

## RESEARCH ARTICLES

## ОРИГИНАЛЬНЫЕ СТАТЬИ

ECOLOGICAL ANALYSIS OF THE HELMINTH FAUNA IN *BUFO BUFO*  
(AMPHIBIA: ANURA) FROM VARIOUS HABITATSIgor V. Chikhlyaev<sup>1</sup>, Alexander B. Ruchin<sup>2</sup>, Alexander A. Kirillov<sup>1</sup><sup>1</sup>*Institute of Ecology of the Volga River Basin of RAS, Russia**e-mail: diplodiscus@mail.ru, parasitolog@yandex.ru*<sup>2</sup>*Joint Directorate of the Mordovia State Nature Reserve and National Park «Smolny», Russia**e-mail: ruchin.alexander@gmail.com*

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In this study is made an ecological analysis of the species composition, structure of the helminth community and the invasion degree of *Bufo bufo* in different habitats in the National Park «Smolny» (Republic of Mordovia, Russia). In 2018–2019, they were collected in five biotopes with different spawning water bodies. Nine helminth species were found, including five Trematoda species and four Chromadorea (Nematoda) species. There are two groups of species. The first of them develops on terrestrial nematodes *Rhabdias bufonis*, *Oswaldocruzia filiformis*, *Oxysomatium brevicaudatum*, rather common, regardless of the habitat, with a high degree of invasion and dominating in the helminth community. The species of the second group develop in aquatic nematode *Cosmocerca ornata*, as well as trematodes which are rarer, locally and with a low infection rate. They are typical parasites of green frogs (genus *Pelophylax*). The diversity of helminthes and the structure of their component community depend on the type, nature of the spawning water body and the presence of green frogs. Individuals of *B. bufo*, spawning in stagnant water bodies where green frogs live, have a more diverse helminth species composition, because there are helminthes of the second group, whose development is related to the aquatic environment. The structure of their community is more complex. *Bufo bufo*, when using flowing water bodies without green frogs for spawning, has a depleted helminth composition and a simplified structure of their community. The obtained results have demonstrated the influence of one allotopic amphibian species on the formation of helminth community of another species under conditions of temporary syntopic occurrence.

**Key words:** European common toad, helminth community, National Park «Smolny», nematodes, parasites, spawning water body, syntopic occurrence, trematodes

**Introduction**

Tailless amphibians (Anura) are very important in biocenoses. They are predators for a complex of aquatic and semi-aquatic invertebrate species (rarely of vertebrates). In addition, they are prey for vertebrate predators of a higher trophic level, represented by intermediate, intercalary (mesocercar), additional, paratenic (metacercar) and final hosts for helminthes of different taxa (Sessions & Ruth, 1990; Thiemann & Wassersug, 2000; González & Hamann, 2007; Chikhlyaev & Ruchin, 2014; Chikhlyaev et al., 2016a,b, 2018a,b, 2019a,b). Habitat conditions significantly influence the formation of a helminth fauna in amphibians. They form the features of its biology and ecology, i.e. lifestyle and breadth of the dietary spectrum (Ruchin et al., 2009; Hamann et al., 2013). Its basis is the biotopic distribution or the nature of the host's habitat. For example, amphibians from the humid lowland forest of Nigeria have a more diverse helminth fauna than those living in a brackish swamp (Aisien et al., 2017), mangroves (Aisien et al., 2015), or savannas

(Imasuen & Aisien, 2015). The *Lysapsus limellum* Cope, 1862, living in permanent ponds of Argentina with stable environmental conditions, has a stable helminth community with a high level of interspecific relationships, rather than just a temporary set of disparate species found in amphibians in drying water bodies (Kehr et al., 2000). This example highlights the importance of the constancy or dynamism of the host's habitat conditions during the formation of its helminth fauna.

*Bufo bufo* (Linnaeus, 1758) is a widespread species in Western Europe to Eastern Siberia. It prefers wet habitats such as woodlands, swamps, wet meadows and ravines, overgrown floodplain terraces of rivers and streams, and avoids open landscapes (Dujsebayaeva, 2006; Ruchin & Chikhlyaev, 2017; Korzikov & Aleksanov, 2018). Its biological and ecological patterns have been studied in detail over the largest part of its range in contrast to its parasite fauna, described only in certain regions of the River Volga basin, River Dnieper basin, as well as in the Urals and Siberia.

