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Xenocylindrocladium guianense and *X. subverticillatum*, two new species of hyphomycetes from plant debris in the tropics

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Two new species of hyphomycetes, *Xenocylindrocladium guianense* and *X. subverticillatum*, are described from plant debris collected in French Guiana and Singapore, respectively. The genus *Xenocylindrocladium* has thus far been known from one species, *X. serpens*, which was described from plant debris collected in Ecuador. The two new taxa are compared with and distinguished from *X. serpens* based on morphology, cultural characteristics and phylogenetic analysis of DNA sequence data of the 5.8S rDNA with flanking ITS1 and ITS2 regions and the 5′ end of the β-tubulin gene. These species are also compared with other closely related hypocrealean taxa. Present collection data suggest that species of *Xenocylindrocladium* could be restricted to the tropics.

Key Words——*Cylindrocladium*; Hypocreales; saprobe; *Xenocylindrocladium*.

During recent collecting trips to French Guiana and Singapore, two unusual hyphomycetes were found sporulating on dead plant materials in rainforests. The hyaline, penicillate conidiophores had the tendency to cluster in sporodochia. Conidia were septate and cylindrical, thus suggesting them to be representative of the *Cylindrocladium* complex. However, stipe extensions were hyaline, smooth, avesiculate and curved, becoming septate at maturity, thus not resembling *Cylindrocladium* Morgan *sensu stricto*, but rather *Xenocylindrocladium* Decock, Hennebert & Crous. Although there are many similarities between the two genera, they are phylogenetically distinct, and also have different teleomorphs: i.e. *Calonectria* De Not. for *Cylindrocladium*, and *Xenocalonectria* Crous & C.L. Schoch for *Xenocylindrocladium* (Schoch et al., 2000).

The monotypic genus *Xenocylindrocladium* was established for *X. serpens* Decock, Hennebert & Crous collected from the bark of a rainforest tree in Ecuador (Decock et al., 1997). The teleomorph, *Xenocalonectria serpens* (Decock, Hennebert & Crous) Crous & C. L. Schoch, is a homothallic species, and is distinguished from *Calonectria* by having long cylindrical asci with flattened apices, refractive apical apparati, and 1-septate, ellipsoidal ascospores. Furthermore, a phylogeny derived from ITS1 and ITS2 data (Schoch et al., 2000) showed *Xenocylindrocladium* to be closely related to *Curvicladium* Decock & Crous (1998), but distinct from *Cylindrocladium*.

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The aim of the present study was thus to name the new hyphomycetes from French Giuana and Singapore, and also to confirm their phylogeny to other taxa in the *Cylindrocladium* complex.

Materials and Methods

Isolate collection and morphology Isolation of the cylindrocladium-like fungi were performed from single conidia under a stereo microscope immediately after collection. Isolates were first cultured on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa), and then plated onto carnation-leaf agar (CLA) (Fisher et al., 1982; Crous et al., 1992). They were incubated at 25°C under near-ultraviolet light, and examined after 7 d. Sporulating cultures were treated as explained in Crous et al. (2000). Growth and cultural characteristics were determined after 6 d on MEA at 25°C in the dark, using procedures described by Crous and Wingfield (1994). Colony colours were coded according to Rayner (1970). Cryoscanning electron microscopy (cryo-SEM) was used to observe the nature of the stipe and terminal vesicle. Specimens were flash frozen (−212°C) in liquid nitrogen under vacuum for cryo-SEM, transferred to the preparation chamber, and then to the SEM chamber where the frozen samples were sublimated (−80°C) to remove ice particles. Samples were sputter coated with gold in the preparation chamber for 75 s under 1.2 kV at −170°C. Specimens were viewed under 5 kV at −188°C with a Phillips XL20 scanning electron microscope using an Oxford cryosystem.

