Blood flukes of Asiatic softshell turtles: revision of Coeuritrema Mehra, 1933 (Digenea: Schistosomatoida) and a new species infecting Chinese softshell turtles, Pelodiscus sinensis (Trionychidae), from Vietnam

Jackson R. Roberts¹, Raphael Orélis-Ribeiro¹, Binh T. Dang², Kenneth M. Halanych³ and Stephen A. Bullard¹

¹Auburn University, School of Fisheries, Aquaculture & Aquatic Sciences and Aquatic Parasitology Laboratory, Auburn, AL, USA; ²Nha Trang University, Department of Biological Sciences, Institute for Biotechnology and Environment, Nha Trang, Vietnam; ³Auburn University, Department of Biological Sciences and Molette Biology Laboratory for Environmental & Climate Change Studies, Auburn, AL, USA

Abstract: Coeuritrema Mehra, 1933, previously regarded as a junior subjective synonym of Hapalorhynchus Stunkard, 1922, herein is revised to include Coeuritrema lyssimus Mehra, 1933 (type species), Coeuritrema rugatus (Brooks et Sullivan, 1981) comb. n., and Coeuritrema platti Roberts et Bullard sp. n. These genera are morphologically similar by having a ventral sucker, non-fused caeca, two testes, a pre-testicular cirrus sac, an intertesticular ovary, and a common genital pore that opens dorsally and in the sinistral half of the body. Phylogenetic analysis of the D1–D3 domains of the nuclear large subunit ribosomal DNA (28S) suggested that Coeuritrema and Hapalorhynchus share a recent common ancestor. Coeuritrema is morphologically most easily differentiated from Hapalorhynchus by having ventrolateral tegumental papillae and a definitive metraterm that is approximately 3–7× longer than the uterus. Coeuritrema comprises species that reportedly infect Asiatic softshell turtles (Testudines: Trionychidae) only, whereas Hapalorhynchus (as currently defined) comprises blood flukes that reportedly infect those hosts plus North American musk turtles (Sternotherus Bell in Gray) and mud turtles (Kinosternidae), North American snapping turtles (Chelydridae), Asiatic hard-shelled turtles (Geoemydidae) and African pleurodirans (Pelemodusidae). Coeuritrema platti sp. n. infects the blood of Chinese softshell turtles, Pelodiscus sinensis (Wiegmann), cultured in the Da Rang River Basin (Phu Yen Province, Vietnam). It differs from C. lyssimus by having a narrow hindbody (< 1.6× forebody width), ventrolateral tegumental papillae restricted to the hindbody, a short cirrus sac (<10% of corresponding body length), a transverse ovary buttressing the caeca, a short, wholly pre-ovarian metraterm (~10% of corresponding body length), and a submarginal genital pore. It differs from C. rugatus by having small ventrolateral tegumental papillae, testes without deep lobes, and a Laurer’s canal pore that opens posterior to the vitelline reservoir and dorsal to the oviducal seminal receptacle. The new species is only the second turtle blood fluke reported from Vietnam.

Keywords: taxonomy, Spirorchia, 28S rDNA, molecular phylogeny, Griphobilharzia

The turtle blood flukes (TBFs; paraphyletic ‘Spirorchidae’) comprise 84 accepted species (54 freshwater; 30 marine) assigned to 20 genera and that infect 37 freshwater and three marine turtle species (Platt 1993, 2002, Smith 1997a,b, Tkach et al. 2009, Platt and Sharma 2012, Orélis-Ribeiro et al. 2014, Roberts et al. 2016). Seventeen TBFs of five genera (Hapalorhynchus Stunkard, 1922; Vasotrema Stunkard, 1926; Coeuritrema Mehra, 1933; Enterohaematotrema Mehra, 1940, Cardiotrema Dwivedi, 1967) infect softshell turtles (Testudines: Trionychidae) (Table 1), but only nine of 31 (29%) trionychids host a TBF, suggesting that perhaps numerous uninnominate TBFs infect this turtle lineage. Softshell turtles of Pelodiscus Fitzinger (P. axenaria [Zhou, Zhang et Fang], P. maackii [Brandt], P. parviformis Tang, P. sinensis [Wiegmann]) are commercially prized and cultured for food and magic throughout Asia (Alves et al. 2008, Fritz et al. 2010, Stickas and Fritz 2011). A survey of Chinese aquaculture facilities indicated that > 300 million Chinese softshell turtles are in captivity now and that ~ 125 million individuals are available for sale now in China alone (Haitao et al. 2008). Even though they are abundant in southeast Asian aquaculture, several of these species have become scarce in the wild (van Dijk et al. 2000). Despite their commercial importance, the taxonomy of their blood parasites remains as undereported as the infectious diseases that afflict these turtles in aquaculture.
During a recent parasitological expedition to Vietnam, we encountered a new TBF species infecting Chinese softshell turtles, which led to a consideration of all TBFs infecting softshell turtles. Herein, we revise Coeuritrema, emend its diagnosis, redescribe Coeuritrema rugatus (Brooks et Sullivan, 1981) comb. n. (formerly Hapaloryynchus) based on museum specimens, describe a new species, and provide an updated phylogeny for the TBFs inclusive of the new taxon. This is the second TBF species reported from Vietnam.

MATERIALS AND METHODS

Turtles were purchased from commercial turtle trappers in Nha Trang, Vietnam and from a turtle farm in Phu Yen Province during 1-16 June 2015. Turtles were killed by decapitation. Necropsies were performed using 7.0 g/l sodium citrate saline solution. Living flukes intended as whole-mounts were killed with a butane hand lighter under little or no coverslip pressure and transferred to a vial of 5% neutral buffered formalin (n.b.f.). TBFs were maintained in 5% n.b.f. until staining. After washing with distilled water, specimens intended as whole mounts were stained in Van Cleave’s hematoxylin with several additional drops of Ehrlich’s hematoxylin, dehydrated using an ethanol series, cleared in clove oil, and permanently mounted in Canada balsam.

Whole mounts were examined using a Leica DM 2500 microscope with differential interference contrast optical components. Parasite measurements are reported in micrometres (μm) followed by the mean and number of specimens measured in parentheses. Turtle scientific names and taxonomic authorities follow van Dijk et al. (2014). Classification and anatomical terms for TBF’s follow Roberts et al. (2014). Classification and anatomical terms for TBF’s follow Roberts et al. (2014).

Specimens for molecular analyses were handled with camel-hair brushes, pipettes or fine forceps, and immediately preserved in a vial of absolute EtOH and stored at -20°C. Total genomic DNA (gDNA) was extracted using DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the manufacturer’s protocol, except for the incubation period and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer’s protocol. DNA sequencing was performed by GENEWIZ with ABI Prism 3730xl DNA analyser (GENEWIZ, Inc., South Plainfield, New Jersey, USA). Primers used in sequencing of 28S rDNA included the PCR primers and the internal forward primers 300F (5'-CAA GTACCTGAGGAAAATTTG-3') and 900F (5'-CCGCTTGAACCAACGCAAG-3') and reverse primer 1200R (5'-GATAGTTCAACATTCTCCTG-3') (Lockyer et al. 2003). Sequence assembling and analysis of chromatograms were conducted using BioNumerics version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium).

