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ANIMAL SCIENCE

Microcotylidae (Monogena) parasites of snappers (Perciformes: Lutjanidae) collected from the northeast coast of Brazil

ANDRÉ M. ALVES, GEZA THAIS R. SOUZA, RICARDO M. TAKEMOTO, CLÁUDIA M. DE MELO, RUBENS R. MADI & VERÓNICA DE LOURDES S. JERALDO

Abstract: Monogeneans are a diverse group of flatworms, being ectoparasites of marine and freshwater fish, with great morphological and ecological variety. Analyzes of monogenetics in fish with great habitat diversity such as snappers are scarce in the literature, which already emphasizes the need for an update in this regard. The presente study found, morphologically characterized and mapped the geographic distribution and in known hosts the species *Microcotyloides incisa* and *Microcotyloides impudicus*, describing the first occurrence of these monogeneans for South America and in new hosts. The survey of hosts in the literature shows that *M. incisa* seems to have a greater Affinity with congener species of Lutjanidae and fish from other families, which may indicate a preference or specificity for fish of this family because they are congeners of their host type. The introduction of these parasites into South American waters may have occurred due to the migratory behavior of snappers, which are well distributed along the Mexican coast, where the parasites are usually reported. Here, we also bring the first occurrence of *M. incisa* for *Lutjanus analis* and *Lutjanus jocu* and *M. impudicus* for *Ocyurus chrysurus* and *Lutjanus synagris*, helping in the mapping and distribution of these monogenetic species in the Americas.

Key words: Ectoparasites, fish, lutjanids, monogenean, snappers.

INTRODUCTION

Monogenea is one of the most common and diverse classes of Platyhelminthes, they are ectoparasites that can be found parasitizing teleost and elasmobranch fish in marine and freshwater environments, but can also be found in amphibians and mammals (Whittington et al. 2000, Eiras et al. 2010). Them diversity is not restricted only to the number of described species, but also in relation to the morphological variety and ecology (Poulin 2002). Most are considered "specific", being able to parasitize only one host species, or hosts that are phylogenetically close (Eiras et al. 2010).

The Perciformes is the most diverse order and one of the largest among fish, in which the Lutjanidae family is located, composed of teleost fish of several species (more than 17 genera and 109 species) considered as important fishing resources and which are distributed throughout all the seas of the world. world (mainly tropical and subtropical regions), in addition to having a wide variety of feeding behaviors (generalista carnivores), migratory habits (for reproduction or searching for resources) and habitat diversity such as: coral reefs, estuaries, freshwater, mangroves and open sea (Allen 1985, Rezende et al. 2003, Frédou & Ferreira 2005, Begossi et al. 2011, Cavalcanti et al. 2013).

In Brazil, these fish are popularly known as "Pargos" or "reds", with several species exploited by fishing activities, which means that there is a comprehensive line of research with the fish of this family (Rezende et al. 2003), however there are still few parasitological analyzes of these fish, when compared to other groups of fish such as Scombrids and Serranids. In this sense, fish of this family receive special attention, due to their ecological diversity, which can provide diversified and interesting parasitological data. During the analysis of the gills of snapper species, two species of monogeneans were found and identified. In this sense, the present work aimed to describe the first occurrence of *Microcotyloides incisa* (Linton, 1910) and *Microcotyloides impudicus* Caballero, Bravo-Hollis & Grocott, 1954 for South America and for Brazil, bringing a complete morphological description of the findings, as well as such as occurrence mapping and new hosts.

