INTRODUCTION

The genus *Achroceratosphaeria* was described for perithelial ascomycetes that are morphologically similar to *Ceratosphaeria* and *Pseudohalonectria* of the *Magnaporthaceae* (Réblová et al. 2010).

In the phylogeny inferred from sequences of the small and large subunits of nuclear ribosomal DNA (nuc18S and nuc28S rDNA) *Achroceratosphaeria* has been placed within *Sordariomycetes* incertae sedis; it was nested in a weakly supported clade as sister to the *Lulworthiales* and *Korallonastetales* containing fungi from predominantly marine habitats (Kohlmeyer 1997, Kohlmeyer et al. 2000, Campbell et al. 2005, 2008). *Achroceratosphaeria* comprises two freshwater and one terrestrial species characterised by minute, immersed, subhyaline to pale brown ascomata with a fragile, hyaline to pale brown protruding neck, tapering paraphyses, un disruptive stipe ticate asci with a non-amyl oid apical annulus and eight hyaline, septate, ellipsoidal to fusiform ascos pores. The asexual morph is unknown.

Four specimens of an unidentified fungus were collected on deciduous wood submerged in fresh water in France and Belgium during the years 2006–2014. They are characterised by non-stromatic, immersed to superficial perithecial ascomata, persistent paraphyses, unitunicate asci with an amyloid apical annulus and hyaline, fusiform, cylindrical to cym biform, transversely multiseptate ascospores with conspicuous guttules. No conidia were formed in vitro or on the natural substrate. The clade containing *Achroceratosphaeria* and *Pisorisporium* is introduced as the new order *Pisorisporiales*, family *Pisorisporaceae* in the class *Sordariomycetes*. It represents a new lineage of aquatic fungi. A sister relationship for *Pisorisporiales* with the *Lulworthiales* and *Korallonastetales* is weakly supported by Bayesian inference and maximum likelihood analyses. The systematic position of *Pisorisporium* among morphologically similar perithelial ascomycetes is discussed.

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Key words

*Achroceratosphaeria*

freshwater

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*Lulworthiales*

multigene analysis

systematics

Abstract

Four morphologically similar specimens of an unidentified perithelial ascomycete were collected on decaying wood submerged in fresh water. Phylogenetic analysis of DNA sequences from protein-coding and ribosomal nuclear loci supports the placement of the unidentified fungus together with *Achroceratosphaeria* in a strongly supported monophyletic clade. The four collections are described as two new species of the new genus *Pisorisporium* characterised by non-stromatic, black, immersed to superficial perithecial ascomata, persistent paraphyses, un disruptive stipe ticate asci with an amyloid apical annulus and hyaline, fusiform, cylindrical to cym biform, transversely multiseptate ascospores with conspicuous guttules. The asexual morph is unknown and no conidia were formed in vitro or on the natural substrate. The clade containing *Achroceratosphaeria* and *Pisorisporium* is introduced as the new order *Pisorisporiales*, family *Pisorisporaceae* in the class *Sordariomycetes*. It represents a new lineage of aquatic fungi. A sister relationship for *Pisorisporiales* with the *Lulworthiales* and *Korallonastetales* is weakly supported by Bayesian inference and maximum likelihood analyses. The systematic position of *Pisorisporium* among morphologically similar perithelial ascomycetes is discussed.

