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Helminthofauna of *Pelophylax* kl. *esculentus* (Linne, 1758) from Petrovaradinski Rit Marsh (Serbia)

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Summary

The helminth fauna of lungs, small intestine, and rectum of 83 female specimens of *Pelophylax* kl. *esculentus* was investigated. Three helminth groups, Trematoda, Nematoda, and Acanthocephala were recorded. The sample was dominated by the class Trematoda. This class was represented by 8 adult and 2 larval species. *Opisthioglyphe ranae* and *Pleurogenoides medians* were the dominant fluke species. The data show the occurrence of *Acanthocephalus ranae* in frogs inhabiting Vojvodina Province.

The most frequent Nematoda was *Oswaldocruzia filiformis*. The greatest number of parasitic species and individuals was isolated from the small intestine. Infestation of individual parasitic groups was greater than that of mixed groups. The most frequent combination was T/N while analysed organs were most frequently invaded by a parasitic species only. Antagonism between genus *Rhabdias* and *Haematoloechus* and between species *Aplectana acuminata* and *Opisthioglyphe ranae* was ascertained.

Keywords: helminthofauna; genus *Pelophylax*; Petrovaradinski Rit Marsh

Introduction

Frogs are widespread and numerous aquatic vertebrates representing a very important link in many food chains. Generally, they have extensive biocoenosis effects. *Pelophylax* kl. *esculentus* is a hybrid species between *P. lessonae* and *P. ridibundus*. Evidence suggests that it has a lower fertility, high vigour, and a broad ecological valence, and that it is associated with running and stagnant waters such as ponds, lakes and marshes with thick vegetation (Engelmann, W. E. *et al.*, 1993).

In Vojvodina Province, the anuran helminth fauna was intensively investigated during the last fifteen years (Kostić, 1999; Popović, 1992; Popović & Šimić, 1995; Popović *et al.*, 1997, 1998a, b, 2003). Despite these systematic studies, there are areas requiring additional revision or a new, comprehensive approach. Petrovaradinski Rit marsh is a completely unknown study area. This locality is subjected to periodical floods of the Danube. The marsh is situated at an elevation between 74 and 94m where human impact prevails. This area is overgrown by wet medows including communities of *Carex gracilis-Poa palustris* Ilijanić, 1967, *Bidenteto-Potentilum* anserine Babić, 1971 and *Scirpo-Phragmitetum* W. Koch 1926 and souranded by forests with dominanting communities *Salicetum triandrae* Malcuit, 1929 and *Salicetum albo-Amigdaline* Slavnić, 1952.

This paper provides information about the parasite infestation (infestation extensity and intensity values) of *Pelophylax* kl. *esculentus* from Petrovaradinski Rit marsh.

Material and methods

The sample consisted of 83 female individuals of the species *Pelophylax* kl. *esculentus* from the Petrovaradinski Rit marsh. The analysis of Trematofauna was carried out on the lungs, digestive tract (stomach, intestine and rectum) and bladder. Parasites were preserved in 70 % ethanol, stained with Carmine and fixed in Canada balsam. The material was analyzed with the use of a microscope.

Specimens of Nematoda and Acanthocephala were destained in lactic acid for 1.5 – 2 h and 3 – 4 h, respectively, and analysed microscopically in a drop of lactic acid for species and sex determination.

Results

Helminthic analysis of 83 representatives of the species *P.* kl. *esculentus* shows an infestation extensity of 67.78 %, namely the occurrence of 272 individuals of recorded

Fig. 1. *Paralepoderma cloacicola*

parasites. Three helminth groups, Trematoda, Nematoda, and Acanthocephala, were present. The sample was dominated by the class Trematoda (Fig. 2). This class was represented by 8 adult and 2 larval species. So we recorded lung parasites, *Haematoloechus variegatus variegatus* (Rudolphi, 1819), *H. odeningi* (Boschkow, 1964), and *H. schulzei* (Wundsch, 1911), small intestine parasites, *Pleurogenoides medians* (Olsson, 1876), *Prosotocus confusus* (Looss, 1894), *Pleurogenes claviger* (Rudolphi, 1819), and *Opisthioglyphe ranae* (Frolich, 1791; Looss, 1899), and rectum parasite *Diplodiscus subclavatus* (Goeze, 1872), as well as intestinal metacercaria *Neodiplostomum spathoides* (Dubois, 1937) and *Paralepoderma cloacicola* (Luhe, 1909) (Fig. 1).

