OSWALDOCRUZIA FILIFORMIS SENSU LATO (NEMATODA: MOLINEIDAE) FROM AMPHIBIANS AND REPTILES IN EUROPEAN RUSSIA: MORPHOLOGICAL AND MOLECULAR DATA

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Nematodes of the genus *Oswaldocruzia* parasitise the small intestine of amphibians and reptiles. Their biodiversity remains unknown. We studied *Oswaldocruzia* nematodes from nine species of amphibians and reptiles (*Pelophylax ridibundus*, *Rana arvalis, Rana temporaria, Bufo bufo, Lacerta agilis, Zootoca vivipara, Anguis fragilis, Natrix natrix*, *Vipera berus*) at six localities in European Russia in 2018–2019. To identify their nematode species, we analysed the morphological characters traditionally used in the taxonomy of nematodes of this genus together with new molecular phylogenetic data. The results of partial sequencing and molecular phylogenetic analysis of CoxI mtDNA gene showed that all *Oswaldocruzia* specimens in this study belonged to the same species. We observed a broad morphological variability of nematodes both from different host species and from the same host individual. Morphological variation in nematodes from various host species could be host-induced, while that in nematodes from the same host individuals could be due to phenotypic plasticity of the species. Molecular data indicate that only one species of the genus *Oswaldocruzia*, *O. filiformis* s.l., which has a broad morphological variability, parasitises amphibians and reptiles in European Russia. The results of our study highlight the necessity of studying the diversity of morphologically similar *Oswaldocruzia* spp. from the Western Palearctic by molecular genetic methods.

Key words: CoxI mtDNA, molecular phylogenetic analysis, morphological variability, trichostrongylids, Western Palearctic

Introduction

Nematodes of the genus *Oswaldocruzia* Travassos, 1917 are parasites inhabiting the small intestine of amphibians and reptiles. They are most common in anurans but also parasitize caudate amphibians, lizards and, rarely, snakes. These nematodes are distributed worldwide. The genus comprises about 90 species (Ben Slimane et al., 1995, 1996a,b; Ben Slimane & Durette-Desset, 1996a,b; Durette-Desset et al., 2006; Bursey et al., 2007; Schotthoefer et al., 2009; Bursey & Goldberg, 2011; Svitin, 2017).

Oswaldocruzia filiformis Goeze, 1782 was the first species of the genus described in the Palearctic, from amphibians of the genera *Bufo* and *Rana*. The life cycle of *O. filiformis* is direct. Its invasive larvae occur on soil or on plants (Hendrikx & van Moppes, 1983; Tarasovskaya, 2009; Svitin, 2016). Amphibians and lizards are infected with these nematodes when they occasionally ingest their larva along with the food. Findings of *O. filiformis* in snakes should be considered as cases of post-cyclic parasitism (Bertman & Okulewicz, 1987; Kirillov, 2000, 2010; Novokhatskaya, 2008; Svitin & Gorobchishin, 2015; Svitin, 2016). Snakes become infected after eating amphibians, which are the main hosts of *Oswaldocruzia* spp.

Since the first description of *Oswaldocruzia filiformis* was rather short and most *Oswaldocruzia* species in the Palearctic are morphologically similar, most of the nematodes found in amphibians and reptiles in Russia and other European countries have been reported as *O. filiformis* (Ryzhikov et al., 1980; Murvanidze et al., 2008; Bjelić-Čabrilo et al., 2009; Popiołek et al., 2011; Okulewicz et al., 2014; Herczeg et al., 2016). Therefore, the host list of *O. filiformis* includes many amphibian and reptilian species from different genera and even fish, *Lota lota* (Linnaeus, 1758) and *Salmo trutta fario* Linnaeus, 1758 (Skrjabin et al., 1954; Ryzhikov et al., 1980; Baker, 1981; Anderson, 2000; Sanchis et al., 2000; Galli et al., 2001; Popiołek et al., 2004; Novokhatskaya, 2008).

Travassos (1937) suggested that specimens identified as *Oswaldocruzia filiformis* could in fact belong to several different species. Early descriptions of *Oswaldocruzia* spp. were based on the differences in the structure of spicules and the caudal bursa of males (Travassos, 1937; Skrjabin et al., 1954; Sharpilo, 1976; Ryzhikov et al., 1980).

Durette-Desset & Chabaud (1981) proposed the classification of the types of the caudal bursa of trichostrongylids. Durette-Desset (1985) and Ben Slimane et al. (1993) suggested taking into account the structure of the synlophe in identifying the species of *Oswaldocruzia*.

Based on differences in the structure of the synlophe and the type of the caudal bursa, many *Oswaldocruzia* species have been described and redescribed from different, mainly amphibian hosts, and dichotomous keys for *Oswaldocruzia* spp. from the Western Palearctic have been created in recent decades (Ben Slimane & Durette-Desset, 1993, 1997; Durette-Desset et al., 1993; Ben Slimane et al., 1993, 1995, 1996a; Svitin & Kuzmin, 2012; Svitin, 2016, 2017). These studies indicated that most *Oswaldocruzia* spp. of the Western Palearctic are oligo- and monohostal. *Oswaldocruzia filiformis* is considered a specific parasite of *Bufo bufo* Linnaeus, 1758 (Svitin, 2016). To note, new species were described based on morphological criteria only, without the use of molecular methods.

Previously we identified *Oswaldocruzia* nematodes from various amphibians and reptiles of the Middle Volga region as *Oswaldocruzia filiformis* (Kirillov, 2000; Chikhlyaev & Ruchin, 2014; Kirillov et al., 2015, 2019; Chikhlyaev et al., 2016, 2018, 2019; Kirillov & Kirillova, 2018).

The purpose of this study was to combine morphological and molecular phylogenetic data to solve the question of the species affiliation of the *Oswaldocruzia* nematodes parasitising different amphibian and reptilian hosts in several geographically distant localities of the European Russia.

Material and Methods

Oswaldocruzia nematodes were collected from the small intestine of amphibians and reptiles in six localities in the European part of Russia in 2018 and 2019 (Fig. 1).

The hosts of the nematodes collected in this study belonged to nine species of eight genera of amphibians and reptiles, namely: *Pelophylax ridibundus* (Pallas, 1771), *Rana arvalis* Nilsson, 1842, *Rana temporaria* Linnaeus, 1758 (Amphibia: Ranidae), *Bufo bufo* (Amphibia: Bufonidae), *Lacerta agilis* Linnaeus, 1758, *Zootoca vivipara* (Jacquin, 1787) (Reptilia: Lacertidae), *Anguis fragilis* Linnaeus, 1758 (Reptilia: Anguidae), *Natrix natrix* Linnaeus, 1758 (Reptilia: Colubridae), and *Vipera berus* (Linnaeus, 1758) (Reptilia: Viperidae) (Table 1).

Fig. 1. The map showing sampling localities. Designations of red circles, sampling sites of amphibians and reptiles: 1 – Zvenigorod Biological Station of Moscow University; 2 – Uzola River floodplain; 3 – Mordovia State Nature Reserve; 4 – National Park «Smolny»; 5 – National Park «Samarskaya Luka»; 6 – Ural River floodplain.

