

## A Report about Helminth Parasites of Some Amphibians (Anura: Ranidae, Bufonidae) from the North and Northeast of Iran

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### Abstract

In the present study, 53 specimens of toads and frogs were collected by hand during March 1994 to October 1995 from different sites in north and northeastern of Iran, mainly in Semnan province. Amongst 47 green toads, *Bufo viridis*, 95.7% were infected with one or more parasite species. There was no significant difference between snout-ventral lengths of infected (n=45) and uninfected (n=2) toads by t-test ( $p=0.243$ ), and between infection of males and females by  $\chi^2$  test ( $p=0.778$ ). Helminth parasites of *B. viridis* were the monogenean, *Polystoma viridis*, (mean intensity  $3.65\pm 3.33$ ); the cyclophyllid cestode *Nematotaenia dispar* (mean intensity  $2.78\pm 1.79$ ); and the nematodes *Rhabdias bufonis* and *Cosmocerca* sp. There was significant difference between males and females infected with *P. viridis* ( $\chi^2=9.08$ ,  $p=0.003$ ) and *N. dispar* ( $\chi^2=7.55$ ,  $p=0.006$ ), but there was no significant difference between males and females ( $\chi^2=0.002$ ,  $p=0.968$ ) infected with *R. bufonis*, by  $\chi^2$  test. There was no significant difference between snout-ventral lengths of *B. viridis* samples infected with *N. dispar* and uninfected samples ( $p=0.248$ ), and for infection with *P. viridis* ( $p=0.872$ ), and *R. bufonis* ( $p=0.128$ ), by t-test. One of the three marsh frogs, *Rana ridibunda ridibunda*, was infected with the trematode, *Skrjabenoeces similis*. All three Iranian wood frogs, *Rana macrocnemis pseudodalmatina* were infected with *Haplometra cylindracea*, and one of them with *Oswaldocruzia filiformis*, as well.

**Keywords:** Parasite, Helminth, *Bufo*, *Rana*, Iran.

### Introduction

Helminth parasites of the different species of amphibians of Iran are studied in recent years. Mashaii (2005) has reported one monogenean, one digenean, two cestodes and four nematode species from the green toad, *Bufo viridis*; one digenean and one nematode species from *Rana ridibunda ridibunda*; one monogenean, one digenean and one cestode species from *Hyla arborea savignyi* collected from south west of Iran. Also seven digenean species, two metacercarian cysts and one nematode species were recorded from Astara, Anzali and Aghbaba region from the north of Iran (Mashaii *et al.* 2000), three trematode parasite species from *Rana r. ridibunda* and *R. camerani* in Chahar Mahal-o-Bakhtiari province at southwest of Iran (Mashaii 1999). Massoud and Farahnak (1994) reported four species of trematode parasites from frogs. They didn't mention the host species. Combes and Knoepffler (1972) studied helminth parasites of the marsh frog, *Rana r. ridibunda* from lagoon of Anzali in north of Iran. They reported five digeneans and one acanthocephalan species. They

also mentioned four nematode species without giving of their names. Their work was the only reference about helminth fauna of Iranian frogs till 1994.

The green toad, *Bufo viridis* Laurenti 1768, is the common toad of Iran and has a wide distribution in most provinces. This toad is rarely found in central and eastern areas of Iran. The marsh frog, *Rana ridibunda ridibunda* Pallas 1771, is the common frog of Iran which can be found all over the country except for some small areas at the southeast. The Iranian long-legged wood frog, *Rana macrocnemis pseudodalmatina* is common to Iran and found only in some areas of northern provinces (Anderson 1963). Some helminth parasites including trematodes, cestodes and nematodes are reported from *B. viridis* and *R. ridibunda ridibunda* in different areas of Iran, but there was no report about helminth fauna of *R. macrocnemis pseudodalmatina* from Iran before this survey.

The present study contains new records about helminth parasites of *Bufo viridis*, *Rana ridibunda ridibunda* and *R. macrocnemis pseudodalmatina*

from north and northeast of Iran, mainly from Semnan province.

