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Helminth parasites of the eastern spadefoot toad, *Pelobates syriacus* (Pelobatidae), from Turkey

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Abstract: Ninety-one eastern spadefoot toads, *Pelobates syriacus*, were collected from 3 localities in Turkey between 1993 and 2003 and examined for helminths. One species of Monogenea (*Polystoma* sp.) and 3 species of Nematoda (*Aplectana brumpti, Oxysomatium brevicaudatum, Skrjabinelazia taurica*) were found. *Pelobates syriacus* represents a new host record for *Polystoma* sp. and *S. taurica*.

Key words: Monogenea, Nematoda, eastern spadefoot toads, Pelobates syriacus, Turkey

Türkiye'den toplanan toprak kurbağası (*Pelobates syriacus*)'nın (Pelobatidae) helmint parazitleri

Özet: 1993-2003 yılları arasında Türkiye'den 3 değişik yerden 91 toprak kurbağası helmintleri belirlenmek üzere toplanmıştır. İnceleme sonucunda 4 helmint türüne rastlanmıştır. Bunlardan biri Monogenea (*Polystoma* sp), 3'ü (*Aplectana brumpti, Oxsyomatium brevicaudatum, Skrjabinelazia taurica*) Nematoda'ya aittir. *Pelobates syriacus*, *Polystoma* sp. ve *S. taurica* için yeni konak kaydıdır.

Anahtar sözcükler: Monogen, Nematoda, toprak kurbağası, *Pelobates syriacus*, Türkiye

Introduction

The eastern spadefoot toad, *Pelobates syriacus* Boettger, 1889, a fossorial species from Israel, Syria, and Turkey to Transcaucasica, lives in self-constructed burrows in loose and soft soil at elevations up to 1600 m, except during the breeding periods. In Turkey, it is found in suitable habitats in Anatolia and Turkish Thrace (Baran and Atatür, 1986). To our knowledge, there are 2 published reports of helminths in *P. syriacus*: Schad et al. (1960) reported an occurrence of *Aplectana brumpti* and Yıldırımhan et al. (1997a) found *Oxysomatium brevicaudatum*. The purpose of this paper is to present a formal list of helminth species harbored by *P. syriacus*.

Materials and methods

Ninety -one eastern spadefoot toads, *Pelobates syriacus*, (31 female, 60 male; mean snout-vent length range [SVL] = 51-74 mm) were collected by hand

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between 1993-2003 from 3 localities in Turkey: Bursa, Osmangazi district, (40°12´N, 29°00´E; elevation 120 m; N = 25); Konya province, Seydişehir district (37°17´N, 32°04´E; elevation 1090 m; N = 42); Edirne province, Büyükdöllük Marsh (41°45´N, 26°35´E; elevation 53 m; N = 24).

The toads were examined within 1 week of capture. They were over-anaesthetized with sodium pentobarbital and the body cavity was opened by an incision from vent to throat. The abdominal cavity, stomach, intestine, heart, lungs, liver, urinary bladder, and mouth were examined separately for helminths using a dissecting microscope. Helminths were killed in hot saline solution; nematodes were fixed in 70% ethanol and mounted in glycerol; monogenans were fixed in 70% ethanol, stained with iron-carmine (per Georgiev et al., 1986), cleared in clove oil, and mounted in Entellan® for examination with a compound microscope. Identifications are based upon the reference keys of Ryzhikov et al. (1980) and Prudhoe and Bray (1982). Helminth voucher specimens were deposited in the helminth collection of Uludağ University Museum of Zoology, Bursa, Turkey; Toad specimens were deposited in the Department of Biology, Uludağ University, Bursa, Turkey. Anuran nomenclature follows Frost (2008).

Results

***Pelobates syriacus* Boettger, 1889**

Ninety-one *Pelobates syriacus* from 3 localities in Turkey were utilized for this study: Bursa, Osmangazi district (12 female, 13 male, SVL = 65.1 ± 4.3 mm, range 58-74 mm) collected in June 1993; Edirne, Büyükdöllük Marsh (3 females, 21 males, SVL = 58.7 ± 4.11 mm, range 61-73 mm) collected in May 2000; Konya, Seydişehir county (16 females, 26 males, SVL = 57.3 ± 4.26 mm, range 51-64 mm) collected in July 2000 (N = 19), August 2002 (N = 7), June 2003 (N = 16).

