Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 1, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NEW DATA FOR HELMINTH FAUNA OF BUFONIDAE (AMPHIBIA) IN THE REPUBLIC OF MOLDOVA

Elena GHERASIM, Dumitru ERHAN

State University of Moldova, Institute of Zoology, 1 Academiei Street, MD-2028, Chișinău, Republic of Moldova

Corresponding author email: gherasimlenuta@gmail.com

Abstract

The paper presents data on the identification of the helminth fauna structure of Bufo bufo Linnaeus, 1758 and Bufotes viridis Laurenti, 1768 species, ecaudata amphibian from Bufonidae families, and the determination of its role as bioindicators and as vectors for parasitic agents specific to animals. As result of helminthological investigations during 2013-2023 years, in Bufonidae species 19 helminths species was established. The helminthological research carried out on the species Bufo bufo highlighted the presence of 19 species of helminths, of which the predominant parasitic agents are trematodes which make up 47.4%, nematodes with 31.5%, acanthocephals with 15.8% and monogeneans with 5.3%. In Bufotes viridis species, the presence of 18 species of helminths was established, of which helminth species from the trematode class represent the predominant group with 44.4%, nematodes with 38.9%, acanthocephales with 11.1% and monogeneans with 5.6%. The helminthological researches were carried out depending on the age of the host, the type of habitat and the area. The bioindicator significance of the established parasite species was presented for an ecological assessment of the studied area.

Key words*: Bufo bufo, Bufotes viridis, helminth fauna, Republic of Moldova.*

INTRODUCTION

Anurans 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; Chikhlyaev et al., 2016b, Chikhlyaev et al., 2018a, Chikhlyaev et al., 2018b; Gherasim, 2020).

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 & Fayzulin, 2019a; Ruchin & Fayzulin, 2019b).

The diversity of the parasitic fauna specific to the amphibian species *Bufo bufo* and *Bufo viridis* from the Bufonidae family is a component of both terrestrial and aquatic biotopes (only for the reproduction period) and forms certain specific ecological interrelations. The parasitic factor is one of the main biotic factors, which determine the numerical effectively of the hosts, and through their numerical regulation, the structure and functioning of the ecosystem as a whole is influenced.

In the context of assessing the state of ecosystems and recognize the sources of spread of parasitosis in domestic animals, wild and humans, for the first time in the Republic of Moldova a complex batraco-helminthological study of the *Bufo bufo* and *Bufo viridis* amphibian species from the Bufonidae family was performed, as well as identifying their role as hosts, as vectors, but also their role in the formation and maintenance of foci of parasitic agents.

MATERIALS AND METHODS

The study area includes natural and anthropogenic, aquatic and terrestrial ecosystems specific to the *Bufo bufo* and *Bufo viridis* amphibian species in the Central, Northern and Southern area of the Republic of Moldova. The collection of the material was

carried during the active period of their annual life cycle (March-October) and of all age categories: embryos, larvae, juveniles, subadult and adult individuals of toads. Helminthological investigations were carried out over a period of 10 years (2013-2023).

The determination of amphibian species, both of adult forms, embryonic and larval stages, was carried by classical deductive methods, which refer to parameters, morphometric indices and body coloration (Bannikov et al., 1971 Bannikov et al., 1977; Arnold & Burton, 1986).

The helminthological analysis of biological samples was performed according to the standard method proposed by Skrjabin, which involves the examination of all the internal organs of the animal (Skrjabin, 1928). Helminthological research of the parenchymal organs was performed with the help of compressors, and the digestive tract - by successive washes. The collection, fixing, determination and processing of the helminthological material was carried after the methods proposed by various authors (Gashev et al., 2006; Kuzmin, 2012; Petrocenko, 1956; Ryzhikov et al., 1980; Serghiev, 2001; Sudarikov, 1964). The determination of the helminthological material was performed after standard methods (Ryzhikov et al., 1980).

To quantify the contamination characteristic by helminthes, the Intensity indice was calculated (*II, exemplars*) – the minimum and maximum number of parasites of a species and the extensivity of invasion (*EI, %*) – the percentage of host contamination by a species of parasite.

Laboratory helminthological investigations of biological samples of *Bufo bufo* and *Bufo viridis* to the presence of helminths or helminthic elements (eggs, larvae), allowed to obtain data of special value in order to determine the importance of amphibians in the formation and maintenance of outbreaks of common parasitic organisms in wild animals, pets and human.

RESULTS AND DISCUSSIONS

Given the fact that toads, as well as the other species of amphibians on the territory of our country, are amphibians species which live in two types of characteristic habitats: terrestrial and aquatic during the entire annual and vital life cycle, they are represent some of the main vectors of parasitic agents from the aquatic life environment to the terrestrial one, and vice versa. One of the main factors that essentially influence the activity of toads is represented by environmental factors, which are directly reflected on the structure of their helminthic fauna.

