

Helminths of the Eastern Tree Frog, *Hyla orientalis*, Bedriaga, 1890 (Anura: Hylidae), collected from Denizli Province, Inner-West Anatolia Region, Turkey

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Summary

In this investigation, *Hyla orientalis* (eastern tree frog) was collected in different localities from Denizli province (Inner-west Anatolia Region – the eastern part of Aegean Region) Turkey, between 2008 and 2011 and examined for the first time for helminths. Also, this study contains first detailed SEM imaging attempt of the some amphibian helminths from Turkey. Of 17 *Hyla orientalis* 8 (47.05 %) were infected with one or more helminths. *Hyla orientalis* harbored one species of Monogenea 1 (*Polystoma skrjabini*), one species of Digenea (*Pleurogenoides medians*), three species of nematoda (*Oswaldocruzia filiformis*, *Cosmocerca ornata* and *Abbreviata* sp.), and one species of Acanthocephala (*Acanthocephalus ranae*). All helminths recorded first time for *Hyla orientalis*.

Keywords: Acantocephala; Denizli; Digenea; Helminth; *Hyla orientalis*; Hylidae; Monogenea; Nematoda; SEM; Turkey

Introduction

Previously, the eastern tree frog *H. orientalis* was not distinguished from *H. arborea*, this species is formerly known as *H. arborea* (Linnaeus, 1758) in Turkey and vicinity; Stöck *et al.* (2008) stated that south-eastern European and western Anatolian *H. arborea* populations should be considered a separate species. So, they split *H. arborea* into three species based on molecular data, and resurrected the name *H. orientalis* Bedriaga, 1890 for the eastern populations (e.g. Bulgaria, Ukraine, Turkey and Iran) (Gvoždík, 2010; Gvoždík *et al.*, 2010; Gül *et al.*, 2012).

The eastern tree frog *H. orientalis* is a small arboreal species. It is nocturnal in habit and shelters under leaves in the daytime; it goes to water only in the breeding season, preferring clean, deep, heavily-vegetated water. In Turkey, this species is known in west, north and south-western Anatolia (Baran *et al.*, 2012). To our knowledge, the first

helminthological study on a Hylid member (*H. arborea*) was published by Düşen and Öz (2004) in Turkey. They recorded six nematode species from southwestern Turkey. Yıldırımhan *et al* (2006a) is reported three helminths in *Hyla arborea* from north-western Turkey.

So far, there has been no published study on helminths of eastern tree frog (*H. orientalis*) from Denizli province, and its vicinity (Inner-west Anatolia Region – the eastern part of Aegean Region) in Turkey. This is the first helminthological investigation which has been done in this province.

Materials and methods

Seventeen *H. orientalis* (7 ♂♂, 8 ♀♀, 2 juv., mean± SD snout-vent length (SVL) = 47.56 ± 7.13 mm, a range 26.79 – 52.16 mm), were collected between 2008 – 2011 in Denizli province (38°29' - 38° 52' N - 28°38' - 30° 05' E) and vicinity (Fig. 1), within 24 hr, toads were overdosed with ether.

The body cavities were 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 and organ were each mixed with 0.5 % saline solution and were poured into petri dishes for

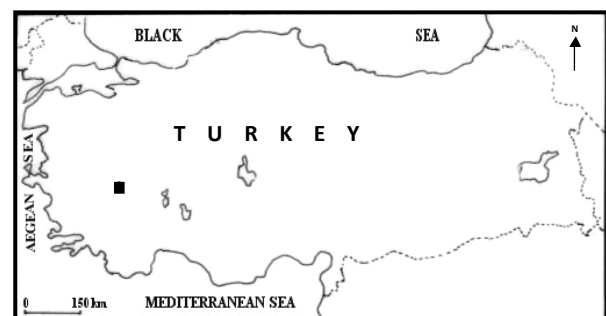


Fig. 1. The collecting locality of *H. orientalis* from inner-west Anatolia Region in Turkey

