

Rapid Communication**First record of the worm snail *Eualetes tulipa* (Rousseau in Chenu, 1843) in Southeast Asia**Siong Kiat Tan¹, Rebecca K. Loh² and Ting Hui Ng^{1,*}¹Lee Kong Chian Natural History Museum, Faculty of Science, National University of Singapore, 2 Conservatory Drive, 117377, Singapore²Department of Biological Sciences, Faculty of Science, National University of Singapore, 14 Science Drive 4, 117543, SingaporeAuthor e-mails: nhmtsk@nus.edu.sg (SKT), dbsrlk@nus.edu.sg (RKL), nhmnth@nus.edu.sg (THN)

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OPEN ACCESS**Abstract**

The Vermetidae is a family of sessile marine gastropods whose members are difficult to identify accurately, but one species, *Eualetes tulipa* is widely documented as an introduced species globally. In Asia, there is only one known record of this species from India to date. We report the presence of *E. tulipa* in Singapore based on DNA barcoding. This is the second known record from Asia and the first confirmation of a non-native vermetid for Southeast Asia.

Key words: alien species, DNA barcoding, establishment, Gastropoda, Vermetidae, Singapore**Introduction**

The Vermetidae is a family of sessile marine gastropods that live permanently cemented on, or embedded in, hard substrata after larval settlement and are characterised by their worm-like shells. The shell growth and direction of the distal ends may frequently change in response to damage, water currents, and obstacles (Schiaparelli and Cattaneo-Vietti 1999), resulting in their typically irregularly or haphazardly coiled appearance. The inherent high variability of their shell shape and characteristics make taxonomic identification challenging (Bieler and Petit 2011; Golding et al. 2014).

Despite problems with proper species identification, one vermetid species has been widely documented in recent years. *Eualetes tulipa* (Rousseau in Chenu, 1843) has been recorded from both sides of the Americas in Panama, Florida, Costa Rica, Venezuela and Brazil (Keen 1971a, b; Miloslavich and Penchaszadeh 1992; Rawlings et al. 2010; Cortés 2012; Spotorno-Oliviera et al. 2018; CABI 2020; Wells and Bieler 2020), in the Pacific Ocean in Hawaii (Coles and Eldredge 2002) and the Indian Ocean in Tamil Nadu, India (Jebakumar et al. 2015). The type locality of *E. tulipa* remains unknown, but the species is considered to be introduced to Hawaii, Brazil and India, based on a number of factors, including the perception that it is native to Panama where it is known since the early 1800's, its relatively recent

appearance in local habitats and its occurrence in predominantly human-disturbed areas or on man-made structures along the coast (see Spotorno-Oliviera et al. 2018).

Vermetids are known from Southeast Asia but remain largely understudied. The genera *Dendropoma*, *Petalconchus* and *Thylacodes* have been recorded from Thailand and Myanmar (Oo 2019; Wells et al. 2021), while most recent records from Singapore have been identified as *Vermetus* sp. (Tan and Chou 2000; Tan and Woo 2010; Tan and Yeo 2010; Chou et al. 2020), with the exception of a single record that was identified as *Serpulorbis* sp. (Chuang 1973). No vermetid species have been recorded as being introduced in Southeast Asia to date.

Despite being an international shipping hub, Singapore has an unexpectedly low number of introduced marine species – only 22 species have been recorded to date (Wells et al. 2019). Of these, only four species of molluscs have been recognised as non-native species that are established in Singapore (see Wells et al. 2019). In this article, we add a new record of an introduced marine mollusc to Singapore by confirming the establishment of *E. tulipa* here, which also appears to be the first confirmation of its presence in Southeast Asia.

Materials and methods

We examined vermetid specimens deposited at the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore, including material from the Comprehensive Marine Biodiversity Survey of Singapore (see Tan et al. 2016). Morphological characters were compared to figures within Chenu (1843), and descriptions in Keen (1971b), and Hadfield et al. (1972, as *Vermetus alii*, a widely accepted junior subjective synonym [see Golding et al. 2014; Spotorno-Oliviera et al. 2018]).

