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Taxonomic position of *Melomastia italica sp. nov.* and phylogenetic reappraisal of Dyfrolomycetales

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Abstract – *Melomastia* is a genus of saprobic fungal species found on wood, with 29 species epithets listed in Index Fungorum. The classification of species in the genus has been a challenge due to a high degree of morphological overlap and a lack of DNA based phylogenies. The present study clarifies the phylogenetic placement of the genus and with an additional new species based on a fresh collection from Italy. The new species, *Melomastia italica*, is described based on morphological and relationships inferred from phylogenetic analyses of SSU and LSU sequence data. *Melomastia* is accommodated within the family *Pleurotremaceae* in the class Dothideomycetes. The phylogenetic relationships and intergeneric taxonomy within the family *Pleurotremaceae* are revisited, while *Dyfrolomyces maolanensis* is transferred to the genus *Melomastia*.

Genera *incertae sedis* / Dothideomycetes / Molecular analyses/ Morphology / Saprobic fungi

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INTRODUCTION

The genus *Melomastia* Nitschke ex Sacc. was described by Nitschke (1875) to accommodate *Melomastia mastoidea* (Fr.) J. Schröt (= *Melomastia friesii* Nitschke) (Saccardo, 1875). There have been several taxonomic controversies pertaining to the ordinal/familial placement of this genus within the Ascomycota (Barr, 1989, 1994; Hawksworth *et al.*, 1995; Kirk *et al.*, 2008; Hu *et al.*, 2010; Lumbsch & Huhndorf, 2010). It is presently placed in Ascomycetes, genera *incertae sedis* (Lumbsch & Huhndorf, 2010; Maharachchikumbura *et al.*, 2015, 2016; Wijayawardene *et al.*, 2017). Barr (1989) accommodated *Melomastia* in *Clypeosphaeriaceae*, which is related to *Amphisphaeriaceae*. Later, Barr (1994) assigned three families with unitunicate asci (*Amphisphaeriaceae*, *Clypeosphaeriaceae* and *Pleurotremataceae*) to the order Xylariales and placed five genera within *Pleurotremaceae* (*Daruvedia*, *Melomastia*, *Phomatospora*, *Pleurotrema*, *Saccardoella*) based on the non-fissitunicate ascus character.

Hawksworth *et al.* (1995) retained *Pleurotrema* in the order Pyrenulales. Hu *et al.* (2010) agreed with Kirk *et al.* (2008) and placed *Daruvedia* in the Pleosporales (Dothideomycetes) based on type specimen and fresh collections of *Daruvedia*. Senanayake *et al.* (2016) referred the genus *Phomatospora* to a new family *Phomatosporaceae* (Phomatosporales) based on DNA sequence data analyses. Presently, *Melomastia* and *Saccardoella* are referred to Ascomycota genera, *incertae sedis* (Kirk *et al.*, 2001; Lumbsch & Huhndorf, 2010). Suetrong *et al.* (2009) analyzed DNA sequence data of *Saccardoella rhizophorae*, a species known from marine environments, and currently positioned it within the class Dothideomycetes. However, placement of *S. rhizophorae* at an appropriate higher taxonomic level was difficult as the latter did not group with any well-established order/family within the Dothideomycetes (Hyde, 1992; Tsui *et al.*, 1998; Suetrong *et al.*, 2009). Thereafter, Pang *et al.* (2013) introduced a new family, *Dyfrolomycetaceae* (Dothideomycetes) to accommodate *Dyfrolomyces tiomanensis,* which grouped with *Saccardoella rhizophorae* based on combined DNA sequence data analyses. This resulted in three species of *Saccardoella* (*S. mangrovei*, *S. marinospora*, *S. rhizophorae*) being transferred to *Dyfrolomyces* (Pang *et al.*, 2013; Hyde *et al.*, 2013).

Maharachchikumbura *et al.* (2015) accepted *Pleurotrema* as monotypic in *Pleurotremataceae* within Chaetosphaeriales without taxonomic justifications and referred *Melomastia* to Sordariomycetes, genera *incertae sedis*. Later, Maharachchikumbura *et al.* (2016) synonymised *Dyfrolomycetaceae* under *Pleurotremataceae* and excluded it from Sordariomycetes based on morphology of the *Pleurotrema* isotype*,* which are similar to species of *Saccardoella* and *Dyfrolomyces* in Dothideomycetes. To date, 29 epithets of *Melomastia* are recorded in Index Fungorum (2017). During our fungal diversity survey in Italy, we recovered an unusual *Melomastia* species which we described as a new species herein. Phylogenetic investigations also revealed some interesting taxonomic insights pertaining to the intergeneric relationships of *Dyfrolomyces* and *Melomastia*. We provide a more robust phylogeny and resolve some of the taxonomic confusion surrounding the family *Pleurotremataceae*.

MATERIAL AND METHODS

Sample collection and examination of specimens

The samples were collected from dead branches of *Vitis vinifera* in Italy in 2015. The specimens were returned to the laboratory in paper bags, examined and descriptions were carried out following Norphanphoun *et al.* (2016). Micro-morphological characters were studied using a Motic SMZ 168 dissecting microscope for fungal fruiting bodies. Hand sections of the fruiting structures were mounted in water and examined for morphological details. Fungi were also examined using a Nikon Ni compound microscope and photographed with a Canon EOS 600D digital camera fitted to the microscope. Photo-plates were made using Adobe Photoshop CS6 Extended version 13.0 × 64 (Adobe Systems, USA), while Tarosoft (R) Image Frame Work program v. 0.9.7 was used for measurements. The contents inside the ascomata, which comprised asci, hamathecium and ascospores, were removed with a sterile needle and soaked in sterile water in a glass container prior to examination.

Cultures were obtained by single spore isolation, following the description in Chomnunti *et al.* (2014). Spore germination was observed and photographed using a Nikon Ni compound microscope fitted with Canon EOS 600D digital camera. Geminated spores were transferred aseptically to fresh malt extract agar (MEA) and incubated at room temperature (18-25°C).

Herbarium specimens are deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU) and duplicated in New Zealand Fungarium (PDD). Living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Culture Collection (KUMCC). Index Fungorum numbers are registered (Jayasiri *et al.*, 2015). New species are established as recommended by Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

DNA extraction process was followed by Norphanphoun *et al.* (2017) with fresh fungal mycelia growing on MEA at room temperature (18-25°C) for three weeks. The genomic DNA was obtained using a E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) following the manufacturer's instructions.

