

Morphometric Features of Three Lungworms in Materials from Wild Boars from Bulgaria

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A necropsy of wild boars from some regions of Southwest Bulgaria showed an infection with three lungworm species from family Metastrongylidae - *Metastrongylus pudendotectus*, *M. salmi* and *M. elongatus*. The collected parasites were used for a morphometric description of the species. The results were compared with the existing ones in the literature. It was established that as a whole the morphometric features of the species correspond to such described by other authors in materials from Bulgaria and abroad.

Key words: *Metastrongylus elongatus*, *Metastrongylus pudendotectus*, *Metastrongylus salmi*, wild boars, morphometric description

Introduction

Lungworms in wild boars are nematode parasites from family Metastrongylidae. They are believed to be the most important parasites influencing the health status of the wild boar [12]. The infection with metastrongylids leads to verminous pneumonia and secondary disorders in the hosts, loss of weight, abortion and higher mortality, especially in young animals. Because of their importance, many studies on different aspects of the induced by them illness have been performed [2, 3, 4, 8, 10, 13]. The systematic position of these parasites, however, is still unclarified. Kontrimavichus et al. [9] include in Metastrongylidae family two subfamilies – Heterostrongylinae (parasites on marsupials and insectivores) and Metastrongylinae. These authors [9] associate only one genus with subfamily Metastrongylinae – *Metastrongylus* in which include 7 species (*M. madagascariensis*, *M. pudendotectus*, *M. asymmetricus*, *M. salmi*, *M. confusus*, *M. elongatus*, *M. tschiauricus*) infected domestic swine and wild boars. Anderson et al. [1], however, believe that the above system is in error and Metastrongylidae should be restricted to *Metastrongylus* of swine, the genus being characterized by a pair of large, lateral trilobed labia, thick-shelled sculptured eggs, an atypical bursa and earthworm intermediate hosts. It is necessary to collect great quantity of information in order to solve

such kind of contradictions, in particular information about morphology of the parasites in materials from different hosts around the world. During a study on the etiology of helminthoses in wild boar in Bulgaria we have established an infection with three lung nematode species - *Metastrongylus pudendotectus*, *M. salmi* and *M. elongatus*. In connection with the above mentioned we set a goal to supply morphometric descriptions of these species in our materials.

Materials and Methods

The internal organs of 6 wild boars that were hunted during the shooting season in 2016 in three regions of Southwest Bulgaria were helminthologically necropsied. The discovered helminths were collected in a saline solution and after cleaning they were stored in 70% ethyl alcohol. Some of the helminths were enlightened with an alcohol-glycerol sequence so that the taxonomic important features could be easier observed. After that the helminths were included in gelatin-glycerin and the edges of cover glasses were coated with Canadian balm for longer storage. The measurements were performed with the help of the classical parasitological methods or after shooting of the separate structures of the parasites with a Web camera Logitech 4000, which was attached to the “Amplival” microscope, and their measuring with the picture analyzing computer program Image-Pro Plus-Version 6. Pictures were taken using a light microscope Leica DM5000 B, supplied with a camera and software (Leica Application Suite LAS v. 3.1). The obtained data were statistically analyzed according to Georgieva et al. [6]. Measurements in the tables are in μm .

Results and Discussion

We found metastrongylids in the lungs of 4 wild boars. They were *Metastrongylus pudendotectus*, *M. salmi* and *M. elongatus*. Further down data about their morphological and metrical features are supplied.

***Metastrongylus* spp. – general features**

Metastrongylids have a thread-like form and whitish color. The mouth opening is encircled with two trilobed lips; the oesophagus is slowly widening distally (**Fig. 1a**). The males have a small bursa copulatrix with poorly-developed and sometimes hard visible rays. The spicules have spongiform, comb-like structure. The females have a prevulvar cuticular swelling (valve). The eggs in the vagina are oval and contain fully developed larvae.

***M. pudendotectus* Vostokov, 1905**

Male. Bursa copulatrix (**Fig. 1b**): The dorsal ray is short and consists of two branches that look like a pair of pincers. The exterodorsal rays are detached, thin and short. The lateral rays come from one trunk. The anterolateral ray is the longest. It is the first that separates from the trunk, after that gradually becomes narrow and ends with roundness. The medio- and posterolateral rays remain connected longer. The mediolateral ray is wider and longer than posterolateral one and ends with an enlargement. The posterolateral ray also ends with an enlargement. The ventral rays are wide and begin from one trunk. The spicules are long and thin. Their wings are narrow and do not reach to the end of the stem. The distal end of the spicule stems is bifurcated and it looks like an anchor (**Fig. 1c**). The gubernaculum is small and looks like a shield (**Fig. 1d**).

