ISSN Versión impresa 2218-6425 ISSN Versión Electrónica 1995-1043

aphia
ASOCIACIÓN PERUANA DE HELMINTOLOGÍA E INVERTEBRADOS AFINES

ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

ACANTHOSTOMUM GNERII SZIDAT, 1954 (DIGENEA: CRYPTOGONIMIDAE) FROM SILVER CATFISH *RHAMDIA QUELEN* (QUOY & GAIMARD, 1824)

ACANTHOSTOMUM GNERII SZIDAT, 1954 (DIGENEA: CRYPTOGONIMIDAE) DE *RHAMDIA QUELEN* (QUOY & GAIMARD, 1824)

Maurício L. Martins[1*]; Karen R. Tancredo[1]; Natália C. Marchiori[2]; Joaber Pereira Jr.[3]; Luis A.S de Castro[4]; Patrícia Garcia[1] & Juan Esquivel[5]

[1] AQUOS Laboratório de Sanidade de Organismos Aquáticos, Departmento de Aquicultura, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga 1346, 88040-900, Florianópolis, SC, Brasil.
[2] Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Campo Experimental de Piscicultura de Camboriú, Rua Joaquim Garcia, s/nº, Centro, CEP 88340-000, Camboriú, SC, Brasil.
[3] Laboratório Biologia de Parasitos de Organismos Aquáticos, Caixa postal 474, Cassino, Rio Grande, RS, Brasil.
[4] Laboratório de Imunologia, EMBRAPA Clima Temperado, BR 392, km 78
Monte Bonito, Pelotas, RS, Brasil.
[5] Piscicultura Panamá Ltda., Est. Geral Bom Retiro, km 8, 88490-000, Paulo Lopes, SC, Brasil.
*Correspondence to author: mauricio.martins@ufsc.br

Neotropical Helminthology, 2016, 10(2), jul-dic: 189-203.

ABSTRACT

Acanthostomum gnerii is a digenean frequently reported from intestine of Siluriformes and it is well distributed over Central and South America. The aim of this study is to provide additional information on morphological features of *A. gnerii* by histological sections and scanning electron microscopy, as well as to provide comparative measurements of *Acanthostomum* spp. In contrast to previous reports of this helminth in *Rhamdia* sp., specimens in the present study showed two arms in the anterior portion of the body, just after the pharynx. They join and terminate in one lateral pore on the left side of the body similar to that reported in *A. proctophorum* and *A. minimum*. Such morphology was not described as a valid characteristic for Acanthostomidae. This morphological variation could be associated with different populations of the same species. New studies using molecular techniques are recommended to determine if this character corresponds to a diversity of *A. gnerii* found in other fish species.

Keywords: Acanthostominae – Cryptogonimidae – Digenea – histology – parasite – silver catfish

RESUMEN

Acanthostomum gnerii és un digenea frecuentemente reportado en el intestino de Siluriformes y bien distribuida en el centro y suramérica. El objetivo de este estudio es proporcionar información adicional sobre las características morfológicas de la *A. gnerii* por secciones histológicas y microscopio electrónico de barrido, así como para proporcionar mediciones comparativas de *Acanthostomum* spp.. Diferente de registros anteriores de este helminto en *Rhamdia* sp., los espécimes de presente estudio presentearon dos brazos en la porción anterior del cuerpo luego después de la faringe. Ellos se juntan en un poro lateral en el lado esquierdo del cuerpo semelhante al observado en *A. proctophorum* y *A. minimum*. Esta morfología no hay sido descripta como característica válida para Acanthostomidae. La variación morfológica podria estar asociada a diferentes poblaciones de la misma especie. Nuevos estudios usando tecnicas moleculares son recomendados para certificar se esta característica coresponde a la diversidad de *A. gnerii* encuentrada en otras especies de peces.

Palabras clave: Acanthostominae – bagre – Cryptogonimidae – Digenea – histología – parásito

INTRODUCTION

The silver catfish *Rhamdia quelen* (Pimelodidae), commonly known as "jundia" is one of the most cultivated freshwater fish in Southern Brazil due to its wide distribution, easy management, hardness and good feed conversion. This catfish is found from the South to North of Mexico and Central to South of Argentina (Silvergip, 1996; Fracalossi et al., 2004).

It lives in lagoons and rivers bottomless pits, preferring lentic environment with sandy bottom and mud, near border vegetation (Baldisserotto *et al.*, 2010). It has also a benthic behaviour (Bizerril & Lima, 2000) which facilitates the Digenea recruitment (Eiras *et al.*, 1999).

Acanthostomum gnerii is a digenetic fluke commonly found in the digestory tract of freshwater Siluriformes (including Heptapteridae and Pimelodidae). It is well distributed along Central and South America (; Lamothe-Argumedo & Ponciano-Rodriguez, 1985; Watson, 1976; Kohn & Fróes, 1986; Ostrowski de Nuñes & Gil de Pertierra, 1991). In South and Southeast Brazil, it has been reported from *Rhamdia quelen* (=*R. sapo*) (Brandão, 1977; Kohn & Fróes, 1986; Fortes & Hoffman, 1995) and *Pseudoplatystoma corruscans* (Corrêa & Brasil-Sato, 2008).

The aim of this study is to provide additional information on morphological features of the *A. gnerii* by histological sections and scanning electron microscope, as well as provides comparative measurements of *Acanthostomum* spp.

