Egypt. J. Vet. Sci. **Vol. 55,** No. 1, pp. 175-182 (2024)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Morphology and Molecular Phylogeny of *Tetrancistrum sigani* (Monogenea: Dactylogyridae) and *Haliotrema banana* (Monogenea: Ancyrocephalidae), Parasites of Mullidae and Siganidae Fish of the Red Sea, Egypt.

Fathy A. Abdel-ghaffar, Manal F. Elgarhy, Nahed M. Saed* and Kareem Morsy
Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt.

THIS STUDY aimed for the description of two monogenean parasites isolated from two marine fish. Worms were classified morphologically as well as by molecular analysis. Monogeneans are ectoparasitic flatworms that live on the gills, fins, and skin of fish. The Diplectanidae are a family of monopisthocotylean monogeneans. They are all parasitic on the gills of marine fish. In the present study, a total of 25 of the white-spotted spine foot, *Siganus canaliculatius* (Siganidae), and 35 of the cinnabar goatfish, *Parupeneus heptacanthus* (Mullidae), were captured from the Red Sea near Egypt's Hurghada coast. Ten spinefoot fish and 16 goatfish were infected. Morphological characterization for the isolated parasites according to the light micrographs of the hard parts as well as the copulatory organ revealed that the infected fish parasitized by *Tetrancistrum sigani* (Dactylogyridae) and *Haliotrema banana* (Ancyrocephalidae). Also, ribosomal DNA of the isolated parasites was processed and sequenced where the recovered sequences were compared with previously deposited species in the gene bank, it was observed from the constructed phylogenetic tree that the species of *Tetrancistrum* sequences (present study) isolates were closely related to *T. sigani* (accession no. MN179335.1) deposited in GenBank, while the sequences of *Haliotrema* isolates of the present study were closely related to the same species by 96%.

Keywords: Monogenea, External parasites, Morphology, Molecular analysis, Red Sea.

Introduction

Marine fish supplies are critical to the global food chain, especially for many of the world's poorest people [1]. The Egyptian economy benefits greatly from fish, including improved income, a variety of livelihoods, animal-based protein nutrition, and foreign exchange earnings. Fish are among the main animals in marine ecosystems with considerable taxonomic and functional diversity. They are critical components of the aquatic food chain at all levels of consumption, from primary consumers to apex predators and decomposers [2-3]. The Red Sea is home to 1078 different fish species [4]. Monogenea are parasitic trematodes that live mostly on the exterior surfaces and gills of freshwater and marine fish [5]. Due to damage to tissues, respiratory affection and distress, and secondary bacterial and fungal infections, they have the potential to cause severe fish mortality. Most monogeneans are highly host-specific [6, 7] making it easier to identify worms from their specific host. The variety and geographic distributions of monogenean parasites infesting Red Sea fish in Egypt are unknown. Most of them are parasites that live primarily on fish gills and can demonstrate both host and location specificity [8, 9], generating economic losses and, in some cases, becoming pathogenic [10]. The cinnabar goatfish *Parupeneus heptacanthus* of the family Mullidae and the white-spotted spinefoot, *Siganus canaliculatius* of the family Siganidae, holds

*Corresponding author: Nahed M. Saed, e-mail: naheds@sci.cu.edu.eg . Tel.: 01007882520
(Received 17/07/2023, accepted 18/09/2023)
DOI: 10.21608/EJVS.2023.223240.1542

economic importance in coastal communities. It is sought after by the fishing industry for its commercial value as a food fish. Local markets and restaurants often feature this species due to its delicate flavor and appealing appearance. The sustainable management of *P. heptacanthus* populations is crucial to support livelihoods and ensure the continued availability of this valuable resource for both local consumption and export. In the present study, the gills of *P. heptacanthus* and *S. canaliculatius* collected from the Hurghada coast along the Red Sea in Egypt were examined for external parasitic infection. The taxonomy of the isolated parasites was determined by light microscopic examination of their hard parts including haptor, hamuli, bars, and the copulatory organ as well as molecular phylogeny.

