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Flammeascoma ryskae sp. nov. (Pleosporales, Anteagloniaceae) as a new pigment—producing species of European floodplain forest habitats

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Abstract

The relatively recently described family *Anteagloniaceae* has four genera—*Anteaglonium, Flammeascoma*, *Purpureofaciens* and a newly defined monotypic genus *Neolophiotrema*. The newly described species ***Flammeascoma ryskae sp. nov***. is the first species of this genus found in Europe so far. It was found during an ecological study of fungal diversity in floodplain forests at selected locations in the Czech Republic. As one of the four species of this family so far known, this new species is characterized by the production of a reddish pigment in the agar medium. In this study, in addition to the description of the new species, the current knowledge about the family *Anteagloniacea*e is summarized, including a discussion of their secondary metabolism.

Key words: *Anteaglonium*, anteaglonialides, didymosporous, fungal diversity, fungal pigments, hysterothecia, lignicolous, *Neolophiotrema*, phylogeny, *Purpureofaciens*, taxonomy

Introduction

The Family *Anteagloniaceae* was descripted by Hyde *et al.* (2013) on the base collection of *Anteaglonium* (=*Hysterium*) *abbreviatum*. In this family are recently four genera—*Anteaglonium* Mugambi & Huhndorf, *Flammeascoma* Phook. & K.D. Hyde, *Neolophiotrema* G.C. Ren & K.D. Hyde and *Purpureofaciens* W. Dong, H. Zhang & K.D. Hyde. The last genus, *Purpureofaciens*, is phylogenetically related to *Flammeascoma*, but by Dong *et al.* (2020) clusters with *Flammeascoma* species with low bootstrap support and morphologically separates them as distinct genera. The family belongs to the very diversified order Pleosporales, where it is phylogenetically situated in the evolutionarily most original branches together with the families *Lophiotremataceae, Lophiostomataceae, Tetraplosphaeriaceae*, etc. (Hyde *et al.* 2013; Jaklitsch, Fournier & Voglmayr 2018; Jayasiri *et al.* 2016).

The genus *Anteaglonium* is the type and simultaneously the most comprehensive genus of the family *Anteagloniaceae* (Hyde *et al*. 2013). Ecologically, species from this genus are associated with decorticated wood (Jayasiri *et al*. 2016). For this genus are characterized hysterothecial ascomata with hyaline didymospores and look quite similar to hysterothecial species of the genus *Glonium* (Boehm *et al.* 2009; Mugambi & Huhndorf 2009a; etc.). However, this is not a universal characteristic of the genus, as evidenced by the species *Anteaglonium rubescens* described from Greece, which has dark brown, 2-cell ascospores, later disarticulating on the distal and proximal part (Jaklitsch, Fournier & Voglmayr 2018).

The genera *Flammeascoma* and *Purpureofaciens* are typical mainly for freshwater habitats, where they grow on decaying wood (Ariyawansa *et al.* 2015; Dong *et al.* 2020). The genus *Purpureofaciens* is characterized by the production of reddish pigmentation at the apex and, unlike the genus *Flammeascoma*, cylindrical asci with olivaceous, ellipsoidal ascospores (Dong *et al.* 2020).

The genus *Flammeascoma* is well-defined based on the presence of orange-brown pigments in the ascostroma and hyaline, didymosporous to phragmosporous ascospores (Liu *et al.* 2015). The genus *Flammeascoma* contains two species—*Flammeascoma bambusae* Phook. & K.D. Hyde, which was described from Thailand from the dead stems of bamboo (Liu *et al.* 2015) and *Flammeascoma lignicola*, which was also described from Thailand, but from dead wood of *Pinus* (Ariyawansa *et al.* 2015).

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The last monotypic genus *Neolophiotrema* was described in 2021 from decaying wood in China (Ren *et al.* 2021). It differs from the previous genera based on coriaceous ascomata and hamathecium structure, which has narrow cellular pseudoparaphyses (similar to genus *Flammeascoma* which has trabeculate pseudoparaphyses) (Ren *et al.* 2021).

A very typical character of some species of the family *Anteagloniaceae* is the production of secondary metabolites that color the surrounding substrate more or less purple to red (Dong *et al.* 2020; Jaklitsch, Fournier & Voglmayr 2018; Ren *et al.* 2021). These metabolites are also produced on the agar medium *in vitro*, which is also indicated by their coloration (Dong *et al*. 2020; Jaklitsch, Fournier & Voglmayr 2018; Ren *et al.* 2021). It was indicated typically in case of *Anteaglonium rubescens* (Jaklitsch, Fournier & Voglmayr 2018), *Purpureofaciens aquaticus* (Dong *et al.* 2020) and *Neolophiotrema xiaokongense* (Ren *et al.* 2021). The biochemical nature of these metabolites has so far been analyzed in the case of one unspecified strain of *Anteaglonium* sp. FLO768, which was isolated from *Selaginella arenicola* (Xu *et al.* 2015). In this case, 22 new metabolites from a group of pigments were detected, which were named anteaglonialides A–F (Xu *et al.* 2015). All isolated substances were tested for their potential cytotoxic activity against the human Ewing's sarcoma cell line CHP-100 as part of this study. Only Anteaglonialide F showed cytotoxic activity (Xu *et al.* 2015).

