

Research Note

Morphological characterization of *Dujardinascaris* spp. (Nematoda: Anisakidae) from the striped red mullet *Mullus surmuletus* in the Mediterranean Sea

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Summary

The striped red mullet *Mullus surmuletus* (Linnaeus, 1758) (Perciformis: Mullidae) has a high commercial value and therewithal is a common demersal fish of the Mediterranean Sea, therefore studying the helminth parasites of this fish is required. Anisakids nematodes are common parasites of animals including human causing economic losses and different parasitic diseases. During the present study, the nematode *Dujardinascaris* spp. (Anisakidae) was described from the body cavity and small intestine of *Mullus surmuletus* in the Alexandria, Mediterranean Sea, Egypt as new host and new geographical record. Forty-five (37.5%) fish out of (120) were found infected by the parasite. The morphological features of the collected nematode were investigated by both light and scanning electron microscopy. The study revealed that the specimens were characterized from other species of the genus by the presence of two large lateral pouches attached to each cephalic lip, the different large-sized papillae on the cephalic region and on the dorsal surface of the nematode cuticle.

Keywords: light microscopy; *Mullus surmuletus*; Nematode; scanning electron microscopy (SEM)

Introduction

The striped red mullet *Mullus surmuletus* (Linnaeus, 1758) (Perciformis: Mullidae) is one of the most abundant and widely distributed fish in the sublittoral zone along the Eastern Atlantic, from the North Sea to the northern part of West Africa and the Mediterranean Sea (Klimpel *et al.*, 2008). Several authors studied the helminths of *M. surmuletus* such as Figus *et al.* (2005) who identified 18 species of helminths with total infection rate (65.5 %) in Italy. Ferrer *et al.* (2005) from the Spanish Western Mediterranean Coast. Bayoumy *et al.* (2008) from Syrt Coast, Libya. Also, Klimpel *et al.* (2008) in the North Sea and Mediterranean Sea. *Mullus surmuletus* occurs on broken and rough grounds in less than 100 m water depth. It is highly infected in the M. Sea hosting about 28 different species of parasites (Bartoli & Bray, 1996; Fer-

rer *et al.*, 2005). Mediterranean Sea is characterized by an unusual high species diversity for a temperate sea. It contains around 7 % of the total global marine fish species with a wide range of both tropical and temperate species. Nematodes considered as one of the largest and most diverse group of helminth parasites infect marine, freshwater and even brackish water fish (Klimpel *et al.*, 2011; Morsy *et al.*, 2012). Their infections cause great morbidity in humans and animals. Numerous cases of human anisakiosis especially of genera *Anisakis* and *Pseudoterranova* occur due to the consumption of undercooked fish (Chaligiannis *et al.*, 2012; Guardone *et al.*, 2018). Food-borne zoonosis in aquatic animals are most linked to anisakid nematodes. They are common parasites of mammals, reptiles, fish and fish eating birds with a worldwide distribution causing diseases and important economic losses (Dadar *et al.*, 2016). Among this group members of

