First record of *Lernaea cyprinacea* (Linnaeus, 1758) (Copepoda: Cyclopoida) on *Betta splendens* in Brazil

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**ABSTRACT**

The aim of this work is to bring this first record of *L. cyprinacea* in *B. splendens*. The anchor worm, also known as *L. cyprinacea* is an ectoparasitic copepod that can result in the mortality of the host by causing hemorrhages, ulcerations, and secondary infections. *L. cyprinacea* is widely distributed in world. Previous reports are restricted cyprinacaea on other fishes. The study detected, for the first time, the occurrence of lernaeosis in *Betta splendens* of the Alagoas state, Brazil. The occurrence was *L. cyprinacea* was detected on the narines, gills, soft parts the dorsal and anal fin of *Betta splendens*. It brings concern the occurrence of the anchor worms *Lernaea cyprinacea* in a cultivate because this exotic species is able to spread and to parasitize other species of fishes.

**Keywords:** Anchor worms. Copepod. ornamental fish. ectoparasite.

**RESUMO**

O objetivo deste trabalho é trazer este primeiro registro de *L. cyprinacea* em *B. splendens*. O *L. cyprinacea*, também conhecido como: verme âncora, é um copépode ectoparasitário que pode causar mortalidade do hospedeiro, causando hemorragias, ulcerações e infecções secundárias. O *L. cyprinacea* é amplamente distribuído no mundo. Relatos anteriores são restritos a cyprinacaea em outros peixes. O presente estudo encontrou pela primeira vez, a ocorrência de lernaeose in *Betta splendens* of the Alagoas state, Brazil. A ocorrência de *L. cyprinacea* foi detectada nas narinas, brânquias, partes moles da barbatana dorsal e anal de *Betta splendens*. Preocupa a ocorrência da verme âncora, *Lernaea cyprinacea*, em um cultivo, porque esta espécie exótica é capaz de se espalhar e parasitar outras espécies de peixes.

**Palavras-chave:** vermes âncora. copepodo. peixe ornamental. ectoparasita.
1 Introduction

With the rapid developments of commercial aquaculture farming, diseases become the major constraint of the development of aquaculture globally. According to Santos et al. (2013), most of the pathogens are opportunistic to infect cultured fish once the environment is deteriorated. Parasitic diseases constitute impact significant of diseases affecting warm water fish in world, that mainly affect the fish health, growth and survivability (Salinas et al., 2019).

In recent decades, the relevance of studies related to parasites and other pathogens of aquatic organisms, especially those hosts with potential for cultivation and marketing, given the significant increase in these activities in Brazil and worldwide (Luque, 2004; Santos et al., 2017).

Lernaea cyprinacea (Linnaeus, 1758) is among most known species of the copepods exotic that parasite fish in Brazil (Tavares-Dias et al., 2015). The parasite L. cyprinacea and most exotic species have been a worldwide environmental concern.

Introduced during the 80’s via the common carp Cyprinus carpio Linnaeus, 1758, L. cyprinacea diffusion began at the northeast area, spreading to southeastern, mid-west and reaching the all regions of Brazil (Fortes; Hoffmann; Scariot, 1998). Its economic importance in Brazilian fish farm is increasing due to numerous epizootics occurring among the most important farmed fish species. However, there are no official reports of L. cyprinacea induced fish mortalities from Brazil.

Lernaeids occur in freshwater fishes both in natural water systems and in aquaculture environments. They are notorious killers specifically of small fishes, and re the caused of great economic loss. They are suspected of transmitting viroses and/or bacteria which result in secondary infection.

Soares et al. (2018) reports that L. cyprinacea is one of the most common parasites in wild and aquaculture fishes of Brazil South region and produces serious economic losses to the fish industry. Therefore, the extensive geographical distribution can not be directly atributed to dispersion by natural means. The human activity could have an indirect influence, by the scaering of L. cyprinacea when using live specimens of fish as bait in other lakes and rivers.

Lernaea cyprinacea is an opportunistic ectoparasite of many freshwater fish species and non-fish hosts like amphibians. Adult females of L. cyprinacea attach to exposed body surfaces of host fish including the head, dorsal, ventral, and caudal regions, but most frequently to the bases of fins. Their aggregation on body sites induces severe local damage with adverse consequences to host function and survival (Barson; Mulonga; Nhiwatiwa, 2008).