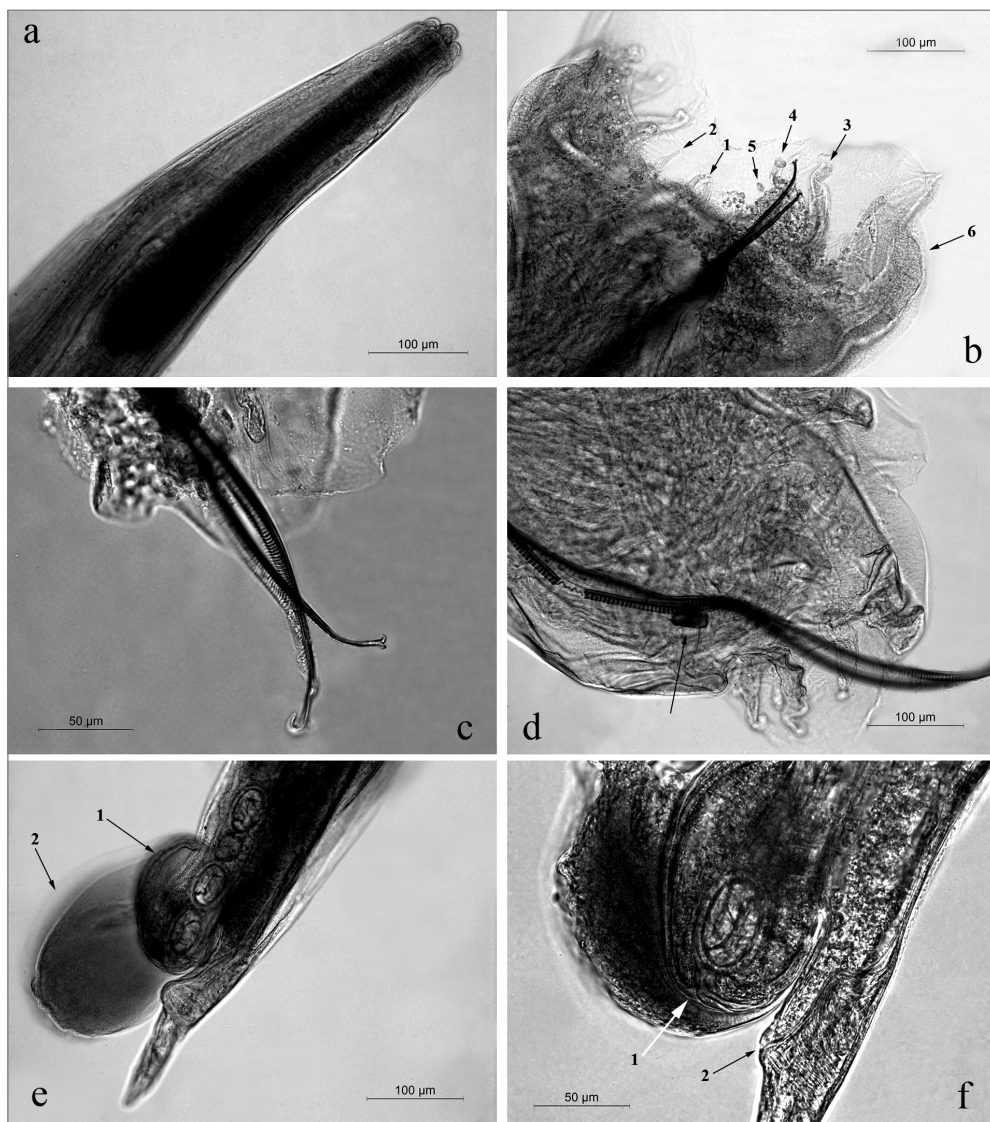


Fig. 1. Morphologic features of *Metastrongylus pudentotectus* in materials from wild boars from Bulgaria: a) anterior end; b) bursa copulatrix: 1- dorsal ray, 2 - exterdorsal ray; 3 - anterolateral ray, 4 - mediolateral ray, 5 - posterolateral ray, 6 - ventral rays; c) distal spicule end; d) gubernaculum; e) posterior end of female: 1- prevulvar cuticular valve, 2 - cuticular dilatation; f) posterior end of female: 1- vulva, 2 - anus

Female. The vagina is situated parallel to the body. The prevulvar cuticular valve is well-developed and surrounded by spherical cuticular dilatation (**Fig. 1e**). The vulva is opened at the distal end of the valve (**Fig. 1f**). The metric data about this species in our materials as well as those available in the literature are given in **Table 1**. Some of our metric data are outside the limits given by Kontrimavichus et al. [9], who have summarized the literature data up to that point. Our specimens have shorter oesophagus and gubernaculum.