Table 1. Nematode specimens examined in this study, with museum specimen numbers and GenBank accession numbers according to the geographical origin. Specimens with accession numbers MT300256–MT300275 were collected and sequenced by the authors of this study

Nematode species, isolate no.	Locality	Co-ordinates	Host	GenBank acc. no.	Museum specimen no.	Source
Oswaldocruzia filiformis, 116	National Park «Samarskaya Luka», Samara region	53.176111° N, 49.436667° E	*Pelophylax ridibundus*	MT300271	Nem-Os-116	This study
Oswaldocruzia filiformis, 120	National Park «Samarskaya Luka», Samara region	53.176111° N, 49.436667° E	*Pelophylax ridibundus*	MT300269	Nem-Os-120	This study
Oswaldocruzia filiformis, 183	National Park «Samarskaya Luka», Samara region	53.176111° N, 49.436667° E	*Pelophylax ridibundus*	MT300272	Nem-Os-183	This study
Oswaldocruzia filiformis, 155	National Park «Smolny», Republic of Mordovia	55.835278° N, 45.378611° E	*Bufo bufo*	MT300259	Nem-Os-155	This study
Oswaldocruzia filiformis, 156	National Park «Smolny», Republic of Mordovia	55.76° N, 45.405833° E	*Rana arvalis*	MT300268	Nem-Os-156	This study
Oswaldocruzia filiformis, 159	Zvenigorod Biological Station of Moscow State University, Moscow region	55.700556° N, 36.722222° E	*Rana temporaria*	MT300258	Nem-Os-159	This study
Oswaldocruzia filiformis, 157	Uzola River floodplain, Nizhniy Novgorod region	56.603333° N, 43.585° E	*Rana arvalis*	MT300257	Nem-Os-157	This study
Oswaldocruzia filiformis, 158	Ural River floodplain, Orenburg region	51.218056° N, 58.556111° E	*Rana arvalis*	MT300256	Nem-Os-158	This study
Oswaldocruzia filiformis, 16	Mordovia State Nature Reserve, Republic of Mordovia	54.713611° N, 43.2275° E	*Lacerta agilis*	MT300266	Nem-Os-16	This study
Oswaldocruzia filiformis, 8	Mordovia State Nature Reserve, Republic of Mordovia	54.713611° N, 43.2275° E	*Lacerta agilis*	MT300262	Nem-Os-18	This study
Oswaldocruzia filiformis, 10	Mordovia State Nature Reserve, Republic of Mordovia	54.713611° N, 43.2275° E	*Zootoca vivipara*	MT300260	Nem-Os-10	This study
Oswaldocruzia filiformis, 16_1	Mordovia State Nature Reserve, Republic of Mordovia	54.713611° N, 43.2275° E	*Zootoca vivipara*	MT300273	Nem-Os-16_1	This study
Oswaldocruzia filiformis, 88_4	National Park «Smolny», Republic of Mordovia	54.746944° N, 45.262778° E	*Zootoca vivipara*	MT300267	Nem-Os-88_4	This study
Oswaldocruzia filiformis, 107_17	National Park «Smolny», Republic of Mordovia	54.732778° N, 45.271111° E	*Lacerta agilis*	MT300265	Nem-Os-107_17	This study
Oswaldocruzia filiformis, 95_5	National Park «Smolny», Republic of Mordovia	54.732778° N, 45.271111° E	*Lacerta agilis*	MT300270	Nem-Os-95_5	This study
Oswaldocruzia filiformis, 89_4	National Park «Smolny», Republic of Mordovia	54.746944° N, 45.262778° E	*Lacerta agilis*	MT300275	Nem-Os-89_4	This study
Oswaldocruzia filiformis, 190_1	National Park «Smolny», Republic of Mordovia	54.744722° N, 45.502222° E	*Anguis fragilis*	MT300264	Nem-Os-190_1	This study
Oswaldocruzia filiformis, 76_1	National Park «Smolny», Republic of Mordovia	54.744722° N, 45.502222° E	*Natrix natrix*	MT300261	Nem-Os-76_1	This study
Oswaldocruzia filiformis, 113	National Park «Smolny», Republic of Mordovia	54.746944° N, 45.262778° E	*Vipera berus*	MT300263	Nem-Os-113	This study
Oswaldocruzia filiformis, 123_4	National Park «Smolny», Republic of Mordovia	54.746944° N, 45.262778° E	*Vipera berus*	MT300274	Nem-Os-123_4	This study
Chabertia ovina	Jingyang County, China*	34.529167° N, 108.84° E	*Capra aegagrus hircus*	KF279335	–	Zhao et al. (2013)
Ancylostoma ceylanicum, m30	Guangzhou, Guangdong, China*	23.130278° N, 113.259167° E	*Felis catus* (faeces)	KP072073	–	Hu et al. (2016)
Ancylostoma ceylanicum, cm107	Guangzhou, Guangdong, China*	23.130278° N, 113.259167° E	*Felis catus* (faeces)	KP072079	–	Hu et al. (2016)
Rhabditis sp., B VSS-2017	India*	22.351111° N, 78.667778° E	No data	MF742401	–	Direct submission

Note: * – mean geographical co-ordinates.

Statement of the welfare of animals

Our study was conducted in compliance with ethical standards of humane handling of animals. The animals were collected and processed according to the recommended practices described in Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The amphibians and reptiles collected in this study belong to widespread and abundant species. Many of them were dead by the time of collection (dead from natural causes, road-killed or killed by rural residents or their pets). Some specimens were provided by the researchers from the IEVB of the RAS, Mordovia State Nature Reserve, National Park «Smolny» and National Park «Samarskaya Luka».

Twenty nematode specimens were recovered from amphibians or reptiles and preserved in 96% ethanol for further molecular phylogenetic analysis. For the morphological examination the nematodes were killed by heating in water and cleared in lactic acid. In total, we studied and measured 189 specimens of *Oswaldocruzia filiformis*, of which 99 were females and 90 were males. The data on the geographic origin and the final hosts of the studied nematodes are provided in Fig. 1 and Table 1.

Drawings of nematodes were made using an MBI-9 light microscope with the Levenhuk M500 BASE Digital Camera and drawing tube RA-7. All the measurements are given in mm. Transverse and apical sections of nematodes were made manually with a razor blade. The synlophe was studied according to Durette-Desset (1985). The nomenclature of the caudal bursa followed Durette-Desset & Chabaud (1981). The number of dissected vertebrates, prevalence (P, %), mean abundance of helminths (A, specimens) and range (R) are given to estimate the infection of amphibians and reptiles with the parasites. If the geographical co-ordinates of the study sites are not indicated in the papers, we characterised them by their mean geographical coordinates obtained using the Geocode Finder (https://www.mapdevelopers.com/geocode_tool.php). All *Oswaldocruzia filiformis* vouchers were deposited in the parasitological collection of Saint Petersburg State University (Russia). Isolate numbers are given in Table 1.

DNA extraction, amplification and sequencing, phylogenetic analysis

The JB3/JB4.5 primer pair (Bowles et al., 1992) was successfully used for barcoding and partial phylogenetic reconstruction in species of *Ancylostoma* Dubini, 1843 (Hu et al., 2016) and in species identification and phylogenetic reconstruction of nematode parasites of *Lissotriton vulgaris* Linnaeus, 1758 (Sinsch et al., 2019). In order to obtain partial CoxI mtDNA sequences, specimens of ethanol-fixed *Oswaldocruzia filiformis* were dried at 37°C in dry block heater for 3 h. Then the specimens were moved to clear 500 µl tubes with a mixture of 49 µl 0.1% Chelex-100 and 1 µl Proteinase K (concentration 10 mg/mL) and incubated for 3.5 h at 55°C and 25 min. at 95°C to stop proteinase activity. After that, the water solution of the total DNA was moved to sterile 500 µl tube and frozen at -80°C.

The amplification primer pair was JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and JB4,5 (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′) (Bowles et al., 1992). The PCR was performed in 25 µl each in BioRad C1000 thermal cycler. The following parameters were used: initial denaturation (3 min. at 95°C) followed by 35 cycles of 20 s. at 95°C, 20 s. at 53°C and 40 s. at 72°C, followed by 5 min. at 72°C for final extension. Amplicons were directly sequenced using ABI-PRISM 3500xl with the same primers.

The newly obtained sequences were aligned against the full mitochondrial genome of *Trichostrongylus vitrinus* (GenBank no. NC_013807.1) using Muscle algorithm as implemented at CIPRES Portal (Miller et al., 2010). According to the nucleotide numbering of *T. vitrinus* mitochondrial genome, the newly obtained sequences were located between positions 720-1162. Other *Oswaldocruzia* sequences available in GenBank were located between nucleotides 49-741 (the numbering is given according to the same mitogenome no. NC_013807.1). Based on the results of this preliminary alignment, we excluded the sequences of the genus *Oswaldocruzia* obtained by other authors from further phylogenetic analysis.

