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Morphological variability of *Oswaldocruzia filiformis* (Nematoda: Molineidae) in reptiles inhabiting the protected areas of the Republic of Mordovia (Russia)

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Morphological variability of *Oswaldocruzia filiformis* (Nematoda: Molineidae) in reptiles inhabiting the protected areas of the Republic of Mordovia (Russia)

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Abstract. The morphological variability of the nematode *Oswaldocruzia filiformis* Goeze, 1782 from five reptile species (*Lacerta agilis, Zootoca vivipara, Anguis fragilis, Natrix natrix, Vipera berus*) was studied in 2018–2020 in the Smolny National Park and the Mordovia State Nature Reserve. We analyzed the morphological characters traditionally used in the taxonomy of *Oswaldocruzia* spp. A wide morphological variability of nematodes has been found, both in different host species and within the same host species. Variation of the morphological characters of *O. filiformis* from different reptile species was due to the host-induced variability. The morphological variability of nematodes from the same host species could be due to the phenotypic plasticity of *O. filiformis*.

1. Introduction

Nematodes of the genus *Oswaldocruzia* Travassos, 1917 are common and widespread parasites of the amphibians and reptiles. The type of caudal bursa and the structure of the synlophe (system of longitudinal cuticular ridges) are taken into account when identifying *Oswaldocruzia* species [1–3]. In recent decades, the taxonomic keys were developed for the genus *Oswaldocruzia* for the Western Palaearctic; many species have been described and re-described [3–10]. Most *Oswaldocruzia* species in the Western Palaearctic are considered the specific parasites of particular amphibian and reptilian species in which they have been found for the first time [3, 7, 9, 11–13].

Original molecular phylogenetic analysis of *Oswaldocruzia* spp. from the amphibians and reptiles of the European Russia evidenced that only one species, *Oswaldocruzia filiformis* Goeze, 1782, parasitized in all the species studied so far [14].

The study aims to assess the variability of the morphological characters of *O. filiformis* parasitizing in reptiles of the Smolny National Park and the Mordovia State Nature Reserve (Russia).

2. Methods

The study of the morphological variability of *O. filiformis* was carried out in the Smolny National Park and the Mordovia State Nature Reserve (Republic of Mordovia, Russia) in 2018–2020 (figure 1). Nematodes were collected from five reptilian species: slow worm *Anguis fragilis* Linnaeus 1758, sand lizard *Lacerta agilis* Linnaeus 1758, viviparous lizard *Zootoca vivipara* (Jacquin, 1787), common

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European viper *Vipera berus* (Linnaeus, 1758) and grass snake *Natrix natrix* Linnaeus 1758. A total of 102 mature specimens of *O. filiformis* were studied, 55 males and 47 females.

Figure 1. Schematic map of *O. filiformis* sampling locations (indicated by red stars): 1 – Mordovia State Nature Reserve; 2 – Smolny National Park.

The mature nematodes were undergone the morphological examination; the parasites were killed by heating in water and cleared in lactic acid. Apical and transverse sections of nematodes were made with the help of a razor blade. Drawings of nematodes were made using MBI-9 light stereomicroscope with the drawing unit RA-7. The nomenclature of the caudal bursa is given in accordance to the classification of M.C. Durette-Desset and A.G. Chabaud [1], of the synlophe, according to M.C. Durette-Desset [2].

3. Results

The main morphological characters of *O. filiformis* males and females from different reptilian species have been studied; the measurements are presented in the tables 1 and 2.

Table 1. Morphometry of *Oswaldocruzia filiformis* males

Charac-ters [a]	*A. fragilis* [b] (11)		*L. agilis* [b] (20)		*Z. vivipara* [c] (10)		*N. natrix* [b] (3)		*V. berus* [b] (3)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean	min–max
BL	8.51	7.75–9.50	7.61	6.65–9.25	7.53	6.90–8.25	8.79	8.50–9.16	8.16	7.22–8.75
BW	0.157	0.134–0.185	0.144	0.118–0.181	0.143	0.125–0.161	0.125	0.118–0.130	0.159	0.145–0.173
LV	0.080	0.073–0.083	0.081	0.071–0.089	0.078	0.069–0.085	0.078	0.076–0.081	0.076	0.075–0.077
WV	0.041	0.035–0.049	0.043	0.037–0.049	0.042	0.035–0.047	0.038	0.037–0.039	0.040	0.039–0.041
OL	0.395	0.354–0.433	0.384	0.354–0.417	0.389	0.370–0.409	0.466	0.448–0.489	0.453	0.437–0.472
TL	0.130	0.129–0.136	0.129	0.114–0.142	0.130	0.118–0.139	0.130	0.122–0.138	0.113	0.106–0.118
SL	0.218	0.197–0.236	0.208	0.188–0.232	0.200	0.185–0.212	0.217	0.200–0.232	0.212	0.208–0.217
NC	36–39		37–45		39–41		34–36		40–45	

