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# Helminth Parasites of *Bufo viridis*, *Rana ridibunda* and *Hyla arborea* Collected from the Different Regions of Turkey

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**Abstract**
A total of 170 frogs were examined for helminth infection: 97 specimens of the night frog *Bufo viridis* Laurenti, 1768, 50 of the marsh frog *Rana ridibunda* Pallas, 1771 and 23 of the tree frog *Hyla arborea* Linneaus, 1758. During this study *Polystoma viridis* Euzet, Combes, Batchvarov, 1974, *Nematotaenia dispar* (Goeze, 1782) (Included Plathelminthes), *Rhabdias bufonis* (Schrank,1788), *Cosmocerca commutata* (Dies, 1851), *Oswaldocruzia filiformis* (Goeze, 1782) (Included Nemathelminthes), *Acanthocephalus ranae* (Schrank, 1788) (Included Acanthocephala) were found to infest *Bufo viridis*; *Diplodiscus subclavatus* (Pallas, 1760) Diesing, 1836, *Gorgodera vitelliloba* (Olsson, 1876), *Prosotocus confusus* (Looss, 1894), *Haematoleochus breivance* (Sudarikov, 1950), *Opisthioglyphe ranae* (Frölic, 1791) (Included Plathelminthes), *Cosmocerca ornata* (Dujardin, 1845), *Eustronglydes excisus* Jagerskiöld, 1903 (Included Nemathelminthes), *Acanthocephalus ranae* (Included Acanthocephala) were found to infest *Rana ridibunda* and *Cosmocerca ornata*, *Oswaldocruzia filiformis* (Included Nemathelminthes) were found to infest *Hyla arborea*.

Keywords: Amphibians, helminth, infection, Plathelminthes, Nemathelminthes, Acanthocephala.

# Türkiye'nin Farklı Bölgelerinden Toplanmış *Bufo viridis*, *Rana ridibunda* ve *Hyla arborea*'nın Helmint Parazitleri

**Özet:**
Gece kurbağası *Bufo viridis* Laurenti, 1768'den 97, bataklık kurbağası *Rana ridibunda* Pallas, 1771'dan 50 ve ağaç kurbağası *Hyla arborea* Linneaus, 1758'dan 23 örnek olmak üzere toplam 170 kurbağa helmint enfeksiyonu için incelenmiştir. Bu çalışma süresince *Polystoma viridis* Euzet, Combes, Batchvarov, 1974, *Nematotaenia dispar* (Goeze, 1782) (Plathelminthes'e dahil), *Rhabdias bufonis* (Schrank,1788), *Cosmocerca commutata* (Dies, 1851), *Oswaldocruzia filiformis* (Goeze, 1782) (Nemathelminthes'e dahil), *Acanthocephalus ranae* (Schrank, 1788) (Acanthocephala'ya dahil)'nin *Bufo viridis*'i enfekte ettiği; *Diplodiscus subclavatus* (Pallas, 1760) Diesing, 1836, *Gorgodera vitelliloba* (Olsson, 1876), *Prosotocus confusus* (Looss, 1894), *Haematoleochus breivance* (Sudarikov, 1950), *Opisthioglyphe ranae* (Frölic, 1791) (Plathelminthes'e dahil), *Cosmocerca ornata* (Dujardin, 1845), *Eustronglydes excisus* Jagerskiöld, 1903 (Nemathelminthes'e dahil), *Acanthocephalus ranae* (Acanthocephala'ya dahil)'nin *Rana ridibunda*'yı enfekte ettiği ve *Cosmocerca ornata*, *Oswaldocruzia filiformis* (Nemathelminthes'e dahil)'in *Hyla arborea*'yı enfekte ettiği bulunmuştur.

Anahtar Kelimeler: Amfibiler, helmint, enfeksiyon, Plathelminthes, Nemathelminthes, Acanthocephala.

