

New studies on *Apiospora* (Amphisphaeriales, Apiosporaceae): epitypification of *Sphaeria apiospora*, proposal of *Ap. marianiae* sp. nov. and description of the asexual morph of *Ap. sichuanensis*

Ángel Pintos^{1*}, Pablo Alvarado^{2*}

1 *Interdisciplinary Ecology Group, Universitat de les Illes Balears, Ctra. Valldemossa Km 7,5, 07122 Palma de Mallorca, Spain* **2** *ALVALAB, Dr. Fernando Bongera st., Severo Ochoa bldg. S1.04, 33006 Oviedo, Spain*

Corresponding author: Pablo Alvarado (pablo.alvarado@gmail.com)

Academic editor: S. Maharachchikumbura | Received 9 June 2022 | Accepted 27 July 2022 | Published 23 August 2022

Citation: Pintos Á, Alvarado P (2022) New studies on *Apiospora* (Amphisphaeriales, Apiosporaceae): epitypification of *Sphaeria apiospora*, proposal of *Ap. marianiae* sp. nov. and description of the asexual morph of *Ap. sichuanensis*. MycoKeys 92: 63–78. <https://doi.org/10.3897/mycokeys.92.87593>

Abstract

In the present work, an epitype for *Sphaeria apiospora*, the basionym of the type species of the genus *Apiospora*, *Apiospora montagnei*, is selected among collections growing in the host plant species reported in the original protologue, *Arundo micrantha*. Most samples obtained from localities near that of the lectotype (Perpignan, France) belong to the same species, which is not significantly different from the clade previously named *Ap. phragmitis*, suggesting that this name is a later synonym of *Ap. montagnei*. In addition, the name *Ap. marianiae* is here proposed to accommodate a newly discovered species found in the Balearic Islands (Spain), and the asexual state of *Ap. sichuanensis* is described for the first time from samples growing in the same islands.

Keywords

Apiosporaceae, *Ascomycota*, *Sordariomycetes*

* These authors contributed equally.

Introduction

Apiospora Sacc. is the type genus of family *Apiosporaceae* K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr. It occurs worldwide, and includes important pathogens and saprophytes of animals, plants and seaweeds (Heo et al. 2018, Park et al. 2018, Wang et al. 2018, Kwon et al. 2021). Genus *Apiospora* was built around *Apiospora montagnei* Sacc. (Saccardo 1875), a replacement name for *Sphaeria apiospora* Durieu & Mont. (Bory de Saint-Vincent and Durieu de Maisonneuve 1849). For a long time, *Apiospora* was considered a sexual state of genus *Arthrinium* Kunze, and both were even formally synonymized by Crous and Groenewald (2013), but recently shown to represent independent clades and separated again by Pintos and Alvarado (2021). These authors concluded that although the morphology of the original collections of *S. apiospora* (\equiv *Ap. montagnei*) does not allow to link them with a unique phylogenetic clade, they should nest inside the clade containing most other species with basauxially-generated rounded/lenticular conidia that occur mainly on Poaceae (but also many other plant families, seaweeds and animals) worldwide (including tropical and subtropical regions), and differ from species in the clade of *Arthrinium*, which have variously shaped conidia, a narrower host range (mainly, but not exclusively, Cyperaceae and Juncaceae), and occur in temperate, cold or alpine (but not tropical or subtropical) regions. This way, Pintos and Alvarado (2021) selected a lectotype for *S. apiospora* (\equiv *Ap. montagnei*), and fixed the phylogenetic limits of *Apiospora*, proposing the necessary combinations at species rank. Later authors followed this approach (Crous et al. 2021; Tian et al. 2021), and genomic analyses seem to confirm their taxonomic decision (Sørensen et al. 2022). A third group of species formerly placed within *Arthrinium*, including *Ar. urticae* M.B. Ellis (Ellis 1965) and *Ar. trachycarpi* C.M. Tian & H. Yan (Yan et al. 2019), are probably unrelated to *Arthrinium* or *Apiospora* (Tang et al. 2021), and therefore deserve to be classified in a different genus.

Despite these important taxonomic changes, the exact identity of the type species of *Apiospora*, *Ap. montagnei*, still remains uncertain. Pintos and Alvarado (2021) discussed the host plants mentioned by Bory de Saint-Vincent and Durieu de Maisonneuve (1849), concluding that the lectotype (collected near Perpignan, France) was found on *Arundo micrantha* or *Aru. donaciformis*. Only four species of *Apiospora*, *Ap. iberica* (Pintos & P. Alvarado) Pintos & P. Alvarado, *Ap. italica* (Pintos & P. Alvarado) Pintos & P. Alvarado, *Ap. marii* (Larrondo & Calvo) Pintos & P. Alvarado and *Ap. phragmitis* (Crous) Pintos & P. Alvarado had been recorded in *Arundo* spp. (Pintos et al. 2019, Pintos and Alvarado 2021), but since *Ap. iberica* and *Ap. italica* are relatively rare, *Ap. marii* and *Ap. phragmitis* were considered the most probable synonyms of *Ap. montagnei* (Pintos and Alvarado 2021).

In the present work, several collections of *Apiospora* growing on *Arundo* aff. *micrantha* in northeastern Spain and the Balearic Islands were analyzed, and an epitype of *Ap. montagnei* selected among them to fix the identity of this species. In addition,

a newly discovered species found in the same region is described and given a new name, and the asexual state of *Ap. sichuanensis* Samarak., Jian K. Liu & K.D. Hyde is described for the first time.

Materials and methods

Isolates

Methods employed to isolate the sexual and asexual states are described in Pintos and Alvarado (2021). The samples were deposited in the fungarium of the Muséum National d'Histoire Naturelle (**PC**; Paris, France) and the Fungarium of the University of Vienna (**WU**; Vienna, Austria). Living cultures were deposited in Fungal collection at the Westerdijk Fungal Biodiversity Institute (**CBS**; Utrecht, The Netherlands).

Morphology

Samples were studied with a Zeiss Axioscope compound microscope operating with differential interference contrast (DIC). Images were obtained with a FLIR camera using open source software Microscopia Oberta (A. Coloma). Measurements were taken with FIJI win64 ImageJ software, and reported as follows: maximum value in parentheses, range between the mean plus and minus the standard deviation, minimum value in parentheses, and the number of elements measured in parentheses. For some images of conidiophores, the image stacking software Zerene Stacker v. 1.04 (Zerene Systems LLC, Richland, WA, USA) was employed. Morphological descriptions were based on fertile cultures growing on 2% MEA (20 g/L malt extract, 20 g/L soy peptone, 15 g/L agar, pH 7) at room temperature.