MATERIALS AND METHODS

The fish were purchased on the northeast coast of Brazil, monthly and according to the supply and availability of fish, directly from artisanal fishermen at the Public Fishing Terminal in the city of Aracaju/SE (10°54'17"S37°2'56"W) from 2014 to 2019. In this study, 69 specimens of *Lutjanus analis* (Cuvier, 1828), 30 of *Ocyurus chrysurus* (Linnaeus, 1758), 30 of *Lutjanus synagris* (Linnaeus, 1758) and 23 of *Lutjanus jocu* (Bloch & Schneider, 1801) were analyzed. The specimens were stored in thermal boxes and transported to the Tropical Biology Laboratory, where they were identified according to Allen 1985. Subsequently, biometry was performed, measuring the total length and weight of the specimens. parasitological analysis was performed according to Eiras et al. 2006. The gills were removed and the branchial arches separated in Petri dishes containing saline solution, the monogeneans found were carefully collected with the aid of a stylus and fixed in 70% alcohol for later identification of the species.

Specimens were mounted on slides, according to the methodology by Eiras et al. 2006 for this taxonomic group of parasites: slides mounted with Gray & Wess solution medium - for visualization of internal organs and slides mounted with Hoyer - for visualization of sclerotized parts. The parasites were measured (in millimeters - mm) and illustrations were made using a Coleman microscope (model N-120) with an attached camera lucida. The species were identified according to Fuji (1944), Mendoza-Garfias & Pérez-Ponce de León 1998 and Claxton et al. 2017. The parasitological indices of Prevalence (P%), Mean Intensity (Im) and Mean Abundance (Am) were calculated according to Bush et al. 1997.

RESULTS

Thirty-one monogeneans of the species *Microcotyloides incisa* (Linton, 1910) Fujii, 1944 (18 specimens) collected from *L. analis* and *L. jocu* and *Microcotyloides impudicus* Caballero, Bravo-Hollis & Grocott, 1954 (15 specimens) collected from *O. chrysurus* were collected. and *L. synagris*.

Family: Microcotylidae Taschenberg, 1879

Genus: *Microcotyloides* Fujii, 1940

Species: *Microcotyloides incisa* (Linton, 1910) Fujii, 1944 (Figure 1) (described by Linton, 1910 as *Microcotyle incisa*, later redescribed as *Microcotyloides incisa* by Fujii, 1944)

Description of the specimens: Long body (6 mm), large amount of vitelline glands, distributed from the anterior region below the pharynx to the posterior region of the body before the haptor (Figure 1a). The anterior part of the body has two large elliptical oral suckers

