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First data on the helminth fauna of a locally distributed mountain frog, "Tavas frog" *Rana tavasensis* Baran & Atatür, 1986 (Anura: Ranidae), from the inner-west Anatolian region of Turkey

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Abstract: A total of 8 specimens of *Rana tavasensis* Baran & Atatür, 1986 (Tavas frog) were collected in Denizli Province (inner-west Anatolia, or the eastern part of the Aegean region), Turkey, in 2008 and 2009 and were examined for the first time for helminths. All 8 frogs were infected with 1 or more helminths. The helminth fauna of *R. tavasensis* comprised 3 species: 1 species of Digenea [*Haplometra cylindracea* (Zeder, 1800) Looss, 1899], 1 species of Nematoda [*Cosmocerca ornata* (Dujardin, 1845)], and 1 species of Acanthocephala [*Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911)]. *R. tavasensis* represents a new host record for each of the observed helminth species in Turkey.

Key words: Denizli, helminth, Kızılcabölük, *Rana tavasensis*, Tavas frog, Turkey

İç-Batı Anadolu yöresinde lokal yayılış gösteren dağ kurbağası *Rana tavasensis* Baran ve Atatür, 1986 (Anura: Ranidae)'nın (Tavas Kurbağası) helmint faunası hakkında ilk kayıt

Özet: Bu çalışmada, Türkiye'nin Ege Bölgesinin doğusunda yer alan İç-Batı Anadolu yöresindeki Denizli'den 2008-2009 yılları arasında toplanan Tavas Kurbağası (*Rana tavasensis* Baran ve Atatür, 1986)'nın helmintleri ilk kez incelenmiştir. Sekiz kurbağa örneğinin tamamının bir ya da daha fazla helmintle infekte olduğu tespit edilmiştir. *R. tavasensis*'te gözlenen 3 helmint türünden biri Trematoda'dan *Haplometra cylindracea* (Zeder, 1800) Looss, 1899, biri Nematoda'dan *Cosmocerca ornata* (Dujardin, 1845) ve diğeri de Acanthocephala'dan *Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911) olup, *R. tavasensis*, tespit edilen helmint türleri için Türkiye'den yeni konak kaydıdır.

Anahtar sözcükler: Denizli, helmint, Kızılcabölük, *Rana tavasensis*, Tavas kurbağası, Türkiye

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Introduction

Rana tavasensis was originally described by Baran and Atatür in 1986, from Akdağ-Çakıroluk (near Kızılcabölük-Tavas, in Denizli Province) in Turkey. *R. tavasensis* usually inhabits slow-flowing streams in open fields and wet grasses in forested areas (Budak and Göçmen, 2008). *R. tavasensis* has "carnivorous" feeding habits; it feeds on suitable crustaceans, arachnids, insects, and gastropods in its vicinity (Düşen and Arslan, 2010). *R. tavasensis* is threatened by the general pollution and drainage of breeding sites and wetlands. This species is listed as Endangered in the IUCN Red List (IUCN, 2010).

