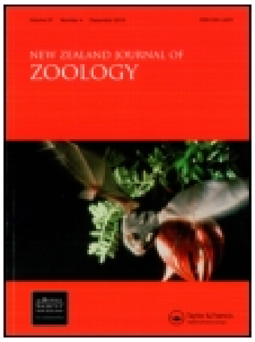

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Analyses of tuatua populations – *Paphies subtriangulata* and *P. donacina*

J. R. RICHARDSON[1]
A. E. ALDRIDGE[2]
P. J. SMITH[3]
[1]New Zealand Oceanographic Institute
Department of Scientific and Industrial Research
P.O. Box 12–346, Wellington North, New Zealand
[2]Applied Mathematics Division
Department of Scientific and Industrial Research
Mt Albert Research Centre
Private Bag, Auckland, New Zealand
[3]Fisheries Research Division
Ministry of Agriculture and Fisheries
P.O. Box 19062, Wellington, New Zealand

Abstract Populations of tuatua, *Paphies subtriangulata* and *P. donacina*, occur in the same beds in sandy beaches in New Zealand. The species may be differentiated on external and internal shell characters; living individuals may also be separated specifically by the colour of the adductor muscles. The invariable presence of algae and hydroids on the posterior shell surfaces of *P. subtriangulata* indicates a habitat difference (depth of substrate occupied) from *P. donacina*, on which epibionts are rare or absent. These conclusions, made from association and multivariate analyses, are supported by differences in esterase phenotypes which indicate that the species do not interbreed.

Keywords Bivalvia; Mesodesmatidae; *Paphies subtriangulata*; *Paphies donacina*; distribution; association analysis; multivariate analysis; esterase phenotype; character discrimination.

INTRODUCTION

The genus *Paphies* as used by Beu (1971) contains the toheroa and tuatua, the edible bivalves characteristic of sandy beaches on New Zealand's open coasts. The name tuatua is commonly used to refer to 2 species, and Beu & De Rooij-Schuiling (1982) show that they should be called *P. subtriangulata* and *P. donacina*. These species have been regarded as separate by Deshayes (1832), Lamy (1914), Iredale (1914), and Finlay (1927), who clearly differentiated them on shell outline.

Dawson (1959) and Paul (1959) have questioned this classification, with conflicting accounts of distribution. Dawson (1959) considered that the 2 forms of tuatua are not species but subspecies that represent the ends of a cline, *P. subtriangulata* being characteristic of northern New Zealand beaches and *P. donacina* of southern ones. Paul (1959) analysed populations collected from beds at Raumati Beach, on the Wellington west coast. He found that both species were present in the same beds and that, if factors additional to shell outline are considered, 3 forms could be differentiated visually.

Collections recently made by us at Paraparaumu and neighbouring beaches (Fig. 1) consisted of individuals of both *P. subtriangulata* and *P. donacina*, as defined on shell outline by the earlier authors cited above. Since subspecies are defined (Mayr 1970) as geographically isolated populations (which would intergrade if they were to overlap geographically), Dawson's interpretation cannot be accepted.

Therefore, the question arises as to whether more than 1 species is present in populations of tuatua on beaches of the south-western North Island. To answer this question, analyses were made of all the

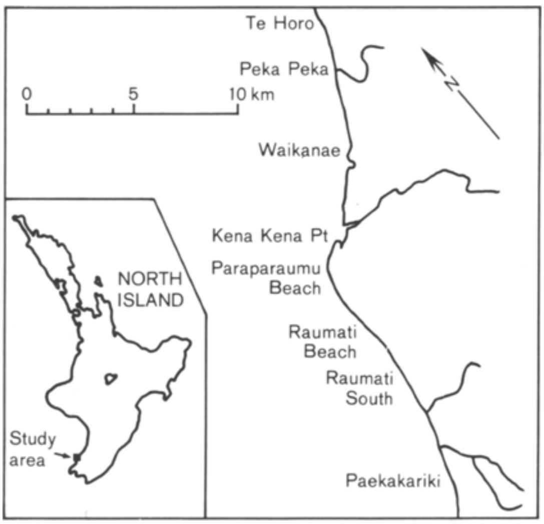

Fig. 1 Location of beaches at which populations of tuatua (*Paphies subtriangulata* and *P. donacina*) were sampled.

