

Research Note

 Helminths of the Eurasian marsh frog, *Pelophylax ridibundus* (Pallas, 1771)
 (Anura: Ranidae), from the Shiraz region, southwestern Iran

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Summary

Fourty seven specimens of *Pelophylax ridibundus* were collected in the vicinity of Shiraz, Fars Province, Iran in 1972. Fourteen helminth species were found, eight digeneans (*Diplodiscus subclavatus*, *Halipegus alhaussaini*, *Haematoloechus similis*, *Codonocephalus urniger*, and four species of metacercariae) and 6 nematodes (*Cosmocerca ornata*, *Rhabdias bufonis*, *Abbreviata* sp., *Eustrongylides* sp., Onchocercidae gen. sp. and one species of larval nematodes). Of these, only six are adults, while 8 are in their larval stage. The most prevalent helminths were the metacercariae of *Codonocephalus urniger* (61.7%) and the larvae *Abbreviata* sp. (55.32%). The adults with the highest prevalence are the digenean *Halipegus alhaussaini*, and the nematode *Cosmocerca ornata* (34% in both cases).

Keywords: Amphibians; Platyhelminthes; Nematoda; Parasites

Introduction

Helminths of Iranian amphibians have been scarcely studied. Of the 14 recognized anuran species inhabiting this country (Safaei-Mahroo *et al.*, 2015), only 5 have been examined for helminths: *Bufotes variabilis* Pallas, 1769 (recorded as *Bufo viridis* Laurenti, 1768), *Hyla savignyi* Audouin, 1827 (recorded as *Hyla arborea savignyi*), *Pelophylax ridibundus* (Pallas, 1771), *Rana macrocnemis* Boulenger, 1885 (reported as *Rana camerani* Boulenger, 1886) and *R. pseudodalmatina* Eiselt and Schmidtler, 1971 (reported as *R. macrocnemis pseudodalmatina*) (Combes & Knoepffler, 1972; Massoud & Farahnak, 1994; Mashaii, 1999, 2005; Mashaii *et al.*, 2000, 2008; Rakhshandehroo *et al.*, 2017) (Table 1). The marsh frog *Pelophylax ridibundus* is considered to be widely distributed in Iran, and it has been the most extensively studied species from the helminthological perspective (Combes & Knoepffler, 1972; Mashaii, 1999, 2005; Mashaii *et al.*, 2000, 2008) (Table 1), nev-

ertheless, the specific identity of the marsh frogs in Iran has been recently questioned based on molecular evidence (Pesarakloo *et al.*, 2017). The goal of this study is to contribute to the knowledge of the helminth fauna of *Pelophylax ridibundus* of Iran.

Materials and Methods

Unidentified helminths recovered from 47 specimens of *Pelophylax ridibundus* (Pallas, 1771) (but see Pesarakloo *et al.*, 2017) collected in the vicinity of Shiraz, Fars Province, Iran in 1972, were borrowed from the Canadian Museum of Nature Parasites Collection in 2016 by the author. Platyhelminthes were stained with Mayer's paracarmine or Gomori's trichrome, dehydrated, cleared in methyl salicylate, and mounted in Canada balsam. Some specimens were permanently mounted between cover slips and held in Cobb slides. Nematodes were cleared with Amann's lactophenol and mounted in temporary slides for microscopical study. For

Table 1. Helminth record of amphibians of Iran. A = adult; L = larvae; bc = body cavity; i = intestine; k = kidneys; li = liver; lu = lungs; me = mesenteries; mo = mouth cavity floor; mu = muscle; o = ovary; r = rectum; t = testes; u = urinary bladder. 1 = Combes and Knoepffler, 1972; 2 = Massoud and Farahnak, 1994; 3 = Mashaii, 1999; 4 = Mashaii *et al.*, 2000; 5 = Mashaii, 2005; 6 = Mashaii *et al.*, 2008; 7 = Rakhshandehroo *et al.*, 2017.

