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MORPHOLOGICAL AND MOLECULAR-GENETIC CHARACTERIZATION OF PETASIGER EXAERETUS DIETZ, 1909 (TREMATODA: ECHINOSTOMATIDAE)

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Abstract

This article presents the morphological and molecular-genetic classification of Petasiger exaeretus Dietz, 1909, a parasitic trematode species from the genus Petasiger Dietz, 1909, found in the digestive system of the great cormorant (Phalacrocorax carbo) distributed in aquatic habitats of Central Uzbekistan. The molecular-genetic analysis was performed based on the nucleotide sequence of the internal transcribed spacer (ITS) region of the ribosomal DNA of P. exaeretus.

Keywords

Cormorant, parasite, trematode, species, morphology, DNA, ITS.

Introduction. The great cormorant (Phalacrocorax carbo) (Order: Pelecaniformes; Family: Phalacrocoracidae) is a widespread, piscivorous bird species that inhabits numerous countries across various continents, excluding South America and Antarctica. As opportunistic predators, these birds exert considerable pressure on native fish populations, often altering local aquatic ecosystems (Moravec & Scholz, 2016). In the Czech Republic—particularly in regions such as South Bohemia and South Moravia—their high population density has been linked to substantial negative impacts on commercial fisheries (Moravec & Scholz, 2016). Beyond their ecological role as predators, great cormorants serve as ideal definitive hosts for digenean trematodes, a group of parasitic flatworms whose life cycle involves fish as the primary intermediate hosts. Consequently, the digestive tract of these birds typically harbors a diverse community of parasitic helminths, including flatworms, cestodes (tapeworms), and trematodes (flukes), reflecting their central role in the transmission of aquatic parasites.

Digenetic flukes (Class: Trematoda; Subclass: Digenea), members of the phylum Platyhelminthes, are regarded as some of the most impactful parasitic organisms, both in terms of veterinary relevance and their zoonotic potential. These parasites are responsible for a range of diseases affecting wildlife,

domestic animals, and humans alike. Among numerous piscivorous avian species, great cormorants (Phalacrocorax carbo) have been identified as prominent definitive hosts for a variety of digenean trematodes. Their feeding behavior and mobility allow them to act as effective vectors, facilitating the dispersal of parasitic flukes between isolated aquatic habitats. As early as 1972, Edelényi documented several digenean species infecting cormorants in the Hortobágy region of Hungary, including representatives of the genera Hysteromorpha (Lutz, 1931) and Petasiger (Dietz, 1909). Decades later, Moravec and Scholz (2016) reported a high prevalence of Petasiger spp. in cormorants in Czechia, along with sporadic occurrences of Hysteromorpha and Metorchis (Looss, 1899). A large-scale parasitological investigation conducted in Poland further reinforced these findings. The survey revealed the presence of nine distinct species of digenean trematodes, all belonging to the genera Petasiger, Hysteromorpha, and Metorchis (Kanarek et al., 2003), highlighting the ecological importance of great cormorants in the life cycle and transmission of aquatic trematodes.

Genus *Petasiger* (Dietz, 1909) belongs to the family Echinostomatidae (Loss, 1899) and reported from many avian species including *Ardeacinerea*, *A. cocoi, Antigone*, *Podiceps australis, P. poliocephalus*, *P. podiceps, P. ufficollisjaponicus, P. cristatus, P. fluviatilis, P. grisegena, P. dominicus, P. minor, P. major, Anas platyrhynchos, Gavia stellate, Podilymbus podiceps, Casarca ferruginea*, *Puffinus, Colymbusnigricollis*, *C. caspicus, C. grisegena, C. cristatus, C. auritus, C. nigricans, Pedetaithyagrisegena, Nyrocanyroca*, *Dendrocygna javanica*, *Gallinula*

chloropus, larus ichthyaetus, Anhinga melanogaster, *A. ruffa, Tachybaptus dominicus*, *Rollandia rolland, Coragyps atratus*, *Pelecanus occidentalis, Phalacrocorax carbo*, *P. pygmaeus, P. melanoleucus, P. sulcirostris, P. africanus, P. niger, P. capitalus* and *Columba livia.* Examinations of these birds have evealed

33 species of genus *Petasiger.* Out of them 19 are valid species (Abdel-Malek, 1953; Bisseru, 1957; Yamaguti, 1971; Nasicova, 1994; Faltynkova *et al.*, 2008; Lunaschi and Drago, 2010; Pinto *et al.*, 2013).