[a] abbreviations: BL – body length; BW – body width at the mid-length level; LV – length of cephalic vesicle; WV – width of cephalic vesicle; OL – oesophagus length; TL – tail length; SL – spicule length; NC – number of crests at the mid-body

[b] Smolny National Park

[c] Mordovia State Nature Reserve

Table 2. Morphometry of *Oswaldocruzia filiformis* females

Characters [a]	*A. fragilis* [b] (11)		*L. agilis* [b] (20)		*Z. vivipara* [c] (10)		*N. natrix* [b](3)		*V. berus* [b] (3)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean	min–max
BL	14.91	14.00–15.75	12.68	11.00–15.65	11.70	10.52–13.24	10.36	10.05–10.66	11.43	9.25–13.20
BW	0.200	0.165–0.236	0.221	0.173–0.256	0.200	0.177–0.216	0.204	0.193–0.215	0.222	0.213–0.230
LV	0.087	0.085–0.089	0.083	0.078–0.089	0.084	0.077–0.091	0.084	0.081–0.087	0.083	0.079–0.087
WV	0.039	0.037–0.041	0.043	0.039–0.049	0.043	0.038–0.047	0.042	0.041–0.043	0.045	0.043–0.047
OL	0.488	0.465–0.504	0.457	0.421–0.496	0.430	0.390–0.469	0.500	0.496–0.504	0.445	0.390–0.500
AV	9.93	9.45–10.30	8.34	6.85–10.15	7.38	6.70–8.70	6.63	6.25–7.00	7.52	6.25–8.90
EL	0.085	0.082–0.091	0.085	0.080–0.089	0.081	0.079–0,085	0.080	0.077–0.085	0.082	0.078–0.085
EW	0.044	0.043–0.047	0.046	0.043–0.049	0.045	0.043–0.047	0.043	0.041–0.045	0.042	0.040–0.045
TL	0.253	0.236–0.268	0.251	0.228–0.276	0.241	0.228–0.268	0.195	0.189–0.200	0.238	0.224–0.256
NC	58–61		55–61		48–55		40–45		57–60	

[a] abbreviations: AV – distance from anterior end to vulva; EL – egg length; EW – egg width
Other designations as in table 1

The mean values of the studied morphological characters differed significantly when considering the nematodes obtained from different hosts. The mean body size of nematodes varied from 7.53 to 8.79 (body length) and from 0.125 to 0.159 (body width). The largest *O. filiformis* males were observed in *N. natrix*, females, in *A. fragilis*. The smallest males were recorded in *Z. vivipara*, females, in *N. natrix* (tables 1, 2).

The shape of the cephalic vesicle of nematodes, both from one host species and from different hosts, varies greatly. It can be whole (undivided) or consisting of two parts: a wider anterior part and a narrower posterior part. Both parts can be smooth or with transverse folds (figure 2).

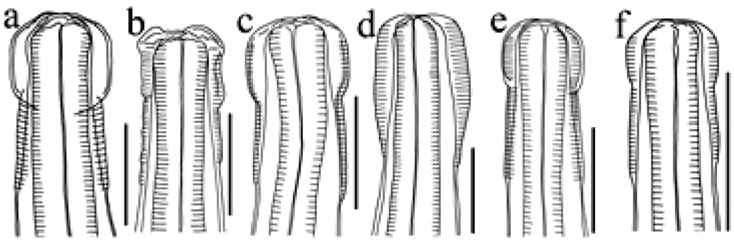

Figure 2. Variability of cephalic vesicle of *O. filiformis*: a – female from *A. fragilis*; b – male from *A. fragilis*; c – female from *L. agilis*; d – male from *Z. vivipara*; e – male from *N. natrix*; f – female from *V. berus*. Scale bars: 0.05 mm

The number of crests on transverse sections of nematodes at the mid-body level varied within wide limits. In particular, the number of crests in *O. filiformis* males varied from 34 to 45, in females, from 40 to 61. The largest number of crests in the middle of the body was noted in males from *V. berus*, in females, from *A. fragilis*. Both male and female nematodes from *N. natrix* had the smallest number of crests at the mid body (figure 3, tables 1, 2).

