An Anatomical and Morphological Study about *Gordius* aquaticus, Linnaeus, 1758 (Nematomorpha) Found in Sarıyer, Istanbul

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Abstract

Nematomorpha also is known as hairworms. About 300 freshwater and 5 marine horsehair worms (Nematomorpha) species have been described to date. They are parasitic in arthropods during their juvenile stage. When they become adult they live in an aquatic environment as free-living organisms. Most aspects of their systematics and biology are currently unknown. The aim of this paper was to investigate the anatomy and morphology of *Gordius aquaticus* (Nematomorpha) found in Sariyer, Istanbul. After observing the morphology, the anatomy of the specimen was investigated under light microscopy to confirm the species as *Gordius aquaticus*.

Keywords: Gordius aquaticus, Nematomorpha, Hairworms, Anatomy, Morphology

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Introduction

The horsehair worms (Nematomorpha) include about 300 freshwater (Gordiida) and 5 marine species (Nectonema) (Bleidorn et al. 2002). They are parasites during the major phase of their life cycle. After development in the host, they emerge to copulate in freshwater or in the seawater (Schmidt-Rhaesa and Ehrmann 2001). The parasitoids usually live their larval stage as endoparasites in the hemocoelic cavity of several invertebrates, especially terrestrial arthropods. After the developmental phase, adult worms kill and leave the host, beginning their free-living period (Brivio et al. 2000).

There are some reports on the presence of Nematomorpha in frogs, fishes, birds and mammals (including humans) (Brivio et al. 2000, Bolek and Coggins 2002). Freshwater hairworms adults, eggs and pre-parasitic larvae occur in ponds, streams, lakes and various manmade structures that retain water. The adults colour can be tan to black. The body shape is longitudial and several centimeters to over a meter in length with diameters of about 1mm (Poinar 2008).

The internal anatomy of gordiid species is little known. But it seems to be more or less uniform. The cuticular structures carry major importance for the taxonomy of Nematomorpha. The basic taxonomic characters for Nematomorpha systematics are based on structures of the body cuticula and on

the posterior end of the males (Schmidt-Rhaesa 2001). The aim of this study is to describe structural and morphological characteristics of the *Gordius aquaticus* found in Sarryer, Istanbul.

MATERIALS AND METHODS

Male specimen of Gordius aquaticus was collected by hand from the man-made structure that retains water in the winter season of 2007 from the Sariyer region of Istanbul, Turkey (41°09'59"N-29°02'07"E). We kept Gordius aquaticus alive in tap water for a week. Some photographs were taken to show the appearance of the head and the two lobed tail by using Nikon Coolpix 4500. Longitudinal measurements of the outstretched worm was made using a ruler. For the investigation by light microscopy (LM) a collected specimen was fixed in Bouin's fluid for 24h, dehydrated through an increasing alcohol series, cleared in xylene, embedded in paraffin and sections taken in thickness 5 μ m were stained with hematoxylin-eosin (H&E), Masson's triple stain (Masson), and Periodic acid - Schiff (PAS). Investigation covered different body areas such as anterior end, mid body and posterior end. The photographs of the light microscopy preparates were taken with Olympus CHO40.

RESULTS

Villalobos et al. (2001) was used in classification of the specimen. This species is listed below:

Phylum: Nematomorpha Family: Gordiidae

Genus: Gordius Linnaeus,1758 Species: Gordius aquaticus

Linnaeus, 1758

Description:

Body is 310 mm in length and 1 mm in diameter (Fig. 1). The colour of the body is light brown on the ventral side and dark brown on the dorsal. The anterior end is rounded with dark collar behind the white tip (Fig. 2). The mouth opening is terminally located. The

posterior end bilobed with 2 lateral lobes. It is present in all *Gordius* species that a semicircular cuticular structure called the postcloacal crescent is located on the posterior part of the round cloacal opening (Fig. 3).