***Polystoma* sp.**

Prevalence, mean intensity, and range: Hosts infected, 11 of 91 (12%, 1.70 ± 1.01, 1-4).

Temporal distribution: Konya-Seydişehir county: July 2000, 5 hosts with 1, 1, 1, 2, 2; August 2002, 1 host with 2; June 2003, 5 hosts with 1, 1, 1, 3, 4 respectively;

Site of infection: Urinary bladder.

Geographic range: The genus *Polystoma* has a cosmopolitan distribution (Diengdoh and Tandon, 1991).

Specimens deposited: Uludağ University Museum of Zoology

Remarks: *P. syriacus* represents a new host record for *Polystoma* sp. Three species of *Polystoma, P. macrocnemis* Biserkov, Yildirimhan and Ugurtas, 2001 in the Uludag frog, *Rana macrocnemis, P. skrjabini* Buchvarov, 1984 in the European treefrog, *Hyla arborea,* and *P. viridis* Euzet, Combes and Batchvarov, 1974 in the European green toad, *Pseudepidalea viridis* (formerly *Bufo viridis*), have been reported from Turkey (Yıldırımhan, 1999; Biserkov et al., 2001; Düşen and Öz, 2004). Euzet et al. (1974) assumed that each species of anuran harbored a distinct species of *Polystoma. Polystoma macrocnemis* and *P. viridis* are so closely related to the type species *P. integerrimum* (Frölich, 1791) with respect to both geographical range and morphometric chartacteristics that separation is difficult (Biserkov et al., 2001). It should also be noted that the brown frogs (*Rana temporaria* group) in Turkey are recognized as 2 species, *R. camerani* and *R. macrocnemis*, by some authors, e.g., Duellman (1999) or as the same species under the name *R. macrocnemis* by other authors, e.g., Anderson (1985), Baran and Atatür (1986). Thus more work on the species of *Polystoma* in Turkey is necessary before our specimens can be properly evaluated either as a new species or a previously described one.

Gallien (1935) studied the general biology of *Polystoma integerrimum* and reported that the adults lay eggs only in the spring, which give rise to larvae which attach themselves to the gills of tadpoles and remain attached through tadpole metamorphosis at which time the larvae migrate through the digestive tract to the urinary bladder.

***Aplectana brumpti* Travassos, 1931**

(Syn: *Aplectana miranda* Ivanitzky, 1940; *Aplectana corti* Lopez-Neyru, 1947; *Aplectana ivanitzkyi* Morkov, Khonyakina, and Grivoreva 1972).

Prevalence, mean intensity, and range: Hosts infected, 25 of 91 (27%, 74.6 ± 61.66, 2-184).

Temporal distribution: Edirne-Büyükdöllük Marsh: May 2000, 10 hosts with 2, 40 , 48, 102, 115, 118, 126, 126, 132, 146, respectively; Konya-Seydişehir county: July 2000, 6 hosts with 14, 20, 21, 32, 36, 48, respectively; August 2002, 3 hosts with 8, 14, 16, respectively; June 2003, 6 hosts with 4, 33, 142, 166, 172, 184, respectively;

Site of infection: Intestine.

Additional Turkish hosts: *Pseudepidalea viridis* (reported as *Bufo viridis* Schad et al., 1960); *Pelobates syriacus*, (Schad et al., 1960).

Type host and type locality: *Pseudepidalea viridis* (reported as *Bufo viridis*), Corsica, Spain (Travassos, 1931).

Other reported hosts: Common European toad, *Bufo bufo* (Fernández et al., 1987); natterjack toad, *Epidalea calamita* (reported as *Bufo calamita* Fernández et al., 1987); marsh frog, *Pelophylax ridibundus* (reported as *Rana ridibunda* Ivanitzky, 1940); *Pseudepidalea viridis* (reported as *Bufo viridis* Travassos, 1931; Lopez-Neyra, 1947; Kozák, 1969; Kozlowska, 1960; Frandsen, 1974; Baker, 1980a); European common frog, *Rana temporaria* (Ivanitzky, 1940); grass snake, *Natrix natrix* (Sharpilo, 1976); dice snake, *Natrix tessellata* (Sharpilo, 1976).