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| Classification   | Taxon                                      | Source                  | GenBank accession numbers |
|------------------|-------------------------------------------|-------------------------|---------------------------|
| **Sordariomycetes** |                                          |                         |                           |
| Annulatascaceae  | Annulatascus velatiporus                 | A70-18                  | Y058734 – –                |
|                  | Annullatascus triseptatus                | CBS 131483, CBS 128631  | JQ425624, JQ425628        |
|                  | Asciulina austriaca                      | CBS 131685              | JQ425624, JQ425628        |
| Boliniaceae      | Camaropella pulgillus                    | SMH 3845                |                           |
|                  | Camarops micropora                       | CBS 649.92              | Y083821, DQ471036, DQ470937 |
|                  | Compinula ellipsoides                    | SMH 1378                | DQ231441 – –              |
| Calosphaeriaceae | Calosphaeria pulchella                   | CBS 11599               | Y071075, Y071071, GQ180661 |
|                  | Jataea algeriensis                      | STE-U 6399, CBS 120871 | EU367457, EU367462, HQ876803 |
| Togninellaceae   | Togninella micropera                     | CBS 113648              | Y071076, Y071071, GQ180661 |
| Chaetosphaeriaceae | Chaetosphaeria ciliata                 | ICMP 18253              | Y083821, Y083816, GQ180661 |
|                  | Chaetosphaeria curvispora                | ICMP 18253              | Y083821, Y083816, GQ180661 |
| Coniochaetaceae | Coniochaetula discoides                  | SANK 12878, CBS 158.80  | Y046297, A875179, Y070911  |
|                  | Coniochaeta ostrea                      | CBS 507.70              | DQ470959, DQ471007, DQ470909 |
| **Coronophorales** |                                              |                         |                           |
| Berta moriformis | SMH 3344, SMH 4320                      | Y085621 – –             | Y170901, Y170912          |
| Chaetosphaerella | Chaetosphaerella phaeostroma             | SMH 4858                | Y046297 – –               |
MATERIALS AND METHODS

Herbarium material and fungal strains
Dry ascomata were rehydrated with water; material was examined with an Olympus SZX12 dissecting microscope, and hand-sectioned centrum material (including asci, ascospores and paraphyses) was mounted in Melzer’s reagent, Lugol, 90 % lactic acid, aqueous cotton-blue (1 mg/mL), and blue or black Waterman ink. Hand sections of the ascomatal wall were studied in 3 % KOH or heated chloral-lactophenol. All measurements were made in Melzer’s reagent. Means ± standard deviation (SD) based on 20—25 measurements, excluding maxima and minima, are given for dimensions of asci and ascospores. Images were captured by differential interference (DIC) or phase contrast (PC) microscopy using an Olympus DP70 Camera operated by Imaging Software Cell on an Olympus BX51 compound microscope.

Multi-ascospore isolates were obtained from fresh material of three collections (PRM 924377-924379) with the aid of a spore isolator (Meopta, Prague, Czech Republic). Ascospores and asci were spread on water agar, ascospores germinated within 48 h. Germinating ascospores were transferred and isolates were grown on water agar, potato-dextrose agar (PDA, Oxoid) and potato-carrot agar (PCA, Gams et al. 1998). Colonies were examined after 7, 21 and 30 d incubation at 25 °C in the dark. The ex-type culture is maintained at CBS (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands). Type and other herbarium material are deposited in PRM herbarium (National Museum in Prague, Czech Republic). The Online auction colour chart (2004) was used as the colour standard.

DNA extraction, amplification and sequence alignment
Cultures used for DNA isolations were grown as previously described by Réblová et al. (2011) and DNA was extracted following the protocols of Lee & Taylor (1990). Procedures for amplifying and sequencing the nuc18S, nuc28S and rpb2 were performed as described in Réblová et al. (2011). Sequences were edited using Sequencher v. 5.0 software (Gene Codes Corp., Ann Arbor, MI, USA).

GenBank accession numbers for newly sequenced taxa and other homologous sequences of members of the Sordariomycetes and Leotiomycetes retrieved from GenBank are listed in Table 1. Sequences were manually aligned in BioEdit v. 7.0.9.0 (Hall 1999). The nuclear ribosomal loci were aligned according to the secondary structure of Saccharomyces cerevisiae Meyen ex E.C. Hansen in order to improve the decisions on homologous characters and introduction of gaps (Gutell 1993, Gutell et al. 1993, www.rna.ccbb.utexas.edu). These procedures and alignment of the rpb2 sequences were performed as described in Réblová & Réblová (2013). The single-locus datasets (nuc28S: 1 923 characters and 77 sequences, nuc18S: 1 805 characters and 68 sequences, rpb2 segments 5—7: 1 213 characters and 48 sequences) were examined for topological incongruence among loci. For each individual locus, 500 bootstrap replicates were generated with RAxML-HPC v. 7.0.3 (Stamatakis et al. 2005, Stamatakis 2006) and compared visually for topological conflict between supported clades in phylogenetic trees. A conflict between two loci was assumed to occur when a clade appeared monophyletic with bootstrap support of ≥ 75 % in one tree, but was supported as non-monophyletic in another (Mason-Gamer & Kellogg 1996). Individual, conflict-free alignments were concatenated to combine sequences for subsequent phylogenetic analyses. The multiple sequence alignment is deposited in TreeBASE (Study no. 16406).

