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Emendation and new species of *Hapalorhynchus* Stunkard, 1922 (Digenea: Schistosomatoidea) from musk turtles (Kinosternidae: *Sternotherus*) in Alabama and Florida rivers

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ABSTRACT

Hapalorhynchus Stunkard, 1922 is emended based on morphological study of existing museum specimens (type and voucher specimens) and newly-collected specimens infecting musk turtles (Testudines: Kinosternidae: *Sternotherus* spp.) from rivers in Alabama and Florida (USA). *Hapalorhynchus conecuhensis* n. sp. is described from an innominate musk turtle, *Sternotherus* cf. *minor*, (type host) from Blue Spring (31°5′27.64″N, 86°30′53.21″W; Pensacola Bay Basin, Alabama) and the loggerhead musk turtle, *Sternotherus minor* (Agassiz, 1857) from the Wacissa River (30°20′24.73″N, 83°59′27.56″W; Apalachee Bay Basin, Florida). It differs from congeners by lacking a body constriction at level of the ventral sucker, paired anterior caeca, and a transverse ovary as well as by having a small ventral sucker, proportionally short posterior caeca, nearly equally-sized anterior and posterior testes, a small cirrus sac, and a uterus extending dorsal to the ovary and the anterior testis. Specimens of *Hapalorhynchus reelfooti* Byrd, 1939 infected loggerhead musk turtles, stripe-necked musk turtles (*Sternotherus peltifer* Smith and Glass, 1947), Eastern musk turtles (*Sternotherus odoratus* [Latreille *in* Sonnini and Latreille, 1801]), and *S.* cf. *minor*. Those of *Hapalorhynchus* cf. *stunkardi* infected *S. minor* and *S. odoratus*. *Sternothorus minor*, *S. peltifer*, and *S.* cf. *minor* plus *S. minor* and *S. odoratus* are new host records for *H. reelfooti* and *H.* cf. *stunkardi*, respectively. This is the first report of an infected musk turtle from the Coosa and Tallapoosa Rivers (Mobile-Tensaw River Basin), Pensacola Bay Basin, or Apalachee Bay Basin. Sequence analysis of the large subunit rDNA (28S) showed a strongly-supported clade for *Hapalorhynchus*.

1. Introduction

Hapalorhynchus Stunkard, 1922 includes 19 accepted species that infect both snapping turtles (Testudines: Chelydridae) as well as musk and mud turtles (Kinosternidae) from the Nearctic realm, side-necked turtles (Pelomedusidae) from the Ethiopian realm, and both pond turtles (Geoemydidae) and softshell turtles (Trionychidae) from the Indomalayan realm (Smith [1,2]; Platt and Snyder [3]; Platt and Sharma [4]; Roberts et al. [5]). Three of the six species of *Hapalorhynchus* reported from North America infect kinosternids: *Hapalorhynchus reelfooti* Byrd, 1939 from the Eastern musk turtle (*Sternotherus odoratus* [Latreille *in* Sonnini and Latreille, 1801]), *Hapalorhynchus stunkardi* Byrd, 1939 from the razor-backed musk turtle (*Sternotherus carinatus* [Gray, 1856]), and *Hapalorhynchus albertoi* Lamothe-Argumedo, 1978 from the white- lipped mud turtle (*Kinosternon leucostomum* Duméril and Bibron *in* Duméril and Duméril, 1851). The present work focuses on musk turtles (*Sternotherus* spp.) in Alabama and Florida because only two of the six North American musk turtles had previously been reported as blood fluke hosts and no infected musk turtle had been reported from Alabama or Florida. Herein, loggerhead musk turtles (*Sternotherus minor* [Agassiz, 1857]), stripe-necked musk turtles (*Sternotherus peltifer* Smith and Glass, 1947), Eastern musk turtles, and specimens of an innominate loggerhead musk turtle (*Sternotherus cf. minor*) were examined for blood fluke infections.

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Table 1
Hapalorhynchus spp. infecting mud and musk turtles (Kinosternidae).

Turtle host	Blood fluke	Site in host	Riverine locality	Accession nos.	Reference
Kinosternon leucostomum Duméril and Bibron in Duméril and Duméril, 1851	*H. albertoi* Lamothe-Argumedo, 1978	blood (adult)	Grijalva River, Villahermosa, Tabasco, Mexico	Colección Helmintológica del Instituto de Biología de la UNAM 229-19, 229-20	Lamothe-Argumedo [7]
Sternotherus carinatus (Gray, 1856) (probably *S. minor*; see Section 3.4.3 Remarks)	*H. stunkardi* Byrd, 1939	pulmonary blood vessels (adult)	North Oconee River, Athens, Georgia, USA	USNM 1321967	Byrd [8]
***Sternotherus minor* (Agassiz, 1857)**	***H. reelfooti* Byrd, 1939**	**mesenteric blood vessels, viscera wash (adult)**	**Wacissa River (tributary of Aucilla River) (30°19′39.80″N, 83°59′6.13″W), Florida, USA**	**Retained by Bullard laboratory**	**Present study**
			Wacissa River (30°20′24.73″N, 83°59′27.56″W)	**USNM 1437606**	**Present study**
	H.* cf. *stunkardi	**heart (adult)**	**Wacissa River (30°20′24.73″N, 83°59′27.56″W)**	**USNM 1437610**	**Present study**
	***H. conecuhensis* n. sp.**	**heart (adult)**	**Wacissa River (30°20′24.73″N, 83°59′27.56″W)**	**GenBank MF568037**	**Present study**
Sternotherus* cf. *minor	***H. reelfooti***	**mesenteric blood vessels, viscera wash, heart (adult)**	**Blue Spring (Yellow River) (31°5′27.64″N, 86°30′53.21″W), Alabama, USA**	**USNM 1437607**	**Present study**
	H. conecuhensis	**heart, viscera wash (adult)**	**Blue Spring (31°5′27.64″N, 86°30′53.21″W)**	**USNM 1437612 – 1437614**	**Present study**
Sternotherus odoratus (Latreille *in* Sonnini and Latreille, 1801)	*H. reelfooti*	blood (adult)	Reelfoot Lake (Mississippi River) (36°21′12.23″N, 89°25′21.50″W), Tennessee, USA	USNM 1321968	Byrd [8]
		viscera wash (adult)	North Chain Lake (Kankakee River) (41°41′57.03″N, 86°22′16.51″W), Indiana, USA	USNM 1393855	Platt and Snyder [3]
		viscera wash (adult)	Westhampton Lake (James River) (37°34′38.01″N, 77°32′26.90″W), Richmond, Virginia, USA	USNM 1393857	Platt and Snyder [3]
		viscera wash (adult)	**Canoe Lake (Coosa River) (33°47′56.16″N, 86°29′25.02″W), Alabama**	**USNM 1437608**	**Present study**
		mesenteric blood vessels (adult)	**Pond off Odom Creek (Tallapoosa River) (32°30′9.58″N, 85°26′6.07″W), Alabama**	**GenBank MF568030**	**Present study**
	H.* cf. *stunkardi	**heart (adult)**	**Pond off Parkerson Mill Creek (Tallapoosa River) (32°35′20.04″N, 85°29′41.51″W), Alabama**	**USNM 1437611**	**Present study**
***Sternotherus peltifer* (Smith and Glass, 1947)**	***H. reelfooti***	**mesenteric blood vessels, lung (adult)**	**Chewacla Creek (Tallapoosa River) (32°31′58.64″N, 85°29′53.09″W), Alabama**	**USNM 1437609**	**Present study**

Bold indicates records comprising the present study.

Hapalorhynchus reelfooti Byrd, 1939, *Hapalorhynchus* cf. *stunkardi*, and a new species of *Hapalorhynchus* were identified. Based on these new collections and borrowed type materials of related blood flukes, we emend *Hapalorhynchus*, describe the new congener (the first new species of the genus described from North America in 29 years; Platt [6]), report the new host and geographic locality records for *H. reelfooti*, and provide a large subunit ribosomal DNA (28S) phylogeny for these taxa.

2. Materials and methods

2.1. Specimen collection and preparation

Turtles were collected from the Coosa River (Canoe Lake), Tallapoosa River (Odom Creek, Parkerson Mill Creek, Chewacla Creek), and Yellow River (Blue Spring) in Alabama as well as the Aucilla River (Wacissa River) in Florida (Table 1). Two loggerhead musk turtles were captured by hand from two sites (30°20′24.73″N, 83°59′27.56″W; 30°19′39.80″N, 83°59′6.13″W, respectively) in the Wacissa River on 23 April 2016. One Eastern musk turtle was captured with a monitored, baited hoop-net from Canoe Lake (33°47′56.16″N, 86°29′25.02″W), one was captured by hand from a pond off Parkerson Mill Creek (32°35′20.04″N, 85°29′41.51″W), and two were captured by hand from a pond off Odom Creek (32°30′9.58″N, 85°26′6.07″W) on 24 June 2015, 7 September 2015, and 22 – 24 September 2015, respectively. Two specimens of the innominate loggerhead musk turtle were captured by hand from Blue Spring (31°5′27.64″N, 86°30′53.21″W) on 19 May 2016. One stripe-necked musk turtle was captured from Chewacla Creek (32°31′58.64″N, 85°29′53.09″W) on 15 July 2015. All turtles were decapitated immediately upon returning to the laboratory. Necropsies were performed with aid of 7.0 g/L sodium citrate saline solution to prevent blood coagulation. Live turtle blood flukes (TBFs) intended as whole mounts for morphology were heat-killed on a glass slide with coverslip (no coverslip pressure) using a butane hand lighter. Resulting specimens were transferred and maintained in 5% neutral buffered formalin for morphology or placed in 95% non-denatured ethanol (EtOH) for molecular biology (see below). Specimens for morphology were rinsed in distilled water, stained in Van Cleave's hematoxylin with several drops of Ehrlich's hematoxylin, dehydrated through an ethanol series, made basic at 70% EtOH with lithium carbonate and butly-amine, cleared in clove oil, and permanently mounted in Canada balsam. The resulting whole mounts were examined and illustrated with Leica DM 2500 and Leica DMR (Leica, Wetzler, Germany) microscopes each equipped with differential interference contrast (DIC) optical components, an ocular micrometer, and a drawing tube. Measurements of the TBF specimens are reported in micrometers (μm) followed by the mean and number of specimens measured in parentheses. Measurements for the new species are reported for the holotype and paratype, respectively. Those for *Hapalorhynchus* cf. *stunkardi* are reported separately for each voucher specimen (National Museum of National History, Smithsonian Department of Invertebrate Zoology [USNM] 1437610 and 1437611, respectively), and those for the holotype of *H. stunkardi* (USNM 1321967) immediately follow in brackets ("n/a" indicates not available due to poor specimen quality). All of the newly-collected specimens were deposited in the USNM. Turtle scientific and common names follow Spinks et al. [9], van Dijk et al. [10], and Guyer et al. [11]. Classification and anatomical terms for TBFs follow Roberts et al. [5,12,13] and Platt [6] except that 'anterolateral diverticula' are herein referred to as 'anterior caeca'.

