

# A renal mycosis of roach (*Rutilus rutilus*) caused by the *Aureobasidium pullulans*

Jiří Řehulka<sup>a</sup>, Vit Hubka<sup>b,c,\*</sup>

<sup>a</sup> Department of Zoology, Silesian Museum, Nadrazní okruh 31, 746 01, Opava, Czech Republic

<sup>b</sup> Department of Botany, Faculty of Science, Charles University, Benatska 2, 128 00, Prague 2, Czech Republic

<sup>c</sup> Laboratory of Fungal Genetics and Metabolism, Institute of Microbiology of the Academy of Sciences of the Czech Republic, v.v.i., Videnska 1083, 142 20, Prague 4, Czech Republic

## ARTICLE INFO

Handling Editor: Dr Adilia Warris

### Keywords:

Black yeasts  
Histopathology  
Molecular diagnosis  
Nuclear erythrocyte abnormalities  
Spontaneous mycosis

## ABSTRACT

Spontaneous mycosis caused by *Aureobasidium pullulans* is documented in roach in a cyprinid-prevalent water reservoir in Czechia. Gross pathological lesions included pale gills and splenomegaly, as revealed during necropsy examination. Histological examination showed extensive foci with fungal elements in the kidney. The isolated fungus was identified through phenotypic and molecular characterization, including phylogeny. This report represents the first case of *A. pullulans* infection in fish and cold-blooded vertebrates, to the best of our knowledge.

## 1. Introduction

*Aureobasidium pullulans* is a versatile melanized yeast-like fungus (*Ascomycota*, *Dothideomycetes*, *Dothideales*) well-documented for its wide distribution in different environments ranging from plant surfaces and soil to water and rock surfaces. It has been reported in cold and warm climates as well as humid and arid regions. Due to its wide spectrum of natural product synthesis, including enzymes and antimicrobial compounds, it is a significant candidate for biotechnological and environmental applications [1,2]. The fungus was described as a pathogen in a variety of human infections usually resulting from traumatic implantation, such as cutaneous and subcutaneous phaeohyphomycosis and keratitis. Additionally, it may cause fungemia or systemic infections affecting various organs in immunocompromised patients [3]. It is rarely reported in veterinary infections, limited to canine otitis [4], subcutaneous [5] and cerebral infections [6]. Cutaneous infections in porcupines (*Erethizon dorsatum*) have also been described [7]. Furthermore, the species was detected in a paraffin-embedded sample from feline nose tissue [8]. Along with *Rhodospiridium* sp., it was isolated from ocular infection in brown shrimp (*Penaeus californiensis*) [9].

The roach, *Rutilus rutilus*, is a non-predatory freshwater ray-finned fish, most commonly found in lowland aquatic ecosystems. As an eurytopic species, it is able to exploit all available habitats. The roach is

one of the dominant species in the stable cyprinid phase of artificial lakes in Europe [10]. To the best of our knowledge, the present study is the first report describing mycosis due to *A. pullulans* not only in fish but also in cold-blooded vertebrates. The fungal pathogen was identified using phenotypic and molecular data.

## 2. Case presentation

During systematic health surveillance in fish stock of a water-supply reservoir 404 m above sea level in the Odra River basin, Czech Republic, mycosis was detected in a roach (*Rutilus rutilus*). The specimen, measuring 182 mm and weighing 140 g, exhibited pallor in the gills. Necropsy identified splenomegaly, and renal wet mounts revealed extensive fungal foci. GMS (Grocott's methenamine silver) stained kidney sections displayed infiltration by fungal hyphae (Fig. 1a and b) amidst cellular inflammation, characterized by a preponderance of neutrophil band forms (Fig. 1c). The fungus was absent in other organs examined (gill, liver, spleen, intestine, heart and brain), and no parasites or bacteria were found in the tissues analyzed.

Erythrogramme abnormalities were dominated by disintegrating nuclei, binucleated cells and eccentric nuclei (Fig. 2a–g). We used histological, bacteriological and mycological procedures described previously [11]. Haematological procedures used were described by Řehulka

\* Corresponding author. Department of Botany, Faculty of Science, Charles University, 128 00, Prague 2, Czech Republic.

