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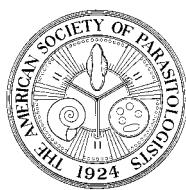
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NEOTROPICAL TURTLE BLOOD FLUKES: TWO NEW GENERA AND SPECIES FROM THE AMAZON RIVER BASIN WITH A KEY TO GENERA AND COMMENTS ON A MARINE-DERIVED PARASITE LINEAGE IN SOUTH AMERICA

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KEY WORDS ABSTRACT

Taxonomy
Systematics
Biogeography
Transitions
Biodiversity
Rainforest
Pebas Mega-Wetland

Two new genera and species of freshwater turtle blood flukes (TBFs) are described herein based on specimens infecting the nephritic and mesenteric blood vessels of “matamatas” (a side-necked turtle, *Chelus fimbriata* [Schneider, 1783] [Pleurodira: Chelidae]) from the Amazon River Basin, Peru. These taxa comprise the first-named species and the first-proposed genera of freshwater TBFs from the continent of South America. A new comparison of all TBF genera produced 6 morphologically diagnosed groups that are discussed in light of previous TBF classification schemes and a novel phylogenetic hypothesis based on the nuclear large subunit ribosomal DNA (28S). Considering external and internal anatomical features, species of the new genera (*Atamatam* Bullard and Roberts n. gen., *Paramatatam* Bullard and Roberts n. gen.) are most similar to each other and are together most similar to those of several marine TBF genera. The 28S phylogenetic analysis supported the monophyly of all 6 morphologically diagnosed groups of genera. Most notably, the freshwater TBFs of South America comprise a derived group nested within the clade that includes the paraphyletic marine TBFs. Not surprisingly in light of morphology, another marine TBF lineage (*Neospiroorchis* Price, 1934) clustered with the freshwater TBFs of *Baracktrema* Roberts, Platt, and Bullard, 2016 and *Unicaecum* Stunkard, 1925. Our results, including an ancestral state reconstruction, indicated that (1) freshwater TBFs have colonized marine turtles twice independently and that (2) the South American freshwater TBFs comprise a marine-derived lineage. This is the first evidence that TBFs have twice independently transitioned from a marine to freshwater definitive host. Marine incursion is considered as a possible mechanism affecting the natural history of marine-derived freshwater TBFs in South America. A dichotomous key to accepted TBF genera is provided.

There are 356 extant turtle species (Sarcopterygii: Testudines Linnaeus, 1758), including 60 polytypic taxa that add 122 subspecies (Rhodin et al., 2017). One of the more diverse groups of metazoan parasites that infect turtles comprises the turtle blood flukes (TBFs) (Digenea: Schistosomatidae Stiles and

Hassel, 1898), including approximately 103 species assigned to 22 accepted genera (Platt et al., 1991; Platt, 1992, 1993, 2002; Platt and Pichelin, 1994; Roberts et al., 2016a, 2016b, 2016c, 2017, 2018a, 2018b, 2019; Roberts and Bullard, 2017). TBFs infect members of both major turtle lineages, sub-order Pleurodira Cope, 1864 (side-necked turtles, withdraw neck by bending in horizontal plane; 93 species) and sub-order Cryptodira Cope, 1868 (hidden-necked turtles, withdraw head by bending neck in

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vertical plane; 263 species) (Gaffney et al., 2006; Pereira et al., 2017; Ferreira et al., 2018). Turtles split into these principal lineages before or during the Early Jurassic period (178–225.4 Ma [million years ago]) (Gaffney et al., 2006; Pereira et al., 2017). Extant cryptodires range in the Northern and Southern hemispheres, whereas extant pleurodires are geographically limited to freshwater habitats of the Southern Hemisphere (van Dijk et al., 2014).

Pleurodires are vastly underexplored for TBF infections compared to the globally distributed cryptodires. A total of 39 cryptodires assigned to 27 genera in 6 families are known as TBF hosts; freshwater cryptodires host 66 nominal TBFs of 11 genera, and marine cryptodires (all Cheloniidae) host 33 TBFs of 10 genera. The most intensive TBF taxon sampling to date clearly has focused on the iconic oceanic cryptodires of Cheloniidae Oppel, 1811 (“sea turtles”). For example, the green sea turtle, *Chelonia mydas* (Linnaeus, 1758), alone is host to 20 TBFs assigned to 8 genera (>1/3 of accepted TBF genera). In stark contrast to this documented diversity, only 5 nominal TBF species (3 in Australia; 2 in Africa) have been documented from 93 species of Pleurodira (Bougat and Kulo, 1987; Goodman, 1987; Platt and Pichelin, 1994; Platt and Blair, 1996). In fact, 90% (310) of the world’s freshwater turtles have yet to be documented or examined as a TBF host.

The South American pleurodires remain especially undersampled for parasites. Recent parasitological surveys focused on ectoparasites and gastro-intestinal helminths have highlighted this gap in knowledge and have begun addressing it (e.g., Mascarenhas et al., 2013, 2016; Fernandes and Kohn, 2014; Viana et al., 2016; Du Preez et al., 2017; Correa et al., 2018; Pereira et al., 2018). In contrast, and perhaps owing to the difficulty in extracting taxonomically useful specimens from turtle blood, the blood parasites of freshwater turtles in South America remain unsampled. To our knowledge from South America, there is no published record of a blood parasite (of any phylum) infecting a freshwater turtle, and no freshwater turtle has been confirmed as a TBF host (Pinto et al., 2015). Platt (1992), who first drew attention to this gap in our knowledge of TBF biodiversity, placed a bold question mark over the figure of the continent of South America to make his point. Twenty-three years later, relatively little progress had been made along these lines when compared to the taxonomic exploration of TBFs in North America and Southeast Asia. Pinto et al. (2015) sequenced 3 innominate cercariae (W810 Spirorchiidae sp. 3 ex. *Pomacea* Brazil; W702 Spirorchiidae sp. 1 ex. *Biomphalaria* Brazil; W811 Spirorchiidae sp. 2 ex. *Biomphalaria* Brazil); W702 and W811 clustered with marine TBFs, and W810 was sister to *Unicaecum* sp. This result was intriguing because it indicated that freshwater TBFs ranged in South America and that they share a recent common ancestor with marine TBFs. Yet, at present, South America remains the only continent holding extant freshwater turtles that has yet to include a record of a blood fluke infection in a turtle. This is remarkable given the diversity of turtles that range there.

A total of 44 freshwater turtles (15% of all extant turtles) range in continental South America (excluding the Galapagos Islands; Bonin et al., 2006; Buhlmann et al., 2009; Mittermeier et al., 2015; Pereira et al., 2017; Rhodin et al., 2017). Pleurodires of South America are assigned to Chelidae Gray, 1825 (4 species of *Acanthochelys* Gray, 1873; *Chelus fimbriata* [Schneider, 1783]; 2 of *Hydromedusa* Wagler, 1830; 10 of *Mesoclemmys* Gray, 1837; 4 of

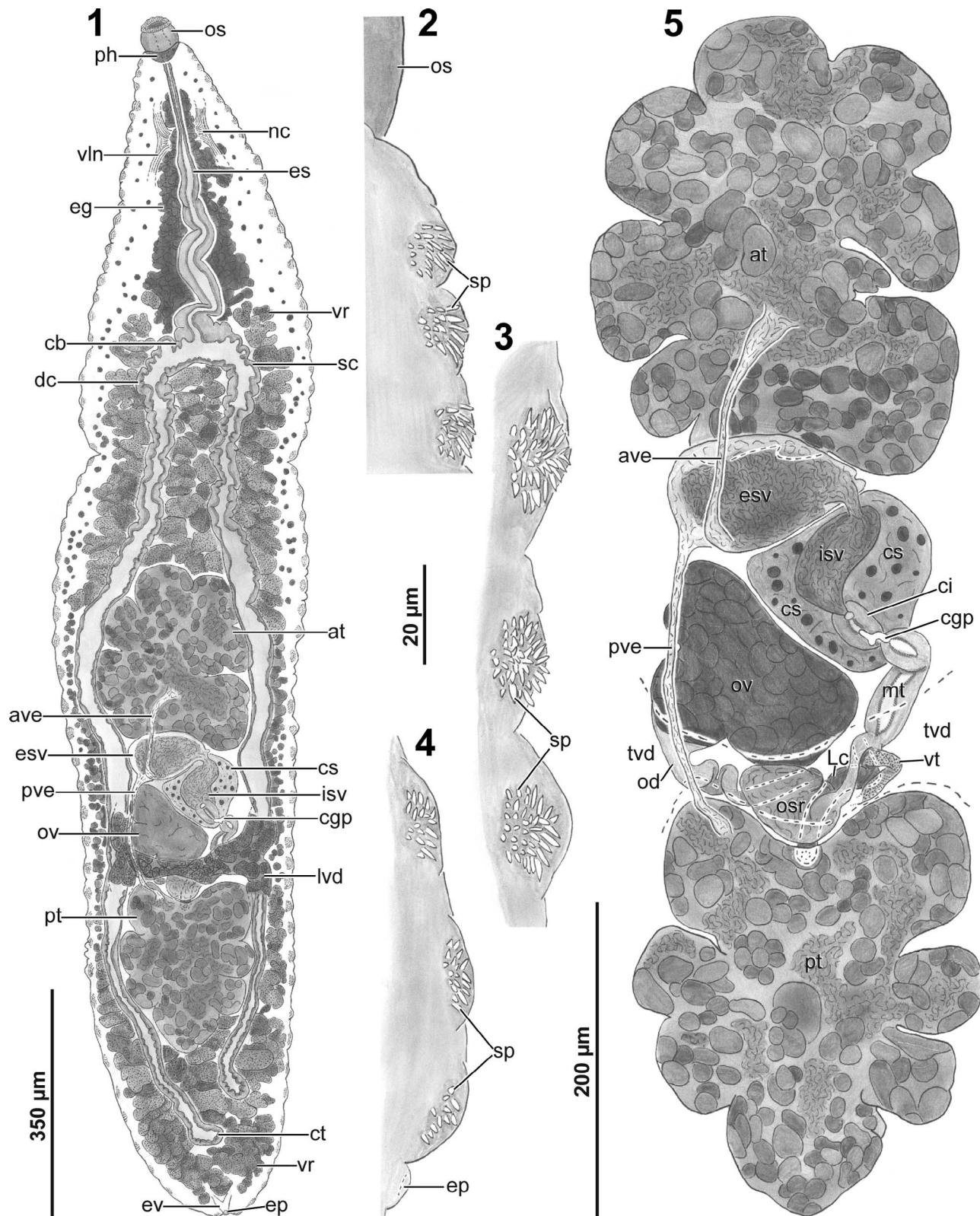
Phrynops Wagner, 1830; *Platemys platycephala* Schneider, 1792; *Rhinemys rufipes* [Spix, 1824]) and Pelomedusidae Cope, 1868 (*Peltoccephalus dumerilianus* [Schweigger, 1812]; 6 of *Podocnemis* [Wagler, 1830]) (Rhodin et al., 2017). Cryptodires of South America are assigned to Emydidae Rafinesque, 1815 (3 of *Trachemys* Agassiz, 1857), Kinosternidae Agassiz, 1857 (3 of *Kinosternon* Spix, 1824), Testudinidae Batsch, 1788 (3 of *Chelonoidis* plus *Chersobius boulengeri* Duerden, 1906), and Geoemydidae (5 of *Rhinoclemmys* [Fitzinger, 1835]).

Chelidae, which includes the new host species reported herein, is the most diverse turtle family with representation in South America and comprises 59 extant species distributed in the Southern Hemisphere only. South America holds 23 chelids (over half of South America’s freshwater turtle fauna), Australia has 26 chelids (11 of *Chelodina* Fitzinger, 1826; 4 of *Elseya* Gray, 1867; *Elusor macrurus* Cann and Legler, 1994; 3 of *Emydura* Bonaparte, 1836; 4 of *Myuchelys* Thomson and Georges, 2009; *Pseudemydura umbrina* Sebenrock, 1901; *Rheodytes leukops* Legler and Cann, 1980), and New Guinea has 10 chelids (4 of *Chelodina*; 5 of *Elseya*; *Emydura subglobosa* [Kreft, 1876]) (Rhodin et al., 2017). Chelidae is monophyletic and comprises South American and Australian/New Guinean sister clades (Pereira et al., 2017), leading some workers to refer to members of Chelidae as “Austro-South American side-necked turtles.” Based on fossil (Smith, 2010; Ferreira et al., 2016, 2018) and molecular (Pereira et al., 2017) evidence, the South American and Australian chelids diverged in the Early Cretaceous (140 Ma). This biogeographic foundation makes the chelids alluring for parasite biodiversity and biogeography studies. At present, only 2 chelids are reported as TBF hosts, and TBF sampling in South America, Africa, and Australia is low (present study; Platt and Pichelin, 1994; Platt and Blair, 1996). A single Australian freshwater turtle (Murray River turtle, *Emydura macquarrii* [Gray, 1830] [Pleurodira: Chelidae]) is known as a TBF host (infected by *Uterotrema australispinosa*, *Uterotrema burnsii* Platt and Blair, 1996, and *Uterotrema kreffti* Platt and Blair, 1996), and the present study comprises the only records of nominal TBFs from South America.

Herein, we describe 2 new species of TBFs that infect the blood of a chelid (matamata, *C. fimbriata*) from the Amazon River Basin and propose a new genus to accommodate each one. The present report comprises the first record of a blood fluke infection in a freshwater turtle in South America and only the second confirmed chelid host for a TBF worldwide. Based on a consideration of generic features across all TBFs, we grouped the accepted TBF genera (22 genera + 2 new genera) into 6 morphologically diagnosed groups that we discuss in light of previous classification schemes for “Spirorchiidae” (*sensu lato*) and in consideration of an updated molecular phylogenetic hypothesis. We also discuss parasite-host cophyly, marine-derived lineages (MDLs), and marine incursions, as well as chelid diversity and its fossil record in light of that phylogenetic hypothesis.

MATERIALS AND METHODS

During July 2016, the viscera of 5 matamatas (*C. fimbriata*) from the Belén Market (3°45'32.08"S, 73°14'53.15"W), Iquitos, Peru (Amazon River), were examined opportunistically for blood fluke infections following previously published procedures (Roberts et al., 2016a, 2016b, 2016c, 2017, 2018a, 2018b, 2019; Roberts and Bullard, 2017). The resulting blood flukes (Figs. 1–35) were



Figures 1–5. *Atamatam amazoniensis* n. g., n. sp. (holotype, USNM 1557310) from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. (1) Body showing oral sucker (os), pharynx (ph), nerve commissure (nc), ventrolateral nerve chords (vln), esophagus (es), esophageal gland (eg), vitellarium (vr), cecal bifurcation (cb), sinistral cecum (sc), dextral cecum (dc), anterior testis (at), anterior vas efferens (ave), external seminal vesicle (esv), cirrus sac (cs), posterior vas efferens (pve), internal seminal vesicle (isv), common genital pore (cg), ovary (ov), lateral vitelline collecting ducts (lvd), posterior testis (pt), cecal terminus (ct), excretory vesicle (ev), and excretory pore (ep). Details of mammillae stylized. Ventral view. (2) Anterior-most ventrolateral tegumental mammillae showing mammillae spines (sp). Sinistroventral view. (3) Midbody ventrolateral

observed microscopically prior to fixation, fixed in 5–10% neutral buffered formalin (nbf) for morphology, or preserved in 95% non-denatured ethanol (EtOH) for DNA extraction. The ovoid TBF specimens with a relatively thick tegument (i.e., specimens of the first new species described below) were routinely heat-killed on glass slides using a butane hand lighter under little or no coverslip pressure. The elongate, delicate blood fluke specimens having a thin tegument (i.e., specimens of the second new species described below) were isolated in a vial half-filled with citrated saline into which a drop of 5% nbf was added every 60 sec between periods of intense shaking. The latter method likewise resulted in relatively flat and straight, fixed specimens without exposure to heat, which evidently damages these delicate flukes such that they do not readily take to staining with conventional methods effective for other blood flukes. Collectively, the resulting specimens were stained in Van Cleave's hematoxylin with several drops of Ehrlich's hematoxylin, dehydrated with a graded EtOH series, made basic at 70% EtOH with lithium carbonate and butyl-amine, dehydrated in absolute EtOH and xylene, cleared with clove oil, and permanently mounted in Canada balsam.

Drawings were made with Leica DM2500 and Leica DMR (Leica, Wetzler, Germany) compound microscopes, each equipped with differential interference contrast optical components and a drawing tube. Measurements were obtained with a calibrated ocular micrometer (as straight lines along the course of each duct) and are herein reported in micrometers (μm) followed by the mean and number of anatomical features measured in parentheses.

Formalin-fixed specimens intended for scanning electron microscopy (SEM) were rinsed in distilled water, dehydrated in a graded ethanol series, critical point dried in liquid CO_2 , mounted on SEM aluminum stubs with double-sided carbon tape, sputter coated with gold-palladium (19.32 g/cm^3 ; 25 mA), and viewed with a Zeiss EVO 50VP SEM (Carl-Zeiss, Oberkochen, Germany). Type specimens of the new species were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (Smithsonian Institution, USNM Collection nos. 1557310–1557319). Classification and anatomical terms for TBFs follow Roberts et al. (2016a, 2016b, 2016c, 2017). Turtle scientific and common names follow van Dijk et al. (2014), and higher classification of turtles follows Pereira et al. (2017).

