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ORIGINAL ARTICLE

Scanning Electron Microscopy of Acanthocephala Isolated from *Saurida tumbil* in Minab City, Hormozgan Province, South Coast of Iran

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Abstract

The Synodontidae family is a group of marine fish that includes four genera and 57 species and is distributed throughout all waters of the world. A total of 150 lizardfish (*Saurida tumbil*) were randomly sampled from various fish markets in Minab city, Hormozgan province, Iran, from June 2022 to May 2023. The fish were dissected, and their digestive tracts were examined for acanthocephalans. Taxonomic identification of the parasites was based on morphological characteristics described using a light microscope and then confirmed by Scanning Electron Microscope (SEM) to clarify ambiguous details. To prepare the parasites for observation using SEM, some samples were placed in 2.5% glutaraldehyde diluted with 0.1 M sodium cacodylate buffer for 2 to 4 hours at 4°C. After primary fixation, washing was performed three times with 0.1 M sodium cacodylate buffer, each wash lasting 15 minutes. For secondary fixation, 1% osmium tetroxide was applied for two hours. Following a repeat of the washing steps, the dehydration stage was performed using ascending grades of alcohol, with each grade applied for 15 minutes. Finally, the samples were dehydrated and dried in a desiccator. The prepared samples were mounted on copper stubs, then coated with a 4 to 7 nm thick layer of gold using a sputter machine to enable imaging. The images were acquired using a Leo scanning electron microscope. The collected specimens exhibited characteristics typical of the genus. Parasite indices of prevalence, intensity, and mean abundance were evaluated for each parasite species. The discovered samples were identified morphologically as *Neoechinorhynchus* spp. and *Quadrigyrus* spp. from ten *Saurida tumbil*. This is the first report of *Neoechinorhynchus* and *Quadrigyrus* parasitizing *Saurida tumbil.*

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Introduction

The waters of the Persian Gulf and the Sea of Oman are connected through the Strait of Hormuz, which is 560 km long and 320 km wide (1). One reason for the great diversity of aquatic life in the southern regions of Iran is the connection between the waters of the southern basin and the open ocean. To date, about 907 species of fish in 157 families have been identified from the Persian Gulf and the Sea of Oman. The lizardfish family (Synodontidae) is a group of marine fish that includes four genera and 57 species, distributed throughout the world's waters (2). One of the most well-known of these species is *Saurida tumbil*, whose primary habitat is the Oman Sea and the Persian Gulf; however, it is also found in parts of the eastern coast of Africa to the Arabian Sea, Southeast Asia, the Red Sea, and Australia (3). Lizardfish are typically found at a depth of 20 to 60 meters (4). One characteristic of this fish is its small, cylindrical size with an elongated shape, measuring between 19 and 35 cm in length. The fish has a light brown and silvery white body on the dorsal and ventral sides, respectively, with pale cross stripes (5). This species inhabits coral reefs, primarily in tropical waters. Crustaceans, squid, and other fish are the main food of lizardfish (4). Parasitic infections represent one of the greatest risks in the aquaculture and fisheries industry (6). Therefore, considering the increase in losses and the decrease in fish population and the value of natural reserves, research on fish parasitic diseases can be of great importance (7). Acanthocephala are parasitic worms that can infect a wide range of animals (8). To date, 157 genera and 1,298 species of acanthocephalans have been studied (9). Acanthocephala are identified by a fixed proboscis as an attached organ. Their sexes are separate and females can be distinguished by their bell-shaped uterus and males by their cement glands (10). The proboscis organ plays an essential role in connecting Acanthocephala to the host's intestinal wall. Consequently, it can cause serious tissue damage and mortality in various hosts worldwide (6). Based on research, some Acanthocephala species can penetrate deep into the intestinal wall and cause irreparable pathological damage (8). Adult Acanthocephala inhabit the intestines of various vertebrates that serve as definitive hosts (fishes, birds, amphibians, and mammals) and are usually infected by ingestion of intermediate hosts such as brine shrimp and cyclops (11). In some cases, the host can serve as a paratenic host for some species, in which no development occurs (12). According to reports, nearly 25% of marine animals, such as bony fish, are infected with Acanthocephala, harboring the mature stages of the parasite (13). While most species are non-zoonotic, some can infect humans and cause disease as paratenic hosts in fish (8). Acanthocephalans of the genus *Neoechinorhynchus* usually infect fish and turtles, with approximately 116 described species divided into two subgenera, *Neoechinorhynchus* and *Hebesoma*, both of which have a worldwide distribution (14). The genus *Quadrigyrus* is another acanthocephala of fish, classified within the family Quadrigyridae and has two subfamilies, Palacentinae and Quadrigyrinae (15). Most species identified in this genus have been reported from Latin American countries such as Brazil and Argentina (15–18). The arrangement of hooks on the proboscis is a key characteristic for identifying the mature stages of acanthocephalans, which can be seen in this group of worms (19). In addition to light microscopy, Scanning Electron Microscopy (SEM) can aid in detecting some overlooked features in acanthocephalans (20). In Iran, there is limited information about the species of fish acanthocephalans in the Persian Gulf and the Sea of Oman. Therefore, this study was conducted for the first time in Minab city, Hormozgan province, located in southern Iran, with the aim of accurately identifying acanthocephalans in *S. tumbil* using SEM.

