

## First record of *Gnathostoma* sp. (Owen, 1836) (Nematoda: Gnathostomatidae) infecting the European Eel *Anguilla anguilla* (Anguillidae) from the Mediterranean Sea, Egypt

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### ABSTRACT

Gnathostomiasis is a fish-borne zoonotic infection caused by eating infected seafood or fish parasitized by third-stage nematode larvae of the genus *Gnathostoma*. Twenty-seven fish specimens of the European eel *Anguilla anguilla* (Anguillidae) were collected from water coasts of the Mediterranean Sea in Egypt. Of the 27 fish that were studied, 14 fish (51.58%) were parasitized with third-stage larvae (L3) of *Gnathostoma* sp. (Gnathostomatidae), these larvae were investigated as encapsulated on the surface of different visceral organs and some embedded in muscles of the infected fish. By using both light and scanning electron microscopes, the morphological examination of the recovered nematodes revealed that the main diagnostic features represented by an elongated body, measured  $6.5 \pm 2$  (7.8–9) mm long, and its greatest width observed at the posterior one-third of the body was  $0.4 \pm 0.02$  (0.38–0.44) mm with narrow lateral alae, the presence of a cephalic bulb measured  $0.26 \pm 0.02$  (0.18–0.28) mm long,  $0.31 \pm 0.02$  (0.28–0.34) wide and armed with six transverse rows of spines, mouth opening surrounded by two simple lateral lips, and conical tail terminated with a pointed mucron. The described species was morphologically and morphometrically compared with some of the previously recorded species of the same genus. Based on our detailed description, the present species was identified as *Gnathostoma* sp. recovered from *A.anguilla* as the first record in Egypt.

### INTRODUCTION

Fish are source of many vital ingredients: protein, carbohydrates, minerals, vitamins, lipids and antioxidants, which are of significant nutritional value to humans (Sarojnalini and Hei, 2019). It constitutes an important component of Egyptian diet. Consumption of fish, has many benefits of promoting healthy vision, skin and immune system, and play an important in the reproductive process, also, enables heart, kidneys, lungs, and other organs to work properly (Singh *et al.*, 2005)

It was documented that about 56 million human parasitic infections are due to consumption of infected fish products (Mostafa *et al.*, 2020). Nematodes are the most common parasitic helminths that have a significant economic impact on fish worldwide.

Adults are usually found in fish intestines, whereas their larval stages are occasionally found on the viscera and flesh, causing serious fish diseases and compromise the profitability and sustainability of fisheries (Mehlhorn *et al.*, 2011; Morsy *et al.*, 2012; Lafferty *et al.*, 2015). The migration of these larvae to the muscles of fish is epidemiologically important, since the muscles are the component of the fish that humans consume (Sohn *et al.*, 2015) resulting in decrease in consumer acceptance of fish (Abdel Ghaffar *et al.*, 2015).

Human gnathostomiasis, a food-borne zoonosis, is caused by the third-stage larvae (L3) of *Gnathostoma* spp. (Chaicumpa, 2010; Tuyen *et al.*, 2019; Liu *et al.*, 2020). Humans are infected by these nematodes through consumption of raw or undercooked fish, frogs or poultry that contain larval stage (Sapp *et al.*, 2019). The most common clinical signs and symptoms of the disease are migratory cutaneous swellings and eosinophilia. In severe cases, L3 also invade internal organs and tissues such as the liver, eyes, nerves, spinal cord and brain, which can result in blindness, nerve pain, paralysis, coma and even death (Diaz, 2015; Chai *et al.*, 2020; Zhang *et al.*, 2021).

Therefore, this study aimed to determine the occurrence of *Gnathostoma* sp. larvae infecting the European eel *Anguilla anguilla* and make full morphological description and morphometric characterization using light and scanning electron microscopes for the first time from this host species in Egypt.

## MATERIALS AND METHODS

The current study was performed following the guidelines approved by the Cairo University Institutional Animal Care and Use Committee (CU-IACUC), and the relevant document (No. CU/I/F/32/19) was approved by the committee.

### 1. Fish collection

A total of 27 fish specimens of European eel *Anguilla anguilla* (F: Anguillidae) were obtained from commercial fishermen and boat landing sites at the coasts of Alexandrina City along the Mediterranean Sea in Egypt during the period from February to November 2021. Fish were transported to the laboratory of Parasitology, Faculty of Science, Cairo University using small containers containing seawater with a good aeration. Fish were necropsied and the helminths were collected using a stereomicroscope. Nematodes were observed encapsulated on the surface of various abdominal organs as well as embedded in host muscles, then, they were isolated and

washed in a physiological saline 0.7 % then, relaxed and preserved in 70% hot ethyl alcohol.

