Nectria serpens sp. nov. and its hyphomycetous anamorph *Xenocylindrocladium* 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, *Xenocylindrocladium*, 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 asci with apical discharge mechanisms. Both the teleomorph and anamorph states are newly described as *Nectria serpens* and *Xenocylindrocladium serpens*.

In the Hypocreales *Nectria* (Fr.) Fr. includes morphologically diverse fungi that have superficial, brightly coloured, uniloculate ascomata and phialidic anamorphs. Rossman (1979, 1983) defined additional anatomical and morphological characters of both teleomorph and anamorph states to segregate *Ophionectria* Sacc. and *Calonectria* De Not. from *Nectria*. She furthermore restricted *Calonectria* to species having warty ascocarps, that turn red to blood-red in 3% KOH, have darkened stromatic bases, and *Cylindrocladium* Morgan anamorphs. Rossman (1989) restricted *Nectria*, on the basis of its type species *N. cinnabarina* (Tode: Fr.) Fr., to only those species that are closely related to *N. cinnabarina* and belong to the *N. cinnabarina*-group. Several other groups of nectriaceous species, which based on their teleomorph and anamorph features are still placed in *Nectria sensu lato*, require a better generic position (Rossman 1993). These include the *Nectria haematococca*-group, *N. rigidiuscula*-group, *N. flammea*-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 (Rossman, 1989, 1993).

Cylindrocladium, initially revised by Boedijn & Reitsma (1950), has subsequently been treated by Peerally (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

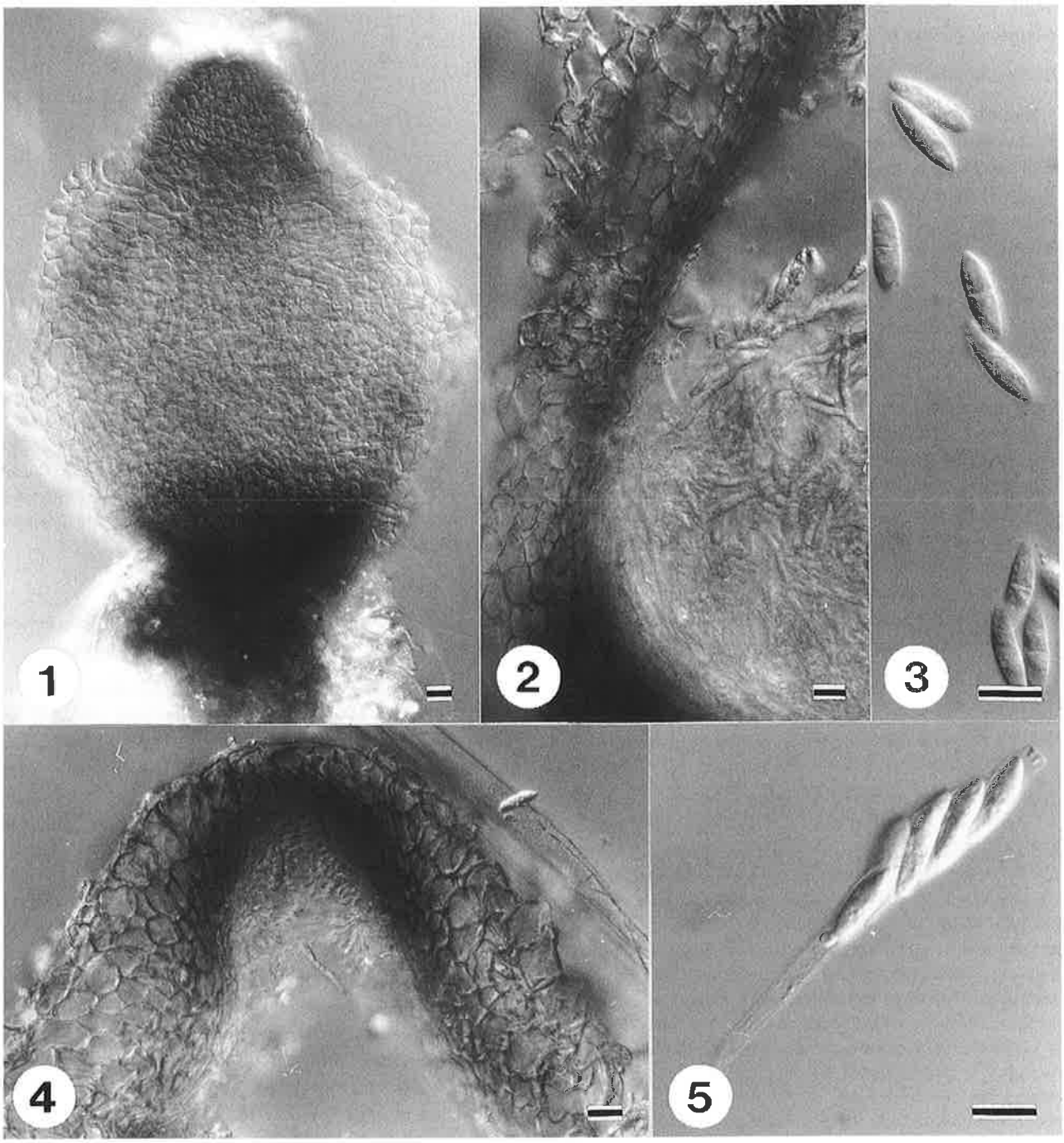
The fungus was collected from the bark of a fallen tree in the Tierra Firme Forest, near PUCE Research Station, north-east of Laguna Grande, on the northern affluent of the Rio Cuyabeno, latitude 0°, longitude 76°W, in the Cuyabeno Reserve, north-eastern Ecuador. Isolation of the associated *Cylindrocladium*-like 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 nuv light, and examined after 7 d. Only material occurring on carnation leaves was examined. Mounts were prepared in lactophenol, and measurements made at 1000 × 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°) 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°) to remove ice particles. Samples were sputter coated with gold in the preparation chamber for 75 s under 1·2 kV at −170°. 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 *Gliocladiopsis* 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 *Calonectria*. The *Nectria* teleomorph associated with the present *Cylindrocladium*-like collection suggests that this holomorph cannot be accommodated in the *Calonectria* 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.