DNA extraction, amplification, primers, and sequencing protocols followed Roberts et al. (2017). Using 2 EtOH-preserved and microscopically identified blood flukes per new taxon, total genomic DNA (gDNA) was extracted using the DNeasyTM Blood and Tissue Kit (Qiagen, Valencia, California) as per the manufacturer's protocol except that the proteinase-K incubation period was extended overnight and the final elution step used 100 μl of elution buffer to increase the final DNA concentration. Amplification and sequencing of the D1–D3 domains of the large subunit ribosomal DNA (28S) used the set of primers described in Orélis-Ribeiro et al. (2017). PCR amplifications were performed using a total volume of 50 μl with 2 μl of DNA template, 0.4 μM of each primer along with 1× buffer, 2.5 mM MgCl₂, 1 mM dNTP mixture, and 0.3 μl Taq polymerase (5 U/ μl) (Promega, Madison, Wisconsin). The thermocycling profile comprised 5 min at 95 °C for denaturation, 40 repeating cycles at 94 °C for 30 sec for denaturation, 61 °C for 30 sec for annealing, and 72 °C for 2 min

for extension followed by a final 10 min at 72 °C for extension. All PCR reactions were carried out in a MJ Research PTC-200 (BioRad, Hercules, California). PCR products (18 μl) were verified on a 1% agarose gel and stained with ethidium bromide. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocols except that the last elution step was performed with autoclaved nanopure H₂O rather than with the provided buffer. The DNA sequencing was performed by ACGT, Inc. (Wheeling, Illinois). Reactions were sequenced using BigDye terminator version 3.1, cleaned with magnetic beads (CleanSeq dye terminator removal kit), and analyzed using an ABI 3730 XL or 3730 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts).

Sequence assembly and analysis of chromatograms were performed with Geneious version 11.0.5 (<http://www.geneious.com>; Kearse et al. [2012]). Newly generated sequences and those retrieved from GenBank (Table I) were aligned using MAFFT (Katoh and Standley, 2013) with default settings. JModelTest 2 version 2.1.10 was implemented to perform a statistical selection of the best-fit models of nucleotide substitution based on Bayesian information criteria (BIC) (Darriba et al., 2012). Aligned sequences were reformatted (from fasta to Nexus and Phylip) using the web application ALTER (Glez-Pena et al., 2010). Bayesian inference was conducted using BEAST 2.5.1 (Bouckaert et al., 2014). Phylogenetic reconstruction in BEAST was done using the GTR+G substitution model and a birth-death tree prior (Gernhard, 2008). BEAST analyses also included a relaxed molecular clock with a lognormal prior distribution (Drummond et al., 2006) and a rate set to one because we were interested more in relative diversification timing than absolute times. Two independent BEAST runs were performed for 10,000,000 Markov chain Monte Carlo (MCMC) generations, sampling the posterior every 1,000 generations. Convergence and stationarity of the BEAST runs were checked with Tracer, revealing a 25% burn-in was sufficient. A maximum clade credibility tree was computed for the BEAST analysis with TreeAnnotater (Bouckaert et al., 2014); support for relationships was measured by posterior probabilities (PPs). Trees were visualized with FigTree (Rambaut et al., 2014) and further edited with Adobe Illustrator (Adobe Systems).

We also ran a maximum likelihood phylogenetic analysis with IQTREE 1.6 (Nguyen et al., 2015). The maximum likelihood tree was inferred using default parameters and the GTR + Γ model. One thousand ultrafast bootstrap (BS) replicates were done to assess nodal support (Minh et al., 2013). The most likely tree, with BS values mapped to nodes, was visualized with FigTree and edited for visualization purposes with Adobe Illustrator.

We reconstructed the ancestral states of taxon habitat preference (freshwater or marine habitat of the definitive host) by performing a stochastic mapping of ancestral states (Huelsenbeck et al., 2003). The host taxa were characterized as having a freshwater or marine habitat type using published accounts for those fishes (Carpenter, 2002; Orélis-Ribeiro et al., 2014) and turtles (van Dijk et al., 2014). The best-fit model of character evolution was inferred with the R (R Core Team, 2017) package Geiger (Harmon et al., 2008) using AICc criteria. Stochastic mapping of character evolution was done in the R package Phytools (Revell, 2012) with 10,000 simulations and the equal rates model of character evolution. Characters were mapped on the BEAST maximum clade credibility tree. The PP of character

states at all points on the phylogeny was visualized by plotting the posterior density of characters on the BEAST maximum clade credibility tree. Nucleotide sequence data reported in this paper are available in the GenBank database under accession numbers MK775718–MK775720 (Table I).

DESCRIPTIONS

Atamatam n. gen. Bullard and Roberts (Figs. 1–17, 36)

Body dorsoventrally flat (not cylindrical), ventrally concave, ovoid (not thread-like), 4–6× longer than wide, having inverse V-shaped anterior end, with posterior end broadly rounded, having spinous ventrolateral mammillae, having tegumental projections distributing across ventral body surface and dorsal body margin; mammillae small, slightly raised, spinous; mammillae spines spike-like, lacking recurved distal tip, on curved apical surface of mammillae. Oral sucker robust, demarcated from body by constriction, spinous, papillate; oral sucker spines (exposed portion) triangular, distributing in single band on anteroventral surface of mouth. Ventral sucker absent. Pharynx present, enveloping anterior extremity of esophagus. Esophagus terminating in anterior one-third of body, ventral to anterior nerve commissure; lateral esophageal diverticula and median esophageal diverticulum absent; esophageal gland surrounding most of esophagus. Intestinal ceca inverse U-shaped, comprising non-fused ceca bifurcating in anterior one-third of body, diverticulate in anterior portion only, extending approximately three-fourths of body length posteriad, terminating near posterior body extremity, asymmetrical. Testes 2 in number, arranged in a column, comprising an anterior testis and a post-ovarian testis, inter-cecal, occupying space immediately anterior and posterior to terminal genitalia, ovoid (slightly longer than wide). Anterior and posterior trunks of vasa efferentia present, ventral to gonads, connecting to vas deferens; posterior vas efferens dextral. Vas deferens comprising a compact external seminal vesicle; external seminal vesicle abutting anterior testis, ovary, and cirrus sac. Cirrus sac enclosing internal seminal vesicle and cirrus, lateral to ovary, directed posterolaterad. Pars prostatica absent. Ovary dextral, inter-cecal, inter-testicular, closely flanked by testes anteriorly and posteriorly, having smooth borders (lacking deep lobes). Oviduct transverse, crossing midline before extending anteriad, convoluted proximally; oviducal seminal receptacle comprising middle portion of oviduct, at level of transverse vitelline duct. Laurer's canal inter-cecal, inter-testicular, with dorsal pore. Vitellarium symmetrical, comprising a series of interconnected large spheroid masses of vitelline follicles, surrounding ceca, distributing from cecal bifurcation to excretory vesicle (extending beyond tips of ceca); transverse vitelline duct inter-testicular, between ovary and posterior testis, ventral to gonads. Uterus short, straight (lacking coils, loops, convolutions), inter-cecal, with distal end comprising metraterm, lacking uterine pouch; metraterm strongly muscular, inter-gonadal. Common genital pore sinistral, lateral to ovary, ventral, inter-cecal, inter-

testicular. Excretory vesicle Y-shaped; excretory pore dorsal, subterminal. Manter's organ absent.

Differential diagnosis: Body flat, 4–6× longer than wide, having inverse V-shaped anterior end, having spinous ventrolateral mammillae, having tegumental projections distributing across ventral body surface and along the dorsal body margin; spines present on curved apical surface of mammillae. Oral sucker robust, spinous, papillate; spines (exposed portion) triangular, distributing in single band on anteroventral surface of mouth. Ventral sucker, lateral esophageal diverticula, and median esophageal diverticulum absent. Intestinal ceca inverse U-shaped, diverticulate anteriorly, terminating near posterior body extremity. Testes 2, inter-cecal, flanking and abutting terminal genitalia. Posterior vas efferens dextral. External seminal vesicle abutting anterior testis, ovary, and cirrus sac. Cirrus sac lateral to ovary, directed posterolaterad. Ovary dextral, flanked by testes anteriorly and posteriorly. Oviduct transverse, crossing midline before extending anteriad, convoluted proximally; oviducal seminal receptacle at level of transverse vitelline duct. Laurer's canal inter-testicular. Vitellarium distributing from cecal bifurcation to excretory vesicle (extending beyond tips of ceca); transverse vitelline duct between ovary and posterior testis. Common genital pore sinistral, lateral to ovary, ventral. Manter's organ absent.

Taxonomic summary

Type and only known species: *Atamatam amazoniensis* n. sp.

ZooBank registration: urn:lsid:zoobank.org:act:2119D3B8-143C-46DA-9E51-FEEB4E7CCB4E.

Etymology: *Atamatam* is a semordnilap of matamata, the type host.

Atamatam amazoniensis n. sp. Bullard and Roberts (Figs. 1–17)

Description of adult (based on 3 whole-mounted specimens):

Body 1,760–2,070 (1,897; 3) long, 350–370 (361; 3) in maximum width at level of cecal bifurcation, 4.8–5.7× (5.2; 3) longer than wide (Fig. 1); ventrolateral tegumental mammillae numbering 118–136 (124; 3) total, with equal number on each body margin or 59–68 (62; 3) per side of body (stylized in Fig. 1), distributing from oral sucker to posterior body end, more narrow and tightly spaced anteriorly, broader and more widely spaced posteriorly; mammillae in anterior body region (anterior mammillae) 10–28 (17; 9) wide at base, with tegumental spines 3–7 (5; 27) long and protruding approximately 1 from tegument (Figs. 2, 15–17); mammillae at level of ovary (middle mammillae) 20–30 (21; 9) wide at base, with tegumental spines 4–6 (5; 27) long and protruding approximately 1 from tegument (Figs. 3, 13); mammillae at level of tips of ceca (posterior mammillae) 26–35 (30; 9) wide at base, with tegumental spines 2–5 (4; 27) long and protruding <1 from tegument (Figs. 4, 14). Body surface having symmetrically arranged tegumental projections (approximately 2–4 long, 0.5 wide) ventrally (Figs. 10, 12) and laterally (Figs. 6, 12, 16, 17).

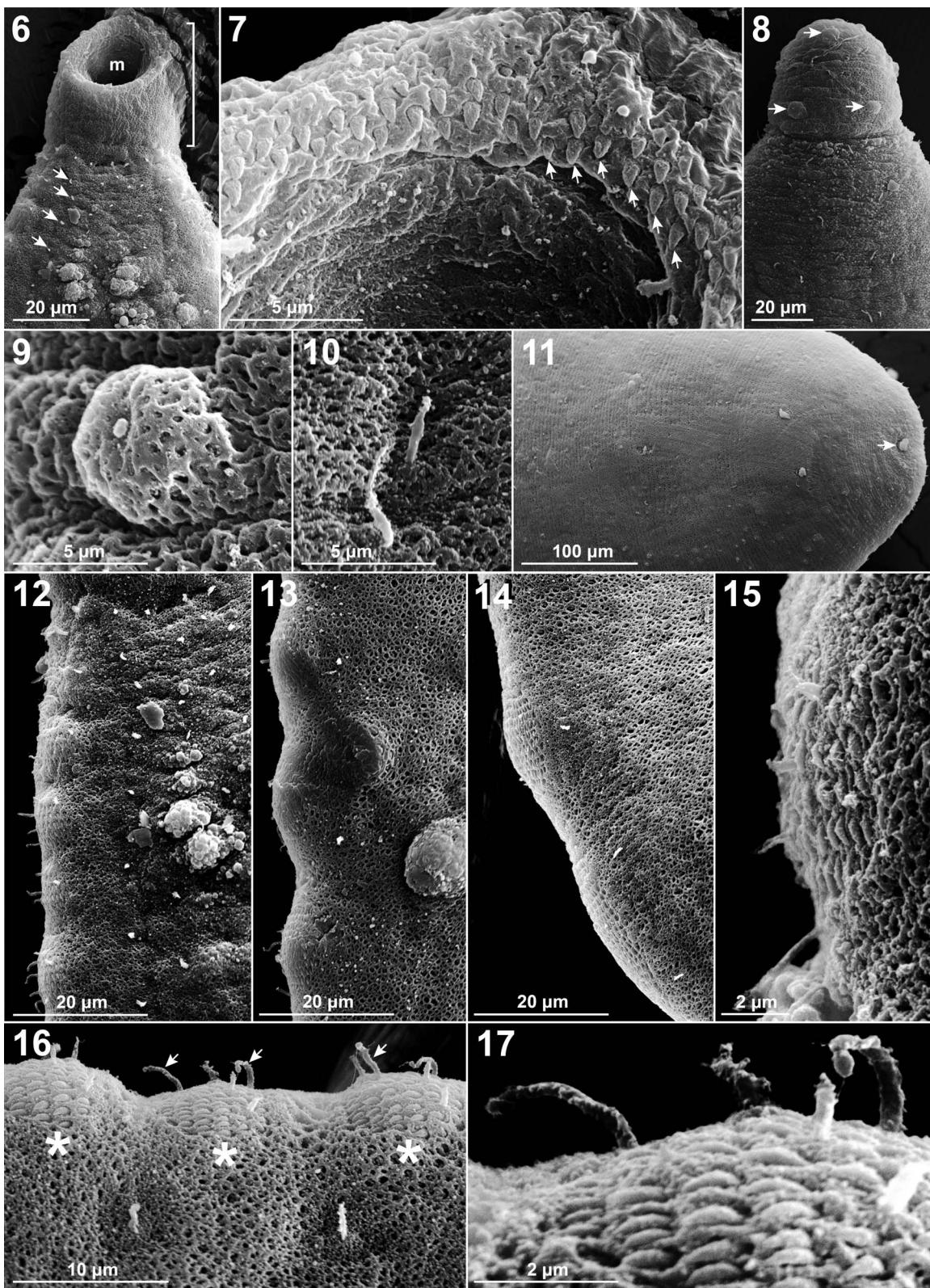
← tegumental mammillae. Sinistroventral view. (4) Posterior-most ventrolateral tegumental mammillae showing excretory pore (ep). Sinistroventral view. (5) Genitalia showing cirrus (ci), metraterm (mt), oviduct (od), primary vitelline duct (vt), oviducal seminal receptacle (osr), Laurer's canal (Lc), and transverse vitelline duct (tvd; dotted to highlight course of female genitalia). Ventral view.

