GENETIC CONFIRMATION OF THE SPECIFIC STATUS OF TWO SPONGES OF THE GENUS *CINACHYRELLA* (PORIFERA: DEMOSPONGIAE: SPIROPHORIDA) IN THE SOUTHWEST ATLANTIC

CRISTIANO LAZOSKI, SOLANGE PEIXINHO, CLAUDIA A.M. RUSSO AND ANTONIO M. SOLÉ-CAVA

Lazoski, C., Peixinho, S., Russo, C.A.M. & Solé-Cava, A.M. 1999 06 30: Genetic confirmation of the specific status of two sponges of the genus *Cinachyrella* (Porifera: Demospongiae: Spirophorida) in the Southwest Atlantic. *Memoirs of the Queensland Museum* **44**: 299-305. Brisbane. ISSN 0079-8835.

In the Caribbean *Cinachyrella alloclada* and *C. apion* are readily distinguished by their different spicule types and sizes, and by the presence of buds in the former. In contrast, in the SW Atlantic both species can reproduce by budding, and also have identical chemical profiles in lectins, fatty acids and steroids. Verification of whether *C. alloclada* and *C. apion* were different biological species or were morphotypes of a single polymorphic species on the Brazilian coast was undertaken using allozyme electrophoresis. Samples collected in the intertidal zone of Pituba beach, Salvador, Brazil, and studied independently by morphological and allozyme analyses, showed a high congruence between morphology and allozymes, and 11 (of 19) loci were diagnostic of each species. *Cinachyrella apion* has smooth oxeas of only one size class, protriaenes of two sizes, anatriaenes of one size, small sigmaspires and raphides. *Cinachyrella alloclada* has smooth oxeas of two or three size classes, protriaenes and anatriaenes with one size, and sigmaspires like those of *C. apion*. The unbiased genetic identity between the two species was very low (I=0.28), as often found for congeneric sponge species. The consistent morphological and genetic differences between the two putative species confirm that, in spite of their high chemical similarity, they are distinct biological species. This indicates that, at least in these species, evolutionary rates for allozymes and secondary metabolites are clearly unrelated. ❐ *Porifera, Demospongiae, Spirophorida, Cinachyrella, morphology, allozymes, molecular systematics, Brazil.*

Cristiano Lazoski, Claudia A.M. Russo & Antonio Solé-Cava (email: sole@centroin.com.br), Departamento de Genética; Instituto de Biologia; Universidade Federal do Rio de Janeiro; Bloco A-CCS-Ilha do Fundão; 21941-490-Rio de Janeiro; Brazil; Solange Peixinho, Departamento de Zoologia; Instituto de Biologia; Universidade Federal da Bahia; Campus de Ondina; 40170-290-Salvador; Brazil; *16 March 1999.*

Cinachyrella apion (Uliczka, 1929) and *C. alloclada* (Uliczka, 1929) are common tetillid marine sponges found in the Caribbean and Brazilian coast (Mothes de Moraes, 1980; Rützler, 1987; Rützler & Smith, 1992). Because of their ubiquity and abundance, they have been the subject of many chemical and pharmacological studies (Atta et al., 1989; Barnathan et al., 1992a; Bergquist & Bedford, 1978; Kaul et al., 1977; Portugal, 1992; Rodriguez et al., 1997). Although the two species can be separated on the basis of reproductive and spicular characters in Caribbean populations (Rützler & Smith, 1992), their differences are less obvious on the Brazilian coast (Peixinho, unpublished results). For example, reproductive buds, reported by Rützler & Smith (1992) as occurring only in *C. apion*, are very common in both species in Brazil. Furthermore, samples of *Cinachyrella* from Brazil, identified on the basis of spiculation as *C. apion* or *C. alloclada*, had identical sterol (Rodriguez et al., 1997), lectin (Portugal, 1992) and fatty acid patterns (Jiménez, unpublished results), as well as proteases with the same chromatographic and electrophoretic profiles (Portugal, 1992). Therefore, it became important to verify whether *C. apion* and *C. alloclada* on the Brazilian coast comprised two species with a high chemical and reproductive similarity, or whether they represented the product of phenotypic polymorphism or plasticity of one single species.

The method of choice for the determination of specific status of sympatric populations is the genetic interpretation of allozyme patterns (Thorpe & Solé-Cava, 1994), a complementary approach to morphology that has been used with great success to identify cryptic species in sponges (Solé-Cava & Thorpe, 1986; Solé-Cava et al., 1991a, 1991b; Bavestrello & Sarà, 1992;

Boury-Esnault et al., 1992; Klautau et al., 1994; Muricy et al., 1996). In this paper we compare electrophoretically sympatric populations of *C. apion* and *C. alloclada* to verify their specific status.

MATERIALS AND METHODS

COLLECTION. Fifteen samples each of *C. alloclada* and *C. apion* were collected in June 1995 from the intertidal zone at Pituba beach, Bahia, Brazil (13°27'S, 38°26'W). After collection, the presence of buds on each individual was verified, and the specimens were transported to the laboratory, where each one was immediately divided into two parts: one part was fixed in ethanol for morphological analysis, at the Federal University of Bahia; and the other was stored at –20°C for electrophoresis, in the Federal University of Rio de Janeiro. Both parts of each sponge were given the same code, prior to their putative identification, and the genetic and morphological analyses of the samples were performed in different laboratories. The electrophoresis laboratory, therefore, did not have any information as to the species identity of the samples. This blind-analysis helped to minimise any possible bias in the interpretation of genetic patterns, which might be critical given the supposed high similarity between the two species.

To verify the consistency of the diagnostic loci for discriminating each species, a second collection from the same locality was made in July 1996, consisting of 33 samples of *C. alloclada* and 66 samples of *C. apion*. These samples were then analysed for 4 of the 11 diagnostic loci found in the first study. The results of the first and second experiments were merged for the final analysis.

ALLOZYME ANALYSES. Horizontal 12.5% starch gel electrophoresis was carried out as previously described for sponges (Solé-Cava & Thorpe, 1986). The buffer systems used were the 0.25M Tris 0.06M citrate, pH 8.0 (Ward & Beardmore, 1977) and the discontinuous 0.03M Tris 0.005M citrate, pH 8.5 (gel), 0.30M borate, pH 8.1 (buffer tank; Poulik, 1957).

Twenty enzyme systems were investigated, of which ten: acid phosphatase (*Acp*; E.C.3.1.3.2); catalase (*Cat*; E.C.1.11.1.6); esterases (*Est*; E.C.3.1.1.1); glutamate dehydrogenase (*Gdh*; E.C.1.4.1.4); hexokinase (*Hk*; E.C.2.7.1.1); leucine aminopeptidase (*Lap*; E.C.3.4.11.1); malate dehydrogenase (*Mdh*; E.C.1.1.1.37); 6-phosphogluconate dehydrogenase (*Pgd*; E.C.1.1.1.44); phosphoglucose isomerase (*Pgi*; E.C.5.3.1.9); and superoxide dismutase (*Sod*; E.C.1.15.1.1), gave reproducible results for 19 loci. The staining of the gels followed standard procedures (Manchenko, 1994).

Genotype frequency data from both species were used to estimate gene frequencies, fits to Hardy-Weinberg equilibrium, and the unbiased gene identity between them (Nei, 1978) using the BIOSYS-1 programme (Swofford & Selander, 1981).

MORPHOLOGY. The overall morphology of the sponge was analysed under a binocular microscope, and the presence of porocalices and sub-ectosomal cavities (vestibules sensu Boury-Esnault & Rützler, 1997) were visualised in histological sections of paraffin-embedded samples.

Small pieces of each sponge were boiled in nitric acid to obtain clean preparations for spicule analysis. A qualitative analysis of mounted spicule preparations was made of every individual collected. Furthermore, 30 measurements of length and width of the eight types of spicule were made, using a light microscope in one individual of each putative species. Spicular and morphological nomenclature follow Boury-Esnault & Rützler (1997).

RESULTS

ELECTROPHORESIS. Of the 19 gene loci observed, 11 (*Acp*-2, *Acp*-4, *Cat*, *Est*-2, *Est*-3, *Est*-5, *Gdh*, *Lap*, *Mdh*-2, *Pgd* and *Sod*-1; Table 1) unambiguously separated the analysed sponges into two groups, which corresponded perfectly well with the species separated by the morphological analyses. These loci were, therefore, diagnostic of each species (sensu Ayala, 1983).

Levels of heterozygosity (h) within each population were high: h=0.13 in *C. apion* and h=0.15 in *C. alloclada*, as often seen in marine sponges (Solé-Cava & Thorpe, 1989, 1991). No significant deviations of genotype frequencies from Hardy-Weinberg expectations were observed at any of the loci studied ($P>0.05$; Fisher's exact test, using a Bonferroni transformation for multiple tests; Lessios, 1992). The unbiased genetic identity (Nei, 1978) observed between the two species was 0.28.

Locus	Allele	*C. alloclada*	N	*C. apion*	N
Acp-1	1	1.00	12	1.00	15
Acp-2	1	1.00	12	0.00	15
	2	0.00		1.00	
Acp-3	1	1.00	12	1.00	15
Acp-4	1	1.00	12	0.00	15
	2	0.00		1.00	
Cat	1	0.00	13	0.29	14
	2	0.00		0.46	
	3	0.00		0.25	
	4	1.00		0.00	
Est-1	1	1.00	13	1.00	14
Est-2	1	1.00	46	0.00	80
	2	0.00		1.00	
Est-3	1	1.00	46	0.00	80
	2	0.00		1.00	
Est-4	1	0.65	13	0.00	14
	2	0.00		0.86	
	3	0.35		0.14	
Est-5	1	0.77	31	0.00	46
	2	0.23		0.00	
	3	0.00		1.00	
Gdh	1	1.00	46	0.00	80
	2	0.00		1.00	
Hk	1	0.00	12	0.42	13
	2	0.00		0.42	
	3	0.25		0.12	
	4	0.75		0.04	
Lap	1	1.00	12	0.00	15
	2	0.00		1.00	
Mdh-1	1	0.25	10	0.03	14
	2	0.50		0.04	
	3	0.15		0.89	
	4	0.10		0.00	
	5	0.00		0.04	
Mdh-2	1	0.50	11	0.00	14
	2	0.50		0.00	
	3	0.00		1.00	
Pgd	1	1.00	12	0.00	15
	2	0.00		1.00	
Pgi	1	0.00	13	0.31	13
	2	0.00		0.04	
	3	0.46		0.61	
	4	0.00		0.04	
	5	0.54		0.00	
Sod-1	1	0.00	13	0.96	14
	2	1.00		0.00	
	3	0.00		0.04	
Sod-2	1	1.00	6	1.00	6

TABLE 1. *Cinachyrella alloclada* and *C. apion*. Gene frequencies at the 19 loci studied. N = number of individuals analysed.

MORPHOLOGY. The two species had similar overall (round) shape, yellow colour, hispid surface and firm consistency, being virtually indistinguishable on the field. Both species had porocalices, as is typical of the genus, but vestibules were only observed in *C. apion*. Calcareous precipitates were observed in both species. The spicular composition of *C. apion* consists of one size class of anatriaenes and oxeas, and two size classes of protriaenes, small sigmaspires and raphides (Table 2). Conversely, the spicular composition of *C. alloclada* consists of two or three size classes of smooth oxeas, one size class of protriaenes and anatriaenes, which varied in abundance from rare to abundant, and sigmaspires (Table 3). The sizes of the sigmaspire microscleres of the two species in Brazil were clearly not different (both had about the same average: 10μm), and more like those of *C. apion* from the Caribbean (Table 2). Reproductive buds were observed on the surface of most specimens of both species.

DISCUSSION

The presence of 11 diagnostic loci and the consequent very low genetic identity, associated with the sympatry of the samples, clearly demonstrate that the Brazilian *C. apion* and *C. alloclada* are reproductively isolated, regardless of their chemical similarity. Therefore, they must be evolving independently and belong, thus, to different biological and phylogenetic species (Mayr, 1981; Cracraft, 1987). The genetic identity (I=0.28) found between these two species is, in fact, as small as that often found between species belonging to different genera in other invertebrate groups (Thorpe, 1982; Knowlton, 1993; Thorpe & Solé-Cava, 1994). Similarly, high levels of gene divergence have been found for some aster-bearing hadromerid genera and *Oscarella* (Boury-Esnault et al., 1992; Sarà et al., 1993; Barbieri et al., 1995; Boury-Esnault et al., 1999; Solé-Cava & Boury-Esnault, 1999, this volume). However, further data are necessary before overall generalisations can be made about gene divergence in sponges, and surely before decisions as to the taxonomic rank (above species level) can be directly inferred from genetic

TABLE 2. Spicule micrometry of *Cinachyrella apion*. Measurements are given in micrometers, as minimum-*mean (standard deviation)*-maximum. Triaene measurements refer only to the rhabdomes. 30 spicules were measured for each type, except in the case of anatriaenes, which were rare so only 9 spicules were measured. (A = absent).

Spicule type	Present data	Rützler & Smith, 1992
Oxeas 1 length	2217 - *3797(660)* - 5478	3500 - *4100* - 4600
diameter	21.7 - *65.9(20.9)* - 108.7	35 - *41* - 45
Oxeas 2 length	A	A
diameter	A	A
Oxeas 3 length	A	A
diameter	A	A
Protriaenes 1 length	1587 - *3907(1045)* - 5761	1800 - *3500* - 8000
diameter	8.6 - *16.9(5.3)* - 25.9	4 - *8.3* - 10
Protriaenes 2 length	588 - *1079(186)* - 1400	400 - *1350* - 1800
diameter	3.6 - *4.3(1.4)* - 7.2	1 - *2.3* - 4
Anatriaenes length	2196 - *2560(222)* - 2880	1800 - *2900* - 3500
diameter	10.8 - *12.8(1.9)* - 14.4	3 - *4.6* - 5
Sigmaspires length	3.4 - *10.0(2.6)* - 15.5	12 - *13.4* - 16
Raphides	212 - *238(14)* - 259	200 - *244* - 270

identities (Solé-Cava & Boury-Esnault, 1999, this volume).

Spicule sizes of the Brazilian specimens of *C. apion* and *C. alloclada* were similar to those described by Rützler & Smith (1992) for Caribbean populations. Conversely, samples of *C. alloclada* were very different from those described by Mothes de Moraes (1980) (see Table 3). Sponges from the SE coast of Brazil identified as *C. alloclada* by Mothes de Moraes, had much smaller protriaenes and larger anatriaenes than those found by us and by other authors (Uliczka, 1929; Wiedenmayer, 1974; Rützler & Smith, 1992). This difference might be judged large enough to warrant the creation of a new species. However, it could also be the result of phenotypic polymorphism, since spicule size can be very variable and dependent of environmental conditions (Simpson, 1978; Bavestrello et al., 1993; Schrönberg & Barthel, 1998). Further studies, possibly using genetic markers, should be done on *Cinachyrella* samples from the same region studied by Mothes-de-Moraes in SE Brazil, to verify their specific status.

The very large genetic divergence (*I*=0.28) observed between *C. alloclada* and *C. apion* contrasts markedly with their overall chemical similarity (Atta et al., 1989; Portugal, 1992; Rodriguez et al., 1997). However, this is to be expected, since the evolutionary rates of the enzymes involved in the housekeeping metabolism (like those used on allozyme analyses) are not necessarily related to those of secondary metabolites. Allozyme polymorphisms are genetically based and usually neutral, since they involve small changes in areas of the protein that do not significantly affect its function (Kimura, 1991). For this reason, these polymorphisms are expected to evolve at constant rates and unlinked to environment conditions, which is very important when dealing

TABLE 3. Spicule micrometry of *Cinachyrella alloclada*. Measurements are given in micrometers, as minimum-*mean (standard deviation)*-maximum. Triaene measurements refer only to the rhabdomes. 30 spicules were measured for each type. (A = absent).

Spicule type	Present data	Rützler & Smith, 1992	Mothes de Moraes, 1980
Oxeas 1 length	1900 - *1932(26)* - 2016	1500 - *3500* - 5900	2644 - *4923* - 6256
diameter	14.4 - *18.2(1.9)* - 21.6	20 - *50* - 65	17 - *38* - 56
Oxeas 2 length	1144 - *1217(78)* - 1440	900 - *1800* - 2800	A
diameter	10.8 - *12.8(1.8)* - 14.4	1 - *13* - 20	A
Oxeas 3 length	756 - *837(81)* - 1008	100 - 355 - 950	74 - *159* - 223
diameter	7.2 - *7.2(0)* - 7.2	2.5 - *5.4* - 8	4 - *7* - 9
Protriaenes 1 length	1296 - *2164(423)* - 3197	2400 - *4200* - 6500	407 - *437* - 462
diameter	3.6 - *4.6(1.6)* - 7.2	4 - *10.7* - 20	3 - *11* - 23
Protriaenes 2 length	A	A	A
diameter	A	A	A
Anatriaenes length	1051 - *1225(102)* - 1440	2200 - *3200* - 4000	7259 - *8289* - 9061
diameter	4.0 - *7(2.0)* - 14.0	3 - *8.3* - 14	5 - *9* - 14
Sigmaspires length	7.0 - *10.1(1.3)* - 11.2	10 - *14.3* - 23	10 - *14* - 22
Raphides	A	A	A

with problems on taxonomic status of organisms. On the other hand, studies with secondary metabolites necessarily deal with the products of enzyme function, which are much more constrained by natural selection and therefore are not expected to evolve in a clock-wise manner (Nei, 1987). Lectins and secondary metabolites are often involved in strong interspecies interactions, playing a very important role in the survival of the species (Kennedy et al., 1995; Hirabayashi & Kasai, 1998). Therefore, most of the time they are likely to be under strong normalising or directional selection, which are highly dependent on environmental conditions. This may explain, for example, why steroids in three species of *Cinachyrella* from W Africa are not overtly different except for small variations in their proportions (Barnathan et al., 1992b), and the presence of cholest-4-en-3-one steroids, both in the Brazilian *Cinachyrella* (Rodriguez, et al., 1997), and in *C. tarentina* (Aiello et al., 1991). A high intrageneric similarity has also been observed in sterols of the genus *Aplysina* (Kelecom & Kannengiesser, 1979) and in lectin composition of *Axinella* spp. (Bretting et al., 1980). Structures or molecules of adaptive importance and, hence, under strong normalising selection can also present large evolutionary shifts due to directional selection. These shifts, albeit rare, can generate homoplasy by evolutionary convergence. It is not uncommon, for example, to find species from different phyla that share a secondary metabolite which is not found in other members of the same family or order (Tursch et al., 1978). At the species level, therefore, secondary metabolites are not indicated for phylogenetic studies, since they can be evolutionarily too conserved at that level (i.e. plesiomorphic) or prone to homoplasy due to convergence. At this taxonomic level, thus, the use of allozymes or neutral DNA genes is more indicated (Hillis et al., 1996).

The two Brazilian species of *Cinachyrella* can be positively identified as the Caribbean *C. apion* and *C. alloclada*, based on morphological data. However, the use of molecular systematics for the study of sponge species has unveiled a large amount of hidden biodiversity in the phylum (reviewed in Solé-Cava & Thorpe, 1994), especially when applied to so-called cosmopolitan species and many of those with very large and/or disjunct geographical distributions. In fact, all allegedly cosmopolitan sponge species studied to date by allozyme electrophoresis have been shown to consist of clusters of morphologically similar, but evolutionarily distinct species. This means that levels of endemism in marine sponges may be much larger than assumed by taxonomists using only morphometric data (Solé-Cava et al., 1991a, 1992; Klautau et al., 1994; Klautau et al., in press). It would be very interesting, therefore, to verify whether *C. alloclada* and *C. apion* from the Bermudas, Senegalese coast (Barnathan et al. 1992a), Brazil (including the samples from Bahia studied here), and those from SE Brazil (Mothes de Moraes, 1980) are indeed conspecific.

ACKNOWLEDGMENTS

We thank Miguel Couceiro, Renata Lellis, and Paulo Vianna for help in the collection of samples and in the electrophoretic work. We are also thankful to Walter Cerqueira and Carla Stringetti for help in the morphological analyses and to Nicole Boury-Esnault for suggestions on the manuscript. This work was supported by grants from CNPq, FAPERJ, and FUJB (Brazilian Government).

LITERATURE CITED

AIELLO, A., FATTORUSSO, E., MAGNO, S., MENNA, M. & PANSINI, M. 1991. Steroids of the marine sponge *Cinachyra tarentina*: isolation of cholest-4-ene-3,6-dione and (24R)-24-ethylcholest-4-ene-3,6-dione. Journal of Natural Products (Lloydia) 54(1): 281-285.

ATTA, A.M., BARRAL NETTO, M., PEIXINHO, S. & SOUSA ATTA, M.L.B. 1989. Isolation and functional characterization of a mitogenic lectin from the marine sponge *Cinachyrella alloclada*. Brazilian Journal of Medical and Biological Research 22(3): 379-385.

AYALA, F.J. 1983. Enzymes as taxonomic characters. Pp. 3-26. In Oxford, G.S. & Rollinson, D. (eds) Protein polymorphism: adaptive and taxonomic significance. (Academic Press: London).

BARBIERI, M., BAVESTRELLO, G. & SARÀ, M. 1995. Morphological and ecological differences in two electrophoretically detected species of *Cliona* (Porifera, Demospongiae). Biological Journal of the Linnean Society 54: 193-200.

BARNATHAN, G., MIRALLES, J., GAYDOU, E.M., BOURY-ESNAULT, N. & KORNPROBST, J.M. 1992a. New phospholipid fatty-acids from the marine sponge *Cinachyrella alloclada* Uliczka. Lipids 27(10): 779-784.

BARNATHAN, G., MIRALLES, J., NJINKOUE, J.M., MANGONI, A., FATTORUSSO, E., DEBITUS, C., BOURY-ESNAULT, N. & KORNPROBST, J.M. 1992b. Sterol composition of 3 marine sponge species from the genus *Cinachyrella*. Comparative Biochemistry and Physiology B -

Biochemistry and Molecular Biology 103(4): 1043-1047.

BAVESTRELLO, G., BONITO, M. & SARÀ, M. 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 Uriz, M.-J. & Rützler, K. (eds) Recent advances in ecology and systematics of sponges. (Instituto de Ciencias del mar, C.S.I.C: Barcelona).

BAVESTRELLO, G. & SARÀ, M. 1992. Morphological and genetic differences in ecologically distinct populations of *Petrosia* (Porifera, Demospongiae). Biological Journal of the Linnean Society 47: 61-77.

BERGQUIST, P.R. & BEDFORD, J.J. 1978. The incidence of antibacterial activity in marine Demospongiae: systematic and geographic considerations. Marine Biology 46: 215-221.

BOURY-ESNAULT, N., KLAUTAU, M., BÉZAC, C., WULFF, J. & SOLÉ-CAVA, A.M. 1999. Comparative study of putative conspecific sponge populations from both sides of the Isthmus of Panama. Journal of the Marine Biological Association of the United Kingdom 79:39-50.

BOURY-ESNAULT, N. & RÜTZLER, K. (eds) 1997. Thesaurus of sponge morphology (Smithsonian Contributions to Zoology, Smithsonian Institution Press: Washington, D.C.).

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.

BRETTING, H., STANISLAWSKI, E., BECKER, W. & KANIGSMANN, K. 1980. A comparative study of snail galactans with the sponge lectins of *Axinella polypoides* revealing some structural peculiarities of the *Helix pomatia* galactan. Comparative Biochemistry and Physiology 65B: 497-503.

CRACRAFT, J. 1987. Species concepts and the ontology of evolution. Biology and Philosophy 2(3): 329-346.

HILLIS, D.M., MORITZ, S. & MABLE, B.K. 1996. Molecular Systematics, Second edition. (Sinauer Associates: Massachusetts).

HIRABAYASHI, J. & KASAI, K.-I. 1998. Evolution of animal lectins. Pp. 45-88. In Müller, W.E.G. (ed.) Molecular evolution: evidence for monophyly of Metazoa. (Springer-Verlag: Berlin).

KAUL, P.N., KULKARNI, S.K., WEINHEIMER, A.J., SCHMITZ, F.J. & KARNS, T.K.B. 1977. Pharmacologically active substances from the sea. II. Various cardiovascular activities found in the extracts of marine organisms. Lloydia 40: 235-268.

KELECOM, A. & KANNENGIESSER, G.J. 1979. Chemical constituents of *Verongia* sponges. I - A comparison between Brazilian and Mediterranean species. Anais da Academia Brasileira de Ciências 51: 633-637.

KENNEDY, J.F., PALVA, P.M.G., CORELLA, M.T.S., CAVALCANTI, M.S.M. & COELHO, L.C.B.B. 1995. Lectins, versatile proteins of recognition: a review. Carbohydrate Polymers 26: 219-230.

KIMURA, M. 1991. The neutral theory of molecular evolution: a review of recent evidence. Japanese Journal of Genetics 66(131): 367-386.

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.

KLAUTAU, M., RUSSO, C., LAZOSKI, C., BOURY-ESNAULT, N., THORPE, J.P., SOLÉ-CAVA, A.M. 1999. Does cosmopolitanism in morphologically simple species result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. Evolution (in press).

KNOWLTON, N. 1993. Sibling species in the sea. Annual Review of Ecology and Systematics 24: 189-216.

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. (CRC Press Inc.: Ann Arbor, Michigan).

MAYR, E. 1981. Biological classification: toward a synthesis of opposing methodologies. Science 214: 510-516.

MOTHES DE MORAES, B. 1980. Esponjas de São Sebastião, litoral de São Paulo, Brasil (Porifera, Demospongiae). Iheringia 56: 75-86.

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) from the Western Mediterranean. Marine Ecology-Progress Series 138(1-3): 181-187.

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

1987. Molecular evolutionary genetics. (Columbia University Press: New York).

PORTUGAL, K. 1992. Isolamento de lectinas e avaliação de proteases das esponjas marinhas *Cinachyrella apion* e *Cinachyrella alloclada* - uma análise comparativa. (Monograph of the Universidade Federal da Bahia Press: Salvador, Brazil).

POULIK, M.D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477-1479.

RODRIGUEZ, J., NUNEZ, L., PEIXINHO, S. & JIMENEZ, C. 1997. Isolation and synthesis of the first natural 6-hydroximino- 4-en-3- one- steroids

from the sponges *Cinachyrella* spp. Tetrahedron Letters 38: 1833-1836.

RÜTZLER, K. 1987. Tetillidae (Spirophorida, Porifera): a taxonomic revaluation. Pp. 187-203. In Vacelet, J. & Boury-Esnault, N. (eds) Taxonomy of Porifera from the N.E. Atlantic and Mediterranean Sea. (Springer-Verlag: London).

RÜTZLER, K. & SMITH, K.P. 1992. Guide to western Atlantic species of *Cinachyrella* (Porifera: Tetillidae). Proceedings of the Biological Society of Washington 105: 148-164.

SARÀ, M., CORRIERO, G. & BAVESTRELLO, G. 1993. *Tethya* (Porifera, Demospongiae) species coexisting in a Maldivian coral reef lagoon - taxonomical, genetic and ecological data. Marine Ecology 14(4): 341-355.

SCHRÖNBERG, C.H.L. & BARTHEL, D. 1998. Unreliability of Demosponge skeletal characters: the example of *Halichondria panicea.* Pp. 41-54. In Watanabe, Y. & Fusetani, N. (eds) Sponge Sciences. (Springer-Verlag: Tokyo).

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. Journal of Experimental Marine Biology and Ecology 35(1): 31-42.

SOLÉ-CAVA, A.M. & BOURY-ESNAULT, N. 1999. Patterns of intra and interspecific divergence in marine sponges. *Memoirs of the Queensland Museum*, this volume.

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: Homosclerомorpha) in the Mediterranean Sea. Marine Biology 113(2): 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(2): 247-253.

1989. Biochemical correlates of genetic variation in marine lower invertebrates. Biochemical Genetics 27(5-6): 303-312.

1991. High levels of genetic variation in natural populations of marine lower invertebrates. Biological Journal of the Linnean Society 44: 65-80.

1994. Evolutionary genetics of marine sponges. Pp. 55-63. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds) Sponges in Time and Space. (Balkema: Rotterdam).

SOLÉ-CAVA, A.M., THORPE, J.P. & MANCONI, R. 1991b. A new Mediterranean species of *Axinella* detected by biochemical genetic methods. Pp. 313-321. In Reitner, J. & Keupp, H. (eds) Fossil and Recent Sponges. (Springer: Berlin).

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. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annual Review of Ecology Systematic 13: 139-168.

THORPE, J.P. & SOLÉ-CAVA, A.M. 1994. The use of allozyme electrophoresis in invertebrate systematics. Zoologica Scripta 23(1): 3-18.

TURSCH, B., BRAEKMAN, J.C., DALOZE, D. & KAISIN, M. 1978. Terpenoids from coelenterates. Pp. 247-296. In Scheuer, J.P. (ed.) Marine Natural Products. (Academic Press: London).

ULICZKA, E. 1929. Die tetraxonen Schwämme Westindiens (auf Grund der Ergebnisse der Reise Kükenthal Hartmeyer). Zoologische Jahrbücher 16: 36-62.

WARD, R.D. & BEARDMORE, J.A. 1977. Protein variation in the plaice (*Pleuronectes platessa*). Genetic Research 30: 45-62.

WIEDENMAYER, F. 1974. Recent marine shallow water sponges of the West Indies and the problem of speciation. Verhandlungen der Naturforschenden Gesellschaft in Basel 84: 361-375.