



## RESEARCH NOTE

# A study of a population of *Canarium (Canarium) urceus* (Mollusca, Strombidae) from Nha Trang, Vietnam

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The Molluscan family Strombidae Rafinesque, 1815 is taxonomically diverse, with a widespread global distribution. However, the population structure of many of these taxa remains enigmatic. There is a growing interest in exploring population structures within species across their distributions to understand factors affecting morphological diversity. In particular, there is an inherent focus on sexual-size dimorphism between females and males in both physiology and shell morphometrics, as well as sexual bias (Mutlu 2004; Maxwell et al. 2017, 2020a, 2021), in order to provide a basis from which models of sexual dimorphism in general might be generated. Furthermore, the expression of pseudohermaphroditism in stromboideans, which is linked to the environmental causal agent tributyltin (TBT), a marine pollutant, has also been the focus of recent studies, with varying findings in expression ranging from absent to 36 % within populations (Mutlu 2004; Maxwell et al. 2020a; Reed 1993a, 1993b; Ruaza 2019). In addition, another population dynamic that has received recent attention is the expression of colour phenotypes within and between populations (Maxwell et al. 2020a, 2021). Despite these recent studies and the development of a general model for sexual dimorphism (Maxwell et al. 2022), many stromboideans still remain unstudied in terms of morphological variability, sex-ratio bias and pseudohermaphroditism, particularly at the population level.

*Canarium (Canarium) urceus* (Linnaeus, 1758) is one of these overlooked species. Until recently, this species has been used as a “holding name” for much of the *Canarium (C.)* complex (Abbott 1960; Kreipl 1999; Liverani 2014). However, recent revisions have provided a more solid taxonomic footing for the species (Maxwell et al. 2020b), and this has resulted in many of the species that had been included within the synonymy of *C. (C.) urceus* being recircumscribed and validated (Maxwell et al. 2020c), or being separated out, resulting in the circumscription of new species (Dekkers & Maxwell 2020; Maxwell & Dekkers 2021a, 2021b; Dekkers et al. 2022). These recent revisions mean that prior studies claiming to have worked on *C. (C.) urceus* have been on sister taxa (*C. (C.) esculentum* Maxwell, Rymer, Congdon & Dekkers, 2020; Ruaza 2019).

The range of *C. (C.) urceus* extends from Taiwan along the coast of Vietnam into the islands at the edges of the central Gulf of Thailand, and then to Singapore (Maxwell et al. 2020b). The northern Gulf of Thailand, with high drainage from rivers such as the Chao-Phraya and the mighty Mekong delta

system, is a biozone with freshwater inflows that affects sea temperatures and variations in salinity and is not suitable for this species.

This paper has four aims to inform on the population characteristics of *C. (C.) urceus* from Nha Trang, Khánh Hòa Province, Vietnam. First, we assessed sexual dimorphism in axial shell length, which has been explored in other stromboideans (Maxwell et al. 2020a, 2021). Second, we assessed whether there was a sex-ratio bias, a phenomenon variable in this family (Maxwell et al. 2017, 2020a, 2021). Third, we determined the ratio of aperture colour morphology between different colour forms, which is largely unexplored in most stromboideans (Maxwell et al. 2020a). Fourth, we assessed whether there was evidence of pseudohermaphroditism in this species, which provides an indication of potential environmental pollution (Maxwell et al. 2020a).

