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NEW DATA FOR HELMINTH FAUNA OF *Rana temporaria* (Linnaeus, 1758) IN THE REPUBLIC OF MOLDOVA

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Abstract

The paper presents data on the identification for the first time in Moldova of the helminth fauna structure of Rana temporaria and the determination of its role as bioindicators and as vectors for parasitic agents specific to animals. As result of helminthological investigations during 2014-2023 years, 19 helminths species was established. The predominant group are trematodes with 57.8% of cases, nematodes with 31.6% of cases, acanthocephala and monogeneans with 5.3% of cases each. According to the assessment of the main helminthological indices, it was established that the species is infested with helminths in 96.3% of cases. When evaluating the data and the composition of helminth species, it was found that adult forms of helminths are predominant over larval forms, so that, when the host species is infected with trematodes, 9.1% are metacercariae, and 90.9% are the adult forms, when the host species is infected with nematodes 33.3% are the larval stages, and 66.7% are the mature forms, when infesting the host species with acanthocephals and monogeneans, it was established that the helminth species are an adult form, each constituting 5.3%.

Key words*: bioindicators, Moldova, parasitic agents, Rana temporaria, vectors*

INTRODUCTION

Rana temporaria (Common frog) is a species of ecaudate amphibians from Ranidae family with a wide distribution area, found throughout Europe, except for Southern Italy and the Southern Balkans. In the Republic of Moldova, the common frog is characterized by a limited distribution, inhabiting only the northern part of the country, up to the border of Ungheni, Călărași (Sipoteni, Bucovăț), Criuleni and Maiac localities (Cozari et al., 2007; Arnold & Burton 1986, Bannikov et al., 1977, Cozari & Gherasim, 2021).

Currently this species is included in the Red Book of the Republic of Moldova (2015) with the status of vulnerable species (VU), but according to IUCN category 2018-1 it has the status of LC (Least Concern).

The reduction of the population of the *Rana temporaria* species represents its response to the direct action of anthropogenic transformation of the environment, which is currently quite advanced, and contributes directly to the destruction of specio-specific habitats, the actual destruction of specimens, as well as the degradation of biodiversity in general (Cozari & Gherasim, 2021).

Another factor that directly contributes to the reduction of the *Rana temporaria* population is represented by the specific biocenotic relationships in the ecosystems populated by it. As a result of these interactions, certain specific relationships are formed in the parasite-host system, which also includes a cryptic ensemble of parasitic agents, and the *Rana temporaria* species serves as a host for at least one developmental stage of the parasitic agents.

Although the parasitic fauna of the *Rana temporaria* species is a constitutive part of both aquatic and terrestrial biotopes, also representing as actual bioindicators of its specific ecosystems, the parasitic agents have a negative impact on the perpetuation of amphibians as living organisms, thus producing certain dysfunctions of the organ systems of amphibians, and respectively to the reduction of the *Rana temporaria* population.

Internationally, helminthological research on this species of ecaudate amphibians from the Ranidae family has been carried out and described by more authors (Griffin, 1989;

Chikhlyaev & Ruchin, 2014; Herczeg et al., 2021; Burakova & Malkova, 2021).
In the Republic of Moldova, the amphibian species *R. temporaria* has been researched from a biological, taxonomic, ecological, genetic, evolutionary, etc. point of view (Cozari & Gherasim, 2021), but complex helminthological research was carried out for the first time, and the results are presented in this scientific paper.

MATERIALS AND METHODS

The study area includes the Center area (Călărași, Strășeni, Codrii Centrali Hâncești, Telenești, Sângerei Districts) of the Republic of Moldova. In the context of the principles of helminthological research of an organism (species) it is extremely important to apply certain appropriate and effective concepts of species-host identification. In this context, the biological and morphological concepts are of major importance, although each of them, taken separately, also presents certain methodological difficulties.
The amphibian species *Rana temporaria* was identified using of morphometric parameters (Arnold & Burton 1986, Bannikov et al., 1977; Kuzmin, 2012).
The helminthological analysis of biological samples was performed according to the standard method proposed by K.I. Skriabin, which involves the examination of all the internal organs of the animal (Skriabin, 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; Petrochenko, 1956; Erhan & Gherasim, 2022; Sergiev 2001; Sudarikov, et al., 2002; Shchepina et al., 2006).
In order to quantify the characteristic of helminthes contamination, the intensity indexes (II, specimens) was calculated - the minimum and maximum number of parasites of a species and the extent of invasion (EI, %) - the percentage of host contamination by a parasite species.