Phylogenetic analysis

Total DNA was extracted from cultured isolates and dried fungarium specimens employing a modified protocol based on Murray and Thompson (1980). Amplification reactions (Mullis and Faloona 1987) included 35 cycles with an annealing temperature of 54 °C. Primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993) were employed to amplify the ITS1-5.8S-ITS2 nrDNA region (ITS), while LR0R and LR5 (Vilgalys and Hester 1990, Cubeta et al. 1991) were used for the 28S nrDNA region (LSU), EF1-728F, EF1-983F and EF1-1567R (Carbone and Kohn 1999, Rehner and Buckley 2005) for the translation elongation factor 1 alpha (*tef1*) gene, and T1, Bt2a, and Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) for the β -tubulin gene (*tub2*). PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors in MEGA v. 5.0 (Tamura et al. 2011), and these were corrected.

A single alignment was made using: 1) ITS1-5.8S-ITS2 nrDNA, 2) 28S nrDNA, 3) *tef1* region between 3' extreme of intron 1 and the 5' extreme of the exon between introns 2 and 3, and 4) *tub2* region between intron 3 and the 5' extreme of the exon between introns 5 and 6. Homologous sequences of selected samples of *Apiospora* available in public databases (International Nucleotide Sequence Database Collaboration, INSDC, Arita et al. 2021) were included, adding also sequences of *Arthrimum* as out-group. The sequences employed (Suppl. material 1) were mainly retrieved from Smith et al. (2003), Singh et al. (2012), Crous and Groenewald (2013), Crous et al. (2015, 2020), Senanayake et al. (2015), Dai et al. (2016, 2017), Wang et al. (2017, 2018), Jiang et al. (2018, 2019, 2020), Liu et al. (2019), Pintos et al. (2019), Yan et al. (2019), Yang et al. (2019), Senanayake et al. (2020), Pintos and Alvarado (2021), Kwon et al. (2021), Phukhamsakda et al. (2022) and Samarakoon et al. (2022). Sequences first were aligned in MEGA software with its Clustal W application and then corrected manually. Gblocks (Castresana 2000) was employed to remove 191 ambiguously aligned positions from ITS rDNA, resulting in a final alignment with 188/463/82 (ITS rDNA), 126/786/64 (28S rDNA), 358/847/52 (*tef1*) and 576/807/47 (*tub2*) variable sites/ total sites/ sequences.

Results

The phylogenetic analysis of sequenced species of *Apiospora* including ITS1-5.8S-ITS2 and LSU rDNA, as well as exon and intron regions from *tef1* and *tub2* genes (Fig. 1) resulted in six significantly supported major clades: 1) /*sorghii* (apparently containing a single species, *Ap. sorghi* (J.D.P. Bezerra, C.M. Gonçalves & C.M. Souza-Motta) X.G. Tian & Tibpromma = *Ar. taeanense* S.L. Kwon, S. Jang & J.J. Kim), 2) /*jatrophae*, 3) /*hysterina*, 4) /*arundinis*, 5) /*montagnei*, and 6) /*phaeospermum*. These clades, identified in the present work for the first time, maybe represent monophyletic lineages that could be interpreted as sections or subgenera inside *Apiospora*. However, this hypothesis should be further tested with additional data from less variable DNA regions, since ITS1 rDNA and introns can be easily misaligned.

Among the samples analyzed in the present study (Suppl. material 2), five collections of *Apiospora* were found growing on *Arundo* aff. *micrantha* (the supposed host plant of the lectotype of *Sphaeria apiospora*) in Girona (northeastern Spain) and Mallorca (Balearic Islands, Spain). Four of them were not genetically different from *Ap. phragmitis*, and one matched *Ap. sichuanensis*. Another seven samples found growing on *Arundo donax* in Mallorca matched *Ap. phragmitis*, and one *Ap. sichuanensis*. Due to these results and the data available from Mediterranean species of *Apiospora*, *Ap. phragmitis* is here considered the most probable synonym of *Ap. montagnei*, and an epitype for this species selected among the samples analyzed in the present work. Finally, two samples found on *Phleum pratense* in Mallorca turned out to represent a previously unknown phylogenetic lineage, which is given a new name below.

