Curvicladiella paphiopedili sp. nov. (Hypocreales, Nectriaceae), a new species of orchid (Paphiopedilum sp.) from Guizhou, China

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Abstract

Background

An asexual fungus, collected from diseased leaves of Paphiopedilum sp. from Guizhou Province, China, and based on the phylogenetic analyses and morphological characters, it was identified as a new species in Curvicladiella. The genus Curvicladiella are recorded for the first time for China.

New information

The morphology of Curvicladiella paphiopedili sp. nov. is characterised by penicillate conidiophores with a stipe, dull, tapering towards the apex, the curved stipe extension and cylindrical conidia. In the phylogenetic analyses of combined cmdA, his3, ITS, LSU, tef1...
and tub2 sequence data, this taxon was clustered as sister to *Curvicladiella cignea* within Nectriaceae.

**Keywords**

*Curvicladiella*, morphology, phylogeny, *Paphiopedilum*, taxonomy

**Introduction**

Nectriaceae (order Hypocreales) includes many important plant and human pathogens and some species have been used as biodegrading and biocontrol agents in industrial and commercial applications (Lombard et al. 2015). Based on molecular studies, many sexual genera in Nectriaceae were placed in *Nectria* sensu lato (Rehner and Samuels 1995, Rossman et al. 1999). However, *Nectria* sensu stricto is restricted to the type species *N. cinnabarina* (Tode) Fr et al. with tubercularia-like asexual morphs (Rossman 2000, Hirooka et al. 2012). A number of studies have treated taxonomic concepts within Nectriaceae, based on multi-gene phylogenetic inference (Lombard et al. 2010a, Lombard et al. 2010b, Chaverri et al. 2011, Gräfenhan et al. 2011, Schroers et al. 2011, Hirooka et al. 2012, Lombard and Crous 2012a, Lombard and Crous 2012b, Lombard et al. 2014a, Lombard et al. 2014b). Lombard et al. (2015) provided a phylogenetic backbone tree for Nectriaceae, based on combined sequence data of 10 gene regions. *Curvicladiella* is one of the genera in the Nectriaceae.

Decock and Crous (1998) established *Curvicladium* (as *Curvicladiella*) with *C. cigneum* (as *Curvicladiella cignea*) as the type species. The genus is distinct from morphologically-similar genera, such as *Cylindrocladium* Morgan, *Cylindrocladiella* Boesew, *Gliocladiopsis* Saksena, *Falcocladium* Silveira, Alfenas, Crous, Wingf and *Xenocylindrocladium* Decock, Hennebert, Crous by having cylindrical conidia and stipe extensions (Decock and Crous 1998). *Curvicladiella cignea* is the only species in the genus.

Based on the phylogenetic analyses and morphological characters, the fungus collected from diseased leaves of *Paphiopedilum* sp. is identified as a new species in *Curvicladiella*, the artificial infection test shows that it is a pathogen and the specific infection process has been described by Song et al. (Song et al. 2020). *Paphiopedilum* is known as “slipper orchids”, has a high ornamental value and can be used as household bonsai and garden plants (Luan et al. 2019).

**Materials and methods**

**Sample collection and isolation**

Diseased orchid leaves were collected from Guizhou Botanical Garden, Guizhou Province, China (in August 2019). The samples were brought to the laboratory in envelopes, photographed and identified. Pieces of leaves (5 × 5 mm), half of which were diseased and...
half healthy, were sterilised by 75% ethanol for 5–10 s, rinsed three times with sterilised distilled water, placed on potato dextrose agar (PDA) and incubated at 25°C for two days (Fang 2001). Mycelia were transferred to PDA and incubated for ten days at 25°C to obtain the pure cultures. The morphological characters of the fungi obtained from the diseased leaves collected in the field and cultured with PDA, and the fungi obtained from the diseased leaves after an artificial infection test were observed using a Nikon SMZ 745 stereomicroscope. Measurements were made using Image Frame Work.

