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Revalidation of *Saccocoelioides bacilliformis* (Digenea, Haploporidae) parasitizing species of *Astyanax* (Characiformes, Characidae) from southern Brazil

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ABSTRACT. In Argentina, *Saccocoelioides bacilliformis* Szidat, 1973 was described from specimens collected probably in *Astyanax bimaculatus* (Linnaeus, 1758), but latter it was synonymyzed with *Saccocoelioides octavus* Szidat, 1970. During the examination of digenetic trematodes collected in different species of *Astyanax* Baird & Girard, 1854 from southern Brazil, we found specimens identified as *Saccocoelioides*. A detailed comparison of the morphology of the two species revealed that they should be considered valid, thus this study proposes the revalidation of *S. bacilliformis*. The traits that allowed the revalidation were: body shape, position of the caecal bifurcation, and the extension of the uterus and vitellarium. Parameters of infections are provided for the first time in addition to records of new hosts and new locality.

KEYWORDS. Chalcinotrematinae, haploporid, characid, redescription, Taxonomy.

RESUMO. Revalidação de *Saccocoelioides bacilliformis* (Digenea, Haploporidae) parasitando espécies de *Astyanax* (Characiformes, Characidae) do sul do Brasil. Na Argentina, *Saccocoelioides bacilliformis* Szidat, 1973 foi descrita a partir de espécimes coletados provavelmente de *Astyanax bimaculatus* (Linnaeus, 1758), mas posteriormente foi sinonimizada com *Saccocoelioides octavus* Szidat, 1970. Durante o exame de trematódeos digenéticos coletados de diferentes espécies de *Astyanax* Baird & Girard, 1854 no sul do Brasil, foram encontrados espécimes identificados como *Saccocoelioides*. Uma comparação detalhada da morfologia das duas espécies revelou que elas devem ser consideradas válidas, portanto este estudo propõe a revalidação de *S. bacilliformis*. Os caracteres morfológicos que permitiram a revalidação foram: formato do corpo, posição da bifurcação cecal e a extensão do útero e vitelário. Os parâmetros das infecções são fornecidos pela primeira vez, bem como o registro de novos hospedeiros e de uma nova localidade.

PALAVRAS-CHAVE. Chalcinotrematinae, haploporídeo, caracídeo, redescrição, Taxonomia.

The genus *Saccocoelioides* Szidat, 1954 was proposed to accommodate seven species of digenetic trematodes which presented vitellarium formed by rounded and large follicles, distributed in lateral fields and extend from the ventral sucker region to the middle posterior region of the body (Szidat, 1954). Latter, Overstreet & Curran (2005) considered that species of *Saccocoelioides* present a combination of the following traits: intestinal caeca small sac-like or moderately long, never ending close to the end of the body; eyespot concentrated or diffuse and miracidium containing eyespot. The species of *Saccocoelioides* have been reported in fishes from different regions of North, Middle, and South America (Curran *et al.*, 2018).

In Argentina, *Saccocoelioides octavus* Szidat, 1970 was described from specimens collected in *Astyanax fasciatus* (Cuvier, 1819) (Szidat, 1970). Latter, Lunaschi (2002) redescribed the species of *S. octavus* and examined specimens of *Saccocoelioides bacilliformis* Szidat, 1973 available and deposited in the "Colección Nacional de Parasitología, Museo Argentino de Ciencias Naturales Bernardino Rivadavia" (MACN-Pa), Buenos Aires, Argentina. The author considered *S. bacilliformis* to be a synonym of *S. octavus*, a proposal followed by Curran *et al.* (2018) after paratypes examination of *S. octavus* deposited in the MACN-Pa.

The Lake Guaíba contributes to the formation of the Guaíba basin, and constitutes the biggest lacustrine complex in South America (Noronha, 1998; Menegat & Kirchheim, 2006). In the lake, four species of *Astyanax* Baird & Girard, 1854 have been reported: *Astyanax eigenmanniorum* (Cope, 1894), *Astyanax* **aff**. *fasciatus* (Cuvier, 1819), *Astyanax henseli* Melo & Buckup, 2006, and *Astyanax lacustris* (Lütken, 1875) (Lucena *et al.*, 2013; 2017). Their helminth fauna is still very poorly known and considering the trematodes, only three species of digenetic trematodes (*Dendrorchis retrobiloba* Volonterio & Ponce de Léon, 2005, *Genarchella parva* Travassos, Artigas & Pereira, 1928 and *Zonocotyle bicaecata* Travassos, 1948) have been reported in *A.* **aff.** *fasciatus* (Fortes & Hoffmann, 1995; Gallas & Utz, 2019). After the examination of the specimens of *Saccocoelioides* found in different species of *Astyanax*, we propose the revalidation

of *S. bacilliformis* due the shape of body, position of caecal bifurcation, uterus and vitellarium. In addition, records of new hosts and a new locality are also provided.

