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Rapid Communication

First record of the cryptic invader *Pyrgophorus platyrachis* Thompson, 1968 (Gastropoda: Truncatelloidea: Cochliopidae) outside the Americas

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Abstract

Anthropogenic removal of natural dispersal barriers and modification of natural habitats have contributed to the spread of non-native species. Potential invaders that are cryptic in appearance and/or behaviour are particularly troublesome as this confounds efforts to detect or manage incipient invasions. Here we report one such invader, the Florida serrate crownsnail, *Pyrgophorus platyrachis* Thompson, 1968, in Singapore reservoirs—only the first record outside the Americas. We identified *P. platyrachis* using morphological and molecular techniques. The Singapore COI mitochondrial sequences were 99–100% matched to *P. platyrachis* from Florida, USA, while the 16S sequences are the first published for this species, and were most closely matched to another confamilial species.

Key words: introduced species, gastropod, freshwater, Singapore, DNA barcoding

Introduction

The removal of natural dispersal barriers and the creation of empty or novel niches in modified natural habitats during the Anthropocene (McKinney 2006; Hulme 2009) has facilitated the propagation and establishment of non-native species in a range of habitats resulting in both direct and indirect impacts (Clavero and García-Berthou 2005; Nghiem et al. 2013). Although biological invasions are nearly ubiquitous, especially in disturbed habitats, not all invaders are equal, with species traits such as high fecundity and generalist feeding habits increasing the likelihood of establishment in adventive habitats (Moyle and Marchetti 2006). These model invaders become an even greater concern if they are difficult to detect as this negates our ability to pro-actively or rapidly address an incipient invasion before eradication becomes a practical impossibility (Bertolino and Lurz 2013).

A group with all the traits of an ideal invader is the New World freshwater snail genus *Pyrgophorus* Ancey, 1888 (Truncatelloidea: Cochliopidae), which is distributed in Florida, the Caribbean islands and Texas southwards to Mexico until Venezuela (Thompson 1968; Hershler and Thompson 1992; Pointier 2015). *Pyrgophorus* snails are known to occur in high densities (up to >15,000 individuals m^{-2} [Dillon et al. 2006]), and capable of live-bearing relatively large numbers of offspring (i.e., 50 per batch) (Thompson 1968). These snails have spread beyond their native ranges, but none have been definitively identified to species owing to the taxonomic complexity of the group (Englund 2002; Mienis et al. 2011; Nasarat et al. 2014). The inability to conclusively identify members of the genus contributes to the cryptic nature of their invasions, particularly in habitats where superficially similar native snails occur. Adding to the confusion, very little is known about the ecology of these cryptic invaders, with physicochemical environmental parameters recorded for a single *Pyrgophorus* species in Venezuela (Nava and Severeyn 2011) representing all we know about the environmental tolerance of this group. This knowledge gap can be hugely detrimental in the assessment of the potential spread and impacts of members of this genus. The sheer densities of their populations may be indicative of their potential invasive impacts. An analogous invasion case study is present in the similarly-sized New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843), a globally invasive species that reaches

similar densities and is known to impact native aquatic communities via competitive exclusion, predation, and habitat modification (Alonso and Castro-Díez 2012).

In a perfect illustration of the high levels of global connectivity in the "New Pangaea" (Baiser et al. 2012), we report the first record of the Florida serrate crownsnail, *Pyrgophorus platyrachis* Thompson, 1968, in Singapore, an equatorial island in Southeast Asia. Singapore's freshwater habitats comprise mainly natural forest streams, and urban man-made canals and reservoirs (Yeo et al. 2010). Freshwater snails in Singapore are found almost exclusively in urban, disturbed habitats—with man-made reservoirs having the highest species richness (Clements et al. 2006; Tan et al. 2012). Although some species are native to the surrounding Southeast Asian region, most of the freshwater snails in Singapore are considered non-native or are at least cryptogenic owing to their exclusive association with human-disturbed habitats (Tan et al. 2012). *Pyrgophorus platyrachis* is native to Florida (Thompson 1968), with a doubtful occurrence recorded in Venezuela (see Pointier 2015), making the established population in Singapore the first to be recorded outside the Americas. We first discovered *P. platyrachis* in Singapore on 31 October 2014 in inland reservoirs and in this study we include mitochondrial sequence analysis to confirm the species' identity.