For the complex characteristic of the helminthic fauna of toads, throughout the country the multiannual phenology of these ecaudate amphibian species was evaluated. (Table 1).

According to the data obtained, it was established that the phenological phases of the annual life cycle of toads depends primarily on air and soil temperature, but atmospheric precipitation and relative air humidity, the distribution and distance of the location of breeding habitats from the habitats of nutrition, rest and hibernation have a complementary, but no less important role in the formation of intra- and interrelationships ecological speciospecific of toads with their living environments, with biological diversity, which on the whole characterize the diversity of their specific helminth fauna.

Table 1. Phenology of toads (*Bufo bufo, Bufo viridis*) in the conditions of the Republic of Moldova

No	Species	Coming out of hibernation	Moving to breeding sites	Ontogenesis			Moving from summer places	Initiation of the hibernation phase
				Embryonic development	Larval development			
				Egg	Larvae	Achieving metamorphosis and exiting in soil or land		
		t^0C	t^0C	Duration / days	Duration / days	Duration / days	t^0C	t^0C
1.	*Bufotes (Bufo) viridis*	+12.7	+15.2	9-10	41-43	41-45	+11.2	+7.0
2.	*Bufo bufo*	+7.1	+8.9	9-15	39-42	42-43	+11.2	+6.8

The helminthological research carried to amphibian species from the Bufonidae family revealed the presence of 22 helminth species, of which 19 species of helminths in *Bufo bufo*

and 18 species of helminths in *Bufo viridis* where established. From a taxonomic point of view, the parasitic agents detected in toads fall into three Phylum (*Platyhelminthes, Nematoda, Acanthocephala*), four classes (*Trematoda, Monogenea, Secernentea, Palaeacanthocephala*), ten orders (*Plagiorchiida, Echinostomida, Diplostomida, Spirurida Ascaridida, Strongylida, Rhabditida, Echinorhynchida, Polymorphida, Polystomatida*), 17 families (*Omphalometridae, Haematoloechidae, Gorgoderidae, Lecithodendriidae, Cyathocotylidae, Pleurogenidae, Diplodiscidae, Diplostomatidae, Strigeidae, Macroderoididae, Cosmocercidae, Spirocercidae, Molineidae, Rhabdiasidae, Echinorhynchidae, Centrorhynchidae, Polystomatidae*) and 21 genera (*Opisthioglyphe, Haematoloechus, Gorgoderina, Pleurogenes, Pleurogenoides, Prosotocus, Diplodiscus, Strigea, Haplometra, Holostephanus, Tylodelphys, Cosmocerca, Oswalcocruzia, Spirocerca, Ascarops, Agamospirura, Rhabdias, Acanthocephalus, Pseudoacanthocephalus, Sphaerirostris, Polystoma*).

In the evaluation of the scientific data obtained and reflected in Table 2, we can mention that each host species of toads from the Bufonidae family is characterized by a specific helminthic fauna structure for which the main helminthological indices are variable, and the extensivity of invasion (*E.I* - %) - oscillates from 1.1% of cases (*Opisthioglyphe ranae* species in *Bufo bufo* host species) to - 75.0% of cases (*Oswaldocruzia filiformis* in *Bufo viridis* host species). At the same time, the intensivity of invasion (I.I - ex.) of helminths in a host is quite fluctuating, it can vary from – 1 ex. (*Diplodiscus subclavatus* in *Bufo bufo* host species, *Pleurogenes claviger* in *Bufo viridis* host species) up to - 630 ex. in a single host specimen (*Ascarops strongylina* in *Bufo bufo* host species) or 145 ex. in *Bufo viridis* host species (Table 2).

In the Common European Toad (*Bufo bufo*) of the 19 detected species of helminths, the predominant group is represented by trematodes (n = 9) which constitute 47.49% of cases, nematodes with 31.6% of cases (n = 6), acanthocephals with 15.8% of cases (n = 3) and monogeneans (n = 1) with 5.3% of cases (Figure 1).

In the European Green Toad (*Bufo viridis*) of the 18 species of detected helminths, the predominant group is also represented by trematodes (n = 8) which constitute 44.4% of cases, nematodes - 38.9% of cases (n = 7), acanthocephals - 11.1% of cases (n = 2) and monogeneans (n = 1) with 5.6% of cases (Figure 1).

