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First report of the parasitic copepod *Lernaea cyprinacea* (Copepoda: Lernaeidae) on gobioid fishes (Teleostei: Gobonellidae) in southern Europe

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Abstract – *Lernaea cyprinacea* is a non host-specific parasitic copepod known to infest many freshwater fish species. Outbreaks of infestations by this ectoparasite may cause mass mortality of parasitized fishes. *L. cyprinacea* has been found mostly on pelagic species. Records on small benthic fish species are less common. Especially rare are infestations of Gobioidei adapted to a benthic life style, with reports restricted to Asia and, in Europe, to the Ponto-Caspian region. Although it is cosmopolitan, *L. cyprinacea* has rarely been found in Italy. One of the few Italian localities with documented infestations is Lake Trasimeno, a lake with an economically important fishery. Although endoparasites of commercially interesting fish species in this lake are well documented, information about ectoparasites is rare. In May 2015, specimens of two gobioids – *Knipowitschia panizzae* and *Pomatoschistus canestrinii* – infested with *L. cyprinacea* were sampled at the south shore of Lake Trasimeno. Both gobies are not native to the lake. This is the first documentation of gobioid fishes as hosts of *L. cyprinacea* in Italy and in Europe (outside of the Ponto-Caspian region). Although both gobies are not optimal hosts (small size, short life expectancy) they have the potential to carry and to transmit the parasite in freshwater habitats, *e.g.* by unintentional introduction with fry of other fish species.

Keywords: *Lernaea* / Copepoda / *Knipowitschia* / *Pomatoschistus* / Italy / River Tiber basin / Lake Trasimeno

Résumé – Premier signalement du copépode parasite *Lernaea cyprinacea* (Copepoda: Lernaeidae) sur des poissons gobioides (Téléostéen: Gobioidei) dans le sud de l'Europe. *Lernaea cyprinacea* est un copépode parasite non spécifique de l'hôte, connu pour infester de nombreuses espèces de poissons d'eau douce. Les foyers d'infestation par cet ectoparasite peuvent causer une mortalité massive de poissons parasités. *L. cyprinacea* a été trouvée principalement sur des espèces pélagiques. Les données sur les petites espèces de poissons benthiques sont moins courantes. Les infestations de Gobioidei adaptés à un mode de vie benthique sont particulièrement rares, avec des signalements limités à l'Asie et, en Europe, à la région de Ponto-Caspienne. Bien que cosmopolite, *L. cyprinacea* a rarement été trouvée en Italie. L'une des rares localités italiennes avec des infestations documentées est le lac Trasimène, un lac avec une pêche économiquement importante. Bien que les endoparasites d'espèces de poissons commercialement intéressantes dans ce lac soient bien documentés, l'information sur les ectoparasites est rare. En mai 2015, des spécimens de deux gobioidés – *Knipowitschia panizzae* et *Pomatoschistus canestrinii* – infestés par *L. cyprinacea* ont été échantillonnés sur la rive sud du lac Trasimène. Les deux gobies ne sont pas indigènes au lac. Il s'agit de la première documentation de poissons gobioides comme hôtes de *L. cyprinacea* en Italie et en Europe (en dehors de la région Ponto-Caspienne). Bien que les deux gobies ne soient pas des hôtes optimaux (petite taille, espérance de vie courte), ils ont le potentiel de transporter et de transmettre le parasite dans les habitats d'eau douce, par exemple par introduction involontaire avec des alevins d'autres espèces de poissons.

