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Reinstating *Dyfrolomyces* and introducing *Melomastia pyriformis* sp. nov. (Pleurotremataceae, Dyfrolomycetales) from Guangdong Province, China

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Abstract

As part of an ongoing investigation into fungal diversity in plant substrates, a novel species was discovered and isolated from the gardens of Zhongkai University of Agriculture and Engineering. The DNA sequence data from our collection were analyzed against the NCBI database, revealing affinities to species within Pleurotremataceae and Dyfrolomycetales. Further, a combined analysis of LSU, SSU, and *tef1-α* DNA sequences was conducted using maximum likelihood and Bayesian methods to elucidate their phylogenetic relationships. The phylogenetic analysis and distinctive morphological characteristics provide support for the establishment of a new species, *Melomastia pyriformis*. *Melomastia pyriformis* is subjected to comparative analysis with other similar taxa, accompanied by a comprehensive morphological description and illustration. In addition to morphological comparison, the classification of *Dyfrolomyces* and *Melomastia* is re-evaluated based on their ascospore morphology and septation. The genus *Dyfrolomyces* was reinstated to accommodate *M. tiomanensis* (type) and *M. chromolaenae*.

Keywords – Ascospore septation – Generic delimitation – Multi-locus phylogeny – Re-evaluation – Saprobe – Taxonomy

Introduction

The family Pleurotremataceae was introduced by Watson et al. (1929) to accommodate a monotypic genus *Pleurotrema* with *P. polysemum* as the type species. Pleurotremataceae is

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characterized by immersed ascomata, with a clypeus on the substrate, cylindrical asci and multi-septate ascospores with or without a sheath (Watson 1929, Barr 1994). Pang et al. (2013) established another family Dyfrolomycetaceae to accommodate its similar genus *Dyfrolomyces* and accepted four species *D. mangrovei*, *D. marinospora*, *D. rhizophorae* and *D. tiomanensis* (type) based on morphological and phylogenetic evidence. Dyfrolomycetaceae is characterized by relatively large, immersed, globose or subglobose ascomata, cylindrical asci and hyaline, symmetrical, multi-septate broadly fusiform ascospores with or without a sheath (Pang et al. 2013). However, Dyfrolomycetaceae was later synonymized under Pleurotremataceae based on the re-examination of the isotype of *Pleurotrema polysemum* (Maharachchikumbura et al. 2016). Currently, Pleurotremataceae consists of three genera, *viz*. *Dyfrolomyces*, *Melomastia* and *Pleurotrema* based on the latest outline of fungi (Wijayawardene et al. 2022).

The genus *Melomastia* was established by Saccardo (1875) to accommodate the species "*Melomastia friesii*", which was informally introduced by Nitschke (1871). Schröter (1894) treated *Sphaeria mastoidea* as the basionym of *M. friesii*, and thus, the type species of *Melomastia* was designated as *M. mastoidea*. *Melomastia mastoidea* is characterized by the presence of ascomata, which appear as raised black dots on the surface of the host. In vertical section, these structures are obpyriform and immersed, featuring a central periphysate ostiolar canal. The peridium is composed of multiple layers of compressed, dark brown cells, with filamentous paraphyses embedded in a gelatinous matrix. The asci are cylindrical, unitunicate, pedicellate and apically rounded with eight spores. The ascospores are uniseriate, ovoid and hyaline with two septa that are constricted at the septum; each cell contains a lipid globule and is surrounded by a gelatinous sheath (Kang et al. 1999). Norphanphoun et al. (2017) demonstrated that *Melomastia italica* and *Dyfrolomyces maolanensis* formed a distinct lineage with robust statistical support, leading to the transfer of *D. maolanensis* to *Melomastia.* Further, Li et al. (2022) have conducted a significant revision of *Dyfrolomyces* and *Melomastia* based on both morphological characteristics and multi-locus phylogeny analysis. They noted the absence of discernible morphological distinctions between the two genera and transferred 11 species of *Dyfrolomyces* to *Melomastia* (Li et al. 2022). Up to date, *Melomastia* has been recorded with 50 species in Index Fungorum (2023). However, only 17 of these species have corresponding molecular data available in GenBank. Notably, the type species *M. mastoidea* is still lacking such information.

Melomastia species seem to have a cosmopolitan distribution, since they have been recorded from various habitats, such as terrestrial, freshwater, marine and mangrove ecosystems (Hyde 1992, Hyde et al. 2017, Dayarathne et al. 2020, Li et al. 2022). Most species were isolated from woody branches, twigs, and culms as saprobes (Norphanphoun et al. 2017, Phukhamsakda et al. 2020, Li et al. 2022). Additionally, they have wide geographical distribution in both temperate and tropical countries, i.e., Africa (Central African Republic, Ivory Coast, South Africa), Asia (Brunei, China, India: Andaman and Nicobar Islands, Iran, Japan, Kazakhstan, Kirgizstan, Malaysia, Philippines, Thailand, Turkmenistan), Australia, Europe (Czech Republic, France, Germany, Italy) and South America (Argentina, Brazil, Chile) (Li et al. 2022, Farr & Rossman 2023). At present, seven *Melomastia* species have been recorded from China, *viz. M. aquatica*, *M. fusispora*, *M. winteri*, *M. maolanensis*, *M. oleae*, *M. sichuanensis* and *M. thamplaensis.*

During an ongoing investigation into the diversity of fungi in plant substrates, we have discovered a noteworthy dothideomycetous species. Its taxonomic position was determined by combining morphological characteristics with the phylogenetic analyses. Additionally, our findings provide new insights into the taxonomy of *Dyfrolomyces* and *Melomastia* based on both morphology and phylogeny.

