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AIMS Report Number 5 1992

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AIMS Report
Number 5

Australian Institute of Marine Science
Townsville
1992

Copies available from:
Production Editor,
Information Services Section,
Australian Institute of Marine Science
PMB No. 3, Townsville M.C.,
Queensland 4810. Australia.

AIMS Report series: ISSN 1033-6974

National Library of Australia Cataloguing-in-Publication data:

Borsa, Philippe.
Methods for allozyme electrophoresis of the top snails *Trochus* and *Tectus* (Prosobranchia : Trochidae).

Bibliography.
ISBN 0 642 17452 0.

1. Prosobranchia - Queensland. 2. Enzymes - Analysis - Technique. 3. Electrophoresis - Technique. 4. Population genetics - Technique.
I. Benzie, John A.H. (John Alexander H.), II. Australian Institute of Marine Science.
III. Title. (Series : AIMS report; no. 5).

594.32

CONTENTS

INTRODUCTION

Allozyme electrophoresis provides data (simultaneous phenotype for multiple enzymes in large numbers of individuals), that can directly be interpreted in genetic terms. These data have proven useful in studies of population genetics, phylogeny and systematics in a wide range of organisms.

Details concerning preliminary screening for enzyme activity, selection of enzymes, choice of buffers, and description of zymograms no longer appear in papers of population genetics using allozyme electrophoresis. This information is however highly desirable because it varies from one group to another: it saves time and resources in studies of a species for which the electrophoretic techniques have already been refined. Such data help to assess the quality of the genetic interpretation of allozyme patterns, which is the basis of subsequent genetic data processing. It is also useful, in the event further developments are attempted, to know the methods tried previously which did not work and which are rarely reported.

The information presented in this report is a record of the development of genetic markers for comparative studies on populations and species of the top snails *Trochus* and *Tectus* (Prosobranchia, Trochidae).

MATERIALS AND METHODS

Sample collection and processing

Samples of *Trochus niloticus, T. maculatus, T. conus* and *Tectus pyramis* (plus, among these, two species subsequently revealed by electrophoresis and referred to as *Trochus (conus) sp*, and *Tectus (pyramis) sp.)* were collected by SCUBA diving on various reefs of the Northern, Central, and Southern sections of the Great Barrier Reef. The reef crest's turf zone was the preferred habitat of *T. niloticus* and *Tectus sp*. The other species were more frequent in less exposed parts of the reef.

Catch per unit of effort of the abundant *Trochus niloticus* varied between 1.00 individual per minute per diver (in some pristine Southern reefs) and 0.03 ind. $min^{-1}.diver^{-1}$ (e.g. in heavily harvested reefs, e.g. Bowden Reef).

Animals were kept alive on board ship in running seawater, until they were dissected. The shell was crushed with a hammer in such a way that columellar muscle and digestive gland and gonad were preserved intact. Shells were first hit sharply on the side, to expose the animal. Soft parts could also be removed by drilling a hole near the apex of the conch and flushing the tissues out with water. This allowed the shell to be preserved for later morphological observations.

Cubes of approximately 0.1 cm^3 were dissected from the tender part of the columellar muscle and from the digestive gland, put into small polypropylene tubes, and immediately frozen in liquid nitrogen. They were then stored at -80°C until analysis.

Before each electrophoretic run, tissues were homogenized in a 0.04% solution of ß-mercaptoethanol, slightly coloured with Bromophenol Blue. Muscle tissue was homogenized with an equal volume of grinding solution in a ceramic dish using a stainless steel pestle and crushed glass. When a paste of even colour and texture was obtained, this was centrifuged at 7000 *g* for 5 minutes and the supernatant used for electrophoresis. Digestive gland tissue was homogenized with an equal volume of grinding solution by squeezing it into a hole in a perspex plate.

Electrophoresis

Electrophoresis was performed according to standard horizontal starch gel methods as described in Pasteur *et al.* (1988), and cellulose acetate gel methods as described in Richardson *et al* (1986). Starch gels were made of 13% Sigma starch in buffer (12% when buffer TEB was employed). Cellulose acetate gels (Cellogel^{TM}) were purchased from Chemetron, Milan, Italy. Recipes of the different electrode and gel buffers used for starch and cellogel are given in Table 1. Those for starch were adapted from Boyer, Fainer & Watson-Williams (1963), Shaw & Prasad (1970) and Soltis *et al.* (1983). Those for cellogel were from Richardson *et al* (1986).

Stain recipes

Staining followed Richardson *et al.* (1986) for all enzymes except AAT and EST-D for which the stain recipes were those of Pasteur *et al.* (1988).

Table 1. Details of buffer systems used with starch gels (12% Sigma starch) or cellulose acetate gels (Cellogel ^{TM}, Chemetron, Milan) in a preliminary screening for enzyme activity in *Trochus niloticus*.

Buffer	pH	Composition (electrodes)		(gel)
Starch				
TC	8.0	172 mM	Tris	1/6(*) electrode concentration
		39 mM	citric acid	
TEB	8.4	150 mM	Tris	1/3 electrode concentration
		3 mM	EDTA	
		117 mM	boric acid	
TEC	7.9	135 mM	Tris	1/6 electrode concentration
		4 mM	EDTA	
		32 mM	citric acid	
TM	7.4	100 mM	Tris	1/10 electrode concentration
		100 mM	maleic acid	
		10 mM	EDTA	
		1 mM	$MgCl_2$	
		12.5 mM	NaOH	
Cellogel				
CP	6.4	10 mM	Na_2HPO_4	-
		2.5 mM	citric acid	
Phos	7.0	11.6 mM	Na_2HPO_4	-
		8.4 mM	NaH_2PO_4	
TM	7.8	50 mM	Tris	-
		20 mM	maleic acid	

(*) or 1/12 for TC 8.0 [1/2]

RESULTS

Activity

Thirty-eight enzymes were tested for activity in *Trochus niloticus*. These included: (1) all enzymes that have been reported active in other allozyme surveys in molluscs and (2) some enzymes commoly reported active in other groups.

Both muscle and digestive gland tissues from the same 4-10 individuals were tested for each enzyme, together on the same gels. Up to 4 buffer systems in starch and 3 buffer systems in cellogel (Table 1) were tested for each enzyme.

Results of this preliminary step are reported in Table 2. Details of activity according to tissue and buffer for enzymes that showed some activity in this preliminary step are reported in Table 3.

Polymorphism

Seventeen enzymes were selected on the basis of their activity and resolution for the next step of the study: screening for polymorphism. This was done using the combinations of tissue and buffer that had given the best results in earlier tests. An average number of 60 individuals were tested, chosen in similar proportions from the Northern, Central, and Southern sections of the Great Barrier Reef (Northern: Evening Reef and Low Islets; Central: Davies Reef; Southern: Hunt Reef and Little Chauvel Reef). Results are reported in Table 4.

Table 2. Enzymes tested for activity in *Trochus niloticus* from the Great Barrier Reef, with abbreviation (Abbr.) and Enzyme Commission number (E.C.#). o Buffer tested. Tissues assayed: M muscle; DG digestive gland. Details of the buffers used are given in Table 1.