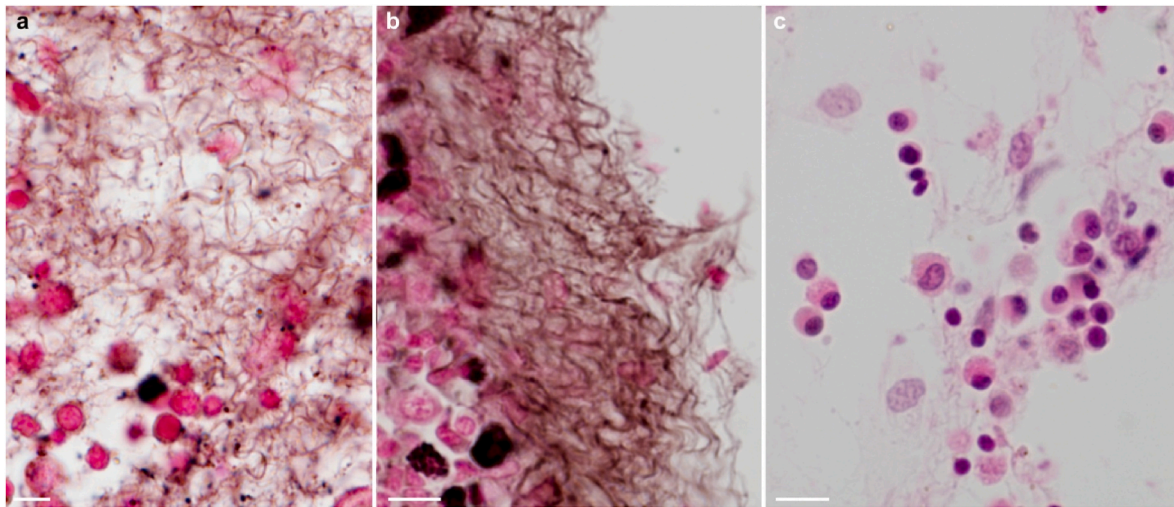
E-mail address: [vit.hubka@gmail.com](mailto:vit.hubka@gmail.com) (V. Hubka).

<https://doi.org/10.1016/j.mmcr.2024.100652>

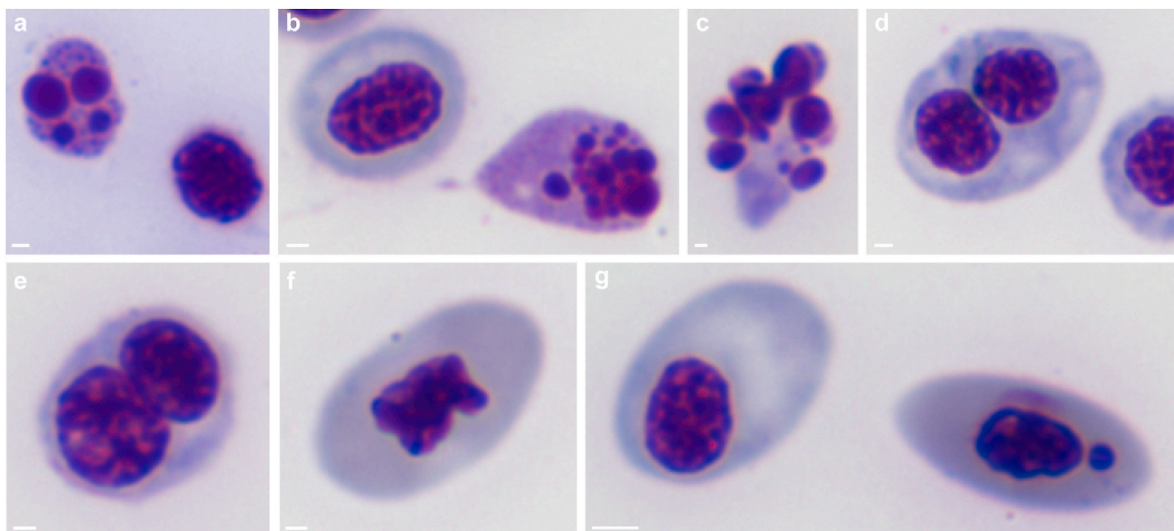
Received 19 April 2024; Accepted 7 May 2024

Available online 8 May 2024

2211-7539/© 2024 The Authors. Published by Elsevier B.V. on behalf of International Society for Human and Animal Mycology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).