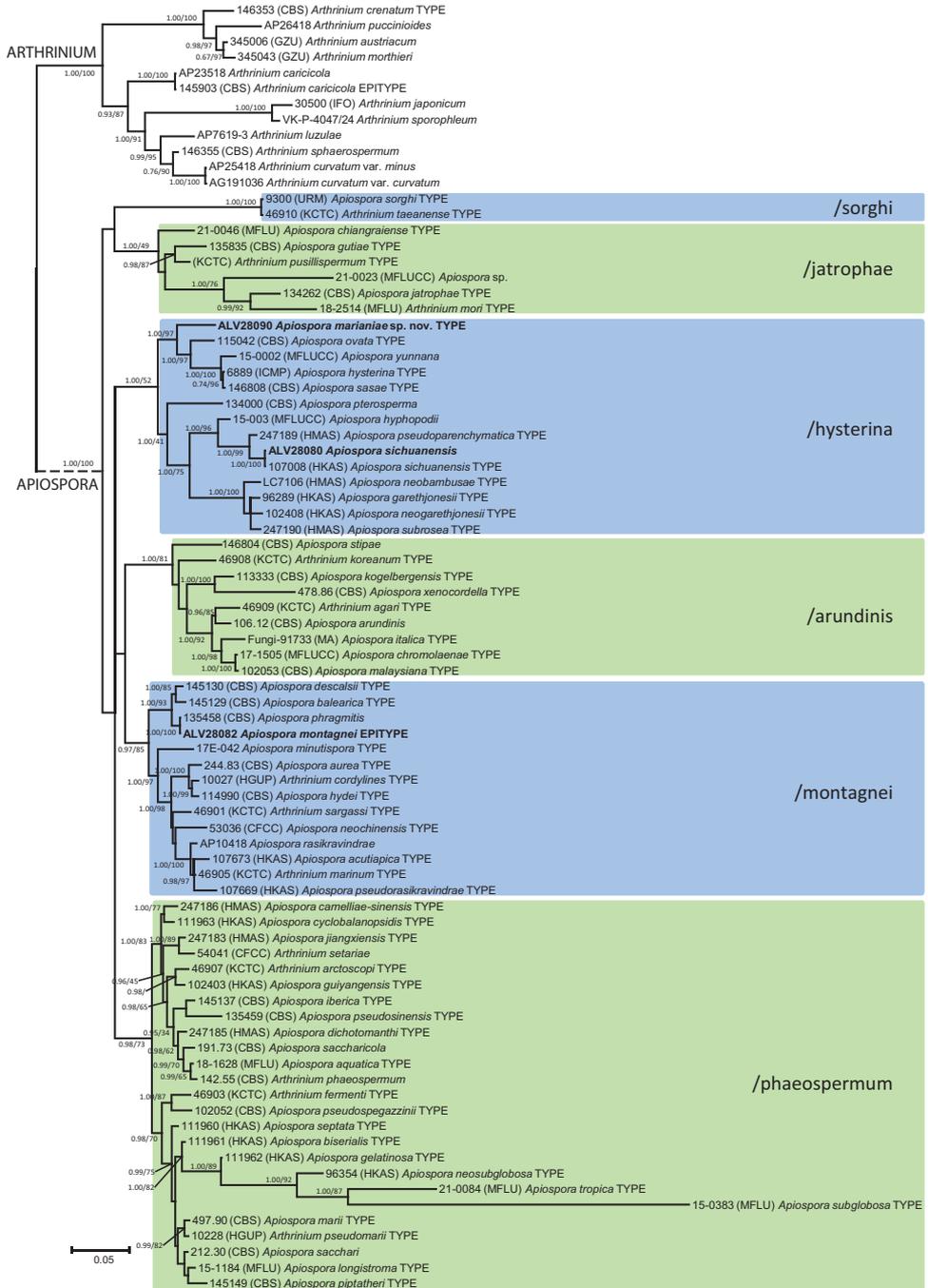


Figure 1. Majority rule consensus (50%) ITS rDNA- 28S rDNA- tef1- tub2 phylogram of the Apiosporaceae obtained in MrBayes from 4 875 sampled trees. Nodes were annotated if supported by > 0.95 Bayesian PP (left) or > 70% ML BP (right).

Taxonomy

Apiospora montagnei Sacc., *Atti Soc. Veneto-Trent. Sci. Nat.* 4: 85. 1875.

Fig. 2C–2N

Sphaeria apiospora Durieu & Mont., *Expl. Sci. Alg., Fl. Algér.* 1, livr. 13: 492. 1849.
[replaced name]

Hypopteris apiospora (Durieu & Mont.) Berk., *Hooker's J. Bot. Kew Gard. Misc.* 6: 227. 1854.

Arthrinium phragmitis Crous, *IMA Fungus* 4: 147. 2013.

Apiospora phragmitis (Crous) Pintos & P. Alvarado, *Fungal Systematics and Evolution* 7: 206. 2021.

Sexual morph. Stromata solitary to gregarious, immersed to erumpent, fusiform, with the long axis broken at the top by one or two cracks, $(0.5\text{--})2.1\text{--}2.9(-4) \times (0.2\text{--})0.25\text{--}0.35(-0.5)$ mm ($n = 20$). Ascomata uniseriate or irregularly arranged beneath stromata, pseudothecial, black, globose to subglobose with a flattened base, $(150\text{--})159\text{--}183(-200)$ μm high \times $(200\text{--})247\text{--}278(-300)$ μm wide ($n = 35$), with a conspicuous periphysate ostiole. Peridium composed of 5 or 6 layers of brown to hyaline cells arranged in textura angularis. Hamathecium paraphyses hyphae-like, up to 4 μm wide. Asci broadly cylindrical, clavate, with an indistinct pedicel, rounded at the apex, lacking apical apparatus, $(72\text{--})99\text{--}111(-115) \times (14\text{--})15.5\text{--}16.5(-18)$ μm ($n = 25$). Ascospores uniseriate or biseriate, clavate to fusiform, straight or slightly curved, with narrowly rounded ends, composed of a large upper cell and a small lower cell, hyaline, smooth-walled, measuring $(21\text{--})23\text{--}24.5(-25) \times (6\text{--})6.3\text{--}7.1(-8)$ μm ($n = 30$).

Asexual morph. Mycelium consisting of hyaline, smooth, branched, septate hyphae 1–4 μm in diam. ($n = 20$). Conidiophore mother cell from hyaline to brown, solitary or aggregated in groups on hyphae, subsphaerical to lageniform or ampuliform, measuring $(4\text{--})6.6\text{--}8(-10) \times (3\text{--})4.5\text{--}5.1(-6)$ μm ($n = 10$). Conidiophores cylindrical, straight to flexuous, some of them branched, hyaline, measuring $(10\text{--})18\text{--}34(-45) \times (1.5\text{--})1.6\text{--}1.8(-2)$ μm ($n = 20$). Conidiogenous cells doliiform to lageniform or ampuliform, hyaline, measuring $(10\text{--})11.5\text{--}13.1(-15) \times (2\text{--})4.3\text{--}5.1(-6)$ μm ($n = 20$). Conidia ellipsoidal to ovoid, smooth to finely roughened, with an equatorial germ slit of paler pigment, measuring $(9\text{--})10.3\text{--}11.3(-12)$ μm in surface view, $(5\text{--})6.2\text{--}7.2(-8)$ μm in side view ($n = 25$). Sterile cells ellipsoidal to clavate, measuring 13–16 μm ($n = 25$).

Culture characteristics. Colonies flat, spreading, with moderate aerial mycelium. On MEA, surface dirty white with pale rose patches, reverse luteous. Occupying an entire 90 mm Petri dish in 14 days at room temperature, sporulating four weeks after culture.

Epitype. Spain: Catalonia, Girona, L'Escala, on *Arundo micrantha*, 30 November 2020, leg. Marc Grañem, AP301120 (epitype selected here PC:0125164, ex-type culture CBS 148707; iso-epitype WU-MYC0044524, ex-type culture CBS 148708).