Sequencing and phylogenetic analysis Single conidial isolates selected for DNA comparison were grown on MEA plates. DNA extraction methods and amplification conditions were as explained by Crous et al. (2000). PCR reactions and conditions were as specified by Schoch et al. (1999, 2000). DNA was amplified using the primers ITS1 and ITS4 (White et al., 1990) for the ITS region, as well as T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) for the β-tubulin gene. The regions amplified were the 5.8S ribosomal RNA gene with the two flanking internal transcribed spacers (ITS1 and ITS2), as well as the 5′ end of the β-tubulin gene. Approximately 540 bp to 600 bp fragments were amplified, and the PCR products were sequenced using the ABI Prism 377 DNA sequencer (Perkin-Elmer, Norwalk, Connecticut) under the conditions as mentioned by Schoch et al. (1999). Sequences were aligned with the computer package by means of the Clustal algorithm with Megalign, forming part of the DNAstar computer package, but also adjusted manually. In order to keep the alignments as objective as possible only minor manual adjustments were made at the tips of the data sets. Phylogenetic analysis of aligned DNA sequences was performed using PAUP* Version 4.0b3a (Swofford, 2000) and printed with the help of TreeView Version 1.5 (Page, 1996). Gaps were treated as missing data. A number of strains representing different species of *Cylindrocladium* and *Xenocylindrocladium* (Schoch et al., 2000) were selected for the generic analysis (Fig. 1). Confidence intervals were determined using 1000 bootstrap replications by means of the branch and bound method in all cases. Partition homogeneity tests were performed by PAUP* Version 4.0b3a with 1000 branch and bound repetitions. The ITS (U34559) and β-tubulin (U34417) DNA sequences of *Fusarium subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas (teleomorph, *Gibberella fujikuroi* (Sawada) Wollenw.) obtained from GenBank were used as outgroup. Additional representative ITS DNA sequences of *Cylindrocladiella infestans* Boesew., *Cylindrocladiella microcylindrica* Crous & D. Victor, *Curvicladium cigneum* Decock & Crous and *Gliocladiopsis tenuis* (Bugnic.) Crous & M. J. Wingf. were taken from Schoch et al. (2000) and included for comparison (Table 1). New sequence data obtained from the 5′ end of the β-tubulin gene for these species, as well as for two new species of *Xenocylindrocladium*, were deposited in GenBank (Table 1). The aligned sequence data were also deposited in TreeBase (S562).

Table 1. Isolates examined in this study.

Anamorph	Teleomorph	Original No.[a]	Collector	Host	Origin	Genbank No. (ITS: β-tubulin)
Cylindrocladium scoparium	*Calonectria morganii*	ATCC 46300	D.M. Benson	*Leucothoe catesbaei*	North Carolina, U.S.A.	AF210888: AF210873
Cylindrocladium floridanum	*Calonectria kyotensis*	ATCC 18882	R.H. Morrison	Peach roots	Florida, U.S.A.	AF220975: AF320193
Cylindrocladiella infestans	*Nectricladiella infestans*	ATCC 44816	H.J. Boesewinkel	*Pinus pinea*	New Zealand	AF220955: AF320190
Cylindrocladiella microcylindrica	*Nectricladiella camelliae*	ATCC 38571	W.A. Shipton	*Pinus pinea*	Australia	AF220960: AF320191
Gliocladiopsis tenuis	*Glionectria tenuis*	IMI 300597	Unknown	*Psidium guajava*	India	AF220980: AF320194
Curvicladium cigneum	Unknown	STE-U 1595	C. Decock	Leaf of angiosperm	French Guiana	AF220973: AF320192
Xenocylindrocladium serpens	*Xenocalonectria serpens*	STE-U 1144	G.L. Hennebert	Bark of unknown tree	Ecuador	AF220982: AF320195
Xenocylindrocladium subverticillatum	Unknown	STE-U 3397	C. Decock	Plant litter	Singapore	AF317347: AF320196
Xenocylindrocladium guianense	Unknown	STE-U 3496	C. Decock	Plant litter	French Guiana	AF317348: AF320197
Xenocylindrocladium guianense	Unknown	STE-U 3497	C. Decock	Plant litter	French Guiana	AF317349: AF320198

a) STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa.

Taxonomy

Xenocylindrocladium guianense Crous & Decock, sp. nov. Figs. 1–5

Teleomorph: Unknown.

Similis *Xenocylindrocladii subverticillati*, sed differt conidiis uni-septatis, (28–)34–38(–40) × 2.5–3 μm et conidiophoris non subverticillatis.

Etymology: Named after the place of collection.

