Evolution, 53(5), 1999, pp. 1414–1422

DOES COSMOPOLITANISM RESULT FROM OVERCONSERVATIVE SYSTEMATICS? A CASE STUDY USING THE MARINE SPONGE *CHONDRILLA NUCULA*

MICHELLE KLAUTAU,[1,2] CLAUDIA A. M. RUSSO,[1] CRISTIANO LAZOSKI,[1] NICOLE BOURY-ESNAULT,[3] JOHN P. THORPE,[4] AND ANTONIO M. SOLÉ-CAVA[1,4]

[1]*Departamento de Genética, Instituto de Biologia, Bloco A, CCS, Universidade Federal do Rio de Janeiro, Ilha do Fundão, CEP 21941-590, Rio de Janeiro, RJ, Brazil*

[2]*E-mail: mkmt@centroin.com.br*

[3]*Centre d'Océanologie de Marseille, Station Marine d'Endoume, Université de la Méditerranée, UMR-CNRS 6540, Marseille, France*

[4]*Port Erin Marine Laboratory, University of Liverpool, Isle of Man, IM9 6JA, United Kingdom*

Abstract.—The sponge species *Chondrilla nucula* has a simple morphology and a very wide geographical distribution. To verify whether the latter might be an artifact of the former, samples of this species were collected across 10,000 km of its range, in the Mediterranean, the Caribbean, and the southwestern atlantic. The classical (spicule morphology) and molecular (allozymes) systematic approaches were compared, to try to define the geographic limits between populations and detect possible cryptic species. We found five distinct genetic forms within *C. nucula* that sometimes showed morphological homogeneity and other times plasticity. The difference in size of spicules could not be related to the clear-cut genetic differences, suggesting that the use of spicule sizes for sponge systematics should be reappraised. The population of one of the genetic forms along 3000 km of the Brazilian coast was highly structured ($F_{ST} = 0.21$; $N_em = 0.96$). Our results reject the null hypothesis of cosmopolitanism of *C. nucula* and indicate that the putative worldwide distribution of some marine sponges, and possibly many other benthic invertebrates, may be the result of overly conservative systematics. Cryptic species appear to be particularly prevalent when genera are well defined but species are characterized by only a few morphological characters.

Key words.—Allozymes, *Chondrilla*, cosmopolitanism, endemism, population structure, taxonomy.

Received June 25, 1998. Accepted April 20, 1999.

Much of the current taxonomy of marine organisms dates back to the last century. Many of the older studies were based on limited phenotypic and geographic information, and it is questionable how adequately these earlier monographs summarize real biological diversity (Knowlton 1993). It has been suggested that the identification of species boundaries in marine invertebrates is particularly problematic because the characters used for species identification are highly biased by the taxonomists' traditional approach of using mainly visible morphological differences, which are quite different from the cues (often chemical) used for mate recognition by the invertebrates themselves (Knowlton 1993). This problem of morphological taxonomy may have led to the worldwide "lumping" of many morphologically similar, but evolutionarily distinct species into single, artificially cosmopolitan morphospecies. In particular, it has been suggested that nominate species that have few defining morphological characteristics (i.e., species that are morphologically simple) generally have a broader apparent geographical distribution than those with more complex morphology, which can therefore be more narrowly defined (Thorpe and Solé-Cava 1994).

There are numerous published examples of allegedly widely distributed "simple" marine invertebrates that have been found to conceal various cryptic species, but the phenomenon is, predictably, a particular problem in various members of the simpler, mainly "lower" invertebrate groups, many of which lack complex morphological characters that could be useful for systematic analyses (e.g., Stobart and Benzie 1994; Amaral et al. 1997; McFadden et al. 1997; Monteiro et al. 1997). A similar problem is also recognized in marine algae, where the allegedly widespread distribution of various morphologically simple species has been attributed to what has been termed the "low morphology problem" (Oppen et al. 1996).

This type of taxonomic problem is particularly well illustrated in sponges (phylum Porifera), where many sibling species have been discovered recently by molecular methods (Solé-Cava and Thorpe 1986; Boury-Esnault et al. 1992; Solé-Cava et al. 1992; Klautau et al. 1994; Muricy et al. 1996). Sponges are an abundant and speciose group (> 6000 species) and are widespread in marine habitats from the poles to the equator and from the intertidal zone to great depths. In marine sponges, systematics is almost entirely based on the architecture of the skeleton and the morphology of the spicules, which can be complex and very diverse, as in most of Poecilosclerida, or relatively simple, as in Hadromerida, Homoscleromorpha, and Chondrosida (see Hechtel 1965; Wiedenmayer 1977; Bergquist 1978).

For a species to be truly cosmopolitan, it must maintain genetic cohesiveness mediated by gene flow throughout its distribution. Thus, it is reasonable to expect that cosmopolitan marine species should be those with highly effective mechanisms for dispersal (Waples 1987; Russo et al. 1994). However, all available data from sponges indicate that the duration of larval life is very short (typically 24–48 h; Borojevic 1970; Fry 1971; Sarà and Vacelet 1973; Uriz 1982). This short planktonic life and the consequent lack of gene flow between sponge populations should lead to high levels of genetic differentiation and thus to enhanced rates of speciation (García-Ramos and Mark 1997).

Molecular markers are well suited to estimate levels of effective gene flow between natural populations (Avise 1994). Between sympatric populations, the presence of fixed allele frequency differences between morphs of a nominal

species indicates that these are likely to belong to reproductively isolated gene pools and thus to different biological species (Thorpe and Solé-Cava 1994). However, the genetic study of what might be termed cosmopolitanism must necessarily deal with divergence in allopatry (Aron and Solé-Cava 1991) and, consequently, with the question of exactly how different populations must be before they can be divided into either separate conspecific populations, when gene flow is restricted (but not absent), or distinct biological species (i.e., reproductively isolated). An approach to this problem has been the use of empirical distributions of levels of gene identity (Nei 1972, 1978), which can be related to levels of systematic differentiation (Thorpe 1982; Nei 1987). A rule of thumb in those cases has been that levels of gene identity above 0.9 indicate conspecificity and below 0.8 correspond to interspecific differentiation, with a gray zone between 0.8 and 0.9 (Thorpe and Solé-Cava 1994). However, these values are based mostly on comparisons of vertebrate species and may be somewhat reduced for marine invertebrates (Knowlton 1993; Thorpe and Solé-Cava 1994).

