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Leptographium elegans: a new species from Taiwan

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Amongst collections of ophiostomatoid fungi occurring on woody hosts in Taiwan a new species, *Leptographium elegans*, was isolated from freshly cut surfaces of *Chamaecyparis formosensis* which had been logged for milling. It is distinguished from other species by its short conidiophores and conidiogenous cells, as well as the presence of a *Sporothrix* synanamorph with prominently denticulate conidiogeneous cells. Although no evidence of a teleomorph has been found, its high tolerance of the antibiotic cycloheximide suggests that it is closely related to other species of *Leptographium* that share this characteristic and have *Ophiostoma* teleomorphs.

Species of *Leptographium* Lagerb. & Melin are best known as being associated with insects, particularly bark beetles that infest trees, especially conifers (Harrington, 1988, 1993). In the strict sense, these fungi are considered to be anamorphs of *Ophiostoma* Syd. & P. Syd. and are thus highly tolerant of the antibiotic cycloheximide (Harrington, 1981) and have both rhamnose and cellulose in their cell walls (Jewell, 1974; Weijman & de Hoog, 1975; Horner, Alexander & Julian, 1986). Numerous species of *Leptographium* are known as root pathogens of conifers (Harrington & Cobb, 1983; Wingfield, Cappretti & MacKenzie, 1988), *L. wageneri* (W. B. Kendr.) M. J. Wingf. being the most virulent species and causal agent of a serious black stain root disease in the western United States (Harrington & Cobb, 1983; Harrington, 1993).

Species of *Leptographium* form a component of what is commonly referred to as the *Leptographium* complex. The genera in this complex have, in common, differentiated dematiaceous conidiophores terminating in a series of metula which subtend conidiogenous cells. Conidia are hyaline amerospores and are produced in a slimy mass which facilitates insect dispersal. The *Leptographium* complex arose after the establishment of *Verticicladiella* S. Hughes to accommodate species resembling *Leptographium* but having sympodial as apposed to percurrent proliferating conidiogenous cells (Hughes, 1953). Likewise, *Phialocephala* W. B. Kendr. was established for species with phialidic conidial development (Kendrick, 1961). Recent studies have shown that both percurrent proliferation and apparent sympodial development are found in most species of *Leptographium*. Similarly, purported phialidic development in the *Leptographium*-like anamorph of *Ophiostoma francke-grosmanniae* (R. W. Davidson) de Hoog & R. J. Scheff. also appears to be typically percurrent (Mounton, Wingfield & Van Wyk, 1992).

The majority of *Leptographium* species presently known have been described from Europe and North America. Very little information is available on this group of fungi from other parts of the world. Given that most species are associated with bark beetles that infest conifers, it is likely, however, that many species have yet to be discovered in other areas where conifers are native.

The present species was found in Taiwan sporulating on freshly cut surfaces of *Chamaecyparis formosensis* which had been logged for milling purposes.

MATERIALS AND METHODS

The fungus was isolated by transferring conidial masses to malt extract agar (MEA) containing 10 g Difco agar and 20 g Oxoid malt extract. Temperature requirements for growth were determined on MEA by transferring colonized agar discs (3 mm diam.) from the periphery of an actively growing colony to the centres of Petri dishes and incubating three replicates at 5° intervals between 5 and 35 °C. Two diagonal measurements of colony diameter were taken for each plate at each temperature and the averages of these six measurements computed.

Tolerance to cycloheximide was tested at 25° in Petri dishes containing MEA amended with the antibiotic to give a range (0, 0·05, 0·1, 0·5, 1·0 and 2·5 g l^{-1}) of concentrations with three replicates for each concentration. Two diagonal colony