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## 1. INTRODUCTION

Many types of helmiths may infect amphibians. But little is known about the amphibian helminth parasites in the Middle East. Madi [1] isolated four trematodes from *Rana bedriagae* Camerano, 1882 and *Bufo viridis* in Jordan: *Gorgodera vitelliloba*, *Prosotocus confusus*, *Pleurogenoides tacapensis* Sonsino, 1894 and *Haematolechus veriegatus* (Rudolphi, 1819) Loossi, 1879. Hassan [2] recovered two trematodes from *Bufo regularis* Reuss, 1833 in Egypt. Fernando [3] isolated two adult digenetic trematodes from *Rana ridibunda* in Saudi Arabia: *Pleurogenoides stromi* Travassos, 1930 and *Pleurogenoides compactus* Strohm, 1940. In Turkey, a recent study was performed on the helminths of *Rana macrocnemis* Boulenger, 1885, in which two species of trematodes, *Pleurogenes claviger* Rudolphi, 1819 and *Gorgoderina vitelliloba*, and one monogenean species, *Polystoma sp.*, were reported [4]. Yildirimhan [5] also 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*, *P. macrocnemis* was described from the long legged wood frog, *Rana macrocnemis* from Turkey [6]. In addition, Yıldırımhan et al. [7] reported on the metazoan parasites of the marsh frog, *Rana ridibunda* from the different regions in Turkey. These included 9 Digenea, 4 Nematoda, 2 Acanthocephala and 1 Hirudinea. And then Yıldırımhan et al. [8] reported 1 monogenia and 2 Nematoda parasitic species from tree frog *Hyla arborea* in Turkey. In Europe, Kuc and Sulgostowska [9,10] showed helmint fauna of *Rana ridibunda* from Goclawski Kanal and helmint fauna of frogs in the forest of Kampinos near Warszawa (Poland). After then, Cedhagen [11] investigated endoparasites in some Swedish Amphibians. After this investigation three review papers summarized all the previous records of helminth infections among the European amphibians [12,13,14].

In this study, helminth parasites of some amphibians collected from the different regions in Turkey were investigated.

## 2. MATERIALS AND METHODS

Amphibians were collected from several localities in Turkey at different time intervals. *Bufo viridis* Laurenti, 1768: (Balıkesir 39° 39.0′ N; 27° 52.2′E), *Rana ridibunda* Pallas, 1771: (Bursa 40° 10.8′N; 29° 01.8′E), *Hyla arborea* Linneaus, 1758: (Yalova 40° 39.0′N; 29° 16.2′E). Three species of frogs belonging to three families (Bufonidae, Ranidae and Hylidae) are known to occur in Turkey (the night frog, *Bufo viridis*; the marsh frog, *Rana ridibunda*; and the tree frog, *Hyla arborea*).

*Isolation of the parasites*

A total of 170 frogs were dissected (97 *Bufo viridis*, 50 *Rana ridibunda*, and 23 *Hyla arborea*). Amphibians 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.

Plathelmint specimens were fixed using Bouin's solution and then removed after 24h and put into 70% alcohol. Nemathelmint and Acanthocephala specimens were fixed using hot water and then put into 70% alcohol included 5% glycerine.

Specimens were stained using acetocarmine and mounted permanently on microscopic slides [15].

For identification of specimens, Yamaguti [16], Prudhoe and Bray [17], Hendriks [18] and Khokhlova [19] were used.

## 3. RESULTS AND DISCUSSION

Ninety-seven specimens of the night frog *B. viridis*, 50 of the marsh frog *R. ridibunda* and 23 of the tree frog *H. arborea* were examined for helminths (table 1). Seven species of adult plathelminthes, five species of adult Nemathelminthes and one species of adult Acanthocephala were found to infect these amphibians.
Of the thirteen helminths, *Cosmocerca commutata* showed the highest prevalence in *B. viridis* (48%). In *R. ridibunda*, *Prosotocus confusus* had the highest prevalence (42%), while in *H. arborea*, *Oswaldocruzia filiformis* had the highest prevalence (13%).