Table I. 28S sequences used herein (bold font indicates the new taxa described herein).

| Blood fluke | Host |
|--|---|
| <i>Amphiorchis</i> sp. KX-109 | <i>Caretta caretta</i> (Linnaeus, 1766), loggerhead sea turtle |
| <i>Amphiorchis</i> sp. KX-111 | <i>Thylaeodus</i> cf. <i>rugulosus</i> (Monterosato, 1878), vermetid gastropod |
| <i>Amphiorchis</i> sp. PAC | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Amphiorchis</i> sp. KC-107 | <i>Caretta caretta</i> (Linnaeus 1766), loggerhead sea turtle |
| <i>Atamatam amazoniensis</i> Bullard and Roberts, n. gen., n. sp. | <i>Chelus fimbriata</i> (Schneider, 1783), matamata |
| <i>Austrobilharzia terrigalensis</i> Johnson, 1916 | <i>Batillaria australis</i> (Quoy and Gaimard, 1834), a batillariid gastropod |
| <i>Baracktrema obamai</i> Roberts, Platt, and Bullard, 2016 | <i>Siebenrockiella crassicornis</i> (Gray, 1831), black marsh turtle |
| <i>Bivitellobilharzia loxdontae</i> Vogel and Minning, 1940 | <i>Loxodonta cyclotis</i> (Matschie, 1900), African forest elephant |
| <i>Carettacola hawaiensis</i> Dailey, Fast, and Balazs, 1991 | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Carettacola hawaiensis</i> Dailey, Fast, and Balazs, 1991 | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Carettacola</i> sp. Manter and Larson, 1950 | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Coeuritrema platti</i> Roberts and Bullard, 2016 | <i>Pelodiscus sinensis</i> (Wiegmann, 1835), Chinese softshell turtle |
| <i>Elopicola nolancribbi</i> Bullard, 2014 | <i>Elops saurus</i> Linnaeus, 1766, ladyfish |
| <i>Elopicola bristowi</i> Orélis-Ribeiro et al., 2017 | <i>Elops hawaiiensis</i> Reagan, 1909, Hawaiian ladyfish |
| <i>Elopicola franksi</i> Orélis-Ribeiro et al., 2017 | <i>Megalops atlanticus</i> Valenciennes, 1847, Atlantic tarpon |
| <i>Griphobilharzia amoena</i> Platt and Blair, 1991 | <i>Crocodylus johnstoni</i> Krefft, 1873, freshwater crocodile |
| <i>Hapalorhynchus conecuhensis</i> Roberts and Bullard, 2017 | <i>Sternotherus minor</i> (Agassiz, 1857), loggerhead musk turtle |
| <i>Hapalorhynchus foliorchis</i> Brooks and Mayes, 1975 | <i>Chelydra serrulata</i> (Linnaeus, 1758), common snapping turtle |
| <i>Hapalorhynchus gracilis</i> Stunkard, 1922 | <i>Chelydra serrulata</i> (Linnaeus, 1758), common snapping turtle |
| <i>Hapalorhynchus reeffooti</i> Byrd, 1939 | <i>Sternotherus odoratus</i> Latreille in Sonnini and Latreille, (1801), common musk turtle |
| <i>Hapalotrema mehrae</i> Rao, 1976 | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Hapalotrema mistroides</i> (Monticelli, 1896) | <i>Caretta caretta</i> (Linnaeus, 1766), loggerhead sea turtle |
| <i>Hapalotrema mistroides</i> (Monticelli, 1896) | <i>Caretta caretta</i> (Linnaeus, 1766), loggerhead sea turtle |
| <i>Hapalotrema</i> sp. CT-2017 | <i>Caretta caretta</i> (Linnaeus, 1766), loggerhead sea turtle |
| <i>Learedius learedi</i> Price, 1934 | <i>Chelonia mydas</i> (Linnaeus, 1758), green sea turtle |
| <i>Macrobilharzia macrobilharzia</i> Travassos, 1922 | <i>Anhinga anhinga</i> (Linnaeus, 1766), anhinga |
| <i>Neospirochis</i> sp. CT-2017 | <i>Caretta caretta</i> (Linnaeus 1766), loggerhead sea turtle |
| <i>Paramatam iquitosiensis</i> Bullard and Roberts, n. gen., n. sp. | <i>Chelus fimbriata</i> (Schneider, 1783), matamata |
| <i>Paramatam iquitosiensis</i> Bullard and Roberts, n. gen., n. sp. | <i>Chelus fimbriata</i> (Schneider, 1783), matamata |
| <i>Platt sinuosus</i> Roberts and Bullard, 2018 | <i>Malayemys subtrijuga</i> (Schlegel and Müller, 1845), Mekong snail-eating turtle |
| <i>Platt snyderi</i> Roberts and Bullard, 2018 | <i>Malayemys subtrijuga</i> (Schlegel and Müller, 1845), Mekong snail-eating turtle |
| <i>Schistosoma haematobium</i> (Bilharz, 1852) | <i>Bulinus nyassanus</i> (E. A. Smith, 1877), a planorbid gastropod |
| <i>Spirhalpum polesianum</i> Ejsmont, 1927 | <i>Emys orbicularis</i> (Linnaeus, 1758), European pond slider |
| <i>Spirhalpum siamensis</i> Tkach, Snyder, and Vaughan, 2009 | <i>Cuora amboinensis</i> (Daudin, 1802), Malayan box turtle |
| <i>Spiroorchis artericola</i> (Ward, 1921) Stunkard, 1921 | <i>Chrysemys picta</i> (Schneider, 1783), painted turtle |
| <i>Spiroorchis collinsi</i> Roberts and Bullard, 2016 | <i>Deirochelys reticularia</i> (Latreille in Sonnini and Latreille, [1801]), chicken turtle |
| <i>Spiroorchis haematobius</i> (Stunkard, 1922) Price, 1934 | <i>Chelydra serrulata</i> (Linnaeus, 1758), common snapping turtle |
| <i>Spiroorchis picta</i> Stunkard, 1923 | <i>Trachemys scripta</i> (Thunberg in Schoepff, [1792]), pond slider |
| <i>Spiroorchis scripta</i> Stunkard, 1923 | <i>Graptemys pulchra</i> (Baur, 1893), Alabama map turtle |
| <i>Spiroorchis</i> cf. <i>scripta</i> | <i>D. reticularia</i> (Latreille in Sonnini and Latreille, [1801]), chicken turtle |
| <i>Trichobilharzia querquedulae</i> McLeod, 1937 | <i>Anas discors</i> (Linnaeus, 1766), blue-winged teal |
| <i>Unicaecum</i> sp. | <i>Trachemys scripta</i> (Thunberg in Schoepff, 1792), pond slider |
| <i>Uterotrema</i> sp. | Indeterminate |
| <i>Vasotrema</i> cf. <i>robustum</i> Stunkard, 1928 | <i>Apalone spinifera aspera</i> (Agassiz, 1857), Gulf Coast spiny softshell turtle |
| TBF sp. W-702 | <i>Biomphalaria</i> sp. Preston, 1910, a planorbid gastropod |
| TBF sp. W-810 | <i>Pomacea</i> sp. Perry, 1810, an ampullariid gastropod |
| TBF sp. W-811 | <i>Biomphalaria</i> sp. Preston, 1910, a planorbid gastropod |
| TBF sp. W-1120 | <i>Biomphalaria sudanica</i> (Martens, 1870), a planorbid gastropod |
| TBF sp. CMA-2009 | <i>Physa acuta</i> Draparnaud, 1805, a physid gastropod |
| TBF sp. CMA-2010 | <i>Physa acuta</i> Draparnaud, 1805, a physid gastropod |

Table I. Extended.

| Locality | GenBank accession no. | Reference |
|---|-----------------------|-----------------------------|
| Florida | 28S | |
| Oceanogràfic Aquarium, Valencia, Spain | KX987109 | Cribb et al., 2017b |
| Queensland, Australia | KX987111 | Cribb et al., 2017b |
| Oceanogràfic Aquarium, Valencia, Spain | KU892017 | Chapman et al., 2015 |
| Upper Amazon River Basin (3°45'32.08"S, 73°14'53.15"W), Belén Market, Iquitos, Peru | KX987107 | Cribb et al., 2017b |
| Rodd Point, Iron Cove, Sydney Harbor, NSW, Australia | MK775718 | Present study |
| Perak, Perak River, Malaysia | AY157249 | Lockyer et al., 2003 |
| Bai Hokou field station (2°51'34"N, 16°28'03"E), Central African Republic | KX061500 | Roberts et al., 2016a |
| Pacific Ocean, Hawaii | JN579949 | Brant and Loker, 2013 |
| Queensland, Australia | AY604709 | Snyder, 2004 |
| Queensland, Australia | KU600068 | Chapman et al., 2016 |
| Da Rang River, Phu yen Province, Vietnam | KU600069 | Chapman et al., 2016 |
| Gulf of Mexico, off Ship Island, Mississippi | KX712243 | Roberts et al., 2016a |
| Vĩnh Hải fish market, Nha Trang (Eastern Sea), Vietnam | KY243880 | Orélis-Ribeiro et al., 2017 |
| Gulf of Mexico, North Captiva Island and Bayboro Harbor, off Florida | KY243881 | Orélis-Ribeiro et al., 2017 |
| Darwin (probably Adelaide River Basin), Australia | KY243882 | Orélis-Ribeiro et al., 2017 |
| Blue Spring, Yellow River drainage, (31°5'27.64"N, 86°30'53.21"W), Alabama | AY899914 | Brant and Loker, 2005 |
| Pond off Saugahatchee Creek (Tallapoosa River) (32°39'1.36"N, 85°29'4.70"W), Alabama | MF568036 | Roberts et al., 2017 |
| Reelfoot Lake (Mississippi River) (36°21'12.23"N, 89°25'21.50"W), Tennessee | KX712242 | Roberts et al., 2017 |
| Pond off Odom Creek (Tallapoosa River), (32°30'9.58"N, 85°26'6.07"W), Alabama | AY604710 | Snyder, 2004 |
| Pond off Saugahatchee Creek (Tallapoosa River) (32°38'52.78"N; 85°29'7.20"W), Alabama | MF568030 | Roberts et al., 2017 |
| Coosa River (32°32'19.68"N; 86°12'22.69"W), Wetumpka, Alabama | AY604708 | Snyder, 2004 |
| Big Beaver Pond (Tallapoosa River) (32°25'44.03"N; 85°38'44.87"W), Alabama | KY499799 | Santoro et al., 2017 |
| Florida | KU892016 | Stacey et al., 2017 |
| Northern Adriatic coast, Italy | LT882715 | Marchiori et al., 2018 |
| Pacific Ocean, Hawaii | AY604707 | Snyder, 2004 |
| Ascension Parish, Sorrento Timberton Hunt Club, Louisiana | AY858885 | Brant et al., 2006 |
| Northern Adriatic coast, Italy | LT882716 | Marchiori et al., 2018 |
| Upper Amazon River Basin (3°45'32.08"S, 73°14'53.15"W), Belén Market, Iquitos, Peru | MK775719 | Present study |
| Upper Amazon River Basin (3°45'32.08"S, 73°14'53.15"W), Belén Market, Iquitos, Peru | MK775720 | Present study |
| Can Tho, Mekong River drainage (10°01'42.15"N, 105°47'14.15"E), Vietnam | MG579527 | Roberts et al., 2018a |
| Can Tho, Mekong River drainage (10°01'42.15"N, 105°47'14.15"E), Vietnam | MF579528 | Roberts et al., 2018a |
| Cape Maclear (Nankumba Peninsula), Lake Malawi-Chembe, Malawi | EU567126 | Stauffer et al., 2008 |
| Lesniki, Kyiv Region, Ukraine | AY604705 | Snyder, 2004 |
| Mae Sot, Moei River (16°42'N; 98°34'W), Thailand | FJ481166 | Tkach et al., 2009 |
| Reelfoot Lake (36°21'43.03"N; 89°25'44.32"W), Tennessee | AY604704 | Snyder, 2004 |
| Big Beaver Pond (Tallapoosa River) (32°25'44.03"N; 85°38'44.87"W), Alabama | KY091664 | Roberts et al., 2016b |
| Fremont, Iowa | FJ481164 | Tkach et al., 2009 |
| Pond off Saugahatchee Creek (Tallapoosa River) (32°38'52.78"N; 85°29'7.20"W), Alabama | KY091665 | Roberts et al., 2016b |
| Coosa River (32°32'19.68"N; 86°12'22.69"W), Wetumpka, Alabama | AY222174 | Olson et al., 2003 |
| Big Beaver Pond (Tallapoosa River) (32°25'44.03"N; 85°38'44.87"W), Alabama | KY091666 | Roberts et al., 2016b |
| Florida | FJ174469 | Brant and Loker, 2009 |
| Reelfoot Lake, Tennessee | AY604711 | Snyder, 2004 |
| Australia | MK878384 | Pinto et al., 2015 |
| Round Lake (Cahaba River) (32°41'50.91"N; 87°14'30.39"W), Cahaba River, Alabama | MH843490 | Roberts and Bullard, 2017 |
| Pampulha Reservoir, Belo Horizonte, state of Minas Gerais (19°51'77"S; 43°58'54"W), Brazil | KT235799 | Pinto et al., 2015 |
| Pampulha Reservoir, Belo Horizonte, state of Minas Gerais (19°51'77"S; 43°58'54"W), Brazil | KT235798 | Pinto et al., 2015 |
| Pampulha Reservoir, Belo Horizonte, state of Minas Gerais (19°51'77"S; 43°58'54"W), Brazil | KT235800 | Pinto et al., 2015 |
| Kazinga Channel, Jetty no. 2 (00°11'31"S; 29°53'53"E), Mwea Safari Lodge, Queen Elizabeth National Park, Uganda | AY858884 | Brant et al., 2006 |
| (35.05"N, 106.41"W) New Mexico | FJ550134 | Kraus et al., 2014 |
| Bosque del Apache, New Mexico | GU270101 | Pinto et al., 2015 |



Figures 6–17. Scanning electron microscopy (SEM) of adult specimens of *Atamatam amazoniensis* n. gen., n. sp. from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. Scale value aside bar. (6) Anterior body end showing anterior sucker (bracket), mouth (m), and putative sensory cilia that are more or less symmetrically distributed on ventral body surface (arrows). Ventral view. (7) Spines along anterodorsal portion of mouth; a selection of spines (arrows) on the ventrosinistral side of the mouth. Ventral view. (8) Anterior body end showing oral sucker and papillae (arrows). Dorsal view. (9) Putative sensory papilla from dorsal surface of oral sucker. Dorsal view. (10) Putative sensory cilium from ventral body surface. Ventral view. (11) Posterior end of body showing dorsal, sub-terminal excretory pore (arrow). Dorsal view. Figures 12–17.

Oral sucker 35–68 (55; 3) long or 3–4% (3%; 3) of body length, 35–55 (47; 3) wide or 16–18% (18%; 3) of maximum body width, goblet-shaped (Figs. 1, 6–8); oral sucker spines projecting from tegument approximately 1 (Fig. 7); dorsal surface of oral sucker papillate, having 2 papillae near posteroventral margin of oral sucker and 1 papilla near anterodorsal margin (Fig. 8); each papilla hemispherical with button-like projection at apex (Fig. 9). Nerve commissure 177–202 (186; 3) or 9–11% (10%; 3) of body length from anterior body end. Pharynx 20–25 (22; 3) or 4–5% (5%; 3) of esophagus length, 27–48 (40; 3) wide or 52–71% (64%; 3) of maximum esophagus width. Esophagus 413–518 (474; 3) long or 22–28% (25%; 3) of body length, 7–11 (9; 3) wide immediately posterior to pharynx and with wall 3 (2) thick, 30–45 (39; 3) wide or 8–13% (11%; 3) of body width at mid-esophagus and with wall 23–31 (27; 3) thick, 52–68 (61; 3) wide or 14–19% (17%; 3) of body width at cecal bifurcation and with wall 41–57 (51; 3) thick; esophageal gland 409–416 (413; 3) long or 20–23% (22%; 3) of body length, 175–186 (179; 3) wide or 48–50% (49%; 3) of body width. Intestine bifurcating 470–504 (490; 3) or 23–28% (26%; 3) of body length from anterior body end, extending posteriad approximately in parallel with lateral body margin, slightly sinuous, having numerous diverticula immediately posterior to cecal bifurcation and in posterior portion (Fig. 1); sinistral posterior cecum 1,120–1,450 (1,257; 3) long or 64–70% (66%; 3) of body length, 34–57 (48; 3) wide or 9–16% (13%; 3) of body width at level of cecal bifurcation, 27–57 (38; 3) wide or 8–16% (11%; 3) of body width at level of ovary, 34–52 (44; 3) wide or 14–25% (19%; 3) of body width at ends of ceca; dextral posterior cecum 1,210–1,510 (1,330; 3) long or 68–73% (70%; 10) of body length, 34–55 (43; 3) wide or 9–15% (12%; 3) of body width at level of cecal bifurcation, 30–34 (31; 3) wide or 9–10% (9%; 3) of body width at level of ovary, 32–50 (41; 3) wide or 14–24% (18%; 3) of body width at level of ends of ceca; ceca terminating 75–111 (87; 3) or 4–6% (5%; 3) of body length from posterior body end.

Anterior testis 236–322 (284; 3) long or 13–16% (15%; 3) of body length or 1.0–1.1× (1.1; 3) posterior testis length, 202–245 (227; 3) wide or 62–70% (67%; 3) of body width at level of ovary or 99–109% (105%; 3) of posterior testis width; inter-testicular space 202–261 (225; 3) long or 11–13% (12%; 3) of body length. Posterior testis 234–291 (267; 3) long or 13–15% (14%; 3) of body length, 186–247 (216; 3) wide or 57–71% (64%; 3) of body width at level of ovary, 214–261 (242; 3) or 12–14% (13%; 3) of body length from posterior body end. Vasa efferentia coalescing anteriorly and posteriorly and connecting to each testis, each appearing to connect directly to external seminal vesicle (vas deferens *sensu stricto* is extremely short, if present); anterior vas efferens emanating from ventromedial surface of anterior testis, extending posterodextrad 95–102 (99; 2), 14–34 (24; 2) wide; posterior vas efferens emanating from the ventral surface of the posterior testis, extending anterodextrad 216–257 (237; 2) or 12% (2) of body length, 7 (2) wide, lateral to or ventral to ovary (Fig. 5). External seminal vesicle transverse (crossing midline), directed sinistrad, 70–111 (88; 3) long or 4–5% (5%; 3) of body length,

116–159 (134; 3) wide, 1.4–1.8× (1.5; 3) wider than long, immediately posterior to anterior testis; internal seminal vesicle longitudinal, slightly sinuous, 89–139 (108; 3) long or 5–7% (6%; 3) of body length, 32–45 (37; 3) wide, 2.6–3.1× (2.9; 3) longer than wide. Cirrus sac ovoid, 118–127 (122; 3) long or 6–7% (6%; 3) of body length, 114–139 (123; 3) wide or 35–40% (36%; 3) body width at level of genital pore; cirrus extending posterolaterad 20–36 (30; 3) or 16–30% (25%; 3) of cirrus sac length, 25–39 (33; 3) wide.

