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The Family Bithyniidae Gray, 1857 (Gastropoda: Truncatelloidea) in Peninsular Malaysia and Singapore

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ABSTRACT. – Freshwater snails of the family Bithyniidae on mainland Southeast Asia are important intermediate hosts of zoonotic parasites. However, bithyniids in the southern tip of the mainland, in Peninsular Malaysia and Singapore, have remained largely overlooked. We review records of the bithyniids from this target region based on literature and museum material to verify species identities, statuses and distribution. Species identification was conducted mainly using shell characters and supplemented by analyses of the COI gene. We recorded four species—*Digoniostoma siamensis siamensis*, *Gabbia minuta*, *Gabbia* cf. *stenothyroides*, *Wattebledia baschi*. In Malaysia, *G. minuta* and *W. baschi* are only known from their type localities. *Digoniostoma siamensis siamensis* occurs in Peninsular Malaysia and was introduced to Singapore in recent decades. *Gabbia* cf. *stenothyroides* has also been introduced to Singapore. Molecular analyses indicated that *D. siamensis siamensis* shares the same clade with mainland Southeast Asian *Bithynia* spp., *Gabbia* cf. *stenothyroides* is sister to a bithyniid from Sulawesi, while *Bithynia* and *Gabbia* were recovered as non-monophyletic groups. Our study clarifies the presence of two introduced bithyniids in Singapore, reveals the lack of knowledge on native (including endemic) Malaysian species, and further emphasises the need for a revision of all bithyniids.

KEYWORDS: freshwater snails, Southeast Asia, endemic species, introduced species

INTRODUCTION

The freshwater snails of the family Bithyniidae Gray, 1857 (Gastropoda: Truncatelloidea) are distributed in Europe, Africa, Australia and Asia (Ponder, 2019). Seven genera are known from throughout Southeast Asia—*Bithynia* Leach, 1818, *Emmericiopsis* Thiele, 1928, *Gabbia* Tryon, 1865, *Hydrobioides* G. Nevill, 1885, *Parabithynia* Pilsbry, 1928, *Petroglyphus* Möllendorf in Quadras and Möllendorf, 1894 and *Wattebledia* Crosse, 1886 (Annandale, 1918; Benthem Jutting, 1956; Brandt, 1974; Ponder, 2019).

Despite some species of *Bithynia* being known since the mid-20th century as intermediate hosts of zoonotic parasites in Thailand (Wykoff et al., 1965), taxonomic and phylogenetic studies of Bithyniidae in Southeast Asia have been somewhat lacking. It was only in the past decade that a few studies were conducted on the genetic diversity of bithyniids, though the focus was largely on species in Thailand and surrounding areas (Kulsantiwong et al., 2013; Tantrawatpan et al., 2020; Bunchom et al., 2021a, b).

Bithyniids have been recorded further south in Peninsular Malaysia and the island nation of Singapore since the late 1800s (Morgan, 1885). Although a bithyniid from Singapore was mentioned in Frauenfeld (1862), the first verifiable record of *Bithynia* species in Singapore was only in the mid-1990s (Chan, 1996). Unlike their congeners in Thailand, parasites have not been recorded from bithyniids in Peninsular Malaysia and Singapore to date (Brandt, 1974; Tropmed, 1986). Owing to their seeming unimportance and small size (most have shell heights less than 10 mm), bithyniids in Peninsular Malaysia and Singapore have thus far remained mostly overlooked. In this paper, we review the records of bithyniids in this target region based on literature and museum material to verify the species identities and distribution, with a brief phylogenetic analysis compared to closely related taxa in Southeast Asia.

MATERIALS AND METHODS

Examination of specimens and morphological identification

Specimens of Bithyniidae from Singapore, Peninsular Malaysia and elsewhere in Southeast Asia that are deposited in the Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (ZRC) were examined. Morphological identification was carried out mainly following Brandt (1974). Photographs of relevant type material were requested from the Natural History Museum, London, UK (NHM), Senckenberg Research Institute Frankfurt am Main, Germany (SMF) and the Smithsonian Institution, National Museum of Natural History, USA (USNM).

Molecular systematic methods

Genomic DNA was extracted from the foot tissue of the selected specimens using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-Tek), following the manufacturer's instructions. Partial fragments of the mitochondrial gene COI were amplified in a 25-µl PCR mix containing 1.5 µL DNA template, 8.0 µL ddH_2O, 12.5 µL exTEN 2X PCR Master Mix (1st Base) and 1.25 µL each 10 µM universal primer LCO1490/HCO2198 (Folmer et al., 1994). Cycling parameters for PCR were as follows: an initial step at 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 50 °C (annealing) for 60 s, and 72 °C (extension) for 60 s; and a final extension step at 72 °C for 10 min.

The PCR products were checked visually on a 1% (w/v) agarose gel. The PCR products were purified, and both DNA strands were cycle-sequenced at Axil Scientific Pte. Ltd. (Singapore). Forward and reverse sequences were assembled and the reading frame was checked using MEGA 7.0.14 (Kumar et al., 2016). Sequences obtained in this study were deposited in GenBank (OR714244–OR714246, Supplementary material 1). A BLAST search (highly similar sequences [megablast]) (Zhang et al., 2000) was conducted in GenBank to compare closely related sequences.

Additional comparative sequences were downloaded from GenBank for the phylogenetic analyses (Wilke et al., 2000, 2023; Kulsantiwong et al., 2013; Ng et al., 2016; Bocxlaer et al., 2018; Tantrawatpan et al., 2020; Bunchom et al., 2021a, b; Supplementary material 1). *Amnicola limosus* (Say, 1817), *Bithynella* cf. *austriaca* (Frauenfeld, 1857) and *Moria kikuchii* (Habe, 1961) were used as outgroups. Sequences were aligned using MAFFT v.7, using default settings (Katoh and Standley, 2013). Phylogenies were reconstructed using maximum likelihood (ML) and Bayesian approaches. The ML tree was estimated using W-IQ-TREE (Trifinopoulos et al., 2016). Branch support was estimated using 10,000 ultra-fast bootstrap replicates (Hoang et al., 2018) and the SH-aLRT test (Anisimova et al., 2011). For Bayesian analysis, the best-fit nucleotide substitution models were estimated using model selection on IQ Tree, and the analysis was conducted with MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) in the CIPRES Science Gateway portal (Miller et al., 2010). Two simultaneous runs of 1 million MCMC generations were performed; trees were sampled every 1000 generations, with the first 25% of trees being discarded as burn-in. A branch/clade was considered to be well supported if ultra-fast bootstrap support values were ≥95%, SH-aLRT support values were ≥80% and posterior probability values were ≥0.95, respectively (Alfaro and Holder, 2006; Anisimova et al., 2011; Hoang et al., 2018).

