

A phylogenetic re-evaluation of *Arthrinium*

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Abstract: Although the genus *Arthrinium* (sexual morph *Apiospora*) is commonly isolated as an endophyte from a range of substrates, and is extremely interesting for the pharmaceutical industry, its molecular phylogeny has never been resolved. Based on morphology and DNA sequence data of the large subunit nuclear ribosomal RNA gene (LSU, 28S) and the internal transcribed spacers (ITS) and 5.8S rRNA gene of the nrDNA operon, the genus *Arthrinium* is shown to belong to *Apiosporaceae* in *Xylariales*. *Arthrinium* is morphologically and phylogenetically circumscribed, and the sexual genus *Apiospora* treated as synonym on the basis that *Arthrinium* is older, more commonly encountered, and more frequently used in literature. An epitype is designated for *Arthrinium pterospermum*, and several well-known species are redefined based on their morphology and sequence data of the translation elongation factor 1-alpha (TEF), beta-tubulin (TUB) and internal transcribed spacer (ITS1, 5.8S, ITS2) gene regions. Newly described are *A. hydei* on *Bambusa tuldoidea* from Hong Kong, *A. kogelbergense* on dead culms of *Restionaceae* from South Africa, *A. malaysianum* on *Macaranga hullettii* from Malaysia, *A. ovatum* on *Arundinaria hindsii* from Hong Kong, *A. phragmites* on *Phragmites australis* from Italy, *A. pseudospegazzinii* on *Macaranga hullettii* from Malaysia, *A. pseudosinense* on bamboo from The Netherlands, and *A. xenocordella* from soil in Zimbabwe. Furthermore, the genera *Pteroconium* and *Cordella* are also reduced to synonymy, rejecting spore shape and the presence of setae as characters of generic significance separating them from *Arthrinium*.

Key words:

Apiospora
Apiosporaceae
 ITS
 LSU
 Ascomycota
Sordariomycetes
 Systematics

Article info: Submitted: 15 May 2013; Accepted: 4 June 2013; Published: 24 June 2013.

INTRODUCTION

The genus *Arthrinium* (sexual morph *Apiospora*; Ellis 1971, Seifert *et al.* 2011) is widespread and ecologically diverse. It commonly occurs as a saprobe on grasses, and also on leaves, stems and roots of a range of different plant substrates (Agut & Calvo 2004). *Arthrinium* is ecologically diverse, and has been reported as a plant pathogen, with *A. arundinis* causing kernel blight of barley (Martinez-Cano *et al.* 1992), and *A. sacchari* causing damping-off of wheat (Mavragani *et al.* 2007). It is reported as an endophyte in plant tissue (Ramos *et al.* 2010), lichens (He & Zhang 2012), and marine algae (Suryanarayanan 2012). *Arthrinium phaeospermum* causes cutaneous infections of humans (Rai 1989, Zhao *et al.* 1990, de Hoog *et al.* 2000).

Isolates of *Arthrinium* produce a range of interesting extrolites in culture, some of which exhibit significant toxicity against human cancer cell lines (Klemke *et al.* 2003), or inhibit a broad range of human pathogenic filamentous fungi, yeasts, and bacteria (Cabello *et al.* 2001, Ramos *et al.* 2010). An endophytic isolate of *A. phaeospermum* produces growth-promoting substances in *Carex kobomugi*, a plant surviving under extreme conditions on sand dunes in Korea (Khan *et al.* 2009).

The genus *Arthrinium* was described in 1817 and has numerous generic synonyms (Seifert *et al.* 2011). One such generic name with uncertain status is *Pteroconium*, introduced in 1892, which Ellis (1971, 1976) and Seifert *et al.* (2011) retained as separate from *Arthrinium*, in spite of its *Apiospora* sexual morph. *Cordella* is another potential synonym of *Arthrinium*, distinguished chiefly by possessing setae. During this study several interesting isolates were collected, including ones of *P. pterospermum*, the type species of *Pteroconium*. The decision to move to a single nomenclature is explained elsewhere (Hawksworth *et al.* 2011, Wingfield *et al.* 2012), and adopted here in accordance with the current *Code*. Although both genera (*Arthrinium* and *Apiospora*) have a similar number of species, *Arthrinium* is older and more commonly encountered and referred to in the literature than *Apiospora* introduced in 1875. Following the principles advocated by Hawksworth (2012) for dealing with names in the present period of transition, we propose that in future *Arthrinium* be used when referring to these taxa. No in-depth phylogenetic analysis has thus far been published on *Arthrinium*, which is placed in *Apiosporaceae* (*Sordariomycetes*) (Hyde *et al.* 1998, Lumbsch & Huhndorf 2010). The aims of the present study were to resolve the potential synonymy of *Arthrinium*, *Cordella*, and *Pteroconium*,

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elucidate the higher classification and phylogeny of *Apiosporaceae*, and at the same time provide a more robust tree for species of *Arthrinium*.

MATERIALS AND METHODS

Isolates

Fresh collections were made from debris of diverse hosts by placing material in damp chambers for 1–2 d. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous *et al.* 1991, 2009b). Additional strains were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands. Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al.* 2009b), and pine needle agar (PNA) (Smith *et al.* 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains are deposited in CBS.

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The primers ITS4 (White *et al.* 1990) and LSU1Fd (Crous *et al.* 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. Part of the translation elongation factor 1-alpha (TEF) was amplified and sequenced using primers EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell *et al.* 1998), while T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) were used for the beta-tubulin gene region (TUB). Amplification conditions for ITS, LSU and TEF followed Crous *et al.* (2013) and for TUB, Lee *et al.* (2004). Megablast searches (Altschul *et al.* 1997) using the ITS and LSU sequences were performed in NCBI's GenBank nucleotide sequence database to identify the closest matching sequences, which were added to the sequence alignment. The sequence alignment and subsequent phylogenetic analyses for all the above were carried out using the methods in Crous *et al.* (2006). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses (only for ITS and TEF; see alignment in TreeBASE: ID 14349); the remaining gaps were treated as "fifth state" data in the parsimony analyses. For the LSU alignment, MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings prior to the Bayesian analysis in MrBayes v. 3.2.1 (Ronquist *et al.* 2012). Sequences derived in this study were lodged at GenBank, the alignments and trees in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al.* 2004).

Morphology

Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Measurements and photographs were made from structures mounted in clear lactic acid. The 95 % confidence intervals were derived from 30 observations ($\times 1000$ magnification), with the extremes given in parentheses. Ranges of the dimensions of other characters are given. Colony characters and pigment production were noted after 2 wk of growth on MEA, PDA and OA (Crous *et al.* 2009b) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on cultures sporulating on PDA.

RESULTS

Phylogeny

Amplicons of approximately 1700 bases were obtained of the partial 18S rRNA, full length ITS and partial 28S rRNA (LSU) genes for the isolates in Table 1, and approximately 750 bp and 450 bp for TUB and TEF, respectively. The LSU alignment was used to resolve the generic placement of strains (Fig. 1) and the ITS to determine species identification (Fig. 2; discussed in species notes where applicable). The combined TEF and TUB alignment (Fig. 3) was used to confirm the species resolution of ITS and that no cryptic species complexes were present. As each alignment addressed a specific research question (LSU: genera, ITS: species as the standard barcode region, and TEF and TUB to resolve species complexes, if any), a combined tree based on all four loci was not generated. In addition, such a combined tree would be based on an alignment which includes some missing sequences and would, therefore, not be as robust as the phylogenetic trees presented in Figs 1–3.

The manually adjusted LSU alignment contained 80 sequences (including the outgroup sequence), and 791 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; the data partition contained 199 unique site patterns. Based on the results of MrModeltest, the following priors were set in MrBayes: dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed. The Bayesian analysis lasted 2 655 000 generations and the 50 % consensus trees and posterior probabilities were calculated from the 3984 trees left after discarding 1328 trees (the first 25 % of generations) for burn-in (Fig. 1). All *Apiospora* and *Arthrinium* strains clustered in a well-supported clade indicated in Fig. 1 as the family *Apiosporaceae*.

The manually adjusted ITS alignment contained 72 sequences (including the outgroup sequence), and 514 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis. Of these characters, 157 were parsimony-informative, 51 variable and parsimony-uninformative, and 306 constant. The parsimony analysis of the ITS alignment yielded 72 equally most parsimonious trees (TL = 552 steps; CI = 0.621; RI = 0.938; RC = 0.583). Some species, e.g. *A. marii* and *A. sacchari*, are not well-

supported in the ITS phylogeny (Fig. 2), but well-supported in the combined TUB and TEF phylogeny (Fig. 3).

The manually adjusted combined TUB and TEF alignment contained 39 sequences (including the outgroup sequence) and 1288 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 565 of these were parsimony-informative, 51 were variable and parsimony-uninformative, and 486 were constant. The parsimony analysis of the ITS alignment yielded four equally most parsimonious trees (TL = 2003 steps; CI = 0.703; RI = 0.875; RC = 0.616). All included species were well-supported in the combined TUB and TEF phylogeny (Fig. 3).

TAXONOMY

The species treated below are those that were available in culture. Several other names exist, but these await to be recollected and subjected to DNA analysis.

Apiosporaceae K. D. Hyde *et al.*, *Sydowia* **50**: 23 (1998).

Description: *Conidiophores* frequently arising from hyphae or aggregated in a brown stroma, forming black sporodochia, brown to dark brown, forming conidia laterally and terminally. *Setae* present or absent, brown, smooth, erect, sparsely septate, intermingled among conidiophores. *Conidiogenous cells* discrete, doliiform to ampulliform to subcylindrical, subhyaline to pale brown, smooth to finely verruculose, aggregated on aerial hyphae, giving rise to clusters of conidia; at times reduced to lateral pegs on hyphae, proliferating sympodially or percurrently. *Conidia* aseptate, brown to dark brown, smooth to verruculose, guttulate to granular, frequently with equatorial slit of lighter pigment. *Stromata* immersed in epidermis, becoming erumpent through a longitudinal split, revealing rows of densely arranged perithecial ascomata. *Paraphyses* broadly filiform, septate, deliquescing early. *Ascomata* globose with papillate ostioles; wall composed of multiple layers of pseudoparenchymatous cells. *Asci* 8-spored, unitunicate, clavate to broadly cylindrical. *Ascospores* bi- to tri-seriate, ellipsoidal, inequilateral, tapered at both ends, apiosporous, 1-septate near the lower end, smooth, hyaline, with or without mucoid sheath.

Type genus: *Apiospora* Sacc. 1875 (syn. *Arthrimum* Kunze 1817).

Note: Based on morphology, Hyde *et al.* (1998) regarded *Dictyoarthrinium*, *Endocalyx*, *Scyphospora* and *Spegazzinia* as possible members of this family, though this remains to be confirmed, pending molecular studies.

Arthrimum Kunze, in Kunze & Schmidt, *Mykol. Hefte* **1**: 9 (1817) : Fr., *Syst. Mycol.* **1**: xlv (1821).

Type species: *A. caricicola* Kunze & J.C. Schmidt 1817
Synonyms: *Apiospora* Sacc., *Atti Soc. Veneto-Trent. Sci. Nat., Padova* **4**: 85 (1875).
Type species: *A. montagnei* Sacc. 1875

Cordella Speg., *Anales Soc. Ci. Argent.* **22**: 210 (1886).

Type species: *C. coniosporioides* Speg. 1886

Pteronium Sacc., *Syll. Fung.* **10**: 570 (1892).

Type species: *P. pterospermum* (Cooke & Masee) Grove 1914

Additional synonyms are listed in Ellis (1965) and Seifert *et al.* (2011).

Description: *Colonies* compact, black to dark brown, superficial to erumpent. *Mycelium* immersed and superficial. *Conidiophores* arising from basal cells that are subcylindrical, subhyaline with refractive, thick transverse septa, brown to dark brown, forming conidia laterally and terminally; conidiophores frequently aggregated in a brown stroma, forming black sporodochia on the host and in culture. *Setae* present or absent, brown, smooth, erect, sparsely septate, tapering to subacute apex, intermingled among conidiophores. *Conidiogenous cells* discrete, doliiform to ampulliform to subcylindrical, subhyaline to pale brown, smooth to finely verruculose, aggregated on aerial hyphae, giving rise to clusters of conidia; at times reduced to lateral pegs on hyphae, holoblastic, proliferating sympodially (at times clearly phialidic with periclinal thickening, rarely with percurrent proliferation). *Conidia* aseptate, brown to dark brown, smooth to verruculose, guttulate to granular, with distinctive shape (round, curved, curved with two horns, oblong, irregular, limoniform, fusiform, navicular, dentate or lobed), at times flattened, with equatorial slit of lighter pigment. *Sterile cells* when formed replace conidia, usually smaller and paler than conidia, with different shape, frequently containing refractive cubical bodies. *Stromata* immersed in epidermis, becoming erumpent through a longitudinal split, revealing rows of densely arranged perithecial ascomata. *Ascomata* globose with papillate ostioles; wall composed of 6–9 layers of pseudoparenchymatous cells. *Paraphyses* broadly filiform, septate, deliquescing early. *Asci* 8-spored, unitunicate (appearing bitunicate when young), clavate to broadly cylindrical. *Ascospores* smooth, hyaline, bi- to tri-seriate, ellipsoidal, inequilateral, tapered at both ends, apiosporous, 1-septate near the lower end, with the lower, smaller cell subglobose; ascospores with or without mucoid sheath.

Notes: The conidiogenesis of *Arthrimum* species is of particular interest. Conidiogenous cells are generally aggregated on a pale brown stroma, forming sporodochia. They tend to be doliiform to subcylindrical, pale brown, with clear periclinal thickening, as illustrated in Ellis (1965). Given moist conditions, they develop further and become ampulliform, with a prominent, elongated neck. The neck can give rise to conidia either sympodially (appearing as holoblastic loci), or in some cases percurrently, with annulations aggregated at the apex. This variation in conidiogenesis makes it difficult to compare these characters among taxa, as conidiophores can either be hyphae with lateral loci, or be reduced to doliiform conidiogenous cells that can be seen to develop further (or not), and are frequently aggregated in sporodochia. Conidia themselves, however, do not appear to differ between those

Table 1. Details of strains included in the phylogenetic analyses.

