ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 4| April 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691 || ISI Value: 1.188

MOLECULAR GENETIC CLASSIFICATION OF THE SPECIES *G. OSCULATA* (GOEZE, 1782) BELONGING TO THE GENUS *GLANITAENIA* (CESTODA: *PROTEOCEPHALIDAE*)

Karomat Sultanbayevna Bazarbayeva[1], Oybek Olimjonovich. Amirov[2]
[1]*Khorezm Mamun Academy, Khorazm, Khiva district Center-1, 100053, Republic of Uzbekistan*
[2]*Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan, 232 Bagishamol st., Tashkent, 100053, Republic of Uzbekistan*

Article DOI: https://doi.org/10.36713/epra20871
DOI No: 10.36713/epra20871

ABSTRACT

In this article, nucleotides belonging to the ITS region of the ribosomal DNA of Glanitaenia osculata (Goeze, 1782) found in the flounder (Silurus glanis. Linnaeus, 1758) were analyzed and the phylogenetic relationships of this species were studied. Glanitaenia was differentiated from the other genera of the subfamily Proteocephalinae by the possession of a well-developed, functional apical sucker with a deep cavity (the apical sucker in different species of the Proteocephalus-aggregate is vestigial, without any cavity, or completely absent – see Scholz et al., 1998). However, some morphological details, including characteristics that are recently considered to be of potential taxonomic and phylogenetic importance, such as the development of the uterus, the relative size of the ovary, the morphology of the eggs and terminal genitalia (the distal part of the vaginal canal), and the course of osmoregulatory canals were not provided.

KEYWORDS: *Cestoda, genus, species, ribosomal DNA, ITS, phylogeny, Glanitaenia osculata, Silurus glanis, bioinformatic.*
Financial Support. *This work was carried out by the Khorezm Mamun Academy under the state program.*

INTRODUCTION

There are synonyms like *Taenia osculata* Goeze, 1782; *Proteocephalus osculatus* (Goeze, 1782) La Rue, 1911; *Gangesia osculata* (Goeze, 1782) Reichenbach-Klinke 1962] of the species *Glanitaenia osculata* (Goeze, 1782) belonging to the genus *Glanitaenia* de Chambrier, Zehnder, Vaucher & Mariaux, 2004. According to the data of this species (S.O. Osmonov, 1971), it belonged to the genus *Proteocephalus* Weinland, 1858, later, as a result of molecular genetic studies of this species, it was transferred to the genus *Glanitaenia* with the help of a phylogenetic tree done by nucleotides of 5.8S + ITS2, 18S, and 28S regions of ribosomal DNA. In the diagnosis of *Glanitaenia*, de Chambrier et al. (2004) relied mainly on the morphological features of *G. osculata* described by previous authors, especially Nybelin (1942), Freze (1965), Scholz & Hanzelová (1998), and Scholz et al. (1998) [1, 2, 3,].

P. osculatus is a parasite of the common wels catfish *Silurus glanis L.* The study of *P. osculatus* is interesting as a parasite of the only European representative of the ancient and thermophilic wels catfish family. The few publications on the biology of this species contain mainly information about its occurrence and life cycle characteristics. It is known that *P. osculatus* is a specific parasite of the common wels catfish and is widespread in Central and Southern Europe. Wels catfish infection in the southern regions is close to 100%. The host's infection intensity has seasonal fluctuations, which are associated with the annual development cycle of the parasite [8, 10].

Therefore, the generic diagnosis of Glanitaenia is amended in the present paper and the current distribution of *G. osculata* is reviewed based on new geographical records from Europe and in relation to the current expansion of its fi sh host, wels catfish, which is classifi ed as an invasive species (Copp et al., 2009).

This research work aims to describe the molecular genetics of the species *Glanitaenia osculata* (Goeze, 1782) collected from the digestive system of the wels catfish in the natural and artificial water ponds of the Khorezm region of the Republic of Uzbekistan.