Polymerase chain reactions (PCR) were carried out using primer pairs of LROR (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) to amplify the large subunit rDNA (28S, LSU) (Vilgalys & Hester 1990), and the small subunit nuclear rDNA (18S, SSU) was amplified with primers (5′-GTAGTCATATGCTTGTCTC-3′) and NS4 (5′-CTTCCGTCAATTCCTTTAAG-3′) (White *et al.* 1990). The amplification reactions were carried out with the following protocol: 50 µl reaction volume containing 2 µl of DNA template, 2 µl of each forward and reverse primers, 25 µl of 2 × Bench Top^{TM}Taq Master Mix (mixture of Taq DNA Polymerase (recombinant): 0.05 units/µL, $MgCl_2$: 4 mM, and dNTPs (dATP, dCTP, dGTP, dTTP): 0.4 mM) and 19 µl of double-distilled water (ddH_2O) (sterilized water). The PCR thermal cycle program for LSU gene amplification are as follows: 95°C for 3 min, followed by 34 cycles of 95°C for 30 s, 51°C for 50 s, 72°C for 1 min, and final extension at 72°C for 10 min. The PCR thermal cycle program for SSU gene amplification were provided as: 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 51°C for 1 min, 72°C for 45 s, and final extension at 72°C for 10 min. Purification and sequencing of PCR product with the same primers mentioned above were carried out at Life Biotechnology Co., Shanghai, China.

Phylogenetic analysis

Blast searches were made to retrieve the closest matches in GenBank and recently published sequences (Suetrong *et al.*, 2009; Pang *et al.*, 2013; Hyde *et al.*, 2016, 2017; Zhang *et al.*, 2017). Combined analyses of SSU and LSU sequence data of 27 taxa (Table 1) were analysed under different optimality criteria as suggested by Jeewon *et al.* (2002, 2003). The combined sequence alignments were obtained

Table 1. GenBank accession numbers of the sequences used in phylogenetic analyses

Taxa	*Strain* [a]	*Genbank accession numbers*	
		SSU	*LSU*
Acrospermum adeanum	M133	EU940031	EU940104
Acrospermum compressum	M151	EU940012	EU940084
Acrospermum gramineum	M152	EU940013	EU940085
Aliquandostipite siamensiae	SS81.02	EF175645	EF175666
Aliquandostipite khaoyaiensis	CBS 118232	AF201453	GU301796
Dendryphiopsis atra	AFTOL-ID 273	DQ677996	DQ678046
Dyfrolomyces thamplaensis	MFLUCC 15-0635	KX925436	KX925435
Dyfrolomyces phetchaburiensis	MFLUCC 15-0951	MF615403	MF615402
Dyfrolomyces rhizophorae	JK 5456A/JK_5349A	GU479766	GU479799
Dyfrolomyces thailandica	MFLUCC 15-0945	KX611367	KX611366
Dyfrolomyces tiomanensis	NTOU3636	KC692155	KC692156
Geoglossum nigritum	AFTOL-ID 56	AY544694	AY544650
Jahnula sangamonensis	A482-1B	EF175640	EF175662
Jahnula seychellensis	SS2113.2	EF175643	EF175664
Kirschsteiniothelia aethiops	CBS 109.53	AY016344	AY016361
Kirschsteiniothelia sp.	MFLUCC 10-0036	HQ441569	HQ441568
Lecanora hybocarpa	AFTOL-ID 639	DQ782883	EF105421
Manglicola guatemalensis	BCC20157	FJ743444	FJ743450
Manglicola guatemalensis	BCC24217	FJ743441	FJ743447
Melomastia italica	MFLUCC 15-0160	MG029459	MG029458
Melomastia maolanensis	GZCC 16-0102	KY111906	KY111905
Petriella setifera	AFTOL-ID 956	DQ471020	DQ470969
Rhizocarpon oederi	AFTOL-ID 1372	DQ983486	DQ986804
Roccellographa cretacea	AFTOL-ID 93	DQ883705	DQ883696
Schismatomma decolorans	UKE:47570	NG_013155	NG_027622
Trichoglossum hirsutum	AFTOL-ID 64	AY544697	AY544653
Xylaria acuta	AFTOL-ID 63	AY544719	AY544676

[a] *A* Carol Shearer, ascomycetes; *AFTOL-ID* Assembling the Fungal Tree of Life: *BCC* Belgian Coordinated Collections of Microorganisms: *CBS* CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; *GZCC* Guizhou Culture Collection Guiyang, China; *JK* J. Kohlmeyer; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; *S* working collection of William Quaedvlieg.

from MEGA7 version 7.0.14 (Kumar *et al.*, 2015) and ambiguously aligned regions were excluded, gaps were treated as missing data and further alignments performed in BioEdit v. 7.2 (Hall, 1999). Phylogenetic trees were inferred under maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI).

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis, 2006) implemented in raxmlGUI v.1.3 (Silvestro & Michalak, 2012). The 1000 rapid bootstrap replicates were run with generalized time reversible GTRGAMMA model of nucleotide substitution and searches for model selected for ML were applied.

Maximum parsimony (MP) analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2003). The trees were inferred using the heuristic search option with tree bisection-reconnection (TBR) as the branch swapping algorithm and 1000 random sequence additions. Maxtrees were setup to 5000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC] and homoplasy index [HI] were calculated for the Maximum Parsimonious Tree (MPT). The robustness of the most parsimonious trees were evaluated by 1000 bootstrap replications, each with ten replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino-Hasegawa tests (KHT) were performed to determine whether the trees were significantly different (Kishino & Hasegawa, 1989).

Bayesian inference (BI) analysis was performed using the Markov Chain Monte Carlo (MCMC) method with MrBayes 3.2.2 (Ronquist *et al.*, 2012). The best-fit nucleotide substitution models for each dataset were separately determined using MrModeltest version 2.2 (Nylander, 2004). GTR+I+G were selected as best-fitting models for the SSU, LSU and TEF datasets. The MCMC analyses, with four chains, were run, started from random tree topology and lasted 5,000,000 generations and sampled every 100 generations (Nylander, 2004). The Tracer v. 1.5.0 program was used to check the effective sampling sizes (ESS) that should be above 200, the stable likelihood plateaus and burn-in value (Rambaut, 2013). The first 5000 generations were excluded as burn-in.