Species	Strain accession number ^{1,2}	Substrate of isolation	Origin	Collector	GenBank accession numbers ³			
					ITS	LSU	TUB	TEF
<i>Arthrinium arundinis</i>	CBS 106.12	—	Germany: Bromberg	E. Schaffnit	KF144883	KF144927	KF144973	KF145015
	CBS 114316	Leaf of <i>Hordeum vulgare</i>	Iran: Shabestar	B. Askari	KF144884	KF144928	KF144974	KF145016
	CBS 124788	Living leaves of <i>Fagus sylvatica</i>	Switzerland: Basel	M. Unterseher	KF144885	KF144929	KF144975	KF145017
	CBS 133509 = NRRL 13883	<i>Aspergillus flavus</i> sclerotium buried in sandy field	USA: Kilbourne	—	KF144886	KF144930	KF144976	KF145018
	CBS 449.92	Culm of cultivated <i>Sasa</i>	Canada: Vancouver	R.J. Bandoni	KF144887	KF144931	KF144977	KF145019
	CBS 450.92	Stem of cultivated bamboo	Canada: Vancouver	R.J. & A.A. Bandoni	AB220259	KF144932	KF144978	KF145020
	CBS 464.83	Dead culms of <i>Phragmites australis</i>	The Netherlands: Harderbos	W. Gams	KF144888	KF144933	KF144979	KF145021
	CBS 732.71	Dung	India	B.C. Lodha	KF144889	KF144934	KF144980	KF145022
<i>Arthrinium aureum</i>	CBS 244.83 ^{ET}	Air	Spain: Barcelona	A. Calvo & J. Guarro	AB220251	KF144935	KF144981	KF145023
<i>Arthrinium hydei</i>	CBS 114990 ^{ET}	Culms of <i>Bambusa tuldooides</i>	Hong Kong: Tai Po Kau	K.D. Hyde	KF144890	KF144936	KF144982	KF145024
<i>Arthrinium kogelbergense</i>	CBS 113332	Dead culms of <i>Cannomolis virgata</i>	South Africa	S. Lee	KF144891	KF144937	KF144983	KF145025
	CBS 113333 ^{ET}	Dead culms of <i>Restionaceae</i>	South Africa	S. Lee	KF144892	KF144938	KF144984	KF145026
	CBS 113335	Dead culms of <i>Resfia quadratus</i>	South Africa	S. Lee	KF144893	KF144939	KF144985	KF145027
	CBS 114734 = UPSC 3251	<i>Juncus gerardi</i>	Sweden: Börstäl par.	K. & L. Holm	KF144894	KF144940	KF144986	KF145028
	CBS 117206	Unknown algae	Croatia	E. Eguereva	KF144895	KF144941	KF144987	KF145029
<i>Arthrinium malaysianum</i>	CBS 102053 ^{ET}	<i>Macaranga hulleitii</i> stem colonised by ants	Malaysia: Gombak	W. Federle	KF144896	KF144942	KF144988	KF145030
	CBS 251.29	Stembase of <i>Cinnamomum camphora</i>	—	—	KF144897	KF144943	KF144989	KF145031
	CBS 113535	Oats	Sweden	C. Svenson	KF144898	KF144944	KF144990	KF145032
<i>Arthrinium marii</i>	CBS 114803 = HKUCC 3143	Culm of <i>Arundinaria hindsi</i>	Hong Kong: Lung Fu Shan	K.D. Hyde	KF144899	KF144945	KF144991	KF145033
	CBS 200.57	Leaf of <i>Beta vulgaris</i>	The Netherlands	Unknown	KF144900	KF144946	KF144992	KF145034
	CBS 497.90 ^{ET} = MUCL 31300	Beach sand	Spain: Barcelona	J.V. Larrondo & A. Calvo	AB220252	KF144947	KF144993	KF145035
	CPC 18902	Stems of <i>Phragmites australis</i>	Italy: Bomarzo	W. Gams	KF144901	KF144948	—	—
	CPC 18904	Stems of <i>Phragmites australis</i>	Italy: Bomarzo	W. Gams	KF144902	KF144949	KF144994	KF145036
<i>Arthrinium ovatum</i>	CBS 115042 ^{ET}	<i>Arundinaria hindsi</i>	Hong Kong	K.D. Hyde	KF144903	KF144950	KF144995	KF145037
<i>Arthrinium phaeospermum</i>	CBS 114314	Leaf of <i>Hordeum vulgare</i>	Iran: Marand	B. Askari	KF144904	KF144951	KF144996	KF145038
	CBS 114315	Leaf of <i>Hordeum vulgare</i>	Iran: Shabestar	B. Askari	KF144905	KF144952	KF144997	KF145039
	CBS 114317	Leaf of <i>Hordeum vulgare</i>	Iran: Marand	B. Askari	KF144906	KF144953	KF144998	KF145040
	CBS 114318	Leaf of <i>Hordeum vulgare</i>	Iran: Marand	B. Askari	KF144907	KF144954	KF144999	KF145041

Table 1. (Continued).

Species	Strain accession number ^{1,2}	Substrate of isolation	Origin	Collector	GenBank accession numbers ³			
					ITS	LSU	TUB	TEF
<i>Arthrinium phragmites</i>	CBS 142.55 CPC 18900 = CBS 135458 ^{ET}	Soil Culms of <i>Phragmites australis</i>	Japan: Tiba prefecture Italy: Bomarzo	K. Tubaki W. Gams	KF144908 KF144909	KF144955 KF144956	KF145000 KF145001	KF145042 KF145043
<i>Arthrinium pseudosinense</i>	CPC 21546 = CBS 135459 ^{ET}	Leaf of bamboo	The Netherlands: Utrecht	U. Damm	KF144910	KF144957	—	KF145044
<i>Arthrinium pseudospegazzinii</i>	CBS 102052 ^{ET}	<i>Macaranga hullettii</i> stem colonised by ants	Malaysia: Gombak	W. Federle	KF144911	KF144958	KF145002	KF145045
<i>Arthrinium pterospermum</i>	CBS 123185 = CPC 15380	Leaf lesion of <i>Machaerina sinclairii</i>	New Zealand: Auckland	C.F. Hill	KF144912	KF144959	KF145003	—
<i>Arthrinium rasikravindrii</i>	CPC 20193 = CBS 134000 ^{EE} CBS 337.61 = MUCL 8428	Leaf of <i>Lepidosperma gladiatum</i> Cissus	Australia: Adelaide The Netherlands	W. Quaedvlieg H.A. van der Aa	KF144913 KF144914	KF144960 KF144961	KF145004	KF145046
<i>Arthrinium saccharii</i>	CPC 21602 CBS 212.30	Rice <i>Phragmites australis</i>	Thailand United Kingdom: Cambridge	P.W. Crous E.W. Mason	KF144915 KF144916	— KF144962	— KF145005	— KF145047
	CBS 301.49	Bamboo	Indonesia	K.B. Boedijn & J. Reitsma	KF144917	KF144963	KF145006	KF145048
	CBS 372.67	Air	—	—	KF144918	KF144964	KF145007	KF145049
	CBS 664.74	Soil under <i>Calluna vulgaris</i>	The Netherlands	H. Linder	KF144919	KF144965	KF145008	KF145050
<i>Arthrinium saccharicola</i>	CBS 191.73 CBS 334.86 CBS 463.83	Air Dead culms of <i>Phragmites australis</i> Dead culms of <i>Phragmites australis</i>	The Netherlands France: Etang d'Hardy The Netherlands: Harderbos	H.A. van der Aa H.A. van der Aa W. Gams	KF144920 AB220257 KF144921	KF144966 KF144967 KF144968	KF145009 KF145010 KF145011	KF145051 KF145052 KF145053
	CBS 831.71 CPC 18977	— <i>Phragmites australis</i>	The Netherlands The Netherlands	M. van Schothorst P.W. Crous	KF144922 KF144923	KF144969 —	KF145012	KF145054
<i>Arthrinium</i> sp.	CPC 21866	Bamboo	Vietnam	U. Damm	KF144924	—	—	—
<i>Arthrinium xenocordella</i>	CBS 478.86 ^{ET} CBS 595.66 = MUCL 10009	Soil from roadway Soil	Zimbabwe: Matopos Austria: Plaseckerjoch	J.C. Krug M.A.A. Schipper	KF144925 KF144926	KF144970 KF144971	KF145013	KF145055

¹ CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; HKUCC: The University of Hong Kong Culture Collection, Hong Kong, China; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL: National Center for Agricultural Utilization Research, Peoria, Illinois, U.S.A.; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden.

² EE: ex-epitype strain; ET: ex-type strain.

³ ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S nrDNA; TEF: translation elongation factor 1- α ; TUB: partial beta-tubulin gene.

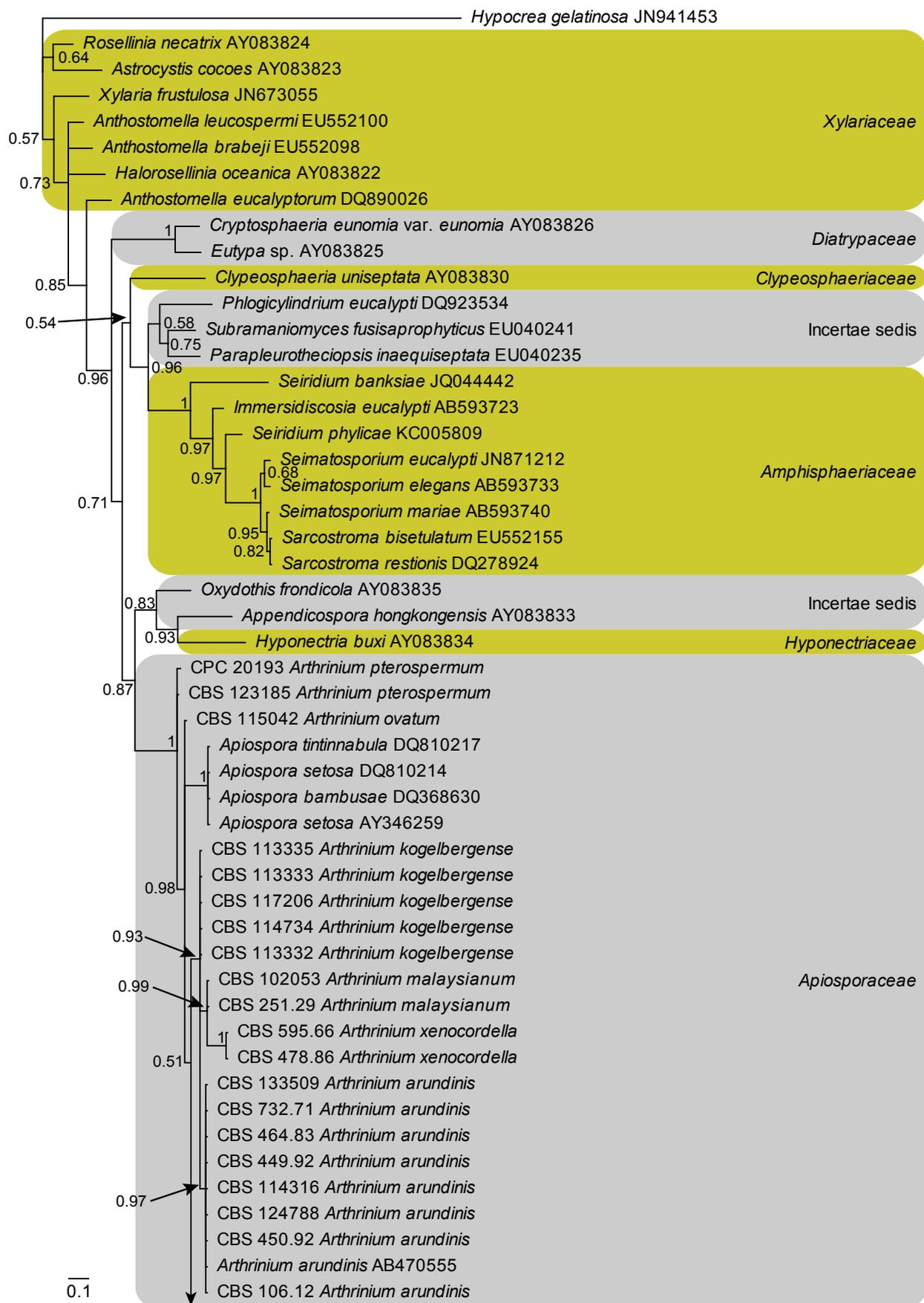


Fig. 1. Consensus phylogram (50 % majority rule) of 3 984 trees resulting from a Bayesian analysis of the LSU sequence alignment using MrBayes v. 3.2.1. Bayesian posterior probabilities are indicated at the nodes and the scale bar represents the expected changes per site. Families are indicated in coloured blocks and species names in black text. GenBank accession numbers for downloaded sequences are shown after species names and culture collection numbers before species names. The tree was rooted to *Hypocrea gelatinosa* (GenBank JN941453).