MATERIALS AND METHODS

Collection of Genetic Material. To carry out this research work, during the years 2023-2024, 17 wels catfish (*Silurus glanis*. Linnaeus, 1758) were collected from the natural and artificial water ponds of the Khorezm region of the Republic of Uzbekistan. Including, 7 wels catfish from the Kushkupir district, 3 wels catfish from the Urganch district, 2 wels catfish from the Yangibazar district, and 5 from the Gurlan district were collected and 43 copies of *Glanitaenia osculata* (Goeze, 1782) species were collected from the wels catfish by complete and incomplete helminthological methods. (1-Figure).

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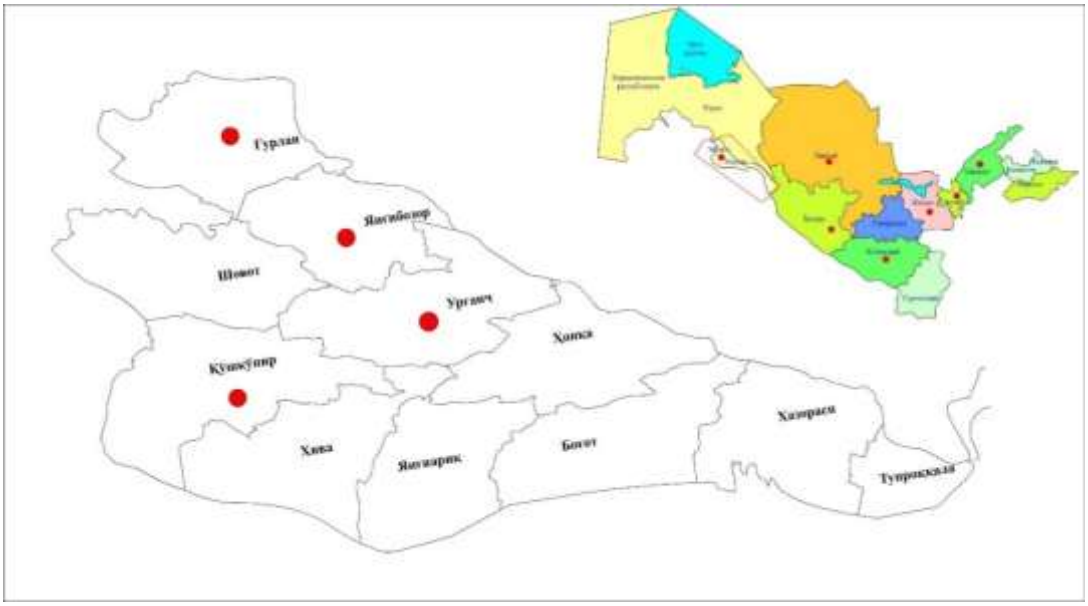


Figure 1. Research areas. **Khorezm region, Republic of Uzbekistan**

Skryabin and Bikhov-Pavlov methods were used to fully dissect the fish, as well as generally accepted methods were used for collecting and processing helminthological samples.

Molecular genetic studies. *Glanitaenia osculata* species belonging to the genus *Glanitaenia* cestode was fixed in 70% ethanol solution and genomic DNA was isolated using head parts for molecular-genetic studies. The GeneJET GENOMIC DNA Reagent was used to extract DNA from selected samples (Vogelstein, B. 1979, Marko, M.A., 1982, Boom, R., 1990, Kuchboev A.E, 2021, Soatov B.B, 2023)

ITS fragments of ribosomal DNA (rDNA), which are widely used in the molecular-genetic characterization of cestodes, trematodes, and nematodes, were isolated using AV28 right (ata tgc tta agt tca gcg ggt) and TW81 reverse (gtt tcc gta ggt gaa cct gc) primers (Curran, J., et al. 1994, Mardanova G., 2023, Turgunov S.N., 2024). PCR recipe: primary DNA denaturation at 94ºC for 3 min, followed by 9 cycles consisting of denaturation at 94ºC – 1 min, annealing at 55ºC – 1 min 30 s and elongation at 72ºC – 1 min 30 s; then 24 cycles, consisting of denaturation at 94ºC – 45 s, annealing at 57ºC – 1 min and chain elongation at 72ºC – 1 min 20 s; followed by final elongation at 72ºC for 5 minutes. The results of the PCR reaction were checked by electrophoresis of 1 µl of the product in a 1% agarose gel (100 V, 80–100 mA, approximately 30-40 min).