Members of the genus Petasiger Dietz, 1909, like all echinostomatid trematodes, are morphologically defined by the presence of a characteristic collar of spines encircling the anterior region. Among the most taxonomically informative features for distinguishing both species and genera within Echinostomatidae is the number of collar spines, which serves as a primary diagnostic trait. In the genus Petasiger, this number typically ranges between 19 and 27. Species within this genus that exhibit 27 collar spines include Petasiger exaeretus Dietz, 1909—subsequently redescribed in detail by Davies (1934)—as well as P. variospinosus, which was initially classified under the genus Echinostomum as E. variospinosum.

Additional representatives include Petasiger nicolli, described by Pande in 1939, and two species introduced by Nigam in 1944: Petasiger yamagutii and Petasiger antigonus.

The purpose of this research is to perform a morphological and molecular-genetic classification of Petasiger exaeretus Dietz, 1909, a parasitic trematode belonging to the genus Petasiger Dietz, 1909, found in the digestive system of the great cormorant (Phalacrocorax carbo) inhabiting the aquatic ecosystems of Central Uzbekistan.

Materials and Methods. During the years 2024–2025, seven specimens of the great cormorant (*Phalacrocorax carbo*) were examined using complete and partial helminthological dissection methods. The birds were collected from aquatic ecosystems in the Jizzakh, Navoi, and Bukhara regions of Uzbekistan. A total of 430 specimens of *Petasiger exaeretus* Dietz, 1909 were isolated from the digestive tracts of the birds. All collected trematodes were fixed in 70% ethanol solution for preservation and further analysis.

DNA Extraction. Genomic DNA was extracted from Petasiger exaeretus specimens preserved in 70% ethanol using the GeneJET Genomic DNA Purification Column kit. The concentration of each DNA sample was measured using a spectrophotometer (Thermo Fisher Scientific, China). The extracted genomic DNA was stored at –20°C until used for polymerase chain reaction (PCR).

Polymerase Chain Reaction (PCR). To carry out the PCR, primers targeting the 16S region of ribosomal DNA (rDNA), commonly used for the molecular-genetic identification of amphibians, were utilized. The PCR mixture consisted of: 26.4 µL bidistilled water, 4 µL 10× Taq buffer, 0.8 µL dNTPs, 2 µL each of the forward primer AB28 (ATA TGC TTA AGT TCA GCG GGT) and reverse primer TW81 (CTT TCC GTA GGT GAA CCT GC), 4 µL DNA template, and 0.8 µL Taq polymerase, making a total volume of 40 µL.

PCR conditions were as follows:

– Initial denaturation at 92°C for 3 minutes

– 35 cycles of: 92°C for 15 seconds

55°C for 30 seconds

72°C for 30 seconds

– Final extension at 72°C for 10 minutes

The presence of DNA in PCR products was verified by electrophoresis in 1.0% agarose gel at 100 V. DNA amplification and extraction from the gel were

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carried out using reagent kits produced by "Sileks M" (Moscow, Russia), according to the manufacturer's instructions.

DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v3.1 kit, and the reaction products were sequenced by GATC Biotech AG. The resulting nucleotide sequences were analyzed using BioEdit, ClustalX2, DNAstar™, and PAUP4 software packages.

RESULTS AND THEIR ANALYSIS

Results of Morphological Examination. The body varies from 1-21 to 1-62 mm. in length with a maximum diameter of 0-34 mm. in the region of the ventral sucker. A maximum depth of 0-19 mm. is attained in the region of the shell gland and ovary. At the anterior end of the body there is a well-developed collar, 0-20 mm. in diameter, bearing 27 oral spines in two alternating rows. Of the four oral spines borne on each lappet the two anterior pairs measure 0-007 mm. and the two posterior 0-004 mm. in length. Towards the dorsal surface of the collar the hooks decrease slightly in size (Fig. 1).

