

**Surface topography of *Hysterothylacium winteri*  
Torres and Soto, 2004 ( Nematoda : Anisakidae ) from  
*Boops boops* marine fishes from Egypt**

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**ABSTRACT**

*Hysterothylacium winteri*. ( Nematoda : Anisakidae ) was collected from the intestine of a marine fish , *Boops boops* (Sparidae), from Alexandria fish market in Egypt. Fourteen ( 50 % ) out of 28 fish were infected ; the intensity was 1-7(mean 3) worms/host. *Hysterothylacium winteri* possessing one double pair of postanal papillae, a lateral pair of phasmids situated near the tip of tail. The present investigation added more details: A groove like structure in the ventral surface which may be serve for mechanical function and a food like material was seen between the flexible lips. *H . winteri* was recorded for the first time from *Boops boops* as well as from Egypt.

**INTRODUCTION**

Adult stage of members of the genus *Hysterothylacium* are normally found in the gut of fishes. Larvae have been reported widely in the tissues of a variety of marine fish and invertebrates which serve as intermediate hosts (Deardorff and Overstreet,1981). In the literature, larvae and adults have frequently been referred to *Contracaecum* and *Thynnascaris* but Deardorff and Overstreet (1980) have distinguished *Hysterothylacium* from *Contracaecum*.

The genus *Hysterothylacium* includes 59 species which are found at the adult stage in the intestine of fishes (

Bruce et al . 1994 , Torres et al . 1998 , Moravec and Nagasawa 2000). A new method for the identification of anisakid nematodes were carried out by Szostakowska et al (2002) and Kijewska et al (2002). In Egypt, *Hysterothylacium* sp. larvae was reported by Abd El-Rahman (1995) from marine fishes *Trachurus mediterraneus*, *Boops boops*, and *Sardinella aurita* caught from the Mediterranean Sea coast . In the present work the adult nematode *Hysterothylacium winteri* was studied by scanning electron microscopy in order to describe the surface topography and to determine the pattern of the fine structures found in the surface and the head of these nematodes which as far as the available literature indicates is not described.

## **MATERIALS AND METHODS**

Twenty eight specimens of the marine fish *Boops boops* were collected from Alexandria fish market in Egypt. Nematodes were removed from the intestine, rinsed in saline , fixed in cold 10 % buffered formalin and cleared in lactophenol for morphological study using light microscopy. Some specimens were postfixed in ethanol , dehydrated in ascending series of ethanol, then transferred through a series of intermediate fluids (3:1, 2:1 and 1:1) for 100% ethanol & amyl acetate, then to liquid CO<sub>2</sub> in Polaron critical point dryer. The dried specimens were then coated with gold/palladium and examined with JEOL scanning electron microscope. Unless otherwise stated ,measurements are given in mm (range followed by the mean in parentheses).

## **RESULTS**

*Hysterothylacium winteri* Torres and Soto, 2004.

Figs.( 1-15)

Type host : *Boops boops* (Sparidae)

Site of infection : Intestine.

Type locality : Mediterranean Sea, Alexandria , Egypt.

Prevalence : 50 % (14 fish infected / 28 fish examined)

Intensity : 1-7 (3) specimens per fish .

**Description:**

The body is elongate, tapering anteriorly and posteriorly (Figs.1&12). The cuticular surface of the body is regularly transversely striated (Figs 3&9). The 3 lips are similar in shape and size , slightly wider than long (Figs. 4&5&7). A food like material was seen protruding from the mouth (Fig.5).The dorsal lip has two large papillae (Fig.6), while each subventral lip possesses a single large papilla, a small lateral papilla and one amphid (Fig.8). The lips are provided with thick cuticular ridges on their external margins (Figs.6&7). Interlabia are triangular and their base is wider than length (Fig7).The two alae run posteriorly from the cervical region to the tail (Figs.3&4&11&15). About 6 longitudinal ridges start from the cervical region and run to near the posterior extremity of the body (Figs.4&12).One pair of postanal papillae is present near the tail (Fig. 14) A groove like structure was seen in the ventral surface running from the anterior extremity to the region close to the posterior end of the body (Figs.9&12).The excretory pore lies slightly posterior to the ventral lips (Fig.7).The tail is ventrally flexed and possesses a conical tip provided with numerous spines (Figs.12&14). One pair of Phasmids situated in the lateral portion of tail near the tip (Figs.12&13&14).

