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Colletotrichum paridis sp. nov., a novel endophytic species on *Paris polyphylla* var. *chinensis*

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Abstract

The genus *Paris* is a crucial medicinal herb, widely cultivated in East Asia and Europe. In 2017 a novel *Colletotrichum* species was isolated from leaf lesions with grey mould of *Paris polyphylla* var. *chinensis* in Sichuan Province of China. Through single-spore technique, four isolates were obtained for characterization. Phylogeny inferred from multi-locus sequences of ITS, *gapdh*, *act*, *tub2*, *chs-1*, and *his3*, showed that these isolates represented a novel sister species to *C. jinshuiense* within the *C. dematium* complex. Morphologically, this new species produces larger conidia than those of *C. jinshuiense* and cultures on PDA show a noticeable pink ring. Tests showed that this species had no pathogenicity to the host plants. Based on phylogenetic, morphological, and pathogenicity data, we propose the name *C. paridis sp. nov.* for this new endophyte from *P. polyphylla* in China.

Key words: *Colletotrichum paridis*, *Paris polyphylla*, phylogeny, taxonomy

Introduction

The genus *Paris* includes about 27 species that are perennial herbs of the family Liliaceae, mainly distributed in East Asia and Europe (Cunningham *et al.* 2018, Thakur *et al.* 2023, Shah *et al.* 2012). Of these, *P. polyphylla*, also called "Chonglou" in Chinese, is well known for its anti-inflammatory and hemostatic activity, resulting in huge demand mostly from Chinese and Indian industries (Thakur *et al.* 2023). Many fungal species have been reported as endophytes or pathogens of *P. polyphylla*, such as *Aspergillus fumigatus*, *Alternaria* spp., *Pichia guilliermondii*, *Fusarium solani*, *Colletotrichum fructicola*, and *Setophoma terrestris* (Li *et al.* 2008, Fu *et al.* 2018, 2019b, Zhao *et al.* 2010, Su *et al.* 2023, Zhou *et al.* 2020, Zhang *et al.* 2022). Moreover, several novel species of fungi have been described from the genus *Paris*, *i.e.*, *Botrytis polyphyllae*, *Cercospora paridis*, *Passalora paridis*, *Septoria paridis*, *Urocystis paridis* (Farr *et al.* 2021).

The genus *Colletotrichum* includes numerous plant endophytes, pathogens, and saprobes with a worldwide distribution, that have been classified into at least 15 species complexes and various singletons (Damm *et al.* 2009, Cannon *et al.* 2012, Liu *et al.* 2022). Accurate identification of the *Colletotrichum* species is vital for biodiversity, evolutionary history, and for control of plant diseases (Liu *et al.* 2022). Due to the lack of reliable morphological characters (Cai *et al.* 2009), the phylogenetic relationships of multiple loci have become essential for species identification within *Colletotrichum* (Hyde *et al.* 2016, 2020, Li *et al.* 2016, Jayawardena *et al.* 2017, Tibpromma *et al.* 2017).

To date, six loci, ITS, *gapdh*, *act*, *tub2*, *chs-1*, and *his3*, have been used for species delimitation in the *C. dematium* complex (Damm *et al.* 2009, Fu *et al.* 2019a, Lee & Jung 2018, Yuan *et al.* 2020). For instance, *C. hemerocallidis*, *C. eryngiicola*, and *C. zhejiangense* have been introduced as new species within the *C. dematium* complex based on

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phylogenetic analyses of these six loci and morphological features (Liu *et al.* 2022, Yang *et al.* 2012, Buyck *et al.* 2017). These data provide a solid foundation for the accurate identification of the new species in this study.

During a survey on fungal diseases from *P. polyphylla* var. *chinensis* in the Beichuan Qiang Autonomous County, Sichuan Province, an unidentified *Colletotrichum* species was isolated from leaves with grey mould (Zhong *et al.* 2019, Fu *et al.* 2021). Therefore, the current study was conducted to identify this new species based on both morphology and multilocus phylogeny, and to determine any pathogenicity towards the host leaves *in vitro*.

Materials and methods

Fungal isolation

Sixteen fresh samples of leaves with grey mould from ten plants of *Paris polyphylla* var. *chinensis* were collected from a cultivated field in Beichuan Qiang Autonomous County, Sichuan Province, China (31.8494°N, 104.3986°E, 1135m elevation) in 2017 (Fig. 1a,b). For isolation, 1 × 0.5 cm sections were cut from the margin of leaf lesions, surface-sterilized in 75% ethanol for 40s, washed 3 times with sterile water, then placed on sterile filter paper to air dry before transferring to potato dextrose agar (PDA) plates. After 2 to 5 days, fungal hyphae that grew on these plates were transferred to fresh PDA plates.