Host species	Helminth species	Stage	Habitat	Locality	Reference
<i>Bufo</i> <i>variabilis</i>	Monogenea				
	<i>Polystoma viridis</i> Euzet, Combes and Batchvarov, 1974	A	u	Khouzestan, W Iran	5
		A	u	Semnan, NE Iran	6
		A	u	Fars, SW Iran	7
	Digenea				
	<i>Haplometra cylindracea</i> (Zeder, 1800) Looss, 1899	A	lu	Khouzestan, W Iran	5
	Cestoda				
	<i>Nematotaenia dispar</i> (Goeze, 1782)	A	i	Khouzestan, W Iran	5
	Lühe, 1899	A	i	Semnan, NE Iran	6
	<i>Diplopylidium acanthotetra</i> Parona, 1886	A	i	Khouzestan, W Iran	5
	Nematoda				
	<i>Aplectana</i> sp.	A	r	Khouzestan, W Iran	5
	<i>Cosmocerca commutata</i> Diesing, 1851	A	r	Khouzestan, W Iran	5
	<i>Cosmocerca ornata</i> Diesing, 1861	A	r	Khouzestan, W Iran	5
	<i>Oswaldocruzia</i> sp.*	A	i	Fars, SW Iran	7
<i>Rhabdias bufonis</i> Schrank, 1788	A	lu	Khouzestan, W Iran	5	
	A	lu	Semnan, NE Iran	6	
<i>Hyla savignyi</i>	Monogenea				
	<i>Polystoma viridis</i>	A	u	Khouzestan, W Iran	5
	Cestoda				
	<i>Nematotaenia dispar</i>	A	i	Khouzestan, W Iran	5
	A	i	Fars, SW Iran	7	
	Nematoda				
	<i>Aplectana</i> sp.	A	r	Khouzestan, W Iran	5
<i>Pelophylax</i> <i>ridibundus</i>	Digenea				
	<i>Diplodiscus subclavatus</i> (Goeze, 1782)	A	r	Anzali, NW Iran	4
	<i>Gorgodera dollfusi</i> Pigulevsky, 1945	A	u	Anzali, NW Iran	4
	<i>Gorgodera microovata</i> Fuhrmann, 1924	A	u	Anzali, NW Iran	1
	<i>Haematoloechus breviansa</i> (Sudarikov, 1950)	A	lu	Anzali, NW Iran	4
	<i>Haematoloechus similis</i> (Looss, 1899)	A	lu	Anzali, NW Iran	1
		A	lu	Chaharmahal and Bakhtiari, W Iran	3
		A	lu	Anzali, NW Iran	4
		A	lu	Semnan, NE Iran	6
	<i>Haematoloechus variegatus</i> (Rudolphi, 1819)	A	lu	Anzali, NW Iran	1
	<i>Opisthioglyphe ranae</i> Frohlich, 1791	A	i	Anzali, NW Iran	1
		A	i	Anzali, NW Iran	4
	<i>Pleurogenoides medians</i> (Olsson, 1876)	A	i	Aghbaba, NE Iran	4
	<i>Prosotocus confusus</i> (Loss, 1894)	A	i	Anzali and Astara, NW Iran	4
	<i>Prosotocus fuelleborni</i> Travassos, 1930	A	i	Anzali, NW Iran	1
	<i>Codonocephalus ridibundus</i> (Rudolphi, 1819) Luhe, 1909	L	o, k, mu, me	Chaharmahal and Bakhtiari, W Iran	3
		L	bc, me	Anzali and Astara, NW Iran	4
		L	o, k, mu, me	Khouzestan, W Iran	5
	<i>Encyclometra colubrimurorum</i> (Rudolphi, 1819)	L	li	Anzali, NW Iran	4
	Nematoda				
	<i>Aplectana</i> sp.	A	r	Khouzestan, W Iran	5
	<i>Cosmocerca ornata</i>	A	r	Astara, NW Iran	4
Acanthocephala					
<i>Acanthocephalus ranae</i> Schrank, 1788	A	i	Anzali, NW Iran	1	
<i>Rana</i> <i>macrocnemis</i>	Digenea				
	<i>Dolichosaccus rastelus</i> (Olson, 1876) Travassos, 1930	A	i	Chaharmahal and Bakhtiari, W Iran	3
<i>Rana</i> <i>pseudodalmatina</i>	Digenea				
	<i>Haplometra cylindracea</i>	A	lu	Khouzestan, W Iran	5
	Nematoda				
	<i>Oswaldocruzia filiformis</i> Goeze, 1782	A	i	Semnan, NE Iran	6
Not specified	Digenea				
	Heterophidae	L	–	Khouzestan, W Iran	2

* This specimens were recorded as *Rhabdias bufonis* by Rakhshandehroo *et al.* (2017), but according to their drawings and the habitat of the helminths, they correspond to *Oswaldocruzia* sp.

