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A new genus and species of turtle blood fluke (Digenea: Schistosomatoidea) from the Mekong snail-eating turtle, *Malayemys subtrijuga* (Schlegel & Müller) (Testudines: Geoemydidae) in Vietnam, with a reassessment of related Asiatic turtle blood flukes and molecular phylogeny

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Abstract *Platt sinuosus* Roberts & Bullard n. g., n. sp. (type-species) infects the kidney and mesenteric blood vessels of Mekong snail-eating turtles, *Malayemys subtrijuga* (Schlegel & Müller), in the Mekong River Basin. Species of *Platt* Roberts & Bullard n. g. are unique by the combination of having a papillate ventral sucker, vasa efferentia that are dorsal to the gonads, a massive cirrus-sac that is directed anteriad or laterad, and a vitellarium that surrounds the intestinal caeca. The new species resembles *Platt ocadiae* (Takeuti, 1942) Roberts & Bullard n. comb. but differs from it by having an external seminal vesicle that overlaps with or is immediately posterior to the level of the ventral sucker. Seven species previously of *Hapalorhynchus* Stunkard, 1922 are reassigned herein to *Platt*: *P. odhnerensis* (Mehra, 1933) Roberts & Bullard n. comb.; *P. yoshidai* (Ozaki, 1939) Roberts & Bullard n. comb.; *P. ocadiae*; *P. oschmarini* (Belous, 1963) Roberts & Bullard n. comb.; *P. sutlejensis* (Mehrotra, 1973) Roberts & Bullard n. comb.; *P. synderi* (Platt & Sharma, 2012) Roberts & Bullard n. comb.; and *P. tkachi* (Platt & Sharma, 2012) Roberts & Bullard n. comb. A dichotomous key to *Platt* spp. is provided. *Hapalorhynchus sheilae* (Mehrotra, 1973) Bourgat, 1990 and *Hapalorhynchus mica* (Oshmarin, 1971) Bourgat, 1990 are considered as *species inquirendae*, and *Hapalorhynchus indicus* (Thapar, 1933) Price, 1934 and *Hapalorhynchus macrotesticularis* (Rohde, Lee, & Lim, 1968) Brooks & Sullivan, 1981 are considered as *species incertae sedis*. Phylogenetic analysis of the large subunit rDNA (28S) showed *P. sinuosus* and *P. snyderi* to be sister taxa distinct from a monophyletic *Hapalorhynchus* and *Coeuritrema platti* Roberts & Bullard, 2016.

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Introduction

Hapalorhynchus Stunkard, 1922 (Digenea: Schistosomatoidea) currently includes 20 nominal species that collectively infect freshwater turtles of North America (Cryptodira: Chelydridae, Kinosternidae), Asia (Trionychidae, Geoemydidae), and Africa (Pleurodira: Pelomedusidae). As such, it is the only genus of freshwater turtle blood fluke (TBF) that includes species infecting turtles on more than one continent (Smith, 1997a, b; Platt, 2002; Platt & Sharma 2012; Roberts et al., 2016a, b, 2017). Eleven of those 20 species were described from Asiatic softshell turtles (Trionychidae) or pond turtles (Geoemydidae); none have been reported since their original description. An opportunistic examination of two Mekong snail-eating turtles, *Malayemys subtrijuga* (Schlegel & Müller), (Testudines: Geoemydidae) from a market in Can Tho, Vietnam, revealed infections by two turtle blood flukes: *Hapalorhynchus snyderi* Platt & Sharma, 2012 and a new species. Herein, we propose a new genus to accommodate the new species and seven species formerly of *Hapalorhynchus*, provide a dichotomous key to all species of the new genus, and provide both a phylogenetic reconstruction based on the large subunit ribosomal DNA (28S rDNA) only and additional sequence data for the internal transcribed region 2 (ITS2 rDNA) for species of *Hapalorhynchus* and *Coeuritrema platti* Roberts & Bullard, 2016.

Materials and methods

In November 2015, during a parasitological survey of aquatic vertebrates in the Mekong River Basin, two individuals of the Mekong snail-eating turtle *Malayemys subtrijuga* were opportunistically sampled from a market in Can Tho, Vietnam (10°01′42.15″N, 105°47′14.15″E). Flukes intended for morphology were fixed, stained, and whole-mounted as per Roberts et al. (2017); illustrated with the aid of Leica DM 2500 and Leica DMR (Leica, Wetzler, Germany) microscopes each equipped with differential interference contrast (DIC) optical components, an ocular micrometer, and a drawing tube; and compared with morphologically similar species. Measurements of turtle blood fluke (TBF) specimens herein are reported in micrometres (μm) as the range followed by the mean and number of specimens measured in parentheses.

Turtle scientific and common names follow van Dijk et al. (2014). Classification and anatomical terms for TBFs follow Roberts et al. (2016a, b, c) and Roberts et al. (2017) except that "ventrolateral tegumental papillae" of Roberts et al. (2016b) is replaced by "ventrolateral tegumental mamillae".

Griphobilharzia amoena Platt, Blair, Purdie & Melville, 1991 served as an outgroup with which to root the phylogeny. Specimens intended for collection of molecular data were placed directly in 95% non-denatured ethanol. Total genomic DNA (gDNA) was extracted using DNeasyTM Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol except that the incubation period with proteinase-K was extended to overnight and that the final elution step was performed using only 100 μl of elution buffer to increase the final DNA concentration. The partial 28S rDNA (domains D1-D3; *c.*1,400 bp) was amplified using the forward primer "U178" (5′-GCA CCC GCT GAA YTT AAG-3′) and the reverse primer "L1642" (5′-CCA GCG CCA TCC ATT TTC A-3′) (Lockyer et al., 2003). The internal transcribed region 2 (ITS2 rDNA) was amplified using the forward primer "GA1" (5′-AGA ACA TCG ACA TCT TGA AC-3; Anderson & Barker, 1998) and the reverse primer "ITS2.2" (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′; Cribb et al., 1998). The PCR amplifications were performed using a total volume of 50 μl with 2 μl of DNA template, 0.4 μM of each primer along with $1\times$ buffer, 2.5 mM $MgCl_2$, 1 mM dNTP mixture, and 0.3 μl Taq polymerase (5 U/μl) (Promega, Madison, Wisconsin, USA). The thermocycling profile for both genes comprised an initial 5 min at 95°C for denaturation, followed by 40 cycles of 94°C for 30 s for denaturation, 63°C for 30 s for annealing, and 72°C for 2 min for extension, followed by a final five min at 72°C for extension. All PCR reactions were carried out in a MJ Research PTC-200 (BioRad, Hercules, California, USA). PCR products (10 μl) were verified on a 1% agarose gel and stained with ethidium bromide. PCR products were purified by microcentrifuge with the QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol, except that the last elution step was performed with autoclaved nanopure H_2O rather than the provided buffer. DNA sequencing was performed by ACGT, Incorporated (Wheeling, Illinois, USA). Reactions were sequenced using BigDye terminator version