Pure cultures were deposited in Guizhou Culture Collection (GZCC) Guizhou, China and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand. Herbarium specimens were deposited in the Guizhou Academy of Agricultural Sciences (GZAAS), Guiyang, China and the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand.

**DNA extraction, PCR amplification and sequencing**

The fungal mycelia were scraped from the pure culture growing on PDA for ten days at 25°C. DNA was extracted using the Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, China). Six gene regions, the 28S large subunit rDNA (LSU), calmodulin (cmdA), histone H3 (his3), internal transcribed spacer region and intervening 5.8S nrRNA gene (ITS), translation elongation factor 1-alpha (tef1) and β-tubulin (tub2) gene were amplified by the primer pairs LR0R and LR5 (Vilgalys and Hester 1990, Rehner and Samuels 1994), CAL-228F and CAL2Rd (Carbone and Kohn 1999, Groenewald et al. 2013), CYLH3F and CYLH3R (Crous et al. 2004), ITS5 and ITS4 (White et al. 1990), EF1-728F and EF2 (O’Donnell et al. 1998, Carbone and Kohn 1999), T1 and CYLTUB1R (O’Donnell and Cigelnik 1997, Crous et al. 2004), respectively. Polymerase chain reaction (PCR) was carried out in 25 µl reaction volume containing 12.5 µl 2 × PCR Master Mix (Sangon Biotech, China), 9.5 µl ddH₂O, 1µl of each primer and 1µl DNA template. The PCR products were examined by using 1.2% agarose electrophoresis gel, stained with ethidium bromide and were purified and sequenced by Sangon Biotech (Shanghai) Co. Ltd, China. The nucleotide sequences were submitted in GenBank.

**Phylogenetic analyses**

Phylogenetic analyses were performed using combined sequence data with six gene regions, LSU, cmdA, his3, ITS, tef1 and tub2. Related strains of *Curvicladiella* (Table 1) were referred to Lombard et al. (2015). Sequences were obtained from GenBank. The sequences were aligned using the online multiple alignment programme MAFFT v.7 ([http://mafft.cbrc.jp/alignment/server](http://mafft.cbrc.jp/alignment/server)) (Standley 2013). The alignments were checked visually and optimised manually by using BioEdit v. 7.2.6.1.

Maximum Likelihood (ML) analysis was performed using RaxmlGUI 1.3.1 (Silvestro and Michalak 2012). The optimal RAxML tree search was conducted with 1000 bootstrap replicates and the default algorithm was used from a random starting tree for each replicate. The final tree was selected from amongst suboptimal trees from each replicate by comparing likelihood scores under the GTR+GAMMA substitution model.
Table 1.
Taxa or selected taxa used in this study and their GenBank accession numbers. The type species have T as superscript and the newly-generated sequences have been highlighted in bold.

