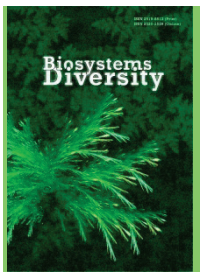

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An overview of helminths of the European fire-bellied toad *Bombina bombina* (Amphibia, Anura) in the Volga River Basin

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The helminthofauna of *Bombina bombina* (Linnaeus, 1761) has been studied to an unequal degree in different parts of the habitat. Thus, it has been studied in more detail in the west of its range (in the countries of central and eastern Europe) and in less detail in the center (in Belarus and Ukraine). There were few data on helminths of this host in the east of its range (in Russia). For the first time, an inventory of the helminthofauna in *B. bombina* was carried out for populations in the Volga River Basin. The results of our own research are presented and supplemented with information from other authors. We summarized scattered data on helminths from 390 specimens of amphibians collected over more than 40 years in the territory of five regions: Kaluga and Samara regions, the Republics of Mordovia, Tatarstan and Bashkortostan. The helminthofauna includes 21 species from three classes: Trematoda (15), Chromadorea (5) and Clitellata (1). For each species, we give the systematic position, localization, places of detection, geographical distribution and characteristics of the life cycle. The leech *Helobdella stagnalis* (Linnaeus, 1758) was first recorded in the European fire-bellied toad in Europe. Four species of trematodes are new to this amphibian species in Russia: *Haematoloechus abbreviatus* (Bychowsky, 1932), *Paralepoderma cloacicola* (Luhe, 1909), larvae, *Tylodelphys excavata* (Rudolphi, 1803), larvae and *Astiotrema monticelli* (Stossich, 1904), larvae. Another species of trematode – *Strigea strigis* (Schrank, 1788), larvae – was first recorded in this host within the boundaries of the Volga Basin. A specific parasite is the trematode *Haematoloechus abbreviatus* (Bychowsky, 1932). The number and composition of the species of helminths of the European fire-bellied toad vary in different regions; the structure of the helminth fauna is generally stable and includes three groups of species: adult and larval stages of trematodes, adult nematodes-geohelminths. The results of the study create a database for further population studies and contribute to the development of ideas about the distribution and formation of the amphibian helminth fauna in Europe, Russia and the Volga Basin.

Keywords: trematodes; nematodes; leeches; Bombinatoridae; Volga region; *Haematoloechus abbreviatus*; *Helobdella stagnalis*.

Introduction

Speaking of the global threats, the conservation of biological diversity is becoming more and more urgent every year. It means that the hundreds of species and millions of populations are disappearing on a planetary scale. During the last century, 400 vertebrate species have become extinct, which in the normal course of evolution would have taken 10 thousand years (Ceballos, 2015). Another 515 species (1.7% of the total number of living organisms) are on the verge of extinction (Ceballos et al., 2020). Amphibians are considered the most vulnerable group of vertebrates experiencing a sharp reduction of the range and numbers of populations around the world (Gardner, 2001; Wake & Vredenburg, 2008; Fayzulin et al., 2018; Litvinchuk & Kidov, 2018; Kurnaz & Kutrup 2019). Perhaps a fifth of their species have already become extinct or is close to it. The amphibian crisis is usually associated with the disappearance, degradation and pollution of habitats because of anthropogenic activity (Blaustein & Kiesecker, 2002; Lebedinskii et al., 2019). According to another version, the global warming and climate change are to blame for this (Tytar et al., 2018).

There are also vulnerable amphibian species in Europe, for example, toads (genus *Bombina* Oken, 1816). The *B. bombina* (Linnaeus, 1761) is distributed on the plains of Central and Eastern Europe from Denmark in the west to the Urals in the east. It inhabits the forest zone; in the forest-steppe and steppe prefers floodplain biotopes in river valleys. It has an aquatic lifestyle; inhabits shallow, heated water bodies with standing water (lakes, old trees, swamps), herbaceous flora as well as man-made water bodies: ponds, flooded ditches, quarries, peat bogs. It survives in polluted waters: sedimentation tanks, rice paddies (Kuzmin, 2012). Destruction and drying of water bodies, recreation, water pollution with chemicals (Sayim, 2010), introduction of alien fish species are the most dangerous threats for this species (Pupina et al., 2018).

