

Morphological variability and molecular phylogeny of the nematophagous fungus *Monacrosporium drechsleri*

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Abstract: An isolate of the nematode-trapping fungus *Monacrosporium drechsleri* was collected from cultures of the root-knot nematode *Meloidogyne arenaria* that had been maintained on tomato roots in greenhouse pots in Beltsville, Maryland. The plant-parasitic nematodes *Heterodera glycines*, *Meloidogyne incognita* and *Pratylenchus zeae* and the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* were placed on colonies of *M. drechsleri* grown in Petri dishes to study ability of the isolate to trap various nematode hosts. None of the nematodes placed near adhesive knobs were motile within 1 d. To determine where *M. drechsleri* fits within the existing phylogeny of nematode-trapping fungi, the ITS1-ITS2 regions of rDNA and the nuclear gene EF1- α were sequenced for the new isolate of *M. drechsleri*, for the species *M. parvicolle* and *M. lysipagum*, and for an isolate of *M. ellipsosporum* distinct from the one listed in GenBank. Parsimony trees were constructed showing the closest molecular relative of *M. drechsleri* to be the newly sequenced isolate of *M. ellipsosporum*; the latter had a highly divergent sequence from the sequence recorded in GenBank for a different isolate of *M. ellipsosporum*. Unique, consistent and discrete morphological characters are absent in these related taxa, so an independent molecular character should be considered essential for their accurate identification.

Key words: *Arthrobotrys*, *Caenorhabditis elegans*, *Dactylella*, *Dactylellina*, *Gamsylella*, *Heterodera glycines*, *Meloidogyne incognita*, *Monacrosporium*, *Monacrosporium ellipsosporum*, *Monacrosporium lysipagum*, *Monacrosporium parvicolle*, nematode, nematophagous fungus, *Panagrellus redivivus*, *Pratylenchus zeae*

INTRODUCTION

The nematophagous fungus *Monacrosporium drechsleri* (Tarjan) R.C. Cooke & C.H. Dickinson first was isolated from roots and soil obtained from a grapefruit grove (Tarjan 1961). Although many nematode-trapping fungi have been examined intensively in ecological studies and as potential biocontrol agents for plant-parasitic and animal-parasitic nematodes, little work has been conducted with this species (Pria et al 1991, Pria and Ferraz 1996, Tarjan 1961). A new and potentially aggressive isolate of *M. drechsleri*, recently collected from greenhouse cultures of the root-knot nematode *Meloidogyne arenaria*, therefore was singled out for more extensive study. The source nematode cultures, which were established originally by A. Morgan Golden in the 1960s, have been maintained on tomato plants in the USDA-ARS Beltsville Nematology Laboratory greenhouse. During summer 2002 it was observed that the *M. arenaria* populations were becoming depleted. Examination of the remaining *M. arenaria* indicated that the second-stage juveniles (J2) were sluggish, and that most J2 were parasitized by fungi. However, none of the stages of the free-living nematode *Diploscapter* spp. in the same pots were visibly affected. Consequently, *M. arenaria* J2 were studied to determine the nature of the infesting fungi, and a fungus was isolated from parasitized nematodes and morphologically characterized as *M. drechsleri*.

Monacrosporium drechsleri is described as having conidia that vary in shape but generally are somewhat fusiform, 22–48.7 μm long \times 9.5–15.4 μm wide, most with three septa (TABLE I; description after Tarjan 1961, Rubner 1996). Conidiophores can be branched or unbranched. The fungus traps nematodes on short-stalked, adhesive knobs. The morphology of *M. drechsleri* somewhat resembles that of *Monacrosporium ellipsosporum* (Preuss) R.C. Cooke & C.H. Dickinson (Rubner 1996), which is described in Rubner's monograph (1996) as having spindle-shaped, mostly 4-septate conidia; measurements are listed (TABLE I). *Monacrosporium ellipsosporum* also traps nematodes on stalked knobs and is of interest because it has been studied widely as a nematophagous fungus. For example, *M. ellipsosporum* was tested for ability to suppress root-knot nematode populations; the fungus was effective in some of the studies (Jaffee and

TABLE I. Conidium measurements and trapping structures of *Monacrosporium drechsleri*, *M. elliposporum*, *M. lysipagum* and *M. parvicolle*. ATCC = American Type Culture Collection; CBS = Centraalbureau voor Schimmelcultures; NL = USDA-ARS Beltsville Nematology Laboratory. Measurements of ATCC and NL cultures were made during the current study^a; all other measurements are reported in Drechsler (1937, 1961), Rubner (1996) or Tarjan (1961)

Species, isolate, and medium used for NL 565 ^b	Length mean \pm standard deviation, range in μm	Width mean \pm standard deviation, range in μm	Septation mean \pm standard deviation, range	Trapping structure for each species
<i>Monacrosporium drechsleri</i> NL 565 ½ CMA	30.2 \pm 3.3 25.0–34.2	7.7 \pm 0.9 6.2–9.2	2.9 \pm 0.3 3, rarely 2	Stalked knobs
<i>M. drechsleri</i> NL 565 CMA	33.3 \pm 5.1 22.5–55.0	10.1 \pm 2.1 6.2–15.7	3.0 \pm 0.4 3, rarely 2–4	
<i>M. drechsleri</i> NL 565 PDA	35.4 \pm 6.2 25.1–48.4	11.7 \pm 1.5 8.7–15.0	3.9 \pm 0.6 3–4 (rarely 5)	
<i>M. drechsleri</i> NL 565 WA + RKN	33.0 \pm 2.4 30.5–37.5	10.0 \pm 1.7 7.5–12.5	2.7 \pm 0.4 Most 3, some 2	
<i>M. drechsleri</i> NL 565 (Results combined for all media above)	33.7 \pm 5.3 22.5–55.0	10.4 \pm 2.1 6.2–15.7	Most 3 (70.5%), some 2 (9.8%), or 4 (16.4%), rarely 5 (3.3%)	
<i>M. drechsleri</i> Reported in Tarjan (1961)	39.6 29.5–48.7	13.1 10.7–15.4	Most 3, some 2, less commonly 1 or 4	
<i>M. drechsleri</i> CBS 549.63 Reported in Rubner (1996)	22–43.5	9.5–13	Most 3, some 4	
<i>Monacrosporium elliposporum</i> ATCC 204100	41.1 \pm 3.7 32.5–46.0	12.2 \pm 1.4 10.0–14.5	4 \pm 0	Stalked knobs
<i>M. elliposporum</i> CBS 302.94 Reported in Rubner (1996)	44 40–50	15 12–16	Most 4, some 3	
<i>Monacrosporium lysipagum</i> ATCC 28265	34.2 \pm 2.8 29.0–38.0	15.3 \pm 1.4 11.5–16.7	3.1 \pm 0.6 Most 3, some 4, less commonly 2	Stalked knobs (non-constricting rings were not observed)
<i>Monacrosporium lysipagum</i> Reported in Drechsler (1937)	40.7 28–55	11.6 9–14	Most 4, some 2 or 3	Stalked knobs, non-constricting rings
<i>Monacrosporium parvicolle</i> ^c Reported in Drechsler (1961) and Rubner (1996: CBS 219.61, 313.94, 314.94)	29–56	6–16	Most 4 or 3, some 2	Unstalked or short-stalked adhesive knobs that can form loops