Geographic range: Western Europe (Baker, 1980a).

Specimens deposited: Uludağ University Museum of Zoology

Remarks: This is the second report of *A. brumpi* in *P. syriacus* from Turkey. Females produce eggs that larvate in utero before release to the environment where hatching occurs; the final host becomes infected orally (Anderson, 2000).

***Oxysomatium brevicaudatum* (Zeder, 1800) Railliet and Henry, 1916**

(Syn: *Oxysoma* Schneider, 1866 (preoccupied); *Neoxysomatium* Ballesteros-Marsquez, 1945; *Fusaria brevicaudata* Zeder, 1800; *Oxysoma contortum* Linstow, 1906; *Oxysomatium longespiculum* Railliet and Henry, 1916).

Prevalence, mean intensity and range: Hosts infected, 28 of 91 (31%, 18.3 ± 10.86, 3-45).

Temporal distribution: Edirne-Büyükdöllük-Marsh: May 2000, 9 hosts with 14, 15, 22, 28, 30, 32, 35, 37, 45, respectively; Bursa-Osmangazi district May 1993, 19 hosts with 3, 6, 7, 7, 8, 8, 9, 10, 11, 12, 14, 14, 15, 16, 18, 20, 21, 24, 32, respectively;

Site of infection: Intestine.

Additional Turkish hosts: *Bufo bufo* (Yıldırımhan et al., 1997a); square-marked toad, *Amietophrynus regularis* (reported as *Bufo regularis*, Schad et al., 1960); *Pseudepidalea viridis* (reported as *Bufo viridis* Schad et al., 1960); *Pelobates syriacus* (Yıldırımhan et al., 1997a); *Rana macrocnemis* (Schad et al., 1960); *Rana ridibunda* (Schad et al., 1960; Yıldırımhan et al., 2005; Sağlam and Arıkan, 2006); *Natrix natrix* (Schad et al., 1960);

Type host and type locality: No type host, Germany

Other reported hosts: Midwife toad, *Alytes obstetricans* (Chabaud and Campana-Rouget, 1955; Baker, 1980b); *Amietophrynus regularis* (reported as *Bufo regularis* Baker, 1980b); European fire-bellied toad, *Bombina bombina* (Baker, 1980b); *Bufo bufo* (Kozlowska, 1960; Vojtkova et al., 1963; Kozak, 1969, 1973; Frandsen, 1974; Prokopic and Krivanec, 1975; Vojtkova, 1976; Baker, 1980b; Soriano et al., 1996; Galli et al., 2001; Shimalov and Shimalov, 2001; reported as *B. vulgaris*, Andre, 1912; Baylis, 1928); *Hyla arborea* (Kozlowska, 1960; Vojtkova, 1976; Baker, 1980b; Galeano et al., 1990); common spadefoot toad, *Pelobates fuscus* (Baker, 1980b); *Pelophylax ridibundus* (reported as *Rana ridibunda* Baker, 1980b; Kirin and Buchvarov, 2002; reported as *Rana esculenta* Kozlowska, 1960; Vojtkova et al., 1963; Prokopic and Krivanec, 1975; Vojtkova, 1976; Baker, 1980b); *Pseudepidalea viridis* (reported as *Bufo viridis*, Kozlowska, 1960; Prokopic and Krivanec, 1975; Vojtkova, 1976; Baker, 1980b); moor frog, *Rana arvalis* (Kozak, 1973; Prokopic and Krivanec, 1975;Vojtkova, 1976; Baker, 1980b; Cedhagen, 1988; reported as *Rana terrestris* Kozlowska, 1960); agile frog, *Rana dalmatina* (Baker, 1980b); Balkan stream frog, *Rana graeaca* (Hristovski, 1974; Baker, 1980b); Iberian frog, *Rana iberica* (Navarro et al, 1988); *Rana macronemis* (Baker, 1980b); *Rana temporaria* (Andre, 1913; Kozlowska, 1960; Vojtkova et al., 1963; Hristovski and Lees, 1973; Kozak, 1973; Prokopic and Krivanec, 1975; Vojtkova, 1976; Sattmann, 1986;