Materials & Methods

Sample collection, morphological studies and isolation

Dead plant specimens were collected from the Garden of Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong Province, China, on June 5th, 2022. The sample was

brought to the laboratory in paper bags and examined with a stereomicroscope (Carl Zeiss Discovery V8). Microscopic mounts of fruiting structures in sterilized tap water were examined and photographed using a stereomicroscope fitted with a camera (ZEISS Axiocam 208). Hand sections of fruiting bodies were made by a razor blade and mounted in a water drop for microscope studies and photomicrography. The micro-morphological characteristics such as peridium, asci, ascospores, sheath were studied and photographed using a compound microscope (Nikon Eclipse 80i) fitted with a digital camera (Canon 450D). All microscopic measurements were made with Tarosoft image framework (v. 0.9.0.7). Images used for figures were combined and edited using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). Ascospores were cultured following the method described by Senanayake et al. (2020). The germinated ascospores were aseptically transferred into fresh potato dextrose agar (PDA) plates and incubated at 25 °C in the dark to obtain pure cultures. Colony characteristics were recorded from PDA cultures after two weeks. Fungarium specimen was deposited at the Herbarium of Zhongkai University of Agriculture and Engineering (MHZU), and the ex-type culture was deposited at the Culture Collection of Zhongkai University of Agriculture and Engineering (ZHKUCC). Index Fungorum numbers (http://www.indexfungorum.org) and Facesoffungi numbers (Jayasiri et al. 2015) were registered for the new species.

DNA extraction, PCR amplification and sequencing

Fresh mycelia growing on PDA was scraped for DNA extraction using a fungal genomic DNA extraction kit (Biospin DNA Extraction Kit, Bioer Technology, Co. Ltd., Hangzhou, China) following the manufacturer's protocols. The genomic DNA was stored at -20 °C. Polymerase chain reactions (PCR) and sequencing were carried out for the following loci: the partial LSU ribosomal DNA locus, amplified and sequenced as a single fragment with primers LR0R/LR5 (Vilgalys & Hester 1990); partial SSU ribosomal DNA locus, amplified and sequenced as a single fragment with primers NS1/NS4 (White et al. 1990), and part of the translation elongation factor 1-alpha (*tef1-α*) with primers EF1-983F/EF1-2218R (Rehner 2001).

The PCR amplification reactions were carried out with the following protocol. The total volume of the PCR reaction was 25 µl containing 1 µl of DNA template, 1 µl of each forward and reverse primer, 12.5 µl of 2 × PCR Master Mix, and 9.5 µl of double-distilled sterilized water (ddH_2O). The reaction was conducted by running for 35 cycles following the conditions in Table 1. The PCR products were observed on 1% agarose electrophoresis gel stained with ethidium bromide. Purification and sequencing of PCR products were carried out at Sunbiotech Company, Beijing, China. Sequence quality was checked, and sequences were condensed with DNASTAR Lasergene v. 7.1 (de Oliveira et al. 2021). Sequences derived in this study were deposited in GenBank and accession numbers were listed in (Table 2).

Table 1 Polymerase chain reactions (PCR) thermal cycle program for each locus*.

Locus	PCR thermal cycle protocols (Annealing temp. in bold)
LSU	94 °C: 5 min; (94 °C: 1min, **56** °C: 50s, 72 °C: 10s) × 35 cycles
SSU	95 °C: 5 min; (95 °C: 1min, **52** °C: 50s, 72 °C: 10s) × 35 cycles
tef1-α	95 °C: 5 min; (94 °C: 1min, 5**5** °C: 90s, 72 °C: 10s) × 35 cycles

*All the PCR thermal cycles include a final hold at 4 °C.

Phylogenetic analyses

The newly obtained sequences were initially subjected to BLASTn searches in GenBank (http://www.ncbi.nlm.nih.gov/) for a preliminary identification. Additional appropriate sequences were downloaded from GenBank based on the blast results and recent studies Norphanphoun et al. 2017, Phukhamsakda et al. 2020, Li et al. 2022. All the ex-type strains of species were included if available, and other authentic strains were selected when sequences from ex-type strains were

unavailable. The concatenated LSU, SSU, and *tef1-α* sequence dataset for Pleurotremataceae comprised 36 strains with *Anisomeridium phaeospermum* (MPN539) and *A. ubianum* (MPN94) selected as the outgroup. The sequences of LSU, SSU, and *tef1-α* gene regions were aligned separately using the online version of MAFFT v. 7.0362 (Katoh et al. 2019) with default settings and manually adjusted using BioEdit 7.1.3 (Hall 1999) when necessary to allow maximum alignment and minimum gaps.

