



Helminth Fauna of the Yellow-bellied Toad *Bombina variegata* (Linnaeus, 1758) (Anura: Bombinatoridae) from North-western Türkiye

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Abstract: A total of 45 individuals (18 females, 20 males and 7 juveniles) of the yellow-bellied toad *Bombina variegata* were collected from Karacahasan Mountain region (Enez District, Edirne Province, NW Türkiye) in 2015–2016 and examined for helminths. Two species of Digenea (*Gorgodera cygnoides* and *Pleurogenoides medians*) and one species of Acanthocephala (*Acanthocephalus ranae*) were found. This study is the first helminthological report on *B. variegata* from Türkiye.

Key words: Toad, Helminth, Parasite, Digenea, Acanthocephala

Introduction

The yellow-bellied toad *Bombina variegata* (L., 1758) was firstly reported in Türkiye by Bülbül et al. (2016) and genetically confirmed by Karış et al. (2017). The species has a wide distribution area throughout Europe (excluding the Iberian Peninsula and Peninsular Italy south of the Po River), east to southern Poland, southwestern Ukraine, Bulgaria, Greece and European Türkiye. It has been introduced into England (Frost 2023). In Türkiye, the species is generally found in broad-leaved tree habitats (*Quercus ilex*, *Fraxinus* sp., *Tilia* sp., *Paliurus aculeatus* and *Rob-*

inia pseudoacacia) containing small breeding ponds and rocky, sandy substrate at elevation of 100-200 m. It is common in the Karacahasan Mountain region of Enez District (Edirne Province) in the northwestern part of Türkiye (Bülbül et al. 2018).

Sixteen anuran species have been recorded from Türkiye (Frost 2023), and with the exceptions of *Bombina variegata* and *Bufo verrucosissimus*, all have been examined helminthologically. Early studies of Turkish populations were published as a checklist by Yıldırımhan & Birlik (2013). Studies conducted after that publication can be found in the publications by Demir et al. (2015), Karakaş (2015),

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Koyun et al. (2015), Yakar et al. (2016), Yıldırımhan et al. (2016) and Tepe & Yılan (2021).

In this report, we provide the first helminthological information on a Turkish population of *B. variegata*.

Materials and Methods

With the special permission (No. 2014–51946) provided by the Ministry of Forestry and Water Affairs (Directorate of Nature Conservation and National Parks) of the Republic of Türkiye, 45 individuals of yellow-bellied toad (18 females, 20 males and 7 juveniles) were collected from the Karacahasan Mountain region, Enez District, Edirne Province, in 2015–2016. The authors received ethical permission from Ege University Animal Experiments Ethic Committee (No. 2014-002). The toads were captured by hand and taken to the Bursa Uludağ University, Faculty of Arts and Sciences, Department of Biology, Zoology Laboratory of Parasitology. Standard post-mortem examination procedures were followed (Garner 2006). During dissection, the abdominal cavity, stomach, intestine, heart, lungs, liver, urinary bladder and mouthparts of the toads were examined separately for helminths. Helminths, when found, were stored in 70% alcohol until identification using the acetocarmine staining technique and studied under both stereo- and light-microscopy. Procedures of Yamaguti (1963) and Yamaguti (1971) were used for helminth identification.

Results

Forty-five toads were captured in the Edirne Province and were examined. Out of them, 39 (87%) were found to harbour helminth parasites. A total of 282 helminth specimens were recorded, identified and assigned to three species.

Two species of Digenea were found. *Gorgodera cygnoides* (n = 39) was isolated from the urinary bladders. Another digenean species, *Pleurogenoides medians* (n = 138), as well as one acanthocephalan species (*Acanthocephalus ranae*, n = 105), were found in the intestines. Six toads harboured no helminths, 33 harboured one species, six harboured two species and one toad harboured three species. On average, there were 6.26 helminth individuals per infected toad (range 1–40).

Digenea

Gorgodera cygnoides (Zeder, 1800) Looss, 1899

Syn. *Distoma cynoides* Zeder, 1800; *Distomum cygnoides* Looss, 1894; *Gorgodera loossi* Sinitzin, 1905.