Ovary having smooth borders, 84–139 (112; 3) long or 5–8% (6%; 3) of body length, 120–161 (141; 3) wide or 37–46% (42%; 3) of body width; post-ovarian space 477–573 (529; 3) or 27–29% (28%; 3) of body length. Oviduct 39–45 (42; 3) long or 2–3% (2%; 3) of body length, 11–20 (16; 3) wide proximally, laterally expanding to form oviducal seminal receptacle at midline, turning anteriad distal to oviducal seminal receptacle; oviducal seminal receptacle 59–95 (76; 3) long or 3–5% (4%; 3) of body length, 34–44 (38; 3) wide or 10–13% (11%; 3) of body width, between ovary and posterior testis. Laurer's canal extending from distal portion of seminal receptacle, extending anterosinistrad 32–44 (38; 3) and posterodextrad 68–81 (76; 3), 14–23 (18; 3) in maximum width, with pore at midline and dorsal to anterior margin of posterior testis. Vitellarium terminating 27–39 (33; 3) or 1–2% (2%; 3) of body length from posterior body end; lateral collecting ducts ventral to ceca, 27–57 (38; 4) wide, between ovary and posterior ceca to form transverse vitelline duct (Figs. 1, 5); transverse vitelline duct 172–216 (187; 3) in breadth 23–39 (30; 3) wide; primary vitelline collecting duct dorsal to transverse vitelline duct, extending anterosinistrad 44–88 (66; 3) before connecting with oviduct, 11–12 (11; 3) wide. Ootype not observed. Uterus 48–68 (61; 3) long or 3–4% (3%; 3) of body length, 23–25 (24; 3) wide, dorsal to transverse vitelline duct; metraterm 36–50 (41; 3) long or 2% (3) of body length, 14–23 (19; 3) wide or 4–7% (6%; 3) of body width; uterine egg not observed. Common genital pore 535–670 (605; 3) or 30–33% (32%; 3) of body length from posterior body end, 11–23 (17; 3) in diameter (Fig. 5).

Excretory pore dorsal, subterminal; excretory vesicle 9–11 (10; 3) wide or 4–5% (4%; 3) of body width at level of cecal termini; excretory pore 14–16 (15; 3) or 1% (3) of body length from posterior body margin (Figs. 1, 11).

Taxonomic summary

Type and only reported host: *Chelus fimbriata* (Schneider, 1783) (Pleurodira: Chelidae), matamata.

Type locality: Upper Amazon River Basin (Belén Market, Iquitos, Peru; 3°45'32.08"S, 73°14'53.15"W).

Specimens and sequences deposited: Holotype (USNM 1557310); paratypes (USNM 1557311; 1557312); 28S sequences (GenBank accession no. MK775718).

Site in host: Blood vessels of kidney and mesentery.

Prevalence and intensity: One of 5 (20%) matamatas was infected with 8 specimens of *A. amazoniensis*.

ZooBank registration: urn:lsid:zoobank.org:act:A77A9B13-A206-49D1-B46F-FAC42B4ED6D3.

← Mammillae and spines of dextral body margin. Ventral view. (12) Anterior portion of body near anterior sucker. (13) Mid-body. (14) Posterior body end near excretory pore. (15) Spinous mamilla in anterior portion of body near anterior sucker. (16) Spinous mammillae in anterior portion of body near anterior sucker showing 3 mammillae (*) and their spines as well as the dorsolateral putative sensory cilia (arrows). (17) Mamilla showing spines and putative sensory cilia.

Etymology: The specific epithet “amazoniensis” refers to the river basin of the type locality and emphasizes that this taxon is the first nominal TBF species described from the Amazon River Basin.

Paratamatam n. gen. Bullard and Roberts

(Figs. 18–35)

Body slightly cylindrical, extremely elongate (thread-like), 50–90 × longer than wide, with posterior end slightly more rounded than anterior end, having spinous lateral mammillae along body margin, lacking minute tegumental projections on body surface; mammillae large, hemispherical in profile, imparting strongly crenulate appearance to lateral body margin; mammillae spines spike-like, lacking recurved tip, distributing in rows wrapping dorsoventrally on each mammilla. Oral sucker spinous, papillate; oral sucker spines (exposed portion) triangular, distributing in dextral and sinistral fields on anteroventral surface of oral sucker. Ventral sucker absent. Pharynx present, enveloping anterior extremity of esophagus. Esophagus terminating in anterior one-eighth to one-sixth of body, ventral to anterior nerve commissure; lateral esophageal diverticula and median esophageal diverticulum absent; esophageal gland surrounding most of esophagus. Intestinal ceca inverse U-shaped, comprising non-fused posterior ceca bifurcating in anterior one-eighth to one sixth of body, lacking intestinal diverticula, extending three-fourths to four-fifths of body length posteriad, terminating far anterior to posterior body end, asymmetrical. Testes 2 in number, comprising an anterior testis and a post-ovarian testis, inter-cecal, occupying space far anterior and posterior to terminal genitalia, markedly elongate. Anterior and posterior trunks of vasa efferentia present, ventral to gonads, connecting to vas deferens; posterior vas efferens sinistral. Vas deferens comprising a compact external seminal vesicle; external seminal vesicle not abutting anterior testis, ovary, or cirrus sac, far anterior to cirrus sac, midway between cirrus sac and anterior testis. Cirrus sac pre-ovarian, longitudinal, directed posteriad, enclosing internal seminal vesicle and cirrus. Pars prostatica absent. Ovary sinistral, inter-cecal, inter-testicular, occupying space between testes (testes not closely flanking ovary), deeply lobed. Oviduct transverse, crossing midline before extending posteriad, convoluted proximally; oviducal seminal receptacle posterior to transverse vitelline duct. Laurer’s canal inter-cecal, inter-testicular, with dorsal pore. Vitellarium symmetrical, comprising a series of interconnected large spheroid masses of vitelline follicles, surrounding ceca, distributing from cecal bifurcation to ends of ceca; transverse vitelline duct inter-testicular, between ovary and posterior testis, ventral to gonads; oötype elongate, inter-testicular, inter-cecal. Uterus elongate, straight (lacking coils, loops, convolutions), inter-cecal, with distal end comprising metraterm, lacking uterine pouch; metraterm lacking strongly muscular wall. Common genital pore medial, pre-ovarian, ventral, inter-cecal, inter-testicular. Excretory vesicle Y-shaped; excretory pore dorsal, subterminal. Manter’s organ absent.

Differential diagnosis: Body slightly cylindrical, thread-like, 50–90 × longer than wide, having spinous lateral mammillae, lacking minute tegumental projections; mammillae large, hemispherical in profile; mammillae spines spike-like, distributing in rows wrapping dorsoventrally on each mammilla. Oral sucker spinous, papillate; oral sucker spines triangular, distributing in

dextral and sinistral fields on anteroventral surface of oral sucker. Ventral sucker, lateral esophageal diverticula, and median esophageal diverticulum absent. Intestinal ceca inverse U-shaped, lacking diverticula, terminating far anterior to posterior body end. Testes 2, including a post-ovarian testis, inter-cecal, occupying space far anterior and posterior to terminal genitalia. Posterior vas efferens sinistral. External seminal vesicle not abutting anterior testis, ovary, or cirrus sac, between cirrus sac and anterior testis. Cirrus sac pre-ovarian, longitudinal, directed posteriad. Ovary sinistral, occupying space between testes, deeply lobed. Oviduct transverse, crossing midline before extending posteriad, convoluted proximally; oviducal seminal receptacle posterior to transverse vitelline duct. Laurer’s canal inter-testicular. Vitellarium distributing from cecal bifurcation to ends of ceca; transverse vitelline duct between ovary and posterior testis. Oötype elongate, inter-testicular, inter-cecal. Common genital pore medial, pre-ovarian. Manter’s organ absent.

Taxonomic summary

Type and only known species: *Paratamatam iquitosiensis* n. sp.

ZooBank registration: urn:lsid:zoobank.org:act:42BDE6BA-FCDB-4731-8C4B-91CB0B3E1156.

Etymology: *Paratamatam* refers to the morphological similarity between this genus and *Atamatam* and to the fact that the same turtle species hosts the type species for both genera.

Paratamatam iquitosiensis n. sp. **Bullard and Roberts**

(Figs. 18–36)

Description of adult (based on 7 whole-mounted specimens): Body 10,770–16,100 (13,320; 4) long, 130–238 (181; 7) in maximum width at level of anterior testis, 55–87× (75; 4) longer than wide (Figs. 18, 19–23); mammillae numbering 328 and 330 (2) total, with equal number on each body margin or 164–165 (2) per side of body (stylized in Figs. 18–23), distributing from just anterior to nerve commissure to excretory vesicle; mammillae in anterior body region (anterior mammillae) 15–57 (36; 9) wide at base, with tegumental spines 2–4 (3; 27) long and protruding <1 from tegument (Figs. 24, 28, 30, 33); mammillae at level of ovary (middle mammillae) 50–91 (65; 9) wide at base, with tegumental spines 2–5 (4; 27) long and protruding <1 from tegument (Figs. 25, 31, 34); mammillae at level of tips of ceca (posterior mammillae) 23–68 (42; 9) wide, with tegumental spines 2–4 (3; 27) long and protruding <1 from tegument (Figs. 26, 32, 35).

Oral sucker 23–41 (30; 4) long or <1% (4) of body length, 20–45 (32; 4) wide or 15–35% (22%; 4) of maximum body width (Figs. 28, 29); oral sucker spines approximately 0.7 wide, projecting from tegument approximately 1.0–1.5 (Fig. 29); tegument immediately adjacent to mouth papillate (Fig. 29); each oral papilla hemispherical with nipple- or spike-like projection at apex (Fig. 29). Nerve commissure 241–295 (269; 4) or 2% (4) of body length from anterior body end (Figs. 18, 19). Pharynx 14–27 (21; 4) or 1% (4) of esophagus length, 34–45 (39; 4) wide or 37–53% (46%; 4) of maximum esophagus width. Esophagus 1,815–2,000 (1,949; 4) long or 12–17% (15%; 4) of body length, 7–11 (10; 4) wide immediately posterior to pharynx and with wall 5–9 (7; 4) thick, 30–50 (41; 5) wide or 21–29% (24%; 5) of body width at mid-esophagus and with wall 19–32 (26; 5) thick, 77–98 (85; 5) wide or 44–66% (52%; 5) of body width at cecal bifurcation and with wall 66–87 (73; 5) thick; esophageal gland

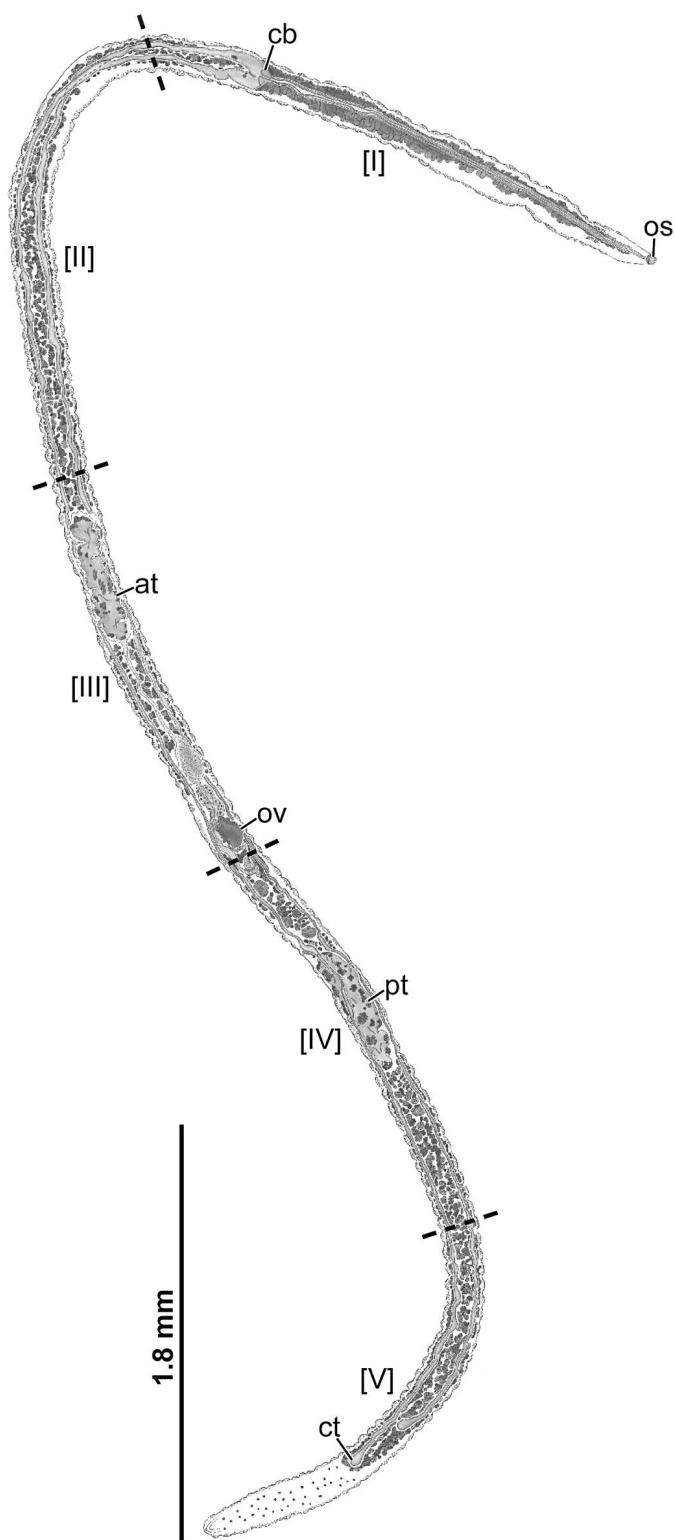
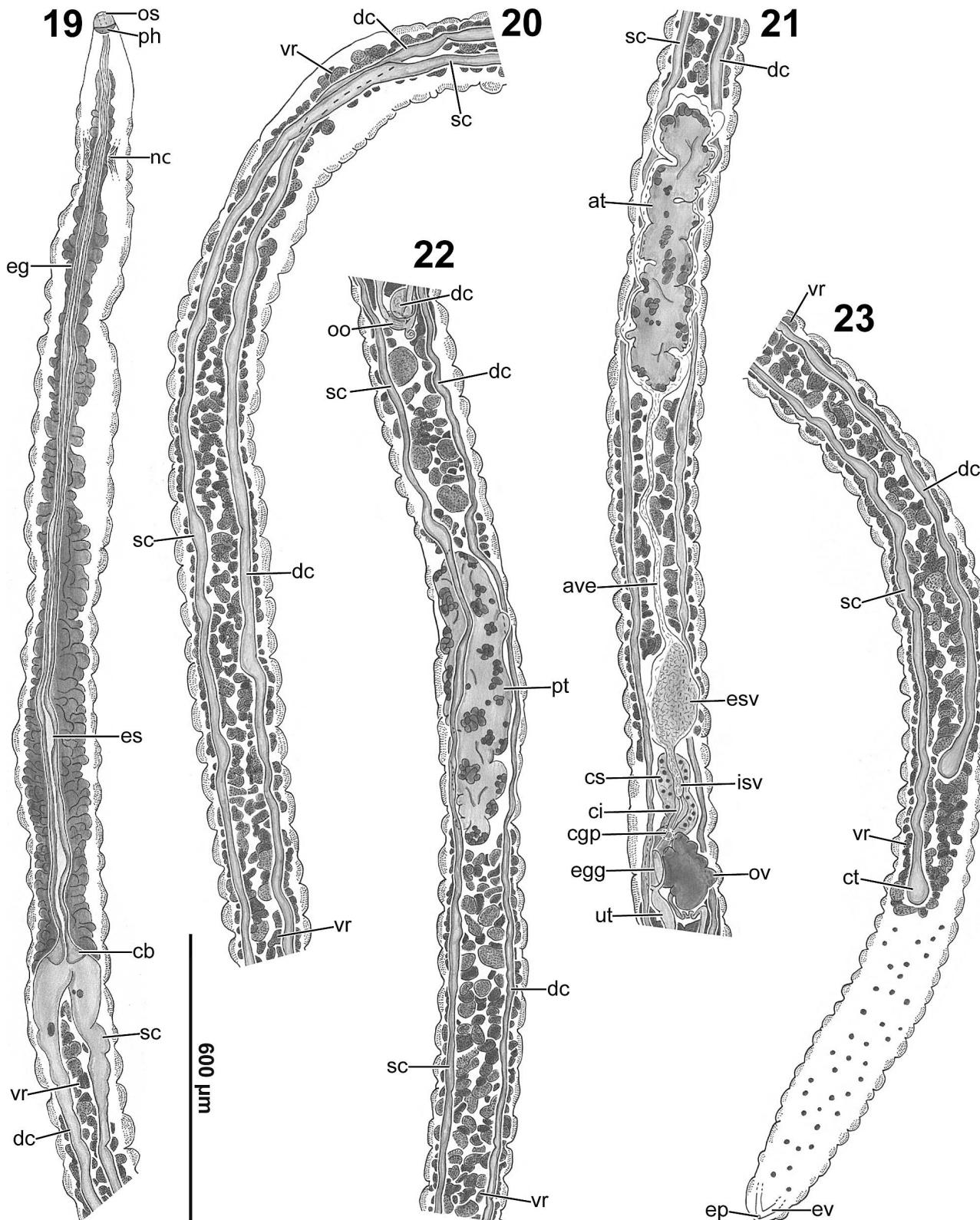


Figure 18. *Paratamatam iquitosiensis* n. gen., n. sp. (holotype, USNM 1557313) from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. Scale value aside bars. Oral sucker (os), cecal bifurcation (cb), anterior testis (at), ovary (ov), posterior testis (pt), and cecal terminus (ct). Roman numerals and dashed lines indicate body segments that are illustrated at higher magnification (see Figs. 19–23). Principally dorsal view; anterior portion (I) is ventral view.