Methods

We recorded *P. platyrachis* from three reservoirs in Singapore—Bedok, Pandan, and Punggol Reservoirs (Figure 1, supplementary material Table S1). All three are artificial habitats located in urbanised areas: Pandan Reservoir was created over mangrove swamps at the mouth of the Pandan River; Bedok Reservoir is a former sand quarry; and Punggol Reservoir was formed by damming the previously estuarine Punggol River (Yeo et al. 2010). To characterize their habitat and environmental tolerance, physicochemical variables—water temperature (°C), pH, dissolved oxygen (mgL^{-1}), conductivity ($mScm^{-1}$), and total dissolved solids (gL^{-1})—were measured using a YSI Professional Plus handheld multiparameter meter (YSI Inc.).

We collected *P. platyrachis* specimens using customised, cage-type samplers (diameter 20.0 cm; height 10.0 cm, 1.3 cm^2 mesh size) as artificial invertebrate colonisers (Loke et al. 2010) and a 15.2 cm^3 Ekman grab (Forestry Suppliers, Inc.). Six colonisers were deployed per reservoir, in areas with soft, muddy substrata at depths of 0.5–2.0 m for two to three weeks. All samples were collected between October 2014 and June 2015, and deposited in the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore (Catalogue numbers ZRC.MOL.005854–005861).

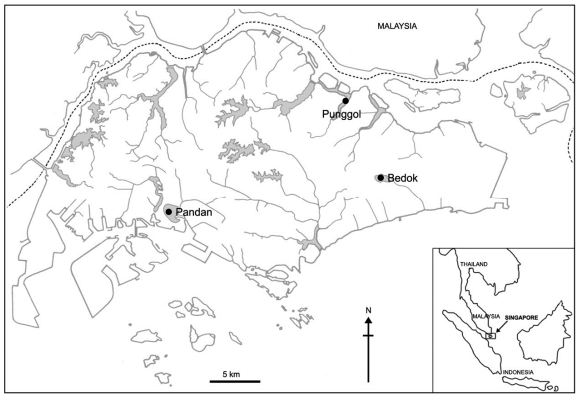

Figure 1. Known distribution of *Pyrgophorus platyrachis* in Singapore (see details in supplementary material Table S1).

Over 1000 snails per coloniser from Pandan and Punggol Reservoirs, and two individuals from Bedok Reservoir were examined. In order to facilitate further identification, we separated the body of selected snails from the shell by placing the specimens inside a microwave oven (800W) for 10–15 seconds (Galindo et al. 2014). The shell and body (including penial morphology) were examined using a stereomicroscope. Identification of species was based on Thompson (1968; 2004).

We extracted total genomic DNA from the foot tissue of selected individuals following a CTAB phenol-chloroform protocol. The mitochondrial COI and 16S genes were amplified in polymerase chain reactions (PCR) with degenerate primers (dgLCO1490 5'–GGTCAACAAATCATAAAGAYATYGG–3', dgHCO2198 5'–TAAACTTCAGGGTGACCAAAR AAYCA–3' [Meyer 2003], mICOIntF 5'–GGWAC WGGWTGAACWGTWTAYCCYCC–3' [Leray et al. 2013], Bivalvia HCO2198 5'–TANACYTCNGGRT GNCCRAARAAYCA–3' [Geller et al. 2013], 16S forward 5'–TRACYGTGCDAAGGTAGC–3', 16S reverse 5'–YTRRTYCAACATCGAGGTC–3' [Zhan et al. 2014]), following which PCR products were examined visually on a 1% agarose gel. Post PCR clean-ups were performed on successfully amplified products using SureClean reagent (Bioline Inc.) and the purified products were sequenced with BigDye Terminator (ThermoFisher) reactions and analysed on the ABI PRISM 3130XL sequencer (Applied Biosystems). We inspected and trimmed sequence chromatograms using Sequencher ver. 4.6 (Genecodes), and aligned them using MAFFT version 7 (Katoh and Standley 2013) with default settings. We then conducted

