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Monochaetia ilexae sp. nov. (Pestalotiopsidaceae) from Yunnan Province in China

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Abstract

Monochaetia is a pestalotiopsis-like genus characterized by 3–5-septate hyaline to brown conidia with single apical and basal appendages. *Monochaetia* species exhibit diverse conidial morphology, but many species lack molecular data and thus it is not clear if the genus is monophyletic. In this paper, combined LSU and ITS sequence data and morphological traits are used to introduce a new *Monochaetia* species, *M. ilexae* from Yunnan Province, China from dead leaves of *Ilex* species. *Monochaetia ilexae* shares similar morphology with the type, *M. monochaeta* and *M. kansensis* having fusiform conidia and has a similar range of conidia. However *M. ilexae* differs from *M. monochaeta* and *M. kansensis* having different conidia length, apical and basal appendage lengths. Phylogeny agrees with morphological differences allowing *Monochaetia ilexae* as a new species that clusters with *Monochaetia* species, but is separated from the main clade with high support.

Key words: coelomycetes, morphology, new species, phylogeny

Introduction

Monochaetia is a pestalotiopsis-like genus in the family Pestalotiopsidaceae which has not been linked to a sexual-morph (Senanayake *et al*. 2015, Maharachchikumbura *et al*. 2015a,b, 2016). Recent studies include the genera *Ciliochorella*, *Lepteutypa*, *Neopestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis* and *Seiridium* in the family Pestalotiopsidaceae (Senanayake *et al*. 2015, Maharachchikumbura *et al*. 2016). *Monochaetia* species are often plant pathogens that cause post-harvest losses and found on different plant hosts such as Coniferales, Salicales, Rosales and Ericales (Guba 1961, Gonthier *et al*. 2006, Wijayawardene *et al*. 2016).

Saccardo (1884) introduced *Monochaetia* as a sub genus of *Pestalotia* (as *Pestolozzia*). The genus *Monochaetia* was introduced by Allescher (1902) who included 23 species, without designating a type. Allescher (1931) designated the type *Monochaetia monochaeta* which has a single apical appendage (Guba 1961, Maharachchikumbura *et al*. 2014, Senanayake *et al*. 2015). Steyaert (1949) transferred numerous *Monochaetia* species to *Pestalotiopsis* or *Truncatella*. More than 40 species of *Monochaetia* were recognized by the monograph of Guba (1961). There are 121 *Monochaetia* epithets in the Index Fungorum (2016) and most have been transferred to other genera such as *Sarcostroma*, *Seimatosporium* and *Seiridium* (Nag Rag 1993, Maharachchikumbura *et al*. 2011, 2014, 2016, Wijayawardene *et al*. 2016).

The family Pestalotiopsidaceae is characterized by immersed, erumpent acervular or pycnidial conidiomata and ellipsoid to clavate, or fusiform, 3–4-euseptate, hyaline, pale olivaceous or brown conidia with cellular appendages (Senanayake *et al*. 2015). Conidiomata of *Monochaetia* species are usually true acervuli, sometimes pycnidia or pseudopycnidia, superficial to subepidermal, usually without a true ostiole. Conidia are coloured, clavate, narrowly fusiform and septate with characteristic hyaline or rarely dilute yellow or faintly coloured single apical and basal appendages (Guba 1961, Senanayake *et al*. 2015, Wijayawardene *et al*. 2016).

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Most of the genera of Pestalotiopsidaceae contain over-lapping morphological characters of conidia such as the number of median cells, colour of median cells, presence of apical and basal appendages (Jeewon *et al.* 2002). However, most of the *Monochaetia* species lack molecular data. In this study, we made a collection of *Monochaetia* species on dead leaves of *Ilex* species in Yunnan Province, China and morphological characters plus multi-gene molecular analyses were used to resolve the species in the genus.

Materials and Methods

The fungus was collected and isolated from dead leaves of *Ilex* sp. in China. Specimens were returned to the laboratory in Zip lock plastic bags and observed with a JNOEC JSZ4 stereomicroscope. Morphological structures were examined with an OLYMPUS SZ61 compound microscope. Images were taken using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 600D digital camera. All microscopic measurements were made with Tarosoft image framework (v. 0.9.0.7) and images used in the paper were processed with Adobe Photoshop CS3 Extended version. Cultures of the fungus were obtained through single spore isolation (Chomnunti *et al*. 2014). Germinating conidia were aseptically transferred to fresh PDA media.