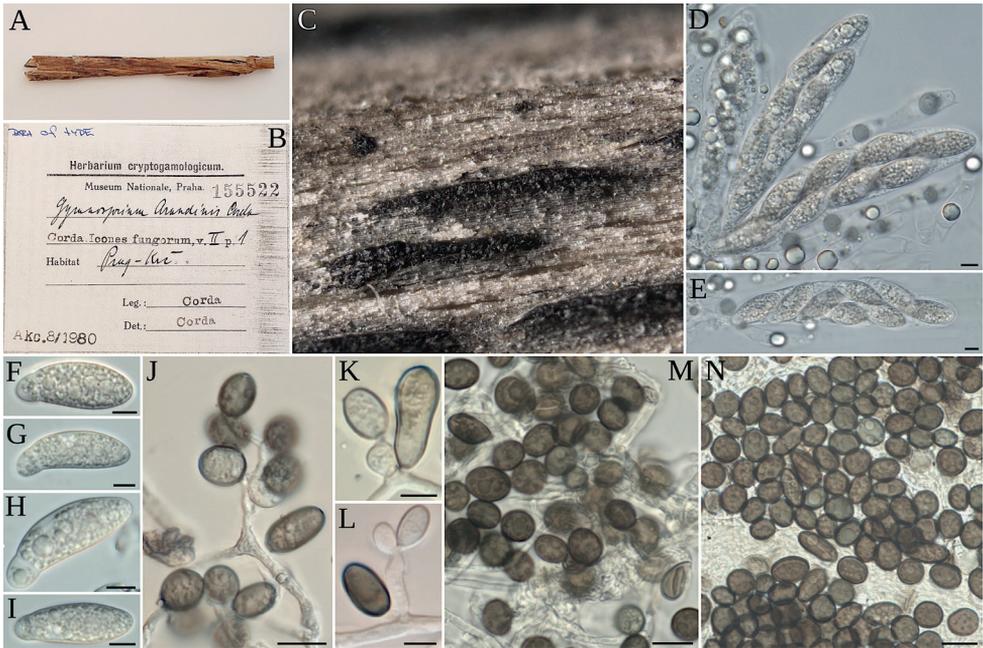


Figure 2. **A, B** *Gymnosporium arundinis* (original material from PRM) **A** substrate of the type **B** label of the original collection of *Gymnosporium arundinis* **C–N** *Apiospora montagnei* (AP301120) **C** stromata on host **D, E** asci and periphyses **F–I** ascospores **J–L** conidiogenous cell with conidia **M–N** conidia in face and side view. Scale bars: 100 μm (**C**); 5 μm (**D–I**); 10 μm (**J–N**).

Other specimens examined. Spain: Balearic Islands, Mallorca, Esporlas, on *Arundo donax*, 14 December 2020, leg. Ángel Pintos, AP141220 (WU-MYC0044527). Balearic Islands, Mallorca, Palma de Mallorca, Torrente de Establiments, on *Arundo micrantha*, 19 April 2021, leg. Ángel Pintos, AP19421 (WU-MYC0044523). Balearic Islands, Mallorca, Puerto de Alcudia, on *Arundo donax*, 2 April 2021, leg. Ángel Pintos, AP2421 (WU-MYC0044522). Balearic Islands, Mallorca, Puerto de Andratx, on *Arundo donax*, 5 April 2021, leg. Ángel Pintos, AP5421 (WU-MYC0044529). Balearic Islands, Mallorca, Puerto de Soller, on *Arundo donax*, 3 April 2021, leg. Ángel Pintos, AP3421 (WU-MYC0044528). Balearic Islands, Mallorca, Puigpunyent, on *Arundo micrantha*, 11 December 2020, leg. Ángel Pintos, AP111220A. Balearic Islands, Mallorca, Soller, on *Arundo donax*, 4 April 2021, leg. Ángel Pintos, AP4421 (WU-MYC0044530). Balearic Islands, Mallorca, Universitat de les Illes Balears (UIB), on *Arundo donax*, 28 December 2020, leg. Ángel Pintos, AP281220 (WU-MYC0044521). Catalonia, Barcelona, Premia de Dalt, on *Arundo donax*, 10 October 2020, leg. Miguel Mir, AP101020. Catalonia, Girona, Bescanó, on *Arundo micrantha*, 29 November 2020, leg. Marc Grañem, AP291120 (WU-MYC0044526).

Notes. The phylogenetic boundaries of *Apiospora* were recently discussed by Pintos and Alvarado (2021), who selected a lectotype (PC:0125160) for *S. apiospora*,

the basionym of the type species *Ap. montagnei*. In the present study, an epitype (PC:0125164) of *Ap. montagnei* is selected among modern collections growing on the same host in Girona, Spain (about 100 km south of the type locality, Perpignan, France). All samples growing on the same host collected in Girona or the Balearic Islands (Spain) are genetically identical to the epitype, and match the phylogenetic clade formerly known as *Ap. phragmitis*, excepting one that matches *Ap. sichuanensis*, but the ascospores of this species (29–48 × 7–10.5 µm, Samarakoon et al. 2022) clearly exceed those of *Ap. montagnei* (21–25 µm, Pintos and Alvarado 2021). Therefore, on the basis of these results, it is here hypothesized that *Ap. montagnei* is a priority synonym of *Ap. phragmitis*.

***Apiospora marianiae* sp. nov. Pintos & P. Alvarado**

MycoBank No: 843732

Fig. 3

Etymology. The epithet refers to Marian Mateu, the person who found the holotype collection and beloved wife of the first author.

Holotype. Spain: Balearic Islands, Palma de Mallorca, on *Phleum pratense*, 18 February 2019, leg. Marian Mateu AP18219 (holotype CBS 148710).

Asexual morph. Mycelium branched, septate, brown to dark brown. Conidiomata sporodochial, punctiform, scattered or confluent, black, (150–)169–203(–220) µm long × (70–)76–88(–100) µm wide (n = 30). Conidiophore mother cells on the surface of the stroma lageniform to ellipsoidal or doliiform, hyaline to brown, measuring (12–)13.4–14.2(–15) × (4–)6–7.2(–8) µm (n = 10). Conidiophores arising from conidiogenous mother cells, basauxic, cylindrical, straight to flexuous, hyaline except the thin transverse septa, smooth, measuring (19–)28–44(–55) × 3–3.6(–4) µm (n = 25). Conidiogenous cells monoblastic, integrated, terminal and intercalary, cylindrical. Conidia brown, solitary; face view: globose to ovate or ellipsoidal, with pale germ slit, (11–)12.1–13.5(–18) µm in diam. (n = 70); side view: lenticular, (8–)8.4–9.2(–10) µm in diam. (n = 30). Sterile cells only seen in culture, brown, granulate, irregularly lobed, (19–)25–31(–35) × (6–)8.15–8.45(–12) µm diam. (n = 40).

Culture characteristics. colonies in MEA white and cottony, with gray patches, reverse gray. Reaching 80–90 mm in diam, in 14 days at room temperature, sporulating after 5 weeks.

Other specimens examined. Spain: Balearic Islands, Palma de Mallorca, Establishments, on *Phleum pratense*, 30 November 2019, leg. Ángel Pintos, AP301119.

Notes. According to phylogenetic inference, *Ap. ovata* is the species most closely related to *Ap. marianiae*, but their ITS rDNA sequences are only 94% similar (including gaps). Their conidia are both oval to broadly ellipsoid, but those of *Ap. marianiae* measure 11–15 µm in diam., while those of *Ap. ovata* are longer, measuring about 18–20 µm in diam. in surface view.

