

## Parasitism with *Acanthocephalus ranae* in frogs (*Pelophylax ridibundus* Pallas 1771), from North-east Romania

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### Summary

The present study was carried out on 10 frogs, *Pelophylax ridibundus* (Pallas, 1771), captured near the river Bahlui, Iasi, between April and May, 2019, for the study of digestive helminths and their effect on the digestive tract. Macroscopic examination of the general cavity revealed distension of the small intestine. The sectioning of the intestinal wall revealed a variable number of parasites (1 – 12) with a whitish cylindrical body, anchored in the duodenal mucosa. The morphological study of the parasites confirmed the species as *Acanthocephalus ranae*. The prevalence of the infection was 60 % and the average intensity was  $4.83 \pm 4.15$ . Lesions of the small intestine were characterized by catarrhal enteritis, hemorrhagic spots and ulcers on the mucosa, occlusion and obstruction of the intestinal lumen. Histologically, eosinophilic inflammatory infiltrate in the glandular crypts, mucosal and submucosal edema, fibrosis and conjunctival hyperplasia, total mucosal atrophy, were found. The infection with *Acanthocephalus ranae* in *Pelophylax ridibundus* caused severe pathological changes, conditioned by the intensity of the infection, aspects revealed for the first time in Romania.

**Keywords:** *Pelophylax ridibundus*; small intestine; *Acanthocephalus ranae*; prevalence; histopathological features

Ranids are an important component of the biodiversity of the aquatic environment in which they also play a host role for many species of parasites. They are of particular interest, as they are used in both biomedical experiments and human nutrition. They are also bioindicators of environmental quality and play an important role in controlling populations of harmful insects, or in the pharmaceutical industry, through the use of biologically active compounds in the skin (Cogalniceanu 2002).

*Pelophylax ridibundus* Pallas, 1771 sin. *Rana ridibunda* Pallas, 1771 (the marsh frog) lives in lakes, stagnant waters, and shallow marshes with a lot of vegetation. It is a diurnal species, of medium size (9 – 15 cm) (Cogalniceanu, 2002), which differs according to sex and geographical area (in males: mean 71.84 mm, range:

49.56 – 110.80 mm, and in females: mean 80.10 mm, range: 51.05 – 104.78 mm) (Ayaz *et al.*, 2007), which feeds opportunistically on terrestrial and aquatic invertebrates (insects, spiders, crustaceans, gastropods, beetles) (Bam-e-Zar *et al.*, 2019), and small vertebrates (freshwater fish), and sometimes by cannibalism. It has a wide geographical distribution (Ciudin, 2004).

Ninety-eight percent of ranids are parasitized (Elmajdoub *et al.*, 2018), being definitive, intermediate or paratenic hosts for many species of parasites (trematodes, cestodes, nematodes, acanthocephalans) (Imkongwapang *et al.*, 2014), which affect their state of health (Imasuen *et al.*, 2012). Frog health is important for ecological balance, for the accuracy of the results obtained in experimental laboratories and for human health (Ciudin, 2004). Among

the parasitic entities that affect the ranids, acanthocephalosis is differentiated by the magnitude of the conflict between the parasite and the host, ending, in general, by triggering the disease state, the depreciation of the host organism to a lethal end (Aznar *et al.*, 2018). *Acanthocephalus ranae* (Schrank, 1788) Lühe, 1911 has a wide specificity and a large geographical distribution (Hackman *et al.*, 2011).

