Mycologia, 96(3), 2004, pp. 548–557.

Leptographium pruni, sp. nov. from bark beetle-infested *Prunus jamasakura* in Japan

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Abstract: *Leptographium* species are anamorphs of *Ophiostoma,* commonly isolated from conifer. There are, however, a small number of these fungi that have been collected from angiosperm hosts. In this study, we describe *Leptographium pruni,* sp. nov. isolated from the bark of *Prunus jamasakura* infested by the bark beetle *Polygraphus ssiori.* This new species is unusual in having a distinct *Sporothrix* synanamorph with ramoconidia. No evidence of a teleomorph was found, but a high level of tolerance to the antibiotic cycloheximide and the presence of a *Sporothrix* synanamorph suggest that *L. pruni* is an *Ophiostoma* anamorph. Analysis of sequence data for the domain 1 region of the LSUrDNA operon also supports the phylogenetic relationship of *L. pruni* with *Ophiostoma.* In addition, sequence data suggest that *L. pruni* is related to other species of *Leptographium* rather than *Pesotum* species with distinct *Sporothrix* synanamorphs.

Key words: *Leptographium, Prunus, Sporothrix* synanamorph, ITS, LSUrDNA, Ophiostomatales

INTRODUCTION

Species of *Leptographium* Largeberg & Melin are characterized by dark mononematous conidiophores terminating in a conidiogenous apparatus made up of a series of branches and conidiogenous cells that produce hyaline 1-celled conidia in mucilaginous masses (Kendrick 1962, Wingfield 1993). This morphology is adapted for dispersal by insects, especially bark beetles (Coleoptera:Scolytidae; Lagerberg et al 1927, Upadhyay 1981, Harrington 1988, Jacobs and Wingfield 2001). Most *Leptographium* species are thought to be anamorphs of *Ophiostoma* H. & P. Sydow (Harrington 1988, Wingfield 1993, Jacobs and Wingfield 2001), although teleomorphs are known only for a small number of the species.

Leptographium includes economically important species that cause blue stain in lumber (Lagerberg et al 1927, Harrington 1988, Gibbs 1993, Seifert 1993, Jacobs and Wingfield 2001). A few species, such as *L. wageneri* (W.B. Kendrick) Wingfield, are important root pathogens of conifers (e.g., Wagener and Mielke 1961, Leaphart 1960, Smith 1967). Most species of *Leptographium* occur on conifers, and only a few occur on angiosperms (Jacobs and Wingfield 2001). This might reflect the relatively few studies on *Ophiostoma* spp. or their anamorphs on hardwoods, or the absence of blue-stain problems in this wood type.

During a survey of ophiostomatoid fungi in Japan, a species of *Leptographium* was isolated from *Prunus* infested with the bark beetle *Polygraphus ssiori* Nijima. This fungus was unusual because of a distinct *Sporothrix* Hektoen & Perkins synanamorph, particularly, with ramoconidia. The well developed *Sporothrix*-like synanamorph and ramoconidia are unknown in other *Leptographium* species or unnamed *Leptographium* anamorphs of *Ophiostoma* (Jacobs and Wingfield 2001). The aim of this study was to describe this species. In addition, its taxonomic position was confirmed based on partial rDNA sequence data.

MATERIALS AND METHODS

Fungus isolations.—Isolations were made from the bark beetle *P. ssiori* and from the walls of its galleries in the bark of dead trees of a *Prunus jamasakura* Seib. The fungus was collected on the same tree species at two localities in northern Japan (Higashidohri Village, Aomori Prefecture, and Morioka City, Iwate Prefecture) in May 2000. A total of 162 beetles and 100 pieces of bark from galleries of the insects were used for isolations.

Adult beetles or two small pieces of bark were placed on the surface of 1% malt-extract agar (Difco malt extract, 10 g; agar, 15 g; distilled water, 1000 mL) without surface ster-

Accepted for publication November 22, 2003.
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ilization, and the plates were incubated at 15 C in the dark. After 2 mo, fungi that had grown on the plates were isolated by transferring hyphal tips or by lifting conidial masses, using a sterilized tungsten needle, to Petri dishes containing 2% MEA (Difco malt extract, 20 g; agar, 15 g; distilled water, 1000 mL). These dishes were incubated at 15 C in the dark for an additional 2 wk.

Morphology.—The isolates were incubated at 15 C in the dark on 2% MA, and after 2 wk small pieces of sterilized twigs or bark of *Prunus* sp. were added to the plates to stimulate sporulation. Conidiophores and conidia that were produced in cultures were mounted on glass slides in 1% lacto-fuchsin and observed and measured with a light microscope. Fifty measurements were made of each structure, and the ranges, averages and standard deviations were computed.

In preparation for scanning electron microscopy (SEM), agar disks 8 mm diam were cut from the colonies and fixed in 2.5% glutaraldehyde overnight and 1% osmium tetraoxide for 1 h. Specimens were dehydrated in a graded ethanol series, passed through ethanol-isoamylacetate and dried with a Hitachi critical-point dryer. The specimens were mounted and coated with gold palladium alloy and examined with a JOEL JSM5310LV scanning electron microscope.

The growth rates of isolates were determined at 4, 10, 15, 20, 25 and 30 C. Agar disks 5 mm diam were cut from actively growing margins of colonies of each isolate to be tested and placed at the center of plates containing 2% MEA. Three replicate plates were prepared for each isolate. In addition, cycloheximide tolerance of isolates was tested at a range (0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 g/L) of concentration. Colony diameter on each plate was measured after 1 wk of incubation at 20 C, and growth rates were calculated as mm/d.