Materials and methods

Sample collection and examination

The material was collected during methodical research on the diversity of floodplain forest fungi carried out in selected small protected areas in the Czech Republic. As part of this project, the diversity of all fungi in association with specific forest biotopes was researched. The material of this species was collected in a remnant fragment of a floodplain forest classified as *a Tilio-Carpinetum* community in the Bařiny Nature Reserve near the meanders of the Odra River. Collecting material was focused on dead decorticated wood of deciduous trees, which contained live sexual morphs.

The morphological study of microscopic characters, including the taking of microphotographs and macrophotographs, was developed according to Mlčoch & Matušinský (2024): The Breser Trino microscope was used for microscopic studies, and microphotographs were made using a USB2.0 YW500 Digital camera (SRATE). Microscopic structures were measured with the PIXIMÉTRE software v5.10R (Henriot 2020) in sterile distilled water. As a standard, it was measured in 15–30 repetitions according to the number of structures that were available. The mathematical parameter Q, indicating the ratio of length and width (QAV for the arithmetic mean of the values), was also used to describe the shape of the spores (Mlčoch & Matušinský 2024). Using sterile dissection, ascomata with asci and spores were removed from the slide after evaluation of microscopic characters and subsequently were moved on sterile PDA, similarly to a previous study (Mlčoch & Matušinský 2024). Subsequently, cultures were incubated at laboratory temperature for 7–10 days. Microscopic study of morphological characteristics of asexual morphs was on the basis of the sporulation *in vitro*. Macro photographs of the ascomata on the host surface were taken with a Panasonic Lumix DMC-FZ300 camera with Raynox DRC-250 conversion lens. Subsequently, the method of focus stacking was used with the help of CombineZP (Hadley 2012) for the digital editing of photos. The result was edited by the Zoner Photo Studio 17 software (ZONER, Brno, the Czech Republic).

Cultivation, DNA extraction, PCR amplification and DNA sequencing

Cultures were prepared and maintained as described previously (Mlčoch & Matušinský 2024): Fungal isolates were grown on Petri dishes containing potato dextrose agar (PDA) with ampicillin (50 $mg.l^{-1}$; Carl Roth GmbH + Co. KG, Germany) and covered with cellophane discs. Fungal mycelia were harvested from the Petri dishes, ground to a fine powder in a mortar using liquid nitrogen, homogenized, and total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA concentration was measured using a Qubit (Thermo Fisher Scientific, Waltham, USA), and DNA was diluted to a concentration of 10 ng μl^{-1}. PCR reactions were performed in a 20 ml volume containing 10 ng of fungal DNA. Phylogenetic analyses were based on partial sequences of the *ITS* region, *large subunit* (*LSU*) of the rDNA and *translation elongation factor 1-alpha* (*TEF-1 alpha*). The reaction mixture consisted of 0.2 mM dNTP, 1 U Taq polymerase (Thermo Fisher Scientific, Waltham, USA), and each primer of the ITS1/ITS4 (White *et al.* 1990), LROR and LR5 (Vilgalys & Hester 1990) and EF1-728F/EF1-2218R (Carbone

& Kohn 1999). The reaction buffer consisted of 75 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, and 2.5 mM $MgCl_2$ (Thermo Fisher Scientific, Waltham, USA). PCR was performed under the following conditions: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation (94 °C, 1 min), annealing (57 °C, 1 min), and final extension at 72 °C for 5 min. PCR fragments were purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) to remove primers, nucleotides, enzymes, mineral oil, salts, and other impurities from the sample. The PCR products were sequenced by Biocev (Czech Republic) using the 3500 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA).

Phylogenetic analyses

Bioinformatic processing of sequences was running standardly as in the previous study (Mlčoch & Matušinský 2024) in the GENEIOUS 10.2.6 software, AliView v1.28 (Larsson 2014), library Ape (Paradis & Schliep 2019) in R Studio software (RStudio team 2020), IQ-Tree v2.2.0 (Trifinopoulos *et al.* 2016) and FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). The obtained sequences of *ITS, LSU*, and *TEF-1 alpha* genes were initially edited in the GENEIOUS software and comparative analyses were further performed in the online algorithm of BLASTn (https://blast.ncbi.nlm.nih.gov). The nucleotide BLASTn search was used to evaluate the similarity and percentage identity of sequences that were situated in the NCBI database. The newly generated sequences were subsequently incorporated into the prepared dataset, including reference sequences obtained from relevant published data (see Table 1). Sequences were first loaded from the NCBI database in R Studio and, then alignments were created for individual genes using the MAFFT v7 algorithm (Katoh & Standley 2013) in the R program. The final matrix was put together using the TextPad and AliView software. For itself, phylogenetic analyses were used for the Maximum Likelihood algorithm with the utilization of the Sequences, which were first loaded from the NCBI database in the R Studio, and then alignments were created for individual genes using the MAFT v7 algorithm (Katoh & Standley 2013) in the R program. The final matrix was put together using the TextPad and AliView software. For itself, phylogenetic analyses were used for the Maximum Likelihood algorithm with the utilization of the HKY+F+I+G4 model and ML heuristic method of Nearest Neighbour Interchange. A test of phylogeny was performed using the ultrafast bootstrap method with 10,000 replications. The phylogram was prepared in IQ-Tree (online version) and final adjustments were performed in FigTree software.