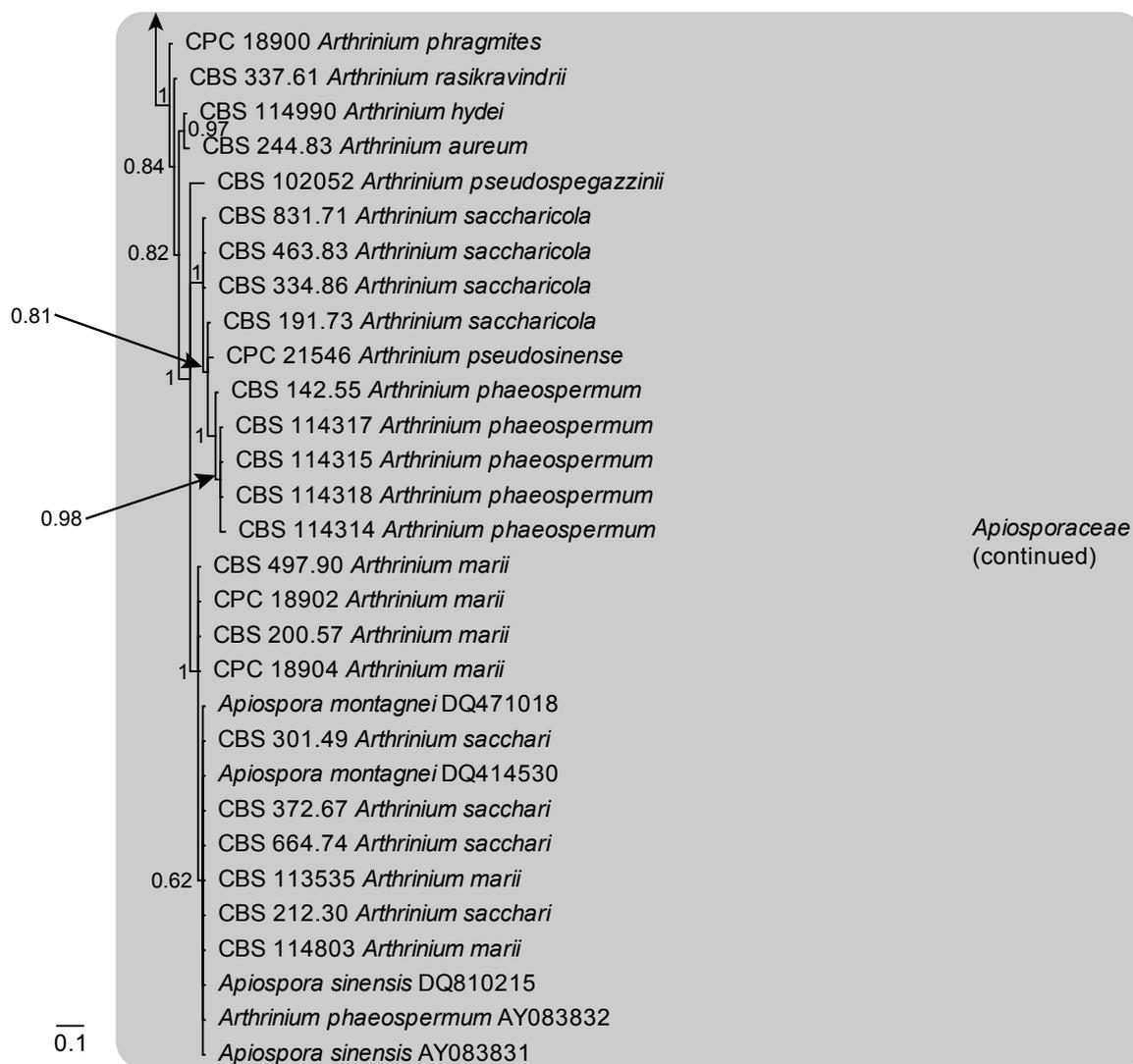


Fig. 1. (Continued).

observed in aerial mycelial strands (conidiophores *sensu* Ellis 1965) or conidiogenous cells situated on a stroma in a black sporodochium.

Arthrinium arundinis (Corda) Dyko & B. Sutton, *Mycotaxon* 8: 119 (1979).

Basionym: *Gymnosporium arundinis* Corda, *Icon. fung.* 2: 1 (1838).

Synonym: *Apiospora montagnei* Sacc., *N. Giorn. bot. Ital.* 7: 306 (1875).

(Fig. 4)

For further synonyms see Ellis (1965).

Description: *Mycelium* consisting of smooth, hyaline, branched, septate, 2–3 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* aggregated in clusters on hyphae, pale brown, smooth, ampulliform, 6–12 × 3–4 µm, apical neck 3–5 µm long, basal part 4–6 µm long. *Conidia* brown, smooth, globose in surface view, (5–)6–7 µm, lenticular in side view, 3–4 µm diam, with pale equatorial slit.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On PDA, MEA and OA surface iron-grey with patches of dirty white, reverse iron-grey.

Specimens examined: **Canada**: *British Columbia*: Vancouver, University of British Columbia campus, culm of cultivated *Sasa*, 13 July 1988, *R. J. Bandoni* (CBS 449.92); *loc. cit.*, stem of cultivated bamboo, 7 May 1992, *R. J. & A. A. Bandoni* (CBS 450.92). – **Germany**: Bromberg, 1912, *E. Schaffnit* (CBS 106.12). – **India**: dung, Dec. 1971, *B.C. Lodha* (CBS 732.71). – **Iran**: Shabestar, leaf of *Hordeum vulgare*, *B. Askari* (CBS 114316). – **The Netherlands**: *Flevoland*: Harderbos, dead culms of *Phragmites australis*, 15 May 1983, *W. Gams* (CBS 464.83). – **Switzerland**: Basel, living leaves of *Fagus sylvatica*, 8 Jan. 2008, *M. Unterseher* (CBS 124788). – **USA**: *Illinois*: Killbourne, *Aspergillus flavus* sclerotium buried in sandy field (NRRL 25634 = CBS 133509; isolate submitted for whole genome sequence analysis; <http://genome.jgi-psf.org/pages/search-for-genes.jsf?organism=Apimo1>).

Notes: The present cultures closely fit the original description and concept of *Arthrinium arundinis*, inclusive of the sexual morph, which is a commonly occurring, widely distributed

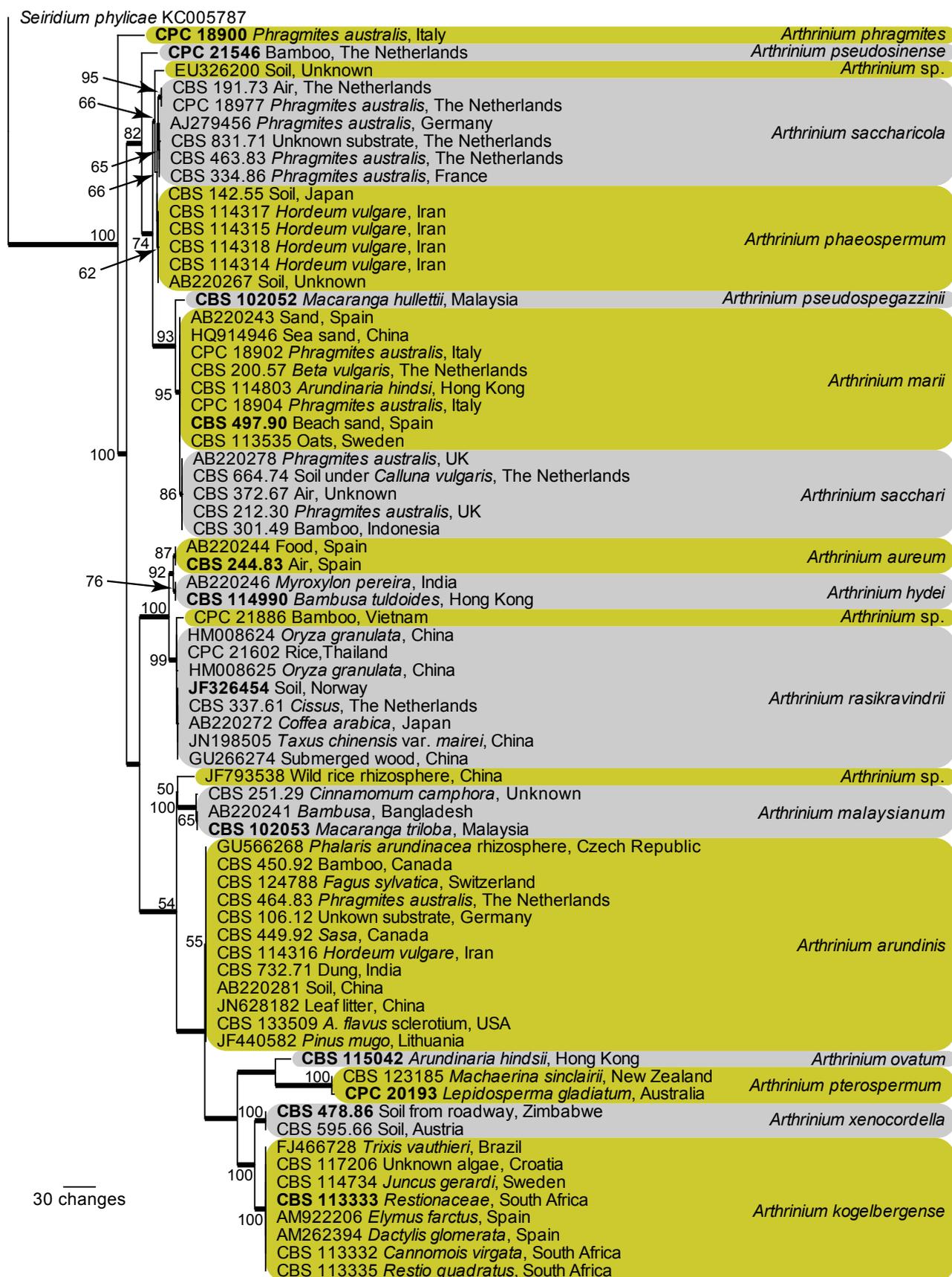


Fig. 2. The first of 72 equally most parsimonious trees obtained from an analysis of the ITS sequence alignment (TL = 552 steps, CI = 0.621, RI = 0.938, RC = 0.583). The numbers at the nodes represent bootstrap support values based on 1000 resamplings and thickened lines indicate those branches present in the strict consensus tree. Type and ex-type strains are indicated in bold and the scale bar indicates 30 changes. The culture collection or GenBank accession number is indicated for each sequence, followed by the isolation source and country of origin. The tree is rooted to *Seiridium phylicae* (GenBank accession KC005787).

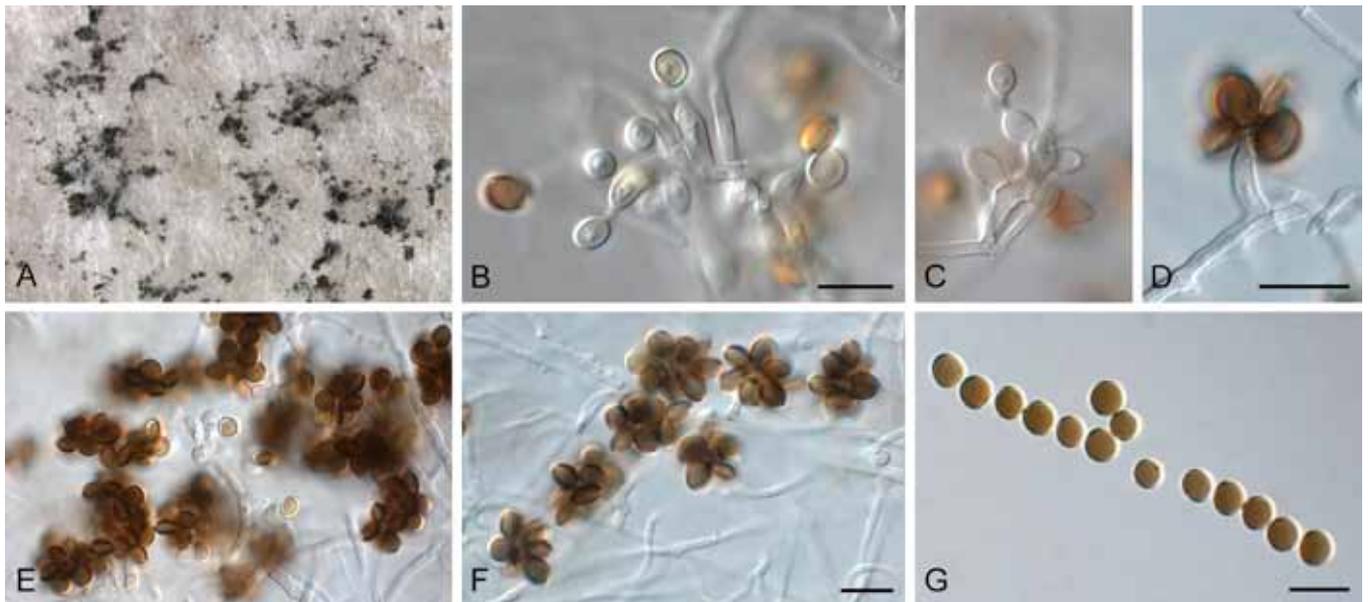


Fig. 4. *Arthrinium arundinis* (CBS 133509). **A.** Colony on PDA. **B–F.** Conidiogenous cells giving rise to conidia. **G.** Globose conidia. Bars = 10 μm ; B = C, D = E, F.

species. Although this present taxon needs to be epitypified, we refrain for doing it here, as we have not yet traced the holotype specimen.

Arthrinium aureum Calvo & Guarro, *Trans. Br. mycol. Soc.* **75**: 156 (1980)
(Fig. 5)

Type: Spain: Barcelona, from air, 1977, A. Calvo & J. Guarro (CBS 244.83 – ex-type culture).

Description: Calvo & Guarro (1980).

Arthrinium hydei Crous, *sp. nov.*

Mycobank MB804339

(Fig. 6)

Etymology: Named in honour of Kevin D. Hyde, who collected this fungus in Hong Kong, and has published extensively on the genus.

Diagnosis: Conidia brown, finely roughened, globose in surface view, lenticular in side view, (15–)17–19(–22) μm diam in surface view, (10–)11–12(–14) μm diam in side view.