Prevalence, mean intensity and range: 11/45 (24.4%); 3.5; 0–10.

Temporal distribution: Karacahasan Mountain, Edirne Province, 2015, 11 hosts.

Site of infection: Bladder.

Type host and type locality: *Rana esculenta*, Europe (Zeder 1800).

Other Türkiye records: *Pelodytes caucasicus* (Yıldırımhan et al. 2009); *Pelophylax ridibundus* (Yıldırımhan et al. 1996, Yıldırımhan et al. 2005, Dusen et al. 2010, Dusen & Oz 2013); *Rana macronemis* identified as *Rana camerani* (Yıldırımhan et al. 2006); *Lacerta trilineata* (Yıldırımhan et al. 2011).

Other reported hosts:

Bombina bombina (Kopriva 1957, Rittichova 1958, Vojtkova & Vojtek 1975, Ryzhikov et al. 1980);

Bombina variegata (Prokopic 1957, Rittichova 1958, Kozak 1966, 1969, 1973, Vojtkova & Vojtek 1975, Buchvarov 1977);

Bufo bufo (André 1912 [identified as *Bufo vulgaris*]; Buchvarov 1962 [reported as *Gorgodera loossi*]);

Bufo viridis identified as *Bufo viridis* (Buchvarov 1977, Ryzhikov et al. 1980, Shimalov & Shimalov 2001);

Hyla arborea (Ryzhikov et al. 1980);

Pelodytes esculenta (André 1913 [identified as *Rana esculenta*]; Bovien 1916 [identified as *Rana esculenta*]; Kopriva 1957 [identified as *Rana esculenta*]; Odening 1957 [identified as *Rana esculenta*]; Prokopic 1957 [identified as *Rana esculenta*]; Rittichova 1958 [identified as *Rana esculenta*]; Vojtkova 1961 [identified as *Rana esculenta*]; Vojtkova et al. 1963 [identified as *Rana esculenta*]; Volna–Nabelkova 1964 [identified as *Rana esculenta*]; Kozak 1966, 1968, 1969, 1973 [identified as *Rana esculenta*]; Cox 1971 [identified as *Rana esculenta*]; Kozak 1968 [identified as *Rana esculenta*]; Frandsen 1974 [identified as *Rana esculenta*]; Prokopic & Krivanec 1975 [identified as *Rana esculenta*]; Vojtkova and Vojtek 1975 [identified as *Rana esculenta*]; Buchvarov 1977 [identified as *Rana esculenta*]; Ryzhikov et al. 1980 [identified as *Rana esculenta*]; Kuc & Sulgostowska 1988b [identified as *Rana esculenta*]; Popovic & Mikes 1988 [identified as *Rana esculenta*]; Popiolek et al. 2011 [reported as hybrid *Pelophylax ridibundus* x *Pelophylax lessonae*];

Pelophylax lessonae (Kopriva 1957 [identified as *Rana lessonae*]; Vojtkova and Vojtek 1975 [identified as *Rana lessonae*]; Popiolek et al. 2011) [identified as *Rana lessonae*];

Pelophylax ridibundus (Cox 1971 [identified as *Rana ridibunda*]; Buchvarov 1962, 1965, 1977,

1983 [identified as *Rana ridibunda*]; Bondy 1967 [identified as *Rana ridibunda*]; Kozak 1969, 1973 [identified as *Rana ridibunda*]; Ryzhikov et al. 1980 [identified as *Rana ridibunda*]; Kuc and Sulgostowska 1988b [identified as *Rana ridibunda*]; Kirin & Buchvarov 2002 [identified as *Rana ridibunda*]; Popiolek et al. 2011 [identified as *Rana ridibunda*];

Pseudepidalea viridis (Buchvarov, 1977 [identified as *Bufo viridis*]; Ryzhikov et al. 1980 [identified as *Bufo viridis*]; Shimalov and Shimalov 2001 [identified as *Bufo viridis*];

Rana arvalis (Rittichova 1958; Kozak 1969, 1973; Vojtkova and Votjek 1975; Ryzhikov et al. 1980);

Rana camerani (Tepe & Yılan 2021);

Rana dalmatina (Buchvarov 1983; Yıldırımhan et al. 2016);

Rana graeca (Buchvarov 1983);

Rana temporaria (John 1875; André 1913; Freund 1933; Kopriva 1957; Rittichova 1958; Kozak 1966, 1967; 1969, 1973; Cox 1971; Hristovski and Lees 1973; Vojtkova and Votjek 1975; Ryzhikov et al. 1980).

