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Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp

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Microevolutionary studies and natural history suggest that host-specialization has promoted the high diversity of tropical sponge-dwelling snapping shrimps (Decapoda, Alpheidae, *Synalpheus*). Yet the taxonomic difficulty of this genus has precluded rigorous tests of this hypothesis. *S. rathbunae* Coutière is among the most abundant invertebrates inhabiting the framework of sponges and dead coral that forms the floor of Caribbean coral reefs. Even within a small area *S. rathbunae* exhibits the apparently wide variation in size, color, and morphology that has long frustrated efforts to identify and define species boundaries within this large (> 100 described species) genus. Here I show that sympatric populations of this nominal species occupying different sponge hosts display clear, concordant differences in allozyme genotypes and in multivariate morphometrics, confirming that the populations represent three distinct biological species. Moreover, careful field sampling revealed that the three *S. rathbunae* taxa and the closely related *S. filidigitus* Armstrong showed almost no overlap in the species of hosts occupied. Interestingly, while there was significant differentiation between Belizean and Panamanian populations of the one taxon that occurred at both sites (~ 1500 km apart), these populations were recognizable as conspecific using both genetic and morphological characters. These results show that (1) diversity of *Synalpheus*, which is already among the most species-rich crustacean genera, is probably several times higher than currently recognized, and (2) species of sponge-dwelling *Synalpheus* are highly host-specific, with related species distinctly segregated among hosts. Together with previous evidence of host race differentiation within shrimp species, these results suggest a primary role for resource specialization in the origin and/or maintenance of this group's characteristically high diversity.

ADDITIONAL KEY WORDS: — coral reefs – diversity – ecological specialization – sibling species – speciation – symbiosis – *Synalpheus*.

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INTRODUCTION

The cessation of gene flow between populations, and their subsequent differentiation into biological species, is a fundamental step in the origin of biological diversity. Yet the relative importance of natural selection versus spatial isolation in driving speciation remains obscure. Comparison of closely related ('sibling') species offers one of the clearest available windows on this process, as recently diverged species are more likely than older species to retain geographic distributions and characters useful in reconstructing the history of their divergence. The more recently a speciation event has occurred, however, the more the resultant sister species are likely to be similar in morphology and in other characters that might be used to identify them. Thus the taxa most promising for macroevolutionary studies of speciation are frequently the most difficult to recognize. This problem is particularly pronounced for marine invertebrates, whose species have traditionally been defined on the basis of conspicuous morphological characters, with the consequence that wide variation in ecology, morphology, and behaviour have generally been interpreted as representing environmentally induced phenotypic plasticity (Knowlton & Jackson, 1994). Recent research has revealed that, in a range of marine invertebrate taxa, many such morphospecies in fact comprise complexes of genetically and ecologically distinct biological species (Knowlton, 1993). Such cases often demand substantial reinterpretations of their ecology and biogeography, and offer important opportunities for tracing the course of speciation.

Here I use the comparative approach to investigate the role of resource use, specifically host affiliation, in the radiation of tropical sponge-dwelling shrimp. The shrimp genus *Synalpheus* (Decapoda: Alpheidae) comprises one of the most species-rich (Bruce, 1976; Chace, 1989) and abundant (Reed *et al.*, 1982; Snelgrove & Lewis, 1989) genera of tropical crustaceans. Most of the Caribbean species (19 of 30 described) in the genus belong to the gambarelloides species group, an apparently monophyletic clade that is largely restricted to the tropical west Atlantic, and consists almost entirely of obligate inhabitants of sponges (Coutière, 1909; Dardeau, 1984; Duffy, 1992). Several *Synalpheus* species are known to feed on host sponge tissue (Rützler, 1976; Erdman & Blake, 1987; Duffy, unpublished observations), and most are probably at least facultative parasites. Because of their sedentary habits, patchily distributed hosts, and the presence of direct development in many *Synalpheus* species (Dobkin, 1965, 1969), populations of these shrimps are often strongly subdivided on scales of km or less (Duffy, 1993, 1995), in marked contrast to typical, planktonically-dispersing marine invertebrates (Palumbi, 1994).

Intensive sampling of sponge-associated faunas in the San Blas islands of Caribbean Panama, and in the vicinity of Carrie Bow Cay on the Belize Barrier Reef (Duffy, 1992 and unpublished) revealed that the closely related species *S. rathbunae* and *S. filidigitus* are among the most common invertebrates inhabiting the sponges and dead coral rubble that fill the interstices of coral reefs in these areas. In this study I show that the nominal species *S. rathbunae* is divisible into three biological species distinguishable by diagnostic allozyme alleles and distinct combinations of morpho-

metric characters. Most importantly, these closely related shrimp species display almost no overlap in the species of host sponges occupied, supporting evidence from population-level studies of other *Synalpheus* species that resource (host) specialization has been important in the radiation of this diverse group.

MATERIAL AND METHODS

As is true of many members of the genus *Synalpheus* (Dardeau, 1984), shrimp belonging to the nominal species *S. rathbunae* display a wide range in colour, body size, host affiliation, and in several morphological characters. I used a combination of genetic, morphometric, and behavioural data to assess whether such variation occurs primarily within or among species, by comparing populations of *S. rathbunae* occupying different host sponge species in Caribbean Panama and Belize.

Collections and documentation of host use patterns

To quantify shrimp host specificity and to obtain specimens for genetic and morphometric analysis, I collected samples of exposed sponges, and rubble derived from dead branching coral, and dissected them in search of shrimp. I obtained sponge and rubble samples from shallow water (< 10 m) on Ulagsukun, Guigalatupo and Mamitupo reefs, and from the Limones Cays, all in the vicinity of the Smithsonian Tropical Research Institute's field station in the San Blas Islands, Panama (9° 34'N 78° 58'W). On the Belize Barrier Reef, I collected samples of sponges growing among the dead basal branches of colonies of the reef coral *Madracis mirabilis* (Duchassaing and Michelotti) on the outer reef ridge (~ 15 m deep) of Carrie Bow Cay (16° 48'N 88° 05'W), and samples of sponge-encrusted branching coral rubble from shallow (< 3 m) patch reefs east of Blue Ground Range.

Virtually all of the shrimp obtained were associated with living sponges, identifications of which were confirmed from tissue samples and photographs. Because many of the sampled sponge species live in cryptic habitats, they were invisible until the rubble samples had been collected, returned to the laboratory and dissected; thus it was impossible to collect equal numbers of each sponge species.

