

## Quantifying shell pattern and colour polymorphism in the button snail *Umbonium vestiarium* (Mollusca: Gastropoda: Trochacea) and comparing morph frequencies between two populations using the Mantel test

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**Abstract.** The button snail, *Umbonium vestiarium*, is a small intertidal snail with a wide geographic range. It displays a high degree of phenotypic polymorphism in shell pattern and colour, but this is poorly researched. To examine morph frequencies, we collected 2845 empty shells and 207 live individuals from Tanah Merah and Changi beaches in Singapore. From these, two 2-dimensional matrices of morph types were constructed. The ‘Comprehensive Matrix’ classifies individuals based on 22 distinct band designs (which differ based on the presence, number and continuity of bands) and nine whorl designs (which differ based on marking colour and type: pink or brown uniform, dotted, wavy-lined, striped and with or without stripes on the lowest whorl). In total, 96 different morphs were observed. These morphs were then pooled into morph-groups to form a 5 × 5 ‘Condensed Matrix’ that represents a useful approach for quickly categorising live individuals and comparing morph frequencies among populations. Using the Mantel test on the Condensed Matrices, morph frequencies for the two populations studied here were statistically compared and found to be similar (p-values of 0.001 for empty shells and 0.01 for live snails). Together, these two matrices form a basis for future investigation into the mechanisms behind the maintenance of phenotypic polymorphism in *Umbonium vestiarium*.

**Key words.** *Umbonium vestiarium*, polymorphism, morph frequency, Mantel test, pattern, Singapore

### INTRODUCTION

Phenotypic polymorphism (herein referred to as ‘polymorphism’) occurs when two or more morphs (i.e., phenotypic variants of the same species that differ, for example, in shape, colour and pattern) occupy the same habitat at the same time within a panmictic population (Ford, 1965; Leimar, 2005). The many different potential colours and patterns on the shell of the terrestrial grove snail, *Cepaea nemoralis*, is a classic example of polymorphism that has attracted a large amount of research as a model organism (see, for example, Cain & Sheppard, 1950; Cain et al., 1960; Jones et al., 1980; Cook, 1998; Ozgo, 2004; Mann & Jackson, 2014). These and other studies have helped to shed light on the mechanisms that maintain polymorphism in different organisms.

In most cases, both genetic and environmental factors are responsible for the development and maintenance of

polymorphism (Leimar, 2009). *Cepaea nemoralis* shell patterns, for example, are genetically controlled by a complex super-gene system: a group of closely-linked genes at various loci (Cain et al., 1960; Jones et al., 1980). Allele series at each locus determine whorl colour (with brown being the dominant colour, yellow the recessive colour, and pink intermediate to the two; Ozgo, 2004), and separate modifier genes control the presence and number of bands through epistasis (Cain et al., 1960; Jones et al., 1980). Environmental factors, such as differential predation pressure due to crypsis and ‘search image effect’ (i.e., the tendency of a predator to disproportionately target morphs that are more common and that they have encountered before), exert selection pressures which result in negative frequency-dependent selection, thereby maintaining rare morphs and promoting a polymorphic population (Cain & Sheppard, 1950; Cain & Sheppard, 1954; Cain & Currey, 1963; Cain & Currey, 1968).

Despite decades of work there is still much to be done in the study of polymorphism, including developing techniques to characterise morph types objectively and to quantify levels of crypsis (e.g. Todd et al., 2005); even for the relatively well-studied *Cepaea nemoralis*, knowledge gaps still exist (Cameron & Cook, 2012; Jones et al., 1977; Mann & Jackson, 2014). Furthermore, most polymorphism research to date has been conducted in temperate regions and many tropical and sub-tropical species are poorly studied if studied at all. The button snail, *Umbonium vestiarium*, is one such

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example. This species is a small intertidal and shallow-subtidal archaeogastropod snail found on sandy coasts from the Mediterranean (Barash & Danin, 1972) eastwards through the Suez Canal to the coasts of Arabia, the Indian subcontinent, Southeast Asia (Kalayanasundaram et al., 1974), and Japan (Tamaki & Kikuchi, 1983). *Umbonium vestiarius* is anecdotally known to display a wide variety of shell patterns and colours, but no published study has examined this high degree of phenotypic variation and the reasons behind it (i.e., its genome and the various environmental forces that may exert selection pressures on morphs). Hence, it is an excellent potential tropical model species for studying shell pattern polymorphism: a marine equivalent of *Cepaea nemoralis*.

As the first step to understanding the mechanisms that maintain the shell polymorphism seen in *Umbonium vestiarius*, the main objective of this study was to identify and categorise the different morphs found in Singapore. The second objective was to develop a technique to quickly, easily and non-destructively classify live *Umbonium vestiarius* into different morphs using a simple morph identification matrix, and to subsequently statistically compare morph frequencies in different populations. For this comparison, the utility of the Mantel test (Mantel, 1967; Mantel & Valand, 1970) was assessed. If successful, the matrices and Mantel test would form a useful framework to aid future studies on *Umbonium vestiarius* and potentially similar research on other species. To achieve these two objectives, two studies were conducted: one on empty shells and one on live samples.

## MATERIAL AND METHODS

**Empty shells.** Empty *Umbonium vestiarius* shells were collected from two sites where populations had previously been reported by Tan (2008) through the WildSingapore website: Tanah Merah Beach (001.316°N, 103.973°E) on the south-east coast of Singapore and Changi Beach (001.384°N, 104.002°E) on the north-east coast, on 30 January 2013 and 1 February 2013 respectively (Fig. 1). At each site, samples were collected from near the high tide line when a high tide had just started to recede. To equalise sampling effort, sampling areas measuring 50 × 2 m<sup>2</sup> were demarcated at both sites and all shells visible on the surface of the sand within each area were collected. Shells newly deposited by waves in areas that had already been searched and shells buried beneath the surface of the sand were not collected.