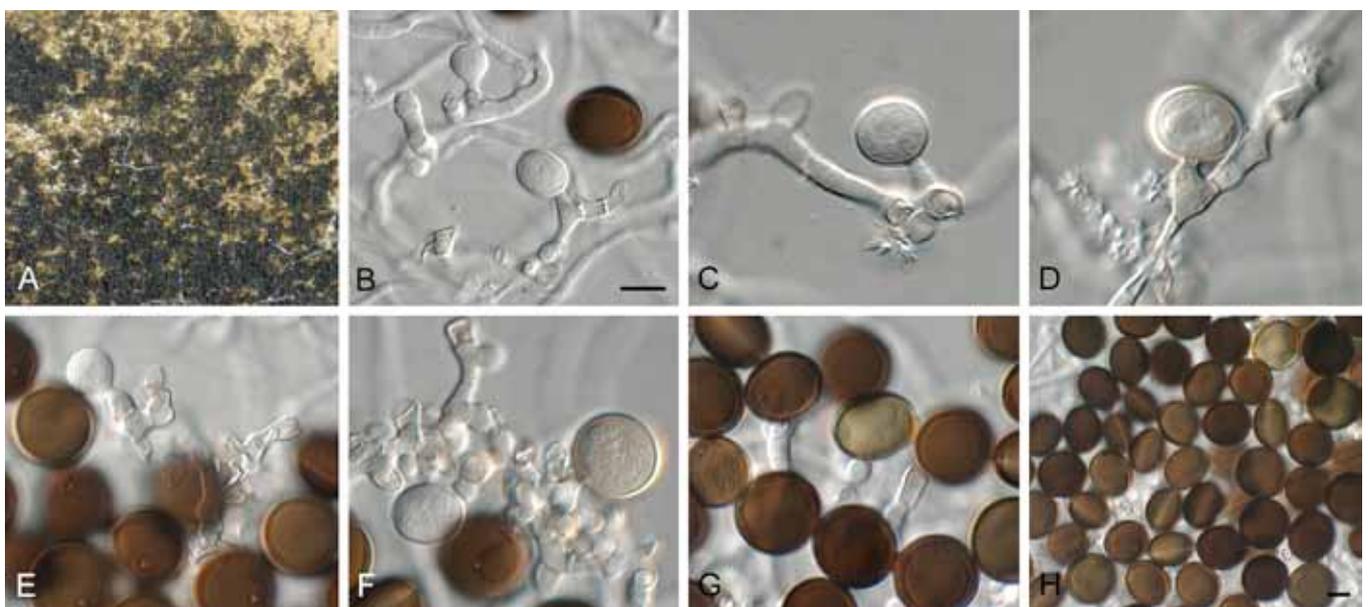


Fig. 5. *Arthrinium aureum* (CBS 244.83). **A.** Colony on MEA. **B–G.** Conidiogenous cells giving rise to conidia. **H.** Conidia. Scale bars = 10 μm ; B = C–G.

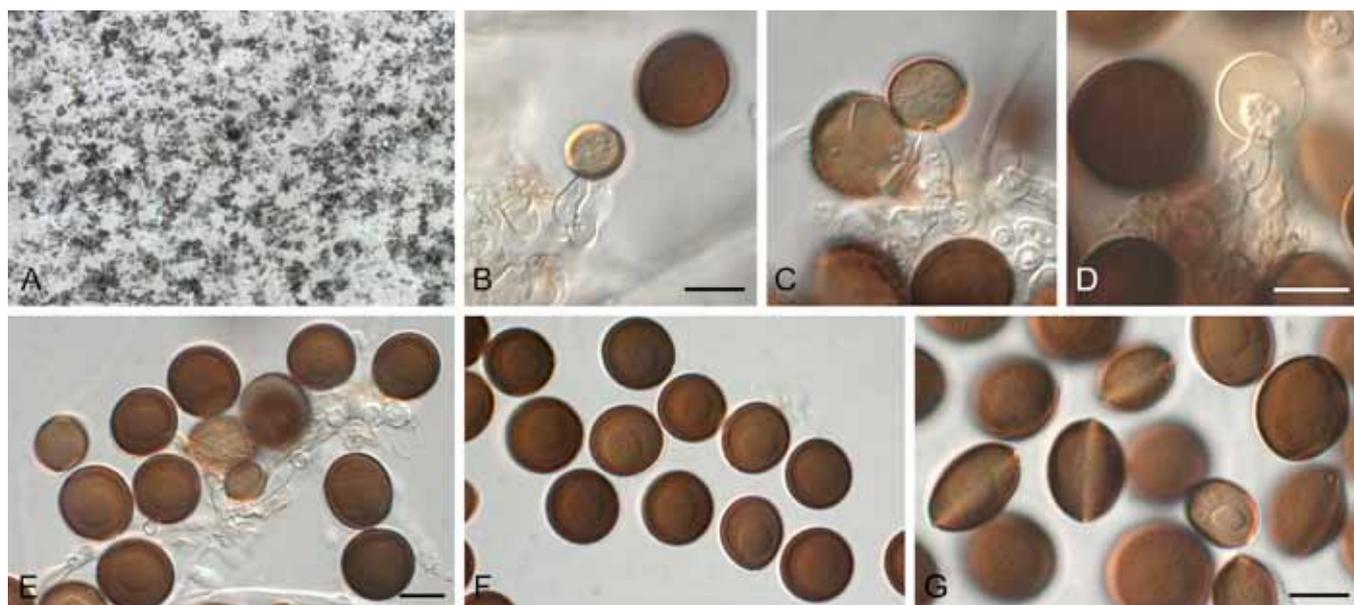


Fig. 6. *Arthrinium hydei* (CBS 114990). **A.** Colony on OA. **B–E.** Conidiogenous cells giving rise to conidia. **F.** Globose conidia in surface view. **G.** Lenticular in side view, with pale equatorial slit. Bars = 10 µm; B = C, E = F.

Type: Hong Kong: New Territories: Tai Po Kau, on culms of *Bambusa tuldooides*, 19 Apr. 1999, K. D. Hyde (CBS H-21272 – holotype; CBS 114990 – ex-type culture).

Description: Mycelium consisting of smooth, hyaline to pale brown, branched, septate, 2–3 µm diam hyphae. Conidiophores pale brown, smooth, subcylindrical, transversely septate, branched, 20–40 × 3–5 µm. Conidiogenous cells aggregated in clusters on hyphae, brown, smooth, subcylindrical to doliiform to lageniform, 5–8 × 4–5 µm. Conidia brown, roughened, globose in surface view, lenticular in side view, with pale equatorial slit, (15–)17–19(–22) µm diam in surface view, (10–)11–12(–14) µm diam in side view, with a central scar, 1.5–2 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. On PDA surface and reverse pale luteous. On OA surface dirty white with patches of olivaceous-grey, reverse pale luteous. On MEA surface and reverse pale luteous.

Notes: Originally identified as *Apiospora sinensis*, a species described from a dead petiole of *Trachycarpus fortunei* collected in China (Hyde *et al.* 1998), but the conidia of *A. hydei* are much larger than that reported for *A. sinensis*, 9–12 × 6–8 µm; those of the latter species fall in the range of *A. phaeospermum*.

***Arthrinium kogelbergense* Crous, sp. nov.**

Mycobank MB804340

(Fig. 7)

Etymology: Named after the Kogelberg Nature Reserve, where the ex-type strain of this fungus was collected.

Diagnosis: Conidia brown, smooth, finely guttulate, globose to ellipsoid in surface view, lenticular in side view, (8–)9–10 × 7–8(–9) µm in surface view, 4–5 µm diam in side view.

Type: South Africa: Western Cape Province: Kogelberg Nature Reserve, dead culms of *Restionaceae*, 11 May 2001,

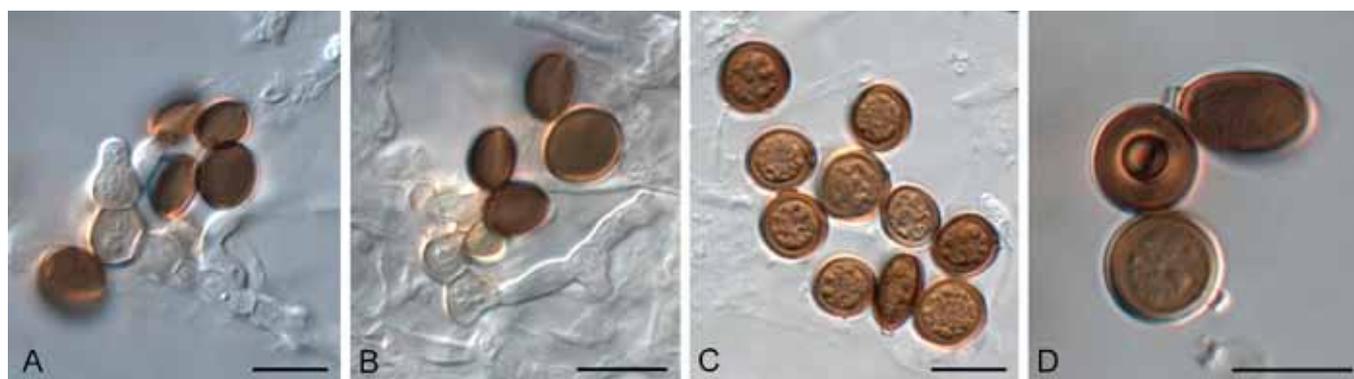


Fig. 7. *Arthrinium kogelbergense* (CBS 113333). **A–C.** Conidiogenous cells giving rise to conidia. **D.** Globose to ellipsoid conidia. Bars = 10 µm.

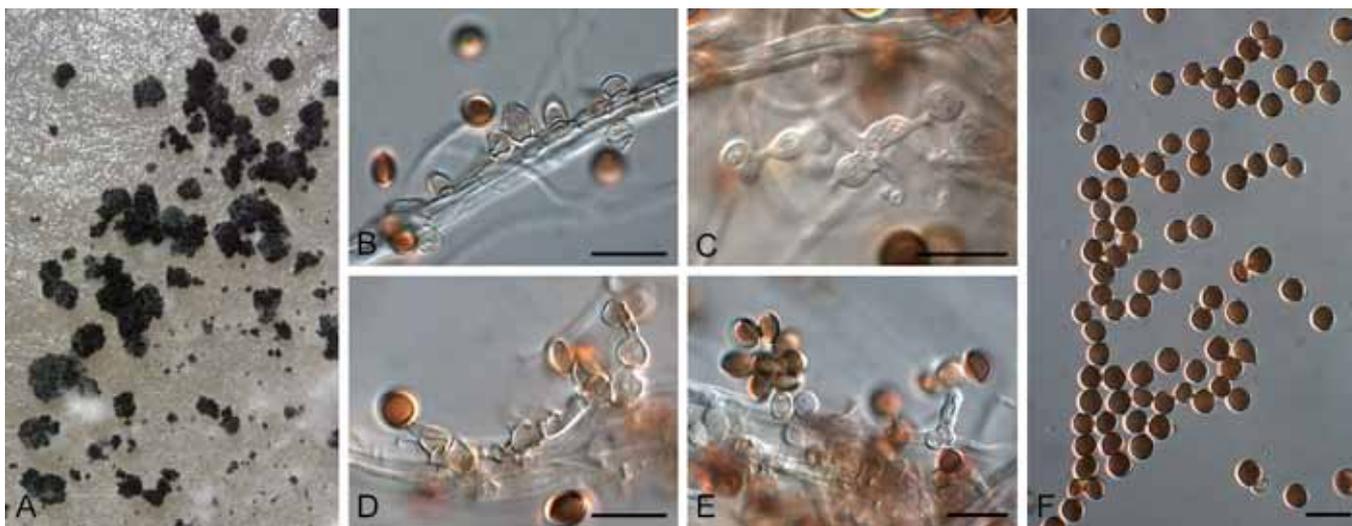


Fig. 8. *Arthrinium malaysianum* (CBS 102053). **A.** Colony on OA. **B–E.** Conidiogenous cells giving rise to conidia. **F.** Globose conidia in surface view. Bars = 10 μ m.

S. Lee (CBS H-21271 – holotype; CBS 113333 – ex-type culture).

Description: Mycelium consisting of smooth, hyaline, branched, septate, 3–5 μ m diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, pale brown, smooth, doliiform to subcylindrical, 5–12 \times 4–5 μ m. Conidia brown, smooth, finely guttulate, globose to ellipsoid in surface view, lenticular in side view, with pale equatorial slit, (8–)9–10 \times 7–8(–9) μ m in surface view, 4–5 μ m diam in side view, with central scar, 1.5–2 μ m diam.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On PDA, MEA and OA surface dirty white, reverse pale luteous to sienna.

Additional specimens examined: **Croatia:** Adriatic Coast, unknown alga, *E. Eguereva* (CBS 117206). – **South Africa:** Western Cape Province: Jonkershoek Nature Reserve, dead culms of *Cannomois virgata*, 15 July 2001, *S. Lee* (CBS 113332; Helderberg Nature Reserve, dead culms of *Restio quadratus*, 13 Apr. 2002, *S. Lee* (CBS 113335). – **Sweden:** Uppland: Börstil par., on *Juncus gerardi*, 2 Aug. 1990, *K. & L. Holm* (CBS 114734 = UPSC 3251).

Notes: *Arthrinium kogelbergense* is morphologically close to *A. phaeospermum*, which has conidia that are slightly longer, (9–)10(–12) μ m diam in surface view, and wider, 6–7 μ m diam in side view.

Arthrinium malaysianum Crous, sp. nov.

MycoBank MB804342

(Fig. 8)

Etymology: Named after the country where one of the strains was collected, Malaysia.

Diagnosis: Conidia brown, smooth, globose in surface view, lenticular in side view, 5–6 diam in surface view, 3–4 μ m diam in side view.

