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## Platyhelminth Parasites of Some Amphibians in Jordan

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**Abstract:** A total of 470 frogs were examined for helminth infection: 220 specimens of the green toad *Bufo viridis*, 65 of Savigny's tree frog *Hyla savignyi* and 185 of the Levantine frog *Rana bedriagae*. Six species of adult platyhelminths (trematodes, monogeneans and cestodes) were found to infest the three frog species: two monogeneans, *Polystoma integerrimum* (23%) and *Polystoma viridis* (8%); two digenetic trematodes, *Pleurogenoides tacapensis* (14.5%) and *Prostotocus confusus* (11%); and two cestodes *Nematotaenia dispar* (35%) and *Nematotaenia chantalae* (8.5%). Of the six helminth parasite species, *Polystoma integerrimum* had the highest prevalence in *B. viridis*. In *R. bedriagae*, *Pleurogenoides tacapensis* had the highest prevalence. *Nematotaenia dispar* was the only parasite found to infest the tree frog *H. savignyi*.

**Key Words:** Amphibians, Helminth, Monogenea, Digenea, Cestoda, Jordan

### Ürdün'deki Bazı Amfibilerin Platelmint Asalakları

**Özet:** Helminth enfeksiyonu açısından toplam 470 kurbağa incelenmiştir. Kurbağa örneklerinin 220'si Gece Kurbağası *Bufo viridis*'se, 65'i Ağaç Kurbağası *Hyla savignyi*'ye ve 185'i ise Ova Kurbağası *Rana bedriagae*'ye aitti. Söz konusu üç kurbağa türünün altı ergin yassıkurt türü tarafından enfekte edildiği görüldü: İki monogenea, *Polystoma integerrimum* (% 23) ve *Polystoma viridis* (% 8); iki digenetik trematod, *Pleurogenoides tacapensis* (% 14.5) ve *Prostotocus confusus* (% 11); ve iki sestod, *Nematotaenia dispar* (% 35) ve *Nematotaenia chantalae* (% 8.5). Altı asalak helminth türünden *Polystoma integerrimum*, *Bufo viridis*'le yaygın olarak gözlenmiştir. *Rana bedriagae*'da ise *Pleurogenoides tacapensis* en sık görülen asalaktır. *Nematotaenia dispar* ağaç kurbağasını enfekte eden tek asalak olarak belirlenmiştir.

**Anahtar Sözcükler:** Amfibiler, Helminth, Monogenea, Digenea, Cestoda, Ürdün

### Introduction

Little is known about the amphibian parasites in the Middle East. In Jordan, Madi (1) isolated four trematodes (*Gorgoderina vitelliloba*, *Prostotocus confusus*, *Pleurogenoides tacapensis* and *Haematolechus variegatus*) from *Rana bedriagae* and *Bufo viridis* in Azraq Oasis. Hassan (2) recovered two trematodes from *Bufo regularis* in Egypt. Fernando (3) isolated two adult digenetic trematodes from *Rana ridibunda* in Saudi Arabia: *Pleurogenoides stromi* and *Pleurogenoides compactus*. In Turkey a recent study was performed on the helminths of *Rana macrocnemis*, in which two species of trematodes, *Pleurogenes caliveger* and *Gorgoderina vitelliloba*, and one monogenean species, *Polystoma* sp., were reported (4). In addition, Yildirimhan (5) reported

on the parasitic helminths of *Bufo viridis*. He recovered *Polystoma viridis*, *Proteocephalus* sp., and *Nematotaenia dispar* as well as other nematodes and one acanthocephalan. Furthermore, a new species of *Polystoma*, *Polystoma macrocnemis*, was described from the longlegged wood frog, *Rana macrocnemis* from Turkey (6).

Two review papers summarized all the previous records of helminth infections among the European amphibians (7,8), with a total of 48 digeneans, one monogenean (*Polystoma integerrimum*), 39 larval stages of digenea, and a single cestode (*Nematotaenia dispar*).

The present study is the first attempt to investigate the helminths associated with three species of amphibians in Jordan.

## Materials and Methods

### Frogs of Jordan

Amphibians were collected from several localities in Jordan at different time intervals. Three species of frogs belonging to three families (Bufonidae, Ranidae and Hylidae) are known to occur in Jordan (the Levantine frog, *Rana bedriagae*; the green toad, *Bufo viridis*; and the tree frog, *Hyla savignyi*).