Herbarium material is deposited in the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Kunming, China and living cultures are deposited at Kunming Culture Collection (KUMCC) and duplicated at Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi and Index Fungorum numbers are processed as described in Jayasiri *et al*. (2015) and Index Fungorum (2016) respectively. Sequence data derived from this study is deposited in GenBank (Table 1).

DNA extraction, PCR amplification and gene sequencing

Fresh fungal mycelium grown on PDA for 7 days, was scraped and used for DNA extraction. DNA extraction was carried out using a Biospin fungus genomic DNA kit (BioFlux®, P.R. China) following the manufacturer's protocol.

PCR amplification of the partial large subunit nuclear rDNA (LSU) was amplified with primer pair LROR and LR5 (Vilgalys *et al*. 1990). The internal transcribed spacers (ITS) were amplified with primer pair ITS5 and ITS4 (White *et al*. 1990). PCR was performed with a 25 µl reaction mixture consisting of 1.0 µl of DNA template, 1 µl each primers, 12.5 µl Taq PCR Master Mix (Bioteke Co., China) and 9.5 µl sterilized water. The PCR amplification was performed with an initial denaturing step of 94 °C for 3 min, followed by 40 amplification cycles of 94 °C for 45 s, 55 °C for 50 s and 72 °C for 1 min and a final extension step of 72 °C for 10 min. The PCR products were checked on 1 % agarose gel stained with ethidium bromide. PCR purification and DNA sequencing of PCR products were carried out at Shanghai Sangon Biological Engineering Technology & Services Co., China.

Phylogenetic analyses

The BLAST search engine of the National Centre for Biotechnology Information (NCBI) was used for the preliminary identification of DNA sequences of the new isolate. Other related sequences from the family Pestalotiopsidaceae were downloaded from GenBank based on recently published data (Senanayake *et al*. 2015, Maharachchikumbura *et al*. 2016).

Phylogenetic analyses were based on combined LSU and ITS sequence data. Multiple sequence alignments were visually prepared with MEGA v. 5.2.2 (Kumar *et al*. 2012) and BioEdit v. 7.0.9 (Hall 1999). The phylogenetic analyses were performed for maximum likelihood in RAxMl GUI v. 1.3 (Silvestro & Michalak 2012). Parameters of the RAxMl GUI v. 1.3 were set to rapid bootstrapping and the analysis carried out using 1000 replicates and GTRGAMMA model of nucleotide substitution. Bootstrap values for maximum likelihood are shown at nodes (ML, black) (Fig 1).

Evolutionary model for phylogenetic analyses was selected using MrModeltest v. 3.7 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC). The GTR+I+G model was used for Bayesian analysis. A Bayesian analysis was conducted using MrBayes v. 3.2.1 (Ronquist *et al*. 2012). Markov chains were run for 1 000 000 generations and trees were sampled every 100th generations (printfreq=100) and 10 000 trees were obtained. Initial trees were discarded (20% burn-in value) and remaining trees were used to evaluate posterior probabilities (PP) in the majority rule consensus tree. Branches with Bayesian posterior probabilities greater than 0.90 are shown as black bold (Fig 1).

A maximum parsimony analysis (MP) was carried out using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Parsimony bootstrap analyses parameters were set-up as heuristic search option, random stepwise addition, and 1000 random sequence additions, with 1000 maxtrees. Gaps were treated as 'missing' data. Descriptive tree statistics for parsimony Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for the Maximum Parsimonious Tree (MPT). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed to determine whether the trees were significantly different. Bootstrap values for maximum parsimony are shown at nodes (MP, red) (Fig 1). Phylogenetic trees were viewed in FigTree v. 1.4.0 (Rambaut & Drummond 2008) and further editing was done using in Microsoft power point (2007).

TABLE 1. GenBank accession numbers and culture accession numbers of isolates included in this study. The newly generated sequence is shown in black bold.