| Taxa                          | Isolate numbers | GenBank Accession numbers |
|-------------------------------|-----------------|---------------------------|
|                               |                 | LSU | CMDA | HIS3 | ITS | TEF1 | TUB2 |
| Aquanectria penicillioides    | CBS 257.54      | KM231613 | KM231275 | –   | KM231743 | KM231865 | KM232000 |
| Aquanectria submersa          | CBS 394.62 T    | KM231612 | –   | KM231458 | HQ897796 | –   | KM231999 |
| Calonectria brassicae         | CBS 111869      | GQ280698 | GQ267382 | DQ190720 | GQ280576 | FJ918567 | AF232857 |
| Calonectria ilicicola         | CBS 190.50 T    | GQ280727 | AY725764 | AY725676 | GQ280605 | AY725726 | AY725631 |
| Calonectria naviculata        | CBS 101121 T    | GQ280722 | GQ267399 | GQ267252 | GQ280600 | GQ267317 | GQ267211 |
| Campylocarpon fasciculare     | CBS 112613 T    | HM364313 | KM231297 | JF735502 | AY677301 | JF735691 | AY677221 |
| Campylocarpon pseudofasciculare | CBS 112679 T | HM364314 | KM231298 | JF735503 | AY677306 | JF735692 | AY677214 |
| Corallonectria jatrophae      | CBS 913.96 T    | KM231611 | KM231273 | KM231457 | KC479758 | KM231863 | KC479787 |
| Curvicladiella cignea         | CBS 101411      | JQ666075 | KM231285 | KM231459 | KM231744 | KM231866 | KM232001 |
| Curvicladiella cignea         | CBS 109168      | JQ666074 | KM231286 | KM231460 | KM231745 | KM231868 | KM232003 |
| Curvicladiella cignea         | CBS 109167 T    | AY793431 | KM231287 | KM231461 | AF220973 | KM231867 | KM232002 |
| Curvicladiella paphiopedili   | MFLUCC 20-0110 T | MT279199 | MT294104 | MT294105 | MT279198 | MT294103 | MT294102 |
| Curvicladiella paphiopedili   | GZCC22-0001     | OM899803 | –   | –   | OM903885 | –   | –   |
| Cylindrocarpostylus gregarius | CBS 101074      | KM231614 | KM231291 | –   | KM231746 | KM231869 | KM232004 |
| Cylindrocarpostylus gregarius | CBS 101072 T   | JQ666084 | KM231292 | –   | KM231747 | KM231870 | KM232005 |
| Cylindrocarpostylus gregarius | CBS 101073      | JQ666083 | KM231293 | KM231465 | KM231748 | KM231871 | KM232006 |
| Cylindrocladiella camelliae   | CPC 234 T       | JN099249 | KM231280 | AY793509 | AF220952 | JN099087 | AY793471 |
| Taxa                     | Isolate numbers | GenBank Accession numbers |
|-------------------------|-----------------|---------------------------|
|                         |                 | LSU | CMDA | HIS3 | ITS  | TEF1 | TUB2 |
| Cylindrocladiella       | CBS 340.92 T    | JN099165 | KM231279 | AY793520 | AF220959 | JN099003 | AY793481 |
| lageniformis            |                 |     |      |      |      |      |      |
| Cylindrocladiella       | CBS 114524 T    | JN099171 | KM231281 | AY793526 | AF220964 | JN099009 | AY793486 |
| parva                   |                 |     |      |      |      |      |      |
| Dematiocladium          | CBS 115994 T    | AY793438 | KM231274 | – | AY793430 | KM231864 | – |
| celtidis                |                 |     |      |      |      |      |      |
| Gliocephalotrichum      | CBS 242.62 T    | AY489732 | KM231283 | KF513326 | – | KM231892 | DQ377831 |
| bulbilium               |                 |     |      |      |      |      |      |
| Gliocephalotrichum      | CBS 902.70T     | JQ666077 | KM231284 | KF513353 | DQ366705 | KF513408 | DQ377841 |
| cylindrosporum          |                 |     |      |      |      |      |      |
| Gliocephalotrichum      | CBS 126571 T    | KM231686 | KM231282 | KF513367 | DQ278422 | KF513435 | DQ377835 |
| longibrachium           |                 |     |      |      |      |      |      |
| Gliocladiopsis          | CBS 755.97 T    | JQ666082 | KM231278 | JQ666023 | AF220977 | KF513449 | JQ666133 |
| irregularis             |                 |     |      |      |      |      |      |
| Gliocladiopsis          | CBS 116074 T    | JQ666080 | KM231277 | JQ666030 | AF220981 | JQ666099 | JQ666140 |
| pseudotenuis            |                 |     |      |      |      |      |      |
| Gliocladiopsis          | CBS 199.55 T    | JQ666078 | KM231276 | JQ666031 | JQ666063 | JQ666106 | JQ666141 |
| sagariensis             |                 |     |      |      |      |      |      |
| Penicillifer            | CBS 420.88 T    | KM231608 | KM231270 | KM231454 | KM231740 | KM231860 | KM231996 |
| bipapillatus            |                 |     |      |      |      |      |      |
| Penicillifer            | CBS 376.59 T    | KM231609 | KM231271 | KM231455 | KM231741 | KM231861 | KM231997 |
| diparietisporus         |                 |     |      |      |      |      |      |
| Penicillifer            | CBS 423.88 T    | KM231607 | KM231269 | KM231453 | KM231739 | KM231859 | KM231995 |
| penicilliferi           |                 |     |      |      |      |      |      |
| Penicillifer            | CBS 560.67 T    | KM231610 | KM231272 | KM231456 | KM231742 | KM231862 | KM231998 |
| pulcher                 |                 |     |      |      |      |      |      |
| Rugonectria              | CBS 125120      | HM364322 | KM231294 | KM231466 | KM231750 | KM231874 | HM352869 |
| neobalansae             |                 |     |      |      |      |      |      |
| Rugonectria              | CBS 129158      | JF832761 | KM231295 | KM231467 | JF832661 | KM231872 | JF832911 |
| rugulosa                |                 |     |      |      |      |      |      |
| Rugonectria              | CBS 126565      | KM231615 | KM231296 | KM231468 | KM231749 | KM231873 | KM232007 |
| rugulosa                |                 |     |      |      |      |      |      |
| Thelonectria             | CBS 125153      | HM364307 | KM231327 | KM231489 | HM364294 | KM231897 | HM352860 |
| discophora              |                 |     |      |      |      |      |      |
| Thelonectria             | CBS 215.67 T    | HM364317 | KM231325 | KM231487 | AY677293 | HM364345 | KM232024 |
| olida                   |                 |     |      |      |      |      |      |
| Thelonectria             | CBS 112467 T    | HM364312 | KM231326 | KM231488 | AY677297 | KM231896 | AY677258 |
| trachosa                |                 |     |      |      |      |      |      |
| Xenocylindrocladium      | CBS 112179 T    | JQ666073 | KM231289 | KM231463 | AY317348 | KM231895 | AY320197 |
| guianense               |                 |     |      |      |      |      |      |
Bayesian analyses were carried out using MrBayes 3.2 (Huelsenbeck 2012). MrModeltest 2.2 was used to choose the best-fit evolutionary model (Nylander 2004). Posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2. Six simultaneous Markov chains were run for 10 million generations and trees were sampled every 1000th generation. The temperature values were lowered to 0.15, burn-in was set to 0.25 and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01.

