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A new *Arthrinium*-like genus of Amphisphaeriales in China

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Abstract

Species of *Arthrinium s. l.* are usually known as endophytes, pathogens or saprobes occurring on various hosts and substrates and are characterised by globose to subglobose, sometimes irregular, dark brown and smooth-walled or finely verruculose conidia, always with a truncate basal scar. Currently, *Arthrinium s. l.* contains two phylogenetically distinct clades, namely, *Apiospora* and *Arthrinium s. s.* However, *Arthrinium trachycarpi* and *Ar. urticae* have still not been properly classified. With new isolates from diseased leaves of *Lithocarpus glaber* collected in China, we propose the new *Arthrinium*-like genus *Neoarthrinium* in Amphisphaeriales. Based on the morphology and phylogeny of multiple loci, the new genus is established with the type species, *N. lithocarpicola* and three new combinations, *N. moseri* (syn. *Wardomyces moseri*), *N. trachycarpi* (syn. *Ar. trachycarpi*) and *N. urticae* (syn. *Ar. urticae*) are added to this genus.

Keywords

Apiospora, *Arthrinium*, *Neoarthrinium*, phylogeny, taxonomy

Introduction

Apiosporaceae, including *Arthrinium*-like taxa, was proposed to accommodate genera with apiosporous hyaline ascospores and a basauxic, *Arthrinium*-like conidiogenesis (Hyde et al. 1998). In a recent outline of Sordariomycetes, Hyde et al. (2020)

accepted five genera viz. *Appendicospora*, *Arthrinium*, *Dictyoarthrinium*, *Endocalyx* and *Nigrospora* in family Apiosporaceae. Soon thereafter, *Dictyoarthrinium* was transferred to Didymosphaeriaceae, based on a multigene phylogeny (Samarakoon et al. 2020). Subsequently, Pintos and Alvarado (2021) separated *Apiospora* from *Arthrinium*, based on the study of the type species of both genera and on multigene phylogeny. Recently, Konta et al. (2021) transferred *Endocalyx* to Cainiaceae, based on morphological and phylogenetic evidence and Samarakoon et al. (2022) described the new family Appendicosporaceae for *Appendicospora*. Therefore, Apiosporaceae currently contains three genera, viz. *Apiospora*, *Arthrinium* and *Nigrospora.*

Until the study of Pintos and Alvarado (2021), the genera *Apiospora* and *Arthrinium* were considered synonymous, the first being used for the sexual morph and the second for the asexual morph in dual nomenclature (Réblová et al. 2016). Following the abandonment of dual nomenclature, the older name *Arthrinium* was recommended for use in unitary nomenclature (Réblová et al. 2016). The genus *Arthrinium* was proposed by Kunze and Schmidt (1817) and validated by Fries (1832) with *Ar. caricicola* as the generic type. *Apiospora*, the type genus of Apiosporaceae, was typified with *Ap. montagnei*, a new name for *Sphaeria apiospora* (Saccardo 1875). However, the phylogenetic identity of *Ap. montagnei* has been confused because multiple names in *Arthrinium* have similar sexual morphs that have been referred to as *Apiospora montagnei* (Hudson et al. 1976; Pintos et al. 2019; Pintos and Alvarado 2021). New collections from the original region and hosts (*Arundo*, *Piptatherum*) of *Ap. montagnei* have been isolated in pure culture and sequenced (Crous and Groenewald 2013; Pintos et al. 2019). Five species, previously placed in *Arthrinium*, are classified in *Apiospora*. Two of these phylogenetically distinct species, *Ap. marii* and *Ap. phragmitis*, are morphologically similar to *Ap. montagnei* (Pintos and Alvarado 2021), but due to a lack of sequence data from the type, it cannot be determined which of these two species should become a synonym of *Ap. montagnei*. Irrespective of these taxonomic uncertainties in species concept, recent multigene phylogenies revealed that *Arthrinium* and *Apiospora* represent two well-supported, distinct lineages close to *Nigrospora* in Apiosporaceae (Pintos and Alvarado 2021; Samarakoon et al. 2022). However, two *Arthrinium* species resembling *Apiospora* in conidial morphology, viz. *Ar. trachycarpi* and *Ar. urticae*, were not considered in these studies.

Arthrinium-like species are globally distributed, inhabiting various substrates, mainly associated with plant tissues as endophytes, pathogens and saprobes (Cooke 1954; Minter 1985; Larrondo and Calvo 1992; Senanayake et al. 2020; Feng et al. 2021; Jiang and Tian 2021; Tian et al. 2021). Some species are important plant pathogens; for example, *Ap. arundinis* causes bamboo brown culm streak, chestnut leaf spot and barley kernel blight (Martínez-Cano et al. 1992; Chen et al. 2014; Jiang et al. 2021), while *Ap. sacchari* causes damping-off of durum wheat (Mavragani et al. 2007). Another species, *Ar. phaeospermum*, can cause dermatomycosis in humans (Zhao et al. 1990).

In the present study, new *Arthrinium*-like isolates were collected and morphologically examined and their phylogenetic affiliation was determined by analyses of a combined matrix of ITS, LSU, *tef1* and *tub2* sequences. The aim of this study was to determine the phylogenetic placement of *Ar. trachycarpi*, *Ar. urticae* and our new isolates within Amphisphaeriales, which resulted in the identification of a new phylogenetic lineage with isolates belonging to neither *Arthrinium* nor *Apiospora*. As a result, a new genus is established for these isolates.

Materials and methods

Isolation and morphology

Diseased leaves of *Lithocarpus glaber* were observed and collected in Guangdong Province of China (39 m elevation; 23°8'52"N, 113°27'18"E), packed in paper bags and transferred to the laboratory for pure culture isolation. The samples were first surface-sterilised for 1 min in 75% ethanol, 3 min in 1.25% sodium hypochlorite and 1 min in 75% ethanol, rinsed for 2 min in distilled water and blotted on dry sterile filter paper. Then, the diseased areas of the leaves were cut into 0.5 × 0.5 cm pieces using an aseptic razor blade, transferred on to the surface of potato dextrose agar plates (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per litre) and incubated at 25 °C to obtain pure cultures. The cultures were deposited in the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/) and the specimen was deposited in the Herbarium of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn/).

