
Jackson R. Roberts and Stephen A. Bullard
Aquatic Parasitology Laboratory, School of Fisheries, Aquaculture, and Aquatic Sciences, College of Agriculture, Auburn University, 203 Swingle Hall, Auburn, Alabama 36849. Correspondence should be sent to Jackson R. Roberts at: jrr0035@auburn.edu

ABSTRACT: Gulf Coast spiny softshell turtles, Apalone spinifera aspera (Agassiz, 1857) (Testudines: Trionychidae) from Canoe Lake (33°47’56.16”N, 86°29’25.02”W; Springville, Alabama) and Round Lake (32°41’50.91”N, 87°14’30.39”W; Perry Lakes State Park, Marion, Alabama), were infected by V. robustum Stunkard, 1928, Vasotrema longitestis Byrd, 1939, and Vasotrema rileyae n. sp. The new species differs from its congeners by having papillate suckers, a short testis, an ovary dextral to the oviduct, and a pre-ovarian genital pore that is lateral to the ventral sucker. We studied the newly collected specimens and museum specimens of all congeners to revise the diagnosis of Vasotrema Stunkard, 1926 and redescribe and provide an updated dichotomous key to all species of the genus.

MATERIALS AND METHODS

Four Gulf Coast spiny softshell turtles were collected with the use of hoop nets baited with store-bought chicken liver and fish on 25 June 2015 and 23 July 2015 from Canoe Lake (33°47’56.16”N, 86°29’25.02”W), Springville, Alabama, and Round Lake (32°41’50.91”N, 87°14’30.39”W), Perry Lakes State Park, Marion, Alabama, respectively. Turtles were transported alive to the laboratory in a cooler with pond water within an air-conditioned vehicle cab, decapitated before necropsy, and examined with the aid of 7.0 g/L sodium citrate saline solution and a stereo-dissection microscope. Each host organ (brain, eye, heart, lung, spleen, liver, intestine, mesentery, kidney, rectum) was isolated in a glass container filled with saline. Portions of each organ then were excised and macerated in a petri dish while being viewed under high magnification with a Meiji Techno RZ (Meiji, Saitama, Japan) dissection microscope until the entire organ had been examined. The sediment from each petri dish and holding container was then examined to gather TBFs that had crawled or fallen from the excised organ/tissue. Living flukes were pipetted from saline dishes, concentrated in a clean glass dish with saline, rinsed in saline, pipetted onto glass slides, cover-slipped (only to ensure the flukes remained flat; no pressure exerted on specimen by coverslip), and killed with a 2-sec exposure to heat emitted by a butane hand lighter. After heat killing, a few drops of a graded ethanol series, dehydrated in absolute EtOH and xylene, with several drops of Ehrlich’s hematoxylin, dehydrated with a balsam.

Whole mounts were examined with the use of both Leica DM 2500 (Leica, Wetzlar, Germany) and Leica DMR microscopes, both equipped with differential interference contrast (DIC). Illustrations were made using both scopes equipped with drawing tubes. Measurements were obtained with a calibrated ocular micrometer (as straight lines along the course of each duct) and...
Table I. Host and geographic locality records and museum specimens of *Vasotrema* spp.

<table>
<thead>
<tr>
<th>Turtle host</th>
<th>Vasotrema sp.</th>
<th>Site in host</th>
<th>Riverine locality</th>
<th>Accession no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apalone ferox (Schneider, 1783)</td>
<td><em>Vasotrema amydae</em> Stunkard, 1926 (type species)</td>
<td>Blood (adult)</td>
<td>None specified, Florida</td>
<td>AMNH 791*</td>
<td>Stunkard (1926, 1928)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema attenuatum</em> Stunkard, 1928</td>
<td>Blood (adult)</td>
<td>None specified, Florida</td>
<td>AMNH 806, 807†</td>
<td>Stunkard (1928)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema robustum</em> Stunkard, 1928</td>
<td>None specified (adult)</td>
<td>None specified, probably Fort Myers, Florida</td>
<td>USNM 37306‡</td>
<td>Wall (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumen of heart, blood vessels of liver, lung, intestine (adult)</td>
<td>Lake Okeechobee, Palm Beach County, Florida</td>
<td>HWML 39326</td>
<td>Foster et al. (1998)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema</em> sp.</td>
<td>None specified (adult)</td>
<td>None specified, probably Fort Myers, Florida</td>
<td>USNM 37306‡</td>
<td>Wall (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HWML 20075</td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td>Apalone mutica (LeSueur, 1827)</td>
<td><em>Vasotrema</em> sp.</td>
<td>Blood (adult)</td>
<td>Ochlockonee River, Leon County, Florida</td>
<td>None specified</td>
<td>Loftin (1960)</td>
</tr>
<tr>
<td></td>
<td><em>V. attenuatum</em></td>
<td>Blood (adult)</td>
<td>None specified, Nebraska</td>
<td>None specified</td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema brevitestis</em> Brooks and Mayes, 1975</td>
<td>Blood (adult)</td>
<td>Missouri River, site (41°31'22.34&quot;N, 96°8'7.30&quot;W) 2.4 km south of Blair, Nebraska</td>
<td>USNM 73817, 73818; HWML 20077</td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td></td>
<td><em>V. robustum</em></td>
<td>None specified (adult)</td>
<td>None specified (possibly Cumberland River, Davidson County, Tennessee)</td>
<td>USNM 37306‡</td>
<td>Wall (1951)</td>
</tr>
<tr>
<td>Apalone spinifera (LeSueur, 1827)</td>
<td><em>V. amydae</em> (type species)</td>
<td>Circulatory system (adult)</td>
<td>None specified, Nebraska</td>
<td>None specified</td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td></td>
<td><em>V. attenuatum</em></td>
<td>Blood (adult)</td>
<td>None specified, Indiana</td>
<td>None specified</td>
<td>AMNH 791*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesenteric blood vessels (adult)</td>
<td>Reelfoot Lake (36°21'12.23&quot;N, 89°25'21.50&quot;W), Tennessee</td>
<td>USNM 9227</td>
<td>Byrd (1939); Platt and Snyder (2007); present study</td>
</tr>
<tr>
<td></td>
<td><em>V. brevitestis</em></td>
<td>Blood (adult)</td>
<td>Huron River, Washtenaw County, Michigan</td>
<td>None specified</td>
<td>Wall (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None specified</td>
<td>None specified, Indiana</td>
<td>None specified</td>
<td>AMNH 806, 807†</td>
</tr>
<tr>
<td></td>
<td><em>V. robustum</em></td>
<td>Blood (adult)</td>
<td>Atkinson Lake (42°32'20.36&quot;N, 99°3'04.92&quot;W), 0.8 km west of Atkinson, Nebraska</td>
<td>USNM 73819; HWML 20076</td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arterial circulation (adult)</td>
<td>Reelfoot Lake (36°21'12.23&quot;N, 89°25'21.50&quot;W), Tennessee</td>
<td>USNM 1322971; HWML 31121</td>
<td>Byrd (1939); Platt and Prestwood (1990)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema longitestis</em> Byrd, 1939</td>
<td>Blood</td>
<td>None specified, Indiana</td>
<td>AMNH 808, 809</td>
<td>Stunkard (1928)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ventricle of heart (adult)</td>
<td>Reelfoot Lake (36°21'12.23&quot;N, 89°25'21.50&quot;W), Tennessee</td>
<td>None specified</td>
<td>Byrd (1939)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumen of heart and other large blood vessels (adult)</td>
<td>Huron River, Washtenaw County, Michigan</td>
<td>USNM 37306*</td>
<td>Wall (1951)</td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema</em> longitestis</td>
<td>Blood (adult)</td>
<td>Cumberland River, Davidson County, Tennessee</td>
<td>None specified, Nebraska</td>
<td>HWML 20075</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None specified (adult)</td>
<td></td>
<td></td>
<td>HWML 45795</td>
</tr>
<tr>
<td>Apalone spiniferaaspera (Agassiz, 1857)</td>
<td><em>V. longitestis</em></td>
<td>Mesenteric blood vessels (adult)</td>
<td>Canoe Lake (33°47'56.16&quot;N, 86°29'25.02&quot;W), Coosa River, Springville, Alabama</td>
<td>USNM 1422437-1422444</td>
<td>Present study</td>
</tr>
</tbody>
</table>
### Table I. Continued.

<table>
<thead>
<tr>
<th>Turtle host</th>
<th>Accession no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. attenuatum</td>
<td>None specified</td>
<td>None specified</td>
</tr>
</tbody>
</table>

#### Description

**Vasotrema Stunkard, 1926 emended**

*(Figs. 1–20)*

Diagnosis: Body dorsoventrally flattened (not cylindrical), 3–20x longer than wide, aspinous; ventral body surface papillate. Oral sucker spheroid, papillate or apapillate, spinous or aspinous; paired terminal papillae present or absent; paired internal mouth papillae present or absent. Ventral sucker papillate or apapillate. Pharynx present, enveloping anterior extremity of esophagus. Esophagus sinuous, extending posteriad approximately 1/10–1/4 of body length, having numerous lateral esophageal diverticula and a single median esophageal diverticulum and associated esophageal gland; lateral esophageal diverticula surrounding esophagus for entire length, becoming larger and more numerous posteriorly; median esophageal diverticulum dorsal to intestinal bifurcation; esophageal gland surrounding esophagus from posterior margin of pharynx to cecal bifurcation, strongly basophilic, widest surrounding mediolateral esophageal diverticulum (Figs. 5, 6). Intestine inverse U-shaped, comprising paired ceca; each cecum extending 1/2–3/4 of body length directly posteriad and in parallel with body margin, terminating in posterior end of body, smooth (lacking diverticula). Testis coiled or not, intercecal. Vas deferens extending anteriad from and ventral to anterior half of testis. External seminal vesicle present or indistinct, pre-testicular, intercecal. Internal seminal vesicle present. Cirrus sac pre-testicular. Cirrus straight. Ovary lobed or not, intercecal, pre-testicular, between cirrus sac and testis. Oviduct emerging from sinistral, dextral, or posterior margin of ovary, extending posteriad or sinistral. Oviducal seminal receptacle comprising middle portion of oviduct, at level of or posterior to ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary, extending posteriorly. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distributing from cecal bifurcation to Manter’s organ. Ootype diminutive, pre-oviduct, opening dorsally. Vitellarium follicular, distributing from ovarian or post-ovarian, extending anteriad or posteriad from ovary. Laurer’s canal intercecal, pre-testicular, pre-ovarian or post-ovarian, extending anteriad or posteriad from oviduct, opening dorsally. Vitellarium follicular, distrib
Table II. Turtle blood fluke specimens examined in the present study.