The sequence data for nuclear 28S rDNA generated during this study were aligned with those for selected blood flukes (Schistosomatoida) available on GenBank. Outgroups were selected from representative fish blood flukes, Aporocotyliidae Odhner, 1912 (see Bullard et al. 2009). The ingroup comprised newly-generated sequence data from the new species (KX7112243) and Hapaloryynchus foliorichis Brooks et Mayes, 1975 (KX7112242) plus publicly-available sequences from BarcodeData obama Roberts, Platt et Bullard, 2016 (KX061500), Griphobilharzia amenoa Platt et Blair, 1991 (AY899914), and ten other TBFs (Orléis-Ribeiro et al. 2014). The specimens of H. foliorichis were collected in Alabama from Chelydra serpentina (Linnaeus) during October 2014 (Roberts et al. 2016). Sequences were aligned using MAFFT (Katoh and Toh 2010) with default settings implemented in the CIPRES Science Gateway V.3.3 (Miller et al. 2010). The resulting alignment was refined by eye using MEGA version 5.2.2 (Tamura et al. 2011) and the ends of each fragment were trimmed to match the shortest sequence. Ambiguous positions were identified and removed using the Gblocks server (Castresana 2000) with settings for a less stringent selection. Bayesian inference (BI) was performed using the Metropolis-coupled Markov chain Monte Carlo method (MC3) in MrBayes version 3.2.6 (Huelsenbeck et al. 2011) and the ends of each fragment were trimmed to match the shortest sequence. Ambiguous positions were identified and removed using the Gblocks server (Castresana 2000) with settings for a less stringent selection. Bayesian inference (BI) was performed using the Metropolis-coupled Markov chain Monte Carlo method (MC3) in MrBayes version 3.2.6 (Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003, Huelsenbeck and Ronquist 2003) plus public sequences available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank.

Sequence assembling and analysis of chromatograms were conducted using BioNumerics version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium).

The sequence data for nuclear 28S rDNA generated during this study were aligned with those for selected blood flukes (Schistosomatidae) available on GenBank. Outgroups were selected from representative fish blood flukes, Aporocotyliidae Odhner, 1912 (see Bullard et al. 2009). The ingroup comprised newly-generated sequence data from the new species (KX7112243) and Hapaloryynchus foliorichis Brooks et Mayes, 1975 (KX7112242) plus publicly-available sequences from BarcodeData obama Roberts, Platt et Bullard, 2016 (KX061500), Griphobilharzia amenoa Platt et Blair, 1991 (AY899914), and ten other TBFs (Orléis-Ribeiro et al. 2014). The specimens of H. foliorichis were collected in Alabama from Chelydra serpentina (Linnaeus) during October 2014 (Roberts et al. 2016). Sequences were aligned using MAFFT (Katoh and Toh 2010) with default settings implemented in the CIPRES Science Gateway V.3.3 (Miller et al. 2010). The resulting alignment was refined by eye using MEGA version 5.2.2 (Tamura et al. 2011) and the ends of each fragment were trimmed to match the shortest sequence. Ambiguous positions were identified and removed using the Gblocks server (Castresana 2000) with settings for a less stringent selection. Bayesian inference (BI) was performed using the Metropolis-coupled Markov chain Monte Carlo method (MC3) in MrBayes version 3.2.6 (Huelsenbeck et al. 2011) and the ends of each fragment were trimmed to match the shortest sequence. Ambiguous positions were identified and removed using the Gblocks server (Castresana 2000) with settings for a less stringent selection. Bayesian inference (BI) was performed using the Metropolis-coupled Markov chain Monte Carlo method (MC3) in MrBayes version 3.2.6 (Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003, Huelsenbeck and Ronquist 2003) plus public sequences available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank. Outgroups were selected from selected blood flukes (Schistosomatidae) available on GenBank.

Sequence assembling and analysis of chromatograms were conducted using BioNumerics version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium).
RESULTS

Coeuritrema Mehra, 1933 emended

Diagnosis. Body dorsoventrally flattened (not cylin-

rical), 3–7× longer than wide, constricted at level of ventral

sucker, having hindbody 1–3× longer than forebody, as-

pinous, papillate; ventrolateral tegumental papillae pres-

cent, distributing from oral sucker posteriord or from ven-

tral sucker posteriord. Dorosilateral and ventrolateral nerve

chords present.

Oral sucker robust, aspinous, demarcated from body by

posterior constriction. Ventral sucker present, aspinous.

Pharynx present, enveloping anterior extremity of oe-

sophagus. Oesophagus extending posteriord <1/4 of body

length, ventral to anterior nerve commissure, lacking di-

verticula, plicate organ, or median oesophageal pouch,

straight or slightly sinuous; oesophageal gland surrounding

verticula, plicate organ, or median oesophageal pouch.

Type species: Coeuritrema lyssimus Mehra, 1933 from

heart ventricle of Indian flapshell turtle, Lissemyys punctata

(Bonnaterre) (Testudines: Trionychidae), in the Ganges River,

India.

Remarks. Mehra (1933) proposed Coeuritrema for

C. lyssimus and Hapalarhynchus odhnerensis (Mehra,

1933) Byrd, 1939. That diagnosis included “body wall with

or without small papillae” (= ventrolateral tegumental pa-

pillae) such that H. odhnerensis, which lacks ventrolateral
tegumental papillae, could be included. As emended here-
in, however, Coeuritrema includes papillate TBFs only.

Excretory pore terminal. Manter’s organ absent. In blood

of Asiatic trionychids.

Differential diagnosis. Body 3–7× longer than wide,
aspinous; ventrolateral tegumental papillae present. Ventral

sucker present. Oesophagus <1/4 of body length, lacking
diverticula, plicate organ, or median oesophageal pouch.

Intestine comprising non-fused caeca. Testes comprising

one anterior and one posterior testis. External seminal ves-

cicle abutting anterodextral margin of cirrus sac and at level

of common genital pore. Ovary intertesticular. Oviducal

semenal receptacle comprising middle portion of oviduct

between ovary and posterior testis. Laurer’s canal interca-

celial, intertesticular, post-ovarian. Vitelline reservoir inter-

esticular. Uterine pouch absent. Egg single, occupying fe-

male reproductive tract proximal to metraterm. Metraterm

massive, approximately 3–7× uterus length, apparently not

storing egg(s). Common genital pore dorsal, sinistral, pre-

dominantly anterior to genitalia.

Ovary single, wider than long, triangular, lacking lobes,
testes comprising, intertesticular. Oviduct emerging from dextral

margin of ovary, directing posteriord; oviducal seminal re-

ceptacle comprising middle portion of oviduct between ovar
dy and posterior testis. Laurer’s canal intercaecal, inter-
esticular, post-ovarian, extending anteriad or posteriord from

oviduct at level of vitelline reservoir, opening dorsally. Vi-

tellarium follicular, occupying space from caecal bifurca-
tion to caecal termination; vitelline reservoir intercaecal,
testes, ventral to ovary. Ootype a weakly glandular

and thin-walled chamber, intercaecal, occupying space be-

tween anterior testis and ovary, posterior to cirrus sac.

Uterus intercaecal, intertesticular, straight (not coiled);
uterine pouch absent. Egg single, occupying female re-

productive tract proximal to metraterm. Metraterm massive,

approximately 3–7× uterus length, longitudinal (extend-

ing anteriad in parallel with body margin), between ventral

sucker and vitelline reservoir, sinistral to anterior testis,

having an obviously muscular thick wall, apparently not

storing egg(s). Common genital pore dorsal, sinistral, pos-
terior to ventral sucker, predominantly anterior to genitalia,
aspinous, lacking suckers.

Excretory vesicle globular, extensively lobed, extending

posteriad from distal ends of caeca to posterior body end.
served more than a single egg ("ovum") in the female reproductive tract of *C. lyssimus* (type species) and that each egg resided "in the proximal portion of the metraterm," which, based on his illustrations (see plate 1, fig. 1 of Mehra 1933) could be interpreted as the putative egg chamber. Brooks and Sullivan (1981) likewise reported that only a single egg occurred in the uterus of ovigerous specimens. Perhaps this is related to the gestation time for the large egg.