MATERIAL AND METHODS

Between 2017 and 2018, a total of 96 specimens belonging to the species of *A. eigenmanniorum* (n = 20), *A.* **aff.** *fasciatus* (n = 46), *A. henseli* (n = 5) and *A. lacustris* (n = 25) were collected with seine nets or fyke nets by fishermen in Pintada Island (30°17'11"S, 51°18'01"W), Lake Guaíba, Porto Alegre city, state of Rio Grande do Sul, Brazil. Fishes were transported in ice coolers and frozen until necropsy in laboratory. The digenetic trematodes found were fixed in A.F.A. (70° GL ethanol – 93 parts; formalin 37% – five parts; glacial acetic acid – two parts) for 48 hours and then stored in 70° GL ethanol (Amato & Amato, 2010). Helminths were stained with Delafield's hematoxylin, clarified in cedarwood oil and mounted using Canada balsam (Amato & Amato, 2010; Gallas & Utz, 2019).

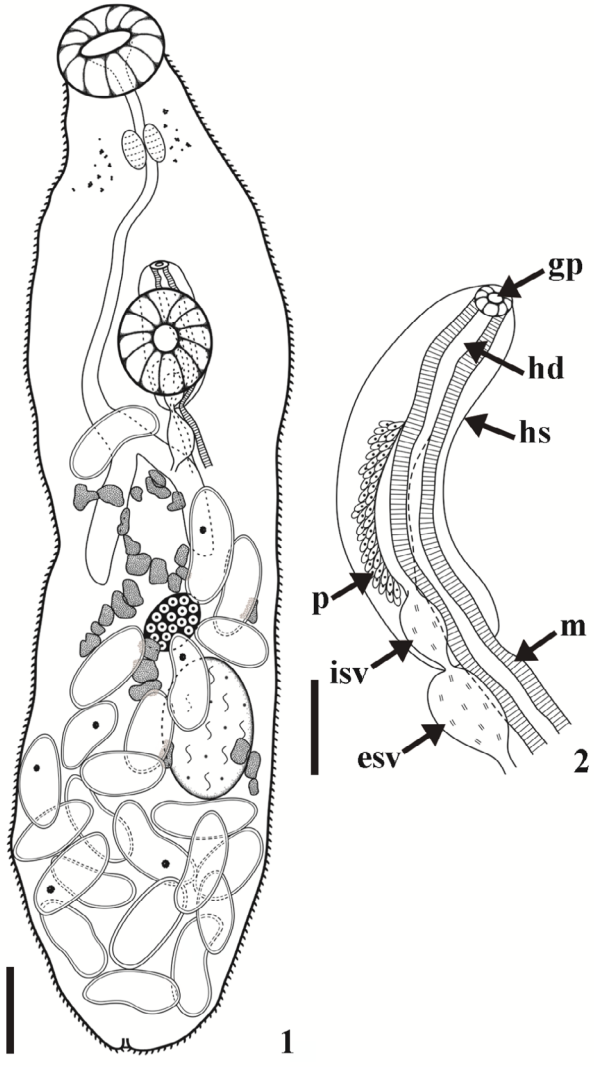
In the description, terms as forebody and hindbody were used according to Yamaguti (1971). Measurements were made using an ocular micrometer and appear in the text in micrometers (µm) unless otherwise indicated, representing the range followed between parenthesis by the mean, the standard deviation, and the sample size. Systematics used for the parasite follows Overstreet & Curran (2005). Line drawings were made with light microscope. Terms as prevalence, mean intensity and mean abundance of infections follow Bush *et al.* (1997). Voucher specimens were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, state of Rio de Janeiro, Brazil, and in the Helminthological Collection of the Natural Sciences Museum (*Coleção Helmintológica do Museu de Ciências Naturais*, CHMU) at the *Universidade Luterana do Brasil* in Canoas, Rio Grande do Sul, Brazil.

