faire 3 photocop

Borsa, Philippe; Benzie, J.A.H.
Genetic relationships among the topshells Trochus and Tectus (Prosobranchia: Trochidae)
1993
Journal of molluscan Studies 59, 275-284.

à vous envoyer cop

Large species of Trochus and Tectus common on the Great Barrier Reef were screened for polymorphism at eleven putative allozyme loci. The samples were first identified as belonging to Trochus niloticus L., 1758, Trochus conus Gmelin, 1791, Trochus maculatus L., 1758 and Tectus pyramis (Born, 1778), but subsequent electrophoretic analysis showed that Trochus conus and Tectus pyramis each consisted of at least two reproductively isolated, sympatric

species. Some of these species matched older descriptions, based on shell morphology, of taxa which had since been synonymised. Allozyme polymorphism ranged from high in Tectus pyramis to nearly zero in Trochus maculatus. Average Nei's genetic distance between Trochus species was 1.726. Genetic distance between Tectus species was 1.510. Nei's genetic distances between Trochus and Tectus ranged from 3.226 to infinity. A phylogenetic tree based on genetic distances grouped together all four Trochus species versus the two Tectus species, confirming the validity of these two genera erected originally on the basis of shell morphology.

Keywords: Topshells, Trochidae, Trochus niloticus, Trochus maculatus, Trochus conus, Trochus virgatus, Tectus pyramis, Tectus tabidus, Tectus coerulescens, allozymes, population genetics, genetic distance, taxonomy, systematics, Great Barrier Reef, Coral Sea

J. Moll. Stud. (1993), **59**, 275–284

GENETIC RELATIONSHIPS AMONG THE TOPSHELLS *TROCHUS* AND *TECTUS* (PROSOBRANCHIA: TROCHIDAE) FROM THE GREAT BARRIER REEF

PHILIPPE BORSA[1] and JOHN A.H. BENZIE
Australian Institute of Marine Science, P.M.B. no.3, Townsville, Queensland 4810, Australia
(Received 22 June 1992, accepted 7 December 1992)

ABSTRACT

Large species of *Trochus* and *Tectus* common on the Great Barrier Reef were screened for polymorphism at eleven putative allozyme loci. The samples were first identified as belonging to *Trochus niloticus* L., 1758, *Trochus conus* Gmelin, 1791, *Trochus maculatus* L., 1758 and *Tectus pyramis* (Born, 1778), but subsequent electrophoretic analysis showed that *Trochus conus* and *Tectus pyramis* each consisted of at least two reproductively isolated, sympatric species. Some of these species matched older descriptions, based on shell morphology, of taxa which had since been synonymised. Allozyme polymorphism ranged from high in *Tectus pyramis* to nearly zero in *Trochus maculatus*. Average Nei's genetic distance between *Trochus* species was 1.726. Genetic distance between *Tectus* species was 1.510. Nei's genetic distances between *Trochus* and *Tectus* ranged from 3.226 to infinity. A phylogenetic tree based on genetic distances grouped together all four *Trochus* species versus the two *Tectus* species, confirming the validity of these two genera erected originally on the basis of shell morphology.

INTRODUCTION

Several large species of the genera *Trochus* and *Tectus* (Prosobranchia: Trochidae) are common on the Australian Great Barrier Reef (GBR). These gastropods are ecologically important as algal grazers in coral reef habitats (Klumpp & Pulfrich, 1989). *Trochus niloticus* is also of economic value on the GBR, where natural populations of this species are exploited for meat and nacre (Nash, 1985).

Trochus and *Tectus* species, including the common taxa currently recognized in the GBR, have been described on the basis of their shell morphology (e.g. Reeve, 1862). Some of these species have since been considered morphological variants of other taxa with which they were synonymised (e.g. Cernohorsky, 1972).

[1] Address for correspondence: ORSTOM, B.P.A5, Noumea, Nouvellé-Calédonie.

The grouping of species into two genera *Trochus* Linné, 1758 and *Tectus* Montfort, 1810, on the basis of shell architecture, is generally accepted (Cernohorsky, 1972; Abbott, 1986). However, there is still confusion as to whether *Trochus niloticus* L., 1758 and *Trochus conus* Gmelin, 1791 should be put into the genus *Tectus* instead of *Trochus* (A.M. Keen, in Moore, 1960; Kira, 1962; Habe, 1964). *Tectus* has also been considered as a sub-genus of *Trochus* (Rippingdale & McMichael, 1961; Wilson & Gillett, 1971). The systematics of these species are therefore in need of some revision.

The present paper reports the phylogenetic relationships inferred from allozyme variation in several species of *Trochus* and *Tectus* common on the GBR, in an attempt to clarify some aspects of the systematics of this group. In the process, taxa currently identified as *Trochus conus* and *Tectus pyramis* were each found to consist of at least two reproductively isolated sympatric species on the GBR, highlighting further taxonomic problems in the Trochidae with respect to the definition of species boundaries.