The metraterm of other whole-mounted blood flukes does hold eggs (Bullard and Overstreet 2006, Bullard et al. 2006, Bullard and Jensen 2008); however, eggs in those species are smaller, much more numerous in the uterus and metraterm, and seemingly less developed. Perhaps this is because they must infect minute branchial vessels before transiting to the gill epithelium and hatching (Bullard and Overstreet 2008). The extent to which the metraterm serves as a storage organ for TBF eggs is indeterminate, and Overstreet 2008). The extent to which the metraterm, and seemingly less developed. Perhaps this is because they must infect minute branchial vessels before transiting to the gill epithelium and hatching (Bullard and Overstreet 2008). The extent to which the metraterm serves as a storage organ for TBF eggs is indeterminate, and Overstreet 2008).

**Coeuritrema and Hapalorhynchus** are similar morphologically by having a ventral sucker, non-fused ceca, two testes, a pre-testicular cirrus sac, an intertesticular ovary, and a common genital pore that opens dorsally and in the sinistral half of the body. Phylogenetic analysis of the D1–D3 domains of the nuclear large subunit ribosomal DNA (28S) suggests that *Coeuritrema* and *Hapalorhynchus* indeed share a recent, common ancestor (Fig. 7; see below). We concur with Platt (2002) in that several apapillate turtle blood fluke species originally assigned to *Coeuritrema* should be assigned to *Hapalorhynchus*: *H. odhnerensis*, *Hapalorhynchus ocadiae* (Takeuti, 1942), *Hapalorhynchus oschmarini* (Belous, 1963), *Hapalorhynchus macrotesticularis* (Rohde, Lee et Lim, 1968), *Hapalorhynchus mica* (Oshmarin, 1971), *Hapalorhynchus sheilae* (Mehrotra, 1973) and *Hapalorhynchus sutlejensis* (Mehrotra, 1973) (see Takeuti 1942, Rohde et al. 1968, Oshmarin 1971, Mehrotra 1973). This brings the total number of accepted species of *Coeuritrema* and *Hapalorhynchus* to 3 and 19, respectively.

Yamaguti (1958) considered *Coeuritrema* a junior synonym of *Tremarhynchus* Thapar, 1933; however, the diagnosis of *Coeuritrema* was published in May 1933 and that of *Tremarhynchus* was published immediately thereafter in June. Hence, the former genus has taxonomic priority (Dwivedi 1967). Byrd (1939) and Platt (2002) considered *Tremarhynchus* (and *Coeuritrema*) a junior subjective synonym of *Hapalorhynchus*. No author has treated *Tremarhynchus* since 2002.

Tremarhynchinae Yamaguti, 1958 included *Enterohaematotrema* and *Tremarhynchus* (type genus) (see Yamaguti 1958). Dwivedi (1967) commented that the subfamily should be renamed Coeuritrematinae Dwivedi, 1967 (type genus *Coeuritrema*) to correct Yamaguti’s (1958) name for the subfamily based on his erroneous acceptance of *Tremarhynchus* as a senior synonym of *Coeuritrema* by taxonomic priority. This decision was probably based upon the International Code of Zoological Nomenclature’s Article 61.1.2. (under “Statement of the Principle of Typification”), which states that “a nominal family-group taxon is the nominal genus on which its name is based.” (International Commission on Zoological Nomenclature 2000). Hence, because post-1967 *Coeuritrema* was correctly understood to have taxonomic priority over *Tremarhynchus*, the subfamily name had to be changed.

Yamaguti (1971) accepted Coeuritrematinae Dwivedi, 1967 (as “1968”) and included *Coeuritrema*, *Enterohaematotrema*, and *Cardiotrema* based on the presence of a ventral sucker, paired ceca not united posteriorly, two testes, a cirrus sac “more or less strongly developed” between the ventral sucker and anterior testis, an intertesticular ovary, a vitellarium as long as ceca, and a genital pore between the ventral sucker and testes. These genera plus *Hapalorhynchus* and *Vasotrema* include species infecting softshell turtles (Trionychidae) (Table 1). No molecular sequence data exist for any species of *Enterohaematotrema* or *Cardiotrema*, and *Vasotrema* does not group with the *Coeuritrema* + *Hapalorhynchus* clade (Fig. 7). Hence, presently, no support exists for monophyly of softshell TBFs, suggesting that TBFs have repeatedly, independently colonised softshell turtles.

*Coeuritrema* differs from *Enterohaematotrema* by having a dorsal, sinistral genital pore (rather than a ventral, medial genital pore), from *Cardiotrema* by having an intertesticular ovary (rather than a sinistral ovary) and a large ventral sucker >1/2 body width (rather than <1/5 body width for *Cardiotrema*), and from *Vasotrema* by having two testes and a dorsal genital pore (rather than one testis posterior to the ovary and a ventral genital pore). Mehra (1934) provided a key to TBF genera and differentiated *Coeuritrema* and *Hapalorhynchus* by the presence or absence of a cirrus sac and cirrus. These features have been the focus of intense study because of their stated value as differential features. Our observations herein confirmed the presence of an obvious cirrus sac among the accepted species of *Coeuritrema*, but this feature may or may not be of generic importance in other TBF genera. For example, within the closely-related *Hapalorhynchus* the presence or absence of the cirrus sac and cirrus have been accepted as generic features by some (Mehra 1939, 1940, Yamaguti 1958, 1971) and rejected by others (Byrd 1939, Brooks and Mayes 1976, Platt 1988, 2002, Bourgat 1990).

Brooks and Mayes (1976) provided supplemental observations of the male genitalia of *Hapalorhynchus stunkardi* Byrd, 1939 and emended *Hapalorhynchus* to include “well-developed or poorly-developed cirrus.” Platt (1988) observed, in histological sections, prostate cells not bound by a limiting membrane but scattered in the surrounding parenchyma in *Hapalorhynchus gracilis* Stunkard, 1922, *H. foliorchis* and *H. stunkardi*. He also observed that the prostatic complex of *Hapalorhynchus albertoi* Lamotho-Argumedo, 1978 and *Hapalorhynchus brooksi* Platt, 1988 was membrane-bound (= cirrus sac) and that the distal portion of the ejaculatory duct (= cirrus) was eversi-
Table 1. Blood flukes infecting softshell turtles (Testudines: Trionychidae)