In this study we compare levels of genetic divergence between populations of a cosmopolitan sponge species over widely varying geographic distances (2 km to 10,000 km) to try to separate the effects of isolation by distance from those that may be related to speciation. To our knowledge, this is the first time that levels of genetic differentiation between populations of a benthic marine invertebrate have been compared over such a large range of geographic scales. The aim of the study was to test the hypothesis that in marine invertebrates with weak dispersal capabilities, particularly in species that are morphologically simple, allegedly cosmopolitan distributions are likely to be mainly an artifact of overconservative systematics.

The sponge chosen for this study was *Chondrilla nucula* Schmidt, 1862 (Demospongiae, Tetractinomorpha, Chondrosida). This chondrosid sponge is the type species of a genus defined solely by a skeleton composed of a single type of spicule. Thus, it is morphologically very simple in terms of the morphological characters conventionally used in sponges. *Chondrilla nucula* is well defined, and there are no described species with which it could reasonably be confused. Its distribution is very wide; it is considered to occur circumglobally in the tropics and subtropics, having been reported from the Atlantic, Pacific, and Indian Oceans, as well as the Mediterranean Sea and a few other areas. The species is gonochoric and oviparous (Liaci et al. 1973), but also reproduces asexually (Gaino and Pronzato 1983).

The method chosen to estimate genetic divergence was allozyme starch gel electrophoresis. This method has now been extensively used in marine sponges, and the techniques are well established and known to give useful results (Solé-Cava and Thorpe 1986; Klautau et al. 1994; Thorpe and Solé-Cava 1994). Also, there is an extensive literature on allozyme genetic distances in invertebrate species, which can be used as a sound basis from which to infer levels of taxonomic divergence. Because spicules are the sole morphological characteristic used in the identification of *C. nucula*, we have included an extensive morphological analysis of the spicules from all populations studied.

Materials and Methods

Sample Sites and Collection

Specimens of the marine sponge *C. nucula* were collected from several localities over a range of geographic distances from the eastern Mediterranean Sea, from which the species was originally described (in the Adriatic). Sampling locations (Fig. 1) and distances were from (1) eastern Mediterranean Sea: Santa Margharita, Ligurian Sea (0 km); La Vesse, French Provence coast (350 km); (2) Caribbean Sea: San Blas Islands, off the Panamanian coast (8850 km); and (3) southwestern Atlantic: Fernando de Noronha, an island 360 km off the Brazilian coast (7200 km); and four locations along the Brazilian coast: Salvador (8350 km), Búzios (9550 km), Picinguaba (10,000 km), and Ilha do Mel (10,600 km). To investigate differentiation on a smaller geographic scale, we sampled *C. nucula* from three localities along the coast of Rio de Janeiro: Búzios (0 km), Arraial do Cabo (30 km), and Itacuruçá (300 km); and two beaches in Arraial do Cabo: Anjos (0 km) and Forno (2 km; Fig. 1). All distances were shortest straight-line distances by sea (i.e., minimum distance a larva could travel) and were estimated by direct measurement from the largest available maps (Christie et al. 1995). Sample sizes are given in Table 1.

Sponges were collected between July 1995 and April 1997 by Scuba diving or snorkeling. The sponges were transported in liquid nitrogen to the Universidade Federal do Rio de Janeiro, where a small part of each specimen was separated for the study of spicule morphology. The samples remained stored in liquid nitrogen until required for genetic work.

Genetic Analysis

Horizontal 13% starch gel electrophoresis was carried out by methods that have become standard for sponges (Solé-Cava and Thorpe 1986). Of the 34 enzyme systems essayed, only 10 met the stringent conditions of clear resolution and reproducibility in individuals from all sampling sites studied. These were: acid phosphatase (*Acp*; EC 3.1.3.2); catalase (*Cat*; EC 1.11.1.6); α-esterase (α-*Est*; EC 3.1.1.1); hexokinase (*Hk*; EC 2.7.1.1); mannose phosphate isomerase (*Mpi*; EC 5.3.1.8); peptidases (*Pep*; EC 3.4.1.1) with three different substrates; *Pep*I (Pro-Phe), *Pep*II (Leu-Tyr), *Pep*III (Pro-Phe-Ala); phosphoglucose isomerase (*Pgi*; EC 5.3.1.9); and phosphoglucomutase (*Pgm*; EC 5.4.2.2). Enzyme staining followed Manchenko (1994). The buffer system used was the Tris-EDTA-maleate pH 7.4 (Hillis et al. 1996).

Genotype frequency data were used to calculate gene frequencies, fit to Hardy-Weinberg equilibrium, inbreeding indices (Wright 1978), and unbiased pairwise gene identities (Nei 1978). The significance of F_{ST} was estimated by using Waples's (1987) χ^2 test: [H_0: $F_{ST} = 0$]: $\chi^2 = 2NF_{ST}(k - 1)$; $df = (k - 1)(s - 1)$; where N is the total number of individuals sampled, k is the number of alleles, and s is the number of subpopulations analyzed for each locus. There are numerous models of gene flow between populations, but a consequence of nearly all of these is that, if populations exchange an average of (at least) one individual per generation, they will not diverge genetically, regardless of the effective population number (Wright 1978). Therefore, the effective number of