3.1, cleaned-up with magnetic beads (CleanSeq dye terminator removal kit), and analyzed using ABI 3730 XL or 3730 Genetic Analyzer. Primers used in sequencing of 28S rDNA included the PCR primers and the reverse primer 1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′) (Lockyer et al., 2003). Sequence assembly and analysis of chromatograms were performed with BioNumerics version 7.0 (Applied Maths, Saint-Martens-Latem, Belgium).

Analysis of sequences, which followed Roberts et al. (2017), is briefly described here. Assembled sequences were aligned with MAFFT 7.310 (Katoh & Standley, 2013) and subsequently corrected by eye in Mesquite 3.2 (Maddison & Maddison, 2017). Regions that could not be unambiguously aligned were excluded form further analyses. Nucleotide models were chosen using jModeltest with the AIC criterion (Darriba et al., 2012). Bayesian Inference was performed with MrBayes 3.5.3 (Ronquist et al., 2012). Using a GTR + Gamma model, 4 runs of 4 chains each were conducted for 1,000,000 generations. Priors were set to default values and 'burn-in' was set to 25% of generations (or 250,000). Chains were run until the average standard deviation of split frequencies was below 0.01. All nucleotide sequence data were deposited in the GenBank database.

Superfamily Schistosomatoidea Stiles & Hassall, 1898
Family Spirorchiidae Stunkard, 1921 (*sensu lato*)

***Platt* Roberts & Bullard n. g.**

Diagnosis
Body dorsoventrally flat (not cylindrical), 3.7–12.0 longer than wide; body constriction at level of ventral sucker present or absent; hindbody 1.6–3.3× longer than forebody, aspinous; ventral body papillae not observed; ventrolateral tegumental mamillae absent. Oral sucker robust, demarcated from body by constriction; oral sucker spines absent; oral sucker papillae absent. Ventral sucker papillate, 1/4–1/2 of body width in diameter, aspinous. Pharynx present, enveloping anterior extremity of oesophagus. Oesophagus long, extending posteriad 1/6–1/4 of body length, ventral to anterior nerve commissure; lateral oesophageal diverticula absent; median oesophageal diverticulum absent; oesophageal gland surrounding oesophagus

from pharynx to caecal bifurcation. Intestine comprising non-fused posterior caeca bifurcating immediately anterior to ventral sucker, smooth, lacking diverticula; anterior caeca absent; posterior caeca inverse U-shaped, extending 1/2–3/4 of body length directly posteriad, not extensively convoluted, terminating in posterior body extremity. Testes 2, comprising one anterior and one posterior testis in posterior 2/3 of body, intercaecal, smooth or lobed. Male terminal genitalia pre-gonadal. Vas deferens ventral to cirrus-sac; anterior and posterior trunks of vasa efferentia dorsal to gonads; external seminal vesicle posterior to ventral sucker, intercaecal, extending anteriad to level of genital pore or level of ventral sucker; internal seminal vesicle present; pars prostatica difficult to discern, enveloping distal extremity of internal seminal vesicle when present. Cirrus-sac robust, extending anteriad (at 45° angle to or parallel with body margin) or laterad (perpendicular to body margin). Ovary sinistral, intercaecal, intertesticular. Oviduct emerging from dextral margin of ovary, extending directly posteriad or sinistrad; oviducal seminal receptacle comprising middle portion of oviduct between ovary and posterior testis. Laurer's canal intercaecal, intertesticular; pore dorsal. Vitellarium comprising large follicles, surrounding caeca (dorsal, ventral and lateral), distributed between oesophagus and excretory vesicle; transverse vitelline duct intertesticular, ventral or dorsal to gonads, comprising lateral collecting ducts ventral or dorsal to caeca. Oötype longitudinal, intercaecal. Mehlis' gland not observed. Uterus intercaecal, straight, lacking coils or extensive convolutions, difficult to discern from oötype, extending anteriad, dorsal to ovary; metraterm difficult to discern from uterus, anterior to ovary, sinistral to anterior testis. Uterine pouch absent. Uterine egg single, tricornute or ovoid (with or without filaments), occupying oötype and uterus proximal to metraterm. Common genital pore sinistral, dorsal, between sinistral cecum and body margin (lateral, extracaecal). Excretory vesicle distinctly Y-shaped or sinuous (lacking multiple laterally-directed lobes). Manter's organ absent. Excretory pore terminal. Blood and tissue parasites of Asiatic softshells (Trionychidae) and pond turtles (Geoemydidae).
Type-species: *Platt sinuosus* Roberts & Bullard n. sp.
Other species: *Platt odhnerensis* (Mehra, 1933) Roberts & Bullard n. comb., *Platt yoshidai* (Ozaki, 1939) Roberts & Bullard n. comb., *Platt ocadiae* (Takeuti, 1942) Roberts & Bullard n. comb., *Platt*

oschmarini (Belous, 1963) Roberts & Bullard n. comb., *Platt sutlejensis* (Mehrotra, 1973) Roberts & Bullard n. comb., *Platt snyderi* (Platt & Sharma, 2012) Roberts & Bullard n. comb., and *Platt tkachi* (Platt & Sharma, 2012) Roberts & Bullard n. comb.