### Materials and Methods

In total, 53 specimens including: 47 *Bufo viridis*, 3 *R. macrocnemis pseudodalmatina* and 3 *Rana ridibunda ridibunda* were collected by hand during March 1992 to September 1993. Specimens of *B. viridis* were collected from suburbs of Semnan (53°25'E, 35°35'N), Parvar and Seidva (52°29'E, 35°59'N), Pishva (51°43'E, 35°12'N) and Karaj (50°58'E, 35°45'N). Specimens of *R. macrocnemis pseudodalmatina*. and *R. ridibunda ridibunda* were collected from Kenet, Behshahr (53°33'E, 36°43'N), and suburb of Semnan (53°25'E, 35°35'N), respectively.

Snout-ventral length (SVL) of each sample was measured and sex was recorded. Specimens were immediately dissected after being euthanised. Body cavity, peritoneum, muscles and mesenteries were examined for cysts of parasites. Then, urinary bladder, lungs, stomach, intestine, rectum and liver of the dissected frogs were examined in separate Petri dishes, containing sodium chloride solution, under a low power light microscope at 12× magnification. Parasites were fixed and preserved in 10% formalin, stained in carmine alum and mounted in Canada balsam on the microscope slides. Nematodes were cleaned in lacto-phenol solution and cross sections of their mouthparts were prepared. Figures were drawn with the aid of a Camera Lucida.

Parasite species were determined based on Jones (1987), Moravec (1985), Prudhoe and Bray (1982), Grabda-Kazubaska and Combes (1981), Smyth and Smyth (1980), and Euzet et al. (1974) works.

Determination of the species was confirmed by the Natural History Museum of London.

Snout-ventral lengths of infected and uninfected hosts, also infection with different parasite species, were compared by t-test ( $p < 0.05$ ). Parasite infection was compared between sexes by  $\chi^2$  test ( $p < 0.05$ ).

### Results

Amongst 47 *B. viridis* samples (mean SVL=5.797±0.28 cm) including 19 females and 28 males, 95.7% were infected with one or more parasite species. There was no significant difference between snout-ventral lengths of infected (n=45) and uninfected (n=2) toads by t-test ( $p=0.243$ ), and between infection of males and females by  $\chi^2$  test ( $p=0.778$ ). Helminth parasites of *B. viridis* were the monogenean, *Polystoma viridis* (mean intensity 3.65±3.33); the cyclophyllid cestode *Nematotaenia dispar* (mean intensity 2.78±1.79) and the nematodes *Rhabdias bufonis* and *Cosmocerca* sp. There was no significant difference between snout-ventral lengths of *B. viridis* samples infected with *N. dispar* and uninfected samples ( $p=0.248$ ), and for infection with *P. viridis* ( $p=0.872$ ) and *R. bufonis* ( $p=0.128$ ), by t-test. Female toads were significantly more infected with *P. viridis* ( $\chi^2=9.08$ ,  $p=0.003$ ) and *N. dispar* ( $\chi^2=7.55$ ,  $p=0.006$ ) than males, but there was no significant difference between males and females ( $\chi^2=0.002$ ,  $p=0.968$ ) infected with *R. bufonis* (n=8), by  $\chi^2$  test. One of the three marsh frogs, *Rana ridibunda ridibunda*, was infected with the trematode, *Skrjabenoeces similis*. All three Iranian wood frog specimens, *R. macrocnemis pseudodalmatina* were infected with *Haplometra cylindracea*, and one of them with *Oswaldocruzia filiformis*, as well. (Tables. 1 and 2).

**Table 1- Helminth parasites of the toads and frogs from areas at the north and northeast of Iran, their hosts and microhabitat.**

Parasites	Sites	Hosts	Microhabitats
<i>Polystoma viridis</i>	Suburbs of Semnan, and Karaj	<i>Bufo viridis</i>	Urinary bladder
<i>Nematotaenia dispar</i>	Suburbs of Semnan and Karaj	<i>Bufo viridis</i>	Intestine
<i>Rhabdias bufonis</i>	Pishva	<i>Bufo viridis</i>	Lungs
<i>Skrjabinoeces similis</i>	Suburb of Semnan	<i>Rana ridibunda ridibunda</i>	Lungs
<i>Haplometra cylindracea</i>	Kenet, Behshahr	<i>Rana macrocnemis pseudodalmatina</i>	Lungs
<i>Oswaldocruzia filiformis</i>	Kenet, Behshahr	<i>Rana macrocnemis pseudodalmatina</i>	Intestine