Type: Malaysia: Gombak, on *Macaranga hullettii* stem colonised by ants, Aug. 1999, *W. Federle* (CBS H-21269 – holotype; CBS 102053 – ex-type culture).

Description: Mycelium consisting of smooth, hyaline, branched, septate, 2–3 μ m diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliiform to clavate to ampulliform, 4–7 \times 3–5 μ m. Conidia brown, smooth, globose in surface view, lenticular in side view, with pale equatorial slit, 5–6 μ m diam in surface view, 3–4 μ m diam in side view.

Culture characteristics: Colonies flat, spreading, with fluffy aerial mycelium. On PDA surface dirty white, with patches of iron-grey due to sporulation, reverse luteous to sienna.

Additional specimen examined: Unknown country: stem base of *Cinnamomum camphora*, CBS 251.29.

Notes: Conidial dimensions are close to, but slightly longer than those of *Arthrinium euphorbiae*, (4–)4.7(–5.5) μ m in surface view, (3–)3.2(–4) μ m in side view (from *Euphorbia*, collected in Zambia; Ellis 1965). *Arthrinium malaysianum* is the second species collected from the same source, namely *Macaranga hullettii* stems colonised by ants in Malaysia (see CBS 102052).

Arthrinium marii Larrondo & Calvo, *Mycologia* 82:

397 (1990).

(Fig. 9)

Type: Spain: Barcelona, from beach sand, Nov. 1990, *J.V. Larrondo* & *A. Calvo* (IMI 326872 – holotype; CBS 497.90 = MUCL 31300 – ex-type culture).

Description: Mycelium consisting of smooth, hyaline, branched, septate, 1.5–4 μ m diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in

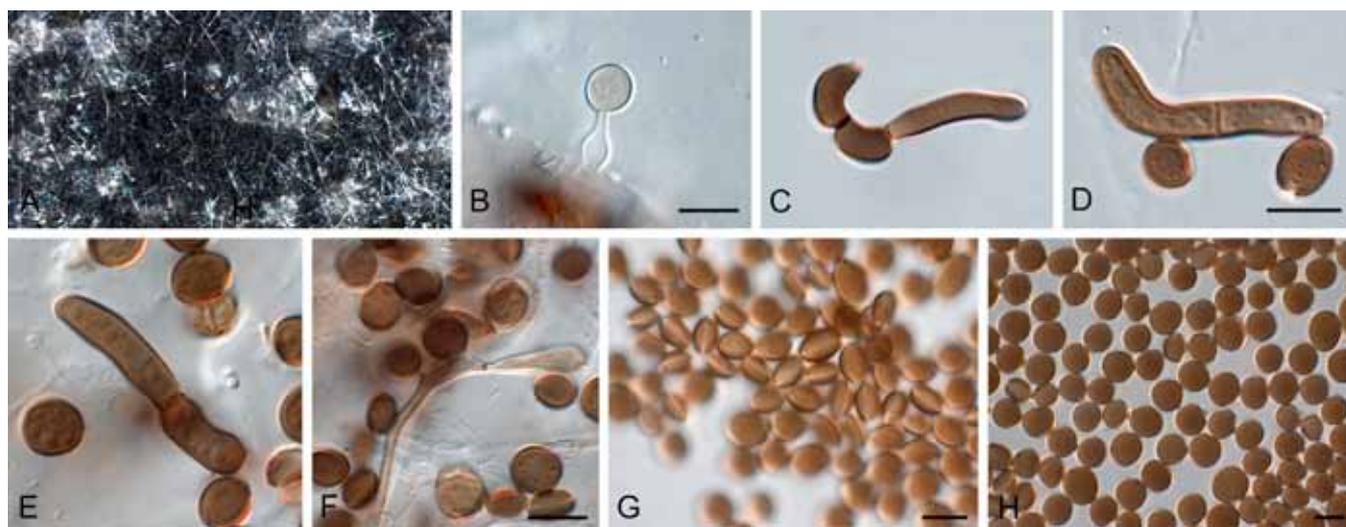


Fig. 9. *Arthrinium marii* (CBS 497.90). **A.** Colony on PDA. **B, F.** Conidiogenous cells giving rise to conidia. **C–E.** Elongated conidia (sterile cells?). **G.** Lenticular conidia in side view. **H.** Globose to ellipsoid conidia in surface view. Bars = 10 μ m; B = C, D = E.

clusters on hyphae, brown, smooth, ampulliform, 5–10 \times 3–4.5 μ m. *Conidia* brown, smooth, granular, globose to elongate ellipsoid in surface view, 8–10(–13) μ m diam, lenticular in side view, with pale equatorial slit, (5–)6(–8) μ m diam in side view; with central basal scar, 1 μ m diam. Brown, elongated cells (sterile cells?) at times intermingled among conidia.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. On OA pale luteous with patches of olivaceous-grey due to sporulation. On PDA olivaceous-grey on surface, reverse smoke-grey with patches of olivaceous-grey. On MEA luteous with patches of umber, reverse sienna with patches of luteous.

Additional specimens examined: **Italy:** Bomarzo, Footpath Santa Lecilia, Mugana, Viterbo, on stems of *Phragmites australis*, 24 Nov. 2010, *W. Gams* (CPC 18904, 18902). – **The Netherlands:** on leaf of *Beta vulgaris*, Apr. 1957, *Gerold* (CBS 200.57). – **Sweden:** oats, Nov. 1985, *C. Svenson* (CBS 113535). – **Hong Kong:** Lung Fu Shan, on culm of *Arundinaria hindsii*, 30 July 1998, *K. D. Hyde* (CBS 114803 = HKUCC 3143).

Note: Based on the results obtained here (Figs 1–3), it appears that *Arthrinium marii* is quite a commonly occurring species on different hosts in Europe, with a single report from Hong Kong.

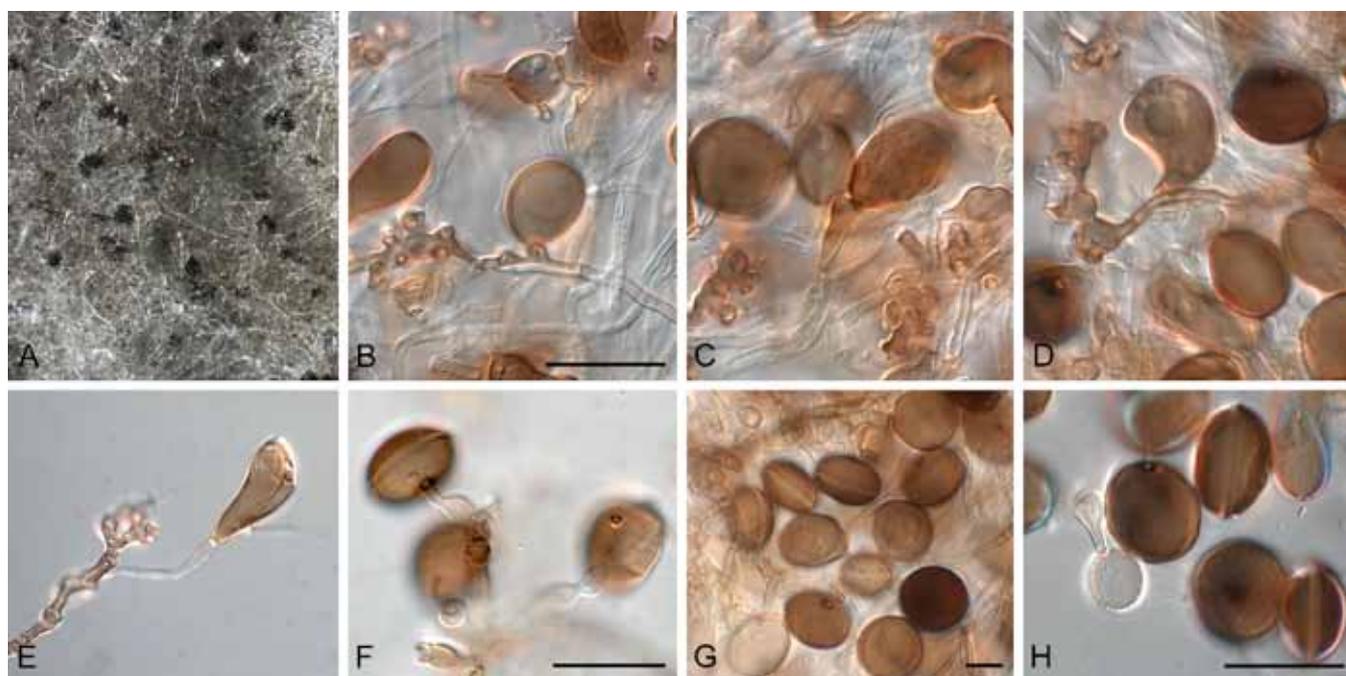


Fig. 10. *Arthrinium ovatum* (CBS 115042). **A.** Colony on PDA. **B–E.** Curved or irregularly angled or lobed sterile cells. **F.** Conidiogenous cells giving rise to conidia. **G, H.** Conidia. Bars = 10 μ m; B = C–E.

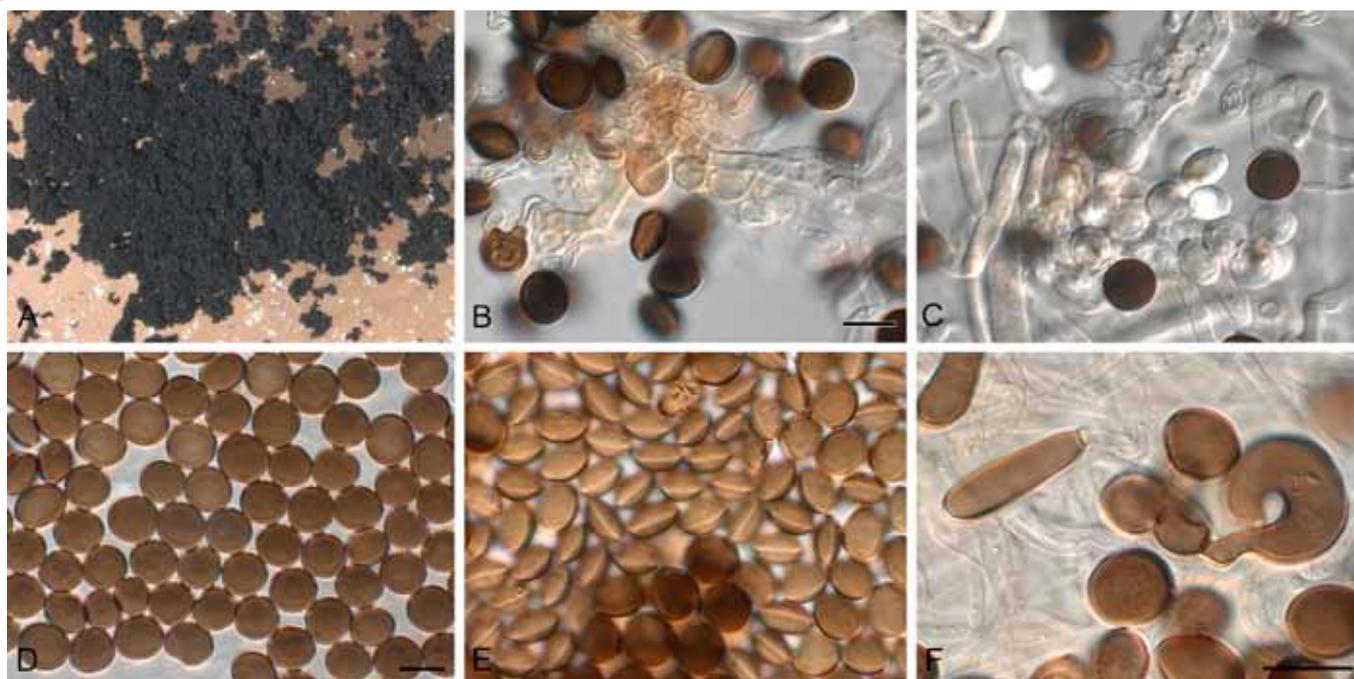


Fig. 11. *Arthrinium phaeospermum* (CBS 142.55). **A.** Colony on OA. **B, C.** Conidiogenous cells giving rise to conidia. **D, E.** Conidia in surface and side view. **F.** Conidia and sterile cells. Bars = 10 µm; B = C, D = E.

Arthrinium ovatum Crous, sp. nov.

MycoBank MB804343

(Fig. 10)

Etymology: Named after the ovoid shape of its conidia.

Diagnosis: Conidia oval to broadly ellipsoid, medium brown, finely roughened, 18–20 µm diam in surface view, 12–14 µm diam in side view.

Type: **Hong Kong:** on *Arundinaria hindsii*, 10 Feb. 2004, K. D. Hyde (CBS H-21273 – holotype; CBS 115042 – ex-type culture).

Description: Mycelium consisting of branched, septate, hyaline, 3–5 µm diam hyphae, becoming brown closer to conidiogenous region. Conidiophores aggregated in black sporodochia, multiseptate, branched, to 60 µm long, 5–7 µm diam. Conidiogenous cells pale brown, smooth, aggregated, ampulliform, 7–12 × 4–6 µm, in clusters on aerial mycelium, or forming black sporodochial conidiomata on agar surface. Sterile cells terminal on hyphae, pale brown, elongated ellipsoidal to clavate, 20–35 × 10–15 µm, or somewhat curved or irregularly angled or lobed, up to 80 µm long, 5–20 µm diam. Conidia oval to broadly ellipsoid, medium brown, finely roughened, 18–20 µm diam in surface view, 12–14 µm diam in side view, with equatorial slit of lighter pigment, and central scar, 2–3 µm diam.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On MEA surface ochreous with patches of dirty white, reverse sienna. On PDA surface and reverse dirty white, with patches of umber. On OA surface dirty white with patches of olivaceous-grey, reverse iron-grey.