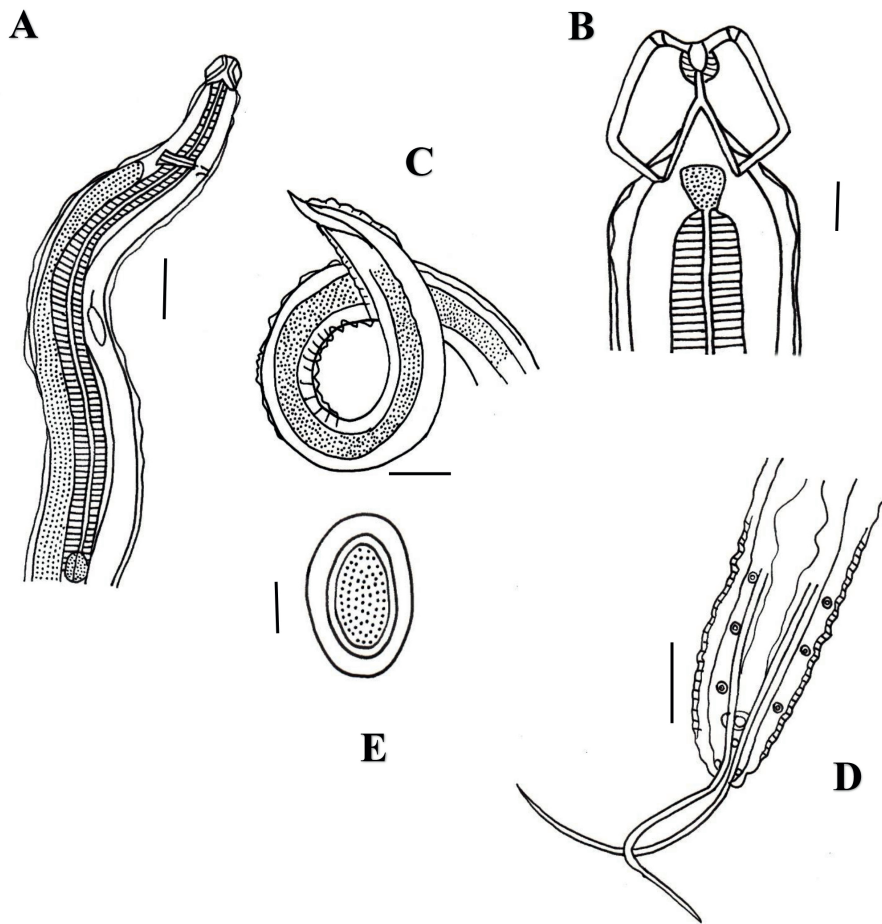


Fig. 1 (A–E): *Dujardinascaris* spp. from Red Mullet *Mullus surmuletus* in Mediterranean Sea. A: Anterior end of male dorsal view, B: cephalic region of male, C: The posterior end of female subventral view, D: The posterior end of male ventral view, E: Egg. Scale bars: A,C 0.2; B 200 μ m; D 0.3; E 50 μ m.

the genera *Anisakis* Dujardin, 1845, *Pseudoterranova* Mozgovoi, 1951 and *Contracaecum* Railliet and Henry, 1912 may be a problem for the commercial fishing industry (Amor *et al.*, 2011; Klimpel & Palm, 2011). Based on light and scanning electron microscope studies, the present nematode was described from the striped red mullet *Mullus surmuletus* in the Alexandria, Mediterranean Sea, Egypt. It represents the first record in the Mediterranean and the first time for recording from the host *M. surmuletus*. The subfamily Heterocheilinae Railliet and Henry, 1912 have species parasitize mainly adult vertebrates such as crocodilians, tortoises, sirenians and some other fish species (Sprent 1990). The nematode genus *Dujardinascaris* was established by Baylis, 1947. It includes about 20 species most of them parasitize lizards and crocodiles but a few number infecting fish (Masova *et al.*, 2014).

Materials and Methods

A total of 120 striped red mullet *Mullus surmuletus* were obtained

from local fishermen operating on Alexandria coast, Mediterranean Sea, Egypt. The hosts were transported to the laboratory for their identification according to (Burgees *et al.*, 2000; Schultz 2003). They were dissected, the abdominal cavities were inspected and internal organs were removed, separated in petri dishes and washed very well by saline solution (0.7%). The intestine was opened carefully searching for helminth parasites and its content was examined using a dissecting stereomicroscope. The collected nematodes were isolated, counted, their sites were recorded and then they washed several times in physiological solution. Seventy-four female and thirty-eight male of the genus *Dujardinascaris* were obtained. The specimens were fixed by 7% formalin for about 12 h. for morphological identification, the fixative was gradually replaced by lactophenol solution, cleared in few drops of lactophenol then mounted by DPX. Photomicrographs were taken using a microscope supplied with digital camera. Drawings were made by Camera Lucida. All measurements were taken in millimeters unless otherwise stated. The nematode specimens were identified