All the fluke species are typical anuran parasites while the trematode *P. medians* invades also tailed amphibians and

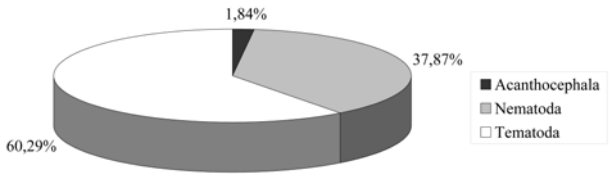

Fig. 2. Percentage of helmintological groups in total sample of analized host - *Pelophylax* kl. *esculentus*

lizards. The species *N. spathoides* parasitizes birds of the order Falconiformes (Edelenyi, 1974) while the species *P. cloacicola* is a parasite of *Natrix natrix* (Vojtkova, 1974) where the frogs are important intermediate hosts.

In total, infestation extensity amounted 43.37 % (164 parasites) while the average number per host was 4.56. According to the infestation extensity, the hosts were dominated by *Opisthioglyphe ranae* (50 %) while according to the number of parasite individuals, *Pleurogenoides medians* (55 individuals) was dominant. The small intestine was the organ where the greatest number of fluke species (6) and also the greatest number of individuals (125) was isolated. The lowest values of infestation extensity were obtained with *H. v. variegatus* (5.56 %), *H. odeningi* (2.78 %) and *H. schulzei* (2.78 %), whereas in the anuran rectum only a species *D. subclavatus* (27.78 % or 20 individuals) was found. Metacercarias of *P. cloacicola* (5.56 % or 5 individuals) and *N. spathoides* (11.11 % or 7 individuals) were isolated from anuran intestine (Tab. 1).

The majority of infested hosts belongs to the 1 – 4 group (1 – 4 parasites per host). Maximum parasite number per host was 26.

Parasites of the Phylum Nematoda infested 31 out of 83 frog females, accounting for the infestation extensity of 37.35 %. With respect to the frog organs, lungs, small intestine, and rectum were invaded by the 4 Nematoda species.

Table 1. Invasion extensity and number of individuals of fluke species by host organs (*P.* kl. e*sculentus*)

Org	Fluke species	Extensity (%)	N° of parasites	Average number
Pul	*H. v. variegatus*	5.56	8	4
	H. odeningi	2.78	1	1
	H. schulzei	2.78	10	10
Int	*P. medians*	30.56	**55**	5
	P. confusus	13.89	8	1.6
	P. claviger	5.56	2	1
	O. ranae	**50**	48	2.67
	P. cloacicola	5.56	5	2.5
	N. spathoides	11.11	7	1.75
Rec	*D. subclavatus*	27.78	20	2
	Total	43.37	164	4.56

The species *Rhabdias bufonis* (Schrank, 1788), represented by 6 individuals (5.82 %), that infested 6 frog females (19.35 %) was found in frog lungs.

The occurrence of 3 Nematoda species was observed in both small intestine and rectum. *Oswaldocruzia filiformis* (Goeze, 1782) Travassos, 1917 was a dominant species occurring much more abundantly in the small intestine. In other words, the rectum of only one host was infested (3.22 %) by one male of Nematoda species, whereas small intestine of even 13 hosts was entered by a parasite, showing an infestation extensity of 41.93 %. Also, 46 parasite individuals (44.66 %) was isolated. On the contrary, *Cosmocerca ornata* (Djuardin, 1845) was more abundant in frog rectum. It was found in 7 hosts (22.58 %) where 18 individuals (17.48 %) were observed. Small intestine of only 3 hosts was infested by 5 nematodes (4.85 %). *Aplectana acuminata* Railliet et Henry, 1916 was also more numerous in rectum. It was represented by 23 individuals (22.33 %) in 9 hosts (29.03 %). Five parasite individuals (4.85 %) were found in small intestine of 4 hosts (12.90 %) (Tab. 2).

Table 2. Percentage of Nematoda species by organs, age, and sex in females of *Pelophylax* kl. *esculentus*

Org.	Nematode species	D.		P.							
				Total		Females.		Males		Juv.	
		No	%	N°	%	N°	%	N°	%	N°	%
Pul.	*R. bufonis*	6	19.35	6	5.82	6	8.82	-	-	-	-
Int.	*C. ornata*	3	9.68	4	3.88	2	2.94	1	3.85	1	11.11
	O. filiformis	13	**41.93**	46	**44.66**	27	**39.71**	19	**73.08**	-	-
	A. acuminata	4	12.90	5	4.85	3	4.41	1	3.85	1	11.11
Rec.	*C. ornata*	7	22.58	18	17.48	12	17.65	3	11.54	3	33.33
	O. filiformis	1	3.22	1	0.97	-	-	1	3.85	-	-
	A. acuminata	9	29.93	23	22.33	18	26.47	1	3.85	4	**44.44**
	Total	31	37.35	103		68	66.02	26	25.24	9	8.74

Irrespective of frog organ, *O. filiformis* invaded the greatest number of hosts (45.63%), then *A. acuminata* (27.18 %) and *C. ornata* (21.36 %) (Fig. 3).

