Research Article

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***Ruavermis mikebarger* gen. et sp. n. (Digenea: Schistosomatoidea) infecting the yellow-headed temple turtle, *Heosemys annandalii* (Cryptodira: Geoemydidae), in Vietnam, including an updated phylogeny for the turtle blood flukes**

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Abstract: *Ruavermis mikebarger* gen. et sp. n. infects the yellow-headed temple turtles *Heosemys annandalii* (Boulenger et Robinson) in the Mekong River Basin. It resembles *Platt* Roberts et Bullard, 2018 and *Coeuritrema* Mehra, 1933 by having the anterior to posterior anatomical sequence of a ventral sucker, external seminal vesicle, cirrus sac, anterior testis, ovary, transverse vitelline duct, and posterior testis. These genera are further similar by having the combination of an elongate/ovoid aspinous body, a ventral sucker at the level of the body constriction, an oesophagus that terminates in the anterior 1/5 of the body and that is ventral to the anterior nerve commissure, intestinal caeca that bifurcate in the anterior 1/3 of the body (not immediately anterior to the ventral sucker), a sinistral caecum that bends toward the midline at level of the cirrus and common genital pore, an external seminal vesicle that abuts the anterodextral margin of the cirrus sac, an oviduct that emerges from the dextral margin of the ovary, and an oviducal seminal receptacle that comprises the middle portion of the oviduct. These genera lack lateral oesophageal diverticulae and a median oesophageal diverticulum. The new genus is unique by having a papillate ventral body surface, an external seminal vesicle lateral to the cirrus sac, vasa efferentia that are ventral to the gonads, an oviduct that is convoluted, a Laurer's canal pore that is preovarian, a Laurer's canal that extends anterolaterad, and an excretory vesicle that is Y-shaped. The 28S rDNA phylogenetic analysis recovered the new species sister to *Coeuritrema platti* Roberts et Bullard, 2016, with that clade sister to *Hapalorhynchus* spp. and *Platt* spp. The new turtle blood fluke is the fourth from Vietnam, second from a Vietnam geomydid, and first from *Heosemys* Stejneger as well as the first endohelminth from the yellow-headed temple turtle.

Keywords: taxonomy, systematics, herpetology, parasitology

The yellow-headed temple turtle, *Heosemys annandalii* (Boulenger et Robinson) (Cryptodira: Geoemydidae), is a large pond- and river-dwelling turtle native to South-East Asia, inhabiting rivers and freshwater marshes as well as estuaries in the lowland areas of southern Vietnam (van Dijk and Palasuwan 2000). Although the population status of this turtle has not been assessed, van Dijk and Palasuwan (2000) asserted that this species is probably vulnerable to habitat loss and likely being overharvested for food, cultural traditions related to magic or natural therapies, and the pet trade. In China, the demand for turtles is high such that adjacent countries may be affected (Ly et al. 2011), contributing to the so-called 'Asian turtle crisis.'

Many Asian turtle species are threatened with extinction in the wild, and an estimated 13,000 metric tons of live turtles (equal to millions of individuals) are annually exported from southeast Asia to China to meet this demand (Ly et al. 2011). At present, the only parasites that have been doc-

umented from the yellow-headed temple turtle comprise an ectoparasitic leech, *Placobdelloides siamensis* (Oka, 1917), (Euhirudinea: Glossiphoniidae), and a blood-dwelling haemogregarine, *Haemogregarine* sp. (Apicomplexa: Adeleina: Haemogregarinidae) (Assawawongkasem et al. 2008, Chiangkul et al. 2018).

Turtle blood flukes (TBFs; Platyhelminthes: Digenea: Schistosomatoidea) infect the cardiovascular system of turtles (Bullard et al. 2019, Dutton et al. 2019). Three of the ten nominal TBFs that range in South-East Asia (excludes India and China) are from Vietnam: one (*Platt sinuosus* Roberts et Bullard, 2018) infects a geomydid and two (*Coeuritrema platti* Roberts et Bullard, 2016 and *Hapalorhynchus mica* Oshmarin, 1971) infect softshelled turtles (Cryptodira: Trionychidae) (Roberts et al. 2016, 2018).