Genetic analysis of shrimp populations

I used horizontal starch gel electrophoresis to assess the extent of gene flow among shrimp populations. In Panama, I collected and analysed shrimp from *Lissodendoryx colombiensis* Zea & van Soest (a total of 100 shrimp from 9 individual sponges) and *Xestospongia rosariensis* Zea & Rützler (100 shrimp from 8 sponges) on four reefs. In Belize I analysed 10–13 shrimp from each of 6 *Xestospongia* cf. *subtriangularis* (Duchassaing), and 13 from a single *Lissodendoryx* cf. *strongylata* van Soest, all from Carrie Bow Cay. Live shrimp were dissected from sponges and maintained alive for up to 9 days before being flash-frozen in liquid nitrogen or on dry ice and stored at –80°C until electrophoresis. Immediately before electrophoresis frozen shrimps were placed in ceramic spot plates with 30–40 µl of 0.25 M sucrose in 2% (v/v) 2-phenoxyethanol, and ground manually with a pestle. Homogenates were absorbed

onto filter-paper wicks and analysed via horizontal starch gel electrophoresis using standard methods (Selander *et al.*, 1971) and staining procedures (Brewer, 1970; Harris & Hopkinson, 1976). In most specimens, seven enzyme loci were resolved: *Pgi*, *Pgm*, *Mdh-1* and *Mdh*-2 on citrate pH 6.0 (Clayton & Tretiak, 1972); *Tpi* on lithium hydroxide pH 8.1/8.4 (System 2 of Selander *et al.*, 1971); and *Idh* and *Mpi* on Tris-citrate pH 8.0 (System 5 of Selander *et al.*, 1971). For a subset of shrimp run later in the study, I also scored *Aat* on Tris-citrate pH 8.0. Samples from different locations and host species were interspersed on a given gel to facilitate comparison of alleles; all alleles were named according to their % mobility relative to the most common allele in *S. brooksi* Coutière from Tiantupo, Panama, which was designated 100 (see Duffy, 1995). Using BIOSYS-1 (Swofford & Selander, 1981), allele frequencies were calculated and used to compute Nei's (1978) unbiased genetic distance between pairs of demes (I use deme to designate the group of conspecific shrimps inhabiting an individual sponge); demes were then clustered phenetically using UPGMA.

Morphometric analysis of shrimp populations

In an attempt to diagnose shrimp taxa from different host species on the basis of characters observable in preserved specimens, I measured a suite of 16 morphological characters (see Chace, 1972 for illustration): (1) carapace length (an index of body size); (2) length of the basicerite, i.e. the lateral spine on the basis, or first article, of the second antenna; (3) length of the scaphocerite (or antennal scale), i.e. the exopod of the second antenna; (4) length of the major chela, i.e. the claw on the larger of the first or anterior pair of chelipeds; lengths of the (5) chela, and (6) carpus (i.e. next proximal segment, or fifth article) of the minor first cheliped; (7) length, and (8) width of the merus (fourth article) of the minor first cheliped; (9) length, and (10) width of the merus of the second cheliped; (11) the mean number of fixed teeth on the lateral branch of the right and left uropods; (12) length, (13) width at anterior margin, and (14) width at posterior margin of the telson (the terminal abdominal segment); and lengths of the (15) lateral, and (16) medial spines on the posterior margin of the telson. I measured a total of 34 shrimp from *Lissodendoryx colombiensis* in Panama (5–7 from each of 6 individual sponges), 38 shrimp from *Xestospongia rosariensis* in Panama (6–8 from each of 6 sponges), 35 shrimp from *Xestospongia* cf. *subtriangularis* in Belize (5–6 from each of 6 sponges), and 5–7 shrimp each from single specimens of *Agelas clathrodes* (Schmidt), *Lissodendoryx* cf. *strongylata*, and an unidentified cryptic sponge in Belize.

I used canonical variates (CV) analysis to assess morphological differences among shrimp populations. I define a population here as the group of demes using a given host species in a given area. CV analysis finds linear combinations of variables, called canonical variates, that maximize differences among groups designated *a priori* (in this case, different shrimp populations). Differences among the centroids (multivariate means) of the groups can then be tested for significance. CV analysis can be very useful in identifying which of a large group of variables contribute most to the separation of groups; however, it also poses several practical problems. First, because the resulting canonical axes are not orthogonal to one another, the coefficients or 'loadings' of the original variables onto the canonical axes cannot be interpreted as straightforwardly as is possible in principal components analysis (Marcus, 1990; Reyment, 1990). Moreover, CV analysis is sensitive to violations of the assumptions

of multivariate normality and equality of variances, neither of which properties can reliably be judged from univariate distributions. For these reasons, I used the canonical coefficients obtained for each of the 16 original variables not as quantitative measures of their importance in discriminating groups of shrimp, but rather as qualitative guides to which of these variables should be examined more closely. I then conducted univariate ANOVAs to test the significance of single characters, or ratios between characters, that were indicated as important in discriminating groups by the CV analysis.

I used the CANDISC procedure of SAS (SAS, 1985) to conduct separate CV analyses at two hierarchical levels. First, all shrimp measured in the study were analysed together to identify morphological differentiation among populations from different sponges and localities. Second, I assessed morphological differentiation among conspecific demes by using shrimp samples from individual, conspecific sponges as the designated groups. In the first analysis, comparing shrimp populations in different sponge species, I used the raw data, unstandardized for body size, as input. In the analysis of differences among demes within species, however, the data for all size variables (i.e. all variables except number of uropod teeth) were divided by carapace length (an index of overall body size), and this standardized variable was used as input for the analysis. The rationale for this different treatment was as follows. The populations from different sponge species appeared to differ consistently in adult body size, such that overall size was considered likely to be a real component of morphological differences among these taxa, and thus a legitimate component of the analysis; in addition, since the total sample of shrimp from a given sponge species consisted of individuals spanning a wide size range and originating from several demes, it seemed likely that the average body size in each sample would approximate that of the population at large. Samples from individual demes, on the other hand, commonly consisted of only 5–6 shrimp and seemed less likely to represent the entire size range of the population. Differences in body size among these demes, therefore, were judged more likely to be affected by sampling error and so measurements were standardized by dividing by carapace length.

Behavioural responses to alternate hosts

As a final source of evidence for host-associated differences among shrimp populations, I conducted behavioural assays in the laboratory to assess whether shrimp from the two sympatric sponges *Lissodendoryx colombiensis* and *Xestospongia rosariensis* in Panama exhibit differential preference for their respective hosts. I chose these two sponges because they were relatively common and easily collected on the same reefs. The assay consisted of offering pieces of each of the two sponges to individual shrimp in the laboratory and recording which one was occupied after several hours. Shrimp used in these assays were obtained from three demes (i.e. individual sponges) of each host species.

