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Genetic and morphometric comparisons of squat lobster, *Munidopsis scobina* (Decapoda: Anomura: Galatheidae) populations, with notes on the phylogeny of the genus *Munidopsis*

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Abstract

Specimens of the galatheid *Munidopsis scobina* were collected from two stations on the continental slope off Oman, at depths of 900 and 1000 m, using an Agassiz trawl. Starch gel electrophoresis, across 10 enzyme loci, was carried out on 427 specimens. Genetic variability was calculated for both populations using a number of parameters. *F*-statistics were used to estimate genetic variance within (F_{IS}) and between (F_{ST}) populations. Significant deviations from Hardy-Weinberg expectations were detected at one locus (*Gotb*). Analyses of F_{IS} revealed significant differences from zero at *Gotb* and *Pgm*, as a result of heterozygote deficiency. No relationship was observed between size of individuals and genotype. The number of genetically effective migrants per deme per generation (N_em) was calculated using both F_{ST} and private alleles methods. N_em values were theoretically sufficient to offset the effects of genetic drift. Additional morphometric analyses were carried out on *Munidopsis scobina* from the two populations. Individuals were sexed ($n = 2476$ individuals) and ten parameters measured ($n = 1238$). All specimens were examined for parasites (either bopyrid isopod or rhizocephalan).

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Significant male-biased sex ratios were observed in both populations ($p < 0.01$). In addition, significant differences in size-frequency distributions ($p < 0.01$) were recorded both within sites between sexes, and within sexes between sites; possibly related to a size-dependent response to hypoxia. Significant differences also were observed in mean cheliped length between sexes ($p < 0.01$), potentially indicating that male *M. scobina* exhibit agonistic behaviour. The genetic relationships of *Munidopsis scobina* to four other species of *Munidopsis* (*M. crassa*, *M. parfaiti*, *M. spinihirsuta* and *M. subsquamosa*) and the confamilial *Galathea squamifera* were also examined using allozyme loci. Within the genus *Munidopsis*, pairwise comparisons of genetic identity were within the normal range expected for congeneric species. Comparisons between *G. squamifera* and *Munidopsis* spp. were within the range expected for confamilial genera.

1. Introduction

Species of the genus *Munidopsis* (Crustacea: Galatheidae) are found in a variety of shallow- and deep-water habitats throughout the Atlantic, Indian and Pacific oceans (Ambler, 1980; Wenner, 1982; Chevaldonné and Olu, 1996). In shallow waters, *Munidopsis polymorpha* is recorded from anchialine pools in caverns in the Canary Islands (Wilkens et al., 1990). However, the majority of *Munidopsis* spp. occur in the deep sea, with many species having been recorded from the continental shelf, slope and rise, and over half of the recorded species having been sampled from depths of 800 m or more (Doflein and Balss, 1913; Ambler, 1980; Wenner, 1982). *Munidopsis* spp. are also the most frequently reported galatheid crabs from deep-sea hydrothermal vents and other reducing environments such as cold seeps and whale carcasses (Williams, 1988; Bennett et al., 1994; Chevaldonné and Olu, 1996).

Despite the cosmopolitan distribution of *Munidopsis*, most of the literature published on the genus consists primarily of original species descriptions (Williams and Turner, 1986). With the increase in deep-sea research in the last two decades, and particularly research undertaken around hydrothermal vent sites, the number of species of *Munidopsis* described has continued to increase (Chevaldonné and Olu, 1996) and probably exceeds the estimated 115 species reported by Chace (1942). However, many species of *Munidopsis* appear to be morphologically very similar, leading to confusion about systematic status (e.g. *M. crassa* – *M. subsquamosa*; Gordon, 1955; Gore, 1983), and this may have led to overestimates or underestimates of diversity within this group. Aspects of the biology of *Munidopsis* spp., such as population dynamics and life history, are also relatively poorly resolved. One area in which data are present is that of parasite infestations. The presence of high bopyrid and rhizocephalan parasite loads in galatheids, and the effects of such parasites on host organisms, are well documented (O'Brien and van Wyk, 1985; Høeg, 1995). Furthermore, the impact of parasites on populations of the host species, and resulting implications for genetic techniques, recently has been reviewed (Poly, 1997).

In 1994, a cruise (*Discovery* 211) was undertaken in order to assess the effects of the oxygen minimum zone on the benthic fauna of the continental slope off Oman, in the northern Arabian Sea. Fauna were sampled and surveyed, using a variety of gear,

across a bathymetric range of 50–3400 m (Gage, 1995). In this region, between 300 and 700 m, the megafaunal community was characterised by low diversity, associated with extremely low oxygen levels. This low diversity community was dominated by large numbers of a majid spider crab (*Encephaloides armstrongi* Wood-Mason, 1891), a cocoon-dwelling bivalve (*Amygdalum* sp.), and an ascidian (*Styela gagetyleri*) (Creasey et al., 1997; Young and Vazquez, 1997). At depths of 900–1000 m, both oxygen levels and megafaunal species diversity increased and various species of galatheid crabs, including large numbers of *Munidopsis* spp., were observed and collected (Gage, 1995).

Examination of the population genetics of the majid spider crab *Encephaloides armstrongi* indicated that populations were highly structured over relatively short geographic distances (Creasey et al., 1997). Furthermore, Creasey et al. (1997) observed that genetic and morphometric structuring within populations of *E. armstrongi* were caused by variable sex ratios and male gender-biased dispersal. One consequence of a highly biased sex ratio is that effective population size may decrease, potentially resulting in differential investment and variable recruitment (Hedgecock and Sly, 1990; Avise, 1994). The collection of large numbers of the galatheid, *Munidopsis scobina* Alcock, 1894 from the oxygen minimum zone presented the opportunity to carry out a comparative study on another decapod crustacean from the same region to that of *E. armstrongi*. In the present study, the biology and genetic differentiation of two populations of *M. scobina* were assessed using both morphometric and biochemical techniques. In addition, species of *Munidopsis* have previously been classified into five distinct genera (*Bathyankyristes*, *Elasmonotus*, *Galathodes*, *Munidopsis* and *Orophorhynchus*; Alcock, 1901; Tirmizi, 1966; see also discussion in Chace, 1942). Reduction in the number of putative genera occurred as a result of a lack of reliable, discernable taxonomic characters (Chace, 1942). In the present study, a phylogenetic comparison is made between *M. scobina* and five other galatheid species (*M. spinihirsuta* Lloyd 1907, *M. crassa* Smith 1885, *M. parfaiti* Milne-Edwards 1885, *M. subsquamosa* Henderson 1885 and *Galathea squamifera* Leach 1815) collected from the Atlantic and Pacific Oceans. Furthermore, specimens of *Munidopsis* spp. used in the current study include species from the previously defined genera *Orophorhynchus* (*M. parfaiti*) and *Munidopsis* (*M. crassa*, *M. scobina*, *M. spinihirsuta*, *M. subsquamosa*).

2. Materials and methods

2.1. Sample collection and measurement

Samples of the squat lobster *Munidopsis scobina* were collected from two sites in the Arabian Sea for population comparisons, during R.R.S. *Discovery* cruise 211. For phylogenetic analysis, a number of other species of *Munidopsis* were collected. Positions and depths of stations sampled along with details of capture gear and the number of specimens of each sampled for electrophoresis are given in Table 1. In addition, *Galathea squamifera* was collected from intertidal rock pools at Wembury beach (approx. 12 km east of Plymouth, Devon, UK).

Table 1
Details of position and depth of stations sampled, capture gear employed and number of specimens electrophoresed. N/A = Not applicable; OTSB = Semi-balloon otter trawl

Number of specimens	Cruise No.	Station No. (date)	Ocean (region)	Latitude	Longitude	Depth (m)	Capture gear
2 *Munidopsis crassa*	R.R.S. *Discovery* 222 Leg B	12930#64 (09/13/96)	Atlantic (Northeast)	48°49′N	16°29′W	4839	OTSB
5 *Munidopsis parfaiti*	*R.*R.S. *Discovery* 222 Leg B	12930#46 (09/09/96) and 12930#78 (09/17/96)	Atlantic (Northeast)	48°49′N to 48°51′N	16°33′W to 16°42′W	4841 to 4836	OTSB
256 *Munidopsis scobina*	R.R.S *Discovery* 211	12702#1 (10/25/94)	Indian (West)	19°15′N	58°27′E	1000	Agassiz Trawl
171 *Munidopsis scobina* 4 *Munidopsis spinihirsuta*	R.R.S. *Discovery* 211	12714#1 (10/29/94)	Indian (West)	19°12′N	58°22′E	900	Agassiz Trawl
4 *Munidopsis subsquamosa*	N.O. *Nadir* HOT 96	13°N Genesis and Parigo	Pacific (Northeast)	12°48′N	103°56′W	2630	Trap
6 *Galathea squamifera*	N/A	N/A	Atlantic (English Channel)	50°19′N	4°5′W	0	Manual collection

Once brought to the surface all specimens were immediately frozen whole at −70°C. Frozen samples were transferred in dry ice to the Marine Biological Association, Plymouth, where they were individually measured and numbered. Carapace length was measured to the nearest millimetre using dial callipers. Individuals were stored at −70°C prior to electrophoresis.

Additional samples of *Munidopsis scobina* and *M. spinihirsuta* also were captured at stations 12702#1 and 12714#1 (see Table 1 for station data) and were immediately fixed in 2% sea-water-buffered formalin. After transfer to the Marine Biological Association, Plymouth, samples were placed into 70% ethanol. 1876 individuals from station 12702#1 and 601 from station 12714#1 were sexed. A number of parameters were measured using dial callipers, to the nearest 0.1 mm, in 777 of the individuals from station 12702#1 and all 601 from station 12714#1. These were: total length, carapace length, carapace width, pereopod 2 (right) total length, cheliped 1 (right) total length. In addition, a number of measurements were made along segments of cheliped 1 (right): carpus length, merus length, propodus length, dactyl length and propodus width. All individuals were provisionally examined for evidence of parasites. Parasitised individuals were assigned to different subpopulations than non-parasitised individuals, according to type of parasite (either bopyrid isopod or rhizocephalan).

2.2. Electrophoresis

A 3 mm cube of abdominal muscle tissue was dissected from the galatheid whilst still frozen. Starch gel electrophoresis was performed by methods described by Creasey et al. (1996,1997) using 12.5% starch gels. Two buffer systems were used: Buffer System I, Tris-versene-borate, continuous, pH 8.0 (Shaw and Prasad, 1970 No. 3); Buffer System II, Tris-citric-boric-lithium hydroxide, discontinuous, electrode pH 8.29, gel pH 8.26 (Redfield and Salini, 1980). Buffer system I was run at 300 V, 40 mA for 7 hours; Buffer System II was run a 185 V, 40 mA for 7 h.

A total of eight enzymes coding for 10 loci were visualised using enzyme-specific stains modified from Manchenko (1994): carbonic anhydrase (CA, E.C. 4.2.1.1, in buffer system II); gluconate dehydrogenase (GNDH, E.C. 1.1.1.69, in buffer system II); glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1, in buffer system I); general protein (GP, in buffer system II); malate dehydrogenase (MDH, E.C. 1.1.1.37, in buffer system I); malic enzyme (ME, E.C. 1.1.1.40, in buffer system I); phosphoglucose isomerase (PGI, E.C. 5.3.1.9, in buffer system II); phosphoglucomutase (PGM, E.C. 5.4.2.2, in buffer system I).