<table>
<thead>
<tr>
<th>Vasotrema sp.</th>
<th>Slide label</th>
<th>Accession no.</th>
<th>No. slides</th>
<th>Specimen</th>
<th>Host</th>
<th>Locality</th>
<th>Notes</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vasotrema amydae</em></td>
<td>AMNH 791</td>
<td>1</td>
<td></td>
<td>Cotype</td>
<td>Not specified (either <em>Apalone ferox</em> [Schneider, 1783] or <em>Apalone spinifera</em> [LeSueur, 1827])</td>
<td>Not specified (either Florida or Indiana river drainage)</td>
<td>Two specimens on slide; specimen mounted center is isotype</td>
<td>Stunkard (1926, 1928)</td>
</tr>
<tr>
<td><em>Vasotrema attenuatum</em></td>
<td>AMNH 806</td>
<td>17</td>
<td></td>
<td>Syntypes</td>
<td>Not specified (either <em>A. ferox</em> or <em>A. spinifera</em>); <em>A. spinifera</em> for slides 806-1, 2, 12, 16</td>
<td>Not specified (either Florida or Indiana river drainage); Indiana, for slides 806-1, 2, 12, 16</td>
<td>Slides 806-1, 2, 12, 16 have <em>Apalone spinifera</em> (labeled as <em>Amyda spinifera</em>)</td>
<td>Stunkard (1928)</td>
</tr>
<tr>
<td><em>Vasotrema robustum</em></td>
<td>AMNH 808</td>
<td>1</td>
<td></td>
<td>Syntype</td>
<td>Not specified (<em>A. spinifera</em>; see Stunkard, 1928)</td>
<td>Not specified, Indiana V. amydae is written, with amydae scratched out</td>
<td></td>
<td>Stunkard (1928)</td>
</tr>
<tr>
<td><em>Vasotrema longitestis</em></td>
<td>USNM 1321971</td>
<td>1</td>
<td></td>
<td>Holotype</td>
<td><em>A. spinifera</em> (labeled as <em>Amyda spinifera</em>)</td>
<td>Reelfoot Lake (36°21'12.23&quot;N, 89°25'21.50&quot;W), Tennessee</td>
<td></td>
<td>Byrd (1939)</td>
</tr>
<tr>
<td><em>Vasotrema brevittestis</em></td>
<td>HWML 20076</td>
<td>4 (loaned 2)</td>
<td></td>
<td>Paratypes</td>
<td><em>A. spinifera</em> (labeled as <em>Trionyx spinifera</em>)</td>
<td>Atkinson Lake (42°32'20.36&quot;N, 99°0'3.04&quot;W), 0.8 km west of Atkinson, Nebraska</td>
<td></td>
<td>Brooks and Mayes (1975)</td>
</tr>
<tr>
<td></td>
<td>HWML 20077</td>
<td>6 (loaned 3)</td>
<td></td>
<td>Paratypes</td>
<td><em>Apalone mutica</em> (LeSueur, 1827) (labeled as <em>Trionyx muticae</em>)</td>
<td>Missouri River, site (41°31'22.34&quot;N, 96° 8'7.30&quot;W) 2.4 km south of Blair, Nebraska</td>
<td></td>
<td>Brooks and Mayes (1975)</td>
</tr>
</tbody>
</table>


Type species: *Vasotrema amydae* Stunkard, 1926

Remarks

Stunkard (1926) proposed *Vasotrema* (misspelled therein as "Vasatrema," which thereby is junior synonym of *Vasotrema* ([International Commission on Zoological Nomenclature, 2000]) to accommodate a new TBF infecting Florida softshell turtles and spiny softshell turtles. Stunkard (1928) later named 2 additional congeners and corrected the genus spelling, an error that he attributed to “transcription error.” The 5 nominal species of *Vasotrema* (*V. amydae, V. attenuatum, V. robustum, V. longitestis, V. brevitestis*) infect softshell turtles (Trionychidae) only, and we accept all of them as distinct taxa (see dichotomous key).

Recent molecular analyses indicate that *Vasotrema* is most closely related to *Spirorchis* MacCallum, 1918 and *Spirhapalum* Ejsmont, 1927 ([Orélis-Ribeiro et al., 2014; Roberts et al., 2016a, 2016c]). *Vasotrema* resembles these TBFs by having an oral sucker, lateral esophageal diverticula, U-shaped ceca terminating in the posterior body end, a putative transverse vitelline duct, and a ventral and sinistral common genital pore (Roberts et al., 2016c). *Vasotrema* can be differentiated from *Spirorchis* by having a ventral sucker, a median esophageal diverticulum dorsal to the cecal bifurcation, a single testis, and a pre-testicular ovary. *Spirorchis* lacks a ventral sucker and has a median esophageal diverticulum ventral to the cecal bifurcation (when present), a testicular column comprising 4–11 testes, and a post-testicular ovary. *Vasotrema* differs from *Spirhapalum* by having a single testis and a pre-testicular ovary. *Spirhapalum* has a pre-ovarian testicular column comprising 4–12 testes with a post-ovarian testis as well. Further comparisons of *Vasotrema* and *Spirhapalum* are impossible without obtaining type materials because their original descriptions are incomplete (Ejsmont, 1927; Rohde et al., 1968).

We herein revise the generic diagnosis for *Vasotrema* to include features associated with spines, papillae, pharynx, esophageal diverticula, vitellarium, and uterus. Some of these features were misinterpreted or omitted from previous concepts of the genus.