Among other dangers for amphibians, we should note the emergence of a new type of threat in the XXI century such as infectious diseases caused by parasites – fungi (Narayan et al., 2011; Meurling, 2019; Sewell et al., 2021) and helminths (Jayawardena et al., 2013). The pathogenic effect of helminthic infection in amphibians has a wide range of manifesttations: from a number of skeletal anomalies (Schotthoefer et al., 2003; Szuroczki et al., 2011) and limb malformations (Svinin et al., 2020a, 2020b) till the dysfunction of internal organs (Vedernikov et al., 2020), suppression of behavioral reactions, life instincts and death (Ivanov et al., 2012). Unfortunately, amphibian helminths are not given due attention nowadays, despite the fact that the relevance of research in this direction is obvious.

The helminthofauna of the European fire-bellied toad has been studied to an unequal degree in different parts of its range. It has been studied more detailed in the west (in the countries of central and eastern Europe) (Vojtková & Roca, 1994, 1996). In the central part of the range, helminths of this species were studied in Belarus (Shimalov, 2008, 2009; Bychkova et al., 2017) and Ukraine (Shevchenko, 1965; Maguza, 1973; Iskova et al., 1995). However, so far, there are few data on helminths of this host in east part of its range in Russia (Ryzhikov et al., 1980). The eastern part of the range is located in the Volga River Basin, which occupies 33% of the territory of European Russia and almost 13% of Europe. The Volga River has a length of 3,690 km and a basin area of about 1.4 million km^2; it flows from north to south through an entire spectrum of biomes from taiga to semi-desert (Litvinov et al., 2009). The European fire-bellied toad is

broadly distributed in the floodplain biocenoses of the Volga River basin: from the Kaluga and Moscow regions (Kuzmin, 2008; Dunaev & Orlova, 2017) in its upper reaches to the environs of Volgograd and the Republic of Kalmykia – in the lower reaches (personal observation).

This research is the first inventory of the helminth fauna of the European fire-bellied toad from the populations in the Volga River Basin and continues a cycle of similar studies of other amphibian species (Chikhlyaev & Ruchin, 2014, 2021; Chikhlyaev et al., 2016, 2018, 2019a, 2019b).

Material and methods

The study is based on the results of our own research and information from other authors for the period from 1974 to 2020. The study material consists of data on helminths from 390 specimens of amphibians collected for more than 40 years in the territory of five regions of the Volga Basin: Kaluga and Samara regions, the Republics of Mordovia, Tatarstan and Bashkortostan (Fig. 1).

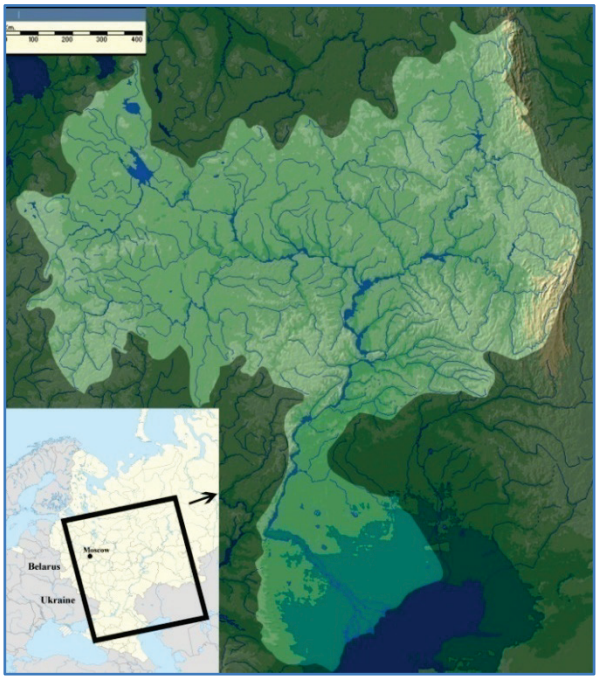

Fig. 1. Location of the Volga Basin

The study of amphibians was carried out using the method of complete helminthological auropsy of vertebrates (Skrjabin, 1928). Collection, fixation and processing of helminthological material were carried out in laboratory conditions (Byhovskaya-Pavlovskaya, 1985). Trematodes were fixed with 70% ethanol, stained with alum or acetic acid carmine, and after placing in dimethyl phthalate as a clearing agent, they were enclosed in Canadian balsam. Nematodes were straightened by heating, fixed with Barbagallo liquid, clarified in lactic acid and enclosed in glycerin-gelatin. Leeches were fixed with 1–2% formalin solution, after which they were clarified in glycerin.

