

Geographic variation among three isolated populations of the hadal amphipod *Hirondellea gigas* (Crustacea: Amphipoda: Lysianassoidea)

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ABSTRACT: The relative homogeneity and continuity of the deep sea may provide a habitat in which species can maintain widespread, genetically homogeneous populations. This study examines the lysianassoid amphipod *Hirondellea gigas* (Birstein & Vinogradov 1955) to determine whether populations from spatially-disjunct habitats in the deep sea maintain genetic homogeneity. *H. gigas* is a scavenger which rapidly responds to and consumes experimental baitfalls at depths of > 10 000 m. It appears to be endemic to western Pacific trenches, having been collected only within the Kurile-Kamchatka, Japan, Izu-Bonin, Volcano, Yap, Mariana, Palau, and Philippine Trenches. To estimate the degree of isolation of populations from the last 3 trenches, morphological variation was quantified through a discriminant analysis of surficial morphometric characters. Discriminant classification results were compared to 'null-populations', generated using a bootstrap randomization procedure, to determine the significance of the discrimination. Variation was sufficient to allow 68.4 to 84.5 % of individuals to be correctly reclassified into their population using discriminant functions. Bootstrapped randomizations of the data indicate that the classification success was significantly greater than random. Results indicate that these geographically-isolated trench populations may have reduced levels of gene flow between them, causing them to diverge morphologically.

INTRODUCTION

A major unresolved question in deep-sea biology involves the geographical extent over which species can maintain interbreeding populations. Questions regarding population structure and the extent of gene flow are critical to understanding species distributions and speciation processes in the deep sea, yet few studies have focused on population-level processes. Geographical patterns of genetic variation can reveal the population structure of a species. Patterns of geographic variation can provide information on the evolutionary history of a species (Gould & Johnston 1972). Thus, to learn more about the extent of gene flow in the deep sea, it is necessary to examine populations.

Abyssal and bathyal species have historically been

assumed to have widespread or cosmopolitan distributions, because the deep sea has generally been viewed as a single, vast, homogeneous habitat (Wilson & Hessler 1987). But on the scale of the organism, the deep sea is not homogeneous and free of dispersal barriers. At a gross level, immense distances within this continuous habitat may restrict gene flow between widespread populations of poor dispersers, although, on a smaller scale, dispersal between adjacent populations may produce a cline in gene frequencies from one end of the range to the other (Endler 1977). Benthic species may be isolated by topographic barriers, such as undersea ridges and continental margins, and/or currents. Differences in temperature (e.g. warm bathyal basins such as the Sulu Sea, Mediterranean Sea), bottom type (hard vs soft), nutrient input (e.g. phytodetritus; Rice et al. 1986), oxygen (e.g. anoxic basins, oxygen minimum zones), and community structure (e.g. biological structures as habitat; Thistle 1979)

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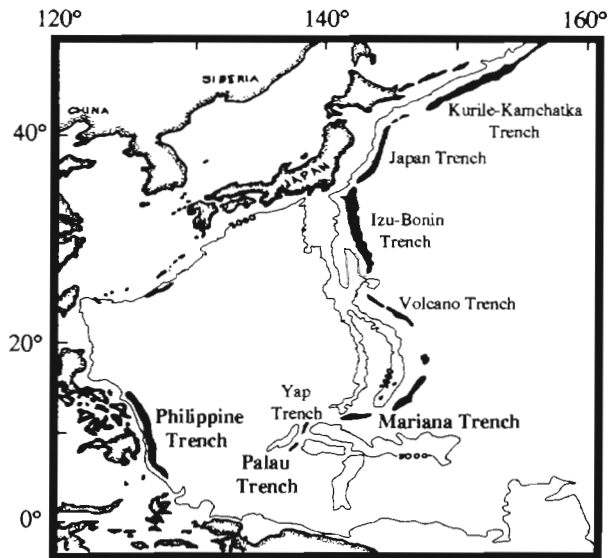


Fig. 1 Bathymetric map of the Philippine Sea region with trench depths below 7000 m shaded black. The 3000 m depth contour is also shown (adapted from Mogi 1972)

may have major effects on the local environment and, therefore, introduce gaps in appropriate habitat. Despite these hypothesized barriers, a number of deep-sea morphospecies nevertheless have broad geographic distributions (Gage & Tyler 1991).

Scavenging lysianassoid amphipods are one group of deep-sea organisms that has received considerable attention, due in part to their ubiquity, importance in energy transfer and ease of capture in baited traps (reviewed by Sainte-Marie 1992). Many lysianassoids have distributions that span multiple abyssal basins (Thurston 1990) and some are worldwide cosmopolites (e.g. *Eurythenes gryllus*, *Alicella gigantea*, *Abyssorchomene abyssorum*). For my initial studies, I have chosen to focus on species that have a disjunct distribution. Benthic fauna restricted to abyssal basins (Barnard 1961), trenches (Belyaev 1966), hydrothermal vents (Van Dover & Hessler 1990) and seamounts (Wilson & Kaufmann 1987), all have patchy distributions within their ranges imposed by patchiness of hab-

itat. For such species, definition of a sampling unit can unambiguously be assigned by geographic isolation. The question then becomes whether genetic homogeneity is being maintained among spatially isolated populations through gene flow, or whether each population is genetically isolated, possibly a relic of a previously continuous distribution.

Hadal faunas would be expected to display disjunct distributions if they are stenobathic; without hadal continuity, such a fauna may be endemic to specific trenches. Previous hadal sampling has shown the scavenging lysianassoid amphipod *Hirondellea gigas* to be present in the trenches of the northwestern Pacific, extending from the Kurile-Kamchatka Trench (Birstein & Vinogradov 1955) south to the Palau Trench (Kamenskaya 1981) and west to the Philippine Trench (Dahl 1959). In the present study, I report on morphometric variation among populations from the Palau, Philippine and Mariana Trenches (Fig. 1), with a goal of determining whether the individuals all belong to a single interbreeding population, thus implying recent gene flow, or whether the population of each trench is morphologically distinct, implying some degree of genetic isolation.