2.2. DNA extraction, amplification, and sequencing

Specimens for molecular analyses were handled with camel hair brushes, fine-tipped forceps, or syringe tips and immediately preserved in a vial of 95% EtOH and stored at − 20 °C. Total genomic DNA (gDNA) was extracted using DNeasyTM Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol except that the incubation period with proteinase-K was extended to overnight and that the final elution step was performed using only 100 μL of elution buffer to increase the final DNA concentration. The partial 28S rDNA (domains D1–D3; ~1400 bp) was amplified using the forward primer "U178" (5′-GCA CCC GCT GAA YTT AAG-3′) and the reverse primer "L1642" (5′-CCA GCG CCATCCATT TTC A-3′) (Lockyer et al. [14]). 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 $MgCl_2$, 1 mM dNTP mixture, and 0.3 μl Taq polymerase (5 U/μl) (Promega, Madison, Wisconsin, USA). The thermocycling profile comprised an initial 5 min at 95 °C for denaturation, followed by 40 repeating cycles of 94 °C for 30 s for denaturation, 50 °C for 30 s for annealing, and 72 °C for 2 min for extension, followed by a final five min at 72 °C for extension. All PCR reactions were carried out in a MJ Research PTC-200 (BioRad, Hercules, California, USA). PCR products (10 μl) were verified on a 1% agarose gel and stained with ethidium bromide. PCR products were purified by microcentrifuge with the QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) according to manufacturer's protocol, except that the last elution step was performed with autoclaved nanopure H_2O rather than the provided buffer. DNA sequencing was performed by ACGT, Incorporated (Wheeling, Illinois, USA). Reactions were sequenced using BigDye terminator version 3.1, cleaned-up with magnetic beads (CleanSeq dye terminator removal kit), and analyzed using ABI 3730 XL or 3730 Genetic Analyzer. Primers used in sequencing of 28S rDNA included the PCR primers and the reverse primer 1200R (5′-GCATAGTTCACCATCTTTCGG-3′) (Lockyer et al. [14]). Sequence assembly and analysis of chromatograms were performed with BioNumerics version 7.0 (Applied Maths, Saint-Martens- Latem, Belgium).

2.3. Sequence alignments and phylogenetic analyses

Assembled sequences (Table 2) were aligned with MAFFT 7.310 (Katoh and Standley [17]) and subsequently corrected by eye in Mesquite 3.2 (Maddison and Maddison [18]). Regions that could not be unambiguously aligned were excluded from further analyses. Bayesian Inference was performed with MrBayes 3.5.3 (Ronquist et al. [19]). Using a GTR + Gamma model, 4 runs of 4 chains each were conducted for 1,000,000 generations. Priors were set to default values and burnin was set to 25% of generations (or 250,000). Chains were run until the average standard deviation of split frequencies was below 0.01. The resulting phylogenetic tree was viewed using FigTree v1.4.3 (Rambaut [20]) and then edited in Adobe Illustrator CC 2015.3 (Adobe Systems).

3. Results

3.1. Hapalorhynchus Stunkard, 1922, emended (Figs. 1-7)

3.1.1. Diagnosis

Body dorsoventrally flat (not cylindrical), 3.7 – 17.0 × longer than wide; body constriction at level of ventral sucker present or absent; hindbody 1.4 – 3.3 × longer than forebody, aspinous; small ventral body papillae present or absent; massive, ventrolateral tegumental papillae absent. Oral sucker robust, demarcated from body by constriction; oral sucker spines present or absent; papillae present or absent. Ventral sucker present, aspinous. Pharynx present, enveloping anterior extremity of oesophagus. Oesophagus extending posteriad 1/10 – 1/4 of body length, ventral to anterior nerve commissure; lateral oesophageal diverticula present or absent; median oesophageal diverticulum absent; oesophageal gland surrounding oesophagus from pharynx to caecal bifurcation. Intestine comprising non-fused posterior caeca bifurcating anterior to ventral sucker; paired anterior caeca present (in *Hapalorhynchus brooksi* Platt, 1988) or absent; posterior caeca inverse U-shaped, extending 1/2 – 3/4 of body length directly posteriad, not extensively convoluted, terminating in posterior body extremity. Testes comprising one anterior and one posterior testis, in

Table 2
Large subunit ribosomal DNA (28S) sequences used herein.

Turtle blood fluke	Turtle host	Locality	GenBank Accession No.	Reference
Coeuritrema platti Roberts and Bullard, 2016	*Pelodiscus sinensis* (Wiegmann, 1835)	Da Rang River Basin, Phu Yen Province, Vietnam	KX712243	Roberts et al. [5]
Hapalorhynchus gracilis Stunkard, 1922	*Chelydra serpentina* Linnaeus, 1758	Reelfoot Lake (Mississippi River) (36°21′12.23″N, 89°25′21.50″W), Tennessee, USA	AY604710	Snyder [15]
Hapalorhynchus foliorchis Brooks and Mayes, 1975	*C. serpentina*	Pond off Saugahatchee Creek (Tallapoosa River) (32°39′1.36″N, 85°29′4.70″W), Alabama, USA	KX712242	Roberts et al. [5]
***Hapalorhynchus reelfooti* Byrd, 1939**	***Sternotherus minor* (Agassiz, 1857)**	**Wacissa River (tributary of Aucilla River) (30°20′24.73″N, 83°59′27.56″W), Florida, USA**	**MF568032**	**Present study**
			MF568033	**Present study**
	Sternotherus* cf. *minor	**Blue Spring (Yellow River) (31°5′27.64″N, 86°30′53.21″W), Alabama, USA**	**MF568034**	**Present study**
			MF568035	**Present study**
	***Sternotherus odoratus* (Latreille *in* Sonnini and Latreille, 1801)**	**Canoe Lake (Coosa River) (33°47′56.16″N, 86°29′25.02″W), Alabama, USA**	**MF568031**	**Present study**
		Pond off Odom Creek (Tallapoosa River), (32°30′9.58″N, 85°26′6.07″W), Alabama, USA	**MF568030**	**Present study**
***Hapalorhynchus conecuhensis* n. sp.**	***Sternotherus* cf. *minor***	**Blue Spring (31°5′27.64″N, 86°30′53.21″W)**	**MF568036hologenophore**	**Present study**
			MF568038	**Present study**
	S. minor	**Wacissa River (30°20′24.73″N, 83°59′27.56″W)**	**MF568037**	**Present study**
Schistosoma japonicum (Katsurada, 1904)	*Mus musculus* Linnaeus, 1758	experimental infection; original isolate from Phillipines	Z46504 AY157607	Lockyer et al. [14]; Olson et al. [16]
Schistosoma mansoni Sambon, 1907	*M. musculus*	experimental infection	AY157173	Lockyer et al. [14]
Schistosoma sinensium Pao, 1959	*M. musculus*	experimental infection; original isolate from Mianzhu, Sichuan, China	AY157251	Lockyer et al. [14]

Bold indicates records comprising the present study.

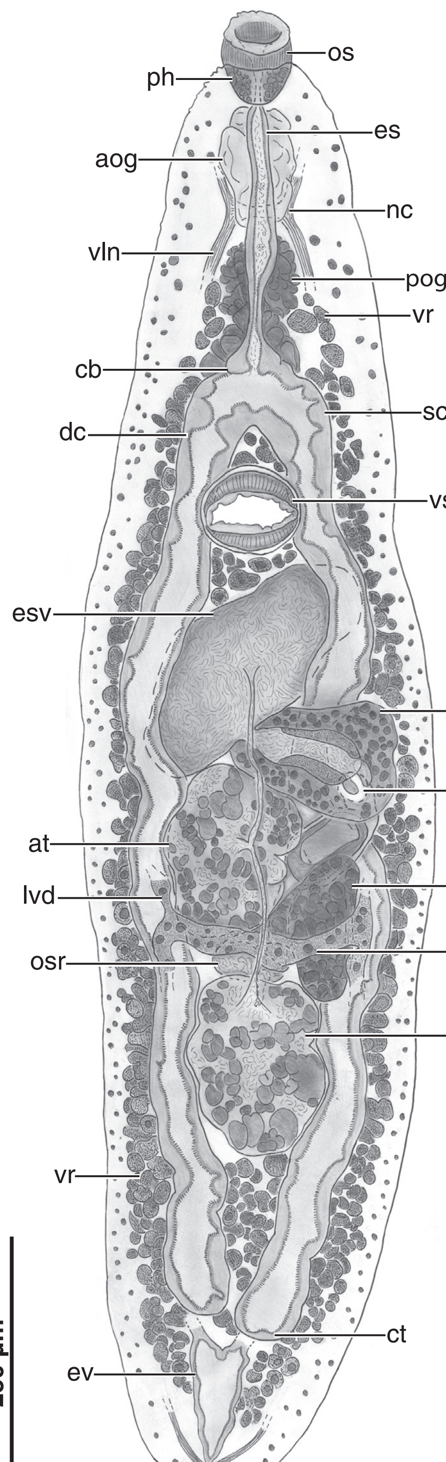

Fig. 1. Adult of *Hapalorhynchus conecuhensis* n. sp. from *Sternotherus* cf. *minor*, from Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama, USA). Scale value aside bar. Holotype (National Museum of National History, Smithsonian Department of Invertebrate Zoology [USNM] 1437612) of *H. conecuhensis* showing oral sucker (os), pharynx (ph), oesophagus (es), anterior oesophageal gland (aog), nerve commissure (nc), ventrolateral nerve chords (vln), posterior oesophageal gland (pog), vitellarium (vr), caecal bifurcation (cb), sinistral posterior caecum (sc), dextral posterior caecum (dc), ventral sucker (vs), external seminal vesicle (esv), cirrus sac (cs), vas deferens (vd), common genital pore (cgp), anterior testis (at), ovary (ov), lateral vitelline collecting duct (lvd), transverse vitelline collecting duct (tvd), oviducal seminal receptacle (osr), posterior testis (pt), caecal terminus (ct), excretory vesicle (ev), and excretory pore (ep). Ventral view.