Sequencing was carried out at the "Genotech" Center for Use (formerly "Genome"), obtaining the result in the form of "ab1" files, which were read in the "Chromas 1.45" program. Further analysis of sequences (construction of alignments, trees, analysis of nucleotide differences) was carried out using the programs "Clustal X version 1.81" (Jeanmougin F, 1998), «Gendoc version 2. 5. 000» и PAUP* 4.0b10, Mega11.

RESULTS AND THEIR ANALYSIS

Results of Molecular Genetic Research. According to the results of the molecular genetic research conducted on the *G. osculata* species belonging to the genus *Glanitaenia*, nucleotides with a length of 709 pairs of bases belonging to the ITS region of rDNA were isolated and for comparative study of these species, the national bioinformatics information marquee (https://blast.ncbi. nlm.nih.gov) type *G. osculata* (Accession number: AY551169) was used.

According to the results of the bioinformatic analysis, it was found that there are differences in 5 nucleotides between the *G. osculata* sample and the *G. osculata* sample obtained from the genebank database (accession number: AY551169) (Figure 2).

These differences are T-thymine in *G. osculata* sample at nucleotides 209, 213 and 432, C- cytosine in *G. osculata* sample (accession number: AY551169), A-adenine in *G. osculata* sample at nucleotides 398 and 631, *G. osculata* (accession no. number: AY551169) and it was found that T-thymine nucleotides were exchanged. The index of difference between the nucleotides of both samples was 0.7%. This identified indicator cannot be used as evidence that the *G. osculata* specimen from the genebank (accession number: AY551169) is a separate species. This difference can be explained by the ecological factors of the place where the species of *G. osculata* is found and the type of host.