RESULTS

Thirty-one lots of bithyniids collected from Peninsular Malaysia and Singapore in the ZRC were examined. Four species of bithyniids have been recorded from Peninsular Malaysia and Singapore to date—*Digoniostoma siamensis siamensis* (I. Lea, 1856), *Gabbia minuta* (Ghosh, 1929), *Gabbia* cf. *stenothyroides* (Dohrn, 1857) and *Wattlebledia baschi* Brandt, 1968 (Fig. 1).

Molecular analysis

The COI gene was successfully amplified in three individuals from Singapore that were morphologically identified as *D. siamensis siamensis* (one individual) and *G.* cf. *stenothyroides* (two individuals). The top matches from the BLAST search for *D. s. siamensis* were eight sequences identified as *Bithynia siamensis siamensis* from Thailand, at 99.24% identity (KY118653, KY118641, KY118640, KY118639, KY118638, KY118637, KY118636, KY118635 from Kulsantiwong et al., 2013). The top match for both *G.* cf. *stenothyroides* was *Bithynia* sp. from the Singapore aquarium trade (KU318328 from Ng et al., 2016).

An additional 40 COI sequences obtained from GenBank were used for ML and Bayesian analysis. The best-fit models were as follows: ML—TNe+G4 for the first, TPM3u+F+I+G4 for the second and HKY+F+G4 for third codon positions, and Bayesian—HKY+F+I+G4 for the first and second codon positions, HKY+F+G4 for the third codon position. The resulting trees largely shared similar topology and support (Figs. 2, 3). The *D. siamensis siamensis* from Singapore shared the same clade with *B. siamensis siamensis*, *B. siamensis goniomphalos* and *B. funiculata* from Thailand and surrounding countries, with the position of *B. siamensis goniomphalos* of Laos being recovered inconsistently between the different analyses (Fig. 2 and 3). *Gabbia* cf. *stenothyroides* was sister to an unknown bithyniid from Lake Poso in Sulawesi. The genera *Bithynia* and *Gabbia* were recovered as non-monophyletic groups.

Systematics

Superfamily TRUNCATELLOIDEA Gray, 1840

Family BITHYNIIDAE Gray, 1857

Genus *Digoniostoma* Annandale, 1920

Type species.— *Paludina cerameopoma* W.H. Benson, 1830: 125 ("A ditch in Banda [India]"; syntypes UMZC I.102180, see Preece et al., 2023).

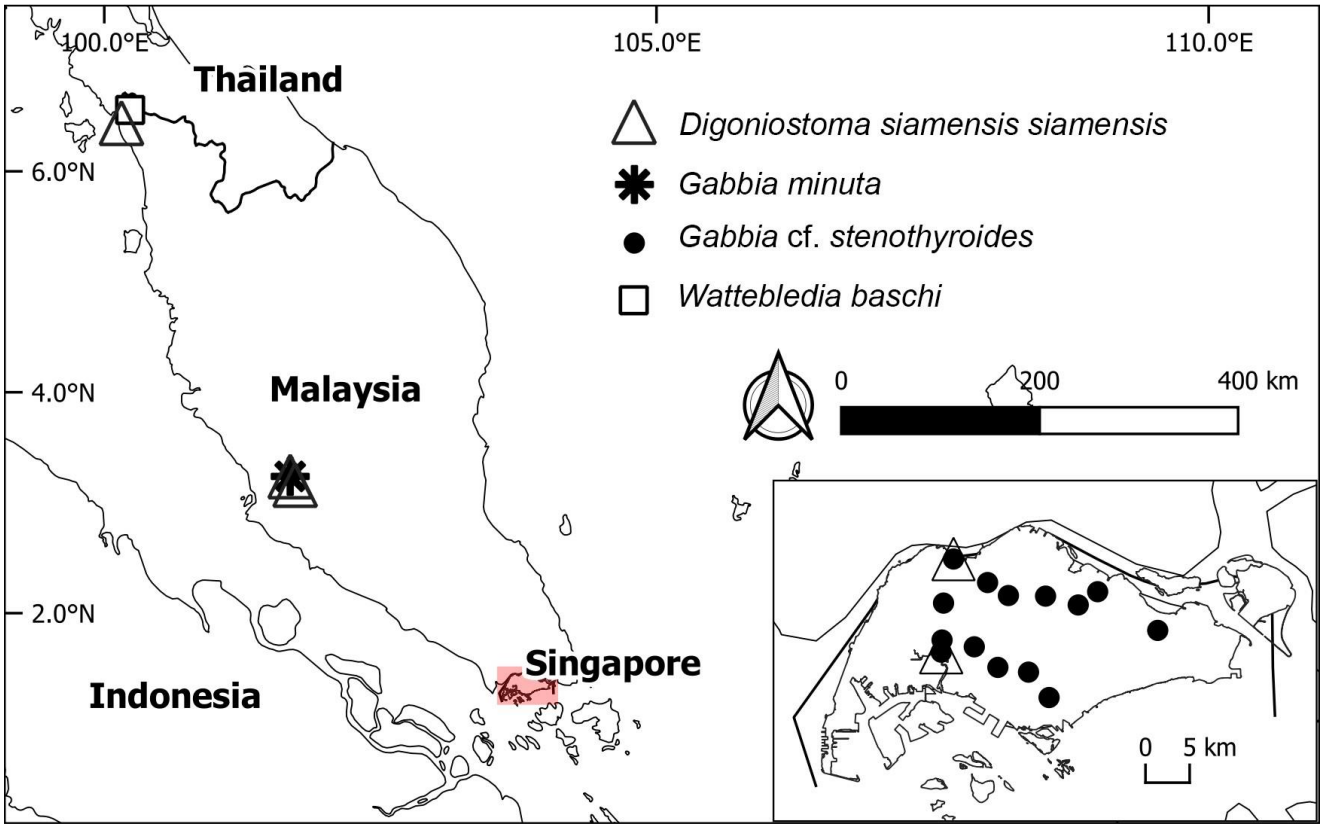

FIGURE 1. Distribution map of species from the family Bithyniidae in Peninsular Malaysia and Singapore based on museum records.