TABLE 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences in this study are indicated in bold.

Taxon	*ITS*	*LSU*	*TEF1-A*	Strain	Citation
Angustimassarina quercicola	KP899133.1	KP888638.1	-	MFLUCC 14-0506	-
Anteaglonium abbreviatum	-	GQ221881.1	GQ221915	GKM219N	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium abbreviatum	-	GQ221877.1	GQ221924	ANM925a	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium brasiliense	KF906410.1	-	-	HUEFS:192250	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium latirostrum	-	GQ221874.1	GQ221937	GKM1119	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium globosum	-	GQ221879.1	GQ221925	ANM925.2	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium globosum	-	GQ221911.2	GQ221919	SMH5283	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium gordoniae	MK347761.1	-	-	MFLUCC 17-2431	-
Anteaglonium gordoniae	OK335788.1	-	-	CD7	-
Anteaglonium gordoniae	NR_163338.1	MK347977.1	MK360042.1	C332	-
Anteaglonium parvulum	MN582759.1	-	KU922919	C 009	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium parvulum	MN608545.1	MN577414.1	-	MFLUCC:11-0511	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018

.....continued on the next page

TABLE 1 (Continued)

Taxon	*ITS*	*LSU*	*TEF1-A*	Strain	Citation
Anteaglonium parvulum	MN608544.1	-	-	MFLUCC:11-0380	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium parvulum	MN608543.1	MN577412.1	-	MFLUCC:11-0374	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium rubescens	MG912910.1	-	MG912914	OR1	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Anteaglonium rubescens	MG912909	-	MG912913	OR	Fournier J. & Voglmayr H. 2018
Anteaglonium rubescens	NR_164489.1	-	-	CBS 143911	-
Antealophiotrema brunneosporum	MH863275.1	MH874799.1	-	CBS:123095	-
Flammeascoma bambusae	NR_132915.1	-	-	MFLUCC 10-0551	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Flammeascoma bambusae	KP744440.1	KP744485.1	-	MFLU 11-0143	Ren G. *et al.* 2021
Flammeascoma lignicola	KT324582.1	KT324583.1	KT324585	MFLUCC 10-0128b	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Flammeascoma ryskae	**PQ859670**	**PQ859671.1**	**PV018829**	**TIL1**	**This study**
Flammeascoma ryskae	**PQ859672**	**PQ859673.1**	**PV018830**	**TIL2**	**This study**
Hermatomyces clematidis	NR_170802.1	MT214556.1	-	MFLUCC 17-2085	-
Hermatomyces iriomotens	LC194483.1	LC194367.1	LC194394	KT2016-1	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Atrocalyx lignicola	MH863205.1	MH874736.1	-	CBS:122364	-
Lophiostoma winteri	JN942969.1	-	LC001763.1	KT 740	-
Lophiotrema eburnoides	NR_138014.1	-	LC194403	KT 1424-1	Ren G. *et al.* 2021
Lophiotrema nucula	OL739258.1	-	-	CBS 627.86	-
Lophiotrema nucula	MW759242.1	MW750373.1	-	MAL47	-
Mytilinidion resinicola	NR_160068.1	MH867038.	FJ161101	CBS 304.34	-
Mytilinidion scolecosporum	NR_160069.1	MH867039.1	FJ161102	CBS 305.34	Ren G. *et al.* 2021
Neolophiotrema xiaokongense	MT957893.1	MT957892	MT968871	KUMCC 20-0173	Ren G. *et al.* 2021
Purpureofaciens aquatica	NR_171968.1	MN913717.1	MT954372.1	MFLUCC 18-1241	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Sigarispora arundinis	AJ496633.1	-	-	CBS 621.86	-
Sigarispora caulium	LC001724.1	-	LC001740.1	KT603	-
Tetraploa sasicola	AB524807.1	AB524631.1	-	KT 563	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018
Triplosphaeria maxima	AB524812.1	AB524637.1	-	KT 870	Jaklitsch W. M., Fournier J. & Voglmayr H. 2018

Results

Phylogeny

Phylogenetic analyses performed in IQ Tree software combined 38 concatenated sequences of ITS, LSU and tef1-alpha regions with 697 columns. Alignment contained 451 distinct patterns, 335 parsimony-informative sites, 167 singleton sites and 1195 constant sites. For phylogram visualization, an HKY+F+I+G4 model was used. The root of the tree was manually set with Mytilinidiales as an outgroup (Fig. 1).

