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Castor, Pollux and life histories of fungi[1]

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Abstract: The literature on teleomorph-anamorph connections in the Orbiliaceae and the position of the family in the Leotiales is reviewed. 18S data show that the Orbiliaceae occupies an isolated position in relationship to the other members of the Leotiales which have so far been studied. The following form genera have been studied in cultures derived from ascospores of Orbiliaceae: *Anguillospora, Arthrobotrys, Dactylella, Dicranidion, Helicoon, Monacrosporium, Trinacrium* and conidial types that are referred to as being *Idriella*-like. Characteristics of the anamorphs are discussed and illustrated. Analyses of the ITS region of several of the isolates indicate that there are several well-supported clades within the Orbiliaceae. These clades can be recognized based on the anamorphs produced. They are: an *Arthrobotrys-Monacrosporium* clade, a *Dicranidion* clade, and a *Helicoon* clade. Outside of these clades is a well-supported clade which contains two *Arthrobotrys* isolates which were derived from conidia produced on natural substrates. The taxonomic and phylogenetic implications of this information are discussed. The Orbiliaceae occur in nature on substrates that are either continually wet or on substrates that periodically dry out. Field observations indicate that those taxa which occur on wet substrates produce perennial mycelia. Some discussion is provided on the way in which scientific information is viewed and can be used.

Key Words: anamorphs, ecology, nematophagous, Orbiliaceae

INTRODUCTION AND HISTORY

Perhaps I should say at the beginning that not everyone believed that such an endeavor as this, a presidential address, was reasonable. Roland Thaxter said of presidential addresses that they were a "foolish requirement and absurd practice" (quoted from Farr, 1982). Nonetheless we have been indulging in this ritual since the beginning when William H. Weston (1933) gave the first presidential address. His topic? Roland Thaxter of course. I want to take the opportunity to talk about the life histories of fungi and especially those we have worked out in the family Orbiliaceae. As a way to focus on the concepts of life histories, I invoke a parable of sorts.

The ancient story of Castor and Pollux, the Dioscuri, goes something like this: They were twin sons of Zeus, arising from the same egg. They carried out many heroic exploits. They were inseparable in life but each developed special individual skills. Castor was renowned for taming and managing horses; Pollux was a boxer. Castor was killed and went to the world below. Pollux was inconsolable. Jupiter, seeing the situation allowed them to live alternately in the world. They became the patron deities of mariners, voyagers, soldiers and the lambent flames.

My topic today centers around other Greek twins—teleomorphs and anamorphs of pleomorphic fungi (for discussions see Hennebert and Weresub, 1979; Weresub and Hennebert, 1979; Weresub 1979; and Weresub and Pirozynski, 1979). I realize that the use of these terms has been called into question and attempts have been made to refine or eliminate them; see for example, *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics* (Reynolds and Taylor, 1993) and the most recent *Ainsworth and Bisby's dictionary of fungi* (Hawksworth et al., 1995). Nonetheless I will use them, not as part of a scheme of naming but in a descriptive sense as a way to organize and discuss life histories and relationships among pleomorphic fungi—phases arising from alternate expressions of the same DNA—arising as it were from the same egg. At the very least the terms impart an organization for the discussion of the biology of fungi (Hennebert and Weresub, 1979). In these days of sensitivity to the nuances of words and meanings they might be challenged. "Teleo-" implies completion or as defined by Hennebert and Weresub (1979) "having perfect achievement, being complete, adult." It gives perhaps the false sense that fructification is always the end result of fungal life cycles or that teleomorphs represent perfection in some evolutionary way. "Ana-" implies movement toward completion or repetition. It also has another

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Donald H. Pfister, President, Mycological Society of America, 1995–1996.

definition unrelated to fungi—producing or having different magnifications of an image in each of two perpendicular directions, that is, a distorted optical image. In the ascomycetes we teach life cycles as spirals through time of a repeating mitotic phase and meiotic phases. Controversy centers on the naming of these phases. With the advent of technologies that allow the assignment of exclusively mitotically reproducing taxa to positions among their ascus-bearing twins, the entire system of recognizing anamorphic taxa has been called into question.

Our research on the Orbiliaceae can be taken as both a cautionary tale about assumptions made about teleomorph-anamorph connections and for the need to study fungal biology and ecology along with laboratory and computer work. It also shows that interesting fungi are not far from home. At the beginning it might be well to look back at the beginning of the study of pleomorphic fungi. The history of these concepts is documented in a number of sources which do not need review here (see Reynolds and Taylor, 1993 and the various articles by Weresub and Hennebert already cited above).

The concept of these connections is traced to Tulasne in a series of papers and ultimately the *Selecta fungorum carpologia.* Pleomorphism was observed and named by Tulasne. It was essentially a concept about the biology of fungi. De Bary (Bary, 1854), who showed a connection between *Aspergillus glaucus* and *Eurotium herbariorum,* gave credit to Tulasne and commented in his text book (Bary, 1887) on subsequent developments: "The researches of Tulasne first led gradually to an understanding of the real condition of things to which he gave the name of pleomorphism, and to him we are chiefly indebted for the distinguishing and naming of the possible forms in the development of a species. These researches rested on the broad foundation of the comparative observation of numerous forms, of their cohabitation, of their anatomical connection, and their succession in time. Pursued in this way they arrived on the whole at the truth, and it is a small diminution of their merits that they should have given rise to some erroneous views on special points, or that they occasionally made a too extensive application of *schemes* drawn from a number of observations. This latter proceeding led indeed to more important mistakes in the hands of some less careful followers." The early master of elucidating teleomorph and anamorph connections through cultural studies was Julius Oscar Brefeld. In his magnificent series variously titled *Botanische Untersuchungen über Schimmelpilze* and *Untersuchungen aus dem Gesammtgebiete der Mykologie* he described and illustrated the results he obtained growing fungi in culture. I remind my students that the "high tech" boom of the 1870s and 1880s that so changed mycology, revolved around petri plates, cotton plugs and tindalization (discontinuous sterilization) and that Brefeld was at the center of the revolution. Of course today it is more or less routine practice to grow the fungi we are studying. We try to assign the twins names and to draw conclusions about the relationships of one by studying the other.

I will turn now to the Orbiliaceae and to specific examples of teleomorph-anamorph connections. The Orbiliaceae is currently under taxonomic revision by H.-O. Baral. It has been considered a family of somewhat isolated position in the Leotiales or, as it is sometimes called, the Helotiales (Nannfeldt, 1932), and it has been suggested that it might be placed with lichenized taxa (Benny et al., 1978; Sherwood in the *Dictionary of Fungi* (Hawksworth et al., 1995)). There are several genera that have been recognized in the literature based on ascocarp color and shape and on the shape and size of ascospores and paraphyses. Most of the taxa that I refer to in this treatment are in the genus *Orbilia* but when it becomes available,

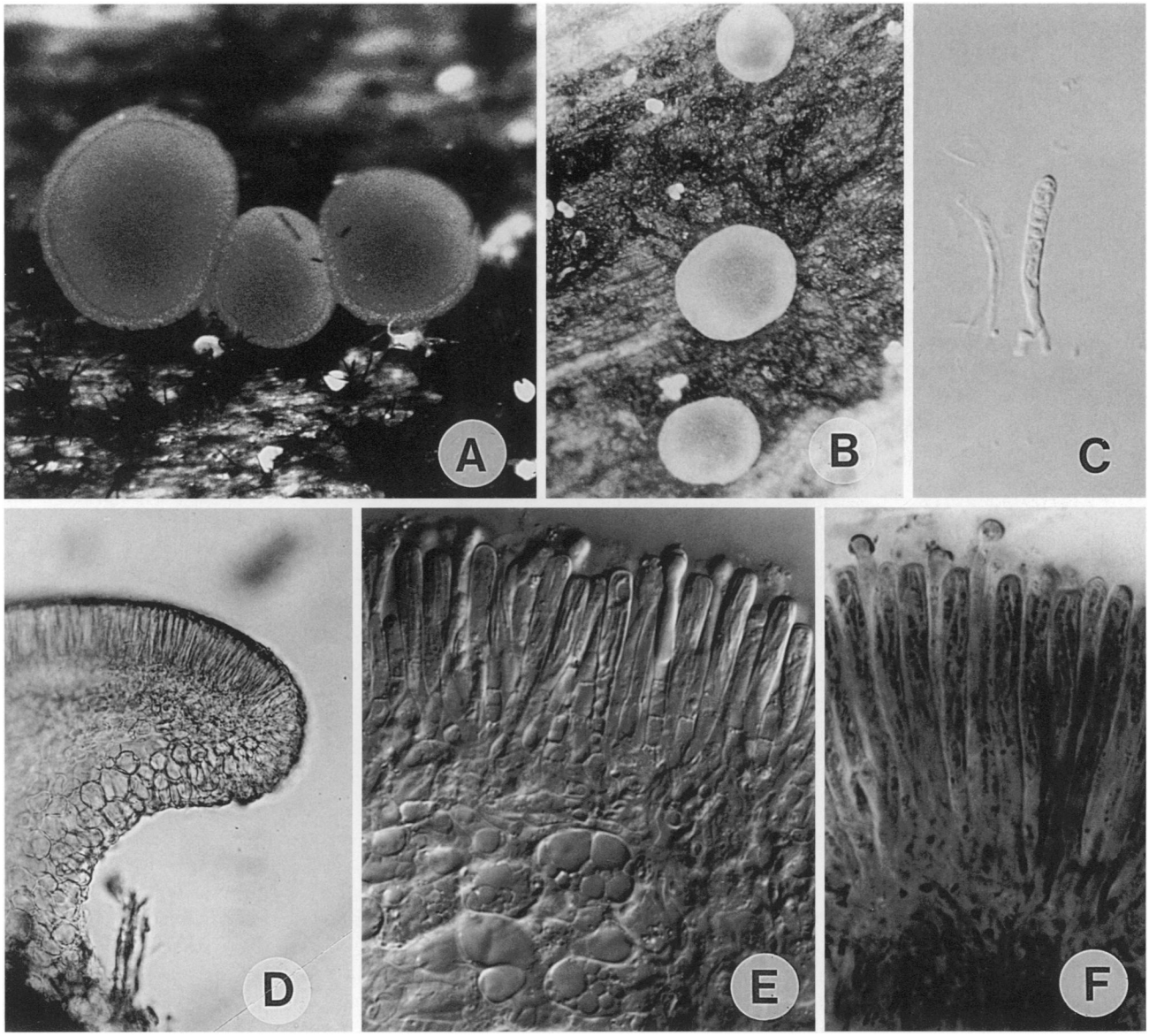

FIG. 1. Some general features of species of *Orbilia*. A. Apothecia of *O. delicatula*, × 70. B. Ascomata of *O. luteorubella*, × 40. C. Ascus of *O. delicatula*, × 1000. D. Cross-section of *O. luteorubella*, × 400. E. Portion of hymenium and excipulum of *O. luteorubella*, × 400. F. Portion of hymenium of *O. fimicola*, × 1000. Microscopic preparation in water except F which is stained in Congo Red in ammonia and is mounted in glycerin.

Baral's monograph should be consulted for proper teleomorphic taxonomy; in the meantime his paper (Baral, 1994) and unpublished poster presentations have informed me and other mycologists of new alignments of the taxa. His monograph will supersede the work of Svrček (1954). Regional treatments have been produced by Korf (1992) and Spooner (1987). The family is a large one. A recent count of epithets used in *Orbilia* and related genera yielded 266.