MATERIAL AND METHODS

Silver catfish total length 23.70 ± 5.01 cm and weight 131.6 ± 94.71 g were obtained from Madre river (27° 57' 38" S, 48° 45' 27" W), Santa Catarina State, Brazil between May 2010 and April 2012. Fish 45 were donated from a local fisherman and the digestory tract was removed, placed in Petri dishes with tap water and then examined for parasites with the aid of a stereomicroscope. Specimens of *A. gnerii* were fixed in AFA (alcohol, formaline

and acetic acid), stained in acetic alum carmine, dehydrated in ethyl alcohol, cleared in beechwood creosote and mounted in Canada balsam. For histological sections, previous fixed specimens were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Cuts of three micrometers sections were made on a microtome. The sections were stained with standard Harris' haematoxylin and eosin (H & E) stain. For scanning electron microscopy, specimens were fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer and 2% osmium tetroxide, dehydrated in a graded series of alcohol, critical-point dried using liquid CO_2 and examined using a scanning electron microscope (Zeiss DSM-940A) at EMBRAPA - Clima Temperado, Pelotas, RS, Brazil. All measurements are expressed in millimeters and are presented as follows: mean ± standard deviation (if n > 30) (minimum and maximum values). The ecological terms "prevalence" (P), "mean intensity" (MI) and "mean abundance" (MA) follow Bush et al. (1997). Vouchers were deposited in the "Helminthological Collection of the Oswaldo Cruz Institute (CHIOC)".

RESULTS

Taxonomic summary

Hosts*: Rhamdia quelen* (= *Rhamdia sapo*) in Szidat (1954); Travassos *et al.* (1969); Brandão (1977); Lunaschi (1986); Ostrowski de Nuñez & Gil de Pertierra (1991); Fortes & Hoffmann (1995); Gil de Pertierra & Ostrowski de Nuñez (1995); present study); *Rhamdia rogersi* in Caballero & Brenes (1958); Brenes-Madrigal (1961); *Rhamdia nicaraguenses* and *Rhamdia managuensis* in Watson (1976); *Rhamdia guatemalensis* in Lamothe-Argumedo & Ponciano-Rodriguez (1985); *Rhamdia* sp. in Kohn & Fróes (1986); *Cyphocharax gilbert* (=*Pseudocurimata gilbert*) in Lunaschi (1986); *Pimelodella laticeps* in Lunaschi (1986); Ostrowski de Nuñez & Gil de Pertierra (1991); *Pseudoplatystoma corruscans* in Corrêa & Brasil-Sato (2008); *Hypostomus commersoni*; *Loricariichthys anus* and *Pimelodus maculatus* in Fortes & Hoffmann (1995).
Site of infection: stomach, intestine.

Locality: Argentine (Type locality, Szidat, 1954; Lunaschi, 1986; Ostrowski de Nuñez & Gil de Pertierra, 1991; Gil de Pertierra & Ostrowski de Nuñez, 1995); Costa Rica (Caballero & Brenes, 1958, Brenes, 1961); Brazil (Brandão, 1977; Kohn & Fróes, 1986; Fortes & Hoffmann, 1995; Corrêa & Brasil-Sato, 2008; present study); Nicaragua (Watson, 1976) and Mexico (Lamothe-Argumedo & Ponciano-Rodriguez, 1985).

Prevalence, mean intensity of infection, mean abundance: P 40%; MI 1.88 ± 1.27; MA 0.75 ± 1.22

Material examined: 34 specimens (21 stained with acetic alum carmine; 8 stained with H&E; 5 analyzed with a scanning electron microscope)

Voucher specimens deposited: CHIOC (2 vouchers).

Description of the studied specimens: Elongate body 1.437 ± 0.443 (0.984-2.840) length by 0.393 ± 0.061 (0.304-0.560) greatest width. Tegumental spines small, rounded and dense in forebody, becoming smaller and less dense until absent towards posterior end of body. Multiple agglomerations of glandular cells intermediating the proteinaceous spines. Two tegumental pits or infoldings close to the ventral sucker, one anterior and the other posterior to ventral sucker. Unicellular glands abundant around the pharynx (seen in histological sections (Fig. 2F); such glands are also present in the body tegument, becoming less dense towards posterior end of body. Retractile oral sucker larger than ventral

sucker, terminal, funnel-shaped 0.256 ± 0.054 (0.168-0.320) long by 0.204 ± 0.046 (0.096-0.264) wide. Its opening is surrounded by a single ininterrupted row of 23-24 large retractile spine-like structures of 0.036 ± 0.008 (0.024-0.056) by 0.016 ± 0.004 (0.008-0.024). Pre-pharynx long 0.069 ± 0.049 (0.004-0.178), but appears shorter when contracted. Pharynx muscular, 0.135 ± 0.017 (0.112-0.176) long by 0.078 ± 0.024 (0.004-0.112) wide. Epitheliated esophagus short. Ventral sucker pre-equatorial 0.138 ± 0.022 (0.088-0.176) long by 0.139 ± 0.030 (0.088-0.232) wide submerged in tegument or elevated above tegument surface (it depends on the state of the worm at fixation). Genital pore immediately pre-acetabular. One sinistral epitheliated intestinal caecum 0.122 ± 0.040 (0.092-0.198) with two arms terminating blindly disposed anterior up to the level of pharynx. Posteriorly, it ends into

Table 1. Comparative measurements of *Acanthostomum gnerii* Szidat, 1954. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. ^{L}Length, ^{w}Width.

