Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 717 – 726 (2024)
www.ejabf.journals.ekb.eg

Morphological, Epizotological, and Molecular-Genetic Description of the Species *Schyzocotyle acheilognathi* (Yamaguti, 1934), a Parasite of the Digestive System of Fishes of Khorezm Region's Reservoirs, Uzbekistan

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ARTICLE INFO

Article History:
Received: Oct. 13, 2024
Accepted: Nov. 20, 2024
Online: Nov. 27, 2024

Keywords:
Terentievi,
Helminth,
Morphology,
Molecular phylogeny,
Ribosomal DNA,
ITS region,
Genbank

ABSTRACT

This article provides information on the morphology, epistemology, and molecular genetics of the cestode species *Schyzocotyle acheilognathi* (Yamaguti, 1934), which belongs to the genus *Schyzocotyle* Akhmerov, 1960. This species is a parasite of the digestive systems of the fish *Cyprinus carpio* Linnaeus, 1758, *Carassius gibelio* Linnaeus, 1758, and *Channa argus* Cantor, 1842, in the water reservoirs of the Khorezm region. As a result of this study, samples of *S. acheilognathi* found in carp and the silver crucian carp were compared. Differences were detected, accounting for 0.36% of the total nucleotide variation. This variation can be attributed to the interaction of environmental factors affecting the host and the environment in which the helminth resides.

INTRODUCTION

Until now, animals of the fauna of our republic, including fish **(Quvatov *et al.*, 2023)**, nematodes **(Aliyev, 2024; Mirzaev, 2024)**, and insects **(Kimyonazarov *et al.*, 2024)** have been studied at the molecular level.

Schyzocotyle acheilognathi (Yamaguti, 1934) of the genus *Schyzocotyle* Akhmerov, 1960 is a cestode species adapted to live in the intestines of fish native to East Asia; originally, this spcies was identified in carp fish in 1934 by Yamaguti and named as *Bothriocephalus acheilognathi* **(Yamaguti 1934; Kuchta *et al.*, 2018)**. This species has 7 close synonyms for the structure and shape of the nipples on the head and the structure of

the jointed genitalia (*Bothriocephalus opsariichthydis, Bothriocephalus gowkongensis, Bothriocephalus sinensis, Bothriocephalus phoxini, Bothriocephalus aegyptiacus, Bothriocephalus kivuensis, Coelobothrium gambusiense*) **(Brabec *et al.*, 2016; Xi *et al.*, 2016)**.

The cestode species *S. acheilognathi* (Yamaguti, 1934) has been recorded in 38 families, 14 genera, and 312 species of fish worldwide **(Kuchta *et al.*, 2018)**. Moreover, this parasite has been detected in other vertebrates, including reptiles, amphibians, and birds **(Garcia-Prieto *et al.*, 1991; Scholz, 1999; Kuchta *et al.*, 2018)**.

This type of parasite entered the Asian countries as a result of bringing carp from Europe and North America in the 1960s and 1970s **(Matey *et al.*, 2015)** and currently found in all continents except Antarctica; the level of damage is high, bringing the hosts to death and causing great economic damage **(Han *et al.*, 2010; Xi *et al.*, 2016)**.

Microecosystems in the gastrointestinal tract of vertebrates, including bacteria, fungi, protozoa, and helminths and helminth larvae, lead to changes in host physiology and homeostasis **(Peachey, 2017)**. Fish infected with *S. acheilognathi* species can cause bothriocephalosis, gastrointestinal obstruction, intestinal mucosal damage, intestinal rupture, abdominal distension, weight loss, protein depletion, anemia, reduced buoyancy, and death **(Davydov, 1978; Scott & Grizzle, 1979; Brouder, 1999; Hansen *et al.*, 2006; Matey *et al.*, 2015)**.

Studies on the systematics and phylogeny of helminths have utilized ribosomal DNA (ITS rDNA) nucleotide sequence analysis **(Bowles, 1995)**. Currently, the taxonomy of parasitic helminths and their larvae in vertebrates is complex, with a diverse species composition. Molecular genetic studies are increasingly employed to identify parasites at various developmental stages **(Kuchboev, 2021; Ikromov, 2023; Soatov, 2023; Turgunov, 2024)**.

Research on the species *S. acheilognathi* was conducted by **Yera *et al.* (2013)**, who characterized the CO1 region of its mitochondrial DNA based on nucleotide sequencing.