Material and Methods

Sample collection

During a recent examination of helminth parasites infecting marine fish obtained from various locations at Hurghada coasts along the Red Sea in Egypt, 25 specimens of the white-spotted spinefoot, *Siganus canaliculatius* (family Siganidae) and 35 specimens of the cinnabar goatfish *Parupeneus heptacanthus* (family Mullidae) were examined for monogenean parasites infection. Fish lived in aquaria filled with the same water source and were evaluated within a few hours to prevent the loss of mobile and transitory ectoparasites.

Parasitological study

Once the gill arches were exposed after the removal of the opercula, each gill was carefully removed from the fish and bathed in normal saline to remove any excess gill mucus. Under a dissecting binocular microscope, monogenean parasites were collected with a Pasteur pipette and 4% formalin. Carleton [11] recommended coloring permanent whole-mount preparations with acetic acid alum carmine for 5-10 minutes. Dehydration was maintained by passing ethyl alcohol in ascending order. Clove oil and xylene were used to clean the specimens before mounting them in Canada balsam [12]. Under a microscope, the hard parts of worms were identified after being mounted on slides in ammonium picrate glycerin and examined via coverslips, as described in [13]. A digital camera was used to take photomicrographs with a Zeiss compound microscope. Prevalence, mean abundance, and morphometric measures were made in accordance with [14]. In parenthesis, measurements were provided as mean standard error followed by range.

Molecular analysis and phylogeny

Genomic DNA (gDNA) was extracted using the DNeasy tissue kit (Qiagen, Germany) per the manufacturer's instructions from samples stored in 70% ethanol. For this amplification, a MJ Research PTC-150 thermocycler was utilized. C1M13(5'GTAAAACGACGGCCAGACCCGC TGAATTTAAGCAT-3') and D2M13(5'CAGG AAACAGCTATGACTCCGTGTTTCAAGAC GG-3') are two universal primers [15] used to amplify a portion of a 28s rRNA sequence by polymerase chain reaction. 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, and 50 mM KCl), 1.5 mM MgCl2, 0.2 mM deoxynucleoside triphosphate mixture (dATP, dCTP, dGTP, and dTTP), 100 pmol of each primer, 2.5 U of Thermus aquaticus (Taq) polymerase, 0.1 µg of extracted parasite genomic DNA, and nuclease-free sterile double-distilled water up to 50.0 µL were used in PCR. The mixture was then subjected to 60 cycles for 30 seconds at 94°C, 10 minutes at 50°C, and 2 minutes at 72°C in a programmed thermocycler (Biometra GmbH, Germany) [16]. The amplified result (volume 10-15 L) was validated by agarose gel electrophoresis (1.5%), and the DNA bands were stained with ethidium bromide (0.5 g/mL) against a ready-to-use GeneRuler 100 bp Plus DNA ladder (molecular weight marker) (Fermentas, Canada) [16]. To extract the PCR amplicons of the appropriate size from the gel, a DNA gel purification kit (Abgene, United Kingdom) was used. The Big Dye Terminator v.3.1 Cycle Sequencing Kit was used for sequencing on an autonomous sequencer (3500 Genetic Analyzer; Applied Biosystems, USA) with the same primer sets. The sequences were aligned and compared to those of other Dactylogyridae species in GeneBank. The sequence identity of the recovered data was confirmed using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nih.gov/BLAST/). The sequence pruning for the recovered congeneric species was performed with Bioedit v 7.2.5, the sequence alignment was performed with CLUSTAL W v2, and the Maximum likelihood analysis-based phylogenetic tree was constructed with the MEGA 7 program.

Results

Tetrancistrum sigani Goto and Kikuchi (1917), Figures 1, 2

Description

The parasite has a leaf-like body with a broad trunk. The cephalic region and peduncle are

