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Research Article

Clarifying the identity of the long-established, globally-invasive *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae) in Singapore

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Abstract

The freshwater snail identified as *Physastra sumatrana* has been recorded in Singapore since the late 1980's. It is distributed throughout the island and commonly associated with ornamental aquatic plants. Although the species has previously been considered by some to be native to Singapore, its origin is currently categorised as unknown. Morphological comparisons of freshly collected specimens and material in museum collections with type material, together with DNA barcoding, show that both *Physastra sumatrana*, and a recent gastropod record of *Stenophysa spathidophallus*, in Singapore are actually the same species—the globally-invasive *Physa acuta*. An unidentified physid snail was also collected from the Singapore aquarium trade.

Key words: *Physastra sumatrana*, *Stenophysa spathidophallus*, taxonomy, DNA barcoding, alien species

Introduction

Changes in freshwater molluscan communities, including the introduction of alien species, have followed habitat modification (e.g., dam construction [Köhler et al. 2012]). In Singapore, urban habitats such as reservoirs and canals, which were mostly created or modified from existing rivers and streams within the past half a century, are the main habitats for freshwater molluscs (Clements et al. 2006; Tan et al. 2012). The pre-modified natural inland waters of Singapore were likely too acidic to sustain a high diversity of freshwater molluscs (Johnson 1967), and many species found in Singapore today have been introduced or are considered cryptogenic (i.e., of unknown origin) (Tan et al. 2012).

One species that was previously considered to be native (Clements et al. 2006), but whose origin is currently categorised as unknown (Tan et al. 2012), is *Physastra sumatrana* (Martens, 1897) of the family Planorbidae. It was first described from Indonesia, and is commonly found throughout Singapore among ornamental aquatic plants. In neighbouring Malaysia, the species was first recorded in the 1970's, and was suspected to have been introduced via the aquarium trade (Sullivan et al. 1977). A species that closely resembles *Physastra sumatrana*, *Stenophysa spathidophallus* Taylor, 2003 (Physidae), was described from Singapore, and first collected in 1985 (Taylor 2003). *Stenophysa spathidophallus* had not been collected in Singapore again since the original record, but was recently 'rediscovered' in a public park in Singapore (Ng and Tan 2013).

In this article, we clarify that the species thus far known as *Physastra sumatrana* in Singapore, and the recent report of *Stenophysa spathidophallus*, are in fact specimens of the globally-invasive *Physa acuta* Draparnaud, 1805.

Figure 1. Type specimen of *Physa acuta* (NHMW Mollusca 102796, coll. Draparnaud 1820.XXVI.45. Copyright of NHMW, photograph by A. Schumacher/NHMW).

Methods

Specimens deposited at the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum (formerly the Raffles Museum of Biodiversity Research), National University of Singapore, were examined. These consisted of specimens collected from various sites in Singapore and Malaysia, including material collected during recent freshwater surveys. Specimens obtained from the Singapore aquarium trade were also examined. Photographs of comparative material were obtained from the Naturhistorisches Museum, Wien (Vienna), Austria (NHWM), Museum of Comparative Zoology, Harvard University, Massachusetts, USA (MCZ), Universitat Humboldt, Museum fur Naturkunde, Berlin, Germany (ZMB), and Universitat Hamburg, Zoologisches Institut und Museum, Hamburg, Germany (ZMH).