Materials and Methods

Study Area

Minab is a city in Hormozgan province located in southern Iran, at geographical location of 57°41' east, 27°6' north. Minab has a hot and humid desert climate. The maximum temperature in summer averages 37 degrees Celsius from April to September, and the minimum temperature in winter can drop to 5 degrees Celsius. The annual rainfall is about 124 mm. Humidity levels in Minab vary throughout the year, with August being the wettest month at 58% humidity and May the driest at 45% humidity.

Sampling Method

This study was conducted over a one-year period between June 2022 and May 2023. A total of 150 specimens of *S. tumbil* were randomly selected from fish supply stores in Minab city to investigate contamination with acanthocephalic parasites. The fish were promptly placed in ice boxes and transferred to the parasitology laboratory of the Faculty of Veterinary Medicine at Shahid Bahonar University of Kerman, Iran. The weight and length of each fish were measured and recorded in the laboratory. Fish species were identified using valid identification keys (21).

Diagnosis and Identification of Acanthocephalans

After recording the data, the fish were dissected, and their digestive systems were examined for the presence of acanthocephalans. Once separated, the acanthocephalans were placed in a saline solution so that, through the process of organ cooling, their proboscises protruded, making the hooks and spines visible. Subsequently, the samples were first fixed in 70% ethanol and 5% formalin, then stained with carmine dye and dehydrated through graded ethanol concentrations. In the next step, the samples were cleared in xylene and mounted with Canada balsam glue. The samples were identified using an optical microscope (Olympus, Japan) and valid identification keys (22, 23).

Preparation of Acanthocephalans for SEM

To prepare the parasites for observation using SEM, some samples were placed in 2.5% glutaraldehyde diluted with 0.1 M sodium cacodylate buffer for 2 to 4 hours at 4°C. After primary fixation, washing was performed three times with 0.1 M sodium cacodylate buffer, each wash lasting 15 minutes. For secondary fixation, 1% osmium tetroxide was applied for two hours. Following a repeat of the washing steps, the dehydration stage was performed using ascending grades of alcohol, with each grade applied for 15 minutes. Finally, the samples were dehydrated and dried in a desiccator. The prepared samples were mounted on copper stubs, then coated with a 4 to 7 nm thick layer of gold using a sputter machine (SC7620, UK) to enable imaging. The images were acquired using a Leo scanning electron microscope (LEO1450VP).