Maximum likelihood analysis was performed by RAxML (Stamatakis & Alachiotis 2010) implemented in raxmlGUIv.1.5 (Silvestro & Michalak 2012) using the ML+rapid bootstrap setting and the GTR+I+G model of nucleotide substitution with 1,000 replicates. For the Bayesian inference (BI) analysis, the optimal substitution model for the combined datasets was determined to be GTR+I+G using the MrModeltest software v. 2.2 (Nylander 2004). The BI analysis was computed in MrBayes v. 3.2.6 (Ronquist et al. 2012) with four simultaneous Markov chain Monte Carlo chains from random trees over 1,000,000 generations (standard deviation of split frequencies less than 0.01) and trees were sampled every 500th generations.

The distribution of log-likelihood scores was observed to check whether sampling was in stationary phase and Tracer v1.5 was used to check if further runs were required to reach convergence (Rambaut & Drummond 2007). The consensus tree and posterior probabilities were calculated after discarding the first 20% of the sampled trees as burn-in. The phylogram was visualized in FigTree v. 1.4 (Rambaut 2009).

Table 2 Taxa used in the present phylogenetic analyses and GenBank numbers of sequences. "N/A" sequence is unavailable. GenBank numbers of newly generated sequences are presented in bold.

Species name	**Culture accession number**	**GenBank accession number**		
		LSU	**SSU**	***tef1-α***
Acrospermum adeanum	M 133	EU940104	EU940031	N/A
Acrospermum compressum	M 151	EU940084	EU940012	N/A
Acrospermum graminum	M 152	EU940085	EU940013	N/A
Anisomeridium phaeospermum	MPN539	JN887394	JN887374	JN887418
Anisomeridium ubianum	MPN94	N/A	JN887379	JN887421
Dyfrolomyces chromolaenae	MFLUCC 17-1434	KY111905	MT214413	MT235800
Melomastia clematidis	MFLUCC 17-2092	MT214607	MT226718	MT394663
Melomastia distoseptata	NFCCI 4377	MH971236	N/A	N/A
Melomastia fulvicomae	MFLUCC 17-2083	MT214608	MT226719	N/A
Melomastia fusispora	CGMCC 3.20618	OK623464	OK623494	OL335189
Melomastia fusispora	UESTCC 21.0001	OK623465	OK623495	OL335190
Melomastia italica	MFLUCC 15-0160	MG029458	MG029459	N/A
Melomastia sp.	ZHKUCC 22-0174	OQ379412	OQ379411	N/A
Melomastia maolanensis	GZCC 16-0102	KY111905	KY111906	KY814762
Melomastia neothailandica	MFLU 17-2589	NG068294	N/A	N/A
Melomastia oleae	CGMCC 3.20619	OK623466	OK623496	OL335191
Melomastia oleae	UESTCC 21.0003	OK623467	OK623497	OL335192
Melomastia phetchaburiensis	MFLUCC 15-0951	MF615402	MF615403	N/A
Melomastia pyriformis	**ZHKUCC 22-0175**	**OP791870**	**OP739334**	**OQ718392**
Melomastia rhizophorae	JK 5439 A	GU479799	GU479766	GU479860
Melomastia sichuanensis	CGMCC 3.20620	OK623469	OK623500	OL335195
Melomastia sinensis	MFLUCC 17-1344	MG836699	MG836700	N/A
Melomastia thailandica	MFLUCC 15-0945	KX611366	KX611367	N/A
Melomastia thamplaensis	MFLUCC 15-0635	KX925435	KX925436	KY814763
Dyfrolomyces tiomanensis	MFLUCC 13-0440	KC692156	KC692155	KC692157
Melomastia winteri	CGMCC 3.20621	OK623471	OK623502	OL335197

Table 2 Continued.

Species name	Culture accession number	GenBank accession number		
		LSU	SSU	*tef1-α*
Muyocopron castanopsis	MFLUCC 14-1108	KU726965	KU726968	MT136753
Muyocopron dipterocarpi	MFLU 17-2608	KU726966	KU726969	MT136754
Muyocopron heveae	MFLUCC 17-0066	MH986832	MH986828	N/A
Muyocopron lithocarpi	MFLUCC 14-1106	KU726967	KU726970	MT136755
Palawania thailandensis	MFLICC 14-1121	KY086494	N/A	N/A
Palawania thailandensis	MFLU 16-1873	KY086493	KY086495	N/A
Stigmatodiscus enigmaticus	CBS 132036	KU234108	KU234130	N/A
Stigmatodiscus labiatus	CBS 144700	MH756065	MH756065	MH756083
Stigmatodiscus oculatus	CBS 144701	MH756069	N/A	MH756086
Stigmatodiscus pruni	CBS 142598	KX611110	KX611110	KX611111

Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Netherlands; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; JK: J. Kohlmeyer personal collection; MFLU: Herbarium at Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MPN: Matthew P. Nelsen personal collection; NFCCI: National Fungal Culture Collection of India; GZCC: Guizhou culture collection, Guizhou, China; UESTCC: University of Electronic Science and Technology Culture Collection, Chengdu, China; ZHKUCC: Zhongkai University of Agriculture and Engineering Culture Collection, Guangzhou, China

Results

Phylogeny

The multi-locus alignment that contains taxa in Pleurotremataceae comprised 2,827 nucleotide characters (918 of LSU, 1,009 of SSU, 900 of *tef1-α*). The best scoring RAxML tree for maximum likelihood analysis yielded (Fig. 1) with the final ML optimization likelihood value of -12748.327734 and the following model parameters: estimated base frequencies A = 0.243752, C = 0.271096, G = 0.287908, and T = 0.197244; substitution rates AC = 0.696378, AG = 1.867564, AT = 1.1893, CG = 0.881385, CT = 6.956797 and GT = 1.0; proportion of invariable sites I = 0.607084; gamma distribution shape parameter: α = 0.210837. The alignment contained a total of 932 distinct alignment patterns and 25.2% of undetermined characters.

