Short communication

# Ecological speciation in a Caribbean marine sponge

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## 1. Introduction

Even though a reduction in the speciation rate could be expected in marine ecosystems because of the lack of geographic barriers due to larval long-range dispersal of many organisms (Palumbi, 1994), coral reefs environments host some of the most diverse species assemblages in the planet. Only a few examples, most of them in fishes, show how selection by habitat in coral reefs might be an important source of diversification (Carlon and Budd, 2002; Duda and Rolán, 2005; Rocha et al., 2005; Streelman et al., 2002). Darwin (1859) was the first to propose that natural selection can lead to speciation through the ecological interactions of organisms with their environment. Lately, there has been a renaissance of interest in ecological speciation and the manner in which natural selection plays a role in the development of reproductive isolation (Rundle and Nosil, 2005; Schluter, 2001; Via, 2002). Only a limited number of examples involving different taxa have been described (Rundle and Nosil, 2005), most of them from terrestrial habitats. These studies suggest that natural selection caused by shifts in ecological parameters or invasions of new habitats can cause extremely rapid divergence (Losos et al., 1997; Reznick et al., 1997; Streelman et al., 2002) and might play an important role in speciation (McKinnon et al., 2004; Schluter, 2001).

Mangals are distributed worldwide on sheltered, tropical coastlines and form islands in the backwaters of most coral reefs. They occur at the boundary between terrestrial and marine environments and may serve as models to study stressful and marginal environments (Rützler and Feller, 1996). In spite of their geographical proximity, mangrove and reef habitats display large differences in ecological,

physical, and chemical factors, and thus pose great potential for studying ecological speciation processes.

Sponges are invertebrates where morphological simplicity and plasticity have led to poor taxonomy, most prominently at the species level (Knowlton, 2000). They are widely distributed in aquatic systems and successfully inhabit hard- and soft-bottom communities, from tropical to polar latitudes, littoral to abyssal habitats, and fresh- to saltwater, being one of the major groups in both biomass and number of species in hard-bottom communities (Sarà and Vacelet, 1973). Genetic studies based on protein electrophoresis have shown prevalence of cryptic species (Solé-Cava and Boury-Esnault, 1999), typically associated with diagnostic but subtle morphological differences, and—in traditional approaches to sponge taxonomy—generally interpreted as intraspecific plasticity.

*Chondrilla* cf. *nucula* Schmidt (Chondrillidae, Chondrosida) is a common Caribbean sponge that grows in both reefs and mangal swamp habitats. It has been suggested that it is an undescribed species because the type material of *C. nucula* is from the Adriatic Sea and the often-cited "cosmopolitan distribution" is now considered unlikely (Boury-Esnault, 2002; Klautau et al., 1999). On coral reefs, *C.* cf. *nucula* encrusts rocks or hard corals in thin (<5 mm) sheets of light golden green coloration and it is known to be an aggressive space competitor (Vicente, 1990; Wiedenmayer, 1977). In mangal swamps it grows among seagrass and envelopes mangrove roots in a thick (>5 cm) lobate mass of gray-green to chestnut brown coloration (Alcolado, 1994; Rützler et al., 2000). Despite these morphological differences in growth form and coloration (see Fig. 1), the spicules and internal structure (Rützler, 1986) of both morphotypes are very similar, and hence they have been considered different growth forms of the same species (Wiedenmayer, 1977). Experimental transplants between habitats (Swearingen and Pawlik, 1998) suggest that the two morphotypes are the result of environmental adaptation. The unusual habitat spectrum for

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Fig. 1. *Chondrilla nucula*, reef morphotype on the left and mangrove morphotype on the right.