MATERIALS AND METHODS

Sampling

Animals were collected by SCUBA on three reefs of the GBR: Escape Reef (15°53'S, 145°48'E), Davies Reef (18°49'S, 147°39'E) and Square Reef (20°03'S, 149°49'E) (Fig. 1). Samples of *Trochus niloticus* L., 1758 were collected at Escape Reef and Square Reef (sample size, $n = 38$ for each sample). Samples first identified as *Tectus pyramis* (Born, 1778) were collected at Escape Reef ($n = 39$), Davies Reef ($n = 27$) and Square Reef ($n = 30$). *Trochus maculatus* L., 1758 ($n = 19$) and a species identified as *Trochus conus* Gmelin, 1791 ($n = 3$) were collected at Davies Reef. One more individual identified as *Trochus conus* was collected at Escape Reef.

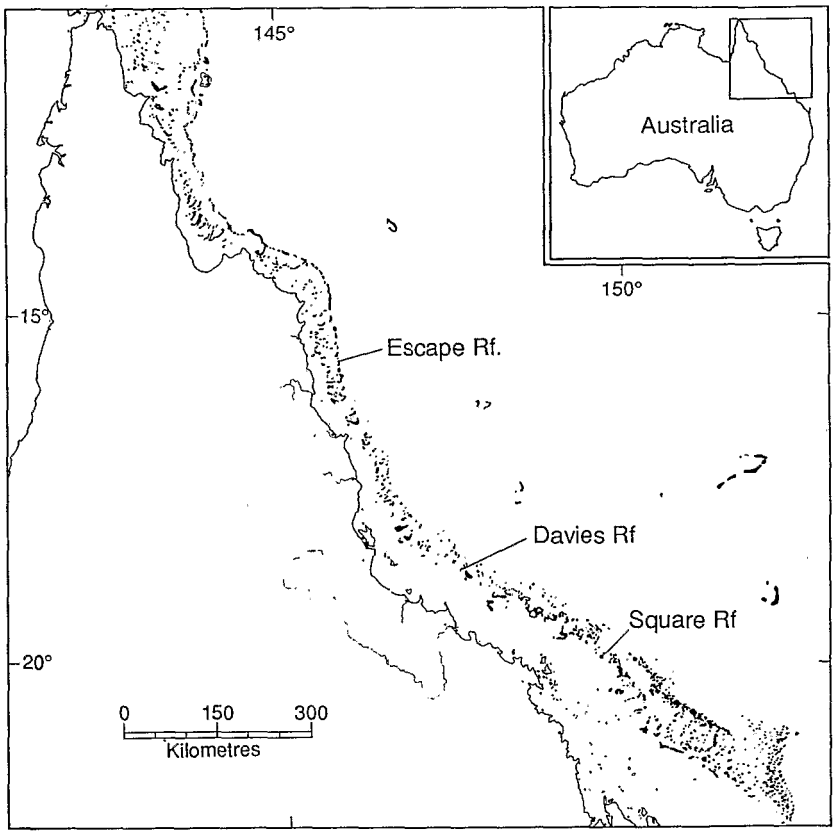

Figure 1. Location of sites sampled for *Trochus* and *Tectus* on the Great Barrier Reef.

Species identification

Identifications of *Tectus pyramis*, *Trochus conus* and *Trochus maculatus* were made by Mr I. Loch (Australian Museum, Sydney), on a few shells of each species which had been preserved (Fig. 2). These shells were deposited at the Australian Museum with registration numbers C.168454 to C.168463 (see Borsa & Benzie, 1992, for details).

The *Tectus pyramis* sample of Escape Reef could be separated into two distinct groups on the basis of shell morphology (see below). Electrophoretic data obtained in the present study indicated that these two groups belonged to two distinct species. One of these ($n = 2$ at Escape Reef) was morphologically similar to that sampled at Davies Reef and Square Reef, with shell pyramidal with a wide, flat base and whorls convexly flattened and tuberculated towards the apex (Fig. 2a). It will be referred to here as *Tectus pyramis*. The other species, collected only at Escape Reef ($n = 37$), had a shell of depressed conoid form with a wide, flat base and whorls flatly sloping and more prominently tuberculated than *Tectus pyramis* (Fig. 2b). It will be referred to here as *Tectus* aff. *pyramis*. The sample of *Trochus conus* ($n = 4$) was also morphologically heterogeneous, with one individual (Davies Reef) corresponding to the description of *Trochus conus* given in e.g. Abbott (1986), still called here *Trochus conus*, and which had a perfectly conical shell and round peristome (Fig. 2d). The shells of the three other individuals (two from Davies Reef, one from Escape Reef) were heavy, tall, slightly onion-shaped, their aperture slightly flattened and their entire surface covered by encircling ridges bearing granules (Fig. 2e, f). Allozymic data showed that these three individuals were of a separate group reproductively isolated from *Trochus conus*, referred to here as *Trochus* aff. *conus*.

The preferred habitat of *Trochus niloticus* was the most exposed part of the reef crest, the habitat of *Tectus* aff. *pyramis* was the reef flat behind the most exposed zone of the reef crest. The habitat of *Tectus pyramis* was on average more protected at Davies reef and Square Reef than that of *Tectus* aff. *pyramis* at Escape Reef. All other species were found on the more protected parts of the reef, on the leeward side or in the lagoon.