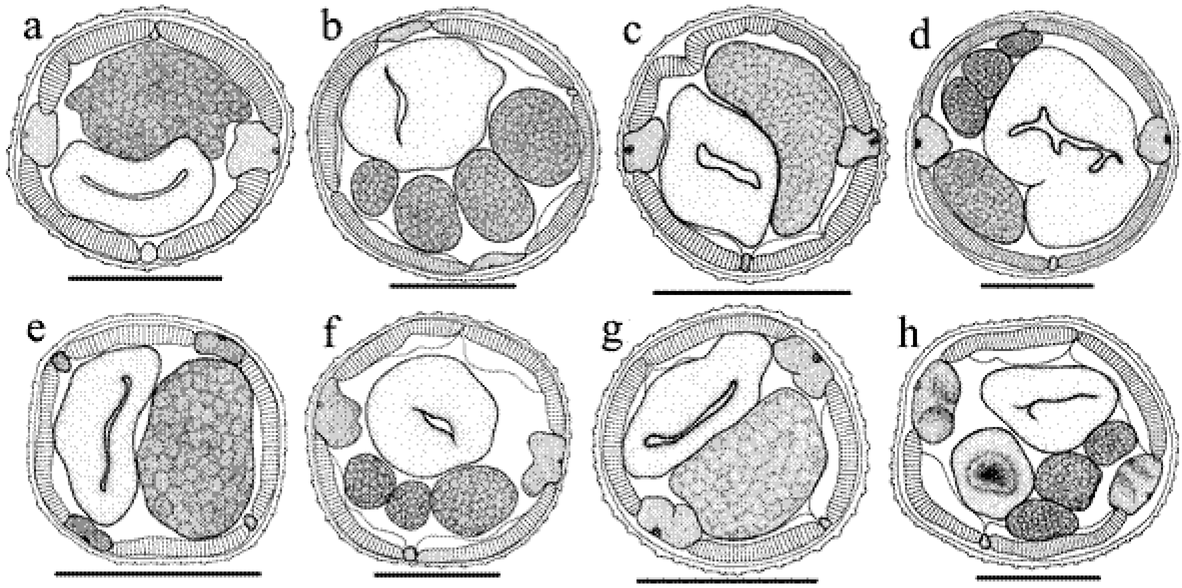

Figure 3. Transverse sections of *O. filiformis* at the mid-body: a – male from *A. fragilis*, 40 crests; b – female from *A. fragilis*, 61 crest; c – male from *L. agilis*, 37 crests; d – female from *L. agilis*, 58 crests; e – male from *N. natrix*, 36 crests; f – female from *N. natrix*, 45 crests; g – male from *V. berus*, 45 crests; h – female from *V. berus*, 57 crests. Scale bars: 0.1 mm.

The degree of development and shape of the lateral alae also varied both in nematodes from different reptilian hosts and in nematodes from the same host species (figure 4). Thus, we found *O. filiformis* female with small narrow lateral alae formed by three slightly enlarged crests (dorsal and ventral crests and a smaller central crest between them) and the male with a more developed ventral crest in the lateral alae at the middle of oesophagus in the same specimen of *N. natrix* [14]. Similar *O. filiformis* male with a more developed ventral crest in the lateral alae was found in *A. fragilis* together with the parasites carrying well-developed lateral alae (figures 4a, 4b).

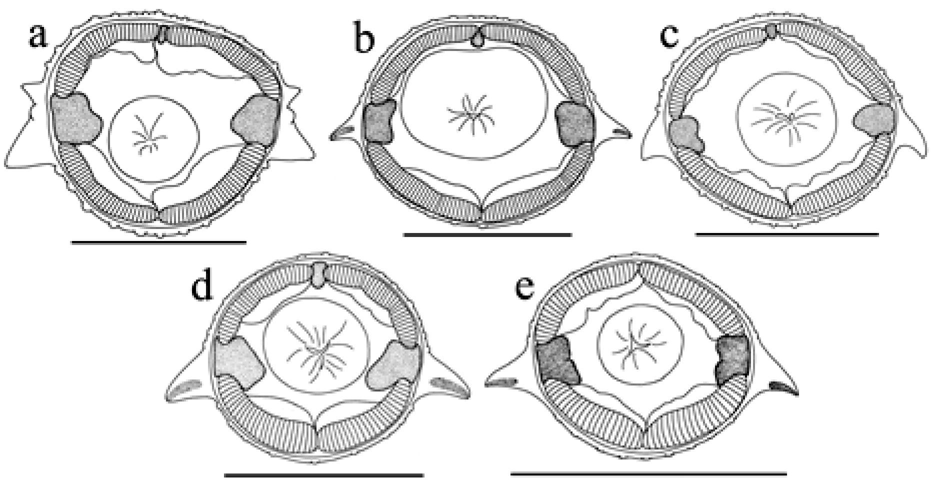

Figure 4. Transverse sections of *O. filiformis* at the middle of oesophagus: a, b – males from *A. fragilis*; c – female from *L. agilis*; d – female from *Z. vivipara*; e – male from *V. berus*. Scale bars: 0.05 mm.