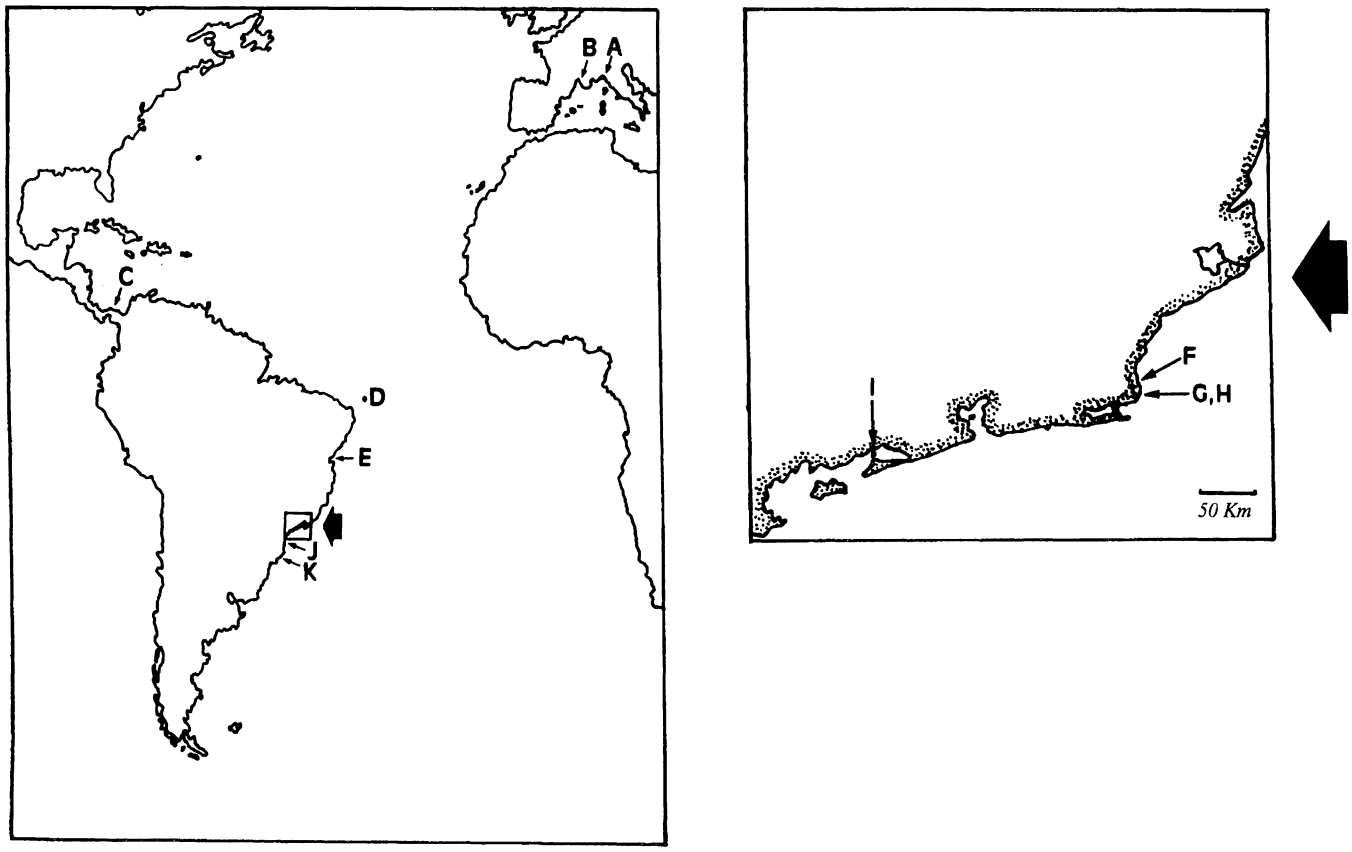

FIG. 1. Collecting sites: A, Santa Margharita, Italy; B, La Vesse, France; C, San Blas, Panama; D, Fernando de Noronha; E, Salvador; F, Búzios; G, Forno; H, Anjos; I, Itacuruçá; J, Picinguaba; K, Ilha do Mel.

migrants among subpopulations (N_em) of *C. nucula* was calculated by two separate approaches: $N_em = [(1/F_{ST}) - 1]/4$ (Wright 1978) and $N_em = e^{-[\ln p(1)+2.44]/0.505}/(N/25)$ (Slatkin 1987), where $p(1)$ is the average frequency of all private alleles (alleles present in only one population) and N the average number of individuals sampled per population.

Because there are large stochastic errors associated with estimating gene identities over small numbers of loci (Nei and Roychoudhury 1974; Nei 1987), we decided to use UPGMA (Sneath and Sokal 1973) to construct a dendrogram of relationships between populations, because this method has been shown to give better estimates of tree topology when the variance is large (Nei et al. 1983).

Spicule Morphology

The single type of spicule present in *C. nucula* is the spheraster, which is a microsclere with short rays and a thick centrum (Boury-Esnault and Rützler 1997). The morphology of spherasters was analyzed under a light microscope and measured using a camera lucida and a digitizing tablet. Forty spicules were measured for each of four individuals for each population. Each spicule had seven measurements taken: overall diameter of the spicule and length and width at the base of three of the spines. A pairwise Pearson's correlation analysis (Sokal and Rohlf 1995) was performed between the different measurements to verify their independence. The statistical significance of population differences in size of spicules was tested by a hierarchical analysis of variance (Sokal and Rohlf 1995).

RESULTS

Genetic Analysis

None of the 10 loci analyzed was entirely monomorphic over all samples (Table 1). One locus (*Mpi*) showed a significant heterozygote deficiency (Fisher's exact test; $P < 0.001$ after Bonferroni transformation; Lessios 1992), and another (*Pep*III) showed a significant ($P < 0.05$) excess of heterozygotes.

A region of nonspecific bands, similar to that observed in various other sponge species (Stoddart 1989; Boury-Esnault et al. 1992; Solé-Cava et al. 1992), was also observed in the gels. They were eliminated from our analysis because the homology of these bands is difficult to ascertain and they may come from endosymbionts.

Heterozygosity levels were usually high (Table 1), as is common in sponge populations (Solé-Cava and Thorpe 1989, 1991). On the basis of gene frequencies, the populations analyzed could be separated into five clearly distinct gene pools using Nei's unbiased genetic identities (Fig 2; Table 2), each separated from all samples in other groups by I-values not higher than 0.64 (between Salvador and Búzios). In most cases, I-values fall between 0.20 and 0.50. The two Mediterranean samples, which are about 300 km apart, show some

TABLE 1. Allele frequencies in populations of *Chondrilla nucula* (Ital, Santa Margharita, Italy; Fra, La Vesse, France; Pan, San Blas, Panama; Nor1, Fernando de Noronha 1; Nor2, Fernando de Noronha 2; Sal, Salvador; Buz, Búzios; For, Forno; Anj, Anjos; Itac, Itacuruçá; Pic, Picinguaba; Mel, Ilha do Mel). N, mean number of alleles sampled per locus; He, mean Hardy-Weinberg expected heterozygosity; Ho, mean observed heterozygosity.

