THE PELOPHYLAX ESCULENTA (*AMPHIBIA*) COMPLEX AND THEIR INFESTATION BY THE TREMATODE CODONCEPHALUS URNIGER (*STRIGEIDA*) SPECIES IN THE REPUBLIC OF MOLDOVA CONDITION

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Abstract. The paper presents data on amphibians' trematodes infestation from Pelophylax esculenta complex (Amphibia, Anura) in natural and anthropized ecosystems of Central Codri forest from the Republic of Moldova. The helminthological researches of amphibians were accomplished for the first time in the Republic of Moldova. As result, the infestation of Pelophylax complex species (Pelophylax ridibundus, P. lessonae, P. esculentus) with trematodes from family Diplostomidae (Codonocephalus urniger, Rudolphi, 1918) was established.

For each species there were established: taxonomic status, including synonyms, the hosts, the location in organs, the geographic spreading, etiology and biologic cycle. There are also mentioned the amount of collected material, the morphologic description of the species, original figures and pictures, the level of infestation depending on age, sex and ecosystem.

Key words: Amphibia, infestation, trematodes, family Diplostomidae.
Universal Decimal Classification: 597.6/.9

COMPLEXUL PELOPHYLAX ESCULENTA (*AMPHIBIA*) ȘI INFESTAREA ACESTORA DE SPECIILE DE TREMATODĂ CODONCEFALUS URNIGER (*STRIGEIDA*) ÎN CONDIȚIA REPUBLICII MOLDOVA

Abstract. Lucrarea prezintă date despre infestarea trematodelor amfibiene din complexul Pelophylax esculenta (Amphibia, Anura) în ecosistemele naturale și antropizate ale Codrilor Centrali din Republica Moldova. Pentru prima dată în Republica Moldova au fost realizate cercetările helminologice ale amfibienilor. Ca rezultat, a fost stabilită infestarea speciilor complexe (Pelophylax ridibundus, P. lessonae, P. esculentus) cu trematode din familia Diplostomidae (Codonocephalus urniger, Rudolphi, 1918).

Pentru fiecare specie au fost stabilite: statutul taxonomic, sinonimele incluse, gazdele, localizarea în organe, răspândirea geografică, etiologia și ciclul biologic. Se menționează, de asemenea, cantitatea de material colectat, descrierea morfologică a speciei, figurile originale și imaginile, nivelul infestării în funcție de vârstă, sex și ecosistem.

Cuvinte cheie: Amfibie, infestare, trematode, familia Diplostomidae.

Introduction

Helminthological researches are frequently focused on study of the infestation level of domestic animals, wild animals, pets and humans. Currently, in different regions of the world most taxa species are poorly studied from helminthological point of view, including amphibians. Amphibians' value as an essential component of biota is quite obvious as the definitive, intermediate, complementary hosts and as host for various groups of helminthes. In some cases, amphibians serve as cause of infections not only for domestic and wild animals, but also are considered as an important agent in maintaining

their circulation in nature and actively participate in the formation of parasitic zoonoses [6].

That is why, it is appropriate for helminthological study of the amphibians from *Pelophylax esculenta* complex, to establish their circulation specificity in the natural and anthropogenic habitat, but also their contact with the host.

This study will contribute to developing the knowledge base necessary to assess the ecological role of amphibians as definitive, intermediate, complementary, reservoir hosts, as well as biological indicators of aquatic and terrestrial habitats, and will determine the parasitological situation, establishing some features in the pathogenesis of outbreaks of parasitic agents and the development of epizootic and epidemiological measures, increasing knowledge of fauna [1].

In terms of taxonomic and systematic research, data on faunal and ecological researches in amphibians (fam. *Ranidae*) can be considered rather complete, but their helminth fauna in the Republic of Moldova for the first time is studied.

Material and methods

Investigations for determining the level of infestation of amphibians from fam. *Ranidae* were performed in the laboratory of Parasitology and Helminthology of the Institute of Zoology of the MECC. The amphibian species were identified by external characters[2].

The amphibians in natural and artificial water basins have been captured during the 2012 - 2018 years, during active life.

237 of amphibians have been captured, including 165 specimens of *Pelophylax ridibundus* species (104 males, 61 females), 44 – *Pelophylax lessonae* (35 males, 9 females) and 28 specimens of *Pelophylax esculentus* (18 males, 10 females).