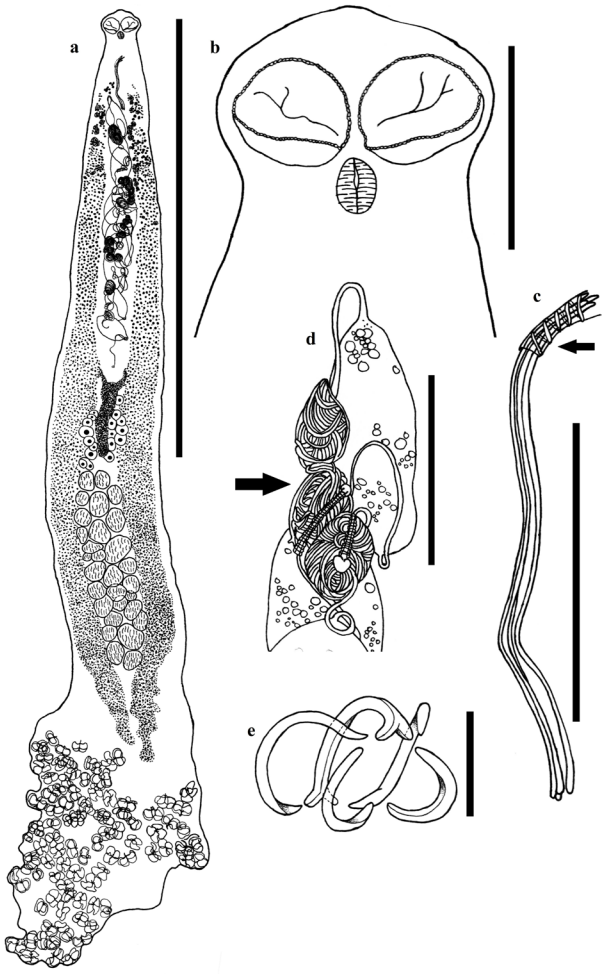

Figure 1. ***Microcotyloides incisa*** **collected from the gills of specimens of** ***Lutjanus analis*** **and** ***Lutjanus jocu*****. a- Species morphotype scale: 3mm; b- Anterior region with ornamented suckers and pharynx, scale: 0.1mm; c- Sclerites of the genital atrium, arrow indicates spiral structure of the prostatic tube, scale: 0.15mm; d- Elliptical eggs, arrow highlights the tangled filaments that unite the eggs, scale: 0.05mm; e-clamps, scale: 0.03mm.**

(0.1 mm wide by 0.08 mm long) each provided with a septum; the edge has a fine ornate line (Figure 1b). Subterminal mouth. Below the suckers is a large, muscular, rounded pharynx. Genital atrium is formed by a prostatic muscular bulb without spines (0.04 mm in length), inside it there are 4-5 sclerotized filaments (0.3 mm in length) whose anterior and posterior ends are papilliform (Figure 1c). Surrounding the bulb is a spiral structure that extends to the anterior part of the organ. Elliptical and filamentous eggs (0.2 mm long by 0.1 mm wide) both in the anterior and in the posterior part of the eggs (Figure 1d). The filaments are long, forming "knots". The posterior filaments of each egg have a sclerotized structure, similar to an anchor, in this structure the anterior filament of the next egg is coiled, interconnecting the eggs, apparently this mechanism serves to prevent the eggs from separating, forming a kind of chain of eggs. Vitelary is composed of dark spots. Yolk sac is large, as is the ovary, which folds behind the yolk sac, taking on a horseshoe appearance. Post-ovarian testes large and rounded, 20-29 in total. In the posterior part of the body it has a short and wide haptor (1 mm wide). It has staples of only one type, without the presence of marginal hooks or anchors. Staples are formed by five sclerites, one of which is central (it has a groove on the central axis, which gives them a concave appearance) that bifurcates at the ends (Figure 1e). Hosts of the present study: *Lutjanus analis* and *Lutjanus jocu*.

Geographical location: Northeast Coast, Aracaju, SE, Brazil.

Parasitological indices for each host: *L. analis* (P= 2.89%; Im= 2.5; Am= 0.07); *L. jocu* (P= 26.08%; Im= 2.16; Am= 0.56).

Site of Infestation: Gills (gill filaments)

Species: *Microcotyloides impudicus* Caballero, Bravo-Hollis & Grocott, 1954 (Figure 2)

Description: Long and long body (5mm), large number of yolk glands, forming dark spots distributed from the anterior region below the pharynx to the posterior region of the body (Figure 2a). The anterior part of the body has two large elliptical oral suckers (0.1 mm wide by 0.08 mm long) each provided with a septum; the edge has a thin, ornamented cuticle armed with numerous small spines (Figure 2b). Subterminal mouth. Below the suckers is a large, muscular, rounded pharynx. Genital atrium formed by

