

Research Paper

*Current address: Escuela Nacional de Estudios Superiores Unidad Mérida, Universidad Nacional Autónoma de México (ENES-Mérida), Km 4.5 Carretera Mérida-Tetiz, Ucu, Yucatán, Mexico.

Cite this article: Hernández-Mena DI, Cabañas-Granillo J, Medina-Hernández E, Pérez-Ponce de León G (2022). Discovery of a new species of *Homalometron* Stafford, 1904 (Digenea: Apocreadiidae) from the stripped mojarra, *Eugerres plumieri* in a coastal lagoon of the Gulf of Mexico. *Journal of Helminthology* **96**, e46, 1–8. <https://doi.org/10.1017/S0022149X22000372>

Received: 26 April 2022
Revised: 10 June 2022
Accepted: 12 June 2022



Key Words:

Homalometron; Mexico; new species; Trematoda; phylogeny

Author for correspondence:

David I. Hernández-Mena,
E-mail: dahernandez.243@gmail.com

Discovery of a new species of *Homalometron* Stafford, 1904 (Digenea: Apocreadiidae) from the stripped mojarra, *Eugerres plumieri* in a coastal lagoon of the Gulf of Mexico

D.I. Hernández-Mena¹ , J. Cabañas-Granillo², E. Medina-Hernández² and G. Pérez-Ponce de León^{2,*} 

¹Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Unidad Mérida, Antigua Carretera Progreso Km 6, Cordemex, 97310 Mérida, Yucatán, Mexico and ²Instituto de Biología, Universidad Nacional Autónoma de México, CP 04510, Apartado Postal 70-153, Ciudad Universitaria, Ciudad de México, Mexico

Abstract

To date, 34 species of the genus *Homalometron* (Apocreadiidae) have been described; five of them in Mexican fresh or brackish water fish, whereas five have been reported as parasites of members of the fish family Gerreidae. While sampling wildlife vertebrates during a field course of parasitology at the Los Tuxtlas Biological Station (Instituto de Biología, Universidad Nacional Autónoma de México) in Veracruz, specimens of digeneans were collected from the intestine of the stripped mojarra, *Eugerres plumieri* in Sontecomapan Lagoon. Specimens were studied morphologically and molecularly, and we discovered that they represented a new species of *Homalometron*. The new species is morphologically like the other four congeners in having three pairs of well-developed oral papillae on the oral sucker: *Homalometron elongatum*; *Homalometron lesliorum*; *Homalometron carapavae*; and *Homalometron papilliferum*. Here, we describe the newly discovered species, increasing our understanding about the parasite diversity of brackish water fishes of Mexico.

Introduction

Homalometron Stafford, 1904 is a species-rich genus of digeneans (with 34 species according to the World Register of Marine Species, 2022) whose species are distributed worldwide. These parasites mature in the digestive tract of actinopterygian fishes with marine, brackish and freshwater habits. Twenty-five out of the 34 described species have been reported in aquatic environments across the Americas. Particularly, six species were described and found as parasites of fish belonging to the family Gerreidae Leach in marine and brackish water environments, namely: *Homalometron elongatum* Manter, 1947 in *Gerres cinereus* (Walbaum) from the United States (see Manter, 1947; see Parker *et al.*, 2010 that includes other localities in the Caribbean, Puerto Rico, Jamaica and Bahamas), in *Eucinostomus californiensis* (Gill) from the Panamanian Pacific (see Sogandares-Bernal, 1959), in *Diapterus peruvianus* (Cuvier) and in *Eucinostomus gracilis* (Gill) from Mexico (see Pérez-Ponce de León *et al.*, 2007); *Homalometron longulum* Travassos, Teixeira de Freitas & Bührnheim, 1965 in *Diapterus rhombeus* Cuvier from Brazil (see Travassos *et al.*, 1965), in *Diapterus peruvianus* and *Gerres cinereus* from Mexico (see Pérez-Ponce de León *et al.*, 2007); *Homalometron carapevae* Amato, 1983 in *Eugerres brasilianus* (Cuvier) from Brazil; *Homalometron lesliorum* Parker *et al.*, 2010 in *Eucinostomus currani* Zahuranec from Costa Rica; *Homalometron stradbrokeense* Cribb & Bray, 1999 in *Gerres subfasciatus* Cuvier from Australia; and *Homalometron palmeri* Curran *et al.*, 2013 was reported in *Eucinostomus argenteus* Baird & Girard and in various sciaenids and fundulids from the northern Gulf of Mexico (Curran *et al.*, 2013).

During a field parasitology course at Los Tuxtlas Biological Station (Instituto de Biología, Universidad Nacional Autónoma de México (UNAM)) in tropical rain forest of Los Tuxtlas in Veracruz state in 2018, several marine and brackish water fish species were necropsied and studied for metazoan parasites. Specimens of the stripped mojarra, *Eugerres plumieri* (Cuvier), were sampled from Sontecomapan lagoon. Specimens of digeneans belonging to the genus *Homalometron* were found in the intestines of their hosts, studied morphologically. DNA sequence data for two molecular markers were used to corroborate, through a phylogenetic analysis, that they represented an undescribed species. We describe a new species of *Homalometron* herein as *Homalometron avis* n. sp., which represents the seventh species of this genus reported in Mexico.

Material and methods

Sample collections

In April 2018, several individuals of *E. plumieri* were collected in Sontecomapan lagoon, Veracruz, Mexico, on the Gulf of Mexico slope. Fish were collected using cast nets, kept alive and necropsied 4 h after capture. The gastrointestinal tract from each fish was removed and placed in Petri dishes with 6.5% saline solution and examined under a stereomicroscope. For morphological study, specimens were fixed in hot (nearly boiling) tap water, some were kept in vials with 4% formalin for scanning electron microscopy, and some in 96% ethanol for staining and mounting. For molecular study, other specimens were placed in vials with 100% ethanol.