The morphology of the isolates was studied, based on sporulating axenic cultures grown on PDA in the dark at 25 °C. The conidiomata were observed and photographed under a dissecting microscope (M205 C, Leica, Wetzlar, Germany). The conidiogenous cells and conidia were immersed in tap water and then the microscopic photographs were captured with an Axio Imager 2 microscope (Zeiss, Oberkochen, Germany), equipped with an Axiocam 506 colour camera using differential interference contrast (DIC) illumination. For measurements, 50 conidiogenous cells and conidia were randomly selected. Culture characteristics were recorded from PDA after 10 d of incubation at 25 °C in the dark.

DNA extraction, PCR amplification and phylogenetic analyses

Genomic DNA was extracted from colonies grown on cellophane-covered PDA using a cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). DNA was checked by electrophoresis in a 1% agarose gel and the quality and quantity were measured using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). The following primer pairs were used for amplification of the gene regions sequenced in the present study: ITS1/ITS4 for the ITS1-5.8S-ITS2 nrDNA region (ITS) (White et al. 1990); LR0R/LR5 for the 28S nrDNA region (LSU) (Vilgalys and Hester 1990); EF1-728F/EF2 for the translation elongation factor 1-α (*tef1*) gene (O'Donnell and Cigelnik 1997; Carbone and Kohn 1999); Bt2a/Bt2b for the beta-tubulin (*tub2*) gene

(Glass and Donaldson 1995). The PCR conditions were set as follows: an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 50 s at 52 °C (ITS and LSU) or 54 °C (*tef1* and *tub2*) and 1 min at 72 °C and a final elongation step of 7 min at 72 °C. The PCR products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyser with a BigDye Terminator Kit v.3.1 (Invitrogen, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

The quality of the chromatograms obtained was checked and the nucleotide sequences were assembled using SeqMan v.7.1.0, the DNASTAR lasergene core suite software (DNASTAR Inc, Madison, WI, USA). Reference sequences were retrieved from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov), based on related publications (Crous and Groenewald 2013; Wang et al. 2018; Liu et al. 2019; Pintos and Alvarado 2021; Samarakoon et al. 2022). Sequences were aligned using MAFFT v. 6 (Katoh and Toh 2010) and corrected manually using MEGA 7.0.21 (Kumar et al. 2016).

The phylogenetic analyses of the combined loci were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML was implemented on the CIPRES Science Gateway portal (https://www.phylo.org) using RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014), employing a GTRGAMMA substitution model with 1000 bootstrap replicates. The Bayesian posterior probabilities (BPP) were determined by Markov Chain Monte Carlo (MCMC) sampling in MrBayes v. 3.2.6 (Ronquist et al. 2012). The six simultaneous Markov chains were run for 1 M generations, starting from random trees and sampling trees every 100th generation and 25% of aging samples were discarded, running until the average standard deviation of the split frequencies dropped below 0.01. The phylogram was visualised in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software) and edited in Adobe Illustrator CS5 (Adobe Systems Inc., USA). The newly-generated nucleotide sequences were deposited in GenBank (Table 1).

Results

Phylogenetic analyses

The combined sequence dataset (ITS, LSU, *tef1* and *tub2*) was analysed to infer the phylogenetic placement of our new isolates within Amphisphaeriales. The dataset consisted of 136 sequences, including two outgroup taxa, *Clypeosphaeria mamillana* (CBS 140735) and *Pseudosporidesmium knawiae* (CBS 123529). A total of 3526 characters, including gaps (793 for ITS, 859 for LSU, 762 for *tef1* and 1112 for *tub2*), were included in the phylogenetic analysis. Of these characters, 1543 were constant, 284 were variable, but parsimony-uninformative and 1699 were parsimony-informative. The best ML tree (lnL = - 72640.48) revealed by RAxML is shown in Fig. 1. The topologies resulting from ML and BI analyses of the concatenated dataset were congruent (Fig. 1). Isolates CFCC 54456 and CFCC 55883 from the present study, together with CFCC

53038, CFCC 53039, CBS 164.80 and IMI 326344, formed a clade distinct from Apiosporaceae and the other families in Amphisphaeriales. Hence, a new genus named *Neoarthrinium* is proposed herein for this clade. *Arthrinium trachycarpi*, *Ar. urticae* and *Wardomyces moseri* are transferred to *Neoarthrinium*. In addition, the two new isolates (CFCC 54456 and CFCC 55883) that form a sister clade to *N. moseri*, *N. trachycarpi* and *N. urticae* are described here as the new species *N. lithocarpicola.*

Table 1. Isolates and GenBank accession numbers used in the phylogenetic analyses.