## 2. Morphological examination of the recovered nematodes

For morphological studies, the recovered nematodes were cleared in lactophenol for examination in temporary mounts by light microscopy (Pritchard and Kruse, 1982). Photomicrographs were obtained with a LEICA DM 750 microscope equipped with a LEICA ICC 50 HD camera. All body measurements are presented as means (mm  $\pm$  S.D) in parentheses. For Scanning electron microscopy (SEM), according to Guo *et al.* (2014) nematodes were fixed in 2.5% glutaraldehyde. After 24 h, samples were post-fixed in 1% osmium tetroxide (OsO<sub>4</sub>) in phosphate buffer for 24 h, then dehydrated through a graded ethanol series (50%, 60%, 70%, 80%, 90% and 100%), and dried at 30°C for 30 min using a critical point dryer (LEICA, EM CPD300). Dried specimens were mounted with carbon tape on aluminum stubs, coated with gold, and examined with a JEOL JSM-5200 SEM (Tokyo, Japan) at the Faculty of Agriculture, Cairo University at an accelerating voltage 25kV.

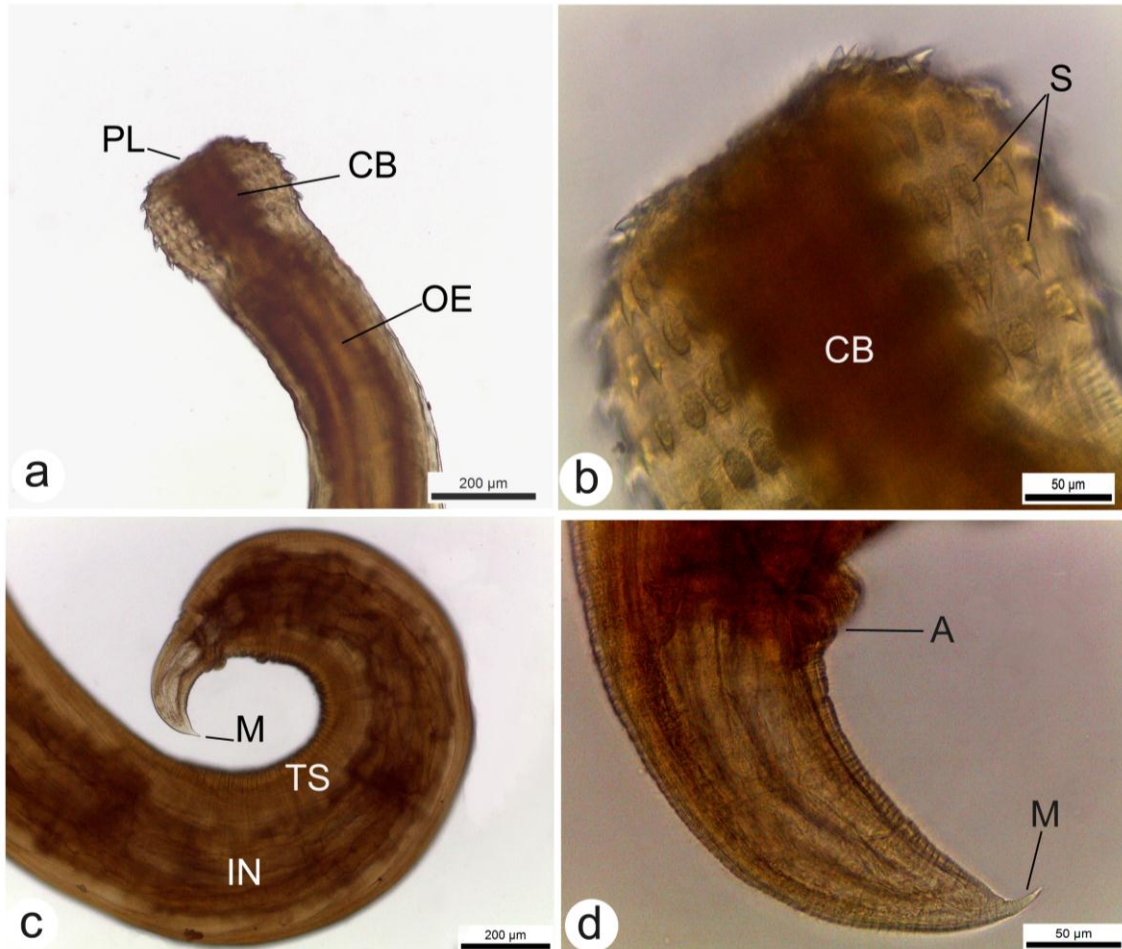
## RESULTS

Fourteen out of 27 examined specimens of the European eel *Anguilla anguilla* (F: Anguillidae) were found to be naturally infected with third stage larvae of *Gnathostoma* sp. (F: Gnathostomatidae) with an infection rate of 51.85 %. The number of isolated larvae per fish ranged from three to seven.