	Present study	**Szidat (1954)**	**Brandão (1977)**	**Kohn & Fróes (1986)**
Hosts	*Rhamdia quelen*	*Rhamdia quelen*	*Rhamdia sapo (=R. quelen)*	*Rhamdia* sp.
Site of infection	Stomach, intestine	Intestine	Intestine	Intestine
Body^{L}	1.437 ± 0.443 (0.984-2.840)	1.64-2.00	1.64-2.0	2.8 (1.9-3.48)
Body^{W}	0.393 ± 0.061 (0.304-0.560)	0.50-0.64	0.50-0.64	0.65 (0.49-0.80)
Oral sucker^{L}	0.256 ± 0.054 (0.168-0.320)	0.245-0.250	0.245-0.250	0.28 (0.24-0.32)
Oral sucker^{W}	0.204 ± 0.046 (0.096-0.264)	0.200	0.200	0.24 (0.21-0.30)
Number of peribuccal spines	23-24	21-23	21-23	20-24
Spine^{L}	0.036 ± 0.008 (0.024-0.056)	0.069	-	0.041-0.060
Spine^{W}	0.016 ± 0.004 (0.008-0.024)	0.012	-	0.012-0.020
Ventral sucker^{L}	0.138 ± 0.022 (0.088-0.176)	0.10	0.10	-
Ventral sucker^{W}	0.139 ± 0.030 (0.088-0.232)	0.15	0.15	0.18 (0.15-0.21)
Prepharynx^{L}	0.069 ± 0.049 (0.004-0.178)	-		-
Pharynx^{L}	0.135 ± 0.017 (0.112-0.176)	0.12	0.12	0.14 (0.11-0.15)
Pharynx^{W}	0.078 ± 0.024 (0.004-0.112)	0.10	0.10	-
Oesophagus^{L}	-	-	-	-
Testis^{L}		0.15-0.18	-	0.28 (0.21-0.35)

a dorsal-lateral anal pore in posterior extremity of body. Ovary compact, entire and spherical 0.113 ± 0.032 (0.064-0.192) long by 0.125 ± 0.035 (0.064-0.176) wide, dextral in most specimens, slightly anterior to testes. Saclike seminal receptacle of variable size 0.104 ± 0.032 (0.072-0.176) by 0.104 ± 0.028 (0.056-0.152) situated between ovary and anterior testis. In fully relaxed specimens, ovary separated from anterior testes by seminal receptacle. Two dextral testes tandem, ovoid, wider than long; anterior testis 0.116 ± 0.037 (0.064-0.232) by 0.171 ± 0.034 (0.112-0.256) somewhat smaller than posterior testis 0.139 ± 0.044 (0.096-0.288) by 0.177 ± 0.037 (0.112-0.288). Seminal vesicle composed of a long tube, strongly coiled in its proximal region and less coiled and more sac-like in its distal region. Vitellaria consisting of compact follicles arranged in two irregular or more or less regular lateral rows, generally 7 on the left side and 6 on the right side extending between the posterior extremity of the uterus to post-ovarian. Uterine loops entirely preovarian, occupying the space from the ventral sucker to the ovary. Operculate eggs 0.028 ± 0.003 (0.023-0.032) by 0.011 ± 0.002 (0.007-0.013). Excretory vesicle Y-shaped very long, bifurcating immediately posterior to ventral sucker and extending close to the level of pharynx (Fig. 2D). Excretory pore terminal.

Table 1. Continued. Comparative measurements of *Acanthostomum gnerii* Szidat, 1954. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. [L]Length, [W]Width.

	Present study	**Szidat (1954)**	**Brandão (1977)**	**Kohn & Fróes (1986)**
Hosts	*Rhamdia quelen*	*Rhamdia quelen*	*Rhamdia sapo (=R. quelen)*	*Rhamdia sp.*
Site of infection	Stomach, intestine	Intestine	Intestine	Intestine
Anterior	0.116 ± 0.037 (0.064-0.232)	-		
Posterior	0.139 ± 0.044 (0.096-0.288)	-		
Testis[W]		0.25-028	-	0.30 (0.21 -0.38)
Anterior	0.171 ± 0.034 (0.112-0.256)	-		
Posterior	0.177 ± 0.037 (0.112-0.288)	-		
Ovary[L]	0.113 ± 0.032 (0.064-0.192)	0.12-0.13	-	0.18 (0.14-0.21)
Ovary[W]	0.125 ± 0.035 (0.064-0.176)	-	-	0.19 (0.15-0.24)
Seminal receptacle[L]	0.104 ± 0.032 (0.072-0.176)	-	-	-
Seminal receptacle[W]	0.104 ± 0.028 (0.056-0.152)	-	-	-
Egg[L]	0.028 ± 0.003 (0.023-0.032)	0.029-0.037	0.029-0.37	0.030 (0.027-0.032)
Egg[W]	0.011 ± 0.002 (0.007-0.013)	0.014-0.016	0.014-0.016	0.014 (0.012-0.015)

