J. Mar. Biol. Ass. U.K. (1999), **79**, 39–50
Printed in the United Kingdom

Comparative study of putative conspecific sponge populations from both sides of the Isthmus of Panama

N. Boury-Esnault*, M. Klautau[†], C. Bézac*, J. Wulff[‡] and A.M. Solé-Cava[†∫]

*Centre d'Océanologie de Marseille, Station Marine d'Endoume, Université de la Méditerranée, UMR–CNRS 6540 'DIMAR' & FR 6106, rue de la Batterie des Lions, 13007-Marseille, France (e-mail: esnault@com.univ-mrs.fr). [†]Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Genética, Bloco A-CCS-Ilha do Fundão, 21941-Rio de Janeiro, Brazil. [‡]Department of Biology, Middlebury College, Middlebury, Vermont, 05753, USA. [∫]Department of Environmental and Evolutionary Biology, University of Liverpool, Port Erin Marine Laboratory, Isle of Man

A morphological, cytological and genetic comparison of putative conspecific populations of *Spirastrella* sp. cf. *mollis* (Porifera: Demospongiae) from both sides of the Isthmus of Panama revealed a very high level of genetic differentiation together with morphological and cytological differences. The main differences were the distribution of the spirasters within the choanosome, the size and shape of spirasters 1, and the size and shape of inclusions within type I cells. Consequently these two populations clearly belong to different biological species. The Atlantic one, *S. hartmani* sp. nov. corresponds to what previous authors have called *S. cunctatrix* in the Caribbean. The finding of this new species raises to three the number of *Spirastrella* spp. known from the Caribbean Sea. The Pacific species is named here *S. sabogae* sp. nov. The levels of gene divergence found between *S. hartmani* and *S. sabogae* (Nei's genetic distance D=2.30) were as high as those found between different genera in other groups of invertebrates. Similarly exceptionally high values of gene divergence have been found between other congeneric sponge species and may be indicative either of a higher rate of molecular evolution or a very slow rate of morphological evolution in the Porifera compared to other metazoans.

INTRODUCTION

The extremely high levels of genetic divergence found between sympatric or allopatric morphs of supposedly cosmopolitan sponge species (Solé-Cava et al., 1991a, 1992; Boury-Esnault et al., 1992; Klautau et al., 1994) indicate that the cosmopolitanism of many (if not most) sponge species can be the result of over conservative systematics. This has also been the conclusion of all recent cytological studies of cryptic or cosmopolitan species (Boury-Esnault et al., 1992, 1994, 1995) as well as precise morphological studies (Soest & Hooper, 1993; Boury-Esnault et al., 1993). A basic question in evolutionary biology and ecology is the effect of the genetic divergence resulting from geographical isolation (Jackson et al., 1993). Although populations in allopatry are expected to diverge and speciate (Mayr, 1963; Lessios, 1981; Weinberg & Starczak, 1989; Knowlton, 1993), the rate of divergence is often difficult to estimate because of the uncertainties in determining the time of their separation. A common vicariant event used to estimate that rate is the rising of the Isthmus of Panama during the Pliocene, which isolated the populations in the Caribbean Sea from those in the tropical eastern Pacific. Morphologically similar remnants of the separation of Atlantic and Pacific are presently found on both sides of the Isthmus of Panama [e.g. sea urchins (Lessios, 1981); barnacles (Laguna, 1987); isopods (Weinberg & Starczak, 1989); molluscs (Jackson et al., 1993); shrimps (Knowlton et al., 1993)]. Unlike madreporarians, for which there is almost no overlap between genera and species of both sides of Panama (Glynn et al., 1973), a relatively high number of similar species have been reported for sponges (Laubenfels, 1936a). A recent morphological and ecological study of four of those species (*Spirastrella* sp. cf. *mollis*, *Haliclona caerulea* and two species of *Halichondria*) concluded that they were indistinguishable cautioning, however, that techniques beyond classical morphological analysis (e.g. cytology and genetics) might be necessary to confirm their identity (Wulff, 1996).

The aims of this paper, were to compare morphologically, cytologically and genetically populations of *Spirastrella* sp. cf. *mollis* from both sides of the Isthmus of Panama, to verify their taxonomic status, and to relate their levels of genetic divergence to the vicariant effect that may have been responsible for their separation.

MATERIALS AND METHODS

Collection of samples

Two sites were chosen on the Atlantic coast of Panama (Figure 1): San Blas Islands and Galeta, close to the mouth of the canal. One site was on the Pacific coast; Saboga Island in Las Perlas Archipelago in the Gulf of Panama. Samples were collected by snorkelling or SCUBA diving on the Atlantic and Pacific coasts in July and August 1995. They were immediately fixed for cytology and frozen for allozyme work.

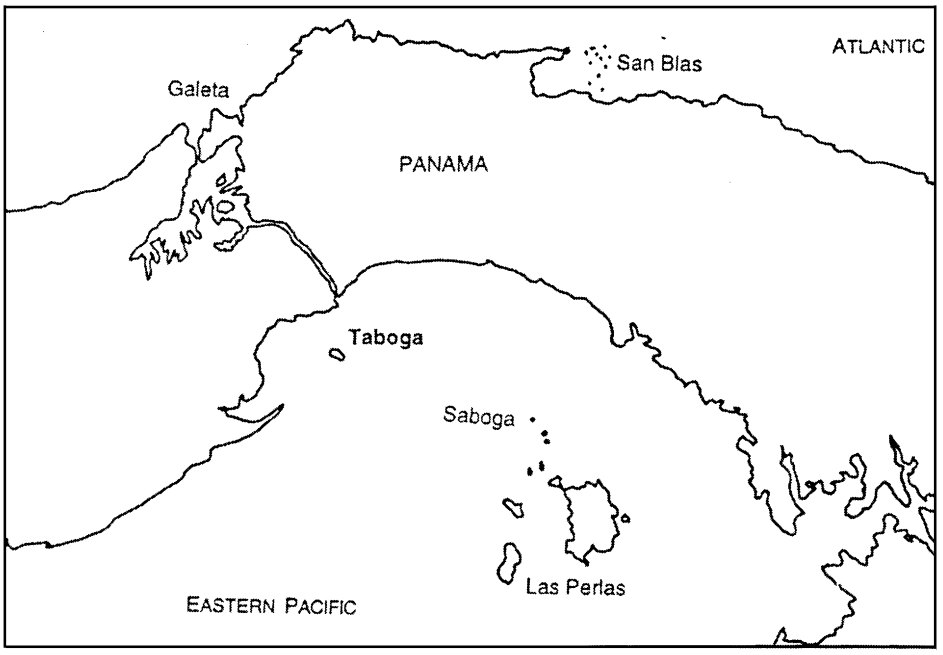

Figure 1. Map of collecting sites in Panama.

Morphology

Detailed morphology of the spicules was observed by scanning electron microscopy (SEM). Clean dehydrated spicules coated with gold-palladium were examined through a Hitachi S570 scanning electron microscope. For each population, spicule measurements (minimum 50 per spicule type) were obtained for five different specimens. Size range, as well as the mean and standard deviation, were calculated for all types of spicules. For spirasters, a histogram of length frequency was made using the algorithm for frequency classifications of Doane (1976). For the study of the architecture of the skeleton, specimens were embedded in Araldite and prepared as polished thin sections.

Cytology

For cytology, specimens were fixed as previously described (Boury-Esnault et al., 1984): glutaraldehyde 2.5% in a mixture of 0.4M cacodylate buffer and seawater (4:5 V:V; 1120 mOsm) and post fixed in 2% osmium tetroxide in seawater. For light and transmission electron microscopy (TEM) the specimens were embedded in Araldite. Semithin sections were stained with toluidine blue. Thin sections of two specimens per population, contrasted with uranyl acetate and lead citrate, were observed under a transmission electron microscope (Hitachi Hu 600).