The purpose of this research work is the molecular-genetic classification of *S. acheilognathi* (**Yamaguti, 1934**) belonging to the genus *Schyzocotyle* Akhmerov, 1960, which was found in the digestive system of the carp, silver sole, and eel found in the Khorezm region of our Republic.

MATERIALS AND METHODS

Helminthological studies

To conduct this research, samples were collected from natural and artificial water reservoirs in the Khorezm region of the Republic of Uzbekistan during 2023-2024. A total of 33 carp (*Cyprinus carpio* Linnaeus, 1758), 42 crucian carp (*Carassius gibelio* Linnaeus, 1758), and 19 snakeheads (*Channa argus* Cantor, 1842) were gathered (Table. 1 & Fig. 1).

Table 1. Number of fish species examined

№	Districts of Khorezm region	Common carp (*Cyprinus carpio*)	Crucian (*Carassius gibelio*)	Snakeheads (*Channa argus*)
1	Koshkopir	7	5	5
2	Urganch	9	10	4
3	Gurlan	11	8	6
4	Bogot	8	7	4
5	Khozorasp	12	3	7
6	Shovat	13	6	4
7	Yangibozor	5	9	5
8	Tuproqqala	6	3	4
Total		**71**	**51**	**39**

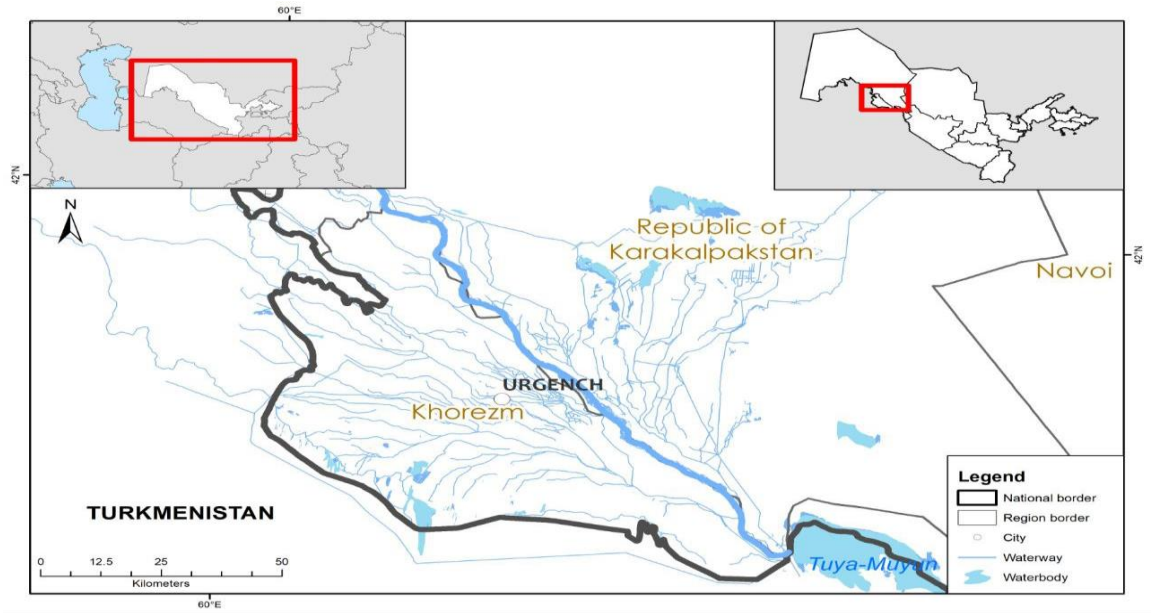

Fig. 1. Research areas

The Scriabin and Bykhovskoy-Pavlovskoy methods for complete fish dissection were employed, and the collection and processing of helminthological samples followed

established protocols **(Markevich, 1951; Dogel, 1962; Bykhovskaya-Pavlovskaya, 1985)**. To assess the impact of helminth infections on fish, we calculated the extent of invasion (IE)—the percentage of infected fish relative to the total examined—and the intensity of invasion (II)—the average number of parasites per infected individual.

