Prevalence and pathology of *Lernaea cyprinacea* (Crustacea: Lernaeidae) parasitizing tadpoles of *Pelophylax bedriagae* (Anura: Ranidae) in Antalya, Turkey

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Abstract

Prevalence and pathology of *Lernaea cyprinacea* (Crustacea: Lernaeidae) parasitizing tadpoles of *Pelophylax bedriagae* (Anura: Ranidae) in Antalya, Turkey. This is a report of the first record of the occurrence of *Lernaea cyprinacea* on tadpoles of *Pelophylax bedriagae*, a frog species native from Karpuzçay Creek, Antalya, Turkey. A total of 20 tadpoles of *P. bedriagae* were examined and 14 (70%) specimens were infected. A maximum of two parasites per host was found, primarily around the cloaca. In general, slight to moderate hyperemia was noticed around the parasite. Typically, granuloma form in the tissues around the parasite. Histopathological examination revealed that hemorrhages and pathological lesions such as dermal erosions, and a chronic inflammatory reaction in skin and muscle were present at the site of parasite attachment.

Keywords: Anchor worms, disease, parasitic copepods, parasitism.

Resumo

Prevalência e patologia de *Lernaea cyprinacea* (Crustacea: Lernaeidae) parasitando girinos de *Pelophylax bedriagae* (Anura: Ranidae) em Antalya, Turquia. Este é o relato do primeiro registro da ocorrência de *Lernaea cyprinacea* em girinos de *Pelophylax bedriagae*, uma espécie de anuro nativa de Karpuzçay Creek, Antalya, Turquia. Foram examinados 20 girinos de *P. bedriagae,* e 14 (70%) espécimes estavam infectados. Um máximo de dois parasitas por hospedeiro foi encontrado, principalmente ao redor da cloaca. Em geral, observou-se hiperemia leve a moderada ao redor do parasita. Normalmente, o granuloma forma-se nos tecidos ao redor do parasita. O exame histopatológico revelou que hemorragias e lesões patológicas, como erosões dérmicas, e uma reação inflamatória crônica na pele e nos músculos estavam presentes no local da fixação dos parasitas.

Palavras-chave: copépodes parasitas, doença, parasitismo, vermes-âncora.

Introduction

Amphibians reflect the global decline of biodiversity (Houlahan *et al.* 2000, Alford *et al.* 2001); they are highly threatened by invasive species, over-exploitation, land-use change, climate change, increased use of pesticides, and infectious diseases (Collins and Storfer 2003). These environmental changes can affect the health of organisms by fostering the spread of diseases, such as parasitic infections. Likewise, environmental change may affect parasite transmission as a result of interactions among hosts, parasites, and biotic and abiotic conditions (Lafferty and Kuris 1999). Parasitic infections causes severe health effects, such as limb malformations (Sessions and Ruth 1990, Johnson *et al.* 1999, 2004, Ming 2001), and tissue damage (Martin and Conn 1990, Schotthoefer *et al.* 2003, Belden 2006, Holland *et al.* 2007). Therefore, it is important to understand the interactions of amphibians and parasites to assess the status of amphibian populations in the changing environmental conditions of today's world.

Ranid water frogs of the genus *Pelophylax* are distributed across the eastern Mediterranean. Three species of *Pelophylax* are found in Turkey—viz., *P. ridibundus* (Pallas, 1771), *P. bedriagae* (Camerano, 1882), and *P. caralitanus* (Arikan, 1988). The Levant Green Frog (*P. bedriagae*) is a largely aquatic species that inhabits permanent wetlands with rich aquatic vegetation; it is categorized as a species of Least Concern (LC) with a decreasing population trend in the Red List of International Union for the Conservation of Nature (IUCN). It is widely distributed in the eastern Mediterranean (Figure 1), especially along the Aegean coasts and southern Anatolian highlands of Turkey (Papenfuss *et al.* 2009). Population declines of *P. bedriagae* have been reported in the western Aegen Region of Turkey (Başkale and Kaya 2012).

According to the report of Amin *et al.* (2012) on helminth parasites in anurans from Turkey,

the most heavily infected species was *Pelophylax ridibundus.* Demir *et al.* (2015) is the first report of helminth parasites in *P. bedriagae* from western Aegean Region in Turkey. Düşen and Öz (2006) also reported helminth infections in *P. ridibundus* (= *Rana ridibunda*) from Antalya; however, these frogs were more likely belonging to *P. bedriagae,* considering the distribution range of the species. Infections of parasites other than helminths have not been reported in *P. bedriagae.* Although infections of *Lernaea cyprinacea* Linnaeus, 1758 are documented for adult and larval anurans worldwide (Baur 1962, Martins and Souza 1996, Alcalde and Batistoni 2005, Kupferberg *et al.* 2009, Salinas *et al.* 2016), the parasite has not been reported in anurans from Turkey.