Selected individuals were subjected to molecular analysis to confirm their identities. Total genomic DNA was extracted from foot tissue following a CTAB phenol-chloroform protocol. The DNA barcodes (mitochondrial CO1) were amplified in polymerase chain reactions (PCR) with LCO1490 and HCO2198 primers (Folmer et al. 1994). The PCR products were checked visually on a 1% agarose gel. Post PCR clean-ups were performed on successfully amplified products using SureClean reagent (Bioline Inc.). The purified products were sequenced with BigDye Terminator reactions and analysed on the ABI PRISM 3130XL sequencer (Applied Biosystems) at the DNA Sequencing Laboratory of the National University of Singapore. Sequence chromatograms were inspected visually and trimmed using Sequencher ver. 4.6 (Genecodes), and aligned using MAFFT version 7 (Katoh and Standley 2013) with default settings. DNA sequences were inspected using the objective clustering in SpeciesIdentifier version 1.7.9 (Meier et al. 2006), with a species delimitation threshold of 3% (Wethington et al. 2009). A BLASTN search (highly similar sequences [megablast]) was carried out on GenBank to identify the most likely species (Zhang et al. 2000). All sequences were deposited in GenBank (Accession numbers KP182981–KP182986).

Results

Fifty-nine lots of specimens from the ZRC (supplementary material Table S1) were examined and identified to be *Physa acuta* based on morphological comparison to the type specimen (Figure 1), and the original description (Draparnaud 1805). The examined material included three specimens from Malaysia, and one from the Singapore aquarium trade.

The earliest records of *Physa acuta* from Singapore in the ZRC (1989–1991) were initially identified as belonging to the morphologically-similar, but dextral, family Lymnaeidae. Our examination of the ZRC museum material revealed that four out of the nine ZRC lots, initially labeled as *Lymnaea* sp. or *Lymnaea rubiginosa*, contained labels showing that they were re-identified as *Physa acuta* by Wai Hoong Ho in 1995. However, this correct identification appears to have been overlooked, and subsequent lots of *Physa acuta* were almost all labeled as *Physastra sumatrana*, except for ZRC.MOL.5656 that was identified as *Stenophysa spathidophallus*. Based on the material from ZRC, *Physa acuta* is distributed throughout Singapore (Figure 2).

The mitochondrial COI fragment was successfully amplified for six individuals from the ZRC material—two from Singapore, one from the aquarium trade, and three from Malaysia. Topotypic material for *Physa acuta* (River Garonne, France, AY282589) was also included in the analysis (Albrecht et al. 2014). Based on objective clustering, the six sequences were shown to belong to two genetic clusters, separated by 21–22% divergence.

