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Research Note

Helminth parasites of the levantine frog (*Pelophylax bedriagae* Camerano, 1882) from the western part of Turkey

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Article info

Summary

Received July 21, 2014 Accepted October 29, 2014 Fifty-four *Pelophylax bedriagae* (Levantine Frog) from Turkey (İzmir and Manisa Provinces) were examined for helminth parasites. The frogs were collected between 2012 and 2014 years. Eight species of helminth parasites were recorded: 3 species of Nematoda (*Rhabdias bufonis*, *Cosmocerca ornata*, *Oswaldocruzia filiformis*), 3 species of Digenea (*Diplodiscus subclavatus*, *Haematoloechus breviansa*, *Gorgoderina vitelliloba*), 1 species of Acanthocephala (*Acanthocephalus ranae*) and 1 species of Hirudinea (*Hirudo medicinalis*). *Pelophylax bedriagae* is a new host record for these parasite species.

Keywords: Pelophylax bedriagae; Nematoda; Digenea; Acanthocephala; Hirudinea

Introduction

Levantine frog (*Pelophylax bedriagae* Camerano, 1882) is distributed widely in the eastern Mediterranean. This species is commonly found along the western and southern parts of Anatolia in Turkey, Greek islands and Cyprus. *Pelophylax bedriagae* is a highly aquatic frog living in permanent ponds, rain pools, streams, rivers, irrigation channels, reservoirs, marshes, springs and fishponds, and sometimes found in surrounding terrestrial habitats (Papenfuss *et al.*, 2009).

There have been several studies about helminth parasites of different frog species in Turkey. Although there are some studies about parasites of other water frogs (*Pelophylax ridibundus*, *Rana tavasensis*, *Rana camerani*, *Rana dalmatina*, *Rana holtzi*, *Rana macrocnemis*), there is no report on the parasites of *Pelophylax bedriagae* in Turkey (Amin *et al.*, 2012). To our knowledge, there is just 1 published report of parasites in *P. bedriagae* from Jordan: Al-Sorkhy and Amr (2003) recorded *Prosotocus confusus*, *Pleurogenoides tacapensis* and *Nematotaenia dispar*.

The aim of this study was to investigate the parasites of *Pelophylax bedriagae*. This is the first detailed helminthological study conducted on *P. bedriagae* from the İzmir and Manisa Provinces in Western Turkey.

Materials and Methods

Fifty-four *Pelophylax bedriagae* (43 male and 11 female) were collected by hand in spring of 2012, 2013 and 2014 years from 7 different localities in Turkey: İzmir Province Bornova district (38°29'18"N, 27°13'26"E; N = 10), Ödemiş district (38°18'43"N, 28°01'37"E; N = 17), Balçova district (38°23'04"N, 27°01'57"E; N = 2), İnciraltı district (38°24'51"N, 27°01'39"E; N = 4), Seferihisar district (38°16'53"N, 26°51'01"E; N = 9), Karagöl Lake (38°33'27"N, 27°13'06"E; N = 5); Manisa Province, Salihli district (38°28'39"N, 28°06'27"E; N = 7) (Fig. 1). The mean \pm SD snoutvent length (SVL) of the specimens was 70.2593 \pm 9.3469 mm, with a range from 50 to 97 mm.

The frogs were euthanized with sodium pentobarbital. The heart, lungs, liver, stomach, body cavity, intestine, kidneys, urinary bladder were removed and placed in petri dishes containing a physiological solution and examined for parasites using a stere-omicroscope. Helminths were killed in a hot saline solution; nematodes were fixed in 70 % ethanol and mounted in glycerol; digeneans and acanthocephalans were fixed in 70 % ethanol, stained with iron-carmine (Georgiev et al., 1986), cleaned with clove oil and mounted in Entellan® for examination with a compound microscope. Prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997). The species

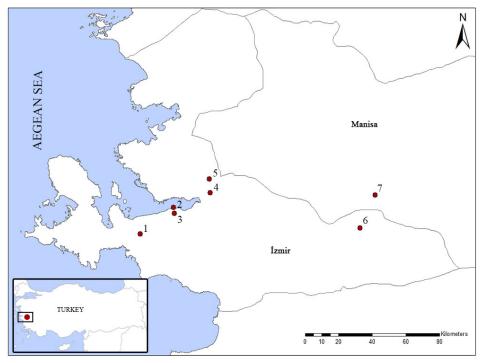


Fig. 1. Collecting localities of Pelophylax bedriagae from Western Part of Turkey: 1. Seferihisar 2. İnciraltı 3. Balçova 4. Bornova 5. Karagöl 6. Ödemiş 7. Salihli

identification was done according to Yorke and Maplestone (1926), Yamaguti (1958, 1961, 1963), Baker (1987), Bray *et al.* (2008). The Kruskal-Wallis test and the Mann-Whitney U test were used to compare differences in parasite abundance between the localities. The significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS version 22.0.