It is identified in many species of amphibians, including *Triturus karelinii* Strauch, 1870, *Bombina variegata* Linnaeus, 1758, *Rana dalmatina* Bonaparte, 1940, *Rana esculenta* Linnaeus, 1758, *Rana temporaria* Linnaeus, 1758, *Rana ridibunda* Pallas, 1771, in Bulgaria (Dimitrova & Dimitrova, 2012; Dimitrova & Marinova 2018), *Pelophylax esculentus* Linnaeus, 1758, in Hungary (Heczeg *et al.*, 2016), *Rhinella icterica* Spix, 1824, in Brazil (Pilati *et al.*, 2013), *Bufo viridis* Laurenti, 1768, *Rana ridibunda* (Karakas, 2015), *Hyla orientalis* Bedriaga, 1890 (Yakar *et al.* 2016), *Rana dalmatina* Bonaparte, 1940 (Dusen, *et al.*, 2009), *Pelophylax ridibundus* Pallas, 1771 (Koyun *et al.*, 2015), *Pelophylax bedriagae* Camerano, 1882 (Demir *et al.*, 2015), in Turkey, *Pelophylax ridibundus* (Leon-Regagnon, 2019), in Iran, and the small intestine, in fish (Sakthivel *et al.*, 2017), in India. The traumatic, irritative, compressive, occlusive, inflammatory, toxic, and spoilage mechanisms used by acanthocephalans during parasitism cause digestive disorders, maldigestion, malabsorption, metabolic disorders, irreversible tissue changes on the gastrointestinal tract, morbidity and mortality (Aznar *et al.*, 2018). *Acanthocephalus ranae* is transmitted through an intermediate host represented by the isopod crustacean, *Caecidotea militaris* (Amin, 1982; Pfennig, 2017) and the species *Asellus aquaticus* (Dimitrova & Dimitrova; 2012, Amin *et al.*, 2019), in which the infectious stage of cystacanth develops. Ingestion of intermediate host crustaceans ensures the development of acanthocephalans in the digestive tract of amphibians, freshwater fish (family Cyprinidae), and other aquatic animals. The continuous decline of amphibian populations is directly or indirectly influenced by human activities. The protection of amphibians is regulated worldwide. According to Law 462/2001 (Regime of natural protected areas, conservation of natural habitats of wild fauna and flora), all species of amphibians are protected. Habitats Directive 92/43 / EEC - consolidated on 01/01/2007 by EU Directive, emphasizes that 10 species are strictly protected and three species (*Rana esculenta*, *R. ridibunda*, and *R. temporaria*) can be economically exploited following impact studies (Cogalniceanu 2002; Herczeg *et al.*, 2016). The purpose of these investigations is to report the species *A. ranae* in the marsh frog species *Pelophylax ridibundus* in the area of the river Bahlui-lasi, and the histopathological effects on the small intestine, aspects not previously reported in Romania.

## Material and Methods

### Study area and sample collection

The investigations took place on frogs collected near the Bahlui River, Iasi (47° 9'44 "N 27° 35'20" E). The Bahlui River, known as

the "muddy river, home of frogs" has a length of 119 km, and an average annual flow of 4.88 m<sup>3</sup>/s; passes through the city of Iasi and has a basin with an area of 2,007 km<sup>2</sup>.

Ten frogs of the species *Pelophylax ridibundus* were captured manually between April and May 2019, for the study of digestive helminths and their effect on the digestive tract. Frogs care and handling were carried out in accordance with institutional guidelines. The frogs were examined individually and euthanized by placing them in an ether container. Through the median incision, the general cavity was opened and the internal organs were examined. In some frogs, the opening of the intestine revealed in variable numbers, cylindrical, whitish formations, fixed in the intestinal wall, blocking the lumen. The parasites were extracted, washed with warm distilled water and deposited in containers with 70 % alcohol, for morphology and prevalence studies. Also, fragments of intestine (duodenum and jejunum) were taken and deposited in containers with 10 % formaldehyde, for histopathological processing.

### Sample examination

The extracted parasites were counted, separated by sex and examined morphologically, to identify the species, in accordance with the literature (Kurbanov, 1978; Norton & Esposito, 1994; Heckmann *et al.*, 2011).

The intestinal fragments were embedded in paraffin, sectioned to 5 µm thick, in serial sections and stained with the Masson trichromic method (Foot, 1933). Tissue examination and microphotography were performed with the Leica 750 DM photon microscope, Leica 750 DM, ICC 750 Camera and Leica Application Suite (LAS), software version 4.20 (Oct. 2012), for image retrieval. (Leica Microsystems GmbH Wetzlar, Germany).