In the lab, I sliced the sponge portions into pieces approximately $4 \times 4 \times 1$ cm in size, removed and set aside all shrimp, then suspended the sponge pieces in a mesh bag submerged in the field for ≥ 30 min prior to assays. After this recovery period one piece of each of the two sponge species was placed in a plastic bowl containing ~ 400 ml of seawater, and a single shrimp was then added to the bowl. The assays began in the evening, and shrimp were allowed to distribute themselves for 9.5 h in

the darkness. After this time, I quickly removed each sponge piece from the bowl, dipped it in freshwater to dislodge the shrimp, and recorded which sponge piece was occupied by the shrimp in each replicate bowl. For each of the two shrimp populations, differences in the numbers of individuals occupying the two offered sponge species were compared with a binomial test (Zar, 1974). Differences in host preference between shrimp originating from the two host species were assessed with Fisher's exact test.

RESULTS

Genetic analysis of shrimp populations

Populations of *S. rathbunae* clustered genetically into three quite distinct groups diagnosed by fixed allelic differences at each of several loci, and clearly representing

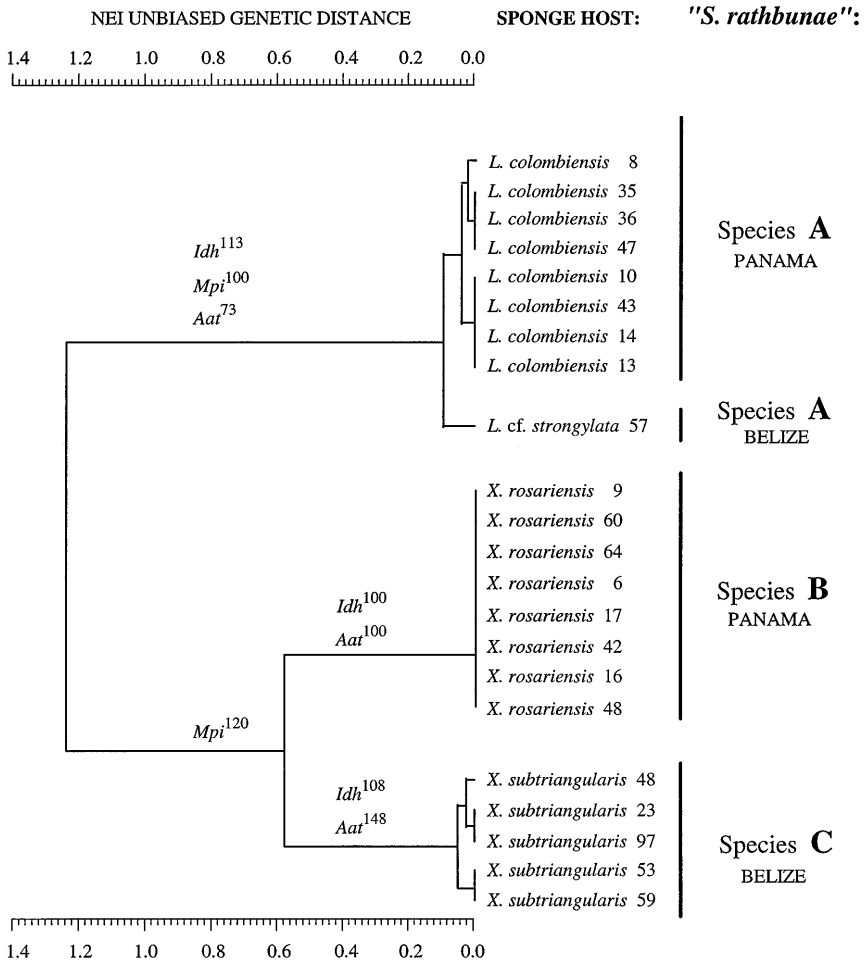

Figure 1. UPGMA phenogram of populations in the *Synalpheus rathbunae* complex, based on Nei's (1978) unbiased genetic distance. Three biological species (A–C) are recognized, based on the indicated fixed allelic differences characterizing the demes on each branch.

separate biological species (Fig. 1, Table 1). Sympatric shrimp populations inhabiting the sponges *Lissodendoryx colombiensis* and *Xestospongia rosariensis* in Panama (designated

TABLE 1. Allele frequencies at eight allozyme loci in the four studied taxa of the *Synalpheu rathbunae* complex.

	Species			
Locus Allele	A (Panama)	A (Belize)	B	C
Pgi				
n	100	13	100	57
119				0.947
108	0.957	0.500		
91				0.053
89			1.000	
87	0.025	0.500		
Pgm				
n	96	12	98	57
100	0.266			
83	0.328	0.375	0.015	
79		0.083		0.553
68		0.417		0.439
58	0.406	0.125	0.082	
50			0.837	
43			0.015	
33			0.051	0.009
Mdh-2				
n	96	13	98	55
164	0.016			
146			0.020	
130	0.984	1.000		
116			0.015	
100			0.959	0.927
66			0.005	0.073
Tpi-2				
n	91	13	95	47
126		0.077	0.068	
119	0.071			
116			0.011	
100	0.907	0.923	0.921	0.840
82				0.160
30	0.022			
Idh-1				
n	71	13	67	57
113	1.000	1.000		
108				1.000
100			1.000	
Mpi				
n	82	13	85	55
120			1.000	1.000
100	0.890	1.000		
96	0.110			
Aat				
n	37	13	33	56
148				1.000
121			0.015	
100			0.985	
73	1.000	1.000		

S. 'rathbunae A' and 'B' respectively) shared no alleles at 5 of the 8 loci scored. The single Belizean population from *Lissodendoryx* cf. *strongylata* clustered together with Panamanian populations from *Lissodendoryx colombiensis*, showed no fixed allelic differences from these populations, and was consequently also designated species A. A third taxon, *S. 'rathbunae* C' from *Xestospongia* cf. *subtriangularis* in Belize, was genetically distinct from both of the other *S. rathbunae* taxa. Each of the three taxa could be reliably diagnosed from the other two by its genotype at the *Pgi, Idh* and *Aat* loci (Table 1), the latter two of which were fixed for species-specific alleles. Accordingly, the three host-specific species were separated by very high values of Nei's (1978) unbiased genetic distance, ranging from 0.55–1.25, with much smaller distances among demes within species.

