Didymocyrtis microxanthoriae (*Phaeosphaeriaceae*, *Dothideomycetes*), a new lichenicolous fungus from France

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Abstract: The new asexual *Didymocyrtis microxanthoriae*, lichenicolous on *Xanthoria parietina*, is described from France. Morphological characters and DNA sequence data (ITS, nuLSU) place the new fungus in the genus *Didymocyrtis*. *Didymocyrtis microxanthoriae* is only distantly related to the other known species of the genus inhabiting *X. parietina*, and is characterized by the particularly small, subspherical to shortly ellipsoid, 1(–2)-guttulate conidia, 3.0–3.7 × 2.6–2.9 µm that distinguish it from all other known species of the genus.

Key words: asexual fungi, lichen-inhabiting fungi, *Phoma*, *Xanthoria*

1. Introduction

In 2020, the second author collected specimens of *Xanthoria parietina* infected by an unknown *Phoma*-like asexual fungus with characters that did not fit any of the so-far known lichenicolous species. Such *Phoma*-like anamorphs may belong to various lichenicolous genera, including *Didymocyrtis* (*Phaeosphaeriaceae*, *Dothideomycetes*), which was the most likely genus for this fungus based on its morphology. Species of *Didymocyrtis* in the asexual stage are characterized by unilocular, ostiolate, dark brown pycnidia with a pseudoparenchymatous wall, the lack of conidiophores, hyaline ampulliform conidiogenous cells that line the pycnidial cavity, an enteroblastic, phialidic conidiogenesis, and hyaline, usually aseptate, smooth-walled conidia with more or less rounded apices (e.g., Ertz et al. 2015, Das et al. 2021). To confirm the systematic position of the unknown fungus, we analysed its morphology as well as fast- and slow-evolving ribosomal gene markers and, based on the results, describe it here as a new species.

2. Material and methods

2.1. Microscopy

The material examined is deposited in BR and TUF, and in the private collection of A. Delhoume. Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5. Macroscopic photographs were done using a Canon 6D camera with a Nikon BD Plan 10× microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. For microscopical examination, squash preparations and vertical sections were examined in water and ammoniacal Congo red. Microscopic photographs were prepared using a Leica DMLB microscope with DIC optics and a Leica EC3 camera, and Helicon Focus for increasing the depth of field. Measure-

ments done in water mounts, followed by the number n, are given as (min.–)X-σ_x–X+σ_x(–max.); the ratio length/breadth of conidia is indicated as L/B and given in the same way.

2.2. DNA extraction, PCR amplification and DNA sequencing

DNA extraction, amplification and purification were carried out at Tartu University. Total genomic DNA was extracted from pycnidia using High Pure PCR Template Preparation Kit (Roche Applied Science®, Penzberg, Germany) following the protocol provided by the manufacturer. The extracted DNA is deposited in the DNA and Environmental Sample Collections of the Natural History Museum in Tartu University (TUE). We amplified two nuclear ribosomal loci – the internal transcribed spacer (ITS) with primer pair ITS0F / LA-W (Tedersoo et al. 2008), and the large subunit (nuLSU) DNA region with primer pair LR0R / LR7 (Vilgalys & Hester 1990). Sample preparation, polymerase chain reaction (PCR) amplification and DNA purification are described in detail in Suija et al. (2017). Both strands were Sanger sequenced at Macrogen Inc. (Amsterdam, the Netherlands). The ITS sequences were sequenced with primer pair ITS4 and ITS5 (White et al. 1990), nuLSU with CTB6 (Garbelotto et al. 1997) and LR7. CodonCode Aligner 8.0.2 (CodonCode Corporation®, Centerville, MA, USA) was used to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in GenBank using blastn algorithm (Altschul et al. 1990)