Taxon	Culture Accession No	Gene Bank Accession	
		LSU	ITS
Bartalinia robillardoides	CBS 122705	KJ710438	NR126145
Ciliochorella castaneae	HHUF 28799	AB433277	-
Lepteutypa cupressi	IMI 052255	AF382379	-
Monochaetia ilexae	**KUMCC 15–0520**	**KX984152**	**KX984153**
M. kansensis	PSHI2004 Endo1031	DQ534036	DQ534045
M. kansensis	PSHI2004 Endo1030	DQ534035	DQ534044
M. monochaeta	CBS 191.82	KF590148	-
Monochaetia sp.	PQM10	LC057625	-
Monochaetia sp.	PQG03	LC057328	-
Monochaetia sp.	PQA12	LC057184	-
Neopestalotiopsis rosae	CBS 101057	KM116245	KM199359
Pestalotiopsis karstenii	ICMP 10669	AF382371	AF405300
(*Monochaetia karstenii)* *Pestalotiopsis knightiae*	CBS 114138	KM116227	KM199310
Pestalotiopsis malayana	CBS 102220	KM116238	KM199306
Pestalotiopsis protearum	CBS 114178	JN712564	JN712498
Pseudopestalotiopsis cocos	CBS 279.29	KM116276	KM199378
Pseudopestalotiopsis theae	MFLUCC 12-0055	KM116282	NR111716
Seiridium cardinale	ICMP 7323	AF382377	AF405305
Seiridium cardinale	CBS 172.56	AF382376	-
Seiridium phylicae	CPC 19970	KC005810	KC005788
Seiridium phylicae	CPC 19965	KC005809	KC005787

Results

Phylogenetic analyses

The combined LSU and ITS sequence data set comprised 22 strains including the new strain *Monochaetia ilexae* (KUMCC 15–0520) in Pestalotiopsidaceae with *Bartalinia robillardoides* as the out-group taxon. The maximum likelihood analysis, maximum parsimony and Bayesian analyses resulted in a tree with similar topology that did not differ significantly from one another (data not shown).

The maximum parsimony analysis comprised 1404 total characters and of this 1190 characters were constant, 68

variable characters were parsimony-uninformative and 146 characters were parsimony-informative. The parsimony analysis resulted in 1000 equally parsimonious trees and the first tree (length = 294 steps with CI = 0.796, RI = 0.881, RC = 0.701and HI = 0.204) is shown here. The new strain clustered in genus *Monochaetia,* but separated from other sequence available species in the genus.

Taxonomy

Monochaetia ilexae N.I. de Silva, Phookamsak & K.D. Hyde**, sp. nov.**
Index Fungorum number: IF552505, Faces of Fungi number: FoF 02622

Etymology: the epithet "*ilexae*" refers to the host, of which the taxon was collected.

Saprobic on dead leaves of *Ilex* sp. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 115–180 µm diam, pycnidial, solitary, scattered, immersed to erumpent, easily broken, visible as brown, flat, scar-like structures on the host, uni-loculate, glabrous, without an ostiole, releasing conidia by breaking the host surface. *Conidiomata wall* multi-layered, thin walled, brown, comprising cells of *textura angularis*. *Conidiophores* indistinct. *Conidiogenous cells* 4–6 × 1–2 µm ($\overline{x}$ =5.4 × 1.4 µm), holoblastic, phialidic, discrete, cylindrical, hyaline, smooth, thin-walled. *Conidia* 20–27 × 3–5 µm diam. ($\overline{x}$ =23.7 × 6 µm), fusiform, tapering at both ends, 4-septate, erect or sometimes slightly curved; apical cell 2.5–4.7 µm long ($\overline{x}$ =3.7 µm), conical, hyaline and smooth-walled; three median cells together 13–18 µm long ($\overline{x}$ =15.8 µm), doliiform, brown, rough-walled, echinulate, upper second cell 4.9–7.1 µm long ($\overline{x}$ =5.7 µm), upper third cell 3.5–5.9 µm long ($\overline{x}$ =5.1 µm), upper fourth cell 4.3–6.9 µm long ($\overline{x}$ =5.6 µm); basal cell 2.6–5.2 µm long ($\overline{x}$ =3.7 µm), conic, hyaline and smooth-walled; apical appendage 6–24 µm long ($\overline{x}$ =15.3 µm), single, tubular, filiform; basal appendage 3–12 µm long ($\overline{x}$ =7.3 µm), single, central, tubular, filiform.