The resulting trees of Maximum Likelihood and Bayesian were visualised with Fig Tree v. 1.4.0. The layouts were undertaken using Microsoft Powerpoint 2010 and Adobe Photoshop CS6.

Taxon treatment

*Curvicladiella paphiopedili* Lian-Chai Song, Jing Yang, Zuo-Yi Liu, 2019, sp. nov.

- IndexFungorum [http://www.indexfungorum.org/names:IF558310](http://www.indexfungorum.org/names:IF558310)
- Species-ID [Facesoffungi number:FOF 09697](https://facesoffungi.com/en/species/Curvicladiella-paphiopedili)

**Materials**

**Holotype:**
- scientificName: *Curvicladiella paphiopedili*; class: Sordariomycetes; order: Hypocreales; family: Nectriaceae; genus: *Curvicladiella*; locationRemarks: China, Gui Zhou Province, Guiyang City, Guizhou Botanical Garden, 26°37'N, 106°43'E, 13 August 2019; habitat: Terrestrial; fieldNotes: diseased leaves of *Paphiopedilum* sp.; recordNumber: zwy-di4-2; recordedBy: Lian Chai Song; type: Stillimage; language: English; collectionID: MFLU 20-0203

**Isotype:**
- scientificName: *Curvicladiella paphiopedili*; class: Sordariomycetes; order: Hypocreales; family: Nectriaceae; genus: *Curvicladiella*; locationRemarks: China, Gui Zhou Province, Guiyang City, Guizhou Botanical Garden, 26°37'N, 106°43'E, 13 August 2019; habitat: Terrestrial; fieldNotes: diseased leaves of *Paphiopedilum* sp.; recordNumber: zwy-di4-2;
Description