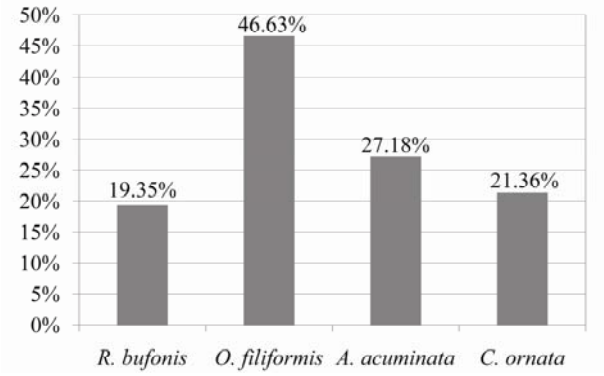

Fig. 3. Infestation extenzity with nematode species irrespective of analysed frog organ

A total of 103 Nematoda specimens dominated by female individuals (66.02 %) were isolated. Also, adults prevailed (91.26 %).

The least present were representatives of Acanthocephala. This phylum was represented by 5 specimens of the species *Acanthocephalus ranae* (Schrank, 1788) Luche, 1911 that invaded only few hosts (4.44 %).

Actually, the analysed helminth groups, Trematoda, Nematoda, and Acanthocephala, were individually present in the host. Most frequently, nematodes were found in combination with trematodes (T/N) (Fig. 4). Also, most hosts were infested by one trematode species. The small intestine was the organ showing an exception (2 fluke species). *O. ranae* was most frequently found in the combination with *P. medians*. Maximum number of parasitic species, *P. medians*, *P. confusus*, *P. claviger*, and *O. ranae*, was isolated from small intestine while maximum number of fluke species per host included *H. v. variegatus*, *P. medians*, *P. confusus*, *P. claviger*, *O. ranae*, and *D. subclavatus*. With respect to Nematoda, also one species per host was intrinsic to most analysed frogs. The organ that was an exception was the rectum where both *A. acuminata* and *C. ornata* were found. The infestation with the genus *Haematoloechus* excluded the invasion of the genus *Rhabdias*. The species *O. ranae* was found in combination with *A. acuminata* in the same host, but no such combination in the same organ was recorded.

Discussion

Most sites in Vojvodina Province are dominated by the species *P. medians* and *O. ranae* (Popović, 1992; Popović & Šimić, 1996; Popović *et al.*, 1998a,b). Their intermediate hosts are snails and insects of order Coleoptera, Odonata, Megaloptera, and Lepidoptera. They play an important role in the diet of *P.* kl. *esculentus* (Paunović, 2000). Moreover, the prevalence of the species *O. ranae* is determined by the elimination of the second intermediate host (Grabda-Kazubska, 1980). The species *D. subclavatus* is also widespread in Vojvodina Province (Popović & Šimić, 1995; 1996; Popović *et al.*, 1997; 1998a,b; Vučković et al. 2002). Its life cycle requires only one intermediate host (Vojtkova, 1982). The second intermediate host is eliminated (Grabda-Kazubska, 1969), enabling direct cercarial invasion that increases the possibility of host infection. Judging by the number of invaded hosts and the number of isolated parasite individuals, the lung trematodes, *H. variegatus variegatus*, *H. odeningi*, and *H. schulzei*, were rare in the sample. Ginecinskaya (1968) stated that the eggs of the representatives of the genus *Haematoloechus* require an environment rich in oxygen. Consequently, lack of oxygen may be a limiting factor affecting development. An insufficient number of intermediate hosts in the host diet is another possible underlying condition limiting their numbers. This assumption may be confirmed by the fact that intermediate hosts of the species *H. v. variegatus* are the representatives of the order Odonata and mosquitoes larvae. Their number in the anuran diet is insignificant

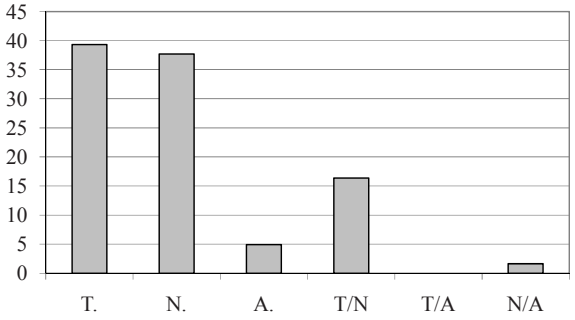

Fig. 4. Individual and mixed invasion with three helminth's groups in females of *P.* kl. *esculentus*

(Paunović, 2000). The species *P. claviger* and *P. confusus* were recorded at the majority of the investigated sites of Vojvodina Province (Popović, 1992; Popović *et al.*, 1997b; 1998a; 2001). In the sample of the edible frog, *P. claviger* and *P. confusus* were scarcely present (2 and 8 individuals, respectively). The available data show that the intermediate host of these trematodes, *Asselus aquaticus* (the second intermediate host of *P. claviger*) and the representatives of the order Odonata and Trichoptera (intermediate host of *P. confusus*) are rare in the diet of the host species (Paunović, 2000).