Navarro et al, 1988; Kirin and Buchvarov, 2002); smooth newt, *Lissotriton vulgaris* (reported as *Trituris vulgaris* Barus and Groschaft, 1962; Barus et al., 1963; Vojtkova, 1976; Baker, 1980b); Alpine newt, *Mesotriton alpestris* (reported as *Trituris alpestris* Kozlowska, 1960; Barus and Groschaft, 1962; Barus et al., 1963; Vojtkova, 1976; Baker, 1980b); Alpine salamander, *Salamandra atra* (Baker, 1980b); European fire salamander, *Salamandra salamandra* (Grabda and Grabda, 1953; Barus et al., 1963; Prokopic and Krivanec, 1975; Baker, 1980b; Bertman, 1986; reported as *S. maculosa* Andre, 1912; Vojtkova, 1976); Monte Albo cave salamander, *Speleomantes flavus* (Ricci, 1987); northern crested newt, *Trituris cristatus* (Barus et al., 1963; Frandsen, 1974; Vojtkova, 1976; Baker, 1980b); slow worm, *Anguis fragilis* (Zeder, 1800; Baylis, 1928; Sharpilo, 1976; Baker, 1980b; Moravec, 1963; Lewin, 1990; Shimalov et al., 2000; Borkovcova and Kopriva, 2004); sand lizard, *Lacerta agilis* (Lewin, 1992a); European legless lizard, *Pseudopus apodus* (reported as *Ophisaurus apodus* Baker, 1980b); *Natrix natrix* (Lukasiak, 1939; Baker, 1980b; Lewin, 1992b; Shimalov and Shimalov, 2000); *Natrix tessellata* (Sharpilo, 1976); northern viper, *Vipera berus* (Sharpilo, 1976; Shimalov and Shimalov, 2000).

Geographic range: Western Europe (Baker, 1987).

Specimens deposited: Uludağ University Museum of Zoology

Remarks: This is the second report of *O. brevicaudatum* in *P. syriacus*. The life history of *O. brevicaudatum* has apparently not been studied. It is assigned to the subfamily Cosmocercinae in which 2 pathways for infection are known, namely infection orally or skin penetration (Anderson, 2000).

***Skrjabinelazia taurica* Sypliaxov, 1930**

(Syn: *Salobrella* Freitas, 1940).

Prevalence, mean intensity, and range: Hosts infected, 1 of 91 (1%, 3).

Temporal distribution: Edirne-Büyükdöllük Marsh: May 2000, 1 host with 3 respectively;

Site of infection: Intestine.

Type host and type locality: Crimean wall lizard, *Podarcis tauricus* (reported as *Lacerta taurica*), Crimea, USSR (Sypliaxov, 1930).

Additional Turkish hosts: *Podarcis tauricus* (reported as *Lacerta taurica* Schad et al., 1960); European green lizard, *Lacerta viridis* (Schad et al., 1960).

Other reported hosts: *Podarcis tauricus* (reported as *Lacerta taurica* Sharpilo, 1976).

Geographic range: Turkey, Ukraine (Baker, 1987).

Specimens deposited: Uludag University Museum of Zoology

Remarks: Species of *Skrjabinelazia* have previously been reported only from lizards belonging to the families Gekkonidae or Lacertidae (Lhermitte, 2008). In some species, females produce 2 types of eggs, namely membranous eggs in which larvae develop and hatch in utero, and thick-shelled eggs, which are released (Lhermitte, 2008). Given the number of *S. taurica* found in this study, the infection reported here may represent an incidental infection acquired through some nontypical behavior of the host. However, *P. syriacus* would represent a new host record for *S. taurica*.

Discussion

In all, 2400 helminths were collected from 50 (55%) of the 91 toads examined. Four helminth species were found: 1 monogenean (N = 19) and 3 nematodes (N = 2381). No individual host contained more than 3 species of helminths; of the infected toads: 36 (72%) harbored 1 species of helminth, 13 (26%) harbored 2 species, and 1 (2%) harbored 3 species. The collection sites yielded different communities of helminths: toads collected in Bursa harbored only *O. brevicaudatum*; toads from Edirne harbored *A. brumpti, O. brevicaudatum,* and *S. taurica*; toads from Konya harbored *Polystoma* sp. and *A. brumpti*. The reasons for the lack of a helminth species at a particular location are currently unknown.

