

## The helminth community of the agile frog, *Rana dalmatina* Bonaparte, 1839 (Anura: Ranidae) collected from Northwest of Turkey

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### Summary

A total of 33 agile frogs (*Rana dalmatina*) were collected from 7 localities in Edirne, Bursa and Adapazarı Provinces (Northwest of Turkey), between 1987 – 2007 and examined for the first time for helminths. *R. dalmatina* harbored one species of Monogenea (*Polystoma* sp.), 3 species of Digenea (*Diplodiscus subclavatus*, *Pleurogenoides medians*, and *Pleurogenes claviger*), 4 species of Nematoda (*Rhabdias bufonis*, *Oswaldocruzia filiformis*, *Cosmocerca ornata*, and *Oxysomatium brevicaudatum*) and one species of Acanthocephala (*Acanthocephalus ranae*). All helminths represent new host records for *Rana dalmatina* in Turkey.

Keywords: helminth; agile frog; *Rana dalmatina*; Turkey

### Introduction

The agile frog *Rana dalmatina* Bonaparte, 1839 is a medium sized strictly terrestrial, anuran species, that lives in deciduous forests, damp grasslands with higher vegetation, and usually quite far away from water bodies up to 1500m elevation. *R. dalmatina* is found in Turkish Thrace and northern parts of Anatolia. The species is mainly nocturnal, its diet consists of various insects (Baran and Atatürk, 1997).

Previous reports have identified various species of Digenea, Nematoda and Acanthocephala from *R. dalmatina* collected from Bulgaria and the former Czechoslovakia (Vojtkova & Vojtek, 1975; Buchvarov *et al.* 1975; Buchvarov, 1977 and Kirin & Buchvarov, 2002). Only one polystome species have been reported from *R. dalmatina* in Bulgaria (Buchvarov, 1980). A leech was also reported by Buchvarov (1977) in Bulgaria. Thus, a total of 21 species of helminths have been reported from *R. dalmatina*.

So far, there has been no published study on helminths of

the agile frog (*R. dalmatina*) in Turkey. We report for the first time helminths of *R. dalmatina* from Turkey.

### Materials and Methods

Frogs were collected by hand, between 1987-2007 from 7 localities in Turkey: Büyükdöllük (120 m) Edirne Province (41° 45' N; 26° 36' E), Uludağ Mountain (1200 m), Bursa Province (40° 09' N; 29° 05' E), Karacabey (50 m), Bursa province (40° 13' N; 28° 21' E), İnegöl (450 m) Bursa province (40° 04' N; 23° 30' E), Kemalpaşa (160 m) Bursa province (40° 02' N; 28° 24' E), Akyazı (200 m), Adapazarı Province (40° 41' N; 30° 41' E), Karasu (35 m) Adapazarı province (41° 05' N; 30° 42' E).

In total, 33 *Rana dalmatina* (6 males, 25 females and 2 semi-adults) were examined for helminth parasites. The mean ± SD snout-vent length (SVL) of specimens was 50.05 ± 10.45 mm, with a range from 27 to 66 mm.

Frogs were overdosed in ether-filled glass containers, fixed by injecting 7 % formalin into the body cavity and preserved in 70 % ethyl alcohol. The body cavity was opened by a longitudinal ventral incision. The alimentary canal was excised and separated into stomach, small intestine, large intestine and rectum. The contents of each part and other organs (lungs, liver, gall bladder, kidneys and urinary bladder) were each mixed with 0.5 % saline solution and poured into Petri dishes for examination under a stereomicroscope. The muscles, plus portions of peritoneum and spinal cord, were teased out with needles and examined under a stereomicroscope. Trematodes were stored in 70 % ethanol. Nematodes were stored in 70 % ethyl alcohol with 5 % glycerol, and Acanthocephalans were stored in 70 % ethyl alcohol. Digeneans and acanthocephalans were stained with acetocarmine, dehydrated, cleared in cedar oil or xylol, and mounted in Canada Balsam; nematodes were

Table 1. Prevalence and intensity of helminths in 33 agile frogs collected from Northwest of Turkey