After discarding the first 20% of generations in the Bayesian analyses, 1,600 trees remained from which the 50% consensus tree and posterior probabilities were calculated (Fig. 1). All individual trees generated under different criteria from single gene datasets were similar in topology and not significantly different from the final trees generated from the concatenated datasets of Pleurotremataceae. The topologies of the ML and Bayesian trees were similar to each other and there are no significant differences. In this analysis, all *Melomastia* species grouped together forming three subclades. Our collection (ZHKUCC 22-0175) forms a sister clade with *M. thamplaensis*, with ML/BI = 89%/0.90 statistical support which is grouped in the *Melomastia* sensu lato subclade.

Taxonomy

Dyfrolomyces K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, Cryptog. Mycol. 34(3): 227 (2013)

Type species – *Dyfrolomyces tiomanensis* K.L. Pang, Alias, K.D. Hyde, Suetrong & E.B.G. Jones, Cryptog. Mycol. 34(3): 228 (2013)

Notes – *Dyfrolomyces* was established to accommodate the type species *D. tiomanensis*, which was discovered in a marine habitat on Tioman Island, Malaysia (Pang et al. 2013). *Dyfrolomyces* shares similar morphological characteristics with *Melomastia* in having globose to subglobose, immersed to semi-immersed or erumpent ascomata, cylindrical asci and hyaline, ellipsoid to fusiform

ascospores with or without a mucilaginous sheath (Barr 1994, Kang et al. 1999, Pang et al. 2013, Norphanphoun et al. 2017, Li et al. 2022). Due to the absence of sequence data of *Melomastia* species including the type, the phylogenetic relationships between these two genera have not been well-resolved yet. Norphanphoun et al. (2017) demonstrated the paraphyletic nature of *Dyfrolomyces* and *Melomastia*, and reclassified *D. maolanensis* to the genus *Melomastia* primarily based on its morphological characteristics. According to the updated multi-locus phylogenetic tree, Li et al. (2022) synonymized *Dyfrolomyces* under *Melomastia*, and transferred 11 *Dyfrolomyces* species to *Melomastia*.

In the present phylogenetic analyses, *M. tiomanensis* and *M. chromolaenae* were found to form a well-supported basal clade with other *Melomastia* species (Fig. 1), which is consistent with previous studies (Mapook et al. 2020, Phukhamsakda et al. 2020, Li et al. 2022). The members of this clade possess spindle-shaped ascospores that are 6–11-septate and have acute ends (Pang et al. 2013, Phukhamsakda et al. 2020), which notably differs from other species in *Melomastia*. Therefore, we propose that they represent a distinct genus and reinstate *Dyfrolomyces* to accommodate both *M. tiomanensis* (type) and *M. chromolaenae*.