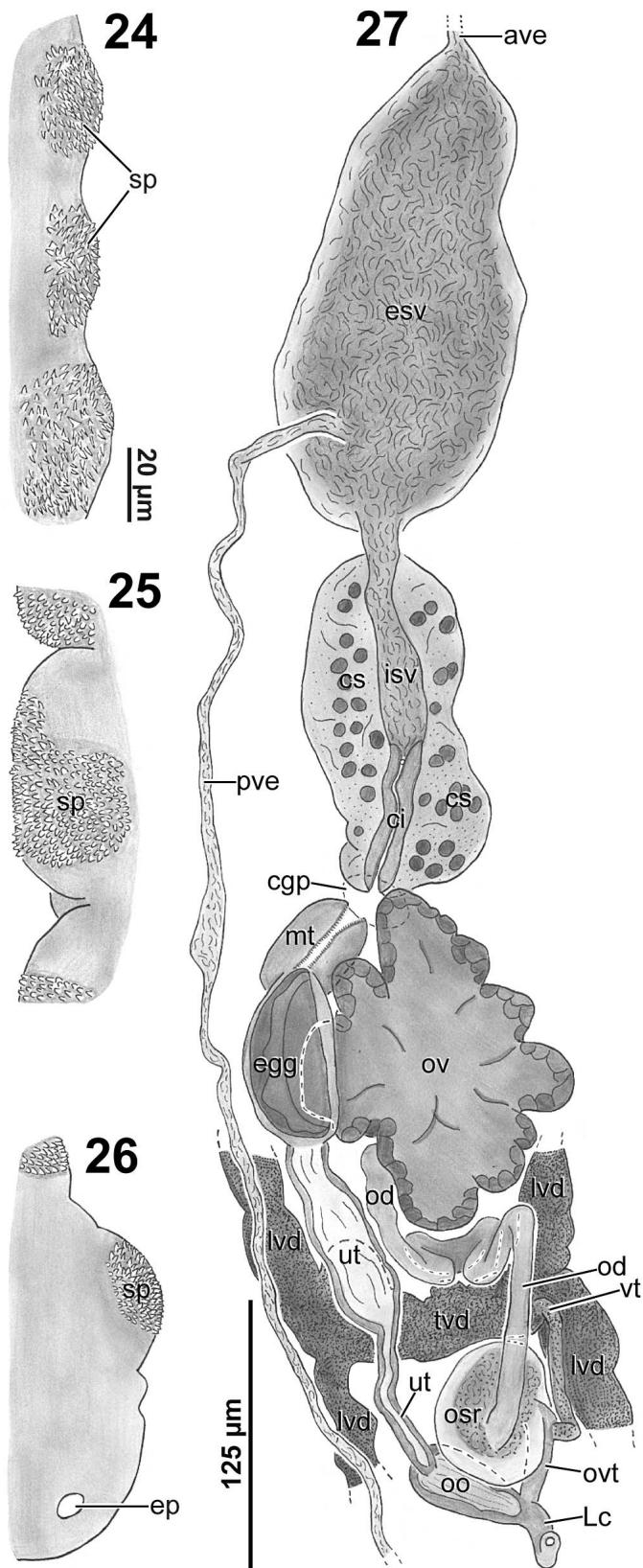
1,670–1,910 (1,813; 4) long or 11–16% (14%; 4) of body length, 102–148 (135; 5) wide or 73–88% (81%; 5) of body width. Intestine bifurcating 1,820–2,005 (1,954; 4) or 13–17% (15%; 4) of body length from anterior body end, each cecum extending sinuously posteriad and tightly apposed to lateral body margin (Figs. 18, 19); sinistral posterior cecum 6,500–13,385 (9,661; 5) long or 75–83% (78%; 4) of body length, 66–91 (75; 5) wide or 39–59% (46%; 5) of body width at level of cecal bifurcation, 9–23 (17; 7) wide or 5–18% (10%; 7) of body width at level of ovary, 30–50 (39; 7) wide or 19–47% (29%; 7) of body width at ends of ceca; dextral posterior cecum 6,730–13,165 (9,777; 5) long or 77–82% (79%; 4) of body length, 45–95 (76; 5) wide or 26–69% (47%; 5) of body width at level of cecal bifurcation, 14–23 (18; 7) wide or 8–11% (10%; 7) of body width at level of ovary, 23–64 (40; 7) wide or 15–45% (30%; 7) of body width at ends of ceca; ceca terminating 390–1,000 (730; 7) or 4–7% (5%; 4) of body length from posterior body end (Fig. 23).

Anterior testis 472–726 (621; 7) long or 4–6% (5%; 4) of body length or 0.9–1.1× (1.0; 7) posterior testis length, 86–175 (134; 7) wide or 64–88% (74%; 7) of body width or 86–107% (96%; 7) of posterior testis width; inter-testicular space 1,320–2,360 (1,859; 7) or 10–18% (15%; 4) of body length (Figs. 18, 19–23, 27). Posterior testis 534–720 (616; 7) long or 4–6% (5%; 4) of body length, 98–164 (138; 7) wide or 69–84% (77%; 7) of body width, 2,350–4,380 (3,013; 7) or 18–32% (24%; 4) of body length from posterior body end (Figs. 18, 27). Vasa efferentia coalescing anteriorly and posteriorly to form a single trunk connecting each testis, each connecting to external seminal vesicle; anterior vas efferens emanating from posterior margin of anterior testis, extending posteriad 522–790 (637; 7), 9–20 (12; 7) wide; posterior vas efferens emanating from anterior margin of posterior testis, extending anterosinistrad and dorsal to sinistral cecum, extending anteriad between sinistral cecum and sinistral body margin for 851–1,000 (918; 3) or 6% (1) of body length before joining external seminal vesicle, 5–11 (8; 3) wide (Fig. 27). External seminal vesicle longitudinal (not crossing midline), 200–395 (273; 7) long or 1–3% (2%; 4) of body length, 75–109 (95; 7) wide or 39–63% (54%; 7) of body width, 1.8–5.3× (3.0; 7) longer than wide; internal seminal vesicle 95–191 (147; 7) long or 1% (4) of body length, 18–45 (27; 7) wide or 3.1–7.8× (5.8; 7) longer than wide. Cirrus sac ovoid, 170–216 (190; 7) long or 1–2% (1%; 4) of body length, 52–82 (67; 7) wide or 35–60% (42%; 7) body width at level of genital pore; cirrus extending posteriad 55–70 (62; 5) or 25–40% (33%; 5) of cirrus sac, 15–30 (23; 7) wide (Figs. 21, 27).

Ovary having lobes or smooth borders, 125–218 (175; 7) long or 1–2% (1%; 4) of body length or 1.1–2.6× (1.7; 7) longer than wide, 80–145 (107; 7) wide or 52–87% (67%; 7) of body width; post-ovarian space 3,470–6,010 (4,287; 7) or 25–44% (35%; 4) of body length (Fig. 27). Oviduct extending posteromediad from ovary 34–64 (48; 5), 11–18 (15; 5) wide proximally, turning ventrally to form a loop before extending posteriad 93–209 (148; 5) and laterally expanding to form oviducal seminal receptacle; oviducal seminal receptacle 48–70 (58; 7) long or 25–42% (34%; 7) of body length, 32–57 (47; 7) wide or 18–39% (30%; 7) of body width, immediately posterior to transverse vitelline duct. Laurer's canal immediately posterior to level of ootype and oviducal seminal receptacle, extending posteriad 23–73 (48; 7) or 14–58% (29%; 7) of ovary length, 9–18 (14; 7) wide, with dorsal pore between oviducal seminal receptacle and posterior testis (Fig. 27). Vitellarium terminating 380–975 (731; 7) or 4–7% (5%; 4) of



Figures 19–23. *Paratamatam iquitosiensis* n. gen., n. sp. (holotype, USNM 1557313) from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. Scale value aside bar, all figures drawn to same scale. (19) Anterior body segment (I) showing oral sucker (os), pharynx (ph), nerve commissure (nc), esophageal gland (eg), esophagus (es), cecal bifurcation (cb), sinistral cecum (sc), vitellarium (vr), dextral cecum (dc). Ventral view. (20) Body segment (II). Principally dorsal view. (21) Body segment (III) showing anterior testis (at), anterior trunk of vasa efferentia (ave), external seminal vesicle (esv), cirrus sac (cs), internal seminal vesicle (isv), cirrus (ci), common genital pore (cg), *in utero* egg (egg), ovary (ov), and uterus (ut). Dorsal view. (22) Body segment (IV) showing oötype (oo), and posterior testis (pt). Dorsal view. (23) Posterior body segment (V) showing cecal terminus (ct), excretory vesicle (ev), and excretory pore (ep). Dorsal view.



Figures 24–27. *Paramatam iquitosiensis* n. gen., n. sp. (holotype USNM 1557313) from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. Scale value aside bars. Figures 24–26 drawn at same scale. (24) Anterior ventrolateral tegumental

body length from posterior body end; lateral collecting ducts ventral to ceca, 18–43 (29; 4) wide, between ovary and seminal receptacle to form transverse vitelline duct (Fig. 27); transverse vitelline duct 1,010–1,600 (1,263; 7) in breadth, 27–68 (48; 7) wide; primary vitelline collecting duct dorsal to transverse vitelline duct, extending posteriad 57–82 (69; 5) before connecting with oviduct, 9–11 (10; 5) wide. Ovo-vitelline duct extending posteriad 41–80 (57; 7), 9–23 (15; 7) wide. Oötype a clearly delineated oblong chamber, posterior to or ventral to oviducal seminal receptacle, extending anterosinistrad, 57–102 (70; 7) long, 14–25 (20; 7) wide or 9–20% (12%; 7) of body width (Fig. 27). Uterus 170–319 (235; 7) long or 2% (4) of body length, 16–45 (25; 7) wide, dorsal to transverse vitelline duct; metraterm short, comprising distal portion of uterus, sinistral and dorsal to ovary, 57–93 (75; 7) long, 23–45 (35; 7) wide or 13–26% (19%; 7) of body width; egg 82 long by 34 wide or 2.4× longer than wide. Common genital pore nearly pre-ovarian, at level of anterior margin of ovary, 3,585–6,220 (4,437; 7) or 26–46% (36%; 4) of body length from posterior body end, 25–45 (35; 7) in diameter (Fig. 27).

Excretory vesicle 7–14 (10; 7) wide or 6–10% (7%; 7) of body width at level of cecal termini; excretory pore 9 and 14 (2) or <1% (2) of body length from posterior body margin (Fig. 26).

Taxonomic summary

Type and only reported host: *Chelus fimbriata* (Schneider, 1783) (Pleurodira: Chelidae), matamata.

Type locality: Upper Amazon River Basin (Belen Market, Iquitos, Peru; 3°45'32.08"S, 73°14'53.15"W).

Specimens and sequences deposited: Holotype (USNM 1557313); paratypes (USNM 1557314–1557319); 28S sequence (GenBank accession nos. MK775719, MK775720).

Site in host: Blood vessels of kidney and mesentery.

Prevalence and intensity: One of 5 (20%) matamata was infected with 33 specimens of the new species, *P. iquitosiensis*.

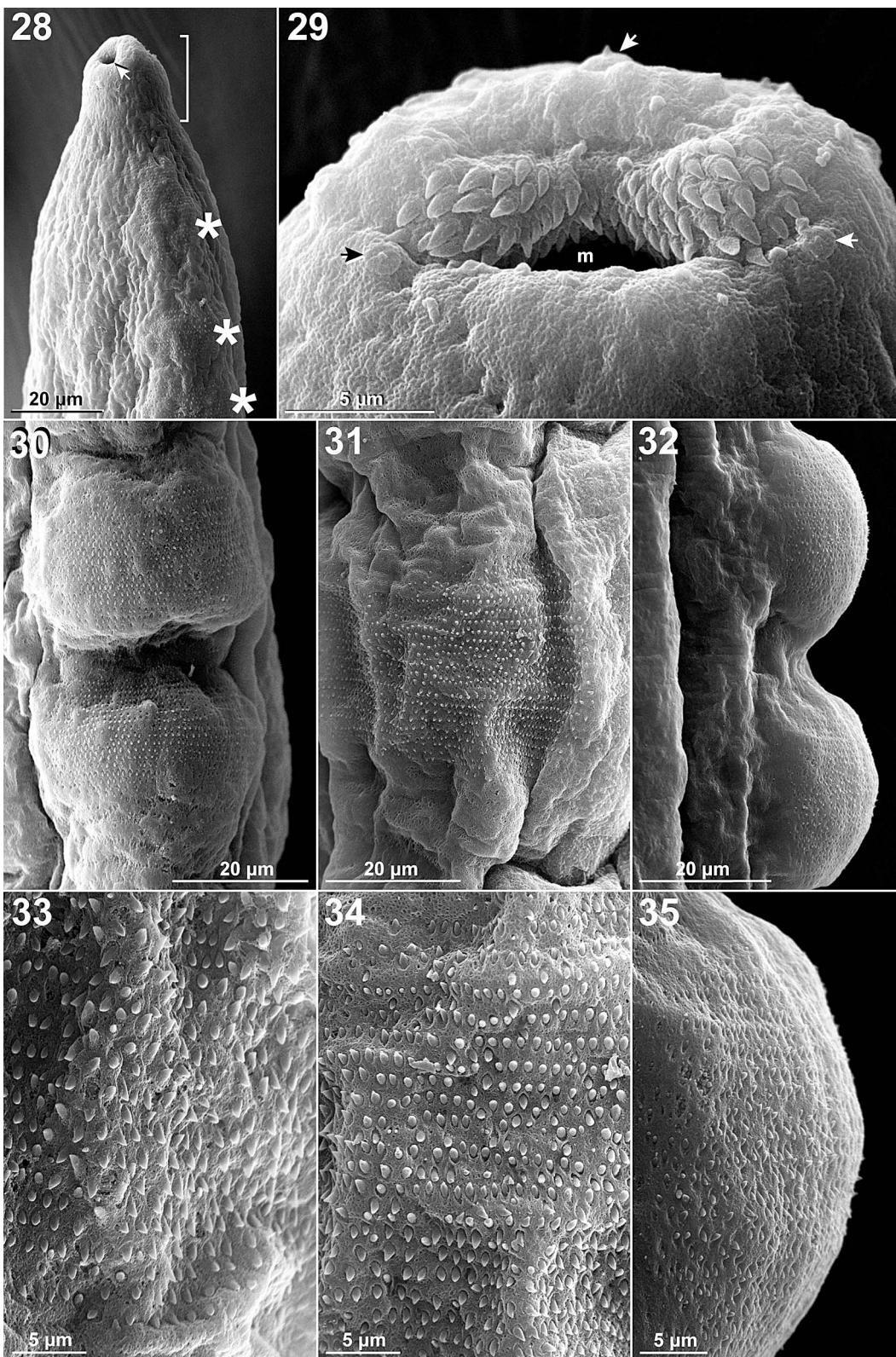
ZooBank registration: urn:lsid:zoobank.org:act:6808F31C-9180-45D0-9BF9-C2146EC14645.

Etymology: The specific epithet “*iquitosiensis*” refers to the type locality, Iquitos, Peru.

Phylogenetic results

The amplified 28S fragments representing the new genera comprised 1,607 and 1,558 nucleotides for *A. amazoniensis* (GenBank accession no. MK775718) and *P. iquitosiensis* (GenBank accession nos. MK775719, MK775720), respectively. That of *A. amazoniensis* differed from that of *P. iquitosiensis* by 21 nucleotides (3.2%) and from those of TBF sp. W702 and TBF sp.

mammillae on sinistral body margin showing mammillae spines (sp). Ventral view. (25) Midbody ventrolateral tegumental mammillae. Ventral view. (26) Posterior ventrolateral tegumental mammillae showing dorsal, sub-terminal excretory pore (ep). Dorsal view. (27) Genitalia showing anterior vas efferens (ave), external seminal vesicle (esv), internal seminal vesicle (isv), cirrus sac (cs), posterior vas efferens (pve), cirrus (ci), common genital pore (cgp), metraterm (mt), in utero egg (egg), ovary (ov), oviduct (od), lateral vitelline collecting ducts (lvd), uterus (ut), transverse vitelline duct (tvd), primary vitelline collecting duct (vt), oviducal seminal receptacle (osr), ovo-vitelline duct (ovt), Laurer's canal (Lc), and oötype (oo). Dorsal view.



