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Additions to Taiwan fungal flora 1: Neomassariaceae fam. nov.

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Abstract: In the course of surveys on dothideomycetous fungal genera associated with various hosts in Taiwan, Neomassaria-like species were collected as saprobes on dead stems of *Rhododendron* sp. Maximum likelihood and Bayesian phylogenetic analyses based on concatenated LSU, *rpb2*, SSU and *tef1* gene matrices indicate that Neomassaria-like isolates generated in our study formed a separate clade in a sister group relationship with *Neomassaria fabacearum* with high statistical support. Hence, the new species *Neomassaria formosana* is described to accommodate the new linage in *Neomassaria.* The new species is characterised by immersed to erumpent, papillate ascomata with a dark brown peridium, fissitunicate, cylindrical to oblong asci and fusoid to ellipsoid, hyaline, bicellular ascospores with 4–6 large guttules. Moreover, *Neomassaria* formed a monophyletic and well-supported lineage with an uncertain phylogenetic placement within the Pleosporales. Therefore, the new family Neomassariaceae is proposed for the genus *Neomassaria* within the order Pleosporales, Dothideomycetes. The morphological characters also support justification for both the new family and the new species.

Dothideomycetes / Multi-gene analysis / New family / Pleosporales / Saprobe

INTRODUCTION

The Dothideomycetes is the largest and most diverse class of the phylum Ascomycota including more than 19,000 species (Hyde *et al.*, 2013; Jaklitsch *et al.*, 2016; Wijayawardene *et al.*, 2018). Currently the class Dothideomycetes contains 33 recognised orders identified by molecular phylogenetic studies in combination with morphological data (Hyde *et al.*, 2013; Wijayawardene *et al.*, 2018). Among the orders of Dothideomycetes, Pleosporales is the largest and most diverse order containing more than 70 families (Wijayawardene *et al.*, 2018). Members of

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Pleosporales are characterised by perithecioid ascomata typically with a papilla and bitunicate, generally fissitunicate asci bearing mostly septate ascospores of different colours and shapes, with or without a gelatinous sheath (Zhang *et al.*, 2009, 2012; Hyde *et al.*, 2013; Jaklitsch & Voglmayr, 2016; Jaklitsch *et al.*, 2018).

The family Massariaceae was introduced by Nitschke (1869) to place the genus *Massaria* and typified with *M. inquinans* (Tode) De Not. (Hyde *et al.*, 2017). *Massaria* species discovered by molecular phylogeny can also be characterised by morphological features such as ascospore shape, ascospore colour in the intact ascus versus post-discharge and staining of the substrate (Voglmayr & Jaklitsch, 2011). Multi-gene phylogenies showed that the family Massariaceae is likely to represent the most basal clade of the order Pleosporales (Schoch *et al.*, 2009; Voglmayr & Jaklitsch, 2011; Zhang *et al.*, 2012; Hyde *et al.*, 2013, 2017). Recently, Hyde *et al.* (2017) introduced the monotypic genus *Neomassaria* to include *N. fabacearum* in the family Massariaceae. This proposal was supported by multi-gene phylogeny coupled with morphology, where the type strain of *Neomassaria fabacearum* formed a distinct clade basal to the monophyletic genus *Massaria* (Hyde *et al.*, 2017).

Taiwan is recognised as an extremely biodiverse country in the tropics due to its warm and humid weather (Sivanesan & Hsieh, 1989; Hsieh & Li, 1991; Tzean *et al.*, 1997). A number of investigations implemented in the course of the last few years have advanced our understanding of the dothideomycetous fungal flora in Taiwan (Chang & Wang, 2009; Pang *et al.*, 2011; Yang *et al.*, 2016; Ariyawansa *et al.*, 2018; Tennakoon *et al.*, 2018). In fact, the number of ascomycete species being revealed is progressively increasing due to increasing investigations of micro-fungi in a great variation of terrestrial and aquatic environments (Sivanesan & Hsieh, 1989; Hsieh & Li, 1991; Hsieh *et al.*, 1997, 1998; Tzean *et al.*, 1997, 1998; Chang *et al.*, 1998; Ju *et al.*, 2004, 2011; Ariyawansa *et al.*, 2018; Tennakoon *et al.*, 2018). During a species diversity study of saprobic fungi in Taiwan, an unidentified dothideomycetous fungus was discovered. The purpose of the present study was to determine the molecular systematics of the discovered fungus based on DNA sequence data aided by morphological features.

MATERIALS AND METHODS

Sample collection and specimen examination

During the survey of dothideomycetous fungi in Taipei, Taiwan, woody samples were collected during 2017–2018 and returned to the laboratory in zip-lock plastic bags. The samples were processed and examined following the method described in Ariyawansa *et al.* (2016a, b).