The phylograms are visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/; Rambaut, 2012) and made in Adobe Illustrator CC and Adobe Photoshop CS6 Extended version 13.1.2 × 64. Sequences data from this study are deposited in GenBank.

RESULTS

Molecular phylogeny

The combined dataset consisted of 27 taxa with a total length of 1909 characters including alignment gaps (1-1007 and 1010-1909 were derived from SSU and LSU sequence data respectively). The result from the partition homogeneity test (PHT) was not significant (level 95%), indicating that the individual data sets were congruent and could be combined. Parsimony analysis indicate that 1909 characters were constant, 146 variable characters were parsimony uninformative and 483 characters were parsimony informative and yielded 1000 most parsimonious trees (TL = 1578, CI = 0.570, RI = 0.749, RC = 0.426, HI = 0.430).

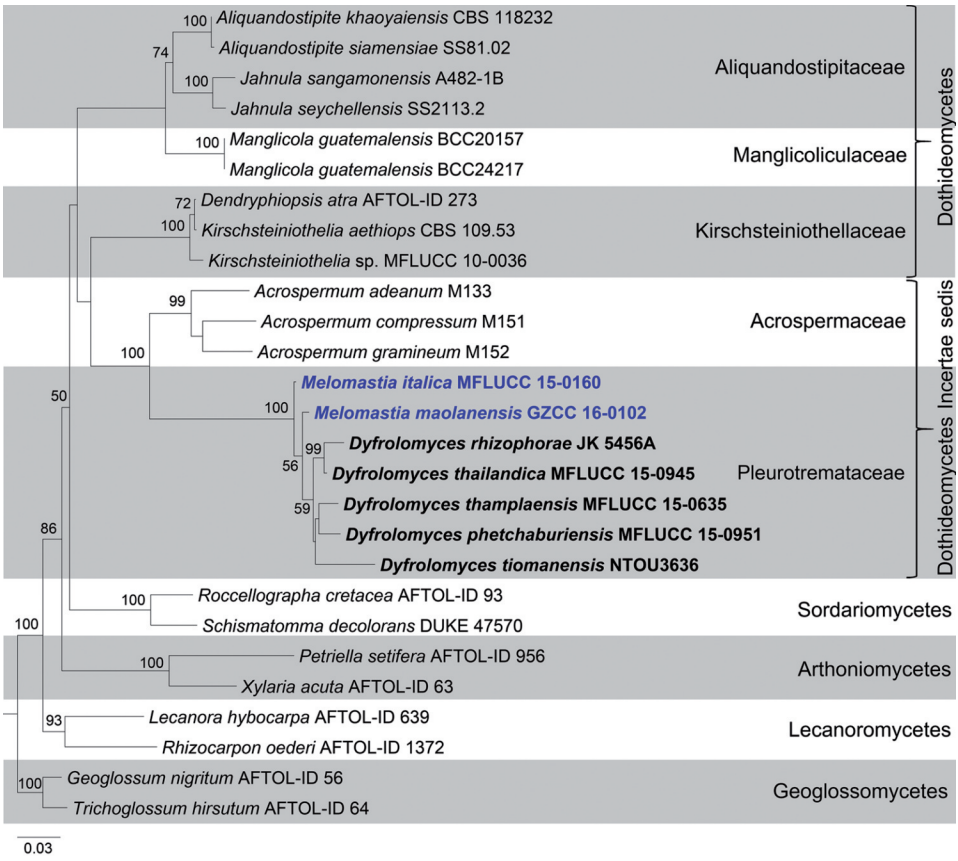


Fig. 1. Phylogram generated from maximum likelihood (RAxML) based on analysis of a combined SSU and LSU sequence data. Bootstrap values ≥ 50% given at the nodes. The species obtained in this study is in blue bold. Ex-type taxa from other studies are in black bold. *Geoglossum* and *Trichoglossum* were used as outgroups.

ML, MP and BY analyses of the combined data resulted in phylogenies that can separate genera within *Pleurotremaceae* (Dothideomycetes) as shown in Figs 1, 2 and 3 respectively. Bootstrap support values of ML, MP (≥ 50%) and Bayesian posterior probabilities (≥ 0.95) are shown on the upper branches (Figs 1, 2, 3). Taxa from Geoglossomycetes were used as outgroups.

Taxonomy

Dyfrolomycetales K.L. Pang, K.D. Hyde & E.B.G. Jones, in Hyde *et al.*, Fungal Diversity 63: 7 (2013)

Pleurotremataceae Walt. Watson, New Phytol. 28: 113 (1929)

= *Dyfrolomycetaceae* K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, in Pang, Hyde, Alias, Suetrong, Guo & Jones, Cryptog. Mycol. 34(3): 227 (2013)

For description see Maharachchikumbura *et al.* (2016)

Type: *Pleurotrema* Müll.