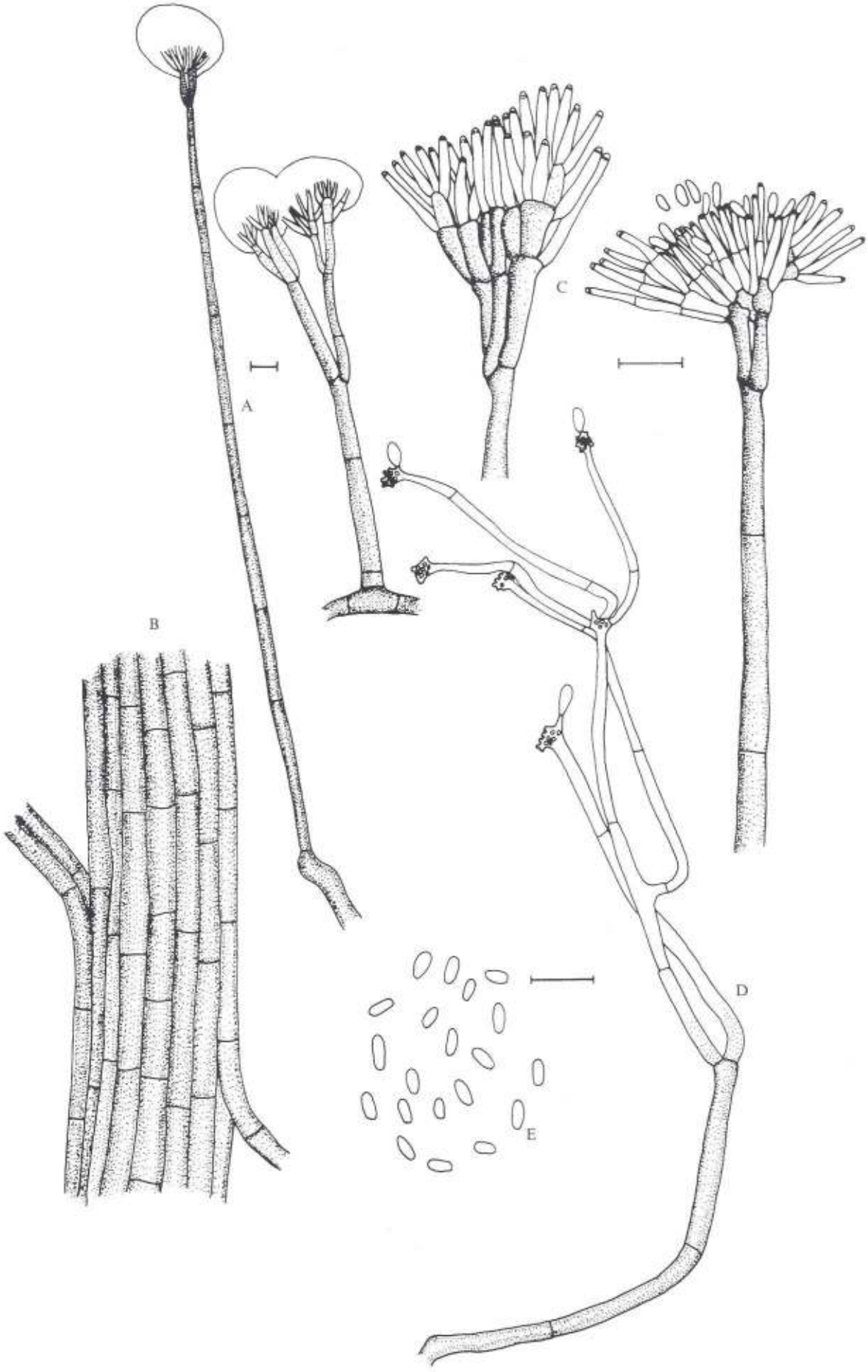


Fig. 1. For caption see facing page.

measurements were taken after eight days and averages computed.

For scanning electron microscopy, discs of MEA bearing fertile conidiophores were fixed in 2·5% glutaraldehyde and 1·5% osmium tetroxide in a 0·1 M phosphate buffer, dehydrated in a graded acetone series, critical-point dried, coated with gold-palladium and examined using a Jeol 6400 scanning electron microscope.