***Pleurogenoides medians* (Olsson, 1876) Travassos, 1921**

Syn. *Distomum medians* Olsson, 1876; *Pleurogenes medians* (Olsson, 1876)

Prevalence, mean intensity and range: 8/45 (17.7%); 17.25; 0–40.

Temporal distribution: Karacahasan Mountain, Edirne Province, 2015, 8 hosts.

Site of infection: Intestine.

Type host and type locality: *Bufo viridis*, Denmark (Olsson 1893).

Other Türkiye records: *Hyla arborea* (Duşen & Öz 2004); *Hyla savignyi* (Yıldırımhan et al. 2012); *Pelophylax ridibundus* (Yıldırımhan et al. 1996; Yıldırımhan et al. 2005; Sağlam & Arıkan 2006; Dusen et al. 2010); *Rana dalmatina* (Düşen et al. 2009); *Rana macrocnemis* (Dusen 2007 [identified as *Rana camerani*]; Yıldırımhan et al. 2006 [identified as *Rana camerani*]).

Other reported hosts:

Bombina bombina (Vojtkova 1961, Vojtkova et al. 1963, Kozak 1973, Vojtkova & Votjek 1975);

Bombina variegata (Vojtkova & Votjek 1975, Buchvarov 1983); Cox 1971, Kozak 1973, Vojtkova & Votjek 1975, Ryzhikov et al. 1980, Fernandez et al. 1986, Shimalov & Shimalov 2001);

Epidalea calamita (Vojtkova & Votjek 1975 [identified as *Bufo calamita*]);

B. viridis (Vojtkova & Votjek 1975, Ryzhikov et al. 1980, Düşen et al. 2010, Düşen & Oğuz 2010);

Hyla arborea (Kozak 1969, 1973, Vojtkova & Votjek 1975, Ryzhikov et al. 1980);

Pelobates fuscus (Ryzhikov et al. 1980);

Pelophylax esculentus (André 1913 [identified as *Rana esculenta*]; Bovien 1916 [identified as *Rana esculenta*]; Kopriva 1957 [identified as *Rana esculenta*]; Odening 1957 [identified as *Rana esculenta*]; Vojtkova 1961 [identified as *Rana esculenta*]; Vojtkova et al. 1963 [identified as *Rana esculenta*]; Volna–Naelkova 1964 [identified as *Rana esculenta*]; Kozak 1966, 1968, 1969, 1973 [identified as *Rana esculenta*]; Combes et al. 1973 [identified as *Rana esculenta*]; Prokopic and Krivanec 1975 [identified as *Rana esculenta*]; Vojtkova & Votjek 1975 [identified as *Rana esculenta*]; Ryzhikov et al. 1980 [identified as *Rana esculenta*]; Kuc & Sulgostowska 1988b [identified as *Rana esculenta*]; Popovic & Mikes 1989 [identified as *Rana esculenta*]; Bjelic–Cabrillo et al. 2009 [identified as hybrid *Pelophylax lessonae* x *Pelophylax ridibundus*];

Pelophylax ridibundus (Combes and Gerbeaux 1970 [identified as *Rana ridibunda*]; Buchvarov 1962, 1977, 1983 [identified as *Rana ridibunda*]; Kozak 1973 [identified as *Rana ridibunda*]; Ryzhikov et al. 1980; Kuc and Sulgostowska 1988b [identified as *Rana ridibunda*]; Popovic & Mikes 1989 [identified as *Rana ridibunda*];

Rana amurensis (Ryzhikov et al. 1980);