Morphological study

Specimens fixed for morphology were dehydrated in a graded ethanol series, stained with Mayer's paracarmine and Gomori's trichrome, cleared in methyl salicylate and processed as permanent mounts in Canada balsam. Specimens were examined using an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan). Measurements were taken using an Olympus Quick-Photo Image-Program, and are presented in micrometres (μm) with the range followed by the mean in parentheses. Drawings were made with a drawing tube attached to the microscope. Additionally, for the scanning electron microscopy (SEM) study, two specimens of the new species were dehydrated through a graded ethanol series and then critical point dried with carbon dioxide. Specimens were mounted on a metal stub with carbon adhesive tabs, then gold coated, and examined at 15 kV with a Hitachi Stereoscan SU1510 SEM (Hitachi Ltd., Tokyo, Japan). Specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM).

Molecular study

Each specimen was digested, and its genomic DNA extracted using the REDExtract-N-Amp Tissue PCR kit (Sigma, St Louis, USA) following the manufacturer's instructions. Proteinase was denaturated at 95 °C for 3 min. A fragment of the 28S gene (the domains D1–D3) and the internal transcribed spacer (ITS) (ITS1, 5.8S, ITS2) of the ribosomal DNA were amplified. For the 28S the forward primer 391 5'-AGCGGAGGAAAAGAAAC TAA-3' (Nadler & Hudspeth, 1998) and the reverse primer 536 5'-CAGCTATCCTGAGGGAAAC-3' (Stock *et al.*, 2001) were used. For ITS the forward BD1 5'-GTCGTAACAAGGTTTCC GTA-3', plus the reverse BD2 5'-TATGCTTAAATTCAGC GGGT-3' (Luton *et al.*, 1992) were used. The polymerase chain reaction (PCR) was performed in a final volume of 12.5 μl containing 1 μl of each primer, 2 μl of extracted DNA, 2.5 μl of 10 \times buffer, 1.5 μl of MgCl_2 at 25 mM, 0.5 μl of dNTPs at 10 mM and 1 U of Taq DNA polymerase. Thermal cycling conditions for both 28S and ITS consisted of an initial denaturation at 95 °C for 5 min followed by 35 cycles at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products for 28S were sequenced with PCR primers plus internal primers 503 5'-CC TTGGTCCGTGTTTCAAGACG-3' (Stock *et al.*, 2001) and 504 5'-CGTCTTGAACACGGACTAAGG-3' (García-Varela & Nadler, 2005). Instead, sequencing reactions for ITS were

performed using the two initial primers and with the following internal primers, BD3 5'-GAACATCGACATCTTGAACG-3' and BD4 5'-ATAAGCCGACCCTCGGC-3' (Hernández-Mena *et al.*, 2014). PCR products were sequenced using an ABI 3730xl Genetic Analyser (Applied Biosystems). The resulting sequences were analysed, and a consensus sequence was obtained for each sequenced specimen, both for 28S and ITS, using the software Geneious Pro 4.8.4 software (Biomatters Ltd., Auckland, New Zealand). Sequences were submitted to GenBank.

Phylogenetic analyses

The identity of the newly generated sequences of 28S and ITS was first checked using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST/), and sequences were aligned with those available in GenBank of other species of Apocreadiidae Skrjabin, 1942 (the accession numbers of the sequences used in this study are in the phylogenetic trees). Two data sets were constructed, one for each analysed gene, in Mesquite 3.62 (<https://www.mesquiteproject.org/>), and these data sets were aligned using ClustalW (Thompson *et al.*, 1994), with the approach 'SLOW/ACCURATE' and weight matrix 'CLUSTALW (for DNA)' in the web site <http://www.genome.jp/tools/clustalw/>. Based on previous phylogenetic analyses (Pérez-Ponce de León & Hernández-Mena, 2019), species of *Schistorchis* Lühe, 1906, *Haintestinum* Pulis, Curran, Andres & Overstreet, 2014, *Megapera* Manter, 1934 and *Thysanopharynx* Manter, were used in 28S and ITS data sets as outgroups for rooting the trees. Phylogenetic analyses were run under maximum likelihood (ML), using the nucleotide evolution models selected with jModelTest v2 (Darriba *et al.*, 2012). The ML trees, model parameters and bootstrap (Bt) support (1000 repetitions) were estimated with RAXML v. 7.0.4 (Stamatakis, 2006). The phylogenetic trees obtained from both analyses were visualized in FigTree v. 1.4.2. Genetic distances were calculated as uncorrected P-distance using MEGA v6 (Tamura *et al.*, 2013).

Results

Family: Apocreadiidae Skrjabin, 1942

Genus: *Homalometron* Stafford, 1904

***Homalometron avis* Hernández-Mena, Cabañas-Granillo, Medina-Hernández & Pérez-Ponce de León n. sp.**

(Figures 1, 2)

ZooBank Life Science Identifier: urn:lsid:zoobank.org:act:03B5E209-A416-4F43-B8F6-E067A32D09D3

Taxonomic summary

Type host: *Eugerres plumieri* (Cuvier, 1830) (Gerreidae)

Type locality: Laguna de Sontecomapan, Veracruz, Mexico (18° 33' 18.3" N, 94° 59' 28.2" W).

Site in host: Intestine.

Prevalence: Five of 15 (33.3%).

Type material: Holotype (CNHE 11667); XX paratypes: (CNHE 11668).

Representative DNA sequences: 28S rDNA (ON814857–ON814858) ITS rDNA (ON815018–ON815019).

Etymology: The epithet of the species refers to the Latin word 'avus,' which means 'grandparents' (or 'abuelos' in Spanish),