examination under a stereomicroscope. The lungs, liver, gall bladder, kidneys and urinary bladder were also searched for helminths. Trematode samples were immobilized by heat, fixed, and stored in 70 % ethanol. Nematodes were straightened by heat, fixed, and stored in 70 % ethanol with 5 % glycerol. Acanthocephalans were relaxed in saline and heat-fixed under slight coverslip pressure in warm alcohol-formalin-acetic acid. Monogenean, digenean and acanthocephalan samples were stained with acetocarmine, dehydrated, cleared in cedar oil, and mounted in Entellan[®]; nematodes were cleared in glycerol and examined. Intesities are presented as mean values followed by the range. Voucher host specimens and parasite specimens were deposited in Pamukkale University, Faculty of Sciences and Arts, Department of Biology, Denizli, Turkey, under the accession number (PAU-HELM-5-10/2013). For SEM (Scanning Electron Microscope), samples of

some helminths had been stored in 70 % ethanol were processed following standard methods, (Schatten & James, 2008) that included critical point drying in porous capsules (pore diameter is 100µm) and mounted on SEM sample mounts using conductive double sided carbon tape. Helminth samples were then gold coated for 3 minutes using a sputter coater establishing an approximate thickness of 20nm. Samples were then placed in a Zeiss-Leo 14320 under low vacuum conditions. Permanent images were obtained with a digital camera at various magnifications.

Results and discussion

Hyla orientalis, Bedriaga, 1890

Seventeen specimens (7 ♂♂, 8 ♀♀, 2 juv.) were collected between 2008 – 2011 years from Denizli province, Turkey.

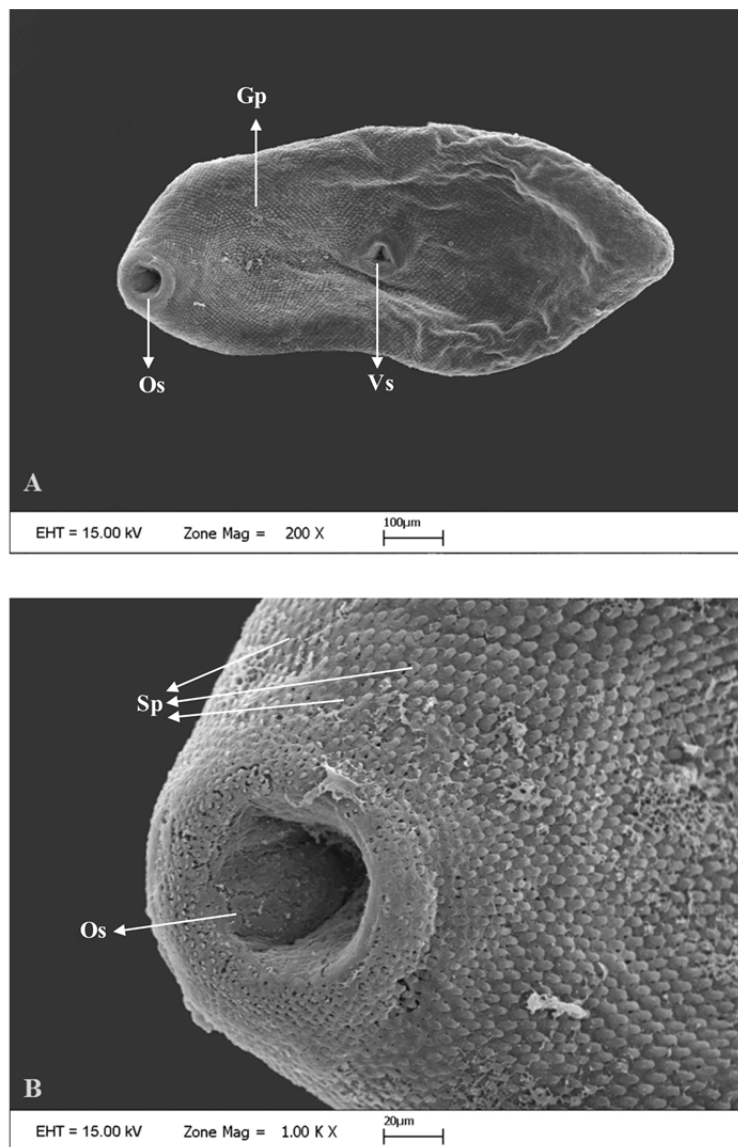


Fig. 2. SEM of specimens of *Pleurogenoides medians* from *H. orientalis*.