Results

In this study, out of 150 fish examined, 10 individuals (6.66%) were infected with acanthocephalans. Three fish (2%) were infected with *Neoechinorhynchus* parasite, and seven fish (4.66%) were infected with *Quadrigyrus* parasite. The frequency of contamination was 0.13 and the intensity of contamination was 2. A total of 20 acanthocephalans were isolated from these fish, of which 12 belonged to the genus *Quadrigyrus* (8 males and 4 females) and 8 belonged to the genus *Neoechinorhynchus* (6 males and 2 females). The characteristics of *Neoechinorhynchus* parasites are as follows: Family: Neoechinorhynchidae, Superfamily: Neoechinorhynchinea, and Genus: *Neoechinorhynchus* (24).

Parasite Description

Both males and females were small to medium in size. The average length of the collected specimens ranged from 7.5 to 10 mm. The shape of the trunk was mostly spindle or rectangular, and in some cases, cylindrical. The surface of the body had a row of wrinkled lines and was covered with spines on the front part. These spines formed 11 to 17 rows in the Acanthocephala of the present study. The spines were short, narrow cones with their tips oriented towards the end of the parasite (Figures 1 and 2).

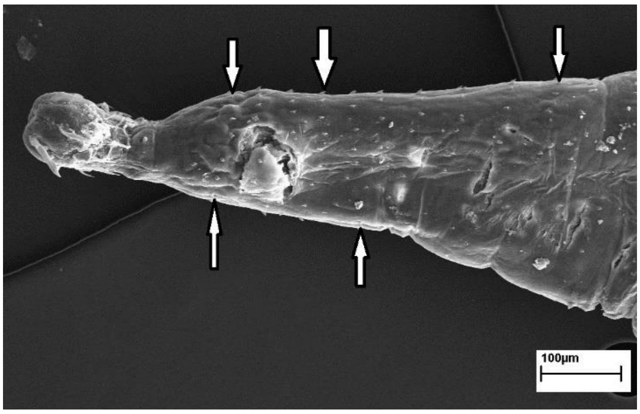

Figure 1. Anterior end of male *Neoechinorhynchus* with 11 rows of spines on trunk (white arrows)

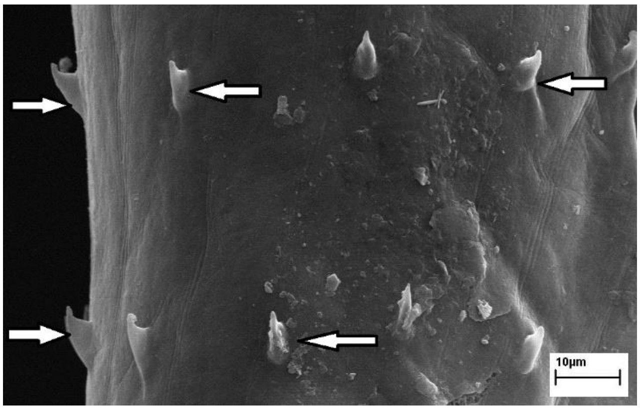

Figure 2. Anterior end of male *Neoechinorhynchus*, higher magnification of some spines (white arrows)

A short, spherical to somewhat cylindrical proboscis protruded from the trunk, with well-defined curved hooks. The apical region was slightly drawn inward, and one of the sensory pits was clearly visible. The proboscis hooks were arranged in three rows, with six hooks, and only the anterior ones had a prominent base. The front ring had long and equal hooks. The hooks of the middle ring were smaller than those of the anterior ring and larger than those of the posterior ring. The hooks of the last two rings lacked bases. The proboscis and indistinct neck were prominent relative to the trunk, with a thick cuticle forming a collar or belt at the anterior end of the trunk (Figure 3).