As a student and collector of discomycetes I admit that *Orbilia* has always seemed a miserable little genus—a kind of weed of the woods. As a graduate student I recall that Dick Korf tried to get students interested in the family, to no avail. Traditional generic names used in the family are *Orbilia, Hyalinia, Habrostictis, Orbiliaster, Patinella* and others that will certainly change in Baral's monograph. The family is traditionally characterized by its small apothecial ascomata (FIG. 1A, B), rarely more than 3 mm in diameter, they often occur in troops and are variously colored ranging from white, beige, yellow, orange, or red to vinaceous and dark brown. Color variation is common within collections of the same taxon. As-

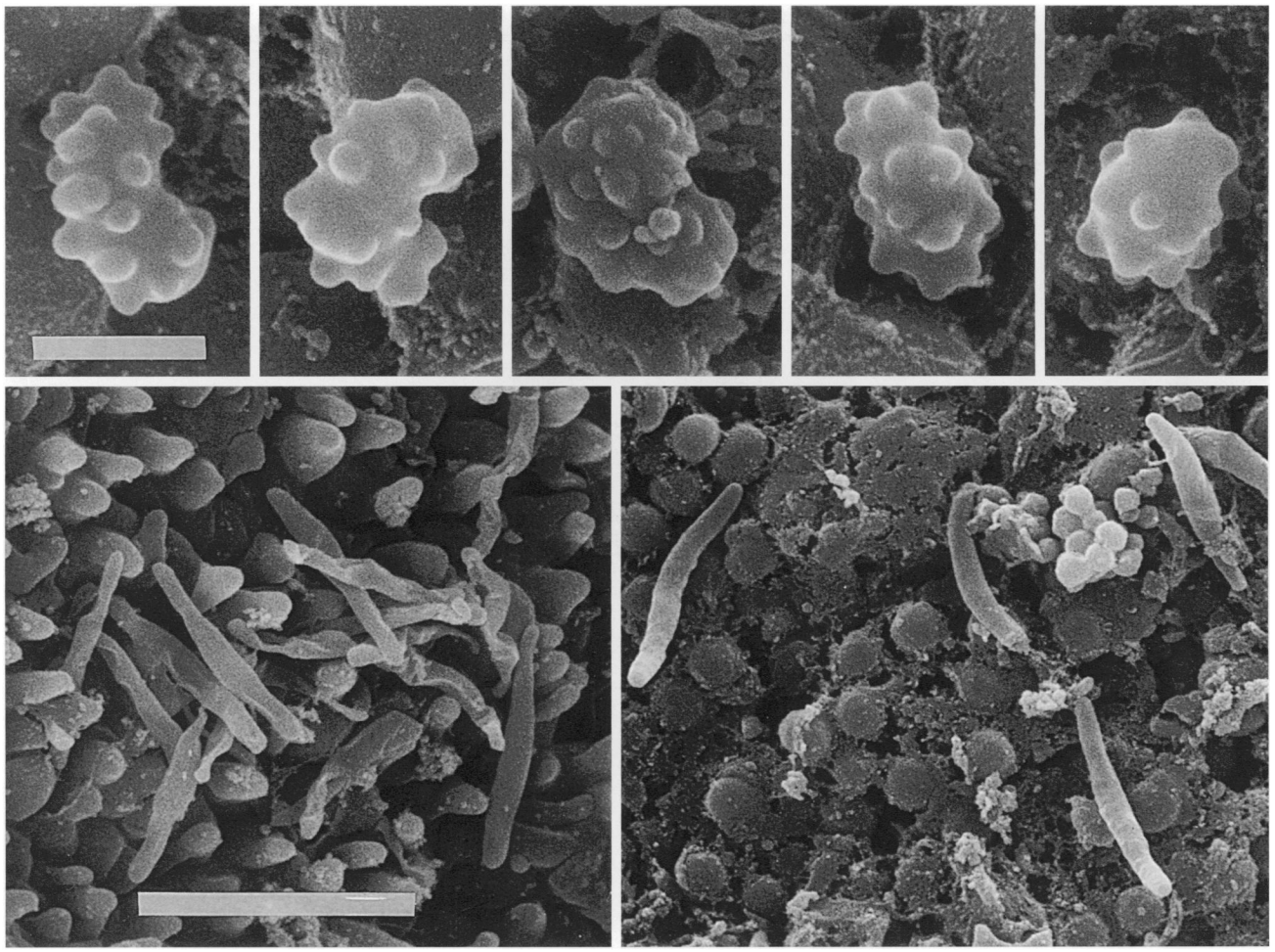

FIG. 2. SEM photographs of ascospores. Top row, ascospores of *O. delicatula,* scale bar = approximately 1.5 μm. Bottom row, ascospores and ascus tips of *Orbilia* sp. (DHP PR 116), scale bar = approximately 10 μm.

comata have a "waxy" appearance. Many species produce small asci and ascospores (asci often hover around 40 μm and ascospores range from 2.5 × 1 to 12 × 2 μm, FIG. 2). Baral and Marson (pers. comm.) have among their unpublished taxa species with much larger asci and ascospores. Asci are uncolored in iodine with an apex frequently truncate and thin-walled. Certain common taxa have distinctive croziers which produce forked bases (FIG. 1C). The excipulum is composed of hyaline or lightly colored, generally thin-walled, globose cells (FIG. 1D) or of thin-walled hyphae running parallel to the outer surface of the ascomata. Hairs are sometimes present and are sometimes encrusted by an amorphous refractive exudate which also frequently covers the whole exterior of the ascocarp. Ascomatal cells often contain inclusions that dissolve in KOH reported by Baral (1994) and used as a character by him. Ascospores are, with one exception (*O. delicatula*), smooth-walled, and in shape they may be nearly globose, allantoid, fusoid, or sigmoid (FIG. 2). They contain a membrane-bound "organelle" or spore body (Benny et al., 1978) that is seen under oil immersion in living spores, disappears in KOH, and selectively stains bright blue in aqueous brilliant cresyl blue (Baral, 1994) and Baral and Marson (pers. comm.). According to Benny et al. (1978) the spore body is derived from a mitochondrion and surrounding rough ER. The paraphyses are often swollen apically; they and the asci are sometimes tenaciously held together by material in the hymenium (FIG. 1E, F). They are found on dead wood and bark, either wet on the ground or hanging in trees, on old fungus sporocarps, on herbaceous stems and culms, on dung (Jeng and Krug, 1977), or on soil (Raitviir and Faizova, 1983).

Perhaps because of their diminutive size, or perhaps because of difficult taxonomy there never has been much interest in cultivating members of this family. Once germination has taken place, growth is

TABLE I. Isolates used in this study

DHP isolate number	Anamorph identification	Collection information	GenBank accession number
55	*Arthrobotrys oligospora*	derived from *O. auricolor,* MA	U72598
60	*A. superba*	derived from *O. fimicola,* MA	U72599
90	*A. cladodes* var. *macroides*	derived from *O. auricolor,* MA	U72593
91	*Dicranidion* sp.	derived from *O. alnea,* MA	U72600
100	*Arthrobotrys* sp.	conidial isolate from horse dung, MA	U72594
107	*Dactylella* sp.	derived from *O.* ? *alnea,* ME	U72601
108	*Dicranidion* sp.	derived from *O. delicatula,* ME	U72595
109	*Arthrobotrys* sp.	conidial isolate from seaweed, ME	U72602
111	*Dicranidion* sp.	derived from *O. delicatula,* ME	U72603
120	*Dicranidion* sp.	derived from *O. delicatula,* NY	U72593
125	non-sporulating	derived from "*O. luteorubella* type," MA	U72604
129	*Helicoon sessile*	conidial isolate from wood, MA	U72605
130	*Dicranidion* sp.	derived from *Orbilia* sp.	U72597
133	*Monacrosporium polybrochum*	derived from *Patinella tenebricosa,* MA	U72606
146	non-sporulating	derived from "*O. luteorubella,* " MA	U72607
204	*M.* ? *doedycoides*	derived from *Orbilia* sp., PR	U72596
209	*Gelatinopulvinella astraeicola*	SANK 14594	U72611
211	*Monacrosporium* sp.	ARSEF 4809, mistakenly as *A. amerospora*	U72611
212	*A. brochopaga*	ARSEF 4815	U72608
213	*M. psychrophilum*	ARSEF 4813	U72609

usually reasonably rapid though with certain taxa the cultures are short lived. The ascospores of some species, particularly *O. inflatula* and those near it, have not yet been coaxed into germination.

In 1994 I obtained a conidial fungus of the form genus *Arthrobotrys* from ascospores of an *Orbilia* species on dung, which I identified as *O. fimicola* Jeng and Krug. There had been no verified reports of a teleomorph for this well-studied and documented nematode-trapping genus. Always something of a mystery, a possible teleomorph was observed and commented upon by Drechsler (1937) for *Arthrobotrys superba.* This, a small inoperculate discomycete, turned up but once in old cultures and never completely matured but judging from Drechsler's detailed description and his illustration, it is indeed referable to *Orbilia.* Later Zachariah (1983) obtained immature ascomata of a putative *Orbilia* in cultures of *Arthrobotrys dactyloides.*

In the literature the anamorph of record for *Orbilia* species is the staurousporous genus *Dicranidion.* It was first isolated from an *Orbilia* by none other than Brefeld (1891). Others had confirmed his findings (Berthet, 1964; Korf, 1992).

Other connections had been at least hinted at through cohabitation of teleomorphs and anamorphs. Müggenburg (1878) described conidia accompanying *Orbilia leucostigma.* These he described as 24–25 μm long and 6 μm broad, spindle to club-shaped with from 1–5 septa. This remains a questionable record and a name of questionable application. Kirschstein (1938) described a new genus, *Orbiliella,* for a subiculate species. The subiculum produced conidia which were referred to in the hyphomycete genus *Trichothecium,* near *T. roseum.* The use of the name *Trichothecium* for nematode-trapping taxa was introduced by Drechsler (1937). *Orbilia* was also implicated as the teleomorph of *Dactylella rhopalota* by Thakur and Zachariah (1989).

The link between *Arthrobotrys* and *Orbilia* and the established connection between *Dicranidion* and *Orbilia* led to a major effort to establish cultures of species of *Orbilia.* It is this work that I report on now along with the presentation of our preliminary analysis of the 18S region of several taxa and the ITS region of the small subunit of rDNA for several of the isolates.

MATERIALS AND METHODS

Culture methods.—Ascospore deposits were obtained from fresh ascomata which were suspended over petri plates. The standard medium used was malt extract yeast extract agar (MEYE). The protocol used is that outlined in Pfister and Liftik (1995). Nematodes used in this study were *Cephalobus* sp. (Carolina Biological Supply K3-L278) maintained on sterile potato plugs.

The isolates used in this study are listed in TABLE I with culture numbers (DHP) and GenBank acces-

sion numbers. Voucher specimens are deposited in the Farlow Herbarium (FH).

Lyophilized mycelia were the source of genomic DNA. For DNA isolation, plugs of mycelial cultures (either from single ascospores or groups of ascospores) were grown in liquid cultures of MEYE broth at room temperature in 250 mL flasks under ambient light for at least three weeks.

DNA extraction, amplification, and sequencing.—Common abbreviations for reagents, solutions and standard measurements follow Ausubel et. al. (1992). Lyophilized mycelia were first ground with a pestle in either a porcelain mortar or a 1.5-mL microcentrifuge tube, then suspended in 600–800 μL of lysis buffer (200 mM Tris/HCl, pH 8.5; 250 mM NaCl; 25 mM Na_2 EDTA, pH 8.0; 0.5% SDS), gently vortexed (Vortex-Genie 2, Scientific Products, McGraw Park, IL) for 10 s before heating in a heat block (VWR Products) or water bath (Fisher Water bath) at 60 C 1 h. This solution was then centrifuged (IEC Micromax microfuge, 14 000 rpm) for five min. The supernatant was transferred to a new 1.5-mL microcentrifuge tube and extracted twice with equal volumes of 25:24:1 phenol/chloroform/isoamyl alcohol. After each extraction the supernatant was transferred to a new 1.5-mL microcentrifuge tube. Protocol from the GENECLEAN II Kit (BIO101, Vista, CA) was then followed, beginning with the step of adding three volumes of NaI to the supernatant from the second extraction. The time on crushed ice was extended to 30 min. The eluted DNA was suspended in 400 μL distilled deionized water and used directly for amplification. The double-stranded amplified products from the PCR reactions were separated electrophoretically in 0.8% agarose gels in TBE. The DNA fragment from the agarose gel was further purified (QIAquick gel extraction kit, QIAGEN Inc., Chatsworth, CA 91311) before quantification. The DNA concentration was estimated with a low DNA mass ladder (GibcoBRL, Life Technologies, Gaithersburg, MD) before use as a template in the PCR reactions. Ten to 100 fmoles of DNA were used as template for sequencing reactions based on the brightness of the band when visualized with ethidium bromide under ultraviolet light. The cycle sequencer used for both amplification and sequencing reactions was Perkin Elmer Cetus' 9600 GeneAmp PCR Instrument system (Perkin Elmer, Norwalk, CT). The ITS regions (ITS1 and ITS2, White et al., 1990), including the intervening 5.8S rRNA gene were amplified with the standard primers ITS4 and ITS5. The 18S regions were amplified according to O'Donnell et al. (1996). The primers were either made by Operon (Alameda, CA) or provided by K. O'Donnell. Twenty-eight cycles of the following thermal program were used for the 50/100 μL reactions (plus control reactions): 30 s at 95 C, 30 s at 55 C, 4 min at 72 C, then hold at 72 C for 7 min.