Fresh materials were examined using a Motic SMZ 168 dissecting microscope to locate ascomata. Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The samples were examined using an Olympus BX51 microscope with differential interference contrast (DIC). Single ascospore isolations were done by following an improved method of Ariyawansa *et al.* (2015). Contents of the sectioned fruiting structures were moved to a drop of sterile water on a flame-sterilized slide. Drops of the spore suspension were pipetted and distributed on a Petri dish containing 2% water agar (WA) and incubated at 25°C. Germinated ascospores were transferred individually to 2% MEA

(Ariyawansa *et al.*, 2015). Voucher specimens were placed in the herbarium of the Department of Plant Pathology and Microbiology, National Taiwan University (NTUH). Living cultures were deposited at the Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection (NTUCC). Taxonomic descriptions and nomenclature information were registered in MycoBank.

DNA extraction, PCR amplification and sequencing

The extraction of genomic DNA was accomplished as described earlier (Ariyawansa *et al.*, 2015, 2016a, b) using the Bioman Fungus Genomic DNA Extraction Kit (Bioman®) following the manufacturer's protocol (BIOMAN SCIENTIFIC CO., LTD). PCR amplification was carried out in a 50 µl reaction volume containing 5–10 ng DNA, 0.8 units Taq polymerase, 1× PCR buffer, 0.2 mM d'NTPs, 0.3 µM of each primer with the addition of 1.5 mM $MgCl_2$. PCR conditions for amplification of the partial SSU (small subunit of the nrRNA gene) and LSU (large subunit of the nrRNA gene) followed the protocol of Ariyawansa *et al.* (2015). Amplification of partial *rpb2* (RNA polymerase II second largest subunit gene) and partial *tef1* (translation elongation factor 1-α gene) followed the procedure of Ariyawansa *et al.* (2018). Primer sets used for these genes were as follows: LSU: LR0R/LR5; SSU: NS1/NS4; (White *et al.*, 1990; Liu *et al.*, 1999; Sung *et al.*, 2007); *tef1*: EF1 983/2218R (Carbone & Kohn, 1999; Hyde *et al.*, 2017) and *rpb2*: fRPB2-SF/ fRPB2-7cR (Ariyawansa *et al.*, 2018). The PCR products were checked on 1.5% agarose gels stained with SYBR safe DNA gel stain. PCR products were purified and sequenced at the Genomics Company, New Taipei, Taiwan using Sanger sequencing method. DNASTAR Lasergene SeqMan Pro v.8.1.3 was used to acquire consensus sequences from sequences produced by forward and reverse primers. Newly obtained sequences were placed at NCBI GenBank under the accession numbers provided in Table 2.

Sequence alignment and phylogenetic analyses

LSU, *rpb2*, SSU and *tef1* genes were included in the phylogenetic analyses. NCBI BLASTn searches were made to reveal the closest matches in GenBank. All sequences obtained from GenBank and used by Voglmayr & Jaklitsch (2011), Hyde *et al.* (2013, 2017), Ariyawansa *et al.* (2015, 2018), Jaklitsch &Voglmayr (2016), Hernandez-Restrepo *et al.* (2017), Hashimoto *et al.* (2017), Wanasinghe *et al.* (2017), Valenzuela-Lopez *et al.* (2018), which were included in the analyses, are listed in Table 2. Multiple sequence alignments were generated with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html). All introns and exons were aligned separately. Regions containing many leading or trailing gaps were removed from the LSU, *rpb2*, SSU and *tef1* alignments prior to phylogenetic inference. The alignments were checked visually and improved manually where necessary. Concordance of the LSU, *rpb2*, SSU and *tef1* genes datasets was estimated with the partition-homogeneity test implemented with PAUP v. 4.0b10 (Swofford, 2003). Single alignments for each locus and the combined four-gene dataset were analysed using different methods of phylogenetic reconstruction.

Evolutionary models for each locus were determined individually using MrModeltest v. 2.3 (Nylander, 2004) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10.

A maximum likelihood analysis (ML) was executed at the CIPRES webportal (Miller *et al.*, 2010) using RAxML-HPC2 on XSEDE (v 8.2.8) with default parameters and bootstrapping with 1000 replicates (Stamatakis, 2014).

Bayesian Markov Chain Monte Carlo (MCMC) analyses were conducted with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The maximum number of generations was set to 10 million and the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. Trees were saved each 100 generations. MCMC heated chain was set with a "temperature" value of 0.15. The distribution of log-likelihood scores was checked with Tracer v 1.5 to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence (Rambaut & Drummond, 2007). All sampled topologies below the asymptote (20%) were discarded as part of a burn-in process, the remaining trees were used to compute posterior probabilities (PP) in the majority rule consensus tree.

Phylogenetic trees and data files were checked and formatted in MEGA v. 5 (Tamura *et al.*, 2011), TreeView v. 1.6.6 (Page, 2001) and FigTree v. 1.4 (Rambaut & Drummond, 2008). ML bootstrap values equal to or higher than 70 % and BP equal to or higher than 0.90 are given at each node in Figure 1. Nodes with a posterior probability (PP) lower than 0.90 or ML bootstrap support lower than 70% were considered uncertain.