Rana arvalis (Volna–Nabelkova 1964; Kozak 1973; Vojtkova & Votjek 1975; Ryzhikov et al. 1980);

Rana dalmatina (Kozak 1973; Prokopic & Krivanec 1975; Buchvarov 1977);

Rana graeca (Buchvarov 1977, 1983);

Rana lessonae (Vojtkova & Votjek 1975);

Rana ridibunda (Buchvarov 1962, 1977, 1983; Combes & Gerbeaux 1970; Cox 1971; Kozak 1973; Ryzhikov et al. 1980; Kuc & Sulgostowska 1988b, Sanchis et al. 2000; Yıldırımhan et al. 1996; Yıldırımhan et al. 2005; Düşen & Öz 2006; Sağlam & Arıkan 2006; Düşen et al. 2010; Düşen & Öz 2013; Koyun et al. 2015; Yıldırımhan et al. 2016; Abdel–Gaber et al. 2017; Kuzmin et al. 2020);

Rana temporaria (Bovien 1916; Kopriva 1957; Vojtkova & Krivanec 1970; Prokopic & Krivanec 1975; Vojtkova & Votjek 1975; Ryzhikov et al. 1980; Cedhagen 1988);

Lissotriton vulgaris (Vojtkova 1963 [identified as *Triturus vulgaris*]; Vojtkova & Votjek 1963, 1972 [identified as *Triturus vulgaris*]; Ryzhikov et al. 1980);

Triturus cristatus (Vojtkova 1963; Vojtkova & Votjek 1972);

Lacerta agilis (Lewin 1992a; Sharpilo et al. 2001);

Podarcis hispanica (Roca and Lluch 1988; Roca et al. 1989).

Acanthocephala

Acanthocephalus ranae (Schrank, 1788) Lühe, 1911

Syn. *Echinorhynchus ranae* Schrank, 1788; *Echinorhynchus haeruca* Rudolphi, 1808.

Prevalence, mean intensity and range: 26/45 (57.7%); 4.03; 0–11.

Temporal distribution: Karacahasan Mountain, Edirne Province, 2015, 27 hosts.

Site of infection: Intestine

Type host and type locality: *Rana esculenta*, Europe (Schrank 1788)

Other Türkiye records: *Bombina bombina* (Yıldırımhan et al. 2001); *Bufo bufo* (Yıldırımhan & Karadeniz 2007, Dusen 2011, Heckmann et al. 2011); *Bufo viridis* (Yıldırımhan 1999 [identified as *Bufo viridis*]); *Hyla arborea* (Düşen & Öz 2004, Heckmann et al. 2011); *Pelophylax ridibundus* (Yıldırımhan et al. 2005 [identified as *Rana ridibunda*]); Yıldırımhan et al. 2005 [identified as *Rana ridibunda*]; Düzen & Oz 2006 [identified as *Rana ridibunda*]; Heckman et al. 2011 [identified as *Rana ridibunda*]; Heckmann et al. 2011; Yıldırımhan et al. 2016); *Rana macrocnemis* (Yıldırımhan et al. 1997; Yıldırımhan et al. 2006 [identified as *Rana camerani*], Düşen 2007; Heckmann et al. 2011); *Rana tavasensis* (Heckmann et al. 2011; Dusen 2012); *Mertensiella caucasica* (Yıldırımhan et al. 2005).

Reported hosts: *Bombina bombina* (Volna–Nabelkova 1964; Frandsen 1974; Amin et al. 2023);

Bombina variegata (Volna–Nabelkova 1964; Prokopic & Krivanec 1975);

Bufo bufo (Goto & Hewer 1949; Cox 1971; Frandsen 1974; Prokopic & Krivanec 1975); Bozkov 1980 [identified as *Bufo viridis*]; Shimalov & Shimalov 2001 [identified as *Bufo viridis*]; Karakaş 2015);

Epidalea calamita (Shimalov & Shimalov 2001 [identified as *Bufo calamita*]);