*Bufo viridis* specimens showed four intestinal parasites (*N. dispar, C. commutata, O. filiformis* and *A. ranae*), one urinary bladder (*P. viridis*), and one lung (*R. bufonis*) parasites. Three were infected with *N. dispar* and *C. commutata*, while seven night frogs harboured both *N. dispar* and *O. filiformis*. *Rana ridibunda* specimens showed five intestinal parasites (*D. subclavatus, P. confuses, O. ranae, C. ornata* and *A. ranae*), one urinary bladder (*G. vitelliloba*), and one lung (*H. breivance*) parasites while one parasite is found in the body cavity. Five *R. ridibunda* specimens were infected with *N. dispar, P. confuses* and *O. ranae* while six marsh frogs infected both *N. dispar* and *O. ranae*. *Hyla arborea* specimens showed only two intestinal parasites (*O. filiformis* and *C. ornata*).

Table 2 shows the site of parasitic infection in the thirteen recovered parasitic species. *Nematotaenia dispar, P. confuses, O. ranae, C.comutata, O. filiformis* and *A. ranae* were recovered from the small intestine, while *D. subclavatus* and *C. ornata* were recovered from the large intestine. *Polystoma viridis* and *G. vitelliloba* were recovered from the urinary bladder. *Haematoleochus breivance* and *R. bifonis* were recovered from the lungs. Only *Eustronglydes excisus* was recovered from the body cavity of *R. ridibunda*.

Many types of helminths may infect amphibians (figure 1). Monogenes, also common parasites of fishes, may externally infect aquatic life stages of amphibians. Some monogenes may also reside on or in adults, generally as internal infections [20]. Heavy infections are more likely to result in health problems due to traumatic injury or creation of a portal for secondary infections at the site of attachment. Diagnosis is usually made through parasite identification in skin scrapings, gill clips, or other tissue samplings.

Trematodes, otherwise known as flukes or digenes, may be the most widely recognized of the amphibian parasites. Amphibians may serve as secondary intermediate or definitive (final) hosts for trematodes. In general, disease is associated only with high numbers of trematodes encysting in, attaching to, or migrating through host tissues. Lesions generally result from trauma, compression, or displacement of normal tissue by encysted metacercariae, an intermediate life stage of trematodes.

Nematodes or roundworms are also common helminths that infect amphibians from egg to adult life stages and affect a variety of organs and tissues. Nematodes of the genus *Rhabdias* are lungworms that are problematic among captive anurans and possibly caudates. The parthenogenic adult worms cause pulmonary damage and eosinophilic pneumonia. The worm, which has a direct life cycle, produces large numbers of larvated eggs that hatch efficiently at room temperature. Some larvated eggs may hatch within the lumen of the lung or while passing through the gastro-intestinal tract. The larvae actively burrow into the skin and deeper organs, and large numbers of burrowing larvae may be fatal to anurans [21]. Larvae are free in the lymphatic sacs and body cavity, and they appear histologically in nearly all organs.

Cestodes, acanthocephalans and hirudineans reported to infect amphibians and occasionally produce disease. Cestodes, or tapeworms, are not commonly isolated from or problematic in amphibian species, but they may produce significant gastro-intestinal lesions, gastro-intestinal obstruction, and death in heavy infections [22].

The present work examines for the helminth parasites in a neglected group of frogs and toads that represent an important group of vertebrates. Such hosts constitute a significant group of tropic levels of any ecosystem. Amphibian parasitism has been used as a model for understanding very important issues pertaining to the evolution of parasites and their hosts, life cycle, host-parasite relationships, etc.

One species of the class Monogenia was isolated during this study: *Polystoma viridis*. It was considered by far the most common amphibian parasite all over the world. It inhabits the urinary bladder of its amphibian hosts. In this study, *B. viridis* is the only host of this parasite with a prevalence of 16%. In Europe, however, it has a wider range of hosts. It was isolated from *Rana temporaria* Linnaeus, 1758 in France, Austria, and Poland with a prevalence not exceeding 45% [23]. *Polystoma viridis* compared to *P. integerrimum* Frölich, 1791 which can be found in species of the family *Ranidae*, while *P. viridis* is found only in *B. viridis* [24]. *Polystoma viridis* was only recorded in Corsica, Bulgaria and Turkey [5,6,24].

