Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Montes MM, Barneche J, Croci Y, Balcazar D, Almirón A, Martorelli S, Pérez-Ponce de León G (2021). Description of a new species of *Auriculostoma* (Digenea: Allocreadiidae) from *Characidium heirmostigmata* (Characiformes: Crenuchidae) from Argentina, using morphological and molecular data. *Journal of Helminthology* **95**, e19, 1–8. https://doi.org/10.1017/S0022149X21000109

Received: 19 December 2020
Revised: 1 March 2021
Accepted: 3 March 2021

Key words:
Auriculostoma; Allocreadiidae; Iguazú; Argentina; Crenuchidae; Characidium heirmostigmata

Author for correspondence:
M.M. Montes,
E-mail: martinmiguelmontes@gmail.com

Description of a new species of *Auriculostoma* (Digenea: Allocreadiidae) from *Characidium heirmostigmata* (Characiformes: Crenuchidae) from Argentina, using morphological and molecular data

M.M. Montes[1], J. Barneche[1], Y. Croci[1], D. Balcazar[1], A. Almirón[2], S. Martorelli[1] and G. Pérez-Ponce de León[3]

[1]Centro de Estudios Parasitológicos y Vectores (CEPAVE), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata (CCT, CONICET-UNLP), Boulevard 120s/n e/60 y 64, La Plata, 1900, Argentina; [2]División Zoología Vertebrados, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900, La Plata, Buenos Aires, Argentina and [3]Escuela Nacional de Estudios Superiores Unidad Mérida (ENES-Mérida), Universidad Nacional Autónoma de México, Km. 4.5 carretera Mérida-Tétiz, Municipio de Ucú, C.P. 97357, Yucatán, Mexico

Abstract

During a parasitological survey of fishes at Iguazu National Park, Argentina, specimens belonging to the allocreadiid genus *Auriculostoma* were collected from the intestine of *Characidium heirmostigmata*. The erection of the new species is based on a unique combination of morphological traits as well as on phylogenetic analysis. *Auriculostoma guacurarii* n. sp. resembles four congeneric species – *Auriculostoma diagonale*, *Auriculostoma platense*, *Auriculostoma tica* and *Auriculostoma totonacapanensis* – in having smooth and oblique testes, but can be distinguished by a combination of several morphological features, hosts association and geographic distribution. Morphologically, the new species can be distinguished from both *A. diagonale* and *A. platense* by the egg size (bigger in the first and smaller in the last); from *A. tica* by a shorter body length, the genital pore position and the extension of the caeca; and from *A. totonacapanensis* by the size of the oral and ventral sucker and the post-testicular space. Additionally, one specimen of *Auriculostoma* cf. *stenopteri* from the characid *Charax stenopterus* (Characiformes) from La Plata River, Argentina, was sampled and the partial 28S rRNA gene was sequenced. The phylogenetic analysis revealed that *A. guacurarii* n. sp. clustered with *A. tica* and these two as sister taxa to *A.* cf. *stenopteri*. The new species described herein is the tenth species in the genus and the first one parasitizing a member of the family Crenuchidae.

Introduction

The Iguazu National Park was established as a biological reserve in 1934 to protect the natural and cultural resources of the Paranaense subtropical forest (Administración de Parques Nacionales, 2020). This area includes the Iguazú River Basin, which is divided by the Iguazu waterfalls into two ecoregions: the Iguazu and the Lower Parana ecoregions, located upstream and downstream of the waterfalls, respectively (Abell *et al.*, 2008). These ecoregions show differences in fish community composition and a high diversity of endemic fish species (Casciotta *et al.*, 2016).

During a parasitological survey of fishes conducted in the Arrechea Stream, which drains into the upstream region of the Iguazú River (Iguazu ecoregion), allocreadiid trematodes were collected from the intestines of the characiform fish *Characidium heirmostigmata* Graça & Pavanelli, 2008. The family Allocreadiidae Looss, 1902 is distributed worldwide and their members are mainly parasites of the digestive tract of freshwater fishes (see Caira & Bogéa, 2004). The studied specimens belong to a group of nine genera of allocreadiids characterized by having muscular lobes associated with the oral sucker, including *Acrolichanus* Ward, 1917; *Auriculostoma* Scholz, *Aguirre-Macedo & Choudhury,* 2004; *Crepidostomum* Braun, 1900; *Creptotrema* Travassos, Artigas & Pereira, 1928; *Creptotrematina* Yamaguti, 1954; *Bunodera* Railliet, 1896; *Bunoderella* Schell, 1964; *Megalogonia* Surber, 1928; and *Pseudoparacreptotrema* Pérez Ponce de León, Pinacho-Pinacho, Mendoza-Garfias, Choudhury & García-Varela, 2016 (Atopkin *et al.*, 2020; Pérez-Ponce de León *et al.*, 2020). However, our specimens conform with the diagnosis of the genus *Auriculostoma* in that they possess a single pair of oral lobes, funnel-shaped oral sucker and pretesticular uterus.

https://doi.org/10.1017/S0022149X21000109 Published online by Cambridge University Press