Enzyme	Abbr.	E.C.#	Buffer (starch)				Buffer (cellogel)			Activity(*) in:	
			TC8	TEB	TM	TEC	CP	Phos	TM	M	DG
Aspartate aminotransferase	AAT	2.6.1.1	o	o	o		o	o	o	-	-
Acid phosphatase	ACP	3.1.3.2	o	o	o					+	+
Adenosine deaminase	ADA	3.5.4.4					o	o	o	-	-
Alcohol dehydrogenase	ADH	1.1.1.1	o	o	o					-	-
Alkaline phosphatase	ALP	3.1.3.1	o	o	o					-	-
Aldehyde dehydrogenase	ALDH	1.2.1.5	o	o	o					-	-
Diaphorase	DIA	1.6.-.-	o	o	o	o				-	+
Enolase	ENOL	4.2.1.11	o	o	o	o	o	o	o	+	-
Esterase (4MU)	EST-D	3.1.1.1	o	o	o	o	o	o	o	+	-
Esterase (Naphthyl acetate)	EST-N	3.1.1.1	o	o	o	o	o	o	o	-	+
Glucose dehydrogenase	GDH	1.1.1.47	o	o	o	o				w	-
Glutamate dehydrogenase	GLDH	1.4.3.1.	o	o	o					-	-
Glyoxalase	GLO	4.4.1.5	o	o	o					w	-
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH	1.2.1.2	o	o	o	o	o	o	o	+	-
α Glycerol-3-phosphate dehydrogenase	$^{\alpha}$GPD	1.1.1.8	o	o	o					w	w
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	o	o	o		o	o	o	w	w
Glucose-phosphate isomerase	GPI	5.3.1.9	o	o	o		o	o	o	+	+
Glutathione reductase	GSR	1.6.4.2	o	o	o					-	-
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30					o	o	o	-	-
Hexokinase	HK	2.7.1.1	o	o	o		o	o	o	w	-
Isocitrate dehydrogenase	IDH	1.1.1.42	o	o	o	o	o	o	o	+	+
Leucine aminopeptidase	LAP	3.4.11.1	o	o	o		o	o	o	-	+
Lactate dehydrogenase	LDH	1.1.1.27	o	o	o					-	-
Malate dehydrogenase	MDH	1.1.1.37	o	o	o		o	o	o	+	w
Malic enzyme	ME	1.1.1.40	o	o	o		o	o	o	+	w
Mannose-phosphate isomerase	MPI	5.3.1.8	o	o	o	o	o	o	o	+	-
Nucleoside phosphorylase	NP	2.4.2.1	o	o	o		o	o	o	w	+
Octopine dehydrogenase	OPDH	1.5.1.11	o	o	o					-	-
Peptidase (Ala Pro)	PEP(AP)	3.4.11.-				o				-	w
Peptidase (Leu Ala)	PEP(LA)	3.4.11.-				o				+	+
Peptidase (Leu Gly Gly)	PEP(LGG)	3.4.11.-	o	o	o	o	o	o	o	+	+
Peptidase (Leu Pro)	PEP(LP)	3.4.13.9				o				-	+
Peptidase (Leu Tyr)	PEP(LT)	3.4.11.-	o	o	o	o	o	o	o	+	+
Peptidase (Val Leu)	PEP(VL)	3.4.11.-	o	o	o	o	o	o	o	+	+
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	o	o	o					w	w
Phosphoglucomutase	PGM	2.7.5.1	o	o	o		o	o	o	+	+
Superoxide dismutase	SOD	1.15.1.1	o	o	o	o	o	o	o	w	+
Xanthine dehydrogenase	XDH	1.2.1.37	o	o	o					-	-

(*) - no activity
w weak
\+ activity sufficient to warrant further investigation

Table 3. Details of buffers showing acceptable results (activity and resolution) for enzyme systems selected from preliminary screening for activity (Table 2). Capitals: starch; small letters: cellogel. Activity and resolution: bold: good; standard: acceptable.

Enzyme	Muscle		Digestive Gland	
	Activity	Resolution	Activity	Resolution
ACP	TEB	TEB	**ALL**	*poor*
DIA	TEB TEC TM	*poor*	**TEB TEC TM**	TEB TEC
ENOL	phos tm cp	phos tm	-	-
EST-D	**TEB TM all**	**TEB TM TEC tm phos**	-	-
EST-N	-	-	**ALL all**	**TEB TEC TM tm**
GDH	*poor*	TEC	-	-
GLO	*poor*	TEB	-	-
G3PDH	**ALL all**	TC tm cp	-	-
αGPD	*poor*	*poor*	*poor*	*poor*
G6PD	*poor*	*poor*	*poor*	*poor*
GPI	**ALL all**	*poor*	**ALL all**	TC phos cp
HK	TC all	TC phos tm	-	-
IDH	**ALL all**	**TC TEC phos TM cp**	**ALL all**	TC
LAP	-	-	*poor*	*poor*
MDH	**ALL tm cp phos**	**TM cp tm phos**	*poor*	*poor*
ME	**ALL all**	TM phos	-	-
MPI	ALL tm	*poor*	-	-
NP	TM tm	*poor*	**TM tm**	*poor*
PEP(LGG)	TEB TEC TC	TEB TEC	ALL	*poor*
PEP(LT)	TEC TC TEC	TEC TEB	ALL	*poor*
PEP(VL)	TC TEB TEC	TEB TEC	ALL	*poor*
6PGD	*poor*	TC	*poor*	*poor*
PGM	**ALL all**	**TC cp TEB TM**	**ALL all**	**TC cp phos TEB TM**
SOD	TEB TEC	TEB TEC	**TEB TEC tm**	TEB TEC tm

Table 4. Results of preliminary screening for variability in samples of *Trochus niloticus* from various regions of the Great Barrier Reef, for 17 enzymes selected from Tables 2 and 3. Choice of buffers was based on the results presented in Table 3. M muscle; DG digestive gland. N Northern; C Central; S Southern Great Barrier Reef; + Enzyme variable; - no variability in the sample.

Enzyme	Buffer used (medium)	Tissue	Number of activity zones		Preliminary screening N	C	S	Total	Variability
DIA	TEB 8.4 (starch)	DG	2	(DIA-1)	20	22	20	62	-
				(DIA-2)	20	22	20	62	-
ENOL	Phos 7.0 (cellogel)	M	1		5	20	20	45	-
EST-D	TM 7.4 (starch)	M	1		20	19	19	58	-
EST-N	TM 7.4 (starch)	DG	1		20	19	19	58	-
GDH	TEC 7.9 (starch)	M	1			12		12	+
G3PDH	TC 8.0 (starch)	M	1		25	20	20	65	-
GPI	TC 8.0 (starch)	DG	1		25	19	19	63	+
HK	TC 8.0 (starch)	M	1		20	14	20	54	-
IDH	TC 8.0 (starch)	M	2	(IDH-1)	20	19	19	58	-
				(IDH-2)	20	19	19	58	-
MDH	TM 7.4 (starch)	M	2	(MDH-1)	20	19	19	58	-
				(MDH-2)	20	19	19	58	-
ME	TM 7.4 (starch)	M	1		20	19	19	58	-
MPI	TC 8.0 (starch)	M	1		20	19	24	63	+(1)
PEP(LT)	TEB 8.4 (starch)	M	3	(PEP-2)	20	20	20	60	+(2)
6PGD	TC 8.0 (starch)	M	1		25	20	20	65	-
PGM	TC 8.0 (starch)	M	1		25	19	19	63	-
SOD	TEB 8.4 (starch)	DG	2	(SOD-1)	20	27	20	67	-

(1)
MPI patterns were variable among individuals, but resolution too poor to allow reliable scoring.

(2)
Only PEP-2, intense and polymorphic, was considered at that stage. PEP-1 and PEP-3 zones of activity were eventually considered too weak to be scored.

Effect of freezing / defreezing

During the phase of screening for polymorphism, a subset of 3-10 individuals of those to be run were ground the day before each run, and their enzyme extracts stored overnight at -80°C. These extracts were then assayed on the same gels as fresh extracts from the same individuals, on the day of the run.

Overnight freezing of the enzyme extract affected enzyme activity in all cases except GDH, for which a clear test was not made. This was of no importance for enzymes showing sharp and intense bands of activity such as GPI, MDH-2, IDH-1 and EST-D, because residual activity was still sufficient for clear interpretation. For the other enzymes, it was preferable to avoid overnight freezing, so grinding was made just before each run. This was in the morning when DG tissue extracts were to be run during the same day, or in the afternoon, prior to overnight runs with muscle tissue, because of the time required to thoroughly grind this tissue (2-3 minutes per individual).

Routine screening

Considerations of maximum return for minimum cost and effort lead us to adopt the schedule given in Table 5.

Table 5. Combinations of buffer and tissue extract for routine screening of 11 enzymes in *Trochus niloticus*. M muscle; DG digestive gland.

Buffer	Tissue extract	
	M	DG
TC 8.0 [1/2]	-	GPI PGM
TEB 8.4	-	SOD DIA EST-N
TEC 7.9	GDH IDH PEP (LT)	-
TM 7.4	EST-D MDH ME	-

Detail of 12 enzyme systems

A detailed description of the zymograms obtained for enzymes routinely scored in *Trochus niloticus*, and other species, follows. Except where indicated, relative mobilities of electromorphs were verified by running individuals of different phenotypes on the same gels. Where more than one zone of activity was observed, these were numbered consecutively from the slower migrating to the faster. Intense enzyme activity was indicated by black spots on the figures. Weaker enzyme activity was shown by dotted spots. Differences in resolution were expressed as differences in spot sizes.