Table 2. The degree of invasion of toads with parasitic agents

No.	Host / Invasion	*Bufo bufo*		*Bufo viridis*	
		EI - %	II – ex.	EI - %	II – ex.
Trematoda					
1.	*Opisthioglyphe rane*	1.1	3	2.27	1-6
2.	*Haematoloechus variegatus*	4.9	1-2	2.27	1-2
3.	*Gorgoderina viteliloba*	12.3	1-4	-	-
4.	*Pleurogenes claviger*	42.6	1-15	2.27	1
5.	*Pleurogenoides medians*	9.55	1-21	4.55	1-13
6.	*Prosotocus confusus*	40.2	1-33	32.8	1-64
7.	*Diplodiscus subclavatus*	1.20	1	-	-
8.	*Strigea sphaerula,* mtc.	-	-	2.27	1-4
9.	*Haplometra cylindracea*	-	-	44.5	1-29
10.	*Holostephanus volgensis,* mtc.	42.3	1-6	-	-
11.	*Tylodelphis excavata,* mtc.	11.3	55	27.7	1-89
Secernentea					
12.	*Cosmocerca ornata*	64.3	1-8	20.00	1-6
13.	*Oswaldocruzia filiformis*	21.4	1-3	75.00	1-9
14.	*Oswaldocruzia duboisi*	28.6	1-5	21.4	1-3
15.	*Spirocerca lupi,* larva	-	-	73.2	1-95
16.	*Ascarops strongylina,* larva	22.5	1-630	12.3	1-145
17.	*Agamospirura sp.,* larva	21.2	8-32	42.3	1-32
18.	*Rhabdias bufonis*	70.0	5-48	59.09	1-18
Palaeacanthocephala					
19.	*Acanthocephalus ranae*	21.4	1-3	10.0	3
20.	*Pseudoacanthocephalus bufonis*	11.9	2-8	26.3	1-11
21.	*Sphaerirostris teres,* larva	1.96	1-3	-	-
Monogenea					
22.	*Polystoma integerrimum*	12.3	1-2	20.0	1-3

Thus, due to the above, the degree of infestation with helminths of ecaudate amphibians from the Bufonidae family, from the territory of our country, is quite high, being recorded in the *Bufo viridis* species in 64.5% of cases, and in the *Bufo bufo* species in 57, 3% of cases.

We can explain this insignificant difference in the degree of helminth infestation of toads as a result of their interaction with the living environments they are inhabit.

Bufo viridis is a species of synanthropic amphibian that lives near humans (orchards, vegetable gardens, vineyards, agricultural land with well-drained soil, parks, etc.), avoiding wooded areas. While *Bufo bufo* is not a

synanthropic species, on the contrary it populates the wooded, forested areas, lowland areas, as far as possible from the human factor (Cozari & Gherasim, 2021).

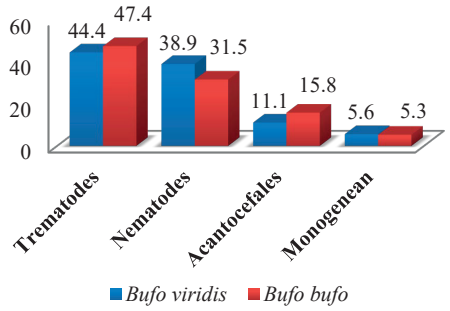

Figure 1. Indices of extensivity of taxonomic classes of helminths in toads

Each of these areas is characterized by a varied diversity of fauna, which directly influences their parasite structure.

In the study of the helminth fauna in amphibians, one of the main factors influencing the diversity of the parasitic fauna of a host is its age, which is accompanied by certain morpho-physiological, ethological and ecological changes, which also leads to a change in the probability of infection (Gherasim & Erhan, 2024).

The biological and ecological peculiarities of toads are very closely related to their age, and in this case the way of life is the same that determines the diversity of the parasitic fauna. Thus, toads are often infected with monogenea and up to two years of age, despite the fact that this parasite are directly develops.

This situation is explained by the fact that the toads infection takes place during reproductive migrations through streams, the environment where monogenean larvae are also found. Then, the extensivity and intensivity of the invasion increases steadily, synchronized, until the toads reach the age of 5 years, after which these indicators approximately at the same level remain.

The reason that the young forms of the toads are infected with this species of monogeneans is due to the fact that the juveniles spend part of the time on the surface of the water in the aquatic pools, the area that represents a real reservoir, where the probability of meeting the larval stages of the parasite, it is higher than in adult amphibians, which may have more contact with the terrestrial habitat, or may go to a greater depth of water. In many cases, the increase of parasite numbers is simply due to the large amount of food ingested by the host (Gherasim & Erhan, 2024).

For a complex assessment of the degree of helminth infestation of *Bufo bufo* and *Bufo viridis* species depending on age, specimens from four ontogenetic periods were evaluated helminthologically: embryos (ponta), larvae, juveniles and adults.

In ponta and larvae of toads, throughout the country, they were not invasive stability elements, but in their juveniles the presence of three species of trematodes (*Opisthiogliphe ranae, Haplometra cyclindracea, Strigea sphaerula*) was established, which constitutes 13.6% of total helminth species and a species of nematode (*Cosmocerca ornata*) which constitutes 4.5% of the cases. Thus, toad juveniles were infected with parasitic agents in 18.2% of cases, while in the adult forms all helminth species represented in table no. two were stable (Table 2, Figure 2).