---

**Figures 1, 2.** *Vasotrema amydae* Stunkard, 1926 (cotype, AMNH Coll. No. 791) from spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae), or Florida softshell turtle, *Apalone ferox* (LeSueur, 1827), from a river in Indiana or Florida. Scale values beside bars. (1) Body (dorsal view) showing oral sucker (os), paired terminal papillae (tp), pharynx (ph), nerve commissure (nc), esophagus (es), esophageal gland (eg), median esophageal diverticulum (med), sinistral cecum (sc), ventral sucker (vs), dextral cecum (dc), common genital pore (cgp), cirrus sac (cs), uterus (ut), external seminal vesicle (esv), ovary (ov), vitellarium (vr), testis (ts), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (2) Genitalia (dorsal view) showing cirrus (ci), metraterm (mt), internal seminal vesicle (isv), ootype (oo), ovi-vitelline duct (ovt), oviduct (od), oviducal seminal receptacle (osr), Laurer’s canal (Lc), primary vitelline duct (vt), and putative vas deferens (pv).
**Figures 3–6.** *Vasotrema attenuatum* Stunkard, 1928 (syntype, AMNH Coll. No. 806-4) from spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae), or Florida softshell turtle, *Apalone ferox* (LeSueur, 1827), from a river in Indiana or Florida. (3) Body (dorsal view) showing oral sucker (os), ventral sucker (vs), common genital pore (cgp), ovary (ov), testis (ts), and cecal terminus (ct). Scale value beside bar; dashed line indicates body segments illustrated at higher magnification in Figures 4 and 5. (4) Anterior portion of body (dorsal view) showing pharynx (ph), esophagus (es), nerve commissure (nc), esophageal gland (eg), median esophageal diverticulum (med), sinistral cecum (sc), dextral cecum (dc), ventral sucker (vs), cirrus sac (cs), common genital pore (cgp), metraterm (mt), cirrus (ci), uterus (ut), internal seminal vesicle (isv), ovary (ov), Laurer’s canal (Lc), oviduct (od), oviducal seminal receptacle (osr), vas deferens (vd), vitelline duct (vt), and testis (ts). (5) Posterior portion of body (dorsal view) vitellarium (vt), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (6) Genitalia (dorsal view) showing ootype (oo) and ovi-vitelline duct (ovt).
**FIGURES 7, 8.** *Vasotrema robustum* Stunkard, 1928 (syntype, AMNH Coll. No. 808) from spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae), from a river in Indiana. Scale values beside bars. (7) Body (ventral view) showing oral sucker (os), pharynx (ph), nerve commissure (nc), esophagus (es), esophageal gland (eg), median esophageal diverticulum (med), dextral cecum (dc), sinistral cecum (sc), ventral sucker (vs), vitellarium (vr), external seminal vesicle (esv), internal seminal vesicle (isv), ovary (ov), common genital pore (cgp), oviducal seminal receptacle (osr), primary vitelline duct (vt), vas deferens (vd), testis (ts), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (8) Genitalia (ventral view) showing cirrus sac (cs), cirrus (ci), oviduct (od), metraterm (mt), uterine egg (egg), uterus (ut), and o¨otype (oo).
FIGURES 9–11. *Vasotrema robustum* Stunkard, 1928 (voucher, USNM 1422436) from Gulf Coast spiny softshell turtle, *Apalone spinifera aspera* (Agassiz, 1857) (Testudines: Trionychidae), from Canoe Lake (33°47′56.16″N, 86°29′25.02″W), Coosa River, Springville, Alabama. Scale values beside bars. (9) Body (ventral view) showing paired terminal papillae (tp), oral sucker (os), pharynx (ph), esophagus (es), nerve commissure (nc), esophageal gland (eg), median esophageal diverticulum (med), dextral cecum (dc), sinistral cecum (sc), ventral sucker (vs), vitellarium (vr), internal seminal vesicle (isv), external seminal vesicle (esv), common genital pore (cgp), ovary (ov), uterus (ut), oviducal seminal receptacle (osr), primary vitelline duct (vt), testis (ts), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (10) Oral sucker (ventral view) showing marginal spines (ms), internal mouth spines (ims), and paired internal mouth papillae (ipa). (11) Genitalia (ventral view) showing cirrus sac (cs), cirrus (ci), metraterm (mt), oviduct (od), ootype (oo), Laurer’s canal (Lc), and vas deferens (vd).
Figures 12–14. *Vasotrema longitestis* Byrd, 1939 from spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae) (12), and Gulf Coast spiny softshell, *Apalone spinifera aspera* (Agassiz, 1857) (Testudines: Trionychidae) (13, 14). Scale values beside bars. (12) Body (holotype, USNM 1321971, from Reelfoot Lake [36°21'12.23"N, 89°25'21.50"W], Tennessee) (ventral view) showing oral sucker (os), pharynx (ph), esophagus (es), common genital pore (cgp), ventral sucker (vs), metraterm (mt), cirrus sac (cs), uterine egg (egg), ovary (ov), testis (ts), and excretory vesicle (ev). (13) Body (voucher, USNM 1422437, from Canoe Lake [33°47'56.16"N, 86°29'25.02"W], Coosa River, Springville, Alabama) (ventral view) showing nerve commissure (nc), median esophageal diverticulum (med), esophageal gland (eg), dextral cecum (dc), sinistral cecum (sc), vas deferens (vd), primary vitelline duct (vt), vitellarium (vr), cecal terminus (ct), Manter’s organ (Mo), and excretory pore (ep). (14) Genitalia (voucher, USNM 1422473, from Canoe Lake [33°47'56.16"N, 86°29'25.02"W], Coosa River, Springville, Alabama) (ventral view) showing cirrus (ci), metraterm (mt), internal seminal vesicle (isv), egg chamber (ec), Laurer’s canal (Lc), oviduct (od), and oviducal seminal receptacle (osr).
FIGURES 15–18. *Vasotrema brevitestis* Brooks and Mayes, 1975 (paratype, HWML Coll. No. 20077) from midland smooth softshell turtle, *Apalone mutica* (LeSueur, 1827) (Testudines: Trionychidae), from the Missouri River, Nebraska. Scale values beside bars. (15) Body (paratype, HWML 20077-1) (ventral view) showing oral sucker (os), pharynx (ph), nerve commissure (nc), esophagus (es), esophageal gland (eg), median esophageal diverticulum (med), dextral cecum (dc), sinistral cecum (sc), ventral sucker (vs), common genital pore (cgp), cirrus sac (cs), uterus (ut), external seminal vesicle (esv), ovary (ov), Laurer’s canal (Lc), vitellarium (vr), testis (ts), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (16) Genitalia (paratype, HWML 20077-1) (ventral view) showing cirrus (ci), metraterm (mt), internal seminal vesicle (isv), ootype (oo), ovi-vitelline duct (ovt), oviduct (od), oviducal seminal receptacle (osr), primary vitelline duct (vt), and vas deferens (vd). (17) Body (paratype, HWML 20077-3) (dorsal view). (18) Genitalia (paratype, HWML 20077-3) (dorsal view).
and descriptions of its species. Regarding spines, Wall (1951) and Brooks and Mayes (1975) reported that tegumental body spines were present on V. robustum and V. brevitestis, respectively; however, we found no evidence of a tegumental spine on any species of Vasotrema. Regarding sucker papillae, none had previously been detailed in any species of TBF (Platt, 2002) but we describe several types: marginal papillae of the oral sucker and ventral sucker (Figs. 1, 7, 9, 10, 13, 19), paired terminal papillae dorsal to the mouth (Figs. 1, 9, 10, 19), and paired papillae within the mouth cavity (Fig. 10). Voucher specimens of Hapalotrema mehrai Rao, 1976, from Thomas R. Platt’s collection indicate that Hapalotrema Looss, 1899 may have a papillate ventral sucker also (J. R. Roberts and S. A. Bullard, unpubl. data). These specimens have a ventral sucker with large spinose crenulations similar to the marginal papillae described herein. Morphologically similar papillae are present in some genera of fish blood flukes (Digenea: Aporocotylidae; Bullard and Overstreet, 2004; Truong and Bullard, 2013; Yong et al., 2016). As these structures are present in phylogenetically unrelated taxa (Orélias-Ribeiro et al., 2014), they likely evolved independently. Perhaps these papillae function to allow the fluke to sense their location within the turtle’s vascular system or to sense the presence of other flukes.

Regarding the pharynx, Stunkard (1926, 1928) and others (Byrd, 1939; Wall, 1951; Brooks and Mayes, 1975; Platt, 2002) diagnosed Vasotrema as lacking a pharynx. As in species of Spirorchis, Unicaecum Stunkard, 1925, Coeuriatrema, and Baracktrema Roberts, Platt, and Bullard, 2016, we suspect that previous workers misinterpreted the pharynx as a component of the oral sucker because it is immediately dorsal to the oral sucker (Roberts et al., 2016a, 2016b, 2016c). Regarding the esophageal diverticula, comprising numerous lateral diverticula plus a median diverticulum dorsal to the cecal bifurcation, previous descriptions detailed the posterior-most diverticula only. The diverticula emanating laterally from the anterior portion of the esophagus are considerably smaller and more difficult to delineate from the esophageal gland; which envelops the esophagus and its diverticula. Similar to Spirorchis, the diverticula surround the esophagus for its entire length and become larger and more numerous posteriorly (Figs. 5, 6; Roberts et al., 2016c). Regarding the median esophageal diverticulum, Stunkard (1928) emphasized that he did not observe a “poche [pouch]” at the esophageal–cecal junction as he did in Spirorchis spp. (Stunkard, 1923). Whereas the median esophageal diverticulum identified in Vasotrema spp. is similar to, and probably homologous to, that described in Spirorchis spp. (Roberts et al., 2016c), it differs by being dorsal to, rather than ventral to, the cecal bifurcation. Our results herein confirmed that the median esophageal diverticulum is present in all species of Vasotrema; however, it is evidently lacking in some species of Spirorchis (i.e., Spirorchis elegans Stunkard, 1923; see Stunkard, 1923; Platt, 1993; Roberts et al., 2016c).

![Figures 19, 20. Vasotrema rileyae n. sp. (holotype, USNM 1422447) from Gulf Coast spiny softshell turtle, Apalone spinifera aspera (Agassiz, 1857) (Testudines: Trionychidae), from Round Lake (32°8'41.00"N, 87°8'14.00"W), Cahaba River, Perry Lakes State Park, Marion, Alabama. Scale values beside bars. (19) Body (dorsal view) showing oral sucker (os), paired terminal papillae (tp), pharynx (ph), esophagus (es), nerve commissure (nc), esophageal gland (eg), median esophageal diverticulum (med), sinistral cecum (sc), ventral sucker (vs), dextral cecum (dc), uterus (ut), cirrus sac (cs), external seminal vesicle (esv), ovary (ov), vitellarium (vr), tesis (ts), cecal terminus (ct), Manter’s organ (Mo), excretory vesicle (ev), and excretory pore (ep). (20) Genitalia (dorsal view) showing common genital pore (cgp), cirrus (ci), metraterm (mt), internal seminal vesicle (isv), uterus (ut), ootype (oo), ovi-vitelline duct (ovt), Laurer’s canal (Lc), oviduct (od), oviducal seminal receptacle (osr), vas deferens (vd), and primary vitelline duct (vt).]
Regarding the vitellarium, Stunkard (1928) diagnosed it as distributing from the ovary posterior to the termination of the ceca (even though he described the vitellarium of _V. robustum_ as extending to ventral sucker). The newly collected voucher specimen of _V. robustum_ had a vitellarium that extended anterior to the cecal bifurcation. Regarding the uterus, Stunkard (1928) defined the ootype as the duct proximal to the metraterm. We observed that in gravid individuals the egg occupied the lumen of the female reproductive tract proximal to the metraterm, making the ootype and uterus difficult to differentiate even in exceptionally well-stained specimens. That these features are difficult to discern in gravid specimens led us to refer to these combined features as an egg chamber. This is similar to the condition in species of _Coeuritrema_ (see Roberts et al., 2016b) and _Spiroorchis_ (see Roberts et al., 2016c).

