

Lung Parasites in Stone Martens (*Martes foina* L.) from Bulgaria

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II. MATERIALS AND METHODS

Abstract—The present work focused on the study of pulmonary helminth-fauna of the stone marten in Bulgaria in terms of which the data are little. For the purpose, four stone martens were helminthologically necropsied according to the common technique. In addition, some of the injured lung parts were investigated after their boiling in lactic acid and subsequent compression. Four nematode species from different families of order Strongylida and Trichocephalida were found in the lungs. These were *Crenosoma petrowi* Morosov, 1939; *Eucoelus aerophilus* Creplin, 1839; *Filaroides martis* Werner, 1782 and *Sobolevingylus petrowi* Romanov, 1952. Some of the parasite structures with taxonomic importance were measured and described. According to our best knowledge, the species *F. martis* and *S. petrowi* are recorded for the first time as a part of the helminth-fauna of Southeast Europe and Bulgaria in particular.

Keywords—Bulgaria, *Crenosoma petrowi*, *Eucoelus aerophilus*, *Filaroides martis*, lung parasites, *Sobolevingylus petrowi*, stone martens.

I. INTRODUCTION

STONE marten (*Martes foina* L.) is a widespread small predator of the Mustelidae family. It occurs both in mountainous and plain areas. The martens have a varied diet – from small vertebrates and insects to fruits and carrion [1] which is a precondition for more potential ways of infection with parasites. They are also well-adapted to inhabit urban areas where they are in close proximity to people and domestic animals. This makes it possible to exchange parasites between them, what in turn increases the interest in studying of their parasitofauna [2]-[8].

During the investigations of stone marten in Bulgaria two nematodes parasitizing in the lungs have been established: Jancev and Genov [9] have found and described *Crenosoma petrowi*, and Kirkova et al. [10] have reported for *Eucoelus aerophilus* under the synonym *Capillaria aerophyla*.

In the present work, we record as these two species, as another two - *Filaroides martis* and *Sobolevingylus petrowi*, that are new for the helminth-fauna of Bulgaria. We also supply some morphometrical data about these species in the present materials.

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Four stone martens were found dead after their accidentally killing on the highway E-79 between the villages Djerman and Dolna Gradeshnitsa, Southwest Bulgaria. In laboratory conditions, they were helminthologically necropsied according to the common technique. To find small, hardly visible worms located in the bronchioles and alveoli, the abnormal lung tissues were examined as follows: 1-2 cm³ parts from the lung lesions were selected and boiled in 40 percent lactic acid in a water bath at 100 °C for 1.5 hour. After that, small (2-3 mm) pieces were compressed between slides and cover glasses and observed under a light microscope at 63, 160, and 400 magnification to visualize sexual structures such as spicules and gubernaculum.

The supplied morphometrical data are based on the following materials: for *C. petrowi* – two caudal ends of male and female specimens, extracted from the lungs and spicules and gubernaculum observed in pulmonary parenchyma by compression after boiling in lactic acid (CBLA); for *F. martis* – parts of the nematodes, including two anterior ends, extracted from well-formed nodules (with a size between 3 and 10 mm, connected with the exterior wall of the bronchi) and spicules observed in these nodules by CBLA; for *S. petrowi* – spicules observed in pulmonary parenchyma by CBLA; for *E. aerophilus* – small parts of the nematodes and great number of eggs observed in pulmonary parenchyma by CBLA.

The identification of the helminths was carried out on the basis of their host species, localization, and morphological characteristics [11]-[13]. Morphological structures of the helminths were measured using the classic methods of parasitology or after shooting of separate structures of 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 specimens were deposited in the collection of the Institute of experimental morphology, pathology and anthropology with museum, Bulgarian Academy of Sciences, Sofia, Bulgaria.

III. RESULTS

In three from the four martens, the following lung nematodes were found: from order Strongylida – *C. petrowi* (family Crenosomatidae), *F. martis* (family Filaroididae), *S. petrowi* (family Angiostrongylidae) and order Trichocephalida – *E. aerophilus* (family Trichuridae) (Table I).

TABLE I
 PULMONARY HELMINTH-FAUNA OF STONE MARTENS (*MARTES FIONA* L.)
 FROM BULGARIA

Infected animal	Parasite species	Localization
First	<i>C. petrowi</i>	Alveoles
	<i>S. petrowi</i>	Alveoles
	<i>E. aerophilus</i>	Alveoles
Second	<i>F. martis</i>	In nodules on the bronchial wall
Third	<i>F. martis</i>	In nodules on the bronchial wall
	<i>E. aerophilus</i>	Alveoles

A. Morphometric Data

1) *Crenosoma petrowi* Morosov, 1939

Bursa copulatrix has three parts and is typical of the species (Fig. 1 (a)). The body width is 149 μm . The spicules (Fig. 1 (b)) are equal in size (between 231-260 μm , mean 247 μm) and gently bent in the ends. In their second part, the spicules have thin hardly visible growth (Fig. 1 (c)). The gubernaculum is well visible and laterally it looks like a boat (Fig. 1 (b)). Its length is 97-105 μm , mean 102 μm . The posterior end of the female is conic without any cuticular peculiarities. The width of the body at the anus is 113 μm . The distance between the anus and the tail tip is 212 μm .