^a *M. parvicolle* ATCC 96680 is not included in TABLE I because it sporulated poorly in the current study and did not form trapping structures.

^b CMA = cornmeal agar, ½ CMA = half strength CMA, PDA = potato dextrose agar, and WA + RKN = 1.5% water agar plus root-knot nematodes (*Meloidogyne incognita*).

^c Compilation of information from multiple isolates.

Muldoon 1995a, b, Persson and Jansson 1999, Santos and Ferraz 2000). *Monacrosporium ellipsosporum* also was active against the lesion nematode *Pratylenchus penetrans* (Timper and Brodie 1993), but in another investigation caused little reduction in populations of the cyst nematode *Heterodera schachtii*, even though the fungus could parasitize *H. schachtii* in soil (Jaffee et al 1992, Jaffee and Muldoon 1995a). However, little is known about the morphologically similar *M. drechsleri*. In laboratory tests *M. drechsleri* growing on agar media was a predator of *Panagrellus* sp. and *Pratylenchus* spp. (Pria et al 1991, Tarjan 1961), while another study indicated that an isolate of this species did not suppress *Meloidogyne incognita* populations significantly on roots (Pria and Ferraz 1996).

In addition to the paucity of biocontrol studies with this species, *M. drechsleri* has not been included in molecular studies conducted on nematode-trapping fungi. Previous phylogenies have demonstrated a general association of fungal clades with trapping structures. The SSU 18S rDNA supported three lineages: nonparasites, fungi that form constricting rings and producers of adhesive structures (Ahrén et al 1998, Ahrén and Tunlid 2003). The resolution of this finding was increased somewhat with an ITS phylogeny (Liou and Tzean 1997). Because of the large database of ITS sequences that already exists for nematode-trapping fungi, we conducted a phylogenetic analysis on the new isolate of *M. drechsleri*, and on isolates of other *Monacrosporium* species, to improve resolution and test or complement diagnosis by morphological measures. The goals of the current study were: (i) to examine the new *M. drechsleri* isolate morphologically; (ii) to look at its molecular relationships with other nematode-trapping fungi; and (iii) to study its ability to trap diverse nematode hosts. This information is needed to determine how this species is related to other nematode-trapping fungi, to understand more about the ecology of the fungus and its interactions with plant-pathogenic and beneficial nematodes and to optimize potential use of the fungus as a biocontrol agent.

MATERIALS AND METHODS

Fungus sources and morphology study.—To obtain the new fungal isolate, specimens of the root-knot nematode *M. arenaria* (Nematology Laboratory greenhouse culture No. C-75-24) were removed from greenhouse-grown tomato (*Lycopersicon esculentum*) plants. Fungal infected J2 then were placed onto potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan). The fungus subsequently was isolated from the J2, identified as *M. drechsleri*, given the USDA-ARS Beltsville Nematology Laboratory (NL) designation 565 and maintained in the laboratory on PDA and cornmeal agar (CMA; Difco Laboratories, Detroit, Michigan). The isolate

also was sent to the American Type Culture Collection (ATCC) where it was designated “MYA-3405”. Morphological and molecular comparisons were made with live cultures of these fungi: (i) *M. drechsleri* NL 565; (ii) *M. ellipsosporum* ATCC 204100; (iii) *Monacrosporium lysipagum* (Drechsler) Subram. ATCC 28265; and (iv) *Monacrosporium parvicolle* (Drechsler) R.C. Cooke & C.H. Dickinson ATCC 96680 (listed as *Monacrosporium parvicollis* in ATCC). *Monacrosporium ellipsosporum* and *M. lysipagum* were selected for study because of morphological similarities to *M. drechsleri*; *M. parvicolle* was selected as a related but somewhat phylogenetically removed species for comparison. It is noted here that Scholler et al (1999) established four groups of nematode-trapping fungi, placing them in the genera *Arthrobotrys* (genus with adhesive networks), *Dactylellina* (stalked adhesive knobs with or without stalked 3-celled nonconstricting rings), *Drechslerella* (stalked 3-celled constricting rings), and *Gamsylella* (adhesive columns and unstalked adhesive knobs). *Monacrosporium drechsleri*, *M. ellipsosporum* and *M. lysipagum* were placed in *Dactylellina* by Scholler et al (1999) and *M. parvicolle* in *Gamsylella*. However, these generic designations have not been used commonly so we have used the older generic designations that are more familiar to many taxonomists and that are entered in GenBank.

Morphology of live cultures was studied on PDA, CMA, and/or half-strength CMA (number of media used varied with species). *Monacrosporium drechsleri* also was grown on 1.5% water agar inoculated with surface-sterilized root-knot nematodes (*M. incognita*), which were obtained from greenhouse cultures (procedures for removing microbes from *M. incognita* described below). Measurements were made with a Leica DMIL microscope. Descriptive statistics and ANOVA were run in an Excel 2002 spreadsheet.

Herbarium specimens of *M. drechsleri* examined were: (i) USA, Florida: Lake Alfred, Citrus Experiment Station. Cornmeal agar, 16 July 1960, A.C. Tarjan, 415091 BPI, and (ii) USA, Florida: Lake Alfred, Citrus Experiment Station. Cornmeal agar, 16 May 1960, A.C. Tarjan, 418234 BPI. Tarjan (1961) stated “Type cultures of *Dactylella drechsleri* are numbered F1636 in The National Fungus Collections, Plant Industry Station, United States Department of Agriculture, Beltsville, Maryland” (p 144). Without trying to settle nomenclatural problems associated with the deposit of the two type specimens, we simply note that both specimens are given the number F1636 and both are labeled “Holotype”.