Diaphorase

Two zones of NADH-dependent diaphorase activity were observed in all 6 species studied (Fig. 1). The only individual of *Trochus conus* analysed showed three bands of activity. The two slower ones were close to one the other, and similar in appearance, whereas the faster one was more intense. The quaternary structure of this enzyme is monomeric in man (Harris & Hopkinson, 1976). We assumed that the two zones of activity therefore corresponded to two distinct loci, with the individual of *Trochus conus* being presumptively heterozygous at the locus encoding the slower enzyme. However additional data (e.g. scores from a larger sample of *Trochus conus*) are required before a clear genetic interpretation of DIA zymograms can be proposed.

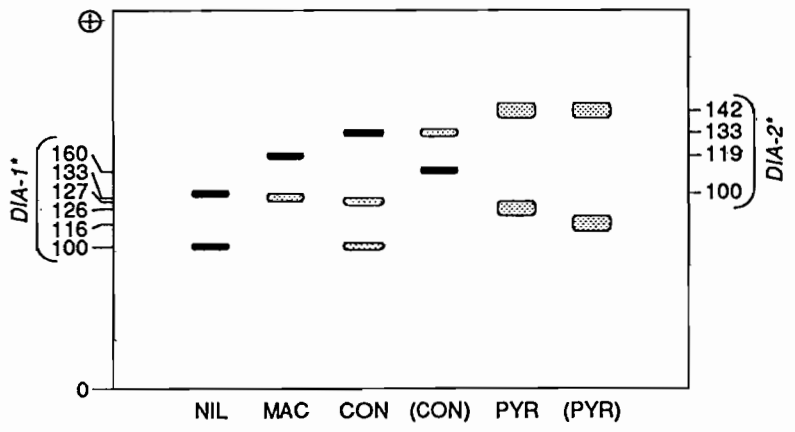

Figure 1. Enzyme variants observed for DIA. *DIA-2** is represented by the fastest migrating band in each lane.

Esterase-D

This enzyme specifically used radical 4-methylumbelliferyl acetate (4MU) as substrate. It did not show any activity with another substrate (α naphthyl acetate) which is hydrolysed by non-specific esterases. Also, its presumed quaternary structure (dimeric, inferred from the number of bands displayed by presumed heterozygotes) is different from that of the latter (monomeric). It therefore can be considered as an analog of the vertebrate EST-D (Hopkinson *et al.*, 1973; Ward & Beardmore, 1977). A 4MU-specific, presumptively dimeric esterase is also present in bivalves (Skibinski, Ahmad & Beardmore, 1978; Borsa & Thiriot-Quievreux 1990).

Staining of this enzyme is inexpensive and simple. It gave very clear and intense bands of activity in all *Trochus* and *Tectus* species studied so far (Fig. 2). It is likely to be useless as a genetic marker in *Trochus niloticus* because it was almost completely

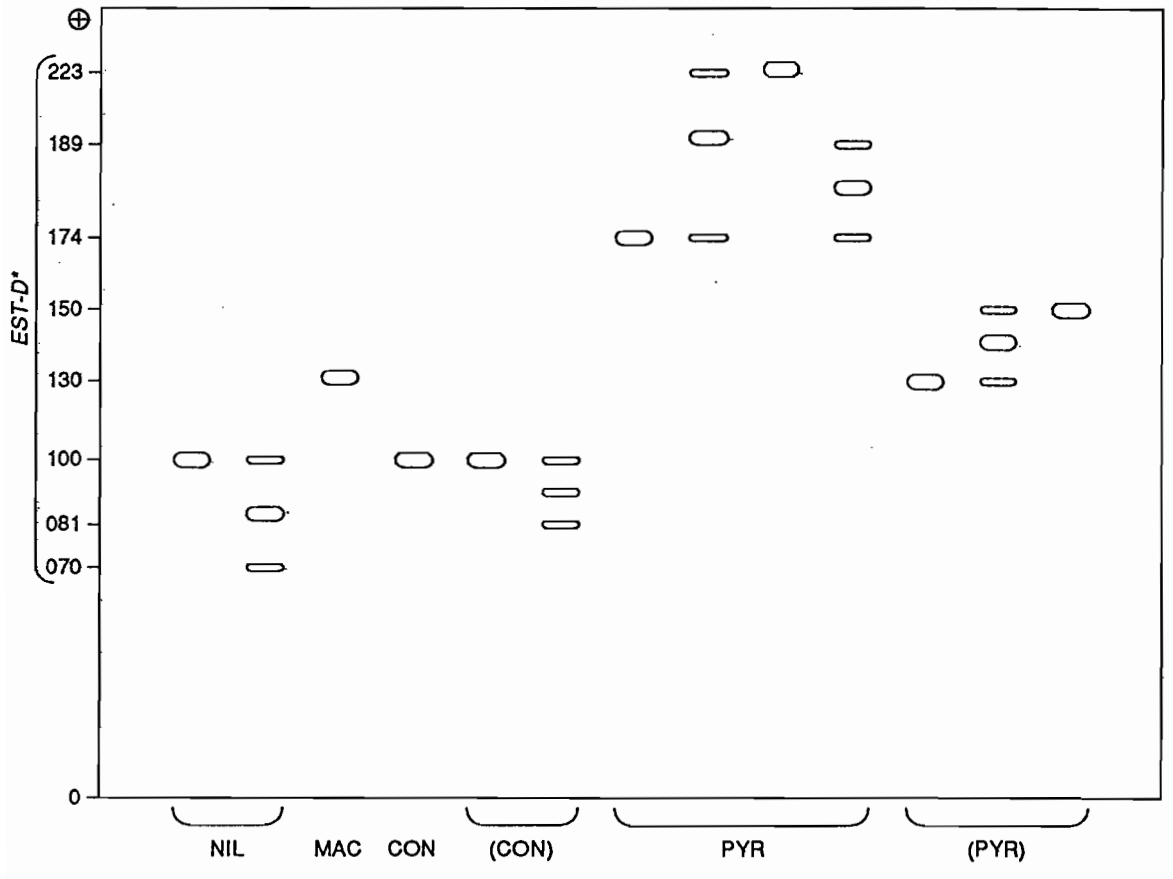

Figure 2. Enzyme variants observed for EST-D. PYR and (PYR) heterozygotes were not symmetrical and the patterns observed on the gels are reproduced exactly in the illustration.

monomorphic in our samples of that species (one single variant over 839 individuals analysed). A higher level of variation was present in *Trochus sp., Tectus pyramis* and *Tectus sp.* Best results were achieved using muscle tissue extract as the source of enzyme. EST-D was also present in digestive gland tissue extracts. When these were run, additional faster bands of non-specific EST-N activity also appeared.

Esterase-N

In presence of radical α-napthyl acetate and a soluble diazo dye (Fast Garnet GBC, Fast Black K), several zones of activity appeared in digestive gland extracts of *Trochus niloticus*, one of which (the fastest one) was sharp and intense. The same result could be obtained with 4MU as substrate, except that bands of EST-D additionally appeared. This fast and sharp band was assumed to correspond to a locus (*EST-N**) totally monomorphic in *T. niloticus*. Bands with habitus and mobilities similar to those of *T. niloticus* were present with DG extracts of other *Trochus* and *Tectus* species (Fig. 3, produced from a run on cellogel TM). The patterns shown in Fig. 3 are typical of a polymorphic locus with co- dominant alleles encoding a monomeric enzyme. The fine resolution of cellogel TM was not always achieved with starch gels.

Glucose dehydrogenase

Preliminary tests using NAD as cofactor showed no GDH activity. New attempts were made later in the study, using NADP as cofactor. These demonstrated weak GDH activity. When present, this enzyme showed two anodal zones of activity in *T. niloticus*. The weaker, slower one was interpreted as the expression of a monomorphic locus (not scored at all), the faster one as that of a polymorphic locus here called *GDH.* GDH** was scored in only those few gels exhibiting clear banding patterns (Fig. 4) for all individuals. TEB and TEC were the only buffers tested for NADP-dependent GDH activity: similar results were obtained with both these buffers.

Glucose-phosphate isomerase

This enzyme exhibited some variation in *Trochus niloticus* (4 allelomorphs in a sample of 839 individuals). It was found to be very variable in *Tectus pyramis*, with 7 allelomorphs in 58 individuals (Fig. 5). Half the concentration of gel buffer (TC8 [1/2]) was preferred because it increased the speed of migration, hence the separation between electromorphs, otherwise low in *T. niloticus*. This did not affect the intensity or the sharpness of the bands of activity which were in any case very good. Good results were also obtained with TEC [1/2]).