DNA sequence comparisons.—Cultures were incubated on 2% MA plates for 4 wk. DNA was amplified directly from the mycelium, using the polymerase chain reaction (PCR) in a GeneAmp 9600 thermal cycler (Perkin-Elmer). The method used was that of Suyama et al (1996) with slight modification. A small amount of aerial mycelium was removed from cultures and crushed in a 100 μm PCR tube with a pipette tip, under a dissection microscope. Fifty μL of reaction mixture containing 5 μL 10× buffer, 6 μL of 25 mM MgCl2, 10 mM of dNTPs, 20 pmol of each primer, ITS1 and ITS4 for ITS1-5.8s-ITS2 region (White et al 1990), NL1 and NL4 for 5′ terminal end of the LSU rDNA gene containing D1/D2 regions (O'Donnell 1993), 10 μL 5× CG-RICH solution (with FastStart Taq DNA polymerase, Roche Molecular Biochemicals), 2.5 unit of Fast Start Taq DNA polymerase (Roche Molecular Biochemicals) were added to the template. The PCR conditions were: initial denaturation at 95 C for 4 min, followed by 40 cycles of denaturation at 94 C for 20 s and annealing at 56 C for 1 min. Extension was done during the change of temperature from annealing and denaturing. Final elongation reaction was done at 72 C for 10 min. The PCR products were purified with Microcon-30 Microconcentrators (Amicon Inc., U.S.A.) and used for sequencing using Big Dye Terminator Cycle Sequencing FS Ready Reaction kit and ABI PRISM 310 genetic analyzer (Perkin Elmer Applied Biosystems). Sequences of three isolates were determined, but all of them were identical. Sequences for the isolate MAFF410951 were deposited in GenBank (AB091219 for ITS and AB104900 for LSrDNA D1/D2 regions).

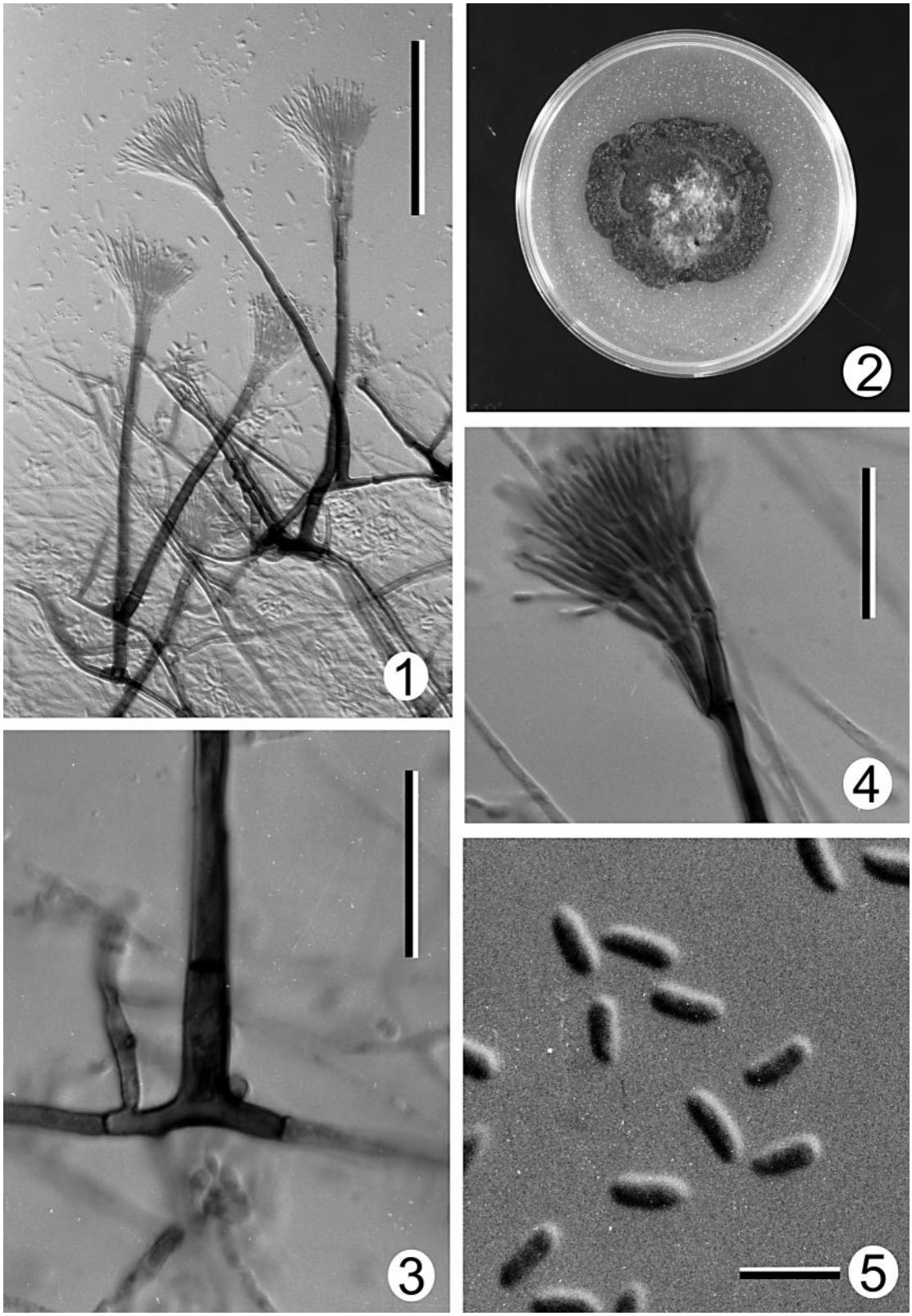
Sequence data for *Leptographium* species are relatively limited, but data for the LSUrDNA D1 and ITS2 regions were available for many species of this genus. Thus sequence data were analyzed together with previously reported sequences for the domain 1 regions of the large-subunit ribosomal DNA operon (Hausner et al 2000) and for the ITS2 region (Jacobs et al 2001). Overall, the dataset for LSrDNA D1 region included 36 sequences, including sequence data produced in this study. The dataset for the ITS2 regions included the 41 sequences for species of *Leptographium* derived from Jacobs et al (2001) as well as the sequence data obtained in this study. Species included in this study and their GenBank accession numbers were those provided in Hausner et al (2000) and Jacobs et al (2001).

