Leptographium costaricense sp. nov., a new species from roots of *Talauma sambuensis* from Costa Rica

G. WEBER[1], F. SPAAIJ[1] AND M. J. WINGFIELD[2]

[1] *Universität Tübingen, Lehrstuhl Spezielle Botanik/Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen, Germany*

[2] *Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa*

Leptographium costaricense, a new species of Hyphomycetes, isolated from the rhizoplane of *Talauma sambuensis* from the lowland rainforest in Costa Rica, is characterized by dark, mononematous, penicillately branched conidiophores, with 2–5 series of metulae and relatively small truncate conidia produced in a slimy brownish matrix. Light and scanning electron microscopy showed that conidial production is both annellidic and sympodial. It is equipped with an ubiquinone system of Co–Q–10H_2. *Leptographium costaricense* is most similar to *L. reconditum* that is known from soil in South Africa. The two species can, however, be distinguished by a more complex conidiogenous apparatus in *L. costaricense* and differences in the mol% G+C values of their DNA. Both species share no DNA relatedness based on DNA–DNA reassociation studies.

Species of *Leptographium* have dark mononematous conidiophores that terminate in a complex conidiogenous apparatus comprised of a series of apical branches (metulae) and ameroconidia produced in slimy masses. These fungi are known as associates of insects and particularly bark beetles (Coleoptera: Scolytidae) that infest conifers (Upadhyay, 1981; Harrington & Cobb, 1988). A number of species have also been associated with root diseases of conifers of which *Leptographium wageneri* (W. B. Kendr.) M. J. Wingf. is the most virulent and important species (Harrington, 1988; Harrington & Cobb, 1988; Wingfield, Capretti & Mackenzie, 1988).

Leptographium species are known as anamorphs of *Ophiostoma* spp. (Wingfield, 1993). In this sense they are typically tolerant to high concentrations of cycloheximide in culture (Harrington, 1981) and also have rhamnose and cellulose in their cells walls (Patik & Rosinski, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & De Hoog, 1975; De Hoog & Scheffers, 1984). *Leptographium* spp. have been known to reside in a complex of genera also including *Verticicladiella* S. Hughes and *Phialocephala* W. B. Kendr., where the three genera were distinguished by annellidic, sympodial and phialidic conidium development, respectively. Wingfield (1985), however, showed that most species of *Leptographium* produce conidia both percurrently and sympodially and, therefore, reduced *Verticicladiella* to synonymy with *Leptographium*.

A single species, *L. reconditum*, possesses the unusual characteristic of occurring in soil and, more specifically, in the rhizosphere of maize plants in South Africa. During the course of ecological studies on populations of microfungi on the roots of various tree species in rain forests of Costa Rica, two isolates of an apparent *Leptographium* sp. were collected from the roots of *Talauma sambuensis* (Magnoliaceae). The aim of this study was to characterize and describe this fungus.

MATERIALS AND METHODS

Strains of *Leptographium costaricense* and *L. reconditum* were examined for their morphological and cultural characteristic on 2% MEA (malt extract agar) and on OA (oatmeal agar) after 7 and 14 d at 25 °C.

Observations using SEM were based on material grown on OA at 25° for 10 d and prepared as described by Weber, Spaaij & Gams (1994).

For the determination of the DNA base composition, cells were grown in 2% Glucose–Yeast–Peptone (Van der Walt & Yarrow, 1984) at 25° on a rotary shaker until the late log phase. Extraction and purification of the DNA were by a combination of methods described by Maniatis, Fritsch & Sambrook (1982) and Cryer, Eccleshall & Marmur (1975). The purity of the DNA preparation was checked by the photospectrical method as recommended by Maniatis, Fritsch & Sambrook (1982). The base composition of the DNA was determined in 0·1 SSC (SSC = 0·15 M sodium chloride and 0·015 M sodium citrate, pH 7·0) from the thermal denaturation profile, according to the method of Owen, Hill & Lapage (1969), with a Gilford Response UV-VIS Spectrophotometer and its Thermal Programming Software heated at a rate of 0·1° min^{-1}. The composition was calculated by the formula mol% G+C = 2·08 × T_m − 106·4. As a control, a standard preparation of DNA of *Candida parapsilosis* CBS 604 (T_m 70·6°) was included in every determination. The values reported reflect the mean and standard deviation of three

determinations. The extent of DNA–DNA reassociation was determined using the same instrument following the procedures described by Seidler & Mandel (1971) as modified by Kurtzman *et al.* (1980).