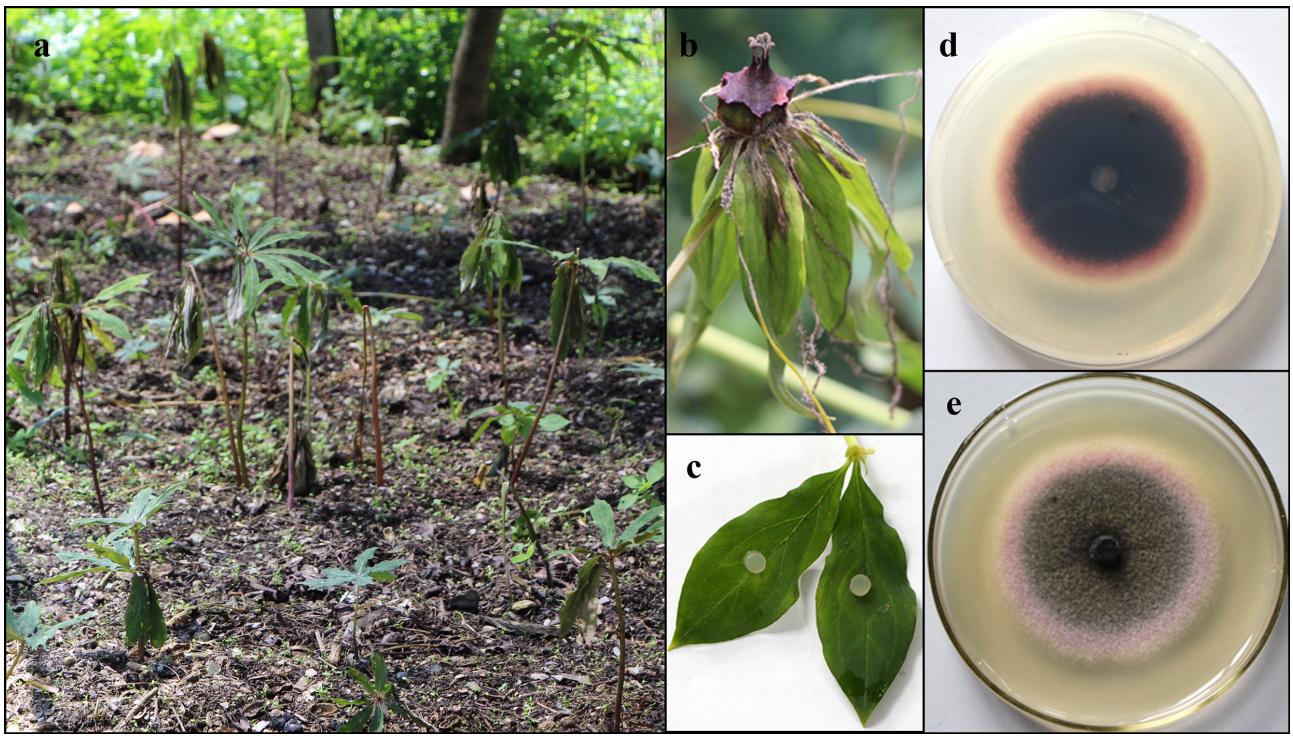

FIGURE 1. a. The collection of samples. b. *Paris polyphylla* with grey mould where *C. paridis* was isolated from. c. No symptoms on inoculated leaves. d, e. Culture of *C. paridis* on PDA after 5 days (d, upper; e, reverse).

Morphological and cultural characterization

Colony characters (diameters, colours, etc.) on PDA at 25 °C in darkness were recorded at days 5, 10 and 14. Colony diameters were measured at day 5 to calculate mycelial growth rates (mm/d). PDA plugs (7 mm diam) with fresh mycelia were transferred to PDA and synthetic nutrient-poor agar (SNA) plates, and incubated under near-ultraviolet (UV) light for about 10 days to produce the asexual morph (conidia, setae, etc.). Appressoria were produced using a slide culture technique, in which 10 mm^2 plugs of PDA were placed in an empty Petri dish, and a sterile cover slip was placed over the agar (Cai *et al.* 2009). After five days, the formation of appressoria was observed on the cover slip. Micro-morphological features were observed using an Olympus BX63 microscope (Japan) and the sizes of each morphological structure were measured. All data in this study were analyzed using IBM SPSS Statistics v.22.

Scanning electron microscopy

Sporulation of the fungus was observed using a microscope (Olympus BX63, Japan) and a 5 mm^2 segment of colony on PDA was selected from an area of spore production. The sample was immersed in 2.5% glutaraldehyde fixative for 30 minutes, after which it was rinsed five times with 10× PBS buffer, each for a period of 10 minutes. A graded series of ethanol was employed to dehydrate the specimens for 15 minutes each in 60%, 70%, 80% and 90% ethanol, followed by two additional dehydration periods in 100% ethanol, each lasting 20 minutes. Subsequently, the sample was immersed in 100% tert-Butanol solution for 40 minutes, with each immersion lasting 20 minutes. Following this, the sample was placed into a freeze-drier (Scientz-12N/A, China) for 24 hours. The dried sample was mounted on aluminium stubs with double-sided copper tape and coated with gold on a coater (DII-29030SCTR, Japan). The sample was observed and photographed with a scanning electron microscope (JSM-IT200(LA), Japan) at the accelerating voltage of 5-15 kV.