Eight geomydid genera include turtle species that host TBFs (*Batagur* Gray, *Cuora* Gray, *Geoclemys* Gray, *Hardella* Gray, *Malayemys* Lindholm, *Mauremys* Gray, *Pang-*

shura Gray, *Siebenrockiella* Lindholm). The new species described herein is the first TBF from a species of *Heosemys* Stejneger and the first endohelminth from *H. annandalii*. We also provide a phylogenetic analysis to corroborate our results using morphology.

MATERIALS AND METHODS

During a parasitological expedition to Vietnam in September 2018, we encountered specimens of a new TBF species infecting the heart of a yellow-headed temple turtle that was opportunistically sampled for TBF infections from the Cao Lanh Farmer's Market (10°27'13.32"N; 105°38'14.97"E), Cao Lanh, Đồng Tháp Province, Vietnam (Mekong River). TBF specimens intended for morphology as whole-mounts were observed microscopically, heat-killed on glass slides using a butane hand lighter under little or no coverslip pressure, fixed in 10% neutral buffered formalin (nbf), rinsed with water, stained in Van Cleave's hematoxylin with several drops of Ehrlich's hematoxylin, dehydrated through a graded series of EtOHs, made basic at 70% EtOH with lithium carbonate and butyl-amine, dehydrated in absolute EtOH and xylene, cleared with clove oil, and permanently mounted on glass slides using Canada balsam (Dutton et al. 2019).

The resulting whole mounts were examined and illustrated with the aid of compound microscopes (Leica DM2500 and DMR Leica, Wetzlar, Germany) equipped with differential interference contrast (DIC) optical components and drawing tubes. Measurements were obtained with a calibrated ocular micrometre (as straight-lines along the course of each duct) and are reported in micrometres (µm) as the range followed by the mean, $\pm$ standard deviation, and sample size in parentheses.

Type specimens of the new species were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (Smithsonian Institution, USNM Collection Nos. 1613430–1613432). Classification and anatomical terms for TBFs follow Dutton et al. (2019). Turtle scientific and common names follow Rhodin et al. (2017), and higher classification of turtles follows Pereira et al. (2017).

Total genomic DNA (gDNA) was extracted from 3 EtOH-preserved and microscopically-identified blood flukes using DNeasy™ Blood and Tissue Kit (Qiagen, Valencia, California) as per the manufacturer's protocol except that the proteinase-K incubation period was extended overnight, and 100 µl of elution buffer was used to increase the final DNA concentration. Amplification and sequencing of the D1–D3 domains of the large subunit ribosomal DNA (28S) used the primer set of Orélis-Ribeiro et al. (2017). PCR amplifications were performed according to Dutton et al. (2019). DNA sequencing was performed by ACGT, Incorporated (Wheeling, Illinois). Reactions were sequenced using BigDye terminator version 3.1, cleaned with magnetic beads (CleanSeq dye terminator removal kit; Beckman Coulter, Brea, California), and analysed using an ABI 3730 XL or 3730 Genetic Analyser (Applied Biosystems, Waltham, Massachusetts). Sequence assembly and analysis of chromatograms were completed with Geneious version 11.0.5 (<http://www.geneious.com>; Kearse et al. 2012). All nucleotide sequence data were deposited in GenBank (MT103553).

The phylogenetic analysis included the taxa included in Dutton et al. (2019) as well as the new taxon. As per Dutton et al. (2019), sequences were aligned using MAFFT (Katoh and Standley 2013). JModelTest 2 version 2.1.10 was implemented to per-

form statistical selection of the best-fit models of nucleotide substitution based on Bayesian information criteria (BIC) (Darriba et al. 2012). Aligned sequences were reformatted (from .fasta to .nexus) using the web application ALTER (Glez-Peña et al. 2010) to run Bayesian inference (BI). BI was performed in MrBayes version 3.2.5 (Ronquist and Huelsenbeck 2003) using substitution model averaging ('nst-mixed') and a gamma distribution to model rate-heterogeneity. Defaults were used in all other parameters. Three independent runs with four Metropolis-coupled chains were run for 5,000,000 generations, sampling the posterior distribution every 1,000 generations. Convergence was checked using Tracer v1.6.1 (Rambaut et al. 2014) and the 'sump' command in MrBayes: all runs appeared to reach convergence after discarding the first 25% of generation as burn-in. A majority rule consensus tree of the post burn-in posterior distribution was generated with the 'sumt' command in MrBayes. The inferred phylogenetic tree was visualised using FigTree v1.4.3 (Rambaut et al. 2014) and further edited for visualisation purposes with Adobe Illustrator (Adobe Systems).