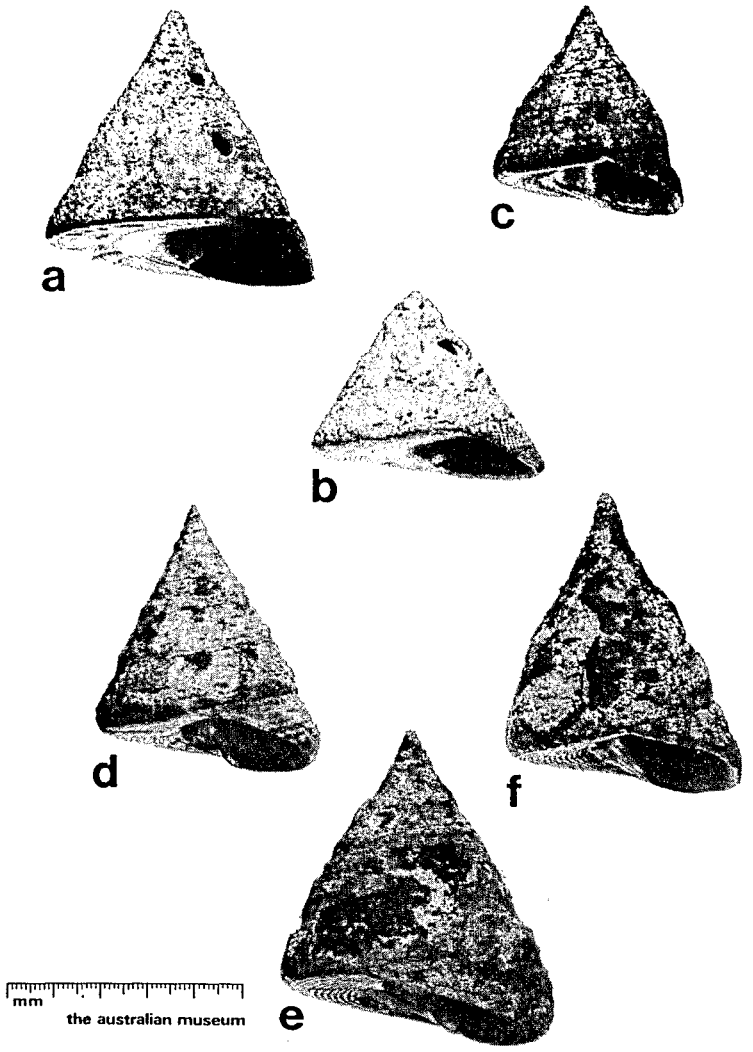

Figure 2. Voucher specimens of *Trochus* and *Tectus* from the Great Barrier Reef: **a.** *Tectus pyramis* (Born, 1778), Davies Reef, Central Great Barrier Reef (AM C.168458); **b.** *Tectus* aff. *pyramis*, Escape Reef, Great Barrier Reef off Cape Tribulation (AM C.168456); **c.** *Trochus maculatus* Linné, 1758, Square Reef, Southern Great Barrier Reef (AM C.168457); **d.** *Trochus conus* Gmelin, 1791, Davies Reef (AM C.168463); **e.** *Trochus* aff. *conus*, Davies Reef (AM C.168462); **f.** *Trochus* aff. *conus*, Escape Reef (AM C.168455). Scale bar = 50 mm.

Processing

Animals were kept alive in running seawater on board the research vessel until they were dissected and processed according to protocols reported in Borsa & Benzie (1992). Portions of digestive gland and columellar muscle tissue were dissected and stored at −80°C. Tissues were homogenised in an equal volume of aqueous β-mercaptoethanol and, in the case of muscle, centrifuged at 7,000*g* for 5 minutes and the supernatant used as the source of soluble enzymes.

Electrophoresis

The methods for electrophoresis were originally developed for *Trochus niloticus* and were extended to all species studied here. Detailed protocols for electrophoresis are given in Borsa & Benzie (1992). Nine

enzymes were assayed, selected on the basis of their electrophoretic intensity and resolution in *Trochus niloticus*: diaphorase (EC 1.6.-.-), esterase-D (EC 3.1.1.1), glucose-phosphate isomerase (EC 5.3.1.9), isocitrate dehydrogenase (EC 1.1.1.42), malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), non-specific dehydrogenase (EC 1.1.1.-), peptidase (EC 3.4.11.-) and phosphoglucomutase (EC 2.7.5.1). Because of (1) the consistency of electrophoretic banding patterns with their known quaternary structures and (2) the presence of all phenotypes expected to be in non-marginal proportions according to a Mendelian model, the genetic basis of the electrophoretic patterns for these enzymes has been ascribed to Mendelian variation at eleven loci, respectively: *Dia-1, Dia-2, Est-D, Gpi, Idh-1, Mdh-1, Mdh-2, Me-2, Ndh, Pep-2* and *Pgm* (Borsa & Benzie, 1992). Bands for *Mdh-1* and *Ndh* in *Tectus pyramis* were not detected on gels stained for these systems even though other species developed bands. The absence of activity was assumed to reflect the presence of an allele unique to *Tectus pyramis* in each case. No data were obtained for *Ndh* in *Trochus niloticus* from Square Reef.

Electromorph frequencies in each population were estimated directly from zymogram data.

Data analysis

The distribution of genotypic frequencies in each sample was compared to Hardy-Weinberg expectations by means of Weir & Cockerham's (1984) *f*-statistic. Single-locus *f* values were tested assuming that $f^2n(k—1)$ has a χ^2 distribution with $k(k—1)/2$ degrees of freedom under the null hypothesis of equilibrium (Waples, 1987), where n = sample size and k = number of alleles in the sample. Mean *f* over all polymorphic loci was compared to zero by Student's *t*-test.