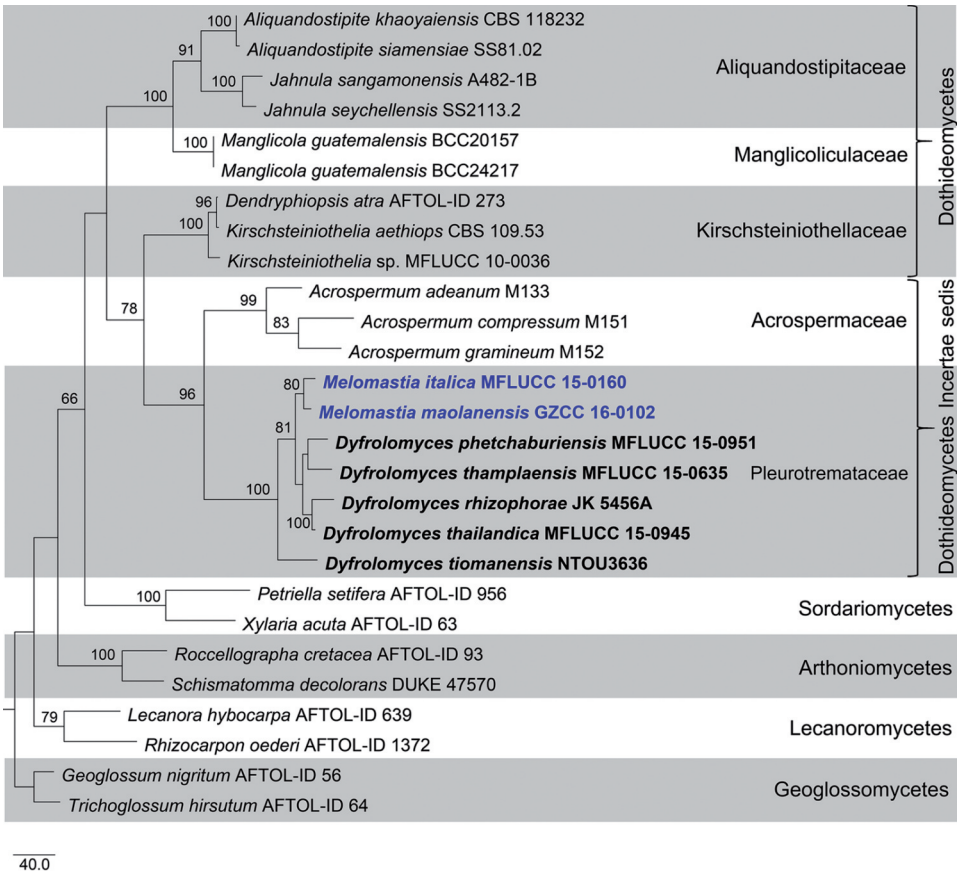


Fig. 2. Phylogram generated from maximum parsimony based on analysis of a combined SSU and LSU sequence data. Bootstrap values ≥ 50% given at the nodes. The species obtained in this study is in blue bold. Ex-type taxa from other studies are in black bold. *Geoglossum* and *Trichoglossum* were used as outgroups.

Melomastia Nitschke ex Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 44: 90 (1875)

Ascomata raised, immersed, globose, black; in vertical section obpyriform, with a central periphysate ostiolar canal. *Peridium* comprising several layers of brown to dark brown, compressed cells. *Paraphyses* filamentous, flexuose, numerous. *Asci* 8-spored, cylindrical, thick-walled, non fissitunicate, pedicellate, apically rounded, with a J-, subapical ring. *Ascospores* uniseriate, ovoid, hyaline, 2-septate, strongly constricted at the septa, with a lipid globule in each cell, surrounded by a gelatinous sheath.

Type species: *Melomastia friesii* Nitschke

Melomastia mastoidea (Fr.) J. Schröt., in Cohn, Krypt.-Fl. Schlesien (Breslau) 3.2(3): 320 (1894) [1908]

= *Melomastia friesii* Nitschke, in Fuckel, Jb. nassau. Ver. Naturk. 25-26: 306 (1871)

Fig. 4

For descriptions see Saccardo (1875) and Kang *et al.* (1999)

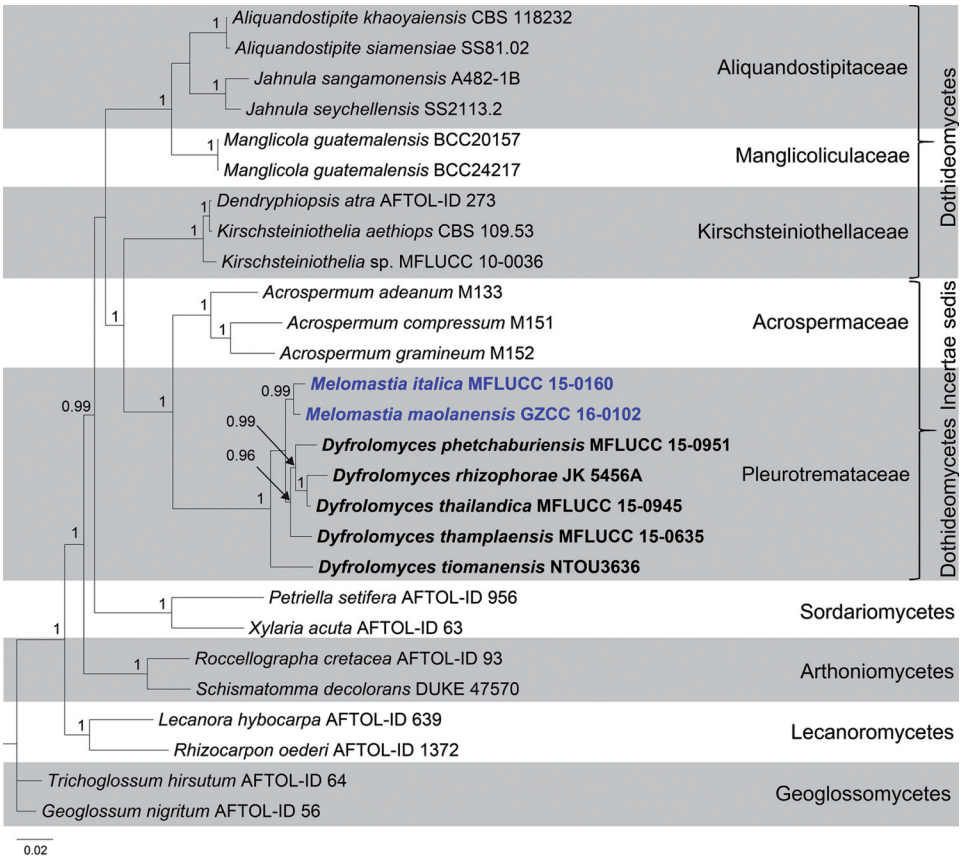


Fig. 3. Phylogram generated from Bayesian analysis based on analysis of a combined SSU and LSU sequence data. Bayesian posterior probabilities ≥ 0.95 (PP) are given at the nodes. The species obtained in this study is in blue bold. Ex-type taxa from other studies are in black bold. *Geoglossum* and *Trichoglossum* were used as outgroups.

Melomastia italica Norphanphoun, E. Camporesi, T.C. Wen & K.D. Hyde, ***sp. nov.***

Index Fungorum number: IF553984; *Facesoffungi number*: FoF 03853

Fig. 5

Etymology: refers to the country where the fungus was collected.

Holotype: MFLU 15-0228

Saprobic on dead branches. **Sexual morph**: *Ascomata* 400-500 µm high, up to 500 µm diam, perithecial, semi-immersed to immersed in host tissue, subglobose to obpyriform, single, scattered, black, with a short ostiolar neck and erumpent ostiole. *Peridium* thick walled, formed of *textura prismatica*. *Paraphyses* simple, apex inflated at tip. *Asci* 120-190 × 5.1-8.9 µm ($\bar{x}$ = 175.5 × 7.5 µm), 8-spored, thick-walled, non fissitunicate, cylindrical or subclavate, undifferentiated at apex, immature asci in Meltzer's reagent showing J-, apical ring. *Ascospores* 8.8-10.5 × 2.8-4.1 µm ($\bar{x}$ = 10.1 × 4 µm), uniseriate, ellipsoid, contents granular, 2-septate, constricted at the septa, hyaline glistening, smooth-walled, surrounded by a gelatinous sheath when immature. **Asexual morph**: Undetermined.

