

# First record of *Pseudoterranova decipiens* (Nematoda, Anisakidae) infecting the Red spot emperor *Lethrinus lentjan* in the Red Sea

Primeiro registro de *Pseudoterranova decipiens* (Nematoda, Anisakidae) infectando o imperador da mancha vermelha *Lethrinus lentjan* no Mar Vermelho

Saleh Al Quraishy<sup>1\*</sup> ; Rewaida Abdel-Gaber<sup>1,2</sup> ; Mohamed Abdel Monem Dkhil<sup>1,3</sup> 

<sup>1</sup> Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

<sup>2</sup> Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

<sup>3</sup> Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

Received May 21, 2019

Accepted June 25, 2019

## Abstract

The current parasitological study was carried out to investigate helminth parasites infecting the Red spot emperor *Lethrinus lentjan* inhabiting Hurghada City at the Gulf of Suez, Red Sea, Egypt. Third-stage larvae of nematode parasite was isolated from the intestine as well as body cavity of the examined fish. Light and scanning electron microscopy revealed that this parasite belonged to Anisakidae family within the genus *Pseudoterranova*. The present species is named *Pseudoterranova decipiens* based on the presence of triangular mouth aperture with prominent boring teeth and soft swellings of the cuticle, long muscular esophagus, ventrally excretory pore, and narrow transverse slit of anal opening followed by a short mucron. The morphological characteristics of this species were confirmed by molecular analysis of 18S rDNA gene region of the present parasite. It demonstrated a close identity  $\geq 89\%$  with taxa under family Anisakidae, 85% with Raphidascarididae, and 79-84% with Toxocaridae. A preliminary genetic comparison between gene sequence of the present parasite and other oxyurid species placed it as a putative sister taxon to other *Pseudoterranova decipiens* described previously. This study demonstrated that the 18S rDNA gene region of *Pseudoterranova decipiens* yielded a unique sequence that confirmed its taxonomic position in Anisakidae.

**Keywords:** Marine fish, *Lethrinus lentjan*, *Pseudoterranova* spp., morphological studies, molecular analysis.

## Resumo

O presente estudo parasitológico foi realizado para investigar os helmintos parasitos que infectam o peixe imperador *Lethrinus lentjan*, que habita a cidade de Hurghada no Golfo de Suez, Mar Vermelho, no Egito. Larvas de terceiro estágio de parasitos nematoides foram isoladas do intestino e da cavidade do corpo do peixe examinado. Microscopia eletrônica de luz e de varredura revelou que este parasita pertence à família Anisakidae dentro do gênero *Pseudoterranova*. A espécie atual é denominada *Pseudoterranova decipiens* baseada na presença de abertura triangular da boca com dentes proeminentes chatos e inchaços moles da cutícula, esôfago muscular longo, poro ventralmente excretor e fenda transversal estreita da abertura anal seguida por um mucron curto. As características morfológicas desta espécie foram confirmadas pela análise molecular da região do gene 18S rDNA do presente parasito. Demonstrou uma identidade próxima  $\geq 89\%$  com taxa sob família Anisakidae, 85% com Raphidascarididae, e 79-84% com Toxocaridae. Uma comparação genética preliminar entre a sequência genética do presente parasito e outras espécies de oxiurídeos coloca-o como um taxon irmão putativo para outros *Pseudoterranova* descritos anteriormente. Este estudo demonstra que a região do gene 18S rDNA de *Pseudoterranova decipiens* produz uma sequência única que confirma sua posição taxonômica em Anisakidae.

**Palavras-chave:** Peixe marinho, *Lethrinus lentjan*, *Pseudoterranova* spp., estudos morfológicos, análise molecular.

## Introduction

Anisakid nematodes have a global distribution among a wide variety of more than 200 marine fish species as intermediate host (MCCLELLAND et al., 1990) or as paratenic hosts (KØIE et al.,

1995; KUHN et al., 2011). Cephalopod, marine mammals, and humans can become accidental hosts for anisakids by ingesting fish infected with third-stage larvae and their prevalence's can be very high (WHARTON et al., 1999; ABOLLO et al., 2001; MCCLELLAND, 2002; SZOSTAKOWSKA et al., 2002). The life cycles of marine ascaridoid nematodes involve a number of stages

\*Corresponding author: Saleh Al Quraishy. Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia. e-mail: [squraishy@ksu.edu.sa](mailto:squraishy@ksu.edu.sa)



and hosts. Adult and other life stages of these anisakid nematodes can be found in almost any part of the fish including the body cavity, internal organs, swim bladder, deeper layers of the skin or fins and external muscle layers (SMITH & WOOTTEN, 1978; MATTIUCCI et al., 2008). Rocka (2004) followed by Nada & Abd El-Ghany (2011) stated that anisakid nematodes commonly found in bony fish are represented by the following genera: *Anisakis* (Dujardin, 1845), *Contracaecum* (Railliet & Henry, 1912), *Hysterothylacium* (Ward & Magath, 1917), *Paranisakiopsis* (Yamaguti, 1941) and *Pseudoterranova* (Mozgovoy, 1951). Anisakid larvae are usually very difficult to identify species using morphology due to the lack of differential characters, but when adults are described and genetically characterized, then such larva assigned to a species based on molecular studies (MATTIUCCI et al., 1997).