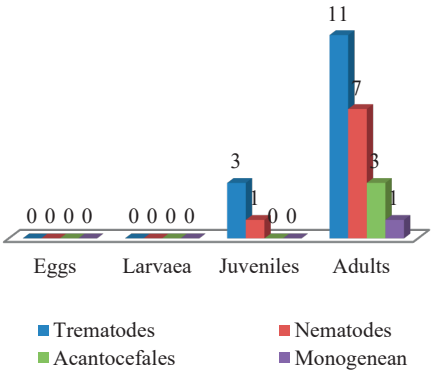

Figure 2. The structure of the helminthic fauna in toads depending on the age host

The helminthological analysis of the data allowed us to establish that one and the same helminth species can be common to toads from different ontogenetic periods, thus 18.2% of the total helminth species detected are specific to both juvenile and adult forms. The infestation both of juveniles and adult forms of amphibians from the Bufonidae family with one and the same species of helminths is

explained by the fact that the young forms (juveniles) of these species, after metamorphosis process, immediately leave the water bodies in which they were developed and inhabits areas specific to adult forms.

In order to determine the diversity of the helminthic fauna in toads depending on their main phenological phases, the amphibians were investigated on the entire annual life cycle during the spring-summer-autumn seasonal periods. Thus, during the course of the helminthological investigations in the spring season, it was established that the toads when they come out of the hibernation phase are characterized by a poor structure of their helminthic fauna, but also a prevalence of invasion of 8.5% of cases. Along with the increase in the environmental temperature, during their movement to the summer places (breeding pools), the infestation toads was recorded in 9.2% of cases. Later, after completing the reproductive process, the toads dont form couples, thus leaving the breeding pools, their nutritio are intensify and the their parasite structure at the end of the summer season is recorded with a prevalence of invasion in 71.0% of cases. Therefore, toads are the species of amphibians that form aggregations, and during the breeding period they usually accumulate many parasitic agents at the same time, because during this short period the probability of contact between the parasite and the host considerably increases.

In the autumn period, when the environmental factors dont represent the ecological optimum for toads, they are leave the summer sites and are characterized by the highest degree of infestation with helminths, which constitute 92.5% of cases. Gradually, with the establishment of the environmental conditions with low temperatures, towards the initiation of the hibernation phase, the prevalence of the invasion takes on lower values, being recorded in 62.0% of cases (Figure 3).

According to the data obtained, it was established that the variation of ecological factors is only a very important aspect not only for the vital activity of toads, but also for their helminthic fauna.

The high degree of helminth infestation of toads during the period of leaving summer sites is the result of the presence of obligate intermediate hosts in the development of life cycle for certain helminth species.

Although during the autumn season, when the toads initiate the hibernation phase, their infestation was established in 62.0% of cases, in the spring when they come out of the hibernation phase, the structure of the helminthic fauna is much reduced. This situation does not allow us to conclude that the reduction of the vital functions of the amphibian body under the influence of low temperatures during the winter season significantly reduces the structure of their helminthic fauna.

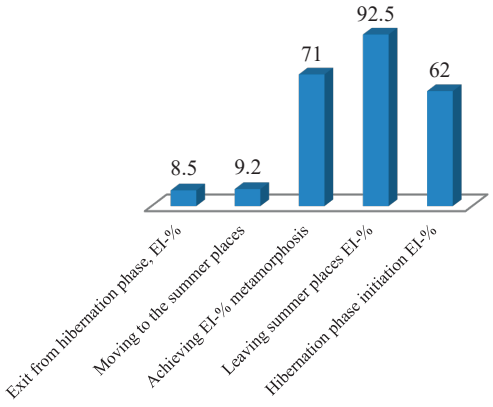

Figure 3. Degree of helminth infestation of toads depending on their phenological phases

At the same time, toads are also characterized by an infestation with different species of helminths, thus delimiting the two habitats of the species. With the post-reproductive migrations, the toads back to the summer sites and they have the capacity to lose certain parasitic agents. The toads returning from the breeding pools to the summer sites carry with them the aquatic parasitic fauna, but, moving up to the hibernation areas, they gradually lose it.

The annual monitoring and forecast of the parasitological situation of toads, makes it possible to assess the risks of invasion of a certain effective of animals, but also taking into account the number of susceptible animals. The forecast for the Republic of Moldova is of a general nature, because the biology of helminths for the different species presents significant differences depending on their

distribution area and their biological cycle.
Although the Republic of Moldova is a country with a small area of 33,846 km^2, the climatic conditions in the Center, North and South areas are different, and the forecasts have their own characteristics regarding the population and diversity structure of the intermediate hosts (for biohelminths), as well as the living environment conditions in a certain area.
According to the evaluation of the helminthological data depending on the area, the structure of the helminthic fauna in toads demonstrates that one and the same host species, analyzed from different points of its range, has a qualitatively and quantitatively different helminthic fauna (Figure 4).