Table 1. Metric data of *Metastrongylus pudentotectus* by different authors

Structure	Kontrimavichus et al., 1976	Gasso et al., 2014	Our data
Body length ♂ (in mm)	14.5-19.25	-	16.5 ± 0.14 (14-21)
Body width in the end of oesophagus ♂	-	-	108 ± 8,7 (90-126)
Oesophagus length ♂	414-520	-	408 ± 15 (385-462)
Max. oesophagus width ♂	-	-	64 ± 5.5 (58-83)
Spicule length	1310-1650	1380 ± 70 (1300-1500)	1500 ± 120 (1300- 1700)
Gubernaculum length	43-55	34 ± 5.55 (27.5-45)	39.7 ± 2.8 (31-46)
Body length ♀ (in mm)	21.5-40		23 ± 0.16 (19-29)
Body width in the end of oesophagus ♀	-	-	142 ± 11 (103-174)
Oesophagus length ♀	540-630	-	454 ± 10.8 (423-486)
Max. oesophagus width ♀	76-108	-	82.5 ± 7.2 (68-104)
Tail length ♀	-	119 ± 14.99 (100-150)	189 ± 8.4 (157-217)
Length of prevulvar cuticular dilatation	-	262.75 ± 42.07 (200-350)	186 ± 9 (165-207)
Width of prevulvar cuticular dilatation	-	254.5 ± 44.05 (200-330)	160 ± 11.4 (139-204)
Length of prevulvar cuticular valve	-	165.25 ± 33.36 (87.5 – 200)	147 ± 6.4 (125-164)
Width of prevulvar cuticular valve	-	87.5 ± 22.21 (50-137.5)	80 ± 4.6 (65-94)
Egg length	60-64	56 ± 2.93 (50-60)	56.3 ± 2.8 (50-67)
Egg width	43-45	43.5 ± 6.26 (37.5-55)	35.8 ± 2.8 (30-47)

The mean length of the spicules measured by us is in the upper limit pointed in previous study in materials from wild boars from Bulgaria [11]. There is also a certain difference with the data provided by Gasso et al. [5] in materials from wild boars from Spain and Poland. The gubernaculum and the tail of the females are shorter in their specimens, whereas the prevulvar cuticular valve, cuticular dilatation and width of the eggs are shorter in our specimens.

***M. salmi* Geddoelst, 1923.**

Male. Bursa copulatrix (**Fig. 2a**): The dorsal ray is short and bifurcated, with a form of pincers. The extrodorsal rays are detached, comparatively thin and short. The lateral rays come from one trunk. The anterolateral one is the longest and widest. Gradually it is narrowed and ends with a major rough thickening which is directed towards the ventral rays. The mediolateral ray is vastly shorter. It also ends with a thickening, which is turned to the posterolateral ray. The posterolateral ray is short – only one third