Parasite (ZDEU Helm. Coll. no.)	Developmental stage	Site of infection	No. of infected (%)	Range	Mean intensity (±SE)
<b>POLYSTOMATIDAE</b>					
<i>Polystoma</i> sp. (ZDEU HELM-16/2007)	Juvenile	UB	3 (9.09%)	1 – 2	1.33 (±0.33)
<b>PARAMPHISTOMIDAE</b>					
<i>Diplodiscus subclavatus</i> (Pallas, 1760) (ZDEU HELM-17/2007)	Adult	LI	3 (9.09%)	1 – 6	2.66 (±1.66)
<b>LECTHODENDRIIDAE</b>					
<i>Pleurogenes claviger</i> (Rudolphi, 1819) Looss, 1899 (ZDEU HELM-18/2007)	Adult	SI	4 (12.12%)	3 – 21	9.25 (±4.25)
<i>Pleurogenoides medians</i> (Olsson, 1876) Travassos, 1921 (ZDEU HELM-19/2007)	Adult	SI	1 (3.03%)	4	4.0
<b>RHABDIASIDAE</b>					
<i>Rhabdias bifrons</i> (Schrank, 1788) Stiles and Hassall, 1905 (ZDEU HELM-20/2007)	Adult	LU	8 (24.24%)	1 – 85	13.12 (±10.33)
<b>MOLINEIDAE</b>					
<i>Oswaldocruzia filiformis</i> (Goeze, 1782) Travassos, 1917 (ZDEU HELM-21/2007)	Adult	SI	18 (54.54%)	1 – 10	3.22 (±0.68)
<b>COSMOERCIDAE</b>					
<i>Oxyssomatium brevicaudatum</i> (Zeder, 1800) Raillet and Henry, 1916 (ZDEU HELM-22/2007)	Adult	SI	11 (33.33%)	1 – 20	4.63 (±1.63)
<i>Cosmocerca ornata</i> (Dujardin, 1845) (ZDEU HELM-23/2007)	Adult	SI, LI	12 (36.36%)	1 – 2	1.18 (±0.12)
<b>ECHINORHYNCHIDAE</b>					
<i>Acanthocephalus ranae</i> (Schrank, 1788) Lühe, 1911 (ZDEU HELM-24/2007)	Adult	SI	15 (45.45%)	1 – 7	2.66 (±2.25)
<i>Acanthocephalus ranae</i>	Larval	MS	4 (12.12%)	1 – 15	6.50 (±6.19)

SI, small intestine; LI, large intestine; LU, lung; UB, urinary bladder; MS, Mesenteries

Table 2. Helminth species recorded in Amphibian and Reptile species from Turkey

Helminth Name	Host Species	Reference
<i>Polystoma viridis</i>	<i>Bufo viridis</i>	Yıldırımhan, 1999a; Düşen, 2003.
<i>Polystoma skrjabini</i>	<i>Hyla arborea</i>	Düşen & Öz, 2004.
<i>Polystoma macrocnemis</i>	<i>Rana macrocnemis</i>	Biserkov <i>et al.</i> , 2001.
<i>Diplodiscus subclavatus</i>	<i>Rana ridibunda</i>	Oğuz <i>et al.</i> , 1994; Yıldırımhan <i>et al.</i> , 1996; Kır <i>et al.</i> , 2001; Düşen and Öz, 2006.
<i>Pleurogenes claviger</i>	<i>Rana macrocnemis</i> <i>Rana ridibunda</i>	Yıldırımhan <i>et al.</i> , 1997b; Yıldırımhan <i>et al.</i> , 2006b. Oğuz <i>et al.</i> , 1994; Yıldırımhan <i>et al.</i> , 1996; Kır <i>et al.</i> , 2001.
<i>Pleurogenoides medians</i>	<i>Hyla arborea</i> <i>Rana macrocnemis</i> <i>Rana camerani</i> <i>Rana ridibunda</i> <i>Lacerta trilineata</i>	Düşen and Öz, 2004. Yıldırımhan <i>et al.</i> , 1997b; Yıldırımhan <i>et al.</i> , 2006b. Yıldırımhan <i>et al.</i> , 2006c; Düşen, 2007. Oğuz <i>et al.</i> , 1994; Yıldırımhan <i>et al.</i> , 1996; Yıldırımhan <i>et al.</i> , 2005b; Düşen and Öz, 2006; Sağlam and Arıkan, 2006. Yıldırımhan, 1999b.
<i>Rhabdias bufonis</i>	<i>Bombina bombina</i> <i>Bufo bufo</i> , <i>Bufo viridis</i> <i>Rana camerani</i> <i>Rana ridibunda</i>	Yıldırımhan <i>et al.</i> , 2001. Yıldırımhan and Karadeniz, 2007. Yıldırımhan, 1999a. Yıldırımhan <i>et al.</i> , 2006c. Yıldırımhan <i>et al.</i> , 1997a; Yıldırımhan <i>et al.</i> , 1997a; Düşen and Öz, 2006; Sağlam and Arıkan, 2006.
<i>Oswaldocruzia flitifformis</i>	<i>Bufo bufo</i> , <i>Bufo viridis</i> , <i>Hyla arborea</i> , <i>Rana camerani</i> , <i>Rana macrocnemis</i> <i>Rana ridibunda</i> <i>Lacerta trilineata</i> , <i>Lacerta viridis</i> , <i>Anguis fragilis</i>	Yıldırımhan and Karadeniz, 2007. Yıldırımhan, 1999a. Yıldırımhan <i>et al.</i> , 2006a. Yıldırımhan <i>et al.</i> , 2006c. Schad <i>et al.</i> , 1960; Yıldırımhan <i>et al.</i> , 1997b. Yıldırımhan <i>et al.</i> , 1996; Yıldırımhan, 1999a; Yıldırımhan, 1999b; Yıldırımhan <i>et al.</i> , 2005b; Sağlam and Arıkan, 2006; Yıldırımhan <i>et al.</i> , 2006b. Yıldırımhan, 1999b. Yıldırımhan, 1999b. Schad <i>et al.</i> , 1960.

