

## ***Sporendocladia fumosa* and *Lauriomyces bellulus* sp. nov. from *Castanea cupules* in Switzerland**

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Two hyphomycetes, one of them a new species, isolated from sweet chestnut cupules collected in Comano, Switzerland, are described in this study. The general morphology easily distinguishes *Lauriomyces bellulus* from other species of the genus. In the case of *Sporendocladia fumosa*, however, several discrepancies were observed in regard to its conidium formation and morphology. Conidia were found to be cuneiform rather than cylindrical, and to have only basal dehiscence scars, indicating apical as opposed to ring wall building conidial development. The genus *Sporendocladia* is therefore heterogeneous, and includes species with conidia arranged in false or true chains, having basal, or basal and apical dehiscence scars, respectively.

Keywords: taxonomy, deuteromycota, hyphomycetes.

Numerous microfungi have been described from cupules of *Castanea sativa* Mill. collected in Britain (Sutton, 1973; 1975), and it appears that this substratum is colonized by many hyphomycetes. Other than in Britain, this substratum has not been studied in detail. We recently have examined a sample of fallen *C. sativa* cupules from a forest in Comano, Switzerland, from which we could isolate *Sporendocladia fumosa* (Ell. & Ev.) Wingfield, and an undescribed species of *Lauriomyces* Castañeda. These species are described and discussed in the present study.

### **Materials and methods**

Cupules of *C. sativa* were cut into small pieces containing 3–5 spines each, surface-sterilized in 1 % NaOCl for 1 min, and rinsed in 70 % ethanol for 30 sec. Surface-sterilized pieces were allowed to dry in a laminar flow bench, plated onto potato-carrot medium (PCA)

(Johnston & Booth, 1983), and incubated at 20-25 C on the laboratory bench. Sporulating hyphomycetes were single-spored, cultured on 2% malt-extract agar (MEA) (20 g Oxoid malt extract, 15 g Difco agar, 1000 ml H<sub>2</sub>O), incubated at 25 C under near-ultraviolet light, and examined after 14 d. Mounts were prepared in lactophenol cotton blue. All measurements were made at 1000 x magnification. To determine the maximum radial growth of species in culture, agar plugs (3 mm diam.) from the periphery of 14-d-old colonies of each fungus were plated at the centre of MEA plates and treated as described in Crous & al. (1994). Conidium development was examined using Scanning Electron Microscopy (SEM). Specimens (approximately 7 mm<sup>2</sup>) for SEM were cut from agar cultures, fixed in glutaraldehyde followed by 1% osmium tetroxide in a 0.1 M phosphate buffer and then dehydrated in a graded acetone series, critical-point dried and mounted. Specimens were coated with gold-palladium and viewed with a JSM 6400 scanning electron microscope.

### Taxonomic part

Kendrick (1961) distinguished species in the *Leptographium* Lagerb. & Melin complex with phialidic conidium development from those with anellidies, and placed the former in their own genus, *Phialocephala* B. Kendrick. This genus was reassessed by Wingfield & al. (1987), who found that it contained a heterogeneous group of species. An examination of conidium development in these species showed that those with ring wall building (Minter & al., 1983) were best placed in the genus *Sporendocladia* Arnaud *ex* Nag Raj & B. Kendrick. These species appeared to have conidia with both basal and apical dehiscence scars, and were arranged in chains. Furthermore, conidia were cylindrical, and were produced from phialides with distinct cylindrical collarettes (Wingfield & al., 1987). Species of *Phialocephala* are characterized by apical wall building conidial development, and oval conidia with basal dehiscence scars (Wingfield & al., 1987), that occur in false or true chains (Carmichael & al., 1980; Kendrick, 1961; Nag Raj & Kendrick, 1975; Sutton, 1975), which is true of the type species of the genus, *P. dimorphospora* B. Kendrick.

Results obtained in the present study have shown that the type species of *Sporendocladia*, *S. fumosa*, is not characterized by ring wall building as suggested by Wingfield & al. (1987). Although conidia of *S. fumosa* occur in chains, they have only basal dehiscence scars, and are formed by apical wall building. Conidia were also observed to be cuneiform rather than cylindrical in shape. Because of these discrepancies, a redescription of *S. fumosa* is given below.