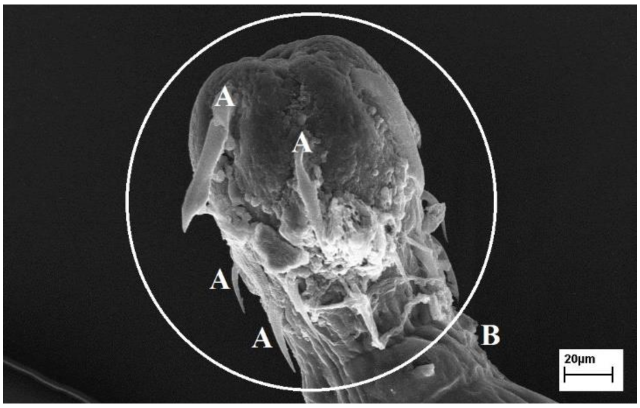

Figure 3. Proboscis (circle) and its hooks (A) of *Neoechinorhynchus* along with cuticular collar (B) at the anterior end of the trunk

Micropores were present up to near the junction of the spines in the middle and caudal parts of the body, but they did not progress on them (Figures 4 and 5).

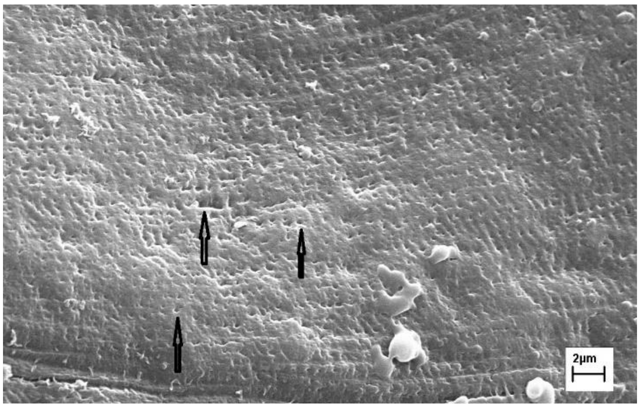

Figure 4. The middle part of *Neoechinorhynchus* body with large and abundant micropores (black arrows)

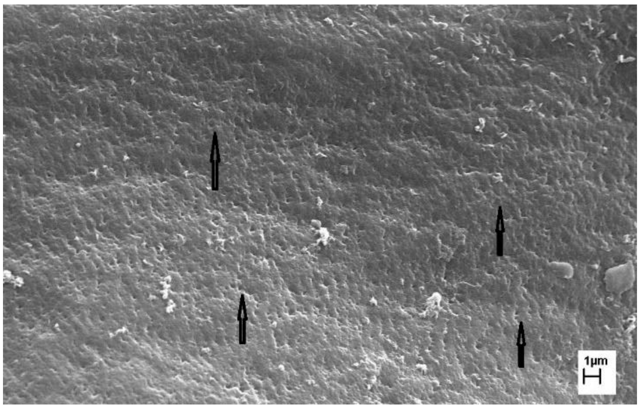

Figure 5. The caudal part of *Neoechinorhynchus* body with smaller micropores (black arrows)

At the caudal end of the male *Neoechinorhynchus*, a short genital bursa was observed, bent towards the ventral side. No sensory plates were present around the bursa, and no distinct sensory receptors were visible in the opening of the bursa (Figure 6). The characteristics of Q*uadrigyrus* parasites are as follows: Family: Quadrigiridae, Superfamily: Quadrigirinae, Genus: *Quadrigyrus* (25).

Figure 6. Posterior end of male *Neoechinorhynchus* with genital bursa (A)

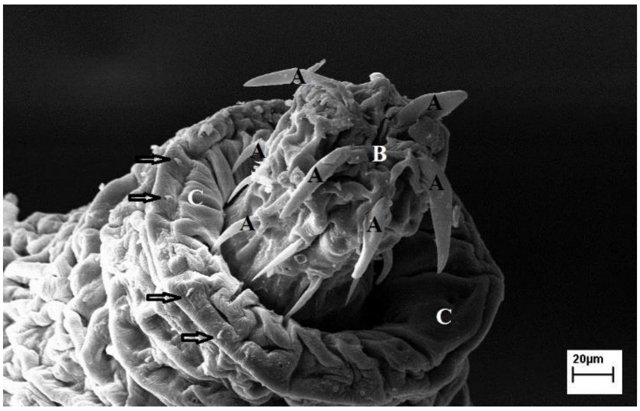

Figure 7. Anterior end of *Quadrigyrus* with a distinct proboscis, hooks (A), and the apical region (B). The first row of trunk spines (black arrows) is visible at the posterior end of collar (C).