A – Ventral view of a whole adult worm of *P. medians*, Os: Oral sucker, Gp: Genital pore, Vs: ventral sucker; B – Oral sucker and the tegument on the antero-ventral spinulate surface, Sp: Spines

Monogenea

Family: Polystomatidae

Polystoma skrjabini Buchvarov, 1984

Prevalence, intensity and range: One of 17 hosts infected (5.88 %, 1, 1).

Polystoma skrjabini is first time observed in the Urinary bladder of *H. arborea* by Buchvarov, 1984 in Bulgaria, Düşen & Öz (2004) and Yildirimhan *et al.* (2006) were reported this species from different localities in (*H. arborea*) from Turkey.

Geographic range: East Europe and the Middle East (Buchvarov, 1984; Düşen & Öz, 2004).

Specimens deposited: PAU-HELM-5/2013 (1 slide)

Digenea

Family: Lecithodendriidae

Pleurogenoides medians (Olsson, 1876) Travassos, 1921

Prevalence, intensity and range: Hosts infected, One of 17 hosts infected (5.88 %, 7, 7).

Other reported hosts: *P. medians* has been reported in various amphibians and reptiles species, *Rana ridibunda* (Satmann, 1990), *Triturus cristatus* (Shimalov *et al.*, 2001), *T. vulgaris* (Vojtková & Vojtek, 1975; Shimalov *et al.*, 2001); *Bombina bombina*, (Vojtková & Vojtek, 1975), *Bo. variegata*, (Vojtková & Vojtek, 1975), *Bufo bufo* (Shimalov & Shimalov, 2001), *B. calamita*, (Vojtková & Vojtek, 1975), *B. vulgaris* (Yamaguti, 1958), *B. viridis* (Düşen *et al.*, 2010; Düşen & Oğuz, 2010), *H. arborea* (Vojtková & Vojtek, 1975; Düşen & Öz, 2004), *H. savignyi* (Yildirimhan *et al.*, 2012), *Rana arvalis* (Vojtková & Vojtek, 1975), *R. camerani* (Yildirimhan *et al.*, 2006b; Düşen, 2007), *R. dalmatina* (Buchvarov, 1977; Düşen *et al.*, 2009), *R. esculenta* (Vojtková & Vojtek, 1975; Buchvarov, 1977; Kuc & Sulgostowska, 1988b), *R. macrocnemis* (Yildirimhan *et al.*, 2006c; Düşen, 2007), *R. arvalis* (Vojtková & Vojtek, 1975), *R. temporaria* (Vojtková & Vojtek, 1975; Cedhagen, 1977), *R. holtzi* (Topçu, 2002), *Lacerta trilineata* (Yamaguti, 1963;