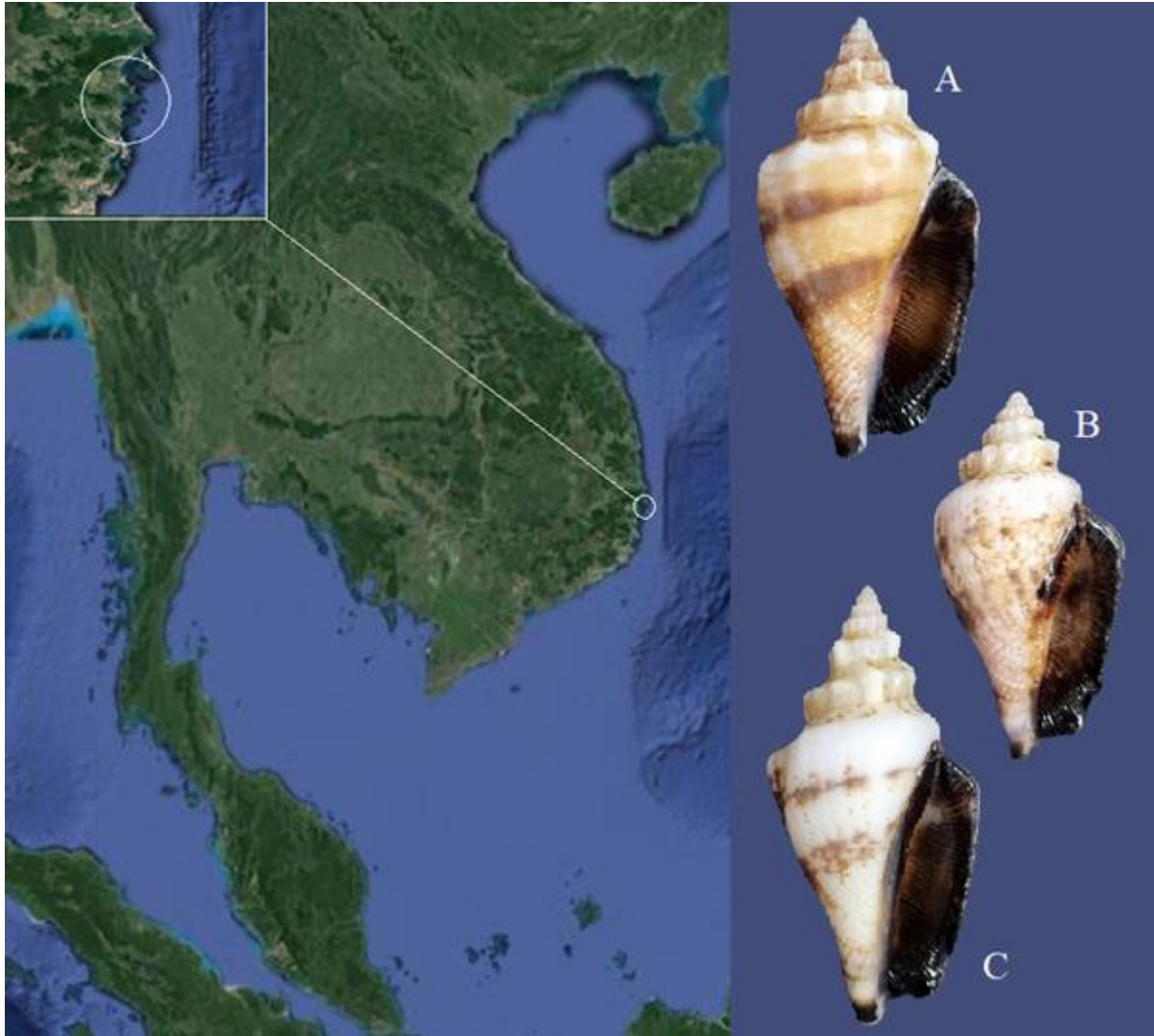
In March 2021, we contracted local shell dealers to obtain a sample of *C. (C.) urceus* from one fisherman from Nha Trang, Khánh Hòa Province, Vietnam (12°11'N 109°14'E; Fig. 1). *C. (C.) urceus* are collected regularly for domestic consumption and sold at local markets as “Ốc nháy môi đen”. The markets of Nha Trang are supplied with fresh seafood from the nearby islands, making the identification of the exact collection site of the material impossible. Therefore, we only purchased samples off one boat, which also avoids the mixing of samples from other locations on sales tables in the markets. After collection, specimens were placed in alcohol and shipped to James Cook University, Cairns, Australia, following the required customs approvals and clearances.

Once in Australia, conformation of the identity of studied material was carried out using the type of *C. (C.) urceus* (Maxwell et al. 2020b) and comparing the material with the types of that species sister taxa (Maxwell et al. 2020c; Dekkers & Maxwell 2020; Maxwell & Dekkers 2021a, 2021b; Dekkers et al. 2022). Once the identity was confirmed, specimens were sorted, and the animals were removed from the shells. Adult specimens can be differentiated from juveniles by the terminal growth at maturity represented by the shell characteristics of a thickened outer lip, colouration of the aperture, and the development of a columella callous. The following information was then documented for each specimen: total axial length, colouration of the aperture, sex (presence of a verge indicated a male, presence of an oviduct indicated a female) and presence or absence of pseudohermaphroditism (recognised by the presence of a deformed vestigial verge on females (Reed 1993a, 1995). Shells were catalogued into the Stephen Maxwell Collection (SMC) for future reference.

The SPLUS v 2007 (Insightment Corp.) package was used to undertake statistical analyses. Size statistics (mean  $\pm$  SE) were calculated and the maximum and minimum of each sex noted. Significant differences in body size between the sexes were tested using an independent-sample t-test. The sex ratio was tested using an exact binominal test with a hypothesised proportion of 0.5. The ratio of aperture colours was calculated as a percentage of the total sample, and the presence of pseudohermaphroditism was simply recorded as either present or absent.