Sequences were aligned using ClustalX version 1.81 (Thompson et al 1997). Alignments were adjusted manually using the program BioEdit version 5.0.9 (Hall 1999). In addition, alignment of LSU rDNA domain 1 region was adjusted based on the alignment of Hausner et al (2000). The alignment of ITS2 sequences included many sites that were difficult to align. These sites in the alignment of ITS2 region were decided using SOAP version 1.2 (Löytynoja and Milinkovitch 2001) for comparing alignments obtained in 20 trials, each with a different gap penalty (2–22), and removed from the alignment. As a result, 250 bp and 75 bp of sequences, including gaps, for each of the LSU rDNA D1 regions and ITS2 region, respectively, were used in the phylogenetic analyses. Aligned dataset was analyzed using the programs PAUP*4.0 beta (Swofford 1999). A parsimony analysis was carried out using heuristic search with random stepwise addition and tree-bisection reconnection (TBR) option of the program. Gaps were treated as missing data. All characters were equally weighted. The MAXTREE option was set to auto-increase. Bootstrap and jackknife values (each 1000 replicates) also were calculated with MAXTREE option set to 1000.

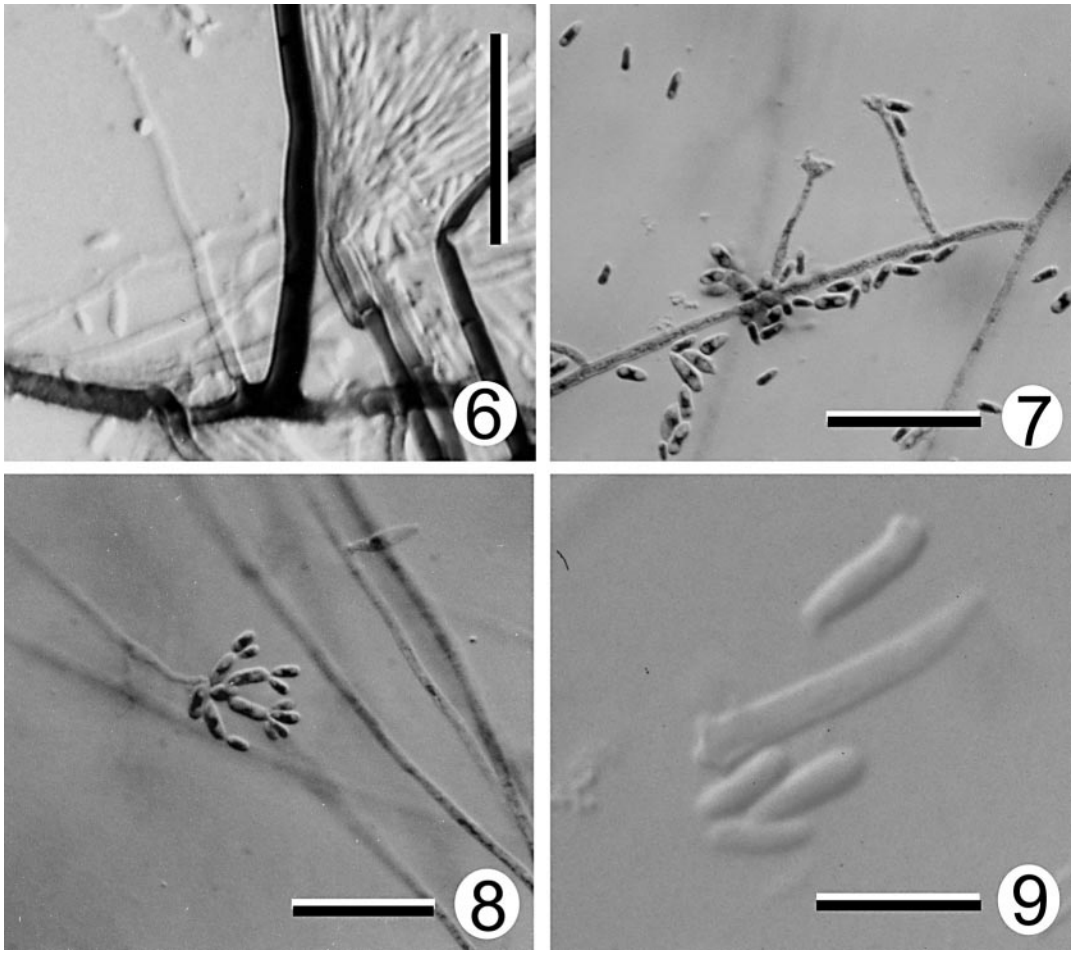
RESULTS

Fungus isolations.—A species of *Leptographium* was isolated from beetles and their galleries but at a relatively low frequency (<5%). The fungus, therefore, does not appear to be a primary associate of *P. ssiori.*

Morphology.—The *Leptographium* species is characterized by dark mononematous, macronematous conidiophores, and 2–3 primary branches, and hyaline 1-celled, oblong to ellipsoidal conidia (FIGS. 1, 4 and 5). These characteristics are typical of the genus *Leptographium,* but the fungus is unusual in having an obvious synanamorph with micronematous conidiophores and conidia arising from sympodialy developing denticles. This synanamorph is typical of spe-



FIGS. 1–5. *Leptographium pruni*. 1. Macronematous conidiophore. 2. Colony characteristics on MEA after 1 mo at 20 C in the dark. 3. Base of a stipe. 4. Conidiogenous apparatus. 5. Conidia. Scale bars: 1 = 50 μm, 3, 4 = 20 μm, 5 = 5 μm.



