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MORPHOLOGICAL CHARACTERISTICS OF NEMATODES OF THE *GLOBOCEPHALUS* GENUS PREVALENT AMONG WILD BOARS FROM VARIOUS REGIONS OF BULGARIA

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Summary

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Sexually mature parasites of the *Globocephalus* genus, collected from wild boars inhabiting Bulgarian State Game Breeding Stations with various ecogeographical characteristics were investigated A complete morphometric description of collected worms was performed and the presence of a single species – *G. urosubulatus*, was observed The data were analyzed and compared to morphological descriptions reported in the literature. Conclusions about the consistency of morphometric criteria used in the recognition of *Globocephalus* species were made.

Key words: *Globocephalus*, morphology, nematodes, wild boars, worm parasites

INTRODUCTION

Globocephalosis is a widely distributed parasitic infection in domestic and wild pigs, inhabiting various geographical areas. Furthermore, it is reported in carnivores and royal stags. The causative agents are nematodes from the *Globocephalus* Molin, 1861 genus. As a consequence of the search for consistent morphological traits for species identi-fication of its representatives, the *Globocephalus* genus had undergone several taxonomical reorganizations and therefore, some of the species became synonymic and others were described as representatives of new genera (Linstow, 1897; Alessandrini, 1909; Railliet *et al.*, 1913; Cameron, 1924; Freitas & Lent, 1936). According to literature data, globocephalosis among swine in Europe is caused by two species – *G. longemucronatus* Molin, 1861 and *G. urosubulatus* (Alessandrini, 1909). The first one is found by Baruztki *et al.* (1990, 1991), and the second – by Henne *et al.* (1978) in wild pigs in Germany. Hartwich (1986) considers the validity only of the *G. longemucronatus* species and the other one – *G. urosubulatus,* is considered as a synonym. At the same time the author suggested *Crassioma urosubulatum* as a synonym of the *G. longemucronatus* Molin, 1861. A number of authors however, do not support the synonymization proposed by Hartwich (1986) and thus *G. urosubulatus* is among of the most frequently reported in various parts of Europe: in Germany by Mennerich-Bunge *et al.* (1993), in Italy by Magi *et al.* (2002), in Spain by Fernandez-de-Mera *et al.*

(2003, 2004), in France by Foata *et al.* (2006) etc.

Gastrointestinal strongylids on wild pigs in Bulgaria are studied by Dimitrova (1964, 1969), Daskalova & Tzvetkov (1995), Mutafova *et al.* (2004) etc. The observed *Globocephalus* parasites were determined as *G. urosubulatus.* Mutafova *et al.* (2004) reported that 80.5% of wild pigs inhabiting mountainous regions with a various ecogeographical characteristics have been infected with helminthes. In 22.27% of cases, the infectious agents were from the *Globocephalus* genus. Higher percentages of parasitic infection were observed in low-mountainous regions in India by Yadav & Tandon (1989).

The aim of the present studies was to determine the species affiliation of *Globocephalus* hookworms after morphological description, to perform a critical analysis of data about some of their morphological traits and to look after criteria that could be used as a reliable trait in species' identification.

MATERIALS AND METHODS

A total of 284 female and 166 male sexually mature hookworms, collected in the partial helminthological necropsy of 11 shot wild boars inhabiting State Game Breeding Stations were studied. The stations were situated in regions with a different ecogeographical characteristics – West Rhodope mountain and East Strandja mountain. The parasites were fixed in solution of Barbagallo (30 g solution of 40% formalin in 1 L physiological saline) and their morphology was studied with a Jenaval microscope. The manuals of Popova (1955) and Hartwich (1986) were used in the species identification, observing the following morphological traits: body dimensions and surface, arrangement of the mouth cavity and mouth capsule, oesophageal length, structure of spicules and the copulatory bursa in male parasites, morphology of the tail end in female parasites.

RESULTS AND DISCUSSION

The isolated and investigated *Globocephalus* parasites had a thick striated cuticle. Body length in male was from 3.5 to 5 mm and in females – from 4.5 to 8 mm (Table 1). According to literature data, body length in male from some *Globocephalus* species varies from 4.3 to 7.9 mm (Hall, 1924; Smit & Notosoediro, 1926; Yamaguti, 1935; Beylis, 1936; Freitas & Lent, 1936; Gildenblat, 1939; Caballero & Cerecero, 1944; Kobulej, 1954). Hence, among the representatives of one and the same species, there is a large variation in body lengths and therefore, this trait should be prudently used in the species identification of this genus. This assumption is supported by the fact that in *G. marsupialis,* according to Caballero & Cerecero (1944), the length of male parasites varies from 7.123 to 7.938 mm, and according to Freitas & Lent (1936) – from 4.4 to 6.18 mm. In the view of Popova (1955), the length of *G. longemucronatus* varies between 4.7–5.7 and 7 mm, and Hartwich (1986) reports body lengths in на *G. urosubulatus* between 3.7 and 7.1 mm.