Nematode motility.—Beltsville Nematology Laboratory cultures of the two free-living nematodes *Caenorhabditis elegans* (N2 strain) and *Panagrellus redivivus* (LKC26) and of the three plant-parasitic nematodes *Heterodera glycines* (Race 3), *M. incognita* (Race 1) and *Pratylenchus zeae* (all plant parasites originally collected in Maryland) were used. *Pratylenchus zeae* (corn lesion nematode) was maintained on root explant cultures of corn (*Zea mays*) cv. Iochief grown on Gamborg's B-5 medium (Gibco, Life Technologies Inc., Grand Island, New York). *Meloidogyne incognita* and *H. glycines* were obtained from greenhouse pot cultures. *Caenorhabditis elegans* and *P. redivivus* were grown in a semi-defined liquid culture without bacteria (from Chitwood et

al 1984, except that the medium had only 10 μg of sterol per mL). To collect *P. zoeae* sterile water was added to root explant cultures and the nematodes were pipetted into a sterile dish. The juveniles then were hand picked and centrifuged (this and other centrifugations in this section were done at $2000 \times g$) to increase the nematode concentration. *Meloidogyne incognita* cultures reared on tomato cv. Orange Pixie in greenhouse pots were collected by rinsing the tomato roots in water to remove soil and debris and hand-picking ripe egg masses, which were placed into water, rinsed and shaken in 6% sodium hypochlorite 2 min to break up egg masses. The eggs then were pipetted onto a 500-mesh sieve, rinsed with 95% EtOH and then with water, pipetted into sterile 1.5 mL microcentrifuge tubes, centrifuged 30 s, gently shaken 8 min in 6% sodium hypochlorite and centrifuged again. The supernatant was replaced with water, and the eggs were shaken, centrifuged again and washed with water. To obtain *H. glycines* (soybean cyst nematode), soil was washed off soybean (*Glycine max*) cv. Essex roots that had been grown in the greenhouse and infested with the nematode. Cysts then were collected on a 60-mesh sieve, centrifuged in water and then in a granulated sugar (sucrose) solution in water (454 g sugar/L). The supernatant containing the cysts was collected, rinsed, placed in a glass homogenizer and crushed to collect the eggs. The eggs then were treated as with root-knot nematode. Water suspensions of *M. incognita* and *H. glycines* were poured onto sterile polypropylene screens with apertures 25 μm diam; sterile broth cultures of *C. elegans* and *P. redivivus* were poured directly onto the screens. Juveniles that passed through the mesh were collected and centrifuged to concentrate the nematodes. *Pratylenchus zoeae* could be used without passage through the screens.

To prepare *M. drechsleri* the fungus was inoculated onto PDA and incubated 4–7 d in the dark at 26 C. Plugs of fungus 1 cm diam were cut from near the peripheries of the fungal colonies, and one plug was placed into the center of each of 15 Petri dishes (60 mm diam) that contained a thin layer of 1.5% water agar with 0.3% penicillin (Sigma, St Louis, Missouri). The fungus grew 1 wk in the dark at 26 C, and each Petri dish then was inoculated with five 25 μL drops, each drop containing ca. 10–20 nematodes. Drops were placed just inside the peripheries of the fungal colonies. Nematodes also were placed on control Petri dishes containing 1.5% water agar with 0.3% penicillin. For each trial of the experiment, each nematode test was replicated on a total of three fungus-treated Petri dishes and three control Petri dishes. The day after the nematodes were placed on the fungus cultures, counts were made of motile and nonmotile nematodes on each Petri dish. The experiment was repeated for a total of six fungus-treated Petri dishes and six control Petri dishes per tested nematode species.

DNA extraction, PCR, sequencing.—Cultures of the fungi were maintained on PDA at 26 C in the dark. Mycelia for DNA extraction were grown in potato dextrose broth (Difco Laboratories, Detroit, Michigan) at 25 C on a rotary shaker at 125 rpm 3–5 d. Mycelia were harvested by filtration or centrifugation, washed once with sterile distilled water, ly-

ophilized and stored at -20 C. Approximately 50 mg of lyophilized mycelium was ground with 0.5 mm glass-zirconia beads (Biospec Products Inc., Bartlesville, Oklahoma) in a FastPrep FP120 tissue homogenizer (Thermo Savant, Holbrook, New York). DNA extractions were performed with modifications of a method by Cambereri and Kinsey (1993). Powdered mycelium was suspended in detergent solution (2 M NaCl, 0.4% w/v deoxycholic acid, 1.0% w/v polyoxyethylene 20 cetyl ether), incubated at 55 C for 15 min and extracted with an equal volume of 24:1 chloroform : isoamyl alcohol; the cellular debris plus glass-zirconia beads were pelleted by a 15 min centrifugation. The supernatant was mixed with an equal volume of 6 M guanidinium thiocyanate, and total nucleic acids were bound to glass powder (Vogelstein and Gillespie 1979), washed twice with ethanol buffer and eluted into sterile distilled water. DNA for PCR amplifications was diluted to 1–2 ng/ μL .

Two nuclear loci, the ribosomal internal transcribed spacers (ITS1, 5.8S rRNA, ITS2) and a partial sequence of the elongation factor 1-alpha (EF1- α) were amplified and sequenced. The ITS locus was amplified and sequenced with primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) (White et al 1990). An approximately 1000 bp segment of EF1- α spanning the 3' two-thirds of the gene was amplified with primers 983F (5'-GCYCCYGGHCAYCGTGAYTTYAT) and 2218R (5'-ATGACACCRACRGCRCRGTYYTG). PCR amplifications were performed in a reaction volume of 50 μL , which included 5 μL of 10 \times PCR buffer (10 mM Tris/HCl pH 8.0, 50 mM KCl, 1.5–2.0 mM MgCl_2), 4 μL of dNTP mix (1.25 mM each dATP, dCTP, dGTP and dTTP), 10 pmol each primer, 0.5 μL *Taq* polymerase (Promega, Madison, Wisconsin) and 5–10 ng genomic DNA. PCR for both loci was performed with touchdown PCR (Don et al 1991). Touchdown PCR amplifications were initiated with 2 min denaturation at 94 C. The annealing temperature in the initial amplification cycle was 66 C after which it successively was reduced 1 C per cycle over the next nine cycles. An additional 36 cycles were performed, each consisting of 30 s denaturation at 94 C, a 30 s annealing step at 56 C, and a 1 min extension at 72 C, concluding with 10 min incubation at 72 C. PCR reaction volumes were reduced to approximately 10 μL by lyophilization and then fractionated on a 1.5% NuSieve agarose gel (BioWhittaker, Rockland, Maine) in a low EDTA Tris-acetate buffer (40 mM Tris-acetate, 0.1 mM EDTA). PCR products were cut from the gel with a scalpel, frozen and thawed, and the DNA was extruded from the gel slice by centrifugation for 10 min at $20\,000 \times g$.