Xenocylindrocladium 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 Nectriae genere.

Similar to *Cylindrocladium*, but distinct in having coiled stipe extensions devoid of vesicles, and a *Nectria* teleomorph.

Type species: *Xenocylindrocladium serpens* Decock, Hennebert & Crous.

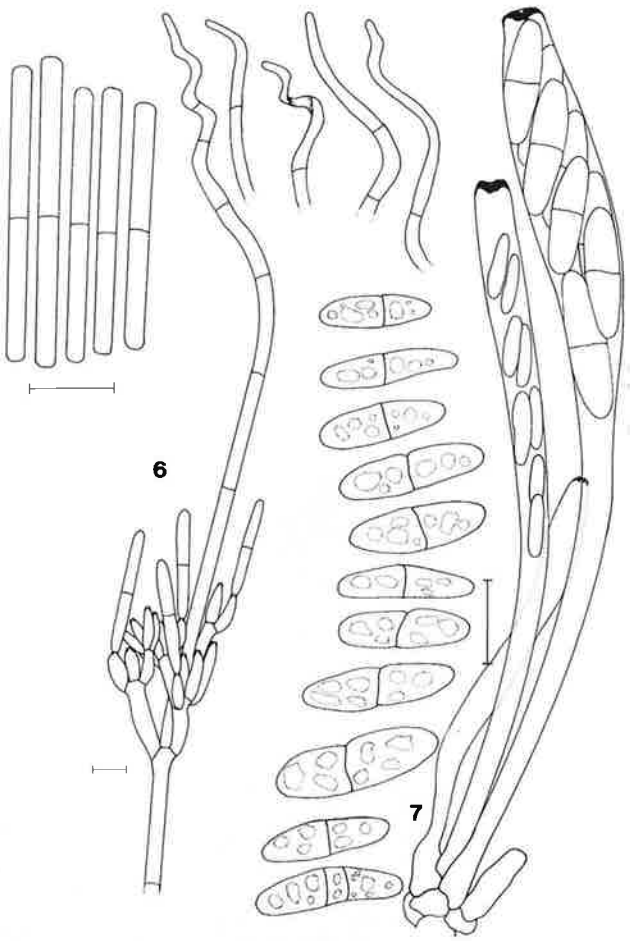
Samuels & Seifert (1987) compared the straight conidia of the *Cylindrocarpon* anamorphs of the *Nectria radicicola*-group to those of *Cylindrocladium*. Samuels & Brayford (1990) further discussed the similarities in perithecial anatomy and morphology of the *N. radicicola*-group to that of *Calonectria*. 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 *Cylindrocarpon* anamorphs.