**Fig. 1.** Mass of melanized fungal hyphae in kidney (a, b). Inflammatory cell population with abundant neutrophil band forms (c). Grocott's methenamine silver stain (a,b), May-Grünwald-Giemsa (c). Scale bars (a) = 5  $\mu$ m, (b,c) = 10  $\mu$ m.



**Fig. 2.** Nuclear abnormalities found during examination of peripheral blood cells. Erythrocytes with disintegrating nuclei (a–c). Binucleated cells (d, e). Lobed nuclei (f). Erythrocyte with eccentric nucleus (left) and micronucleated cell (g). May-Grünwald-Giemsa (a–g). Scale bars (a,b,d,e,f) = 1  $\mu$ m, (c) = 0.5  $\mu$ m, (g) = 2  $\mu$ m.

et al. [12].

A fungal strain was isolated in pure culture from the kidney on Sabouraud's agar at 24 °C. Bacteria were not observed in the lesion material and were not cultivated from samples inoculated on Blood agar (MKM 01011 Columbia agar; Trios), trypticase soya agar (MKM 10061; Trios), Mueller-Hinton agar (MKM 02011; Trios) and Anacker-Ordal agar. Parasitological investigation revealed only minor infestation by *Gyrodactylus prostrae* (the intensity of infestation on fins and gills was two and three specimens).

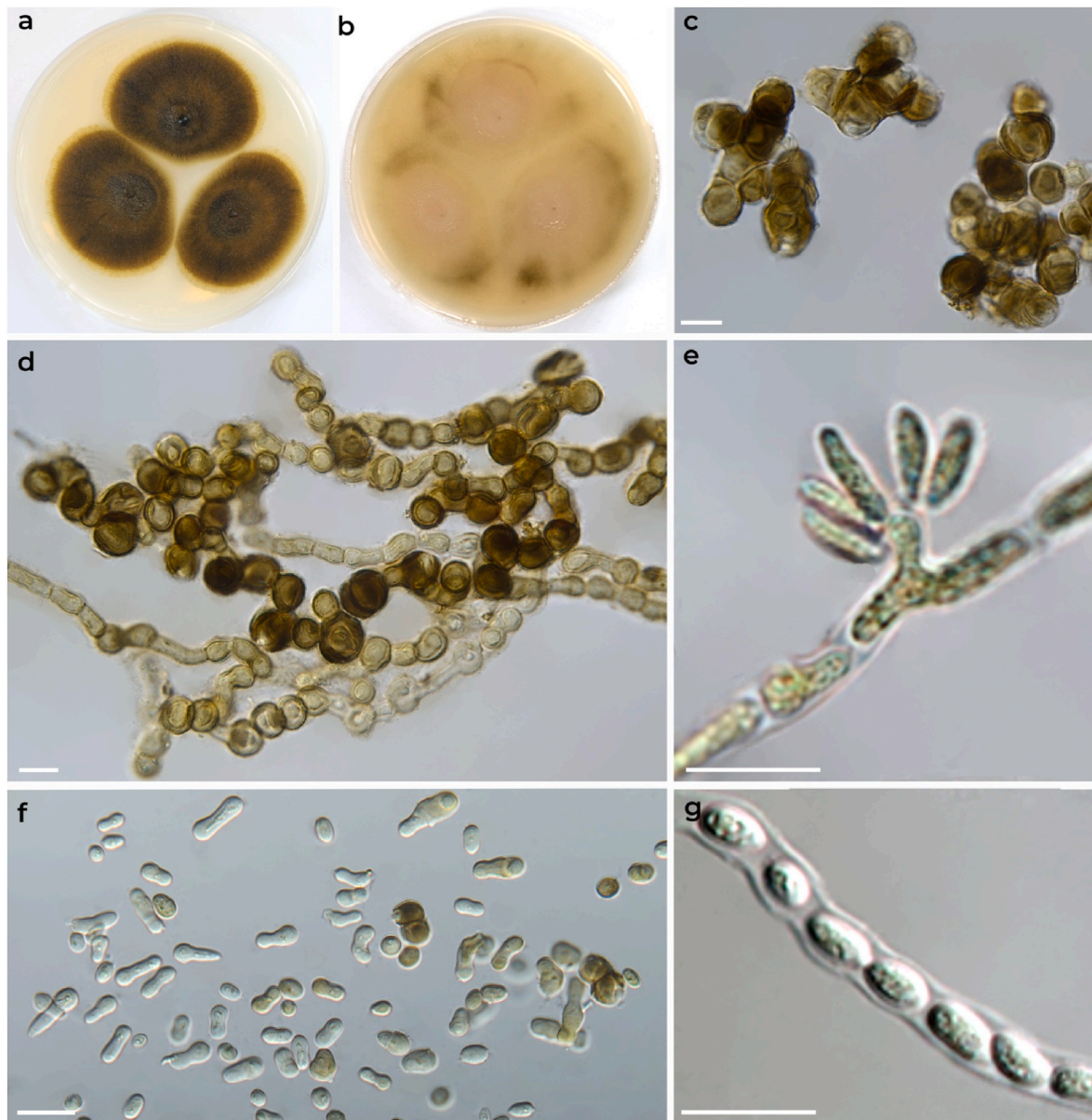
Subcultures of isolated strain were grown on malt extract agar (MEA, Oxoid), potato carrot agar (PCA, potatoes 20 g, carrot 20 g, agar 20 g, water 1000 ml) at 25 and 37 °C (Fig. 3). Colonies incubated at 25 °C on MEA reached a diameter of 23–26 mm after 7 days and 42–46 mm after 14 days. They were yellowish brown to brown, smooth, with a wet appearance and superficial exudate. Colonies on PCA reached a diameter of 32–38 mm after 7 days and 53–55 mm after 14 days. They were cream-coloured, pinkish-white with olive-brown sectors. No growth was observed at 37 °C.