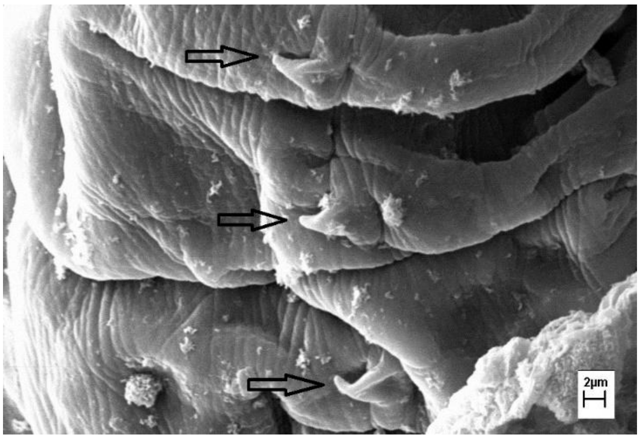

Figure 8. Anterior end of *Quadrigyrus*, higher magnification of a row of spines (black arrows)

This genus of worms exhibited a small to medium-sized body, with a significant increase in the anterior region. The average length of the collected specimens ranged from 5.5 to 7.25 mm. Both male and female proboscises were spherical and contained five hooks in four rows. The front row included the largest hooks, and their size decreased towards the back of the trunk. The hooks were all straight, but the larger hooks of the first row were curved at the base and had a sharp angle with the root. The apical area of the proboscis was bare, and in some specimens, it was slightly drawn inward. Sensory pits were observed near the lowest row of hooks and in the neck of the parasite. The cavity system consisted of irregularly branched chains, and the trunk had four rows of cuticular spines. The first row of trunk spines was located on the posterior edge of the collar. The proboscis sheath had a single muscular wall. The spines were located only in the anterior part of the body and were absent in the posterior part (Figures 7 and 8).

Discussion

This study clearly revealed the occurrence of infection by two species of *Neoechinorhynchus* and *Quadrigyrus* in *S. tumbil* fish from the southern basin of Iran as digestive parasites. Recently, *Neoechinorhynchus* has been reported from different types of marine fishes in some parts of the world (26–35). In this study, we clearly identified *Neoechinorhynchus* through the described characteristics of the studied specimens, including the shape and size of the proboscis, the number and position of hooks on the proboscis, and the shape of the body. This represents our first report on the genus *Neoechinorhynchus* from *S. tumbil*. In addition, we did not find any published research indicating the presence of *Neoechinorhynchus* contamination in this species of fish from the southern coast of Iran. Also, most of the reports from different parts of Iran are related to freshwater species (36). Several factors may influence the spread and prevalence of acanthocephalan infection. One of the main factors is the diversity of aquatic species, which can affect both freshwater and marine fish species. Environmental conditions can be considered a potential factor in the life cycle and spread of *Neoechinorhynchus*, as these factors can ensure the survival of the parasite and the intermediate host (35). Seasonal fluctuations can also influence the prevalence of this parasite in freshwater and saltwater fish (37,38). Ecological changes are another factor that can have a direct effect on the dynamics of the host fish population and an indirect effect on the infection rate of this parasite (39). Although only 2% of *S. tumbil* specimens in this study were infected with *Neoechinorhynchus* parasite, most of the infected fish exhibited no clinical symptoms and appeared clinically healthy. This aligns with findings by Emshiheet et al. (2024) who reported the infection rate of *Neoechinorhynchus* in another species of this fish (*Saurida undosquamis*) to be less than 1% (40). These results prove that this species cannot be considered a suitable host for this parasite. In the present study, the most important feature of *Neoechinorhynchus* was the larger size of the anterior hooks compared to the posterior ones. These larger lateral hooks were always positioned anteriorly to the other hooks on the proboscis (Figure 3). This pattern matches observations in studies by Amin et al. (41,42). The proboscis and neck are separated from the body by a collar at the anterior end of the trunk (Fig. 3). Collars have also been occasionally observed in other *Neoechinorhynchus* species. The trunk of *Neoechinorhynchus* in our study was porous and had numerous large micropores uniformly distributed throughout its length (Figures 4 and 5). These pores are enlarged on the external surface and their function is to absorb food uniformly across different areas of the trunk. A similar structure has been clearly observed in the mid-trunk of *Megarhynchus aspersentis* (43) and *Neoechinorhynchus johni* (42).