PCR products were sequenced bidirectionally with ABI BigDye 2.0 (PE Applied Biosystems, Foster City, California) with 0.5 μL BigDye diluted in 1.5 μL dilution buffer (400 mM Tris/HCl pH 9.0, 10 mM MgCl_2), 3 pMol primer, 75–100 ng gel-purified PCR template in a total volume of 5 μL . ITS amplicons were sequenced with the ITS5 and ITS4 primers. EF1- α amplicons were sequenced with primers 983F and three internal primers, 1567RintB (5'-ACHGTRC CRATACCACCRAT), 1577F (5'-CARGAYGTBTACAAGA TYGGTGG) and 2212R (5'-CCRAACRGCRCRGTYYG TCTCAT). Cycle sequencing was performed according to the manufacturer's instructions but with the total number

of cycles raised to 35. Cycle sequencing products were precipitated by mixing with 4 volumes of 72% ethanol, incubating at room temperature 15 min and centrifuging at $3500 \times g$ 15 min. The ethanol supernatant was removed by inverting the reaction vessel on a paper towel and centrifuging at $250 \times g$ 10 s, air-dried 10 min at room temperature and stored at -20 C. Immediately before sequencing the reactions were suspended in deionized formamide, heat denatured and run on an ABI 3100 Genetic Analyzer. DNA sequences were assembled into contigs and edited with Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan), and multiple sequence alignments were constructed with the MegAlign module of DNASTAR 5 (LaserGene, Madison, Wisconsin), outputted in Nexus format for phylogenetic analysis and concatenated into a single file with MacClade 4 (Maddison and Maddison 2000).

Sequences were submitted to GenBank. GenBank accession numbers for the nuclear gene EF1- α and the ITS1-ITS2 sequences are respectively: AY695062 and AY695063 (*M. drechsleri* NL 565), AY695064 and AY695065 (*M. ellipsosporum* ATCC 204100), AY695066 and AY695067 (*M. lysipagum* ATCC 28265) and AY695068 and AY695069 (*M. parvicolle* ATCC 96680). GenBank accession numbers for other taxa used to make the trees are: U51946 (*Arthrotrichia haptospora*), AY444596 (*Arthrotrichia oligospora*), U51956 (*Dactylella formosana*), AY444697 (*Duddingtonia flagrans*), U51971 (*M. ellipsosporum* CBS 224.54), AF106529 (*Monacrosporium leptosporum*), U51959 (*Monacrosporium mammillatum*), U51970 (*Monacrosporium phymatopagum*), and AF106531 (*Monacrosporium tentaculatum*).

Phylogenetic analysis.—The data were analyzed by maximum parsimony (MP) in PAUP 4.0b10 (Swofford 2001) using the heuristic search option with TBR (tree-bisection-reconnection) branch-swapping under equal character weighting on all characters of Clustal W (Thompson et al 1994) alignments from sequences pruned in GeneDoc (Nicholas et al 1997). Searches included 500 random-addition replicate analyses and heuristic MP bootstrap analysis (Felsenstein 1985) that consisted of 1000 pseudoreplicates with TBR branch-swapping with 10 random-addition replicates per pseudoreplicate and parsimony-uninformative characters excluded. Outgroups were selected based on broader alignments from trees not shown here. Sequence alignments were submitted to TreeBase. The TreeBase study accession number is S1151, and the matrix accession numbers are M2080 (FIG. 4) and M1977 (FIG. 5).

RESULTS

Nematode motility and morphological study.—The new isolate of *M. drechsleri* (NL 565) was similar to the cultures described by Tarjan (1961), with some variation. Conidiophores bearing solitary conidia (FIG. 1A) were common, but conidiophores with one or more branches were observed frequently (FIG. 1B). Conidiophore length was variable, with a mean of 210.3 ± 133 μm (range 20.0–937.5 μm ; 79 conidiophores were measured).

Conidium size and septation are important for categorizing species of these fungi. Consequently 10–44 *M. drechsleri* conidia were measured on each of the four media (TABLE I). The smallest mean lengths and widths for conidia were recorded from one-half CMA, and the largest from PDA. When measurements of NL 565 conidia from all four media were combined, conidium length and width ranges were similar to those reported by Tarjan (1961) and Rubner (1996) (TABLE I), although the length range extended higher and the width range lower than the ranges recorded in those publications. As expected, conidia tended to be smaller than those of the morphologically similar species *M. ellipsosporum* (TABLE I). Measurements of *M. ellipsosporum* ATCC 204100 conidia showed a slightly lower (but overlapping) range when compared to measurements reported by Rubner for *M. ellipsosporum* (TABLE I). Size ranges of conidia produced by *M. lysipagum* and *M. parvicolle* also are included for comparison (TABLE I). Conidial shape can be variable, but general differences have been noted in previous studies: *M. drechsleri* is described as having somewhat fusiform conidia with a truncate base and a rounded distal end, *M. ellipsosporum* as producing broadly spindle-shaped conidia with a rounded tip, *M. lysipagum* with conidia that are spindle-shaped with a somewhat acutely rounded apex and *M. parvicolle* with slender, spindle-shaped conidia (Rubner 1996).

Most of the *M. drechsleri* NL 565 conidia were 3-septate, as also described by Tarjan (1961) and Rubner (1996) (TABLE I), but two conidia with five septa each were observed on PDA. The other species listed (TABLE I) generally produce more 4-septate conidia. However, *M. lysipagum* ATCC 28265 tended to have more 3-septate conidia than expected; the conidia were shorter and wider, with fewer septa, than *M. lysipagum* as reported by Drechsler (1937).