*Nematotaenia dispar* was found to be the most dominant species of Cestodes. It was found to parasite species belonging to Bufonidae. *Nematotaenia dispar* covers a broad geographical region throughout the countries of North Africa, the Middle East, Europa, India and South East Asia [25]. Host records to date imply that *N. dispar* is primarily a parasite of the family Bufonidae [17], and it was also recorded from species of anuran families (Ranidae and Hylidae), and from caudate amphibians [26].

*Diplodiscus subclavatus*, *G. vitelliloba*, *H. breivance*, and *O. ranae* were isolated from *R. ridibunda*. *Rana ridibunda* is the only host of these parasites in Turkey with a prevalence of 16%, 12%, 9%, and 8%. These results are similar to Yildirimhan et al. [7].

*Prosotocus confusus* was isolated from small intestine of *R. ridibunda*. This parasite was isolated from the same body region of *R. bedriagae* in Jordan [27]. *Rana ridibunda* showed a high rate of infection with *P. confusus* (42%). In contrast, neither the night frog nor the tree frog was ever infected by this parasite. The difference between the results obtained from the two anuran hosts may be explained on the basis of the difference in their behaviour. Toads or night frogs are less common inhabitants of water than frogs and thus are less exposed to infection by larval plathelminthes.

*Rhabdias bufonis* was the most wide spread helminth species, found in toads throughout all biotopes, irrespective of the level of urban development. It inhabited the lungs of its amphibian hosts (Vashetko and Siddikov, 1999). Similarly, this parasite was isolated from *B. viridis'* lungs by me.

*Cosmocerca commutata*, *O. filiformis*, and *C. ornata* extracted from intestine. But *C. commutata* and *O. filiformis* were recovered from the small intestine, while *C. ornata* was found to infest the rectum. Vashetko and Siddikov [28] showed that *C. ornata* larvae parasited various organs and tissues, mainly in the mouth.

Only *Eustronglydes excisus* was isolated from body cavity of *R. ridibunda*. Yildirimhan et al. [7] showed that *E. excisus* larvae isolated from body cavity of *R. ridibunda* collected from Istanbul. But in my observations, *E. excisus* was adult form.

*Acanthocephalus ranae* was recorded in the small intestine of *B. viridis* and *R. ridibunda.* It was also recorded in the small intestine and stomachs of toads by Vashetko and Siddikov [28]. This similarity showed that *A. ranae* is a gastro-intestinal parasite of amphibians.

This study on the helminths of amphibians collected from the different regions of Turkey is not the first step for future studies to understand their population dynamics and life histories. But general findings about the parasites of amphibians are consistent with a view that these amphibian hosts a variety of helminth parasites in their native range.