MATERIALS AND METHODS

Amphipods were collected using baited, free-vehicle traps (see Hessler et al. 1978, Ingram & Hessler 1983 for description of trap sets) during 3 separate cruises on board the RV 'Thomas Washington': expedition EURYDICE in February 1975 to the Philippine Trench, expedition INDOPAC IX in January 1977 to the Mariana Trench, and expedition PAPA-TUA in March 1986 to the Palau and Philippine Trench (Table 1). Trap sets, deployment times and some life-history data for the amphipods collected in the Philippine and Mariana Trenches have previously been published (Hessler et al. 1978). All Palau Trench individuals collected were measured and the sex determined. Sex was determined by presence of penes on the sternum of pereon

Table 1. *Hirondellea gigas*. Sampling locations

Date	Locality	Coordinates	Depth (m)	Sample size ^a
Feb 1975	Philippine Trench (PHP)	10° 36.5' N, 126° 36.6' E	9604	95
Jan 1977	Mariana Trench (MAR)	11° 18.8' N, 141° 58.7' E	9144	73
Jan 1977	Mariana Trench (MAR)	11° 22.1' N, 141° 35.4' E	7218	8
Mar 1986	Palau Trench (PAL)	7° 46.7' N, 134° 57.3' E	7997	168
Apr 1986	Philippine Trench (P86)	10° 24.3' N, 126° 40.9' E	8467	25

^a These are sample sizes used in the morphometric analysis; samples collected were greater in all cases except for the Palau Trench, where all individuals were measured

VII for males, and by presence of oostegites on the coxae of pereopod V for females. Individuals without either of these structures were classified as juveniles. Hessler et al. (1978) estimated the developmental stage (instar number) based on the development of sexual characters. For females, I measured the length of the oostegite of pereopod V and for males the number of articles in the flagellum of the second antennae.

Studies of deep-sea amphipods typically present total length data for individuals collected. However, the bodies of preserved individuals are flexed to varying degrees, making measurement of total length time-consuming and imprecise. For the present study, I endeavored to find a measure which could approximate total unflexed body length. I examined 50 individuals from each of the 3 trench populations. For a series of measures collected during the morphometric analysis, I calculated geometric mean regressions on total unflexed body length to see which measurement would be the best estimator of total length. The length of the dorsal edge of the first pereonite (thoracic segment) in lateral view (measure Th3) produced the best estimate of total length (Palau Trench, total length = $9.477 \text{ Th3} - 0.263$, $r^2 = 0.976$). I used Th3 in the remainder of the analyses as an indicator of total size.

To evaluate size and shape variation among *Hirondellea gigas* populations, several different measurements were made covering the lateral surface of the pereon and 3 of the pereopods. Measurements were not made over the pleon and urosome since varying degrees of flexion in preserved specimens could distort comparisons. Pereonal measurements were made by placing individual amphipods in a plasticine well filled with 70 % ethanol under a Wild-M5 dissecting microscope. A camera lucida attached to the microscope allowed visualization of the amphipod on a HIPAD digitizer. Measurements were digitized directly into a computer using the program Sigma-Scan. Pereopods were removed from each individual after thoracic measures were taken, and pereopodal measurements were made as above. In a preliminary study, more than 70 length measurements were made per individual; principal-components analysis of the data demonstrated that most of the information was contained in a set of 13 measurements (Fig. 2), so all analyses described below were of these non-redundant measures. Because I could not determine specific instar stages for each individual, a range of body sizes from each population was included in the analyses. All measurements were log-transformed to meet the assumption of equality of variances. To ensure that the effect of size did not influence the results, I adjusted the data set for possible effects of growth and size differences using Burnaby's method for size correction, as described by Rohlf & Bookstein (1987).

The extent of geographic variation among populations from the 3 trenches was assessed by discriminant analysis of the morphometric measurements. This analysis produces weighted linear combinations of variables (discriminant functions) which maximize discriminating power between groups (Gould & Johnston 1972, Thorpe 1976, Pimentel 1979). To proceed with the analysis, a MANOVA test must first detect a significant difference between groups. The degree of differentiation among groups can be assessed by using the discriminant functions to reclassify all the individuals to population. If the populations are strongly divergent in morphological characters, then it should be relatively easy to discriminate them, and individuals from each population will be reclassified correctly by the discriminant functions. Successful classifications are scored as 'hits' and misclassifications as 'misses'.

In the absence of measurable environmental differences among the trenches (Belyaev 1966), and therefore a selective gradient, one may assume that phenotypic (morphological) variation among populations reflects genetic variation resulting from random processes; morphometric measurements should detect genetic variation to the extent that it is phenotypically expressed. In this case, genetically distinct populations should have high hit percentages whereas subpopulations of a single interbreeding population should have high misclassifications. Thus, one may estimate the degree of genetic isolation of the populations based on the observed proportion of misclassifications. However, this proportion underestimates the true misclassification probability since the individuals being classified are the same ones used to generate the discriminant functions (Afifi & Clark 1990). A better

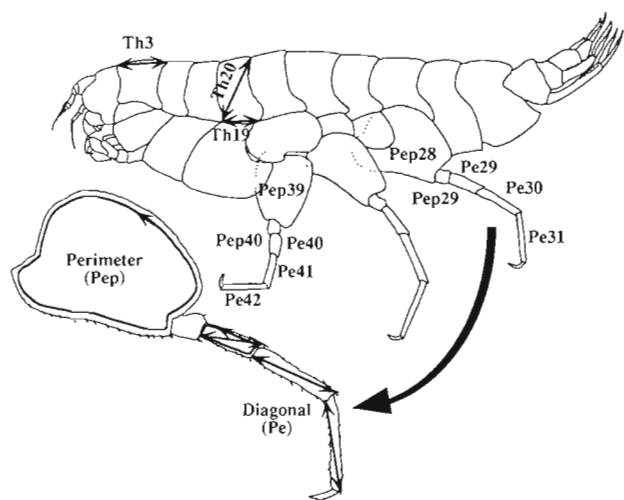


Fig. 2. *Hirondellea gigas*. Thirteen length measures used in this study. There are 3 measures on the thorax, 5 measures each on pereopods V and VII, consisting of 2 perimeter lengths (articles 2 & 4) and 3 diagonal lengths (articles 4 to 6)

estimate of the misclassification probability can be calculated using a cross-validation procedure (e.g. pseudo-jackknifing procedure in BMDP P7M); however Solow (1990) recommends a randomization test rather than cross-validation.

I employed the 'bootstrap' as a randomization test (Marcus 1990, Efron & Tibshirani 1991). In a bootstrap algorithm, one generates a number of new samples by randomly resampling the data set with replacement, and then calculates the desired parameter for each new sample. In theory, individuals are 'replicated' thousands of times to produce a large bootstrapped population which is representative of the actual population from which the sample was collected. An underlying assumption of the bootstrap method is that the original sample is a random, independent sample. The resultant bootstrap-sample mimics a random, independent sample collected from the field from which the desired parameters may be calculated. Repeating this process several hundred times produces a distribution of values of the parameter of interest, from which a grand mean and confidence interval may be computed. I derived estimates from 500 bootstrap samples of each of the 3 trench populations in order to calculate a grand mean and 95 % confidence interval for classification hits and misses in each population. The confidence intervals were empirically derived by removing 2.5 % of values from each tail of the distribution of 500 bootstrapped means.