Electrophoresis

Samples were homogenized with an equal volume of 0.01M Tris, 0.001M EDTA, 0.01M maleic acid, 0.001M $MgCl_2$, pH 7.4, and analysed by horizontal 13% starch gel electrophoresis, using a 0.1M Tris, 0.01M EDTA, 0.1M maleic acid, 0.001M $MgCl_2$, pH 7.4 buffer (for further details see Boury-Esnault et al., 1992; Solé-Cava et al., 1992). Twenty enzyme systems were assayed, of which seven (coding for a total of eight gene loci) gave reproducible results. These were acid phosphatases (ACP; E.C. 3.1.3.2), adenylate kinase (AK, E.C. 2.7.4.3), catalase (CAT; E.C. 1.11.1.6), α-esterases (EST; E.C. 3.1.1.X), glucose 6-phosphate dehydrogenase (G6PDH; E.C. 1.1.1.49), hexokinase (HK, E.C. 2.7.1.1) and phosphoglucose isomerase (PGI, E.C. 5.3.1.9). Enzyme stain recipes and nomenclature were based on Manchenko (1994). Gene frequencies, heterozygosity levels and genetic identities (Nei, 1978) were calculated with the BIOSYS-1 program (Swofford & Selander, 1981).

RESULTS

Ecology

San Blas and Galeta specimens were collected between 1 and 3 m depth in sea grass beds. They were found fixed on small pebbles. The Saboga specimens were collected on coral reefs in the low intertidal zone. Both species appear to be well protected against predators except from some sponge-specialist fish (Wulff, 1994, 1995).

Morphology

Both Atlantic and Pacific populations of *Spirastrella* spp. collected share the same general aspect. They are encrusting sponges with large visible exhalant canals. They are dull salmon-brown at the surface and brick coloured inside. The skeleton consists of tracts of tylostyles perpendicular to the substrate diverging in bouquets at the surface, and ectosomal layers of spirasters. In the Atlantic (San Blas and Galeta) populations the spirasters are densely packed in the ectosome and in a basal layer near the substrate whereas they are scattered in the choanosome (Figure 2A). In contrast, in the Pacific (Saboga) population the spirasters are densely packed not only in the ectosome and in the basal layer, but also in the choanosome (Figure 2B).

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

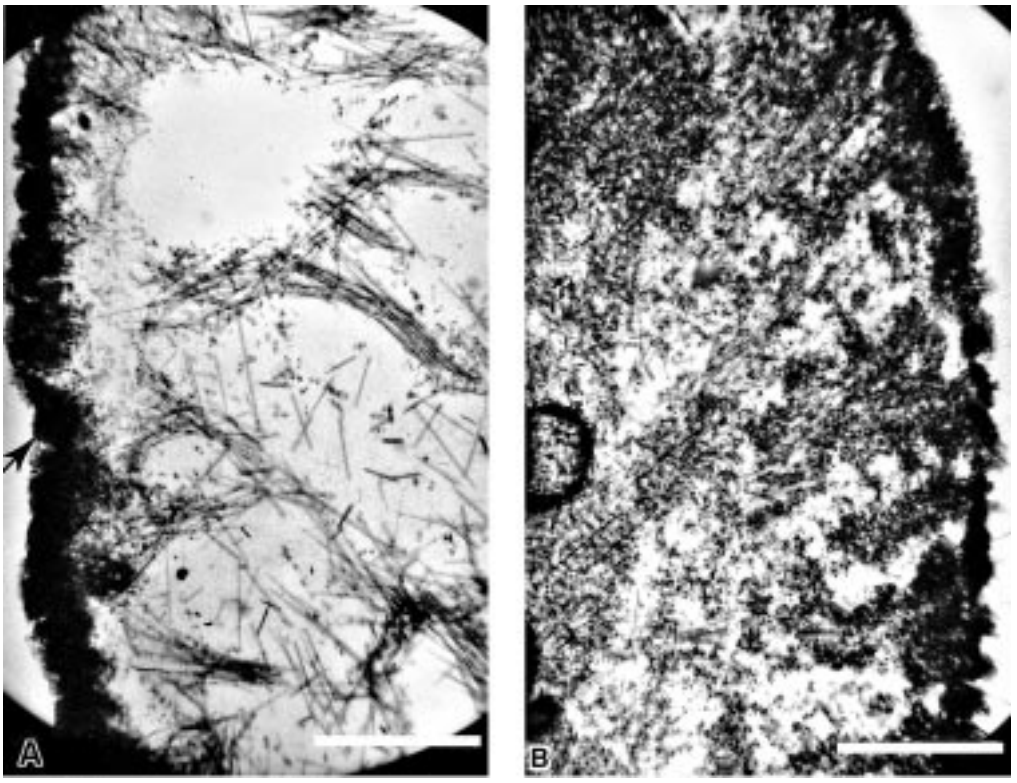

Figure 2. Polished thin section of the skeleton of (A) an Atlantic specimen where an ectosomal crust of spirasters is visible on the left of the micrograph (arrow). Spirasters are scattered in the choanosome. (B) A Pacific specimen where spirasters are densely packed in the ectosome and in the choanosome. Scale bars: A, 425 μm; B, 320 μm.

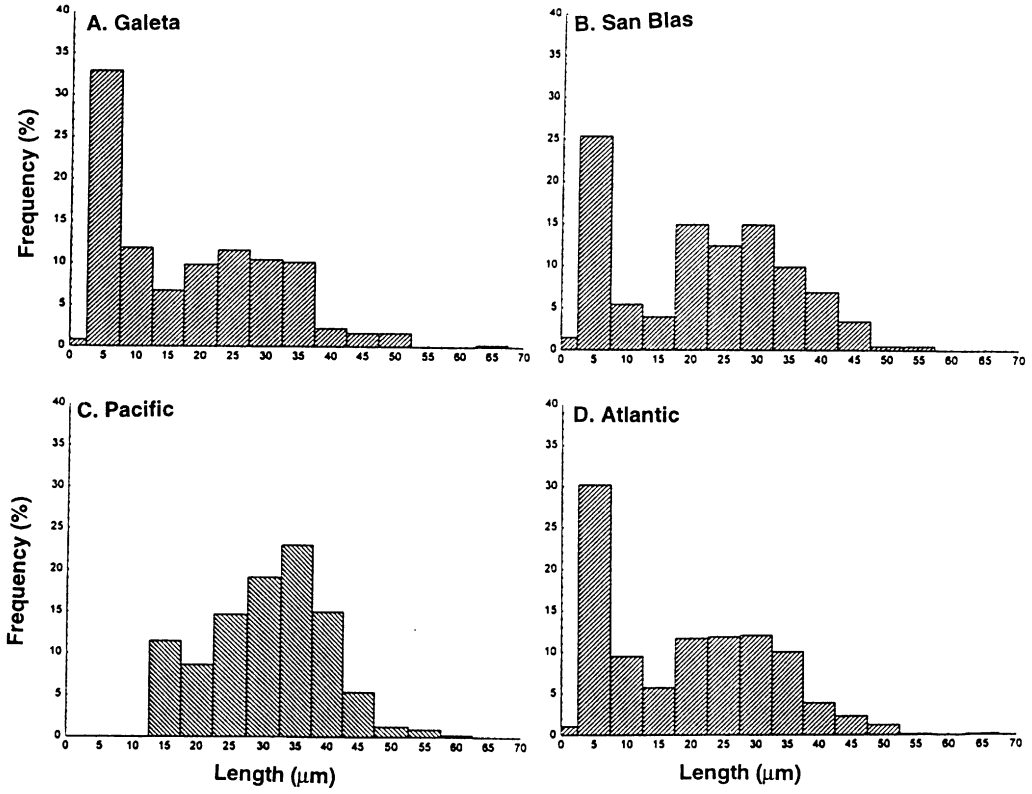

Figure 3. Frequency histogram of size classes of spirasters I and II. Each class has an interval of 5 μm.