RESULTS

Phylogeny of LSU and SSU datasets

The final LSU and SSU dataset comprised 973 and 997 characters from 77 taxa respectively. *Aliquandostipite khaoyaiensis* (CBS 118232) from Jahnulales is selected as an outgroup taxon (Voglmayr & Jaklitsch 2011; Hyde *et al.*, 2013). The best scoring RAxML tree for LSU received the final ML optimization likelihood value of -7677.939473, while the SSU RAxML tree received a likelihood value of -4948.927761. The phylogeny based on the LSU sequences showed that the newly generated strains in the present study (NTUCC 17-007, NTUCC 17-008 and NTUCC 17-013) together with *Neomassaria fabacearum* (MFLUCC 16-1875) formed a basal lineage in a poorly supported sister relationship with the Massariaceae (data not shown). In contrast, *Neomassaria* strains (NTUCC 17-007, NTUCC 17-008, NTUCC 17-013 and MFLUCC 16-1875) formed a distinct but less supported lineage in a sister group position to the clades of Astrosphaeriellaceae and Delitschiaceae in SSU phylogeny (data not shown). However, in general, most of the topologies of the LSU and SSU trees did not receive significant support, and remain therefore inconclusive with respect to the placement of *Neomassaria* but also of other lineages in these single-locus analyses. Thus, to reveal the phylogenetic affiliations of the Neomassariaceae lineage with the other members of the Pleosporales, we conducted multi gene analyses based on LSU, *rpb2*, SSU and *tef1*.

Phylogeny of combined LSU, *rpb2*, SSU and *tef1* datasets

After exclusion of equivocally aligned positions from each locus, the final concatenated dataset comprised 3835 characters (LSU 973, *rpb2* 989, SSU 997 and *tef1* 876) from 77 taxa. Results of the partition-homogeneity test (P = 0.107) indicate that the LSU, *rpb2*, SSU and *tef1* gene trees reflect the same underlying phylogeny. Therefore, these datasets were combined and analysed by using several tree-building programs. *Aliquandostipite khaoyaiensis* (CBS 118232) from Jahnulales is included as an outgroup taxon for rooting the tree (Voglmayr & Jaklitsch, 2011; Hyde *et al.*, 2013). The best scoring RAxML tree is presented in Figure 1, with the likelihood value of -42787.773081. The MCMC analysis of the four combined genes run for 17000000 generations resulted in 170000 trees. The first 34000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used to calculate posterior probabilities in the majority rule consensus tree. Phylogenetic trees obtained from ML and Bayesian analyses showed similar overall topologies at subclass and family level relationships in agreement with previous studies based on ML and Bayesian analysis (Voglmayr & Jaklitsch, 2011; Hyde *et al.*, 2013, 2017; Ariyawansa *et al.*, 2015, 2018; Jaklitsch & Voglmayr, 2016; Hernandez-Restrepo *et al.*, 2017; Hashimoto *et al.*, 2017; Wanasinghe *et al.*, 2017; Valenzuela-Lopez *et al.*, 2018). Synopsis of the alignment properties and nucleotide substitution models are given in Table 1.

The phylogenetic tree revealed three distinct clades corresponding to the classes Hysteriales, Mytilinidiales and Pleosporales. The three newly isolated strains from this study (NTUCC 17-007, NTUCC 17-008 and NTUCC 17-013) formed a distinct sister clade to *Neomassaria fabacearum* (MFLUCC 16-1875) with high BS and PP support in analyses of the single locus and concatenated datasets. Hence, the novel lineage is regarded here as the new species *Neomassaria formosana*. The genus *Neomassaria* with the two species *Neomassaria fabacearum* and *N. formosana* comprised a monophyletic and well supported clade in an uncertain position within members of Pleosporales included in the dataset.

Table 1. Comparison of alignment properties of genes and nucleotide substitution models used in Pleosporales phylogenetic analyses.

	LSU	*SSU*	*rpb2*	*tef1*
Alignment strategy (MAFFT v6)	G-INS-1	G-INS-1	G-INS-1 +manual	G-INS-1 +manual
Nucleotide substitution models for Bayesian analysis (determined by MrModeltest)	GTR+I+G	HKY+I+G	GTR+I+G	GTR+I+G