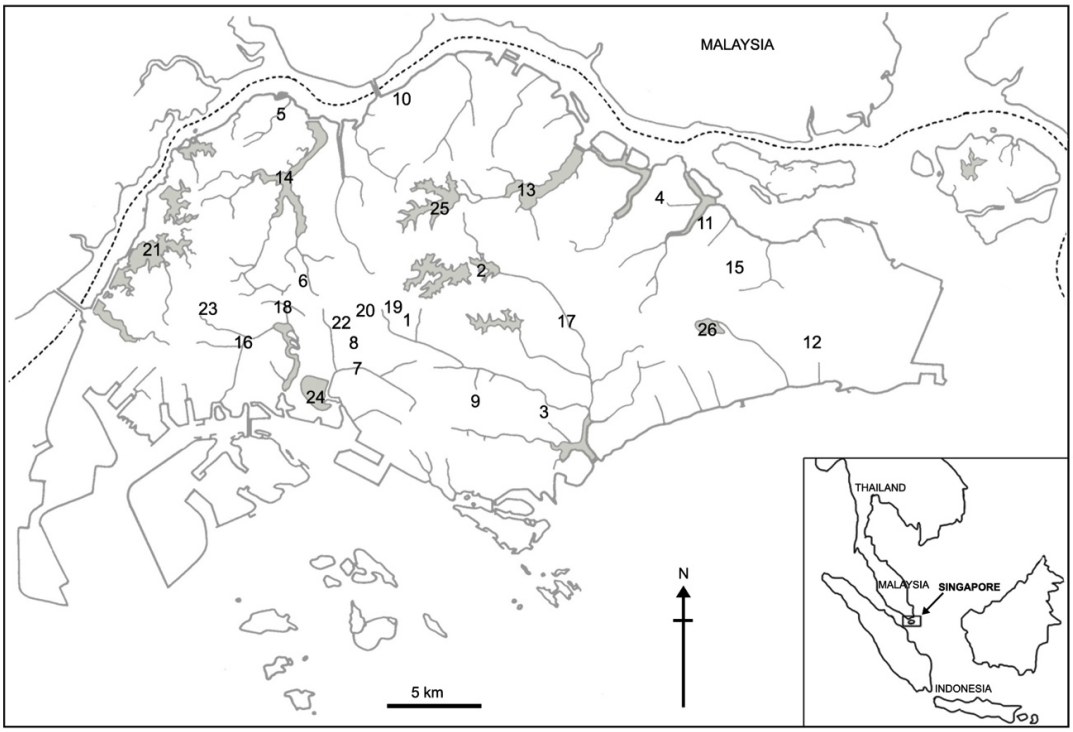

Figure 2. Map of Singapore showing approximate locations where *Physa acuta* has been collected from, based on ZRC material. 1. Hindhede Drive, 2. Lower Peirce Reservoir area, 3. Kampong Java, 4. Punggol, 5. Sungei Buloh area, 6. Sungei Tengah/Choa Chu Kang Avenue 3, 7. Sungei Pandan, Buona Vista area, 8. Clementi Road/Holland Woods, 9. Botanic Gardens, 10. Woodlands Town Park, 11. Lorong Halus area, 12. Upper Changi Road, 13. Lower Seletar Reservoir, 14. Kranji Reservoir area, 15. Pasir Ris Drive 1/Tampines Eco Green, 16. Jurong Central Park, 17. Kallang River, 18. Sungei Jurong, 19. Singapore Quarry, 20. Bukit Batok Town Park, 21. Western Catchment reservoirs, 22. Bukit Batok West Avenue 5, 23. CleanTech Park, 24. Pandan Reservoir area, 25. Upper Seletar Reservoir, 26. Bedok Reservoir.

The BLASTN search confirmed that one genetic cluster (the two Singapore individuals from Jurong Central Park [Figure 3C, ZRC.MOL.5751, KP18 2986] and CleanTech Park [Figure 3D, ZRC.MOL.5761, KP182983], and two individuals from the Malaysian states of Negeri Sembilan [ZRC.MOL.5777, KP182984] and Perak [ZRC. MOL.5778, KP182985]) are *Physa acuta*. The topotypic material clustered with these individuals.

Although all examined material were initially identified as *Physa acuta* based on morphology, the individual from the aquarium trade (Figure 4A, ZRC.MOL.5773, KP182981) and one individual from Malaysia (ZRC.MOL.5776, KP182982) belonged to the other genetic cluster, and did not have any close matches on GenBank. The closest match was *Physa acuta* at 78% identity. Although the morphology of both specimens is similar to *Physa acuta*, the molecular analysis suggests that they may in fact represent a currently unsequenced species of Physidae.

Discussion

The common, sinistral freshwater gastropod in Singapore, previously known as *Physastra sumatrana* (e.g., Clements et al. 2006; Tan and Woo 2010; Tan et al. 2012), is now confirmed to be *Physa acuta*. Blakely et al. (2010) had identified a *Physa* species to be present in Singapore, but the species continued to be regarded as *Physastra sumatrana* (e.g., Tan et al. 2012). Based on the original description (Martens 1897) and on type material (Figure 4B), *Physastra sumatrana* has never been collected from Singapore and all records of it to date (Clements et al. 2006; Tan and Woo 2010; Tan et al. 2012) are very likely misidentifications. The recent record of *Stenophysa spathidophallus* by Ng and Tan (2013; ZRC.MOL. 5656) was reexamined in this study, and based on morphology, is also considered an erroneous identification; it should instead be *Physa acuta*.