Received 9 March 1981; held over pending submission of Beu & De Rooij-Schuiling (1982)

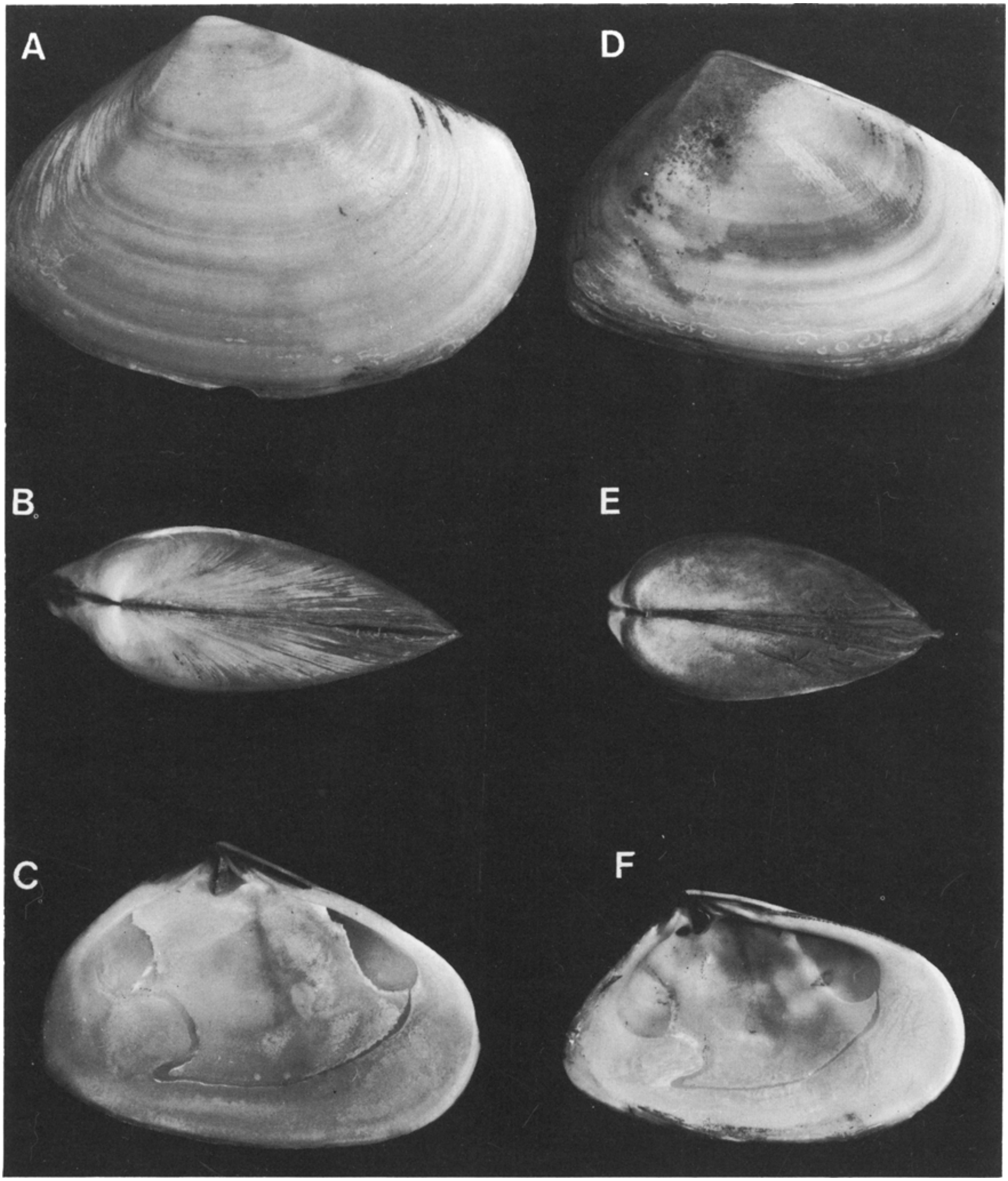

Fig. 2 A–C, *Paphies donacina* and D–F, *P. subtriangulata*, lateral view, posterior view, and interior of right valve respectively.

variables evident in individuals from 2 beaches, at Peka Peka and Waikanae. Gel electrophoresis was used to compare esterase phenotypes between the different forms of tuatua.

DATA

Altogether 361 individuals were collected on 24 April 1978 from the 2 beaches. Population densities are greatest at and beyond Low Water Springs,

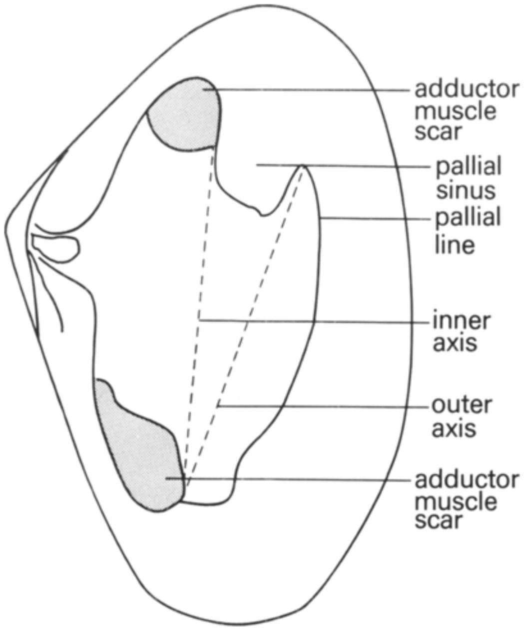

Fig. 3 *Paphies donacina*, interior of left valve; pecked lines indicate measurements used to determine depth of pallial sinus.

where the shells are wedged in the sand with their posterior faces lying at or just below the surface. The distribution of populations at Peka Peka and Waikanae is similar to that described by Paul (1959) for populations near the Wharemauku Stream, approximately 3 km south of Paraparaumu Beach township (Fig. 1).

Regardless of their nature, all recognisable variations in individuals from these populations were recorded; no judgement of the function or permanence of any attribute or character was made. Externally the shells differed in outline, in colour, and in the occurrence on the posterior surface of green algae and hydroid growths. Internally, variations were observed in the colour of the adductor muscles, in the depth of the pallial sinus, and in the curvature of the pallial line.

Those variations which could be determined by visual examination are referred to as 'attributes', and comprise shell and muscle colour, algal and hydroid growth, and curvature of the pallial line. Differences observed in shell outlines were derived from measurements of shell length, width, thickness, and circumference; the depth of the pallial sinus was assessed by measurement of inner and outer axes (Fig. 2 and 3). Hence, the data used in the following analyses are derived from 7 linear measurements and 5 attributes.

STATISTICAL PROCEDURE AND RESULTS

Individuals in collections from the 2 beaches were first separated using the differences in external shell outline noted by previous authors, i.e., thick posteriorly truncated individuals were assigned to *Paphies subtriangulata* while flatter, more ovate individuals were assigned to *P. donacina*. A total of 197 individuals were collected at Peka Peka, 171 *P. subtriangulata* and 26 *P. donacina*; at Waikanae a total of 154 individuals comprised 150 *P. subtriangulata* and 4 *P. donacina*.

In subsequent analyses the above classification was ignored, and only the 5 attributes and 7 measurements were used to define groups of individuals. The individuals were classified first on attributes only, then measurements were used to test the significance of this classification.