	Sampling site											
Locus	Ital	Fra	Pan	Nor1	Nor2	Sal	Buz	For	Anj	Itac	Pic	Mel
Acp												
A	—	—	—	—	—	—	0.04	—	0.08	—	—	—
B	1.00	0.97	1.00	0.83	0.75	—	0.65	0.80	0.58	0.96	1.00	0.62
C	—	0.03	—	0.17	0.25	1.00	0.31	0.20	0.34	0.04	—	0.38
Cat												
A	—	—	0.69	1.00	—	1.00	0.08	0.13	—	—	0.02	0.04
B	—	—	0.31	—	1.00	—	0.92	0.87	1.00	1.00	0.98	0.96
C	1.00	1.00	—	—	—	—	—	—	—	—	—	—
Est												
A	—	—	—	—	0.50	—	—	—	—	—	—	—
B	1.00	0.94	1.00	0.25	0.16	—	0.08	0.04	0.04	0.04	0.09	—
C	—	—	—	0.50	0.17	—	—	—	—	—	—	—
D	—	0.06	—	0.25	0.17	1.00	0.92	0.96	0.96	0.96	0.91	1.00
Hk												
A	—	—	0.81	0.06	0.25	—	0.21	0.28	0.47	0.50	0.39	0.12
B	—	—	—	0.19	—	—	—	—	—	—	—	—
C	—	—	0.19	0.25	0.17	0.21	0.58	0.50	0.50	0.50	0.54	0.72
D	—	—	—	—	—	—	—	—	—	—	0.05	—
E	—	—	—	0.50	0.50	0.79	0.21	0.22	0.03	—	0.02	0.16
F	1.00	1.00	—	—	0.08	—	—	—	—	—	—	—
Mpi												
A	0.60	—	—	—	—	—	—	—	—	—	—	—
B	0.35	—	0.20	—	—	—	—	—	—	—	—	—
C	0.05	0.07	—	—	—	—	—	—	—	—	—	—
D	—	0.93	0.70	—	—	—	0.04	—	0.13	—	—	—
E	—	—	0.10	0.06	0.17	1.00	0.64	0.34	0.33	0.61	0.33	0.65
F	—	—	—	0.94	0.83	—	0.32	0.66	0.54	0.35	0.56	0.25
G	—	—	—	—	—	—	—	—	—	0.04	0.11	0.10
*Pep*I												
A	—	—	1.00	—	—	—	—	—	—	—	—	—
B	1.00	1.00	—	—	—	—	—	—	—	—	—	—
C	—	—	—	—	1.00	0.57	1.00	1.00	1.00	1.00	1.00	1.00
D	—	—	—	0.94	—	0.43	—	—	—	—	—	—
E	—	—	—	0.06	—	—	—	—	—	—	—	—
*Pep*II												
A	—	—	—	—	—	—	—	—	—	0.36	—	—
B	0.05	—	—	0.06	0.50	—	0.63	0.04	—	0.50	0.55	0.57
C	0.45	0.10	0.40	0.06	0.42	—	0.25	0.34	0.45	—	0.45	0.43
D	0.40	0.30	0.40	—	0.08	—	0.08	0.48	0.55	0.07	—	—
E	0.10	0.60	0.20	0.27	—	0.62	0.04	0.14	—	0.07	—	—
F	—	—	—	0.61	—	0.25	—	—	—	—	—	—
G	—	—	—	—	—	0.13	—	—	—	—	—	—
*Pep*III												
A	0.94	1.00	0.30	—	—	—	—	—	—	—	—	—
B	—	—	0.70	—	—	0.12	—	—	—	—	—	—
C	0.06	—	—	0.14	1.00	0.25	0.50	1.00	1.00	0.79	1.00	1.00
D	—	—	—	0.86	—	0.38	0.50	—	—	0.21	—	—
E	—	—	—	—	—	0.25	—	—	—	—	—	—
Pgi												
A	—	—	0.75	—	0.11	—	—	—	—	—	—	—
B	1.00	1.00	0.25	1.00	0.89	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgm												
A	1.00	1.00	1.00	—	0.50	0.50	0.26	0.60	0.89	0.50	1.00	0.29
B	—	—	—	—	—	0.14	—	—	—	—	—	—
C	—	—	—	0.50	0.50	—	0.14	0.25	0.11	0.50	—	—
D	—	—	—	—	—	0.36	0.05	—	—	—	—	—
E	—	—	—	0.50	—	—	0.55	0.15	—	—	—	0.71
N	22	22	12	14	12	12	24	48	26	22	32	32
H_e	0.13	0.09	0.29	0.40	0.37	0.30	0.31	0.30	0.25	0.29	0.19	0.25
H_o	0.14	0.09	0.30	0.34	0.22	0.19	0.30	0.25	0.23	0.29	0.17	0.16

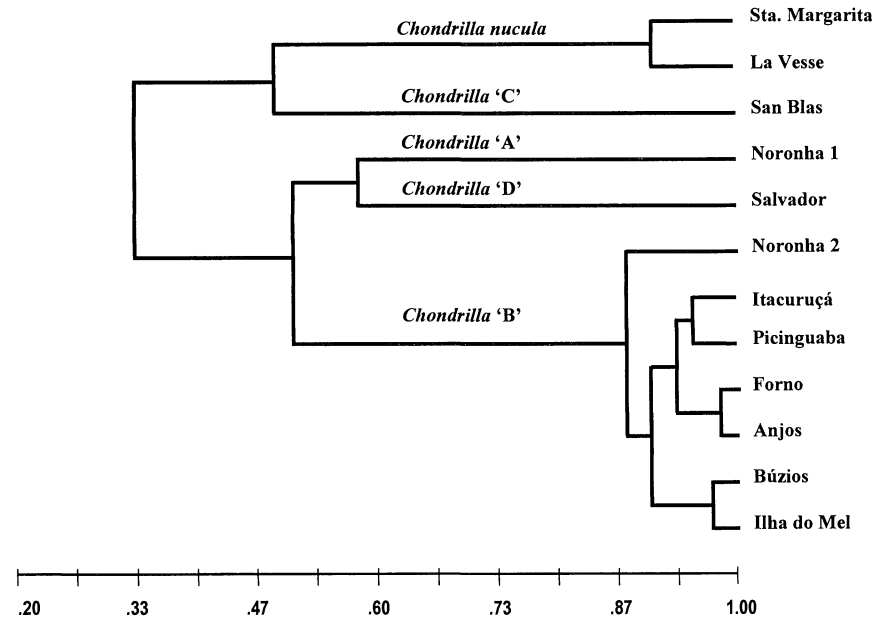

FIG. 2. UPGMA tree based on Nei's (1978) unbiased pairwise distances between the studied populations of *Chondrilla "nucula."* The four new species (A, B, C, and D) identified are indicated.

genetic differences ($I = 0.91$), but these are within the range commonly found between conspecific populations. Similarly, the six more southerly samples from about 1000 km of coastline of Brazil (Búzios, Anjos, Forno, Itacuruçá, Picinguaba, and Ilha do Mel) form a cluster, which can also be linked with one of the two sympatric species found on the Atlantic island of Fernando de Noronha (i.e., Noronha 2). Fernando de Noronha is situated about 2000 km northeast of the northernmost sample of the rest of the cluster (see Fig. 1). However, the remaining three samples, Noronha 1, Salvador, and Panama, are clearly distinct and in the dendrogram separate from each other and from all other samples with *I*-values below 0.6.