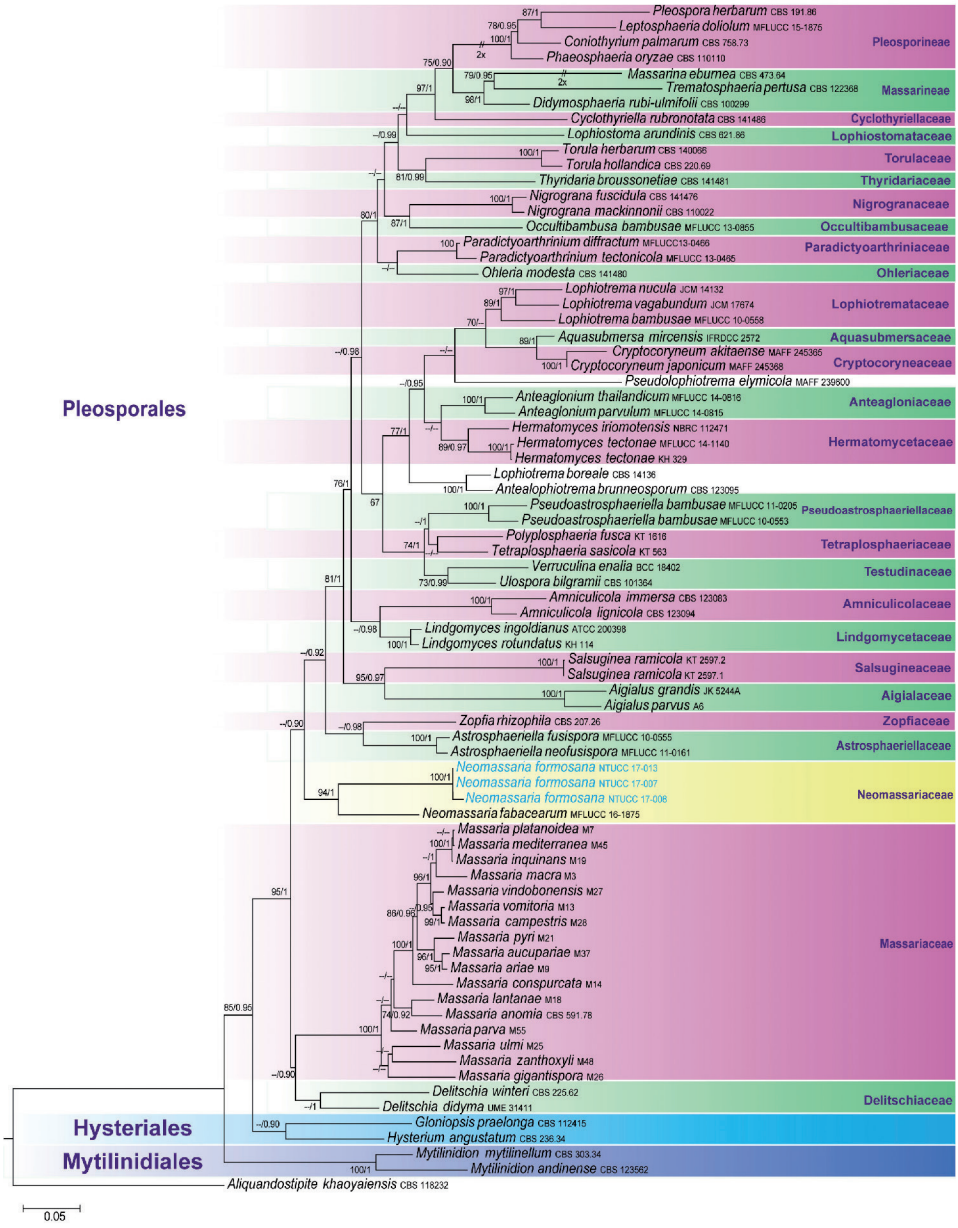

Figure 1. RAxML tree obtained from the concatenated DNA sequence data of LSU and SSU rRNA, *rpb2* and *tef1* genes. The new isolates are shown in blue. ML bootstrap values (BS) ≥70 % and Bayesian posterior probabilities (PP) ≥ 0.90 are presented at the nodes. The branches of the Pleosporineae and *Massaria eburnea* clades were scaled to half to enable a better presentation of the tree. The scale bar indicates the number of estimated substitutions per site. *Aliquandostipite khaoyaiensis* (Aliquandostipitaceae, Jahnulales) was used as outgroup for rooting the tree.

Table 2. Sequence details of the isolates used in the phylogenetic tree. New submissions in bold. En-dash indicates that the sequence is not available.