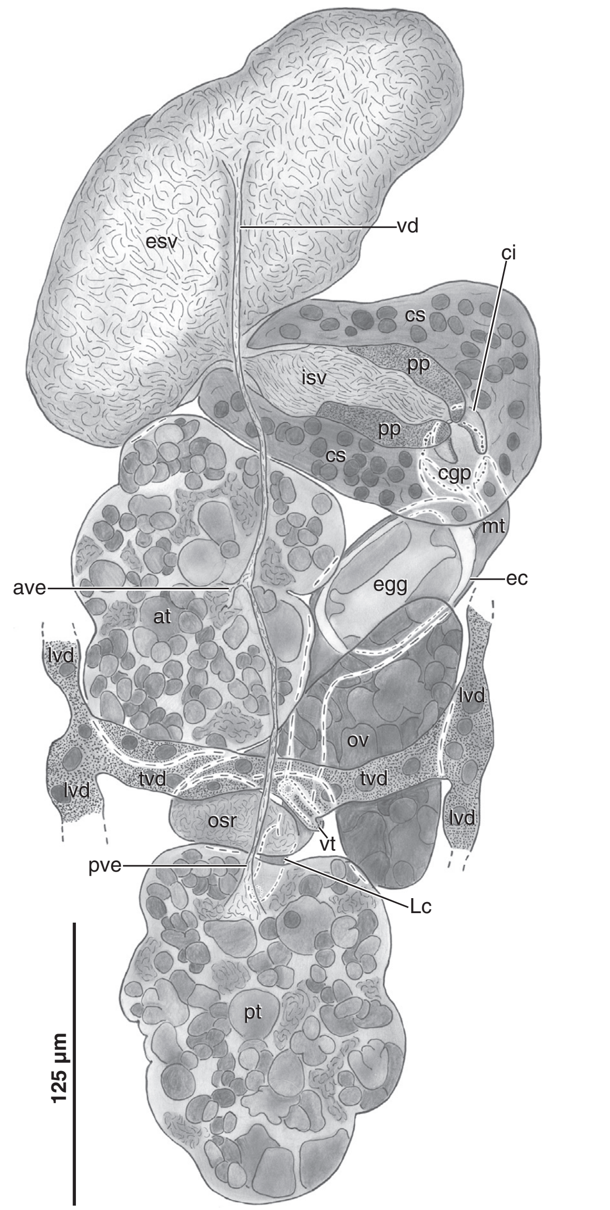

Fig. 2. Genitalia of adult *Hapalorhynchus conecuhensis* n. sp. from *Sternotherus* cf. *minor*, from Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama, USA). Scale value aside bar. Genital complex of holotype (USNM 1437612) showing vas deferens (vd), external seminal vesicle (esv), cirrus (ci), cirrus sac (cs), pars prostatica (pp), internal seminal vesicle (isv), common genital pore (cgp), metraterm (mt), egg chamber (ec), uterine egg (egg), anterior trunk of vasa efferentia (ave), anterior testis (at), lateral vitelline collecting duct (lvd), ovary (ov), transverse vitelline collecting duct (tvd), oviducal seminal receptacle (osr), primary vitelline collecting duct (vt), posterior trunk of vasa efferentia (pve), Laurer's canal (Lc), and posterior testis (pt). Ventral view.

posterior 2/3 of body, intercaecal, smooth or lobed. Vas deferens extending anteriad and ventral to gonads before laterally expanding to form external seminal vesicle; external seminal vesicle posterior to ventral sucker, intercaecal, anterior or dextral to genital pore; internal seminal vesicle present (when cirrus sac present); pars prostatica difficult to discern, enveloping distal extremity of internal seminal vesicle when present. Cirrus sac absent or pre-testicular and directed posteriad if present. Ovary ovoid or triangular in outline, intercaecal, intertesticular, transverse or sinistral. Oviduct emerging from dextral margin of ovary, extending directly posteriad or sinistrad; oviducal seminal receptacle comprising middle portion of oviduct between ovary and posterior testis. Laurer's canal intercaecal, intertesticular; Laurer's canal

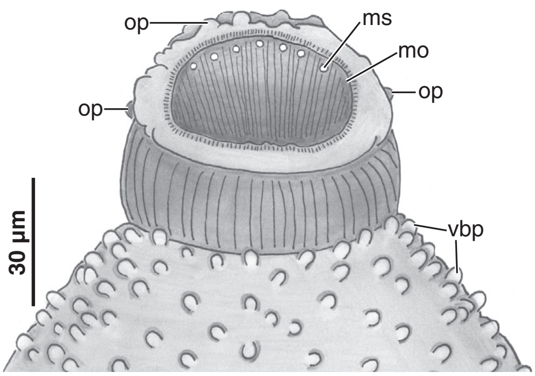

Fig. 3. Oral sucker of *Hapalorhynchus conecuhensis* n. sp. from *Sternotherus* cf. *minor* collected from Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama, USA). Scale value aside bar. Holotype (National Museum of National History, Smithsonian Department of Invertebrate Zoology [USNM] 1437612) showing oral sucker papillae (op), oral sucker spines (ss), mouth (mo), and ventral body papillae (vbp). Ventral view.

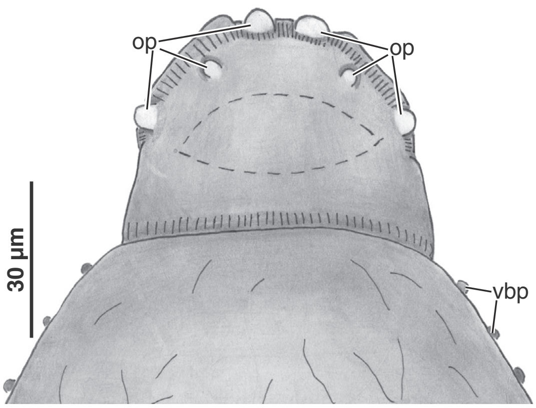

Fig. 4. Oral sucker of *Hapalorhynchus conecuhensis* n. sp. from *Sternotherus* cf. *minor* collected from Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama, USA). Scale value aside bar. Paratype (USNM 1437613) showing oral sucker papillae (op) and ventral body papillae (vbp). Dorsal view.

pore dorsal. Vitellarium follicular, ventrolateral to caeca and gonads, distributing from oesophagus to excretory vesicle or from caecal bifurcation to ends of posterior caeca; transverse vitelline duct intertesticular, ventral to ovary, comprising lateral collecting ducts ventral to caeca. Oötype with longitudinal axis parallel to that of body, intercecal. Mehlis' gland not observed. Uterus intercaecal, intertesticular, straight (not coiled), difficult to discern from oötype in gravid specimens, extending anteriad, dorsal or ventral to ovary; metraterm narrow, anterior to ovary, sinistral to anterior testis. Uterine pouch absent.

Uterine egg single, tricornate or ovoid (with or without filaments), occupying oötype and uterus proximal to metraterm. Common genital pore sinistral, dorsal. Excretory vesicle distinctly Y- shaped or sinuous (lacking multiple laterally-directed lobes). Manter's organ absent. Excretory pore terminal or dorsal.

Type species: *Hapalorhynchus gracilis* Stunkard, 1922

3.1.2. Differential diagnosis

Body dorsoventrally flat (not cylindrical), aspinous; ventrolateral tegumental papillae *sensu* Roberts et al. [5] absent. Oral sucker robust, spinous or aspinous. Pharynx present. Median oesophageal diverticulum absent. Testes comprising one anterior and one posterior testis, intercaecal. Ovary ovoid or triangular in outline, intertesticular, transverse or sinistral. Uterus extending anteriad dorsal or ventral to ovary; metraterm narrow. Common genital pore sinistral, dorsal. Excretory vesicle distinctly Y-shaped or sinuous (lacking multiple laterally-directed lobes). Manter's organ absent.

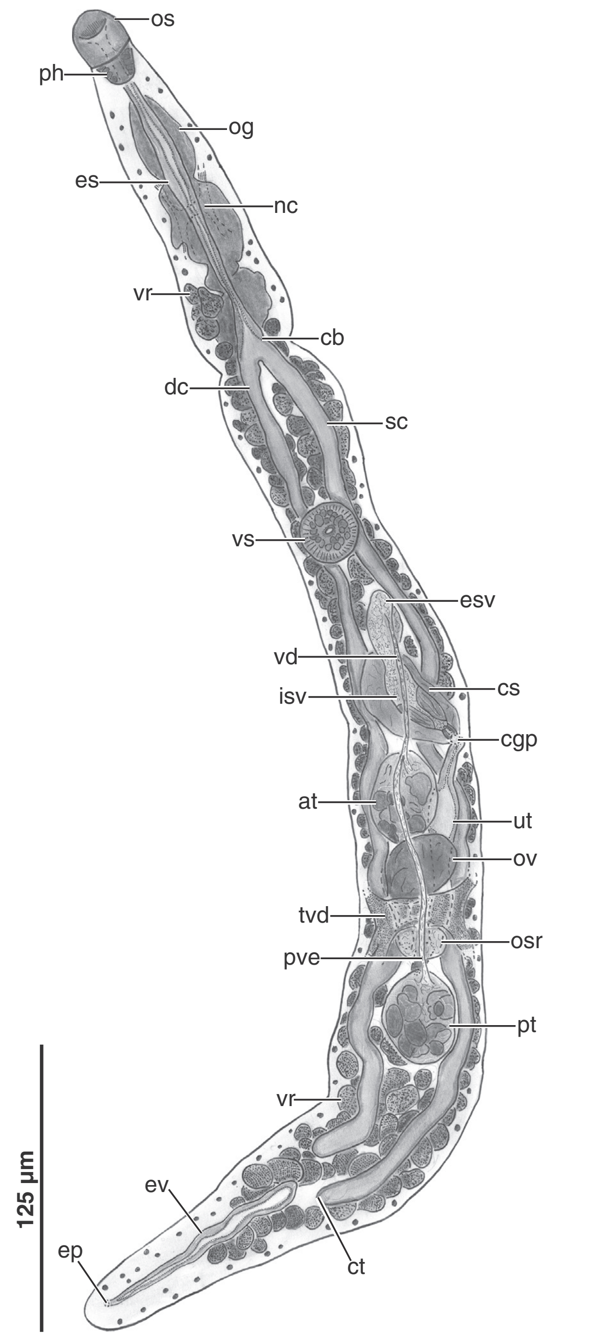

Fig. 5. Adult fluke from loggerhead musk turtles, *Sternotherus minor*, collected from the Wacissa River (30°20′24.73″N, 83°59′27.56″W; tributary of Aucilla River, Florida, USA). Scale value aside bar. *Hapalorhynchus reelfooti* Stunkard, 1922 (National Museum of National History, Smithsonian Department of Invertebrate Zoology [USNM] 1437606) showing oral sucker (os), pharynx (ph), oesophageal gland (og), oesophagus (es), nerve commissure (nc), vitellarium (vr), caecal bifurcation (cb), dextral posterior caecum (dc), sinistral posterior caecum (sc), ventral sucker (vs), external seminal vesicle (esv), vas deferens (vd), cirrus sac (cs), internal seminal vesicle (isv), common genital pore (cgp), anterior testis (at), uterus (ut), ovary (ov), transverse vitelline collecting duct (tvd), oviducal seminal receptacle (osr), posterior trunk of vasa efferentia (pve), posterior testis (pt), caecal terminus (ct), excretory vesicle (ev), and excretory pore (ep). Ventral view.