The largest sample of *Munidopsis scobina* (Station 12702#1) was chosen as the reference population. Alleles were labelled in ascending alphabetical order according to their distance from the origin. The most common allele at each enzyme locus in the reference population was given a relative mobility of 100. Other alleles at each locus were given a mobility relative to the most common allele. Where more than one locus appeared for each enzyme system, the loci were numbered from the slowest to the fastest.

2.3. Data analysis

2.3.1. Population biology

The sex ratios (S_0) of the overall population (i.e. both parasitised and non-parasitised individuals) and the non-parasitised and parasitised sub-populations of *Munidopsis scobina* were determined according to the formula modified from Christiansen et al. (1990):

$$S_0 = \frac{(M_0 - F_0)}{(M_0 + F_0)}, \tag{1}$$

where M_0 is the total number of males, and F_0 is the total number of females in the sample. Size class (0.5 mm intervals of carapace width) in the overall population of *M. scobina* was plotted against sex ratio, and a trend line inserted with a floating mean of three size classes (in order to offset bias owing to small sample numbers in some size classes). Sex ratios of the overall populations and the non-parasitised and parasitised sub-populations of *M. scobina* were analysed using two-tailed, chi-squared tests to determine significant deviations from an expected 1 : 1 sex ratio.

Variation in mean carapace width of bopyrid-, rhizocephalan- and non-parasitised individuals was determined at each of the two stations for each sex. Significance of variation was tested using a one-way analysis of variance between each subpopulation, using a post hoc Tukey hypothesis of pairwise comparisons.

Size frequency of non-parasitised individuals was plotted for both sexes of *Munidopsis scobina* from both stations, using 0.5 mm intervals of carapace width in order to detect modal peaks that may be indicative of discrete developmental instars. A one-way analysis of variance (ANOVA) was used to determine any significant difference in size distribution (carapace width) between non-parasitised male and female *M. scobina* within each population. A one-way ANOVA also was used to determine, for each of the morphometric parameters measured, if there was a significant-different size distribution within and between each sex at the two populations, using a post hoc Tukey hypothesis. Testing was performed under two hypotheses: (1) there was no sexual dimorphism in any parameter at each station (population), and (2) there was no significant variation in parameters within individuals of a single sex from the two stations (populations).

All Analyses of Variance tests were performed using SYSTAT 5.04 (Wilkinson, 1990). Where multiple tests were carried out, all tests were Bonferroni adjusted (Rice, 1989).

2.3.2. Genetics

Allelic frequencies were calculated across all 10 enzyme loci for all samples of *Munidopsis* spp., and across nine enzyme loci for *Galathea squamifera* (see results). Genetic variability was assessed using a number of parameters: the mean number of alleles per locus, the percentage polymorphic loci, the observed heterozygosity and the expected heterozygosity based upon conformity to the Hardy–Weinberg equilibrium. Individuals of both populations of *Munidopsis scobina* also were pooled according to

size class (1 mm carapace length). Allele frequencies and genetic variability were assessed in each of the size classes.

For both populations of *Munidopsis scobina*, the percentage of polymorphic loci, overall genetic differentiation estimates [using Nei's (1972) genetic identity (I) and genetic distance (D)], genetic differentiation among populations (F_{ST}), deviation from random-mating expectations within populations (F_{IS}), F_{ST} and F_{IS} significance level adjustments for multiple testing, number of migrants per deme per generation (N_em) and Wright's (1951,1965) fixation index (F) were calculated as in Creasey et al. (1997). F_{IS} and F_{ST} were analysed by the method given in Creasey et al. (1997) using the FSTAT program (Goudet, 1994). For the remaining species of *Munidopsis* and *Galathea*, the percentage of polymorphic loci and overall genetic differentiation were estimated as per Creasey et al. (1996).

All calculations, apart from estimates of F_{ST}, F_{IS}, and N_em obtained from the FSTAT programme (Goudet, 1994), were calculated using BIOSYS release 1.7 (Swofford and Selander, 1989).

3. Results

3.1. Population biology

The overall sex ratio of both populations of *Munidopsis scobina* in the present study showed significant deviation from a 1 : 1 expected ratio (Table 2), as did the non-parasitised subpopulations. Amongst the parasitised subpopulations, only rhizocephalan-infected individuals from station 12702#1 showed a significant deviation from an expected 1 : 1 sex ratio (Table 2). Between size classes, sex ratio varied, with males dominating in 9–11 mm carapace width size categories and females in 13–15 mm categories in both populations (Figs. 1a and b).

At both stations, significant differences in size distribution (carapace width) between male and female *Munidopsis scobina* were detected in the non-parasitised individuals using a one-way ANOVA for untransformed data ($p < 0.01$ for both stations; see Table 3). In addition, differences in size distribution within sexes were observed between the stations ($p < 0.05$ for both sexes; see Table 3). At both stations, female *M. scobina* were larger than males and individuals from station 12702#1 (1000 m) were smaller than those from station 12714#1 (900 m) (see Table 3; Figs. 2a and b). At both stations, each sex appeared to consist of a single mode (Figs. 2a and b).

Within stations, numerous morphometric parameters were significantly different between the sexes (Table 3). Of 20 comparisons made, only two (one from each population) were not significantly different at the $p < 0.01$ level. Females exhibited larger mean total length, mean carapace width and length than males. However, males generally exhibited larger mean cheliped segment lengths as well as overall mean cheliped and pereopod lengths than females (Table 3). Within each sex, significant variation was observed, but this was markedly less than that between sexes at a single station (Table 3). Carapace width, total length, carpus and merus lengths were all significantly greater in both sexes in individuals from station 12714#1 than in those

Table 2
Details of number of specimens sexed from, and parasitised within, each population. Sex ratio (S_0) and significance values of sex ratio deviation from an expected 1 : 1 ratio are also given for each population (*$p < 0.05$; **$p < 0.01$; d.f. = degrees of freedom)

	Overall population		Bopyrid parasitised		Rhizocephalan parasitised		Non-parasitised	
Station	12702#1	12714#1	12702#1	12714#1	12702#1	12714#1	12702#1	12714#1
Number of males (%)	1140	368	41 (3.6)	16 (4.3)	32 (2.8)	6 (1.6)	1067 (93.5)	346 (94.0)
Number of females (%)	735	233	27 (3.7)	21 (9.0)	88 (12.0)	14 (6.0)	618 (84.1)	198 (85.0)
Sex ratio (S_0)	0.22	0.22	0.21	− 0.14	− 0.47	− 0.40	0.27	0.27
Sex ratio (No. males: 1 female)	1.55	1.58	1.51	0.76	0.36	0.43	1.73	1.75
Deviation from 1 : 1 ratio	$\chi^2 = 44.5$** (d.f. = 1)	$\chi^2 = 15.4$** (d.f. = 1)	$\chi^2 = 1.46$ (d.f. = 1)	$\chi^2 = 0.34$ (d.f. = 1)	$\chi^2 = 13.8$** (d.f. = 1)	$\chi^2 = 1.7$ (d.f. = 1)	$\chi^2 = 60.9$** (d.f. = 1)	$\chi^2 = 20.5$** (d.f. = 1)

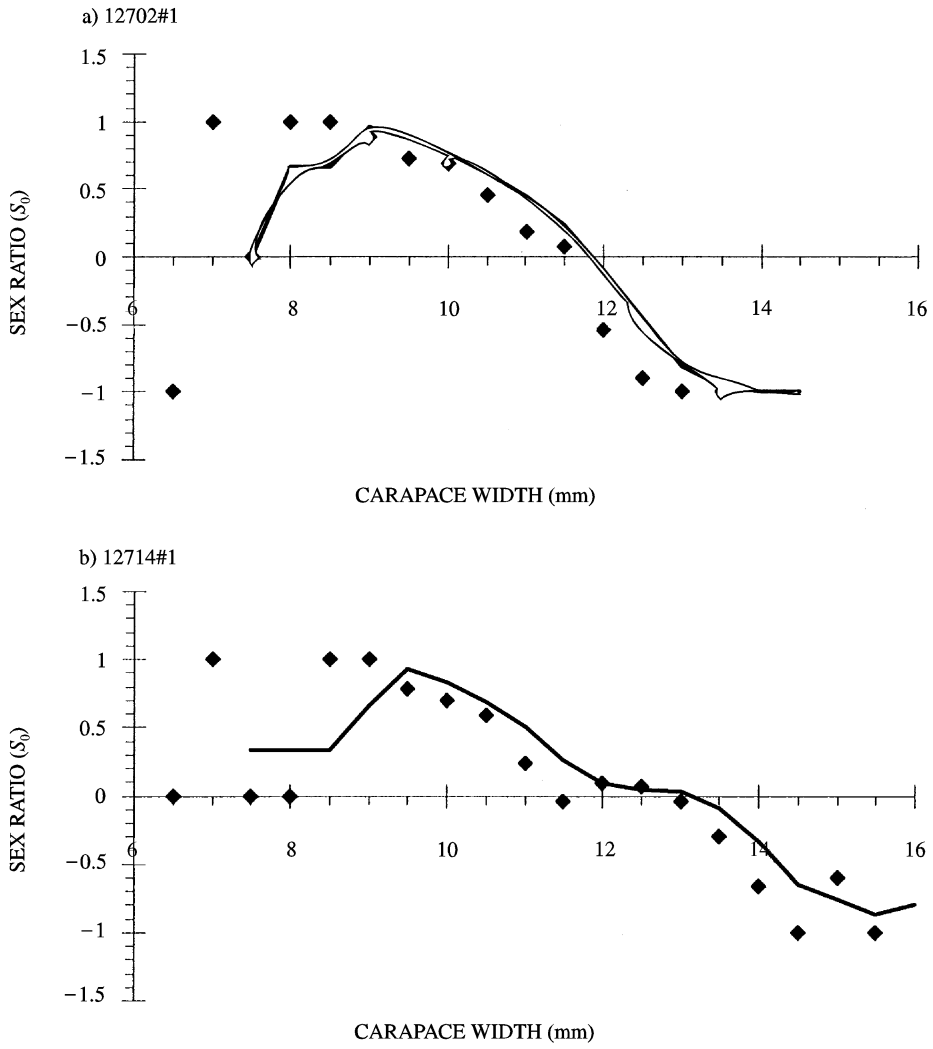

Fig. 1. *Munidopsis scobina*. Graphs of the sex ratio (S_0, denoted by solid diamonds) of non-parasitised individuals against carapace length. (a) Station 12702#1, (b) Station 12714#1.

from 12702#1 (Table 3). In addition, males from station 12714#1 had significantly larger mean propodus widths than those from 12702#1 (Table 3).

