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Rapid Communication

First non-native establishment of the carnivorous assassin snail, *Anentome helena* (von dem Busch in Philippi, 1847)

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Abstract

Anentome helena (von dem Busch in Philippi, 1847) is known among aquarium enthusiasts as the "assassin snail", and is usually kept to prey on other snail species that are considered pests in home aquaria. There have been concerns that, given its prevalence in the ornamental pet trade, it is only a matter of time before this predator is introduced to the wild. We report the first occurrence of established populations of *Anentome helena* in a non-native habitat, and provide CO1 sequences of the species from the wild.

Key words: introduced species, gastropod, freshwater, predator, Singapore

Introduction

Anentome helena (von dem Busch in Philippi, 1847) (Nassariidae, formerly Buccinidae [see Galindo et al. 2016]) is known among aquarium enthusiasts as the "assassin snail", and is usually kept to prey on other snail species that are considered pests in home aquaria (Monks 2010). It was first described from Java, Indonesia (Philippi 1847), and has since also been recorded from Cambodia (Crosse and Fischer 1876; Fischer 1891), Thailand (Fischer 1891; Brandt 1974), Vietnam (Fischer 1891; Madsen and Hung 2014), Laos (Dautzenberg and Fischer 1908; Vongsombath et al. 2009; Sri-Aroon et al. 2015; Attwood and Cottet 2015), Sumatra in Indonesia (van Benthem Jutting 1956, 1959), and northern Peninsular Malaysia (Chan 1997). In Thailand, the species has been known to be a scavenger as well as an active predator of worms and other snails (Brandt 1974).

Predatory invasive species have been known to cause significant, devastating damage to native fauna (Pitt and Witmer 2007). For instance, widespread introduction of the predatory rosy wolf snail, *Euglandia rosea* (Férrusac, 1821), for the purpose of controlling the invasive giant African snail, *Achatina fulica* Bowdich, 1822, resulted in the decline of native, endemic snails instead (Cowie 2001). Owing to its potential to harm native fauna if introduced into the wild, the prevalence of *Anentome helena* in the ornamental pet trade—a known introduction pathway for non-native snails (Mackie 1999; Ting Hui Ng et al., unpublished data)—has been a subject of concern to biologists (Mienis 2011; Bogan and Hanneman 2013).

The ornamental pet trade is suspected to be the source of many non-native species in Singapore (Yeo and Chia 2010; Ng et al. 2015). A high proportion of the country's freshwater snails, found almost exclusively in urban, disturbed habitats, are likely to have been introduced through various pathways including the ornamental trade (Clements et al. 2006; Tan et al. 2012). While *Anentome helena*

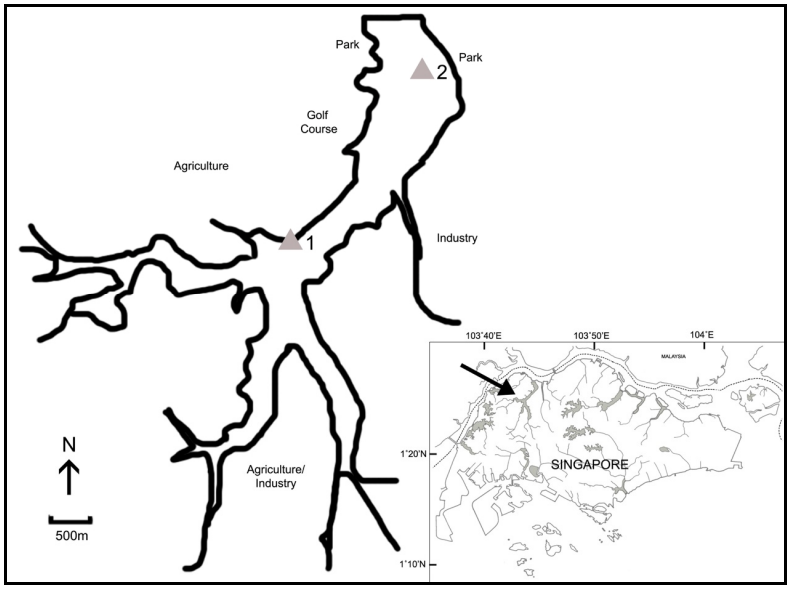

Figure 1. Sites where *Anenetome helena* was recorded from at Kranji Reservoir, indicated by triangles: 1. first record on 24 March 2016; 2. observation on 27 March 2016 (see supplementary Table S1 for localities). Inset: arrow indicates location of Kranji Reservoir in Singapore.

is distributed in the region surrounding Singapore, it has never been reported from the island nation itself despite various surveys being conducted over the past 15 years (Clements et al. 2006; Clews et al. 2014; Peter KL Ng et al. unpublished data), and therefore is not regarded as part of the native malacofauna. We report the first record of *Anentome helena* in Singapore, which is also the first record of the species in a non-native habitat.