Vegetative hyphae were 1.5–15  $\mu$ m thick, consisting of hyaline thin-walled cells as well as yellowish to brown thick-walled cylindrical or

subglobose cells that later disintegrated into 1- to 2-celled fragments (arthroconidia). Chlamydospores were mostly globose or subglobose, brown, thick-walled, intercalary or terminal, sometimes not clearly differentiated from thick-walled vegetative hyphae (continuum). Conidiogenous cells were integrated into vegetative hyphae, intercalary or terminal, with hyaline blastoconidia produced on small denticles and protrusions. Conidia were 1-celled, variable in shape (ellipsoidal, cylindrical, biconcave or pyriform), typically 5–19  $\times$  3–7  $\mu$ m in diameter. Yeast-like budding cells (conidia) were abundantly present, and occasionally endoconidia were found within vegetative hyphae (Fig. 3).

DNA isolation and PCR conditions used in this study were based on Hubka et al. [13]; the primers used for amplification of selected loci were based on Řehulka et al. [14]. A BLAST similarity search using the ITS region of rDNA revealed a 99.6 % similarity (2 bp difference) with *A. pullulans* ex-type CBS 584.75 (AJ244232) and its synonym *A. proteae*, while other species showed a similarity <99 %. Similarly, the *RPB2* gene (RNA polymerase II second largest subunit) showed the highest similarity with the ex-type of *A. pullulans* and *A. proteae* at 98.8 % (8 bp difference), while other species with available sequences in Genbank showed similarity <90 %. Phylogenetic trees constructed from ITS and