RESULTS

Saccocoelioides bacilliformis Szidat, 1973
(Figs 1, 2)

Redescription. Haploporidae, Chalcinotrematinae. Based on 14 mature specimens. Body 1.03–1.85 mm (1.5 ± 0.3 mm; n = 13) long, 0.24–0.45 mm (0.37 ± 0.07 mm; n = 13) wide. Tegument spinose and delicate. Pigmented eyespots scattered in the region of pharynx and hermaphroditic sac. Oral sucker 100–120 (106 ± 6) long, 110–150 (121 ± 10) wide. Ventral sucker 90–110 (94 ± 6) long, 90–110 (96 ± 6) wide, distant 300–500 (344 ± 56) from anterior extremity. Ratio of total body length/oral sucker 1:14.1; ratio of oral sucker/ventral sucker 1:1.13. Prepharynx 20–120 (44 ± 29) long, 10–20 (12 ± 4; n = 13) wide. Pharynx 50–70 (58 ± 7) long, 40–70 (53 ± 9) wide. Esophagus 130–290 (204 ± 56; n = 11) long, caecal bifurcation at the level of posterior limit or behind the ventral sucker, distant 320–560 (411 ± 65; n = 12) from anterior extremity; intestinal caeca sacculate, short, ending about next to the ovary or between ovary and testis.

One single testis, oval to rounded, 107–250 (159 ± 43; n = 13) long, 80–170 (120 ± 26; n = 13) wide, next to the middle of the hindbody. External seminal vesicle longer than internal seminal vesicle. Hermaphroditic sac 105–220 (155 ± 38; n = 13) long, 45–80 (58 ± 10; n = 13) wide, containing internal seminal vesicle, pars prostatic and the final portion of the metraterm, forming the hermaphroditic duct. Genital pore anterior to the ventral sucker, distant 240–430 (308 ± 49) from anterior end. Ovary rounded, usually intercaecal, but always pretesticular, 70–125 (91 ± 17) long, 47–120 (73 ± 18) wide. Laurer's canal not observed, Mehlis'gland not observed. Uterus long, inter and extracaecal, occupying all of the hindbody and reaching the ventral sucker. Numerous eggs, 90–110 (100 ± 6) long, 40–60 (50 ± 5) wide, some of them with developed miracidium, containing pigmented eyespot. Vitellarium constituted by generally spherical



Figs 1, 2. Incomplete diagrams of *Saccocoelioides bacilliformis* Szidat, 1973: 1, composite, ventral view of a specimen *in toto*. Scale bar = 100 µm; 2, detail of the final portion of the reproductive system (gp, genital pore; hd, hermaphroditic duct; hs, hermaphroditic sac; m, metraterm; p, pars prostatic; isv, internal seminal vesicle; esv, external seminal vesicle). Scale bar = 40 µm.

follicles, ranging from the ventral sucker to the posterior limit of the testis, where in some specimens they are divided in two groups: most of them are pretesticular and a few follicles are pos-testicular. Excretory vesicle not observed, excretory pore terminal, in the posterior extremity.

Taxonomic summary
Hosts: *Astyanax* **aff.** *fasciatus* (Cuvier, 1819) and *Astyanax henseli* Melo & Buckup, 2006 – new hosts records.
Locality: Lake Guaíba, city of Porto Alegre, state of Rio Grande do Sul, Brazil – new locality record.
Site of infection: stomach.
Prevalences: 8.7% (*A*. **aff.** *fasciatus*) and 40% (*A*. *henseli*).
Mean intensity of infections: 15.75 helminths/host (*A*. **aff.** *fasciatus*) and 2 helminths/host (*A*. *henseli*).
Mean abundance of infections: 1.37 helminths/host (*A*. **aff.** *fasciatus*) and 0.8 helminth/host (*A*. *henseli*).
Amplitude of the intensity of infections: 1 to 54 helminths in *A*. **aff.** *fasciatus* and 1 to 3 helminths in *A*. *henseli*.
Voucher specimens of helminths deposited: CHIOC 40094; CHMU 291-1-1.

