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Morphology and multi-gene phylogeny reveal a new host record of *Cycasicola coffeae* species from *Artocarpus heterophyllus* in Yunnan, China

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Abstract

Jackfruit (*Artocarpus heterophyllus*) is a tropical fruit tree native to southwest India and is one of the most economically valuable tropical fruits. Jackfruit is affected by various fungal pathogens, but the saprobic and endophytic fungi have been poorly studied. To investigate saprophytic fungi, dead branches of jackfruit with fungal fruiting bodies in Yuxi, Yunnan Province, China, were collected in this study. Multi-gene phylogenetic analyses of SSU, ITS, LSU, *RPB2* and *TEF-1α* sequence data indicated that our collections belonged to *Cycasicola* and formed a sister branch with the species *Cycasicola coffeae* in *Cycasicola*. Thus, we describe our fungus as a new host record of *Cycasicola coffeae* based on the morphology and multi-gene phylogeny. This is the first report of Thyridariaceae species isolated from *Artocarpus heterophyllus* in China. A complete description, micrographs and a phylogenetic tree showing the placement of the new host records are provided.

Keywords – DNA, Jackfruit, one new host record, taxonomy, Thyridariaceae

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a large, evergreen plant of the mulberry family (Moraceae Gaudich) (Prakash et al. 2009). It is one of the most important fruits in the world, which produces a heavier yield than any other tree species, and bears the largest known edible fruit (up to 35 kg) (Mao et al. 2007, Prakash et al. 2009). In addition to the edible value of the fruit, *A. heterophyllus* has important medicinal and other values; for example, the polyphenols in its rind can be used for the

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treatment of hyperglycaemia and as antioxidants (Mao et al. 2007, Prakash et al. 2009, Fang & Zhang 2018). Jackfruit is native to the Western Ghats of India and mainly distributed in tropical and subtropical areas, for example, Australia, Brazil, India, Kenya, Malaysia, Mauritius, Myanmar, Sri Lanka, Southern China, Thailand, and Uganda (Mao et al. 2007, Prakash et al. 2009, Powo 2023). Jackfruit has been cultivated for thousands of years in China as an important tropical fruit and is widely grown in the southern parts of Fujian, Guangdong, Guangxi, Hainan, Sichuan, and Yunnan (Mao et al. 2007).

Several fungal pathogens cause diseases in *Artocarpus heterophyllus* (Prakash et al. 2009). For example, *Phyllosticta artocarpi* Speg. and *Phyllostictina artocarpina* (Syd., P. Syd. & E.J. Butler) Syd. cause leaf spots; *Ganoderma applanatum* (Pers.) Pat. causes white mottled rot and *Fomes lamaoensis* (Murrill) Sacc. & Trotter causes brown root rot (Spaulding 1961, Mathur 1979, Alfieri et al. 1984, Farr et al. 2021). Some of the earliest reports of fungi from *A. heterophyllus* were published by Johnston (1960) and Spaulding (1961) in India and Malaysia. According to the USDA, approximately 75 fungal species have been reported from *A. heterophyllus* worldwide, mostly as saprobes (Farr et al. 2021).

The class Dothideomycetes O.E. Erikss. & Winka was introduced by Eriksson & Winka (1997) as one of the most important and largest classes in the Ascomycota (Nannfeldt 1932, Luttrell 1955, Janex-Favre 1971, Reynolds 1971, Barr 1979a, b, Eriksson 1981, Tehler 1990, 1995, Letrouit-Galinou et al. 1994, Barr & Huhndorf 2001, Liu & Hall 2004, Hongsanan et al. 2020a), and was estimated to consist of more than 19,000 species (Kirk et al. 2008, Hongsanan et al. 2020a). The members of Dothideomycetes are important pathogens, endophytes and saprobes distributed worldwide (Del Prado et al. 2006, Schoch et al. 2009, Hyde et al. 2013, Hongsanan et al. 2020a, b, Wijayawardene et al. 2020). Wijayawardene et al. (2020) listed 39 orders and 211 families in Dothideomycetes. Hongsanan et al. (2020a, b) provided an overall phylogenetic tree of families in Dothideomycetes based on a combined analysis of LSU, SSU, *RPB2* and *TEF1-α*. Subsequent studies introduced six new orders and eight families in Dothideomycetes (Abdollahzadeh et al. 2020, Haridas et al. 2020, Hernández-Restrepo et al. 2020). According to the latest research, the class Dothideomycetes consists of 52 orders and 223 families (Wijayawardene et al. 2022).

Pleosporales Luttr. ex M.E. Barr was introduced by Barr (1987) as the largest order in Dothideomycetes and accounted for one-fourth of all dothideomycetous species (Hyde et al. 2013, Liu *et al.* 2017, Hongsanan et al. 2020a). Pleosporales species are important plant epiphytes, saprobes, endophytes, parasites, hyperparasites on fungi or insects, and lichenized fungi distributed worldwide (Zhang et al. 2012, Hyde et al. 2013, Wanasinghe et al. 2018, Hongsanan et al. 2020a, Mapook et al. 2020). Hongsanan et al. (2020a) showed that Pleosporales diverged 205 million years ago (MYA) and accepted 91 families in Pleosporales. Wijayawardene et al. (2022) listed 91 families and 655 genera in Pleosporales.

Thyridariaceae Q. Tian & K.D. Hyde was introduced by Hyde et al. (2013) to accommodate *Thyridaria* Saccas. Jaklitsch & Voglmayr (2016) introduced Roussoellaceae Jian K. Liu, Phook., D.Q. Dai & K.D. Hyde, which was synonymized with Thyridariaceae, and a new genus *Parathyridaria* Jaklitsch & Voglmayr was introduced in the family. However, Tibpromma et al. (2017) reinstated Roussoellaceae based on morphology and phylogenetic analyses based on LSU, ITS, *TEF-1a* and *RPB2*, following Liu et al. (2014) and treated it as a well-resolved family in Pleosporales. Then, many new genera and new species were introduced from 2018 to 2022 (Devadatha et al. 2018, Wanasinghe et al. 2018, Phookamsak et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Poli et al. 2020,

Boonmee et al. 2021, Wanasinghe & Mortimer 2022). Since the introduction of Thyridariaceae by Hyde et al. (2013), 65 species have been accepted across eight genera, viz., *Chromolaenomyces* Mapook & K.D. Hyde (one species), *Cycasicola* Wanas., E.B.G. Jones & K.D. Hyde (two species), *Liua* Phookamsak & K.D. Hyde (one species), *Parathyridaria* (12 species), *Parathyridariella* Prigione, A. Poli, E. Bovio & Varese (one species), *Pseudothyridariella* Mapook & K.D. Hyde (two species), *Thyridaria* (52 species), and *Thyridariella* Devadatha, V.V. Sarma, K.D. Hyde, D.N. Wanas. & E.B.G. Jones (two species) (Devadatha et al. 2018, Wanasinghe et al. 2018, Phookamsak et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Poli et al. 2020, Boonmee et al. 2021, Wanasinghe & Mortimer 2022, Wijayawardene et al. 2022). The divergence time for Thyridariaceae is estimated to have a crown age of 15 (3–38) millions of years ago (MYA) in the Cenozoic period and a stem age of 95 (60–138) millions of years ago (MYA) in the Cretaceous, based on molecular clock methods (Liu et al. 2017). Most Thyridariaceae species are reported as saprobes or endophytes from *Acer* sp., *Avicennia* sp., *Ephedra* sp., *Fagaceae* sp., *Leucaena* sp., *Lonicera* sp., *Ribes* sp., *Sambucus* sp., and *Ulmus* sp. (Ahmed et al. 2014, Jaklitsch & Voglmayr 2016, Tibpromma et al. 2017, Crous et al. 2018, Wanasinghe et al. 2018, Hongsanan et al. 2020a). Some Thyridariaceae species might be the pathogens; for example, *Parathyridaria percutanea* (Abd. Ahmed, D.A. Stevens, Sande & de Hoog) Jaklitsch & Voglmayr causes human subcutaneous mycoses (Ahmed et al. 2014a, b, Hongsanan et al. 2020a).