narrow and tapered. The body was 1,130–2,183 (1,442) long x 366–535 (455; n = 10) wide. The cephalic area contains well-developed anterior lobes, and huge bilateral sets of cephalic glands are present lateral to the pharynx. The pharynx was muscular, elongated, and oval, and the intestinal caeca was bifurcate, posterior to the pharynx, with diverticula, and terminated at the posterior area. The germarium is pretesticular, flask-shaped, and 88-195 (165; n = 10) long by 120-200 (173; n = 15) wide at the base. The vaginal vestibule is disc-shaped, 35-72 (65; n = 10) wide, with a straight vaginal duct and thick vitellarium. The testis was solitary, big, and subspherical, measuring 100-251 (188; n = 13) x 112-189 (173; n = 13) mm. The copulatory complex consists of a sigmoid MCO tube, a thin anterior basal flange that resembles a handle, and an accessory component that is slightly hinged with MCO. Two connected sections, a tapering rod-shaped anterior portion and a large cylindrical, extended posterior half, complete the complex. The copulatory complex measures 70-116 (100; n = 10) in length. Haptor measures 60-115 (82; n = 12) in length and 48-166 (72; n = 12) in width. Haptoral hooks absent; Ventral anchor 72-99 (82; n = 10) long, dorsal anchor 90-112 (106; n = 12) long, with superficial root tip and fragile shaft. 16-30 (25; n = 15) long stout ventral bar with inwardly pointed tips and projecting central portion. Dorsal bar is straight and grooved, with terminal expansions measuring 22-35 (30; n = 13) in length.

Haliotrema banana Lim and Justine (2007), Figures 3, 4

Body elongate, 489–733 long x 120–180 (166; n= 12) wide, Three pairs of cephalic glands and two pairs of eye spots are located in the anterior area. Subterminal ventral mouth; oval pharynx; The intestine branches out just behind the pharynx and is connected to the testicles and the short peduncle behind them. Testis is single, spherical, larger than the ovary, and postero-dorsal to and overlaps the ovary. The copulatory organ consists of a copulatory tube with a long base and sinuous tapering tube, as well as a thin filament (accessory component) at the distal apex of the tube, with a length of 90-110 (105; n=12). The oviduct emerges from the anterior portion of the ovary and receives a slender vaginal duct from the distal portion (not the tip) of the flask-shaped saccular vagina. Haptor set off from the body, rectangular; 75–130 (100; n= 15) long x 100–160) (155; n= 13) wide, two pairs of anchors; two dorsal anchors 50–66 (59; n= 10) long; two ventral anchors with well-developed roots, 63 (55–72) (70; n= 12) long; 2 connecting bars: dorsal bar, straight, 33–50 (40; n= 10) wide and a ventral bar, U-shaped, 42 (30–53) wide.

Molecular study

The sequence analysis of the PCR amplicons revealed that the 696bp DNA fragments encoding the gene aligned with 49 monogenean species from the family Dactylogyridae obtained from GenBank. To calculate and analyze the evolutionary relationships of the sequences, a phylogenetic tree (Fig. 5) was constructed, with the length of the horizontal line proportional to the estimated genetic distance between the sequences. The tree represents the relationship between the two species in question, and the nucleotide identity percentage between *T. sigani* sequences (present study) and those of other closely related relatives in GenBank ranged from 92 to 97%; 97% of identity with *T. sigani*, accession no. MN179335.1; 96% with *T. polymorphum*, accession no. MT023786.1 and 92% with *T. labyrinthus*. *H. banana* sequences of the present study showed percentages of nucleotide identity ranging between 86-96% with closely related species of the genus *Haliotrema.* A high percentage of infection was observed for *H. magnihamus,* accession no. MG593838.1 with a percentage of identity of 95%, while the lowest percentage was recorded for *H. sicklocirrus,* accession no. KJ571015.1.

Discussion

The presence of a club-shaped accessory piece distinguishes *T. sigani* from other related species. *Tetrancistrum* spp. descriptions compared to show that the detected species' morphometric characteristics are like those of its relatives. This species' MCO can fluctuate, occasionally appearing J-shaped, especially in compressed specimens. *T. sigani* lacks "marginal" hooks, which were previously thought to be a diagnostic feature of the genus, according to Goto and Kikuchi [17]. However, the presence or lack of hooks is not always a defining criterion for diagnosis. *T. sigani's* copulatory complex and haptoral features are like those found in *T. suezicum* and *T. oraminii.* The main things that make *T. sigani* different from *T. suezicum* and *T. oraminii* are the shape of the MCO tube and the anterior basal flange, as well as the shape and make-up of the extra piece. Even though the authors did not compare their species to *T. sigani*, their representations of the copulatory complex, as well as its reported dimensions