A metacercaria of *Paralepoderma cloacocola* was recorded for the first time in Serbia. Also, *Neodiplostomum spathoides* was recorded for the first time in *P. ridibundus* residing at the Tamiš river sites (Popović *et al.*, 1998a). Therefore, *P.* kl. *esculentus* is a new host of *N. spathoides*.

Helminthological analysis shows the occurrence of the the Nematoda species *Rhabdias bufonis* in host lungs while *Cosmocerca ornata*, *Oswaldocruzia filiformis* and *Aplectana acuminata* were found in the small intestine and rectum. All the above species are widespread in Europe (Vojtkova, 1990). In our study, infestation extensity of only 37.35 % was recorded. Higher extensity values were obtained with the same host in the Tamiš river area (Popović *et al.*, 1998a) and Ribnjak (Kostić, 1999). Research suggests that the species *Oswaldocruzia filiformis* is widespread in Vojvodina Province, generally with low infestation values (Popović et al., 1997b, 1998a; Kostić, 1999). Its evident affinity for the small intestine agrees very closely with the data published elsewhere (Kostić, 1999; Popović et al., 1997b, 1998a). The percentage of *Cosmocerca ornata* is in agreement with recent investigations (Popović *et al.*, 1997b, 2000). Nematofauna of edible frog from the Carska Bara pond is dominated by this Nematoda species, showing lower infestation extensity and quantity values (Popović *et al.*, 1997b). It prevails in hosts of the genus *Pelophylax* of the Koviljski Rit marsh (Popović *et al.*, 2000) and in the Tamiš river area (Popović *et al.*, 1998a).

High percentages of *O. filiformis* and *C. ornata* may be attributed to their direct life cycle (Hristovski & Lees, 1973a).

In the papers dealing with *Aplectana acuminata*, lower values of its infestation extensity and quantity were presented (Popović *et al.*, 1997b; 1998a). Its abundance in the host rectum was also reported by other authors (Popović *et al.*, 1997b; 1998a; Kostić, 1999; Popović *et al.*, 2000; 2001).

Rhabdias bufonis, the lung parasite was dominant in all the three frog species of the *Pelophylax* complex in the lokality of Ribnjak (Sremska Kamenica) (Kostić, 1999).

Infestation extensity values obtained in our study show that the majority of hosts belong to the group of smaller parasite numbers that agrees with data published elsewhere (Kostić, 1999; Popović *et al.*, 1997b; 1998a). With the exception of *R. bufonis*, Nematoda species were represented by both male and female individuals where females were predominant. Our results are in accordance with those presented by Mazurmovič (1951) who explained this phenomenon by a shorter life of males.

Quantitatively and qualitatively, the Acanthocephala was represented by the smallest number of individuals that is in agreement with earlier investigations (Rozman, 1976; 1981; Vojtkova, 1982; Hristovski *et al.*, 1985). Our results are close to those published by Rozman (1976) who was the first to record the species *Acanthocephalus ranae* in frogs inhabiting Vojvodina Province. Significantly higher infestation extensity values in edible frog were reported by Bačvarov (1973a), whereas our own data are similar to those presented by Plasota (1969). Infestation intensity is lower than that quoted in most papers (Bačvarov, 1973a,b; 1982a,b; Rožman, 1976). This situation may be explained by the fact that the species *Asselus aquaticus* is known as the intermediate host of *A. ranae*, but this Isopoda is occasionally present in the diet of the analysed host (Paunović, 2000).

Our results associated with the small intestine are in agreement with data presented by Mazurmovič (1951).

The infestation modes including both individual and mixed invasion, point to the characteristics referring to the utilization of an ecological niche. Invasion accomplished by the combination of helminth groups was present only in small intestine and rectum. One realistic record of this phenomenon was pioneered by Plasota (1969). The author assumed that the homogeneous conditions prevailing in lungs give rise to a far greater competition. On the other hand, intestinal heterogeneity enables the presence of more than one helminth species or a group of helminths. Dominance of the individual infestation as well as the presence of mixed invasion only in the small intestine and rectum is in agreement with data presented by Popović and Mikeš (1986) and Popović *et al.* (1989). These authors stated that the most frequent combination in *P.* kl. *esculentus* and *P. ridibundus* is T/N. The antagonism between *Rhabdias* and *Haematoloechus* and between *Opisthioglyphe ranae* and *Aplectana acuminata* is well known (Mazurmovič, 1957; 1963). We could recognize the quoted antagonism in the two species recorded from the same host but not from the same host organ.

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