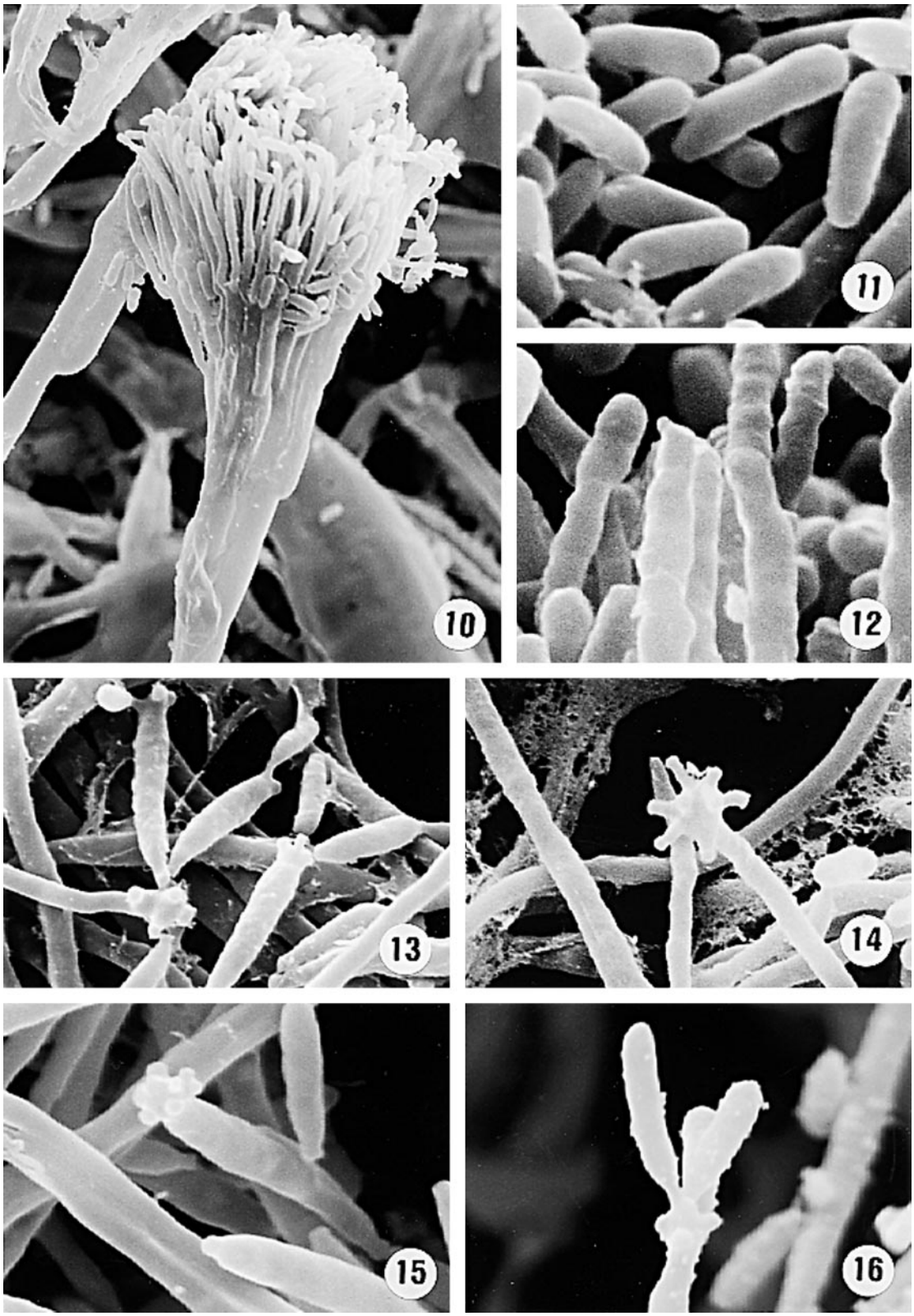
FIGS. 6–9. *Sporothrix* synanamorph of *Leptographium pruni*. 6. Micronematous conidiophore (*Sporothrix*-type) forming from the base of macronematous conidiophore, 7. Micronematous conidiophore (*Sporothrix*-type) with distinct denticles. 8. Micronematous conidiophore (*Hyalodendron*-type) with catenulate conidia. 9. Ramoconidia (arrow) and conidia produced by micronematous conidiophores. Scale bars: 6–8 = 20 μm, 9 = 5 μm.

cies of *Sporothrix* Hektoen & Perkins, with conidia produced on obvious denticles (FIGS. 6, 7, 14 and 16). In addition, these micronematous conidiophores often produced ramoconidia giving rise to secondary conidia (FIGS. 8, 9, 13 and 15). These conidiophores were particularly common in young cultures (about 2 wk old).