and those of the anchors, correspond to the specimens at hand. As a result, *P. granulosum* is regarded as a junior subjective synonym of *T. sigani.* The molecular analysis also supported the current parasite's taxonomy as *T. sigani*. A big flask-shaped vagina, a small medial ovary, a round dorsal testis with the vas deferens circling the left intestinal caecum, a simple copulatory apparatus, a noticeable prostatic reservoir, four anchors, and two bars distinguish *Haliotrema*. Klassen [18] proposed a *Haliotrema* bodiani group that comprises *H. bodiani*, *H. balisticus*, *H. priacanthi*, *H. pacificus*, *H. cornutus* (Mizelle et Kritsky [19], *H. ornatum* Yamaguti [20], and *H. teuthis* (MacCallum [21]). The current species is distinct from the *Haliotrema* species of the bodiani group. The pronounced flask-shaped vagina seen in the current *Haliotrema* species is also seen in *Haliotrema* species outside the bodiani group [18]. *H. spirale*, for example, has a comparable vaginal morphology, copulatory apparatus, and anchoring but is part of the spirale group. *H. cancescens* [20], *H. ctenochaeti* [20], *H. curvicirrus* [20], *H. flexicirrus* [20], *H. macracantha* [20], *H. palmatum* [20], *H. serpenticirrus* [20], and *H. spirale* [20] are other *Haliotrema* species with huge flask-like vaginas. *H. brotulae* (annulocirrus group), *H. chelicirrus* (annulocirrus group), *H. rectangulare* (annulocirrus group), *H. priacanthi* (annulocirrus group), and *H. sigmodocirrus* (annulocirrus group) have smaller vaginal "flasks" and longer vaginal ducts [18]. Based on the combination of vaginal shape, U-shaped ventral bar, and copulatory apparatus, the present species is comparable to *H. spirale* from *Brotula multibarbata* Temminck et Schlegel and *H. minutospirale* [20] from *Parupeneus cyclostomus* Lacépède (junior synonym *P. chryserydros*). *H. spirale*, on the other hand, has a more noticeable and longer filament-like accessory piece. According to morphological analysis, the current specimen is more comparable and closer to the *H. banana* described by Lim and Justine [22], which relied solely on morphological analysis without molecular characterization to be compared with the isolates retrieved from the geneBank.

Conflict of interest: Authors declare that there is no conflict of interest.

*Acknowledgment:*The authors extend their appreciation to the Faculty of Science, Cairo University for supporting this work.