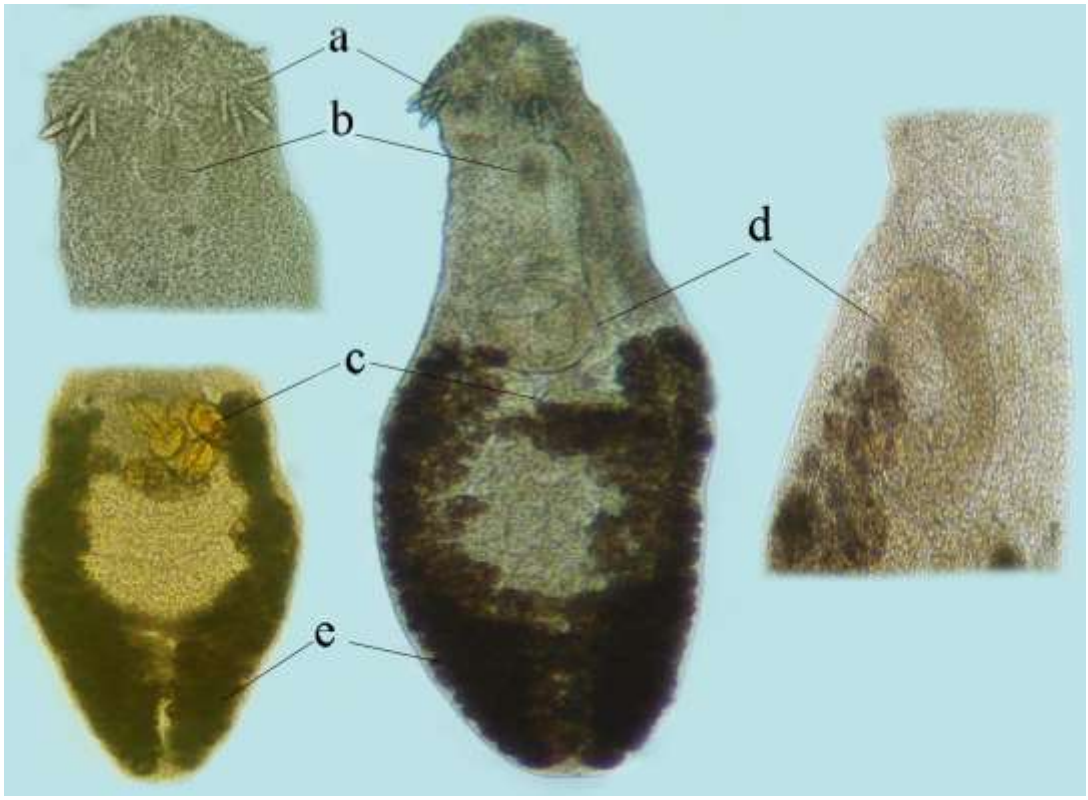

Figure 1. Morphological appearance of *Petasiger exaeretus* Dietz, 1909.

Note: a- Arrangement of collar spines around the oral sucker, b- pharynx, c- eggs, d- acetabulum, e- vitellaria.

The cuticle is armed dorsally and ventrally with rows of spines (5-7/x. long) as far as the ventral sucker. The oral sucker, 0-08-0-09 mm. in diameter, is situated subterminally with its aperture directed ventrally. The ventral sucker lies 0-56 mm. behind the oral and is 0-2 mm.

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Molecular and Genetic Research Results. Based on the molecular-genetic investigations, a 750 base pair sequence of the ribosomal DNA ITS region was isolated from the species *Petasiger exaeretus* Dietz, 1909, which belongs to the genus *Petasiger* Dietz, 1909. The obtained sequence was subsequently analyzed and compared with existing reference data available in the National Center for Biotechnology Information (NCBI) database (see Fig. 2).

According to bioinformatics analysis, the ITS sequence of the studied *P. exaeretus* sample showed a single nucleotide substitution when compared with the *P. exaeretus* sequence available in the NCBI database (Accession No: PP188700). Specifically, an adenine (A) at position 193 in the studied sample was substituted by a guanine (G) in the database sample. The overall genetic divergence between the two sequences was calculated to be 0.13%.

Furthermore, when compared with another species, *P. radiatus* (NCBI Accession No: PP188701), a total of 52 nucleotide differences were identified, corresponding to a 6.9% overall divergence between the sequences.

As a result of the molecular-genetic analyses and bioinformatic processing, the newly obtained ITS sequence data was deposited in the National Center for Biotechnology Information (NCBI), and an accession number was assigned: Petasiger exaeretus–PV449811.

