

Report of Strigeidae (Digenea) metacercariae from leeches (Hirudinida)

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Digenetic flukes are characterized by a multi-host life cycle with molluscs as a first intermediate host and vertebrate as a definitive host. The majority of digeneans also have an invertebrate or a vertebrate second intermediate host that usually harbour an encysted metacercaria. Some digeneans with aquatic life cycles, such as a few representatives of the family Strigeidae Railliet, 1919, may use leeches as a second intermediate host. The strigeids use mainly aquatic birds, especially anatids (ducks, geese, swans) as definitive hosts; these birds often feed on leeches. For example, *Australapatemon burti* (Miller, 1923) described from North American, is one of better known strigeid species that uses leeches as an intermediate host and many reported species of anamid ducks as definitive host. A cosmopolitan species, *Cotylurus cornutus* (Rudolphi, 1808) also uses leeches as the second intermediate host. However, the knowledge about life cycles and host associations of these digeneans is still insufficient.

The aim of this study was to elucidate the role of leeches as intermediate host of strigeid digeneans in Poland. Additionally, adult strigeids obtained from birds were used for molecular comparative studies.

Leeches belonging to 4 species (*Haemopsis sanguisuga*, *Theromyzon tessulatum*, *Glossiphonia complanata*, *Erpobdella octoculata*) were collected in Subcarpathia Province, Lower Silesia and Gdansk Pomerania in 2017 and 2018. All leeches were examined for digeneans. All collected parasites were preserved in 70% alcohol for further research. Metacercariae were photographed prior to DNA extractions. DNA was extracted from single metacercariae with the use of commercial DNA extraction kit (EURx) according to the manufacturer's protocol. Partial nuclear large ribosomal subunit (28S rDNA) gene, internal transcribed spacer (ITS) and mitochondrial gene encoding cytochrome c oxidase subunit I (COI) were amplified. Newly generated sequences from larval and adult stages were identified using BLAST search engine.

The metacercariae found in leeches were identified based on the sequence data as representatives of the genera *Australapatemon* (Sudarikov, 1959) and *Cotylurus* Szidat, 1928 (Strigeida, Diplostomoidea, Strigeidae). The ecological analysis of occurrence of strigeid metacercariae as well as the phylogenetic analysis of the Strigeidae using sequences obtained in this study were conducted.