Anisakid nematodes belonging to the *Pseudoterranova decipiens* species complex (also known as sealworms or codworms) mature and reproduce in the digestive tract of pinnipeds (LAUCKNER, 1985; DI AZEVEDO et al., 2017; IRIGOITIA et al., 2018). As far as it has been known, the life cycle of *Pseudoterranova* species also includes crustaceans as the first hosts, and fish as second hosts (SUKHDEO, 2012). Third-stage larvae (L3) of sealworms have commonly been reported in marine teleosts worldwide (GEORGE-NASCIMENTO, 1987; MATTIUCCI & NASCETTI, 2008). The complex of *Pseudoterranova decipiens* is composed of six sibling species with four species occurring in the Northern Hemisphere are namely *Pseudoterranova azarasi* (Yamaguti & Arima, 1942), *Pseudoterranova bulbosa* (Cobb, 1888), *Pseudoterranova decipiens* sensu stricto (s.s.) (Krabbe, 1868), and *Pseudoterranova krabbei* Paggi et al. (2000). While, Mattiucci & Nascetti (2008) reported that the remaining two species present in the Southern Hemisphere are namely *Pseudoterranova cattani* (George-Nascimento & Urrutia, 2000), and *Pseudoterranova decipiens* Bullini et al. (1997). Additional species in the genus *Pseudoterranova* include *Pseudoterranova cattani*, identified using allozyme markers in the Pacific (SUKHDEO, 2012).

Therefore, the present study aimed to report the natural occurrence of anisakid nematodes in the Red spot emperor *Lethrinus lentjan*. Additionally, identify the recovered worms by using light and scanning electron microscopy in order to determine its characteristic morphology, which may contribute valuable information to knowledge of the anisakids. Also, clarify the taxonomic position of the present anisakid nematodes using molecular phylogenetic analysis.

## Materials and Methods

### *Fish samples collection and parasitological examinations*

Forty two specimens of the Red spot emperor *Lethrinus lentjan* (Family: Lethrinidae) were collected during the period of August 2017–May 2018 from boat landing sites and fishermen of Hurghada City at the Gulf of Suez, Red Sea, Egypt. The collected fish specimens were transported immediately to the Laboratory of Parasitology Research at Zoology Department, Faculty of Science, Cairo University, Egypt, using special boxes for parasitological examination. All procedures contributing to this work comply with the ethical standards authorized by the Institutional Animal Care and Use Committee (IACUC) at Faculty of Science, Cairo University in Egypt with no. CUFS/S/Para/38/2014.

Gross microscopic examinations of all abdominal fish organs were done. The contents of the digestive tract were examined under a binocular microscope for the detection of any parasitic worms, which then removed with a fine forceps or a pipette. After isolation of parasitic worms from the infected fish, washing several times with an isotonic saline solution was done to get rid of any mucous and debris wastes. Isolated worms were fixed in 70% ethanol and subsequently clarified with lactophenol for morphological identification, in accordance with standard reference keys (PETTER & QUENTIN, 2009). Parasite prevalence was calculated according to Bush et al. (1997). Photomicrographs of adult specimens were made with the aid of microscope Leica DM 2500 (NIS ELEMENTS software, ver. 3.8) in the Laboratory of Parasitology Research in Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

For scanning electron microscope, some of nematode worms were fixed with 3% buffered glutaraldehyde, dehydrated in ascending series of ethyl alcohol (70%, 80%, 95%, 100%), infiltrated with amyl acetate, processed in a critical point dryer “LEICA, EM CPD300”, sputter-coated with gold–palladium in a gold sputtering system (JEOL, JEC-3000FC) for 30 s at ~70 m Torr pressure, and finally examined under an Etec Autoscan at 10-kV JEOL scanning electron microscope (JSM-6060LV). Measurements were carried out in millimeters, presented as a range followed by the arithmetic mean±SD in parentheses, and unless otherwise were stated.