RESULTS

Ruavermis gen. n.

Figs. 1–3

ZooBank number for species:

url:lsid:zoobank.org:act:035C275D-8DBE-4E6C-80F9-1D26CC5CE60B

Generic diagnosis. Body dorsoventrally flat (not cylindrical), ventrally concave, ovoid (not thread-like), 4–5 × longer than wide, lanceolate; mammillae absent. Oral sucker robust, demarcated from body by constriction, apapillate, aspinous. Ventral sucker present, crenulate (having papillate rim), at level of body constriction (forebody more narrow than hind body).

Pharynx present, enveloping anterior extremity of oesophagus. Oesophagus straight or slightly sinuous, terminating in anterior 1/5 of body, ventral to anterior nerve commissure, lateral oesophageal diverticula and median oesophageal diverticulum absent; oesophageal gland surrounding oesophagus from posterior margin of pharynx to caecal bifurcation, widest at level of caecal bifurcation. Intestinal caeca inverse U-shaped, comprising non-fused caeca bifurcating in anterior 1/3 of body (not immediately anterior to ventral sucker), lacking diverticulae, not extensively convoluted, sinistral caecum bending mediad at level of cirrus and around common genital pore, extending approximately 3/4 of body length posteriad, terminating near posterior body extremity, slightly asymmetrical.

Testes 2 in number, comprising a preovarian (anterior) testis and a postovarian (posterior) testis, intercaecal, having deep lobes or slightly irregular margins, ovoid (slightly longer than wide). Anterior and posterior trunks of vasa efferentia present, ventral to gonads, connecting to vas deferens; vas deferens extending directly anteriad ventral to cirrus sac, expanding to form external seminal vesicle; external seminal vesicle posterior to ventral sucker, lateral to cirrus sac, intercaecal, extending anteromediad. Cirrus sac robust, directed anterosinistral, enclosing internal seminal

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Fig. 1 *Ruavermis mikebargeri* gen. et sp. n. (Digenea: Schistosomatoidea) from the heart of the yellow-headed temple turtle, *Heosemys annandali* (Boulenger) (Cryptodira: Geoemydidae). Body of holotype (USNM No. 1613430), ventral view. Bar = 500 μ m. **Abbreviations:** at – anterior testis; ave – anterior trunk of vasa efferentia; cb – caecal bifurcation; cgp – common genital pore; cs – cirrus sac; ct – caecal terminus; dc – dextral caecum; ec – eversible cirrus; esv – external seminal vesicle; ev – excretory vesicle; isv – internal seminal vesicle; Lc – Laurer's canal; lvd – lateral vitelline collecting ducts; mt – metraterm; nc – nerve commissure; od – oviduct; oe – oesophagus; og – oesophageal gland; os – oral sucker; osr – oviducal seminal receptacle; ov – ovary; ph – pharynx; pt – posterior testis; pve – posterior trunk of vasa efferentia; sc – sinistral caecum; tvd – transverse vitelline duct; vd – vas deferens; vln – ventrolateral nerve chords; vr – vitellarium; and vs – ventral sucker.

vesicle and cirrus, containing variously sized gland-like cells. Pars prostatica indeterminate.

Ovary primarily sinistral, slightly transverse, non intercaecal, intertesticular, flanked by testes anteriorly and posteriorly, having smooth margins. Proximal portion of oviduct emerging from dextral margin of ovary, directed posteriad, convoluted before connecting to oviducal seminal receptacle; oviducal seminal receptacle comprising middle portion of oviduct, posterior to transverse vitelline duct; distal portion of oviduct straight (not coiled), intercaecal, extending anteriorad, sinuous, dorsal to ovary, with distal end comprising metraterm. Laurer's canal intercaecal, intertesticular, extending anteriorad to posterior margin of anterior testis from oviduct at anterior margin of ovary, with dorsal pore.