Morphology

Overall diameter and the length and width of the rays were measured in 2080 spherasters. A high correlation ($P < 10^{-6}$) was found between the dimensions of those three characters. This strongly indicated a lack of independence of measurements and consequently it was decided to use only the diameter of the spicules for population comparisons. Highly significant ($P < 10^{-8}$) differences in spicule size were observed both between sampling sites and between individuals within each site (Table 3). The populations that diverged most in spicule measurements (Tukey's pairwise comparisons; Sokal and Rohlf 1995) were those of the two populations of Fernando de Noronha, whose spicule sizes were similar to each other but larger than those of all other localities (Table 3).

DISCUSSION

The most striking result of this study is the extremely high level of genetic differentiation found between supposedly conspecific populations. Some pairwise genetic identity values between populations were as low as 0.20 (Table 2). Furthermore, the data indicate that there are two sympatric sibling species (indicated, in this case, by the presence of two diagnostic loci sensu Ayala 1983) at one of the localities

TABLE 2. Pairwise unbiased genetic identities (above diagonal) and distances (below diagonal; Nei 1978). Abbreviations as in Table 1.

	Ital	Fra	Pan	Nor1	Nor2	Sal	Buz	For	Anj	Itac	Pic	Mel
St. Margharita	—	0.91	0.50	0.28	0.36	0.20	0.30	0.37	0.37	0.33	0.40	0.27
La Vesse	0.10	—	0.55	0.29	0.32	0.25	0.28	0.34	0.35	0.32	0.37	0.24
San Blas	0.69	0.61	—	0.32	0.37	0.25	0.31	0.39	0.42	0.37	0.43	0.27
Noronha 1	1.27	1.25	1.14	—	0.50	0.58	0.53	0.49	0.38	0.46	0.41	0.47
Noronha 2	1.01	1.14	0.99	0.69	—	0.45	0.84	0.91	0.88	0.88	0.90	0.84
Salvador	1.60	1.41	1.35	0.54	0.81	—	0.64	0.59	0.56	0.55	0.49	0.60
Búzios	1.21	1.26	1.16	0.64	0.18	0.44	—	0.90	0.87	0.95	0.89	0.98
Forno	1.01	1.07	0.94	0.71	0.10	0.54	0.11	—	0.99	0.94	0.89	0.98
Anjos	0.99	1.05	0.87	0.96	0.13	0.59	0.14	0.01	—	0.92	0.95	0.89
Itacuruçá	1.11	1.14	0.98	0.78	0.12	0.60	0.06	0.06	0.09	—	0.95	0.91
Picinguaba	0.91	1.00	0.84	0.90	0.10	0.71	0.11	0.05	0.05	0.05	—	0.91
Ilha do Mel	1.30	1.42	1.31	0.87	0.18	0.52	0.02	0.09	0.12	0.09	0.09	—

TABLE 3. Spicule measurements of all populations of *Chondrilla* analyzed. Measures are given in micrometers, as mean ± standard error. There was a significant difference between individuals ($F = 48.99$, $df = 12$, $P < 10^{-4}$) and between populations ($F = 5.31$, $df = 39$, $P < 10^{-4}$) in a two-way analysis of variance (Sokal and Rohlf 1995).

Population	Diameter	Ray length	Ray width
St. Margharita	23.97 ± 0.83	5.97 ± 0.26	5.33 ± 0.20
La Vesse	27.86 ± 0.45	7.15 ± 0.17	6.05 ± 0.12
San Blas	26.80 ± 0.77	6.25 ± 0.25	5.52 ± 0.18
Noronha 1	35.51 ± 1.00	6.64 ± 0.26	6.99 ± 0.22
Noronha 2	35.89 ± 1.22	7.55 ± 0.31	7.54 ± 0.28
Salvador	30.66 ± 0.71	6.49 ± 0.19	6.35 ± 0.15
Búzios	24.56 ± 1.00	5.57 ± 0.25	4.99 ± 0.20
Forno	27.04 ± 0.90	6.36 ± 0.28	5.66 ± 0.21
Anjos	26.83 ± 0.79	5.98 ± 0.24	5.41 ± 0.21
Itacuruçá	23.88 ± 0.86	5.16 ± 0.24	4.82 ± 0.18
Picinguaba	27.15 ± 0.49	5.73 ± 0.20	5.48 ± 0.15
Ilha do Mel	26.48 ± 0.55	5.02 ± 0.15	5.27 ± 0.14

(Fernando de Noronha, Brazil). Between sympatric populations, the presence of diagnostic loci strongly indicates differentiation at the species level (Knowlton 1993; Thorpe and Solé-Cava 1994). Decisions as to species boundaries are not as straightforward, however, when comparing populations in allopatry.

From a survey of published data (Solé-Cava and Boury-Esnault 1999), it appears that sponge species show levels of genetic divergence essentially similar to those found in other groups. The mean values obtained from 34 interspecific and 44 intraspecific comparisons in sponges were 0.50 and 0.90 respectively. These compare closely with the corresponding values (0.54 and 0.96) found by Thorpe (1982) for a wide range of plant and animal species. Using these threshold values, in conjunction with the diagnostic loci, it becomes clear that the samples for which gene frequencies are shown in Table 1 can be considered to be from a total of five distinct biological species, but all within the nominate morphospecies *C. "nucula."*

Pending formal description, we will maintain the name *C. "nucula"* for the Mediterranean form (the type locality is the Adriatic sea) and assign the letters A–D for genotypes with increasing geographical distance from it. Thus, the first species from the Island of Fernando de Noronha (Noronha 1) becomes *Chondrilla* sp. A; the second species from Fernando de Noronha (Noronha 2), which is also found at several localities further south on the coast of Brazil, becomes *Chondrilla* sp. B; the Panamanian sample becomes *Chondrilla* sp. C; and the remaining sample, from Salvador, becomes *Chondrilla* sp. D.