Pelophylax esculentus (André 1913 [identified as *Rana esculenta*]; Vojtkova et al. 1963 [identified as *Rana esculenta*]; Kozak 1968 [identified as *Rana esculenta*]; Vojtkova & Krivanec 1970 [identified as *Rana esculenta*]; Cox 1971 [identified as *Rana esculenta*]; Frandsen 1974 [identified as *Rana esculenta*]; Prokopic & Krivanec 1975 [identified as *Rana esculenta*]; Kuc & Sulgostowska 1988b [identified as *Rana esculenta*]; Bjelic–Cabrilo et al. 2009; Popiolek et al. 2011 [identified as *Rana esculenta*]);

Pelophylax lessonae (Popiolek et al. 2011 [identified as *Rana lessonae*]);

Pelophylax ridibundus (Ivanitzky 1940 [identified as *Rana ridibunda*]; Buchvarov 1962, 1965 [identified as *Rana ridibunda*]; Bondy 1967 [identified as *Rana ridibunda*]; Buchvarov et al. 1975 [identified as *Rana ridibunda*]; Bozkov 1980 [identified as *Rana ridibunda*]; Kuc & Sulgostowska 1988a [identified as *Rana ridibunda*]; Düşen & Öz 2013; Koyun et al. 2015; Karakaş 2015; Kuzmin et al. 2020; Tepe & Yılan 2021; Jacob 2021; Amin et al. 2023);

Rana arvalis (Frandsen 1974; Prokopic & Krivanec 1975; Cedhagen 1988; Kuc & Sulgostowska 1988b; Amin et al. 2023);

Rana dalmatina (Frandsen 1974);

Rana macrocnemis (Tepe & Yılan 2021);

Rana temporaria (André 1913; Goto & Hewer 1949; Lees 1962; Bondy 1967; Cox 1971; Hristovski & Lees 1973; Frandsen 1974; Prokopic & Krivanec 1975; Cedhagen 1988; Kuc & Sulgostowska 1988b);

Lissotriton vulgaris (Taranko–Tulecka 1959 [identified as *Triturus vulgaris*]; Cox 1971 [identified as *Triturus vulgaris*]; Frandsen 1974; [identified as *Triturus vulgaris*]);

Triturus cristatus (Cox 1971);

Anguis fragilis (Shimalov et al. 2000);

Natrix natrix (Bertman 1993; Shimalov & Shimalov 2000);

Rana tavasensis (Heckmann et al. 2011; Düşen 2012);

Pelophylax bedriagae (Yıldırımhan et al. 2015);

Hyla orientalis (Yakar et al. 2016);

Caranx ignobilis (Sakthivel & Gopalakrishnan 2021);

Seriolina nigrofasciata (Sakthivel and Gopalakrishnan 2021);

Dayseiaena albida (Sakthivel & Gopalakrishnan 2021; Amin et al. 2023);

Pelophylax esculentus (Amin et al. 2023; Bjelic–Cabrilo et al. 2009; Kuzmin et al. 2020; Herczeg et al. 2016);

Rhinella icterica (Pilati et al. 2013);

Lutra lutra (Rolbiecki & Izdebska 2014).

Discussion

To our knowledge, 27 helminth species have been identified in *B. variegata* collected in Austria, Bulgaria, former Czechoslovakia, Denmark, Greece, Poland and former Yugoslavia. These are *Brandesia turgida* (Prokopic & Krivanec 1975); *Cephalogonimus retusus* (Buchvarov 1962); *Diplodiscus subclavatus* (Prokopic 1957; Volna–Babelkova