Notes: Based on the larger conidia, *Arthrinium ovatum* appears to represent an undescribed species (Ellis 1965, 1976, Gjaerum 1967, Pollack & Benjamin 1969, Hudson & McKenzie 1976, Calvo & Guarro 1980, Khan & Sullia 1980, Samuels *et al.* 1981, von Arx 1981, Koskela 1983, Kirk 1986, Larrando & Calvo 1990, 1992, Müller 1992, Bhat & Kendrick 1993, Hyde *et al.* 1998, Jones *et al.* 2009, Singh *et al.* 2012).

Arthrinium phaeospermum (Corda) M.B. Ellis, *Mycol. Pap.* 103: 8 (1965)

Basionym: *Gymnosporium phaeospermum* Corda, *Icon. fung.* 1: 1 (1837).

Synonym: *Botryconis sanguinea* Tubaki, *Nagaoa* 1: 7 (1952).

(Fig. 11)

For further synonyms see Ellis (1965).

Description: Mycelium consisting of smooth, hyaline, branched, septate, 3–4 µm diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, medium brown, smooth, ampulliform, 5–10 × 3–5 µm, apical neck 2–4 µm long, basal part 3–6 µm long. Conidia brown, smooth, granular, globose to ellipsoid in surface view, (9–)10(–12) µm diam, lenticular in side view, with pale equatorial slit, 6–7 µm diam in side view; with central basal scar, 2 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. Surface iron-grey on OA and MEA, iron-grey with patches of dirty white and sienna on PDA.

Specimens examined: **Iran:** Marand, on leaf of *Hordeum vulgare*, B. Askari, CBS 114314, 114317, 114318; Shabestar, on leaf of *Hordeum*

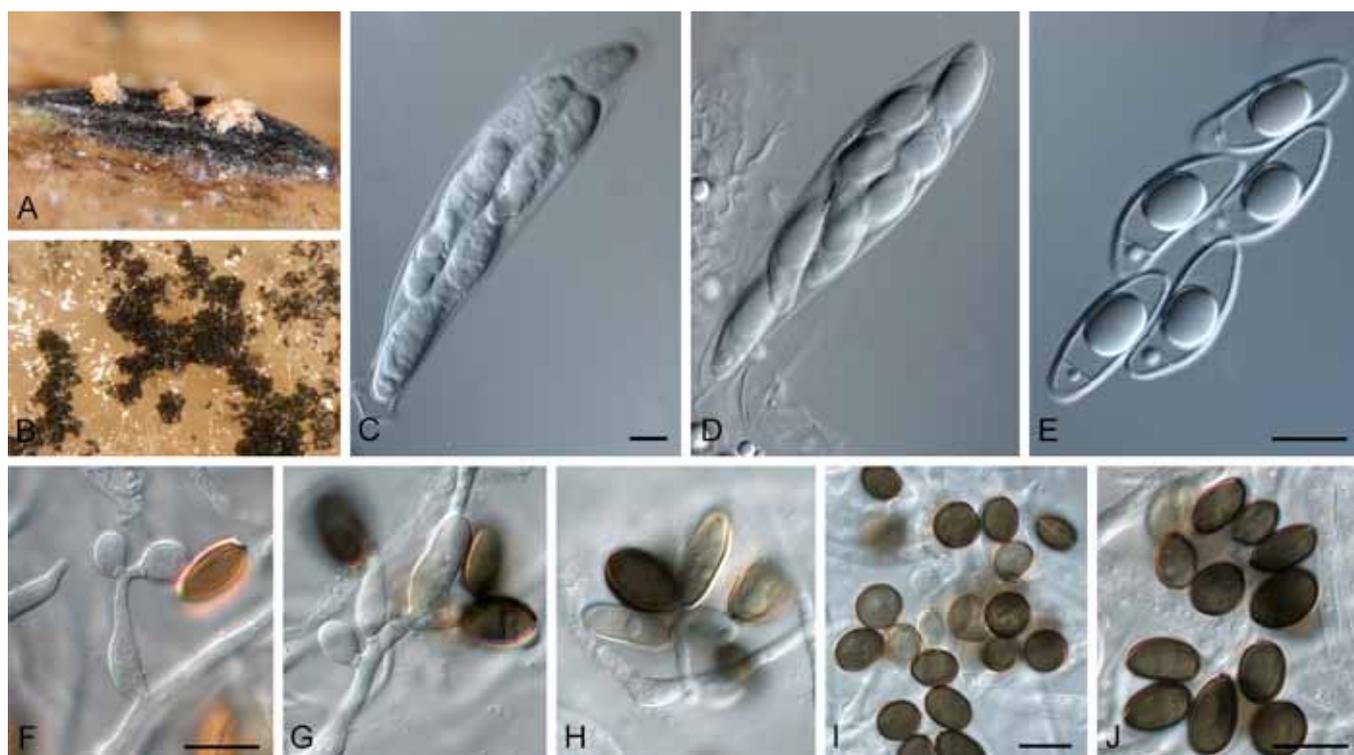


Fig. 12. *Arthrinium phragmites* (CPC 18900). **A.** Ascoma with oozing ascospores. **B.** Colony on OA. **C–E.** Asci with ascospores. **F–H.** Conidiogenous cells giving rise to conidia. **I, J.** Conidia. Bars = 10 µm; C = D, F = G, H.

vulgare, *B. Askari*, CBS 114315. – **Japan:** Tiba Prefecture: soil, 1951, *K. Tubaki* (CBS 142.55 – isotype of *Botryconis sanguinea*).

Notes: Although *Arthrinium phaeospermum* is common and widely distributed, many isolates in the literature have been incorrectly identified as representing this taxon. The present phylogenetic data show that *A. phaeospermum* represents a species complex, and that minute differences in conidial dimensions correlate with distinct taxa. Singh *et al.* (2012) incorrectly cite the isotype strain of *Botryconis sanguinea* as isotype of *A. phaeospermum*, a species to which *B. sanguinea* is synonymous. Although we accept the same clade as representative of *A. phaeospermum*, this species presently does not have any ex-type strains available for study, and needs to be epitypified.

Arthrinium phragmites Crous, *sp. nov.*

Mycobank MB804344

(Fig. 12)

Etymology: Named after the host from which it was isolated, *Phragmites*.

Diagnosis: Conidia brown, smooth, but finely roughened on surface, ellipsoid to ovoid, 9–10(–12) µm in surface view, (5–)6(–7) µm in side view. Ascospores apiosporous, basal cell smaller, hyaline, straight to curved, smooth, lacking mucilaginous sheath, 22–25 × 7–9 µm; basal cell 4–6 µm long.

Type: **Italy:** Viterbo Province: Bomarzo, footpath from Santa Cecilia to Nugnano, on culms of *Phragmites australis*, 24

Nov. 2010, *W. Gams* (CBS H-21267 – holotype; CPC 18901, 18900 = CBS 135458 – ex-type culture).

Description: Occurring on dead stem stalks. *Mycelium* consisting of hyaline, smooth, branched, septate, 2–3 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* erect, ampulliform to doliiform, pale brown, smooth, 12–15 × 3–5 µm. *Conidia* brown, smooth to finely roughened, ellipsoid to ovoid, with equatorial slit of paler pigment, 9–10(–12) µm in surface view, (5–)6(–7) µm in side view. *Sterile cells* forming on solitary loci on hyphae, brown, finely roughened, ellipsoid to clavate, 13–15(–17) × (5–)6 µm. *Ascomata* immersed beneath a pseudostroma, 1–3 mm long, 0.5–1 mm diam, dark brown to black, becoming erumpent, splitting along its length, revealing a row of separate, subglobose, brown ascomata, each exuding a white cirrhous of ascospores; ascomata subglobose, arranged in rows, medium to dark brown, 150–200 µm diam, 200–300 µm tall; wall consisting of 3–4 layers of *textura angularis*; ostiole single, central, 10–25 µm diam, with a periphysate channel 20–40 µm long. *Paraphyses* intermingled among asci, not very prominent, hyphae-like, hyaline, smooth, septate, sparingly branched, thin-walled, up to 4 µm diam, at times breaking into segments. *Asci* hyaline, smooth, clavate with a short basal pedicel, unitunicate, thin-walled, obtusely rounded apex lacking an apical mechanism, 70–110 × 17–25 µm. *Ascospores* hyaline, smooth, 2–3-seriate, apiosporous, straight to curved, ellipsoid to reniform, some ascospores showing remnants of mucoid sheath covering length of spore; ascospores granular or not, widest in middle of apical cell, (20–)22–24(–25) × (7–)8–9(–10) µm; basal cell obtusely rounded, hyaline, smooth, 5–6 × 5 µm.



Fig. 13. *Arthrinium pseudosinense* (CPC 21546). **A.** Erumpent ascomata on host surface. **B–D.** Asci and ascospores. **E–H.** Conidiogenous cells giving rise to conidia. Bars = 10 µm; B = C, E = F, G.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On PDA surface dirty white with patches of pale luteous, reverse luteous.

Notes: Based on its conidial dimensions, *Arthrinium phragmites* is close to *A. phaeospermum*, which has conidia that are 9–12 µm diam in surface view, and 6–7 µm diam in side view. However, conidia of *A. phragmites* are somewhat narrower in side view, and more ellipsoid in shape. The ascospores are also smaller than those attributed to *Apiospora sinensis*, the purported sexual morph of *Arthrinium phaeospermum* (see below). Many published reports of *A. phaeospermum* may however belong to *A. phragmites*.

***Arthrinium pseudosinense* Crous, sp. nov.**

Mycobank MB804347

(Fig. 13)

Etymology: Named after its morphological similarity to *Apiospora sinensis*.

Diagnosis: Conidia brown, smooth, ellipsoid, 8–10 × 7–10 µm diam in surface view, 7–8 µm diam in side view. Ascospores 2–3 seriate, apiosporous, basal cell smaller, hyaline, straight to curved, smooth, surrounded by a thin mucilaginous sheath, (25–)27–30(–33) × (6–)8(–10) µm; basal cell 3–6 µm long.

Type: The **Netherlands:** Utrecht: Utrecht Botanical Garden, on leaves of bamboo, 6 Oct. 2012, *U. Damm* (CBS H-21268 – holotype; CBS 135459 = CPC 21546, CPC 21547 – ex-type culture).

Description: Associated with leaf tip blight, occurring on dead leaf tissue. *Mycelium* consisting of pale brown, smooth, branched, septate, 2–3 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform to doliiform or subcylindrical, pale brown, smooth, 5–12 × 3–5 µm. *Conidia* brown, smooth, ellipsoid, with equatorial slit of paler pigment, 8–10 × 7–10 µm diam in surface view, 7–8 µm diam in side view. *Ascomata* immersed, subepidermal becoming erumpent, solitary or arranged in linear rows, splitting epidermis via longitudinal slit; globose to subglobose, somewhat papillate, to 300 µm diam, brown, with central periphysate ostiole to 50 µm diam. *Paraphyses* hyaline, smooth, septate, prominently constricted at septa, 3–5 µm diam at basal part, apex frequently swollen, to 10 µm diam. *Asci* unitunicate, 8-spored, thin-walled, clavate, stipitate, apex lacking apical mechanism, 85–100 × 15–20 µm. *Ascospores* 2–3 seriate, apiosporous, basal cell smaller, hyaline, straight to curved, smooth, surrounded by a thin mucilaginous sheath, (25–)27–30(–33) × (6–)8(–10) µm; basal cell 3–6 µm long.

Culture characteristics: Colonies flat, spreading. On MEA surface and reverse dirty white with patches of umber, and with sparse aerial mycelium. On OA surface moderately fluffy, with dirty white aerial mycelium. On PDA aerial mycelium sparse, surface concolorous with agar, with patches of umber, reverse umber.

Notes: Morphologically, *Arthrinium pseudosinense* closely resembles *Apiospora sinensis* (ascospores (26–)31(–34) × (6–)7.6(–8.4) µm; conidia ellipsoid, 9–12 × 6–8 µm; Hyde *et al.* 1998), except that the ascospores are on average

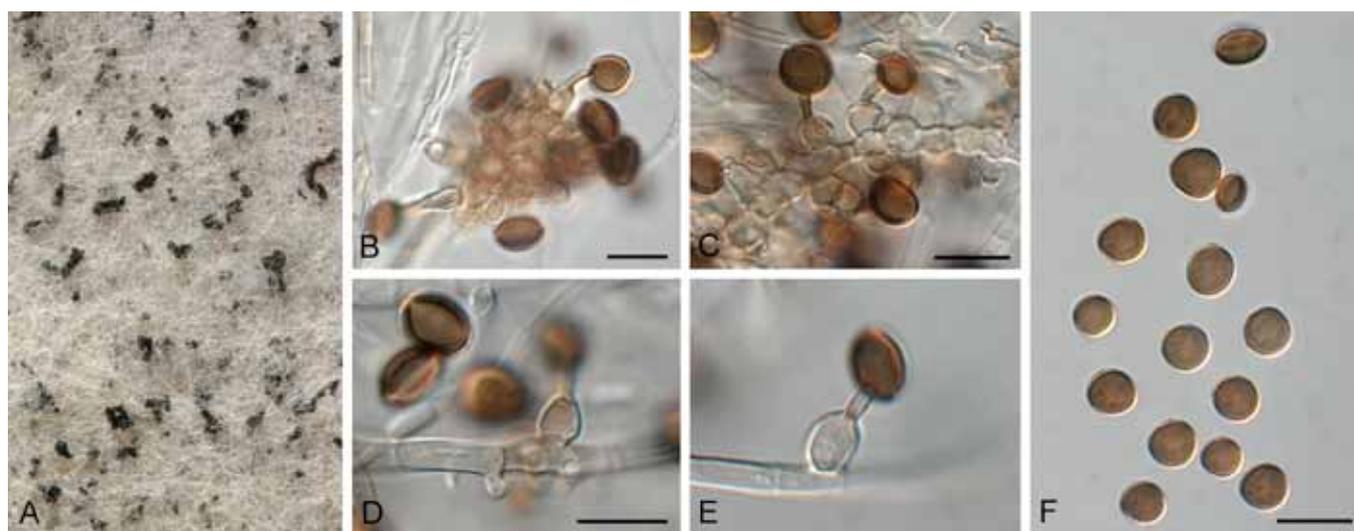


Fig. 14. *Arthrinium pseudospegazzinii* (CBS 102052). **A.** Colony on PDA. **B–E.** Conidiogenous cells giving rise to conidia. **F.** Conidia. Bars = 10 µm; D = E.

shorter and wider, have a less prominent sheath, and the conidia are smaller. A fresh collection of *A. sinensis* from China (south-west Huhei Province, Xuanen, on dead petiole of *Trachycarpus fortunei*) would be needed to facilitate a molecular comparison, with what is obviously a species complex, as other isolates originally identified as *Apiospora sinensis* in the CBS collection also clustered apart.