The diversity within *C. "nucula"* may be even greater than shown by the data here because we also obtained three samples of this morphospecies from Bermuda. Because of the small sample sizes the data are not included here, but the genetic results suggest that two of these were from a sixth species and the other from a seventh species of *C. "nucula"*: These two Bermudan species were highly genetically divergent from all other samples and from each other ($I < 0.6$). Note that, except where I-values are very close to one, small samples sizes have little effect on their variance (Nei 1978; Gorman and Renzi 1979).

All the populations studied had, as expected, only one type of spicule. However, spicule sizes were variable (Table 3) and over all populations the variation was highly significant (two-way ANOVA, $P < 10^{-8}$). Surprisingly, this great variation in spicule size did not correlate with the species boundaries defined genetically. The two sympatric species from Fernando de Noronha had spicules of similar size (Mann-Whitney test, $P > 0.30$) and these were significantly larger (Mann-Whitney test, $P < 0.0001$) than those from other sites; in contrast, the genetic data indicate that what we have termed Noronha 2 is conspecific with several samples from other parts of Brazil (see above). This surprising result suggests that spicule size in *Chondrilla* may be more a result of environmental factors (e.g., temperature, Simpson 1978; growth phase, Schrönberg and Barthel 1998; or silica concentration, Stone 1970) than of genetic differences, as suggested by Bavestrello et al. (1993). In any case, the fact that different species from the same locality had spicules that were more similar than those of conspecific sponges from other localities indicates that spicule size is probably not a sound taxonomic character in this genus and, possibly, also not in other genera of sponges. This conclusion has major implications, because spicule size is widely used to define sponge species.

The finding of a species complex within the nominate species *C. "nucula"* reflects the overall difficulty of delimiting species in sponges based on only one morphological criterion and raises serious doubts as to the identity of other sponges attributed to *C. "nucula"* in other parts of the world. *Chondrilla "nucula"* is considered to be widely distributed across the warmer parts of the Pacific and Indian Oceans. Given the level of differentiation found in the amphiatlantic samples, it is quite likely that over the rest of the vast area over which the species is considered to occur, there will be several cryptic species currently considered to be *C. "nucula."* For example, based on results reported here, it is unlikely that *C. "nucula"* from Sri Lanka (Carter 1881), Africa (Topsent 1918), Australia (Burton 1934), and California (Hofkecht 1978) are all conspecific.

Levels of gene variation in the *Chondrilla* samples studied were unusually high, with many values for Hardy-Weinberg expected heterozygosity in the range of 0.2–0.4 (cf. review by Nevo 1978). However, such high values have been noted previously for sponges and other "lower" marine invertebrates (Solé-Cava and Thorpe 1989, 1991). Despite numerous

hypotheses, the reasons for high genetic variability within species in these groups are unclear.

When this investigation was started, it was intended to use samples over a very wide range of geographical distances to examine structuring of *C.* "*nucula*" with distance. However, this aim has been largely confounded by the extensive cryptic speciation discovered. It is only in *Chondrilla* sp. B, found at six sites along the Brazilian coast and one site on the coast of the island of Fernando de Noronha (2), that there are enough sampling sites for genetic structure analysis. The total distance covering these seven sampling sites is about 2700 km, and over this distance there is considerable genetic heterogeneity ($F_{ST} = 0.21$; $P[H_o: F_{ST} = 0] < 10^{-6}$ for each locus studied; Waples 1987). This gives approximate values of 0.96 (Wright 1978) or 0.33 (Slatkin 1987) for numbers of effective migrants per generation. These values indicate little gene flow even along the Brazilian coast. Similar results have been reported for dictyoceratid sponges in Australia, where *Nm* was also smaller than 1.0 between populations from 10 to 400 km apart (Benzie et al. 1994). Again, this indicates that gene flow in sponges, even over short geographical distances, can be very small and is incompatible with the existence of true cosmopolitanism in the group.

The larvae of *C.* "*nucula*" are likely to be very short lived, given that those of the sister genus *Chondrosia* live only a few days (Lévi and Lévi 1976). Also, because sponge larvae generally are considered to have low survival under environmental stress (Fry 1970), their transport in the ballast water of ships, a mechanism considered important for the distribution of, for example, various molluscs and bryozoans (Carlton and Geller 1993) is unlikely, and has never been reported. Rafting, another alternative for long-distance transport in the sea, is also unlikely because *C.* "*nucula*" occurs on rocks and is not known to encrust potentially mobile substrata (e.g., wood or drift plastic). Overall, considering its life history, the level of subdivision found in *C.* "*nucula*" is not surprising, but it reinforces the suggestion that this species is unlikely to be able to maintain genetic exchange on a transoceanic scale.

From the currently available evidence from other invertebrate groups (Knowlton 1993; Thorpe and Solé-Cava 1994), it seems that the observed lumping of different species within *C.* "*nucula*" is not an exceptional case. Indeed, many marine invertebrate species that were supposed to be cosmopolitan have turned out to be, under close molecular scrutiny, groups of morphologically similar but genetically very divergent species. To try to better understand the factors related to pseudo-cosmopolitanism in sponges we have examined the Mediterranean sponge fauna, which is composed of about 605 species, 85 of which (14%) are cosmopolitan (Vacelet 1980; Pulitzer-Finali 1983; Pansini 1992, 1995). An analysis of this fauna indicates that the factors responsible for this possible inflation of cosmopolitan species seem to include but transcend the usual "low-morphology" equals "high cosmopolitanism" explanation.

There does seem to be a tendency for morphologically complex orders to have fewer cosmopolitan species than morphologically simple orders. For example, Poecilosclerida, which can have up to 10 different types of spicules, have at least a six-fold smaller proportion of putative cosmopolitan species in the Mediterranean (7 of 143, or 4.9%) than the morphologically simple orders (zero to three types of spicules) Hadromerida (34%), Homoscleromorpha (25%), and Chondrosida (66%). However there are exceptions to this tendency. The order Haplosclerida is comparably simple morphologically, but has a surprisingly small proportion of cosmopolitan species (6.8%). The primary difference between the Hadromerida and Haplosclerida is not morphological complexity, but rather the type of characters used to define their genera. Hadromerid genera are defined by obvious and easily accessible morphological characters, whereas haplosclerid genera are only vaguely defined and thus in practice difficult to identify reliably. Taxa from such genera are more likely to be omitted from regional faunistic surveys and thus less likely to produce distributional records suggesting cosmopolitanism.