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1 Introduction

Lernaeidae are cyclopoid freshwater copepods. The females are morphologically highly modified and adapted to a parasitic life style. The only cosmopolitan species is *Lernaea cyprinacea* Linnaeus, 1758, the "anchor worm". It is of Eurasian origin and was unintentionally introduced worldwide with parasitized cyprinid fish stocks (Tidd, 1934; Marina *et al.*, 2008; Avenant-Oldewage, 2012; Walter and Boxshall, 2018). After the females have burrowed into the skin of the host, their cephalothorax develops lateral processes as holdfasts, the name-giving "anchor". Outbreaks can cause mass mortality of parasitized fishes and severe commercial losses (*e.g.* Piasecki *et al.*, 2004; Boxshall and Defaye, 2008; Fast, 2014), but in natural fish populations of temperate regions this parasite is apparently not common (*e.g.* Cloutman, 1903; Piasecki *et al.*, 2004; Bednarska *et al.*, 2009). Nevertheless, global warming by climate change may favor the increasing occurrence of the thermophilic *L. cyprinacea*.

This parasite is adapted to freshwater hosts, generally fishes (Kabata, 1979; Piasecki *et al.*, 2004), but it also infests amphibians (Kupferberg *et al.*, 2009, Salinas *et al.*, 2016) and aquatic insects (McAllister *et al.*, 2011). Most infested fish species have a pelagic life style. Benthic species are less often infested (Djikanovic *et al.*, 2012; Koyun *et al.*, 2015). Especially Gobioidei, a clade of fishes adapted primarily to a benthic life style, are rarely infected by *L. cyprinacea* (*e.g.* Kvach, 2004; Nagasawa *et al.*, 2007; Kvach et al., 2015; Zhokhov *et al.*, 2017). All records to date of infested Gobioidei were from Asia and from the Ponto-Caspian region (Tab. S1).

Reports of *L. cyprinacea* in Italy in general (Fratello and Sabatini, 1972; Macchioni *et al.*, 2015) and from Lake Trasimeno in particular (Fratello and Sabatini, 1972) are scarce. A single record of *L. cyprinacea* from the pelagic fish species *Lepomis gibbosus* (Linnaeus, 1758) dates back to the early 1970s. In the following years no further records were documented. Most fish species of the lake are well studied, including their parasites (Lorenzoni *et al.*, 2002, 2010, 2015; Giovinazzo *et al.*, 2006; Pompei *et al.*, 2012). The finding of *L. cyprinacea* in Lake Trasimeno and in particular on the small benthic gobionellid fishes *Knipowitschia panizzae* (Verga, 1841) and *Pomatoschistus canestrinii* (Ninni, 1883) is therefore remarkable.

The present study reports (1) the first documentation of an infection with *L. cyprinacea* of two gobionellid species endemic to the Adriatic basin, (2) the first infestation with this parasite of gobioid species in Europe outside the Ponto-Caspian region and finally (3) an extension of host species for *L. cyprinacea*.

2 Materials and methods

2.1 Study locality

Fisheries are a highly important economic sector in Lake Trasimeno (43°06′ N, 12°07′ E), which boasts the largest group of professional fishers in inland Italian lakes and is an aquaculture center for fish breeding. It is the fourth largest lake in Italy (124 km^2) and meso-eutrophic with low water transparency (average Secchi disk depth = 1 m) (Giardino *et al.*, 2010). Because the lake is shallow (average depth: 4.7 m), thermal stratification is usually absent and the water temperature is always about the same as the air temperature: it can exceed 30 °C during summer (Lorenzoni *et al.*, 2010; Ludovisi and Gaino, 2010). The fish assemblage comprises 19 species, most of which are alien species and is dominated by cyprinids including tench (*Tinca tinca* (Linnaeus, 1758)), carp (*Cyprinus carpio* Linnaeus, 1758), goldfish (*Carassius* sp.) and rudd (*Scardinius hesperidicus* Bonaparte, 1845). Other common species are southern pike (*Esox cisalpinus* Bianco and Delmastro, 2011), perch (*Perca fluviatilis* Linnaeus, 1758), largemouth bass (*Micropterus salmoides* (Lacepéde, 1802)) and sandsmelt (*Atherina boyeri* Risso, 1810) (Lorenzoni *et al.*, 2010).