Results

Eight species of helminth parasites were detected in levantine frog. These species were *Rhabdias bufonis*, *Cosmocerca ornata*, *Oswaldocruzia filiformis* (Nematoda); *Diplodiscus subclavatus*, *Haematoloechus breviansa*, *Gorgoderina vitelliloba* (Digenea); *Acanthocephalus ranae* (Acanthocephala) and *Hirudo medicinalis* (Hirudinea). The infection sites included the lungs, intestine, urinary bladder and skin. *C. ornata* was the most prevalent species, followed by *R. bufonis*. Infection prevalence, abundance and mean intensity of the parasites in *P. bedriagae* were given in Table 1.

Discussion

Eight helminth parasites infected *P. bedriagae* in the present study. All of these parasite species were previously reported from different frogs in Turkey (Table 2).

Of 54 Pelophylax bedriagae 48 (88.88 %) were infected with one or more parasites. Eight parasite species totaling 530 individuals were collected from 48 levantine frogs. Of the infected levantine frogs, 17 (35.41 %) harbored 1 parasite species, 23 (47.91 %) harbored 2 parasite species, 4 (8.33 %) harbored 3 parasite species, 3 (6.25 %) harbored 4 parasite species, 1 (2.08 %) harbored 5 parasite species.

Aho (1990) compiled distributional patterns for anurans in general and reported the total number (mean \pm SE) of helminth species per host species as 3.54 ± 0.24 (range: 0-9). Thus, the infection rates for *P. bedriagae* are much lower than for anurans in general. Determining whether permanent ponds, rain pools, streams, rivers, irrigation channels, reservoirs, marshes, springs and fishponds, being sometimes found in surrounding terrestrial habitats

Table 1. Prevalence, mean intensity and abundance of parasites in Pelophylax bedriagae

| Parasite species | Site | Number of infected | Mean | Min-Max | Abundance |
|--------------------------|-----------------|--------------------|-----------|---------|-----------|
| - | of infection | frogs (%) | intensity | | |
| Rhabdias bufonis | Lungs | 20 (37.03) | 6.40 | 1 – 30 | 2.37 |
| Cosmocerca ornata | Intestine | 28 (51.85) | 7.10 | 1 – 19 | 3.68 |
| Oswaldocruzia filiformis | Intestine | 8 (14.81) | 4.62 | 1 – 13 | 0.68 |
| Diplodiscus subclavatus | Intestine | 16 (29.62) | 4.25 | 1 – 22 | 1.25 |
| Haematoloechus breviansa | Lungs | 4 (7.40) | 1.50 | 1 – 2 | 0.11 |
| Gorgoderina vitelliloba | Urinary bladder | 9 (16.66) | 1.88 | 1 – 4 | 0.31 |
| Acanthocephalus ranae | Intestine | 5 (9.25) | 14.60 | 1 – 53 | 1.35 |
| Hirudo medicinalis | Skin | 2 (3.70) | 1.00 | 1 | 0.03 |