Culture characteristics: Colonies on PDA 35 mm diameter after 7 days at 25 °C, circular, raised, dense surface with lobate edge, zonate with different sector light brown at the margin, brown at the center and; reverse brown at the margin, dark brown at the center.

Material examined: CHINA, Yunnan Province, Shangri-La, on a dead leaf of *Ilex* sp. (Aquifeliaceae), July 2014, R. Phookamsak, NI009 (HKAS 92492, **holotype**, MFLU 16–1429 **isotype**), ex-type living culture KUMCC 15–0520, MFLUCC 16–0829.

Notes: The new strain, *Monochaetia ilexae* clusters with *Monochaetia* species, but is separated from the main clade with high bootstrap support (100% ML, 98% MP, 1.00 PP, Fig. 1). *Monochaetia ilexae* shares similar morphology with the type, *M. monochaeta* (Guba 1961) in having fusiform conidia and has a similar range of conidia (20–27 µm) with *M. kansensis* (18–26 µm) (Guba 1961). However, *M. ilexae* differs from *M. monochaeta* as the former has fusiform, brown, 20–27 µm long conidia with a 6–24 µm long, single, apical appendage, whereas *M. monochaeta* has pale olivaceous, 15–21 µm long conidia, with a 5–19 µm long, single, apical appendage (Guba 1961). *Monochaetia kansensis* differs in its erect, slightly curved, olivaceous or umber conidia, with a 10–38 µm long, single, apical appendage.

In Table 2 we tabulate the morphological data for different *Monochaetia* species (Guba 1961). *Monochaetia* species have been recorded from range of host species, including *Alnus* sp., *Bellota* sp., *Camellia* sp., *Castanea* sp., *Corylus* sp., *Cryptomeria* sp., *Osyris* sp., *Pteridium* sp., *Quercus* sp., *Rosa* sp., *Russelia* sp., *Schinus* sp, (Guba 1961) and this is the first record from *Ilex* sp. in China (Guba 1961, Farr & Rossman 2016).

Monochaetia bicornis, *M. hysteriiformis*, *M. macropoda*, *M. miersii*, *M. monochaeta*, *M. osyrella* and *M. osyridella* have fusiform conidia, which are similar to those of *M. ilexae*. Among them, *M. hysteriiformis* and *M. osyridella* have overlapping conidial lengths with *M. ilexae*. However, *M. hysteriiformis* has umber-coloured conidia, with 19–24 µm median cells and *M. osyridella* has chestnut brown conidia, with 15–18 µm long, basal appendage, that differs from *M. illexae*. *Monochaetia alnea*, *M. cryptomeriae*, *M. kansensis*, *M. phyllostictea*, *M. rosae-caninae* and *M. schini* do not have fusiform conidia.

