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PARASITOLOGY OF HYDROBIONTS

Helminths of Gray Toad (*Bufo bufo*) Fingerlings in Specially Protected Natural Areas of the Urals and Analysis of Its Parasite Fauna on the Territory of Russia

A. V. Burakova[a, *] **and E. A. Malkova**[a]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia*
**e-mail: annabios@list.ru*

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Abstract—The helminths of fingerlings of the gray toad *Bufo bufo* (Linnaeus, 1758) have been studied in some specially protected natural areas (protected areas (PAs)) of the Urals. Only four species of helminths belonging to a Nematoda type are found. The nematodes *Oswaldocruzia filiformis* and *Rhabdias bufonis* are common for the studied populations of amphibians. The species composition of geohelminths does not differ in fingerlings or sexually mature amphibians; the infection in adult toads is higher than in fingerlings. The analysis of helminth fauna in the gray toad demonstrated that the nematodes *Oswaldocruzia filiformis* and *Rhabdias bufonis* dominate in *Bufo bufo* in most regions of Russia. The richest species composition of trematodes is noted in the gray toads of Mordovia.

Keywords: helminths, fingerlings, gray toad, *Bufo bufo*, species diversity, PA, Urals, regions of Russia

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INTRODUCTION

As the most common and diverse form of life on Earth, parasites are an integral part of biocenosis and serve as one of the factors of the dynamics of host animal population size, as well as an instrument of natural selection, affecting different parameters of the host (Blanchet et al., 2009). Tailless amphibians act as predators for a number of invertebrate species and are involved in the circulation of a large number of helminths typical for freshwater and terrestrial ecosystems (Garanin, 1997; Chikhlyaev and Ruchin, 2014).

As a rule, specially protected natural areas (protected areas (PAs)) are characterized by a rich biodiversity of flora and fauna (not excluding parasites) and can serve as standards for natural and minimally disturbed ecosystems.

The gray toad *Bufo bufo* (Linnaeus, 1758) covers a vast area and is widespread in Europe and Western Siberia and is found in Eastern Siberia. The northern border runs from the northern coast of the White Sea through Arkhangelsk oblast, the Komi Republic, the Urals, Tyumen oblast, Krasnoyarsk krai, and Irkutsk oblast. The southern border runs along the southwest of Belgorod oblast, north of Saratov and Orenburg oblasts, and further to the east (Vershinin, 2007).

The gray toad belongs to hygrophilic amphibian species, prefers mixed and swampy coniferous forests, avoids open spaces far from reservoirs, which is associated with its low migratory activity. Terrestrial forms of invertebrates (slugs, mollusks, sedentary insect larvae and imago; myrmecophagy (eating ants) is common) are mainly the food objects (Vershinin, 2007; Kuz'min, 2012).

The helminth fauna of amphibians is formed depending on a number of factors (ecological and morphological peculiarities of a certain host species and ecology and biology of intermediate hosts and helminths), including the host age. It is known that the extensiveness and intensity of infection, as well as the diversity of parasites, increase with the host age (the older the animal, the longer it contacts the parasite) (Kennedi, 1978). Fingerlings are mainly invaded by nematodes (helminths, the life cycle of which proceeds without the involvement of intermediate hosts (except for Monogenea)), which is associated with changes in food preferences and lifestyle at different stages of amphibian development (Dogel', 1962; Kennedi, 1978).

The parasite fauna of the gray toad in Russia (Chikhlyaev et al., 2020) and, particularly, the Urals, remains insufficiently studied. Previously, data were obtained on the species composition of helminths of

Abbreviations: PA, protected areas; *A*, abundance index; *I*, invasion intensity; *P*, invasion extensiveness.

gray toad fingerlings in the territory of the Middle Urals (Lake Peschanoe (Sverdlovsk oblast)) (Burakova and Vershinin, 2016) and on the helminths of its sexually mature individuals living in the vicinity of the Visimsky State Natural Biosphere Reserve (Burakova and Baitimirova, 2017).