There is also a tendency for cosmopolitan taxa to be the type species of their genera or at least be species described before 1900. Among cosmopolitan Mediterranean species, for example, 60% are types for their genera and 98.8% were described before 1900. In the Haplosclerida and Homoscleromorpha, 58% and 80%, respectively, of the type species of the genera are cosmopolitan, compared to 0% and 13% respectively for the noncosmopolitan species in these orders ($\chi^2 = 18.2$; df = 1, $P < 10^{-4}$). Cosmopolitan species in these orders are also much more likely to have been described before 1900 ($\chi^2 = 33.1$, df = 1, $P < 10^{-8}$). Early descriptions typically provide only limited details, making it easier for later taxonomists to assign material from elsewhere to these species. The reasons for the prevalence of type species among cosmopolitan taxa are not so clear, but may include the absence of obvious and accessible characters to discriminate species of some genera and a tendency of early systematists to emphasize diagnostic and conspicuous characters of the type species. Samples from elsewhere sharing those features would thus tend to be assigned to the type species, resulting in an apparently cosmopolitan distribution.

In summary, the cosmopolitanism of many sponges, and perhaps many marine invertebrates, may stem not only from the normal difficulty of distinguishing taxa with few morphological characters, but also because of factors related to taxonomic traditions. Among species with limited powers of dispersal, well-defined genera and poorly defined species, an alleged cosmopolitan distribution should be regarded with great suspicion and cryptic speciation with large levels of endemism must be expected.

Acknowledgments

We thank R. Lellis, B. Lemos, G. Muricy, M. Oliveira, P. Paiva, M. Pansini, S. Peixinho, M. Tavares, J. Wulf, and P. Vianna for their help in the collection of samples; B. Bitner-Mathé, I. Nascimento, and P. Paiva for technical and statistical assistance; and N. Knowlton for valuable suggestions on the manuscript. This work was supported by grants from CNPq, FAPERJ, and FUJB (Brazil) and CNRS (France).

Literature Cited

Amaral, F. D. S., L. Maurício-Da-Silva, and A. M. Solé-Cava. 1997. Molecular systematics of *Millepora alcicornis* Linnaeus, 1758

and *M. brasiliensis* Verrill, 1868 (Hydrozoa: Milleporidae) from Brazil. Pp. 1577–1579 *in* Proceedings of the 8th International Coral Reef Symposium. Vol. 2. Smithsonian Institution Press, Panama.

Aron, S., and A. M. Solé-Cava. 1991. Genetic evaluation of the taxonomic status of two varieties of the cosmopolitan ascidian *Botryllus niger* (Ascidiacea: Botryllidae). Biochem. Syst. Ecol. 19:271–276.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, London.

Ayala, F. J. 1983. Enzymes as taxonomic characters. Pp. 3–26 *in* S. Oxford and D. Rollinson, eds. Protein polymorphism: adaptive and taxonomic significance. Academic Press, London.

Bavestrello, G., M. Bonito, and M. Sarà. 1993. Silica content and spicular size variation during an annual cycle in *Chondrilla nucula* Schmidt (Porifera, Demospongiae) in the Ligurian Sea. Pp. 421–425 *in* M.-J. Uriz and K. Rützler, eds. Recent advances in ecology and systematics of sponges. Instituto de Ciencias del Mar, C.S.I.C, Barcelona.

Benzie, J. A. H., C. Sandusky, and C. R. Wilkinson. 1994. Genetic structure of dictyoceratid sponge populations on the western Coral Sea reefs. Mar. Biol. 119:335–345.

Bergquist, P. R. 1978. Sponges. Hutchinson and Co., London.

Borojevic, R. 1970. Différentiation cellulaire dans l'embryogénèse et la morphogénèse chez les spongiaires. Pp. 467–490 *in* W. G. Fry, ed. The biology of the Porifera. Academic Press, London.

Boury-Esnault, N., and K. Rützler. 1997. Thesaurus of sponge morphology. Smithsonian Institution Press, Washington, DC.

Boury-Esnault, N., A. M. Solé-Cava, and J. P. Thorpe. 1992. Genetic and cytological divergence between colour morphs of the Mediterranean sponge *Oscarella lobularis* Schmidt (Porifera, Demospongiae, Oscarellidae). J. Nat. Hist. 26:271–284.

Burton, M. 1934. Sponges. Pp. 513–621 *in* Scientific report of the Great Barrier Reef expedition. Vol. 4. British Museum of Natural Histroy, London.

Carlton, J. T., and J. B. Geller. 1993. Ecological roulette: the global transport of indigenous organisms. Science 261:78–82.

Carter, H. J. 1881. Supplementary report on specimens dredged up from the Gulf of Manaar, together with others from the sea in the vicinity of the Bass Rocks and from Bass's Straits, respectively, presented to the Liverpool Free Museum by Capt. H. Cawne Warren. Ann. Mag. Nat. Hist. 7:361–385.

Christie, J., A. M. Ewington, H. A. G. Lewis, P. Middleton, and B. Winkleman. 1995. The Times concise atlas of the world. Bartholomew and Times Books, London.

Fry, W. G. 1970. The sponge as a population; a biometric approach. Pp. 135–162 *in* W. G. Fry, ed. The biology of the Porifera. Academic Press, London.

———. 1971. The biology of the larvae of *Ophlitaspongia seriata* from two North Wales populations. Pp.155–178 *in* D. J. Crisp, ed. Fourth European marine biology symposium. Cambridge Univ. Press, Cambridge.

Gaino, E., and R. Pronzato. 1983. Etude en microscopie électronique du filament des formes étirées chez *Chondrilla nucula* Schmidt (Porifera, Demospongiae). Ann. Sci. Nat. Zool. Biol. Anim. 5:221–234.

García-Ramos, G. K., and A. Mark. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51: 21–28.

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

Hechtel, G. J. 1965. A systematic study of the Demospongiae of Port Royal, Jamaica. Bull. Peabody Mus. Nat. Hist. 20:1–103.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. 2d ed. Sinauer, Sunderland, MA.

Hofkecht, G. 1978. Descriptions and key to the intertidal sponges of the Puerto Peñasco area in the Gulf of California. J. Ariz. Nev. Acad. Sci. 13:51–56.