3.1.3. Remarks

Our emended diagnosis of *Hapalorhynchus* adds or further details numerous features that help differentiate it from that of other TBF genera: details of the body surface and oral sucker, pharynx, anterior

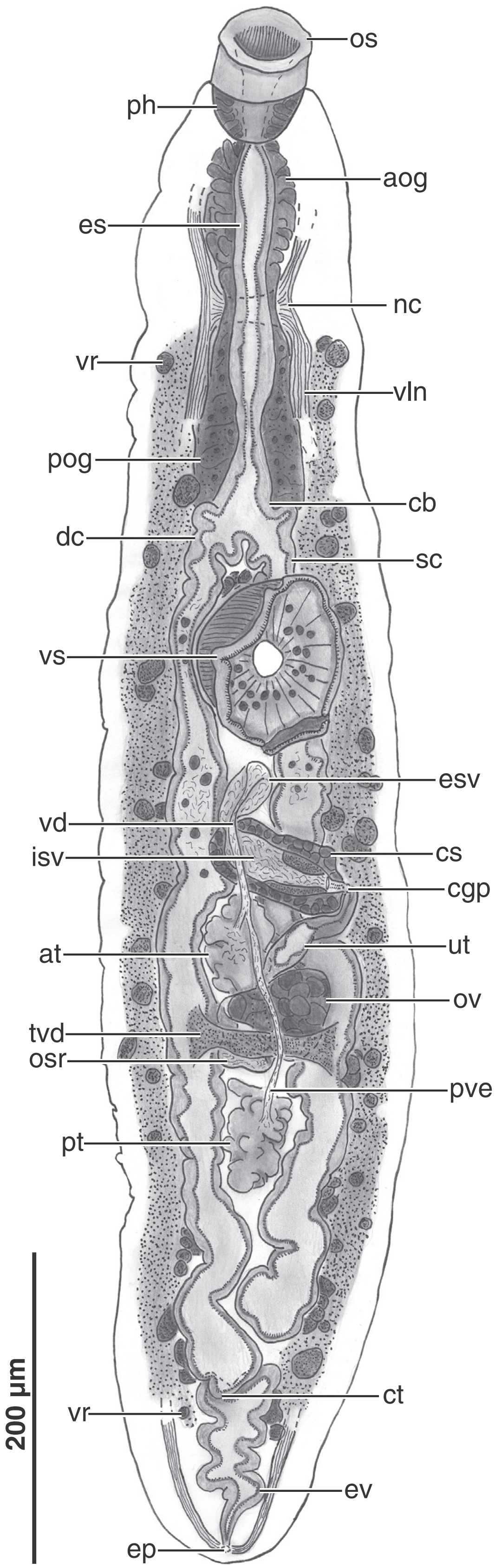

Fig. 6. Adult fluke from loggerhead musk turtles, *Sternotherus minor*, collected from the Wacissa River (30°20′24.73″N, 83°59′27.56″W; tributary of Aucilla River, Florida, USA). Scale value aside bar. *Hapalorhynchus* cf. *stunkardi* (USNM 1437610) showing oral sucker (os), pharynx (ph), anterior oesophageal gland (aog), oesophagus (es), nerve commissure (nc), vitellarium (vr), ventrolateral nerve chords (vln), posterior oesophageal gland (pog), caecal bifurcation (cb), dextral posterior caecum (dc), sinistral caecum (sc), ventral sucker (vs), external seminal vesicle (esv), vas deferens (vd), cirrus sac (cs), internal seminal vesicle (isv), common genital pore (cgp), uterus (ut), anterior testis (at), ovary (ov), transverse vitelline collecting duct (tvd), oviducal seminal receptacle (osr), posterior trunk of vasa efferentia (pve), posterior testis (pt), caecal terminus (ct), excretory vesicle (ev), and excretory pore (ep). Ventral view.

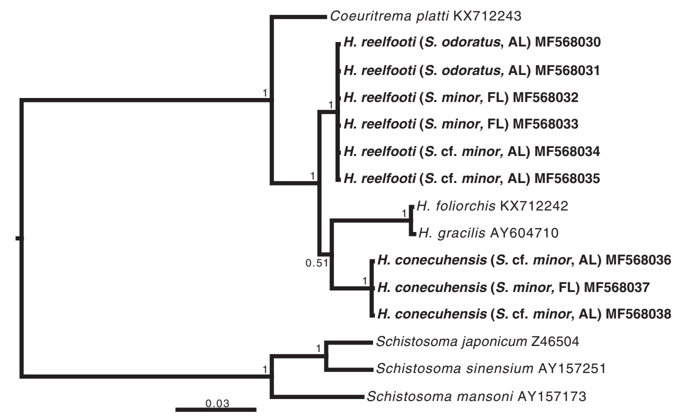

Fig. 7. Phylogenetic relationships of blood flukes reconstructed by Bayesian inference and based on partial D1–D3 domains of the large subunit ribosomal DNA (28S) sequences from selected blood flukes (15 TBFs, three schistosomes). Numbers aside tree nodes indicate posterior probability.

caeca, oesophageal gland, vas deferens, internal seminal vesicle, pars prostatica, oviduct, transverse vitelline duct, oötype, metraterm, and excretory pore. Yet, the genus likely needs additional revisionary work: its 19 accepted species are unusual among TBF genera in that their collective geographic distributions include North America, Asia, and Africa (Platt and Sharma [4]). The North American species of *Hapalorhynchus* infect snapping turtles (Chelydridae, infecting one of four species belonging to one of two genera) as well as the mud and musk turtles (Kinosternidae, infecting three of 26 species belonging to both genera; Table 1): *Hapalorhynchus gracilis* Stunkard, 1922 (type species), *H. reelfooti*, *H. stunkardi*, *Hapalorhynchus foliorchis* Brooks and Mayes, 1975, *H. albertoi*, and *H. brooksi*.

The Asian species of *Hapalorhynchus* infect pond turtles (Geoemydidae, infecting five of 69 species belonging to four of 19 genera) and soft-shelled turtles (Trionychidae, infecting four of 31 species belonging to four of 13 genera; all previously *Coeuritrema* unless otherwise noted): *Hapalorhynchus odhnerensis* (Mehra, 1933) Byrd, 1939, *Hapalorhynchus indicus* (Thapar, 1933) Price, 1934 (as *Tremarhynchus* Thapar, 1933), *Hapalorhynchus yoshidai* Ozaki, 1939, *Hapalorhynchus ocadiae* (Takeuti, 1942) Platt, 2002, *Hapalorhynchus oschmarini* (Belous, 1963) Platt, 2002, *Hapalorhynchus macrotesticularis* (Rohde, Lee and Lim, 1968), Brooks and Sullivan, 1981, *Hapalorhynchus mica* (Oshmarin, 1971) Platt, 2002, *Hapalorhynchus sheilae* (Mehrotra, 1973) Platt, 2002, *Hapalorhynchus sutlejensis* (Mehrotra, 1973) Platt, 2002, *Hapalorhynchus snyderi* Platt and Sharma, 2012, and *Hapalorhynchus tkachi* Platt and Sharma, 2012. All of these species except *H. snyderi* and *H. tkachi* lack complete taxonomic descriptions and their type specimens either do not exist or reside in museums or research collections that cannot or choose not to loan materials outside of the Indian subcontinent (Brooks and Sullivan [21]; Platt [22]; T. Platt, pers. comm.). The uniqueness of these taxa needs to be reassessed.

Hapalorhynchus snyderi and *H. tkachi* (for which we examined type material) differ from North American species of *Hapalorhynchus* by having a papillate ventral sucker (similar to *Vasotrema* Stunkard, 1926;

see Roberts and Bullard [23]), an external seminal vesicle abutting the anterior margin of the cirrus sac, and a cirrus sac posterior to the genital pore and directed anteriad).

The African species of *Hapalorhynchus* infect African side-necked turtles (Pelomedusidae, infecting two of 18 species belonging to one of two genera) and comprise *Hapalorhynchus beadlei* Goodman, 1987 and *Hapalorhynchus tchalimi* Bourgat and Kulo, 1987. These infections were reported only once for both *H. beadlei* and *H. tchalimi* (Goodman [24]; Bourgat and Kulo [25]). Goodman [24] described *H. beadlei* based on specimens collected from the Albert Nile mud turtle, *Pelusios williamsi lutescens* Laurent, 1965, but lost all of the original type materials before the work was accepted for publication. Goodman returned to the type locality and sampled the type host; however, according to Platt [26,27], these newer specimens (represented by voucher USNM 79375) were not conspecific with those Goodman originally illustrated, measured, and named but rather were conspecific with those of *H. tchalimi* (Royal Museum for Central Africa, Tervuren; see Bourgat and Kulo [25]). This matter was resolved in a petition (case no. 2653) by Platt to the International Commission on Zoological Nomenclature (Platt [26]). Several reported features distinguish *H. beadlei* and *H. tchalimi* from congeners: lateral oesophageal diverticula present, cirrus sac absent in *H. beadlei* (see Goodman [24]), and Laurer's canal originating near the proximal margin of the seminal receptacle (Fig. 2, p. 438; Bourgat and Kulo [25]). Further, given the uniqueness of these taxa regarding their geographic origin and their hosts' ancestry, additional specimens of these taxa should be collected and studied so that their membership in *Hapalorhynchus* can be confirmed.

Hapalorhynchus resembles *Coeuritrema* Mehra, 1933, *Enterohaematotrema* Mehra, 1940, and *Cardiotrema* Dwivedi, 1967 by having a ventral sucker, inverse U-shaped posterior caeca, two testes, and pre-gonadal male terminal genitalia. *Hapalorhynchus* differs from *Coeuritrema* by lacking ventrolateral tegumental papillae and by having a diminutive metraterm that is difficult to discern from the uterus. *Coeuritrema* has massive, mound-like, ventrolateral tegumental papillae (*sensu* Roberts et al. [5]) and a robust metraterm (1/10–1/7 of body length) that is easily differentiated from the uterus, even in gravid specimens. *Hapalorhynchus* can be differentiated from *Enterohaematotrema* by having a moderately-sized cirrus sac (1/10-1/7 of body length when present), a diminutive metraterm, and a dorsosinistral genital pore. *Enterohaematotrema* has a massive cirrus sac (1/3 of body length), a massive metraterm (1/3 of body length), and a ventromedial genital pore posterior to the ventral sucker. Lastly, *Hapalorhynchus* differs from *Cardiotrema* by having a longer oesophagus (1/10–1/4 of body length), a larger ventral sucker (1/4 – 1/2 of body width), and an intertesticular ovary. *Cardiotrema* has a short oesophagus (< 1/10 of body length), a small ventral sucker (< 1/5 of body width), and an ovary that is either lateral to the anterior testis or intertesticular.

