A reevaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data

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Hagedorn, G. & M. Scholler (1999). A reevaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data. – Sydowia 51(1): 27–48.

A 1.2 kb long fragment of ribosomal DNA, including the 3' half of the 18S rDNA, the ITS1 region, the 5.8S rDNA, and the ITS2 region was sequenced in 20 strains of predatory hyphomycetes and allied species. Additional sequences were obtained from GenBank and a conspectus of the evolution of the predatory orbiliaceous fungi is provided. Amongst the species studied so far, no secondary loss of predacity could be detected. The predatory taxa were found to have evolved from within the genus *Orbilia*. Two major monophyletic clades were identified: species possessing constricting rings (group I), and species with various adhesive trapping devices. The latter clade could be subdivided into at least three monophyletic groups, corresponding to species with adhesive networks (group II), adhesive columns or unstalked adhesive knobs (group III), and stalked adhesive knobs (group IV). Species with non-constricting rings appeared as a terminal development within group IV. The species possessing constricting rings (group I) had a basal position relative to species with adhesive trapping devices, but this relationship had no significant bootstrap support.

Keywords: molecular evolution, nematophagous fungi, predatory hyphomycetes, *Orbiliaceae*, systematics, trapping devices

Fungi capable of trapping nematodes are fascinating organisms. The diversity of trapping organs and the ecological and evolutionary implications of this mode of nutrition have attracted the attention of many mycologists for a long time. A major group of these fungi are the predatory hyphomycetes with teleomorphs in the *Orbiliaceae* (Ascomycota). The taxonomic concepts of this group are still primarily based on the morphology of conidia and conidiophores. The morphology of the trapping organs is not adequately taken into account. Species with diverse types of trapping organs are currently assigned to one genus, and the same trapping organ occurs in several genera. Recent molecular studies (Pfister, 1997, Liou & Tzean, 1997, Ahrén & al., 1998) have made it increasingly clear that the trapping organs are phylogenetically more informative than the morphology

of the dispersive structures. These studies used different DNA regions (ITS regions or 18S rDNA) and came to contradictory conclusions regarding the monophyly of the predatory orbiliaceous fungi. The purpose of the present study is to clarify the picture by providing additional data and synthesizing the available information. Our findings lead us to propose a new generic concept, which is presented in a separate article (Scholler & al., 1999).

Materials and methods

The mycelium of 20 strains of nematophagous and related fungi (See Tab. 1 at the end of the publication) was frozen with liquid nitrogen directly on agar media and scraped from the plates. The frozen mycelium was pulverized in a precooled mortar and the DNA was isolated and purified according to the method described by Hering (1997). A region of the ribosomal DNA was amplified using the primers NS5 and ITS4 (White & al., 1990) and the length of the fragments was determined on agarose gels. PCR conditions were optimized until all strains yielded a single PCR product, which was purified using 12 µl of Prep-A-Gene matrix (BioRad), according to the manufacturer's instructions. The purified double-stranded PCR products were directly sequenced using Amersham Thermo-Sequenase reactions (6 µl scale) and IRD-labeled primers (NS5, NS6, NS7, NS8, ITS5, ITS1, and ITS4; White & al., 1990). The reactions were separated on a LI-COR 4000L automated DNA sequencer using 66 cm gels with 4.3% acrylamide (Amersham RapidGel XL), obtaining an average reading length of 850 bases. The entire product was sequenced bidirectionally. The assembled and corrected sequences have been deposited in GenBank (accession numbers AF106519-AF106538, see Tab. 1). Additional sequences were obtained from GenBank using direct queries for the genera involved. A BLAST 2.0 similarity search (Altschul & al., 1997) on the consensus sequence of the 5.8S region did not yield any additional sequences.

Sequences were aligned using ClustalX 1.64b/ClustalW (Thompson & al., 1994, Thompson & al., 1997) with gap opening : extension parameters of 15 : 1 and the structure of blocks and inserts was manually corrected using GeneDoc 2.4 (Nicholas & Nicholas, 1997). The alignment was then split into multiple files according to the block/insert structure, each of which was realigned in ClustalX with gap opening : extension parameters of 15 : 6, merged into a single alignment and finally manually corrected again. A region 5 bases long, starting 18 bases downstream of the NS5 primer, was excluded from the analyses, since it regularly showed a strong band compression that made the sequences unreliable.

Since the availability of different DNA regions varied among the strains, separate analyses were performed for the following regions: (a) the partial 18S rDNA (primer NS5 to NS8), (b) the region containing the ITS1, 5.8S rDNA, and ITS2 region (called ITS-region in the following; primer ITS1 to ITS4), (c) the insert between primer ITS5 and NS8, and (d) the 5.8S rDNA region alone. Terminal parts of the alignments were trimmed to increase the reliability of the analyses. Neighbor-joining analyses were performed using TreeCon vers. 1.3b (Van de Peer & De Wachter, 1994). Distances were calculated under the Kimura two-parameter model with insertions separately accounted for; all bootstrap analyses were performed with 1,000 replicates. The bootstrap replicate trees were re-rooted using *Gliocladium roseum* as an outgroup before the bootstrap consensus was computed. Bootstrap values below 75% were considered not significant and are usually not shown.