Phylogenetic analysis
Phylogenetic relationships of the unidentified fungus were resolved by an analysis of nuc18S, nuc28S and rpb2 sequences of representatives of 19 orders or individual families of the Sordariomycetes. We analysed the first 2/3 of the 5’ half of the nuc28S, the almost entire nuc18S, and segments 5—7 of rpb2. Bases 1—148 of the nuc18S, 1—85 of the nuc28S, and 1—58 of the rpb2 alignments at the 5’-end and 1 457—1 923 of the nuc28S alignment at the 3’-end were excluded from analyses because of incompleteness of the majority of the available sequences. The combined dataset was partitioned into several subsets of nucleotide sites, i.e. nuc28S, nuc18S, and first, second and third codon positions of rpb2. Two members of the Leotiomycetes, Leotia lubrica and Microglossum rufum were used to root the multilocus phylogeny.

The program MrModeltest2 v. 2.3 (Nylander 2008) was used to infer the appropriate substitution model that would best fit the model of DNA evolution for each sequence dataset and each partition of the combined datasets. Maximum likelihood (ML) and Bayesian inference (BI) analyses were used to estimate phylogenetic relationships. ML analysis was performed with RAxML-HPC v. 7.0.3 with a GTRCAT model of evolution. Nodal support was determined by non-parametric bootstrapping (BS) with 1 000 replicates.

BI analysis was performed in a likelihood framework as implemented in MrBayes v. 3.0b4 software package to reconstruct phylogenetic trees (Huelsenbeck & Ronquist 2001). For the combined nuc18S, nuc28S and rpb2 dataset we used for each partition the GTR+I+G substitution model. Two Bayesian searches were performed using the default parameters. Analyses were run for 10 M generations, with trees sampled every 1 000 generations. Tracer v. 1.6.0. (Rambaut et al. 2013) was used to confirm convergence of trees and burn-in. The first 50 000 trees, which represented the burn-in phase of the analysis, were discarded. The remaining trees were used for calculating posterior probabilities (PP) of recovered branches (Larget & Simon 1999).

RESULTS
Phylogenetic results
In the ML analyses (conducted by RAxML) of individual nuc28S, nuc18S and rpb2 loci, the three strains of the unidentified fungus grouped always with two species of Achroceratosphaeria in a strongly supported monophyletic clade distantly related to the known orders and families of the Sordariomycetes. The clade is introduced as the new order Pisorisporiales. Analyses of individual nuclear ribosomal and protein-coding loci place Pisorisporiales at different positions in the Sordariomycetes, however none of the internodes received significant statistical support. The nuc28S locus supports the placement of Pisorisporiales as a basal group in the Sordariomycetes. The phylogenies derived from individual nuc18S and rpb2 loci consistently place Pisorisporiales within the Sordariomycetes. In the nuc18S tree, the Pisorisporiales are located basal to the Hypocreomycetidae, while in the rpb2 locus they are at the base of the Sordariomycetidae. All other families and orders of the Sordariomycetes formed well-supported monophyletic clades in analyses of all three individual loci.

The final alignment consisted of 79 combined nuc18S, nuc28S and rpb2 sequences of members of the Sordariomycetes, each with 4 941 characters after introduction of gaps. The alignment had 2 551 distinct alignment patterns (ML analysis); the ML tree is shown in Fig. 1. The Sordariomycetes are shown as a robust monophyletic clade (100 % MLBS / 1.0 PP) comprising
three strongly supported lineages, the Sordariomycetidae, Hypocreomycetidae and Xylariomycetidae. The Pisorisporales are nested in a weakly supported clade as sister to the Lulworthiales (100/1.0) and Koralionastetales (100/1.0). The whole clade is situated basal (65/0.87) to the Hypocreomycetidae. The other three taxonomic groups of the Hypocreomycetidae that contain predominantly fungi from aquatic habitats form strongly supported monophyletic clades, i.e. the Halosphaeraceae (100/1.0), Savoryellales (100/1.0), and the complex of marine genera (95/1.0) comprising the Ethicsporaceae (100/1.0), Juncigenaceae (100/1.0) and Torpedosporaceae (100/1.0) of the TBM clade (76/1.0).