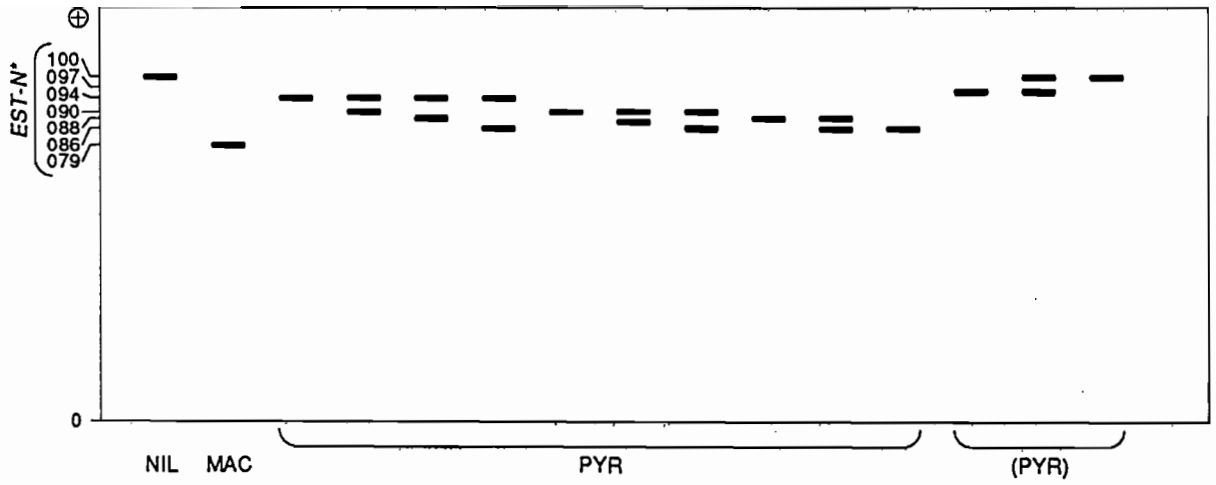

Figure 3. Enzyme variants observed for EST-N. No activity was observed for CON and (CON).

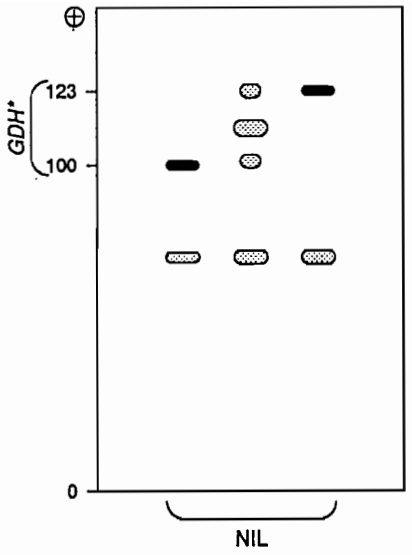

Figure 4. Enzyme variants observed for GDH.

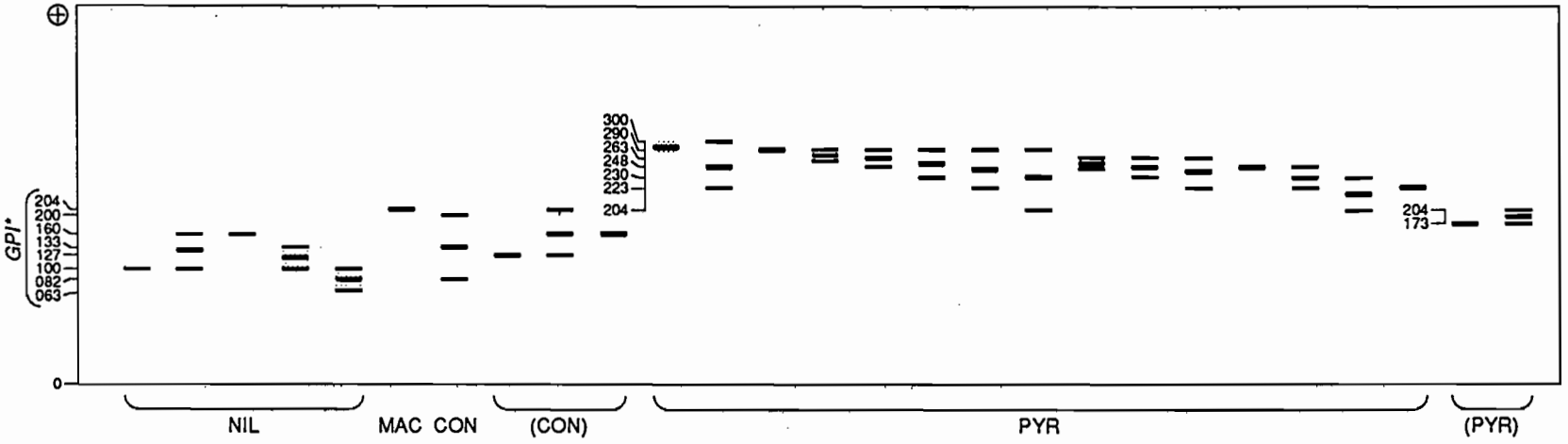

Figure 5. Enzyme variants observed for GPI.

Isocitrate dehydrogenase

Two isocitrate dehydrogenase zones of activity were usually present. The patterns shown in Fig. 6 suggest that *IDH-1** and *IDH-2** were encoded by two different loci. *IDH-1** of *Trochus niloticus* exhibited sub-bands whose presence did not interfere with the clarity of the IDH zymograms. Whether electromorph **030* of *Trochus conus* was different from the rare electromorph **021* of *T. niloticus* could not be verified by running both samples side-by-side.

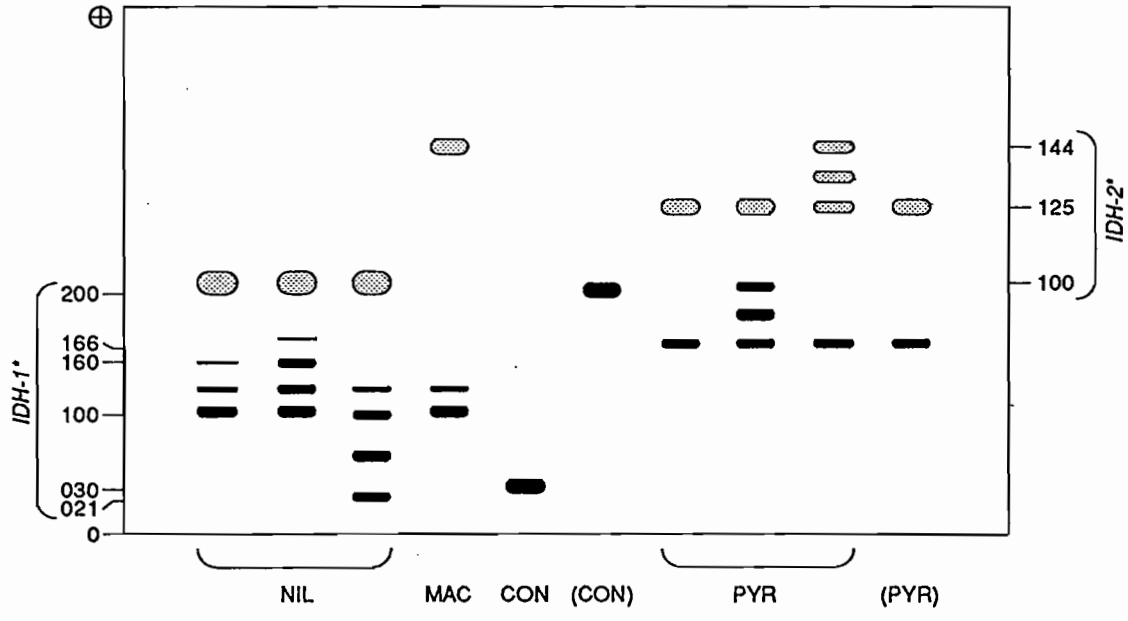

Figure 6. Enzyme variants observed for IDH.

Malate dehydrogenase

Two malate dehydrogenase zones of activity were present in all 6 species except *Tectus pyramis* (Fig. 7). These were clearly distinct, and could be ascribed to two different loci, thanks to the occurrence of distinct heterozygous patterns in *Trochus niloticus*. Because of intense sub-banding, heterozygous phenotypes usually were not of the classical three-banded type as would have been expected for that presumptively dimeric enzyme, although three-banded heterozygous individuals were observed on some gels for *Tectus pyramis*. *MDH-2** was highly polymorphic in *Tectus pyramis*, but was monomorphic in *Tectus sp.*, *Trochus maculatus* and almost monomorphic in *T. niloticus*.