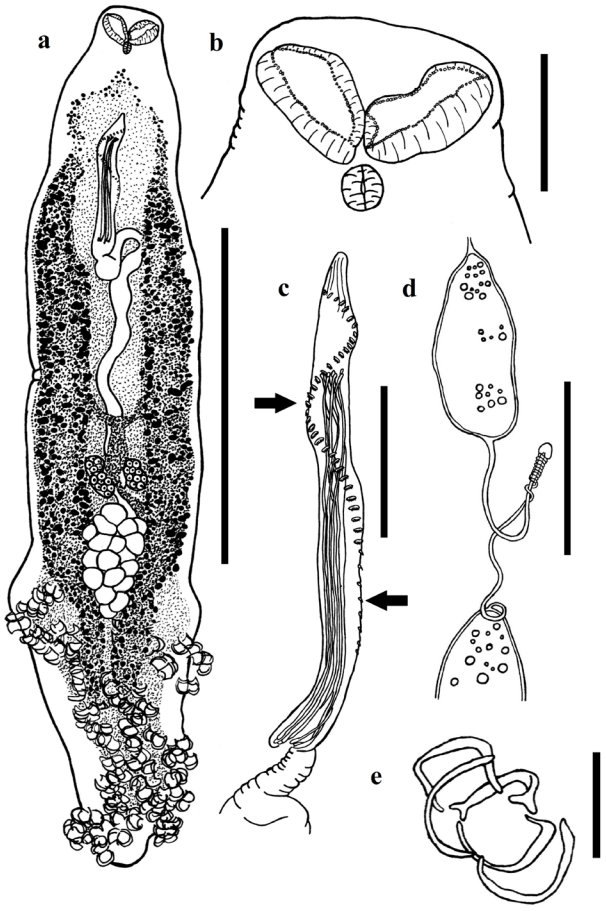

Figure 2. ***Microcotyloides impudicus*** **collected from the gills of specimens of** ***Ocyurus chrysurus*** **and** ***Lutjanus synagris*****; a- Parasite morphotype, scale: 0.1mm; b- Anterior region with ornamented suckers and pharynx, scale: 0.1mm; c- Genital Atrium, scale: 0.1mm; d- Elliptical eggs, with tangled filaments that unite the eggs, scale: 0.05mm; e- clamps, scale: 0.03mm .**

a prostatic muscular bulb (Figure 2c). It has 6-8 sclerite filaments inside the bulb. The tube is surrounded by 36-40 small sclerotized structures, which surround the tube in a spiral and in the posterior-lateral region of the tube there are eight small spines (Figure 2b, arrows). The eggs are elliptical and filamentous (Figure 2d) presenting the same "chain mechanism" as that presented by *M. incisa*. Haptor also only has one type of hairpin. Staples are formed by five sclerites, one of which is central (it has a groove on the central axis, which gives it a concave appearance) that bifurcates at the ends (Figure 2e).

Hosts of the present study: *Ocyurus Chrysurus* and *Lutjanus synagris*.

Geographic location of the study: Northeast Coast, Aracaju, SE, Brazil.

Parasitological indices for each host: *O. chrysurus* (P= 23.42%, Im= 2.00; Am= 0.46); *L. synagris* (P= 3.33%; Im= 1.00; Am= 0.03).

Site of Infection: Gills (gill filaments).

Remarks: Claxton et al. (2017) describe that species can be differentiated by characteristics such as the number of testes (20-30 testes in *M. incisa* and 9-18 testes in *M. impudicus*) and the number of clamps of the species (64-72 pairs in *M. incisa* and 70-74 pairs in *M. impudicus*). Other characteristics, such as the copulatory organ in the form of a muscular bulb and the morphology of the male and female apparatus, and the shape of the hooks, are in accordance with the characteristics described by Fujii (1944). Even so, in the specimens found here, some structural morphological characteristics not observed in previous works were observed and which also help in the differentiation of the two species. The genital atrium for the genus is described as a prostatic tube, without counting the internal sclerites or spiny projections for the two species, being important characteristics in the distinction of both. Claxton et al. (2017) refers that specimens of *Microcotyloides* spp. have prostatic reservoirs with a 2-3 piece fixed, sclerotic, rod-like sheath associated with the male distal apparatus. The specimens of *M. incisa* showed 4-5 sclerotized filaments inside the prostatic bulb, without projections or thorns around it, the specimens still have a small structure similar to a spiral "tape", in the anterior region of the bulb. While specimens of *M. impudicus* had 6-8 filaments inside the prostatic bulb, in addition to 36-40 small sclerites that extend from the anterior region

and along the tube forming a spiral, ending in 8 tiny spines in the region posterolateral side of the bulb. Such characteristics are not specified in the literature and allow to clearly differentiate the two species.