### Statistical analysis

The statistical parameters regarding body size in acanthocephalans (Mean, Standard Error, Standard Deviation, Sample Variance, Minimum, Maximum) were analyzed through the descriptive statistics program, Microsoft Office 2016, Microsoft Excel 2016 application.

### Ethical Approval and/or Informed Consent.

Animal care and handling were carried out in accordance with institutional guidelines.

## Results

### Necropsy examination

Macroscopic examination revealed in the small intestine of six frogs, a variable (1 – 12) number of parasites, strongly anchored in the duodenal wall, which completely occluded and obstructed the intestinal lumen (Fig. 1). On the mucosa, hemorrhagic spots, edema of the tissues at the place of fixation of the parasites, thicken-



Fig. 1. *Pelophylax ridibundus*: parasites anchored in the intestinal mucosa (digital photo).

ing of the wall and abundant mucus, were observed. Twenty-nine specimens were collected from the small intestine of frogs, of which 11 males and 18 females, with a prevalence of 60 % and an average intensity of  $4.83 \pm 4.15$ . The parasites collected have an elongated, cylindrical, whitish-yellow body with relatively small dimensions. Males measured on average  $11.16 \pm 0.51$  mm in length and  $1.37 \pm 0.55$  mm in width. Females measured on average  $25.31 \pm 3.87$  mm in length and  $2.06 \pm 0.54$  mm in width. According to the literature (Kurbanov, 1978; Norton & Esposito, 1994; Heckmann *et al.*, 2011), the collected specimens belonged to the species *A. ranae*.

#### Histopathological results

Infected frogs showed variable intestinal lesions, determined by the intensity of parasitism. Histologically, areas of desquamation and detachment of the epithelium from the intestinal villi, epithelial hyperplasia, associated with inflammatory cell infiltrate, arranged at the base of the villi, in the glandular crypts was observed (Fig. 2a). The inflammatory infiltrate was rich in lymphocytes, fibro-

blasts, and granulocytes in which eosinophils predominated in association with erythrocytes released from hemorrhages induced by parasite fixation (Fig. 2b). In other cases, the histological lesions were more serious, characterized by partial or total destruction of the villi, the disappearance of the mucosal epithelium and discovery of the conjunctival axis (Fig. 2c). The intensity of the infection had severe consequences on the intestinal mucosa, causing fibrosis, and conjunctival hyperplasia of microvilli, complete disappearance of intestinal epithelium, abundant inflammatory infiltrate in glandular crypts (Fig. 2b). Also, catarrhal inflammation of the intestinal mucosa was observed (Fig. 2d), in which, hyperplasia and edematous infiltration of the submucosa and muscosa, ulcerative areas and inflammatory infiltrate, necrobiosis and cell necrosis processes, and areas with total atrophy of the intestinal mucosa were found.

In the intestinal contents, cellular detritus, acanthocephalans eggs, and erythrocytes, and also, a section through the proboscis, and the body of a female with the uterus full of eggs, were observed.