Cycasicola was established by Wanasinghe et al. (2018), with *C. goaensis* Wanas., E.B.G. Jones & K.D. Hyde as the type species. This genus is characterized by solitary, gregarious or confluence, immersed, unilocular, globose conidiomata, thick-walled cells of *textura angularis* composed of brown peridium, phialidic, ampulliform, hyaline, smooth-walled conidiogenous cells, and hyaline or pale brown, smooth-walled, fusiform to cylindrical, straight or slightly curved, guttulate, aseptate conidia, obtuse at the apex and base, and sometimes slightly truncated at the base (Wanasinghe et al. 2018). Currently, there are three species (*C. goaensis, C. leucaenae* Jayasiri, E.B.G. Jones & K.D. Hyde and *C. coffeae* L. Lu & Tibpromma) in *Cycasicola*, introduced based on morphology and multi-gene phylogeny of SSU, ITS, LSU, *RPB2* and *TEF-1α*, which were saprobic on *Cycas* sp. and *Leucaena* sp. and distributed in China, India and Thailand (Wanasinghe et al. 2018, Jayasiri et al. 2019, Lu et al. 2024).

In this study, we introduce a new host record, *Cycasicola coffeae*, isolated from the dead branch of *Artocarpus heterophyllus* in Yuxi, Yunnan Province in China. The new record was confirmed based on the morphological and multi-gene phylogenetic analyses.

Materials and methods

Sample collection and isolation

Specimens with fungal fruiting bodies were collected from Yuxi, Yunnan Province, China, in September 2022, stored in sealable plastic bags and taken to the mycology laboratory in the Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Yunnan, China. The specimens were later examined under a stereomicroscope (OLYMPUS SZ61). Single spore isolation and morphological observations were carried out following Senanayake et al. (2020). Morphological structures were carried out using an OPTEC SZ650 dissecting stereomicroscope and photographed using an OLYMPUS SZ65 and Nikon camera series EOS 600D (Canon, Japan) microscope imaging system. Adobe Photoshop CS3 Extended version 10.0 software was used to measure and process the photo plates. Germinated

ascospores were aseptically transferred to potato dextrose agar (PDA) plates, obtained pure cultures, and incubated at room temperature (25 ℃) in the constant temperature incubator. Herbarium specimens were deposited at the Zhongkai University of Agriculture and Engineering (ZHKU), China, and living cultures were deposited at the culture collection of Zhongkai University of Agriculture and Engineering (ZHKUCC), China. Index Fungorum numbers were registered as instructed in Index Fungorum (2024), and Facesoffungi numbers were registered as described in Jayasiri et al. (2015). The species descriptions and illustrations were deposited in the GMS Microfungi database (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) was used to extract DNA from pure fungal mycelia (25 days old culture), following manufacturer protocol. The extracted DNA was maintained at -20 ℃ for long-term storage. The polymerase chain reactions (PCR) were performed for five genes, small subunits ribosomal RNA (SSU), internal transcribed spacer (ITS), nuclear large subunit region (LSU), RNA polymerase II subunit 2 (*RPB2*) and translation elongation factor 1-alpha (*TEF-1α*) gene. The SSU, ITS, LSU, *RPB2* and *TEF-1α* genes were amplified using the primers, NS1 and NS4 (White et al. 1990), ITS5 and ITS4 (White et al. 1990), LR0R and LR7 (Vilgalys & Hester 1990), fRPB2-5F and fPB2-7R (Liu et al. 1999), and EF1-983F and EF1-2218R (Rehner & Samuels 1994), respectively.

Amplification reactions were performed in a 25 μL reaction volume, composed of 2 μL DNA, 12.5 μL 2×FastTaq Premix (a mixture of FastTaq TM DNA Polymerase, buffer, dNTP Mixture, and stabilizer) (Beijing Qingke Biological Technology Co., Ltd., Beijing, P.R. China), 1 μL of each reverse and forward primers (Beijing Kinco Biotechnology Co., Ltd. Kunming Branch, P.R. China) and 8.5 μL double distilled water (ddH_2O). For SSU, ITS, LSU and *TEF-1α*, the following conditions were used: initial denaturation of 2 min at 94 ℃, followed by 35 cycles of denaturation at 95 ℃ for 30 sec, primer annealing at 55 ℃ for 50 sec, primer extension at 72 ℃ for 90 sec and a final extension at 72 ℃ for 10 minutes. For the *RPB2* region, PCR amplification conditions were as follows: initial denaturation at 94 ℃ for 2 minutes, followed by 35 cycles of denaturation at 95 ℃ for 45 sec, primer annealing at 57 ℃ for 50 sec, primer extension at 72 ℃ for 90 sec, and a final extension of 10 minutes at 72 ℃. The quality of PCR products was checked on 1% agarose gel electrophoresis stained with TS-GelRed (TSJ002, Beijing Kinco Biotechnology Co., Ltd. Kunming Branch, P.R. China). Purification and sequencing of PCR products were performed by Tsingke Biological Engineering Technology and Services Co., Ltd (Yunnan, P.R. China).

Phylogenetic analyses

Phylogenetic analyses followed the methods in Dissanayake et al. (2020). Newly generated sequence data were blasted to select the closest matches through a BLAST search in GenBank (http://www.ncbi.nlm.nih.gov). Sequences generated from this study were obtained from GenBank and recent publications (Jaklitsch & Voglmayr 2016, Wanasinghe et al. 2018, Jayasiri et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Poli et al. 2020, Das et al. 2021, Mattoo & Nonzom 2022). Forward and reverse sequences were assembled using Geneious Pro.v4.8.5. Single gene sequence datasets were aligned with MAFFT V. 7.215 (https://mafft.cbrc.jp/alignment/server/; Katoh & Standley 2016), trimmed in Trimal.v1.2rev59 and edited manually where necessary in BioEdit V.7.0.5.2 (Hall 1999). The sequence datasets were combined using SequenceMatrix and AliView or

BioEdit V.7.0.5.2 (Hall 1999). FASTA datafile formats were transferred to PHYLIP and NEXUS formats by the online tool available on the ALTER website (http://sing.ei.uvigo.es/ALTER/) (Glez-Peña et al. 2010). Phylogenetic trees were based on Randomized Accelerated Maximum Likelihood (ML) and Bayesian inference analyses (BI).

Maximum likelihood trees were generated via RAxML-HPC BlackBox (8.2.4) (Stamatakis 2006, 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using the GTR+I+G model of evolution. BI analysis was conducted with MrBayes v. 3.1.2 (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform to evaluate posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (MCMC). The GTR+I+G evolution model was also applied in the BI analyses for all gene regions. Six simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled at every 100th generation. The resulting trees were visualized in FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and annotated in Microsoft PowerPoint 2019. The ML bootstrap supports greater than or equal to 70%, and Bayesian posterior probabilities greater than or equal to 0.90 are shown in the tree and presented under each relevant description. Sequence data of the new strains generated in this study are deposited in the GenBank, and the accession numbers are listed in Table 1.