Samples were cleaned, examined and sorted. Excessively broken or faded shells (i.e., those where the shell pattern and colouration could no longer be discerned) were discarded. The remainder were classified into morphs based on two criteria: band design (lines that follow shell sutures) and whorl design (Fig. 2). Due to the continuous nature of the variation in these patterns, the classifications were necessarily arbitrary. The frequency of each morph at each site was then recorded in a 2-dimensional Comprehensive Matrix with different band designs on the vertical axis and different whorl designs on the horizontal axis. The designs in the Comprehensive Matrix were subsequently pooled to create a

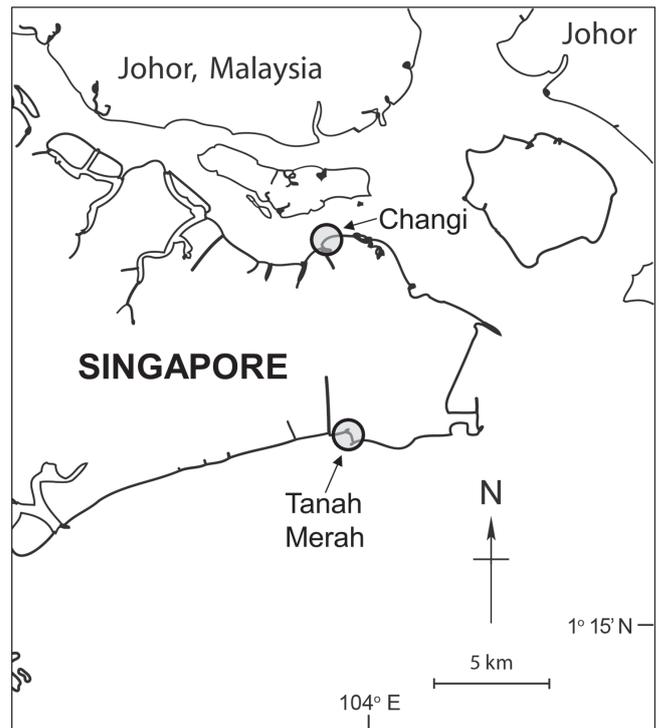


Fig. 1. Location of sampling sites.

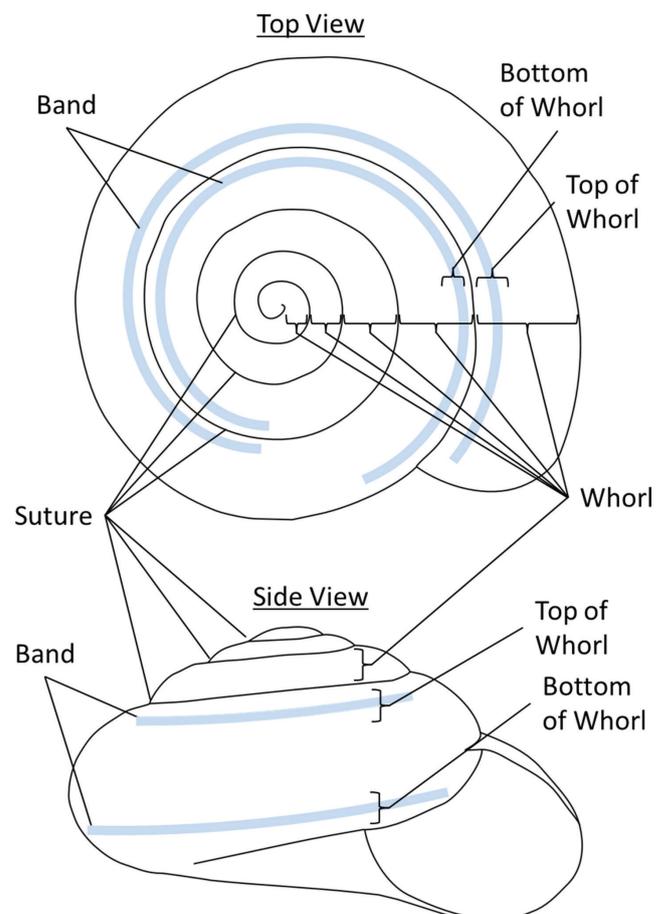


Fig. 2. Illustration of the terms used to describe different aspects of *Umbonium vestiarius* shell patterns.