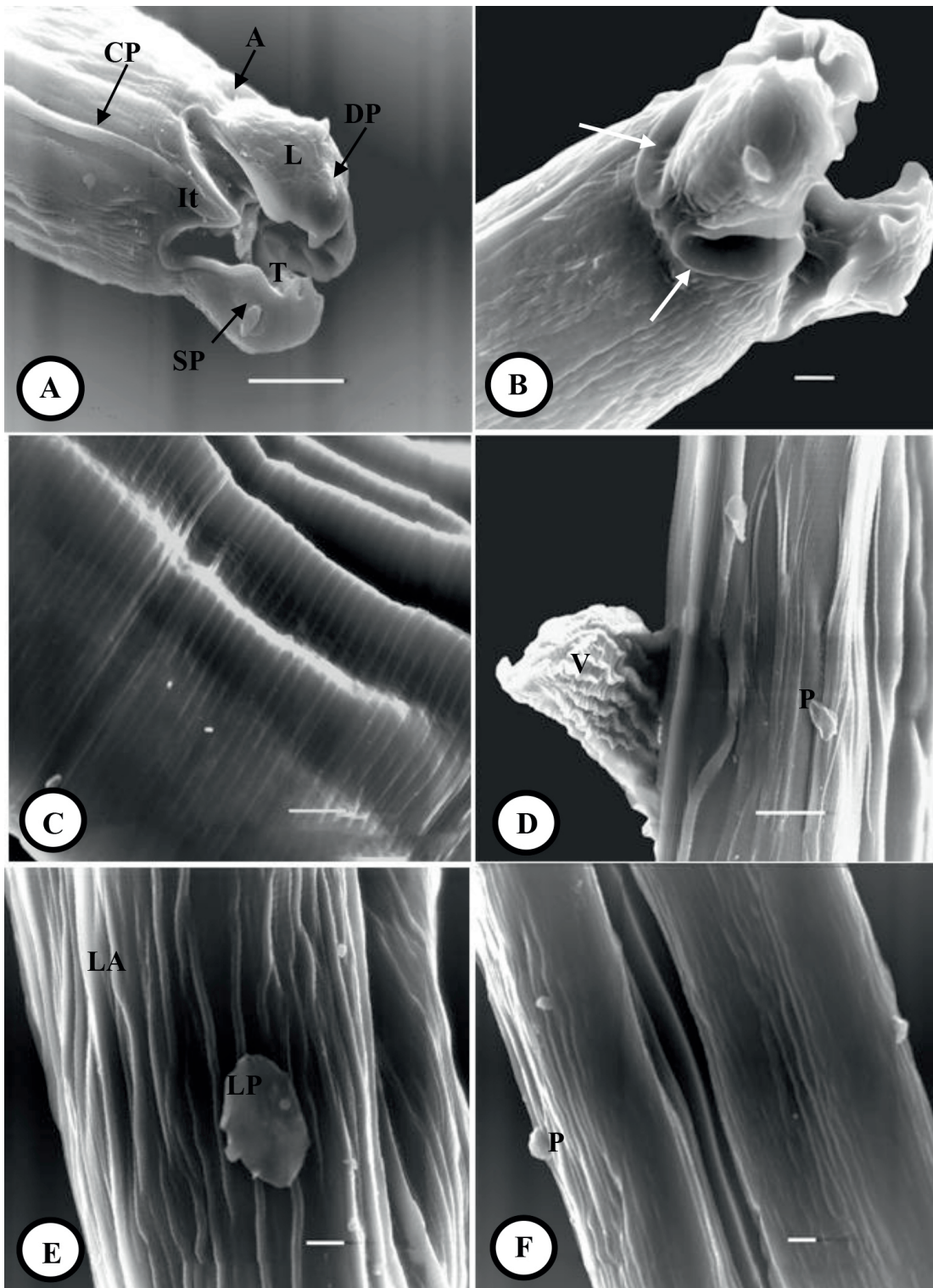


Fig. 2 (A – F): Scanning Electron Microscope of *Dujardinascaris* spp. (A): The cephalic region of the worm with interlocked processes, A amphid, L lip, It interlabium, T teeth, DP double papillae, SP single papilla, CP cuticular prolongation. (B): the anterior end showing the cephalic lateral pouches (arrows). (C): The middle region of nematode body showing the cuticular transverse striations. (D): The vulvar region with protruded vagina V. (E): The dorsal view of somatic region showing the longitudinal annulation LA, large rounded-like papilla LP. (F): The post-equatorial part of nematode body showing papillae P. Scale bars: A,D,H,I,K,L = 50 μ m, B,C,EF,G,J = 10 μ m.