Table 2. Parasite species previously reported from different frogs in Turkey

| Parasite species | Host | Reference | |
|--|--|------------------------------|--|
| Cosmocerca ornata | Bufo viridis | 1, 2, 3, 4 | |
| | Rana macrocnemis | 1, 5, 6 | |
| | Rana ridibunda | 1, 2, 3, 7 | |
| | Hyla arborea | 8 | |
| | Rana camerani | 6, 9 | |
| | Rana holtzi | 5 | |
| | Bufo bufo | 2, 4, 10 | |
| | Pelodytes caucasicus | 11 | |
| | Rana dalmatina | 12 | |
| | Rana tavasensis | 13 | |
| 5/ / " / / / | | | |
| Rhabdias bufonis | Rana ridibunda | 14, 15, 16, 17, 18 | |
| | Bufo bufo | 4, 10, 15 | |
| | Bufo viridis | 4, 19 | |
| | Bombina bombina | 20 | |
| | Rana camerani | 9 | |
| | Rana macrocnemis | 5 | |
| | Rana dalmatina | 12 | |
| | Pelodytes caucasicus | 11 | |
| Oswaldocruzia filiformis | Rana macrocnemis | 1, 5, 21 | |
| | Bufo regularis | 1 | |
| | Bufo viridis | 2, 3, 4, 19, 22, | |
| | Rana ridibunda | 3, 7, 17 | |
| | Hyla arborea | 8 | |
| | | 9 | |
| | Rana camerani | • | |
| | Bufo bufo | 3, 4, 10 | |
| | Triturus vittatus | 23 | |
| | Pelodytes caucasicus | 11 | |
| | Rana dalmatina | 12 | |
| Diplodiscus subclavatus | Rana ridibunda | 2, 7, 14, 16, 18, 22, 24 | |
| | Rana holtzi | 25 | |
| | Rana dalmatina | 12 | |
| | Hyla savignyi | 26 | |
| Gorgoderina vitelliloba | Rana ridibunda | 2, 3, 7, 14, 17, 18 | |
| | Rana macrocnemis | 5, 21 | |
| | Rana camerani | 9 | |
| | Rana holtzi | 5 | |
| | | | |
| Haematoloechus breviansa | Rana ridibunda | 3, 7, 14, 18 | |
| Acanthocephalus ranae | Rana ridibunda | 3, 7, 14, 16, 17, 18, 22, 24 | |
| | Rana macrocnemis | 5, 6, 21 | |
| | Bufo viridis | 19 | |
| | Bombina bombina | 20 | |
| | Hyla arborea | 27 | |
| | Mertensiella caucasica | 28 | |
| | Rana camerani | 9 | |
| | Bufo bufo | 4, 10 | |
| | Rana dalmatina | 12 | |
| | Rana tavasensis | 13 | |
| | Rana ridibunda, Rana tavasensis, Rana macrocnemis, | 13 29 | |
| | Rana dalmatina, Hyla arborea, Bufo bufo | ۷J | |
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| Hirudo medicinalis | Rana ridibunda Düsen and Oğuz. 2010: 4. Düsen. 2011: 5. Yıldırımhan et al 2006b | 7, 14, 16 | |

^{1.} Schad et al., 1960; 2. Düşen et al., 2010; 3. Düşen and Oğuz, 2010; 4. Düşen, 2011; 5. Yıldırımhan et al., 2006b; 6. Düşen, 2007; 7. Yıldırımhan et al., 2005b; 8. Yıldırımhan et al., 2006c; 10. Yıldırımhan and Karadeniz, 2007; 11. Yıldırımhan et al., 2009; 12. Düşen et al., 2009; 13. Düşen, 2012; 14. Yıldırımhan et al., 1996; 15. Yıldırımhan et al., 1997b; 16. Kır et al., 2001; 17. Sağlam and Arıkan, 2006; 18. Düşen and Öz, 2006; 19. Yıldırımhan, 1999; 20. Yıldırımhan et al., 2001; 21. Yıldırımhan et al., 2012; 27. Düşen and Öz, 2004; 28. Yıldırımhan et al., 2005a; 29. Heckmann et al., 2011

of *P. bedriagae* is responsible for this difference will require additional work.

Helminths are the most common invertebrate parasites of amphibians (Smyth & Smyth, 1980). The digeneans generally utilize either a molluscan first intermediate host from which the cercariae leave and penetrate a frog host directly, or a variety of invertebrate hosts, which are then eaten by the final host (Smyth & Smyth, 1980). Cestodes are not common parasites in the gastrointestinal tract of amphibians, but when present may persist for a long time (Nickol, 1985). In our study, we didn't record any cestode. Adult acanthocephalans adhere to the mucosa of the stomach or intestine. They require at least 2 hosts in the life cycle; arthropods are the usual intermediate hosts in which the infective stage develops, and when eaten by an appropriate final host, the parasite develops to maturity in the digestive tract (Nickol, 1985). Finally, nematodes are particularly abundant in the digestive tract, lungs and blood vessels of vertebrates (Pough et al., 2001). The species recorded from anurans collected in Turkey include: Aplectana brumpti, Cosmocerca ornata, Oswaldocruzia filiformis, Oxysomatium brevicaudatum, and Rhabdias bufonis, directly infect the host (Anderson, 2000). Thus, for nematodes, habitat is more important than diet in determining rates of infection. In our study, Cosmocerca ornata was found to be the most dominant species of nematodes. Amphibians are also hosts to other groups of less common parasitic invertebrates, such as annelids, pentastomids and arthropods (copepods, ticks, insects) (Tinsley, 1995). In our study, we recorded Hirudo medicinalis from 2 of 54 P. bedriagae (3.70 %). In previous studies this species was recorded from Pelophylax ridibundus (formerly known as Rana ridibunda) (Yıldırımhan et al., 1996, 2005b; Kır et al., 2001) in Turkey.