Conidiophores separate or aggregated in sporodochia, comprised of a stipe, a penicillate arrangement of fertile

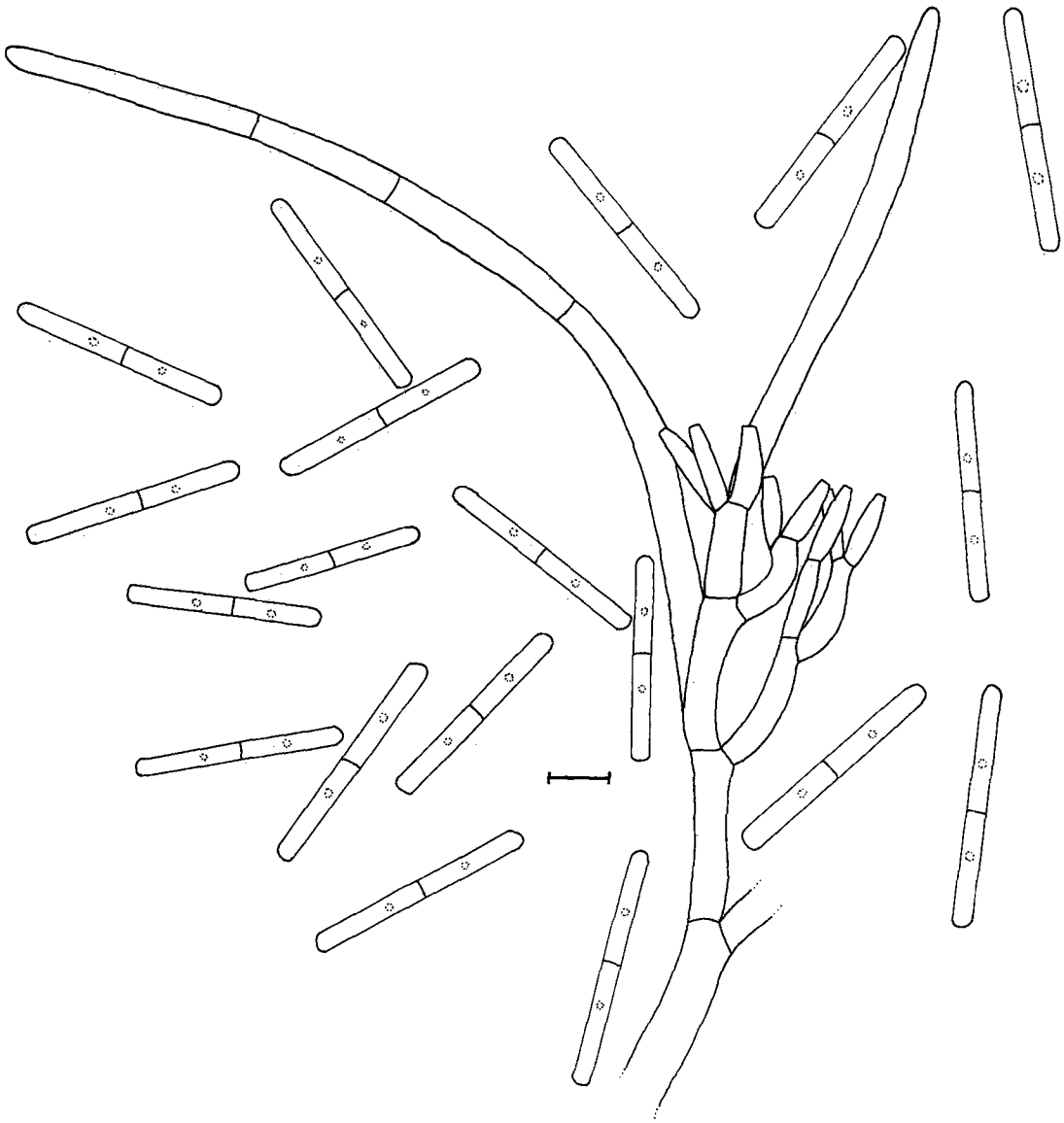


Fig. 1. Penicillate conidiophore and conidia of *Xenocylindrocladium guianense* (STE-U 3494, ex-type). Scale bar = 10 μm.

branches and an avesiculate stipe extension; stipe septate, hyaline, smooth, 60–80 × 4–5 μm; stipe extensions with one basal septum, developing more septa with age, flexuous, with a bluntly rounded apex, 60–150 μm long, 3.5–4 μm wide at the most apical septum, avesiculate, but frequently with a longer apical cell; 1–4 stipe extensions per conidiophore. Conidiogenous apparatus with aseptate or 1-septate primary branches, 20–35 × 4–5 μm; secondary branches aseptate, 10–25 × 3–4 μm; tertiary branches aseptate, 10–13 × 3–4 μm, each terminal branch producing 2–6 phialides; phialides elongate doliiform to reniform, hyaline, aseptate, 10–17 × 2.5–4 μm, apex with minute periclinal thickening and inconspicuous collarette. Conidia cylindrical, rounded at both ends, straight, (28–)34–38(–40) × 2.5–3 μm, 1-septate, lacking a visible abscission scar, held in cylindrical clusters by colourless slime.