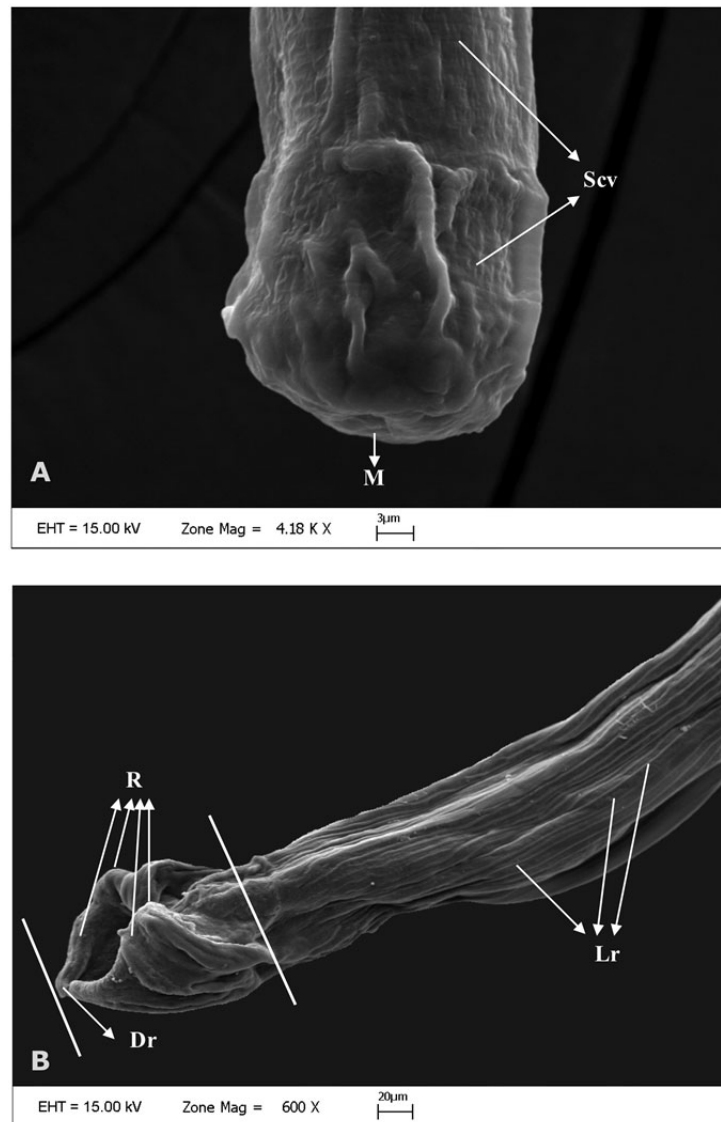


Fig. 3. SEM of specimens of *Oswaldocruzia filiformis* from *H. orientalis*

A – Cephalic end of male M: mouth and tegument, Scv: Simple cuticular vesicle; B – Caudal end of male, in lateral view of the caudal bursa (Between in two parallel white lines), Lr: longitudinal ridges; R: Rays; Dr: Dorsal ray

Yıldırımhan *et al.*, 2011), *L. agilis* (Sharpilo *et al.*, 2001); *Natrix natrix* (Kirin, 2002a) and *Atheris hispida* (Hassl, 2010; Hassl *et al.*, 2010), *Pelophylax ridibundus* (Düşen & Öz, 2013).

Geographic range: Europe and Asia (Yamaguti, 1958); Australasian Regions (Prudhoe & Bray, 1982).

Specimens deposited: PAU-HELM-6/2013 (1 slide)

The tegument surface is covered with regularly arranged flattened hand like spines, the spines are more densely arranged towards the anterior end and more sparsely distributed towards the posterior end. The genital pore is also observed. The SEM images of this species is presented in Fig. 2.

Nematoda

Family: Molineidae

Oswaldocruzia filiformis (Goeze, 1782) Travassos, 1917

Prevalence, intensity and range: one of 17 hosts infected (5.88 %, 4, 4).

Oswaldocruzia filiformis is recorded from various amphibian and reptile species, including *S. salamandra* (Buchvarov, 1977), *T. alpestris* and *T. karelini* (Buchvarov, 1977; Cedhagen 1988; Kirin & Buchvarov, 2002), *T. vulgaris* (Buchvarov, 1977; Satmann, 1990; Shimalov *et al.*, 2001), *T. vittatus* (Yıldırımhan, 2008), *Bo. bombina* and *B. variegata* (Buchvarov, 1977, Kirin & Buchvarov, 2002), *B. regularis* (probably *B. viridis*) (Schad *et al.*, 1960), *B. viridis* (Buchvarov, 1977; Yıldırımhan, 1999; Shimalov & Shimalov, 2001; Düşen *et al.*, 2010a; Düşen & Oğuz, 2010), *B. viridis* (Buchvarov, 1977; Yıldırımhan, 1999; Shimalov & Shimalov, 2001; Topçu & Bayrak, 2000; Düşen *et al.*, 2010a; Düşen & Oğuz, 2010; Mohammad *et al.*, 2010), *B. bufo* (Buchvarov, 1977; Yıldırımhan & Karadeniz, 2007; Düşen & Oğuz, 2010; Düşen, 2011); *Pseudepidalea viridis* (Düşen, 2011), *Pelodytes caucasicus* (Yıldırımhan *et al.*, 2009), *H. arborea* (Buchvarov, 1977; Yıldırımhan *et al.*, 2006a), *R. camerani*, *R. dalmatina*, (Buchvarov *et al.*, 1975; Buchvarov, 1977; Kirin & Buch-