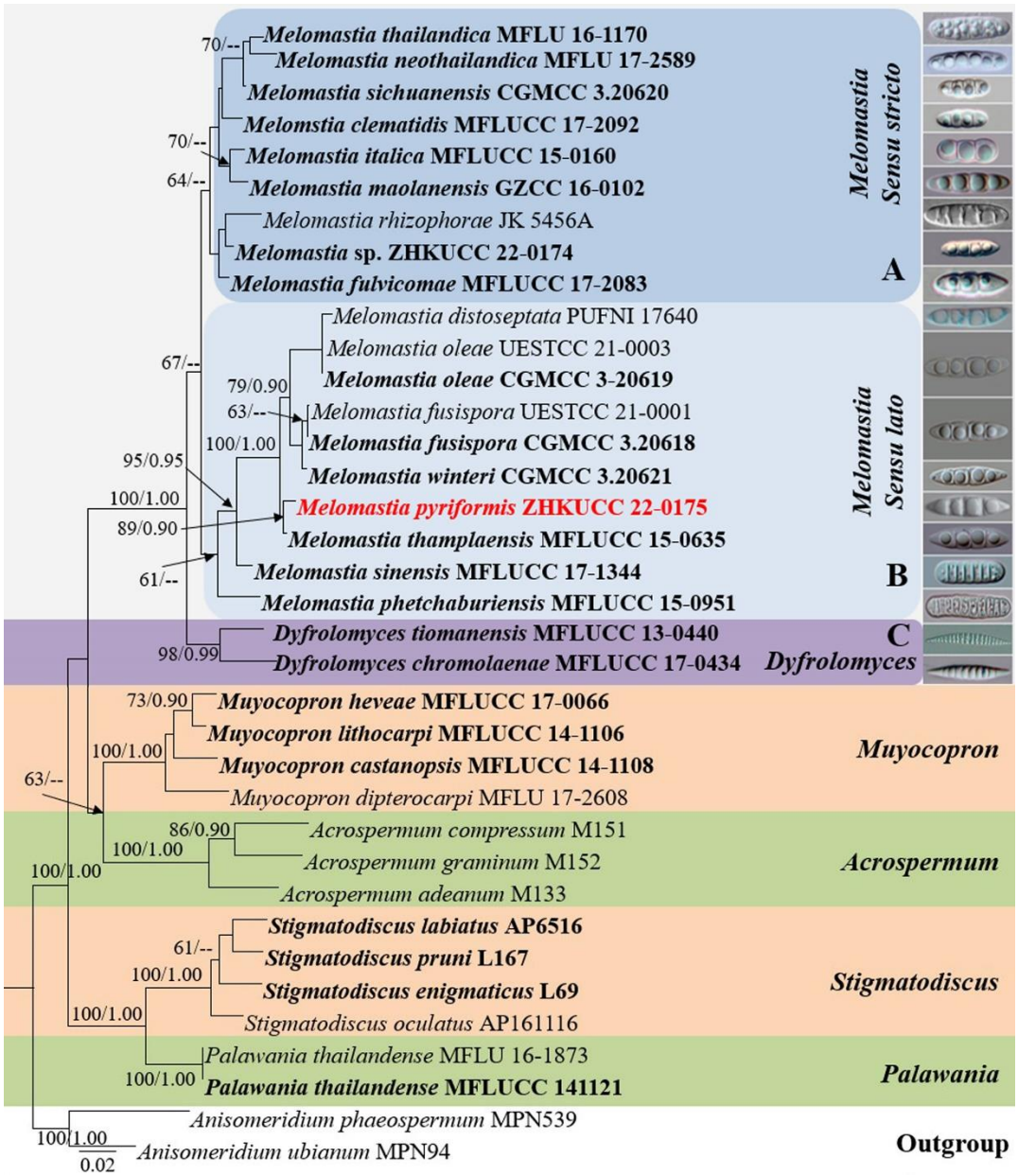

Fig. 1 – Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, and *tef1-α* sequence alignment. Maximum likelihood bootstrap support values greater than 60% and

Bayesian posterior probabilities greater than 0.90 are given at the nodes. The tree is rooted with *Anisomeridium phaeospermum* MPN539 and *A. ubianum* MPN94. Ex-type cultures are presented in bold and the newly generated sequences are indicated in red bold.

Dyfrolomyces tiomanensis K.L. Pang, Alias, K.D. Hyde, Suetrong & E.B.G. Jones, Cryptog. Mycol. 34(3): 228 (2013)

= *Melomastia tiomanensis* (K.L. Pang, Alias, K.D. Hyde, Suetrong & E.B.G. Jones) W.L. Li, Maharachch. & Jian K. Liu, Journal of Fungi 8(1, no. 76): 17 (2022)

Index Fungorum number: IF804661

Holotype – MFLU 13-0063

Distribution – Malaysia (Tioman Island)

Description and illustration – Cryptogamie Mycologie. 34(1): 228–229p, Figs 2–8, September 2013.

Notes – Li et al. (2022) have reclassified *D. tiomanensis*, the type species of *Dyfrolomyces*, as *Melomastia* due to their overlapped morphological characteristics and close phylogenetic relationships. However, in this study, we propose reinstating *Dyfrolomyces* as a distinct genus to accommodate *D. tiomanensis* (please see the notes of *Dyfrolomyces*).

Dyfrolomyces chromolaenae Mapook & K.D. Hyde, Fungal Diversity 101: 1–175, 2020.

= *Melomastia chromolaenae* (Mapook & K.D. Hyde) W.L. Li, Maharachch. & Jian K. Liu, Journal of Fungi 8(1, no. 76): 16 (2022)

Index Fungorum number: IF557290

Holotype – MFLU 20-0311

Distribution – Thailand

Description and illustration – Fungal Diversity. 101: 118,120p, Fig. 105, April 2020.

Notes – *Dyfrolomyces chromolaenae* exhibits morphological characteristics highly similar to those of the type species *D. tiomanensis*, including spindle-shaped, 6–11-septate ascospores that are noticeably distinct from those of *Melomastia* species. Furthermore, our phylogenetic analysis clearly demonstrates that *D. chromolaenae* and *D. tiomanensis* form a distinct lineage, representing a separate genus from *Melomastia* (please see the notes of *Dyfrolomyces*).

Melomastia Nitschke ex Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 4 4: 90 (1875)

Index Fungorum number: IF3118; Facesoffungi number: FoF 07673

Type species – *Melomastia mastoidea* (Fr.) J. Schröt., Krypt. -Fl. Schlesien (Breslau) 3.2(3): 320 (1894) [1908]

Melomastia pyriformis Kular. & Senan. sp. nov. Fig. 2

Index Fungorum number: IF558382; Facesoffungi number: FoF 13244

Etymology – Species epithet derived from the pyriform ascomata.