**TAXONOMY**

DNA sequences of nuclear ribosomal and protein-coding loci of specimens obtained from freshwater habitats in this study were shown to represent a new genus Pisorisporum and order in the Sordariomycetes based on phylogenetic analysis. Morphological examination showed that two species were present, described here as *P. cymbiforme* and *P. glaucum*. For the latter species DNA sequences could not be obtained but morphologically it fits clearly within the newly described genus, while it is morphologically distinct from the first species.

**Pisorisporales** Réblová & J. Fourn., ord. nov. — MycoBank MB810338

*Type family.* Pisorisporaceae Réblová & J. Fourn.

Ascomata perithelial, non-stromatic. Ostiole periphysate. *Ascomatal wall* leathery to fragile, brown, partly carbonaceous. *Hamathecium* of true paraphyses. Asci unitunicate, persistent, with an amyloid or non-amyloid apical ring. Ascospores hyaline, transversely multisepate. Asexual morph unknown. Saprobic on wood.

**Pisorisporaceae** Réblová & J. Fourn., fam. nov. — MycoBank MB810339

*Type genus.* Pisorisporum Réblová & J. Fourn.

Ascomata non-stromatic, immersed to superficial, papillate or with a long neck, venter subglobose to conical, upright or lying obliquely or horizontally, neck central rarely eccentric. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, partly carbonaceous in the outer layers, pigmented dark brown, opaque to light brown to subhyaline, comprising two layers. *Paraphyses* abundant, persistent, cylindrical. *Asci* unitunicate, 8-spored, with a pronounced amyloid or non-amyloid apical annulus, cylindrical-clavate, persistently attached to the ascogenous hyphae at maturity. *Ascospores* fusiform, cylindrical to cymbi-
Fig. 2 Pisorisporium cymbiforme. a, b. Ascomata arranged in small groups or in rows; c, d. vertical sections of the ascomatal wall; e–g. asci; h, i. ascospores; j. paraphyses (a, b, g, i from PRM 924377 holotype; c, d, h from PRM 924379; e, f from PRM 924378); e–i: DIC; j: PC. — Scale bars: a, b = 200 µm; c, d = 20 µm; e–j = 10 µm.
form slightly tapering towards the ends, hyaline, transversely multisepitate, lacking a mucilaginous sheath or appendages, often with numerous guttules. Asexual morph unknown.

**Pisorisporium** Réblová & J. Fourn., gen. nov. — MycoBank MB810340

Type species. *Pisorisporium cymbiforme* Réblová & J. Fourn.

**Etymology.** *Pisum* (Latin), meaning boat-shaped (a long rowboat), referring to the ascomata.

**Ascobata** non-stromatic, immersed, gradually erumpent to superficial, solitary or in small groups or rows, papillate or with a short beak, glabrous, venter subglobose to broadly conical, laterally or basally flattened, upright or lying obliquely or horizontally. **Ostiole** periphysate. **Ascomatal wall** fragile, partly carbonaceous in the outer layer, 2-layered. **Paraphyses** persistent, septate, hyaline, arising from the bottom and sides in the ascomatal cavity. **Ascus** (180–)190–207 £ 5.4 μm (mean ± SD = 19.9 ± 5.4 μm), cylindrical to subcylindrical, central to lateral, opening by a rounded pore. **Ostiole** subglobose, dark brown to black, sometimes laterally or basally flattened, glabrous, upright or lying obliquely to horizontally, papillate or with a beak 50–180 μm high, conical or subcylindrical, central to lateral, opening by a rounded pore. **Asci** 2–3 layers of subhyaline to pale brown, polyhedral to angular cells of the neck 20–30 μm, 2-layered; outer layer consisting of brown, polyhedral cells of *textura prismatica* with opaque walls and lumina reduced to occluded; onwards grading into 2–3 layers of subhyaline to pale brown, polyhedral to angular cells of *textura angularis*. **Ascospore** (52–)55–67 £ 4.5–5.5 μm (mean ± SD = 36.9 ± 4.4 μm), ± SD = 5.4 μm, cylindrical, slightly longer, 187–210 £ 7 ± 12.8 μm. **Spores** persistent, septate, hyaline, sparsely branched in the upper half and intertwined, c. 3.0–5.5 μm wide, tapering to 2.0–2.5 μm.