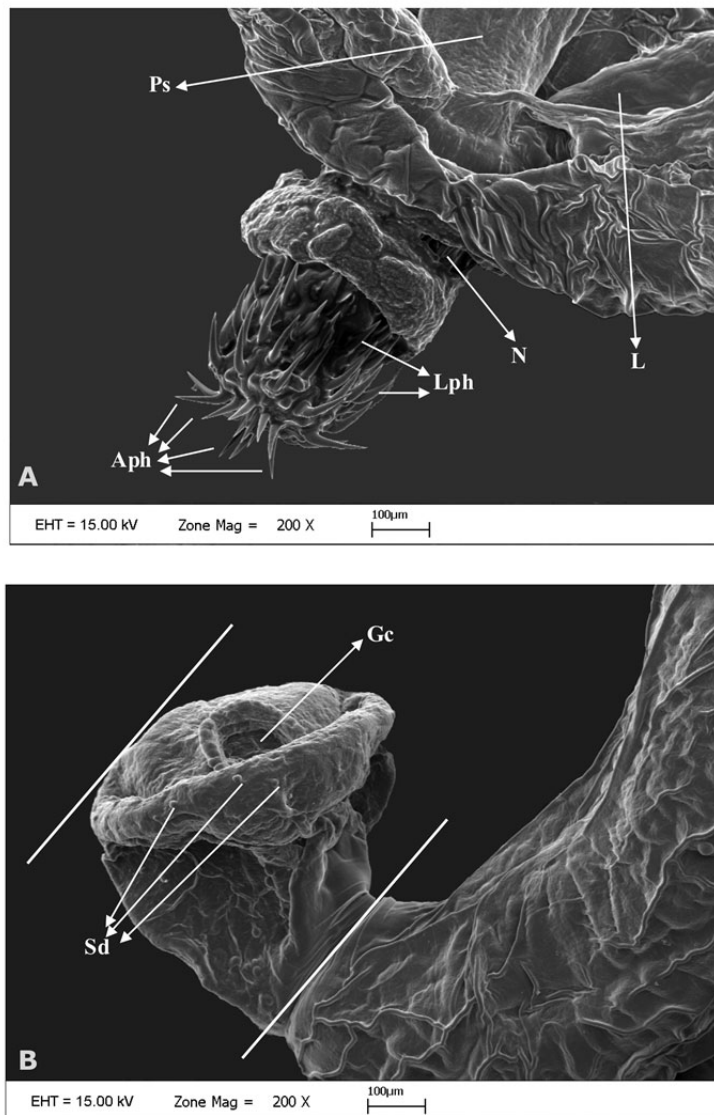


Fig. 4. SEM of pecimens of *Acanthocephalus ranae* (male) from *H. orientalis*.

