotrys*, *Dactylellina* and *Drechslerella*, are retained in the redefinition of the circumscription of three genera of nematode-trapping Orbiliaceous fungi. Species forming constricting rings (always with three cells) are placed in *Drechslerella*. *Arthrobotrys* is characterized by species forming adhesive net-

works. Taxa with unstalked adhesive knobs that grow out to form simple adhesive networks also are assigned to *Arthrotrys*. Species that capture nematodes mainly by stalked adhesive knobs are placed in *Dactylellina*. Taxa with both nonconstricting rings and stalked adhesive knobs and taxa with unstalked adhesive knobs that grow out to form loops also are assigned to *Dactylellina*.

KEYS TO GENERA OF NEMATODE-TRAPPING FUNGI

- 1 Trapping-device a constricting ring, which consists of three inflated cells with a short, strong stalk *Drechslerella*
- 1' Trapping-device not a constricting ring, but various adhesive trapping devices 2
- 2 Trapping device unstalked adhesive knobs that develops into an adhesive network or adhesive networks only. *Arthrotrys*
- 2' Trapping device stalked adhesive knobs, some with non-constricting rings, or unstalked adhesive knobs which grow out to form adhesive branches and loops *Dactylellina*

Taxonomic changes

Arthrotrys arcuata (Scheuer & J. Webster) Y. Li, comb. nov.

Basionym: *Dactylella arcuata* Scheuer & J. Webster, Mycol Res 94:718. 1990

? *Monacrosporium arcuatum* (Scheuer & J. Webster) A. Rubner, Stud Mycol 39:49. 1996

? *Gamsylella arcuata* (Scheuer & J. Webster) M. Scholler, Sydowia 51:108.1999.

Arthrotrys gephyropaga (Drechsler) Y. Li, comb. nov.

Basionym: *Dactylella gephyropaga* Drechsler, Mycologia 29:512. 1937

≡ *Monacrosporium gephyrophagum* (Drechsler) Subram., J Indian Bot Soc 42:293. 1963

≡ *Golovinia gephyropaga* (Drechsler) Mekht., Mikol Fitopatol 1:276. 1967

≡ *Gamsylella gephyropaga* (Drechsler) M. Scholler, Sydowia 51:108.1999.

= *Dactylella cionopaga* Drechsler, Mycologia 42:30. 1950

≡ *Monacrosporium cionopagum* (Drechsler) Subram., J Indian Bot Soc 42:293. 1963

≡ *Golovinia cionopaga* (Drechsler) Mekht., Khishchnye nematofagovye Griby-Gifomitsety: 166. 1979.

Dactylellina candidum (Nees: Fr.) Y. Li, comb. nov.

Basionym: *Dactylum candidum* Nees, Syst Pilze Schw 58. 1817: Fries Syst Mycol 3:44. 1829

≡ *Dactylaria candida* (Nees : Fr.) Sacc., Sylloge Fungorum 4: 195. 1886

≡ *Candelabrella candida* (Nees: Fr.) Rifai., Reinwardtia 7:369. 1968

≡ *Arthrotrys candida* (Nees: Fr.) S. Schenck, W.B. Kendr. & Pramer, Can J Bot 55:982. 1977

≡ *Dactylella candida* (Nees: Fr.) de Hoog & Oorschot, Stud Mycol 26:102. 1985

≡ *Monacrosporium candidum* (Nees: Fr.) Xing Z. Liu & K.Q. Zhang, Mycol Res 98:864. 1994

≡ *Golovinia capitulopaga* Mekht., Khishchnye nematofagovye Griby-Gifomitsety: 169. 1979

= *Dactylaria haptotyla* Drechsler, Mycologia 42:48. 1950

≡ *Golovinia haptotyla* (Drechsler) Mekht, Mikol Fitopatol 1:277. 1967

≡ *Candelabrella haptotyla* (Drechsler) Rifai, Reinwardtia 7:369. 1968

≡ *Arthrotrys haptotyla* (Drechsler) S. Schenck, W.B. Kendr. & Pramer, Can J Bot 55:983. 1977

≡ *Dactylella haptotyla* (Drechsler) de Hoog & Oorschot, Stud Mycol 26:111. 1985

≡ *Monacrosporium haptotylum* (Drechsler) Xing Z. Liu & K.Q. Zhang, Mycol Res 98:865. 1994.

≡ *Dactylellina haptotyla* (Drechsler) M. Scholler, Sydowia 51:108. 1999.

= *Dactylaria sclerohypha* Drechsler, Mycologia 42:57. 1950.

≡ *Monacrosporium sclerohyphum* (Drechsler) Xing Z. Liu & K.Q. Zhang, Mycol Res 98:865. 1994.

= *Monacrosporium yunnanense* K.Q. Zhang, Xing Z. Liu & L. Cao, Mycol Res 100:275. 1996.

≡ *Dactylellina yunnanensis* (K.Q. Zhang, Xing Z. Liu & L. Cao) M. Scholler, Sydowia 51:108. 1999.

Dactylellina lobata (Dudd.) Y. Li, comb. nov.