Some, but not all, of the genetically distinct taxa could be recognized by body color in life, although the elaborate color patterns useful in discriminating sibling species in the related genus *Alpheus* (e.g. Knowlton & Keller, 1985) and some other decapods (Huber, 1985; Knowlton, 1986) are not present in most *Synalpheus* species. *S. rathbunae* A and C were both translucent orange overall, more intensely so in the distal part of the major chela; ovaries and embryos of *S. rathbunae* A were bright orange whereas those of *S. rathbunae* C were pale green. *S. rathbunae* B and *S. filidigitus* were both translucent whitish with a brown wash to the distal part of the major chela; ovaries and embryos of *S. rathbunae* B were whitish, whereas they were pale yellow to pale green in *S. filidigitus*.

Morphometric analysis of shrimp populations

All of the shrimp populations examined were similar in morphology, and when taken singly, none of the morphological characters measured was entirely reliable in assigning a specimen to one of the three species recognized genetically (Table 2). Although the taxa showed overlapping distributions for all of the characters, canonical variates analysis separated the three *S. rathbunae* taxa clearly (Fig. 2, Wilks' lambda = 0.0034, $P < 0.0001$). Canonical coefficients of the 16 morphometric measurements suggested that the major contribution to canonical variate (CV) I came from the proportions of the elements of the minor first cheliped. Univariate tests confirmed this, showing that the distinctness of Panamanian populations from *Xestospongia rosariensis* (i.e. species 'B') on this axis (Fig. 2) results from the relatively short chela, and long carpus and merus in these shrimp, compared with the other species (Table 2). High scores on CV II appeared due primarily to a relatively slender (long, narrow) merus on the second cheliped, and a long scaphocerite. This axis separated demes collected from *Lissodendoryx colombiensis* in Panama, and from *L. cf. strongylata, Agelas clathrodes*, and the unidentified encrusting sponge in Belize (all species 'A') from populations in *Xestospongia* cf. *subtriangularis* in Belize (species 'C', Fig. 2). The Panamanian and Belizean populations designated as species A clustered together in morphospace (Fig. 2), corroborating their genetic similarity.

Intraspecific analyses revealed significant, and in some cases strong, differentiation among demes within species (Fig. 3). Not surprisingly, the strongest intraspecific differentiation occurred among the geographically distant populations of *S. rathbunae* A. Populations of this species from Panama and Belize were clearly separated along CV I ($P < 0.0001$), with Belizean demes characterized by a stouter (shorter, wider) telson, and proportionally longer scaphocerite than the Panamanian demes (Table

2). Differentiation among demes of species B was not as marked ($P = 0.016$) and appeared attributable primarily to differences in relative lengths of the second cheliped's merus, and the telson. Interdemic differentiation in species C was surprisingly high ($P = 0.0007$), resulting mainly from differences in relative lengths of the merus and chela of the minor first cheliped.

Host specificity in the field

The three *S. rathbunae* species, along with the closely related species *S. filidigitus*, were distinctly segregated by host species in the field (Fig. 4). In Panama, the two sympatric taxa *S. rathbunae* A and B showed no overlap in host occupation: of 24 specimens of *Lissodendoryx colombiensis* examined, 83% (20) were occupied by *S. rathbunae* species A, while none harboured *S. rathbunae* B. Apart from these collections, examination of a large number of Panamanian sponge samples representing at least 20 species (Duffy, 1992 and unpublished) found *S. rathbunae* A only in 2 of 8 sampled specimens of *Hymeniacidon* cf. *caerulea* Pulitzer-Finali (Fig. 4), and in a single specimen of an unidentified sponge growing among coral rubble. None of these sponge species harboured *S. rathbunae* B, which appears to be a specialist on *Xestospongia rosariensis* in Panama, where it occupied 100% ($n = 28$) of the sponges sampled (Fig. 4), and was found in no other host.

Segregation of the three Belizean shrimp species by host was also quite marked. *S. rathbunae* C was found primarily in *Xestospongia* cf. *subtriangularis*, occupying 91% of

TABLE 2. Morphometrics of the four studied populations in the *Synalpheus rathbunae* species complex. All measurements (except carapace length and number of uropod teeth) have been size-standardized by dividing the raw measurement by carapace length. Data are expressed as mean ± 1 SD. The last four rows present ratios; populations sharing the same superscript letter do not differ significantly at α=0.05 (one-way ANOVA followed by Ryan's Q test). A=anterior; Chel=Cheliped; L=length; P=posterior; W=width.

Character	Species A Panama (n=34)	Species A Belize (n=18)	Species B (n=38)	Species C (n=35)
1. Carapace length	3.34±0.43	2.85±0.30	2.75±0.15	2.57±0.21
2. Basicerite	0.196±0.024	0.228±0.025	0.193±0.025	0.164±0.017
3. Scaphocerite	0.285 ± 0.027^{B}	0.326 ± 0.030^{A}	0.298 ± 0.029^{B}	0.247 ± 0.016^{C}
4. Major chela length	1.144±0.144	1.190±0.130	1.211±0.208	1.191±0.147
5. Minor Chel 1 carpus length	0.195±0.020	0.201±0.015	0.229±0.016	0.209±0.013
6. Minor Chel 1 chela length	0.420±0.021	0.432±0.019	0.390±0.027	0.432±0.018
7. Minor Chel 1 merus length	0.430±0.030	0.449±0.018	0.455±0.024	0.463±0.017
8. Minor Chel 1 merus width	0.140±0.013	0.155±0.010	0.131±0.010	0.140±0.010
9. Chel 2 merus length	0.362±0.020	0.377±0.016	0.356±0.019	0.366±0.014
10. Chel 2 merus width	0.076±0.003	0.079±0.004	0.079±0.005	0.082±0.005
11. Telson length	0.284±0.014	0.278±0.026	0.276±0.020	0.290±0.020
12. Telson anterior width	0.226±0.008	0.230±0.012	0.233±0.014	0.235±0.011
13. Telson posterior width	0.078±0.007	0.092±0.012	0.093±0.012	0.083±0.012
14. Telson lat. spine length	0.034±0.006	0.029±0.004	0.033±0.005	0.031±0.005
15. Telson med. spine length	0.056±0.005	0.056±0.009	0.070±0.009	0.049±0.008
16. Number of uropod teeth	2.37±0.57	1.72±0.46	1.22±0.38	3.01±0.51
Minor Chel 1 carpus/chela	0.46 ± 0.04^{C}	0.47 ± 0.04^{BC}	0.59 ± 0.03^{A}	0.48 ± 0.03^{B}
Minor Chel 1 merus L/W	3.09 ± 0.25^{B}	2.90 ± 0.17^{C}	3.50 ± 0.21^{A}	3.32 ± 0.26^{A}
Chel 2 merus L/W	4.75 ± 0.06^{A}	4.77 ± 0.06^{A}	4.49 ± 0.06^{B}	4.47 ± 0.06^{B}
Telson A/P width	2.91 ± 0.29^{A}	2.55 ± 0.37^{B}	2.53 ± 0.27^{B}	2.91 ± 0.56^{A}