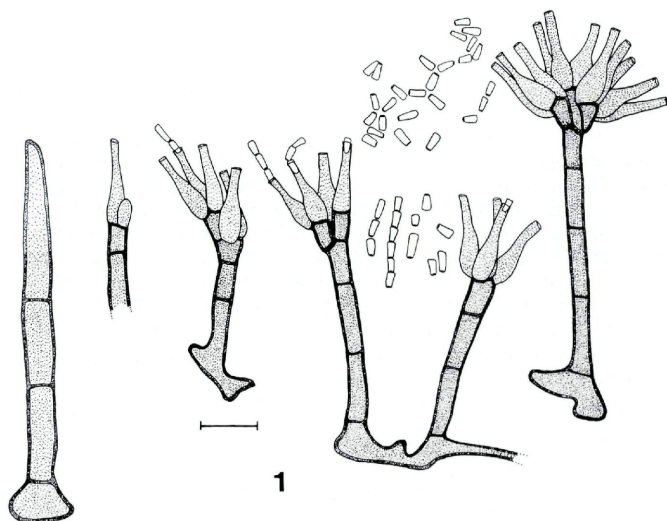


Fig. 1. - Developing macronematous conidiophores of *Sporendocladia fumosa* giving rise to cuneiform conidia arranged in false chains (bar = 10  $\mu$ m).

***Sporendocladia fumosa*** (Ell. & Ev.) Wingfield, Trans. Br. mycol. Soc.

89: 515 (1987). - Figs. 1-5.

= *Spicaria fumosa* Ell. & Ev., Bull. Torrey Bot. Club 10: 97 (1883).

= *Phialocephala fumosa* (Ell. & Ev.) Sutton, Trans. Br. mycol. Soc. 64: 411 (1975).

= *Sporendocladia castaneae* Arnaud, Bull. Soc. Mycol. Fr. 69: 279 (1954), nom. inval.

= *Sporendocladia castaneae* Arnaud ex Nag Raj & B. Kendrick, A monograph of *Chalara* and allied genera, p. 162 (1975).

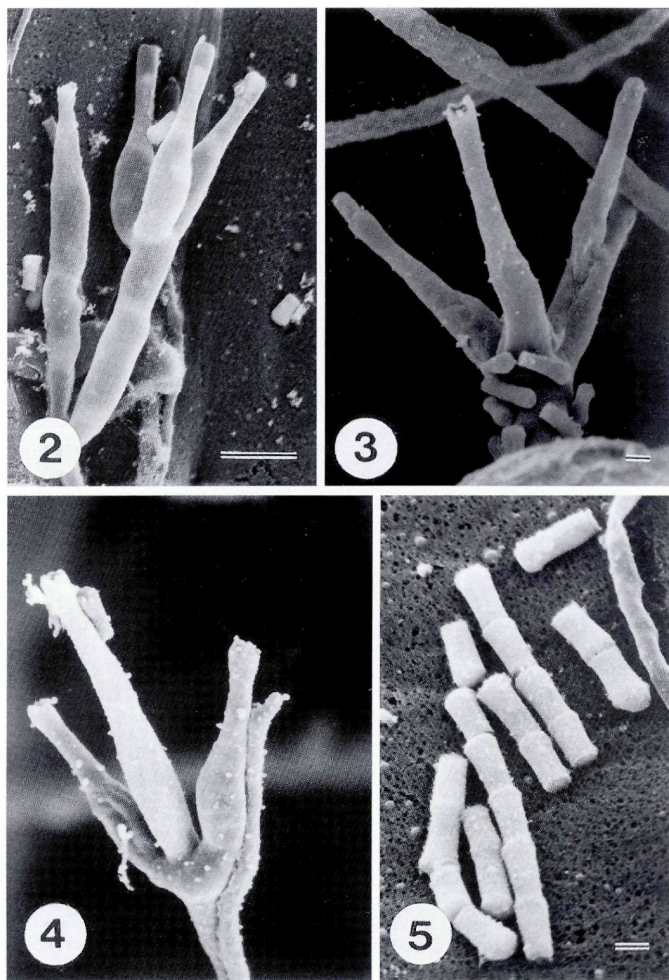
Colonies on MEA effuse, hairy in the middle, becoming smooth towards the even, felty margin, honey to primrose, 21 "b-23" d, (reverse) (Rayner, 1970). - Mycelium immersed and superficial, consisting of septate, branched, smooth, hyaline hyphae, 1.5-2.5  $\mu$ m wide, becoming brown near the conidiophores. - Conidiophores macronematous, mononematous, unbranched, solitary, erect, straight, smooth, dark brown, becoming paler towards the apex, thick-walled, 40-110  $\mu$ m high, 3-5  $\mu$ m wide at the first septum above the swollen base, 4-7-septate, 3-5  $\mu$ m wide below the conidiogenous branches (Figs. 1, 2). - Conidiogenous apparatus consisting of branches with terminal phialides in a loose arrangement with a mono-