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	*tub2*	*tef1*
Allelochaeta acuta	CPC 16629	*Eucalyptus dives*	Australia	MH554086	MH554297	MH554758	MH554519
Allelochaeta neoacuta	CBS 115131	*Eucalyptus smithii*	South Africa	JN871200	JN871209	MH704627	MH704602
Amphisphaeria micheliae	MFLUCC 20-0121	*Michelia alba*	China	MT756626	MT756620	MT774371	NA
Apiospora acutiapica	KUMCC 20-0209	*Bambusa bambos*	China	MT946342	MT946338	MT947365	MT947359
Apiospora acutiapica	KUMCC 20-0210	*Bambusa bambos*	China	MT946343	MT946339	MT947366	MT947360
Apiospora arundinis	CBS 114316	*Hordeum vulgare*	Iran	KF144884	KF144928	KF144974	KF145016
Apiospora aurea	CBS 244.83	Air	Spain	AB220251	KF144935	KF144981	KF145023
Apiospora balearica	CBS 145129	Poaceae	Spain	MK014869	MK014836	MK017975	NA
Apiospora biserialis	CGMCC 3.20135	Bamboo	China	MW481708	MW478885	MW522955	MW522938
Apiospora camelliae-sinensis	LC8181	*Brassica campestris*	China	KY494761	KY494837	KY705229	NA
Apiospora camelliae-sinensis	CGMCC 3.18333	*Camellia sinensis*	China	KY494704	KY494780	KY705173	KY705103
Apiospora cyclobalanopsidis	CGMCC 3.20136	*Cyclobalanopsis glauca*	China	MW481713	MW478892	MW522962	MW522945
Apiospora descalsii	CBS 145130	*Ampelodesmos mauritanicus*	Spain	MK014870	MK014837	MK017976	NA
Apiospora dichotomanthi	LC8175	*Dichotomanthes tristaniiaecarpa*	China	KY494755	KY494831	KY705223	KY705151
Apiospora dichotomanthi	CGMCC 3.18332	*Dichotomanthes tristaniiaecarpa*	China	KY494697	KY494773	KY705167	KY705096
Apiospora esporlensis	CBS 145136	*Phyllostachys aurea*	Spain	MK014878	MK014845	MK017983	NA
Apiospora gelatinosa	GZAAS 20-0107	Bamboo	China	MW481707	MW478889	MW522959	MW522942
Apiospora guizhouensis	LC5318	Air	China	KY494708	KY494784	KY705177	KY705107
Apiospora guizhouensis	CGMCC 3.18334	Air	China	KY494709	KY494785	KY705178	KY705108
Apiospora hydei	CBS 114990	Bamboo	China	KF144890	KF144936	KF144982	KF145024
Apiospora iberica	CBS 145137	*Arundo donax*	Portugal	MK014879	MK014846	MK017984	NA
Apiospora intestini	CBS 135835	Gut of a grasshopper	India	KR011352	MH877577	KR011350	NA
Apiospora italica	CBS 145138	*Arundo donax*	Italy	MK014880	MK014847	MK017985	NA
Apiospora jiangxiensis	CGMCC 3.18381	*Maesa* sp.	China	KY494693	KY494769	KY705163	KY705092
Apiospora kogelbergensis	CBS 113332	*Cannomois virgata*	South Africa	KF144891	KF144937	KF144983	KF145025
Apiospora kogelbergensis	CBS 113333	Restionaceae	South Africa	KF144892	KF144938	KF144984	KF145026
Apiospora malaysiana	CBS 102053	*Macaranga hullettii*	Malaysia	KF144896	KF144942	KF144988	KF145030
Apiospora marii	CBS 497.90	Air	Spain	AB220252	KF144947	KF144993	KF145035
Apiospora neobambusae	CGMCC 3.18335	Bamboo	China	KY494718	KY494794	KY705186	KY806204
Apiospora neobambusae	LC7107	Bamboo	China	KY494719	KY494795	KY705187	KY705117
Apiospora obovata	CGMCC 3.18331	*Lithocarpus* sp.	China	KY494696	KY494772	KY705166	KY705095
Apiospora obovata	LC8177	*Lithocarpus* sp.	China	KY494757	KY494833	KY705225	KY705153
Apiospora ovata	CBS 115042	*Arundinaria hindsii*	China	KF144903	KF144950	KF144995	KF145037
Apiospora phragmitis	CBS 135458	*Phragmites australis*	Italy	KF144909	KF144956	KF145001	KF145043
Apiospora phyllostachydis	MFLUCC 18-1101	*Phyllostachys heteroclada*	China	MK351842	MH368077	MK291949	MK340918
Apiospora pseudoparenchymatica	CGMCC 3.18336	Bamboo	China	KY494743	KY494819	KY705211	KY705139
Apiospora pseudospegazzinii	CBS 102052	*Macaranga hullettii*	Malaysia	KF144911	KF144958	KF145002	KF145045
Apiospora pterosperma	CBS 134000	*Machaerina sinclairii*	Australia	KF144913	KF144960	KF145004	KF145046
Apiospora saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145009	KF145051
Apiospora septata	CGMCC 3.20134	Bamboo	China	MW481711	MW478890	MW522960	MW522943
Apiospora serenensis	IMI 326869	NA	Spain	AB220250	AB220344	AB220297	NA