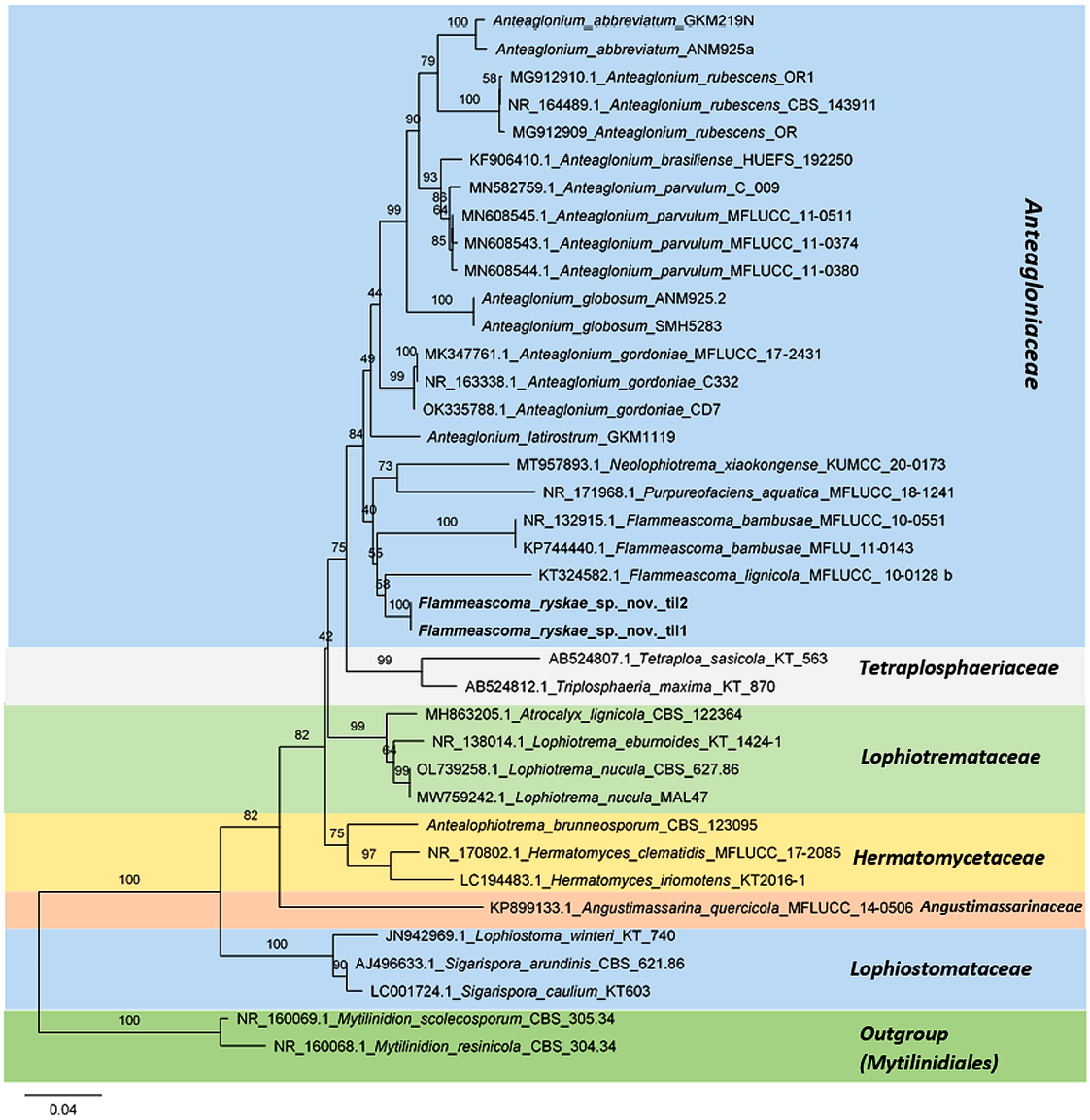

FIGURE 1. Phylogenetic tree constructed based on analyses of a combined *ITS, LSU* and *TEF1* dataset. Bootstrap support values for maximum likelihood (ML) with 10,000 replications for IQtree UltraFast algorithm equal to or higher than 80 %, and Bayesian posterior probabilities (PP) equal to or greater than 0.75 are indicated above the nodes. The new isolate from this study is shown in bold italic. The tree is rooted with *Mytilinidion scolecosporum* and *M. resinicola*.