Klautau, M., A. M. Solé-Cava, and R. Borojevic. 1994. Biochemical systematics of sibling sympatric species of *Clathrina* (Porifera: Calcarea). Biochem. Syst. Ecol. 22:367–375.

Knowlton, N. 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24:189–216.

Lessios, H. A. 1992. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Mar. Biol. 112:517–523.

Lévi, C., and P. Lévi. 1976. Embryogenèse de *Chondrosia reniformis* (Nardo), démosponge ovipare, et transmission des bactéries symbiotiques. Ann. Sci. Nat. Zool. 18:367–380.

Liaci, L. S., M. Sciscioli, and A. Matarrese. 1973. Sexual reproduction in some sponges: *Chondrilla nucula* O.S. and *Chondrosia reniformis* Nardo (Tetractinomorpha). Rapp. Comm. Int. Mer Médit. 22:129–130.

Manchenko, G. P. 1994. Handbook of detection of enzymes on electrophoretic gels. CRC Press Inc., Ann Arbor, MI.

McFadden, C. S., R. K. Grosberg, B. B. Cameron, D. P. Karlton, and D. Secord. 1997. Genetic relationships within and between clonal and solitary forms of the sea anemone *Anthopleura elegantissima* revisited: evidence for the existence of two species. Mar. Biol. 128:127–139.

Monteiro, F. A., A. M. Solé-Cava, and J. P. Thorpe. 1997. Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. Mar. Biol. 129:425–433.

Muricy, G., A. M. Solé-Cava, J. P. Thorpe, and N. Boury-Esnault. 1996. Genetic evidence for extensive cryptic speciation in the subtidal sponge *Plakina trilopha* (Porifera, Demospongiae, Homoscleromorpha) from the western Mediterranean. Mar. Ecol. Prog. Ser. 138:181–187.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283–292.

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

———. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nei, M., and A. K. Roychoudhury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379–390.

Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. J. Mol. Evol. 19:153–170.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Popul. Biol. 13:121–177.

Oppen, M. J. H. V., H. Klerk, J. L. Olsen, and W. T. Stam. 1996. Hidden diversity in marine algae: some examples of genetic variation below the species level. J. Mar. Biol. Assoc. U.K. 76: 239–242.

Pansini, M. 1992. Considérations biogéographiques et systématiques pour une mise à jour des données sur le peuplement de spongiaires méditerranéens. Bull. Inst. Océanogr. 9:43–51.

———. 1995. Porifera. Pp. 1–23 *in* A. Minelli, S. Ruffo, and S. La Posta, eds. Checklist delle specie della fauna italiana. Calderini, Bologna.

Pulitzer-Finali, G. 1983. A collection of Mediterranean Demospongiae (Porifera) with, in appendix, a list of the Demospongaie hitherto recorded from the Mediterranean Sea. Ann. Mus. Civ. Stor. Nat. Genova 84:445–621.

Russo, C. A. M., A. M. Solé-Cava, and J. P. Thorpe. 1994. Population-structure and genetic-variation in two tropical sea-anemones (Cnidaria, Actinidae) with different reproductive strategies. Mar. Biol. 119:267–276.

Sarà, M., and J. Vacelet. 1973. Ecologie des Démosponges. Pp. 462–576 *in* P. P. Grassé, ed. Spongiaires. Masson, Paris.

Schrönberg, C. H. L., and D. Barthel. 1998. Unreliability of demosponge skeletal characters: the example of *Halichondria panicea*. Pp. 41–54 *in* Y. Watanabe and N. Fusetani, eds. Sponge Sciences. Springer-Verlag, Tokyo, Japan.

Simpson, T. L. 1978. The biology of the marine sponge *Microciona prolifera* (Ellis and Solander). 3. Spicule secretion and the effect of temperature on spicule size. J. Exp. Mar. Biol. Ecol. 35:31–42.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco, CA.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. Freeman, San Francisco, CA.

Solé-Cava, A. M., and N. Boury-Esnault. 1999. Levels of inter- and intraspecific differentiation in marine sponges. Mem. Qld. Mus. 44:591–602.

Solé-Cava, A. M., and J. P. Thorpe. 1986. Genetic differentiation between morphotypes of the marine sponge *Suberites ficus* (Demospongiae: Hadromerida). Mar. Biol. 93:247–253.

———. 1989. Biochemical correlates of genetic variation in marine lower invertebrates. Biochem. Genet. 27:303–312.

———. 1991. High levels of genetic variation in natural populations of marine lower invertebrates. Biol. J. Linn. Soc. 44:65–80.

Solé-Cava, A. M., N. Boury-Esnault, J. Vacelet, and J. P. Thorpe. 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. Mar. Biol. 113:299–304.

Stobart, B., and J. A. H. Benzie. 1994. Allozyme electrophoresis demonstrates that the scleractinian coral *Montipora digitata* is two species. Mar. Biol. 118:183–190.

Stoddart, J. A. 1989. Foliose dictyoceratida of the Australian Great Barrier Reef. III. Preliminary electrophoretic systematics. Mar. Ecol. 10:167–178.

Stone, A. R. 1970. Seasonal variations of spicule size in *Hymeniacidon perleve*. J. Mar. Biol. Assoc. U.K. 50:343–348.

Thorpe, J. P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annu. Rev. Ecol. Syst. 13:139–168.

Thorpe, J. P., and A. M. Solé-Cava. 1994. The use of allozyme electrophoresis in invertebrate systematics. Zool. Scr. 23:3–18.

Topsent, E. 1918. Eponges de San Thomé. Essai sur les genres *Spirastrella, Donatia* et *Chondrilla*. Arch. Zool. Exp. Gén. 57: 535–618.

Uriz, M. J. 1982. Reproducción en *Hymeniacidon sanguinea* (Grant, 1926): biología de la larva y primeros estadios postlarvarios. Invest. Pesq. 46:29–39.

Vacelet, J. 1980. Les affinités du peuplement de Spongiaires de la Méditerranée. Journées Études Systém. Biogéogr. Médit. C.I.E.S.M. 29:29–30.

Waples, R. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400.

Wiedenmayer, F. 1977. Shallow-water sponges of the western Bahamas. Experientia 28:1–287.

Wright, S. 1978. Evolution and the genetics of populations. Vol. 4. Univ. of Chicago Press, Chicago.

Corresponding Editor: J. Neigel