The genus *Auriculostoma* currently contains nine species distributed in Neotropical freshwater fishes across the Americas. Two species are found in Mexico: *Auriculostoma lobata* Hernández-Mena, Lynggaard, Mendoz-Garfias & Pérez-Ponce de León, 2016 in *Brycon guatemalensis* Regan (Characiformes: Bryconidae); and *Auriculostoma totonacapanensis* Razo-Mendivil, Mendoza-Garfias, Pérez-Ponce de León & Rubio-Godoy, 2014 in *Astyanax mexicanus* (de Filippi) (Characiformes: Characidae) (Razo-Mendivil *et al*., 2014; Hernández-Mena *et al*., 2016). Two species are found in Central America: *Auriculostoma astyanace* Scholz, Aguirre-Macedo & Choudhury, 2004 in *Astyanax fasciatus* Cuvier (= *Astyanax aeneus*) (Characiformes: Characidae) from Nicaragua; and *Auriculostoma tica* Hernández-Mena, Pinacho-Pinacho, García-Varela, Mendoza-Garfias & Pérez- Ponce de León, 2018 in *Gymnotus maculosus* (Albert & Miller) (Gymnotiformes: Gymnotidae) from Costa Rica (Scholz *et al*., 2004; Hernández-Mena *et al*., 2019). The remaining five species occur in South America: *Auriculostoma diagonale* Curran, Tkach & Overstreet, 2011 in *Stethaprion* cf. *erythrops* Cope (Characiformes: Characidae) from Peru; *Auriculostoma foliaceum* Curran, Tkach & Overstreet, 2011 in *Bryconops* cf. *caudomaculatus* (Günther) (Characiformes: Characidae) from Peru; *Auriculostoma stenopteri* (Mañé-Garzón & Gascón, 1973) Scholz, Aguirre-Macedo & Choudhury, 2004 in *Charax stenopterus* Cope (Characiformes: Characidae) from Uruguay; *Auriculostoma platense* (Szidat, 1954) Scholz, Aguirre-Macedo & Choudhury, 2004 in *Pimelodus maculatus* Lacépède (= *Pimelodus clarias*) from Brazil; and *Auriculostoma macrorchis* (Szidat, 1954) Scholz, Aguirre-Macedo & Choudhury, 2004 in *Pachyurus bonariensis* Steindachner (Perciformes: Sciaenidae) from Argentina (Mañé-Garzón & Gascón, 1973; Kohn *et al*., 2007; Curran *et al*., 2011; Ostrowski de Nuñez *et al*., 2017).

In Argentina, two species of *Auriculostoma* have been reported: *A. macrorchis* and *A. platense*. The former species has been mainly found in siluriforms, particularly in *Ageneiosus inermis* (L.), *Ageneiosus militaris* Valenciennes, *Auchenipterus nigripinnis* (Boulenger), *Auchenipterus osteomystax* (Miranda Ribeiro) (=*Auchenipterus nuchalis* (Spix & Agassiz)) (Siluriformes: Auchenipteridae); also in *Luciopimelodus pati* (Valenciennes) (Siluriformes: Pimelodidae), *Rhinodoras dorbignyi* (Kner) (Siluriformes: Doradidae) from La Plata River, Buenos Aires City; Middle Paraná River, Corrientes Province; Colastiné River (tributary of the Paraná River), Santa Fe Province; and Paraná-Guazú River, Entre Ríos Province. *Auriculostoma platense* has been reported also from siluriforms, in *Iheringichthys labrosus* (Lütken), *Pimelodus albicans* (Valenciennes), *Pimelodus argenteus* Perugia, *P. maculatus* Lacépède (= *P. clarias*) (Siluriformes: Pimelodidae) and *R. dorbignyi* (Siluriformes: Doradidae); in *Rhamphichthys rostratus* (Linnaeus) (Gymnotiformes: Rhamphichthyidae) from La Plata River, Buenos Aires City and Colastiné River, Santa Fe Province (Szidat, 1954; Ostrowski de Nuñez *et al*., 2017). The newly sampled specimens of *Auriculostoma* are different from the previously described congeners. The main objective of this study is to describe a new species of *Auriculostoma* from characiforms of the Iguazu National Park by using molecular and morphological evidence.

Materials and methods

Sample collections and morphological study

The Arrechea Stream runs near the Macuco Path, ending in a 23-m waterfall in the Iguazu National Park, Misiones Province, Argentina, Iguazu Ecoregion. Specimens of *C. heirmostigmata* were sampled from this stream in 2018 and 2019. Fish were collected with hand nets and transported alive to the field laboratory where they were necropsied. Digeneans found in the intestine were heat-killed and preserved in a 10% formalin. Whole-mount specimens were stained with hydrochloric carmine and mounted in Canada balsam according to standard parasitological techniques (Pritchard & Kruse, 1982) and one specimen was used for scanning electron microscope (SEM) studies. Digital images of specimens were taken using an AmScope MU 1000 10 MP digital camera (Irvine, USA)attached to the microscope and structures were measured using the ImageJ software (Schneider *et al*., 2012). Drawings were made using a light microscope Olympus BX53 (Tokyo, Japan) fitted with differential interference contrast and equipped with a camera lucida. Measurements are given in micrometres (μm) as the mean ± standard deviation followed by the minimum and maximum in parenthesis.

Some specimens were preserved in 96% molecular grade ethanol for DNA analysis. In addition, a single specimen morphologically identified as *Auriculostoma* cf. *stenopteri* found in *C. stenopterus* from Punta Lara, Buenos Aires Province, Argentina, was preserved in 96% ethanol and subsequently used for DNA analysis.

Molecular analysis

Partial sequences of 28S rDNA (28S rRNA gene) were obtained through the polymerase chain reaction (PCR), using the forward primer LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) (Littlewood *et al*., 2000) and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach *et al*., 2003). The PCR reactions were carried out in an Eppendorf Mastercycler thermal using Green GoTaq 5X Buffer (Promega, Madison, Winsconsin, USA), 2.5 mM magnesium chloride (Promega), 0.2 mM of New England Biolabs (Ipswich, Massachusetts, USA) Nucleotide Mix and Flexi GoTaq polymerase enzyme (Promega). The thermocycling profile followed that of Tkach *et al*. (2003).