Electromorph **128* of *Trochus sp.* might be identical to the rare electromorph **125* of *T. niloticus* but this could not be verified by side-by-side sample running.

Despite potentially confusing sub-banding, assessing the presence of heterozygotes at one or the other *MDH** loci in *T. niloticus* was not problematical.

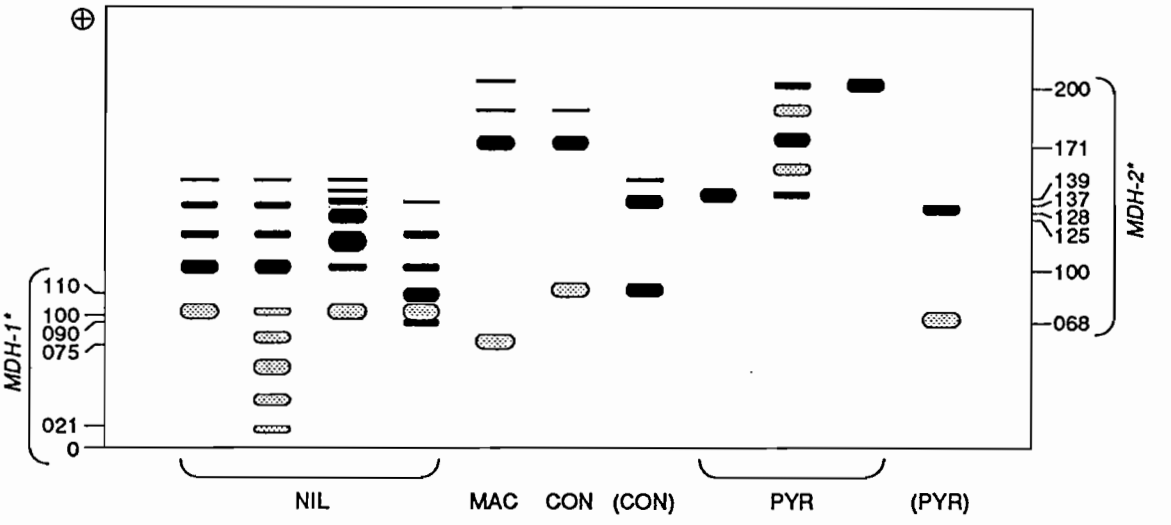

Figure 7. Enzyme variants observed for MDH

Malic enzyme

Staining for this dehydrogenase required the same substrate as MDH (L- malic acid) and NADP as cofactor. Its quaternary structure is tetrameric in vertebrates (Nevaldine, Bassel & Hsu 1974; Hopkinson *et al.*, 1976). Heterozygotes are expected to have a five-banded phenotype in the best case, or a roughly beer barrel-shaped smear if resolution of the gel is not perfect. The latter patterns were observed in *Trochus* and *Tectus* (Fig. 8). The presence of both homozygotes and heterozygotes for the two presumptive alleles in *Tectus pyramis* reinforced the reliability of our genetic interpretation.

A slower migrating faint band (designated "*ME-1**" in Fig. 8) was often observed on gels of *T. niloticus* stained for ME. In *Trochus niloticus,* homozygotes for the rare **087* presumptive allele were not observed. It is therefore possible that the latter was identical to allele **092* of *T. maculatus*.

Non-specific dehydrogenase

Weak but clear-cut bands were sometimes found to appear after 24 hrs of incubation on gels stained for both GDH and IDH, thus identified as NADP-dependent non-specific dehydrogenase (NDH) zones of activity. This enzyme showed electrophoretic

variation between species, but not within species (Fig.9), although numbers of individuals scored for *NDH** in each of them were low (see Appendix). In *Trochus niloticus* and *Tectus sp.*, *NDH** bands overlapped those of *IDH-1.**

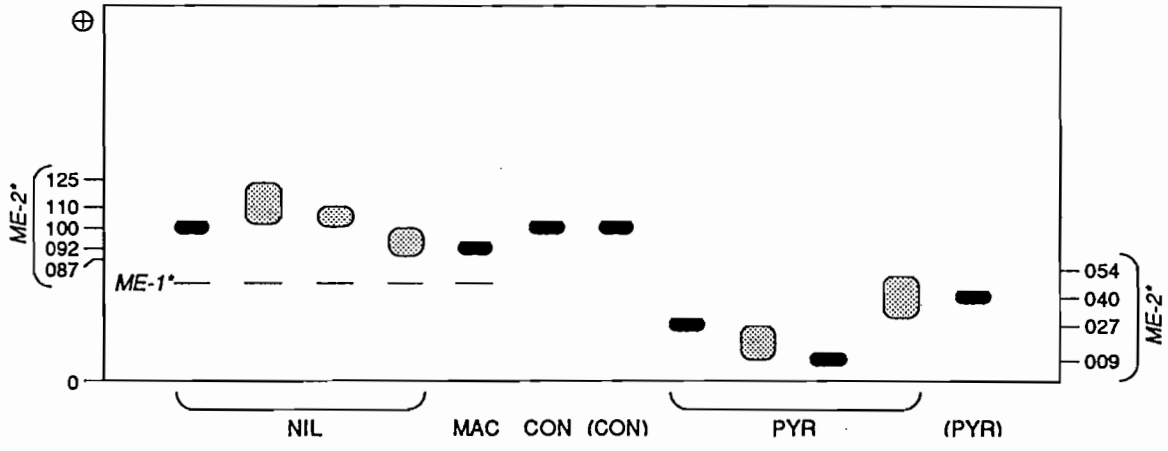

Figure 8. Enzyme variants observed for ME

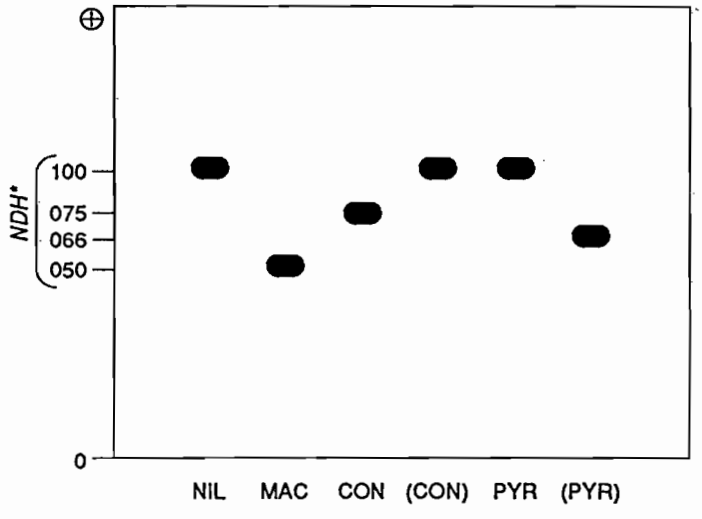

Figure 9. Enzyme variants observed for NDH

Peptidase (LeuTyr)

In *Trochus niloticus*, a comparative run involving the muscle tissue extracts of 10 individuals and different combinations of buffer (TEB 8.4, TEC 7.9) and substrate (Leu-Gly-Gly, Leu-Tyr, Val-Leu) was made in addition to the preliminary screening described above. Results for intensity, resolution and variability are summarized in Table 6.

Table 6. Intensity (I), resolution (R) and enzyme polymorphism (P) in the three peptidase zones of activity observed in 6 combinations of buffer and enzyme substrate. See text for details.

Buffer	Substrate		
	LeuGlyGly	LeuTyr	ValLeu
TEB 8.4			
*PEP-1**	I: weak R: poor P: present (slight)	I: intense R: not sufficient P: present (same as LGG)	I: weak R: ok P : absent
*PEP-2**	I: good R: poor P: present	I: good R: borderline P: present (same as LGG)	I: good R: ok P: absent
*PEP-3**	I: good R: good P: absent	I: good R: good P: absent	I: very weak R: ok P: absent
TEC 7.9			
*PEP-1**	I: good R: good P: present (same as above)	I: sufficient R: poor P: present (same as above)	I: very weak R: poor P: ?
*PEP-2**	I: good R: borderline P: present (same as above)	I: intense R: good P: present (same as above)	I:very weak R: poor P: ?
*PEP-3**	I: weak R: poor P: ?	I: weak R: poor P: absent	I: very weak R: poor P: ?

Three zones of peptidase activity were present with buffers TEB 8.4 and TEC 7.9 when gels were stained using either Leu-Gly-Gly, Leu-Tyr or Val-Leu as substrates. The relative mobilities of each zone were identical when compared on slices of the same gel, stained using these different substrates. However, the relative intensities, sharpness, and electromorph mobilities within each zone were variable. Resolution was better with TEC than with TEB for LGG and LT. Intensity of PEP (VL) was greater with TEB.