The sequences were mounted in general alignment with other nematode species (Table 1). The sequences were automatically aligned using Muscle algorithm (Edgar, 2004) as implemented in SeaView 4.0 (Gouy et al., 2010); the alignment was then trimmed manually. The phylogenetic analysis was performed using the maximum likelihood method at Cipres portal (Miller et al., 2010) with GTR + G + I model and a non-parametric bootstrap with 1000 pseudoreplicates. Bayesian analysis was performed with the help of MrBayes on XSEDE 3.2.7a, the GTR model with gamma correction for intersite rate variation (eight categories) and the covarion model were used. Trees were run as two separate chains (default heating parameters) for 15 000 000 generations, by which time they had ceased converging (the final average standard deviation of the split frequencies was less than 0.01). The quality of the chains was estimated using built-in MrBayes tools and, additionally, using Tracer 1.6 package (Rambaut et al., 2018); based on the estimates by Tracer, the first 6000 generations were discarded for burn-in.

Results

The nematode *Oswaldocruzia filiformis* s.l. was found in the samples taken from all nine amphibian and reptilian species examined in our study. The infection indices are shown in Table 2.

Among amphibians, the highest rates of infection with nematodes were registered in *Rana arvalis*. The infection of *Bufo bufo* and *Rana temporaria* was comparatively lower. The highest rates of nematode infection in reptiles were recorded in *Lacerta agilis*, while the lowest were recorded in snakes (Table 2).

Table 2. Indices of infection of amphibians and reptiles with *Oswaldocruzia filiformis* s.l.

Host	Number of host infected/examined	Prevalence, %	Mean abundance	Range	Locality
Pelophylax ridibundus	27/56	48.21	2.57	1–21	National Park «Samarskaya Luka»
Rana arvalis	8/16	50.00	2.13	1–9	National Park «Smolny»
Rana arvalis	2/4	50.00	6.00	2–10	Uzola River floodplain
Rana arvalis	5/6	83.33	3.60	2–7	Ural River floodplain
Rana temporaria	14/21	66.67	2.65	1–12	Zvenigorod Biological Station of Moscow State University
Bufo bufo	11/16	68.75	3.19	1–12	National Park «Smolny»
Lacerta agilis	7/21	33.33	2.00	1–12	Mordovia State Nature Reserve
Lacerta agilis	31/100	31.00	0.96	1–11	National Park «Smolny»
Zootoca vivipara	5/16	31.25	1.63	1–12	Mordovia State Nature Reserve
Zootoca vivipara	1/35	2.86	0.07	2	National Park «Smolny»
Anguis fragilis	6/18	33.33	1.11	1–9	National Park «Smolny»
Natrix natrix	3/33	9.11	0.18	1–3	National Park «Smolny»
Vipera berus	3/18	16.67	0.50	1–6	National Park «Smolny»

Description of nematodes

General morphology

Body is thin, elongated, with maximum width at mid-length. Cephalic vesicle presents on cuticle of anterior end. Cephalic vesicle is variable in shape: whole (undivided) or consisting of two parts, a wider anterior part and a narrow posterior part, the latter smooth or with transverse folds (Fig. 2C, Fig. 3A). Vesicle shape is variable even in nematodes from one host individual. Cuticle forms uninterrupted longitudinal crests beginning behind cephalic vesicle and running along entire body. Crests are invisible on ventral side of body on transverse sections of anterior part of oesophagus region in some nematode individuals.

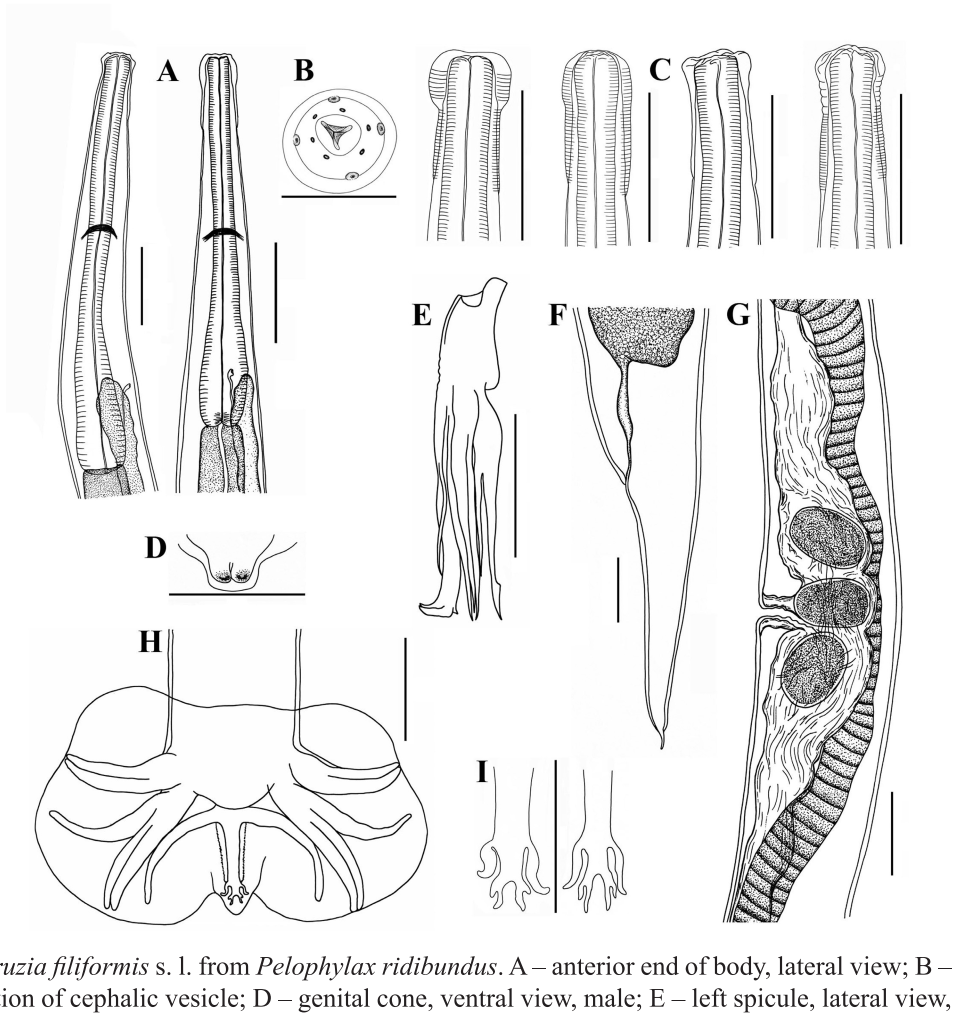

Fig. 2. *Oswaldocruzia filiformis* s. l. from *Pelophylax ridibundus*. A – anterior end of body, lateral view; B – head apical view, female; C – variation of cephalic vesicle; D – genital cone, ventral view, male; E – left spicule, lateral view, male; F –tail, left lateral view, female; G – vulvar and ovejector regions of female, left lateral view; H – caudal bursa of male, ventral view; I – variation of dorsal ray of bursa. Scale bars: A, C, E–H = 0.1 mm; B, D, I = 0.05 mm.