Phylogenetic analysis

Sequences obtained in this study were assembled using the platform Geneious Pro version 5.1.7 (Drummond *et al*., 2016), and used to search for homologous sequences in GenBank (table 1) using a BLAST search. Sequences (table 1) were aligned using the online version of MAFFT version 7 (Katoh & Standley, 2013) and a matrix of 1246 bp constructed. The online program Gblocks version 0.91 (Castresana, 2000; Talavera & Castresana, 2007) using a less stringent selection (allowing smaller final blocks, gap position within the final blocks and less strict flanking positions) was applied to detect ambiguously aligned, hypervariable regions in the 28S rDNA dataset, according to a secondary structure model. The best partitioning scheme and substitution model for the DNA partition were chosen under the Bayesian Information Criterion (Schwarz, 1978) using the 'greedy' search strategy in Partition Finder version 1.1.1 (Lanfear *et al*., 2012, 2014). The obtained sequences were aligned with previously published sequences of *Auriculostoma* spp. in the GenBank database along to species of other three genera of allocreadiids, and the callodistomid *Prosthenhystera obesa* (Diesing, 1850) Travassos, 1922 as outgroup for rooting the trees (table 1); the appropriate nucleotide substitution model implemented for the 28S rDNA matrix was TVM + I+G.

The phylogenetic reconstruction was carried out using Bayesian inference through MrBayes version 3.2.3 (Ronquist *et al*., 2012). Phylogenetic trees were constructed using two

Table 1. Collection data and GenBank accession numbers for allocreadiid species analysed in this study. New species in bold.

Digenean species	GenBank accession numbers	Host species	Locality	Reference
Allocreadiidae Looss, 1902				
Auriculostoma astyanace Scholz, Aguirre-Macedo & Choudhury, 2004	HQ833707	*Astyanax aeneus* (Günther)	Tempisquito River, Guanacaste, Costa Rica	Curran *et al.* (2011)
	KF631422	*Astyanax faxciatus* (Cuvier)	Sapoa River, Guanacaste, Costa Rica	Razo-Mendivil *et al.* (2014)
Auriculostoma lobata Hernández-Mena, Lynggaard, Mendoza-Garfías & Pérez-Ponce de León	KX954170			
	KX954174	*Brycon guatemalensis* Regan	El Managal Lagoon, Tenosique, Mexico	Hernández-Mena *et al.* (2016)
Auriculostoma tica Hernández-Mena, Pinacho-Pinacho, García-Varela, Mendoza-Garfias & Pérez-Ponce de León, 2018	MH997001–02	*Gymnotus maculosus* Albert & Miller	Orosí River, Costa Rica	Hernández-Mena et al. (2019)
Auriculostoma totonacapenensis Razo-Mendivil, Mendoza-Garfias, Pérez-Ponce de León & Rubio-Godoy, 2014	KF631417			
	KF631418	*Astyanax mexicanus* (De Filippi)	Filipinas, Veracruz, Mexico	Razo-Mendivil *et al.* (2014)
Creptotrematina aguirrepequenoi Jiménez-Guzmán, 1973	HQ833708			
	HQ833709	*Astyanax aeneus* (Günther)	Tempisquito River, Guanacaste, Costa Rica	Curran *et al.* (2011)
***Auriculostoma guacurarii* n. sp.**	MN822004	*Characidium heirmostigmata* Graça & Pavanelli	Iguazú National Park, Arrechea Stream, Argentina	This study
***Auriculostoma* cf. *stenopteri* (Mañé-Garzón & Gascón, 1973) Scholz, Aguirre Macedo & Choudhury, 2004**	MN822005	*Charax stenopterus* Cope	Punta Lara, Rio de La Plata, Argentina	This study
Creptotrema funduli Mueller, 1934	JQ425256	*Fundulus notatus* Rafinesque	Harrison County, Biloxi River, USA	Curran *et al.* (2012)
Wallinia anindoi Hernández Mena, Pinacho-Pinacho, García-Varela, Mendoza-Garfias & Pérez-Ponce de León, 2018	MH535504	*Astyanax aeneus* (Günther)	San Juan del Río, Oaxaca, Mexico	Hernández-Mena et al. (2019)
Wallinia brasiliensis Dias, Müller, de Almeida, da Silva, de Azevedo, Pérez-Ponce de León & Abdallah, 2018	MH520995	*Astyanax fasciatus* (Cuvier) A. lacustris (Lütken)	State of São Paulo, Brazil	Dias et al. (2018)
Wallinia chavarriae Choudhury, Daverdin & Brooks, 2002	HQ833703	*Astyanax aeneus* (Günther)	Animas River, Guanacaste, Costa Rica	Curran *et al.* (2011)
Wallinia mexicana Pérez-Ponce de León, Razo-Mendivil, Mendoza-Garfias, Rubio-Godoy & Choudhury, 2015	KJ535504	*Astyanax mexicanus* (De Filippi)	Covadonga River, Durango, Mexico	Pérez-Ponce de León *et al.* (2015)
Outgroup taxa Callodistomidae Odhner, 1910				
Prosthenhystera obesa (Diesing, 1850) Travassos, 1922	AY222206	*Hoplias* sp. Gill	Rio Itaya, Iquitos, Peru	Olson *et al.* (2003)

parallel analyses of Metropolis-coupled Markov chain Monte Carlo for 20 million generations each, to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations and the average standard deviation of split frequencies was observed to be less than 0.01 (Ronquist *et al.*, 2012). The robustness of the clades was assessed using Bayesian PP, where PP > 0.95 was considered strongly supported. A majority consensus tree with branch lengths was reconstructed for each run after discarding the first 25% of sampled trees.

Additionally, the uncorrected *p*-distance (Nei & Kumar, 2000) was obtained to compare the genetic distance between lineages. The *P*-value matrix was obtained using MEGA X (Kumar *et al.*, 2018), with the bootstrap method (1000 replicates). Newly generated sequences were submitted to the National Centre for Biotechnological Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov), under the GenBank accession numbers MN822004–MN822005.