2.3. Phylogenetic analyses

We compiled DNA alignments for both gene sequences by including a representative set of *Didymocyrtis* and top-match sequences downloaded from NCBI and UNITE databases, several of which represent sequences from environmental samples. All environmental sequences were annotated as belonging to *Phaeosphaeriaceae* (*Dothideomycetes*) in the UNITE database (Kõljalg et al. 2013). The DNA sequences were aligned with the on-line version of Mafft ver. 7 (Katoh et al. 2019) using default options and corrected and trimmed manually with SeaView ver. 4.6 (Gouy et al. 2010) and AliView ver. 1.27 (Larsson 2014). Phylogenetic relationships and tree confidence were inferred using two different methodologies: Metropolis coupled Markov Chain Monte Carlo (MCMC, Bayesian) approach implemented in MrBayes ver. 3.2.1. (Ronquist et al. 2012) in CIPRES Science Gateway ver. 3.3 (Miller et al. 2010) and Maximum Likelihood (ML) implemented in IQTree (Trifinopoulos et al. 2016). We show here only the results based on the more comprehensive ITS alignment containing 53 sequences. The ITS alignment included 596 nucleotide positions of which 102 were informative (23.7%). As the next step, we calculated the best-fit nucleotide substitution model using jModeltest ver. 2.1.6. (Darriba et al. 2012). According to AIC (and AICc) criterion, the best-fit model was GTR + I + G and this was implemented in both analyses. To find branch support, ultrafast bootstrapping over 1000 bootstrap alignments was applied in ML. In Bayesian analyses, the following settings were used: two parallel simultaneous runs over 1 million generations starting with a random tree and employing four simultaneous chains; sampling after 1000 steps. The analysis was run until the average standard deviation of split frequencies across runs reached 0.01, and the average potential scale reduction factor (PSRF) for all models and parameters was 1. The first 25 % of saved data was discarded as 'burnin'; the consensus tree and posterior probabilities (PP) were calculated from the rest. The clades with posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 0.75 were regarded as significantly supported. The phylogenetic tree was visualized using FigTree ver. 1.4.2 (Rambaut 2014), and Adobe Illustrator CS3® was used for artwork.

3. Results and Discussion

Based on the blastn search (Altschul et al. 1990), no identical or similar ITS and nuLSU DNA sequences were found from either the NCBI or the UNITE nucleotide data-