Stalked adhesive knobs were formed on mycelium (FIG. 1C), on germinating conidia (FIG. 1D), and on hyphal coils (FIG. 1E). Stalk lengths, which are used as one character for separating the genera established by Scholler et al (1999), were measured in the current study. Lengths for *M. drechsleri* NL 565 were 9.4 ± 5.1 μm , ranging from 5.0–38.6 μm long (82 stalks measured). The mean length is similar to that reported by Tarjan (1961): stalks 7.9 μm (4.4–14.0 μm). Some NL 565 stalks were branched (FIG. 1F). Drechsler (1937) reported *M. ellipsosporum* stalk lengths to range most often from 5–10 μm (with extremes of 3–25 μm) and *M. lysipagum* stalk lengths to be 5–35 μm . We recorded stalk lengths of 7.9 ± 2.3 μm (5.0–12.5 μm) for *M. ellipsosporum* ATCC 204100 and 7.8 ± 2.5 μm (5.0–12.5 μm) for *M. lysipagum* ATCC 28265 (10 stalks were measured for

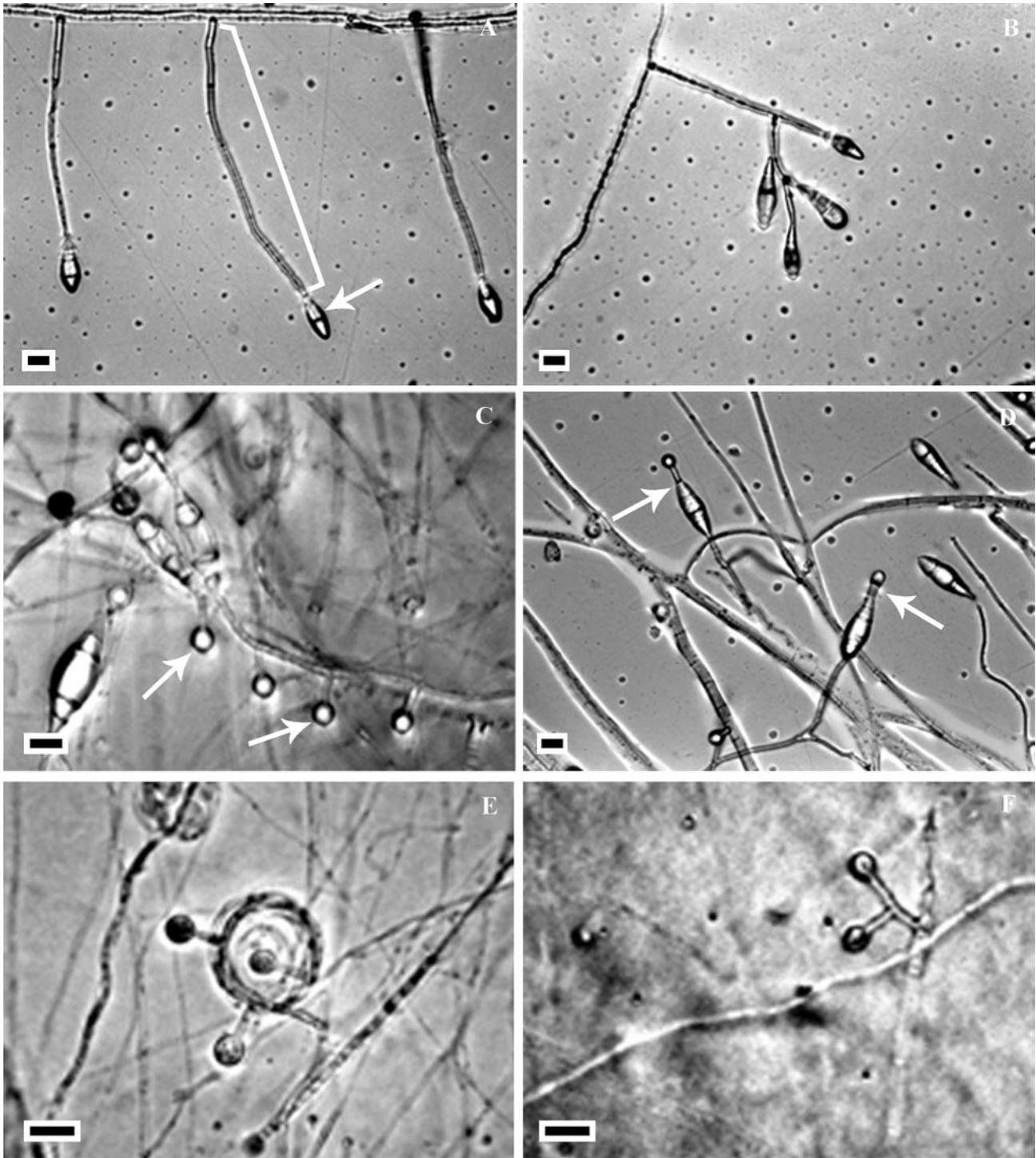


FIG. 1. Conidia, conidiophores and trapping structures of *Monacrosporium drechsleri*. A. Conidiophores (one conidiophore indicated with bar) bearing solitary conidia (arrow pointing to one conidium). B. Branched conidiophores. C. Stalked adhesive knobs (two indicated with arrows). D. Germinating conidia bearing stalked knobs (arrows). E. Coiled hypha bearing stalked knobs. F. Adhesive knobs with branched stalk. Bars = 10 μ m.

each fungus). Stalks from *M. lysipagum* ATCC 28265 tended to be shorter than generally recorded for *M. lysipagum*. *Monacrosporium parvicolle* adhesive knobs were described as forming laterally on hyphae or on stalks 0.5–3.5 μm long (Drechsler 1961); adhesive knobs were not observed on *M. parvicolle* ATCC 96680 in our study.

Formation of chlamydo-spores can depend on culture age and condition of nematode-trapping fungi and is not common in the new genus *Dactylellina* that includes nematode-trapping species with stalked knobs (Scholler et al 1999). However, chlamydo-spores consisting of rounded, thick-walled cells were observed in cultures of the new isolate (FIG. 2A), in the holotype specimen (FIG. 2B) and in *M. ellipso-sporum* (FIG. 2C).