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

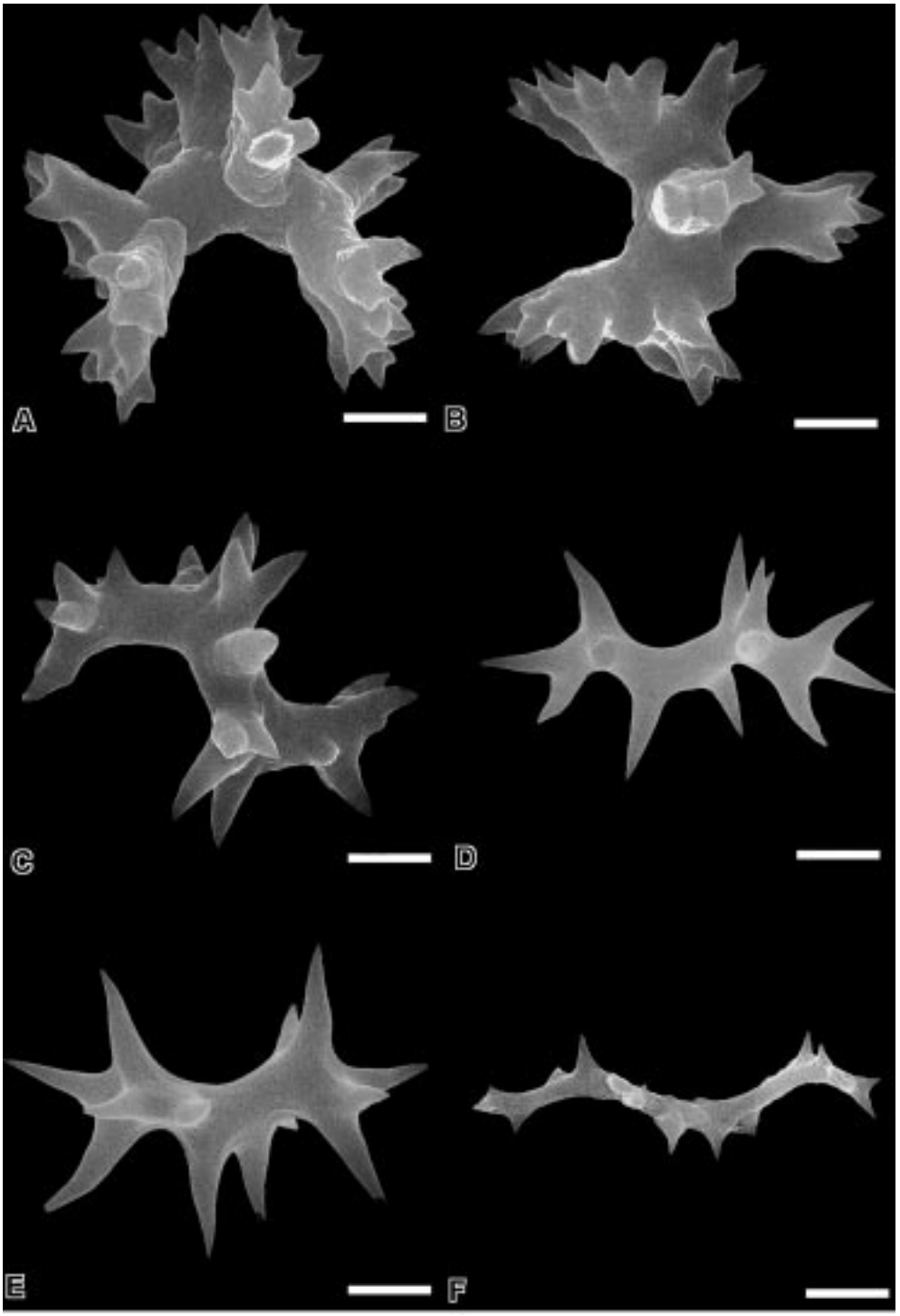

Figure 4. Electron micrographs of: (A) spiraster I San Blas specimen; (B) spiraster I Galeta specimen; (C) spiraster I Saboga specimen; (D) spiraster II San Blas specimen; (E) spiraster II Saboga specimen; (F) young form of spiraster II. Scale bars: A, 1.5 μm; B, 1.4 μm; C, 2.3 μm; D, 8 μm; E, 5.2 μm; F, 5.7 μm.

Tylostyles of the San Blas and Galeta populations are straight and slightly fusiform. The largest width is not immediately below the head but at a certain distance beyond it. The head is spherical, sometimes irregularly shaped, with an annular swelling below the neck. The sharp point is slightly stepped and often blunt or rounded. Spicule dimensions (length × width) in both populations were: San Blas 223–541 × 5.3–15.9 μm (mean $400 \times 9.9 \pm 52 \times 2$) and Galeta 307–546 × 5.3–15.9 μm (mean $429 \times 9.7 \pm 62 \times 2.6$). The head of the tylostyles in the San Blas and Galeta populations measured, respectively, 6.8–15.3 μm (mean 11.2 ± 1.7) and 8.5–15.3 μm (mean 11.3 ± 1.9). The difference of tylostyle size between the Atlantic populations was not significant (Student's *t*-test: $P < 0.05$).

In the Saboga population, tylostyles are straight, sometimes slightly flexuous, and the head is ovoid, sometimes irregularly shaped. The largest width is immediately below the head. The shaft gradually tapers to a sharp point. The dimensions of tylostyles