**Male** ( 8 specimens ) . Body 24.6- 44.3 ( 33.3 ) long and 0.356 - 0.598 (0.466 ) maximum width . Dorsal lip 0.067-0.110 (0.088 ) long and 0.070-0.117 (0.100)

maximum width . subventral lip 0.071-0.111 (0.092 ) long and 0.071-0.113 (0.095 ) wide . Interlabia 0.022-0.048 ( 0.034 ) long and 0.042-.0.058 ( 0.051 ) wide . Nerve ring lies at 0.352-0.497 ( 0.423 ) from the anterior end . Excretory pore 0.412-0.678 ( 0.550 ) from the anterior end . Oesophagus 4-6.6 (4.9) long and 0.155-0.180 ( 0.158 ) maximum width . Oesophagus about 15.9 % length of body . Ventriculus 0.090-0.168 (0.136 ) long and 0.099-0.179 (0.150 ) wide. Ventricular appendix 1.0-1.8 ( 1.5 ) long and 0.175-0.356 ( 0.216 ) maximum width . Intestinal caecum 0.269-0.388 ( 0.332) long and 0.075-0.088 ( 0.085 ) maximum width . Length of ventricular appendix represents about 29 % of oesophagus length . Length ratio of intestinal caecum to ventricular appendix 1 : 3-5.3 ( 1 : 4.2 ) . Intestinal caecum about 7.6 % of oesophagus length . Ejaculatory duct 1.2-2.2 (1.7 ) long and 0.168-0.279 (0.222 ) wide , about 5.3 % length of body . Spicules are embedded inside the body, equal in size , slightly curved and measures 0.323-0.404 (0.381 ) long and 0.022-0.045 (0.033) maximum width , about 1.3 % of body length . The tail is conical and measures 0.129-0.206 ( 0.169) long , with a tip measures 0.037-0.046 (0.037) long. Phasmids lies at 0.013-0.025 from the anterior border of the tip.

**Female** (10 specimens ) . Body 31-50.3 ( 40) long and 0.469-0.832 ( 0.635 ) maximum width . Dorsal lip 0.088-0.161 (0.120 ) long and 0.091-0.163 ( 0.128 ) maximum width . Subventral lip 0.087-0.160 ( 0.119 ) long and 0.100- 0.164 ( 0.131 ) wide . Interlabia 0.031-0.060 ( 0.047 ) long and 0.063-0.114 ( 0.085 ) wide . Nerve ring lies at 0.430-0.750 ( 0.570 ) from the anterior end . Excretory pore 0.517-0.847 ( 0.660 ) from the

anterior end . Oesophagus 4.4-7.1 ( 5.6 ) long and 0.126-0.181 (0.155) maximum width. Oesophagus about 15 % length of body . Ventriculus 0.141-0.198 ( 0.161 ) long and 0.098-0.208 (0.164) maximum width . Ventricular appendix 1-1.9 ( 1.6 ) long and 0.143-0.298 ( 0.233 ) maximum width . Intestinal caecum 0.309-0.544 (0.410) long and 0.085-0.115 ( 0.098 ) maximum width . Length of ventricular appendix represents about 27 % of oesophagus length . Length ratio of intestinal caecum to ventricular appendix 1 : 2.5-5.7(1 : 3.6) . Intestinal caecum about 7.7 % length of oesophagus . Vulva preequatorial and lies at 12-18.8 ( 16.3 ) from the anterior end. Vagina muscular , directed posteriorly . Uteri opposed . The tail is conical and measures 0.298-0.431 (0.345 ) long , with a tip measures 0.022-0.034 (0.038 ) long and covered by numerous spines. Phasmids 0.016-0.029 ( 0.023 ) from the anterior border of the tip.