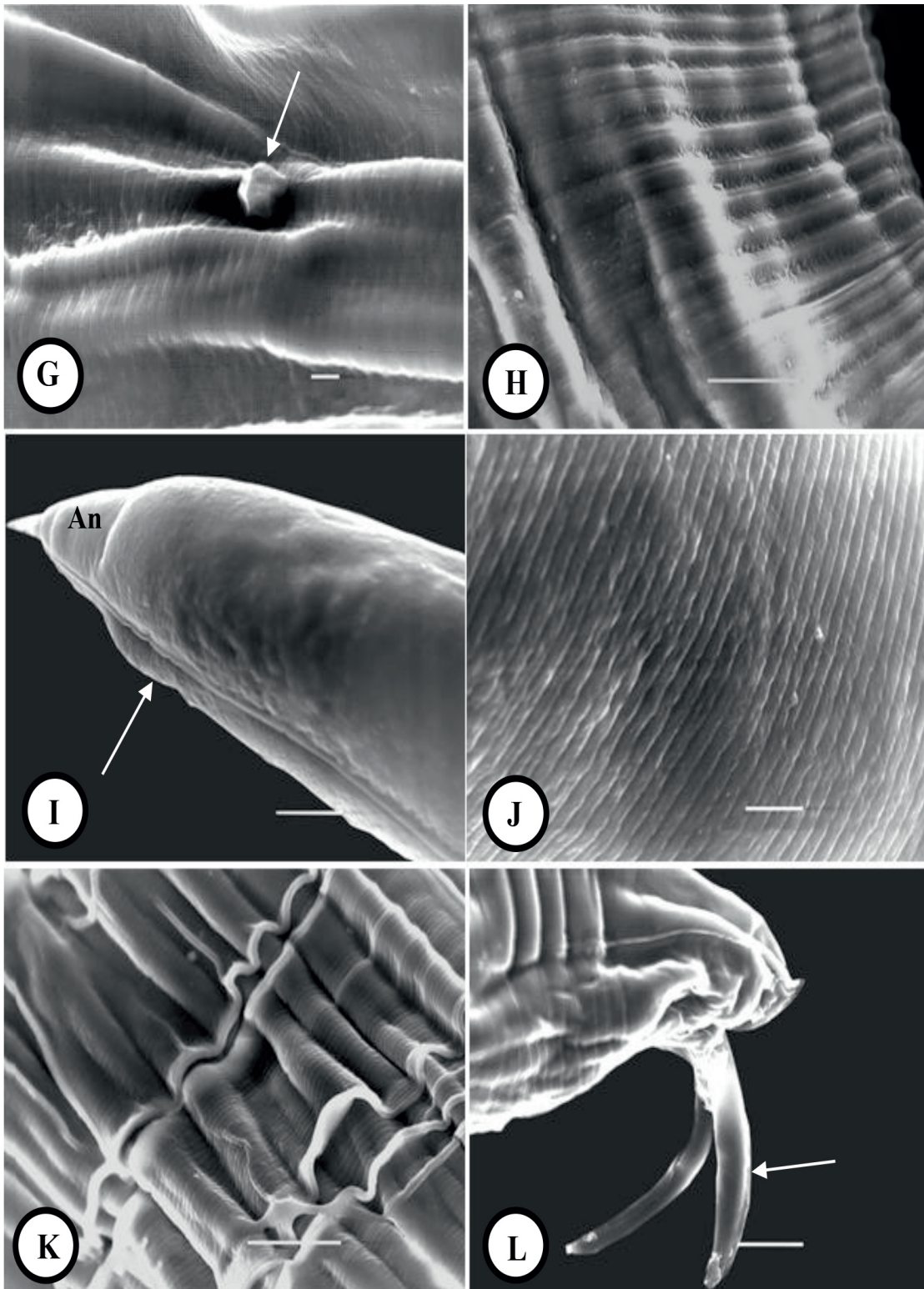


Fig. 2 (G – M): Scanning Electron Microscope of *Dujardinascaris* spp. (G): The ventral surface of the worm at cuticular region showing button-like papilla (arrow). (H): The cuticle at the posterior region of female worm showing the transverse striations. (I): The posterior end of female showing anus An & the caudal alae (arrows). (J): The cuticle at the anal region showing the transverse striations. (K): The posterior end of male showing the cuticular transverse striations. (L): The posterior end of male showing spicules (arrows). Scale bars: A,D,H,I,K,L = 50 μ m, B,C,E,F,G,J = 10 μ m.

according to (Yamaguti 1961; Anderson *et al.*, 2009). The techniques used in the present study help in the observation as well as the identification of parasites. For scanning electron microscopy, the nematodes were fixed in 2 % glutaraldehyde in 0.1 M. sodium cacodylate buffer (PH 7.2), dehydrated in ethyl alcohol, critical point dried, mounted on stubs carefully and coated with gold then they were examined and photographed at varying magnifications using a JOEL JSM-5400LV scanning electron microscope at an accelerating voltage of 15 KV at Electron Microscopic Lab of the Atomic Energy Agency, Nasr City, Cairo, Egypt.

Ethical Approval and/or Informed Consent

The research related to animals has been complied with all the relevant national regulations and institutional policies for the care and use of animals. For this study formal consent was not required.

Results

Dujardinascaris spp.

Family: Anisakidae Railliet & Henry, 1912

Subfamily: Heterochilinae Railliet & Henry, 1912

Type-host: The striped red mullet *Mullus surmuletus* Linnaeus, 1758