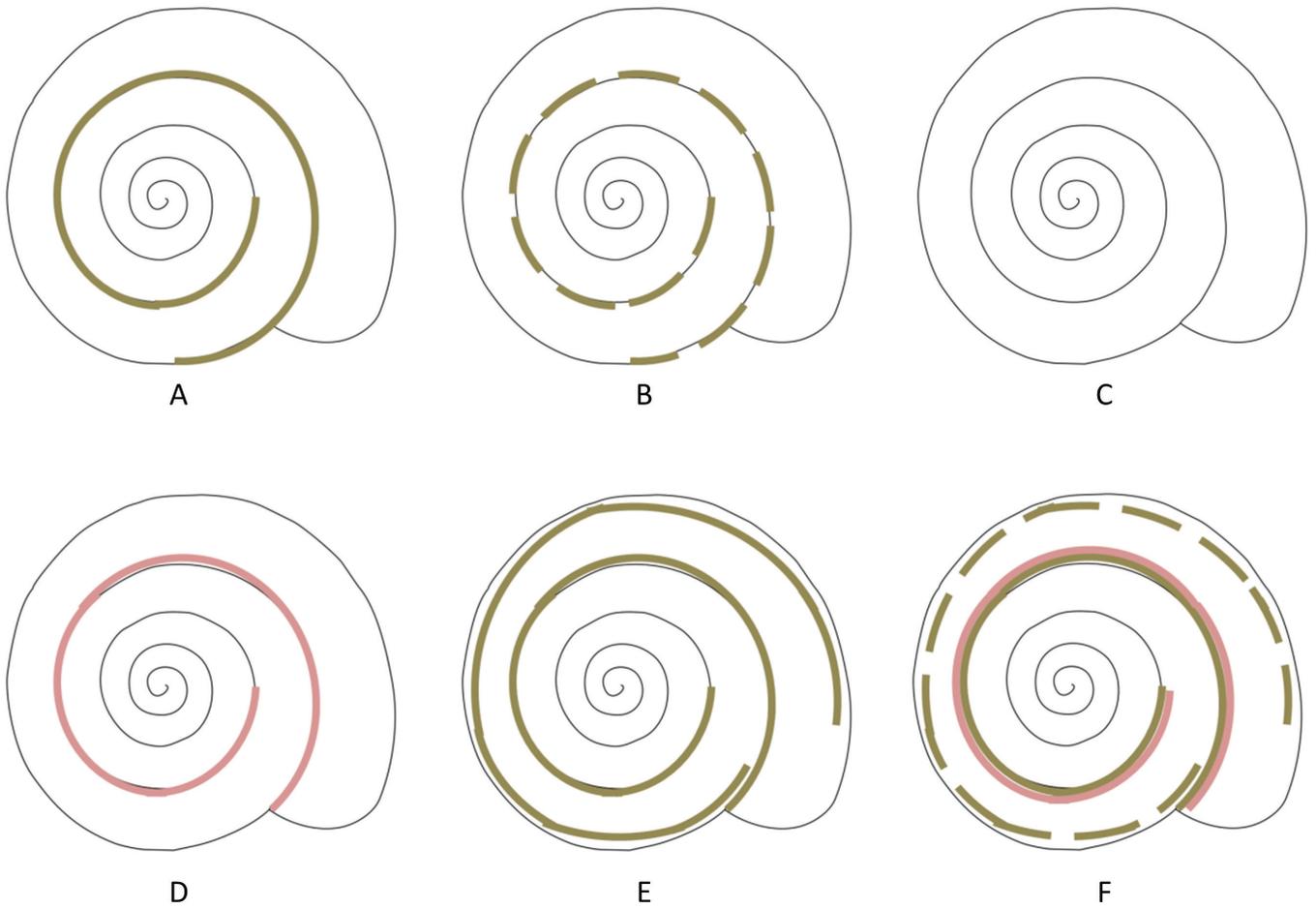


Fig. 3. Illustration of the different types of band designs observed. Example A has a brown solid band, B has a brown dashed band, and C has no band; D has a pink solid band, and E depicts an example with two brown solid bands, one at the top and one at the bottom of each whorl (band shown is truncated); F depicts an example with three bands, one solid brown, one solid pink at the top of whorls, and one dotted brown at the bottom.

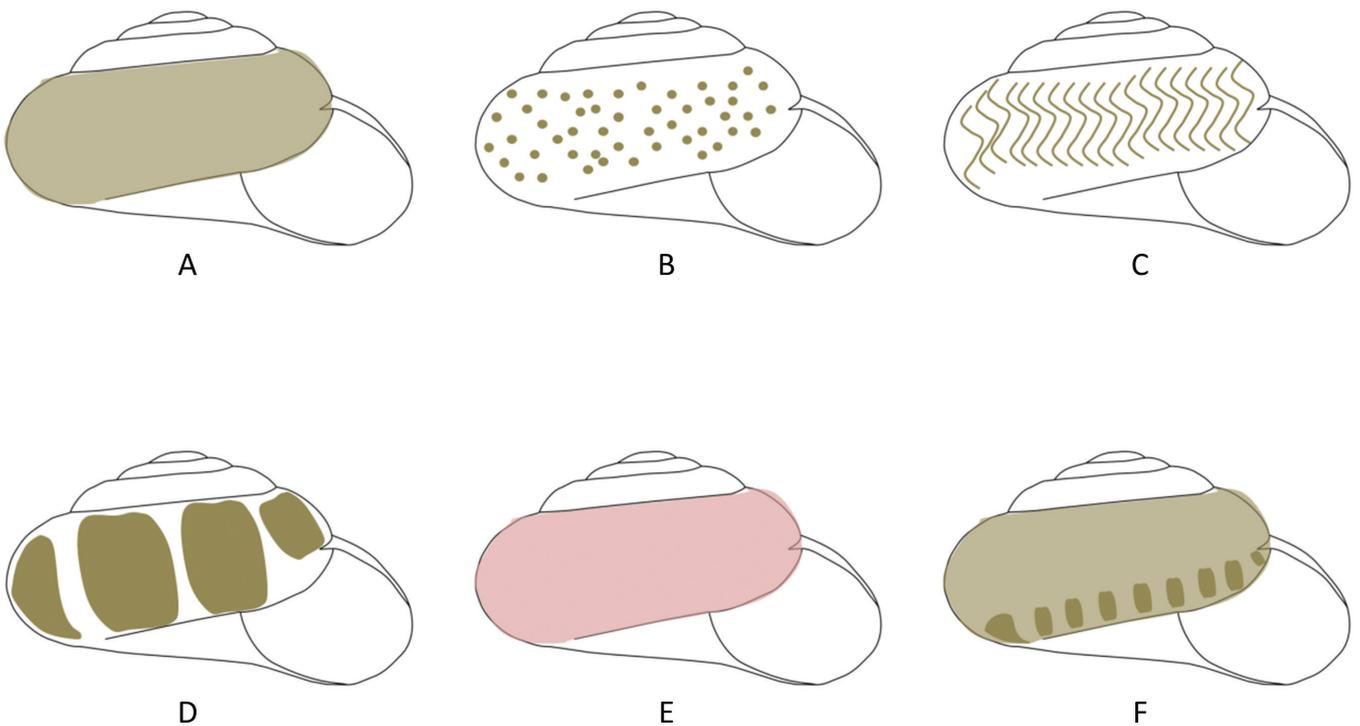


Fig. 4. Illustration of the different types of whorl designs observed. Example A is a brown uniform design, B is a brown dotted design, C is a brown wavy-lined design, D is a striped design, E depicts a pink uniform design, and F depicts a brown uniform design with stripes at the bottom of the lowest whorl.

Condensed Matrix that fulfilled two criteria: (1) the different band and whorl designs allow quick and easy identification in the field; and (2) there are equal numbers of band and whorl designs (the Mantel test requires a square matrix). The condensed matrices for the two sites were then statistically compared using the Mantel test (10,000 permutations) with the Vegan package in R (Oksanen et al., 2013).

**Live snails.** Live button snails were collected at the same two sites as above, with each site being searched for a total of nine hours over three days in November and December 2013 (actual dates were assigned randomly). Each search session was conducted at low tide, and plots along the low tide line measuring  $20 \times 5 \text{ m}^2$  were searched for three hours; specimens were either unearthed using a shovel or picked off the surface of the sand when visible. They were then categorised according to the morph-groups in the Condensed Matrix, photographed for record-keeping, and later returned to separate bays further down the shoreline at the end of each sampling session (to prevent resampling). Morph frequencies at each site were compared using the Mantel test (10,000 permutations) with the Vegan package in R (Oksanen et al., 2013).

## RESULTS

**Empty shells.** A total of 5372 shells were collected, of which 47% were discarded. Examination of the remaining 2845 found that both band and whorl designs vary according to multiple criteria. Band designs could be classified using four primary criteria (illustrated in Fig. 3):

- 1) The number of bands, from a minimum of zero to a maximum of four. For example, compare A, E and F in Fig. 3.
- 2) The continuity of the band. Three types were observed: no line, dashed lines and solid lines. For example, compare A to C in Fig. 3.
- 3) The position of the band. Two positions were observed. In the first case (called 'Top') the band is at the top portion of the whorl, just below the suture. In the second case ('Bottom'), it is at the bottom of the whorl, just above the suture. See examples E and F in Fig. 3.
- 4) The colour of the band. Three colours were observed: pink, brown and white. Compare examples A and D in Fig. 3.