**Notes** — The paraphyses were present abundantly, they are fragile, easily broken in squash mounts, making it difficult to determine their length. They are cylindrical, arranged in parallel at the bottom of the ascomata and among the asci, tapering, sparsely branched and often intertwined in the upper half. The outer ascomatal wall is carbonaceous, grading outwards into 2–3 layers of subhyaline to pale brown, polyhedral to angular cells that probably account for the finely roughened appearance of the wall in both species.

**Pisorisporium cymbiforme** Réblová & J. Fourn., sp. nov. — MycoBank MB810341; Fig. 2

**Etymology.** *Cymbiform* (Latin), meaning boat-shaped (a long rowboat), referring to the shape of the ascosporae.

**Ascobata** non-stromatic, immersed, gradually erumpent to superficial, solitary or in small groups of 2–4, or in rows, venter (240–)290–380 μm diam, 220–280 μm high, subglobose to broadly conical, dark brown to black, sometimes laterally or basally flattened, glabrous, finely roughened, upright or lying obliquely to horizontally, papillate or with a beak 30–110 μm high, conical or subcylindrical, central to lateral, opening by a rounded pore. **Ostiole** periphysate. **Ascomatal wall** fragile, carbonaceous, (12–)14–26 μm thick, becoming thicker in the neck c. 45–58 μm, 2-layered; outer layer consisting of brown, polyhedral cells of *textura prismatica* with opaque walls and lumina reduced to occluded; onwards grading into 2–3 layers of subhyaline to pale brown, polyhedral to angular cells of *textura angularis*. **Ascus** 2–3 layers of subhyaline to pale brown, polyhedral to angular cells of *textura angularis*. **Ascospore** 4.6–6 μm thick, collapsing in old ascobata and forming a persistent subhyaline amorphous coating; inwards grading into several layers of thin-walled, pale brown to hyaline, flattened cells. **Paraphyses** abundant, persistent, septate, hyaline, sparsely branched in the upper half and intertwined, c. 3.5–5.0 μm wide, tapering to c. 3.0 μm. **Ascospore** (180–)190–207 £ 11–13 ± 14 μm (mean ± SD = 199.7 ± 5.4 μm), cylindrical-clavate, obtuse to broadly rounded apically, 8-spored; apex with an amyloid apical annulus 3.0–3.2 μm wide, 1.9–2.3 μm high. **Ascospores** 40–45 £ 3.8–4.3–4.8–5.0 μm (mean ± SD = 43.7 ± 1.9 ± 4.5 ± 0.3 μm), cymobal to fusiform to cylindrical, slightly tapering towards the ends, hyaline, smooth, (8–)12–16-septate, non-constricted at the septa, each cell with a large guttule, arranged 2-seriately in the ascus.

**Notes** — *Pisorisporium glaucum* is easily distinguishable from *Pisorisporium* by longer and slightly wider ascosporae and longer ascus. The number of septa of the ascospore is in both species comparable and varies from 10 to 16. This species has not been cultivated at the time of its collection and DNA sequences could not be obtained due to insufficient number of ascomata that would be required for successful DNA extraction. Such procedure would cause destruction of the type material.
Fig. 3 Pisorisporium glaucum. a, b. Ascomata arranged in small groups or in rows; c. vertical section of the ascomatal wall; d. ascogenous hypha with attached bases of asci; e–g. asci; h. thimble-shaped apical annulus staining blue in Lugol; i–k. ascospores (a–k from PRM 924380 holotype); e, f, h, i, k: DIC; d, g, j: PC. — Scale bars: a, b = 200 µm; c = 20 µm; d–g, i–k = 10 µm; h = 5 µm.
DISCUSSION

The combined analysis of nuc18S-nuc28S-rpb2 sequences (Fig. 1) led to the discovery that the three strains of P. cymoforme and Achoroceraspheraea form a strongly supported monophyletic clade (100/1.0), which is distinctly related to fresh-water and marine ascomycetes of the Annulatascaceae, Halosphaeriaceae, Papulosaceae, Savoryellales, marine genera of the TBM clade, now classified as the Ethereophoraceae, Juncigenaceae, Torpedosporaceae and Falcocladiaeae (Jones et al. 2014), and other morphologically similar fungi. The newly recognised clade containing Achoroceraspheraea and Pisorisporium represents a distinct taxonomic group at the ordinal level within the Sordariomycetes based on the evidence of molecular sequence data. However, its relationship with other orders could not be elucidated with good statistical support. Pisorisporiales is nested in an unsupported clade as sister to the Lulworthiales and Koralionastetales situated basal to the Hypocreomycetaeidae.

