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ZOOLOGY

Analysis of Parasitofauna and Endosymbionts of *Lissotriton vulgaris* L., 1758 (Caudata, Salamandridae) in Natural and Urban Gradients of the Environment

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Abstract—The analysis of the parasitic and endosymbiontofauna of the common newt (*Lissotriton vulgaris* L., 1758) in the natural and urban landscapes of the Middle Urals is presented for the first time. Four species of helminths belonging to the Nematoda type were found, including one taxon indeterminate to the species (*Nematoda* sp.) and one species of endosymbionts (phylum Chromista). The works devoted to the species composition of *L. vulgaris* parasites on the territory of Eurasia are analyzed. The fauna of the helminths of the common newt on the eastern slope of the Urals is depleted, which is probably determined by the limited distribution of the species outside Europe, the temperature regime in the north, and the availability of moisture in the south.

Keywords: common newt, helminths, urbocenosis, natural gradient of the environment

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The common newt (*Lissotriton vulgaris* L., 1758) is a widespread and ecologically flexible species of tailed amphibians (Vershinin, 2007), inhabiting the forest zone of the European part and second in number only to brown frogs. This species is found in anthropogenic landscapes (mainly in forested and suburban areas) (Vershinin, 2007; Kuzmin, 2012). Threat of extinction of local populations *L. vulgaris* relatively small compared to *Triturus cristatus* (Laurenti, 1768) (Kinne, 2006). However, in the landscapes of megacities, *L. vulgaris* may have the status of a rare and even endangered species (Fayzulin and Kuzovenko, 2015; Kidov et al., 2021).

The number and distribution of many species of amphibians have been declining globally in recent decades for various reasons: chemical pollution, destruction and transformation of habitats, various infections, and the emergence of invasive species of fish and amphibians (Berger et al., 1998; Houlahan et al., 2000; Reshetnikov, 2001; Blaustein and Kiesecker, 2002; Kinne, 2006; Wake and Vredenburg, 2008).

One of the significant biotic factors that regulate the number of hosts, and therefore affect the survival of animals, is parasitic invasions. Amphibians can be biological reservoirs of parasites and distributors of helminth infections in natural ecosystems and can serve as definitive, intermediate, facultative, and reservoir hosts for helminths. Some types of trematodes can accelerate puberty in newts and cause a reduction in life expectancy (Sinsch et al., 2018a) and various anomalies (Sessions and Ruth, 1990), making them most vulnerable to predators (Caffara et al., 2014), and thereby reduce the survival rate of amphibians and lead to population decline.

Within Russia, the parasitic fauna *L. vulgaris* has been described for Vologda oblast (Shabunov and Radchenko, 2012) and the Republic of Mordovia (Ruchin and Chikhlyaev, 2016). The most complete study of the helminth fauna of *L. vulgaris* was undertaken in Samara oblast (Chikhlyaev, 2007; Chikhlyaev et al., 2018).

For the Ural region, information on the parasites *L. vulgaris* is absent. In this regard, the purpose of this work is a comparative analysis of the parasitic fauna *L. vulgaris* in the European part of the range and in natural and urban landscapes of the Middle Urals.

MATERIALS AND METHODS

This study was carried out in May 2020 and 2021 in natural and urban areas of the Middle Urals (Fig. 1). The study of parasitic fauna along an urbanization gradient was carried out using the example of the city of Yekaterinburg, located on the eastern slope of the Middle Urals.

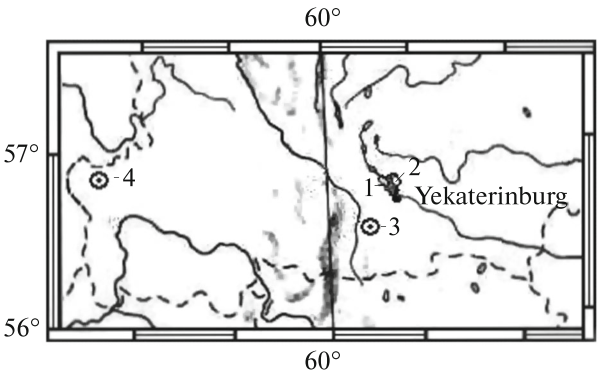

Fig. 1. Map-scheme of collecting material (1, residential part of the city of Yekaterinburg; 2, forest–park part of the city of Yekaterinburg; 3, village of Mramorskoye; 4, village of Bolshoye Koshaevo).