We obtained a DNA barcode of the mitochondrial *c* oxidase gene (COI) from one *E. tulipa* specimen from a next-generation sequencing project, which aimed to build a local DNA barcode database for the marine gastropods of Singapore (Loh 2016). A fresh sample of foot tissue was obtained from an individual collected off St John's Island in 2013 and subsequently stored in cryogenic conditions at the ZRC. We performed DNA extraction using the QuickExtract DNA Extraction Solution (Epicentre), according to the manufacturer's protocol, followed by DNA amplification and sequencing according to the methods of Meier et al. (2016) and Yeo et al. (2018). In short, the primers mlCO1intF (5'-GGWACWGGWTGAACWGTWTAYCCYCC-3') and jgHCO2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3') (Geller et al. 2013; Leray et al. 2013) were used to amplify a 313-bp fragment of COI. Successful amplifications were verified by gel electrophoresis and PCR products were pooled and purified using SureClean (Bioline) according to the manufacturer's instructions. The final sample was

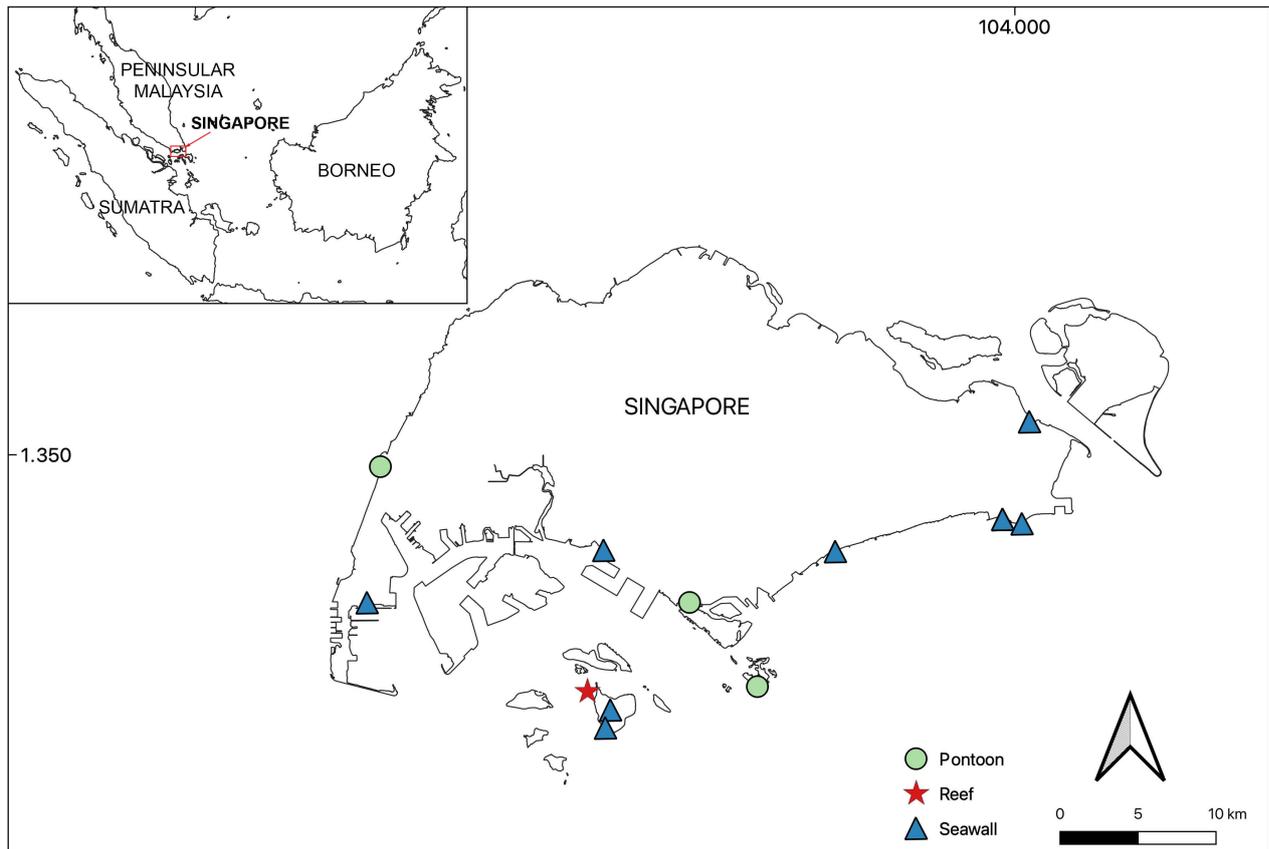


Figure 1. Records of *Eualetes tulipa* in Singapore from material deposited in the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore according to habitat type: pontoon (circle), reef (star) and seawall (triangle). Inset shows location of Singapore within Southeast Asia.

submitted to AITbiotech (Singapore) for DNA library preparation and sequencing on an Illumina Miseq platform (Illumina Inc., San Diego, CA, U.S.A.) using a 2×300 -bp paired end kit.

Raw paired-end reads were merged and put through a bioinformatics pipeline as described in Yeo et al. (2018). After a series of quality checks, we obtained the final COI barcode for the vermetid specimen and conducted a BLAST search against NCBI GenBank to identify similar sequences. The COI barcode is deposited in GenBank (accession number MW29053).

Results

Morphological identification

