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Curvicladiella paphiopedili sp. nov., a new species on orchid (*Paphiopedilum* sp.) from Guizhou, China

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Abstract

Background

Paphiopedilum is known as “slipper orchids”, which has a high ornamental value and can be used as household bonsai and garden plants. *Paphiopedilum* is also one of the most beautiful plants in the world due to their exotic and unique flowers. An asexual fungus was collected from diseased leaves of *Paphiopedilum* sp. from Guizhou Province, China, is described and illustrated on the basis of morphological characters and molecular evidence.

New information

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

Keywords

morphology, phylogeny, taxonomy

Introduction

Nectriaceae (Hypocreales, class) includes many important plant and human pathogens, and some species were used as biodegraders 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, Lombard and Crous 2012a, Lombard et al. 2014a, Lombard et al. 2014b, Lombard and Crous 2012b, Chaverri et al. 2011, Gräfenhan et al. 2011, Schroers et al. 2011, Hirooka et al. 2012). Lombard et al. (2015) provided a phylogenetic backbone tree for Nectriaceae based on a combined sequence data of 10 gene regions.

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. was identified as a new species in *Curvicladiella,* which has been proved to be a plant pathogen (Song et al. 2020).

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 laboratory in envelopes, photographed and identified. Pieces of leaves (5 × 5 mm), each with half part diseased and half healthy, were sterilized by 75% ethanol for 5–10 s, rinsed three times with sterilized distilled water, placed on potato dextrose agar (PDA) and incubated at 25°C for two days (Fang 2001). Mycelia were transferred to PDA, incubated for ten days at 25°C to get the pure cultures. Morphological characters were observed using 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 25ºC. DNA was extracted using Ezup Column Fungi Genomic DNA Purification Kit

(Sangon Biotech, China). Gene sequences were determined for 28S large subunit (LSU) nrDNA, 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). Primer pairs used for amplifying each gene region were listed in Table 1. Polymerase chain reaction (PCR) was carried out in 25 ul reaction volume containing 12.5 ul 2 × PCR Master Mix (Sangon Biotech, China), 9.5 ul ddH_2O, 1ul of each primer and 1ul 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 a combined sequence data with six gene regions, LSU, cmdA, his3, ITS, tef1 and tub2. Related strains of *Curvicladiella* (Table 2) were referred to Lombard et al. (2015). Sequences were obtained from GenBank. The sequences were aligned using the online multiple alignment program MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Standley 2013). The alignments were checked visually and optimized 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 among suboptimal trees from each replicate by comparing likelihood scores under the GTR+GAMMA substitution model.

Maximum parsimony (MP) analysis was performed with the heuristic search in PAUP v. 4.0b10 (Swofford 2002). All characters were equally weight and unordered. Gaps were treated as missing in the alignment. Maxtrees were unlimited. All multiple, equally parsimonious trees were saved. The zero length of branches were collapsed. Clade stability was assessed by using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis and Bull 1993).

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 100000000 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, maximum parsimony and bayesian were visualized with Fig Tree v1.4.0. The layouts were done in the program of 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
- Species-ID Facesoffungi number:FOF 09697

Materials

Holotype:

a. 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-dl4-2; recordedBy: Lian Chai Song; type: StillImage; language: English; collectionID: MFLU 20-0203

Isotype:

a. collectionID: GZAAS 19-2061

Description

Pathogenic fungi on *Paphiopedilum* sp. through an artificial infection test. **Asexual morph:** Colonies 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 aseptate of conidiogenous apparatus, 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.

Culture characters: 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 aseptate of conidiogenous apparatus, 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, ($\bar{x}$= 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, ($\bar{x}$= 16.3 × 10.8 µm, n = 20) (Fig. 2).

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

Etymology

Refers to the host name *Paphiopedilum* sp.

Analysis

Phylogenetic analyses

The alignment of combined LSU, cmdA, his3, ITS, tef1 and tub2 sequence data comprised a total of 3872 characters with gaps (840bp for LSU, 734bp for cmdA, 529bp for his3, 611bp for ITS, 548bp for tef1 and 610bp for tub2). The dataset composed 38 taxa with *Campylocarpon fasciculare* and *C. pseudofasciculare* as the outgroup taxa. The best scoring RAxML tree was shown in Fig. 3, the MP and bayesian tree (not shown) had a similar topology with the ML tree. *Curvicladiella paphiopedili* was clustered as sister taxon to *C. cignea* within Nectriaceae with high support (99/98/1.00) (Fig. 3).