Holotype – MHZU 22-0092

Saprobic on twigs of an unidentified plant. Sexual morph: *Ascomata* 330–640 × 275–420 µm ($\bar{x}$ = 510 × 342 µm, n = 10), solitary or gregarious, erumpent to superficial when mature, pyriform, dark brown to black, coriaceous, papillate, ostiolate. *Clypeus* 10–40 µm thick, extending outwards around the ascomata, thicker around the papilla, composed of dense, melanized cells. *Papilla* 105–110 × 105–115 µm ($\bar{x}$ = 108 × 109 µm, n = 10), central, wide, ostiolar canal internally covered by filiform periphyses. *Peridium* 20–50 µm ($\bar{x}$ = 22 µm, n = 10), thin at the base and become thick towards sides, comprised of brown, thick-walled, cells of *textura intricata* in sides; and thin-walled, pale brown, cells of *textura angularis* in base. *Hamathecium* comprising numerous, dense, filiform, unbranched, septate, 1.8–2.5 µm wide pseudoparaphyses, anastomosing between and above the asci. *Asci* 135–160 × 5.5–7.5 µm ($\bar{x}$ = 138 × 6.3 µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, apically round, with an indistinct ocular chamber, short pedicellate, straight or slightly curved. *Ascospores* 20–25 × 4.5–7 µm ($\bar{x}$ = 21 × 5.3 µm, n = 10), uniseriate to overlapping uniseriate,

fusiform with acute ends, hyaline, 3-septate, not constricted at the septa, with guttules in each cell, smooth-walled without a sheath or appendages. Asexual morph: undetermined.

Colony characters – *Colonies* on PDA reaching 2 cm after 2 weeks incubating at 25 °C in dark, irregular, umbonate, filiform margin, slightly raised in the center, off-white, covered with wooly areal mycelial clots, reverse yellowish-brown. No sporulation and pigmentation observed in agar medium within 30 days.

Material examined – China, Guangdong Province, Guangzhou City, Zhongkai University of Agriculture and Engineering (23°06'28.4"N 113°16'51.6"E), on dead twigs of an unidentified plant, June 5th 2022, N.D. Kularathnage, NDK 58 (MHZU 22-0092, **holotype**); ex-type culture ZHKUCC 22-0175.