Of 2477 *Munidopsis scobina* sexed, the overall parasite load of the two populations ranged from 9.5% (12714#1) to 10.2% (12702#1). The overall parasite load in each sex was relatively uniform (c.6% in males, 15% in females), although the level of infestation by each of the parasite types varied both between populations and sexes (Table 2). The incidence of rhizocephalan infestation in both sexes of *M. scobina* was

Table 3
Details of morphometric parameters measured in *Munidopsis scobina* and significance values (p) within populations (n = number of observations; N.S. = not significant)

	Within stations, Between sexes						Within sexes	
Station	12702#1			12714#1			Males	Females
Sex	Male	Female	p	Male	Female	p	p	p
Carapace width	10.47 ± 0.05 ($n = 464$)	11.52 ± 0.07 ($n = 230$)	0.01	10.72 ± 0.06 ($n = 346$)	11.85 ± 0.08 ($n = 198$)	0.01	0.05	0.05
Carapace length	18.07 ± 0.10 ($n = 464$)	19.12 ± 0.14 ($n = 230$)	0.01	18.17 ± 0.11 ($n = 346$)	19.50 ± 0.15 ($n = 198$)	0.01	N.S.	N.S.
Total length	32.13 ± 0.17 ($n = 464$)	34.63 ± 0.24 ($n = 230$)	0.01	33.40 ± 0.19 ($n = 346$)	36.08 ± 0.26 ($n = 198$)	0.01	0.01	0.01
Carpus length	4.77 ± 0.04 ($n = 403$)	4.14 ± 0.05 ($n = 196$)	0.01	5.28 ± 0.06 ($n = 147$)	4.41 ± 0.08 ($n = 91$)	0.01	0.01	0.05
Merus length	9.70 ± 0.07 ($n = 405$)	7.90 ± 0.10 ($n = 196$)	0.01	10.08 ± 0.12 ($n = 147$)	8.50 ± 0.15 ($n = 91$)	0.01	0.05	0.01
Propodus length	13.74 ± 0.10 ($n = 379$)	10.44 ± 0.14 ($n = 186$)	0.01	14.06 ± 0.16 ($n = 144$)	10.93 ± 0.20 ($n = 91$)	0.01	N.S.	N.S.
Propodus width	1.39 ± 0.01 ($n = 391$)	1.36 ± 0.01 ($n = 187$)	N.S.	1.52 ± 0.02 ($n = 145$)	1.42 ± 0.02 ($n = 91$)	0.01	0.01	N.S.
Dactyl length	7.98 ± 0.06 ($n = 379$)	5.63 ± 0.09 ($n = 186$)	0.01	8.12 ± 0.1 ($n = 144$)	5.89 ± 0.13 ($n = 91$)	0.01	N.S.	N.S.
Cheliped length	36.43 ± 0.24 ($n = 378$)	30.55 ± 0.34 ($n = 186$)	0.01	36.81 ± 0.38 ($n = 146$)	31.43 ± 0.48 ($n = 91$)	0.01	N.S.	N.S.
Pereopod length	27.35 ± 0.17 ($n = 390$)	26.11 ± 0.24 ($n = 186$)	0.01	26.87 ± 0.27 ($n = 152$)	26.52 ± 0.34 ($n = 96$)	N.S.	N.S.	N.S.

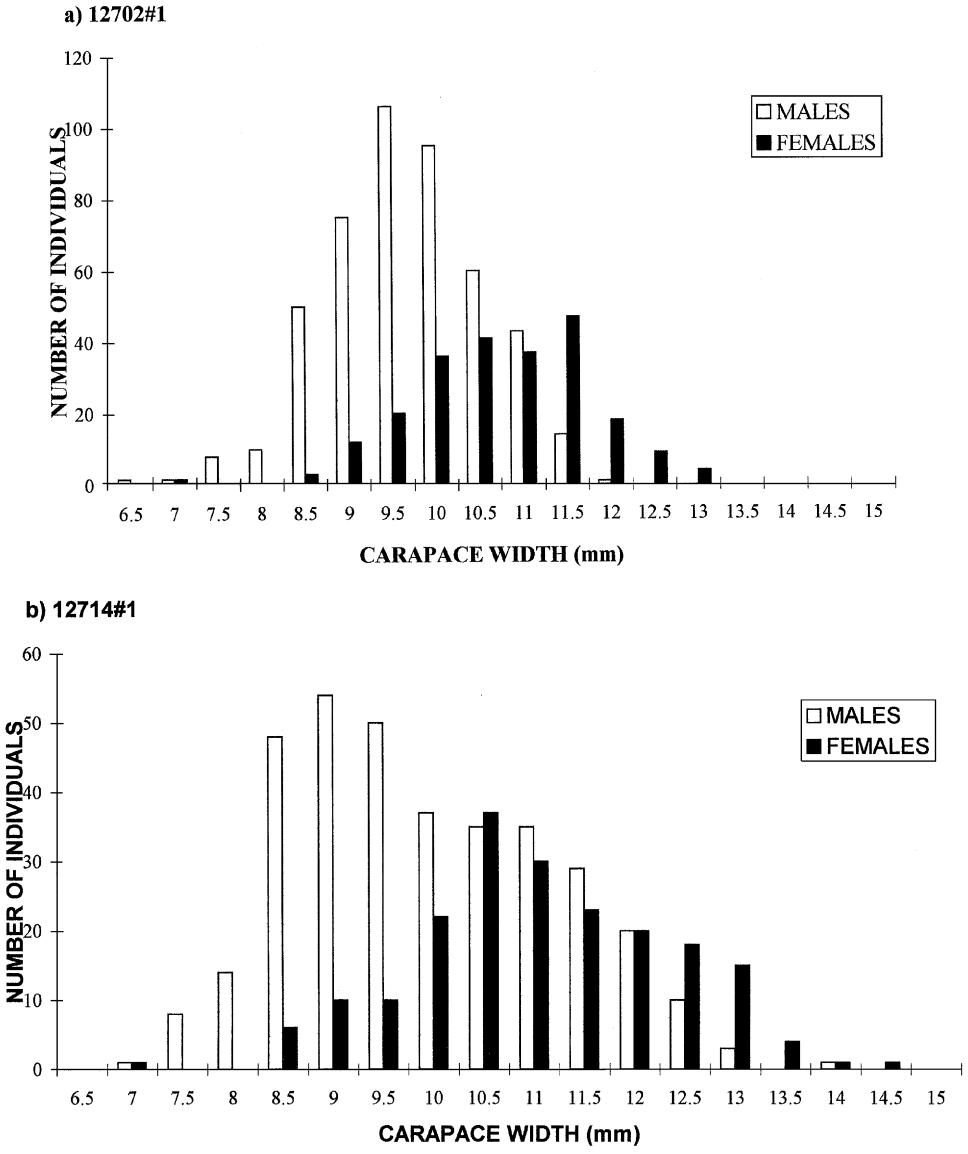

Fig. 2. *Munidopsis scobina*. Histograms of size frequency for non-parasitised male (open bars) and female (hatched bars) squat lobsters. (a) Station 12702#1, (b) Station 12714#1.

higher in individuals from station 12702#1 than in those from 12714#1. The opposite trend was seen in bopyrid-parasitised individuals (Table 2). Bopyrid-parasitised individuals at station 12714#1 were observed to be significantly larger than either rhizocephalan- ($p < 0.01$) or non-parasitised ($p < 0.01$) individuals at the same

Table 4
Mean carapace width (mm) in parasitised and non-parasitised individuals at each population (Standard deviation, number of observations)

Station	12702#1		12714#1	
Sex	Male	Female	Male	Female
Non-parasitised	10.47 [0.88, 464]	11.52 [1.01, 230]	10.72 [1.31, 346]	11.84 [1.28, 198]
Bopyrid-parasitised	10.66 [0.65, 20]	12.22 [1.03, 18]	11.28 [1.45, 16]	13.72 [1.41, 21]
Rhizocephalan-parasitised	10.44 [0.98, 14]	11.34 [1.00, 31]	11.13 [0.76, 6]	11.36 [0.89, 14]

station (Table 4). No other significant differences were observed in any of the subpopulations at each station, in either sex. In addition to the data presented in Table 2 three individuals (one male, two female) from station 12702#1 were parasitised by both bopyrids and rhizocephalans, which is similar to that statistically expected (one male, three females).

3.2. Population genetics

All enzyme loci produced well-resolved staining patterns consistent with known enzyme subunit structures for all individuals with the following exceptions. The gluconate dehydrogenase locus was scored as *Gndh*, although, despite following the staining protocol given in Manchenko (1994), the characteristic blue, formazan-precipitated banding was not observed. Instead, translucent bands against a blue background were noted, which are neither characteristic of gluconate dehydrogenase nor D-2-hydroxyacid dehydrogenase (2-HADH-A; E.C. 1.1.99.6), which may be visualised using this stain (Tobler and Grell, 1978). The carbonic anhydrase locus was scored as *Cab*. One other locus (*Caa*), which was closer to the origin, showed poor resolution with low activity. In addition, several other (2 +) loci were detected on the gel if the stain was incubated overnight. However, these additional loci were often poorly resolved, and probably represented a mixture of other carbonic anhydrase and esterase (E.C. 3.1.1.1) loci. These additional loci were not used in the present study. Two loci were also expressed in the GOT stain system. However, owing to a combination of poor resolution and activity, *Gota* was not scored. All individuals were scored for *Gotb*. In *Galathea squamifera*, no third general protein (*Gpc*) locus was detected.

In both populations of *Munidopsis scobina* allele frequencies were similar (Table 5), with all polymorphic loci in both populations exhibiting diallellism. Allele frequencies

between the two populations of *Munidopsis scobina* and those of other *Munidopsis* species show major differences between two (*M. scobina* vs. *M. parfaiti*) to six (*M. scobina* vs. *M. spinihirsuta*) of the 10 enzyme loci (Table 5).

Genetic variation in both populations of *Munidopsis scobina* was similar (Table 6), both in terms of the percentage of polymorphic loci and number of alleles per locus. In *M. scobina*, observed heterozygosity (H_0) ranged from 0.088 to 0.108 and was lower than expected heterozygosity (H_e). H_0 was larger in the largest sample (12702 # 1), although sample numbers were relatively high in both populations (Table 6). There was no significant relationship between observed heterozygosity and size for *M. scobina*. In *M. parfaiti*, the only other population in which enzyme polymorphism was detected (Table 7), the percentage of polymorphic loci (44.4%) was similar to that observed in *M. scobina* (40.0%). In *M. parfaiti*, unlike *M. scobina*, H_0 (0.156) exceeded H_e (0.126).

Individuals of *Munidopsis crassa, M. spinihirsuta*, *M. subsquamosa* and *Galathea squamifera* showed no polymorphic loci and hence heterozygosity could not be estimated. The uniformity in genotype frequencies across all loci resulted from the low sample size and low number of enzyme loci screened. Similarly, the heterozygote excess shown in *M. parfaiti* is likely to be a result of the low sample size and number of enzyme loci scored.

Analyses of Nei's (1972) genetic identity (I) and genetic distance (D) (Table 7) indicate that both populations of *Munidopsis scobina* are genetically almost identical ($I = 0.996$). The I values obtained between different species of *Munidopsis* ranged from 0.400 to 0.712. I values between *Galathea squamifera* and *Munidopsis* spp. ranged from 0.222 to 0.333. However, as a result of the low number of individuals for all species, except *M. scobina*, and relatively low number of enzyme loci screened, sampling error may be high (Gorman and Renzi, 1979). The relationships between the species, based on UPGMA analysis of Nei's (1978) unbiased genetic distance, in the present study can be seen in Fig. 3.