Sampling was conducted in May 2015 at the southeast shore of the lake near the large aquaculture center, where submerged vegetation dominates the bottom (Giovanardi *et al.*, 2010).

Comparative material was investigated from two natural populations: *K. panizzae* from the brackish waters of the abandoned salt-works of Zaule (45° 36′ N, 13° 46′ E) directly south of Trieste and *P. canestrinii* from the lower reaches of the karst river Jadro at Solin (43° 32′ N, 16° 29′ E) near Split. These samples were collected at the end of the 19th century, are deposited at the Ichthyological collection of the First Zoological Department (Vertebrata) of the Natural History in Vienna (NMW), and registered by the numbers NMW 29806-807, NMW 29809 (*K. panizzae*) and NMW 30371-373 (*P. canestrinii*).

2.2 Material

We investigated a total of 427 specimens of *K. panizzae* and *P. canestrinii*: 391 gobies from Lake Trasimeno (Italy), 86 from Zaule near Trieste (Italy) and 50 from the Jadro River near Split (Croatia). Samples from Lake Trasimeno were captured in May ($n = 182$) and November 2015 ($n = 209$).

From the specimens collected in Lake Trasimeno during the reproductive period in May 2015, 42 specimens were adult *K. panizzae* (mean 22.4 mm SL) (23 males and 19 females) and 140 specimens were adult *P. canestrinii* (mean 27.3 mm SL) (79 males and 61 females). Sex and maturity were determined based on the shape of the urogenital papilla (longer than wide in males, shorter and about as long as wide in females) and by inspecting the gonads.

Fish samples were caught under the permission of the state and regional institutions and were in accordance with Italian state laws on fisheries.

2.3 Methods

The fishes were captured using landing nets with a mesh width of 5 mm. They were anesthetized and subsequently euthanized by an overdose of 2-phenoxyethanol and stored in 6% formalin for several weeks. After passing through an ascending series of ethanol, the specimens were stored in 70% ethanol.

Fish were examined for the presence of parasites under a stereo microscope. Crustacean parasites found were carefully removed from the fish, transferred to a 1:1 mixture of

glycerine-ethanol (glycerol 99.5% AnalaR Normapur; ethanol 80%). The samples were kept in an incubator (Memmert, Germany) for 24 h at 40 °C to evaporate ethanol and condense glycerol. Objects were then placed on microscopic glass slides and mounted in pure glycerine under a cover glass. Micrographs and measurements were taken under a Nikon Eclipse Ni transmitted light microscope with a Nikon DS Ri2 camera.

Collected specimens of *L. cyprinacea* are deposited in the Collection Crustacea of the Natural History Museum in Vienna with the register number NHMW 26.266.

Information on infestation of gobioid species with *L. cyprinacea* and of gobioid species not infested but co-occurring with infested species listed in Table S1 was retrieved from the literature.

Parasitological prevalence (percentage of hosts infected with parasites) is based on Bush *et al.* (1997).

3 Results

We found 4.8% of *K. panizzae* ($n = 2$) and 5% of *P. canestrinii* ($n = 7$), collected in Lake Trasimeno in May 2015 to be infested by *L. cyprinacea*.

Two females of *K. panizzae* were infested. The parasites were attached (i) at the right side of the anal fin base close to the origin of the fin ($n = 1$); (ii) between the urogenital papilla and the origin of the anal fin (Fig. 1). Five male and two female *P. canestrinii* were infested. The parasites were attached to the males at the (i) ventral side of the head ($n = 2$); (ii) ventral side of the abdomen at the origin of the pelvic fins (sucking disc) ($n = 1$) (Fig. 1); (iii) internal origin of the pectoral fin ($n = 1$) and (iv) in the opercular chamber ($n = 2$). The parasites were attached to the females at the internal origin of the pectoral fin ($n = 2$). No host was infested by more than one parasite.

Measurements of the four *L. cyprinacea* specimens are provided in Table 1.