The identification of helminths was made according to the reports of Ryzhikov et al. (1980) and Sudarikov et al. (2002). The paper reflects current views on the taxonomy of trematodes (Tkach et al., 2001; Gibson et al., 2002; Olson et al., 2003; Bray et al., 2008) and nematodes (Hodda, 2011). The study was carried out in the laboratory of Population Ecology of the Institute of Ecology of the Volga River Basin of the Russian Academy of Sciences of the Samara Federal Research Scientific Center of the Russian Academy of Sciences (Togliatti, Russia).

Results

We present an annotated list of helminth species of *B. bombina* from biocenoses of the Volga River basin. For each species, we present data on systematic position, localization, places of detection and geographical distribution. Table 1 shows the composition of helminths of the European fire-bellied toad in different regions of the Volga basin. Table 2 demonstrates the information about the life cycle of the helminths.

Phylum Platyhelminthes Schneider, 1873
Class Trematoda Rudolphi, 1808
Order Hemiurida Skrjabin et Guschanskaja, 1956
Family Derogenidae Nicoll, 1910
1. *Halipegus ovocaudatus* (Vulpian, 1859)
Localization: tongue, mouth.

Areas of detection: The Republic of Tatarstan (Shaldybin, 1977) and Samara region (Chikhlyaev, 2009; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1).

Biology: common parasite of the tongue and mouth of anurans (Anura). Tetraxenic life cycle (Table 2).

Distribution: Europe.

Table 1
Helminths of the European fire-bellied toad *Bombina bombina* in the Volga Basin regions

Helminth species	KL	MR	SM	TT	BS
Halipegus ovocaudatus	–	–	+	+	–
Diplodiscus subclavatus	+	–	+	+	–
Gorgodera cygnoides	–	+	+	–	+
Gorgoderina vitelliloba	–	–	+	+	+
Opisthioglyphe ranae	–	–	+	+	+
Haematoloechus asper	–	–	+	+	–
Haematoloechus abbreviatus	+	+	+	–	–
Haematoloechus variegatus	–	–	+	+	+
Pleurogenes claviger	–	–	–	+	–
Pleurogenoides medians	–	–	–	–	+
Paralepoderma cloacicola, larvae	–	–	+	–	–
Strigea strigis, larvae	+	–	+	–	–
Strigea sphaerula, larvae	+	–	+	–	+
Tylodelphys excavata, larvae	+	+	+	–	–
Astiotrema monticelli, larvae	–	–	+	–	–
Rhabdias bufonis	–	–	+	+	+
Oswaldocruzia filiformis	–	–	+	–	+
Aplectana acuminata	–	–	–	+	–
Cosmocerca ornata	+	+	+	+	–
Cosmocerca commutata	–	–	–	+	–
Helobdella stagnalis	–	–	+	–	–
Species in total	6[3]	4[1]	17[6]	11	8[1]
Trematoda	5[3]	3[1]	13[6]	7	6[1]
Chromadorea	1	1	3	4	2
Clitellata	–	–	1	–	–
Examined, specimens	37	15	235	36	67

Notes: KL – Kaluga region (Chikhlyaev et al., 2019; our data); MR – The Republic of Mordovia (Chikhlyaev et al., 2015; Ruchin et al., 2016; our data); SM – Samara region (Evlanov et al., 2001, 2002; Chikhlyaev, 2004, 2009, 2019; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018; our data); TT – The Republic of Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978; Smirnova et al., 1987); BS – The Republic of Bashkortostan (Ayupov et al., 1974; Bayanov, 1992; Yumagulova, 2000; Bayanov et al., 2015); "+" – helminth detected; "–" – no helminth detected.

Order Paramphistomida Skrjabin et Schulz, 1937
Family Diplodiscidae Cohn, 1904
2. *Diplodiscus subclavatus* (Pallas, 1760)
Localization: rectum, small intestine.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Chikhlyaev, 2004, 2009; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) regions, the Republic of Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978; Smirnova et al., 1987) (Table 1).

Biology: a common parasite of the intestines of amphibians (Amphibia). Dixenic life cycle (Table 2).

Distribution: cosmopolite.