The length of the mouth capsule in studied parasites was from 0.140 to 0.200 mm and its width – from 0.150 to 0.170 mm. The ratio between length and width was variable and depended on the location of the parasite at the moment of its fixation and thus, the capsule's shape was more or less elongated or oval. These observations supported the comments of Kobulej (1954), who disagrees with some

Table 1. Metric characteristics of *G. urosubulatus* Alessandrini, 1909, prevalent in wild boars from various regions in Bulgaria (data of ours)

Parameters		Dimensions (mm)
Male	length	3.5–5.0
	width	0.36–0.37
Female	length	4.5–8.0
	width	0.42–0.50
Mouth capsule	length	0.14–0.20
	width	0.14–0.17
Oesophagus	length	0.56–0.69
	width	0.12–0.15
Distance between cervical papillae and the head end		0.43–0.61
Distance between the nervous ring and the head end		0.38–0.52
Length of spicules		0.42–0.58
Gubernaculum		0.07–0.08
Length of tail spike in female parasites		0.04
Distance between the vulva and the posterior body end		2.20–2.40
Distance between the anal orifice and the end of tail		0.12–0.18

authors as Cameron (1924), affirming that some species of the genus had a mouth capsule with round, and others – with elongated shape. On this ground, we believe that the shape of the mouth capsule was an inconsistent trait for species identification.

The diameter of the mouth was 0.08 to 0.09 mm, bordered by an annular thickening connected with capsule's base by four well visible ribs (Fig. 1a). The same morphology was communicated by Hall, cited by Popova (1955) for *G. longemucronatus*.

Subventrally, at the bottom of the mouth capsule, there were two triangular-shaped teeth with pointed edge, large base, length of 0.04–0.05 mm and width of 0.03–0.07 mm (Fig. 1b, c). The dimensions of both teeth were almost identical, with variations in the length and width of single parasites within the limits specified by Kobulej (1954) for *G. longemucronatus* and by Smit & Ile (Popova, 1955) for *G. amucronatus*. According to Popova (1955) the absence of two teeth in the mouth capsule was the cause for the announcement of the *G. longemucronatus* Molin (1861) species. On the contrary, Hartwich (1986) believes that Molin had not noticed the teeth due to the bad lightening.

Mediodorsally, on the inner side of the mouth capsule, a glandular groove, reaching closely the anterior part of the capsule, was observed (Fig. 1c). This trait was found out in all *Globocephalus* species and therefore it could be affirmed that its solely use could not serve as a trait of species identification.

The oesophagus had a length of 0.560 to 0.690 mm, a well-developed muscular layer and enlargement at the distal end

