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 ocurrence 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 cultive 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 em Betta splendens, provinda do Estado de Alagoas, Brasil. A ocorrência de L. cyprinacea foi detectada nas narinas, brânquias, partes moles da barbatana dorsal e anal de Betta splendens. Preocupa a ocorrência do 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 to become the major constraint of the development of aquaculture globally. According Santos *et al.* (2013) most of the pathogens are opportunist 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* difusion 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 ocurring 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 secundary 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 (THONGPRAJUKAEW *et al.*, 2011; ZUANON; SALARO; FURUYA, 2011; TORRES-VALENCIA; IMUES-FIGUEROA; BUCHELI-FUELANTALA, 2016), reproduction (GIANNECCHINI; 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 (Belonitiidae). *B. splendens* in Latin refers to brilliant warrior, indeed a suitable name, since males of the colourful species

perform an elaborate aggressive display when provoked (MOTLAGH *et al*., 2012).

The species naturals 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.

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 ºC 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 coletive 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.

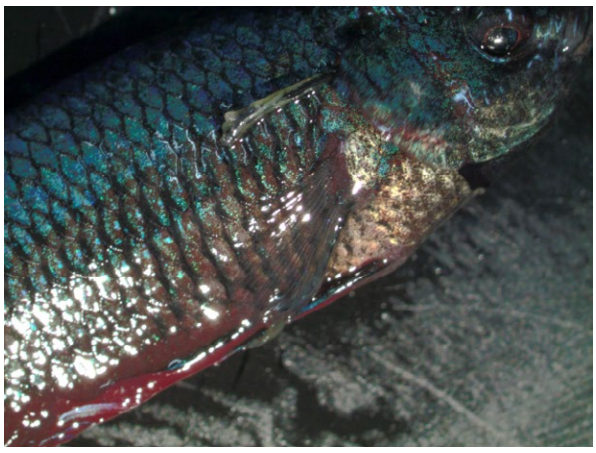
Water quality parameters were measured at the surface at each field tank, in original local of animals management. Temperature (°C), nitrite, pH and dissolved oxygen concentration were determined by using a'YSI water meter (Professional Plus).

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

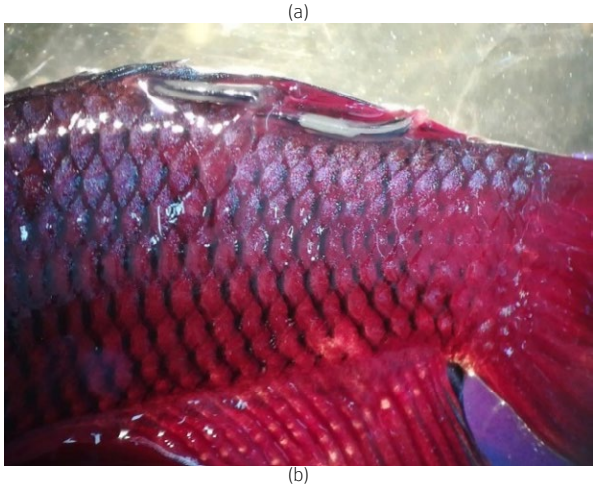
3 Results and discussion

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).



(a)



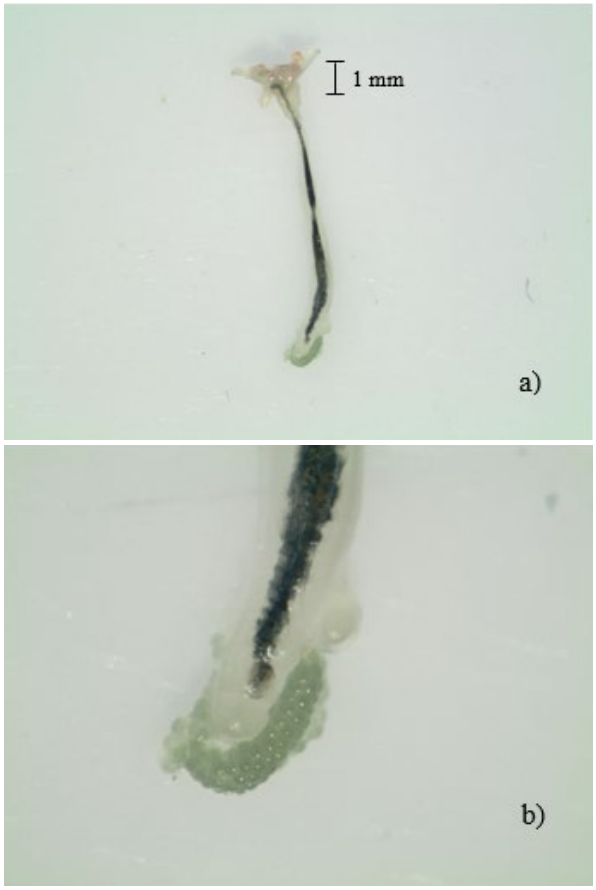
(b)