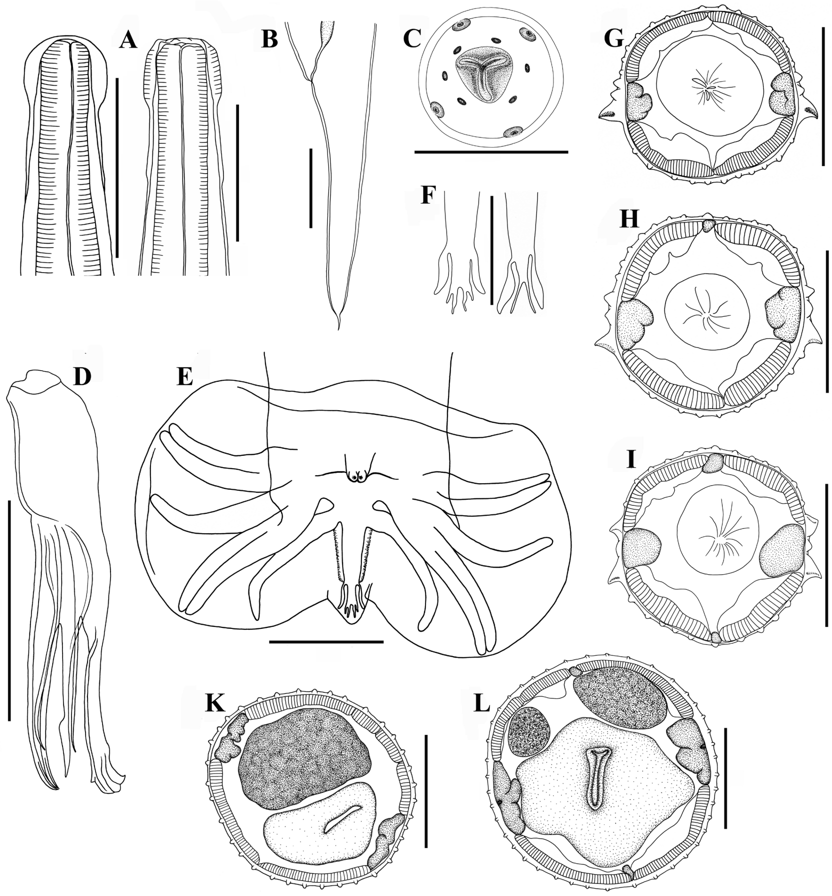

Fig. 3. *Oswaldocruzia filiformis* s.l. from *Bufo bufo*. A – variation of cephalic vesicle; B – female, tail, left lateral view; C – head apical view, female; D – right spicule, lateral view, male; E – caudal bursa of male, ventral view; F – variation of dorsal ray of bursa; G – transverse section at mid-oesophagus level, male; H – transverse section at mid-oesophagus level, male; I –mid-oesophagus level, female; K – transverse section at mid-body, 36 crests, male; L – transverse section at mid-body, 42 crests, female. Scale bars: A, B, D, E, K, L = 0.1 mm; C, F–I = 0.05 mm.

Anterior end is rounded. Oral opening is triangular, surrounded by four large cephalic papillae and six not always distinguishable externo-labial papillae (Fig. 2B, Fig. 3C). Oesophagus is thin, club-shaped, cylindrical in anterior part and widening posteriorly. Posterior end of oesophagus is rounded, forming posterior bulb (Fig. 2A). Position of excretory pore varies within posterior third of oesophagus. Nerve ring surrounding oesophagus in the middle part is somewhat closer to its anterior third (Fig. 2A).

Synlophe is symmetrical. Triangular cervical alae are clearly visible on transverse sections at mid-oesophagus level (Fig. 3G,H,I, Fig. 4B, Fig. 5A,B). Lateral alae include three crests, with ventral crest always well-developed; two much smaller (dorsal and central) crests are above large ventral crest. Lateral alae begin approximately at level of second third of oesophagus. Lateral alae are with varying degrees of development in different nematode individuals. Some nematodes from *Bufo bufo* and *Natrix natrix* were found to have small narrow cervical alae formed by three somewhat enlarged crests. On transverse sections at mid-length of oesophagus, the lateral alae include dorsal and ventral crests and smaller central crest between them (Fig. 3G,H,I, Fig. 5A,B). At level of anterior part of intestine, lateral alae become simple crests. Number of crests at body mid-length varies depending on sex, age and host of nematodes (Fig. 3K, L, Fig. 4D,F,G,H, Table 3, Table 4, Table 5, Table 6).

Male

Body ends with wide caudal bursa. Caudal bursa is symmetrical, three-lobed, belonging to type II according to Durette-Desset & Chabaud (1981). Rays 2 and 3 are joined along their entire length: ray 4 is joined to ray 5 in its proximal part; rays 5 and 6 are joined along their entire length; rays 6 and 8 are joined at mid-length; rays 9 and 10 form a wide dorsal ray. Rays 9 are always with S-shaped bend. Ray 10 is always with extra branch of variable size (Fig. 2H, Fig. 3E, Fig. 5D). Only one male from *Bufo bufo* was with no extra branches on rays 10 (Fig. 2F), and one male from *Lacerta agilis* was with third extra branch on inner side of one ray 10.

Genital cone is well developed, with two papillae (Fig. 2D). Gubernaculum is absent. Spicules are approximately equal, long, surrounded by thin membrane, with three branches: blade is divided into two branches at the end; distal parts of each branch split into two thin branches, which are not always clearly visible; fork is divided into two branches approximately at mid-length. Shoe is with thin branch at mid-length and bent at distal end (Fig. 2E, Fig. 3D, Fig. 5C). Shoe shape slightly varies in different individuals. Morphometric measurements are presented in Table 3 and Table 5.

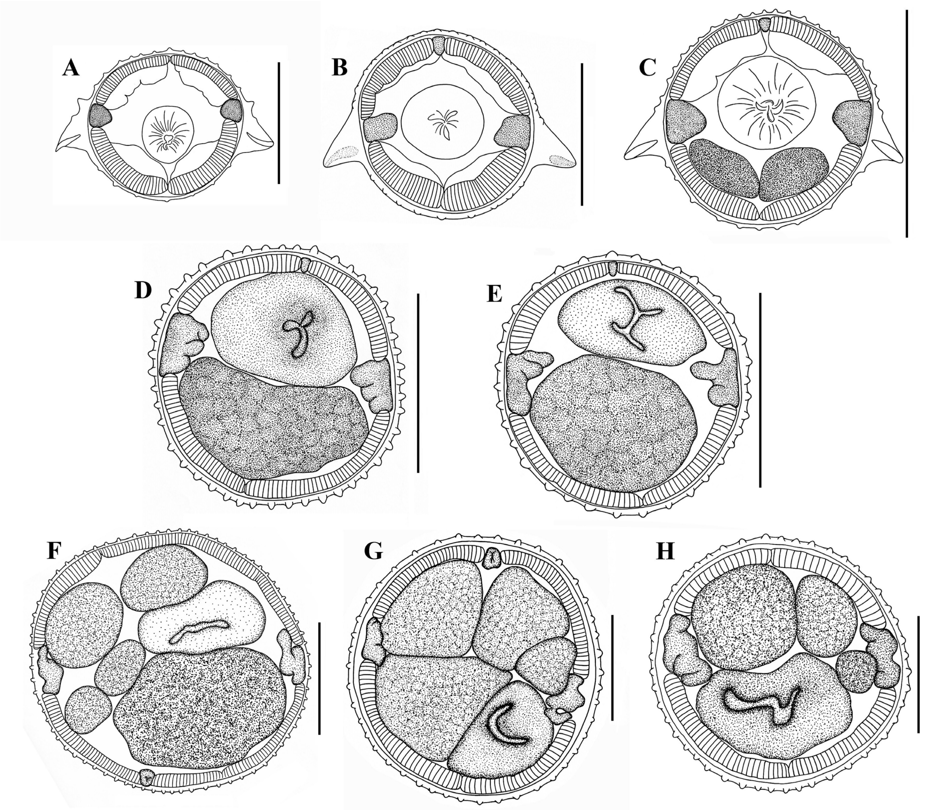

Fig. 4. *Oswaldocruzia filiformis* s. l. from *Pelophylax ridibundus*. Transverse sections. A – anterior part of oesophagus, female; B – mid-oesophagus level, female; C – posterior part of oesophagus, female; D – mid-body level, 46 crests, male; E –mid-body level, 35 crests, male; F – mid-body level, 77 crests, female; G – mid-body level, 43 crests, female; H – mid-body level, 47 crests, female. Scale bars: A, B = 0.05 mm; C–H = 0.1 mm.