Data from various tracers were utilized to determine the species composition of the identified parasites **(Osmanov, 1961; Avdeev *et al.*, 1987)**. The identification of helminth species was conducted using ML 2000 (Meiji) and Olympus CK 2, Zeiss AX 10 microscopes. For identifying helminths found in fish, we referred to several resources, including the "Parasites of Fish in Uzbekistan" **(Osmanov, 1971)**, "Identifier of Parasites of Freshwater Fish Fauna" **(USSR, 1984, 1985, 1987)**, and "Metacercariae of Trematodes—Parasites of Aquatic Organisms of Russia" **(Sudarikov *et al.*, 2002)**. This was along with other literature **(Osmonov, 1971; Sudarikov *et al.*, 1974; Ryssai *et al.*, 1984)**. Statistical analysis of species morphometric data was conducted using Biostat 2007 and Microsoft Office Excel 2010.

Molecular genetic studies

For molecular genetic studies, the cestode *S. acheilognathi* **(Yamaguti, 1934)**, belonging to the genus *Schyzocotyle* Akhmerov, 1960, was preserved in a 70% ethanol solution, and genomic DNA was isolated from the head parts. The GeneJET Genomic DNA Reagent Kit was used for DNA extraction **(Vogelstein, 1979; Marko, 1982; Boom, 1990)**.

We isolated ITS fragments of ribosomal DNA (rDNA), commonly used for the molecular genetic characterization of cestodes, trematodes, and nematodes, using AV28 forward (ata tgc tta agt tca gcg ggt) and TW81 reverse (gtt tcc gta ggt gaa cct gc) primers **(Curran *et al.*, 1994)**. The PCR protocol included initial DNA denaturation at 94°C for 3 minutes, followed by 9 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute 30 seconds, and elongation at 72°C for 1 minute 30 seconds. This was followed by 24 cycles of denaturation at 94°C for 45 seconds, annealing at 57°C for 1 minute, and chain elongation at 72°C for 1 minute 20 seconds, concluding with a final elongation at 72°C for 5 minutes. The results of the PCR reaction were verified by electrophoresis of 1.0µl of the product in a 1.0% agarose gel (100 V, 80–100 mA, approximately 30–40 minutes).

Sequencing was performed at the Genotech Center for Collective Use (formerly Genome), producing results in the form of AB1 files, which were analyzed using the Chromas 1.45 program. Further sequence analysis, including alignment, phylogenetic tree construction, and nucleotide difference analysis, was conducted using Clustal X version 1.81 **(Jeanmougin, 1998)**.

RESULTS

The cestode was identified as *S. acheilognathi* based on the following combination of characteristics (measured in micrometers):

- Body length: approximately 2550
- Scolex: heart-shaped, measuring 581.3 long × 723.4 wide, with narrow, deep dorsal and lateral bothrias that are short and very deep
- Neck: absent, with the first proglottid immediately posterior to the scolex
- Immature proglottids: much narrower than the scolex, measuring 196.7 long × 203.97 wide
- Mature proglottids: 224.2 long × 534.1 wide
- Gravid proglottids: 316.7 long × 769.5 wide
- Immature, mature, and gravid segments: rounded edges
- Cirrus sac: 77 long × 70 wide
- Medullary testes: present
- Lobed ovary: 365.5 long × 179 wide, located near the posterior margin of the proglottids
- Eggs: 47-59 long × 31-37 wide (Fig. 2).