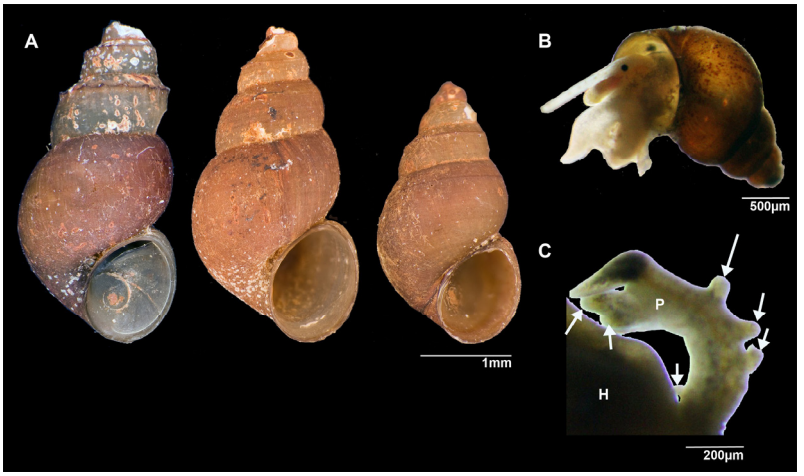

Figure 2. *Pyrgophorus platyrachis* from Singapore. A. different morphs; B. live specimen from Pandan Reservoir; C. penis, arrows showing papillae, H: head of the snail, P: penis. Photographs by: YC Ang (A), TH Ng (B & C).

a BLASTN search (highly similar sequences [megablast]) on GenBank to create a shortlist of probable species (Zhang et al. 2000). Finally, the DNA sequences obtained were inspected together with published COI and 16S sequences (obtained from GenBank), using the objective clustering in SpeciesIdentifier version 1.7.9 (Meier et al. 2006) with a species delimitation threshold of 2% (Meier et al. 2008). All sequences were deposited in GenBank (Accession numbers KT326188, KT326189, KT326190, KT372180).

Results

Morphological identification

The species is diagnosed by the following morphological characters: shell ovate-conic, spire elongated, suture deeply impressed, with 4–6 whorls, peristome slightly detached and complete in mature individuals, dextral; operculum elongate-ellipsoidal, paucispiral, thin and flexible (Figure 2A); penis with 4 papillae on the right margin, tip tapered and pigmented, two papillae on the left margin, immediately below the tip, smaller papilla on the proximal left margin (Figure 2C) (sensu Thompson 1968; 2004). Living snails were semi-transparent and yellow-speckled (Figure 2B). Three distinct morphs were recorded: i) relatively smooth-shelled, ii) shells with raised spiral threads, and iii) shells with spines on the periphery of the threads (Figure 2A). Shell height 2.5–4.6 mm; shell width 1.5–2.5 mm.

Molecular analysis

In addition to morphological assessment of the specimens, we also successfully sequenced both COI and 16S genes from two individuals, one individual each from Pandan and Punggol Reservoirs. The COI sequences (KT326190 and KT372180) matched *Pyrgophorus platyrachis* (99–100% identity) AF129327 and AF367632 (Hershler et al. 1999; Wilke et al. 2001), while both 16S sequences (KT326188 and KT326189) were most closely matched to the confamilial *Spurwinkia salsa* (Pilsbry, 1905) (97% identity) EU573991 (Ponder et al. 2008). Based on objective clustering of five COI sequences (two from this study, the two top hits from GENBANK, and *S. salsa* AF367633 [Ponder et al. 2008]), two genetic clusters were formed. The Singapore individuals and the published *P. platyrachis* sequences from GENBANK formed one cluster, while *S. salsa* clustered differently, separated by a 6.6% divergence. For 16S, both sequences from this study clustered together, while the published sequence of *S. salsa* recorded a divergence value of 3%, similar to the BLASTN results. No other published *P. platyrachis* 16S sequences were available for comparison.