In the present study, we have estimated for the first time the influence of several environmental factors on the formation of a helminth community of *B. bufo* in natural populations. The correct assessment of parasite-host relationships and describing their dynamics and trends are possible only without any anthropogenic impact, highlighting the importance and necessity of parasitological research in Protected Areas (Kirillova & Kirillov, 2017a; Chikhlyayev et al., 2018a; Orlova & Orlov, 2019; Zharkikh et al., 2019). This study is aimed to characterise and analyse the species composition and structure of the helminth community and the invasion level of *B. bufo* in different habitats.

### Material and Methods

For this study, we collected helminthes from 85 *B. bufo* individuals, which were caught in five habitats in the National Park «Smolny» (Republic of Mordovia, Russia) in August 2018 and May 2019. The habitat characteristics of spawning water bodies are presented below.

Locality I (54.747027° N, 45.263173° E): Tashkinskiy pond has an area of 2000 m<sup>2</sup>. It is located on the River Chernushka (quarter 85 of the Kemlyanskoe forestry district). It is surrounded by a mixed forest (*Pinus sylvestris* L., *Betula pendula* Roth, *Alnus glutinosa* (L.) Gaertn.). The southern shore of the pond has a sandy beach. The water is clear. The aquatic flora is represented by *Typha latifolia* L., *Carex* spp., *Bidens tripartita* L. It is a habitat of *Pelophylax lessonae* (Camerano, 1882) and *P. esculentus* (Linnaeus, 1758), as well as a spawning site of *Rana temporaria* Linnaeus, 1758, *R. arvalis* Nilsson, 1842, *B. bufo*, *Pelobates vespertinus* (Pallas, 1771), *Lissotriton vulgaris* (Linnaeus, 1758), *Triturus cristatus* (Laurenti, 1768). The pond is surrounded by wood buildings and recreation area. It is experiencing a recreational load.

Locality II (54.795825° N, 45.356672° E): fire-prevention pond (quarter 22 of the Kemlyanskoe forestry district) is watered by the stream Rodnikovyi. In the north and west parts, it is separated from the highway «Saransk – Romodanovo – Bolshoe Ignatovo» by *Pinus sylvestris* forest and mixed forest. In the southern part, it is separated by a stream and *Alnus glutinosa* forest. The water mirror is hidden by a cover of *Lemna* spp. The shoreline vegetation is formed by *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia*, *Filipendula ulmaria* (L.) Maxim., *Urtica dioica* L., *Carex* spp. It is a habitat of *P. lessonae*, and a spawning site of *R. temporaria*, *R. arvalis*, and *B. bufo*. There is a spring and a forest

glade equipped with buildings and recreation places are represented nearby the pond.

Locality III (54.770598° N, 45.373335° E): a beaver (*Castor fiber* Linnaeus, 1758) dam is located on the stream Kuzoley (quarter 49 of the Kemlyanskoe forest district) in the vicinity of the non-residential village Vasilyevka. In the west part, the water body is surrounded by *Alnus glutinosa* thickets. In the east part, there are floodplain meadows. The water is covered by *Lemna* spp. *Carex* species grow along the shoreline. It is a spawning place of *R. arvalis* and *B. bufo*.

Locality IV (54.833494° N, 45.374085° E): this private pond is located in the village Obrezki (near the quarter 63 of the Lvovskoe forestry district). The pond's shores are steep. The western shoreline is overgrown with *Alnus glutinosa*, *Salix* sp., *Betula* sp., herbaceous plants (grasses (Poaceae), *Carex* spp., *Phragmites australis*, *Urtica dioica*). It is a habitat of *P. lessonae*, *P. esculentus*, *P. ridibundus* (Pallas, 1771), as well as a spawning site of *R. temporaria*, *R. arvalis*, *B. bufo*. This pond is stocked with fish. It is partially surrounded by residential low-rise and other buildings. It is affected by some recreational load.

Locality V (54.877361° N, 45.473218° E): it is the highway section extending about 1 km from the village Lesnoy to the highway 89K-12 «Saransk – Romodanovo – Bolshoe Ignatovo» (between quarter 2 and quarter 3 of the Alexandrovskoe forest district). Southward and parallel to the road, there is a stream overgrown with *Alnus glutinosa*, *Betula pendula*, *Salix* sp. and single *Pinus sylvestris*. In this place, amphibians often die under the vehicle wheels, especially during their mass evening migrations in the rain after a long drought (personal observation).