Cultural characteristics: Colonies on MEA orange to sienna, 13b–13i in reverse; chlamydospores moderate to extensive, throughout medium, forming microsclerotia; aerial mycelium extensive, but not dense, off-white.

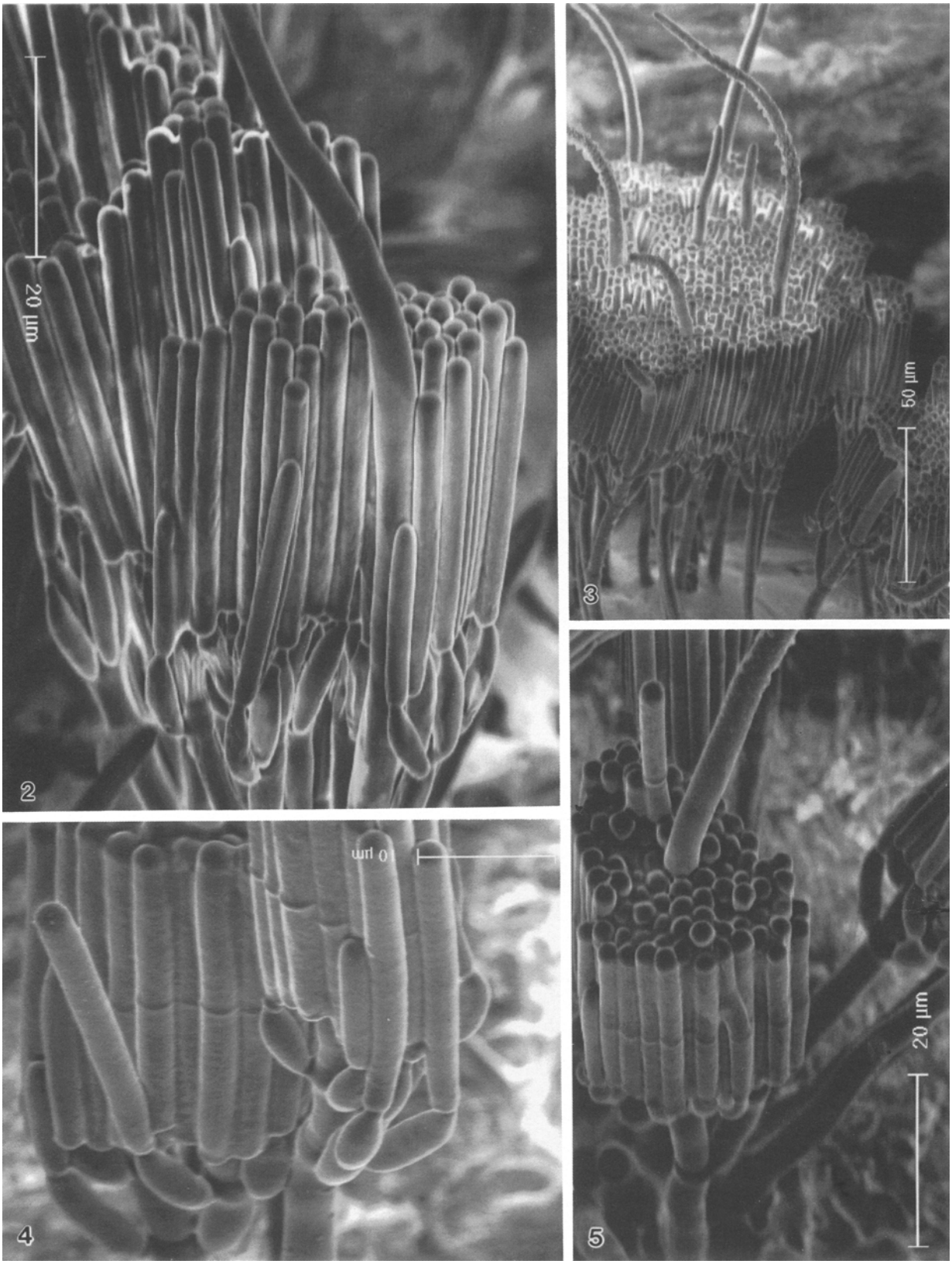
Cardinal temperatures for growth: Min above 10°C, max below 35°C, opt 25°C. This is a moderate temperature species, with medium sporulation on aerial mycelium.