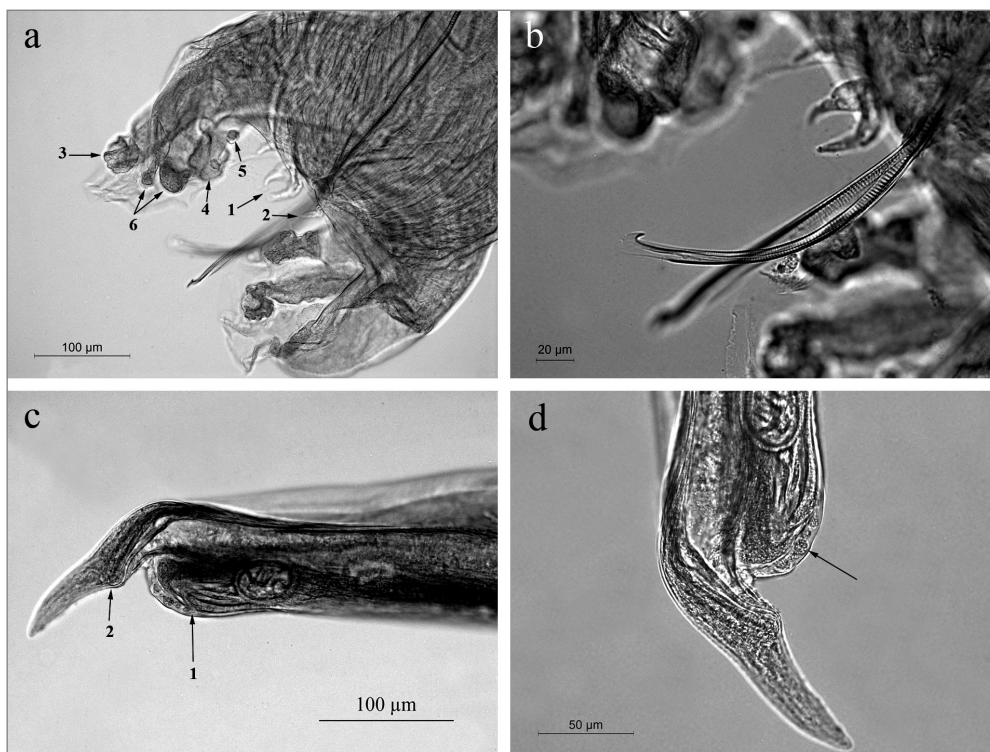


Fig. 2. Morphologic features of *Metastrongylus salmi* in materials from wild boars from Bulgaria. a) Bursa copulatrix: 1- dorsal ray, 2 - exterodorsal ray; 3 - anterolateral ray, 4 -mediolateral ray, 5 - posterolateral ray, 6 - ventral rays; b) distal spicule end; c) posterior end of female: 1- vulva, 2 - anus; d) posterior end of female: marginal sickle-like line of light-refracting formations

of the length of mediolateral ray, it begins from the base of mediolateral ray, can hardly be observed and ends with a thickening too. The ventral rays begin from one trunk. One of them has an obtuse end and another ends with a big thickening. The spicules end with a single growth with a form of hook (**Fig. 2b**). The gubernaculum is small and hard to be observed.

Female. The vagina is parallel to the body to the middle of the valve, after which it turns perpendicular towards the cuticle and passes into the vulva that opens on the free side of the valve (**Fig. 2c**). In the distal end of the cuticular valve a marginal sickle-like line can be observed, which consists of large light-refracting formations (**Fig. 2d**). The tail has a cone-like form. The metric data about this species in our materials as well as those available in the literature are given in **Table 2**.

It is clear from the table that our results correspond with those by Hollo [7] in materials from Hungary. On the other hand, it is visible that our mean values for most structures are either lower or around the lower limits of those pointed by Kontrimavichus et al. [9], and Gasso et al. [5]. An exception of this relation is the gubernaculum – in our measurements its mean size is 26.4 µm and according to Kontrimavichus et al. [9] it is 22 µm. The small size of the gubernaculum makes its observation difficult. Perhaps, this is the reason because of which why gubernaculum has not been established by Gasso et al. [5] in this species.

Table 2. Metric data of *Metastrongylus salmi* by different authors

Structure	Kontrimavichus et al., 1976	Hollo, 1965	Gasso et al., 2014	Our data
Body length ♂ (in mm)	14-17	11-15	-	14.3 ± 0.12 (11.6-18)
Body width in the end of oesophagus ♂	120-160	-	-	98 ± 5 (87-108)
Oesophagus length ♂	396-468	-	-	367 ± 14 (332-408)
Max. oesophagus width ♂	100	-	-	60.7 ± 3 (56-70)
Spicule length	2120-2370	1900 -3460	2120±220 (1600–2400)	2290 ± 80 (2150-2450)
Gubernaculum length	22	-	No gubernaculum	26.4±3.1 (23-29)
Body length ♀ (in mm)	39-40	25-39	-	29.5 ± 0.26 (25.2-42.8)
Body width in the end of oesophagus ♀	-	-	-	136 ± 19 (90-179)
Oesophagus length ♀	594-638	-	-	454 ± 4.6 (407-486)
Max. oesophagus width ♀	-	-	-	77.8 ± 6.8 (64-98)
Distance vulva- tail tip	-	-	-	90 ± 5.8 (78-105)
Distance anus-tail tip	-	71.3-108.5	71 ± 9.59 (62.5–92.5)	71 ± 4.4 (54-80)
Length of prevulvar cuticular valve	-	-	102.92±17.78 (75–125)	72.7 ± 6.8 (60-93)
Width of prevulvar cuticular valve	-	-	58.5 ± 10.35 (50-82.5)	61 ± 5.6 (47-78)
Egg length	82	46.5-52.7	51.75 (47.5 -60)	48.9± 2 (46-58)
Egg width	42	31-37.2	36.26 (35-37.5)	31.1± 2 (28-36)

***Metastrongylus elongatus* Gmelin, 1790**

Male. We found only one male specimen. The bursa copulatrix is small with hardly distinguishable parts (**Fig. 3a**). The distal parts of the spicules were cut, the length of the rest ones was 4.4 mm. There is no gubernaculum.