*C.* cf. *nucula* in the Caribbean—from reef to lagoon to mangal—provides an exceptional scenario for the investigation of levels of reproductive isolation between sponge populations from different habitats. It is well known that ecological speciation requires a mechanism of divergent selection to generate reproductive isolation, such as environmental differences, sexual selection, and ecological interactions (Rundle and Nosil, 2005). Environmental differences and disparity of ecological interactions can be demonstrated for mangal and reef habitats, indicating a great potential for ecological speciation between both habitats, especially for sessile species whose habitat choice is irreversible once their larvae have settled and metamorphosed. A possible reduction in gene flow between mangal and reef populations of *C.* cf. *nucula* has been detected by sequencing a mitochondrial DNA (mtDNA) fragment of the cytochrome *c* oxidase subunit I (COI) gene and by comparing both the genetic composition and the morphological divergence between and within the two habitat types from three locations in the Caribbean.

## 2. Materials and methods

### 2.1. Sampling and data collection

We collected a total of 111 sponge specimens from three geographically distant localities in the Caribbean (Florida Keys, Belize, and Panama) separated by ca. 1000–1700 km.

For each locality, populations from reefs and mangal habitats (separated by 4–18 km) were sampled (Table 1 and Fig. 2), and stored in absolute ethanol until processed.

Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) following the instructions of the supplier. A fragment of the mtDNA COI gene was amplified using the universal primers LCO1490 and HCO2198, described in Folmer et al. (1994). Amplifications were carried out in a 20-μL volume reaction, following procedures described in Duran et al. (2004). The PCR-amplified samples were purified with the QIAquick PCR Purification Kit (Qiagen). The sequencing reaction was carried out in a 10 μL volume reaction: 2 μL of Terminator Ready Reaction Mix (ABI PRISM BigDye version 3.0 Terminator Cycle Sequencing Ready Reaction Kit), 2 μL of 5× sequencing buffer (supplied with BigDye), 10–30 ng/mL of PCR product, 5 pmol of primer and distilled water (10 μL). The cycle-sequencing program consisted of an initial step at 94 °C for 3 min, 25 sequencing cycles (94 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min) and a rapid thermal ramp to 4 °C and hold. The BigDye-labeled PCR products were cleaned with AGTC Gel Filtration Cartridges (Edge BioSystems). Chromatograms obtained from the automated sequencer were read and contigs assembled using the sequence editing software SEQUENCER version 4.0 (Gene Codes Corporation). Sequences were edited and aligned with BIOEDIT Sequence Alignment Editor (Hall, 1999). No indels were

Table 1
Diversity measures for the populations of *Chondrilla* cf. *nucula*

| Population | Code | GPS | $N$ | Nh | $h$ | $\pi$ |
|---|---|---|---|---|---|---|
| Florida Mangrove | FL MG | 24°49.411N 80°48.743W | 16 | 2 | 0.125 (0.106) | 0.0004 (0.0005) |
| Belize Mangrove 1 | BZ MG1 | 16°40.047N 88°11.530W | 17 | 2 | 0.395 (0.158) | 0.0021 (0.0016) |
| Belize Mangrove 2 | BZ MG2 | 16°50.092N 88°06.134W | 14 | 4 | 0.529 (0.045) | 0.0009 (0.0008) |
| Panama Mangrove | PN MG | 9°15.916N 82°9.516W | 4 | 2 | 0.500 (0.265) | 0.0025 (0.0022) |
| Florida Reef | FL RF | 24°43.395N 80°51.683W | 15 | 5 | 0.676 (0.105) | 0.0063 (0.0038) |
| Belize Reef 1 | BZ RF1 | 16°48.327N 88°05.216W | 15 | 2 | 0.704 (0.088) | 0.0055 (0.0034) |
| Belize Reef 2 | BZ RF2 | 16°46.800N 88°06.915W | 15 | 5 | 0.247 (0.131) | 0.0025 (0.0018) |
| Panama Reef | PN RF | 9°20.015N 82°13.127W | 15 | 1 | 0.000 (0.000) | 0.0000 (0.0000) |