Order Fasciolida Skrjabin et Schulz, 1935
Family Gorgoderidae Looss, 1899
3. *Gorgodera cygnoides* (Zeder, 1800)
Localization: bladder.

Table 2
Life cycles of helminths of the European fire-bellied toad (*Bombina bombina*)

Helminths	Life cycle	Autors
Halipegus ovocaudatus (Vulpian, 1859)	gastropods (Planorbidae)[1] – cyclops (Cyclopidae)[3] – dragonflies (Calopterygidae, Coenagrionidae, Lestidae, Libellulidae)[3] – amphibians (Amphibia)[5]	Keshemir, 1976
Diplodiscus subclavatus (Pallas, 1760)	gastropods (Planorbidae)[1] – amphibians (Amphibia)[5]	Skrjabin, 1949; Grabda-Kazubska, 1980
Gorgodera cygnoides (Zeder, 1800)	bivalves (Sphaeriidae)[1] – dragonflies (Corduliidae)[3] – frogs (Ranidae)[5]	Pigulevsky, 1952
Gorgoderina vitelliloba (Olsson, 1876)	bivalves (Sphaeriidae)[1] – tadpoles, alderflies[3] – anurans (Anura)[5]	Pigulevsky, 1953; Vojtková, 1974; Kalabekov, 1976
Opisthioglyphe ranae (Frölich, 1791)	gastropods (Lymnaeidae)[1] – gastropods (Lymnaeidae), tadpoles (Anura)[3] – anurans (Anura)[5]	Dobrowolsky, 1965a; Grabda-Kazubska, 1969
Paralepoderma cloacicola (Lühe, 1909), larvae	gastropods (Planorbidae)[1] – anurans (Anura)[3] – snakes (Colubridae)[5]	Dobrowolsky, 1969; Grabda-Kazubska, 1975
Haematoloechus asper Looss, 1899	gastropods (Planorbidae)[1] – dragonflies (Calopterygidae, Lestidae)[3] – anurans (Anura)[5]	Dobrowolsky, 1965b
Haematoloechus abbreviatus Looss, 1899	gastropods (Planorbidae)[1] – mosquitoes (Culicidae), dragonflies (Calopterygidae, Libellulidae)[3] – anurans (Anura)[5]	Skrjabin & Antipin, 1962; Thiel, 1930
Haematoloechus variegatus (Rudolphi, 1819)	gastropods (Planorbidae)[1] – mosquitoes (Culicidae), dragonflies (Calopterygidae, Libellulidae)[3] – anurans (Anura)[5]	Skrjabin & Antipin, 1962; Thiel, 1930
Pleurogenes claviger (Rudolphi, 1819)	gastropods (Bithyniidae)[1] – dragonflies, bugs, mayflies, caddis flies, alderflies, crustaceans (Gammaridae, Asellidae)[3] – anurans (Anura)[5]	Khotenovsky, 1970; Grabda-Kazubska, 1971
Pleurogenoides medians (Olsson, 1876)	gastropods (Bithyniidae)1 – dragonflies, bugs, mayflies, caddis flies, alderflies, mosquitoes, crustaceans (Gammaridae, Asellidae)[3] – anurans (Anura)[5]	Neuhaus, 1940; Khotenovsky, 1970
Strigea strigis (Schranck, 1788), larvae	gastropods (Planorbidae)[1] – tadpoles (Anura)[2] – anurans (Anura)[3,4] – snakes (Colubridae), mammals (Eulipotyphla, Mustelidae, Canidae)[4] – owls (Strigiformes)[5]	Odening, 1966a, 1967; Sudarikov, 1984
Strigea sphaerula (Rudolphi, 1803), larvae	gastropods (Planorbidae)[1] – tadpoles (Anura)[2] – anurans (Anura)[3,4] – snakes (Colubridae)[4] – crows (Corvidae)[5]	Odening, 1966b, 1967; Sudarikov, 1984
Tylodelphys excavata (Rudolphi, 1803), larvae	gastropods (Planorbidae)[1] – frogs (Anura)[3] – long-legged birds (Ciconiiformes)	Sudarikov, 1960
Astiotrema monticelli Stossich, 1904, larvae	gastropods (Bithyniidae)[1] – anurans (Anura)[3] – snakes (Colubridae, Viperidae)[5]	Shevchenko & Vergun, 1960; Sharpilo, 1976
Rhabdias bufonis (Schranck, 1788)	soil – oligochaetes, gastropods[4] – anurans (Anura)[5]	Schaake, 1931; Savinov, 1963; Hartwich, 1975
Oswaldocruzia filiformis (Goeze, 1782)	soil – amphibians (Amphibia)[5]	Hendrix, 1983; Moravec & Vojtkova, 1975
Aplectana acuminata (Schrank, 1788)	unknown	none
Cosmocerca ornata (Duiardin, 1845)	water – anurans (Anura)[5]	Kirillova & Kirillov, 2021
Cosmocerca commutata (Diesing, 1851)	soil – anurans (Anura)[1,5]	Skrjabin et al., 1961; Yumagulova, 1999
Helobdella stagnalis (Linnaeus, 1758)	water – insects, crustaceans, oligochaetes, leeches [5]	Sawyer, 1986