Female. The prevulvar cuticular valve is oval. The vagina goes parallel to the body, its distal part in the valve makes a light S-form curve and is opened in the vulva near the anus (**Fig. 3b**). The metric data about this species in our materials as well as those available in the literature are given in **Table 3**. The sizes of our specimens are lower or around the lower limit of those pointed by Kontrimavichus et al. [9]. Our metric data are within the limits given by Hollo [7], and Gasso et al. [5] with one exception: the width of eggs that is smaller in our specimens. The specimens measured in present study are smaller than those from other populations of the species from Bulgaria [11].

Table 3. Metric data of *Metastrongylus elongatus* by different authors

Structure	Kontrimavichus et al., 1976	Hollo, 1965	Mutafova, 2005	Gasso et al., 2014	Our data
Body length ♂ (in mm)	14 - 19	11-20	16.4 (14-18)	-	11
Body width in the end of oesophagus ♂	104 - 160	-	148 (117-169)	-	86
Oesophagus length ♂	414 - 504	-	490 (465-504)	-	415
Max. oesophagus width ♂	-	-	-	-	62
Spicule length	3870 - 5530	3710-5360	3600-3800	4200 ± 210 (3900-4500)	> 4400
Body length ♀ (in mm)	28 - 48.5	23-48	37.38 (29-43)	-	33.1 ± 0. (29.2-36.2)
Body width in the end of oesophagus ♀	-	-	-	-	163 ± 16 (131-180)
Oesophagus length ♀	576 - 774	-	593 (465-625)	-	510.6 ± 44 (431-581)
Max. oesophagus width ♀	-	-	-	-	82.6 ± 8.4 (71-99)
Distance vulva-tail tip	-	-	-	-	87.2 ± 20.8 (60-109)
Distance anus-tail tip	-	75-117	-	71.75 ± 9.59 (57.5 - 87.5)	74.5 ± 5.5 (68-82)
Length of prevulvar cuticular valve	-	-	-	100.25±24.09 (77.5 - 160)	93.7 ± 16 (76-119)
Width of prevulvar cuticular valve	-	-	-	50 ± 11.67 (35 - 70)	63 ± 4.7 (54-69)
Egg length	40	46,5-52.7	-	53 ± 2.84 (47.5-57.5)	47.5± 1.1 (46- 49)
Egg width	32-44	34.1-43.4	-	36.5 ± 1.75 (35-40)	29.5±1.8 (27-32)

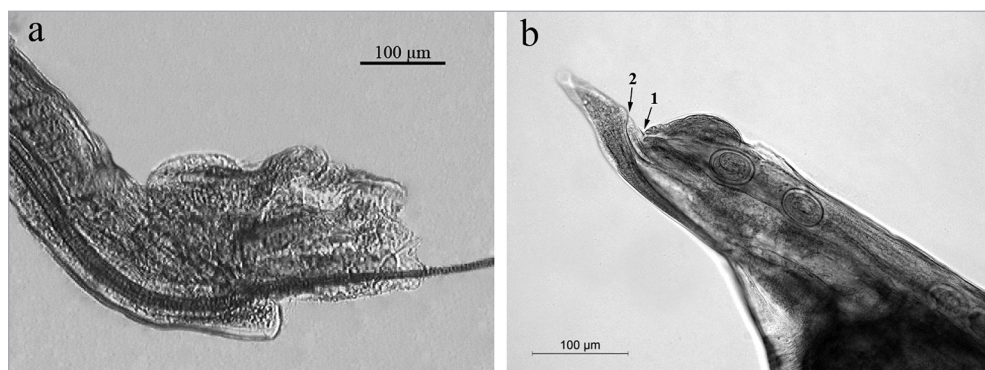


Fig. 3. Morphologic features of *Metastrongylus elongatus* in materials from wild boars from Bulgaria. a) bursa copulatrix; b) posterior end of female: 1 - vulva, 2 - anus

Conclusion

As a whole, morphological features of the described in the present work three metastrongylid species correspond to those pointed in previous descriptions by other authors in materials from domestic pigs and wild boars from different parts of the world. Variation regarding the metric characteristic is observed. It could be due to differences in methods of preparation and observation of the helminths as well as to the peculiarities of the separate parasite populations from different parts of the world.

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