Site in host: Intestine and body cavity

Locality: Depth ranges from 5 – 60 at tropical level 3.5, Mediterranean Sea, Alexandria, Egypt.

Prevalence and intensity: 45 infected out of 120 examined fish with prevalence (37.5 %), 1 – 4 specimens per fish.

Deposition of voucher specimens: Parasite specimens are deposited in the helminthes collection in the Zoology Department, Faculty of Education, Ain Shams University.

Description: light and scanning electron microscopy

It is medium to large in size, white in color, females are longer and more pointed posteriorly than males. Cuticle is transversally striated. Cervical and caudal alae present, the cephalic region has three large lips with distinct interlocked processes. The dorsal lip has two convex processes for articulating with the ventrolateral lips. Each lip is supported at its internal edge with row of 3 – 4 small pointed teeth. Interlabia large and well developed. Two lateral amphids, double cephalic sensory papillae on the dorsal lips and a single one on the sub ventral lips were found (Fig. 2 A&B). A cervical ala was extended from the mouth opening along the nematode body. Dentigerous ridges absent. In addition, SEM revealed a pair of large symmetrical wide pouches located at the base of each lip (Fig.2B). A morphological variation of the transverse striations was observed along the whole body worm (Fig.2 C,E,J,K). Different shapes of papillae were found on the vulvar and post-vulvar region of the nematode body (Fig.2 D&E). Also, a large flattened-like papilla was observed on the middle part of dorsal body worm (Fig.2G). A pair of sensory lateral papillae were