*PEP-1** and *PEP-2** each showed allozyme variability with LGG and LT, but not with VL. PEP-1 (VL) had the same mobility as the most common electromorph of *PEP-1** (LGG or LT). *PEP-2**(VL) had the same mobility as electromorph **100* of *PEP-2** (LGG or LT). *PEP-3** was monomorphic in the 10 individuals for all

combinations tested. It was intense in TEB, but weak enough in TEC to prevent being confused with any possible fast electromorph of PEP-2.

Only *PEP-2** (LT) was therefore considered for further analysis, and it was routinely scored on TEC 7.9 (Fig. 10).

*PEP-2** (LT) was also the only peptidase scored in the five other *Trochus* and *Tectus* species. Because of sub-banding, and resolution not sufficient to separate overlapping electromorphs with suspected different mobilities, assessment of *PEP-2** phenotypes was more difficult than with *T. niloticus*. We feel that refining procedures similar to those developed for *T. niloticus* (Table 6) might result in increased resolution in these other species.

As stressed by Richardson *et al.* (1986), peptidases are usually ambiguous markers: their electrophoretic patterns may be determined by an unknown number of loci and the isozyme products of separate loci often overlap. However, gels obtained for *T. niloticus* usually exhibited clear *PEP-2** (LT) one-banded or two-banded patterns, in accordance with expectations for a monomeric enzyme encoded by several co-dominant alleles.

Phosphoglucomutase

Interpretation of zymograms of this presumptively monomeric enzyme was straightforward (Fig.11). Caution should be taken, however, in assessing the mobilities of the different electromorphs of PGM in the highly polymorphic *Tectus pyramis*. Ideally, one would use a large proportion of the different available phenotypes as reference markers on each gel, but this was not always possible (see Appendix).

In *T. niloticus,* only one intense zone of activity was present in digestive gland tissue extracts. Two zones appeared in muscle. One had the same mobility as that of digestive gland and was therefore assumed to correspond to the same locus, although only monomorphic samples of *T. niloticus* could be compared between these tissues. The other slower zone was considered to correspond to another locus, whose existence in other molluscs (bivalves) has been inferred from zymograms in which variation was displayed at both loci (Worms & Pasteur, 1982; Borsa & Thiriot-Quievreux, 1990).

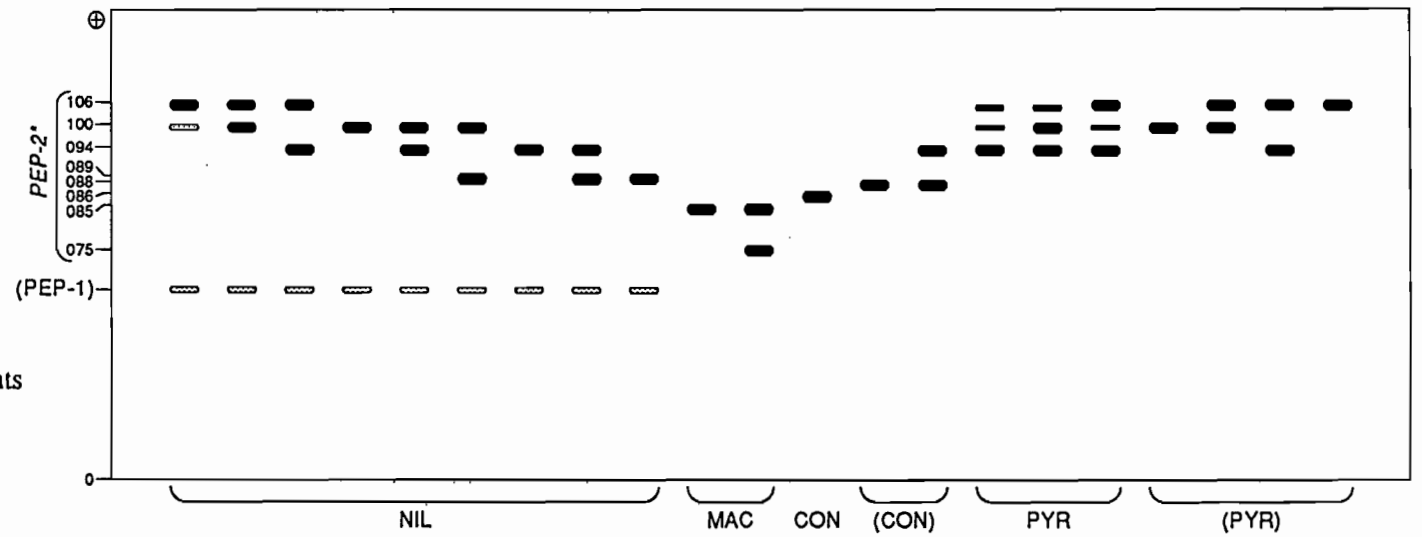

Figure 10.
Enzyme variants observed for PEP-2 (LT).

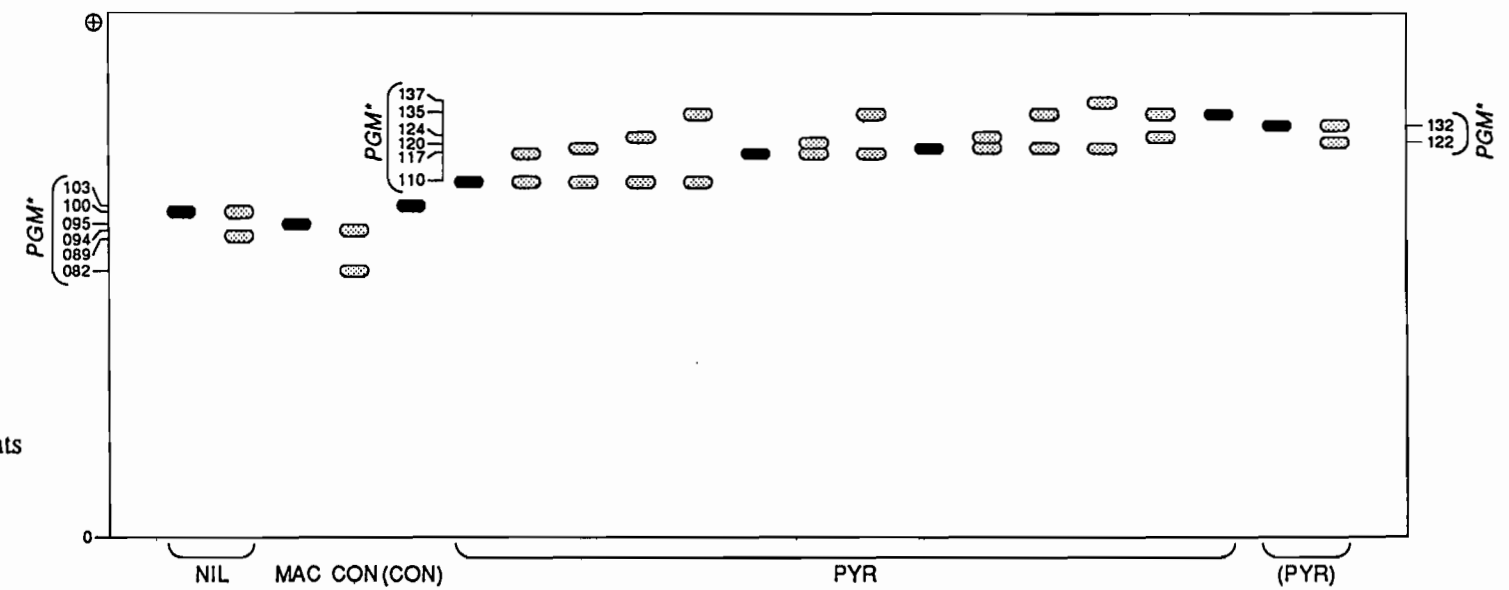

Figure 11.
Enzyme variants observed for PGM.

Superoxide dismutase

Two zones of SOD activity, best visualized with digestive gland tissue extract, were observed. The faster one was intense but not sharp enough for reliable interpretation. The weaker, slower one (*SOD-1**) was clear enough for comparisons between species, and for assessing polymorphism in *Tectus sp.* (Fig. 12). In *Trochus niloticus*, faster sub-bands were often present. These may have masked possible rare heterozygotes.