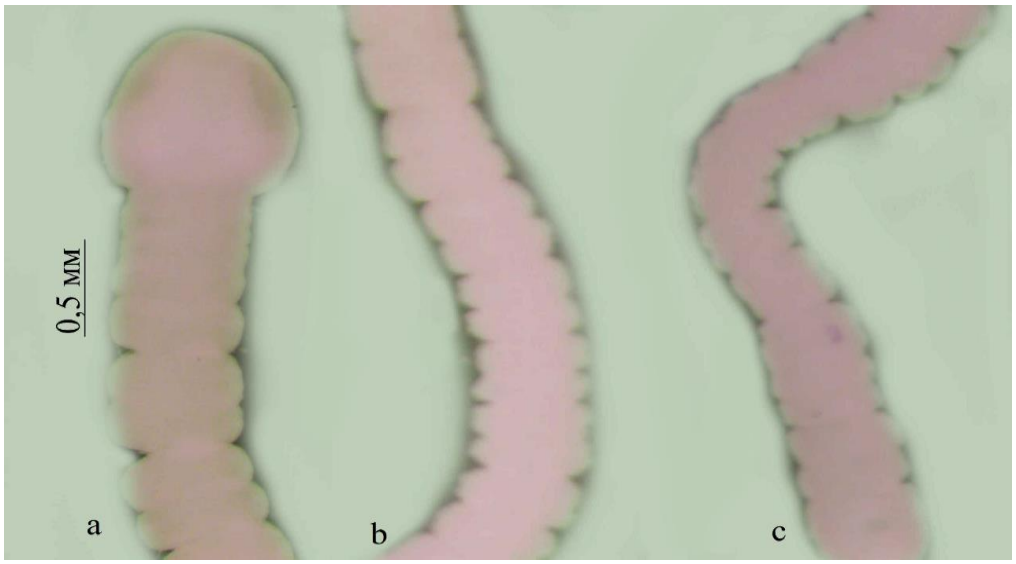

Fig. 2. Morphological appearance of the species *S. Acheilognathi* showing: **a-** Head part; **b-**Joint part; **c-**Tail part

Results of epistemological research

According to the results of helminthological research conducted on the carp, crucian carp, and snakeheads in the Khorezm region, out of 71 carp examined, 32 were found to be infected, resulting in an average infection extent (IE) of 45.07% and an intensity of invasion (II) of 1-21. In the case of the crucian carp, 18 out of 51 were infected, yielding an average IE of 35.3% and an II of 1-15. Among 39 examined snakeheads, 13 were infected, with an average IE of 33.3% and an II of 1-11. The samples infected with *S. acheilognathi* are summarized in Table (2).

Table 2. Damage indicators of the species *S. acheilognathi*

№	Districts of Khorezm region	Number of fish examined; number of injured fish; (IE%), in copy II		
		Cyprinus carpio	***Carassisus gibelio***	***Channa argus***
1	Kushkpir	7/4, (57.14), 1-12	5/1, (20), 5	5/3, (60), 1-7
2	Urganch	9/3, (33.3), 1-9	10/3, (33.3), 1-9	4/1, (25), 5
3	Gurlan	11/5, (45.4), 1-17	8/3, (37.5), 1-12	6/2, (33.3), 1-9
4	Bogot	8/3, (37.5), 1-9	7/4, (57.5), 1-15	4/1, (25), 3
5	Khozorasp	12/6, (41.6), 2-14	3/1, (33.3), 3	7/2, (28.6), 1-5
6	Shovot	13/7, (53.8), 1-21	6/2, (33.3), 1-8	4/1, (25), 3
7	Yangibozor	5/2, (20), 1-7	9/3, (33.3), 2-6	5/2, (40), 1-11
8	Tuprokkala	6/2, (33.3), 1-5	3/1, (33.3), 4	4/1 (25), 4

Results of molecular genetic research

According to the results of the molecular genetic research conducted on samples of *S. acheilognathi* belonging to the genus *Schyzocotyle* Akhmerov, 1960, identified in the carp, crucian carp, and snakehead, nucleotides with a length of 821 base pairs from the ITS region of rDNA were isolated. The type sequence of *S. acheilognathi* (accession number: MN341860) obtained from the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov) was used to compare the nucleotides of the samples.

Bioinformatic analysis revealed no differences between the nucleotides of *S. acheilognathi* samples detected in the carp and crucian carp and the sample from NCBI (accession number: MN341860) (Fig. 3). However, the *S. acheilognathi* specimen identified in the snakehead exhibited three nucleotide differences:

1. A-adenine at the 152nd nucleotide was present in the carp and crucian carp samples, but S-cytosine was found in the snakehead sample.
2. S-cytosine at the 601st nucleotide was observed in the carp and crucian carp samples, while T-thymine was present in the snakehead sample.
3. G-guanine at the 624th nucleotide was found in the carp and crucian carp samples, whereas S-cytosine was identified in the snakehead sample.

These differences in nucleotides between the snakehead and the other two samples accounted for a 0.36% variation. However, the differences observed do not provide conclusive evidence that the specimens from the snakehead, crucian carp, and carp

represent separate species. These variations can be explained by environmental factors affecting the *S. acheilognathi* population found in the snakeheads and the type of host.

The nucleotide sequences obtained from this molecular genetic research have been submitted to the international bioinformatics information center, and accession numbers were obtained (Accession number: PQ358427).