*Bufo bufo* was examined by the method of complete helminthological autopsy (Skrjabin, 1928). Collection, fixation and cameral processing of the material were performed according to Byhovskaya-Pavlovskaya (1985). The helminth species were identified using Ryzhikov et al. (1980).

The prevalence (P, %), intensity range (R, min-max, specimens), helminth abundance (A, specimens) are given to estimate the *B. bufo* infection by parasites. The degree of parasite dominance is estimated by the percentage of each species in the structure of the component community. We used the following scale per host sample: dominant has  $\geq 30\%$  of the total number of helminthes; subdominant has 10–30% of the total number of helminthes; common has 1–10% of the total number of helminthes; rare has 0.1–1.0% of the total number

of helminthes sample; single has 0.01–0.10% of the total number of helminthes (Kirillov, 2011; Burakova & Baytimirova, 2017). The Shannon ( $H'$ ) and Simpson (1-D) indices were used to estimate the species diversity of helminths. The similarity of helminth compositions was determined by cluster analysis on the basis of the Jaccard similarity index coefficient ( $C_j$ ) (Magurran, 1992). Statistical data processing was performed using the software Statistica 6.1 and Microsoft Excel 2003.

### Results

In the National Park «Smolny», the helminth fauna of *B. bufo* includes nine species from nine genera, six families, four orders and two classes, including five Trematoda species and four species of Chromadorea (Nematoda). The table presents values of contamination indicators and the proportion of each helminth species in the structure of the component community (Table). Eight species are widespread, polyhostal parasites of anurans. At the same time, one species, *Gorgoderina microovata* Fuhrmann, 1924, is an oligohostal trematode specific for Ranidae family. All revealed species parasitise on adult stages of animals. Amphibians serve as final hosts of these parasites. The trematode *Diplodiscus subclavatus* (Pallas, 1760) has

been registered for the first time in *B. bufo* in the Republic of Mordovia.

The helminth fauna structure of *B. bufo* includes two groups of helminthes. The first group consists of biohelminthes (trematodes), which develop with changing of hosts. The second groups contents geo-helminthes (nematodes), which have a direct development cycle. Biohelminthes are represented by five trematodes living in the bladder (*Gorgoderina microovata*, *Gorgoderina vitelliloba* (Olsson, 1876)) and intestinal tract (*Pleurogenes claviger* (Rudolphi, 1819), *Pleurogenoides medians* (Olsson, 1876), *Diplodiscus subclavatus*). Infection occurs through trophic chains and consumption of additional (metacercar) hosts. For *Gorgoderina microovata*, these additional hosts are larvae and imago of Odonata (Pigulevsky, 1952). For *Gorgoderina vitelliloba*, these hosts are tadpoles and froglets of amphibians (Pigulevsky, 1953; Kalabekov, 1976). For *Pleurogenes claviger* and *Pleurogenoides medians*, they are Coleoptera, Trichoptera, Ephemeroptera, Megaloptera, Diptera, Isopoda, and Amphipoda (Neuhaus, 1940; Khotenovsky, 1970; Grabda-Kazubska, 1971). *Bufo bufo* could be accidentally infested by the trematode *Diplodiscus subclavatus* by ingesting encysted adolescariae with water, silt or peeling epidermis (Skrjabin, 1949; Grabda-Kazubska, 1980).