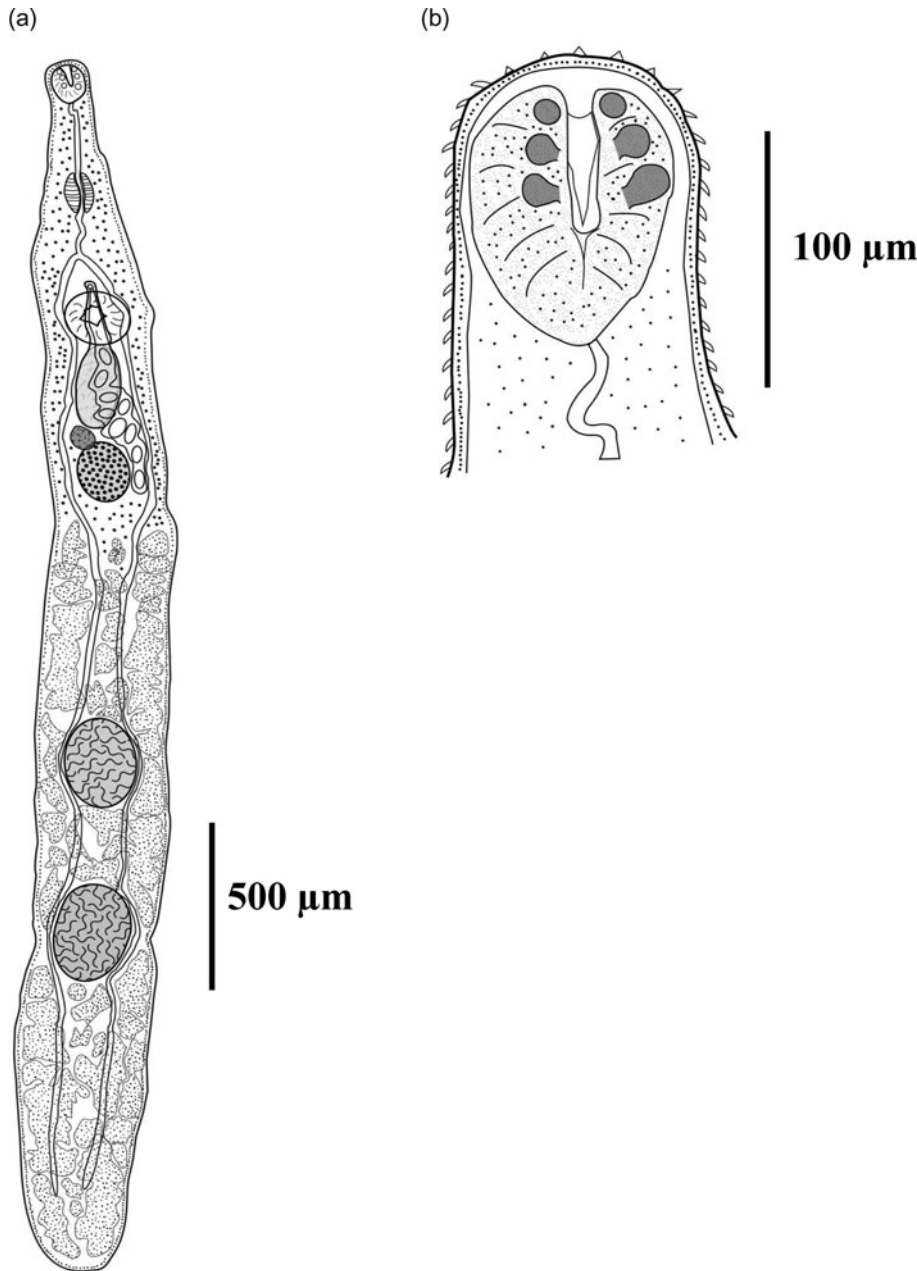


Fig. 1. *Homalometron avis* n. sp. (a) ventral view of holotype; and (b) ventral view of ventral sucker.

since the name of the new species is dedicated to the relatives of the first author.

Description

Based on five adult specimens, and on additional observations of two specimens through SEM. Body elongated, 1907–3665 (2921) long, narrow at oral sucker level; maximum width 212–443 (286) at testes level. Body tegument covered by scale-like spines denser at anterior half of body, decreasing their numbers towards posterior end. Each spine formed by a solid base covered by minute pectinate spines. Forebody length 580–925 (703), 18–35% (25%) of body length; unobservable eyespots. Oral sucker subterminal, 89–117 (102) long, 66–97 (81) wide, with three pairs of well-developed opposing papillae surrounding mouth opening. Ventral sucker 110–192 (155) long, 100–186 (147) wide. Oral sucker/ventral sucker length ratio 1:1.22–2.13 (1:1.56). Oral sucker/ventral sucker width ratio 1:1.41–2.64

(1:1.85). Mouth subterminal. Prepharynx long, elongated, 158–357 (249), with anterior end curved and posterior end slightly enveloping anterior end of pharynx; pharynx muscular, subglobular, 65–133 (102) long, 49–84 (63) wide, slightly surrounded by gland cells. Oesophagus shorter than prepharynx, 83–187 (132) long. Intestinal bifurcating 526–775 (627) from anterior body end, approximately two-thirds of distance between oral and ventral suckers and approximately one-half between pharynx and ventral sucker, ending blindly 140–241 (189) from posterior body end. Postcaecal space representing 5.4–8.1% (6.3%) of body length.

Two testes of similar size (slightly smaller anterior testis), subspherical, entire, in tandem, median, post-ovarian, in posterior half of body; anterior testis 122–301 (226) long, 106–310 (207) wide; posterior testis 135–352 (251) long, 127–352 (230) wide. Inter-testicular space 34–245 (131) long. The post-testicular

