Nectria serpens sp. nov. and its hyphomycetous anamorph
Xenocylinodrocladium gen. nov.

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A homothallic species of Nectria producing a Cylindrocladium-like anamorph was collected from bark of a fallen tree in the Amazonian forest in Ecuador. The anamorph, which is placed in a new genus, Xenocylinodrocladium, is characterized by forming straight, cylindrical, 1-septate conidia borne on penicillate conidiophores with coiled, avesiculate stipe extensions. The teleomorph, which is best accommodated in Nectria, is distinct in forming yellow-orange perithecia with red ostiolar regions, ellipsoidal, smooth, hyaline, 1-septate ascospores, and long-stalked, cylindrical ascii with apical discharge mechanisms. Both the teleomorph and anamorph states are newly described as Nectria serpens and Xenocylinodrocladium serpens.

In the Hypocreales Nectria (Fr.) Fr. includes morphologically diverse fungi that have superficially, brightly coloured, uniloculate ascomata and phialidic anamorphs. Rossmann (1979, 1983) defined additional anatomical and morphological characters of both teleomorph and anamorph states to segregate Ophiocordyceps Sacc. and Calonectria De Not. from Nectria. She furthermore restricted Calonectria to species having warty ascosporas, that turn red to blood-red in 3% KOH, have darkened stromatic bases, and Cylindrocladium Morgan anamorphs. Rossmann (1989) restricted Nectria, on the basis of its type species N. cinabarina (Tode: Fr.) Fr., to only those species that are closely related to N. cinabarina and belong to the N. cinabarina-group. Several other groups of nectriaceous species, which on their teleomorph and anamorph features are still placed in Nectria sensu lato, require a better generic position (Rossmann 1993). These include the Nectria haematozoa-group, N. rigidiceps-group, N.flammans-group, N. subg. Dialonectria Sacc., all with Fusarium Link anamorphs, and the N. radicicola-group with Cylindrocarpon Wollenw. anamorphs. Several nectriaceous species have since been clustered in separate groups based on their teleomorph and anamorph characteristics (Rossmann, 1989, 1993).

Cylindrocladium, initially revived by Boedijn & Reitsma (1950), has subsequently been treated by Peerrally (1991), and recently by Crous & Wingfield (1994) who redefined it as having hyaline, penicillate conidiophores, giving rise to whorls of phialides bearing cylindrical, straight or curved, 1-9-septate conidia. The stipes of these conidiophores extend above the phialides, and terminate in thin-walled vesicles of characteristic shape. All teleomorphs presently known for Cylindrocladium are placed in Calonectria. Several species are known also to produce a microconidial anamorph (Crous & Wingfield, 1994).

During the British Mycological Society's expedition in the Cuyabeno rainforest, Ecuador, in July 1993, a nectriaceous species associated to a Cylindrocladium-like anamorph was collected by G. L. Hennebert from the bark of a dead tree trunk (GLH 2504 F and GLH 2504 G). This fungus appears to be undescribed. It is here described and named as new.

MATERIALS AND METHODS

The fungus was collected from the bark of a fallen tree in the Tierra Firme Forest, near PUCF Research Station, north-east of Laguna Grande, on the northern affluent of the Rio Cuyabeno, latitude 0°, longitude 76°W, in the Cuyabeno Reserve, north-eastern Ecuador. Isolation of the associated Cylindrocladium-like anamorph was performed from single conidia under a stereo microscope immediately after collection. Single-conidial isolates were cultured on 2% malt extract agar (MEA) (Oxoid), plated onto carnation-leaf agar (CLA) (Crous, Phillips & Wingfield, 1992), incubated at 25 °C under nut light, and examined after 7 d. Only material occurring on carnation leaves was examined. Mounts were prepared in lactophenol, and measurements made at 1000x magnification. Wherever possible, each measurement represents at least 50 observations, and extremes are given in parentheses.

Scanning electron microscopy (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° with a Jeol JSM 6100 scanning electron microscope.
RESULTS AND DESCRIPTION OF SPECIES

In a study comparing the Cylindrocladium spp. with 3-septate conidia and ovoid-like vesicles (Crous et al., 1993), it was stated that all species of Cylindrocladium have stipe extensions terminating in vesicles of characteristic shape. Based on several features including its characteristic stipe morphology and Nectria teleomorph, Cylindrocladiella Boesew. can easily be distinguished from Cylindrocladium (Samuels et al., 1991; Crous & Wingfield, 1993). Furthermore, the absence of stipe extensions and the characteristic branching habit of its conidiophores also separated Ghoiadiopsis S. B. Saksena from Cylindrocladiella.

Among the 20 species of Cylindrocladium presently recognized (Crous & Wingfield, 1994; Victor et al., 1997; P. W. Crous, unpublished), no species has yet been linked to a teleomorph other than Colocteria. The Nectria teleomorph associated with the present Cylindrocladium-like collection suggests that this holomorph cannot be accommodated in the Colocteria complex, which represents a well-defined anamorph/teleomorph relationship. Furthermore, the absence of characteristic terminal vesicles, as well as the coiled stipe

Figs 1–5. Nectria serpens. Fig. 1. Yellow perithecium with dark red base and red ostiolar region (bar, 20 μm). Fig. 2. Vertical section through perithecium, showing various wall layers. Fig. 3. Ellipsoidal 1-septate ascospores. Fig. 4. Ostiolar region with periphyses. Fig. 5. Long-stemmed, cylindrical ascus with apical discharge mechanism (bars, 10 μm).
extensions, suggests that the anamorph cannot be accommodated in Cylindrocladium. As none of the genera with septate, cylindrical conidia, penicillate conidiophores and stipe extensions is suitable for this collection, it is accommodated in a new hyphomycete genus proposed below.