The placement of the Lulworthiales, including Spathulosporales, and Koralionastetales within the Sordariomycetes based on DNA data had been ambiguous (Ericsson & Wink 1997, Spatafora et al. 1998, Kohlmeyer et al. 2000, Jones et al. 2009). Their sister relationship with the Hypocreomycetidae is supported in the 3-, 4- and 6-gene phylogenies by BI and ML methods (Schoch et al. 2007, Spatafora et al. 2007, Zhang et al. 2007), whereas the maximum parsimony, weighted parsimony and ML methods of the 4-gene analysis support their placement as a basal group in the Sordariomycetes (Zhang et al. 2007). The current position in combination with the Pisorisporales may suggest a new subclass lineage in the Sordariomycetes.

Without molecular data, it is, in fact, challenging to place Pisorisporium in any of the accepted families and genera of the Sordariomycetes. Members of Pisorisporum grow on decaying deciduous wood submersed in fresh water. They are characterised by minute, immersed ascomata arranged in small groups or in rows oriented with the grain of wood, gradually erumpent by water erosion of the substrate and becoming superficial. Ascomata are upright but often grow obliquely or almost horizontally, which may be caused by the water flow. Paraphyses are fragile, arranged parallel among the asci, continuously tapering, becoming sparsely branched and intertwined above the ascal apices (Fig. 4). In ascospore and to some extent ascus morphology, Pisorisporum resembles members of Ceratosphaeria, Ceratosphaerella and Pseudohalolenticulae of the Magnaporthaceae. They are similar in overall morphology of fusiform, cylindrical to cymbiform, multiseptate, hyaline ascospores, but the three latter genera differ from Pisorisporum in a non-amyloid reaction of the apical annulus, long, sometimes flexuous protruding necks of ascomata and asexual morphs, i.e. harpophora-like and phialophora-like asexual morphs experimentally linked to Ceratosphaeria and the presumed Didymobotryum-like asexual morph of Ceratosphaeraellae (Shearer 1989, Réblová 2006, Huhndorf et al. 2008). Moreover, species of Pseudohalolenticulae and Ceratosphaeria philidica differ from Pisorisporium by cylindrical to cymbiform asci with ascospores arranged in a fascicle or rarely 4-seriately, while in Pisorisporium, Ceratosphaeria and Ceratosphaeraellae the ascospores are predominantly 2-seriate within the ascus.

The amyloid reaction of the apical annulus is not quite consistent among orders of ascomycetes; in the Sordariomycetes it occurs predominantly in members of the Xylariales, i.e. Amphisphaeriaceae, Diatrypaceae and Xylariaeaceae. The positive blue to dark reaction of iodine solutions, i.e. Lugol and Melzer’s reagents, due to the presence of starch-like polysaccharides in fungal microscopic structures is generally termed amyloid or euamyloid. The apical annulus of both species of Pisorisporum can be termed amyloid; it turns blue in Melzer’s reagent and in Lugol’s solution irrespective of whether a pre-treatment with KOH was applied. Regarding the amyloidity of the ascus, apical structures and chemical reactions with other dyes like Congo red, toluidine blue or blue ink, we noticed a difference between Pisorisporium on one hand and members of the Xylariaeaceae and other taxa on the other. Only in Pisorisporum the apical annulus is readily stained by these chemicals (Fig. 4). However, such coloration, commonly encountered in many sordariaceous genera with chitinoid (non-amyloid) apical annulus, does not occur in genera with a known amyloid apical annulus. Our observation may imply that the apical annulus of Pisorisporum is composed of other components than commonly encountered in members of the Xylariales. Clarification of the chemical compounds responsible for this discrepancy is beyond the scope of the present paper. However, the fact itself is interesting and worth being reported.