Results

Allocreadiidae (Looss, 1902)
Auriculostoma Scholz, Aguirre-Macedo & Choudhury, 2004

https://doi.org/10.1017/S0022149X21000109 Published online by Cambridge University Press

Auriculostoma guacurarii *n. sp.*

Taxonomic summary

Host. *Characidium heirmostigmata* (Graça & Pavanelli) (Characiformes: Crenuchidae).

Locality. Arrechea Stream, Iguazu River above the waterfalls, Iguazu National Park, Misiones, Argentina (25°39′29″S, −54° 27′15″W).

Site of infection. Intestine.

Prevalence, intensity and abundance. 40% (12/30), 1.08, 0.44.

Type material. Museo de Ciencias Naturales de La Plata, Helminthology Collection: holotype (MLP-He 7713) and paratypes (MLP He 7714).

ZooBank registration. urn:lsid:zoobank.org:pub:C4F63F63-0EA4-4711-B8FC-C078F43134B5.

Etymology. The species name *guacurarii* is given in honour of Andresito Guacurari, a forgotten hero from the Indigenous Guarani People, who bravely fought against the Portuguese invasion of Argentina in the 19th century. It also honours the jaguar (*Panthera onca* Linnaeus, 1758) Guacurarí, which was killed by poachers in the Urugua-í Reserve, Misiones Province, Argentina. We aim to raise awareness about the importance of forests in Misiones as biodiversity hotspots and the need for their protection.

Description

Based on six adult specimens (fig. 1a). Body elongate, 1254 ± 147 (1084–1439) long, 372 ± 83 (259–456) wide; maximum width at the ventral sucker level; tegument smooth, eyespot remnants present. Forebody 30–32% of body length. Oral sucker subterminal, with one pair of muscular oral lobes on both sides, 112 ± 19 (93–137) long, 129 ± 23 (106–160) wide, bearing small dome-like papillae (arrangement not clearly seen (fig. 2a, b). Ventral sucker spherical, muscular, slightly larger than oral sucker, 138 ± 15 (122–157) long, 132 ± 14 (120–148) wide. Oral sucker/ventral sucker length and width ratio 1:1.15–1.23, 1:0.92–1.06, respectively. Prepharynx absent. Pharynx muscular, globular 51 ± 9 (41–60) long, 58 ± 10 (50–69) wide. Oesophagus straight, 62 ± 19 (41–76) long. Intestinal bifurcation in forebody, half distance between ventral and oral suckers. Caeca long, ending between posterior testis and posterior end of body. Post-caecal space length 219 ± 25 (200–256). Testes two, oblique, smooth, post-equatorial, intercaecal. Anterior testis dextral, 134 ± 6 (128–139) long, 71 ± 3 (68–74) wide. Posterior testis larger than anterior testis, 152 ± 22 (126–173) long, 74 ± 8 (69–83) wide. Post-testicular space length 340 ± 56 (270–401), representing 27 (25–30) per cent of body length. Cirrus sac 241 ± 79 (158–316) long, dorsal, medial, slightly curved, extending from the genital pore to the anterior margin of ovary, containing elongated and bi-partite seminal vesicle, with constriction between pars prostatica and seminal vesicle (fig. 1b). Cirrus not observed. Genital pore opening medially on forebody, close to intestinal bifurcation. Ovary oval, equatorial, sinistral, pretesticular, 125 ± 22 (101–145) long, 88 ± 17 (77–104) wide. Seminal receptacle post-ovarian, subspherical, 54 ± 9 (47–63) long, 66 ± 24 (45–92) wide. Mehlis' gland and Laurer's canal not observed. Vitelline follicles extending from level of intestinal bifurcation to almost reach posterior end of body, filling intra and extracaecal space. Uterus pre- and inter-testicular, extending from mid-level of testes to the genital atrium; eggs few in number, 67 ± 2 (65–70) long, 40 ± 1 (38–42) wide. Excretory vesicle I-shaped, 127 ± 17 (103–143).