DISCUSSION

The Microcotylidae family currently comprises 51 genera. The Genus *Microcotyloides* comprises two valid species: *M. incisa* and *M. impudicus*. Only a few authors discuss and present taxonomic data regarding the species (see Fujii 1944, Mendoza-Garfias & Pérez-Ponce de León 1998, Claxton et al. 2017, Mendoza-Franco et al. 2018). The species *M. incisa* was recorded parasitizing fish from the families Lutjanidae, Sciaenidae, Cirrhitidae. After the description made by Linton (1910) this species was re-described by Fujii 1944 with specimens collected from the gills of its type host, *Lutjanus griseus* (Linnaeus, 1758) collected in Tortuga, Florida and Bermuda Islands. Later the same species was found by Mendoza-Garfias & Pérez-Ponce de León 1998 occurring in *L. argentiventris* (Peters, 1869), *L. guttatus* (Steindachner, 1869) and *L. jordani* (Gilbert, 1898) in Bahía de Chamela, Mexico. Then in *O. chrysurus* by Montoya-Mendoza et al. 2014 in the region of Vera Cruz, Mexico and was recently found by Claxton et al. 2017 in Puerto Rico, USA in the host *Rhomboplites aurorubens* (Cuvier, 1829) and again in *L. griseus* by Mendoza-Franco et al. 2018 at Banco Campeche (southwest Gulf of Mexico). Therefore, there was a record of this species only in North America and Central America, its occurrence in South America has not yet been registered, and it is also found in two new hosts: *L. analis* and *L. jocu* (See Figure 3).

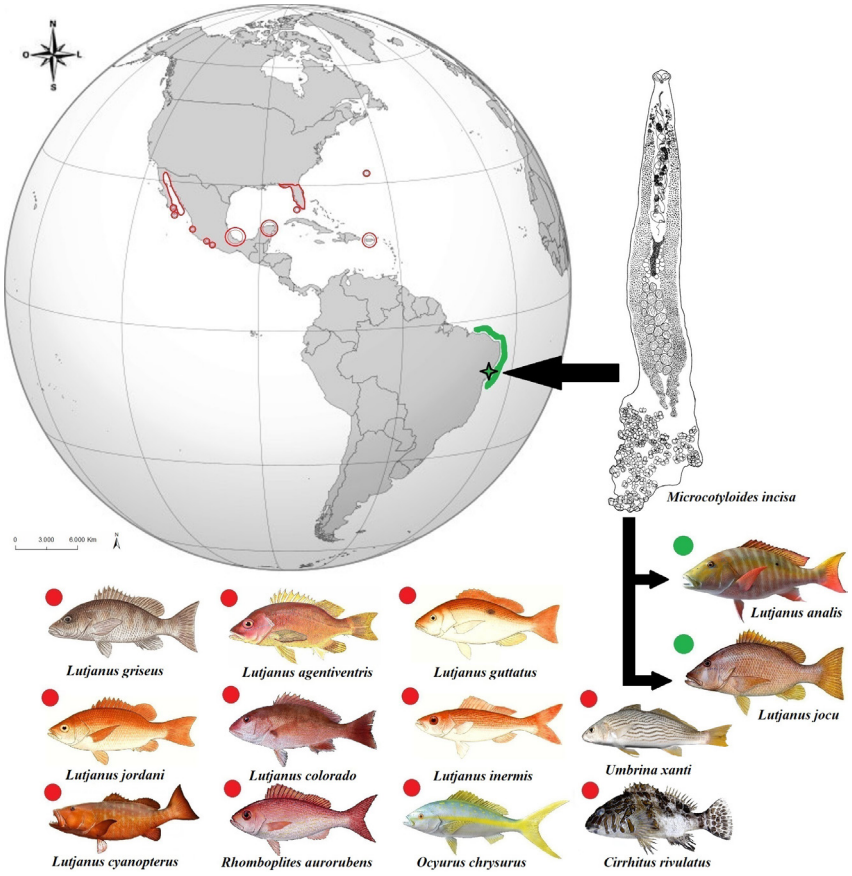

Figure 3. Distribution of *Microcotyloides incisa*, specifically in the Americas. In red, the areas of North and Central America and hosts where the parasite is already registered are highlighted; in green the new location: South America, Brazil and new hosts where the same parasite was found in the present study. Source: Image prepared by the authors and adapted from Google.