The earliest report of helminth parasites of anurans in Turkey was published by Schad et al. (1960); they reported helminths of 5 species of anurans (*Bufo viridis*, *B. regularis*, *Pelobates syriacus*, *R. macrocnemis*, and *R. ridibunda*) collected from different localities of Turkey. Several studies of helminth fauna of Turkish anurans have been published by different researchers consecutively: Saygı and Başıbüyük (1990) reported helminth parasites of *R. ridibunda* collected from Sivas Province in central Anatolia; Oğuz et al. (1994) studied *R. ridibunda* collected from Bursa and Edirne provinces in the northwest of Turkey; Yıldırımhan et al. (1996) presented a detailed investigation of the helminths of *R. ridibunda* in the same region; Yıldırımhan et al. (1997b) studied helminths of *R. macrocnemis* collected from Bursa Province; Yıldırımhan et al. (1997a) reported helminths of *R. ridibunda*, *B. bufo*, and *P. syriacus* collected from Bursa Province; Yıldırımhan (1999) reported helminths of *B. viridis* collected from Bursa Province; Yıldırımhan et al. (2001) recorded helminths of *Bombina bombina* collected from Bursa and Edirne provinces; Kır et al. (2001) reported helminths of *R. ridibunda* collected from Eğirdir Lake in Isparta Province in the southwest of Turkey; Düşen and Öz (2004) studied helminth fauna of *Hyla arborea* collected from Antalya Province in the southwest of Turkey; Yıldırımhan et al. (2005) reported helminths of *R. ridibunda* collected from Bursa, İstanbul, Kütahya, and Rize provinces; Yıldırımhan et al. (2006b) collected *R. camerani* from Kayseri and Kars provinces; Yildirimhan et al. (2006a) recorded helminth parasites from *R. holtzi* and *R. macrocnemis* collected from Niğde, Eskişehir, Bursa, and Ordu provinces; Yıldırımhan et al. (2006) reported helminth parasites of *H. arborea* collected from Bursa and Edirne provinces; Düşen and Öz (2006) studied helminths of *R. ridibunda* collected from Antalya Province; Saglam and Arıkan (2006) studied helminths of *R. ridibunda* collected from Hazar Lake in eastern Turkey; Düşen (2007) studied helminths of *R. macrocnemis* and *R. camerani* collected from Antalya Province; Yildirimhan and Karadeniz (2007) studied helminths of *B. bufo* collected from Trabzon Province in northeastern Turkey; Düşen and Oğuz (2008) studied helminths of *R. ridibunda* collected from Denizli and Amasya provinces (inner-west Anatolian region and mid-Black Sea region); Düşen et al. (2009) reported helminth fauna of *R. dalmatina* collected from Bursa and Sakarya provinces in the northwest of Turkey; Yildirimhan et al. (2009) studied helminths of *Pelodytes caucasicus* collected from the eastern Black Sea region; Yıldırımhan and Bursey (2010) reported helminths of *Pelobates syriacus* collected from 3 localities (Bursa, Edirne, and Konya provinces in northwest and central Turkey); Düşen et al. (2010) recorded helminths of *B. bufo*, *B. viridis*, and *R. ridibunda* collected from Çanakkale Province in the northwest of Turkey; and Düşen and Oğuz (2010) reported helminths of *B. bufo*, *B. viridis*, and *R. ridibunda* collected from the central Black Sea region.

According to the given literature, several helminthological studies have been done on the helminth fauna of different frog species in Turkey. So far, however, there have been no published studies on helminths of *R. tavasensis* in Turkey. In this study, helminths of *R. tavasensis* in Turkey are being reported for the first time. This study is important for adding new information on the Turkish anuran helminth fauna.

Materials and methods

In 2008 and 2009, frogs were collected by hand from the Akdağ-Çakıroluk district (1660 m a.s.l.) of Denizli Province, Turkey (37°41′N, 29°02′E; Figure). In total, 8 *R. tavasensis* specimens (6 males, 2 females) were examined for helminth parasites. The mean ± SD snout-vent length (SVL) of the specimens was 59.12 ± 3.86 mm, with a range from 50.91 to 64.07 mm, calculated according to the methods of Bush et al. (1997).

