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Prevalence, Morphological and Molecular Characterization of *Anisakis simplex* Larvae in Commercially Important Fishes from Egyptian Markets

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Abstract

ANISAKIASIS, caused by nematodes from the Anisakidae family, has become a notable global health issue, primarily due to the increased consumption of raw or undercooked seafood. This study investigated the occurrence and prevalence of *Anisakis* larvae in commonly sold fish in Egyptian markets. Morphological and molecular techniques were employed to identify the larvae. Our findings revealed a high prevalence of *Anisakis* larvae, with 85% of Atlantic herring (*Clupea harengus*) and 52% of Atlantic mackerel (*Scomber scombrus*) being infected. The mean intensity of infection was 11.17 larvae per infected herring and 4.03 larvae per infected mackerel, while the mean abundance was 9.5 larvae per herring and 2.1 larvae per mackerel. Detailed morphological analysis identified the larvae as *Anisakis simplex* type I, characterized by specific anatomical features visible under light and scanning electron microscopy. DNA sequencing of the Internal Transcribed Spacer (ITS) region was aligned with GenBank data, confirming the identity of the larvae as *A. simplex*. This study highlights the significant presence of *Anisakis* larvae in the Egyptian fish supply, emphasizing the need for public health interventions to mitigate the risks associated with consuming raw or undercooked fish. The application of molecular techniques has enhanced our understanding of the species composition and potential health impacts of *Anisakis* in Egypt, contributing to global efforts to manage anisakiasis.

Keywords: *Anisakis simplex, Atlantic herring, Atlantic mackerel, zoonosis, molecular identification*.

Introduction

Anisakiasis, a zoonotic disease caused by nematodes of the family Anisakidae, has emerged as a significant global health concern [1]. The causative agents, larvae belonging to genera such as *Pseudoterranova*, *Anisakis*, and *Contracaecum*, are widely distributed in aquatic ecosystems worldwide [2]. The rising incidence of anisakiasis is linked to the increasing popularity of consuming raw or minimally processed seafood, with Japan accounting for over 90% of the nearly 20,000 reported cases globally [3, 4]. The life cycle of anisakid nematodes involves multiple host species [5]. Adult worms inhabit the gastrointestinal tracts of marine mammals, producing eggs that are shed into the ocean via fecal matter [6]. Upon hatching, these larvae invade small crustaceans where they develop into third-stage larvae (L3) [7]. These L3 larvae infect various fish and cephalopod species, serving as intermediate or paratenic hosts [8]. Human infection occurs when people consume raw or inadequately cooked fish harboring these infective L3 larvae [9,10]. While unable to complete their life cycle in humans, these larvae can cause significant pathological changes, leading to clinical manifestations of anisakiasis [11]. The complex nature of this life cycle contributes to the challenge of controlling anisakid infections and highlights the importance of understanding the ecology of these parasites in different regions [12]. The Mediterranean region has witnessed an increasing trend towards consuming raw or undercooked seafood, influenced by the global popularity of dishes such as sushi [13,14]. This cultural change has led to a rise in reported anisakiasis cases in countries

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like Italy, Spain, and France [15,16]. Egypt, traditionally not associated with raw fish consumption, has also observed this trend emerge, with sushi and other raw fish products becoming increasingly available in urban areas [17,18]. The growing popularity of imported fish species like Atlantic salmon (*Salmo salar*) and Atlantic mackerel (*Scomber scombrus*) for raw consumption in Egypt raises concerns about the potential risk of anisakid infections [19]. This shift in dietary preferences underscores the need for comprehensive studies to assess the prevalence of anisakid parasites in the Egyptian fish supply and the potential health risks to consumers [20].

Despite its public health implications, there is a paucity of comprehensive studies on anisakiasis in Egypt. Some studies have identified *Anisakis* larvae in fish sold in Egyptian markets, but significant knowledge gaps persist [3, 6, 11, 12]. The occurrence, prevalence, and molecular identification of *Anisakis* larvae in ready-to-eat fish products and imported fishes in Egypt remain understudied [3]. Epidemiological data on anisakiasis infections among the Egyptians are limited and inconclusive [3]. This lack of data hampers efforts to implement effective prevention strategies and may leave the population vulnerable to an emerging health threat [6]. The prevalence of anisakid larvae in commonly consumed, locally caught fish species such as Atlantic herring (*Clupea harengus*), Mediterranean horse mackerel (*Trachurus trachurus*), and Atlantic mackerel has received minimal scientific attention [3, 6, 10, 11]. Understanding the parasite burden in these species is crucial for assessing the risk to Egyptian consumers and for developing targeted interventions [12, 13].

While morphological examination can identify *Anisakis* larvae to the genus level, species-level identification requires molecular techniques such as DNA sequencing [3, 6]. The internal transcribed spacer (ITS) gene has been proven to be an effective molecular marker for distinguishing between *Anisakis* species [3]. Sequencing this gene region from isolated parasites can provide more accurate species identifications compared to morphology alone, offering valuable insights into the epidemiology and potential health impacts of different *Anisakis* species [6]. The application of these molecular techniques in the Egyptian context could enhance our understanding of the anisakid species present in the local fish supply and their potential impact on human health [3].

This study aimed to investigate the occurrence and prevalence of *Anisakis* larvae in commonly sold fish in Egyptian markets. This study employed morphological and molecular identification techniques to accurately identify *Anisakis* spp present in the sampled fish. This approach allowed for a more nuanced understanding of the parasite species composition and potential health risks. These objectives provided a comprehensive understanding of *Anisakis* infections in the Egyptian fish supply, informed public health interventions, and offered insights into the emergence of anisakiasis as a public health concern in the context of evolving food culture.