Results

The sequences of the chosen region measured between 1202 and 2317 bp, with the variation being due to the occasional presence of one or two long inserts. The first insert started 15 bases after the NS5 primer (= insert position 943, Gargas & al., 1995) and was between 376 and 416 bases long. It was present in *Monacrosporium arcuatum* and *Dactylella cylindrospora*. The second insert started one base after the ITS5 primer (= insert position 1506, Gargas & al., 1995) and was between 385 and 416 bases long. It was present in *Monacrosporium leptosporum*, *Arthrobotrys conoides*, and *Dactylella cylindrospora*. This second insert was homologous to the insert observed by Liou & Tzean (1997). Fig. 3 displays the relationships among the sequences of the second insert. The sequences of the first insert aligned very poorly with those of the second insert and did not seem to be related. Both inserts were removed from subsequent analyses.

A phylogram based on the ITS region is depicted in Fig. 1. The predatory taxa evolved from within the *Orbilia* complex (Helotiales/ 'Leotiales') and formed a single clade that was the sister group of the clade containing *Dactylella cylindrospora*. Neither the sister group relationship nor the monophyly of the predatory taxa as a whole were supported by a significant bootstrap value. Within the predatory taxa, four major groups were identified (Fig. 1, groups I–IV; details of group II in Fig. 2). Each group corresponded to a unique type of trapping device. With the exception of two species of uncertain position (*M. phymatopagum* and *M. bembicodes*), the monophyly of each group was supported by bootstrap values between 93 and 99%. In addition, groups II to IV formed a separate clade supported by a bootstrap value of 91%. The exact relationships among

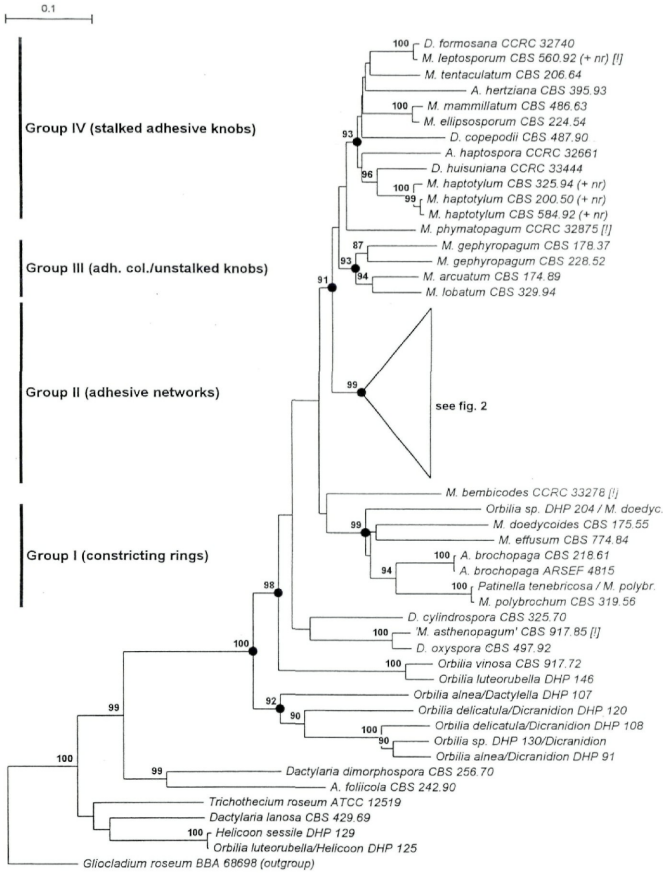

Fig. 1. – Bootstrapped neighbor-joining analysis of strains where sequences of the ITS1 region, the 5.8S rDNA, and the ITS2 region are available. Only bootstrap values >75% are shown. Horizontal branch lengths represent mean distances of all bootstrap samples. Major clades discussed in the text are marked with a dot. Species whose identification or association with one of the major groups is problematic are marked with "[!]". Within group IV, species with non-constricting rings in addition to adhesive knobs are marked with "(+ nr)" after the species name. Species of group II are shown in Fig. 2. – Abbreviations: *A.* = *Arthrobotrys*, *M.* = *Monacrosporium*, *D.* = *Dactylella*; for abbreviations of culture collections see Tab. 1.

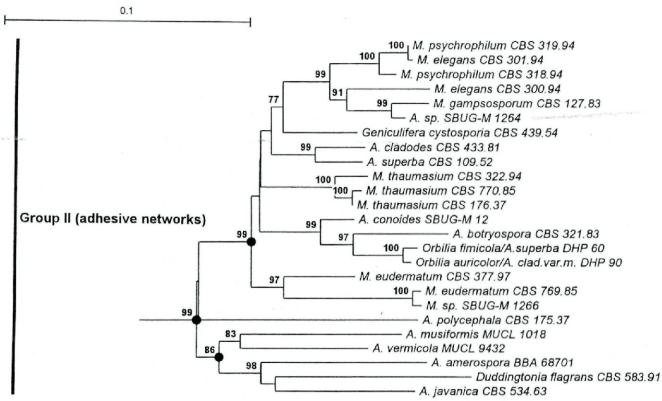

Fig. 2. – Detailed phylogram of group II from the bootstrapped neighbor-joining analysis depicted in Fig. 1 (note the enlarged distance scale).

the major groups and the evolution of trapping devices, however, could not be resolved with confidence. No bootstrap values were available to support sister group hypotheses between the four major groups of trapping devices.