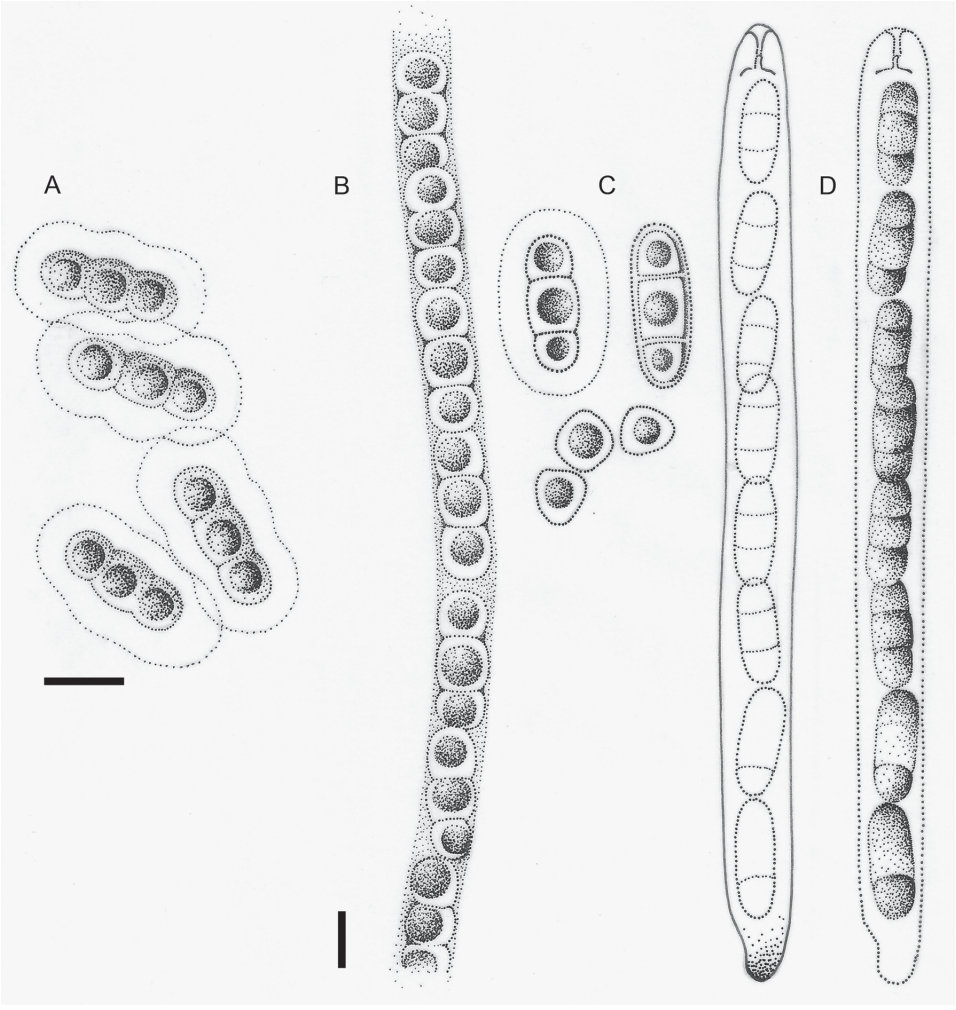
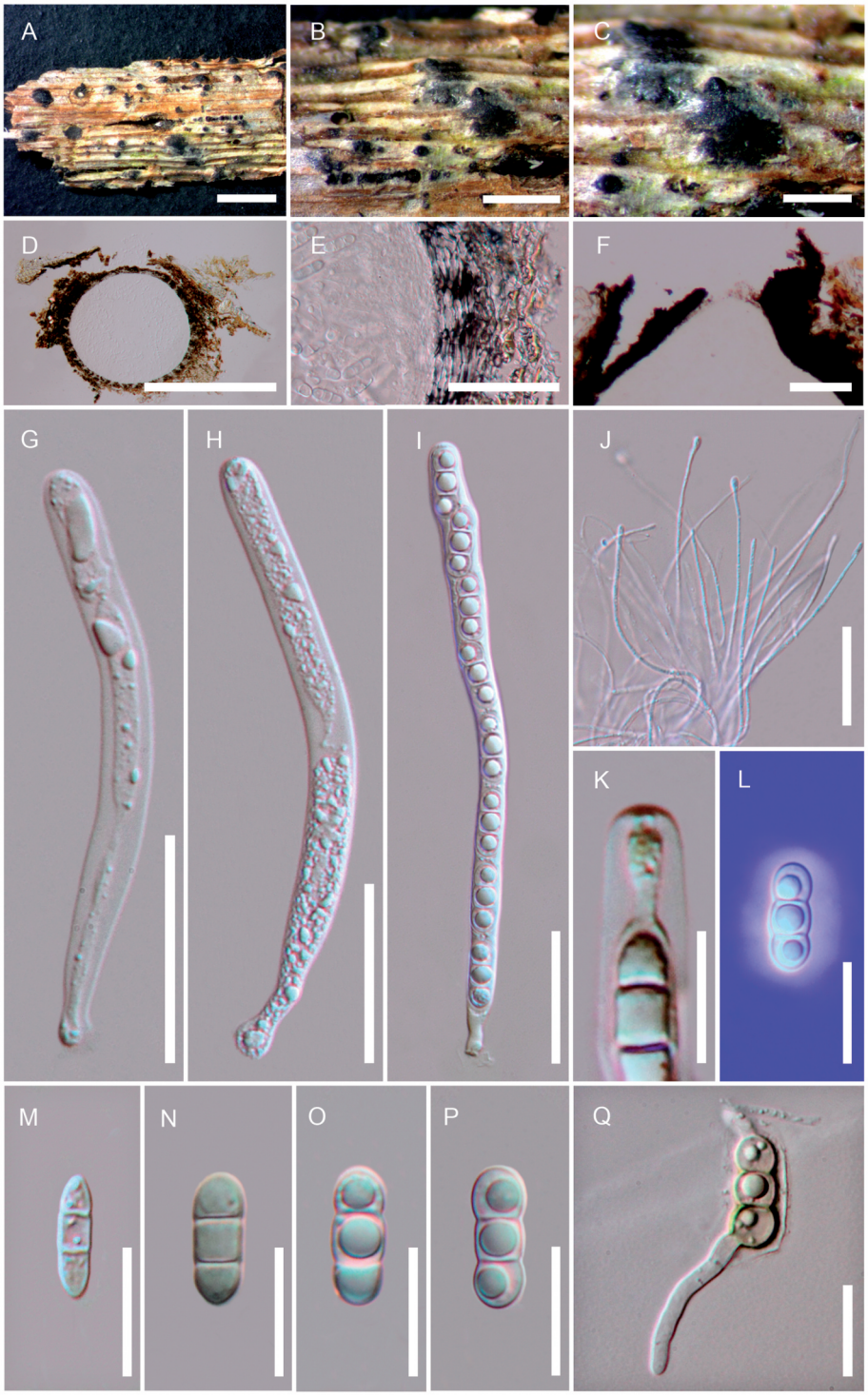