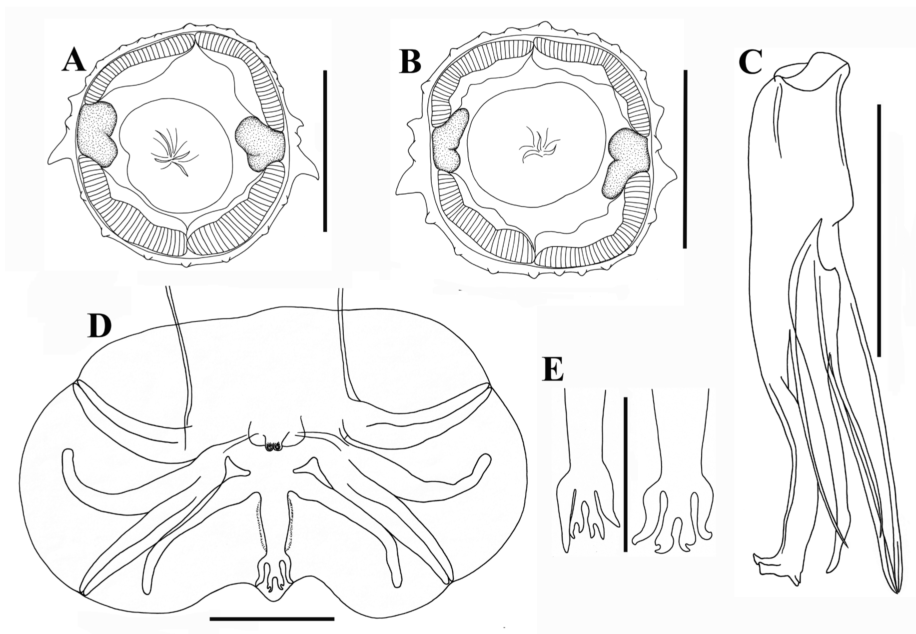

Fig. 5. *Oswaldocruzia filiformis* s. l. from *Natrix natrix*. A – transverse section at mid-oesophagus level, male; B – transverse section at mid-oesophagus level, female; C – left spicule, lateral view, male; D – caudal bursa of male, ventral view; E – variation of dorsal ray of bursa. Scale bars: A, B, E = 0.05 mm; C, D = 0.1 mm.

Table 3. Morphometry of *Oswaldocruzia filiformis* s.l. males from amphibians

Characters	*Pelophylax ridibundus*[1] (20 specimens)		*Rana arvalis*[2] (12 specimens)		*Rana temporaria*[3] (6 specimens)		*Bufo bufo*[2] (10 specimens)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	8.98	6.75–12.50	10.28	8.00–12.85	8.72	7.60–9.85	10.89	9.40–13.25
Width of body	0.161	0.126–0.205	0.178	0.165–0.236	0.154	0.141–0.165	0.213	0.196–0.230
Length of cephalic vesicle	0.080	0.071–0.087	0.086	0.079–0.091	0.091	0.087–0.094	0.096	0.087–0.110
Width of cephalic vesicle	0.038	0.035–0.041	0.039	0.037–0.040	0.039	0.037–0.041	0.046	0..039–0.071
Length of oesophagus	0.436	0.358–0.559	0.508	0.457–0.571	0.479	0.464–0.496	0.527	0.496–0.567
Length of oesophagus in % of body length	4.9	4.2–5.5	5.0	4.4–5.7	5.6	4.7–6.2	4.9	4.3–5.8
Width of oesophageal bulb	0.060	0.047–0.071	0.063	0.055–0.071	0.052	0.045–0.059	0.066	0.059–0.075
Distance from anterior end of oesophagus to nerve ring	0.193	0.160–0.236	0.215	0.207–0.224	0.189	0.177–0.205	0.216	0.205–0.230
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	44.3	39.9–47.7	42.4	39.3–45.7	42.6	40.8–45.2	41.0	38.9–43.1
Distance from anterior end of oesophagus to excretory pore	0.364	0.313–0.457	0.425	0.370–0.492	0.408	0.390–0.425	0.442	0.406–0.465
Distance from anterior end of oesophagus to excretory pore in % of oesophagus length	83.6	75.0–89.4	83.8	77.4–89.3	85.2	83.2–86.9	83.7	79.9–88.9
Length of tail	0.134	0.118–0.156	0.125	0.118–0.142	0.121	0.110–0.130	0.130	0.118–0.150
Length of spicules	0.223	0.195–0.248	0.223	0.216–0.234	0.212	0.201–0.220	0.213	0.189–0.230
Number of crests at mid-body level	35–47		40–45		34–39		36–40	

Note: 1 – National Park «Samarskaya Luka» (Samara region), 2 – National Park «Smolny» (Republic of Mordovia); 3 – Zvenigorod Biological Station (Moscow region).

Table 4. Morphometry of females of *Oswaldocruzia filiformis* s.l. from amphibians

Characters	*Pelophylax ridibundus*[1] (20 specimens)		*Rana arvalis*[2] (10 specimens)		*Rana temporaria*[3] (12 specimens)		*Bufo bufo*[2] (10 specimens)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	17.26	10.95–24.50	16.63	13.25–21.50	15.39	12.75–19.25	19.63	12.85–26.70
Width of body	0.228	0.189–0.256	0.219	0.201–0.256	0.229	0.213–0.244	0.254	0.217–0.315
Length of cephalic vesicle	0.094	0.087–0.106	0.091	0.083–0.098	0.090	0.081–0.106	0.109	0.102–0.116
Width of cephalic vesicle	0.046	0.039–0.055	0.044	0.039–0.053	0.042	0.039–0.047	0.066	0.049–0.067
Length of oesophagus	0.517	0.465–0.575	0.536	0.492–0.590	0.516	0.484–0.551	0.586	0.543–0.630
Length of oesophagus in % of body length	3.0	2.4–4.2	3.3	2.7–4.0	3.4	2.9–4.0	3.1	2.4–4.2
Width of oesophageal bulb	0.068	0.055–0.075	0.074	0.059–0.094	0.063	0.055–0.075	0.081	0.073–0.091
Distance from anterior end of oesophagus to nerve ring	0.223	0.197–0.260	0.220	0.197–0.236	0.218	0.209–0.224	0.254	0.244–0.268
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	43.1	39.8–45.7	41.1	38.9–43.2	42.1	40.0–45.5	43.4	40.8–46.3
Distance from anterior end of oesophagus to excretory pore	0.423	0.370–0.475	0.450	0.413–0.480	0.432	0.402–0.453	0.503	0.465–0.571
Distance from anterior end of oesophagus to excretory pore in % of oesophagus length	81.6	77.7–85.4	84.0	79.6–85.9	78.4	73.0–88.5	84.5	78.7–90.6
Distance from anterior end to vulva	10.76	6.92–15.0	10.31	7.70–12.75	9.81	7.25–12.50	12.98	7.2–18.75
Distance to vulva in % of body length	62.6	58.7–67.2	62.1	56.9–68.6	63.6	56.9–67.0	65.7	56.0–70.2
Length of eggs	0.085	0.071–0.096	0.082	0.078–0.089	0.083	0.075–0.091	0.087	0.077–0.099
Width of eggs	0.048	0.041–0.053	0.046	0.043–0.051	0.042	0.039–0.047	0.046	0.039–0.051
Length of tail	0.312	0.236–0.433	0.266	0.232–0.303	0.264	0.224–0.311	0.306	0.276–0.354
Length of tail in % of body length	1.9	1.4–2.4	1.6	1.4–1.9	1.8	1.6–1.9	1.6	1.2–2.4
Number of crests at mid-body level	39–77		45–73		40–45		40–51	

Note: 1 – National Park «Samarskaya Luka» (Samara region), 2 – National Park «Smolny» (Republic of Mordovia); 3 – Zvenigorod Biological Station (Moscow region).

Table 5. Morphometry of *Oswaldocruzia filiformis* s.l. males from reptiles

Characters	*Anguis fragilis*[1] (11 specimens)		*Lacerta agilis*[1] (20 specimens)		*Zootoca vivipara*[2] (10 specimens)		*Natrix natrix*[1] (3 specimens)		*Vipera berus*[1] (3 specimens)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	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
Width of body	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	0.145–1.173
Length of cephalic vesicle	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
Width of cephalic vesicle	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
Length of oesophagus	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
Length of oesophagus in % of body length	4.7	4.4–4.9	5.1	4.5–5.5	5.2	5.0–5.5	5.3	5.2–5.4	5.6	5.3–6.1
Width of oesophageal bulb	0.057	0.047–0.069	0.049	0.047–0.051	0.050	0.047–0.054	0.051	0.047–0.055	0.053	0.051–0.055
Distance from anterior end of oesophagus to nerve ring	0.179	0.169–0.199	0.180	0.159–0.197	0.174	0.157–0.196	0.190	0.185–0.197	0.184	0.173–0.194
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	45.4	42.5–47.7	46.7	44.1–48.6	44.1	41.1–47.9	40.8	40.2–41.3	40.6	39.6–41.1
Distance from anterior end of oesophagus to excretory pore	0.290	0.256–0.314	0.289	0.262–0.323	0.293	0.269–0.323	0.394	0.386–0.403	0.357	0.340–0.378
Distance from anterior end of oesophagus to excretory pore in % of oesophagus length	73.3	70.9–77.3	75.1	70.5–82.4	75.2	72.7–79.0	84.5	82.4–87.5	78.8	77.8–80.1
Length of tail	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
Length of spicules	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
Number of crests at mid-body level	36–39		37–45		39–41		34–36		40–45	

Note: 1 – National Park «Smolny» (Republic of Mordovia); 2 – Mordovia State Nature Reserve (Republic of Mordovia).