Discussion

Morphologically, *Curvicladiella paphiopedili* 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, e, Fig. 2e–g), and curved extension stipes (Fig. 1f, 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 paphiopedili* is different from the type species *Curvicladiella cignea* with in the size of extension stipes, conidia and chlamydospores, without swollen cell below the apical septum, on the other hand, the curved position of stipes are different. In the phylogenetic analyses, the two taxa of *Curvicladiella* formed a well-supported monoclade and *Curvicladiella aphiopedili* represented a distinct lineage (Fig. 3).

Acknowledgements

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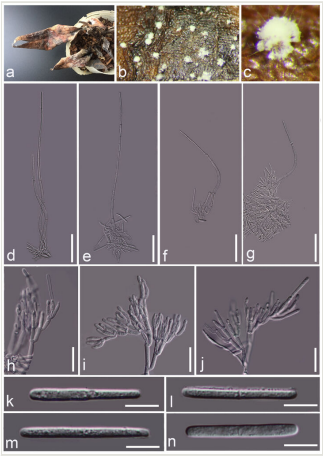

Figure 1.

Curvicladiella paphiopedili. **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.

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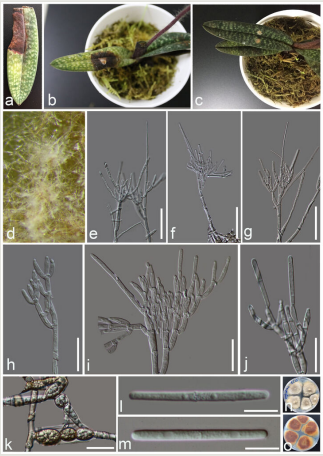

Figure 2.

Curvicladiella paphiopedili. (MFLU 20-0203, holotype) **a** *Paphiopedilum* diseased leaf in the field **b** *Curvicladiella paphiopedili* caused *Paphiopedilum* leaf diseased through an artificial infection test **c** The contrast **d** Colonies on PDA producing conidia masses **e–j** Conidiophores, conidiogenous cells and stipes extension **k** Chlamydosporae **l, m** Conidia **n, o** Culture on PDA, (**n**) from above, (**o**) from blow. Scale bars: **e–g**=50 µm, **h–k**=20 µm, **l, m**=10 µm.

Figure 3.

The RAxML tree based on analysis of LSU, cmdA, his3, ITS, tef1 and tub2 sequnces data. Bootstrap support values for ML, MP and Bayesian greater than 75%, 75% and 0.95 were given near nodes respectively. The tree was rooted with *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare*. The new isolate was marked in red.

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Table 1.

Primers pairs used in this study.

Locus	Primers	References
LSU	LR0R	Rehner and Samuels 1994
	LR5	Vilgalys and Hester 1990
CMDA	CAL-228F	Carbone and Kohn 1999
	CAL2Rd	Groenewald et al. 2013
HIS3	CYLH3F, CYLH3R	Crous et al. 2004
ITS	ITS5, ITS4	White et al. 1990
TEF1	EF1-728F	Carbone and Kohn 1999
	EF2	O'Donnell et al. 1998
TUB2	T1	O'Donnell and Cigelnik 1997
	CYLTUB1R	Crous et al. 2004

Table 2.

Isolated taxa used in this study and their GenBank accession numbers. The type species are superscripted T and the newly generated sequences are indicated in red.