specimens (32 of 35) sampled, and occurred in a few *Hyattella intestinalis* (Lamarck) as well. *S. rathbunae* A was rare in Belize and appeared opportunistic in host use there, being found only in a single specimen of *Agelas clathrodes* (n = 28 sampled), 3 of 18 *Lissodendoryx* cf. *strongylata* (n = 11), and in the single sampled specimen of an uncommon, unidentified encrusting sponge. Of these sponge species, none supported any of the other species in the *S. rathbunae* complex. Finally, the closely related *S. filidigitus* occurred primarily in *Xestospongia* cf. *proxima* (Duchassaing & Michelotti) and *Oceanapia* sp., neither of which supported any of the *S. rathbunae* taxa; however, *S. filidigitus* also occupied a small percentage of the *X.* cf. *subtriangularis* sampled (Fig. 4). Even for the latter sponge, the only host species in which more than one of these shrimp species occurred, I found no case in which an individual sponge was occupied by more than one shrimp species.

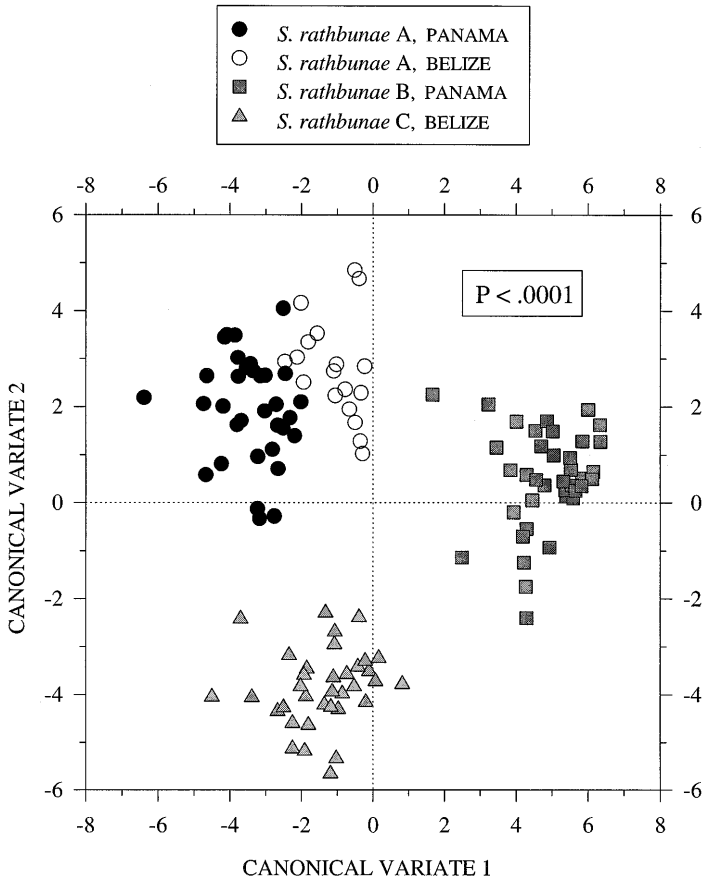

Figure 2. Morphological differentiation among taxa in the *Synalpheus rathbunae* complex, as illustrated by their scores on the first two canonical variate axes derived from measurements of 16 morphological characters. The *P* value is from Wilks' lambda test of the null hypothesis that the centroids (multivariate means) of the four groups do not differ significantly (SAS, 1985). Each symbol represents an individual shrimp. The three distinct clusters correspond to the three biological species diagnosable by fixed allozyme differences (Fig. 1, Table 1).

Behavioural responses to alternate hosts

In the laboratory, host choice by the two sympatric Panamanian taxa of the *Synalpheus rathbunae* complex generally corroborated the specificity of their host associations in the field (Fig. 5). *S. rathbunae* from *Lissodendoryx colombiensis* (species A) tended to prefer this sponge over *Xestospongia rosariensis* ($P = 0.053$, binomial test).

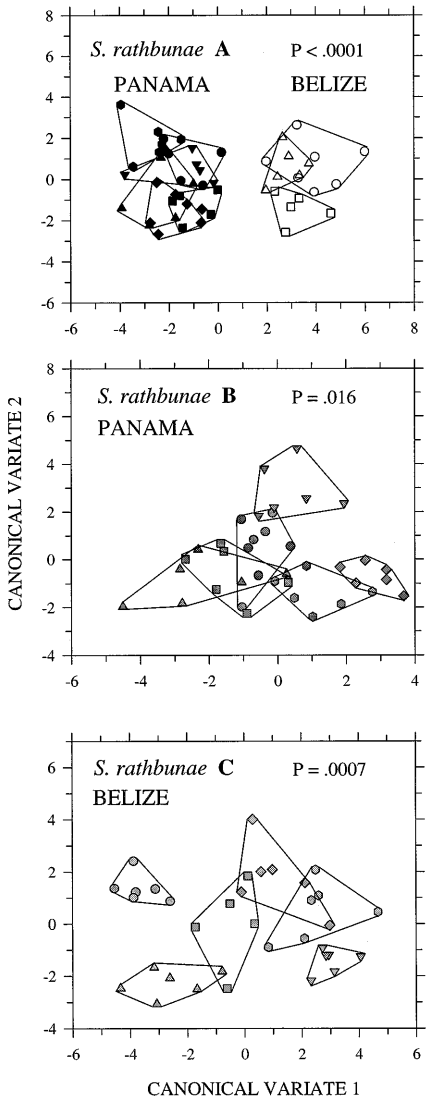

Figure 3. Morphological differentiation among demes (i.e. samples of shrimp from different individual sponges) within each of the three taxa of the *S. rathbunae* complex distinguished in Figs 1, 2. Symbols of the same shape represent shrimp from the same deme, and are bounded by lines. For species A, filled and unfilled symbols represent shrimp from Panama and Belize, respectively. Analysis is as in Fig. 2 except that the morphological variables were standardized to body size by dividing each value (with the exception of uropod tooth number) by the individual's carapace length (see 'Methods: morphometric analysis of shrimp populations' for explanation).

Conversely, *S. rathbunae* from *X. rosariensis* (species B) occupied this sponge almost exclusively ($P < 0.001$, binomial test), as it did in the field. The difference between the two shrimp species in host choice was highly significant ($P < 0.001$, Fisher's exact test).