Ubiquinone isoprenologues were extracted and purified as described by Yamada & Kondô (1971, 1973). For the determination of the type of Co–Q, reverse-phase thin layer chromatography was employed, using Merck HPTLC RP-18 F254s plates and a mixture of acetonitrile (80:20, v/v) as the developing solvent (Nakase & Suzuki, 1985). The separated components were detected under uv light at 254 nm.

The ability to tolerate cycloheximide in culture is an important taxonomic characteristic of *Leptographium* spp. (Harrington, 1981). An isolate of the species from Costa Rica was, therefore, tested for this capacity by transferring it to 1% Malt Extract Agar (Merck) in Petri dishes amended with 0, 0·05, 0·1, 0·5, 1·0, 1·5 and 2·5 g l^{-1} of this antibiotic. Three plates were used for each of the test concentrations and isolates of *Ceratocystis fimbriata* and *Ophiostoma piceae* were included in the experiment for comparative purposes. Plates were incubated at 25° for 8 d, whereafter two measurements of colony diameter were taken for each colony, at right angles to each other. The experiment was repeated.

RESULTS

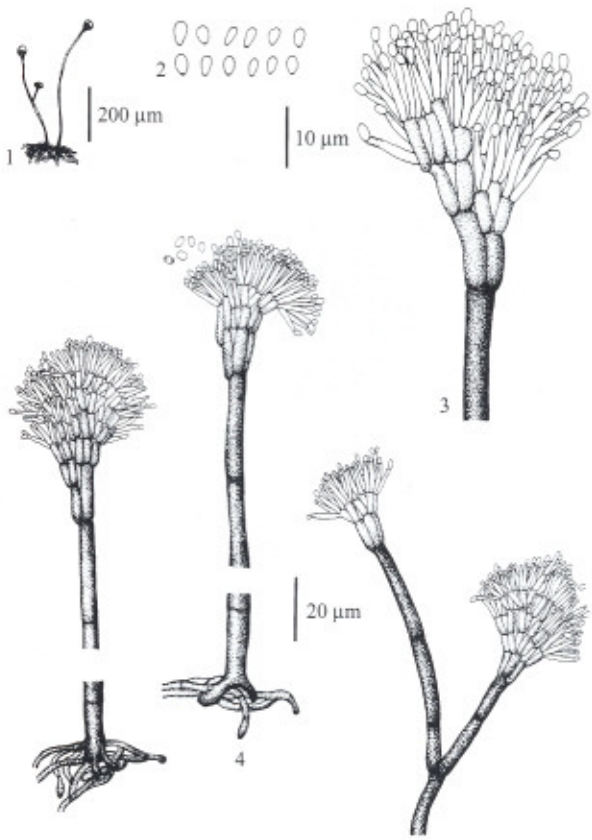
Taxonomy

Leptographium costaricense G. Weber, Spaaij & M. J. Wingf. sp. nov. (Figs 1–9)