<table>
<thead>
<tr>
<th>Turtle</th>
<th>Fluke</th>
<th>Site in host</th>
<th>Interpretation/Alias</th>
<th>Locality</th>
<th>Museum nos.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apalone ferox</em> (Schneider), Florida softshell</td>
<td><em>Vasotrema amydae</em> (Stunkard, 1926) (type species)</td>
<td>blood</td>
<td>probably a Gulf of Mexico river drainage, Florida, USA</td>
<td>AMNH 791</td>
<td>Brooks and Sullivan (1981)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema attenuatum</em> (Stunkard, 1928)</td>
<td>blood</td>
<td>probably a Gulf of Mexico river drainage, Florida, USA</td>
<td>AMNH 791</td>
<td>Brooks and Sullivan (1981)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema sp.</em></td>
<td>not specified</td>
<td>Apalachicola River, Franklin County, Florida, USA</td>
<td>not specified</td>
<td>Loftin (1960)</td>
<td></td>
</tr>
<tr>
<td><em>Apalone mutica</em> (LeSueur), smooth softshell</td>
<td><em>Vasotrema attenuatum</em> (Brooks et Mayes, 1975)</td>
<td>blood</td>
<td>Missouri River Drainage, Nebraska, USA</td>
<td>USNM 73817−73819; HWML 20077</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema robustum</em> (Brooks et Mayes, 1975)</td>
<td>blood</td>
<td>Missouri River Drainage, Nebraska, USA</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema amydae</em> (type species)</td>
<td>blood</td>
<td>Ohio River Drainage, Indiana, USA</td>
<td>AMNH 791</td>
<td>Brooks and Sullivan (1981)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema attenuatum</em></td>
<td>mesenteric blood vessels</td>
<td>Missouri River Drainage, Indiana, USA</td>
<td>AMNH 806, 807</td>
<td>Stunkard (1928)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema brevitestis</em> (type species)</td>
<td>not specified</td>
<td>Missouri River Drainage, Nebraska, USA</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema robustum</em> (type species)</td>
<td>blood</td>
<td>Ohio River Drainage, Indiana, USA</td>
<td>AMNH 806, 807</td>
<td>Stunkard (1928)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema longitrox</em> (Byrd, 1939)</td>
<td>arterial circulation</td>
<td>Reelfoot Lake, Tennessee, USA</td>
<td>HWML 9227</td>
<td>Byrd (1939) (see also Platt and Snyder 2007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema brevitestis</em></td>
<td>not specified</td>
<td>Reelfoot Lake, Tennessee, USA</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema longitrox</em></td>
<td>not specified</td>
<td>Atkinson Lake, Atkinson Recreation Area, 0.8 km west of Atkinson, Nebraska, USA</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema robustum</em></td>
<td>heart, large blood vessels</td>
<td>Missouri River Drainage, Nebraska, USA</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vasotrema longitrox</em></td>
<td>heart arteries near heart</td>
<td>unknown drainage, Kuala Lumpur (purchased from Chinese merchant), Malaysia</td>
<td>not specified</td>
<td>Brooks and Mayes (1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hapalorhynchus macrostolaridis</em> (Roehde, Lee et Litm, 1968)</td>
<td>heart, arteries near heart</td>
<td>Reelfoot Lake, Tennessee, USA</td>
<td>HWML 2207</td>
<td>Byrd (1939) (see Platt and Prewoood 1990)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lissensmys punctata</em> (Bonna- terre), Indian flagshell</td>
<td><em>Cardiotrema raparissis</em> (Mehrotra, 1973)</td>
<td>not specified</td>
<td>Sutlej River, Ropar, Punjab State, India</td>
<td>not specified</td>
<td>Mehrotra (1973)</td>
</tr>
<tr>
<td></td>
<td><em>Enterochaetonotremora palaearcticum</em> Mehra, 1940 (type species)</td>
<td>body wash, hepatic blood vessels</td>
<td>not specified</td>
<td>Sutlej River, Ropar, Punjab State; Yamuna River, Karnal, Haryana State, India; Ganges River, Allahabad, Uttar Pradesh State, India</td>
<td>not specified</td>
<td>Mehrotra (1940)</td>
</tr>
<tr>
<td></td>
<td><em>Coeuriotrema lyssinus</em> Mehra, 1940 (type species)</td>
<td>small intestine</td>
<td>Ganges River, Allahabad, Uttar Pradesh State, India</td>
<td>not specified</td>
<td>Mehrotra (1933)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hapalorhynchus advenensis</em> (Mehra, 1940)</td>
<td>ventricle of heart</td>
<td>Ganges River, Allahabad, Uttar Pradesh State, India</td>
<td>not specified</td>
<td>Tandon and Gupta (1982)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hapalorhynchus shielae</em> (Mehrotra, 1973)</td>
<td>ventricle of heart</td>
<td>Ganges River, Allahabad, Uttar Pradesh State, India</td>
<td>not specified</td>
<td>Mehrotra (1973)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hapalorhynchus sutleujensis</em> (Mehrotra, 1973)</td>
<td>not specified</td>
<td>Patiala River (Ghaggar River Drainage), Patiala, Punjab State; Sutlej River, Ropar, Punjab State; Ghanggar River, Sangur, Punjab State; Ganges River Drainage, Radirpur, Uttarakhanda State, India</td>
<td>not specified</td>
<td>Mehrotra (1973)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Delosiotrema indicus</em> (Thapar, 1933)</td>
<td>heart, large blood vessels</td>
<td>Gomti River, Lucknow, Uttar Pradesh State; Ghanggar River, Sangur, Punjab State, India</td>
<td>not specified</td>
<td>Tandon and Gupta (1982)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Nylenia hawii</em> (Gray), Indian peacock softshell</td>
<td><em>Hapalorhynchus indicus</em></td>
<td>heart, large blood vessels</td>
<td>Gomti River, Lucknow, Uttar Pradesh State; Ghanggar River, Sangur, Punjab State, India</td>
<td>not specified</td>
<td>Thapar (1933)</td>
</tr>
<tr>
<td></td>
<td><em>Pelodius simplex</em> (Wieghmann), Chinese softshell</td>
<td><em>Coeuriotrema platii</em> sp. n.</td>
<td>heart, mesentery, lung</td>
<td>Da Rang River, Phuyn Province, Vietnam</td>
<td>USNM 1411790, 1411791</td>
<td>Present study (Prestwood 1990)</td>
</tr>
<tr>
<td></td>
<td><em>Hapalorhynchus oschmarini</em> (Belous, 1963)</td>
<td>hepatic blood vessels</td>
<td>Lake Khanka, Russia</td>
<td>not specified</td>
<td>Belous (1963)</td>
<td></td>
</tr>
</tbody>
</table>
ble (Lamothe-Argumedo 1978, Platt 1988). As a result, he emended the diagnosis as “cirrus sac present or absent.” As additional species of Coeuritrema are documented, special care should be taken to assess the cirrus sac and cirrus.

Coeuritrema rugatus (Brooks et Sullivan, 1981) comb. n. Figs. 1, 2


Redescription (based on light microscopy of eight whole-mounted adult specimens, including paratypes): Body 1250–1540 (1423; 7) long or 5.3–7.2× (6.5; 7) longer than wide, 150–200 (177; 8) wide or 12–13% (12%; 7) of body length at level of caecal bifurcation, 200–265 (219; 8) wide or 14–19% (15%; 7) of body length at ovary (typically maximum width of specimen), 150–205 (174; 8) wide or 11–15% (12%; 7) of body length at caecal termination; forebody (middle of ventral sucker to anterior end) 340–470 (439; 7) long or 24–34% (31%; 7) of body length, lacking ventrolateral tegumental papillae; hindbody (middle of ventral sucker to posterior end) 840–1090 (968; 8) long or 66–76% (69%; 7) of body length, having ventrolateral tegumental papillae (Fig. 1). Ventrolateral tegumental papillae 17–20 (19; 8) along dextral body margin, 18–20 (19; 8) along sinistral body margin, 35–39 (38; 8) total (Fig. 1); anterior papillae 8–35 (20; 8 [3 papillae measured per specimen]) long, with base 15–38 (24; 8) wide, 0.6–2.5× (1.3; 8) wider than long; middle papillae 25–43 (32; 8) long, with base 35–53 (43; 8) wide, 1.0–1.8× (1.3; 8) wider than long; posterior papillae 8–38 (23; 8) long, base 13–45 (29; 8) wide, 0.7–2.9× (1.3; 8) wider than long.

Oral sucker 60–75 (67; 7) long or 4–5% (5%; 7) of body length, 78–100 (93; 7) wide or 38–50% (43%; 7) of maximum body width (Fig. 1). Ventral sucker 103–125 (114; 8) long or 7–9% (8%; 7) of body length, 103–133 (120; 8) wide or 57–75% (68%; 8) of body width at caecal bifurcation, 1.3–1.5× (1.3; 7) wider than oral sucker (Fig. 1). Nerve commissure 193–225 (202; 7) or 13–16% (14%; 7) of body length from anterior body end.