A – Partially sectioned presoma, N: Neck; Ps: Proboscis sheath, L: Lemniscus, Aph: Apical proboscis hooks, Lph: Lateral proboscis hooks, B – Partially opened Bursa (Between in two parallel white lines), Sd: Sensory discs; Gc: Genital canal

varov, 2002; Düşen *et al.*, 2009), *R. kurtmuelleri* (Hristovski *et al.*, 2006), *R. macrocnemis* (Schad *et al.*, 1960; Yıldırımhan *et al.*, 1997; Yıldırımhan *et al.*, 2006c), *R. ridibunda* (Buchvarov, 1977; Yıldırımhan *et al.*, 1996; Buchvarov *et al.*, 1975; Kirin & Buchvarov, 2002; Yıldırımhan *et al.*, 2005a; Sağlam & Arıkan 2006; Düşen & Oğuz, 2010), *R. temporaria* (Buchvarov, 1977; Cedhagen, 1988; Kirin & Buchvarov, 2002), *R. graeca* (Božkov & Stojkova, 1970; Buchvarov, 1977), *Pe. ridibundus* (Düşen & Öz, 2013); *Lacerta agilis*, (Sharpilo *et al.*, 2001; Shimalov *et al.*, 2000; Mihalca *et al.*, 2007), *L. trilineata* (Yıldırımhan *et al.*, 2011), *L. viridis* (Biserkov & Kostadinova, 1998; Kirin, 2002b; Borkovcová & Kopřiva, 2005), *L. vivipara* (Shimalov *et al.*, 2000); *Anguis fragilis* (Schad *et al.*, 1960; Bertman & Okulewicz, 1987; Shimalov *et al.*, 2000; Borkovcová & Kopřiva 2005; Düşen *et al.*, 2010b), *Zootoca vivipara* (Sanchis *et al.*, 2000), *N. natrix* (Bertman & Okulewicz, 1987; Shimalov & Shimalov, 2000; Kirin 2002b) and *V. berus* (Shimalov & Shimalov, 2000).

Schad *et al.* (1960) were first time reported *O. filiformis* in *Bufo regularis* (probably this species is *Pseudepidalea viridis* in Turkey (formerly known as *Bufo viridis*)) and *R. macrocnemis* from Turkey. *O. filiformis* was observed in small intestines from *H. orientalis* samples in this study.

Geographic range: Europe and Asia (Yamaguti, 1961).

Specimens deposited: PAU-HELM-7/2013 (2 slides)

The body has longitudinal ridges and these longitudinal ridges, some disappearing and others appearing near the caudal bursa, ridges not continuous along the nematode body. The SEM images of this species is presented in Fig. 3.

Family: Cosmocercidae

Cosmocerca ornata (Dujardin, 1845)

Prevalence, intensity and range: Two of 17 hosts were infected (11.76 %, 1, 1 – 3).

There are several papers reporting *Cosmocerca ornata* from many species of amphibians and reptiles, including *Bufo*, *Hyla*, *Rana*, *Triturus* (Yamaguti, 1961), *T. alpestris* (Walton, 1933; Buchvarov, 1977; Shimalov *et al.*, 2000), *T. cristatus* (Walton, 1933; Shimalov *et al.*, 2001), *T. vulgaris* (Shimalov *et al.*, 2001), *Bo. bombina*, (Buchvarov, 1977; Grabda-Kazubska & Lewin, 1989), *Bo. variegata* (Buchvarov, 1977; Grabda-Kazubska & Lewin, 1989; Satmann, 1990; Kirin & Buchvarov, 2002), *B. viridis* (Buchvarov *et al.*, 1975; Buchvarov, 1977; Vashetko & Siddikov, 1999; Masshahi, 2005; Düşen *et al.*, 2010a, Düşen & Oğuz, 2010), *Pseudepidalea viridis* (Düşen, 2011), *B. bufo* (Düşen, 2011), *Pe. caucasicus* (Yıldırımhan *et al.*, 2009), *H. arborea* (Buchvarov, 1977, Yıldırımhan *et al.*, 2006a), *Pelobates syriacus* (Shimalov *et al.*, 2000), *R. esculenta* (Walton, 1933; Buchvarov, 1977), *R. arvalis* (Cedhagen, 1988; Kuc & Sulgostowska, 1988b), *R. temporaria*, (Walton, 1933; Buchvarov, 1977; Kuc & Sulgostowska, 1988b), *R. graeca* (Božkov & Stojkova, 1970; Buchvarov, 1977), *R. holtzi* (Yıldırımhan *et al.*, 2006c), *R. macrocnemis* (Yıldırımhan *et al.*, 2006c; Düşen,