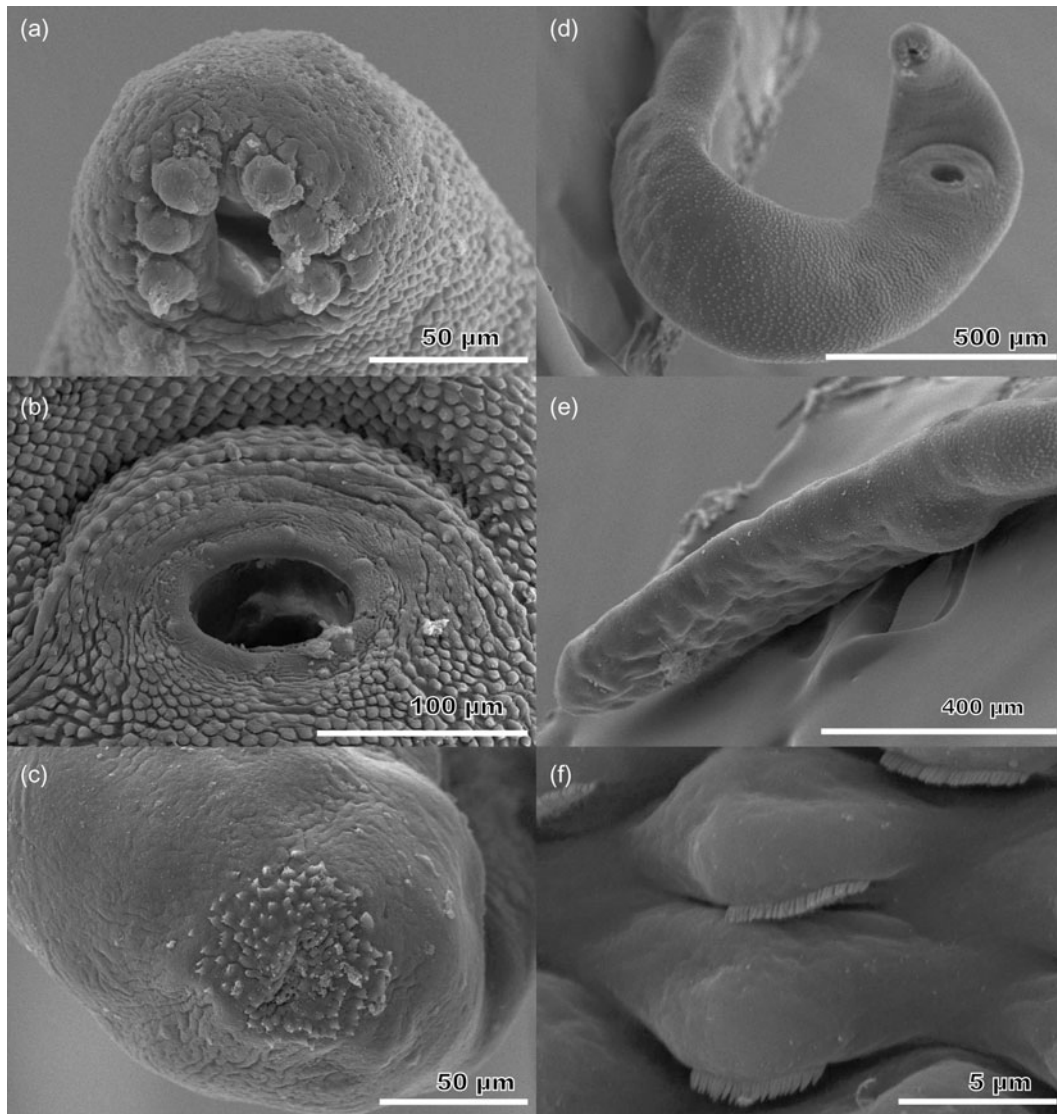


Fig. 2. Scanning electron micrographs of *Homalometron avis* n. sp. (a) ventral view of oral sucker with three lateral papillae; (b) ventral sucker surrounded by scale-like spines; (c) posterior end of the body where the excretory pore is located surrounded by small tegumental spines; (d) ventral view of the forebody and the anterior part of the hindbody showing the decrease in tegumental spines as they approach the posterior end of the body; (e) ventral view of the hindbody where the external relief of the posterior testicle is observed; and (f) scale-like spines formed by a solid base and pectinate minute spines.

space 307–881 (612), representing 14.1–24.5% (19.5%) of body length. Male terminal genitalia consisting of seminal vesicle, pars prostatica, and ejaculatory duct; seminal vesicle saccular, median, dorsal to ventral sucker, extending into hindbody, 169–279 (231) long, 71–144 (106) wide, opening into spindle-shaped prostatic region; pars prostatica opening into slender elongated ejaculatory duct; ejaculatory duct merging with muscular elongated female duct forming a muscular dorsoventral hermaphroditic duct; hermaphroditic duct opening through ventral genital pore, median, immediately anterior to ventral sucker.

Ovary globular, 83–179 (142) long, 67–159 (125) wide, median, pre-testicular, approximately one-third the distance between ventral sucker and anterior testis. Distance between ovary and anterior testis 251–844 (599). Seminal receptacle globular, anterior to ovary, opening into oviduct near posterior margin of ovary. Mehlis' gland posterior to ovary and seminal receptacle. Laurer's canal not observed. Vitellarium consisting of one confluent field of large follicles in hindbody; anteriorly, vitelline follicles

post-ovarian, and at 50–60 (57) from posterior margin of ovary; posteriorly, follicles extend to posterior end of body; follicles intercaecal and extracaecal and dorso-ventral in post-ovarian region and in intertesticular space. Vitelline reservoir ventral to Mehlis' gland. Ootype immediately post-ovarian. Uterus short, pre-testicular, intercaecal, between posterior end of ovary and genital pore. Eggs 52–84 (71) long, 35–51 (44) wide. Excretory vesicle I-shaped, terminating in post-testicular region; excretory pore terminal, surrounded by spines.

Remarks

Homalometron avis n. sp. conforms to the diagnosis of *Homalometron* and Apocreadiidae as given by Cribb (2005). The new species can be easily distinguished from 30 of the 34 congeneric species by having an elongated body and possessing three pairs of oral papillae. Four species in the genus share these morphological characters, namely *Homalometron*

carapevae, *Homalometron elongatum*, *Homalometron lesliorum* and *Homalometron papilliferum* (Szidat, 1956) Ostrowski de Nuñez *et al.*, 2000. Interestingly, the first three species are also found in fish of the family Gerreidae, whereas *H. papilliferum* parasitizes *Percichthys trucha* (Valenciennes) (Ostrowski de Nuñez *et al.*, 2000; Shimazu *et al.*, 2000). *Homalometron avis* n. sp. can be readily distinguished from these congeneric species by having a smaller oral sucker (89–116 × 66–97 vs. 120–140 × 108–136 in *H. carapevae*, 156–223 × 162–218 in *H. elongatum*, 125–207 × 162–213 in *H. lesliorum*, and 189–254 × 185–263 in *H. papilliferum*). The new species further differs from *H. elongatum* and *H. lesliorum* by the anterior distribution of the vitelline follicles, and by the oral to ventral sucker length and width ratios; the vitelline follicles in the new species are post-ovarian, and they have a ventral sucker larger than the oral sucker of 1:1.21–2.13 × 1:1.40–2.64, whereas in *H. elongatum* and *H. lesliorum* the vitelline follicles reach into pre-ovarian zone, and the ventral sucker is smaller than the oral sucker (1:0.76–0.88 × 1:0.79–0.88 and 1:0.90–1.05 × 1:0.81–0.98, respectively). The new species is also readily distinguished from *H. papilliferum* because in this species the oral and ventral suckers are almost similar in size, although with a tendency for the oral sucker to be larger (1:0.71–1.21 × 1:0.67–1.20), the prepharynx is shorter (34–143 in *H. papilliferum* vs. 158–357 in the new species), the eggs of *H. papilliferum* are larger (77–110 × 48–65 vs. 52–84 × 35–51 in the new species), and finally, the vitelline follicles of *H. papilliferum* reach the posterior margin of the ovary and are absent in the inter-testicular space, whereas in the *H. avis* n. sp. follicles never extend beyond the ovary and occupy the inter-testicular space.