**Table.** Helminths of *Bufo bufo* in the National Park «Smolny» (Russia)

Parasite	Pond Tashkinskiy (I)	Fire-prevention pond (II)	Village Vasilyevka (III)	Village Obrezki (IV)	Village Lesnoy (V)
<i>Gorgoderina microovata</i>	–	–	–	6.25(2)0.13 0.74	–
<i>Gorgoderina vitelliloba</i>	–	5.56(1)0.06 0.50	–	–	–
<i>Pleurogenes claviger</i>	5.00(39)1.95 5.42	–	–	–	–
<i>Pleurogenoides medians</i>	5.00(2)0.10 0.28	–	–	–	–
<i>Diplodiscus subclavatus</i>	–	16.67(1–1)0.17 1.49	–	–	–
<i>Rhabdias bufonis</i>	80.00(2–37)9.70 26.98	88.89(1–22)4.72 42.29	87.50(1–17)5.50 28.39	100(1–34)9.50 56.51	100(1–63)26.53 51.87
<i>Oswaldocruzia filiformis</i>	85.00(1–21)6.85 19.05	72.22(1–8)2.17 19.40	100(1–44)11.69 60.32	68.75(1–12)3.19 18.96	100(1–47)19.27 38.64
<i>Oxysomatium brevicaudatum</i>	85.00(1–114)17.35 48.26	66.67(1–18)4.06 36.32	62.50(1–13)2.19 11.29	56.25(1–30)3.00 17.84	33.33(2–8)1.87 3.74
<i>Cosmocerca ornata</i>	–	–	–	12.50(5–11)1.00 5.95	93.33(1–5)2.87 5.75
Number of helminth species	5	5	3	5	4
Trematoda	2	2	–	1	–
Nematoda	3	3	3	4	4
Number of <i>Bufo bufo</i> studied	20	18	16	16	15
Shannon diversity index, $H'$	1.20	1.16	0.90	1.15	0.99
Simpson index, 1-D	0.34	0.34	0.46	0.39	0.42

Note: prevalence (P, %) is in front of brackets; intensity range (R, specimens) is in brackets; abundance (A, specimens) is behind the brackets; the proportion of species in community (%) is on bottom.

Geohelminthes include four nematode species infecting *B. bufo* during the activity period with a break for wintering. Species such as *Oswaldocruzia filiformis* (Goeze, 1782), *Oxysomatium brevicaudatum* (Zeder, 1800) parasitise in the intestinal tract by ingesting them by oral contact between the host and invasive larvae on land (Hendrikx, 1983). There the nematode *Rhabdias bufonis* (Schrank, 1788) processes by several ways. Firstly, invasive larvae percutaneously penetrate from the soil by entering in the host's lungs with lymph and blood flows (Hartwich, 1975). The second way occurs through reservoir hosts, i.e. Oligochaeta, Mollusca (Savinov, 1963). The invasive *Cosmocerca ornata* (Dujardin, 1845) larvae infect *B. bufo* in water bodies, penetrating into the host at the surface water layer through the conjunctiva of the lower eyelid and migrating to a place of permanent localisation in the rectum (Kirillov & Kirillova, 2016; Kirillova & Kirillov, 2017b).

In amphibians from the Tashkinskiy pond (I), five helminth species were registered: two trematodes and three nematodes (Table). The total infestation rate was 100%. The overall abundance was 35.95 specimens. *Oswaldocruzia filiformis* (85.00%; 6.85 specimens) and *Oxysomatium brevicaudatum* (85.00%; 17.35 specimens) had the highest rates of invasion. *Oxysomatium brevicaudatum* was dominant (48.26%) in the helminth fauna of *B. bufo*. Other nematodes were subdominants. In the mentioned biotope, there were two trematodes, one common (*Pleurogenes claviger*, 5.42%) and one rare (*Pleurogenoides medians*, 0.28%), considered as parasites of *B. bufo* (Table).

In amphibians from the fire-prevention pond (II), five helminth species were found, including two trematodes and three nematodes (Table). The total infestation rate was 88.89%. The total abundance was minimal (11.17 specimens). *Rhabdias bufonis* was found most frequently (88.89%; 4.72 specimens). This species and the nematode *Oxysomatium brevicaudatum* were dominant parasites (42.29 and 36.32%, respectively). One more geohelminth belongs to the subdominant group. The trematodes are characterised as common (*Diplodiscus subclavatus*, 1.49%) and rare (*Gorgoderina vitelliloba*, 0.50%) parasites of the *B. bufo* (Table).

In *B. bufo* from the beaver dam near the village Vasilyevka (III), a minimal number of helminth species was found, including three nematodes (Table). The total infestation rate was maximal, 100%. The total abundance was on average at 19.38 specimens.

The nematode *Oswaldocruzia filiformis* had the highest rate of invasion (100%; 11.69 specimens). It also serves as a dominant parasite (60.32%). The remaining species are subdominants. The structure of the helminth community is simplified due to the absence of trematodes (Table).