DNA extraction, amplification and sequencing

The isolates were cultured on PDA at 25 °C for 5 days in darkness, before the marginal mycelia of the colonies were scraped off with a sterile scalpel. Total DNA was extracted using an Omega Fungal DNA Extraction Kit (Georgia, USA). The ITS, *gapdh*, *act*, *tub2*, *chs-1*, and *his3* sequences were amplified using the primer pairs ITS1/ITS4 (White *et al.* 1990), GDF1/GDR1 (Guerber *et al.* 2003), ACT-512F/ACT-783R (Carbone & Kohn 1999), T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), CHS-79F/CHS-345R (Carbone & Kohn 1999), and CYLH3F/CYLH3R (Crous *et al.* 2004), respectively. Sequencing was performed by Tsingke Biological Technology Co. (Chengdu, China).

Phylogenetic analysis

BLAST analyses of each gene in NCBI (http://blast.ncbi.nlm.nih.gov/) to find species matches and similarities. These similar and reported sequences of representative isolates were collected from GenBank. Phylogenetic trees were constructed as previously explained (Xue *et al.* 2023). Briefly, the sequences of each gene were aligned with MAFFT v.7 (Katoh & Standley 2013) and manually adjusted by MEGA v.6 (Tamura *et al.* 2013); the six loci alignments were then combined with SequenceMatrix 1.8 (Vaidya *et al.* 2011); the Bayesian inference (BI) and randomized axelerated maximum likelihood (RAxML) analyses were performed using both MrBayes v.3.2.6 (Ronquist *et al.* 2012) and raxmlGUI v.2.0 (Edler *et al.* 2021), respectively. The RAxML analyses were based on 1,000 replicates. For BI analysis, the best-fit nucleotide substitution models of each gene were assessed by MrModeltest v.2.3 (Nylander 2004); analyses of MCMC chains based on the full dataset were run for two million generations and sampled every 100 generations; the first 25% of the generations were discarded as burn-in. Only the RAxML tree of the combined dataset is shown in this study. Bootstrap values for RAxML higher than 50% and Bayesian posterior probabilities (BP) surpassing 0.9 are given at the nodes (RAxML/BP). The single-locus trees of each gene in both BI and RAxML analyses were also compared by the same methods.

Pathogenicity tests

Young, healthy and consistent leaves were obtained from 2-year-old *P. polyphylla* var. *chinensis* plants in the cultivated field. Conidial suspensions of single-spore isolates were adjusted to a concentration of around 1×10^6 conidia/ml in sterile water with 0.01% (vol/vol) Tween 80. PDA plugs (4 mm diam.) were taken from the edge of fresh colonies. For each isolate, six detached leaves were inoculated with a 20 µl conidial suspension, and another six leaves were inoculated with the PDA plugs. Another set of six leaves were inoculated with sterile water containing Tween 80 or with PDA plugs without the fungus were used as controls. Inoculated leaves were transferred to an enamelware tray and each tray was then covered with transparent plastic films to maintain high relative humidity in a greenhouse at 16–25 °C. Pathogenicity assays were performed four times as described above. Five days after inoculation, the diameters of leaf lesions were measured to evaluate virulence.

Results

Isolation and preservation of Colletotrichum

In total, 47 fungal strains were isolated from leaves with grey mould, and preliminarily identified by ITS sequence. Of these, two isolates were of *Colletotrichum*, 32 of *Botrytis*, 3 of *Pythium*, and 10 other fungal isolates. Only the two

isolates of *Colletotrichum* were systematically studied, and their cultural characteristics on PDA and ITS sequences were consistent.

Four single-spore cultures (BCTJB1–4) were finally obtained from the above *Colletotrichum* isolates by the single-spore technique and were kept in a refrigerator (4 ℃) for several months. Of these cultures, holotype BCTJB1 (=GMCC000018, CGMCC 3.19921, ACCC 35519) was deposited in three microbiological culture collection centers. CGMCC is China General Microbiological Culture Collection Center (https://cgmcc.net), GMCC is Grassland Microbiome Culture Collection (https://gmcc.lzu.edu.cn/login) and ACCC is Agricultural Culture Collection of China (http://www.accc.org.cn),

Phylogenetic analysis

The sequences of all six loci of the four single-spore isolates generated in this study were deposited in GenBank [the ITS sequences of pure isolates of the other genera were also submitted to NCBI: *Botrytis* spp. (MF614072 to MF614075) and *Pythium* sp. (PP218425)]. BLAST analyses of each gene showed that the four isolates belonged to the *C. dematium* complex. The corresponding sequences of another 35 reported isolates in the *C. dematium* complex were acquired (Table 1). After processing, the combined dataset comprised 2158 characters (518 for ITS, 249 for *gadph*, 512 for *tub2*, 248 for *act*, 239 for *chs-1*, and 392 for *his3*, including alignment gaps). For BI analysis, the best-fit models were determined: K80+I+G for ITS; HKY+I for *act*; K80+G for *gapdh*; GTR+G for *tub2*; SYM+G for *chs-1*; GTR+I+G for *his3*. The tree generated from both RAxML and BI analyses of the combined dataset (Fig. 2) showed the four isolates did not cluster well with any other species of the *C. dematium* complex, but formed a monophyletic clade well-supported by RAxML/BP value (100/1). In addition, phylogeny inferred from single loci demonstrated that this new species was also distinguished from other species of the *C. dematium* complex by *gapdh*, with highly supported subclades (99/1). Ultimately, the combined alignment and its corresponding tree were deposited in TreeBASE (Submission ID: 30907).

