Mycologia, 98(5), 2006, pp. 771–780.

Leptographium piriforme sp. nov., from a taxonomically diverse collection of arthropods collected in an aspen-dominated forest in western Canada

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Abstract: During a survey of fungi associated with arthropods collected in a southern boreal mixedwood forest in Alberta we obtained 29 isolates of a unique species of *Leptographium.* This species displayed a distinct combination of characteristics, including curved conidia on short-stipitate conidiophores, a secondary micronematous conidial state, stalked pear-shaped cells and an optimal growth rate at 35 C, and is described as *Leptographium piriforme* sp. nov. The isolates were most similar morphologically to *L. crassivaginatum,* but ITS sequence comparisons indicate that our isolates cannot be assigned to this or any other sequenced species in the genus. Initial observations on the pear-shaped cells in feeding experiments with *Sancassania berlesei* show that these structures may act as a nutritional incentive for visiting arthropods. Most arthropods carrying this new species were caught in traps baited with dung which, in light of its optimum growth temperature, suggests a coprophilous phase in the life cycle of this species. Additional isolates from woody species typical of the survey area might clarify whether *Leptographium piriforme* in its forest habitat occurs as a plant pathogen or saprobe.

Key words: Bromatia, Coprophilous, *Leptographium,* ITS, Morphology, Ophiostomatales

INTRODUCTION

Leptographium Lagerb. & Melin is a genus accommodating anamorphs affiliated with the Ophiostomatales and is defined on the basis of its macronematous, melanized conidiophores bearing brush-like clusters of annellidic conidiogenous cells that produce slimy masses of conidia. Each conidium is pushed aside after its formation by a pronounced percurrent proliferation of the annellide. Delayed secession of the conidia gives the conidiogenous axis a sympodial appearance (Wingfield 1985, Jacobs and Wingfield 2001).

There are 52 named species of *Leptographium* based on morphological and molecular characters (Wingfield 1985, van Wyk et al 1988, Hausner et al 2000, Jacobs and Wingfield 2001, Masuya et al 2004, Kim et al 2004, Kim et al 2005). Many are saprobes on plant material, particularly of coniferous origin, some are weak plant pathogens (Harrington 1988, Jacobs et al 1998, Jacobs and Wingfield 2001, Six and Bentz 2003) and others cause economically important timber diseases, such as black-stain root disease and white pine root decline (Harrington and Cobb Jr. 1986, Lewis and Alexander 1986, Lewis et al 1987, Harrington 1993). Arthropods are the presumptive carriers of these fungi and bark beetles are well documented vectors of the conifer pathogens (Wingfield 1985, van Wyk et al 1988, Hausner et al 2000, Jacobs and Wingfield 2001, Masuya et al 2004, Kim et al 2004, Kim et al 2005).

In a survey of the microfungi associated with arthropods visiting different types of organic debris in a broadleaf forest, dominated by *Populus tremuloides* Michx., in western Canada, 29 isolates of a unique species of *Leptographium* were obtained from a collection of trapped insects, spiders, and mites (Greif and Currah 2006). Its distinct morphology, along with the range of arthropod carriers and the nonconiferous habitat, suggested the taxon was new. Additional physiological and molecular characters supported the supposition that the species had not been described previously. In this paper we describe these unique isolates under the name *Leptographium piriforme* sp. nov.

MATERIALS AND METHODS

Arthropods were trapped May–Aug 2002 and 2003 in a broadleaf boreal forest dominated by *Populus tremuloides* 100 km east of Edmonton, Alberta. Traps were baited with dung (coyote and moose), rotted wood (brown- and white-rotted aspen), and fiberglass (Greif and Currah 2006). Trapped arthropods, 1687 in total, were identified and streaked on Mycobiotic™ agar (35.6 g Difco Mycobiotic agar [BD Biosciences, Mississauga, Ontario] in 1 L dH_2O) which contains 0.05 g/L chloramphenicol and 0.5 g/L

Accepted for publication 18 Aug 2006.
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cycloheximide. Colonies developing under ambient temperature and light of the laboratory in 4–6 mo were subcultured and identified. From among approximately 1700 isolates of microfungi, 29 conspecific isolates of *Leptographium* were identified. Six of these were deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH) (TABLE II). These six isolates were cultured in triplicate on Petri plates of cornmeal agar (CMA, 17 g Acumedia™ cornmeal media (Neogen Corp., Lansing, Michigan) and 1 L dH_20) and malt-extract agar (MEA, 15 g Difco™ malt extract (BD Biosciences, Mississauga, Ontario), 20 g Invitrogen™ select agar (Invitrogen Canada Inc., Burlington, Ontario) and 1 L dH_20) and incubated at 5 C increments (5–40 C) to determine growth rates and temperature optima. Colony diameters were measured twice at right angles at 5 d post inoculation, with the exception of isolates grown at 5 C and 10 C, which were measured at 15 d. Growth rates in mm/d ± standard deviation (SD) were calculated with the average diameter from both measurements for all three replicates of each of the six isolates.

Morphological data were obtained from isolates grown on CMA and MEA at room temperature under ambient light. The range and mean ± standard error (SE) for dimensions, rounded to the nearest decimal place, were based on measurements of 25 structures selected at random. Photographs of direct mounts stained with lactofuchsin (0.1 g acid fuchsin in 100 mL 85% lactic acid) were taken with a Canon Powershot A75™ digital camera (Canon Canada Inc., Mississauga, Ontario) through the eyepiece of an Olympus BH-2 light microscope (LM) (Olympus America Inc., Melville, New York).