Whorl designs could be classified using three primary criteria (illustrated in Fig. 4):

- 1) Pattern. Four patterns were observed: uniform colouration (referred to as 'uniform'), dotted, wavy-lined or striped. See examples A to D in Fig. 4.
- 2) Colour. Two colours were observed: pink and brown. The only pattern present in both pink and brown was the uniform pattern. The other three patterns (dotted, wavy-lined and striped) were observed in only brown. Compare examples A and E in Fig. 4.
- 3) Presence of stripes at the bottom of the lowest whorl. See examples A and F in Fig. 4.

In total, 22 different band designs (labelled '1' to '22') and nine different whorl designs (labelled 'A' to 'J', not inclusive of 'I') were identified. The different designs were combined to form a Comprehensive Matrix with a total of 198 possible different morphs, of which 96 (48.5%) were actually observed. The top and side views of these morphs are presented in Fig. 5. Table 1 shows the number of shells from each morph collected at each site, and overall frequencies are plotted in Fig. 6. The overall three most common morphs are G22 (7.62%), H21 (6.87%) and H22 (6.74%), and are generally combinations of the most common band designs (20, 21 and 22) and whorl designs (G, H and J).

To create the Condensed Matrix, the 22 band designs were pooled based on number of bands into five groups: 'no band', 'one-band', 'two-band', 'three-band' and 'four or more bands'. Whorl designs were pooled into five groups: pink uniform (a combination of morph designs A and E), brown uniform (B and F), dotted (C and G), wavy-lined (D and H) and striped (morph design J only). The resulting  $5 \times 5$  matrix is presented in Fig. 7 and the overall three most common morph-groups were CG1 (dotted whorl design with one band; 18.6%), DH1 (wavy-lined whorl design with one band; 17.9%) and J1 (striped whorl design with one band; 10.9%). Table 2 shows the number of snails from each morph-group collected at each site and relative frequencies for all groups at each site are provided in Fig. 8. The results of the Mantel test comparing these two  $5 \times 5$  Condensed Matrices suggest that there is correlation (i.e., no statistical difference) between the morph frequency of *Umbonium vestiarium* shells collected from both sites (two-tailed p-value = 0.001).

**Live snails.** A total of 207 live specimens were collected, 123 from Tanah Merah and 84 from Changi. Overall, the same three morph-groups (CG1, DH1 and J1) were found to be the most common at both sites, although the most common group at Tanah Merah (CG1) was the second most common at Changi (where DH1 was most common). Relative morph frequencies at the two sites (Fig. 9) were found to be statistically correlated (Mantel test two-tailed p-value = 0.01), agreeing with results from the study on empty shells.

## DISCUSSION

Understanding polymorphism in mollusc populations continues to be a challenge for ecologists and evolutionary biologists. The results in this paper quantify what had already been anecdotally reported for *Umbonium vestiarium*, i.e., that they are highly pattern and colour polymorphic within populations. From the 2845 shells used to create the matrices in this study, 96 distinct morphs were observed. However, more sampling may result in an increase in this number. Using a more compact ( $5 \times 5$ ) Condensed Matrix, data on morph frequencies of live populations could be quickly and easily collected and relative frequencies between the two sites could be compared, demonstrating that the Mantel test is a suitable tool for such analyses.

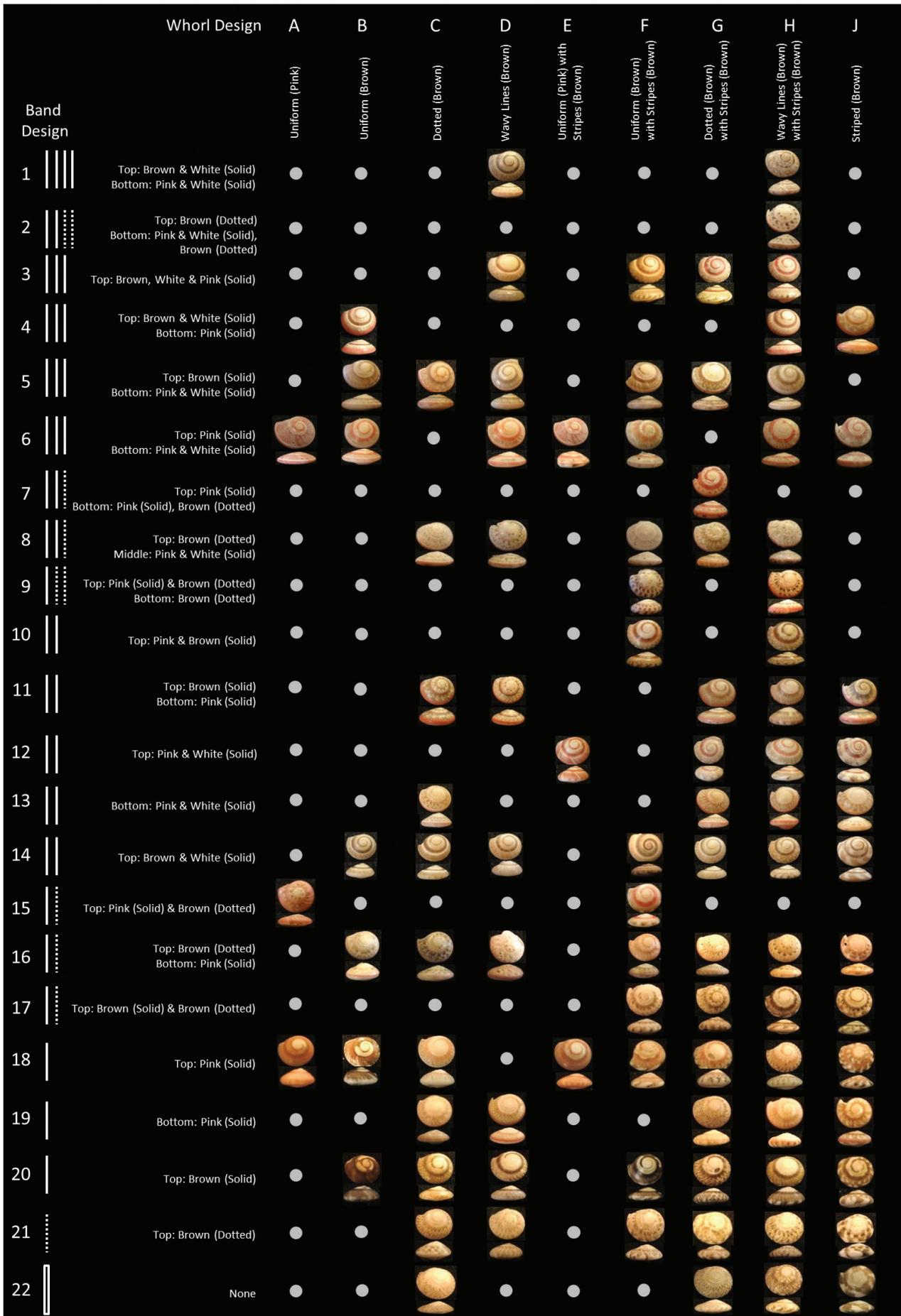


Fig. 5. Comprehensive Matrix classifying the phenotypic polymorphism of *Umbonium vestiarium* shells according to 22 different band designs (on the vertical axis) and nine different whorl designs (on the horizontal axis). Morphs not observed are represented by grey circles.