To test the significance of the observed (and bootstrapped) discriminant classifications from random, I generated 'null' bootstrap samples. Individuals from all 3 trench populations were pooled prior to replication. Sampled individuals were then randomly assigned to 'null' populations of sizes equal to those of the original trench samples (i.e. $n = 95$, $n = 81$, or $n = 168$). Each of these null populations is made up of individuals randomly sampled from all 3 trenches and therefore should be indistinguishable from one another. In comparison to geographic populations, the null populations should include more misclassifications if the former are morphometrically differentiated. If there is no difference in the classification hits, then we can conclude that based on the characters measured, there is no detectable systematic variation in these populations. The bootstrap routine (a C-language program written by Alex Leonard of the Scripps Institution of Oceanography) was run concurrently with the statistical package SYSTAT, which performed the discriminant analyses, on a Compaq PC. Discriminant analyses were repeated using the mainframe package SAS to calculate Mahalanobis' distances (D^2) between populations. The Mahalanobis' D^2 is a non-dimensional normalized distance between centroids and is useful as a relative estimate of the distance between multi-

variate means of each population. The distance, or difference, among populations is graphically represented by plotting each individual's discriminant scores on canonical axes.

A final estimate of differentiation between populations comes from the percent-overlap statistic. This statistic was developed by Royce (1957, 1964) to estimate the proportion of one group having characteristics identical to those of another. Inferences, based on the similarity of the groups compared, can be made of the degree to which the groups 'intermingle', or interbreed. Although a high percent overlap cannot be used as proof of extensive interbreeding, very low overlap values can provide evidence of little or no interbreeding (Mais 1972). Studies in fisheries stock assessments have used a percent-overlap critical value of 25 % or less to indicate clearly separate groups of fish (Sharp et al. 1978, Haug & Fevolden 1986, Christensen et al. 1990). In this study, percent overlap is calculated from an adjusted Mahalanobis' D^2 estimate derived from the discriminant analysis. This adjustment accounts for a small bias in D^2 due to unequal sample sizes by subtracting the value:

$$P = \frac{n_1 + n_2}{n_1 n_2}$$

where P = no. of characters in the analysis; and n_1 and n_2 are sample sizes (Mais 1972).

RESULTS

Trapping results

The free-vehicle trap sets deployed in the Palau Trench captured 174 individuals of *Hirondellea gigas* and 11 individuals of a second undescribed lysianassoid, tentatively assigned to the genus *Tmetonyx*. Previous baited-trap sampling of trenches of the western Pacific had found that *H. gigas* was the only amphipod responding to bait (except for a single unidentified individual in the Mariana Trench reported by Hessler et al. 1978). An interesting feature of the undescribed species of *Tmetonyx* is that 7 of the 11 specimens were brooding females. Brooding deep-sea lysianassoid females are not typically captured in baited traps, an indication that they do not feed, possibly because they are avoiding cannibalism of embryos (Hessler et al. 1978, Ingram & Hessler 1983)

The range in total length for the Palau Trench specimens (11 to 40 mm, estimated by regression equation from Th3 length) is comparable to that of the Philippine Trench (11 to 43 mm) and that of the Mariana Trench (10.5 to 43 mm) specimens. That the maximum total length measured in the Palau Trench is slightly smaller than in the other 2 trenches may be a

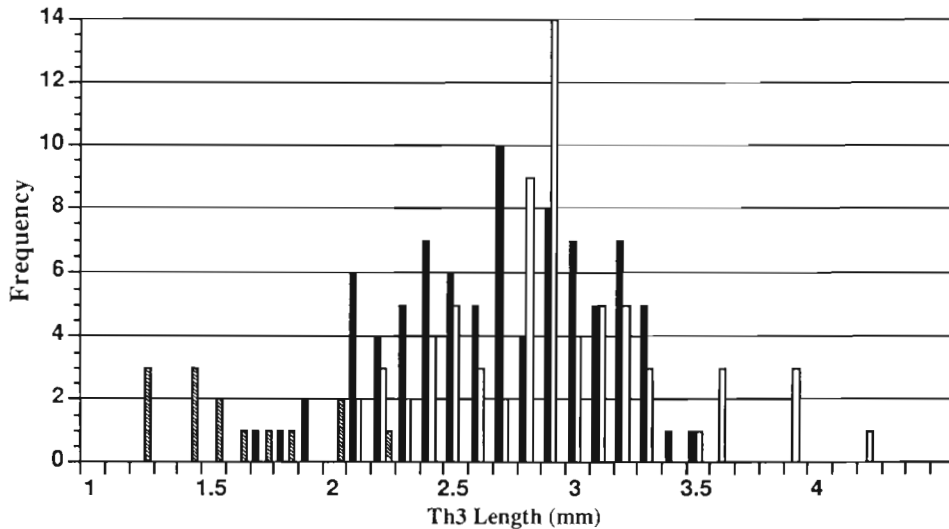


Fig. 3. *Hirondelella gigas*. Frequency distribution of individuals from the Palau Trench. Total body length = $9.477 \text{ Th3} - 0.263$ (see 'Results'). Solid bars: males; open bars: females; hatched bars: juveniles (see text for definitions)

sampling artifact. The largest individuals are final instar females, the brooding stage. Brooding females are not attracted to baited traps and therefore larger females are under-represented in the sample. In the original Philippine-Trench sample, only 1 % of females trapped belonged to the final instar (Hessler et al. 1978). However, although the Marianas and Philippine Trench individuals showed a slightly longer upper size, the mean lengths of the individuals used in this study were not significantly different (Variable Th3, ANOVA, $p = 0.1842$). Fig. 3 shows a length-frequency histogram of Palau Trench individuals by size and sex. The sample consisted of 14 juveniles (11 to 21 mm), 85 males (16 to 33 mm), and 69 females (20 to 40 mm). The number of individuals available is too small to allow for a modal analysis; all that can be said is that most of the

Table 2. *Hirondelella gigas*. Mean values (SD) in mm for the 13 variables measured in the 3 populations

Variable	Philippine Trench	Mariana Trench	Palau Trench
Th3	2.56 (0.58)	2.69 (0.66)	2.63 (0.55)
Th19	1.96 (0.43)	1.96 (0.50)	1.94 (0.44)
Th20	4.17 (0.96)	4.39 (1.14)	4.14 (0.94)
Pep28	13.17 (3.10)	13.84 (3.75)	13.23 (2.85)
Pep29	3.96 (0.93)	4.04 (1.02)	3.90 (0.84)
Pe29	1.61 (0.39)	1.66 (0.43)	1.56 (0.35)
Pe30	2.15 (0.58)	2.21 (0.63)	2.16 (0.53)
Pe31	2.41 (0.63)	2.45 (0.66)	2.38 (0.59)
Pep39	10.87 (2.55)	11.45 (3.06)	11.05 (2.38)
Pep40	3.37 (0.78)	3.47 (0.93)	3.40 (0.74)
Pe40	1.34 (0.32)	1.37 (0.38)	1.36 (0.30)
Pe41	1.62 (0.43)	1.67 (0.46)	1.72 (0.42)
Pe42	1.94 (0.49)	2.01 (0.57)	2.08 (0.52)

females trapped were young instars, i.e. only 11 % of the oostegites were longer than 0.6 mm, which corresponds to instar 3 (out of 6) based on the Philippine-Trench sample analyzed by Hessler et al. (1978). No brooding females were trapped. Multiple modes which could be related to an instar could not be detected for males. The number of articles on the flagellum of the antennae does not appear to be a useful character for instar estimation.