Fourteen lots of specimens from the ZRC were examined and identified as *E. tulipa* from various intertidal coastal sites in Singapore (Figure 1; Table 1). The species was identified by the following characters: Shell white with variable brown streaks, with a maximum apertural diameter of around 11 mm; juvenile shells darker and uniformly reddish brown; distal ends of large specimens always white; cancellate sculpture present on much of earlier whorls, often only a few fine radial ribs present on latter whorls that disappear towards the distal ends, which are usually sculptured with growth lines; early coils usually somewhat wedge-shaped on flatter and somewhat

Table 1. *Eualetes tulipa* from Singapore deposited in the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore.

Museum Accession Number	Locality	Latitude	Longitude	Collection Date	Habitat Type
ZRC.MOL.20342	East Coast Park, Fort Road, on rocky bund	1.293878	103.896495	27 September 1992	Seawall
ZRC.MOL.6732	Tanah Merah Ferry Terminal, on rock at seawall	1.312577	103.992910	27 January 1998	Seawall
ZRC.MOL.15459	Changi, Tanah Merah Ferry Terminal, attached on breakwater rocks	1.310001	104.004040	27 January 1998	Seawall
ZRC.MOL.15462	Tanah Merah Ferry Terminal, among rocks at seawall	1.312577	103.992910	5 December 1998	Seawall
ZRC.MOL.7549	Tanah Merah Ferry Terminal, on breakwater rock	1.312577	103.992910	26 October 1999	Seawall
ZRC.MOL.15461	Tanah Merah Ferry Terminal	1.312577	103.992910	4 June 2000	Seawall
ZRC.MOL.20285	Tuas, Raffles Marina	1.343137	103.634443	November 2000	Pontoon
ZRC.MOL.13159	Tuas	1.264191	103.626711	1 March 2005	Seawall
ZRC.MOL.12445	Tanah Merah Ferry Terminal	1.312577	103.992910	18 June 2006	Seawall
ZRC.MOL.12519	Changi, North Bay	1.369216	104.008317	13 August 2006	Seawall
ZRC.MOL.20424	Semakau Landfill, Phase 2, on muddy rocks at rock bund and on rocks on muddy sand areas	1.201867	103.766945	2–3 March 2011	Seawall
ZRC.MOL.20343	Keppel Bay Marina, subtidal	1.264302	103.812551	July 2012	Pontoon
ZRC.MOL.20341	Pulau Semakau Phase 2	1.191460	103.764041	6 August 2012	Seawall
ZRC.MOL.16624	Terumbu Raya, submerged reef	1.212617	103.753783	30 May 2013	Reef
ZRC.MOL.20425	St John's Island, Lagoon pontoon, under pontoon/fouling agents	1.215467	103.85165	30 May 2013	Pontoon
ZRC.MOL.8538	West Coast Park	1.294601	103.763021	17 October 2016	Seawall