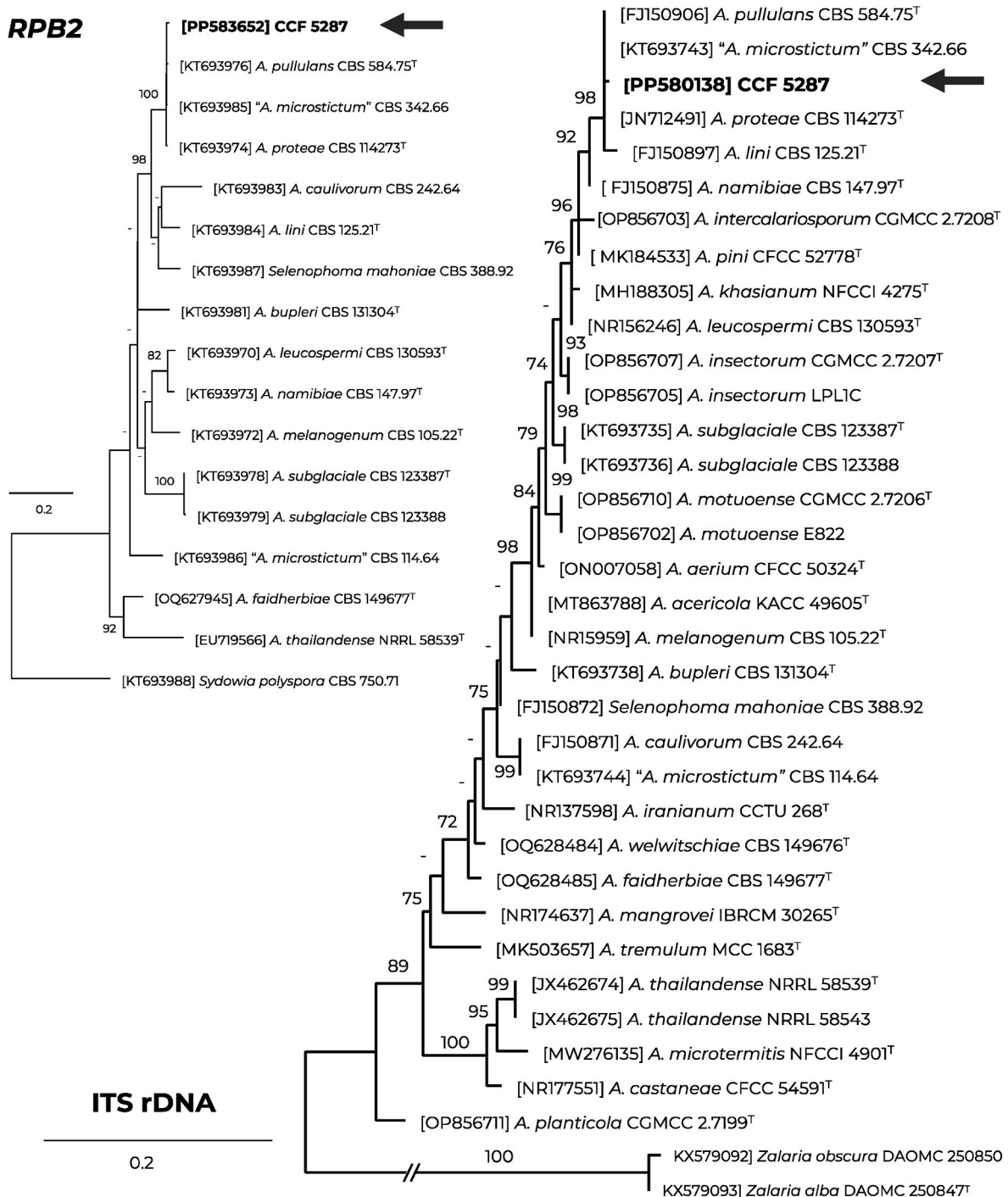
**Fig. 3.** Macromorphology and micromorphology of *Aureobasidium pullulans* CCF 5287. Colonies incubated for 14 days on MEA (a) and PCA (b) at 25 °C. Clusters of chlamydospores (c); hyaline, pale yellow to brown vegetative hyphae with brown intercalary and terminal chlamydospores (d); conidiogenous cell integrated into vegetative hypha with hyaline conidia produced on a lateral protrusion (e); free yeast-like budding cells, conidia and chlamydospores (f); endoconidia (g). Scale bars: c-g = 10 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

*RPB2* loci confirmed the clustering of the case isolate with *A. pullulans* (Fig. 4). Other amplified markers were excluded from analyses due to low representation of sequences in GenBank (*tub2*;  $\beta$ -tubulin) or low variability between species (LSU rDNA). The sequences were deposited into the GenBank database under the following accession numbers: PP580138 (ITS), PP580189 (LSU), PP583653 (*tub2*) and PP583652 (*RPB2*). The fungus was deposited into the Culture Collection of Fungi, Charles University, Department of Botany, Prague, Czech Republic as CCF 5287.