The new species closely resembles *H. carapevae* which presents more similar oral/ventral sucker ratios, as well as pre-pharynx and eggs sizes. The new species also share with *H. carapevae* the anterior distribution of the vitelline follicles; however, they can be distinguished because in *H. carapevae* follicles at the anterior end reach the posterior margin of the ovary, whereas in *H. avis* n. sp. follicles never extend to reach the ovary. There are also differences in host association and geographical distribution since *H. carapevae* was described by Amato (1983) from the intestine of the Brazilian mojarra, *Eugerres brasiliensis* in Brazil, whereas the new species was found in *Eugerres plumieri* further north, in the Gulf of Mexico.

Phylogenetic affinities

The aligned data set for the 28S gene was 1414 base pairs (bp) long and consisted of 25 species of Apocreadiidae. The substitution model selected for this data set used to infer the ML phylogenetic hypothesis was GTR+I, nucleotide frequencies were $A = 0.221$, $C = 0.222$, $G = 0.313$ and $T = 0.243$, and a likelihood value of $\ln = -5535.184152$. The phylogenetic tree resolved three clades which are consistent with subfamilies accepted within Apocreadiidae, that is, Schistorchiinae Yamaguti, 1942, Megaperinae Manter, 1934 and Apocreadiinae Skrzabin, 1942, with high bt support value (fig. 3a). Within the Apocreadiinae clade, the genus *Homalometron* was resolved as not monophyletic since three species were grouped separately, that is, *Homalometron mexicanum* + *Homalometron* sp. (which was the sister group of the other representative genera of Apocreadiinae), and *Homalometron synagris* (Yamaguti, 1953) Cribb & Bray, 1999 (grouped in an independent clade along with *Neoapocreadium* Siddiqi & Cable, 1960 and *Callohelms*

Cribb & Bray, 1999). Moreover, species of *Crassicutis* Manter, 1936 were recovered as the sister clade of *Homalometron*, with the exception of *Crassicutis archosargi* Sparks & Thatcher, 1960, a species that nested within the *Homalometron* clade.

Particularly, *H. avis* n. sp. was resolved as a member of *Homalometron*, as the sister species of *C. archosargi*, with high bt support value (98). In addition, within the *Homalometron* clade other subclades were formed albeit some with low nodal support values: a subclade grouping *Homalometron mesoamericanum* + (*Homalometron octopapillatum* + *Homalometron cupuloris*); *Homalometron pseudopallidum* separated independently as the sister group to the other species of the genus; another subclade grouping *Homalometron elongatum* + *Homalometron lesliorum*; and finally, a subclade formed by *Homalometron armatum* + ((*Homalometron manteri* + *Homalometron palmeri*) + (*Homalometron frocioneae* + (*Homalometron robisoni* + *Homalometron pallidum*))). *Homalometron elongatum* and *H. lesliorum* are morphologically similar species to the new species as mentioned above, due to the elongated body and the presence of three pairs of oral papillae, and they all infect gerreid fishes, but interestingly they were not grouped together in the molecular phylogenetic analysis.

Furthermore, the aligned data set for the ITS was 1111 bp long and had 20 species of apocreadiids. The selected model for the ML analysis was GTR+G+I, and nucleotide frequencies were $A = 0.200$, $C = 0.256$, $G = 0.271$ and $T = 0.273$. The ML tree had a likelihood value of $\ln = -5592.505050$. In this data set as in that of 28S, it also included representative species of Schistorchiinae and Megaperinae. The phylogenetic relationships of the species with the ITS were similar to those of 28S, where *H. mexicanum* was not grouped with the rest of the species of its genus, since *Crassicutis cichlasomae* remained as the sister species of the *Homalometron* clade. Instead, *C. archosargi* was grouped within the *Homalometron* clade. Within the *Homalometron* clade, *H. pseudopallidum* remained the sister species of the remaining sequenced species, and 3 subclades were formed (fig. 3b): one containing *H. mesoamericanum* + (*H. octopapillatum* + *H. cupuloris*); another containing *H. elongatum* + *H. lesliorum*; and finally another containing (((*H. avis* + *C. archosargi*) + (*H. frocioneae* + (*H. robisoni* + *H. pallidum*))) + (*H. manteri* + *H. palmeri*)) + *H. armatum*. Therefore, the new species also turned out to be sister to *C. archosargi*, and as in the 28S tree, the ITS tree topology indicated that *H. avis* n. sp. was not a sister taxon of *H. elongatum* and *H. lesliorum*.

In terms of genetic variation, the 28S gene showed *H. avis* n. sp. and *C. archosargi*, its sister species, differing by 1.91%, whereas the genetic divergence between the new species and those of *Homalometron* within the same clade varied from 1.64 to 4.45% (table 1). The divergence between the new species and the remaining species of *Homalometron* grouped in independent clades was very high and varied between 6.32 and 7.15%. Intergeneric genetic divergence was also high among genera of the subfamily Apocreadiinae, with values ranging from 5.67 to 6.59% (table 1). For ITS sequences, the genetic variation between *H. avis* n. sp. and *C. archosargi* was 1.37%, and with the congeneric species within the same clade ranged from 2.47 to 5.27% (table 1); the new species differed between 6 and 6.42% from *H. elongatum* and *H. lesliorum*, respectively. Finally, variation between the new species and the remaining species was from 6.94 to 13.06%; the highest variation was found with respect to *H. mexicanum* (table 1).