Diagnosis.— Shell small, ovately conical, basal end of peristome angular, with a corresponding keel around the open umbilicus.

***Digoniostoma siamensis siamensis* (I. Lea, 1856)**
Fig. 4A
Bithinia siamensis I. Lea, 1856: 110 ("Takrong River, Siam" [Thailand]).
Bithinia kintana Morgan, 1885: p. 416, pl. VII, fig. 7 ("le S. Bertam, affluent du S. Kinta, à Batou-Gadja et dans un ruisseau près de Penkalan-Bahrou (Kinta)" [Perak]; syntypes MNHN-IM-2000-32676).
Bithinia laevis Morelet, 1875: pp. 313–314, pl. XIII, fig. 2 ("royaume de Siam, ainsi que dans la Cochin-chine").
Bythinia kintana—Möllendorf, 1891: p. 347 ("Kinta valley" [Perak; after Morgan, 1885]).
Paludomus baccula—Ghosh, 1929a [=*Parafossorulus striatulus/Paludina* (*Bithynia*) *striatula*; see Laidlaw, 1940]: p. 334 ("Batu Caves, Selangor"); Ghosh, 1929b: p. 392 ("ponds near Kuala Lumpur, Selangor") [not Reeve, 1854].
Bithynia pulchella—Benthem Jutting, 1949: p. 58, fig. 1, ("Batu Caves, Dark Caves, Selangor"); Berry, 1974: p. 357 ("Kuala Lumpur, Selangor"); Chan, 1996: p. 185, fig. 1 ("Jurong Lake" [Singapore]) [not Benson, 1836].
Digoniostoma pulchellum—Benthem Jutting, 1960: p. 14 ("swamp near Batu Caves, Selangor"); Berry, 1963: p. 4, pl. 2, fig. 6 ("Dark Caves at Batu Caves, Selangor") [not Benson, 1836].
Paludomus buccula [sic]—McClure et al. 1967: p. 413 (Dark Cave, Batu Caves) [not Reeve, 1854].
Bithynia (*Digoniostoma*) *laevis*—Brandt, 1968: p. 223 ("in the ponds outside the caves" [Batu Caves, Peninsular Malaysia]).
Bithynia (*Digoniostoma*) *siamensis siamensis*—Brandt, 1974: p. 59, pI. 4, figs 66–67 ("…the whole Malaccan Peninsula").
Bithynia (*Digoniostoma*) *pulchella*—Chan, 1997: p. 37 ("Selangor, West Malaysia") [not Benson, 1836].
Bithynia siamensis siamensis—Maassen, 2001: pp. 47–48 (West Malaysia).
Digoniostoma pulchella—Clements et al., 2006: p. 144 (estuarine reservoir" [Singapore]); Tan and Woo, 2010: p. 37 (Singapore); Tan et al., 2012: p. 140, (Singapore) [not Benson, 1836].

Material examined.— PENINSULAR MALAYSIA: Kuala Lumpur, Batu Caves, date unknown, coll. unknown, ZRC.MOL.023067; lake at Kuala Lumpur, June 1955, coll. D.S. Johnson, ZRC.MOL.023069; old mining pond, 4½ miles from Kuala Lumpur on