DNA sequence analyses analysis.—About 560 bp were amplified with the primers ITS1 and ITS4 for ITS1-5.6SrDNA-ITS2 region in rDNA. Five hundred forty-six bp of ITS sequence were determined, and 179 bp from the ITS2 region were used for comparison with other species of *Leptographium*. Five hundred eight bp of LSU rDNA D1/D2 regions were determined and 244 bp from the D1 region were used for comparisons with other species of *Ophiostoma, Leptographium* and their relatives.

Phylogenetic analysis of LSU rDNA D1 region showed that the *Leptographium* sp. from *Prunus jamasakura* is related to *Ophiostoma,* particularly those species with *Leptographium* states. The fungus was not related specifically to species of *Ophiostoma* that are characterized by *Pesotum* states and having distinct *Sporothrix* synanamorphs. This relationship was supported by high bootstrap value (FIG. 18). In the phylogenetic analysis of LSU rDNA D1 region, from a total of 250 characters, 154 characters were constant, 35 variable characters were parsimony uninformative and 61 were informative.

Although the phylogenetic tree based on the ITS2 region had low resolution, *Leptographium* species with sympodial synanamorphs do not appear in the same clade (FIG. 19). We obtained other parsimony and neighbor-joining trees with alignments based on different gap penalties or using the Elision method (Wheeler et al 1995), and none of them supported the monophyly of *Leptographium* species with sympodial synanamorphs (data not shown). The *Lepto-*



FIGS. 10–16. *Leptographium pruni* (SEM) 10. Conidiogenous apparatus of macronematous conidiophore. 11. Conidia from macronematous conidiophores. 12. Conidiogenous cells showing percurrent proliferation. 13. Catenulate conidiophore and ramoconidia with distinct denticles. 14. Developed denticles of micronematous conidiophore. 15. Ramoconidia with distinct denticles. 16. Conidiogenous cells showing sympodial ontogeny. Scale bars: 10 = 20 μm, 11, 15, 16 = 5 μm, 12–14 = μm.

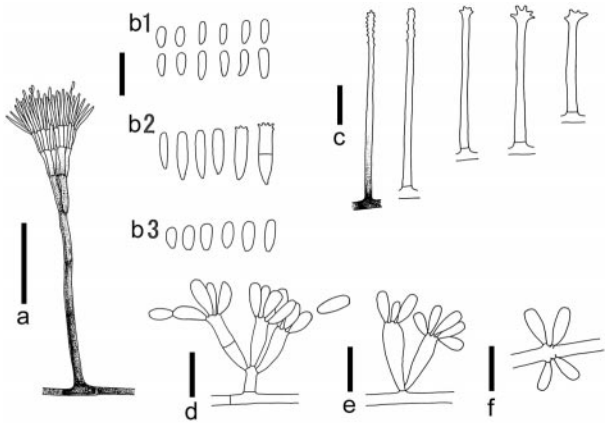


FIG. 17. *Leptographium pruni*. a. Macronematous conidiophore. b. Conidia (b1. Conidia developing from macronematous conidiophore. b2. Conidia from micronematous conidiophore and ramoconidia. b3. Secondary conidia). c. Micronematous (*Sporothrix*-type) conidiophore. d and e. Micronematous (*Hyalodendron*-type) conidiophore. f. Laterally formed secondary conidia on undifferentiated hyphae. Scale bars: a = 30 μm, b–f = 5 μm.

graphium sp. from *Prunus* was different from but most closely related to *L. grandifoliae* and *L. robustum*. In the phylogenetic analysis of ITS2 sequence data, from a total of 75 characters, 47 characters were constant, 15 variable characters were parsimony uninformative and 13 were informative.

TAXONOMY

Based on distinct morphology and clear differences in DNA sequence data, we conclude that the *Leptographium* sp. from *Prunus* in Japan represents an undescribed taxon. The species thus is described as new:

***Leptographium pruni*, sp. nov.** H. Masuya & M. J. Wingfield. FIGS. 1–17

Coloniae in 2% agaro maltoso 5.5–6.5 mm/d ad 25C, pallide olivaceae (21‘‘‘d). Conidiophora macronematosa et micronematosa; conidiophora macronematosa, mononematosa, e latere hyphorum singulariter vel saepe laxe aggregatim exorientia. Rhizoidaceae structurae absentes. Stipites erectus, pallide brunnei vel atro-brunnei, 1–6-septati, 32–190(–320) μm longi, ad basim 3–10.5 lati. Apparatus conidiogeni 22–80 μm longi massa conidica exclusi, ex ramis 3–5 (plerumque 3–4) seriatis constantes; rami primarii usque quatuor sed plerumque duo inter se adjacentes, ramus centralis distinctus nullus, 6–24 × 2–6.5 μm. Cellulae conidiogenae discretae, hyalinae, sursum attenuatae, 5–13(–16) × 1.0–2.5 μm. Conidiogenesis holoblastica, percurrens, per separationem retardatam ut in proliferatione sympodiali visa. Conidia hyalina, oblonga vel ellipsoidea, interdum clavata, apice rotundata, basi rotundata vel subtruncata, 2.5–8.5(–13) × 1–3 μm. Conidiophora micronematosa, simili *Sporotrichi* in denticules conspicui ubi sporae portatae et quoque in catena acropeta conidia holoblastica efferens.