Habitat characteristics and density

The sites from which *P. platyrachis* were collected had the following environmental characteristics: 1) temperatures 29.55–30.48˚C; 2) pH 8.31–8.64; 3) dissolved oxygen 5.85–6.93mgL^{-1}; 4) conductivity 0.14–0.74mScm^{-1}; and 5) total dissolved solids 0.08 – 0.44gL^{-1}. The highest abundances of *P. platyrachis* occurred in muddy substrate but individuals were also found on submerged walls of floating structures (pontoons) in the reservoirs. The highest average *P. platyrachis* densities (individuals per coloniser) were observed in Pandan Reservoir (306.0±746.7 individuals), followed by Punggol Reservoir (109.7±194.5 individuals), and then Bedok Reservoir (0.3±0.8 individuals).

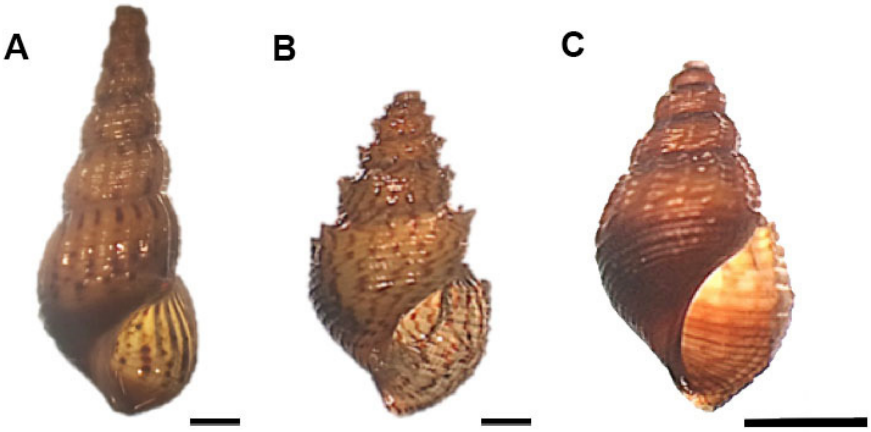

Figure 3. Juvenile Thiaridae commonly found in Singapore: A. *Melanoides tuberculata*, B. *Mieniplotia scabra*, C. *Tarebia granifera*. Scale bar = 1mm. Photographs by JZE Song.

Discussion

Species identification

Identifying members of *Pyrgophorus* is problematic, with populations introduced to the Middle East and Hawaii yet to be definitively identified to species level (Englund 2002; Mienis et al. 2011; Nasarat et al. 2014). The taxonomic confusion is exemplified by the identification of *P. parvulus* in Venezuela as a junior synonym of *P. platyrachis* by Nava et al. (2011) though others consider *P. parvulus* a valid species (Pointier 2015). Here we used a combination of morphological and molecular evidence to verify the identity of the present aquatic snail species belonging to a complex and taxonomically unresolved genus (Thompson 1968; Hershler and Thompson 1992) to confirm the first record of *P. platyrachis* outside the Americas. The three distinct shell morphs are typical of the genus (Hershler and Thompson 1992), and were similar to the morphs recorded from Florida (Thompson 2004). The top hits for the COI gene also supported the identity of the species as *P. platyrachis*. The close match of the 16S gene to the confamilial *S. salsa* is not unexpected given that interspecific variability can differ very widely, and nuclear markers would be required to better delimit the cochliopids (Meier 2008).