Etymology: *Platt* honors Dr Thomas Reid Platt (Professor Emeritus, Saint Mary's College, Indiana, USA) for his contributions to turtle blood fluke taxonomy and systematics.

Differential diagnosis

Body dorsoventrally flattened (not cylindrical), 3.7–12.0× longer than wide. Ventral sucker papillate, 1/4–1/2 of body width in diameter. Oesophagus long, extending posteriad 1/6–1/4 of body length. Posterior caeca inverse U-shaped. Testes 2, comprising a single anterior testis and single posterior testis in posterior 2/3 of body. Male terminal genitalia pre-gonadal. Vas deferens ventral to cirrus-sac; anterior and posterior trunks of vasa efferentia dorsal to gonads. Cirrus-sac robust, extending anteriad (at 45° angle to or parallel with body margin) or laterad (perpendicular to body margin). Vitellarium surrounding caeca (dorsal, ventral and lateral). Common genital pore sinistral, dorsal.

Platt sinuosus Roberts & Bullard n. sp.

Type-host: *Malayemys subtrijuga* (Schlegel & Müller), (Testudines: Geoemydidae), Mekong snail-eating turtle.

Type-locality: Mekong River (10°01′42.15″N, 105°47′14.15″E), Can Tho, Vietnam.

Type-material: Holotype (USNM 1422452) and 4 paratypes (USNM 1422453–1422456).

Site in host: Kidney and mesenteric blood vessels.

Prevalence and intensity of infection: Two Mekong snail-eating turtles were infected with 4 and 13 specimens of the new species.

Representative DNA sequences: 28S sequence (GenBank: MF579527); ITS2 sequence (GenBank: MF589230).

Comparative material examined: Holotype of *Hapalorhynchus gracilis* Stunkard, 1922 (AMNH 125); holotype of *Hapalorhynchus stunkardi* Byrd, 1939 (USNM 1321967); holotype of *Hapalorhynchus reelfooti* Byrd, 1939 (USNM 1321968); vouchers of *H. reelfooti* (USNM 1393855, 1393857); vouchers of *Hapalorhynchus foliorchis* Brooks & Mayes, 1975 (USNM 1422462–1422464); holotype of *Hapalorhynchus brooksi* Platt, 1988 (USNM 1375720); holotype of *P. snyderi* (as *Hapalorhynchus snyderi* Platt & Sharma, 2012; USNM 105194); holotype of *P. tkachi* (as *Hapalorhynchus tkachi* Platt & Sharma, 2012; USNM 105196); holotype of *Hapalorhynchus conecuhensis* Roberts & Bullard, 2017 (USNM 1437612); paratype of *H. conecuhensis* (USNM 1437613); hologenophore of *H. conecuhensis* (1437614) (Table 1).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new genus and species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Platt* n. g. is urn:lsid:-zoobank.org:act:F1E3190A-987D-4B2A-8858-AECC5D470BEF and the LSID for *Platt sinuosus* n. sp. is urn:lsid:zoobank.org:act: 9C8FFF14-AF27-4E12-B230-D5326B78A3CD.

Etymology: The Latin specific epithet *sinuosus* refers to the elongate, S-shaped cirrus-sac.

Description (Fig. 1A, B)

[Based on 10 whole-mounted specimens unless otherwise indicated; USNM coll. nos. 1422452–1422456.] Body 1,130–1,420 (1,250) long or 9.2–11.4× (10.1) longer than wide, 75–107 (93) wide at level of caecal bifurcation [or 6–8% (8%) of body length], 89–114 (102) wide at level of ventral sucker [or 7–9% (8%) of body length], 95–136 (115) wide at level of genital pore [or 8–11% (9%) of body length], 105–139 (123) wide at level of ovary [or 9–11% (10%) of body length], 86–109 (99) wide at level of caecal termini [or 7–9% (8%) of body length]. Forebody (middle of ventral sucker to anterior body end) 322–438 (369) long [or 26–31% (29%) of body length]; hindbody (middle of ventral sucker to posterior body end) 798–982 (882) long [or 69–74% (71%) of body length], 2.2–2.8× (2.4) longer than forebody; small ventral body papillae not observed.

Oral sucker 43–68 (52) long or 4–5% (4%) of body length, 43–64 (50) wide or 45–70% (53%) of body width at level of caecal bifurcation; oral sucker spines not observed; papillae not observed. Ventral sucker 34–68 (58) long or 3–6% (5%) of body length, 0.7–1.4× (1.1) longer than oral sucker, 52–77 (62)

Table 1 Species of *Platt* n. g. and related Asiatic turtle blood flukes[a]