In this regard, carrying out parasitological work in PAs is of a particular importance, because they allow one to obtain not only information on the species composition of helminths in the protected areas, but also conduct a control in order to identify epidemiologically dangerous parasite species. This study on the diversity of helminths of *B. bufo* fingerlings in some PA is a continuation and addition to the work on the study of the helminth fauna of different sizes and age groups of the gray toad living at the territory of the Urals (Burakova and Baitimirova, 2017). The use of only fingerlings in the work makes it possible to exclude the negative effect on the reproductive nucleus of the gray toad population in PAs.

The aim of this work was to study the helminths of the gray toad (*B. bufo*) fingerlings in some PAs of the Urals and analyze its parasite fauna in Russia according to the literature data.

MATERIALS AND METHODS

Habitats. The material was collected in July and August 2016 and 2017 in the vicinity of the village of Bol'shie Galashki (57°28′14″ N, 59°29′40″ E), habitat 1 (H1); in the territory of the Taganay National Park (55°13′14.57″ N, 59°44′7.13″ E), habitat 2 (H2); and in the vicinity of Lake Tavatui (57°08′18″ N, 60°11′21″ E), habitat 3 (H3). In H1, 25 toad specimens were caught; in H2, 14 specimens were caught; and, in H3, 41 specimens were caught.

H1 is adjacent to the protected zone of the Visimsky State Natural Biosphere Reserve, which has a federal significance. The reserve is located in the subzone of southern taiga of the Middle Urals (Sverdlovsk oblast) in the upper reaches of right tributaries of the Chusovaya, Sulem, Dar'a, and Shishim rivers. In the valleys and on lower parts of the slopes, there are boreal swampy spruce forests and spruce–birch forests.[1] Animal captures were carried out in the village of Bol'shie Galashki along the banks of the upper reaches of the Sulem River, where amphibians spawn. The Sulem River originates from a series of short sources in the highest, eastern part of the reserve. The water is carbonate type, slightly mineralized (Arkhipova, 2001). The village is surrounded by the reserve. This is a protected area with limited access (access system).

The territory of the Taganay National Park (H2) is located in the northern part of the Zlatoustovsky and Kusinsky districts of Chelyabinsk oblast.[2] This park of federal significance belongs to the Ural mountain–forest vegetation region of the forest zone and includes a subzone of mountainous southern taiga dark coniferous forests with fragments of mixed deciduous–dark coniferous forests (Rusyaeva, 2007). Animal captures were carried out in the vicinity of the Central Estate on the side of the tourist path, where animals go out to feed at dusk. The path borders on the central car parking and a tourist meadow with tourist houses and campfires located on it. The nearest small reservoir (area 1771 m^2), which amphibians use for breeding, is located 130 m in a straight line (to the south) from the place the animals were captured.

H3 has a high recreational significance (without an access system). This is the Lake Tavatui with Surrounding Forests State Landscape Nature Reserve, which has a regional significance.[3] Lake Tavatui is located in the Nevyansky district of Sverdlovsk oblast in the subzone of southern taiga of mountain forest region of the Middle Urals and includes spruce–fir and mixed forests (Kolesnikov, 1969). The lake belongs to the Neyva River basin and forms a single system with Verkh-Neyvinsky Pond. The drain into the pond is located in the north of the lake. The animal captures were carried out in the northern part of Lake Tavatui at the territory of the Tavatui children's recreational camp. The coastline in this part of the lake is partially swampy. The amphibians were caught on the border of two environments (water and land), as well as on the nearest paths.

Infestation with macroparasites. Helminths were identified using standard methods (Ryzhikov et al., 1980). The infestation of amphibians was estimated according to the following indices: *P*, portion of infected host individuals in the studied sample (%); *A*, average number of parasites of a certain species or group of parasites in all host individuals, specimens/host individual; *I*, average number of helminths per one studied host individual, specimens/host individual (Breev, 1976).

The dominance structure was analyzed using a previously described approach (Kirillov, 2011): dominants are species reaching ≥30% of the total number of animals in the composition of the component community; subdominants, 10–30%; ordinary, 1–10%; rare, 0.1–1%; and single, 0.01–0.1%. Statistical data processing was carried out using a Quantitative Parasitology program (Rozsa et al., 2000). For pairwise

[1] Cadastral report on the Visimsky State Natural Biosphere Reserve PA, 2022, PAs of Russia. http://www.oopt.aari.ru/oopt/ [Cited February 24, 2022].