Table 1. Number of shells from each morph collected at Tanah Merah (first number) and Changi (second number).

Band Design**	Whorl Design*									
	A	B	C	D	E	F	G	H	J	
1	0, 0	0, 0	0, 0	2, 8	0, 0	0, 0	0, 0	1, 4	0, 0	
2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	1, 1	0, 0	
3	0, 0	0, 0	0, 0	0, 0	0, 0	1, 1	1, 0	1, 0	0, 0	
4	0, 0	1, 0	0, 0	4, 0	0, 0	0, 0	0, 0	4, 0	1, 0	
5	0, 0	0, 3	2, 1	4, 5	0, 0	2, 0	4, 0	4, 0	0, 0	
6	0, 2	0, 1	0, 0	0, 1	0, 1	0, 3	0, 0	0, 3	0, 1	
7	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 1	0, 0	0, 0	
8	0, 0	0, 0	0, 2	0, 5	0, 0	0, 1	0, 4	0, 9	0, 0	
9	0, 0	0, 0	0, 0	0, 0	0, 0	0, 2	0, 0	0, 1	0, 0	
10	0, 0	0, 0	0, 0	0, 0	0, 0	1, 0	0, 0	1, 0	0, 0	
11	0, 0	0, 0	1, 3	1, 1	0, 0	0, 0	1, 1	5, 2	3, 1	
12	0, 0	0, 0	0, 0	0, 0	0, 1	0, 0	0, 1	1, 1	0, 1	
13	0, 0	0, 0	18, 7	0, 0	0, 0	0, 0	45, 6	5, 0	2, 0	
14	0, 0	15, 6	10, 11	16, 17	0, 0	17, 16	34, 15	76, 37	17, 20	
15	0, 1	0, 0	0, 0	0, 0	0, 0	11, 0	0, 0	0, 0	0, 0	
16	0, 0	1, 0	8, 2	0, 3	0, 0	6, 1	23, 6	25, 2	3, 0	
17	0, 0	0, 0	0, 0	0, 0	0, 0	4, 1	13, 8	29, 5	8, 4	
18	24, 25	4, 0	15, 3	0, 0	51, 42	11, 0	41, 6	41, 22	61, 46	
19	0, 0	0, 0	9, 4	3, 3	0, 0	0, 0	31, 1	17, 3	10, 1	
20	0, 0	4, 19	22, 15	18, 16	0, 0	26, 49	117, 40	106, 49	81, 42	
21	0, 0	0, 0	19, 0	13, 9	0, 0	26, 5	144, 62	125, 85	37, 31	
22	0, 0	0, 0	25, 0	0, 0	0, 0	0, 0	205, 28	106, 15	101, 58	

\*A: Uniform (Pink), B: Uniform (Brown), C: Dotted (Brown), D: Wavy Lines (Brown), E: Uniform (Pink) with Stripes (Brown), F: Uniform (Brown) with Stripes (Brown), G: Dotted (Brown) with Stripes (Brown), H: Wavy Lines (Brown) with Stripes (Brown), J: Striped (Brown).

\*\* 1: Brown & White (Solid) on top and Pink & White (Solid) on bottom, 2: Brown (Dotted) on top and Pink & White (Solid) & Brown (Dotted) on bottom, 3: Brown, White & Pink (Solid) on top, 4: Brown & White (Solid) on top and Pink (Solid) on bottom, 5: Brown (Solid) on top and Pink & White (Solid) on bottom, 6: Pink (Solid) on top and Pink & White (Solid) on bottom, 7: Pink (Solid) on top and Pink (Solid) & Brown (Dotted) on bottom, 8: Brown (Dotted) on top and Pink & White (Solid) on bottom, 9: Pink (Solid) & Brown (Dotted) on top and Brown (Dotted) on bottom, 10: Pink & Brown (Solid) on top, 11: Brown (Solid) on top and Pink (Solid) on bottom, 12: Pink & White (Solid) on top, 13: Pink & White (Solid) on bottom, 14: Brown & White (Solid) on top, 15: Pink (Solid) & Brown (Dotted) on top, 16: Brown (Dotted) on top and Pink (Solid) on bottom, 17: Brown (Solid) & Brown (Dotted) on top, 18: Pink (Solid) on top, 19: Pink (Solid) on bottom, 20: Brown (Solid) on top, 21: Brown (Dotted) on top, 22: None.

Table 2. Number of live individuals from each morph-group collected from Tanah Merah (first number) and Changi (second number).

Number of Bands	Whorl Design*				
	AE	BF	CG	DH	J
≥4	0, 0	0, 0	0, 0	0, 1	0, 0
3	0, 1	1, 0	1, 1	2, 2	1, 0
2	0, 1	2, 3	8, 7	9, 8	3, 3
1	5, 2	4, 2	27, 13	19, 16	13, 12
0	0, 0	0, 0	11, 5	10, 3	7, 4

\*AE: Uniform (Pink) and Uniform (Pink) with Stripes (Brown), BF: Uniform (Brown) and Uniform (Brown) with Stripes (Brown), CG: Dotted (Brown) and Dotted (Brown) with Stripes (Brown), DH: Wavy Lines (Brown) and Wavy Lines (Brown) with Stripes (Brown), J: Striped (Brown).