For scanning electron microscopy (SEM), disks of agar, 5 mm diam and bearing conidiophores were fixed in buffered 2% glutaraldehyde 4 h, placed in phosphate buffer overnight at 5 C, and postfixed in 2% OsO_4 4 h at room temperature. Fixed material was dehydrated in an ethanol series, critical-point dried with carbon dioxide (SeeVac Inc., Hialeah, Florida), coated with gold using an Edwards S150 B gold coater (BOC-Edwards Inc., Crawley, Britain) and examined under a Hitachi S-2500 scanning electron microscope (Hitachi Ltd., Tokyo, Japan).

DNA sequences for the internal transcribed spacer region (ITS) of the nuclear rDNA region were obtained from subcultures of three isolates (UAMH 10680, 10682 and 10681) (TABLE II) on MEA overlaid with sterile Cellophane™ sheets (UCB Films, Somerset, Britain). DNA extraction was done following the method described by (Cubero et al 1999) with some modification. Approximately 100 mg of mycelium was scraped off the cellulose and ground in 2× CTAB extraction buffer (10% CTAB, NaCl, 0.25 M EDTA, 1 M Tris-HCl pH 8.0, 2% PVP, and dH_2O). After incubation for 2 h in a 65 C water bath, genomic DNA was extracted with a chloroform:isoamyl alcohol (24:1v/v) solution. Crude DNA was purified with a QIAquick DNA purification kit (QIAGEN Inc., Mississauga, Ontario). Amplification of the ITS region was done with the primers ITS1 (White et al 1990) and BMB-CR (Lane et al 1985). PCR was run 30 cycles in a PE GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, California) set to these parameters: denaturation at 94 C for 1 min, annealing at 55 C for 1 min, extension at 72 C for 2 min, final extension at 74 C for 7 min followed by a cool down stage at 4 C for 10 min. The amplicon was purified with the QIAquick DNA purification kit and DNA concentration was determined with a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, Delaware). Cycle sequencing was done with internal primers ITS1, ITS2, ITS4, and BMB-CR (White et al 1990, Lane et al 1985) and BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, California). The amplicons were run on an ABI 377 automated DNA sequencer (Amersham Pharmacia Biotech Inc, Piscataway, New Jersey). A consensus sequence was constructed and edited with Sequencher version 4.0.2 (Gene Codes Corp., Ann Arbor, Michigan). The newly determined ITS sequence, was subjected to a BLAST search (Altschul et al 1997) to find related sequences in GenBank (www.ncbi.nlm.nih.gov).

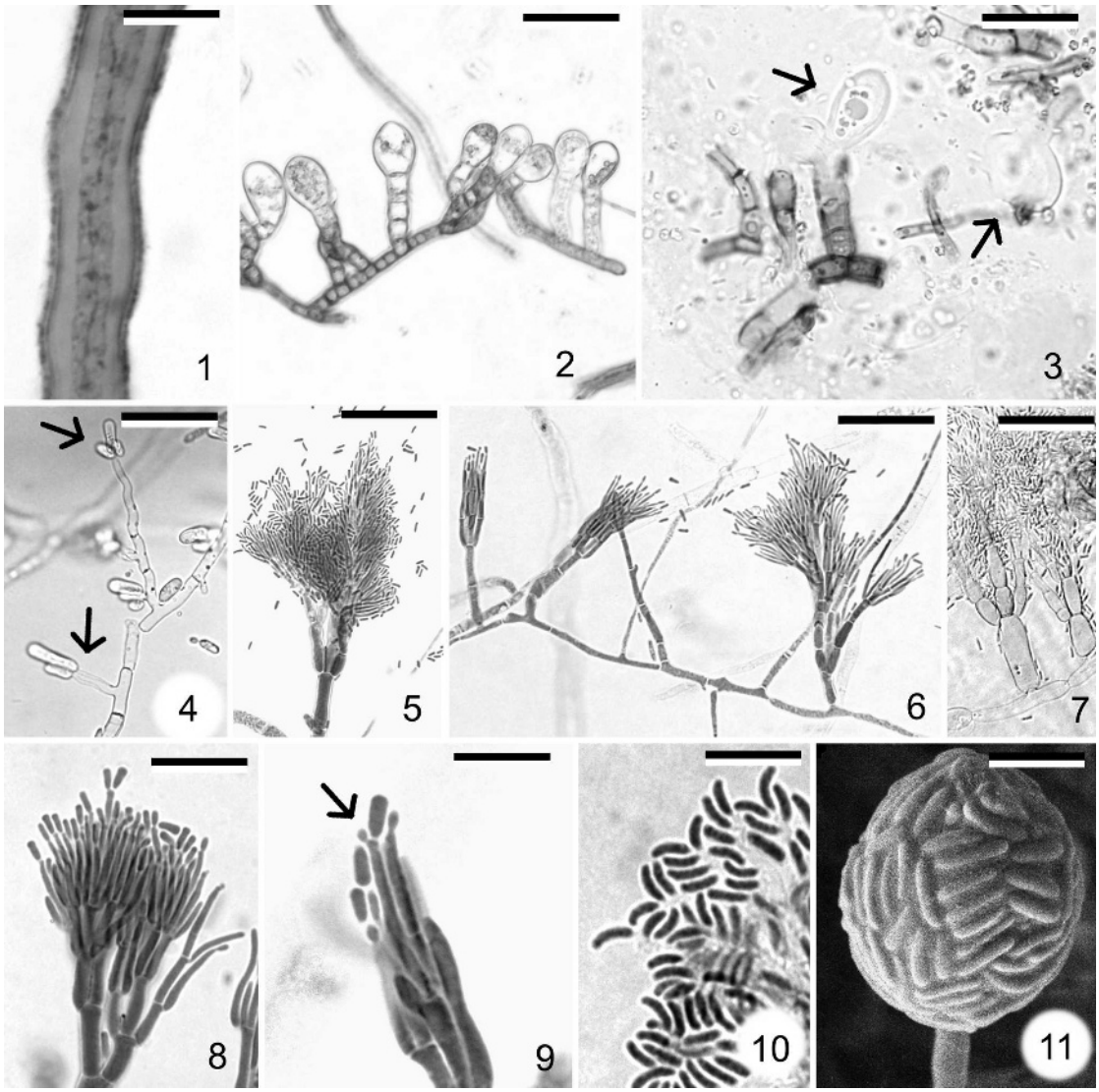
The newly determined ITS sequence (GenBank DQ885241) was manually aligned with 22 ITS sequences of representative species of *Leptographium* and *Ophiostoma* retrieved from GenBank (www.ncbi.nlm.nih.gov) (FIG. 16) with Se-Al v2.0a11 Carbon (http://evolve.zoo.ox.ac.uk/). *Peziza varia* (GenBank AF491568) was used as outgroup. The aligned sequence matrix was analyzed with PAUP v 4.0 b10 (Swofford 2002). A heuristic search was done with parsimony as the optimality criterion. Gaps were treated as missing data. Starting trees were obtained at random via stepwise addition with tree-bisection-reconnection as the branch-swapping algorithm. Confidence in the branches of the resulting trees was evaluated by bootstrap analysis (Felsenstein 1985) with 100 replicates.