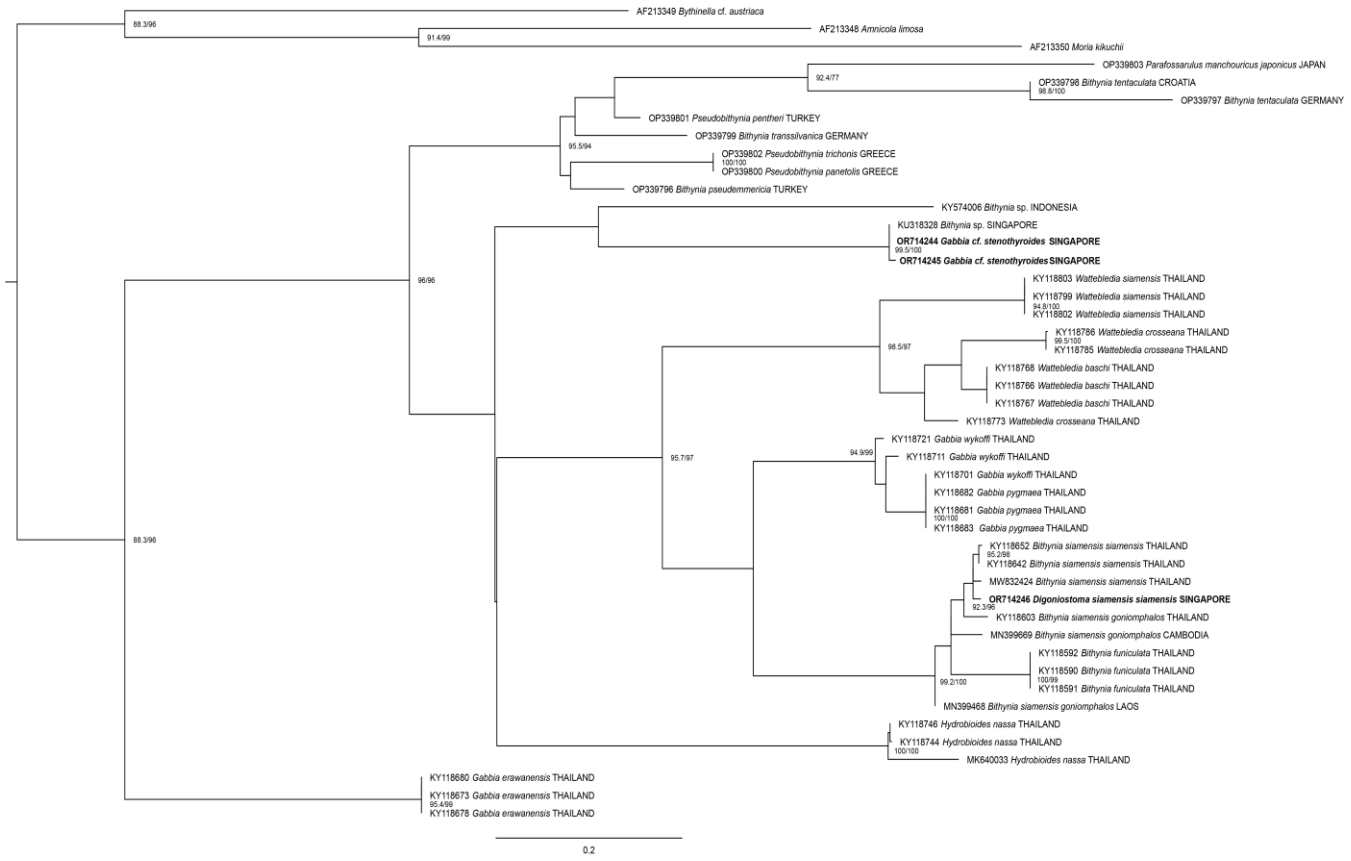

FIGURE 2. Maximum likelihood tree of Bithyniidae based on mitochondrial COI gene sequences. The outgroups are *Bithynella* cf. *austricia*, *Amnicola limosa* and *Moria kikuchi* (Amnicolidae). Numbers by the nodes are the support values for SH-alrt/ultra-fast (UF) bootstrap; only values >80% for SH-alrt and ≥ 95% for UF are shown. Sequences generated in this study are indicated in **bold**, GenBank accession numbers are indicated to the left of the species names, locations of the sequenced bithyniids are indicated to the right of the species names in capital letters.

Ampang Road, June 1955, coll. D.S. Johnson, ZRC.MOL.023070; circa 2 miles Kuala Lumpur on Klang Road, June 1955, coll. D.S. Johnson, ZRC.MOL.023071; big pond at foot of Batu Caves, 14 Dec. 1957, coll. D.S. Johnson, ZRC.MOL.023072; pool at foot of Batu Caves, 13 Dec. 1958, coll. D.S. Johnson(?), ZRC.MOL.023073; pool by limestone hill and quarry, north of Kuala Perlis, 18 Dec. 1958, coll. D.S. Johnson, ZRC.MOL.023074. **SINGAPORE:** Jurong Lake, date unknown, coll. W.H. Ho, ZRC.MOL.023075; Jurong Lake, 9 Jul. 1994, coll. W.H. Ho, ZRC.MOL.023076; Jurong Lake,11 Jul. 2002, coll. R. Clements, ZRC.MOL.000023; Jurong Lake, 3 Sep. 2021, coll. W.T. Lim, ZRC.MOL.28603.

Diagnostic description.— Shell ovately conic, small to medium sized, shell height to 11 mm. Spire moderately high, apex often eroded in adults. Whorls moderately rounded, suture relatively shallow. Umbilicus narrow, bordered by a blunt, sometimes indistinct, keel. Aperture height approximately 1/3 shell height. Operculum nucleus positioned towards inner lip.

Distribution.— Along the west coast of Peninsular Malaysia, from northernmost state of Perlis to the central region of Selangor and Kuala Lumpur. Possibly elsewhere throughout Peninsular Malaysia and Singapore, and in Thailand, Cambodia, southern Vietnam and Myanmar southwards from Mandalay (Brandt, 1974). Introduced to Singapore.

Remarks.— One lot of four specimens labelled as possible syntypes of *Bithinia siamensis* is present in the USNM (USNM IZMOL 121376), and appears to consist of at least one specimen of a different species. Material from Peninsular Malaysia and Singapore were previously identified as *B. pulchella* (e.g., Benthem Jutting, 1949; Chan, 1997; Clements et al., 2006). Based on comparison to the type specimen of *B. pulchella* (see Fig. 43B of Preece et al., 2022), the material from Peninsular Malaysia and Singapore are larger and generally have less convex whorls. The