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	*tub2*	*tef1*
Apiospora subrosea	LC7291	Bamboo	China	KY494751	KY494827	KY705219	KY705147
Apiospora subrosea	CGMCC 3.18337	Bamboo	China	KY494752	KY494828	KY705220	KY705148
Apiospora xenocordella	CBS 595.66	Soil	Austria	KF144926	KF144971	KF145013	KF145055
Arthrinium caricicola	CBS 145127	*Carex ericetorum*	Germany	MK014871	MK014838	MK017977	NA
Arthrinium crenatum	CBS 146353	Grass	France	MW208931	MW208861	MW221923	MW221917
Arthrinium curvatum	CBS 145131	*Carex* sp.	Germany	MK014872	MK014839	MK017978	NA
Arthrinium japonicum	IFO 30500	*Carex despalata*	Japan	AB220262	AB220356	AB220309	NA
Arthrinium japonicum	IFO 31098	*Carex despalata*	Japan	AB220264	AB220358	AB220311	NA
Arthrinium luzulae	AP7619-3	*Luzula sylvatica*	Spain	MW208937	MW208863	MW221925	MW221919
Arthrinium morthieri	GZU 345043	*Carex digitata*	Austria	MW208938	MW208864	MW221926	MW221920
Arthrinium puccinioides	CBS 549.86	*Lepidosperma gladiatum*	Germany	AB220253	AB220347	AB220300	NA
Arthrinium sphaerospermum	CBS 146355	Poaceae	Norway	MW208943	MW208865	NA	NA
Arthrinium sporophleum	CBS 145154	*Juncus* sp.	Spain	MK014898	MK014865	MK018001	NA
Bartalinia bella	CBS 125525	*Maytenus abbottii*	South Africa	GU291796	MH554214	MH554663	MH554421
Bartalinia pini	CBS 143891	*Pinus patula*	Uganda	MH554125	MH554330	MH554797	MH554559
Beltrania pseudorhombica	CBS 138003	*Pinus tabulaeformis*	China	MH554124	KJ869215	NA	MH554558
Beltrania rhombica	CBS 123.58	Sand near mangrove swamp	Mozambique	MH553990	MH554209	MH704631	MH704606
Beltraniopsis neolitseae	CPC 22168	*Neolitsea australiensis*	Australia	KJ869126	KJ869183	NA	NA
Broomella vitalbae	HPC 1154	NA	NA	MH554173	MH554367	MH554846	MH554608
Castanediella cagnizarii	CBS 542.96	Leaf litter	Cuba	MH862597	MH874222	NA	NA
Ciliochorella phanericola	MFLUCC 12-0310	Dead leaves	Thailand	KF827444	KF827445	KF827478	KF827477
Clypeophysalospora latitans	CBS 141463	*Eucalyptus* sp.	Portugal	NR_153929	NG_058958	NA	NA
Clypeosphaeria mamillana	CBS 140735	*Cornus alba*	France	KT949897	MH554225	MH704637	MH704610
Cylindrium elongatum	CBS 115974	*Fagus* sp.	The Netherlands	KM231853	KM231733	KM232123	KM231989
Diploceras hypericinum	CBS 109058	*Hypericum* sp.	New Zealand	MH553955	MH554178	MH554614	MH554373
Disaeta arbuti	CBS 143903	*Acacia pycnantha*	Australia	MH554148	MH554346	MH554821	MH554583
Discosia artocreas	CBS 124848	*Fagus sylvatica*	Germany	MH553994	MH554213	MH554662	MH554420
Discosia brasiliensis	MFLUCC 12-0429	Dead leaf	Thailand	KF827432	KF827436	KF827469	KF827465
Distononappendiculata banksiae	CBS 131308	*Banksia marginata*	Australia	JQ044422	JQ044442	MH554670	MH554428
Distononappendiculata casuarinae	CBS 143884	*Casuarina* sp.	Australia	MH554093	MH554303	MH554766	MH554527
Diversimediispora humicola	CBS 302.86	Soil	USA	MH554028	MH554247	MH554705	MH554463
Heterotruncatella acacigena	CBS 143880	*Acacia pedina*	Australia	MH554084	MH554295	MH554756	MH554517
Heterotruncatella aspera	CBS 144140	*Acacia glaucoptera*	Australia	MH554156	MH554352	MH554829	MH554591
Hyalotiella spartii	MFLUCC 13-0397	*Spartium junceum*	Italy	KP757756	KP757752	NA	NA
Hyalotiella transvalensis	CBS 303.65	Leaf litter and topsoil of *Acacia karroo* community	South Africa	MH554029	MH554248	MH554706	MH554464
Hymenopleella austroafricana	CBS 143886	*Gleditsia triacanthos*	South Africa	MH554115	MH554320	MH554788	MH554549
Hymenopleella hippophaëicola	CBS 113687	*Hippophaë rhamnoides*	Sweden	MH553969	MH554188	MH554628	MH554387
Immersidiscosia eucalypti	NBRC 104195	*Quercus myrsinifolia*	Japan	AB594790	AB593722	NA	NA
Lepteutypa fuckelii	CBS 140409	*Tilia cordata*	Belgium	NR_154123	KT949902	MH554677	MH554435
Lepteutypa sambuci	CBS 131707	*Sambucus nigra*	UK	NR_154124	MH554219	MH704632	MH704612
Monochaetia monochaeta	CBS 115004	*Quercus robur*	Netherlands	AY853243	MH554198	MH554639	MH554398
Monochaetia quercus	CBS 144034	*Quercus eduardi*	Mexico	MH554171	MH554365	MH554844	MH554606
Morinia acaciae	CBS 137994	*Acacia melanoxylon*	France	MH554002	MH554221	MH554673	MH554431
Morinia crini	CBS 143888	*Crinum bulbispermum*	South Africa	MH554118	MH554323	MH554791	MH554552
Neoarthrinium lithocarpicola	**CFCC 54456**	***Lithocarpus glaber***	**China**	**ON427580**	**ON427582**	**ON456914**	**NA**
Neoarthrinium lithocarpicola	**CFCC 55883**	***Lithocarpus glaber***	**China**	**ON427581**	**ON427583**	**ON456915**	**NA**
Noarthrinium moseri	CBS 164.80	Dead petiole	Colombia	LN850995	LN851049	LN851154	NA
Neoarthrinium trachycarpi	CFCC 53038	*Trachycarpus fortunei*	China	MK301098	NA	MK303394	MK303396