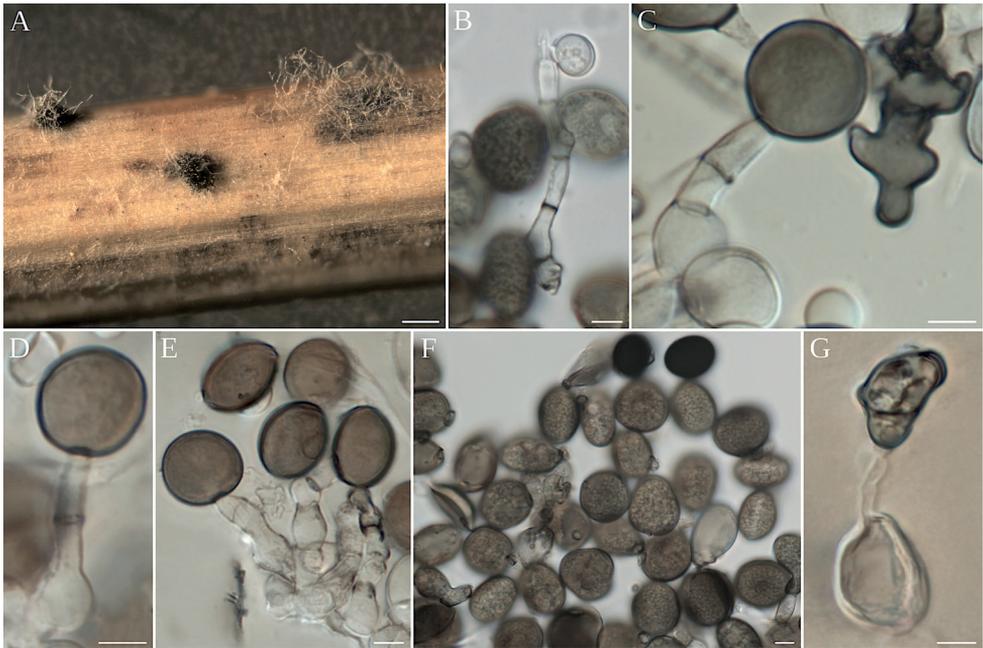


Figure 3. *Apiospora marianiae* (AP18219) **A** colony on culture **B–E** conidiophore mother cell with septate conidiophore giving rise to conidia, in **C** irregularly lobate sterile cell **F** conidia in face and side view **G** conidiophore mother cell with irregular conidia from agar. Scale bars: 100 μm (**A**); 5 μm (**B–G**).

Apiospora sichuanensis Samarak., Jian K. Liu & K.D. Hyde, in Samarakoon, Hyde, Maharachchikumbura, Stadler, Gareth Jones, Promptuttha, Suwannarach, Camporesi, Bulgakov & Liu, *Fungal Diversity* 112: 21. 2022.

Fig. 4

Asexual morph. Mycelium branched, septate, brown. Conidiomata on host parallel to the longitudinal axis of the stem, subepidermal, opening after the dehiscence of the host epidermis, containing a black conidial mass, measuring (400–)600–950(–1000) \times (275–)300–550(–600) μm ($n = 40$). Conidiophore mother cells arising from the stroma, lageniform to ampuliform, pale brown, with superficial granular depositions, (5–)6–10(–16) \times (3–)5–7(–8) μm ($n = 30$). Conidiophores basauxic, cylindrical, straight or flexuous, sometimes with a thin septum, hyaline to brown, smooth, with granular pigments, (20–)43–67(–80) μm in length \times (2–)2.2–3.4(–4) μm wide ($n = 50$). Conidia globose, subcylindrical to ovate, polygonal or obpyriform, with a lateral germ slit over the entire length, brown, smooth, irregularly lobed, measuring (10–)23–31(–35) \times (5–)9–13(–14) μm ($n = 30$).

Culture characteristics. colonies on MEA 70–90 mm in diam. after 14 days at room temperature, flat, spreading, first white and cottony, later becoming gray, reverse

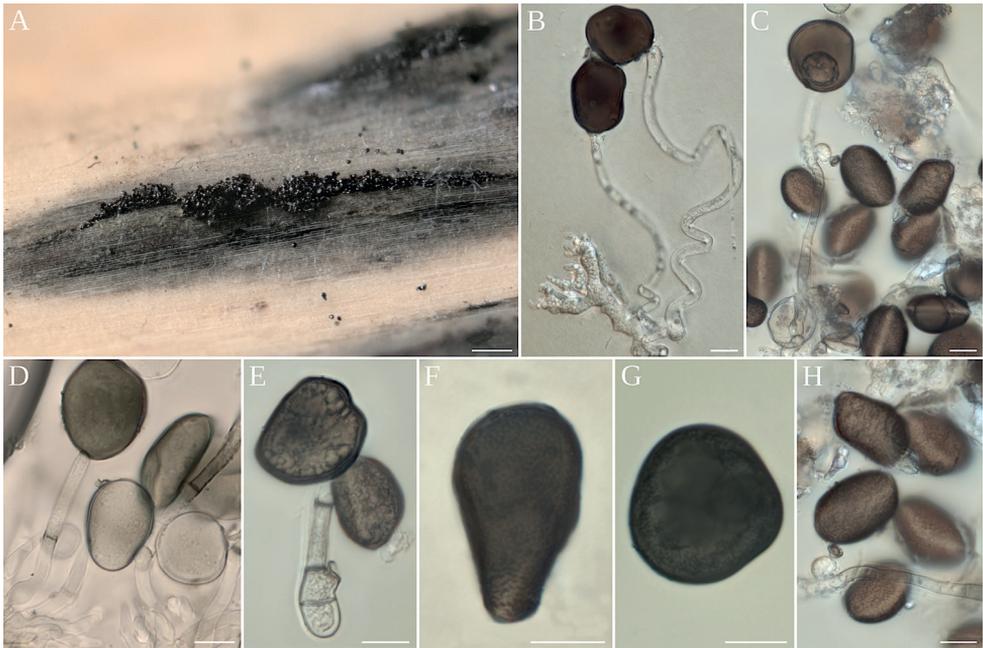


Figure 4. *Apiospora sichuanensis* (AP121220) **A** conidiomata on host **B** germinated conidia **C–E** conidiophore mother cell giving rise to septate conidiophore with conidia **F–H** conidia. Scale bars 100 μm (**A**); 10 μm (**B–H**).

dark gray. On PDA (200 g/L potato, 20 g/L dextrose, 20 g/L agar, pH 7.0), 80–90 mm in diam, after 14 days at room temperature, sporulating after 4–5 weeks, white cottony at first, then becoming gray with luteous patches, reverse dark gray.