No species of *Hapalorhynchus* had previously been described as having papillae on the body surface (Figs. 3, 4). Vouchers (USNM 1393855, 1393857) and newly collected specimens of *H. reelfooti* (USNM 1437606 – 1437609), newly collected specimens of *H.* cf. *stunkardi* (USNM 1437610, 1437611), and specimens of the new species (USNM 1437612 – 1437614) have papillae on the body surface. Papillae were indistinct in the holotype of *H. gracilis*, *H. stunkardi*, and *H. brooksi* (AMNH 125; USNM 1321967, 1375720, respectively) as well as vouchers of *H. foliorchis* (USNM 1422462 – 1422464). We suspect these papillae are present in other species of *Hapalorhynchus*; however, they are quite difficult to see without exceptionally well-stained material. Regarding the pharynx, like *Spirorchis* MacCallum, 1918, *Unicaecum* Stunkard, 1925, *Vasotrema*, *Coeuritrema*, and *Baracktrema* Roberts, Platt, and Bullard, 2016, species of *Hapalorhynchus* have a pharynx located in the extreme anterior end of the oesophagus such that it appears in fixed, whole-mounted specimens as immediately dorsal to the mouth. This position makes it easily missed, shrouded by the muscular oral sucker, or mistaken for a component of the oral sucker itself (Figs. 1, 5, 6; Stunkard [28]; Platt [22]; Roberts et al. [5,12,13]; Roberts and Bullard [23]). Regarding the oral sucker spines, no species of *Hapalorhynchus* has been described as having spines associated with the oral sucker (Stunkard [28]; Platt [22]) but the holotype (USNM 1437615) of the new species has spines on the dorsal rim of the mouth (Fig. 3). Similar spines have been described on the surface of the ventral concavity of the oral sucker of *Vasotrema robustum* Stunkard, 1928 (see Roberts and Bullard [23]). No spines were observed in specimens of any other species examined herein. Oral sucker papillae were herein observed in the new species and *Hapalorhynchus* cf. *stunkardi* (Figs. 3, 4). Such papillae have not been reported previously for any species of *Hapalorhynchus*; however, they have been observed in species of *Vasotrema* (see Roberts and Bullard [23]) and some fish blood flukes (Aporocotylidae) (Bullard and Overstreet [29]; Truong and Bullard [30]; Yong et al. [31]).

Because these papillae are present in species assigned to evidently unrelated genera (Orélis- Ribeiro et al. [32; Roberts et al. [12]), they likely evolved independently; perhaps as a result of strong 'selection pressure' for the fluke being able to detect its position in the host or to locate conspecifics.

Regarding the cirrus sac, we accept it as being present or absent among members of the genus (Platt [6,22]): *H. gracilis*, *H. foliorchis*, and *H. beadlei* lack a cirrus sac. The holotype of *H. gracilis* (American Museum of Natural History [AMNH] Coll. No. 125) and vouchers of *H. foliorchis* (USNM 1422462 – 1422464) lack a cirrus sac. As stated above, type materials of *H. beadlei* were lost, with Goodman's [24] description designated as the lectotype (Platt, 26; 27).

Examination of newly-collected specimens from the type host (*Pelusios* sp.) and type locality (Kampala, Uganda) are needed to assess this feature in *H. beadlei* (Platt [26,27]).

Hapalorhynchus stunkardi was originally described as having a "thin-walled and non-muscular (*sic*)" cirrus sac. Platt [6] reported that the prostatic cells of *H. stunkardi* were irregularly scattered about the cirrus; however, our observations of that region in the holotype of *H. stunkardi* (USNM 1321967) agree with Byrd's [8] description, i.e., the cirrus sac is present, thin- walled, and non-muscular.

3.2. *Hapalorhynchus conecuhensis* Roberts and Bullard, n. sp. (Figs. 1-4, 7)

3.2.1. Diagnosis of adult (based on light microscopy of two intact specimens [holotype, USNM 1437612; paratype, USNM 1437613] and two partial specimens [hologenophore, USNM 1437614; paratype, SAB's collection]).

Body 1390 and 1340 long or 4.3 × (2) longer than wide, 250 and 230 wide or 18% and 17% of body length at level of caecal bifurcation, 223–275 (253; 3) wide or 19% and 21% of body length at level of ventral sucker, 245–320 (288; 4) wide or 23% and 24% of body length at level of external seminal vesicle, 260–335 (295; 4) wide or 22% and 25% of body length at level of ovary, 200–250 (224; 4) wide or 17% and 15% of body length at level of caecal terminus; forebody (middle of ventral sucker to anterior body end) 470 (2) long or 34% and 35% of body length; hindbody (middle of ventral sucker to posterior body end) 920 and 870 long or 66% and 65% of body length or 2.0 × and 1.9 × longer than forebody; small ventral body papillae present (Figs. 3, 4). Oral sucker 58 and 73 long or 4% and 5% of body length, 65 and 60 wide or 26% (2) of body width at level of caecal bifurcation; oral sucker spines present; papillae present (Figs. 3, 4). Ventral sucker 75 and 95 long or 5% and 7% of body length or 1.3 ×(2) longer than oral sucker, 88 and 100 wide or 34% and 26% of body width at level of ventral sucker or 1.4 × and 1.7 × wider than oral sucker. Nerve commissure 190 and 165 long or 14% and 12% of body length from anterior body end. Pharynx 40 and 50 long or 13% and 16% of oesophagus length, 63 and 58 wide or 1.9 × and 1.8 × wider than maximum oesophagus width. Oesophagus 310 and 308 long or 22% and 23% of body length, 20 and 13 wide posterior to pharynx with wall 10 and 8 thick, 33 (2) wide or 13% and 14% of body width at mid-oesophagus, with wall 15 and 20 thick, 65 (2) wide or 26% and 28% of body width at caecal bifurcation, with wall 55 and 47 thick; anterior oesophageal gland 130 and 114 long or 42% and 37% of oesophagus length, 75 and 57 wide or 30% and 25% of body width at level of caecal bifurcation; posterior oesophageal gland 125 and 107 long or 40% and 35% of oesophagus length, 77 and 80 wide or 31% and 35% of body width at level of caecal bifurcation. Intestine bifurcating 335 and 325 or 24% (2) of body length from anterior body end; sinistral posterior caecum 830 – 920 (870; 3) long or 66% and 64% of body length, 50 – 63 (56; 3) wide or 22% and 27% of body width at level of caecal bifurcation, 25 – 43 (33; 4) wide or 10 – 13% (11%; 4) of body width at level of ovary, 38 – 63 (51; 4) wide or 17 – 30% (23%; 4) of body width at terminus; dextral posterior caecum 775 – 890 (852; 3) long or 64% and 66% of body length, 38 – 63 (55; 3) wide or 25% and 27% of body width at level of caecal bifurcation, 25 – 45 (39; 4) wide or 10 – 15% (13%;4) of body width at level of ovary, 38 – 58 (48; 4) wide or 18 – 25% (21%; 4) of body width at terminus; caecal terminus 140 (2) or 10% (2) of body length from posterior body end.

Anterior testis lobed, follicular, 100 – 178 (150; 4) long or 12% and 13% of body length or 88 – 95% (92%; 4) of posterior testis length, 83 – 150 (127; 4) wide or 32 – 52% (43%; 4) of body width at level of ovary or 98 – 106% (101%; 4) of posterior testis width; intertesticular space 28 – 73 (45; 4) or 3% (2) of body length. Posterior testis lobed, follicular, 113 – 188 (161; 4) long or 12% and 14% of body length, 78 – 153 (128; 4) wide or 30 – 52% (43%; 4) of body width at level of gonads, 243 – 313 (282; 3) or 23% and 22% of body length from posterior body end. Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending 13 – 50 (26; 3) or 1% (2) of body length anteriad, 5 – 8 (6; 3) wide; posterior trunk of vasa efferentia emanating from ventral surface of posterior testis, ventral to gonads, extending 150 – 255 (206; 3) or 11% and 16% of body length anteriad, 5 (4) wide, meeting anterior trunk of vasa efferentia ventral and posterior to anterior testis to form vas deferens. Vas deferens ventral, extending 133 – 195 (156; 3) or 14% and 10% (2) of body length anteriad, 5 – 10 (8; 4) wide, expanding to form external seminal vesicle. External seminal vesicle 63 – 113 (94; 4) long or 8% and 7% of body length, 103 – 258 (189; 4) wide or 42 – 81% (64%; 4) of body width or 1.6 – 2.3 × (2.0; 4) wider than long, dorsal to caeca (Figs. 1, 2); internal seminal vesicle 102 – 150 (118; 4) long or 70 – 80% (74%; 4) of cirrus sac length, 25 – 43 (35; 4) wide or 2.4 – 4.1 × (3.4; 4) longer than wide. Pars prostatica surrounding distal portion of internal seminal vesicle, 60 – 100 (72; 4) long or 52 – 67% (61%; 4) of internal seminal vesicle length, 45 – 63 (50; 4) wide or 1.3 – 1.6 × (1.4; 4) longer than wide (Fig. 2). Cirrus sac obvious, enveloping entirety of internal seminal vesicle and cirrus, 128 – 205 (159; 4) long or 11% and 15% of body length, 75 – 113 (101; 4) wide or 31 – 39% (35%; 4) body width at level of external seminal vesicle; cirrus 18 – 28 (24; 4) long, 18 – 50 (34; 4) wide (Fig. 2).