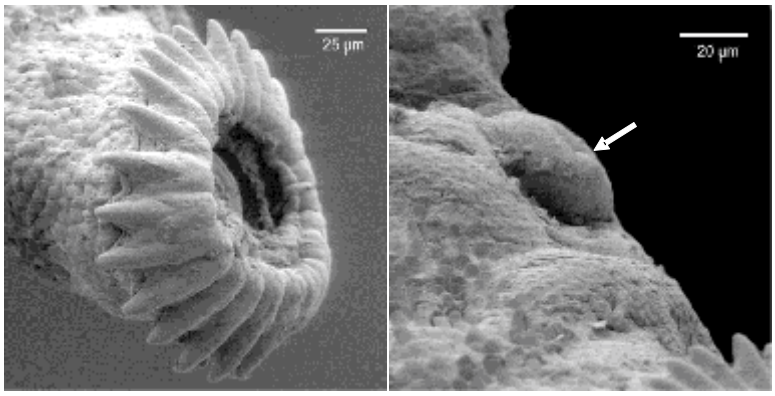

Figure 1. Scanning electron micrographs of the external morphology of *Acanthostomum gnerii,*. **A.** Everted oral sucker surrounded by circum-oral spines. Note the presence of spines in the tegument surface on anterior-dorsal region of the body. **B.** Region of the elevated ventral sucker (white arrow).

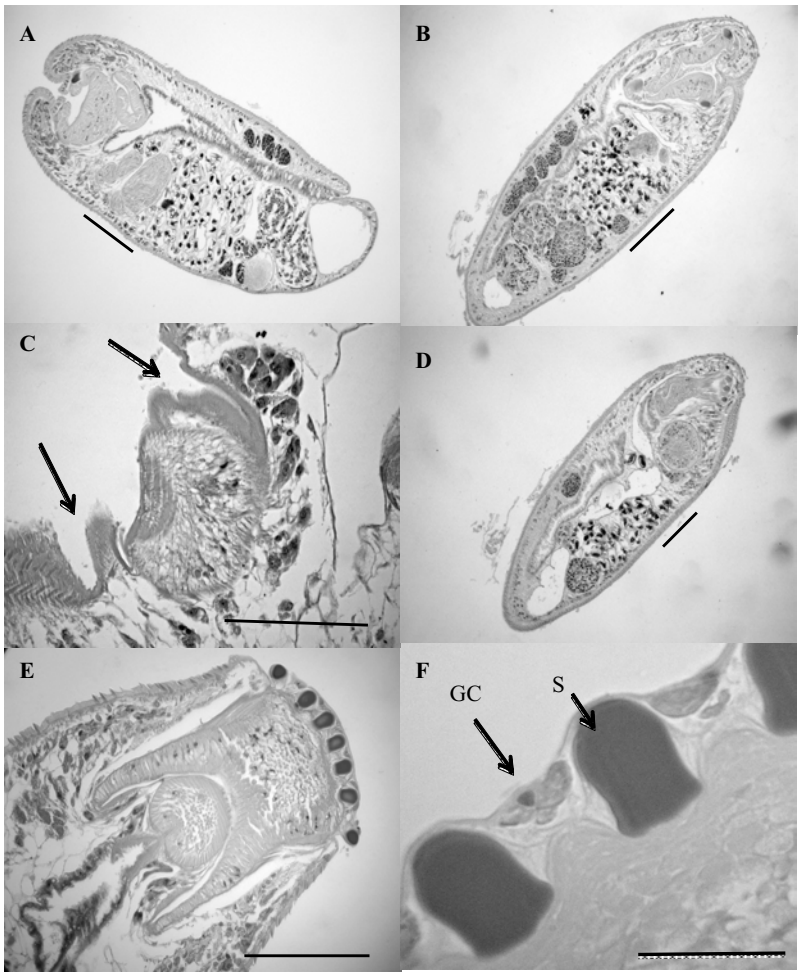

Figure 2. Light micrographs of histological sections of adult *Acanthostomum gnerii* stained with haematoxylin & eosin. **A, B.** Longitudinal section of the body. **C.** Region of the ventral sucker showing preacetabular and postacetabular pits (arrows). **D.** Longitudinal section showing the excretory vesicle. **E.** Section of the anterior end. **F.** Longitudinal section of the anterior sucker revealing the proteinaceous spines (S) with an agglomerated of glandular cells between two of them (GC). Bar: A: 0.15; B: 0.25; C: 0.10; D: 0.15; E: 0.20 F: 0.035 mm.

Table 2. Comparative measurements of *Acanthostomum* spp. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. [L]Length, [W]Width.

	A. gnerii **Szidat (1954) in Lunaschi (1986)**	***A. gnerii*** **Szidat (1954) in Ostrowski de Nuñes & Gil de Pertierra (1991)**	***A. astorquii*** **Watson (1976)**
Hosts	*Rhamdia quelen* (=*R. sapo*), *Cyphocharax gilbert* (=*Pseudocurimata gilberti*) and *Pimelodella laticeps*	*R. sapo*	*R. nicaraguensis*
Site of infection	Stomach and intestine	-	Intestine
Body^{L}	0.434-0.990	1.538 ± 0.372 (0.900 2.484)	0.825 (0.750-0.875)
Body^{W}	0.171-0.389	0.356 ± 0.091 (0.18-0.576)	0.300 (0.255-0.390)
Oral sucker^{L}	0.094-0.187	0.212 ± 0.033 (0.130-0.284)	0.169 (0.147-0.180)
Oral sucker^{W}	0.092-0.214	0.169 ± 0.0317 (0.087-0.232)	0.130 (0.119-0.135)
Number of peribuccal spines	20 (19-23)	20 (19-21)	20
Spine^{L}		0.0509 ± 0.010 (0.029-0.066)	0.033 (0.032-0.036)
Spine^{W}		0.012 ± 0.001 (0.008-0.014)	0.011 (0.009-0.013)
Ventral sucker^{L}	0.051-0.124	0.128 ± 0.022 (0.088-0.188)	0.099 (0.086-0.107)
Ventral sucker^{W}	0.064-0.131	0.129 ± 0.022 (0.087-0.182)	0.103 (0.090-0.114)
Prepharynx^{L}	0.053-0.059		0.046
Pharynx^{L}	0.071-0.148	0.139 ± 0.023 (0.087-0.185)	0.104 (0.100-0.107)
Pharynx^{W}	0.055-0.080	0.100 ± 0.024 (0.055-0.150)	0.088 (0.057-0.118)
Oesophagus^{L}	-		-

Table 2. Continued. Comparative measurements of *Acanthostomum* spp. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. [L]Length, [W]Width.