Table 2. (continued)

<i>Oxyssomatium brevicaudatum</i>	<i>Pelobates syriacus</i> , <i>Bufo viridis</i> , <i>Rana macrocnemis</i> , <i>Rana ridibunda</i> , <i>Anguis fragilis</i> , <i>Natrix natrix</i>	Yildirimhan, 1997a. Yildirimhan, 1999a. Schad <i>et al.</i> , 1960. Yildirimhan, 1997b; Yildirimhan <i>et al.</i> , 2005b; Sağlam and Arıkan, 2006. Schad <i>et al.</i> , 1960. Schad <i>et al.</i> , 1960.
<i>Cosmocerca ornata</i>	<i>Bufo viridis</i> <i>Hyla arborea</i> <i>Rana holtzi</i> , <i>Rana camerani</i> <i>Rana macrocnemis</i> <i>Rana ridibunda</i> <i>Anguis fragilis</i>	Schad <i>et al</i> 1960. Yildirimhan <i>et al.</i> , 2006a Yildirimhan <i>et al</i> , 2006b. Yildirimhan <i>et al</i> , 2006c; Düşen 2007. Schad <i>et al</i> 1960. Yildirimhan <i>et al.</i> , 2005b. Schad <i>et al</i> 1960.
<i>Acanthocephalus ranae</i>	<i>Mertensiella caucasica</i> <i>Bombina bombina</i> <i>Bufo bufo</i> <i>Bufo viridis</i> <i>Hyla arborea</i> <i>Rana camerani</i> <i>Rana macrocnemis</i> <i>Rana ridibunda</i>	Yildirimhan <i>et al.</i> , 2005b. Yildirimhan <i>et al.</i> , 2001. Yildirimhan and Karadeniz, 2007. Yildirimhan, 1999a. Düşen and Öz, 2004. Yildirimhan <i>et al</i> , 2006c. Yildirimhan <i>et al.</i> , 1997b; Yildirimhan <i>et al</i> , 2006b; Düşen, 2007. Oğuz <i>et al.</i> , 1994; Yildirimhan <i>et al.</i> , 1996; Kir <i>et al</i> , 2001; Yildirimhan <i>et al.</i> , 2005b; Düşen and Öz, 2006; Sağlam and Arıkan, 2006.

cleared in glycerol and examined. Intensities are presented as mean values ( $\pm 1$  SE) followed by the range.

Voucher specimens of parasites were deposited in the Ege University, Museum of Zoology, Izmir, Turkey (ZDEU HELM-16-24/2007); host specimens were deposited in the Uludağ University, Faculty of Arts and Sciences Department of Biology, Bursa Turkey.

## Results and Discussion

In summary, 344 individuals of 9 helminth species were collected from 33 anurans examined. Helminths were collected from the large and small intestine, urinary bladder, lungs and body cavity mesenteries (Table 1).