_Xenocylinbrocladium_ Decock, Hennebert & Crous gen. nov.  
(Figs 6, 8–11)

**Teleomorph:** _Nectria_.

Cylindrocladio similis, sed dissimilis per sterilem stipitis extentionem non vesiculatum, spiris tortam et teleomorphosis e _Nectria_ genere.

Similar to _Cylindrocladium_, but distinct in having coiled stipe extensions devoid of vesicles, and a _Nectria_ teleomorph.

**Type species:** _Xenocylinbrocladium serpens_ Decock, Hennebert & Crous.

Samuels & Seifert (1987) compared the straight conidia of the _Cylindrocladium_ anamorphs of the _Nectria radicicola_-group to those of _Cylindrocladium_. Samuels & Brayford (1990) further discussed the similarities in perithecial anatomy and morphology of the _N. radicicola_-group to that of _Calonectria_. Although these similarities exist, species of _Calonectria_ have a very conserved ascal morphology, which is distinct from that of the _N. radicicola_-group. Although the _N. radicicola_-group resembles _Calonectria_ in perithecial anatomy and general cultural characteristics, it is distinct with regard to the flat abscission scars on the conidia, shape of its microconidia, and conidiophore branching patterns. The _N. radicicola_-group is furthermore also distinct from the other _Nectria_ groups with _Cylindrocladium_ anamorphs.

Perithecia associated with _Xenocylinbrocladium_ are not as warded as those of the _N. radicicola_-group or _Calonectria_. The perithecial morphology is, however, closer to that of the _N. radicicola_-group than _Calonectria_, while the anamorph is more similar to _Cylindrocladium_ than _Cylindrocarpon_. Based on the anamorphs, therefore, it is possible that our collection represents yet another group in this complex of nectriaceous fungi.

_Nectria serpens_ Decock, Hennebert & Crous sp. nov.  
(Figs 6, 8–11)

**Anamorph:** _Xenocylinbrocladium serpens_ Decock, Hennebert & Crous sp. nov.  
(Figs 6, 8–11)


Perithecia on CLA in vitro, superficial, solitary or in clusters of 2–3, globose to subglobose, 400–450 μm high, 350–400 μm wide, warty, yellow, becoming yellow-orange, with a red, papillate ostiolar region and a dark red stromatic base, 60–160 μm wide; perithecial body becoming light red in 3% KOH, ostiolar region red, and stromatic base dark red. Perithecium wall consisting of two regions: outside layer of thick-walled _textura globulosa_, up to 100 μm wide, cells 16–28 μm wide, becoming darkened, compressed, _textura angularis_ towards the centrum; hymenium layer of _textura prismaticata_, hyaline, up to 10 μm wide. Ostiolar _periphyses_ tubular with rounded ends, up to 3 μm wide and 30 μm long. _Asci_ uniloculate, 8-spored, cylindrical with long basal stalks, a flattened apex, and a refractive apical apparatus (visible in immature and mature _asci_), 70–120 × 7–11 μm; _asci_ swelling slightly in apical part when ascospores are mature. _Ascospores_ aggregated in upper third of the ascus, hyaline, broadly to narrowly ellipsoidal, smooth, with a granular content, (8–)15.5(–25) × (4–)4.5(–6) μm, medially 1-septate, becoming constricted at the septum, and developing up to 2 septa with age.
Macroconidiophores penicillate. Stipe septate, hyaline, straight, becoming sinuous, and coiled in upper part; avesiculate, tapering towards a terminal cell (2–)2.5(–3) μm diam.; stipes (140–)180(–250) μm long. Conidiophore branches: primary branches non-septate or rarely 1-septate, (15–)20(–35) × (3–)3.5(–4) μm; secondary branches non-septate, (10–)15(–19) × 3(–4) μm. Phialides doliform to reniform, hyaline, non-septate, (10–)12(–15) × 3(–4) μm. Conidia cylindrical, hyaline, straight with rounded ends, 1-septate, (24–)30(–36) × (2.5–)3(–3.5) μm. Colony colour (reverse) 13K, amber brown (Rayner, 1970). Chlamydospores in extensive numbers, with medium to extensive sporulation on aerial mycelium. Cardinal temperature requirements for growth: minimum above 5°, optimum 25–30°, maximum below 35°.

Nectria serpens is distinguishable from species of Calonectria based on its cylindrical asci, refractive apical ascal rings, and Xenozylophoracladium anamorph. Within Nectria it is similar to N. venusta Syd., but has smaller ascospores (8–25 × 4–6 μm) than those of N. venusta (17–28 × 6–9 μm) (Sydow, 1930). Within Calonectria, it is also easily separable from known species based on its smaller ascospore dimensions and ascus morphology.

Calonectria should not be expanded to include fungi with anamorphs other than Cylindrocladium sensu stricto. If more species with a perithecial anatomy, ascus morphology and anamorph similar to N. serpens are collected, further attention can be given to elucidate the relationship between Calonectria, the N. radicicola-group and N. serpens.

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Xenocyndrocladium gen. nov.

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REFERENCES


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