Five species of helminths were found in amphibians from the pond in the village Obrezki (IV), including one trematode and four nematodes (Table). The total infestation rate was maximal – 100%; the total abundance was insignificant (16.81 specimens). The most common species is *Rhabdias bufonis* (100%; 9.50 specimens), which is the only dominant species in this biotope (56.51%); two other species of geohelminths are subdominants. The nematode *Cosmocerca ornata* (5.95%) and the trematode *Gorgoderina microovata* (0.74%) belong to the groups of common and rare parasites, respectively (Table).

In the vicinity of the village Lesnoy (V), four helminth species were found in *B. bufo*, including four nematodes (Table). The total infection rate was 100%. The total abundance was also maximal (49.60 specimens). *Rhabdias bufonis* (100%; 26.53 specimens) and *Oswaldocruzia filiformis* (100%; 19.27 specimens) reached the peak of infestation. They are also the dominant parasites of this host (51.87 and 38.64%, respectively). Other nematodes, *Cosmocerca ornata* and *Oxysomatium brevicaudatum*, are common parasites (5.75% and 3.74%, respectively) of *B. bufo*. The structure of the helminth community is also simplified due to the absence of trematodes and subdominant species (Table).

The highest variety of helminths (five species) was observed in amphibians in the pond Tashkinskiy (I), fire-prevention pond (II) and pond in the village Obrezki (IV). A smaller number (four species) has been recorded in the vicinity of the village Lesnoy (V). The lowest number of helminthes (three species) was found in *B. bufo* living near the village Vasilyevka (III) (Table).

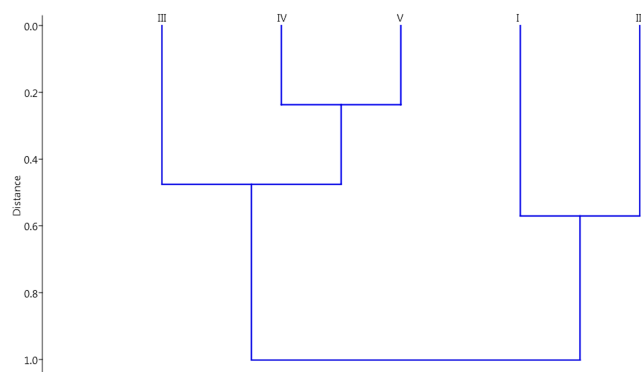
Only three nematodes were recorded in all studied biotopes. These were *Rhabdias bufonis*, *Oswaldocruzia filiformis* and *Oxysomatium brevicaudatum*. The nematode *Cosmocerca ornata* was found only in the village Obrezki (IV) and the village Lesnoy (V). Both habitats are located in the northern part of the National Park «Smolny». On the other hand, some trematodes have a strict narrow distribution. *Pleurogenes claviger* and *Pleurogenoides medians* were recorded in *B. bufo* in the pond Tashkinskiy (I). *Gorgoderina vitelliloba* and *Diplodiscus*

*subclavatus* were recorded in the fire-prevention pond (II). *Gorgoderia microovata* was recorded in the pond in the village Obrezki (IV). No trematodes were found in other locations (Table).

In the pond Tashkinskiy (I), *B. bufo* had the highest species diversity of helminthes ( $H' = 1.20$ ). In the fire-prevention pond (II), amphibians had a lower species diversity ( $H' = 1.16$ ), as well as those from the village Obrezki (IV) ( $H' = 1.15$ ). The helminth fauna had the least diversity and higher evenness in the vicinity of the village Lesnoy (V) (Simpson's index = 0.42) and in the stream Kuzoley nearby the village Vasilyevka (III) (Simpson's index = 0.46) (Table). The detected differences are significant at the level of 95%.

We compared the helminth compositions of *B. bufo* from different habitats using a cluster analysis on the basis of the Jaccard similarity coefficient ( $C_j$ ) (Fig.). The dendrogram shows that the pond Tashkinskiy (I) and the fire-prevention pond (II) were the most different from the others ( $C_j = 0.43$ ). This is due to a very specific composition of trematodes (Table). On the other hand, the village Obrezki (IV) and the village Lesnoy (V) are the most similar ( $C_j = 0.80$ ) in helminth composition. The presence of all four nematodes contributes to this result (Table).

To assess completely the helminth fauna of *B. bufo*, we took into account not only the qualitative and quantitative composition of its helminths, but also the values of infection indicators. A comparative analysis of trematode infestations is not possible, since their records are very rare, while the infection rate of *B. bufo* by most of their species, except *Diplodiscus subclavatus*, did not exceed 10% (Table).