noted on the cuticle of somatic region that follow the vulvar region (Fig.2F). Esophagus is muscular, long and a small rounded ventriculus is present (Fig. 1A). Nerve ring encircling esophagus at about 30 % of its length. Excretory pore is located approximately at the same level of the nerve ring. Vulva is pre-equatorial in the first third of the body. The cuticle of the present nematode exhibited distinctive transverse striations at various areas along the nematode body. The dorsal surface that follow the cephalic region has an irregular longitudinal annulations interrupted by papillae-like structures while the region that follow the vulva has regular transverse striations. The annuli at the last third of male specimen has a distinct pattern as the cuticular annuli are parallel to each other and subdivided transversally. The cuticular surface of the last part of female body is characterized by narrowed transverse striations separated by fine grooves (Fig.2 C,E,H,J,&K). Male: (Based on six specimens): Body 10 – 14.2 long and 0.33 – 0.47 wide. Lips 0.06 – 0.085 long, interlabia 0.033 – 0.041 long. Esophagus 1.21 – 1.66 long with 0.062 – 0.071 wide representing (10 %) of body length. Nerve ring and excretory pore at 0.35 – 0.38 and 0.37 – 0.39 respectively from the anterior extremity. Intestinal caecum 0.71 – 1.33 long representing (8.5 %) of body length. Spicules are long, slender, equal in size measure 2.23 – 3.45 μm . long. Gubernaculum is present 0.08 – 0.11 long. Tail 0.11 – 0.17. Caudal papillae are observed by light microscope in the form of four pairs: three pairs of precloacal, one pair of post cloacal papillae and one median cloacal papilla. A pair of lateral phasmids was observed (Figs.1D). Female: (Based on six specimens): Body 13 – 22.5 long and 0.18 – 0.24 wide. Lips 0.09 – 0.15 long. Interlabia 0.05 – 0.062 long. Esophagus 1.67 – 2.1 long and 0.07 – 0.082 wide representing (12 %) of body length. Nerve ring and excretory pore located at 0.41 – 0.52 and 0.46 – 0.57 respectively from anterior extremity. Intestinal caecum 1.11 – 1.43 long. Vulva is post-equatorial (0.093 – 1.15) from the anterior end. The muscular vagina was extruded outside from vulva on the ventrolateral surface at the first third of nematode body (Fig.2D). Tail 0.34 – 0.52 long, it is ended by small caudal spike (Fig.1C). The distal end of female is provided by an elevating transverse outgrowth (Fig.2I). The cuticle of last third of female body has transverse striations that separated by internal fine grooves. The cuticle surrounding the anus region characterized by the absence of annulations. Eggs have smooth surface, oval shaped and measure 52 – 90 x 40 – 78 μm (Fig.1E).

Discussion

Dujardinascaris (Table 1) parasite currently includes 20 nominal species recorded from different hosts of lizards and fishes (Li *et al.*, 2015). It was firstly established by Baylis, 1947 from crocodiles. Previous studies indicated that most species of that genus were isolated from crocodiles while few of them parasitize fishes (Sprent, 1990, Li *et al.*, 2015). Yamaguti, 1941 has been listed 13 species in genus *Dujardinascaris*, two of them have been reported from fishes and 11 species from reptiles (Morsy *et al.*, 2013).

Table 1. Morphometric comparison of some species of nematode *Dujardinascaris* from fishes with the present species. (The data for the previous described species are taken from the original descriptions).