### *Molecular analysis*

gDNA was extracted from ethanol-preserved samples by using Qiagen DNeasy™ tissue kit according to the manufacturer's protocol. PCR reaction was carried out to amplify the target gDNA using the previously mentioned primers of NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') by Nadler et al. (2005). PCR reactions (25 µl) were performed in 2 mM MgCl<sub>2</sub>, 0.2 mM each of dNTPs, 2.5 µl 10× rTaq DNA buffer, 2.5 µM of each primer, 1.25 U rTaq polymerase buffer, 1 µl of DNA sample and completed to 25 µl with dist. H<sub>2</sub>O in a thermocycler (BioRad) under the following conditions: 94°C for 5 min (initial denaturation), then 35 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (annealing), and 1 min at 72°C (extension) and finally post-PCR extension was carried out for 7 min at 72 °C. Amplicons were sequenced using ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) with 310 Automated DNA Sequencer (Applied Biosystems, USA). To identify related sequences, a BLAST search was carried out on NCBI database. GenBank accession numbers of additional sequences utilized in the analyses were *Pseudoterranova decipiens* (gb| KF806033.1, U94766.1, JQ673263.1), *Pseudoterranova* sp. (gb| KC013597.1), *Terranova caballeroi* (gb| U94767.1), *Anisakis typica* (gb| HF911524.1), *Anisakis simplex* (gb| LL324431.1), *Phocascaris* sp. (gb| AF226575.1), *Contracaecum radiatum* (gb| AF226577.1), *Contracaecum multipapillatum* (gb| AF226574.1), *Hysterothylacium fortalezae* (gb| U94760.1), *Raphidascaris acus* (gb| AY821772.1), *Toxocara cati* (gb| JN256994.1), and *Toxocara canis* (gb| JN256996.1). Data of DNA sequences were aligned using CLUSTAL-X multiple sequence alignment. The alignment will be corrected manually using the alignment editor of

software BioEdit 4.8.9 (HALL 1999). A phylogenetic tree was reconstructed using MEGA ver. 7.0 by using maximum parsimony (neighbour-interchange [CNI] level 3, random addition trees 100). To evaluate the robustness of the tree topologies, bootstrap analysis was performed based on 1000 replicates.

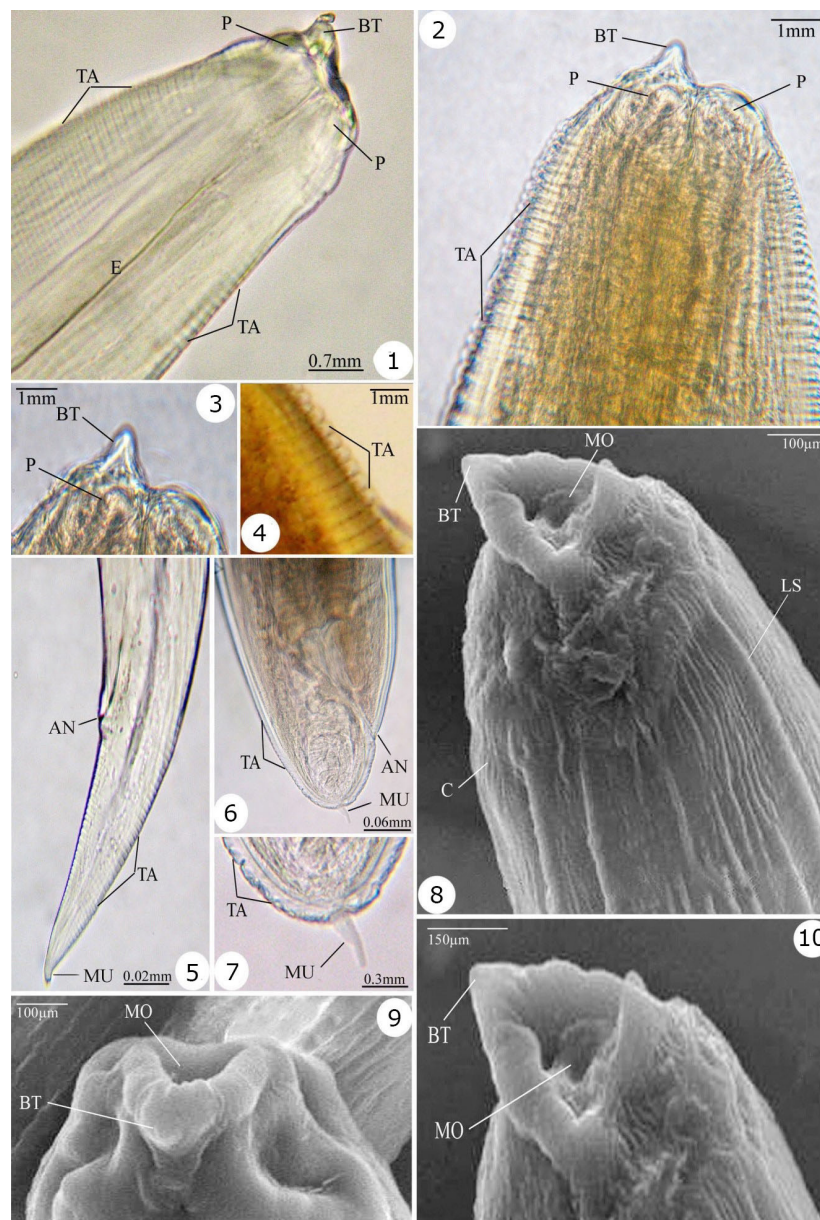
## Results

Thirty one (73.80%) out of forty two specimens of the Red spot emperor *Lethrinus lentjan* (Family: Lethrinidae) were found to be infected with anisakid nematode parasite. The infection

was recorded in the intestine as well as the body cavity of the examined fish. The infection was increased during summer to be 95.23% (20 out of 21) followed by winter season to be 52.38% (11 out of 21). The number of parasites per fish was ranged from 8 to 16.