Perithecia associated with *Xenocylindrocladium* are not as warted 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 1–5, 7)

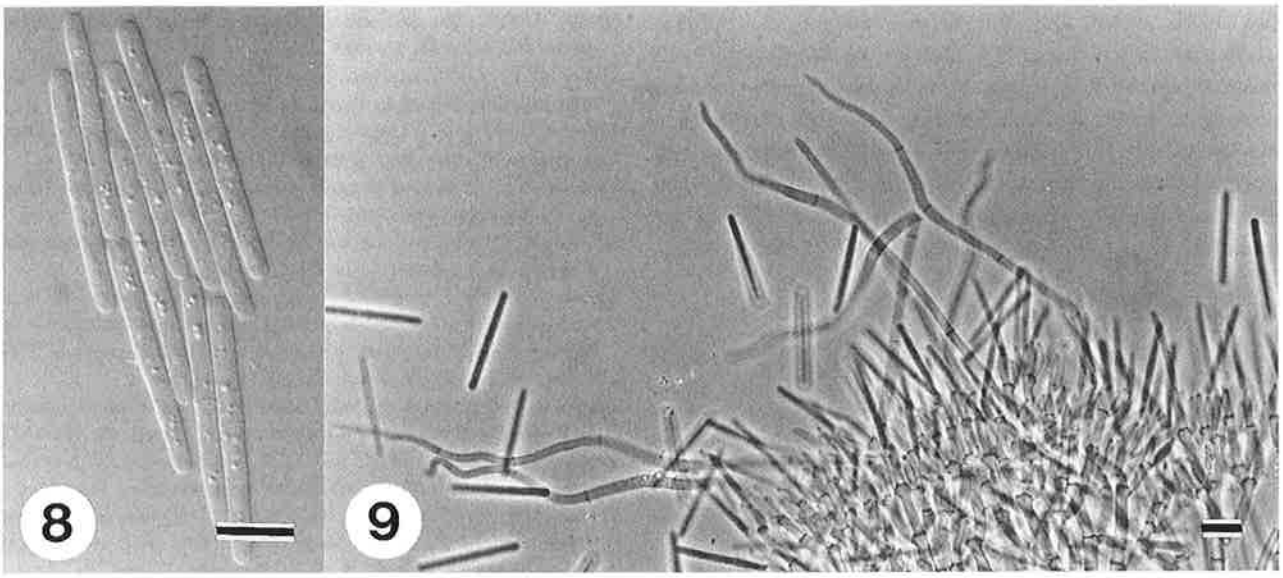
Anamorph: *Xenocylindrocladium serpens* Decock, Hennebert & Crous sp. anam. nov. (Figs 6, 8–11)

Perithecia superficialia, solitaria vel aggregata, globosa ad pyriformia, 400–450 × 350–400 µm, verrucosa, lutea usque ad luteo-aurantiaca, cum rubro papillato ostiolo et umbroso-rubra stromatica 60–160 µm lata basi, pariete et ostiolo rubris in 3% KOH, externa pariete 100 µm, textura globulosa, crasso tunicata, media pariete textura angularis, compressa, interna pariete 10 µm hyalina, textura prismatica. *Periphysae* cylindricae, 30 × 3 µm. *Asci* unitunicati, octospori, hyalini, cylindrici, inflati in maturitate, cum elongato basi et applanato apice refracto annello praedito, 70–120 × 7–11 µm. *Ascosporae* in superiore parte asci, hyalinae, ellipticae, leves, medio uni-septatae, 8–25 × 4–6 µm, medio 15·5 × 4·5 µm. *Conidiophori* penicillati, stipite septato, hyalino, erecto, cum sinuoso vel helico, cylindrico, 140–250 µm longo, 3–4 µm basaliter, 2–3 µm apicaliter lato. Primis ramis non septatis, 20 × 3·5 µm, secundis ramis verticillatis 15 × 3 µm. *Phialides* doliformes vel reniformes, curvatae, hyalinae, 10–15 × 3–4 µm. Conidia cylindrica, hyalina, recta, apice rotundato, uniseptata, 24–36 × 2·5–3·5 µm, medio 30 × 3 µm. *Chlamydosporae* in cultura productae numerosae.