**Fourth-stage larva** ( 9 specimens ) . Body 12.7-19.1 ( 15.0 ) long and 0.209-0.313 ( 0.260 ) maximum width . Dorsal lip 0.045-0.090 ( 0.055 ) long and 0.043-0.078 ( 0.054 ) wide . Subventral lip 0.042-0.082 ( 0.060 ) long and 0.042-0.061 ( 0.050 ) wide . Interlabia 0.019-0.030 ( 0.025 ) long . Nerve ring lies at 0.318-0.430 (0.370 ) from the anterior end. Excretory pore 0.387-0.500 ( 0.437 ) from the anterior end . Oesophagus 1.8-3.4 ( 2.5 ) long and 0.150-0.325 ( 0.197 ) maximum width. Oesophagus 13.1-21.5 ( 18.4 ) % length of body . Ventriculus 0.070-0.085 (0.081) long and 0.074-0.105 (0.087 ) wide . Ventricular appendix 0.789-0.960 ( 0.848 ) long and 0.066-0.195 (0.125 ) maximum width. Intestinal caecum 0.195-0.290 ( 0.250 ) long and 0.070-0.193 (0.122 ) maximum width. Length of ventricular appendix represents about 37.4 % of oesophagus length .

Length ratio of intestinal caecum to ventricular appendix 1 : 2.7-4.6 ( 1 : 3.6 ) . Intestinal caecum about 10.5 % length of oesophagus. The tail measures 0.106-0.183 (0.154) long with a tip covered by numerous spines.

## DISCUSSION

Species of the genus *Hysterothylacium* which were reported in fishes from marine regions in South America include : *H. aduncum* in Chile (Carvajal et al., 1995) and Argentina (Sardella et al., 1998) ; *H. reliquens* ( Norris and Overstreet , 1975 ) at the coast of Brazil ( Deardorff and Overstreet , 1981 ; Vicente et al ., 1985 ) , being also reported for Guyana and Colombia ; and *H. corrugatum* Deardorff and Overstreet , 1981 in Ecuador ( Deardorff and Overstreet 1981 ) . *H. rhamdiae* Brizzola and Tanzola, 1995 and *H. patagonense* Moravec , Urawa and Coria , 1997 in Argentina and *H. geschei* in Chile (Brizzola and Tanzola 1995 , Moravec et al . 1997 , Moravec 1998 , Torres et al . 1998 ) have been described in fishes from freshwater ecosystems of South America .

Other species reported from marine and/or estuarine fishes from the South Pacific Ocean include:, *H. pelagicum* Deardorff and Overstreet , 1982 , *H. scomberomori* ( Yamaguti , 1941 ) , *H. leptaspi* Bruce , 1990 , *H. chrysostomi* Bruce , 1990 , *H. sebae* Bruce , 1990 *H. murrayense* ( Johnston and Mawson , 1940 ) , *H. thalassini* Bruce , 1990 in Australia , *H. scomberoidei* Bruce and Cannon , 1989 and *H. tasmaniense* (Johnston and Mawson , 1945 ) and *H. zenis* ( Baylis , 1929 ) in New Zealand ( Deardorff and Overstreet 1982 , Bruce and Cannon 1989 , Bruce 1990a , b , Bruce et al . 1994 ) .

*Hysterothylacium winteri* belong to the group of species possessing one double pair of postanal papillae , namely *H . habena* ( Linton , 1901 ) , *H . geschei* , *H . tasmaniense* , *H . reliquens* , *H . ogcocephali* ( Olsen , 1952 ) , *H . chaunaxi* ( Olsen , 1952 ) , and *H . zenis* (Norris and Overstreet 1975 , Deardorff and Overstreet 1981 , Bruce 1990b , Torres et al . 1998 ) . By possessing a lateral pair of phasmids situated near the tip of tail .

*H . winteri* most closely resembles *H . habena* . The last characteristic has not been found in some other species (Soleim and Berland 1981, Deardorff and Overstreet 1981 , 1982 , Bruce and Cannon ,1989 , Bruce 1990a , b , Bruce et al . 1994 , Moravec 1994 , 1998 , Moravec and Nagasawa 2000 ) . Cuticular flanges of *H . winteri* are different from *H . habena* , *H . ogcocephali* and *H . reliquens* , ( indented ) , *H . zenis* ( approximately rectangular ) and *H . geschei*(wider beneath of lip papillae ) . *Hysterothylacium winteri* differs clearly from *H . habena* , *H . geschei* , *H . zenis* , *H . tasmaniense* , *H . reliquens* and *H . chaunaxi* in having short spicules and smaller percentage of spicules length with respect to the body length; it is also distinguished from *H . ogcocephali* by tip of the tail covered by numerous spines. Numbers of pairs of postanal papillae are greater in *H . zenis* ( 6 to 7 pairs ) and *H . chaunaxi* ( 7 pairs ) , than in *H . winteri* ( 4 pairs) (Torres and Soto,2004) .