### Description (Figures 1-10)

The body of the recovered third-stage larvae was yellowish to reddish in color, medium-sized, and elongated. Body measured 6.8-8.6 ( $7.2 \pm 1.1$ ) mm in length and 0.16-0.21 ( $0.18 \pm 0.01$ ) mm in width. The anterior end is rounded with triangular mouth aperture. Lips were absent with prominent boring teeth at the



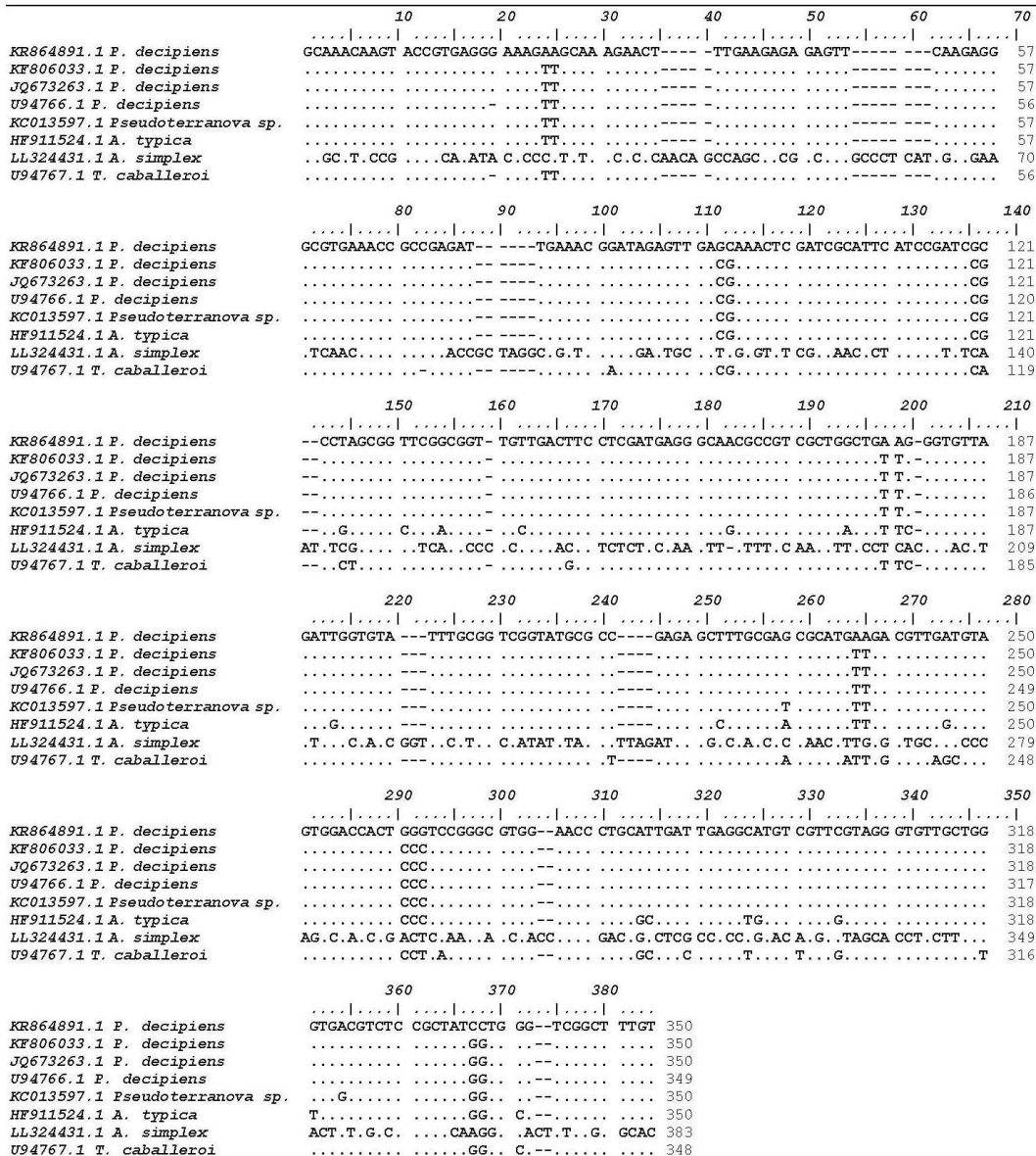
**Figures 1-10.** Photomicrographs of *Pseudoterranova decipiens* third-stage larvae recovered during the period of this study from *Lethrinus lentjan* inhabiting Hurghada City at the Gulf of Suez, Red Sea, Egypt; showing high magnifications of: **1-3** Anterior end with boring tooth (BT), papillae (P), esophagus (E) and clear appearance of transverse annulations (TA) of the cuticle (C). **4** Transverse annulations (TA) of the cuticle. **5** Posterior end of juvenile with anus (AN) and mucron (MU). **6,7** Posterior ends of larva with short mucron (MU). **Figs. 8-10** Scanning electron micrographs for the anterior end of *Pseudoterranova decipiens* showing boring tooth (BT), mouth opening (MO), and clear appearance of longitudinal striations (LS) of the cuticle.