Primary PCR products were used directly for the sequencing procedures (Perkin Elmer's ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit, Norwalk, CT). Sequencing reaction products were first cleaned (ethanol precipitation protocol 1, Perkin Elmer's ABI PRISM Dye Terminator Cycle Sequencing) then electrophoresed in either 6% (Long Ranger Gelling Solution, FMC, Rockland, ME) or 5.7% (SequaGel-6, National Diagnostics, Atlanta, GA) acrylamide gels on an automatic sequencer (ABI DNA sequencer 370A). The gels were then analysed and the sequences edited using SeqED (v1.03).

Sequence data entry and phylogenetic analyses.—After editing, the sequence gels were aligned using MALIGN version 1.01 (Wheeler and Gladstein, 1993). The sections of the ITS and 18S regions were aligned using the "pair" and "build" options with the following parameters: internal gap cost 9, extra gaps cost 8, change cost 5, leading gap cost 6, and trailing gap cost 4. Cladistic analyses using unweighted parsimony were performed with the programs NONA (ver 1.16, Goloboff, 1993) and Paup (Swofford, 1993). In NONA, the tree-searching command was mult*45, which, for 45 replicates searches for trees using random taxon addition and TBR branch swapping. In Paup, heuristic searches were performed with 500 random taxon addition replicates and TBR branch-swapping with mulpars in effect. Phylogenetic bootstrapping (Felsenstein, 1985; 500 replicates) was implemented in PAUP (ver. 4.d48, Swofford, 1993) using simple addition sequence with TBR branch-swapping; mulpars was in effect.

RESULTS AND COMMENTARY

Placement of the Orbiliaceae.—Support for the inclusion of the Orbiliaceae within the Leotiales comes from several sources. Our isolates are fast growing and most produce various conidial anamorphs in culture. This suggests that the Orbiliaceae would be out of place in groups closely allied to lichen forming fungi. The suggestion of affinities with lichenized taxa was based in one case on the occurrence of algae in the excipulum of one taxon (Benny et al., 1978) and in the other on the similarities in apothecial anatomy and ascus morphology between *Orbilia* and the Gyalectales (see entry in *The dictionary of Fungi* (Hawksworth et al., 1995). Hyphomycetous anamorphs are rarely encountered in lichenized fungi and certainly those which have been studied do not

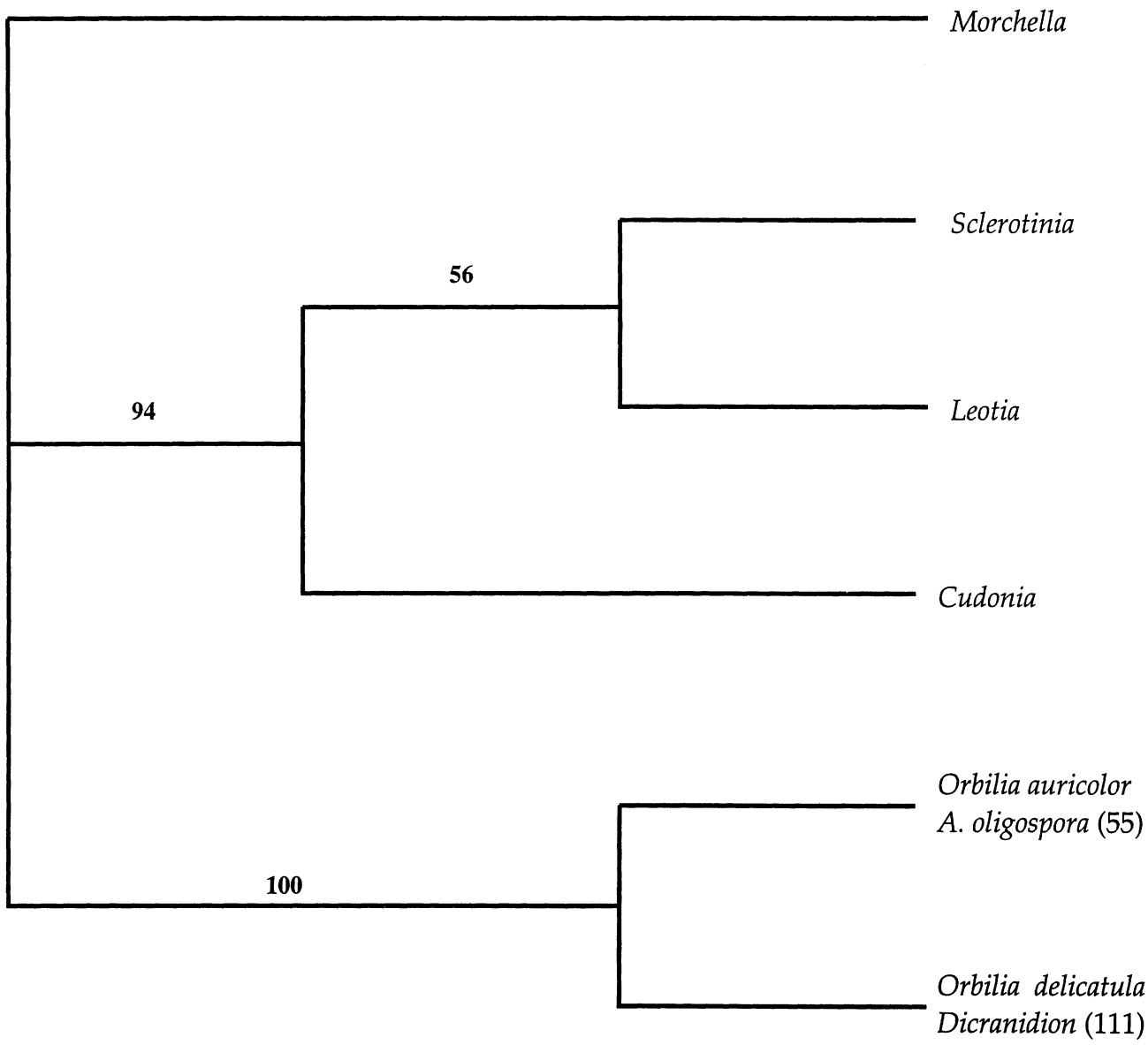

FIG. 3. Single most parsimonious tree based on 1786 aligned sites of 18S rDNA, 67 of which were phylogenetically informative (Length = 233, consistency index = .88, retention index = .65).

demonstrate the rapid growth characteristic of the isolates we have studied. As an example, in a comparative study of mycobionts of the genus *Cladonia* I (Pfister, 1996) based my observations on cultures that were two years old. These covered less than half the radius of 20 mm petri plates. None produced conidia. There is evidence for maintaining the Orbiliaceae within the Leotiales which comes from molecular data. We have to date sequenced the 18S rDNA for two species of *Orbilia*. An analysis including these two taxa and other available sequences indicate that they fall outside the paraphyletic Leotiales (FIG. 3). Landvik (1996) provided a "discomycete" tree in which some of the taxa are included that we have used in our analysis but there are few sequences available to which one can appropriately compare these sequences within the Leotiales since few of the 8 families commonly recognized have been sampled. This indicates the lack of attention that has been given to this complex order. The Orbiliaceae to date still seem distant from the Leotiaceae and the Sclerotiniaceae. Nothing else definitive can be said.

Anamorphs of some members of the Orbiliaceae.

Anguillospora Ingold FIG. 4

Species of *Anguillospora* are commonly encountered in fresh water samples. The conidia are filamentous, multiseptate, hyaline and are holoblastic.

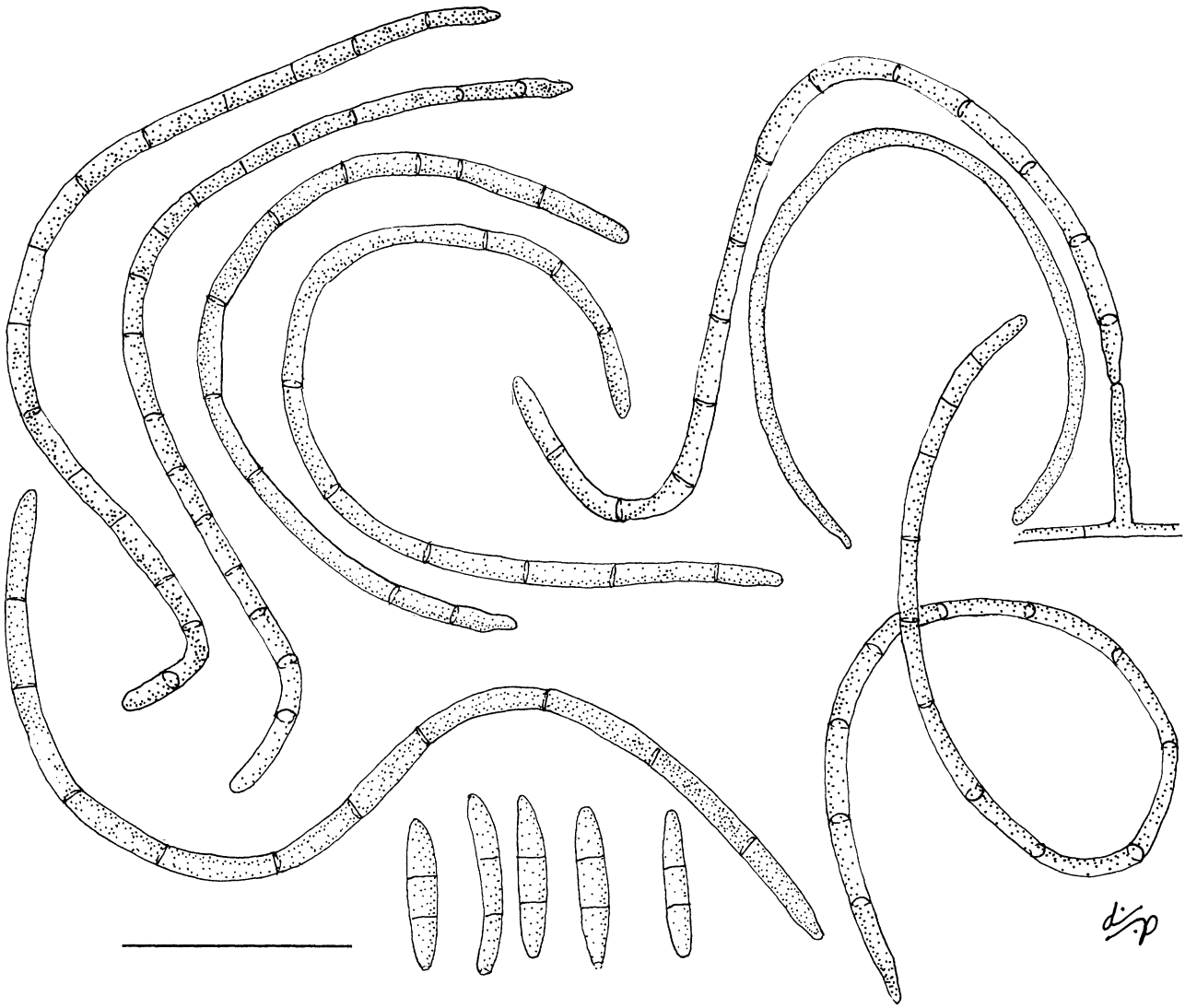

FIG. 4. *Anguillospora* sp. Macro- and microconidia illustrated from culture (isolate DHP 115), scale bar = 20 μm.

The mode of release of the conidia vary from species to species being either rhexolytic or via the fracture of the conidiogenous cell. The genus *Anguillospora* is problematic taxonomically as has already been recognized by Webster and Descals (1979). They reported an *Anguillospora* that produced an *Orbilia* in culture. This was referred to as *Anguillospora* sp. 1, later as *A. rosea* (Webster, 1992). Another *Anguillospora* was reported with a *Massaria* teleomorph (Webster and Descals, 1979) and yet another with a *Mollisia* teleomorph (Webster, 1961). All these fungi produce long, curved conidia. They are frequently found in foam samples and are perhaps the most common aquatic spore type. Such parallel morphologies in unrelated ascomycetes point to convergent evolution in aquatic or semi-aquatic habitats.