Vitellarium distribution symmetrical, comprising a series of dispersed large asymmetrical spheroid masses of vitelline follicles, surrounding caeca, distributing from caecal bifurcation to caecal tips (surrounding caeca); transverse vitelline duct intertesticular, approximately at level of ovary, comprising lateral collecting ducts ventral to caeca, ventral to gonads. Metraterm anterior to ovary, longitudinal (extending anteriorad in parallel with body margin), sinistral to anterior testis, intergonadal, having obvious muscular thick walls; uterine pouch absent; egg not observed. Common genital pore dorsal, sinistral, marginal (extracaecal or near body margin), between sinistral caecum and body margin, posterior to ventral sucker, predominately anterior to genitalia. Excretory vesicle Y-shaped, extending to tips of caeca; excretory pore subterminal. Manter's organ absent.

Differential diagnosis (see Discussion): Body flat, 4–5 $\times$ longer than wide, mammillae absent, papillate. Oral sucker aspinous. Ventral sucker crenulate, at level of body constriction. Lateral oesophageal diverticula and median oesophageal diverticulum absent. Intestinal caeca inverse U-shaped, bifurcating in anterior 1/3 of body, sinistral caecum bending toward midline at level of cirrus around common genital pore. Testes 2, intercaecal. Male terminal genitalia pregonadal. Vas deferens ventral to cirrus sac; anterior and posterior trunks of vasa efferentia ventral to gonads. External seminal vesicle lateral to cirrus sac. Cirrus sac directed anterosinistral. Ovary intertesticular. Proximal portion of oviduct emerging from dextral margin of ovary, convoluted before connecting to oviducal seminal receptacle; oviducal seminal receptacle comprising middle portion of oviduct, posterior to transverse vitelline duct. Laurer's canal intertesticular; pore preovarian, extends anterolaterad. Transverse vitelline duct intertesticular, ventral to gonads. Metraterm anterior to ovary, sinistral to anterior testis. Common genital pore sinistral, dorsal, between sinistral caecum and body margin. Excretory vesicle Y-shaped, extending to tips of caeca; Manter's organ absent.

Taxonomic summary

Type species: *Ruavermis mikebargeri* sp. n. (Digenea: Schistosomatoidea: 'Spirorchidae') - note that the family is in quotation marks to indicate its provisional status as a polyphyletic taxon.

Figs. 2, 3 *Ruavermis mikebarger* Dutton et Bullard gen. et sp. n. (Digenea: Schistosomatoidea) from the heart of the yellow-headed temple turtle, *Heosemys annandalii* (Boulenger) (Cryptodira: Geoemydidae). **Fig. 2.** Anterior end of holotype (USNM No. 1613430), ventral view. **Fig. 3.** Genitalia of holotype (USNM No. 1613430), ventral view. Bar = 100 µm. **Abbreviations:** at – anterior testis; ave – anterior trunk of vasa efferentia; cb – caecal bifurcation; cgp – common genital pore; ci – cirrus; cs – cirrus sac; dc – dextral caecum; ec – eversible cirrus; esv – external seminal vesicle; isv – internal seminal vesicle; Lc – Laurer's canal; mt – metraterm; nc – nerve commissure; od – oviduct; oe – oesophagus; og – oesophageal gland; os – oral sucker; osr – oviducal seminal receptacle; ov – ovary; ph – pharynx; pt – posterior testis; pve – posterior trunk of vasa efferentia; sc – sinistral caecum; tvd – transverse vitelline duct; vd – vas deferens; vln – ventrolateral nerve chords; vr – vitellarium; and vs – ventral sucker.

Type host: The yellow-headed temple, *Heosemys annandalii* (Cryptodira: Geoemydidae).

Etymology: 'Rua' is the Vietnamese word for hard shelled turtle, and 'vermis' is for worm.

Ruavermis mikebarger sp. n.