Specimens examined. Spain: Balearic Islands, Mallorca, Palma de Mallorca, Torrenete de Soller, on *Arundo donax*, 12 December 2020, leg. Ángel Pintos, AP121220 (WU-MYC0044525). Catalonia, Girona, Bescanó, on *Arundo micrantha*, 15 November 2020, leg. Marc Grañem. AP151120.

Notes. *Ap. sichuanensis* is genetically close to *Ap. pseudoparenchymatica* (M. Wang & L. Cai) Pintos & P. Alvarado, but the fruiting body of the former is an acervulus and that of the latter a sporodochium. In addition, the conidia of *Ap. sichuanensis* are 10–35 \times 5–14 μm , longer and narrower than those of *Ap. pseudoparenchymatica*, which measure 13.5–27.0 \times 12.0–23.5.

Discussion

In the present work, several samples of *Apiospora* growing on *Arundo* aff. *micrantha* (the most probable host plant of the lectotype collection of *S. apiospora*, PC:0125160, Pintos and Alvarado 2021) were analyzed in order to clarify the identity of the type species of *Apiospora*, *Ap. montagnei* (\equiv *Sphaeria apiospora*). In previous works (Crous and Groenewald 2013, Pintos et al. 2019), four species of *Apiospora* were found on

Arundo spp.: *Ap. iberica*, *Ap. italica*, *Ap. marii*, and *Ap. phragmitis*. In the present work, the first record of *Ap. sichuanensis* in Europe was found growing also on *Arundo* spp. However, the ascospore dimensions reported in the protologues of *Ap. iberica* (29–34 × 6–8 µm, Pintos et al. 2019) and *Ap. sichuanensis* (29–48 × 7–10.5 µm, Samarakoon et al. 2022), clearly exceed those measured on the lectotype of *S. apiospora* (PC 0125160, 21–25 µm, Pintos and Alvarado 2021), and other original material of this species (23–28 µm, Hyde et al. 1998). On the contrary, ascospores of *Ap. italica*, *Ap. marii* and *Ap. phragmitis* are not significantly different from those of the lectotype of *S. apiospora*.

All samples of *Apiospora* found on *Arundo* aff. *micrantha* with an ascospore size matching that of *Ap. montagnei* are genetically identical to *Ap. phragmitis*. A single collection of *Ap. italica* (MA-Fungi 91733, Pintos et al. 2019), and another one of *Ap. marii* (MA-Fungi 91735, Pintos et al. 2019) were previously found on *Arundo donax*, a host plant where *Ap. phragmitis* and *Ap. sichuanensis* can occur too. Despite the lack of collections confirming it, it is certainly possible that *Ap. italica* and *Ap. marii* grow also on *Arundo* aff. *micrantha*, as these species have been found also on other host plants, especially *Phragmites*, but also *Ampelodesmos* and many others (*Arundinaria*, *Beta*, oats, seaweeds). *Apiospora marii* has been found in southern, central and northern Europe (Spain, Italy, Austria, The Netherlands, Sweden) and Asia (China, Korea), being most probably a widespread species. By way of contrast, *Ap. italica* and *Ap. phragmitis* have been found only in the Mediterranean region.

Given the wide host plant range observed in *Apiospora*, other species which have not been found yet on *Arundo* could be collected on this host plant genus in the future, reducing the reliability of this character for diagnosis. Of those species occurring in the Mediterranean region, some present ascospores differing in size from *Ap. montagnei* (i.e., *Ap. balearica* (Pintos & P. Alvarado) Pintos & P. Alvarado, *Ap. hysterina* (Sacc.) Pintos & P. Alvarado). Others, such as *Ap. descalsii* (Pintos & P. Alvarado) Pintos & P. Alvarado, are apparently rare, and the probability of a synonymy with *Ap. montagnei* is therefore low. The sexual state of *Ap. rasikravindrae* (Shiv M. Singh, L.S. Yadav, P.N. Singh, Rah. Sharma & S.K. Singh) Pintos & P. Alvarado produces ascospores measuring 21.5–24.5 × 7–9.5 µm (Dai et al. 2017), therefore fitting the size range observed in *S. apiospora* lectotype, but the synonymy is here rejected because this species has never been found yet on *Arundo* sp. (only known to grow on ornamental *Phyllostachys* and bamboo plants in Mallorca). Unfortunately, the sexual state of multiple species occurring in the Mediterranean region (i.e., *Ap. aurea* (Calvo & Guarro) Pintos & P. Alvarado, *Ap. esporlensis* (Pintos & P. Alvarado) Pintos & P. Alvarado, *Ap. hispanica* (Larrondo & Calvo) Pintos & P. Alvarado, *Ap. mediterranea* (Larrondo & Calvo) Pintos & P. Alvarado, *Ap. piptatheri* (Pintos & P. Alvarado) Pintos & P. Alvarado, *Ap. serenensis* (Larrondo & Calvo) Pintos & P. Alvarado, as well as the new species introduced in the present work, *Ap. marianiae*) is still unknown, and therefore they cannot be compared yet with the lectotype of *S. apiospora* (≡ *Ap. montagnei*). However, these seem to be rare species, and they have never been found on *Arundo* yet, so the synonymy is here considered much less probable.

A classical candidate synonym of *Ap. montagnei*, *Ap. arundinis* (Corda) Pintos & P. Alvarado (Crous and Groenewald 2013), has not been found yet in the western

Mediterranean region (Pintos et al. 2019, Pintos and Alvarado 2021), but it seems to be widespread elsewhere, occurring in temperate, cold and also subtropical countries (Crous and Groenewald 2013). In Spain, it has been found in ornamental *Bambusa* plants in Galicia (north-western Spain), but not in the Mediterranean border with France (closer and ecologically more similar to Perpignan, the type locality of *Ap. montagnei*). Sequenced samples of *Ap. arundinis* found growing in *Arundo* are currently lacking, but the type collection of its basionym, *Gymnosporium arundinis* Corda was reported to grow on reeds and grasses near Prague by Corda (1838). An original sample kindly loaned by the Prague herbarium (PRM 155522) was found to present globose conidia 5–7 µm in diam., a size compatible with that observed in the clade identified as *Ar. arundinis* by Crous and Groenewald (2013), but also others, such as *Ap. descalsii*, *Ap. italicum*, *Ap. jiangxiensis* (M. Wang & L. Cai) Pintos & P. Alvarado, *Ap. malaysiana* (Crous) Pintos & P. Alvarado, *Ap. pseudospegazzinii* (Crous) Pintos & Alvarado or *Ap. sacchari* (Speg.) Pintos & P. Alvarado. Interestingly, the host plant of this original collection of *G. arundinis* loaned by PRM was not *Arundo*, but very probably *Phalaris arundinacea*. The identity of *Ap. arundinis* needs to be further investigated, and an epitype from Prague selected, to ascertain if the name is being correctly applied.