Coloniae post 14 dies ad 25° in agaro farinae avenaceae 38–40 mm diametro; velutinae, atrobrunneae; in agaro farinae avenaceae mycelium aerium deest, in agaro malti sparsim formatur. *Hyphae* septatae, tenuitunicatae, brunneolae, 1·5 ad 3·5 µm latae. *Conidiophora* frequentiora plerumque in nonnullis zonis angustis et concentricis, atrobrunnea, macronematosa, mononematosa, erecta, raro ramosa, solitaria vel quaterna ad sena aggregata. Stipites 210–750(–900) µm longi, erecti, basi (5–)7–12(–16) µm, infra penicillium 4·5–6(–8) µm lati, multiseptati (plerumque 7–9 septati), crassitunicati (pariete 1–2 µm crasso), deorsum brunnei, sursum pallescentes, glomeribus rhizoideorum prope basim. *Caput conidiogenum* complexum, hyalinum, 30–50 µm longum, e 2–5 seriebus ramorum, metularum phialidumque constat. Rami primarii pauci ad 4–6, stipite principali angustiores, (10–)15–17 µm longi (3–)3·5–4(–5) µm lati. Rami secundarii bini ad quini aggregati, 6–11 × 2·5–3 µm metientes. Ram tertiari quartariique 5–7 × 2–2·5 µm, bini ad quaterni aggregati. *Cellulae conidiogenae* hyalinae, subcylindricae, 7–10 µm longae, 2–2·5 µm latae, in colonis vetustioribus nonnumquam apice collarettiformi, conidia et annelidice et holoblastice sympodialiter procreant. *Conidia* unicellularia, primum hyalina, deinde brunneola, laevia, basi paulo truncata, 3·0–4·5 × 2–2·5 µm metientia, saepe utrimque inclusione globulosa, in guttas magnas brunneolas mucosas congregantur.

Holotypus: GW-CR-243 in M; isotypus in TUB. Cultura viva, TUMY-GW-CR-243, CBS 409·94, MAFF 237157. isolata e radice *Talauma sambuensis*, Reserva Biologica 'La Selva', Costa Rica.