Taxon	*Culture code*	*SSU*	*LSU*	*tef1*	*rpb2*
Aigialus grandis	JK 5244A	NG016503	GU301793	GU479839	GU479814
Aigialus parvus	A6	GU296133	GU301795	GU349064	GU371771
Aliquandostipite khaoyaiensis	CBS 118232	AF201453	GU301796	GU349048	FJ238360
Amniculicola immersa	CBS 123083	GU456295	FJ795498	GU456273	GU456358
Amniculicola lignicola	CBS 123094	EF493863	EF493861	GU456278	EF493862
Anteaglonium parvulum	MFLUCC 14-0815	KU922912	KU922911	KU922919	-
Anteaglonium thailandicum	MFLUCC 14-0816	KU922910	KU922909	KU922920	-
Antealophiotrema brunneosporum	CBS 123095	LC194298	LC194340	LC194382	LC194419
Aquasubmersa mircensis	IFRDCC 2572	JX276956	JX276955	-	-
Astrosphaeriella fusispora	MFLUCC 10-0555	KT955443	KT955462	KT955425	-
Astrosphaeriella neofusispora	MFLUCC 11-0161	KT955444	KT955463	KT955426	-
Coniothyrium palmarum	CBS 758.73	AY642513	EU754153	DQ677903	KT389592
Cryptocoryneum akitaense	MAFF 245365	LC194306	LC194348	LC096136	LC194430
Cryptocoryneum japonicum	MAFF 245368	LC194312	LC194354	LC096145	LC194436
Cyclothyriella rubronotata	CBS 141486	KX650507	KX650544	KX650519	KX650574
Delitschia didyma	UME 31411	AF242264	AY853366	-	-
Delitschia winteri	CBS 225.62	DQ678026	DQ678077	-	-
Didymosphaeria rubi-ulmifolii	CBS 100299	KJ436587	JX496124	-	-
Gloniopsis praelonga	CBS 112415	FJ161134	FJ161173	FJ161090	FJ161113
Hermatomyces iriomotensis	NBRC 112471	LC194325	LC194367	LC194394	LC194449
Hermatomyces tectonae	MAFF 245731	LC194326	LC194368	LC194395	LC194450
Hermatomyces tectonae	MFLUCC 14-1140	KU712465	KU764695	KU872757	KU712486
Hysterium angustatum	CBS 236.34	GU397359	FJ161180	FJ161096	FJ161117
Leptosphaeria doliolum	MFLUCC 15-1875	GQ387515	KT454719	GU349069	KY064035
Lindgomyces ingoldianus	ATCC 200398	AB521719	AB521736	-	-
Lindgomyces rotundatus	KH 114	AB521725	AB521742	-	-
Lophiostoma arundinis	CBS 621.86	DQ782383	DQ782384	DQ782387	DQ782386
Lophiotrema bambusae	MFLUCC 10-0558	KX672159	KX672154	KX672162	KX672161
Lophiotrema nucula	JCM 14132	AB618703	AB619021	LC194410	LC194465
Lophiotrema vagabundum	JCM 17674	LC194336	LC194378	LC194414	LC194469
Lophiotrema boreale	JCM 14136	LC194333	LC194375	LC194402	LC194457
Massaria anomia	CBS 591.78	GU296169	GU301839	-	GU371769
Massaria ariae	M9	HQ599458	HQ599381	HQ599321	-

Taxon	*Culture code*	*SSU*	*LSU*	*tef1*	*rpb2*
Massaria aucupariae	M37	HQ599451	HQ599383	HQ599323	-
Massaria campestris	M28	HQ599449	HQ599385	HQ599325	HQ599459
Massaria conspurcata	M14	HQ599441	HQ599393	HQ599333	-
Massaria gigantispora	M26	HQ599447	HQ599397	HQ599337	-
Massaria inquinans	M19	HQ599444	HQ599402	HQ599342	HQ599460
Massaria lantanae	M18	HQ599443	HQ599406	HQ599346	-
Massaria macra	M3	HQ599450	HQ599408	HQ599348	-
Massaria mediterranea	M45	HQ599452	HQ599417	HQ599357	-
Massaria parva	M55	HQ599467	HQ599418	-	-
Massaria platanoidea	M7	HQ599457	HQ599420	HQ599359	HQ599462
Massaria pyri	M21	HQ599445	HQ599424	HQ599363	-
Massaria ulmi	M25	HQ599446	HQ599428	HQ599367	-
Massaria vindobonensis	M27	HQ599448	HQ599429	HQ599368	HQ599464
Massaria vomitoria	M13	HQ599440	HQ599437	HQ599375	HQ599466
Massaria zanthoxyli	M48	HQ599454	HQ599439	HQ599377	-
Massarina eburnea	CBS 473.64	GU296170	GU301840	GU349040	FJ795466
Mytilinidion andinense	CBS 123562	FJ161159	FJ161199	FJ161107	FJ161125
Mytilinidion mytilinellum	CBS 303.34	FJ161144	FJ161184	FJ161100	FJ161119
Neomassaria fabacearum	MFLUCC 16-1875	KX524147	NG059708	KX524149	-
Neomassaria formosana	**NTUCC 17-013**	**MH714759**	**MH714756**	**MH714762**	**MH714765**
Neomassaria formosana	**NTUCC 17-007**	**MH714760**	**MH714757**	**MH714763**	**MH714766**
Neomassaria formosana	**NTUCC 17-008**	**MH714761**	**MH714758**	**MH714764**	**MH714767**
Nigrograna fuscidula	CBS 141476	KX650509	KX650547	KX650522	KX650576
Nigrograna mackinnonii	CBS 110022	GQ387552	GQ387613	KF407986	KF015703
Occultibambusa bambusae	MFLUCC 13-0855	KU872116	KU863112	KU940193	KU940170
Ohleria modesta	CBS 141480	KX650513	KX650563	KX650534	KX650583
Paradictyoarthrinium diffractum	MFLUCC13-0466	KP753960	KP744498	-	-
Paradictyoarthrinium diffractum	MFLUCC 13-0465	KP753961	KP744500	-	-
Phaeosphaeria oryzae	CBS 110110	GQ387530	KM434279	-	KF252193
Pleospora herbarum	CBS 191.86	DQ247812	DQ247804	DQ471090	KC584471
Polyplosphaeria fusca	KT 1616	AB524463	AB524604	-	-
Pseudoastrosphaeriella bambusae	MFLUCC 11-0205	KT955455	KT955475	KT955437	KT955414
Pseudoastrosphaeriella thailandensis	MFLUCC 10-0553	KT955456	KT955477	KT955439	KT955411
Pseudolophiotrema elymicola	MAFF 239600	LC194339	LC194381	LC194418	LC194473
Salsuginea ramicola	KT 2597.2	GU479768	GU479801	GU479862	GU479834