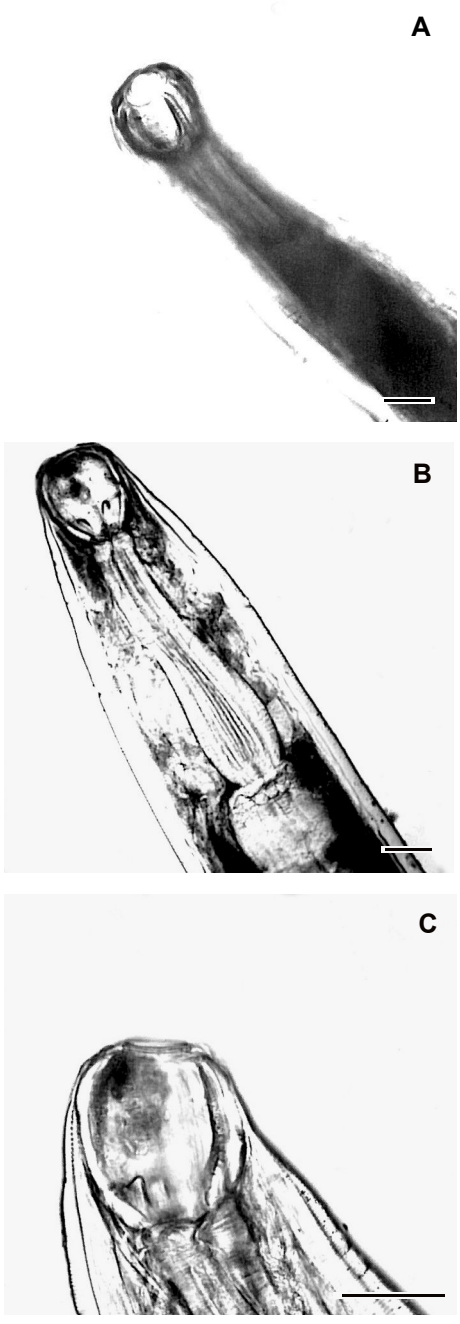

Fig. 1. *Globocephalus urosubulatus*. Male: anterior end (A, B, C). Bar=50 μm.

(Fig. 1b). At the boundary between the oesophagus and the intestine, 4–5 glands with rounded shape could be found out, described in all species from the genus and determined by some authors as valves.

The spicules were two, with similar shape and dimensions, slightly striated, with a length of 0.420–0.580 mm. Significantly bigger dimensions were reported by Kobulej (1954) (0.600–0.650 mm); Yamaguti (1935) (0.69–0.72 mm) etc. for *G. longemucronatus* spicules, by Popova (1955) for *G. lutrae* spicules, by Smit & Ihle (1928) for *G. amucronatus* spicules. The length of the gubernaculum was 0.07–0.08 mm, with a wider median part and thinner end parts (Fig 1a). Similar morphology of the gubernaculum was described in *G. longemucronatus* by Hall (1924). After Popova (1955), the values obtained by us corresponded to those reported about the gubernaculum of *G. urosubulatus* (from 0.06 to 0.105 mm) and were with similar to those of *G. connorfilii* (0.09 mm), although with lower values. In other species as *G. marsupialis,* the shape and dimensions of the gubernaculum are different. In accordance with Freitas & Lent (1936) it was composed by two wide branches bended as a horseshoe and was considerably smaller (0.034–0.038 mm).

The copulatory bursa was well developed, with large prebursal papillae. The genital cone was strongly prominent, with dimension of 0.100 mm when the bursa was dorsally situated. Similarly to the description of Hartwich (1986) for *Globocephalus* parasites found in Europe and identified as *G. urosubulatus*, it looked like a thinning sheath. The cloaca opened in the base of the sexual cone. On its both sides, two papillae were present, one under the other. The distal ones were with a