No individual host harbored more than 6 helminth species. Of the infected anurans, 1 (3.03 %) harbored 6 species of helminth, 1 (3.03 %) harbored 5 species of helminth, 2 (6.06 %) harbored 4 species of helminth; 13 (39.39 %), harbored 3 species of helminth; 8 (24.24 %), harbored 2 species of helminth; and 8 (24.24 %), harbored 1 species of helminth.

There were  $2.48 \pm 1.20$  helminth species per infected host and  $10.60 \pm 3.4$  helminth individuals per infected host. Of the 9 helminth species found in this study, 6 helminths had prevalences greater than 10 %, *A. ranae* was the most prevalent helminth, occurring in 19 of 33 hosts (57.6 %), followed by *O. filiformis* with 18 of 33 hosts (54.54 %), *C. ornata* with 12 of 33 hosts (36.36 %), *O. brevicaudatum* with 11 of 33 hosts (33.33 %), *R. bufonis* with 8 of 33 hosts (24.24 %) and *P. claviger* with 4 of 33 hosts (12.12 %).

This is the first published study of helminths of *R. dalmatina* from Turkey. All helminths collected are new host records for *Rana dalmatina* in Turkey. Buchvarov *et al.* (1975) reported 3 species of nematodes (*Cosmocerca ornata*, *C. commutata*, *Oswaldocruzia filiformis*) in Velinograd district (Bulgaria); Vojtkova and Vojtek (1975) reported 6 species of trematodes (*Diplodiscus subclavatus*, *Opisthophyllum ranae*, *Haplometra cylindracea*, *Euryhelminth squamula*, and metacercarian stages of *Enyclometra colubriformis*, *O. ranae* and *Echinoparyphium recurvatum*) in the *R. dalmatina* from the former Czechoslovakia. Buchvarov (1977) recorded 8 species of trematodes (*Diplodiscus subclavatus*, *Opisthophyllum ranae*, *Pneumonoeces variegatus*, *Cephalogonimus retusus*, *Gorgoderia pagenstecheri*, *Gorgoderina vitelliloba*, *Pleurogenoides medians*, and *Tylodelphis rhachiaea*), 6 species of nematodes (*Cosmocerca ornata*, *C. commutata*, *Oswaldocruzia filiformis*, *O. bialata*, *O. brevicaudatum*, and *Rhabdias bufonis*), one species of acanthocephalan (*Acanthocephalus ranae*) and one species of leech (*Batrachobdella algira*) in *R. dalmatina* collected from Bulgaria. Also, Kirin and Buchvarov (2002) observed one species of nematode (*O. filiformis*) from Britsa riverside in Bulgaria. Similarly, we observed *D. subclavatus*, *P. medians*, *R. bufonis*, *C. ornata*, *Oswaldocruzia filiformis*, *O. brevicaudatum*, and *A. ranae* in this study in *R. dalmatina* in Turkey. No leech species were found in this investigation.

*R. dalmatina* represents a new host record for *Polystoma*

sp. There are 3 species of *Polystoma* that have been reported from Turkey: *Polystoma macrocnemis* in the Uludağ frog (*Rana macrocnemis*), *Polystoma skrjabini* in the European tree frog (*Hyla arborea*), and *Polystoma viridis* in the European green toad, (*Bufo viridis*) (Yıldırımhan, 1999a; Biserkov *et al.*, 2001; Düşen & Öz, 2004). Buchvarov (1980) reported a new species, *Polystoma mazurmovici* in *R. dalmatina* from Bulgaria. We were unable to identify the species of *Polystoma* collected in this study, because the specimens are juvenile forms.

The other helminth species (*D. subclavatus*, *Pleurogenes claviger*, *P. medians*, *R. bufonis*, *Oswaldocruzia filiformis*, *O. brevicaudatum*, *C. ornata* and *A. ranae*) found in this study are common parasites of European anurans (see Yamaguthi, 1963; Buchvarov, 1977; Prudhoe & Bray, 1982; Anderson, 2000) and these species have been observed in several amphibians and some reptiles species in Turkey (Table 2).

This study has expanded the geographical and host range distribution of various helminth species. Future studies should also expand the host-parasite list from Turkey.

This information may then be used to increase our understanding of parasite-host relationships.

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