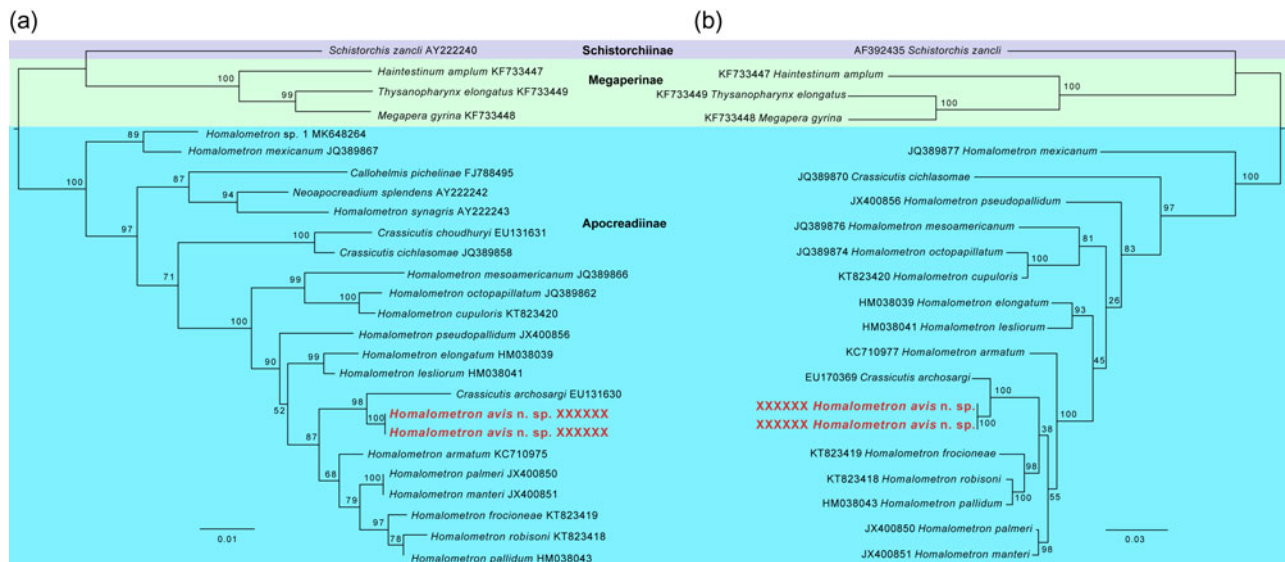


Fig. 3. Maximum likelihood phylogenetic trees for *Homalometron* species, showing the position of the new species that is highlighted in bold red font. (a) phylogenetic tree of the 28S gene; and (b) phylogenetic tree of the internal transcribed spacer (ITS) gene. Purple rectangle = schistorchiinae; green rectangle = megaperinae; blue rectangle = apocreadiinae. Numbers near internal nodes indicate bootstrap support values.

Table 1. Genetic distances between the new species and congeneric species used for phylogenetic analyses. In the centre column is the comparison with the 28S gene and in the right column is the comparison with the internal transcribed spacer (ITS) gene. DA = data not available.

Species	<i>Homalometron avis</i> n. sp.	
	28S	ITS
<i>Crassicutis archosargi</i>	1.91	1.37
<i>Homalometron pallidum</i>	2.73	4.24
<i>Homalometron robisoni</i>	2.78	4.24
<i>Homalometron frocioneae</i>	2.60	4.55
<i>Homalometron palmeri</i>	2.15	3.72
<i>Homalometron manteri</i>	2.15	3.93
<i>Homalometron armatum</i>	1.67	3.93–5.27
<i>Homalometron elongatum</i>	2.98	6.00
<i>Homalometron lesliorum</i>	2.40	6.42
<i>Homalometron pseudopallidum</i>	3.22	6.94
<i>Homalometron octopapillatum</i>	4.24	7.58
<i>Homalometron cupuloris</i>	4.22	7.44
<i>Homalometron mesoamericanum</i>	4.45	7.81
<i>Homalometron synagris</i>	6.32	DA
<i>Homalometron mexicanum</i>	7.15	13.06
<i>Homalometron</i> sp. 1	6.54	DA
<i>Crassicutis choudhuryi</i>	5.86	DA
<i>Crassicutis cichlasomae</i>	5.74	12.95
<i>Callohelms pichelinae</i>	6.59	DA
<i>Neopocreadium splendens</i>	5.67	DA

Discussion

The new species described in this study is the seventh described as parasite of the Gerreidae, and the seventh for Mexico. The newly discovered species is readily distinguishable from most congeners excepting a group of four species that possess three pairs of prominent oral papillae on the oral sucker. Still, the new species can be diagnosed by the combination of characters such as the distribution of the vitelline follicles, the size of the oral sucker, the oral sucker/ventral sucker length ratio, the size of the eggs and the length of the pre-pharynx. Amato (1983), Ostrowski de Nuñez *et al.* (2000), and Parker *et al.* (2010) distinguished the species they described possessing three pairs of oral papillae from their congeners using these characters. These authors also pointed out that the tegument of *H. carapevae*, *H. elongatum* and *H. lesliorum* were completely covered by small spines, whereas Ostrowski de Nuñez *et al.* (2000) described that *H. papilliferum* possessed scale-like spines distributed only on the anterior region of the parasite. Our observations of the tegument of the new species through SEM revealed that specimens possessed scale-like spines distributed along most body surfaces, more abundant in the forebody and particularly in the region of the ventral sucker, but their number decreasing towards the posterior end of body. Furthermore, SEM photomicrographs showed that each one of the scale-like structures comprises a pectinate structure of smaller spines at their distal end (see fig. 2f). Unfortunately, we cannot corroborate the existence of these types of spines on *H. carapevae*, *H. elongatum*, *H. lesliorum* and *H. papilliferum*. We emphasize here the need to use SEM as a common practice in future descriptions of trematodes, and we look forward for researchers to show the spines of the tegument of these species in the future in case they are sampled in the localities where they have been reported.