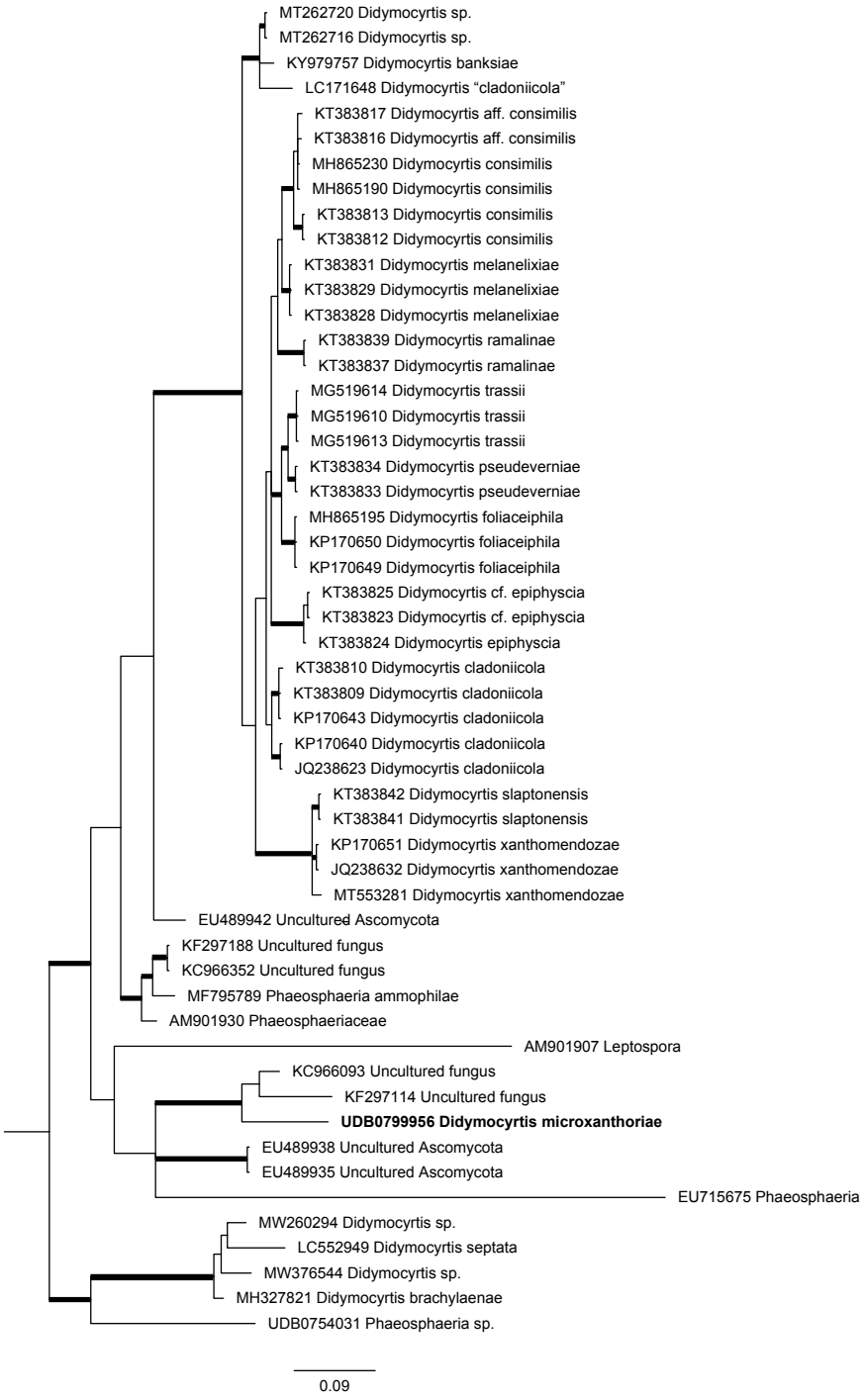


Fig. 1. The ITS-based Bayesian phylogeny showing the position of *Didymocyrtis microxanthoriae* (in bold) within *Phaeosphaeriaceae* (*Pleosporales*, *Dothideomycetes*). The supported branches with posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 75 % are marked with a thicker line. NCBI and UNITE accession numbers (UDBxxx) are given at the branches. The obviously incorrect annotation of the sequence LC171648 is marked in quotation marks.

bases. However, the closest match for both sequences belonged to *Phaeosphaeriaceae* (*Pleosporales*, *Dothideomycetes*). The closest match for ITS was KC966093 (uncultured fungus, percentage of identity 96.11%, 31 variable positions) and for nuLSU KF636781 (*Phaeosphaeriaceae* sp., 99.54%, six variable positions). Sequence LC171648 is obviously incorrectly annotated as *D. cladoniicola* in the NCBI database and is therefore marked using quotation marks in Fig. 1. In the ITS tree, the new sequence clustered together with two sequences of uncultured *Ascomycota* inherited from soil samples (BP=96, PP=1). The analysis also showed that the specimen is only distantly related to the other known *Didymocyrtis* species inhabiting *Xanthoria parietina* (*D. epiphyscia* Ertz & Diederich, *D. slaptonensis* (D. Hawksw.) Hafellner & Ertz). The specimen is morpho-anatomically distinct from the abovementioned species allowing us to describe it as a new species.

Didymocyrtis microxanthoriae Poumarat, Delhoume, Diederich & Suija, sp. nov. (Fig. 2)

Diagnosis: Lichenicolous asexual fungus growing on *Xanthoria parietina*, distinguished from all other known *Didymocyrtis* species by the particularly small, subspherical to shortly ellipsoid, 1(–2)-guttulate conidia, mainly 3.0–3.7 × 2.6–2.9 µm.

Etymology: From *micro* (small) and *Xanthoria* (host genus), denoting a species with very small conidia growing on *Xanthoria*.

Typus: France, Grand Est, Marne, Giffaumont-Champaubert, lac du Der, port de plaisance de Giffaumont-Champaubert, 48.55°N, 4.77°E, 140 m, on ascomata and thallus of corticolous *Xanthoria parietina*, 4 Jan. 2020, A. Delhoume s. n. (TUF 091958 – holotypus; BR, herb. Delhoume – isotypi).