Figs. 1–3

ZooBank number for species:

[urn:lsid:zoobank.org:act:3E2E38A3-D401-4255-A20D-62F3657A2C6B](https://zoobank.org/act:3E2E38A3-D401-4255-A20D-62F3657A2C6B)

Diagnosis of adult (based on three whole-mounted specimens; USNM coll. nos. 1613430–1613432):

Body 1,800–1,952 ($1,881 \pm 76$; 3) long, 400–469 (439 ± 35 ; 3) in maximum width at level of caecal bifurcation, 4–5 (4 ± 0 ; 3) longer than wide; ventrolateral tegumental

mammillae absent. Body surface having asymmetrically arranged tegumental projections (approximately 1–2 long, 1 wide) ventrally and dorsally on the anterior end.

Oral sucker 109–114 (112 ± 3 ; 3) long or 6% ($6\% \pm 0\%$; 3) of body length, 64–77 (68 ± 8 ; 3) wide or 14–17% ($16\% \pm 2\%$; 3) of maximum body width, oral sucker spines absent. Ventral sucker 191–222 (210 ± 17 ; 3) long or 10–12% ($11\% \pm 1\%$; 3) of body length, 164–182 (176 ± 10 ; 3) wide or 62–68% ($66\% \pm 4\%$; 3) of maximum body width. Nerve commissure 227–236 (233 ± 5 ; 3) or 12–13% ($12\% \pm 1\%$; 3) of body length from anterior body end.

Pharynx 82–91 (85 ± 5 ; 3) or 24–27% ($25\% \pm 2\%$; 3) of oesophagus length, 100–111 (105 ± 6 ; 3) wide or $1.8\text{--}2.3\times$ (2.1 ± 0.3 ; 3) wider than maximum oesophagus width. Oesophagus 336–345 (341 ± 5 ; 3) long or 18–19% ($18\% \pm$

1%; 3) of body length, 23 (23 ± 0 ; 3) wide immediately posterior to pharynx and with wall 9 (9 ± 0 ; 3) thick, 45–52 (47 ± 4 ; 3) wide or 19–27% ($22\% \pm 4\%$; 3) of body width at mid-oesophagus and with wall 16–18 (17 ± 1 ; 3) thick, 25–45 (35 ± 10 ; 3) wide or 8–19% ($13\% \pm 5\%$; 3) of body width at caecal bifurcation and with wall 11–16 (14 ± 3 ; 3) thick; oesophageal gland 252–295 (281 ± 25 ; 3) long or 13–16% ($15\% \pm 3\%$; 3) of body length, 157–170 (162 ± 7 ; 3) wide or 33–40% ($37\% \pm 3\%$; 3) of body width. Intestine bifurcating 102–123 (111 ± 11 ; 3) or 6–7% ($6\% \pm 0\%$; 3) of body length from anterior body end, anterior caeca absent; posterior caeca extending posteriad approximately in parallel with lateral body margin, slightly sinuous; sinistral posterior caecum 1,219–1,333 ($1,273 \pm 57$; 3) long or 65–70% ($68\% \pm 3\%$; 3) of body length, 45–57 (51 ± 6 ; 3) wide or 15–24% ($19\% \pm 4\%$; 3) of body width at level of caecal bifurcation, 45–66 (53 ± 11 ; 3) wide or 11–14% ($12\% \pm 2\%$; 3) of body width at level of ovary, 32–57 (43 ± 13 ; 3) wide or 13–23% ($17\% \pm 5\%$; 3) of body width at ends of caeca, caeca terminating 184–238 (216 ± 29 ; 3) or 11–13% ($12\% \pm 1\%$; 3) of body length from posterior body end; dextral posterior caecum 1,238–1,285 ($1,260 \pm 24$; 3) long or 65–70% ($67\% \pm 2\%$; 3) of body length, 43–50 (46 ± 4 ; 3) wide or 16–19% ($17\% \pm 1\%$; 3) of body width at level of caecal bifurcation, 48–61 (56 ± 7 ; 3) wide or 11–15% ($13\% \pm 2\%$; 3) of body width at level of ovary, 27–43 (34 ± 8 ; 3) wide or 11–17% ($14\% \pm 3\%$; 3) of body width at level of ends of caeca; caeca terminating 193–279 (237 ± 43 ; 3) or 11–15% ($13\% \pm 2\%$; 3) of body length from posterior body end.