### 3. Discussion

This case report documents the first known mycosis in *Rutilus rutilus* caused by the “black yeast” *A. pullulans*, a versatile environmental saprophyte and occasional pathogen with a significant presence in various aquatic ecosystems [15,16]. The observed pathology in roach primarily manifested as renal fungal foci, illustrating a novel aspect of

*A. pullulans* pathogenic potential in poikilothermic animals. However, other clinical signs and hematological findings (disintegrating nuclei, binucleated cells and eccentric nuclei) cannot be explicitly attributed to fungal infection as no virological examination was performed. Such an examination could confirm the etiology of the morphological lesions of erythrocytes, which were similar to those seen in sockeye salmon infected with infectious hematopoietic necrosis virus (IHN) [17,18]. Binucleated cells have been previously described in young coho salmon [19] and young rainbow trout deficient in folic acid [20]. A tendency to produce binucleated cells was also observed in rainbow trout experimentally infected with the fungus *Bradomyces oncorhynchi* [12]. This finding prompts further exploration into the etiology of morphological lesions in erythrocytes and the potential role of the secondary metabolites produced by *A. pullulans* in terms of their toxicity, teratogenicity and mutagenicity. The described erythrocyte lesions, aside from micronucleated cells, were not observed in the other five examined roach from the same dam lake habitat.



**Fig. 4.** The best-scoring maximum-likelihood trees based on the ITS region (right) and the RNA polymerase II second largest subunit (*RPB2*) gene (left), illustrating the relationships of the case isolate of *Aureobasidium pullulans* CCF 5287 to species of *Aureobasidium*. The substitution models were selected and the tree was constructed using IQ-TREE v. 2.3.2 (ITS alignment: 35 taxa, 620 positions, 134, variable positions, TIM2e + I + G4 model; *RPB2* alignment: 17 taxa, 753 positions, 300 variable positions, TN + F + G4 model). Only bootstrap supports  $\geq 70\%$  are shown, with lower supports indicated by a dash; the ex-type strains are designated by a superscript "T".

The identification of *A. pullulans* in a wild freshwater fish expands its known opportunistic pathogenicity to cold-blooded vertebrates in natural settings, emphasizing the importance of comprehensive aquatic health monitoring. The specific pathogenic mechanisms of *A. pullulans* in *Rutilus rutilus* remain to be elucidated. While surface lesions on fish may serve as infection gateways, further research is needed to determine the exact dissemination routes, including potential transmission through contaminated food.

**CRedit authorship contribution statement**

**Jirí Řehulka:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Writing – original draft. **Vit Hubka:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – review & editing.

## Declaration of competing interest

None.

## Acknowledgements

This work was financially supported by the Ministry of Culture of Czech Republic through institutional financing of long-term conceptual development of the research institution (the Silesian Museum, MK000100595) and internal grant of the Silesian Museum No. IGS 201708/2017. Contribution of VH was supported by project Strategie AV21'VP33 Houby kolem nás i v nás' of the Czech Academy of Sciences and Czech Academy of Sciences Long-term Research Development Project (RVO: 61388971).