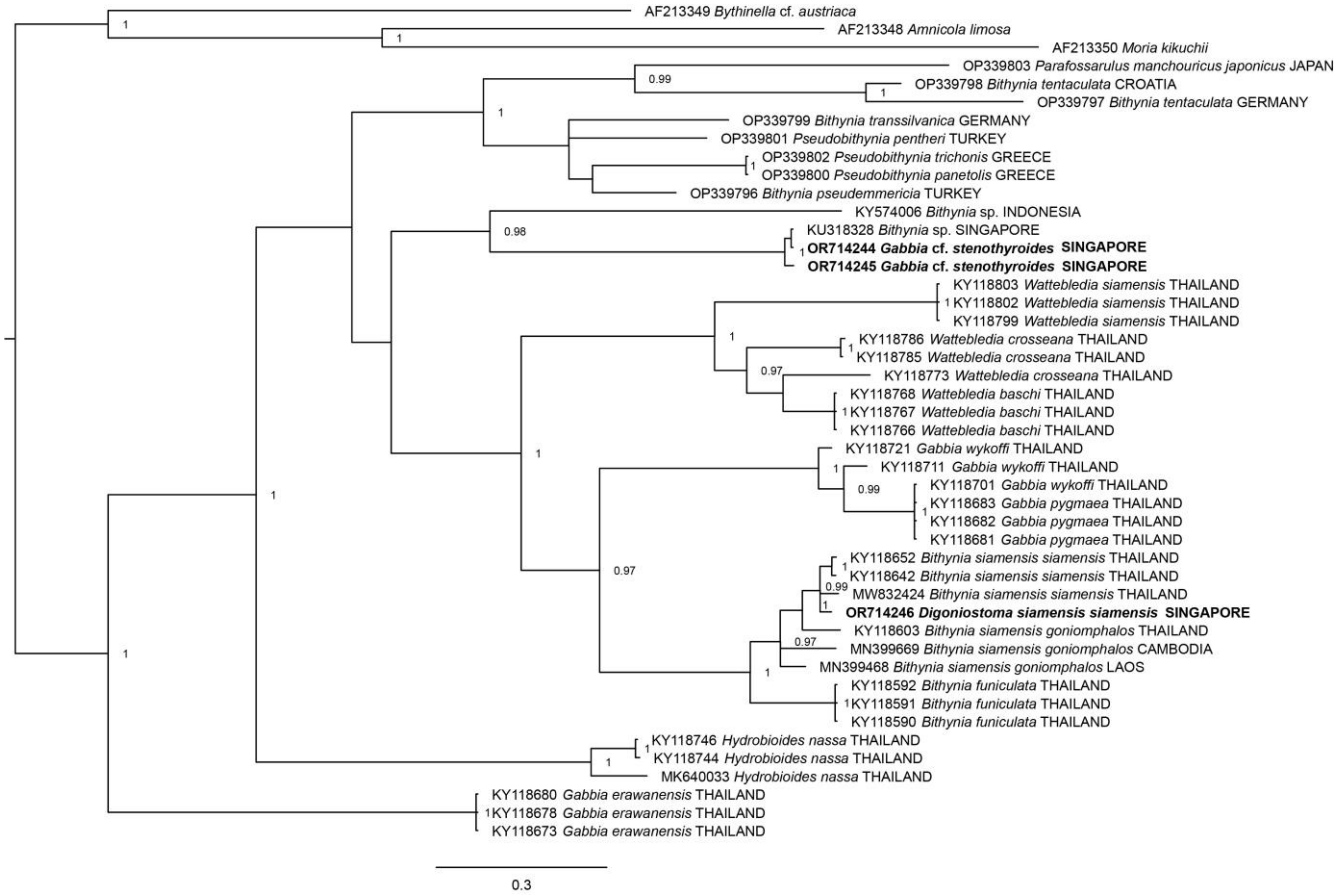

FIGURE 3. Bayesian inference tree of Bithyniidae based on mitochondrial COI gene sequences. The outgroups are *Bithynella* cf. *austricia*, *Amnicola limosa* and *Moria kikuchi* (Amnicolidae). Numbers by the nodes are the posterior probabilities; only values ≥ 0.95 are shown. Sequences generated in this study are indicated in **bold**, GenBank accession numbers are indicated to the left of the species names, locations of the sequenced bithyniids are indicated to the right of the species names in capital letters.

molecular data from Singapore confirms the assessment by Brandt (1974) that *D. siamensis siamensis* is distributed throughout Peninsular Malaysia to Singapore. Our phylogenetic results (Figs. 2, 3) indicate that the status of the subspecies, *D. siamensis siamensis* and *D. siamensis goniomphalos*, would require more rigorous taxonomic assessment than relying on publicly-available sequences alone. Nonetheless, pending future revisions, we follow Brandt in identifying our material as *D. siamensis siamensis*, since the results indicate that it matches the subspecies elsewhere in mainland Southeast Asia. Furthermore, the Malayan material have more convex body whorls than the syntypes of *Paludina goniomphalos* (see Natural History Museum, 2021) and the *D. siamensis goniomphalos* material from Cambodia in the ZRC (ZRC.MOL.18278–1828, 15696–15701).

We conclude that the species was introduced to Singapore because it was first recorded in 1994 from Jurong Lake, a reservoir created from the damming of Jurong River, and it has since only been recorded from one other reservoir, Kranji. On mainland Peninsular Malaysia, the species is not distributed beyond the central region, including the southern regions closest to Singapore.

Genus *Gabbia* Tyron, 1865

Type species.— *Gabbia australis* Tyron, 1865 (by monotypy; = *Bithynia vertiginosa* Frauenfeld, 1862) (New South Wales).

Diagnosis.— Shell small, ovately conic to globose, umbilicus narrow or absent.

Gabbia minuta (Ghosh, 1929)

Fig. 4B, 5

Paludomus baccula var. *minuta* Ghosh, 1929a: 334–335, Fig. 2 (“Batu Caves (Dark Cave) [Selangor]).

Digoniostoma pulchellum—Laidlaw, 1940: P. 133 (“Batu Caves”) [not Benson, 1836].

Paludomus buccula [sic] var. *minuta*—McClure et al., 1967: p. 413 (Dark Cave, Batu Caves).