Anterior testis 214–232 (224 ± 9 ; 3) long or 11–13% ($12\% \pm 1\%$; 3) of body length or $1.0\text{--}1.1 \times$ (1.1 ± 0.1 ; 3) posterior testis length, 136–159 (151 ± 13 ; 3) wide or 29–40% ($35\% \pm 5\%$; 3) of body width at level of ovary or 75–85% ($81\% \pm 6\%$; 3) of posterior testis width; intertesticular space 80–95 (86 ± 8 ; 3) long or 4–5% ($5\% \pm 0\%$; 3) of body length. Posterior testis 202–207 (205 ± 3 ; 3) long or 11% ($11\% \pm 0\%$; 3) of body length, 182–193 (187 ± 6 ; 3) wide or 39–47% ($43\% \pm 4\%$; 3) of body width at level of ovary, 227–546 (358 ± 167 ; 3) or 13–28% ($19\% \pm 8\%$; 3) of body length from posterior body end (Fig. 3).

Anterior trunk of vasa efferentia emanating from ventral surface of anterior testis, extending antieriad 20–25 (23 ± 3 ; 3), 5 (5 ± 0 ; 3); posterior trunk of vasa efferentia emanating from the ventral surface of the posterior testis, extending antieriad 216–250 (239 ± 20 ; 3), or 11–14% ($13\% \pm 1\%$; 3), of body length, 4–5 (5 ± 1 ; 3) wide, ventral to ovary, meeting anterior trunk posterior to genital pore to form vas deferens; vas deferens extending antieriad 170–193 (184 ± 12 ; 3) of body length, 5 (5 ± 0 ; 3) wide before laterally expanding and turning dorsally to form external seminal vesicle (Fig. 3). External seminal vesicle longitudinal (not crossing midline), directed anterosinistrad 107–140 (122 ± 17 ; 3) long or 6–7% ($6\% \pm 1\%$; 3) of body length, 45–67 (56 ± 11 ; 3) wide, 2–3 $\times$ (2 ± 1 ; 3) wider than long, immediately posterior to anterior testis; internal seminal vesicle transverse, 159–170 (163 ± 6 ; 3) long or 8–9% ($9\% \pm 0\%$; 3) of body length, 52–64 (60 ± 7 ; 3) wide, 2–3 $\times$ (3 ± 0 ; 3) longer than wide. Cirrus sac ovoid,

270–295 (287 ± 14 ; 3) long or 14–16% ($15\% \pm 1\%$; 3) of body length, 111–114 (113 ± 2 ; 3) wide or 24–29% ($26\% \pm 2\%$; 3) body width at level of genital pore; cirrus extending posterolaterad 227–272 (246 ± 23 ; 3) or 81–92% ($86\% \pm 6\%$; 3) of cirrus sac length, 23–53 (40 ± 16 ; 3) wide.

Ovary smooth and lobed, 177–205 (192 ± 14 ; 3) long or 9–11% ($10\% \pm 1\%$; 3) of body length, 120–134 (127 ± 7 ; 3) wide or 28–30% ($29\% \pm 1\%$; 3) of body width; postovarian space 614–791 (718 ± 93 ; 3) or 34–42% ($38\% \pm 4\%$; 3) of body length. Proximal oviduct 107–114 (112 ± 4 ; 3) long or 6% ($6\% \pm 0\%$; 3) of body length, 16–18 (17 ± 1 ; 3) wide proximally, laterally expanding to form oviducal seminal receptacle; oviducal seminal receptacle 70–100 (86 ± 15 ; 3) long or 4–5% ($5\% \pm 1\%$; 3) of body length, 45–57 (50 ± 6 ; 3) wide or 10–13% ($11\% \pm 1\%$; 3) of body width, between ovary and posterior testis. Laurer's canal extending anterior from ascending portion of distal oviduct 45–50 (47 ± 3 ; 3), and 7–9 (8 ± 1 ; 3) in maximum width, with pore dextral to oviduct and ventral to posterior margin of anterior testis.