Note: [1] – intermediate host; [2] – intercalary host; [3] – additional host; [4] – paratenic host; [5] – definitive host.

Areas of detection: The Republics of Mordovia (Chikhlyaev et al., 2015; Ruchin et al., 2016) and Bashkortostan (Bayanov, 1992; Yumagulova, 2000), Samara region (Chikhlyaev, 2009; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1).

Biology: common parasite of the bladder of anurans (Anura). Trixenic life cycle (Table 2).

Distribution: Palearctic.

4. *Gorgoderina vitelliloba* (Olsson, 1876)

Localization: bladder.

Areas of detection: The Republics of Tatarstan (Shaldybin, 1977; Smirnova et al., 1987) and Bashkortostan (Bayanov, 1992; Yumagulova, 2000), Samara region (Chikhlyaev, 2009; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1).

Biology: common parasite of the bladder of anurans (Anura). Trixenic life cycle (Table 2).

Distribution: Palearctic.

Order Plagiorchiida La Rue, 1957

Family Telorchiidae Looss, 1899

5. *Opisthioglyphe ranae* (Frohlich, 1791)

Localization: small intestine.

Areas of detection: The Republics of Tatarstan (Shaldybin, 1977; Smirnova et al., 1987) and Bashkortostan (Bayanov, 1992; Yumagulova, 2000), Samara region (Chikhlyaev, 2009; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1).

Biology: a common intestinal parasite of amphibians (Amphibia) at the adult stage. Trixenic life cycle (Table 2).

Distribution: Palearctic.

Family Leptophallidae Dayal, 1938

6. *Paralepoderma cloacicola* (Luhe, 1909), larvae

Localization: musculature, mesentery.

Areas of detection: Samara region (Evlanov et al., 2001, 2002; Chikhlyaev, 2004, 2009; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1). This is the first finding of the parasite in the European fire-bellied toad in Russia and the Volga basin.

Biology: in the metacercariae stage, a common parasite of the musculature and mesentery of amphibians (Amphibia). Trixenic life cycle (Table 2).

Distribution: Palearctic.

Family Haematoloechidae Freitas et Lent, 1939

7. *Haematoloechus abbreviatus* (Bychowsky, 1932)

Localization: lungs.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Chikhlyaev, 2019) regions, the Republics of Mordovia (Chikhlyaev et al., 2015; Ruchin et al., 2016) (Table 1).

Biology: specific amphibian lung parasite of the genus *Bombina* (Oken, 1816). Trixenic life cycle (Table 2).

Distribution: Europe.

8. *Haematoloechus asper* Looss, 1899

Localization: lungs.

Areas of detection: The Republic of Tatarstan (Shaldybin, 1977; Smirnova et al., 1987) and Samara region (Chikhlyaev, 2004, 2009; Fayzulin et al., 2013) (Table 1).

Biology: typical parasite of frog (Ranidae) lungs. Trixenic life cycle (Table 2).

Distribution: Europe.

9. *Haematoloechus variegatus* (Rudolphi, 1819)

Localization: lungs.

Areas of detection: Samara (Chikhlyaev, 2009; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) region, the Republics of Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978; Smirnova et al., 1987) and Bashkortostan (Ayupov et al., 1974; Bayanov, 1992; Yumagulova, 2000) (Table 1).

Biology: common parasite of anurans (Anura) lungs. Trixenic life cycle (Table 2).

Distribution: Palearctic.

Family Lecithodendridae Odhner, 1911

10. *Pleurogenes claviger* (Rudolphi, 1819)

Localization: small intestine.