**Fig.** Dendrogram of helminth community similarity on the basis of the Jaccard similarity index ( $C_j$ ). Designations: I – pond Tashkinskiy; II – fire-prevention pond on the stream Rodnikovyi; III – beaver dam on the stream Kuzoley near the former village Vasilyevka; IV – pond in the village Obrezki; V – village Lesnoy.

The infestation by nematodes depends on the biotope features. *Rhabdias bufonis* occurs in all five biotopes. It has the highest rate of infestation in *B. bufo* in the pond in the village Obrezki (IV) and the village Lesnoy (V) (80.00–100.00%; 4.72–26.53 specimens). *Oswaldocruzia filiformis* has lower infection values (68.75–100.00%; 2.17–19.27 specimens). It reaches a peak in the village Vasilyevka (III) and the village Lesnoy (V). *Oxysomatium brevicaudatum* is an example of a wide variation in the degree of infestation from the smallest in the village Lesnoy (V) to the highest in the pond Tashkinskiy (I) (33.33–85.00%; 1.87–17.35 specimens). The infection of *B. bufo* with *Cosmocerca ornata* differs sharply in different habitats. It varies from low values in the village Obrezki (IV) to high values in the village Lesnoy (V) (12.50–93.33%; 1.00–2.87 specimens) (Table).

Depending on the biotope, the proportion of individual helminth species varies in the structure of the *B. bufo* helminth fauna. The number and composition of dominant, subdominant, common and rare species varies. Helminths belonging to the category of single species are absent in *B. bufo* (Table). Three trematodes represent a group of rare parasite species. Each of them was registered only one time in one of the following habitats: the pond Tashkinskiy (I), fire-prevention pond (II), the village Obrezki (IV). The group of common parasites consists of four species of trematodes and nematodes. One to two species of them occur in all habitats, except for the village Vasilyevka (III). Three nematodes are subdominants. Of them, one to two species are present in four habitats, except for the village Lesnoy (V). The group of dominant species is represented by three species of nematodes, which are present in *B. bufo* in all habitats in the amount of 1–2 species (Table).

Of the parasite species known in all five locations, there is no species, which is dominant in all these habitats (Table). All these species vary in the degree of dominance from the dominant helminth to the common one in different habitats. *Rhabdias bufonis* is dominant in three studied locations, while it is a subdominant in the pond Tashkinskiy (I) and the village Vasilyevka (III). *Oswaldocruzia filiformis* is dominant in the village Vasilyevka (III), village Lesnoy (V), being a subdominant in the other three habitats. *Oxysomatium brevicaudatum* is even more variable. It is a dominant parasite in the pond Tashkinskiy (I) and fire-prevention pond (II), while it is a subdominant in the village Vasilyevka (III), village Obrezki (IV), and, finally,

it is a common helminth species in the vicinity of the village Lesnoy (V). *Cosmocerca ornata* is a common parasite in the village Obrezki (IV) and the village Lesnoy (V) (Table).

### Discussion

The helminth fauna of *B. bufo* in the National Park «Smolny» is not numerous (nine species), typical for this host species. However, the studied *B. bufo* helminth fauna is more diverse than in other parts of the species range, such as in the upper Volga region (Chikhlyayev et al., 2016b) or in the Urals (Burakova & Vershinin, 2016; Burakova & Baytimirova, 2017).

There are two groups of species forming the helminth fauna of *B. bufo*. They differ in the life cycle and the invasion way of their host, infestation parameters, the degree of dominance and distribution in habitats. The nematodes-geohelminthes represent the first group. They are found on a regular basis and regardless of the habitat. Three of the four species (*Rhabdias bufonis*, *Oswaldocruzia filiformis* and *Oxysomatium brevicaudatum*) were recorded in all five habitats. Their prevalence and infestation vary by habitat but remain at a high level. These species are dominant and subdominant in the structure of the helminth community and form the core of the helminth fauna of *B. bufo*. This is caused by the terrestrial lifestyle of *B. bufo* under conditions of moisture forest on the one hand, and the specific life cycle of nematodes and the way of their invasion into the host on the other hand. The nematodes-geohelminthes have a direct life cycle, the initial development phase of which occurs on the soil surface. Therefore, terrestrial amphibians are able to closely contact and become infected with invasive larvae.