*Hysterothylacium winteri* was originally described by Torres and Soto ,2004 from *Eleginops maclovinus* in Chile. The present material agreed fully with the original description, but in the present description one pair only of postanal papillae was seen near the tail. The present investigation added more details such as a groove like structure in the ventral surface which may be serve for

mechanical function to support the worm in the intestine of the host, a food like material was seen protruding from the mouth between the flexible lips. The latter not only for feeding but also for anchoring function. Moreover the present work extend the geographical distribution of this species to Egypt.

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## EXPLANATION OF FIGURES

Fig. 1 : Light micrograph of the anterior region of *Hysterothylacium winteri*. Scale bar = 450  $\mu\text{m}$  .

Fig. 2 : Light micrograph of the vulval region of *Hysterothylacium winteri* . Scale bar = 558  $\mu\text{m}$  .

Fig. 3 : Scanning electron micrograph of *Hysterothylacium winteri*. a, Ala ; ts ,Transverse striations. Scale bar = 7 $\mu\text{m}$  .

Fig. 4 : Scanning electron micrograph of the anterior region of *Hysterothylacium winteri* a, Ala ; lr-Longitudinal ridges. Scale bar = 450 $\mu\text{m}$  .

Fig. 5 : Scanning electron micrograph of the anterior region of *Hysterothylacium winteri* dorsal view . f, Food ; dl ,Dorsal lip ; i, Interlabium. Scale bar = 98 $\mu\text{m}$  .

Fig. 6 : Scanning electron micrograph of *Hysterothylacium winteri* for showing dorsal lip. d, Large papilla. Scale bar = 25  $\mu\text{m}$  .

Fig. 7 : Scanning electron micrograph of the anterior region of *Hysterothylacium winteri* ventral view vl, Ventral lip ; ex, Excretory pore . S. bar = 98 $\mu\text{m}$  .

Fig. 8: Scanning electron micrograph of *Hysterothylacium winteri* subventral lip. am, Amphid ; sm, Small papilla ; d, large papilla . Scale bar = 25  $\mu\text{m}$  .

Fig. 9 : Scanning electron micrograph of the anterior region of *Hysterothylacium winteri* Lateral view. vg, Ventral groove ; ts- transverse striations . Scale bar = 235 $\mu\text{m}$  .

Fig. 10 : Scanning electron micrograph of the posterior region of the premature male *Hysterothylacium winteri*. m , male genital opening . Scale bar = 150  $\mu\text{m}$  .

Fig. 11 : Scanning electron micrograph of the middle region of *Hysterothylacium winteri* dorsolateral view . a, ala Scale bar = 144  $\mu\text{m}$  .

Fig. 12 : Scanning electron micrograph of the posterior region of *Hysterothylacium winteri* fourth stage larvae lateral view. Ct, conical tip ; ph, phasmid . Scale bar = 180  $\mu\text{m}$  .

Fig. 13 : Scanning electron micrograph of the posterior region of *Hysterothylacium winteri* premature female apical view. Ct, conical tip ; ph, Phasmid . Scale bar = 170  $\mu\text{m}$  .

Fig. 14 : Scanning electron micrograph of the posterior region of *Hysterothylacium winteri* mature female ventral view. An, anus ; p, postanal papilla ; ph, Phasmid. Scale bar = 197  $\mu\text{m}$  .

Fig. 15 : Scanning electron micrograph of *Hysterothylacium winteri* cross section. a ala ; i, intestinal caecum . Scale bar = 197  $\mu\text{m}$  .