### Isolation of the parasites

A total of 470 frogs were dissected (65 *Hyla savignyi*, 185 *Rana bedriagae*, and 220 *B. viridis*). Frogs and toads were sacrificed using chloroform and then dissected and examined for their helminth parasites. The stomach, intestine, lungs and urinary bladder were dissected and placed separately in a Petri dish containing amphibian saline. Contents were cleaned in saline and examined under a dissecting microscope.

Cestode specimens were fixed using Bouin's solution and then removed after 24 h and put into 70% alcohol. Trematodes were kept in 70% ethyl alcohol, while monogeneans were treated with hot AFA before being transferred into 70% alcohol. Specimens were stained using acetocarmine and mounted permanently on microscopic slides.

For identification, Yamaguti (9) and Prudhoe and Bray (10) were used. All recovered parasites were confirmed by specialists.

## Results

Two hundred and twenty specimens of the green toad *B. viridis*, 65 of the tree frog *H. savignyi* and 185 of the Levantine frog *R. bedriagae* were examined for helminths (Table 1). Six species of adult platyhelminths were found to infect these amphibians: two monogeneans, two digenetic trematodes and two cestodes species. Of the six helminths, *Polystoma integerrimum* showed the highest prevalence in *B. viridis* (16%). In *R. bedriagae*, *Pleurogenoides tacapensis* had the highest prevalence (12%), while in *H. savignyi*, *Nematotaenia dispar* was the only cestode recovered.

Five *R. bedriagae* specimens showed three intestinal parasites (*Pl. tacapensis*, *Pr. confusus*, and *N. dispar*), two were infected with *Pl. tacapensis* and *Pr. confusus*, six with *N. dispar* and *Pl. tacapensis* and three with *N. dispar* and *Pr. confusus*. Twenty green toads were infected with *P. integerrimum* and *N. dispar*, while six toads harboured both *P. viridis* and *N. dispar*.

Table 2 shows the site of parasitic infection in the six recovered parasite species. Both *P. integerrimum* and *P. viridis* were recovered from the urinary bladder, while the other parasites were found to infest the small intestine.

## Discussion

The present work examines for the first time helminth parasites in a neglected group of frogs and

Table 1. Prevalence of helminth parasites in three amphibian species.

Parasite	Host								
	<i>Bufo viridis</i>			<i>Rana bedriagae</i>			<i>Hyla savignyi</i>		
	No. Examined	No. Infected	% Infected	No. Examined	No. Infected	% Infected	No. Examined	No. Infected	% Infected
Class Monogenea									
<i>Polystoma viridis</i>	220	12	5%	185	0	0%	65	0	0%
<i>Polystoma integerrimum</i>	220	35	16%	185	0	0%	65	0	0%
Order Digenea									
<i>Prosotocus confusus</i>	220	0	0%	185	17	9%	65	0	0%
<i>Pleurogenoides tacapensis</i>	220	0	0%	185	22	12%	65	0	0%
Class Cestoda									
<i>Nematotaenia chantalae</i>	220	13	6%	185	0	0%	65	0	0%
<i>Nematotaenia dispar</i>	220	25	11%	185	20	10%	65	9	14%

Table 2. Site of infection for helminth parasites recovered from Jordanian frogs.

Parasite	Host	Site of Infection
<i>Polystoma integerrimum</i>	<i>Bufo viridis</i>	Urinary bladder
<i>Polystoma viridis</i>	<i>Bufo viridis</i>	Urinary bladder
<i>Pleurogenoides tacapensis</i>	<i>Rana bedriagae</i>	Small intestine
<i>Prosotocus confusus</i>	<i>Rana bedriagae</i>	Small intestine
<i>Nematotaenia dispar</i>	<i>Bufo viridis</i> , <i>Rana bedriagae</i> and <i>Hyla savignyi</i>	Small intestine
<i>Nematotaenia chantalae</i>	<i>Bufo viridis</i>	Small intestine

toads that represent an important group of vertebrates in Jordan. Such hosts constitute a significant group of trophic levels of any ecosystem. This work comes in time to open the door for further research on amphibian parasitism. Amphibian parasitism has been used as a model for understanding very important issues pertaining to the evolution of parasites and their hosts, life cycles, host-parasite relationships, etc.

*Nematotaenia dispar* was found to be the most dominant species of cestodes; it was found to parasite species belonging to Bufonidae, Ranidae, and Hylidae. *Nematotaenia dispar* covers a broad geographical region throughout the countries of North Africa, the Middle East, Europe, India, and South-East Asia (11). Host records to date imply that *N. dispar* is primarily a parasite of the family Bufonidae (10), and it was also recorded from species of anuran families (Hylidae and Ranidae), and from caudate amphibians (12). In the Middle East, it was reported only in Turkey, Iraq, Iran and Yemen (5,10), while Fernando (3) failed to record it from Saudi Arabia. *Nematotaenia viride* is a synonym of *N. dispar* and it was recorded from Tunisia (13). However, Jones (11) considered this taxon a senior synonym.