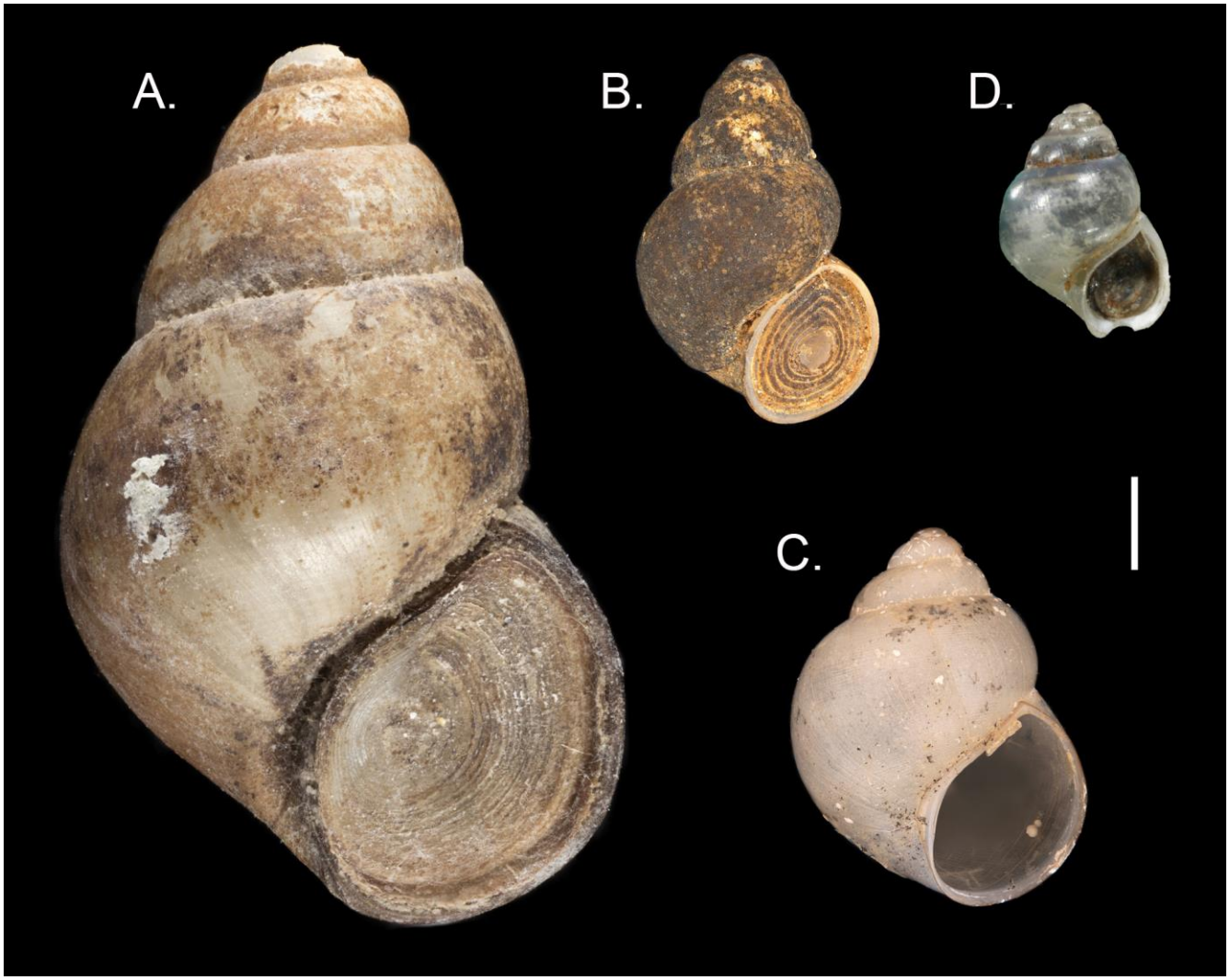

FIGURE 4. Bithyniidae species recorded from Peninsular Malaysia and Singapore. **A.** *Digoniostoma siamensis siamensis* MOL.23070, Kuala Lumpur, Malaysia, **B.** *Gabbia minuta* ZRC.MOL.23066 Batu Caves, Malaysia, **C.** *Gabbia* cf. *stenothyroides* ZRC.MOL.23077, Seletar East Farmway 2, Singapore, **D.** *Wattebledia baschi* holotype SMF197314, Kampong Padang Malau, Malaysia. Photographs by: A–C, Renee SL Ong, D, Sigrid Hof. Scale bar = 1 mm.

Bithynia (*Gabbia*) *minuta*—Brandt, 1968: 222–223, Pl. 8, Fig. 7 ("known from type locality only", "in ponds in the dark part of the cave").
Bithynia sp.—Moseley et al., 2012: p. 87 ("GC [Ganesh Cave]: d.th [deep threshold]. In shallow pool").
Gabbia minuta—Foon and Marzuki, 2023: p. 135 (Dark Cave, Batu Caves).

Material examined.— PENINSULAR MALAYSIA: Kuala Lumpur, Batu Caves, taken inside cave underwater, 22 Mar. 1928, ZRC.MOL.023066.

Diagnostic description.— Shell ovately conic, small, shell height to 5.1 mm. Spire moderately high. Whorls rounded, suture deep. Umbilicus narrow, often partly obscured by edge of peristome. Aperture height slightly less than ½ shell height. Operculum nucleus slightly positioned towards outer lip.

Distribution.— Only known from the type locality.

Remarks.— Although the material in the ZRC dates back to the time of its original description, we do not have sufficient evidence to verify that the shells are type material. According to Laidlaw (1940) specimens identified by Ghosh (1929) as *Paludomus baccula* var. *minuta*, are in the collection of the then Raffles Museum (predecessor of the ZRC). Nonetheless, he did not explicitly mention that these were also the ones examined by Ghosh, as he did for the species that Ghosh had called *P. baccula*. The living animal appears translucent and occurs in high abundance at the type locality (JK Foon, M Moseley, pers. comm.; Fig. 5).