Table 6. Morphometry of *Oswaldocruzia filiformis* s.l. females from reptiles

Characters	*Anguis fragilis*[1] (5 specimens)		*Lacerta agilis*[1] (20 specimens)		*Zootoca vivipara*[2] (10 specimens)		*Natrix natrix*[1] (2 specimens)		*Vipera berus*[1] (5 specimens)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	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
Width of body at mid-length	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
Length of cephalic vesicle	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
Width of cephalic vesicle	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
Length of oesophagus	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
Length of oesophagus in % of body length	3.3	3.2–3.4	3.5	3.1–3.9	3.7	3.5–3.8	4.8	4.7–4.9	3.9	3.7–4.2
Width of oesophageal bulb	0.065	0.059–0.071	0.064	0.053–0.079	0.063	0.055–0.076	0.061	0.059–0.063	0.062	0.055–0.067
Distance from anterior end of oesophagus to nerve ring	0.203	0.177–0.220	200.3	0.190–0.217	0.193	0.181–0.213	0.209	0.205–0.213	0.201	0.181–0.217
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	42.9	40.3–45.0	43.8	41.9–46.8	44.9	43.1–46.4	41.9	41.3–42.5	45.2	43.4–46.4
Distance from anterior end of oesophagus to excretory pore	0.415	0.396–0.440	0.357	0.326–0.398	0.315	0.295–0.339	0.409	0.405–0.413	0.371	0.315–0.413
Distance to excretory pore in % of oesophagus length	85.0	81.2–87.3	78.1	71.5–82.0	73.3	70.6–75.6	81.9	80.4–83.3	83.2	80.8–85.6
Distance from anterior end to vulva	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
Distance from anterior end to vulva in % of body length	66.7	63.5–68.5	63.1	58.3–67.5	63.1	58.5–66.7	64.0	62.2–65.7	65.8	63.9–67.6
Length of eggs	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
Width of eggs	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
Length of tail	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
Length of tail in % of body length	1.7	1.6–1.8	1.9	1.7–2.1	2.0	1.9–2.2	1.9	1.9–1.9	2.1	1.9–2.4
Number of crests at mid-body level	58–61		55–61		48–55		40–45		57–60	

Note: 1 – National Park «Smolny» (Republic of Mordovia); 2 – Mordovia State Nature Reserve (Republic of Mordovia).

Female

Body is larger than in males, always tapering to elongated tail with needle-shaped tip (Fig. 2F, Fig. 3B). Vulva is wide, opening with a transverse slot postequatorially (behind mid-length of female body). Muscular vagina passes into paired ovejector with powerful sphincters (Fig. 2G). Anterior ovary forms numerous loops and bends in anterior part of body, reaching oesophagus level and returning back. Posterior ovary extends to the end of the body, bending strongly and extending forward with slight twisting, going beyond vulva level. Uterus of mature females is filled with eggs. All eggs were found in uterus, ovejector and vulva at morula stage. Morphometric measurements are given in Table 4 and Table 6.

Molecular phylogenetic analysis

All *Oswaldocruzia filiformis* specimens were successfully amplified with JB3 and JB4, five primers without non-specific PCR-products. The sequence lengths were of about 442 bp after contigs assembly. Alignment of the newly obtained sequences against the full mitochondrial genome of *T. vitrinus* (GeneBank № NC_013807.1) confirmed that *Oswaldocruzia filiformis* sequences belonged to CoxI mtDNA region.

Maximum Likelihood and Bayesian inference trees were identical in terms of recognised relationships among species of *Ancylostoma* and *Chabertia*. Bootstraps of clades in ML analysis among *Oswaldocruzia filiformis* subclades were extremely low, and their topology disagreed between ML and BI analysis. Because of this, the relationships of representatives of *O. filiformis* isolates are described only after BI analysis (Fig. 6).

Seven isolates (numbers 88-4, 16, 107-17, 76-1, 190-1, 113, 155) were combined in a polytomic clade. They were collected from a broad range of vertebrate hosts, such as *Zootoca vivipara*, *Lacerta agilis*, *Natrix natrix*, *Anguis fragilis*, *Vipera berus* and *Bufo bufo*. Most of the isolates were collected in National Park «Smolny» (Republic of Mordovia), but *Oswaldocruzia filiformis* isolate 16 was collected in the Mordovia State Nature Reserve (Republic of Mordovia). *Oswaldocruzia filiformis* isolate 8 (ex *Lacerta agilis*, Republic of Mordovia) and isolate 10 (ex *Zootoca vivipara*, Republic of Mordovia) form a polytomic clade with a middle-supported deep subclade of two *O. filiformis* isolates 159 (ex *Rana temporaria*, Moscow region) and 157 (ex *Rana arvalis*, Nizhniy Novgorod region). Isolates 95-5 (ex *Lacerta agilis*), 120 (ex *Pelophylax ridibundus*), 156 (ex *Rana arvalis*) were collected from geographically distant sites in the Republic of Mordovia and Samara region. The most basal polytomic clades were represented by six isolates: no. 89-4 (ex *Lacerta agilis*, National Park «Smolny», Republic of Mordovia), no. 116 (ex *Pelophylax ridibundus*, National Park «Samarskaya Luka», Samara region), no. 16-1 (ex *Zootoca vivipara*, Mordovia State Nature Reserve, Republic of Mordovia), no. 123-4 (ex *Vipera berus*, National Park «Smolny», Republic of Mordovia), no. 183 (ex *Pelophylax ridibundus*, National Park «Samarskaya Luka», Samara region), no. 158 (ex *Rana arvalis*, Ural River floodplain, Orenburg region) (Fig. 6).

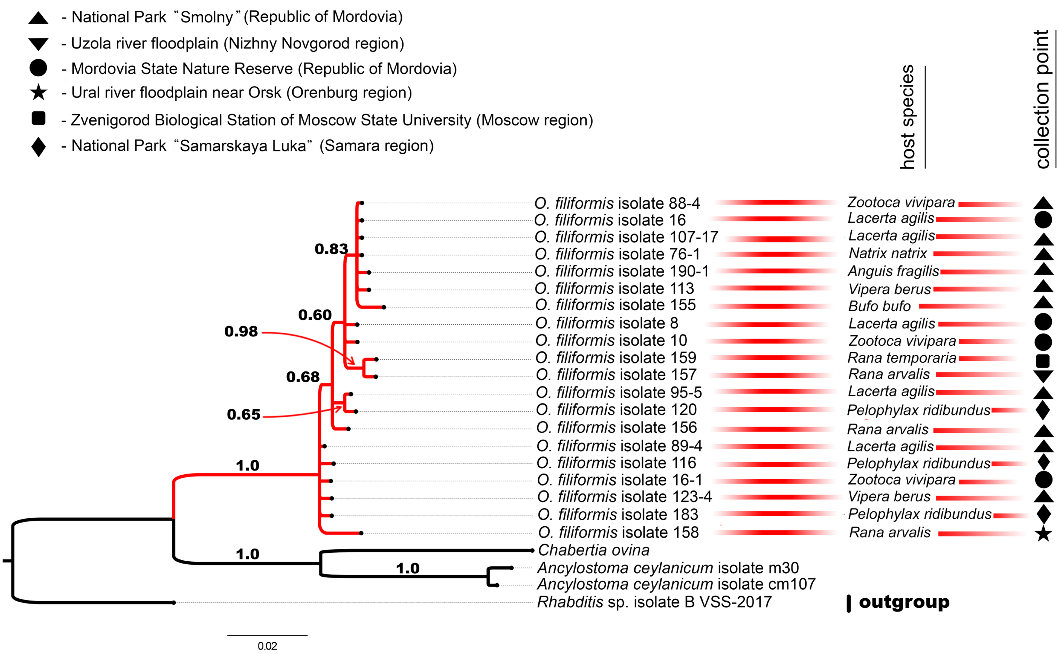

Fig. 6. Results of molecular phylogenetic analysis of *Oswaldocruzia* nematodes based on CoxI mtDNA gene sequences. Only posterior probabilities are shown.