Table 1. Names, strain numbers and corresponding GenBank accession numbers of the taxa included in the phylogenetic analyses.

Species	Strain	ITS	LSU	SSU	*TEF*-1*α*	*RPB2*
Arthopyrenia salicis	CBS 368.94	KF443410	AY538339	AY538333	KF443404	KF443397
Chromolaenomyces appendiculatus	MFLUCC 17-1455 [T]	NR_168862	NG_068705	MT214394	MT235770	MT235806
Cycasicola coffeae	ZHKUCC 23-0640	OR428409	OR428431	OR428387	OR509761	_
Cycasicola coffeae	ZHKUCC 23-0641 [T]	OR428410	OR428432	OR428388	OR509762	_
Cycasicola coffeae	**ZHKUCC 23-1024**	**PP734606**	**PP734602**	**PP734604**	**PP728355**	**PP728353**
Cycasicola coffeae	**ZHKUCC 23-1025**	**PP734607**	**PP734603**	**PP734605**	**PP728356**	**PP728354**
Cycasicola goaensis	MFLUCC 17-0754 [T]	MG828885	MG829001	MG829112	MG829198	–
Cycasicola goaensis	MFLU 17-0581	NR_157510	NG_059057	–	–	–
Cycasicola leucaenae	MFLUCC 17-0914 [T]	NR_163322	NG_070071	NG_065771	MK360046	MK434900
Cycasicola leucaenae	C215	MK347726	MK347942	MK347833	–	–
Liua muriformis	KUMCC 18-0177 [T]	MK433599	MK433598	MK433595	MK426798	MK426799
Neoroussoella alishanense	AKW 11 FU31018	MK503818	MK503824	MK503830	MK336182	MN037757
Neoroussoella alishanense	AKW 03 FU31016 [T]	MK503816	MK503822	MK503828	MK336181	MN037756
Neoroussoella entadae	MFLUCC 18-0243	MK347786	MK348004	MK347893	MK360065	MK434866
Neoroussoella leucaenae	MFLUCC 18-1544 [T]	MK347767	MK347984	MK347874	MK360067	MK434876
Occultibambusa bambusae	MFLUCC 11-0394	KU940124	KU863113	–	KU940194	KU940171
Occultibambusa bambusae	MFLUCC 13-0855 [T]	KU940123	KU863112	KU872116	KU940193	KU940170
Ohleria modesta	MGC	KX650562	KX650562	–	KX650533	KX650582
Ohleria modesta	OM	KX650563	KX650563	KX650513	KX650534	KX650583
Parathyridaria clematidis	MFLUCC 17-2154	MT310645	MT214601	MT226712	MT394657	MT394712
Parathyridaria clematidis	MFLUCC 17-2157	MT310644	MT214600	MT226711	MT394656	MT394711
Parathyridaria clematidis	MFLUCC 17-2160	MT310643	MT214599	MT226710	MT394655	MT394710
Parathyridaria clematidis	MFLUCC 17-2185 [T]	MT310642	MT214598	NG_070668	MT394654	MT394709
Parathyridaria ellipsoidea	KNU-JJ-1829 [T]	LC552950	LC552952	–	–	–
Parathyridaria flabelliae	MUT 4886	KR014358	KP671720	KT587317	MN605910	MN605930
Parathyridaria flabelliae	MUT 4859 [T]	KR014355	KP671716	KT587315	MN605909	MN605929
Parathyridaria percutanea	CBS 128203	KF322117	KF366448	KF366450	KF407988	KF366453
Parathyridaria percutanea	CBS 868.95 [T]	KF322118	KF366449	KF366451	KF407987	KF366452
Parathyridaria philadelphi	CBS 143432 [T]	MH107905	NG_063958	–	–	–
Parathyridaria ramulicola	MUT 4397	KC339235	KF636775	MN556311	MN605913	MN605933
Parathyridaria ramulicola	CBS 141479 [T]	NR_147657	KX650565	KX650514	KX650536	KX650584

Parathyridaria robiniae	MUT 2452	MG813183	MG816491	MN556312	MN605903	MN605923
Parathyridaria robiniae	MUT 4893	KM355998	MN556328	KM355993	MN605904	MN605924
Parathyridaria robiniae	MFLUCC 14-1119 [T]	KY511142	KY511141	–	KY549682	–
Parathyridaria rosae	MFLU 17-0623 T	NR_157530	NG_059873	–	–	–
Parathyridaria serratifoliae	MFLUCC 17-2210 [T]	MT310646	MT214602	NG_070669	MT394658	MT394713
Parathyridaria tyrrhenica	MUT 4966	KR014366	KP671740	KT587309	MN605911	MN605931
Parathyridaria tyrrhenica	MUT 5371 [T]	KU314951	MN556329	KU314952	MN605912	MN605932
Parathyridaria virginianae	MFLUCC 17-2163 [T]	MT310647	NG_073853	NG_070670	MT394659	MT394714
Parathyridariella dematiacea	MUT 4419	KC339245	KF636786	MN556313	MN605905	MN605925
Parathyridariella dematiacea	MUT 5310	KU255057	MN556330	MN556314	MN605907	MN605927
Parathyridariella dematiacea	MUT 5381	KU314959	MN556331	KU314960	MN605908	MN605928
Parathyridariella dematiacea	MUT 4884 [T]	MN556317	KP671726	KT587329	MN605906	MN605926
Pseudothyridariella chromolaenae	MFLUCC 17-1472 [T]	NR_168863	NG_068706	MT214395	MT235771	MT235807
Pseudothyridariella mahakoshae	NFCCI 4215 [T]	MG020435	MG020438	MG020441	MG023140	MG020446
Roussoella hysterioides	CBS 546.94 [T]	KF443405	KF443381	AY642528	KF443399	KF443392
Roussoella intermedia	CBS 170.96	KF443407	KF443382	KF443390	KF443398	KF443394
Roussoella japanensis	MAFF 239636 [T]	KJ474829	AB524621	AB524480	AB539114	AB539101
Roussoella margidorensis	MUT 5329 [T]	KU314944	MN556322	MN556309	MN605897	MN605917
Roussoella mediterranea	MUT 5306 [T]	KU255054	MN556323	MN556310	MN605898	MN605918
Roussoella padinae	MUT 5341	KU158153	MN556325	KU158176	MN605900	MN605920
Roussoella pustulans	KT 1709	KJ474830	AB524623	AB524482	AB539116	AB539103
Roussoella siamensis	MFLUCC 11-0149 [T]	KJ474837	KJ474845	KU872125	KJ474854	KJ474861
Thyridaria acaciae	CBS 138873 T	KP004469	KP004497	–	–	–
Thyridaria aureobrunnea	MFLUCC 21-0090 [T]	MZ538528	MZ538562	–	–	–
Thyridaria broussonetiae	TB [T]	KX650567	KX650567	–	KX650538	KX650585
Thyridaria broussonetiae	TB1a	KX650569	KX650569	–	–	–
Thyridaria broussonetiae	TB2	KX650570	KX650570	–	KX650540	KX650587
Thyridaria broussonetiae	TB1	KX650568	KX650568	KX650515	KX650539	KX650586
Thyridaria jonahhulmei	KUMCC 21-0816 [T]	ON007041	ON007037	ON007046	ON009131	ON009135
Thyridaria jonahhulmei	KUMCC 21-0817	ON007042	ON007038	ON007047	ON009132	ON009136
Thyridariella mangrovei	NFCCI 4214	MG020436	MG020439	MG020442	MG020444	MG020447
Thyridariella mangrovei	NFCCI 4213 [T]	MG020434	MG020437	MG020440	MG020443	MG020445
Torula herbarum	CBS 111855	KF443409	KF443386	KF443391	KF443403	KF443396
Torula herbarum	CBS 595.96 [T]	KF443408	KF443385	KF443387	KF443402	KF443395

— indicates data unavailability; T indicates the ex-type; newly generated sequences are in bold.