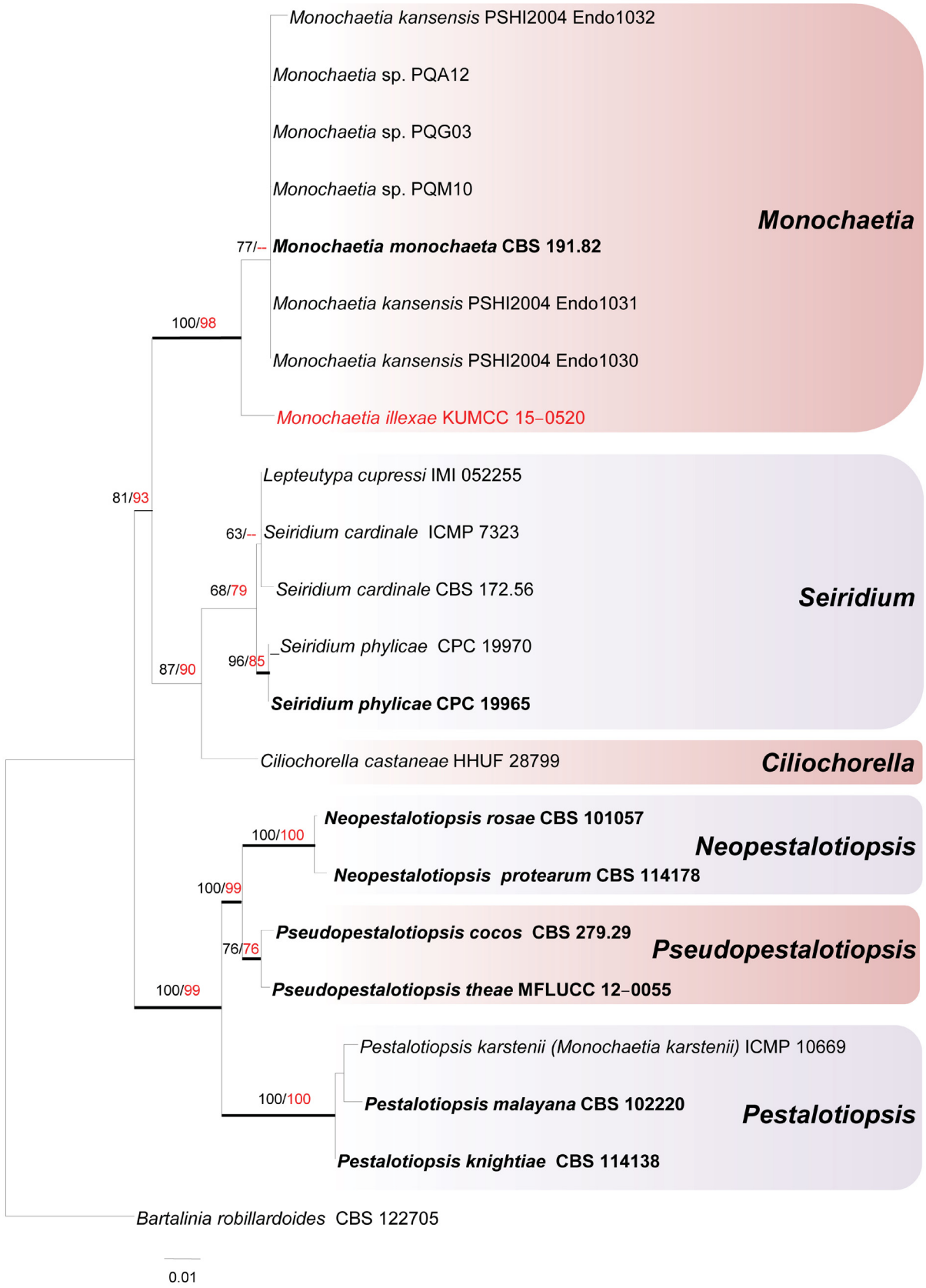

FIGURE 1. Phylogenetic tree resulting from maximum likelihood analysis of the combined LSU and ITS sequence alignment. Bootstrap values for maximum likelihood (ML, black) and maximum parsimony (MP, red) are shown at the nodes (ML/MP). Bayesian posterior probabilities, greater than 0.90 are indicated by thickened lines. The tree is rooted with *Bartalinia robillardoides* (CBS 122705). The new strain is indicated in red bold and type strains are indicated as black bold.