RESULTS AND DISCUSSIONS

Rana temporaria Linnaeus, 1758 species is a species of ecaudate amphibians of the Ranidae family, the group of brown frogs, which emerges very early from the hibernation phase, when the environmental temperature is not ecologically optimal for a wide range of invertebrate animals, which are obligate intermediate hosts (insects, artropods, crustaceans, etc.), for its specific parasitic fauna.
As a result of the helminthological investigations carried out on the common frog during its entire annual and life cycle, the presence of 19 species of helminths was established, which from a taxonomic point of view are included in 3 phylums (Platyhelminthes, Nematoda, Acanthocephala), four classes (Trematoda, Monogenea, Secernentea, Palaeacanthocephala), eight orders (Plagiorchiida, Echinostomida, Ascaridida, Strongylida, Spirurida, Rhabditida, Echinorhynchida, Polystomatida), 15 families (Omphalometridae, Cepahlogonimidae, Gorgoderidae, Lecithodendriidae, Pleurogenidae, Diplodiscidae, Plagiorchiidae, Diplostomatidae, Macroderoididae, Cosmocercidae, Molineidae, Spirocercidae, Rhabdiasidae, Echinorhynchidae, Polystomatidae) and 18 genres (*Opisthioglyphe, Cephalogonimus, Gorgodera, Gorgoderina, Pleurogenes, Pleurogenoides, Prosotocus, Diplodiscus, Haplometra, Plagiorchis, Tylodelphys, Cosmocerca, Oswalcocruzia, Ascarops, Agamospirura, Rhabdias, Acanthocephalus, Polystoma*).
Therefore, in the *Rana temporaria* species from the 19 detected species of helminths, the predominant group are trematodes (n=11) which constitute 57.9% of cases, nematodes with 31.6% of cases (n = 6) and acanthocephals (n = 1) and with monogeneans (n = 1) with 5.3% of cases each (Figure 1).
To evaluating of the data and the structure of helminth species, it was found that the adult forms of helminths are predominant over the larval forms, so that when the host species are infected with trematodes, 9.1% are metacercariae (*Tylodelphys excavata*), and 90.9% are the mature forms (*Opisthioglyphe ranae, Cephalogonimus retusus, Gorgodera*

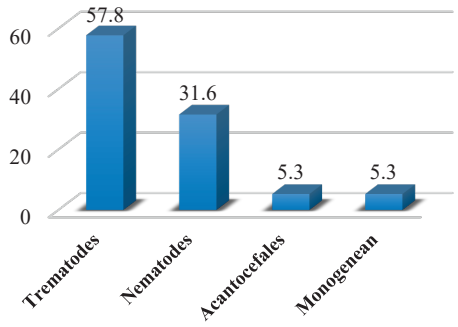

Figure 1. Indices of extensivity of the taxonomic classes of helminths detected in the *Rana temporaria* species

varsoviensis, Gorgoderina vitelliloba, Pleurogenes claviger, Pleurogenoides medians, Prosotocus confusus, Diplodiscus sbclavatus, Haplometra cylindracea, Plagiorchis elegans), when the host species is infected with nematodes 33.3% are the larval stages and 66.7% are the mature forms, to the infestation of the host species with acanthocephals and monogenean it was established that the helminth species are an adult form, each constituting 5.3% (Figure 2).

