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Research article

Parasitic nematodes of Pool Frog (*Pelophylax lessonae*) in the Volga Basin

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ABSTRACT

Objetive. Present a modern review of the nematodes fauna of the pool frog *Pelophylax lessonae* (Camerano, 1882) from Volga basin populations on the basis of our own research and literature sources analysis. **Materials and methods.** Present work consolidates data from different helminthological works over the past 80 years, supported by our own research results. During the period from 1936 to 2016 different authors examined 1460 specimens of pool frog, using the method of full helminthological autopsy, from 13 regions of the Volga basin. **Results.** In total 9 nematodes species were recorded. Nematode *Icosiella neglecta* found for the first time in the studied host from the territory of Russia and Volga basin. Three species appeared to be more widespread: *Oswaldocruzia filiformis, Cosmocerca ornata* and *Icosiella neglecta*. For each helminth species the following information included: systematic position, areas of detection, localization, biology, list of definitive hosts, the level of host-specificity. **Conclusions.** Nematodes of pool frog, excluding *I. neglecta*, belong to the group of soil-transmitted helminthes (geohelminth) and parasitize in adult stages. Some species (*O. filiformis*, *C. ornata*, *I. neglecta*) are widespread in the host range. The latter two are able to reach high invasion indices and also to be the background parasites of the pool frog. It happens due to these nematode species life cycle specificity and the long-term connection of the amphibian with water.

Keywords: *Aplectana, Cosmocerca, Icosiella neglecta, Oswaldocruzia filiformis* (*Source: CAB*).

RESUMEN

Objetivo. Presentar una revisión moderna de la fauna de nematodos de la rana esculenta *Pelophylax lessonae* (Camerano, 1882) de las poblaciones de la cuenca del Río Volga **Materiales y métodos.** Este trabajo consolida los datos de diferentes trabajos helmintológicos de los últimos 80 años, respaldados por los resultados de nuestras propias investigaciones. Durante el período de 1936 a 2016 diferentes autores examinaron 1460 especímenes de rana esculenta, utilizando el método de autopsia helmintológica completa de 13 regiones de la cuenca del Río Volga. **Resultados.** En total se registraron 9 especies de nematodos. Nematodo *Icosiella neglecta* encontrado por primera vez en el huésped estudiado en el territorio de Rusia y la cuenca del Río Volga. Tres especies parecían estar más extendidas: *Oswaldocruzia filiformis, Cosmocerca ornata* e *Icosiella neglecta*. Para cada especie de helmintos se incluyó la siguiente información: posición sistemática, áreas de detección, localización, biología, lista de hospederos definitivos, nivel de especificidad del hospedero. **Conclusiones.** Los nematodos de la rana esculenta, excepto *I. neglecta*, pertenecen al grupo de helmintos transmitidos por el suelo (geohelmintos) y parasitan en estadios adultos. Algunas especies (*O. filiformis*, *C. ornata*, *I. neglecta*) están muy extendidas en el área de distribución del hospedador. Estos dos últimos son capaces de alcanzar altos índices de invasión y también de ser los parásitos subyacentes de la rana esculenta. Esto sucede debido a la especificidad del ciclo de vida de estas especies de nematodos y a la conexión a largo plazo del anfibio con el agua.

Palabras clave: *Aplectana, Cosmocerca, Icosiella neglecta, Oswaldocruzia bialata* (*Fuente: CAB*).

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INTRODUCTION

The pool frog *Pelophylax lessonae* (Camerano, 1882) (Amphibia: Anura) is common in Europe from southern France in the west to Tatarstan and Bashkortostan (Russia) in the east (1,2,3,4). It is restricted to the forest zone, in which it inhabits plain broad leaved and mixed forests till the south taiga. In the forest the frog prefers shallow standing water bodies with dense vegetation: lakes, ponds, former river-beds, marshes; it occasionally occurs in the shallows of rivers and streams, in flood meadows and islands (1,5,6). Through riverine thickets and forested floodplains, this frog penetrates the forest-steppe and steppe zones; adults are able to migrate to neighboring water bodies. It is common in the anthropogenic landscapes where it inhabits temporary and drying reservoirs like: road tracks, roadside pits, ditches and quarries with water (7,8,9,10,11). The helminths fauna of the pool frog on the territory of the habitat has been studied unevenly and insufficiently. Reviews on parasites of frogs and toads for European countries clearly confirm this (12,13,14,15,16).