Taxonomy

Anteagloniaceae

Flammeascoma ryskae Mlčoch ***sp. nov.***—fig. 2, 3.
Holotype. BRNM 847516
MycoBank no. 857366

Etymology. The species name is named after a famous Czech phytopathologist, Anna Ryšková.

Sexual morph. *Ascoma* subglobose to globose, in numerous groups, partially nested in the wood to superficial, 210–290 µm in diameter. *Ostioles* central, very broad to conical, irregular. *Ascomal wall* composed of several layers of pseudoparenchymatous cells, composed of dark brown polygonate cells of *textura angularis* to *textura epidermoidea*. (3.5) 4–4.5 (6) × 3–3.5 (4.5) µm, N=10. *Hamathecium* is composed of numerous, narrow, cellular hyaline pseudoparaphyses. *Asci* bitunicate, 8-spored, cylindrical to cylindric-clavate, short pedicellate, 63.7–76.6 (80)

× 6.2–7.4 (7.5) µm, N=10. *Ascospores* 1 to 2-seriate in asci, fusiform, subhyaline to brownish, becoming brown, when released from asci and germinating, 1 to 3-septate, second cells enlarged, without gelatinous sheath, appendages and ornamentation, with several globose lipid drops, (15) 16–20 (20.6) × (3) 3.5–4.7 (5) µm, Q=3.9–5.4, Q_{AV}=4.6, N=23. **Asexual morph.** Undetermined.

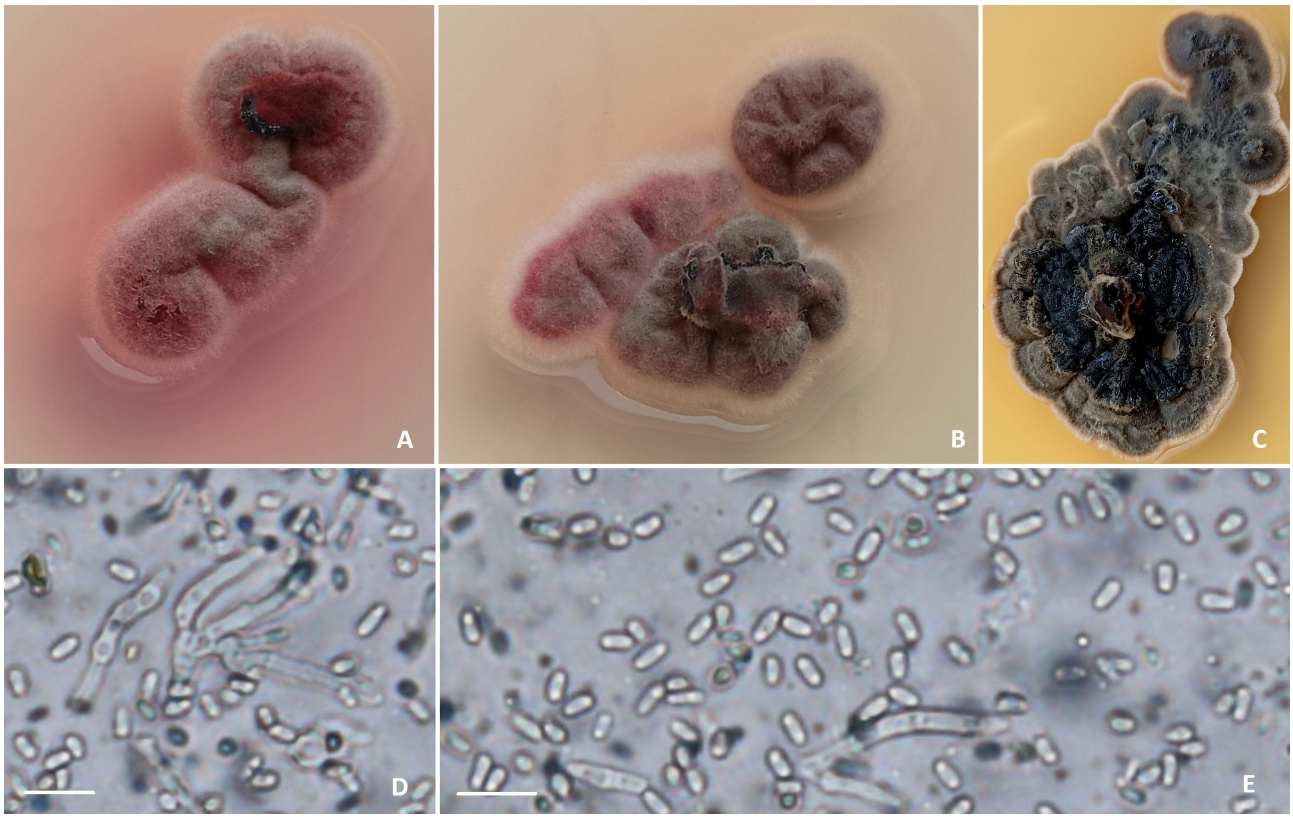

FIGURE 2. *Flammeascoma ryskae sp. nov*. in vitro on PDA at 25 °C. A, B—culture after 2 weeks; C—culture after 1 month; D, E—conidiogenous cells and conidia. Scale: 10 µm.