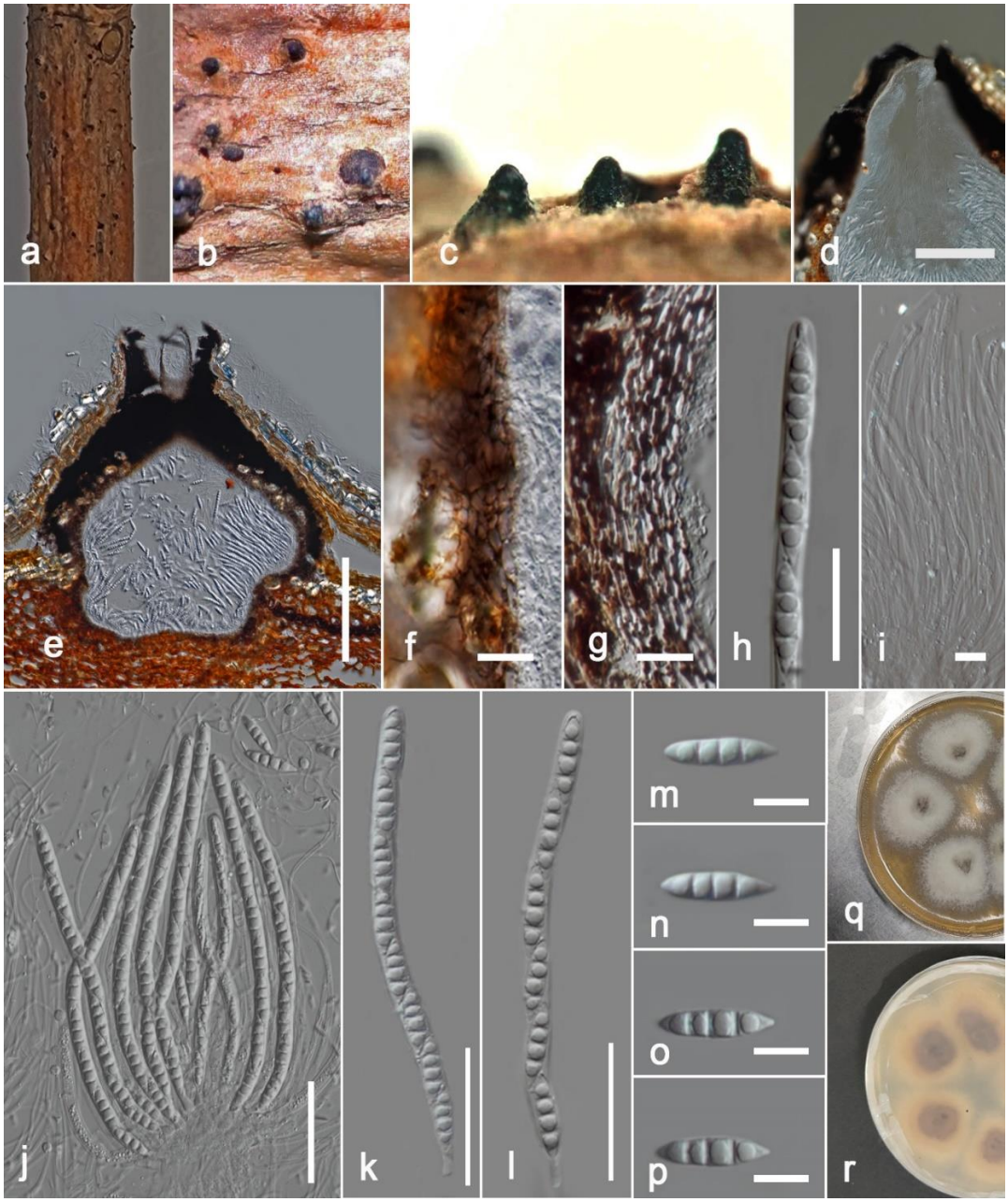

Fig. 2 – *Melomastia pyriformis* (MHZU 22-0092, holotype). a Dead twig of an unidentified plant. b, c Appearance of ascomata on the substrate. d Vertical section through ostiole cannel. e Vertical section of an ascoma. f, g Peridium (f at base, g at sides). h Apical chamber of an ascus. i Pseudoparaphyses. j–l Asci. m–p Ascospores. q Front view of colony on PDA. r Reverse view of colony on PDA. Scale bars: d, e = 200 μm, f, g = 20 μm, i = 5 μm, h, j–l = 40 μm, m–p = 10 μm.

Table 3 A morphological comparison of all *Melomastia* and *Dyfrolomyces* species, the novel species proposed herein is indicated in bold.

Species	Asci size (µm)	Ascospores				Habitats/Host	Locality	References
		Shape	Size (µm)	Septation	Sheath			
Clade A (*Melomastia sensu stricto*)								
Melomastia clematidis	115–160 × 4–7	Broad fusiform with acute ends	13–20 × 3.8–5	3	Yes	Terrestrial/*Clematis sikkimensis*	Thailand	Phukhamsakda et al. (2020)
M. fulvicomae	70–90 × 4–6	Broad fusiform with rounded/ acute ends	9–15 × 3.5–5.5	2–3	Yes	Terrestrial/*Clematis fulvicoma*	Thailand	Phukhamsakda et al. (2020)
M. italica	120–190 × 5.1–8.9	Ellipsoidal	8.8–10.5 × 2.8–4.11	2	Yes	Terrestrial/*Vitis vinifera*	Italy	Norphanphoun et al. (2017)
M. mastoidea	160–288 × 6–10	Ovoid with rounded ends	16–19 × 5–6	2	Yes	Terrestrial/*Metasphaeria macounii*	Canada, British Columbia	Kang et al. (1999)
M. maolanensis	103–118 × 4–5.5	Fusiform with round ends	13.5–18 × 3.5–4.5	3	No	Terrestrial/Unknown	China	Zhang et al. (2017)
M. marinospora	190–240 × 10–12	Cylindrical with acute poles	25–31 × 7.5–10	3	Yes	Intertidal/*Kandelia candel*	Brunei	Hyde et al. (2013)
M. neothailandica	165–190 × 10–12	Ellipsoidal	26–28 × 7.2–8	5	Yes	Marine/*Rhizophora* sp.	Thailand	Dayarathne et al. (2020)
M. rhizophorae	135–160 × 8–10	Ellipsoidal	19–26 × 6–8	4–6	Yes	Intertidal/*Rhizophora*	Thailand	Hyde (1992)
M. sichuanensis	101–112 × 6.5–7.6	Broad fusiform with rounded ends	15–17.5 × 4.7–5.1	3	Yes	Terrestrial/*Olea europaea*	China	Li et al. (2022)
M. thailandica	146–158 × 7–9	Ellipsoidal	24–32 × 6–8	3–5	Yes	Marine/*Marina cvicennia*	Thailand	Hyde et al. (2016)
Clade B (*Melomastia sensu lato*)								
Melomastia aquatica	185–230 × 7–9	Fusiform	26–34 × 6–8	3	Yes	Freshwater/Unknown	China	Hyde (1992)
M. distoseptata	127–146 × 4.7–6.3	Fusoid, obtuse ends	19.7–24.9 × 4.3–5	3	No	Terrestrial/Unknown	India	Hongsanan et al. (2020)
M. fusispora	200–231 × 7.6–9.2	Fusiform	27.5–32 × 6.5–7.5	3	Yes	Terrestrial/*Olea europaea*	China	Li et al. (2022)
M. mangrove	154–216 × 8.5–14	Fusiform	26–33 × 6–8	7–9	Yes	Intertidal/*Rhizophora* sp.	Thailand	Hyde et al. (2013)
M. oleae	209–237 × 7.5–9	Fusiform, obtuse ends	28–34 × 6–7	3	No	Terrestrial/*Europaea olea*	China	Li et al. (2022)

Table 3 Continued.