#### Discussions

The parasitism with *Acanthocephalus ranae* (Schrank, 1788) Luhe, 1911 is studied by many authors (Kurbanov, 1978; Norton & Esposito, 1994; Heckmann *et al.* 2011; Dimitrova & Dimitrova, 2012; Koyun *et al.*, 2015; Pfenning, 2017; Aznar *et al.*, 2018), from all over the world. The prevalence of *A. ranae* infection in *Pelophylax ridibundus* reported in this paper is in agreement with data from the literature. In general, the prevalence of *A. ranae* infection in amphibians ranged from 2 % in *Bufo viridis* (Karakas, 2015), to 39 % – 100 % in *Rhinella icterica* (Pilati *et al.*, 2013). Yakar *et al.* (2016), report an infection prevalence of 6.66 % in *Hyla orientalis*, and Demir *et al.* (2015), report a 9.25 % prevalence in *Pelophylax bedriagae*, while Dusen *et al.* (2009), report prevalence of 45.45 % in *Rana dalmatina*. The prevalence of *A. ranae* is similar to the prevalence of another species of acanthocephalans reported in amphibians, in other geographical areas (*Corynosoma* spp. 2.04 %, in *Rana saharica*) (Elmajdoub *et al.*, 2018). It is known that *A. ranae* parasitizes amphibians in the small intestine, but Karakas *et al.* (2015), report both intestinal and gastric localization of *A. ranae* species, *Rana ridibunda* and *Bufo viridis*. In the present work, *A. ranae* was found only in the intestine. Pilati *et al.* (2013), showed that the mean intensity of small intestine parasitism in *Rhinella icterica* with *A. ranae* was 25 specimens, and the maximum intensity, 53 specimens, in *Pelophylax bedriagae* (Demir *et al.*, 2015). In most studies, the mean intensity of acanthocephalans infection ranged from 1.50 in *Hyla orientalis* (Yakar *et al.*, 2016), to 2.66 in *Rana dalmatina* (Dusen *et al.*, 2009), or 14.60 in *Pelophylax bedriagae* (Demir *et al.*, 2015). In the current study, the mean intensity of *A. ranae* infection in *Pelophylax ridibundus* was 4.83 (1 – 12). The number of acanthocephalans and long-term parasitism influenced the development of the pathological condition. The irritative, compressive, occlusive, toxic, spoilage mechanisms