Within the urban agglomeration, a residential part and a forest park zone were distinguished (Vershinin et al., 2015). Suburban populations were used as controls for *L. vulgaris* (Fig. 1).

191 individuals of the common newt were studied, of which 119 specimens lived within the urban agglomeration (residential zone, 34 specimens; forest park zone, 85), and 72 specimens were from rural areas.

Identification of parasites was carried out according to standard methods (Ivashkin et al., 1971; Ryzhikov et al., 1980). The infestation of amphibians is assessed according to the following indicators: *P* (extensiveness of invasion), the proportion of infected host individuals in the studied sample (%); *A* (abundance index), the average number of parasites of a certain species or group of parasites in all individuals of the host, specimen/individual of the host (Breev, 1976). The assessment of the dominance structure was carried out using the approach of A.A. Kirillov (Kirillov, 2011). Statistical data processing was carried out using the Quantitative Parasitology program (Rozsa et al., 2000) and Statistica 7.0.

RESULTS

In the intestines of the common newts studied, four species of nematodes were found (one taxon was not identified to the species) and one species of endosymbiotic protozoa. The species composition and infestation rates are presented in Tables 1 and 2.

In natural areas *L. vulgaris* nematodes are represented by three species of the order Rhabditida; specimens of unknown species were also noted (*Nematoda* sp.). In urban communities of *L. vulgaris,* two types of nematodes were identified (Table 1). Along with the presence of common species (*Oswaldocruzia filiformis* and *Megalobatrachonema terdentatum*) on the studied territories of the Middle Urals, for *L. vulgaris* only on the eastern slope was a nematode larva of *Neoxysomatium brevicaudatum* noted, and specimens of *Nematoda* sp. were found on the western slope. The endosymbiotic protozoa *Cepedea dimidiata* was identified in *L. vulgaris* from the eastern slope of the Middle Urals and in urban communities.

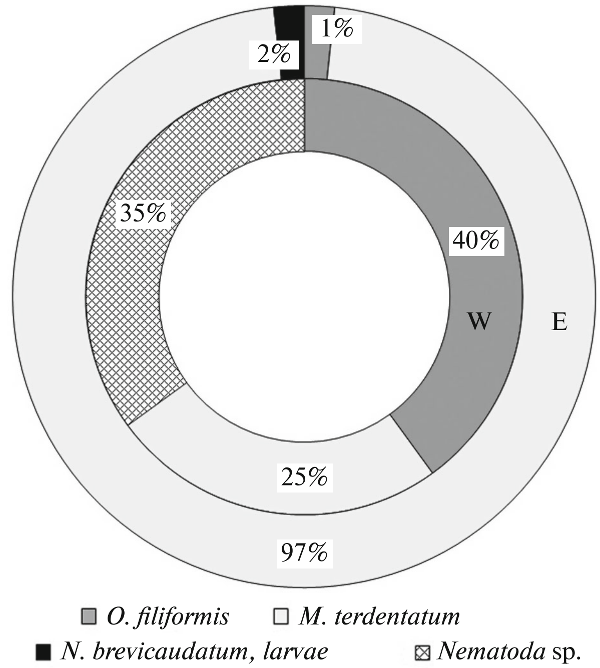

Fig. 2. The ratio of parasite species in *L. vulgaris* on the western and eastern slopes of the Middle Urals (W, Western slope; E, Eastern slope).

In Central Ural populations of *L. vulgaris,* on the eastern slope, in terms of the share in the component community, the dominant species is *M. terdentatum* (97%), compared with the western slope (25%) (Fig. 2). In natural populations of *L. vulgaris* on the western slope, a bidominant structure is noted—*O. filiformis* (40%) and *Nematoda* sp. (35%) (Fig. 2). The extensiveness of invasion of *M. terdentatum* is also significantly higher for amphibians on the eastern slope compared to the western slope (Table 1).

For amphibians of the urban agglomeration (residential and forest–park zones) and natural areas, two general types of parasites have been noted—*O. filiformis* and *M. terdentatum* (Table 2). On the territory of the urban community and natural areas of the eastern slope, *M. terdentatum* dominates; its share in the urbanization gradient remains at a high level (from 96.67% for amphibians in suburban areas to 97.44% for animals in residential areas). The share of *O. filiformis* is low and increases along the urbanization gradient (from 1.67% in suburban areas to 2.56% in residential areas) (Fig. 3).