Genetic variation in every population was estimated using three parameters: (1) genetic diversity,

$$H = 1/l\sum_{j}^{l} 2n_j(1 - \sum_{i}^{k} x_{ij}^2)/(2n_j - 1)$$

where l = number of loci and x_{ij} = frequency of electromorph i at locus j; (2) Percentage (P) of loci polymorphic, *i.e.* those for which the largest electromorph frequency was less than 0.95; (3) A = mean number of allelomorphs per locus, whose frequency was larger than 0.05.

Standard deviations around each estimation of mean *f*, *H*, *P* and *A* were estimated by jackknifing (Miller, 1974) the corresponding set of single-locus values.

Genetic distances were estimated using the indices of Nei (1972) and Rogers (1972) and a Wagner tree was built from the matrix of Rogers' genetic distances using Swofford & Selander's (1981) BIOSYS package. The assumption that the absence of activity in *Tectus pyramis* for *Mdh-1* and *Ndh* reflected the presence of an allele unique to *Tectus pyramis* in each case is the most conservative interpretation. It effectively treats these characters as autapomorphies which will not affect the branching pattern of the cladistic tree produced, but extend the length of only the branch from the last node connecting *Tectus pyramis* to the rest of the network.

The sample sizes for *Trochus conus* and *Trochus* aff. *conus* were respectively $n = 1$ and $n = 3$. Gorman & Renzi (1979) have empirically shown that such sample sizes could be considered large enough for approximately estimating genetic distances between species and genetic diversity.

RESULTS

Electromorph frequencies at eleven putative enzyme loci in samples of *Trochus maculatus*, *Trochus conus*, *Trochus* aff. *conus*, *Trochus niloticus*, *Tectus pyramis* and *Tectus* aff. *pyramis* are reported in Table 1. Raw genotypic data for the two individuals of *Tectus pyramis* collected at Escape Reef (reported in Borsa & Benzie, 1992) did not indicate differences with the two other samples (Davies Reef and Square Reef). Gene frequencies were similar in different populations of a single species, but differed markedly between species. Several fixed gene differences were observed between the taxa, including the cryptic *Tectus pyramis* and *Tectus* aff. *pyramis*, and *Trochus conus* and *Trochus* aff. *conus*. Of the nine loci surveyed in common in both species, four (*Dia-1, Est-D, Mdh-2* and *Me-2*) were diagnostic between *Tectus pyramis* and *Tectus* aff. *pyramis*. At three other loci (*Gpi*, *Pep-2* and *Pgm*), the most frequent electromorph in one species was not represented, or had a very low frequency in the other species. Six loci (*Dia-1*, *Gpi*, *Mdh-2*, *Ndh*, *Pep-2* and *Pgm*) out of eleven, were diagnostic between *Trochus conus* and *Trochus* aff. *conus*.

Heterozygote deficiencies (large positive values of Weir & Cockerham's *f*) were noted at locus *Est-D* in both *Tectus pyramis* and *Tectus* aff. *pyramis*, at loci *Me-2* and *Pgm* in *Tectus pyramis* and at *Pep-2* in *Tectus* aff. *pyramis*. Heterozygote excesses were observed at *Gpi* and *Pep-2* in each of the two samples of *Trochus niloticus* (Table 2). However, only one single-locus value among a total of twenty-eight and only one mean *f* value out of seven had a probability of occurrence of less than 0.05, results which might be expected by chance alone. The extreme values of heterozygote deficiency present in *Trochus* aff. *conus*, were due to the presence of one individual from Davies Reef (Fig. 2e) differentiated from the two others [one from Davies Reef, the other one (Fig. 2f) from escape Reef] by a fixed gene difference at each of three loci (*Dia-1*, *Gpi* and *Idh-1*). For

Table 1. Electromorph frequencies in eight populations of *Trochus* and *Tectus* from the Great Barrier Reef. **NIL1**, *Trochus niloticus*, Escape Reef; **NIL2**, *Trochus niloticus*, Square Reef; **MAC** *Trochus maculatus*, Davies Reef; **PYR1**, *Tectus pyramis*, Davies Reef; **PYR2**, *Tectus pyramis*, Square Reef; **(PYR)SP**, *Tectus* aff. *pyramis*, Escape Reef; **CON**, *Trochus conus*, Davies Reef; **(CON)SP**, *Trochus* aff. *conus*, Davies Reef and Escape Reef. Each electromorph numerotated as its mobility relative to the most common electromorph in *Trochus niloticus*. *n*, sample size.