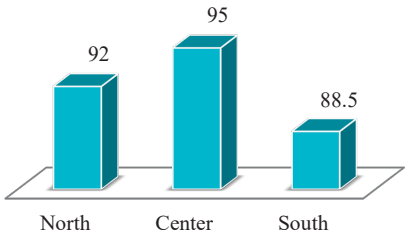

Figure 4. The structure of the helminthic fauna in toads depending on the area

So, these divergences regarding the structure of the helminthic fauna and the degree of helminth infestation of amphibians depending on the area, are not only to the trophic factor, but also to the zonal preference of amphibians and their adaptability to abiotic factors.
In order to evaluate the degree of helminth infestation of toads in the aspect of mono- and polyinvasions during their annual and life cycle, it was established that both in the *Bufo bufo* species (Figure 5) and in the *Bufo viridis* species the infestation in the aspect of monoinvasion was established (Figure 6).

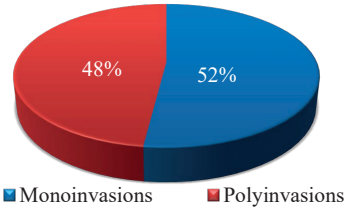

Figure 5. The structure of the helminth fauna in the *Bufo bufo* species in aspects of mono- and polyinvasion

The diversity of the helminthic fauna in amphibians depending on the biological cycle of the parasitic agents allowed us to realize their evolutionary characteristic.

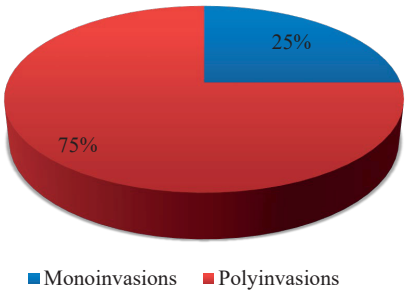

Figure 6. The structure of the helminth fauna in the*Bufo viridis* species in aspects of mono- and polyinvasion

Thus, of the 22 species of helminths detected in amphibians, 22.7% (n = 5 species) develop according to the monoxen model (*Cosmocerca ornata, Oswaldocruzia filiformis, Oswaldocruzia duboisi, Rhabdias bufonis, Polystoma integerrimum*), 18.2% of the species (n = 4 species) develop according to the dixen model (*Diplodiscus subclavatus, Agamospirura* sp., *Ascarops strongylina, Acanthocephalus ranae*), 54.5% of the species (n = 12 species) develop according to the trixen model (*Gorgoderina vitelliloba, Haematoloechus variegatus, Pleurogenes claviger, Pleurogenoides medians, Prosotocus confusus, Haplometra cylindracea, Opisthioglyphe rane, Tylodelphis excavata, Holostephanus volgensis, Spirocerca lupi, Pseudoacanthocephalus bufonis, Sphaerirostris teres*, larva) and 4.5% of the species (n = 1 species) develop according to the tetraxen model (*Strigea sphaerula*, mtc.) (Figure 7).

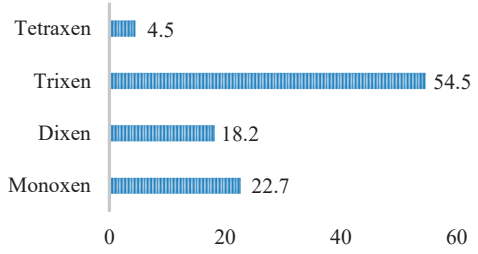

Figure 7. Evolutionary characteristics of the parasitic agents detected in toads

When evaluating the helminthological data obtained, it was established that adult forms of parasitic agents are predominate in the structure of the helminthic fauna of *Bufo bufo* species, so that when the host species is infected with trematodes, 22.2% are metacercariae, and 77.8% are adult forms of parasitic agents, when the host species are infected with nematodes 33.3% are the larval stages, and 77.7% are the adult forms, when the host species are infested with acanthocephals, it was established that the adult and larval forms are in a 1:1 report, both adult and larval forms they each constitute 50.0%, and the monogeneans are represented by a single species, and this is an adult form (100.0%) (Figure 8).

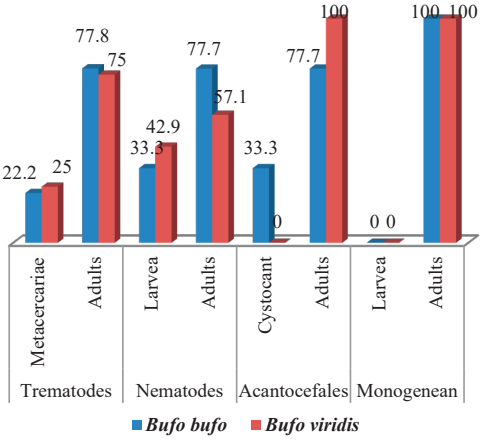

Figure 8. Helminthological indices of the *Bufo bufo* and *Bufo viridis* species depending on the ontogenesis of the parasitic agents