Meloidogyne arenaria from greenhouse pots was parasitized by the new isolate of *M. drechsleri* (FIG. 3A–C), and the fungus was seen emerging from some infected specimens of *M. arenaria* (FIG. 3A, B). In laboratory tests *Monacrosporium drechsleri* NL 565 was effective in trapping all of the tested nematode species; this is illustrated with *M. incognita* (FIG. 3D). The mean percentages of motile nematodes from each control treatment were: (i) *C. elegans* (free-living nematode) 54.9%; (ii) *H. glycines* (plant-parasitic nematode) 35.4%; (iii) *M. incognita* (plant-parasitic nematode) 57.2%; (iv) *P. redivivus* (free-living nematode) 65.3%; (v) *P. zaeae* (plant-parasitic nematode) 50.0%. The mean percentage of motile nematodes on each *M. drechsleri* culture was 0% for all nematodes but *P. zaeae*; 0.7% was recorded for this nematode because one *P. zaeae* J2 was in an area on a culture without adhesive knobs and was motile. Many nematodes on the cultures appeared to be parasitized and rapidly disintegrating; this was especially noticeable with the smaller free-living nematodes.

Phylogenetic analysis.—Lengths of ITS sequences for *M. lysipagum*, *M. parvicolle*, *M. ellipso-sporum* and *M. drechsleri* respectively were 966, 621, 591 and 593 bp. The added length of the *M. lysipagum* sequence was due to a large insert in the ITS1, which was trimmed from the sequence to facilitate the alignment. A 7 bp indel was present in *M. ellipso-sporum* but absent in *M. drechsleri*. The EF1- α sequence compared was 983 bp of exon sequence and was colinear for *M. lysipagum*, *M. ellipso-sporum* and *M. drechsleri*. *Monacrosporium parvicolle* was 1038 bp; the increased sequence length was due to a unique 54 bp intron, but this sequence otherwise aligned perfectly with other sequences. Alignment of the sequences showed a close similarity between *M. ellipso-sporum* and *M. drechsleri*, which differed by a single base pair in EF1- α sequence. *Monacrosporium lysipagum* and *M. parvicolle*

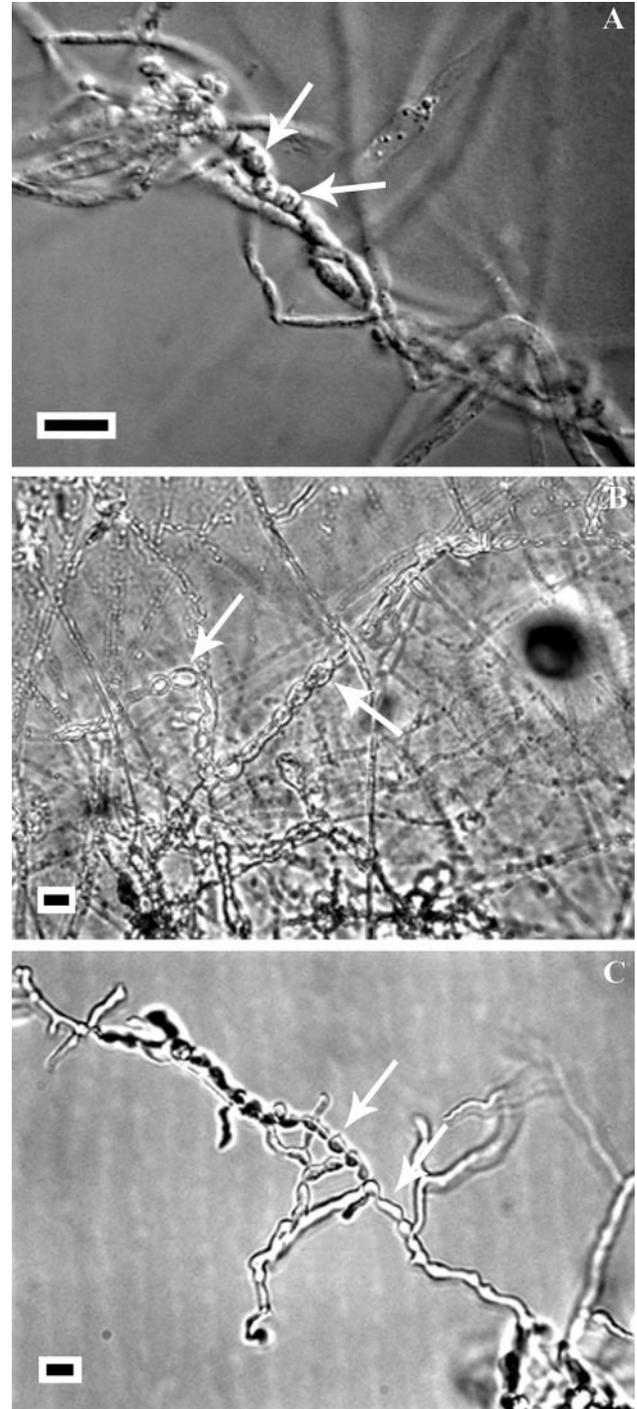


FIG. 2. Chlamydo-spores (arrows indicate examples). A. *Monacrosporium drechsleri*; irregularly sized chlamydo-spores on PDA. B. Chlamydo-spores from Tarjan's type material of *Dactylella drechsleri* (= *M. drechsleri*). C. *Monacrosporium ellipso-sporum* ATCC 204100 chlamydo-spores. Bars = 10 μm .

differed significantly from each other and from *M. ellipso-sporum* and *M. drechsleri*.