[2] Cadastral report on Taganay Natural Park PA, 2022, PAs of Russia. http://www.oopt.aari.ru/oopt/ [Cited February 24, 2022].

[3] Cadastral report on the State Landscape Nature Reserve of regional significance "Lake Tavatui with surrounding forests" PA, 2022, PAs of Russia. http://www.oopt.aari.ru/oopt/ [Cited February 24, 2022].

Table 1. Infestation of fingerlings of gray toads with parasites in some specially protected natural areas of the Urals (M1 and M2)

Parasite taxon	P (CI)/A (CI)		I (CI)		I min–max	
	H1	H2	H1	H2	H1	H2
Nematoda	$\frac{36.0^{*}\,(17.97\text{–}57.48)}{1.6\,(0.72\text{–}3.44)}$	$\frac{28.6\,(8.38\text{–}58.11)}{0.64\,(0.14\text{–}1.43)}$	4.44 (2.44–8.22)	2.25 (1.25–3.25)	1–14	1–4
Oswaldocruzia filiformis (Goeze, 1782)[1]	$\frac{24.0\,(9.35\text{–}45.13)}{0.40\,(0.12\text{–}1.04)}$	$\frac{28.6\,(8.38\text{–}58.11)}{0.43\,(0.07\text{–}1.0)}$	1.67 (1.0–2.33)	1.5 (1–2)	1–5	1–3
Neoraillietnema praeputiale (Skrjabin, 1916)[1]	$\frac{4.0\,(0.1\text{–}20.36)}{0.04\,(0\text{–}0.12)}$	0	1	0	0–1	0
Cosmocerca ornata (Dujardin, 1845)[1]	$\frac{36.0\,(17.97\text{–}57.48)}{0.8\,(0.36\text{–}1.52)}$	0	2.22 (1.33–3.44)	0	1–5	0
Rhabdias bufonis (Schrank, 1788)[2]	$\frac{24.0^{*}\,(9.35\text{–}45.13)}{0.36^{*}\,(0.12\text{–}0.72)}$	$\frac{21.4\,(4.65\text{–}50.80)}{0.21\,(0\text{–}0.43)}$	1.5* (1–2.17)	1	1–3	0–1

P, invasion extensiveness, %; *A*, abundance index, specimens/host individual; *I*, invasion intensity, specimens/host individual; CI, 95% confidence interval; *I* min-max, the minimum and maximum number of specimens of a parasite of a certain species in a single host individual; [1] parasites of intestinal localization; and [2] parasites of lung localization. No helminths were noted in toads inhabiting H3.
* Significant differences between populations H1 and H2 ($p < 0.05$).

comparisons of the number of nematodes in sexually mature individuals and fingerlings, a Mann–Whitney test (U-test) (Statistica 8.0) was applied.

RESULTS

In general, four helminth species belonging to the Nematoda type (Table 1), which are widespread in terrestrial cold-blooded vertebrates of the Palearctic, were found in *B. bufo* in the studied territories.

The infestation of fingerlings of toads with nematodes is significantly higher in the population from the territory H1 when compared with H2 (Table 1). Two nematode species (*Oswaldocruzia filiformis* (Goeze, 1782) and *Rhabdias bufonis* (Schrank, 1788)) are common for the amphibian populations of H1 and H2.

Four nematode species were identified at in H1 (Table 1). *Cosmocerca ornata* (Dujardin, 1845) dominates by the infestation indices and portion (50%) in the component community. Subdominant species in H1 include *Oswaldocruzia filiformis* (25%) and *Rhabdias bufonis* (22.5%). Fingerlings of toads are significantly higher invaded by a lung helminth *R. bufonis* at the territory H1 (Table 1) as compared with H2. The nematode *Neoraillietnema praeputiale* (Skrjabin, 1916) (2.5%) is presented as a common species.