Worldwide, the popularity of fish keeping has grown by 14% annually since the 1970s and currently over 1 billion individual fish are traded internationally each year (Maceda-Veiga et al., 2016). The global aquarium fish trade (freshwater and marine ornamental fish plus accessory products) is worth c. US $15–30 billion (Penning et al., 2009). Around 500 million fishes are traded annually by 145 countries, of which 80-85% is tropical species (Kumar et al., 2016).

Several studies have been developed to improve the knowledge on the biology and management during the rearing of ornamental species, such as: feeding management (Santos et al., 2014; Santos et al., 2019), techniques and system of production (Sales et al., 2016), nutrition (Thongprajukaeuw et al., 2011; Zuanon; Salaro; Furuya, 2011; Torres-Valencia; Imues-FIGUEROA; Bucheli-Fuelantala, 2016), reproduction (Gianecchini; Massago; Fernandes, 2012), behavior (Maclaren; Wisniewski; Maclaren, 2018), laviculture management (Fabregat et al., 2017), among others.

The number of species for ornamental purposes is high, and there is lack of information about diseases and parasites, even for those produced globally on a large scale. However, despite the variety in fresh water fish species, certain species are extremely popular amongst hobbyists, including the tropical fish Betta splendens Regan, 1910)

This fish is an extremely popular ornamental fish among hobby aquarists. It has na interesting behavioral repertoire, particularly where male aggression and territoriality are concerned (Pleeging; Moons, 2017).

Betta splendens is an important species in the Brazil and international ornamental fish trade due its coloured patterns and singular behaviour. Reproduction and larviculture are a challenge for many producers due to the lack of scientific information, mainly on the diseases and parasites.

The Siamese fighting fish (Betta splendens) is a member of the Labyrinth fish family (Belontiidae). B. splendens in Latin refers to brilliant warrior, indeed a suitable name, since males of the colourful species
dissolved oxygen concentration were determined by using a YSI water meter (Professional Plus).

All experimental procedure followed the Animal Ethics Committee of the Federal University of Alagoas recommendations (n° 07/2018- CEUA/UFAL).

3 Results and discussion

A detailed description of *L. cyprinacea* is presented, including the corresponding measurements (Figure 1 and 2).

**Figure 1** – *Betta splendens* parasitized by anchor worm, *Lernaea cyprinacea* (arrows) in gills (a) and dorsal fin (b).

In this study, the parasite considered as the cause of lernaeosis in ornamental exotic fish species, *Betta splendens*, was *L. cyprinacea*. The morphological characteristics observed here are typical of *L. cyprinacea* (DEMAREE JR, 1967; THATCHER, 2006; KOYUN; ULUPINAR; MART, 2015, HOSSAIN; FERDOUSHI; RUPOM, 2018). It has an elongate, tubular body, which is differentiated into

2 Materials and methods

Our study area is located in the municipality of Maribondo, Alagoas State, Brazil, at local from which *Betta splendens* has been collected (-9.591395 N, -36.307179 E). The local study is characterized by a humid, tropical climate. The maximum temperature is 33 °C with an average minimum 18 °C in moth of the july of 2018.

The Fishes, samples of *Betta splendens* (n = 24), only males, were colleted of collective field tank of fishes, in Maribondo, Alagoas, Brazil. Posteriorly, examined for the presence of *L. cyprinacea* after collection. Areas around the fins, nostril, operculum and buccal cavity were examined with a dissection microscope. Fishes were put on ice and immediately transported to the laboratory.

To extract crustaceans, fish were subjected to immersion for 10-30 minutes with 1% sodium chloride. Parasites that did not detach on their own were removed with dissecting forceps. Once removed, the affected area was treated with a mild disinfectant.

The ectoparasites were preserved in ethanol 70% for their subsequent identification in the Laboratory of the Aquaculture, Federal University of Alagoas (UFAL, Alagoas, Brasil). The *L. cyprinacea* was identified based on published references following Thatcher (2006).

The review of the hosts and geographical distribution in range of the anchor worm was made from national and international scientific journals. In the case of Brazil, available technical reports, theses, and congress summaries were also included.

Water quality parameters were measured at the surface at each field tank, in original local of animals management. Temperature (°C), nitrite, pH and perform an elaborate aggressive display when provoked (MOTLagh et al., 2012).

The species natural habitats are shallow freshwater ponds with muddy bottoms or flooded rice paddy fields. This vegetation provides cover against fish-eating birds, like egrets, herons, kingfishers and others predators (BERTucci; MATOS; DABELSTEEN, 2013). They can breathe oxygen from the surface (due to their labyrinth organ), which enables the species to survive in low oxygen waters.