All *Oswaldocruzia filiformis* isolates form a sister clade to *Chabertia ovina* with full posterior probability. Two isolates of *Ancylostoma ceylanicum* are closely related with full Bayesian support. The branch length of *A. ceylanicum* isolates is comparable with that of *O. filiformis* isolates (Fig. 6).

Discussion

In this study, we described the morphology of several isolates of the nematodes from the genus *Oswaldocruzia* and obtained new molecular phylogenetic data on them. Here we present an analysis of morphological characters traditionally used in the taxonomy of this genus.

The nematodes that we found belong to the Palearctic group of *Oswaldocruzia* species. They are characterized by «idiomorphic» spicules with three main branches («blade», «fork» and «shoe»), with the spicular «fork» divided above the level of its distal third (Ben Sliman et al., 1996a). Based on type II of the caudal bursa (Durette-Desset & Chabaud, 1981), the nematodes examined by us could be assigned to any of the four species: *Oswaldocruzia filiformis*, *O bialata* Molin, 1860, *O. duboisi* Ben Slimane, Durette-Desset & Chabaud, 1993 or *O. skrjabini* Travassos, 1937.

Oswaldocruzia filiformis was re-described by Ben Slimane et al. (1993) on the basis of the structure of the synlophe, spicules, and the caudal bursa of males. On the basis of the structure of spicules and the caudal bursa of males, Travassos (1937) described *Oswaldocruzia skrjabini* from *Zootoca vivipara*. The description of this nematode is also contained in Svitin (2015, 2016). *Oswaldocruzia duboisi* was described by Ben Slimane et al. (1993), taking into account the structure of the synlophe and spicules. The description of this parasite is also contained in Svitin & Kuzmin (2012), Svitin (2016). The re-description of *Oswaldocruzia bialata*, taking into account the structure of the synlophe, is contained in Durette-Desset et al. (1993). The description of this nematode is also contained in Svitin (2016).

The body sizes of *Oswaldocruzia* nematodes taken from various species of amphibians and reptiles in our study were different (Table 3, Table 4, Table 5, Table 6). The mean body size of the *Oswaldocruzia* males collected by us was bigger than of males of *Oswaldocruzia filiformis*, *O. bialata*, *O. duboisi* and *O. skrjabini* (Ben Slimane et al., 1993; Durette-Desset et al., 1993; Svitin & Kuzmin, 2012; Svitin, 2015, 2016). The males collected from *Lacerta agilis* and *Zootoca vivipara* were an exception. Their mean size was about the same as that of *Oswaldocruzia filiformis* in Ben Slimane et al. (1993) and Svitin (2016) (Table 3, Table 5, Table 7).

In our study, the mean body size of the *Oswaldocruzia* females was also bigger than of females of the mentioned above four species in comparison. *Oswaldocruzia* females from lizards and snakes were an exception. Their mean size was slightly smaller than of *Oswaldocruzia filiformis* (Ben Slimane et al., 1993; Svitin, 2016) (Table 4, Table 6, Table 8). Intra- and interpopulational variability of nematodes from vertebrates and, in particular, amphibians was also noted by Tarasovskaya (2009, 2011), Tarasovskaya & Pashkevich (2011), Kirillova et al. (2012), Kirillov & Kirillova (2015), who showed that the body size of nematodes varied under the influence of such factors as sex, age, phenotype and host species, number of parasites in the host, seasonal changes and others.

Table 7. Morphometry of *Oswaldocruzia* spp. males (literature data)

Characters	*Oswaldocruzia filiformis* (Ben Slimane et al., 1993)		*Oswaldocruzia duboisi* (Svitin & Kuzmin, 2012)		*Oswaldocruzia bialata* (Svitin, 2016)		*Oswaldocruzia skrjabini* (Svitin, 2015)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	6.84	4.00–11.05	5.63	3.54–7.28	7.29	4.93–10.07	6.14	5.49–6.71
Width of body	0.124	0.050–0.200	0.168	0.090–0.530	0.134	0.080–0.180	0.110	0.100–0.120
Length of cephalic vesicle	0.090	0.050–0.120	0.064	0.053–0.083	0.080	0.060–0.093	0.073	0.068–0.080
Width of cephalic vesicle	0.050	0.040–0.055	0.038	0.028–0.048	0.038	0.035–0.043	0.040	0..035–0.050
Length of oesophagus	0.460	0.255–0.565	0.396	0.283–0.583	0.429	0.358–0.495	0.377	0.345–0.418
Length of oesophagus in % of body length	–	–	7.1	4.8–9.9	6.0	4.8–6.4	6.12	5.55–6.69
Width of oesophageal bulb	–	–	0.050	0.033–0.058	0.059	0.043–0.073	0.044	0.038–0.053
Distance from anterior end of oesophagus to nerve ring	0.210	0.140–0.280	0.178	0.115–0.268	0.203	0.180–0.230	0.202	0.183–0.238
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	–	–	45.1	35.4–57.8	47.4	44.2–51.9	53.4	49.7–56.9
Distance from anterior end of oesophagus to excretory pore	0.360	0.190–0.550	0.260	0.198–0.348	0.330	0.265–0.388	0.275	0.270–0.280
Distance from anterior end of oesophagus to excretory pore in % of oesophagus length	–	–	66.1	49.1–87.4	77.0	67.0–88.1	73.5	65.9–81.2
Length of tail	–	–	0.118	0.98–0.168	0.116	0.075–0.158	0.117	0.108–0.128
Length of spicules	0.210	0.155–0.240	0.176	0.155–0.250	0.192	0.173–0.210	0.173	0.170–0.175
Number of crests at mid-body level	21–58		71		50		–	

Table 8. Morphometry of females of *Oswaldocruzia* spp. (literature data)

Characters	*Oswaldocruzia filiformis* (Ben Slimane et al., 1993)		*Oswaldocruzia duboisi* (Svitin & Kuzmin, 2012)		*Oswaldocruzia bialata* (Svitin, 2016)		*Oswaldocruzia skrjabini* (Svitin, 2015)	
	mean	min–max	mean	min–max	mean	min–max	mean	min–max
Length of body	11.51	7.55-18.30	8.89	2.29–13.62	11.80	8.21–14.92	8.84	7.24–10.13
Width of body	0.180	0.100-0.280	0.173	0.100–0.250	0.185	0.110–0.230	0.129	0.100–0.180
Length of cephalic vesicle	0.096	0.065-0.120	0.071	0.050–0.095	0.078	0.065–0.098	0.077	0.055–0.108
Width of cephalic vesicle	0.055	0.045-0.060	0.044	0.038–0.050	0.041	0.033–0.053	0.039	0.035–0.043
Length of oesophagus	0.540	0.370–0.700	0.437	0.375–0.508	0.465	0.403–0.543	0.305	0.286–0.316
Length of oesophagus in % of body length	–	–	5.2	3.5–17.7	4.0	3.2–5.8	3.5	3.0–4.3
Width of oesophageal bulb	–	–	–	–	0.069	0.050–0.083	0.047	0.040–0.055
Distance from anterior end of oesophagus to nerve ring	0.230	0.100–0.340	0.189	0.160–0.245	0.206	0.183–0.230	0.180	0.145–0.208
Distance from anterior end of oesophagus to nerve ring in % of oesophagus length	–	–	43.3	37.4–51.2	44.4	36.9–48.2	59.1	46.2–68.7
Distance from anterior end of oesophagus to excretory pore	0.440	0.200–0.580	0.270	0.190–0.433	0.357	0.285–0.418	0.278	0.243–0.328
Distance from anterior end of oesophagus to excretory pore in % of oesophagus length	–	–	61.5	46.6–86.1	77.1	64.9–86.4	91.3	77.2–107.0
Distance from anterior end to vulva	–	–	5.62	2.76–8.40	7.54	5.42–9.98	5.27	4.07–6.25
Distance to vulva in % of body length	–	–	62.3	44.0–68.6	63.9	61.2–66.9	59.5	50.3–63.7
Length of eggs	0.090	0.070–0.100	–	0.157–0.162	–	0.078–0.093	–	0.075–0.095
Width of eggs	0.054	0.040–0.065	–	0.095–0.117	–	0.045–0.063	–	0.053–0.055
Length of tail	0.265	0.200–0.580	0.206	0.148–0.273	0.200	0.145–0.283	0.195	0.165–0.243
Length of tail in % of body length	–	–	2.4	1.7–7.4	1.7	1.3–2.3	2.2	1.8–2.7
Number of crests at mid-body level	34–75		71		50		–	