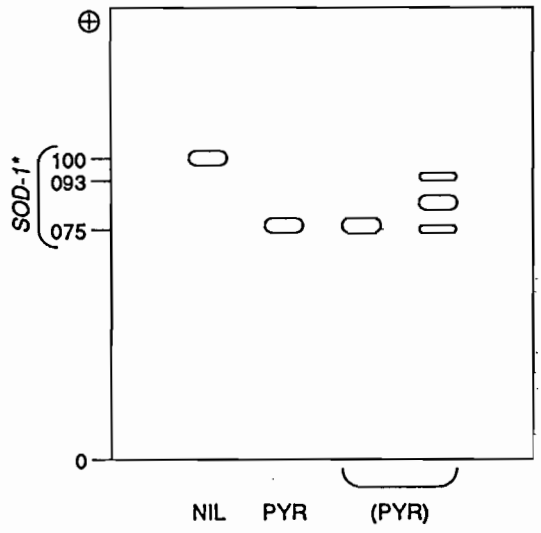

Figure 12. Enzyme variants observed for SOD.
No activity was observed for MAC, CON or (CON).

DISCUSSION

The methods presented here were originally selected to screen for variation in populations of *Trochus niloticus* from the Great Barrier Reef. They were extended later to other species belonging to the genera *Trochus* and *Tectus* (both Trochidae). In several instances, this comparative approach was useful in reinforcing the reliability of our genetic interpretation of zymograms for *T. niloticus*: (1) Some enzyme loci monomorphic in *T. niloticus* (e.g. *EST-N** and *IDH-2**) and some others (*EST-D**, *ME** and *PGM**) slightly polymorphic in *T. niloticus* and lacking the phenotype expected for the rare homozygote, displayed polymorphism in other trochids, which was in accordance with the expectations derived from a simple genetic model and the known enzyme's quaternary structure. (2) An enzyme (*NDH**) revealed using IDH staining, and appearing to have the same mobility as *IDH-1** in *T. niloticus* was clearly distinct from *IDH-1** in another species (*Tectus sp.*), thus confirming its separate status.

No breeding experiments have been attempted yet to demonstrate that the observed allozyme banding patterns in *Trochus* and *Tectus* have a simple genetic basis. In most cases of polymorphism, the zymograms conformed to typical patterns of codominance. Breeding experiments performed in another prosobranch species, *Littorina saxatilis*, have demonstrated that the patterns observed for enzymes *GPI**, *IDH-1**, *IDH-2**, *PEP-2** and *PGM** are inherited in a simple Mendelian fashion (Ward, Warwick & Knight, 1991). Similar results have been obtained in a few other molluscan (bivalve) species (Beaumont, Beveridge & Budd, 1983; Hvilsom & Theisen, 1984; Foltz, 1986), adding *EST-D** and one *MDH** to the above set of enzymes.

A variety of factors other than genetic polymorphism can result in banding variation: environmental effects (Oxford, 1975), protein degradation (Harris & Hopkinson, 1976), uncontrolled variation in gel quality (Borsa, Jousselin & Delay, 1991, reinterpreting Monti *et al.*, 1986) or other stain artifacts (Harris & Hopkinson, 1976). However, the two following criteria suggested by Selander *et al.* (1970) that are usually considered acceptable among population geneticists (Burton, 1983), were met in *Trochus* and *Tectus* with polymorphic enzymes for which no breeding data are available: (1) Consistency of banding patterns with known quaternary structure and (2) presence of all phenotypes expected to be in non-marginal proportions according to a Mendelian model.

Nevertheless, definitive assessment of the genetic control of banding variation requires formal genetic analyses. In *Trochus* and *Tectus,* this requires that pairs of individuals be induced to spawn and their offspring cultivated, prior to comparing the allozyme phenotypes of offspring and parents. Heslinga & Hillmann (1981) have shown that experimental spawning of *Trochus niloticus* and subsequent rearing of juveniles could easily be achieved in standard mariculture conditions. Sexing might be possible prior to cross-breeding by carefully drilling a hole in the apical region of the shell, through which it is then possible to visualize the gonad's colour and texture (green, granulated for females; creamy for males). Since no harmless way of assessing each parent's phenotype seems to be available, the pairs to be cross-bred can only be randomly chosen. A few such random crossings would be expected to give valuable results with sufficiently variable enzymes like *PEP-2** in *Trochus niloticus, EST-N*, GPI*, MDH** and *PGM** in *Tectus pyramis* and *EST-D*, EST-N** and *PEP-2** in *Tectus sp.*

ACKNOWLEDGEMENTS

This study was part of a one-year post-doctoral project by P. Borsa, funded by a *Lavoisier* fellowship from the French Ministere des Affaires Etrangeres, and in part by AIMS project 4.17. We gratefully acknowledge the service of the crews of Research Vessels *Harry Messel, Sirius* and *Pegasus,* and the participation of L. Cusack, D. Hosking, A. Karl-Medeiros, M. Oliveira da Silva, C. Sandusky, L. Schwarzwalder, D. Smith, G. Stockdale, J. True, D. Woo and J. Yeatman as volunteer divers, during the phase of sampling, which was done in compliance with GBRMPA permit # G91/098.

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APPENDIX

Table A1 Key to allelomorphs in Table A2

Locus	Allelomorphs
*DIA-1**	A=100, B=116, C=126, D=127, E=132
*DIA-2**	A=100, B=119, C=133, D=142
*EST-D**	A=070, B=081, C=100, D=130, E=150, F=174, G=189, H=223
*EST-N**	A=079, B=086, C=088, D=090, E=094, F=097, G=100
*GDH**	A=100, B=123
*GPI**	A=082, B=100, C=127, D=160, E=173, F=200, G=204, H=223, I=230, J=236, K=248, L=263, M=290, N=300
*IDH-1**	A=030, B=100, C=120, D=127, E=160, F=166, G=200
*IDH-2**	A=100, B=125, C=144
*MDH-1**	A=075, B=090, C=100, D=110
*MDH-2**	A=100, B=128, C=137, D=139, E=171, F=200
*NDH**	A=050, B=066, C=075, D=100
*ME-2**	A=009, B=027, C=040, D=092, E=100, F=054
*PEP-2**	A=075, B=085, C=086, D=088, E=089, F=094, G=100, H=106
*PGM**	A=082, B=094, C=095, D=100, E=103, F=110, G=117, H=120, I=122, J=124, K=132, L=135, M=137
*SOD-1**	A=075, B=093, C=100

Table A2 Individual multilocus genotypes in four species of genus *Trochus*, *T. niloticus* (L. 1758), *T. maculatus* (L. 1758), *T. conus* (Gmelin 1791) and *Trochus* sp., and two species of genus *Tectus*, *T. pyramis* (Lmk. 1822) and *Tectus sp.* from the Great Barrier Reef.

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**	*GDH**
***Trochus niloticus*, Escape Reef**															
01	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
02	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	EE	CC	
03	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FE	CC	
04	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
05	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
06	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FF	CC	
07	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
08	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
09	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
10	AA	AA	CC	AA	EB	AA		DD	BD	GG	CA	EE	FF	CC	
11	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
12	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
13	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
14	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
15	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FF	CC	
16	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
17	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
18	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FE	CC	
19	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
20	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GG	CC	
21	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
22	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
23	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
24	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
25	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**	*GDH**
26	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	FE	CC	AA
27	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	BB
28	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GE	CC	AA
29	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	AA
30	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	AA
31	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	AA
32	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GE	CC	AA
33	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	BB
34	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GG	CC	AA
35	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GG	CC	AA
36	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	AA
37	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GF	CC	AA
38	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	FF	CC	AA
***Trochus maculatus*, Davies Reef (*Square Reef)**															
01	EE	BB	AA	EE	BB	CC	AA	CC	GG	AA	DD	DD	DD		
02	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
03	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
04	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DA		
05	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
06	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
07	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
08	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
09	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
10	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
11	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**
12	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD	
14	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD	
15	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD	
16	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
17	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
18	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
V3*	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
V4	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
***Trochus conus*, Davies Reef**														
V5	DA	CC	DD	EE	AA		CC	BA	FA		CC	EE	CC	
***Trochus (conus)* sp., Davies Reef (*Escape Reef)**														
01	GG	CC	DD	CC	GG		DD	EE	CC		CB	EE	FD	
V6	FF	CC	DD	CC	AA		DD	EE	JD		CC	EE	DD	
V7*	GG	CC	DD	CC	GG		DD	EE	GC		CC	EE	DD	
***Tectus pyramis*, Davies Reef**														
01	CC	DD		FD	FF	BB		FF	ML	BB	FF	BB	FF	AA
02	CC	DD		DD	FF	BB		HF	MH	BB	FF	BB	FF	AA
03	CC	DD		DD	FF	BB		FF	KK	EE	FF	BB	FF	AA
04	CC	DD		DD	FF	BB		GF	NH	EB	FF	BB	FF	AA
05	CC	DD		FD	FF	BB		JH	MM	DD	FF	BB	FF	AA
06	CC	DD		DD	FF	BB		FF	MH	EE	FF	BB	FF	AA
07	CC	DD		FF	FF	BB		FF	KH	EC	FF	BB	FF	AA
08	CC	DD		DD	FF	BB		HG	MK	EB	FF	BB	FF	AA