Results

Phylogenetic analyses

The combined SSU, ITS, LSU, *RPB2* and *TEF-1α* dataset comprised sequences from 65 strains representing 39 taxa, including Ohleriaceae Jaklitsch & Voglmayr (two strains, one taxa), Roussoellaceae (13 strains, 12 taxa), Thyridariaceae (44 strains, 24 taxa), Torulaceae Corda (two strains, one taxa), and the outgroup taxa of *Occultibambusa bambusae* D.Q. Dai & K.D. Hyde (MFLUCC 11-0394 and MFLUCC 13-0855).

The RAxML analysis of the combined dataset yielded a best-scoring tree (Fig. 1) with a final ML optimization likelihood value of -30369.626325. The matrix had 1615 distinct alignment patterns, with 17.11% of undetermined characters or gaps. Parameters for the GTR+I+G model of the combined ITS, *TEF-1α* and *TUB* were as follows: Estimated base frequencies; A = 0.247883, C = 0.257410, G = 0.26684, T = 0.228024; substitution rates AC = 1.499502, AG = 3.832147, AT = 1.645291, CG = 0.982750, CT = 7.568015, GT = 1.000000; proportion of invariable sites I = 0.435011; gamma distribution shape parameter α = 0.400126.

Four families (Ohleriaceae, Roussoellaceae, Thyridariaceae and Torulaceae) of Pleosporales formed well-separated and well-supported clades (Fig. 1). The topology structure of ML and BI analyses (Fig. 1) is consistent with the phylogenetic results of Poli et al. (2020) and other phylogenies of Thyridariaceae (Mapook et al. 2020, Phukhamsakda et al. 2020, Wanasinghe & Mortimer 2022). Our new strains (ZHKUCC 23-1024 and ZHKUCC 23-1025) grouped with *Cycasicola Coffeae* (ZHKUCC 23-0640 and ZHKUCC 23-0641 (Type)), regarded as a new host record.

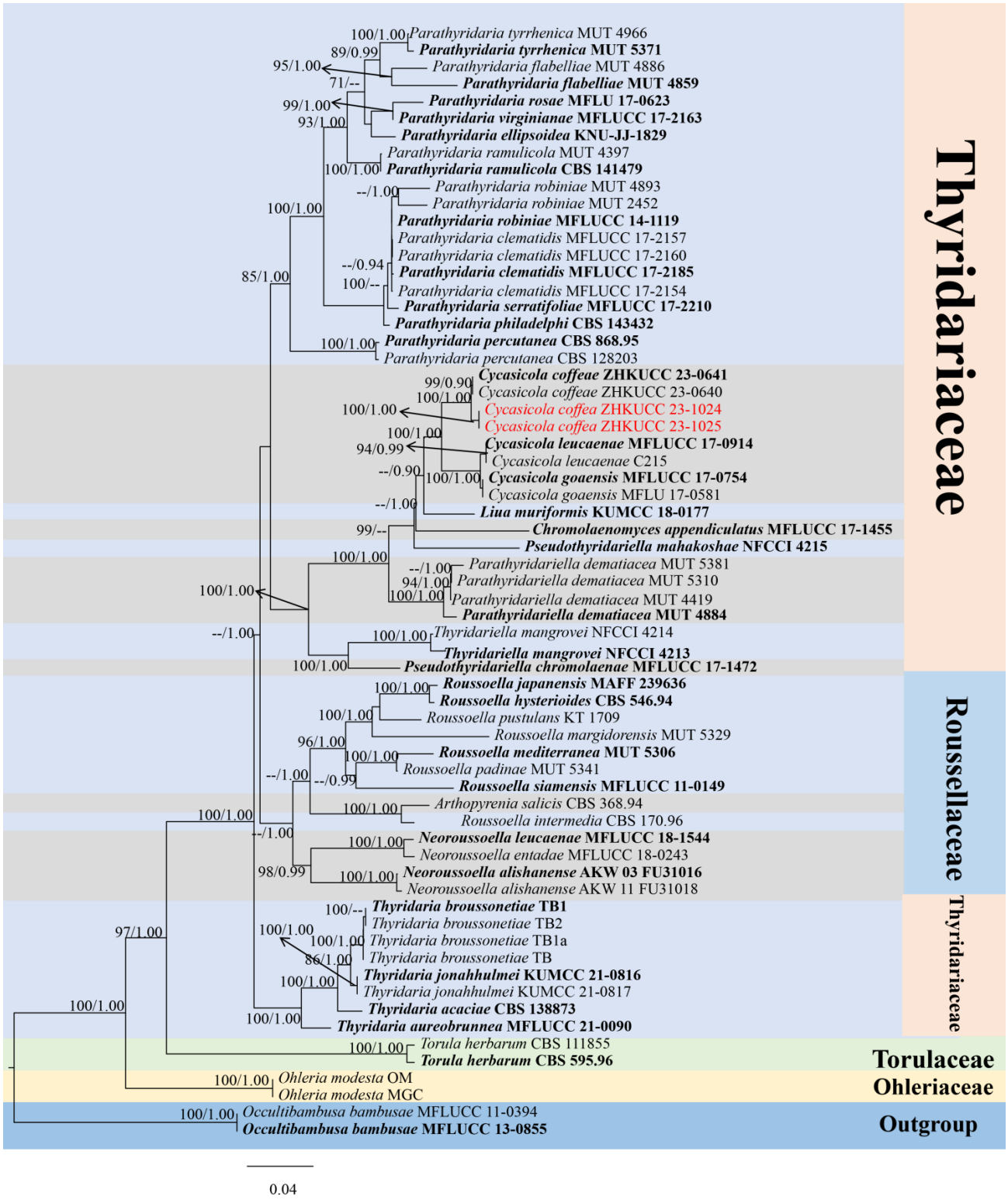

Figure 1. RAxML tree based on a combined dataset of SSU, ITS, LSU, *RPB2* and *TEF-1α* partial sequences. Bootstrap support values for maximum likelihood (ML) equal to or higher than 70% and Bayesian probability (BYPP) equal to or higher than 0.90 are mentioned above the branches (ML/BI). Newly generated sequences are shown in red, and type strains are in bold.