The helminthological analysis of the obtained data revealed that in the structure of the helminthic fauna of the Bufo viridis species the adult forms of the helminths predominate, so that when the host species are infected with trematodes, 25.0% are metacercariae, and 75.0% are the adult forms, when the infestation of the host species with nematodes, 42.9% are the larval stages, and 57.1% are the adult forms, when the host species was infested with acanthocephals, it was established that the helminth specimens are as adult forms and represent 100.0%, and the monogeneans are represented by only one species, and this is an adult form (100.0%) (Figure 8). Therefore, according to the evaluation of the structure of helminthic fauna in amphibians from the Bufonidae family depending on the ontogenetic phases of the their helminths detected in the host species and establishing their role as vectors for various helminths species common other vertebrates animals, it was found that both the *Bufo bufo* species and *Bufo viridis* species are an increased interest in the vectorization of parasitic agents common to fish in 9.1% of cases (*Tylodelphis excavata*, mtc., *Holostephanus volgensis*, mtc.), birds in 22.7% of cases (*Ascarops strongylina*, larva, *Tylodelphis excavata*, mtc.), mammals in 9.1% of wild cases: wild boars, insectivorous rodents (*Ascarops strongylina*, larva), carnivorous mammals (dog, fox, wolf), occasionally domestic mammals: goats, horses, bulls, pigs (*Spirocerca lupi*, larva) and human in 4.5% of cases (*Pseudoacanthocephalus bufonis*) (Figure 9).

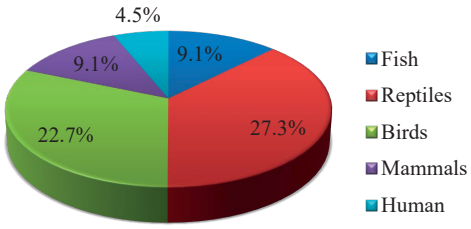

Figure 9. The degree of vectorization of parasitic agents by toads to various groups of animals

Although in toads the larval forms of parasitic agents don't have been determined as predominant forms, though the *Bufo bufo* and *Bufo viridis* species have a very important role in the vectoring of parasitic agents common to domestic animals, wild, pets and humans.

The increased degree of vectorization of the parasitic agents by toads to vertebrate animals is due to the trophic relations in the ecosystem (prey-predator), but also the possibility of simultaneous infestation of a single host with more species of parasitic agents.

In *Bufo bufo* species, their infestation with until to three species of helminths simultaneously was determined. According to the assessments, it was found that amphibians in 52.4% of cases were infested with a single species of helminths, in 37.6% of cases - with 2 species of

helminths and 10.3% of cases - with 3 species of helminths (Figure 10).

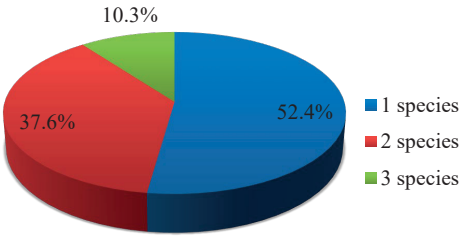

Figure 10. The degree of co-infection of the *Bufo bufo* species

In the *Bufo viridis* species, their infestation with until to four species of helminths simultaneously was established. According to the assessments, it was found that the amphibians in 25.0% of cases were infested with only one species of helminths, in 35.0% of cases - with 2 species of helminths, 35.0% of cases - with 3 species of helminths and 5.0% of cases - with 4 species of helminths (Figure 11). It is known that amphibians have a very important role in the functioning of ecosystems as consumers and in regulating the population of invertebrates in an ecosystem.

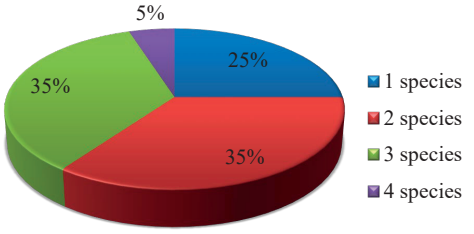

Figure 11. The degree of co-infection of the *Bufo viridis* species

At the same time, according to our data obtained on the territory of our country, the role of toads as definitive hosts was determined in 63.6% of cases (*Cosmocerca ornata, Oswaldocruzia filiformis, Oswaldocruzia duboisi, Rhabdias bufonis, Polystoma integerrimum, Diplodiscus subclavatus, Acanthocephalus ranae, Gorgoderina vitelliloba, Haematoloechus variegatus, Pleurogenes claviger, Pleurogenoides medians, Prosotocus confusus, Haplometra cylindracea, Opisthioglyphe rane*), intermediate in 22.7% of cases (*Agamospirura* sp., *Tylodelphis excavata, Holostephanus volgensis, Spirocerca lupi, Strigea sphaerula*) and as paratenic hosts in 13.7% of cases (*Ascarops strongylina, Pseudoacanthocephalus bufonis, Sphaerirostris teres*) for a large diversity of helminth species common to fish, reptiles, birds, mammals and humans (Figure 12).