Pharynx 53–68 (59; 7) long or 20–25% (23%; 7) of oesophagus length, 60–83 (77; 7) wide or 0.8–1.5× (1.1; 7) maximum oesophagus width (Fig. 1). Oesophagus 225–295 (267; 7) long or 17–21% (18%; 7) of body length, 15–25 (19; 7) wide posterior to pharynx, with wall 10–18 (14; 7) thick, 53–95 (73; 8) maximum width or 29–53% (41%; 8) of body width at caecal bifurcation, with wall 10–50 (24; 8) thick (Fig. 1), narrowing to 13–25 (20; 8) width or 7–14% (11%; 8) of body width at caecal bifurcation, with wall 10–20 (15; 8) thick; oesophageal gland 205–245 (223; 8) long or 14–17% (16%; 7) of body length and 81–93% (86%; 7) of oesophagus length, 100–135 (121; 8) wide or 56–84% (70%; 8) of body width at caecal bifurcation.

Intestine bifurcating 262–330 (285; 7) or 17–23% (20%; 7) of body length from anterior end; sinistral caecal 850–1050 (962; 8) long or 67–72% (69%; 7) of body length, 73–100 (87; 7) wide at bifurcation or 46–54% (49%; 7) of body width, 20–30 (26; 8) wide at ovary or 9–15% (12%; 8) of body width, 23–45 (31; 8) wide at termination or 13–24% (18%; 8) of body width; dextral caecal 820–1100 (953; 8) long or 65–73% (68%; 7) of body length, 65–90 (77%; 8) wide at bifurcation or 38–50% (43%; 8) of body width, 15–30 (24; 8) wide at ovary or 7–13% (11%; 8) of body width, 25–45 (32; 8) wide at termination or 15–24% (18%; 8) of body width; caecal termination 115–145 (130; 8) or 8–10% (9%; 7) from posterior end.

Anterior testis lobed, intensely follicular marginally, 118–188 (143; 8) long or 9–13% (10%; 7) of body length, 70–115 (87; 8) wide or 35–44% (40%; 8) of body width at gonads; intertesticular space 63–90 (83; 8) or 4–7% (6%; 7) of body length (Figs. 1, 2). Posterior testis as anterior testis, 138–208 (167; 8) long or 10–15% (12%; 7) of body length, 70–135 (91; 8) wide or 35–51% (41%; 7) of body width at gonads; post-testicular space 340–430 (391; 8) or 26–30% (28%; 7) of body length from posterior end. Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending anteriad 20–28 (22; 8) or <1% (7) of body length from testis, 5 (8) wide or 2–3% (2%; 8) of body width (Fig. 2); posterior trunk of vasa efferentia emanating from ventral surface of posterior testis, extending anteriad 200–273 (234; 8) or 16–19% (17; 7) of body length, 3–5 (4; 8) wide or 1–3% (2%; 8) of body width, coalescing with anterior trunk of vasa efferentia ventral to anterior portion of anterior testis (Fig. 2).

Vas deferens 88–153 (111; 8) long or 6–10% (8%; 7) of body length, 5–8 (5; 8) in maximum width, extending anteroventrally. External seminal vesicle 40–80 (60; 8) long or 3–6% (4%; 7) of body length, 25–115 (46; 8) wide, 0.7–2.0× (1.5; 8) longer than wide, narrowing posteriorly and proximal to internal seminal vesicle (Figs. 1, 2); internal seminal vesicle 75–88 (80; 8) long or 1.0–2.1× (1.4; 8) external seminal vesicle length, 25–35 (31; 8) wide or 2.2–3.1× (2.6; 8) longer than wide or 22–132% (81%; 8) of external seminal vesicle width (Figs. 1, 2). Pars prostatica surrounding distal portion of internal seminal vesicle, 48–58 (52; 8) long, 30–35 (32; 8) wide or 1.5–1.9× (1.6; 8) longer than wide (Fig. 2). Cirrus 5–10 (8; 8) long, 3–5 (4; 8) wide; cirrus sac enveloping most of internal seminal vesicle and entirety of pars prostatica, 88–160 (119; 8) long or 6–11% (8%; 7) of body length, 55–95 (67; 8) wide or 24–44% (31%; 8) of maximum body width, containing large putative secretory cells (Figs. 1, 2).

Ovarian trunk, with broadest portion in sinistral half of body, 260–355 (310; 8) or 21–25% (22%; 7) of body length from middle of ventral sucker buttressing caeca, 58–100 (77; 8) long or 5–6% (6%; 7) of body length, 93–140 (112; 8) wide or 44–58% (51%; 7) of body width, 1.3–1.8× (1.5; 8) wider than long; post-ovarian space 495–640 (581; 8) or 40–45% (41%; 7) of body length (Fig. 1); oocytes largest and most basopholic near oviduct (Fig. 2). Oviduct extending posteriorly, turning dorsally, proceeding counterclockwise for 48–65 (54; 8) or 3–5% (4%; 7) of body length, 5–10 (8; 8) in maximum width, expanding laterally to form oviducal seminal receptacle; oviducal seminal receptacle extending sinistrad for 53–80

doi: 10.14411/fp.2016.031

Roberts et al.: Coeuritrema spp.
**Figs. 1−2. Coeuritrema rugatus** (Brooks et Sullivan, 1981) comb. n. (Digenea: Schistosomatoidea) from the mesenteric vessels of *Amyda cartilaginea* (Boddaert) (Testudines: Trionychidae) from Malaysia, paratype (HWML Coll. No. 213199). **Fig. 1.** Total view, ventrally. **Fig. 2.** Genitalia, ventral view. **Abbreviations:** at – anterior testis; ave – anterior trunk of vasa efferentia; cb – caecal bifurcation; cgp – common genital pore; cs – cirrus sac; dc – dextral caecum; dln – dorsolateral nerve chord; ec – eversible cirrus; egg – egg in utero; ep – excretory pore; esv – external seminal vesicle; ev – excretory vesicle; isv – internal seminal vesicle; Lc – Laurer’s canal; lvd – lateral vitelline collecting duct; mt – metraterm; nc – nerve commissure; od – oviduct; oe – oesophagus; og – oesophageal gland; oo – ootype; os – oral sucker; osr – oviducal seminal receptacle; ov – ovary; ph – pharynx; pp – pars prostatica; pt – posterior testis; pve – posterior trunk of vasa efferentia; sc – sinistral caecum; tvd – transverse vitelline duct; ut – uterus; vd – vas deferens; vlp – ventrolateral tegumental papillae; vr – vitellarii; vrs – vitelline reservoir; vs – ventral sucker; vt – vitelline duct.
(63; 8) or 58–112% (84%; 8) of ovary width, 25–33 (28; 8) in maximum width at origin or 9–16% (13%; 8) of maximum body width, narrowing before turning dorsal, extending anterodextral 58–95 (82; 8) or 5–7% (6%; 7) of body length, 10–13 (12; 8) in maximum width or 5–7% (5%; 8) of body width (Fig. 2). Laurer’s canal a narrow duct extending 23–43 (31; 8) anterosinistrad from middle portion of oviduct, 8–13 (11; 8) wide, opening dorsally at level of middle portion of ovary (Fig. 2).