In H2, two species of nematodes (*Oswaldocruzia filiformis* (66.67%) and *Rhabdias bufonis* (33.33%)) were noted in the parasite fauna of the gray toad population. No species *Cosmocerca ornata* and *Neoraillietnema praeputiale* were found. Despite the absence of significant differences, the infestation of *Oswaldocruzia filiformis* in gray toads is higher here than in H1. No helminths were noted in the toads living in H3. Since *Cosmocerca ornata* was noted in the toad fingerlings in H1; no estimation of territorial differences in the infestation of amphibians with this helminth in comparison with other territories was carried out.

Based on the analysis of literature data on the helminth fauna of the gray toad in Russia, 17 species, including 8 nematode species and 9 trematode species, were identified (Table 2). The greatest diversity in the species composition of helminths was registered in the Republic of Mordovia. Twelve species (4 nematode species and 8 trematode species) were found here.

For most of the considered regions of the Russian Federation, the nematodes *Oswaldocruzia filiformis* and *Rhabdias bufonis* are assigned to the dominant species; the nematode *Cosmocerca ornate* is the third most common species in Russia (Table 2). Species found only in one territory were identified: the trematode *Strigea strigis* was noted in gray toads inhabiting Kaliningrad oblast; the nematode *Spauligodon pseudoeremiasi* was found in Perm krai.

DISCUSSION

Part of the life cycle of *Oswaldocruzia filiformis, Rhabdias bufonis,* and *Neoraillietnema praeputiale* takes place in the soil and on plants; these are live larvae or eggs. Gray toads are invaded with *O. filiformis* orally in the case of accidental contact with infective larvae on the land (Hendrikx, 1983). The toads can be infected with the nematode *Rhabdias bufonis* when the larvae penetrate through the skin from the soil that then enter the host lungs with lymph and blood flows (Hartwich, 1975), as well as through the reservoir hosts (oligochaetes and mollusks) (Savinov, 1963).

Table 2. Helminth fauna of the gray toad inhabiting Russia

Region	Nematoda								Trematoda									Literature source
	RB	OF	OG	AA	CO	NB	NP	SP	SS	GC	GA	GM	GVi	PC	PM	AM	DS	
Kaliningrad oblast	+		+		+		+		+									Golikova, 1960
Republic of Karelia	+	+			+													Fakhrutdinova, 2020
Kaluga oblast	+	+			+													Chikhlyaev et al., 2016
Vologda oblast	+	+		+	+	+	+							+				Radchenko and Shabunov, 2008
Republic of Mordovia	+	+			+	+				+	+	+	+	+	+	+	+	Lukiyanov et al., 2009; Chikhlyaev et al., 2015, 2016, 2020; Chikhlyaev and Ruchin, 2020
Kostroma oblast	+	+		+	+		+						+					Radchenko and Budalova, 1980; Churkina and Kilesova, 2020; Volkova, 2021
Chuvash Republic	+	+		+	+	+				+			+					Chikhlyaev et al., 2010
Republic of Bashkortostan		+		+		+												Bayanov, 1992; Petrova and Bayanov, 2000; Yumagulova, 2000
Chelyabinsk oblast	+																	Yumagulova, 2000
Perm krai	+		+					+										Golubchikova et al., 2019
Sverdlovsk oblast	+	+			+		+											Burakova and Vershinin, 2016; Burakova and Baitimirova, 2017
Tomsk oblast	+	+																Kuranova, 1988

RB, *Rhabdias bufonis* (Schrank, 1788); OF, *Oswaldocruzia filiformis* (Goeze, 1782); OG, *Oswaldocruzia goezei* Skrjabin et Schulz, 1952; AA, *Aplectana acuminata* (Schrank, 1788); CO, *Cosmocerca ornata* (Dujardin, 1845); NB, *Neoxysomatium brevicaudatum* (Zeder, 1800); NP, *Neoraillietnema praeputiale* (Skrjabin, 1916); SP, *Spauligodon pseudoeremiasi* (Markov et Bogdanov, 1961); SS, *Strigea strigis* (Schrank, 1788), mtc. (=*Tetracotyle strigis* (Schrank, 1788)); GC, *Gorgodera cygnoides* (Zeder, 1800); GA, *G. asiatica* Pigulewski, 1943; GM, *G. microovata* Fuhrmann, 1924; GVi, *Gorgoderina vitelliloba* (Olsson, 1876); PC, *Pleurogenes claviger* (Rudolphi, 1819); PM, *Pleurogenoides medians* (Olsson, 1876); AM, *Astiotrema monticelli* Stossich, 1904, mtc.; and DS, *Diplodiscus subclavatus* (Pallas, 1760).

