1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)
Project title:

Transmission efficiency of bird flukes of the genera: Diplostomum and Trichobilharzia in the face of global warming - life cycle, biology of invasive larvae and assessment of the risk of invasion

1.1. Project goals

We intend to study the distribution of bird flukes of the genera: Diplostomum and Trichobilharzia in populations of snail-host species, which play a key role in maintaining parasites in aquatic ecosystems and at the same time are a source of cercariae - larvae released into the water, which pose a threat to fish farming or human health. We plan to check the presence and species composition of cercariae in planktonic samples using molecular diagnostic methods and to learn the impact of thermal conditions on the productivity, survival and behavior of larvae released from snails. We will also examine the reactions of host snails to changes in thermal conditions (survival, fertility, etc.). We propose a thesis that parasites with a shorter (dual-host) life cycle (Trochobilharzia: snail-bird), under the conditions of climate warming, will gain an advantage over three-host (snail-fish-bird) flukes of the genus Diplostomum due to the possibility of faster gene recombination (inside birds – definitive hosts). We expect that the presence of an essential second intermediate host - ectothermic vertebrate (fish) in the Diplostomum cycle, under climate warming conditions, may significantly reduce the transmission of these flukes to the definitive host.

1.2. Outline

Bird flukes of the genera Diplostomum and Trichobilharzia use snails of the family Lymnaeidae as key intermediate hosts (Schwelm et al., 2021; Marszewska et al., 2018). In both cases, the cercariae leaving the snails are an important risk factor for the transmission of parasites in the environment. *Diplostomum* spp. larvae attack fish and lodge in their eyes, causing blindness (McCloughlin, 2016), and *Trichobilharzia* spp. pose a threat to human health, causing swimmers itch (Horák et al. 2002; Schets et al., 2010; Soldánová et al., 2013) when they accidentally penetrate human skin while bathing or wading in water. Trichobilharzia flukes belong to the

Schistosomatidae and are related to human blood flukes, which are the cause of over 200 million cases of schistosomiasis in Asia, Africa and South America (WHO, 2019). Initially, bird schistosomes were perceived as the etiological agents of sporadic dermatoses, but studies have shown that they can generate a serious health problem, due to the species of the so-called nasal schistosomes such as *Trichobilharzia regenti*, whose larvae lodge in the nervous system of experimentally infected mammals (Machácek et al., 2022). In turn, flukes of the genus Diplostomum, commonly found in water reservoirs, are an important factor regulating the populations of fish-host species (Kudlai et al., 2017). Losses in fish farming caused by the transmission of these flukes are a serious economic problem, particularly acute in developing countries (Chibwana, 2018). Representatives of both types of flukes have in common: the low usefulness of cercariae morphology in the diagnosis of species and classical cercariometry as a method of assessing the risk of parasite transmission in the environment. In the face of climate warming, it can be expected that the adaptations of ectothermic organisms, both flukes and their hosts - snails and fish, will play a key role in increasing or decreasing the threat of parasite invasion in the environment.

1.3. Work plan

- Recognition of the distribution of Diplostomum and Trichobilharzia fluke larvae in populations of Lymnaeidae representatives from selected lakes of the Polish Lowlands - application of molecular diagnostics of parasite species - comparison of the algorithm of the tested species of parasites

- Thermal experiments on survival rate, fertility, number and rate of release of parasitic larvae from naturally infected snails - comparison of the strategies of the tested species of parasites

- Thermal experiments on the chronology of release and survival of Diplostomum and Trichobilharzia

- Field studies on the occurrence of Diplostomum and Trichobilharzia cercariae in planktonic samples from lakes where the presence of infected first intermediate hosts were found - molecular identification of parasites

- After each of the above-mentioned stages of research, the obtained results will be analyzed, and the results of the analysis will be presented during scientific conferences and prepared for publication in scientific journals

1.4. **Literature** *(max. 10 listed, as a suggestion for a PhD candidate)*

- Chibwana F.D., 2018. African Diplostomum (sensu Dubois 1961): Minireview on taxonomy and biology. Int J Aquac Fish Sci 4(3): 31-38

- Horák P., Kolářová L., Adema C.M., 2002. Biology of the schistosome genus Trichobilharzia. Adv Parasitol 52: 155–233

- Kudlai O., Oros M., Kostadinova A., Georgieva S., 2017. Exploring the diversity of Diplostomum (Digenea: Diplostomidae) in fishes from the River Danube using mitochondrial DNA barcodes. Parasites & Vectors 10:592

- Machácek T., Leontovyč R., Šmídová B., Majer M., Vondráček O., Petrásek T., Horák P., 2022. Mechanisms of the host immune response and helminth-induced pathology during Trichobilharzia regenti (Schistosomatidae) neuroinvasion in mice. PLoS Pathog 18, e1010302

- Marszewska A., Strzała T., Cichy A., Dąbrowska G.B., Żbikowska E., 2018. Agent of swimmer's itch – dangerous minority in the Digenea invasion of Lymnaeidae in water bodies and the first report of *Trichobilharzia regenti* in Poland. Parasitol Res 117: 3695-3704

- McCloughlin T., 2016. A sight for sore eyes: Diplostomum and Tylodelphys in the eyes of fish. Iran J Parasitol 11(3): 429-430

- Schets F.M., Lodder W.J., De Roda Husman A.M., 2010. Confirmation of the presence of Trichobilharzia by examination of water samples and snails following reports of cases of cercarial dermatitis. Parasitology 137: 77–83

- Schwelm J., Georgieva S., Grabner D., Kostadinova A., Sures B., 2021. Molecular and morphological characterisation of *Diplosomum phoxini* (Faust, 1918) with a revised classification and an updated nomenclature of the species-level lineages of Diplostomum (Digenea: Diplostomidae) sequenced worldwide. Parasitology 148: 1648-1664

- Soldánová M., Selbach C.., Kalbe M, Kostadinova A., Sures B., 2013. Swimmer's itch: Etiology, impact, and risk factors in Europe. Trends Parasitol 29: 65–74

- World Health Organization, 2019. Wkly Epidemiol Rec 94: 601–612

1.5. Required initial knowledge and skills of the PhD candidate

- Knowledge in invertebrate zoology and hydrobiology
- Experience in work with aquatic invertebrates (preparing and sustaining aquarium cultures, conducting observations, manipulating animals and aquarium equipment)
- Knowledge of molecular species identification techniques
- Critical thinking
- English communication skills (reading, speaking and writing)

1.6. Expected development of the PhD candidate's knowledge and skills

- In-depth knowledge in the fields of host-parasite interaction on the basis of snail-Digenea larvae
- Designing and conducting thermal experiments
- Analysis of experimental data (tools for recording and analysing the influence of parasites on snail biology, statistical data analysis)
- Presentation of scientific data (writing scientific papers, conference presentations)
- Team work: cooperation within the research team, external scientific contacts