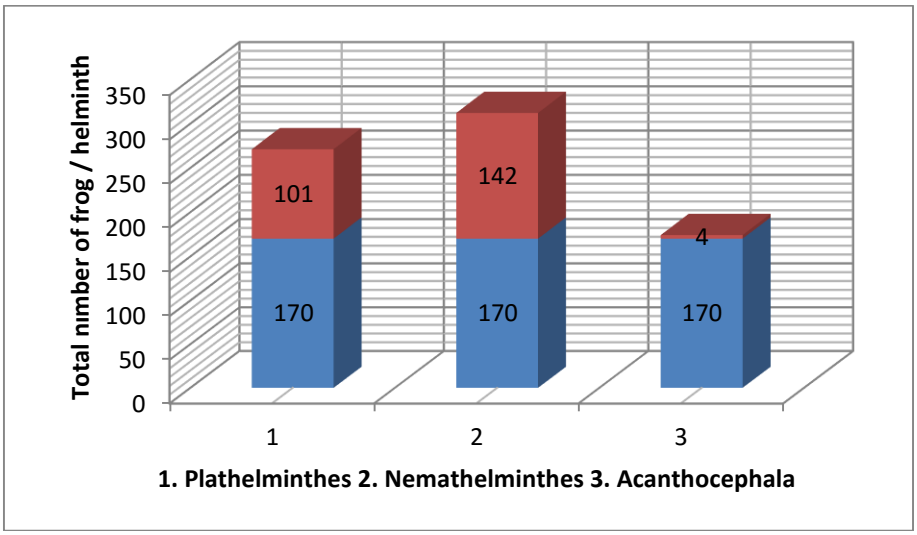


**Figure 1.** Column chart. Upper: Total number of helmint parasites of frogs. Lower: Total number of examined frogs.

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

| | HOST | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Bufo viridis* | | | *Rana ridibunda* | | | *Hyla arborea* | | |
| **Parasite** | **No. Examined** | **No. Infected** | **% Infected** | **No. Examined** | **No. Infected** | **% Infected** | **No. Examined** | **No. Infected** | **% Infected** |
| **Plathelminthes** | | | | | | | | | |
| *Polystoma viridis* | 97 | 16 | 16% | 50 | 0 | 0% | 23 | 0 | 0% |
| *Nematotaenia dispar* | 97 | 19 | 20% | 50 | 0 | 0% | 23 | 0 | 0% |
| *Diplodiscus subclavatus* | 97 | 0 | 0% | 50 | 16 | 32% | 23 | 0 | 0% |
| *Gorgoderina vitelliloba* | 97 | 0 | 0% | 50 | 12 | 24% | 23 | 0 | 0% |
| *Prosotocus confuses* | 97 | 0 | 0% | 50 | 21 | 42% | 23 | 0 | 0% |
| *Haematoleochus breivance* | 97 | 0 | 0% | 50 | 9 | 18% | 23 | 0 | 0% |
| *Opisthiopglyphe ranae* | 97 | 0 | 0% | 50 | 8 | 16% | 23 | 0 | 0% |
| **Nemathelminthes** | | | | | | | | | |
| *Rhabdias bufonis* | 97 | 36 | 37% | 50 | 0 | 0% | 23 | 0 | 0% |
| *Cosmocerca commutate* | 97 | 47 | 48% | 50 | 0 | 0% | 23 | 0 | 0% |
| *Oswaldocruzia filiformis* | 97 | 31 | 32% | 50 | 0 | 0% | 23 | 3 | 13% |
| *Cosmocerca ornate* | 97 | 0 | 0% | 50 | 14 | 28% | 23 | 1 | 4% |
| *Eustronglydes excises* | 97 | 0 | 0% | 50 | 10 | 20% | 23 | 0 | 0% |
| **Acanthocephala** | | | | | | | | | |
| *Acanthocephalus ranae* | 97 | 2 | 2% | 50 | 2 | 4% | 23 | 0 | 0% |

**Table 2.** Site of infection for helminth parasites recovered from amphibians

| **Parasite** | **Host** | **Site of infection** |
|---|---|---|
| **Plathelminthes** | | |
| *Polystoma viridis* | *Bufo viridis* | Urinary bladder |
| *Nematotaenia dispar* | *Bufo viridis* | Small intestine |
| *Diplodiscus subclavatus* | *Rana ridibunda* | Large intestine |
| *Gorgoderina vitelliloba* | *Rana ridibunda* | Urinary bladder |
| *Prosotocus confuses* | *Rana ridibunda* | Small intestine |
| *Haematoleochus breivance* | *Rana ridibunda* | Lung |
| *Opisthiopglyphe ranae* | *Rana ridibunda* | Small intestine |
| **Nemathelminthes** | | |
| *Rhabdias bufonis* | *Bufo viridis* | Lung |
| *Cosmocerca commutate* | *Bufo viridis* | Small intestine |
| *Oswaldocruzia filiformis* | *Bufo viridis, Hyla arborea* | Small intestine |
| *Cosmocerca ornate* | *Rana ridibunda, Hyla arborea* | Large intestine |
| *Eustronglydes excises* | *Rana ridibunda* | Body cavity |
| **Acanthocephala** | | |
| *Acanthocephalus ranae* | *Bufo viridis, Rana ridibunda* | Small intestine |

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