Areas of detection: The Republic of Tatarstan (Shaldybin, 1977; Smirnova et al., 1987) (Table 1).

Biology: a common parasite of the intestines of amphibians (Amphibia). Trixenic life cycle (Table 2).

Distribution: cosmopolite.

11. *Pleurogenoides medians* (Olsson, 1876)

Localization: small intestine.

Areas of detection: The Republic of Bashkortostan (Ayupov et al., 1974; Bayanov, 1992; Yumagulova, 2000) (Table 1).

Biology: a common parasite of the intestines of anurans (Anura). Trixenic life cycle (Table 2).

Distribution: Palearctic.

Order Strigeidida (La Rue, 1926)

Family Strigeidae Railliet, 1919

12. *Strigea strigis* (Schrank, 1788), larvae

Localization: serous coat of inner organs, mesentery, musculature.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Chikhlyaev, 2004, 2009; Fayzulin et al., 2013; Kirillov et al., 2018) regions (Table 1). The parasite has been recorded for the first time in the European fire-bellied toad in the Volga basin.

Biology: in the metacercariae stage, a common parasite of the body cavity, internal organs, musculature and mesentery of anurans (Anura). Tetraxenic life cycle (Table 2).

Distribution: Palearctic.

13. *Strigea sphaerula* (Rudolphi, 1803), larvae

Localization: serous coat of inner organs, pericardium, musculature.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Chikhlyaev, 2004, 2009; Fayzulin et al., 2013; Kirillov et al., 2018) regions, the Republic of Bashkortostan (Ayupov et al., 1974; Bayanov, 1992; Yumagulova, 2000) (Table 1).

Biology: in the metacercariae stage, a common parasite of the body cavity, internal organs, musculature and mesentery of anurans (Anura). Tetraxenic life cycle (Table 2).

Distribution: Europe.

Family Diplostomidae Poirier, 1886

14. *Tylodelphys excavata* (Rudolphi, 1803), larvae

Localization: spinal cord canal.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Evlanov et al., 2001, 2002; Chikhlyaev, 2004, 2009; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) regions, the Republic of Mordovia (Chikhlyaev et al., 2015; Ruchin et al., 2016) (Table 1). The parasite was first noted in the European fire-bellied toad in Russia and the Volga basin.

Biology: typical parasite of the spinal canal of anurans (Ranidae, Discoglossidae) at the metacercariae stage. Trixenic life cycle (Table 2).

Distribution: Palearctic.

Incertae sedis group

15. *Astiotrema monticelli* Stossich, 1904, larvae

Localization: intestine mesentery.

Areas of detection: Samara region (Chikhlyaev, 2004; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1). This is a new parasite for the European fire-bellied toad of Russia and the Volga basin.

Biology: in the metacercariae stage, the common parasite of the mesentery and body cavity of anurans (Anura). Trixenic life cycle (Table 2).

Distribution: Europe.

Phylum Nematoda Cobb, 1932

Class Chromadorea Inglis, 1983

Order Panagrolaimida Hodda, 2007

Family Rhabdiasidae Railliet, 1915

16. *Rhabdias bufonis* (Schrank, 1788)

Localization: lungs.

Areas of detection: The Republics of Tatarstan (Shaldybin, 1977; Smirnova et al., 1987) and Bashkortostan (Ayupov et al., 1974; Yumagulova, 2000), Samara region (Chikhlyaev, 2009, 2019; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1).

Biology: common parasite of anurans (Anura) lungs. Monoxenic life cycle (geohelminth) (Table 2).

Distribution: Holarctic.

Family Trichostrongylidae Leiper, 1908

17. *Oswaldocruzia filiformis* (Goeze, 1782)

Localization: small intestine.

Areas of detection: Samara region (Chikhlyaev, 2019) and the Republic of Bashkortostan (Bayanov, 1992; Yumagulova, 2000) (Table 1).

Biology: a common parasite of the intestines of amphibians (Amphibia). Monoxenic life cycle (geohelminth) (Table 2).

Distribution: Palearctic.

Order Spirurida Railliet, 1914

Family Cosmocercidae Travassos, 1925

18. *Aplectana acuminata* (Schrank, 1788)

Localization: intestine.

Areas of detection: The Republic of Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978; Smirnova et al., 1987) (Table 1).