The helminth communities of P. bedriagae presented few differences in 7 localities. We observed differences in parasite species distribution between the localities studied. Cosmocerca ornata was recorded from all localities except two localities (Balçova and İnciraltı). Within these localities there were significant differences according to mean abundances (Kruskal-Wallis, p = 0.002). The highest mean abundance was observed at Seferihisar (8.00 ± 6.61). Rhabdias bufonis was recorded from four localities. This species was not observed from the other three localities (Ödemiş, Inciralti and Karagöl). There were no significant differences between mean abundances of these four localities (Kruskal-Wallis, p = 0.157). Oswaldocruzia filiformis was observed only Bornova. Seferihisar and Salihli. Significant differences were not found between mean abundances of these three localities (Kruskal-Wallis, p = 0.064). Acanthocephalus ranae was observed at 4 localities (Ödemiş, Balçova, Karagöl and Manisa). In these 4 localities species mean abundances were similar (Kruskal-Wallis, p = 0.297). Diplodiscus subclavatus was recorded from 2 localities (Ödemiş and Seferihisar districts). Between these two localities, Ödemiş had higher mean abundance than Seferihisar (Mann-Whitney U, p = 0.001). While Gorgoderina vitelliloba was recorded in Ödemis district, there was no record of this species in other 6 localities (Kruskal-Wallis, p = 0.001). Haematoloechus breviansa was recorded from Bornova, Ödemiş, İnciraltı and Karagöl. Within these four localities significant difference was not observed (Kruskal-Wallis, p = 0.630). Hirudo medicinalis was recorded only two localities (Bornova and Salihli). Mean abundances of these localities were similar (Mann-Whitney U, p =

0.793). The total richness in 4 localities was very similar (5 species in Bornova, Salihli and Ödemiş district, 4 species in Seferihisar district).

To our knowledge, there is only one study about parasites of P. bedriagae in the world. Al-Sorkhy and Amr (2003) reported Prosotocus confusus, Pleurogenoides tacapensis and Nematotaenia dispar from Jordan. We didn't record these parasite species in our study. The afore-mentioned study in Jordan was conducted in the same ecozone with the present study, but different helminth species was reported. Climatic or ecological factors may play a role in this situation. On the other hand, 2 of these parasites by Al-Sorkhy and Amr (2003) were recorded from Turkey from different host frogs. P. confusus was recorded from Pelophylax ridibundus from Edirne, Bursa, İstanbul and Antalya provinces of Turkey (Yıldırımhan et al., 1996; Yıldırımhan et al., 2005b; Düşen & Öz, 2006). N. dispar was recorded from Bufotes variabilis (formerly known as Bufo viridis), Rana camerani and Hyla savignyi from Bursa, Kayseri, Kars, Çanakkale, Amasya, Çorum, Tokat, Denizli and Hatay provinces of Turkey (Yıldırımhan, 1999; Yıldırımhan et al., 2006c; Düşen et al., 2010; Düşen & Oğuz, 2010; Düşen, 2011; Yıldırımhan et al., 2012). One of these parasites -P. tacapensis- wasn't recorded from Turkey, previously. Generalist helminths infect Turkish anurans and may vary within a particular host over time and space. However, within its population of hosts, a helminth species is persistent. We predict that as more subpopulations of these hosts are studied, parasite lists will become more similar.

The present work examines for the first time helminth parasites in non-studied host frog *P. bedriagae* from Turkey that represent an important group of vertebrates in Turkey. These parasite species were all new records for *Pelophylax bedriagae*.

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