Taxonomy

Cycasicola coffeae L. Lu and Tibpromma, Mycology X: XX (2024), **Figs. 2, 3**

Index Fungorum Number: IF 901007; Facesoffungi Number: FoF 14733

Saprobic on a branch of jackfruit (*Artocarpus heterophyllus*). **Asexual morph**: Coelomycetous. *Conidiomata* 150–400 × 100–350 µm, semi-immersed, solitary or gregarious, globose to subglobose, uni or muti-locule, black to brown, shiny, without hair, smooth-wall. *Conidiomatal wall* 16–55 µm wide, outer layer composed of multi-layered, brown to light brown cells of *textura angularis*, lined with a hyaline layer bearing conidiogenous cells, or reduced to conidiogenous cells. *Conidiophores* single, short, densely aggregated, straight or flexuous, cylindrical, branched, hyaline, septate, smooth-walled. *Conidiogenous cells* 2–15.5 × 0.5–5 µm ($\bar{x}$ = 7.03 × 2.76 µm; n = 60), holoblastic, phialidic, ampulliform to cylindrical, unbranched, hyaline, and smooth-walled. *Conidia* 2–8 × 1–4 µm ($\bar{x}$ = 5.26 × 2.55 µm; n = 60), oval or subglobose, slightly curved, smooth-walled, 1–3 (–5)-granulated, hyaline when immature, pale brown at maturity, aseptate, without out a sheath. **Sexual morph**: Undetermined.

Culture characters – Cultures on PDA, colonies slow-growing, raised at centre, radially sulcate, umbonate, smooth, brown edges, dark brown. *Mycelia* 2.5–6 µm wide, hyaline to brown, septate; *conidiomata* on the PDA pycnidial, 200–400 × 180–350 µm, semi-immersed, solitary, gregarious, glabrous, variable in shapes and sizes, mostly globose to subglobose, black to brown; *conidiogenous cells* 3–15 × 1–7 µm ($\bar{x}$ = 7.78 × 3.51 µm; n = 30), holoblastic, phialidic, discrete, determinate, ampulliform to subglobose, unbranched, aseptate, hyaline, smooth; *conidia* 3.5–6 × 1–3.5 µm ($\overline{x}$ = 4.78 × 2.49 µm, n = 60), oval to subglobose, 1–3 (–5)-granulated, hyaline when immature, pale brown at maturity, aseptate, smooth-walled (Fig. 3).

Material examined – CHINA, Yunnan Province, Yuxi, Yuanjiang County, on a dead branch of *Artocarpus heterophyllus*, 18 September 2022, Xian Zhang, YY1 (MHZU 23-0277, living culture ZHKUCC 23-1024; *ibid.* YY1-B, living culture ZHKUCC 23-1025.

Notes – *Cycasicola coffeae* was introduced by Lu et al. (2024) from a branch of coffee in China, Yunnan Province, Dali City. Our new isolates form a sister clade to *C. coffeae* in the phylogenetic analyses, with high statistical support (100 ML/1.00 BYPP, Fig. 1). The morphology of the new isolate (MHZU 23-0277) is very similar to the holotype *C. coffeae* (ZHKUCC 23-0640) in size and color of conidia (compared in Table 3). Based on blast results, search results of sequence data, ITS, LSU, SSU and *TEF*-1α are similar to *C. cofffeae* (ZHKUCC 23-0640, 98.3%, 99.77%, 99.81%, 99.67%), respectively. Based on both morphology and phylogenetic analyses, Therefore, the new isolate (MHZU 23-0277) is identified as *C. coffeae,* and this is a new host record of *C. coffeae*, isolated from on a dead branch of *Artocarpus heterophyllus.*

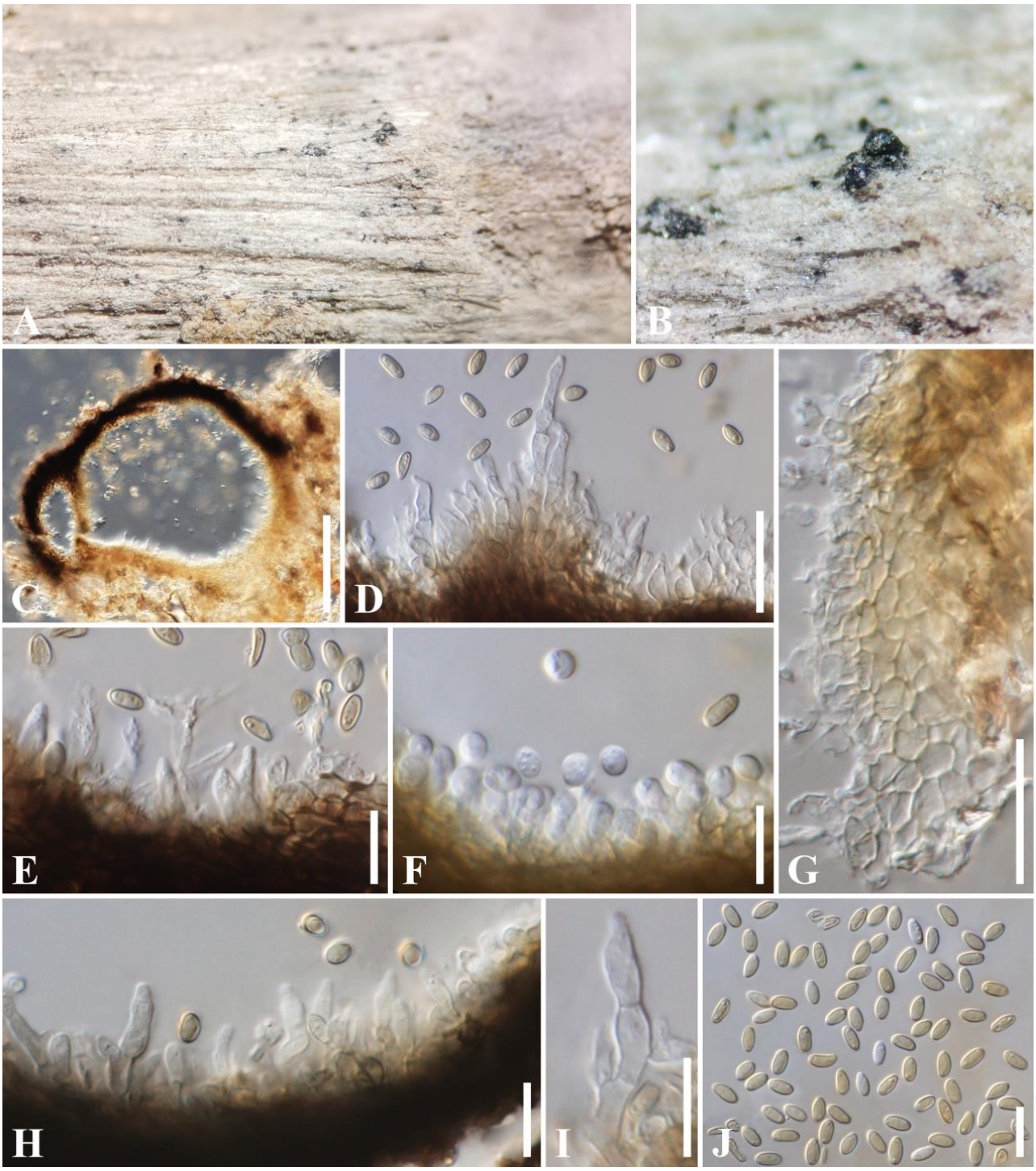

Figure 2. *Cycasicola coffeae* (MHZU 23-0277). A, B Conidiomata on the substrate. C Vertical section through a conidioma. D–F, H Conidiogenous cells and conidia. G Section of a conidioma wall. I Conidiogenous cells. J Conidia. Scale bars: C = 100 μm, D, G = 20 μm, E, F, H–J = 10 μm.