## References

- [1] C. Gostinčar, R.A. Ohm, T. Kogej, S. Sonjak, M. Turk, J. Zajc, et al., Genome sequencing of four *Aureobasidium pullulans* varieties: biotechnological potential, stress tolerance, and description of new species, *BMC Genom.* 15 (2014) 1–29.
- [2] D. Bozoudi, D. Tsaltas, The multiple and versatile roles of *Aureobasidium pullulans* in the vitivinicultural sector, *Fermentation* 4 (2018) 85.
- [3] G. de Hoog, J. Guarro, J. Gené, S. Ahmed, A. Al-Hatmi, M. Figueras, et al., Atlas of Clinical Fungi, fourth ed., Foundation Atlas of Clinical Fungi, Hilversum, 2020.
- [4] J.J. Campbell, K.S. Coyner, S.C. Rankin, T.P. Lewis, A.E. Schick, A.K. Shumaker, Evaluation of fungal flora in normal and diseased canine ears, *Vet. Dermatol.* 21 (2010) 619–625.
- [5] S.B. Waller, M.R.A. Ferreira, A. dos Reis Gomes, M.K. Ripoll, A.L. Silva, O. de Almeida Martins, et al., Subcutaneous phaeohyphomycosis due to *Aureobasidium pullulans* infection in a dog, *Vet. Ital.* 57 (2021) 233–237.
- [6] M. Perkins, P. Martin, M. Krockenberger, K. Weeks, R. Malik, Cerebral phaeohyphomycosis in a young female German Shepherd, *Aust. Vet. Pract.* 34 (2004) 110–116.
- [7] I. Salkin, M. Gordon, W. Stone, Cutaneous infection of a porcupine (*Erethizon dorsatum*) by *Aureobasidium pullulans*, *Sabouraudia* 14 (1976) 47–49.
- [8] A. Bernhardt, W. von Bomhard, E. Antweiler, K. Tintelnot, Molecular identification of fungal pathogens in nodular skin lesions of cats, *Med. Mycol.* 53 (2014) 132–144.
- [9] C. Alarcón-González, R. Vázquez-Juárez, R. López-Trinidad, N. Hernández-Saavedra, Yeast infections in brown shrimp (*Penaeus californiensis*) in Baja California Sur, Mexico: isolation, identification and sensitivity to fungicides, *Rev. Latinoam. Microbiol.* 32 (1990) 121–125.
- [10] M. Říha, J. Kubečka, M. Vašek, J. Sedá, T. Mrkvička, M. Prchalová, et al., Long-term development of fish populations in the Rímov Reservoir, *Fish. Manag. Ecol.* 16 (2009) 121–129.
- [11] J. Řehulka, A. Kubátová, V. Hubka, *Cephalotheca sulfurea* (Ascomycota, Sordariomycetes), a new fungal pathogen of the farmed rainbow trout *Oncorhynchus mykiss*, *J. Fish. Dis.* 39 (2016) 1413–1419.
- [12] J. Řehulka, M. Kolařík, V. Hubka, Clinical and histopathological changes in rainbow trout *Oncorhynchus mykiss* experimentally infected with fungus *Bradomyces oncorhynchi*, *Folia Microbiol.* 65 (2020) 1025–1032.
- [13] V. Hubka, A. Nováková, Ž. Jurjevič, F. Sklenář, J.C. Frisvad, J. Houbraeken, et al., Polyphasic data support the splitting of *Aspergillus candidus* into two species; proposal of *Aspergillus dobrogensis* sp. nov., *Int. J. Syst. Evol. Microbiol.* 68 (2018) 995–1011.
- [14] J. Řehulka, A. Kubátová, V. Hubka, Swim bladder mycosis in farmed rainbow trout *Oncorhynchus mykiss* caused by *Phoma herbarum* and experimental verification of pathogenicity, *Dis. Aquat. Org.* 138 (2020) 237–246.
- [15] E. Sláviková, R. Vadkertiová, A. Kocková-Kratochvílová, Yeasts isolated from artificial lake waters, *Can. J. Microbiol.* 38 (1992) 1206–1209.
- [16] S. Meyers, D. Ahearn, W. Gunkel, F. Roth, Yeasts from the North Sea, *Mar. Biol.* 1 (1967) 118–123.
- [17] D.F. Amend, L. Smith, Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout: hematological and blood chemical changes in moribund fish, *Infect. Immun.* 11 (1975) 171–179.
- [18] M.E. Watson, R.W. Guenther, R.D. Royce, Hematology of healthy and virus-diseased sockeye salmon, *Oncorhynchus nerka*, *Zoologica* 41 (1956) 27–38.
- [19] C.E. Smith, Hematological changes in coho salmon fed a folic acid deficient diet, *J. Fish Board. Can.* 25 (1968) 151–156.
- [20] C.B. Cowey, B. Woodward, The dietary requirement of young rainbow trout (*Oncorhynchus mykiss*) for folic acid, *J. Nutr.* 123 (1993) 1594–1600.