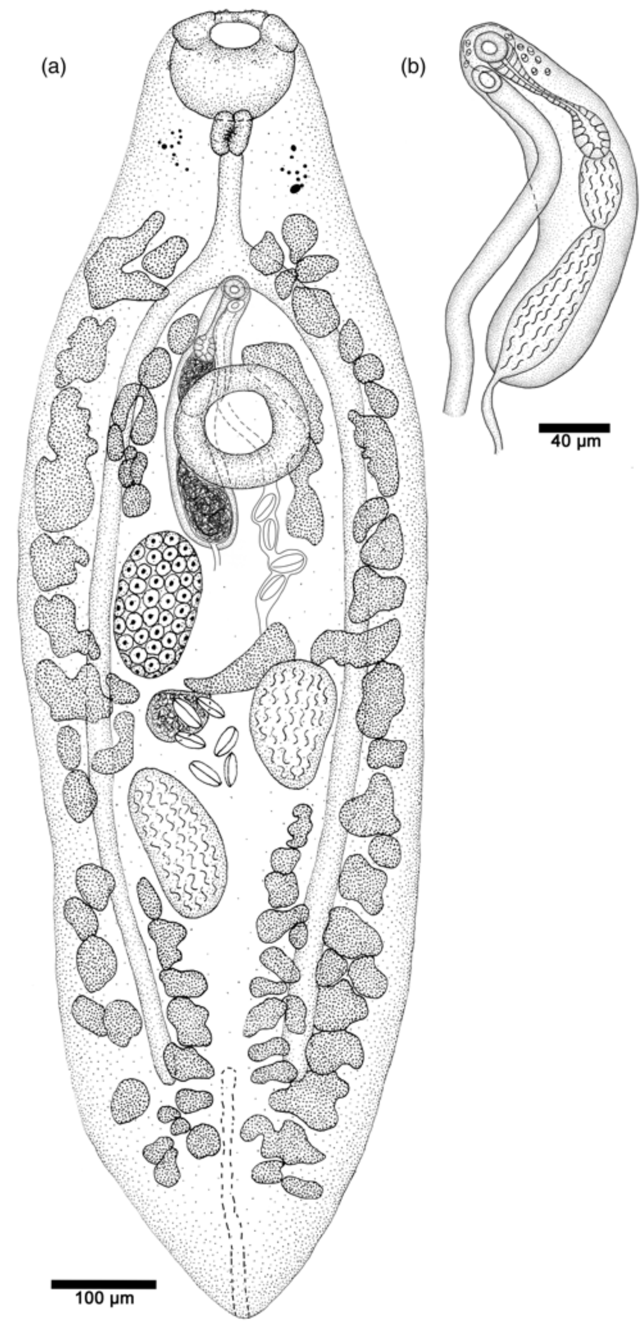

Fig. 1. (a) *Auriculostoma guacurarii* n. sp., ventral view of holotype; (b) detail of the genital terminalia in dorsal view.

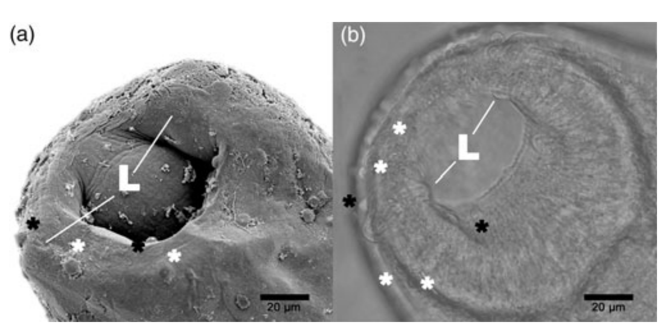

Fig. 2. Detail of *Auriculostoma guacurarii* n. sp. oral sucker. (a) SEM photo. (b) Optical microscope photo. White asterisk = papillae only observable in that photo. Black asterisk = papilla observed on both photos. L, muscular lobes.

https://doi.org/10.1017/S0022149X21000109 Published online by Cambridge University Press

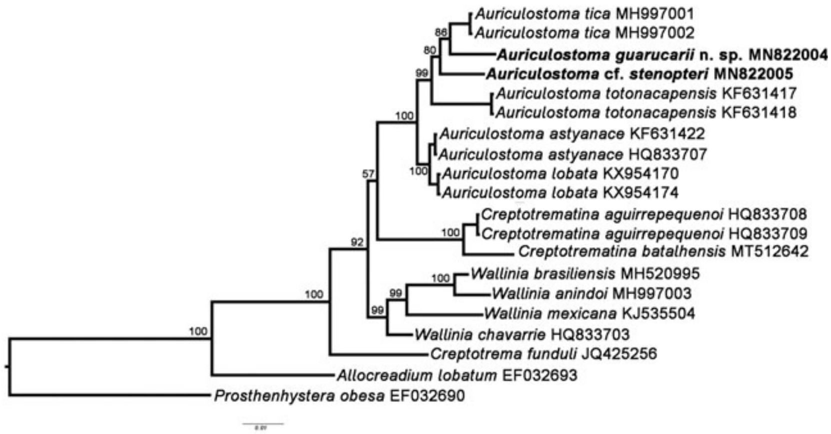

Fig. 3. Phylogram resulting from Bayesian inference of partial 28S rDNA gene sequences of *Auriculostoma guacurarii* n. sp. and *Auriculostoma* cf. *stenopteri* among allocreadiids rooted with *Prosthenhystera obesa*. Scale bar shows the number of substitutions per site.

Taxonomic remarks

The new species agrees with the diagnosis of *Auriculostoma* by possessing a single pair of muscular lobes on either side of the oral sucker (Scholz *et al*., 2004; Razo Mendivil *et al*., 2014; Hernández-Mena *et al*., 2019). Unfortunately, the specimen used for SEM was in poor shape and the structure of the oral lobes is not clearly seen. The new species is part of a group of six species of *Auriculostoma* that possess testes with smooth margins, including *A. astyanace*, *A. diagonale*, *A. platense*, *A. tica* and *A. totonacapanensis* (Scholz *et al*., 2004; Curran *et al*., 2011; Razo Mendivil *et al*., 2014; Hernández-Mena *et al*., 2019). *Auriculostoma astyanace* differs from *A. guacurarii* n. sp. by having testes in tandem, a genital pore located between the anterior margin of the ventral sucker and the caecal bifurcation, and by parasitizing fishes of the family Characidae in Costa Rica and Nicaragua (Scholz *et al*., 2004). The remaining four species possess oblique testes as in the new species.