vertical surfaces before straightening with the distal ends rising above the substrate. Coils of lone individuals on more or less horizontal surfaces tend to be rather planorboid in outline. Head-foot generally purplish red (Figure 2A); foot and cephalic tentacles purplish pink, cephalic tentacles and anterior-ventral region smattered with pale yellow or white spots; eyes small, black, near base of cephalic tentacles; border of mantle collar purplish pink, fading to pale pinkish white posteriorly, resulting in a distinct reddish tint around the rim of the aperture in living examples. Operculum circular, shallowly concave, corneous (Figure 2B).

Molecular analysis

The top match to our vermetid barcode on GenBank was to *Eualetes tulipa* (99.63% identity and 100% query cover) from Florida, USA (GenBank accession number HM174254, Rawlings et al. 2010), while the second closest-match, was to *E. tulipa* (89.3% identity and 85% query cover) from Tamil Nadu, India (GenBank accession number KP241014, Jebakumar et al. 2015).

Discussion

Our confirmation of the presence of *Eualetes tulipa* in Singapore increases the total number of non-native marine molluscs to five species. Most of the other introduced species have been recorded from artificial marine habitats, similar to the localities where *E. tulipa* has been observed. Incidentally, *Eualetes tulipa* is also one of the few non-native molluscs recorded from southern Florida, which, similar to Singapore, has a relatively low number of established non-native molluscs (see Wells and Bieler 2020). *Eualetes tulipa* appears to be well-established and is the largest and