anterior extremity provided with soft swellings of the cuticle at the level of papillae surrounding the tri-radiate mouth opening. The worm's esophagus had a long anterior muscular part, 0.79-0.96 (0.91±0.01) mm long. The excretory pore opened ventrally, below the boring teeth at the anterior end. The covering cuticle was rigid with transverse annular striations. The anal opening was in the form of a fairly long and narrow transverse slit. Tail was short, conical, pointed, 0.10-0.20 (0.15±0.01) mm long. The worm body ended by a short mucron, 0.01-0.03 (0.02±0.002) mm long.

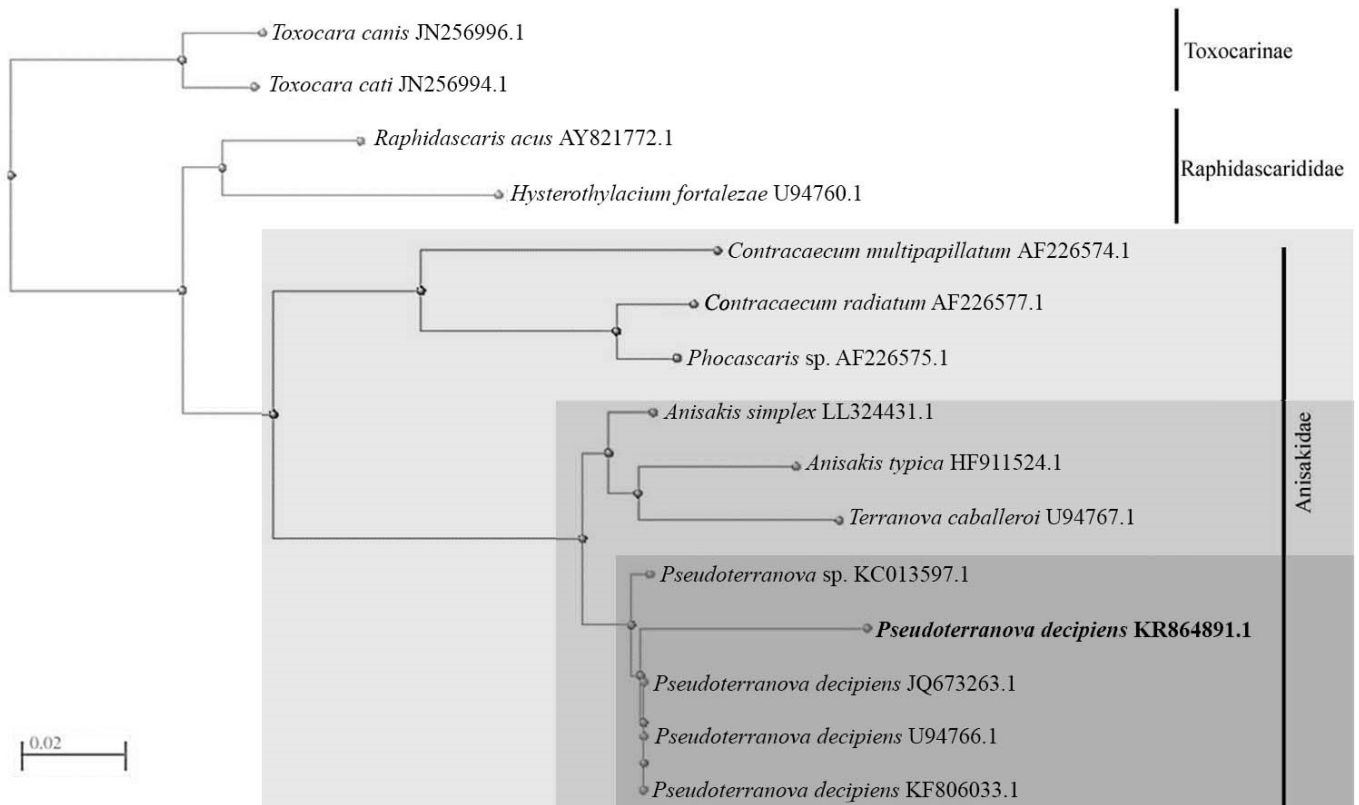
*Molecular analysis*

A total of 350 bp of 18S rDNA gene sequence with 54.6% GC content was recovered from the present anisakid species and deposited in GenBank (gb| KR864891.1). Pairwise comparison of