MycoBank: MB841458

DNA barcode/reference sequence (rDNA ITS): UDB0799956; nuLSU: UDB0818902

Ascomata unknown. *Conidiomata* pycnidial, immersed in the host apothecia, more rarely in the host thallus, first only the ostiolar region, later the upper third visible, black, subspherical to ovoid or obpyriform, ostiolate, 70–130 µm diam.; vegetative mycelium immersed, partly attached to the lower pycnidial wall, pale brown, smooth-walled, constricted at the septa, cells 2.5–5 µm thick and 5–15 µm long; pycnidial wall brown, pseudoparenchymatous, basally 20–25 µm thick, laterally 12–18 µm thick, composed of 3–5 layers of tangentially flattened polyhedral cells, 7–12 µm diam., outer cells dark brown, inner cells pale to medium brown. *Conidiophores* absent. *Conidiogenous cells* lining the inner wall of the pycnidial cavity, subglobose to broadly ampulliform, not proliferating, hyaline, smooth-walled, (4.8–)7.5–9.4(–10.0) µm tall, (4.8–)5.7–8.3(–9.0) µm diam. (n=10), conidiogenesis enteroblastic. *Conidia* abundantly produced, arising singly, subspherical to shortly ellipsoid, apically rounded, basally rounded or indistinctly truncate, hyaline, simple, smooth-walled, 1(–2)-guttulate, (2.5–)3.0–3.7(–4.0) × (2.4–)2.6–2.9(–3.0) µm, L/B (1.0–)1.1–1.4(–1.6) (n=100).

Distribution, ecology and host. Known only from the type locality in France, on the apothecia and more rarely the thallus of corticolous *Xanthoria parietina*. Infected host thalli are slightly bleached, which facilitated the detection of them in the field.

Notes. The new species is characterized by the particularly small, subspherical to shortly ellipsoid conidia. Amongst the *Didymocyrtis* species keyed out by Ertz et al. (2015), the closest taxon is *D. melanelixiae* (Brackel) Diederich, R. C. Harris & Etayo, a common and widespread species growing on *Parmeliaceae* hosts, with conidia mainly 3.8–5.1 × 3.2–3.8 µm. *Didymocyrtis consimilis* Vain. (in the hymenium of *Caloplaca* species) and *D. epiphyscia* Ertz & Diederich s. str. (on the thallus of *Physcia aipolia*) have similarly shaped, but distinctly larger conidia, the former 4.5–6.5 × 2.5–4.5 µm, and the latter 4.6–6.1 × 3.5–4.2 µm. Additional species described or combined in *Didymocyrtis* after 2015 differ either by the larger or narrower conidia: *D. banksiae* Crous & P. A. Barber (Crous et al. 2017), *D. brachylaenae* Crous (Crous et al. 2018), *D. grumantiana* (Zhurb. & Diederich) Zhurb. & Diederich (Diederich et al. 2018), *D. septata* K. Das, S. Y. Lee & H. Y Jung (Das et al. 2021), *D. peltigerae* (Fuckel) Hafellner (Hafellner 2019), *D. rhizoplacae* Y. Joshi & K. Bisht (Joshi et al. 2018), *D. trassii* Suija, Darmostuk & Khodos. (Khodosovtsev et al.