Nematode larvae of *N. brevicaudatum* were noted in animals in natural populations; the proportion was 1.67% (Table 2, Fig. 3).

Table 1. Species composition and occupancy rates by parasites and endosymbionts in *L. vulgaris* in ecosystems of the Middle Urals

Parasite/Endosymbiont	$P \pm SE/A \pm SE$		
	Middle Urals (n = 191)		
	natural areas (n = 72)		Urban cenosis (n = 119)
	Western slope (n = 37)	Eastern slope (n = 35)	
Phylum Nematoda Cobb, 1932			
Class Chromadorea Inglis, 1983	24.30 ± 7.05 / 0.54 ± 0.21	40.0 ± 8.28 / 1.71 ± 0.60	29.40 ± 4.18 / 1.04 ± 0.23
Order Rhabditida Chitwood, 1933 *Oswaldocruzia filiformis* (Goeze, 1782)	8.10 ± 4.46 / 0.22 ± 0.16	2.90 ± 2.84 / 0.03 ± 0.02	1.70 ± 1.19 / 0.03 ± 0.02
Megalobatrachonema terdentatum (Linstow, 1890)	5.40 ± 3.72 / 0.14 ± 0.11	34.3^{A} ± 8.03 / 1.66 ± 0.60	28.60 ± 4.14 / 1.02 ± 0.21
Neoxysomatium brevicaudatum, larvae Zeder, 1800	–	2.90 ± 2.84 / 0.03 ± 0.02	–
Nematoda sp.	24.30 ± 7.05 / 0.19 ± 0.09	–	–
Phylum Chromista Cavalier-Smith, 1987			
Class Opalinea Wenyon, 1926 *Cepedea dimidiata* (Metcalf, 1923)	–	17.10 ± 6.36 / 9.06 ± 4.37	31.1 ± 4.24 / 17.33 ± 2.93
Total species	3/0	3/1	2/1

Above the line, P, extent of invasion, %; below the line, A, abundance index, host specimen/individual; SE, mathematical error; in the column "Total species," the number of species of parasites and endosymbionts is indicated through a fraction; ^{A} is significantly higher compared to the western slope ($P < 0.05$); "–" means no data.

Endosymbiotic protozoa have been observed both in residential areas and in natural landscapes. The extensiveness of invasion for *C. dimidiata* is significantly higher in *L. vulgaris* in the residential area, in comparison with the forest park area and natural area (Table 2).

An analysis of the literature data regarding the fauna of parasites of the common newt on the territory of Eurasia showed that the most diverse in the species composition of helminths is the Republic of Belarus (eight species, including five species of trematodes and three species of nematodes) and the Samara region (eight species, including six species of trematodes and two types of nematodes). The fewest helminth species were observed in populations from Italy (Sesto Fiorentino, Tuscany region), Northern Greece, and the Republic of Mordovia (one species). Moreover, trematodes were detected in the first two European regions, and nematodes were detected in newts on the territory of the Republic of Mordovia (Table 3).

For most of the regions of Europe and the Russian Federation considered, the two most common nematodes are *O. filiformis*, marked in *L. vulgaris* in Europe in the southwestern part of Germany (Rhineland-Palatinate), in Northern Greece, in the Republic of Belarus, and in the regions of the Russian Federation (Vologda and Samara oblasts, Republic of Mordovia), and the nematode *M. terdentatum* identified in *L. vulgaris* in Germany, in the southwestern part of England (Somerset County), and in Vologda and Samara oblasts.

A number of species are known only for certain areas: larval forms of trematodes: *Paralepoderma cloacicola* (Luhe, 1909), met., *Pharyngostomum cordatum* (Diesing,1850), met., *Strigea* sp., met., marked in *L. vulgaris* from the territory of Samara oblast; trematodes: *Strigea sphaerula* (Rudolphi, 1803) Szidat, 1928 and *Opisthioglyphe ranae* (Frohlich, 1791); nematodes: *Cosmocerca ornata* (Dujardin, 1845) Diesing, 1861 and *Agamospirura* Henry, Sisoff 1913 sp., larvae, found for common newts from the territory of the Republic of Belarus. The fluke *Clinostomum* spp. Leidy, 1856 was identified in *L. vulgaris*, in Italy (Tuscany region); the fluke *Parastrigea robusta* Szidat, 1928 and the nematode *Cosmocerca longicauda* (Linstow, 1885) were found in a common newt from Germany. Skreben *Acanthocephalus anthuris* (Dujardin, 1845) was found in *L. vulgaris* inhabiting the southwestern part of England (Table 3).