TABLE 1. Sources of *Colletotrichum* strains and GenBank accession numbers of isolates used in this study.

Species	Strain	GenBank accession number					
		ITS	act	gapdh	tub2	chs-1	his3
C. anthrisci	CBS 125334*	GU227845	GU227943	GU228237	GU228139	GU228335	GU228041
C. anthrisci	CBS 125335	GU227846	GU227944	GU228238	GU228140	GU228336	GU228042
C. circinans	CBS 221.81*	GU227855	GU227953	GU228247	GU228149	GU228345	GU228051
C. dematium	CBS 125.25*	GU227819	GU227917	GU228211	GU228113	GU228309	GU228015
C. dematium	CBS 125340	GU227820	GU227918	GU228212	GU228114	GU228310	GU228016
C. dematium	CBS 123728	GU227822	GU227920	GU228214	GU228116	GU22831	GU228018
C. dematium	CBS 115524	GU227826	GU227924	GU228218	GU228120	GU228316	GU228022
C. dematium	IMI 350847	GU227825	GU227923	GU228217	GU228119	GU228315	GU228021
C. eryngiicola	MFLUCC 17-0318*	KY792726	KY792717	KY792723	KY792729	KY792720	…
C. fructi	CBS 346.37*	GU227844	GU227942	GU228236	GU228138	GU228334	GU228040
C. hemerocallidis	CBS 130624*	JQ400005	JQ399991	JQ400012	JQ400019	JQ399998	…
C. insertae	MFLU 15-1895	KX618686	KX618682	KX618684	KX618685	KX618683	…
C. jinshuiense	PAFQ26*	MG748077	MG747767	MG747995	MG748157	MG747913	…
C. jinshuiense	PAFQ26a	MG874830	MG874807	MG874822	MG874838	MG874814	…
C. jinshuiense	PAFQ26b	MG874831	MG874808	MG874823	MG874839	MG874815	…
C. jinshuiense	PAFQ26c	MG874832	…	MG874824	MG874840	MG874816	…

.....continued on the next page

TABLE 1. (Continued)

Species	Strain	GenBank accession number					
		ITS	act	gapdh	tub2	chs-1	his3
C. jinshuiense	PAFQ26d	MG874833	MG874809	MG874825	MG874841	MG874817	…
C. jinshuiense	KCTC 46679*	LC324781	LC324785	LC324787	LC324791	LC324783	LC324789
C. jinshuiense	KCTC 46680	LC324782	LC324786	LC324788	LC324792	LC324784	LC324790
C. jinshuiense	LC8509	MZ595860	MZ664158	MZ664123	MZ673981	MZ799341	MZ673880
C. jinshuiense	EsH8	MW440484	MW676252	MW676256	MW676254	MW676258	…
C. jinshuiense	EsH11	MW440485	MW676253	MW676257	MW676255	MW676259	…
C. lineola	CBS 125337*	GU227829	GU227927	GU228221	GU228123	GU228319	GU228025
C. lineola	CBS 125339	GU227830	GU227928	GU228222	GU228124	GU228320	GU228026
C. lineola	CBS 125332	GU227831	GU227929	GU228223	GU228125	GU228321	GU228027
C. menispermi	MFLU 14-0625*	KU242357	KU242353	KU242356	KU242354	KU242355	…
C. orchidis	MFLUCC 17-1302*	MK502144	MK496854	MK496858	MK496860	MK496856	…
C. paridis	BCTJB1* = GMCC000018, ACCC 35519, CGMCC 3.19921	MF775292	MF775300	MF775312	MF775304	MF775316	MF775320
C. paridis	BCTJB2	MF775293	MF775301	MF775313	MF775305	MF775317	MF775321
C. paridis	BCTJB3	MF775294	MF775302	MF775314	MF775306	MF775318	MF775322
C. paridis	BCTJB4	MF775295	MF775303	MF775315	MF775307	MF775319	MF775323
C. parthenocissicola	MFLUCC 17-1098*	MK629452	MK639358	MK639362	MK639360	MK639356	…
C. quinquefoliae	MFLU 14-0626*	KU236391	KU236389	KU236390	KU236392	…	…
C. sambucicola	MFLUCC16-1388*	KY098781	KY098778	KY098780	KY098782	KY098779	…
C. sedi	MFLUCC14-1002*	KM974758	KM974756	KM974755	KM974757	KM974754	…
C. sonchicola	MFLUCC17-1299*	KY962757	KY962748	KY962754	…	KY962751	…
C. spinaciae	CBS 128.57	GU227847	GU227945	GU228239	GU228141	…	…
Colletotrichum sp.	CGMCC 3.15172	HM751816	KC843547	KC843522	KC244162	GU228337	GU228043
C. zhejiangense	NN076215	MZ595912	MZ664210	MZ664124	MZ674030	MZ799342	MZ673932
Outgroup							
C. chlorophyti	IMI 103806*	GU227894	GU227992	GU228286	GU228188	GU228384	GU228090