the nucleotide sequences and divergence showed that the present anisakid species revealed sequence identities ≥89% with taxa under family Anisakidae, 85% with Raphidascairididae, and 79-84% with Toxocaridae. Among Anisakidae members, the maximum identity with low divergent values was recorded as 96% with the previously studied *Pseudoterranova decipiens* (gb| KF806033.1, U94766.1, JQ673263.1), followed by 95% with *Pseudoterranova* sp. (gb| KC013597.1), 93% with *Anisakis simplex* (gb| LL324431.1), 91% with *Anisakis typica* (gb| HF911524.1), and 89% with *Terranova caballeroi* (gb| U94767.1), which had the highest blast scores with small number of nucleotide differences (Figure 11). Phylogenetic analysis produced a neighbor-joining tree constructed with partial sequences consistently formed two major lineages (Figure 12). The first major clade represent monophyletic origin for Ascaridoidea species and consisted of two larger subclades, showed



**Figure 11.** Sequence alignment of 18S rDNA gene region of *P. decipiens* with the most closely related ansakid species. Only variable sites are shown. Dots represent bases identical to those of the first sequences, and dashes indicate gaps.



**Figure 12.** Molecular Phylogenetic analysis of the present *Pseudoterranova decipiens* third-stage larvae by Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-2014.41) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

that Raphidascarididae (sampled genera included *Raphidascaris* and *Hysterothylacium*) being sister to Anisakidae (sampled genera included *Anisakis*, *Pseudoterranova*, *Contraecum*, and *Phocascaris*). The other clade represents the monophyletic origin of Toxocaridae represented by genus *Toxocara*. Phylogenetic relationship showed that the present anisakid species is deeply embedded in the genus *Pseudoterranova* with close relationship to all previously studied species of *Pseudoterranova* species especially *Pseudoterranova decipiens* as a more related sister taxon.

## Discussion

The anisakid nematode *Pseudoterranova decipiens* was recovered during the current study to infect the intestine as well as the body cavity of the Red spot emperor *Lethrinus lentjan* fish with 73.80% as a percentage of infection. This observation agreed with data obtained by Klimpel & Palm (2011) followed by Najda et al. (2018) whom stated that the digestive tract considered as the preferred site of anisakid infection in marine mammals. The rate of parasitic infection increased during summer and fall during winter; these results coincided with data obtained by Ólafsdóttir & Hauksson (1998) who stated that the common seal infected by *Pseudoterranova decipiens* with a percentage of 44.7% and development of ascaridoid larvae are highly influenced by temperature and the generation time likely to be longer in the warm water.

The morphology of the present parasite has the same diagnostic generic features of genus *Pseudoterranova* by having an elongated body provided with a rounded anterior end with triangular mouth aperture provided with lateral boring teeth and ended by a short pointed tail with mucron. It was compared morphologically and morphometrically to other *Pseudoterranova* species recorded previously. Description of the present parasite species agreed much more with *Pseudoterranova decipiens* described previously by Timi et al. (2001) from *Engraulis anchoita* in morphological and morphometric data of the different body parts. In addition, it resembled to records of Felizardo et al. (2009) from *Paralichthys isosceles*, Piña-Vázquez et al. (2012) from *Lophius gastrophysus* with little difference in measurements.

Molecular approaches to delimiting and identifying anisakid nematodes have markedly influenced our understanding of their systematics and biodiversity (NADLER & HUDSPETH, 1998, 2000; NADLER et al., 2000; KELLERMANNNS et al., 2007; BRUNET et al., 2017). In the present study, a nuclear rDNA region of 350 bp was amplified by using NC5 and NC2 primers and revealed sequence similar to homologous regions within the nuclear ribosomal sequence of other *Pseudoterranova decipiens* described previously. Apparently, the tree estimated in this study strongly supported several of the higher taxonomic groups. Phylogenetic tree constructed with partial sequences consistently formed two major lineages one represent monophyletic origin

for Ascaridoidea species and consisted of two larger subclades, showed that Raphidascarididae is a sister group to Anisakidae with low genetic variations between them; while, the other clade represent the monophyletic origin of Toxocaridae, these results are in accordance with that obtained by Paggi et al. (2000) followed by Abollo et al. (2003), Nadler et al. (2005), Kellermanns et al. (2007) and Madanire-Moyo & Avenant-Oldewage (2013). The MP tree supported the taxonomic position of the present *Pseudoterranova* species which is deeply embedded in the genus *Pseudoterranova* with a close relationship with other *Pseudoterranova decipiens* described previously as a more related sister taxon. Therefore, this anisakid species has a unique genetic sequence for one of *Pseudoterranova* species. Therefore, it could be concluded that the parasite species found in *Lethrinus lentjan* was identified as *Pseudoterranova decipiens* with a unique genetic sequence and having new locality records in Egyptian water.

## Acknowledgements

Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no (RG-002).