According to the structure of spicules, the nematodes found in amphibians and reptiles could only be assigned to *Oswaldocruzia filiformis*. In our nematodes, the spicular «blade» is divided at the end into three branches, while the «fork» and the «shoe» did not have extra branches. On the contrary, in *Oswaldocruzia bialata* and *O. duboisi*, the blade is sharp at the end and not divided into branches (Ben Slimane et al., 1993; Durette-Desset et al., 1993; Svitin & Kuzmin, 2012; Svitin, 2016). *Oswaldocruzia skrjabini* has extra branches on the «fork» and the «shoe», and the blade is divided at the end into four branches (Svitin, 2015). In our study, all nematodes have an additional branch on each ray 10, which is also present in *Oswaldocruzia skrjabini*, but not found in *O. filiformis*, *O. bialata* and *O. duboisi*.

There are differences in the number of crests at the mid-body of the nematodes we studied with the existing descriptions of the four species mentioned above (Table 3, Table 4, Table 5, Table 6, Table 7, Table 8). So, in our study, the number of crests in *Oswaldocruzia* males varied from 34 to 47, while in females it varied from 39 to 77. For comparison, both males and females of *Oswaldocruzia bialata* have about 50 ridges in the middle of the body (Durette-Desset et al., 1993; Svitin, 2016). *Oswaldocruzia duboisi* has about 70 ridges (Ben Slimane et al., 1993; Svitin & Kuzmin, 2012), while *O. filiformis* has about 40 ridges (Ben Slimane et al., 1993; Svitin, 2016) (Fig. 3, Fig. 4, Fig. 5, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8). *Oswaldocruzia bialata* is characterised by the absence of crests on the dorsal and ventral body sides at the oesophagus level (Durette-Desset et al., 1993). In our study, nematodes always had crests at the oesophagus level. In our study, the number of crests varied in nematodes of the same sex, even those taken from one host individual. The number of crests in young and adult nematodes of the same sex also differed.

The degree of development and the shape of the lateral alae were also variable, both in nematodes from hosts belonging to different species and in different nematodes recovered from one and the same host individual (Fig. 3, Fig. 4, Fig. 5). So, in one *Bufo bufo* individual, we found one *Oswaldocruzia* female with small narrow cervical alae formed by three slightly increased crests (dorsal and ventral crests and a smaller central crest between them) and two males with more developed ventral crest in lateral alae at the level of the middle of the oesophagus (Fig. 3G,H,I). Moreover, the structure of the spicules of these *Oswaldocruzia* males was identical with that of the nematode males from *Pelophylax ridibundus* and *Natrix natrix* (Fig. 2E, Fig. 3D, Fig. 5C).

It should be noted that there are little data in the literature on the variability of the number of crests, the shape and the degree of development of lateral alae in *Oswaldocruzia* spp. depending on the population structure of the parasites and the characteristics of their hosts (e.g., sex, age, phenotype). Only Ben Slimane & Durette-Desset (1996a,b) Ben Slimane et al. (1993, 1996b), Durette-Desset et al. (2006) and Guerrero (2013) indicated differences in the number of crests in nematodes of different sexes.

Thus, in our material, we observed a broad morphological variability of nematodes both from different host species and from one host individual. Variability was observed in the size of the nematode body and individual organs, in the shape and size of the cephalic vesicle, in the shape and development degree of the lateral alae, in the number of crests on the transverse sections at the mid-body level and in the shape of rays 9 and 10 of the caudal bursa. Variation of all these characters in nematodes from various host species can be attributed to the host-induced morphological variability as has been shown on other species of helminths (Amin, 1975; Roytman & Kazakov, 1977; Machado-Silva et al., 1994; Anikieva, 2004; Kirillova et al., 2012; Nadler et al., 2013; Catalano et al., 2015). Variation of features in one host individual may be phenotypic (Anikieva, 2005, 2008; Kirillov & Kirillova, 2010; Viney & Diaz, 2012).

The nematodes collected in our study could not be identified with certainty as any of the *Oswaldocruzia* species, whose descriptions are available in the recent literature. However, they corresponded well to the morphological description of *Oswaldocruzia filiformis* (= *O. goezei* Skrjabin & Schulz, 1952) in «older» reviews by Skrjabin et al. (1954), Sharpilo (1976) and Ryzhikov et al. (1980), who did not take into account the structure of the synlophe.

The molecular phylogenetic analysis also showed that *Oswaldocruzia* nematodes collected in this study from different species of amphibians and reptiles in European Russia belonged to the species *Oswaldocruzia filiformis* s.l. Thus, our data contradict the opinion that several species of this genus parasitise different host species in the Western Palearctic (Durette-Desset et al., 1993; Ben Slimane et al., 1993, 1995, 1996a; Svitin, 2015, 2016, 2017).

The phylogenetic distance between nematode individuals collected in geographically distant regions was approximately the same as that between parasites from geographically close habitats. Unfortunately, there are still little data on the molecular phylogeny based on the CoxI mtDNA gene for this nematode genus and it is thus impossible to make extensive conclusions.

Conclusions

Our morphological and molecular phylogenetic data indicated that amphibians and reptiles in European Russia harbour only one species of the genus *Oswaldocruzia*, *O. filiformis* s.l. The analysis of the morphological features of *Oswaldocruzia* nematodes both from a single host species and from different host species showed a broad morphological variability. They are in conflict with the literature records, according to which most *Oswaldocruzia* spp. noted in the Western Palearctic are specific of amphibian and reptilian species, in which they are found. The results of our study highlight the necessity to study the diversity of morphologically similar *Oswaldocruzia* spp. from the Western Palearctic by genetic methods.

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OSWALDOCRUZIA FILIFORMIS SENSU LATO (NEMATODA: MOLINEIDAE) ИЗ АМФИБИЙ И РЕПТИЛИЙ ЕВРОПЕЙСКОЙ ЧАСТИ РОССИИ: МОРФОЛОГИЧЕСКИЕ И МОЛЕКУЛЯРНЫЕ ДАННЫЕ

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Нематоды рода *Oswaldocruzia* паразитируют в тонком кишечнике амфибий и рептилий. Их биоразнообразие остается до сих пор невыясненным. Мы изучили нематод рода *Oswaldocruzia* из девяти видов земноводных и пресмыкающихся (*Pelophylax ridibundus, Rana arvalis, R. temporaria, Bufo bufo, Lacerta agilis, Zootoca vivipara, Anguis fragilis, Natrix natrix, Vipera berus*), собранных в шести точках европейской части России в 2018–2019 гг. Для идентификации видов мы проанализировали морфологические признаки, традиционно используемые в таксономии нематод этого рода, а также новые молекулярные филогенетические данные. Результаты секвенирования участка гена CoxI мтДНК и молекулярно-филогенетический анализ полученных данных показал, что все экземпляры нематод рода *Oswaldocruzia* в этом исследовании относятся к одному виду. Мы наблюдали широкую морфологическую изменчивость нематод, как из разных видов хозяев, так и из одной особи хозяина. Морфологические различия нематод из разных видов хозяев могут быть обусловлены гостальной изменчивостью, в то время как у нематод из особей одного вида хозяина это может быть связано с фенотипической пластичностью вида. Генетические данные показали, что у амфибий и рептилий европейской части России паразитирует только один вид рода *Oswaldocruzia*, *O. filiformis* s.l., который имеет широкую морфологическую изменчивость. Результаты нашего исследования выявили необходимость проверки многообразия морфологически близких видов рода *Oswaldocruzia* Западной Палеарктики молекулярно-генетическими методами.

Ключевые слова: CoxI mtDNA, Западная Палеарктика, молекулярно-филогенетический анализ, морфологическая изменчивость, трихостронгилиды