An investigation on endoparasites of *Rana ridibunda* complex (s. 1.) with dermal parasite (metacercariae) in Dalaman (Turkey)

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Abstract: In this study, endoparasites of the Rana ridibunda specimens collected from Dalaman area, southwestern Turkey were investigated. In the intestine of 17 specimens of *R. ridibunda*, five different parasites, represented by three species of the trematoda (Diplodiscus sp, Pleurogenoides sp, Plagiorychis sp) and two genera of round worms (Cosmocerca sp, Foleyella sp) were recognized. The trematoda and one of the round worms were found in the rectal region whereas the other round worm species are observed in the abdominal cavity.

Key words: *Rana ridibunda*, Intestinal parasites

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Introduction

*R. ridibunda*, considered as a monotypical species until recent years, was first described from Atyrau (Western Kazakhstan) (terra typica restricta). The species is distributed in Central and South Europe, North Africa and West Asia. In a previous study dealing with the classification of *Rana ridibunda* complex (s.l.) collected from Dalaman region situated the southwestern Turkey, the presence of certain black spots on the ventral sides of these specimens were recognised (Tok et al., 2000). These black spots were considered to be the most important criteria in differentiating between Dalaman population and all other populations. They indicated that in these samples, no parasitic invasion of the epidermis or the dermis and the accumulation of a lipid-like material in the dermis was observed. However, they directed attention to the fact that these black spots could be formed from groupings of well developed chromatophores and therefore further studies on the histological, cytotoxic and ecological studies on the this population are necessary to establish as to whether this unique character stems from environmental factors or is a congenital feature of the population.

In the previous study by the authors, it was noted that in Dalaman population, a trematoda at a metacercaria stage (immature) was recognized within a cyst located beneath the black spots in ventral region and the black spots forms from groupings of the melanocytes comprising melanin.

As it is known, the species of anura can be an intermediate or final host to parasites depending upon their habitats. There are number of publications related to various groups of parasites obtained from different body parts of *R. ridibunda* specimens collected from different regions of Turkey (Saygi and Basibuyuk, 1990; Yildirimhan et al., 1996; Kir et al., 2001; Yildirimhan et al., 2005). However, a trematod at a metacercaria stage in skin of *R. ridibunda* collected from Dalaman region was first recognised in this country. Furthermore, the presence of the black spots was related to the fact that the Dalaman area is characterised by different environmental factors.

The primary aim of this study is to investigate as to whether the samples *R. ridibunda* with dermal parasites collected from Dalaman region indicate different endoparasites.

Materials and Methods

Seventeen frogs with dermal parasite (10 females, 7 males) were etherized and their internal organs were dissected out and placed into physiological saline. The body cavities of these frogs were also filled with physiological saline and then examined. Some of the parasites were stained directly with Carmin and Giemsa dyes and the others were fixed in 10% formalin before staining with the same stains. The nematodes detected in the internal organs were fixed in 95 ml of 70% ethyl alcohol and 5 ml glycerine. Then alcohol was evaporated at a room temperature and the specimens were cleared in glycerine and mounted in glycerine jelly. The other parasites were fixed either in ethyl alcohol or in formalin, and were stained with Semichon’s Carmin (Saygi, 2000). The preparations were microscopically examined and micro-photographs were taken. Certain features of the trematodes such as general morphology, the structure of their teguments, the number, location and size of suckers, the number of testes as well as their positions with respect to each other, location of the ovarium and the shape of the eggs, if there was any, are described and the study is confined to genus level only. As for the nematodes, features such as general morphology, determination of their sexes and the formation of the esophagus were dealt with.
Results and Discussion

The investigation of the parasites of the intestinal tract and body cavity of the *R. ridibunda* specimens with metacercariae on their skin collected from the Dalaman area led to five different parasites.

Examinations revealed no parasites in the lungs, the liver and the reproductive organs. However, three species of trematodes and one nematode species were found in different parts of the intestinal tract and one nematode species in the body cavity. These are dealt with some details according to their groups.

**Fig. 1.** *Diplodiscus subclavatus* (a) General view, (b) Anterior region, (c) Adult form,

C = Cecum, O = Ovary, T = Testis, A = Acetabulum, Os = Oral sucker, S = Pharyngeal sacs, * = Oocytes

**Fig. 2.** (a) Anterior region of *Pleurogenoides* sp, (b) The anterior region of another *Pleurogenoides* sp. Os = Oral sucker, P = Pharynx, C = Cecum, Cs = Cirrus sac, T = Testes, O = Ovary, A = Acetabulum, * = Vitelline glands (Carmin)
**Endoparasites of Rana ridibunda**

**Trematodes:**

*Diplodiscus subclavatus*: From 6 of the 17 frogs (35.3%) that were examined, a total of 9 *D. subclavatus*, one or two in each sample, were recognized. The tegument covering the body had a wavy appearance. There was a wide sucker the ventral surface of which was covered with papillae, at the posterior end of the body (Fig. 1a). At the anterior part, the mouth was surrounded with a sucker and it opened into a muscular pharynx connected with two short pharyngeal pouches (Fig. 1b). The intestine was split into two branches ending in blind pouches (ceca) after the esophagus, whose tip had a muscular projection which was extending all the way down to the posterior sucker (Fig. 1a). Vitellus glands were located on the side and along the branches of the intestine. While the testes lied close to one another beneath the point where the intestine was split into two, the ovarium was in the middle of the intestinal pouches. In adult, few large and roundish eggs with thin shells were observed in the uterus (Fig. 1c). Based on these features (Hyman, 1951; Noble and Noble, 1976; Saygi, 1999; Schmidt and Roberts, 2000), this particular parasite was identified as the *Diplodiscus subclavatus* species belonging to the Family Paramphistomidae of the Phylum Trematoda.