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

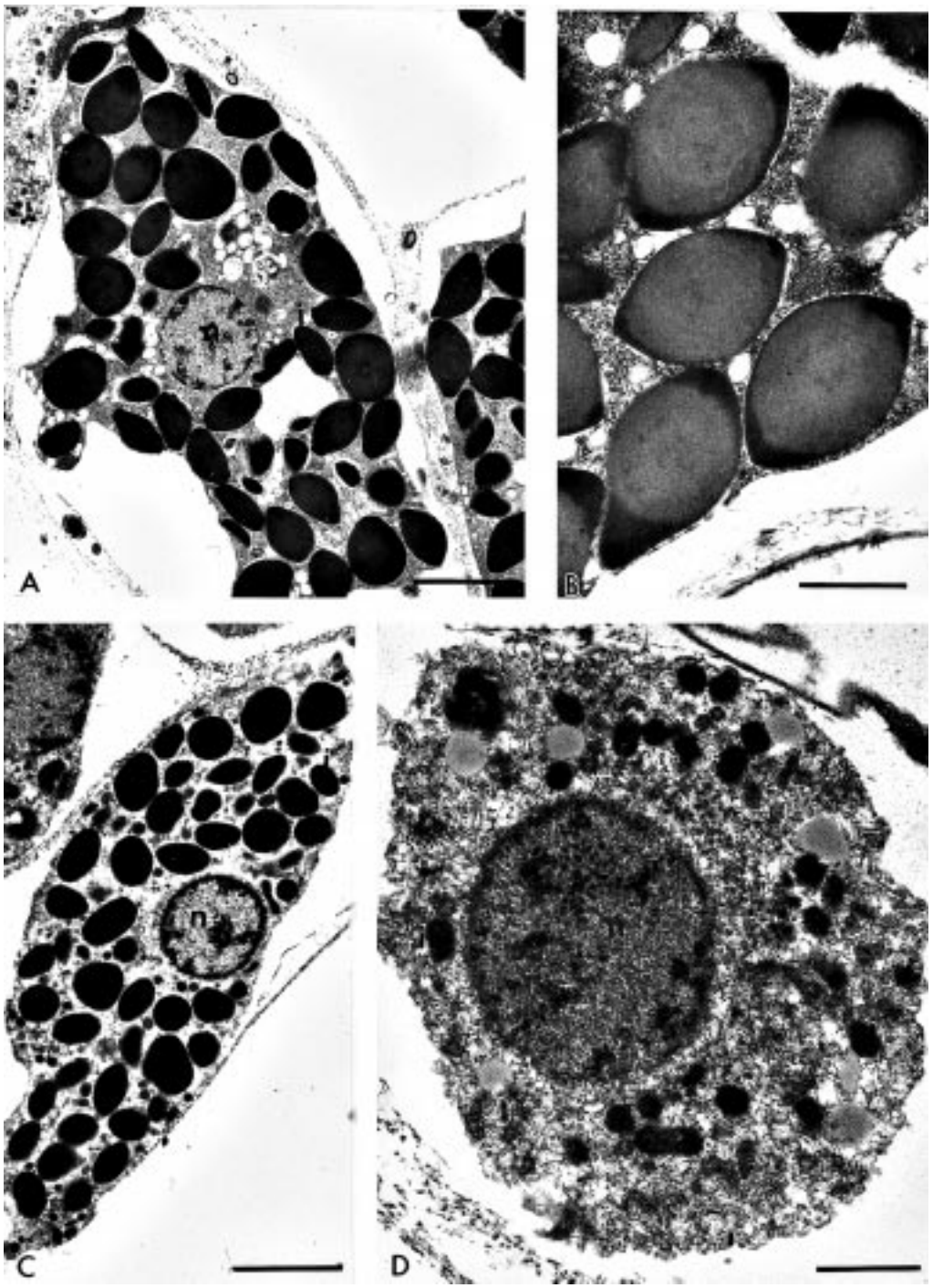

Figure 5. Electron micrographs showing: (A) Atlantic *Spirastrella* cell type I; (B) details of the ovoid inclusions of cell type I of Atlantic *Spirastrella*; (C) Atlantic *Spirastrella* cell type II; (D) Atlantic *Spirastrella* cell type III. i, inclusions; n, nucleus. Scale bars: A, 2.4 μm; B, 0.7 μm; C, 1.7 μm; D, 0.76 μm.

in the Saboga population were: 297–578 × 2.6–15.9 μm (mean 450 × 8 ± 50 × 2.6). The diameter of the head was 6.8–13.6 μm (mean 9.9 ± 1.6).

Spirasters: the histogram of length frequency shows a bimodal distribution (Figure 3). In the populations from Galeta and San Blas, the lengths of the first size-class were very similar: respectively 2–14 μm (mean 8.5 ± 2 μm) and 4–14 μm (mean 9 ± 2.8 μm). They have a very short shaft, generally bent only once; a second bend is rarely present. Two groups of spines are present at each extremity and a third one on the convex part. These are robust spines that are often fused. The extremities only are free and often ramified (Figure 4A,B). In the Pacific population the size of the first class of spirasters 10–20 μm (mean 15 ± 3 μm), is almost twice the size of that observed in Atlantic specimens. The spirasters of the Pacific population have a long shaft bent two to three times and the robust spines, present on the convex part, are free or fused only at the base. The extremities are sometimes ramified (Figure 4C).

The second size-class of spirasters had the same dimensions in the three populations: Atlantic 15–66 μm (mean 30 ± 9 μm); Pacific 21–57 μm (mean 32 ± 7 μm). They show two to four bends and are covered by free robust

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

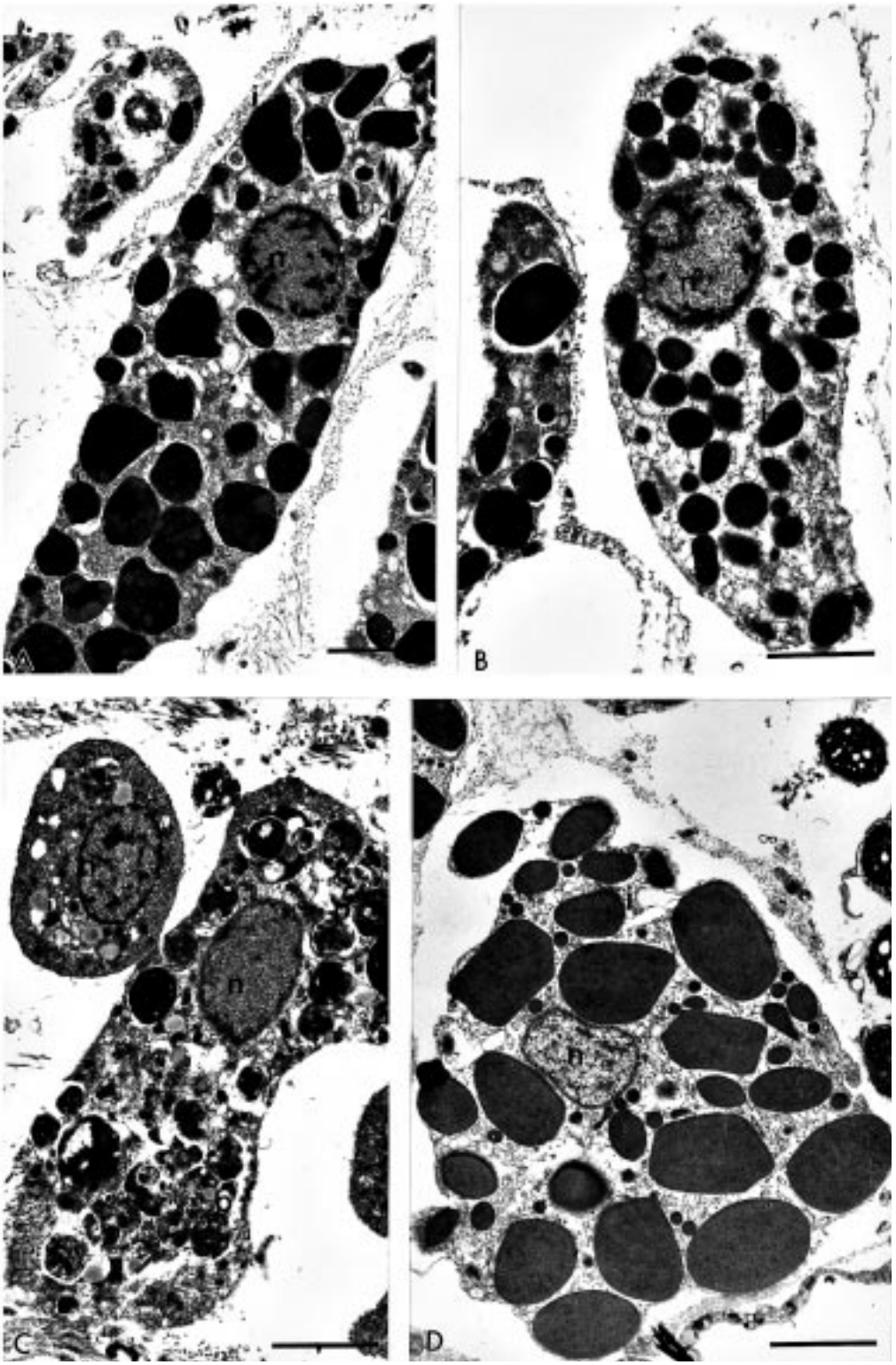

Figure 6. Electron micrographs showing: (A) Pacific *Spirastrella* cell type I; (B) Pacific *Spirastrella* cell type II; (C) Pacific *Spirastrella* cell type III and archaeocyte; (D) Mediterranean *Spirastrella cunctatrix* cell type I. i, inclusions; n, nucleus; p, phagosome. Scale bars: A, 2.4 μm; B, 1.1 μm; C, 1.4 μm; D, 2.3 μm.

spines starting from the convex part of the bends (Figure 4D,E). The spirasters I are very easily distinguished from young forms of spirasters II (Figure 4F).