Four GenBank accessions covering the ITS region were excluded from the analysis shown in Fig. 1 and 2 because they aligned extremely poorly with the other sequences. The first is U51958,

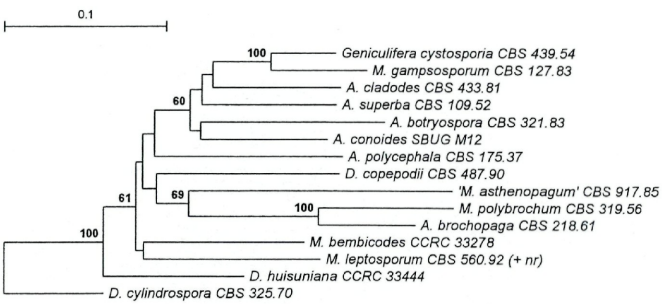

Fig. 3. – Bootstrapped neighbor-joining analysis of the alignable part (alignment length 453, sequences are 362 to 405 bp long) of the second insert (see text); rooted at *Dactylella cylindrospora* (U51962) to make the tree comparable with Fig. 1. Only bootstrap values >50% are shown. The gene tree of the insert deviates from the phylogenetic tree as determined from the remaining sequences.

Monacrosporium lobatum (= *Dactylella lobata*), which was submitted by Liou & Tzean but not used in Liou & Tzean (1997). We assume that this indicates doubts as to the identity of the sequence. Since a well defined strain of *Monacrosporium lobatum* was sequenced in the current study, U51958 has been excluded. The other 3 accessions belong to unidentified *Arthrobotrys* (U72594 = D.H.P. 100, U72602 = D.H.P. 109) and *Monacrosporium* species (U72608 = D.H.P. 211 [quoted as 'U72611' in Pfister, 1997]) submitted by Pfister (1997). D. Pfister (pers. comm.) himself considers the identifications uncertain. Until further evidence is available, these four sequences should be considered with caution.

Regarding the question of monophyly of the predatory taxa as a whole, the results of an analysis of the 5.8S region alone were similar to the results of the analysis of the ITS region (including the 5.8S rDNA). The clade containing the predatory taxa was consistently present, but supported only by low bootstrap values: 42% when all available sequences were included, and 57% when only those sequences published by Liou & Tzean (1997) were included (phylogram not shown).

A phylogram based on the partial 18S sequences is presented in Fig. 4. This analysis allowed the comparison of our data with those

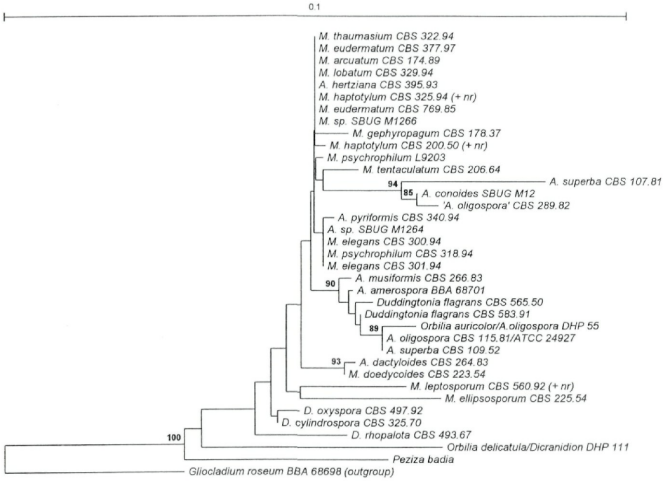

Fig. 4. – Bootstrapped neighbor-joining analysis of strains where partial 18S rDNA sequences (primer NS5 to NS8) are available. Inserts and doubtful regions are excluded. Only bootstrap values >75% are shown.

published by Ahrén & al. (1998). The monophyly of the predatory taxa together with some non-nematophagous *Dactylella* species was again evident. The *Dactylella* species had a basal position, followed by two species possessing constricting rings (equivalent to group I in Fig. 1). The monophyly of the latter group was supported by a bootstrap value of 93% (Fig. 4). The taxa possessing adhesive trapping structures were poorly resolved. A group containing *A. musiformis*, *A. amerospora* and *Duddingtonia flagrans* could also be found in the ITS analysis within group II (Fig. 2). The position of the two strains of *A. oligospora* (CBS 115.81 and CBS 289.82) and the two strains of *A. superba* (CBS 109.52 and CBS 107.81) was particularly interesting. The first strain of each species appeared in a clade arising from within the *musiformis-amerospora-flagrans* group (bootstrap value 89%), while the second strain of each species was paired in an entirely separate clade together with *A. conoides* (bootstrap value 94%).

Discussion

The original rationale for sequencing the 18S rDNA in addition to the ITS region was to elucidate the systematic position of nematophagous fungi within the ascomycetes. ITS data are expected to be of limited use in this respect, because they may be saturated with mutations, making it impossible to distinguish between synapomorphic and heteroplasic similarities. While this work was in progress, the publication by Ahrén & al. (1998) made this intention obsolete, and the sequencing was stopped when only the 3' half of the 18S rDNA was sequenced. The phylogram in Fig. 4 combines all available sequences for this part of the 18S rDNA. It is of limited value because of the low number or phylogenetically informative sites present. It does, however, allow the placement of several species for which no ITS sequences are available, such as *Arthrobotrys dactyloides*, *A. oligospora*, *A. superba* (CBS 107.81) and *Dactylella rhopalota*. Additionally, the 18S rDNA analysis supports the conclusions drawn from the analysis of the ITS region, by proving that the taxa in question are closely related and an analysis of the ITS region is warranted.