to biverticillate branching pattern (Pitt, 1979); primary branches cylindrical, 5–7  $\mu\text{m}$  long, 3–5  $\mu\text{m}$  wide, brown, smooth, thick-walled, giving rise to terminal phialides. – *Phialides* thick-walled, light brown, smooth, lageniform, consisting of an ellipsoid venter 7–10  $\mu\text{m}$  long, 3–4  $\mu\text{m}$  wide, and a cylindrical collarete 5–7  $\mu\text{m}$  long, 1.5–2  $\mu\text{m}$  wide (Figs. 1, 2–4). – *Conidial ontogeny* by apical wall building; secession schizolytic. – *Conidia* catenate, arranged in false chains with only basal dehiscence scars, borne in a mucous droplet at the apex of conidiophores, non-septate, smooth, hyaline, cuneiform with rounded apices and truncate bases, widest at their apices, tapering inconspicuously to narrower bases, 2–3(–5)  $\times$  1–1.5  $\mu\text{m}$  (Figs. 1, 5). In culture on MEA, conidia become up to 7  $\mu\text{m}$  long, and more prominently basal tapered, with swollen, rounded apices. – *Cardinal temperatures*: minimum 15 C, maximum below 30 C, optimum 25 C, reaching an average radial growth of 3.5 mm after 10 d at 25 C in the dark.

*Specimens examined*. – SWITZERLAND: Comano, *Castanea sativa* cupules, P.W. Crous, M.J. Wingfield & O. Petrini, May 1993, PREM 51633 (culture CPC 650, CBS 518.93); Comano, *C. sativa* stump, M.J. Wingfield, May 1993, PREM 51634. – UNITED KINGDOM: Nr. Dorking, Surrey, *C. sativa* cupules, B.C. Sutton & M. Gumel, 11 Jul. 1973, IMI 176928b. – FRANCE: *C. vesca*, G. Arnaud, Sept. 1945, PC 2230 (lectotype of *S. castaneae*). – USA: Philadelphia, Fairmont Park, *Castanea* cupules, H. Bilgram, Jul. 1883, NY 5266 (type of *Spicaria fumosa*).

*S. fumosa* resembles the type species of *Phialocephala*, *P. dimorphospora*, in having conidia with only basal scars arranged in false chains. Despite this similarity, however, conidia of *S. fumosa* are cuneiform, whereas those of *Phialocephala* species are mostly oval. Furthermore, Mouton & al. (1993) also found that the mode of conidium development in *P. dimorphospora* might be distinct from other species of *Phialocephala*.

In showing that phialides of *S. fumosa* form conidia via apical wall building, this criterion can no longer be used to distinguish species of *Phialocephala* from those of *Sporendocladia*. The latter genus can, however, still be distinguished from *Phialocephala* by having species with cylindrical or slightly tapered conidia produced within phialides with cylindrical collarettes. Furthermore, it is our opinion that the genus *Phialocephala* still includes species that are distantly related. This contention was recently reinforced by Siegfried & al. (1992) in their description of *P. virens* Siegfried & Seifert. This species has distinctive green colonies, whereas *Phialocephala* species usually produce grey, black or brown colonies (Siegfried & al., 1992). In addition, *S. fumosa* has honey to primrose colonies, which seem to add a third group to the genus.



Figs. 2-5. - Scanning electron micrographs of *Sporendocladia fumosa*. - 2. conidiophore with phialides showing apical wall conidial development (bar = 5 μm). - 3, 4. conidiophores with phialides showing tubular collarettes and cuneiform conidia (bar = 1 μm). - 5. cuneiform conidia in false chains with thickened basal hila, indicating basal dehiscence scars (bar = 1 μm).

*Phialocephala dimorphospora* has recently been shown to exhibit an unique pattern of conidium development which is fundamentally different from the typical apical wall building development found in *P. virens* (Mouton & Wingfield, 1993; Mouton & al., 1993). These findings, together with those of the present study, support the view that this group of fungi need revision. We are of the opinion that a more intensive study of conidium development and molecular comparisons in additional species of *Phialocephala* should precede any further treatment of this group.