Discriminant analyses

Mean values of the 13 measured variables in each of the populations are presented in Table 2. Four MANOVA tests are performed by SYSTAT prior to calculating the discriminant functions. All the tests rejected the hypothesis ($p < 0.001$) that there is a common mean vector of the 13 variables for the 3 populations. This result does not require that all populations be different from one another, but that at least 1 differs from the others. Formal rejection of the null hypotheses allows the use of the discriminant analysis (Pimentel 1979).

Results of the discriminant analysis indicate that each population is sufficiently distinctive to be classified with high success rates. There were only minor differences in classification success (Table 3) and Mahalanobis' D^2 values between log-transformed data and the size-adjusted data. Only the log-transformed data were used in the remainder of this study. The discriminant functions correctly classify individuals into their respective populations with high frequency

Table 3. *Hirondelella gigas*. Summaries of successful classifications (hits) from discriminant analysis of the 3 populations, using untransformed, log-transformed and size-corrected data [Burnaby's method (Rohlf & Bookstein 1987)]. All analyses calculated using SAS program software

Individuals from	% Hits		
	Untransformed data	Log-transformed data	Size-corrected data
Philippine	73.7	68.4	70.5
Mariana	75.3	77.8	77.8
Palau	83.4	84.5	83.9

(Table 4). The Palau Trench population (PAL) has the highest classification success (84.5 %) and the Philippine population (PHP) has the lowest (68.4 %). A greater percentage of individuals from the Mariana Trench (MAR) are misclassified into PHP (16.0 %) than into PAL (6.2 %), while almost equal numbers of PHP individuals misclassified into the remaining 2 populations (16.8, 14.7 % respectively). All 8 amphipods from the shallower trapping site within the Mariana Trench were correctly reclassified into the Mariana population. Classification tables calculated for each of the 500 replicated sets of the 3 bootstrapped populations produced mean classification hit percentages similar to the value determined for the original sample: Philippine 72 vs 68 % original, Mariana 77 vs 78 % original, Palau 84 vs 84 % original (Table 5). The distinctness of the 3 populations is visu-

Table 4. *Hirondellea gigas*. Classification summary from discriminant analysis of the 3 populations. Classified to: no. of individuals classified to a population; % Hits: percent successful classifications (hits)

Individuals from	Classified to:			% Hits
	Philippine Trench	Mariana Trench	Palau Trench	
Philippine	65	16	14	68.4
Mariana	13	63	5	77.8
Palau	11	15	142	84.5
Total	89	94	161	78.5

Table 5. *Hirondellea gigas*. Mean classification summary from discriminant analysis of 500 bootstrap iterations of the 3 populations. Each value in the table is the mean of 500 bootstrap iterations, and is presented as a percentage of individuals classified [(mean no. classified ÷ sample size) × 100], and is accompanied in parentheses by 95 % confidence intervals (also presented as a percentage). Successful classifications (hits) are shown in bold

Individuals from	Percent classified to:		
	Philippine Trench	Mariana Trench	Palau Trench
Philippine	72 (62–80)	15 (5–23)	7 (4–11)
Mariana	15 (9–22)	77 (64–88)	9 (4–13)
Palau	13 (5–20)	8 (2–15)	84 (76–91)

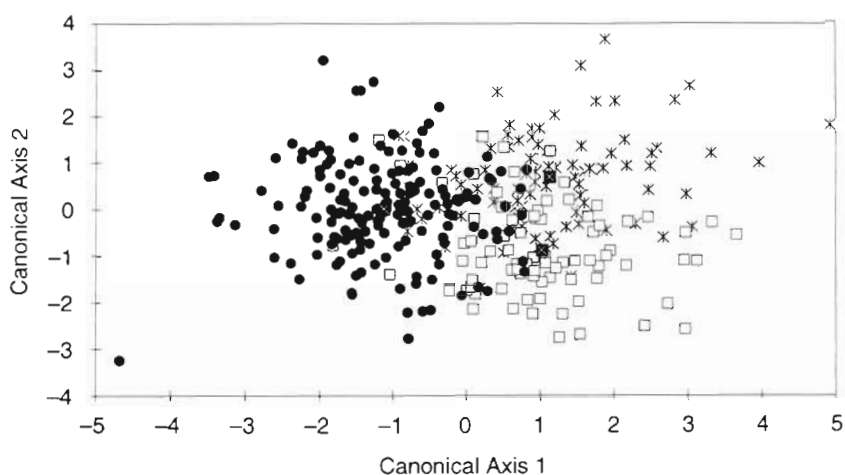


Fig. 4. *Hirondellea gigas*. Plot of discriminant scores on canonical discriminant axes. All individuals are plotted along with the mean value for each population (■) (□) Mariana Trench; (●) Palau Trench; (×) Philippine Trench

ally illustrated when discriminant scores generated by the functions are plotted for each individual; it is apparent that PAL individuals are more distinct from PHP and MAR than the latter are from each other (Fig. 4). This is also reflected by the Mahalanobis distance (D^2), which between PAL and either of the other populations is more than double that between PHP and MAR (Table 6).

Discriminant analyses were also performed separately in 2 runs using male-only and female-only data sets. The discriminant functions were somewhat better at reclassifying Philippine Trench males than females (82 % hits for males, 61.8 % hits for females) and performed more poorly with Mariana Trench females (58.1 % hits) than males (80 % hits); there was no difference in classification success between sexes from the Palau Trench (ca 85 % hits).