The infection with *Neoraillietnema praeputiale* is carried out without the involvement of intermediate hosts.

A high infestation with *Oswaldocruzia filiformis* in the populations of gray toads in H1 and H2 is probably caused by a synchronization of the metamorphosis of fingerlings and the maturation of invasive larvae from a new generation of helminths. Both processes occur towards the end of July or in the beginning of August (Vershinin, 2007; Aralkhanova, 2010). The difference in the level of infestation of fingerlings in these habitats is obviously caused by the ecological peculiarities of the habitat.

It was demonstrated (Vakker, 2018) that the development of relatively large eggs of the nematode *O. filiformis* requires optimal conditions: biotopes with sufficient soil moisture, air temperature ~14 to 15°C, the presence of a well-moistened coastal strip, dense vegetation cover on it, free standing trees creating a shade, and shrubs and reeds required for the movement of the larvae of this nematode. On the contrary, in biotopes in which the temperature in July reaches 22 to 25°C, the reservoirs with open sandy shores slightly overgrown with willow are the conditions preventing the implementation of the life cycle of *O. filiformis*. The eggs and larvae of the nematode *Rhabdias bufonis* ripen in the soil when there is a large amount of precipitation; that is, they also need a sufficient substrate moisture to implement the life cycle of the species (Barton, 1998).

Thus, environment humidity, as well as the presence of abundant vegetation cover, are important for maintaining the viability of the eggs and larvae of helminths, which is directly associated with the probability of infecting amphibians with them (Vakker, 2018; Silva et al., 2018). The captures of toads in H1 were carried out in the floodplain of the Sulem River, where the soil humidity is high. In H2, the animals were caught on the side of the tourist path, near the central parking and the tourist meadow; the nearest reservoir was located 130 m from the place of capture, and the ground here was less moist as compared with H1. The high infestation of *Bufo bufo* fingerlings with nematodes in H1 is probably associated with that. Taking into account the above peculiarities of H2, the effect of additional factors cannot be excluded: traffic, tourism, the development of the path network, the increased anxiety of the animals (Vershinin, 1997), etc.

Despite the capture of toads in close proximity to the reservoir (habitat with a sufficient moisture), no helminths were found in the toads in H3. Lake Tavatui is located within the Upper Iset granite massif. The granites come to the surface; therefore, the bottom and shores are mostly rocky or sandy. Such conditions can interfere with the implementation of the helminth life cycle (Vakker, 2018; Silva et al., 2018). Since the lake shore is a popular recreation area, a complex of unfavorable anthropogenic factors can be an additional reason for the reduction in the species diversity of helminths (as well as in H2) (Chikhlyaev et al., 2017).

The larvae of *Cosmocerca ornata* (age III) of the gray toad fingerling invade the host in the near-surface water layer, penetrating through the conjunctiva of the lower eyelid (Kirillova and Kirillov, 2021). It was established (Kirillova and Kirillov, 2021) that the arrival of new generations of *C. ornata* larvae occurs when the reservoir is heated up already at a temperature of 16 to 17°C and reaches a maximum infection at high temperatures of 24 to 29°C. Previously, *C. ornata* was also found in adult individuals of *Bufo bufo* and *Rana temporaria* Linnaeus, 1758 inhabiting H1 (Burakova and Baitimirova, 2017; Burakova and Malkova, 2021). The average value of the invasion extensiveness in adult toads in this habitat is two times higher than in fingerlings (72.4 and 36.0%, respectively) (Burakova and Baitimirova, 2017; Burakova and Malkova, 2021).

According to the literature data, the infestation of *Bufo bufo* with trematodes is low, which is associated with a predominantly terrestrial way of life and a short-term association with the water environment (Chikhlyaev et al., 2009). In the studied habitats, the trematodes were found only in H1 in the common frog (lung trematode *Haplometra cylindracea* (Zeder, 1800) (Burakova and Malkova, 2021)). However, according to literary sources (Ryzhikov et al., 1980; Kuz'min, 2012), no infection of gray toads with this trematode was noted.