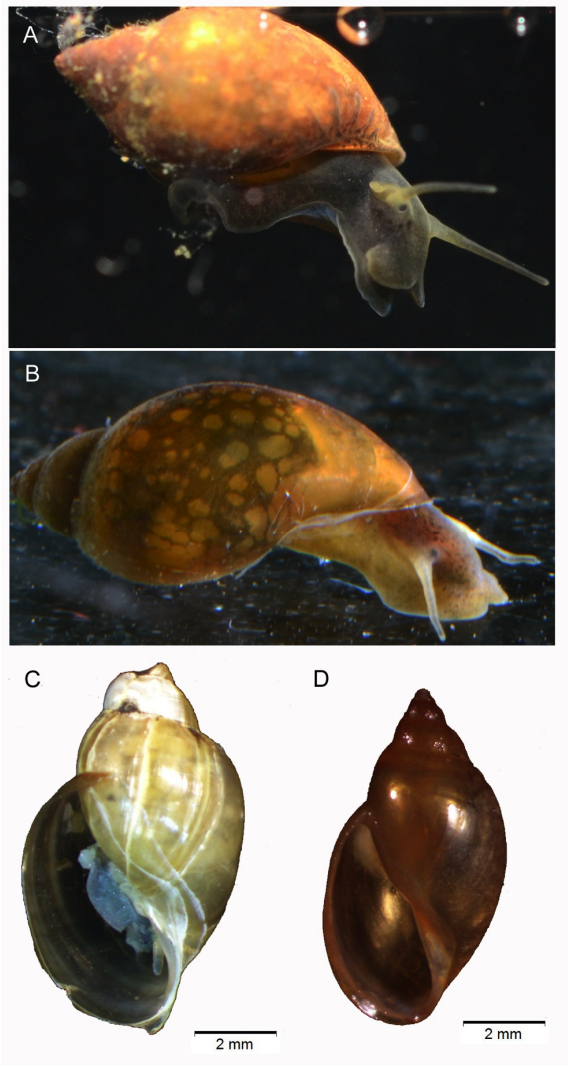

Figure 3. *Physa acuta* from Singapore—A. and B. live animals, C. collected from Jurong Central Park (from the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, ZRC.MOL.5751) and D. CleanTech Park (ZRC.MOL. 5761) (photographs by TH Ng).

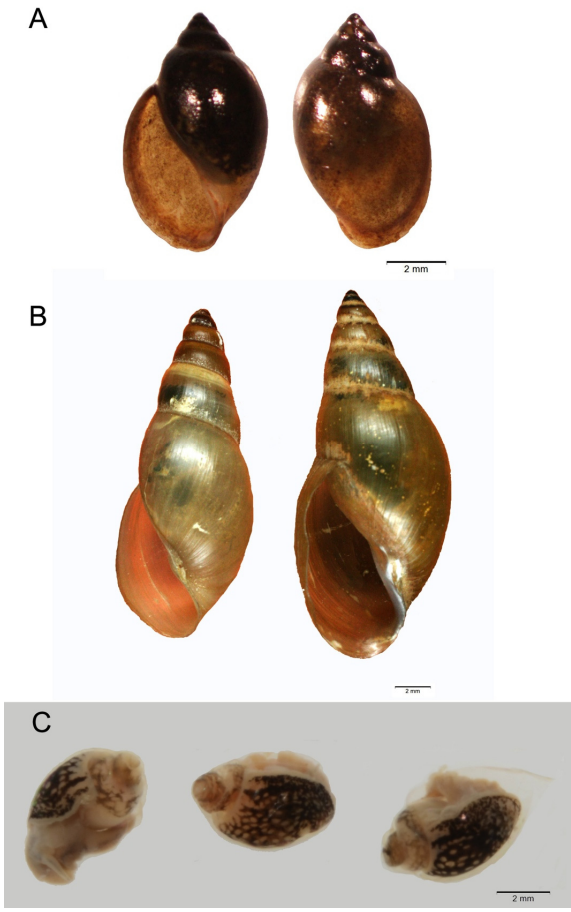

Figure 4. A. Physidae sp. collected on 17 June 2014, from a Singapore aquarium shop (ZRC.MOL.5773) (photographs by TH Ng). B. Paratypes of *Physastra* sumatrana (from the Museum fur Naturkunde, Berlin, Germany, ZMB104535, coll. M Weber. Copyright of ZMB, photograph by C Zorn/ZMB). C. Specimens collected from Selangor, Malaysia in 1977 (from the Zoologisches Institut und Museum, Hamburg, ZMH16984, leg. JR Palmieri, coll. R Brandt) (photograph by TH Ng). Fragile condition of shells likely owing to initial preservation in formalin (JR Palmieri, pers. comm.).