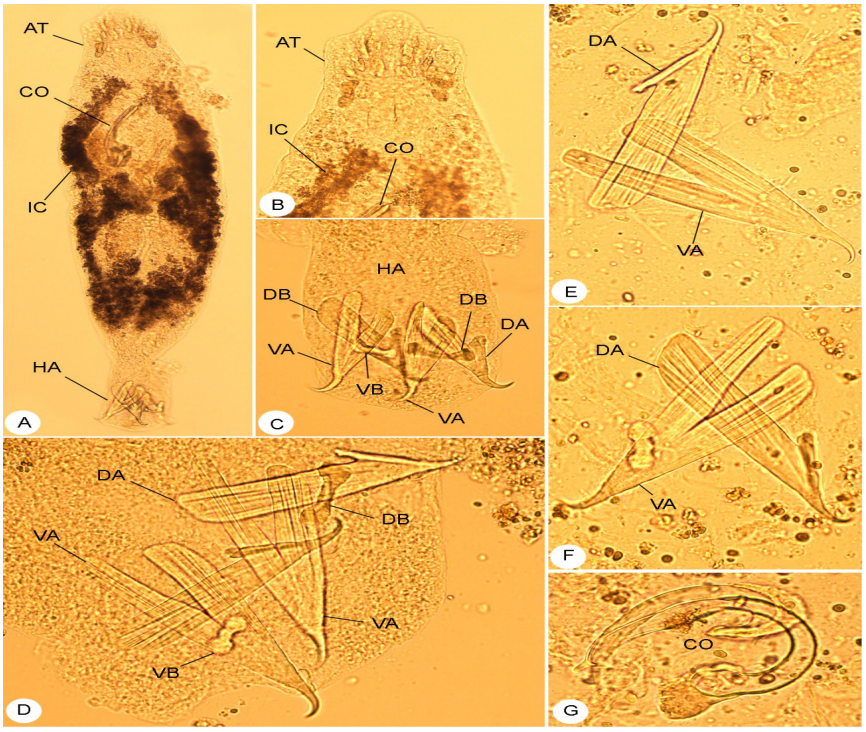

Fig.1. Photomicrographs of *T. sigani* isolated from the gills of *S. canaliculatius* showing: (A) Whole-mount preparation of adult worm, dorsal view, AT anterior attachment organ, IC intestinal ceca, CO copulatory organ, HA haptor, Bar 30µm; (B) the anterior part of the adult worm, Bar 5µm; (C-F) Hard parts of haptor, Bar 5µm; DA dorsal anchor, VA ventral anchor, DB dorsal bar, VB ventral bar. (G) Copulatory organ (CO).

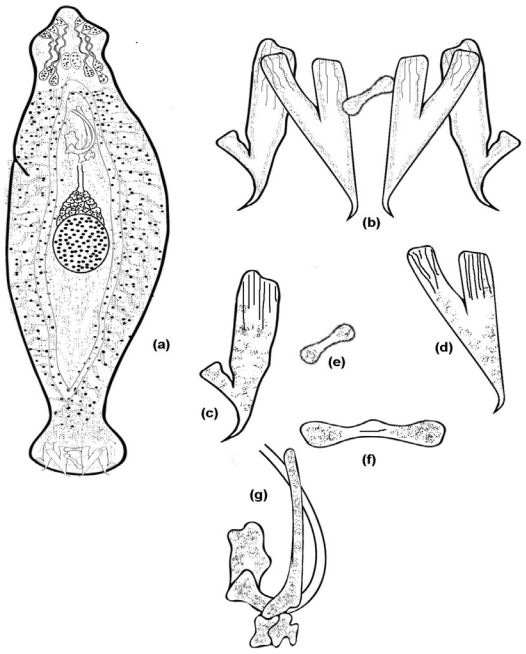

Fig. 2. Line diagram of T. sigani isolated from the gills of *S. canaliculatius* showing: (a) whole-mount preparation of adult worm, dorsal view, (b) haptor (c) dorsal anchor, (d) ventral anchor, (e) dorsal bar, (f) ventral bar. (g) Copulatory organ.