Culture characters. Growth is very slow and variable, colony only 4–5 mm in diameter. After 2 weeks on PDA at 25 °C and 12–16 mm, after 1 month on PDA at 25 °C. Colony irregular, primarily whitish to greyish on the surface, with rosy to reddish zones at the edge, often dyes of medium, later the color disappears. The oldest colony after one month on PDA irregular with convex to umbonate elevation, margin undulate, greyish at the edge, dark grey to black at the centre, concentrically colored. *Pycnides* in culture globose, to 350 µm in diam., with a very unremarkable ostiole. *Pycnidial wall* composed of several layers of dark brown pseudoparenchymatous cells, 5–7.5 × 5–5.5 µm, N=10. Interior lined with palisade of hyaline to pinkish lageniform to cylindric conidiogenous cells, 17–24 × 3 µm, N=10. *Conidia* subglobose to globose, often ellipsoid to oblong, hyaline, 1-celled, with rounded ends, smooth, with 2 lipid oils, (3) 3.7–4.5 (5) × (1.5) 2–2.5 (2.7) µm, Q=1.8–2.5, Q_{AV}=2.1, N=30.

Habitat. On dead wood of deciduous trees, known from the dead wood of *Tilia cf. cordata*. Found in the floodplain forest of the *Tilio-Carpinetum* association in the lowland grade.

Distribution. Known from the Czech Republic.

Material examined. Czechia, Moravian Gate, Suchdol nad Odrou, Bařiny nature reserve, floodplain forest, in *Tilio-Carpinetum*, 260 m a.s.l., on the dead wood of *Tilia cf. cordata,* 16. September 2021, Col. P. Mlčoch, 847516 (BRNM, **holotype**), GPS: 49.6324322N, 17.9576669E.

Notes. Phylogenetic analyses of the sequence data of the *ITS* and *LSU* regions indicated that this collection of new taxa belongs in *Anteagloniaceae*. This taxon is closely related to the genera *Anteaglonium* and *Flammeascoma*. Morphologically, it is related to the *Flammeascoma*, because ascoma it isn't hysterothecial and ascospores it isn't small, ellipsoid to ellipsoid fusoid, didymosporous, as in the case of the genus *Anteaglonium* (Jaklitsch, Fournier & Voglmayr 2018; Jayasiri *et al.* 2016). Fusoid face and overall appearance of young spores rather resemble *Flammeascoma bambusae* (Liu *et al.* 2015) and *Flammeascoma lignicola* (Ariyawansa *et al.* 2015). *Flammeascoma ryskae* is different from these two species on the basis of other dimensions of ascospores (less than 25 µm in length), the presence of 3-septate spore cells and brownish pigment in the old spores. This taxon also colors the cultivation medium red, as phylogenetically distant *Anteaglonium rubescens* (Jaklitsch, Fournier & Voglmayr 2018).