	A. gnerii **Szidat (1954) in Lunaschi (1986)**	***A. gnerii*** **Szidat (1954) in Ostrowski de Nuñes & Gil de Pertierra (1991)**	***A. astorquii*** **Watson (1976)**
Hosts	*Rhamdia quelen* (=*R. sapo*), *Cyphocharax gilbert* (=*Pseudocurimata gilberti*) and *Pimelodella laticeps*	*R. sapo*	*R. nicaraguensis*
Site of infection	Stomach and intestine	-	Intestine
Testis[L]			
Anterior	0.064-0.092	0.141 ± 0.032 (0.084-0.24)	0.105 (0.096-0.121)
Posterior	0.057-0.104	0.165 ± 0.038 (0.096-0.300)	0.114 (0.107-0.121)
Testis[W]			
Anterior	0.099-0.198	0.192 ± 0.050 (0.096-0.324)	0.122 (0.116-0.132)
Posterior	0.085-0.193	0.194 ± 0.052 (0.108-0.336)	0.125 (0.107-0.144)
Ovary[L]	0.053-0.110	0.120 ± 0.030 (0.072-0.252)	0.086 (0.082-0.093)
Ovary[W]	0.044-0.113	0.1159 ± 0.027 (0.060-0.216)	0.079 (0.052-0.100)
Seminal receptacle[L]	-		0.050
Seminal receptacle[W]	-		0.063 (0.054-0.071)
Egg[L]	0.025-0.034	0.030 ± 0.002 (0.026-0.037)	0.030 (0.029-0.032)
Egg[W]	0.016-0.022	0.014 ± 0.002 (0.011-0.020)	0.019 (0.018-0.020)

Table 3. Comparative measurements of *Acanthostomum* spp. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. [L]Length, [W]Width.

	A. minimum **Stunkard (1938)**	***A. minimum*** **Stunkard (1938)** (=*Stunkardiella minima) in* Lamothe-Argumedo & Ponciano-Rodriguez (1985)	***A. proctophorum*** (Dwivedi, 1966) Yamaguti, 1971 *in* Dwivedi (1966)
Hosts	*Rhamdia guatemalensis*	*Rhamdia guatemalensis*	*Trop idonotus piscator*
Site of infection			
Body[L]	0.63-1.18	1.271-2.270	1.98-2.85
Body[W]	0.39-0.42	0.241-0.418	0.18-0.21
Oral sucker[L]	0.17	0.165-0.285	0.135-0.150
Oral sucker[W]	0.18	0.191-0.234	0.105-0.130
Number of peribuccal spines	18-20	19-20	20-22
Spine[L]	0.04	0.037-0.52	0.052-0.054
Spine[W]		0.015-0.018	0.013-0.014
Ventral sucker[L]	0.12	0.101-0.146	0.075-0.090
Ventral sucker[W]	0.13	0.093-0.150	0.060-0.075
Prepharynx[L]		0.157-0.255	0.075-0.150
Pharynx[L]	0.10-0.11	0.086-0.135	0.060-0.090
Pharynx[W]	0.068-0.085	0.067-0.136	0.060-0.075
Oesophagus[L]		0.037-0.048	0.018-0.021
Testis[L]	0.14-0.22		
Anterior		0.138-0.176	0.090-0.135

Table 3. Continued. Comparative measurements of *Acanthostomum* spp. The averages ± standard deviation are followed by minimum and maximum values and the number of structures or specimens measured in parenthesis. [L]Length, [W]Width.

	***A. minimum* Stunkard (1938)**	***A. minimum* Stunkard (1938)** (=*Stunkardiella minima) in* Lamothe-Argumedo & Ponciano-Rodriguez (1985)	***A. proctophorum*** (Dwivedi, 1966) Yamaguti (1971) *in* Dwivedi (1966)
Hosts	*Rhamdia guatemalensis*	*Rhamdia guatemalensis*	*Tropidonotus piscator*
Site of infection			
Posterior		0.097-0.243	0.112-0.165
Testis[W]	0.07-0.15		
Anterior		0.146-0.217	0.090-0.105
Posterior		0.112-0.281	0.090-0.105
Ovary[L]	0.07-0.15 in diameter	0.090-0.120	0.080-0.100
Ovary[W]		0.037-0.131	0.064-0.080
Seminal receptacle[L]	0.08-0.13 in diameter	0.071-0.136	0.076-0.084
Seminal receptacle[W]		0.112-0.138	0.076-0.080
Egg[L]	0.028-0.030	0.026-0.033	0.032
Egg[W]	0.016-0.018	0.011-0.015	0.012