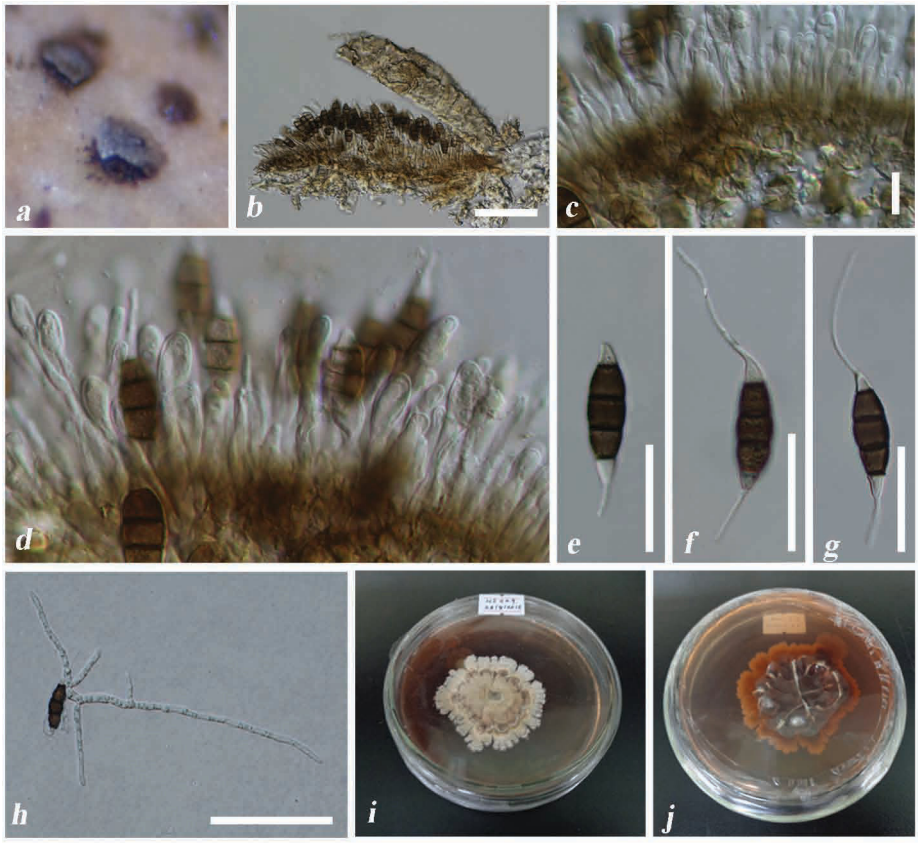

FIGURE 2. *Monochaetia ilexae* (HKAS 92492, **holotype)** a Conidiomata on the host. b, c Sections through conidiomata. d Conidia with conidiogenous cell. e–g Conidia. h Germinating spore. j Upper view of culture. k Lower view of culture. Scale bars: b, h = 50 µm, c = 10 µm, e–g = 20 µm.

TABLE 2. Synopsis of *Monochaetia ilexae* and related species

Monochaetia species	Conidia	Length of conidia (µm)	Width of conidia (µm)	Colour of median cells	Length of 3 median cells (µm)	Length of apical appenda-ge (µm)	Length of basal append-age (µm)	Host
M. alnea (Har. & Briard) Sacc. & D. Sacc.	ovate-elongate	16–20	6–8	brown	present	12–16	present	*Alnus glutinosa*
M. bicornis (Durieu & Mont.) Sacc. & D. Sacc.	narrow fusiform	13–18	4–5	pale olivaceous	9–13	4–15	3–16	*Quercus* sp.
M. camelliae Miles	-	18–20	4–4.5	dark olivaceous	present	12–14	present	*Camellia japonica*
M. concentrica (Berk. & Broome) Sacc. & D. Sacc.	-	20–26	6.5–8.5	dark or umber	15–19	15	present	*Castanea* sp., *Quercus* sp., *Corylus* sp.
M. cryptomeriae M. Wilson	fusoid	22–30	7.5–10	chocolate brown	present	20–32	4–20	*Cryptomeria japonica*
M. hysteriiformis (Berk. & M.A. Curtis) Guba	long fusiform	20–25	6.5–9.5	umber or darker	19–24	9–13	3–10	*Quercus* sp.

...*Continued on next page*

TABLE 2. (Cotninued)