Sequence data available for two of the four species allows us to explore their phylogenetic relationships with respect to the new species herein described. The 28S and ITS phylogenetic trees

revealed that *H. elongatum* and *H. lesliorum* are sister taxa, they both are parasites mainly of Gerreidae and one of them, *H. elongatum* occurs in localities nearby the type locality of the new species in the Gulf of Mexico (Veracruz), including Tortugas, Florida, and other areas of the Caribbean Sea (see Parker *et al.*, 2010). Interestingly, *H. elongatum* and *H. lesliorum* are not close relatives to the new species. They do not conform a monophyletic group; therefore, the presence of three pairs of oral papillae cannot be considered as a synapomorphy for the group of species, at least the ones for which sequences are available. Sequence data for *H. papilliferum* and *H. carapevae* are needed to corroborate this hypothesis. The new species is morphologically very similar to *H. carapevae* and both are found in gerreid fishes, although the latter was described from Brazil; these species possess qualitative and quantitative characters that allow us to differentiate them, but the lack of molecular data for *H. carapevae* limits our understanding of their potential phylogenetic relationships. One possible hypothesis is that due to their morphological similarities and their host affinities (*Eugerres plumieri* for the new species and *Eugerres brasiliensis* for *H. carapevae*) they might be sister species as in the case of *H. elongatum* and *H. lesliorum* even though they are found in the Gulf of Mexico/Caribbean Sea, and Pacific Ocean, respectively (Manter, 1947; Amato, 1983; Parker *et al.*, 2010). As in the case of SEM, molecular data are also required to confirm this hypothesis.

Overall, the phylogenetic trees also show that the genus *Homalometron* needs a detailed taxonomic revision. The species sequenced up to now show that they do not conform a monophyletic assemblage. The morphological concept of the genus *Homalometron* requires further evaluation, incorporating not only genetic but also host association and geographical data. Species identified as *Homalometron* sp. 1, *Homalometron mexicanum* and *H. synagris* are not resolved as monophyletic in relation to the remaining sequenced species of *Homalometron*. They were originally allocated as belonging to the genus *Apocreadium* Manter, 1947 but later transferred to *Homalometron* by Cribb & Bray (1999) and validated in Cribb (2005). *Homalometron* sp. 1 (GenBank No. MK648264) represents a potential new species found in *Balistes vetula* L. from Puerto Morelos reef in the Caribbean Sea of Mexico (Pérez-Ponce de León & Hernández-Mena, 2019); *H. mexicanum* was described from several species of Balistidae, Labrisomidae and Monacanthidae in the Gulf of Mexico, Caribbean Sea and Pacific coast of Mexico (see records in Cribb & Bray, 1999); finally, *H. synagris* was described as a parasite of *Scolpis monogramma* (Cuvier) (Nemapteridae) from Malaysia, Indonesia and Australia (Cribb & Bray, 1999). Clearly, more sequence data of apocreadiines are required to further understand the interrelationships of members of the Apocreadiidae; that includes adding data on other species of *Homalometron* as well as data on other genera of the family.

Our phylogenetic analyses based on the 28S rRNA gene unequivocally demonstrated that *Crassicutis archosargi*, a species described from a sparid fish in the Gulf of Mexico (*Archosargus probatocephalus* Walbaum) (Sparks & Thatcher, 1960; Overstreet, 1976), is resolved as the sister taxon of the new species. This may indicate the species is not a member of *Crassicutis*, but a species of *Homalometron*. Previous phylogenetic studies have shown that the genus *Crassicutis* represents a monophyletic assemblage (see Razo-Mendivil *et al.*, 2010; Pantoja *et al.*, 2021). Some morphological traits indicate that *C. archosargi* falls within the concept of *Homalometron*. We refrain now from considering the synonymy of *C. archosargi* with *Homalometron*, as *H. archosargi* until new

samples are available for morphological comparison that includes SEM observations of the tegument. In *C. archosargi* the tegument is highly modified, bearing refractile bodies and was described by Sparks & Thatcher (1960) (and later redescribed by Overstreet, 1976) as lacking tegumental spines. Additionally, the fact that vitelline follicles in *C. archosargi* extend to the forebody make their inclusion in the current concept of *Homalometron* incorrect. The synonymy requires further morphological verification that spines might be present on the tegument, or probably lost after modification of a rugose and thick tegument, and a probable modification of the diagnosis of the genus *Homalometron* to include species with vitelline follicles extending into the forebody.

To conclude, it is important to emphasize that the comparison of morphological, genetic and phylogenetic information has allowed us to discover a new species in a coastal lagoon of Veracruz, Mexico. The discovery expands our knowledge about the biodiversity of brackish water fishes, although some species of *Homalometron* were previously reported or described from freshwater habitats. This is the seventh species of the genus currently reported from Mexico, along with *H. pallidum* Stafford, 1904, *H. mesoamericanum* Pérez-Ponce de León, Razo-Mendivil, & García-Magaña, 2012; *H. octopapillatum* Pérez-Ponce de León, Razo-Mendivil, & García-Magaña, 2012; *H. caballeroi* (Bravo-Hollis, 1954) Cribb & Bray, 1999; *H. verrunculi* (Lamothe-Argumedo, 1965) although see Cribb & Bray (1999) who suggested this was a nom. nov. for *H. caballeroi*; and *H. mexicanum* (Manter, 1937) Cribb & Bray, 1999 (see Cribb & Bray, 1999; Cribb, 2005; Pérez-Ponce de León *et al.*, 2012; Pérez-Ponce de León & Aguilar-Aguilar, 2019). Therefore, seven species of *Homalometron sensu lato* have been reported from Mexico. Our study also emphasizes the view that current taxonomic studies must use various sources of information that allow us to recognize and find support for the discovery of new taxa.

Acknowledgements. We thank Luis García-Prieto, Alejandro Ocegüera-Figeroa, David Osorio-Sarabia and Martín García-Varela, for their help during field work, Berenit Mendoza, Laboratorio Nacional de Biodiversidad (LANABIO), for obtaining scanning electron microscopy photomicrographs, and Laura Márquez and Nelly López (LANABIO) for their help with the use of the automatic sequencer.

Financial support. This project was partially funded by the Consejo Nacional de Ciencia y Tecnología (CONACYT) A1-S-21694, and by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN212621 to G.P.P.L.

Conflict of interest. None.

Ethical standards. Specimens in Mexico were collected under the Cartilla Nacional de Colector Científico (FAUT 0202 and 0057) issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), to M.G.V. and G.P.P.L., respectively.