The genus *Haplographium* Berk. & Broome contains species with unbranched, erect, brown conidiophores with a compact apical apparatus that gives rise to hyaline conidiogenous cells and conidia in slimy heads. Castañeda & Kendrick (1990) erected a similar genus, *Lauriomyces* Castañeda, for species bearing conidia in dry persistent chains; those of *Haplographium* are not arranged in chains, and are borne in slimy droplets. In establishing the genus *Lauriomyces*, Castañeda & Kendrick (1990) noted its similarity to genera such as *Haplographium*, *Leptographium*, *Phialocephala* and *Verticicladium* Preuss. Castañeda & Kendrick (1990) recognized four species of *Lauriomyces*, of which one, *L. heliocephalus* (Rao & de Hoog) Castañeda & Kendrick, resembles the species collected in this study. An examination of the type specimen of *L. heliocephalus* (CBS-H 3920), however, showed that these two species could easily be distinguished. Conidiophores of *L. heliocephalus* are shorter, 110–140 µm long, whereas those of the species from *Castanea* cupules are up to 200 µm long. Furthermore, the setiform conidiophores observed in our collection were not present in the type of *L. heliocephalus*, and the conidiophores did not regenerate enteroblastically as in our species. Conidia of *L. heliocephalus* are also shorter and narrower than those of the *Castanea* collection. The *Lauriomyces* species from *C. sativa* cupules is therefore described as new.

***Lauriomyces bellulus*** P. W. Crous & M. J. Wingfield anam. sp. nov. – Figs. 6–13.

Coloniae in agarò maltoso effusae, planae et leves, in medio ob intensam conidiationem candidae, margine levi, flavobrunneae. Mycelium superficiale et immersum, hyphis septatis, hyalinis, ramosis, levibus, conidiophora versus brunneis, 3–4 µm crassis. Conidiophora macronemata, mononemata, simplicia, solitaria, erecta, recta vel curvata, levia, atrobrunnea, fertilia vel setae instar sterilia, apicem versus pallidiora, crassitunicata; conidiophora apicem versus anguste rotundata, ad 600 µm alta, 4–7 µm ad primum septum supra basim crassa. Conidiophora ramosa ad triseriata, cellulae ramorum tenuitunicatae, cylindraceae, apice paululum inflato, denticulatae, e denticulis ramiconidii orientibus. Ramiconidia primaria 8–13 x 4–5 µm, secundaria 5–8 x 3–4 µm, tertiaria 5–6 x 2–4 µm. Ramiconidiorum ontogenesis holoblastica, acropetaliter catenata, catenis ramosis