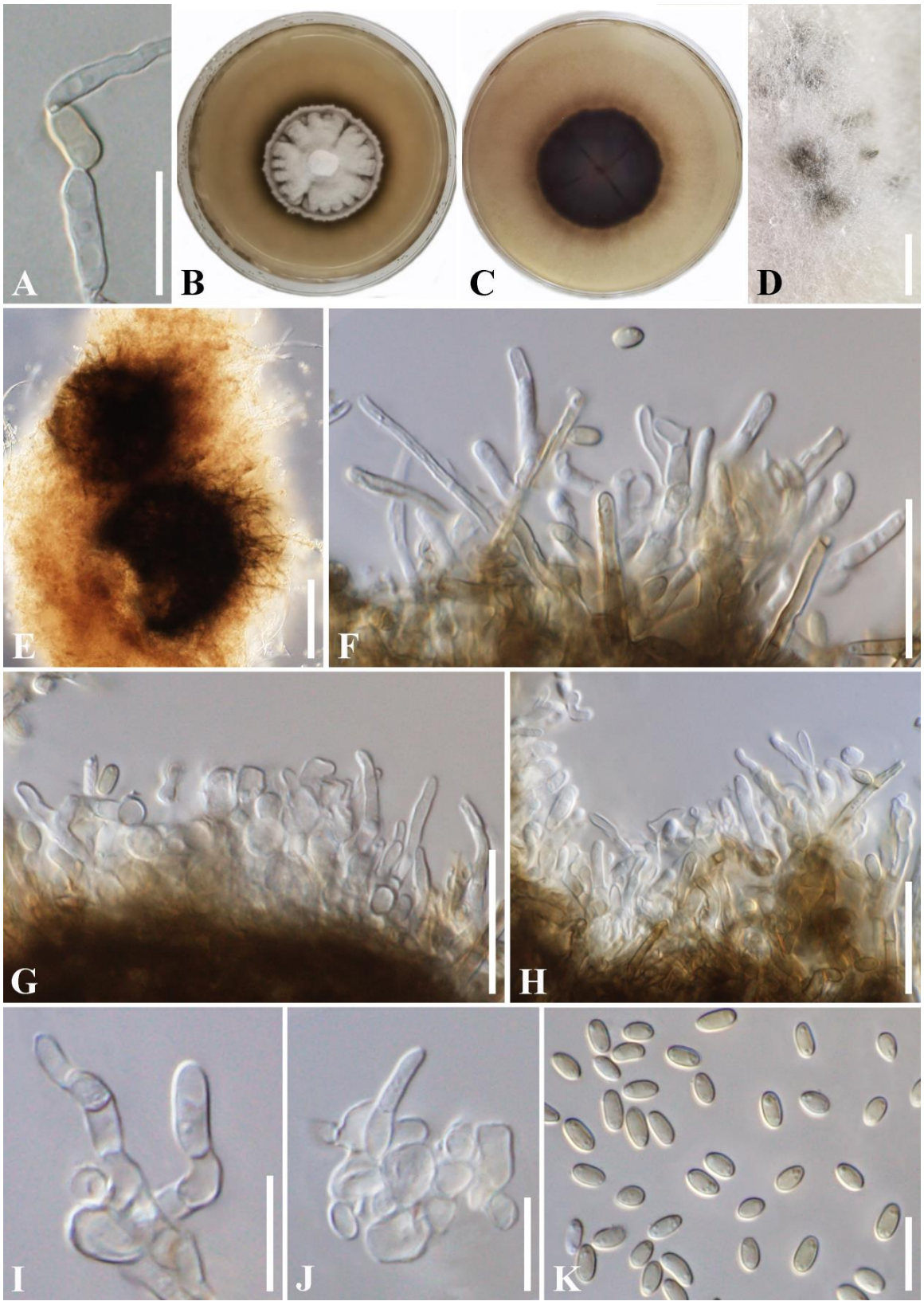

Figure 3. *Cycasicola coffeae* (ZHKUCC 23-1024) on PDA. A A germinated conidium. B, C Upper and lower view of cultures on the PDA. D Conidiomata on PDA medium. E Conidiomata. F–H Conidiogenous cells and conidia. I–J Conidiogenous cells. K Conidia. Scale bars: A, I–K = 10 μm, D = 500 μm, E = 100 μm, F–H = 20 μm.

Discussion

Artocarpus heterophyllus appears to be a highly susceptible host for fungi, as many have been reported on this host. However, these reports were mainly published before the year 2000, thus, many fungal names are incorrect. The need for an updated checklist of fungal species associated with *A. heterophyllus* is fulfilled in this study (Table 2). According to previous reports, 87 fungi species and six fungus-like organisms associated with *A. heterophyllus* belonging to four phyla (Ascomycota, Basidiomycota, Mucoromycota and Oomycota) have been reported worldwide (Prakash et al. 2009, Borines et al. 2013, Farr et al. 2021). There are approximately 37 pathogens among the 93 known fungi and fungus-like organisms isolated from *A. heterophyllus*, causing leaf spots, stems, fruits, male inflorescence rots, gray blight, and pink disease (Table 2) (Johnston 1960, Spaulding 1961, Mathur 1979, Alfieri et al. 1984, Prakash et al. 2009, Farr et al. 2021). The highest numbers of fungal species isolated from *A. heterophyllus* were reported from China, India and Thailand, followed by Brazil, Malaysia, USA, Sierra Leone and Sri Lanka (Johnston 1960, Spaulding 1961, Mathur 1979, Alfieri et al. 1984, Prakash et al. 2009, Borines et al. 2013, Farr et al. 2021). The fungal families most frequently reported from *A. heterophyllus* belong to Glomerellaceae Locq. ex Seifert & W. Gams (13 species), followed by Mycosphaerellaceae Lindau (6 species), Botryosphaeriaceae Theiss. & Syd. (5 species), Nectriaceae Tul. & C. Tul. (5 species) and Peronosporaceae de Bary (5 species) (Table 4) (Spaulding 1961, Mathur 1979, Alfieri et al. 1984, Prakash et al. 2009, Borines et al. 2013, Farr et al. 2021).

Thyridariaceae is usually a saprophyte on dead wood and is mainly distributed in Asia (Ahmed et al. 2014, Jaklitsch & Voglmayr 2016, Tibpromma et al. 2017, Crous et al. 2018, Wanasinghe et al. 2018, Hongsanan et al. 2020a, Farr et al. 2021). Since its establishment in 2013, an increasing number of new record have been introduced into this family (Ahmed et al. 2014, Jaklitsch & Voglmayr 2016, Tibpromma et al. 2017, Crous et al. 2018, Wanasinghe et al. 2018, Hongsanan et al. 2020a). In this study, we introduce a host record, *Cycasicola coffeae*, this is the first report of a *Cycasicola* or even Thyridariaceae taxon isolated from *Artocarpus heterophyllus*.

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TABLE 2. Fungal and fungus-like organisms isolated from *Artocarpus heterophyllus*.