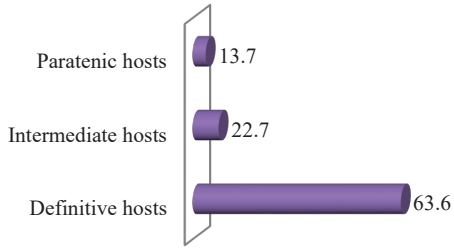

Figure 12. The role of toads as hosts of parasitic agents

Along with determining the role of toads as hosts for a large diversity of helminth species that are specific to fish, reptiles, birds, mammals and humans, at the same time, the data obtained also show their importance as bioindicators of ecosystems populated by these toads.

The distribution and the appearance dynamics of parasites in a certain environment, time and in different hosts, and the factors that are found during the host's relationship with the parasite at the individual level, or at the population level represent a complex study with a deep approach to various biological, ecological and helminthological aspects both to the host organisms and for the parasites.

The presence of obligatory stages of finding parasites in the external environment (natural) creates additional some impediments because the parasite must adapt to two different living environments - inside the host organism and outside it. Therefore, in addition to spreading in the environment, the parasitic agents must have the ability to spread over time and survive the direct influence of adverse climatic conditions while waiting for their improvement, or finding a new host. This activity is achieved by including in the life cycle of the parasite either an inactive or latent stage with a number of

protective adaptations, or an intermediate host, or even both. Both the latent stage and the intermediate host enable to the parasite, to some extent, to avoid the dangers associated with fluctuations conditions in environmental and to prolong its existence over time.

Any biological cycle of a parasitic agent is highly dependent on the obligate hosts involved in its realization, and the differences tend to relate to the processes of transmission and synchronization of the life cycles of the parasite and the host, which ensure the survival of the next generation.

Therefore, the monitoring of the helminth fauna in the ecaudate amphibians of *Bufo bufo* and *Bufo viridis* from the family Bufonidae, in various biotopes, depending on the intrinsic and extrinsic factors, it has of particular bioecological, medical and veterinary importance, and the data obtained have a particular contribution to the prevention of transmission parasitic agents to humans and animals involved in the biological cycles of parasites agents with a zoonotic and epizootic role.

So, according to the batraco-helminthological research carried on toads, we can conclude that the helminthic fauna of these host organisms, with the amphibious mode of life, which populate the most diverse living environments and with the highest degree of anthropization, are of particular importance not only theoretical, but also practical in science, because these species of amphibians actively participate in the formation and maintenance of foci of parasitic agents common to fish, birds, mammals (domestic, wild) and the human.

CONCLUSIONS

The study of the helminthic fauna in the ecaudate amphibians of *Bufo bufo* and *Bufo viridis* in the Republic of Moldova revealed the presence of 22 species of helminths, of which 19 species of helminths in *Bufo bufo* and 18 species of helminths in *Bufo viridis*. Taxonomic the parasitic agents detected in toads fall into three phylum, four classes, 10 orders, 17 families and 21 genera.

In this study, a complex ecological analysis of toads was carried out in which the structure of the parasite communities and the degree of invasion in relation to the phenology of the hosts was determined. These results allowed us to conclude that the variation of ecological factors during an annual cycle of toads represents a very important aspect on the formation of helminth fauna, but the divergent degree of invasion recorded in different phenological phases is the result of the presence of obligatory intermediate hosts in the biological cycle of the specific agents parasites.

At the same time, toads are also characterized by a certain infestation with different species of helminths, thus delimiting those two habitats of the species, terrestrial and aquatic (only during the reproduction period). Along with the post-reproductive migrations, towards the summer sites, the toads carry the aquatic parasitic fauna, but, moving towards the summery areas, they gradually lose it. Monitoring and annual predict of the parasitological situation of toads, makes it possible to assess the risks of invasion of a certain number of hosts.

It was determined that the biological and ecological peculiarities of the toads are very closely related to their age criterion, which determines the diversity of their parasitic fauna as well as the formation of the relationship in the parasite-host system from the juvenile stage.

The role of toads in the vectorization, formation and maintenance of foci of parasitic agents common to fish, reptiles, birds, mammals and human was determined.

ACKNOWLEDGEMENTS

This research work was carried out with the support of framework of the postdoctoral project “Helminthic fauna of amphibians (Amphibia), their importance as vectors in the formation and maintenance of parasitic zoonoses” no. 23.00208.7007.05/PDII and the project ”Evaluation of the structure and functioning of animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population no. 010701

REFERENCES

Arnold, E. N., & Burton, J. A. (1986). *Guida dei rettilii e degli anfibi d'Europa. Atlante illustrato a colori*. Roma, I: Franco muzzio and editori, 244 p. [In Italian]

Bannikov A. G., Darevskiy I.S., & Ishchenko V.G. (1977). *Guide to Amphibians and Reptiles of the USSR Fauna*. Moscow, RU: Prosveshchenie Publishing House. [In Russian]