### Description (based on 5 larvae)

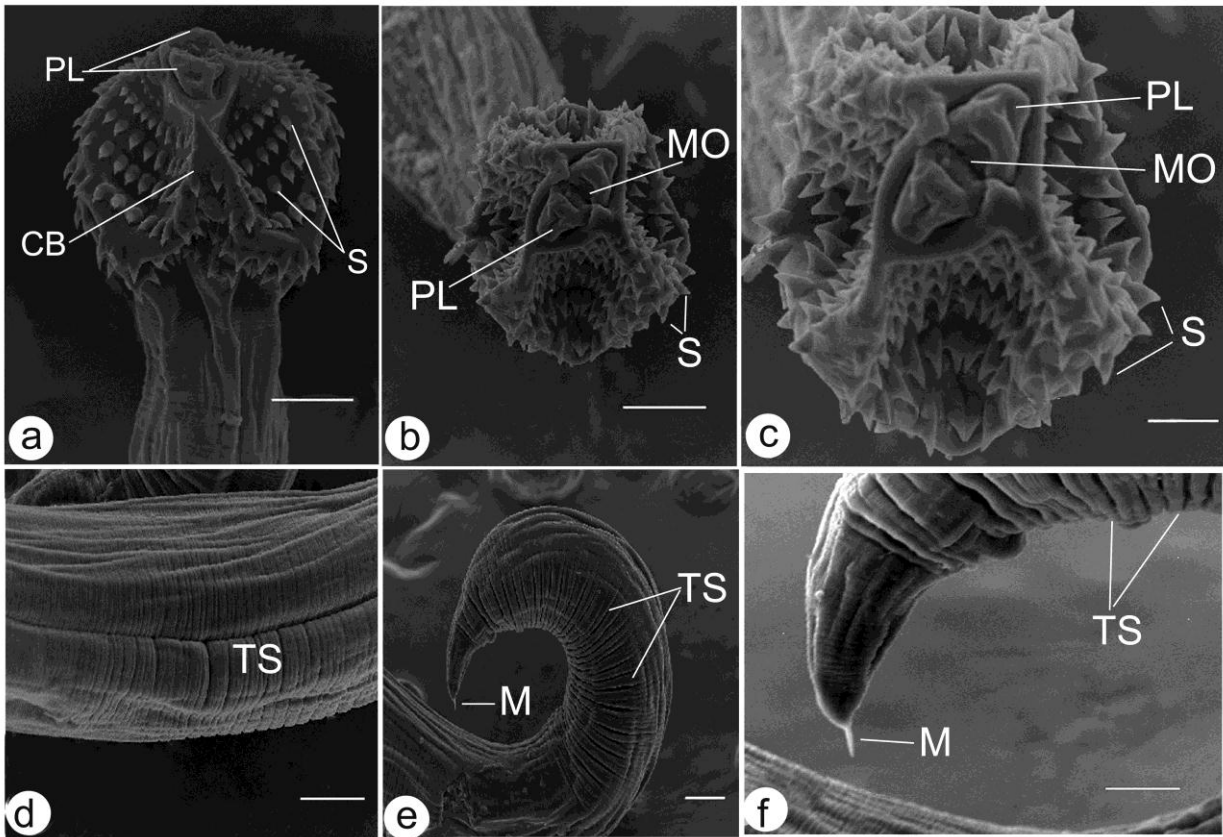
The present larval stage was long, yellowish in color measured  $6.5 \pm 2$  (7.8–9) mm long, and its greatest width observed at the posterior one third of the body was  $0.4 \pm 0.02$  (0.38–0.44) mm. The mouth opening was guarded by two terminal bulbous lips, each was  $0.04 \pm 0.02$  (0.02–0.06) mm long. A cephalic bulb measured  $0.26 \pm 0.02$  (0.18–0.28) mm long,  $0.31 \pm 0.02$  (0.28–0.34) wide and armed with six transverse circular rows of spines or hooklets [Fig. 1 (a), (b) & Fig. 2 (a)] projecting from the cuticle were somewhat curved posteriorly and had a sharp-pointed end Cephalic papillae could not be observed. Body was covered by a transversely striated cuticle [Fig.1 (c) & Fig.2 (d), (e), (f)]. The esophagus was  $2.32 \pm 0.2$  (2.12–2.72) mm long, open into intestine by a valve; the ventricular appendix was narrow, usually somewhat shorter than the intestine. The intestinal caecum was  $7.1 \pm 0.2$  (6.8–7.5) mm long and  $0.15 \pm 0.02$  (0.13– 0.17) mm wide, opened at a midventral anus approximately located 0.03 mm from the posterior tip of the

body which terminated at a pointed mucron measuring  $0.148 \pm 0.02$  (0.120-0.183) mm long [Fig.1 (c), (d) & Fig.2 (e), (f)]. No papillae were observed in the pre- or postanal regions.