This paper continues a series of publications devoted to the modern characterization of the helminth fauna of certain amphibian species in the Volga basin (17,18,19,20,21). Data on the fauna of trematodes (Trematoda) of this species of amphibians from the Middle Volga region are presented in the works of Chikhlyaev et al (22,23). The aim of the study is to present a modern review of the nematodes (Nematoda) fauna of the pool frog from Volga basin populations on the basis of our own research and literature sources analysis.

MATERIALS AND METHODS

Site study. The Volga basin covers an area of 1360 thousand km^2, which is almost 13% of the area of Europe (Figure 1). In Russia, 41 regions belong fully or partially to this basin. The channel of the Volga and its tributaries are located in lowlands, and only in some places the river flows through elevations (for example, the Samarskaya Luka near the Zhiguli Mountains). The vast basin is characterized by a significant variety of landscapes. Forest ecosystems predominate in the northern part of the basin. At the same time, there are the mixed and coniferous forests in the northwestern part, while the forests of the taiga type with larch and spruce prevail in the northeastern part. In the middle part of the Volga basin, the forest ecosystems gradually pass into the zone of the forest-steppe landscapes, then to the south, the steppe and semi-desert ecosystems are more expressed. Beyond the southern margin of the dry steppes the flat and anhydrous Caspian semi-desert with its saline soil cover, drainage rivers, low-lying and sparse vegetation begins.

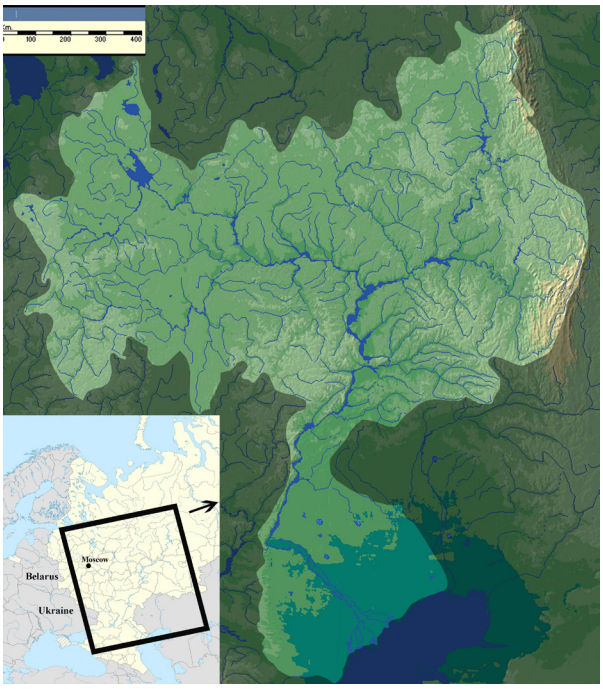

Figure 1. Location of the Volga basin.

In the period from 1936 to 2016 different authors have examined 1460 specimens of pool frog from 13 regions of the Volga basin, among which are: Kaluga, Moscow, Ivanovo, Kostroma, Nizhny Novgorod, Tambov, Ulyanovsk and Samara regions, Republic of Mordovia, Republic of Chuvashia, Republic of Mari El, Republic of Tatarstan and Republic of Bashkortostan (Table 1). Own researches are conducted in 8 regions of Russia. In total, more than 568 specimens of *P. lessonae* have been studied. Studies were performed by the method of full helminthological autopsy. The collection, fixation and processing of the material were carried out according to the standard methods (24).

For species determination of helminths, were used reports of Ryzhikov et al (24). To refer nematodes species to according systematic taxones, we used systems developed by Hodda (25).