Characters	<i>D. melapteruri</i>	<i>D. mujibi</i>	<i>D. mormyropsis</i>	<i>Dujardinascaris</i> spp.
Length ♂	14.8	17 – 24	11.5 – 15.33	10 – 14.2
♀	12.3 – 35.0	23 – 38	26 – 43.22	13 – 22.5
Width ♂	0.3	0.25 – 0.65	0.38 – 0.54	0.33 – 0.47
♀	0.25 – 0.68	0.76 – 0.84	0.089 – 0.1	0.18 – 0.34
Lips L. ♂	70 µm	–	75 – 90 µm	70 – 98 µm
♀	50 – 110 µm	–	135 – 159 µm	50 – 90 µm
Interlabia L. ♂	–	–	30 – 45 µm	33 – 41 µm
♀	–	–	78 – 96 µm	40 – 60 µm
Esophagus L. ♂	1.7	2.25 – 2.75	1.32 – 1.84	1.21 – 1.66
♀	1.3 – 2.6	4.45 – 5.84	2.2 – 3.07	1.67 – 2.1
Esophagus W. ♂	–	0.11 – 0.15	0.08 – 0.11	0.062 – 0.071
♀	–	0.15 – 0.18	0.09 – 0.12	0.07 – 0.082
Nerve ring * ♂	0.35	–	0.42 – 0.56	0.35 – 0.37
♀	0.27 – 0.41	–	0.58 – 0.77	0.41 – 0.52
Excretory pore * ♂	0.36	–	0.42 – 0.56	0.36 – 0.39
♀	0.4	–	0.58 – 0.81	0.46 – 0.57
Intestinal canal ♂	1.1	–	0.68 – 1.11	0.71 – 1.33
♀	0.77 – 2	–	1.19 – 1.73	1.11 – 1.43
Tail L.	0.08	–	0.12 – 0.17	0.11 – 0.17
Spicule L.	1.4 µm	1.13 – 1.34 µm	1.7 – 1.81 µm	2.23 – 3.45 µm
Gubernaculum L.	–	–	0.186 – 0.2	0.08 – 0.11
Vulva *	–	–	9.13 – 15.92	0.093 – 1.15
Egg L.	–	–	0.06 – 0.09	0.052 – 0.09
Egg W.	–	–	0.052 – 0.072	0.04 – 0.078
Caudal alae	Absent	Absent	Absent	present
Host species	<i>Malapterurus electricus</i>	<i>Pagrus pagrus</i>	<i>Mormyrops anguilloides</i>	<i>Mullus surmuletus</i>
Locality	Khartoum, Sudan	Red Sea, Egypt	Central Africa	Mediterranean Sea, Egypt
Author	Sprent, (1990)	Morsy <i>et al.</i> (2013)	Moravec & Jirku, (2014)	Present study (2019)

Abbreviations: L.=length; W.=width; ♂=male; ♀=female; * From anterior extremity

Sprent (1977) also listed 12 species of that genus from crocodilians worldwide. Machida *et al.* (1992) described *D. philippinensis* from *Crocodylus porosus* in Philippines. Sprent *et al.* (1998) described five species of the present genus from crocodiles in Australia and New Guinea; *D. patterae*, *D. blairi*, *D. harrisae*, *D. westonae* and *D. angusae*. Moravec *et al.* (2014) reported *D. madagascariensis* from *C. niloticus*. Li *et al.* (2015) discovered *D. gigantea* from *Alligator sinensis* in China. Species of genus *Dujardinascaris* that reported from fishes included *D. cybii* by Lakshmi and Sudha (2000) from intestine of *Mugil cephalus* as new host in India. It was characterized by slender pointed tail with cuticular ring-shaped striations and absence of caudal alae. *D. mujibii* described by Morsy *et*

al. (2013) from the intestine of *P. pagrus* in the Red Sea. It is similar to the present species in the shape of lips as each one has 3 – 4 small pointed teeth. It differs from it in the situation of the vulva in the first third of the body in the present species (vs post-equatorial), in the body measurements and in the absence of caudal alae in *D. mujibii*. Also, *D. mormyropsis* described by Moravec and Jirku (2014) from stomach of *Mormyrops anguilloides* in central Africa. It was mainly distinguished from the present species by the number of teeth (10) that oriented from the lips, the presence of denticulous ridges on lips, in the absence of cervical and caudal alae and finally in the measurements of caecum and esophagus length. From the other hand, the present species agrees with *D. mormy-*