There were 1.30 ± 0.07 (X ± 1 SE) (range 1-3) helminth species per infected toad and 48.00 ± 8.72 (range 1-184) helminth individuals per infected toad. Aho (1990) compiled distributional patterns for anurans in general and reported the mean (± SE) total number of helminth species per host species as 3.54 ± 0.24 (range 0-9). Thus the infection rates for *P. syriacus* are lower than these seen for anurans in

Table. Reported helminths of Turkish anurans.

	Bombina bombina	*Bufo bufo*	*Pseudepidalea viridis*	*Hyla arborea*	*Pelobates syriacus*	*Pelodytes caucasius*	*Rana camerani*	*Rana holtzi*	*Rana macrocnemis*	*Pelophylax ridibundus*
Monogenea										
Polystoma sp.	–	–	–	–	6	–	8	–	10	–
Polystoma macrocnemis	–	–	–	–	–	–	–	–	9,11	–
Polystoma skrjabini	–	–	–	5	–	–	–	–	–	–
Polystoma viridis	–	–	4	–	–	–	–	–	–	–
Digenea										
Bucephalus polymorphis	–	–	–	–	–	–	–	–	–	16
Candidotrema loossi	–	–	–	–	–	–	–	–	–	13
Diplodiscus sp.	–	–	–	–	–	–	–	–	–	15
Diplodiscus subclavatus	–	–	–	–	–	–	–	–	–	12,13,14,16,18
Gorgodera sp.	–	–	–	–	–	–	–	–	–	15
Gorgodera cygnoides	–	–	–	–	–	7	8	–	–	13,14,18
Gorgoderina sp.	–	–	–	–	–	–	–	–	–	15
Gorgoderina vitelliloba	–	–	–	–	–	–	8	9	9,10	13,14,17,18
Haematoloechus breviansa	–	–	–	–	–	–	–	–	–	13,14,18
Haematoloechus variegatus	–	–	–	–	–	–	–	–	–	13,17
Haplometra cylindracea	–	–	–	–	–	–	8,19	9	9,19	–
Opisthioglyphe ranae	1	–	–	–	–	–	–	–	–	13,14,18
Opisthioglyphe rastellus	–	–	–	–	–	–	8,19	9	9	–
Plagiorchis sp.	–	–	–	–	–	–	–	–	–	15
Pleurogenes claviger	–	–	–	–	–	–	–	–	9,10	12, 16
Pleurogenoides medians	–	–	–	5	–	–	8,19	–	9	12,13,14,17,18
Pleurogenoides stromi	–	–	–	–	–	–	–	–	–	14
Prosotocus confusus	–	–	–	–	–	–	–	–	–	13,14,18
Brachycoelium salamandrae	–	–	–	–	–	–	–	–	–	18
Rauschiella sp.	–	–	–	–	–	–	–	–	–	13
Encyclometra colubrimurorum (metacercariae)	–	–	–	5	–	–	–	–	–	18
Codonocephalus urnigerus (metacercariae)	–	–	–	–	–	–	–	–	–	13,14,18
Cestoda										
Nematotaenia dispar	–	–	4	–	–	–	8	–	–	–
Proteocephalus sp. (juvenile)	–	–	4	5	–	–	–	–	–	–
Nematoda										
Agfa tauricus	–	–	–	–	–	7	–	–	–	–
Aplectana brumpti	–	–	3	–	3,6	7	–	–	–	–
Cosmocerca sp.	1	–	–	–	–	–	–	–	10	2,13,15
Cosmocerca ornata	–	–	3	–	–	7	8,19	9	3,9,19	3,14
Cosmocerca commutata	–	–	3,4	5	–	–	–	–	–	18
Cosmocercoides sp.	–	–	4	–	–	–	–	–	–	17
Oswaldocruzia sp.	–	2	3	–	–	–	–	–	–	13
Oswaldocruzia filiformis	–	–	4	–	–	7	8	–	3,9,10	14,17
Neoxysomatium sp.	–	–	–	–	–	–	–	–	–	18
Oxysomatium brevicaudatum	–	2	3,4	–	2,6	–	–	–	3	3,14,17
Rhabdias sp.	–	–	3	–	–	–	–	–	3	–
Rhabdias bufonis	1	2	4	–	–	7	8	–	9	2,13,16,17,18
Skrjabinelazia taurica	–	–	–	–	6	–	–	–	–	–
Eustrongylides sp.	–	–	–	–	–	–	–	–	–	18
Eustrongylides excisus (larvae)	–	–	–	–	–	–	–	–	–	14,17
Abbreviata sp.	–	–	–	–	–	–	–	–	–	18
Acanthocephala										
Acantocephalus sp.	–	–	–	–	–	–	–	–	–	15
Acantocephalus ranae	1	–	4	5	–	–	8	–	9,10,19	2,13,14,16,17,18
Centrorhynchus sp.	–	–	–	–	–	–	–	–	–	14
Pseudoacanthocephala caucasicus	–	–	–	–	–	7	–	–	–	–