Our results are consistent with previously published data on helminths of the gray toad fingerlings from the territory of the Middle Urals (in Lake Peschanoe (Sverdlovsk oblast) (Burakova and Vershinin, 2016)) and on helminths of its sexually mature individuals living in the vicinity of the Visimsky State Natural Biosphere Reserve (Burakova and Baitimirova, 2017). The same species were noted in amphibians in these territories, while the dominant *Oswaldocruzia filiformis* and *Rhabdias bufonis* were maintained. At the same time, the abundance index of adult animals (Burakova and Baitimirova, 2017) is naturally higher (mean ± SD: 47.0 ± 19.9 for adults; 2.2 ± 6.7 for fingerlings), and the differences are statistically significant ($U = 15.5$; $p < 0.001$), which is associated with the increase in the size of animals and the accumulation of parasites with age. Additionally for the Urals, it can be noted that *Aplectana acuminata* (Schrank, 1788) and *Neoxysomatium brevicaudatum* (Zeder, 1800) (which were not recorded in our samples (Yumagulova, 2000)) were found in *Bufo bufo* in the Republic of Bashkortostan.

In the territories of all the regions of the Russian Federation under consideration, nematode species *Oswaldocruzia filiformis* and *Rhabdias bufonis* act as dominants. These are nematodes that are widespread in the Palearctic, parasitizing on a wide range of amphibians and reptiles (Ryzhikov et al., 1980; Chikhlyaev et al., 2016; Vacker, 2018) and found in different habitats of the same region (Roitman and Kazakov, 1977). Since the development of the *Oswaldocruzia filiformis* and *Rhabdias bufonis* larvae occurs in the soil, these species mainly invade amphibians more connected to the land and, to a lesser extent, animals that permanently or predominantly live in water (Kirin and Buchvarov, 2002). It was noted that the eggs of the members of the Trichostrongylidae family, which includes the nematode *Oswaldocruzia filiformis,* are characterized by a resistance to chemical effects due to their dense membrane, which consists of four layers but is well permeable to water (Skryabin et al., 1954). The relatively large size of adult individuals of the hosts themselves is considered one more factor contributing to the dominant position of *O. filiformis* among parasites of gray toads. In the Russian Federation, the gray toad is one of the amphibian species that is large in size and leads a predominantly terrestrial lifestyle. It was demonstrated (Kirillova et al., 2021) that nematodes of the largest size are found in *Bufo bufo* as compared with smaller amphibian species. The authors (Kirillova et al., 2021) suggest that the gray toad is a more ancient (possibly a primary) host of *Oswaldocruzia filiformis*. The above facts cause the almost ubiquitous infestation of gray toads by these nematode species.

The Republic of Mordovia is distinguished by a rich species composition of trematodes in *Bufo bufo,* which is probably associated with the climatic peculiarities of this region. In addition, the presence of a native species of amphibian leading a water lifestyle (the lake frog) in the reservoirs of Mordovia contributes to the vast spread of trematodes that infect animals in water.

Primary data on the diversity of helminths of *B. bufo* fingerlings at the studied territories suggest that their species composition depends on a complex of biotopic factors.

CONCLUSIONS

The species composition of helminths of *B. bufo* fingerlings in the studied PAs is represented by nematodes, which is associated with the terrestrial lifestyle of this species. In the gray toad fingerlings, four nematode species were detected in H1, two in H2, and none in H3. The *Cosmocerca ornata* dominates in amphibians in H1 by infestation indices and portion in the component community. In H2, the dominance structure changes to a bidominant (*Oswaldocruzia filiformis* and *Rhabdias bufonis*); the *Cosmocerca ornata* and *Neoraillietnema praeputiale* disappear. The species composition of fingerlings and adult animals does not differ, but the abundance index in sexually mature animals is significantly higher than in fingerlings. A complex analysis of data on the species composition of the gray toad helminths from different regions of Russia demonstrated that the nematodes *Oswaldocruzia filiformis* and *Rhabdias bufonis* dominate in most regions. The Republic of Mordovia stands out among other regions of Russia by the presence of a diverse trematode fauna.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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