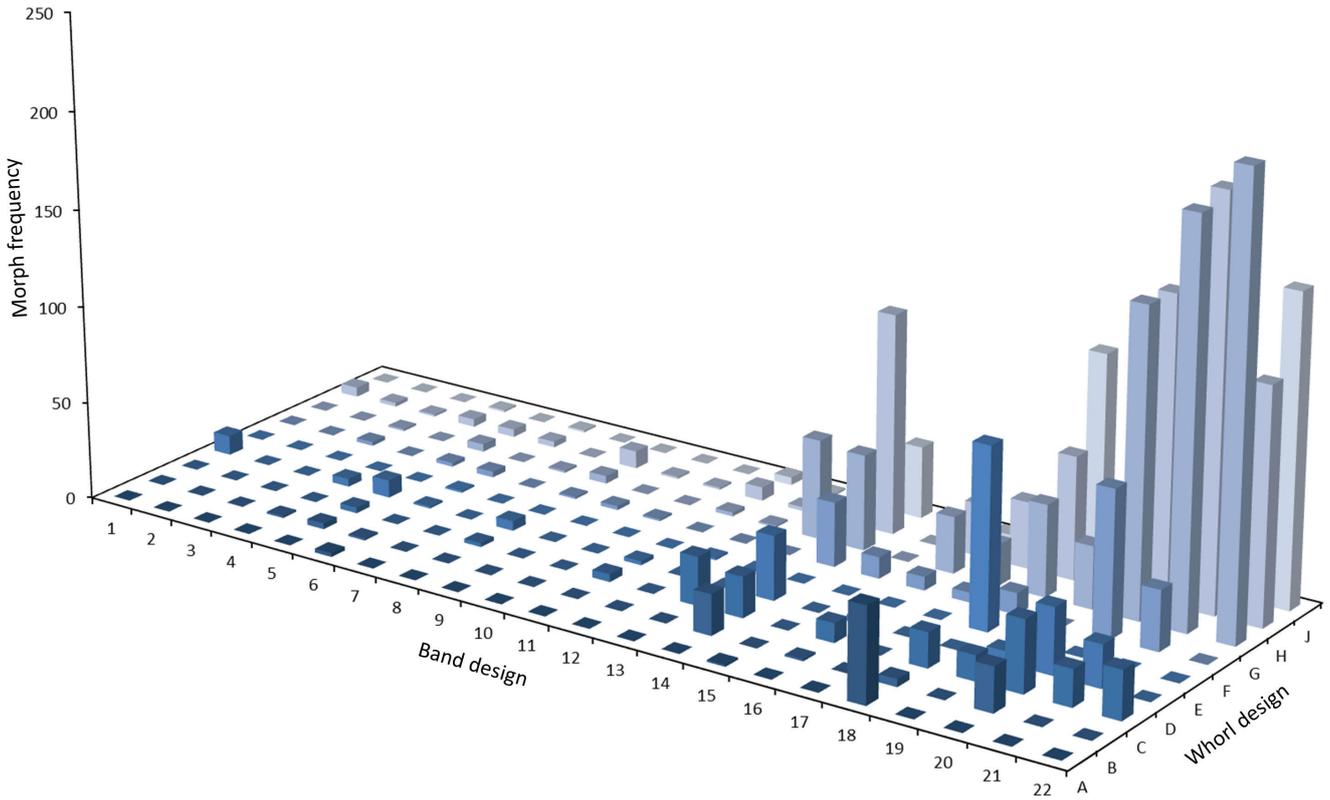


Fig. 6. Overall frequencies of each morph. Note how the majority of individuals are clustered to the top right of the chart, showing the prevalence of band designs 20, 21, and 22 and whorl designs G, H, and J.

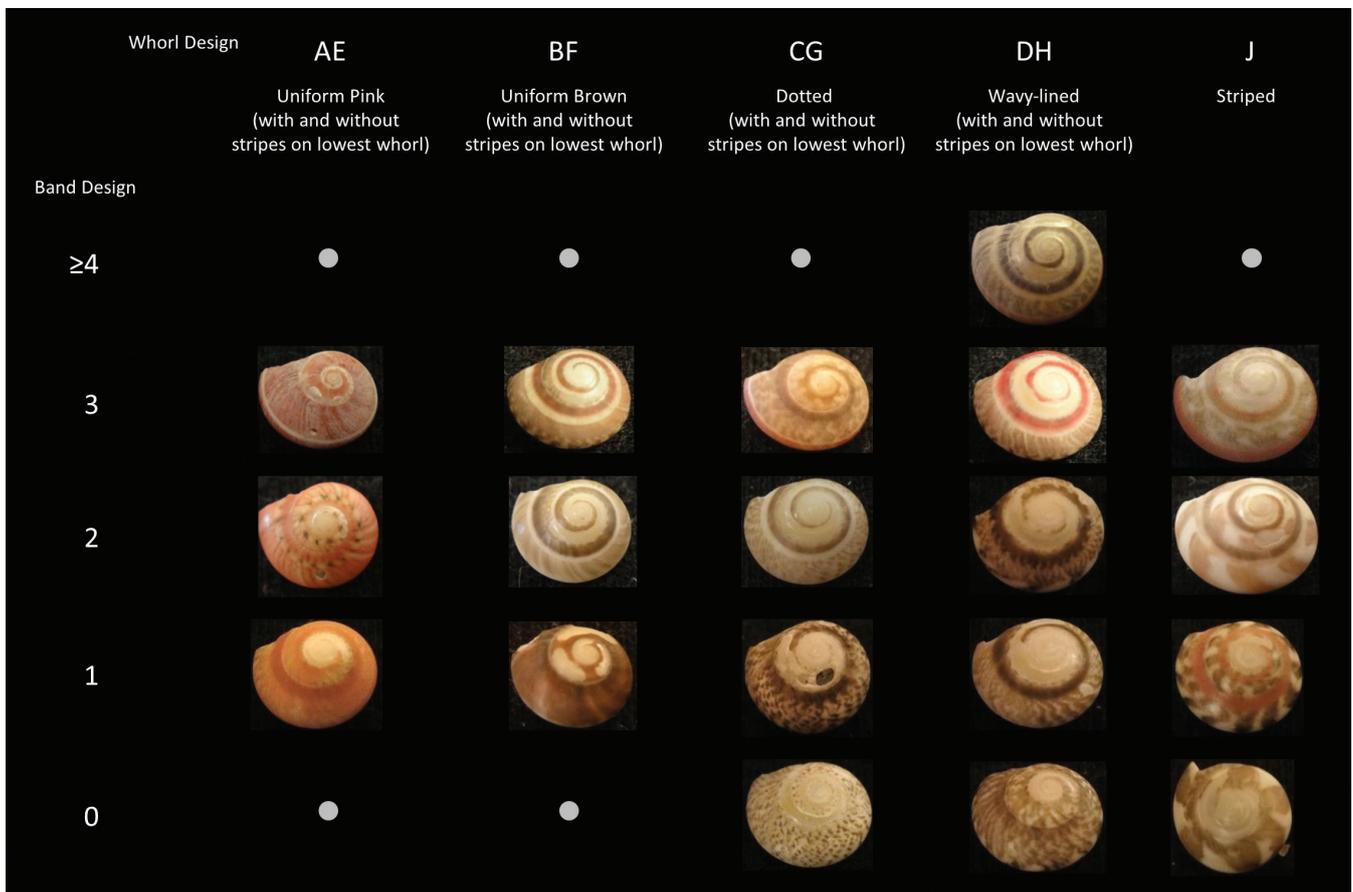


Fig. 7. Condensed Matrix classifying the phenotypic polymorphism of *Umbonium vestiarium* shells according to five different band designs: no band (0), one (1), two (2), three (3), and four or more (≥4) bands; and five whorl designs: uniform pink (AE), uniform brown (BF), dotted (CG), wavy-lined (DH), and striped (J). Morphs not observed are represented by grey circles. Morph-group frequencies from this matrix were used for the Mantel test.