Taxon	*Culture code*	*SSU*	*LSU*	*tef1*	*rpb2*
Salsuginea ramicola	KT 2597.1	GU479767	GU479800	GU479861	GU479833
Tetraplosphaeria sasicola	KT 563	AB524490	AB524631	-	-
Thyridaria broussonetiae	CBS 141481	KX650515	KX650568	KX650539	KX650586
Torula herbarum	CBS 140066	KR873260	KR873288	-	-
Torula hollandica	CBS 220.69	KF443389	KF443384	KF443401	KF443393
Trematosphaeria pertusa	CBS 122368	FJ201991	FJ201990	KF015701	FJ795476
Ulospora bilgramii	CBS 101364	DQ678025	DQ678076	DQ677921	DQ677974
Verruculina enalia	BCC 18402	GU479771	GU479803	GU479864	GU479836
Zopfia rhizophila	CBS 207.26	DQ384086	DQ384104	-	-

TAXONOMY

As shown by the molecular phylogenetic results, *Neomassaria fabacearum* (MFLUCC 16-1875) together with the novel taxon introduced in this study, *N. formosana* (NTUCC 17-007, NTUCC 17-008 and NTUCC 17-013) formed a highly supported lineage with uncertain affinities to other families of Pleoporales. Therefore, we propose the novel family Neomassariaceae for *Neomassaria*, because it does not fit with any known family in Pleoporales.

Neomassariaceae Ariyawansa, Jaklitsch & Voglmayr**, fam. nov.**

MycoBank: MB827113

Diagnosis: Sexual morph: Ascomata immersed, solitary or scattered, globose to subglobose, brown to dark brown with central ostiole. *Peridium* comprising brown cells of *textura angularis*. *Hamathecium* comprising cylindrical to filiform pseudoparaphyses. *Asci* 8-spored, bitunicate, oblong to cylindrical, pedicellate. *Ascospores* overlapping, ellipsoid to fusiform, 1-septate, hyaline, with or without a gelatinous sheath. *Asexual morph* undetermined.

Type genus: *Neomassaria* Mapook, Camporesi & K.D. Hyde in Hyde *et al.*, Fungal Diversity 80: 74. 2016

Neomassaria Mapook, Camporesi & K.D. Hyde in Hyde *et al*., Fungal Diversity 80: 74. 2016.

Diagnosis: Sexual morph: *Ascomata* immersed, solitary or scattered, coriaceous, globose to subglobose, brown to dark brown. *Ostiole* central. *Peridium* comprising brown *textura angularis*. *Hamathecium* comprising cylindrical to filiform, septate, branched pseudoparaphyses. Asci 8-spored, bitunicate, fissitunicate, oblong to cylindrical, pedicellate, with an ocular chamber. *Ascospores* overlapping 1–2-seriate, hyaline, ellipsoid to broadly fusiform, 1-septate, constricted at the septa, with or without guttules, with or without a gelatinous sheath. *Asexual morph* undetermined. Saprobic on dead stems.

*Type species***:** *Neomassaria fabacearum* Mapook, Camporesi & K.D. Hyde, in Hyde *et al.*, Fungal Diversity: 80:77. 2016.

Neomassaria formosana Ariyawansa, Jaklitsch & Voglmayr, **sp. nov.** **Fig. 2**

MycoBank: MB 827114

*Etymology***:** The epithet refers to Formosa, another name used for Taiwan, where this species was collected.

Sexual morph: *Ascomata* 100–200 × 100–370 µm ($\bar{x}$ = 145 × 255 µm, n = 10) solitary, sometimes gregarious, immersed with erumpent neck, visible as black dots on the host surface, unilocular, globose, coriaceous, brown to dark brown, with centrally opening ostiole. *Ostioles* 20–38 µm ($\bar{x}$ = 27 µm, n = 10) diam, papillate to depressed; ostiolar canal filled with periphyses. *Peridium* 13–40 µm ($\bar{x}$ = 23 µm, n = 10) wide, comprising 3–4 layers of dark brown, relatively thick-walled cells of *textura angularis*, thinner-walled and lighter to the inner side, outside fusing with the host. *Hamathecium* composed of 1–2 µm ($\bar{x}$ = 1.5 µm, n = 20) wide, septate, cellular pseudoparaphyses surrounding the numerous asci, embedded in a gelatinous matrix. *Asci* 80–125 × 14–17 µm ($\bar{x}$ = 100 × 15 µm, n= 20), bitunicate, fissitunicate, cylindrical or oblong, with a short pedicel, apically rounded, with an ocular chamber, containing 8 biseriate ascospores. *Ascospores* 20–30 × 3–7 µm ($\bar{x}$ = 25 × 5 µm, n = 30), fusoid to ellipsoid, 2-celled, constricted at the septum, hyaline, with 4–6 large guttules and narrowly rounded ends, without a mucilaginous sheath.