Methods

Anentome helena was collected from Kranji Reservoir, a coastal reservoir formed in 1975 by the damming of the mouth of the Kranji River (Ng et al. 2011) on the north coast of Singapore (Figure 1, supplementary Table S1). The reservoir catchment area consists of mixed land use areas, i.e., residential estates, agricultural and industrial areas, and undeveloped land (Te and Gin 2011). Specimens were first found on 24 March 2016 among the roots and stems of water spinach, *Ipomoea aquatica* Forsskål 1775, which grows along the banks of the reservoir, and were deposited in the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore (Catalogue number ZRC.MOL.6450). On 27 March 2016, *Anentome helena* was recorded from another location in the reservoir, approximately 2.5 km away from the first site (Figure 1 and 2B). An empty shell was collected from the same site on 3 May 2016. Physicochemical variables—water temperature (°C), pH, dissolved oxygen (mgL^{-1}), conductivity ($mScm^{-1}$), and total dissolved solids (gL^{-1})—were measured at the collection sites using a YSI Professional Plus handheld multiparameter meter (YSI Inc.).

Ten specimens from Kranji Reservoir were examined and identified based on morphology by comparing with the original description (Philippi 1847), images of the lectotype (Knipper 1958), and material from the ZRC. For molecular validation, we also extracted total genomic DNA from the foot tissue of selected individuals from Kranji Reservoir and the ornamental pet trade using QuickExtract™ DNA Extraction Solution (Epicentre), following the manufacturer's protocol. The mitochondrial COI gene was amplified in polymerase chain reactions (PCR) with degenerate primers dgLCO1490 5'–GGTCAACAAATCATAA AGAYATYGG–3' and dgHCO2198 5'–TAAACTT CAGGGTGACCAAARAAYCA–3' (Meyer 2003), and sequenced. 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 a BLAST search (highly similar sequences [megablast]) (Zhang et al. 2000) in GenBank and in BOLD to compare to closely related species. Finally, the DNA sequences obtained were inspected using objective clustering based on uncorrected distances in SpeciesIdentifier version 1.7.9 (Meier et al. 2006), at 2%, 3%, and 4% thresholds