The differences between the populations can be further elucidated by calculating percent overlap between the populations from pairwise D^2 's (Table 6). Empirical studies with fisheries stocks have indicated that a percent overlap of 25 % or less is indicative of populations that do not intermingle on breeding grounds, i.e. no gene flow (see references in 'Methods' section). Percent overlap between PHP and MAR

Table 6. *Hirondellea gigas*. Unadjusted Mahalanobis' D^2 (above diagonal) and percent overlap (below diagonal) between trench populations

	Philippine Trench	Mariana Trench	Palau Trench
Philippine	·	2.443	5.513
Mariana	46.5	·	5.365
Palau	24.2	24.6	·

(46.5 %) is well above this critical value, whereas overlap between PAL and PHP (24.2 %) or MAR (24.6 %) is at the level that would be taken – according to the fisheries criterion – as indicating little or no interbreeding.

To assess the significance of the observed discrimination, I computed classification matrices for random, or null, populations, using the 'bootstrap' randomization technique, and compared these to the classification matrix generated from the original data. The distribution of classification scores for null populations is presented in Table 7. The mean hit percentage ranged from 41 to 47 %, meaning more than half of the individuals in any null population were incorrectly assigned. In no case did the distribution of classification hits from the null data sets overlap with confidence

Table 7. *Hirondellea gigas*. Discriminant analysis classification summary of random groups formed from pooled populations. Each value in the table is the mean of 500 bootstrap iterations, and is presented as a percentage of individuals classified [(mean no. classified ÷ sample size) × 100] and is accompanied in parentheses by 95 % confidence intervals (also presented as a percentage). Successful classifications (hits) are in bold

Individuals from	Percent classified to:		
	Group 1 n = 95	Group 2 n = 81	Group 3 n = 168
Group 1	42 (31–54)	26 (19–33)	29 (23–34)
Group 2	29 (17–40)	47 (36–56)	30 (23–39)
Group 3	29 (19–33)	28 (19–38)	41 (32–48)

Table 8. *Hirondellea gigas*. (A) Philippine Trench individuals collected in 1986, classified with the linear discriminant functions calculated using the other 3 populations; results presented as percent of total individuals (n = 25). (B): Mahalanobis D^2 (above diagonal) and percent overlap (below diagonal) calculated from discriminant analysis run with all 4 populations

A. Classification summary				
Individuals from	Philippine Trench	Classified to:		
		Mariana Trench	Palau Trench	
Phil. 1986	64	28	8	
B. Mahalanobis' D^2 and percent overlap				
	Phil. Trench 1986	Philippine Trench	Mariana Trench	Palau Trench
Phil. 1986	•	2.752	4.257	5.460
Philippine	47.2	•	2.421	5.703
Mariana	34.2	46.5	•	5.554
Palau	27.1	24.2	25.0	•

intervals from bootstrapped data of non-pooled populations (Table 5), indicating the observed discrimination is significantly different from random.

Because the samples were not collected at the same time, one may argue that the observed differences among individuals from the 3 populations result from temporal, rather than spatial, phenomena. Samples collected in the Philippine Trench during the 1986 PAPA-TUA cruise allowed me to test whether individuals from a single location had undergone significant morphometric changes over the 10 yr interim. Twenty-five individuals from the 1986 sample (P86) were measured and subsequently classified using the discriminant functions generated in the analysis of the PHP, MAR, and PAL populations. Sixteen of the twenty-five individuals were correctly assigned to the Philippine Trench (Table 8); only 2 amphipods were classified to PAL, the remaining being assigned to MAR. In order to compute D^2 and percent overlap between P86 and PHP, a second discriminant analysis was performed which included P86 as a separate group. All but 2 of the P86 individuals were classified as either P86 (76 %) or PHP (16 %). The adjusted D^2 between PHP and P86 (2.09) was lower than any other pairwise comparison, and translated to a percent overlap of 47.2 % (Table 8). This value is well above the 25 % critical value indicative of non-interbreeding populations. Overlap of P86 with MAR was almost as great (34.2 %) and was low for P86 vs PAL (27.1 %). These results suggest that the differences observed between PAL and the other populations are a result of spatial isolation rather than a temporal effect.

DISCUSSION

This is the first report of baited-trap collections of scavenging crustaceans from the Palau Trench. The 2 free-vehicle trap sets deployed captured 174 individuals of the lysianassoid amphipod *Hirondellea gigas* and 11 individuals of a second, as yet undescribed, lysianassoid. The known distribution of *H. gigas* is restricted to trenches of the western Pacific (Japan, Kurile-Kamchatka, Izu-Bonin, Volcano, Mariana, Yap, Palau, and Philippine Trenches) (Birstein & Vinogradov 1958, Kamenskaya 1981). Deployments of baited traps in the Aleutian Trench failed to capture *H. gigas*, although other lysianassoid amphipod species were collected (C. Ingram unpubl. data). This suggests that the northward extent of distribution is the Kurile-Kamchatka Trench. The Galathea Expedition conducted several pelagic and benthic trawls in the Sunda, Banda, New Britain, and Kermadec Trenches (Bruun 1959), but no *H. gigas* were collected (Dahl 1959), and there has apparently been no baited-trap

sampling of the many trenches south of the Palau Trench (ca 5° N). Thus the southern extent of distribution is unknown.

Discriminant analysis of the 3 trench populations of *Hirondellea gigas* reveals a significant amount of morphometric differentiation among them. Each population forms a discrete group as indicated by the classification success rate (Table 4) and the discriminant plot (Fig. 4). Classification success of the individual amphipods is significantly greater than classifications of random groupings of the same individuals (Table 7). It is also evident that the Palau Trench population is more clearly differentiated from both the Philippine and Mariana Trench populations. Nevertheless, it is difficult to gauge the phylogenetic significance, or attribute causality, to the differences.

One way to place the degree of among-population divergence into systematic perspective is to make a comparison with a congeneric deep-sea species. I measured the same 13 morphometric variables on 10 individuals of *Hirondellea brevicaudata* collected from the Hatteras abyssal plain in the NW Atlantic. The discriminant analysis was repeated as above including this fourth group. The resultant mean Mahalanobis' D^2 between *H. gigas* and *H. brevicaudata* was 81.78, compared to a mean D^2 of 4.16 among the 3 trench populations of *H. gigas*. This suggests that although significantly morphometrically differentiated, the trench populations are conspecific.