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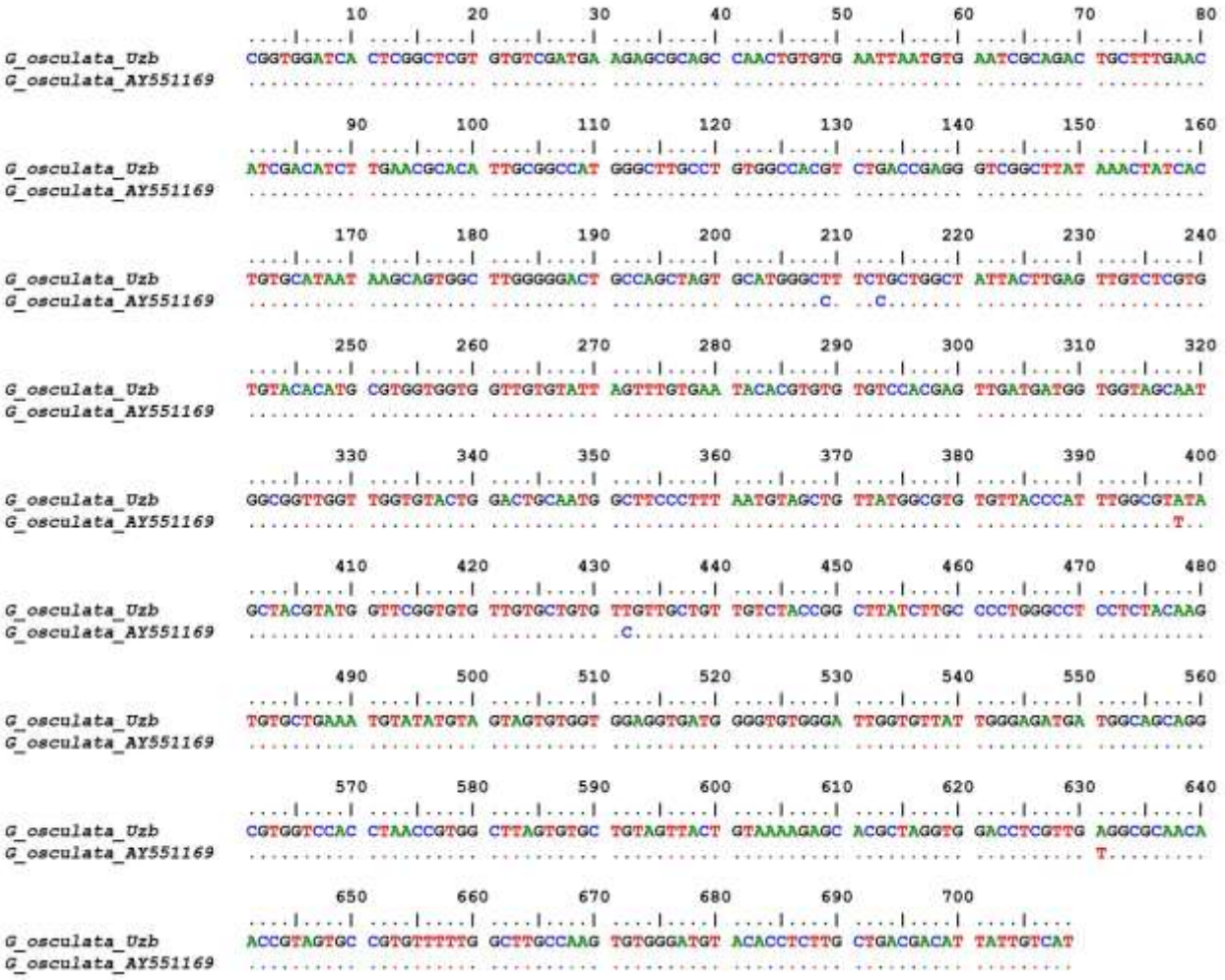


Figure 2. Comparison of nucleotide sequence of rDNA ITS region of *G. osculata* species from *Glanitaenia* genus and *G. osculata* (accession number: AY551169) samples from Genbank database based on sequence materials.

Phylogenetic Tree. The rRNA ITS gene sequence of the *G. osculata* Uzb was compared with 28S rDNA sequences of the related species available in the GenBank database using blast analysis at NCBI (www.ncbi.nlm.nih.gov/). The phylogenetic tree was reconstructed using the Maximum Likelihood method in the mega X software package. The evolutionary distances were computed using the maximum composite likelihood method. Bootstrap analysis with 1000 replicates was performed to estimate the support of clusters (**Figure 3**).