F_{ST} values for individual loci in the two populations of *Munidopsis scobina* vary from 0.000 to 0.033 (Table 8). F_{ST} values for *Cab*, *Gotb*, *Pgia* and the mean F_{ST} are all significantly different from zero at the $p < 0.01$ level. When these data were bootstrapped (500 permutations), F_{ST} values were still significant at the $p < 0.01$ level, for the *Cab* and *Gotb* loci and the overall F_{ST}. This would appear to indicate a significant degree of genetic differentiation between the two populations.

The number of migrants per deme per generation ($N_e m$) calculated from the mean F_{ST} indicate a moderate level of genetically effective migration between the two populations (Table 8). Analysis of F_{IS} for the two populations of *Munidopsis scobina*, which indicates the level of correlation between homologous alleles within a population, showed significant values at the $p < 0.01$ level for *Gotb*, *Pgma* and the mean F_{IS} (Table 8). When data were bootstrapped, F_{IS} was significant at the *Pgma* ($p < 0.05$), *Gotb* ($p < 0.01$) loci and overall F_{IS} value ($p < 0.01$). Significant values of F_{IS} at the *Gotb* and *Pgma* loci resulted from a heterozygote deficiency, as indicated by the large positive values for Wright's (1951,1965) Fixation index (F) at both loci in both populations of *M. scobina* (Table 9). Heterozygote deficiency also was responsible for a significant deviation from genotype frequencies expected under Hardy–Weinberg

Table 5
Allele frequencies for loci for all samples of *Munidopsis* spp. and *Galathea squamifera*. Relative mobilities of alleles are in parentheses. Most common allele in reference population (*Munidopsis scobina*, station 12702#1) was nominated a mobility of 1.00. *n* = number of samples

Locus	*M. crassa*	*M. parfaiti*	*M. scobina*		*M. spinihirsuta*	*M. subsquamosa*	*G. squamifera*
Station			12702#1	12714#1	12714#1		
n	4	5	256	171	4	4	6
Cab							
A (0.62)	—	—	—	—	1.000	—	—
B (0.80)	—	0.100	0.004	—	—	—	1.000
C (0.93)	1.000	—	0.021	0.003	—	1.000	—
D (0.97)	—	—	0.029	0.003	—	—	—
E (1.00)	—	0.900	0.945	0.994	—	—	—
Gndh							
A (1.00)	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gotb							
A (0.94)	—	—	0.387	0.193	—	—	—
B (1.00)	—	—	0.605	0.751	—	—	1.000
C (1.06)	—	0.900	0.008	0.053	1.000	—	—
D (1.12)	1.000	0.100	—	0.003	—	1.000	—
Gpa							
A (1.00)	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpb							
A (1.00)	1.000	1.000	1.000	1.000	—	1.000	—
B (1.12)	—	—	—	—	1.000	—	—
C (1.14)	—	—	—	—	—	—	1.000

Gpc							
A (0.84)	—	—	—	—	—	—	—
B (1.00)	1.000	1.000	1.000	1.000	—	—	—
C (1.10)	—	—	—	—	1.000	1.000	—
Mdh							
A (1.00)	—	—	1.000	1.000	1.000	—	—
B (1.26)	—	1.000	—	—	—	1.000	—
C (1.47)	1.000	—	—	—	—	—	1.000
Me							
A (1.00)	1.000	1.000	1.000	1.000	—	1.000	—
B (1.15)	—	—	—	—	1.000	—	—
C (1.42)	—	—	—	—	—	—	1.000
Pgi							
A (0.10)	—	—	0.002	—	—	—	—
B (0.89)	—	0.400	0.004	0.006	—	—	1.000
C (0.95)	—	—	0.252	0.325	—	—	—
D (1.00)	1.000	0.600	0.742	0.670	—	1.000	—
E (1.13)	—	—	—	—	1.000	—	—
Pgm							
A (0.47)	—	—	—	—	—	—	1.000
B (0.84)	—	—	—	0.003	—	—	—
C (0.94)	1.000	—	0.160	0.164	—	1.000	—
D (1.00)	—	0.900	0.836	0.825	1.000	—	—
E (1.03)	—	0.100	—	—	—	—	—
F (1.06)	—	—	0.004	0.006	—	—	—
G (2.58)	—	—	—	0.003	—	—	—

Table 6
Measures of genetic variability [means (S.E.)] in *Munidopsis* spp. and *Galathea squamifera*. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99 (H_0 = observed heterozygosity [direct-count]; H_e = Hardy–Weinberg expected heterozygosity).

Population	*M. crassa*	*M. parfaiti*	*M. scobina*		*M. spinihirsuta*	*M. subsquamosa*	*G. squamifera*
Station			12702#1	12714#1	12714#1		
Mean sample size per locus	4	5	256	171	4	4	6
Mean no. of alleles per locus	1.0 (0.0)	1.4 (0.2)	2.0 (0.4)	2.1 (0.5)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Percentage polymorphic loci	0.0	44.4	40.0	40.0	0.0	0.0	0.0
Observed Heterozygosity (H_0)	0.000 (0.000)	0.156 (0.087)	0.108 (0.049)	0.088 (0.045)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Expected Heterozygosity (H_e)	0.000 (0.000)	0.126 (0.060)	0.125 (0.059)	0.115 (0.059)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)

Table 7
Pairwise comparisons of Nei's (1972) genetic identity and distance between samples of *Munidopsis* spp. and *Galathea squamifera*. Above diagonal: Nei's (1972) Genetic identity (*I*). Below diagonal: Nei's (1972) Genetic distance (*D*). All comparisons between *Munidopsis* spp. are based upon 10 enzyme loci. Comparisons including *Galathea squamifera* are based upon nine enzyme loci

Population (Station)	*M. crassa*	*M. parfaiti*	*M. scobina* (12702 # 1)	*M. scobina* (12714 # 1)	*M. spinihirsuta*	*M. subsquamosa*	*G. squamifera*
M. crassa	—	0.602	0.706	0.691	0.400	0.700	0.313
M. parfaiti	0.508	—	0.712	0.712	0.517	0.602	0.328
M. scobina (12702 # 1)	0.349	0.340	—	0.996	0.411	0.526	0.222
M. scobina (12714 # 1)	0.370	0.339	0.004	—	0.412	0.514	0.222
M. spinihirsuta	0.916	0.660	0.889	0.887	—	0.400	0.333
M. subsquamosa	0.357	0.508	0.642	0.665	0.916	—	0.295
G. squamifera	1.099	1.221	1.162	1.115	1.504	1.504	—

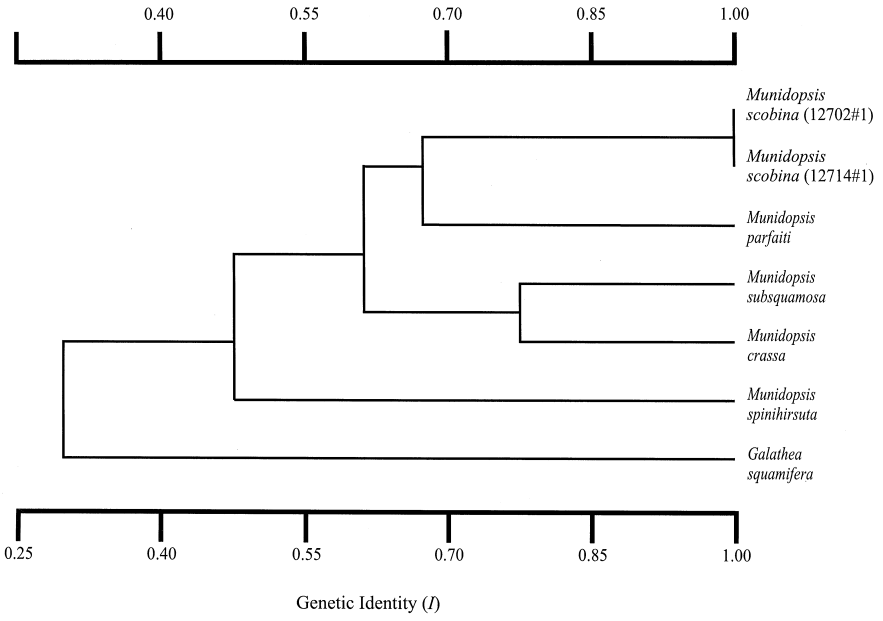

Fig. 3. *Munidopsis* spp. and *Galathea squamifera*. Unweighted pairgroup method analysis (UPGMA) dendrogram calculated from Nei's (1978) genetic identity (*I*).

Table 8
F-statistics (analysis of variance of genotype frequencies within and between populations) for two populations of *Munidopsis scobina* (*$p < 0.01$).

Locus	F_{IS}	F_{ST}
Cab	0.029	0.015*
Gotb	0.314*	0.033*
Pgi	0.102	0.006*
Pgm	0.135*	0.000
Mean	0.184*	0.015*
$N_e m$	16.4	

equilibrium at the *Gotb* locus. At the *Gotb*, *Pgia* and *Pgma* loci there was a higher heterozygote deficiency in the population from station 12714#1 (Table 9).

4. Discussion

4.1. Population biology

The exact geographic distribution of *Munidopsis scobina* is uncertain, both in terms of horizontal and vertical ranges. The species was initially described from specimens

Table 9
Fixation index (F) for variable loci at each sample station for populations of *Munidopsis scobina*. Significant deviations from Hardy–Weinberg expectations are indicated. ($*p < 0.01$).

Locus	Station	
	12702#1	12714#1
Cab	0.033	−0.004
Gotb	0.273*	0.364*
Pgi	0.048	0.148
Pgm	0.064	0.202

which were captured in the Bay of Bengal (Northeast Indian Ocean) at depths of approximately 350–750 m, during the cruise of H.M.S. *Investigator* (Alcock, 1894,1901). *M. scobina* also was collected during the *John Murray* expedition (1933–1934) from the western Arabian Sea at a depth of 1046 m (Tirmizi, 1966). In the present study, specimens were collected off the coast of Oman, extending the known western/southern range of the species by approximately 325 km. There are no reports of *M. scobina* between the Bay of Bengal (Alcock, 1894,1901) and Arabian Sea (Tirmizi, 1966; present study), so the distribution of the species between these regions is uncertain. Furthermore, there is a possibility that the Bengal population is a separate species to those from the Arabian Sea (Tirmizi, 1966; also see later).

The vertical range of *Munidopsis scobina* is also difficult to assess, since specimens collected by Alcock (1894,1901) were from shallower depths than those collected by Tirmizi (1966) and in the present study. In the present study, neither *M. scobina* nor *M. spinihirsuta* were collected or observed with photographic gear above 900 m (Gage, 1995). At depths where *M. Scobina* were captured, oxygen concentrations were recorded as ranging from 0.24 ± 0.30 ml l^{-1} to (850 m) to 0.30 ± 0.33 ml l^{-1} (1000 m) (Levin et al., 2000). Neyman et al. (1973) reported that in the Indian Ocean the oxygen minimum zone (OMZ) may extend to 1200 m depth, and hydrogen sulphide may be present at depths of up to 600 m. During *Discovery* Cruise 211, to the northern Arabian Sea, the OMZ was observed between 300 and 1250 m depth (Gage, 1995; Creasey et al., 1997; Levin et al., 2000). It is possible that the OMZ fluctuates, both spatially and temporally. If the OMZ does fluctuate in both severity and depth range, then populations such as those in the present study that are normally found where oxygen levels are greater than approximately 0.2 ml l^{-1}, may be periodically exposed to more severe hypoxia, resulting in localised extinctions. This would imply that neither species could tolerate extreme hypoxia. It is therefore possible that the upper distribution limits of both *M. scobina* and *M. spinihirsuta* are regulated by oxygen concentrations. The oxygen concentration at which this response occurs may be similar to the minimum critical level reported (0.13 ml l^{-1}) for aerobic respiration in midwater Crustacea (Childress, 1975; Wishner et al., 1990). Such a response would be consistent with the poor tolerance to hypoxia observed in other galatheids of the genera *Munida* and *Galathea* (Wishner et al., 1990; Zainal et al., 1992), although some

galatheids, such as *Pleuroncodes planipes*, are adapted to more hypoxic conditions (Kashkina and Kashkin, 1993). Note that mortality caused by fluctuations in spatial and temporal variations of the OMZ may not occur just in the adult population but could arise in earlier life-history stages that might be more susceptible to hypoxia (Marcus et al., 1997; see later).