Colonies on 2% MEA hyaline at first becoming

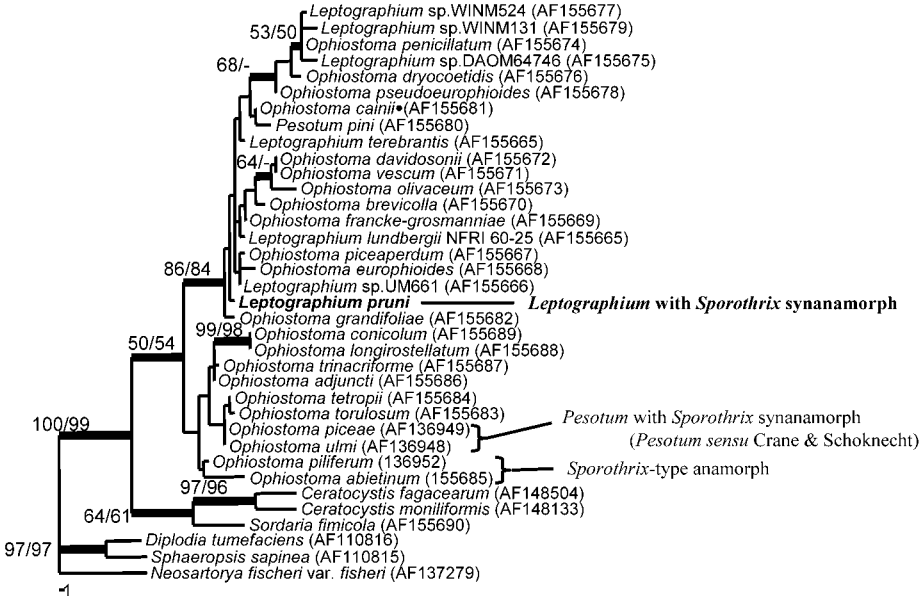


FIG. 18. One of 1651 most-parsimonious trees of *Ophiostoma, Leptographium* and other Ascomycetous fungi based on 250 characters, including gaps, of D1 region of large subunit rDNA operon. The tree is unrooted. One thousand replicates of bootstrap values >50% and jackknife values >50% indicated above the branches (bootstrap value/jackknife value). Tree length = 205, consistency index (CI) = 0.6585, homoplasy index (HI) = 0.3415, retention index (RI) = 0.8006.

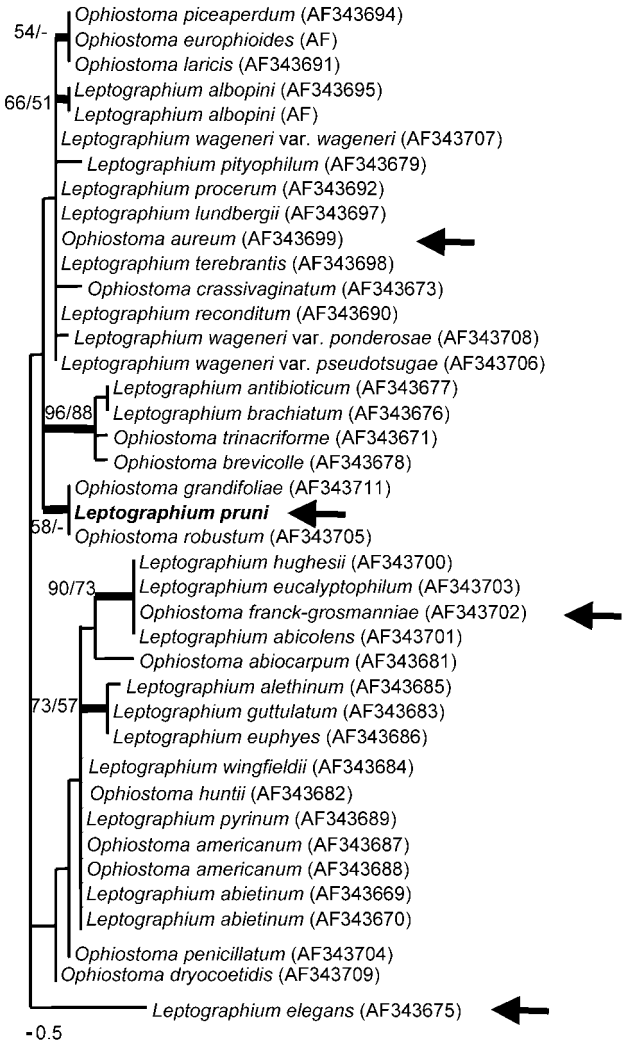


FIG. 19. One of the most-parsimonious trees of *Leptographium* species based on 75 characters, including gaps, of ITS-2 regions of rDNA operon. The tree is unrooted. One thousand replicates of bootstrap values >50% and jackknife values >50% indicated above the branches (bootstrap value/jackknife value). Arrows show the *Leptographium* species reported to have *Sporothrix* synanamorphs. Tree length = 41, Consistency index (CI) = 0.8049, Homoplasy index (HI) = 0.1951, Retention index (RI) = 0.9286.

smoke gray (21‘‘‘d, Rayner 1970), aerial hyphae abundant. Mycelium straight or curved, spreading radially on the medium and immersed in the medium, hyaline to pale brown, sometimes brown, verrucose, 1.5–12 μm diam, occurring singly or aggregated in strands of 2–5 hyphae. Aerial hyphae hyaline to pale brown, sometimes verrucose. Conidiophores mononematous and both macronematous and micronematous forms present.