**Fig. 1.** Photomicrographs of third-stage larvae *Gnathostoma* sp. parasitizing *A. anguilla*

- a) Anterior region of worm showing cephalic bulb (CB) provided with bulbous lip (BL) and long muscular esophagus (OS), scale bar = 200 µm.
- b) High magnification of anterior extremity showing cephalic bulb (CB) armed with circular rows of spines (S), scale bar = 50 µm.
- c) Posterior region showing transverse striations of cuticle (TS), intestine (IN), and terminated with pointed mucron (M), scale bar = 200 µm.
- d) High magnification of posterior extremity showing anal opening (A) and pointed mucron (M), scale bar = 50 µm.



**Fig. 2.** Scanning electron micrograph of third-stage larvae *Gnathostoma* sp.

- a)** Anterior region of worm revealed a cephalic bulb (CB) armed with rows of spines (S), and terminated anteriorly by two pseudolabia (PL). scale bar = 100 $\mu$ m,  
**b, c)** Top view of head region showing mouth opening (MO), pseudolabia (PL) and cephalic bulb (CB) armed with spines (S), scale bar =100  $\mu$ m & 50  $\mu$ m  
**d)** Body cuticle showing transverse striations (TS), scale bar = 100  $\mu$ m respectively.  
**e, f)** Posterior extremity showing the characteristic pointed mucron (M), and transverse striations (TS) scale bar = 100  $\mu$ m & 50  $\mu$ m respectively.

### Taxonomic summary

**Species:** *Gnathostoma* sp. (Owen, 1836)

**Family:** Gnathostomatidae (Railliet, 1895).

**Host:** European eel *Anguilla anguilla* (F: Anguillidae) Linnaeus, 1758

**Site of infection:** The larvae were found encapsulated either on the surface of various organs in the abdominal cavity and some embedded in host muscles.

**Locality:** Alexandrina coasts of the Mediterranean Sea, Egypt.

**Prevalence:** 14 out of 27 (51.85%) specimens of the examined fish were naturally infected.

**Specimen deposition:** Specimens were deposited in the Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

## DISCUSSION

Genus *Gnathostoma* Owen (1836) comprises 17 species, their adult specimens are found in the stomach serosa of animals that consume raw fish (Nawa *et al.*, 2015; Pinheiro *et al.*, 2017) at least six species have been reported parasitizing humans causing a zoonotic disease, gnathostomiasis (Cole *et al.*, 2014; Hem *et al.*, 2015); these include *G. spinigerum* Owen, 1836, mainly in Thailand, Korea and Mexico, and *G. dorolesi* Tubangui, 1925; *G. hispidum* Fedtschenko, 1872; *G. malaysiae* Miyazaki & Dunn, 1965; *G. nipponicum* Yamaguti, 1941 and *G. binucleatum* Almeyda-Artigas, 1991 in other parts of the world.

The morphological characterization and morphometry of the cephalic bulb are the main characteristics used to distinguish Gnathostomatidae members (Moravec, 2007; Jung *et al.*, 2008; Gaspar-Navarro *et al.*, 2013). Also, Chai *et al.* (2015) proposed that the number and distribution of spines on the cephalic bulb play a major role in differentiation of *Gnathostoma* larvae, which can only be analysed using a scanning electron microscope rather than a light microscope. SEM proved to be a very valuable tool for studying the structure pseudo-labia, cephalic bulb hooklets number and distribution, genital papillae, pre-cloacal ventral cuticular ornamentations, and caudal papillae in males (Bertoni-Ruiz *et al.*, 2011; Pinheiro *et al.*, 2017).

There are few reports of Gnathostomatid nematodes naturally infecting fish in Egypt represented by Abdel-Ghaffar *et al.* (2013) who identified *Echinocephalus carpioe* n.sp. from the intestine of the common carp *Cyprinus carpio* inhabiting Burullus Lake, Kafr el-Sheikh Governorate. Also, larval stages (L4) of *E. overstreeti* were recorded from *Saurida undosquamis* (Morsy *et al.*, 2015) and from the common sea bream *Pagrus pagrus* (Adam *et al.*, 2020) from water coasts at Hurghada City, Red Sea.