We have confirmed Webster and Descals's report, having isolated an *Anguillospora* anamorph from an *Orbilia* tentatively identified as *O. luteorubella*. This came from our collecting site in Concord, Massachusetts. Cultures yielded microconidia as did Webster and Descals's material. You will notice later that *O. luteorubella* is implicated in another connection regarding another aquatic taxon, *Helicoon*.

Arthrobotrys Corda FIG. 5

Corda (1839) described *Arthrobotrys superba* without reference to trapping. Corda's report is noteworthy because it shows that one did not and still does not need to go far from home to find interesting fungi. His material of *Arthrobotrys superba* was collected among the flower pots on the parapet outside the window of his room. Zopf (1888) first reported *Arthrobotrys* (along with other fungi) as a nematode-trapping fungus.

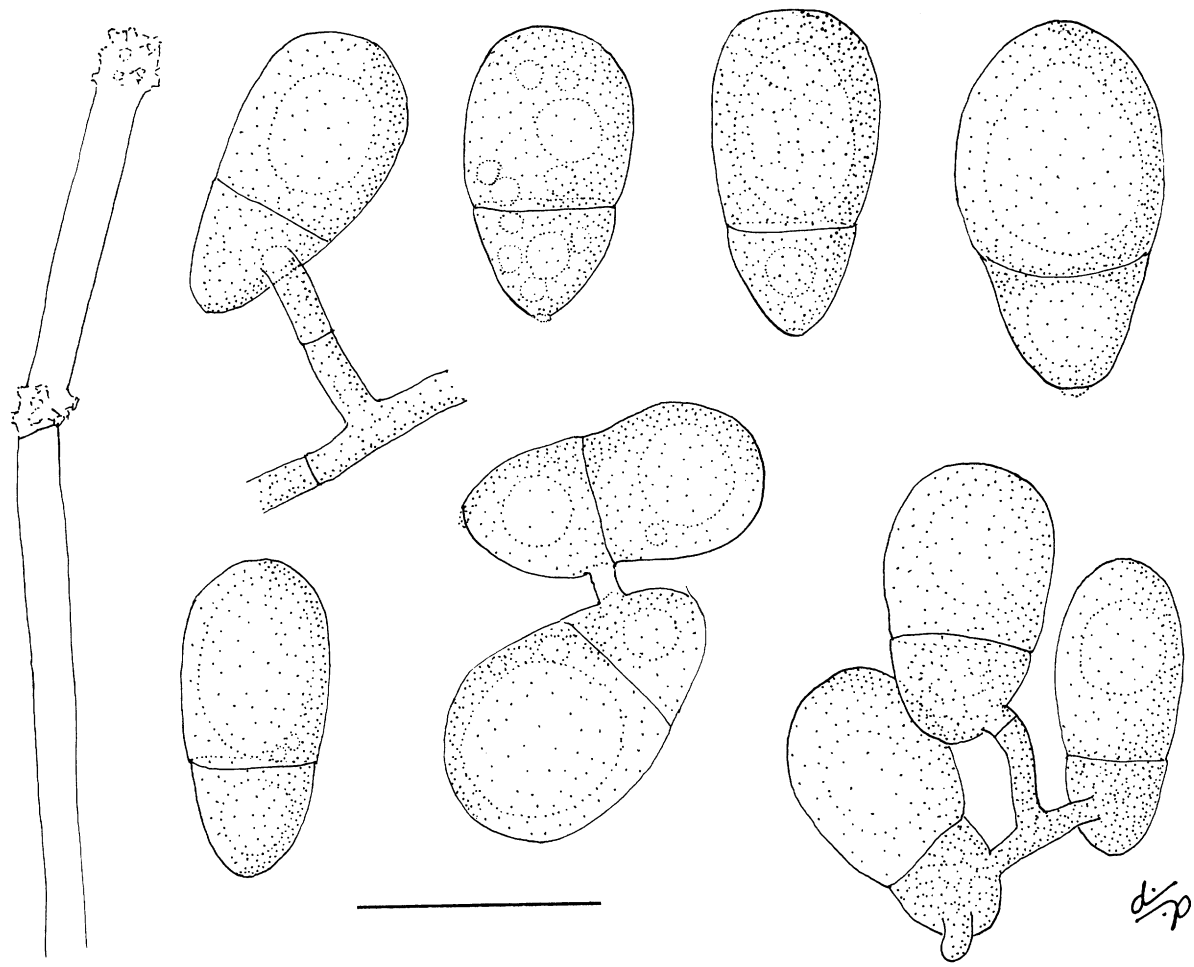

FIG. 5. *Arthrobotrys* sp. Conidia and conidiophores illustrated from culture, conidia with fusion hyphae (isolate DHP 199), scale bar = 20 μm.

In *Arthrobotrys* conidia are usually, but not exclusively, two-celled (1-celled and multicelled conidial forms have been included (Shenk et al. 1977); they are holoblastic. All species produce trapping mechanisms (Oorschot, 1985) when nematodes or in some cases other organisms, e.g. copepodes or mites, are present. Traps are of the multiloop bail-type or are single loop constricting traps.

As previously stated, Drechsler (1937) first reported a discomycete connection for *Arthrobotrys* but his report was not verified. Zachariah (1983) found ascomatal primordia in cultures of *Arthrobotrys dactyloides* which required several amino acids for growth. There are several treatments of the genus *Arthrobotrys* (Haard, 1968; Oorschot, 1985; Schenk et al., 1977). There are several generic synonyms: *Didymozoophaga, Candelabrella, Dactylariopsis, Geniculifera, Nematophagus, Woroninula.* In most recent treatments these are now considered synonyms. There are about 50 species reported.

Based on cultural studies, *Arthrobotrys* and *Orbilia* are unequivocally anamorph-teleomorph pairs. To date *Arthrobotrys* anamorphs have been isolated from those *Orbilia* species that are on moist substrates and that have curved or tear-shaped ascospores. This constitutes a group of species that have nearly indistinguishable teleomorphs. The names used for this group, *O. auricolor, O. curvatispora* and *O. fimicola,* have been variously applied and it is not clear that there will be resolution of the teleomorph taxonomy. It is unclear what teleomorphic characters can be used to distinguish taxa but it is clear that anamorphs differ.

Dactylella Grove FIG. 6

The genus was reviewed along with *Monacrosporium* by Rubner (1996). It has been the subject of much revision and restriction. In its restricted sense it is characterized as producing holoblastic multiseptate conidia which proliferate sympodially. It is not considered nematode-trapping by Rubner (1996) but

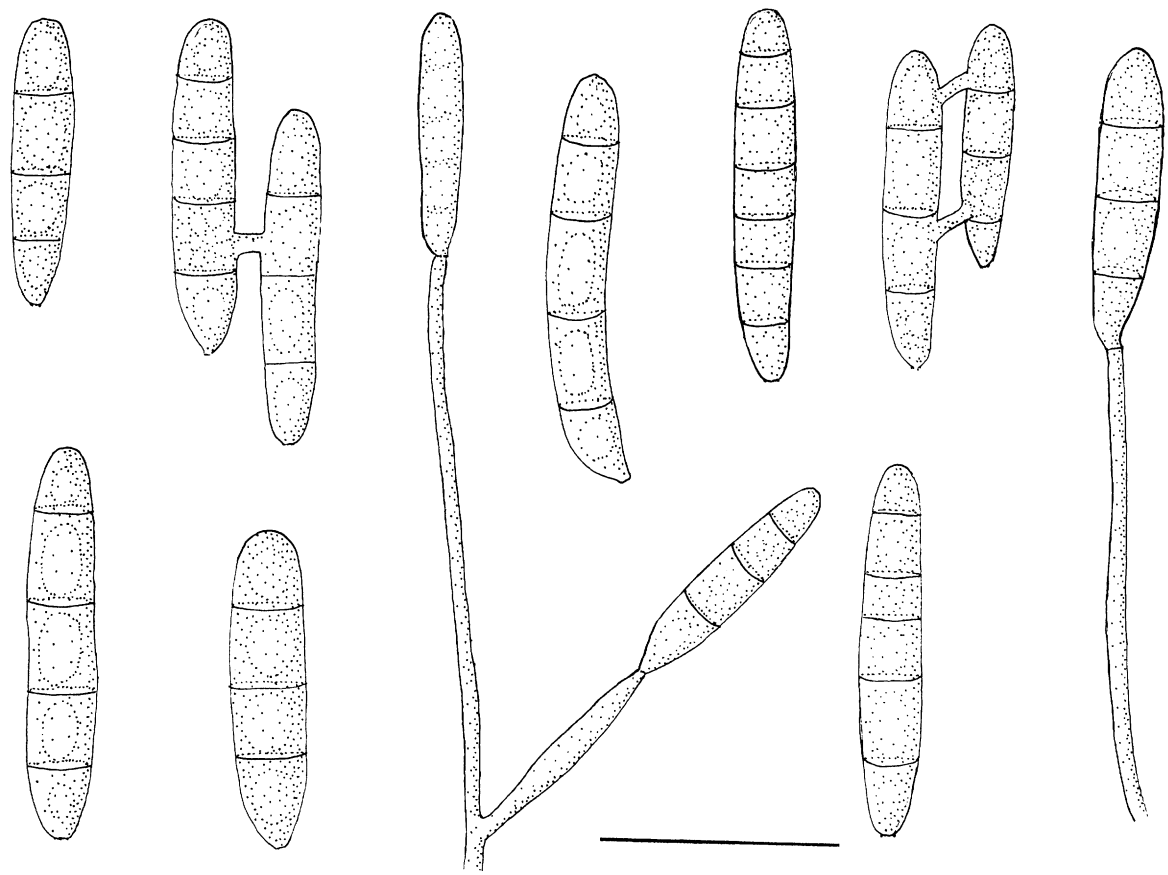

FIG. 6. *Dactylella* sp. Conidia and conidiogenous cells illustrated from culture (isolate DHP 184), scale bar = 20 μm.

is restricted by her to those taxa parasitic on oogonia of oomycetes, nematode cysts or those that are usually saprophytes. Most of the species placed in *Dactylella* by Drechsler are now treated as *Monacrosporium*. An alternative view is expressed by Zhang et al. (1994). The difficulty in assigning these fungi even to the proper class is illustrated by the amoebae-capturing *D. tylopaga* which has been shown to be a basidiomycete (Saikawa et al., 1994).

The *Orbilia* connection to *Dactylella* was reported by Thakur and Zachariah (1989). They found what was apparently a primordial *Orbilia* in cultures of *D. rhopalota*.

We have found only one *Dactylella* in our studies of cultures derived from *Orbilia* ascospores. This has been tested for its ability to trap nematodes and has failed.

Dicranidion Harkness FIG. 7, 8

Harkness (1885) described the genus which was later reviewed by Peek and Solheim (1958). The type material was from decaying wood. The genus is characterized by branched, "Y-shaped," septate, hyaline, holoblastic conidia. In culture the conidiogenous cells are scattered, sometimes aggregated, but on natural substrates are said to form sporodochia.

There are no reported trapping structures in *Dicranidion* and our tests with isolates of *Dicranidion* using nematodes resulted in no trapping. A species of the closely allied genus *Pedilospora,* often placed in the synonymy of *Dicranidion,* was isolated from decaying rootlets by Drechsler (1934). *Pedilospora dactylopaga* captures and consumes testate amoebae by means of knoblike hyphal branches. To date we have been unable to demonstrate such trapping with our isolates of *Dicranidion* to which we added the rhizopod *Diflugia.* It is likely that *Pedilospora* is indeed synonymous with *Dicranidion* and trapping capabilities will be further explored.