Therefore, on the basis of the data currently available (host plants, ascospore sizes, abundances, distributions), it is here hypothesized that the lectotype of *S. apiospora* (\equiv *Ap. montagnei*) is not genetically different from the clade of *Ap. phragmitis*. An epitype of *S. apiospora* (\equiv *Ap. montagnei*) from Girona (Spain, about 100 km south of Perpignan, the locality where the lectotype was found) is here chosen, and a synonymy between *Ap. montagnei* and *Ap. phragmitis* is suggested.

Acknowledgements

The authors wish to thank Juan Planas for assembling the image plates, as well as Jan Holec, curator of PRM herbarium for kindly allowing the authors to study original collections of *Gymnosporium arundinis*. Finally, Marc Grañem, Marian Mateu and Miguel Mir are thanked also for collaborating in finding samples of *Apiospora*.

References

- Arita M, Karsch-Mizrachi I, Cochrane G (2021) The international nucleotide sequence database collaboration. *Nucleic Acids Research* 49: D121–D124. <https://doi.org/10.1093/nar/gkaa967>
- Bory de Saint-Vincent JBG, Durieu de Maisonneuve MC (1849) *Exploration scientifique de l'Algérie pendant les années 1840, 1841, 1842*. Sciences Physiques, Botanique. Flore d'Algérie. Imprimerie Royale, Paris.
- Carbone I, Kohn L (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91(3): 553–556. <https://doi.org/10.1080/00275514.1999.12061051>

- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17(4): 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Corda ACJ (1838) *Icones Fungorum hucusque cognitorum*. Tomus II. Apud J. G. Calve, Prague.
- Crous PW, Groenewald JZ (2013) A phylogenetic re-evaluation of *Arthrinium*. *IMA Fungus* 4(1): 133–154. <https://doi.org/10.5598/imafungus.2013.04.01.13>
- Crous PW, Wingfield MJ, Le Roux JJ, Richardson DM, Strasberg D, et al. (2015) Fungal Planet description sheets: 371–399. *Persoonia* 35(1): 264–327. <https://doi.org/10.3767/003158515X690269>
- Crous PW, Schumacher RK, Wood AR, Groenewald JZ (2020) The genera of Fungi – G5: *Arthrinium*, *Ceratospaeria*, *Dimerosporiopsis*, *Hormodochis*, *Lecanostictopsis*, *Lembosina*, *Neomelanconium*, *Phragmotrichum*, *Pseudomelanconium*, *Rutola* and *Trullula*. *Fungal Systematics and Evolution* 5(1): 77–98. <https://doi.org/10.3114/fuse.2020.05.04>
- Crous PW, Hernández-Restrepo M, Schumacher RK, Cowan DA, Maggs-Kölling G, Marais E, Wingfield MJ, Yilmaz N, Adan OCG, Akulov A, Duarte EÁ, Berraf-Tebbal A, Bulgakov TS, Carnegie AJ, de Beer ZW, Decock C, Dijksterhuis J, Duong TA, Eichmeier A, Hien LT, Houbraken JAMP, Khanh TN, Liem NV, Lombard L, Lutzoni FM, Miadlikowska JM, Nel WJ, Pascoe IG, Roets F, Roux J, Samson RA, Shen M, Spetik M, Thangavel R, Thanh HM, Thao LD, van Nieuwenhuijzen EJ, Zhang JQ, Zhang Y, Zhao LL, Groenewald JZ (2021) New and Interesting Fungi. 4. *Fungal Systematics and Evolution* 7(1): 255–343. <https://doi.org/10.3114/fuse.2021.07.13>
- Cubeta MA, Echandi E, Abernethy T, Vilgalys R (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* 81(11): 1395–1400. <https://doi.org/10.1094/Phyto-81-1395>
- Dai DQ, Jiang HB, Tang LZ, Bhat DJ (2016) Two new species of *Arthrinium* (Apiosporaceae, Xylariales) associated with bamboo from Yunnan, China. *Mycosphere: Journal of Fungal Biology* 7(9): 1332–1345. <https://doi.org/10.5943/mycosphere/7/9/7>
- Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote E (2017) Bambusicolous fungi. *Fungal Diversity* 82(1): 1–105. <https://doi.org/10.1007/s13225-016-0367-8>
- Ellis MB (1965) Dematiaceous Hyphomycetes. VI. *Mycological Papers* 103: 1–46.
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes—Application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2): 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61(4): 1323–1330. <https://doi.org/10.1128/aem.61.4.1323-1330.1995>
- Heo YM, Kim K, Ryu SM, Kwon SL, Park MY, Kang JE, Hong J-H, Lim YW, Kim C, Kim BS, Lee D, Kim J-J (2018) Diversity and ecology of marine Algalicolous *Arthrinium* species as a source of bioactive natural products. *Marine Drugs* 16(12): 508. <https://doi.org/10.3390/md16120508>
- Hyde KD, Fröhlich J, Taylor JE (1998) Fungi from palms. XXXVI. Reflections on unitunicate ascomycetes with apiospores. *Sydowia* 50: 21–80.