The classification from attributes alone was made using an association analysis with a summed χ^2 criterion (Williams & Lambert 1959). This analysis led to a breakdown of the collections into 2 groups, the first (Group A; $n = 40$) consisting of individuals with cream shells, cream muscles, a curved pallial line, and some with hydroid growths on the posterior surface of the shell, the second ($n = 321$) consisting of individuals with grey shells, blue muscles, and a flattened pallial line. This latter group could be further split into 2 (groups B and C) according to the presence or absence of hydroids and algae. Group B ($n = 114$) comprises shells all with hydroids, 71 of which also carry algal growths. Group C comprises 207 individuals in which hydroids are absent, but algae are present on 70 shells. Thus, the analysis of attributes only gives the same shell groupings as result from the classification using variables. In Table 1 the percentage of individuals in each group is matched with species designation, attributes, and beaches. All individuals in Group A had earlier been attributed to *P. donacina* and all in groups B and C to *P. subtriangulata*.

It was possible to determine the influence of geographical position on shell dimensions by further analysis of groups A–C, each split according to beach of origin. Linear measurements were used to test the significance of differences between the 6 groups so derived. Length, and the other 6 shell measurements divided by length—i.e., length and 6 ratios—were calculated to eliminate the influence of age and beach profile position. Two combinations (canonical variates or discriminant functions) were formed, from length and the 6 ratios, that best highlighted differences in shape and size between the groups. A plot of class means and associated 95% circular confidence regions on these 2 combinations is given in Fig. 4.

Table 1 Percentages of *Paphies* individuals in groups defined from analysis of attributes, species designation, and beach of origin.

GROUP	ATTRIBUTES PRESENT (%) Algae on shell	Muscle colour	Pallial line	Shell colour	Hydroids on shell	SPECIES	BEACH (Peka Peka: Waikanae)	*n*
A	0	97 cream	100 curved	100 cream	15	100 *P. donacina*	65:35	40
B	62	100 blue	100 flattened	98 grey	100	100 *P. subtriangulata*	25:75	114
C	34	100 blue	100 flattened	100 grey	0	100 *P. subtriangulata*	69:31	207