1964; Kozak 1966, 1969, 1973; Prokopic & Krivanec 1975; Grabda–Kazubaska and Lewin 1989); *Euryhalmis squamula* (Vojtkova and Vojtek 1975); *Gorgoderina cygnoides* (Prokopic 1957; Rittichova 1958; Kozak 1966, 1969, 1973; Vojtkova & Vojtek 1975; Buchvarov 1977;); *Gorgoderina alobata* (Lees & Mitchell 1966; Vojtkova & Krivanec 1970; Kozak 1973; Prokopic & Krivanec 1975; Grabda–Kazubaska & Lewin 1989); *Gorgoderina vitelliloba* (Vojtkova et al. 1963; Volna–Nabelkova 1964); *Haematoloechus abbreviatus* (Kozak 1966, 1973; Vojtkova & Krivanec 1970; Grabda–Kazubaska & Lewin 1989); *Haematoloechus asper* (Prokopic & Krivanec 1975); *Haematoloechus schulzei* (Buchvarov 1965); *Haematoloechus similis* (Buchvarov 1962); *Haematoloechus variegatus* (Rittichova 1958; Volna–Nabelkova 1964; Buchvarov 1965); *Halipegus ovocaudatus* (Vojtkova & Vojtek 1975); *Haplometra cylindracea* (Vojtkova & Vojtek 1975); (Vojtkova & Vojtek 1975); *Lepiophallus nigrovenosus* (Vojtkova & Vojtek 1975); *Opisthioglyphe ranae* (Prokopic 1957; Kozak 1973; Prokopic & Krivanec 1975); *Opisthioglyphe rastellus* (Prokopic 1957, Vojtkova et al. 1963; Vojtkova & Krivanec 1970; Kozak 1973; Prokopic & Krivanec 1975; Grabda–Kazubaska & Lewin 1989; Sattmann 1990); *Plagiorchis elegans* (Prokopic & Krivanec 1975); *Pleurogenoides medians* (Vojtkova & Vojtek 1975; Buchvarov 1983); *Prosotocus confusus* (Prokopic & Krivanec 1975); *Nematotaenia dispar* (Prokopic 1957; Buchvarov 1963; Prokopic & Krivanec 1975; Hristovski & Riggio 1971; Ryzhikov et al. 1980); *Cosmocerca ornata* (Prokopic 1957; Kozłowska 1960; Buchvarov 1962, 1965; Volna–Nabelkova 1964; Kozak 1969, 1973; Hristovski 1973; Moravec & Vojtkova 1974; Vojtkova 1976; Grabda–Kazubaska & Lewin 1989; Sattmann 1990); *Hedruris androphora* (Vojtkova 1976); *Oswaldocruzia filiformis* (Prokopic 1957; Buchvarov 1962; Volna–Nabelkova 1964; Kozak 1969, 1973; Prokopic & Krivanec 1975; Vojtkova 1976; Rozman 1976; Grabda–Kazubaska & Lewin 1989; Kirin & Buchvarov 2002); *Oswaldocruzia ukrainae* (Vojtkova 1976); *Rhabdias bufonis* (Prokopic 1957; Buchvarov 1965); *Nematotaenia dispar* (Prokopic 1957; Buchvarov 1963; Hristovski and Riggio 1971; Prokopic & Krivanec 1975; Ryzhikov et al. 1980); *Acanthocephalus ranae* (Volna–Nabelkova 1964; Grabda–Kazubaska & Lewin 1989).

In comparison with other countries, the number of helminth parasites in *B. variegata* collected in Türkiye (n = 3) is rather low: Austria (1), Bulgaria (9), former Czechoslovakia, (22), Denmark (1), Greece, (2), Poland (7) and former Yugoslavia (2).

However, this may be a result of the number of published reports. Further study of the Turkish population of *B. variegata* should expand the parasite list.

Notably, a recent study (Dufresnes et al. 2025) has shown that the populations of *Bombina variegata* in southeastern Bulgaria, northeastern Greece and Türkiye constitute a distinct subspecies, *Bombina variegata rhodopensis* Lukanov, Denoël, Jablonski & Dufresnes, 2025. The present report represents the first documented study on helminth parasites from this newly-recognised subspecies. Perhaps some of the studies from Bulgaria (Buchvarov 1962, 1963, Buchvarov et al. 1975) were also related to this subspecies but the localities are not clearly described in the publications. Consequently, the current findings provide the first verified helminthological data for the newly-recognised subspecies *B. v. rhodopensis*.

Ethical Approval: All applicable international, national, and/or institutional guidelines for animal testing, animal care, and the use of toads were followed by the authors. Ethical approval for animal experimentation is provided in the Materials and methods section.

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