A large maximum-parsimony ITS1 and ITS2 tree of 18 nematode-trapping fungal taxa showed a clade

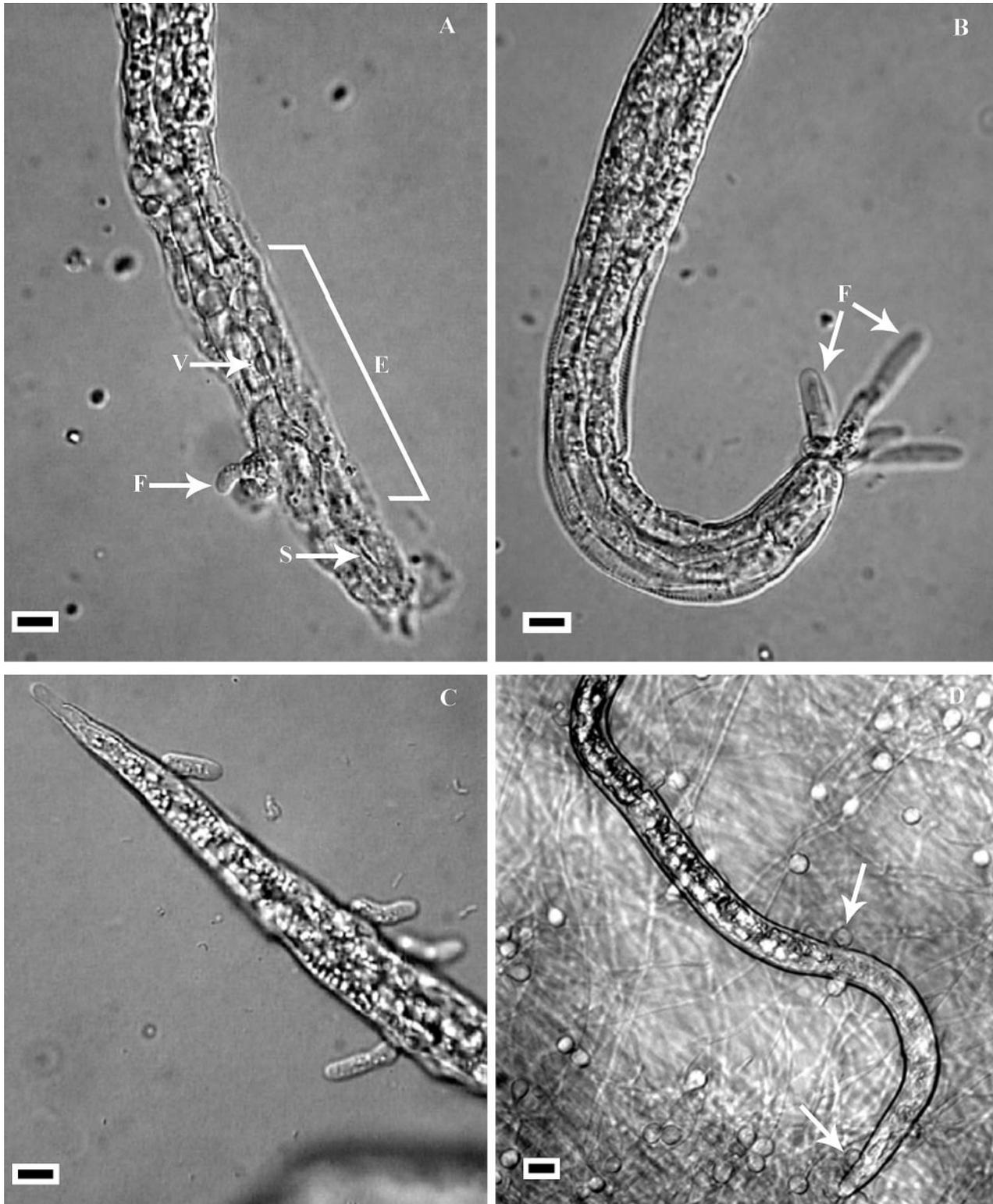


FIG. 3. Second-stage juveniles (J2) parasitized by *Monacrosporium drechsleri*. A–C. *Meloidogyne arenaria*. A. A J2 with internal hyphae and with the fungus (F) emerging from the side of the esophagus (E) region; the stylet (S) and median bulb valve (V) are visible. B. A J2 with internal hyphae and with the fungus (F) emerging from the mouth opening. C. A parasitized J2 from greenhouse tomato. D. A *Meloidogyne incognita* J2 held by *M. drechsleri* adhesive knobs (one knob indicated with arrow). Bars = 10 μ m.

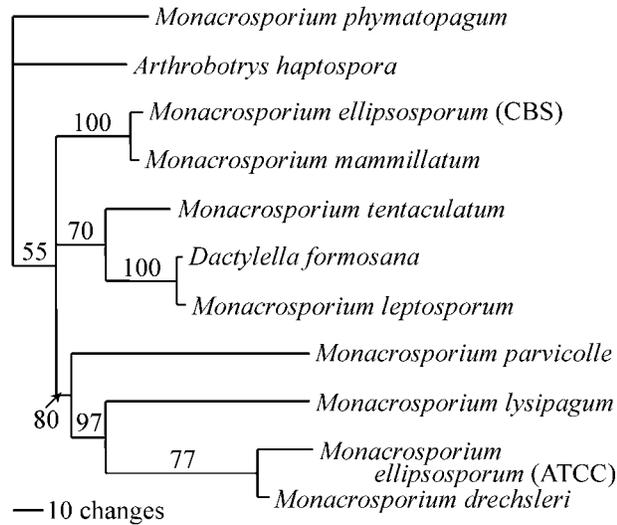


FIG. 4. Maximum parsimony ITS 50% majority rule consensus phylogenetic tree generated in PAUP*4.0 of 10 taxa recorded in GenBank that are the most closely related to *Monacrosporium drechsleri* based on a Clustal W alignment of 603 characters. *Monacrosporium phymatopagum* and *Arthrobotrys haptospora* are designated as outgroups. Heuristic bootstrapped search employing TBR branch-swapping with 1000 pseudoreplicates. Tree length = 344, Consistency index = 0.762, parsimony informative characters = 119. New sequences for *M. drechsleri*, *M. elliposporum* (ATCC 204100), *M. lysipagum* and *M. parvicolle* formed a single clade separate from *M. elliposporum* (CBS 224.54) and *M. mammillatum*.

of *M. drechsleri* with *M. elliposporum* and *M. lysipagum* (not shown). This allowed selection of outgroups *A. haptospora* (Drechsler) S. Schenck, W.B. Kendr. & Pramer and *M. phymatopagum* (Drechsler) Subram. for the taxa of interest to generate a tree of intermediate resolution. To compare the live *M. drechsleri* NL 565 culture with a live culture of the closest known molecular relative, *M. elliposporum* (ATCC 204100) was included in our study. The GenBank sequence (Liou and Tzean 1997) of the UK isolate of *M. elliposporum* (Centraalbureau voor Schimmelcultures [CBS] 224.54) identified by S.M. Dixon (Rubner 1996) was greatly divergent from that of California *M. elliposporum* ATCC 204100 identified by B. Jaffe and sequenced in our study (FIG. 4). *Monacrosporium elliposporum* CBS 224.54 was more similar to *M. mammillatum* (isolate CBS 486.63, which originally was preserved as *Dactylella lysipaga* and later identified as *M. mammillatum* by Rubner 1996), a species that also produces stalked knobs.