In the current study, SEM observations showed the characteristic cephalic bulb, a circular mouth opening surrounded by two lips located anteriorly, and thick transversely striations of body cuticle forming muscle bundle which suggest these larvae herein belonged to genus *Gnathostoma*. the present species revealed certain specific features such as six rows of cephalic spines or hooklets were somewhat curved posteriorly and had a sharp-pointed end.

**Table 1.** Morphometric comparison (in mm) of third-stage larvae of some different *Gnathostoma* spp.

Characters	Third-stage larvae of <i>Gnathostoma</i> spp.				
	<i>Gnathostoma</i> sp.	<i>Gnathostoma</i> sp.	<i>Gnathostoma spinigerum</i>	<i>Gnathostoma spinigerum</i>	<i>Gnathostoma lamothei</i>
References	Present study	Pinheiro <i>et al.</i> (2017)	Chai <i>et al.</i> (2015)	Chai <i>et al.</i> (2020)	Gaspar-Navarro <i>et al.</i> (2013)
Host	<i>Anguilla anguilla</i>	<i>Colomesus psittacus</i>	<i>Monopterus albus</i> ,	<i>Monopterus albus</i>	<i>Poeciliopsis gracilis</i>
Locality	Alexandria, Mediterranean Sea	Soure, state of Pará, Eastern Amazon, Brazil	Yangon, Myanmar	Cambodia	México
Body Length	6.5±2 (7.8–9)	12 (10–14)	2.300–4.400 (3.347)	2.575–3.825 (3.250)	4.48 (3.58–5.09)
Body width	0.4±0.02 (0.38–0.44)	0.415 (0.333–0.480)	0.250–0.425 (0.366)	0.375–0.425 (0.386)	0.288 (0.236–0.318)
Cephalic bulb (L)	0.26±0.02 (0.18–0.28)	0.313 (0.277–0.387)	0.075–0.115 (0.093)	0.095–0.115 (0.104)	0.084 (0.069–1.06)
Cephalic bulb (W)	0.31±0.02 (0.28–0.34)	0.340 (0.293–0.386)	0.165–0.250 (0.221)	0.175–0.235 (0.218)	0.188 (0.167–0.216)
Cephalic rows of spines	six transverse rows of spines	six transverse rows of spines	4 transverse rows of hooklets	4 transverse rows of hooklets	4 transverse rows of hooklets
Body rows of spines	absent	absent	present	present	present
Tail	0.148±0.02 (0.120–0.183) terminated at a pointed mucron	0.175 (0.100–0.216) including terminal mucron,	The tail was very short and rounded	The tail was very short and rounded 0.030–0.060 (0.044)	The tail was short and blunt 0.059.16 ± 0.011 (0.36.72–0.075)

Based on morphological and morphometric comparison of the present nematode material with those previously recorded of this genus as shown in (Table 1), it was found that these larvae were more similar to those of *Gnathostoma* sp. reported by **Pinheiro *et al.* (2017)** from *Colomesus psittacus* in Brazil particularly in the number rows of head bulb spines (six rows of spines, Figs. 1b, 2a), a tail ending in a pointed terminal mucron (Figs. 1c,d and 2e,f), and absence of body spines with slightly few morphometric differences such as shorter body length and a smaller cephalic bulb. But it differed from *Gnathostoma spinigerum* (**Chai *et al.*, 2015, 2020**) which was isolated from Asian swamp eels, *Monopterus albus* in Myanmar and Cambodia and *G. lamothei* (**Gaspar-Navarro *et al.*, 2013**) in México, both have possessed four rows of cephalic spines, presence of body spines, and blunt tail without mucron. Furthermore, these nematode larvae were found encysted on the visceral organs of the peritoneal cavity and some embedded in host muscles of *A. anguilla* suggesting that may cause pathological changes in consistence with **Paperna (1986)**. From these findings, all recovered larvae from *A. anguilla* as were identified *Gnathostoma* sp. as a new host record in the Egyptian waters.

## CONCLUSION

The morphological data of the parasite studied herein are of importance because the present study is the first report of genus *Gnathostoma* parasitizing fish in Egypt.

These data introduce an important addition to the previous reports on gnathostomatids infecting fish in Egypt which may be highly relevant to the public health threat. They also contributed to our knowledge of pathogens that may affect aquatic hosts. Further genetic studies are recommended to identify this described parasite in the present study taxonomically at the species level.

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