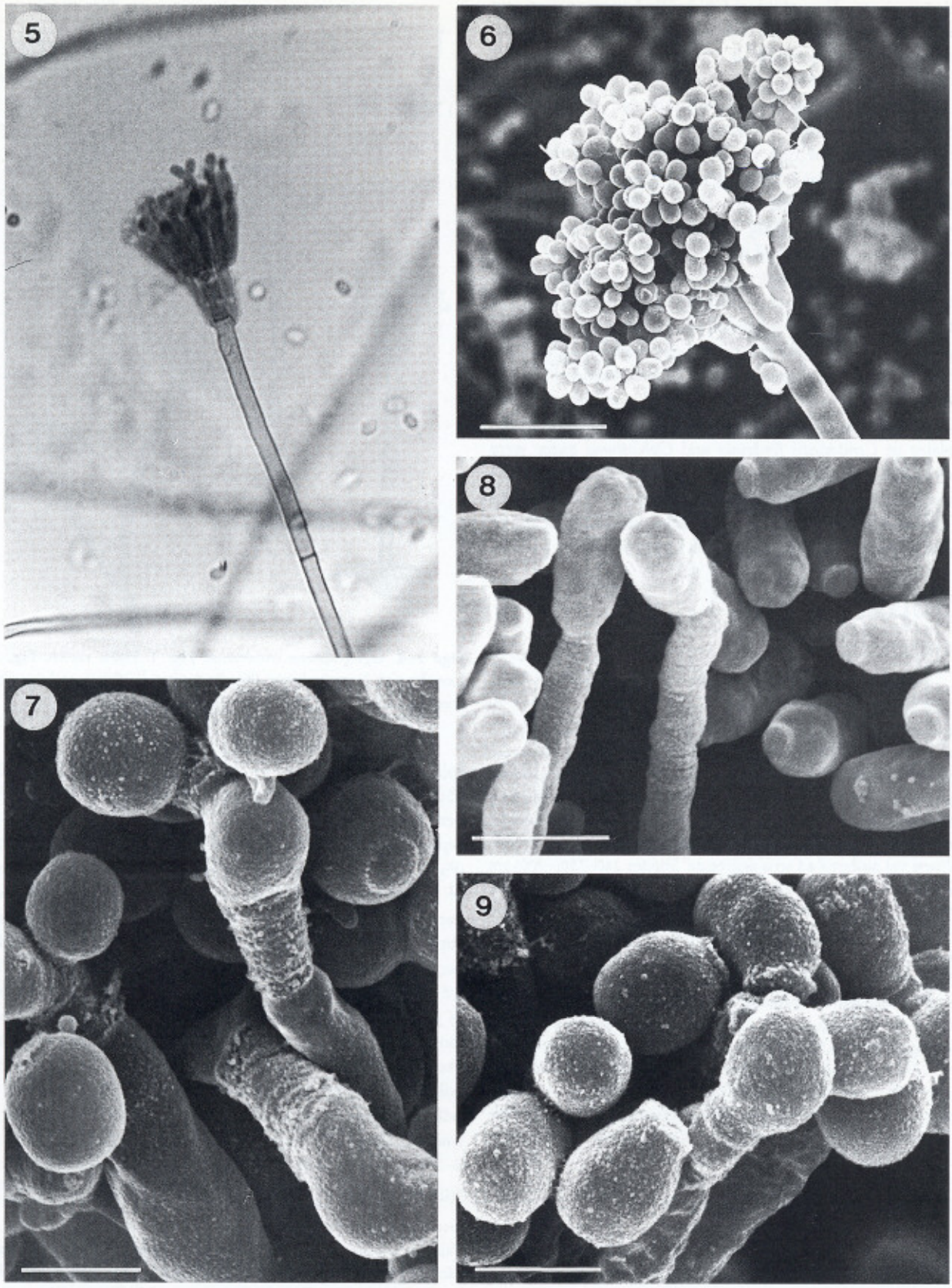
Colonies on OA reaching 38–40 mm diam after 14 days at 25°, velvety, dark brown, aerial mycelium absent on OA, but sparse on MEA, hyphae light brown, 1·5–3·5 µm wide. *Conidiophores* fairly numerous, mostly occurring with increased density in a few narrow concentric zones, dark-brown, macronematous, mononematous, erect, seldom branched, solitary or clustered in groups of 4–6, stipes 210–750(–900) µm tall, near the base (5–)7–12(–16) µm, below the penicillus 4·5–6(–8) µm wide, multiseptate (mostly 7–9 septate), thick-walled, with basal clusters of rhizoidal hyphae (Figs 1, 4). *Conidiogenous complex* hyaline, 30–50 µm, consisting of 2–5 (mostly 2–3) series of branches. Primary branches varying in number from few to 4–6, narrower than the main stipe, (10–)15–17 µm long and (3–)3·5–4(–5) µm wide. Secondary branches in groups of 2–5, measuring 6–11 × 2·5–3 µm. Tertiary and quaternary branches, 5–7 × 2–2·5 µm, in groups of 2–4. *Conidiogenous cells* subcylindrical, 7–10 µm long, 2–2·5 µm wide, in older cultures some with collarette-like apices, producing conidia by annellidic as well as by holoblastic, sympodial conidiogenesis (Figs 3, 7–8). *Conidia* 1-celled, hyaline, later in age becoming slightly pale brown, smooth, slightly truncate at the base, measuring 3·0–4·5 × 2–2·5 µm, often with two globules at each end, becoming aggregated in a large, brownish, slime drop (Figs 2, 6, 7, 9).



Figs 1–4. *Leptographium costaricense*. **Fig. 1.** Habit sketch. **Fig. 2.** Conidia with truncate bases. **Figs 3, 4.** Conidiophores and complex conidiogenous heads with several series of metulae.

Species examined: Holotype: Cultures on OA, isolated from roots of *Talauma sambuensis*, Biological Station of 'La Selva', Sarapiqui, Costa Rica, March 1992, G. Weber, GW-CR-243 in M; isotype in TUB. Living cultures GW-CR-243, CBS 409.94, MAFF 237157.

Additional characters: Major ubiquinone system: Co–Q–10H_2; mol% G+C: *Leptographium costaricense* (GW-CR-243): 57·8±0·3, *Leptographium reconditum*: 52·9±0·4; extent of DNA complementary between *Leptographium costaricense* and *Leptographium reconditum*: 0%.