Table 2. Population by parasites and endosymbionts in *L. vulgaris* in the urbanization gradient

Parasite/Endosymbiont	$P \pm SE/A \pm SE$		
	Residential territory ($n = 34$)	Forest–park area ($n = 85$)	Natural areas ($n = 72$)
Phylum Nematoda Cobb, 1932			
Class Chromadorea Inglis, 1983	26.50 ± 7.57 / 1.15 ± 0.42	31.80 ± 5.05 / 1.40 ± 0.26	42.90 ± 5.83 / 1.89 ± 0.59
Oswaldocruzia filiformis (Goeze, 1782)	5.30 ± 3.84 / 0.05 ± 0.03	2.50 ± 1.69 / 0.05 ± 0.03	2.90 ± 1.98 / 0.03 ± 0.02
Megalobatrachonema terdentatum (Linstow, 1890)	23.50 ± 7.27 / 1.12 ± 0.42	31.80 ± 5.05 / 0.98 ± 0.25	34.30 ± 5.59 / 1.66 ± 0.60
Neoxysomatium brevicaudatum, larvae Zeder, 1800	–	–	2.90 ± 1.98 / 0.03 ± 0.02
Phylum Chromista Cavalier-Smith, 1987			
Class Opalinea Wenyon, 1926 *Cepedea dimidiata* (Stein, 1860) (Metcalf, 1923)	47.10^{ab} ± 8.56 / 18.82 ± 4.99	24.70 ± 4.68 / 16.73 ± 3.59	17.10 ± 4.43 / 9.06 ± 4.37

Above the line, *P*, extent of invasion, %; below the line, *A*, abundance index, host specimen/individual; SE, mathematical error; [a], significantly higher compared to forest–park area ($P < 0.05$); [b], significantly higher compared to natural territory ($P < 0.05$); "–" means no data.

DISCUSSION

The parasitic fauna of common newts in the study areas is characterized by both widespread species and specialized helminths of tailed amphibians.

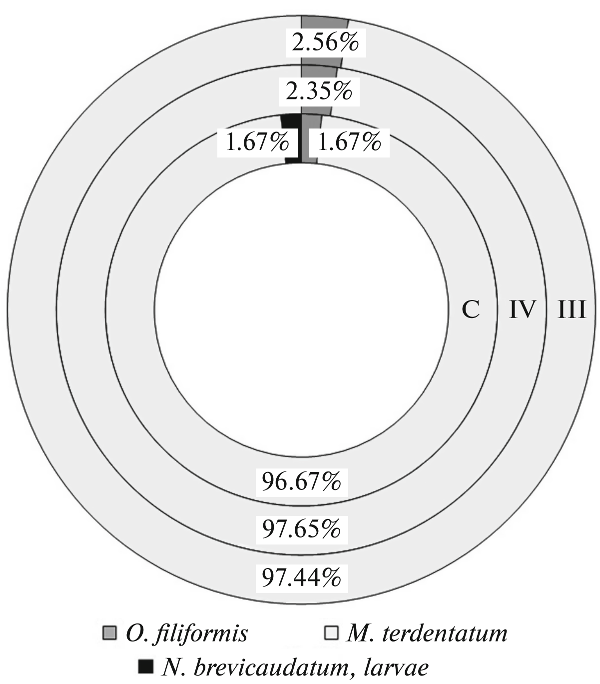

Fig. 3. The ratio of parasite species for *L. vulgaris* in urbanized and natural areas of the eastern slope of the Middle Urals (III, residential area of the city of Yekaterinburg; IV, forest park zone of the city of Yekaterinburg; C, natural areas).

The nematode *M. terdentatum* is a European species, a highly specific parasite, characteristic of the common newt in the western regions of Ukraine, Belarus, and Czechoslovakia, noted in *L. vulgaris* on the territory of Vologda oblast (Shabunov and Radchenko, 2012) and the Volga region (Samara) (Kirillov et al., 2018). Infection by *M. terdentatum* occurs orally in water or through reservoir hosts (Petter and Chabaud, 1971). There is the opinion that the presence of some species of nematodes may be associated with the isolation of the ecological niche of caudates, which influenced the independent formation of the helminth fauna of this group of hosts during evolution (Ryzhikov et al., 1980).