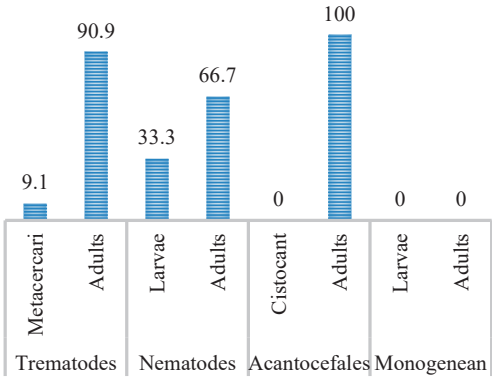

Figure 2. Helminthological indices of the *Rana temporaria* species depending on ontogeny parasitic agents

Therefore, during the helminthological investigation of *Rana temporaria* species it was established that this is a host species that is predominantly infested with invasive elements of adult form.

Therefore, according to the evaluation of the structure of the helminthic fauna in the host species *Rana temporaria* depending on the ontogenetic phases of the detected helminths, it was found that this host for 15.8% of the total number of specific helminth species constitutes a truthful vector.

At the same time, the degree of vectorization of parasitic agents by the ecaudate amphibian species *Rana temporaria* from the Raniade family to wild, domestic, companion animals and humans is explained not only thanks to the trophic relations in the ecosystem (prey-predator), but also the possibility of its simultaneous infestation with several species of parasitic agents.

Thus, according to our data for the *Rana temporaria* species, it was established that in 7.7% of the cases the specimens were infested with a single species of helminths, 46.1% of the cases - with 2 species of helminths, 23.1% of the cases - with 3 species of helminths, 15.4% of cases - with 4 species of helminths and 7.7% of cases - with 5 species of helminthes (Figure 3).

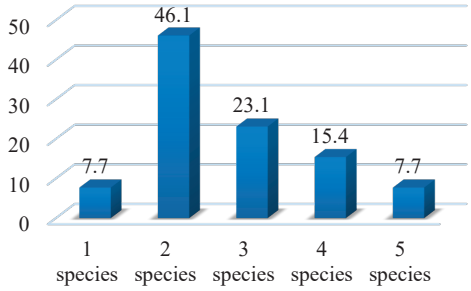

Figure 3. Degree of co-infection of the *Rana temporaria* species

It is known that amphibians have a big important role in the functioning of ecosystems as consumers and in regulating the population of invertebrates in an ecosystem.

According to our obtained data, the role of the ecaudate amphibian species *Rana temporaria* was determined as the definitive host for 84.2% of cases of helminth species (*Cosmocerca ornata, Oswaldocruzia filiformis, Oswaldocruzia duboisi, Rhabdias bufonis, Polystoma integerrimum, Diplodiscus subclavatus, Acanthocephalus ranae, Gorgodera varsoviensis, Gorgoderina vitelliloba, Pleurogenes claviger,*

Pleurogenoides medians, Prosotocus confusus, Cephalogonimus retusus, Haplometra cylindracea, Opisthioglyphe ranae, Plagiorchis elegans), intermediate for 10.5% of cases (*Agamospirura sp., Tylodelphis excavata*) and as a paratenic host for 5.3% of cases (*Ascarops strongylina*) for helminth species common to fish, reptiles, birds, mammals and humans (Figure 4).

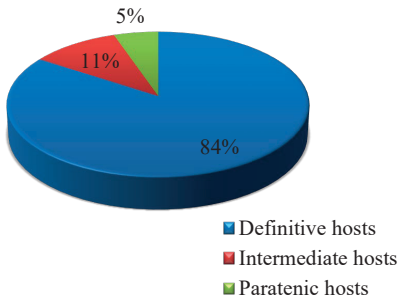

Figure 4. The role of *Rana temporaria* species as a host of the parasitic agents