2007), *R. ridibunda* (Buchvarov *et al.*, 1975; Buchvarov, 1977; Kuc & Sulgostowska, 1988a; Masshahi *et al.*, 2000; Kirin & Buchvarov, 2002; Kirin, 2003a, b; Yıldırımhan *et al.*, 2005a; Düşen & Öz, 2006; Düşen & Oğuz, 2010; Düşen *et al.*, 2010), *R. camerani* (Yıldırımhan *et al.*, 2006b; Düşen, 2007), *R. tavasensis* (Düşen, 2012), *Chiasmocleis capixaba* (Van Sluys *et al.*, 2006) and *A. fragilis* (Shimalov *et al.*, 2000; Düşen *et al.*, 2010b).

Schad *et al.* (1960) were first time recorded *C. ornata* in *Pseudepidalea viridis* (formerly known as *Bufo viridis*), *R. macrocnemis* and *R. ridibunda* in Turkey. In this study, *C. ornata* was observed in small and large intestines of *H. orientalis* specimens.

Geographic range: New and Old Worlds (Baker, 1987).

Specimens deposited: PAU-HELM-8/2013 (2 slides)

Family: Physalopteridae

Abbreviata sp. (larvae - Encapsulated larvae in submucosa of stomach and small intestine).

Prevalence, intensity and range: Hosts infected, 2 of 17 hosts (11.76 %, Uncountable).

Other reported hosts: Fernando (1989) observed this nematode larvae in *R. ridibunda* from Saudi Arabia; Fernando (1989) reported that parasite larvae in brown cysts were located subcutaneously on body wall, heavy infestations also occurred in the submucosae of stomach, small intestine and mesenteries and embedded deeply in them muscle. Düşen and Öz (2006) observed this encapsulated larvae in submucosa of stomach and small intestines of *R. ridibunda* from Southwest of Turkey (in Antalya region); Düşen and Öz (2013) also reported *Pe. ridibundus* from Innerwest part of Turkey (in Denizli province); Borkovcová and Kopřiva (2005) recorded this nematode species in some lizards (*A. fragilis*, *L. viridis* and *L. agilis*) and one snake (*Coronella austriaca*) species in South Moravia (Czech Republic). In this investigation, the larvae of *Abbreviata* sp. were observed numerous, and deeply embedded in submucosa of the stomach and small intestine in *H. orientalis*, therefore counting was difficult.

Geographic range: Europe Asia, and Western Australia (Anderson, 2000).

Specimens deposited: PAU-HELM-9/2013 (1 slide)

Acanthocephala

Family: Echinorhynchidae

Acanthocephalus ranae (Schrank, 1788) Lühe, 1911

Prevalence, intensity and range: Two of 17 hosts were infected (11.76 %, 2, 2).

Other reported hosts: *Rana* sp., *Bombinator* sp., *Hyla* sp., *Triturus* sp., *Salamandra* sp., *Diemictylus viridescens* (Yamaguti, 1963), *Bo. bombina* (Buchvarov, 1977; Grabda-Kazubska & Lewin, 1989; Yıldırımhan *et al.*, 2001a); *B. variegata* (Grabda-Kazubska & Lewin, 1989); *B. viridis* (Buchvarov, 1977; Yıldırımhan, 1999; Vashetko & Siddikov, 1999; Shimalov & Shimalov, 2001); *B. calamita* (Shimalov & Shimalov, 2001); *B. bufo* (Düşen, 2011), *H. arborea* (Düşen & Öz, 2004), *R. arvalis*, *R. dalmatina* (Buchvarov, 1977; Düşen *et al.*, 2009); *R. temporaria*

Table 1. Prevalence, Mean intensity, infection sites and range of helminths in *H. orientalis*