Table 1. Nematodes of pool frog *Pelophylax lessonae* in the Volga basin regions

Nematodes species	KL	MS	IV	KS	NN	TM	UL	SM	RM	CH	ME	TT	BS
Phylum Nematoda Cobb, 1932 Class Chromadorea Inglis, 1983 Order Panagrolaimida Hodda, 2007 Family Rhabdiasidae Railliet, 1916													
Rhabdias bufonis				+	+			+	+			+	
Order Rhabditida Chitwood, 1933													
Family Trichostrongylidae Leiper, 1908													
Oswaldocruzia filiformis	+	+	+	+	+	+	+	+	+	+	+	+	+
Oswaldocruzia bialata				+								+	
Order Spirurida Railliet, 1914 Family Cosmocercidae Travassos, 1925													
Aplectana acuminata				+	+							+	
Cosmocerca commutata					+							+	
Cosmocerca ornata	+	+	+	+	+	+		+	+		+	+	
Neoraillietnema praeputiale				+									
Oxysomatium brevicaudatum		+		+	+							+	
Family Onchocercidae (Leiper, 1911)													
Icosiella neglecta	+				+	+		+	+	+	+	+	
Species in total	3	3	2	7	7	3	1	4	4	2	3	8	1
Examined, specimens	22	121	132	134	418	40	17	203	53	38	8	257	17

Notes: KL – Kaluga region (32,our data); MS – Moscow region (33); IV – Ivanovo region (34); KS – Kostroma region (26); NN – Nizhny Novgorod region (35,36,our data); TM – Tambov region (37,our data); UL – Ulyanovsk region (38); SM – Samara region (39,40,our data); RM – The Republic of Mordovia (18,41,our data); CH – The Republic of Chuvashia (our data); ME – The Republic of Mari El (our data); TT – The Republic of Tatarstan (42,our data); BS – The Republic of Bashkortostan (43).

RESULTS

Annotated list of pool frog nematodes species with an indication of their systematic position, areas of detection, localization, biology and geographic distribution is given below. The information on degree of specificity of parasites to hosts is also provided. For each species of nematodes, a list of their definitive hosts within Russia is indicated, corrected according to literary sources analysis (18,19,22-24).

Phylum: Nematoda Cobb, 1932
Class: Chromadorea Inglis, 1983
Order: Panagrolaimida Hodda, 2007
Family: Rhabdiasidae Railliet, 1916
Rhabdias bufonis (Schrank, 1788)
Localization: lungs.
Areas of detection: Kostroma, Nizhny Novgorod and Samara regions, the Republics of Mordovia and Tatarstan.
Description (n=2): The shape of the buccal capsule is close to cylindrical. The size of the buccal capsule, which is 10–11 μm long and 9 μm wide. The shape of the tail is slender; body length, which is 9–10 mm.
Biology: It is a soil-transmitted parasite (geohelminth). Infection of amphibians results from percutaneous penetration of invasive larvae which migrate with a blood flow in the host's lungs on the land (26). Participation of paratenic hosts - land mollusks, oligochaetes is probable (24). Is a widely specific parasite of anurans.
Definitive hosts: different species of anurans amphibians of the genera *Pelophylax*, *Rana*, *Bufo*, *Pelobates* and *Hyla*, *Bombina bombina* (Linnaeus, 1761), *Bufotes viridis* (Laurenti, 1768).
Distribution: Holarctic.