According to A.A. Kirillov et al. (Kirillov et al., 2018), the nematode *M. terdentatum* in the common newt living in the city of Samara was noted for the first time. Infection of animals with this species was also low and amounted to 25% and 0.5 helminths per individual. Similar data are shown for *L. vulgaris* from the territory of Vologda oblast (infestation is two specimens per individual) (Shabunov and Radchenko, 2012).

Domination of *M. terdentatum* in common newts on the Eastern slope of the Middle Urals in natural ecosystems and urban communities is probably due to the specific ecology of the host itself (*L. vulgaris*), as well as the biology of this helminth. Infection with *M. terdentatum* occurs orally during the aquatic phase of life of the common newt during active feeding when the reservoir hosts eaten are gastropods and oligochaetes (Petter and Chabaud, 1971). It was established (Petter and Chabaud, 1971) that the third invasive stage of *M. terdentatum* develops in an aquatic envi-

Table 3. Species composition of parasites in *L. vulgaris* according to the literature data

Parasite	Region							
	England	Germany	Italy	Northern Greece	Republic of Belarus	Vologda oblast	Republic of Mordovia	Samara oblast
Phylum Plathelminthes Gegenbaur, 1859								
Diplodiscus subclavatus (Pallas, 1760)					+			+
Paralepoderma cloacicola (Luhe, 1909), met.								+
Pharyngostomum cordatum (Diesing, 1850), met.								+
Strigea sp., met. Abildgaard, 1790								+
Strigea sphaerula (Rudolphi, 1803) Szidat, 1928					+			
Clinostomum spp. Leidy, 1856			+					
Opisthioglyphe ranae (Frohlich, 1791)					+			
Pleurogenoides medians (Olsson, 1876)					+			+
Alaria alata (Schrank, 1788) Krause, 1914					+			+
Parastrigea robusta Szidat, 1928		+						
Phylum Nematoda Cobb, 1932								
Oswaldocruzia filiformis Goeze, 1782		+		+	+	+	+	+
Megalobatrachonema terdentatum (Linstow, 1890)	+	+				+		+
Cosmocerca ornata (Dujardin, 1845) Diesing, 1861					+			
Agamospirura Henry, Sisoff 1913 sp., larvae					+			
Cosmocerca longicauda (Linstow, 1885)		+						
Phylum Acanthocephala Kölr., 1771								
Acanthocephalus anthuris (Dujardin, 1845)	+							
Author	Avery, 1971	Sinsch et al., 2018a, Sinsch et al., 2018b	Caffara, 2014	Sattmann, 1990	Shimalov, 2009	Shabunov and Radchenko, 2012	Ruchin and Chikhlyaev, 2016	Faizulin et al., 2011, Chikhlyaev et al., 2018, Kirillov et al., 2018

ronment at a temperature of about 20°C. It has been shown (Berzin and Burakova, 2022) that infection of sexually mature animals of *Triturus cristatus* (Laurenti, 1768) by the nematode *M. terdentatum* begins already at a water temperature of 16.9°C and above. Unlike the crested newt, *L. vulgaris* is a less stenotopic species and its reproduction begins earlier at lower temperatures ($t = +8$°C) (Vershinin, 2007). In urban communities, spawning reservoirs warm up faster (Vershinin, 2007), which creates optimal conditions for the development of both newts and the nematodes *M. terdentatum*. Thus, most likely, favorable temperature conditions, as well as the presence of active nutrition in the aquatic phase of the life cycle, affect the likelihood of infection by *L. vulgaris* of this species.

Another type of helminth is the nematode *O. filiformis,* which is widespread in the Palearctic, parasitizing a wide range of amphibians and reptiles (Ryzhikov et al., 1980; Wacker, 2018). Common newts become infected by *O. filiformis* orally through accidental contact with infective larvae on land (Hendrikx, 1983).

Infestation of *L. vulgaris* by the nematode *O. filiformis* is low, but this type of helminth is in second place in terms of its share in the component community. It has been shown (Kirillova et al., 2021) that the greatest infestation was noted for the common toad (*Bufo bufo* Linnaeus, 1758), as a species of large size and leading a predominantly terrestrial lifestyle. Most likely one of the reasons for the low infestation rates is the small size of the common newt and the relatively long duration of the aqueous phase.