What factors may have led to the existing level of population differentiation? Previous studies with freshwater and shallow-water amphipods have related phenotypic and genotypic differentiation to environmental gradients or geographic isolation (e.g. Sheader 1975, Siegismund 1985, Siegismund et al. 1985, Bulnheim & Scholl 1986, McDonald 1987, Patarnello et al. 1989, Gooch 1990, Scheepmaker 1990, Kane et al. 1992). It is difficult to determine if there are selective differences that would lead to alternate phenotypes among the three trenches. The Palau Trench is 2000 m shallower than the Mariana or Philippine Trench, but all 3 share similar environmental characteristics (Belyaev 1966). Additionally, 8 individuals captured from the Mariana Trench slope, almost 2000 m shallower than the other Marianas individuals, were correctly reclassified as belonging to the Mariana population, suggesting depth itself is not a factor causing divergence in these characters. Biotic factors influencing *Hirondellea gigas* are largely unknown. Amphipods are typically the dominant scavenger arriving at experimental baitfalls in trenches (Hessler et al. 1978), and in the Philippine Trench *H. gigas* was the sole species observed. While *H. gigas* was the numerical dominant in the Palau Trench trap sets, the presence of a second lysianassoid species suggests that this population may be subjected

to competitive pressures from other scavengers, although we can only speculate on the degree of selection due to this competition. In the absence of differing selective pressures, and assuming mutation is low and constant across all 3 populations, random processes, such as genetic drift, can lead to genetic differentiation among small populations that remain isolated from one another over time. Using the assumption that phenotypic differences allow us to infer genetic differentiation (which may not always be the case, e.g. Scheepmaker 1990), the high percent overlap found between the Philippine and Mariana populations does not allow us to conclude that they are actively interbreeding, but the low percent overlap between the Palau Trench and the other populations strongly suggests that gene flow between them is restricted.

Although the distances separating the 3 trench populations are immense relative to the size of the amphipods, the dissimilarity between the Mariana (MAR) or Philippine Trench (PHP) populations and the Palau Trench (PAL) population cannot be solely attributed to geographic isolation restricting dispersal due to distance. PAL is located roughly midway between MAR and PHP and approximately 320 km south (Fig. 1). MAR is approximately 1477 km from PHP, but only 755 km from PAL. If morphometric similarity were simply a function of dispersal distances, then populations from PHP and MAR should be most distinct and the PAL population more similar to them both. While geographic distance is an important factor for isolation, the intervening topography and associated current structure may be more critical to the dispersal/migration of the amphipods. Because the Mariana Trench is actually subdivided into 3 troughs by intervening sills of less than 7000 meters depth (Mogi 1972), it would be of great interest to sample amphipod populations from one of the more northerly troughs to test the effect of topography vs distance.

The topography of the ocean floor of this region of the western Pacific is extremely complex (Mogi 1972). The trenches are associated with island arcs along their western boundaries. These island arcs are topographic highs of ridges that, for the Mariana and Palau Trench populations, present additional barriers to dispersal toward the Philippine Trench, located at the southwestern edge of the Philippine Sea (Fig. 1). Moreover, the abyssal basin of the Philippine Sea is subdivided by the north-south running Kyushu-Palau Ridge. Such ridges will impede dispersal of *Hirondellea gigas* only if it is stenobathic. The finding of *H. gigas* exclusively within trenches suggests that it is adapted to hadal depths. Yayanos (1981) has shown that deep-sea amphipods appear physiologically capable of vertical migrations of more than 3000 m. Hessler et al. (1978) did not capture any specimens of

H. gigas at 5861 meters in the abyssal hills on the eastern lip of the Philippine Trench. Birstein & Vinogradov (1955, 1958, 1970) report capturing *H. gigas* in pelagic trawls, possibly > 1000 m above the bottom, within the Kurile-Kamchatka Trench; however, none were caught shallower than 7000 m depth (Table 3 in Birstein & Vinogradov 1970), thus there is no indication individuals rise above the sill depth. Due to the uncertainty of trawl fishing depths relative to bottom depth, it is unclear at what height above the bottom the specimens were caught; however, capture by trawling suggests that this species is a capable swimmer. Many abyssal scavenging lysianassoid amphipods can evidently swim into the water column several hundred meters above the bottom (Thurston 1990). For example, *Paracallisoma alberti* has been trawled from greater than 3650 m above the Iberian Abyssal Plain (Thurston 1990). *Eurythenes gryllus*, the most extensively studied abyssal necrophage, has been trapped up to 1800 m above bottom (Baldwin & Smith 1987) and inhabits a range of depths from 1320 to 7230 m (Ingram & Hessler 1987). However, swimming at great heights above the bottom is not a requirement for dispersal out of a trench. Demersal and benthic organisms can remain close to the bottom and travel up the slope of trench walls. With an observed distribution across 8 non-continuous trenches of the western Pacific, it seems at least plausible that some individuals of *H. gigas* are able to migrate over a considerable depth (vertical) gradient from the floor of 1 trench into the water column and over topographic features to another trench.

Amphipods may be carried by currents as they search for odor plumes from food sources. Uehara & Taira (1990) have inferred, from temperature and salinity profiles, that abyssal bottom-water flows northward into the Philippine Sea through a narrow gap at the Yap-Mariana Junction. This junction separates the Mariana Ridge and Trench to the north from the Yap Ridge and Trench to the south; the Palau Trench is south of the Yap Trench. Yoshioka et al. (1988) measured an average southward current flow of speed 0.8 cm s^{-1} at 4000 m (bottom depth of 4604 m) at a station 200 km NW of the Yap-Mariana Junction. The mean flow fluctuated in direction and speed in subsequent years, but remained weak (Uehara & Taira 1990). Such a gentle current is much slower than those measured within the Philippine Trench ($\bar{x} = 1.8$ to 7.5 cm s^{-1} ; Hessler et al. 1978), and measurements of swimming speeds of deep-sea lysianassoids (Laver et al. 1985) have shown them to be sufficiently fast to swim upstream in these weak currents; thus this current need not 'sweep' an amphipod along its course. However, bottom topography strongly influences current flow and can enhance current speed (Genin et al.

1986). Accelerated current flow through the junction may be able to carry animals into the West Mariana Basin. In the absence of stimulatory cues indicating the presence of food, it seems conceivable that individuals of *Hirondellea gigas* emerging from the southwestern end of the Mariana Trench may drift with the current, rather than swimming upstream. Once in the West Mariana Basin, individuals may be transported to the Philippine Trench; however the distance is great and the topographical barriers numerous, e.g. Kyushu-Palau Ridge. Based on an average swimming speed of 6 cm s^{-1} (= low average speed of *Eurythenes gryllus*; Laver et al. 1985) and no currents, a constantly swimming amphipod would take roughly 289 d to reach the Philippine Trench in a straight line. Without feeding, this requires an energy expenditure that would make the 'trip' unlikely at best. This purely speculative estimate can not be judged until we know more about the life history of *H. gigas*.