Figs 5–9. *Leptographium costaricense*. **Fig. 5.** LM of conidiophore with few series of metulae and conidiogenous cells. **Figs 6–9.** SEM of conidiogenous elements. **Fig. 6.** Complex conidiogenous head with conidia. **Figs 7, 8.** Conidiogenous cells with both annellidic and sympodial conidiogenesis. **Fig. 9.** Conidia with truncate base and annellide. Bars in Fig. 5 = 20 µm; Fig. 6 = 10 µm; Figs 7–9 = 2 µm.

DISCUSSION

Morphologically, *L. costaricense* is a typical *Leptographium* species, having an erect dematiaceous stipe subtending a penicillate conidiogenous apparatus, with conidia aggregated in a slimy head. *L. costaricense* was, however, relatively sensitive to even low concentrations of cycloheximide in culture (Table 1), which tends to suggest that it does not have Ophiostomatoid affinities. Given its morphological characteristics, we still believe that it is best placed in *Leptographium* for the present, although this situation might need modification in the future as generic concepts for the group become more precise.

Most *Leptographium* species are associated with dark beetles and woody substrates which make the soil habitat of *L. costaricense* unusual. *Leptographium reconditum* Jooste is the only other species that has been isolated from the rhizosphere. This was first described from South Africa associated with *Zea mays* roots (Jooste, 1978). The two fungi, which are morphologically very similar, can, be distinguished relatively easily based on the fact that the conidiogenous apparatus of *L. reconditum*, which has between one and three series of metulae, is considerably less elaborate than that of *L. costaricense*.

The comparison of the mol% G+C value of the DNA of *Leptographium costaricense* and *L. reconditum*, which differ by 4·9%, implies that the two species represent genetically isolated populations (Kurtzmann & Phaff, 1987). This assumption was also reconfirmed based on the low percentage of DNA–DNA reassociation between the two species.

In the most narrow sense, species of *Leptographium* are typically anamorphs of *Ophiostoma*, and these fungi are most commonly found in a close association with bark beetles that infest trees, particularly conifers (Upadhyay, 1981; Harrington, 1993; Wingfield, 1993). In this sense, *L. costaricense* and *L. reconditum* are most unusual. Although they are morphologically similar to *Leptographium*, their generic affinities may lie outside this group. These similarities may be a result of convergent evolution, possibly in an association with soil arthropods.

Convergence has been common in a broad range of insect associated fungi, and is particularly well recognized in the group broadly defined as *Ceratocystis sensu lato*. It would not be unusual in a species with a *Leptographium*-like morphology (Wingfield, 1993; Blackwell, 1994). Although *Leptographium costaricense* is not able to tolerate high concentrations of cycloheximide, and probably does not have ophiostomatoid affinities, its morphology is suggestive of an ecological habitat similar to that of more typical *Leptographium* species. Another typical characteristic of these fungi, and one unusual for the Ascomycetes, is the presence of cellulose and rhamnose in the cell walls (Smith *et al.*, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & De Hoog, 1975; De Hoog & Scheffers, 1984), which is to be tested in an ongoing study for *L. costaricense*.

At this stage we invisage *L. costaricense* with a tropical distribution. The fungus is unusual not only taxonomically, but also in terms of its association with *Talauma sambuensis* roots and its occurrence in the soil. It is hoped that further studies, particularly on the ecology of *L. costaricense*, will expand our knowledge of this interesting group of fungi.

Table 1. Cycloheximide tolerance of *Leptographium costaricense*, *Ceratocystis fimbriata* and *Ophiostoma piceae*

	Cycloheximide concentration (g l^{-1})					
	0·05	0·1	0·5	1·0	1·5	2·5
Leptographium costaricense	+	−	−	−	−	−
Ceratocystis fimbriata	−	−	−	−	−	−
Ophiostoma piceae	+	+	+	+	+	+

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