In-situ identification of newly introduced species can be difficult in the presence of morphologically-similar native or established species. *Pyrgophorus platyrachis* may have first been collected as early as 2013 (JHL pers. obs.), but were likely mistaken for juveniles of the native Thiaridae (Figure 3), which are common in Singapore's fresh waters (Tan et al. 2012). Thiarids here, however, can be easily distinguished from *Pyrgophorus* spp. by the presence of prominent shell sculpture, e.g., axial ribs and/or spiral grooves (versus absence of obvious shell sculpture in *Pyrgophorus*) (Figure 3), and incomplete peristome interrupted at the parietal area (versus the slightly detached and complete peristome seen in mature *Pyrgophorus* individuals) (Figure 2A). Cochliopidae are also superficially similar in size and shape to the globally-invasive New Zealand mud snail, *Potamopyrgus antipodarum* (not recorded in Singapore to date), and recently introduced cochliopids elsewhere have been mistakenly identified as the mud snail (Hershler et al. 2015). The quickest way to distinguish these species is by comparing morphs with spines—spines of *Potamopyrgus* are not calcified and are part of the periostracum, while spines of *Pyrgophorus* are protrusions of the shell itself (Winterbourn 1970; Mienis et al. 2011).

Habitat characteristics

We encountered the highest abundances of *P. platyrachis* at sites with muddy bottoms. This is consistent with the snail's typical habitat in its native range in Florida, where they are most commonly found on floating plants and in fresh waters with soft, muddy sediment, although some populations are known to occur in mangroves (Thompson 1968). While there appears to be no available environmental variables recorded from its native habitats in Florida for comparison, ecological studies have been

conducted on a congener, *P. parvulus*, in Venezuela (Nava and Severyn 2011 [as *P. platyrachis*]; Pointier 2015), and the physicochemical conditions of all the *P. platyrachis* collection sites in Singapore are within the pH and dissolved oxygen ranges of the sites in Venezuela (Nava and Severyn 2011). Our findings do not reflect the plasticity in habitat preference or tolerance of environmental variables sometimes reported in biological invaders.

Possible routes of introduction

We did not find evidence to shed more light on the introduction pathways of *P. platyrachis* into Singapore. Birds are known vectors of invasive snails (Hershler et al. 2015), but *P. platyrachis* has not been recorded anywhere else along the East Asia-Australasian Flyway, the path taken by migratory birds passing through Singapore (Wang and Hails 2007). Therefore, we postulate that the snails may instead have been accidentally introduced to Singapore via the ornamental trade, a key introduction pathway for aquatic plants and animals in Singapore (Yeo and Chia 2010). In both native and introduced habitats, *Pyrgophorus* species have been reported to be associated with floating macrophytes like water hyacinth *Eichhornia crassipes* (Mart.) Solms, and water lettuce *Pistia stratiotes* L. (Thompson 1968; Mienis et al. 2011; Nava et al. 2011)—both of which have been introduced into Singapore (Yeo et al. 2010). It is possible that the snails were inadvertently brought in with ornamental aquatic plants and introduced indirectly via indiscriminate disposal of aquarium water into the waterways (Duggan 2010), or planting of ornamental plants for landscaping purposes in the vicinity of water bodies.

Implications and conclusions

Owing to its propensity to be undetected because of its small size and unremarkable appearance, the actual introduced range of *Pyrgophorus platyrachis* outside its native distribution may be wider than current literature suggests. Moreover, even when noticed, the snails may be mistaken for another morphologically similar invasive species that is more widespread and well-known, or even juveniles of native species. Our study highlights the importance of rapid identification of potential invaders using a combination of morphological and molecular assessments as this, along with the ecology of the organism in adventive habitats, can be crucial in managing and preventing the further spread of biological invaders (Pyšek et al. 2013).