Herein, we describe a parasitic copepod infection in larvae of *Pelophylax bedriagae* and the histopathological effects of the parasite on infection areas. We also consider the possible effects of the parasite on populations of *P. bedriagae* in response to future environmental changes.

Materials and Methods

Larvae of *Pelophylax bedriagae* were collected with a dip net on 07 October 2017 from Karpuzçay Creek (36°42'56.84" N, 31°33'0.95" E; Figure 1), which flows into the Antalya Gulf (Mediterranean Sea, Turkey). Immediately after collection, the tadpoles during were examined for the presence of *Lernaea cyprinacea,* which subsequently was identified in the laboratory with a dissecting microscope and Bauer (1987) as a reference. The terms *prevalence* (percentage of hosts infected with a particular parasite species or taxonomic group) and *intensity* (number of individuals of a parasite species in/on a single infected host) are used sensu Bush *et al.* (1997).

Tissue samples were collected during the necropsy. Transverse sections of areas where parasites were attached cut and fixed in 10%

Figure 1. Distribution of *Pelophylax bedriage* and the study site (indicated by *) at Karpuzçay Creek, Antalya-Turkey.

neutral formalin for histopathological studies. After routine processing of the formalin-fixed samples using automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany), the samples were embedded in paraffin, and 5-μm serial sections of the attached parasite bodies were prepared with a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). These sections were stained with hematoxylin and eosin (HE) and examined under light microscope. Morphometric evaluation and microphotography were performed using the Database Manual cellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Results

Of the 20 larvae of *Pelophylax bedriagae* that were examined, 14 (70%) were infected by *Lernaea cyprinacea* (Figure 2). A total of 16 *L. cyprinacea* were obtained from the 14 parasitized tadpoles. A mean intensity of 1.14 *L. cyprinacea* with 1 or 2 specimens per host was recorded; 63.5 % were attached around the tadpole cloaca, with the remaining parasites being attached elsewhere on the body.

Usually, the larval tissue around the parasite is grossly hyperemic. Histopathological examination reveals obvious pathological lesions in the area of parasite attachment. Microscopical

examination of sections revealed that the lesions are located in the epidermis, dermis, and muscles. Typically, ulcers are found in the epidermis at the site of parasite attachment (Figure 3 A–C). In most cases, the epidermis presented mild to severe acanthosis and spongiosis around the parasite. The most common symptom in the subdermal muscle is a chronic inflammatory reaction. In response to the presence of the parasite, there are infiltrated groups of cells that primarily comprise lymphocytes, histiocytes, and eosinophils. Fibrous tissue formation and granuloma-like lesions are common at the sites of parasite attachment. Chronic granulomatous myositis characterized by degenerative or, in some cases, necrotic lesions were noted in skeletal muscles (Figure 3 D–F). Other lesions in skin and muscle tissues are edema, congestion and small hemorrhages were also observed.

