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COMMUNICATION

SHORT

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Lernaea cf. *cyprinacea* infection in agile frog (*Rana dalmatina*) tadpoles in Hungary

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ABSTRACT

The ectoparasitic copepod *Lernaea cyprinacea* (anchor worm) has more than 100 host species among teleost fishes and affects cyprinids both in fish farms and natural waters. In addition, while *L. cyprinacea* infection in amphibians has been recorded in Asia, North and South America, there is no data available in the literature on their presence in Europe. In this study, we first reported *L. cf. cyprinacea* parasitising an anuran tadpole in Europe. Specimens of *L. cf. cyprinacea* were observed attached to a tadpole of the agile frog (*Rana dalmatina*), which was caught during fishing of crucian carp fingerlings (*Carassius carassius*) from a small fish pond in Hungary during the summer of 2012. The infected tadpole was collected from a rearing pond, where juvenile crucian carps were kept. The tadpole was inspected in the laboratory, and digital photos were taken. The parasites were found attached to the body-tail junction and to the leg of the tadpole (at Gosner stage 41). The parasite species was identified as *L. cf. cyprinacea* based on morphological traits.

KEYWORDS

fish fingerlings, freshwater, tadpole, anuran, Europe

The parasitic species of the *Lernaea* family belongs to the subclass Copepoda. The family Lernaeidae includes nearly 120 species (Walter and Boxshall, 2018). *Lernaea* spp. are distributed worldwide from Europe to Asia, through North and South America (Kabata et al., 1979; Hoffman et al., 1999).

The anchor worm *Lernaea cyprinacea* L., 1758 is native to Eurasia. Nowadays, the species has a wider global distribution including Africa, Australia, North and South America, where it is considered as an invasive species (Kabata, 1979; Hall 1983; Kennedy, 1993; Hoffman 1999). *L. cyprinacea* can infect a wide range of teleost fish species (Baur, 1962; Hoffman, 1999). In Hungarian waters, this species most frequently infects common carp *Cyprinus carpio* L., grass carp *Ctenopharyngodon idella* (Valenciennes), silver carp *Hypophthalmichthys molitrix* (Valenciennes) and bighead carp *H. nobilis* (Richardson) in fish farms, especially under polyculture rearing conditions. Besides, heavy infestations may occur on both wild and 2-3-year-old fish in aquaculture facilities (Molnár and Baska, 2017).

Although teleost fishes are the most common hosts of anchor worms, there are published data on the occasional occurrence of *Lernaea* infection in amphibians (anurans and caudates) (Table 1). To the best of our knowledge, there are no reports of such infestations in Europe.

Water temperature between 23 and 30 °C is the most suitable for the development of *L. cyprinacea* (Baur, 1962). First, free-floating nauplii develop from the released eggs.

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| umphibiums | | |
|---|-----------|---------------------------------|
| Anuran host | Country | Reference |
| Ambystoma mexicanum Shaw & Nodder, 1798 | Uruguay | Carnevia and Speranza (2003) |
| <i>Boana cordobae</i> Barrio, 1965 | Argentina | Alcalde and Batistoni (2005) |
| Boana faber Wied-Neuwied, 1821 | Brazil | De Lima et al. (2021) |
| <i>Hylarana chalconota</i> Schlegel, 1837 | Indonesia | Ming (2001) |
| Aquarana catesbeiana Shaw, 1802 | Argentina | Salinas et al. (2016) |
| Aquarana catesbeiana Shaw, 1802 | USA | Matson (2019) |
| <i>Aquarana clamitans</i> Latreille, 1801 | USA | Stunkard and Cable (1931) |
| <i>Lithobates pipiens</i> Schreber, 1782 | USA | Tidd and Shields (1963)* |
| Pelophylax bedriagae Camerano, 1882 | Turkey | Innal et al. (2019) |
| <i>Physalaemus cuvieri</i> Fitzinger, 1826 | Brazil | De Lima et al. (2021) |
| Amerana boylii Baird, 1854 | USA | Kupferberg et al. (2009) |

 Table 1. Published data of Lernaea cyprinacea infection in amphibians

* = artificial infestation.

This developing stage is followed by copepodit stages, which have five moulting cycles. During the last two developing stages, the cyclopoid larvae are partly free-living, but after two moults, they can infect hosts attaching mostly to the gills (Grabda, 1963). Cyclopoid males fertilise the females, then all male individuals die. Only females can further develop in hosts. Females undergo significant morphological changes after attaching to the host. This includes body elongation and the formation of two ovaries at the tail end (Grabda, 1963; Molnár, 1977, 2003; Molnár and Baska, 2017). Typically, an inflammatory process of the affected tissues of the host starts where the female copepods are attached, followed by the formation of small ulcers. These lesions may approach ulcerative dermatitis, especially after the detachment of Lernaea sp. (Molnár and Baska, 2017).

The cyclopoid larva of *Lernaea* sp. can attach to various body parts of the fish host e.g. the skin, fins or gills. The infection can cause haemorrhages, ulceration, muscle necrosis and intense inflammatory response of the affected organs (Khalifa and Post, 1976; Berry et al., 1991; Silva-Souza et al., 2000; McAllister et al., 2011; Sayyadzadeh and Roudbar, 2014). In addition, the wounds can expose the fish to secondary viral and bacterial infections (Khalifa and Post, 1976; Berry et al., 1991).

This is the first report of lernaeosis in a wild tadpole in Europe. The affected tadpoles were collected during fishing for juvenile crucian carps using tulle nets in a small rearing pond with a foil bed $(2 \times 7 \times 0.7 \text{ m})$ in Hungary near Gödöllő $(47^{\circ}59'27'' \text{ N}, 19^{\circ}36'98'' \text{ E}, 244 \text{ m} \text{ above sea level})$ on 21 August 2012.

Among the fish fry (~200 individuals, body length between 2 and 3 cm), we accidentally captured one agile frog tadpole at Gosner stage 41 (Gosner et al., 1960) with already developed hindlimbs that had three ovipositing copepod parasites on the body surface. The tadpole was separated from the fish and several photographs were taken with Nikon Coolpix 4500 digital camera.

Two parasite individuals were attached to the left-ventral side of the body-tail junction, while the third individual was anchored in the lower fifth of the right leg, above the tibia (Fig. 1). The inguinal attachment site reported here correspond to the "dead water zone" – the region on the body-tail junction where the hindlimbs develop (Liu et al., 1997). No visible tissue damage was observed on the frog. However, it is important to note that the crucian carp fingerlings in the foil-system pond were heavily infected with the parasite.

The parasite specimens were identified at the species level by using morphological traits. Based on the morphological characteristics, the adult female parasite on the body surface of the agile frog most closely resembled *L. cyprinacea* (Hoffman, 1976; Boxshall and Halsey, 2004). As the identification of the species cannot be confirmed without DNA-sequence-based characterization, we name the detected parasite *L. cf. cyprinacea*.

According to our knowledge, this was the first observation and description of *L*. cf. *cyprinacea* infection in an



Fig. 1. Lernaea cf. *cyprinacea* females with egg sacs on the surface of the body-tail junction (ellipse) and the right leg (arrow) of *Rana dalmatina* tadpole

amphibian species in Europe. Amphibians are among the most endangered taxa on Earth (Ceballos et al., 2020; Button and Borzée 2021) and such parasitic host extensions may put further pressure on their populations, particularly in aquacultural landscapes.

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