Biology: typical intestinal parasite of tadpoles, less commonly adult anurans (Anura). Monoxenic life cycle (geohelminth, Table 2).

Distribution: Europe.

19. *Cosmocerca ornata* (Dujardin, 1845)

Localization: rectum.

Areas of detection: Kaluga (Chikhlyaev et al., 2019) and Samara (Chikhlyaev, 2004, 2009, 2019; Fayzulin et al., 2013; Kirillov et al., 2018) regions, the Republics of Mordovia (Chikhlyaev et al., 2015; Ruchin et al., 2016) and Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978; Smirnova et al., 1987) (Table 1).

Biology: a common parasite of the intestines of amphibians (Amphibia). Monoxenic life cycle (geohelminth) (Table 2).

Distribution: Europe.

20. *Cosmocerca commutata* (Diesing, 1851)

Localization: rectum.

Areas of detection: The Republic of Tatarstan (Shaldybin, 1977; Smirnova & Sizova, 1978).

Biology: In the adult stage of development a specific parasite of the intestines of green toads (*Bufotes viridis* Laurenti, 1768, Table 1). Monoxenic life cycle (geohelminth) with obligatory amphixeny (Table 2).

Distribution: Palearctic.

Phylum Annelida Lamarck, 1809

Class Clitellata Michaelsen, 1919

Order Rhynchobdellida Blanchard, 1894

Family Glossiphonidae Vaillant, 1890

21. *Helobdella stagnalis* (Linnaeus, 1758)

Localization: skin.

Areas of detection: The Samara region (Chikhlyaev, 2004; Kirillov & Chikhlyaev, 2011; Fayzulin et al., 2013; Kirillov et al., 2018) (Table 1). This is the first and the only find of leeches in the European fire-bellied toad in Europe, Russia and the Volga basin. There were no deviations from the original description. Earlier discoveries have been made in the yellow-bellied toad (*Bombina variegata* (Linnaeus, 1758)) in Bosnia and Herzegovina (Zimić, 2015), the common frog (*Rana temporaria* Linnaeus, 1758) in Northwestern Italy (Tiberti & Gentilli, 2010) and the palmate newt (*Lissotriton helveticus* Razoumowsky, 1789) in the Netherlands (Stark et al., 2017).

Biology: accidental parasite of amphibians (Amphibia). A common parasite (other reports say it is a predator, sucking out the body fluids and soft parts of its prey) of freshwater molluscs, arthropods, oligochaetes and other leeches. Monoxenic life cycle (Table 2).

Distribution: Cosmopolite.

Discussion

Therefore, there are currently known 21 species of helminths parasitizing in *B. bombina* in the Volga River basin. Taxonomically, they belong to 17 genera, 13 families, 8 orders and 3 classes: Trematoda – 15, Chromadorea – 5, Clitellata – 1. The leech *Helobdella stagnalis* (Linnaeus, 1758) was first recorded in this amphibian species in Europe. Four species of trematodes are new to the European fire-bellied toad in Russia: *Haematoloechus abbreviatus* (Bychowsky, 1932), *Paralepoderma cloacicola* (Luhe, 1909), larvae, *Tylodelphys excavata* (Rudolphi, 1803), larvae and *Astiotrema monticelli* Stossich, 1904, larvae. Another species of trematode – *Strigea strigis* (Schrank, 1788), larvae – was first recorded in this host within the boundaries of the Volga Basin.

Species of helminths differ in the degree of hostal specificity. Most of the species (18) of trematodes and nematodes are poly-hostal parasites of tailless amphibians. The nematode *Cosmocerca commutata* is a monohost-specific parasite of *Bufotes viridis* Laurenti, 1768. However, according to some scientists, Shaldybin (1977) and Smirnova & Sizova (1978), it occurs in amphibians of other genera and families. The leech *Helobdella stagnalis* is an obligate ectoparasite of freshwater invertebrates. This find can be regarded as a rare example of accidental parasitism in the conditions of joint habitation of the natural hosts of leeches in the same water bodies.

The trematode *Haematoloechus abbreviatus* (Bychowsky, 1932) is a specific oligohostal parasite of amphibians of the genus *Bombina* (Oken, 1816). Previously known as a subspecies of *Pneumonoeces variegatus* abbreviatus Bychowsky, 1932, now it is listed as an independent species. This is due not only to anatomical differences (Skryabin & Antipin, 1962), but also to the results of molecular genetic analysis (Leon-Regagnon & Brooks, 2003).