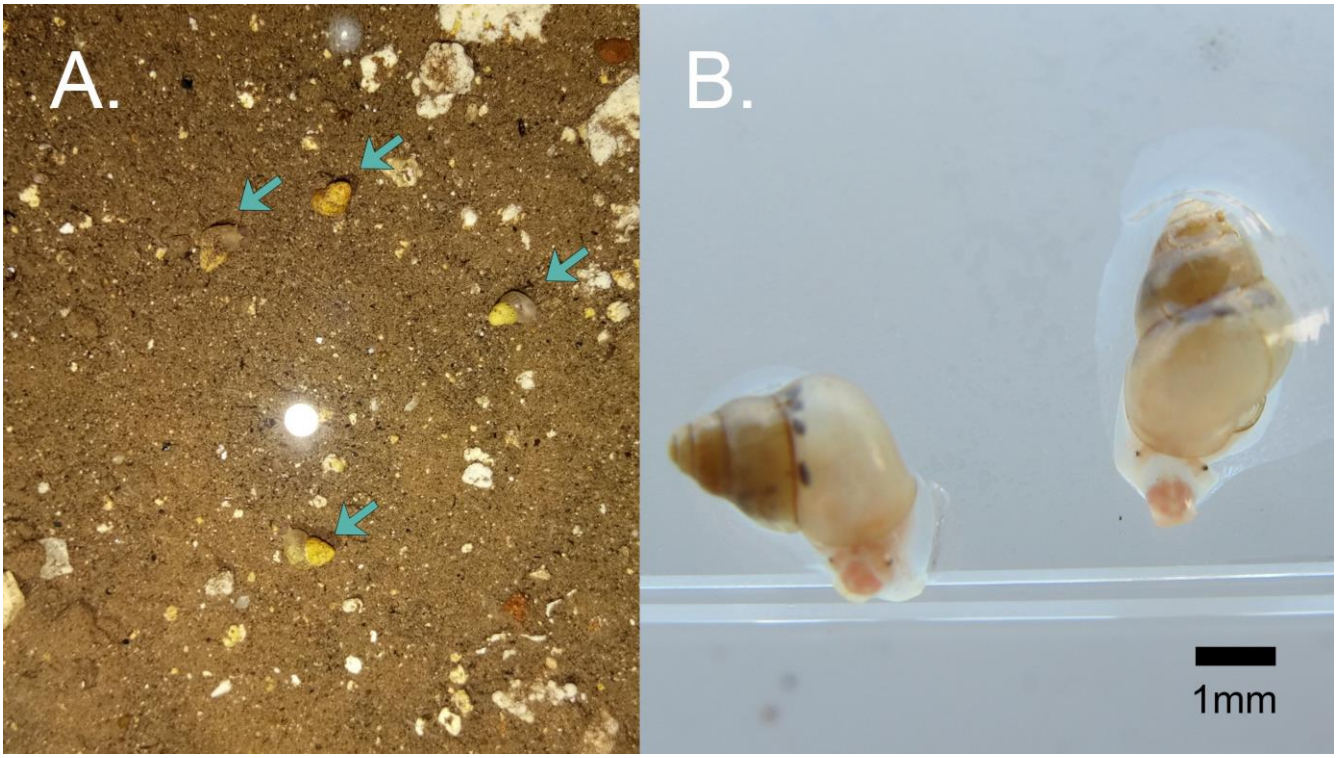

FIGURE 5. *Gabbia minuta* from Batu Caves, Selangor, Peninsular Malaysia, **A.** in-situ, individuals indicated by arrows; **B.** live individuals. Photographs by: A. J.K. Foon, B. M. Moseley.

***Gabbia* cf. *stenothyroides* (Dohrn, 1857)**
Fig. 4C
Bithynia stenothyroides Dohrn, 1857: p. 123 ("Ceylon, Nilgherries').
Bithynia sp.— Chan, 1996: p. 185, fig. 1 ("ornamental fish shop… Botanical Gardens and a waterweed farm" [Singapore]); Clements et al., 2006: p. 144 ("rural streams…ponds" [Singapore]); Tan and Woo, 2010: p. 37, locality= Singapore; Tan et al., 2012: p. 141 (Singapore); Ng et al., 2016: p. 5, fig. 1 (ornamental trade, Singapore).
Gabbia wykoffi—Chan and Lau, 2020: p. 153, figs. 1–4 ("pond near Buangkok Green Medical Park, Singapore) [not Brandt, 1968].

Material examined.— SINGAPORE: River Valley Road, 15 Nov. 1982, coll. Unknown, ZRC.MOL.023095; Seletar East Farmway 2, fish pond, 15 May 1995, coll. W.H. Ho, ZRC.MOL.023077; Botanic Gardens, 26 Jul. 2002, coll. R. Clements, ZRC.MOL.000024; Botanic Gardens pond, 25 Aug. 2007, coll. C.F. Lim, ZRC.MOL.002941; Upper Seletar Reservoir, 4 Dec. 2008, coll. T.H. Ng, ZRC.MOL.009845, ZRC.MOL.009935; Lower Seletar Reservoir, 18 Mar. 2009, coll. not stated, ZRC.MOL.028602; canal along Lentor Road, tributary of Lower Seletar Reservoir, 11 Nov. 2010, coll. S.K. Tan et al., ZRC.MOL.009934; ornamental pet trade, 24 Aug. 2011, coll. H.H. Tan, ZRC.MOL.005800; Sungei Tengah Road canal, 26 Apr. 2012, coll. T.H. Ng, ZRC.MOL.009937; Kranji Reservoir, Sep. 2012, coll. J. Tay, ZRC.MOL.009938; Holland Woods, 25 Sep. 2012, coll. R. Yue and M. Lim, ZRC.MOL.009850; Sungei Seletar, 25 Oct. 2012, coll. T.H. Ng, ZRC.MOL.009933; canal at Jurong Road, 26 Nov. 2012, coll. J. Ho et al., ZRC.MOL.009945; canal at Jurong Road., 27 Nov. 2012, coll. T.H. Ng, ZRC.MOL.009939; Kranji Marshes, 23 Dec. 2011, coll. T.H. Ng, ZRC.MOL.008495; Jurong Lake, 25 Mar. 2013, coll. T.H. Ng, ZRC.MOL.009846; Tampines Eco Green, 6 Aug. 2013, coll. T.H. Ng, ZRC.MOL.010234; Bukit Batok Nature Reserve Park, 30 Oct. 2013, coll. T.H. Ng, ZRC.MOL.009847; Kranji Marshes, Turut Track, 30 Jul. 2014, coll. T.H. Ng, ZRC.MOL.009849; Punggol Reservoir, 27 Jun. 2016, coll. W.Q. Ng and T.H. Ng, ZRC.MOL.009848; Lorong Asrama, 31 Mar. 2017, coll. H.H. Tan and B.W. Low, ZRC.MOL.009611; Singapore Botanic Gardens, 22 Aug. 2007, coll. C.F. Lim, ZRC.MOL.29005; Clementi Forest, 27 Sep. 2021, coll. W.T. Lim and H.L.C. Tang, ZRC.MOL.28705; Clementi Forest, 27 Sep. 2021, coll. W.T. Lim and M. Chua, ZRC.MOL.28704.