Note: ACCC, Agricultural Culture Collection of China; CBS, Culture collection of the Centraalbureau voor Schimmelcultures; CGMCC, China General Microbiological Culture Collection Center; GMCC, Grassland Microbiome Culture Collection; IMI International Mycological Institute; KCTC, Korean Collection for Type Cultures; MFLU, Herbarium of Mae Fah Luang University; MFLUCC, Mae Fah Luang University Culture Collection; Sequences generated in this study are indicated in bold; ex-type strains are marked with an asterisk (*); …, absent.

Pathogenicity

Five days after inoculation, both conidial suspensions and PDA plugs of BCTJB1–4 caused no symptoms on detached leaves of *P. polyphylla* var. *chinensis* (Fig. 1c).

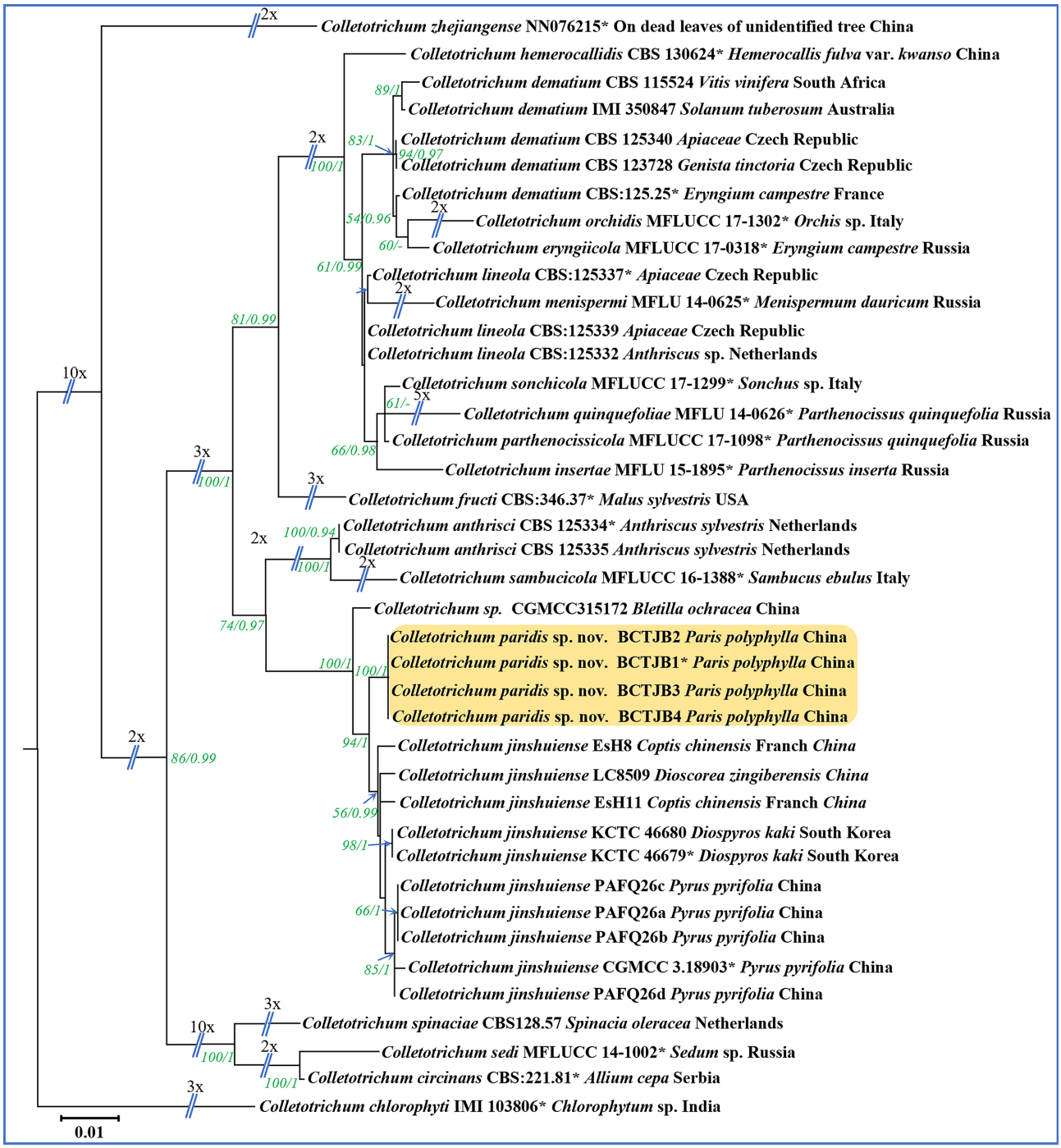

FIGURE 2. Phylogenetic tree generated from randomized axelerated maximum likelihood (RAxML) analysis based on the combined ITS, *gapdh*, *act*, *tub2*, *chs-1* and *his3* alignment of isolates from *C. dematium* complex. Bootstrap values for RAxML higher than 50% and Bayesian posterior probabilities (BP) higher than 0.9 are given at the nodes (RAxML/BP). *Colletotrichum chlorophyti* IMI 103806 is outgroup. Ex-type or holotype specimens are marked with an asterisk (*).

Taxonomy

Colletotrichum paridis L.H. Xue, H.W. Cui & X.K. Wei, *sp. nov*. Figs 1d,e, 3.
Mycobank no. MB 850803

***Type*:**—CHINA. Sichuan Province: Beichuan Qiang Autonomous County; 31.8494°N, 104.3986°E, 1135m elevation, isolated from leaf lesions with grey mould of cultivated *Paris polyphylla* var. *chinensis*, 2017, L.H. Xue. **Holotype**

GMCC000018, stored in a metabolically inactive state; ex-type living culture BCTJB1 (= GMCC000018, CGMCC 3.19921, ACCC 35519). GenBank: MF775292 (ITS); MF775312 (*gapdh*); MF775300 (*act*); MF775304 (*tub2*); MF775316 (*chs-1*); MF775320 (*his3*).

Etymology:—Named after the host genus from which it was isolated, *Paris*.

Diagnosis:—Phylogenetically, *C. paridis* differs (by 6 bp or more) in *gapdh* sequence from other species within the *C. dematium* complex.