Basionym: *Dactylella lobata* Dudd, Trans Br Mycol Soc 34:94. 1951b

≡ *Monacrosporium lobatum* (Dudd.) A. Rubner, Stud Mycol 39:80. 1996

≡ *Gamsylella lobata* (Dudd.) M. Scholler, Sydowia 51:108.1999.

Dactylellina parvicolle (Drechsler) Y. Li, comb. nov.

Basionym: *Dactylella parvicollis* Drechsler, Sydowia 15:13. 1962 (1961)

≡ *Monacrosporium parvicolle* (Drechsler) R.C. Cooke & C.H. Dickinson [as "*parvicollis*"], Trans Br Mycol Soc 48:622. 1965

≡ *Golovinia parvicollis* (Drechsler) Mekht., Khishchnye nematofagovye Griby-Gifomitsety: 162. 1979

≡ *Gamsylella parvicollis* (Drechsler) M. Scholler, Sydowia 51:109.1999.

Dactylellina phymatopaga (Drechsler) Y. Li, comb. nov.

Basionym: *Dactylella phymatopaga* Drechsler, Mycologia 46:775. 1954

- ≡ *Monacrosporium phymatopagum* (Drechsler) Subram., J Indian Bot Soc 42:293. 1963
- ≡ *Golovinia phymatopaga* (Drechsler) Mekht., Khishch-nye nematofogovye Griby-Gifomitsety: 165. 1979
- ≡ *Gamsylella phymatopaga* (Drechsler) M. Scholler, Sydowia 51:109.1999.

Dactylellina robusta (J.S. McCulloch) Y. Li, comb. nov.

- Basionym: *Monacrosporium robustum* J.S. McCulloch, Trans Br Mycol Soc 68:177. 1977
- ≡ *Gamsylella robusta* (J.S. McCulloch) M. Scholler, Sydowia 51:109.1999.

Evolution of trapping-devices.—The concept that predatory nematode-trapping fungi evolved from nonpredatory taxa was proposed by Rubner (1996) who provided a hypothetical model for the development of trapping devices. Pfister (1997) also showed that the trapping Orbiliaceae formed a clade within the nonpredatory members of the family. Scholler et al (1999) further concluded that, based on 18S and ITS data, predatory species originated from nonpredatory species within the genus *Orbilia*. Our findings support these previous studies.

Ahrén et al (1998) analyzed 18S rDNA and found that species with constricting rings are monophyletic and distinct from species with other adhesive trapping devices and nonpredatory species. They therefore suggested that the ability to capture nematodes have arisen at least twice, once in a lineage with species forming constricting rings and once in a lineage leading to the formation of adhesive trapping structures. Further molecular studies showed that taxa with adhesive networks were monophyletic and could be separated from other taxa with various adhesive-trapping devices (Scholler et al 1999, Kano et al 2004).

Liou and Tzean (1997) suggested that species with adhesive knobs should be treated as sister groups and taxa with constricting rings and those with adhesive networks were derived from these sister groups (Liou and Tzean 1997). The monophyly of species characterized by adhesive knobs is supported in our datasets (FIG. 3, clade A). Our data also show that the nonpredatory taxa, *Dactylella rhombospora* and *D. oxyspora* are closely related to three taxa with adhesive knobs (FIG. 3). This indicates that species with adhesive knobs have closer phylogenetic affinities to nonpredatory fungi than to species with adhesive networks and constricting rings (FIG. 3).

Adhesive knobs, adhesive networks and constricting rings are the three main nematode-trapping devices and they capture nematodes based on different mechanisms. Constricting rings comprise three cells

that can inflate in a short time to strangle nematodes. Nematodes wriggle in networks and become trapped in the adhesive material lining the networks until they become exhausted and die. Adhesive knobs, however, trap nematodes at only one point and are not as efficient because nematodes can easily escape, while nematodes must wriggle into nonconstricting rings which can then break off and be carried away by the nematode. It suggested that possess constricting rings and adhesive networks should be more complex and better strategies for capturing nematodes and obtaining nutrition than adhesive knobs.

Species with more than one type of trapping device provide some evidence into how trapping-devices evolved in nematode trapping fungi from adhesive knobs to adhesive networks and constricting rings. One possible hypothesis for the evolution of various trapping devices based on phylogenetic analyses and large numbers of morphological observations is provided (FIG. 4).

As we know, nonconstricting rings usually are formed as a type of trapping device along with adhesive knobs in some species (e.g. *Monacrosporium lysipagum*). Our analyses also place species with nonconstricting rings and stalked adhesive knobs within clade A (FIG. 3). Species that form unstalked adhesive knobs then grow out to form loops (e.g. *Monacrosporium parvicolle*, *M. phymatopagum* and *M. robustum*), appear to be related to clade A (FIG. 3). These findings imply that ancestral adhesive knobs may grow out to form loops which then evolved into nonconstricting rings without adhesive materials (e.g. *Monacrosporium lysipagum*, *D. leptospora* and *M. candidum*). Both nonconstricting rings and constricting rings have three cells, stalks and no adhesive materials, even though nonconstricting rings cannot inflate and can be broken easily because of the long slender stalk. This similar structure suggested constricting rings might evolve from non constricting rings (FIG. 4, Branch A).