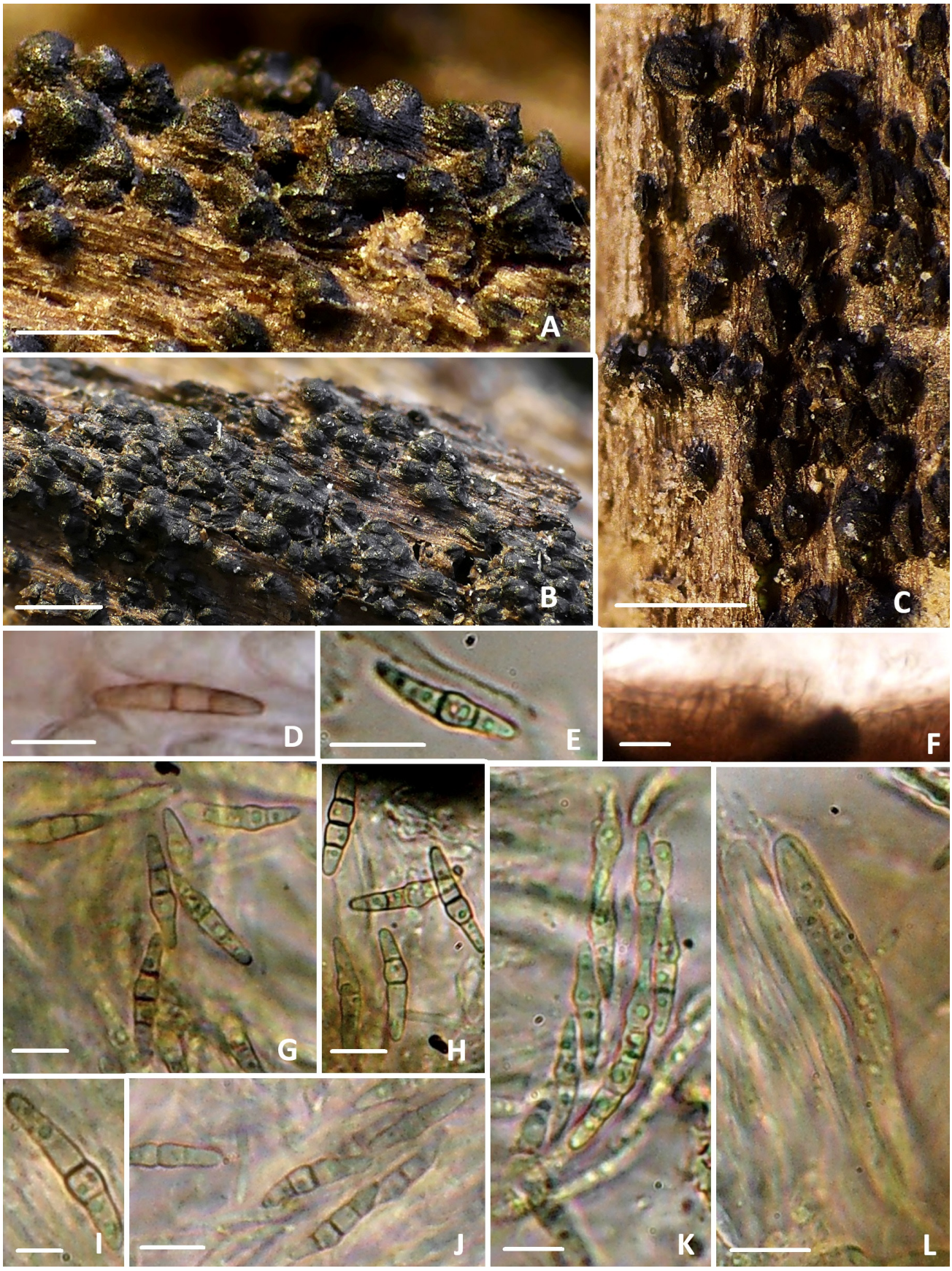

FIGURE 3. *Flammeascoma ryskae sp. nov*. A–C—ascoma on dead wood; F—ascomal wall; L—asci; D–E, G–K—ascospores. Scale: A—250 µm; B, C—500 µm; F—15 µm; I—5 µm; D, E, G, H, J, K, L—10 µm.

TABLE 2. Synoptic table of known species of the family *Anteagloniaceae*.

Species	Ascospores (µm)	Number of septum	**Color**	**Mucilaginous sheet**	**Asci (µm)**	**Habitat, Distribution**	**Pigment production *in vitro***	**Citation**
Anteaglonium abbreviatum	6–7 × 2–3	1	Hyaline	Absent	No data	Wood, North America, Europe, East Africa	No data	Shini *et al*. (2016)
Anteaglonium brasiliense	9–13 × 2–4	1	Hyaline	Absent	34.5–47 × 4–5.5	Twig, Brazil	Not	De Almeida, Gusmao & Miller (2014)
Anteaglonium globosum	6–7 × 2–3	1	Hyaline	Absent	No data	North America	No data	Shini *et al*. (2016)
Anteaglonium gordoniae	20–22 × 1.5–3	1	Hyaline	Absent	60–70 × 7–10	Decaying cupule of *Gordonia* sp., Thailand	Not	Jayasiri *et al* (2019)
Anteaglonium hydei	19–24 × 4.5–6	1	Hyaline	Absent	87–105 × 9–10.5	Wood, China	Not	Zhang et al (2023)
Anteaglonium latirostrum	22–28 × 4–6	1–4	Hyaline	Yes	115–124 × 9–10	Wood, Kenya	Not	Mugambi & Huhndorf (2009)
Anteaglonium lusitanicum	7.4–10 × 2.9–3.4	1	Hyaline	Absent	53–86.8 × 4–5.6	Wood of *Prunus lusitanica*, Spain	Not	Tan *et al* (2022)
Anteaglonium parvulum	5–8 × 2–3.5	1	Hyaline	Absent	30–52 × 3.2–5.8	Wood, Thailand	Not	Jayasiri *et al.* (2016)
Anteaglonium queenslandicum	No data[1]	No data[1]	No data[1]	No data[1]	No data[1]	No data[1]	No data[1]	Tan *et al*. (2023)
Anteaglonium rubescens	5–7 × 3–4.5	1	Hyaline to brown	Absent	72–87 × 4.7–5.7	Twings of *Pistacia*, Greece	Reddish	Jaklitsch, Fournier & Voglmayer (2018)
Anteaglonium thailandicum	6.5–8 × 2.5–3	1	Hyaline	Absent	45–55 × 3.5–5.5	Wood, Thailand	Not	Jayasiri *et al.* (2016)
Flammeascoma bambusae	40–48 × 15–17	1	Hyaline	Yes	130–160 × 15–17	Bamboo, Thailand	Not	Liu *et al.* (2015)
Flammeascoma lignicola	46–55 × 10–13	1	Hyaline	absent	128–163 × 18–24.5	Wood of Pinus, Thailand	Not	Ariyawansa *et al.* 2015
Flammeascoma ryskae	**16–20 × 3.5–4.7**	**1–3**	**Hyaline to brown**	**absent**	**63–76** × 6.2–7.4	**Wood, Czechia**	**Reddish**	**This study**
Neolophiotrema xiaokongense	20–30 × 6–7	1–3	Hyaline	Yes	110–170 × 15–25	Wood, China	reddish brown to light brown	Ren *et al.* (2021)
Purpureofaciens aquatica	15–22 × 7–10	1	hyaline	Yes	135–150 × 8.5–11	Submerged wood, Thailand	reddish brown to light brown	Dong *et al.* (2020)