Blood fluke	Turtle host	Site in host	Locality	Accession nos.	Reference
Hapalorhynchus mica (Oshmarin, 1971) Bourgat, 1990[b]	*Pelodiscus sinensis* (Wiegmann), (Geoemydidae)	Liver	Not specified (purchased from a market), Haiphong, Vietnam	Not reported	Oshmarin (1971)
Hapalorhynchus sheilae (Mehrotra, 1973) Bourgat, 1990[b]	*Pangshura tecta* (Gray) (Geoemydidae)	Heart, liver, blood vessels	River Sutlej, Ropar, Punjab State, India	Not reported	Mehrotra (1973); Tandon & Gupta (1982)
	Lissemys punctata punctata (Bonnaterre) (Trionychidae)	Heart, liver, blood vessels	River Ganges, Rudrapur, Uttarakhand State; River Patiala (Ghaggar River Basin), Patiala, Punjab State; River Ghaggar, Sangrur, Punjab State, India	Not reported	Mehrotra (1973); Tandon & Gupta (1982)
Platt occadiae (Takeuti, 1942) Roberts & Bullard n. comb.	*Mauremys sinensis* (Gray) (Geoemydidae)	Blood	Not specified (shipped to laboratory in Japan from Taiwan) (as Formosa)	Not reported	Takeuti (1942)
Platt odhnerensis (Mehra, 1933) Roberts & Bullard n. comb.	*Lissemys punctata* (Bonnaterre) (Trionychidae)	Heart	River Ganges, Allahabad, Uttar Pradesh State, India	Not reported	Mehra (1933)
Platt oschmarini (Belous, 1963) Roberts & Bullard n. comb.	*Pelodiscus maackii* (Brandt)(Trionychidae)	Liver	Lake Khanka (River Songacha) (44°59′45.62″N, 132°23′28.78″E), Russia	Not reported	Belous (1963)
Platt sinuosus n. sp. (type-species)	*Malayemys subtrijuga* (Schlegel & Müller) (Geoemydidae)	Kidney, mesenteric Blood vessels	River Mekong (10°01′42.15″N; 105°47′14.15″E), Can Tho, Vietnam	USNM 1422452–1422456	Present study
Platt snyderi (Platt & Sharma, 2012) Roberts & Bullard n. comb.	*M. subtrijuga* (Geoemydidae)	Viscera wash	River Perlis, Kangar, Perlis, Malaysia	USNM 105194, 105195[c]	Platt & Sharma (2012)
		Kidney, mesenteric blood vessels	River Mekong (10°01′42.15″N; 105°47′14.15″E) Can Tho, Vietnam	USNM 1422457–1422461	Present study
Platt sutlejensis (Mehrotra, 1973) Roberts & Bullard n. comb.	*Pangshura sylhetensis* Jerdon (Geoemydidae)	Heart	River Sutlej, Ropar, Punjab State, India	Not reported	Mehrotra (1973); Tandon & Gupta (1982)
	L. p. punctata	Heart	River Gomti, Lucknow, Uttar Pradesh State; River Ghaggar, Sangrur, Punjab State, India	Not reported	Mehrotra (1973); Tandon & Gupta (1982)

Table 1 continued

Blood fluke	Turtle host	Site in host	Locality	Accession nos.	Reference
Platt tkachi (Platt and Sharma, 2012) Roberts & Bullard n. comb.	*Siebenrockiella crassicollis* (Gray) (Geoemydidae)	Viscera wash	Kinta River Basin, Ipoh, Perak, Malaysia	USNM 105196, 105197	Platt & Sharma (2012)
Platt yoshidai (Ozaki, 1939) Roberts & Bullard n. comb.	*M. sinensis*	Blood	River Yangtze, Shanghai, China	Not reported	Ozaki (1939)

[a] *Hapalorhynchus indicus* (Thapar, 1933) and *Hapalorhynchus macrotesticularis* (Rohde, Lee, & Lim, 1968) are *species incertae sedis*. [b] *species inquirenda* perhaps a junior subjective synonym of *P. oschmarini*; [c] Platt & Sharma (2012) incorrectly reported these accession numbers as 104195 and 104197, respectively

wide or 53–75% (61%) of body width at level of ventral sucker, 1.1–1.5× (1.3) wider than oral sucker (Fig. 1A). Nerve commissure 155–202 (172; n = 4) or 12–16% (13%; n = 4) of body length from anterior body end. Pharynx 34–52 (42) or 14–20% (17%) of oesophagus length, 25–45 (36) wide, or 0.9–1.7× (1.4) wider than maximum oesophagus width. Oesophagus 216–272 (245) long or 18–22% (20%) of body length, 5–27 (15; n = 9) wide posterior to pharynx, with wall 4 (n = 1) thick, 18–34 (26) wide or 19–37% (28%) of body width at mid-esophagus, with wall 9–19 (12; n = 8) thick, 18–34 (26) wide or 19–35% (28%) of body width at caecal bifurcation; oesophageal wall 22 and 27 (2) thick. Oesophageal gland 193 and 198 (n = 2) long or 15% and 18% (n = 2) of body length, 55 and 75 (n = 2) wide or 58% and 70% (n = 2) of body width at level of caecal bifurcation. Intestine bifurcating 236–307 (267) or 19–24% (21%) of body length from anterior body end; sinistral posterior caecum 685–1,070 (792) long or 58–75% (63%) of body length, 32–45 (39; n = 8) wide at level of caecal bifurcation [or 34–60% (43%; n = 8) of body width], 16–20 (17; n = 5) wide at level of ovary [or 12–16% (14%; n = 5) of body width], 11–25 (19) wide at terminus [or 13–23% (19%) of body width]; dextral posterior cecum 705–1,105 (809) long or 57–78% (65%) of body length, 32–45 (38; n = 8) wide at level of caecal bifurcation [or 34–57% (41%; n = 8) of body width], 16–23 (18; n = 6) wide at level of ovary [or 12–19% (15%; n = 6) of body width], 11–35 (19) wide at terminus [or 10–41% (19%) of body width]; caecal termini at 173–236 (207) from posterior body end [or 14–19% (17%) of body length].

Testes 2. Anterior testis smooth, lacking lobes, 86–118 (96) long or 7–10% (8%) of body length or 42–66% (56%) of posterior testis length, 55–84 (67) wide or 41–69% (55%) of body width at level of ovary or 63–120% (89%) of posterior testis width; intertesticular space 41–91 (58) or 3–6% (5%) of body length (Fig. 1A, B). Posterior testis lobed, follicular, 148–236 (172) long or 12–17% (14%) of body length, 64–91 (77) wide or 55–67% (62%) of body width at level of ovary, at 318–441 (366) from posterior body end or 25–31% (29%) of body length (Fig. 1A, B). Anterior trunk of vasa efferentia emanating from dorsal surface of anterior testis, extending anteriad 58 (n = 1), 9 wide (Fig. 1B); posterior trunk of vasa efferentia emanating from the dorsal surface of the