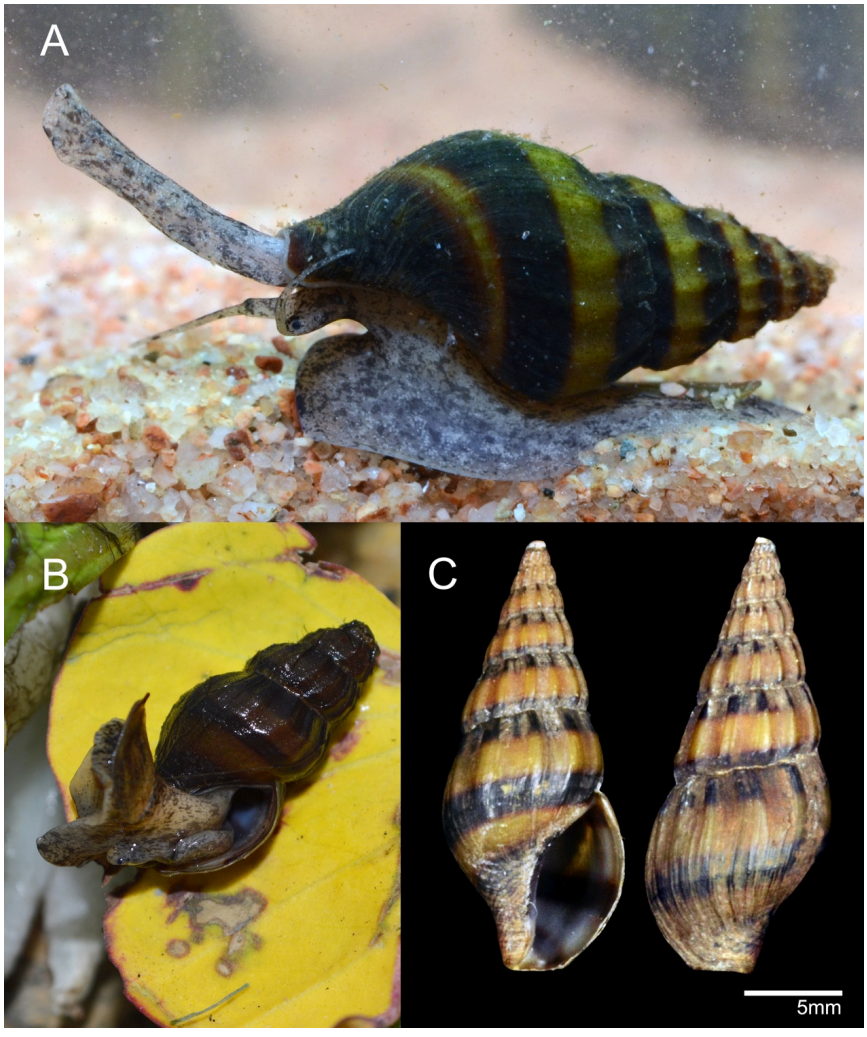

Figure 2. *Anentome helena* from Kranji Reservoir (A and B), and from the ornamental pet trade (C). Photographs by TH Ng (A and C) and Mark KK Chan (B).

following Meier et al. (2008). Objective clusters are groups of sequences that have at least one other sequence below the threshold. Voucher sequences were deposited in GenBank (Accession numbers KU318329, KX132092).

Results

Material examined. — *Anentome helena*: ZRC.1975.2.18.72–84 Sungai Chuping, Perlis, Malaysia, March 1936; ZRC1990.11473–11474 Kangar, Perlis, Malaysia, 31 July 1957; ZRC.MOL.6447 Ayutthaya, Thailand, 16 July 2012; ZRC.MOL.6448 Beseri, Perlis, Malaysia, 2 May 14; ZRC.MOL.6449 Kota Bharu, Kelantan, Malaysia, 4 Aug 2014; ZRC.MOL.5919 Singapore ornamental pet trade, 10 June 2014; ZRC.MOL.6304 Singapore ornamental pet trade, 19 June 2014; ZRC.MOL.6305 Singapore ornamental pet trade, 23 August 2011; ZRC.MOL.6306 Singapore ornamental pet trade, 9 December 2011; ZRC. MOL.6450 Kranji Reservoir, Singapore, 24 March 2016; ZRC.MOL.6494 Kranji Reservoir, Singapore, 3 May 2016.

Conchological description. — Shell elongately conic, to more than 20 mm in shell height, dextral. Whorls 7–8, somewhat convex, sculptured with axial ribs that become indistinct at the anterior part of the body whorl. Aperture oval, outer lip slightly thickened in mature specimens; siphonal canal short and wide. Shell straw-coloured with dark brown bands, usually 3 on body whorl (Figure 2).

Genetic identification. — COI was successfully sequenced from five specimens from Kranji, and three from the ornamental pet trade. The top hits on GENBANK and BOLD were matched to confamilial (Nassariidae) marine species at < 90% identity. The

eight sequences revealed three haplotypes with pairwise distances of 0.00%–3.24%. All Kranji sequences belonged to a single haplotype, and were 0.2%–0.3% distant genetically from the ornamental pet trade specimens, which were represented by two haplotypes.

Habitat characteristics. — The sites from which *Anentome helena* were collected had the following environmental characteristics: temperatures 30.5–31.6 ºC; pH 7.11–7.29; dissolved oxygen 1.17–2.75 mgL^{-1}; conductivity 0.2555–0.2610 $mScm^{-1}$; and total dissolved solids 0.1475–0.1534 gL^{-1}.