Fig. 4. Morphology of *Melomastia mastoidea* (redrawn from Barr, 1994; Kang *et al.*, 1999). **A, C.** Ascospores. **B, D.** Asci. Scale bars: A, B = 10 µm.

Material examined: ITALY, Province of Forlì-Cesena, near Monte Cavallo - Meldola on dead aerial branches of *Vitis vinifera* L., 1 January 2015, E. Camporesi IT1578 (MFLU 15-0228, **holotype**; KUN, **isotype**); ex-type-living cultures, MFLUCC 15-0160, KUMCC.

Notes: *Melomastia* was introduced by Nitschke (1875) with *M. friesii* as the type species found on *Viburnum opulus* L. (*Adoxaceae*) in Germany. Later, the species was synonymized under *Melomastia mastoidea* (Schröter, 1894). *Melomastia italica* resembles *Melomastia* due to its ellipsoid, granular, 2-septate, hyaline and glistening ascospores, surrounded by a gelatinous sheath (Barr, 1994; Kang *et al.*, 1999). It is most similar to *M. antarctica*, *M. chilensis* and *M. mastoidea*. However, they differ in ascospore size and presence or absence of a gelatinous sheath.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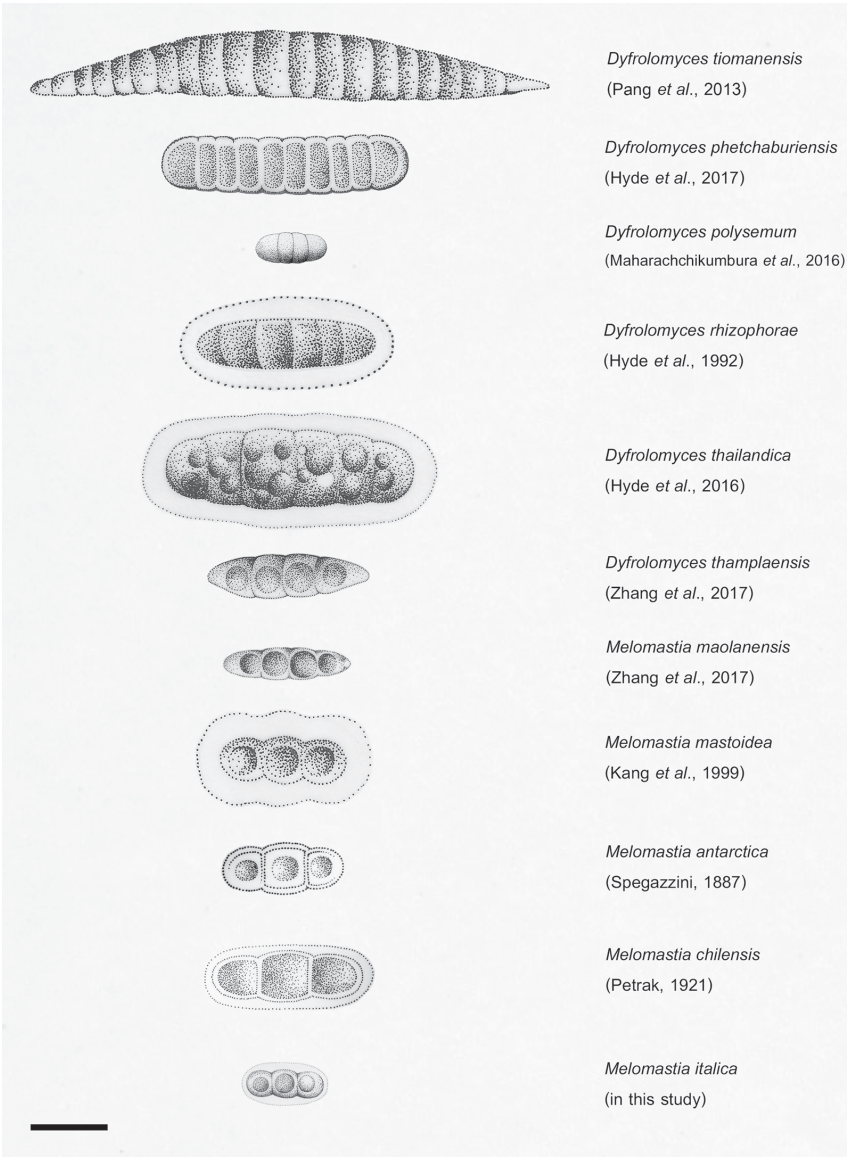

Fig. 6. Ascospore of species of *Pleurotremaceae* discussed in the text (redrawn from Petrak (1921), Spegazzini (1887), Hyde (1992), Pang *et al.* (2013), Hyde *et al.* (2016, 2017), Maharachchikumbura *et al.* (2016) and Zhang *et al.* (2017)). Scale bar 10 µm.

◀ Fig. 5. **Morphology of *Melomastia italica*** (MFLUCC 15-0160, **holotype**). **A.** Habitat. **B, C.** Habit of ascomata in host. **D.** Section through ascoma. **E.** Peridium. **F.** Ostiole. **G-H.** Immature asci. **I.** Mature ascus. **J.** Paraphyses. **K.** Immature ascus in Meltzer's reagent showing J+, apical apparatus. **L.** Mature ascospore stained in Indian ink. **M-N.** Immature ascospores. **O-P.** Development of mature ascospores. Q. Germinated ascospore, Scale bars: A = 2 mm, B = 1 mm, C = 500 µm, D = 400 µm, E = 50 µm, F = 100 µm, G-J = 30 µm, K- Q = 10 µm.