Global positioning system coordinates (GPS), sample size ($N$), number of haplotypes (Nh), haplotype diversity ($h$), and nucleotide diversity ($\pi$). Standard deviations are given in parentheses.
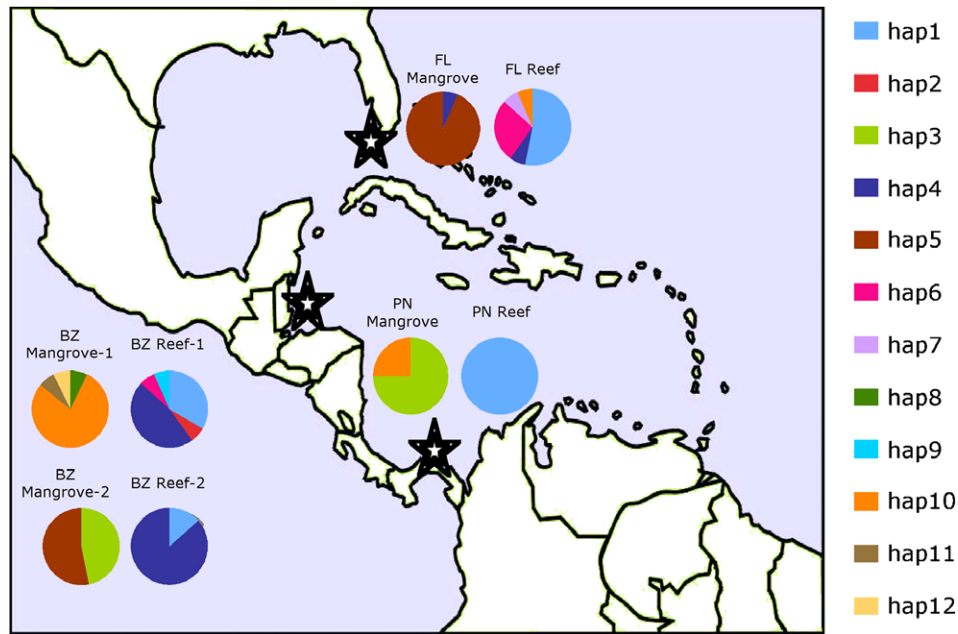
Fig. 2. Haplotype geographic distribution. Map of the Caribbean area showing the populations sampled with the geographic distribution of the haplotypes and haplotype frequencies per populations (FL, Florida; PN, Panama, BZ, Belize).

observed. All the sequences have been submitted to Gen-Bank under the Accession Nos. DQ086802–DQ086813.

### 2.2. Genetic variability and population genetic parameters

Haplotype frequencies and their distribution among samples as well as haplotype and nucleotide diversity values (Nei, 1987) were calculated using ARLEQUIN version 2.001 (Schneider et al., 2000). An analysis of molecular variance (AMOVA) was performed to examine hierarchical population structure pooling the populations in reef and mangle groups. We executed 16,000 permutations to guarantee having less than 1% difference with the exact probability in 99% of cases (Guo and Thompson, 1992). The same program was used to calculate the pairwise genetic distances ($Fst$) and their significance by performing 10,000 permutations among the individuals between populations. An exact test of population differentiation based on haplotype frequencies (Raymond and Rousset, 1995) was performed to test the null hypothesis that observed haplotype distribution is random with respect to sampling location. The significance of individual tests was esti-

mated by comparison with simulated distributions constructed from 10,000 random permutations of the original data matrix.

Relationships among haplotypes were analyzed in a parsimony network estimated with TCS version 1.18 (Clement et al., 2000) using the statistical parsimony procedure. This method estimates the unrooted tree and provides a 95% plausible set for all sequence type linkages within the unrooted tree.