Helminth fauna diversity was determined according to standard method proposed by Academician K.I. Skrjabin, that involves examination all the internal organs of the animal [5].

The collection, fixation, determination and helminthological material processing were performed by the methods proposed by various authors [4].

To establish the veracity of data were used the methods of mathematics and statistics.

Results and discussions

The conducted studies denote that amphibians from Ranidae family that inhabit the natural and anthropic ecosystems of the Central Codrii from the Republic of Moldova are widespread. Unlike brown European species (*Rana dalmatina*, *R.temporaria*, *R.arvalis* s.a.), the green ranide, or *Pelophylax esculenta* complex, have, preponderantly, a green

color body and during the annual life cycle depend largely on aquatic environment, therefore they, usually, inhabiting aquatic habitats and their immediate vicinity.

Due to the fact that water basins in the Republic of Moldova have rich aquatic-terrestrial vegetation, hydrologic and thermal regime, depth, configuration and height of the banks, creates optimal conditions for the whole annual cycle of green ranide life with wide spread both in natural ecosystems, as well as in artificial ones.

The aim of the researches was to establish amphibian infestation from *Pelophylax esculenta* complex with trematodes from *Strigeida* order.

Systematic classification:

(www.faunaeur.org/index.php)

Order – STRIGEIDA La Rue, 1926, Subordo, Sudarikov, 1959

Infraorder – DIPLOSTOMOIDEA

Etiology. The trematode *Codonocephalus urniger* Rudolph, 1819 (Figure 1, Photo. 1) parasites under the skin, into the body cavity, muscles and other organs of the amphibians.

The body length varies from 0.507 to 1.149 mm.

The anterior segment of body has cup-shaped form. The edge opening is wavy or lobed. At the top of the small oval protrusion the cup mouth is located with a diameter of 0.077 to 0.104 mm.

Directly behind the suction cup is the pharynx, whose diameter is 0.127- 0.125 mm. Intestinal thin branches stretch to the rear end of the body. Ventral suction cup with a diameter of 0.192 - 0.208mm, it is located on a flexible stem. The posterior segment is cylindrical and in size - more than the previous one.

Part of the genital atrium is differentiated by a moderate narrowing. The testicles are round, with diameter of 0.286 - 0.369 x 0.239 - 0.358mm.

The seminal vesicle is present. The ovary has dimensions of 0.151 - 0.156mm. Vitelogene glands occupy the space between the previous segments up to genital cone. The uterus is free of eggs. Genital cone is bulky and penetrated by hermaphrodite channels.

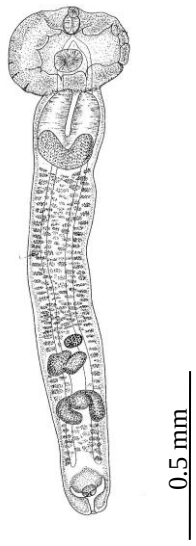

Figure 1. Codonocephalus urniger, larvae - general aspect. Original.

Foto 1. Codonocephalus urniger, larvae - general aspect. Original.

Table 1. Morphometric parameters of the species Codonocephalus urniger Rudolphi, 1819, n =15

Characters	Mean, mm	MS	σ	CV	sdCV	Minim, mm	Maxim, mm
Surface	2.851	0.149	0.577	20.2	3.8	1.364	3.447
Body length	5.053	0.208	0.807	16.0	3.0	3.586	5.921
Body width	0.545	0.022	0.085	15.5	2.9	0.337	0.687
Suction cups length	0.077	0.007	0.023	30.1	7.3	0.050	0.117
Suction cups width	0.104	0.005	0.016	15.4	3.5	0.070	0.123
Pharynx length	0.127	0.006	0.0237	18.7	3.7	0.078	0.170
Pharynx width	0.125	0.006	0.021	16.5	3.2	0.094	0.165
Ventral suction cup length	0.192	0.014	0.048	24.8	5.4	0.116	0.279
Ventral section cup width	0.208	0.011	0.037	17.7	3.7	0.154	0.272
First testicle length	0.286	0.029	0.110	38.4	8.3	0.122	0.468
First testicle width	0.369	0.266	0.099	26.9	5.4	0.158	0.568
Second testicle length	0.239	0.019	0.068	28.5	6.0	0.142	0.381
Second testicle width	0.358	0.021	0.076	21.1	4.3	0.208	0.506
Ovary length	0.151	0.012	0.015	29.5	6.0	0.099	0.027
Ovary width	0.156	0.009	0.033	20.9	4.1	0.093	0.215

Note: MS – average error, *σ* – standard deviation, *CV* – variation coefficient, *sdCV* – error of variation coefficient.