Description:—Endophytic on leaves of *P. polyphylla* var. *chinensis*. Asexual morph on PDA. *Setae* dark brown to black, smooth-walled, cylindrical to conical, tip acute or rounded, 1–4-septate, 51–193 µm (mean±SD=110±35 µm) long. *Conidiophores* hyaline to light brown, smooth-walled, septate. *Conidia* solitary, hyaline, curved or falcate, apex narrow and acute, base subtruncate, with many guttules, 21.7–31.7 × 3.7–5.5 µm (mean±SD = 24.9±2.3 × 4.6±0.5 µm; *n* = 50), length/width (L/W) ratio=5.4. *Appressoria* light brown, smooth-walled, ellipsoidal to irregular, sometimes slightly lobed, 6.2–13.5 × 4.7–11.1 µm (mean±SD = 10.2±1.7 × 6.5±1.7 µm; *n* = 26), length/width (L/W) ratio = 1.6. Sexual morph: undetermined.

Asexual morph on SNA. *Conidia* solitary, hyaline, curved, apex acute, base subtruncate, with many guttules, 24.3–30.8 × 4.2–5.7 µm (mean±SD = 27.5±1.6 × 4.7±0.3 µm; *n* = 50), length/width (L/W) ratio = 5.9.

Colonies on PDA flat with entire margin, aerial mycelium dense, cottony, surface reddish-brown to black with white to pink margin; reverse black with white to pink margin; mycelial growth rate 5.8–6.0 mm/d.

Additional material examined:—CHINA. Sichuan Province: Beichuan Qiang Autonomous County, 31.8494°N, 104.3986°E, 1135m elevation, from grey mould of *P. polyphylla* var. *chinensis*, 2017, L.H. Xue, living cultures BCTJB2 (ITS accession no. MF775293), BCTJB3 ((ITS: MF775294), and BCTJB4 (ITS: MF775295).

Notes:**—**Phylogenetically, this species is a sister group to both *C. jinshuiense* and *Colletotrichum* sp. isolate CGMCC 3.15172, but it differs at 9–18 positions from reported isolates of these two related species (Table 2). Morphologically, *C. paridis* produces larger conidia on SNA than those of *C. jinshuiense* (av. 27.5 × 4.7 vs. 24 × 4 µm) (Lee & Jung 2018). In addition, cultures of *C. paridis* on PDA show a noticeable pink ring, and have slightly faster mycelial growth rate (5.8–6 vs. 3.3–5.8 mm/d) (Table 3).

TABLE 2. Different nucleotide bases between *Colletotrichum paridis*, *C. jinshuiense* and *Colletotrichum* sp. strain CGMCC 3.15172 at different gene loci.