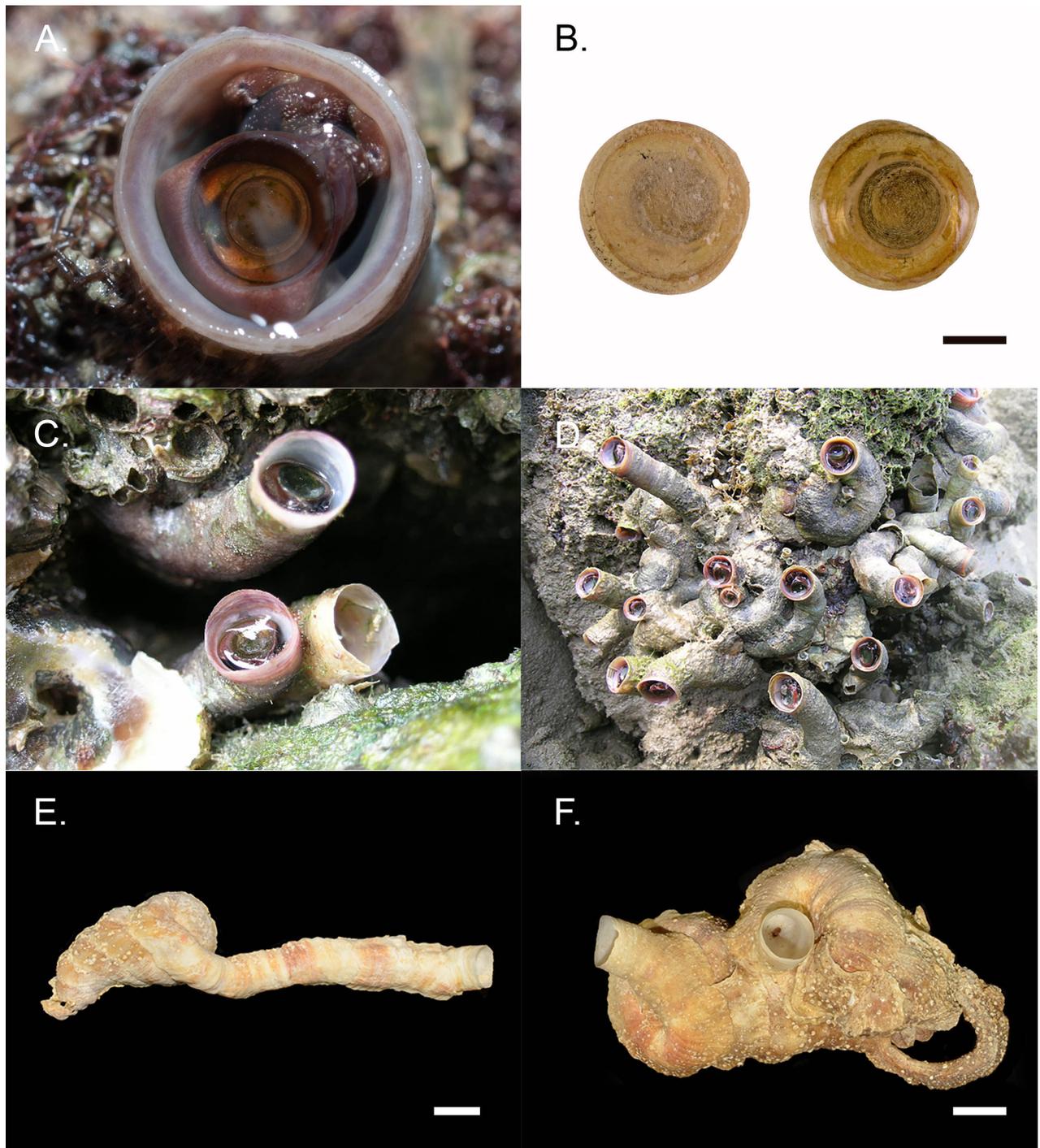


Figure 2. *Eualetes tulipa* from Singapore: A. close-up of the head-foot region; B. exterior (left) and interior (right) views of the operculum; in-situ at C. Tuas and D. Pulau Semakau; specimens from Tanah Merah Ferry Terminal, Changi, ZRC.MOL.15459 (E, F). Photographs by Ria Tan (A), Iffah Iesa (B) and Siong Kiat Tan (C–F). Scale bars = 1 mm (B); 10 mm (E, F).

predominant vermetid on seawalls and amongst fouling communities on pontoons in marinas in Singapore (Figure 1; SKT *unpubl. data*). The species tends to occur in clusters, but it is not uncommon to see lone individuals in the general vicinity (Figure 2).

Prior to the 1990s, no vermetid species corresponding to *Eualetes tulipa* had been deposited in the ZRC. The presence of vermetids in Singapore only appeared in publications from 2000 onwards, which were all identified

as *Vermetus* sp. (Tan and Chou 2000: 81 [1 unnumbered figure]; Tan and Woo 2010: 40; Tan and Yeo 2010: 294; Chou et al. 2020: 38). Based on our examination of museum specimens, the earliest verifiable record of the species in Singapore is based on material collected in 1992 (Table 1). It is therefore possible that *E. tulipa* has been present in Singapore for around three decades.

Based on our findings, the species recorded as *E. tulipa* and known to be introduced world-wide (Spotorno-Oliveira et al. 2018; CABI 2020), may in fact be more than one species. While our COI sequence was a match to the specimen from Florida (Rawlings et al. 2010), the uncorrected *p*-distance was more than 10% when compared to the sequence that was identified as *E. tulipa* from India (Jebakumar et al. 2015). In order to resolve the true identity of *E. tulipa* and obtain sufficient information to detect further instances of introduction within Southeast Asia, an integrated approach to combine molecular and morphological analyses of material from across its current cosmopolitan distribution would be necessary.

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