TAXONOMY

Leptographium piriforme Greif, Gibas, and Currah, sp. nov. FIGS. 1–15

Latin Diagnosis: Conidiophora macromatea cum stirpe brevi, 7.2–45.6 µm longitudine. Apparatus conidiogenosus 28.8–93.6 µm a basi ramorum primariorum ad apicem cellularum conidiogenosarum, cum 2–3 (rare 4) ordinibus ramorum. Rami primarii 2–3 numero in conidiophoris quibusque, pigmentati, leves, aliquando septati, 3.4–15.4 × 1.9–4.8 µm. Rami secundarii pigmentati, aliquando septati, leves, 7.2–17.3 × 1–4.8 µm. Rami tertiani et quartani hyalini, leves, 3.8–11.5 × 1–1.9 µm. Annelida orientia a ramis secundariis vel tertianis vel quartanis, saepe solitaria in ramis secundariis vel in catenis 2–4 continentibus in ramis tertianis vel quartanis, fastigata ad apicem, 8.6–15.4 × 1.0–1.4 µm. Conidia aseptata, hyalina, levia, curvata, truncata ad extremum proximum, (2–)4–4.6 × 1.0–1.4 µm, accumulantia in stilla limosa. Conidiophora micronematea abundantia, simplicia, claviformia, orientia directe a hyphis vegetativis, producent conidia hyalina et tenuiter curvata, 4.8–14.4 × 2.4–4.8 µm. Cellulae piriformes abundantes, 14.4–31.2 × 7.2–16.8 µm, portatae in culmis 1–4 cellulas continentibus, 7.2–45.6 × 4.8–7.2 µm. Incrementum optimum ad 35 C.

Holotypus: Cultura exsiccata preparata a UAMH 10680, isolata a musca capta in laqueo excrementum canis latrantis continenti, collecta 16 Iul 2003 in silva populorum