Locus	Population							
	NIL1	NIL2	MAC	PYR1	PYR2	(PYR)SP	CON	(CON)SP
Dia-1								
100	1.000	1.000	0	0	0	0	0.500	0
116	0	0	0	0	0	1.000	0	0
126	0	0	0	1.000	1.000	0	0	0
127	0	0	0	0	0	0	0.500	0
132	0	0	1.000	0	0	0	0	0
136	0	0	0	0	0	0	0	0.333
160	0	0	0	0	0	0	0	0.667
n	38	38	19	27	30	37	1	3
Dia-2								
100	1.000	1.000	0	0	0	0	0	0
119	0	0	1.000	0	0	0	0	0
133	0	0	0	0	0	0	1.000	1.000
142	0	0	0	1.000	1.000	1.000	0	0
n	38	38	19	27	30	37	1	3
Est-D								
070	0.013	0	0	0	0	0	0	0
081	0	0	0	0	0	0	0	0.167
100	0.987	1.000	0	0	0	0	1.000	0.833
130	0	0	1.000	0	0	0.297	0	0
150	0	0	0	0	0	0.703	0	0
174	0	0	0	0.981	0.900	0	0	0
189	0	0	0	0.019	0.017	0	0	0
223	0	0	0	0	0.083	0	0	0
n	38	38	19	26	30	37	1	3
Gpi								
082	0	0	0	0	0	0	0.500	0
100	0.842	0.934	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0.500
160	0.158	0.066	0	0	0	0	0	0.167
173	0	0	0	0	0	0.946	0	0
200	0	0	0	0	0	0	0.500	0
204	0	0	1.000	0.019	0.021	0.054	0	0.167
223	0	0	0	0.204	0.083	0	0	0
230	0	0	0	0.019	0.063	0	0	0
236	0	0	0	0	0	0	0	0.167
248	0	0	0	0.185	0.375	0	0	0
263	0	0	0	0.111	0.042	0	0	0
290	0	0	0	0.444	0.396	0	0	0
300	0	0	0	0.019	0.021	0	0	0
n	38	38	19	27	24	37	1	3
Idh-1								
030	0	0	0	0	0	0	1.000	0.333
100	0.987	1.000	1.000	0	0	0	0	0
120	0	0	0	0	0	0.014	0	0
127	0	0	0	0	0	0.014	0	0
160	0.013	0	0	0	0	0	0	0
166	0	0	0	1.000	0.983	0.973	0	0
200	0	0	0	0	0.017	0	0	0.667
n	38	38	19	27	30	37	1	3

Table 1. *continued*

Locus	Population							
	NIL1	NIL2	MAC	PYR1	PYR2	(PYR)SP	CON	(CON)SP
Mdh-1								
075	0	0	1.000	—	—	0	0	0
090	0	0	0	—	—	1.000	0	0
100	1.000	1.000	0	—	—	0	0	0
110	0	0	0	—	—	0	1.000	1.000
n	38	38	19	0	0	2	1	3
Mdh-2								
100	1.000	1.000	0	0	0	0	0	0
128	0	0	0	0	0	1.000	0	0
137	0	0	0	0	0	0	0	1.000
139	0	0	0	0.778	0.750	0	0	0
171	0	0	1.000	0	0	0	1.000	0
200	0	0	0	0.222	0.250	0	0	0
n	38	38	19	27	30	37	1	3
Me-2								
009	0	0	0	0	0.083	0	0	0
027	0	0	0	1.000	0.917	0	0	0
040	0	0	0	0	0	1.000	0	0
092	0	0	1.000	0	0	0	0	0
100	1.000	1.000	0	0	0	0	1.000	1.000
n	38	38	19	27	30	37	1	3
Ndh								
050	0	—	1.000	—	—	0	0	0
066	0	—	0	—	—	1.000	0	0
075	0	—	0	—	—	0	1.000	0
100	1.000	—	0	—	—	0	0	1.000
n	17	0	6	0	0	3	1	3
Pep-2								
075	0	0	0.026	0	0	0	0	0
085	0	0	0	0	0	0	0	0
086	0	0	0	0	0	0	1.000	0
088	0	0	0.974	0	0	0	0	0.833
089	0.092	0.066	0	0	0	0	0	0
094	0.368	0.382	0	1.000	0.967	0.068	0	0.167
100	0.539	0.539	0	0	0.017	0.351	0	0
106	0	0.013	0	0	0.017	0.581	0	0
n	38	38	19	27	30	37	1	3
Pgm								
082	0	0	0	0	0	0	0.500	0
094	0	0	0	0	0	0	0.500	0
095	0	0	1.000	0	0	0	0	0
100	1.000	1.000	0	0	0	0	0	0
103	0	0	0	0	0	0	0	1.000
110	0	0	0	0.542	0.400	0.041	0	0
117	0	0	0	0.208	0.200	0	0	0
120	0	0	0	0.208	0.250	0	0	0
122	0	0	0	0	0	0	0	0
124	0	0	0	0.042	0	0	0	0
132	0	0	0	0	0	0.959	0	0
135	0	0	0	0	0.150	0	0	0
n	38	38	19	12	10	37	1	3

Table 2. Weir and Cockerham's (1984) fixation index (f) values in seven populations of *Trochus* and *Tectus* species from the Great Barrier Reef. ND, no data; *SD*, jackknife estimate of standard deviation; other abbreviations as in Table 1.

Locus	Population						
	NIL1	NIL2	MAC	(CON)SP	PYR1	PYR2	(PYR)SP
Dia-1	—	—	—	1.000	—	—	—
Dia-2	—	—	—	—	—	—	—
Est-D	0.000	—	—	0.000	0.000	0.286	0.237
Gpi	−0.175	−0.057	—	0.200	−0.020	0.000	−0.044
Idh-1	0.000	—	—	1.000	—	−0.067	−0.007
Mdh-1	—	—	—	—	ND	ND	—
Mdh-2	—	—	—	—	−0.053	0.039	—
Me-2	—	—	—	—	—	0.360*	—
Ndh	—	ND	—	—	ND	ND	—
Pep-2	−0.118	−0.306	0.000	0.000	—	−0.009	0.237
Pgm	—	—	—	—	0.364	0.212	−0.029
mean f	−0.060	−0.261	0.000	0.529	0.116	0.104	0.184*
SD	0.084	0.126	—	0.238	0.155	0.090	0.051

* $p < 0.05$.