Numerous studies have reported descriptions of galatheids (Chace, 1942; Chevaldonné and Olu, 1996 and references therein), but relatively few studies have captured large numbers of specimens to allow an adequate assessment of the population structure in the target species. Wenner (1982) reported that in six species of *Munidopsis* and *Munida*, significant female-biased sex ratios were observed in *Munida longipes* and *Munida valida*, whilst Wilkens et al. (1990) also recorded a similar sex ratio bias in the anchialine *Munidopsis polymorpha*. Male-biased sex ratio was observed in *Munida iris* (Bursey, 1978), *Munida sarsi* (Attrill, 1989) and *Munidopsis bairdii* (Wenner, 1982), although in the last case this was not significant. In the present study, the overall sex ratio of both *Munidopsis scobina* populations deviated significantly from an expected 1 : 1 ratio (Table 2), as a result of an excess of males, although parasitised subpopulations also exhibited some deviation from a 1 : 1 ratio as a result of a deficit of males (see below). Sex ratio data are not recorded in any of the previous records of *M. scobina* (Alcock, 1894,1901; Tirmizi, 1966), and Tirmizi (1966) only noted '… several specimens of both sexes'.

Crustacea are thought to have a pair of heteromorphic sex chromosomes, which determine the sex of individuals (Ginsburger-Vogel and Charniaux-Cotton, 1982). It is therefore expected that an equal sex ratio should occur in eggs of Crustacea (Hartnoll et al., 1993). A significant male-biased sex ratio has been recorded in the galatheids *Munida iris* and *M. sarsi* (see above; Bursey, 1978; Attrill, 1989), and also in the majid *Encephaloides armstrongi* (Creasey et al., 1997), which was captured from the same region as the *Munidopsis scobina* in the present study. There are a number of possible explanations for the observed sex ratio in the present study: (1) sex reversal, (2) differential behaviour and (3) differential growth. Sex reversal in galatheids has been reported in response to parasitic infestation (Wenner, 1982; Attrill, 1989). No sexually indeterminate individuals were observed in the present study, and sex reversal is therefore considered to be unlikely amongst non-parasitised individuals (however, see below).

Differential behaviour also has been cited as a reason for variable sex ratios. If males and females have temporally or spatially different migratory patterns, then segregation may result (Allen, 1966; Creasey et al., 1997). Also, if behavioural differences between males and females cause differential mortality, then a variable sex ratio may result. In crabs this is usually expressed as an increased mortality of males as a result of increased competition (see below) or predation (see discussion in Creasey et al., 1997). Differential behaviour however, may cause a difference in gear selectivity between the sexes (see below).

The sex ratio in the present study also varied with carapace size class (Figs. 1a and b). Male *Munidopsis scobina* were dominant in both populations in the 9–11 mm size classes, and females in the 13–15 mm size classes. In the 6.0–9.5 mm size classes, only 12 females were measured, of which six were ovigerous. It is therefore possible that the

10 mm size class represents the typical size at which females mature. Furthermore, it is possible that in the smaller, and presumably younger size classes, immature females may be difficult to accurately sex as they may exhibit characters more typical of males, and a bias in sex ratio may result. The sex ratio distribution curves of both populations are similar to that termed as an 'intermediate' pattern by Wenner (1972), whereby the proportion of males is relatively uniform in small size classes and then decreases sigmoidally as size classes increase. Wenner (1972) proposed five explanations for this relationship between sex ratio bias and size. These explanations are similar to those proposed for the overall sex ratio variation (see above). Protandry has been proposed in populations that exhibit a predominance of males in small size classes, which decreases sigmoidally into larger, predominantly female size classes (Wenner, 1972), with sexually indeterminate specimens occurring in intermediate size classes. However, this pattern also has been observed in species in which sexually intermediate individuals did not appear, and therefore were not believed to exhibit protandry (see data on *Hippa pacifica*; Wenner, 1972). In the present study, no sexually indeterminate individuals were observed, whilst a number of small (< 9.5 mm size classes) females were captured. It is therefore unlikely that protandry occurs in *M. scobina*.

Differential mortality also is considered unlikely (see above) to explain the observed trend in sex ratio. Differential migration between the sexes is unlikely, since male-biased sex ratios may be related to spawning aggregations (Creasey et al., 1997). The presence of large numbers of ovigerous females, with two size classes of eggs (Creasey et al., 2000), would indicate that egg laying would have already occurred, unless eggs are extruded immediately after release of the previous cohort and the present study sampled individuals during such a period. Furthermore, if ovigerous females exhibited behavioural responses to avoid predation it would be unexpected to obtain the large proportion of ovigerous individuals as seen in the present study. Differential mortality also may not explain the observed variation. If large males suffered higher mortalities than females, larger size classes would be predominantly female. However smaller size classes would not contain large proportions of males unless the sex ratio in these classes was biased from a 1 : 1 ratio because of an earlier event. The most probable explanation for the observed trend is differential growth, with females growing at faster rates and possibly to larger sizes than male siblings. This is consistent with the observed variation in size-frequency distribution between the sexes (Table 3; Figs. 2a and b).

The size distribution (carapace width) data for *Munidopsis scobina* indicated significant differences between both male and female squat lobsters at both stations (Table 3; Figs. 2a and b). In addition, there were significant differences in size distribution within each sex, between the two stations (Table 3). At both stations each sex probably consisted of a single mode (Figs. 2a and b), although it is not known whether this is a reflection of capture gear bias, or if each mode represents a terminal moult. In both sexes, individuals from station 12714#1 were larger than those from 12702#1 (Table 3) and female *M. scobina* were larger than males (Table 3, Figs. 2a and b). These data are consistent with those observed in other galatheid Crustacea. In five out of six species of the genera *Munida* and *Munidopsis*, captured from the Middle Atlantic

Bight, Wenner (1982) reported ovigerous females were significantly larger than males in the same population. The larger size of individuals from the shallower station (900 m; 12714 # 1) may suggest that individuals of *M. scobina* may migrate up the continental slope as they mature, as has been reported for the deep-sea crab *Geryon trispinosus* (Attrill et al., 1990). Allopatry per se is considered unlikely because of the high estimates of migrants ($N_e m$) between the two populations (see later). Particle flux in this region has been reported as decreasing with increasing depth (see below; Nair et al., 1989), and individuals from Station 12714 # 1 (900 m) therefore may have access to increased amounts of food with limited interspecific competition and hence may have higher growth rates. However, individuals at this population, and populations in general, at these depths may risk aperiodic extinctions as a result of increased hypoxia in the oxygen minimum zone (see above; see also later). Coupled with this hypothesis is the possibility that there is a size-dependent respiration tolerance to hypoxia, with larger individuals being able to survive in areas of lower oxygen concentration. Such a relationship has previously been observed in galatheids (e.g. Burd and Brinkhurst, 1984).

Female *Munidopsis scobina* exhibited larger carapace lengths and total lengths than males from the same station (Table 2). However, male squat lobsters in both populations exhibited larger cheliped lengths, as well as greater lengths along a number of sections along the cheliped. The most probable explanation for this is that increased cheliped length is a secondary sexual characteristic in *M. scobina* and that males possibly exhibit agonistic behaviour when mating, as has been reported in a number of other Crustacea (Lee, 1995). It is therefore possible that in *M. scobina*, as in other Crustacea, cheliped size may be perceived by interacting males as being correlated with physical strength (Lee and Seed, 1992). However, an alternative explanation is that cheliped dimorphism is related to differential foraging strategy, although this is more common in predatory Crustacea (Lee and Seed, 1992).

Within the two populations of *Munidopsis scobina* examined in the present study, approximately 10% of specimens were parasitised in each population, although the incidence of each type of parasite varied (Table 2). The incidence of both bopyrid and rhizocephalan parasitisation varied both between station and sex, from 3.6% (males, 12702 # 1) to 9.0% (females, 12714 # 1) by bopyrids, and from 1.6% (males, 12714 # 1) to 12.0% (females, 12702 # 1) by rhizocephalans. Whilst the overall levels of infection by the parasites were uniform within each host sex ($\pm$ 1% in both males and females; see Table 2), the level of infection differed markedly between different host sexes ($\pm$ 10% between males and females), and was higher in females at both populations. A number of hypotheses may explain this observation, but the most probable cause for the deviation in sex ratio bias in parasitised *M. scobina* is that infected males undergo feminisation (see later).

The level of bopyrid parasitisation observed in the present study on *Munidopsis scobina* is consistent with that observed previously in galatheids, where infestation varied between 2 and 10% (Bursey, 1978; Wenner and Windsor, 1979; Squires, 1990). In the present study the absolute number of males parasitised was higher than females, but the proportion of infection was higher in females. However, the sex ratio of parasitised individuals was not significantly different from either a 1 : 1 expected sex

ratio (Table 2) or the 1.6 : 1 sex ratio observed in non-parasitised individuals (12702#1: $\chi^2 = 0.003$, d.f. = 1, $p > 0.95$; 12714#1: $\chi^2 = 3.13$, d.f. = 1, $p > 0.05$). Similarly, the level of rhizocephalan parasitisation observed in the present study is similar to those previously reported (1–5%) for galatheids (Wenner, 1982; Lützen, 1985; Attrill, 1989). The rhizocephalan in the present study has been identified as belonging to the genus *Cyphosaccus* (J.T. Høeg, University of Copenhagen, personal communication), species of which have a bathyal or abyssal distribution, and have been previously recorded as parasitising *Munidopsis* spp. (Lützen, 1985). The sex ratio of *Cyphosaccus*-parasitised individuals differed significantly from a 1 : 1 expected ratio (Table 2), a result of an excess of females. Of the possible causes for this bias (see above), the most probable is feminisation of males, as has frequently been reported in other rhizocephalan-infected Crustacea (see references, above). The most probable explanation for feminisation is that it increases parasite survivorship as a result of induced host behavioural changes caused by "egg mimicry" (Hartnoll, 1967; Høeg, 1995). Hartnoll (1967) and Ritchie and Høeg (1981) observed that the rhizocephalan externae occupy the position and role of the host egg mass. As a result, the host will exhibit brood-caring behaviour (Høeg, 1995). Furthermore, when the parasite spawns the host typically exhibits the spawning behaviour of flapping its abdomen to increase larval dispersal (Høeg, 1995). By inducing feminisation in parasitised males, brood-caring behavioural responses can be induced in hosts that would not normally exhibit such behaviour (Ritchie and Høeg, 1981; Høeg, 1995). Such an effect on male hosts reduces the level of parasite mortality by inducing brood-care behaviour and also ensuring that the parasite does not have to preferentially select potential hosts (i.e. females) in order to optimise reproductive conditions. This situation may be exacerbated when potential hosts may be temporally or spatially susceptible to parasitisation for limited periods (e.g. during ecdysis).