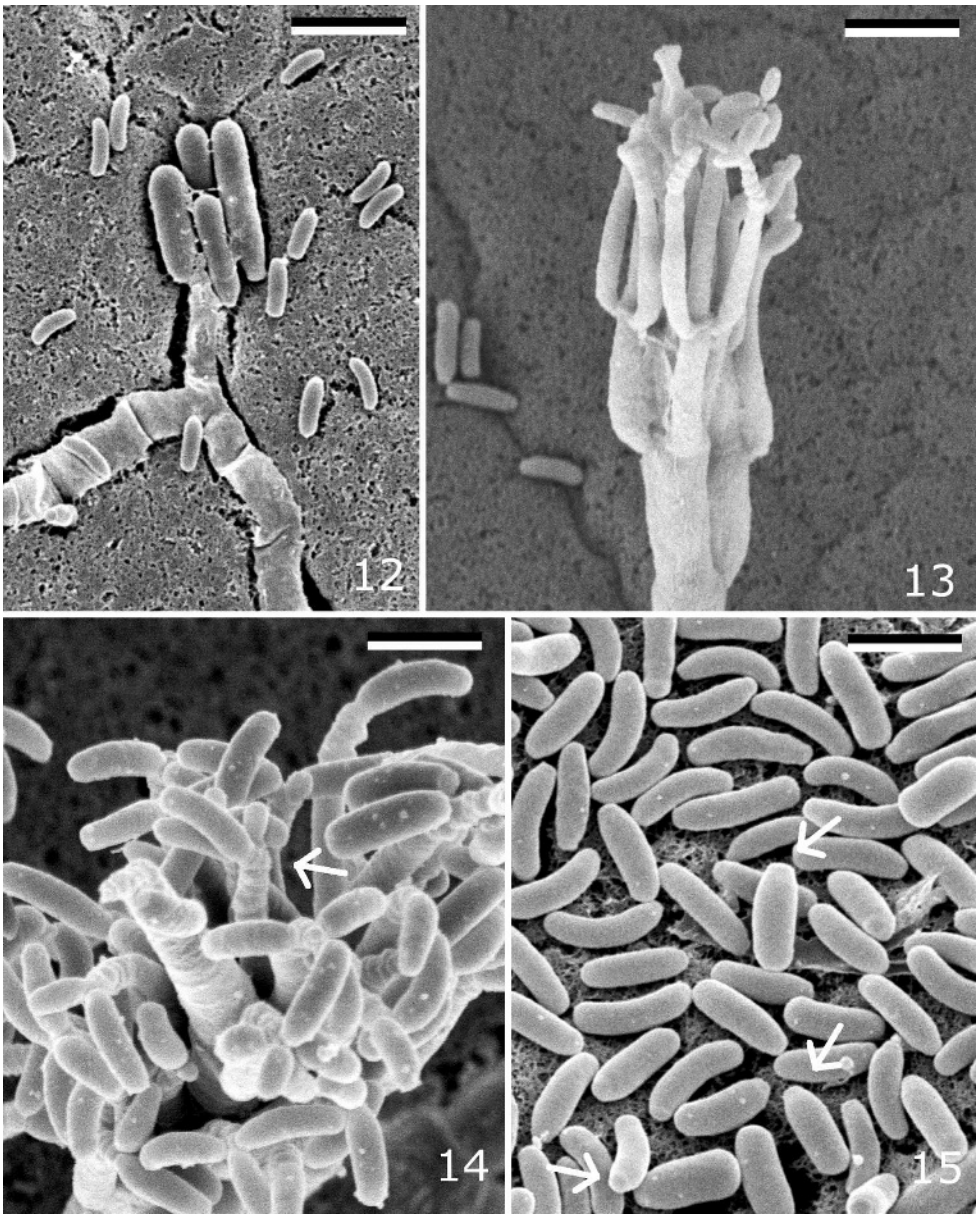


FIGS. 1–11. *Leptographium piriforme*. 1. Vegetative hypha with a granular surface on MEA. Bar = 8.5 µm. 2. Pear-shaped cells borne on septate stalks on MEA. Bar = 22 µm. 3. Gut contents of *Sancassania berlesei* after grazing on colony. Arrows indicate remnants of pear-shaped cells. Bar = 26 µm. 4. Micronematous conidiophores on CMA. Conidia aggregate in loose clusters on tips of peg-like conidiophores (arrows). Note that vegetative hyphae are smooth-walled on CMA. Bar = 26 µm. 5. Macronematous conidiophore displaying a characteristic branching head. Bar = 26 µm. 6. Macronematous conidiophores arising from a vegetative hypha. Note variation in stipe length and septation. Bar = 38 µm. 7. Conidiophores with symmetrical conidiogenous apparatus. Bar = 26 µm. 8. Conidiogenous apparatus with primary, secondary, and tertiary branches. Conidiogenous cells arise from both secondary and tertiary branches. Bar = 22 µm. 9. Close up of conidiogenous cells. Conidiogenesis annellidic with delayed succession (arrow). Bar = 7 µm. 10. Mature conidia are curved, aseptate, and smooth walled. Bar = 7 µm. 11. Conidia form a slimy mass at the tip of a conidiogenous apparatus (SEM). Bar = 6 µm.

tremuloidum 2(km a Elk Island National Park, Alberta, Canada.

Etymology: Species epithet is derived from the Latin word for pear (*pirum*) in reference to the pear-shaped cells formed in culture.

On CMA mycelium sparse, hyaline and submerged; conidia forming in white to beige droplets on agar surface. On MEA mycelium on surface of agar abundant, light brown, dense, floccose; submerged mycelium dark brown, conidia forming in white to beige droplets among the aerial hyphae. Vegetative hyphae septate, 2–10 µm wide, smooth-walled on CMA and roughened on MEA with a granular surface (FIG. 1). Pear-shaped cells 14.4–31.2(23.4 ± 0.8) × 7.2–16.8(12 ± 0.4) µm, borne on a one- to four-celled stalk, 7.2–45.6(27.4 ± 2.6) × 4.8–7.2(4.2 ± 0.3)



FIGS. 12–15. Scanning electron micrographs of *L. piriforme*. 12. Conidia produced by micronematous conidiophores are two to three times larger than conidia produced by macronematous conidiophores. Bar = 6 µm. 13. Macronematous conidiophore displaying conidiogenous cells and conidia. Bar = 5 µm. 14. Conidiogenous cells displaying annellations at tips and signs of delayed conidial succession (arrow). Bar = 3 µm. 15. Conidia produced by macronematous conidiophores display a distinct curvature and are truncate at the proximal end (arrows). Bar = 3 µm.

µm (FIGS. 2–3) abundant 18 d post inoculation on MEA only.

Conidiophores micronematous and macronematous, forming simultaneously. Micronematous conidiophores abundant, simple and peg-like, arising directly from vegetative hyphae and producing hyaline, slightly curved conidia, 4.8–14.4(9.7 ± 0.5) × 2.4–4.8(2.6 ± 0.1) µm (FIGS. 4, 12). Macronematous conidiophores with short stipes, 7.2–45.6(29.9 ± 3.9) µm long and up to five cells long (FIGS. 5–6). Basal cell cylindrical, pigmented, 7.2–1.6(12.2 ± 0.9) × 2.4–7.2(5.2 ± 0.3) µm. Apical cell cylindrical, pigmented, 9.6–21.6(14.5 ± 0.9) × 2.4–7.2(5.2 ± 0.3) µm.