Figures 28–35. Scanning electron microscopy (SEM) of adult specimens of *Paratamatam iquitosensis* n. gen., n. sp. from matamata, *Chelus fimbriata* (Pleurodira: Chelidae) from the Amazon River in Iquitos, Peru. Scale value aside bar. (28) Anterior body end showing anterior sucker (bracket), mouth (arrow), and spinous mammillae (*). Ventrolateral view. (29) Spines along anteroventral portion of mouth (m) and probable sensory papillae (arrows). Ventral view. Figures 30–35. Spinous mammillae and spines of dextral body margin. (30) Anterior portion of body near anterior sucker. Lateral view. (31) Mid-body. Lateral view. (32) Posterior portion of body. Ventrolateral view. (33) Spines from Figure 30. (34) Spines from Figure 31. (35) Spines from Figure 32.

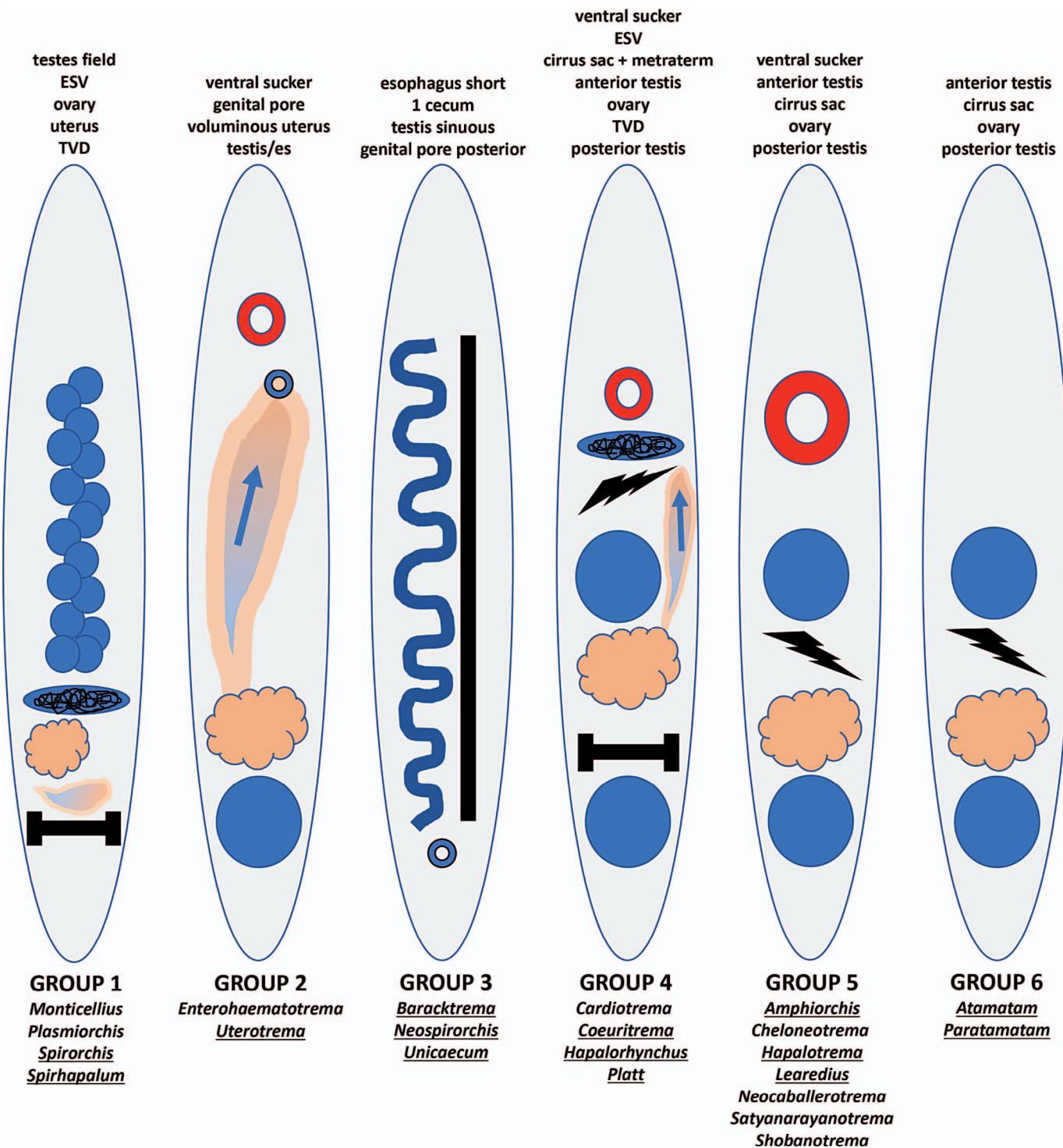


Figure 36. Diagrammatic representation of morphological features of turtle blood fluke genera that are elaborated on in the text. Red circle (ventral sucker), lightning bolt (cirrus sac), club with arrow (uterus; arrow points distally), cream cloud (ovary), blue circle (testis), blue oval with black lines (external seminal vesicle), straight black line with bar ends (transverse vitelline duct), blue ring (genital pore), wavy blue line (testis), straight black line (cecum). The sequence of anatomical features from anterior (top of figure) to posterior are indicated above each diagram, and the genera having that phenotype are listed below. Underlined taxa comprise those that are represented in the phylogenetic analysis (Fig. 37). Abbreviations: ESV, external seminal vesicle; TVD, transverse vitelline duct; es, esophagus. Color version available online.

811 by 23 (3.5%) and 24 (3.7%) nucleotides, respectively. The 28S sequences from 2 specimens of *P. iquitosiensis* were identical (100% similar) to each other and differed from TBF sp. 702 and TBF sp. 811 by 22 (3.4%) and 23 (3.6%) nucleotides, respectively.

The phylogenetic analyses (Maximum Likelihood using IQ-TREE [Fig. 37] and Bayesian Analysis using BEAST [Fig. 38]) produced concordant tree topologies with exception to the placement of *Coeuritrema* Mehra, 1933. The topology of the clade that includes the new taxa and related marine turtle blood flukes was identical in both analyses. In addition, the position of the marine TBF *Neospiorchis* sp. (sister to *Baracktrema obamai* Roberts, Platt, and Bullard, 2016 and within a clade including *Unicaecum* sp. and TBF sp. W-810) was recovered by both analyses (BS \geq 88; PP \geq 0.84). Regarding *Coeuritrema*, the Maximum Likelihood analysis (Fig. 37) recovered it as sister to Platt, Roberts, and Bullard, 2018 but within a clade including *Hapalorhynchus* Stunkard, 1922, whereas the Bayesian analysis (Fig. 38) recovered it as sister to *Hapalorhynchus*. Nodal support for the monophyly of the schistosomes plus all marine TBFs (except *Neospiorchis* sp.) plus the new taxa described herein was high (1.0 PP and 100% BS) in both analyses. Specifics of the tree topology are treated below (see Remarks) aside the relevant morphological comparisons.

The ancestral state reconstruction indicated that the most recent common ancestor of the newly described species plus TBF sp. W811 and TBF sp. W702 had a freshwater definitive host (Fig. 38). The clade comprising these species is nested within a clade of species that mature in marine turtles, indicating a marine to freshwater transition. Such a transition was also recovered among other TBFs (the marine TBF *Neospiorchis* sp. nested within a clade of freshwater TBFs) and Schistosomes (*Macrobilharzia macrobilharzia*) (Fig. 38).

Remarks

The 2 new genera proposed herein bring the total number of accepted TBF genera to 24 (Fig. 36). To morphologically compare the new genera with the other TBF genera, and in lieu of extant type materials and more complete diagnoses and descriptions for several related taxa (many from the Indian subcontinent), we use attributes related to the ventral sucker, esophagus, intestine, gonads, cirrus sac, vitelline duct, and genital pore (Fig. 36). Because some TBF genera include species that need re-description or re-assignment to other genera, we base our comparisons on the type species of each genus principally. Previous work from Roberts and Bullard have treated revisions of several TBF genera (Roberts et al., 2016a, 2016b, 2016c, 2017, 2018a, 2018b, 2019; Roberts and Bullard, 2017), but, where appropriate, we indicate exceptions to generic diagnoses that suggest needed revisionary work.

Based on these comparisons, we identify 6 morphological groups (Groups 1–6; Fig. 36) that are useful in diagnosing TBF genera as well as provide a dichotomous key to all accepted TBF genera. Below we morphologically diagnose each group, report the number of accepted species, list the type species, indicate the freshwater or marine affinity of the definitive host, and discuss their phylogenetic placement in light of an ancestral state reconstruction (Figs. 37, 38).

Group 1: *Monticellius* Mehra, 1939 (monotypic, *Monticellius indicum* Mehra, 1939), *Plasmiorchis* Mehra, 1934 (5 species; type

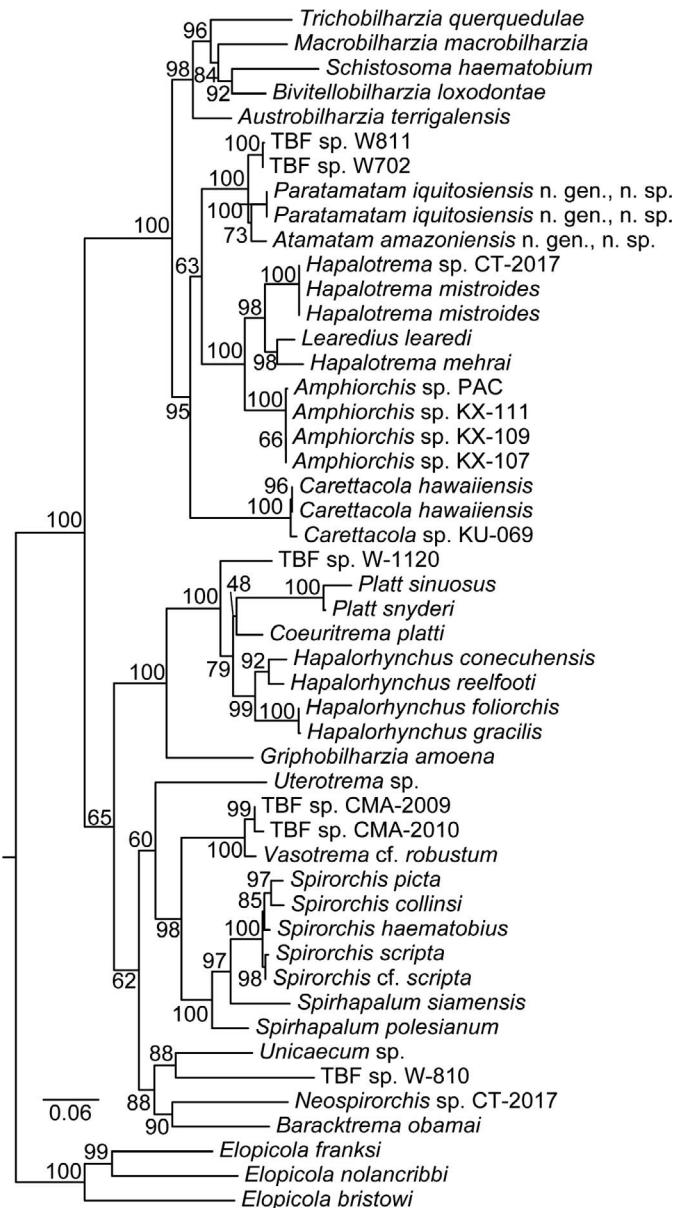


Figure 37. Maximum likelihood phylogeny inferred with IQ-TREE. Labels in front of nodes are ultrafast bootstrap support values. Scale bar in substitutions per site.

species *Plasmiorchis orientalis* Mehra, 1934), *Spirorchis* MacCallum, 1918 (12 species; type species *Spirorchis innominatus* Ward, 1921; see Platt [1993]; Roberts et al. [2018b, 2019]), and *Spirhapalum* Ejsmont, 1927 (3 species; type species *Spirhapalum polesianum* Ejsmont, 1927) (Fig. 36). These genera have the anatomical sequence (anterior to posterior) of an inter-cecal testicular column/field (*Spirhapalum* has an additional testis posterior to the transverse vitelline duct), an external seminal vesicle (lateral to ovary in *P. orientalis*), ovary, uterus, and transverse vitelline duct (Fig. 36). In addition to this combination of features, these genera have an aspinous body (*Monticellius* is reportedly spined), an aspinous ventral sucker (*Spirorchis* lacks a ventral sucker; *Monticellius* reportedly has ventral sucker spines), lateral esophageal diverticulae (“plicate organ”; indeterminate for

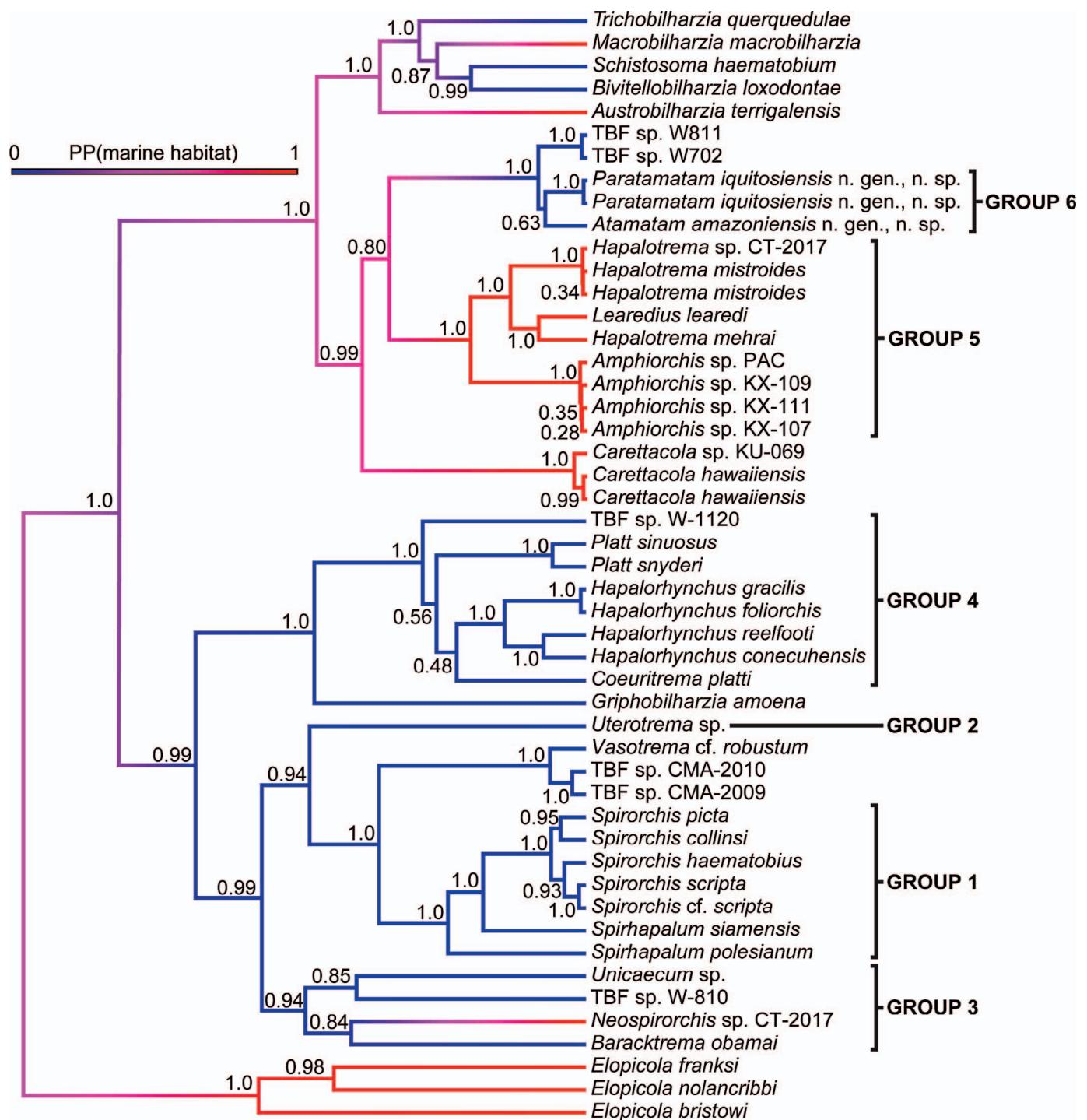


Figure 38. Bayesian phylogeny inferred with BEAST. Labels in front of nodes are PP support values. Colors represent PP of a marine host character state inferred through stochastic mapping. Redder shades represent higher PPs. Morphological groups indicated aside clades. Color version available online.

Monticellius), a medial esophageal diverticulum (indeterminate for *Monticellius*), ceca that terminate in the extreme posterior end of the body (overlapping with the excretory vesicle), >2 testes that are ovoid (no testis is coiled nor elongated), a longitudinal cirrus directed posteriad (laterad in *Spirorchis*), a post-ovarian Lauer's canal, an oviducal seminal receptacle, a straight uterus (lacking convolutions), and a Y-shaped excretory bladder. *Spirhalpalum* is unique among these genera by having a testis posterior to the transverse vitelline duct (Ejsmont, 1927; Tkach et al., 2009). *Spirhalpalum*, *Spirorchis*, and *Plasmiorchis* are unique by having M-shaped ceca and a Manter's organ, which is evidently absent in *Monticellius*. *Spirhalpalum* and *Plasmiorchis* are differentiated by having a transverse vitelline duct that is inter-testicular and post-testicular, respectively. With exception to monotypic *Monticellius*, these genera include species that infect freshwater turtles only.