***Arthrinium pseudospegazzinii* Crous, sp. nov.**

Mycobank MB804346

(Fig. 14)

Etymology: Named after its morphological similarity to *A. spegazzinii*.

Diagnosis: Conidia brown, guttulate, roughened, globose in surface view, lenticular in side view, (7–)8–9 µm diam in surface view, 5–6 µm diam in side view.

Type: Malaysia: Gombak, on *Macaranga hullettii* stem colonised by ants, Aug. 1999, *W. Federle* (CBS H-21276 – holotype; CBS 102052 – ex-type culture).

Description: Mycelium consisting of smooth, hyaline to pale brown, branched, septate, 3–4 µm diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, brown, smooth, ampulliform with elongated neck, 8–13 µm long, basal part 3–5 × 3–5 µm, neck 3–7 × 1.5–2 µm. Conidia brown, guttulate, finely roughened, globose in surface view, lenticular in side view, with pale equatorial slit, (7–)8–9 µm diam in surface view, 5–6 µm diam in side view, with central scar, 1.5–2 µm diam.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On MEA surface pale olivaceous-grey, reverse luteous. On OA surface dirty white with patches of olivaceous-grey, reverse olivaceous-grey. On MEA

surface dirty white, with patches of grey-olivaceous, reverse olivaceous-grey.

Notes: Although conidia were observed to be finely roughened, they were not as rough, more globose in surface view, and were much smaller than those of *Arthrinium spegazzinii* (5–8 × 3–6 µm; Ellis 1965).

***Arthrinium pterospermum* (Cooke & Masee) Arx, Gen. Fungi Spor. Pure Cult, 3rd edn: 331 (1981).**

Basionym: *Coniosporium pterospermum* Cooke & Masee, *Hedwigia* 19: 90 (1880).

Synonym: *Pteroconium pterospermum* (Cooke & Masee) Grove, *Hedwigia* 55: 146 (1914).

(Fig. 15)

Type: Australia: Victoria: on *Lepidosperma* sp., *Martin* 778 (K (M) 179237 – holotype, ex herb. M. C. Cooke as *Coniosporium pterospermum* and annotated by W. G. Grove); Adelaide, on leaf of *Lepidosperma gladiatum*, 4 Jan. 2012, *W. Quaedvlieg* (CBS H-21275 – **epitype designated here** "MBT 175265"; CPC 20194, 20193 = CBS 134000 – cultures ex-epitype).

Description: Mycelium consisting of branched, septate, hyaline, 2–4 µm diam hyphae, becoming brown closer to conidiogenous region. Conidiophores aggregated in black sporodochia, transversely multiseptate, branched, brown, smooth, to 150 µm long, 3–5 µm diam. Conidiogenous cells lateral and terminal on conidiophores, brown, finely roughened, aggregated, doliiform to ampulliform, 5–10 × 4–5 µm. Conidia brown, finely roughened, with equatorial slit of lighter pigment, and central scar, polygonal, lobed or dentate, irregular in surface view, 15–25 µm diam; in side view, 8–10 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. On MEA surface pale olivaceous-grey, reverse olivaceous-grey. On OA surface olivaceous-grey, with patches of dirty white, reverse olivaceous-grey.

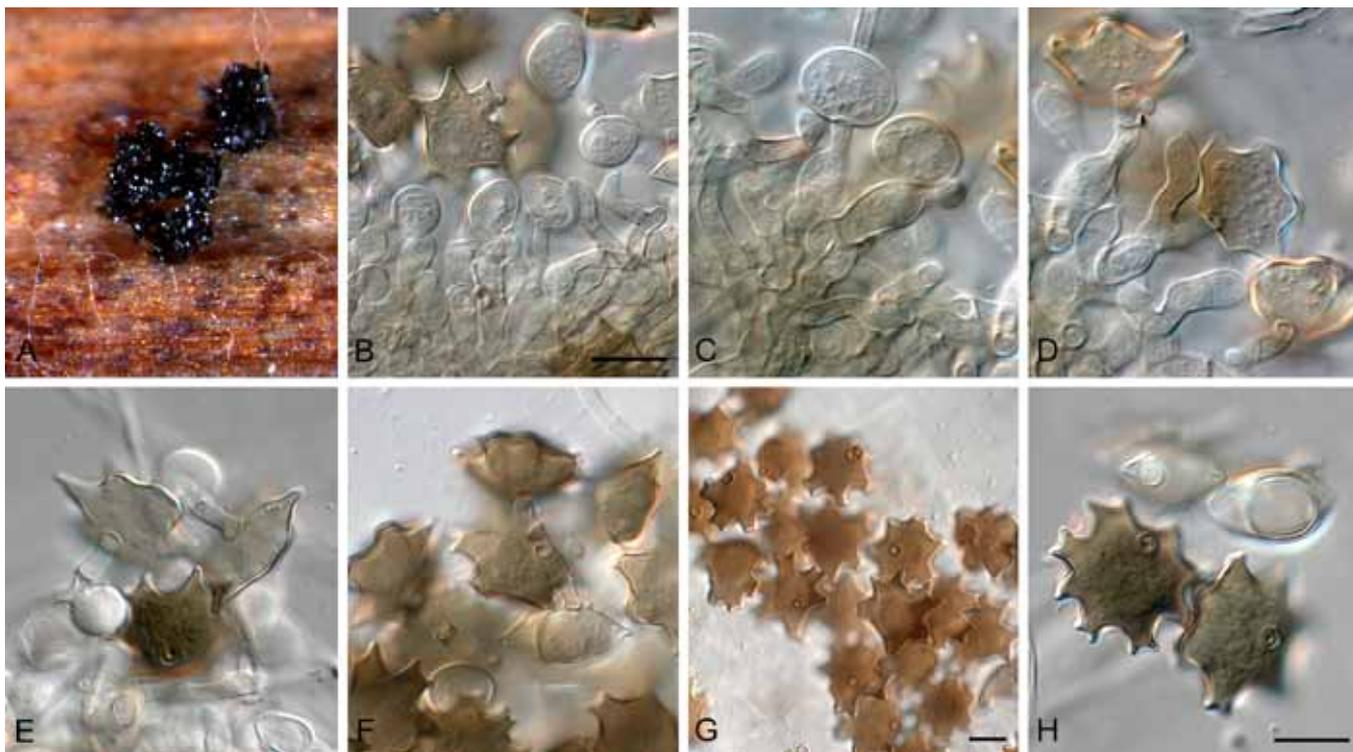


Fig. 15. *Arthrinium pterospermum* (CPC 20194). **A.** Sporodochium on host surface. **B–F.** Conidiogenous cells giving rise to conidia. **G, H.** Dentate conidia. Bars = 10 µm; B = C–F.

Additional specimen examined: New Zealand: Auckland, Auckland University, leaf lesion of *Machaerina sinclairii*, 27 Jan. 2008, C. F. Hill (CBS 123185 = 2008/423-X = CPC 15380).

Notes: From the Australian specimens available of this fungus in BRIP and VPRI, it seems that *Arthrinium pterospermum* is common on leaves of *Lepidosperma gladiatum* (Cyperaceae). The decision by von Arx (1981) to dispose *Pteroconium pterospermum* to *Arthrinium* is supported by

the present phylogenetic analysis (Fig. 1), which widens the circumscription of *Arthrinium* to also include conidia with irregular, lobed or dentate conidia.

***Arthrinium sacchari* (Speg.) M.B. Ellis, *Mycol. Pap.* 103: 11 (1965).**

Basionym: *Coniosporium sacchari* Speg., *Revista Fac. Agron. Univ. Nac. La Plata* 2(19): 248 (1896). (Fig. 16)

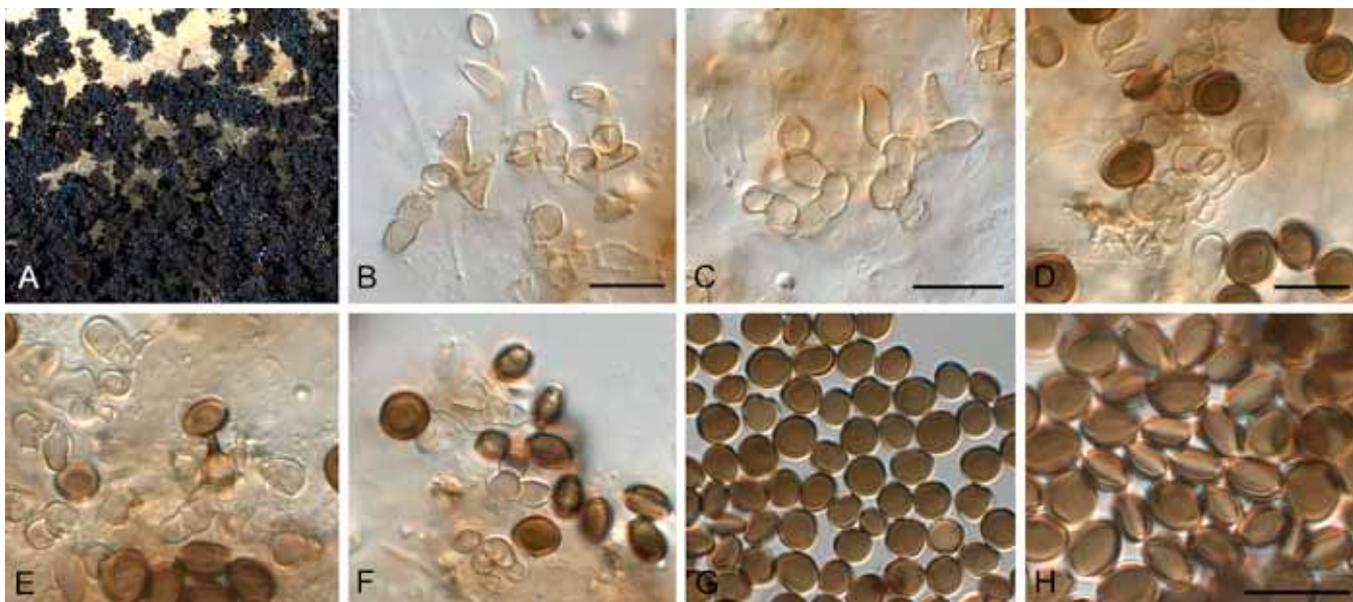


Fig. 16. *Arthrinium sacchari* (CBS 301.49). **A.** Colony on PDA. **B–F.** Conidiogenous cells giving rise to conidia. **G, H.** Conidia. Bars = 10 µm; D = E–G.

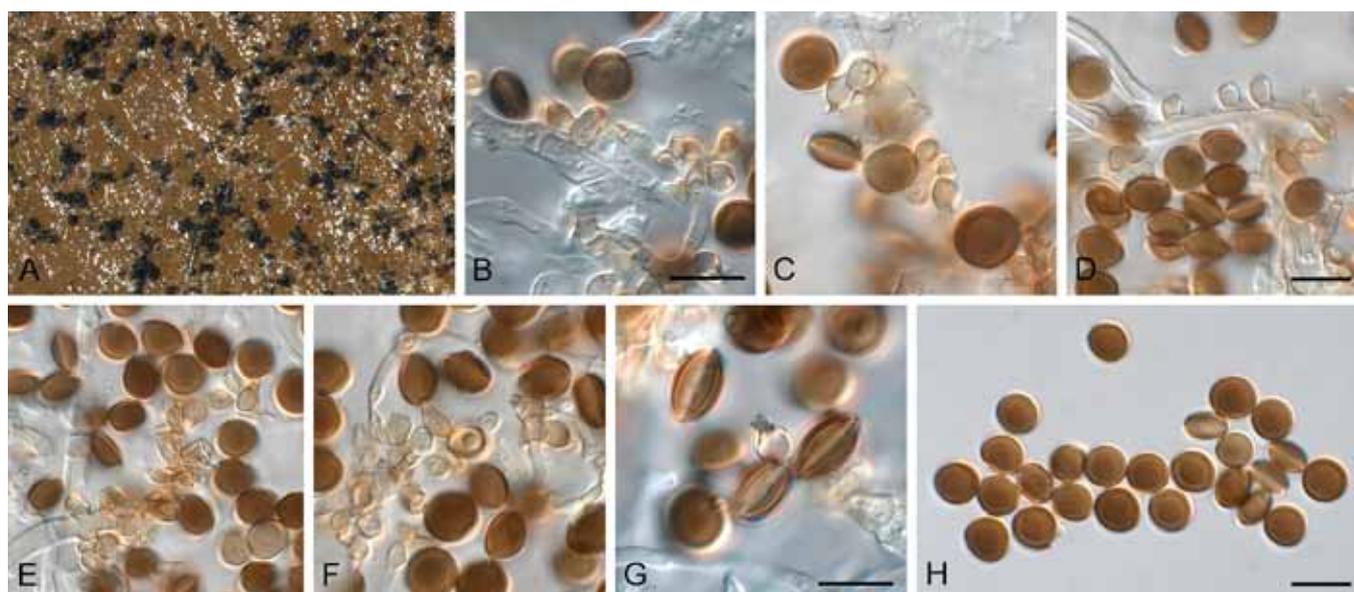


Fig. 17. *Arthrinium saccharicola* (CBS 831.71). **A.** Colony on MEA. **B–G.** Conidiogenous cells giving rise to conidia. **H.** Globose conidia. Bars = 10 µm; B = C, D = E, F.