*Cosmocerca ornata* is an exception. It differs from the previous three nematodes by the way of invasion in the aquatic environment. Therefore, it is closer to the second group of helminthes. Its favourite hosts are aquatic amphibian species (*Pelophylax*, *Bombina*), while it occurs rarely and with variable infestation in *B. bufo* (Grabda-Kazubska & Tenora, 1991; Düşen, 2011; Chikhlyayev et al., 2019b).

Biohelminthes are trematodes belonging to the second group. They are found sporadically and locally. They are not typical parasites of *B. bufo* but make the helminth community more unique. Each of the five trematode species (*Gorgoderina microovata*, *Gorgoderina vitelliloba*, *Pleurogenes claviger*, *Pleurogenoides medians*, and *Diplodiscus subclavatus*) was found in only one of the five

studied habitats. Their number and infestation are low, so the structure of the helminth community is mainly occupied by rare species of parasites. *Bufo bufo*'s lifestyle is different from the trematode life cycle. This is the main reason for such low values of infestation by them. The development of trematodes is closely related to the aquatic environment. It is performed with the change of hosts, which are aquatic and semi-aquatic animals. *Bufo bufo* is known to prey on ground-crawling groups of invertebrates (Formicidae, Myriapoda, Coleoptera, Arachnida, Mollusca). When this amphibian is located in the water during the breeding season, *B. bufo* is characterised by a «mating diet» (Cornish et al., 1995; Crnobrnja-Isailović et al., 2012; Ruchin & Chikhlyayev, 2017; Čadenović et al., 2018). Available data on *B. bufo*'s diet after wintering and before spawning in water bodies (Kołenda et al., 2019) do not contradict to the above mentioned. This assumption that some amphibians have a «mating diet» is common in the literature, but it is not proven yet (Bókony et al., 2018). This may refer to casual observations (Tinsley & Jackson, 1988), or to studies of the water period before or during spawning (Jørgensen et al., 1978). Consumption of terrestrial invertebrates, which are not a part of the development cycles of trematodes, does not affect the infestation of *B. bufo*. However, the presence of several trematodes in the helminth community indicates that the infestation occurs somehow, but its ways are unclear. Probably, during the «mating dances» *B. bufo* accidentally swallows adolestercariae cysts or small planktonic invertebrates (e.g. Amphipoda) infected with metacercariae of trematodes. The low rate of invasion indirectly indicates the randomness of this process.

All trematodes were recorded in *B. bufo* only in stagnant water bodies (ponds) where their common hosts (*Pelophylax* species) inhabit. *Bufo bufo* living in flowing water bodies (streams) or caught near them were infected exclusively with nematodes. This is caused by the fact that the existence of trematodes is determined by the presence of their intermediate (Mollusca) and additional (Arthropoda) hosts in water bodies. The diversity, population density and infestation degree in invertebrates were higher in habitats rich on macrophytes (Frolova, 1975), growing massively in stagnant water bodies with a muddy bottom and warm waters. This type of water bodies (ponds, lakes, oxbows) is the typical habitat of *Pelophylax* frogs which are the final hosts of trematodes. Infestation by these parasites occurs by consumption of

aquatic and semi-aquatic invertebrates (Krizmanić & Ivanović, 2010; Paunović et al., 2010; Askenderov et al., 2018; Čavlović et al., 2018; Fayzulin et al., 2018; Ivanov et al., 2019). The diversity of vertebrates (final hosts) in a habitat determines the species composition of trematodes by ensuring the penetration of invasive eggs into environment (Altman, 2010; Johnson et al., 2016).

Flowing water bodies are less suitable for development of lifecycles of trematodes, as the current washes away the eggs, cysts and invasive planktonic larvae, and the unheated water slows down their development. The sandy littoral is poorly populated by both aquatic vegetation and intermediate invertebrate hosts (Ginetsinskaya, 1968, 1983; Frolova, 1975). Stream-type habitats are also ignored by the final hosts (*Pelophylax* frogs) due to a lack of food. All these factors ultimately create unfavourable conditions for the development of trematodes in flowing water bodies and prevent their infestation of amphibians in general.