The 28S trees (Figs. 37, 38), like others published previously (e.g., Pinto et al., 2015; Roberts et al., 2016c), each hinted at a monophyletic Spirorchinae Stunkard, 1921 (sensu Platt [1992]) because they recovered paraphyletic *Spirhalpalum* and monophyletic *Spirorchis* in the same clade (no 28S sequence data are available for *Monticellius* nor *Plasmiorchis*). Platt (1992) hypothesized that *Spirhalpalum* was a stem lineage (pleiomorphic member) of the Spirorchinae; however, the recovered 28S phylogeny and that of Tkach et al. (2009) indicate that *Spirhalpalum* is paraphyletic with the *Spirorchis* crown group (Fig. 37), which together comprise a clade that is sister to *V. cf. robustum*. While no 28S sequence exists for monotypic *Monticellius* (infecting a marine turtle), its morphological similarity to TBFs of freshwater turtles hints at a freshwater to marine transition.

Group 2: *Enterohaematotrema* Mehra, 1940 (2 species; type species *Enterohaematotrema palaeorticum* Mehra, 1940) and *Uterotrema* Platt and Pichelin, 1994 (3 species; type species *Uterotrema australispinosa* Platt and Pichelin, 1994) (Fig. 36). These morphologically bizarre species are unique in having a massive, inter-cecal uterus/metraterm that is longitudinal (parallel with long axis of body) and located between the ventral sucker and testis(es) (Fig. 36). Mehra (1940) referred to the distal portion of the female reproductive tract as a metraterm, whereas Platt and Pichelin (1994) and Platt and Blair (1996) referred to it as a uterus. These genera also have a common genital pore that is far anterior in the body (immediately posterior to the ventral sucker, far pre-testicular). *Enterohaematotrema* is aspinous and has 2 testes and an external seminal vesicle immediately anterior to the anterior testis. *Uterotrema* has a spinous ventral sucker, rows of lateral tegumental spines in the hindbody (posterior to ventral sucker), a single inter-cecal testis, and an external seminal vesicle between the ventral sucker and genital pore. Both genera include species that infect phylogenetically unrelated freshwater turtles of the Indian subcontinent and Australia, respectively. The 3 freshwater Australian TBFs comprise *Uterotrema* (no congener is known from elsewhere) and infect 2 endemic chelids (Pleurodira). *Enterohaematotrema* comprises TBFs that infect a geomydid and 2 trionychids (Cryptodira) on the Indian subcontinent only. Related to the present study and aside from the new taxa described herein from *C. fimriatus* (Chelidae), the 3 species of *Uterotrema* comprise the only other nominal TBFs to infect a chelid, which is among the most species-rich turtle families. A morphological comparison between *Uterotrema* and the new genera is below.

Pinto et al. (2015) presented a 28S phylogeny that recovered *Uterotrema* sp. sister to *Vasotrema* + Spirorchinae. Our results largely corroborated this finding (Figs. 37, 38). To date, no molecular sequence data exist for *Enterohaematotrema*, but, given the aforementioned morphological similarities between these genera, we predict that it will be sister to *Uterotrema*. Including a species of *Enterohaematotrema* and additional species of *Uterotrema* will likely be necessary to fully resolve the placement of this lineage.

Group 3: *Baracktrema* Roberts, Platt, and Bullard, 2016 (monotypic, *Baracktrema obamai* Roberts, Platt, and Bullard, 2016 [see Roberts et al., 2016a]), *Neospiroorchis* Price, 1934 (2 species, type species *Neospiroorchis schistosomatoides* Price, 1934), and *Unicaecum* Stunkard, 1925 (2 species; type species *Unicaecum ruszkowskii* Stunkard, 1925) (Fig. 36). These genera are distinctive in lacking the paired ceca typical of most blood flukes. *Unicaecum* and *Baracktrema* have a single cecum, whereas *Neospiroorchis* has a cyclocoel (ceca that are fused posteriorly). *Baracktrema* has terminal genitalia that are post-cecal, whereas *Unicaecum* has terminal genitalia that are anterior to the tips of the ceca. In addition, these genera have an abbreviated esophagus as well as a sinuous testis that is lateral to the intestine (Fig. 36). *Neospiroorchis* spp. infect marine turtles, and species of *Baracktrema* and *Unicaecum* infect freshwater turtles only.

Both 28S analyses (Figs. 37, 38) recovered *Unicaecum* sister to the clade that includes *Baracktrema*, *Neospiroorchis*, and a cercarial sequence (W810, from *Pomacea* sp. in Brazil [Pinto et al., 2015]), having long branches suggesting a long separated lineage from the other TBFs. The recovered relationships correspond to the assignment of *Unicaecum* and *Neospiroorchis* to their own subfamilies as *Unicaecuminae* Yamaguti, 1971 and *Neospiorchinae* Yamaguti, 1971, respectively (Platt, 1992). If one accepts those subfamilies, monotypic *Baracktrema* should be assigned to its own subfamily or should be combined with *Unicaecum* and *Neospiroorchis* as a single subfamily. Each of these genera is starkly unique from other TBFs based on the anatomy of their gut. This result is interesting because it demonstrates that the marine TBFs are both paraphyletic but also rather distantly related (i.e., species of *Neospiroorchis* have a freshwater turtle TBF ancestor).

Group 4: *Cardiotrema* Dwivedi, 1967 (2 species; type species *Cardiotrema vaidya* Dwivedi, 1967), *Coeuritrema* (3 species; type species *Coeuritrema lyssimus* Mehra, 1933 [see Roberts et al., 2016b]), *Hapalorhynchus* (13 species; type species *Hapalorhynchus gracilis* Stunkard, 1922; see Roberts et al. [2017]), and *Platt* Roberts and Bullard, 2018 (8 species; type species *Platt sinuosus* Roberts and Bullard, 2018; see Roberts et al. [2018a]). These genera have the anatomical sequence (anterior to posterior) of a ventral sucker, external seminal vesicle, cirrus sac (lateral to the metraterm), anterior testis, ovary, transverse vitelline duct (inter-testicular), and posterior testis (Fig. 36). In addition to this combination of features, these genera also have an inflated esophagus, U-shaped ceca, 2 testes (which are ovoid), a pars prostatica, a transverse cirrus sac (crosses midline, directed laterad), a sinistral, dorsal common genital pore, an inter-testicular ovary and Lauer's canal, an oviducal seminal receptacle (ambiguous in *Cardiotrema*), a straight uterus directed anteriad only, a strongly muscular metraterm, pre-gonadal terminal genitalia, and a massive, globular excretory vesicle (Manter's organ absent). This clade is likely representative of the freshwater

“*Hapalotrematinae* Yamaguti, 1971” of Platt (1992), including *Hapalorhynchus*, *Cardiotrema*, *Coeuritrema*, and *Enterohaemato-trema* Mehra, 1940 while excluding *Hapalotrema* (see below). Like *Hapalotrema*, *Enterohaemato-trema* stands out as a morphologically distinctive lineage that is separate from the other genera that Yamaguti (1971) assigned to the “*Hapalotrematinae*. Together these examples add further support for rejecting this subfamily. All of these genera comprise species that infect freshwater turtles only. *Platt* differs from *Hapalorhynchus* by having a papillate ventral sucker and a massive cirrus sac that is directed anteriad or laterad, whereas *Hapalorhynchus* has an apapillate ventral sucker and a diminutive cirrus sac, if present (Roberts et al., 2018a). *Platt* differs from *Coeuritrema* by lacking ventrolateral tegumental mammillae and by having a diminutive metraterm that is not readily distinguished from the distal portion of the uterus. *Coeuritrema* has ventrolateral mammillae and a massive metraterm. *Platt* can be readily differentiated from *Cardiotrema* by having a large ventral sucker (one-fourth to one-half body width) that is immediately posterior to the cecal bifurcation, whereas *Cardiotrema* has a rudimentary ventral sucker far posterior to the cecal bifurcation (Roberts et al., 2018a).

Concordant with the morphological similarities observed between these genera, the resulting 28S analyses recovered a clade comprising *Coeuritrema*, *Platt*, and *Hapalorhynchus* (Figs. 37, 38). The position of *Coeuritrema* differs between analyses and is sister to either *Platt* (Fig. 37) or *Hapalorhynchus* (Fig. 38). These results also strongly suggest that the cercarial sequence (W-1120, from *Biomphalaria sudanica* in Uganda [Brant et al., 2006]), which is sister to the clade including *Coeuritrema*, *Platt*, and *Hapalorhynchus*, likely represents a TBF species that should be assigned to a new genus and that would belong to this morphological group (Group 4).

Group 5: *Amphiorchis* Price, 1934 (7 species; type species *Amphiorchis amphiorchis* Price, 1934), *Cheloneotrema* Simha and Chattopadhyaya, 1980 (monotypic, *Cheloneotrema testicaudata* Simha and Chattopadhyaya, 1980), *Hapalotrema* Price, 1934 (7 species; type species *Hapalotrema loossi* Price, 1934), *Learedius* Price, 1934 (5 species; *Learedius learedi* Price, 1934), *Neocaballerotrema* Simha, 1977 (monotypic, *Neocaballerotrema caballeroi* Simha, 1977), *Satyantarayano-trema* Simha and Chattopadhyaya, 1980 (monotypic, *Satyantarayano-trema satyanarayani* Simha and Chattopadhyaya, 1980), and *Shobanotrema* Simha and Chattopadhyaya, 1980 (monotypic, *Shobanotrema shobanae* Simha and Chattopadhyaya, 1980). These genera are unique in having the anatomical sequence (anterior to posterior) of a ventral sucker, anterior testis(es), cirrus sac, ovary, and posterior testis(es) (Fig. 36). In addition to this combination of features, most have the combination of U-shaped ceca (*Learedius* has M-shaped ceca), ovoid, inter-cecal testes, external and internal seminal vesicles (indeterminate for *Cheloneotrema* and *Satyantarayano-trema*), inter-gonadal terminal genitalia and common genital pore (*Learedius* has post-ovarian terminal genitalia and common genital pore), and a Y-shaped excretory vesicle (Manter’s organ absent). *Hapalotrema* differs from the other genera of this group by having an anterior and posterior field of testes, whereas *Learedius* has a pre-ovarian field of testes only. The remaining genera (*Amphiorchis*, *Satyantarayano-trema*, *Shobanotrema*, *Neocaballerotrema*, and *Chelonotrema*) have 2 testes flanking the ovary anteriorly and posteriorly. *Amphiorchis* and *Satyantarayano-trema* are the only genera of this group that reportedly lack

tegumental body spines. *Amphiorchis* can be differentiated by having a slender body, smooth ceca, and a vitellarium that extends anteriad to the cecal bifurcation, whereas *Satyantarayano-trema* has a broad body, diverticulate ceca anteriorly, and a vitellarium that is reportedly in the posterior half of the body only. *Shobanotrema* differs from the other genera in this group by having a cyclocoel (present also only in *Neospirorchis*), and *Neocaballerotrema* and *Chelonotrema* can be differentiated by the pre-testicular and post-testicular location of the genital pore, respectively. All of these genera include species that infect marine turtles only.

The 28S phylogeny recovered a monophyletic Group 5. Noteworthy again is that *Hapalotrema* was paraphyletic: *Learedius learedi* was sister to *Hapalotrema mehrai*, and that clade was sister to the other *Hapalotrema* taxa in the analyses. As indicated by Chapman et al. (2015), because the type species of *Learedius* is nested within species of *Hapalotrema*, these data objectively indicate that the former genus should be regarded as a junior subjective synonym of *Hapalotrema*. At least this result indicates that *Hapalotrema* needs revision or that some specimens for sequencing need taxonomic confirmation (Snyder, 2004; Marchiori et al., 2017; Stacey et al., 2017; Santoro et al., 2017).

Group 6: *Atamatam* and *Paramatam* share numerous morphological similarities with the marine TBFs (Fig. 36), and this likeness is reflected in the recovered 28S tree (Figs. 37, 38). The new genera and the marine TBF genera *Amphiorchis*, *Cheloneotrema*, *Hapalotrema*, *Learedius*, *Neocaballerotrema*, *Satyantarayano-trema*, and *Shobanotrema* are unique among TBFs in having the anatomical sequence (from anterior to posterior) of anterior testis, cirrus sac, ovary, and posterior testis. Further, in addition to that combination of features, the new genera are similar to *Amphiorchis*, *Hapalotrema*, and *Learedius* by having well-developed external (pre-ovarian) and internal seminal vesicles, a ventral common genital pore, an inter-cecal, post-ovarian Laurer’s canal pore, an oviducal seminal receptacle, a straight uterus that is directed anteriad only, an external seminal vesicle and oviducal seminal receptacle that flank the ovary anteriorly and posteriorly (respectively), and a Y-shaped excretory bladder. *Caretacola* has those features as well; however, it is easily differentiated from those genera by having an inter-cecal field of numerous testes and a ventral sucker, among many other morphological differences (see above). *Atamatam* and *Paramatam* differ from all marine TBF genera (*Amphiorchis*, *Caretacola*, *Cheloneotrema*, *Hapalotrema*, *Learedius*, *Monticellius*, *Neocaballerotrema*, *Neospirorchis*, *Satyantarayano-trema*, and *Shobanotrema*) by lacking a ventral sucker (present in all but *Neospirorchis*) and a strongly muscular Laurer’s canal (present in *Caretacola* and *Hapalotrema*). They further differ from all marine TBF genera by the combination of having a spinous oral sucker, spinous mammillae distributing along the lateral body margin, a pharynx, U-shaped ceca (non-fused ceca), 2 testes in a column, a post-ovarian testis, a convoluted oviduct, an inter-testicular Laurer’s canal, a strongly muscular metraterm, and inter-gonadal terminal genitalia.

The only other nominal TBFs reported from an Austro-South American side-necked turtle (Chelidae) comprise the aforementioned 3 species of *Uterotrema* in Australia. The new genera share few morphological similarities with *Uterotrema*, indicating that they perhaps do not share a recent common ancestor. This is also reflected in the 28S analyses herein that suggest *Uterotrema* is

sister to the clade including *Vasotrema*, *Spirorchis*, and para-phyletic *Spirhalum*.

Considering all accepted TBF genera, *Atamatam* is most similar to *Paratamatam* but differs from it by having an ovoid (vs. thread-like in *Paratamatam*) body; spines distributing in a single band (vs. dextral and sinistral fields) on the anteroventral surface of the oral sucker; tegumental projections (probable sensory structures) distributing across the ventral body surface and along the dorsal body margin (vs. absent); ventrolateral (vs. lateral) and small, slightly raised (vs. large, hemi-spherical in profile) mammillae; inter-cecal (vs. extra-cecal) testes; an external seminal vesicle abutting the anterior tests, ovary, and cirrus sac (vs. not abutting, midway between cirrus sac and anterior testis); a cirrus sac that is anterolateral to the ovary and directed posterosinistrad (vs. pre-ovarian, longitudinal, and directed posteriad); a dextral (vs. sinistral) ovary; a transverse oviduct that crosses the midline before extending anteriad (vs. extending posteriad); and a sinistral common genital pore that is lateral (vs. medial, pre-ovarian) to the ovary. These genera differ further in that *Atamatam* has diverticulate ceca anteriorly; a dextral, posterior vas efferens; a vitellarium that extends posteriad beyond the tips of the ceca; and an oviducal seminal receptacle at level of the transverse vitelline duct. *Paratamatam* has smooth ceca anteriorly (lacking diverticula), a sinistral posterior vas efferens, a vitellarium terminating at the tips of the ceca, and an oviducal seminal receptacle posterior to the transverse vitelline duct.

The 28S analyses recovered the new genera as sister taxa sharing a recent common ancestor with all marine TBFs except *Neospiorchis* (Figs. 37, 38). As part of the ancestral state reconstruction (Fig. 38), stochastic mapping of character states suggested that there have been multiple reversal changes in habitat type across the evolutionary history of tetrapod blood flukes, i.e., *Neospiorchis* and the lineage comprising *Atamatam* and *Paratamatam* (Fig. 38).