Infection by *O. filiformis* in common newts occurs during the terrestrial phase of the life cycle, through accidental ingestion of food items. The most favorable conditions for the existence of common newts on land are special microclimatic conditions provided by the height and density of the grass stand (Vershinin, 2007). It has been shown (Wakker, 2018) that for the development of eggs of the nematode *O. filiformis,* optimal conditions are needed: these are biotopes with sufficient soil moisture, air temperature about +14...+15°C, dense vegetation cover on it, free-standing trees that create shade, and shrubs and reeds necessary for the movement of the larvae of this nematode. Thus, another reason for the infection of common newts by the nematode *O. filiformis*, in addition to the size of the animal, is the height of the grass stand along which the nematode larvae migrate.

N. brevicaudatum, larvae Zeder, 1800 is one of the parasites found in representatives of the herpetofauna of Europe (Borkovcová and Kopriva, 2005; Yildirimhan et al., 2005; Saglam and Arikan, 2006; Jones et al., 2012), noted in amphibians of the genera *Bombina* Oken, 1816; *Bufo* Laurenti, 1768; *Hyla* Laurenti, 1768; *Rana* Linne, 1758; and *Triturus* Rafinesque, 1815 and sometimes reptiles of the genera *Anguis* Linnaeus, 1758 and *Natrix* Laurenti, 1768 (Shimalov and Shimalov, 2000; Jones et al., 2012). A nematode that has a direct life cycle (first-stage larvae hatch from eggs outside the host and then develop and molt twice until the third stage of infection). The final host becomes infected orally, and larvae can be found in the tissues (Vashetko and Siddikov, 1999; Saeed et al., 2007).

Infestation with larvae of *N. brevicaudatum* is small, which is probably related, as in the case of the nematode *O. filiformis*, with the small size of the host itself.

One of the simplest amphibians that inhabits the digestive tract is *C. dimidiata* (Wahab et al., 2008). It is believed that this species has an endosymbiotic relationship with its hosts, without exerting any negative effects, despite their detection in large numbers (Poynton and Whitaker, 2001 cited in Mohammad et al., 2013).

It was shown (McConnachie, 1960) that the colonization of amphibians by representatives of the class Opalinea occurs during the breeding season and depends on the secretion of host hormones. After emerging from hibernation, amphibians begin to feed actively; with increasing daylight hours, the production of gonadotropin increases. Another factor influencing the activity of the pituitary gland, which secretes gonadotropin, in sexually mature individuals is an increase in the environmental temperature in the spring (McConnachie, 1960).

The temperature of the environment, as well as of the hosts themselves, influences the morphological and physiological characteristics of protozoa (Mikhalchenko, 1958; Sukhanova, 1953, 1963, quoted from Sukhanova, 1968).

For example, *Opalina ranarum* (Purkinje et Valentin, 1835) showed that in tadpoles, young of the year, and mature frogs, the average survival time of protozoan cysts is higher in a reservoir with a higher water temperature (Sukhanova, 1968).

High rates of invasion by the endosymbiotic protozoa *C. dimidiata* are probably associated with the synchronization of the life cycles of the host and protozoa during the period of reproduction, including the temperature regime of the reservoir; in May the water begins to warm up, which affects the reproduction and survival of protozoan cysts.

There is a trend towards an increase in the rates of invasion by endosymbionts in common newts in the urbanization gradient (toward zone III), which may be due to the thermal pollution characteristic of urban communities. In urban areas, approximately 1–2°C higher than outside the city, accordingly, in the residential area, the May average monthly temperatures of water bodies are approximately 3°C higher than in the forest park zone and natural areas (Vershinin, 2014).

The specificity of the species composition of the parasitic complex of the common newt on the eastern slope of the Urals is determined by the limited distribution of the species outside of Europe (Skorinov et al., 2008), the temperature regime in the north, and moisture supply in the south (Terentyev and Chernov, 1949; Kuzmin, 2012).

CONCLUSIONS

As the range of the common newt moves eastward, climatic zones and plant communities change, and the temperature and moisture supply of the territories change. The eastern slope of the Urals in this sense is characterized by an increasingly continental climate, which affects the species composition and diversity of the parasitic fauna of the common newt. Anthropogenic modifications of communities differ from natural ones in their greater thermal supply, but insufficient humidity, which affects the survival of helminths with a direct life cycle (*O. filiformis*, *N. brevicaudatum*, larvae) compared to *M. terdentatum,* the development of which occurs with a change in reservoir hosts.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Permission to collection of this species in Russia is not required. Animals were collected, handled and euthanized in accordance with Russian Federation national rules from 1977 and the second part of Working Party Report DGXT of the EU (1997).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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