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**
09	CC	DD		FD	FF	BB		FF	MH	BB	FF	BB	FF	AA
10	CC	DD		FD	FF	BB		GF	MM	DD	FF	BB	FF	AA
11	CC	DD		FD	FF	BB		GG	MK	ED	FF	BB	FF	AA
12	CC	DD		DD	FF	BB		HH	MH		FF	BB	FF	AA
13	CC	DD		DD	FF	BB			LK	EC	FF	BB	FF	
14	CC	DD		FD	FF	BB			ML	EE	FF	BB	FF	
15	CC	DD		DD	FF	BB			LK	DB	FF	BB	FF	
16	CC	DD		FD	FF	BB			MM	EB	FF	BB	FF	
17	CC	DD		DD	FF	BB			LI	EE	FF	BB	FF	
18	CC	DD		DD	FF	BB			HH	ED	FF	BB	FF	
19	CC	DD		DD	FF	BB			MM	DD	FF	BB	FF	
20	CC	DD		FD	FF	BB			MH	EC	FF	BB	FF	
21	CC	DD		DD	FF	BB			MK	EE	FF	BB	FF	
22	CC	DD		DD	FF	BB			MH	ED	FF	BB	FF	
23	CC	DD		FD	FF	BB			MG	DD	FF	BB	FF	
24	CC	DD		DD	FF	BB			MK	DD	FF	BB	FF	
25	CC	DD		DD	FF	BB			MK	EC	GF	BB	FF	
26	CC	DD		DD	FF	BB			MM	EE	FF	BB	FF	
V1	CC	DD		FD	FF	BB			LH	CC		BB	FF	
***Tectus pyramis*, Square Reef**														
01	CC	DD		DD	FF	BB		LG	KK	EB	FF	BB	FF	
02	CC	DD		FD	FF	BB		HH		EC	FF	BB	GF	
03	CC	DD		DD	FF	BB		HH	MK	CC	FF	BB	FF	
04	CC	DD		FF	FF	BB		LF	MK	EE	HH	BB	FF	
05	CC	DD		DD	FF	BB		GF	IG	EB	HF	BB	FF	
06	CC	DD		FD	FF	BB		FF	MM	EC	FF	BB	FF	
07	CC	DD		FD	FF	BB		LG	MI	DD	FF	BB	FF	

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**
08	CC	DD		DD	FF	BB		GF	KI	DB	HF	BB	FF	
09	CC	DD		FD	FF	BB		FF	MM	BB	FF	BB	FF	
10	CC	DD		DD	FF	BB		HF	MK	ED	FF	BB	FF	
11	CC	DD		FD	FF	BB			MM	DC	FF	BB	FF	
12	CC	DD		DD	FF	BB			KK	DD	FF	BB	FF	
13	CC	DD		DD	FF	BB				CC	FF	BA	FF	
14	CC	DD		DD	FF	BB				EB	FF	BB	FF	
15	CC	DD		DD	FF	BB			MK	EE	FF	BA	FF	
16	CC	DD		DD	GF	BB			KH	DB	FF	BB	FF	
17	CC	DD		FF	FF	BB				DD	FF	BA	FF	
18	CC	DD		DD	FF	BB				BB	GF	BB	FF	
19	CC	DD		FD	FF	BB				CB	FF	BB	FF	
20	CC	DD		FD	FF	CB			NM	EB	FF	AA	FF	
21	CC	DD		FD	FF	BB			MK	EC	FF	BB	FF	
22	CC	DD		DD	FF	BB			MK	DC	FF	BB	FF	
23	CC	DD		DD	FF	BB			MK	DD	FF	BB	FF	
24	CC	DD		DD	FF	BB			KK	DB	FF	BB	FF	
25	CC	DD		FD	FF	BB			KH	DB	FF	BB	FF	
26	CC	DD		FD	FF	BB			MK	CC	FF	BB	FF	
27	CC	DD		DD	FF	BB			MH	DC	FF	BB	FF	
28	CC	DD		FD	FF	BB			MM	BB	FF	BB	HF	
29	CC	DD		DD	FF	BB			LK		HF	BB	FF	
30	CC	DD		DD	FF	BB			LH	DD	FF	BB	FF	
***Tectus pyramis*, Escape Reef**														
02	CC	DD		DD	FF	BB	DD	GF	KH	DD	FF	FB	FF	AA
06	CC	DD		FF	FF	BB	DD	GF	MK		FF	BB	FF	AA

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**
***Tectus (pyramis)* sp., Escape Reef**														
01	BB	DD	BB	BB	FF	BB	BB	KK	EE	FF	ED	CC	GG	AA
03	BB	DD		BB	FF	BB	BB	KK	EE		DD	CC	HG	AA
04	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	AA
05	BB	DD		BB	FF		BB	KK	EE		EE	CC	GG	AA
07	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	AA
08	BB	DD		BB	FF		BB	KK	EE		ED	CC	GG	AA
09	BB	DD		BB	FF		BB	KK	EE		ED	CC	HH	AA
10	BB	DD		BB	FF		BB	KF	EE	FF	EE	CC	HG	AA
11	BB	DD		BB	FD		BB	KK	EE		DD	CC	HF	BA
12	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	BA
13	BB	DD		BB	FF		BB	KK	EE		DD	CC	HH	AA
14	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	BA
15	BB	DD		BB	FF		BB	KK	EE		ED	CC	HG	AA
16	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	AA
17	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	AA
18	BB	DD		BB	FF		BB	KK	EE		DD	CC	GG	AA
19	BB	DD		BB	FF		BB	KK	EE		EE	CC	GG	AA
20	BB	DD		BB	FF		BB	KK	EE		ED	CC	GG	AA
21	BB	DD		BB	FF		BB	KK	GE	GG	EE	CC	GG	AA
22	BB	DD		BB	FF		BB	KK	EE	GG	EE	CC	HG	AA
23	BB	DD		BB	FF		BB	KK	EE	GF	EE	CC	HH	BA
24	BB	DD		BB	FF		BB	KK	EE	GG	DD	CC	HH	BA
25	BB	DD		BB	FF		BB	KK	EE	FF	EE	CC	HH	AA
26	BB	DD		BB	FF		BB	KK	EE	GF	EE	CC	HG	AA
27	BB	DD		BB	FC		BB	KK	EE	GF	ED	CC	HG	BA
28	BB	DD		BB	FF		BB	KF	EE	FF	ED	CC	HH	BA
29	BB	DD		BB	FF		BB	KK	EE	GG	ED	CC	HG	AA

Table A2 (continued)

#	*DIA-1**	*DIA-2**	*MDH-1**	*MDH-2**	*IDH-1**	*IDH-2**	*NDH**	*PGM**	*GPI**	*EST-N**	*EST-D**	*ME-2**	*PEP-2**	*SOD-1**
30	BB	DD		BB	FF		BB	KF	EE	FF	ED	CC	HG	AA
31	BB	DD		BB	FF		BB	KK	GE		EE	CC	HH	
32	BB	DD		BB	FF		BB	KK	GE		EE	CC	HH	
33	BB	DD		BB	FF		BB	KK	EE		ED	CC	HH	
34	BB	DD		BB	FF		BB	KK	GE		ED	CC	HF	
35	BB	DD		BB	FF		BB	KK	EE		EE	CC	HG	
36	BB	DD		BB	FF		BB	KK	EE		ED	CC	HG	
37	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	
38	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	
V2	BB	DD	BB	BB	FF	BB	BB	KK	EE		EE	CC	GG	

Voucher specimens

The shells of individuals # V1 to V7 were sent as voucher specimens to the Australian Museum, Sydney, with registration numbers C.168458, C.168454, C.168457, C.168459, C.168463, C.168462 and C.168455, respectively. The shells of individuals *Trochus maculatus* # 01 and 15 were registered as C.168460 and C.168461, respectively.

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ISSN 1033-6974