Cytology

The aquiferous system and the anatomy of Atlantic and Pacific populations of *Spirastrella* are quite similar. The eurypilous choanocyte chambers are spherical and have a volume of $\sim$8000 μm^3. A central cell is present at the apopyle. Two layers can be distinguished in the ectosome: the external layer is $\sim$70 μm thick and is essentially constituted by T-shaped exopinacocytes, collencytes and collagen. The inner layer is $\sim$120–150 μm thick and is mostly constituted by aggregates of large cells with inclusions. These cells are present with other types of cells

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

Table 1. *Gene frequencies and heterozygosity levels for the populations studied. Ten alleles were sampled from each population. H_o and H_e-direct count and Hardy–Weinberg mean heterozygosities, respectively.*

Locus	San Blas	San Blas	Galeta	Pacific
ACP				
A	0.00	0.00	0.00	0.10
B	0.00	0.00	0.00	0.20
C	0.00	0.00	0.00	0.70
D	0.13	0.10	0.00	0.00
E	0.87	0.90	1.00	0.00
AK				
A	0.00	0.00	0.10	0.00
B	0.13	0.30	0.10	0.00
C	0.87	0.70	0.80	0.00
D	0.00	0.00	0.00	0.70
E	0.00	0.00	0.00	0.30
CAT				
A	0.00	0.10	0.00	1.00
B	0.38	0.90	1.00	0.00
C	0.62	0.00	0.00	0.00
EST-1				
A	0.00	0.00	0.30	0.00
B	0.88	1.00	0.70	0.00
C	0.12	0.00	0.00	0.00
D	0.00	0.00	0.00	0.60
E	0.00	0.00	0.00	0.40
EST-2				
A	0.63	0.20	0.10	0.70
B	0.00	0.00	0.20	0.00
C	0.00	0.40	0.00	0.00
D	0.37	0.40	0.70	0.20
E	0.00	0.00	0.00	0.10
G6PDH				
A	0.00	0.00	0.00	1.00
B	0.87	0.90	0.90	0.00
C	0.13	0.10	0.10	0.00
HK				
A	0.75	0.67	0.20	0.00
B	0.25	0.33	0.80	0.70
C	0.00	0.00	0.00	0.30
PGI				
A	1.00	1.00	1.00	0.00
B	0.00	0.00	0.00	1.00
H_o	0.31	0.31	0.28	0.35
H_e	0.31	0.29	0.24	0.31

with inclusions in the collagen fibrils surrounding the tylostyle tracts. Three types of cells with inclusions could be distinguished by their ultrastructure.

Atlantic populations: type I cells, (Figure 5A), are very large, up to $15 \times 7\ \mu$m, and filled by more than 40 osmiophilic ovoid inclusions of $\sim 1.6 \times 1.3\ \mu$m. These inclusions are homogeneous but a more osmiophilic zone is visible at the extremities and sometimes all around the inclusions (Figure 5B). Type II cells, (Figure 5C), measure $\sim 6 \times 5\ \mu$m, and contain about 15–30 ovoid to spherical osmiophilic 0.6 μm-diameter inclusions. Type III cells, (Figure 5D), are smaller ($\sim 4.7 \times 4.6\ \mu$m) and rarer. The cytoplasm contains spherical or ovoid osmiophilic inclusions and lipid droplets of ~ 0.2–$0.5\ \mu$m.

Pacific population: type I cells, (Figure 6A), are elongated and measure up to $18 \times 5\ \mu$m. They are filled with up to 50 irregularly shaped inclusions ~ 0.9–$1.5\ \mu$m large. These inclusions are osmiophilic, homogeneous but with some clearer areas irregularly distributed. Type II cells, (Figure 6B), are more or less ovoid in shape ($6 \times 3\ \mu$m). The cytoplasm contains about 30–40 ovoid to spherical osmiophilic, homogeneous inclusions of ~ 0.5–$0.6\ \mu$m in diameter. Type III cells, (Figure 6C), are smaller up to $5 \sim 2\ \mu$m and rarer. The cytoplasm contains osmiophilic inclusions of $\sim 0.2\ \mu$m in diameter, vesicles and lipid droplets of 0.3–0.4 μm.

Allozymes

Levels of heterozygosity were high in all populations analysed (0.24–0.31; Table 1). No significant deviations from Hardy–Weinberg equilibrium were found in any population [Fisher's exact test (Sokal & Rohlf, 1969), with a Bonferroni transformation of significance levels,

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

Table 2. *Pairwise unbiased genetic identities (Nei, 1978; above diagonal) and distances (Nei, 1978; below diagonal) for the populations studied.*

	San Blas 1	San Blas 2	Galeta	Pacific
San Blas 1	–	0.95	0.87	0.13
San Blas 2	0.05	–	0.95	0.10
Galeta	0.14	0.05	–	0.13

(Lessios, 1992)]. The genetic identities found between Atlantic populations varied between 0.87 and 0.95, which are in the range of identities usually found between conspecific populations (Table 2). In contrast, the genetic identities between Atlantic populations and the one from the Pacific varied between 0.10 and 0.13, (Table 2) which is much lower than the lowest values obtained for conspecific populations of any organism (Thorpe & Solé-Cava, 1994).

Table 3. *Main characters used to distinguish between the three Caribbean* Spirastrella *spp., the Pacific Panamanian one and* Spirastrella cunctatrix *from the Mediterranean. All dimensions are given in micrometres.*

	Spirastrella spp.					
Characteristics	*S. mollis*	*S. coccinea*	*S. cunctatrix* Car.	*S. hartmani* sp. nov.	*S. cunctatrix* Med.	*S. sabogae* sp. nov.
Colour	?	orange red	reddish brown	dull salmon/brick	orange salmon	dull salmon/brick
Choanosomal spirasters	?	densely packed	scattered	scattered	densely packed	densely packed
Tylostyle size	310	518/6.1	404/6.4	414/9.8	510/6	450/8
Spirasters	24–48	25–52	7–40	2–14; 15–66	15–41	10–20; 21–57
Spiraster 2 shape	abrupt angle	twisted	twisted	twisted	twisted	twisted
Spine shapes	?	simple	bifid, plurifid	bifid, plurifid	simple	bifid
Inclusion cell I	?	?	?	ovoid, 1.6–1.3	irregular, 2.2–2.7	irregular, 0.9–1.5
Ecology	on corals	outer lagoon	inner lagoon	sea grass beds	cave, dark biotopes	corals

Car., Caribbean; Med., Mediterranean.