### 3. Results and discussion

mtDNA sequences (584 nucleotides) from the cytochrome $c$ oxidase subunit I gene show a mosaic distribution of haplotypes (Fig. 2, Supplementary Table 1), where out of a total of 12 haplotypes, 5 are unique to mangal habitats while other 5 are only found in reef habitats. Only two haplotypes were shared between the mangal and reef habitats, by one individual each, suggesting high levels of reproductive isolation between mangal and reef populations. Nevertheless, all the individuals collected from mangal swamps (from mangrove roots to seagrass beds) shared the thicker morphology and darker coloration, while all the individuals

Table 2
Analysis of molecular variance

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | Fixation indices |
|---|---|---|---|---|---|
| Among groups | 1 | 9.426 | 0.11831 Va | 23.99[a] | FCT:0.23990 |
| Among populations within groups | 6 | 15.905 | 0.18170 Vb | 36.84[a] | FST:0.60834 |
| Within populations | 103 | 19.894 | 0.19315 | 39.17[a] | FSC:0.48473 |
| Total | 110 | 45.225 | 0.49315 | | |

Groups correspond to mangal and reef habitats. Va, Vb, and Vc are the associate covariance components. FCT, FST, and FSC are the *F*-statistics.
[a] Significant values at $P < 0.05$ after 16,000 permutations.

collected in the coral reef habitat shared the thinner and lighter coloration.

AMOVA based on haplotype frequencies (Table 2) statistically supports high genetic isolation between the two habitats, and strong genetic differentiation among populations within habitats. All pairwise *Fst* values were higher than 0.1 and only two distances (from comparisons within the same kind of habitats) were non-significant, after the permutation process (Table 3). Similarly, exact tests on population differentiation showed significant differences ($P < 0.05$) for every pair of populations compared except for Belize Mangal1 vs. Panama Mangal and Belize Reef2 vs. Belize Reef1 (data not shown). The significant isolation between reef habitats spread across the Caribbean, as well as between mangal habitats spread across the same region can be explained by the short mobile life span of sponge larvae (Maldonado and Uriz, 1999) as in general, sponge

dispersal by means of lecitotrophic larvae is very limited. However, populations from the same habitat (mangal or reef) in different locations separated by more than 1000 km have a similar haplotype composition, while populations from the same location but from different habitats show very distinct haplotype composition. These results suggest that strong ecological barriers exist between reefs and mangal swamps for the sponge *C.* cf. *nucula* which has adapted in distinct ways to those environmental differences. The malleability of body shape raises the possibility that multiple mechanisms underlie reproductive isolation between populations differing in growth form. Nevertheless, the maintenance of the two phenotypes in geographically distant but otherwise identical habitat types suggests that certain morphological traits, like growth form, coloration, or osculum size, are principally adaptations to environmental conditions. Examples of such determinants are hydrody-

Table 3
Pairwise *Fst* values between populations of *Chondrilla* cf. *nucula*

|  | FL MG | BZ MG2 | BZ MG1 | PN MG | FL RF | BZ RF2 | BZ RF1 | PN RF |
|---|---|---|---|---|---|---|---|---|
| FL MG | 0 | | | | | | | |
| BZ MG2 | 0.3449[a] | 0 | | | | | | |
| BZ MG1 | 0.7472[a] | 0.5334[a] | 0 | | | | | |
| PN MG | 0.7944[a] | 0.1978 | 0.4683[a] | 0 | | | | |
| FL RF | 0.6039[a] | 0.3995[a] | 0.4317[a] | 0.3695[a] | 0 | | | |
| BZ RF2 | 0.8048[a] | 0.6053[a] | 0.6804[a] | 0.6901[a] | 0.4697[a] | 0 | | |
| BZ RF1 | 0.5791[a] | 0.3856[a] | 0.4468[a] | 0.3610[a] | 0.1071 | 0.1359[a] | 0 | |
| PN RF | 0.9354[a] | 0.7219[a] | 0.8083[a] | 0.9005[a] | 0.2755[a] | 0.8571[a] | 0.4714[a] | 0 |