Order: Rhabditida Chitwood, 1933
Family: Trichostrongylidae Leiper, 1908
Oswaldocruzia filiformis (Goeze, 1782)
Syn.: *Oswaldocruzia goezei* Skrjabin et Schulz, 1952
Localization: small intestine.
Areas of detection: Kaluga, Moscow, Ivanovo, Kostroma, Nizhny Novgorod, Tambov, Ulyanovsk and Samara regions, the Republics of Mordovia, Chuvashia, Mari El, Tatarstan and Bashkortostan.
Biology: Geohelminth. Infection of amphibians occurs by oral penetration of invasive larvae on the land. Experiments on percutaneous infections did not reveal actual penetration of larvae in or through the skin nor a subsequent migration through host tissues. The percutaneous route of infection is not plausible for *O. filiformis* (27). Is a widely specific parasite of amphibians.
Definitive hosts: different species of amphibians of the genera *Pelophylax*, *Rana*, *Bufo*, *Pelobates*, *Bombina* and *Hyla*, *Pelodytes caucasicus* Boulenger, 1896, *B. viridis*, *Lissotriton vulgaris* (Linnaeus, 1758), *Salamandra salamandra* (Linnaeus, 1758).
Distribution: Palearctic.

Oswaldocruzia bialata (Molin, 1860)
Localization: small intestine.
Areas of detection: Kostroma region, Republic of Tatarstan.
Biology: Geohelminth (28). The development cycle is probably similar to that *O. filiformis*.
Definitive hosts: anurans amphibians of family Ranidae, *Bufo bufo* (Linnaeus, 1758).
Distribution: Palearctic.

Order: Spirurida Railliet, 1914
Family: Cosmocercidae Travassos, 1925
Aplectana acuminata (Schrank, 1788)
Localization: intestine.
Areas of detection: Moscow, Kostroma and Nizhny Novgorod regions, the Republic of Tatarstan.
Biology: Geohelminth. Is widely specific parasite of tadpoles amphibians whom they catch very much early. Own finds of nematodes at tadpoles of green frogs testify to the water route of infection with a parasite.
Definitive hosts: amphibians of genera *Pelophylax*, *Rana*, *Pelobates* and *Hyla*, *B. bombina*, *B. bufo*, *B. viridis*, *Triturus cristatus* (Laurenti, 1768).
Distribution: Europe.

Cosmocerca ornata (Dujardin, 1845)
Localization: rectum.
Areas of detection: Kaluga, Moscow, Ivanovo, Kostroma, Nizhny Novgorod, Tambov and Samara regions, the Republics of Mordovia, Mari El and Tatarstan.
Biology: Geohelminth. Infection of amphibians occurs in water at a stage of tadpoles. However nematode larvae in intestines of tadpoles don't develop and in process a metamorphosis of the host are eliminated (29). Possibly, development of nematodes happens only at infection of adult frogs. Is widely specific parasite of anurans amphibians.
Definitive hosts: amphibians of genera *Pelophylax*, *Rana*, *Bufo*, *Pelobates* and *Hyla*, *B. bombina*, *B. viridis*, *L. vulgaris* (?).
Distribution: Europe.

Cosmocerca commutata (Diesing, 1851)
Syn.: *Cosmocercoides skrjabini* (Ivanitzky, 1940)
Localization: rectum (adult specimens); musculature of throat, body cavity (larval stages).
Areas of detection: Nizhny Novgorod region and the Republic of Tatarstan.
Biology: Geohelminth. The nematode larvae, as a result of peroral penetration are localized in capsules of the oral cavity, where they develop into adults and, after the encapsulation, migrate in rectum (30). A narrow specific parasite of the *B. viridis*.
Definitive hosts: *B. viridis*. Findings from other species of amphibians (especially frogs) require confirmation.
Distribution: Palearctic.

Oxysomatium brevicaudatum (Zeder, 1800)
Syn.: *Neoxysomatium brevicaudatum* (Zeder, 1800)
Localization: rectum.
Areas of detection: Moscow, Kostroma and Nizhny Novgorod regions, the Republic of Tatarstan.
Biology: Geohelminth. The frequent occurrence of a nematode at terrectrial amphibians, according to us, testifies in favor of a land route of infection. Is widely specific parasite of anurans amphibians.
Definitive hosts: amphibians of genera *Pelophylax*, *Rana* and *Pelobates*, *B. bombina*, *B. bufo*, *B. viridis*, *L. vulgaris* (?).
Distribution: Holarctic.