[1]This species was described only on the basis of differences in molecular data, morphological description absents in the original study (Tan *et al.* 2023)

Discussion

Alluvial forests are one of the most valuable biotopes in Central Europe in terms of functional ecology and species diversity, after high-mountain areas. However, these biotopes are already very fragmented in the Czech Republic and are threatened by anthropogenic agents. Their species composition in terms of fungi has not yet been fully explored. *Flammeascoma ryskae*, which is described as a new species in this study, was found in a natural fragment of *Tilio-Carpinetum* floodplain community. This biotope is characteristic for the Poodří region (Meixnerová & Mlčoch 2021). For example, at the studied locality, a greater diversity of macro-fungi (Basidiomycota and Ascomycota) was found in the *Tilio-Carpinetum* community than in other compared biotopes at the locality (*Glycerietum maximae*, *Carici elongatae-Alnetum glutinosae, Carici acutiformis-Alnetum glutinosae* and *Pruno-Fraxinetum*) (Meixnerová & Mlčoch 2021).

Based on a study of morphological and molecular data, a collection of Pleosporales fungi from dead wood of a deciduous tree was described as a new species in this study. Because the other two species from the genus *Flammeascoma* are so far known only from type localities in Thailand (Ariyawansa *et al*. 2015; Dong *et al.* 2020), the collection of the newly described species is the first finding of this genus in Europe.

Similar to some other species of the family *Anteagloniaceae*, this species is also characterized by the production of reddish pigment in agar medium. The chemical nature of these pigments was not examined in this study. However, taking into account the study by Xu *et al.* (2015), it is likely that the chemical structure of the pigment in *F. ryskae* will be related to the described anteaglonialides, as in the case of the unidentified strain *Anteaglonium* sp. FLO768. When describing the culture of *Flammeascoma ryskae*, a decreasing tendency of their production was observed inversely proportional to the growth of the mycelium. In the primary culture, it was most pronounced after 2 weeks of growth on PDA at 20 °C, later it decreased, and in their clones on the same medium and under the same culture conditions, the production was already much weaker. As we can see in the table 2. The production of pigments in agar medium was not detected in all species from the genera *Anteaglonium* and *Flammeascoma*, but only in two related species of the monotypic genera *Purpureofaciens* and *Neolophiotrema*, further only in one species from the genus *Anteaglonium* (only at *A. rubescens*) and newly one species from the genus *Flammeascoma* (only *F. ryskae*). Due to the nature of the evolutionary relatedness of pigment-producing species and the existence of a number of phylogenetic branches with pigment-lacking taxa, pigment production can be considered a homoplasy character. However, a closer analysis of this idea would require a comparative biochemical analysis of the pigments in *Flammeascoma ryskae, Purpureofaciens aquatica, Neolophiotrema xiakongense,* and *Anteaglonium rubescens* (it may not be identical to the endophytic strain *Anteaglonium* sp. FLO768, this strain with GenBank acc. no JQ760428.1 has 98 % similarity index with *Anteaglonium gordoniae* in BLASTn, note of author). In the evolution of the Pleosporales, the differentiation and functional expression of different pigments appears to have occurred many times in different species and is usually not a feature common to a particular genus. In other basal Pleosporales, the production of pigments is proven, e. g. in the case of *Nigrograna rubescens* (*Nigrograna*ceae) (Mack *et al.* 2024) or *Lignosphaerella diospyrosa* (*Phaeoseptaceae*) (Hyde *et al*. 2020), in evolutionarily more derived Pleosporales e.g. in the diversified endophytic genus *Epicoccum* (*Didymellaceae*) (Kaliňák *et al.* 2013; Elkhateeb & Daba 2023) or some species from the genera *Pyrenophora* (*Pleosporaceae*) and *Alternaria* (*Pleosporaceae*) (Elkhateeb & Daba 2023). The importance of these metabolites is probably related to the increase of their competitive abilities during the colonization of the substrate (Alam *et al.* 2021).

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