The ITS phylogeny (based on ITS1, ITS2, and 5.8S rDNA, Figs. 1 and 2) is much better resolved than the one based on the 18S data. Many clades are supported by high bootstrap values. It should be noted, however, that the alignment of outlier sequences (strains below *Dactylaria dimorphospora* in Fig. 1) was difficult and could be challenged. The relationships of these sequences presented in Fig. 1, therefore, should not be interpreted beyond the statement that they are not closely related with the core group of orbiliaceous taxa under

study. The endophytic, non-predatory fungus *Arthrobotrys foliicola* CBS 242.90 is only morphologically similar to other *Arthrobotrys* species and already Liou & Tzean (1997) suggested that it should be placed in a different genus.

Within the anamorphic or teleomorphic ascomycete species investigated, the predatory taxa form a distinct monophyletic clade. This clade, however, has no significant bootstrap support, neither in the analysis of the ITS region (Fig. 1) nor in the analysis of the partial 18S rDNA sequences (Fig. 4). This contrasts with the results of Liou & Tzean (1997), who found a significant bootstrap support for a monophyletic origin of the predatory taxa using maximum parsimony analysis of only the 5.8S rDNA sequences. Our own analyses of 5.8S rDNA sequences alone (using neighbor-joining) do not corroborate these results, regardless of whether all available sequences were analyzed or only those used by Liou & Tzean (1997).

The monophyletic origin of the taxa forming various types of adhesive trapping organs (groups II–IV) is strongly supported by a bootstrap value of 91% (Fig. 1). This clade is the sister group to group I forming constricting rings (no bootstrap support for sister group relationship). This result is in concordance with the findings of Ahrén & al. (1998), who detected a similar dichotomy between constricting and adhesive trapping organs based on complete 18S rDNA sequences. The exact relationships among groups II, III, and IV could not be clarified in our study. Judging from the small distances between the relevant clades in Fig. 1, the groups seem to be very closely related. This makes it likely that the evolution of adhesive trapping devices by an ancestor of these fungi was followed by a period of evolutionary radiation and the development of a variety of trapping devices in a relatively short period of time.

Ahrén & al. (1998) found that the non-nematophagous species *Dactylella oxyspora* and *D. rhopalota* were placed between the group with constricting rings and the group with adhesive devices. They conclude that either the two groups have obtained the capability to capture hyphomycetes independently, or some *Dactylella* species have subsequently lost that capability. Although we cannot refute this conclusion, it is based on weak statistical support. The relevant node (labeled 'B' in their analysis) is supported by bootstrap values of 45 and 52% in their maximum parsimony and neighbor-joining analyses, respectively, and is not resolved at all in their maximum likelihood analysis. In our opinion, such low bootstrap values should not be considered significant.

Within the *Dactylella* clade (below group I in Fig. 1), *Monacrosporium asthenopagum* CBS 917.85 seems to be misplaced. *M. asthenopagum* is described as a nematophagous species forming adhesive knobs. The strain sequenced (CBS 917.85), however, seems to

be neither *M. asthenopagum* nor a nematophagous species. Rubner (1996: 52) studied this isolate and mentioned that it was "sporulating abundantly, not producing trapping organs, resembling *Dactylella cylindrospora*". These morphological observations are congruent with our molecular analysis, where this strain is a sister group of *Dactylella oxyspora*, which in turn is a sister group of *D. cylindrospora* (Fig. 1).

The association of two predatory species (*Monacrosporium bembicodes* and *M. phymatopagum*) with the major groups is not supported by bootstrap analysis. The constricting ring former *Monacrosporium bembicodes* appears to be related to group I, but has a basal position. Given that the remaining three groups are held together with a high bootstrap support (91%), and considering the unique nature of the trapping device, it is likely that *M. bembicodes* belongs to group I. The sequence for this strain has been deposited in GenBank by Liou & Tzean, but was not mentioned in their publication (Liou & Tzean, 1997). The authors may have had some doubts about the validity of the strain identification. New sequences for *M. bembicodes* are therefore needed. The second species with an uncertain position, *M. phymatopagum*, forms unstalked adhesive knobs. Rubner (1996) proposed a model for the evolution of trapping devices, according to which species with unstalked adhesive knobs are considered primitive. In our analysis, this species is associated with group IV possessing stalked adhesive knobs, but it has a basal position between groups III and IV, and no bootstrap support is available for its association with group IV. Rubner's hypothesis may therefore still be valid.

Although Liou & Tzean (1997) came to similar conclusions regarding the importance of trapping devices, the topology of their phylogram based on ITS sequences seems to be quite different from Fig. 1. This is mainly due to the fact that they used *M. phymatopagum* as outgroup in the analysis of the ITS1 and ITS2 regions. Rerooting their phylogram produces a topology generally similar to the phylogram depicted in Fig. 1. The remaining differences, especially regarding the strength of bootstrap support, can be explained by the fact that Liou & Tzean (1997) analyzed fewer species, used a different method for phylogenetic inference, excluded areas they considered ambiguously aligned from the analyses, and did not analyse all base positions available (they split them into two separate analyses for the 5.8S rDNA and the two ITS regions, respectively).