**Pleurogenoides sp:** The surface of the body was covered with spined tegument; oral and ventral suckers were almost of the same size; a small, round and muscular pharynx right next to the oral sucker followed by the esophagus; and the branches of the intestine were short, ending at the same level as the ventral sucker (Fig. 2a, b). The well developed cirrus pouch was opening to outside from the side. Vitellus glands were located on either side at the level of the pharynx and the esophagus, and the ovarium was situated among the intestinal pouches. Some specimens had their testes above the intestinal pouches, and their ovariums were beneath these pouches (Fig. 2a), whereas others had their testes beneath the intestinal pouches and their ovaries were above one of the branches of the intestine (Fig. 2b). The esophagus was shorter in specimens that had their testes in front of the intestinal pouches as compared to those that had them beneath the intestinal pouches. Adult individuals had many shuttle-shaped eggs. The excretory pouch was Y-shaped. Based on these observations it was concluded that these parasites belonged to the species of the genus *Pleurogenoides* belonging to the Family Lechiodendriidae of the Phylum Trematoda. Yildirimhan et al. (1996, 2005) were determined at the posterior end of the male specimens, which were comparatively shorter and thinner; also, there were five big papillae, of which, the second was largest (Fig. 5). Based on the location and the morphological features of these nematodes, it was concluded that these specimens most probably belonged to the genus *Foleyella* of the Family Filariidae and Phylum Nematoda (Yorke and Mapleston, 1962; Noble and Noble, 1976; Saygi, 1999). There are studies showing the presence of similar species from this family in the digestive tracts of other frogs (Saygi and Basibuyuk, 1990; Yildirimhan et al., 1996; Kırın, 2003a; Kırın, 2003b; Vashetko et al., 1999).

**Foleyella sp:** Nematode specimens in different numbers, with different lengths and thicknesses were found in the abdominal cavity of 14 frogs of 17 (82.3%) that were examined. A large number of embryonated eggs were observed in the uterus of females with their lengths ranged between 4 to 4.5 cm. Furthermore, apparently some of the eggs were hatched in the uterus; so there were free larvae side by side with the eggs (Fig. 4). A couple of spicules, of which one was thin and quite long and the other was thick and long, were determined at the posterior end of the male specimens, which were comparatively shorter and thinner; also, there were five big papillae, of which, the second was largest (Fig. 5). Based on the location and the morphological features of these nematodes, it was concluded that these specimens mostly live in the muscles and subcutaneous connective tissues of such vertebrates. Its presence in the body cavities might have occurred during the investigation process. *Foleyella* species lives in the body cavity of various amphibians and other animals. They encyst in intestinal mesenteries and mesenteries about kidneys. Adult worms produce sheated microfilariae which circulate in peripheral blood. Apparently

**Plagiorchis sp:** The third trematode found in the intestine was relatively small, almost cylindrical leaf shaped and had a barely distinguishable ventral sucker which was almost of the same size as the oral sucker; it was located close to the center of the ventral side. The testes were located posteriorly in tandem position; the ovarium right beneath the ventral sucker; posteriorly directed vitellus glands were on either side, running along the whole body. Accordingly, this parasite was identified as a species of the genus *Plagiorchis* belonging to the Family Plagiorchidae of the Phylum Trematoda (Hyman, 1951; Noble and Noble, 1976; Saygi, 1999; Schmidt and Roberts, 2000).

Presence of various species belonging to genera of trematodes has also been reported in the digestive tract of *R. ridibunda* specimens living in different areas of Turkey (Saygi and Basibuyuk, 1990; Yildirimhan et al., 1996; Kir et al., 2001; Yildirimhan et al., 2005). However, Saygi and Basibuyuk (1990) appeared to have made an error in their publication as the figure given as *Diplodiscus* sp belonged to *Plagiorchis* species belonging to these trematod genera were also encountered in other frog species (Liuch et al., 1986; Madhavi et al., 1987; Kırın, 2003a; Kırın, 2003b).

**Nematodes:**

**Cosmocerca sp:** A large number of round worms were found in the rectum. The cuticle of these worms had horizontal stripes: their long esophagus toward the end narrowed slightly first and then widened taking a bulb-like shape (Fig. 3). The diameter of the intestine was wider in the area where the esophagus and the intestine met. The posterior end of the female specimens narrows down to the thickness of a bristle especially posterior to the anus, and ends in a needle-like prolongation. Unfortunately no male individuals were encountered among the collected specimens. Based on these findings, especially the structure of the esophagus, it was concluded that these nematodes belonged to the genus *Cosmocerca*, Family Oxyuridae within the Phylum Nematoda (Yorke and Mapleston, 1962; Noble and Noble, 1976; Saygi, 1999). There are studies showing the presence of similar species from this family in the digestive tracts of other frogs (Saygi and Basibuyuk, 1990; Yildirimhan et al., 1996; Kırın, 2003a; Kırın, 2003b; Vashetko et al., 1999).

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mosquitoes are the vectors, at the same time intermediate host of this filaria. Studies have shown that adult worms die before frog enters hibernation but microfilariæ overwinter in the same host.

In conclusion, it is suggested that endoparasites of *R. ridibunda* with dermal parasite collected from Dalaman area of the Turkey are identical to that of the endoparasites in the other regions. However, it should be pointed out here that the endoparasite species number in this *R. ridibunda* samples is less than other regions.

**References**