the identification of specimens, specialized literature (Anderson *et al.*, 2009; Gibson *et al.*, 2002; Prudhoe & Bray, 1982) and original descriptions were used. Host nomenclature follows Frost (2018). Helminth specimens were deposited in the Canadian Museum of Nature Parasites Collection, 1740 Pink Road, Gatineau, Quebec, Canada, with the Accession numbers CMNPA2019-0001 - CNMPA2019-0207.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Results and Discussion

Fourteen helminth species were found in *Pelophylax ridibundus* in the Shiraz region, eight digeneans and 6 nematodes (Table 2). Of these, only six are adults, while 8 are in their larval stage. The most prevalent helminths were the metacercariae of *Codonocephalus urniger*, found in diverse organs of 61.7 % of the examined frogs, followed by the larvae of the nematode *Abbreviata* sp., present in the stomach wall of 55.32 % of the hosts. The adults with the highest prevalence are the digenean *Halipegus alhaussaini* in the stomach, and the nematode *Cosmocerca ornata* in the rectum, in 34 % of the frogs.

Diplodiscus subclavatus (Goeze, 1782) (Digenea: Paramphistomidae) has been recorded in *P. ridibundus* in the North East of Iran

(Mashai *et al.*, 2000), and in a variety of anuran hosts in Europe and Africa (Amin *et al.*, 2012; Bakhroum *et al.*, 2011; Düşen & Öz, 2006; Düşen *et al.*, 2009, 2010; Galeano *et al.*, 1996; Grabda-Kazubska, 1980; Herczeg *et al.*, 2016; Honer, 1961; Kir *et al.*, 2001; Oğuz *et al.*, 1994; Salami-Cadoux & DeGregorio, 1976; Yildirimhan *et al.*, 1996, 2005, 2012; Yildirimhan & Incedogan, 2013). The life cycle of species of this genus include a freshwater snail of the family Planorbidae as intermediate host; cercariae encyst on diverse surfaces including the skin of tadpoles. Frogs get infected through ingestion of encysted metacercariae, grazing on surfaces in their tadpole stage, or during moulting, when they eat their cast-off skin. The metacercariae mature in the rectum of the frog (Herber, 1939; Yamaguti, 1975).

Halipegus alhaussaini Saoud and Roshdy, 1970 (Digenea: Derogenidae) was described from *Rana esculenta* Linnaeus, 1758 (= *Pelophylax ridibundus*) in Al-Basrah, Southwestern Iraq (Saoud & Roshdy, 1970) and has never been recorded afterwards. This species differs from other *Halipegus* species in the region by the small body size (2.47 – 2.61 mm), the postacetabular distribution of the vitelline glands, and the length of the egg filament (twice the length of the egg length). This is the first report of this species in Iran. First and second intermediate hosts of *Halipegus* spp. are freshwater snails and arthropods (copepods and ostracods) respectively; tadpoles get the infection when they eat infected copepods or ostracods and helminths mature in the upper digestive tract after metamorphoses (Yamaguti, 1975).

Haematoloechus similis (Looss, 1899) (Digenea: Haematoloechi-

Table 2. Helminths of *Pelophylax ridibundus* from the Shiraz region, southwestern Iran; n = 47.

	Habitat	Stage	Prevalence
Digenea			
<i>Diplodiscus subclavatus</i>	Rectum	Adult	21.28
<i>Halipegus alhaussaini</i>	Stomach, anterior intestine	Adult	34.04
<i>Haematoloechus similis</i>	Lungs	Adult	23.40
<i>Codonocephalus urniger</i>	Ovary, testes, mouth cavity floor, muscle, kidneys	Metacercariae	61.7
Diplostomidae (Neodiplostomulum type)	Kidneys	Metacercariae	12.77
Diplostomidae (Diplostomulum type)	Liver	Metacercariae	4.26
Strigeidae (Tetracotyle type)	Ovary	Metacercariae	8.51
Digenea	Muscle, heart, mesenteries, urinary bladder	Metacercariae	14.89
Nematoda			
<i>Cosmocerca ornata</i>	Rectum	Adults	34.04
<i>Rhabdias bufonis</i>	Lungs	Adults	38.30
<i>Abbreviata</i> sp.	Stomach walls	Larvae	55.32
<i>Eustrongylides</i> sp.	Mesenteries	Larvae	10.64
Onchocercidae	Body cavity	Juvenile	2.13
Nematoda	Intestine wall, mesenteries, testes	Larvae	8.51

Table 3. Comparative helminth record of *Rana ridibunda* in different regions of Iran.