Ovary triangular in outline, sinistral, 305 – 335 (316; 3) or 24% and 23% of body length posterior to middle of ventral sucker, 25 – 55 (45; 4) or 4% and 3% of body length posterior to genital pore, 100 – 138 (118; 4) long or 10% and 8% of body length, 80 – 110 (92; 4) wide or 26 – 34% (31%; 4) of body width; post-ovarian space 460 and 475 or 33% and 35% of body length. Oviduct turning dorsad and extending posteriad 30 – 50 (44; 4) or 4% and 3% (2) of body length, 8 – 15 (12; 4) in maximum width, laterally expanding to form seminal receptacle; oviducal seminal receptacle extending sinistrad for 50 – 73 (63; 4) or 57 – 85% (69%; 4) of ovary width, 20 – 35 (30; 4) wide or 7 – 12% (10%; 4) of body width, constricting and turning dorsad, extending anteriad for 68 – 100 (87; 3) or 7% (2) of body length before joining oötype, 13 – 18 (15; 3) wide or 4 – 7% (7%; 3) of body width (Figs. 1, 2). Laurer's canal originating immediately distal to seminal receptacle terminus, extending posteriad 25 – 35 (32; 3) or 1% (2) of body length, 8 (3) wide, opening dorsally over posterior testis (Fig. 2). Vitellarium comprising a series of interconnected spheroid masses of follicles (Fig. 1), ventro-lateral to caeca (illustrated as lateral to emphasize course of caeca), distributing from level of oesophagus median or 285 and 205 or 21% and 15% of body length from anterior body end to excretory vesicle or 55 and 88 or 4% and 7% of body length from posterior body end; lateral collecting ducts 8 – 20 (15; 16 [4 ducts per specimen]) wide, ventral to caeca, coalescing at level of posterior margin of ovary to form transverse vitelline duct; transverse vitelline duct ventral to ovary, 370 – 390 (378; 3) or 28% (2) of body length from middle of ventral sucker; primary vitelline collecting duct extending anterodextrad and dorsal 26 – 53 (35; 4) or 3% and 2% of body length before connecting with oviduct (Fig. 2), 9 – 14 (11; 4) wide or 3 – 5% (4%; 4) of body width. Oötype difficult to discern in gravid individuals, forming egg chamber with proximal portion of uterus, 33 and 28 long, 18 and 20 wide, dorsal to ovary and anterior testis. Uterus originating 300 or 22% (1) of body length posterior from middle of ventral sucker. In gravid individuals (2 of 4), egg chamber originating 305 and 335 or 22% (1) of body length posterior from middle of ventral sucker, 98 and 100 long or 7% (1) of body length, 55 and 50 wide or 18% (2) of body width; metraterm extending anterosinistrad, 35 – 52 (41; 4) long or 3% and 4% of body length, 11 – 30 (18; 4) wide or 3 – 10% (6%; 4) of body width, 1.1 × and 0.9 × longer than uterus. Uterine egg ovoid, 73 and 90 long or 5% (1) of body length, 45 and 43 wide or 15% and 16% of body width. Common genital pore 225 – 250 (238; 3) or 18% and 17% of body length posterior to middle of ventral sucker, 30 – 40 (35; 4) in diameter.

Excretory vesicle 25 – 65 (48; 3) wide or 23% and 13% of body width at level of caecal terminus; wall 10 – 17 (14; 3) thick (Fig. 2); excretory pore dorsal, subterminal, 13 and 18 or 1% (2) of body length from posterior body margin.

3.2.2. Taxonomic summary

Type host: *Sternotherus cf. minor* (Testudines: Kinosternidae), innominate musk turtle; other host: *Sternotherus minor*, loggerhead musk turtle.

Type locality: Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama, USA; *S.* cf. *minor*); other locality: Wacissa River (30°20′24.73″N, 83°59′27.56″W; tributary of Aucilla River, Florida, USA; *S. minor*).

Site in host: Heart, viscera wash.

Prevalence and intensity of infection by adults: Two innominate musk turtles from Blue Spring had two (whole-mounted specimens) and four (MF568036 [hologenophore], MF568038, three whole-mounted specimens) specimens of *H. conecuhensis*. One of two specimens of loggerhead musk turtle from the Wacissa River had one specimen of *H. conecuhensis* (MF568037).

Specimens deposited: Holotype (USNM 1437612); Paratype (USNM 1437613); Hologenophore (USNM 1437614); GenBank (Nos. MF568036 – MF568038).

Specimens examined: Holotype of *H. gracilis* (AMNH 125); Vouchers of *H. foliorchis* (USNM 1422462 – 1422464); Holotype of *H. stunkardi* (USNM 1321967); Holotype of *H. reelfooti* (USNM 1321968); Vouchers of *H. reelfooti* (USNM 1393855, 1393857); Holotype of *H. brooksi* (USNM 1375720).

Etymology: The specific epithet *conecuhensis* is for the type locality, Conecuh National Forest, Andalusia, Alabama.

3.2.3. Remarks

The new species resembles *H. brooksi* and *H. stunkardi* by having (in addition to all diagnostic characters of the genus) proportionally long posterior caeca (post-caecal space < 14% of body length), a transverse external seminal vesicle (abutting posterior caeca), and lobed testes that are > 40% of body width. It most closely resembles *H. stunkardi* by lacking a marked body constriction at level of the ventral sucker, paired anterior caeca, and a transverse ovary as well as by having a vitellarium distributing from the oesophagus to the excretory vesicle and an ovoid uterine egg that lacks polar filaments. *Hapalorhynchus brooksi* has a marked body constriction at level of the ventral sucker, paired anterior caeca, a transverse ovary, a vitellarium distributing from the caecal bifurcation to the caecal terminus, and an egg with polar filaments. The new species differs from *H. stunkardi* by the combination of having a relatively small ventral sucker (34 – 36% of body width), short posterior caeca (64 – 66% of body length), nearly equal-sized anterior and posterior testes (anterior testis is 88 – 95% and 98 – 106% of posterior testis length and width, respectively), a small cirrus sac (31 – 39% of body width), and a uterus that extends dorsal to the ovary and the anterior testis (Fig. 2). *Hapalorhynchus stunkardi* has a large ventral sucker (43% of body width), long posterior caeca (72% and 74% of body length), a markedly smaller anterior testis relative to the posterior testis (< 80% of posterior testis length and width), a large cirrus sac (47% of body width), and a uterus that extends ventrolateral to the anterior testis and anterior margin of ovary.

The type host for *H. conecuhensis*, *S.* cf. *minor* (see Guyer et al. [11]; pp. 21, 22), is an innominate species of musk turtle, and its taxonomic description is forthcoming (P. Scott [University of California, Los Angeles [UCLA]; Los Angeles, California, USA], personal communication to JRR).

3.3. *Hapalorhynchus reelfooti* Byrd, 1939 (Figs. 5, 7)

3.3.1. Diagnosis of adult (based on light microscopy of 13 intact specimens [vouchers USNM 1437606 – 1437609])

With features of *H. reelfooti* (see Byrd, [8] and Platt and Snyder, [3]) except for the following. Body 40–82 (62; 13) wide or 5–11% (8%; 13) of body width at level of caecal bifurcation, 33–75 (57; 13) wide or 5–10% (7%; 13) of body width at level of ventral sucker, 43–111 (78; 13) wide or 7–14% (9%; 13) of body length at level of ovary, 35–98 (66; 13) wide or 6–11% (8%; 13) at level of caecal terminus; small ventral body papillae present. Oral sucker 21–36 (26; 13) long or 2–5% (3%; 13) of body length, 23–34 (29; 13) wide or 33–63% (48%; 13) of body width at level of caecal bifurcation. Pharynx 13–36 (24; 13) long or 7–18% (13%; 13) of body length, 20–33 (26; 13) wide or 0.7–1.4 × (1.2; 13) wider than maximum oesophagus width. Oesophagus 5–14 (8; 13) wide posterior to pharynx with wall 3–8 (5; 13) thick, 11–25 (18; 13) wide or 16–49% (30%; 13) of body width at mid-oesophagus, with wall 7–16 (11; 13) thick, 16–32 (21; 13) wide or 23–45% (35%; 13) of body width at caecal bifurcation, with wall 8–24 (15; 13) thick; oesophageal gland 96 – 175 (133; 10) long or 12 – 18% (15%; 10) of body length, 26 – 44 (36; 10) wide or 39 – 71% (54%; 10) of body width at level of caecal bifurcation. Intestine bifurcating 153 – 245 (195; 13) or 19 – 27% (24%; 13) of body length from anterior body end; sinistral posterior caecum 297 – 786 (497; 13) long or 44 – 89% (60%; 13) of body length, 8 – 23 (16; 13) wide or 18 – 35% (26%; 13) of body width at level of caecal bifurcation, 5 – 14 (8; 13) wide or 5 – 21% (11%; 13) of body width at level of ovary, 8 – 14 (11; 13) wide or 11 – 28% (17%; 13) of body width at terminus; dextral posterior caecum 292 – 761 (495; 13) long or 44 – 89% (60%; 13) of body length, 8 – 23 (16; 13) wide or 14 – 35% (26%; 13) of body width at level of caecal bifurcation, 3 – 11 (8; 13) wide or 5 – 21% (11%; 13) of body width at level of ovary, 3 – 16 (11; 13) wide or 9 – 28% (17%; 13) of body width at terminus.

Intertesticular space 43 – 84 (60; 13) or 5 – 9% (7%; 12) of body length; posterior testis 190 – 311 (240; 12) or 21 – 32% (29%; 12) of body length from posterior body end. Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending 5 and 9 (2) or 1% (2) of body length anteriad, 2 and 5 (2) wide; posterior trunk of vasa efferentia emanating from ventral surface of posterior testis, ventral to gonads, extending 118 and 141 (2) or 15% and 16% (2) of body length anteriad, 2 and 5 (2) wide, meeting anterior trunk of vasa efferentia ventral and posterior to anterior testis to form vas deferens. Vas deferens ventral, extending 80 and 95 (2) or 10% and 11% (2) of body length anteriad, 2 and 5 (2) wide, expanding to form external seminal vesicle. Internal seminal vesicle 42 – 54 (50; 10) long or 61 – 84% (71%; 10) of cirrus sac length, 16 – 28 (21; 10) wide or 1.8 – 3.3 × (2.4; 10) longer than wide. Pars prostatica surrounding distal portion of internal seminal vesicle, 18 – 26 (21; 7) long or 33 – 49% (42%; 7) of internal seminal vesicle length, 16 – 25 (19; 7) wide or 0.8 – 1.4 × (1.1; 7) longer than wide. Cirrus sac transverse, abutting both caeca, enveloping entirety of internal seminal vesicle and cirrus, 40 – 81 (66; 12) long or 6 – 10% (8%; 12) of body length, 20 – 53 (40; 12) wide or 45 – 63% (54%; 12) body width at level of external seminal vesicle; cirrus 11 – 16 (13; 10) long, 4 – 11 (7; 10) wide.

Ovary ovoid in outline, transverse, 137 – 223 (171; 10) or 16 – 24% (20%; 10) of body length posterior to middle of ventral sucker, 28 – 55 (42; 12) or 4 – 7% (5%; 12) of body length posterior to genital pore; post-ovarian space 238 – 400 (326; 12) or 30 – 43% (39%; 12) of body length.