The characters of pathogenic fungi on the leaves were identified through an artificial infection test. **Asexual morph:** Conidiomata white, scattered, hairy. Conidiophores straight to flexuous, consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe septate, hyaline, smooth; stipe extensions septate, straight or curved, dull and tapering towards the apex, 128.5–549.9 µm long, (x̄ = 288.1 µm, n = 20). The primary branches of conidiogenous apparatus aseptate, 9.3–17.5 × 2.6–3.7 µm; secondary branches aseptate, 9.9–19.1 × 2.5–3.9 µm; tertiary branches aseptate, 9.5–17.6 × 2.6–3.7 µm; quaternary and additional branches (–6) aseptate, 11–16.3 × 2.5–3.9 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, apex with minute periclinal thickening and inconspicuous collarette. Conidia cylindrical, rounded at both ends, straight, 1-septate, hyaline, (30.5–) 31.2–37.2 (–42.0) × (2.6–) 2.9–3.5 (–3.9) µm, (x̄ = 34.2 × 3.2 µm, n = 20) (Fig. 1). **Sexual morph:** not observed.

**Figure 1.** *Curvicladiella paphiopedilli.* a The diseased leaves were withered; b, c Conidiomata; d–g Stipes extension and conidiogenous cells; h–j Conidiogenous cells and conidiophores; k–n Conidia. Scale bars: d–g=50 µm, h–j=20 µm, k–n=10 µm.
The characters of fungus obtained from the diseased leaves collected in the field that were cultured with PDA: after 10 days at 25°C on PDA, colonies reached 47 mm in diam. Beige to pale yellow colony on the surface, brown in reverse with irregular margins, extensive sporulation on the medium surface. Conidiophores straight to flexuous, consisting of a stipe bearing a penicillate arrangement of fertile branches, stipe extensions septate, straight or slightly flexuous, 104.4–153.0 µm long, (x̄= 128.7 µm, n = 10). The primary branches of conidiogenous apparatus aseptate, 8.9–17.8 × 2.7–3.4 µm; secondary branches aseptate, 7.8–14.0 × 2.5–5.9 µm; tertiary branches aseptate, 8.9–17.7 × 2.3–3.5 µm; quaternary and additional branches (–6) aseptate, 9.3–16.7 × 2.3–3.7 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, apex with minute periclinal thickening and inconspicuous collarette. Conidia cylindrical, rounded at both ends, straight, 1-septate, hyaline, (38.5–) 45.2–56.6 (–63.2) × (2.2–) 2.9–4.2 (–4.9) µm, (x̄= 50.9 × 3.5 µm, n = 40). Chlamydospores thick-walled, ellipsoidal or sphaeropedunculate, brown to hyaline,
(9.0–) 11.9–20.7 (–23.1) × (8.1–) 8.9–12.8 (–15.4) µm, (x̄= 16.3 × 10.8 µm, n = 20) (Fig. 2).

Material: ex-type living culture, MFLUCC 20-0110.

Etymology

Refers to the genus name *Paphiopedilum*.

Analysis

Phylogenetic analyses

The final alignment consists of the new species and the fungus obtained from the diseased leaves after use of the new species to infect the healthy *Paphiopedilum* and other genera of the families Nectriaceae. Additionally, the alignment of combined cmdA, his3, ITS, LSU, tef1 and tub2 sequence data comprised a total of 3877 characters with gaps (734bp for cmdA, 529bp for his3, 616bp for ITS, 840bp for LSU, 548bp for tef1 and 610bp for tub2).
The dataset comprised 39 taxa with *Campylocarpon fasciculare* and *C. pseudofasciculare* as the outgroup taxa. The best scoring RAxML tree is shown in Fig. 3, with the Bayesian tree (not shown) having a similar topology with the ML tree. *Curvicladiella paphiopedilii* was clustered as sister taxon to *C. cignea* within Nectriaceae with high support (99/1.00) (Fig. 3).