The type of parasites recorded in the present study have three major effects on host organisms: (i) differential host size, (ii) parasitic castration, and (iii) Host feminisation. Host size is related to parasite size in bopyrids (Nelson et al., 1986). This may be an adaptation that results in increased fitness of the parasite as a result of increased host growth rate or size, or decreased host mortality (Baudoin, 1975). Such a relationship is seen in the present study, where bopyrid-parasitised *Munidopsis scobina* are larger than non-parasitised individuals, and significantly so in females from station 12714#1 (Table 4). In contrast, amongst the *Cyphosaccus*-parasitised individuals, mean size (carapace width) was less than that observed in non-parasitised individuals of the same sex, from the same station, except in males from station 12714#1 (Table 4). This is consistent with the observation of reduced mean host size in sacculinid-parasitised Crustacea (O'Brien and van Wyk, 1985). However, it is atypical of the positive correlation between host size and infection rate observed in peltogastrid-parasitised Crustacea (O'Brien and van Wyk, 1985). Overall, it is not possible to determine if these results are direct parasite-host interactions, per se, without further sampling and ideally observation of live, parasitised and non-parasitised hosts (see discussion in O'Brien and van Wyk, 1985).

Previous studies also have reported decreased fecundities in bopyrid-parasitised Crustacea. Nelson et al. (1986) observed that there was no significant difference in

respiratory rate between parasitised and non-parasitised grass shrimp (*Crangon franciscorum*). However, both Abu-Hakima (1984) and Nelson et al. (1986) stated that the parasite may have a significant effect on the reproductive output of the host, with fecundity decreasing by as much as 40%. In the present study, parasitised ovigerous females had fecundities that were 60–65% of those observed in non-parasitised, ovigerous *Munidopsis scobina*. It is therefore possible that the energetic demands and effects of bopyrids on *M. scobina* are similar to those observed in other decapod Crustacea. Bopyrid parasites also have been recorded as causing parasitic castration, by interfering with the uptake of vitellogin into the oocytes (Abu-Hakima, 1986 and references therein). In the present study no ovigerous, *Cyphosaccus*-parasitised, female *Munidopsis scobina* were recorded, and this is consistent with the complete "parasitic sterilisation" observed in rhizocephalan-infected Crustacea (Høeg, 1995). A number of hypotheses have been proposed to assess the evolutionary significance of parasitic castration on host growth: increased host growth, reduced host size, timing of parasite reproduction, and mortality during ecdysis (O'Brien and van Wyk, 1985). Increased host growth may result in increased host fecundity, survivorship or competitive advantage compared to non-parasitised individuals and as a result increased parasite survivorship. Evidence is equivocal for rhizocephalan-parasitised Crustacea (see O'Brien and van Wyk, 1985). Reduced host size at first reproduction may allow parasites to increase fecundity by reducing generation time. In addition, parasitised hosts may mature earlier than non-parasitised hosts in order to allow parasites to reproduce prior to the hosts reaching a more vulnerable part of their life cycle (e.g. ecdysis). Finally, limited host moulting could be selected for if parasite survivorship decreased as a result of host ecdysis, again resulting in reduced host size in comparison to non-parasitised individuals. In the present study, it is not possible to determine which of the above hypotheses are likely to be correct, without further investigation.

The role of parasites in affecting electrophoretic data of host organisms has been reviewed by Poly (1997). Parasites have been recorded as contributing detectable enzyme activity in a number of studies, including those on invertebrates (e.g. Wright et al., 1979; see Poly (1997) and references therein for full discussion). In the present study no evidence of parasite-derived enzymatic activity was observed. However, it is possible that some specific host genotypes are more susceptible to parasites than others and genetic structuring between populations of *Munidopsis scobina* may therefore be a result of parasite-induced mortality (e.g. Dybdahl and Lively, 1998).

4.2. Population genetics

In deep-sea Crustacea, observed heterozygosity (H_0) has varied from 0.000 to 0.158, with a mean of 0.086 (see Creasey and Rogers, 1999, for review). Hedgecock et al. (1982) reported that expected heterozygosity (H_e) values for galatheids were in the range 0.070–0.123. Two previous studies on the population genetics of *Munidopsis* spp. have also given similar estimates of genetic variation with $H_e = 0.123$ for *M. diomedeae* (Gooch and Schopf, 1972) and $H_0 = 0.079$ for *M. hamata* (Costa and Bisol, 1978). The observed heterozygosities for the two populations of *M. scobina*

(H_0 = 0.088–0.108), and for *M. parfaiti* (H_0 = 0.156) in the present study are therefore characteristic of both galatheids and deep-sea Crustacea in general.

The genetic identity (*I*) value obtained for the two populations of *Munidopsis scobina* is extremely high (I = 0.996). Thorpe (1982,1983) showed that there is only a small level of overlap between *I* values for conspecific populations and those for congeneric species, with 97% of species-level *I* values falling below 0.85. It is therefore highly probable that the two populations of *M. scobina* are conspecific. The genetic identity (*I*) values between different species of *Munidopsis* (I = 0.400–0.712) are typical of values obtained for comparisons of populations of congeneric species, whilst the values obtained for comparisons between *Munidopsis* spp. and *Galathea squamifera* (0.222–0.333) are characteristic of a confamilial level of taxonomic separation (Thorpe, 1982,1983; Thorpe and Solé-Cava, 1994). Thorpe (1982) and Thorpe and Solé-Cava (1994) concluded that an *I* value of 0.35 was critical in distinguishing between congeneric and intergeneric level distinctions. Approximately 85% of all *I* values between congeneric species are above 0.35, whilst between genera 77% of all values fall below 0.35 (Thorpe and Solé-Cava, 1994). *I* values obtained in the present study are subject to error because of the low sample sizes of both the number of loci scored and the number of individuals scored in the study species (Gorman and Renzi, 1979).

The phylogenetic relationship amongst the galatheid species screened in the present study (Fig. 3), based upon unweighted pair group method analysis (UPGMA), supports the hypothesis that all species of the genus *Munidopsis* screened are putatively monophyletic and that *Galathea squamifera* represents an outgroup. Five subgenera, *Munidopsis*, *Bathyankyristes*, *Elasmonotus*, *Galathodes* and *Orophorhynchus*, have been proposed, based on morphological variation of species within the genus *Munidopsis* (Alcock, 1901; Tirmizi, 1966). *M. crassa*, *M. scobina*, *M. spinihirsuta* and *M. subsquamosa* are all representative of the sub-genus *Munidopsis*, whilst *M. parfaiti* is assigned to the sub-genus *Orophorhynchus*. From the species screened in the present study, it is apparent that *M.* (*Orophorhynchus*) *parfaiti* is genetically similar to the four species from the sub-genus *Munidopsis*, and may be closely related to *M. scobina*. *M. spinihirsuta* appears to be more distantly related to other species assigned to the same sub-genus.

Munidopsis crassa and *M. subsquamosa* have both been recorded from hydrothermal vents (Chevaldonné and Olu, 1996), and their separate specific status has been questioned (Gordon, 1955; Gore, 1983). The genetic distance estimated in the present study between *M. crassa* and *M. subsquamosa* is not consistent with conspecific status, and supports morphological studies that concluded these species are separate (Gordon, 1955; Gore, 1983). It is notable that these two species appear to be more closely related to each other than to the other species of *Munidopsis* in the present study that have not been recorded from reducing environments (Fig. 3). However, since the present study only screened five species of *Munidopsis*, across a relatively low number of enzyme loci, the phylogenetic relationships of species in the genus *Munidopsis* are subject to a large degree of error. While these results are suggestive, a more thorough study of the phylogeny of the genus *Munidopsis*, including an increased number of species and other informative genetic markers, would enable a more

definitive assessment of the monophyletic or polyphyletic origins of the genus, as well as the relationship between species able, or not, to tolerate reducing environments.

A possible error in the identification of *Munidopsis* spp. in the present study is that the species identified as *Munidopsis scobina* may represent a different species to that described as *M. scobina* by Alcock (1894,1901). Tirmizi (1966) reported consistent morphological differences between the Bay of Bengal and Arabian Sea populations of *M. scobina*. Whether these morphological differences are the result of phenotypic variation as a result of environmental differences between the eastern and western regions of the northern Indian Ocean, or whether these differences have a genetic basis (and if so are genetic differences merely indicative of different populations) are open to question. Until further sampling is undertaken in these regions, it is not possible to determine the systematic status of *M. scobina* morphotypes.

Chi-squared analyses of departure of F_{ST} from zero indicated significant genetic differentiation between the two populations of *Munidopsis scobina* (Table 8). The number of migrants per deme per generation ($N_e m$) calculated from F_{ST} indicated a moderate level of gene-flow between the two populations (Table 8). The $N_e m$ estimate is in excess of the theoretical 1 individual per generation that is required to prevent divergence between populations owing to random genetic drift (Wright, 1940).

Munidopsis scobina had a level of gene flow between the two study populations, similar to the deep-sea, amphipods *Abyssorchomene* spp. (France, 1994) and *Eurythenes gryllus* (Bucklin et al., 1987) but a level of genetic differentiation lower, though still significant, than populations of the majid spider crab *Encephaloides armstrongi* from the northern Arabian Sea (Creasey et al., 1997). However, the study on *Eurythenes gryllus* is not strictly comparable as it was carried out on populations separated by over 1500 km, as opposed to 10s km in the present study, whilst genetic differentiation between populations of *Encephaloides armstrongi* was thought to result from mixing of genetically distinct populations and/or gender-biased dispersal (Creasey et al., 1997).

There are a variety of explanations for significant genetic differentiation between sample sites for *Munidopsis scobina* in the present study. Gene-flow may not be sufficient to maintain a homogenous genetic structure in the sampling region. *M. scobina* may not exhibit lecithotrophy as has been hypothesised for many of its congeners (Van Dover and Williams, 1991). Furthermore, larval development may be direct or abbreviated (as in *M. tridentata* (Sars, 1889), or *M. polymorpha* (Wilkens et al., 1990)). Data on population biology (Creasey et al., 2000) indicate that some or all larvae may be lecithotrophic or that eggs may hatch directly into post-larval stages. Alternatively, genetic differentiation between populations may be a result of adults being relatively sedentary in comparison to mobile species such as *Eurythenes gryllus*. Another possible reason for limited gene-flow across small geographic distances, particularly in the present study, is the presence of hydrographic or topographic barriers that limit dispersal in the study region. Other explanations for significant genetic differentiation between sampled populations of *M. scobina* are not related to dispersal (see below).

Significant heterozygote deficiencies have been recorded in many marine invertebrates, notably molluscs (Volckaert and Zouros, 1989; Borsa et al., 1991), but have

also been recorded in deep-sea Crustacea including hydrothermal vent-endemic species (Creasey et al., 1996). Five explanations are generally given for heterozygote deficiencies in a population: (1) inbreeding; (2) mixing of genetically different populations (Wahlund effect); (3) differential mortality of heterozygotes as a result of selection or assortative mating; (4) bias in sampling, and (5) incorrect scoring of genotypes owing to the presence of null alleles or loss of chromosomes.