Physastra sumatrana was first recorded from Malaysia in the 1970's (Sullivan et al. 1977), but the snails that were collected during that study and later lodged at ZMH were examined and also appear to be *Physa acuta* (Figure 4C).

Based on the material in the ZRC, *Physa acuta* was first collected from Singapore in 1989 (ZRC.1991.19309–19342). Despite early attempts by Wai Hoong Ho to correctly identify this introduced species, *Physa acuta* had been repeatedly misidentified and was even once presumed to be native to Singapore (as *Physastra sumatrana* in Clements et al. 2006). The initial introduction was almost certainly earlier, as *Physa acuta* was already observed on aquatic plants in home aquariums in the mid-1980's (second author pers. obs.). The aquarium trade is a main introduction pathway of aquatic snails, and is the most likely source of *Physa acuta* to multiple countries, including Singapore (Madsen and Fransen 1989; Appleton 2003; Tan et al. 2012 [as *Physastra sumatrana*]). Singapore is a global hub for the aquarium trade, and many freshwater species have been introduced via this pathway (Yeo and Chia 2010). *Physa*

acuta has to date been collected from ornamental aquatic plants in landscaped gardens as well as from the reservoirs and canals (supplementary material Table S1), and also found as hitchhikers in home aquariums (unpublished data). The unidentified physid sequenced in this study was collected from the pot of an ornamental aquatic plant being sold at a local aquarium shop. As ornamental aquatic plants are both cultivated locally in Singapore and imported (e.g., http://www.orientalaquarium.com/oo_singapore.html), it is uncertain if this unidentified species originated from a local or foreign population.

The family Physidae, native to the Americas with the highest diversity in North America, has been introduced worldwide (Wethington and Lydeard 2007). *Physa* species have been recorded in Southeast Asia from Malaysia (Ali 1993; Hill et al. 1997), Indonesia (Mienis 2005), Vietnam (Madsen and Hung 2014), and Brunei (first author pers. obs.). Although *Physa acuta* is a globally-invasive species (Madsen and Frandsen 1989; Zukowski and Walker 2009), besides its distribution in Malaysia (Ali 1993), nothing is known about its ecology and impacts in this region. Although shells of *Physa* species can be highly plastic depending on the environment (Gustafson et al. 2014), our genetic data from the Singapore aquarium trade and from Malaysia suggest that there may be more than one species of Physidae introduced to the region (unpublished data).

In introduced ranges in South Africa and Australia, *Physa acuta* has been shown to have various biological advantages over native species (e.g., higher fecundity and temperature tolerance), potentially impacting native communities (Brackenbury and Appleton 1991; Zukowski and Walker 2009). Further investigation needs to be carried out to determine the impacts of *Physa acuta* on other species, especially in the Southeast Asian region where it may overlap ecologically with native species belonging to the genera *Lymnaea* and *Physastra*. Unfortunately in Southeast Asia, basic information (e.g., ecology and distribution) on many native species is sorely lacking (Köhler et al. 2012). The globally-invasive *Physa acuta* species has established and has been spreading throughout Singapore for the last two decades. As a result of being repeatedly misidentified, the impacts of its invasion remain completely unknown, and this highlights the urgency for increasing research efforts into the malacofauna of this region.

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Physa acuta* material examined from the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore, Sungei/Sungai—River.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2015/Supplements/BIR_2015_Ng_etal_Supplement.xls