The present study reports the presence of *L. cyprinacea* parasitizing *Betta splendens* under captivity in the State of Alagoas, Brazil. A detailed description and previous records in other fishes in the Brazil, are provided.
a cephalothorax, thoracic region and abdomen. The worm’s head is distinctly round with antennae and antennules attach laterally on the anterior portion and a posterior anchor processes. Oral appendages and the first pair of swimming legs are situated in the ventral surface of the cephalothorax.

The second, third and fourth pairs of swimming legs are attached to the thorax, whereas the abdomen bears the fifth and sixth pairs of swimming legs. The abdomen is slightly broadened and curved at the posterior region. In similiar that reports from Magalhães (2006) studying *Astyanax bimaculatus* Linnaeus, 1758, in Minas Gerais, Brazil.

Figure 2 – Parasite anchor worm *Lernaea cyprinacea* (a) with eggs (b).

Gervasoni et al. (2018) observed the description general morphology, post metamorphic females like: elongated body segmented into cephalothorax, neck and genito-abdomen. In relation to cephalothorax composed of head, mouth structure, four cylindrical projections, lobes or horns and first pair of legs. Corroborating also the present study.

Already, Waicheim et al. (2017) related that the specimens of *L. cyprinacea* can be distinguished from other species of the genus by the following combination of characters: head with 4 large anchors frequently branched, dorsal pair much larger than ventral, dividing into 2 branches some distance from their bases, ventral pair usually simple, neck consisting of second to fourth leg-bearing segments, more or less circular in cross section, and genital pore near posterior extremity, fourth legs on hind body, abdomen conical usually displaced dorsally, and total length 10–20 mm.

Future changes in the aquatic environment may stress fish population even further which may lead to an increase in parasite load in up seven parasites per host) in this species, as Mancini et al. (2008) suggested. The current study supports the notion that *L. cyprinacea* can cause death of the hosts, due to intense inflammatory reaction in the subcutaneous tissue as observed. Mirzaei (2015) related that anchor worms can cause intense inflammation, leading to secondary bacterial and fungal infections. These secondary infections sometimes worsen and kill the fish. Larger numbers of parasites on the gill can interfere with respiration, causing death.

Therefore, future studies on parasitic fauna of in ornamental fishes on Brazil are essential to record host and parasite biology and the spread of this pathogen to natural water sources and to other ornamental fishes. Probablly, the infection caused in the bettas were caused by parasites that were parasitizing other fish and that were in the same environment, as observed in *Carassius auratus* and *Poecilia sphenops*, in the same fish farming environment. The fluctuation of temperature may contribute also to the parasitic outbreak.

The parasites can be removed manually by using tweezers as it can be seen with naked eye. However, this method is not practical and unrealistic if fish are heavily infested with huge amount of parasites.

Corroborating this statement, Vasagan et al. (2006) report that, *L. cyprinacea* is a common parasite infecting numerous species of freshwater fish and the common method of control is by using removed manually. However, the adult parasite penetrates the fish host and becomes deeply embedded in the tissues making it extremely difficult to remove them (FURTADO et al., 2019).

In the present study, a large parasite infestation in the gills region was verified. Notably harming the health and development of animals. However, *Lernaea cyprinacea* also was detected on the others parts:
naries, soft parts the dorsal and anal fin of *Betta splendens*.

Carriel (2014) reports that gills are of fundamental importance being involved in health maintenance, oxygen uptake and carbon dioxide release, as well as nitrogen waste and osmoregulation processes. Also, the gills are responsible for the basic acid balance and sensory function in tasting. Thus, they have a delicate structure and exposed to environmental changes. Thus, any change in gill structure will affect fish survival and performance. Gill infections cause some localised hyperplasia of the epithelial tissues and also cause intense epithelial proliferation which may seriously interfere with respiration and also support spread of bacterial infection (Shariff, 1986).

It was also observed that parasite aggregations around the eye cause lens destruction and blindness. Innal et al. (2017) report that Lernaeid copepods are among the most harmful parasites of freshwater fish, especially young fish, which may be killed by only a few parasites.

Diseased fish not only disturbs the nutrient balance in fish body but also make it unfit for nutritionally and pathologically. In Brazil there are few descriptions of parasitic fauna of farmed ornamental fish, as well as the pathogenesis caused by the parasites in their hosts.

In *Betta splendens*, the presence of *Lernaea cyprinacea* has never been reported in the scientific literature. This research is the first report of this parasite in *Betta splendens* in the world. Information such as this is necessary to ensure the production of healthy animals as a subsidy to prevent losses in the activity of ornamental fish farming.