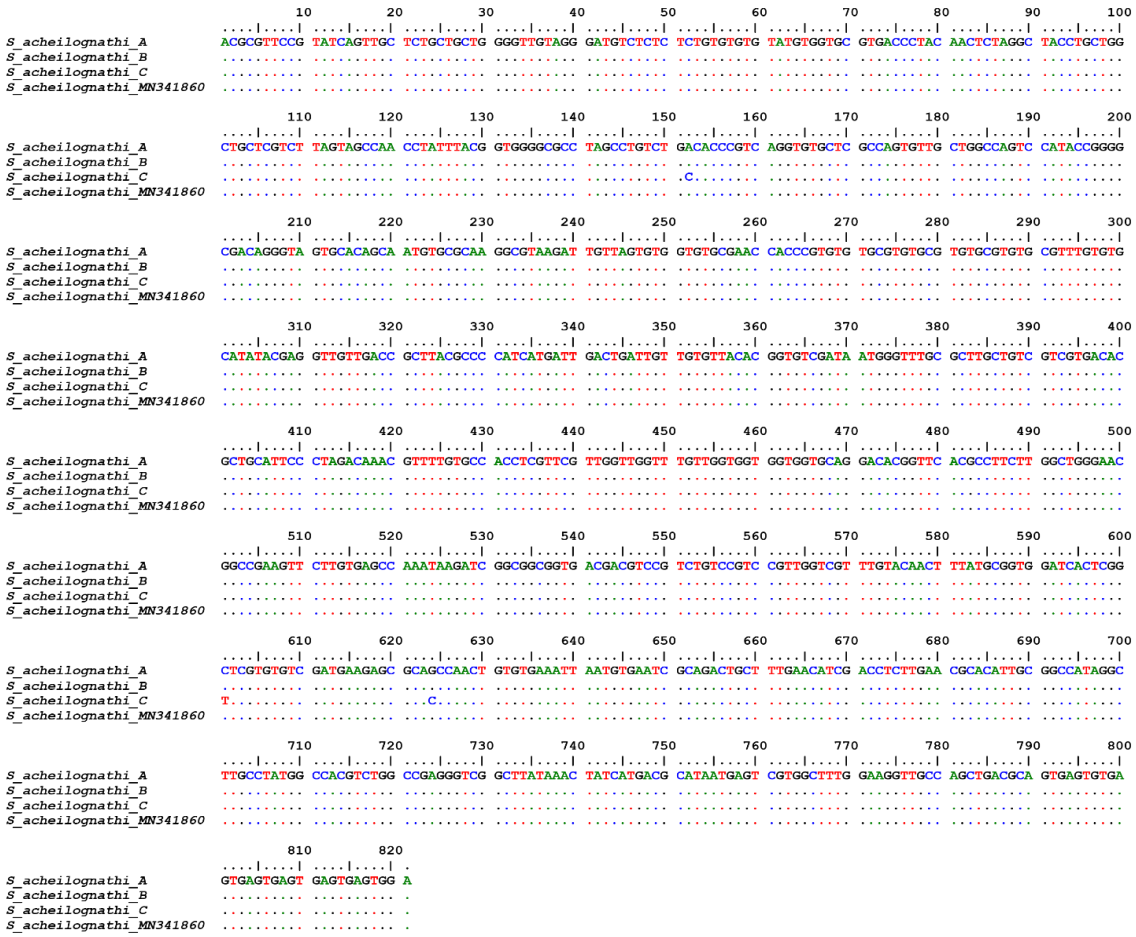

Fig. 3. Comparison of rDNA ITS region nucleotide sequences of *S. acheilognathi* species from the genus *Schyzocotyle*Akhmerov, 1960 and *S. acheilognathi* (accession number: MN341860) from the Genbank database based on sequence material

Notes:

- **A:** Nucleotide of the identified species *S. acheilognathi* from the carp.
- **B:** Nucleotide of the identified species *S. acheilognathi* from the crucian carp.
- **C:** Nucleotide of the identified species *S. acheilognathi* from the snakehead.

CONCLUSION

Morphometric indicators of *S. acheilognathi* (Yamaguti, 1934), belonging to the genus *Schyzocotyle* Akhmerov, 1960, were determined in specimens found in the

digestive systems of the carp, crucian carp, and snakeheads. These morphometric indicators are consistent with those reported by other researchers.

In terms of epizootiology, it was noted that the crucian carp and snakeheads exhibit lower infection rates compared to carp.

The molecular genetic research revealed that the *S. acheilognathi* sample identified in the snakehead showed nucleotide differences compared to those from the carp and crucian carp. This difference can be attributed to the species' dependence on the type of host and the environmental factors influencing their living conditions.

GRATITUDE

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.

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