The genus *Dicranidion* has never been comprehensively reviewed although now the number of taxa stands at eleven due mostly to the work of Matsushima (1971, 1975, 1981, 1987, 1993, 1995) and Tubaki and coauthors (Tubaki and Yokoyama, 1971; Ando and Tubaki, 1984). Distinctions are drawn between species in conidial arm length, septation, and length of the basal cell. Species distinctions are at best difficult. Butterfield (1973) presented cultural studies which indicated that *D. inaequalis* represented a conidial variant of *D. fragile.*

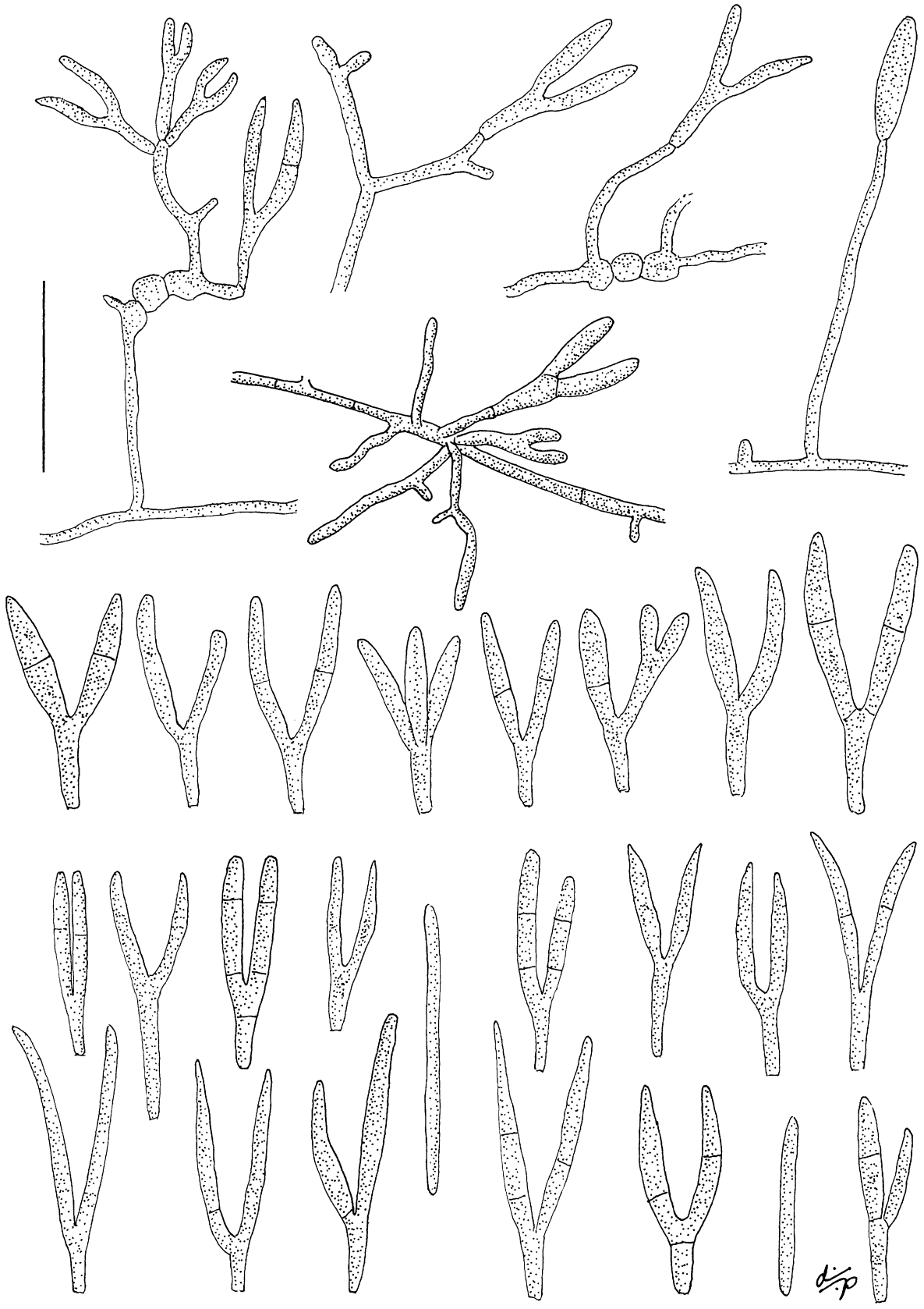

FIG. 7. *Dicranidion* sp. Conidia and conidiogenous cells of *Dicranidion* species of the gracile type from isolates of *Orbilia delicatula,* isolate DHP 111 and 105, scale bar = 20 μm.

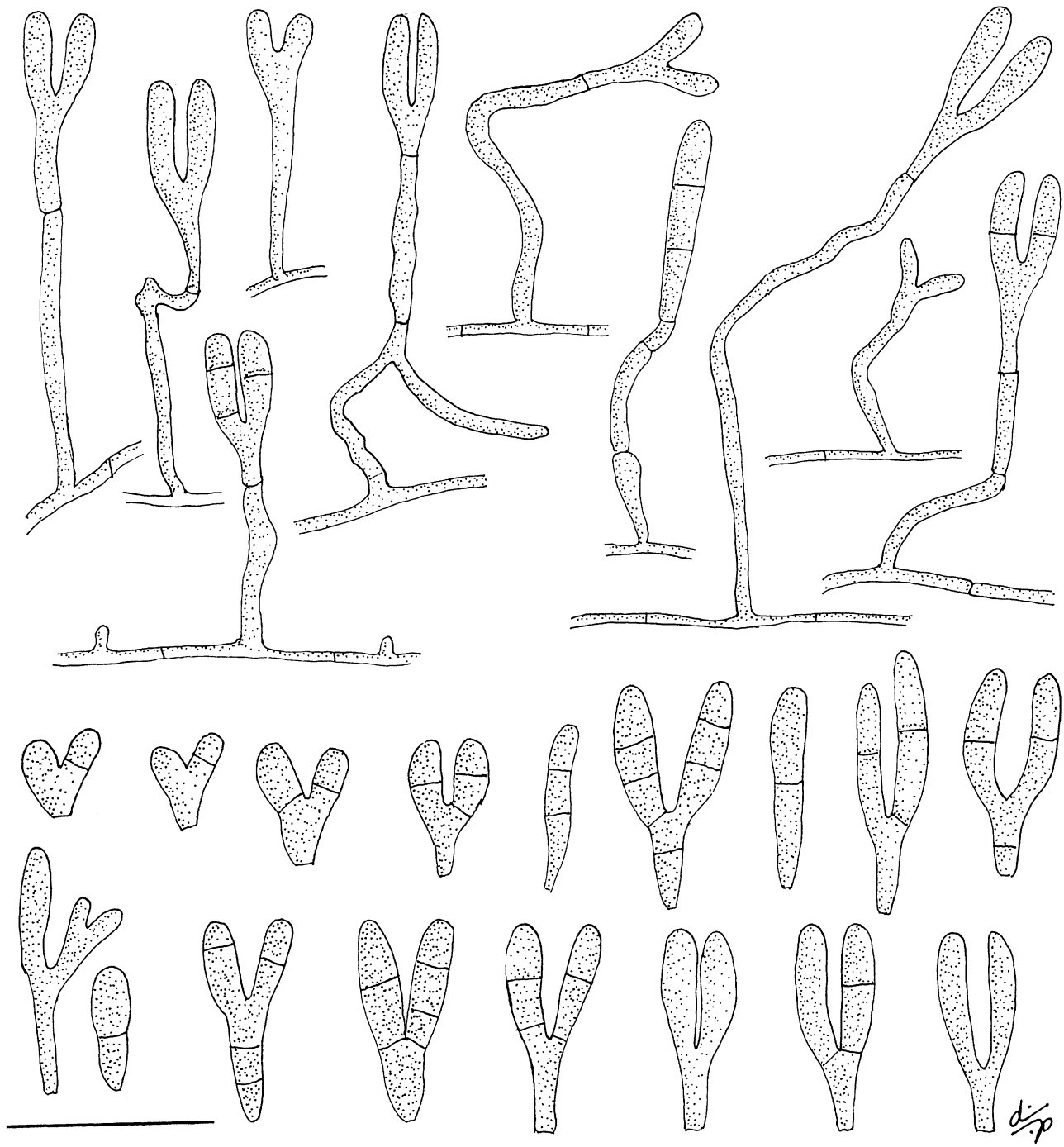

FIG. 8. *Dicranidion* sp. Conidia and conidiogenous cells of *Dicranidion* species of the fragile type from isolates of *Orbilia alnea,* isolate DHP 81, scale bar = 20 μm.

Isolates reported in the literature are mostly derived from decaying wood or palm parts. *Dicranidion fissile* was isolated from rainwater draining from intact trees (Ando and Tubaki, 1984). The spore morphology of *Dicranidion* species is suggestive of an aquatic habitat but the taxon is generally absent from reports of aquatic hyphomycetes from foam samples. An undesignated species was listed from aquatic samples by Descals and Chauvet (1992). Foam samples from our collecting sites have not contained *Dicranidion* conidia but these conidia are often found on the substrates on which certain *Orbilia* collections occur.

The first report of a *Dicranidion* derived from an *Orbilia* species was that of Brefeld (1891) which was subsequently verified by Berthet (1964). Other re-

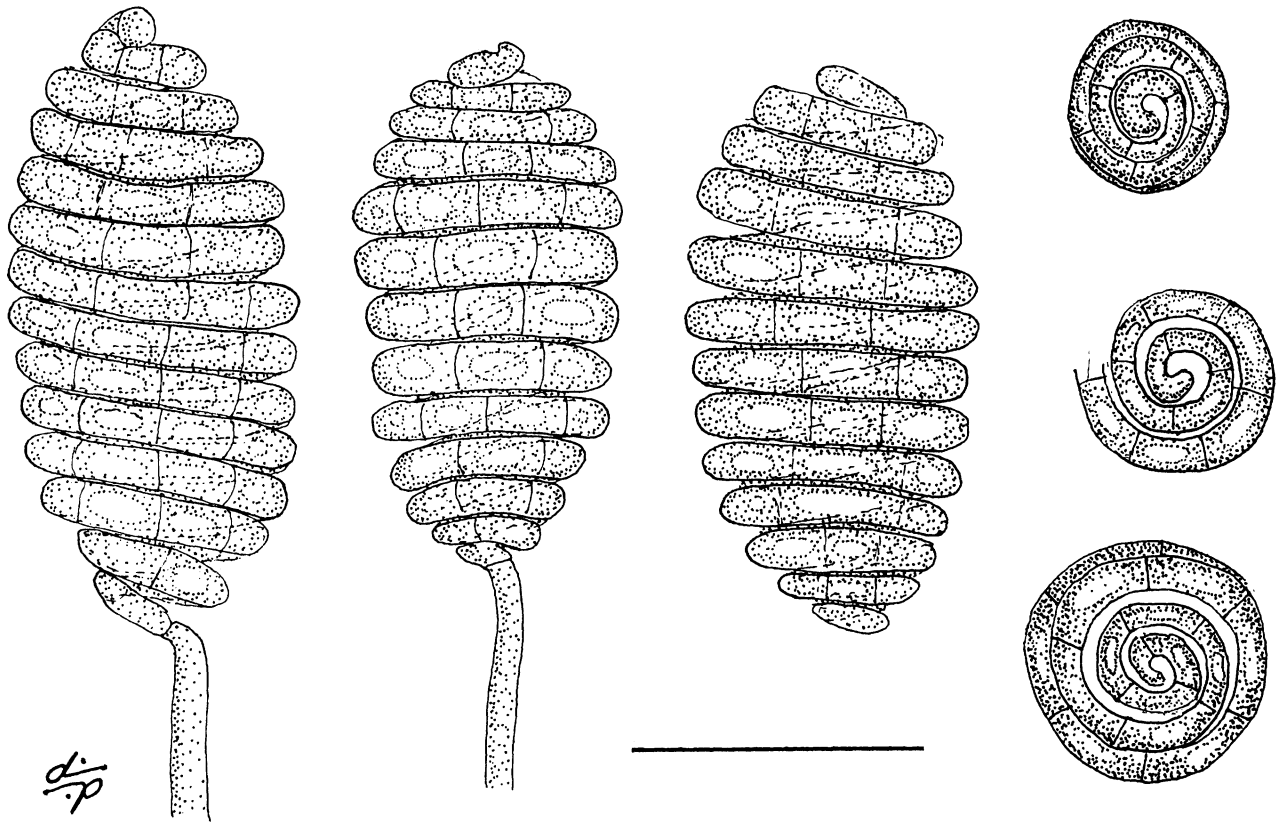

FIG. 9. *Helicoon sessile.* Conidia in various orientations (isolate DHP 79) illustrated from culture, scale bar = 20 μm.

ports of *Dicranidion* species being grown from *Orbilia* ascospores include Korf (1992), and G. Marson (Baral, pers. comm.). The identity of Brefeld's *Orbilia* is questionable but many of our isolates are derived from small orange or yellow orbilias often under the name *O. xanthostigma,* a taxon dropped in modern treatments because of confusions in the application of the name. The *O. alnea* group is also connected to *Dicranidion.*

There are two groups of species of *Dicranidion* that we have noted, which I refer to as the *fragile* (FIG. 7) and the *gracile* (FIG. 8) group. These are distinguished based on the length of the arms of the conidia and the basal cells and to some extent on the septation pattern.