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
Ascomycota	Amphisphaeriaceae	*Ellisiopsis*	*Ellisiopsis gallesiae*	*Beltraniella portoricensis*	Sierra Leone	—	Pirozynski (1963)
	Anteagloniaceae	*Glonium*	*Glonium abbreviatum*	*Anteaglonium abbreviatum*	India	—	Pande (2008)
	Aplosporellaceae	*Aplosporella*	*Aplosporella artocarpi*	*Aplosporella artocarpi*	Thailand	—	Trakunyingcharoen et al. (2015), Zhu et al. (2018)
	Beltraniaceae	*Beltrania*	*Beltrania rhombica*	*Beltrania rhombica*	India and Sierra Leone	—	Heredia-Abarca (1994), Pirozynski & Patil (1970)
	Botryosphaeriaceae	*Botryodiplodia*	*Botryodiplodia theobromae*	*Lasiodiplodia theobromae*	Malaysia, China	Leaf spot	Liu (1977), Zhuang (2001), Borines et al. (2013)
		Diplodia	*Diplodia artocarpi*	*Diplodia artocarpi*	Thailand	—	Giatgong (1980)
			Diplodia theobromae	*Lasiodiplodia theobromae*	Unknow	Diplodia fruit and collar rot	Sangchote et al. (2003), Borines et al. (2013)
		Lasiodiplodia	*Lasiodiplodia theobromae*	*Lasiodiplodia theobromae*	China and Trinidad and Tobago	—	Ni (2008), Rampersad (2008)
		Macrophoma	*Macrophoma* sp.	*Macrophoma* sp.	India	—	Mathur (1979)
		Physalospora	*Physalospora rhodina*	*Lasiodiplodia theobromae*	China and India	—	Richardson (1990), Chen (2002)
	Capnodiaceae	*Capnodium*	*Capnodium* sp.	*Capnodium* sp.	Brazil	—	Mendes et al. (1998)
	Ceratobasidiaceae	*Rhizoctonia*	*Rhizoctonia* sp.	*Rhizoctonia* sp.	Unknow	Root rot	Haq (2006), Borines et al. (2013)
	Ceratostomataceae	*Chlamydomyces*	*Chlamydomyces palmarum*	*Harzia palmara*	China	—	Zhuang (2001), Liu (1977)
	Corynesporascaceae	*Corynespora*	*Corynespora cassiicola*	*Corynespora cassiicola*	Vietnam	Corynespora leaf spot	Mel'nik (2011), Borines et al. (2013)
	Cyphellophoraceae	*Cyphellophora*	*Cyphellophora artocarpi*	*Cyphellophora artocarpi*	China	—	Gao et al. (2015)
	Diaporthaceae	*Phomopsis*	*Phomopsis artocarpi*	*Phomopsis artocarpi*	India	Leaf spot	Mathur (1979), Prakash et al. (2009)
			Phomopsis sp.	*Phomopsis* sp.	Unknow	Miscellaneous	Haq (2006), Borines et al.

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
						leaf diseases	(2013)
	Didymellaceae	*Phoma*	*Phoma* sp.	*Phoma* sp.	Brazil and USA	—	Alfieri (1984), Mendes et al. (1998)
	Dipodascaceae	*Geotrichum*	*Geotrichum candidum*	*Geotrichum candidum*	China	—	Zhuang (2001)
	Elsinoaceae	*Sphaceloma*	*Sphaceloma spondiadis*	*Elsinoe spondiadis*	Brazil	—	Mendes et al. (1998)
	Glomerellaceae	*Colletotrichum*	*Colletotrichum artocarpi*	*Colletotrichum artocarpi*	USA	—	Bhunjun et al. (2019)
			Colletotrichum artocarpicola	*Colletotrichum artocarpicola*	Thailand	—	Bhunjun et al. (2019)
			Colletotrichum fructicola	*Colletotrichum fructicola*	Australia	—	Bhunjun et al. (2019)
			Colletotrichum gloeosporioides	*Colletotrichum gloeosporioides*	China and Thailand	Anthracnose	Borines et al. (2013), Bhunjun et al. (2019)
			Colletotrichum gloeosporioides var. minor	*Colletotrichum gloeosporioides var. minor*	Australia	—	Simmonds (1966)
			Colletotrichum lagenarium	*Colletotrichum lagenarium*	Unknow	Leaf spot	Prakash et al. (2009)
			Colletotrichum orbiculare	*Colletotrichum orbiculare*	Unknow	Leaf spot	Borines et al. (2013)
			Colletotrichum siamense	*Colletotrichum siamense*	Australia	—	Shivas (1989), James et al. (2014), Bhunjun et al. (2019)
		Gloeosporium	*Gloeosporium artocarpi*	*Gloeosporium artocarpi*	Thailand	—	Giatgong (1980)
			Gloeosporium mangiferae	*Colletotrichum coccodes*	China	—	Zhuang (2001)
			Gloeosporium sp.	*Gloeosporium* sp.	India	—	Mathur (1979)
		Glomerella	*Glomerella cingulata*	*Colletotrichum gloeosporioides*	Malaysia	—	Liu (1977)
			Glomerella cingulata var. minor	*Glomerella cingulata var. minor*	Australia	—	Simmonds (1966)
	Hyaloscyphaceae	*Haplographium*	*Haplographium* sp.	*Haplographium* sp.	Malaysia	—	Liu (1977)
	Incertae sedis	*Brooksia*	*Brooksia tropicalis*	*Brooksia tropicalis*	Sierra Leone	—	Deighton & Pirozynski (1966)
		Grallomyces	*Grallomyces portoricensis*	*Grallomyces portoricensis*	Sierra Leone	—	Deighton & Pirozynski (1966)
		Gyrothrix	*Gyrothrix circinata*	*Gyrothrix circinata*	India and Sierra Leone	—	Pirozynski & Patil (1970)
			Gyrothrix podosperma	*Gyrothrix podosperma*	India	—	Pirozynski & Patil (1970)

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
		Nigrospora	*Nigrospora sphaerica*	*Nigrospora oryzae*	Unknow	Leaf spot	Borines et al. (2013)
		Pestalozziella	*Pestalozziella artocarpi*	*Pestalozziella artocarpi*	India	—	Mathur (1979)
		Berkleasmium	*Berkleasmium atrovirens*	*Berkleasmium atrovirens*	China	—	Zhuang et al. (2009)
	Meliolaceae	*Meliola*	*Meliola artocarpi*	*Meliola artocarpi*	India and Thailand	—	Giatgong et al. (1980), Hosagoudar & Goos (1991), Hosagoudar & Abraham (2000)
			Meliola integrifolii	*Meliola integrifolii*	India	—	Hosagoudar & Abraham (2000)
	Micropeltidaceae	*Chaetothyrina*	*Chaetothyrina artocarpi*	*Chaetothyrina artocarpi*	Thailand	—	Hyde et al. (2017)
	Mycosphaerellaceae	*Cercospora*	*Cercospora mehran*	*Cercospora mehran*	China and Pakistan	—	Khan & Kamal (1974), Kamal (2010)
			Cercospora sp.	*Cercospora* sp.	Unknow	Leaf spot	Borines et al. (2013)
		Colletogloeum	*Colletogloeum artocarpi*	*Colletogloeum artocarpi*	India and Nepal	—	Mathur (1979), Sutton (1980)
		Passalora	*Passalora artocarpi*	*Passalora artocarpi*	China	—	Crous & Braun (2003), Zhuang (2001)
		Pseudocercospora	*Pseudocercospora artocarpi*	*Pseudocercospora artocarpi*	India	Leaf spot	Kamal (2010), Borines et al. (2013)
		Septoria	*Septoria artocarpi*	*Septoria artocarpi*	—	Leaf spot	Prakash et al. (2009), Borines et al. (2013)
	Nectriaceae	*Fusarium*	*Fusarium merismoides var. merismoides*	*Fusarium merismoides var. merismoides*	China	—	Zhuang (2001)
			Fusarium oxysporum	*Fusarium oxysporum*	Sri Lanka	—	Adikaram & Yakandawala (2020)
			Fusarium sp.	*Fusarium* sp.	Unknow	Root rot	Haq (2006), Borines et al. (2013)
		Neocosmospora	*Neocosmospora solani*	*Fusarium solani*	Sri Lanka	—	Adikaram & Yakandawala (2020)