Bannikov, A. G., Darevskiy, I. S., & Rustamov, A. K. (1971). *Zemnovodnyye i presmykayushchiyesya SSSR*. Moscow, RU: Prosveshchenie Publishing House. [In Russian]

Chikhlyaev, I.V., Korzikov, V.A., & Fayzulin, A.I. (2016a). Materials for the helminth fauna of Pool frog, Pelophylax lessonae and European common toad, *Bufo bufo* (Amphibia, Anura) in the Kaluga region. *Proceedings of Samara Scientific Centre RAS*, *18*(5–2), 377–381. [In Russian]

Chikhlyaev, I.V., Kirillova, N.Y., & Kirillov, A.A. (2018a). Ecological analysis of trematodes (Trematoda) of marsh frog *Pelophylax ridibundus* (Ranidae, Anura) from various habitats of the National Park «Samarskaya Luka» (Russia). *Nature Conservation Research, 3* (Suppl.1), 36–50.

Chikhlyaev, I.V., & Ruchin, A.B. (2014). The helminth fauna study of European common brown frog (*Rana temporaria Linnaeus*, 1758) in the Volga basin. *Acta Parasitologica, 59*(3), 459–471.

Chikhlyaev, I.V., Ruchin, A.B., & Fayzulin, A.I. (2016b). The helminth fauna study of European common toad in the Volga Basin. *Nature Environment and Pollution Technology, 15*(3), 1103–1109.

Chikhlyaev, I.V., Ruchin, A.B., & Fayzulin, A.I. (2018b). An overview of the trematodes fauna of Pool frog *Pelophylax lessonae* (Camerano, 1882) in the Volga Basin, Russia: 1. Adult stages. *Nusantara Bioscience, 10*(4), 256–262.

Cozari, T., & Gherasim, E. (2021). *Biology, ecology and ethology of ecaudate amphibians (Ranidae, Bufonidae) from the ecosystems of the Republic of Moldova*. Chisinau, MD: Tipografia Universității de Stat din Tiraspol Publishing House.

Erhan, D., & Gherasim, E. (2022). *Helminthic fauna of amphibians and reptiles from the Republic of Moldova. Trematode*, vol. 1. Chișinău, MD: Tipografia Centrala Publishing House.

Gashev, S. N., Zhigyleva, O. N., & Sazonova, N. A. (2006). Zoo Indicators in the System of Tyumen Region Environmental Monitoring: Technique of Use. Tyumen, RU: Tyumen State University.

Gherasim, E. (2020) *Pelophylax ridibundus* (Amphibia: Ranidae) as paratenic host of *Spirocerca lupi* species (Secernentea: Spirocercidae) in the Republic of Moldova. *Life science today for tomorrow. International Congress, 63*(1), 18-24.

Gherasim, E., & Erhan, D. (2024). *Helminthic fauna of amphibians and reptiles from the Republic of Moldova. Trematode,* vol. 2. Chișinău, MD: Tipografia Centrala Publishing House

González, C.E., & Hamann, M.I. (2007). The first record of amphibians as paratenic hosts of *Serpinema larvae* (Nematoda; Camallanidae). *Brazilian Journal of Biology, 67*(3), 579–580.

Kuzmin, S.L. (2012). *Zemnovodnyye byvshego SSSR. Izdaniye vtoroye, pererabotannoye*. Moscow, RU: Prosveshchenie Publishing House. [In Russian]

Petrochenko, V. I. (1956). *Akantotsefaly domashnikh i dikikh zhivotnykh*. Moscow, RU: Akad. Nauk SSSR.

Ruchin, A.B., & Fayzulin, A.I. (2019a). An overview of the trematodes fauna of Pool frog *Pelophylax lessonae* (Camerano, 1882) in the Volga Basin, Russia: 2. Larval stages. *Nusantara Bioscience, 11*(1), 106-111.

Ruchin, A.B., & Fayzulin, A.I. (2019b). Parasitic nematodes of Pool frog (*Pelophylax lessonae*) in the Volga Basin. *Revista MVZ Cordoba, 24*(3), 7314-7321.

Ryzhikov, K. M., Sharpilo, V. P., & Shevchenko, H. H. (1980). Gelminti amfibii fauni SSSR. Moscow, RU: Prosveshchenie Publishing House. [In Russian].

Sessions, S.K., & Ruth, S.B. (1990). Explanation for naturally occurring supernumerary limbs in amphibians. *Journal of Experimental Zoology, 254*(1), 38-47.

Sudarikov, V.Y. (1964). Nekotoryye osobennosti biologii i ontogeneza trematod otryada Strigeidida. V: Eksperimental'naya i ekologicheskaya gel'mintologiya. *GELAN*, 14, 201-220.

Thiemann, G.W., & Wassersug, R.J. (2000). Biased distribution of trematode metacercariae in the nephric system of *Rana tadpoles*. *Journal of Zoology, 252*(4), 531–547.