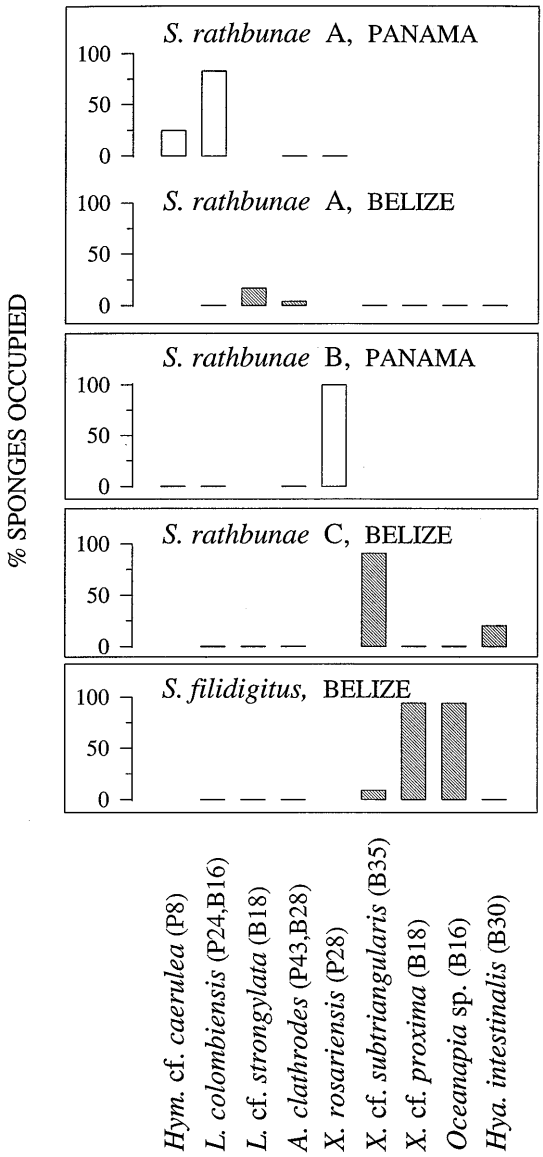

Figure 4. Host sponges occupied by members of the *S. rathbunae* complex and the closely related *S. filidigitus* in the field. Species A, B and C correspond to the genetically distinct taxa in Fig. 1. Plotted is the percentage of sampled sponges in which shrimp of a given species were found; the number of sponges sampled is in parentheses after the sponge name (P and B refer to sample sizes in Panama and Belize respectively). Open bars represent samples from Panama, and hatched bars samples from Belize.

DISCUSSION

This study demonstrates that the nominal species *Synalpheus rathbunae* actually comprises at least three genetically distinct biological species, and that these species, along with the closely related *S. filidigitus*, are highly host-specific and strongly segregated among the sponge species that they collectively occupy. Thus an apparently widespread, morphologically plastic, and ecologically generalized species is in fact a complex of more narrowly distributed ecological specialists. The critical evidence for the status of the three *S. rathbunae* taxa as biological species is their genetic distinctness. There has clearly been little or no gene flow between sympatric

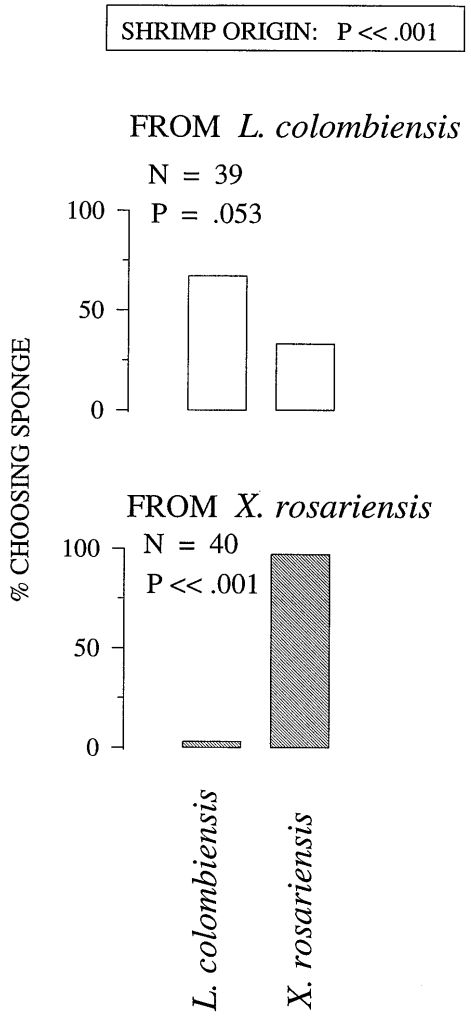

Figure 5. Results of laboratory assays testing differential host preference by the two sympatric taxa of the *Synalpheus rathbunae* complex in Panama. Assays tested choice between the sponges *Lissodendoryx colombiensis* and *Xestospongia rosariensis* as a function of the host species from which shrimp were collected. The *P* values shown separately for the two shrimp taxa are from binomial tests measuring non-random choice between the two sponges. The *P* value for shrimp origin is from Fisher's exact test of the difference in host choice between the two shrimp taxa.

populations of *S. rathbunae* A and B for some time, as evidenced by the lack of shared alleles at 5 of 8 allozyme loci scored. Indeed, the pronounced genetic differentiation between these species implies that they are relatively old, despite comparatively subtle morphological differentiation. This result recalls quite similar findings of large genetic distances accompanied by little morphological evolution in other marine crustaceans (Palumbi & Benzie, 1991; Bucklin, Frost & Kocher, 1992), and in marine invertebrates generally (Knowlton, 1993), emphasizing the value of incorporating ecology, body color, and genetic characters, as well as morphology, into the recognition of species boundaries in these animals.