Material and Methods

Fish Sampling

To comprehensively investigate the occurrence and prevalence of *Anisakis* larvae in commonly sold fish in Egyptian markets, a total of 200 fish specimens were collected over four months from July to October 2022. The sample comprised 100 Atlantic herring (*Clupea harengus*) and 100 Atlantic mackerel (*Scomber scombrus*), representing fish species commonly consumed in Egypt. These species were chosen due to their popularity in Egyptian markets. Atlantic herring specimens had an average length of 38 ± 5.2 cm and weight of 695 ± 45.4 g, while Atlantic mackerel averaged 31 ± 2.3 cm in length and 425 ± 25.3 g in weight. The fish were randomly collected from retail markets across various regions of Egypt. To maintain sample integrity, the fish specimens were transported to the laboratory, in insulated boxes with ice. This method of transportation is crucial for preserving the condition of potential parasites. Upon arrival at the laboratory, each fish underwent a thorough external and internal examination. The specimens were eviscerated, and portions of the muscles, pyloric caeca, and other internal organs were meticulously analyzed for the presence of parasitic larvae. This comprehensive examination approach allows for the detection of *Anisakis* larvae in various anatomical locations, providing a more accurate assessment of parasite distribution within the host.

Parasitology Examination

The collection and preservation of *Anisakis* larvae is a critical step in both morphological and molecular analyses. Third-stage larvae (L3) of *Anisakis* spp. were extracted from both fish species. These L3 larvae are in the infective stage for humans and are typically found in fish muscles or viscera. The larvae were observed either free in the body cavity or encapsulated in the mesentery of the pyloric caeca, intestine, or ovaries, consistent with known *Anisakis* infection patterns. A stereoscopic dissecting microscope was employed for the precise removal of encapsulated larvae from infected organs. This method ensures minimal damage to the larvae, which is crucial for subsequent morphological and molecular analyses. Following extraction, the larvae were rinsed with phosphate-buffered saline (pH 7.2) to remove host tissue debris and preserved at 4°C. This preservation method allows for larval relaxation, facilitating more accurate morphological identification.

Morphological identification

To assess the extent of *Anisakis* infections in the sampled fish, key parasitological parameters were calculated using the Parasitology web version [14]. These parameters included prevalence (percentage of infected hosts), mean intensity (average number of parasites per infected host), and mean abundance (average number of parasites across all examined hosts, including uninfected individuals). These metrics provide valuable insights into the infection dynamics and potential risks to human consumers. Morphological identification of *Anisakis* larvae is an essential first step in species determination. Isolated larvae were cleared with lactophenol and slide-mounted in glycerin jelly for microscopic examination [15]. This technique enhances the visibility of key morphological features. Light microscopy was used to examine specific characteristics, including the structure of lips surrounding the anterior end, the presence or absence of a boring tooth, the shape of the ventriculus in the esophagus, and the configuration of the postanal tail and its terminal mucron or spine [16]. These features are crucial for distinguishing *Anisakis* from other nematode genera and for preliminary species-level identification.

Scanning Electron Microscopy (SEM)

Some larvae were fixed in 2.5% glutaraldehyde for analysis under Scanning Electron Microscopy (SEM). SEM analysis offers high-resolution imaging of surface structures, which can reveal subtle morphological differences between *Anisakis* species that are not visible under light microscopy. This technique allows for a more comprehensive examination of the larvae's external features, providing additional criteria for species identification and comparative morphological studies [17,18].

Genomic DNA Extraction

Molecular techniques are essential for accurate species-level identification of *Anisakis* larvae. Genomic DNA was extracted from L3 larvae using a Real Pure kit (Ref RBMEG01), following the manufacturer's protocol. Spectrophotometric analysis was performed using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA) to determine DNA concentration and purity. The integrity of the extracted DNA was evaluated using agarose gel electrophoresis. The presence of a high molecular weight band with minimal smearing indicated high-quality, intact genomic DNA suitable for PCR amplification. This method ensures high-quality DNA extraction, which is crucial for subsequent PCR amplification and sequencing.

Molecular characterization

The ITS region was targeted for PCR amplification using *Anisakis*-specific primers [19]. The primers used were Forward primer (NC5): 5'-GTAGGTGAACCTGCGGAAGGATCATT-3' Reverse primer (NC2): 5'-TTAGTTTCTTTTCCTCCGCT-3'. The initial denaturation phase was set at 95°C for 10 minutes, followed by 35 cycles of denaturation (95°C for 30 seconds), annealing (58°C for 30 seconds), and elongation (72°C for 30 minutes). The final elongation step was conducted at 72°C for 10 minutes, with the samples subsequently maintained at 4°C [21]. The amplicons were then separated on a 1.0% agarose gel, stained with 1% ethidium bromide, and examined under UV light. Two samples representing Anisakidae L3 larvae were selected for sequencing - one from Atlantic herring and one from Atlantic mackerel. These samples were purified and sequenced by Macrogen Incorporation (Seoul, Korea) using BigDye technology on an automated ABI3730XL DNA sequencer (Applied Biosystems, Foster City, USA).

The DNA sequences underwent manual editing using BioEdit version 7.0 [22]. These sequences were then aligned with other ITS regions available in GenBank, utilizing the NCBI Local Alignment Search Tool. Following this analysis, the finalized sequences were submitted to the DNA GenBank nucleotide sequence database. For phylogenetic analysis, the neighbor-joining method with 1000 bootstrap replications was employed, using MEGA X [23]. The parameters included the maximum composite likelihood for the ITS region. Both transitions and transversions were considered in substitutions, homogeneous patterns among lineages were assumed, partial deletion was applied, and uniform variation rates among sites were used. *Toxascaris leonina* was selected as the outgroup for this analysis.

Results

Incidence of Anisakid Larval Infections

This study examined a total of 200 commercially important fish specimens, equally divided between Atlantic herring and Atlantic mackerel, for the presence of anisakid nematode larvae. The prevalence of infection in Atlantic herring was found to be 85 %, with a mean intensity of 11.17 larvae per infected fish and an overall mean abundance of 9.5 larvae per fish sampled. In contrast, Atlantic mackerel showed a lower infection rate, with 52 % of specimens infected, a mean intensity of 4.03 larvae per infected host, and a total mean abundance of 2.1 larvae per mackerel examined (Table 1).

Parasitological analysis

Upon gross external examination, the naturally infected fish exhibited no discernible lesions or abnormalities, which is consistent with the typically asymptomatic nature of anisakid infections in fish hosts. However, detailed postmortem investigations revealed the presence of *Anisakis* spp. larvae in

various locations within the fish. The parasites were predominantly found in the abdominal cavity and visceral organs, including the liver, spleen, and intestines. The larvae were observed in two distinct states: either encapsulated on the serosal surfaces of organs or freely floating within the body cavity. This distribution pattern is characteristic of anisakid infections and reflects the larval migration patterns within the fish host (Fig. 1).

Microscopic Morphology

The larvae isolated from both fish hosts were identified as *A. simplex* type I (Nematoda: Anisakidae; Dujardin, 1845) based on microscopic examination. These nematodes exhibited an elongated cylindrical body tapered at both ends, characteristic of their species. At the anterior end, three inconspicuous lips surrounded the oral opening, accompanied by an identifiable boring tooth - a feature typical of third-stage anisakid larvae. Light microscopy revealed this prominent boring tooth at the anterior end (Fig. 2A, B), which was further confirmed by ultrastructure microscopy (Figure 3A, B, C). The esophagus displayed a clear division into two sections: an anterior muscular portion measuring 1.95 ± 0.3 mm in length, followed by a posterior glandular ventriculus portion of 0.95 ± 0.2 mm length. Between the esophagus and intestine, an obliquely oriented junction (ventriculus) was observed, measuring 1.3 ± 0.12 mm in length (Figure 2C, D). The outer cuticle of the larvae was distinctly striated transversely along the entire body length, providing a key diagnostic feature. This characteristic striated cuticle was visible under both light microscopy (Fig. 2F) and ultrastructure microscopy (Fig. 3D, E). Posteriorly, the larvae demonstrated the typical anatomy of *A. simplex* L3 larvae, including one spicule and a short mucron or spine at the tail end, measuring 0.030 ± 0.03 mm in length (Fig. 2E). The pointed anterior end of the larvae was particularly well-illustrated in the ultrastructure micrograph (Fig. 3F). These morphological characteristics, observed through both light and ultrastructure microscopy, collectively supported the identification of the larvae as *A. simplex* type I, aligning with established taxonomic descriptions for this species at the third larval stage.

Molecular identification of Anisakis sp.

The ITS gene sequences extracted from *Anisakis* larvae type I isolates in herring and mackerel were submitted to GenBank, receiving accession numbers OP702963 and OP702964, respectively. Sequence alignment firmly positioned these two isolates within the Anisakidae family, confirming their identity as *A. simplex*. Intraspecific comparison of the two *A. simplex* isolates revealed a high similarity of 99.45%, with only five nucleotide differences, indicating minimal genetic variation. BLAST analysis against existing *Anisakis* spp. sequences demonstrated varying degrees of homology: highest similarity (99.11-97.52%) with *A. simplex* (MT355317; OM418763; MH819241, MH819242, LC745133, MG272325), followed by 96.89% with *A. berlandi* (MK325187), 94.76% with *A. pegreffii* (AY603531), and 92.80% with *A. typica* (MT271946; MN420659). Lower homologies were observed with *A. ziphidarum* (90.69%, JN005767; JN005766), *A. brevispiculata* (88.33%, KC342887), *A. paggiae* (88.17%, EU624345), *A. nascettii* (87.91%, JX486104), and *A. physeteris* (87.44%, EU327691).

Phylogenetic analysis of A. simplex

Phylogenetic tree analysis elucidated the evolutionary relationships among *Anisakis* spp, with particular emphasis on *A. simplex* (Fig. 4). The two *A. simplex* isolates from this study (OP702963 and OP702964) formed a robust subclade with *A. simplex* (MT355317), supported by a high bootstrap value of 99%. This *A. simplex* subclade is embedded within a larger, strongly supported group (bootstrap value 100%) that includes *A. berlandi* (KY524216; MK325187) and *A. pegreffii* (AY603531), illustrating the intricate phylogenetic associations within the genus. The tree topology delineates the *A. simplex* subclade from other *Anisakis* spp., including *A. ziphidarum, A. nascettii, A. brevispiculata, A. paggiae, A. physeteris*, and *A. typica*, each forming distinct branches or subclades. The phylogenetic tree was rooted using *T. leonina* (MN175138) as an outgroup, providing context for interrelationships within the *Anisakis* genus. This comprehensive molecular and phylogenetic analysis confirms the taxonomic classification of the *A. simplex* isolates from this study and provides valuable insights into species relationships within the *Anisakis* genus.

Discussion

The global decline in animal protein availability, particularly in developing countries, underscores the critical importance of fish as an economical protein source for growing populations. Fish and shellfish contribute approximately 25% of the total animal protein consumed by humans worldwide [4]. However, parasitic infections, especially those caused by anisakid nematodes, significantly compromise the quality, quantity, and economic value of fish catches [24]. The World Health Organization (WHO) estimates that nearly 56 million cases of foodborne parasites originate annually from fish consumption [7]. In this context, our study provides crucial insights into the prevalence and molecular characterization of *Anisakis* simplex in Atlantic herring (*C. harengus*) and Atlantic mackerel (*S. scombrus*) sold in Egyptian markets, with significant implications for public health and food safety in Egypt, particularly considering evolving dietary habits and the increasing consumption of raw or undercooked fish [25].

Our investigation revealed a high prevalence of *Anisakis* infection, especially in Atlantic herring (85%), which is alarming and consistent with reports from other regions. For instance, infection rates of up to 90% in Atlantic herring were reported from the North Sea [5]. The lower prevalence in Atlantic mackerel (52%) aligns with previous findings [7], which observed variable infection rates in mackerel depending on fishing grounds and seasons. The distribution of *Anisakis* larvae within the fish hosts, primarily in the abdominal cavity and visceral organs, corroborates the typical infection pattern [8]. This distribution pattern has significant implications for food safety, as improper evisceration or preparation of fish may lead to the migration of larvae into the muscle tissue, thereby increasing the risk of human infection.

The morphological characteristics observed through light microscopy and scanning electron microscopy (SEM), including the presence of a boring tooth, obliquely oriented ventriculus, and striated cuticle, are consistent with the previous description of *A. simplex* third-stage larvae [9, 17]. The use of SEM in this study allowed for more detailed observation of surface structures, enhancing the accuracy of morphological identification. However, recognizing that morphological features alone are insufficient for accurate species-level identification, we employed molecular techniques for definitive characterization.

The molecular analysis using the ITSer region confirmed the identity of the larvae as A. simplex, with high similarity (99.45%) between isolates from herring and mackerel. This finding supports the reliability of the ITS region as a molecular marker for *Anisakis* species identification [3]. The high homology with A. simplex sequences from other geographical regions suggests a widespread distribution of this species, which is concerning from a global food safety perspective. Our phylogenetic analysis revealed close clustering of our *A. simplex* isolates with previously reported sequences (e.g., MT355317), supporting the accuracy of the identification. The clear separation from other *Anisakis* species in the phylogenetic tree, such as *A. pegreffii* and *A. physeteris*, underscores the genetic distinctiveness of *A. simplex* and the importance of molecular techniques in species-level identification. The varying degrees of homology observed with other *Anisakis* species (e.g., 96.89% with *A. berlandi*, 94.76% with *A. pegreffii*) reflect the complex evolutionary relationships within the genus. These findings align with comprehensive phylogenetic studies which demonstrated the existence of distinct genetic lineages within the *A. simplex* complex [25].

The high prevalence of *A. simplex* in commonly consumed fish species in Egypt raises significant public health concerns. Anisakiasis, caused by the accidental ingestion of live *Anisakis* larvae, can lead to gastrointestinal symptoms and allergic reactions [27]. The increasing popularity of raw fish dishes in Egypt [3], combined with the high infection rates observed in this study, suggests a potential rise in anisakiasis cases if proper preventive measures are not implemented. Moreover, the presence of *A. simplex* in fish products can pose risks even when the fish is cooked, as heat-stable allergens can trigger allergic reactions in sensitized individuals [31]. This aspect is particularly relevant in the Egyptian context, where awareness of anisakiasis and its associated risks may be limited.

The changing food preferences and ecological patterns revealed through this study necessitate urgent multidimensional preventative interventions. These should engage diverse stakeholders across production-to-consumption pathways to transform risky perceptions, attitudes, and practices related to this neglected parasitic disease. Expanding epidemiological understanding can strengthen evidence-based development of integrated fish-borne zoonoses control programs within local contexts.

In terms of remedies, most processing focuses on killing larvae via freezing, salting, smoking, or heating fish adequately before consumption. However, practical challenges constrain adoption feasibility for small producers. Visual inspection offers a cheaper alternative to identify and reject visibly infected fish but cannot detect internal encysted larvae. While such measures combined can reduce risks, implementing them significantly adds to production costs, threatening the sustainability of small fishery enterprises across developing countries [31]. An alternative strategy targets interrupting the passage of larvae from crustaceans to fish hosts through integrated parasite management of fishing areas. This requires further research into environmental factors favoring transmission to identify water bodies with lower infection levels for fishing. Concurrently, public health education should address safe traditional recipe preparation, appropriate cooking methods, and hygienic handling among communities [32]. At wholesale and retail levels, suppliers and vendors represent key channels for consumer engagement through awareness creation and transparent trade practices like visible parasite disclaimers.

Conclusions

Our study reveals widespread *A. simplex* larvae infestation in popular Egyptian table fish, posing zoonotic risks through consumption habits. The high prevalence in edible tissues and the absence of clear signs hinder detection by fishers and consumers. This emerging health challenge necessitates integrated approaches across the production-to-consumption chain to ensure food safety as Egyptian dietary habits evolve. Future research should expand to more fish species, seasons, and locations, employing multiple

genetic markers for comprehensive *Anisakis* distribution and population insights.

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Conflict of interest

The authors declare that they have no conflicts of interest related to this research.

Ethical Approval

This study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine at Cairo University

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Author contributions

Marwa Attia: Conceptualization, Methodology, Writing draft; Olfat A. Mahdy: Data curation, Formal analysis; Mai A. Salem: Investigation, Validation; Asmaa W. Soliman: Resources, Genetic Analysis; Mohamed Abdelsalam: Writing - Review & Editing.

TABLE 1. Prevalence (P), Mean Intensity (MI), and Mean Abundance (MA) of Isolated Anisakid Larvae in the Examined Fish.

Fish host	Fish sample	No. inf. fish	No. isolated larvae	P	MI	MA
Atlantic herring	100	85	950	85.00%	11.17	9.5
Atlantic mackerel	100	52	210	52.00%	4.03	2.1

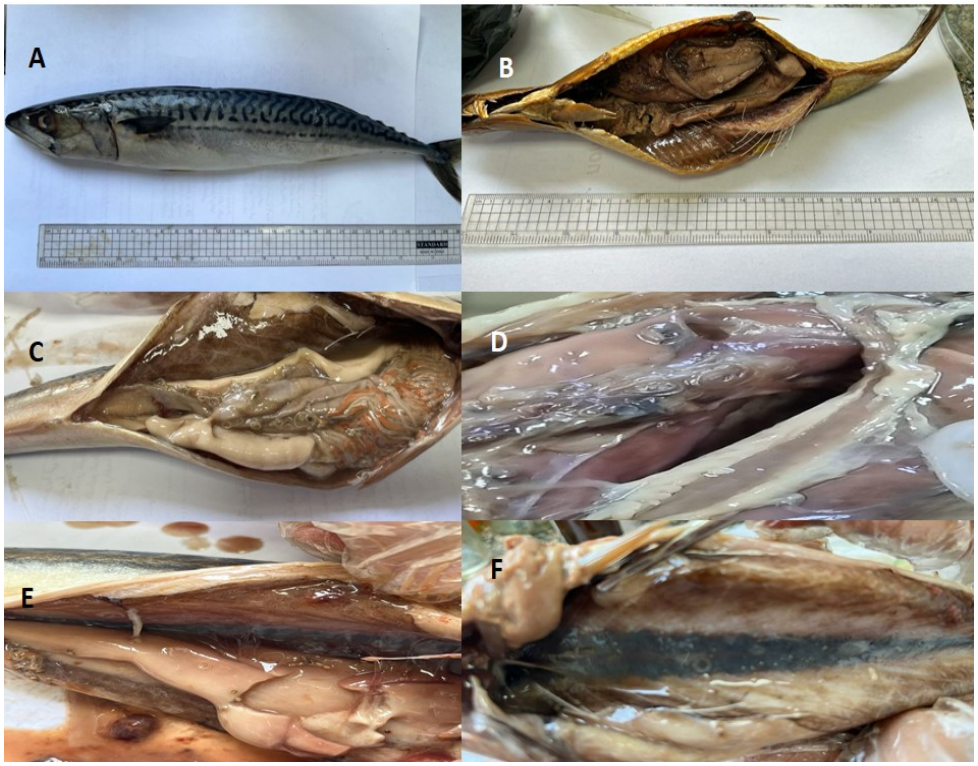

Fig. 1. A: The naturally infected fish showed no discernible external lesions. B: postmortem investigations revealed *Anisakis* spp. larvae in the abdominal cavity. C: *Anisakis* spp. larvae on the serosal surfaces of visceral organs. D: *Anisakis* spp. larvae encapsulated in the liver. E: *Anisakis* spp. larvae freely floating in the body cavity. F: *Anisakis* spp. larvae in the intestines.

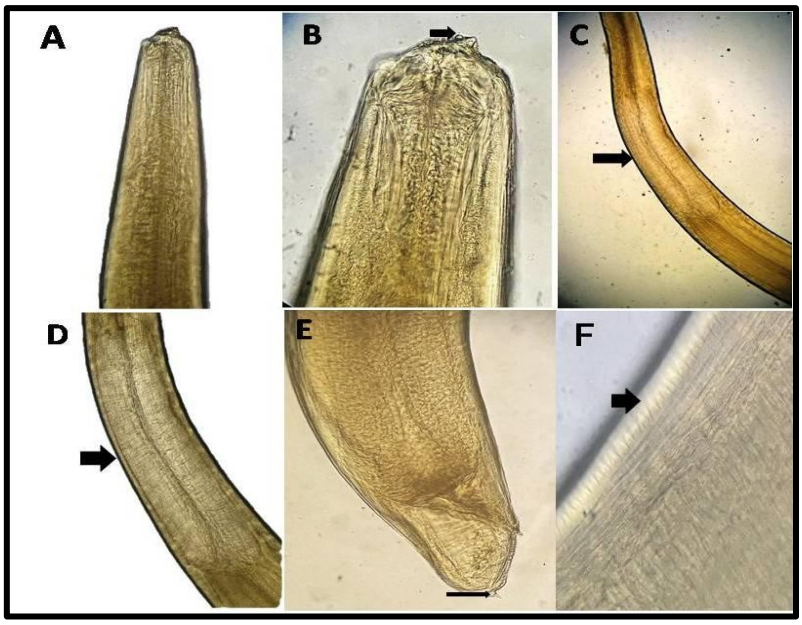

Fig. 2. Light Microscopic Micrograph Illustrating the Anterior and Posterior Ends of *A. simplex* Type I. A, B: Anterior end of *A. simplex* Type I, featuring a prominent boring tooth. C, D: Depiction of the oblique esophago-intestinal junction (ventriculus). E: The posterior end of the third-stage larvae, displaying one spicule and a short mucron or spine. F: Whole larva covered with a transverse striated cuticle.

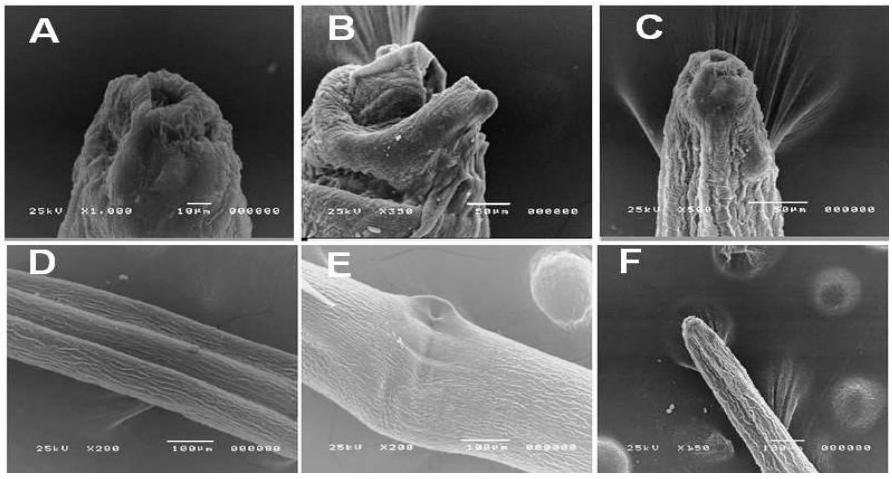

Fig. 3. Ultrastructure Microscopic Micrograph Revealing the Anterior and Posterior Ends of *A. simplex* Type I. A, B, C: Anterior end of *A. simplex* Type I, highlighting a prominent boring tooth. D, E: Whole larva covered with a transverse striated cuticle. F: Pointed anterior end of the larva.

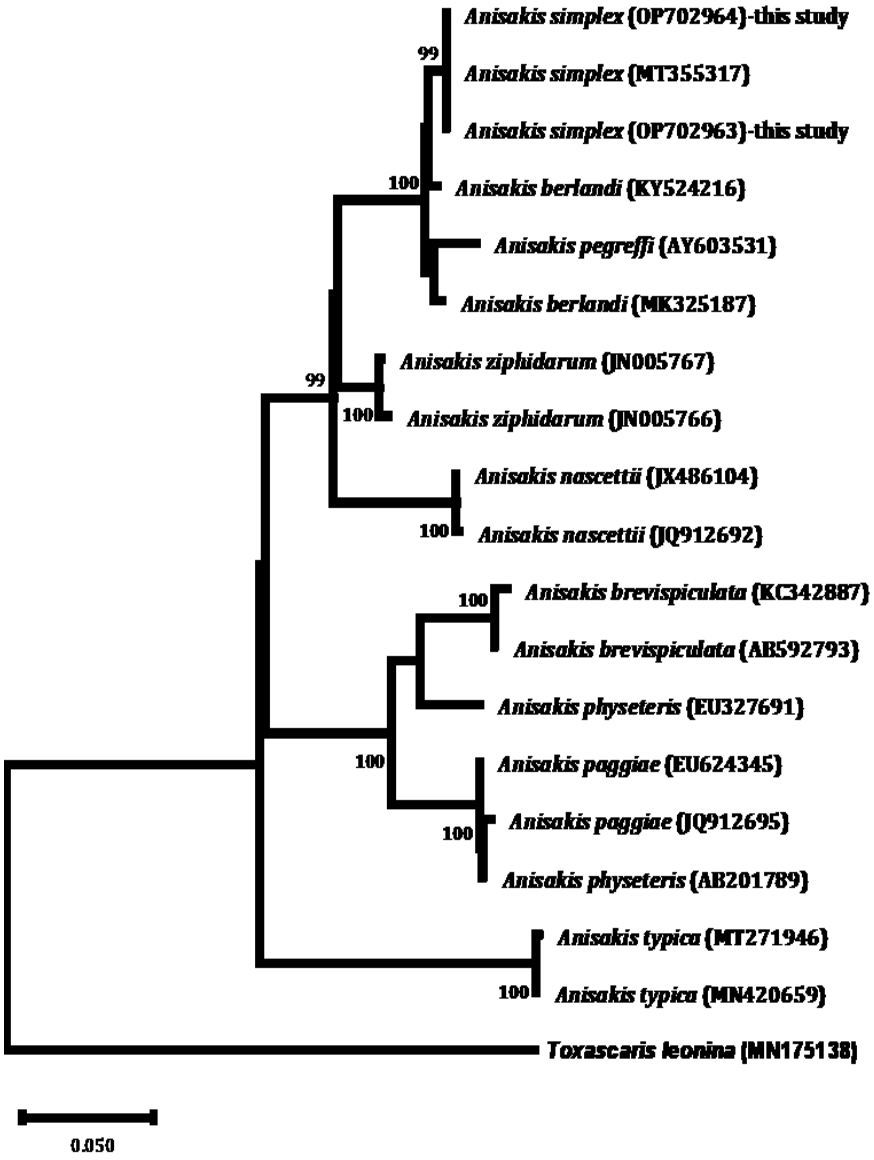

Fig. 4. ***A. simplex*** **phylogenetic analysis using ITS region. The Neighbor-Joining method was used to build the tree. The number of nucleotide substitutions per site is shown by the scale bar at the base of the tree. The GenBank accession numbers of the strains were provided. Outgroups included** ***T. leonina*****.**

References

1. Aibinu, I. E., Smooker, P. M. and Lopata, A. L. *Anisakis* Nematodes in Fish and Shellfish—from Infection to Allergies. *IJP: Parasites and Wildlife,* **9**, 384–393 (2019).
2. Nieuwenhuizen, N. E. and Lopata, A. L. Allergic Reactions to *Anisakis* Found in Fish. *Curr. Allergy Asthma Rep.,* **14**(8), 455 (2014). doi: 10.1007/s11882-014-0455-3.
3. Eissa, A.E., Abdelsalam, M., Abumhara, A., Kammon, A., Gammoudi, F.T., Ben Naser, K.M., Borhan, T. and Asheg, A. First record of *Vibrio vulnificus/anisakis pegreffi* concurrent infection in black scorpionfish (*Scorpaena porcus*) from the south Mediterranean basin. *Res. J. Pharma. Biol. Chem. Sci.,* **6**(3), 1537–1548 (2015).
4. World Health Organization (WHO). Soil-Transmitted Helminths. Geneva: WHO (2012).
5. Levsen, A. and Karl, H. *Anisakis* simplex (s.l.) in Grey Gurnard (*Eutrigla gurnardus*) from the North Sea: Food Safety Considerations in Relation to Fishing Ground and Distribution in the Flesh. *Food Control,* **36**(1), 15-19 (2014).
6. Moustafa, E., Omar, M., Shimaa, S., Hassan, Sh., and Samir, M. Occurrence and Molecular Identification of *Anisakis* Larval Type 1 (Nematoda: Anisakidae) in Marketed Fish in Egypt. J. Parasit. Dis., 44, 536–545 (2020).

7. Cipriani, P., Sbaraglia, G. L., Palomba, M., Giulietti, L., Bellisario, B., Bušelić, I., Mladineo, I., Cheleschi, R., Nascetti, G. and Mattiucci, S. *Anisakis pegreffii* (Nematoda: Anisakidae) in European Anchovy *Engraulis encrasicolus* from the Mediterranean Sea: Fishing Ground as a Predictor of Parasite Distribution. *Fish. Res.,* **202**, 59-68 (2018).

8. Lymbery, A. J. and Cheah, F. Y. Anisakid Nematodes and Anisakiasis. In: Food-borne Parasitic Zoonoses: Fish and Plant-borne Parasites. Springer US, Boston, MA, pp. 185-207 (2007).

9. Hamouda, A.H. and Younis, A.E. Molecular characterization of zoonotic anisakid *Contracaecum* spp. larvae in some fish species from Lake Nasser, Egypt. *Aquacult. Res.,* **53**(6), 2548-2561 (2022).

10. Arafa, W. M., Hassan, A. H. A., et al. Occurrence and Molecular Characterization of Zoonotic *Anisakis simplex* s.s. and *Anisakis pegreffii* Larvae in Retail-Marketed Fish. J. Food Saf., 39, 1–7 (2019).

11. Samir, M., Amin, M. A., Hassan, A. O., Merwad, A. M., and Awadallah, M. A. I. Research Note. Prevalence, Protein Analysis and Possible Preventive Measures Against Zoonotic Anisakid Larvae Isolated from Marine Atherina Fish. Helminthologia, 52, 375–383 (2015). doi: 10.1515/helmin-2015-0060.

12. Sohn, W. M., Na, B. K., Kim, T. H., and Park, T. J. Anisakiasis: Report of 15 Gastric Cases Caused by *Anisakis* Type I Larvae and a Brief Review of Korean Anisakiasis Cases. Korean J. Parasitol., 53(4), 465–470 (2015).

13. Song, H., Jung, B. K., Cho, J., Chang, T., Huh, S., and Chai, J. Y. Molecular Identification of *Anisakis* Larvae Extracted by Gastrointestinal Endoscopy from Health Check-up Patients in Korea. Korean J. Parasitol., 57, 207–211 (2019). doi: 10.3347/kjp.2019.57.2.207.

14. Ibrahim, M.M., Baghdadi, H.B., Shahin, K., Abdel-Glil, M., Thabit, H., Attia, M.M. and Abdelsalam, M. *Dasyrhynchus giganteus* plerocercoids encysting in the musculature of Indian halibut (*Psettodes erumei*): seasonal prevalence, morpho-molecular characterization, and histopathological alterations. BMC Vet. Res., 20(1), 332 (2024).

15. Eissa, A.E., Abdelsalam, M., Attia, M., Ismail, G., Qorany, R., Prince, A., El Zlitne, R. and Abdel-moneam, D. Morphological, molecular and clinical assessment of different *Anisakis* species infecting horse Mackerel *Trachurus trachurus* from South Mediterranean. Egypt. J. Aquat. Biol. Fish., 25(4), 1085–1098 (2021).

16. Abdelsalam, M., Attia, M. M., and Mahmoud, M. A. Comparative Morphomolecular Identification and Pathological Changes Associated with *Anisakis* simplex Larvae (Nematoda: Anisakidae) Infecting Native and Imported Chub Mackerel (*Scomber japonicus*) in Egypt. Reg. Stud. Mar. Sci., 39, 101469 (2020).

17. Mostafa, N.A., Abdel-Ghaffar, F., Fayed, H.O. and Hassan, A.A. Morphological and molecular identification of third-stage larvae of *Anisakis typica* (Nematoda: Anisakidae) from Red Sea coral trout, *Plectropomus areolatus*. *Parasitol. Res.,* **122**(3), 705-715 (2023).

18. Mahdy, O. A., Abdelsalam, M., Abdel-Maogood, S. Z., Shaalan, M. and Salem, M. A. First Genetic Confirmation of Clinostomidae Metacercariae Infection in *Oreochromis niloticus* in Egypt. *Aquac. Res.,* **53**(1), 199–207 (2022).

19. Nogami, Y., Fujii-Nishimura, Y., Banno, K., Suzuki, A., Susumu, N., Hibi, T., Murakami, K., Yamada, T., Sugiyama, H., Morishima, Y. and Aoki, D. Anisakiasis Mimics Cancer Recurrence: Two Cases of Extragastrointestinal Anisakiasis Suspected to Be Recurrence of Gynecological Cancer on PET-CT and Molecular Biological Investigation. *BMC Med. Imaging,* **16**, 31 (2016). doi: 10.1186/s12880-016-0134-z.

20. Gómez-Mateos, M., Valero, A., Morales-Yuste, M. and Martín-Sánchez, J. Molecular Epidemiology and Risk Factors for *Anisakis simplex* s.l. Infection in Blue Whiting (*Micromesistius poutassou*) in a Confluence Zone of the Atlantic and Mediterranean: Differences Between *A. simplex* s.s. and *A. pegreffii*. *Int. J. Food Microbiol.,* **232**, 111–116 (2016).

21. Mattiucci, S., Acerra, V., Paoletti, M., Cipriani, P., Levsen, A., Webb, S. C., Canestrelli, D. and Nascetti, G. No More Time to Stay 'Single' in the Detection of *Anisakis pegreffii*, *A. simplex* (s.s.) and Hybridization Events Between Them: A Multi-marker Nuclear Genotyping Approach. *Parasitol.,* **143**(8), 998-1011 (2016).

22. Hall, T. A. Bio Edit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.,* **41**, 95–98 (1999).

23. Kumar, S., Stecher, G. and Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.,* **33**(7), 1870–1874 (2016).

24. Labony, S. S., Alim, M. A., Hasan, M. M., Hossain, M. S., Islam, A., Alam, M. Z. and Tsuji, N. Fish-borne Trematode Infections in Wild Fishes in Bangladesh. *Pathog. Glob. Health*, **114**(2), 91–98 (2020). https://doi.org/10.1080/20477724.2020.1727217

25. Mattiucci, S., Cipriani, P., Webb, S.C., Paoletti, M., Marcer F., Bellisario, B., Gibson, D.I. and Nascetti, G. Genetic and morphological approaches distinguishing the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). *J. Parasitol.,* **100**, 199–214 (2014).

26. Cavallero, S., El Sherif, R.A., Pizzarelli, A., El Fituri, A.A., El Showhdi, M., Benmosa, F. and D'Amelio, S. Occurrence of *Anisakis* and *Hysterothylacium* nematodes in Atlantic chub mackerels from Libyan coasts. *Helminthol.,* **56**(4), 347–352 (2019).

27. Hamada, Y., Sugano, S., Kamide, Y., Sekiya, K. and Fukutomi, Y. Anisakis allergy versus gastric anisakiasis: A case of repeated Anisakis-associated

symptoms. *J. Allergy Clin. Immunol. Glob.,* **3**(2), 100207 (2024).

28. Abou-Rahma, Y., Abdel-Gaber, R. and Kamal A.A., First record of *Anisakis simplex* third-stage larvae (Nematoda, Anisakidae) in European hake *Merluccius merluccius* lessepsianus in Egyptian waters. *J. Parasitol. Res.,* **2016**(4), 1–8 (2016). https://doi.org/10.1155/2016/9609752.
29. Bak, T.J., Jeon, C.H. and Kim, J.H., Occurrence of anisakid nematode larvae in chub mackerel (*Scomber japonicus*) caught off Korea. *Int. J. Food Microbiol.,* **191**, 149–156 (2014). https://doi.org/10.1016/j.ijfoodmicro.2014.09.002.
30. Garcia-Sanchez, B., Masiá, P., Garcia-Vazquez, E., Ardura, A. and Dopico, E. Detecting Gaps in Knowledge: The Case of the *Anisakis* in Northwestern Spain. *J. Mar. Sci. Eng.,* **12**(8), 1333 (2024).
31. Polimeno, L., Lisanti, M.T., Rossini, M., Giacovazzo, E., Polimeno, L., Debellis, L., Ballini, A., Topi, S. and Santacroce, L. *Anisakis* Allergy: Is Aquacultured Fish a Safe and Alternative Food to Wild-Capture Fisheries for *Anisakis simplex*-Sensitized Patients? *Biology,* **10** (2), 106 (2021).
32. Di Azevedo, I. .M. .N., Knoff, M., Carvalho, V.L., Mello, W.N., Lopes Torres, E.J., Gomes ,D.C. and Iniguez, A.M., Morphological and genetic identification of *Anisakis paggiae* (Nematoda: Anisakidae) in dwarf sperm whale Kogia sima from Brazilian waters. *Dis. Aquat. Org.,* **113,** 103–111 (2015).

انتشار وتوصيف شكلي وجزيئي لأطوار يرقات الديدان الأنيساكيس سيمبلكس في الأسماك ذات الأهمية التجارية في مصر

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الملخص

أصبحت الإصابة بالأنيساكيازيس، التي تسببها الديدان الخيطية من عائلة الأنيساكيداي، مشكلة صحية عالمية بارزة، ويرجع ذلك بشكل رئيسي إلى زيادة استهلاك المأكولات البحرية النيئة أو غير المطهية جيداً. تستهدف هذه الدراسة الكشف عن ظهور وانتشار يرقات الأنيساكيس في الأسماك التي تُباع بشكل شائع في الأسواق المصرية. وقد تم استخدام تقنيات شكلية وجزيئية لتحديد هوية هذه اليرقات. أظهرت النتائج انتشارًا مرتفعًا ليرقات الأنيساكيس، حيث تبين أن 85% من سمك الرنجة الأطلسي و52% من سمك الماكريل الأطلسي مصابة بها. بلغ متوسط شدة العدوى 11.17 يرقة لكل سمكة رنجة مصابة و4.03 يرقات لكل سمكة ماكريل مصاب، في حين بلغ متوسط الوفرة 9.5 يرقة لكل سمكة رنجة و2.1 يرقة لكل سمكة ماكريل. كشف التحليل الشكلي التفصيلي أن اليرقات تنتمي إلى نوع الأنيساكيس سيمبلكس من النوع الأول، والتي تتميز بسمات تشريحية محددة واضحة تحت المجهر الضوئي والإلكتروني الماسح. كما أكدت تقنية تسلسل الحمض النووي لمنطقة المستنسخة الداخلية (ITS)، بعد مطابقتها مع بيانات بنك الجينات، هوية اليرقات على أنها من نوع الأنيساكيس سيمبلكس. تسلط هذه الدراسة الضوء على الانتشار الكبير لليرقات الأنيساكيس في المعروض السمكي المصري، مما يؤكد على الحاجة إلى تدخلات صحية عامة للتخفيف من المخاطر المرتبطة باستهلاك الأسماك النيئة أو غير المطبوخة جيداً. كما عززت التقنيات الجزيئية المستخدمة في هذه الدراسة فهمنا لانتشار الأنواع والآثار الصحية المحتملة للإصابة بالأنيساكيازيس في مصر، مما يدعم الجهود العالمية الرامية لمكافحة هذا المرض.

الكلمات الدالة: الأنيساكيس سيمبلكس، سمك الرنجة الأطلسي، سمك الماكريل الأطلسي، الأمراض المشتركة بين الإنسان والحيوان، التحديد الجزيئي.