Figure 1: External view of G. aquaticus



Figure 2: Anterior end of the body

In the posterior end the cuticle around the cloacal opening and the post cloacal crescent is pigmented darker than the surrounding cuticle. The pigmentation of cuticle around the cloacal opening is lighter than the other body parts (Fig. 3). The cuticle is smooth and slippery.

Histological observations:

In transverse sections, the body wall of Gordius aquaticus is composed of three components namely cuticle, epidermis, and muscle layers (Fig. 4a, 5). The parenchyme tissue fills the rest of the body and surrounds

the visceral organs such as intestine, testis tubes and ventral nerve cord (Fig. 4a).

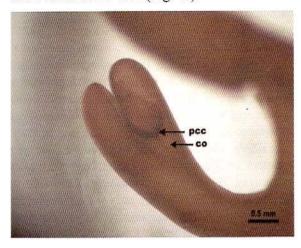


Figure 3: Bilobed posterior end of the body. co, cloacal opening; pcc, posteloacal crescent

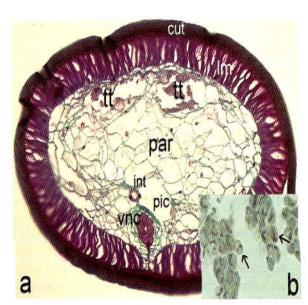


Figure 4 a: The transverse section of male Gordius aquaticus. Cut: cuticle, int: intestine, lm: longitudinal muscle layer, par: parenchyme, pic: periintestinal cavity, tt: testis tubes, vnc: ventral nerve cord (270x). b: The testis tube filled with round shape and aflagellate spermatozoa (arrows) (540x). Masson's triple stain.

The cuticle secreted by epidermis appears as thick and being composed of radial fibrillar layers. The cuticle is eosinophilic and only distal fibrilar layers give PAS positive reaction (Fig. 5, 6). The epidermis is very thin, formed by single low cuboidal cells and has a connection with the ventral nerve cord through the ventral neural lamella (Fig. 5). The nuclei of the epithelial cells were basophilic, flattened in shape and have a dense chromatin. The cytoplasma of the epithelial cells is eosinophilic (Fig. 5). The epidermis is separated from the muscular layer by a basal membrane which gives PAS positive reaction (Fig. 6). A peripheral system of ventral nerve cord which appears to innervate the underlying musculature constitutes the nervous system (Fig. 5, 6).

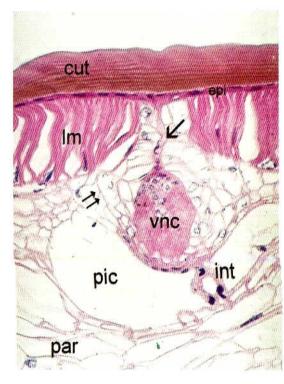


Figure 5: The body wall in higher magnification (540x The single arrow indicates the ventral neur lamella. The double arrows indicate the processes which are sent to ventral nerve cord muscle Epi: epidermis and other abbreviation are as in Fig. 4. Hematoxylin-eosin.

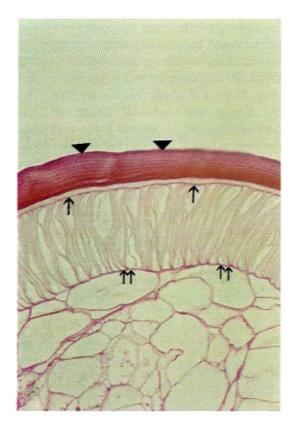


Figure 6: The distal cuticular layers (arrowheads), the basal membrane beneath the epidermis (arrows), and the basal membrane beneath the muscle cells (double arrows) give PAS positive reaction. (540x).

The muscle layer consists of longitudinal, thin and long muscle cells which send processes to the ventral nerve cord (Fig. 5). The muscle layer is separated by a PAS-positive basal membrane from the parenchyme tissue which consists of mostly poliangular cells with flattened nucleus at the edge (Fig. 6). In the ventral region of the body, the intestine is above the ventral nerve cord and surrounded with a cavity called "periintestinal cavity". The intestine is small in diameter (Fig. 5).