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References

Alonso Á, Castro-Díez P (2012) The exotic aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca): state of the art of a worldwide invasion. *Aquatic Sciences* 74: 375–383, http://dx.doi.org/10.1007/s00027-012-0254-7

Baiser B, Olden JD, Record S, Lockwood JL, McKinney ML (2012) Pattern and process of biotic homogenization in the New Pangaea. *Proceedings of the Royal Society* B 279: 4772–4777, http://dx.doi.org/10.1098/rspb.2012.1651

Bertolino S, Lurz PWW (2013) *Callosciurus* squirrels: worldwide introductions, ecological impacts and recommendations to prevent the establishment of new invasive populations. *Mammal Review* 43: 22–23, http://dx.doi.org/10.1111/j.1365-2907.2011.00204.x

Clavero M, García-Berthou E (2005) Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* 20: 110, http://dx.doi.org/10.1016/j.tree.2005.01.003

Clements R, Koh LP, Lee TM, Meier R, Li D (2006) Importance of reservoirs for the conservation of freshwater molluscs in a tropical urban landscape. *Biological Conservation* 128: 136–146, http://dx.doi.org/10.1016/j.biocon.2005.09.023

Dillon RT Jr., Watson BT, Stewart TW, Reeves WK (2006) The freshwater gastropods of North America. http://www.fwgna.org (Accessed 18 February 2016)

Duggan IC (2010) The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biological Invasions* 12: 3757–3770, http://dx.doi.org/10.1007/s10530-010-9768-x

Englund RA (2002) The loss of native biodiversity and continuing nonindigenous species introductions in freshwater, estuarine, and wetland communities of Pearl Harbor, Oahu, Hawaiian Islands. *Estuaries* 25: 418–430, http://dx.doi.org/10.1007/BF02695984

Galindo LA, Puillandre N, Strong EE, Bouchet P (2014) Using microwaves to prepare gastropods for DNA barcoding. *Molecular Ecology Resources* 14: 700–705, http://dx.doi.org/10.1111/1755-0998.12231

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome C oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources* 13: 851–861, http://dx.doi.org/10.1111/1755-0998.12138

Hershler R, Liu H-P, Carlton JT, Cohen AN, Davis CB, Sorensen J, Weedman D (2015) New discoveries of introduced and cryptogenic fresh and brackish water gastropods (Caenogastropoda: Cochliopidae) in the western United States. *Aquatic Invasions* 10: 147–156, http://dx.doi.org/10.3391/ai.2015.10.2.03

Hershler R, Liu H-P, Mulvey M (1999) Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American Southwest. *Molecular Phylogenetics and Evolution* 13: 377–391, http://dx.doi.org/10.1006/mpev.1999.0659

Hershler R, Thompson FG (1992) A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). *Malacological Review* (Supplement 5): 1–140

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18, http://dx.doi.org/10.1111/j.1365-2664.2008.01600.x

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780, http://dx.doi.org/10.1093/molbev/mst010

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology* 10: 34, http://dx.doi.org/10.1186/1742-9994-10-34

Loke L, Clews E, Low E, Belle CC, Todd PA, Eikaas HS, Ng PL (2010) Methods for sampling benthic macroinvertebrates in tropical lentic systems. *Aquatic Biology* 10: 119–130, http://dx.doi.org/10.3354/ab00274

McKinney ML (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation* 127: 247–260, http://dx.doi.org/10.1016/j.biocon.2005.09.005

Meier R, Kwong S, Vaidya G, Ng PKL (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728, http://dx.doi.org/10.1080/10635150600969864

Meier R (2008) DNA sequences in taxonomy: opportunities and challenges. In: Wheeler QD (ed), New Taxonomy. CRC Press, New York, pp 95–127, http://dx.doi.org/10.1201/9781420008562.ch7

Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. *Systematic Biology* 57: 809–813, http://dx.doi.org/10.1080/10635150802406343

Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* 79: 401–459, http://dx.doi.org/10.1046/j.1095-8312.2003.00197.x

Mienis HK, Rittner O, Vaisman S (2011) Another riddle from Israel: How can we explain the presence of a *Pyrgophorus* species in the Tanninim River Basin? *Ellipsaria* 13: 17–18

Moyle PB, Marchetti MP (2006) Predicting invasion success: Freshwater fishes in California as a model. *BioScience* 56: 515–524, http://dx.doi.org/10.1641/0006-3568(2006)56[515:PISFFI]2.0.CO;2