Auriculostoma guacurarii n. sp. can be distinguished from *A. diagonale*, a species described from characids in Peru, by having larger eggs (65–70 vs. 55 µm) vitelline follicles extending anteriorly to the level of the caecal bifurcation instead of reaching anteriorly to the pharynx level, and by having a cirrus sac extending posteriorly to the level of ovary, and not to the anterior testis level. The new species differs from *A. platense*, a species occurring in siluriform fishes of Brazil and Argentina, in the extension of the vitelline follicles (caecal bifurcation level vs. mid-level of the oesophagus), genital pore position near or over the caecal bifurcation vs. between the anterior margin of the ventral sucker and caecal bifurcation, egg size (65–70 vs. 75 µm) and extension of cirrus sac (reaching to the level of the ovary vs. not reaching the posterior margin of the ventral sucker). In addition, in Argentina *A. guacurarii* n. sp. is found in characiforms of the family Crenuchidae in the Iguazu River, whereas *A. platense* occurs in Siluriformes of the Parana River. *Auriculostoma guacurarii* n. sp. differs from *A. tica*, a species recently described from a gymnotid freshwater fish in Costa Rica, in the length of body (1084–1439 vs. 1551–2118), in the position of the genital pore, which opens at the level of the caecal bifurcation rather than between the pharynx and the caecal bifurcation, and in the extension of the caeca (half-distance between posterior testis and posterior end of body vs. almost reaching the posterior end of body). Finally, *A. guacurarii* n. sp. closely resembles *A. totonacapanensis* in the size of most structures, in having smooth and oblique testes, in the position of the genital pore (at the level of the caecal bifurcation), in having a cirrus sac originating in the ovarian region, in having vitelline follicles not confluent in the post-testicular area and in the fact that both are parasites of characiforms, although *A. totonacapanensis* is found in the species of characid with the most northern distribution range, the Mexican tetra, *A. mexicanus* De Filippi, in central Mexico. However, in the new species, the oral and ventral suckers are smaller, the post-testicular space is larger, the eggs are larger and the intestinal caeca extend beyond the posterior testis (in *A. totonacapanensis* intestinal caeca reach the level of the posterior margin of posterior testis).

Two species of *Auriculostoma* have been reported from freshwater fishes in Argentina – *A. macrorchis* and *A. platense.* The new species differs from *A. macrorchis* by having oblique testes rather than testes in tandem, by the anterior extension of the vitelline follicles that reach the level of the caecal bifurcation rather than to pharynx level, and by host association because *A. macrorchis* is mainly a parasite of siluriforms whereas the new species is a parasite of characiforms. The differences between the new species and *A. platense* are mentioned above. We report the presence of *A. stenopteri* in Argentina for the first time. We obtained a single specimen of this species from the intestine of *C. stenopterus*; the specimen was identified morphologically and the whole specimens was used for DNA. We decided to refer the species as *Auriculostoma* cf. *stenopteri* provisionally until more specimens are collected from the same host and locality, and vouchers can be deposited in a parasite collection.

Molecular characterization

The results of the morphological study were further corroborated by sequencing the 28S rRNA gene. Only the reverse sequence and one forward and reverse sequence pair of the partial fragment of 28S rDNA gene were recovered for *A. guacurarii* n. sp. (1139 bp) and *A.* cf. *stenopteri* (1178 bp), respectively. The obtained phylogram provides strong support for the monophyly of the genus *Auriculostoma* (fig. 3). Two main clades are formed within *Auriculostoma*, one containing *A. lobata* and *A. astyanace*, a highly supported group (PP 100%), and other clade containing *A. totonacapanensis* as the sister species of *A.* cf. *stenopteri* and *A. guacurarii* n. sp. + *A. tica*, the clade is supported with a high PP value (99%). The genetic divergence of the 28S rDNA gene between the new species and the other congeners for which sequences are available varied between 2.11 and 3.46% (table 2).

https://doi.org/10.1017/S0022149X21000109 Published online by Cambridge University Press

Table 2. Genetic divergence among species of allocreadiids relevant to the study, estimated through of uncorrected *p*-distance of the 28S rDNA gene and expressed as percentage. Intraspecific divergence in bold.

		1	2	3	4	**5**	6	**7**	8	9	10	11	12	13	14	15
1	*Prosthenhystera obesa (OG)*	**n/c**														
2	*Auriculostoma astyanace*	10.85	**0**													
3	*Auriculostoma lobata*	10.76	0.29	**0**												
4	*Auriculostoma tica*	10.95	1.34	1.44	**0**											
5	***Auriculostoma guacurarii* n. sp.**	11.72	2.69	2.79	2.11	**n/c**										
6	*Auriculostoma totonacapanensis*	12.01	2.21	2.11	2.31	3.46	**0**									
7	***Auriculostoma* cf. *stenopteri***	11.43	1.34	1.25	1.34	2.50	2.40	**n/c**								
8	*Wallinia chavarriae*	11.62	2.40	2.50	3.07	4.23	3.94	3.07	**n/c**							
9	*Wallinia mexicana*	12.39	3.46	3.75	4.51	5.48	5.09	4.51	3.17	**n/c**						
10	*Wallinia brasiliensis*	12.20	3.36	3.46	4.23	4.90	4.80	4.13	2.69	3.65	**n/c**					
11	*Wallinia anindoi*	12.49	4.13	4.23	4.90	5.57	5.57	4.90	3.17	4.23	1.34	**n/c**				
12	*Creptotrema funduli*	11.62	4.23	4.32	5.09	5.96	5.48	4.90	4.23	5.09	4.71	5.09	**n/c**			
13	*Creptotrematina aguirrepequenoi*	11.34	3.55	3.65	3.94	4.80	4.51	4.13	3.75	4.61	4.90	5.48	5.48	**0**		
14	*Creptotrematina batalhensis*	11.91	4.32	4.42	4.71	5.57	5.28	4.90	4.61	5.38	5.67	6.44	6.34	1.63	**n/c**	
15	*Allocreadium lobatum*	10.47	6.53	6.34	7.11	8.07	7.30	7.01	7.30	8.17	8.45	8.84	7.01	7.40	7.78	**n/c**

OG, outgroup; n/c, not calculated.

The results of the molecular analysis further corroborated the independence of the new species.

The topology of the phylogenetic tree, regarding on the position of *Auriculostoma*, *Creptotrematina* and *Wallinia*, agrees with previous studies by Curran *et al.* (2011), Razo-Mendivil *et al.* (2014) and Alves Dias *et al.* (2020), but disagrees with Pérez-Ponce de León *et al.* (2015), Hernández-Mena et al. (2016, 2019) and Da Silva *et al.* (2021). There is no doubt about the monophyly of each genus, but the phylogenetic relationships between them are not well supported and require further scrutiny.