Previous studies of other deep-sea invertebrates have found little variation among populations living within an area free of major topographic discontinuities or habitat gaps. Bucklin et al. (1987) studied the large, highly-mobile amphipod *Eurythenes gryllus* and found very low levels of genetic variation across a 4000 km transect within a single abyssal basin of the eastern North Pacific. Conversely, when samples of *E. gryllus* were taken from a topographic high (a seamount crest) at the western edge of the northeastern Pacific basin, considerable genetic differentiation, relative to the basin population, was detected (Bucklin et al. 1987). Rex and coworkers (Rex et al. 1988, Etter & Rex 1990, Rex & Etter 1990) also found little variation in gastropod populations across large (100's of km) horizontal scales within an abyssal basin in the North Atlantic. Within a basin, low levels of geographic variation may result from unvarying selection pressures and unimpeded dispersal potential (Rex & Etter 1990). Populations of *Hirondellea gigas*, which show considerable geographic variation, live in spatially discontinuous habitats within the relatively continuous environment of the deep sea. Similar findings of geographic variation have been detected in other patchily-distributed, deep-sea populations. The hydrothermal vent-endemic amphipod *Ventiella sulfuris*, whose habitat is an archipelago of vent sites along the axes of eastern Pacific ridges, showed species-level differentiation between sites on disjunct ridge axes (France et al. 1992). Moreover, David (1983) reported morphological variation among populations of irregular urchins living in abyssal basins on opposite sides of the mid-Atlantic ridge in the Norwegian Sea. Taxonomic studies of deep-sea holothurians has shown that trench endemic species are almost without exception restricted to a single trench, indicating that once a species has in-

vaded the hadal zone, the populations become isolated and develop into their own species (Hansen 1967). Together these studies suggest that topographical features of the deep ocean floor may present a barrier to dispersal of some benthic and demersal fauna. These barriers to gene flow may sufficiently isolate populations to the extent that they undergo significant differentiation.

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LITERATURE CITED

- Afifi, A. A., Clark, V. (1990). Computer-aided multivariate analysis. Van Nostrand Reinhold, New York
- Baldwin, R. J., Smith, K. L. Jr (1987). Temporal variation in the catch rate, length, color, and sex of the necrophagus amphipod, *Eurythenes gryllus*, from the central and eastern North Pacific. *Deep Sea Res.* 34: 425–439
- Barnard, J. L. (1961). Gammaridean Amphipoda from depths of 400 to 6000 meters. *Galathea Rep.* 5: 23–128
- Belyaev, G. M. (1966). Hadal bottom fauna of the World Ocean. Academy of Sciences of the USSR, Institute of Oceanology. Israel Program for Scientific Translations, Jerusalem
- Birstein, Y. A., Vinogradov, M. E. (1955). Pelagicheskie gammaridy (Amphipoda-Gammaridea) Kurilo-Kamchatskoi Vpadiny. *Trudy Inst. Okeanol., Akad. Nauk SSSR* 12: 210–287
- Birstein, Y. A., Vinogradov, M. E. (1958). Pelagic gammarids (Amphipoda, Gammaridea) of the northwest Pacific Ocean (English translation, 1959). *Fish. Res. Bd Can., Trans. Ser.* 27: 219–257
- Birstein, Y. A., Vinogradov, M. E. (1970). On the fauna of pelagic gammarids in the Kurile-Kamchatka area of the Pacific Ocean. In: Bogorov, V. G. (ed.) *Fauna of the Kurile-Kamchatka Trench and its environment*. Israel Program for Scientific Translations, Jerusalem, p. 419–438
- Bruun, A. F. (1959). General introduction to the reports and list of deep-sea stations. *Galathea Rep.* 1: 7–48
- Bucklin, A., Wilson, R. R., Smith, K. L. Jr (1987). Genetic differentiation of seamount and basin populations of the deep-sea amphipod *Eurythenes gryllus*. *Deep Sea Res.* 34: 1795–1810
- Bulnheim, H.-P., Scholl, A. (1986). Genetic differentiation between populations of *Talitrus saltator* and *Talorchestia deshayesi* (Crustacea: Amphipoda) from coastal areas of the north-western European continent. *Mar. Biol.* 92: 525–536
- Christensen, I., Haug, T., Wiig, Ø. (1990). Morphometric comparison of minke whales *Balaenoptera acutorostrata* from different areas of the north Atlantic. *Mar. Mam. Sci.* 6: 327–338
- Dahl, E. (1959). Amphipoda from depths exceeding 6000 meters. *Galathea Rep.* 1: 211–241
- David, B. (1983). Early stages in isolation of abyssal benthic populations *Pourtalesia jeffreysi* (Echinoidea, Holasteroidea) in the Norwegian Sea. *Oceanol. Acta* 6: 13–20
- Efron, B., Tibshirani, R. (1991). Statistical data analysis in the computer age. *Science* 253: 390–395
- Endler, J. A. (1977). Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton
- Etter, R. J., Rex, M. A. (1990). Population differentiation decreases with depth in deep-sea gastropods. *Deep Sea Res.* 37: 1251–1261
- France, S. C., Hessler, R. R., Vrijenhoek, R. C. (1992). Genetic differentiation between spatially-disjunct populations of the deep-sea, hydrothermal vent-endemic amphipod *Ventiella sulfuris*. *Mar. Biol.* 114: 551–559
- Gage, J. D., Tyler, P. A. (1991). *Deep sea biology*. Cambridge Univ. Press, Cambridge
- Genin, A., Dayton, P. K., Lonsdale, P. F., Spiess, F. N. (1986). Corals on seamount peaks provide evidence of current acceleration over deep-sea topography. *Nature* 322: 59–61
- Gooch, J. L. (1990). Spatial genetic patterns in relation to regional history and structure: *Gammarus minus* (Amphipoda) in Appalachian watersheds. *Am. Midl. Nat.* 124: 93–104
- Gould, S. J., Johnston, R. F. (1972). Geographic variation. *A. Rev. Ecol. Syst.* 3: 457–498
- Hansen, B. (1967). The taxonomy and zoogeography of the deep-sea holothurians in their evolutionary aspects. *Stud. trop. Oceanogr.* 5: 480–501
- Haug, T., Fevolden, S. E. (1986). Morphology and biochemical genetics of Atlantic halibut, *Hippoglossus hippoglossus* (L.), from various spawning grounds. *J. Fish Biol.* 28: 367–378
- Hessler, R. R., Ingram, C. L., Yayanos, A. A., Burnett, B. R. (1978). Scavenging amphipods from the floor of the Philippine Trench. *Deep Sea Res.* 25: 1029–1047
- Ingram, C. L., Hessler, R. R. (1983). Distribution and behavior of scavenging amphipods from the central North Pacific. *Deep Sea Res.* 30: 683–706
- Ingram, C. L., Hessler, R. R. (1987). Population biology of the deep-sea amphipod *Eurythenes gryllus*: inferences from instar analyses. *Deep Sea Res.* 34: 1889–1910
- Kamenskaya, O. E. (1981). The amphipods (Crustacea) from deep-sea trenches in the western part of the Pacific Ocean. *Trans. P. P. Shirshov Inst. Ocean, Acad. Sci. USSR* 115: 94–107 (in Russian)
- Kane, T. C., Culver, D. C., Jones, R. T. (1992). Genetic structure of morphologically differentiated populations of the amphipod *Gammarus minus*. *Evolution* 46: 272–278
- Laver, M. B., Olsson, M. S., Edelman, J. L., Smith, K. L. Jr (1985). Swimming rates of scavenging deep-sea amphipods recorded with a free-vehicle video camera. *Deep Sea Res.* 32: 1135–1142
- Mais, K. F. (1972). A subpopulation study of the Pacific sardine. *Calif. Fish Game* 58: 296–314
- Marcus, L. F. (1990). Traditional morphometrics. In: Rohlf, F. J., Bookstein, F. L. (eds.) *Proceedings of the Michigan Morphometrics Workshop*. Univ. of Michigan Museum of Zoology, Ann Arbor, p. 77–122
- McDonald, J. H. (1987). Repeated geographic variation at three enzyme loci in the amphipod *Platorchestia platensis*. *Evolution* 41: 438–441
- Mogi, A. (1972). Bathymetry of the Kuroshio region. In:

- Stommel, H., Yoshida, K. (eds.) Kuroshio: Physical aspects of the Japan Current. Univ. of Washington Press, Seattle, p. 53–80
- Patarnello, T., Bisol, P. M., Battaglia, B. (1989). Studies on differential fitness of PGI genotypes with regard to temperature in *Gammarus insensibilis* (Crustacea: Amphipoda). *Mar. Biol.* 102: 355–359
- Pimentel, R. A. (1979). Morphometrics: the multivariate analysis of biological data. Kendall/Hunt Publ. Co., Dubuque
- Rex, M. A., Etter, R. J. (1990). Geographic variation in two deep-sea gastropods, *Benthonella tenella* (Jeffreys) and *Benthomangelia antonia* (Dall). *Deep Sea Res.* 37: 1229–1249
- Rex, M. A., Watts, M. C., Etter, R. J., O'Neill, S. (1988). Character variation in a complex of rissoid gastropods from the upper continental slope of the western north Atlantic. *Malacologia* 29: 325–339
- Rice, A. L., Billett, D. S. M., Fry, J., John, A. W. G., Lampitt, R. S., Mantoura, R. F. C., Morris, R. J. (1986). Seasonal deposition of phytodetritus to the deep-sea floor. *Proc. R. Soc. Edin.* 88B: 265–279
- Rohlf, F. J., Bookstein, F. L. (1987). A comment on shearing as a method for 'size correction'. *Syst. Zool.* 36: 356–367
- Royce, W. F. (1957). Statistical comparison of morphological data. In: Marr, J. C. (ed.) Contributions to the study of subpopulations of fishes. *Spec. Sci. Rep. U.S. Fish. Wildl. Ser., Fish. No. 208*, p. 7–28
- Royce, W. F. (1964). A morphometric study of yellowfin tuna *Thunnus albacares* (Bonnaterre). *Fish. Bull. U.S.* 63: 395–433
- Sainte-Marie, B. (1992). Foraging of scavenging deep-sea lysianassoïd amphipods. In: Rowe, G. T., Pariente, V. (eds.) Deep-sea food chains and the global carbon cycle. Kluwer Academic Publishers, Dordrecht, p. 105–124
- Scheepmaker, M. (1990). Genetic differentiation and estimated levels of gene flow in members of the *Gammarus pulex*-group (Crustacea, Amphipoda) in western Europe. *Bijdr. Dierk.* 60: 3–30
- Sharp, J. C., Able, K. W., Leggett, W. C., Carscadden, J. E. (1978). Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. *J. Fish. Res. Bd Can.* 35: 124–130
- Shearer, M. (1975). Factors influencing change in the phenotype of the planktonic amphipod *Parathemisto gaudicaudi* (Guerin). *J. mar. biol. Ass. U.K.* 55: 887–891
- Siegismund, H. R. (1985). Genetic studies of *Gammarus*. II. Geographical variation at polymorphic enzyme loci in *Gammarus salinus* and *Gammarus oceanicus*. *Hereditas* 102: 15–23
- Siegismund, H. R., Simonsen, V., Kolding, S. (1985). Genetic studies of *Gammarus*. I. Genetic differentiation of local populations. *Hereditas* 102: 1–13
- Solow, A. R. (1990). A randomization test for misclassification probability in discriminant analysis. *Ecology* 71: 2379–2382
- Thistle, D. (1979). Harpacticoid copepods and biogenic structures: implications for deep-sea diversity maintenance. In: Livingston, R. J. (ed.) Ecological processes in coastal and marine systems. Plenum Pub. Corp., New York, p. 217–231
- Thorpe, R. S. (1976). Biometric analysis of geographic variation and racial affinities. *Biol. Rev.* 51: 407–452
- Thurston, M. H. (1990). Abyssal necrophagus amphipods (Crustacea: Amphipoda) in the northeast and tropical Atlantic Ocean. *Prog. Oceanogr.* 24: 257–274
- Uehara, K., Taira, K. (1990). Deep hydrographic structure along 12° N and 13° N in the Philippine Sea. *J. Oceanogr. Soc. Japan* 46: 167–176
- Van Dover, C. L., Hessler, R. R. (1990). Spatial variation in faunal composition of hydrothermal vent communities on the East Pacific Rise and Galapagos Spreading Center. In: McMurray, G. R. (ed.) Gorda Ridge: a seafloor spreading center in the United States Economic Zone. Springer-Verlag, New York, p. 253–264
- Wilson, G. D. F., Hessler, R. R. (1987). Speciation in the deep sea. *A. Rev. Ecol. Syst.* 18: 185–207
- Wilson, R. R. Jr, Kaufmann, R. S. (1987). Seamount biota and biogeography. In: Keating, B., Fryer, P., Batiza, R., Boehlert, G. (eds.) Seamounts, islands, and atolls. American Geophysical Union, Geophysical Monograph No. 43, Washington, DC, p. 355–377
- Yayanos, A. A. (1981). Reversible inactivation of deep-sea amphipods (*Paralicella caperesca*) by a decompression from 601 bars to atmospheric pressure. *Comp. Biochem. Physiol.* 69A: 563–565
- Yoshioka, N., Endoh, M., Ishizaki, H. (1988). Observation of the abyssal current in the West Mariana Basin. *J. Oceanogr. Soc. Japan* 44: 33–39

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