Melomastia antarctica ascospores are 15 × 4.2 µm (without a gelatinous sheath), *M. chilensis* 16-18 × 6-7 µm, *M. mastoidea* 18.1 × 5.4 µm, and *M. italica* 10.1 × 4 µm (Spegazzini, 1887; Petrak, 1921; Barr, 1994; Kang *et al.*, 1999) (Table 2).

New combination

Melomastia maolanensis (J.F. Zhang, J.K. Liu, K.D. Hyde & Z.Y. Liu) Norphanphoun, T.C. Wen & K.D. Hyde, *comb. nov.*

= ***Dyfrolomyces maolanensis*** J.F. Zhang, J.K. Liu, K.D. Hyde & Z.Y. Liu, *Phytotaxa* 313: 267-277 (2017)

Index Fungorum number: IF554041

Genera included in family *Pleurotremaceae*

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

Type: *Dyfrolomyces tiomanensis* K.L. Pang *et al.*

For description see Pang *et al.* (2013)

Melomastia Nitschke ex Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 44: 90 (1875)

Type: *Melomastia mastoidea* (Fr.) J. Schröt.

Pleurotrema Müll. Arg., Bot. Jb. 6: 388 (1885)

Type: *Pleurotrema polysemum* (Nyl.) Müll. Arg., Bot. Jb. 6: 389 (1885)

For description see Spegazzini (1879) and Maharachchikumbura *et al.* (2016)

DISCUSSION

Melomastia was introduced by Nitschke (1875) and is characterized by globose ascomata with erumpent apex, cylindrical asci and 2-septate ascospores with a gelatinous sheath (Barr *et al.*, 1994; Kang *et al.*, 1999). The genus was re-examined by Barr *et al.* (1994) and Kang *et al.* (1999) based on type material, but taxonomic relationships and the position within the Ascomycota remained uncertain. This study, which incorporates a morphological and a phylogenetic approach based on DNA sequence data, partially resolves the taxonomic confusion of *Dyfrolomyces* and *Melomastia* with the genus *Pleurotrema* based on its type species (*Pleurotrema polysemum* (Nyl.) Müll.). Phylogenies clearly indicate that they do not belong in Sordariomycetes as previously postulated by earlier mycologists (Maharachchikumbura *et al.*, 2015; Hyde *et al.*, 2016; Feng *et al.*, 2017). Instead they belong in Dothideomycetes. Our phylogenetic results also corroborate those that pertain to the familial placement of *Dyfrolomyces* and *Melomastia*. *Dyfrolomyces, Melomastia* and *Pleurotrema* are hereby accommodated in the family *Pleurotremaceae* and the latter is closely related to the family *Acrospermaceae* (Figs 1, 2, 3). This relationship is

Table. 2. Synopsis of species of *Pleurotremaceae* discussed in the paper

Species	*Habitat/ Host*	*Ascomata (μm)*	*Asci (μm)*	*Ascospores (μm)*	*Reference*
Dyfrolomyces aquatica = *Saccardoella aquatica*	–	560-700	204.2 × 7.9	29.2 × 7, 3-septate with inconspicuous gelatinous sheath	Tsui *et al.* (1998)
Dyfrolomyces mangrovei ≡ *Saccardoella mangrovei*	Intertidal tertidal mangrove wood	380-585 × 390-485	216 × 8.5-14	26-33 × 6-8, 7-9-septate with mucilaginous sheath	Hyde (1992)
Dyfrolomyces marinospora ≡ *Saccardoella marinospora*	Intertidal mangrove wood	780-1040 × 650-960	190-240 × 10-12	25-31 × 7.5-10, 3-septate with mucilaginous sheath	Hyde (1992)
Dyfrolomyces tiomanensis	On mangrove wood	565-(615)-667 × 283-(374)-446	316-(323)-333 × 12-(16)-17	69-(74)-82 × 9-(10)-11, 20-24-septate without sheath	Pang *et al.* (2013)
Dyfrolomyces phetchaburiensis	Wood of *Rhizophora apiculata*	300 × 264	230 × 10.2	37 × 7.0, 10-septate	Hyde *et al.* (2017)
Dyfrolomyces rhizophorae	Intertidal mangrove wood of *Rhizophora apiculata*	325-455 × up to 364	135-160 × 8-10	19-26 × 6-8, 4-6-septate with thin sheath	Hyde (1992)
Dyfrolomyces thailandica	–	430-350 × 242-280	152 × 8.2	28 × 6.5, 3-5-septate with gelatinous sheath	Hyde *et al.* (2016)
Dyfrolomyces thamplaensis	Dead branch	–	137 × 7	22 × 5.5, 3-septate without sheath	Zhang *et al.* (2017)
Melomastia antarctica	*Gaultheria mucronata*	400-900	130-150 × 6	15 × 4.2, 2-septate without sheath	Spegazzini (1887)
Melomastia chilensis	*Sophora macrocarpa*	250-400	160-180 × 9-10	16-18 × 6-7, 2-septate with gelatinous sheath	Petrak (1921)
Melomastia italica	*Vitis vinifera*	400-500 × up to 500	175.5 × 7.5	10.1 × 4, 2-septate with gelatinous sheath	In this study
Melomastia maolanensis = *Dyfrolomyces maolanensis*	Dead branch	–	110.9 × 4.9	16.2 × 3.9, 3-septate without sheath	Zhang *et al.* (2017), In this study
Melomastia mastoidea	*Rosa*	500-762 × 350-435	198.7 × 8.3	18.1 × 5.4, 2-septate with gelatinous sheath	Barr (1994) and Kang (1999)