The genus *Synalpheus* has long been notorious for the difficulty of diagnosing its species (Coutière, 1909; Chace, 1972; Banner & Banner, 1975; Christofferson, 1979; Dardeau, 1984). Useful morphological characters are few, gradation between forms appears common, and wide variation in body size and host affiliations among putative conspecifics from different geographic areas is the rule. The sometimes pronounced morphometric differentiation found among conspecific demes in this study (Fig. 3) illustrates this problem. Such small-scale variability is probably due in large part to the presence of direct development in the species of the *S. rathbunae* complex which, together with the sedentary habit of adults, results in low gene flow and strong population subdivision (as in *S. brooksi*, Duffy, 1995). The more important finding, however, is that, at least for the one nominal species studied here, the greater part of the phenotypic variation is attributable to the existence of several previously unrecognized biological species which are genetically, ecologically and demographically distinct, but morphologically similar. Likewise, several other nominal species of *Synalpheus* each comprise three or more biological species (J.E. Duffy, unpublished data). Although the morphological differences among these species are fairly subtle, the good news for practical taxonomy is that, as in many such cases (Knowlton, 1993), they are readily observed in preserved specimens once they have been discovered with the aid of ecological and/or genetic data (Fig. 2, Table 2). In fact, it seems especially significant, given the morphological plasticity frequently attributed to species of *Synalpheus*, that populations of *S. rathbunae* A from sites ~ 1500 km apart, and occupying different host species, are recognizable as conspecific using both genetic and morphometric data (Figs 1,2). Specifically, while Panamanian and Belizean populations of this species differed somewhat in allele frequencies, they showed no fixed allozyme differences (Table 1), and were separated by a much smaller genetic distance than were species A, B and C (Fig. 1). Similarly, the two populations of species A, despite significant differentiation between them (Fig. 3), were closer in morphometric space than were any of the other taxa (Fig. 2). Thus, in spite of significant geographic divergence, diagnostic morphological characters for these species appear reliable at least across this part of the species range. This evidence cautions against the wholesale synonymization of species within this genus based on small series of preserved specimens (e.g. Christofferson, 1979), and bolsters the claim (Knowlton & Jackson, 1994) that species diversity of many coral reef taxa has been underestimated by a factor of 3 to 5.

The significance of these results, and indeed much of the controversy surrounding the question of how many species exist, might be seen as an arcane academic argument between taxonomic 'lumpers' and 'splitters'. It is not. The important point is not simply that unrecognized species exist, but rather that a complex of biologically distinct and demographically independent biological species is ecologically very different from a single, widespread and ecologically generalized species. The host use

patterns of species in the *S. rathbunae* complex (Figs 4,5) provide a striking example. Such ecological differences among species often result in responses to habitat alteration (e.g. changes in composition of the sponge assemblage) or other ecological disturbances that could not be predicted based on the assumption of a single, ecologically generalized species. For example, Knowlton *et al.* (1992) demonstrated significant differences in growth rate and oxygen isotopic ratios among previously unrecognized sibling species of the coral *Montastrea annularis*, a commonly used environmental indicator on coral reefs. Since the geographic ranges, and often the population densities, of biological species are frequently considerably smaller than those of the previously recognized morphospecies, they are also likely to be more vulnerable to extinction through habitat destruction and disturbance than a widespread and abundant species would be.

Finally, a complex of biological species implies a potentially very different evolutionary history than a widespread generalist species. In the case of the *S. rathbunae* complex, the host use patterns of the four shrimp species support a significant role for host-specialization in their radiation. Intimate association with other organisms is widely believed to be an important driver of speciation in phytophagous and zooparasitic insects (Bush, 1975; Price, 1980; Mitter, Farrell & Wiegmann, 1988), and this hypothesis is supported for several phytophagous taxa by the existence of closely related insects using different host species (Bush, 1969; Guttman, Wood & Karlin, 1981; Knerer & Atwood, 1973; Menken, Herrebout & Wiebes, 1992; Roininen *et al.*, 1993). A similar situation has been postulated for symbiotic marine animals (Bruce, 1978; Duffy, 1992, 1995), and is particularly likely for sponge-dwelling *Synalpheus* species, many of which are strongly host-specific (Duffy, 1992, figs 4,5). For example, populations of *S. brooksi* occupying different host species show significant differences in demographic structure, behaviour, and allozyme frequencies on a scale of 10s to 100s of m (Duffy, 1993, 1995). The present study provides evidence from a macroevolutionary perspective that is consistent with such population-level patterns, and implicates host specialization in driving the radiation of symbiotic shrimp. Specifically, the almost complete lack of overlap in host use by the *S. rathbunae* species (Fig. 4) is precisely what would be expected if host shifts have been important in their radiation, either by initiating speciation or by preventing competitive exclusion of recently diverged sister species through character displacement in host use. Moreover, these shrimp display a degree of conservatism in host use reminiscent of that common in phytophagous insects (Ehrlich & Raven, 1964; Mitter, Farrell & Futuyma, 1991): three of the four shrimps studied (species B, C and *S. filidigitus*) were associated primarily or exclusively with sponges in the genera *Xestospongia* and *Oceanapia*, which are placed in the related families Petrosiidae and Oceanapiidae (van Soest, 1980). Similarly, *S. rathbunae* A was found most often in species of *Lissodendoryx* in both Panama and Belize.

The generality of the pattern of resource partitioning found in the *S. rathbunae* complex is supported by recent studies of other marine taxa, which document host-specific cryptic species of hydroids encrusting hermit crab shells (Buss & Yund, 1989), pea crabs living in bivalves (Stevens, 1990), and coral-dwelling mussels (Mokady *et al.*, 1994), as well as by earlier findings of assortment of shrimp 'color morphs' by host species (Bruce, 1978). Although such purely descriptive patterns can provide no definitive evidence for mechanisms of origin or coexistence, the collective weight of such examples supports an important role for resource specialization in the origin and/or maintenance of the characteristically high diversity of coral reefs. As

illustrated by the *S. rathbunae* complex, merely identifying these patterns depends critically on careful taxonomy.

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NOTE ADDED IN PROOF

Synalpheus "rathbunae C" has recently been formally described as *Synalpheus regalis* (Duffy 1996).

Duffy JE. 1996. *Synalpheus regalis*, new species, a sponge-dwelling shrimp from the Belize barrier reef, with notes on host specificity in *Synalpheus*. *Journal of Crustacean Biology* (in press).

REFERENCES

Banner DM, Banner AH. 1975. The alpheid shrimp of Australia. Part 2: the genus *Synalpheus*. *Records of the Australian Museum* **29:** 267–389.

Brewer GJ. 1970. *Introduction to Isozyme Techniques*. New York: Academic Press.

Bruce AJ. 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. In: Jones OA, Endean R, eds. *Biology and Geology of Coral Reefs. Volume III: Biology 2*. New York: Academic Press, 37–94.

Bruce AJ. 1978. The evolution and zoogeography of shallow-water tropical shrimps. *Information Series Department of scientific and industrial Research, New Zealand* **137:** 337–355.

Bucklin A, Frost BW, Kocher TD. 1992. DNA sequence variation of the mitochondrial 16S rRNA in *Calanus* (Copepoda; Calanoida): intraspecific and interspecific patterns. *Molecular Marine Biology and Biotechnology* **1:** 397–407.

Bush GL. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* **23:** 237–251.

Bush GL. 1975. Modes of animal speciation. *Annual Review of Ecology and Systematics* **6:** 339–364.

Buss LW, Yund PO. 1989. A sibling species group of *Hydractinia* in the northeastern United States. *Journal of the Marine Biological Society of the United Kingdom* **69:** 857–874.