Base (bp) Species	Strain	ITS	act	gapdh	tub2	chs-1	his3	Summary	Literature cited
C. paridis	BCTJB1*	Check	Check	Check	Check	Check	Check	Check	This study
C. paridis	BCTJB2	0	0	0	0	0	0	0	This study
C. paridis	BCTJB3	0	0	0	0	1	0	1	This study
C. paridis	BCTJB4	0	0	0	0	1	0	1	This study
C. jinshuiense	PAFQ26*	0	2 (2gaps)	6	0	3	…	11	(Fu *et al.* 2019a)
C. jinshuiense	PAFQ26a	0	1 (1gap)	6	0	3 (1gap)	…	10	(Fu *et al.* 2019a)
C. jinshuiense	PAFQ26b	0	1 (1gap)	6	0	3 (1gap)	…	10	(Fu *et al.* 2019a)
C. jinshuiense	PAFQ26c	0	…	6	0	3 (1gap)	…	9	(Fu *et al.* 2019a)
C. jinshuiense	PAFQ26d	0	1 (1gap)	6	0	3	…	10	(Fu *et al.* 2019a)
C. jinshuiense	KCTC 46679*	0	0	8 (1gap)	4	4	2	18	(Lee & Jung 2018)
C. jinshuiense	KCTC 46680	0	0	8 (1gap)	4	4	2	18	(Lee & Jung 2018)
C. jinshuiense	LC8509	0	0	7	3	4	2	16	(Liu *et al.* 2022)
C. jinshuiense	EsH8	0	0	8	3	1	…	12	(Wang *et al.* 2023)
C. jinshuiense	EsH11	1 (1gap)	0	7	2	1	…	11	(Wang *et al.* 2023)
Colletotrichum sp.	CGMCC 3.15172	0	0	9 (2gaps)	4 (1gap)	…	…	13	(Tao *et al.* 2013)

Note: Sequences generated in this study are indicated in bold; ex-type strains are marked with an asterisk (*); …, absent.

TABLE 3. Morphological characteristics of *Colletotrichum paridis* compared with *C. jinshuiense.*

Species	Strain (Host)	Colony characters on PDA		Conidia		Appressoria		Setae	
		Colonial morphology	Growth rate (mm/d)	Size (μm); L/W ratio	Shape	Size (μm); L/W ratio	Shape	Septate	Length (μm)
C. jinshuiense	PAFQ26* PAFQ26a-d (Pyrus pyrifolia)	Surface pale grey-black with white margin; reverse black to dark grey-green in center with white margin	28 °C: 5.6–5.7	on host: 24.4–27.1 × 4; 6.1–6.8	Curved, apex acute	10.7 × 6; 1.8	Ellipsoidal to clavate	1–4	59–363
C. jinshuiense (= *C. kakivorum*)	KCTC 46679*, KCTC 46680 (Diospyrus kaki)	Surface grey-olivaceous; reverse olivaceous-grey with smoke-grey concentric rings	25 °C : 3.3–3.5	on SNA: 24 × 4; 6.0	Curved, apex acute	10.9 × 4.7; 2.3	Clavate or ellipsoidal to slightly lobed	3–6	77–125
C. jinshuiense	EsH8, EsH11 (Coptis chinensis)	Surface white to pale grey; reverse yellowish to olive	25 °C: 5.6–5.8	on host: 25.4 × 4.5; 5.6	Curved, apex acute	8.3 × 7.6; 1.1	Globose or obovoid	1–3	78–134
C. paridis	BCTJB1*, BCTJB2–4 (Paris polyphylla)	Surface reddish-brown to black with a noticeable pink ring; reverse black with white to pink margin	25 °C: 5.8–6	on PDA: 24.9 × 4.6; 5.4 on SNA: 27.5 × 4.7; 5.9	Curved, apex acute	10.2 × 6.5; 1.6	Ellipsoidal to irregular	1–4	51–193

Note: The new species in this study are indicated in bold; ex-type strains are marked with an asterisk (*).

Discussion

Colletotrichum species were isolated from *Paris quadrifolia* in Poland (Mulenko *et al.* 2008). Recently, *C. fructicola* and *C. spaethianum* were reported as pathogens on *P. polyphylla* in China (Zhou *et al.* 2020, Zhong *et al.* 2020). In the present study, *C. paridis* is introduced as a new species isolated from *Paris* and identified by a polyphasic approach combining colony features, morphology, and phylogenetic analyses of multi-locus sequences. Phylogenetically, *C. paridis* is most closely related to *C. jinshuiense* and *Colletotrichum* sp. isolate CGMCC 3.15172, but has notable differences in *gapdh* sequence. Morphologically, *C. paridis* and *C. jinshuiense* could be distinguished by cultural and conidial features. Unfortunately, morphology features were not given for isolate CGMCC 3.15172 (Tao *et al.* 2013).

Colletotrichum jinshuiense was first isolated from leaves of *Pyrus pyrifolia* in 2016, and described as a new species causing anthracnose disease of this host in China (Fu *et al.* 2019a). *C. kakivorum*, which was described as a new species causing leaf spots on *Diospyros kaki* in Korea (Lee & Jung 2018) was later synonymysed with *C. jinshuiense* (Liu *et al.* 2022). Most recently, *C. jinshuiense* was reported to cause anthracnose of *Coptis chinensis* in China (Wang *et al.* 2023). Isolate CGMCC 3.15172 was reported as an endophyte of *Bletilla ochracea* in China (Tao *et al.* 2013).