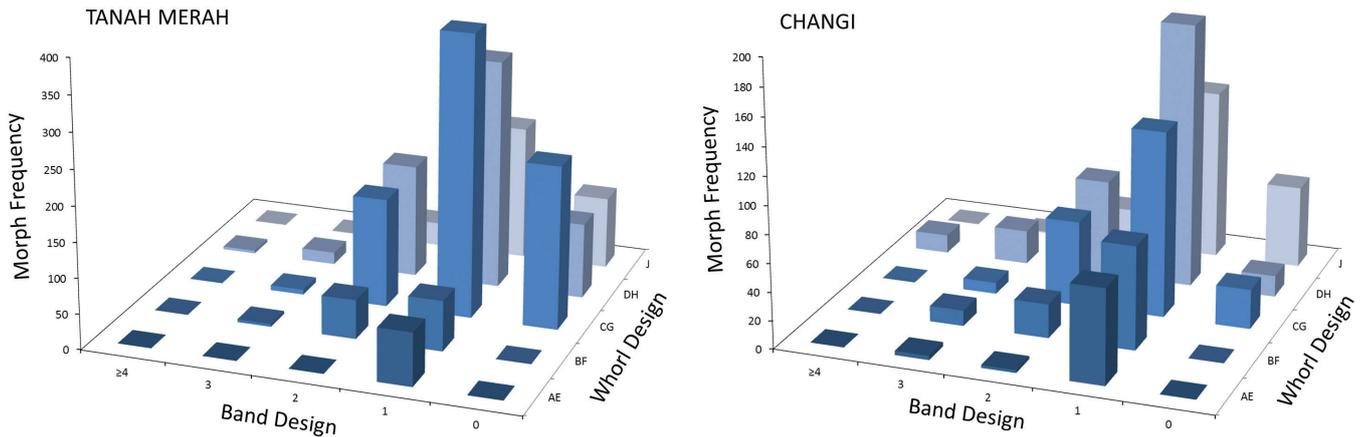


Fig. 8. Frequencies of empty *Umbonium vestiariium* shells categorised into each morph-group at Tanah Merah and Changi. Frequencies observed at both sites were statistically similar (Mantel test two-tailed p-value = 0.001).

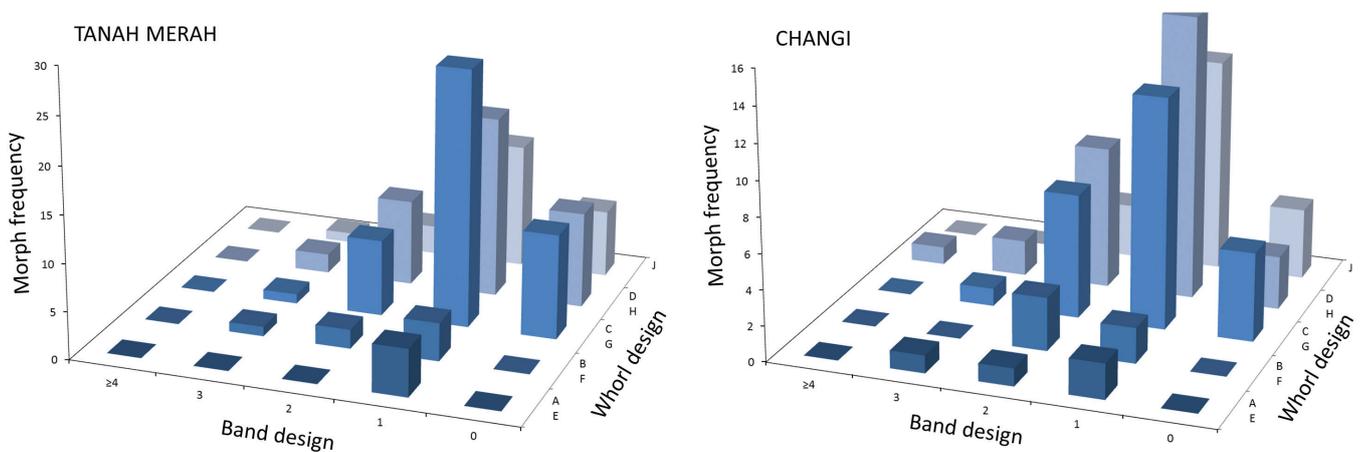


Fig. 9. Frequencies of live *Umbonium vestiariium* categorised into each morph-group at Tanah Merah and Changi. Frequencies observed at both sites were statistically similar (Mantel test two-tailed p-value = 0.01).

The degree of polymorphism found in *Umbonium vestiariium* shells by this study is high; a total of 96 morphs were observed, compared to an estimated 21 morphs in the intertidal snail, *Littorina saxatilis* (Byers, 1990), 18 in the terrestrial snail *Cepaea nemoralis* (Ozgo, 2004), eight in the boring giant clam, *Tridacna crocea* (Todd et al., 2009), and 30 in the colouration of the butterfly *Papilio memnon* (Clarke et al., 1968; Clarke et al., 1971; Clarke & Sheppard, 1973). The primary reason for this is the high variability in band design: over 1500 permutations of band designs alone are theoretically possible, assuming a maximum of four bands, each either solid or dashed, coloured brown, white or pink and positioned either at the top or bottom of whorls. It is unclear what mechanisms maintain this polymorphism as many of the environmental factors cited as important in other studies do not seem to apply to *Umbonium vestiariium*.

One of the most commonly-cited mechanisms is apostatic selection through differential predation rates on morphs (Poulton, 1884; Allen & Clarke, 1984). In these studies, prey morphology affects predation pressures because predators locate prey visually and are therefore affected by phenomena such as crypsis. However, the identified predators of *Umbonium vestiariium* (naticid snails, olivid snails and

sea stars; Berry, 1984) hunt primarily by chemotaxis. It is therefore unlikely that polymorphism in *Umbonium vestiariium* shells is maintained through differential predation rates. Even though it is possible that different morphs release different chemicals into the water and are thereby distinguishable by predators, there is no support for this in the literature, and if *Umbonium vestiariium* had visual predators common enough to exert selection pressure on button snail populations we suggest that these would already be known.