## References

- Abollo E, D'Amelio S, Pascual S. Fitness of the marine parasitic nematode *Anisakis simplex* s. str. in temperate waters of the NE Atlantic. *Dis Aquat Organ* 2001; 45(2): 131-139. <http://dx.doi.org/10.3354/dao045131>. PMID:11463100.
- Abollo E, Paggi L, Pascual S, D'Amelio SD. Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. *Infect Genet Evol* 2003; 3(3): 175-181. [http://dx.doi.org/10.1016/S1567-1348\(03\)00073-X](http://dx.doi.org/10.1016/S1567-1348(03)00073-X). PMID:14522181.
- Brunet J, Pesson B, Royant M, Lemoine JP, Pfaff AW, Abou-Bacar A, et al. Molecular diagnosis of *Pseudoterranova decipiens* s.s in human, France. *BMC Infect Dis* 2017; 17(397): 1-5. <http://dx.doi.org/10.1186/s12879-017-2493-7>. PMID:28583155.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revised. *J Parasitol* 1997; 83(4): 575-583. <http://dx.doi.org/10.2307/3284227>. PMID:9267395.
- Di Azevedo MIN, Carvalho VL, Iñiguez AM. Integrative taxonomy of anisakid nematodes in stranded cetaceans from Brazilian waters: an update on parasite's hosts and geographical records. *Parasitol Res* 2017; 116(11): 3105-3116. <http://dx.doi.org/10.1007/s00436-017-5622-8>. PMID:28975458.
- Felizardo NN, Menezes RC, Tortelly R, Knoff M, Pinto RM, Gomes DC. Larvae of *Hysterothylacium* sp. (Nematoda: Anisakidae) in the sole fish *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) from the littoral of the State of Rio de Janeiro, Brazil. *Vet Parasitol* 2009; 166(1-2): 175-177. <http://dx.doi.org/10.1016/j.vetpar.2009.08.004>. PMID:19713041.
- George-Nascimento M. Ecological helminthology of wildlife animal hosts from South America: a literature review and a search for patterns in marine food webs. *Rev Chil Hist Nat* 1987; 60: 181-202.
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-98.
- Irgoitia MM, Braicovich PE, Lanfranchi AL, Farber MD, Timi JT. Distribution of anisakid nematodes parasitizing rajiform skates under commercial exploitation in the Southwestern Atlantic. *Int J Food Microbiol* 2018; 267: 20-28. <http://dx.doi.org/10.1016/j.ijfoodmicro.2017.12.009>. PMID:29277002.
- Kellermanns E, Klimpel S, Palm HW. Molecular identification of ascaridoid nematodes from the deep-sea onion-eye grenadier (*Macrourus berglax*) from the East Greenland Sea. *Deep Sea Res Part I Oceanogr Res Pap* 2007; 54(12): 2194-2202. <http://dx.doi.org/10.1016/j.dsr.2007.09.001>.
- Klimpel S, Palm HW. Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change? In: Mehlhorn H, editors. *Progress in Parasitology. Parasitology Research Monographs, Volume 2*. Heidelberg: Springer; 2011. p. 201-222.
- Koie M, Berland B, Burt MDB. Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Can J Fish Aquat Sci* 1995;52(S1 Suppl.1): 134-139. <http://dx.doi.org/10.1139/f95-519>.
- Kuhn T, García-Márquez J, Klimpel S. Adaptive radiation within marine anisakid nematodes: a zoogeographical modeling of cosmopolitan, zoonotic parasites. *PLoS One* 2011; 6(12): e28642. <http://dx.doi.org/10.1371/journal.pone.0028642>. PMID:22180787.
- Lauckner G. Diseases of mammalia: Pinnipedia. In: Kinne O, editor. *Diseases of marine animals. Volume IV, Part 2- Introduction, Reptilia, Aves, Mammalia*. Hamburg: Biologische Anstalt Helgoland; 1985. p. 683-793.
- Madanire-Moyo GN, Avenant-Oldewage A. On the development of a Parasitic Copepod, *Lamproglana clariae* Fryer, 1956 (Copepoda, Lernaieidae) infecting the sharp tooth cat fish, *Clarias gariepinus*. *Crustaceana* 2013; 86(4): 416-436. <http://dx.doi.org/10.1163/15685403-00003165>.
- Mattiucci S, Farina V, Campbell N, MacKenzie K, Ramos P, Pinto AL, et al. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: their genetic identification and use as biological tags for host stock characterization. *Fish Res* 2008; 89(2): 146-151. <http://dx.doi.org/10.1016/j.fishres.2007.09.032>.
- Mattiucci S, Nascetti G, Cianchi R, Paggi L, Arduino P, Margolis L, et al. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *J Parasitol* 1997; 83(3): 401-416. <http://dx.doi.org/10.2307/3284402>. PMID:9194819.
- Mattiucci S, Nascetti G. Advances and trends in the molecular systematic of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Adv Parasitol* 2008; 66: 47-148. [http://dx.doi.org/10.1016/S0065-308X\(08\)00202-9](http://dx.doi.org/10.1016/S0065-308X(08)00202-9). PMID:18486689.
- McClelland G, Misra RK, Martell DJ. Larval anisakine nematodes in various fish species from Sable Island Bank and vicinity. *Can Bull Fish Aquat Sci* 1990; 222: 83-118.
- McClelland G. The trouble with sealworms (*Pseudoterranova decipiens*) species complex, (Nematoda): a review. *Parasitology* 2002;124(7 Suppl): 183-203. <http://dx.doi.org/10.1017/S0031182002001658>. PMID:12396224.
- Nada MSM, Abd El-Ghany AM. Anisakid nematodes in marine fishes. *J Am Sci* 2011; 7(9): 1000-1005.
- Nadler SA, Adams BJ, Lyons ET, DeLong RL, Melin SR. Molecular and morphometric evidence for separate species of Uncinaria (Nematoda: Ancylostomatidae) in California sea lions and northern fur seals: hypothesis testing supplants verification. *J Parasitol* 2000; 86(5): 1099-1106. [http://dx.doi.org/10.1645/0022-3395\(2000\)086\[1099:MAMEFS\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2000)086[1099:MAMEFS]2.0.CO;2). PMID:11128487.

- Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA. Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern pacific marine mammals. *J Parasitol* 2005; 91(6): 1413-1429. <http://dx.doi.org/10.1645/GE-522R.1>. PMID:16539026.
- Nadler SA, Hudspeth DSS. Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. *Mol Phylogenet Evol* 1998; 10(2): 221-236. <http://dx.doi.org/10.1006/mpev.1998.0514>. PMID:9878233.
- Nadler SA, Hudspeth DSS. Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. *J Parasitol* 2000; 86(2): 380-394. [http://dx.doi.org/10.1645/0022-3395\(2000\)086\[0380:POTANA\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2000)086[0380:POTANA]2.0.CO;2). PMID:10780561.
- Najda K, Kijewska A, Kijewski T, Plauška K, Rokicki J. Distribution of ascaridoid nematodes (Nematoda: Chromadorea: Ascaridoidea) in fish from the Barents Sea. *Oceanologic Hydrobiologic stud* 2018; 47(2): 128-139. <https://doi.org/10.1515/ohs-2018-0014>.
- Ólafsdóttir D, Hauksson E. Anisakidae nematodes in the common seal (*Phoca vitulina* L.) in Icelandic waters. *Sarsia* 1998; 83(4): 309-316. <http://dx.doi.org/10.1080/00364827.1998.10413690>.
- Paggi L, Mattiucci S, Gibson DI, Berland B, Nascetti G, Cianchi R, et al. *Pseudoterranova decipiens* species A and B (Nematoda, Ascaridoidea): nomenclatural designation, morphological diagnostic characters and genetic markers. *Syst Parasitol* 2000; 45(3): 185-197. <http://dx.doi.org/10.1023/A:1006296316222>. PMID:10768762.
- Petter AJ, Quentin JC. Oxyuroidea. In: Anderson RC, Chabaud AG, Willmott S. *Keys to the nematode parasites of vertebrates: archival volume*. London: CAB International; 2009. p. 218-247. <http://dx.doi.org/10.1079/9781845935726.0218>.
- Piña-Vázquez C, Reyes-López M, Ortiz-Estrada G, de la Garza M, Serrano-Luna J. Host-Parasite Interaction: parasite-derived and induced proteases that degrade human extracellular matrix. *J Parasitol Res* 2012; 2012: 1-63. <http://dx.doi.org/10.1155/2012/748206>. PMID:22792442.
- Rocka A. Nematodes of the Antarctic fishes. *Pol Polar Res* 2004; 25(2): 135-152.
- Smith JW, Wootten R. Anisakis and anisakiasis. *Adv Parasitol* 1978; 16: 93-163. [http://dx.doi.org/10.1016/S0065-308X\(08\)60573-4](http://dx.doi.org/10.1016/S0065-308X(08)60573-4). PMID:364959.
- Sukhdeo MVK. Where are the parasites in food webs? *Parasit Vectors* 2012; 5(239): 1-17. <http://dx.doi.org/10.1186/1756-3305-5-239>. PMID:23092160.
- Szostakowska B, Myjak P, Kur J. Identification of anisakid nematodes from the southern Baltic Sea using PCR-based methods. *Mol Cell Probes* 2002; 16(2): 111-118. <http://dx.doi.org/10.1006/mcpr.2001.0391>. PMID:12030761.
- Timi JT, Sardella NH, Navone GT. Parasitic nematodes of *Engraulis anchoita* Hubbs et Marini, 1935 (Pisces, Engraulidae) off the Argentine and Uruguayan coasts, South West Atlantic. *Acta Parasitol* 2001; 46(3): 186-193.
- Wharton DA, Hassall ML, Aalders O. *Anisakis* (Nematoda) in some New Zealand inshore fish. *N Z J Mar Freshw Res* 1999; 33(4): 643-648. <http://dx.doi.org/10.1080/00288330.1999.9516907>.