Discussion

The combined morphological features along with the genetic differences exhibited by the specimens of *Auriculostoma* collected from *C. heirmostigmata* allow us to validate them as a new species. *Auriculostoma guacurarii* n. sp. is the tenth species described for this genus, the sixth record of this genus from South America and the first one from the Iguazu Basin. Moreover, it is the first species of *Auriculostoma* found parasitizing the family Crenuchidae.

Although the sequenced specimen of *A.* cf. *stenopteri* was immature, it possessed a single pair of muscular oral lobes, and testes in tandem with irregular margins. Interestingly, it was collected from the type-host species (*C. stenopterus*) and type locality (La Plata River) where the species was originally described (Mañé-Garzón & Gascón, 1973; Ostrowski de Nuñez *et al.*, 2017). This was important in deciding that this specimen was conspecific with *A. stenopteri*, even though we observed that both ventral and oral suckers were almost identical in size; although, in the original description, *A. stenopteri* was diagnosed as having a ventral sucker larger than the oral sucker. Nevertheless, we only sampled one individual that was also genetically distinct from the new species we describe in this study. The phylogenetic tree and genetic divergence values unequivocally show that the new species represent an independent lineage. Additionally, the tree suggests that testes shape (smooth or lobed) and position (oblique or in tandem), irrespective of their diagnostic value, are characters that do not define natural groupings since they are found independently within the two well-defined monophyletic clades. Nevertheless, we are aware that DNA sequences are only available for six out of the ten species of the genus. The full understanding of the evolutionary and biogeographical history of this Neotropical genus of trematode will benefit from obtaining DNA sequence data of the remaining four species – that is, *P. macrorchis*, *P. platense* (both from Argentina), *P. diagonale* and *P. foliaceum* (both from Peru). The genus seems to have evolved along with two major groups of Neotropical freshwater fishes, the characiforms and the siluriforms, especially the former, since seven of the ten species are parasites of characiforms, two of siluriforms and one of gymnotids (see Kohn *et al.*, 2007; Choudhury *et al.*, 2016; Ostrowski de Nuñez *et al.*, 2017). Previously, Razo-Mendivil *et al.* (2014) proposed that *Auriculostoma*, along with two other genera of allocreadiids, *Wallinia* and *Creptotrematina*, formed a group associated with characiforms. We expect more species of these three genera to be described from South America as more species of characiforms and their parasites are investigated in this region using integrative taxonomy.

Acknowledgements. We are grateful to the Administración de Parques Nacionales, Argentina, for granting us the permit for fieldwork at Iguazu National Park; the Facultad de Ciencias Naturales y Museo (UNLP) and Centro de Estudios Parasitológicos y de Vectores (CEPAVE) for providing us with the laboratory facilities; Jorge Casciotta, Liliana Ciotek, Pablo Giorgis and the staff of CIES and Delegación Regional Noreste for field assistance; and Patricia Sarmiento and Maria Marcia Montes for the SEM photos and line drawings, respectively.

Financial support. This work was partially funded by Consejo Nacional de Investigación Cienífica y Tecnológica (CONICET) (S.R.M., PIP-0015), Fondo para la Investigación Científica y Tecnológica (FONCyT) (M.M.M., BID PICT 2016-4153) and CEPAVE (PUE 3334/16), and also partially funded by the Consejo Nacional de Ciencia Y Tecnología, Mexico (G.P.P.L., CONACyT number A1-S-21694).

Conflicts of interest. None.

Ethical approval. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Abell R, Thieme ML, Revenga C, *et al.* (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience* **58**, 403–414.

Administración de Parques Nacionales (2020) Sistema de Información de Biodiversidad, Parque Nacional Iguazu. Available at https://sib.gob.ar/area/apn*ig*IGUAZU (accessed 17 May 2020).

Alves Dias KG, Pérez-Ponce de León G, de Almeida Camargo A, Müller MI, da Silva RJ, Kozlowiski de Azevedo R and Abdallah VD (2020) A new species of *Creptotrematina* (Trematoda: Allocreadiidae) from characid fishes of brazil: morphological and molecular data. *Journal of Helminthology* **94**, e163.

Atopkin DM, Sokolov SG, Vainutis KS, Voropaeva EL, Shedko MB and Choudhury A (2020) Amended diagnosis, validity and relationships of the genus *Acrolichanus* Ward, 1917 (Digenea: Allocreadiidae) based on the 28S rRNA gene, and observations on its lineage diversity. *Systematic Parasitology* **97**, 143–156.

Caira JN and Bogéa T (2004) Family Allocreadiidae Looss, 1902. pp. 417–436 *in* Jones A, Bray RA and Gibson DI (*Eds*) *Keys to the Trematoda*. London, CAB International and the Natural History Museum.

Casciotta J, Almirón A, Ciotek AL, *et al.* (2016) Visibilizando lo invisible. Un relevamiento de la diversidad de peces del Parque Nacional Iguazú, Misiones, Argentina. *Revista Historia Natural* **6**, 5–80.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540–552.

Choudhury A, Aguirre-Macedo ML, Curran SS, Ostrowski de Nuñez M, Overstreet RM, Pérez-Ponce de León G and Portes Santos C (2016) Trematode diversity in freshwater fishes of the globe II: 'New world'. *Systematic Parasitology* **93**, 271–282.

Curran SS, Tkach VV and Overstreet RM (2011) Phylogenetic affinities of *Auriculostoma* (Digenea: Allocreadiidae), with descriptions of two new species from Peru. *Journal of Parasitology* **97**, 661–670.