Discussion

The record of *Anentome helena* in Singapore is the first occurrence of the species in the wild from a non-native habitat. Despite its widespread distribution in Southeast Asia, too little is known about *Anentome helena* to accurately distinguish natural from human-mediated distribution, but its non-native status in Singapore is certain because 1) there have never been suitable natural habitats in the past (Johnson 1967; Yeo and Lim 2011; see below), 2) it has never been found in historical records nor detected in recent intensive surveys (Clements et al. 2006; Tan and Woo 2010; Tan et al. 2012; Clews et al. 2014; Peter KL Ng et al. unpublished data), and 3) it has established in a human-modified habitat (Crees and Turvey 2015). The Kranji individuals shared a single mitochondrial CO1 haplotype that was 0.2–0.3% genetically-different from specimens obtained from the ornamental pet trade in 2014. Given that the catchment area of Kranji Reservoir includes housing estates and farms (including major ornamental pet distributors), it is highly likely that *Anentome helena* was introduced into the reservoir as escapees from the ornamental pet trade, or via improper disposal of aquarium water or plants. *Anentome helena* first appeared in the Singapore ornamental pet retail shops in early 2008 (SKT pers. obs.), and is still commonly sold at less than US$1 per individual (THN pers. obs.).

Although *Anentome helena* has been recorded from Peninsular Malaysia (north of Singapore), the species appears to be restricted to northern regions (>700 km away from Singapore; see Material examined, Chan 1997). It has never been found from the east and south of the peninsula (closest to Singapore), where at least two other species of *Anentome* have been recorded instead (Adams 1861; Basch and Solem 1971). *Anentome helena* appears to be the only species of its genus that is found in both lotic habitats (rivers and streams) as well as lentic habitats (freshwater ponds and lakes) in the Indo-Burmese region and Indonesia (van Benthem Jutting 1956; Brandt 1974). In contrast, other species of *Anentome* are found only in fast-flowing streams (Basch and Solem 1971; Brandt 1974; JKF pers. obs.).

While the species seems to be more tolerant of a wider range of conditions compared to its congeners, the natural distribution of *Anentome helena* may be limited by the lack of hard water habitats (e.g., limestone streams) in the southern Malay Peninsula (including Singapore) (Johnson 1967; Chan 1997). The natural fresh water habitats (forest streams and freshwater swamp) of Singapore are largely soft and acidic in nature (Johnson 1967; Yeo and Lim 2011). Conversely, many present-day urban freshwater habitats, including reservoirs and man-made canals, have hard waters and higher pH (Clements et al. 2006; Yeo and Lim 2011; Clews et al. 2014). The urban freshwater environments, including Kranji Reservoir where *Anentome helena* was found, are the main habitats for freshwater molluscs (Clements et al. 2006; Tan et al. 2012). Many species in these disturbed habitats are believed to have been introduced, especially via the ornamental pet trade (Ng et al. 2015; Ting Hui Ng et al., unpublished data).

At least seven other species of freshwater molluscs have been recorded previously from Kranji Reservoir based on intensive surveys over the past 15 years, but no *Anentome helena* was detected before the present records (Clements et al. 2006; Tay 2013; Clews et al. 2014; Peter KL Ng et al. unpublished data). Thus, the time of introduction of *Anentome helena* into Kranji Reservoir could not be more than five years ago. Its natural diet has not been studied in detail, but ex-situ experiments and observations by aquarists have recorded *Anentome helena* actively hunting and preying on smaller-sized Planorbidae, Physidae, and Thiaridae species (van Benthem Jutting 1956; Monks 2010; Coelho et al. 2013; Newel and Bourne 2013), and even larger snails in the Ampullariidae and Viviparidae (Mienis 2011; Bogan and Hanneman 2013). Thus, the other species of freshwater snails in Kranji Reservoir, including juveniles of the rare apple snails *Pila scutata* (see Tan et al. 2013), could be potentially at risk if the spread of *Anentome helena* remains unchecked.

As it appears that *Anentome helena* has only recently been introduced into Kranji Reservoir, it is imperative that further rapid surveys be conducted to investigate the extent of its distribution in Singapore and potential impact on native fauna. Too little is known about its ecological requirements in a natural setting (Coelho et al. 2013). In any case, the popularity of *Anentome helena* as a form of biological control in home aquaria worldwide, could mean multiple potential sources of introduction (i.e., irresponsible aquarists).

This could result in high propagule pressure and frequency, which could in turn lead to more extensive establishment and further spread (Lockwood et al. 2005). Since the invasion in Singapore seems to be at an early stage of introduction and localised establishment, further local studies of *Anentome helena* may help to prevent introductions and inform future eradication or management strategies elsewhere (Simberloff et al. 2013).

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Anentome helena* material collected from Singapore.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2016/Supplements/BIR_2016_Ng_etal2_Supplement.xls