Description: *Mycelium* consisting of smooth, hyaline, branched, septate, 1.5–4 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* aggregated in clusters on hyphae, brown, smooth, ampulliform to doliiform, 5–12 × 2.5–4 µm; conidiogenous cells proliferating sympodially and also percurrently. *Conidia* brown, smooth, granular, globose in surface view, (6–)7(–8) µm diam, lenticular in side view, with pale equatorial slit, (3.5–)4 µm diam in side view; with central basal scar, 1 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. Surface iron-grey on OA and MEA, umber on PDA.

Specimens examined: **Indonesia:** on bamboo, Feb. 1949, K. B. Boedijn & J. Reitsma (CBS 301.49). – **The Netherlands:** soil under *Calluna vulgaris*, June 1974, H. Linde (CBS 664.74). – **UK: England:** near Cambridge, on *Phragmites australis*, Oct. 1930, E. W. Mason (CBS 212.30). – **Unknown country:** from air, Aug. 1967, collector unknown (CBS H-8805, CBS 372.67).

Notes: Morphologically, *Arthrinium arundinis* (syn. *Apiospora montagnei*) and *Arthrinium sacchari* are very similar, and best distinguished by the *A. sacchari* having wider conidiophores (1–1.5 µm) than *A. arundinis* (0.5 µm). Unfortunately, this feature was not useful in culture. However, based on the slightly larger conidia and wider hyphae with conidiogenous loci, we chose to apply the name *A. sacchari* to this clade, rather than the clade we attribute to *A. arundinis*.

Arthrinium saccharicola F. Stevens, *J. Dept. Agric. Porto Rico* 1(4): 223 (1917). (Fig. 17)

Description: *Mycelium* consisting of smooth, hyaline, branched, septate, 3–5 µm diam hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells*

aggregated in clusters on hyphae, medium brown, finely verruculose, ampulliform, 5–10 × 3–5 µm, apical neck 2–4 µm long, basal part 3–6 µm long. *Conidia* brown, smooth, granular, globose to ellipsoid in surface view, (7–)8–9(–10) µm diam, lenticular in side view, with pale equatorial slit (at times appearing like a ridge of paler pigment), (4–)5(–6) µm diam in side view; with central basal scar, 2 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. Surface iron-grey on OA, on MEA and PDA umber, with patches of olivaceous grey.

Specimens examined: **France:** Landes, Seignosse, Etang d'Hardy, on dead culms of *Phragmites australis*, 11 June 1986, H. A. van der Aa (CBS 334.86). – **The Netherlands:** Dec. 1971, M. van Schothorst (RIVM, CBS H-8889, CBS 831.71); on *Phragmites australis*, Jan. 2011, P. W. Crous (CPC 18977); from air, Sept./Oct. 1972, H. A. van der Aa (CBS 191.73); Z. Flevoland, Harderbos, on dead culms of *Phragmites australis*, 15 May 1983, W. Gams (CBS 463.83).

Notes: Conidial morphology and dimensions of isolates in this clade (Fig. 1) closely match those ascribed to *Arthrinium saccharicola*. Unfortunately, no flexuous conidiophores developed in culture, thus the width of conidiophores could not be confirmed. However, hyphae are similar in width to that observed by Ellis (1965) for this species, 2–5 µm thick, which is wider than that observed in other species of *Arthrinium*.

Arthrinium xenocordella Crous, *sp. nov.*
Mycobank MB804348
(Fig. 18)

Etymology: Not a member of the genus *Cordella*.

Diagnosis: Conidia brown, smooth, guttulate, globose to somewhat ellipsoid in surface view, lenticular in side view, (7–)9–10(–11) µm diam in surface view, 6–7 µm diam in side

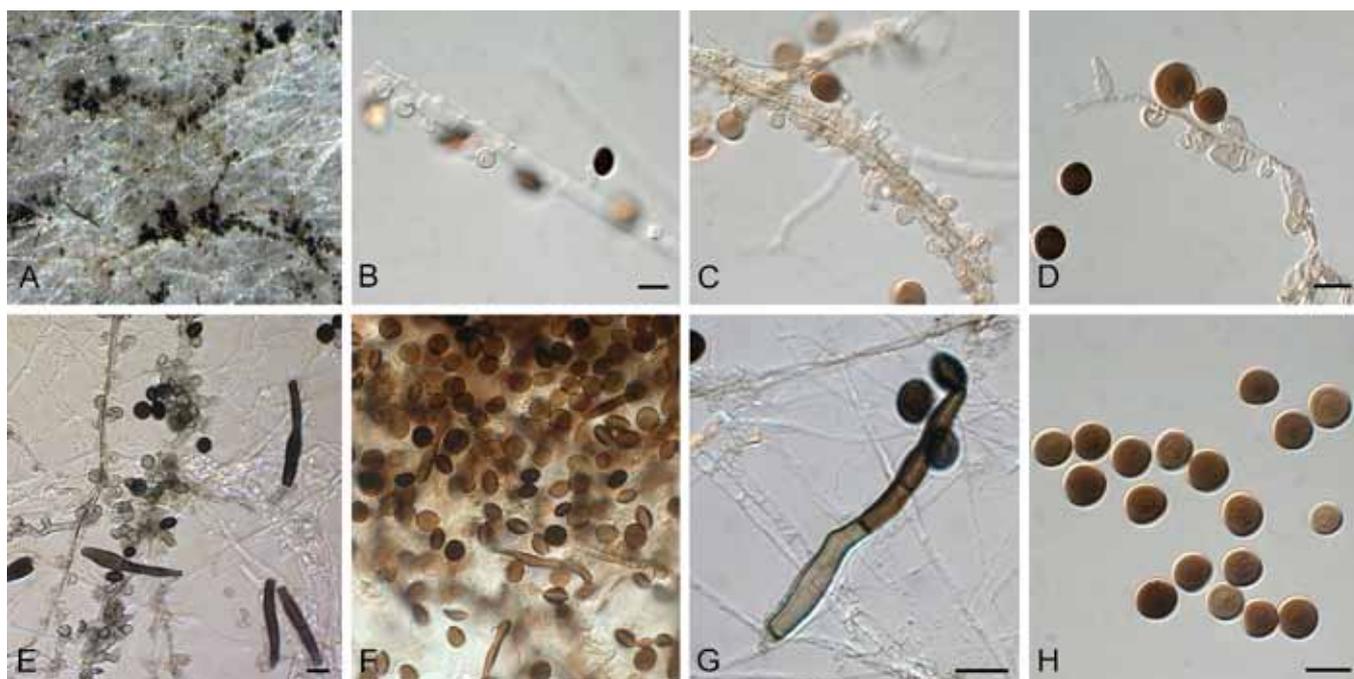


Fig. 18. *Arthrinium xenocordella* (CBS 478.86). **A.** Colony on PDA. **B–D.** Conidiogenous cells giving rise to conidia. **E–G.** Setae intermingled among conidia on agar surface. **H.** Conidia. Bars = 10 µm; B = C, E = F.

view. Setae erect, brown, smooth, subcylindrical, tapering in apical cell to subobtuse or obtuse apex, 1-septate, base truncate, to 100 µm tall, 5–8 µm diam.

Type: **Zimbabwe:** Pomongwe Cave, Matopos, soil from roadway, 21 Dec. 1985, *J. C. Krug* (CBS H-21274 – holotype; CBS 478.86 – ex-type cultures).

Description: Mycelium consisting of smooth to finely verruculose, hyaline to pale brown, branched, septate, 3–5 µm diam hyphae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, brown, verruculose, globose to clavate to doliiform, 5–7 × 4–5 µm. Conidia brown, smooth, guttulate, globose to somewhat ellipsoid in surface view, lenticular in side view, with pale equatorial slit, (7–)9–10(–11) µm diam in surface view, 6–7 µm diam in side view, with central scar, 1.5–2 µm diam. Setae erect, brown, smooth, subcylindrical, tapering in apical cell to subobtuse or obtuse apex, 1-septate, base truncate, to 100 µm tall, 5–8 µm diam, straight to irregularly curved.

Culture characteristics: Colonies flat, spreading, with moderate aerial mycelium. On PDA surface pale luteous with patches of olivaceous-grey, reverse pale luteous. On OA surface dirty white, reverse pale luteous. On MEA surface pale luteous, reverse luteous.

Additional specimen examined: **Austria:** Plaseckerjoch, soil, Aug 1966, *M. A. A. Schipper* (CBS H-8885, CBS 595.66 = MUCL 10009).

Notes: *Arthrinium xenocordella* is presently known from two strains, both isolated from soil. Based on morphology, *A. xenocordella* closely resembles *A. phaeospermum*, but the conidia tend to be globose to ellipsoid in surface

view, and also form brown setae, which are not present in *A. phaeospermum*. That a species with setae clusters in *Arthrinium*, suggests that the generic name *Cordella* (Ellis 1965, Seifert *et al.* 2011), which has *Apiospora* sexual morphs (Samuels *et al.* 1981), should be reduced to synonymy with *Arthrinium*.

DISCUSSION

The higher phylogenetic classification of *Arthrinium* (syn. *Apiospora*) has been the topic of much debate. Theissen & Sydow (1915) placed it in *Dothideales*, Müller & von Arx (1962) assigned it to *Amphisphaeriaceae* (*Xylariales*), and at first Barr chose *Hyponectriaceae* (Barr 1976), but later *Lasio-sphaeriaceae* (*Sordariales*; Barr 1990). Following this debate, Hyde *et al.* (1998), introduced the family name *Apiosporaceae* to accommodate *Apiospora* and *Appendicospora*, based on the unique sexual morphology and their unusual asexual morphs (i.e. basauxic conidiophores with terminal and intercalary polyblastic conidiogenous cells, and unicellular conidia with germ slits). Data derived from a phylogenetic study (SSU and LSU rDNA) incorporating species of *Apiospora* and *Appendicospora*, led Smith *et al.* (2003) to conclude that *Apiosporaceae* represented one of seven families which, at that time could be resolved in *Xylariales*, namely *Amphisphaeriaceae*, *Apiosporaceae*, *Clypeosphaeriaceae*, *Diatrypaceae*, *Graphostromataceae*, *Hyponectriaceae*, and *Xylariaceae*. However, in the latest outline of the *Ascomycota*, Lumbsch & Huhndorf (2010) still list *Apiosporaceae* as *fam. incertae sedis* (*Sordariomycetes*). Based on the results we obtained in this study (Fig. 1), *Apiosporaceae* is confirmed as a family within *Xylariales*, and a sister to *Amphisphaeriaceae*.

The generic name *Appendicospora* (asexual morph unknown; Hyde 1995) was introduced to accommodate *Apiospora coryphae* (Rehm 1913). *Appendicospora* chiefly differs from *Apiospora* in having ascospores with bifurcate appendages. A second species, *A. hongkongensis*, was subsequently introduced to accommodate a taxon occurring on *Livistona chinensis* in Hong Kong (Yanna *et al.* 1997). Our results suggest, however, that although *Appendicospora* is a member of *Xylariales*, it does not belong to *Apiosporaceae*, but represents an as yet undefined family within the order.

The generic circumscription of *Arthrinium* has for some time been regarded as too narrow, ignoring the similar sexual morphology exhibited by various other asexual genera (von Arx 1981). The decision to reduce both *Cordella* and *Pteroniconium* to synonymy with *Arthrinium* here is based on newly available molecular data (Fig. 1). From these data we can conclude that features such as conidium shape and the presence of setae do not appear to be reliable at the generic level in this complex.

We also introduce eight novel species here, most of which would have formerly been treated as belonging to *Arthrinium arundinis* (syn. *Apiospora montagnei*) or *Arthrinium phaeospermum*, two commonly occurring species that have evidently been too widely circumscribed morphologically. *Arthrinium malaysianum* and *A. pseudospegazzinii* are two novel co-occurring species on *Macaranga hullettii* from Malaysia. Species of bamboo have always been known as good substrates for *Arthrinium*, and three species are described from this host here: *A. hydei* and *A. ovatum* from Hong Kong, and *A. pseudosinense* from The Netherlands. In general most grasses and reeds appear to harbour species of *Arthrinium* as endophytes, and hence it is not surprising that the additional novel species include *A. kogelbergense* on dead culms of *Restionaceae* from South Africa, and *A. phragmites* on *Phragmites australis* from Italy. Furthermore, species of *Arthrinium* are also commonly isolated from soil, as demonstrated by the description of *A. rashikravindrii* from soils in Norway (Singh *et al.* 2012), but also now shown to occur on diverse substrates in China, Japan, Thailand, and The Netherlands, and *A. xenocordella* from soil in Austria and Zimbabwe.

This study shows that isolates representing distinct species of *Arthrinium* can co-occur on the same substrate, meaning that links between sexual and asexual morphs need to be confirmed by DNA or the culture of single spores. Furthermore, *Arthrinium* species are highly variable morphologically, depending on the substrate and period of incubation, and the morphological features exhibited *in vitro* do not always match those observed *in vivo*. Fresh collections are therefore required to stabilise the application of many older, well-established names. As a further complication, many well-known taxa unfortunately also appear to represent species complexes.

ACKNOWLEDGEMENTS

We thank the technical staff, Arien van Iperen (cultures), Marjan Vermaas (photographic plates), and Mieke Starink-Willems (DNA isolation, amplification, and sequencing) for their invaluable assistance.

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