At the rear part of genital atrium, around the genital conus the ring crease is well developed. In the posterior segment is a dense network of subcutaneous channels containing fat droplets. Concomitantly with the posterior segment, there are two collecting channels which up to excretory pore are united in one. (*Table 1*).

Biological cycle. *Codonocephalus urniger* Rudolphi, 1819 is a trematode with trixen development cycle. As intermediate hosts serve aquatic snails: *Lymnaea stagnalis* and *L. palustris*, amphibians for this trematode are complementary hosts. In their organism the larval form parasitize, at the stage of trematodes metacercaries *Codonocephalus urniger* Rudolphi, 1819.

Definitive hosts are various species of birds as: *Botaurus slellaris* Linnaeus, 1758, *Ixobrychus minutus* Linnaeus, 1766, *Ardea purpurea* Linnaeus, 1766, *Egretta garzetta* Linnaeus, 1766 etc.

Amphibians' infestation takes place beginning with stage of tadpoles and finishing with adult specimens.

According to performed helminthological investigation on the species *Pelophylax esculenta* (*Pelophylax ridibundus, Pelophylax lessonae, Pelophylax esculentus*) complex in the Republic of Moldova during the 2013 - 2018 years there was established the presence of the species *Codonocephalus urniger* in the body cavity, subcutaneous cellular-adipose tissue, muscles and various internal organs.

At the locating metacercaries in the sexual organs and at the high intensity the sterility of amphibians was established.

Our research performed during the 2013-2018 demonstrates that the level of helminth infestation in *Pelophylax esculenta* complex depending on intrinsic and extrinsic factors depends both on the helminth species, as well as the host species.

In all the species of the complex *Pelophylax ridibundus, Pelophylax lessonae* and *Pelophylax esculentus* infestation with *Codonocephalus urniger* was registered only in the summer and only at the specimens of the complex *Pelophylax esculenta* collected from natural water basin Ghidighici and basins from the Valea Trandafirilor park (Chisinau).

Helminthological investigations in dependence on the type of host, demonstrated that the level of infestation depends both on the species invasion, as well as hormonal factor of the host. So, the highest level of infestation with *Codonocephalus urniger* was registered at females of the *Pelophylax* complex, but higher intensity was established in males (Table 2).

Table 2. Extensivity and intensivity of invasion in species from Pelophylax esculenta complex depending on the host type

Host	Males			Females		
	EI, %	***II, ex.***		***EI, %***	***II, ex.***	
		Min.	**Max.**		**Min.**	**Max.**
Phelophylax ridibundus	3.2	2	52	5.6	14	33
Pelophylax lessonae	7.1	1	36	10.0	1	9
Pelophylax esculentus	6.4	1	4	9.1	1	1

Another important factor on which depends the helminth fauna diversity in amphibians and also a frequent question in the literature is the age factor. According to our helminthological research there wasn't registered any infestation of juvenile with trematods species *C.urniger*. Thus, the level of helminths infestation in amphibians increases with the host age and dependend on its trophicity.

Conclusions

1. For the first time in the Republic of Moldova's fauna has been established and described a new species of trematode *Codonocephalus urniger* with medico-veterinary importance.
2. It was established that at the *Pelophylax esculenta* species complex, *Codonocephalus urniger* trematodes species has its organic specificity in the body cavity, cellular-adipose subcutaneous tissue, muscles and various internal organs, and at the metacercaries location in the sexual organs with a high intensity the amphibian sterility was established.
3. The level of amphibian infestation with helminthes depending on seasonal factors, depends both on the helminthes species, as well as the host species.
4. Following the helminthological researches of the *Pelophylax esculenta* complex depending on host sex different values were established, so females are characterized by a higher infestation level than males.
5. It has been estimated that the level of amphibians infestation with helminthes captured from natural basins is higher, in comparison with artificial ones, and this divergence occur depending on biotope, on faunistic condition (presence of definitive hosts, intermediate, reservoir) as well as on condition of their environment.

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