DISCUSSION

When redescribed *S. octavus*, Lunaschi (2002) compared the species with specimens of *S. bacilliformis* and considered that the latter was described based on specimens too compressed. Thus, the compared measurements of the specimens of *S. octavus* and *S. bacilliformis* were similar, leading the author to consider them identical, resulting in the synonymy of *S. bacilliformis*. This propose was based only on five and eight specimens of *S. octavus* and *S. bacilliformis*, respectively, from the material of Szidat (1970, 1973) deposited in the MACN-Pa. The two species present oral and ventral sucker with similar sizes or, the oral is slightly higher than the ventral sucker (Szidat, 1970, 1973; Lunaschi, 2002). However, the examination of specimens found in the present study revealed substantial differences that support to consider them distinct species.

The specimens found in the present study and the specimens identified as *S. bacilliformis* by Szidat (1973), later studied by Lunaschi (2002) present a more slender body, with practically the same width. However, the specimens identified as *S. octavus* and illustrated by Szidat (1970), Lunaschi (2002) and Curran *et al.* (2018) present greater width in the equatorial region of the body, forming a typically fusiform body. This trait was also observed and described by Lunaschi (2002).

In the examined specimens and those identified as *S. bacilliformis* by Szidat (1973) and Lunaschi (2002), the caecal bifurcation is in the equatorial region or below to the ventral sucker. This position is different when compared with the specimens of *S. octavus* illustrated by Szidat (1970), Lunaschi (2002) and Curran *et al.* (2018), where the caecal bifurcation is above the equatorial region, or above the anterior limit of the ventral sucker. This difference was also found in the specimens examined by Lunaschi (2002).

In the specimens identified as *S. bacilliformis* the uterus always extends to the posterior end of the body (Szidat, 1973; Lunaschi, 2002), which is different in specimens of *S. octavus*. The uterus in *S. octavus* seems to range, in most specimens, from the ventral sucker to the posterior end of the testis (Szidat, 1970; Lunaschi, 2002; Curran *et al.* 2018) and to the posterior end of the body. This character was observed in only one drawing provided by Szidat (1970). In the drawings provided by Lunaschi (2002), and Curran *et al.* (2018) the uterus of the observed specimens does not extend to the posterior end, which may suggest that the specimen observed by Szidat (1970) is not available anymore.

The vitellaria follicles range in the two species from the ventral sucker to the posterior limit of the testis (Szidat, 1970, 1973; Lunaschi, 2002) to close or in the posterior end of the body (Lunaschi, 2002; Curran *et al.*, 2018). In addition, in the specimens of *S. octavus* observed by Szidat (1970), the vitellaria follicles were located at both sides of the ovary. The vitellaria follicles are drop-shaped and distributed in both sides of second and third quarters of the body in *S. bacilliformis* (Szidat, 1973). In the species redescription, Lunaschi (2002) described specimens of *S. octavus* and *S. bacilliformis* presenting two lateral pretesticular and a median post-testicular distribution. In the present study, the specimens presented a vitellarium distribution similar to those specimens described by Szidat (1973), but not usually in two groups as described by Lunaschi (2002). In most of the specimens of *S. octavus*, the vitellarium reaches the posterior end, while in *S. bacilliformis*, eggs are always filling the posterior end of the body.

In general, the measurements obtained from specimens analyzed here were similar to those found by Szidat (1973) and Lunaschi (2002). However, the specimens of *S. bacilliformis* examined present a testis smaller (107–250 [159] long by 80–170 [120] wide) when compared to the specimens measured by Szidat (1973) (250 long by 150 wide). In addition, the ventral sucker of the specimens examined here is smaller (90–110 [90]) than the specimens measured by Szidat (1973) (120) and Lunaschi (2002) (103–140 [126]). The hermaphroditic sac of the individuals measured in the present study (105–220 [155] long by 45–80 [58] wide) is also smaller than the specimens (172–262 [223] long by 69–112 [94] wide) examined by Lunaschi (2002). These measurements suggest that specimens of *S. bacilliformis* presents morphological variation, which could be due to procedures of preparation, or a lack of previous observation.

The eggs measured in the specimens found in this work are in the range reported by Szidat (1973) and Lunaschi (2002). Eggs with a developed miracidium with eyespot were observed in the specimens from this study, in the individuals examined by Szidat (1973) and Lunaschi (2002), and in the specimens previously identified as *S. bacilliformis*. The presence of miracidium developed with eyespot seems to be a trait that must be checked in the specimens of *S. octavus*, given the different descriptions (Szidat, 1970; Lunaschi, 2002; Curran *et al.*, 2018).