OBSERVATIONS AND DISCUSSION

Leptographium elegans M. J. Wingf., Crous & Tzean sp. nov. Figs 1–8

Culturae optime crescunt in MEA and 25°, 300 mm diam, octo diebus in tenebris. Mycelium sub 10° vel supra 30° non-crescens. Fungus resistit solutioni 2·5 g l^{-1} cycloheximide antibiotici continenti, auctu ad 25° deminuto circa 33% in MEA. *Coloniae* effusae, divaricatae, nigrae in MEA. *Hyphae* submersae, aerio mycelio sparso, pallide brunneae ad brunneae, verrucosae, 2·5–4·5 µm diam., singulae vel gregariae, fila 4–13 hypharum explandentia e centro inoculationis patellae formantia. *Conidiophora* singula, vel gregaria usque ad terna, macronematosa, mononematosa, parum aspera, rhizoideis plerumque absentibus ad basim haud tumidam. *Stipites* erecti, brunnei, simplices, 5–11-septati, 90–300 µm longi, 6–9 µm lati ad basim. *Apparatus conidiogenus* 25–45 µm longus, massa conidica exclusa; una ad tres metulae primae, medio-brunneae, metulis centralibus paulo maioribus quam ceteris, 17–35 × 3–3·5 µm; metulae secundariae sub-brunneae, 9–14 × 2·5–3·5 µm. *Cellulae conidiogenae* discretae, ad apicem attenuatae, 10–17 × 2–2·5 µm. *Conidiogenesis* holoblastica proliferatione percurrenti, secessione retardata reddenti faciem fallacem proliferationis sympodialis. *Conidia* hyalina, oblonga vel ellipsoidea, apicibus obtuse rotundatis, rotundata, basi subtruncata 4–6 × 1·5–2·5 µm. Conidia accumulata circa apparatum conidiogenum in massa hyalina mucilaginosa. Nonnullae hyphae fiunt subbrunneae et denique hyalinae, 1·5–2·5 µm latae, *Sporothricem* synanamorphosin formantes. *Cellulae conidiogenae* sparsae, terminales vel in rama lateralia brevia integratae, constantes latitudine, vel latissimae at apicem tumidum, 15–60 µm longae, 1·5–2·5 µm latae sub apice tumido. *Conidia* sympodialiter e fasciculo denticulorum in tumidis apicibus; denticuli cylindracei, extantes, 1–2 µm longi et 1 µm lati.

Cultures growing optimally on MEA at 25°, reaching 30 mm diam. after eight days in the dark, no growth occurring below 10° or above 30°, tolerant of high concentrations of cycloheximide, with growth at 25° reduced by approximately 33% on MEA containing 2·5 g l^{-1}. *Colonies* effuse, spreading, black on MEA. *Hyphae* mainly immersed in the medium, aerial mycelium sparse, brown, verrucose, 2·5–4·5 µm diam., occurring singly or aggregated in strands of 4–13 hyphae, spreading in a radiating fashion (Fig. 1). *Conidiophores* single, or in groups of up to three, macronematous, mononematous, finely roughened, with rhizoids mostly absent at the unswollen base. *Stipe* erect, brown, simple, 5–11-septate, 90–300 µm long, 6–9 µm wide at the base. *Conidiogenous apparatus* 25–45 µm long excluding the conidial mass; one to three medium brown primary metulae, central metulae slightly larger than the others, 17–35 × 3–3·5 µm; secondary metulae pale brown, 9–14 × 2·5–3·5 µm; tertiary metulae hyaline, 6–11 × 2·5–3·5 µm (Figs 1–6). *Conidiogenous cells* discrete, tapering distally, 10–17 × 2–2·5 µm. *Conidium development* replacement wall building with holoblastic ontogeny and percurrent proliferation with delayed secession giving a false appearance of sympodial proliferation. *Conidia* hyaline, oblong to ellipsoid, with a bluntly rounded apex and rounded to subtruncate base, 4–6 × 1·5–2·5 µm, accumulating around the conidiogenous apparatus in a hyaline mucilaginous mass. *Synanamorph* referrable to *Sporothrix* formed on light brown or hyaline hyphae, 1·5–2·5 µm wide (Figs 1, 7). *Conidiogenous cells* scattered, terminal or integrated in short side branches, of uniform width or widest at swollen fertile apex, 15–60 µm long, 1·5–2·5 µm wide below swollen apex, denticulate, denticles cylindrical, protruding. *Conidia* borne sympodially on the denticles, 1–2 µm long and 1 µm wide.

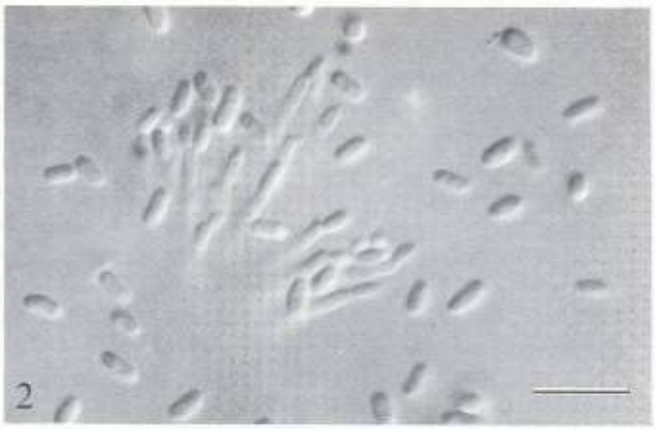
Isolated from wood of *Chamaecyparis formosensis*, Taiwan, Lotung Ilan County, 1992, M. J. Wingfield, PREM 51442, holotype.