In males of Gordius aquaticus, two dorsalateral testicular tubes extend along the whole length of the worm as in other species of Gordiida and are surrounded with only the paranchyme tissue. The testis tubes can be very voluminous and densely packed with spermatozoa or narrow with a few scattered spermatozoa in different region of the body (Fig. 4a). The spermatozoa in the testis tubes are aflagellate and rod-shaped (Fig. 4b).

DISCUSSION

Knowledge of the systematics and distribution of the European Nematomorpha is still uncertain. There is no record from some areas and numerous taxonomic problems are in suspense (Schmidt-Rhaesa 1997; Villalobos et al. 1999). The situation is not much better with respect to Turkish fauna. The Nematomorpha fauna of Turkey is unknown. According to Aydemir et al. (1996) only one species of Nematomorpha, *Gordius aquaticus* has been reported to date from Ankara, Samsun, Istanbul and Antalya regions of Turkey.

In all Gordius species, there is a semicircular cuticular structure called postcloacal crescent which is located posterior part of the round cloacal opening (Schmidt-Rhaesa 2002). The taxonomically important characters are entirely cuticular structures, the general shape of posterior end of the male and postcloacal crescent (Schmidt-Rhaesa 2002, Villalobos et al. 2001). We identified our Nematomorpha specimen as Gordius aquaticus according to these characteristics.

On the other hand, the anatomy of our specimen is similar to the Schmidt-Rhaesa (1997)'s observations on Gordius aquaticus. In only this study, fibrillar lavers distinguished. A thick layer of large fibers is the main element of the cuticle. There are many fiber sheets within the cuticular layer of G. aquaticus. The number of cuticular layer varies between 24-37 individually. This may be dependent on ontogenetic changes, intraspecific variation or regional changes in the cuticle and the host in the larval stage. The function of the cuticle is protection during the free-living phase, because no indication of transcuticular absorption is present. In most Nematomorpha species, the cuticle is structured into elevations called areoles. These areaoles carry importance for determination of the Nematomorpha species. Schmidt-Rhaesa and Gerke (2006) did not observe these areoles in

Gordius aquaticus. Similarly, we could not observe these cuticular structures in our specimen.

We observed that the distal fibrillar layers stain with eosin more lightly than proximal layers and give PAS positive reaction. We considered that this may be related to a continuous maturation process of the cuticle components from the moment of being secreted by the epidermal cells until their exocytosis at the level of the surface and increasing mucopolysaccharides content.

Different points of view exists regarding whether the testes of adult forms of the various Gordiida species are internally bounded by discontinuous or continuous epithelium (Villalobos et al. 2005). Valvassori et al. (1999) had pointed out that the testicular epithelium is continuous in the initial stages of the epithelium spermatogenesis and that discontinuous when mature gametes fill the testicular cavity in the Gordius villoti. Schmidt-Rhaesa (1997) had determined that this epithelium is continuous and that the cells rarely contact each other in Gordius aquaticus. On the other hand, Schmidt-Rhaesa (1997) did not observe this epithelium in some individuals of G. aquaticus, consistent observations.

The morphology of the spermatozoa in the testis tubes is similar with other species of Gordidae (Hanelt et al. 2005). We could not observe any sensory structures in this study. In general, these kinds of structures have not been described from freshwater Nematomorpha, (Gordiida), except a single observation about photoreceptive structure (Schmidt-Rhaesa 2004).

There are some reports about the presence of gordiids in water system within urban areas. Gordiid worms do not cause any danger to humans, but are simply an indication that insect definitive hosts are capable of getting into the water source. So this can be associated with the water quality (Aydemir et al. 1996; Hanelt et al. 2005). Since these worms have been detected in humans digestive tract through the contaminated water from a stream or a river

(Villalobos et al. 2003), in several literatures it has been concluded that humans may serve as regulator host.

There is limited data about the Nematomorpha species found in our country. We considered that this anatomical and morphological study will contribute to the knowledge and distribution of the horsehair worms in Turkey.

Acknowledgements

Many thanks to Yasemin Tunalı for taking the total photographs, and also to Taylan Çam for collecting specimen.