In view of these different considerations, the two species can be distinguished based on the following traits: *S. octavus* presents a fusiform body while *S. bacilliformis* presents body with only the anterior end more tapered. *Saccocoelioides octavus* has caecal bifurcation above or in the middle of the ventral sucker, while in *S. bacilliformis* the caecal bifurcation is in the middle or below the ventral sucker. In specimens of *S. octavus* the uterus does not reach the posterior end, while in *S. bacilliformis* it does. Vitellaria follicles in *S. octavus* reach the posterior end of the body, while in *S. bacilliformis* this part of the body is occupied by the uterus. The vitellaria follicles in *S. bacilliformis* is located right below the middle of the body. The measurements of the two species are similar (Lunaschi, 2002), except those for body's length and hermaphroditic sac's length and width, thus no substantial differences were found to allow the differentiation of *S. bacilliformis* and *S. octavus* based on the measurements.

The specimens of *S. bacilliformis* were found in the pyloric caeca and the anterior portion of the intestine of *Astyanax bimaculatus* (Linnaeus, 1758), previously identified as *Astyanax bipunctatus* (Szidat, 1973; Lunaschi, 2002). In the present study the specimens were collected from the stomach of the examined hosts, maybe as result of a migration after host death. In addition, the specimens found here presented a delicate tegument, making the preparation sometimes difficult and impairing their staining and mounting (Overstreet & Curran, 2005; Aguirre-Macedo & Violante-González, 2008). This feature cannot be avoided due the methodology of fish sampling for posterior examination in laboratory.

The intensity of infection found in the present study was higher in *A*. **aff.** *fasciatus* (15.75 helminths/host) than in *A*. *henseli* (2 helminths/host). There is no data about the parameters of infections such as prevalence, intensity or abundance of infections for *S. bacilliformis*. In the life cycle of *S. octavus*, the fish becomes infected after the ingestion of encysted metacercariae as previously reported by Szidat (1970). It is possible that the reason of high intensity of infection in *A*. **aff.** *fasciatus* is the ingestion of random material in comparison with *A*. *henseli*. This non-selective feeding habit may increase the possibility of *A*. **aff.** *fasciatus* to become infected by *S. bacilliformis*.

Kohn *et al.* (2007) listed 15 valid species of *Saccocoelioides* from South America: *Saccocoelioides antonioi* Lunaschi, 1984, *S. bacilliformis*, *Saccocoelioides carolae* Lunaschi, 1984, *Saccocoelioides elongatus* Szidat, 1954, *Saccocoelioides godoyi* Kohn & Fróes, 1986 (= *Saccocoelioides szidati* (Szidat, 1954) Travassos, Freitas & Kohn, 1969), *Saccocoelioides magniovatus* Szidat, 1954, *Saccocoelioides magnorchis* Thatcher, 1978, *Saccocoelioides magnus* Szidat, 1954, *Saccocoelioides nanii* Szidat, 1954 (type species), *S. octavus*, *Saccocoelioides quintus* Thatcher, 1978, *Saccocoelioides rotundus* Thatcher & Jégu, 1996, *Saccocoelioides saccodontis* Thatcher, 1978 and *Saccocoelioides tarpazensis* Diaz & Gonzáles, 1990, but eight (*S. elongatus*, *S. godoyi*, *S. magniovatus*, *S. magnorchis*, *S. magnus*, *S. nanii*, *S. rotundus* and *S. saccodontis*) were reported in Brazil (Guidelli *et al.*, 2006; Kohn *et al.*, 2007; Takemoto *et al.*, 2009; Azevedo *et al.*, 2010). However, the validity of some species of *Saccocoelioides* has been discussed by some authors (Curran *et al.*, 2018; Andrade-Gómez *et al.*, 2019).

Until now, only *Saccocoelioides nanii* Szidat, 1954 and *S. szidati* (reported as *S. godoyi*) were found in *Leporinus elongatus* Valenciennes, 1850 (= *Megaloporinus obtusidens* Valenciennes, 1837) and *M. obtusidens* from Lake Guaíba (Kohn & Fróes, 1986; Fortes & Hoffmann, 1995; Wendt *et al.*, 2018). This is the first report of *S. bacilliformis* in *A*. **aff.** *fasciatus* and *A. henseli* from Lake Guaíba, contributing to the knowledge of their helminth fauna and to the biodiversity of digenetic trematodes in fish from Brazil. Moreover, additional observations on the morphology of *S. bacilliformis* and *S. octavus* were provided in order to distinguish both species.

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