Helicoon Morgan FIG. 9

At the 1995 meeting of the Mycological Society of America I reported our isolation of *Helicoon sessile* from ascospores of an *Orbilia* tentatively referred to *O. luteorubella.* Helicosporous hyphomycetes have been reported as conidial states of various ascomycetes. So far as *Helicoon* is concerned, no teleomorphs have been reported for any member of the genus. Goos et al. (1986), Goos (1987), Linder (1929), Moore (1955), van der Aa and Sampson (1994) and Webster (1992) have summarized information on *Helicoon* and teleomorphs of helicosporous aquatic taxa.

Helicoon is characterized by its multicelled, coiled holoblastic conidia that coil tightly toward the poles to provide an irregular ellipsoidal conidial body.

Helicoon sessile is a beautiful fungus when grown on MEYE. It is low growing and soon becomes distinctively pink. I have been able to establish cultures from conidia found on natural substrates and I have received a culture from Roger Goos for comparison. *Helicoon sessile* is commonly identified from aquatic samples (Shearer, 1972).

My impression is that *Helicoon* is an heterogeneous assemblage of taxa. Differences in the conidial morphology, culture growth characteristics and color of hyphae, conidia, and cultures all suggest that *Helicoon* is another genus built around species which share biologies not genealogies. The biology of such conidial types is discussed by Fisher (1977) and Michaelides and Kendrick (1982). These fungi sporulate above water but water serves to carry their spores which trap air bubbles, their growth and development may take place when the substrate is submerged. The *Orbilia* species from which cultures were derived were collected from verges of streams.

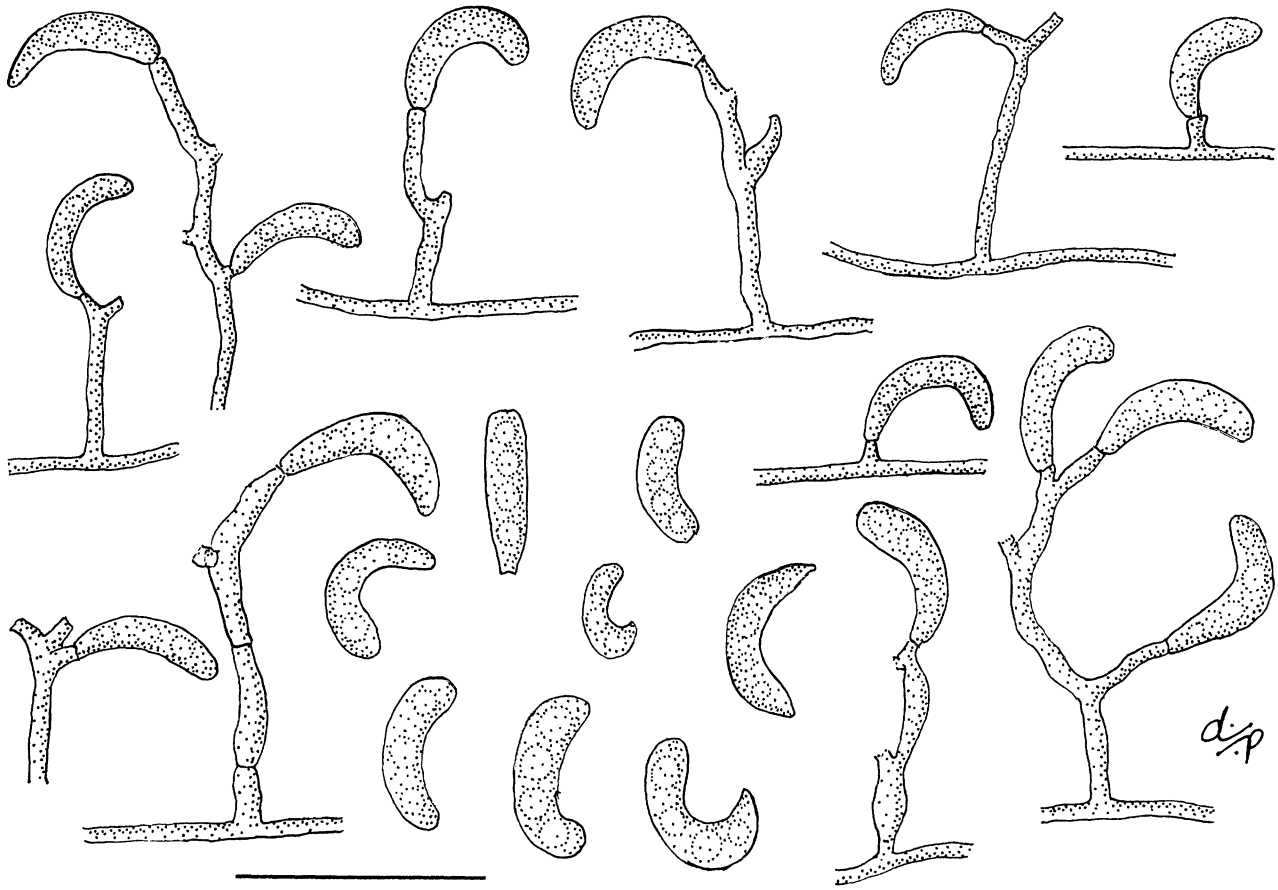

FIG. 10. Conidia, conidiophores and conidiogenous cells of the *Idriella*-like anamorph of *Orbilia piloboloides* (isolate DHP 192), scale bar = 20 μm.

Idriella-like anamorphs FIG. 10

This is a somewhat problematic anamorph. It was reported by Haines and Egger (1982) for an *Orbilia* described as *O. piloboloides*. The isolates do not fit neatly into the existing circumscription of *Idriella*. Several similar anamorphs have been derived from *Orbilia* spp. or have been found in association with them on natural substrates (Baral, pers. comm.).

The small curved conidia of these fungi have suggested to some that this fungus is *Harposporium* (Pfister, 1994) and Baral (pers. comm.), an endoparasite of nematodes. This is based on the fact that *Harposporium* species have small, curved or irregularly shaped conidia, produced from a bulbous swelling, each of which has a barb or hook on one end. Such spores become lodged in the buccal cavities or elsewhere in the alimentary tract of the nematode (Barron, 1981). The so-called *Idriella* anamorph lacks any hooks or barbs. We have added nematodes to sporulating cultures of *O. piloboloides* and have found no evidence of trap formation and there was no evidence that the fungus acted as a pathogen of any type. Nematodes remained alive and active within the sporulating culture for several weeks.

The broader biology and ecology of these fungi is unknown at this time. These anamorphs seem primarily associated with a series of drought tolerant species of *Orbilia*. It should be noted that the substrates on which these species are found are extremely complex. Algae, protozoans and a wide range of fungi are present on them or with them. That these fungi might be associated with other organisms is, of course, possible. Looking at and trying to identify some of these organisms gives even an experienced taxonomist serious doubts about the effectiveness of any biodiversity study.

Monacrosporium Oudem. FIG. 11

Three *Orbilia* species have been connected with *Monacrosporium* species. Rubner (1994; 1996) found *Orbilia auricolor* in cultures of *M. psychrophilum*. This teleomorph material was studied and identified by H. O. Baral. In our studies we have isolated and identified *M. polybrochum* from *Patinella tenebricosa* (iden-

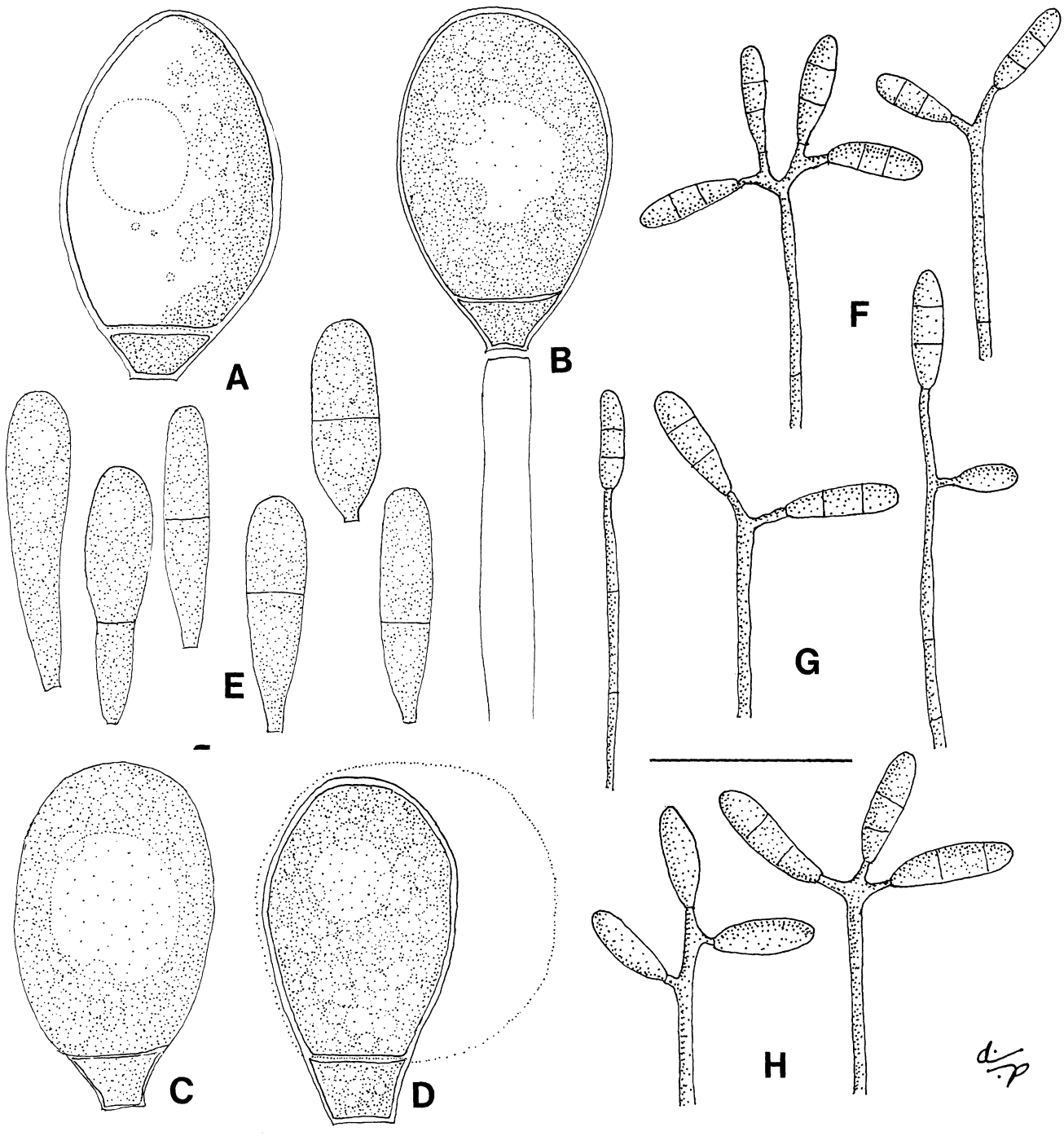

FIG. 11. *Monacrosporium polybrochum*. A–D. Macroconidia. A and D showing gelatinous sheaths surrounding the conidia. E. Microconidia. F–H. Microconidia and conidiogenous cells. Scale bar for A–D = 20 μm, scale bar for F–H = 25 μm.

tified by Baral) and we have isolated *M. doedycoides* from an *Orbilia* collection from Puerto Rico.

Monacrosporium has been recently reviewed by Liu and Zhang (1994) and Rubner (1996) with somewhat different results. All are considered nematode trappers. Conidia are holoblastic, multiseptate, often with one median cell of the conidium being larger than the others. Many of the taxa produce microconidia.

Monacrosporium polybrochum has only rarely been seen or studied in detail judging from the literature, all of which is based on comments on Drechsler's (1937) description and illustrations (see Rubner, 1996; Meckhtieva, 1964). The species has been much transferred. Drechsler had placed it in *Trichothecium*; it has also been placed in *Dactylella* and *Golovinia*. Rubner (1996) chose what she considered a some-

what problematic placement of this species in *Monacrosporium*. *Monacrosporium polybrochum,* which produces constricting traps and micro- and macroconidia, differs from other *Monacrosporium* species in two ways. First, the macroconidia are two celled—unlike most of the other species which have three or more cells. The proximal cell of the macroconidia of *M. polybrochum* is large and the basal cell relatively much smaller. Perhaps more important biologically, the macroconidia are surrounded by a thick gelatinous sheath. No other species of *Monacrosporium* has been described as having a gelatinous sheath, and indeed it is not known in other related nematode-trapping genera. These conidia are easily picked up on coverglass shards, and I presume they are arthropod dispersed in nature.