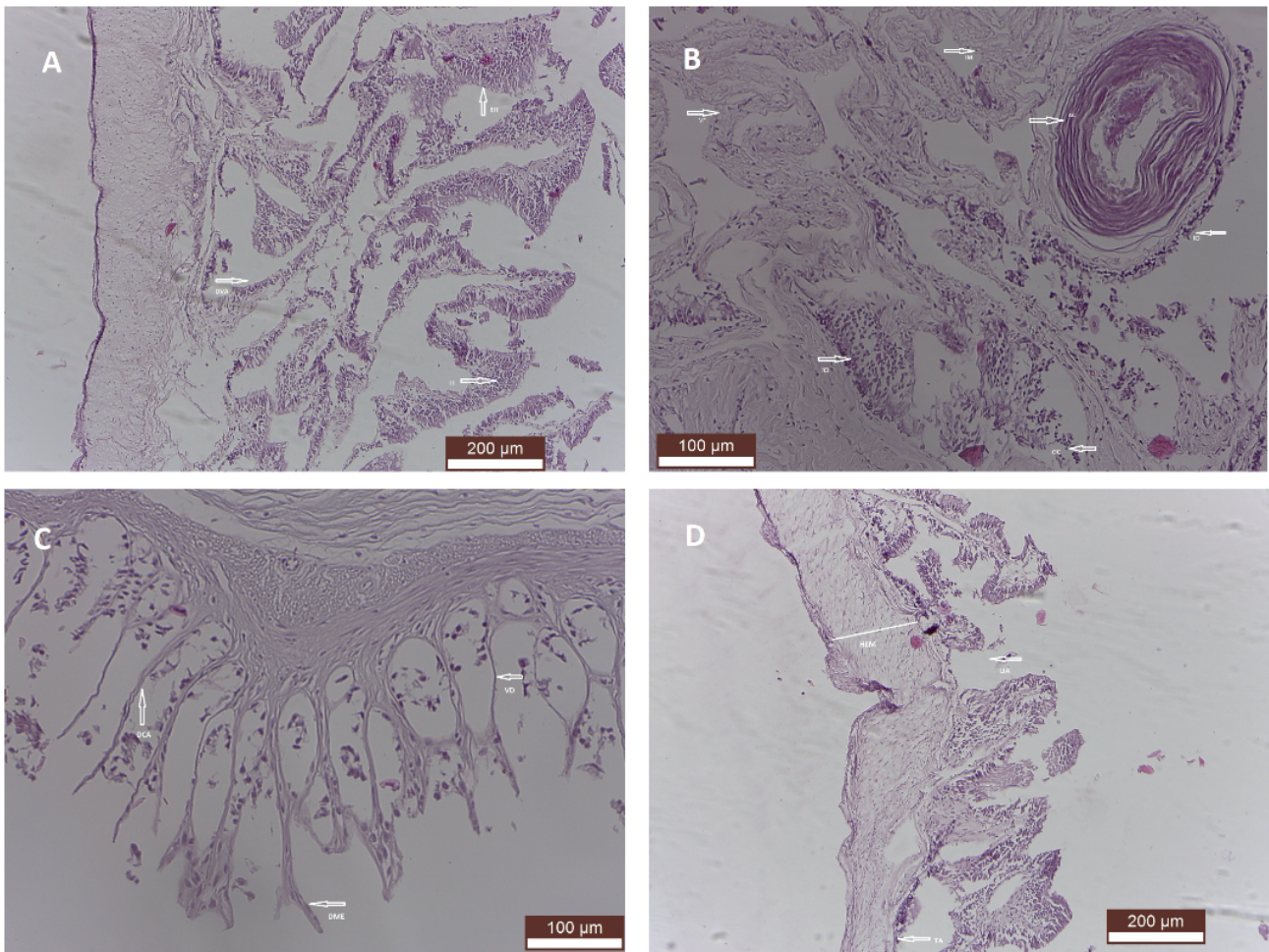


Fig. 2 a, b, c, d. *Pelophylax ridibundus* infected with *A. ranae*. Small intestine. a. Destruction of villi tissue architecture (DVA), epithelial hyperplasia (EH), inflammatory infiltrate (II). b. Around the acanthocephalans (Ac.), the mucosa is injured (IM), with abundant inflammatory cell infiltrate (ICI) in glandular crypts (GC) and villous fibrosis (VF). c. The effect of parasitism consists in partial or total destruction of the villi (VD) the disappearance of the mucosal epithelium (DME) and discovery of the conjunctival axis (DCA), and general atrophy. d. Catarrhal jejunitis: hyperplasia and edematous infiltration of the submucosa and musculosa (HEM), ulcerative areas (UA) and inflammatory infiltrate, necrobiosis and cell necrosis processes, and areas with total atrophy (TA) of the intestinal mucosa (Trichormic Masson stain x 100, 200).

used by acanthocephalans during parasitism cause inflammation of the intestinal mucosa, irreversible tissue damage to the intestinal mucosa, and mortality, aspects also described by Heckmann *et al.* (2011). The macroscopic and microscopic lesions described in this paper are in agreement with the study done by Heckmann *et al.* (2011), which highlights the complete obstruction of the intestinal lumen with acanthocephalans and necrotic tissue, in *Rana ridibunda*. The same authors described, histologically, extensive hemorrhagic areas and many free erythrocytes in the lumen space and granulocytes visible in the inflammatory infiltrate, in high concentrations. The cells of the intestinal villi are destroyed around the extremity of the acanthocephalan penetrated into the intestinal wall, aspects similar to those described in this paper. Recently, Sakthivel *et al.* (2017) reported the intestinal parasitism of freshwater fish, *Caranx ignobilis* species with *A. ranae*, describing tis-

sue changes similar to those presented in *Pelophylax ridibundus*. So, in fish, in the intestine, at the site of fixation of the parasite, tissue damage, congestion, edema, erosions, ulcers, and thickening of the intestinal wall were observed. The same authors highlighted histologically, hyperplasia of the intestinal villi and lamina propria, cellular infiltrated areas around the proboscis, aggregation of lymphocytes, and fibroblasts at the site of fixation and inflammation, suggesting that long-term parasitism increases cell infiltration (Sakthivel *et al.*, 2017). The examination of the *Pelophylax ridibundus* frogs collected near the Bahlui lasi river revealed, for the first time, the parasitism of the small intestine with *Acanthocephalus ranae* and severe tissue changes induced on the intestinal mucosa, aspects not previously reported in Romania. Future research, conducted on a larger number of amphibians collected from other geographical areas of the country, will contribute to a better knowl-

edge of the helminthic fauna of amphibians in Romania and the impact induced on aquatic populations.

### Conflict of Interest

Author state no conflict of interest

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