How vitelline material is collected is a perplexing anatomical problem with species of _Vasotrema_. This problem likely requires the study of serially sectioned specimens, not whole mounts. In our newly collected materials and museum specimens, a large duct passes ventral to the cecum, extends mediad, curves slightly posteriorly, and expands (passes ventral to the cecum, extends mediad, curves slightly our newly collected materials and museum specimens, a large duct of the study of serially sectioned specimens, not whole mounts. In our newly collected materials and museum specimens, a large duct passes ventral to the cecum, extends mediad, curves slightly posteriorly, and expands (passes ventral to the cecum, extends mediad, curves slightly our newly collected materials and museum specimens, a large duct of the study of serially sectioned specimens, not whole mounts. In our newly collected materials and museum specimens, a large duct

_Vasotrema amydae_ Stunkard, 1926 (type species) (Figs. 1, 2)

_Description of adult (based on light microscopy of a cotype [AMNH 791]):_ Body 1,310 long or 10.1× longer than wide, 105 wide or 8% of body length at level of cecal bifurcation, 120 wide or 9% of body length at level of ventral sucker, 130 wide or 10% of body length at level of ovary, 130 wide or 10% of body length at level of testis, 100 wide or 8% of body length at level of cecal terminus; forebody (middle of ventral sucker to anterior body end) 340 long or 26% of body length; hindbody (middle of ventral sucker to posterior body end) 970 long or 74% of body length, 2.9× longer than forebody (Fig. 1). Oral sucker putatively spinous, papillate, 38 long or 3% of body length, 45 wide or 43% of body width at level of cecal bifurcation; paired terminal papillae 2 in number, dorsal to mouth, together 13 long by 28 wide (Fig. 1); paired internal mouth papillae not observed. Ventral sucker papillate, 45 long or 3% of body length, 50 wide or 42% of body width. Nerve commissure 120 or 9% of body length from anterior body end. Pharynx 35 long or 12% of esophagus length, 50 wide or 10.0× esophagus width immediately posterior to pharynx, 1.4× wider than long. Esophagus extending posteriorly 295 long or 23% of body length from mouth to posterior margin of median esophageal diverticulum, 5 wide posterior to pharynx, with wall 3 thick, 10 wide at esophagus median or 10% of body width at level of cecal bifurcation, with wall 5 thick; posteriormost lateral esophageal diverticula radially expanded, 100 long or 8% of body length or 34% of total esophagus length, 70 wide or 67% of body width at level of cecal bifurcation, 15 or 14% of body width from dextral body margin, 13 or 12% of body width from sinistral body margin; median esophageal diverticulum 38 long or 13% of esophagus length, 20 wide or 19% of body width at level of cecal bifurcation, with wall 12 thick; esophageal gland 245 long or 19% of body length, 100 wide or 95% of body width at level of cecal bifurcation (Fig. 1). Intestinal bifurcation 295 from anterior body end or 23% of body length; sinistral cecum 830 long or 63% of body length, 13 wide or 12% of body width at level of cecal bifurcation, 13 wide or 10% of body width at level of ovary, 13 wide or 13% of body width at level of cecal terminus; dextral cecum 810 long or 62% of body length, 18 wide or 17% of body width at level of cecal bifurcation, 15 wide or 12% of body width at level of ovary, 13 wide or 13% body width at level of cecal terminus; post-cecal distance 210 or 16% of body length (Fig 1).

Testis turning 11 times, 365 long or 28% of body length, 25 wide or 19% of body width at level of testis, 14.6× longer than wide, 195 or 15% of body length to cecal terminus, 375 or 29% of body length from posterior body end (Fig. 1). Vas deferens not observed, putatively ventral to gonads; external seminal vesicle discernible from vas deferens, intercecal, 123 long or 9% of body length, 43 wide or 33% of body width at level of testis, 2.9× longer than wide; internal seminal vesicle 40 long or 3% of body length, 15 wide or 12% of body width at level of testis, 2.7× wider than wide (Figs. 1, 2). Cirrus sac 75 long or 6% of body length, 20 wide or 15% of body width at level of testis, 1.3× wider than internal seminal vesicle; cirrus straight, 28 long or 2% of body length, 8 wide or 6% of body width (Figs. 1, 2).

Ovary spheroid or weakly lobate, intercecal, nearly abutting sinistral cecum, abutting posterior margin of external seminal vesicle, 190 or 15% of body length posterior to middle of ventral sucker, 38 long or 2% of body length, 25 wide or 19% of body width, 0.7× wider than long (Fig. 1); post-ovarian distance 765 or 58% of body length (Figs. 1, 2). Oviduct extending dextrad 10 long or 1% of body length, 13 wide; oviducal seminal receptacle 20 long or 2% of body length, 23 wide or 18% of body width at level of ovary, oviduct continuing anteromedially and dextral to ovary 105 or 8% of body length along midline of external seminal vesicle, 13 wide or 10% of body width before connecting with ootype (Fig. 2). Laurer’s canal a narrow duct extending 18 or 1% of body length sinnistral near distal margin of seminal receptacle, 8 wide (Fig. 2). Vitellarium difficult to discern, comprising a series of interconnected spheroid masses of small follicles, 400 or 31% of body length from anterior body end, distributing from common genital pore to Manter’s organ, ventrolateral to testis and ceca, terminating 95 or 7% of body length from posterior body end, coalescing into primary vitelline duct anterior to anterior margin of testis (Fig. 2); primary vitelline duct difficult to discern, 58 long or 6% of body length 81% of oviduct length from seminal receptacle to ootype, 10 wide or 8% of body width at level of ovary. Ootype difficult to discern, 15 long or 1% of body length, 18 wide or 14% of body width. Mehlis
gland not observed. Uterus 85 long or 6% of body length, 28 wide or 22% of maximum body width; egg chamber not observed; metraterm straight, 28 long or 2% of body length, 15 wide or 12% of body width; uterine egg not observed. Common genital pore opening ventral, sinistral, 43 or 3% of body length posterior to middle of ventral sucker (Fig. 1).

Excretory vesicle difficult to discern, 30 long or 2% of body length, 5 wide or 5% of body width at cecal terminus; Manter’s organ sinuous, turning 3 times, extending to cecal terminus, 130 long or 10% of body length, 25 wide or 23% of body width at cecal terminus, joining excretory vesicle (Fig. 1); excretory pore terminal.

Taxonomic summary

Type host: Not specified; Stunkard (1926) reported infections in both spiny softshell turtles, *A. spinifera* (LeSueur, 1827), and Florida softshell turtles, *A. ferox* (Schneider, 1783).

Other hosts: None.

Sites: Adults in blood.

Type locality: Not specified; Stunkard (1928) reported infected Florida softshell turtles and spiny softshell turtles from unspecifi- ced rivers in Florida and Indiana, respectively.

Other localities: Table I. Reelfoot Lake (36°19′0″N, 89°25′21.50″W), Tennessee (Byrd, 1939); Huron River, Washtenaw County, Michigan (Wall, 1951).

Specimens examined: Cototype (AMNH 791) (Stunkard, 1926, 1928).

Remarks

*Vasotrema amydae* differs from its congeners by having papillate suckers, a genital pore opening ventrally and anterior to the ovary, and a short testis that does not extend to the level of the tips of the posterior ceca.

The type of *V. amydae* (Figs. 1, 2; AMNH 791) is, to our knowledge, the only extant specimen that remains from the type series. Stunkard (1928) considered the oötype to comprise the entirety of what we consider the “egg chamber” (= uterus and oötype). As reported in specimens of *Coccotrema* and *Spiroechis* (see Roberts et al., 2016b, 2016c), we identified a distinct oötype, uterus, and metraterm in *V. amydae*. In an apparent lapse, Stunkard (1928) described the oviduct as extending anteriorly dorsal to the external seminal vesicle (as we did), but illustrated it as ventral to the seminal vesicle (Fig. 1: p. 306). The type locality and type host for *V. amydae* are indeterminate, and Stunkard’s (1926, 1928) slide labels do not help resolve the matter, which likely makes it impossible to ever confirm the origin of the only existing type material for *V. amydae*. He necropsied spiny and Florida softshell turtles taken from rivers in Indiana and Florida but failed to indicate where the turtles were captured and which TBF specimens came from which turtle species. Further potentially complicating matters is that a second unidentified TBF specimen shares a slide with the cotype. The specimen is poorly stained and not worth much attention but we identified it as *Vasotrema* sp. by having diagnostic features of the genus but also having elongate esophageal diverticula and a testis that extends posteriorly near the tips of the ceca.

Specimens identified as *Hapalorhynchus evaginatus* Byrd, 1939 infect spiny softshell turtles from Reelfoot Lake, Tennessee. Byrd (1939) comprises the only record of a TBF infection in a species of *Apalone* that is not a species of *Vasotrema*. Aware of this, Platt and Snyder (2007) borrowed Byrd’s holotype (USNM 9227) and considered it to be *V. attenuatum*. In examining Byrd’s holotype, Platt and Snyder (2007) stated that the specimen resembled *Vasotrema* spp. by having large posterior-most esophageal diverticula, a single testis, and a sinistral and dorsal genital pore. We agree with Platt and Snyder (2007) that the illustration resembles *Vasotrema* rather than *Hapalorhynchus*, but we think the authors incorrectly reported the position of the genital pore as dorsal rather than ventral, because the genital pore is dorsal in *Hapalorhynchus* spp. and ventral in *Vasotrema* spp. Byrd’s (1939) original description does not resemble *V. attenuatum*, but certainly likens the specimens to *V. amydae*: the body is elongate and the posterior-most lateral esophageal diverticula surround nearly half of the esophagus length. Based on that, we suspect that *H. evaginatus* is a junior subjective synonym of *V. amydae*.