Host: Unidentified plant material on soil surface.

Distribution: French Guiana.

Holotype: French Guiana, Route de l'Est (N2), close to the "Auberge des Orpailleurs", on plant debris in litter layer, rain forest, C. Decock FG 2084, 20 January 2000, PREM 57193, cultures ex-type MUCL 41973–41976 = STE-U 3494–3497.

Xenocylindrocladium subverticillatum Decock & Crous,



Figs. 2–5. *Xenocylindrocladium guianense* (STE-U 3494, ex-type).
2. Penicillate conidiophore with cylindrical spore mass. 3. Penicillate conidophores with avesiculate stipe extensions (note sticky exudate on stipe extensions). 4. Phialides giving rise to 1-septate conidia. 5. Conidiophore with stipe extension.

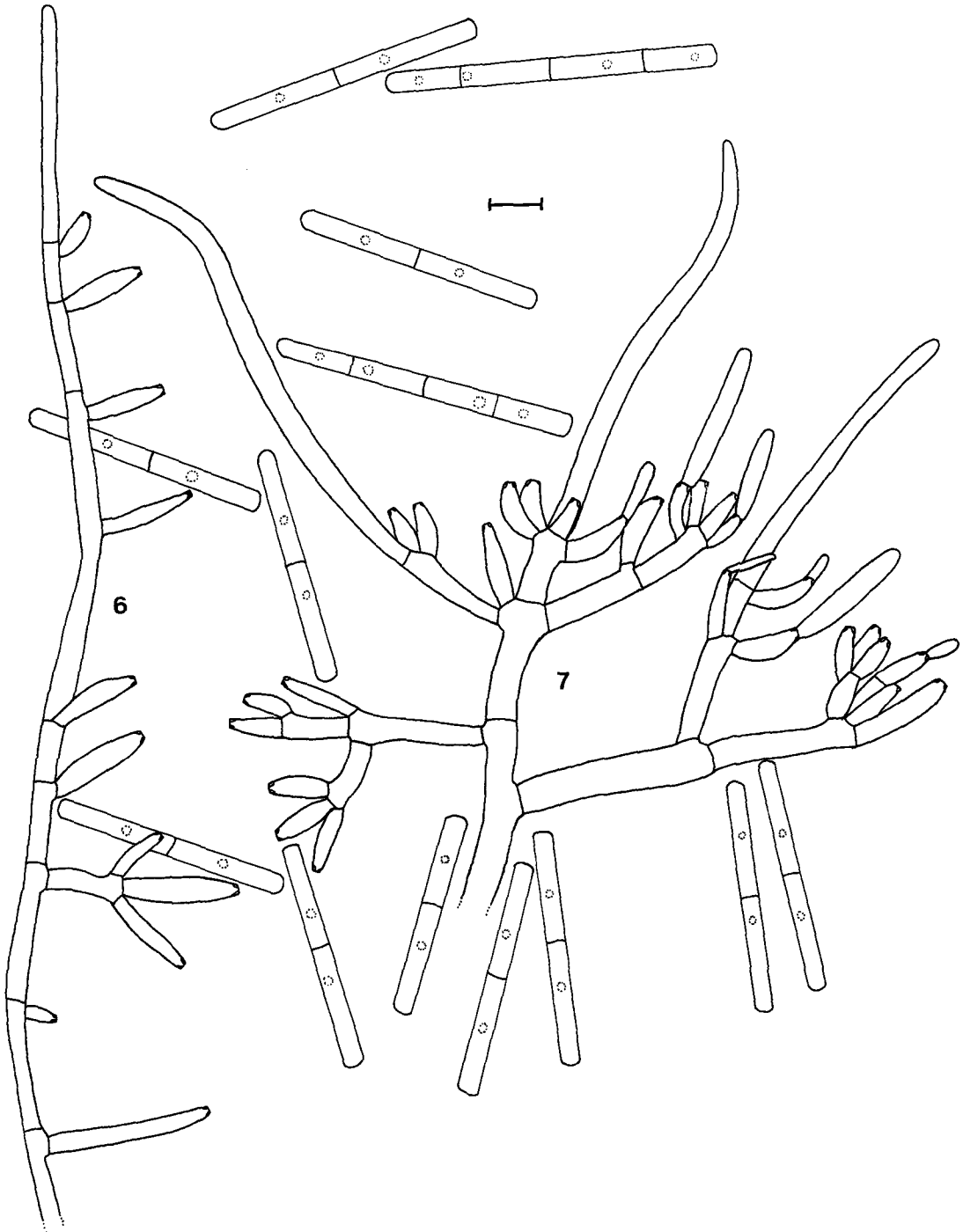
sp. nov. Figs. 6–9

Teleomorph: Unknown.