Nasarat H, Amr Z, Neubert E (2014) Two invasive freshwater snails new to Jordan (Mollusca: Gastropoda). *Zoology in the Middle East* 60: 46–49, http://dx.doi.org/10.1080/09397140.2014.892333

Nava M, Severeyn H (2011) Morphological variations in the shell of the gastropod *Pyrgophorus platyrachis* as a possible response to physical-chemical factors in the Maracaibo system, Venezuela. *Ecotrópicos* 24: 145–163

Nava M, Severeyn H, Machado N (2011) Distribución y taxonomía de *Pyrgophorus platyrachis* (Caenogastropoda: Hydrobiidae), en el Sistema de Maracibo, Venezuela. *Revista de Biologia Tropical* 59: 1165–1172

Nghiem LTP, Soliman T, Yeo DCJ, Tan HTW, Evans TA, Mumford JD, Keller RP, Baker RHA, Corlett RT, Carrasco LR (2013) Economic and environmental impacts of harmful non-indigenous species in Southeast Asia. *PloS ONE* 8: e71255, http://dx.doi.org/10.1371/journal.pone.0071255

Pointier JP (ed) (2015) Freshwater Molluscs of Venezuela and their Medical and Veterinary Importance. ConchBooks, Harxheim, 228 pp

Ponder WF, Wilke T, Zhang WH, Golding RH, Fukuda H, Mason, RAB (2008) *Edgbastonia alanwillsi* n. gen & n. sp. (Tateinae: Hydrobiidae s.l.: Rissooidea: Caenogastropoda); a snail from an artesian spring group in western Queensland, Australia, convergent with some Asian Amnicolidae. *Molluscan Research* 28: 89–106

Pyšek P, Hulme PE, Meyerson LA, Smith GF, Boatwright JS, Crouch NR, Figueiredo E, Foxcroft LC, Jarošík V, Richardson DM, Suda J, Wilson JRU (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AoB PLANTS* 5: plt042, http://dx.doi.org/10.1093/aobpla/plt042

Tan SK, Chan SY, Clements GR (2012) A Guide to Snails and Other Non-Marine Mollucs of Singapore. Science Centre Singapore, Singapore, 176 pp

Thompson FG (1968) The Aquatic Snails of the Family Hydrobiidae of Peninsular Florida. University of Florida Press, Gainesville, 268 pp

Thompson FG (2004) An Identification Manual for the Freshwater Snails of Florida. https://www.flmnh.ufl.edu/malacology/fl-snail/snails1.htm (Accessed 24 June 2015)

Wang LK, Hails CJ (2007) An annotated checklist of the birds of Singapore. *Raffles Bulletin of Zoology* (Supplement 15): 1–179

Wilke T, Davis GM, Falniowski A, Giusti F, Bodon M, Szarowska M (2001) Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. *Proceedings of the Academy of Natural Sciences of Philadelphia* 151: 1–21, http://dx.doi.org/10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

Winterbourn M (1970) The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia* 10(2): 283–321

Yeo DCJ, Chia CS (2010) Introduced species in Singapore: an overview. *COSMOS* 6: 23–37, http://dx.doi.org/10.1142/S0219607710000486

Yeo DCJ, Wang LK, Lim KKP (2010) Private Lives: An Exposé of Singapore's Freshwaters. The Raffles Museum of Biodiversity Research, Singapore, 258 pp

Zhan A, Bailey SA, Heath DD, MacIsaac HJ (2014) Performance comparison of genetic markers for high-throughput sequencing-based biodiversity assessment in complex communities. *Molecular Ecology Resources* 14: 1049–1059, http://dx.doi.org/10.1111/1755-0998.12254

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203–214, http://dx.doi.org/10.1089/10665270050081478

Supplementary material

The following supplementary material is available for this article:

Table S1. *Pyrgophorus platyrachis* collected from Singapore and deposited at the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2016/Supplements/BIR_2016_Ng_etal_Supplement.xls