Three TBF genera (*Carettacola* Manter and Larson, 1950; *Vasotrema* Stunkard, 1926; *Griphobilharzia* Platt and Blair, 1991) are difficult to treat because morphological similarity does not seem concordant with the resulting 28S maximum likelihood and Bayesian trees (Figs. 37, 38). *Carettacola* (3 species; type species *Carettacola bipora* Manter and Larson, 1950; see Platt [2002]) and *Vasotrema* (6 species; type species *Vasotrema amydae* Stunkard, 1926; see Roberts and Bullard [2017]) share some morphological similarities but genetically are distantly related. These genera have the anatomical sequence (anterior to posterior) of a ventral sucker, cirrus sac + uterus/metraterm (both approximately in parallel, longitudinal, extending anteriad), ovary, and testes(is) (testes numerous in *Carettacola*; single in *Vasotrema*). *Carettacola bipora*, *Carettacola stunkardi* (Martin and Bamberger, 1952) Dailey, Fast, and Balazs, 1991 (as *Haemoxenicon*), and *Carettacola hawaiiensis* Dailey, Fast, and Balazs, 1991, plus all species of *Vasotrema* are unique among TBF genera in having a pre-testicular ovary and pre-gonadal terminal genitalia (except *Vasotrema robustum* Stunkard, 1928, which has terminal genitalia lateral to the ovary): no other TBF genus has either of those features. Further, these genera also have the combination of a ventral sucker immediately posterior to the cecal bifurcation, a proportionally short esophagus (relative to body length), a medial esophageal diverticulum, asymmetrical and inverse U-shaped ceca that nearly overlap with the excretory vesicle, inter-cecal testes(is), a ventral, sinistral common genital pore, a pre-testicular Laurer's

canal (*Carettacola* is unique in having a massive, muscular Laurer's canal that is absent in *Vasotrema*: the specific epithet "bipora" is in reference to the large pore associated with the Laurer's canal, which Manter and Larson [1950] referred to as a "vagina-like organ"), an oviducal seminal receptacle comprising the middle portion of the oviduct, a straight (lacking convolutions) uterus that is directed anteriad only, and a Y-shaped excretory bladder. *Carettacola* spp. and *Vasotrema* spp. infect marine and freshwater turtles, respectively. Noteworthy also is that the 2 isolates of TBF sp. CMA-2009 and TBF sp. CMA-2010 likely represent un-sequenced or unnamed species of *Vasotrema* (see Kraus et al., 2014; Pinto et al., 2015).

The dioecious crocodilian blood fluke *Griphobilharzia amoena* Platt and Blair, 1991, was excluded from the morphological comparisons above because its original description is somewhat incomplete and because it represents a bit of a conundrum (Platt et al., 1991, 2013; Khalil, 2002; Brant and Loker, 2005; Loker and Brant, 2006; Orélis-Ribeiro et al., 2014). It appears morphologically as a schistosome because it has a gynocophoral canal and is dioecious; however, genetically it is a TBF (clading with *Coeuritrema* and *Hapalorhynchus*; Fig. 37). Despite it being arguably the most intriguing of blood flukes because of its host affiliation (Platt et al., 1991; Brant and Loker, 2005), this critical species has yet to be completely morphologically characterized and has been collected only once for morphology (Platt et al., 1991, 2013) and again for phylogenetic study (Loker and Brant, 2006). The original (Platt et al., 1991) and subsequent (Khalil, 2002; Platt et al., 2013) morphological accounts of this species lacked detail of the genital pore(s) and terminal genitalia because (as oftentimes happens with delicate, minute, blood flukes) the specimens stained poorly (T. R. Platt, pers. obs.). It has a ventral sucker and U-shaped ceca, but those are features common to many blood fluke genera, including schistosomes and most early branching fish blood flukes (Aporocotylidae Odhner, 1912) (see Bullard et al., 2008; Bullard and Jensen, 2008; Orélis-Ribeiro et al., 2013, 2014; Warren et al., 2017, 2019). As a result, a detailed morphological comparison of this species must await the study of additional whole mounts.

DISCUSSION

Neotropical TBFs comprise an MDL

The phylogenetic position of the new genera and results of the ancestral state reconstruction (Figs. 37, 38) together suggest that the known South American freshwater TBFs comprise a marine-derived lineage (MDL: "lineages endemic to continental freshwaters but derived from clades predominantly and ancestrally distributed in marine environments" [p. 1927 of Bloom and Lovejoy, 2017]). The new genera are morphologically most similar to the majority of marine TBFs (see Remarks; Fig. 36) and, along with the innominate cercarial sequences of Pinto et al. (2015), comprise a crown group nested within the clade that includes all marine TBFs except *Neospiorchis*. All of the other freshwater TBFs are sister to the clade including marine TBFs (excluding *Neospiorchis*), the South American TBFs, and schistosomes (Figs. 37, 38). Again, *Neospiorchis*, comprising marine TBFs, was recovered in a clade with freshwater TBFs (*Unicaecum*, *Baractrema*, cercarial sequence W810), suggesting that this marine taxon has a freshwater ancestor.

Pebas Mega-Wetland effect?

The South American freshwater TBFs, as an MDL, could have diversified during or after the Miocene (24–11 Ma) marine incursions into South America and the subsequent formation of the Pebas Mega-Wetland (Hoorn et al., 2010; Bloom and Lovejoy, 2011, 2017). This would add TBFs to the expansive literature treating various metazoan taxa whose natural history is linked to, or at least partly explained by, the Miocene Marine Incursion Hypothesis (Lovejoy et al., 2006; Bloom and Lovejoy, 2011). The Pebas Mega-Wetland (essentially what is now the central Amazon Basin) occupied >1 million km², included a variety of marine, estuarine, and freshwater habitats (lakes, wetlands, deltas, estuaries), and connected to the Caribbean Sea (Bloom and Lovejoy, 2017). These marine incursions have been linked to the diversification of several fish MDLs (e.g., freshwater stingrays [Potamotrygonidae] and their rhinebothriidean cestodes, needlefishes [Belonidae], anchovies [Engraulidae], herrings [Clupeidae], pufferfishes [Tetraodontidae], and drums [Sciaenidae]) (Lovejoy, 1996; Lovejoy and Collette, 2001; Lovejoy et al., 2006; Reyda and Marques, 2011) as well as non-fish MDLs (e.g., river dolphins, manatee, shrimps, crabs, sponges, and molluscs) (Nuttall, 1990; Wesselingh et al., 2002; Lovejoy et al., 2006). Noteworthy also is that although extant pleurodires are freshwater species geographically limited to the Southern Hemisphere, some extinct pleurodires (Bothremydidae and Stereogenyinidae) had a much wider distribution (Eurasia, India, and North America) and likely occurred in marine waters (Gaffney et al., 2006; Ferreira et al., 2018). Perhaps extant descendants of these marine and estuarine pleurodires harbor TBF MDLs as well.

Chelids colonized twice independently by TBFs

The present study reveals that chelids comprise a turtle lineage that harbors freshwater (*Uterotrema* spp.) and marine-derived TBFs. We hypothesize that the MDL (*Atamatam* and *Paramatam*) is the younger (Neotropical) lineage, whereas the ancestor of *Uterotrema* spp. and related TBFs is the older (Gondwanan) lineage. If the marine-derived TBFs of South America diverged during the Miocene Marine Incursion, we can estimate the age of this lineage at <25 Ma. This is relatively young when compared to their chelid hosts, which diverged from other pleurodires during the late Jurassic Period (149–168 Ma) (Gaffney et al., 2006; Pereira et al., 2017). South American and Australian chelids diverged thereafter (Early Cretaceous) and prior to or during the breakup of Gondwana (Aptian Period; 120 Ma) (Smith, 2010; Pereira et al., 2017; Ferreira et al., 2018). If *Uterotrema* spp. comprise the much older lineage, perhaps these and related TBFs co-diversified with chelids, beginning in the late Jurassic Period on the supercontinent of Gondwana. That hypothesis would be supported by the recovery of monophyletic but sister TBF clades in South America and Australia, respectively, mirroring that of chelids (Krenz et al., 2005; Pereira et al., 2017; Ferreira et al., 2018). Inadequate taxon sampling in pleurodire hosts is a barrier to exploring this hypothesis. Aside from the present study, the TBFs reported from pleurodires total 5 species of 2 genera: the aforementioned 3 species of *Uterotrema* in Australia plus 2 nominal species that were assigned to *Hapalorhynchus* in Africa (Bougat and Kulo, 1987; Goodman, 1987; Platt and Pichelin, 1994; Platt and Blair,

1996). The African pleurodire TBFs suffer from incomplete descriptions and a lack of type materials. Because of this, Roberts et al. (2017) doubted their identity and refrained from including them within *Hapalorhynchus* as revised and emended therein.

TBF intermediate hosts

The discussion above omits detail about the molluscan and polychaete intermediate hosts and their impact on TBF diversification. Fish blood flukes seem to clade by the intermediate host group they infect, i.e., flukes infecting chondrichthyan use bivalves; those of many freshwater ray-finned fishes use snails; and those of marine ray-finned fishes use polychaetes (Orélis-Ribeiro et al., 2014; Cribb et al., 2017a, 2017b; Warren et al., 2019). Freshwater TBFs infect snails of Planorbidae, Physidae, and Ampullariidae, whereas marine TBFs infect those of Fissurellidae (limpets) and Vermetidae (worm snails) as well as polychaetes (De Buron et al., 2018). Schistosomes have thus far been found only to utilize aquatic snails and have secondarily colonized marine habitats at least once (Brant et al., 2017). The use of a wide range of snail family diversity in the schistosomes has likely contributed to their diversification (Brant and Loker, 2013). No robust example of independent acquisitions of freshwater intermediate or definitive hosts yet exists among the fish blood flukes. The blood flukes of sharks, rays, and chimaeras (Chondrichthyes) are monophyletic and seem to be the earliest branching lineage, with separate lineages for the blood flukes of early branching ray-finned fishes (Acipenseriformes) and those infecting marine ray-finned fishes (Orélis-Ribeiro et al., 2014; Warren et al., 2019). In this regard, the fish blood flukes appear to have originated in marine hosts and invaded freshwater once. This contrasts to the emerging pattern among the blood flukes infecting turtles, birds, and mammals wherein a freshwater-marine transition has occurred repeatedly.

Blood flukes and turtles as a model for studying co-diversification

We anticipate that South American and African turtles will be explored for infections, revealing new TBF taxa for morphological studies and molecular phylogenetic analyses. The connection of these parasites to a reasonably small group of extant tetrapods, which have a well-documented and fascinating fossil record dating to the Triassic Period (Pangea), should provide some interesting questions. A good, taxonomically robust, precedent for sustained and focused study of turtle and parasite cophyly and adaptation are the series of papers detailing the turtle-infesting monogeneoids of Polystomatidae Gamble, 1896, especially members of Polystomatinae Yamaguti, 1963, which are the only monogeneoids known to infect sarcopterygians, including turtles (Verneau et al., 2002; Badets et al., 2011; Héritier et al., 2015; Tinsley and Tinsley, 2016; Tinsley, 2017). Comparable studies, those similarly exploring host specificity, biogeography, and cophyly, focused on any single parasite group that has a complex (2-host) life cycle and that matures in turtles are absent from the literature. We expect that the blood flukes of turtles will be a good endohelminth model that contributes additional insights to this turtle-parasite co-diversification/biogeography system.

Genetic and morphological comparisons within and between blood fluke genera

At present, no universally accepted, clear-cut genetic “yardstick” exists for differentiating blood fluke species and genera (present study; Roberts et al., 2018b, 2019). The proportion of inter-specific and inter-generic differences in the 28S and morphology is not consistent across all blood fluke lineages, and TBFs seem especially challenging in this regard. For example, some TBF genera include species that are morphologically extremely similar but have large 28S differences, whereas species look nothing alike but have slight 28S differences. Regarding inter-generic comparisons in the 28S, the new species of *Atamatam* and *Paramatamatam* differ by 3%, species of *Coeuritrema* and *Hapalorhynchus* by 7%, *Spirhapalum* and *Spirorchis* by 8%, *Amphiorchis* and *Hapalotrema* by 8%; and *Austrobilharzia* and *Bivitellobilharzia* by 10%. Regarding inter-specific comparisons in the 28S, species of *Spirhapalum* (*S. polesianum* vs. *S. siamensis*) differ by 10%, and those of *Hapalorhynchus* (*H. conecuhensis* vs. *H. gracilis*) differ by 9%. Some fish blood flukes exhibit large inter-specific (intra-generic) 28S differences but are extremely morphologically similar, e.g., *Elopicola* spp. that are diagnosed by slight morphological differences but differ by as much as 22% in the 28S (Bullard, 2014; Orélis-Ribeiro et al., 2017). All of these comparisons do not detract from the seemingly strong (concordant with morphology) phylogenetic signal of the 28S in resolving inter-generic relationships among blood flukes, but they do suggest the need for the development of additional markers (in addition to 18S and ITS2) or genomic comparisons.

Longstanding paraphyly problem with “Spirorchiidae”—A path ahead?

Our work herein is a step in the direction of systematically resolving the long-standing “paraphyly problem” with “Spirorchiidae” (see Snyder, 2004; Orélis-Ribeiro et al., 2014). The present study is the only taxonomic work that has used morphology to group TBF genera, reconciling the broadly accepted phylogeny for blood flukes in light of morphology. Despite essentially diagnosing several suprageneric taxa (families) herein, we refrain from naming them or resurrecting (nomenclaturally) at this time because (1) a cladistic analysis of all blood flukes based on morphology is pending, (2) several blood fluke genera need revision still, (3) a more complete review and nomenclatural assessment of the early literature treating systematic interrelationships of blood flukes is ongoing, and (4) sequence data from many genera is presently lacking. There is no doubt that the known morphological and genetic diversity warrants splitting “Spirorchiidae” into separate families. This work is ongoing and will come to fruition after the above challenges are addressed, but doing so now is premature. This ultimate work is important because it could provide new insights on the evolution of schistosomes, for example. Moreover, that phylogenetic scaffolding should provide an interesting backdrop with which to test hypotheses concerning the evolution of life history attributes, intermediate host use, freshwater-marine transitions, biogeography, host-parasite cophyly, and virulence across all blood flukes.

Key to genera of turtle blood flukes

- 1a. Cirrus sac and uterus/metraterm between ventral sucker and ovary (ovary pre-testicular)..... 2
- 1b. Male and female genitalia not as in 1a 3
- 2a. Testes numerous..... *Carettacola*
- 2b. Testis single *Vasotrema*
- 3a. Testes column pre-ovarian; ESV and ovary between uterus and testicular column 4
- 3b. Testes column absent; terminal genitalia not as in 3a... 7
- 4a. Anterior portion of ceca at bifurcation U-shaped; Manter's organ absent *Monticellius*
- 4b. Anterior portion of ceca at bifurcation M-shaped; Manter's organ present 5
- 5a. Ventral sucker absent..... *Spirorchis*
- 5b. Ventral sucker present 6
- 6a. Transverse vitelline duct inter-testicular ... *Spirhapalum*
- 6b. Transverse vitelline duct post-testicular ... *Plasmiorchis*
- 7a. Uterus/metraterm massive, occupying large portion of intercecal space 8
- 7b. Uterus/metraterm not massive, not occupying large portion of intercecal space..... 9
- 8a. Ventral sucker and body aspinose, ovary inter-testicular; 2 testes..... *Enterohaematotrema*
- 8b. Ventral sucker and body spinose, ovary pre-testicular; 1 testis *Uterotrema*
- 9a. Ovary post-testicular 10
- 9b. Ovary inter-testicular 12
- 10a. Cyclocoel present, ceca fused posteriorly *Neospiroorchis*
- 10b. Cyclocoel absent, a single cecum present..... 11
- 11a. Terminal genitalia post-cecal..... *Baracktrema*
- 11b. Terminal genitalia at level anterior to posterior ends of ceca *Unicaecum*
- 12a. Cirrus sac and external seminal vesicle pre-testicular 13
- 12b. Cirrus sac and external seminal vesicle inter-testicular 16
- 13a. Ventral sucker papillate; cirrus sac massive, directed anteriad or laterad *Platt*
- 13b. Ventral sucker apapillate 14
- 14a. Ventrolateral tegumental mammillae present *Coeuritrema*
- 14b. Ventrolateral tegumental mammillae absent..... 15
- 15a. Ventral sucker minute, far posterior to cecal bifurcation; esophagus short *Cardiotrema*
- 15b. Ventral sucker large, typically immediately post-cecal bifurcation; esophagus long *Hapalorhynchus*
- 16a. Testes >2 in number 17
- 16b. Testes 2 in number 18
- 17a. Testes divided into pre- and post-ovarian fields *Hapalotrema*
- 17b. Testes pre-ovarian *Learedius*
- 18a. Body aspinose 19
- 18b. Body spinose 20
- 19a. Body slender; ceca smooth; vitellarium anterior to cecal bifurcation..... *Amphiorchis*
- 19b. Body broad; ceca diverticulate anteriorly; vitellarium post-ventral sucker *Satyantarayananotrema*
- 20a. Cyclocoel present..... *Shobanotrema*

- 20b. Cyclocoel absent 21
 21a. Ventral sucker present, oral sucker aspinose 22
 21b. Ventral sucker absent, oral sucker spinose 23
 22a. Genital pore anterior to anterior testis
 *Neocaballerotrema*
 22b. Genital pore post-testicular (posterior to posterior testis) *Chelonotrema*
 23a. Body ovoid; ESR abutting anterior testis; OSR at level of TVD *Atamatam*, n. gen.
 23b. Body thread-like; ESR not abutting anterior testis; OSR posterior to TVD *Paramatam*, n. gen.

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