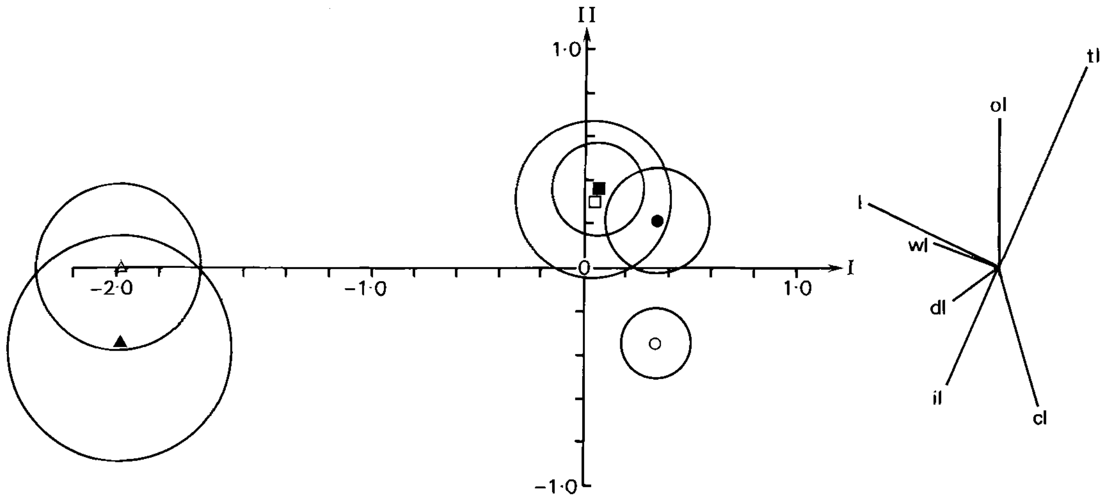

Fig. 4 **A,** Results of multivariate analysis of individuals of *Paphies* species. Group means and associated 95% confidence circles are plotted on the first 2 canonical variates (I and II). (□, *P. subtriangulata* with hydroid; ○, *P. subtriangulata* without hydroid; △, *P. donacina*; open symbols denote Peka Peka beach, solid symbols Waikanae beach). **B,** Relative weightings of shell length, and ratios of the other measurements to length, on the 2 axes (cl, circumference–length ratio; dl, sinus–length ratio; il, inner axis–length ratio; l, shell length; ol, outer axis–length ratio; tl, thickness–length ratio; wl, width–length ratio).

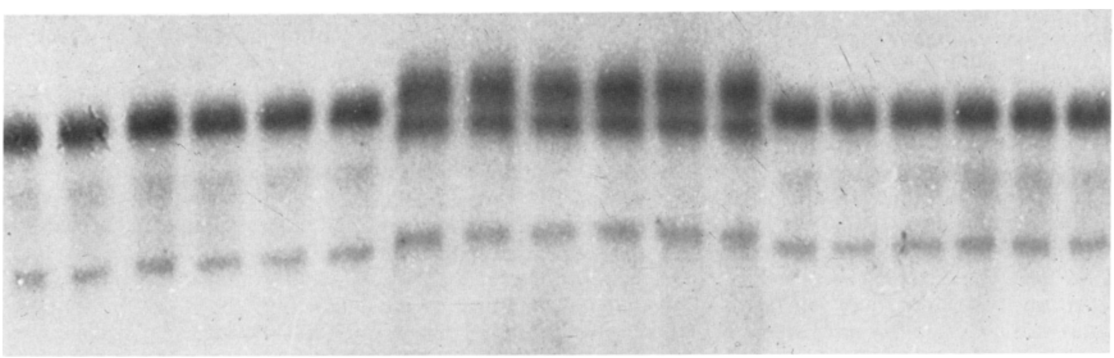

Fig. 5 Esterase phenotypes in *Paphies donacina* and *P. subtriangulata*. Gels 1–6 *(from left)*, *P. subtriangulata* without hydroids; 7–12, *P. donacina*; 13–18, *P. subtriangulata* with hydroids.

Results from this multivariate analysis were significant ($\Lambda = 0.43$; F = 9.4 on 35, 1471 dfj, $P \ll 0.01$). Most (88%) of the differences between groups could be accounted for by- differences between *P. subtriangulata* and *P. donacina*. The group of individuals from Peka Peka without hydroids (group C) appeared only slightly different (though statistically significant) from other individuals attributed to *P. subtriangulata*. Linear measurements showed that beach differences within any group are small relative to the large difference between Group A and Groups B and C combined. Thus, the 6 groups separated on beach of origin and attributes were reduced to 2 significantly different groups of individuals that coincide with the current classification of individuals into 2 groups according to shell outline.

FIELD RECOGNITION

The range of attributes and measurements defined may be used in the identification of living and fossil juveniles of the 2 species.

Living individuals may be differentiated specifically in the field on shell colour and the presence or absence of green algae, using the following key.

1 Algae present on posterior surface ..... *P. subtriangulata*
—No algae on posterior surface 2
2 Shell colour grey ..... *P. subtriangulata*
—Shell colour cream ..... *P. donacina*

In dried or fossil individuals that lack colour and epibionts, weighted combination of the length, width, and thickness of a shell (i.e., a single number or score) may be used to assign it to species. The method of computing this score and the shell classification is given in Appendix 1.

An attribute of great value in the rapid identification of juveniles is the colour of the adductor muscles, which in *P. subtriangulata* are invariably blue (changing to red when the flesh is heated).