If inbreeding or population mixing was responsible for the heterozygote deficiency, the effect should be observed across all variable loci (Tables 8 and 9). This was observed for the majid spider crab *Encephaloides armstrongi* from the same geographic region as *Munidopsis scobina* (Creasey et al., 1997). In contrast, for *M. scobina* in the present study, F_{IS} values for only two loci (*Gotb*, *Pgmb*) were significantly different from zero as a result of heterozygote deficiency in the two sample populations. It is notable that F_{IS} is positive across all loci for the two populations of *M. scobina* and *F* is positive in three out of four polymorphic loci in both populations. Inbreeding may contribute to heterozygote deficiency but its overall effect is unlikely to be significant in view of the results.

Bias in sampling is a potential source of error in the current study, though identical capture gear was employed at both stations. Wenner (1982) noted that *Munidopsis* exhibit avoidance responses to sample gear by burying themselves in the substratum. Therefore, specimens obtained for the present study may not represent random samples.

No homozygotes for null alleles were detected at any locus in the current study, although one heterozygous individual was detected for a null allele at the *Pgi* locus (allele A). The effect of null alleles on the heterozygote deficiency is not thought to be significant, because of the rare occurrence of such alleles (for *Pgi*, ca. 1 in 1000; data from present study and Creasey et al., 1996).

Selection is also a potential factor that may influence heterozygote deficiency. Analysis of genotype data for *Munidopsis scobina* indicated significant heterozygote deficiencies were a result of the presence of rare alleles. Significant heterozygote deficiencies have been recorded at these two enzyme loci in a number of other species (for references see Creasey et al., 1996). Allelic distributions in the majority of these organisms, and deep-sea organisms in general, are also similar to those in the present study upon *M. scobina*. Most polymorphic loci in these studies have been observed to share two common alleles (i.e. diallelism) with relatively high frequencies, and a number of rare alleles (see Creasey et al., 1996). This pattern of allele distribution has been proposed as occurring in populations that undergo frequent bottlenecks but exhibit a subsequent rapid population increase (Black et al., 1994). Bottlenecks and localised extinctions may occur in populations along the continental slope in the northern Arabian Sea because of the variation in the range and severity of the oxygen minimum zone (OMZ; see above). Such variation may act in two different ways producing significant heterozygote deficiencies within, and significant genetic differences between the sample populations. Firstly, mortality in the two *Munidopsis scobina* populations may be a result of severe hypoxia and could have randomly altered the genetic structure of the populations. In this case, random mortality effects are likely to be more pronounced in the population located at 12714#1, in closer proximity to the central part of the OMZ (i.e. water with the lowest oxygen

concentrations). Alternatively, selection may be responsible for the observed heterozygote deficiencies and population structuring. Jollivet et al. (1995) have shown that allelic variants of *Pgm* and *Got* have different thermostabilities in vent-endemic polychaetes, and may therefore be subject to selection in different thermal environments. The consistent deficiency of heterozygotes at these loci reported in vent-endemic organisms (see references in Creasey et al., 1996) may therefore be a result of selection imposed by major environmental factors (e.g. Oxygen concentration and temperature). In the current study, three major environmental factors that may act as selective agents are: hydrostatic pressure, oxygen concentration, and hydrogen sulphide, the last of which had previously been recorded in OMZ's in the Indian Ocean (Neyman et al., 1973). High pressures have been shown to have an effect upon enzymatic integrity of poorly pressure-adapted fish of the genus *Sebastolobus* (see Siebenaller and Somero, 1989 and references therein). However, Siebenaller (1978) did not support the hypothesis of populations exhibiting different allelic variants according to depth. Low oxygen levels and/or the presence of hydrogen sulphide are likely to be the principal selective factors in the present case. Oxygen concentrations have been cited previously as a potential factor in promoting speciation between allopatric populations (White, 1987). Both these factors also may cause selection at hydrothermal vents along with or separately from high temperatures. This may account for some of the heterozygote deficiencies recorded at *Pgm* and *Got* in vent-endemic organisms (see references above). Differences in the frequency and magnitude of severe hypoxic conditions at sample sites in the present study may produce different levels of selection within sample populations and cause both the observed genetic differentiation and size-frequency variations (see above).

Alternatively, the observed significant variation in allelic frequencies may be related to life-history strategy. Shuster and Sassaman (1997) showed that *Pgm* was closely linked with male phenotype at a single locus (the *alternative mating strategy* locus, *Ams*) in the marine isopod *Paracereis sculpta*. Similar linkages in *Munidopsis scobina* could account for some of the observed variations in sex ratio and deviations from Hardy-Weinberg expectations. If in *M. scobina*, reproductive success of males varies according to genotype at the *Pgm* locus, then different individuals would exhibit differing reproductive fitnesses. Furthermore, if this linkage was also related to an extrinsic parameter (e.g. oxygen, temperature, nutrient flux), with different alleles expressing different fitnesses under the environmental variables, then populations may be comprised of individuals whose fitnesses varies temporally and spatially. Under such a scenario, mating within a population may not be random, and consequently allelic frequencies may not be in Hardy–Weinberg equilibrium.

Lewontin (1974) suggested that in populations located in marginal environments where different genotypes are selected for at different times there is an advantage in recombination and high genetic variation. In the present study, the more marginal population located at sample site 12714#1 exhibited a lower observed heterozygosity than the population located at 12702#1. This agrees with previous studies which have shown marginal populations to have a lower observed heterozygosity and genetic diversity than central populations (Street and Montagna, 1996). Selection and

genetic drift have been hypothesised as the causative agents for such decreased heterozygosity (Shumaker and Babble, 1980).

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References

Abu-Hakima, R., 1984. Preliminary observations on the effects of *Epipenaeon elegans* Chopra (Isopoda: Bopyridae) on reproduction of *Penaeus semisulcatus* de Haan (Decapoda: Penaeidae). International Journal of Invertebrate Reproduction and Development 7, 51–62.

Alcock, A., 1894. On the results of deep-sea dredging during the season 1890–91. Annals and Magazine of Natural History 6 (13) [76], 321–331.

Alcock, A., 1901. A descriptive catalogue of the Indian deep-sea Crustacea Decapoda and Anomala in the Indian Museum. Calcutta, 286pp., 3pl.

Allen, J.A., 1966. The rhythms and population dynamics of decapod Crustacea. Oceanography and Marine Biology: an Annual Review 4, 247–265.

Ambler, J.W., 1980. Species of *Munidopsis* (Crustacea, Galatheidae) occurring off Oregon and in adjacent waters. Fishery Bulletin 78 (1), 13–34.

Attrill, M.J., 1989. A rhizocephalan (Crustacea; Cirripedia) infestation of the deep-sea galatheid *Munida sarsi* (Crustacea; Decapoda), the effects on the host and influence of depth upon the host-parasite relationship. Journal of Zoology 217, 663–682.

Attrill, M.J., Hartnoll, R.G., Rice, A.L., Thurston, M.H., 1990. A depth-related distribution of the red crab, *Geryon trispinosus* (Herbst) [= *G. tridens* Krøyer]: indications of vertical migration. Progress in Oceanography 24, 197–206.

Avise, J.C., 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, London.

Baudoin, M., 1975. Host castration as a parasitic strategy. Evolution 29, 335–352.

Bennett, B.A., Smith, C.R., Glaser, B., Maybaum, H.L., 1994. Faunal community structure of a chemoautotrophic assemblage in the deep north east Pacific Ocean. Marine Ecology Progress Series 108, 205–223.

Black, M.B., Lutz, R.A., Vrijenhoek, R.C., 1994. Gene flow among vestimentiferan tube worm (*Riftia pachyptila*) populations from hydrothermal vents of the eastern Pacific. Marine Biology 120, 33–39.

Borsa, P., Zainuri, M., Delay, B., 1991. Heterozygote deficiency and population structure in the bivalve *Ruditapes decussatus*. Heredity 66, 1–8.

Bucklin, A., Wilson, R.R., Smith, K.L., 1987. Genetic differentiation of seamount and basin populations of the deep-sea amphipod *Eurythenes gryllus*. Deep-Sea Research 34 (11), 1795–1810.

Burd, B.J., Brinkhurst, R.O., 1984. The distribution of the galatheid crab *Munida quadraspina* (Benedict 1902) in relation to oxygen concentrations in British Columbia fjords. Journal of Experimental Marine Biology and Ecology 81, 1–20.

Bursey, C.R., 1978. Histopathology of the parasitization of *Munida iris* (Decapoda: Galatheidae) by *Munidion irritans* (Isopoda: Bopyridae). Bulletin of Marine Science 28 (3), 566–570.

Chace Jr., F.A., 1942. Reports on the scientific results of the Atlantic expeditions to the West Indies, under the joint auspices of the university of Havana and Harvard university. Torreia 11, 1–106.

Chevaldonné, P., Olu, K., 1996. Occurrence of Anomuran crabs (Crustacea: Decapoda) in hydrothermal vent and cold-seep communities: a review. Proceedings of the Biological Society of Washington 109 (2), 286–298.

Childress, J.J., 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off California. Comparative Biochemistry and Physiology 50A, 787–799.

Christiansen, B., Pfannkuche, O., Thiel, H., 1990. Vertical distribution and population densities of the necrophagous amphipod *Eurythenes gryllus* in the West European basin. Marine Ecology Progress Series 66, 35–45.

Costa, R., Bisol, P.M., 1978. Genetic variability in deep-sea organisms. Biological Bulletin 155, 125–133.

Creasey, S.S., Rogers, A.D., 1999. Population genetics of bathyal and abyssal organisms. Advances in Marine Biology 35, 1–151.

Creasey, S., Rogers, A.D., Tyler, P.A., 1996. A genetic comparison of two populations of the deep-sea vent shrimp *Rimicaris exoculata* (Decapoda: Caridea: Bresiliidae) from the Mid-Atlantic Ridge. Marine Biology 125, 473–483.

Creasey, S., Rogers, A.D., Tyler, P.A., Young, C.M., Gage, J.D., 1997. The population biology and genetics of the deep-sea spider crab *Encephaloides armstrongi* Wood-Mason 1891 (Decapoda: Majidae). Philosophical Transactions of the Royal Society 352B, 365–379.

Creasey, S.S., Rogers, A.D., Tyler, P.A., Gage, J.D., Rice, A.L. 2000. Reproductive biology of the bathyal squat lobster, *Munidopsis scobina* (Decapoda: Anomura: Galatheidae), from the continental slope of the northern Indian Ocean, in preparation.

Doflein, F., Balss, H., 1913. Die Galatheiden der Deutschen Tiefsee-Expedition. Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer Valdivia 1898–1899 XX, 125-184.

Dybdahl, M.F., Lively, C.M., 1998. Host-parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. Evolution 52, 1057–1066.

France, S.C., 1994. Genetic population structure and gene-flow among deep-sea amphipods, *Abyssorchomene* spp., from six California continental borderland basins. Marine Biology 118, 67–77.