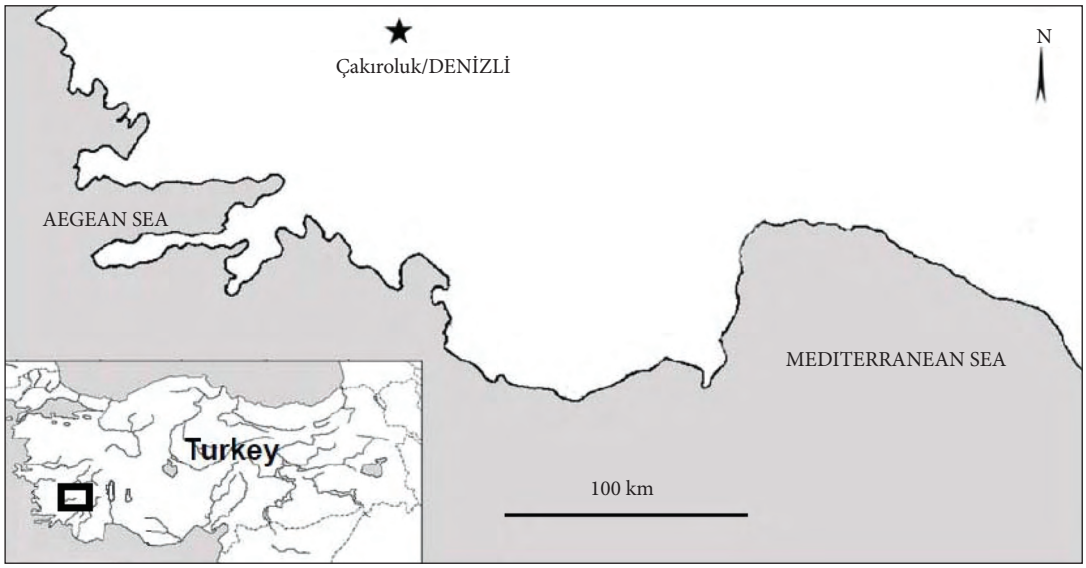

Figure. The collection location of *Rana tavasensis* from the inner-west Anatolian region of Turkey.

The frogs were overdosed in ether-filled glass containers. Each body cavity was opened with a longitudinal ventral incision. The alimentary canal was excised and the stomach, small intestine, large intestine, and rectum were individually separated. 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 the peritoneum and spinal cord, were teased out with needles and examined under a stereomicroscope. Digenea samples were immobilized by cover slip pressure in 70% ethanol, fixed, and stored in 70% ethanol. Nematode samples were straightened by heat, fixed, and stored in 70% ethanol with 5% glycerol. Acanthocephalan samples were relaxed in saline and heat-fixed under slight cover slip pressure in warm ethanol-formalin-acetic acid. Digenea and Acanthocephala samples were stained with acetocarmine, dehydrated, cleared in cedar oil or xylol, and mounted in Canada balsam. Nematode samples were cleared in glycerol and examined. Intensities are presented as mean values followed by the range.

Voucher host and parasite specimens were deposited in the Department of Biology of the Faculty of Arts and Sciences of Pamukkale University, Denizli, Turkey, under the accession number HELM-1-3/2009.

Results and discussion

In summary, 99 individuals of 3 helminth species were collected from 8 frogs. Helminths were observed in the lungs, large intestine, and small intestine of the frog samples (Table 1). Half of the infected frogs harbored 3 helminth species and the other half harbored 2 helminth species.

There were 12.37 ± 10.16 helminth individuals per infected host. The 3 helminth species found in this study all had a prevalence greater than 50%; *Haplometra cylindracea* and *Acanthocephalus ranae* were the most prevalent helminths, occurring in 7 of 8 hosts (87.50%), followed by *Cosmocerca ornata* in 6 of 8 hosts (75%).

H. cylindracea is a common parasite of frogs throughout Europe and northern Asia as far as eastern Siberia, encountered in lowlands and at altitudes of 2000 m or higher in the eastern Pyrenees and central Caucasus. According to Prudhoe and Bray (1982), Popov (1957) found that above about 2000 m in the Caucasus, 66% of *R. macrocnemis* were infested with *H. cylindracea*, and at lower altitudes, the percentage became progressively lower. Yildirimhan et al. (2006b) recorded *H. cylindracea* in *R. camerani* at up to 2000 m in 2 different localities, Yildirimhan et al. (2006a) reported *H. cylindracea* in *R. holtzi* collected at an altitude of 2600 m and *R. macrocnemis* collected at 1600-1750 m from 3 different localities,

Table 1. Occurrence and intensity of helminths in *Rana tavasensis* collected in Turkey.

Parasite (Helm. Coll. No.)	Developmental stage	Site of infection	Number infected (%)	Range	Mean intensity
LECITHODENDRIIDAE					
Haplometra cylindracea (Zeder, 1800) Looss, 1899 (HELM-1/2009)	Adult	LU	7 (87.50%)	1-6	3
COSMOCERCIDAE					
Cosmocerca ornata (Dujardin, 1845) (HELM-2/2009)	Adult	SI, LI	6 (50%)	1-25	6.16
ECHINORHYNCHIDAE					
Acanthocephalus ranae (Schrank, 1788) Lühe, 1911 (HELM-3/2009)	Adult	SI	7 (87.50%)	1-12	5.85

SI: Small intestine; **LI**: Large intestine; **LU**: Lung

and Düşen (2007) reported *H. cylindracea* in *R. camerani* collected at 1850 m and *R. macrocnemis* collected at 1515 m in Turkey. Similarly, in this study, *H. cylindracea* was observed in *R. tavasensis* at an altitude of 1660 m.

It is likely that the eggs of *H. cylindracea* are swallowed by a suitable freshwater snail host and hatch in its intestine, and the miracidia seek their required habitat in that host (Prudhoe and Bray 1982). According to Prudhoe and Bray (1982), Grabda-Kazubska (1970) found that under experimental conditions, cercariae likewise penetrate into frogs of various ages, settle, and develop to maturity in the lungs. On the other hand, Lühe (1909, in Prudhoe and Bray, 1982) and Wesenberg-Lund (1934, in Prudhoe and Bray, 1982) both found that the cercariae of *H. cylindracea* encysted the aquatic larvae of insects, which suggests that frogs and toads might also become infested by ingesting such intermediate hosts. Similarly, *R. tavasensis* could be infected with *H. cylindracea* during its tadpole stage or through feeding activity.

Cosmocercids are parasites of the gut in amphibians and reptiles. Members of the subfamily Cosmocercinae, the females produce thin-shelled eggs that larvate in utero or develop in the external environment into first-stage larvae. Expelled eggs hatch outside the host and first-stage larvae develop and molt twice to an infective third stage. The final hosts become infected either orally or by skin penetration (Anderson, 2000). Hence, the transmission of *C. ornata* could be explained by feeding activity or by contact with skin on the substrate of *R. tavasensis*.

The intermediate hosts of *A. ranae* are *Gammarus* sp. and *Asellus* sp. (Yamaguti, 1963). During our field observations in the research area, *Gammarus* sp. was observed; it could be ingested by *R. tavasensis*, thus completing the transmission of *A. ranae*.

The infection data comparison between *R. tavasensis* and other mountain frogs distributed in Turkey is given in Table 2. According to our observations, *H. cylindracea* and *A. ranae* were more commonly found in *R. tavasensis* (87.50%) than in the other 3 mountain frog species. *C. ornata* had second-order prevalence in *R. tavasensis* (50%), after *R. holtzi* (85%). The high occurrence of the 2 species *H. cylindracea* and *A. ranae* could be negative for the development of *R. tavasensis* in its habitat.

This is the first detailed study of helminths of *R. tavasensis* from Turkey. *R. tavasensis* represents a new host record for *H. cylindracea*, *C. ornata*, and *A. ranae*. All of the observed helminth species in this study are common parasites of European anurans (Yamaguti, 1958, 1961, 1963; Buchvarov, 1977; Prudhoe and Bray, 1982; Anderson, 2000; Düşen and Öz, 2006; Düşen, 2007). In addition, these species were previously observed in several other amphibian species in Turkey (Table 3).

Table 2. Infection data comparison between *Rana tavasensis* and other mountain frogs distributed throughout Turkey.