Table. 3. Occurrence of *Melomastia* species on hosts and references

Species	*Year*	*Host records*	*Location*
Melomastia antarctica Speg.	1887	*Pernettya mucronata*	Argentina
Melomastia aquilegiae Domashova	1960	*Aquilegia karelini*	Kirghiz SSR
Melomastia calami Syd.	1928	*Calamus* sp.	Philippine
Melomastia calligoni Kravtzev	1960	*Calligonum* sp.	Central Asia
Melomastia carinata Petr.	1942	*Erhedra*	Iran
Melomastia chilensis Speg.	1921	*Sophora macrocarpa*	Chile
Melomastia clypeata Petr.	1923	*Salix martiana*	Brazil
Melomastia coffeae Saccas	1981	*Coffea robusta*	Central African Republic
Melomastia constricta Frolov	1967	*Malus turkmenorum*	Turkmen SSR
		Cydonia oblonga	Central Asia
Melomastia corylina Feltgen	1901	*Corylus*	Luxemburg
Melomastia graminicola Saccas	1954	*Sorghum vulgare*	French
Melomastia haloxyli Kravtzev	1955	*Haloxylon aphyllum*	Kazakh SSR
Melomastia heteroderma Syd.	1936	–	Cuba
Melomastia heveae Saccas	1954	*Hevea brasiliensis*	Africa
Melomastia hyalostoma Luc	1951	*Cola vera*	Ivory Coast
Melomastia italica Norph. *et al.*	2015	*Vitis vinifera*	Italy
	2013	*Arundo* spp.	Italy
Melomastia jaapiana Kirschst.	1911	*Betulaceae*	Germany
Melomastia kazachstanica Kravtzev	1955	*Ammodendron conollyi* *Haloxylon persicum*	Central Asia
Melomastia lignicola Kirschst.	1910	*Betula pendula*	Germany
Melomastia mastoidea (Fr.) J. Schröt.	1894	*Chaenomeles speciosa*	Ukraine
		Cornus sanguinea	Denmark
		Deutzia corymbosa	India
		Fraxinus excelsior	Poland
		Fraxinus sp.	Denmark
		Lantana involucrata	Bermuda
		Lonicera periclymenum	Denmark
		Lonicera quinquelocularis	India
		Lonicera xylosteum	Denmark
			Poland
			Russia
		Osmanthus fragrans	Ukraine
		Populus tremula	Denmark
		Rubia peregrina	Portugal
		Sambucus nigra	Denmark
		Symphoricarpos sp.	Denmark
		Syringa sp.	Denmark
			England
		Viburnum opulus	Denmark
			Poland
Melomastia metasequoiae Gucevič	1960	*Metasequoia glyptostroboides*	Ukraine
Melomastia maolanensis (Zhang *et al.*) Norph., T.C.Wen & Hyde	2016	–	China
Melomastia nigrificans Feltgen	1903	*Salicis*	Luxemburg
Melomastia pallidispora Kirschst.	1911	*Trematosphaeria pallidispora*	Italy
Melomastia popuschoji Frolov	1967	*Amygdalus turcomanica*	Turkmen SSR
Melomastia prorumpens (Rehm) Kirschst. ≡ *Trematosphaeria prorumpens* Rehm. = *Zignoëlla prorumpens* (Rehm) Sacc.	1939	Pine	Germany
Melomastia salicicola (Fabre) Feltgen ≡ *Zignoëlla salicicola* Fabre.	1901	*Salix alba*	Vaucluse Galliae
Melomastia saxauli Kravtzev	1955	*Haloxylon persicum* *Salsola arbuscula* *Salsola rigida*	Central Asia
Melomastia sedi Gucevič	1967	*Sedum acre*	Crimean SSR
Melomastia shastensis Earle	1905	*Abies magnifica* var. *shastensis*	California
Melomastia yezoensis I. Hino & Katum.	1960	*Sasa kurilensis*	Japan

strongly supported and consistent even when the same DNA dataset was subjected to different types of analyses (Figs 1, 2, 3). From a morphological perspective, all of these genera are characterized by thick-walled, non fissitunicate asci, and uniseriate, hyaline ascospores which are constricted at the septum, with a gelatinous sheath, which are typical of the *Pleurotremaceae*. In all phylogenies, all *Acrospermum* species constitute a well-supported independent lineage, which supports its generic rank in the family *Acrospermaceae*, sister to the *Pleurotremaceae*. In addition, results herein confirm that *Melomastia* should be accommodated in *Pleurotremaceae* (Barr, 1994; Hyde, 1992; Kang *et al.*, 1999; Lumbsch & Huhndorf, 2010; Maharachchikumbura *et al.*, 2015, 2016; Hyde *et al.*, 2016).

Our molecular study revealed interesting taxonomic relationships of the genus *Melomastia*. Both MP and BA derived phylogenies are congruent and indicate that *Melomastia* should be given generic status. Our species sampled herein cluster together with high support and are nested in between *Dyfrolomyces* species (Figs 2, 3). Analyses of the individual LSU datas*et als*o yielded similar topologies (results not shown). The only discrepancy we noted with respect to *Melomastia* was the placement of the two *Melomastia* species in the ML analyses (Fig 1). *Melomastia italica* segregates in an independent lineage with high support and a similar scenario is observed for *Dyfrolomyces maolanensis* which is nestled between *Melomastia italica* and other *Dyfrolomyces* species, albeit with weak support (Fig 1). Either this depicts a paraphyletic nature of *Melomastia* or simply an artifact during phylogenetic analyses. However, this anomaly reinforces our taxonomic assumption that *Melomastia italica* is a new taxon given its distinct phylogeny. On the other hand, it is worth mentioning that analyses of the LSU gene region only recovered similar phylogeny as the combined dataset (results not shown) and hence we rely mostly on the MP and BA for further taxonomic discussion. Despite a close affinity between *Dyfrolomyces maolanensis* and *Melomastia italica* in other analyses, we consider that *Melomastia italica* is a novel species given its distinct morphological features as compared to *Dyfrolomyces maolanensis*. The latter possesses, *Melomastia italica* is characterized by 2-septate, hyaline and contents granular of ascospores. In addition, comparison of the LSU nucleotides sequenced revealed striking differences in 16 base pairs that justifies it as a new taxon (Jeewon & Hyde, 2016).

There have also been some taxonomic irregularities with respect to the generic status of *Dyfrolomyces, Melomastia* and *Pleurotrema*. In fact, if we take a conservative approach we could combine *Dyfrolomyces* and *Pleurotrema* under the earlier *Melomastia.* However, despite some morphological overlap, our phylogeny supplemented with accurate morphological definitions, indicate that these should be considered as distinct genera. While our molecular data clearly indicates that *Dyfrolomyces* are phylogenetically apart, they are not significantly distinct in morphological characteristics such as septate, variously size ascospores (Fig. 6). However, we predict that many more taxa in these genera will be found and they will be supported as distinct in both phylogeny and morphology. Further studies with more *Pleurotremaceae* collections, especially of *Pleurotrema polysemum* are essential to resolve taxonomic relationships within this family.

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