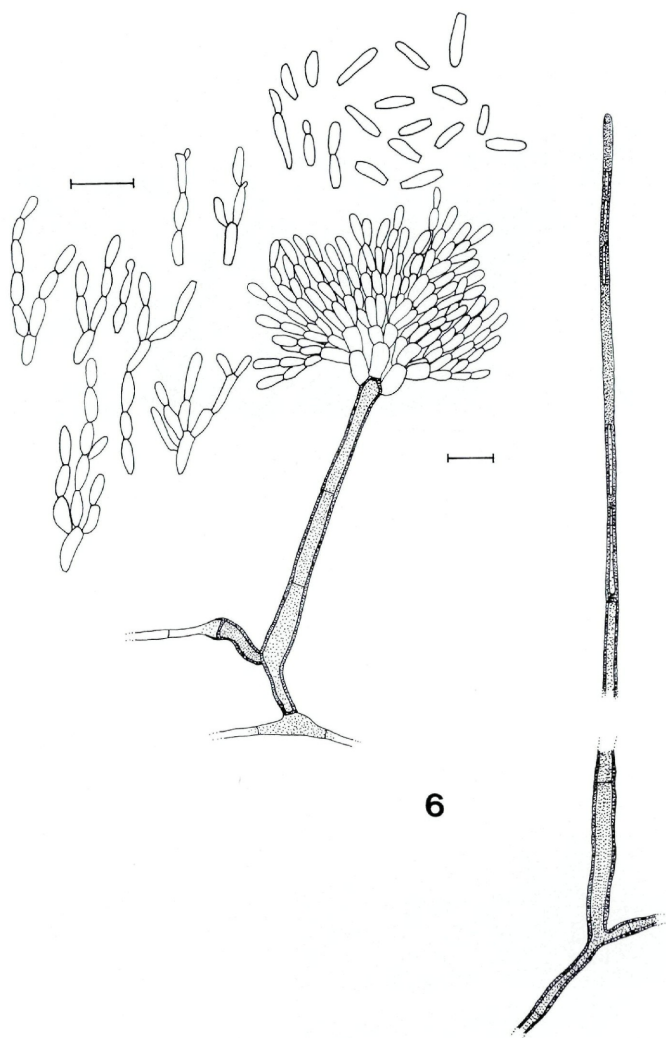
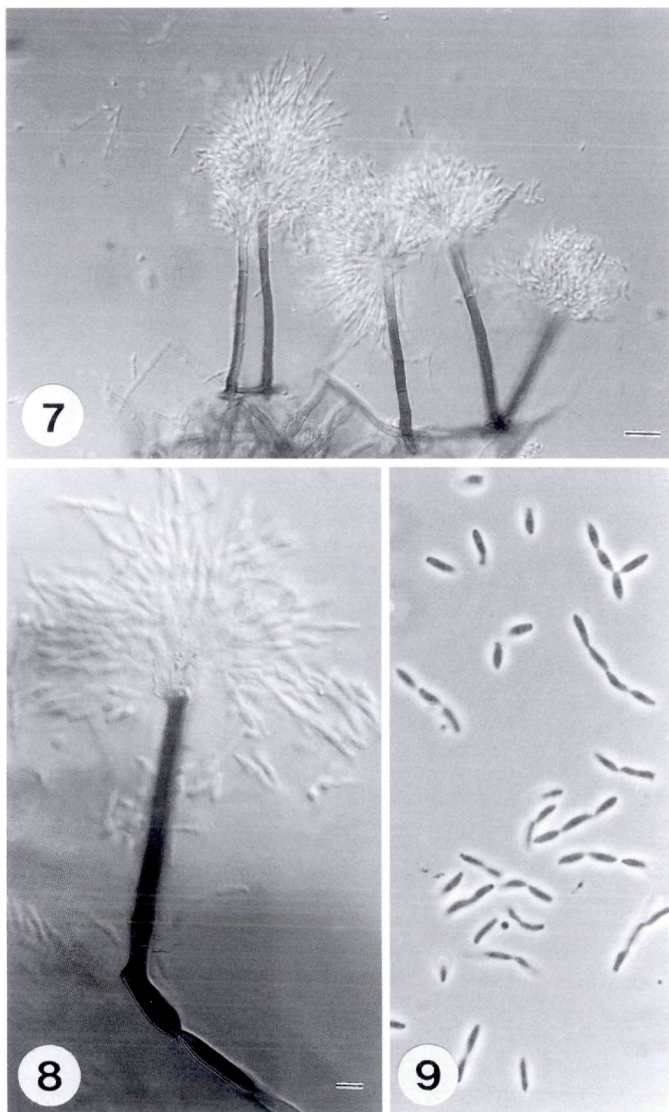
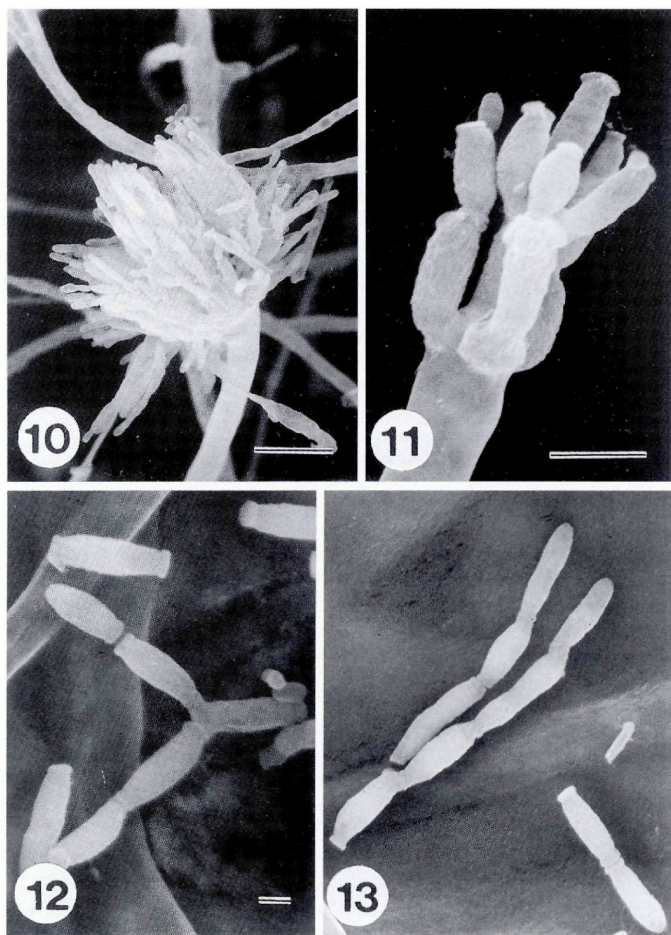


Fig. 6. - Seta and conidiophore of *Lauriomyces bellulus* giving rise to branched conidial chains arranged in dry heads (bar = 10  $\mu$ m).



