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 tuldoides from Hong Kong, *A. kogelbergense* on dead culms of Restionaceae from South Africa, *A. malayansiam* on Macaranga hulletti from Malaysia, *A. ovatum* on Arundinaria hindsi from Hong Kong, *A. phragmites* on *Phragmites australis* from Italy, *A. pseudospagazzini* on *Macaranga hulletti* 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*.

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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 (Martínez-Cano et al. 1992), and *A. sacchari* causing damping-off of wheat (Mavragani et al. 2011) or as an endophyte in plant tissue (Ramos et al. 2010), lichens (He & Zhang 2012), and marine algae (Suryanarayanan 2011). *Arthrinium pterospermum* 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. pterospermum* 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*,...
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 & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify the nuclear rDNA operon to 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 1991-alpha (TEF) was amplified and sequenced using primers 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. 1991), 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 (< 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.


*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, doliform 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* asceptate, 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. *Arthrinium* Kunze 1817).

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


*Type species:* *A. caricicola* Kunze & J.C. Schmidt 1817


*Type species:* *A. montagnei* Sacc. 1875


*Type species:* *C. coniosporioides* Speg. 1886


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

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

*Description:* Colonies compact, black to dark brown, superficial to erumpent. *Myceium* 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 subcute apex, intermingled among conidiophores. *Conidiogenous cells* discrete, doliform 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* asceptate, 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. Ascospore 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, ellipsoid, inequilateral, tapered at both ends, apiosporous, 1-septate near the lower end, with the lower, smaller cell subglobose; ascospores with our without mucoid sheath.

*Notes:* The conidiogenesis of *Arthrinium* species is of particular interest. Conidiogenous cells are generally aggregated on a pale brown stroma, forming sporodochia. They tend to be doliform 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 annelations 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 doliform 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...
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<td><em>Phragmites australis</em></td>
<td>United Kingdom: Cambridge</td>
<td>E.W. Mason</td>
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<td>CBS 664.74</td>
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<td>Dead culms of <em>Phragmites australis</em></td>
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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-alpha; 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).
observed in aerial mycelial strands (conidiophores sensu Ellis 1965) or conidiogenous cells situated on a stroma in a black sporodochium.


(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.


**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. 3.** The first of four equally most parsimonious trees obtained from an analysis of the combined TUB and TEF sequence alignment (TL = 2003 steps, CI = 0.703, RI = 0.875, RC = 0.616). 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. The scale bar indicates 30 changes. The culture collection number is indicated for each sequence, followed by the isolation source and country of origin. The tree is rooted to *Seiridium phylicae* (strain CPC 19965; GenBank accessions KC005821 and KC005817 for TUB and TEF, respectively).
species. Although this present taxon needs to be epitypified, we refrain for doing it here, as we have not yet traced the holotype specimen.

(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.

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**Fig. 4.** *Arthriniun 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.*

**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.*

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 doliform 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 fortune 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.

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

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, pale brown, smooth, doliiform to subcylindrical, 5–12 × 4–5 µm. Conidia brown, smooth, finely guttulate, globose to ellipsoid in surface view, lenticular in side view, with pale equatorial slit, (8–)9–10(–7) × 7–8(–9) µm in surface view, 4–5 µm diam in side view, with central scar, 1.5–2 µm diam.

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


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

Arthrinium malaysianum Crous, sp. nov.

MycoBank MB804342

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 µm.

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 µm 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 × 3–5 µm. Conidia brown, smooth, globose in surface view, lenticular in side view, with pale equatorial slit, 5–6 µm diam in surface view, 3–4 µm 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) µm in surface view, (3–)3.2(–4) µm 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).


(Fig. 9)


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, 5–10 × 3–4.5 µm. Conidia brown, smooth, granular, globose to elongate ellipsoid in surface view, 8–10(–13) µm diam, lenticular in side view, with pale equatorial slit, (5–)6(–8) µm diam in side view; with central basal scar, 1 µm 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.


**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.
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.


Description: Mycelium consisting of branched, septate, hyaline, 3–5 µm diam hyphae, becoming brown closer to conidiogenous region. Conidiophores aggregated in black sporodochia, multisepitate, 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.


Arthrinium phaeospermum (Corda) M.B. Ellis, Mycol. Pap. 103: 8 (1965)
Basionym: Gymnosporium phaeospermum Corda, Icon. fung. 1: 1 (1837).
(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...

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 Botryoconis 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 Nungano, 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 cirrhus 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, uniseriate, 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.

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, apioporous, 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.


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 doliform 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, apioporous, 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...
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.


*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 sparse aerial mycelium. On PDA surface pale luteous, reverse luteous. On OA 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).


(Fig. 15)


*Description:* Mycelium consisting of branched, septate, hyaline, 2–4 µm diam hyphae, becoming brown closer to conidiogenous region. Conidiophores aggregated in black sporodochia, transversely multisepitate, 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.

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.


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.

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.
re-evaluation of Arthrinium (syn. Apiospora)

**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 doliform, 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.


**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.


(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.


**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 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.


**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, 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*.

**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 *Lasiosphaeriaceae* (*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*, *Hyponecciaceae*, 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 *Xylariaceae*, 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 *Pteroconium* 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 arunidis* (syn. *Apiospora montagnei*) or *Arthrinium phaeospermum*, two commonly occurring species that have evidently been too widely circumscribed morphologically. *Arthrinium malaysianum* and *A. pseudospagazzinii* are two novel co-occurring species on *Macaranga hulletti* 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.

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REFERENCES