ELECTROPHORESIS

Collections were made at Peka Peka beach in October 1978 and again in November 1980. Specimens were divided into the 3 groups described above on the evidence of external shell outline, muscle colour, and presence or absence of hydroids. Twelve specimens of group A (*Paphies donacina*), 6 of Group B with hydroids (*P. subtriangulata*), and 6 of Group C without hydroids (*P. subtriangulata*) from each collection were used for electrophoretic analysis.

For electrophoretic preparation individuals were removed from the shell and homogenised in an equal volume of distilled water. The homogenate was centrifuged at 30 000 g for 10 min, and the supernatant was frozen at –70°C. Twelve percent electrostarch gels were made up in a buffer system described by Ridgway et al. (1970) and run at 230 V for 3 h. The gels were stained for esterases by adding 1 ml of 1% alpha-naphthyl acetate in acetone followed by 25 mg of fast blue BB salt in 20 ml of 0.1M tris HCl, pH 8.0.

Two distinct esterase phenotypes (Fig. 5) were found in both the 1978 and 1980 samples. *P. donacina* gave 2 strongly staining esterase bands and 1 faint band. *P. subtriangulata* gave 1 strongly staining band of slight but consistently different mobility to the strong bands of *P. donacina* and 1 faint band of slight but consistently different mobility to the faint band of *P. donacina* (Fig. 5). No differences were apparent between the specimens of *P. subtriangulata* with and without hydroids, in either collection. The difference in esterases of *P. donacina* and *P. subtriangulata* indicates that they are not interbreeding. Such electrophoretic differences are typical of those between species and not of those between populations (Avise 1975).

DISCUSSION

In this study, statistical and electrophoretic techniques have been used to support the observation that populations of *Paphies* that occur in the study areas consist of 2 species. Two statistical techniques, association and discriminant function analysis, showed that of 12 variables considered, 5—shell colour, length, width, thickness, and presence of algae—were effective in separating living members of the species.

Populations of tuatua from 2 beaches were analysed. Other beaches (Raumati, Te Horo, Kena Kena Point) are also known to contain both species. These were not included in our study because our aim was to determine the taxonomic status of populations, not to establish their geographic distribution. It was necessary to study the populations from only a single beach to verify that populations comprised species, not subspecies. Two beaches were included in the study in order to determine whether the characters analysed are influenced by geographic position.

Differences in shell outline used previously to define the species are valid, but study of living populations has shown that other attributes may also be used to distinguish the species. These attributes—shell colour, and the presence of algae and hydroids—are not necessarily evident after the death of the animal. However, apart from their diagnostic value in living individuals, they indicate slight differences in habitat that are difficult to assess by other means. The invariable presence of green algae and hydroids on the shell of *P. subtriangulata* indicates that individuals lie closer to the surface of the sand than do those of *P. donacina*, few of which bear hydroids and none algae.

This interpretation gains support from the fact that all other characters used to differentiate the 2 species appear to relate to the depth of substrate occupied. The flattened contour of the posterior shell surface of *P. subtriangulata* shows that this species can lie at the substrate surface, a position that would result in fracture of the posteriorly tapering valves of *P. donacina* (Fig. 2B,E). The inhalant and exhalant siphons emerge through the posterior gape of the valves, and vary in length according to the animal's position in the substrate. Thus, the deeper pallial sinus and greater length of shell posterior to the hinge observed in *P. donacina* give greater internal space for retracted siphons, and also protection for exserted siphons.

The significance of variability in pallial line curvature and muscle colour is unknown at present. The consistent difference in colour of the adductor muscles in *P. subtriangulata* and *P. donacina* indicated that protein analysis would be productive. Colour in the adductor muscles has been described previously only for a surface-dwelling bivalve, *Mercenaria mercenaria* (L.), and is not evident in the toheroa *P. ventricosum*, which lies deep in the sediments on many beaches where *P. subtriangulata* and *P. donacina* occur. The pallial line is the line of attachment of the pallial muscles, which anchor the mantle and also control the amount of water pumped in and out of the mantle cavity. The pallial sinus is an embayment of the pallial line, and is deeper in *P. donacina* that in *P. subtriangulata*. Consequently, the greater curvature of the pallial line in *P. donacina* may balance the size of the mantle cavity bounded by the pallial line and sinus. If the mantle cavity is not relatively constant in size, then amounts of water pumped in and out may vary between animals occupying surface and subsurface positions.