*Vasotrema attenuatum* Stunkard, 1928
(Figs. 3–6)

Description of adult (based on light microscopy of 10 adult specimens comprising paratypes [AMNH 806]): Body 1,430–1,900 (1,620; 10) long or 12.0–20.1% (16.6; 10) longer than wide, 45–75 (57; 10) wide or 2–5% (4%; 10) of body length at level of cecal bifurcation, 45–65 (56; 10) wide or 3–5% (4%; 10) of body length at level of ventral sucker, 60–115 (82; 10) wide or 4–8% (5%; 10) of body length at level of ovary, 85–120 (99; 10) wide or 5–8% (6%; 10) of body length at level of testis (typically maximum width), 30–85 (67; 10) wide or 3–6% (4%; 10) of body length at level of cecal terminus; forebody (middle of ventral sucker to anterior body end) 295–395 (339; 10) long or 20–22% (21%; 10) of body length; hindbody (middle of ventral sucker to posterior body end) 1,110–1,510 (1,281; 10) long or 78–80% (79%; 10) of body length, 5–4.0× (3.8; 10) longer than forebody (Figs. 3–5). Oral sucker spines not observed, ap papillate, 18–30 (24; 10) long or 1–2% (1%; 10) of body length, 25–33 (28; 10) wide or 33–62% (50%; 10) of body width at level of cecal bifurcation (Figs. 3, 4). Ventral sucker papillae not observed, 30–48 (39; 10) long or 2–3% (2%; 10) of body length, 30–50 (38; 9) wide or 58–91% (69%; 9) of body width. Nerve commissure 100–135 (108; 9) or 6–7% (7%; 9) of body length from anterior body end. Pharynx 30–38 (34; 10) long or 11–18% (13%; 10) of esophagus length, 18–30 (23; 10) wide or 2.3–4.6× (3.3; 10) esophagus length immediately posterior to pharynx, 1.1–2.1× (1.5; 10) longer than wide. Esophagus extending posteriorly 213–325 (263; 10) long or 14–19% (16%; 10) of body length from mouth to posterior margin of median esophageal diverticulum, 5–10 (7; 10) wide immediately posterior to pharynx, with wall 3–5 (3; 10) thick, 8–18 (13; 10) wide at esophagus median or 15–30% (23%; 10) of body width at level of cecal bifurcation, with wall 5–15 (6; 10) thick; posterior-most lateral esophageal diverticula laterally expanded, 43–60 (50; 10) long or 3–4% (3%; 10) of body length or 15–22% (19%; 10) of total esophagus length, 20–40 (29; 10) wide or 40–62% (52%; 10) of body width at level of cecal bifurcation, 10–20 (14; 8) from dextral body margin, 10–20 (14; 8) from sinistral body margin (Figs. 5, 6); median esophageal diverticulum 18–38 (28; 10) long or 7–14% (11%; 10) of esophagus length, 13–28 (20; 10) wide or 24–51% (35%; 10) of body width at level of cecal bifurcation, with wall 3–12 (7; 8) thick (Figs. 5, 6); esophageal gland 203–270 (222; 7) long or 13–15%
at level of ovary; ovi-vitelline duct difficult to discern, 33–65 (46; 7) wide or 11–73% (37%; 7) of body width difficult to discern, 173–175 (174; 3) long or 11–12% (11%; 3) of body length terminating 85–175 (130; 9) or 6–10% (8%; 9) of body length distributing from ovary to Manter’s organ, ventrolateral to ceca, or 22–42% (36%; 8) of body length from anterior body end, interconnected spheroid masses of small follicles, 350–700 (586; 8) long, noncoiled testis that extends posteriad to the tips of the ceca. Genital pore that opens ventrally and anterior to the ovary and a genital pore that opens anteriorly and ventrally to the ovary and a genital pore that opens anteriorly and ventrally to the ovary and a genital pore that opens anteriorly and ventrally to the ovary.

Remarks

**Taxonomic summary**

*Type host:* Not specified; Stunkard (1928) reported infections in both spiny softshell turtles, *A. spinifera* (LeSueur, 1827), and Florida softshell turtles, *A. ferox* (Schneider, 1783).

*Other hosts:* *Apalone sp.* (Wall, 1951); midland softshell turtle, *A. mutica* (LeSueur, 1827) (see Brooks and Mayes, 1975).

*Sites:* Adults in blood (Stunkard, 1928).

*Type locality:* Not specified; Stunkard (1928) reported infected Florida softshell turtles and spiny softshell turtles from unspecifed rivers in Florida and Indiana, respectively.

*Other localities:* Table I; not specified, USA (Wall, 1951); not specified, Nebraska (Brooks and Mayes, 1975).

*Species examined:* Cotype (AMNH 806, 17 slides with 25 whole mounted specimens).

**Remarks**

*Vasotrema attenuatum* differs from its congeners by having a genital pore that opens ventrally and anterior to the ovary and a long, noncoiled testis that extends posterior to the tips of the ceca.

As with the cotype of *V. amydae* and for the same reasons, we cannot confirm the type host or type locality for *V. attenuatum*. Of the extant whole-mounted specimens comprising the type series, 13 of 17 slides have no host information and 4 slides (AMNH 806-1, 2, 12, 16) indicate the host as spiny softshell turtle (*A. spinifera*). Likewise, Wall’s (1951) records of *V. attenuatum, V. robustum*, and *V. amydae* in softshell turtles from the Huron River (Michigan) and Cumberland River (Tennessee) are equally ambiguous (Table I).

Stunkard’s (1928) generally incomplete description omitted several taxonomically important features for *V. attenuatum*; however, our observations of his specimens confirmed most of the features that he did happen to describe. A significant exception is the distal portion of the female genitalia. He illustrated the uterus and metraterm as ventral to the cirus sac (fig. 4; p. 310) but both are dorsal to the cirus sac. Stunkard (1928) also failed to differentiate the oötype, uterus, and thick-walled metraterm; misinterpreting these structures as together comprising the oötype. We agree that these structures function as a sort of egg chamber; however, we confirmed distinct portions of the female reproductive tract corresponding to the oötype, uterus,
and metraterm in nongravid specimens. In fact, we regard the esophagus length, 90\[90\] wide or 1.6\[1.6\] of body length at level of testis, 200\[160\] wide or 12\[8\]% of body length at level of cecal terminus; forebody (middle of ventral sucker to anterior body end) 510\[620\] or 30\[30\]% of body length, 174\[223\] wide or 60\[90\]% of body width (Figs. 9, 11).

Testis 270\[475\] long or 16\[22\]% of body length, 115\[185\] wide or 34\[57\]% of body width at level of testis, 2.3\[2.6\] longer than wide, 215\[250\] or 13\[12\]% from cecal terminus, 395\[385\] or 24\[18\]% of body length from posterior body end (Figs. 7, 9). Vas deferens 130\[200\] long or 8\[9\]% of body length, 8\[10\] wide, ventral to testicular column, laterally expanding before joining external seminal vesicle; external seminal vesicle discernible from vas deferens, intercecal, 158\[270\] long or 9\[13\]% of body length, 125\[160\] long or 7\[8\]% of body length running anteriad, 33\[110\] long or 2\[5\]% of body length running posteriad, 80\[120\] wide or 23\[33\]% of body width at level of ovary, 2.0\[2.0\] longer than wide; internal seminal vesicle 63\[85\] long or 5\[4\]% of body length, 35\[43\] wide or 10\[12\]% of body width at level of ovary, 1.8\[2.0\] longer than wide (Figs. 7–9, 11). Circus sac 103\[150\] long or 6\[7\]% of body length, 40\[63\] wide or 11\[18\]% of body width at level of ovary, 1.1\[1.5\] wider than internal seminal vesicle; circus straight, 35\[33\] long or 2\[2\]% of body length, 20\[13\] wide or 6\[4\]% of body width (Figs. 7–9, 11).

Ovary lobate, 3\[3\] lobes, intercecal, abutting and slightly dorsal to external seminal vesicle, dextrolateral to circus sac, 375\[500\] or 22\[24\]% of body length posterior to middle of ventral sucker, 55\[83\] long or 3\[4\]% of body length, 80\[120\] wide or 23\[33\]% of body width at level of ovary, 1.5\[1.4\] longer than wide (Figs. 9, 11); post-ovarian distance 740\[910\] or 44\[43\]% of body length (Figs. 7–9, 11). Oviduct extending posteriad 30\[63\] long or 2\[3\]% of body length, 15\[18\] wide; oviducal seminal receptacle 48\[43\] long or 3\[2\]% of body length, 45\[48\] wide or 13\[13\]% of maximum body width, oviduct continuing anterorsinistrad 88\[88\] or 5\[4\]% of body length, 25\[20\] or 7\[6\]% of body width before connecting with ootype (Figs. 8, 11). Laurer’s canal a narrow duct extending 75\[88\] or 4\[4\]% of body length sinistrad near distal margin of seminal receptacle, 20\[13\] wide or 6\[4\]% of maximum body width (Figs. 8, 11). Vitellarium comprising a series of interconnected spheroid masses of small follicles, 350\[415\] or 21\[20\]% of body length, distributing from cecal bifurcation [ventral sucker] to Manter’s organ, ventrolateral to testes and ceca (not fully illustrated to highlight paths of ceca), terminating 70\[95\] or 4\[4\]% of body length from posterior body end, coalescing from dextral body margin into primary vitelline duct posterior to oviduct (Figs. 7, 9); primary vitelline duct coalescing and extending posteriad 60\[80\] before turning and extending anteriad 88\[103\], 148\[183\] long or 9\[9\]% of body length, 25\[40\] wide or 7\[11\]% of body width, inserting at proximal margin of ootype. Ootype difficult to discern, 25\[50\] long or 1\[2\]% of body length, 38\[33\] wide or 11\[9\]% of body width (Figs. 8, 11). Meghils gland not observed. Uterus 50\[35\] long or 3\[2\]% of body length, 38\[23\] wide or 11\[6\]% of body width; metraterm straight, 50\[45\] long or 3\[2\]% of body length, 45\[45\] wide or 13\[13\]% of body length (Figs. 8, 11). Uterine egg (not observed in voucher) 35 long or 2\% of body length, 20 wide or 6\% of body width at level of ovary, 1.8\[2.0\] longer than wide (Fig. 8). Common genital pore opening ventral, sinistrad (Figs. 7–9, 11), 375\[580\] or 22\[27\]% of body length posterior to middle of ventral sucker.