Conidiogenous apparatus 28.8–93.6(57.4 ± 3.8) µm from base of primary branches to tip of conidiogenous cells, with 2–3 (rarely 4) tiers of cylindrical branches. Primary branches symmetrically arranged, 2–3 per conidiophore, pigmented, smooth, occasionally septate, 3.4–15.4(8.8 ± 0.7) × 1.9–4.8 (3.4 ± 0.2) µm (FIG. 7). Secondary branches pig-

TABLE I. Mean growth rates of *Leptographium piriforme* on malt extract agar incubated at 5 degree increments from 5 to 40 C

Temperature (C)	Growth rate (mm/d ± SD)
5	0
10	0.9 ± 0.3
15	1.6 ± 0.6
20	3.7 ± 1.1
25	6.6 ± 1.6
30	9.8 ± 1.2
35	10.3 ± 0.4
40	0

mented, occasionally septate, smooth, 7.2–17.3(10.9 ± 0.5) × 1–4.8(2.7 ± 0.2) µm. Tertiary and quaternary branches hyaline, smooth, 3.8–11.5(7.6 ± 0.4) × 1–1.9(1.4 ± 0.1) µm. Conidiogenous cells annellides, developing from secondary, tertiary, and quaternary branches, often solitary on secondary branches and in groups of 2–4 on tertiary/quaternary branches, tapering toward apex, 8.6–15.4(11.7 ± 0.4) × 1.0–1.4(1 ± 0.02) µm (FIGS. 8–9, 13).

Conidia aseptate, hyaline, smooth, curved, truncate at the proximal end, 2.4–4.6(3.8 ± 0.1) × 1.0–1.4(1 ± 0.05) µm, slow to secede from annellides and accumulate in a mass at the tip of conidiogenous cells to form a slimy droplet (FIGS. 10–11, 14–15).

Optimal growth at 35 C (10.3 mm/d ± 0.4 mm) on MEA. No growth detected below 5 C or above 40 C (TABLE I).

Specimens examined. Canada. Alberta: Edmonton, Elk Island National Park. From a fly captured in a trap baited with coyote dung, 16 Jul 2003, collected by M.D. Greif, *Greif UAMH 10680*, (Holotype. UAMH).

Canada. Alberta: Edmonton, Elk Island National Park. From a springtail caught in trap baited with brown-rotted wood, 28 May 2003, collected by M.D. Greif, *Greif UAMH 10682* (B-182), (Paratype. UAMH). Canada. Alberta: Edmonton, Elk Island National Park. From a beetle caught in a trap baited with coyote dung, 23 Jul 2003, collected by M.D. Greif, *Greif UAMH 10681* (C-439), (Paratype. UAMH). Canada. Alberta: Edmonton, Elk Island National Park. From a fly caught in trap baited with coyote dung, 23 Jul 2003, collected by M.D. Greif, *Greif UAMH 10683* (C-445), (Paratype. UAMH). Canada. Alberta: Edmonton, Elk Island National Park. From an ant caught in a trap baited with moose dung, 16 Jul 2003, collected by M.D. Greif, *Greif UAMH 10685* (M-273), (Paratype. UAMH). Canada. Alberta: Edmonton, Elk Island National Park. From a caterpillar caught in a trap baited with fibreglass, 20 Aug 2003, collected by M.D. Greif, *Greif UAMH 10684* (Ct-111), (Paratype. UAMH).

Provenance data for the 29 isolates of *Leptographium piriforme* are provided (TABLE II).

Molecular analysis.—Sequences of the ITS region of the nuclear rDNA were compared among 24 isolates. The final alignment generated a total of 561 characters, 211 of which were constant, 164 were parsimony uninformative and 186 were parsimony informative. Maximum parsimony analysis yielded two most parsimonious trees (MPT) one of which is shown (FIG. 16). The consistency index (CI) was 0.760, the homoplasy index (HI) 0.240 and the retention index (RI) was 0.790

Leptographium piriforme formed a distinct basal lineage within a clade with bootstrap support of 77% containing two isolates of *L. lundbergii* Lagerb. and Melin (one of which had been submitted as *L. truncatum*), *L. terebrantis* S.J. Barras & T.J. Perry, *L. wingfieldii* M. Morelet, *Ophiostoma clavigerum, L. guttulatum* M.J. Wingf. & K. Jacobs, *L. wageneri* (W.B. Kendr.) M.J. Wingf., *L. procerum* (W.B. Kendr.) M.J. Wingf., *O. laricis* K. van der Westh., Yamaoka and M.J. Wingf., and *O. europhioides* (E.F. Wright & Cain) H. Solheim (FIG. 16). This clade was distinct from other clades containing *Ophiostoma* species with known *Leptographium* anamorphs, such as *Ophiostoma penicillatum* (Grosmann) Siemaszko (anamorph = *L. penicillatum* Grosmann), *O. americanum* K. Jacobs & M.J. Wingf. (anamorph = *L. americanum* K. Jacobs & M.J. Wingf.), and *O. dryocoetidis* (W.B. Kendr. & Molnar) de Hoog and R.J. Scheff (anamorph = *L. dryocoetidis* M.J. Wingf.). The subgeneric affinity of the new taxon, specifically among *Leptographium* species, however, was unresolved.

DISCUSSION

Leptographium piriforme most closely resembles *L. crassivaginatum* M.J. Wingf. in having short stipitate conidiophores and similar, although larger, pear-shaped cells (TABLE III) (Griffin 1968, Jacobs and Wingfield 2001). Conidia produced by *L. piriforme*, however, are curved rather than oblong and conidiogenous cells are approximately half the width of their counterparts in *L. crassivaginatum* (TABLE III). In addition *L. crassivaginatum* grows more slowly in culture and shows little growth at 35 C (Jacobs and Wingfield 2001) which is the optimum for *L. piriforme* (TABLES I and III). *Leptographium piriforme* is unique in the genus in having an unusually high optimum growth rate at 35 C (TABLE I). Most other species grow slowly or not at all at this temperature (Jacobs and Wingfield 2001) although *Ophiostoma grandifoliae* (R.W. Davidson) T.C. Harr. and *O. leptographioides* (R.W. Davidson) Arx are reported to show ''some'' and ''significant'' growth, respectively, at 35 C (Jacobs and Wingfield 2001), and *L. calophylli* (Wiehe) J. Webber, K. Jacobs & M.J. Wingf. can grow at temperatures just under 40 C (Webber et al 1999).