Monochaetia species	Conidia	Length of conidia (μm)	Width of conidia (μm)	Colour of median cells	Length of 3 median cells (μm)	Length of apical appenda-ge (μm)	Length of basal append-age (μm)	Host
M. ilexae	fusiform	20–27	5–8	brown	13–18	6–24 usually 10–20	3–12	*Ilex* sp.
M. kansensis (Ellis & Barthol.) Sacc. & D. Sacc.	erect, slightly curved	18–26	6–8	olivaceous or umber	12–17	10–38 usually 15–26	3–15	*Castanea* sp., *Quercus* sp.
M. macropoda (Speg.) Allescher	fusiform	35–38	7	olivaceous	present	15	45–50	*Pteridium aquilinum*
M. miersii Speg.	fusiform, clavate-fusiform	30–35	7–10	dark	present	8–10	present	*Bellota miersii*
M. monochaeta (Desm.) Allescher	elliptic, or narrow fusiform	15–21	5–8	pale olivaceous	10–15	5–19	present	*Quercus* sp.
M. osyrella (Tassi) Sacc. & D. Sacc.	fusiform	18–20	7–8	yellowish	14–15	present	present	*Osyris alba*
M. osyridella Bubák	long-fusiform	18–29	5.5–6.5	chestnut brown	present	12–15.5	15–18	*Osyris alba*
M. phyllostictea (Sacc.) Allescher	elliptic fusoid	19–22	6–8	umber	14–16	5	present	*Rubus fruticosus*
M. rosae-caninae Unamuno	oblong	16–18	5–7	-	present	present	present	*Rosa canina*
M. russeliae Guba	elliptic-fusoid	20–23	6–9.5	chocolate coloured	15.5–19	9–13	present	*Russelia equisetiformis*
M. schini M.T. Lucas & Sousa da Camara	ellopsoid	20–23	8–9.5	brown	present	8–9.5	present	*Schinus* sp.

Discussion

The distinct morphology of the conidia is the main feature that initiated the interest to study *Monochaetia* species (Guba 1961, Maharachchikumbura *et al*. 2014). *Monochaetia* species are morphologically diverse in conidial morphology. The number and colour of median cells of conidia and other biometric measurements are used to differentiate and define *Monochaetia* species (Guba 1961).

Monochaetia ilexae, *Monochaetia monochaeta* (type species) and *Monochaetia kansensis* are 5-celled conidia forms that cluster together in the current phylogenetic analysis (Guba 1961). Guba (1961) designated a three section classification of *Monochaetia* species including, Quadriloculatae, Quinqueloculatae, and Sexloculatae for three-septate, four-septate, and five-septate conidia, respectively. According to Guba's classification, the main character he used to distinguish *Monochaetia* and *Pestalotiopsis* is that *Monochaetia* possess a single apical appendage, whereas *Pestalotiopsis* possess more than one apical appendage (Jeewon *et al*. 2002, 2003). *Monochaetia* and *Pestalotiopsis* show a close relationship because of similar conidial morphology of the euseptate nature of the three median cells (Jeewon *et al*. 2002). However previous studies have shown that they are phylogenetically distinct (Jeewon *et al*. 2002, 2003, Maharachchikumbura *et al*. 2014) and the current study is in agreement.

The presence of a single apical appendage is not a unique character for *Monochaetia* (Jeewon *et al*. 2002). Single apical appendages are also present in *Pestalotiopsis*, *Seiridium* and *Seimatosporium* species (Jeewon *et al*. 2002, Maharachchikumbura *et al*. 2014). *Monochaetia karstenii* has a single apical appendage (Jeewon *et al*. 2002). Nag Rag (1993) synonymized *Monochaetia karstenii* with *Pestalotiopsis karstenii* because of their similar conidial morphology, such as an apical appendage arising as a tubular extension from the apical cell and median cells having thin walls (Jeewon *et al*. 2002). Phylogenetic analyses of Jeewon *et al*. (2002) and the current study show *Monochaetia karstenii* clustering with *Pestalotiopsis* species.

According to the present phylogenetic analysis (Fig. 1), *Seridium* and *Ciliochorella* form a sister clade to *Monochaetia*. *Seridium* species share the character with *Monochaetia* in having a single apical appendage (Jeewon *et al*. 2002). The species also have different morphological characters. Conidia of *Seridium* are distoseptate, whereas in *Monochaetia* they are euseptate (Jeewon *et al*. 2002). Another difference between *Seridium* and *Monochaetia* is that *Seridium* has thick-walled median cells, whereas those of *Monochaetia* have a less elaborate structure with thinner-walled and lighter-colored median cells (Jeewon *et al*. 2002). These morphological differences are supported by the current phylogenetic analysis, showing separate clades for *Monochaetia* and *Seridium*. The sexual morph of *Seridium* species has been reported to be *Lepteutypa*, whereas no sexual morph is known for *Monochaetia* species (Jeewon *et al*. 2002).

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