If in the life cycle of helminths specific to amphibians or other groups of animals (reptiles, birds, mammals) there is an intermediate host (mollusks, insects, amphibians), then it does not participate at all in the search for the next host (definitive host). Of course, the probability of infection increases if the parasite hits the right host and exerts some influence on that host, making it more attractive to a next potential host. According to the ethological analysis of amphibians, it was observed that those amphibian specimens that were infested with metacercariae had a delayed response to the action of biological factors (predators) in the environment. But the insight of the parasite into the next host depends entirely on its nutritional biology, which the parasite cannot influence in any way. If, however, the parasite is found in a suitable (specific) intermediate host, then, due to the nutritional relationships existing in the given habitat, it is limited to a certain set of possibilities and consequences. Thus, the completion of the entire life cycle of the parasite is predetermined and, thanks to this fact, the probability of meeting the appropriate definitive host increases impressively. The parasite does not choose its next host and does not find it as such, but must be able to recognize it as a potential host. If a parasite found in an unsuitable host, it either leaves it immediately or lives in it for a limited period of time and then dies.

The role of amphibians as paratenic hosts, according to our data, represents another strategy of parasite adaptation to the living environment. This occurs when parasite larvae or intermediate hosts infected with it are consumed not by obligate hosts of this parasite, but by amphibians, representing a nutritional necessity of animals. Unlike definitive or intermediate hosts, which are obligatory for the life cycle of a particular parasite, these accidental hosts are facultative, in which, parasites do not develop but simply live for a certain period of time without losing their infectivity to the next host (Gherasim & Erhan, 2024).

Facultative hosts can never replace intermediate hosts, but they can intervene in the life cycle of the parasite at almost any of its stages. The importance of amphibians as facultative hosts in the transmission of parasites to a suitable host is determined by how close are the connection of the food spectrum between successive hosts of the parasite (invertebrates - amphibians - vertebrates) (Gherasim & Erhan, 2024).

For many parasites, the probability of encountering a new host may be negligible if the host population is small or highly dispersed. In such cases, the infection is possible only when hosts form short-term aggregations, such as during the breeding season in amphibians. At the same time, it is absolutely necessary for some parasites to find and infect specimens (hosts) belonging to a new generation of the host. Therefore, the life cycle of such parasites is often synchronized with that of the hosts, with infective larvae developing and being released just as the host specimens form groups or produce offspring. Such synchronization is achieved by the same response of both host and parasite to a physical factor, either because the parasite's reproduction depends by the reproduction of the host or it is directly regulated of this.

Polystoma integerrimum species is a parasite of amphibians, which behaves similarly. Its sexual organs mature just as the amphibians get in the

water to breed, and this parasite only lays eggs during the period (which lasts about a week) when the amphibians are in the water and forming couples (specific only to amphibians that use water pools only for breeding, except for the *Pelophylax esculenta* amphibian complex: *Pelophylax ridibundus, Pelophylax lessonae, Pelophylax esculentus*). Necessary time for eggs to develop into larvae is equal to the necessary time for amphibian eggs to develop into tadpoles and the parasite to reach the stage where they develop in the gills. Thus, the oncomiracidia capable of invasion and the hosts they presumably infect arrive at the same place at the same time.

In this sense, the amphibians being intermediate or paratenic hosts for a series of larval stages of helminths (eggs, cercariae, metacercariae, cystocanth, larvae, etc.), they fulfill a particularly important role in the epidemiology and epizootology of helminthes infestations of wild animals, domestic, companion and human, and their impact on the definitive hosts (vertebrates) is little studied, or even completely missing.

Although scientific research is characterized by an impossible to control scale, still, until now there are species of helminths whose biological cycle is still unknown, and their hosts could be animals of economic importance (cattle, goats, horses, sheep etc.) as well as human. Once helminthological research is carried out in amphibians, their role in the vectorization of helminthiasis in wild animals, pets, farm animals and humans can be identified.

When evaluating the degree of vectorization of parasitic agents by the host species *Rana temporaria* in the areas populated by it, it was determined that of the total of helminth species detected, 2 species (*G. varsoviensis, T. excavata*) are common to fish, which constitutes 10.5%, 2 species are common to reptiles (*P. elegans, A. strongylina*) which represents 10.5% of cases, 3 species of helminths detected in the host *Rana temporaria* (*P. elegans, T. excavata, A. strongylina*) are specific to birds and constitute 15.8% of cases, 3 species are common to mammals (*P. elegans, A. strongylina, Agamospirura* sp.) representing 15.8% of cases and one species can also be found in humans (*P. elegans*) which represents 5.3% of cases (Figure 5).