With 25 sequences available, group II (adhesive networks, Fig. 2) is the largest group in the analysis. Several clades within this group are supported by high bootstrap values. With the exception of the ambiguously placed *A. polycephala*, two major clades can be distinguished. While the monophyly of the clade containing *Dudding-*

tonia flagrans was already visible in the analysis by Liou & Tzean (1997), the monophyly of the remaining species was not evident. No unique morphological character could yet be identified for either clade. At the specific level it is interesting to note how relatively close the two *Orbilia* species are placed. Similarly, the identification of the *M. elegans* strains CBS 301.94 and CBS 300.94 should be reviewed; the first strain appears to be genetically very close to *M. psychrophilum*, and distinct from the second isolate.

In group IV, two species are present that form non-constricting rings in addition to stalked adhesive knobs. Soprunov (1958) assumed that species with constricting rings evolved from species with non-constricting rings. Although Rubner (1996) put forward morphological arguments supporting this hypothesis, it seems unlikely in the light of the recent molecular studies. Species with both stalked adhesive knobs and non-constricting rings do not appear closely related with either the group forming adhesive networks or with the group forming constricting rings. Non-constricting rings appear to be a terminal development rather than a missing link.

In one aspect our new data obscure the picture rather than clarify it. From the taxa forming stalked adhesive knobs in combination with non-constricting rings, Liou & Tzean (1997) studied two strains of *Monacrosporium haptotylum* (under the name *Dactylella candida*). Upon sequencing yet another strain of *M. haptotylum* as well as a strain of *M. leptosporum* (CBS 560.92) supposedly having the same combination of trapping devices, we found that the different species belong to two widely separated groups. Bootstrap values of 97 and 100%, respectively, tie them to other species possessing only stalked adhesive knobs. The immediate interpretation of our result is that this combination of trapping devices has evolved at least twice independently. There is some doubt, however, about the correct identification of CBS 560.92. Rubner (1996: 79) observed that it produces adhesive knobs, but she did not find non-constricting rings. We restudied this strain and confirmed her observation. G. L. Barron (pers. comm.), who isolated this strain (under the name *Dactylaria dasguptae*; considered synonymous to *M. leptosporum* in Rubner, 1996) cannot ascertain whether it originally had or did not have non-constricting rings. It is therefore possible that strain CBS 560.92 is a species different from *M. leptosporum*. If this is the case, non-constricting ring formers could be considered a monophyletic group derived from stalked adhesive knob formers. To verify this, however, more studies on trapping organ ontogeny, and molecular data are necessary. It is unfortunate that CBS 560.92 has been proposed as epitype of *M. leptosporum*.

Pfister (1997) produced a study of anamorph-teleomorph connections within the *Orbiliaceae*. His *Orbilia auricolor-curvatispora*

group corresponds to our group II (anamorphs capable of producing adhesive networks, Fig. 1) and his group including *Patinella tenebricosa* corresponds to our group I (anamorphs capable of producing constricting rings). It is interesting to note that so far no teleomorphs have been identified for groups III and IV. Given the high bootstrap support for a monophyly of groups II-IV, more anamorph connections of *Orbilia* species need to be studied.

Within the group of *Orbilia* species without known predatory anamorphs, two distinct lineages have a high bootstrap support (Fig. 1): The group of *Orbilia vinosa* and *O. luteorubella* D.H.P. 146 is clearly distinct from the *Orbilia coccinella-alnea-delicatula* group with a *Dicranidion* anamorph. Pfister (1997) reported a *Helicoon sessile* anamorph for *O. luteorubella*. In Fig. 1, the isolate D.H.P. 125 is indeed a sister group to an independently sequenced *H. sessile* isolate. It is obvious, however, that this isolate is entirely unrelated to the remaining *Orbilia* species, which include the isolate D.H.P. 146 of *O. luteorubella*. It is plausible that the isolate D.H.P. 125 belongs to a genus that is genetically different from *Orbilia*, though morphologically similar. These problems have to be considered in a future monograph of the *Orbiliaceae*.

Webster & al. (1998) published *Orbilia fimicoloides* with an anamorph identified as *Dactylella* cf. *oxyspora*. Thus, pending further molecular studies, a potential teleomorph for the *Dactylella* clade (containing *D. oxyspora* and *D. cylindrospora*, see Fig. 1) is now available.

Beside our investigation of the ITS and 18S regions we were also interested in whether the insert at position 1506 (Gargas & al., 1995) contains phylogenetic information. As shown in the phylogenetic analysis of this insert (Fig. 3), the topology of the group with *Geniculifera cystosporia* as basal branch is roughly comparable to the phylogeny based on the ITS region (Figs. 1 and 2). Also, the grouping of *M. polybrochum* and *A. brochopaga* is found in both phylograms. The association of *M. asthenopagum* with this group, however, is unexpected and could be due to the possible mobile nature of this element. The general congruence of the two gene phylogenies is astonishing as the insert is optional and completely lacking in the majority of species. Three hypotheses are conceivable: a) The insert evolves consistently at the current position and the lack of the insert in most species is due to multiple, independent deletion events. b) The insert evolves at the current position; in species apparently lacking it, it is present only in a very low number of copies of the rDNA and inapparent on agarose gels after exponential amplification of insertless copies. c) The insert evolves at a different position in the genome, and its presence at position 1506 in some species is due to multiple independent insertions or transpositions. These hy-

potheses could be tested in the future by designing insert-specific primers to test species lacking an insert at position 1506.