Populations coded as in Table 1.
[a] Significant values at $P = 0.05$ after a 10,000 permutation of haplotypes between localities.
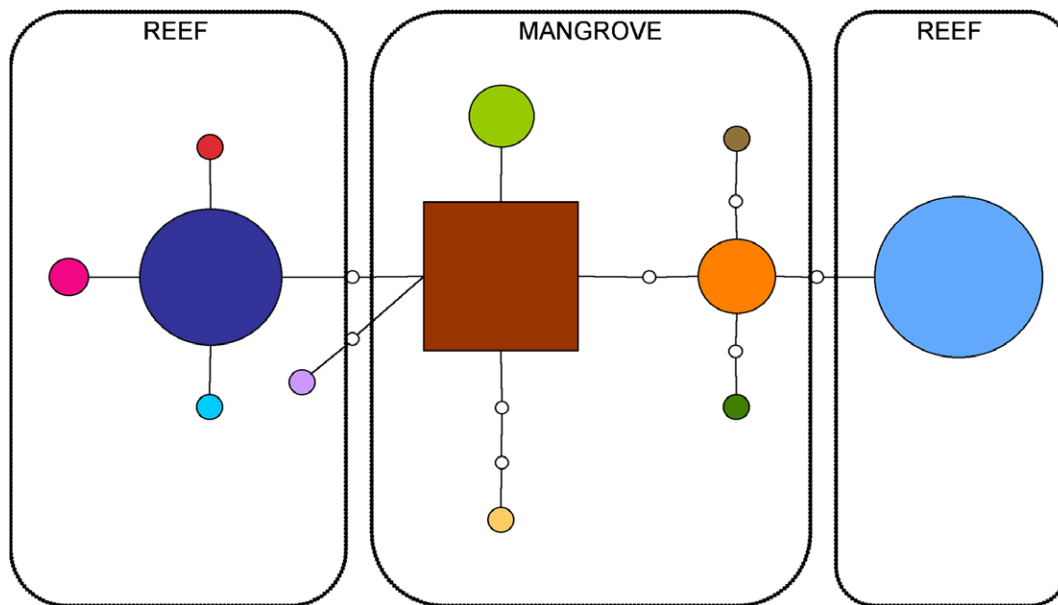


Fig. 3. Statistical parsimony network. Each haplotype is defined by its corresponding color (see Fig. 2). The area of the circles is proportional to the frequency of the haplotypes; the square denotes the inferred ancestral haplotype. Small empty circles indicate intermediate haplotypes that are not present in the samples but are necessary to link the observed haplotypes in the network. Each line represents one mutational step. Haplotypes have been grouped regarding their original habitat.

namics, sedimentation, suspended and dissolved organic substances, and ecological interactions, for instance, pressures from interspecific competition or predation.

Following coalescent theory predictions (Crandall, 1996), the haplotype network (Fig. 3) suggests that mangal populations are ancestral and that at least two main colonization events of reef habitats may have occurred independently. Invasion of the new habitat and adaptation to the different microclimate of the reef environment might have led to divergent selection and reproductive isolation between the two morphotypes, thus causing the changes in color and shape. Whether the invasion of open-water reefs from mangal and other lagoon settings have occurred in a parallel way at different locations, or if the event happened once and then populations expanded geographically remains unknown. Because of the high haplotype relatedness of the two *C.* cf. *nucula* ecotypes and due to the fact that at least two haplotypes are shared between habitats, (in Florida, one mangal individual has a haplotype typically found in reefs (hap4), and one reef individual has a haplotype typically found in mangal (hap10), see Fig. 2) we assume that a slight degree of gene flow still exists between the two forms, reducing the possibility of the two ecotypes being sister taxa (Mediterranean and Atlantic forms). Pending on proper taxonomic description, we suggest designating the two morphotypes as formae of the same species (*mangle* and *hermatypica*), subspecific entities commonly used in sponge systematics (Wiedenmayer, 1977). A detailed understanding of the processes leading to reproductive isolation between *C.* cf. *nucula* populations from mangal and reef habitats still eludes us. Long-term transplant experiments correlated with microanatomical studies would contribute valuable information. Cross-fertilization studies should be attempted although our understanding of reproductive processes in Chondrillidae is still poor, except for the fact that they are gonochoristic (Usher et al., 2004).