Worth mentioning that, parasites are normally present in the environment or in the body of the fish, and when there is an imbalance in the environment-host-parasite relationship, outbreaks of disease occur (Martins et al., 2002). However, it is known that the practice of introducing exotic species is frequent in aquariums and may be responsible for the spread of parasites, including anchor worm, endangering the entire population of farmed fish.

The average values for temperature, pH, nitrite, and dissolved oxygen during the collection period were 27°C ± 0.5°C; 6.8 ± 0.3; 0.15 mg.L⁻¹ ± 0.05 and 6.50 mg.L⁻¹ ± 0.25, respectively, at each field tank, in original local of animals management. These values remained stable and within the range recommended for the tropical species.

The monitoring of aquatic environments by measuring the density of parasite vectors in the water is very important. Unsustainable aquaculture, commercial and ornamental, has great potential to damage aquatic biodiversity and ecosystem functions and services.

Agostinho; Gomes; Pelicice (2007) account that exotic species can have a negative impact by transmitting diseases or etiological agents into native communities. Therefore, colonization of exotic pathogens in aquatic systems constitutes a permanent threat to the integrity of natural environments.

This ectoparasite can colonized native fish species and is dangerously invasive when environmental conditions are favorable, as reported in this work. Further research efforts are needed to prevent the role currently played by *L. cyprinacea* in ornamental fish.

The review of the hosts and geographical distribution in range of the anchor worm are expressed in table 1.

**Table 1** – Previous records of hosts for *Lernaea cyprinacea* in Brazil.

| Province/State | Location (Original name in portuguese) | Popular and scientific name Hosts | References |
|----------------|----------------------------------------|----------------------------------|------------|
| Alagoas        | municipality of Porto Real do Colégio and Igreja Nova | Tambaqui - Colossoma macropomum Cuvier, 1818 | FUJIMOTO et al. (2019) |
| Rio Grande do Sul | municipality of Antonio Prado | Lambari do rabo amarelo - Astyanax bimaculatus Linnaeus, 1758 | GALLIO et al. (2007) |
| Rio Grande do Sul | Pardinho River | Lambari do rabo vermelho - Astyanax fasciatus Cuvier, 1819 | DÜPONT; LOBO (2011) |
| Rio Grande do Sul | Arroio Creek | Carpa comum - Cyprinus carpio Linnaeus, 1758; Biru - Cyprinidae sp. | QUEROL et al. (2005) |
|                |                                        | Biru - Cyphocharax spliotus Vari, 1987; Biru - Cyphocharax sp. Hensel, 1870 |            |
|                |                                        | Biru - Steindacherina bioriata Braga & Azpelicueta, 1987 |            |

(Continue)
| Province/ State | Location (Original name in portuguese) | Popular and scientifc name Hosts | References |
|----------------|----------------------------------------|-----------------------------------|------------|
| Minas Gerais   | Paraíba do Sul river                   | Lambiri do rabo amarelo - Astyanax bimaculatus | MAGALHÃES (2006) |
| Minas Gerais   | San Francisco river (barragem três Marias) | Cumbaca - Franciscodoras marmoratus Reinhardt, 1874 | SANTOS; BRASIL-SATO (2006) |
| Paraíba        | municipality of Sumé                   | Traíra - Hoplias malabaricus Bloch, 1794 | BERNARDINO et al. (2016) |
| Paraná         | Tibagi river                           | Pacu - Piaractus mesopotamicus Holmberg, 1886 Carpa comum - Cyprinus carpio Linneaus, 1758 Piauçu - Leporinus macrocephalus Garavello & Britski, 1987 Piava quatro-pintas - Schizodon intermedius Garavello and Britski, 1990 Amboré - Schizodon nasutos Kner, 1858 Piranha amarela - Serrasalmus spiopleura Kner, 1858 Piau verdadeiro - Leporinus elongatus Valenciennes, 1850 Piau três-pintas - Leporinus friderici Bloch, 1794 Mandi bicus - Ichthyogaster labrosus Lüken, 1874 Curimbatá - Prochilodus lineatus Valenciennes, 1847 Tambacu - C. macropomum (fêmea) x P. mesopotamicus Bagre africano - Clarias gariepinus Burchell, 1822 Tilápia do Nilo - Oreochromis niloticus Linneaus, 1758 Matrinxã - Brycon cephalus Gunther, 1869 Pintado - Pseudoplatystoma corruscans Spix and Agassiz, 1829 | GABRIELLI; ORSI (2000) |
| Paraná         | municipality of Londrina               | Piava quatro-pintas - Schizodon intermedius Garavello and Britski, 1990 | SILVA-SOUZA; ALMEIDA; MACHADO (2000) |
| Paraná         | municipality of Laranjeiras do Sul     | Jundiá - Rhamdia quelen Quoy & Gaimard, 1824 Carpa comum - Cyprinus carpio Linneaus, 1758 Piavu – Leporinus sp. Tambacu - (C. macropomum (fêmea) x P. mesopotamicus) | CARRIEL (2014) |
| Paraná         | municipality of Cornélio Procópio      | Piraputangas - Brycon sp. | VALENTIM et al. (2003) |
| Rio de Janeiro | municipality of Itaocara                | Piabinha - Brycon insignis Steindachner, 1876 | FERNANDES et al. (2006) |
| São Paulo      | municipality of Franca                 | Pacu - Piaractus mesopotamicus Holmberg, 1886 Tambacu (C. macropomum (fêmea) x P. mesopotamicus) | TAVARES-DIAS et al. (2001) |
| São Paulo      | municipality of Franca                 | Piauçu – Leporinus macrocephalus Garavello & Britski, 1988 Pacu - Piaractus mesopotamicus Holmberg, 1887 | TAVARES-DIAS et al. (1999) |