In our study, all males of *O. filiformis* had significant variability of the structure of the dorsal rib. In particular, the shape of the ninth and tenth rays, forming the dorsal rib, varied greatly. The shape, number, and length of extra branches of the tenth ray also varied significantly (figure 5).

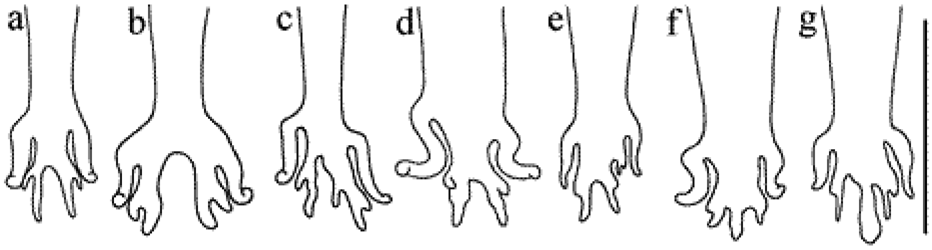

Figure 5. Variability of dorsal ray of the caudal bursa of *O. filiformis*: a – from *A. fragilis*; b, c – from *L. agilis*; d, e – from *Z. vivipara*; f, g – from *V. berus*. Scale bar: 0.05 mm

4. Discussion

The life cycle of *O. filiformis* is direct. The main hosts (amphibians and lizards) are infected with nematodes when they ingest the infecting larvae of *O. filiformis* along with the food [12]. Findings of *O. filiformis* in snakes are the cases of a post-cyclic parasitism [12, 16, 17].

Snakes can be infected by three *Oswaldocruzia* species: *O. duboisi* Ben Slimane, Durette-Desset et Chabaud, 1995, *O. filiformis*, and *O. bialata* Molin, 1860, depending on the amphibian species they have consumed. *V. berus* also can get nematodes from viviparous lizards, which are the food objects for this snake species.

In our opinion, there is also the possibility of direct infection of snakes by getting the infecting larvae of *O. filiformis* from the environment. The morphological characters of nematodes from *V. berus*, in particular, the unique shape and the structure of dorsal rib of the caudal bursa, indicate that the parasite development took place in the snake organism (figures 5f, 5g). Egg-excreting female of *O. filiformis* has been found in *N. natrix*. Therefore, in the snake body, the nematode is able both to survive (and to live) and to complete its life cycle.

The study of the variability of *O. filiformis* from various reptilian species evidences on significant differences in the body size and certain organs and in the nematode morphology. Probably, the degree of variability of the morphological characters of *O. filiformis* is preconditioned by the differences in the physiology and ecology of different reptilian species.

According to our study, the number of crests at the mid-body of nematode varies widely in specimens obtained both from one host species and from different hosts. This feature was considered earlier to be an important diagnostic character in the description of Trichostrongylidae and, in particular, of the nematodes of the genus *Oswaldocruzia* [2]. However, our data do not support this idea. We argue that this extremely variable feature cannot be used as a diagnostic one.

There are scarce data on the variability of the shape and the degree of development of lateral alae, as well as on the number of crests in *Oswaldocruzia* spp. depending on the population structure of the parasites and their hosts. There was reported only on the differences in the number of crests in nematodes of different sexes [4, 5, 8, 10, 15]. The intra- and inter-population variability of nematodes parasitizing in vertebrates and, in particular, of *O. filiformis*, was reported earlier; in particular, it was noted that the body size of nematodes varied depending on sex, age, phenotype, and host species, the number of parasites in the host, season of the year, etc. [18–20].

The structure of the caudal bursa (type II under classification of Durette-Desset and Chabaud [1]) and of the spicules of *O. filiformis* males are relatively constant in different host species, when comparing the variability of this feature and of other morphological characters. It should be noted that the structure and shape of the caudal bursa and spicules of *O. filiformis* males in reptiles and amphibians were similar [14]. No significant variability was observed in the structure of the reproductive system of *O. filiformis* females.

5. Conclusion

A broad morphological variability of nematodes both from different reptiles and from the same host species has been observed, namely, in the size of the nematode body and of certain organs, in the shape and size of the cephalic vesicle, in the number of crests on the transverse sections at the mid-body, in the shape and degree of development of the lateral alae and in the shape of the ninth and tenth rays of the caudal bursa of males. Such a variation of morphological features of *O. filiformis* from different reptilian species may be due to the host-induced variability. In the nematodes obtained from the same host species, such a variability may be due to the phenotypic plasticity of *O. filiformis*. Probably, the differences in the physiology and ecology of reptilian hosts precondition the degree of variability of morphological characteristics of the nematode *O. filiformis*.

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