Differences in depth preference between *P. subtriangulata* and *P. donacina* are slight, and probably amount to only 1–2 cm. However, even this is sufficient for the influence of substrate position to be apparent. More characters appear to be influenced by the difference between surface and subsurface positions than by differences in depth of substrate occupied, however.

In this respect it is profitable to compare the habitat and the characters of *P. ventricosum* with those of *P. subtriangulata* and *P. donacina*. *P. ventricosum* is found on one of the beaches sampled (Peka Peka), at similar positions in the beach profile but buried in the sand to depths of approximately 18–24 cm. The same characters that differentiate *P. subtriangulata* and *P. donacina* may also be used to distinguish *P. subtriangulata* from *P. ventricosum* (algae, hydroids, pallial sinus depth, pallial line curvature, shell outlines)—characters related to the differences between surface and subsurface positions. Differences in those characters related to siphon length are greater in *P. ventricosum* than in *P. donacina*, and *P. ventricosum* also shows differences in characters associated in other bivalves with the occupation of deep substrate positions, namely hinge tooth size and ligament position.

Paul's (1959) separation of populations from Paraparaumu Beach into 3 groups is confirmed in part by our analyses of populations from other beaches, which show that Paul's groups correspond to *P. donacina* plus 2 groups within *P. subtriangulata*. These latter groups could be separated on attributes, but not on measurements or on esterase phenotypes. Their differences are not sufficiently marked to warrant their formal separation, but their existence does indicate the manner in which slight differences in habitat may show the nature of incipient speciation. Analysis of habitat and definition of selection pressures operating on populations are of some importance in view of the commercial value of members of the genus.

ACKNOWLEDGMENTS

This study was begun with information supplied by C. J. Hall, who had observed and recorded differences in populations of tuatua for many years at Raumati Beach. Similarly, O. Smuts-Kennedy and D. Neale assisted us with their observations and supplemented our collections of populations from Waikanae Beach. G. Harrison and C. Verburg are responsible for the photographs. M. Riley participated in all phases of collecting and measuring, and we are also grateful to L. J. Paul for making his unpublished report on tuatuas available. The incontrovertible logic of the young led N. Richardson to suggest that 'wanawana' would be an appropriate popular name for *P. donacina*.

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Appendix 1 Morphometric classification of tuatua shells as *Paphies subtriangulata* or *P. donacina*.

Length, width, and thickness are measured (to the nearest 0.01 cm), and these measurements are then combined according to the following rule to form a score:
score = 4.68–0.906(length) – 0.124(width as % length) + 0.262(thickness as % length)
A positive score indicates that the shell may be classified as *P. subtriangulata*, and a negative score denotes *P. donacina*. In this study the average of all scores from *P. subtriangulata* was + 1.23 and that from *P. donacina* was – 0.81. Scores near zero may lead to misclassification of shells. In this study misclassifications were 2% for *P. subtriangulata* and 13% for *P. donacina*, but larger collections are required from which to estimate realistic rates of misclassification.

Example

A shell in this study had a measured length of 6.25 cm, width 4.35 cm, and thickness 2.21 cm. Applying the above rule:
width as % length = (100 × 4.35)/6.25 = 69.6
thickness as % length = (100 × 2.21)/6.25 = 35.36
score =
4.68 – (0.906 × 6.25) – (0.124 × 69.6) + (0.262 × 35.4)
= –0.348
This negative score meant that the shell was classified (correctly) as *P. donacina*.