The European fire-bellied toad performs a different role in relation to certain helminths species. For example, it is the definitive host for species at the adult stage of development (12); additional or reservoir host for metacercariae of trematodes (5) (Table 1). Another three species of helminths (*G. vitelliloba*, *O. ranae* and *C. commutata*) parasitize in the body of amphibians during their larval and adult stages of the development simultaneously. This characterizes the European fire-bellied toad as an amphixenic host.

The number of helminth species in amphibians in some regions of the Volga Basin is not the same and decreases in the following order: Samara region (17 species) – Republic of Tatarstan (11) – Republic of Bashkortostan (8) – Kaluga Region (6) – Republic of Mordovia (4) (Table 1). The number of helminth species is influenced by the following factors: the volume of studied amphibians, the type and nature of water bodies, the geographical location and climate of the area, the level of anthropogenic impact.

The composition of helminths of the European fire-bellied toad also differs in different regions of the Volga Basin. None out of 21 helminth species were observed in all the samples. The reason for this is probably the biotopic features in a particular region of the Volga Basin. Only one species, the nematode *C. ornata*, was found in four of the five regions. It is a characteristic parasite of aquatic amphibian species. Infection with it occurs in the water, when the host comes into contact with free-floating invasive larvae. Most helminth species (*H. abbreviatus*, *D. subclavatus*, *G. cygnoides*, *G. vitelliloba*, *O. ranae*, *S. sphaerula*, larvae, *T. excavata*, larvae and *Rh. bufonis*) were found in three regions. This group of species can be characterized as common and widespread parasites of this host. Helminths found only in two regions (*H. ovocaudatus*, *H. variegatus*, *H. asper*, *S. strigis*, larvae and *O. filiformis*) are rare and occur sporadically. The remaining trematodes (*P. claviger*, *P. medians*, *P. cloacicola*, larvae and *A. monticelli*, larvae), nematodes (*A. acuminata*, *C. commutata*) and leech (*H. stagnalis*) were recorded strictly locally. These species of helminths are not characteristic of the European fire-bellied toad and are among the accidental parasites.

Conclusion

The biology and ecology of the European fire-bellied toad has a number of distinctive features:

1) aquatic lifestyle in small, shallow and overgrown floodplain water bodies with stagnant water;

2) feeding mainly on small aquatic invertebrates (mollusks, diptera larvae);

3) rarely visits land, except for migrations to wintering sites and reverse migrations to water bodies in spring;

4) they do not feed during mating.

These factors determine the species composition and structure of the helminthofauna for this amphibian species.

The structure of the helminthofauna of the European fire-bellied toad is mainly stable in different regions and includes three groups of species: 1) adult biohelminths (trematodes); 2) adult geohelminths (nematodes); 3) larval biohelminths (trematodes). Most adult trematodes (10 species) are common parasites of this host, with characteristic low rates of invasion. Trematode infestation is limited by the small size of the amphibian's body and mouth, which narrows the spectrum of its nutrition. Nematodes (5), except for *C. ornata*, are rare and have low invasion values. Most species of nematodes develop in soil, which is at odds with the aquatic lifestyle of the European fire-bellied toad. Helminths at the larval stage (5) are recorded sporadically with different indicators of invasion. The determining factor for them is the presence or absence of predators (their definitive hosts) and the degree of habitat transformation.

The European fire-bellied toad performs a special biocenotic role, being a valuable food object for a number of aquatic and near-water predators. Pike, green frogs, grass snakes, marsh turtles, herons and some duck species, insectivorous mammals and mustelids eat adults. The larval stages of helminths use trophic connections to circulate development and indicate the presence of certain predators in the biocenosis. For example, the findings of metacercariae of trematodes *P. cloacicola* and *A. monticelli* indicate grass snakes; *S. strigis*, *S. sphaerula* and *T. excavata* indicate owls, ravens and herons, respectively. In addition, this amphibian species is able to transmit metacercariae of trematodes of the family Strigeidae to their reservoir hosts: vipers and lizards, diurnal birds of prey, gulls and chickens, rodents and canine mammals. The results of the study create a database for further population studies and contribute to the development of ideas about the distribution and formation of the amphibian helminth fauna in Europe, Russia and the Volga basin.

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