posterior testis, extending anteriad 236 (n = 2) or 19% (n = 1) of body length, 5 (n = 1) wide, meeting anterior trunk 104 (n = 1) or 8% (n = 1) of body length posterior to genital pore to form vas deferens; vas deferens extending anteriad 193 (n = 1) or 15% (n = 1) of body length, 9 (n = 1) wide before laterally expanding and turning dorsad to form external seminal vesicle (Fig. 1B). External seminal vesicle 139–250 (175) long or 12–18% (14%) of body length, 34–59 (40) wide or 28–49% (35%) of body width, 3.1–7.4× (4.5) longer than wide, overlapping (n = 8) or at 4 and 20 (n = 2) from posterior margin of ventral sucker or < 1% and 2% (n = 2) of body length, ventral to caeca (Fig. 1A, B); internal seminal vesicle 68–125 (105) long or 6–10% (8%) of body length, 18–52 (26) wide, 2.3–6.9× (4.4) longer than wide. Pars prostatica difficult to discern, surrounding distal portion of internal seminal vesicle, 45–100 (70; n = 5) long or 39–80% (63%; 5) of internal seminal vesicle length, 20–30 (25; n = 5) wide, 2.1–4.3× (2.8; n = 5) longer than wide (Fig. 1B). Cirrus-sac massive, 150–223 (189) long or 12–18% (15%) of body length, 50–95 (69) wide or 42–94% (60%) of body width at level of genital pore; cirrus extending anterosinistrad 39–57 (44) or 20–30% (24%) of cirrus-sac, 9–23 (14) wide (Fig. 1B).

Ovary wedge-shaped in outline, sinistral, 232–391 (273) or 19–28% (22%) of body length posterior to middle of ventral sucker, 120–238 (165) or 10–17% (13%) of body length posterior to genital pore, 68–100 (80; n = 9) long or 5–8% (6%; n = 8) of body length, 36–45 (40; n = 9) wide or 28–39% (32%; n = 9) of body width or 39–62% (51%; n = 9) of ovary length; post-ovarian space 468–738 (579; n = 9) or 40–52% (46%; n = 9) of body length. Oviduct turning dorsad and extending posteriad 25–55 (42; n = 4) or 2–4% (3%; n = 4) of body length, 7–18 (15; n = 4) in maximum width, laterally expanding to form seminal receptacle; oviducal seminal receptacle extending sinistrad for 39–48 (43; n = 4) or 40–60% (51%; n = 4) of ovary length, 18–23 (20; n = 4) wide or 13–19% (16%; n = 4) of body width, constricting and turning dorsad, extending anteriad for 114 and 116 (n = 2) or 9% (n = 2) of body length before joining oötype, 9 and 16 (n = 2) wide or 6% and 13% (n = 2) of body width (Fig. 1A, B). Laurer's canal originating at distal margin of seminal receptacle, extending

posterosinistrad 19 and 34 (n = 2) or 3% (n = 2) of body length, 5 and 8 (n = 2) wide, opening dorsally sinistral to posterior testis (Fig. 1B). Vitellarium comprising a series of interconnected large spheroid masses of follicles (Fig. 1A), dorsal and ventral to caeca (illustrated as dorsolateral to emphasize course of caeca; Fig. 1A), distributing from level of caecal bifurcation or 250 (n = 1) or 20% (n = 1) of body length from anterior body end to excretory vesicle or 82 (n = 1) or 6% (n = 1) of body length from posterior body end; transverse vitelline duct ventral to gonads (dashed in Fig. 1B to illustrate course of oviduct and primary vitelline duct), between ovary and posterior testis, 295–454 (360; n = 5) or 24–32% (28%; n = 5) of body length from middle of ventral sucker, 18–43 (34; n = 5) wide or 16–33% (26%; n = 5) of body width; primary vitelline collecting duct turning dorsad to transverse vitelline duct, extending sinistrad 63 (n = 1) or 5% (n = 1) of body length before connecting with oviduct, 20 (n = 1) wide or 14% (n = 1) of body width. Ovi-vitelline duct extending anteriad 96 (n = 1) or 8% of body length, 12 (n = 1) wide (Fig. 1B). Oötype difficult to discern, 23 (n = 2) long, 23 and 25 (n = 2) wide, dorsolateral to anterior testis (Figs. 1, 2). Uterus originating 186 or 227 (n = 2) or 15% and 18% (n = 2) of body length posterior to middle of ventral sucker, 57 and 68 (n = 2) long or 5% (n = 2) of body length, 20 and 23 (n = 2) wide or 19% (n = 2) of body width; metraterm extending anterosinistrad, 39–68 (51; n = 9) long or 3–6% (4%; n = 9) of body length or 60% and 68% (n = 2) longer than uterus, 14–23 (19; n = 9) wide or 12–20% (17%; n = 9) of body width. Uterine egg not observed. Common genital pore 68–136 (100) or 5–10% (8%) of body length posterior to middle of ventral sucker (Fig. 1A).

Excretory vesicle 157–211 (178) long or 12–17% (14%) of body length, 36–64 (49) wide or 36–61% (49%) of body width at level of caecal termini; wall 9–21 (16) thick (Fig. 1A); excretory pore terminal.