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	*tub2*	*tef1*
Neoarthrinium trachycarpi	CFCC 53039	*Trachycarpus fortunei*	China	MK301099	NA	MK303395	MK303397
Neoarthrinium urticae	IMI 326344	Leaf litter	India	AB220245	AB220339	NA	NA
Neopestalotiopsis cubana	CBS 600.96	Leaf litter	Cuba	KM199347	KM116253	KM199438	KM199521
Neophysalospora eucalypti	CBS 138864	*Corymbia henryi*	Mozambique	KP004462	MH878627	NA	NA
Nigrospora aurantiaca	CGMCC 3.18130	*Nelumbo* sp.	China	KX986064	KX986098	KY019465	KY019295
Nigrospora camelliae-sinensis	CGMCC 3.18125	*Camellia sinensis*	China	KX985986	KX986103	KY019460	KY019293
Nigrospora chinensis	CGMCC 3.18127	*Machilus breviflora*	China	KX986023	KX986107	KY019462	KY019422
Nigrospora gorlenkoana	CBS 480.73	*Vitis vinifera*	Kazakhstan	KX986048	KX986109	KY019456	KY019420
Nigrospora guilinensis	CGMCC 3.18124	*Camellia sinensis*	China	KX985983	KX986113	KY019459	KY019292
Nigrospora hainanensis	CGMCC 3.18129	*Musa paradisiaca*	China	KX986091	KX986112	KY019464	KY019415
Nigrospora lacticolonia	CGMCC 3.18123	*Camellia sinensis*	China	KX985978	KX986105	KY019458	KY019291
Nigrospora musae	CBS 319.34	*Musa* sp.	Australia	MH855545	KX986110	KY019455	KY019419
Nigrospora oryzae	LC2693	*Neolitsea* sp.	China	KX985944	KX986101	KY019471	KY019299
Nigrospora osmanthi	CGMCC 3.18126	*Osmanthus* sp.	China	KX986010	KX986106	KY019461	KY019421
Nigrospora pyriformis	CGMCC 3.18122	*Citrus sinensis*	China	KX985940	KX986100	KY019457	KY019290
Nigrospora rubi	LC2698	*Rubus* sp.	China	KX985948	KX986102	KY019475	KY019302
Nigrospora sphaerica	LC7298	*Nelumbo* sp.	China	KX985937	KX986097	KY019606	KY019401
Nigrospora vesicularis	CGMCC 3.18128	*Musa paradisiaca*	China	KX986088	KX986099	KY019463	KY019294
Nonappendiculata quercina	CBS 116061	*Quercus suber*	Italy	MH553982	MH554199	MH554641	MH554400
Parabartalinia lateralis	CBS 399.71	*Acacia karroo*	South Africa	MH554043	MH554256	MH554719	MH554478
Parapleurotheciopsis inaequiseptata	MUCL 41089	Rotten leaf	Brazil	EU040235	EU040235	NA	NA
Parapleurotheciopsis caespitosa	CBS 519.93	*Syzygium cordatum*	South Africa	MH862437	NG_066263	NA	NA
Pestalotiopsis adusta	CBS 263.33	*Rhododendron ponticum*	Netherlands	KM199316	KM116198	KM199414	KM199489
Pestalotiopsis australasiae	CBS 114126	*Knightia* sp.	New Zealand	KM199297	KM116218	KM199409	KM199499
Phlogicylindrium eucalypti	CBS 120080	*Eucalyptus globulus*	Australia	NR_132813	DQ923534	MH704633	MH704607
Phlogicylindrium eucalyptorum	CBS 120221	*Eucalyptus globus*	Australia	EU040223	MH554204	MH704635	MH704608
Pseudopestalotiopsis ampullacea	LC6618	*Camellia sinensis*	China	KX895025	KX895039	KX895358	KX895244
Pseudopestalotiopsis camelliae-sinensis	LC3009	*Camellia sinensis*	China	KX894935	KX895050	KX895267	KX895152
Pseudosarcostroma osyridicola	CBS 103.76	*Osyris alba*	France	MH553954	MH554177	MH554613	MH554372
Pseudosporidesmium knawiae	CBS 123529	NA	NA	MH863299	MH874823	NA	NA
Robillarda africana	CBS 122.75	NA	South Africa	KR873253	KR873281	MH554656	MH554414
Robillarda terrae	CBS 587.71	Soil	India	KJ710484	KJ710459	MH554734	MH554493
Sarcostroma africanum	CBS 143879	*Pelargonium cucullatum*	South Africa	MH554078	MH554289	MH554752	MH554513
Sarcostroma australiense	CBS 144160	*Daviesia latifolia*	Australia	MH554138	MH554340	MH554811	MH554573
Seimatosporium germanicum	CBS 437.87	NA	Germany	MH554047	MH554259	MH554723	MH554482
Seimatosporium luteosporum	CBS 142599	*Vitis vinifera*	USA	KY706284	KY706309	KY706259	KY706334
Seiridium cancrinum	CBS 226.55	*Cupressus macrocarpa*	Kenya	LT853089	MH554241	LT853236	LT853186
Seiridium cupressi	CBS 224.55	*Cupressus macrocarpa*	Kenya	LT853083	MH554240	LT853230	LT853180
Sporocadus biseptatus	CBS 110324	NA	NA	MH553956	MH554179	MH554615	MH554374
Sporocadus cornicola	CBS 143889	*Cornus sanguinea*	Germany	MH554121	MH554326	MH554794	MH554555
Sporocadus trimorphus	CBS 114203	*Rosa canina*	Sweden	MH553977	MH554196	MH554636	MH554395
Strickeria kochii	CBS 140411	*Robinia pseudoacacia*	Austria	NR_154423	KT949918	MH554679	MH554437
Subramaniomyces fusisaprophyticus	CBS 418.95	Leaf litter	Cuba	EU040241	EU040241	NA	NA
Synnemapestaloides juniperi	CBS 477.77	*Juniperus phoenicea*	France	MH554053	MH554266	MH554729	MH554488
Truncatella angustata	CBS 113.11	*Picea abies*	Germany	MH553966	MH554185	MH554625	MH554384
Xenoseimatosporium quercinum	CBS 129171	*Rhododendron* sp.	Latvia	MH553997	MH554216	MH554666	MH554424
Xyladictyochaeta lusitanica	CBS 143502	*Eucalyptus* sp.	Australia	MH107926	MH107972	MH108053	MH108033

Note: NA, not applicable. Strains in this study are marked in bold.