Figs. 7-9. - Light micrographs of *Lauriomyces bellulus*. - 7. mononematous conidiophores (bar = 15  $\mu\text{m}$ ). - 8, 9. conidiophore arising directly from the mycelium and giving rise to hyaline, non-septate, branched conidial chains (bar = 5  $\mu\text{m}$ ).





Figs. 10-13. - Scanning electron micrographs of *Lauriomyces bellulus*. - 10. conidiophore with conidia arranged in dry heads (bar = 10 µm). - 11. conidiogenous apparatus showing conidiophore branches (bar = 5 µm). - 12 & 13. branched conidial chains showing thickened conidial dehiscence scars (bar = 1 µm).

vel eramosis, eseptata, levia, hyalina, cylindracea vel ellipsoidea, apice rotundata, basi subtruncata, 5-7(-9) x 1.5-2  $\mu\text{m}$ .

Holotypus: ad cupulas *Castaneae sativae*, pago Comano, in Helvetia, a P.W. Crous, M. J. Wingfield & O. Petrini lectus, Mayo 1993, PREM 51635, CBS 518.93.

Colonies on MEA effuse, smooth, with profuse white sporulation towards the centre, margin smooth, straw-coloured (reverse), 21'f (Rayner, 1970), aerial mycelium absent, and point of inoculation darkening with age. - Mycelium immersed and superficial, consisting of septate, branched, smooth hyphae, hyaline, becoming brown near the conidiophores, 3-4  $\mu\text{m}$  wide. - Conidiophores macronematous, mononematous, simple, solitary, erect, straight or curved, smooth, dark brown, fertile or sterile and setose (Fig. 6), becoming lighter brown towards the apex, thick-walled; setiform conidiophores tapering to acutely rounded apices, up to 600  $\mu\text{m}$  long, 4-7  $\mu\text{m}$  wide at the first basal septum; fertile conidiophores 1-5-septate, up to 200  $\mu\text{m}$  long, 4-5  $\mu\text{m}$  wide at the first basal septum (Figs. 6, 7, 8, 10). - Sporogenous apparatus complex, consisting of a series of 1-3 branches or conidiogenous cells arising from the stipe apex, branches hyaline, thin-walled, subcylindrical, slightly swollen at apices, with inconspicuous denticles on which other branches are situated, in whorls of 4-8; primary branches 8-13 x 4-5  $\mu\text{m}$ ; secondary branches 5-8 x 3-4  $\mu\text{m}$ ; tertiary branches 5-6 x 2-4  $\mu\text{m}$ ; each branch can also act separately as a ramoconidium (Figs. 6, 8, 11). - Ramoconidia blastical-acropetal, catenate, in branched or unbranched chains, hyaline, smooth, cylindrical to ellipsoidal, rounding towards flattened, subtruncate ends, 5-7(-9) x 1.5-2  $\mu\text{m}$ , with up to 10 conidia in the main branches (Figs. 6, 9, 12, 13). - Cardinal temperatures: minimum 5-10 C, maximum 25-30 C, optimum 25 C, having an average radial growth of 13 mm after 10 d at 25 C in the dark.

Holotype. - SWITZERLAND: Comano, *Castanea sativa* cupules, P.W. Crous, M.J. Wingfield & O. Petrini, May 1993, PREM 51635 (culture CPC 649, CBS 517.93).

Since the broader definition of the *Leptographium* complex has in recent years been reassessed (Castañeda & Kendrick, 1990; Wingfield & al., 1987; Wingfield, 1985), it has become obvious that numerous collections of this group lodged in herbaria worldwide require reconsideration. Our studies of *Phialocephala* and *Lauriomyces* strains have revealed considerable variation among species in these genera. These findings suggest that generic concepts in this group should be re-evaluated using molecular and ultrastructural techniques.

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