None of the specimens sampled in Lake Trasimeno in November 2015 and none from the natural habitats (*K. panizzae* from Zaule near Trieste and *P. canestrinii* from Jadro River near Split) were infested.

The literature survey revealed that nine gobioid species from Asia and from the Ponto-Caspian region were reported as being infested by *L. cyprinacea*. At the same time, five gobioid species sympatrically occurring with parasitized fish species were not infested (Tab. S1). Nearly all these documentations of infestations of Gobioidei were based on single records only and not recorded a second time.

4 Discussion

K. panizzae and *P. canestrinii* are euryhaline (5–20‰) and short-lived (about one year) gobies endemic to the Adriatic region (summarized in Miller, 2004). The two species were introduced unintentionally into Lake Trasimeno, where they established large reproducing populations (Borroni, 1976; Freyhof, 1998). We found a low prevalence (~5%) of the ectoparasite *L. cyprinacea* for both goby populations. There are no records on infestation of these two gobionellid species in their natural distribution area. This is probably because both prefer habitats of low salinity, an environment not suitable for the strictly freshwater parasite *L. cyprinacea*.

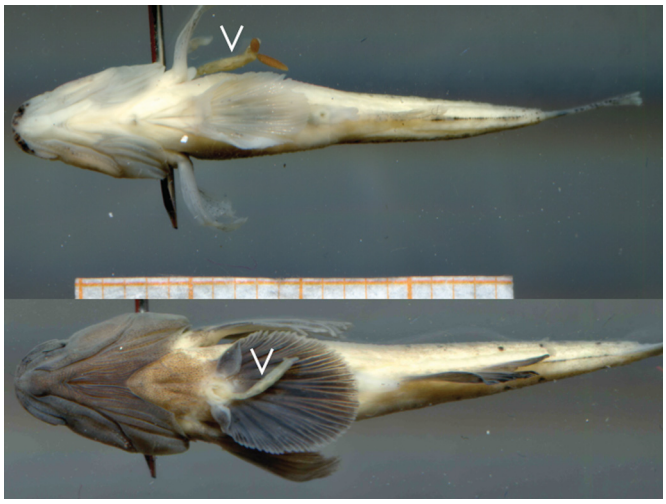

Fig. 1. *Lernaea cyprinacea* (white arrow heads) attached to host *Pomatoschistus canestrinii*; above female, below male. Female: parasite is attached medially on left pectoral fin base. Male: parasite is attached on origin of pelvic fins (united to a sucking disc). Hosts are shown in ventral view. Scale = 20 mm. Photos: A. Gabriel.

A first record of an unidentified Lernaeidae (as *Lernaea* sp.) for Lake Trasimeno was reported in the 1960s from the South European roach *Sarmarutilus rubilio* (Bonaparte, 1837) (as *Rutlius rubilio*) as a host (Cianficconi, 1966). The first and only confirmed occurrence of *L. cyprinacea* in this lake dates back to the early 1970s, when this ectoparasite was detected on the introduced pumpkinseed *L. gibbosus* (Fratello and Sabatini, 1972). Unidentified Lernaeidae were found recently on introduced largemouth bass (*M. salmoides*) (Dr. M. Natali, pers. communication). From these few records we conclude that outbreaks of laerneosis are rather uncommon in Lake Trasimeno. This corresponds to our finding of an apparently low (5%) infestation rate of the two gobionellid lake populations.

Apart from unclear synonymies, the present material was assigned to *L. cyprinacea*. It can be easily distinguished from *L. parasiluri* Yamaguti, 1939 by its second pair of head processes, from *L. esocina* (Burmeister, 1833) by its distinctly unequal anterior and posterior head processes (anchor) (comp. Bykhovskaya-Pavlovskaya *et al.*, 1964).