Two genera of the Amphisphaeriaceae, Crossoascus and Iodosphaeria, can be compared with Pisorisporium based on morphology of ascospores, asci and the amyloidity of the apical annulus (Samuels et al. 1987, Barrasa et al. 1993). Members of Crossoascus differ from Pisorisporum by the flat apical annulus and fusiform, multiseptate, versicolorous ascospores with brown middle cells and hyaline end-cells, sometimes with hyaline cap-like appendages (Barr 1993, Barrasa et al. 1993, Catania & Romero 2012). Iodosphaeria can be distinguished from Pisorisporum by non-papillate ascomata associated with a repent, spreading network of brown hyphae, with a flat top from which radiate numerous, flexuous, unbranched hairs, asci with a flat apical annulus and subballantioid, rarely ellipsoid, non-septate ascospores and asexual morphs belonging to Ceratosporum and Selenospora (Samuels et al. 1987, Barr 1993, Hisieh et al. 1997, Catania & Romero 2012).

Iodosphaeria aquatica is the only species that does not conform to the description of that genus; it resembles Pisorisporum with regard to the aquatic habitat, glabrous ascomata and septate, fusiform ascospores arranged 2–3-seriately within the ascus (Hyde 1995). Iodosphaeria aquatica differs from Pisorisporum by ascomata that are immersed beneath a blackened clypeus, the ascomatal wall, which is composed of thin-walled, brown angular cells and by 1-septate ascospores that have mucilaginous appendages at each pole. Molecular analysis of partial nuc28S rDNA sequences of I. aquatica revealed that the fungus is unrelated to the Sordariomycetes and it is preliminarily placed in the Dothideomycetes among genera with ascolocular development of the ascomata (strain HKUCC 166, nuc28S GenBank accession: AF452044, Jeewon et al. 2003). However, no information is available about whether this is a sequence obtained from the ex-type strain or the DNA was isolated from different material of I. aquatica.

Based on morphological characters and habitat, it is difficult to find similarities among members of the Koralionastetales, Lulworthiales and Pisorisporales. Lulworthiales was established by Kohlmeyer et al. (2000), when it was discovered that the Halosphaeriales are polyphyletic comprising two distinct evolutionary lineages of marine fungi with terrestrial ancestors (Spatafora et al. 1998). Members of the Lulworthiales are predominantly marine ascomycetes, but some also inhabit niches in estuarine environments. They include saprobes on driftwood and intertidal wood, sea grasses, saltmarsh plants, coral rocks or parasites of uncalcified Rhodophyta or Phaeophyta. They are characterised by filamentous, one- to multiseptate ascospores with apical mucus-containing chambers or gelatinous sheath (except species of Lindra), early deliquescing asci and the absence of a hamathecium in mature ascomata, while young ascomata contain pseudoparenchyma (Kohlmeyer 1997, Naka­giri & Tadayoshi 1997, Campbell et al. 2005, Koch et al. 2007).
Asexual morphs of *Lulworthiales* belong to nine hyphomycetous dematiaceous genera with usually coiled conidia; they were assigned to the order based on molecular DNA data or the link between sexual and asexual morph was proven experimentally (Nakagiri & Tubaki 1983, Nakagiri 1984, Campbell et al. 2005, Jones et al. 2008, 2009, Abdel-Wahab et al. 2010).

The *Koralionastetales* were separated from the *Lulworthiales* by Campbell et al. (2008) to include fungi occurring obligatorily in marine habitats. They are characterised by a centrum containing paraphyses and periphyses and ellipsoid, fusiform to filiform ascospores without any apical structures and with typical formation of antheridia on germ tubes. Members of *Koralionastes* live on coral rocks, while *Pontogeneia* is a parasite of marine *Phaeophyta*. Their asexual morphs are unknown.

The type species of *Achroceratosphaeria* (*A. potamia*) and *Pisorisporium* (*P. cymbiforme*), originate in the same territory in the Ariège department in Midi-Pyrénées less than 1 km apart. The La Maille locality, where *P. cymbiforme* was repeatedly collected in 2013 and 2014, is a deep valley bordering steep slopes at the foot of the Arize massif, with a thick deciduous forest with high humidity. Trees and shrubs grow densely also along the shadowy La Maille brook. Decaying branches, twigs and larger logs fall regularly in the water flow, which provide a rich substrate in this locality. The La Maille brook may dry up at the end of season, leaving the otherwise submerged wood and driftwood exposed to air for several weeks or even months. Not far from here, in the Le Baup stream, of which the La Maille brook is a tributary, was collected *A. potamia*, another member of the *Pisorisporiales*.

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