Oviduct extending posteriad 15 – 44 (33; 11) or 2 – 6% (4%; 11) of body length, 5 – 14 (8; 11) in maximum width, laterally expanding to form seminal receptacle; oviducal seminal receptacle extending sinistrad for 25 – 41 (33; 11) or 57 – 85% (69%; 4) of 32 – 46% (39%; 11) of body width, 11 – 23 (19; 11) wide or 17 – 36% (23%; 11) of body width, constricting and turning dorsad, extending anteriad for 26 – 65 (43; 10) or 3 – 7% (5%; 10) of body length before joining oötype, 7 – 12 (10; 10) wide or 7 – 18% (11%; 9) of body width (Fig. 5). Laurer's canal originating immediately distal to seminal receptacle terminus, extending posteriad 9 – 30 (19; 10) or 1 – 3% (2; 10) of body length, 5 – 11 (8; 10) wide, opening dorsally over posterior testis. Vitellarium comprising a series of interconnected spheroid masses of follicles (Fig. 5), ventro-lateral to caeca (illustrated as lateral to emphasize course of caeca), distributing from level of oesophagus median or 109 – 189 (141; 11) or 14 – 19% (16%; 11) of body length from anterior body end to excretory vesicle or 48 – 107 (68; 11) or 5 – 12% (8%; 11) of body length from posterior body end; lateral collecting ducts 5 – 23 (11; 40 [4 ducts per specimen]) wide, ventral to caeca, coalescing at level of posterior margin of ovary to form transverse vitelline duct; transverse vitelline duct ventral to ovary, 158 – 234 (195; 11) or 19 – 25% (23%; 11) of body length from middle of ventral sucker; primary vitelline collecting duct extending anteriad and dorsal 12 and 16 (2) or 1% and 2% (2) of body length before connecting with oviduct, 4 and 5 (2) wide or 5% and 6% (2) of body width. Oötype difficult to discern, 9 – 18 (12; 10) long, 9 – 16 (12; 10) wide, dorsal to ovary. Uterus originating 149 – 195 (176; 9) posteriad from middle of ventral sucker, 19 – 56 (32; 10) long or 2 – 7% (4%; 10) of body length, 11 – 28 (18; 10) wide or 10 – 35% (21%; 10) of body width; metraterm extending anterosinistrad, 14 – 35 (25; 10) long or 2 – 4% (3%; 10) of body length, 5 – 11 (8; 10) wide or 6 – 13% (9%; 10) of body width, 0.3 – 1.6 × (0.8; 10) of uterus length. Common genital pore 66 – 136 (111; 13) or 11 – 16% (13%; 13) of body length posterior to middle of ventral sucker, 4 – 10 (6; 13) in diameter.

Excretory vesicle 14 – 40 (25; 13) wide or 19 – 71% (39%; 13) of body width at level of caecal terminus; wall 4 – 12 (8; 13) thick (Fig. 5); excretory pore dorsal, subterminal, 8 – 16 (11; 13) or 1 – 2% (1%; 13) of body length from posterior body margin.

3.3.2. Taxonomic summary

Host(s): *Sternotherus minor* (Agassiz, 1857) (Testudines: Kinosternidae), loggerhead musk turtle; *Sternotherus odoratus* (Latreille in Sonnini and Latreille, 1801) (Kinosternidae), Eastern musk turtle; *Sternotherus peltifer* Smith and Glass, 1947 (Kinosternidae), stripe-necked musk turtle; *Sternotherus cf. minor* (Kinosternidae), innominate musk turtle.

Locality(ies): Wacissa River (30°19′39.80″N, 83°59′6.13″W; tributary of Aucilla River, Florida, USA); Wacissa River (30°20′24.73″N, 83°59′27.56″W); Canoe Lake (33°47′56.16″N, 86°29′25.02″W; Coosa River, Alabama, USA); Pond off Odom Creek (32°30′9.58″N, 85°26′6.07″W; Tallapoosa River, Alabama); Chewacla Creek (32°31′58.64″N, 85°29′53.09″W; Tallapoosa River, Alabama); Blue Spring (31°5′27.64″N, 86°30′53.21″W; Yellow River, Alabama).

Site in host: Mesenteric blood vessels, heart, lung, viscera wash.

Prevalence and intensity of infection by adults: Two of two (100%) *S. minor* were infected with four and 42 individuals; two of three (67%) *S. odoratus* individuals were infected both with two specimens; one *S. peltifer* individual was infected with five specimens; two of two (100%) *S.* cf. *minor* individuals were infected with 10 and 26 specimens.

Specimens deposited: Vouchers (USNM 1437606 – 1437609); GenBank (Nos. MF568030 – MF568035).

Specimens examined: Holotype of *H. reelfooti* (USNM 1321968); Vouchers of *H. reelfooti* (USNM 1393855, 1393857).

3.3.3. Remarks

This report comprises the first record of *H. reelfooti* from a river in Alabama or Florida, and its previous geographic distribution was limited to Reelfoot Lake (Mississippi River, Tennessee) as well as rivers in Virginia and Indiana (Table 1). It is the third species of *Hapalorhynchus* reported from Alabama, along with *H. conecuhensis* and *H. foliorchis* (see Roberts et al. [5]). It is the first species of *Hapalorhynchus* reported from Florida. Additionally, all but *S. odoratus* comprise new host records for *H. reelfooti*. This is the first record of both *S. minor* and *S. peltifer* as TBF hosts.

Because the holotype of *H. reelfooti* (USNM 1321968) is in poor condition, apparently destained and strongly contracted (Platt and Snyder [3]), we compared our newly collected specimens to Platt and Snyder's [3] vouchers (USNM 1393855, 1393857) rather than relying primarily on the holotype. All of these specimens have minute, spine-like projections in the forebody and hindbody. These projections are distributed over the nearly the entire body surface and appear to be the distal tips of minute sensory papillae. *Hapalorhynchus conecuhensis*, *H. reelfooti*, and *H.* cf. *stunkardi* (below) are the only species of *Hapalorhynchus* confirmed to have them.

3.4. *Hapalorhynchus cf. stunkardi* (Figs. 6, 7)

3.4.1. Diagnosis of adult (based on light microscopy of 2 specimens [vouchers USNM 1437610, 1437611])

With features of *H. stunkardi* (see Byrd [8]; Platt [6]) except for the following. Forebody (middle of ventral sucker to anterior body end) 415 and 368 [460] or 42% and 49% [32%] of body length; hindbody (middle of ventral sucker to posterior body end) 575 and 382 [980] or 58% and 51% [68%] of body length or 1.4 × and 1.0 × [2.1 ×] longer than forebody. Oral sucker 60 and 48 [70] long or 6% (2) [5%] of body length, 60 and 58 [93] wide or 38% and 37% [29%] of body width at level of caecal bifurcation. Ventral sucker 113 and 95 [136] long or 11% and 13% [9%] of body length or 1.9 × and 2.0 × [1.9 ×] longer than oral sucker, 88 and 93 [141] wide or 49% and 52% [43%] of body width at level of ventral sucker or 1.5 × and 1.6 × [1.5 ×] wider than oral sucker. Nerve commissure 183 and 160 [168] long or 18% and 21% [12%] of body length from anterior body end. Pharynx 38 (2) [39] long or 14% and 15% [15%] of oesophagus length, 58 and 60 [91] wide or 2.1 × and 3.3 × [1.6 ×] wider than maximum oesophagus width. Oesophagus 13 (2) [20] wide posterior to pharynx with wall 8 and 10 [15] thick, 28 and 18 [57] wide or 18% and 11% [18%] of body width at mid-esophagus with wall 18 and 15 [34] thick, 38 and 43 [64] or 24% and 27% [20%] of body width at caecal bifurcation, with wall 23 and 35 [50] thick; anterior oesophageal gland 115 and 75 [73] long or 80% and 29% [27%] of oesophagus length, 58 and 45 [141] wide or 36% and 28% [43%] of body width at level of caecal bifurcation; posterior oesophageal gland 125 and 100 [123] long or 47% and 39% [46%] of oesophagus length, 75 and 50 [143] wide or 47% and 32% [44%] of body width at level of caecal bifurcation. Intestine bifurcating 320 and 238 [297] or 32% (2) [21%] of body length from anterior body end; sinistral posterior caecum 535 and 450 [1042] long or 54% and 60% [72%] of body length, 48 and 28 [68] wide or 30% and 18% [21%] of body width at level of caecal bifurcation, 20 and 15 [41] wide or 10% (2) [14%] of body width at level of ovary, 28 and 18 [48] wide or 19% and 13% [18%] of body width at terminus; dextral posterior caecum 570 and 435 [1069] long or 58% (2) [74%] of body length, 43 and 30 [80] wide or 27% and 19% [25%] of body width at level of caecal bifurcation, 30 and 20 [18] wide or 15% and 13% [6%] of body width at level of ovary, 40 and 20 [57] wide or 27% and 14% [21%] of body width at terminus.

Anterior testis 45 and 38 [127] wide or 23% and 25% [43%] of body width at level of ovary or 100% and 76% [76%] of posterior testis width; intertesticular space 58 and 40 [86] or 6% and 5% [6%] of body length. Posterior testis 45 and 50 [168] wide or 23% and 33% [56%] of body width at level of ovary. Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending 18 and 21 [n/a] or 2% and 1% [n/a] of body length anteriad, 7 and 4 [n/a] wide; posterior trunk of vasa efferentia emanating from ventral surface of posterior testis, ventral to gonads, extending 126 and 123 [n/a] or 13% and 16% [n/a] of body length anteriad, 7 and 4 [n/a] wide, meeting anterior trunk of vasa efferentia ventral and posterior to anterior testis to form vas deferens. Vas deferens ventral, extending 72 and 39 [n/a] long or 7% and 5% [n/a] of body length anteriad, 5 and 7 [n/a] wide, expanding to form external seminal vesicle. External seminal vesicle 53 and 28 [57] long or 5% and 4% [4%] of body length, 33 and 13 [202] wide or 17% and 7% [64%] of body width or 1.6 × and 2.2 × [0.3 ×] longer than wide, intercaecal (Fig. 6); internal seminal vesicle 60 and 47 [136] long or 68% and 75% [59%] of anterior testis length, 28 and 12 [43] wide or 2.1 × and 3.9 × [3.2 ×] longer than wide. Pars prostatica surrounding distal portion of internal seminal vesicle, 28 and 20 [91] long or 47% and 43% [67%] of internal seminal vesicle length, 25 and 13 [59] wide or 1.1 × and 1.5 × [1.5 ×] longer than wide. Cirrus sac obvious, abutting both caeca or medial, enveloping entirety of internal seminal vesicle and cirrus, 88 and 63 [232] long or 9% and 8% [16%] of body length, 45 and 35 [150] wide or 23% and 20% [47%] of body width at level of external seminal vesicle; cirrus 20 and 9 [27] long, 25 and 8 [30] wide.