Macronematous conidiophores arising laterally from hyphae, single but often also in loosely arranged groups on the hyphae, without rhizoidal hyphae at the bases. Stipes erect, pale brown to dark brown, 1–6-septate, tapered, 32–190(–324) (x = 86.3) μm long and 3–10.5 (x = 6.2) μm wide at base. Conidiogenous apparatus 22–80 (x = 46.5) μm long (excluding conidial mass) consisting of 3–5 but mostly three or four series of branches. Up to four primary branches present but mostly two adjacent to each other without a distinct central branch∈ 6–24 × 2–6.5 (x = 13 × 4) μm. Arrangement of the primary branches on the stipe-type B (Jacobs and Wingfield 2001). Conidiogenous cells discrete, hyaline, tapering from base to apex, 5–13(–16) × 1–2.5 (x = 10 × 1.5) μm. Conidium development replacement wall building with holoblastic, percurrent proliferation but with delayed secession, giving a false appearance of sympodial proliferation. Conidia hyaline, oblong to ellipsoid, sometimes clavate, with rounded apices and rounded to subtruncate bases, 2.5–8.5(–13) × 1–3 (x = 5 × 2) μm, accumulating around the conidiogenous apparatus in a hyaline mucilaginous mass.

Micronematous conidiophores, arising orthotropically from undifferentiated hyphae, often in terminal position or integrated in short side branches, sometimes developed on aerial hyphae, unbranched. Conidiogenous cells, subcylindrical, variable in shape and size, usually widest at the basal part and slightly tapering, bearing distinct denticles at their tip, 1–40 (x = 16.5) μm long, 0.75–2.5 (x = 1.25) μm width at the base. Conidia hyaline, 1-celled, oblong to ellipsoidal, 2–5 × 0.5–4 (4 × 2.5) μm width, sometimes developing into ramoconidia, which are larger than normal conidia, producing distinct denticles and secondary conidia. Ramoconidia hyaline, 1-celled, sometimes septate, 4–11.5 × 1.5–2.5 (x = 7 × 2) μm. Secondary conidia, hyaline, 1-celled, subglobe to ellipsoidal, 1.5–5 × 0.5–2 (x = 3.5 × 1.5) μm.

The growth rate of colonies on 2% MEA 5.5–6.5 (x = 5.9 ± 0.36) mm/d at 20 C. Growth reduced at temperatures below 20 C and above 30 C (TABLE I). The fungus is tolerant to cycloheximide, with growth at 20 C reduced by approximately 60% on 2% MEA containing 5.0 g/L cycloheximide (TABLE II).

TABLE I. Mean growth rates of *L. pruni* under different temperatures on 2% malt extract agar

Temperature (C)	Growth rate (mm/d ± SD)
4	0.40 ± 0.1
10	3.40 ± 0.17
15	4.40 ± 0.17
20	5.90 ± 0.36
25	6.57 ± 0.93
30	6.83 ± 0.29
35	1.93 ± 0.51

TABLE II. Growth rates of *L. pruni* on different concentration of cycloheximide at 20 C on 2% malt extract agar

Concentration (g/L)	Growth rate (mm/d ± SD)
0	5.90 ± 0.36
0.05	4.6 ± 0.36
0.1	4.6 ± 0.36
0.5	4.13 ± 0.12
1	3.83 ± 0.58
2.5	2.53 ± 0.25
5	2.23 ± 0.25

Specimens examined. JAPAN. AOMORI PREFECTURE: Higashidori, on *Prunus* jamasakura infested with bark beetle *Polygraphus ssiori* Nijima, 14 Jun 2000, *H. Masuya,* (HOLOTYPE; FPH: TFM 7594; ex-type culture: MAFF410951 (JCM11708)). JAPAN. IWATE PREFECTURE: Morioka, on *Prunus* jamasukura infested with bark beetle *Polygraphus ssiori* Nijima, 9 May 2000, *H. Masuya,* (PARATYPE: FPH: TFM 7592; ex-type culture: MAFF410949 (JCM11709)). JAPAN. IWATE PREFECTURE: Morioka, on *Prunus* jamasakura infested with bark beetle *Polygraphus ssiori* Nijima, 9 May 2000, *H. Masuya,* (PARATYPE: FPH:TFM 7593; ex-type culture: MAFF410950 (JCM11710)).

Etymology. pruni referring to *Prunus,* the host on which this fungus was found.

DISCUSSION

Macronematous conidiophores of *L. pruni* are similar to those of *Leptographium grandifoliae* Wingfield, *L. sibiricum* Jacobs & Wingfield and *L. elegans* Wingfield et al. However, unlike *L. grandifoliae,* the *Leptographium* state of the fungus has no rhizoids at the bases of conidiophores, which are typical in the former species. *Leptograhium pruni* morphologically is similar to *L. sibiricum* and *L. elegans,* and the three species have overlapping shapes and sizes of conidiophores and conidia. However, *L. sibiricum* lacks the typical synanamorph found in the *L. pruni* and *L. elegans* has an indistinct and rarely produced *Sporothrix* synanamorph (Wingfield et al 1994). *L. sibiricum* and *L. elegans* also occur on conifers, and there is no precedence for species of *Leptographium* on conifers being found on hardwoods (Jacobs and Wingfield 2001).

Four *Leptographium* species have sympodial synanamorphs that might be considered *Sporothrix*-like and thus potentially confused with the *L. pruni*. These are *L. aureum* Wingfield, *L. elegans* Wingfield et al, *L. francke-grosmanniae* Jacobs & Wingfield and the unnamed anamorph of *O. valdiviana* Butin. Of these, *L. aureum* and *L. francke-grosmanniae* rarely produce a synanamorph and, when these structures are formed, conidia are produced directly on a superficial mycelium and in the absence of distinct conidiophores (Upadhyay 1981, Mouton et al 1992). In addition, their mononematous *Leptographium* morphs are quite different and easily distinguished from *L. pruni*. *Ophiostoma valdiviana* was described as having a reasonably well-developed *Sporothrix* synanamorph, but the *Leptographium* morph has stipes that are sometimes synnematous and that vary in length (Butin and Aquilar 1984) when compared to those of *L. pruni*.