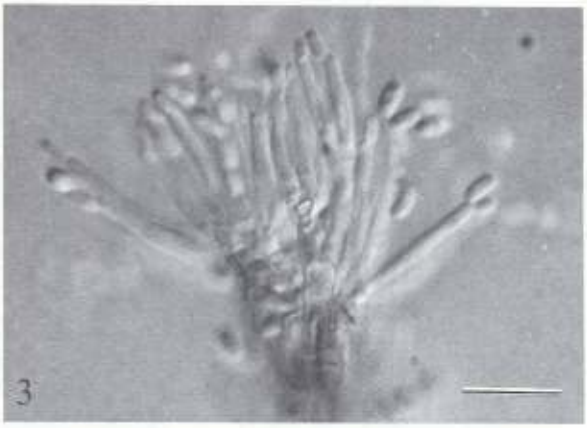
Leptographium elegans is distinguished from other species by the unusual and consistent presence in culture of a distinct *Sporothrix* Hektoen & C. F. Perkins synanamorph and by its relatively short conidiophores and conidiogenous cells. In these characteristics it resembles the currently undescribed *Leptographium* anamorph of *Ophiostoma francke-grosmanniae* which has also recently been shown to have a *Sporothrix* synanamorph (Mouton, Wingfield & Van Wyk, 1992). However, this is rare in cultures and never results in complex denticulate conidiogenous cells typical of the synanamorph of *L. elegans*. *Sporothrix* synanamorphs are a common feature of many *Graphium* anamorphs of *Ophiostoma* (Upadhyay, 1981; Seifert & Okada, 1993) and, given that *Leptographium* is the mononematous analogue of *Graphium*, the absence of *Sporothrix* synanamorphs in *Leptographium* has been considered unusual (Mouton *et al.*, 1992).

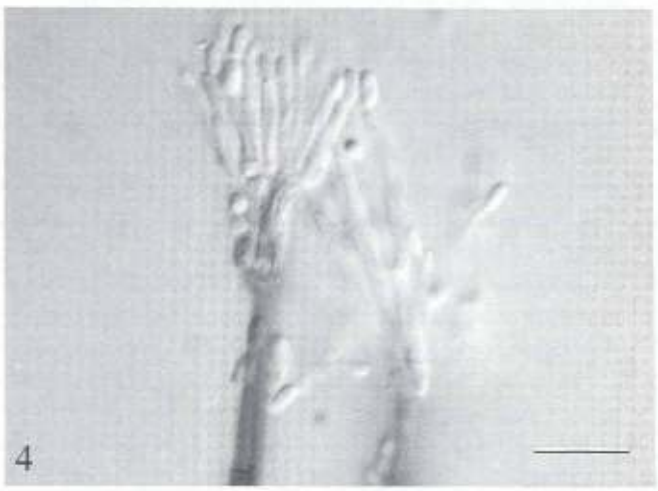
It is our view that *Leptographium* should be reserved for proven or purported anamorphs of *Ophiostoma*. Although we have no evidence of a teleomorph for *L. elegans*, the fact that this fungus can tolerate high concentrations of cycloheximide in culture suggests that it is closely related to species of *Ophiostoma* and *Leptographium* sharing this characteristic (Harrington, 1981).

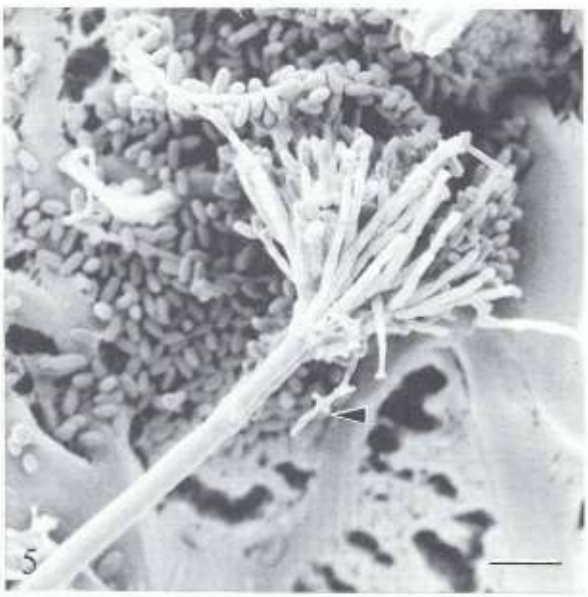
Species of *Leptographium* have previously been separated from other members of the *Leptographium* complex based on different patterns of conidium development. In recent years this characteristic has been shown to be unreliable and misleading (Wingfield, 1985; Wingfield, Van Wyk & Wingfield, 1987; Van Wyk, Wingfield & Marasas, 1988; Mouton, Wingfield & Van Wyk, 1992). As has been found in other species of *Leptographium*, conidium development in *L. elegans* appears to be sympodial when observed using a light microscope. However, conidiogenous cells can clearly be seen to proliferate percurrently when observed using a scanning electron microscope with the illusion of sympodial development resulting from delayed secession of conidia. This has been found to be typical of all other species of *Leptographium* with apparent sympodial development (Wingfield, 1985).