	Combes and Knoepffler, 1972 Anzali, NW Iran	Mashai, 1999 Chaharmahal and Bakhtiari, W Iran	Mashai et al., 2000 Anzali, NW Iran	Mashai, 2005 Khouzestan, W Iran	Mashai et al., 2008 Semnan, NE Iran	This study Shiraz, SW Iran
Digenea						
<i>Diplodiscus subclavatus</i>			X			X
<i>Gorgodera dollfusi</i>			X			
<i>Gorgodera microovata</i>	X					
<i>Halipegus ahaussaini</i>						X
<i>Haematoloechus breviansa</i>			X			
<i>Haematoloechus similis</i>	X	X	X		X	X
<i>Haematoloechus variegatus</i>	X					
<i>Opisthioglyphe ranae</i>	X		X			
<i>Pleurogenoides medians</i>			X			
<i>Prototocus confusus</i>			X			
<i>Prototocus fueleborni</i>	X					
<i>Codonocephalus ridibundus</i>		X	X	X		X
<i>Encyclometra colubrimurorum</i>			X			
<i>Neodiplostomulum</i>						X
<i>Diplostomulum</i>						X
Tetracotyle						X
Digenea Metacercariae						X
Nematoda						
<i>Aplectana</i> sp.				X		
<i>Cosmocerca ornata</i>			X			X
<i>Rhabdias bufonis</i>						X
<i>Abbreviata</i> sp.						X
<i>Eustrongylides</i> sp.						X
Onchocercidae						X
Nematoda larvae						X
<i>Acanthocephala</i>						X
<i>Acanthocephalus ranae</i>	X					

dae) has been recorded in *P. ridibundus* in the North East of Iran (Combes & Knoepffler, 1972) and in several species of *Pelophylax* and *Rana* in Europe: *P. kl. esculentus* Linnaeus, 1758 (Bailenger & Chanseau 1954; Bjelic-Cabrilo *et al.* 2009; Chikhlaev *et al.* 2009; Looss, 1899; Odening, 1960; Prokopic & Krivanec, 1974); *P. ridibundus* (Odening, 1960; Romanova & Matveeva 2010; Saeed *et al.*, 2007), *Rana arvalis* Nilsson, 1842 (Odening 1960; Tkach *et al.* 2000); *R. temporaria* Linnaeus, 1758 (Chikhlyayev & Ruchin 2014; Odening, 1960); *Rana* sp. (Travassos & Darriba 1930). *Haematoloechus similis* first intermediate host is the snail *Planorbis planorbis* Linnaeus, 1758; dragonfly nymphs act as second intermediate hosts and frogs are infected through the ingestion of infected dragonflies (Grabda, 1960).

Codonocephalus urniger has been recorded as metacercariae in marsh frogs in Europe and Middle East (Amin *et al.*, 2012; Dollfus & Patay, 1956; Düşen & Öz, 2006; Murvandize *et al.*, 2008; Saeed *et al.*, 2007; Yildirimhan *et al.*, 1996, 2005). Particularly in Iran, *C. codonocephalus* has been recorded parasitizing *P. ridibundus* in the northeast and in the southwest (Mashaii, 1999, 2005; Mashaii *et al.*, 2000). The life cycle of this species involves the snail *Stagnicola palustris* Müller, 1774 as first intermediate host, marsh frogs as second intermediate hosts and ardeiform birds as definitive hosts (Kostadinova, 1993; Niewiadomska, 1964; Prudhoe & Bray, 1982).

We found three types of metacercariae of Diplostomoidea: Neodiplostomum type, Diplostomulum type (Diplostomidae), and Tetracotyle type (Strigeidae), being the first record of this kind of metacercariae in *P. ridibundus* in this region. These helminth species may use aquatic birds or mammals as definitive hosts (Prudhoe & Bray, 1982).

Metacercariae infecting heart, muscle, mesenteries and urinary bladder were impossible to identify due to their lack of diagnostic characters, and poor preservation conditions.

Cosmocerca ornata is widely distributed in Europe, Asia and Africa in various anuran and some lizard species (Aisien *et al.*, 2004; Amin *et al.*, 2012; Baker, 1981; Bursey & Goldberg, 2011; Düşen, 2007, 2011, 2012; Düşen & Oğuz, 2010; Düzen & Yaka, 2014; Düşen *et al.*, 2009, 2010; Galeano *et al.*, 1990; Galli *et al.*, 2001; Kirillov & Kirillova, 2016; Moravec & Barus, 1990; Moravec *et al.*, 1987; Norval *et al.*, 2013; Sey & Al-Ghaith, 2000; Schad *et al.*, 1960; Yildirimhan & Karadeniz, 2007; Yildirimhan *et al.*, 2005, 2006a, 2006b, 2006c, 2009); it has been recorded in *Bufoles variabilis* and *Pelophylax ridibundus* in the South West and North East of Iran (Mashaii, 2005; Mashaii *et al.*, 2000). *Cosmocerca* spp. have a direct life cycle; eggs are released to the environment with the faeces, larvae hatch in the soil and infect definitive hosts through skin penetration (Anderson, 2000).