The body dimensions of females are highly variable in *L. cyprinacea*. This has led to different suggestions about (sub) species or geographic delimitation by some authors (comp. Demaree, 1967; Nagasawa *et al.*, 2007; Stavrescu-Bedivan *et al.*, 2014). The dimensions measured in our sample are within the range given for North America (Tidd, 1933; Demaree, 1967), *i.e.* 1.4–11.7 mm total length, and also similar to Japanese forms (5.8–8.0 mm; comp. Demaree, 1967). Both are assumed to be smaller than those from Europe and Asia, which Bykhovskaya-Pavlovskaya *et al.* (1964) reports as 9–22 mm, and other authors (for Europe) as 9–11.7 mm (comp. Demaree, 1967). In the future we hope to study a higher number of these parasites from both fish species based on newly collected material and also to obtain material suitable for molecular studies to compare it with sequences from other findings.

Sympatrically occurring fish species often show divergent infestation rates by *L. cyprinacea*. This was linked to several

Table 1. Measurements of four individuals of *Lernaea cyprinacea* of the host *Pomatoschistus canestrinii* from Lake Trasimeno, Italy; in mm.

	Ind. A	Ind. B	Ind. C	Ind. D
Lateral extent anchor	1.55	1.82	2.39	1.93
Length cephalothorax + trunk	3.65	5.64	4.92	6.49
Length abdomen	0.57	0.63	1.09	0.52
Total body length	4.22	6.27	6.01	7.01
Width posterior end of trunk	0.85	0.91	1.39	0.99
Length egg sac	1.55	–	–	1.95
Length egg sac	1.43	–	–	–
Length progentital prominence	–	–	–	0.31

extrinsic and intrinsic factors such as resistance against infection (Shariff and Roberts, 1989; Stavrescu-Bedivan *et al.*, 2014), different trophic behavior (Alam *et al.*, 2012; Iqbal *et al.*, 2012), temperature fluctuations (Raissy *et al.*, 2013; Stavrescu-Bedivan *et al.*, 2014), hydrodynamic conditions (Medeiros and Maltchik, 1999), microhabitat differences (Kadlec *et al.*, 2003; Stavrescu-Bedivan *et al.*, 2014; Innal *et al.*, 2017) or small size (Piasecki *et al.*, 2004; Stavrescu-Bedivan *et al.*, 2011; Innal *et al.*, 2017). Our literature survey revealed that predominantly pelagic fish species were targets of *L. cyprinacae*, less often small benthic species such as Cobitidae, Cottidae or Gobiidae (*e. g.* Pónyi and Molnár, 1969; Amin, 1981; Djikanovic *et al.*, 2012; Koyun *et al.*, 2015). This possibly reflects a combination of different size and microhabitat segregation of sympatrically occurring fish species. This is supported by Raibaut *et al.* (1998), who attributed a low copepod-host ratio to a combination of a benthic life style and small size of fishes, specifically also referring to Gobiidae. This combination of life style and size probably explains why freshwater gobies are rarely infected by *L. cyprinacea* (*e.g.* Kvach, 2002; Nagasawa *et al.*, 2007; Zhokhov *et al.*, 2017) (Tab. S1). This ectoparasite, however, has been found on many freshwater species of several fish families (Amin, 1981; Koyun et al., 2015, Sánchez-Hernández, 2017; Waicheim *et al.*, 2017) but only rarely on gobioid species (Tab. S1). Although occurring sympatrically with infected fishes, gobioid fishes were often not infested by *L. cyprinacea* (*e.g.* Kritscher, 1975; Kvach, 2004; Dalu *et al.*, 2012; Krasnovyd *et al.*, 2012; Kvach *et al.*, 2014, 2015; Zhokhov *et al.*, 2017) (Tab. S1).