Neoraillietnema praeputiale (Skrjabin, 1916)
Syn.: *Aplectana praeputialis* (Skrjabin, 1916)
Localization: rectum.
Area of detection: Kostroma region.
Biology: Geohelminth. Life cycle of a nematode is not studied. Is widely specific parasite of anurans.
Definitive hosts: anurans amphibians of genera *Pelophylax*, *Rana* and *Hyla*, *B. bombina*, *B. bufo* (?), *B. viridis*.
Distribution: Europe.

Family: Onchocercidae (Leiper, 1911)
Icosiella neglecta (Diesing, 1851)
Localization: muscles, subcutaneous tissue.
Areas of detection: Kaluga, Nizhny Novgorod, Tambov and Samara regions, the Republics of Mordovia, Chuvashia, Mari El and Tatarstan. First observed in the pool frog of Russia and the Volga basin.
Biology: Develops with change of hosts (biohelminth). The intermediate hosts are the Ceratopogonidae dipterans – *Forcipomyia velox* and *Sycorax silacea*. Infection with a parasite occurs at percutaneous penetration of invasive larvae through a sting of midges during feeding by blood of frogs (31). Is a specific parasite of anurans amphibians family Ranidae.
Definitive hosts: anurans amphibians of family Ranidae.
Distribution: Palearctic.

DISCUSSION

In the pool frog on the territory of Volga basin there are 9 species of nematodes from 7 genera, 4 families, 3 orders and 1 class (Table 1). Of these, 6 species are broadly specific, polyhostal parasites of anurans, one (*I. neglecta*) – are specific, oligohostal for the family Ranidae Rafinesque, 1814, and one (*C. commutata*) is a narrow specific, monohostal parasite of toads of the genus *Bufotes* Rafinesque, 1815. Nematode *I. neglecta* are indicated for the

first time for the pool frog of Russia and the Volga basin. Among all nematodes, 8 species parasitize in the adult stage and use frogs as obligatory definitive hosts. And only nematode *C. commutata* combine different stages of development in one individual or individuals of different ages, and use amphibians as amphyxenic hosts.

The largest species diversity of nematodes was recorded in pool frogs in the Republic of Tatarstan (8 species), Nizhny Novgorod (7 species) and Kostroma (7 species) regions; lower number of species was registered in Samara (4 species), Kaluga, Moscow, Tambov (on 3 species) and Ivanovo (2 species) regions, Republic of Mordovia (4 species), Republic of Mari El (3 species) and Republic of Chuvashia (2 species); the minimum – in Ulyanovsk (1 species) region and the Republic of Bashkortostan (1 species) (Table 1). These differences depend on the nature of conditions of dwelling of amphibians in separate biotopes, their geographical location, extent of anthropogenic transformation and different volumes of selections.

The nematodes composition of the pool frog varies in the Volga basin. Of the recorded 9 species, only one was observed in all regions (100% of occurrence): it is the nematode *O. filiformis*. Very close to this index were *C. ornata* and *I. neglecta*, found in 10 and 8 samplings, respectively, out of 13 examined. Three more species of helminths (*Rh. bufonis*, *O. brevicaudatum* and *A. acuminata*) were less common in the habitat range of this host, and their findings were of a sporadic nature. The rest of the species, in particular, nematodes *O. bialata*, *C. commutata* and *N. praeputiale* were found locally and they were observed in 1-2 samplings.

In conclusion the fauna of helminths of the amphibians depends on their way of life, the nature of a biotope, duration of stay in water / on the land and food spectrum. Nematodes of pool frog, excluding *I. neglecta*, belong to the group of soil-transmitted helminthes (geohelminth) and parasitize in adult stages. Some species (*O. filiformis*, *C. ornata*, *I. neglecta*) are widespread in the host range. The latter two are able to reach high invasion indices and also to be the background parasites of the pool frog. It happens due to these nematode species life cycle specificity (the availability of free-floating invasive larval stages) and the long-term connection of the amphibian with water.

Conflict of interests.

The authors declare no conflict of interest with publication of this manuscript.

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