Leptographium elegans is the species most similar to the *L. pruni*. Although *L. elegans* has a *Sporothrix* synanamorph, its conidia are similar to those produced by the *Leptographium* morph (Wingfield et al 1994, Jacobs and Wingfield 2001). This is in contrast to *L. pruni,* in which conidia produced by the *Sporothrix* morph are rounded at both ends and morphologically different from those of the *Leptographium* morph, which have rounded apices and truncate bases. In addition, the mycelium of *L. elegans* sometimes aggregates in strands of 4–13 hyphae, distinct from aggregations of 2–5 hyphae in *L. pruni*. To the best of our knowledge, there are no other species of *Leptographium* that have synanamorphs that produce ramoconidia.

Leptographium pruni originates from a host not previously associated with this group of fungi. The *Prunus* host is different from other *Leptographium* species that might be considered similar to it. *L. grandifoliae* was isolated from *Fagus grandifolia* in the United States (Davidson 1976), *L. sibiricum* from *Abies sibirica* in Russia (Jacobs et al 2000), *L. aureum* from *Pinus* spp. in the United States (Harrington 1988), *L. francke-grosmanniae* from *Quercus* sp. in Germany (Davidson 1971), *O. valdiviana* from bark and wood of *Nothofagus alpina* (Poepp. & Endl.) Oerst. and *N. dombeyi* (Mirb.) Oerst. in Chile (Butin and Aquilar 1984) and *L. elegans* from *Chamaecyparis formosensis* Matsum. in Taiwan (Wingfield et al 1994). In general, *Leptographium* spp. are typified by having distinct hosts and the bark beetles that carry them tend to be relatively host specific (Jacobs and Wingfield 2001). Thus, in addition to robust morphological differences, distinct host differences provide us with convincing evidence that *L. pruni* represents a distinct taxon.

We were unable to find evidence of a teleomorph for *L. pruni*. If it occurs frequently, we would have expected it to be present in the galleries of *P. ssiori*. We also failed to make successful crosses between our isolates of *L. pruni* isolate. However, the fact that the fungus is highly tolerant to cycloheximide in culture provides good evidence to suggest that it is an anamorph of *Ophiostoma* (Harrington 1981). Our DNA sequence data also have shown that *L. pruni* is relat-

ed to other species in the Ophiostomataceae and support the views of previous authors (Zambino and Harrington 1992, Hausner et al 2000, Jacobs et al 2000) that the genus *Leptographium* should be reserved for anamorphs of *Ophiostoma*.

The most distinct characteristic of *L. pruni* is its *Sporothrix* synanamorph. The presence of acropetal chains of ramoconidia particularly is characteristic of this species. This synanamorph is more strongly developed than in the reduced *Sporothrix* states in other *Leptographium* spp. and is more similar to those observed in some *Ophiostoma* species with *Pesotum* anamorphs. Phylogenetic analyses in this study have shown that *L. pruni* is closely related to *Ophiostoma* with *Leptographium* anamorphs. Thus, despite its distinct *Sporothrix* state and ramoconidia, it is specifically not related to *Ophiostoma* species having similar *Sporothrix* anamorphs. This suggests that the presence of a distinct *Sporothrix* anamorph can occur widely among the Ophiostomataceae and supports the view of Hausner et al (1993), who suggested a polyphyletic origin for the *Sporothrix*-like morphology in *Ophiostoma*. Mouton et al (1992) also suggested that *Sporothrix* synanamorphs increasingly might be found as new *Leptographium* spp are discovered. Our discovery of the first *Leptographium* sp. having a distinct *Sporothrix* synanamorph with catenulate conidia, therefore, might reflect a poorly collected genus, rather than an unusual characteristic.

Recent DNA sequence-based studies have suggested that separation of the synnematous *Pesotum* and the mononematous *Leptographium* do not reflect phylogenetic relationships in *Ophiostoma* (Okada et al 1998, Hausner et al 2000). Harrington et al (2001) suggested that *Pesotum* should be restricted to the *Ophiostoma* species with synnematous anamorphs and *Sporothrix* synanamorphs. This suggestion was based on the fact that *Pesotum*-like species with *Sporothrix* synanamorphs have a monophyletic origin and reside in the *Ophiostoma piceae* (Münch) H. & P. Sydow complex, separate from other *Pesotum*-like anamorphs without *Sporothrix* synanamorphs. Thus, the *Sporothrix* synanamorph appears to be an important characteristic in the delimitation of *Pesotum* species from other synnematous anamorphs of *Ophiostoma*. In contrast, results of our study suggest that the *Sporothrix* synanamorph in *Leptographium* may have less value for generic treatment, than has been found in *Pesotum*. Our study also suggests that *Sporothrix* synanamorphs may not be unusual in members of *Leptographium* and that its generic concept might be modified to include this view.

Leptographium recently has been subjected to a monographic study by Jacobs and Wingfield (2001). DNA-based comparisons also have been undertaken (Hausner et al 2000, Jacobs et al 2001), and they already are proving to be useful for the identification of known species. However, little evidence has emerged for phylogenetic relationships linked to morphological characters (Hausner et al 2000, Jacobs et al 2001). This might be due to the fact that data for variable regions of the genome for this group of fungi are relatively limited and sequences for additional genes are required. Sequence data also are not available for all species of *Leptographium,* and the addition of taxa could greatly improve the value of phylogenetic trees for this group. Thus surveys and collection of additional species of *Leptographium* from new environments and particularly from Angiosperms should add valuable insight into the taxonomic concepts and evolution of *Leptographium* species.

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