ropsis in the distribution of papillae on the cephalic region. *Dujardinascaris malapteruri* Baylis, 1947 syn. *D. graberi* Troncy, 1969 is the only known valid species of the present genus parasitizing African freshwater fishes as adults (Moravec & Jirku, 2014), it was described by Sprent (1990) in Chad from *Malapterurus electricus*. He distinguished that species by the anterior prolongation of the dorsal lip, ten pairs of caudal papillae on male tail. Bannai *et al.* (2016) studied *D. sphyraenaii* and its pathological effects on fish *Psettodes erumei* in Arabian Gulf. Sood (1989) had proposed a key for species of *Dujardinascaris* Baylis 1947 from fishes in south Asia included five species; *D. magna* Khan & Begum, 1971; *D. ritai* Zaidi & Khan, 1975 from *Rita rita* in Lahore; *D. quadrii* Zubari & Farooq, 1976; *D. sciaena* Bilqees *et al.* 1977 from *Sciaena* sp. in Karachi Coast and *D. cybii* Arya & Johnson, 1978 from *Cybbium guttatum* in India. Moravec and Jirku (2017) redescribed *D. malapteruri* from fish *Malapterurus monsembeensis* in Congo River, they studied it by scanning electron microscope and characterized the species by the presence of dentigerous ridges on lips and absence of caudal alae. Although the lizards and crocodiles are more exposed to the infection by the present nematode, it was clear that genus *Dujardinascaris* had the ability to infect and adapt in a variety of fish hosts and may infect more and more of fish species. The present finding of *Dujardinascaris* from *Mullus surmuletus* fish represents a new host record and a new geographical record from Mediterranean Sea. Moravec and Jirku (2014) suggested that freshwater fish play a role of the intermediate hosts, in which the third stage larvae of the worm may occur. The present genus rarely infects marine water fishes, this may be due to different reasons such as the distribution of the final and intermediate hosts, thus these parasites can overcome wide distances if they infect migratory species. Parasites of fishes have been used as biological indicators for ecology of hosts and their migration in successful way (Klímpel *et al.*, 2008). Scanning electron microscope of the cephalic capsule and distal end of the present species ensures that it is belonging to genus *Dujardinascaris*. The presence of three large cephalic lips each one is supported by a number of small chitinous pointed teeth, this is a taxonomic character for the genera of subfamily Heterochilinae and consequently for genus identification. The number of teeth differs through different species of genus *Dujardinascaris*. The number of teeth vary from three to ten in nematode species that infect fish hosts while in case of species that infecting lizards and crocodiles the number of teeth ranged from 10 – 20 (examples: *D. philippinensis* from *Crocodylus porosus* Machida *et al.*, 1992 and *D. gigantea* from *Alligator sinensis* Li *et al.*, 2015). Teeth seem to have a penetration function in the intestinal wall of the host (Bayoumy *et al.*, 2008). The cuticle of the present nematode exhibited distinctive transverse striations at various areas along the nematode body. Such cuticle striations were previously recorded in many species of nematode fishes (*Hysterothylacium alatum* Moravec and Justine, 2015; *Eustrongylidae exciscus* Gupta, 2019). The usefulness of cuticle morphology for the identification of Anisakids and their developmental stages

(Molina-Fernandez *et al.*, 2018). The pair of phasmids at the caudal end of male considered to have glandular and sensory function. The surface topography of parasites is an important character to understand the intricate relationship between them and their hosts. In all respects the present described nematode is similar to great extent to the specimens that described by Morsy *et al.* (2013) except some differences included the distribution of papillae on the cephalic region, the large pouches that are attached to lips, also, the distribution of different shapes of papillae.

The helminth parasites of the striped red mullet *Mullus surmuletus* have been studied by several parasitologists due to its economic importance. Le Pommelet *et al.* (1998) listed a high diversity of trematodes infecting Mulletts from Western Mediterranean and Adriatic Sea. Ferrer *et al.* (2005) in the Spanish Western Mediterranean Coast. Bayoumy *et al.* (2008) studied the ultrastructure of four species of helminth parasites infecting *M. surmuletus* in Syrt coast, Libya with parasitic prevalence (67.6 %). Also, Klímpel *et al.* (2008) demonstrated that the mullets of the North Sea are more infected with helminthes than those from the Mediterranean Sea. Hassani *et al.* (2015) carried out an epidemiological survey of helminths infecting *M. surmuletus* in Algeria, Western Mediterranean, they recorded 14 species of helminths included trematodes, nematode, cestode and acanthocephalan. These differences in the parasite fauna composition of Mulletts may be regarded to historical and geographical reasons (Hassani *et al.*, 2015).

Conflict of Interest Statement:

Author declare that she has no conflict of interest pertaining to this submission.

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