**Table 2- Prevalence, number of infected hosts and mean intensity ( $\pm$ SD) of parasites from toads and frogs, and results of  $\chi^2$  test for parasite infection of the green toads between sexes ( $p < 0.05$ ).**

Parasites	Prevalence	Num. of hosts	Mean intensity ( $\pm$ SD)	P ( $\chi^2$ )
<i>Polystoma viridis</i>	64	30	3.65 $\pm$ 3.33	0.003* ( $\chi^2=9.08$ )
<i>Nematotaenia dispar</i>	81	38	2.78 $\pm$ 1.79	0.006* ( $\chi^2=7.55$ )
<i>Rhabdias bufonis</i>	32	15	7.33 $\pm$ 3.2	0.968 ( $\chi^2=0.002$ )
<i>Skrjabinoeces similis</i>	33.3	1	3	-
<i>Haplometra cylindracea</i>	100	3	1,1,2	-
<i>Oswaldocruzia filiformis</i>	33.3	1	2	-

**1) *Nematotaenia dispar* (Goeze 1782) Lühe 1899**

**Hosts (and sites):** *Bufo viridis* (Suburb of Semnan, Suburb of Karaj)

**Localization:** Intestine

**2) *Polystoma viridis* Euzet, Combes & Batchvarov, 1974**

**Hosts (and sites):** *Bufo viridis* (Suburb of Semnan, Suburb of Karaj)

**Localization:** Urinary bladder

**3) *Haplometra cylindracea* (Zeder 1800) Looss 1899**

**Host (and site):** *Rana macrocnemis pseudodalmatina* (Kenet)

**Localization:** Lungs

**4) *Skrjabinoeces similis* (Looss 1899) Sudarikov 1950**

**Host (and site):** *Rana ridibunda ridibunda* (Suburb of Semnan)

**Localization:** lungs

**5) *Rhabdias bufonis* Schrank 1788**

**Host (and site):** *Bufo viridis* (Pishva)

**Localization:** Lungs

*Nematotaenia dispar*, *Polystoma viridis*, *Haplometra cylindracea* and *Rhabdias bufonis* were previously described from southwest of Iran (Mashaii 2005). *Skrjabinoeces similis* was described from the north (Mashaii *et al.* 2000, Combes & Knoepffler 1972) and southwest of Iran (Mashaii 1999), as well. So they are not described here repeatedly. Some of the *B. viridis* toads collected from suburb of Semnan were infected with *Cosmocerca* sp. that were localized in the rectum. Description of the *Cosmocerca* sp. needs to study more specimens.

**6) *Oswaldocruzia filiformis* Goeze 1782**

**Synonyms:** *Ascaris tenuissima* Schrank 1988, *A. intestinalis* Gmelin 1790, *A. bufonis* Gmelin 1790, *Strongylus auricularis* Zeder 1800, *Strongylus dispar* Dujardin 1845, *Oswaldocruzia insulae* Morishita 1926.

**Hosts (and sites):** *Rana macrocnemis pseudodalmatina* (Kenet)

**Localization:** Intestine

**Description:** Body elongate, whitish and filiform;

oesophagus straight, a little projecting at the end; cervical alae small; genital spicules of males dissimilar but of the same size, well sclerotized; genital pore of the female a little posterior to midbody; tail conical with a short spine at the end in females; uterus long and coiled; eggs oval, small and numerous (Figs. 1 and 2).



**Figure 1- Body end of female *Oswaldocruzia filiformis* (x200).**



**Figure 2- Body end of male *Oswaldocruzia filiformis* (x200).**

## Discussion

One cyclophillid cestode, one monogenean, two digeneans and one nematode species are reported as parasites of frogs and toads from the north and northeast of Iran.