1. Yıldırımhan et al., 2001; 2. Yıldırımhan, Oğuz and Uğurtaş, 1997a; 3. Schad, 1960; 4. Yıldırımhan, 1999; 5. Düşen and Öz, 2004; 6. this study; 7. Yildirimhan, 2009; 8. Yildirimhan et al, 2006a; 9. Yildirimhan et al, 2006b; 10. Yıldırımhan, Uğurtaş and Altunel, 1997b; 11. Biserkov et al. 2001; 12. Oğuz et al., 1994; 13. Yıldırımhan et al., 1996; 14. Yıldırımhan et al., 2005; 15. Saygı and Başıbüyük, 1990; 16. Kır et al., 2001; 17. Sağlam and Arıkan, 2006;18. Düşen, 2006; 19. Düşen, 2007.

general. Whether the more terrestrial habitat of *P. syriacus* is responsible for this difference will require additional work.

Fourteen species of anurans occur in Turkey (Bombinatoridae: *Bombina bombina* (Linnaeus, 1758); Bufonidae: *Bufo bufo* (Linnaeus, 1758), *Pseudepidalea viridis* (Laurenti, 1768) formerly *Bufo viridis* (Laurenti, 1768); Hylidae: *Hyla arborea* (Linnaeus, 1758), *Hyla savignyi* (Audoin, 1827); Pelobatidae: *Pelobates fuscus* (Laurenti, 1768), *P. syriacus* (Boettger, 1889): Pelodytidae; *Pelodytes caucasicus* (Boulenger, 1896): Ranidae: *Rana camerani* Boulenger, 1886, *R. dalmatina* Bonaparte, 1840, *R. holtzi* F. Werner, 1898, *R. macrocnemis* Boulenger, 1885, *Pelophylax bedriagae* (Camerano, 1882) previously *Rana levantina* Schneider and Sinsch, 1992, *P. ridibundus* (Pallas, 1771) (Baran and Atatür 1998). To our knowledge, Turkish population of *Hyla savignyi, Pelobates fuscus*, *Pelophylax bedriagae,* and *Rana dalmatina* have not been examined for helminths.

The Table presents reported helminths from Turkish anurans. It is of interest to note that the nematodes commonly infecting Turkish anurans, namely, *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. Acanthocephalans require at least 2 hosts in the life cycle; arthropods are the usual intermediate hosts in which the infect stage develops and when eaten by an appropriate final host develops to maturity in the digestive tract (Nickol, 1985). Species of *Polystoma* infect directly (Gallien, 1935). The digeneans generally utilize a molluscan first intermediate host from which cercariae leave and penetrate a frog host directly or a variety of invertebrate hosts, which are then eaten by the final host (Smyth and Smyth, 1980). Presence of the helminths in *P. syriacus* in some areas but not others is most likely the result of patchy distribution on the part of a particular helminth species as well as the discontinuous distribution patterns of the host. The conclusion that we draw is that generalist helminths may vary within a particular host over time and space, but within its population of hosts, the helminth species is persistent.

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