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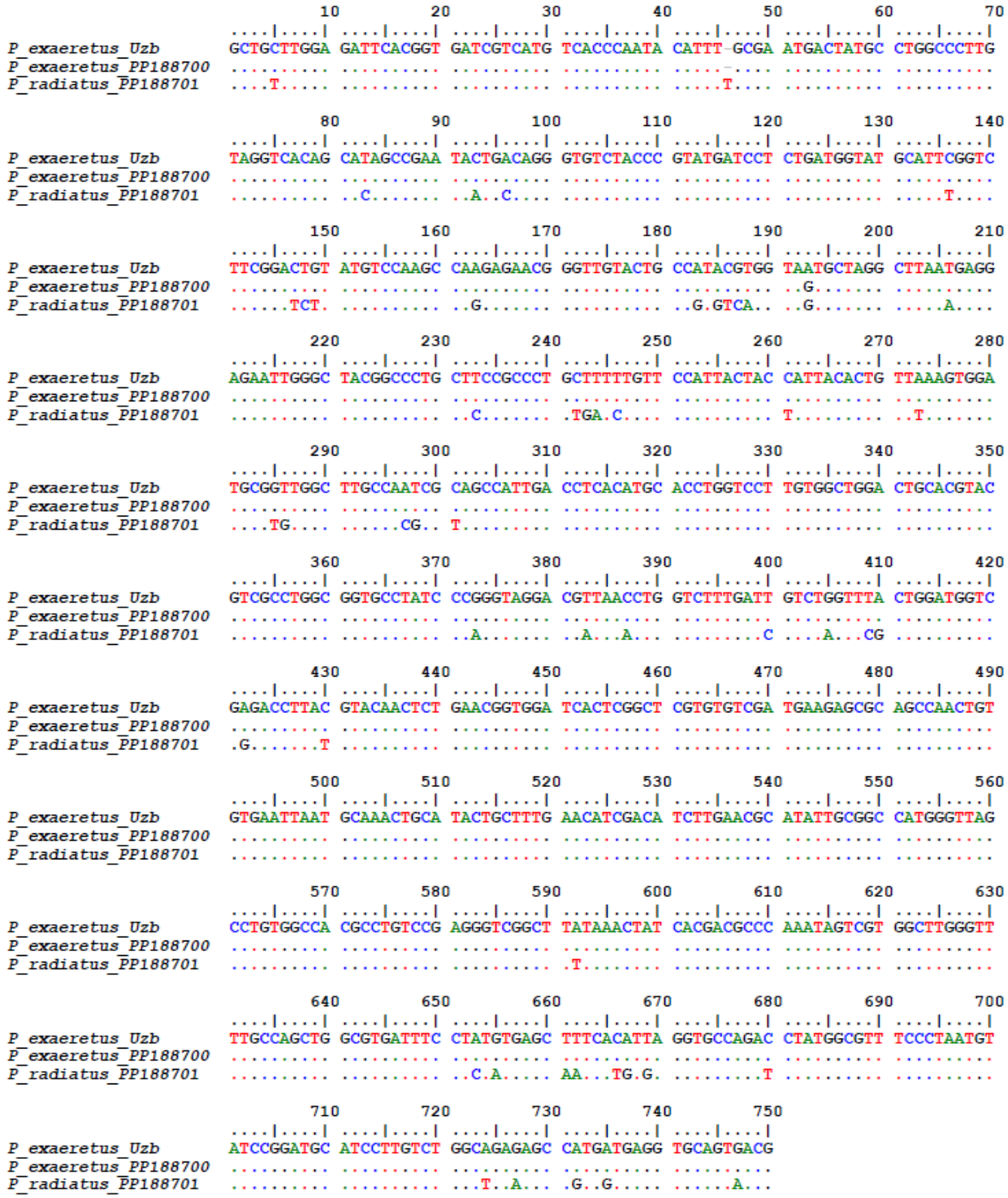

Figure 2. Comparison of nucleotide sequences in the ITS region of ribosomal DNA (rDNA) among species of the genus *Petasiger*, based on sequencing data.

Conclusion. The results of the morphological examination revealed that the body shape variability, along with the arrangement and number of collar spines on the anterior end, represent key diagnostic morphological features for identifying *Petasiger exaeretus*.

Molecular-genetic analyses demonstrated a single nucleotide difference between the *P. exaeretus* sample studied and the reference sequence retrieved from

the NCBI database (*P. exaeretus*, Accession No: PP188700). This difference is most likely attributable to ecological variations in host environments, suggesting that environmental factors may play a role in shaping genetic microdiversity within the species.

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