Based on overall similarity comparison for fragment size and sequence, *M. drechsleri* was related most closely to *M. elliposporum* ATCC 204100 (91% identity versus 88% identity with *M. elliposporum*

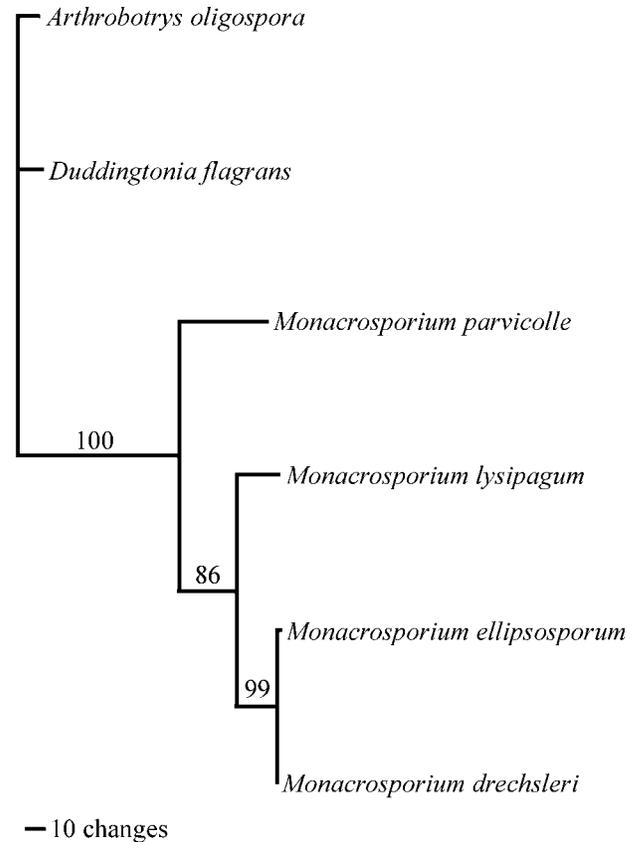


FIG. 5. Maximum parsimony EF1- α 50% majority rule consensus phylogenetic tree generated in PAUP*4.0 of Clustal W-aligned sequences for *Monacrosporium drechsleri*, *M. elliposporum* (ATCC 204100), *M. lysipagum*, *M. parvicolle* and two designated outgroups, *Arthrobotrys oligospora* and *Duddingtonia flagrans*. Heuristic bootstrapped search employing TBR branch-swapping with 1000 pseudoreplicates. Tree length = 128, Consistency index = 0.961, parsimony informative characters = 83.

CBS 224.54). This similarity was reflected in the ITS parsimony tree (FIG. 4) where *M. drechsleri* was positioned as sister taxon of *M. elliposporum* ATCC 204100. *Monacrosporium lysipagum* ATCC 28265 was positioned outside these two species, and *M. parvicolle* ATCC 96680 was positioned outside these three species (FIG. 4). The smaller EF1- α tree (FIG. 5) had identical topology for this clade of taxa but with higher bootstrap support (99 versus 77%) for *M. drechsleri* + *M. elliposporum*, lower support (86 versus 97%) for *M. lysipagum* outside, and higher support for *M. parvicolle* (100 versus 80%) outside the three species.

DISCUSSION

Five nematode taxa were used in our assay of nematode trapping by *M. drechsleri* NL 565, and the isolate trapped and immobilized 100% of all nematodes that

contacted the adhesive knobs. The *M. drechsleri* strain that was isolated by Tarjan (1961) also trapped *P. redivivus*, while another isolate of *M. drechsleri* trapped 98% of *Pratylenchus* spp. (i.e., *Pratylenchus brachyurus* and *Pratylenchus zaeae*) individuals, but only 51.8% of *Panagrellus* sp. individuals (Pria et al 1991). The low rate of trapping observed with *Panagrellus* sp. in the 1991 publication differs from our study, in which 100% of *P. redivivus* individuals were trapped. However, Pria et al (1991) speculated that the low trapping rate of the *Panagrellus* sp. in their investigation might have occurred because this nematode was added to the fungal cultures in much higher numbers than the *Pratylenchus* spp. Although an investigation with an isolate of *M. drechsleri* indicated that the fungus was not effective as a biocontrol agent against *M. incognita* (Pria and Ferraz 1996), *M. drechsleri* NL 565 was isolated from pots with declining populations of *M. arenaria*, introducing the possibility that the fungus under certain circumstances might be involved in suppression of nematode populations. Further studies in the soil would be needed to determine whether the fungus was primarily responsible for suppression of *M. arenaria* populations and, if it was a key factor, to ascertain whether this activity is correlated with efficacy as an applied biocontrol agent.

The new isolate of *M. drechsleri* (NL 565) varies morphologically somewhat from the original description in the frequency of 4-septate conidia (and by the observations of 5-septate conidia), the low numbers of bisepate conidia and the formation of chlamydospores (although these were observed on the holotype).

In the ITS tree it is notable that two isolates identified as *M. ellipso sporum*, ATCC 204100 and CBS 224.54, were phylogenetically so distant. While a distinguishing feature of mostly 4-septate conidia in *M. ellipso sporum* ATCC 204100 was present, the range of conidial measurements recorded for this isolate tended to be somewhat lower than the range reported by Rubner (1996) for *M. ellipso sporum* (TABLE I), although the ranges were overlapping. The conidial measurements for the *M. ellipso sporum* CBS 224.54 isolate were unavailable for a direct comparison with the other isolates. The CBS 224.54 isolate did not sporulate at the time it was examined by Rubner (1996), and morphological measurements were not provided with the original ITS tree (Liou and Tzean 1997). Thus we cannot be certain that the CBS isolate is any closer to type than the ATCC isolate. The differences between the *M. ellipso sporum* pair of morpho-species might indicate a species complex requiring further taxonomic consideration. Some variation is to be expected among ITS sequences within populations of a species, so other molecules and taxa are

needed to better interpret the phylogeny in this group. Because there are no unique, discrete morphological characters associated with these trees at this phylogenetic level, for identification purposes it is clear that morphology should be supplemented with at least one molecular identifier for this group of fungi.

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