DISCUSSION

Acanthostomum gnerii was first described by Szidat (1954) parasitizing *R. quelen* from Argentina. In his original description, Szidat (1954) described *A. gnerii* with 21 to 23 circumoral spines and two intestinal ceca, each with an anal pore at distal end. Later, Lunaschi (1986) re-observed the three existed specimens and stated that one caecum was completely atrophied, promoting therefore a redescription of the species. Surprisingly, most reports of *A. gnerii* made before the redescription promoted by Lunaschi (1986) characterized the species with 2 caeca, following the findings of the original author. In this way, Caballero & Brenes (1958) identified *A. gnerii* from *R. rogersi* in Panama with 19-20 circumoral spines and two intestinal ceca

(however, only one caecum was drawn). Brandão (1977) recognized *A. gnerii* from South Brazil and described it with two intestinal ceca, each with an anal pore at distal end. Kohn & Fróes (1986) defined *A. gnerii* from *Rhamdia* sp. in Rio de Janeiro, Brazil, with 20 to 24 circumoral spines and represented the species in their schematic illustration with two intestinal ceca. From Nicaragua, Watson (1976) reported *A. gnerii* from *R. nicaraguensis* and *R. managuensis* and also described a new species, *A. astorquii* from *R. nicaraguensis* with 20 circumoral spines and one atrophied caeca: the left one five to six times as broad as the right one, each cecum with anus at posterior end of body.

From the 20 species comprising this genus (Brooks, 1980; Catto & Amato, 1993; Brooks, 2004), *Acanthostomum proctophorum* and *A. minimum* closely resembles *A. gnerii* in inumerous characters. However, they mainly differ in the extent of the vitelline follicles (on both species they are distributed laterally in the body from the lower margin of the seminal vesicle up to the posterior level of the ovary, while in *A. gnerii* they are restricted to the posterior portion of the uterus to post-ovarian).

According to Ostrowski de Nunes & Gil de Pertierra (1991), although Stunkard (1938) reported "the digestive ceca arise just behind the pharynx and open to the surface of the body on either side near the posterior end" for *A. minimum*, the author illustrated only one caecum in its representative drawing for the species. The same author also compared *A. gnerii* with *A. minimum* and realized that the latter also had only one caecum present. Additionally, Brooks (1980) analysed holotypes of both *A. minimum* and *A. astorquii* and concluded that the two appeared indistinguishable. However, the author alerted for the need of further examination on larger series of specimens to better elucidate their status. Indeed, although Brooks & Holcmann (1993) recognized *A. minimum* and *A. astorquii* as valid species, the authors did not present morphological distinction between the two, both defined as "having testes oblique; vitelline follicles sparse and one caecum atrophied".

Despite the similarities shared by these species, the present authors prefer to maintain them as separate species until further studies prove otherwise. In the present study, it was possible to analyse specimens of *A. gnerii* in various states of contraction and yet, all of them presented the same disposal of testes (tandem) unlike *A. astorquii*, which is oblique and the same extent of vitellaria, never extending anteriorly to posterior margin of seminal vesicle (as in *A. minimum* and *A. proctophorum*).

Miller & Cribb (2008) stated once the importance of the relative length and termination of caeca as usefull taxonomic characters in the classification of the group. The absence of one caecum in some acanthostomines led Simha (1958) to create *Haplocaecum*, later considered valid by Dwivedi (1966). For Khalil (1963) and Carter & Etges (1972), *Haplocaecum* was considered a subgenus of *Acanthostomum*; as a synonymous of the subgenus *Atrophecaecum* according to Yamaguti (1971) and finally as a synonymous of *Atrophecaecum* by Brooks (1980). It is known a great diversity on the patterns of organization of the gut, especially within the acanthostomines (*sensu* Brooks, 1980). Usually, they end blindly, but there may be separate ani, a uroproct, a cyclocoel, one caecum ending blindly and one opening at an anus, one atrophied caecum, or only a single caecum that ends blindly or at an anus.

However, through histological sections made in the present study, it was possible to comprehend a new pattern of organization for *A. gnerii*, not registered so far. The intestine of the present studied specimens is composed of a single cecum which bifurcates and originates

two arms terminating blindly oriented anteriorly (Figs. 2 A, B); these branches are joined together to form only one intestinal cecum, which ends into only one lateral anal pore. Ibraheem (2006) studied the morphology of *Acanthostomum spiniceps* (Looss, 1896) through histological sections and showed that the short oesophagus clearly bifurcates into two lateral intestinal caeca for this species. It shows, however, two very discrete extensions of the intestine oriented anteriorly arising just after the oesophagus (maybe these branches are atrophied for this species).

Ostrowski de Nuñes & Gil de Pertierra (1991) studied the life cycle of *A. gnerii* collected in Argentina and defined the metacercariae stage as possessing one intestinal caecum in one side well developed, opening into an anal pore at the end of the body and another one in the other side of the body in variable degrees of atrophy. However, in metacercariae older than 45 days post-infection the atrophied caecum was not observed anymore by the authors. With respect to the adult stage, all analysed specimens presented one caecum completely atrophied. Furthermore, these authors, concluded that 64.3% of the specimens had their functional caecum on the left side of the body, while 35.7% had it on the right size.

As they stated once, "because of the great similarity of Argentine, Central American and Mexican species it is doubtful that they are really diferent species". In the same way with specimens collected from Brazil, the present authors believe that it may be a matter of morphological variation of different populations of the same species. The presence of two anterior arms associated with the digestory tract initialy intrigued the present authors: this character was only seen through histological sections and a doubt had arisen if they did not correspond to the excretory vesicle, as suggested by Lunaschi (1986). However, its internal composition differs greatly from the vesicle composition (Figure 2, A, B and D), leaving no doubt that those branches refer to the intestinal tract.