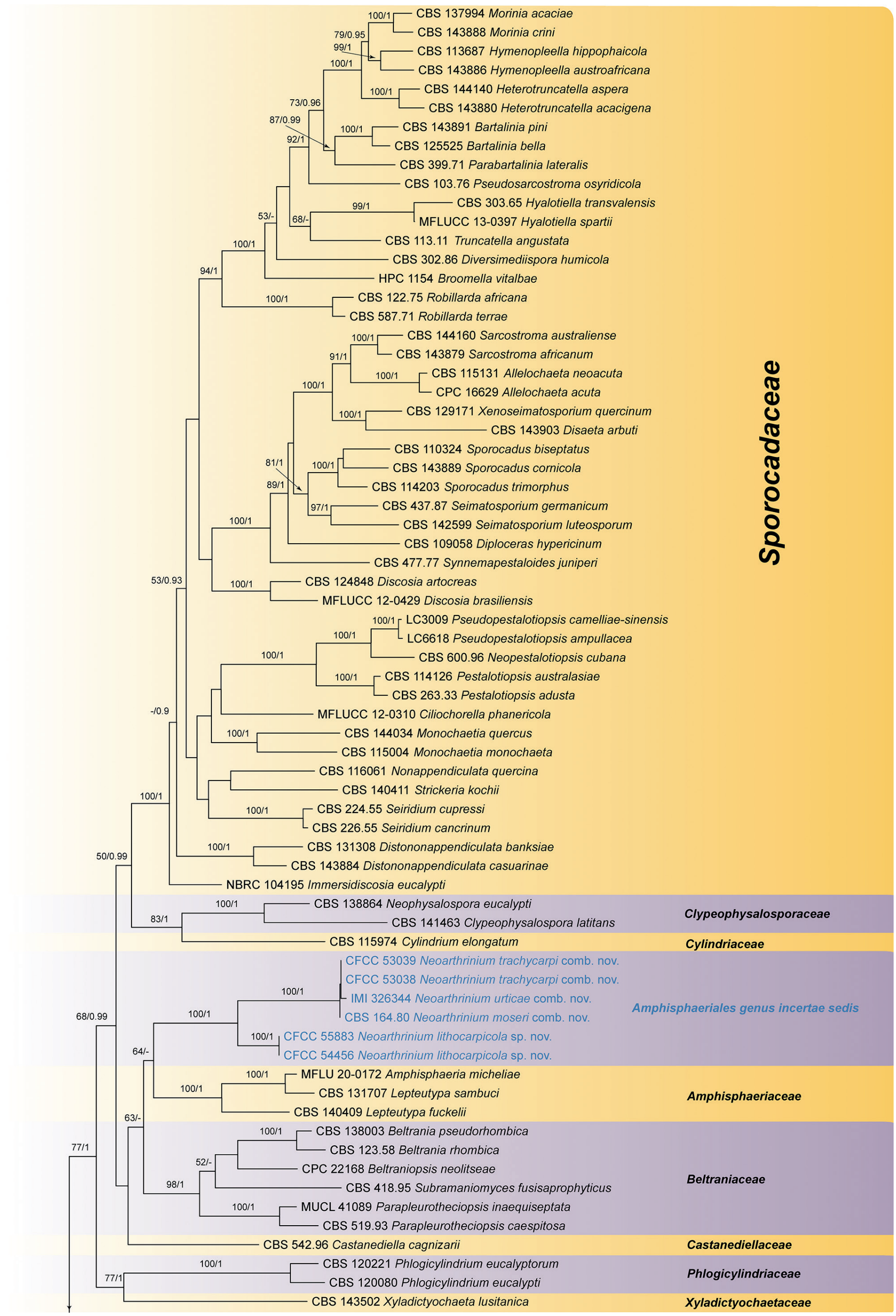

Figure 1. Phylogram of Amphisphaeriales resulting from a Maximum Likelihood analysis, based on a combined matrix of ITS, LSU, *tef1* and *tub2*. Numbers above the branches indicate ML bootstraps (left, ML BS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.90). The tree is rooted with *Clypeosphaeria mamillana* (CBS 140735) and *Pseudosporidesmium knawiae* (CBS 123529). New species and combinations proposed in the present study are marked in blue.

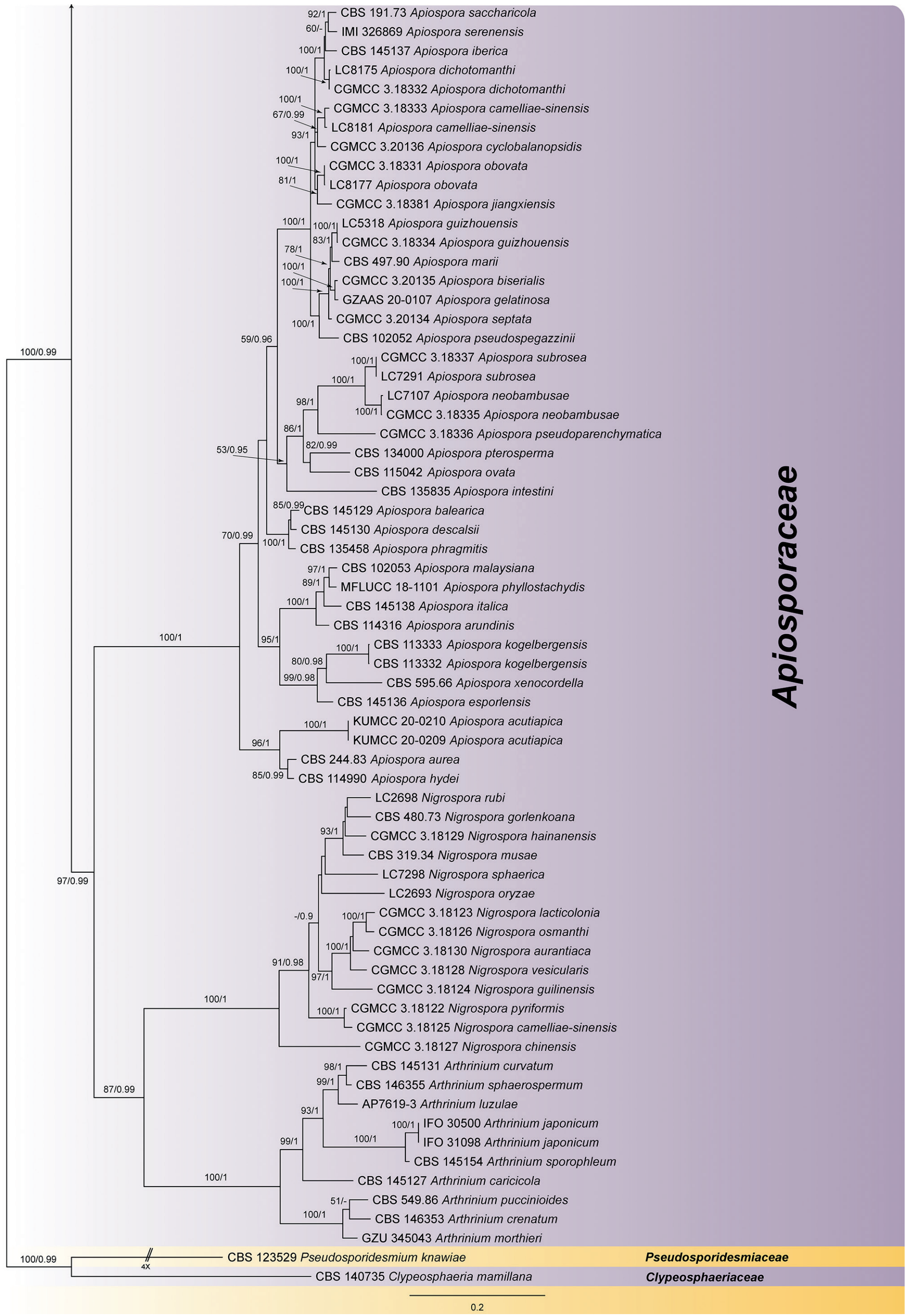

Figure 1. Continued.

Taxonomy

Neoarthrinium Ning Jiang, gen. nov.

MycoBank No: 843845

Etymology. Named after its morphological similarity to *Arthrinium*.

Type species. *Neoarthrinium lithocarpicola* Ning Jiang

Description. ***Hyphae*** formed on PDA hyaline, branched, septate. Asexual morph: ***Conidiophores*** cylindrical, septate, verrucose, flexuous, sometimes reduced to conidiogenous cells. ***Conidiogenous cells*** erect, blastic, aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliiform, subglobose to lageniform, branched. ***Conidia*** brown to dark brown, smooth to finely roughened, subglobose, ellipsoid to lenticular, with a longitudinal germ slit, occasionally elongated to ellipsoidal. Sexual morph: Undetermined.

Neoarthrinium lithocarpicola Ning Jiang, sp. nov.