The size and curvature of conidia in *L. piriforme*

TABLE II. Provenance data for isolates of *Leptographium piriforme* obtained from arthropods. Isolates marked with an asterisk were used to measure morphological and physiological characteristics

Isolate number	UAMH Number	Bait type	Date collected	Arthropod
*182	10682	Brown-rotted wood	May 28/03	Collembola (Springtail)
190		Brown-rotted wood	May 28/03	Hymenoptera: Formicidae (Ant)
138b		Coyote dung	July 18/02	Coleoptera (Beetle)
140		Coyote dung	July 18/02	Trichoptera (Caddisfly)
195		Coyote dung	Aug 21/02	Hemiptera (Bug)
196		Coyote dung	Aug 21/02	Diptera (Fly)
197		Coyote dung	Aug 21/02	Hymenoptera: Formicidae (Ant)
262		Coyote dung	May 28/03	Coleoptera (Beetle)
312		Coyote dung	June 25/03	Diptera (Fly)
313		Coyote dung	June 25/03	Coleoptera (Beetle)
365		Coyote dung	July 2/03	Coleoptera (Beetle)
367		Coyote dung	July 2/03	Acari (Mite)
368		Coyote dung	July 2/03	Pscoptera (Booklice)
369		Coyote dung	July 2/03	Lepidoptera (Moth)
375		Coyote dung	July 9/03	Hymenoptera: Formicidae (Ant)
379		Coyote dung	July 9/03	Acari (Mite)
405		Coyote dung	July 16/03	Hemiptera (Bug)
406		Coyote dung	July 16/03	Hymenoptera: Formicidae (Ant)
*421	10680	Coyote dung	July 16/03	Diptera (Flies)
*439	10681	Coyote dung	July 23/03	Coleoptera (Beetle)
*445	10683	Coyote dung	July 23/03	Diptera (Fly)
448		Coyote dung	July 23/03	Acari (Mite)
536		Coyote dung	Aug 27/03	Hymenoptera: Formicidae (Ant)
537		Coyote dung	Aug 27/03	Hymenoptera: Formicidae (Ant)
79		Fibregass	July 2/03	Araneae (Spider)
*111	10684	Fibregass	Aug 20/03	Lepidoptera (Moth)
*273	10685	Moose dung	July 16/03	Hymenoptera: Formicidae (Ant)
85		White-rotted wood	July 24/02	Araneae (Spider)
186		White-rotted wood	July 2/03	Araneae (Spider)

make the species somewhat similar to *L. abietinum, L. hughesii* and *L. penicillatum*, but the conidiophores of all three have a longer stipe and the conidia of *L. penicillatum* are considerably larger than those of *L. piriforme* (TABLE III). Stipe length varies in *L. abietinum* (35–440 µm), overlapping slightly at the lower end with the 7.2–45.6 µm stipe length of *L. piriforme*. However the stipe of *L. abietinum* is 2–7 cells long (Jacobs and Wingfield 2001) and one-celled stipes (as observed in *L. piriforme*) are not produced (TABLE III). *Leptographium yunnanensis* X.D. Zhou, K. Jacobs & M.J. Wingf., *L. robustum* M.J. Wingf. and *L. calophylli* also produce short stipitate conidiophores (8–85 µm, 9–39 µm and 5–30 µm respectively) but these species have oblong conidia and lack micronematous conidiophores (Jacobs and Wingfield 2001). Finally, *L. pruni* H. Masuya & M.J. Wingf. is also similar to *L. piriforme* in being short stipitate (32–190 um) and producing micronematous conidiophores but differs in the generation of oblong to ellipsoidal conidia, and in having a *Sporothrix* synanamorph (Jacobs and Wingfield 2001, Masuya et al 2004).

The unusual stalked pear-shaped cells in *Leptographium piriforme* on MEA also are found in *L. crassivaginatum* (Griffin 1968, Jacobs and Wingfield 2001). Griffin (1968) did not suggest a function for these structures, but they bear a striking resemblance to the gongylidia (or bromatia, fide Kirk et al 2001) produced by *Leucoagaricus gongylophorus* (A. Møller) Singer, a basidiomycete actively cultivated on piles of masticated plant material by leaf cutter ants (North et al 1997). Gongylidia are snipped off and eaten by tending ants or fed to larvae (Bass and Cherrett 1996). In *L. piriforme* these structures might function as a nutritious reward or attractant for presumed arthropod carriers, increasing the chances for spore contact and dispersal. Casual experiments (data not shown) done with reared mites (*Sancassania berlesei* Michael) showed that more individuals moved toward inoculated blocks of agar bearing mycelium with pear-shaped cells than without. The abundant fragments of