Species with adhesive knobs and simple networks (*Monacrosporium gephyrophagum* and *Dactylella arcuata*) appear to be closely related to species with networks based on molecular datasets (FIGS. 1, 2). Unstalked adhesive knob could be observed to grow out to form branches and several branches that grew up from same hyphae could fuse simple two-dimension network in *M. gephyrophagum* (CBS 178.37). Similar process could be observed in *D. arcuata* (CBS 174.89) but the branches grew up from different hyphae and finally fused three-dimension network. These results imply that ancestral adhesive knobs might grow out to form two-dimension networks and later evolve into species with three-dimension networks (e.g. *M. thaumasium*) (FIG. 4,

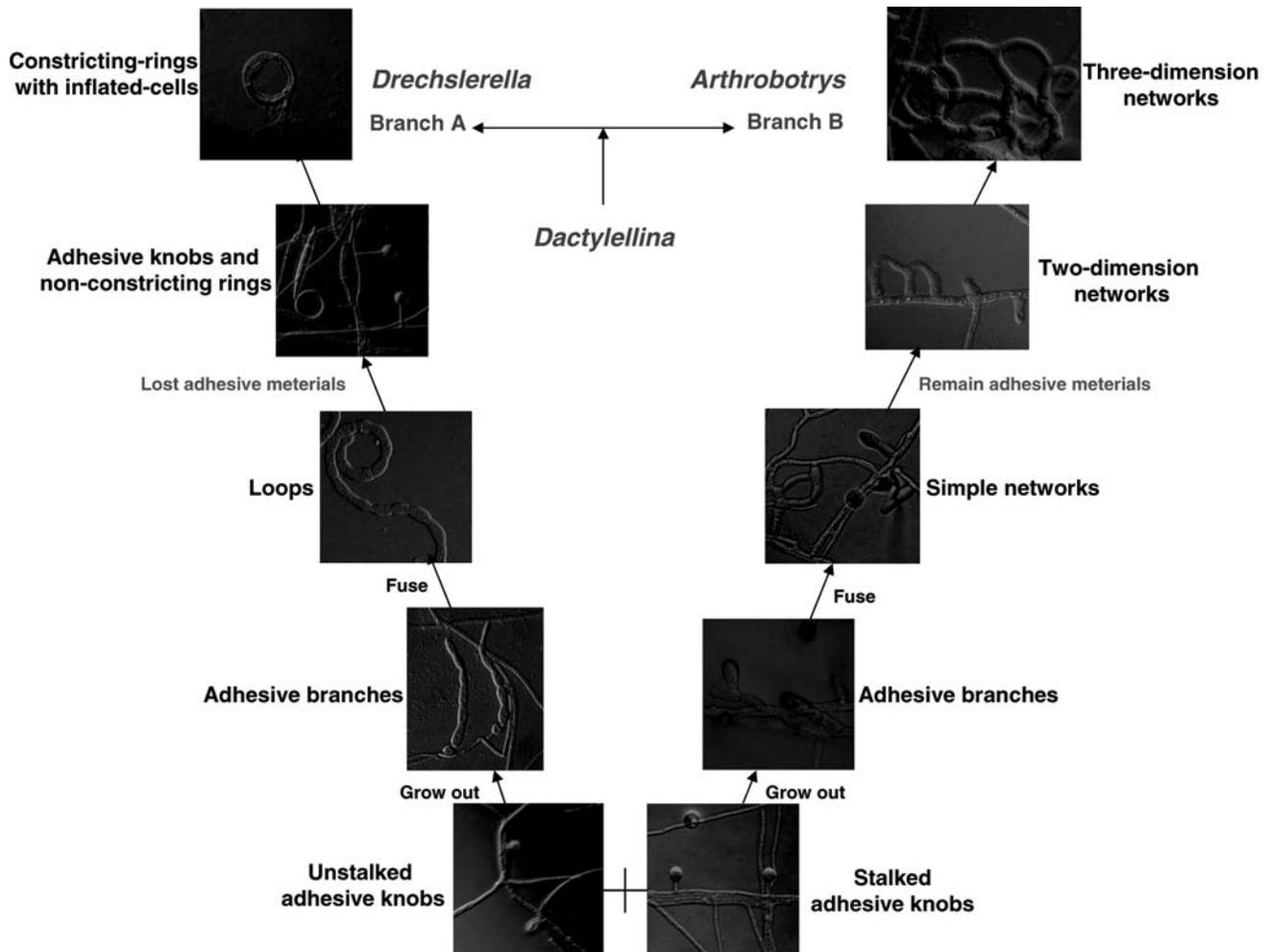


FIG. 4. Hypothetical evolution model of trapping devices in nematode-trapping fungi (Orbiliales) based on molecular data and phylogenetic analyses.

Branch B). Adhesive materials play an important part in the evolution of trapping devices. Unfortunately there is insufficient molecular evidence herein to provide an overview about a general pattern of the evolution of adhesive material. Further studies are needed.

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