Ovary triangular in outline, sinistral, 193 and 165 [382] or 19% and 22% [27%] of body length posterior to middle of ventral sucker, 38 (2) [70] or 4% and 5% [5%] of body length posterior to genital pore; post-ovarian space 325 and 235 [473] or 33% and 31% [33%] of body length. Oviduct extending posteriad 38 and 48 [111] or 4% and 6% [8%] of body length, 8 and 13 [16] in maximum width, laterally expanding to form seminal receptacle; oviducal seminal receptacle extending sinistrad for 35 and 38 [68] or 18% and 25% [23%] of ovary width, 20 and 18 [39] wide or 10% and 12% [13%] of body width, constricting and turning dorsad, extending anteriad for 63 and 50 [125] or 6% and 7% [9%] of body length before joining oötype, 8 and 10 [25] wide or 4% and 7% [8%] of body width (Fig. 6). Laurer's canal originating immediately distal to seminal receptacle terminus, extending posteriad 30 and 47 [45] or 3% and 6% [3%] of body length, 5 and 11 [25], opening dorsally over posterior testis. Vitellarium weakly developed in specimens (Fig. 6), distributing from level of oesophagus median or 214 and 125 [184] or 22% and 17% [13%] of body length from anterior body end to excretory vesicle or 73 and 43 [45] or 7% and 6% [3%] of body length from posterior body end; lateral collecting ducts difficult to discern [n/a]; transverse vitelline duct ventral to ovary, 227 and 184 [491] or 23% and 25% [34%] of body length from middle of ventral sucker; primary vitelline collecting duct not observed [n/a]. Oötype 23 (2) [34] long, 18 and 13 wide [27], ventrolateral to ovary and anterior testis.

Uterus originating 180 and 150 [386] or 18% and 20% [27%] of body length posteriad from middle of ventral sucker; metraterm extending anterosinistrad, 20 (2) [45] long or 2% and 3% [3%] of body length, 13 and 10 [23] wide or 7% (2) [8%] of body width, 80% and 71% [49%] of uterus length. Uterine egg not observed [ovoid], [86] long by [50] wide. Common genital pore 150 and 125 [309] or 15% and 17% [21%] of body length posterior to middle of ventral sucker, 10 and 8 [11] in diameter.

Excretory vesicle 43 and 40 [109] wide or 29% (2) [40%] of body width at level of caecal terminus; excretory pore dorsal, subterminal, 15 and 8 [7] or 2% and 1% [< 1%] of body length from posterior body margin (Fig. 6).

3.4.2. Taxonomic summary

Host(s): *Sternotherus minor* (Agassiz, 1857) (Testudines: Kinosternidae), loggerhead musk turtle; *Sternotherus odoratus* (Latreille in Sonnini and Latreille, 1801) (Kinosternidae), Eastern musk turtle.

Locality(ies): Wacissa River (30°20′24.73″N, 83°59′27.56″W; Florida, USA); Pond off Parkerson Mill Creek (32°35′20.04″N, 85°29′41.51″W; Tallapoosa River, Alabama, USA).

Site in host: Heart, viscera wash.

Prevalence and intensity of infection by adults: One loggerhead musk turtle had one specimen and one of three (33%) Eastern musk turtles had one specimen.

Specimens deposited: Vouchers (USNM 1437610, 1437611).

Specimens examined: Holotype of *H. gracilis* (AMNH 125); Vouchers of *H. foliorchis* (USNM 1422462 – 1422464); Holotype of *H. stunkardi* (USNM 1321967); Holotype of *H. reelfooti* (USNM 1321968); Vouchers of *H. reelfooti* (USNM 1393855, 1393857); Holotype of *H. brooksi* (USNM 1375720).

3.4.3. Remarks

This is the first record of *H.* cf. *stunkardi* from a river in Alabama or Florida, and this species was previously known only from an aquatic locality probably within the North Oconee River (near Athens, Georgia, USA). It is the fourth species of *Hapalorhynchus* reported from an Alabama river. Both of the reported hosts comprise new host records for *H.* cf. *stunkardi*.

Our specimens of *H.* cf. *stunkardi* resembled the holotype (USNM 1321967) and published descriptions (Byrd [8]; Platt [6]) of *H. stunkardi* by having a similar ventral sucker/anterior sucker ratio (1.9 × and 2.0 × anterior sucker length vs. 2.4 × for *H. stunkardi*), an obvious cirrus sac, a vitellarium extending from the oesophagus to the excretory vesicle, and a uterus that is lateral and slightly ventral to the anterior testis and ovary. Specimens of *H.* cf. *stunkardi* differed from those of *H. stunkardi* in numerous subtle ways: having a longer forebody (42% and 49% vs. 32% of body length), shorter caeca (52 – 60% of body length vs. 72 – 74%), smaller testes (ant. testis: 23% and 25% of body width vs. 43%; post. testis: 23% and 33% of body width vs. 56%), a narrower external seminal vesicle (1.6 × and 2.2 × longer than wide vs. 0.3 ×), a smaller cirrus sac (9% and 8% of body length vs. 16%; 23% and 20% of body width vs. 47%), and a longer metraterm (80% and 71% of uterus length vs. 49%). Despite these morphological differences, and because Byrd's [8] original description is incomplete and we lack molecular sequence data from specimens of *H. stunkardi* collected from the type host and type locality, we do not think that the specimens of *H.* cf. *stunkardi* warrant a new species description at this time. Moreover, the two specimens comprising the description above appear to be young adults with developing gonads and genitalia (Fig. 6). Larger adults from these hosts and localities are needed to address intra- or interspecific variation among these closely related species of *Hapalorhynchus*. Future workers should rely upon Platt [6] and the description herein to identify newly-collected TBFs that resemble *H. stunkardi*.

Recent taxonomic revisions and geographic range studies of species of *Sternotherus* suggest that the type host for *H. stunkardi* must be reconsidered. Byrd's [8] description and the holotype slide label indicated that the host was a razor-backed musk turtle (*S. carinatus*) from Athens (see above; Table 1). This turtle ranges throughout the Gulf Coastal Plain from southeastern Oklahoma, central Arkansas, and into Mississippi from the Chickasawhay River south to the Gulf of Mexico (Ernst and Lovich [33]). Blankenship et al. [34] extended the range eastward to include the Escatawpa River, Mobile County, Alabama. If Byrd [8] necropsied a wild-caught turtle near Athens (North Oconee River), that host was likely the loggerhead musk turtle; not the razor-backed musk turtle as he reported. The loggerhead musk turtle was once considered to be a subspecies of the razor-backed musk turtle and ranges from east central Georgia and east central and southeastern Alabama south to central Florida (Iverson [35]; Lindeman [36]; Ernst and Lovich [33]; van Dijk et al. [10]). Unpublished records of loggerhead musk turtles from Athens exist (G. Brown, personal communication JRR).

3.5. Phylogenetic analysis

The reconstructed 28S rDNA phylogeny (Fig. 7) did not refute our morphology-based conclusions regarding respective conspecificity of our specimens of *H. conecuhensis* and *H. reelfooti*. The clade including the blood flukes (*H. foliorchis* and *H. gracilis*) that infect the common snapping turtle, *Chelydra serpentina* (Linnaeus, 1758), and those (*H. conecuhensis* and *H. reelfooti*) infecting musk turtles was strongly supported (posterior probably, pp = 1.00). Isolates of *H. reelfooti* were identical, and an isolate of *H. conecuhensis* (MF568038) differed by 1 base pair (an inserted T at alignment position 1592) from the otherwise identical 28S sequences for the other two conspecific specimens of *H. conecuhensis* (MF568036, MF568037). An updated, broad-scale molecular phylogeny that treats generic interrelationships of TBFs is in preparation, but that *Coeuritrema* and *Hapalorhynchus* share a recent common ancestor has been proposed previously (Orélis-Ribeiro et al. [32]).

4. Discussion

4.1. Future directions for kinosternid TBF discovery

This is the first report of an infected musk turtle from the Coosa and Tallapoosa rivers (Mobile-Tensaw River Basin), Yellow River (Pensacola Bay Basin), or Wacissa River (Apalachee Bay Basin). Despite the broad sampling of species of *Sternotherus* for the present study, kinosternids as a whole remain largely unsampled for TBF infections, with only five of the 26 species (19%) reported as hosts. We report four of the six species of *Sternotherus* as TBF hosts, with the razor-backed musk turtle (*S. carinatus*) and the flattened musk turtle (*S. depressus*) still unsampled for blood flukes. The flattened musk turtle is a threatened species that can only be found in permanent streams of the Black Warrior River system above the Fall Line in Alabama (Guyer et al. [11]). Future sampling of razor-backed musk turtles is likely, but sampling of flattened musk turtles for TBFs is highly unlikely due to their federal and state listing (Guyer et al. [11]). The TBFs infecting mud turtles (*Kinosternon* Spix, 1824) are largely still unknown, with only one of 19 species reported as a TBF host (*H. albertoi* ex. white-lipped mud turtle [*K. leucostomum*]). *Hapalorhynchus albertoi* is a unique congener not only because it is the only TBF known from a mud turtle, but also because it is the only species of *Hapalorhynchus* reported from a kinosternid with large radially extending lateral oesophageal diverticula, resembling those of some species of *Vasotrema* (see Lamothe-Argumedo [7]; Roberts and Bullard [23]). Of the 19 species of *Kinosternon*, only two are reported as ranging in the Southeastern United States: *Kinosternon baurii* Garman, 1891 and *Kinosternon subrubrum* (Bonnaterre, 1789), with the rest (17 species) ranging in the Southwestern United States down through to South America (van Dijk et al. [10]). Continued parasitological surveying of previously unsampled mud turtle species will help us better understand the true diversity of kinosternid blood flukes and their phylogenetic position within *Hapalorhynchus*.

4.2. Interrelationships of Hapalorhynchus spp.

Gene sequence data analysis suggested that the species of *Hapalorhynchus* infecting musk turtles are closely related to *H. gracilis* (type species) and *H. foliorchis* (Fig. 7), i.e., the only other congeners for which 28S sequence data are published. As the node grouping *H. gracilis* and *H. foliorchis* with *H. conecuhensis* is weakly supported (pp = 0.51), testing monophyly of the musk turtle TBFs is not possible without additional data. Despite some morphological similarities between this genus and *Coeuritrema*, these molecular data do not reject our morphology-based acceptance of *H. reelfooti* and *H. conecuhensis* as congeneric with *H. gracilis* and *H. foliorchis*. Our molecular analysis included four of the seven North American species of *Hapalorhynchus* (Fig. 7). *Hapalorhynchus conecuhensis* and *H. reelfooti* (blood flukes of *Sternotherus* spp. [Kinosternidae]) differ from *H. gracilis* and *H. foliorchis* (blood flukes of common snapping turtles [Stunkard [28]; Brooks and Mayes [37]) by having an obvious (well-developed) cirrus sac. Sequence data from *H. brooksi*, which infects common snapping turtles not musk turtles, would be a valuable addition to future analyses because *H. brooksi* is the only congener that reportedly has paired anterior caeca in addition to an obvious cirrus sac. Whereas we only have gene sequence data coverage for just over half of the known North American species of *Hapalorhynchus*, we lack a gene sequence for an Asian or African species of *Hapalorhynchus*. Future discovery and new collections of species of *Hapalorhynchus* from Asian and African turtles is imperative to better understand the taxonomy and systematics of this geographically wide-ranging TBF genus.

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