2) *Filaroides martis* Werner, 1782

The length of the longest nematode part that was extracted from a nodule was 22 mm. The cuticle has well pronounced transverse furrows. The oesophagus is tube-like and faintly dilated at the end (Fig. 2 (a)). Its length measured in the parts from male and female specimens was 260 μm and 280 μm , respectively. The spicules are equal in size (between 218-233 μm , mean 224 μm) and bent like an arc. Their proximal end is wider and has two small growths. In the distal end, spicules are split into two sharp parts (Fig. 2 (b)). Only in one case, the gubernaculum was visible (Fig. 2 (c)) and its length was 33 μm . Larvae were observed in the lung tissue under compression. Their length varied between 300 μm and 369 μm , mean 347 μm .

3) *Sobolevingylus petrowi* Romanov, 1952

The spicules are equal in size (between 84-88 μm , mean 86 μm) and have a peculiar form (Fig. 3). Proximally they start with a spherical enlargement and distally they are flexed in three points. The first flexion is the most pronounced. It is situated between the first and second third of the spicule length. The spicule angle in this part can reach 90°. The spicule part from the enlargement to the first flexion is triangular. Distally spicules are narrowed. There is no gubernaculum.

4) *Eucoleus aerophilus* Creplin, 1839

The maximum width of the nematode parts was 104 μm . The eggs have a typical structure with a form of barrel and well pronounced polar plugs. They are lightly asymmetrical and embryonated (Fig. 4). Their length was from 60 μm to 72 μm , mean 65.9 μm and width was from 30 μm to 36 μm , mean 31.8 μm .



Fig. 1 *Crenosoma petrowi* found in the lungs from a stone marten from Bulgaria: a) Posterior end of the body. b) Spicules (1) and gubernaculum (2) under compression. c) Distal spicule ends. The arrow - spicule growth

IV. DISCUSSION

Stone martens are pointed as a definitive host of eight lung nematodes: *Thominx* (syn. *Eucoleus*) *aerophilus*, *Crenosoma petrowi*, *C. schachmatovae*, *C. vulpis*, *Filaroides martis*, *Mustelivingylus skrjabini*, *Perostrongylus falciformis*, *Sobolevingylus petrowi* [6], [11]. In the present study, four of them have been established: *E. aerophilus*, *C. petrowi*, *F. martis*, and *S. petrowi*.

On the territory of Europe in martens, *E. aerophilus* most often has been found. It is recorded in Germany [14], Italy [2], Spain [3], Austria and Switzerland [4], Bulgaria [10], Lithuania [6], Russia [7] and Ukraine [8]. In the present investigation, *E. aerophilus* was established in two from the three parasitized martens that support its wide spreading. This species can infect various wild animals as well as domestic dogs and cats [15], a fact which has to be taken into account from an environmental point of view.

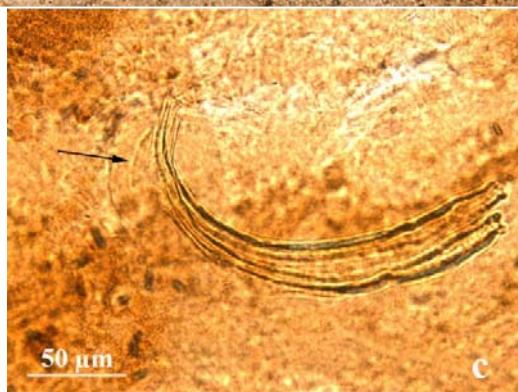


Fig. 2 *Filaroides martis* found in the lungs from a stone marten from Bulgaria: a) Anterior end. b) Spicules (under compression) – proximal end (1), distal end (2). c) Spicules and gubernaculum (the arrow) under compression



Fig. 3 Spicules of *Sobolevingylus petrowi* found in the lungs from a stone marten from Bulgaria (under compression)



Fig. 4 An egg of *Eucoleus aerophilus* found in the lungs from a stone marten from Bulgaria (under compression)

C. petrowi has been established in Bulgaria [9], Italy [2], Spain [3] and former Czechoslovakia [4].

Infections with *F. martis* are reported in Spain [3], Switzerland, and former Czechoslovakia [4].

S. petrowi has been found in Italy [2] and Spain [3].

The literature reference about spreading of these four species on the territory of Europe indicates that *E. aerophilus* and *C. petrowi* are known for the parasitofauna of stone marten in our country whereas another two species (*F. martis* and *S. petrowi*) are established for the first time.

C. petrowi is described by Janchev and Genov [9]. The morphometric data about spicules, gubernaculum and caudal ends of the nematodes in our materials correlate with theirs. However, the sexual structures of our specimens are smaller than those pointed at description by Kozlov [11] where the spicules are 301 µm and gubernaculum is 120 µm in length.

Our data regarding the length and width of eggs of *E. aerophilus* are in fully agreed with those by Zajac and Conboy [13] after who the length of eggs is 58-79 µm and the width is 29-40 µm.

The spicules of *F. martis* are fairly longer in our data (218-233 µm) compared to the data by Kozlov [11] - 151-185 µm. The same is related also to spicules of *S. petrowi*. They are 84-88 µm in length according to our measuring and 69-78 µm in data by Kozlov [11].

The established metric deviations are most probably due to peculiarities of the different populations of the species, object of the different studies.

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