Helminth name	Host species	N	Number of helminth species found	Occurrence and mean intensity	Range	Reference
Haplometra cylindracea	*Rana macrocnemis*	84	10	13%, 6.0	2-15	Yildirimhan et al., (2006a)
		20	3	65%, 5.53	2-11	Düşen (2007)
	Rana camerani	144	11	50%, 6.1	1-30	Yildirimhan et al., (2006b)
		15	4	60%, 5.33	1-27	Düşen (2007)
	Rana holtzi	54	4	44%, 4.1	1-26	Yildirimhan et al., (2006a)
	Rana tavasensis	8	3	87.5%, 3.0	1-6	This study
Cosmocerca ornata	*Rana macrocnemis*	84	10	45%, 7.4	1-40	Yildirimhan et al., (2006a)
		20	3	15%, 1.33	1-2	Düşen (2007)
	Rana camerani	144	11	72%, 4.8	1-24	Yildirimhan et al., (2006b)
		15	4	13.3%, 25.5	1-50	Düşen (2007)
	Rana holtzi	54	4	85%, 4.3	1-12	Yildirimhan et al., (2006a)
	Rana tavasensis	8	3	50%, 6.16	1-25	This study
Acanthocephalus ranae	*Rana macrocnemis*	84	10	56%, 3.9	1-23	Yildirimhan et al., (2006a)
		20	3	40%, 18.42	2-46	Düşen (2007)
	Rana camerani	144	11	1%, 1.4	1-3	Yildirimhan et al., (2006b)
		15	4	Not observed	–	Düşen (2007)
	Rana holtzi	54	4	Not observed	–	Yıldırımhan et al., (2006a)
	Rana tavasensis	8	3	87.5%, 5.85	1-12	This study

Table 3. Records of helminths observed in this study from other amphibian species in Turkey.

Helminth name	Host species	Reference
Haplometra cylindracea	*Rana macrocnemis*	Yildirimhan et al., 2006a; Düşen, 2007
	Rana camerani	Yildirimhan et al., 2006b; Düşen, 2007
	Rana holtzi	Yildirimhan et al., 2006a
Cosmocerca ornata	*Bufo viridis*	Schad et al., 1960; Düşen et al., 2010
	Hyla arborea	Yıldırımhan et al., 2006
	Rana holtzi	Yildirimhan et al., 2006a
	Rana camerani	Yildirimhan et al., 2006b; Düşen, 2007
	Rana macrocnemis	Schad et al., 1960; Yildirimhan et al., 2006a; Düşen, 2007
	Rana ridibunda	Schad et al., 1960; Yıldırımhan et al., 2005; Düşen and Oğuz, 2010; Düşen et al., 2010
	Pelodytes caucasicus	Yildirimhan, et al., 2009
	Rana dalmatina	Düşen et al., 2009
Acanthocephalus ranae	*Mertensiella caucasica*	Yildirimhan et al., 2005
	Bombina bombina	Yıldırımhan et al., 2001
	Bufo bufo	Yildirimhan and Karadeniz 2007
	Bufo viridis	Yıldırımhan, 1999
	Hyla arborea	Düşen and Öz 2004
	Rana camerani	Yildirimhan et al., 2006b
	Rana macrocnemis	Yıldırımhan et al., 1997b; Yildirimhan et al., 2006a; Düşen, 2007 Yildirimhan et al., 2006a
	Rana holtzi	Oğuz et al., 1994; Yıldırımhan et al., 1996; Kır et al., 2001; Yıldırımhan et al., 2006b;
	Rana ridibunda	Düşen and Öz, 2006; Saglam and Arıkan, 2006; Düşen and Oğuz, 2010
	Rana dalmatina	Düşen et al., 2009

There have been several related reports on helminth species of the different anurans from neighboring countries of Turkey: Kirin and Buchvarov (2002) reported *C. ornata* in *R. ridibunda* and *Bombina variegata* in Bulgaria, and Kirin (2003a, 2003b) reported *C. ornata* and *A. ranae* in 2 different localities in Bulgaria. Mashaii et al. (2000) reported *C. ornata* in *R. ridibunda* from northern Iran, and Mashaii (2005) recorded *H. cylindracea* and *C. ornata* in *B. viridis* from southwestern Iran. Saeed et al. (2007) observed *H. cylindracea* in *R. ridibunda* and *C. ornata* in *B. viridis* from Iraq.

These results reinforce the importance of carrying out further studies, which could also expand the host-parasite list from Turkey, in order to better understand the ecological relationship between anurans and their helminth parasites.

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