Gpi this individual was heterozygous for two alleles absent in the other two individuals (Borsa & Benzie, 1992).

Values of mean genetic diversity per locus (H), percentage of loci polymorphic (P) and mean number of allelomorphs per locus (A) with their jackknife estimates of standard deviation are reported for each species in Table 3. Two species were very polymorphic, namely, *Trochus* aff. *conus* and *Tectus pyramis*, and one species almost totally monomorphic (*Trochus maculatus*). The values of H, P and A for the other species were intermediate.

A Wagner tree (Fig. 3) was built from the matrix of Rogers' genetic distances presented in Table 4 and rooted at the midpoint of the largest segment. This was used to represent the hypothetical phylogenetic relationships among the six species from the family Trochidae. The first node of this tree separated all four *Trochus* species from the two *Tectus* species. *Trochus niloticus* and *Trochus conus* clearly were grouped within the *Trochus* branch.

Rogers' distances between populations within a species (*Trochus niloticus* and *Tectus pyramis*) were one to two orders of magnitude less than those between species (Table 4). Values of Nei's standard genetic distance are also provided to assist comparisons with results in the literature. Nei's genetic distance between

Table 3. Genetic variation in eight populations of *Trochus* and *Tectus* species from the Great Barrier Reef, based on the electromorph frequency data at 9 loci scored in all populations (see Table 1); n, mean sample size per locus; H, mean genetic diversity per locus; P, percentage of loci polymorphic (0.95 level); A, mean number of alleles per locus (only alleles whose frequency > 0.05 are considered); *SD*, jackknife estimate of standard deviation; other abbreviations as in Table 1.

Population	n	$H \pm SD$	$P \pm SD$	$A \pm SD$
Trochus niloticus (NIL1)	38.0	0.099 ± 0.066	0.22 ± 0.15	1.33 ± 0.24
Trochus niloticus (NIL2)	38.0	0.077 ± 0.063	0.22 ± 0.15	1.33 ± 0.24
Trochus maculatus	19.0	0.006 ± 0.006	0.00 ± 0.00	1.00 ± 0.00
Trochus conus	1.0	0.222 ± 0.111	0.33 ± 0.17	1.33 ± 0.17
Trochus aff. *conus*	3.0	0.281 ± 0.100	0.56 ± 0.18	1.78 ± 0.32
Tectus pyramis (PYR1)	25.2	0.196 ± 0.100	0.33 ± 0.17	1.67 ± 0.37
Tectus pyramis (PYR2)	27.1	0.253 ± 0.098	0.56 ± 0.18	2.00 ± 0.41
Tectus aff. *pyramis*	37.0	0.133 ± 0.068	0.33 ± 0.17	1.44 ± 0.24

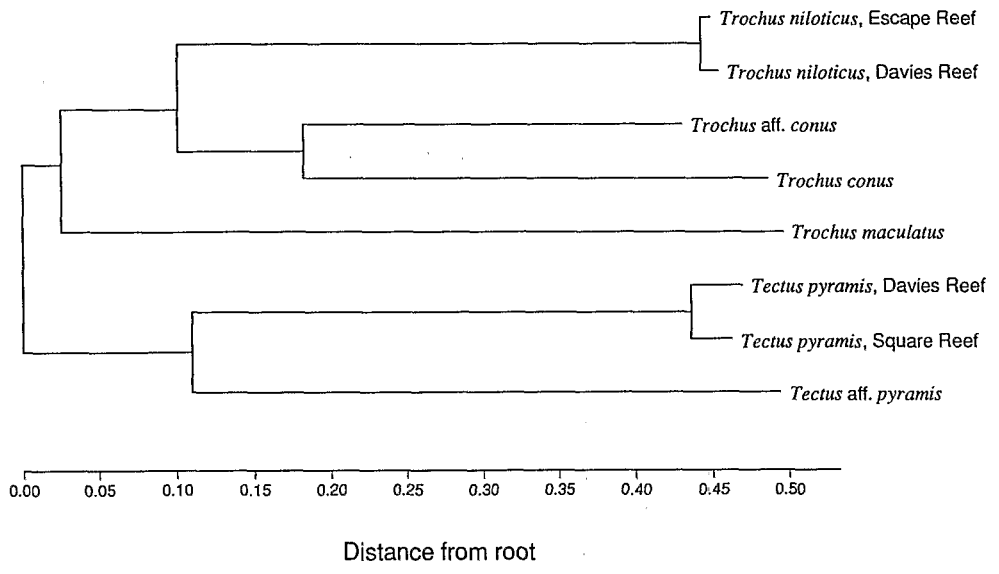

Figure 3. Wagner tree illustrating the hypothetical phylogenetic relationships among 6 taxa from the genera *Trochus* and *Tectus*. Total tree distance was 2.419.

Trochus and *Tectus* ranged between 3.226 and infinity. The average genetic distance between species within genus *Trochus* was 1.726, and 1.510 between *Tectus pyramis* and *Tectus* aff. *pyramis*.