- Jiang N, Li N, Tian CM (2018) *Arthriniium* species associated with bamboo and red plants in China. *Fungal Systematics and Evolution* 2: 1–9. <https://doi.org/10.3114/fuse.2018.02.01>
- Jiang HB, Hyde KD, Doilom M, Karunarathna SC, Xu JC, et al. (2019) *Arthriniium setostromum* (Apiosporaceae, Xylariales), a novel species associated with dead bamboo from Yunnan, China. *Asian Journal of Mycology* 2(1): 254–268. <https://doi.org/10.5943/ajom/2/1/16>
- Jiang N, Liang YM, Tian CM (2020) A novel bambusicolous fungus from China, *Arthriniium chinense* (Xylariales). *Sydowia* 72: 77–83. <https://doi.org/10.12905/0380.sydowia72-2020-0077>
- Kwon SL, Park MS, Jang S, Lee YM, Heo YM, Hong J-H, Lee H, Jang Y, Park J-H, Kim C, Kim G-H, Lim YW, Kim J-J (2021) The genus *Arthriniium* (Ascomycota, Sordariomycetes, Apiosporaceae) from marine habitats from Korea, with eight new species. *IMA Fungus* 12(1): 13. <https://doi.org/10.1186/s43008-021-00065-z>
- Liu F, Bonthond G, Groenewald JZ, Cai L, Crous PW (2019) Sporocadaceae, a family of coelomycetous fungi with appendage bearing conidia. *Studies in Mycology* 92(1): 287–415. <https://doi.org/10.1016/j.simyco.2018.11.001>
- Mullis K, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155: 335–350. [https://doi.org/10.1016/0076-6879\(87\)55023-6](https://doi.org/10.1016/0076-6879(87)55023-6)
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8(19): 4321–4325. <https://doi.org/10.1093/nar/8.19.4321>
- O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7(1): 103–116. <https://doi.org/10.1006/mpev.1996.0376>
- Park MS, Oh S-Y, Lee S, Eimes JA, Lim YW (2018) Fungal diversity and enzyme activity associated with sailfin sandfish egg masses in Korea. *Fungal Ecology* 34: 1–9. <https://doi.org/10.1016/j.funeco.2018.03.004>
- Phukhamsakda C, Nilsson RH, Bhunjun CS, Gomes de Farias AR, Sun Y-R, Wijesinghe SN, Raza M, Bao D-F, Lu L, Tibpromma S, Dong W, Tennakoon DS, Tian X-G, Xiong Y-R, Karunarathna SC, Cai L, Luo Z-L, Wang Y, Manawasinghe IS, Camporesi E, Kirk PM, Promputtha I, Kuo C-H, Su H-Y, Doilom M, Li Y, Fu Y-P, Hyde KD (2022) The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Diversity* 114(1): 327–386. <https://doi.org/10.1007/s13225-022-00502-3>
- Pintos Á, Alvarado P (2021) Phylogenetic delimitation of *Apiospora* and *Arthriniium*. *Fungal Systematics and Evolution* 7(1): 197–221. <https://doi.org/10.3114/fuse.2021.07.10>
- Pintos Á, Alvarado P, Planas J, Jarling R (2019) Six new species of *Arthriniium* from Europe and notes about *A. caricicola* and other species found in *Carex* spp. hosts. *MycKeys* 49: 15–48. <https://doi.org/10.3897/mycokeys.49.32115>
- Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97(1): 84–98. <https://doi.org/10.3852/mycologia.97.1.84>
- Saccardo PA (1875) *Conspectus generum pyrenomycetum italicorum additis speciebus fungorum Venetorum novis vel criticis, systemate carpologico dispositorum*. *Atti della Società Veneto-Trentina di Scienze Naturali* 4: 77–100.

- Samarakoon MC, Hyde KD, Maharachchikumbura SSN, Stadler M, Jones EBG, Promputtha I, Suwannarach N, Camporesi E, Bulgakov TS, Liu J-K (2022) Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of Xylariomycetidae (Sordariomycetes). *Fungal Diversity* 112(1): 1–88. <https://doi.org/10.1007/s13225-021-00495-5>
- Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD, Jones EBG, McKenzie EHC, Dai DQ, Daranagama DA, Dayarathne MC, Goonasekara ID, Konta S, Li WJ, Shang QJ, Stadler M, Wijayawardene NN, Xiao YP, Norphanphoun C, Li Q, Liu XZ, Bahkali AH, Kang JC, Wang Y, Wen TC, Wendt L, Xu JC, Camporesi E (2015) Towards unravelling relationships in Xylariomycetidae (Sordariomycetes). *Fungal Diversity* 73(1): 73–144. <https://doi.org/10.1007/s13225-015-0340-y>
- Senanayake IC, Bhat JD, Cheewangkoon R, Xie N (2020) Bambusicolous *Arthrinium* species in Guangdong province, China. *Frontiers in Microbiology* 11: 602773. <https://doi.org/10.3389/fmicb.2020.602773>
- Singh SM, Yadav LS, Singh PN, Hepat R, Sharma R, Singh SK (2012) *Arthrinium rasikravindrii* sp. nov. from Svalbard, Norway. *Mycotaxon* 122(1): 449–460. <https://doi.org/10.5248/122.449>
- Smith GJD, Liew ECY, Hyde KD (2003) The Xylariales: A monophyletic order containing 7 families. *Fungal Diversity* 13: 185–218.
- Sørensen T, Petersen C, Fechete LI, Nielsen KL, Sondergaard TE (2022) A highly contiguous genome assembly of *Arthrinium puccinoides*. *Genome Biology and Evolution* 14(1): 1–5. <https://doi.org/10.1093/gbe/evac010>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Tang X, Goonasekara ID, Jayawardena RS, Jiang HB, Li JF, Hyde K, Kang J (2021) *Arthrinium bambusicola* (Fungi, Sordariomycetes), a new species from *Schizostachyum brachycladum* in northern Thailand. *Biodiversity Data Journal* 8: e58755. <https://doi.org/10.3897/BDJ.8.e58755>
- Tian X, Karunaratna SC, Mapook A, Promputtha I, Xu J, Bao D, Tibpromma S (2021) One new species and two new host records of *Apiospora* from bamboo and maize in Northern Thailand with thirteen new combinations. *Life (Chicago, Ill.)* 11(10): 1071. <https://doi.org/10.3390/life11101071>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172(8): 4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Wang M, Liu F, Crous PW, Cai L (2017) Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia* 39(1): 118–142. <https://doi.org/10.3767/persoonia.2017.39.06>
- Wang M, Tan X-M, Liu F, Cai L (2018) Eight new *Arthrinium* species from China. *MycoKeys* 34: 1–24. <https://doi.org/10.3897/mycokeys.34.24221>
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al.

(Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, 315–332. <http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>

Yan H, Jiang N, Liang L-Y, Yang Q, Tian C-M (2019) *Arthriniium trachycarpum* sp. nov. from *Trachycarpus fortuneii* in China. Phytotaxa 400(3): 203–210. <https://doi.org/10.11646/phytotaxa.400.3.7>

Yang C-L, Xu X-L, Dong W, Wanasinghe DN, Liu Y-G, Hyde KD (2019) Introducing *Arthriniium phyllostachium* sp. nov. (Apiosporaceae, Xylariales) on *Phyllostachys heteroclada* from Sichuan province, China. Phytotaxa 406(2): 91–110. <https://doi.org/10.11646/phytotaxa.406.2.2>

Supplementary material 1

Table S1

Authors: Ángel Pintos, Pablo Alvarado

Data type: Sequences.

Explanation note: Sequences produced in the present work (in bold) and retrieved from databases.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/mycokeys.92.87593.suppl1>

Supplementary material 2

Table S2

Authors: Ángel Pintos, Pablo Alvarado

Data type: Sequences.

Explanation note: Samples analyzed in the present work.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/mycokeys.92.87593.suppl2>