Curran SS, Pulis EE, Hugg DO, Brown JP, Manuel LC and Overstreet RM (2012) Phylogenetic position of *Creptotrema funduli* in the Allocreadiidae based on partial 28S rDNA sequences. *Journal of Parasitology* **98**, 873–875.

Da Silva BAF, Dias KGA, da Silva RJ and Yamada FH (2021) A new species of *Wallinia* Pearse, 1920 (Digenea: Allocreadiidae), in *Astyanax bimaculatus* (Linnaeus, 1758) (Characidae) in northeast Brazil, based on morphology and DNA sequences. *Parasitology Research* **120**, 37–40.

Dias KGA, Müller MI, De Almeida AC, Da Silva RJ, De Azevedo RK, Pérez-Ponce de León G and Abdallah VD (2018) A new species of Wallinia Pearse, 1920 (Digenea: Allocreadiidae) collected from Astyanax fasciatus (Cuvier, 1819) and A. lacustris Lucena and Soares, 2016 (Characiformes: Characidae) in Brazil based on morphology and DNA sequences. *Parasitology Research* **117**, 2847–2854.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C and Wilson A (2016) Geneious v5.0.4. Available at http://www.geneious.com/ (accessed 17 November 2017).

Hernández-Mena D, Lynggaard C, Mendoza-Garfias B and Pérez-Ponce de León G (2016) A new species of *Auriculostoma* (Trematoda: Allocreadiidae) from the intestine of *Brycon guatemalensis* (Characiformes: Bryconidae) from the Usumacinta River Basin, Mexico, based on morphology and 28S rDNA sequences, with a key to species of the genus. *Zootaxa* **4196**, 261–277.

Hernández-Mena DI, Pinacho-Pinacho CD, García-Varela M, Mendoza-Garfias B and Pérez-Ponce de León G (2019) Description of two new species of allocreadiid trematodes (Digenea: Allocreadiidae) in middle American freshwater fishes using an integrative taxonomy approach. *Parasitology Research* **118**, 421–432.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**, 286–298.

Kohn A, Fernandes BMM and Cohen SC (2007) *South American trematodes parasites of fishes*. 318 pp. Rio de Janeiro, FIOCRUZ, Instituto Oswaldo Cruz.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) Mega X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

Lanfear R, Calcott B, Ho S and Guindon S (2012) Partition finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**, 1695–1701.

Lanfear R, Calcott B, Kainer D, Mayer C and Stamatakis A (2014) Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* **14**, 1–82.

Littlewood DTJ, Curini-Galletti M and Herniou EA (2000) The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution* **16**, 449–466.

Mañé-Garzón F and Gascon A (1973) Digenea de peces de agua dulce del Uruguay, I. Una nueva especie del genero *Crepidostomum* Braum [sic!], 1990 del intestino de *Asiphonichthys stenopterus*. *Revista de Biologıa de Uruguay* **1**, 11–14.

Nei M and Kumar S (2000) *Molecular evolution and phylogenetics*. 333 pp. New York, Oxford University Press.

Olson PD, Cribb TH, Tkach VV, Bray RA and Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**, 733–755.

Ostrowski de Nuñez M, Arredondo NJ and Gil de Pertierra A (2017) Adult trematodes (Platyhelminthes) of freshwater fishes from Argentina: a checklist. *Revue Suisse de Zoologie* **124**, 91–113.

Pérez-Ponce de León G, Razo-Mendivil U, Mendoza-Garfias B, Rubio-Godoy M and Choudhury A (2015) A new species of Wallinia Pearse, 1920 (Digenea: Allocreadiidae) in Astyanax mexicanus (Characidae) from Mexico revealed by morphology and sequences of the 28S ribosomal RNA gene. *Folia Parasitologica* **62**, 018.

Pérez-Ponce de León G, Sereno-Uribe AL, García-Varela M, Mendoza-Garfias B, Hernández-Mena DI, Pinacho-Pinacho CD and Choudhury A (2020) Disentangling the evolutionary and biogeographical history of the freshwater fish trematode genus *Creptotrema* (Digenea: Allocreadiidae) using an integrative taxonomy approach: the case of *Creptotrema agonostomi* in middle American mountain mullets. *Journal of Helminthology* **94**, e171.

Pritchard MH and Kruse GOW (1982) *The collection and preservation of animal parasites*. 141 pp. Nebraska, University of Nebraska Press.

Razo-Mendivil U, Mendoza-Garfias B, Pérez-Ponce de León G and Rubio Godoy M (2014) A new species of *Auriculostoma* (Digenea: Allocreadiidae) in the Mexican tetra *Astyanax mexicanus* (Actinopterygii: Characidae) from central Veracruz, Mexico, described with the use of morphological and molecular data. *Journal of Parasitology* **100**, 331–337.

Ronquist F, Teslenkovan M, van der Mark P, *et al.* (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across large model space. *Systematic Biology* **61**, 539–542.

Schneider CA, Rasband WS and Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–675.

Scholz T, Aguirre-Macedo ML and Choudhury A (2004) *Auriculostoma astyanace* n. gen., n. sp. (Digenea: Allocreadiidae), from the banded astyanax, *Astyanax fasciatus* (Characiformes: Characidae), from Nicaragua, with a reevaluation of Neotropical *Crepidostomum* spp. *Journal of Parasitology* **90**, 1128–1132.

Schwarz G (1978) Estimating the dimension of a model. *The Annals of Statistics* **6**, 461–464.

Szidat L (1954) Trematodes nuevos de peces de agua dulce de la República Argentina y un intento para aclarar su carácter marino. *Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"* **3**, 1–85.

Talavera G and Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**, 564–577.

Tkach V, Littlewood T, Olson P, Kinsella JM and Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56**, 1–15.