**Discussion**

Morphologically, *Curvicladiella paphiopedilii* is similar to species in *Calonectria*, *Cylindrocladium* and *Xenocylindrocladium*, but distinct in having ellipsoidal or sphaeropedunculate chlamydospores (Fig. 2k), dull, tapering towards the apex (Fig. 1d and e, Fig. 2e–g) and curved extension stipes (Fig. 1f and g), without obpyriform, ovoid, ellipsoidal or sphaeropedunculate vesicles (Lombard et al. 2010a, Pham et al. 2019) or coiled stipes (Decock et al. 1997). The morphology of *Curvicladiella paphiopedilii* is different from the type species *Curvicladiella cignea* in the size of stipe extensions and conidia, without the swollen cell below the apical septum; on the other hand, the stipe extensions of *Curvicladiella cignea* is curved obviously, while *Curvicladiella paphiopedilii* is not. The stipe extensions of *Curvicladiella paphiopedilii* are 128.5–549.9 µm long, the *Curvicladiella cignea* is 110–200 µm long. The conidia of *Curvicladiella paphiopedilii* are (38.5–) 45.2–56.6 (–63.2) × (2.2–) 2.9–4.2 (–4.9) µm, the *Curvicladiella cignea* are (28–) 33–36 (–38) × 2.5–3µm. In the phylogenetic analyses, the two taxa of *Curvicladiella* formed a well-supported monoclade and *Curvicladiella aphiopedilii* represented a distinct lineage (Fig. 3).

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**References**

- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3): 553-556. https://doi.org/10.2307/3761358
- Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ (2011) Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. Studies in Mycology 68 (1): 57-78. https://doi.org/10.3114/sim.2011.68.03
- Crous PW, Groenewald JZ, Risede J, Hyweljones N (2004) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50 (2004): 415-430. https://doi.org/10.1023/B:MYCO.0000012225.79969.29
• Decock C, Hennebert GL, Crous PW (1997) *Nectria serpens* sp. nov. and its hyphomycetous anamorph *Xenocylindrocladium* gen. nov. Mycological Research 101 (7): 786-790. https://doi.org/10.1017/S0953756296003334

• Decock C, Crous PW (1998) *Curvicladium* gen. nov., a new hyphomycete genus from French Guiana. Mycologia 90 (2): 276-281. https://doi.org/10.1080/00275514.1998.12026907

• Fang ZD (2001) Research method of plant pathology. China Agriculture Press, Beijing, 427 pp. [In Chinese].

• Gräfenhan T, Schroers HJ, Nirenberg HI, Seifert KA (2011) An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora, Acremonium, Fusarium, Stilbella*, and *Volutella*. Studies in Mycology 68 (68): 79-113. https://doi.org/10.3114/sim.2011.68.04

• Groenewald JZ, Nakashima C, Nishikawa J, Shin HD, Crous PW (2013) Species concepts in *Cercospora*. Studies in Mycology 75 (1): 115-170. https://doi.org/10.3114/sim0012

• Hirooka Y, Rossman AY, Samuels GJ, Lechat C, Chaverri P (2012) A monograph of *Allantonecctria, Nectria*, and *Pleonectria* (Nectriaceae, Hypocreales, Ascomycota) and their pycnidial, sporodochial, and synnematous anamorphs. Studies in Mycology 71 (1): 1-210. https://doi.org/10.3114/sim0001

• Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61 (3): 539-542. https://doi.org/10.1093/sysbio/sys029

• Lombard L, Crous PW, Wingfield BD, Wingfield MJ (2010a) Phylogeny and systematics of the genus *Calonectria*. Studies in Mycology 66 (1): 31-69. https://doi.org/10.3114/sim.2010.66.03