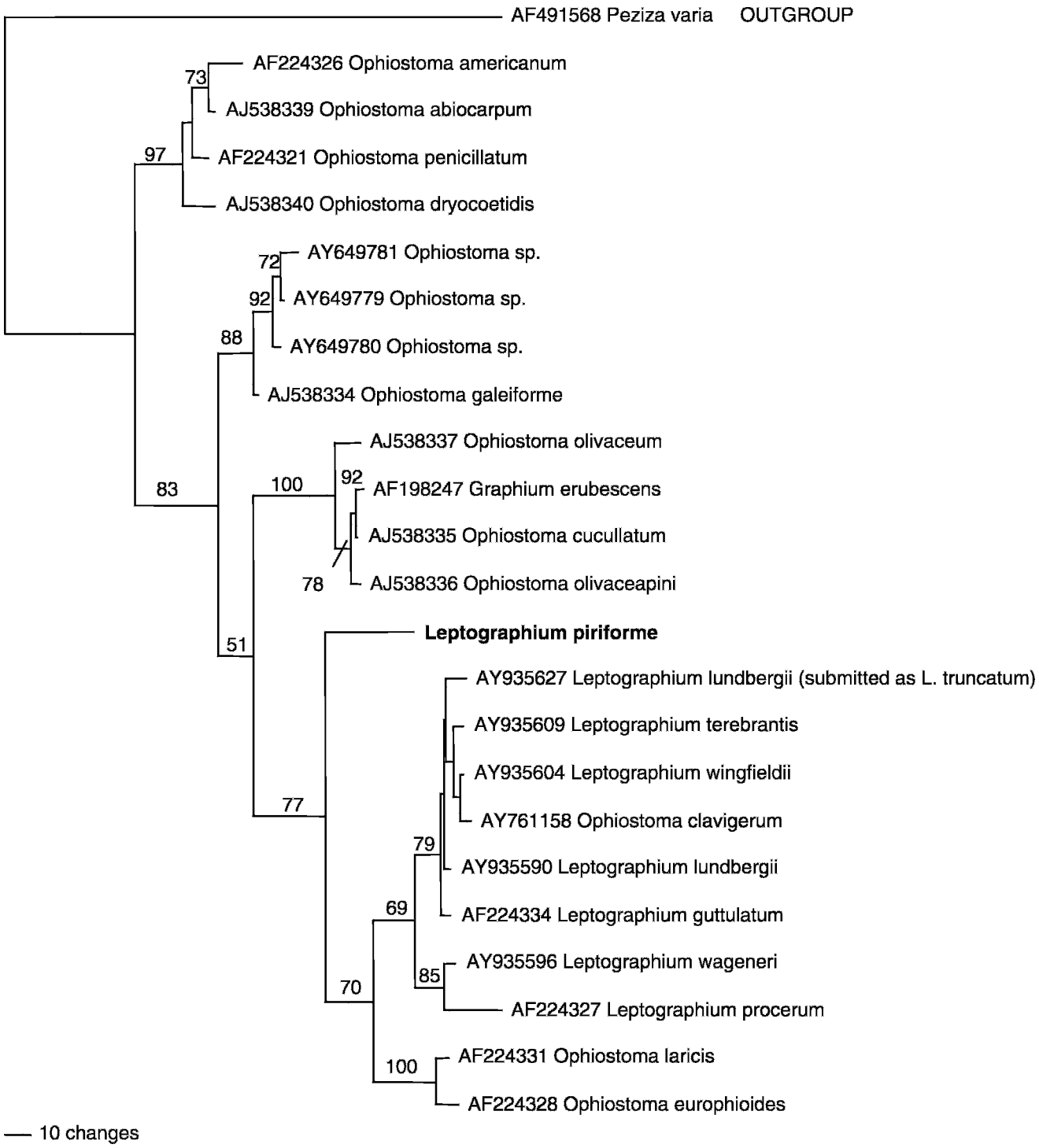


FIG. 16. One of two most-parsimonious trees generated from ITS sequences of representative species of *Leptographium*, *Ophiostoma*, and *Graphium*. *Leptographium piriforme* forms a basal lineage in a clade predominantly composed of *Leptographium* species. Genbank accession numbers are listed beside species. Bootstrap values >50% are shown above branches.

the pear-shaped cells in expressed gut contents (FIG. 3) confirmed that the animals did graze on them. If these structures do form in nature, their role in attracting or at least prolonging the length of visitation by arthropods presumably would lead to larger numbers of the sticky conidia being picked up for transport to new habitat.

Sequence data (ITS) for 34 named species of

TABLE III. Characteristics of *Leptographium piriforme* and other morphologically similar *Leptographium* species

	Leptographium piriforme	*Leptographium crassivaginatum* M.J. Wingf.*	*Leptographium hughesii* K. Jacobs, W.J. Wingf. and T.C. Harr.*	*Leptographium abietinum* (Peck) M.J. Wingf. *	*Leptographium penicillatum* Grossmann*
Conidial shape	Curved	Ellipsoidal	Curved	Curved	Curved
Conidial size	2.4–4.6 × 1–1.4 µm	4–5 × 1–2 µm	3–5 × 1–2 µm	(3–) 4–5 (–7) × 1–2 µm	(4–) 6–7 (–10) × 2–3 µm
Conidiogenous cell dimensions	8.6–15.4 × 1–1.4 µm	(7–) 8–10 (–12) × 2–3 µm	(8–) 9–15 (–18) × 1–2 µm	10–23 (–25) × 1–2 µm	(10–) 12–16 (–25) × 2–3 µm
Conidial apparatus length	28.8–93.6 µm	15–55 (–60) µm	(30–) 67–89 (–175) µm	(25–) 45–50 (–100) µm	(35)– 51–87 (–110) µm
Stipe length	7–45.6 µm	8–60 (–85) µm	(210–) 484–711 (–1130) µm	35–440 (–470) µm	(75–) 199–248 (–340) µm
Number of cells per stipe	One to four	One to six	Four to eighteen	Two to seven	One to ten
Secondary conidial state	Micronematous conidiophores	None reported	None reported	None reported	None reported
Teleomorph	None reported	*Ophiostoma crassivaginatum* H.D. Griffin) T.C. Harr.	None reported	None reported	*Ophiostoma penicillatum* (Grossmann) Siemaszko
Pear-shaped cells	Stalk 7.2–45.6 × 4.8–7.2 µm Tip 14.4–31.2 × 7.2–16.8 µm	12–20 × 8–12 µm**	None reported	None reported	None reported
Arthropod associates	Coleoptera, Diptera, Araneae, Acari, Hemiptera, Lepidoptera, Collembola, Pscoptera, Trichoptera, and Hymenoptera: Formicidae	Coleoptera: *Trypodendron retusus*	None reported	Coleoptera: *Dendroctonus, Hylastes,* and *Hylurgops* sp.	Coleoptera: *Dendroctonus, Hylastes, Hylurgops, Dryocoetus, Ips, Pityogenes, Polygraphum, Tetropium,* and *Trypodendron* sp.
Optimal growth temp on MEA	35 C	30 C	25 C	25 C	30 C
Growth rate at optimum	51 mm in 5 d	31 mm in 9 d	8 mm in 8 d	39 mm in 8 d	12 mm in 8 d
Hosts	Unknown	Angiosperms: *Populus grandidentata, P. tremuloides* Conifers: *Picea* and *Pinus* sp.	Angiosperms: *Parashorea plicata, Aquilana* sp.	Conifers: *Picea* and *Pinus* spp.	Conifers: *Abies, Picea,* and *Pinus* spp.
Distribution	Alberta, Canada	Canada	Vietnam	N.W. United States and W. Canada	Europe and Japan