Discussion

In this study, we reported the first record and description of the parasitic infection of *Lernaea cyprinacaea* on *Pelophylax bedriagae* in Turkey and also in its known distribution range. Parasites can affect populations of anurans, the sizes of which may decrease resulting from infectious diseases caused by fungi, viruses, and parasites (Murray and Skerratt 2012). Little is known about amphibian diseases and parasitic infections in Turkey. The anchor worm, *L. cyprinacea,* is a parasitic copepod that lives in freshwater. The host range of *L. cyprinacea* is wide (Hassan 2008, Avenant-Oldewage 2012), and infections can cause pathologies, developmental malformations, and death in fish and amphibian hosts (Avenant-Oldewage 2012, Kupferberg *et al.* 2009). The mortality can occur by hemorrhages, ulcerations, and secondary infections (Salinas *et al.* 2016). Infections of *L. cyprinacea* had been reported only in species of fish in Turkey (Geldiay and Balık 1974, Burgu *et al.* 1988, Innal and Avenant-Oldewage 2012, Innal *et al.* 2017). Since the first description of *L. cyprinacea* in Turkey, several studies have
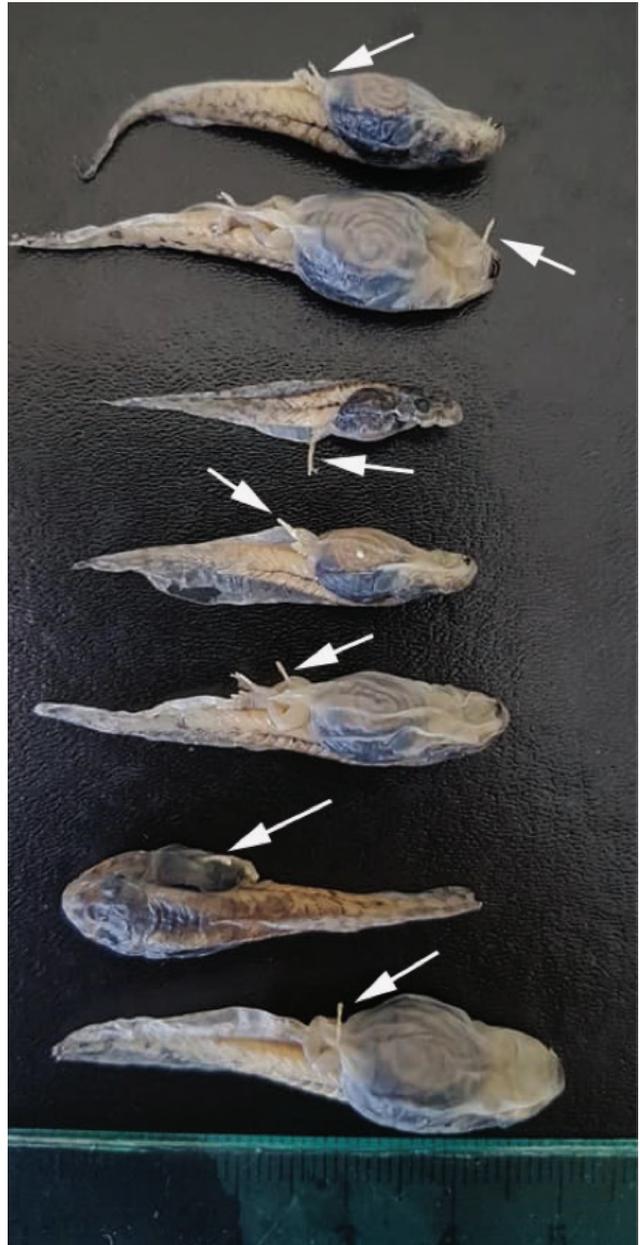

Figure 2. *Pelophylax bedriage* parasitized by anchor worm *Lernaea cyprinacea* (arrows).

demonstrated that the parasite is widespread and it has been identified as a possible threat to the native fish stocks. Our pathological findings were in agreement with those of previous studies.