MycoBank No: 843846

Fig. 2

Etymology. Named for its host genus "*Lithocarpus*" and "*-cola*" = inhabiting.

Description. ***Hyphae*** 1.5–4.5 μm diam., hyaline, branched, septate. Asexual morph: ***Conidiophores*** cylindrical, septate, verrucose, flexuous, sometimes reduced to conidiogenous cells. ***Conidiogenous cells*** erect, blastic, aggregated in clusters on hyphae, hyaline to pale brown, smooth, globose to subglobose, branched, (4–)5.5–8 × 2.5–3.5(–4) μm, mean ± SD = 6.6 ± 1.3 × 3.1 ± 0.4 μm, n = 50. ***Conidia*** brown to dark brown, smooth to finely roughened, subglobose to lenticular, with a longitudinal germ slit, occasionally elongated to ellipsoidal, (5–)6–8(–8.5) × (4.5–)5–5.5(–6) μm, mean ± SD = 7 ± 0.8 × 5.3 ± 0.5 μm, L/W = 1.1–1.8, n = 50. Sexual morph: Undetermined.

Culture characters. ***Colonies*** on PDA flat, spreading, with flocculent aerial mycelium forming concentric rings, edge entire, mouse grey to greyish-green, reaching 60 mm diam. after 10 d at 25 °C, forming abundant conidiomata.

Specimens examined. China. Guangdong Province, Guangzhou City, on leaf spots of *Lithocarpus glaber* (Thunb.) Nakai, *Shang Sun* (holotype CAF800050 = JNH0046; ex-type living culture: CFCC 54456; other living culture: CFCC 55883).

Notes. Two isolates of *Neoarthrinium lithocarpicola* from *Lithocarpus glaber* (Thunb.) Nakai formed a well-supported monophyletic clade, distinct from *N. moseri*, *N. trachycarpi* and *N. urticae* (Fig. 1). Morphologically, *N. lithocarpicola* is distinguished from *N. moseri* in smaller conidia (5–8.5 × 4.5–6 μm in *N. lithocarpicola* vs. 10–14 × 3–4.5 μm in *N. moseri*; Gams 1995). *Neoarthrinium lithocarpicola* is different from *N. urticae* by lacking thick blackish septa in conidiophores (Ellis 1965). *Neoarthrinium lithocarpicola* is similar to *N. trachycarpi* in the size of its conidiogenous cells and conidia, but it can be distinguished by its globose to subglobose conidiogenous cells (Yan et al. 2019).

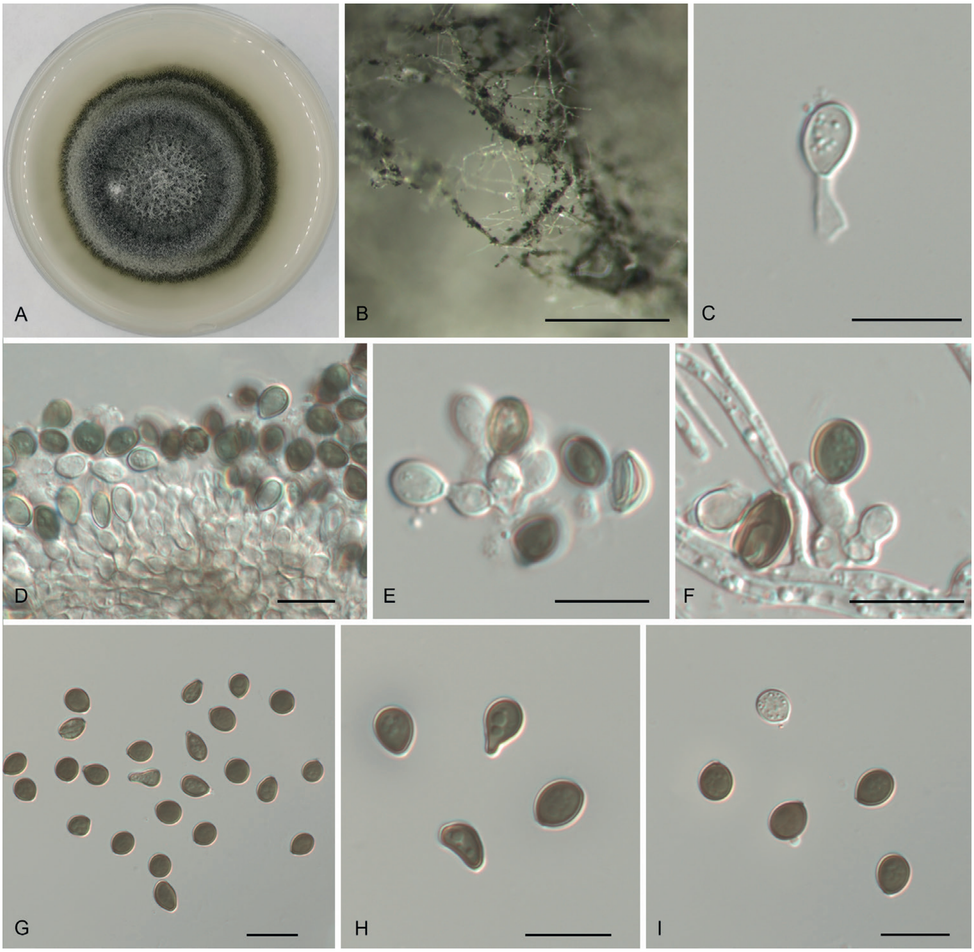

Figure 2. *Neoarthrinium lithocarpicola* **A** colony on PDA **B** conidiomata formed in culture **C–F** conidiogenous cells giving rise to conidia **G–I** conidia. Scale bars: 500 µm (**B**), 10 µm (**C–I**).

Neoarthrinium moseri **(W. Gams) Voglmayr, comb. nov.**
MycoBank No: 844772

Basionym. *Wardomyces moseri* W. Gams, Beih. Sydowia 10: 67 (1995)

Notes. Based on a placement within Xylariales in phylogenetic analyses, Sandoval-Denis et al. (2016) excluded this species from the genus (Microascales); however, they did not suggest an alternative generic classification. The blastic hyaline, smooth, lageniform conidiogenous cells aggregated in clusters and the subglobose to ellipsoid dark brown conidia with a longitudinal germ slit (Gams 1995) fully matched the genus *Neoarthrinum*. The ITS, LSU and *tub2* sequences of the ex-holotype strain of *N. moseri* (CBS 164.80) are almost identical to those of *N. trachycarpi*, indicating that they may be synonymous. Both species were isolated from petioles of palms: *N. moseri* from *Mauritia minor* Burret in Colombia and *N. trachycarpi* from *Trachycarpus fortunei*

(Hook.) H.Wendl. in China. However, the two species were reported to differ in conidial size (10–14 × 3–4.5 µm in *N. moseri* vs. 6.1–8.5 × 4.2–5.8 µm in *N. trachycarpi*; Gams 1995; Yan et al. 2019) and for the time being, we therefore kept them separate.