*Data from Jacobs and Wingfield 2001.

**Additional data from Griffin 1968.

Leptographium, and 101 named species of its teleomorphic counterpart *Ophiostoma*, were available in GenBank (www.ncbi.nlm.nih.gov) but none matched *L. piriforme*. *L. wageneri* was the closest, but it has a much longer stipe (503–757 μm), oblong rather than curved conidia, and an optimal growth rate at 20 C (Jacobs and Wingfield 2001). A partial sequence of the ITS region (200 bp) of *L. crassivaginatum* was available but was not used in the sequence matrix. However a BLAST search comparing corresponding sequences of *L. piriforme* and *L. crassivaginatum* resulted in only 93% similarity. The use of the ITS region in constructing a phylogenetic hypothesis, while confirming the generic placement of *L. piriforme*, was unable to imply affinities at the species level. Better resolution of the relationships among *L piriforme* and other members of the genus, in particular the morphologically similar *L. crassivaginatum*, might be resolved by an examination of the β-tubulin gene (Kim et al 2004, Kim et al 2005).

An association with beetles is often cited as a characteristic of species of *Leptographium* (e.g. *L. wageneri, L. procerum, L. abietinum* and *L. terebrantis*) (Cobb Jr. 1988, Lewis and Alexander 1988, Jacobs and Wingfield 2001) and some have evolved mutualisms with these animals. For example bark beetles in the family Scolytidae carry inoculum in mycangia to uninfested trees where both eggs and fungi are deposited (Jacobs and Wingfield 2001). The fungus becomes established in its new environment and can affect the nutrition and survival of larvae (Jacobs and Wingfield 2001, Six and Bentz 2003). However, of 1687 arthropods obtained in the survey (Greif and Currah 2006), none was from the family Scolytidae, which might account for the lack of other *Leptographium* species among isolated fungi. Some *Leptographium* species have been reported from a wider range of arthropods, indicating a casual or nonspecific association (Olchowecki and Reid 1974, Harrington 1988). Twenty-nine isolates of *L. piriforme* were obtained from arthropods not generally associated with the genus. While cross contamination might account for some of these isolates, the diversity of arthropods carrying *L. piriforme* was greater than any reported for other *Leptographium* species and suggests this species has a much less specific type of relationship.

The production of pear-shaped cells might serve to attract a diverse range of arthropod carriers to sites where the fungus is sporulating, but the preferred host or substrate of *L. piriforme* is unknown. Because most isolates (i.e. 23 of 29, TABLE II) were from arthropods caught in traps baited with animal dung, it is possible that the fungus is coprophilous. The unusually high optimum growth temperature would suggest that composting dung piles indeed might be a place to look for this species. On the other hand inoculum might have been picked up from soil, colonized plant material or through casual transfer from other arthropods. At any rate the habitat is unique; few species of *Leptographium*, or *Ophiostoma* with *Leptographium* anamorphs, have been described previously as associates of the dominant plants in the southern Boreal broadleaf forests of western Canada where Salicaceae (e.g. *Populus tremuloides, Salix* spp.) and Betulaceae (e.g. species of *Alnus, Betula* and *Corylus*) are the dominant components of the canopy (Farr et al 1989). Most species of *Leptographium* are associated with conifers or conifer wood; only two are known from soil (*L. reconditum* Jooste and *L. costaricense* G. Weber, Spaaij, & M.J. Wingf.) (Jooste 1978, Weber et al 1996), and eight from broadleaf trees (*O. leptographioides, L. francke-grosmannia* K. Jacobs & M.J. Wingf., *O. grandifoliae, L. eucalyptophilum* K. Jacobs, M.J. Wingf. & J. Roux, *O. brevicolle* [R.W. Davidson] de Hoog & R.J. Scheff, *L. hughesii, L. pruni*, and *O. crassivaginatum* [see Farr et al 1989 and Jacobs and Wingfield 2001]). Additional sampling directly from various types of substrate including soil, plant material and dung might result in additional isolates of *L. piriforme*, which will help clarify the life history of this species.

ACKNOWLEDGMENTS

This work was made possible by a Challenge Grant in Biodiversity to MDG and by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to RSC. Technical assistance with SEM images was provided by Dr Ming Chen. The authors thank Marcie Plishka and Ben Wilson for assistance with field work. We also thank Michael Hertwig-Jaksch for providing the Latin translation.

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