(Continuation)
### Province/State | Location (Original name in Portuguese) | Popular and scientific name | Hosts | References
---|---|---|---|---
São Paulo | municipality of Guariba | Pauçu - Leporinus macrocephalus Garavello & Britski, 1987 Pacu – Piaractus mesopotamicus Holmberg, 1887 Carpa comum - Cyprinus carpio Linnaeus, 1758 Tambacu - (C. macropomum (fêmea) x P. mesopotamicus) | | SCHALCH; MORAES (2005)
São Paulo | municipality of Jaboticabal | Pacu – Piaractus mesopotamicus Holmberg, 1887 | | MARTINS et al. (2002)
São Paulo | Taguari river, municipality of Taquarituba | Lambari – Piararchus stramineus Eigenmann, 1908 | | NARCISO et al. (2019)
São Paulo | Taguari river, municipality of Taquarituba | Saguarí curto - Steinadchernerina insculpta Fernandez– Yepez, 1948 | | ACOSTA et al. (2013)
São Paulo | municipality of Pirassununga | Tambacu - Colossoma macropomum Cuvier, 1818 | | BASTOS; SÃO CLEMENTE; LIMA (1996)
Santa Catarina | municipality of Florianópolis | Plati - Xiphophorus maculatus Günther, 1866 Molínemia negra – Poecilia sphenops Valenciennes, 1846 | | PIAZZA et al. (2006)
Santa Catarina | municipality of Joinville, Blumenau e Ituporanga | Carpa comum - Cyprinus carpio Linnaeus, 1758 | | GHIRALDELLI et al. (2006)
Santa Catarina | municipality of Itajai | Jundia - Rhamdia quelen Quoy & Gaimard, 1824 | | FURTADO et al. (2019)
Sergipe | municipality of Neópolis and Propriá | Lambiri - Astyanax bimaculatus Linnaeus, 1758 | | VASCONCELOS et al. (2013)
Sergipe | municipality of Aracaju | Peixe japonês - Carassius auratus Linnaeus, 1758 Cascudo- hypostomus sp. Molínesia negra – Poecilia sphenops Valenciennes, 1846 Guppy - Poecilia reticulata Peters 1859 Cauda de espada - Xiphophorus helleri Haeckel 1848 | | ASSIS; CAVALCANTE; BRITO (2014)
Sergipe | municipality of Propriá (San Francisco river) | Tambacu – Colossoma macropomum | | FUJIMOTO et al. (2019)
Mato Grosso do Sul | Great Dourados region | Patinga – (Piaractus mesopotamicus x Piaractus brachyptomus Cuvier 1817) Tambacu – (C. macropomum (fêmea) x P. mesopotamicus) | | ISHIKAWA et al. (2016)
Mato Grosso do Sul | Great Dourados region | Tambacu – (C. macropomum (fêmea) x P. mesopotamicus) | | PEREIRA (2015)

### 4 Conclusion

In conclusion, this research on the parasite fauna of *Betta splendens* is the first report conducted on an *Lernaea cyprinacea* was detected on the narines, soft parts the dorsal and anal fin of this host. This new occurrence and the review presented on the previous records of the parasite in Brazil will provide the basis for further scientific studies on parasites fish tropical.

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