Neoarthrinium trachycarpi **(C.M. Tian & H. Yan) Ning Jiang, comb. nov.**

MycoBank No: 843847

Basionym. *Arthrinium trachycarpi* C.M. Tian & H. Yan [as '*trachycarpum*'], Phytotaxa 400(3): 208 (2019)

Neoarthrinium urticae **(M.B. Ellis) Ning Jiang, comb. nov.**

MycoBank No: 843848

Basionym. *Arthrinium urticae* M.B. Ellis, Mycol. Pap. 103: 16 (1965)

Notes. The possibility that *Apiosporella urticae* (Rehm) Höhn. is the sexual morph of *Arthrinium urticae* is raised by the fact that both share the same host (*Urtica*) and are classified as members of the Apiosporaceae (Index Fungorum, accessed 4 July 2022). This evidence would have far reaching nomenclatural consequences not only for species, but also for generic classification, as *Apiosporella* (Höhnel 1909) may then qualify for an older genus name to be used for *Neoarthrinium*. However, according to L. Holm, the holotype specimen of its basionym, *Apiospora urticae* (S-F12119), represents a very different fungus, *Didymella eupyrena* (Didymellaceae, Pleosporales, Dothideomycetes; https://herbarium.nrm.se/specimens/F12119, accessed 4 July 2022). The status of the genus *Apiosporella* is still unclear because Höhnel (1909) did not choose a type from the six different species included in the genus. However, none of the original species is a close relative of Apiosporaceae or *Neoarthrinium*; therefore, *Apiosporella* should be excluded from Apiosporaceae.

No sequence data are available for isolates from the type host *Urtica dioica* L. (Urticaceae). The single culture sequenced (IMI 326344) was isolated from unidentified leaf litter collected in India. Additional molecular studies on verified isolates from *Urtica* collected in Europe are necessary to reveal whether IMI 326344 represents true *N. urticae*. However, *N. urticae* appears to be very rare and we are unaware of any additional collections with the exception of the type.

Discussion

Arthrinium and related genera are important fungal taxa whose concepts and classification have undergone many changes and additions (e.g. Cooke 1954; Samuels et al. 1981; Larrondo and Calvo 1990; Hyde et al. 1998; Jaklitsch and

Voglmayr 2012; Crous and Groenewald 2013; Singh et al. 2013; Sharma et al. 2014; Dai et al. 2016, 2017; Hyde et al. 2016; Jiang et al. 2018, 2020; Wang et al. 2018; Pintos et al. 2019; Pintos and Alvarado 2021). In recent years, substantial changes in classification were implemented in the course of unitary nomenclature. A large number of newly-discovered species have been described as a result of extensive sampling of new isolates, based on multigene phylogenies (e.g. Crous and Groenewald 2013; Wang et al. 2018; Pintos and Alvarado 2021). Currently, *Arthrinium*-like asexual morphs are shared by three distinct lineages within Amphisphaeriales, viz. *Apiospora*, *Arthrinium s. s.* and *Neoarthrinium* as shown in Fig. 1. *Arthrinium s. s.* is the sister genus to *Nigrospora*, which morphologically differs from *Apiospora*, *Arthrinium* and *Neoarthrinium* in conidial ontogeny (Wang et al. 2017). The phylogram shown in Fig. 1 is consistent with that shown in Tian et al. (2021) in placing *Apiospora*, *Arthrinium* and *Nigrospora* within a clade that is distinct from the new genus *Neoarthrinium*, although *Apiospora* and *Arthrinium* share conidial morphology similar to that of *Neoarthrinium.*

Morphologically, *Apiospora*, *Arthrinium* and *Neoarthrinium* are similar in having basauxic conidiogenesis. Conidia of *Apiospora* and *Neoarthrinium* are generally more or less rounded in face view and lenticular in side view, while those of *Arthrinium* are variously shaped, viz. globose, angular, polygonal, curved, fusiform or navicular (Yan et al. 2019; Pintos and Alvarado 2021). However, the conidiophores of several *Arthrinium* and *Neoarthrinium* species have thick blackish septa, which are rarely observed in *Apiospora* (Ellis 1965; Wang et al. 2018; Pintos and Alvarado 2021). Hence, these three genera are difficult to distinguish by only asexual morphology.

Regarding their hosts, there are some tendencies in host preferences, while *Arthrinium* species are predominantly found in Cyperaceae and Juncaceae (Pintos and Alvarado 2021) and species of *Apiospora* primarily occur on Poaceae (but also on many other hosts; Wang et al. 2018). Four *Neoarthrinium* species were discovered on four hosts from three distantly-related host families (i.e. *N. lithocarpicola* from *Lithocarpus glaber* (Thunb.) Nakai, Fagaceae; *N. moseri* from *Mauritia minor* Burret, Arecaceae; *N. trachycarpi* from *Trachycarpus fortune* (Hook.) H.Wendl., Arecaceae; and *N. urticae* from *Urtica dioica* L., Urticaceae; Ellis 1965; Yan et al. 2019). Hence, host association is not a fully reliable feature to distinguish *Apiospora*, *Arthrinium* and *Neoarthrinium.*

Compared to species, generic delimitation is much more subjective. However, there is a broad agreement that genera, along with all taxonomic classification units at all ranks, should be monophyletic. As morphology is frequently insufficient for phylogenetic classification, molecular evidence is regarded as significant data or even an essential characteristic in the classification and identification of fungal taxa. In the present study, *Neoarthrinium* is proposed as a new genus for a group of species phylogenetically distinct from *Apiospora*, *Arthrinium* and *Nigrospora* to maintain monophyletic *Arthrinium*-like genera. Using morphological and phylogenetic data, however, we need more samples to improve our understanding of *Arthrinium*-like taxa and genera in the Amphisphaeriales.

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