The sample contained only adults (total:  $n = 37$ ; females:  $n = 22$ ; males:  $n = 15$ ; dead:  $n = 5$ ). Females were significantly larger than males ( $t_{2,35} = 3.14$ ,  $p = 0.0034$ ; females =  $40.83 \pm 0.51$  mm, maximum = 43.47 mm, minimum = 36.84 mm; males =  $38.12 \pm 0.73$  mm, maximum = 41.11 mm, minimum = 34.09 mm). More females were collected than males (1:0.68), but this difference was not statistically significant ( $p = 0.1755$ ;  $\alpha = 0.95$ ). All individuals in the sample presented a black aperture. There was no evidence of pseudohermaphroditism in visible genitalia.

As for other populations of stromboideans (Abbott 1949, 1960; Reed 1993a; Mutlu 2004; Maxwell et al. 2017, 2020a), we also found sexual size dimorphism in the axial length of the shell in this population, with females being statistically larger than males. Sexual size dimorphism in favour of females is a symplesiomorphic state in stromboideans, with the expression of this phenomenon established in fossil taxa (Geary & Allmon 1990).



**Figure 1.** Nha Trang, Khánh Hòa Province, Vietnam, and a sample of the *Canarium (Canarium) urceus* (Linné, 1758) collected. Image modified from Google Earth, accessed August 2021. **A.** Female, 42 mm (SMC 19.009a). **B.** Male, 39 mm (SMC 19.009a). **C.** Female, 43 mm (SMC 19.009c).

There was a no significant sex ratio bias in this population, although more females were observed in the sample. There is often a level of non-significant sex ratio bias in stromboidean samples (Maxwell et al. 2020a). The determination and expression of sex ratio bias in stromboidean populations is affected by the type of clustering that the sample is drawn from (Brownell 1977; Catterall & Poiner 1983; Maxwell 2017). While there were no juveniles in the sample, the lack of information on mating pairs makes the type of adult cluster impossible to discern.

All specimens presented a black columella and aperture, which contrasts other members of *Canarium* that only intermittently express this phenotype. In particular, *C. (C.) anatellum* (Duclos, 1844) from Indonesia, and *C. (C.) esculentum* and *C. (Elegantum) radians* (Duclos, 1844) from the Philippines all have atypical specimens within their populations that exhibit the dark colouration of the columella and aperture (Kreipl et al. 1999; Raven 2002; Liverani 2014). There are three possible causes of this colour anomaly in this population. Firstly, it may simply be a consequence of a lag in divergence and complete morphological differentiation (Baum et al. 2009). Secondly, the trait has become fixed within the population (Baum et al. 2009). Thirdly, the interconnectivity of bioregions means that genetic exchange seeds surrounding species populations through hybridisation (Delgado et al. 2006; Paris et al. 2006, 2008), which is not uncommon in the stromboideans (Maxwell et al.

2019). More re-research is needed to establish which of these three hypotheses best explains the expression of this morphological colour trait.

There is a high degree of variability in the expression of pseudohermaphroditism in the stromboideans, with no evidence found in the target species. This contrasts with studies on its sister taxon *C. (C.) esculentum* (Ruaza 2019). There are three reasons why pseudohermaphroditism was not detected (Maxwell et al. 2020a): the causal agent TBT is absent; the relatively small sample size could indicate that specimens were missed, particularly if pseudohermaphroditism is rare; or pseudohermaphroditism is genuinely absent in this population. We suggest that pseudohermaphroditism does not occur in this population. Typically, individuals exhibiting pseudohermaphroditism are larger than normal females (Reed 1993a; Cardenas et al. 2005), but we did not observe any larger female outliers, nor did we observe any vestigial penile structures on the bodies of females.

Here, we provide further insights into the population dynamics of *C. (C.) urceus* and support that stromboideans are sexually dimorphic, with females being larger than males. As for other studies on this species, we found no significant sex ratio bias in favour of either sex, although we found marginally more females in the sample. Unlike other populations of *C. (C.) urceus* that exhibit a black aperture in some specimens, all individuals in the sample exhibited the black aperture colouration. No pseudohermaphroditism was observed, indicating that the presence of this phenomenon is likely highly variable between stromboidean populations, and may generally be rare over-all. This study adds to the growing body of literature illustrating the considerable morphological diversity of *C. (C.) urceus* in particular, and stromboidean molluscs more broadly.

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