Vitellarium comprising a series of interconnected large spheroid masses of follicles, distributing from level of caecal bifurcation to excretory vesicle 148–205 (186 ± 33 ; 3) or 8–11% ($10\% \pm 1\%$; 3) from posterior body end; lateral collecting ducts ventral to gonads, 23–25 (24 ± 1 ; 3) wide; transverse vitelline duct 34–57 (47 ± 12 ; 3) in width 227–250 (235 ± 13 ; 3) wide; primary vitelline collecting duct not observed. Oötype not observed. Distal sinuous oviduct 243–284 (262 ± 21 ; 3) long or 12–15% ($14\% \pm 1\%$; 3) of body length, 20–30 (25 ± 5 ; 3) wide, dorsal to transverse vitelline duct; metraterm 141–152 (147 ± 6 ; 3) long or 7–8% ($8\% \pm 1\%$; 3) of body length, 57–89 (78 ± 18 ; 3) wide or 14–20% ($18\% \pm 3\%$; 3) of body width; uterine egg not observed. Common genital pore 873–1,085 (991 ± 108 ; 3) or 51–59% ($54\% \pm 4\%$; 3) of body length from posterior body end, 41–52 (46 ± 6 ; 3) in diameter.

Excretory pore dorsal, subterminal; excretory vesicle 102–136 (117 ± 17 ; 3) wide or 41–54% ($47\% \pm 7\%$; 3) of body width at level of caecal termini; excretory vesicle 238–300 (270 ± 31 ; 3) or 14–16% ($15\% \pm 1\%$; 3) of body length from posterior body margin.

Taxonomic summary

Type and only reported host: Yellow-headed temple turtle, *Heosemys annandalii* (Boulenger et Robinson), (Cryptodira: Geoemydidae).

Site in host: Heart.

Type locality: Mekong River (Cao Lãnh Farmers Market, Cao Lãnh, Đồng Tháp Province, Vietnam; $10^{\circ}27'13.32''\text{N}$; $105^{\circ}38'14.97''\text{E}$).

Prevalence and intensity of infection: Two of four (50%) yellow-headed temple turtles were infected with 26 specimens of *R. mikebargeri*.

Specimens deposited: Holotype (USNM 1613430), paratypes (USNM 1613431, 1613432).

Etymology: The specific epithet '*mikebargeri*' honours Michael A. Barger (School of Arts and Sciences, Peru State College, Peru, Nebraska, USA) for his contributions to helminthology, and it is in gratitude for introducing HRD to parasitology.

Fig. 4. Bayesian phylogeny based on the large subunit ribosomal DNA (28S). Values aside nodes are posterior probability. Scale bar is in substitutions per site.

Phylogenetic relationships

The amplified 28S fragment representing the new species comprised 1,454 nucleotides and was included in an alignment comprising 968 nucleotides. The 28S sequences from two specimens of *R. mikebarger* were identical (100% similar) to each other and differed from *Coeuritrema platti* 50 (4.1%), *Hapalorhynchus reelfooti* Byrd, 1939, 77 (6.2%), *H. conecuhensis* Roberts et Bullard, 2017 82 (6.6%), *H. foliorchis* Brooks et Mayes, 1975 and *H. gracilis* Stunkard, 1922 96 (7.8%), *P. snyderi* (Platt et Sharma, 2012) 94 (7.6%), and *P. sinuosus* Roberts et Bull-

ard, 2018 104 (8.4%) nucleotides, respectively. The Bayesian analysis herein recovered the new species sister to *C. platti*, with that clade sharing a recent common ancestor with species of *Hapalorhynchus* Stunkard, 1922 (albeit with low nodal support) (see Roberts et al. 2018, Bullard et al. 2019, Dutton et al. 2019). Bullard et al. (2019; maximum likelihood) and Dutton et al. (2019; Bayesian analysis) recovered *Coeuritrema* and *Platt* as sister taxa. However, the present study added a new lineage that groups with *Coeuritrema* and recovered species of *Platt* sister to all other TBFs within that clade.