Molecular results

The sequences incorporated into the phylogenetic analyses herein are presented in Table 2. The 28S rDNA data set consisted of 1,615 aligned nucleotide
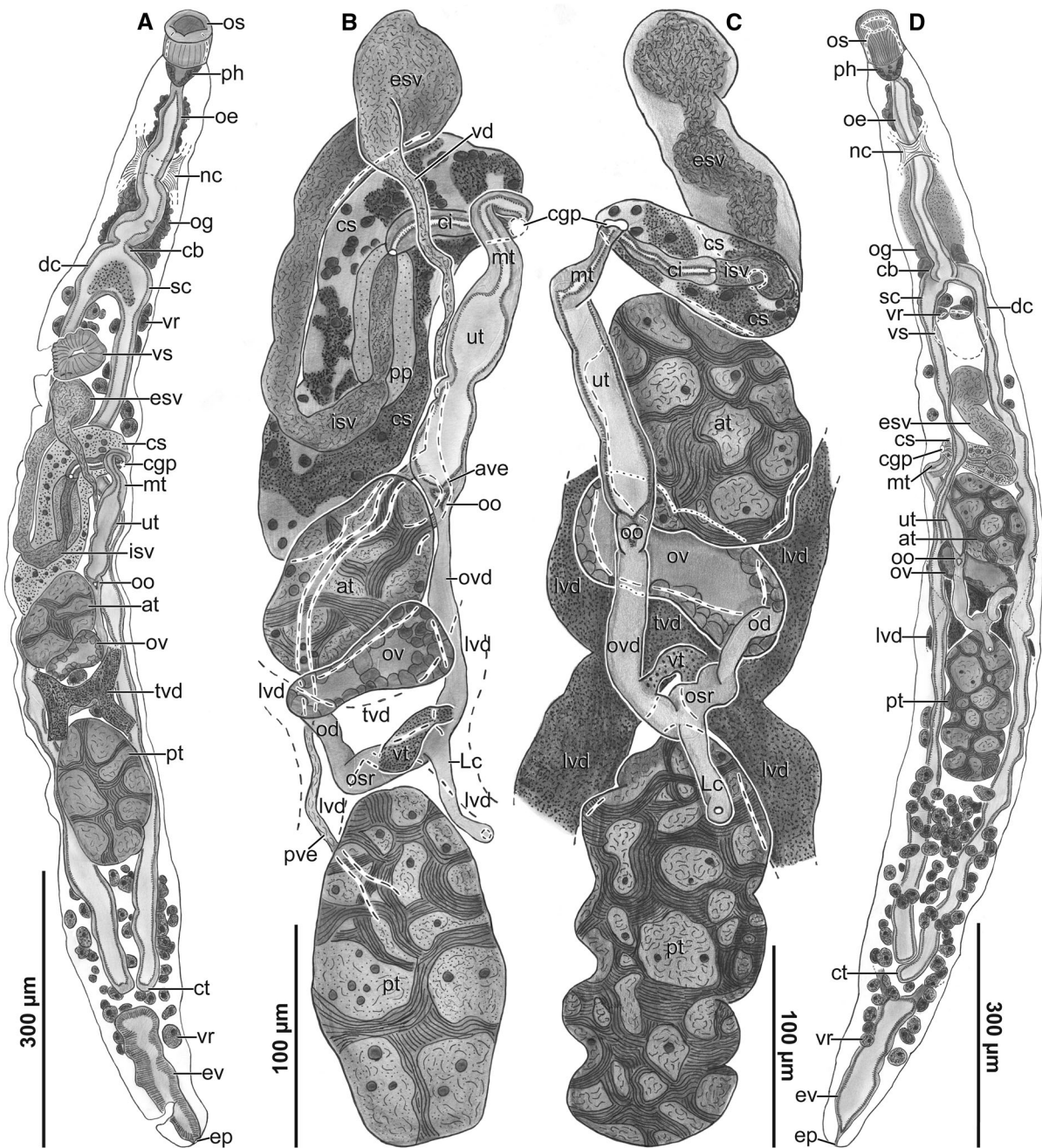
Fig. 1 Blood flukes ex kidney and mesenteric blood vessels of *Malayemys subtrijuga*. A, Body of *Platt sinuosus* n. g., n. sp. (holotype, USNM 1422452), ventral view. B, Genitalia of *Platt sinuosus* n. g., n. sp. (holotype, USNM 1422452), ventral view. C, Genitalia of *Platt snyderi* Roberts & Bullard n. comb. (voucher, USNM 1422457), dorsal view. D, Body of *Platt snyderi* Roberts & Bullard n. comb. (voucher, USNM 1422457), dorsal view. *Abbreviations*: os, oral sucker; ph, pharynx; oe, oesophagus; nc, nerve commissure; og, oesophageal gland; cb, caecal bifurcation; dc, dextral caecum; sc, sinistral caecum; vr, vitellarium; vs, ventral sucker; esv, external seminal vesicle; cs, cirrus-sac; cgp, common genital pore; mt, metraterm; ut, uterus; isv, internal seminal vesicle; oo, oötype; at, anterior testis; ov, ovary; tvd, transverse vitelline duct; pt, posterior testis; ct, caecal termini; ev, excretory vesicle; ep, excretory pore; vd, vas deferens; ci, cirrus; pp, pars prostatica; ave, anterior trunk of vasa efferentia; ovd, ovi-vitelline duct; lvd, lateral collecting vitelline duct; od, oviduct; osr, oviducal seminal receptacle; Lc, Laurer's canal; pve, posterior trunk of vasa efferentia; vt, primary vitelline duct
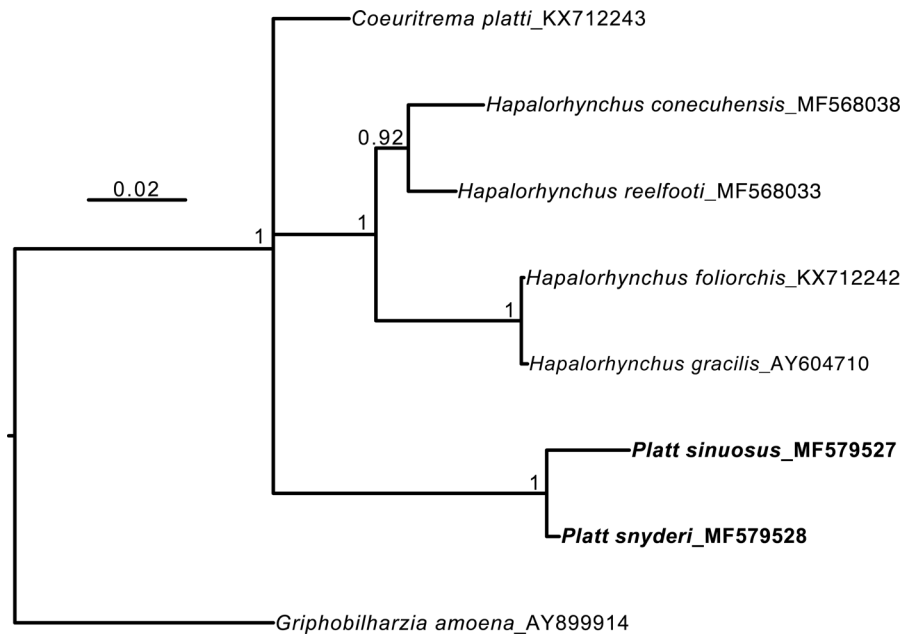
Fig. 2 Phylogenetic relationships of blood flukes reconstructed by Bayesian inference. Numbers aside tree nodes indicate posterior probability. Phylogeny reconstructed based on sequences from the partial D1-D3 domains of the large subunit ribosomal DNA (28S)