Excretory vesicle difficult to discern, 3\[3\] a\[a\] long or 4\[4\]% of body length, 10\[10\] a\[a\] wide or 5\[5\]% of body width at cecal terminus; Manter’s organ coiled, turning 4\[2\] times, extending past cecal terminus, 200\[200\] long or 12\[9\]% of body length,
opened specimen (USNM 1422436) has a vitellARIum that extends anteriad beyond the ventral sucker to the cecal bifurcation.

The type host and locality for *V. robustum* is unclear. Stunkard (1928) did not specify a host but indicated that at least 8 specimens of *V. robustum* were recovered from spiny softshell turtles. Wall (1951) stated that the description (Stunkard, 1928) was based on 13 specimens from that host in Indiana, but it is unclear where that information came from. The AMNH database lists spiny softshell turtle as the type host for *V. robustum* (syntypes 808 and 809) and Indiana as the type locality, but published host and locality records and slide labels for the extant specimens do not unambiguously confirm that.

**Vasotrema longistestis** Byrd, 1939
(Figs. 12–14)

Description of adult (based on light microscopy of the holotype [USNM 1321971] and 10 newly collected vouchers [USNM 1224347-1422446]): Body 730–1,420 (1,144; 10) [635] long or 3.6–7.2× (5.8; 10) [2.1×] longer than wide, 100–145 (115; 10) [160] wide or 8–19% (10%; 10) [25%] of body length at level of cecal bifurcation, 100–155 (121; 10) [180] wide or 9–17% (11%; 10) [28%] of body length at level of ventral sucker, 150–285 (177; 10) [285] wide or 13–22% (17%; 10) [45%] of body length at level of ovary, 155–340 (203; 10) [300] wide or 14–28% (18%; 10) [47%] of body width at level of testis (typically maximum width), 130–250 (156; 9) [160] wide or 11–22% (14%; 10) [25%] of body length at level of cecal terminus; forebody (middle of ventral sucker to anterior body end) 195–370 (254; 10) [140] long or 18–29% (22%; 10) [22%] of body length; hindbody (middle of ventral sucker to posterior body end) 517–1,050 (890; 10) [495] long or 71–82% (78%; 10) [78%] of body length, 2.4–4.7× (3.6; 10) [3.5×] longer than wide (Figs. 12, 13). Oral sucker spines not observed, papillate [papillate], 20–35 (29; 10) [25] long or 2–5% (3%; 10) [4%] of body length, 33–48 (41; 10) [33] wide or 29–48% (36%; 10) [21%] of body width at level of cecal bifurcation (Figs. 12, 13); paired terminal papillae not observed; paired internal mouth papillae not observed. Ventral sucker papillae [not observed], 50–70 (59; 10) [53] long or 5–7% (5%; 10) [8%] of body length, 48–70 (59; 10) [70] wide or 40–60% (51%; 10) [44%] of body width (Figs. 12, 13). Nerve commissure 80–140 (97; 9) [90] or 7–12% (9%; 10) [14%] of body length from anterior body end. Pharynx 30–55 (39; 10) [28] long or 16–25% (19%; 10) [n/a] of esophagus length, 30–43 (36; 10) [35] wide or 3.5–6.6× (4.7; 8) [n/a] esophagus width immediately posterior to pharynx, 0.9–1.3× (1.1; 9) [0.8×] longer than wide. Esophagus extending posteriorly 155–298 (205; 10) [n/a] long or 13–25% (18%; 10) [n/a] of body length from mouth to posterior margin of median esophageal diverticulum, 5–10 (8; 9) [n/a] wide posterior to pharynx, with wall 2–7 (5; 9) [n/a] thick, 10–20 (13; 10) [n/a] wide at esophagus median or 7–18% (12%; 10) [n/a] of body width at level of cecal bifurcation, with wall 5–10 (7; 10) [n/a] thick; posterior-most lateral esophageal diverticula radially expanded, 25–60 (40; 10) [n/a] long or 2–4% (3%; 10) [n/a] of body length and 16–22% (19%; 10) [n/a] of total esophagus length, 30–55 (39; 10) [n/a] wide or 29–41% (33%; 10) [n/a] of body width at level of cecal bifurcation; 23–38 (32; 10) [n/a] or 21–38% (28%; 10) [n/a] of body width from dextoral body margin, 25–38 (30; 10) [n/a] or 25–29% (26%; 10) [n/a] of body width from sinistral body margin;
vesicle comprised entirely of vas deferens; internal seminal vesicle 100–313 (185; 10) [n/a] long or 13–22% (16%; 10) [n/a] of body length from posterior body end (Figs. 12, 13). Vas deferens terminus, 120–263 (214; 10) [95] or 14–22% (19%; 10) [15%] of body length, 10–18 (10; 10) [n/a] wide or 4–9% (6%; 10) [n/a] of body width at level of ovary, 10–15 (13; 10) [n/a] long or 5–9% (7%; 10) [n/a] of body length, 8–18 (14; 10) [n/a] wide or 6–16% (12%; 10) [n/a] of body width at level of cecal bifurcation; dextral cecum 450–1,065 (764; 10) [n/a] long or 61–75% (66%; 10) [n/a] of body length, 10–15 (14; 10) [n/a] wide or 8–17% (13%; 10) [n/a] of body width, 163–310 (207; 10) [n/a] from anterior body end or 15–24% (18%; 10) [n/a] of body length; intestinal excrescence 125–255 (159; 10) [n/a] long or 3–11% (5%; 10) [n/a] of esophagus length, 20–30 (25; 10) [n/a] wide or 6–15% (12%; 10) [n/a] of body length, 18–68 (43; 10) [n/a] long or 5–11% (3%; 10) [n/a] of body length anteriad, 8–23 (13; 9) [n/a] wide or 2–10% (5%; 10) [3%] of body width at level of testis, 1.5–1.9 (1.5; 10) [53%] long or 4–9% (7%; 10) [8%] of body length, 5–15 (9; 10) [8] wide or 2–10% (5%; 10) [3%] of body width at level of testis (Figs. 12–14).

Ovary spherical or weakly lobate, 2 (5) [0] lobes when present, intercecal, abutting dexteral cecum, 118–255 (165; 10) [78] or 12–18% (14%; 10) [12%] of body length posterior to middle of ventral sucker, 33–55 (43; 10) [20] long or 3–5% (4%; 10) [3%] of body length, 18–68 (43; 10) [60] wide or 11–38% (24%; 10) [21%] of body width, 0.4–1.8x (1.0; 10) [3.0x] longer than wide (Figs. 12, 13). Cirrus sac 105–190 (139; 10) [150] long or 9–19% (12%; 10) [24%] of body length, 23–33 (27; 10) [25] wide or 8–18% (14%; 10) [8%] of body width at level of testis, 1.5–1.9x (1.7; 10) [1.7x] wider than internal seminal vesicle; cirrus straight, 40–105 (75; 10) [53] long or 4–9% (7%; 10) [8%] of body length, 5–15 (9; 10) [8] wide or 2–10% (5%; 10) [3%] of body width at level of testis (Figs. 12–14).

Ovary spherical or weakly lobate, 2 (5) [0] lobes when present, intercecal, abutting dexteral cecum, 118–255 (165; 10) [78] or 12–18% (14%; 10) [12%] of body length posterior to middle of ventral sucker, 33–55 (43; 10) [20] long or 3–5% (4%; 10) [3%] of body length, 18–68 (43; 10) [60] wide or 11–38% (24%; 10) [21%] of body width, 0.4–1.8x (1.0; 10) [3.0x] longer than wide (Figs. 12, 13). Cirrus sac 105–190 (139; 10) [150] long or 9–19% (12%; 10) [24%] of body length, 23–33 (27; 10) [25] wide or 8–18% (14%; 10) [8%] of body width at level of testis, 1.5–1.9x (1.7; 10) [1.7x] wider than internal seminal vesicle; cirrus straight, 40–105 (75; 10) [53] long or 4–9% (7%; 10) [8%] of body length, 5–15 (9; 10) [8] wide or 2–10% (5%; 10) [3%] of body width at level of testis (Figs. 12–14).
Remarks

Vasotrema longitestis differs from its congeners by having a genital pore that opens ventrally and anterior to the ovary and a long, coiled testis that extends posterior to the tips of the ceca. The holotype of V. longitestis (USNM 1321971; fig. 9, p. 157; Byrd, 1939) is the only extant specimen that is unambiguously from the type series (see discussion presented in Platt and Prestwood [1990] regarding additional specimens that may belong to the type series). However, the holotype is strongly contracted, poorly stained (perhaps it destained), and apparently strongly flattened (Fig. 12). An outline sketch of that specimen is provided herein mostly for general comparative purposes; however, some additional fine structures were evident in that specimen that had not been previously characterized. For example, we observed papillae around the mouth of the holotype and our specimens as well as papillae associated with the ventral sucker of our specimens (Fig. 13). Also, the 2 posterior esophageal dilations indicated by Byrd (1939) appear to comprise lateral esophageal diverticula. The common genital pore is immediately anterior to the ventral sucker in the holotype and our specimens (Figs. 12, 13), not at level of the posterior margin of the ventral sucker as indicated by Byrd (1939). Although Byrd (1939) described the metraterm as “short,” it is in fact proportionally the longest of any accepted species of Vasotrema, extending 7–15% of body length vs. 1–3% (V. amydae), 2–4% (V. attenuatum), 2–3% (V. robustum), and 2–3% (V. brevitestis). The path of the metraterm in V. longitestis is unique among congeners: extending posteriorly before curving anteriad toward the genital pore (Figs. 12, 14).