This is the only example of a possible arthropod-dispersed nematode-trapping fungus that I have been able to document, and it proves an example of the complexity in natural systems. Here we have conidia held high above the surface of the substrate. The conidia adhere to and thus are transferred to passing organisms. Perhaps the nematodes are transferred concurrently since nematodes often are seen on substrates perched on conidiophores and setae rising above the surface of a substrate.

One might expect this to be a dung-inhabiting species but the teleomorph was collected on charred wood on the ground. The full explanation of the life history of this fungus awaits further study but it is a tantalizing glance at interactions in nature: Castor and Pollux again taking their alternate moments and showing their alternate skills.

An isolate we are calling *Monacrosporium doedycoides* is also a curious fungus that produces constricting traps and produces what Drechsler (Drechsler, 1941) called conidial bodies. Rubner (1996) was unable to verify the presence of these in cultures at her disposal but they are undeniably present in our cultures. Why did Drechsler choose to call these conidial bodies rather than conidia? In our cultures we have observed their germination and would consider them to represent microconidia such as are present in other species of *Monacrosporium*. They develop in culture exactly as illustrated by Drechsler except that they are produced on longer conidiophores than he mentions.

Trinacrium Riess.

Orbilia trinacriifera was described by Matsushima (1995) as having a *Trinacrium* anamorph. The teleomorph was produced in culture. The hyaline conidia are holoblastic, branched in a "T-form," the branches are cut off from the main axis by a septum and the base is 2- or 3-septate. This anamorph appears to be very similar to *Dicranidion* except for the reflexed arms of the conidia. Species are considered saprophytes except *T. subtile* Riess which Drechsler (1938) described as parasitizing oomycetes. Tzean and Chen (1989) provide a brief synopsis of the genus.

Several other *Orbilia* species have *Trinacrium* anamorphs according to Baral (pers. comm.) and we have grown one isolate that has shown characteristics one might associate with the form genus.

Relationships within the genus Orbilia *based on ITS sequences.*—Evidence from the 18S rDNA studies indicates that at least with those species studied to date the Orbiliaceae is monophyletic. The isolates used in the ITS study were alignable and indicate again that the family is monophyletic. The unique nature of the ascospores of Orbiliaceae provides a morphological character that distinguishes this family from all other discomycetes. The inclusion of what Benny, et al. indicated was an elaborated mitochondrion or spore body is unknown in any other group of the Leotiales or any other fungus that I know; Baral (pers. comm.) confirms this view.

The outgroup for this analysis is an isolate of *Gelatinipulvinella astraeicola* which is a member of the tribe Calloriae of the family Leotiaceae (Hosoya and Otani, 1995). A culture of this fungus was kindly provided by Hosoya. This is a small discomycete with simple ascomatal anatomy, and asci are I^-. It was found in Japan on the decaying peridium of *Astraeus* and other earth stars. Other members of the tribe are parasitic on other fungi (Pfister, 1976). *Gelatinipulvinella* produces filamentous and yeastlike growth in culture and produces conidia on annellate conidiogenous cells, an exceptional character in the Leotiales.

The ITS data shows several well-supported groups within the family (FIG. 12). One is an *Arthrobotrys/Monacrosporium* clade. This clade includes taxa derived from ascospore isolates and from conidial iso-

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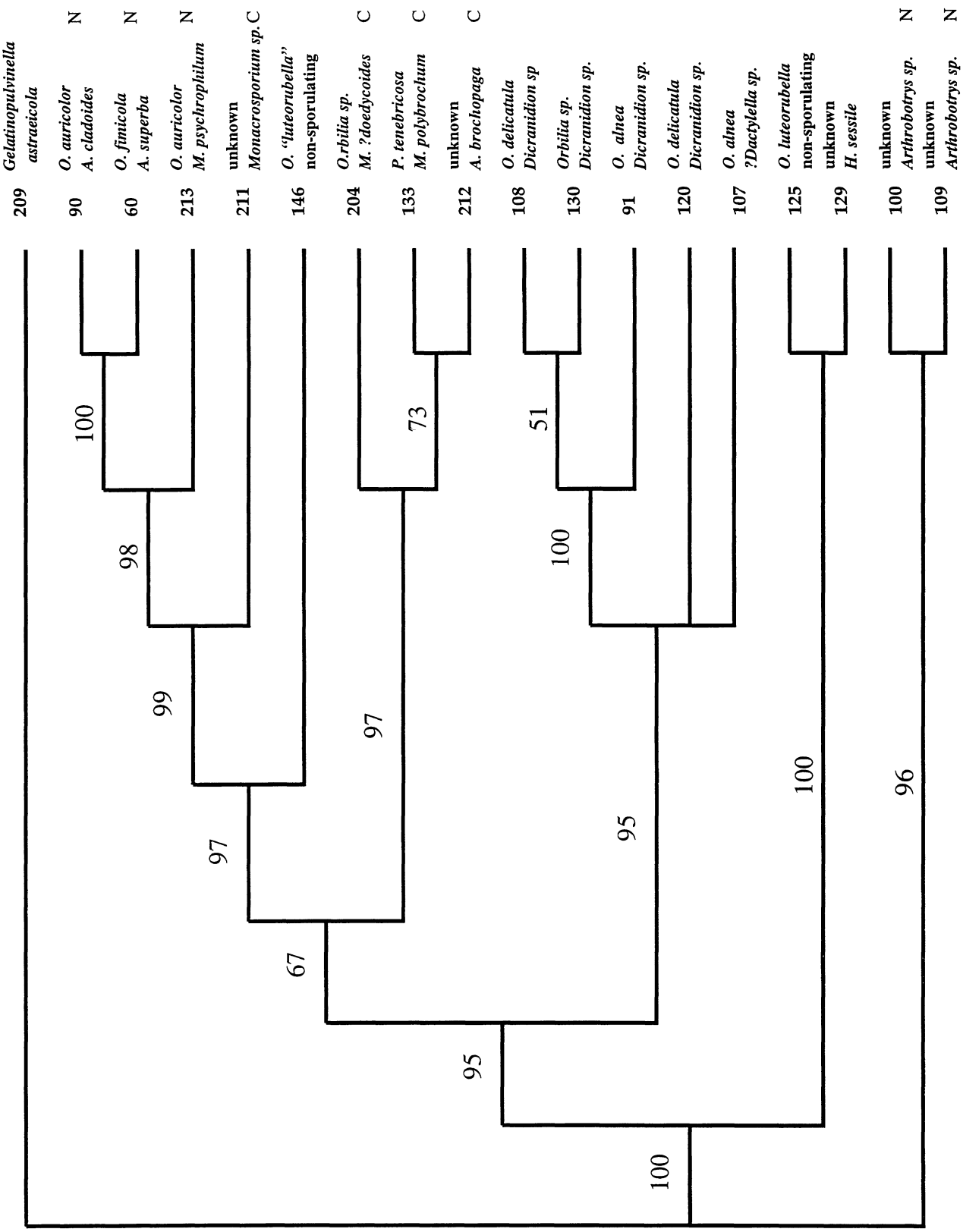
FIG. 12. Strict consensus tree for four equally parsimonious trees (length = 485, consistency index = .62, retention index = .74) based upon 384 unambiguously aligned sites from the ITS region, 159 of which were phylogenetically informative with an outgroup *Gelatinopulvinella astraeusicola*. The number associated with each branch represents the percentage of 500 bootstrap replicates in which that branch was supported. Strain numbers are explained in table I. "N" indicates that network type traps are formed; "C" indicates that constricting traps are formed.



lates. All but one, isolate no. 146, trap nematodes, both constricting and nonconstricting traps are present. In the first group we have three different conidial anamorphs represented which are placed in two form genera—*Arthrobotrys* and *Monacrosporium*. These have teleomorphs that at best are difficult to distinguish from the *O. auricolor—curvatispora* group. Number 90 is an isolate we identified as *A. cladodes* var. *macroides*; it was derived from a collection we identified as *O. auricolor*. This isolate, along with another (DHP no. 55) that produced *A. oligospora* var. *oligospora*, was commented upon by Pfister and Liftik (1995). Isolate number 60 is documented by Pfister (1994) and was called *A. superba* and its teleomorph was considered *O. fimicola*. Number 213 is a culture identified by Rubner as *M. psychrophilum*. She (Rubner, 1994, 1996) reported that this produced *O. auricolor* in culture, identified by Baral. As previously indicated the teleomorphs in this case are difficult to distinguish. Baral re-examined the three samples from which I obtained the three different anamorphs, and found no convincing differences among the teleomorphs. Our molecular information places them within a well-supported clade. Along with these, supported at a high bootstrap value, is a constricting trap-forming *Monacrosporium* isolate with unknown teleomorph and a nonsporulating but nontrapping isolate from an *Orbilia* (no. 146). That this isolate is nonsporulating indicates perhaps that we have just not yet provided proper conditions for it, likewise we may also not have provided proper nematodes or other organisms to stimulate trap formation.

Allied to this group is one consisting of three taxa. First is an isolate of an *Orbilia* species which we have not yet identified collected in Puerto Rico and producing a *Monacrosporium* anamorph that we have tentatively identified as *M. doedycoides*. Next is *Patinella tenebricosa*; the anamorph is *M. polybrochum*. The third is a conidial isolate, teleomorph unknown, and was identified as *A. brochopaga* (DHP no.212 = ARSEF 4815). I have commented previously on *M. polybrochum* and *M. doedycoides*. *Arthrobotrys brochopaga* is assigned to several genera because of the production of multiseptate conidia. Drechsler described it in *Dactylella*. Schenk, et al. (1977) moved it to *Arthrobotrys*. Rubner (1996) used it as an intermediate state between *Arthrobotrys* and *Monacrosporium* in her intuitive phylogeny. All of the fungi of this clade trap by means of constricting rings.

A third group contains taxa that produce a *Dicranidion* anamorph. All of the cultures were derived from ascospore isolates and the teleomorphs belong to *O. delicatula* or what at this point can only be referred to as the *alnea-coccinella* complex. One (no. 107) of the isolates produces what could only be called a *Dactylella* in culture. This may be no more than a one-armed *Dicranidion*. This clade is well-supported and seems to represent a distinct lineage.

Clade four is the *Helicoon* group. Isolate number 125 was derived from ascospores; number 129 was a single conidium isolate made from field collected material of *H. sessile*.

Clade five represents two conidial isolates of *Arthrobotrys*: 100 from dung and 109 from rotting seaweed. These are well removed from the other isolates within this analysis that form *Arthrobotrys*-type conidia or traps.

These results are preliminary and there are certainly holes to be filled. They do indicate that at least two groups of anamorphs form well-supported clades that agree with current concepts of conidial morphology. These are the *Dicranidion* clade and the *Helicoon* clade. The primary *Arthrobotrys* and *Monacrosporium* clades show a mix of morphologies suggesting that naming the form genera in this complex is difficult. One could, for example, question whether *Arthrobotrys brochopaga* should be included in *Arthrobotrys* or in *Monacrosporium*. One might want to revisit Drechsler's earlier work in which he placed most of what we now call *Monacrosporium* and *A. brochopaga* in the same genus, *Dactylella*. Certainly there is support for recognition of only one group based on this preliminary study.

In the Orbiliaceae, at least as it stands at present, there is some correlation of teleomorph and anamorph. The *Dicranidion* formers seem to all belong to a single clade and might be separated morphologically on the basis of their tiny subglobose to ellipsoid to reniform ascospores. The biology of this group needs critical evaluation. They may be capable of trapping small animals. The teleomorphs are in the *O. coccinella-alnea-delicatula* group. The *Helicoon*-type anamorphs seem associated with the *O. luteorubella*-type teleomorphs. Of course much of the difficulty encountered in studying these connections lies in the lack of knowledge about teleomorphs. Baral's analysis will be of great use in this way.

The presence of an *Arthrobotrys* group outside and well removed from the demonstrably sexually reproducing set of *Arthrobotrys* taxa requires some comment. In other cases that have been documented, conidial states that lack teleomorphs have been shown to intergrade among the sexually reproducing taxa (Lobuglio and Taylor, 1993). Such is not the case with two isolates of *Arthrobotrys*. It is too early to definitively comment on these relationships.