References

Aydemir M., Yorgancıgil B., Demirci M., Akkaya H. (1996) İsparta şehir içme suyunda saptanan bir Gordius aquaticus Linneaus, 1758 Olgusu. Türkiye Parazitoloji Dergisi 20(3-4):361-365.

Bleidorn C., Schmidt-Rhaesa A., and Garey J. R. (2002) Systematic relationships of Nematomorpha based on molecular and morphological data. *Invertabrate Biology* 121(4): 357-364.

Bolek M. G. and Coggins J. R. (2002) Seasonal occurrence, morphology and observations on the life history of Gordius difficilis (Nematomorpha: Gordioidea) from Southeastern Wisconsin, United States. The Journal of Parasitology, 88:287-294.

Brivio M. F., de Eguileor M., Grimaldi A., Vigetti D., Valvassori R. and Lanzavecchia G. (2000) Structural and biochemical analysis of the parasite *Gordius villoti* (Nematomorpha, Gordiacea) cuticle. *Tissue & Cell*, 32(5):66-376.

Hanelt B., Thomas F. and Schmidt-Rhaesa A. (2005) Biology of the Phylum Nematomorpha. Advances in Parasitology, 59:244-293.

Poinar Jr. G. (2008) Global diversity of hairworms (Nematomorpha: Gordiaceae) in freshwater. Hydrobiologia, 595:79-83).

Schmidt-Rhaesa A. (1997) Ultrastructural observations of the male reproductive system and spermatozoa of Gordius aquaticus L., 1758. Invertabrate Reproduction and Development 32(1):31-40.

Schmidt-Rhaesa A. (2001) Problems and perspectives in the systematics of

- Nematomorpha. Organisms Diversity & Evolution, 1:161-163.
- Schmidt-Rhaesa A. (2002) Are the genera of Nematomorpha monophyletic taxa? Zoologica Scripta, 31:185-200.
- Schmidt-Rhaesa A. (2004) Ultrastructure of an integümental organ with probable sensory function in Paragordius varius. Acta Zoologica, 85:15-19.
- Schmidt-Rhaesa A. and Ehrmann R. (2001) Horsehair worms (Nematomorpha) as parasites of praying mantids with a discussion of their life cycle. Zoologischer Anzeiger, 240:167-179.
- Schmidt-Rhaesa A. and Gerke S. (2006) Cuticular ultrastructure of *Chordodes nobilii* Camerano, 1901, with a comparision of cuticular ultrastructure in horsehair worms (Nematomorpha). *Zoologischer Anzeiger*, 245:269-276.
- Valvassori R., Eguileor M. de., Grimaldi A. and Lanzavecchia G. (1999) Nematomorpha. In Jamieson BGM (ed) Progress in male gamete ultrastructure and phylogeny, reproductive biology of invertebrates. Oxford University Press 9:213-228.

- Villalobos L. C., Ribera I. and Downie I. S. (1999) Hairworms found in Scottish agricultural land, with descriptions of two new species of Gordionus Müller (Nematomorpha,: Gordiidae). Journal of Natural History 33:1767-1780.
- Villalobos C. D., Ribera I., and Bilton D. T. (2001) First data of Iberian Nematomorpha, with description of Gordius aquaticus Linnaeus, G. plicatulus Heinze, Gordionus wolterstorffii (Camerano) and Paragordius tricuspidatus (Dufour). Contributions to Zoology 70 (2):73-84.
- Villalobos C. D., Schmidt-Rhaesa A. and Zanca F. (2003) Revision of the Genus Betagordius (Gordiida, Nematomorpha). II. South American species with description of two new species. Nem Inst Oswaldo Cruz, Rio de Janeiro, 98(1):115-128.
- Villalobos L. C., Restelli M., Schmidt-Rhaesa A. and Zanca F. (2005) Ultra structural observations of the testicular epithelium and spermatozoa of *Pseudochordodes bedriagae* (Gordiida, Nematomorpha). Cell Tissue Res 321:251-255.