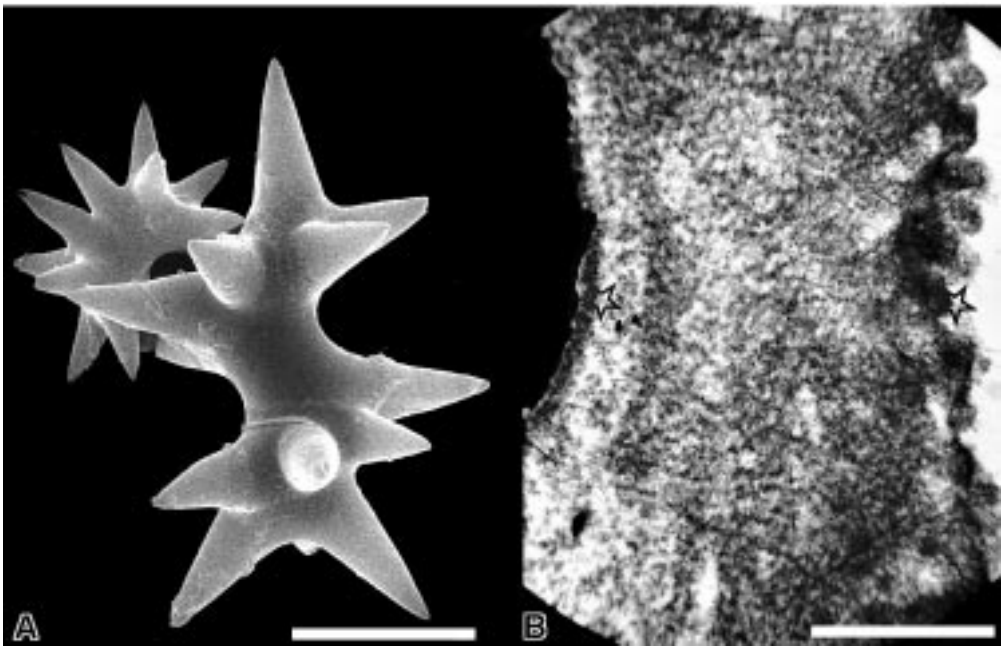

Figure 7. (A) Spirasters I and II of *Spirastrella cunctatrix* from the Mediterranean Sea; (B) polished thin section of the skeleton of *Spirastrella cunctatrix* from the Mediterranean Sea. Spirasters are densely packed in the choanosome and ectosome. They constituted layers at the surface and at the basis (stars). Scale bars: A, 10.5 μm; B, 425 μm.

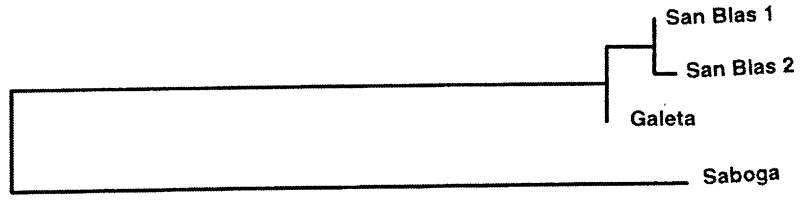

Figure 8. Neighbour joining tree based on Nei's (1978) unbiased pairwise distances between the studied populations of *Spirastrella*.

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

Table 4. *Levels of gene divergence observed between sponge species.*

Genus	Type	Occurrence	I	D	Reference
Axinella	A	sympatric	0.32	1.14	1
Clathrina	A	sympatric	0.58	0.54	2
Corticium	A	sympatric	0.45	0.80	3
Petrosia	A	sympatric	0.62	0.48	4
Plakina	A	sympatric	0.70	0.36	5
Suberites	A	sympatric	0.66	0.41	6
Tethya	A	sympatric	0.19	1.66	7
Clathrina	A	allopatric	0.21	1.56	8
Oscarella	A	allopatric	0.32	1.14	9
Phyllospongia	A	allopatric	0.47	0.75	10
Plakina	A	allopatric	0.63	0.46	5
Spirastrella	A	allopatric	0.12	2.30	This study
Carteriospongia	B	allopatric	0.62	0.48	11
Phyllospongia	B	allopatric	0.64	0.44	11
Average	A		0.44	0.97	
Average	B		0.63	0.46	

A, different species; B, same species; 1, Solé-Cava et al., 1991b; 2, Klautau et al., 1994; 3, Solé-Cava et al., 1992; 4, Bavestrello & Sarà, 1992; 5, Muricy et al., 1996; 6, Solé-Cava & Thorpe, 1986; 7, Sarà, 1990; 8, Solé-Cava et al., 1991a; 9, Boury-Esnault et al., 1992; 10, Stoddart, 1989; 11, Benzie et al., 1994.

DISCUSSION

The levels of genetic differentiation found between the Atlantic and Pacific populations of the putative species *Spirastrella* sp. cf. *mollis* were extremely high (Nei's genetic distance, D=2.30; Figure 8). This genetic differentiation, together with cytological and morphological differences, clearly demonstrates that they must belong to different biological species (Table 3).

The spicules of Atlantic and Pacific populations of *Spirastrella* sp. cf. *mollis*, are consistently different. The difference in size of the tylostyles is not significant but the shape shows consistent differences especially for the tip, which is irregular in the Atlantic populations and regular in the Pacific one; and for the head, which is respectively spherical and ovoid. Two size-classes of spirasters were found. Spiraster II, the largest class, are identical in size and shape in the Atlantic and Pacific populations whereas spiraster I have significant differences in size (Figure 3) and shape (Figure 4A–C). The spirasters are packed in the ectosome of all studied populations, but they are much more abundant in the choanosome of the Pacific population than on the Atlantic ones (Figure 2A,B).

The main cytological difference is observed comparing the type I cells: in Atlantic populations, they are spherical to ovoid, with a dark zone at the extremities or all around the inclusions (Figure 5A,B). In the Pacific population the inclusions have an irregular polygonal shape, with clear zones irregularly distributed (Figure 6A). These inclusions are larger in the Atlantic populations.

Wulff (1996) pointed out the extreme confusion in the literature for the allocation of West Indies *Spirastrella* specimens to a species. The problem is mostly linked to citations without precise descriptions of skeleton and spicules, with the noteworthy exception of Topsent (1918). Some authors (Laubenfels, 1936b; 1950; Hechtel, 1965) considered there to be only one Caribbean *Spirastrella* sp. similar to the Mediterranean *S. cunctatrix* Schmidt, 1868 and which they named *S. coccinea* (Duchassaing & Michelotti, 1864) because of the law of priority. Hechtel (1965) synonymized also *S. mollis* Verrill, with this putative species. Other authors (Wiedenmayer, 1977; Pulitzer-Finali, 1986) discriminated between two Caribbean closely related species, allocated to *S. coccinea* and *S. cunctatrix* (Table 3). Rützler (1986) considered that the Caribbean specimens allocated to *S. cunctatrix* could be better placed in *S. mollis* to distinguish between Mediterranean and Caribbean species. The description of *S. mollis* is very brief and no dimensions of spicules are given. The type specimen seems to be lost. However, the shape of the spirasters and tylostyles figured in Verrill's paper (1907) and those re-observed by Wulff (1996) on a spicule preparation of a *Spirastrella* specimen from the type locality (Bermuda) indicate that *S. mollis* seems to be a good species, different from the Caribbean '*S. cunctatrix*'.

Following the hypothesis of Wulff (1996) we consider that the Atlantic specimens reported here, as well as those allocated to *S. cunctatrix* by Topsent (1918), Wiedenmayer (1977) and Pulitzer-Finali (1986) and allocated to *S. coccinea* by Hechtel (1965), are representatives of a third species of Western Atlantic species. *Spirastrella cunctatrix* from the Mediterranean Sea differs in the aspect of the cells with inclusions (type I) (Figure 6D), by the shape of the small spirasters (Figure 7A) which have simple spines never fused and ramified and by the spirasters densely packed in the choanosome as for the Pacific population (Figure 7B, Table 3). *Spirastrella cunctatrix,* considered a cosmopolitan species and with referred specimens from the Mediterranean Sea as well as from the Caribbean Sea and Indian Ocean (Topsent, 1918) is a species complex which needs a precise revision.

On the Pacific coast there are few records of *Spirastrella* species. From the Gulf of California, Dickinson (1945) mentioned *Spirastrella coccinea* in the sense of Laubenfels (1950) and Hechtel (1965), and from southern California Bakus & Green (1987) cited *Spirastrella* sp. Obviously, the *Spirastrella* populations from both sides of the Panama Isthmus have to be assigned to two new species (Table 3).

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

DESCRIPTIONS OF THE NEW SPECIES

Spirastrella hartmani sp. nov.

Synonymy

See Wiedenmayer 1977, *Spirastrella cunctatrix*.

Material

Holotype deposited at the Museum National d'Histoire Naturelle de Paris: MNHN-NBE-D.1469; type locality: San Blas Island (09°39′N 78°45′W); paratypes at Peabody Museum, Yale University, USA: YPM 21026 and 21027.

Habitat distribution

Sea grass beds and reef flat area, 1–5 m depth, San Blas Islands and Galeta.

Description

Shape. Encrusting species on small pebbles, largest specimens $\sim 15\,cm^2$ *and* ~ 3–5 mm thick.

Colour. They are dull salmon on the surface and brick-coloured inside.

Skeleton (Figure 2A). The ectosomal skeleton is composed of two layers of spirasters. A very thin external layer is made of spirasters I and an inner one is composed mostly of spirasters II. The spirasters formed also a basal layer in contact with the substrate. Tracts of tylostyles run perpendicularly from the substrate to the surface where they are plumose. The spirasters are also scattered in the choanosome.

Megascleres. Tylostyles straight with a spherical head sometimes irregularly shaped with annular swelling below the neck. They are slightly fusiform, the largest width being somewhat distant from the neck. The sharp point is slightly stepped, often blunt or rounded. Dimensions: 223–546×5.3–$15.9\,\mu m$ (mean $414 \times 9.8 \pm 60 \times 2.4\,\mu m$); head 6.8–15.3 μm (mean $11.3 \pm 1.8\,\mu m$).

Microscleres. Spirasters I have a very short shaft bent generally only once; a second bend may be present. Two groups of spines are present at each extremity and a third one on the convex part. Spines are robust and often appear fused. The extremities are always free and often ramified (Figure 4A,B). Dimensions: 2–14 μm (mean $9 \pm 2.6\,\mu m$). Spirasters 2 show two to four bends and are covered by free robust spines starting from the convex part of the bends (Figure 4E). Dimensions: 15–66 μm (mean $30 \pm 9\,\mu m$).

Etymology

The name is given in honour to the great spongologist, Professor Willard Hartman.

Distribution

San Thomé, Jamaica, Bahamas.

Spirastrella sabogae sp. nov.

Material

Holotype: MNHN: D.NBE.1470; type locality: Saboga Island (08°37.5′N 70°04′W).

Habitat distribution

Extreme low intertidal zone on coral pinnacles in Saboga.

Description

Shape. Encrusting on corals, largest specimens $\sim 10\,cm^2$ and ~ 3–5 mm thick.

Colour. They are dull salmon on surface and brick-coloured inside.

Skeleton (Figure 2B). The ectosomal skeleton is constituted by two layers of spirasters. A very thin external layer is made of spirasters I and an inner one is constituted mostly of spirasters II. The spirasters are packed also throughout the choanosome. Tracts of tylostyles run perpendicularly from the substrate to the surface, where they are plumose.

Megascleres. Tylostyles are straight sometimes slightly flexuous, the head is ovoid or irregularly shaped. The largest width is below the head. The shaft gradually tapers to a sharp point. Dimensions: 297–578×2.6–$15.9\,\mu m$ (mean $450 \times 8 \pm 50 \times 2.6\,\mu m$). The diameter of the head is 6.8–13.6 μm (mean 9.9 ± 1.6).

Microscleres (Figure 4C). Spirasters I have a long shaft bent two–three times. The robust spines, present on the convex part, are free or fused only at the base. The extremities are sometimes ramified. Dimensions: 10–20 μm (mean $15 \pm 3\,\mu m$). The spirasters II (Figure 9) show two to four bends and are covered by free robust spines starting from the convex part of the bends. Dimensions: 21–57 μm (mean $32 \pm 7\,\mu m$).

Etymology

The name is taken from the island of Saboga (Las Perlas Archipelago), in the Gulf of Panama.

Distribution

Taboga, Gulf of Panama.

CONCLUSION

The levels of gene divergence found between *Spirastrella sabogae* sp. nov. and *S. hartmani* sp. nov. were as high as those usually found between different genera in other groups of invertebrates (Thorpe & Solé-Cava, 1994). Similarly exceptionally high values of gene divergence have been found between other congeneric sponge species (Table 4). Two possible scenarios can be proposed for this phenomenon: (a) sponges have a much higher rate of molecular (at least for allozyme loci) evolution than other metazoans; (b) sponges display a rate of morphological evolution so slow (at least for the set of morphological data generally used in sponge systematics) that the taxonomic rank of 'genus' has a completely different meaning when applied to sponges than to most other organisms (e.g. the 'Ornithology for the copepodologist' discussion in Knowlton, 1993). If the vicariant event that led to the speciation of *Spirastrella sabogae* sp. nov. and *S. hartmani* sp. nov. was indeed the rising of the Isthmus of Panama during the Pliocene (i.e. $\sim 3 \times 10^6$ y before

https://doi.org/10.1017/S0025315498000046 Published online by Cambridge University Press

present), then it would appear that the rates of molecular evolution in sponges are indeed among the highest of metazoans (i.e. scenario (a) above). This could also be related to the extreme levels of gene variation reported for sponge species (Solé-Cava & Thorpe, 1991), since rates of gene divergence seem to be positively correlated with heterozygosity (Ward & Beardmore, 1977). The barrier to gene flow related to the raising of the Isthmus of Panama may have become effective well before its complete closure, due to changes in sea circulation patterns that were already present in the Miocene (Duque-Caro, 1990). These supposed barriers seem to have had important and differentiated consequences to the restriction to gene flow and consequent speciation in many shallow-water species (Jackson et al., 1993; Knowlton et al., 1993). However, even if we take the earlier date for the appearance of biogeographic reproductive barriers (around 13 million years before present; Duque-Caro, 1990) as the moment of separation of the two species of *Spirastrella*, the resulting rate of allozyme divergence is still very high ($1\ D = 5.65 \times 10^6$ y), among the highest recorded for any animal (Nei, 1987; Thorpe & Solé-Cava, 1994). Possibly both scenarios given above are occurring in the evolution of sponge populations, and their ultimate synergistic effect for their taxonomy is the overview, by morphological systematists, of what is turning out to be a much richer biodiversity in sponges (and possibly other 'lower' marine invertebrates) than considered before. Given the limited number of morphological characters available to the study of marine sponges, such high diversity will probably only be discovered if we pay more attention to detail, within an eclectic framework of systematics methods, including of course the classical methods of morphological measurement, but also with the extensive use of cytological, genetic and ecological approaches.

The authors are thankful to Nancy Knowlton for help in obtaining a grant and for helpful discussions, Karl Kaufmann for considerable logistic support during field work of two of the authors in Panama, Cristiano Lazoski for help with electrophoresis work and to the Kuna Nation and the Panama Government for permission to collect samples in San Blas. This work was supported by grants of the Smithsonian Institution (USA) and a binational collaboration programme of 'Centro Nacional de Pesquisas', Brazil and 'Centre National de la Recherché Scientifique', France.

REFERENCES

Bakus, G.J. & Green, K.D., 1987. The distribution of marine sponges collected from the 1976–1978 Bureau of Land Management Southern California Bight Program. *Bulletin of the Southern California Academy of Science*, **86**, 57–88.

Bavestrello, G. & Sarà, M., 1992. Morphological and genetic differences in ecologically distinct populations of *Petrosia* (Porifera, Demospongiae). *Biological Journal of the Linnean Society*, **47**, 49–60.

Benzie, J.A.H., Sandusky, C. & Wilkinson, C.R., 1994. Genetic structure of dictyoceratid sponge populations on the western Coral Sea reefs. *Marine Biology*, **119**, 335–345.

Boury-Esnault, N., De Vos, L., Donadey, C. & Vacelet, J., 1984. Comparative study of the choanosome of Porifera: I. The Homoscleromorpha. *Journal of Morphology*, **180**, 3–17.

Boury-Esnault, N., Hajdu, E., Klautau, M., Custodio, M. & Borojevic, R., 1994. The value of cytological criteria in distinguishing sponges at the species level: the example of the genus *Polymastia*. *Canadian Journal of Zoology*, **72**, 795–804.

Boury-Esnault, N., Muricy, G., Gallissian, M.-F. & Vacelet, J., 1995. Sponges without skeleton: a new Mediterranean genus of Homoscleromorpha (Porifera, Demospongiae). *Ophelia*, **43**, 25–43.

Boury-Esnault, N., Pansini, M. & Uriz, M.J., 1993. Cosmopolitanism in sponges: the "complex" *Guitarra fimbriata* with description of a new species of *Guitarra* from the northeast Atlantic. In *Recent advances in ecology and systematics of sponges* (ed. M.-J. Uriz and K. Rützler), pp. 367–373. Barcelona: Instituto Ciencias del Mar, CSIC. [*Scientia Marina*, vol. 57.]

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

Dickinson, M.G., 1945. Sponges of the Gulf of California. *Allan Hancock Pacific Expeditions*, **11**, 1–251.

Doane, D.P., 1976. Aesthetic frequency classifications. *The American Statistician*, **30**, 181–183.

Duque-Caro, H., 1990. Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South-America and the evolution of the Panama seaway. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **77**, 203–234.

Glynn, P.W., Stewart, R.H. & Cosker, J.E.M., 1973. Pacific coral reefs of Panama: structure, distribution and predators. *Geologische Rundschau*, **61**, 483–519.

Hechtel, G.H., 1965. A systematic study of the Demospongiae of Port Royal, Jamaica. *Bulletin of the Peabody Museum of Natural History, Yale University*, **20**, 1–103.

Jackson, J.B.C., Jung, P., Coates, A.G. & Collins, L.S., 1993. Diversity and extinction of tropical American mollusks and emergence of the Isthmus of Panama. *Science, New York*, **260**, 1624–1626.

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

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

Knowlton, N., Weigt, L.A., Solórzano, L.A., Mills, D.K. & Bermingham, E., 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the isthmus of Panama. *Science, New York*, **260**, 1629–1632.

Laguna, J.E., 1987. *Euraphia eastropacensis* (Cirripedia, Chthamaloidea), a new species of barnacle from the tropical eastern Pacific: morphological and electrophoretic comparisons with *Euraphia rhizophorae* (de Oliveira) from the tropical western Atlantic and molecular evolutionary implications. *Pacific Science*, **41**, 132–140.

Laubenfels, M.W. de, 1936a. A comparison of the shallow water sponges near the Pacific end of the Panama Canal with those at the Caribbean end. *Proceedings of the United States National Museum*, **83**, 441–466.

Laubenfels, M.W. de, 1936b. A discussion of the sponge fauna of the Dry Tortugas in particular and the West Indies in general, with material for a revision of the families and orders of the Porifera. *Papers from the Department of Marine Biology of the Carnegie Institution. Washington, DC*, **30**, 1–225.

Laubenfels, M.W. de, 1950. The Porifera of the Bermuda Archipelago. *Transactions of the Zoological Society*, **27**, 1–201.

Lessios, H.A., 1981. Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. *Evolution*, **35**, 618–634.

Lessios, H.A., 1992. Testing electrophoretic data for agreement with Hardy–Weinberg expectations. *Marine Biology*, **112**, 517–523.

Manchenko, G.P., 1994. *Handbook of detection of enzymes on electrophoretic gels*. Boca Raton: CRC Press.

Mayr, E., 1963. *Animal species and evolution*. Cambridge: Harvard University Press.

Muricy, G., Solé-Cava, A.M., Thorpe, J.P. & Boury-Esnault, N., 1996. Genetic evidence for extensive cryptic speciation in the subtidal sponge *Plakina trilopha* (Porifera: Demospongiae: Homoscleromorpha). *Marine Ecology Progress Series*, **138**, 181–187.

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

Nei, M., 1987. *Molecular evolutionary genetics*. New York: Columbia University Press.

Pulitzer-Finali, G., 1986. A collection of West Indian Demospongiae (Porifera). In appendix, a list of the Demospongiae hitherto recorded from the West Indies. *Annali del Museo Civico di Storia Naturale di Genova*, **86**, 65–216.

Rützler, K., 1986. Phylum Porifera (sponges). In *Marine fauna and flora of Bermuda* (ed. W. Sterrer), pp. 111–128. New York: John Wiley & Sons, Inc.

Sarà, M., 1990. Divergence between the sympatric species *Tethya aurantium* and *Tethya citrina* and speciation in sponges. In *New perspectives in sponge biology* (ed. K. Rützler), pp. 338–343. Washington: Smithsonian Institution Press.

Soest, R.W.M. van & Hooper, J.N.A., 1993. Taxonomy, phylogeny and biogeography of the marine sponge genus *Rhabderemia* Topsent, 1890 (Demospongiae, Poecilosclerida). In *Recent advances in ecology and systematics of sponges* (ed. M.J. Uriz and K. Rützler), pp. 319–351. Barcelona: Instituto de Ciencias del mar, CSIC. [*Scientia Marina*, vol. 57.]

Sokal, R.R. & Rohlf, F.J., 1969. *Biometry. The principles and practices of statistics in biological research*, 2nd ed. San Francisco: W.H. Freeman & Co.

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

Solé-Cava, A.M., Klautau, M., Boury-Esnault, N., Borojevic, R. & Thorpe, J.P., 1991a. Genetic evidence for cryptic speciation in allopatric populations of two cosmopolitan species of the calcareous sponge genus *Clathrina*. *Marine Biology*, **111**, 381–386.

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

Solé-Cava, A.M. & Thorpe, J.P., 1991. High levels of genetic variation in natural populations of marine lower invertebrates. *Biological Journal of the Linnean Society*, **44**, 65–80.

Solé-Cava, A.M., Thorpe, J.P. & Manconi, R., 1991b. A new Mediterranean species of *Axinella* detected by biochemical genetic methods. In *Fossil and Recent sponges* (ed. J. Reitner and H. Keupp), pp. 313–321. Berlin: Springer-Verlag.

Stoddart, J.A., 1989. Foliose Dictyoceratida of the Australian Great Barrier Reef. III. Preliminary electrophoretic systematics. *P.S.Z.N.I: Marine Ecology*, **10**, 167–178.

Swofford, D.L. & Selander, R.B., 1981. BIOSYS-1: a FORTRAN programme for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity*, **72**, 281–283.

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

Topsent, E., 1918. Eponges de San Thomé. Essai sur les genres *Spirastrella*, *Donatia* et *Chondrilla*. *Archives de Zoologie Expérimentale et Générale*, **57**, 535–618.

Verrill, A.E., 1907. Porifera of the Bermuda Islands. *Transactions of the Academy of Arts and Science, New Haven*, **12**, 330–344.

Ward, R.D. & Beardmore, J.A., 1977. Protein variation in the plaice (*Pleuronectes platessa*). *Genetical Research*, **30**, 45–62.

Weinberg, J.R. & Starczak, V.R., 1989. Morphological divergence of eastern Pacific and Caribbean isopods: effects of a land barrier and the Panama Canal. *Marine Biology*, **103**, 143–152.

Wiedenmayer, F., 1977. *Shallow-water sponges of the western Bahamas*. Basel & Stuttgart: Birkhauser. [*Experientia*, supplement, no. 28.]

Wulff, J.L., 1994. Sponge feeding by Caribbean angelfishes, trunkfishes, and filefishes. In *Sponges in time and space; biology, chemistry, palaeontology* (ed. R.W.M. van Soest et al.), pp. 265–271. Rotterdam: A.A. Balkema.

Wulff, J.L., 1995. Sponge-feeding by the Caribbean starfish *Oreaster reticulatus*. *Marine Biology*, **123**, 313–325.

Wulff, J.L., 1996. Do the same sponge species live on both the Caribbean and eastern Pacific sides of the Isthmus of Panama. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie*, **66**, 165–173.

Submitted 22 August 1997. Accepted 9 February 1998.