*Nematotaenia chantalae* showed a higher level of host specificity; it was found only in *B. viridis* with a narrower geographical distribution. It has not been recorded before in the Middle East. *Nematotaenia chantalae* is known only from the coast of North Africa. Its host range includes species of Bufonidae and Ranidae. Soler (14) recorded a specimen of *N. chantalae* from *B. bufo* in Morocco. It was also isolated from *Bufo* sp. in Algeria and Yemen.

*Pleurogenoides tacapensis* was found in the small intestine of *R. bedriagae* in the Al Ghawr area only. Madi (1) isolated this species from the same host from Azraq. It was taken from *R. ridibunda* in Tunisia (15). This

species deviates from other species of the genus by its excretory vesicle, which is definitely Y-shaped while those of other species of *Pleurogenoides* are V-shaped (16). On this basis, Balozet and Callot (17) placed *Pleurogenoides tacapensis* in a separate genus.

Despite the extensive field studies and collecting in the Azraq pools, this parasite was not recovered. Perhaps the drastic changes in the water level and the continuous water extraction affected the breeding of the intermediate host snail. In this regard, such changes can be indicators of ecological changes in certain habitats.

*Prosotocus confusus* was isolated from *R. bedriagae* inhabiting the same locality from which *P. tacapensis* was recovered. Similarly, this parasite was not isolated from frogs originating from Azraq.

Two species of the class Monogenea were isolated during this study: *Polystoma integerrimum* and *Polystoma viridis*. *Polystoma integerrimum* was considered by far the most common amphibian parasite all over the world. It inhabits the urinary bladder of its amphibian hosts.

*Polystoma integerrimum* is a parasite of Old World frogs. It has a particular reproductive cycle, since it is synchronized with that of its host's hormones. Furthermore, two different types of adults develop: normal and neotenic (17). *Bufo viridis* is the only host of this parasite in Jordan, with a prevalence of 16%. In Europe, however, it has a wider range of hosts. It was isolated from *R. temporaria* in France, Austria, Poland, the former USSR, and the Czech Republic with a prevalence not exceeding 45%. On the other hand, *P. integerrimum* was found to parasitize *R. esculenta* from the Czech Republic. It was also recovered from *B. viridis* inhabiting the former USSR and Poland (18).

*Polystoma integerrimum* and *P. viridis* exhibit many morphological similarities. Differences in measurements

are not considered differential parameters (19). The core difference between these two species is in the morphology of the hamuli (hooks). The hamuli of *P. viridis* can be distinguished from those of *P. integerrimum* by their guard and large handle (or handle-like enlargements). Another distinguishing character is the strict specificity of *P. viridis* compared to *P. integerrimum*, which can be found in species of the family Ranidae, while *P. viridis* is found only in *B. viridis*. This is a very rare species; it was only recorded in Corsica, Bulgaria and Turkey (5,6,19). This is the first record in Jordan and the Middle East. In our study, single infection with either *P. integerrimum* or *P. viridis* was observed within individuals of *B. viridis* within the same locality.

*Rana bedriagae* showed a high rate of infection with both recovered digenetic trematodes. In contrast, neither the green toad nor the tree frog were ever infected by any of these trematodes. The difference between the results obtained from the two anuran hosts may be explained on the basis of the difference in their behaviour. Toads are less common inhabitants of water than frogs and thus are less exposed to infection by larval trematodes.

Species of the genus *Nematotaenia* occur in three biogeographical regions, namely the Palaearctic, Oriental and Australian regions. Members of this genus infect the anuran families Bufonidae, Hylidae and Ranidae, plus the saurian families Gekkonidae, Varanidae and Scincidae in the Palaearctic region (10).

Savage (20) proposed that the Ranidae originated in what is now Africa and moved through the Palaearctic

and Oriental regions into the Nearctic region. The Bufonidae also originated in Africa; one line evolved in that continent and the second spread into South America and radiated from there into the Nearctic, Oriental and Palaearctic regions (20). The Hylidae evolved in South America and then spread as the second line of Bufonidae.

This preliminary study on the platyhelminths of amphibians in Jordan is the first step for future studies to understand their population dynamics and life histories and other nematodes infecting amphibians.

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