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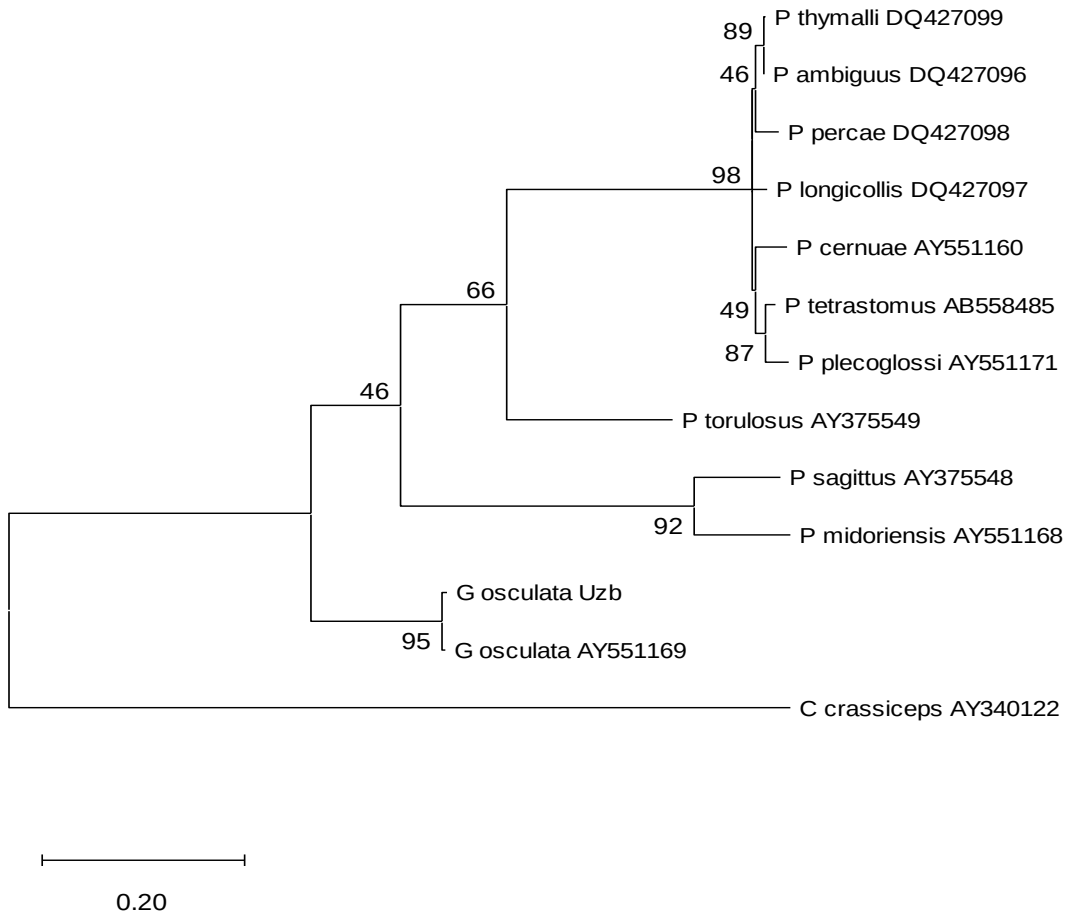


Figure 3. Phylogenetic tree generated by the Maximum Likelihood method using rRNA ITS gene sequence of the *Glanitaenia osculata* Uzb and closely related species, belonging to the subfamily *Proteocephalinae* Mola, 1929.

Phylogenetical analysis of the rRNA ITS gene sequence shows, that *G. osculata* Uzb is most closely related to *G. osculata* AY551169 (bootstrap support 95%, see Figure 1) and forms separate cluster with this organism. Another cluster form Paraproteocephalus sagittus and Paraproteocephalus midoriensis (bootstrap support 92%, Figure 1). Species *Paraproteocephalus thumalli, P. ambiguous, P. percae, P. longicollis, P. cernuae, P. tetrastomus* and *P. plecoglossi* are united into one common clade (bootstrap support 98%, Figure 1). Close to this clade and to cluster formed of Paraproteocephalus sagittus and Paraproteocephalus midoriensis, but separately from them is located Paraproteocephalus torulosus (Figure3).

CONCLUSIONS AND RECOMMENDATIONS

Nucleotides belonging to the ITS region of the ribosomal DNA of *G. osculata* species found in the flounder found in the Khorezm region of our republic were analyzed, and it was found that there were 4 differences between the nucleotides of the sample of *G. osculata* (accession number: AY551169) in the NCBI database, and there was a difference of 0.7%.

Acknowledgements

The work was carried out within the framework of the program "Molecular identification of hoofed animals and their parasitic nematodes" implemented by the Academy of Sciences of the Republic of Uzbekistan.

Conflict of Interest

The authors have declared no conflict of interest.

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Information about The Authors

Karomat Sultanbayevna Bazarbayeva - PhD student In Khorezm Mamun Academy. Lecturer, Department of Biotechnology, Urgench State University. Hamid Olimjon street, Urgench city 14. 220100 ORCID iD: 0009-0004-4664-7501