positions, of which 1,593 were unambiguously aligned and included in the analysis. The resultant topology (Fig. 2) revealed *P. sinuosus* and *P. snyderi* to be sister taxa (posterior probability of 1) distinct from a monophyletic *Hapalorhynchus* (posterior probability of 1). Due to extreme nucleotide and length variation across genera in the ITS2 sequences, we were not able to reliably align the ITS2 data (430 nt in 'aligned' data) and perform a phylogenetic analysis. We did note, however, that sequences of *P. sinuosus* and *P. snyderi* were clearly more similar to each other (K2 distance of 0.042) than to those of other the species. Also, the reported ITS sequences for *Hapalotrema pambanensis* Mehrotra, 1973 (GenBank: KM652626) and *Learedius learedi* Price, 1934 (GenBank: KM652619) are identical.

Key to the species of *Platt*

1a Transverse vitelline duct dorsal to gonads ………………................………… *P. sutlejensis*
1b Transverse vitelline duct ventral to gonads …………………………………………………… 2
2a Cirrus-sac directed laterad (perpendicular to body margin) ……………………… *P. yoshidai*
2b Cirrus-sac directed anteriad (at 45° angle to or parallel with body margin) ………………….. 3
3a Genital pore at level of or immediately posterior to level of ventral sucker ……… *P. oschmarini*
3b Genital pore posterior to level of ventral sucker ……………………………………………… 4
4a Ovary ovoid; both caeca indented at level of genital pore …………………… *P. odhnerensis*
4b Ovary wedge-shaped; sinistral caecum indented at level of genital pore ……………………… 5
5a Cirrus-sac short, closest to anterior testis, orienting at 45° angle to body margin ………… 6
5b Cirrus-sac long, extending anteriad >1/2 distance between anterior testis and ventral sucker, orienting parallel to body margin …………… 7
6a Testes lobed; posterior testis far anterior to caecal termini ……………………… *P. snyderi*
6b Testes smooth; posterior testis immediately anterior to caecal termini …………… *P. tkachi*
7a External seminal vesicle at level of genital pore ……………………………………….. *P. ocadiae*
7b External seminal vesicle overlapping or immediately posterior to ventral sucker ………… …………………………………… *P. sinuosus*

Table 2 Turtle blood fluke sequences used in the present study

Blood fluke	Turtle host	Locality	GenBank accession nos.		Reference
			28S	ITS2	
Coeuritrema platti Roberts & Bullard, 2016	*Pelodiscus sinensis* (Wiegmann)	Da Rang River Basin, Phu Yen Province, Vietnam	KX712243	MF589226	Roberts et al. (2016b); present study
Hapalorhynchus conecuhensis Roberts & Bullard, 2017	*Sternotherus* cf. *minor*	Blue Spring (31°5′27.64″N, 86°30′53.21″W), Yellow River, Alabama, USA	MF568038	MF589227	Roberts et al. (2017); present study
Hapalorhynchus foliorchis Brooks & Mayes, 1975	*Chelydra serpentina* (Linnaeus)	Pond off Saugahatchee Creek (River Tallapoosa) (32°39′1.36″N, 85°29′4.70″W), Alabama	KX712242	MF589228	Roberts et al. (2016b); present study
Hapalorhynchus gracilis Stunkard, 1922	*C. serpentina*	Lake Reelfoot (River Mississippi) (36°21′12.23″N, 89°25′21.50″W), Tennessee, USA	AY604710	–	Snyder (2004)
Hapalorhynchus reelfooti Byrd, 1939	*Sternotherus minor* (Agassiz)	River Wacissa (tributary of Aucilla River) (30°20′24.73″N, 83°59′27.56″W), Florida, USA	MF568033	MF589229	Roberts et al. (2017); present study
Platt sinuosus Roberts & Bullard n. g., n. sp.	*Malayemys subtrijuga* (Schlegel & Müller)	River Mekong (10°01′42.15″N; 105°47′14.15″E), Can Tho, Vietnam	MF579527	MF589230	Present study
Platt snyderi (Platt & Sharma, 2012) Roberts & Bullard n. comb.	*M. subtrijuga*	River Mekong (10°01′42.15″N; 105°47′14.15″E), Can Tho, Vietnam	MF579528	MF589231	Present study
Griphobilharzia amoena Platt & Blair, 1991	*Crocodylus johnstoni* Krefft	Darwin (probably Adelaide River Basin), Australia	AY899914	–	Brant & Loker (2005)
Hapalotrema pambanensis Mehrotra, 1973	*Chelonia mydas* (Linnaeus)	indeterminate[a]	–	KM652626[a]	Chapman et al. (2015)
Learedius learedi Price, 1934	*C. mydas*	Coolum, Queensland, Australia	–	KM652619	Chapman et al. (2015)

[a]Mislabelled as a 28S sequence for *Learedius learedi* in Chapman et al. (2015)

Discussion

Species of *Platt* resemble those of *Hapalorhynchus* Stunkard, 1922, *Coeuritrema* Mehra, 1933, *Enterohaematotrema* Mehra, 1940 and *Cardiotrema* Dwivedi, 1967 by having a ventral sucker, inverse U-shaped posterior caeca, an anterior testis and a posterior testis, and pre-gonadal male terminal genitalia. They differ from species of *Hapalorhynchus* by having a papillate ventral sucker, vasa efferentia that are dorsal to the gonads, a massive cirrus-sac that is directed anteriad or laterad, and a vitellarium that surrounds the intestinal caeca, i.e. distributing dorsal, ventral, and lateral to the caeca. Species of *Hapalorhynchus* have an apapillate ventral sucker, vasa efferentia that are exclusively ventral to the gonads, a diminutive cirrus-sac that is directed posteriad (if