In this study, the parasite considered as the cause of lernaeosis in ornamental exotic fish species, *Betta splendens,* was *L. cyrpinacea*. 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

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 abdômen bears the fifth and sixth pairs of swimming legs. The abdomen is slightly broadened and curved at the posterior region. In similary 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.

Problably, 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:

narines, 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 unfits 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 colection 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 agentes into native communities. Therefore, colonization of exotic pathogens in aquatic systems constitutes a permanente 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 cientific 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 - Cyphocharax spilotus Vari, 1987* *Biru - Cyphocharax voga Hensel,1870* *Biru - Steindachnerina biornata Braga & Azpelicueta, 1987*	QUEROL *et al.* (2005)

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Province/ State	Location (Original name in portuguese)	Popular and cientific name Hosts	References
Minas Gerais	Paraíba do Sul river	*Lambari 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 nasutus Kner, 1858* *Piranha amarela - Serrasalmus spilopleura Kner, 1858* *Piau verdadeiro - Leporinus elongatus Valenciennes, 1850* *Piau três-pintas - Leporinus friderici Bloch, 1794* *Mandi bicudo - Iheringichthys labrosus Lütken, 1874* *Curimbatá - Prochitodus lineatus Valenciennes, 1847* *Tambacu - C. macropomum (fêmea) x P. mesopotamicus* *Bagre africano - Clarias gariepinus Burchell, 1822* *Tilápia do Nilo - Oreochromis niloticus Linnaeus, 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 & Gaimardm, 1824* *Carpa comum - Cyprinus carpio Linnaeus, 1758* *Piau – 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)

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Province/ State	Location (Original name in portuguese)	Popular and cientific name Hosts	References
São Paulo	municipality of Guariba	*Piauçu - 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 - Piabarchus stramineus Eigenmann, 1908*	NARCISO *et al.* (2019)
São Paulo	Taguari river, municipality of Taquarituba	*Saguiri curto - Steindachnerina insculpta Fernandez– Yepez, 1948*	ACOSTA *et al.* (2013)
São Paulo	municipality of Pirassununga	*Tambaqui - Colossoma macropomum Cuvier, 1818*	BASTOS; SÃO CLEMENTE; LIMA (1996)
Santa Catarina	municipality of Florianópolis	*Plati - Xiphophorus maculatus* Günther, 1866 *Molinésia 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 Itajaí	*Jundiá - Rhamdia quelen Quoy & Gaimardm, 1824*	FURTADO *et al.* (2019)
Sergipe	municipality of Neopólis and Propriá	*Lambari - Astyanax bimaculatus Linnaeus, 1758*	VASCONCELOS *et al.* (2013)
Sergipe	municipality of Aracaju	*Peixe japonês - Carassius auratus Linnaeus, 1758* *Cascudo- hypostomus sp.* *Molinesia negra - Poecilia sphenops Valenciennes, 1846* *Guppy - Poecilia reticulata Peters 1859* *Cauda de espada - Xiphophorus hellerii Haekel 1848*	ASSIS; CAVALCANTE; BRITO (2014)
Sergipe	municipality of Propriá (San Francisco river)	*Tambaqui – Colossoma macropomum*	FUJIMOTO *et al.* (2019)
Mato Grosso do Sul	Great Dourados region	*Patinga – (Piaractus mesopotamicus x Piaractus brachypomus 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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