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
		Tubercularia	*Tubercularia vulgaris*	*Nectria cinnabarina*	China	—	Zhuang (2001)
	Pestalotiopsidaceae	*Pestalotiopsis*	*Pestalotiopsis palmarum*	*Pestalotiopsis palmarum*	Malaysia	—	Liu (1977)
			Pestalotiopsis versicolor	*Pestalotiopsis versicolor*	Malaysia	Leaf spot	Liu (1977), Borines et al. (2013)
	Phyllachoraceae	*Phyllachora*	*Phyllachora microcenta*	*Phyllachora microcenta*	India	—	Hosagoudar (1989)
		Phyllosticta	*Phyllosticta artocarpi*	*Phyllosticta artocarpi*	USA	Leaf spot	Alfieri et al. (1984)
			Phyllosticta artocarpina	*Phyllosticta artocarpina*	Unknow	Leaf spot	Borines et al. (2013)
			Phyllosticta sp.	*Phyllosticta* sp.	China and India	—	Mathur (1979), Zhuang (2001)
		Phyllostictina	*Phyllostictina artocarpina*	*Phyllostictina artocarpina*	India and USA	Leaf spot	Spaulding (1961), Mathur (1979)
	Pythiaceae	*Pseudofusicoccum*	*Pseudofusicoccum artocarpi*	*Pseudofusicoccum artocarpi*	Thailand	—	Trakunyingcharoen et al. (2015), Dissanayake et al. (2016), Netto et al. (2017),
	Sclerotiniaceae	*Botrytis*	*Botrytis cinerea*	*Botrytis cinerea*	USA	—	Alfieri et al. (1984)
		Sclerotinia	*Sclerotinia sclerotiorum*	*Sclerotinia sclerotiorum*	Bangladesh	—	Rahman et al. (2015)
	Sporocadaceae	*Pestalotiopsis*	*Pestalotia elasticola*	*Pestalotiopsis elasticola*	—	Gray blight	Prakash et al. (2009), Borines et al. (2013)
	Xylariaceae	*Rosellinia*	*Rosellinia arcuata*	*Dematophora arcuata*	—	Collar rot	Prakash et al. (2009)
			Rosellinia bunodes	*Dematophora bunodes*	Sri Lanka	—	Spaulding et al. (1961)
		Ustulina	*Ustulina zonata*	*Kretzschmaria zonata*	—	Charcoal rot	Prakash et al. (2009)
Basidiomycota	Atheliaceae	*Athelia*	*Athelia rolfsii*	*Athelia rolfsii*	Bangladesh	Fruit rot	Elahi et al. (2021)
		Sclerotium	*Sclerotium rolfsii*	*Athelia rolfsii*	Thailand	—	Giatgong et al. (1980)
	Corticiaceae	Corticium	*Corticium salmonicolor*	*Necator salmonicolor*	—	Pink disease	Spaulding et al. (1961)
		Erythricium	*Erythricium salmonicolor*	*Necator salmonicolor*	Unknow	Pink disease	Borines et al. (2013)
		Pellicularia	*Pellicularia koleroga*	*Corticium koleroga*	Brazil	—	Mendes et al. (1998)
			Pellicularia rolfsii	*Athelia rolfsii*	Sri Lanka	—	Spaulding et al. (1961)
		Phanerochaete	*Phanerochaete salmonicolor*	*Phanerochaete salmonicolor*	Papua New Guinea	—	Shaw (1984)
	Hymenochaetaceae	*Phellinus*	*Phellinus noxius*	*Pyrrhoderma noxium*	China	Brown root and	Ann et al. (2002), Borines et

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
						crown rot	al. (2013)
	Meripilaceae	*Fomes*	*Fomes annularis*	*Ganoderma australe*	Philippines	—	Spaulding et al. (1961)
			Fomes lamaoensis	*Pyrrhoderma lamaoense*	India	Brown root rot	Spaulding et al. (1961)
			Fomes lignosus	*Rigidoporus microporus*	Brazil and Equatorial Guinea	—	Spaulding et al. (1961)
	Pleosporaceae	*Alternaria*	*Alternaria* sp.	*Alternaria* sp.	Unknow	Leaf spot	Borines et al. (2013)
		Curvularia	*Curvularia* sp.	*Curvularia* sp.	Unknow	Miscellaneous leaf diseases	Haq (2006), Borines et al. (2013)
	Phakopsoraceae	*Uredo*	*Uredo artocarpi*	*Physopella artocarpi*	Venezuela	Rust	Spaulding et al. (1961), Borines et al. (2013)
	Polyporaceae	*Ganoderma*	*Ganoderma applanatum*	*Ganoderma applanatum*	India	White mottled rot	Spaulding et al. (1961)
		Polyporus	*Polyporus zonalis*	*Rigidoporus lineatus*	Brazil	—	Spaulding et al. (1961)
Mucoromycota	Rhizopodaceae	*Rhizopus*	*Rhizopus artocarpi*	*Rhizopus stolonifer*	China, Malaysia, and Thailand	Stem, fruit, and male inflorescence rot	Johnston (1960), Giatgong (1980), Chen (2002), Prakash et al. (2009), Deng et al. (2015)
			Rhizopus oryzae	*Rhizopus arrhizus*	Unknow	Rhizopus rot or transit rot	Borines et al. (2013)
		Rhizopus	*Rhizopus* sp.	*Rhizopus* sp.	Cuba	—	Arnold (1986)
			Rhizopus stolonifer	*Rhizopus stolonifer*	Australia, Cook Islands, Dominican Republic, Fiji, Japan, Mexico, Niue and Samoa	Fruit rot and transit rot	Dingley et al. (1981), Shivas (1989), Lenne (1990), Kobayashi (2007), Borines et al. (2013), Garcia-Estrada et *al*. (2019)
Oomycota	Peronosporaceae	*Phytophthora*	*Phytophthora arecae*	*Phytophthora palmivora*	India	Phytophthora root, fruit and stem rot	Erwin & Ribeiro (1996), Borines et al. (2013)

Phylum	Family	Genus	Fungi species	Current name	Country	Disease	References
			Phytophthora citrophthora	*Phytophthora citrophthora*	Unknow	Phytophthora root, fruit and stem rot	Haq (2006), Borines et al. (2013)
			Phytophthora palmivora	*Phytophthora palmivora*	India, Philippines and Vietnam	—	Spaulding (1961), Borines et al. (2014), Azni et al. (2017)
			Phytophthora palmivora var. palmivora	*Phytophthora palmivora var. palmivora*	India, Philippines and Sri Lanka	—	Erwin & Ribeiro (1996)
			Phytophthora sp.	*Phytophthora* sp.	Unknow	Root rot.	Haq (2006), Borines et al. (2013)
	Pythiaceae	*Pythium*	*Pythium splendens*	*Globisporangium splendens*	USA	Root rot.	Alfieri et al. (1984), Haq (2006), Borines et al. (2013)

— = Data not available.

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