Endophytic *Colletotrichum* species have potential as biological control agents of plant diseases. For example, an endophytic isolate of *C. gloeosporioides* (6174) significantly decreased pod loss of *Theobroma cacao* caused by *Phytophthora palmivora* in both greenhouse experiments and field trials (Mejía *et al.* 2008). Also, grey mould infections of *P. polyphylla* caused by *Botrytis cinerea* were significantly reduced by *Paenibacillus terrae* PY8 (Tang *et al.* 2022). Normally, grey mould on *P. polyphylla* is a common disease in southwest China, resulting in up to 50% mortality (Zhong *et al.* 2019, Fu *et al.* 2021). Therefore, it is suggested that studies on the inhibitory activity and control efficacy of *C. paridis* against pathogens, especially against grey mould of *P. polyphylla* should be evaluated.

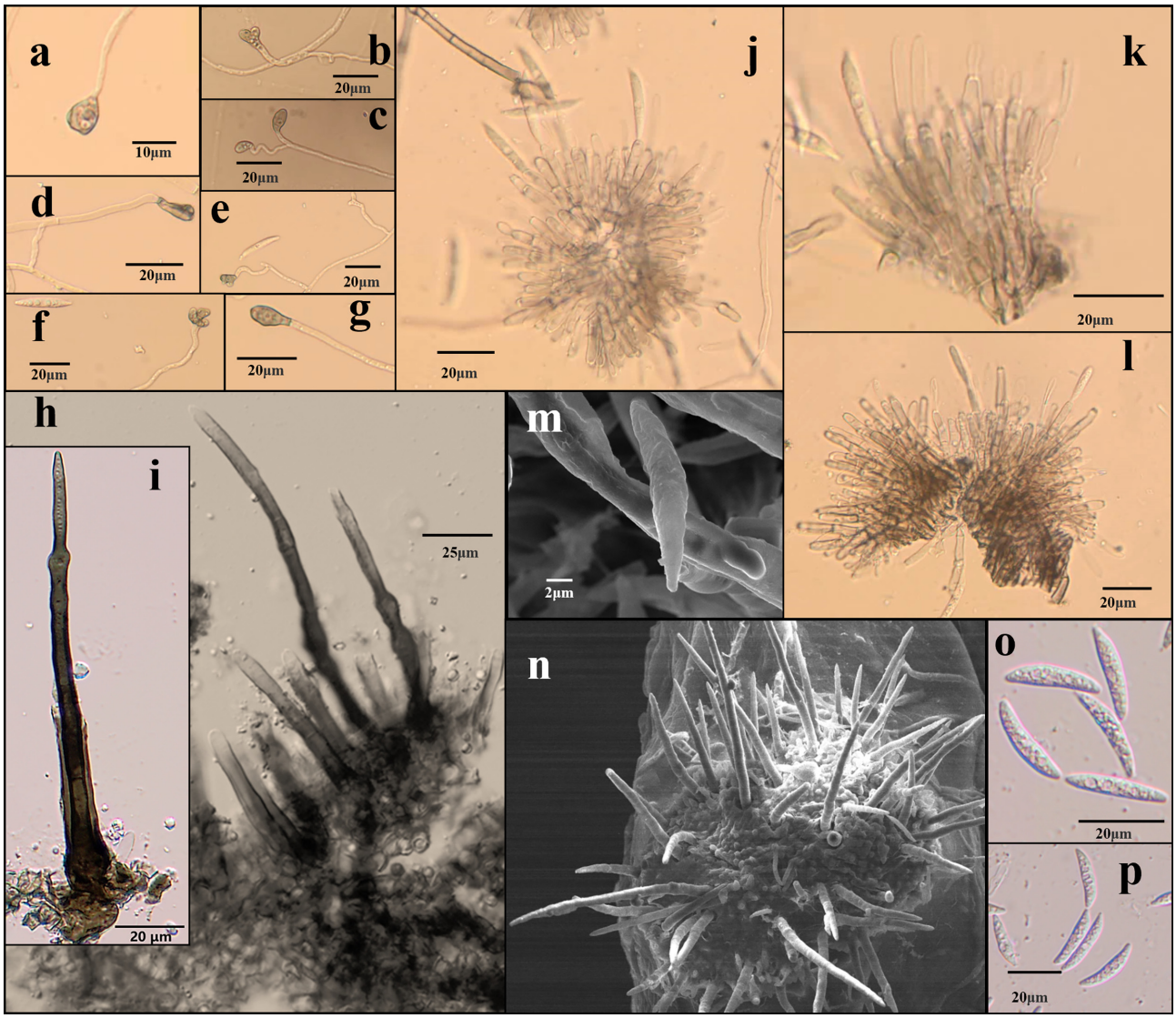

FIGURE 3. *Colletotrichum paridis* (GMCC000018, holotype). a–g. Appressoria. h, i. Setae. j–l. Conidiophores and conidia. m, n. Scanning electron micrographs of (m) a conidium and (n) setae. o, p. Conidia.

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