Vitellarium comprising a series of interconnected spheroid masses of follicles, distributing from level of caecal bifurcation to distal ends of caeca, lateral collecting ducts coalescing at level of posterior margin of ovary to form transverse vitelline duct; transverse vitelline duct ventral to ovary, 315–415 (369; 8) or 25–29% (27%; 7) of body length from middle of ventral sucker (Figs. 1, 2); vitelline reservoir sac-like, ventral to oviducal seminal receptacle; vitelline duct extending anterodextrad and dorsal 55–85 (64; 8) or 4–6% (5%; 7) of body length before connecting with oviduct at ootype (Fig. 2). Ootype difficult to discern in gravid specimens, 30–43 (36; 8) long, 28–43 (37; 8) wide, dorsal to anterior margin of ovary (Fig. 2).

Uterus comprising proximal portion and metraterm (Fig. 2), with a single egg in seven of eight specimens; proximal portion of uterus extending anterosinistrad from ootype, 28–50 (39; 8) long or 2–4% (3%; 7) of body length, 18–30 (27; 8) wide or 9–15% (12%; 8) of maximum body width; metraterm extending anterodextrad, 148–218 (175; 8) long or 11–15% (12%; 7) of body length, maximum width of 25–45 (34; 8) or 12–21% (16%; 8) of maximum body width, 3.2–6.6× (4.7; 8) proximal uterus length. Uterine egg ovoid, 63–165 (144; 8) long or 10–12% (10%; 8) of body length, 23–33 (27; 7) wide or 9–16% (12%; 7) of maximum body width, 1.9–3.3× (2.7; 7) longer than wide (Fig. 2). Common genital pore 95–150 (123; 8) or 8–10% (9%; 7) of body length posterior to middle of ventral sucker (Fig. 1).

Excretory vesicle 130–165 (144; 8) long or 10–12% (10%; 7) of body length, 78–100 (87; 8) wide or 45–55% (50%; 8) of body width at caecal termination; wall 8–13 (10; 8) thick (Fig. 1).

**Type and only known host:** Asiatic softshell turtle, *Amyda cartilaginea* (Boddart) (Testudines: Trionychidae).

**Type locality:** Sungei Jempol, Ulu Jempol, State of Negeri Sembilan, Malaysia.

**Site in host:** Mesenteric blood vessels.

**Specimens examined:** *Hapalorhynchus rugatus* – Harold W. Manter Laboratory (HWML) of Parasitology Coll. No. 21339, paratypes, eight slides comprising eight whole-mounted adult specimens: Body 1 035–1 410 (1218; 6) long or 5.5–6.9× (6.0; 6) longer than wide, 110–170 (147; 6) wide or 11–15% (12%; 6) of body length at level of caecal bifurcation, 150–225 (203; 6) wide or 14–18% (17%; 6) of body length at ovary (typically maximum width of specimen), 120–180 (165; 6) wide or 12–16% (14%; 6) of body length at caecal termination; forebody 390–519 (444; 6) long or 32–39% (36%; 6) of body length, hindbody 660–930 (783; 6) long or 61–68% (64%; 6) of body length, having ventrolateral tegumental papillae (Figs. 3, 5). Ventrolateral tegumental papillae extending posteriorly from middle of ventral sucker to posterior end 18–19 (18; 5) along dextral body margin, 17–20 (18; 5) along sinistral body margin, 35–39 (36; 5) total (Figs. 3, 5); anterior papillae 8–25 (17; 3) long, with base 10–33 (21; 5) wide, 0.7–2.5× (1.2; 3) wider than long; middle papillae 10–25 (17; 3) long, with base 18–35 (26; 5) wide, 1.0–2.8× (1.7; 3) wider than long; posterior papillae 5–25 (14; 3) long, with base 8–28 (17; 5) wide, 0.6–2.0× (1.3; 3) wider than long.

**Oral sucker** 45–70 (58; 4) long or 4–6% (5%; 4) of body length, 50–100 (80; 6) wide or 33–45% (39%; 6) of maximum body width (Figs. 3, 5). Ventral sucker 75–138 (115; 6) long or 7–13% (10%; 6) of body length, 65–138 (114; 5) wide or 59–95% (77%; 5) of body width at caecal bifurcation, 1.3–1.6× (1.4; 5) wider than oral sucker (Figs. 3, 5). Nerve commissure 150–238 (188; 6) or 13–17% (15%; 6) of body length from anterior body end.

**Pharynx** 45–63 (58; 6) long or 20–25% (23%; 6) of oesophagus length, 45–83 (65; 6) wide or 1.6–2.2× (1.7; 6) maximum oesophagus width (Figs. 3, 5). Oesophagus
Figs. 3, 4. Coeuritrema platti sp. n. (Digenea: Schistosomatoida) from viscera of Pelodiscus sinensis (Wiegmann) (Testudines: Trionychidae) from Vietnam, holotype (USNM Coll. No. 1411790). **Fig. 3.** Total view, dorsally. **Fig. 4.** Genitalia, dorsal view. **Abbreviations:** at − anterior testis; ave − anterior trunk of vasa efferentia; cb − caecal bifurcation; cgp − common genital pore; cs − cirrus sac; dc − dextral caecum; ec − eversible cirrus; egg − egg in utero; ep − excretory pore; esv − external seminal vesicle; ev − excretory vesicle; isv − internal seminal vesicle; Lc − Laurer’s canal; lvd − lateral vitelline collecting duct; mt − metraterm; nc − nerve commissure; od − oviduct; oe − oesophagus; og − oesophageal gland; os − oral sucker; osr − oviducal seminal receptacle; ov − ovary; ph − pharynx; pp − pars prostatica; pt − posterior testis; pve − posterior trunk of vasa efferentia; sc − sinistral caecum; tvd − transverse vitelline duct; vd − vas deferens; vln − ventrolateral nerve chord; vlp − ventrolateral tegumental papillae; vr − vitellarium; vrs − vitelline reservoir; vs − ventral sucker; vt − vitelline duct.
Figs. 5, 6. Coeuritrema platti sp. n. (Digenea: Schistosomatoida) from viscera of Pelodiscus sinensis (Wiegmann) (Testudines: Trionychidae) from Vietnam, paratype (USNM 1411791). Fig. 5. Total view, ventrally. Fig. 6. Genitalia, ventral view. Abbreviations: at = anterior testis; ave = anterior trunk of vasa efferentia; cb = cæcal bifurcation; cgp = common genital pore; cs = cirrus sac; dc = dextral caecum; ec = eversible cirrus; ep = excretory pore; esv = external seminal vesicle; ev = excretory vesicle; isv = internal seminal vesicle; Lc = Laurer’s canal; lvd = lateral vitelline collecting duct; mt = metraterm; nc = nerve commissure; od = oviduct; oe = oesophagus; og = oesophageal gland; os = oral sucker; oo = ootype; osr = oviducal seminal receptacle; ov = ovary; ph = pharynx; pp = pars prostatica; pt = posterior testis; pve = posterior trunk of vasa efferentia; sc = sinistral caecum; tvd = transverse vitelline duct; ut = uterus; vd = vas deferens; vln = ventrolateral nerve chord; vlp = ventrolateral tegumental papillae; vr = vitellarium; vrs = vitelline reservoir; vs = ventral sucker; vt = vitelline duct.
straight, 229−301 (259; 6) long or 18−23% (21%; 6) of body length, 10−15 (13; 6) wide posterior to pharynx, with wall 7−10 (9; 6) thick, 25−50 (39; 6) maximum width or (26%; 6) of body width at caecal bifurcation, with wall 10−27 (20; 6) thick, constricting to 13−18 (16; 6) width or 8−16% (11%; 6) of body width at caecal bifurcation, with wall 10−15 (12; 6) thick; oesophageal gland 198−288 (254; 6) long or 17−24% (21%; 6) of body length and 0.9−1.1× (1.0; 6) oesophagus length, 75−125 (104; 6) wide or 68−74% (71%; 6) of body width at caecal bifurcation (Figs. 3, 5). Intestine bifurcating 235−365 (285; 6) or 17−27% (24%; 6) of body length from anterior end; sinistral caecum 855−940 (754; 6) long or 51−68% (62%; 6) of body length, 35−45 (39; 6) wide or 24−32% (27%; 6) of body width at bifurcation, 13−30 (20; 6) wide or 6−15% (10%; 6) of body width at ovary, 20−35 (27; 6) wide or 12−19% (16%; 6) of body width at termination; dextral caecum 855−940 (743; 6) long or 52−67% (61%; 6) of body length, 38−45 (41; 6) wide or 24−36% (29%; 6) of body width at bifurcation, 18−35 (25; 6) wide or 10−18% (12%; 6) of body width at ovary, 18−35 (27; 6) wide or 11−20% (16%; 6) of body width at termination; caecal termination 92−140 (120; 6) or 8−14% (10%; 6) from posterior end.