This is the first study about helminth fauna of the Iranian wood frog, *R. macrocnemis pseudodalmatina*. Also, the nematode *Oswaldocruzia filiformis* is reported from Iranian frogs for the first time. The cyclophillid cestode *N. dispar* was reported as a parasite of the toad, *B. viridis* and the tree frog, *Hyla arborea savignyi*, from Khuzestan and Ilam provinces, at southwest of Iran. The tree frog, *Hyla arborea savignyi* was introduced as the host of *N. dispar*, for the first time. The monogenean *Polystoma viridis* was reported from Khuzestan province as a parasite of *B. viridis* and *H. arborea savignyi*, as well. The digenean *Haplometra cylindracea*, and the nematode *Rhabdias bufonis* were previously reported as a parasite of *B. viridis* from Khuzestan at southwest of Iran (Mashaii 2005). These species are reported from the northern areas of Iran for the first time. *N. dispar* has a wide distribution in amphibians and reptiles overall the world except Nearctic region (Prudhoe & Bray 1982). *H. cylindracea* that is found in *R. macrocnemis pseudodalmatina* is a common trematode of amphibians in Europe and has a wide host spectrum in different parts of the world (Smyth & Smyth

1980). The digenetic trmatode *Skrjabinoeces similis* was reported from *Rana ridibunda ridibunda* of different areas of Guilan and Chahar Mahal-o-Bakhtiari provinces at the north and southwest of Iran (Mashaii et al. 2000, Mashaii 1999, Combes & Knoepffler 1972).

Female toads were significantly more infected with *P. viridis* and *N. dispar* than males. Sex differences in ecological interactions, feeding habits and reproductive behaviours that may affect infections to some parasites, must be detected. Also, the life cycle of the parasites and intermediate hosts must be mentioned. For example, females are larger than males and need more food. So, females would be more often in the exposure of infestation. Sex, season, breeding and age can affect the infection of parasites, because of changes of the hormone levels in blood of the host (Smyth & Smyth 1980).

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## References

- Anderson S.C. 1963: Amphibians and reptiles from Iran. *Proceed. Calif. Acad. Sci.* **4**:417-498.
- Combes C., Knoepffler L.P. 1972: Helminthes parasites de *Rana ridibunda ridibunda* Pallas, 1771 sur les rives Iraniennes de la mer Caspienne. *Vie Milieu.* **23**:329-334.
- Euzet L., Combes C., Batchvarov G. 1974: Sur un nouveau Polystomatidae Européen, parasite de L'Amphibien *Bufo viridis*. *Vie Milieu.* V:XXIV, fasc. **1**: 129-140.
- Grabda-Kazubska B., Combes C. 1981: Morphological variability of *Haplometra cylindracea* (Zeder, 1800) in populational and geographic aspects. *Acta Paras. Polon.* V: XXVIII, fasc. **5**: 39-63.
- Jones K.M. 1987: A taxonomic revision of the Nematotaeniidae Luhe, 1910 (Cestoda : Cyclophillidea). *Sys. Parasi.* **10**: 165-245.
- Mashaii N. 1999: New records of trematode parasites (Digenea) in the banded frog, *Rana camerani* and marsh frog, *Rana ridibunda ridibunda* (Anura : Ranidae), from Southwest of Iran. *Iran. J. Fish. Sci.* **1(2)**: 41-47.
- Mashaii N. 2005: Helminth parasites of green toad, *Bufo viridis* (Anura: Bufonidae); tree frog, *Hyla arborea savignyi* (Anura: Hylidae) and marsh frog, *Rana ridibunda ridibunda* (Anura: Ranidae) from southwest of Iran. *Iran. J. Vet. Res.* **6(3)**: 67-73.
- Mashaii N., Balouch M., Mobedi I. 2000: New records about helminth parasites of the marsh frog, *Rana ridibunda ridibunda* (Anura : Ranidae), from the north of Iran. *Iran. J. Fish. Sci.* **2(2)**: 77-88.

- Massoud J., Farahnak A. 1994: Study on heterophid trematodes in Khuzestan, Southwest of Iran. *Abs. 8<sup>th</sup> Inter. Cong. Parasi., Izmir, Turkey.* **2**: 363.
- Moravec F. 1985: Some nematode parasites of frogs from north Viet Nam. *Paras. Hung.* **18**: 63-77.
- Prudhoe S., Bray R.A. 1982: Platyhelminth parasites of the amphibia. *Brit. Muse. (Natu. Hist.) & Oxfo. Univ. Press, London.* 217 p.
- Smyth J.D., Smyth M.M. 1980: Frogs as host-parasite system, **I** : An introduction to parasitology through the parasites of *Rana temporaria*, *R. esculenta* and *R. pipiens*. *Univ. Lond. Press, London.* 112 p.