Larger fishes often carry many parasites (*e.g.* Whitaker and Schlueter, 1975). We attribute the low host-parasite ratio in Lake Trasimeno in general, and the infestation of the gobies by only a single parasite in particular, primarily to the small size of the fish (<5 cm) and less to their benthic life style. All infested gobies of Lake Trasimeno were adults. An infestation by more than one parasite may well be lethal for these small fishes because the tissue penetration by the parasite causes severe damage (Piasecki *et al.*, 2004; Boxshall and Defaye, 2008; Avenant-Oldewage, 2012; Stavrescu-Bedivan *et al.*, 2014). Moreover, the life span of both species is too short (about one year) to allow these female parasites to hibernate on their hosts as they do on perennial species (Hossain *et al.*, 2013). We therefore we assume that *K. panizzae* and *P. canestrinii* are not optimal hosts for *L. cyprinacea*.

Because juvenile *L. cyprinacea* are unable to complete their development at temperatures below 20 °C, outbreaks generally occur at higher temperatures (Kupferberg *et al.*, 2009; Hossain *et al.*, 2013). Most favorable for the development of the parasite are temperatures ranging from 25 to 28 °C (Piasecki *et al.*, 2004), 24–29 °C after Shields and Tidd (1968) and 26–30 °C after Hossain *et al.* (2013). Similar high water temperatures are reached in Lake Trasimeno from May to September but may extend into October (Ludovisi and Gaino, 2010; ARPA Umbria, 2018). The annual average water temperature in the lake has increased since the late 1980s by 1 °C in summer and by 1.3 °C in spring (March–May) (0.65 °C per decade from 1988 to 2006) (Ludovisi and Gaino, 2010). Annual average air temperature in the Lake Trasimeno basin is predicted to rise between 2 °C (min.) and 4.5 °C (max.) until 2090 (Ludovisi *et al.*, 2013), which is in agreement with temperature changes predicted for southern Europe (Intergovernmental Panel on Climate Change (IPCC), 2007). Therefore, infestation periods may last distinctly longer in shallow lakes such as Lake Trasimeno, where water temperature is nearly identical to the air temperature (Lorenzoni *et al.*, 1993; Ludovisi and Gaino, 2010). Host-parasite interactions are affected both by temperature and other climate-driven variables such as changing water level, reduced precipitation, eutrophication or habitat loss (Marcogliese, 2001, 2008, Marcogliese *et al.*, 2016). All these effects are currently already distinctly altering the environment around Lake Trasimeno (Ludovisi and Gaino, 2010; Ludovisi *et al.*, 2013). Nevertheless, temperature is the master factor for ectotherms in aquatic environments (Webb and Nobilis, 2007; Narum *et al.*, 2013). This makes it likely that, due to rising temperatures, more generations and larger populations of parasites will influence host-parasite interactions in Lake Trasimeno; higher temperatures are believed to favor higher infestation levels of *L. cyprinacea* (Kupferberg *et al.*, 2009).

Conclusions

Two single records of *L. cyprinacea* in Lake Trasimeno date back to the late 1960s and early 1970s, respectively, and because the host-parasite ratio documented in this study is also low, we assume that the parasite is still rare in the lake. The period of successful development of *L. cyprinacea* in this lake is limited to May–October. As a result of climate change, a combination of climate-driven effects such as temperature, changing water level, eutrophication or habitat loss will likely influence host-parasite interactions in Lake Trasimeno. Increasing temperatures will extend the reproductive period and, consequently, infestation rates of this parasite could

increase. Although *K. panizzae* and *P. canestrinii* are not optimal hosts for this parasite based on their small size and short life span, they are carriers, potential transmitters and a potential refuge for the copepod ectoparasite *L. cyprinacea* because of their cryptic life-style.

Author's contributions

HA conceived the study, provided ichthyological advice and wrote the manuscript. RK and HS morphologically identified the parasite specimens and contributed to writing the manuscript. AG and AB identified the fish specimens, contributed to the data sampling and prepared photographs. LP and ML collected the specimens and contributed to writing the manuscript. All authors read and approved the final version of the manuscript.

Supplementary Material

Supplemenatary data.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2018022/olm.

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