Rhabdias bufonis has a palearctic geographic distribution and has been recorded in numerous anuran species, *Bombina bombina* (Yildirimhan *et al.*, 2001); *Bufo bufo* (Düşen, 2011; Yildirimhan *et al.*, 1997; Yildirimhan & Karadeniz, 2007); *Bufo (Pseudepidalea) viridis* (Düşen, 2011; Yildirimhan, 1999); *Rana camerani* (Yildirim-

han *et al.*, 2006c); *R. dalmatina* (Düşen *et al.*, 2009); *R. macrocnemis* (Yildirimhan *et al.*, 1996); *R. ridibunda* (Düşen & Öz, 2006; Kir *et al.*, 2001; Sağlam & Arıkan, 2006; Yildirimhan *et al.*, 1996; 1997); *Pelodytes caucasicus* (Yildirimhan *et al.*, 2009). Nevertheless, Kuzmin (2013) considers that it is not unlikely that this is a complex of cryptic species; in Iran it has been recorded only in *Bufoles variabilis* (Mashaii, 2005; Mashaii *et al.*, 2008). *Rhabdias bufonis* life cycle shows a free gonochoristic generation and a generation of parasitic hermaphrodites; amphibians get the infection by skin penetration or ingestion of larvae (Kuzmin, 2013).

Abbreviata sp. larvae are commonly found encapsulated in the stomach walls of amphibian and reptiles (Anderson, 2000), but this is the first record in Iran. In the Middle East, larvae of *Abbreviata* sp. have been recorded in *P. ridibundus* and *Hyla orientalis* Bedriaga 1890 in Turkey (Düzen & Öz, 2006; Düzen & Yaka, 2014; Heckmann *et al.*, 2010), and in *Ophisaurus apodus* Daudin, 1803 (Lacertilia) and *Coluber jugularis* Linnaeus, 1758 (Serpentes) in Georgia (Murvandize *et al.*, 2008). The adults of *Abbreviata adonisi* Sulahian and Schacher, 2009 were described from the lizard *Agama stellio* in Lebanon (Sulahian & Schacher, 1968). The life cycle of nematodes in the genus *Abbreviata* includes an arthropod first intermediate host, amphibians and small reptiles as paratenic hosts and the majority of species use reptiles as definitive hosts (Anderson, 2000; Gafurov *et al.*, 1970; King *et al.*, 2013).

Adult *Eustrongylides* inhabit the proventriculus of aquatic birds, whereas the infective larval stage is found in the tissues and body cavity of fishes, amphibians and reptiles (Anderson, 2000). This is the first record of *Eustrongylides* sp. larvae in amphibians in Iran, although they have been previously recorded in anurans and fish from the Middle East region (Düşen & Öz, 2006; Sağlam & Arıkan, 2006; Sattari *et al.*, 2002; Yildirimhan *et al.*, 2005)

Onchocercid nematodes that parasitize amphibians belong to the subfamilies Icosiellinae, Waltonellinae or Driofilariinae. As adults they live in the body cavity and mesenteries, females release the larvae (microfilariae) into the blood stream and these are taken by hematophagous vectors, which transmit them to other host after some development (Anderson, 2000). Specimens in this study were collected in a juvenile phase and were poorly preserved, which prevented the identification to a lower level. This is the first record of an onchocercid nematode of amphibians from Iran.

Nematode larvae encysted in the intestine wall, testes and mesenteries of marsh frogs were impossible to identify because their lack of diagnostic characters and poor preservation conditions.

The helminth record of the marsh frogs of the Shiraz Region studied herein presents the highest number of species compared to studies performed in other regions (14 species in this study vs 1 to 10 species in other studies) (Table 3). The second richest helminth record is the one from frogs in Anzali (Mashaii, 2005) with ten species, with the difference that most of those species were adults (8 species), while in our study only 6 species were adults and 8 species were larval stages, many of them parasites of birds in their adult stage. This indicates the presence of abundant aquatic birds

in the area at the time of collection and the important role that these frogs played in those birds diet.

Conflict of Interest

Author states no conflict of interest.

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