Anterior testis follicular throughout, lacking lobes, 60−143 (106; 6) long or 5−11% (9%; 6) of body length, 50−95 (71; 6) wide or 25−43% (35%; 6) of body width at gonads; intertesticular space 30−103 (74; 6) or 3−7% (6%; 6) of body length (Figs. 3−6). Posterior testis as anterior testis, 90−158 (130; 6) long or 8−15% (11%; 6) of body length, 55−125 (89; 6) wide or 28−57% (44%; 6) of body width at gonads; post-testicular space 293−335 (310; 6) or 24−28% (27%; 6) of body length from posterior end. Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending anteriad 10−35 (26; 5) or 1−3% (2%; 5) of body length from testis, 3 (5) wide or 1−2% (1%; 5) of body width (Figs. 4−6); posterior trunk of vasa efferentia emanating from ventral surface of posterior testis, extending anteriad 188−255 (224; 5) or 17−21% (18%; 5) of body length, 3−5 (4; 5) wide or 1−3% (2%; 5) of body width, coalescing with anterior trunk of vasa efferentia ventral to anterior portion of anterior testis (Figs. 4−6).

Vas deferens 80−138 (101; 5) long or 6−10% (8%; 5) of body length, 3−5 (4; 3) in maximum width, extending anteroventrally. External seminal vesicle 63−88 (75; 5) long or 6% (5) of body length, 25−38 (33; 5) wide, 1.9−2.5× (2.3; 5) longer than wide, constricting posteriorly to internal seminal vesicle (Figs. 3−6); internal seminal vesicle 88−118 (100; 5) long or 1.1−1.6× (1.4; 5) external seminal vesicle length, 13−35 (23; 6) wide or 3.4−6.8× (4.4; 5) longer than wide or 52−92% (73%; 5) of external seminal vesicle width (Figs. 3−6). Pars prostatica surrounding distal portion of internal seminal vesicle (Figs. 4, 6), 53−55 (54; 3) long, 20−30 (25; 3) wide or 1.8−2.7× (2.2; 3) longer than wide. Cirrus 5−8 (6; 3) long, 3 (3) wide; cirrus sac enveloping most of internal seminal vesicle and entirety of pars prostatica, 78−128 (100; 6) long or 7−9% (8%; 6) of body length, 45−65 (52; 6) wide or 22−30% (26%; 6) of maximum body width, containing large putative secretory cells (Figs. 3−6).

Ovary triangular, with broadest portion in sinistral half of body, 160−320 (246; 6) or 15−23% (20%; 6) of body length from middle of ventral sucker, buttressing caeca, 35−115 (84; 6) long or 3−9% (7%; 6) of body length, 65−153 (111; 6) wide or 42−70% (54%; 6) of body width, 1.1−1.9× (1.4; 6) wider than long; post-ovarian space 425−510 (458; 6) or 35−45% (28%; 6) of body length (Figs. 3−6); oocytes uniform in size and basophilic throughout ovary (Figs. 4, 6). Oviduct extending posteriorly, turning dorsally, proceeding sinistrad, turning dorsal again before extending posteriad for 35−105 (61; 6) or 3−7% (5%; 6) of body length, 5−10 (8; 6) in maximum width, expanding laterally to form oviducal seminal receptacle; oviducal seminal receptacle extending sinistrad for 65−80 (71; 3) or 63−78% (73%; 3) of ovary width, 25−40 (34; 5) in maximum width at origin or 11−20% (16%; 5) of maximum body width, narrowing before turning dorsal, extending anterodextrad 78−80 (79; 3) or 6−7% (7%; 3) of body length, 10−13 (11; 3) in maximum width or 5−6% (5%; 3) of body width (Figs. 3−6). Laurer’s canal a narrow duct extending 15−25 (21; 3) anterodorsad from middle portion of oviduct, 5−8 (6; 3) wide, opening dorsal and over proximal portion of oviducal seminal receptacle (Figs. 4, 6).

Vitellarium comprising a series of interconnected spheroid masses of follicles, distributing from level of caecal bifurcation to distal ends of caeca, lateral collecting ducts coalescing at level of posterior margin of ovary to form transverse vitelline duct; transverse vitelline duct ventral to ovary, 245−385 (297; 3) or 21−27% (24%; 3; 5) of body length from middle of ventral sucker, lobed dorsally (Figs. 3−6); vitelline reservoir sac-like, ventral to oviducal seminal receptacle; vitelline duct extending anterodextrad and dorsal 30−58 (47; 5) or 2−5% (4%; 5) of body length before connecting with oviduct at ootype (Figs. 3−6). Ootype difficult to discern in gravid specimens, 25−40 (32; 3) long, 33−43 (38; 3) wide, dorsal to dextral half of ovary (Fig. 6).

Uterus comprising proximal portion and metraterm (Figs. 3−6), with a single egg in four of seven specimens; proximal portion of uterus extending anterodorsad from ootype, 25−45 (36; 3) long or 2−4% (3% 3) of body length, 23−38 (29; 3) wide or 12−18% (14%; 3) of maximum body width; metraterm extending anterodorsad, 128−163 (147; 5) long or 11−13% (12%; 5) of body length, 45−63 (53; 3) wide or 13−32% (23%; 3) of maximum body width, 2.8−5.8× (4.3; 3) proximal uterus length. Uterine egg ovoid, 80−95 (86; 4) long or 6−8% (7%; 4) of body length, 30−38 (35; 4) wide or 14−17% (16%; 4) of maximum body width, 2.3−2.7× (2.5; 4) longer than wide (Fig. 4). Common genital pore 63−138 (103; 6) or 6−11% (8%; 6) of body length posterior to middle of ventral sucker.

Excretory vesicle 120−180 (165; 6) long or 12−17% (14%; 6) of body length, 38−90 (74; 6) wide or 32−53% (44%; 6) of body width at caecal termination, with wall 5−15 (9; 4) thick (Figs. 3, 5).

Type and only known host: Chinese softshell turtle, *Pelodiscus sinensis* (Wiegmann) (Testudines: Trionychidae).
Type locality: Da Rang River Basin, Phu Yen Province, Vietnam.

Site in host: Heart, mesentery, lung.

Prevalence and intensity of infection: Two (100%) *P. sinensis* from an aquaculture facility in the Da Rang River Basin had four and five specimens of *C. platti*. None of two *P. sinensis* collected from the Cai River Basin in Nha Trang yielded specimens.