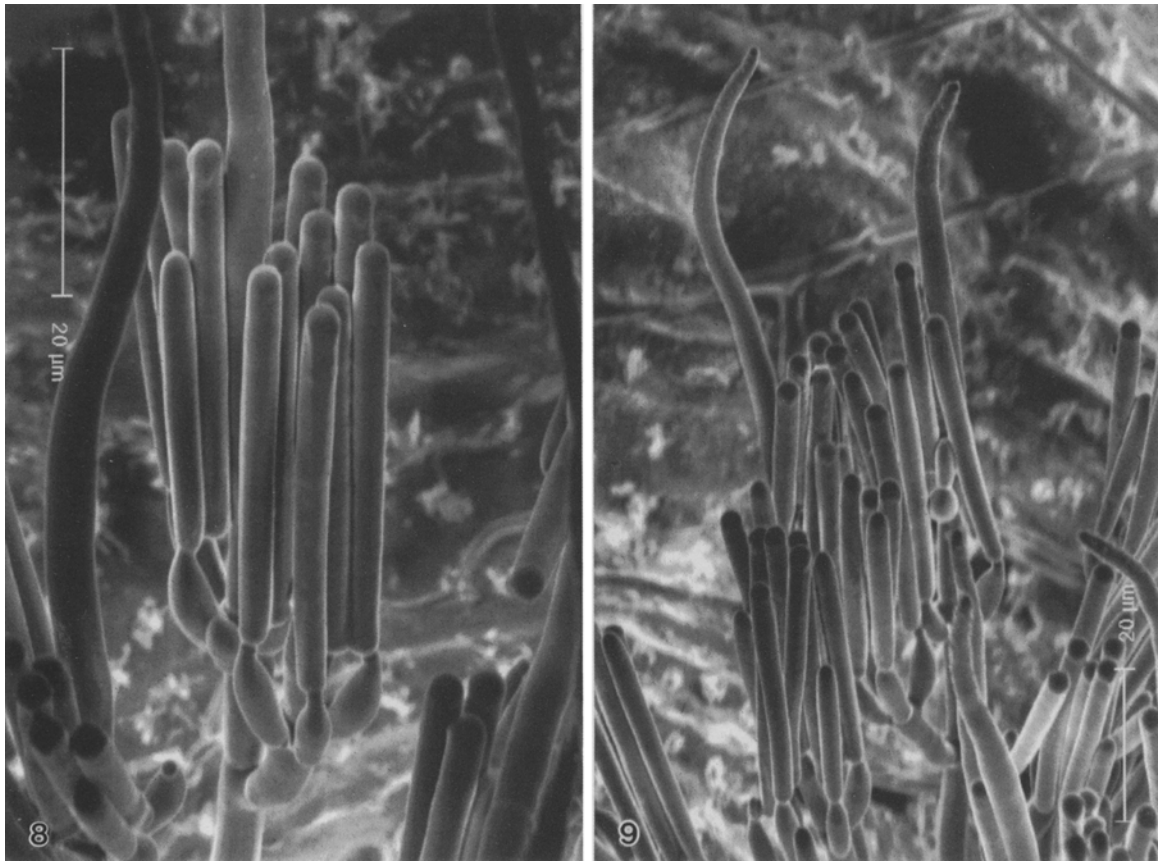
Similis typi generis, *Xenocylindrocladii serpenstis*, sed differt conidiis 1(–3)-septatis, (23–)32–38(–45)× (2.5–)3–4 μm et conidiophoris subverticillatis.

Etymology: Named after its subverticillate conidiophores.