DISCUSSION

The allozyme survey of several taxa confirmed two basic, monophyletic, groupings of trochid species into the genera *Trochus* and *Tectus* erected originally on the basis of shell architecture (Hickman & McLean, 1990). *Trochus niloticus* and *Trochus conus* were each placed within the genus *Trochus* establishing their generic placement which had been subject to some doubt.

The survey also revealed that *Tectus pyramis* and *Trochus conus*, each consist of two species on the Great Barrier Reef. Given the high proportion of diagnostic loci and the sympatric occurrence of the taxa, there is no doubt that reproductive isolation between the latter is total. It is also pertinent to note that genetic distances among the 'sibling' taxa were orders of magnitude greater than inter-populational differences within species, but of the same order as those among the recognised trochid species, and as inter-specific distances in other molluscs (e.g. Skibinski *et al.*, 1980; Buroker, 1982).

Table 4. Values of genetic distances among 8 populations of *Trochus* and *Tectus* species from the Great Barrier Reef. Above diagonal: Rogers' (1972) estimator; below diagonal: Nei's (1972) estimator. Abbreviations as in Table 1.

Population		NIL1	NIL2	MAC	CON	(CON)SP	PYR1	PYR2	(PYR)SP
Trochus niloticus	NIL1	—	0.013	0.888	0.728	0.668	0.911	0.899	0.930
Trochus niloticus	NIL2	0.001	—	0.891	0.731	0.675	0.916	0.903	0.934
Trochus maculatus	MAC	2.367	2.364	—	0.871	0.869	0.955	0.943	0.951
Trochus conus	CON	1.372	1.376	2.322	—	0.545	0.913	0.902	0.934
Trochus aff. *conus*	(CON)SP	1.181	1.192	2.311	0.791	—	0.890	0.879	0.916
Tectus pyramis	PYR1	3.270	3.245	6.300	*infinity*	3.976	—	0.051	0.745
Tectus pyramis	PYR2	3.251	3.226	6.154	*infinity*	3.918	0.007	—	0.732
Tectus aff. *pyramis*	(PYR)SP	3.839	3.810	3.385	*infinity*	6.133	1.532	1.508	—

Heterozygote deficits and, in some instances, excesses, are commonly found in marine molluscs (Zouros & Foltz, 1984; Koehn *et al.*, 1988). Minor departures from expected genotype frequencies in all species studied here except *Trochus* aff. *conus* were not significant (Table 2) and may reflect stochastic effects of sampling. There was no evidence of inbreeding of an extent that might indicate the occurrence of other sibling taxa. On the other hand, the extreme values of heterozygote deficiency in *Trochus* aff. *conus*, although based on only three individuals, and due to one individual genetically very different from the two others, may indicate the occurrence of another, third, sibling species within the group currently recognised as *Trochus conus*.

Each taxon could also be distinguished on shell morphology and some habitat separation was also observed (see above). When comparing sample shells with older descriptions from the literature, there was no ambiguity concerning the identification of *Trochus niloticus*, *Trochus maculatus* and *Trochus conus*. One of the species referred to here as *Trochus* aff. *conus* (Fig. 2e) fitted the description of *Trochus virgatus* Gmelin, 1791 (Abbott, 1986). The species referred to here as *Tectus pyramis* corresponded to *T. coerulescens* originally described by Lamarck (1822), and illustrated by Kiener & Fischer (1850). The species referred to here as *Tectus* aff. *pyramis* fitted the original description of *T. tabidus* given by Reeve (1862; Plate XIII, species 74).

We suggest that the name *Tectus tabidus* (Reeve, 1862) (our *Tectus* aff. *pyramis*) be used again. Further research is needed to decide which of the names *Tectus pyramis* or *Tectus coerulescens* should in fact be retained for the *Tectus* commonly found at Davies Reef and Square Reef (our *Tectus pyramis*).

The estimated value of genetic diversity in *Trochus maculatus* (H = 0.006) was uncommonly low for a marine gastropod. For example, the mean observed heterozygosity value was H_0 = 0.158 with no individual value lower than 0.017 among fifteen marine gastropod species listed by Brown & Richardson (1988). Because other biological and historical parameters are unknown, no clear model can be invoked to account for the low genetic diversity in *Trochus maculatus*, whether based on the hypothesis of recent population bottlenecks (Nei *et al.*, 1975), directional selection (Soulé, 1976) or trophic resource stability (Redfield *et al.*, 1980). At least, this result is inconsistent with the claim that shallow water tropical marine invertebrates have high levels of heterozygosity in relation to their environment (Valentine, 1976). There is a marked contrast between the low genetic diversity (as estimated by enzyme electrophoresis), and the high morphological variability that has been reported for *Trochus maculatus* (Cernohorsky, 1972).

The present results confirm that some older descriptions of trochid species based on shell morphology were correct and indicate that some of the morphological variation presently considered intraspecific instead reflects the occurrence of separate species. The genera *Trochus* and *Tectus* may require further taxonomic revision.