Noteworthy, there is a lack of helminth larvae represented by metacercariae of trematodes in amphibians. This is explained by patterns of *B. bufo*'s biology and ecology. The dense skin and the toxic effect of skin secretions serve as a barrier to the cercariae coming from the water. The barriers for cercariae coming through the feed with reservoir hosts are the «mating diet», terrestrial lifestyle and feeding on terrestrial food objects, which are not included in the development cycles of trematodes.

### Conclusions

The study of *B. bufo* helminth fauna in the National Park «Smolny» revealed habitat differences between helminth communities in individual host populations. We found that amphibians spawning in stagnant water bodies, where *Pelophylax* frogs inhabit, have a more diverse species composition of helminths and a more complex community structure (four categories: dominants, subdominants, common, rare). Amphibians using flowing water bodies without *Pelophylax* for spawning have a depleted helminth composition and a simplified structure of their community (two categories: dominants, subdominants). Thus, the helminth diversity and the complexity of their community structure in *B. bufo* depend on the type and nature of the water body (i.e. with stagnant or flowing environmental conditions) used as a spawning site. The differences are caused in the presence or absence of the helminth species developing in the aquatic environment (trematodes, nematode *Cosmocerca ornata*).

Their main final hosts are *Pelophylax* frogs. Therefore their presence in the water body is important for the formation of the helminth fauna of *B. bufo*. The mentioned amphibians are allotopic in relation to each other due to lifestyle patterns. Their ecological niches are separated spatially. But they may partially overlap during the breeding season because *B. bufo* spawns earlier and usually leaves the spawning pond before or rarely at the time of the emergence of *Pelophylax* frogs in the water body. Thus, it is possible to assert the influence of one allotopic amphibian species on the formation of helminth fauna in another species under conditions of temporary syntopic occurrence.

The results of the study generally repeat and complement the conclusions made earlier in relation to *Pelophylax ridibundus*, *Natrix natrix* (Linnaeus, 1758), and *N. tessellata* (Laurenti, 1768) in different habitats in the National Park «Samar-skaya Luka» (Chikhlyayev et al., 2018a; Kirillov & Kirillova, 2019). This shows the unity of formation patterns of helminth fauna in different species of lower terrestrial vertebrates under the influence of environmental factors.

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## ЭКОЛОГИЧЕСКИЙ АНАЛИЗ ГЕЛЬМИНТОФАУНЫ *BUFO BUFO* (AMPHIBIA: ANURA) ИЗ РАЗНЫХ МЕСТООБИТАНИЙ

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Приводится экологический анализ видового состава, структуры сообщества гельминтов и степени инвазии ими у *Bufo bufo* из разных местообитаний в национальном парке «Смольный» (Республика Мордовия, Россия). В основе – материалы собственных исследований авторов, собранные в 2018–2019 гг. из пяти биотопов, различающихся по особенностям нерестового водоема. Обнаружено девять видов гельминтов, в т.ч. пять видов Trematoda и четыре вида Chromadorea (Nematoda). Выделяются две группы видов. Первая – развивающиеся на суше – нематоды (*Rhabdias bufonis*, *Oswaldocruzia filiformis*, *Oxysomatium brevicaudatum*), которые встречаются регулярно, независимо от места обитания, с высокой степенью инвазии и доминируют в структуре сообщества гельминтов. Вторая – развивающиеся в воде – нематода *Cosmocerca ornata* и трематоды, попадающиеся спорадично, локально и с низкой зараженностью. Виды второй группы являются типичными паразитами зеленых лягушек (род *Pelophylax*). Разнообразие состава гельминтов и структура их компонентного сообщества зависит от типа, характера нерестового водоема и наличия в них лягушек рода *Pelophylax*. *Bufo bufo*, нерестящиеся в стоячих водоемах, где обитают лягушки рода *Pelophylax*, обладают более разнообразным видовым составом гельминтов (за счет присутствия гельминтов второй группы, развитие которых связано с водной средой), а структура их сообщества сложнее. *Bufo bufo*, использующие для икрометания проточные водоемы без лягушек рода *Pelophylax*, имеют обедненный состав гельминтов и упрощенную структуру их сообщества. Полученные результаты демонстрируют влияние одного аллотопичного вида земноводных на формирование гельминтофауны другого вида в условиях временной синтопичности.

**Ключевые слова:** обыкновенная жаба, национальный парк «Смольный», нематоды, нерестовый водоем, паразиты, синтопичность, сообщество гельминтов, трематоды