Conidiophores frequently clustered in sporodochia, comprised of a stipe, a penicillate arrangement of fertile branches, and a stipe extension without a terminal vesicle; stipe 0–1-septate, hyaline, smooth, 25–50×3.5–5 μm; stipe extensions with one basal septum, developing more septa with age, flexuous, 80–150 μm long, 3.5–5 μm wide at basal or most apical septum, avesiculate, terminating in an acutely rounded apex; 1–4 stipe extensions per conidiophore. Conidiogenous apparatus with



Figs. 6, 7. *Xenocylindrocladium subverticillatum* (STE-U 3397, ex-type). 6. Subverticillate conidiophore. 7. Penicillate conidiophore with stipe extensions and 1–3-septate conidia.
Scale bar = 10 μm.



Figs. 8, 9. Conidiophores with conidia and avesiculate stipe extensions of *Xenocylindrocladium subverticillatum* (STE-U 3397, ex-type).

aseptate or 1-septate primary branches, 20–45 × 3.5–4.5 µm; secondary branches aseptate, 10–22 × 3.5–4 µm; tertiary branches aseptate, 10–15 × 3.5–4 µm, each terminal branch producing 2–4 phialides; phialides on penicillate conidiophores doliiform to reniform, hyaline, aseptate, 10–20 × 3–4 µm, apex with minute periclinal thickening and inconspicuous collarette; phialides on subverticillate conidiophores doliiform, reniform to subcylindrical, 12–30 × 2–3 µm. Conidia cylindrical, rounded at both ends, but widest at the apex, straight, rarely slightly curved, (23–)32–38(–45) × (2.5–)3–4 µm, 1(–3)-septate, lacking a visible abscission scar, held in cylindrical clusters with colourless slime, but spore mass frequently becoming red-purple on CLA or MEA with age.

Cultural characteristics: Colonies on MEA sienna to umber, 11i–13m in reverse, sienna on surface; chlamydospores extensive throughout medium; aerial mycelium extensive; conidial masses turning red-purple with age.

Cardinal temperatures for growth. Min above 5°C, max 35–40°C, opt 25°C. This is a high temperature species, with extensive sporulation on aerial mycelium.

Host: Unidentified plant material on soil surface.

Distribution: Singapore.

Holotype: Singapore, Peirce Reservoir, on a decaying twig of an herbaceous plant, secondary rain forest, C. Decock and O. Laurence, November 1999, PREM 57194, culture ex-type MUCL 41834 = STE-U 3397.

The curved, thin-walled, hyaline, smooth, avesiculate stipe extensions suggested both species to be members of the genus *Xenocylindrocladium*, and not *Cylindrocladium*. This was also confirmed by means of the sequence data (Fig. 10), which supported *Xenocylindrocladium* as closely related, but distinct from *Cylindrocladium*.

Discussion

The genus *Xenocylindrocladium* has hitherto been monotypic, based on *X. serpens* (Decock *et al.*, 1997). The collection of two additional species from French Guiana and Singapore suggests that this genus may be well represented in the tropics. *Xenocylindrocladium subverticillatum* can easily be distinguished from *X. serpens* by having 1(–3)-septate, (23–)32–38(–45) × (2.5–)3–4 µm conidia, in comparison to those of *X. serpens*, which are 1-septate, (24–)27–33(–36) × 2.5–3(–3.5) µm. Furthermore, *X. subverticillatum* is also unique in having subverticillate conidiophores, spore masses that frequently turn reddish purple in older cul-

tures, and stipe extensions that are predominantly aseptate. *Xenocylindrocladium subverticillatum* is more similar to *X. guianense*, but the latter species lacks subverticillate conidiophores, has 1-septate conidia, and spore masses that do not become red-purple with age.

The phylogenetic relationships of a number of genera containing anamorphs with cylindrical macroconidia were recently discussed by Schoch et al. (2000). The current study provided the opportunity to assess whether the ITS based phylogenetic tree previously obtained would be supported by an additional analysis of β-tubulin gene sequences. The *P* value of 0.34 obtained after a partition homogeneity test performed with β-tubulin and ITS data sets indicated support for congruence of the phylogenies obtained from these sequence alignments. A final analysis was made with a combination of the β-tubulin-coding sequences and the ribosomal area containing the ITS1 and ITS2 regions, as well as the 5.8S ribosomal RNA gene. This provided a data set with 1109 characters, with 257 variable characters being parsimony informative. A branch and bound search yielded one most parsimonious tree (Fig. 10). As found in the previous study (Schoch et al., 2000, Fig. 1), no strong statistical support for higher level phylogeny between the different genera could be observed, although species of *Gliocladiopsis* S. B. Saksena grouped closer to *Cylindrocladiella* Boesew. with a bootstrap support of 88% (Fig. 10). In addition to this, the phylogenetic distance among the three *Xenocylindrocladium* species is similar to that observed among species of other genera used for comparison. This finding confirms the morphological observations in that the *Xenocylindrocladium* species are congeneric, and not conspecific. Although no teleomorph has yet been observed for *X. guianense* and *X. subverticillatum*, these data also suggest that their teleomorphs, if found, would be species of *Xenocalonectria.*