HELMINTH GROUP	Developmental stage	Site of infection	No. of infected (%)	Mean intensity	Range
Monogenea					
<i>Polystoma skrjabini</i> Batcharov, 1984	Adult	UB	1 (5.88)	1	1
Digenea					
<i>Pleurogenoides medians</i> (Olsson, 1876) Travassos, 1921	Adult	SI	1 (5.88)	7	7
Nematoda					
<i>Oswaldocruzia filiformis</i> (Goeze, 1782) Travassos, 1917	Adult	SI	1 (5.88)	4	4
<i>Cosmocerca ornata</i> (Dujardin, 1845) <i>Abbreviata</i> sp.	Adult	LI, SI ST, SI	2 (11.76)	1	1 – 3
(Encapsulated larvae in submucosa of stomach and small intestine)	Larvae		2 (11.76)	–	Uncount.
Acanthocephala					
<i>Acanthocephalus ranae</i> (Schränk, 1788) Lühe, 1911	Adult	SI	2 (11.76)	1	2

LI: Large intestine, ST: Stomach, SI: Small intestine; UB: Urinary bladder

(Buchvarov, 1977; Cedhagen, 1988; Kuc & Sulgostowska, 1988b); *R. esculenta* (Buchvarov, 1977; Kuc & Sulgostowska, 1988b); *R. macrocnemis* (Yıldırımhan *et al.*, 1997; Yıldırımhan *et al.*, 2006c; Düşen, 2007); *R. camerani* (Yıldırımhan *et al.*, 2006b), *R. kurtmuelleri* (Hristovski *et al.*, 2006); *R. tavasensis* (Düşen, 2012), *Pe. ridibundus* (Düşen & Öz, 2013); *Mertensiella caucasica* (Yıldırımhan *et al.*, 2001b; 2005b); *A. fragilis* (Shimalov *et al.*, 2000). *N. natrrix* (Yamaguti, 1963; Shimalov & Shimalov, 2000). Dimitrova *et al.* (2008) were reported in mammal species (Eurasian otter, *Lutra lutra*) as a paratenic host.

Also, *A. ranae* is reported in *H. arborea* from Turkey by Düşen & Öz (2004).

Geographic range: Europe, U.S.A., Russia (Yamaguti, 1963); Turkey (Oğuz *et al.*, 1994).

Specimens deposited: ZDEU HEL-10/2013 (1 slide)

The neck is wide. Apical hooks were observed as acutely curved compared to other proboscis hooks. The sensory discs and genital canal were also observed the inside of bursa. The SEM images of this species is presented in Fig. 4.

Six helminth species were found infecting *H. orientalis* in this investigation. The site of infection in the frogs and the data on infection parameters for each hosts, are shown in Table 1. In summary, 20 individuals of 6 helminth species were collected from the 17 *H. orientalis* examined. Monogenea was observed in Urinary bladder, Digenans was observed in small intestines, Nematodes were observed in large-small intestines, and cysts on the surface of the intestinal wall; Acanthocephala was also observed in small intestine of this species. According the data obtained 8 (47.06 %) *H. orientalis* harbored the one or more species of helminths and the remaining 7 (41.18 %) were uninfected. There were 1.12 ± 0.35 (1 – 2) helminth species per infected host and there were 3.33 ± 2.42 (1 – 7) helminth

individuals per infected host (the total number of larvae of *Abbreviata* sp. is not included this calculation, because counting was difficult).

The helminths that were observed *H. orientalis* are generally common parasites of European anurans, except *Polystoma skrjabini* (Yamaguthi, 1961; 1963; Buchvarov, 1977; Anderson, 2000; Yıldırımhan, 1999; Yıldırımhan *et al.*, 2007, Düşen & Öz, 2006, Düşen *et al.*, 2010a; Düşen and Öz, 2013), and first time reported for *H. orientalis* from Turkey.

Acknowledgements

This study is supported by the Pamukkale University Scientific Research Projects Unit Project number: 2008BSP005. We also thank, for permission and helps, the Department of National Parks and Wildlife of the Republic of Turkey Ministry of Forestry and Water Affairs.

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RECEIVED DECEMBER 5, 2013

ACCEPTED FEBRUARY 25, 2014