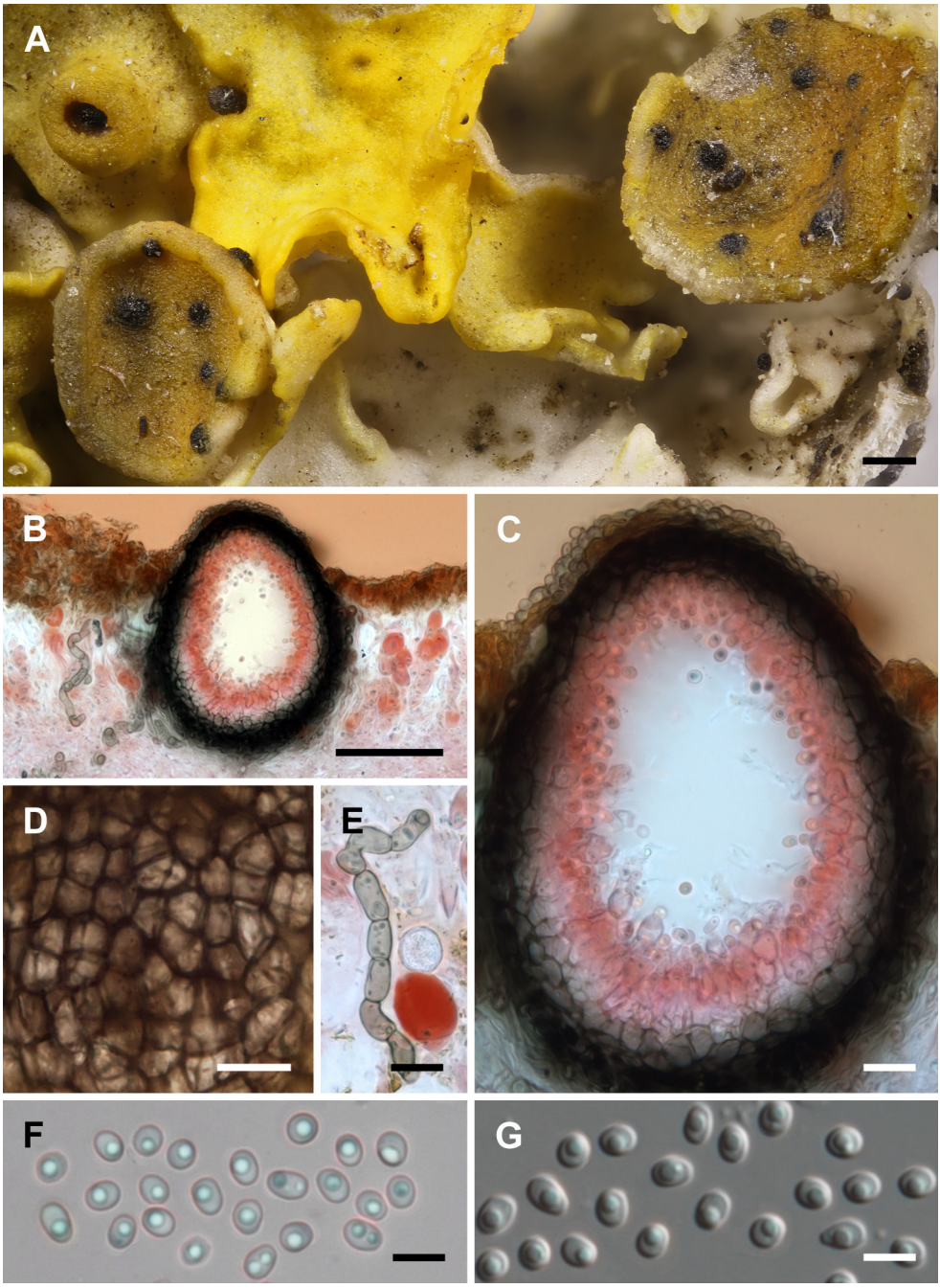


Fig. 2. *Didymocyrtis microxanthoriae*, isotype (BR). A, Pycnidia parasitizing the apothecia of *Xanthoria parietina*. B, Section through host hymenium with one pycnidium; note the brownish vegetative hyphae, some fixed to the pycnidial wall on the left. C, Section through pycnidium, showing wall layer, conidiogenous layer, conidiogenous cells (best visible on the right) and conidia. D, Surface view of pycnidial wall. E, Vegetative hypha. F, Conidia. G, Conidia, using DIC optics. B, C and E stained in ammoniacal Congo red, D, F and G in water. Scale bars: A = 200 µm, B = 50 µm, C–E = 10 µm, F–G = 5 µm.

2018); or by the unknown asexual stage: *D. azorica* Etayo & Pino-Bodas (Etayo & Pino-Bodas 2021), *D. canariensis* van den Boom & Etayo (van den Boom & Clerc 2017), *D. graphidacearum* van den Boom & Ertz (van den Boom et al. 2017), *D. micropunctum* Etayo (Etayo 2017).

This is the third species of *Didymocyrtis* known to grow on *Xanthoria parietina* (Diederich et al. 2018). Populations on this host attributed to *Didymocyrtis epiphyscia* s. lat. (differing from s. str. by the narrower conidia) have much longer, biguttulate or rarely multiguttulate conidia, 4.6–6.4 × 2.5–3.1 µm. *D. slaptoniense* (D. Hawksw.) Hafellner & Ertz has been described based on sexual populations; co-occurring pycnidia have longer, biguttulate conidia, 6–8 × 2.5–3.5 µm (Ertz et al. 2015).

In the current circumscription, the genus *Didymocyrtis* includes both lichenicolous fungi (Ertz et al. 2015) and non-lichenicolous species occurring on leaves (Crous et al. 2017, Crous et al. 2018) and in soil (Das et al. 2021). However, it is well possible that the generic concept should be extended even further, as suggested by the highly similar environmental sequences (KC966093, KF297114) clustering together with the new species (Fig. 1).

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