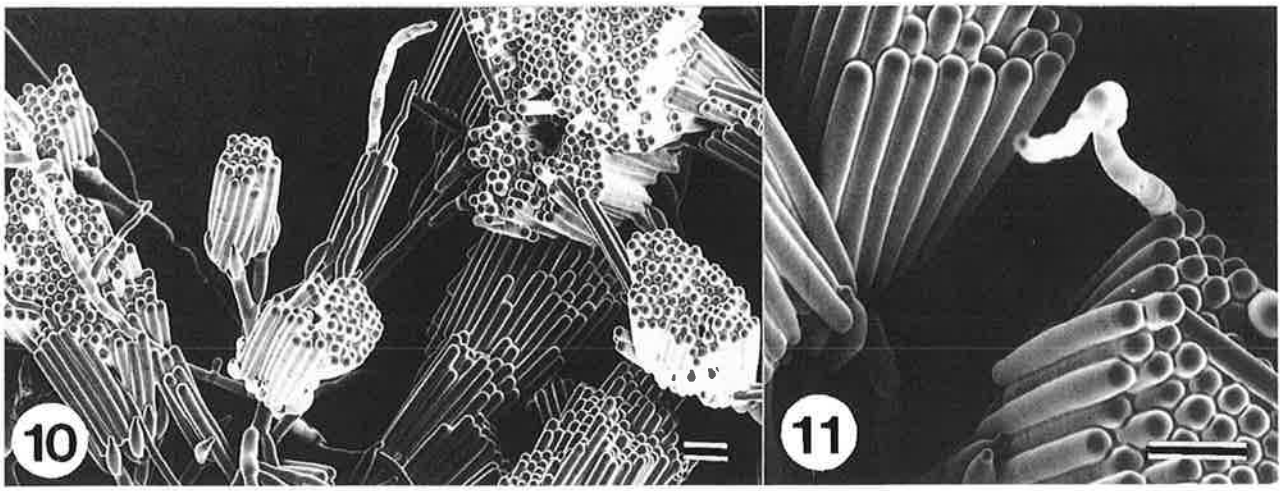


Figs 6, 7. *Nectria serpens* and its anamorph *Xenocylindrocladium serpens*. **Fig. 6.** Penicillate conidiophore, 1-septate conidia and coiled or variously curved vesicles. **Fig. 7.** Cylindrical asci and 1-septate, ellipsoidal ascospores (bars, 10 µm).

Perithecia on CLA *in vitro*, superficial, solitary or in clusters of 2–3, globose to subglobose, 400–450 µm high, 350–400 µm wide, warted, 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. Perithecial 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 prismatica*, hyaline, up to 10 µm wide. Ostiolar *periphyses* tubular with rounded ends, up to 3 µm wide and 30 µm long. *Asci* unitunicate, 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, medianly 1-septate, becoming constricted at the septum, and developing up to 2 septa with age.



Figs 8, 9. *Xenocylindrocladium serpens.* **Fig. 8.** One-septate conidia. **Fig. 9.** Conidiophores with twisting stipe extensions (bars, 10 µm).



Figs 10, 11. Penicillate conidiophores with irregular, twisting stipe extensions of *Xenocylindrocladium serpens* (bars, 10 µm).

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* doliiform 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°.

Specimen examined: Ecuador, Prov. Sucumbios, Reserva de Producción Faunistica, Cuyabeno, Tierra Firme, bark of a fallen trunk, G. L. Hennebert, 2504 G, Jul. 1993, MUCL 39315 (Holotype) in MUCL herbarium, living culture preserved at MUCL (MUCL 39315) and STE-U 1144.

Nectria serpens is distinguishable from species of *Calonectria* based on its cylindrical asci, refractive apical ascal rings, and *Xenocylindrocladium* 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 × 6–9·5 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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