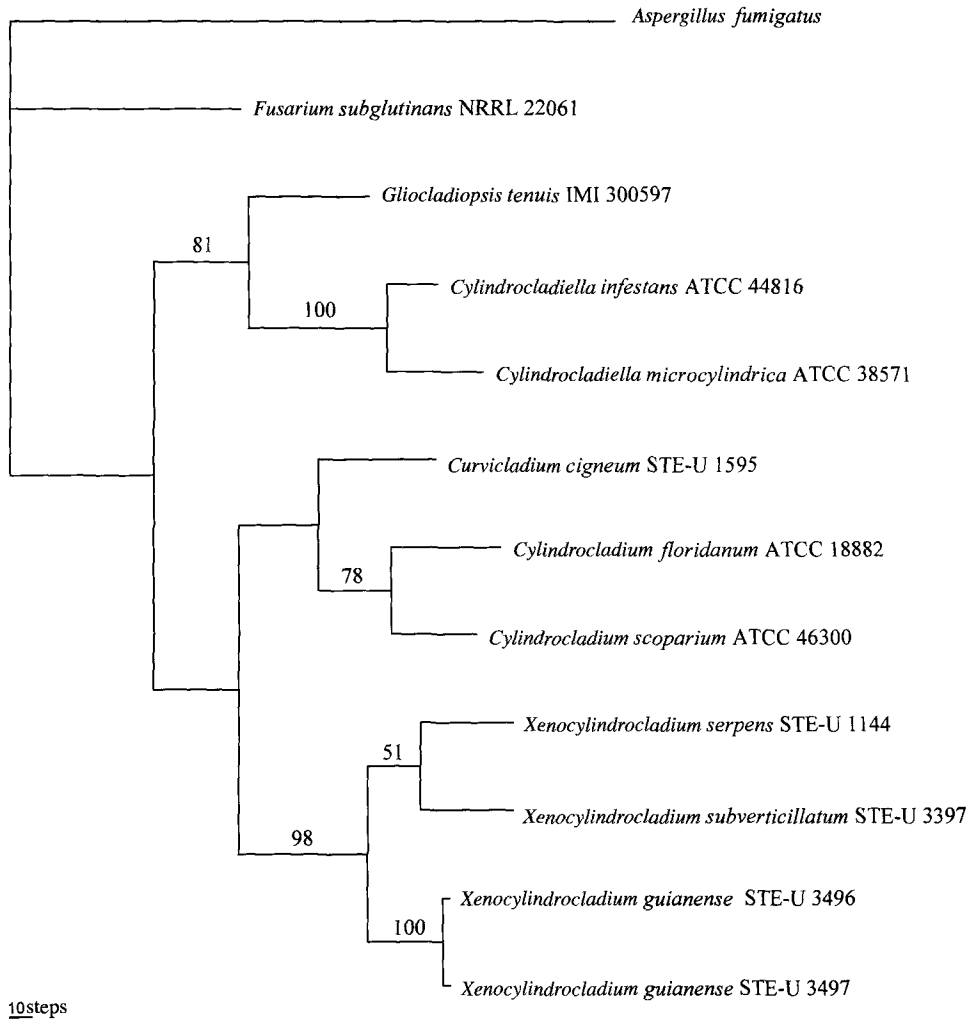


Fig. 10. Most parsimonious tree indicating *Xenocylindrocladium guianense*, *X. subverticillatum* and a number of hypocrealean species with cylindrical macroconidia. The tree was generated by a branch and bound analysis in PAUP* using the combined data set of DNA sequences (1109 bp) obtained from the ITS1, ITS2 and 5.8S rDNA, as well as the 5′ end of the β-tubulin gene. Bootstrap values from 1000 replicates are indicated above the branches (851 steps, CI=0.744, RI=0.579, RC=0.431).

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