Usually, the parasites tend to have a complex life cycles, and it is unlikely that only one host is required in which to grow and therefore reproduce and form new generations. If all parasitic agents were to survive, then their abundance would far exceed that of their hosts, thus creating a potential threat to the existence of the host population.

Over-infestation of the host is always a potential danger to the parasite. Any parasite population, which overuses the available resources, has a chance to survive, and it is the host that provides the parasite with essential resources for survival, such as food and favorable conditions for growth, development and formation of new generations.

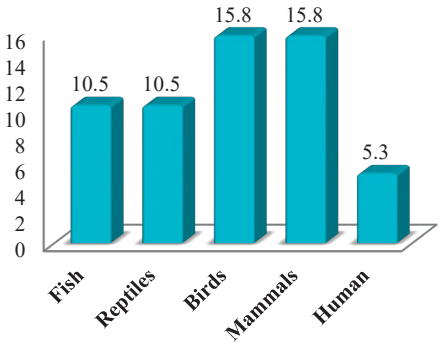

Figure 5. The degree of vectorization of parasitic agents by the amphibian species *Rana temporaria*

At the basis of the perpetuation of any species is its certain ethological criteria regarding leaving the parental habitats, or a population by populating new areas, but avoiding its overpopulation, which usually leads to mortality (Gherasim & Erhan, 2024).

For parasites, the dispersal plays a particularly important role, as over infestation of hosts is a permanent threat to them. Therefore, both adult parasites and their larval stages must possess the ability to leave the host organism and at the same time find other new hosts. Sometimes the new host may belong to the same species, but the need for the parasite to be in another host organism of another generation is constant.

In both cases, the parasite spends a certain stage of its life cycle outside the host organism, in the environment. Dispersal often occurs at this stage, but since the probability of finding a

new host is generally extremely low and parasite mortality during the dispersal period can be very high. To compensate for this mortality, the parasites must have a very high fecundity, and this is also facilitated by their asexual or parthenogenetic reproduction.
The intermediate hosts, thanks to their own mobility, contribute to the spread of the parasite in the environment and, thanks to the ecological relationship's that exist between them and the definitive hosts, facilitate its penetration into the latter's body. Parasite behavior in an intermediate host or the behavior of the host itself may also increase the probability of finding a definitive host.
Therefore, the monitoring of the parasite fauna in amphibians, in various biotopes, depending on the intrinsic and extrinsic factors, it has a bioecological, medical and veterinary importance in preventing the transmission of the parasitic agents to humans and animals involved in the biological cycles of parasites with a zoonotic and epizootic role.
Therefore, the helminthological research in amphibians allows us to conclude that the helminthic fauna of these host organisms, with the amphibiotic way of life, is of particular importance not only theoretically, but also practically, actively participating in the formation and maintenance of foci of parasitic agents common to fish, birds, mammals and human.