Although the great diversity on the patterns of organization of the gut of *A. gnerii*, all other biometric characters of the present studied specimens remained constant with previous reports for the species (Tables 1 and 2). However, the occurrence of intestinal arms disposed anteriorly has, so far, never been associated to this digenetic family and should therefore be included as a valid feature for the Acanthostomidae.

An interesting feature that seems to be associated with all acanthostomes is their ability to retract the entire oral sucker and circum-oral spines into the anterior part of the body. Tkach & Snyder (2003) identified such ability in *Proctocaecum macroclemidis* (Tkach & Snyder, 2003) (=*Acanthostomum macroclemidis*) and concluded that the spines associated with muscles and the tegumental surface form what the authors called a collar like structure that can be retracted almost entirely. Ibraheem (2006) also found this capacity in *A. spiniceps* (Looss, 1896) and *A. absconditum* (Looss, 1901) and correlated this capacity either to anchorage of spines for attachment into host's gut wall or to making a firm attachment during forward movement of the worm. The same author also showed numerous secretory granules on the outer openings of definite pores on the tegumental crown of early juveniles of *A. absconditum.* These granules were also reported in the present study in the form of multiple agglomerations of glandular cells intermediating the proteinaceous spines (Figure 2, E, F).

This study provides additional information on morphological features of the *A. gnerii*, however it is needed to make new studies using molecular tools to determine whether characters found have correspondence with the diversity of species mentioned in the literature.

BIBLIOGRAPHIC REFERENCES

Baldisserotto, B, Radünz Neto, J & Barcellos, LJG. 2010. *Jundiá (*Rhamdia *sp).* In: Baldisserotto, B & Gomes, LC. (Orgs.). Espécies nativas para piscicultura no Brasil. 2. ed. Santa Maria. Ed. da UFSM, 2010. pp. 301-333.

Brandão, DA. 1977. *Trematódeos digenéticos de Rhamdia sapo (Valenciennes, 1840) (jundiá) no estuário do Guaíba, Rio Grande do Sul, Brasil. Porto Alegre – RS*, 32 pp. Dissertação (Mestrado em Medicina Veterinária) – Curso de Pós-graduação em Medicina Veterinária, Universidade Federal do Rio Grande do Sul.

Brenes, RR. 1961. *Catálogo de los helmintos parásitos de Costa Rica.* Revista de Biologia Tropical, vol. 9, pp. 67-95.

Brooks, DR. 1980. *Revision of the Acanthostominae Poche, 1926 (Digenea: Cryptogonomidae).* Zoological Journal of the Linnean Society, vol. 70, pp. 313-382.

Brooks, DR & Holcman, B. 1993. *Revised Classification and Phylogenetic hypothesis for the Acanthostominae Looss, 1899 (Digenea: Opistorchiformes: Cryptogonomidae).* Proceedings of the Biological Society of Washington, vol. 106, pp. 207-220.

Brooks, DR. 2004. *Comments on the gonotyl of* Proctocaecum macroclemidis *(Tkach and Snyder, 2003) n. comb. (Digenea: Acanthostomidae: Acanthostominae), with a key to the genera of Acanthostominae and new phylogenetic tree for* Proctocaecum *Baugh, 1957.* Journal of Parasitology, vol. 90, pp. 594-597.

Bush, AO, Lafferty, KD, Lotz, JM & Shostak, AW. 1997. *Parasitology meets ecology on it own terms:* Margolis *et al*. revisited. The Journal of Parasitology, vol. 83, pp. 575-583.

Caballero, CE & Brenes-Madrigal, R. 1958. *Helmintos de la República de Costa Rica VII. Trematódos de algunos vertebrados salvajes, con descripción de uma nueva especie de* Acanthostomum *Looss, 1899.* Anais do Instituto de Biologia, Universidade Nacional Autónoma de México, vol. 29, pp. 165-179.

Catto, JB & Amato, JFR. 1993. *Digenetic trematodes (Cryptogonomidae: Acanthostominae) parasites of the Caiman,* Caiman crocodilus *yacare (Reptilia, Crocodylia) from the Pantanal Mato-Grossense, Brazil, with the description of a new species.* Memórias do Instituto Oswaldo Cruz, vol. 88, pp. 435-440.

Correa, RSS & Brasil-Sato, MC. 2008. *Digenea in the surubim* Pseudoplatystoma corruscans *(Spix and Agassiz, 1829) (Siluriformes: Pimelodidae) of the upper São Francisco river, State of Minas Gerais, Brazil.* Brazilian Archives of Biology and Technology, vol. 51, pp. 1181-1185.

Carter, S & Etges, FJ. 1972. *A redescription of* Acanthostomum marajoarum *(Teixeira de Freitas and Lent, 1938) with notes on the subfamily Acanthostominae (Nicoll, 1914) Hughes Higginbotham and Clary, 1942 (Trematoda, Acanthostomidae*). Proccedings of the Helminthological Society of Washington, vol. 39, pp. 234-239.

Dwivedi, MP. 1966. *On a new species of* Haplocaecum *Simha, 1958, (Trematoda, Digenea) from a water snake* Tropidonotus piscator. Revista de Biología Tropical, vol. 14, pp. 87–91.