Diagnostic description.— Shell globosely conic, small, shell height to 7 mm. Spire relatively small compared to body whorl. Whorls very rounded, rapidly increasing, suture deep. Umbilicus closed. Aperture slightly descending anteriorly. Aperture height approximately 1/2 shell height.

Distribution.— Known from Sri Lanka, India and Nepal (Budha and Daniel, 2010). Introduced to Singapore.

Remarks.— Based on the earliest record in the ZRC (15 Nov. 1982), *Gabbia* cf. *stenothyroides* was probably first introduced to Singapore more than 40 years ago, and has since spread in various artificial and human-modified freshwater habitats throughout the island. It has previously been recorded as a hitchhiker in the ornamental pet trade (as *Bithynia* sp. in Ng et al., 2016). This species was also recently reported from Singapore as *Gabbia wykoffi* (Brandt, 1968), but that species differs in having a slightly smaller and relatively slimmer shell, with a larger, more prominent, spire (see Brandt, 1968, 1974). In addition, our molecular results show that the *Gabbia* species in Singapore is not closely related to any of the Thai species, including *G. wykoffi* (see Figs. 2 and 3). The species present in Singapore is determined to be closest in identity to *G. stenothyroides* (Dohrn, 1857) based on comparisons of its shell morphology to the original description and an examined syntype of *Bithynia stenothyroides* from Nilgiris, India (NHMUK 20230892, see Natural History Museum, 2024). Corroboration with material from its native range would allow for confirmation of its identity.

Genus *Wattebledia* Crosse, 1886

Type species.— *Bithynia crosseana* Wattebled, 1884 (type by original designation).

Diagnosis.— Shell very small, basal margin of peristome sinuous.

***Wattebledia baschi* Brandt, 1968**

Fig. 4D

Wattebledia baschi Brandt, 1968: p. 227–228, pl. 8, fig. 11, text fig. 9 (“Trench along the road at Kampong Padang, Malau, 12 km north of Kanga, Perlis, North Malaya”; holotype SMF 197314, paratypes SMF 317889).
Wattebledia baschi—Brandt, 1974: p. 65, pl. 5, fig. 80 (“Perlis in Malaya”).

Diagnostic description.— Shell ovately conic, very small, shell height to 2.7 mm (Brandt, 1974). Whorls slightly convex, suture relatively shallow. Umbilicus closed, bordered by a keel. Peristome base with flap-like sinus and deep incision. Aperture height slightly less than ½ shell height.

Distribution.— In Peninsular Malaysia, known only from type locality in the northernmost state of Perlis. Elsewhere, in the southern Thai provinces of Krabi, Nakhon Srithammarat and Surat Thani (Brandt, 1974; Kulsantiwong et al., 2013).

Remarks.— The only known specimens in a reference collection are the type material in the SMF. Brandt (1974) had mentioned that *Wattebledia siamensis* Möllendorf, 1902, was known from the Thai province of Satun, which borders with the Malaysian state of Perlis, where *W. baschi* was collected from. More recent work in Thailand appears to have found only *W. baschi* in southern Thailand (Kulsantiwong et al. 2013). Further surveys of northern Peninsular Malaysia will need to be conducted to confirm if both species are extant and if they are syntopic.

DISCUSSION

The earliest mention of a bithyniid purportedly from Singapore, was *Bithynia tentaculata* (Linnaeus, 1758) in Cuming’s collection, as mentioned in Frauenfeld (1862). Based on current understanding of bithyniid taxonomy however, *B. tentaculata* is a widely distributed European species, that has been introduced to north America (e.g., Mills et al., 1993), but has not been reported with certainty from Southeast Asia. The location of the specimen from Frauenfeld’s (1862) record cannot be located in the collected of the NHM (J. Ablett, pers. comm.). We are thus unable to ascertain the reliability of that record, nor can we speculate upon its possible identity. Pending further information, we consider it prudent to disregard this questionable record.

Based on museum records, we confirm that four species of bithyniids have been recorded from Peninsular Malaysia and Singapore, including two species that are limited in distribution—*G. minuta*, which appears to be endemic to central Peninsular Malaysia, and *W. baschi*, which is distributed in northern Peninsular Malaysia and southern Thailand. Although it has been almost a century and more than half a century since the original descriptions of both species, respectively (Ghosh, 1929; Brandt, 1968), there remains a dearth of information on their distribution within Peninsular Malaysia and their ecology. This is particularly concerning for *G. minuta*, which has to date only been recorded from a single

cave system that is largely exposed to anthropogenic influences (Kiew et al., 2023).

Two other species, *D. siamensis siamensis* and *G.* cf. *stenothyroides* are almost certainly introduced to Singapore, probably accidentally via the ornamental trade of aquatic plants (Ng et al., 2016). While uncertain if it is conspecific with the Singapore species, a '*G. stenothyroides*' has once been intercepted by biosecurity border authorities in Australia (see Ponder et al., 2023). It is possible that one or more species of bithyniids are more widely introduced via pathways such as the ornamental trade but have thus far remained unnoticed.

Our results show a clear separation between *B. tentaculata*, which is the type species for the genus, and other Southeast Asian genera, including species previously placed within the *Bithynia*. Based on our data, it is clear that *Digoniostoma*, which was previously treated as a subgenus of *Bithynia* (Brandt, 1974), should be elevated to genus level and be applied to the *siamensis-funiculata* group in Southeast Asia. The genus *Gabbia* appeared in three separate clades and would require comparative material and molecular data from the Indian subcontinent and Oceania to resolve the complexities. Within the scope of this study, we have only analysed the COI gene. The analysis of more molecular markers from material obtained from a wider range may help to clarify the non-monophyly of this group.

Our study provides new data (species descriptions and DNA sequences) that may help detect the presence of conspecific or closely related bithyniids, which may have been anthropogenically introduced elsewhere. Furthermore, our molecular analyses indicate that the Southeast Asian bithyniids are non-monophyletic and in need of revision. Comparative material from beyond Southeast Asia would be important for resolving the taxonomic uncertainties in this long-neglected group.

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