CONCLUSIONS

For the first time in the Republic of Moldova, helminthological investigations were carried out on the species of ecaudate amphibians *Rana temporaria* Linnaeus, 1758.
As a result of the helminthological research carried out, the presence of 19 species of helminths was established, which from a taxonomic point of view are included in three phylum (Platyhelminthes, Nematoda, Acanthocephala), four classes (Trematoda, Monogenea, Secernentea, Palaeacanthocephala), eight orders (Plagiorchiida, Echinostomida, Ascaridida, Strongylida, Spirurida, Rhabditida, Echinorhynchida, Polystomatida), fifteen families (Omphalometridae, Cepahlogonimidae, Gorgoderidae, Lecithodendriidae, Pleurogenidae, Diplodiscidae, Plagiorchiidae, Diplostomatidae, Macroderoididae, Cosmocercidae, Molineidae, Spirocercidae, Rhabdiasidae, Echinorhynchidae, Polystomatidae) and eighteen genres (*Opisthioglyphe, Cephalogonimus, Gorgodera, Gorgoderina, Pleurogenes, Pleurogenoides, Prosotocus, Diplodiscus, Haplometra, Plagiorchis, Tylodelphys, Cosmocerca, Oswalcocruzia, Ascarops, Agamospirura, Rhabdias, Acanthocephalus, Polystoma*).
The structure of the helminthic fauna was studied depending on the direct influence of ecological factors during the entire annual and life cycle of *Rana temporaria* Linnaeus, 1758, species hitch allowed the appreciation of the degree of knowledge of the period, stations and habitats where it is possible to vectorize parasitic agents to the definitive hosts of detected parasitic agents.
It was found that the host species *Rana temporaria* from the area of the Republic of Moldova plays an important role in the formation and maintenance of foci of parasitic agents common to fish in 10.5% of cases, reptiles - 10.5% of cases, birds - 15.8% of cases, mammals - 15.8% of cases and humans in 5.3% of cases.

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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 Publishing House, 244 p.

Bannikov, A. G., Darevskiy, I.S., & Ishchenko, V.G. (1977). Opredelitel zemnovodnykh i presmykayushchikhsya fauny SSSR (Guide to

Amphibians and Reptiles of the USSR Fauna) Moscow, RU: Prosveshchenie Publishing House, 414 s.

Burakova, A.V., & Malkova, E.A. (2021). Species composition of parasites *Rana temporaria* Linnaeus, 1758 (Amphibia: Ranidae) in the vicinity of the Visim State Nature Biosphere Reserve. *Russian Journal of Parasitology*, 15(1), 16-24 (In Russ.)

Chikhlyaev, I., & Ruchin, A. (2014). The helminth fauna study of European common brown frog (*Rana temporaria* Linnaeus, 1758) in the Volga basin. *Acta Parasitol., 59*(3), 459-71.

Cozari, T., & Gherasim, E. (2021). *Biology, ecology and ethology of ecaudate amphibians (Ranidae, Bufonidae) from the ecosystems of the Republic of Moldova*, p. 240. https://ancd.gov.md/sites/default/files/TODERAS%20Ion.pdf

Cozari, T., Usatâi, M., & Vladimirov, M. (2007). The animal world of Moldova. Fish, amphibians, reptiles, vol. 2. Chisinau, MD: Știința 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., & Erhan, D. (2024). Helminthic fauna of amphibians and reptiles from the Republic of Moldova. Trematode, vol. 2. Chișinău, MD: Tipografia Centrala Publishing House.

Griffin, C.T. (1989). Oswaldocruzia filiformis (Nematoda: Trichostrongyloidea) in frogs (*Rana temporaria*) from three locations in Ireland. *Journal of Helminthology*, 63(1), 53-62.

Herczeg, D., Ujszegi, J., Kásler, A., et al. (2021). Host–multiparasite interactions in amphibians: a review. *Parasites Vectors,* 14, 296 https://doi.org/10.1186/s13071-021-04796-1

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. Publishing House.

Red Book of the Republic of Moldova (2015). Min. Environment of the Rep. Moldova, Academy of Sciences of Moldova, Botanical Garden & Inst. of Zoology; National Commission Chisinau, MD: Știința Publishing House.

Shchepina, N.A., Baldonova, D.R., & Dugarov, Z.H.N. (2006). *Gel'mintofaune beskhvostykh amfibii Zabaikal'ya. Teoreticheskiye i prakticheskiye voprosy parazitologii.* V: Sbornik dokladov Nauchnoi konferentsii, posvyashchennoi 50-letiyu kafedry obshei biologii s genetiki i parazitologii i 80-letiyu so dlya rozhdeniya pervogo zaveduyushchego kafedroi biologicheskikh nauk, 22 dek. Kemerovo, 186-189.

Sudarikov V.Y., Shigin A. A., Kurochkin YU. V. (2002). The metacercariae of trematodes - the parasites of gydrobionts in Russia. Moscow, RU: Nauka Publishing House, 298 p.