DISCUSSION

Bullard et al. (2019) assigned all accepted TBF genera to six morphologically-diagnosed groups, and, by that classification scheme, the new genus belongs to Group 4. This is confirmed by the phylogenetic results in that the new taxon clades with Group 4 taxa. It resembles *Cardiotrema* Dwivedi, 1967, *Coeuritrema*, *Hapalorhynchus*, and *Platt* by having the anatomical sequence (anterior to posterior) of a ventral sucker, external seminal vesicle, cirrus sac (lateral to metraterm), anterior testis, ovary, transverse vitelline duct (intertesticular), and posterior testis. In addition to this combination of features, these genera also have an inflated oesophagus, U-shaped caeca, two testes (which are ovoid), a pars prostatica, a transverse cirrus sac (crosses midline, directed laterad), a sinistral, dorsal common genital pore, an intertesticular ovary and Laurer's canal, an oviducal seminal receptacle (ambiguous in *Cardiotrema*), a straight uterus directed anteriorly only, a strongly muscular metraterm, pregonadal terminal genitalia, and a massive, globular excretory vesicle (Manter's organ absent) (Bullard et al. 2019).

The new genus is most similar to *Platt* and *Coeuritrema* by having the combination of an elongate/ovoid aspinous body, a ventral sucker at the level of the body constriction, an oesophagus that terminates in the anterior 1/5 of the body and that is ventral to the anterior nerve commissure, intestinal caeca that bifurcate in the anterior 1/3 of the body (not immediately anterior to ventral sucker), a sinistral caecum that bends toward the midline at level of the cirrus and common genital pore, an external seminal vesicle that abuts the anterodextral margin of the cirrus sac, an oviduct that emerges from the dextral margin of the ovary, and an oviducal seminal receptacle that comprises the middle portion of the oviduct. These genera lack lateral oesophageal diverticulae and a median oesophageal diverticulum.

The new genus differs from *Coeuritrema* and *Platt* by having a papillate (vs apapillate) ventral body surface, a proximal portion of the external seminal vesicle that is lateral to (vs anterior to) the cirrus sac, an oviduct that is convoluted (vs straight), a Laurer's canal origin that is preovarian (vs postovarian), a Laurer's canal that extends anterolaterad (vs posterosinistral), and an excretory bladder that is Y-shaped (vs globular/sinuous, I-shaped, or branched).

The new genus further differs from *Platt* by having a cirrus that is ventral (vs dorsal) to the sinistral caecum, a Laurer's canal that is demarcated from the oviducal seminal

receptacle by the sinuous distal portion of the oviduct (vs immediately distal to oviducal seminal receptacle), an excretory bladder that extends anterior to the tips of the caeca, vasa efferentia that are ventral (vs dorsal) to the gonads, anterior and posterior vasa efferentia that unite dextral (vs dorsal) to the metraterm, an oviducal seminal receptacle that is posterior to (vs at level of) the transverse vitelline duct, and a vitellarium that is dispersed (vs in clusters).

The new genus further differs from *Coeuritrema* by having a tegument without mammillae (vs mammillae present; tegument with verrucae), a ventral sucker that is crenulate (papillate) (vs smooth), caeca that bifurcate proportionately farther anterior to the ventral sucker (vs immediately anterior to the ventral sucker), a common genital pore between the anterior testis and the ventral sucker (vs common genital pore immediately posterior to ventral sucker), and a vitellarium that extends posteriad beyond the caeca (vs from the caecal bifurcation to the caecal tips; surrounding caeca).

Coeuritrema was long regarded as a junior subjective synonym of *Hapalorhynchus* until Roberts et al. (2016) re-assigned several species using the presence of mammillae, external seminal vesicle position (abutting anterodextral margin of the cirrus sac), and presence of an obvious and muscular metraterm ($3-7 \times$ uterus length). *Coeuritrema* and *Platt* have a uniquely positioned external seminal vesicle, abutting the anterodextral margin of the cirrus sac; however, *Coeuritrema* has a dispersed vitellarium like *Hapalorhynchus* (see Dutton et al. 2019).

Vietnam is the ninth most diverse country for turtle biodiversity, including 32 accepted turtle species. Habitat loss and overharvest of turtles in South-East Asia probably threaten most hard shelled turtle populations there but population-level data are lacking for nearly all species (van Dijk et al. 2000, Ly et al. 2011). Only four TBFs are known from three turtle species in Vietnam, indicating that the diversity of TBFs (along with other parasites from these turtles) there is vastly underestimated.

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