Quadrigyrus was the other acanthocephala isolated from this fish for the first time, with a prevalence rate of 4.66%. However, in previous studies by Tavakol et al. (2015) on Iranian acanthocephalans, no cases of *Quadrigyrus* parasite infection were observed (36). In fact, *Quadrigyrus persicus* was first isolated from the cowfish (*Boleophthalmus dussumieri*) living on the shores of Bandar Abbas and was reported as a new species, demonstrating that cowfish can be definitive hosts or carriers for acanthocephalans (44). In the report by Chemes and Brusa (2013) in Argentina, *Quadrigyrus machadoi* was isolated from *Hoplias malabaricus* and *Pimelodus maculatus* fish, with prevalence rates of 20.31% and 28.6%, respectively (18). In the research conducted in southern Brazil on Astyanax fish, the larval stage of *Quadrigyrus torquatus* was isolated, which can be distinguished from other species based on the morphology of the proboscis hooks and spines (15). Differences in parasite prevalence can be attributed to the characteristics of the life cycle of the host and its feeding. Different species of *Quadrigyrus* may exhibit significant variation in the number of spines on the trunk. In *Quadrigyrus persicus*, studied by Solaimani et al. (2014), body spines were located only in the anterior part, while the posterior part was without spines, but the number of rows was not mentioned (44). In the study conducted on ornamental fish, three species of *Quadrigyrus brazilians*, *Quadrigyrus nickoli* and *Quadrigyrus torquatus* were separated, and the species were identified based on the number of rings and spines on the front part of the body (45). In the present study, four rows of spines were observed in the anterior part of the body, consistent with the findings of Gallas and Utz (15).

Conclusion

This was our first study on these two genera, *Neoechinorhynchus* and *Quadrigyrus,* from *S. tumbil,* in the city of Minab, using SEM. The results of the SEM analysis provide a basis for an in-depth understanding of these parasites. This study demonstrated that organs such as the proboscis, the number and position of hooks and spines, and the shape of the body can significantly aid in the identification of these acanthocephalans. By elucidating the structure and surface organs, SEM leads to a better recognition of the species and reveals their minor differences. More SEM studies should be conducted on acanthocephalan species, alongside pathological tests, to obtain more comprehensive and accurate information about the damage of these parasites to fish hosts. It is recommended that future studies include larger sample collections to confirm the novelty of these species with more certainty and also to determine their frequency and spatial-temporal dynamics in lizardfish from the Synodontidae family. In addition, studies on other marine fish species are also necessary, as they may serve as hosts for similar or closely related species to *Neoechinorhynchus* and *Quadrigyrus*.

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Authors' Contributions

Yousef Ghahvei: Conceptualization, Data curation, Formal analysis, Investigation, Writing original draft, **Mohammad Mirzaei**: Conceptualization, Methodology, Supervision, **Shadi Hashemnia:** Supervision, Conceptualization, Methodology, Validation, Visualization, Writing – review & editing.

Data Availability

All data generated or analyzed during this study are included in this published article [and its additional files].

Ethical Approval

Experiments were approved by the Research Ethics Committees of Shahid Bahonar University of Kerman, Kerman, Iran.

Conflict of Interest

The authors declare that there is no potential conflict of interest.

Consent for Publication

Not applicable.

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