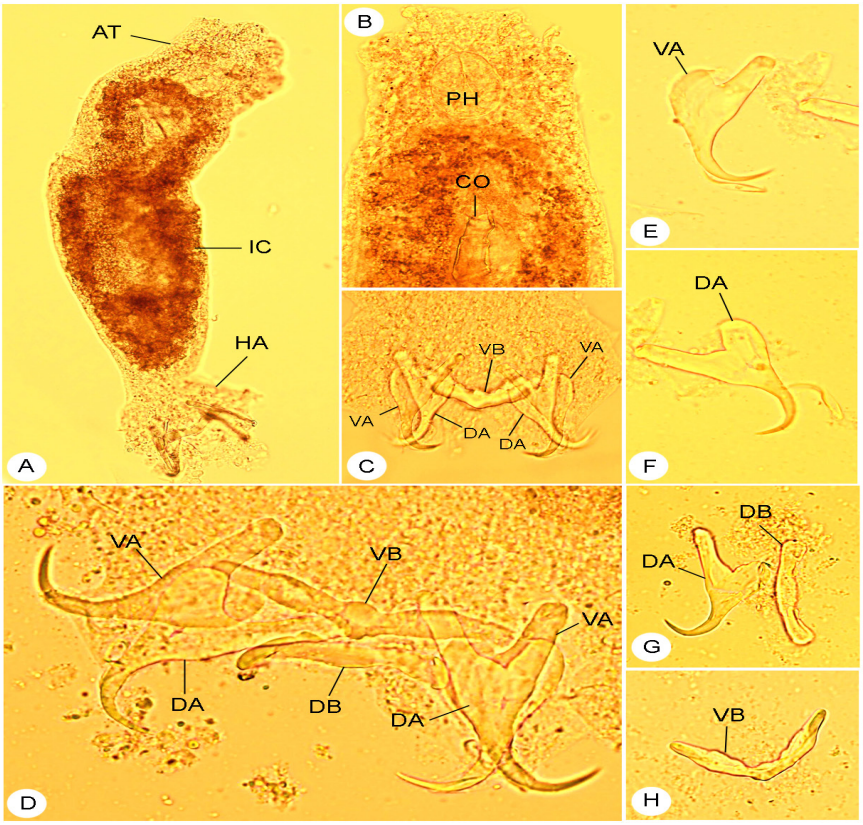

Fig. 3. Photomicrographs of *H. banana* isolated from the gills of *P. heptacanthus* showing: (A) Whole-mount preparation of adult worm, dorsal view, AT anterior attachment organ, IC intestinal ceca, HA haptor, Bar 30µm; (B) the anterior part of the adult worm, Pharynx, CO copulatory organ, Bar 5µm; (C-H) Hard parts of haptor, Bar 5µm; DA dorsal anchor, VA ventral anchor, DB dorsal bar, VB ventral bar.

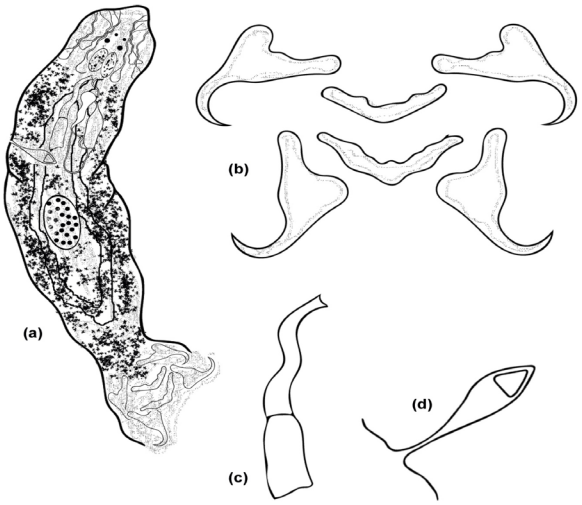

Fig. 4. Photomicrographs of *H.banana* isolated from the gills of *P. heptacanthus* showing: (a) whole-mount preparation of adult worm, dorsal view, (b) haptor (c) Copulatory tube (d) Vagina.

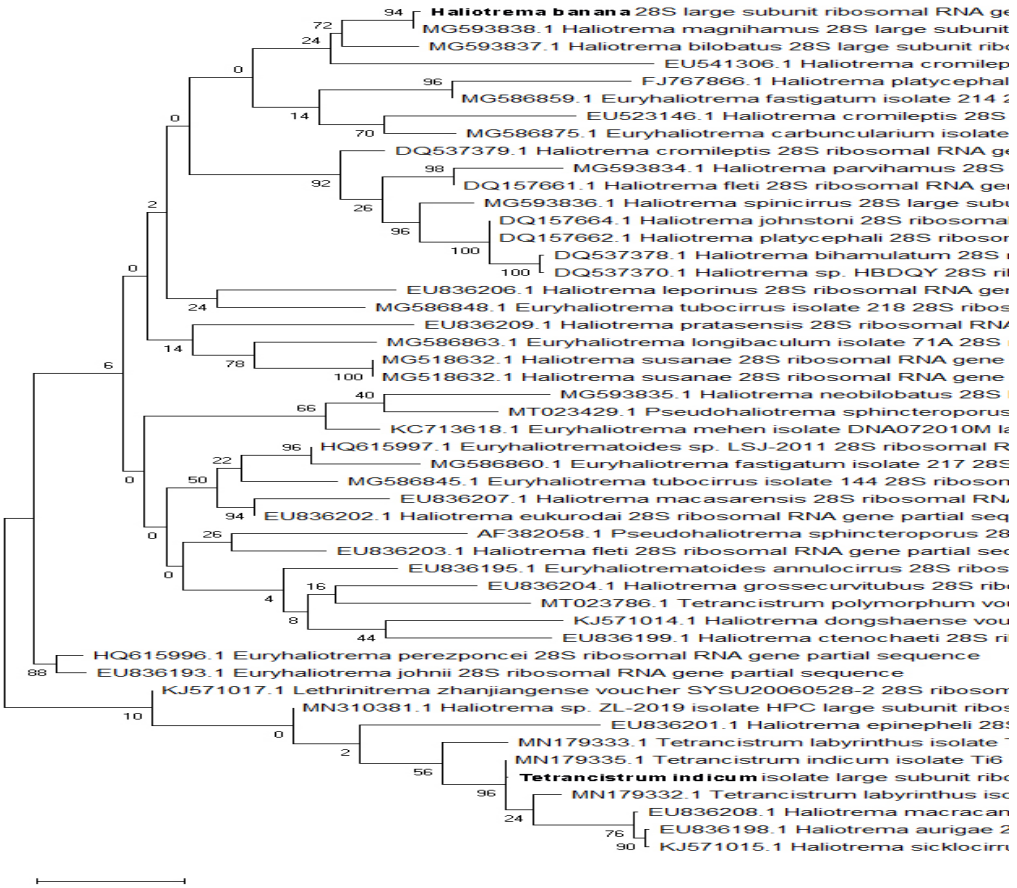

Fig. 5. Phylogenetic tree showing the Evolutionary analysis by Maximum Likelihood method: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 49 nucleotide sequences. There were a total of 696 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

References

1. Hilborn, R., Amoroso, R.O. and Anderson, C.M. Effective fisheries management instrumental in improving fish stock status. *Proceedings of the National Academy of Sciences,* **19**, 1-7 (2020).
2. Specziár, A. and Erős, T. Development of a fish-based index for the assessment of the ecological status of Lake Balaton in the absence of present-day reference condition. *Knowledge and Management of Aquatic Ecosystems*, **421**, 1-11 (2020).
3. Wootton, R.J. Ecology of teleost fishes. Second edition. *Fish and Fisheries Series,* **24**. Dordrecht: Kluwer Academic Publishers (1998).
4. Golani, D. and Bogorodsky, S. The fishes of the Red Sea-reappraisal and updated checklist. *Zootaxa*, **2463,** 1–135 (2010).
5. Mushagalusa Mulega, A., Van Steenberge, M., Kmentová, N., Muterezi Bukinga, F., Rahmouni, I., Masilya, P.M., Benhoussa, A., Pariselle, A. and Vanhove, M.P. Monogeneans from Catfishes in Lake Tanganyika. II: New Infection Site, New Record, and Additional Details on the Morphology of the Male Copulatory Organ of Gyrodactylus transvaalensis Prudhoe and Hussey, 1977. *Pathogens*, **12** (2), 200 (2023).
6. Hargis, W. A new species of Benedenia from *Girella nigricans* of opaleye. *Journal of Parasitology*, **41**, 40-50 (1955).
7. Lawler, A.R. Zoogeography and host specificity of superfamily Capsalodiea Price, 1936 (Monogenea: Monopisthocatylea). An evaluation of the host-parasite locality records of superfamily capsaloidea price, 1936, and their utility in determinations of host specificity and zoogeography. Special papers in Marine Science No. **6**, 650 pp. Gloucester Point, Virginia, USA: *Virginia Institute of Marine Science and School of Marine Science,* College of William and Mary (1981).
8. Kardousha, M.M. Monogenea of Arabian Gulf fishes 1. Descriptions of three *Capsala* spp. (Capsalidae) including *Capsala naffarin*. sp. infecting mackerel tuna *Euthynnus affinis* from coasts of Emirates. *Parasitology International*, **51**, 327-335 (2002).
9. Bayoumy, E.M. and Abu-Taweel, G.M. Metazoan Parasites of Some Arabian Gulf Fish, Off Dammam, Saudi Arabia: 2- Associations of external and internal parasites. *Global Veterinary*, **9**(5), 512-516 (2012).
10. Rückert, S.W., Hagen, A.T., Yuniar. and Palm, H.W. Metazoan parasites of fishes and their potential use as biological indicators in the Segara Anakan Lagoon, Indonesia. *Regional Environmental Change*, **9**, 315-328 (2009).
11. Carleton, H. Carleton's histological technique. Fourth, edition - *Oxford University Press*. New York. Toronto. (1967).
12. Ergens, R. The suitability of ammonium picrate-glycerin in preparing slides of lower Monogenoidea. *Folia Parasitologica*, **16,** 320-328 (1969).
13. Vidal-Martínez, V.M., Osorio-Sarabia, D. and Overstreet, R.M. Experimental infection of *Contracaecum multipapilatum* (Nematoda: Anisakinae) from Mexico in the domestic cat. *Journal of Parasitology*, **80**, 576-579 (1994).
14. Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. Parasitology meets ecology in its own terms: Margolis et al. revisited. *Journal of Parasitology*, **83**, 575-583 (1997).
15. Jovelin, R. and Justine, J.L. Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *International Journal of Parasitology*, **31**, 393-401 (2001).
16. Catalano, S.A., Goloboff, P.A. and Giannini, N.P. Phylogenetic morphometrics (I): the use of landmark data in a phylogenetic framework. *Cladistics*, **26** (5), 539-549 (2010).
17. Goto, S. and Kikuchi, H. Two new trematodes of the family Gyrodactylidae. *Journal of the College of Science*, Imperial University of Tokyo, **39**, 1-22 (1917).
18. Klassen, G.J. Phylogeny of *Haliotrema* species (Monogenea: Ancyrocephalidae) from boxfishes (Tetraodontiformes, Ostracidae): are *Haliotrema* from boxfishes monophyletic? *Journal of Parasitology*, **80**, 596–610 (1994).
19. Mizelle J. D. and Kritsky, D. C. Studies on monogenetic trematodes. XL, New species from marine and freshwater fishes. *American Midland Naturalist*, **82**, 417-428 (1969).