• Lombard L, Crous PW, Wingfield BD, Wingfield MJ (2010b) Species concepts in *Calonectria* (Cylindrocladium). Studies in Mycology 66 (66): 1-13. https://doi.org/10.3114/sim.2010.66.01

• Lombard L, Crous PW (2012a) Phylogeny and taxonomy of the genus *Gliocladiopsis*. Persoonia 28: 25-33. https://doi.org/10.3767/003158512X635056

• Lombard L, Crous PW (2012b) Phylogeny and taxonomy of the genus *Cylindrocladiella*. Mycological Progress 28 (1): 25-33. https://doi.org/10.1007/s11557-011-0799-1

• Lombard L, Serrato-Diaz LM, Cheewangkoonm R (2014a) Phylogeny and taxonomy of the genus *Gliocephalotrichum*. Persoonia 32 (3): 127-140. https://doi.org/10.3767/003158514X680261

• Lombard L, Merwe N, Groenewald JZ, Crous PW (2014b) Lineages in Nectriaceae: re-evaluating the generic status of *Ilyonectria* and allied genera. Phytopathologia Mediterranea 53 (3): 340-357. https://doi.org/10.14601/Phytopathol_Mediterr-14976

• Lombard L, Merwe N, Groenewald JZ, Crous PW (2015) Generic concepts in Nectriaceae. Studies in Mycology 80: 189-245. https://doi.org/10.1016/j.simyco.2014.12.002

• Luan VQ, Cuong LK, Tung HT, Hien VT, Hieu T, Nhut DT (2019) Effects of shoot tip removal, wounding manipulation, and plant growth regulators on shoot regeneration and plantlet development in Paphiopedilum species. Scientia Horticulturae 256 https://doi.org/10.1016/j.scienta.2019.108648

• Nylander J (2004) MrModeltest v.2. Program distributed by the author. Bioinformatics 24: 581-58. URL: http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html
• O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics & Evolution 7 (1): 103-116. https://doi.org/10.1006/mpev.1996.0376

• O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial genealogies. Proceedings of the National Academy of Sciences of the United States of America 95 (5): 2044-2044. https://doi.org/10.1073/pnas.95.5.2044

• Pham NQ, Barnes I, Chen SF, Liu FF, DangQN, Pham TQ, Lombard L, Crous PW, Wingfield MJ (2019) Ten new species of *Calonectria* from Indonesia and Vietnam. Mycologia 111: 1-25.

• Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43: 304-311. https://doi.org/10.1007/BF02338839

• Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98 (6): 625-634. https://doi.org/10.1016/S0953-7562(09)80409-7

• Rehner SA, Samuels GJ (1995) Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. Canadian Journal of Botany 73 (S1): 816-823. https://doi.org/10.1086/175815

• Rossman AY, Samuels GJ, Rogerson CT, Lowen R (1999) Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42 (42): 1-248.

• Rossman AY (2000) Towards monophyletic genera in the holomorphic Hypocreales. Studies in Mycology 45 (45): 27-34.

• Schroers H-J, Gräfenhan T, Nirenberg HI, Seifert KA (2011) A revision of *Cyanonectria* and *Geejayessia* gen. nov., and related species with *Fusarium*-like anamorphs. Studies in Mycology 68 (68): 115-138. https://doi.org/10.3114/sim.2011.68.05

• Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12 (4): 335-337. https://doi.org/10.1007/s13127-011-0056-0

• Song LC, Feng Y, Liu ZY (2020) First report of leaf blight on *Paphiopedilum* caused by *Curvicladiella* sp. (GZCC19-0342) in China. Plant Disease 104: 3079-3079. https://doi.org/10.1094/PDIS-02-20-0223-PDN

• Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772-780. https://doi.org/10.1093/molbev/mst010

• Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172 (8): 4238-4246.

• White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, New York, 315 pp.

• Zhaxybayeva O, Gogarten JP (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3 (1). https://doi.org/10.1186/1471-2164-3-4