The species *M. impudicus* was recorded in locations such as Nayarit, Oaxaca, Sinaloa and Jalisco (Bahía de Chamela), Mexico, in the hosts *Polydactylus octonemus* (Girard, 1858), *Polydactylus approximans* Lay & Bennett, 1839 and *Chanos chanos* (Forsskal, 1775) (Mendoza-Garfias et al. 2017). Therefore, there was a record of this species only in Central America, and just as his congener species has not yet been recorded for South America, in addition, there are no previous records of this species parasitizing the gills of lutjanids, unlike its congener species. Thus, the present study brings the first occurrence of *M. impudicus* to South America, more specifically to the coast of northeastern Brazil, and two new hosts: *O. chrysurus* and *L. synagris* (See Figure 4), but for the latter, only one fish was parasitized by a specimen, so it can be considered as an accidental infection.

Most monogeneans have been described parasitizing only a single species of fish, however, species are considered common to be related or to be found parasitizing fish of certain genera or host families. The species *M. incisa* was described from *L. griseus* and through the number of occurrences in the surveys shown, the parasite seems to have a greater affinity with congener species of Lutjanidae and fish from other families. According to Whittington et al. 2000 this can be explained by the possible reasons: First, the similarity and phylogenetic proximity of the hosts. Since snappers have species that are very close in terms of family and genus, this may allow for the possible availability of a common resource among the hosts, in this way the parasites acquire more forms of dispersion in the environment. The specificity of the microhabitat - because there are species recorded only in certain regions of the gill arches. In the present study, most of the monogeneans were present in the median area of the branchial arches. Thus, with phylogenetically close hosts, similar conditions of microhabitats favorable to the development and fixation of the parasites in the branchial arches would be provided.

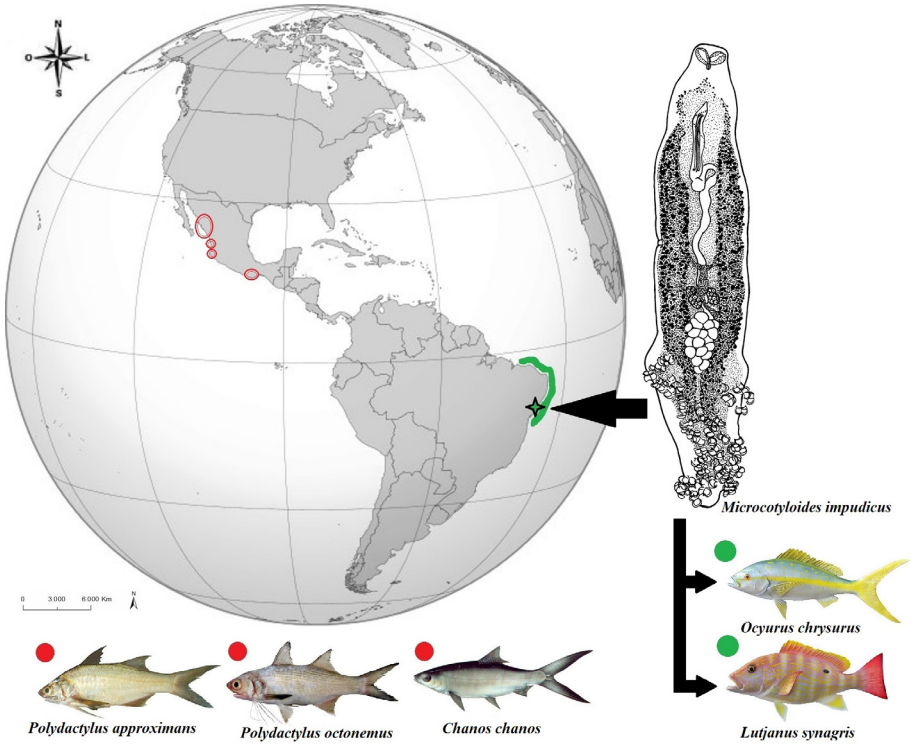

Figure 4. Distribution of *Microcotyloides impudicus* around the globe, specifically in Central America. In red, the areas and hosts where the parasite is already registered are highlighted; in green the new locality (South America – Brazil) and hosts where the same parasite was found in the present study. Source: Image prepared by the authors and adapted from Google.