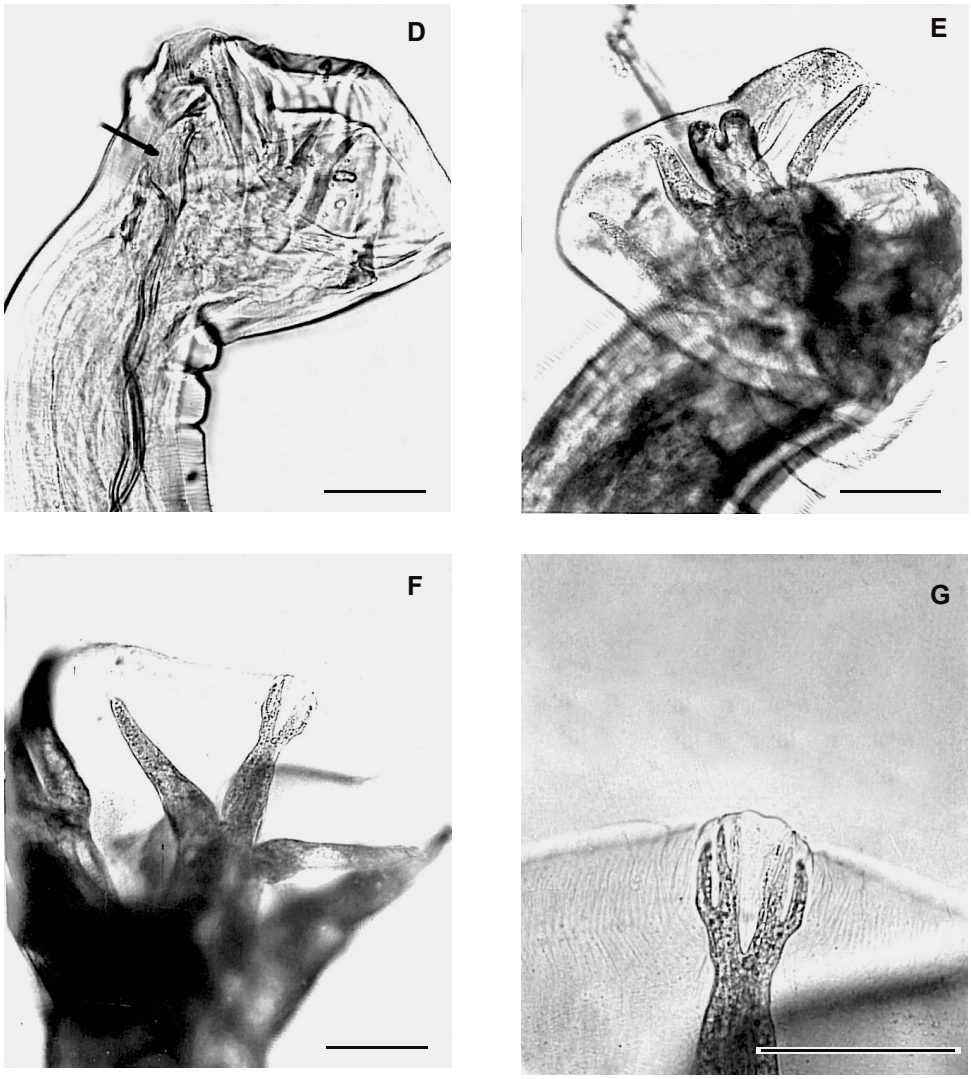

Fig. 1 (cont'd). *Globocephalus urosubulatus*. Male: posterior end (D, E, F, G). Bar=50 μm.

larger base and length of 0.025 to 0.030 mm, and proximal ones were delicate, with a narrow base and length of 0.017 mm. These papillae were hardly visible when the studied parasite was extended. This hinders their detection and partly explains the unstable behaviour of proximal papillae as well as the difference between length data, reported by Kobulej (1954) and Hartwich (1986).

The ventral and the lateral ribs of the copulatory bursa originated from a common trunk, respectively, strong at the latter. Antero- and posteromedial ribs were in close proximity, with a length from 0.140 to 0.170 mm. The posterolateral rib

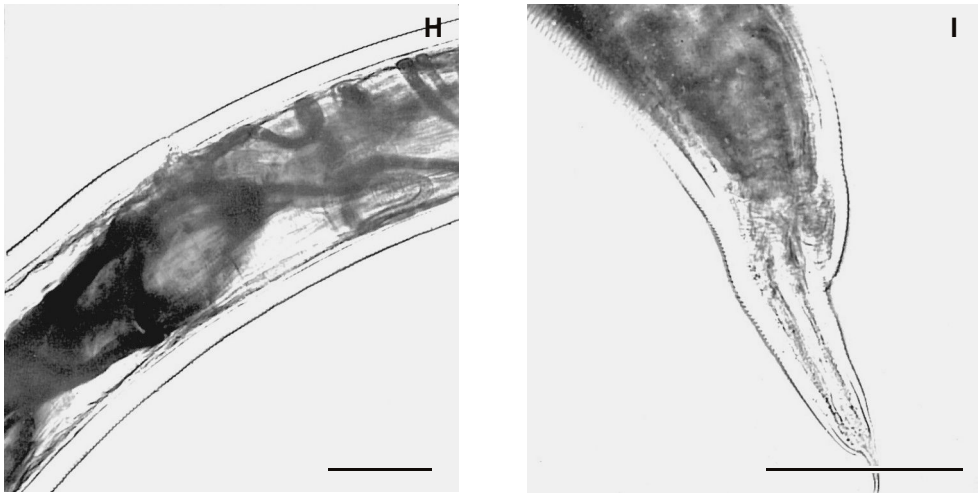

Fig. 1 (cont'd). *Globocephalus urosubulatus*. Female – vulva (H); tail end (I). Bar=50 µm.

was more developed, with dimensions between 0.120 and 0.160 mm and separated from the medio- and anterolateral ones at a distance of 0.030 mm from the beginning of the stem. Mediolateral and anterolateral ribs separated at 0.060 mm from trunk's beginning and then, had almost equal dimensions from 0.130 to 0.160 mm. Dorsal and exterodorsal ribs had a common trunk in the beginning. Exterodorsal ribs separated at 0.070–0.080 mm from trunk's beginning. In some parasites, exterodorsal ribs were asymmetrically situated vs the common stem, and often were with a different length (from 0.158 to 0.144 mm on the average). At 0.150–0.160 mm after the separation of exterodorsal ribs, the dorsal rib divided into two branches that on their part split into 3 projections. The terminal separation of the dorsal rib and the length ratio between the various branches were, in the opinion of Yamaguti (1935), an important taxonomic trait. Later, Kobulej (1954) supported this assumption. Our observations on this feature however, showed variations in the different specimens. Most commonly, there were parasites where the outer branches separated sooner and were considerably longer, whereas the median and inner ones were more delicate and with equal dimensions. This morphology of the terminal end of the dorsal ray was described in *G. longemucronatus* and *G. urosubulatus* by Kobulej (1954). In single parasites from one population, we found out that the median branches were longer than the inner ones, as described by Smit & Notosoediro in *G. amucronatus* (cited by Kobulej, 1954). The length of terminal projections between two branches was also found to vary in one parasite. A similar picture could be seen on the drawings of Freitas & Lent (1936) for *G. marsupialis* and of Lane (1922), cited by Popova (1955) for *G. connorfilii*. According to Alessandrini, cited by Kobulej (1954), the end of dorsal ribs had a similar structure in *G. longemucronatus, G. connorfilii, G. urosubulatus*. On the basis of obtained results and literature data though, we consider that there is no consistent morphological evidence supporting the view of Kobulej (1954) about the taxonomic sig-

nificance of terminal projections of the dorsal rib in *G. longemucronatus, G. connorfilii, G. urosubulatus* and *G. marsupialis.*

In all studied female parasites, the tail end was found to be sharp, cone-shaped, with a thin fine 0.04 mm spike. This morphological sign corresponds to the description about *G. urosubulatus* (Popova, 1955; Hartwich, 1986) and about *G. connorfilii* (Popova, 1955). According to Popova (1955) the length of the spike in *G. amucronatus* was 0.02 mm. The presence of a tail spike in female parasites is a characteristic trait in most *Globocephalus* species (Kobulej, 1954). In our belief, the similarity in spike length in the different *Globocephalus* species could be hardly used as a reliable taxonomic sign.

The literature data about *G. longemucronatus* (Hall, 1924 cited by Yamaguti, 1935, Kobulej, 1954), *G. amucronatus* (Smit & Notosoediro, 1926), *G. connorfilii* (Lane, 1922; Beylis, 1936), *G. urosubulatus* (Alessandrini, 1909) about the dimensions (in mm) of body length in males (4.3–5.7–7; 5; 4.5–5; 4.97–7.35 respectively) and females (5.7–6.8–8; 6.0–7.0; 4.1–6.5; 3.67–9.35 respectively), the oesophagus length (0.52–0.76; 0.49–9.67; 0.625–0.75, 0.485–0.992), the distance from cervical papillae to head end (0.42–0.53; –; 0.375; 0.57) and from the nervous ring to head end (0.38–0.48; –; 0.323–0.48), length of spicules (0.600–0.72; 0.7; 0.275–0.54; 0.46–0.66) and length of the gubernaculum (-; –; 0.9; 0.6–0.105) vary within similar ranges. Therefore, the comparison and the analysis of listed data as well the results of the present study (Table 1) allowed to assume that the independent use of these traits as taxonomic criteria in species' identification is not reliable.

The morphological characteristics of *Globocephalus* spp. isolated from wild boars in Bulgaria confirmed the existence of only one species. The obtained morphometric data corresponded to the *G. urosubulatus* description given by Alessandrini (1909) and Hartwich (1986). On this ground, we presumed that the studied wild boars from two different ecogeographical regions in Bulgaria, were infected with parasites of a single species – *G. urosubulatus*.

CONCLUSIONS

It was found out that 21.35% of studied wild boars in Bulgaria, inhabiting mountainous regions with a various ecogeographical characteristics, were infected with *Globocephalus* organisms. The morphology of studied helminthes corresponds to the description of *G. urosubulatus* (Alessandrini, 1909) according to Popova (1955).

The analysis of data showed that taxonomic traits of the *Globocephalus* genus varied within a broad range and frequently, their values overlapped (Table 1). There are conflicting opinions with regards to some taxonomic traits. All facts suggested that the correct identification of species from this genus require the use of other methods of investigations together with the recognition of morphological differences.

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