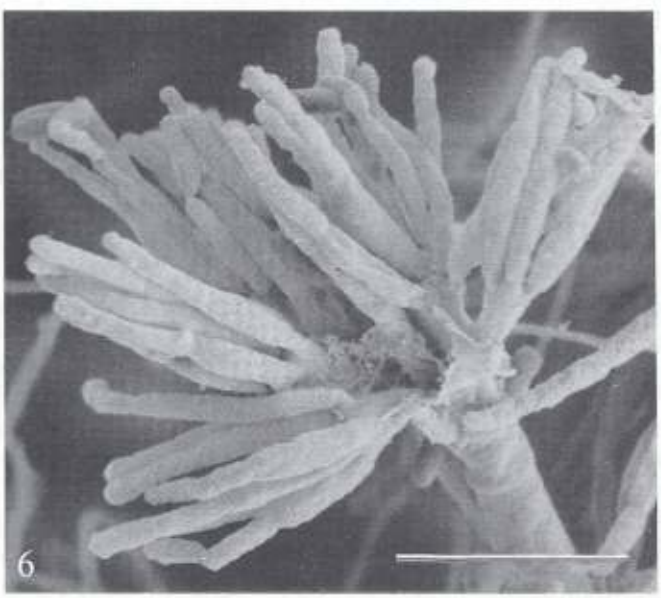
Fig. 1. *Leptographium elegans* (bars = 10 µm). A, Mononematous, dematiaceous conidiophores without basal rhizoids; B, superficial mycelial strand consisting of several dark brown, septate, verruculose hypha; C, conidiogenous apparatus showing several series of metulae; D, dark brown hypha giving rise to a *Sporothrix* synanamorph with denticulate conidiogenous cells; E, hyaline, oblong to ellipsoid conidia with bluntly rounded bases.

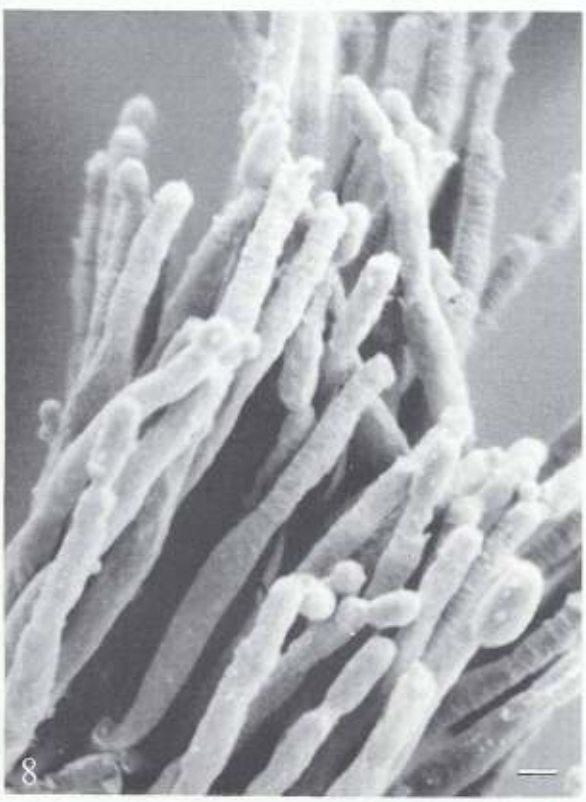












Figs 2–8. For captions see facing page.

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Figs 2–8. *Leptographium elegans* and its *Sporothrix* synanamorph. **Fig. 2.** Conidiogenous cells and oblong to ellipsoid conidia (bar = 10 μm). **Figs 3, 4.** Conidiogenous apparatus of *L. elegans* with apparent sympodial proliferation (bar = 10 μm). **Fig. 5.** SEM micrograph of differentiated *Leptographium* anamorph and integrated *Sporothrix* (arrowed) synanamorph (bar = 10 μm). **Fig. 6.** Conidiogenous cells on mononematous conidiophore of *Leptographium* anamorph. **Fig. 7.** Denticulate conidiogenous cell of the *Sporothrix* synanamorph (bar = 10 μm). **Fig. 8.** Conidiogenous cells of *Leptographium* anamorph showing percurrent proliferations (bar = 1 μm).