Heat management is another factor that has been used to explain morphological features in other gastropods. Nodulation in the intertidal and tropical *Littorina malacanna* (Lee & Lim, 2009) and shell patterns in the temperate *Cepaea nemoralis* (Jones et al., 1977) have been shown to affect their respective thermal properties. In the tropics where solar radiation and temperatures are relatively high (Dodson, 2010), lighter-coloured morphs of *Umbonium vestiariium* potentially have an advantage in terms of avoiding overheating (shown in littorinid snails by Miller & Denny, 2011; and in other species by Cole, 1943). Indeed, most button snail morphs observed in this study were light in colour, but the degree of polymorphism found within these lightly-coloured morphs remains unexplained.

Other factors that may contribute to maintaining the observed polymorphism include the presence of microclimates in the living environment (Blaustein et al., 1999), heterozygote advantage (Kalmus, 1945), and pseudo-polymorphism resulting from developmental plasticity (Gruneberg, 1980). In particular, the clear dominance of certain designs (band designs 20, 21 and 22 account for nearly 60% of all shells, and whorl designs G, H and J account for over 75%) and the presence of intergrades between the major designs observed fits the model of pseudo-polymorphism described by Gruneberg (1980) as ‘basic patterns that merge step by step into each other’. This suggests that plasticity plays a part in the observed polymorphism, but the question of what environmental variables are inducing the plastic responses remains. Furthermore, from morph frequencies alone, it is not possible to determine to what degree this polymorphism is caused by environmental factors versus genetic differences, or to elucidate its evolutionary origin. Therefore, additional research is required, such as testing for environmental effects via transplant experiments (e.g., Todd et al., 2004; Genton et al., 2005) and rearing of morphs under different conditions (e.g., Imbert et al., 1997) as well as studies of the genetic controls behind the different morphs of *Umbonium vestiarium*.

The Comprehensive Matrix constructed in this study provides a first step to understanding the potential genetic controls for shell polymorphism in *Umbonium vestiarium*. Relative phenotype frequencies have been used to predict genetic controls (e.g., Mendel, 1866; Maestri & Beaty, 1992; Jarvik et al., 1994) and the frequencies observed in this matrix may be similarly used to propose and test models of genetic control. However, there is a high possibility that morphs that are unrepresented in the current matrix exist, e.g., due to rarity or variation amongst geographically isolated populations. Future studies should collect live snails from more locations in order to ascertain if there are additional morphs (both in Singapore and globally) and, eventually, to investigate possible geographical variation in terms of morphs, morph frequencies, and genetic material. These locations should ideally be spread out along the species’ geographical range and should include sites such as the offshore islands of Singapore, Cox’s Bazar in Bangladesh, and especially the Mediterranean and Japan (the last two being the extremes of its geographic range, and therefore the most likely to have different morphs and populations that will result in a Mantel test that shows no correlation).

In addition to the Comprehensive Matrix, our study has shown that the Condensed Matrix is useful for categorising live *Umbonium vestiarium* into morphs in situ. Formulated based on two principles: (i) compactness, and (ii) grouping based on easily and quickly recognisable traits, the matrix we constructed categorises morphs based on number of bands and whorl pattern and has only five rows and five columns. This makes it possible to categorise live samples in the field rapidly and non-destructively. The low environmental impact of this manner of data collection makes this technique very appealing, especially for threatened polymorphic species around the world.

The Mantel test has also proven a useful tool for comparing *Umbonium vestiarium* morph frequencies between two sites. Originally designed by Mantel (1967) and Mantel & Valand (1970) to identify time-space clustering of leukemia, the test is a multi-response permutation procedure (Mielke, 1988) that statistically compares the correlation of two square matrices of the same size by calculating the correlation coefficients between one matrix and multiple permutations of the rows and columns of the other matrix (Mantel, 1967). Under the null hypothesis (that the matrices are not correlated), it is expected that approximately half the permutations would give rise to a higher correlation coefficient; on the other hand, if the original matrices are correlated, significantly fewer permutations would result in a higher correlation. Hence, the p-value reported is the proportion of permutations that lead to a higher correlation and a significant p-value indicates that the matrices are correlated (Mantel, 1967). Calculating more permutations enables higher levels of confidence: approximately 1000 permutations for a 95% confidence level and 5000 for 99% (Manly, 1991).

Traditionally, each matrix contains pair-wise distances between all data points; in Mantel (1967), for example, one matrix contains the spatial distances between occurrences of leukaemia and the other contains the temporal distances (i.e., time between the two occurrences). Over time, however, the Mantel test has come to be employed in such areas as analysing  $\beta$  diversity within an ecosystem (Legendre et al., 2005; Tuomisto & Ruokolainen, 2006), analysing the genetic variation caused by different environmental variables such as geographical distance or temperature (Manel et al., 2003), and in population genetics (Diniz-Filho et al., 2013). Using the Mantel test to compare morph frequencies as suggested in this paper is, to our knowledge, a novel application because the elements of morph frequency matrices are not distance measures. The test employs a permutational procedure because the matrices that it was designed to compare contained elements (i.e., distance measures) that were not independent (Mantel, 1967; Mantel & Valand, 1970). However, the mathematical procedures employed by Mantel & Valand (1970) do not restrict the test from handling matrices of independent values.

In addition, the Mantel test has notable advantages over the other statistical methods currently used for comparing morph frequencies such as MANOVA and the Chi-squared test (though ordination methods could also be used these are, *sensu stricto*, for exploratory analysis and not hypothesis testing; Birks, 1998). It is more statistically amenable, as the other methods mentioned (e.g., the Chi-squared test) require replicate datasets, and need certain assumptions on the distribution of the data to be met, whereas the Mantel test circumvents these requirements by comparing numerous permutations of one dataset (Mantel, 1967). Furthermore, zero values, which are common in morph frequency data, interfere with the proper functioning of the other methods but not with the Mantel test. Hence, together with both the Comprehensive and Condensed matrices, the Mantel test forms a useful framework for further research into polymorphism in the shells of *Umbonium vestiarium*.

## CONCLUSIONS

The present study found a high degree of polymorphism in *Umbonium vestiarium* shells: from 2845 shells, 96 distinct morphs were identified and classified within a Comprehensive Matrix based on 22 band and nine whorl designs. This matrix represents the first step towards a framework for examining the genetic controls and environmental factors maintaining the observed polymorphism. To aid in categorisation and inter-site comparisons in future studies, we propose a 5 × 5 Condensed Matrix (based on number of bands and whorl pattern) for quick and easy, non-destructive in situ sampling of live communities, and suggest using the Mantel test as a novel means to statistically compare morph frequencies at different sites.

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