The various sequences studied thus far (18S rDNA, ITS, 5.8S rDNA) all belong to a single linked gene complex. As long as no unlinked genes like beta-tubulin or mitochondrial RNA have been studied, our phylogenetic conclusions remain somewhat doubtful. The high degree of congruence with complex morphological characters like trapping devices, however, makes us confident that the true species tree will only differ in minor details.

Molecular identification methods are equally applicable to both anamorphic and teleomorphic taxa. This may open a door into a future where the artificial distinction between teleomorph and anamorph taxonomy will be dropped in favor of a "holomorphic" taxonomy. However, since it is not yet foreseeable that routine identification of fungi will be based on molecular methods, the practicality of taxonomic changes should remain the taxonomist's primary concern. Molecular studies are a valuable tool in revising the taxonomy of anamorphic taxa (O'Donnell, 1996), but it should not be a goal of anamorph taxonomy to develop a phylogenetic classification system beyond and apart from an integration with the system of teleomorphic taxa. Wherever possible, anamorphic taxa should be defined in such a way that they are congruent with teleomorphic taxa or phylogenetic clades. In the case of the nematophagous anamorphs of the *Orbiliaceae*, we regard it as taxonomic progress to include the morphology of the trapping organs in the definition of taxa, obtaining naturally delimited anamorphic taxa that can be easily recognized without recourse to molecular identification methods.

Acknowledgments

We thank A. Hansen for technical assistance, W. Jooste and the Centraalbureau voor Schimmelcultures, Baarn, for providing strains, and U. Braun, W. Gams, M. Grube, M. Fischer, and D. Maltais for critical revisions of the manuscript. We are grateful to the Studienstiftung Mykologie, which supported the study with a grant that enabled the second author to work as a visiting scientist in Berlin, and Dr. G. Deml, who supported this study in his institute.

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(Manuscript accepted 30th January 1999)

Tab. 1. – Orbiliaceous and related fungi used in the present study. Abbreviations: uk = unstalked adhesive knobs, ak = stalked adhesive knobs (prolif. = proliferating), aknr = stalked adhesive knobs plus non-constricting rings, an = adhesive networks, nn = no trapping organs, ac = adhesive columns, ah = adhesive hyphae, cr = constricting rings; ARSEF = UDSA ARS Collection of Entomopathogenic Fungal Cultures [Ithaca, NY], ATCC = American Type Culture Collection [Rockville, Maryland], BBA = Biologische Bundesanstalt [Germany], CBS = Centraalbureau voor Schimmelcultures [Baarn, Netherlands], CCRC = Culture Collection and Research Center [Hsinchu, Taiwan], MUCL = Mycothèque de l'Université Catholique de Louvain-la-Neuve [Belgium], SBUG-M = Sektion Biologie der Universität Greifswald, Myzelpilze [Germany], A.R. = private collection A. Rubner, D.H.P. = private collection D. H. Pfister.

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Arthrobotrys amerospora S. Schenck, W. B. Kendr. & Pramer	an	BBA 68701, SBUG-M 1257	–	AF106533	–	Germany
Arthrobotrys botryospora G. L. Barron	an/ah	CBS 321.83T, CCRC 32906	–	–	U51955	Canada,Ontario
Arthrobotrys brochopaga (Drechsler) S. Schenck, W. B. Kendr. & Pramer	cr	ARSEF 4815, D.H.P. 212,CBS 332.94, A.R. 9314	–	–	U72609[a]	Ecuador
"	"	CBS 218.61, CCRC 32702	–	–	U51950	USA, FL
Arthrobotrys cladodes Drechsler var. *cladodes*	an	CBS 433.81, CCRC 32697	–	–	U51945[b]	UK
Arthrobotrys conoides Drechsler	an	SBUG-M 12	–	AF106534	–	Germany
Arthrobotrys dactyloides Drechsler	cr	CBS 264.83	AJ001997	–	–	Canada

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Arthrobotrys foliicola Matsush.	nn	CBS 242.90, CCRC 32937	–	–	U51954	UK (?)
Arthrobotrys haptospora Drechsler	ak	CCRC 32661	–	–	U51946	Taiwan
Arthrobotrys hertziana M. Scholler & A. Rubner	ak	CBS 395.93^{T}, SBUG-M 1245	–	AF106519	–	Canary Islands
Arthrobotrys javanica Rifai & R. C. Cooke	an	CBS 534.63^{T}, CCRC 32695	–	–	U51947	Indonesia, Java
Arthrobotrys musiformis Drechsler	an	CBS 266.83	AJ001985	–	–	Nigeria
"	"	MUCL 1018, CBS 110.37, CCRC 32665	–	–	U51948	USA, VA
Arthrobotrys oligospora Fresen.	an	CBS 115.81, ATCC 24927	AJ001986	–	–	Sweden
'Arthrobotrys oligospora' Fresen.	(an/ah)	CBS 289.82	AJ001987	–	–	France
Arthrobotrys polycephala (Drechsler) Rifai	an	CBS 175.37^{T}, CCRC 32910	–	–	U51951	USA, MD
Arthrobotrys pyriformis (Juniper) S. Schenck, W. B. Kendr. & Pramer *(= Arthrobotrys robusta)*	an	CBS 340.94, A.R. 9327	AJ001988^{S,c}	–	–	Germany