ACKNOWLEDGEMENTS

We are grateful to Mr I. Loch and to Dr P. Coleman, Australian Museum, Sydney, for identification of the specimens, to Dr P. Bouchet, Muséum National d'Histoire Naturelle, Paris, for bibliographic assistance and for pointing out the description of *T. tabidus* and to P. Chongprasith, M. Raymond, Laboratoire de Génétique et Environnement and Laboratoire Génome et Populations, Montpellier, for support during the preparation of the manuscript. Field work was possible thanks to the staff at AIMS Marine Operations and the crews of Research Vessels *Harry Messel*, *Sirius* and *Pegasus*. Sample collections were done under Great Barrier Reef Marine Park Authorities permit n°G91/098. P.B. was supported by a postdoctoral Lavoisier fellowship from the French Ministère des Affaires Etrangères. This is contribution number 624 from the Australian Institute of Marine Science.

REFERENCES

ABBOTT, R.T. 1986. *Compendium of seashells*. Crawford House Press, Bathurst.

BORSA, P. & BENZIE, J.A.H. 1992. Methods for allozyme electrophoresis of the topsnails *Trochus* and *Tectus* (Prosobranchia: Trochidae). *Australian Institute of Marine Science Reports*, **5**: 1-31.

BROWN, K.M. & RICHARDSON, T.D. 1988. Genetic polymorphism in gastropods: a comparison of methods and habitat scales. *American Malacological Bulletin*, **6**: 9-17.

BUROKER, N.E. 1982. Allozyme variation in three non-sibling *Ostrea* species. *Journal of Shellfish Research*, **2**: 157-163.

CERNOHORSKY, W.O. 1972. *Marine shells of the Pacific*, **2**. Pacific Publications, Sydney.

GORMAN, G.C. & RENZI, J., JR 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effect of sample size. *Copeia*, **2**: 242-249.

HABE, T. 1964. *Shells of the western Pacific in color*, **2**. Hoikusha, Osaka.

HICKMAN, C.S. & MCLEAN, J.H. 1990. *Systematic revision and suprageneric classification of trochacean gastropods*. Natural History Museum, Los Angeles.

KIENER, L.-C. & FISCHER, P. 1850. *Spéciès général et iconographie des coquilles vivantes, comprenant la collection du Museum d'Histoire naturelle de Paris, la collection Lamarck, celle du Prince Masséna (appartenant maintenant à M. B. Delessert) et les découvertes récentes des voyageurs*, **15**. J.-B. Baillère et fils, Paris.

KIRA, T. 1962. *Shells of the western Pacific in color*. Hoikusha, Osaka.

KLUMPP, D.W. & PULFRICH, A.W. 1989. Trophic significance of herbivorous macroinvertebrates of the Central Great Barrier Reef. *Coral Reefs*, **8**: 135-144.

KOEHN, R.K., DIEHL, W.J. & SCOTT, T.M. 1988. The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulinia lateralis*. *Genetics*, **118**: 121-130.

LAMARCK, J.-B. DE 1822. *Histoire naturelle des animaux sans vertèbres*, **9**. Librairie Verdière, Paris.

MILLER, R.G. 1974. The jackknife—a review. *Biometrika*, **61**: 1-15.

MOORE, R.C. (Ed.) 1960. *Mollusca I. Treatise on invertebrate paleontology*. Geological Society of America, University of Kansas Press, Lawrence.

NASH, W.J. 1985. Aspects of the biology of *Trochus niloticus* and its fishery in the Great Barrier Reef region. Unpublished report to the Queensland Department of Primary Industries and the Great Barrier Reef Marine Park Authorities.

NEI, M. 1972. Genetic distance between populations. *American Naturalist*, **106**: 283-292.

NEI, M., MARUYAMA, T. & CHAKRABORTY, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution*, **29**: 1-10.

REDFIELD, J.A., HEDGECOCK, D., NELSON, K. & SALINI, J.P. 1980. Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis. *Marine Biology Letters*, **1**: 303-313.

REEVE, L.A. 1862. *Conchologia iconica*, **13**. Lovell Reeve, London.

RIPPINGDALE, O.H. & MCMICHAEL, D.F. 1961. *Queensland and Great Barrier Reef shells*. Jacaranda Press, Brisbane.

ROGERS, J.S. 1972. Measures of genetic similarity and distance. *Studies in Genetics VII. University of Texas Publications*, **11**: 736-741.

SKIBINSKI, D.O.F., CROSS, T.F. & AHMAD, M. 1980. Electrophoretic investigation of systematic relationships in the marine mussels *Modiolus modiolus* L., *Mytilus edulis* L., and *Mytilus galloprovincialis* Lmk. (Mytilidae, Mollusca). *Biological Journal of the Linnean Society*, **13**: 65-73.

SOULE, M.E. 1976. Allozyme variation: its determinants in space and time. In: *Molecular evolution* (F.J. Ayala, ed.), 60-77. Sinauer, Sunderland.

SWOFFORD, D.L. & SELANDER, R.K. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity*, **72**: 281-283.

VALENTINE, J.W. 1976. Genetic strategies of adaptation. In: *Molecular evolution* (F.J. Ayala, ed.), 78-94. Sinauer, Sunderland.

WAPLES, R.S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution*, **41**: 385-400.

WEIR, B.S. & COCKERHAM, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358-1370.

WILSON, B.R. & GILLETT, K. 1971. *Australian shells*. A.H. & A.W. Reed, Sydney.

ZOUROS, E. & FOLTZ, D.W. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, **25**: 583-591.