Both reasons above explain the occurrence of *M. incisa* in so many other congener species of Lutjanidae recorded previously and now in two more congeners. In the present research it was also noted that *M. incisa* occurred with greater intensity in *L. jocu*; and *M. impudicus* obtained greater intensity in *O. chrysurus*, which allows us to infer that these two fish were perhaps preferred hosts for each of the parasite species.

The occurrence of these parasites in South America can be explained by the migratory behavior of snappers, fish that are well distributed along the Mexican coast, the location from where the parasites are usually reported. Cocheret de la Morinière et al. 2003 and Freitas et al. 2011 explain that the migratory behavior of these fish is due to a change in diet during their ontogenetic development, *L. analis* for example, the juvenile stages feed on crustaceans and the sub-adults have a more varied diet, both inhabiting sandy bottoms, algal reefs, bays, mangroves and estuaries, while adults feed on smaller fish, inhabiting hard substrates, deep reefs and coastal environments. In this sense, such habitat changes and probable natural aggregations that occur among many species of fish, may favor the dissemination of ectoparasites (which at the same time have a direct transmission cycle, such as monogenetic ones) to other regions and introducing them to new environments. The findings bring the first occurrence of *M. incisa* and *M. impudicus* to South America and Brazil, in addition to two new hosts for each of the species: *L. analis* and *L. jocu* for *M. incisa* and *O. chrysurus* and *L. synagris* for *M. impudicus*. Furthermore, in addition the present work also assists in the mapping and distribution of these monogenetic species in the Americas.

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ANDRÉ M. ALVES[1]
https://orcid.org/0000-0002-9150-5287

GEZA THAIS R. SOUZA[2]
https://orcid.org/0000-0002-1039-6710

RICARDO M. TAKEMOTO[3]
https://orcid.org/0000-0001-7592-2083

CLÁUDIA M. DE MELO[1]
https://orcid.org/0000-0001-9331-003X

RUBENS R. MADI[1]
https://orcid.org/0000-0002-1526-0687

VERÓNICA DE LOURDES S. JERALDO[1]
https://orcid.org/0000-0001-9813-7969

[1]Universidade Tiradentes (UNIT), Instituto de Tecnologia e Pesquisa (ITP), Av. Murilo Dantas, 300, Farolândia, 49032-490 Aracaju, SE, Brazil

[2]Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), Av. Professor Célso Ferreira da Silva, 1333, Jardim Europa II, 18707-150 Avaré, SP, Brazil

[3]Universidade Estadual de Maringá (UEM), Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Av. Colombo, 5790, Vila Esperança, 87020-900 Maringá, PR, Brazil

Correpondence to: **André Mota Alves**
E-mail: andremta@outlook.com

Author contributions

André Mota Alves: Study planning, data collection and Manuscript preparation (Writing and Illustrations), Obtaining, Analyzing and Interpreting data; Geza Thais Rangel e Souza: Study planning, Analysis and interpretation of data, Approval of the final version; Ricardo Massato Takemoto: Study planning and preparation of the manuscript, Analysis and interpretation of data, Approval of the final version; Cláudia Moura de Melo: Study planning and preparation of the manuscript, Approval of the final version; Rubens Riscala Madi: Study planning and preparation of the manuscript, Approval of the final version; Veronica de Lourdes Sierpe Jeraldo: Study planning and preparation of the manuscript, Approval of the final version.