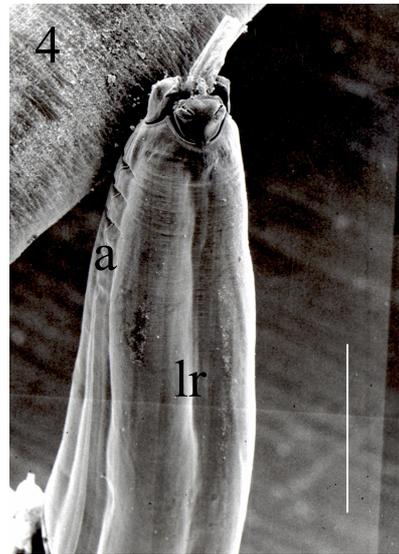
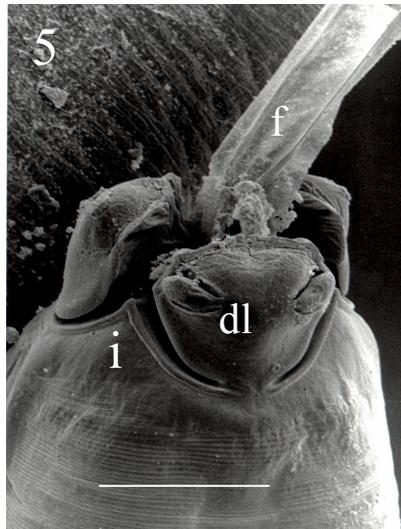
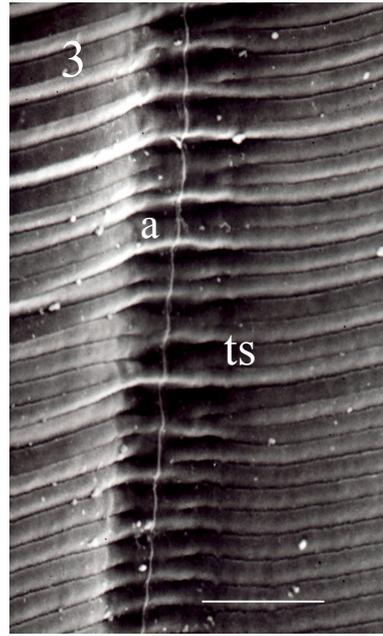
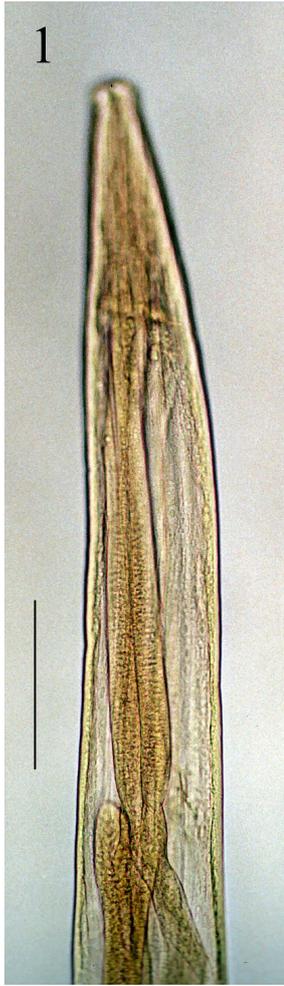
دراسة الشكل الظاهري لدودة هيستيروثيلاثيم وينتيري (نيماتودا-  
انيساكيدى) المستخرجة من اسماك البوقا البحرية من الاسكندرية  
بمصر

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الملخص العربى

تم جمع ٢٨ سمكة من اسماك البوقا البحرية من منطقة الاسكندرية بمصر  
ووجدت ١٤ سمكة مصابة بديدان النيماتودا من نوع هيستيروثيلاثيم  
وينتيري (انيساكيدى) وكانت كثافة الاصابة (١-٧) متوسط (٣) دودة لكل  
سمكة مصابة. هذا النوع يتميز بأحتوائية على زوج من الحلمات خلف  
الاست مع احتوائية على زوج من الفاسميد بالقرب من نهاية الزيل وقد  
اضافة الدراسة الحالية مزيد من الصفات مثل وجود ميزاب بطنى ممتد  
بطول الجسم يعتقد ان له وظيفة ميكانيكية فى تثبيت الدودة بأمعاء العائل  
وظهور شىء يشبه الطعام بارز من فتحة الفم بين الشفاة هذا بالاضافة  
الى أن هذا النوع يوصف لأول مرة من اسماك البوقا ولأول مرة من  
مصر .



*Ultrastructure of Hysterothylacium nematodes*