Gage, J. D., 1995. Benthic community and fluxes in relation to the oxygen minimum zone in the Arabian Sea. Cruise Report: R.R.S. Discovery 211/94 9 October–11 November 1994 Muscat to Owen Basin and adjacent continental slope off Masirah Island to Muscat. Scottish Association for Marine Sciences, Oban, Scotland, U.K. 71pp.

Ginsburger-Vogel, T., Charniaux-Cotton, H., 1982. Sex determination. In: Abele, L.G. (Ed.), The Biology of Crustacea. Vol. 2, Embryology, Morphology and Genetics. Academic Press, New York, pp. 257–281.

Gooch, J.L., Schopf, T.M., 1972. Genetic variability in the deep sea: relation to environmental variability. Evolution 26, 545–552.

Gordon, I., 1955. Crustacea Decapoda. Reports of the Swedish Deep-Sea Expedition. Zoology 2(19), 239–245, 1 Plate.

Gore, R.H., 1983. Notes on rare species of *Munidopsis* (Anomura: Galatheidae) and *Ethusina* (Brachyura: Dorippidae) collected by USNS Bartlett in the Venezuela Basin, Caribbean Sea. Proceedings of the Academy of Natural Sciences of Philadelphia 135, 200–217.

Gorman, G.C., Renzi Jr., J., 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 2, 242–249.

Goudet, J., 1994. FSTAT, a program for the IBM PC compatibles to calculate Weir and Cockerham's 1984. estimators of *F*-statistics. Version 1.2., Dorigny: Université de Lausanne, Switzerland.

Hartnoll, R.G., 1967. The effects of sacculinid parasites on two Jamaican crabs. Zoological Journal of the Linnaean Society 46, 275–295.

Hartnoll, R.G., Bryant, A.D., Gould, P., 1993. Size distribution in spider crab populations- spatial and temporal variation. Journal of Crustacean Biology 13, 647–655.

Hedgecock, D., Sly, F., 1990. Genetic drift and effective population sizes of hatchery-reared propagated stocks of the pacific oyster. *Crassostrea gigas*. Aquaculture 88, 21–38.

Hedgecock, D., Tracey, M.L., Nelson, K., 1982. Genetics. In: Abele, L.G. (Ed.), The Biology of Crustacea. Vol. 2, Embryology, Morphology and Genetics. Academic Press, New York, pp. 283–403.

Høeg, J.T., 1995. The biology and life cycle of the Rhizocephala Cirrepedia. Journal of the Marine Biological Association of the United Kingdom 75 (3), 517–550.

Jollivet, D., Desbruyères, D., Bonhomme, F., Moraga, D., 1995. Genetic differentiation of deep-sea hydrothermal vent alvinellid populations (Annelida: Polychaeta) along the East Pacific Rise. Heredity 74, 376–391.

Kashkina, A.A., Kashkin, N.I., 1993. Structure of the distribution range of the red crab *Pleuroncodes planipes* Stimpson 1860 (Crustacea: Galatheidae). Okeanologiya 33, 397–405.

Lee, S.Y., 1995. Cheliped size and structure: the evolution of a multi-functional decapod organ. Journal of Experimental Marine Biology and Ecology 193, 161–176.

Lee, S.Y., Seed, R., 1992. Ecological implications of cheliped size in crabs: some data from *Carcinus maenas* and *Liocarcinus holsatus*. Marine Ecology Progress Series 84, 151–160.

Levin, L.A., Gage, J.D., Martin, C., Lamont, P.A., 2000. Macrobenthic community structure within and beneath the oxygen minimum zone, NW Arabian Sea. Deep-Sea Research II 47, 189–226.

Lewontin, R.C., 1974. The Genetic Basis of Evolutionary Change. Columbia University Press, New York.

Lützen, J., 1985. Rhizocephala (Crustacea: Cirripedia) from the Deep Sea. Galathea Report 16, 99–112.

Manchenko, G.P., 1994. Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, London.

Marcus, N.H., Lutz, R.V., Chanton, J.P., 1997. Impact of anoxia and sulfide on the viability of eggs of three planktonic copepods. Marine Ecology Progress Series 146, 291–295.

Nair, R.R., Ittekkot, V., Manganini, S.J., Ramaswamy, V., Haake, B., Degens, E.T., Desai, B.N., Honjo, S., 1989. Increased particle flux to the deep ocean related to monsoons. Nature 338, 749–751.

Nei, M., 1972. Genetic distance between populations. American Naturalist 106, 283–292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nelson, S.G., Simmons, M.A., Knight, A.W., 1986. The energy burden of the bopyrid parasite *Argeia purpurata* (Crustacea, Isopoda) on the grass shrimp *Crangon franciscorum* (Crustacea, Crangonidae). Comparative Biochemistry and Physiology 83A, 121–124.

Neyman, A.A., Sokolova, M.N., Vinogradova, N.G., Pasternak, F.A., 1973. Some patterns of the distribution of bottom fauna in the Indian Ocean. In: Zeitzschel, B. (Ed.), The Biology of the Indian Ocean. Springer, Berlin, pp. 467–473.

O'Brien, J.J., van Wyk, P., 1985. Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. In: Wenner, A.M. (Ed.), Crustacean Issues 3. Crustacean growth: Factors in Adult Growth. A.A. Balkema, Rotterdam, pp. 191–218.

Poly, W.J., 1997. Nongenetic variation, genetic-environmental interactions and altered gene expression. II. Disease, parasite and pollution effects. Comparative Biochemistry and Physiology 117B, 61–74.

Redfield, J.A., Salini, J.P., 1980. Techniques of starch-gel electrophoresis of penaeid prawn enzymes (*Penaeus* spp. and *Metapenaeus* spp.). CSIRO. Australia, Division of Fisheries and Oceanography. Reports 116, 1–20.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43 (1), 223–225.

Ritchie, L.E., Høeg, J.T., 1981. The life history of *Lernaeodiscus porcellanae* (Cirripedia Rhizocephala) and co-evolution with its porcellanid host. Journal of Crustacean Biology 1, 334–347.

Sars, G.O., 1889. Bidrag til Kundskaben om Decapodernes Forvandlinger. II. *Lithodes- Eupagurus- Spiropagurus- Galathodes- Galathea- Munida- Porcellana-Nephrops*. Archiv for Mathematik og Naturvidenskab 3, 133–201.

Shaw, C.R., Prasad, R., 1970. Starch gel electrophoresis – a compilation of recipes. Biochemical Genetics 4, 297–320.

Shumaker, K.M., Babble, G.R., 1980. Patterns of allozymic similarity in ecologically central and marginal populations of *Hordeum jubatum* in Utah. Evolution 34 (1), 110–116.

Shuster, S.M., Sassaman, C., 1997. Genetic interaction between male mating strategy and sex ratio in a marine isopod. Nature 388, 373–377.

Siebenaller, J.F., 1978. Genetic variation in deep-sea invertebrate populations: the bathyal gastropod *Bathybembix bairdii*. Marine Biology 47, 265–275.

Siebenaller, J.F., Somero, G.N., 1989. Biochemical adaptation to the deep sea. CRC Critical Reviews in Aquatic Sciences 1, 1–25.

Squires, H. J., 1990. Decapod Crustacea of the Atlantic coast of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences 221, 540 pp.

Street, G.T., Montagna, P.A., 1996. Loss of genetic diversity in Harpacticoida near offshore platforms. Marine Biology 126, 271–282.

Swofford, D. L., Selander, R. B., 1989. Biosys 1. A computer programme for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign, Illinois.

Thorpe, J.P., 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annual Review in Ecology and Systematics 13, 139–168.

Thorpe, J.P., 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In: Oxford, G.S., Rollinson, D. (Eds.), Protein Polymorphism: Adaptive and Taxonomic Significance. Academic Press, London, pp. 131–152.

Thorpe, J.P., Solé-Cava, A., 1994. The use of allozyme electrophoresis in invertebrate systematics. Zoologica Scripta 23, 3–18.

Tirmizi, N.M., 1966. Crustacea: Galatheidae. The John Murray Expedition 1933–34, Scientific Reports, Zoology 11(2), 167–234. Published by the Trustees of the British Museum (Natural History), London, 1970.

Tobler, J.E., Grell, E.H., 1978. Genetics and physiological expression of β-hydroxy acid dehydrogenase in *Drosophila*. Biochemical Genetics 16 (3/4), 333–342.

Van Dover, C.L., Williams, A.B., 1991. Egg size in squat lobsters (Galatheoidea): constraint and freedom. In: Wenner, A., Kuris, A. (Eds.), Crustacean Issues 7. Crustacean Egg Production. A.A. Balkema, Rotterdam, pp. 143–156.

Volckaert, F., Zouros, E., 1989. Allozyme and physiological variation in the scallop *Placopecten magellanicus* and a general model for the effects of heterozygosity on fitness in marine molluscs. Marine Biology 103, 51–61.

Wenner, A.M., 1972. Sex ratio as a function of size in marine Crustacea. American Naturalist 106, 321–350.

Wenner, E.L., 1982. Notes on the distribution and biology of Galatheidae and Chirostylidae (Decapoda: Anomura. from the Middle Atlantic Bight. Journal of Crustacean Biology 2 (3), 360–377.

Wenner, E.L., Windsor, N.T., 1979. Parasitism of galatheid crustaceans from the Norfolk Canyon and Middle Atlantic Bight by bopyrid isopods. Crustaceana 37 (3), 293–303.

White, B.N., 1987. Oceanic anoxic events and allopatric speciation in the deep sea. Biological Oceanography 5, 243–259.

Wilkens, H., Parzefor, J., Ribowski, A., 1990. Population biology and larvae of the anchialine crab *Munidopsis polymorpha* (Galatheidae) from Lanzarote (Canary Islands). Journal of Crustacean Biology 10 (4), 667–675.

Wilkinson, L., 1990. SYSGRAPH: The system for graphics. SYSTAT, Evanston, II.

Williams, A.B., 1988. New marine decapod crustaceans from waters influenced by hydrothermal discharge, brine, and hydrocarbon seepage. Fishery Bulletin 86 (2), 263–287.

Williams, A.B., Turner, R.D., 1986. Squat lobsters (Galatheidae: *Munidopsis*) associated with mesh-enclosed wood panels submerged in the deep-sea. Journal of Crustacean Biology 6 (3), 617–624.

Wishner, K., Levin, L., Gowing, M., Mullineaux, L., 1990. Involvement of the oxygen minimum in benthic zonation on a deep seamount. Nature 346, 57–59.

Wright, C.A., Rollinson, D., Goll, P.H., 1979. Parasites in *Bulinus senegalensis* (Mollusca: Planorbidae) and their detection. Parasitology 79, 95–105.

Wright, S., 1940. Breeding structure of population in relation to speciation. American Naturalist 74, 232–248.

Wright, S., 1951. The genetical structure of populations. Annals of Eugenics 15, 323–354.

Wright, S., 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19, 395–420.

Young, C.M., Vazquez, E., 1997. *Agnezia monnioti* and *Styela gagetyleri*, new deep-sea ascidians specialized for life within and below the oxygen minimum layer in the Arabian Sea. Invertebrate Biology 116 (3), 262–276.

Zainal, K.A.Y., Taylor, A.C., Atkinson, R.J.A., 1992. The effect of temperature and hypoxia on the respiratory physiology of the squat lobsters, *Munida rugosa* and *Munida sarsi* (Anomuram, Galatheidae). Comparative Biochemistry and Physiology 101A, 557–567.