Material examined: *Hapalorhynchus gracilis* – American Museum of Natural History (AMNH) Coll. No. 125, holotype, one slide comprising one whole-mounted specimen, ex *Chelydra serpentina* from North Judson, Indiana (Stunkard 1922); AMNH 1269, paratypes, four slides (three of the four labeled *Hapalorhynchus gracilis*) comprising four whole-mounted specimens, ex *Chelydra serpentina* from North Judson, Indiana (Stunkard 1922); *Vasotrema attenuatum* – AMNH 806, syntype, 17 slides comprising 25 whole-mounted specimens, ex *Apalone ferox* and *A. spinifera* from Florida and Indiana respectively (Stunkard 1928). *Hapalorhynchus rugatus* – HWML 21339.

Etymology: The specific epithet *platti* honours our friend and colleague Thomas R. Platt (Professor Emeritus, Saint Mary’s College, Notre Dame, Indiana) for his extensive, sustained contributions to the taxonomy and systematics of turtle blood flukes.

Remarks. *Coeuritrema platti* is most similar to *C. rugatus* by the combination of having ventrolateral tegumental papillae restricted to the hindbody as well as a hindbody <1.6× forebody width. The new species is most easily distinguished from *C. rugatus* by having small ventrolateral tegumental papillae (35 μm maximum base width), tests without deep lobes, and a Laurer’s canal pore opening posterior to the vitelline reservoir and dorsal to the oviducal seminal receptacle. *Coeuritrema rugatus* has large ventrolateral tegumental papillae (53 μm maximum base width), deeply lobed tests, and a Laurer’s canal pore opening anterior to the vitelline reservoir and oviducal seminal receptacle. *Coeuritrema platti* differs from *C. lyssimus* by having a narrow hindbody (1.1–1.4× forebody width), ventrolateral tegumental papillae restricted to the hindbody, a short cirrus sac extending 7–9% of body length, a transverse ovary buttressing the caeca, a short, wholly pre-ovarian metraterm (11–13% of body length), and a submarginal genital pore. *Coeuritrema lyssimus* has a wide hindbody (1.7× forebody width), ventrolateral tegumental papillae distributing from the oral sucker to the excretory pore, a long cirrus sac extending 10–13% of body length, a sinistral ovary that does not buttress the caeca, a metraterm that begins lateral to the ovary and extends anteriad 17–18% of the body length, and a marginal genital pore.
Molecular phylogenetic results

The results of our phylogenetic analysis of the D1–D3 domains of the nuclear large subunit ribosomal DNA (28S) produced three clades: the chondrichthyan blood fluke *Chimaerohemeces trondheimensis* van der Land, 1967, the blood flukes of bony fishes (*Aporocotyle spinosicanalis* Williams, 1958, *Plethorchis acanthus* Martin, 1975 and *Neoparacardicola nasonis* Yamaguti, 1970), and the TBFs, including the enigmatic crocodilian blood fluke *Griphobilharzia amoena* (Fig. 7). Within TBFs, and as already reported elsewhere (Snyder 2004, Orélis-Ribeiro et al. 2014, Roberts et al. 2016), the marine (Carettacola Manter et Larson, 1950 clade) and freshwater TBFs clustered separately and with high nodal support. Within the freshwater TBFs, we recovered separate *Hapalorhynchus* and *Spiorchis* clades. The new species was sister to *Hapalorhynchus* spp., and that clade was sister to *G. amoena*. The phylogenetic distance (branch lengths) within the *Hapalorhynchus* clade supported our conclusions from morphology in that *Coeuritrema* is distinctive from other accepted TBF genera. Additional sequences from *Enterothaematotrema* and *Cardiotrema* are required to test monophyly of Coeuritremae (see above).

DISCUSSION

Our morphological descriptions and comparisons coupled with our molecular phylogenetic results indicated that *Coeuritrema* shares a recent common ancestor with *Hapalorhynchus* (Fig. 7). However, definitive host ecology, phylogenetic affiliation and geographic distribution do not explain the observed sister-taxa relationships. In specific, both *H. gracilis* (type species) and *H. foliorchis* infect the common snapping turtle, *Chelydra serpentina* (Testudines: Chelydridae), whereas *C. platti* infects *P. sinensis* (Trionychidae). *Chelydra serpentina* and *P. sinensis* are assigned to different families and those families are not phylogenetically closely related (Guillon et al. 2012, Crawford et al. 2015). Moreover, none of these TBFs nor their turtle hosts have overlapping geographic distributions: *H. gracilis* and *H. foliorchis* range in North America only, whereas *C. platti* ranges in Vietnam only (Table 1). Excluding definitive host ancestry and biogeography, we predict that knowledge of the life cycles of species of *Hapalorhynchus*, *Coeuritrema* and *Griphobilharzia* could help explain the observed topology. Unfortunately, no life cycle is known for any species of the *Hapalorhynchus* clade, precluding a deeper discussion of this matter herein. Orélis-Ribeiro et al. (2014) concluded that blood fluke clades can be identified by their molluscan intermediate hosts, with marine flukes infecting bivalves plus freshwater and estuarine flukes infecting snails. Additional morphological, life history and sequence data sourced from the other 17 accepted species of *Hapalorhynchus* and additional species of *Coeuritrema* are required to further test the phylogenetic pattern we recovered herein (Fig. 7).


The stark discordance between comparative morphology and gene sequence analysis reiterate the need for additional molecular gene sequence data from specimens identified as *G. amoena*. *Griphobilharzia amoena* differs from all other TBFs by being dioecious and by maturing in the freshwater crocodile, *Crocodylus johnstoni* Krefft. *Griphobilharzia* resembles all TBFs, except *Baracktrema* (single caecum), *Neospirorchis* Price, 1934 (fused caeca) and *Unicaecum* Stunkard, 1925 (single caecum), by having two non-fused caeca. *Griphobilharzia* resembles *Baracktrema*, *Neospirorchis*, *Unicaecum*, *Uterotrema* Platt et Pichelin, 1994, and *Vasotrema* by having a single testis.

Regarding the systematics of blood flukes sensu lato, for now, this clade as well as the marine TBF clade (*Carettacola*, *Hapalotrema* Looss, 1899 and *Learedius* Price, 1934), must continue to remain in systematic limbo, without a familial assignment (Looss 1899, Price 1934, Manter and Larson 1950, Orélis-Ribeiro et al. 2014, Roberts et al. 2016).

Acknowledgements. We thank Dang Nguyen Anh Tuan and Tran Quang Sang (Nha Trang University) as well as Matthew R. Womble (National Oceanic and Atmospheric Administration, Washington, DC) for helping collect turtles and blood flukes in Vietnam, and Gabor Racz (HWML) and Estefania Rodriguez (AMNH) for loaning museum specimens. The present study is a contribution of the Southeastern Cooperative Fish Parasite and Disease Project (Auburn University) and was supported in part by a grant from the York International Scholars Program (Auburn University) awarded to JRR, ROR and SAB and by the National Science Foundation Division of Environmental Biology via grant nos. 1112729, 1051106 (also with KHM) and 1048523 awarded to SAB. We thank and are indebted to Tom Platt for his donation of his library and helminthological collection to SAB, which made possible the revisionary systematics work presented herein and that which is forthcoming.
REFERENCES


BULLARD S.A., OVERSTREET R.M. 2009. Historical account of the two family-group names in use for the single accepted family comprising the “fish blood flukes.” Acta Parasitol. 54: 78–84.


MEHRA H.R. 1940: A new distome *Enterohaematotrema* n. g. and a new blood fluke *Hemorchis bangalensis* n. sp. belonging to the family Spirorchidae Stunkard, and a new species of the ge- nus *Dendritobilharzia* Skrjabin and Zakharow belonging to the family Schistosomatidae Poche, with remarks on the evolution


Received 13 May 2016
Accepted 5 July 2016
Published online 6 September 2016