Species	Asci size (µm)	Ascospores				Habitats/Host	Locality	References
		Shape	Size (µm)	Septation	Sheath			
M. phetchaburiensis	190–300 × 8–12	Ellipsoidal	35–40 × 5–10	1–10		Marine/*Apiculata rhizophora*	Thailand	Hyde et al. (2017)
M. pyriformis	**135–160 × 5.5–7.5**	**Fusiform with acute ends**	**20–25 × 4.5–7**	**3**	**No**	**Terrestrial/unknown**	**China**	**This study**
M. sinensis	160–220 × 8–10	Cylindrical	18–30 × 5–8	6–7	No	Terrestrial/*Sinensis camellia*	Thailand	Hyde et al. (2018)
M. thamplaensis	114–160 × 6–8.5	Fusiform with acute angular ends	19.5–23.5 × 5–6.5	3	No	Terrestrial/Unknown	Thailand	Zhang et al. (2017)
M. winteri	165–189 × 7–8.5	Fusiform with acute ends	25–30 × 5–6.5	3	No	Terrestrial/*Olea europaea*	China	Li et al. (2022)
Clade C (*Dyfrolomyces*)								
Dyfrolomyces chromolaenae	135–160 × 7–8	Fusiform	29–35 × 4.5–6	9–11	No	Terrestrial/*Chromolaena odorata*	Thailand	Mapook et al. (2020)
D. tiomanensis	316–333 × 12–17	Spindle-shaped	69–82 × 9–11	6–7	No	Terrestrial/*Rhizophora* sp.	Malaysia	Pang et al. (2013)

Table 4 The key morphological differences of species in the subclade A, B and C.

Subclade	Ascospore septation	Ascospore size (µm)	Ascospore shape	References
A (*Melomastia sensu stricto*)	2–5	9–32 × 3–8	Fusiform to oblong with rounded ends. Consist with gelatinous sheath	Norphanphoun et al. (2017), Zhang et al. (2017), Dayarathne et al. (2020), Phukhamsakda et al. (2020), Li et al. (2022)
B (*Melomastia sensu lato*)	1–10	18–40 × 4–10	Fusiform with acute ends	Hyde et al. (2017, 2018), Zhang et al. (2017), Hongsanan et al. (2020), Li et al. (2022)
C (*Dyfrolomyces*)	6–11	29–82 × 9–11	Spindle-shaped with acute ends	Pang et al. (2013), Mapook et al. (2020)

Notes – the phylogenetic analysis revealed that *M. pyriformis* formed a well-supported clade with *M. thamplaensis* (89% ML and 0.90 PP) (Fig. 1). The comparison of DNA sequences at the SSU and *tef1-α* loci between *M. pyriformis* and *M. thamplaensis* revealed base pair differences of 0.69% and 1.22%, respectively, indicating that they are genetically distinct species (Jeewon & Hyde 2016). Morphologically, *M. pyriformis* can be distinguished from *M. thamplaensis* by its thinner ascomatal base (267–314 µm), asci lacking a distinct ocular chamber, and ascospores without constriction at the septa and have angular ends. In contrast, *M. thamplaensis* exhibits a thicker ascomatal base (275–420 µm), asci with an evident apical ring, and ascospores that are distinctly constricted at the septa and have acute ends (Zhang et al. 2017). In addition to *M. thamplaensis*, a comparative analysis of the morphological characteristics of *M. pyriformis* and other accepted species in *Melomastia* is presented in Table 3; however, none of the extant species exhibit similar morphology to our new collection. Therefore, we propose the recognition of *M. pyriformis* as a novel species based on the species delineation criteria discussed in Maharachchikumbura et al. (2021).

Discussion

Li et al. (2022) synonymized all species of *Dyfrolomyces* under *Melomastia* due to the inefficiency of 2-septate, oblong ascospores in distinguishing between the two genera. However, in our phylogenetic analysis, *Melomastia* species are classified into three subclades (A, B and C, Fig. 1), representing at least two distinct morphotypes (Table 4). Species in subclade A possess fusiform ascospores that are 2–5-septate and have rounded ends. Species in subclade B possess fusiform ascospores with 3 septa and acute ends, except for *M. phetchaburiensis* and *M. sinensis* which have narrowly oblong ascospores with 1–10 septa. Based on the morphological features presented in Table 4 and a combined phylogenetic analysis in Fig. 1, it is likely that subclade A and B represent two distinct genera. However, there are currently no remarkable morphological characteristics that can distinguish between the two genera in a significant manner. Presently, we designate subclade A as *Melomastia sensu stricto* due to its close resemblance to the type species *M. mastoidea* (Kang et al. 1999), while subclade B is referred to as *Melomastia sensu lato*. Further conclusions regarding Clade B cannot be made until new collections, sequences and phenotypic data from published species are available. Therefore, in this study, we tentatively identify our new species *M. pyriformis* within *Melomastia sensu lato*. The ascospores of species in subclade C, i.e., *M. tiomanensis* (as *Dyfrolomyces tiomanensis*) and *M. chromolaenae* (as *Dyfrolomyces chromolaenae*), are spindle-shaped, 6–11-septate with tapering ends, which is a distinct contrast to those of the species in subclade A and B. We, thus, propose the reinstating of *Dyfrolomyces* to include *M. tiomanensis* (type) and *M. chromolaenae*.

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