present), and a vitellarium that is exclusively ventral to the intestinal caeca. Species of the new genus are easily differentiated from species of *Coeuritrema* by having a smooth lateral body margin that lacks ventrolateral tegumental mamillae and by having a diminutive metraterm that is morphologically indistinct from the uterus; whereas, as emended by Roberts et al. (2016b), species of *Coeuritrema* have massive, mound-like ventrolateral tegumental mamillae and a massive metraterm (1/10–1/7 of body length) that is markedly distinct from the uterus. Species of *Platt* differ from those of *Enterohaematotrema* by having a dorsosinistral genital pore; whereas, species of *Enterohaematotrema* have a ventromedial genital pore. Species of the new genus differ from those of *Cardiotrema* by having a relatively large ventral sucker (1/4–1/2 of body width) that is at level

immediately posterior to the caecal bifurcation, an elongate oesophagus (1/6–1/4 of body length), and an intertesticular ovary; whereas, species of *Cardiotrema* have a small (rudimentary) ventral sucker (< 1/5 of body width) that is far posterior to the cecal bifurcation, a short oesophagus (< 1/10 of body length), and an ovary that is lateral to the anterior testis or intertesticular. Platt (2002) and Roberts et al. (2017) accepted that the ovary of species of *Cardiotrema* was sinistral to the anterior testis, not intertesticular; however, the ovary is reportedly sinistral to the anterior testis in *Cardiotrema vaidya* Dwivedi, 1967 and intertesticular in *Cardiotrema roparensis* Mehrotra, 1973 (see Tandon & Gupta, 1985). As a consequence, and pending an examination of found type-materials or newly collected vouchers, the generic diagnosis for *Cardiotrema sensu* Platt (2002) should be emended to include ovary sinistral to anterior testis or intertesticular (Dwivedi, 1967; Mehrotra, 1973; Tandon & Gupta, 1985). Based on available sequences, the 28S rDNA topology is consistent with the hypothesis that the species of *Platt* are distinct from those of other blood fluke genera examined, including those of *Hapalorhynchus*.

Platt snyderi (USNM 1422457–1422461; Fig. 1C, D) and the new species infected kidney and mesenteric blood vessels (Fig. 1C, D) of both Mekong snail-eating turtles examined herein. The specimens of *P. snyderi* were diagnosed by having the combination of the morphological features specified in the key as well as by having an elongate body (8.1–12.4× longer than wide), a short forebody (approximately 1/3 of body length), and an external seminal vesicle nearly reaching the level of the posterior margin of the ventral sucker. These specimens differed from the holotype of *P. snyderi* (USNM 105194) and its published description (Platt & Sharma, 2012) by having a vitellarium that extended to the level of the excretory vesicle (Fig. 1C) but in all other details matched the published description of this species by Platt & Sharma (2012). This is the first report of *P. snyderi* infecting a turtle from Vietnam (Table 1).

The new species resembles *P. snyderi*, *P. tkachi*, and *P. ocadiae* by having the combination of a sinistral caecum that is indented at level of the genital pore, a cirrus-sac directed anteriad, a transverse vitelline duct that is ventral to the gonads, an intertesticular ovary that is wedge-shaped, and a genital pore that is posterior to the ventral sucker (see Key above). The

new species can be readily distinguished from these species by the combination of having an external seminal vesicle that overlaps with or is immediately posterior to the level of the ventral sucker as well as a cirrus-sac that is massive (extending anteriad > 1/2 distance between the anterior testis and ventral sucker) and that is oriented parallel to the long axis of the body. *Platt snyderi* and *P. tkachi* have a moderately sized cirrus-sac that is closest to the anterior testis and that is oriented at a 45° angle to the long body axis, and *P. ocadiae* has an external seminal vesicle at level of the genital pore.

Several Asiatic species of *Hapalorhynchus* need to be reassessed. *Hapalorhynchus sheilae* (Mehrotra, 1973) is herein considered a *species inquirenda* because its original description (Mehrotra, 1973) and redescription (Tandon & Gupta, 1982) are incomplete, no type-material is extant, and it has not been treated again since its incomplete redescription. We deduce that the collection treated by Mehrotra (1973) is the same as that treated by Tandon & Gupta (1982) because the type-host and type-locality information for both *P. sutlejensis* and *H. sheilae* (*species inquirenda*) are identical, respectively, between these published works. Components of the description of *H. sheilae* are especially dubious. For example, Tandon & Gupta (1982) described the genital pore of *P. sheilae* as "may be inter or extracaecal (since inward bending of the intestinal caeca has been found to be variable, depending upon the flattened state of the fluke)". If the genital pore is dorsal and if the intestinal caecum is dorsal to the terminal genitalia (i.e. the terminal genitalia loop around the caecum), and in the absence of intraspecific variation, then the only way in which the genital pore could be intercaecal is if the specimen was severely damaged or partially destroyed when being mounted. *Hapalorhynchus mica* (Oshmarin, 1971) may be a junior subjective synonym of *P. oschmarini*. We regard it herein as *species inquirenda* until specimens of *P. mica* can be recollected and examined. Published descriptions of these taxa are both incomplete, no type- or voucher material exists, and Oshmarin (1971) did not specify a characteristic that differentiated *P. mica* from *P. oschmarini*. *Hapalorhynchus indicus* (Thapar, 1933) and *Hapalorhynchus macrotesticularis* (Rohde, Lee & Lim, 1968) are herein considered as *species incertae sedis* because they possess morphological features that differentiate them from all accepted species of

Hapalorhynchus (see Roberts et al., 2017) and *Platt* (present study). For example, *H. indicus* has a cirrus-sac that is reportedly anterior to the external seminal vesicle, and *H. macrotesticularis* has diverticulate posterior caeca and, reportedly, a blind-ending seminal receptacle rather than an oviducal seminal receptacle (Thapar, 1933; Rohde et al., 1968; Platt, 2002). No other species of *Hapalorhynchus* or *Platt* reportedly has a seminal receptacle that does not comprise an oviducal seminal receptacle [figures 2 and 4 in Platt (2002) and Roberts et al. (2017), respectively].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national, and international guidelines for the care and use of animals were followed.

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