Ecology of Orbiliaceae.—There is perhaps no other group in which there is such a varied selection of

anamorphs that, because of morphology, can be so precisely placed with regard to their ecology. Two main types of habitats are colonized: wet areas and those areas exposed to drying. Baral and his collaborator Guy Marson have collected extensively on the dry habitats. These are dead branches at eye level or above that may be partly decorticated. The species that occur in these habitats are drought tolerant in that they remain viable through successive wettings. Marson should be credited with opening an entirely new habit to mycologists; he is a consummate collector of members of the Orbiliaceae in this habitat. The nondrought-tolerant species are found on more or less permanently wet or watersoaked substrates. It is the latter group that I have concentrated on in this study. Such wet, semi-aquatic habitats not only support nematode populations but are also the habitats in which one might expect to find teleomorphs of aquatic hyphomycetes as now seems apparent. It is likely that all of these taxa are saprobic and that some of them are trappers of nematodes and other organisms. As indicated by Benny et al. (1978) there are algae associated with some apothecia. There are, in addition, many different types of organisms on these substrates. Some of the species are likely fungicolous. Even *Arthrobotrys* is mycoparasitic (Jeffries and Young, 1994).

Since 1994 my assistants and I have collected some of the same spots in Harvard's Estabrook Woods and adjacent conservation land in Concord, Massachusetts. These forests were fields when Thoreau roamed Concord. They are today second growth forests of mixed hardwood and conifers. In 1995 we marked certain downed trees on which *Orbilia* species were prolific. The specific area was chosen because, in a preliminary survey, eight *Orbilia* taxa were found in a small area. We marked, with colored pins, exact areas on those logs on which *Orbilia* species were collected. We collected a few apothecia for identification purposes and then returned periodically through the growing season and into the next to follow the "fruiting period" and reoccurrence of the species. We found two things. First, the *Orbilia* apothecia are individually relatively long-lived. We had already observed this in moist chamber studies. Additionally we determined that most apothecial "colonies" were of mixed age—that is, they contained both young and old apothecia concurrently. Thus, each cup lasted for several weeks or up to a month and within a "colony" there were always mature apothecia. "Colonies" persisted for most of the growing season. Second, our preliminary survey of the sites in the second year, 1996, indicates that the same species seem to reoccur in the same spot. This suggests that the mycelium is perennial.

This information is preliminary but it may answer the question where these fungi over winter. This is of specific interest regarding the nematode-trapping fungi in which successful biological control depends on survival of the fungus. Neither thin-walled conidia nor the traps themselves can be used to fully explain the reoccurrence of these fungi from one growing season to the next and not all are known to produce chlamydospores.

What's to be done?—There is much to be done. More isolates need to be incorporated into the data for the ITS and perhaps more genes need to be examined. We need to include fungi that produce sticky knobs and lax networks but I point out that we have not yet isolated any of these fungi from Orbiliaceae. A more complete picture of the 18S sequences in the Leotiales needs to be produced. Means need to be found for testing cultures for trapping capacities of nonnematodes. Taxa trapping other small animals need to be sought and studied. *Tridentaria* and *Trisporina* are examples of trapping taxa which have not been connected with *Orbilia* species but which are likely candidates. The biology of amoeba-trapping fungi needs to be studied. Barron's work in this field, which so elegantly supplements Drechsler's, needs to be continued.

With all these caveats there is at least one take-home message. By knowing life histories and natural history a wealth of literature can be accessed which would otherwise not seem to be pertinent. In this case a vast literature on *Arthrobotrys* and related genera are opened to discomycete studies by knowing the *Orbilia* connection. Some examples from the literature might be important to bring the point home. For example, from 1989 to 1996 *Biological Abstracts* shows 125 references related to *Arthrobotrys*. These range from surveys of soils, competition studies, control experiments of plant and animal parasites, biochemistry and mycoparasitism. During the same period there are 11 references to *Orbilia* taxa, all of which relate to taxonomic studies. On *Medline* from 1983 to 1996, 56 *Arthrobotrys* records were found; there were no *Orbilia* records. We are better informed when we know both the twins.

We will be continuing to isolate anamorphs and will continue our ITS studies. One goal of this work will be to try to resolve the situation regarding the species we know only from conidia and to continue to try to elucidate the life histories of these fascinating fungi.

Some ways to look at historical data.—At the end of this talk on this specific mycological topic, I hope you will indulge me for a moment so that I might comment as an historian on how these results and indeed

all results might be viewed in the future. What I have presented may not all be true. I say this not to qualify everything that I have said but rather as a caution. I hope not to be judged as W. G. Farlow, in jest, joked about himself when discussing a certain impression he had. He said that the impression came not from anything anyone had said or anything he had heard but rather ". . . from my own immense capacity for drawing inferences at sight . . . ." What I have presented are snapshots of natural history and phylogeny. A full portrait is yet to be produced. Parts of this work may ultimately prove to be wrong. Most of the previous work I have outlined for you was not definitive. Tulasne did not get it all right. Brefeld opened the field of cultural studies but his work was not a final word. Much as we appreciate Drechsler's work it has been followed by many studies that have broadened or clarified it. We advance by steps dictated and determined by technology, by previous work, by experience and by insight. Too often we can find examples in the literature where the most important statement is not a new interpretation or a new insight but a repeat of previous work or its dismissal as flawed. A useful case comes to mind and I have selected it because essentially none of those involved are here to comment. This is one of the major differences between history and criticism.

The case is the hypothesis of brachymeiosis; the primary advocate of the theory was Dame Helen Gwynne-Vaughan (born Fraser). What exactly is the nuclear behavior within the ascogenous system and the ascus? Is there a single nuclear fusion within the ascus or are there two nuclear fusions—one in the ascogonium and one in the young ascus? Of course we all know the answer—there is one fusion, that in the young ascus. Meiosis occurs in the ascus but from Fraser's (1908) work until well into the 20th century there was not agreement. Fraser (later Gwynne-Vaughan) became one of the major proponents of the hypothesis that the nucleus of the young ascus was tetraploid, being the product of a fusion within the ascogonium followed by the formation of a diploid dikaryotic ascogenous system, which nuclei ultimately fuse in the ascus. Gwynne-Vaughan's detailed and critical cytological and developmental observations—mostly involving discomycetes—provided evidence, with then available techniques, that a fusion took place within the ascogonium. She observed what she considered to be double pairs of chromosomes in these nuclei. Gäumann and Dodge (1928) and Bessey (1950) discuss the hypothesis and provide background as does the text by Gwynne-Vaughan and Barnes (1937). Gwynne-Vaughan and her students promoted the idea based on their observations. Ultimately they were proven wrong—brachymeiosis is no more than a mitotic division and the nucleus in the ascus is not routinely tetraploid. Were their observations wrong? Were Gwynne-Vaughan and her followers charlatans? Does their work join some dust heap of only "historical interest?" Perhaps, but Gwynne-Vaughan was partially correct in what she saw. Rossen and Westergaard (1966) discovered that premeiotic, prefusion, haploid nuclei have "two multiples" of DNA–that is, that replication has taken place. Gwynne-Vaughan saw chromatids after replication. What she saw was real but they were chromatids not chromosomes; they were not evidence of fused haploid nuclei. For all the confusion she and her students may have created, her observation was correct—meiotic chromosome replication in a fungus begins before karyogamy.

I need to point out that there was more to Gwynne-Vaughan's work than the cytology. The developmental work on discomycetes spawned by her genetic work is some of the most complete that we have. Much of the cytological work on fungi from the early part of the 20th century contains information that is pertinent for many of our studies.

I wish I could say that I learned about brachymeiosis because I wanted to or that I discovered it on my own but such is not the case. Dick Korf made us learn about it because of the historical importance and the use of discomycetes in these studies. I must admit that today citing Rossen and Westergaard's 1966 paper strikes me as vaguely historical. But I will ask you to consider whether anyone today thinks that it is important to learn what is not true?

Mark Twain said, "Get your facts first, and then you can distort them as much as you please." Getting the facts and getting the framework is often the hard part of getting the story straight.

ACKNOWLEDGMENT AND APPRECIATION

Even though some of what I have told you may not be definitive, the supportive labor was shouldered by many and I want to recognize some of them. I do this not to share any errors which may occur—ultimately I am the only one responsible—but to give due credit. Credit comes rarely and I want it to be well dispersed. At the outset I want to mention those who have been directly responsible in the project. A series of undergraduates have been involved in field and culture work. They are Michael Liftik, Seth Goldbarg, and Keith Day. The molecular work was done by postdoctoral fellows Francis Harrington, David Hibbett and undergraduate Elizabeth Pine. Daniel Potter, University of California at Davis, more than discharged any debt he may have owed his former teacher. He helped, only out of the goodness of his heart, in the analysis of the molecular data. Over many years I have been aided in secretarial tasks and other MSA duties by Carolyn Hesterberg. I also owe much

to the past and present staff of the Harvard Botany Libraries, particularly the current librarian Judith Warnament.

It is necessary for me to look further back in history to fully discharge my debts. A most important part of my education occurred at Miami University in Ohio. There a band of loyal teachers in the Botany Department took me, a young, scared and clueless freshman and made me want to be a botanist (I should point out that they did this more or less routinely; at least one other past president of the MSA was their student). Ethel Belk, Charles Heimsch, T. J. Cobb, and most of all William E. "Prof." Wilson were teachers who made me want to work hard. They made the world seem an open and wonderful place to explore—to tramp through the forest, to make discoveries under the microscope, to draw and illustrate, to learn about biologists. In a fateful seminar with Ethel Belk I first read Hunter Dupree's biography of Asa Gray. How could I have suspected that one day Dupree would be my friend in Cambridge and I would be the Asa Gray Professor? My first discomycete, a *Sarcoscypha,* was collected in the woods on the slopes behind old Fisher Hall in Oxford, Ohio and was identified that spring day by Prof. Wilson. A more beautiful and wonderful introduction to discomycetes could hardly be imagined. Dick Korf taught me everything else about discomycetes and much about approaching history. At Harvard, Reed Rollins, long time Director of the Gray Herbarium, helped me get settled. His model of directoral patience helped me in my turn as Director of the Harvard Herbaria to get through more committee meetings and personnel discussions than I care, or perhaps am able, to recall. The joy of discovering history for myself by looking at archives and manuscripts was introduced to me by my friend and colleague in the Farlow Herbarium, Geneva Sayre. She was a person of solid integrity, and devotion to and passion for learning. My students of various stripes have taught me more than they may suspect. These all have been my teachers in and out of the classroom.

For the last 25 years I have made my way, as many of you have, as a teacher and for the last 15 as an administrator in addition. I hate the word administrator. It conjures images of ruthless disregard, benign neglect, and too often of dithering incompetence. To say that this has been a time of uninterrupted pleasure would be more exaggeration than truth but it has put me in the midst of several exciting and interesting groups of people, not the least of whom are the Harvard undergraduates. How should I describe a Harvard student? Each is different, each brings his or her own skills, each is special. Some are as scared and clueless as I was once. As we turn them loose at graduation I cannot but think that a tidal wave is about to hit the unsuspecting observers on the beach. One hopes that no one will be swept out to sea, them or us. Of the strong and hearty swimmers I offer my particular thanks to Michael Liftik who served as my undergraduate assistant for all of this project and for most of his undergraduate career. No one could ask for a better assistant or for a better field companion. I will miss his steady and reliable presence.

Fourteen years of my life have been spent living and working in Kirkland House, one of the Harvard residential colleges. As Housemasters, my wife (and helpmate in many projects) and I have at least nominally overseen between 330 and 370 undergraduates each year. What we have seen, heard, overseen and overheard might make a notable memoir. I am sometimes amused that certain Kirkland House students seemed to think we were put on this planet just to be in Kirkland House, although such was never the case. Between parties and dinners, receptions and introductions I was teaching and tending my research. As the perpetual undergraduate, the one who has said farewell to so many undergraduates every commencement yet who never left, I find inspiration in some of these students. During their 16 hour days and often fully scheduled weekends I came to depend upon the good will and good cheer of generations of Kirkland House students. Although it has little to do with a presidential address I want now to acknowledge in a way I have not done before, the richness they have added to my life.

It is said that in education one makes friends just to have them leave. Indeed, in education we need always to be prepared for loss—our students leave; we continue. Like Castor and Pollux, like teleomorphs and anamorphs, like students and teachers—we take our turns.

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