Vasotrema brevitestis Brooks and Mayes, 1975

(Figs. 15–18)

Description of adult (based on light microscopy of 5 paratypes [HWML 20076, 20077]): Body 1,130–1,330 (1,232; 5) long or 8.3–11.0× (10.0; 5) longer than wide, 110–148 (126; 5) wide or 9–11% (10%; 5) of body length at level of cecal bifurcation, 100–148 (120; 5) or 9–11% (10%; 5) of body length at level of ventral sucker, 95–155 (118; 5) wide or 8–12% (10%; 5) of body length at level of ovary, 105–160 (125; 5) wide or 9–12% (10%; 5) of body length at level of testis, 80–100 (88; 5) wide or 6–8% (7%; 5) of body length at level of cecal terminus; forebody (middle of ventral sucker to anterior body end) 300–430 (360; 5) long or 27–32% (29%; 5) of body length; hindbody (middle of ventral sucker to posterior body end) 830–930 (872; 5) long or 68–73% (71%; 5) of body length, 2.1–2.8× (2.5; 5) longer than forebody (Figs. 15, 17). Oral sucker spinous, apapillate, 30–38 (34; 5) long or 3% (3%; 5) of body length, 38–48 (43; 5) wide or 28–44% (35%; 5) of body width at level of cecal bifurcation (Figs. 15, 17). Ventral sucker apapillate, 45–55 (52; 5) long or 4–5% (4%; 5) of body length, 30–63 (48; 5) wide or 30–46% (39%; 5) of body width. Nerve commissure 93–125 (107; 5) or 8–10% (9%; 5) of body length from anterior body end or 19–25% (22%; 5) of body length; cirrus straight, 23–28 (25; 4) long or 2% (4) of body length, 8–10% (9%; 3) of body length, 13–10 (10; 3) wide, ventral to gonads, laterally expanding before joining external seminal vesicle; external seminal vesicle discernible from vas deferens, intercecal, 70–113 (83; 4) long or 6–9% (7%; 4) of body length, 25–30 (28; 4) wide or 25–27% (26%; 4) of body width at level of testis, 2.5–3.8× (2.9; 4) longer than wide; internal seminal vesicle 30–43 (38; 4) long or 2–4% (3%; 4) of body length, 13–18 (15; 4) wide or 11–17% (13%; 4) of body width; 2.2–2.9× (2.5; 4) longer than wide (Figs. 15–18). Cirrus sac 58–83 (68; 4) long or 5–7% (6%; 4) of body length, 25 (4) wide or 20–23% (22%; 4) of body width at level of testis, 1.4–1.9× (1.7; 4) wider than internal seminal vesicle; cirrus straight, 23–28 (25; 4) long or 2% (4) of body length, 5–8 (6; 4) wide or 4–8% (5%; 4) of body width at level of testis (Figs. 15, 17).

Ovary spheroid, intercecal, abutting dextral cecum and posterior margin of external seminal vesicle, 30–38 (34; 5) long or 2–3% (3%; 5) of body length, 43–55 (47; 5) wide or 32–48% (41%; 5) of body width, 1.2–1.5× (1.4; 5) wider than long (Figs. 15–18); post-ovarian distance 630–740 (687; 5) or 48–64% (56%; 5) of body length. Oviduct extending posteriorly 18–33 (24; 4) long or 2–3% (2%; 4) of body length, 13–15 (14; 4) wide; oviducal seminal receptacle 18–25 (22; 4) long or 2% (4) of body length, 15–30 (21; 4) wide or 13–26% (19%; 4) of body width at level of ovary, oviduct continuing anteriorly 55–75 (63; 3) or 5–
6% (5%; 3) of body length, 13–18 (15; 3) wide or 12–16% (14%; 3) of body width before connecting with oötype (Figs. 16, 18); o-vitelline duct difficult to discern, 33–58 (46; 2) long or 3–5% (4%; 2) of body length, 13–15 (14; 2) wide or 11–14% (13%; 2) of body width at level of ovary (Figs. 16, 18). Laurer’s canal a narrow duct extending 18–50 (31; 3) or 2–4% (3%; 3) of body length posteriadi, 8–13 (10; 3) wide (Figs. 16, 18). Vitellarium difficult to discern, comprising a series of sparse interconnected large spheroid masses of small follicles, 360–575 (460; 5) or 32–43% (37%; 5) of body length from anterior body end, distributing from common genital pore to testis terminus, dorso- and ventrolateral to testis and ceca, terminating 250–438 (358; 5) or 20–37% (29%; 5) of body length from posterior body end, coalescing into primary vitelline duct posterior to oviduct and anterior to anterior margin of testis (Figs. 16, 18); primary vitelline duct difficult to discern, a narrow duct 88–113 (101; 2) long or 7–9% (8%; 2) of body length, 18–20 (19; 2) wide, merging with female genitalia before oötype (Figs. 16, 18). Oötype difficult to discern, 23–30 (27; 4) long or 2–3% (2%; 4) of body length, 20–30 (26; 4) wide or 21–26% (24%; 4) of body width. Melhids gland not observed. Uterus 30–40 (37; 4) long or 2–4% (3%; 4) of body length, 23–33 (28; 4) wide or 22–29% (25%; 4) of body width; metraterrin straight, 20–38 (28; 4) long or 2–3% (2%; 4) of body length, 10–13 (12; 4) wide or 8–14% (11%; 4) of body width at level of ovary; uterine egg not observed. Common genital pore opening ventral, sinistral (Figs. 15–18), 33–58 (40; 4) or 3–5% (3%; 5) of body length posterior to middle of ventral sucker (Figs. 15, 17).

Excretory vesicle difficult to discern, 28–48 (35; 4) long or 2–4% (3%; 4) of body length, 3–20 (11; 4) wide or 4–22% (12%; 4) of body width at cecal terminus; Manter’s organ sinuous, turning 4–6 (5; 3) times, extending to cecal terminus, 8–20 (13; 10) or 5–13% (11%; 10) of body width at level of cecal bifurcation (Fig. 19). Intestinal bifurcation 235–315 (272; 10) long or 16–21% (17%; 10) of body length, 50–78 (64; 10) wide or 25–38% (31%; 10) of body width at level of cecal bifurcation; paired terminal papillae 2 in number, dorsal to mouth, together 5–23 (10; 10) long by 8–30 (19; 10) wide (Fig. 19); paired internal mouth papillae not observed. Ventral sucker papillate, 45–53 (50; 10) long or 3–5% (4%; 10) of body length, 38–63 (46; 10) wide or 30–42% (36%; 10) of body width. Nerve commissure 95–120 (108; 10) or 8–10% (9%; 10) of body length from anterior body end. Pharynx 38–58 (46; 10) long or 15–23% (18%; 10) of esophagus length, 33–53 (41; 10) wide or 2.9–8.6× (5.1; 10) esophagus width immediately posterior to pharynx, 0.9–1.8× (1.1; 10) wider than long. Esophagus extending posteriad 218–305 (260; 10) long or 19–23% (21%; 10) of body length from mouth to posterior margin of median esophageal diverticulum, 5–13 (9; 10) wide posterior to pharynx, with wall 3 (10) thick, 13–18 (15; 10) wide at esophageal median or 10–15% (12%; 10) of body width at level of cecal bifurcation, with wall 8–15 (11; 10) thick; posterior-most lateral esophageal diverticula radially expanded, 75–150 (101; 10) long or 6–11% (8%; 10) of body length or 31–52% (39%; 10) of total esophagus length, 60–85 (74; 10) wide or 48–70% (59%; 10) of body width at level of cecal bifurcation, 15–30 (21; 10) or 13–24% (17%; 10) of body width from dextral body margin, 13–30 (21; 10) or 11–24% (17%; 10) of body width from sinistral body margin; median esophageal diverticulum 28–45 (35; 10) long or 11–16% (14%; 10) of esophagus length, 30–40 (36; 10) wide or 25–38% (29%; 10) of body width at level of cecal bifurcation, with wall 2–10 (5; 10) thick; esophageal gland 163–250 (214; 10) long or 16–21% (17%; 10) of body length, 50–78 (59; 10) wide or 16–21% (17%; 10) of body width at level of cecal bifurcation (Fig. 19). Intestinal bifurcation 235–315 (272; 10) from anterior body end or 20–24% (22%; 10) of body length; sinistral cecum 505–840 (692; 10) long or 51–62% (56%; 10) of body length, 8–18 (15; 10) wide or 6–18% (12%; 10) of body width at level of cecal bifurcation, 8–20 (13; 10) wide or 5–13% (9%; 10) of body width at level of ovary, 8–25 (17; 10) wide or 7–
21% (13%; 10) of body width at level of cecal terminus; dextral cecum 540–820 (702; 10) long or 54–59% (57%; 10) of body length, 13–15 (14; 10) wide or 10–13% (11%; 10) of body width at level of cecal bifurcation, 8–20 (13; 10) wide or 3–10% (7%; 10) of body width at level of ovary, 13–25 (18; 10) wide or 10–19% (14%; 10) body width at level of cecal terminus; post-cecal distance 195–275 (240; 10) or 17–22% (20%; 10) of body length (Fig. 19). Testsis turning 4–11 (6; 10) times, 225–335 (279; 10) long or 21–26% (23%; 10) of body length, 43–75 (63; 10) wide or 28–48% (39%; 10) of body width at level of testis, 3.2–6.5× (4.5; 10) longer than wide, 75–205 (143; 10) or 6–15% (12%; 10) of body length to cecal terminus, 295–490 (394; 10) or 25–36% (32%; 10) of body length from posterior body end (Fig. 19). Vas deferens 73–133 (97; 10) long or 6–10% (8%; 10) of body length, 5–15 (10; 10) wide, ventral to gonads, laterally expanding before joining external seminal vesicles; external seminal vesicle discernible from vas deferens, intercecal, 48–100 (79; 10) long or 4–8% (6%; 10) of body length, 25–73 (52; 10) wide or 15–46% (33%; 10) of body width at level of testis; 1.0–2.3× (1.6; 10) longer than wide; internal seminal vesicle 45–63 (56; 10) long or 4–6% (5%; 10) of body length, 10–23 (16; 10) wide or 6–15% (10%; 10) of body length, 2.5–6.3× (3.9; 10) longer than wide (Figs. 19, 20). Cirrus sac 88–115 (102; 10) long or 7–9% (8%; 10) of body length, 18–38 (26; 10) wide or 10–23% (16%; 10) of body width at level of testis, 1.2–2.5× (1.7; 10) wider than internal seminal vesicle; cirrus straight, 30–50 (41; 10) long or 2–4% (3%; 10) of body length, 8–13 (10; 10) wide or 5–8% (6%; 10) of body width (Figs. 19, 20).