The prevalence and mean intensity levels of *Lernaea cyprinacea* in *Pelophylax bedriagae* from Karpuzçay Creek were 70% and 1.14 parasites per infected tadpoles, respectively.
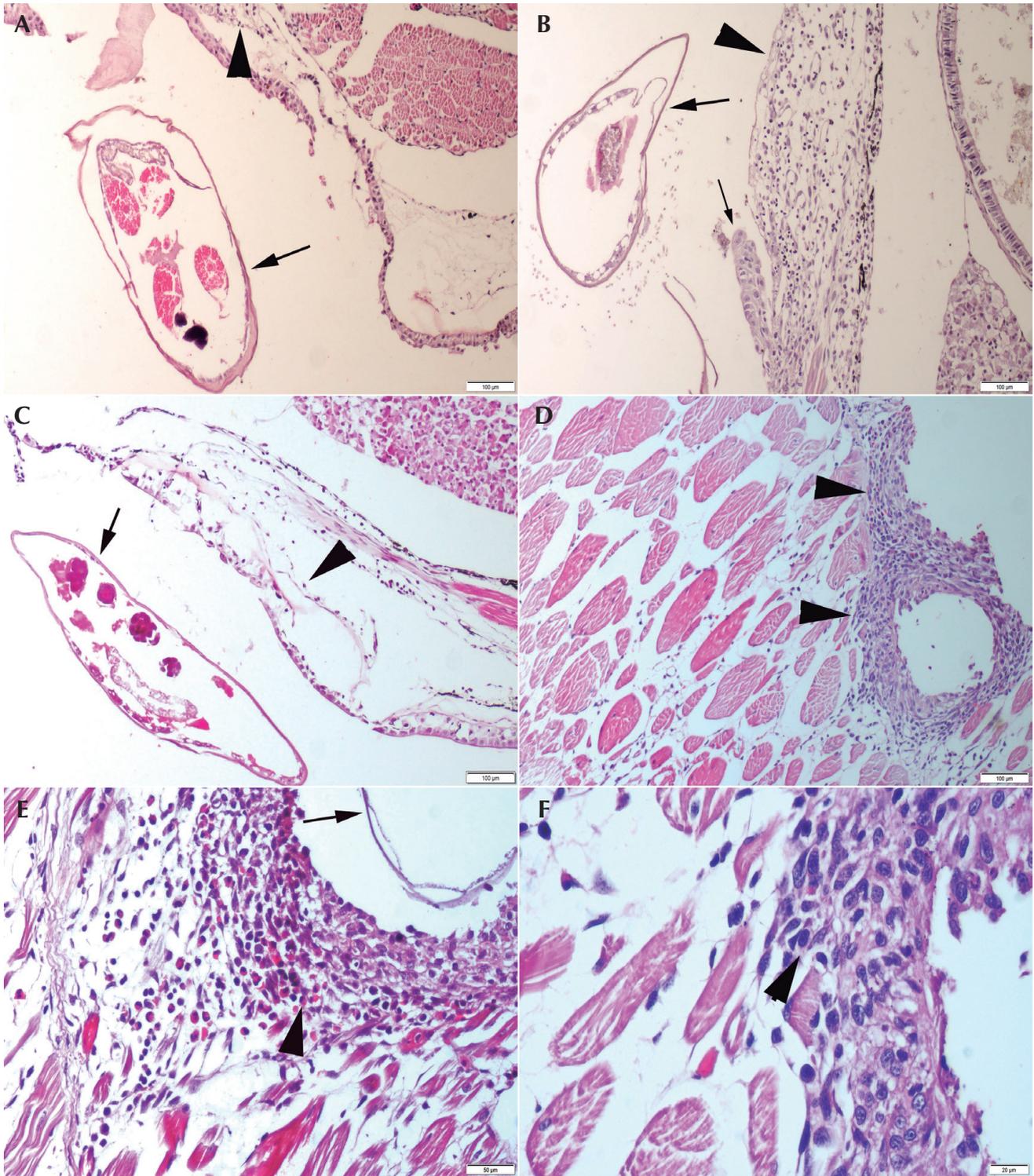
Figure 3. Histopathological appearance of the sites of parasite attachment. (**A**) Parasite section (arrow) and inflammatory reaction (arrow head) with ulcer (thin arrow) in the skin of the young frog, HE, Bar = 100 µm. (**B**) Parasite (arrow) attached to the abdominal skin causing marked inflammatory reaction (arrow head), gut and liver section, HE, Bar = 100 µm. (**C**) Parasite (arrow) and inflammation (arrow head, HE, Bar = 100 µm. (**D**) Two granulomas (arrow heads) in muscle, HE, Bar = 100 µm. (**E**) Marked inflammatory reaction composed by mononuclear cells and eosinophil leucocytes (arrow head) around the parasite (arrow), HE, Bar = 50 µm. (**F**) Higher magnification of the classical granuloma and fibrous tissue (arrow head) around the parasite, HE, Bar = 20 µm.

Levels of *L. cyprinacea* infestation in anuran species are highly variable, with a prevalence of 9.2% in 2006 and 10.5% in 2008 in *Rana boylii* Baird, 1854 in Northern California (Kupferberg *et al.* 2009). The prevalence of the parasite in *Lithobates catesbeianus* (Shaw, 1802) from the Río de los Sauces and Toledo Stream in Argentina was reported to be 53.3% and 58.33%, respectively (Salinas *et al.* 2016).

A general decreasing population sizes of *Peylophylax bedriagae* (Papenfuss *et al.* 2009) has been reported in Turkey (Başkale and Kaya 2012). We have demonstrated that *Lernaea cyprinacaea* has an adverse effect on the health of members of the population of *P. bedriagae* from Karpuzçay Creek. It has been long known that changing environmental conditions influence parasite-host interactions. Kupferberg *et al.* (2009) suggested that changing hydrology during the warm periods followed by lowered water levels and reduced daily discharge may expose high densities of frog larvae to parasite transmission. Increased prevalence of parasite infection may cause a reduction in individuals host fitness, and eventually may reduce the survival rates. Anthropogenic environmental changes have led to increased prevelance of diseases and the global decline in amphibians; clearly, further studies are required to understand the complex interactions between parasites and their amphibian hosts.

Acknowledgments

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