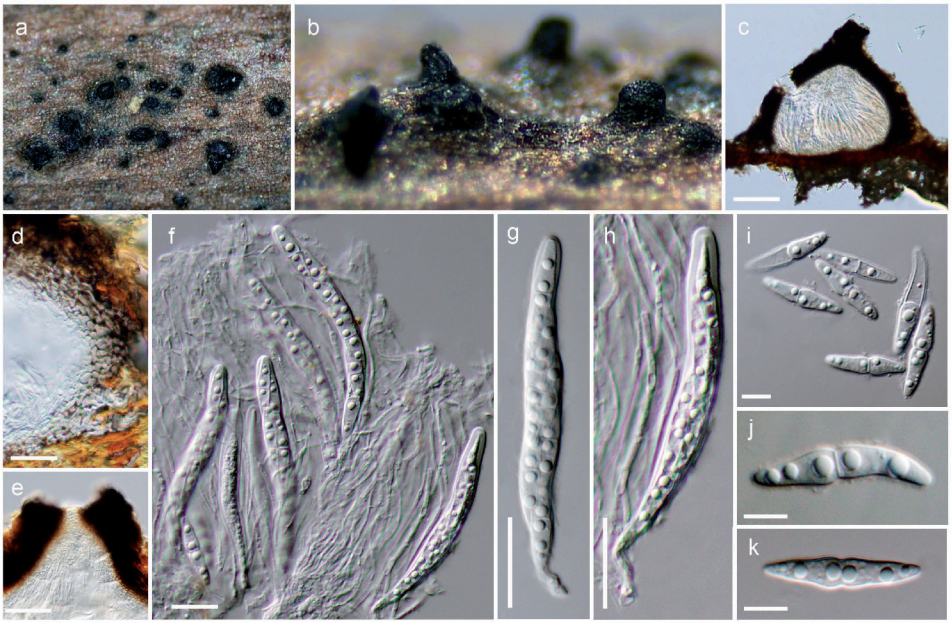

Figure 2. Morphology of *Neomassaria formosana* (NTUH 17-007). **a** Appearance of ascomata on the natural host. **b** Close up of erumpent ostiolar necks and upper parts of ascomata. **c** Vertical section of ascoma. **d** Section through peridium. **e** Ostiolar canal filled with periphyses. **f** Pseudoparaphyses surrounding the asci. **g–h** Asci. **i–k** Ascospores. **Scale bars:** c = 15 µm, d–e = 5 µm, f–h = 10 µm, i–k = 5 µm

Asexual morph: undetermined.

Ecology: *Saprobic* on dead stems of *Rhododendron* sp. *Culture characteristics***:** Colony on PDA reaching 17 mm diam after 20 days at 25 °C, circular, umbonate, radially striate, with slightly lobate edge; surface smooth, with cottony texture, whitish grey to pale brown; reverse dark green expanding from the centre; near margin pale brown.

*Distribution***.** Taiwan

*Holotype***:** TAIWAN. Taipei city, Xinyi District, on a dead stem of *Rhododendron* sp., 5 Dec. 2017, H.A. Ariyawansa (**holotype:** NTUH 17-007, ex-holotype culture NTUCC 17-007).

*Additional material examined***:** TAIWAN. Taipei city, Xinyi District, on dead stem of *Rhododendron* sp., 10 Jan. 2018, H.A. Ariyawansa (NTUH 17-008, living culture NTUCC 17-008); ibidem, same host, 22 Apr. 2018, H.A. Ariyawansa (NTUH 17-013, living culture: NTUCC 17-013);

Notes: In molecular phylogenies based on LSU, *rpb2*, SSU, and *tef1* genes the strains NTUCC 17-007, NTUCC 17-008 and NTUCC 17-013 generated in the present study formed a distinct lineage in a sister group relationship to *Neomassaria fabacearum* with high statistical support. Therefore, the new species *Neomassaria formosana* is here described.

Neomassaria formosana differs from the generic type of *Neomassaria*, *N. fabacearum*, by larger and more fusoid ascospores, which contain 4–6 large guttules and lack a mucilaginous sheath. Furthermore, *Neomassaria formosana* can be clearly differentiated from *N. fabacearum* by the host (*Rhododendron* versus *Hippocrepis*) and the distribution (Taiwan versus Italy).