Ovary spheroid or weakly lobate, intercecal, abutting dextral cecum, abutting posterior margin of external seminal vesicle, 95–131 (114; 10) long or 10–14% (12%; 10) of body length posterior to middle of ventral sucker, 23–55 (31; 10) long or 2–5% (3%; 10) of body length, 45–70 (55; 10) wide or 29–45% (35%; 10) of body width at level of ovary, a short testis that does not extend to the level of tips of the posterior ceca, and a Laurer’s organ, ventrolateral to testis and ceca, terminating 63–100 (83; 10) long or 2–4% (3%; 10) of body length from seminal receptacle to oötype, 13–25 (18; 10) wide or 10–14% (12%; 10) of body width at level of ovary. Mehlis gland not observed. Uterus 30–73 (45; 8) long or 2–5% (4%; 8) of body length, 15–35 (21; 8) wide or 10–18% (13%; 8) of maximum body width; egg chamber (in gravid specimens) 75 and 88 (2) long or 6% and 7% of body length, 50 and 60 (2) wide or 29% and 44% of maximum body width; metraterm straight, 20–40 (29; 10) long or 1–3% (2%; 10) of body length, 13–25 (18; 10) wide or 8–17% (11%; 10) of body width; uterine egg 70 and 83 (2) long or 60% and 61% (2) of body length, 40 and 48 (2) wide or 31% and 36% (2) of maximum body width, 1.7× and 1.8× (2) longer than wide. Common genital pore opening ventral, sinistral, 8–18 (10; 10) or 1–2% (1%; 10) of body length posterior to middle of ventral sucker (Figs. 19, 20).

Excretory vesicle difficult to discern, 13–38 (29; 10) long or 1–3% (2%; 10) of body length, 3–10 (6; 10) wide or 3–7% (4%; 10) of body width at cecal terminus; Manter’s organ sinuous, turning 4–8 (6; 10) times, extending to cecal terminus, 150–225 (179; 10) long or 13–18% (15%; 10) of body length, 15–38 (22; 10) wide or 11–25% (16%; 10) of body width at cecal terminus, joining excretory vesicle (Fig. 19); excretory pore terminal.

**Taxonomic summary**

*Type host:* Gulf Coast spiny softshell turtle, *Apalone spinifera aspera* (Agassiz, 1857).

*Sites:* Mesenteric blood vessels.

*Type locality:* Round Lake (32°41′50.91″N, 87°14′30.39″W), Cahaba River, Perry Lakes State Park, Marion, Alabama.

*Other locality:* Canoe Lake (33°47′56.16″N, 86°29′25.02″W), Coosa River, Springville, Alabama.

*Specimens deposited:* Holotype (USNM 1422447), paratypes (USNM 1422448-1422451).

*Prevalence of infection (present study):* Three of 3 (100%) and 1 of 1 (100%) spiny softshell turtles were infected by *V. rileyae* from Canoe Lake and Round Lake, respectively.

*Etymology:* Specific epithet *rileyae* honors JRR’s wife, Megan Riley Roberts.

**Remarks**

*Vasotrema rileyae* differs from its congeners by having papillate suckers, a genital pore opening ventrally and anterior to the ovary, a short testsis that does not extend to the level of the tips of the posterior ceca, and a dextral ovary. *Vasotrema rileyae* most closely resembles *V. amydae* by having papillate suckers, paired terminal papillae dorsal to the mouth, a genital pore opening ventrally and anterior to the ovary, and a short testsis that does not extend posterior to the level of the tips of the posterior ceca. *Vasotrema rileyae* can be further differentiated from *V. amydae* by the combination of having a genital pore at level of ventral sucker, a wider and shorter testsis (3.2–6.5× longer than wide), an ovary dextral to the oviduct, and a Laurer’s canal extending posterior to the lateral excretory pore. *Vasotrema amydae* has a genital pore posterior to the ventral sucker, a more elongate testsis (14.6× longer than wide), an ovary sinistral to the oviduct, and a Laurer’s canal extending directly sinistrally from the oviduct (Figs. 2, 20).

**DISCUSSION**

Some new species of *Vasotrema* may infect other North American softshell turtles and their subspecies. The Eastern spiny
Softshell turtle, *Apalone spinifera spinifera* (LeSueur, 1827) and Gulf Coast spiny softshell turtle (see Weisrock and Janzen, 2000; Guyer et al., 2015) range in the Mississippi River (north of Louisiana) and Coastal Plain river drainages (east of the Mississippi River), respectively. Herein, we sampled the latter subspecies, describing a new TBF species from it, but all previous records source from the other subspecies. Likewise, the Gulf Coast smooth softshell turtle, *A. calvata*, which is endemic to lower Coastal Plain rivers and ranges from southeastern Louisiana to eastern Alabama and the Florida panhandle, has yet to be sampled for infections by *Vasotrema* spp. This turtle species was recently accepted but long considered a subspecies of the midland softshell turtle, *A. mutica* (see Weisrock and Janzen, 2000; Guyer et al., 2015). Three TBFs (*V. attenuatum*, *V. brevitestis*, *V. robustum*) infect its close relative *A. mutica* in Nebraska (Brooks and Mayes, 1975) (Table I). Also noteworthy biology and morphology, respectively.

Before being preserved or fixed in EtOH and n.b.f. for molecular mounting. If the specimens are too small for that, specimens preserving it in 95% EtOH for molecular biology while saving the posterior 1/3 of the body (posterior to the testis) and is ideal and can be obtained, if specimen size permits, by cutting taxonomic assignments of molecular sequences, a hologenophore softshell turtle, *A. spinifera* could also leave some softshell turtles relatively undersampled. Because they are dietary generalists (Guyer et al., 2015).

Sample because they must be caught by hand, whereas spiny softshell turtles can be captured with baited traps, perhaps because they are dietary generalists (Guyer et al., 2015).

Concurrent infections by *Vasotrema* spp., including congeners infecting the same tissue of the same host individual, are not uncommon. This underscores the importance of morphological vouchers that accompany published molecular sequence data. The only infection records reporting a single-species infection by a species of *Vasotrema* is that turtle diet and behavior coupled with the gear used to trap them could also leave some softshell turtles relatively undersampled. For example, that *A. spinifera* is the most intensively sampled softshell turtle in North America (Table I) could be related to sampling bias associated with baited hoop-net trapping. Smooth softshell turtles (*A. calvata* and *A. mutica*) are more difficult to sample because they must be caught by hand, whereas spiny softshell turtles can be captured with baited traps, perhaps because they are dietary generalists (Guyer et al., 2015).

Key to species of *Vasotrema*

1. Genital pore at level of ovary ............... *V. robustum*
   Genital pore anterior to ovary ..................... 2

2. Testis long, extending posterior to tips of ceca ........ 3
   Testis short, not extending to posterior to tips of ceca ................................................................ 4

3. Testis not coiled .................................... *V. attenuatum*
   Testis coiled ....................................... *V. longitestis*

4. Suckers papillate ................................... 5
   Suckers apapillate ................................ 6

5. Ovary sinistral to oviduct ....................... *V. amydae*
   Ovary dextral to oviduct ........................... *V. rileyae*

Acknowledgments

We thank Paul Johnson (Alabama Department of Conservation and Natural Resources [ADCNR]) and Dean Black for access to sampling sites (Perry Lakes State Park, Marion, Alabama; Canoe Pond, Springville, Alabama, respectively); Nathan R. Roberts (AU), Matt Womble (AU), and Thomas Tarpley (ADCNR) for their assistance with trapping; Estefania Rodriguez (AMNH), Gabor Racz (HWML), Anna Philips (USNM), Kathryn Ahlfield (USNM), and William Moser (USNM) for loaning museum specimens. This is a contribution of the Southeastern Cooperative Fish Parasite and Disease Project (Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences).

Literature Cited