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Arthrobotrys spec.	ak	SBUG-M 1264	–	AF106536	–	S. Africa
Arthrobotrys superba Corda	an	CBS 107.81, ATCC 28922	AJ001989	–	–	?
"	"	CBS 109.52, CCRC 32698	AJ001983[d]	–	U51949	UK
Arthrobotrys vermicola (R. C. Cooke & Satchuth.) Rifai	an	MUCL 9432, CBS 513.66[T], CCRC 32666	–	–	U51944	Uganda
Dactylaria dimorphospora Veenb.-Rijks	nn	CBS 256.70[T], CCRC 32916	–	–	U51980	Netherlands
Dactylaria lanosa Malla & W. Gams	nn	CBS 429.69[T], CCRC 32914	–	–	U51979	Denmark
Dactylella copepodii G. L. Barron	ak	CBS 487.90[T], CCRC 33300	–	–	U51964	New Zealand
Dactylella cylindrospora (R. C. Cooke) A. Rubner (= *Arthrobotrys cylindrospora)*	nn	CBS 325.70, CCRC 32913	–	AF106538[f]	U51953[S,e]	Samoa
Dactylella oxyspora (Sacc. & Marchal) Matsush.	nn	CBS 497.92, A.R. 922	AJ001993	AF106537[g]	–	USA
Dactylella formosana J. Y. Liou, G. Y. Liou & Tzean	ak	CCRC 32740[T]	–	–	U51956	Taiwan
Dactylella huisuniana J. L. Chen, T. L. Huang & Tzean	ak	CCRC 33444[T?]	–	–	U51965	Taiwan

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Dactylella rhopalota Drechsler	nn	CBS 493.67	AJ001992	–	–	Netherlands
Duddingtonia flagrans (Dudd.) R. C. Cooke	an	CBS 583.91, SBUG-M 1234	–	AF106520	–	Germany
"	"	CBS 565.50, CCRC 32917	AJ001991[j]	–	U51961[h]	UK, England
Geniculifera cystosporia (Dudd.) Rifai & R. C. Cooke	an	CBS 439.54, CCRC 32918	–	–	U51966	?
Gliocladium roseum Bainier	nn	BBA 68698	–	AF106532	–	Germany
Helicoon sessile Morgan	nn	D.H.P. 129	–	–	U72605	USA, MA
Monacrosporium arcuatum (Scheuer & J. Webster) A. Rubner	ak (prolif.)	CBS 174.89[T], SBUG-M 1254	–	AF106527	–	UK
'Monacrosporium asthenopagum' (Drechsler) A. Rubner *(= Dactylella asthenopaga)*	(nn!)	CBS 917.85, CCRC 32938	–	–	U51962[S,e]	Netherlands
Monacrosporium bembicodes (Drechsler) Subram.	cr	CCRC 33278	–	–	U51976[e]	?
Monacrosporium doedycoides (Drechsler) R. C. Cooke & C. H. Dickinson	cr	CBS 223.54	AJ001994[k]	–	–	UK
"	"	CBS 175.55, CCRC 32851	–	–	U51969	UK

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Monacrosporium elegans Oudem.	an	CBS 301.94^{ET}, SBUG-M 1227	–	AF106521	–	Germany
"	"	CBS 300.94, SBUG-M 1230	–	AF106522	–	Germany
Monacrosporium effusum (Jarow.) Xing-Z. Liu & K. Q. Zhang(= *Geniculifera effusa)*	cr	CBS 774.84^{T}, CCRC 32920	–	–	U51967^{S}	Canada, Manitoba
Monacrosporium ellipsosporum (Grove) R. C. Cooke & C. H. Dickinson	ak	CBS 224.54, CCRC 32921	AJ001995	–	U51971	UK
Monacrosporium eudermatum (Drechsler) Subram.	an	CBS 377.97, SBUG-M 1252	–	AF106528	–	Burkina Faso
"	"	CBS 769.85, SBUG-M 1253	–	AF106530	–	?
Monacrosporium gampsosporum (Drechsler) A. Rubner(= *Dactylella gampsospora)*	an	CBS 127.83^{S}, CCRC 32871	–	–	U51960^{S}	USA, FL
Monacrosporium gephyropagum (Drechsler) Subram.	ac	CBS 178.37^{T}, CCRC 32923	AJ001996	–	U51974	USA
"	"	CBS 228.52, CCRC 32694	–	–	U51968^{S}	UK

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Monacrosporium haptotylum (Drechsler) Xing-Z. Liu & K.-Q. Zhang	aknr	CBS 200.50, CCRC 32852	AJ001990	–	U51957^{S}	UK, England
(= Dactylella candida)	"	CBS 584.92^{S}, CCRC 33299	–	–	U51963^{S}	Netherlands
"	"	CBS 325.94 SBUG-M 1232	–	AF106523	–	Canary Islands
Monacrosporium leptosporum (Drechsler) A. Rubner (= *Dactylella dasguptae*)	ak ('aknr')	CBS 560.92^{ET}, SBUG-M 1252	–	AF106529	–	USA
Monacrosporium lobatum (Dudd.) A. Rubner	ak	CBS 329.94^{ET}, SBUG-M 1226	–	AF106524	–	Germany
Monacrosporium mammillatum (S. M. Dixon) R. C. Cooke & C. H. Dickinson (= *Dactylella lysipaga*)	ak	CBS 486.63, CCRC 32855	–	–	U51959^{S}	Canada, Ontario
Monacrosporium phymatopagum (Drechsler) Subram.	uk	CCRC 32875	–	–	U51970	Taiwan
Monacrosporium polybrochum (Drechsler) Subram.(= *Dactylella polybrocha*)	cr	CBS 319.56, CCRC 32872	–	–	U51973^{S}	USA, VA