20. Yamaguti, S. Monogenetic trematodes of Hawaiian fishes. *University of Hawaii Press*, Honolulu, Hawaii, **287**, (1968).

21. MacCallum, G. A. Some new species of ectoparasitic trematodes. *Zoologica*, **1**, 395-410 (1915).

22. Lim, L. H. S. and Justine, J. L. *Haliotrema banana* sp. n. (Monogenea: Ancyrocephalidae) from *Bodianus perditio* (Perciformes: Labridae) off New Caledonia. *Folia Parasitologica,* **54**(3), 203(2007).

المورفولوجي والتطور الجزيئي لتيترانسيسترم سيجناي وهاليوتريما بانانا المتطفلة على أسماك السيجان والبربون بالبحر الأحمر في مصر

فتحي عبد الغفار ، منال فاروق الجارحي ، ناهد محمد سعيد* و كريم مرسي
قسم علم الحيوان - كلية العلوم - جامعة القاهرة - القاهرة - مصر.

طفيليات وحيدة العائل (طائفة مونوجينيا) هي كائنات تتطفل على خياشيم وجلد الأسماك مستغلة مكان التطفل في الحصول على الغذاء والحماية مما قد يسبب أضررًا نسيجية وصحية على الأسماك مما يؤي إلى إختناقها وموتها. هدفت الدراسة الحالية في عمل وصف تفصيلي من حيث الدراسة الشكلية وأيضا بإستخدام تقنية التحليل الجزيئي للحمض النووي لنوعين من الطفيليات الخارجية على خياشيم وجلد أسماك السيجان والبربون والتي تم الحصول عليها من سواحل البحر الأحمر بمنطقة الغردقة، جمهورية مصر العربية. وقد أثبتت الدراسة الشكلية التصنيف الدقيق لنوعين الطفيليات التي تم عزلها من الأسماك على أنها طفيل تيترانسيسترم سيجناي والذي تم عزله من سمكة السيجان وطفيل هاليوتريما بانانا والذي تم عزله من سمكة البربون، كما تم إستنتاج مدى تطابق الحمض النووي بنسبة تتعدى 90% مع أنواع تم تسجيلها لنفس الجنس من هذه الطفيليات.

الكلمات الدالة: مونوجينيا، الطفيليات الخارجية، دراسة شكلية، تحليل جزيئي، البحر الأحمر.