References

- Amato JFR (1983) Digenetic trematodes of percoid fishes of Florianópolis, southern Brazil – Homalometridae, Lepocreadiidae, and Opcoelidae, with the description of seven new species. *Revista Brasileira de Biologia* 43(1), 73–98.
- Cribb TH (2005) Family apocreadiidae skrjabin, 1942. pp. 621–639. In Jones A, Bray RA, Gibson DI (Eds) *Keys to the Trematoda*, Vol. 2. Wallingford, CABI Publishing and the Natural History Museum.
- Cribb TH and Bray RA (1999) A review of the Apocreadiidae Skrjabin, 1942 (Trematoda: Digenea) and description of Australian species. *Systematic Parasitology* 44(1), 1–36.

- Curran SS, Tkach VV and Overstreet RM (2013) A new species of *Homalometron* (Digenea: Apocreadiidae) from fishes in the northern Gulf of Mexico. *Journal of Parasitology* **99**(1), 93–101.
- Darriba D, Taboada GL, Doallo R and Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.
- García-Varela M and Nadler SA (2005) Phylogenetic relationships of Palaecanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology* **91**(6), 1401–1409.
- Hernández-Mena DI, García-Prieto L and García-Varela M (2014) Morphological and molecular differentiation of *Parastrigea* (Trematoda: Strigeidae) from Mexico, with the description of a new species. *Parasitology International* **63**(2), 315–323.
- Luton K, Walker D and Blair D (1992) Comparisons of ribosomal internal transcribed spacer from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Molecular and Biochemical Parasitology* **56**(2), 323–328.
- Manter HW (1947) The digenetic trematodes of marine fishes of Tortugas, Florida. *American Midland Naturalist* **38**(2), 257–416.
- Nadler SA and Hudspeth DSS (1998) Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. *Molecular Phylogenetics and Evolution* **10**(2), 221–236.
- Ostrowski de Nuñez M, Brugni N and Viozzi G (2000) *Polylekithum percai* n. sp. (Trematoda: Allocreadiidae) from *Percichthys trucha* (Perciformes: Percichthyidae) in Patagonia, Argentina, and a redescription of *Homalometron papilliferum* (Szidat, 1956) n. comb. *Systematic Parasitology* **47**(1), 51–57.
- Overstreet RM (1976) A redescription of *Crassicutis archosargi*, a digenean exhibiting an unusual tegumental attachment. *Journal of Parasitology* **62** (5), 702–708.
- Pantoja C, Scholz T, Luque JL and Perez-Ponce de León G (2021) Molecular and morphological evidence of a new species of *Crassicutis* Manter 1936 (Digenea), a parasite of cichlids in South America. *Parasitology Research* **120**(7), 2429–2443.
- Parker JH, Curran SS, Overstreet RM and Tkach VV (2010) Examination of *Homalometron elongatum* Manter, 1947 and description of a new congener from *Eucinostomus currani* Zahuranec, 1980 in the Pacific Ocean off Costa Rica. *Comparative Parasitology* **77**(2), 154–163.
- Pérez-Ponce de León G and Aguilar-Aguilar R (2019) Helminth community structure of some freshwater fishes of the Cuatro Ciénegas Basin: patterns and processes. pp. 11–27. In Álvarez F, Ojeda M (Eds) *Animal diversity and biogeography of the Cuatro Ciénegas Basin*. Cham, Springer.
- Pérez-Ponce de León G and Hernández-Mena DI (2019) Testing the higher-level phylogenetic classification of Digenea (Platyhelminthes, Trematoda) based on nuclear rDNA sequences before entering the age of the 'next-generation' Tree of Life. *Journal of Helminthology* **93**(3), 260–276.
- Pérez-Ponce de León G, García-Prieto L and Mendoza-Garfias B (2007) Trematode parasites (Platyhelminthes) of wildlife vertebrates in Mexico. *Zootaxa* **1534**(1), 1–247.
- Pérez-Ponce de León G, Mendoza-Garfias B and García-Prieto L (2012) Biodiversidad de helmintos parásitos de peces marinos y estuarinos de México [Biodiversity of helminths parasites of marine and estuarine fish from Mexico]. pp. 109–120. in Del Moral FF, Martínez JA, Franco J, Ramírez AJ, Tello JL (Eds) *Investigaciones ictiológicas en México. Tópicos selectos en honor al Dr. José Luis Castro Aguirre* [Ichthyological investigations in Mexico. Selected topics in honour of Dr José Luis Castro Aguirre], Vol. 1. Mexico D.F, Facultad de Estudios Superiores-Iztacala y Sociedad Ictiológica Mexicana. [In Spanish.]
- Razo-Mendivil U, Vázquez-Domínguez E, Rosas-Valdez R, Pérez-Ponce de León G and Nadler SA (2010) Phylogenetic analysis of nuclear and mitochondrial DNA reveals a complex of cryptic species in *Crassicutis cichlasomae* (Digenea: Apocreadiidae), a parasite of Middle-American cichlids. *International Journal of Parasitology* **40**(4), 471–486.
- Shimazu T, Urawa S and Coria CO (2000) Four species of digeneans, including *Allocreadium patagonicum* sp. n. (Allocreadiidae), from freshwater fishes of Patagonia, Argentina. *Folia Parasitologica* **47**(2), 111–117.
- Sogandares-Bernal F (1959) Digenetic trematodes of marine fishes from the Gulf of Panama and Bimini, British West Indies. *Tulane Studies in Zoology* **7**(3), 69–117.
- Sparks AK and Thatcher VE (1960) A new species of *Crassicutis* (Trematoda, Allocreadiidae) from a sparid fish (*Archosargus probatocephalus*) in the northern Gulf of Mexico. *Transactions of the American Microscopical Society* **79**(2), 341–342.
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**(21), 2688–2690.
- Stock SP, Campbell JF and Nadler SA (2001) Phylogeny of *Steinernema travassos*, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *Journal of Parasitology* **87**(4), 877–889.
- Tamura K, Stecher G, Peterson D, Filipiński A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**(12), 2725–2729.
- Thompson JD, Higgins DG and Gibson TJ (1994) CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**(22), 4673–4680.
- Travassos L, Teixeira de Freitas JF and Bührnheim PF (1965) Trematodeos de peixes do litoral Capixaba: *Homalometron longulum* sp. n., parasito de caratinga. *Atas da Sociedade de biologia do Rio de Janeiro* **9**, 95–97.
- World Register of Marine Species (2022) *Homalometron* Stafford, 1904. Available at <https://www.marinespecies.org/aphia.php?p=taxdetails&id=108557> (accessed 18 April 2022).