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Monacrosporium psychrophilum (Drechsler) R. C. Cooke & C. H. Dickinson	an	ARSEF 4813, D.H.P. 213, CBS 318.94, A.R. 938, SBUG-M 1228	–	AF106525	U72610[a,m]	Ecuador
"	"	CBS 319.94, CCRC 33301	–	–	U51977	Ecuador
"	"	'L9203'	AJ001998	–	–	Sweden
Monacrosporium sp.	an	SBUG-M 1266	–	AF106535	–	S. Africa
Monacrosporium tentaculatum A. Rubner & W. Gams *(= Laridospora appendiculata)*	ak	CBS 206.64[T]	–	AF106531	–	Hawaii
Monacrosporium thaumasium (Drechsler) de Hoog & Oorschot	an	CBS 322.94 SBUG-M 1221	–	AF106526	–	Germany
"	"	CBS 176.37, CCRC 32922	–	–	U51972	USA
"	"	CBS 770.85, CCRC 33052	–	–	U51975[n]	?
Orbilia alnea Velen.	nn	D.H.P. 91	–	–	U72600	USA, MA
"	"	D.H.P. 107	–	–	U72601	USA, ME

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Orbilia auricolor (Bloxam ex Berk.) Sacc. / *A. cladodes* var. *macroides*	an	D.H.P. 90	–	–	U72592[a]	USA, MA
Orbilia auricolor (Bloxam ex Berk.) Sacc. / *A. oligospora*	an	D.H.P. 55	U72598	–	–	USA, MA
Orbilia delicatula (P. Karst.) P. Karst. / *Dicranidion*	nn	D.H.P. 120	–	–	U72593	USA, NY
"	"	D.H.P. 108	–	–	U72595	USA, ME
"	"	D.H.P. 111	U72603	–	–	USA, ME
Orbilia fimicola Jeng & J. C. Krug/ *A. superba*	an	D.H.P. 60	–	–	U72599	USA, MA
Orbilia luteorubella (Nyl.) P. Karst.	nn	D.H.P. 146	–	–	U72607	USA, MA
"	"	D.H.P. 125	–	–	U72604	USA, MA
Orbilia spec. 'D.H.P. 130'/ *Dicranidion*	nn	D.H.P. 130	–	–	U72597	?
Orbilia spec. 'D.H.P. 204'/ *M. doedycoides*	cr	D.H.P. 204	–	–	U72596	USA, PR
Orbilia vinosa (Alb. & Schwein. : Fr.) P. Karst.	nn	CBS 917.72, CCRC 33149	–	–	U51981	?
Patinella tenebricosa Svrček/ *M. polybrochum*	cr	D.H.P. 133	–	–	U72606	USA, MA

Species (common synonym)	Trapping device	Strain numbers	GenBank accession numbers			Geographic origin of isolate
			18S rDNA	partial 18S, ITS regions & 5.8S rDNA	ITS regions & 5.8S rDNA	
Peziza badia Pers.	nn	?	L37539	–	–	?
Trichothecium roseum (Pers. : Fr.) Link	nn	ATCC 12519, CCRC 30587	–	–	U51982	?

[T] or [ET] (in column 'Strain numbers'): ex type or ex epitype culture.
[S] (in columns 'GenBank accession' or 'Strain numbers'): The GenBank accession or strain is deposited under the name listed as a synonym in the first column.
[a] Erroneous GenBank citations in Pfister (1997): U72609 quoted as 'U72608', U72592 quoted as 'U72593', and U72610 quoted as 'U72609'.
[b] U51952 and U51945 (*A. cladodes*, Liou & Tzean, 1997) are duplicate submissions of identical sequences.
[c] AJ001988 (*A. pyriformis*, Ahrén & al., 1998) erroneously cites the strain number CBS 289.82 in the GenBank submission.
[d] AJ001983 under misapplied name *A. conoides* in GenBank and Ahrén & al. (1998); reidentified at the CBS since 1985.
[e] Sequence submitted to GenBank by Liou & Tzean, but not analyzed in Liou & Tzean (1997).
[f] The ITS part of our *D. cylindrospora* sequence (CBS 325.70) was previously submitted by Liou & Tzean as an unpublished sequence (GenBank accession U51953); our sequence differs at one base position.
[g] The 18S part of our *D. oxyspora* sequence (CBS 497.92) was already published by Ahrén & al. (1998); our sequence differs at one base position.
[h] The ITS region of the *D. flagrans* CBS 565.50 sequence (U51961, Liou & Tzean, 1997) differs only at two base positions from the ITS region of *D. flagrans* CBS 583.91 (AF106520, this study) and was therefore not included in the neighbor-joining analyses.
[j] AJ001991 (*D. flagrans*, Ahrén & al., 1998) is a reverse complement sequence, without being indicated as such. It is otherwise identical with AJ001895 submitted by the same authors.
[k] AJ001994 (*M. doedycoides*, Ahrén & al., 1998) erroneously cites the strain number CBS 233.54 in the GenBank submission.
[m] The ITS part of our *M. psychrophilum* sequence (AF106525, CBS 318.94) was previously submitted by Pfister (1997, GenBank accession U72610); our sequence differs at one base position.
[n] U51975 in GenBank under misapplied name *M. eudermatum*, compare Rubner (1996).