Taxa	Isolate numbers	GenBank Accession numbers					
		LSU	CMDA	HIS3	ITS	TEF1	TUB2
Penicillifer penicilliferi	CBS 423.88T	KM231607	KM231269	KM231453	KM231739	KM231859	KM231995
Penicillifer bipapillatus	CBS 420.88T	KM231608	KM231270	KM231454	KM231740	KM231860	KM231996
Penicillifer diparietisporus	CBS 376.59T	KM231609	KM231271	KM231455	KM231741	KM231861	KM231997
Penicillifer pulcher	CBS 560.67T	KM231610	KM231272	KM231456	KM231742	KM231862	KM231998
Corallonectria jatrophae	CBS 913.96T	KM231611	KM231273	KM231457	KC479758	KM231863	KC479787
Dematiocladium celtidis	CBS 115994T	AY793438	KM231274	–	AY793430	KM231864	–
Aquanectria submersa	CBS 394.62T	KM231612	–	KM231458	HQ897796	–	KM231999
Aquanectria penicillioides	CBS 257.54	KM231613	KM231275	–	KM231743	KM231865	KM232000
Gliocladiopsis sagariensis	CBS 199.55T	JQ666078	KM231276	JQ666031	JQ666063	JQ666106	JQ666141
Gliocladiopsis pseudotenuis	CBS 116074T	JQ666080	KM231277	JQ666030	AF220981	JQ666099	JQ666140
Gliocladiopsis irregularis	CBS 755.97T	JQ666082	KM231278	JQ666023	AF220977	KF513449	JQ666133
Cylindrocladiella lageniformis	CBS 340.92T	JN099165	KM231279	AY793520	AF220959	JN099003	AY793481
Cylindrocladiella camelliae	CPC 234T	JN099249	KM231280	AY793509	AF220952	JN099087	AY793471
Cylindrocladiella parva	CBS 114524T	JN099171	KM231281	AY793526	AF220964	JN099009	AY793486
Gliocephalotrichum longibrachium	CBS 126571T	KM231686	KM231282	KF513367	DQ278422	KF513435	DQ377835

Gliocephalotrichum bulbilium	CBS 242.62T	AY489732	KM231283	KF513326	–	KM231892	DQ377831
Gliocephalotrichum cylindrosporum	CBS 902.70T	JQ666077	KM231284	KF513353	DQ366705	KF513408	DQ377841
Calonectria ilicicola	CBS 190.50T	GQ280727	AY725764	AY725676	GQ280605	AY725726	AY725631
Calonectria brassicae	CBS 111869	GQ280698	GQ267382	DQ190720	GQ280576	FJ918567	AF232857
Calonectria naviculata	CBS 101121T	GQ280722	GQ267399	GQ267252	GQ280600	GQ267317	GQ267211
Curvicladiella cignea	CBS 101411	JQ666075	KM231285	KM231459	KM231744	KM231866	KM232001
Curvicladiella cignea	CBS 109168	JQ666074	KM231286	KM231460	KM231745	KM231868	KM232003
Curvicladiella cignea	CBS 109167T	AY793431	KM231287	KM231461	AF220973	KM231867	KM232002
Curvicladiella paphiopedili	MFLUCC 20-0110^{T}	MT279199	MT294104	MT294105	MT279198	MT294103	MT294102
Xenocylindrocladium subverticillatum	CBS 113660T	KM231687	KM231288	KM231462	AF317347	KM231893	AF320196
Xenocylindrocladium guianense	CBS 112179T	JQ666073	KM231289	KM231463	AF317348	KM231895	AF320197
Xenocylindrocladium serpens	CBS 128439T	KM231688	KM231290	KM231464	AF220982	KM231894	AF320196
Thelonectria olida	CBS 215.67T	HM364317	KM231325	KM231487	AY677293	HM364345	KM232024
Thelonectria trachosa	CBS 112467T	HM364312	KM231326	KM231488	AY677297	KM231896	AY677258
Thelonectria discophora	CBS 125153	HM364307	KM231327	KM231489	HM364294	KM231897	HM352860
Cylindrocarpostylus gregarius	CBS 101074	KM231614	KM231291	–	KM231746	KM231869	KM232004
Cylindrocarpostylus gregarius	CBS 101072T	JQ666084	KM231292	–	KM231747	KM231870	KM232005
Cylindrocarpostylus gregarius	CBS 101073	JQ666083	KM231293	KM231465	KM231748	KM231871	KM232006

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Rugonectria rugulosa	CBS 129158	JF832761	KM231295	KM231467	JF832661	KM231872	JF832911
Rugonectria rugulosa	CBS 126565	KM231615	KM231296	KM231468	KM231749	KM231873	KM232007
Rugonectria neobalansae	CBS 125120	HM364322	KM231294	KM231466	KM231750	KM231874	HM352869
Campylocarpon fasciculare	CBS 112613T	HM364313	KM231297	JF735502	AY677301	JF735691	AY677221
Campylocarpon pseudofasciculare	CBS 112679T	HM364314	KM231298	JF735503	AY677306	JF735692	AY677214

[T] Ex-type and ex-epitype cultures.1 CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: P.W. Crous collection.2 LSU: 28S large subunit; cmdA: calmodulin; his3: histone H3; ITS: the internal transcribed spacer region and intervening 5.8S nrRNA; tef1: translation elongation factor 1-alpha; tub2:β-tubulin.