Chace FR Jr. 1972. The shrimps of the Smithsonian-Bredin expeditions with a summary of West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology* **98:** i–179.

Chace FA Jr. 1989. The caridean shrimps (Crustacea: Decapoda) of the *Albatross* Phillipine expedition, 1907–1910, Part 5: Family Alpheidae. *Smithsonian Contributions to Zoology* **46:** 1–99.

Christofferson ML. 1979. Campagne de la Calypso au large des côtes Atlantiques de l'Amérique du sud (1961–1962). I. Decapoda Crustacea: Alpheoidea. *Résultats Scientifiques des Campagnes de la Calypso* **11:** 297–377.

Clayton JW, Tretiak DN. 1972. Amine citrate buffers in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* **29:** 1169–1172.

Coutière H. 1909. The American species of snapping shrimps of the genus *Synalpheus*. *Proceedings of the United States National Museum* **36:** 1–93.

Dardeau MR. 1984. *Synalpheus* shrimps (Crustacea: Decapoda: Alpheidae). I. The Gambarelloides group, with a description of a new species. *Memoirs of the Hourglass Cruises* **7**, Part 2:1–125.

Dobkin SR. 1965. The first post-embryonic stage of *Synalpheus brooksi* Coutière. *Bulletin of Marine Science* **15:** 450–462.

Dobkin SR, 1969. Abbreviated larval development in caridean shrimps and its significance in the artificial culture of these animals. *FAO Fisheries Reports* **57:** 935–946.

Duffy JE. 1992. Host use patterns and demography in a guild of tropical sponge-dwelling shrimps. *Marine Ecology Progress Series* **90:** 127–138.

Duffy JE. 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. *Marine Biology* **116:** 459–470.

Duffy JE. 1995. Resource-associated population subdivision in a symbiotic coral-reef shrimp. *Evolution* **50:** 360–373.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18:** 586–608.

Erdman RB, Blake NJ. 1987. Population dynamics of the sponge-dwelling alpheid *Synalpheus longicarpus*, with observations on *S. brooksi* and *S. pectiniger*, in shallow-water assemblages of the eastern Gulf of Mexico. *Journal of Crustacean Biology* **7:** 328–337.

Guttman SI, Wood TK, Karlin AA. 1981. Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex (Homoptera: Membracidae). *Evolution* **35:** 205–217.

Harris H, Hopkinson DA. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. New York: American Elsevier.

Huber ME. 1985. Population genetics of eight species of *Trapezia* (Brachyura: Xanthidae), Symbionts of corals. *Marine Biology* **85:** 23–36.

Knerer G, Atwood CE. 1973. Diprionid sawflies: polymorphism and speciation. *Science* **179:** 1090–1099.

Knowlton N. 1986. Cryptic and sibling species among the decapod Crustacea. *Journal of Crustacean Biology* **6:** 356–363.

Knowlton N. 1993. Sibling species in the seas. *Annual Review of Ecology and Systematics* **24:** 189–216.

Knowlton N, Jackson JBC. 1994. New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology and Evolution* **9:** 7–9.

Knowlton N, Keller BD. 1985. Two more sibling species of alpheid shrimps associated with the Caribbean sea anemones *Bartholomea annulata* and *Heteractis lucida*. *Bulletin of Marine Science*. **37:** 893–904.

Knowlton N, Weil E, Weigt LA, Guzmán HM. 1992. Sibling species in *Montastrea annularis*, coral bleaching, and the coral climate record. Science **255:** 330–333.

Marcus LF. 1990. Traditional morphometrics. In: Rohlf FJ, Bookstein FL, eds. Proceedings of the Michigan Morphometrics Workshop. *University of Michigan Museum of Zoology, Special Publication* **2:** 77–122.

Menken SBJ, Herrebout WM, Wiebes JT. 1992. Small ermine moths (*Yponomeuta*): their host relations and evolution. *Annual Review of Entomology* **37:** 41–66.

Mitter C, Farrell B, Wiegmann B. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *American Naturalist*: **132:** 107–128.

Mitter C, Farrell B, Futuyma DJ. 1991. Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. *Trends in Ecology and Evolution* **6:** 290–293.

Mokady O, Rozenblatt S, Graur D, Loya Y. 1994. Coral-host specificity of Red Sea *Lithophaga* bivalves: interspecific and intraspecific variation in 12S mitochondrial ribosomal RNA. *Molecular Marine Biology and Biotechnology* **3:** 158–164.

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

Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* **25:** 547–572.

Palumbi SR, Benzie J. 1991. Large mitochondrial DNA differences between morphologically similar Penaeid shrimp. *Molecular Marine Biology and Biotechnology* **1:** 27–34.

Price PW. 1980. *Evolutionary Biology of Parasites*. Princeton: Princeton University Press.

Reed JK, Gore RH, Scotto LE, Wilson KA. 1982. Community composition, structure, areal and trophic relationships of decapods with shallow- and deep-water *Oculina varicosa* coral reefs. Studies on decapod Crustacea from the Indian River Region of Florida, XXIV. *Bulletin of Marine Science* **32:** 761–786.

Reyment RA. 1990. Reification of classical multivariate statistical analysis in morphometry. In: Rohlf FJ, Bookstein FL, eds. Proceedings of the Michigan Morphometrics Workshop. *University of Michigan Museum of Zoology, Special Publication* **2:** 123–144.

Roininen H, Vuorinen J, Tahvanainen J, Julkunen-Tiitto R. 1993. Host preference and alloyzme differentiation in shoot galling sawfly. *Euura atra*. *Evolution* **47:** 300–308.

Rützler K. 1976. Ecology of Tunisian commercial sponges. *Tethys* **7:** 249–264.

SAS 1985. *SAS User's Guide: Statistics. Version 5 Edition.* Cary: SAS Institute.

Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JB. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Studies in Genetics IV. *University of Texas Publication* **7103:** 49–90.

Snelgrove PVR, Lewis JB. 1989. Response of a coral-associated crustacean community to eutrophication. *Marine Biology* **101:** 249–257.

Stevens PM. 1990. A genetic analysis of the pea crabs (Decapoda: Pinnotheridae) of New Zealand. I. Patterns of spatial and host-associated genetic structuring in *Pinnotheres novaezelandiae* Filhol. *Journal of Experimental Marine Biology and Ecology* **141:** 195–212.

Swofford DL, Selander RK. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72:** 281–283.

van Soest RWM. 1980. Marine sponges from Curaçao and other Caribbean localities. Part II. Haplosclerida. *Studies on the Fauna of Curaçao and other Caribbean Islands* **62**(191): 1–173.

Zar JH. 1974. *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall.