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Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea

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Abstract. The sponge sub-class Homoscleromorpha is generally considered to include just two families, the Oscarellidae (without spicules) and the Plakinidae (with simple spicules). In May 1990, an unusual sponge was found deep inside a submarine cave in the western Mediterranean Sea. On the basis of externally visible characters this sponge appeared indistinguishable from the common plakinid species *Corticium candelabrum* Schmidt, 1862. However, on closer examination in the laboratory the new sponge proved to be devoid of spicules. Therefore, despite great morphological similarities to *C. candelabrum*, the new sponge should, by taxonomic convention, have been placed in the Oscarellidae. On the basis of other criteria, the similarities to *C. candelabrum* were great and the new sponge was at first considered to be conspecific. Thus, the taxonomic position of the new sponge and its relationship to *C. candelabrum* are highly confusing. It could be an aspiculate morph of *C. candelabrum*, or a new and undescribed related species or, lacking spicules, it could justifiably be placed in a different family (Oscarellidae). The relationship of the new sponge to *C. candelabrum* and also to two species of *Oscarella* (Oscarellidae) was assessed by the use of enzyme electrophoresis to estimate genetic divergence between species. It was found that the new sponge was reproductively isolated from sympatric *C. candelabrum*, with 6 of 16 loci proving diagnostic. Thus it is clear that the new sponge belongs to a different biological species. Surprisingly it was also found that, although this new species was fairly closely related to *C. candelabrum* (level of genetic identity, $I \approx 0.47$), the two *Oscarella* species were similarly closely related to *C. candelabrum* ($I \approx 0.31$ to 0.41) and rather less closely to the new species ($I \approx 0.17$ to 0.28). Indeed from genetic identity estimates, *O. tuberculata* is more closely related to *C. candelabrum* than it is to *O. lobularis*. It is concluded that all homoscleromorph sponges should be placed in the single family Plakinidae.

Introduction

Sponges are a taxonomically very difficult group, where even the higher level systematics are still far from clear (Lévi 1979). In many sponge taxa, morphological plasticity is common and hence systematic work has been based largely upon skeletal features (i.e., spicules), which are considered to be less variable. One of several particularly difficult groups is the Homoscleromorpha, now considered to be a sub-class of the class Demospongiae, but until recently placed as an order within another sub-class (Tetractinomorpha) (Lévi 1973). The Homoscleromorpha are generally divided into just two families, the Oscarellidae (without spicules) and the Plakinidae (with simple spicules). Recently one of us (J. V.) found in a Mediterranean cave examples of a sponge which from field characters appeared indistinguishable from the common plakinid species *Corticium candelabrum* Schmidt, 1862. However, on closer examination in the laboratory this new sponge proved to be devoid of spicules. Therefore, despite great morphological similarities to *C. candelabrum*, the new sponge should, by taxonomic convention, have been placed in the Oscarellidae. On the basis of other criteria, the similarities to *C. candelabrum* were so great that the new sponge was initially considered conspecific with it. Thus, the taxonomic position of the new sponge and its relationship to *C. candelabrum* are highly confusing. It could be an aspiculate cave-dwelling morph of *C. candelabrum*, or a new and undescribed related species, or, lacking spicules, it could justifiably be placed in a different family (Oscarellidae). It was considered that the most useful approach to this problem would be to assess the relationship of the new sponge to *C. candelabrum* and to species of Oscarellidae (Boury-Esnault et al. 1992) by the use of enzyme electrophoresis to estimate genetic divergence between species.

This technique is now commonly used to investigate the genetic structure of natural populations in a wide range of plant and animal species (reviewed by, e.g., Nei 1987, Ryman and Utter 1987). It is particularly powerful

for solving taxonomic problems, and in studies of marine invertebrates it has been useful in distinguishing species (e.g. Grassle and Grassle 1976, Thorpe et al. 1978, Solé-Cava et al. 1985) and for quantifying divergence between populations or species (e.g. Macleod et al. 1985, Todd et al. 1988). The major advantages of the technique are the objectivity of the analysis, the ease with which large amounts of genetic information can be acquired quickly, and the relatively unbiased results compared to those obtained from morphological studies (Avise 1974, Solé-Cava and Thorpe 1987). It is surprising, thus, that this technique has only recently started to be used in such a taxonomically confused group as the sponges (e.g. Solé-Cava and Thorpe 1986, Stoddart 1989, Solé-Cava et al. 1991 a, b). From the genetic data available it would appear that sponges can be genetically highly variable (Balakirev and Manchenko 1985, Solé-Cava and Thorpe 1990) and that conventional criteria for the identification of sponge species are not necessarily reliable (Solé-Cava and Thorpe 1986, Solé-Cava et al. 1991 a, b).

Materials and methods

Samples of *Corticium candelabrum* Schmidt, 1862 and of the aspiculate sponge which otherwise looked like *C. candelabrum* were collected by SCUBA diving in May 1990, from the Mediterranean Sea. For genetic comparison, samples of the oscarellid sponges *Oscarella lobularis* (Schmidt, 1862) and *O. tuberculata* (Schulze, 1868) were also collected. All the specimens came from La Vesse, a few kilometres west of Marseilles, or from Riou a few kilometres east of Marseilles. At each site the sponges collected were sympatric. Samples of *C. candelabrum* and *O. lobularis* were collected from both localities, but the aspiculate "*C. candelabrum*" and *O. tuberculata* were obtained from Riou only. At both localities the samples were collected under overhangs or near to the openings of caves from about 6 to 25 m. The exception was the aspiculate "*C. candelabrum*", samples of which were collected from a large population found in the darkest part of a cave about 50 m from the entrance and at a depth of about 15 m.

The sponges were taken to the laboratory in seawater in an insulated container and frozen rapidly by immersion in liquid nitrogen. They were then stored at −20 °C for 1 wk before being packed in dry ice and transported to the Port Erin Marine Laboratory (Isle of Man), where the experimental work was carried out. Pieces of each specimen were preserved in case they were required as an aid to future species identification in subsequent cytological studies.

Electrophoresis was carried out based on standard methods (reviews by Richardson et al. 1986, Murphy et al. 1990) using horizontal 12.5% starch gels. Samples were homogenised in distilled water and run on a continuous Tris-citrate buffer system, pH 8.0 (Ward and Beardmore 1977). The staining of the gels followed standard procedures (Harris and Hopkinson 1978). Further details of methods for sponges are described by Solé-Cava and Thorpe (1986). Samples from each species or population were run on the same gel, to allow the correct identification of alleles. Twenty-four enzyme systems were investigated, of these, 14 (adenylate kinase, AK – E.C. 2.7.4.3; alkaline phosphatase, AP – E.C. 3.1.3.2; catalase, CAT – E.C. 1.11.1.6; α-esterases, EST – E.C. 3.1.1.1; hexokinase, HK – E.C. 2.7.1.1; isocitrate dehydrogenase, IDH – E.C. 1.1.1.42; leucine aminopeptidase, LAP – E.C. 3.4.11.–; malate dehydrogenase, MDH – E.C. 1.1.1.37; malic enzyme, ME – E.C. 1.1.1.40; peptidases, PEP – E.C. 3.4.11.1; phosphoglucose isomerase, PGI – E.C. 5.3.1.9; 6-phosphogluconate dehydrogenase, PGD – E.C. 1.1.1.44; phosphoglucomutase, PGM – E.C. 2.7.5.1; superoxide dismutase, SOD – E.C. 1.15.1.1) could be reliably scored in all samples of *Corticium*, although some did not show good resolution for the species of *Oscarella*.

Not included in the results is a zone of nonspecific activity which was of high mobility and polymorphic in all the species used. This activity gave formazan staining with MTT even in the absence of a cofactor (NAD or NADP). This zone also appeared to display activity on peptidase and esterase gels. Such zones have been previously described in certain other sponge species (Stoddart 1989), although they seem to be absent from species of the calcareous sponge genus *Clathrina* (Solé-Cava et al. 1991 a) and in various species of the demosponge *Suberites* (Solé-Cava and Thorpe 1986). This activity may be caused by bacteria present in the sponge, but further investigation is required.

Genetic results were analysed using the programme BIOSYS-1 (Swafford and Selander 1981). To compensate for small sample sizes, Nei's unbiased genetic identity was used in the comparison on populations (Nei 1978), and Fisher's exact probabilities were used to test for fits to Hardy-Weinberg equilibrium (Swafford and Selander 1981).

Results

Samples of each of the sponges used were typed successfully for 14 enzymes coded for a total of 16 gene loci, although, for technical reasons, not all loci were studied for all sponges from each sample. Allele frequency data are summarised in Table 1. No significant (Fisher's exact test; $P > 0.05$) genetic differences were found between the two allopatric samples of "normal" (i.e., with spicules) *Corticium candelabrum* or between the two samples of *Oscarella lobularis*. Of the 16 loci typed in both *C. candelabrum* and the aspiculate "*Corticium*" only 4 (leucine aminopeptidase, *Lap*; malic enzyme, *Me*; peptidase-2, *Pep-2*; phosphoglucomutase, *Pgm*) were monomorphic and identical in all samples, although 3 others (alkaline phosphatase, *Ap*; isocitrate dehydrogenase, *Idh*; peptidase-1, *Pep-1*) showed some polymorphism, but no significant differences. A further 6 loci (catalase, *Cat*: esterase, *Est*: malate dehydrogenase, *Mdh*; peptidase-3, *Pep-3*; phosphoglucose isomerase, *Pgi*; superoxide dismutase, *Sod*) had no alleles in common between the two morphotypes of *Corticium*, whilst the remaining 3 loci (adenylate kinase, *Ak*; hexokinase, *Hk*; 6-phosphogluconate dehydrogenase, *Pgd*) were polymorphic, and showed significant ($P < 0.05$) differences between the two morphotypes. Both *C. candelabrum* and the aspiculate "*Corticium*" showed some genetic similarities to *O. tuberculata*, whilst the two *Oscarella* species also had some genes in common.

Data summarising levels of genetic variation within each population sampled are given at the bottom of Table 1. No locus showed significant (Fisher's exact test: $P > 0.05$) deviations from Hardy-Weinberg expectations for any sample. However, it should be noted that statistical tests of fit to Hardy-Weinberg equilibrium are weak unless sample sizes are large (Lewontin 1958, Fairbairn and Roff 1980, Valenzuela 1985).

Discussion

The results of the enzyme electrophoresis indicate that the aspiculate sponge which otherwise resembles *Corticium candelabrum* has numerous genetic differences from

Table 1. *Corticium candelabrum*, *Oscarella lobularis* and *O. tuberculata*. Gene frequencies for 16 enzyme loci from La Vesse and Riou populations. H_e and H_0: estimate mean expected and observed heterozygosity per locus respectively. (*n*): number of individuals analysed –: samples not analysed for this locus. Alleles are numbered in order of increasing electrophoretic mobility

Locus, allele	*Corticium*			*Oscarella*		
	candelabrum		aspiculate,	*lobularis*		*tuberculata*,
	La Vesse	Riou	Riou	La Vesse	Riou	Riou
Ak						
1	0.000	0.000	0.000	0.000	0.000	1.000
2	0.786	1.000	0.000	1.000	1.000	0.000
3	0.071	0.000	0.000	0.000	0.000	0.000
4	0.143	0.000	1.000	0.000	0.000	0.000
(*n*)	(7)	(2)	(9)	(15)	(6)	(5)
Ap						
1	0.000	0.000	0.000	0.000	0.000	1.000
2	0.000	0.000	0.000	1.000	1.000	0.000
3	0.500	1.000	1.000	0.000	0.000	0.000
4	0.500	0.000	0.000	0.000	0.000	0.000
(*n*)	(2)	(2)	(2)	(15)	(6)	(5)
Cat						
1	1.000	1.000	0.000	–	–	–
2	0.000	0.000	1.000			
(*n*)	(7)	(2)	(9)			
Est						
1	0.000	0.000	0.000	1.000	1.000	0.000
2	0.000	0.000	1.000	0.000	0.000	0.000
3	1.000	1.000	0.000	0.000	0.000	1.000
(*n*)	(7)	(2)	(9)	(15)	(6)	(5)
Hk						
1	0.000	0.000	0.000	0.000	0.000	0.917
2	0.786	1.000	0.000	0.000	0.000	0.083
3	0.000	0.000	0.000	1.000	1.000	0.000
4	0.214	0.000	1.000	0.000	0.000	0.000
(*n*)	(7)	(2)	(9)	(5)	(2)	(6)
Idh						
1	0.786	1.000	0.833	–	–	–
2	0.214	0.000	0.167	–	–	–
(*n*)	(7)	(2)	(9)			
Lap						
1	0.000	0.000	0.000	0.031	0.083	0.000
2	0.000	0.000	0.000	0.906	0.917	1.000
3	0.000	0.000	0.000	0.063	0.000	0.000
4	1.000	1.000	1.000	0.000	0.000	0.000
(*n*)	(7)	(2)	(2)	(16)	(6)	(5)
Mdh						
1	0.000	0.000	0.000	0.000	0.000	1.000
2	0.000	0.000	0.000	0.100	0.000	0.000
3	1.000	1.000	0.000	0.833	1.000	0.000
4	0.000	0.000	1.000	0.067	0.000	0.000
(*n*)	(7)	(2)	(10)	(15)	(2)	(5)
Me						
1	1.000	1.000	1.000	1.000	1.000	1.000
(*n*)	(15)	(6)	(5)	(7)	(7)	(9)
Pep-1						
1	0.000	0.000	0.000	1.000	1.000	1.000
2	0.000	0.250	0.000	0.000	0.000	0.000
3	0.929	0.750	1.000	0.000	0.000	0.000
4	0.071	0.000	0.000	0.000	0.000	0.000
(*n*)	(7)	(2)	(9)	(5)	(2)	(5)
Pep-2						
1	0.000	0.000	0.000	1.000	1.000	0.000
2	0.000	0.000	0.000	0.000	0.000	1.000
3	1.000	1.000	1.000	0.000	0.000	0.000
(*n*)	(5)	(2)	(5)	(7)	(2)	(9)
Pep-3						
1	0.000	0.000	0.000	0.000	0.250	0.000
2	0.000	0.000	0.000	1.000	0.750	0.000
3	0.000	0.000	0.056	0.000	0.000	0.900
4	0.000	0.000	0.944	0.000	0.000	0.100
5	1.000	1.000	0.000	0.000	0.000	0.000
(*n*)	(7)	(2)	(9)	(5)	(2)	(5)
Pgi						
1	0.571	1.000	0.000	0.000	0.000	1.000
2	0.429	0.000	0.000	0.700	0.500	0.000
3	0.000	0.000	1.000	0.300	0.500	0.000
(*n*)	(7)	(2)	(9)	(5)	(1)	(5)
Pgd						
1	0.714	1.000	0.389	–	–	–
2	0.286	0.000	0.611			
(*n*)	(7)	(2)	(9)			
Pgm						
1	1.000	1.000	1.000	1.000	1.000	1.000
(*n*)	(16)	(6)	(5)	(7)	(2)	(9)
Sod						
1	0.000	0.000	1.000	1.000	1.000	0.000
2	1.000	1.000	0.000	0.000	0.000	1.000
(*n*)	(7)	(2)	(9)	(15)	(6)	(5)
H_e	0.180	0.031	0.057	0.069	0.123	0.028
H_0	0.170	0.031	0.049	0.071	0.128	0.028

sympatric *C. candelabrum*. Between sympatric populations of a single species (e.g. conspecific morphs), there should be no significant difference in gene frequency at any locus since, by definition, conspecific individuals should be freely interbreeding and thus part of a single population. Therefore, among sympatric populations any significant variation in gene frequency indicates the probability of the existence of a barrier to gene flow and, consequently, that the two populations are likely to be different species. Between the aspiculate sponge and *C. candelabrum* 6 out of the 16 loci analysed were fixed for different alleles and 3 others differed significantly. The presence of 6 diagnostic loci (*sensu* Ayala 1983), provides a clear indication of lack of gene flow between populations, thus indicating that the sympatric populations are reproductively isolated. Therefore, it follows that the two are not conspecific.

Overall levels of genetic divergence between populations or species can be reduced to a single value using any of various published statistical indices of genetic similarity, identity or distance (see, e.g. Thorpe 1979, 1982, Nei 1987). The most commonly used measure is the genetic identity, *I*, of Nei (1972), which ranges from 1 (populations identical) to 0 (total dissimilarity). There are many studies available giving levels of genetic divergence observed between various populations and species (reviews by Thorpe 1982, 1984, Nei 1987) over a very wide range of phyla. Generally, conspecific populations have *I* values above ~0.9 and rarely as low as 0.8, whilst identity levels between congeneric species can be expected to

range from ~0.3 to 0.8, and species from different, but confamilial, genera usually show I values below 0.4.

For the six populations studied in the present work, values of genetic identity for all possible pairwise comparisons are given in Table 2. Also shown are values for Nei's (1972) genetic distance, D, which is the converse of his genetic identity and ranges from 0 (no difference) to infinity (no genes in common). D can be calculated from I using $D = \log_e I$. A feature of Nei's I and D is that sampling errors for estimates of genetic divergence between species are little affected by the numbers of individuals used and depend almost entirely on the number of loci analysed (Nei and Roychoudhury 1974, Thorpe 1979). For the estimation of divergence between species, even sample sizes as small as 1 individual will give fairly robust estimates of I or D (Nei 1978, Gorman and Renzi 1979, Thorpe 1982).

The genetic identity values between the samples are shown in the form of a dendrogram in Fig. 1. From this and the genetic identity values in Table 2, the genetic interrelationships of the six populations studied can be estimated. It is not surprising that a high level of similarity is found between the two allopatric populations (from La Vesse and from Riou) of *Corticium candelabrum*. The I value (0.971) falls comfortably within the range expected for conspecific populations (Thorpe 1982). The same is true for the similarly high level of similarity ($I = 0.998$) found between the populations of *Oscarella lobularis* from the same two localities. The genetic identities between these populations and *O. tuberculata* are low ($I \approx 0.32$), and towards the lower end of the range expected for congeneric species (Boury-Esnault et al. 1992).

The aspiculate "*Corticium*" has genetic identity values of 0.45 and 0.49 with the two populations of *C. candelabrum* and is thus within the range usual for congeneric species. This level of difference further confirms the specific distinctness demonstrated by the loci diagnostic between this species and the sympatric *C. candelabrum*. Thus, the aspiculate "*Corticium*" would appear to be a new species which should be congeneric with *C. candelabrum*.

However, the really surprising result is that this new species and *Corticium candelabrum* both appear to be fairly closed related to the two species of *Oscarella*. All the I values between the two samples of *C. candelabrum* and all three samples of *Oscarella* (two of *O. lobularis* and one of *O. tuberculata*) fall within the range expected for congeneric species (i.e., $I > 0.30$). The new *Corticium* sp. has genetic identities just below 0.30 with the two samples of *O. lobularis* and 0.17 with *O. tuberculata*. However, even these lower values are within the range found between species of other sponge genera. For example, within the well-studied calcareous sponge genus *Clathrina*, a number of species pairs gave estimates of $I < 0.30$ (Solé-Cava et al. 1991 a), and indeed some allegedly conspecific populations gave values as low as this. Two allopatric populations of *C. clathrus* had an I value between them of only 0.13. Identity values as low as 0.13 were also found between sympatric congeneric species of the demosponge genus *Axinella* (Solé-Cava et al. 1991 b). Similarly low values have been found between species in various other genera of several phyla (see Thorpe 1982, 1983, Nei 1987).

From Table 2 it can be seen that whilst the new *Corticium* sp. is most closely related to *C. candelabrum*, *C. candelabrum* is rather more closely related to *Oscarella tuberculata* (mean $I = 0.40$) and to *O. lobularis* (mean $I = 0.33$) than *O. lobularis* is to *O. tuberculata* (mean $I = 0.32$). Thus, both *Oscarella* species (family Oscarellidae) appear to be more closely related to *C. candelabrum* (family Plakinidae) that they are to each other. This is illustrated in an UPGMA (unweighted pair-group mean dendrogram analysis) (Fig. 2) which shows that if the data for the new *Corticium* sp. are excluded, both populations of *C. candelabrum* form a cluster with *O. tuberculata*, which subsequently clusters with *O. lobularis* (thus indicating that the genus *Oscarella* may be polyphyletic). It should be borne in mind that the sampling errors of I are considerable (Nei and Roychoudhury 1974, Thorpe 1982, Nei 1987) and that, therefore, many of the I values between the four species used here may not be significantly different, but even so our data make it difficult to support any major separation of the four species. It is clear that *O. lobularis* and *O. tuberculata* should not be placed in a different family from *C. candelabrum* and the new *Corti-*

Table 2. *Corticium candelabrum*, *Corticium* sp., *Oscarella lobularis* and *O. tuberculata*. Matrix of unbiased genetic identities, I (above diagonal, Nei 1978), and distances, D (below diagonal, Nei 1978) for populations from La Vesse and Riou. *Corticium* sp. is an aspiculate sponge otherwise resembling *C. candelabrum*

Population	1	2	3	4	5	6
O. lobularis Vesse (1)		0.998	0.317	0.342	0.312	0.270
O. lobularis Riou (2)	0.002		0.320	0.352	0.329	0.283
O. tuberculata Riou (3)	1.150	1.138		0.395	0.405	0.168
C. candelabrum Vesse (4)	1.074	1.044	0.929		0.971	0.493
C. candelabrum Riou (5)	1.164	1.112	0.905	0.030		0.456
Corticium sp. Riou (6)	1.309	1.261	1.783	0.708	0.786	

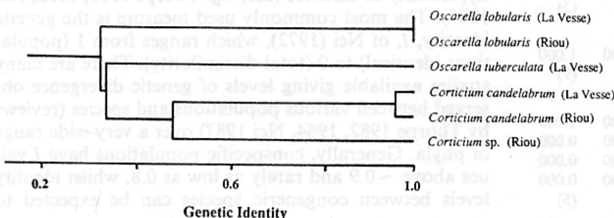

Fig. 1. *Corticium candelabrum*, *Corticium* sp., *Oscarella lobularis* and *O. tuberculata*. Dendrogram of genetic identities of populations of four homoscleromorph sponge species from La Vesse and Riou (both near Marseilles). The dendrogram is constructed using UPGMA method (Swafford and Selander 1981)

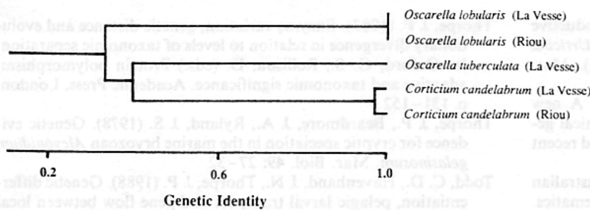

Fig. 2. *Corticium candelabrum, Oscarella lobularis* and *O. tuberculata*. Dendrogram (UPGMA) of genetic identities of populations of three sponge species from La Vesse and Riou. Note that in absence of aspiculate *Corticium* sp., *O. tuberculata* clusters with the two populations of *C. candelabrum* and not with *O. lobularis*

cium sp. We propose that, at least for the present, this problem should be solved by placing the oscarellid and plakinid sponges into a single family. Since the two family names (Oscarellidae Lendenfeld, 1887 and Plakinidae Schulze, 1880) then become synonyms, we suggest that priority should be given to the older name and thus the sole family of homoscleromorph sponges is the Plakinidae. Our genetic data indicate that all four species would best be placed within a single genus, but we wish to leave such decisions pending a morphological and taxonomic revision of the genera of homoscleromorph sponges (Boury-Esnault and Vacelet in preparation). This future work will also provide a detailed description and name for the new *Corticium* sp. It should also be borne in mind that there are other genera (e.g. *Plakina, Plakortis*) within the Plakinidae for which data on genetic divergence may also be relevant.

The genetic results clearly also throw doubt upon the presence or absence of spicules as a useful taxonomic character, at least within this group. The aspiculate *Oscarella tuberculata*, for example, appears more closely related to *C. candelabrum* (which has spicules) than it is to either of the other two aspiculate species, *Corticium* sp. or *O. lobularis*. Unfortunately, this is not the first biochemical genetic study to lead to a questioning of the utility of spicules in sponge taxonomy and systematics (see Solé-Cava and Thorpe 1986, Solé-Cava et al. 1991 a, b) and it is becoming apparent that, as previously pointed out by Hartman (1958) and Bergquist (1979) amongst others, there is a considerable need to find and appraise other characters for taxonomic use with sponges.

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