DISCUSSION

Phylogenetic analyses based on DNA sequence data of LSU, *rpb2*, SSU and *tef1* revealed that the genus *Neomassaria* forms a lineage distinct from all other families of Pleosporales, and is therefore placed in the new family Neomassariaceae. Moreover, by revealing a new species of *Neomassaria*, our investigation adds to the knowledge of the diversity of saprobic pleosporalean fungi growing on dead stems of *Rhododendron* sp. in Taiwan.

Even though phylogeny inferred through DNA sequence data plays a crucial part in discovering and understanding the diversity of pleosporalean fungi, relationships among many basal lineages of Pleosporales remain poorly supported, as shown in previous studies (Voglmayr & Jaklitsch, 2011; Schoch *et al.*, 2012; Zhang *et al.*, 2012; Hyde *et al.*, 2013, 2017; Ariyawansa *et al.*, 2015, 2018; Jaklitsch & Voglmayr, 2016; Hashimoto *et al.*, 2017; Hernandez-Restrepo *et al.*, 2017; Wanasinghe *et al.*, 2017; Valenzuela-Lopez *et al.*, 2018). For instance, monophyletic pleosporalean families with poorly supported sister relationships, such as Aigialaceae, Delitschiaceae, Lindgomycetaceae, Ohleriaceae and Testudinaceae have been observed in many recent studies (Voglmayr & Jaklitsch, 2011; Schoch *et al.*, 2012; Zhang *et al.*, 2012; Hyde *et al.*, 2013; Ariyawansa *et al.*, 2015, 2018; Jaklitsch & Voglmayr, 2016; Hashimoto *et al.*, 2017; Hernandez-Restrepo *et al.*, 2017; Wanasinghe *et al.*, 2017; Valenzuela-Lopez *et al.*, 2018).

The most significant problem of molecular phylogeny is that the phylogeny inferred from a single gene may not always reveal the precise evolutionary history

of the organism (Ariyawansa *et al.*, 2014, 2018; Uilenberg *et al.*, 2004). Therefore, phylogenetic evidence should be based on more than one gene (Vellinga *et al.*, 2015). Single locus analysis of LSU showed that our strains together with *Neomassaria fabacearum* formed a poorly supported basal lineage close to the Massariaceae, while they formed a separate lineage in a sister group position to the clades of Astrosphaeriellaceae and Delitschiaceae in the SSU phylogeny (data not shown). However, these positions did not receive significant support, which was generally true for most basal nodes. Therefore, to reveal the phylogenetic relationships of the Neomassariaceae clade with the other members of the Pleosporales, we finally conducted multi gene analyses based on LSU, *rpb2*, SSU and *tef1*. We obtained a tree with topologies similar to many recent studies (Voglmayr & Jaklitsch, 2011; Schoch *et al.*, 2012; Zhang *et al.*, 2012; Hyde *et al.*, 2013; Ariyawansa *et al.*, 2015; Jaklitsch & Voglmayr, 2016; Hashimoto *et al.*, 2017), while our new strains together with *Neomassaria fabacearum* formed a basal lineage to all other families of Pleosporales, except for Massariaceae and Delitschiaceae. This further confirms the distinctness of the new family Neomassariaceae within the order Pleosporales. The phylogenetic affinities of the family Massariaceae was revised by Voglmayr and Jaklitsch (2011) via phylogenetic analyses based on sequence data of four genes (LSU, *rpb2*, SSU, and *tef1*) coupled with morphology. The family is characterised by large immersed, globose, subglobose to pyriform ascomata with pseudoparenchymatous peridium comprising cells forming *textura angularis*, oblong to cylindrical asci with a wide ocular chamber and a refractive ring containing oblong to ellipsoid ascospores enclosed by a gelatinous sheath (Voglmayr & Jaklitsch, 2011; Hyde *et al.*, 2013, 2017). In their study, Voglmayr & Jaklitsch (2011) concluded that species of Massariaceae are highly host-specific (mainly occurring on *Acer* and Rosaceae species). Morphologically *Neomassaria* differs from the genus *Massaria* in having small globose to subglobose ascomata, small asci lacking a refractive ring and small hyaline, 1-septate ascospores. *Massaria* has large subglobose to broadly pyriform ascomata, large oblong, fusoid or clavate asci with a refractive ring, containing bi- to triseriate large, oblong, narrowly ellipsoidal or fusoid, hyaline, light, medium, dark umber to blackish brown, 3-septate ascospores (Voglmayr & Jaklitsch, 2011). Furthermore, species of *Neomassaria* can be distinguished from *Massaria* species by the hosts (*Rhododendron* and *Hippocrepis* versus primarily *Acer* and Rosaceae species). *Neomassaria* has overlapping morphologies with some of the other genera in Lophiotremataceae (i.e. *Lophiotrema*, *Atrocalyx* and *Pseudolophiotrema*), as well as with some other members of Pleosporales such *Lindgomyces* and *Amniculicola*. Therefore, identification of species sharing similar morphological characters is impossible without molecular data together with ecological considerations (terrestrial versus aquatic occurrence, host range, pathogenicity), distribution or physiology (Crous *et al.*, 2015; Tsai *et al.*, 2018).

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