Eiras, J.C., Dias, M.L., Pavanelli, G.C. & Machado, M. 1999. *Histological studies on the effects of* Clinostomum marginatum *(Digenea, Clinostomidae) in the second intermediate host* Loricariichthys platymetopon *(Osteichthyes, Loricariidae), of the upper Paraná River, Brazil.* Acta Scientiarium, v. 21, pp. 237-241.

Fortes, E & Hoffmann, RP. 1995. *Survey of the parasite fauna of fishes from lake Guaiba, Porto Alegre, Rio Grande do Sul, Brazil*. Revista Brasileira de Medicina Veterinária, vol. 17, pp. 107-111.

Fracalossi, DM, Meyer, G., Santamaria, F.M., Weingartner, M. & Zaniboni Filho, E. 2004. *Desempenho do jundiá,* Rhamdia quelen*, e do dourado,* Salminus brasiliensis*, em viveiros de terra na região Sul do Brasil*. Acta Scientiarum, vol. 26, p. 345-352.

Gil De Pertierra, AA & Ostrowski De Nuñez, M. 1995. *Ocurrencia estacional de* Acanthostomum gnerii *Szidat, 1954 (Acanthostomidae, Acanthostominae) y de dos espécies de Derogenidae, Halipegidae, parásitos del bagre sapo* Rhamdia sapo *Valenciennes, 1840 (Pisces, Pimelodidae) em Argentina.* Revista Brasileira de Biologia, vol. 55, pp. 305-314.

Ibraheem, MH. 2006. *On the morphology of* Acanthostomum spiniceps *(Looss, 1896) and* A. absconditum *(Looss, 1901) (Digenea: Cryptogonimidae: Acanthostominae) with particular reference to the juvenile stage.* Acta Zoologica, vol. 87, pp. 159-169.

Khalil, LF. 1963. *On* Acanthostomum gymnarchi *(Dollfus, 1950) with notes on the genera* Acanthostomum *Looss, 1899,* Atrophocaecum *Bhalerao, 1940,* Gymnatotrema *Morosov, 1955 and* Haplocaecum *Simha, 1958*. Journal of Helminthology, vol. 37, pp. 207–214.

Kohn, A & Fróes, OM. 1986. Saccocoelioides godoyi *n. sp. (Haplosporidae) and other trematodes parasites of fishes from the guaiba estuary, RS, Brazil.* Memórias do Instituto Oswaldo Cruz, vol. 81, pp. 67-72.

Lamothe-Argumedo, R & Ponciano-Rodriguez, G. 1985. *Estudio helmintologico de los animales silvestres de la estacion de Biologia Tropical "Los Tuxtlas", Veracruz. Trematoda III. Redescripcion de Stunkardiella minima (Stunkard, 1938) Lamothe y Ponciano, 1985.* Anales del Instituto. Biologico de la Universidad. Nacional. Autonoma del México Serie Zoologia, vol. 56, pp. 323-336.

Lunaschi, L. 1986. *Helmintos parasitos de peces da Água Dulce de la Argentina. V. Redescripcion de* Acanthostomum *(*Atrophocaecum*)* gnerii *(sic) Szidat 1954 (Trematoda: Acanthostomidae).* Neotropica, vol. 32, pp. 35-42.

Miller, TL & Cribb, TH. 2008. *Family Cryptogonimidae Ward, 1917. In: Keys to the Trematoda,* vol. 3, eds Bray, R.A.; Gibson, D.I.; Jones, A. The Natural History Museum, London, UK. 848p.

Ostrowski De Nuñez, M & Gil De Pertierra, A. 1991. *The life history of* Acanthostomum gnerii *Szidat 1954 (Trematoda: Acanthostomide), from the catfish Rhamdia sapo in Argentina.* Zoologischer Anzeiger, vol. 227, 1/2, pp. 58-71.

Silvergrip, AMC. 1996. *A systematic revision of the neotropical catfish genus* Rhamdia *(Teleostei, Pimelodidae).* 156 pp. Thesies (PhD), Swedish Museum Natural History, Stockholm, Sweden.

Simha, SS. 1958. *Studies on the trematode of reptiles found in Hyderabad state*. Parasitenkunde, 18: 1610218.

Stunkard, HW. 1938. *Parasitic flatworms from Yucatan.* Publications of Carnegie Institute of Washington, vol. 491, pp. 33–50.

Szidat, L. 1954. *Trematodes nuevos de peces de agua dulce de la Republica Argentina y um intento para aclarar su origen marino.* Revista del Instituto Nacional de Investigacion de las Ciencias Naturales, Bernardino Rivadavia, vol. 1, pp. 1-85.

Tkach, VV & Snyder, S. 2003. Acanthostomum macroclemidis *n. sp. (Digenea: Cryptogonimidae) from the*

alligator snapping turtle, Macroclemys temmincki. Journal of Parasitology, vol. 89, pp. 159-167.

Travassos, L, Freitas, JFT & Kohn, A. 1969. *Trematódeos do Brasil*. Memórias do Instituto Oswaldo Cruz, vol. 76, pp. 1-886.

Watson, DE. 1976. *Digenea of fishes from lake Nicaragua.* Papers in the Biological Sciences. Investigations of the Ichtyofauna of Nicaraguan lakes. University of Nebraska, Lincoln.

Yamaguti, S. 1971. *Synopsis of digenetic trematodes of vertebrates*. Keigaku, Tokyo, vol. I, 1074 pp.; vol. II, 349 pp.

Received June 15, 2016.
Accepted August 21, 2016.