Understanding the role of ecology in speciation will require greater integration of ecological, evolutional, and behavioral aspects, and a better understanding of the selective pressures operating in natural populations. Until now, there has been little evidence that ecological selection has limited interbreeding between populations of coral reef organisms. Our study typifies a recent shift in focus of evolutionary studies away from speciation models based solely on allopatry, presenting one of the first examples of ecologically driven gene flow reduction across a habitat boundary in coral reef habitats.

## Acknowledgments

## Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ympev.2006.02.018.

## References

Alcolado, P., 1994. General trends in coral reef sponge communities in Cuba. In: van Soest, R.W.M., van Kempen, T., Braekman, J. (Eds.), Sponges in Time and Space. AA Balkema, Rotterdam, pp. 251–255.

Boury-Esnault, N., 2002. Order Chondrosida Boury-Esnault & Lopès, 1985. Family Chondrillidae Gray, 1872. In: Hooper, J.N.A., van Soest, R.W.M. (Eds.), *Systema Porifera*, vol. 1. Kluwer Academic/Plenum, New York, pp. 291–297.

Carlon, D.B., Budd, A.F., 2002. Incipient speciation across a depth gradient in a scleractinian coral? Evolution 56, 2227–2242.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.

Crandall, K.A., 1996. Multiple interspecies transmissions of human and simian T-cell leukemia/lymphoma virus type I sequences. Mol. Biol. Evol. 13, 115–131.

Darwin, C., 1859. On the Origin of Species by Means of Natural Selection. J. Murray, London.

Duda, T.F., Rolán, E., 2005. Explosive radiation of Cape Verde *Conus*, a marine species flock. Mol. Ecol. 14, 267–272.

Duran, S., Pascual, M., Turon, X., 2004. Low levels of genetic variation in mtDNA sequences over the biogeographic range of the sponge *Crambe crambe*. Mar. Biol. 144, 31–35.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48, 361–372.

Hall, T.A., 1999. Bioedit: a user-friendly biological sequence alignment, editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Klautau, M., Russo, C.A.M., Lazoski, C., Boury-Esnault, N., Thorpe, J.P., Solé-Cava, A.M., 1999. Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. Evolution 53, 1414–1422.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420, 73–90.

Losos, J., Warheit, K., Schoener, T., 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387, 70–73.

Maldonado, M., Uriz, M.J., 1999. Sexual propagation by sponge fragments. Nature 398, 476.

McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L., Chou, J., Schluter, D., 2004. Evidence for ecology's role in speciation. Nature 429, 294–298.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Palumbi, S.R., 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst. 25, 547–572.

Raymond, M., Rousset, F., 1995. An exact test for population differentiation. Evolution 49, 1280–1283.

Reznick, D.N., Shaw, F.H., Rodd, F.H., Shaw, R.G., 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275, 1934–1937.

Rocha, L.A., Robertson, D.R., Roman, J., Bowen, B.W., 2005. Ecological speciation in tropical reef fishes. Proc. R. Soc. Lond. B. Biol. Sci. 272, 573–579.

Rundle, H., Nosil, P., 2005. Ecological speciation. Ecol. Lett. 8, 336–352.

Rützler, K., 1986. Phylum Porifera. In: Sterrer, W. (Ed.), Marine Fauna and Flora of Bermuda. John Wiley & Sons, New York, pp. 110–127.

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Rützler, K., Diaz, M.C., van Soest, R.W.M., Zea, S., Smith, K.P., Alvarez, B., Wulff, J., 2000. Diversity of sponge fauna in mangrove ponds, Pelican Cays, Belize. Atoll Res. Bull. 476, 229–248.

Rützler, K., Feller, I.C., 1996. Caribbean mangrove swamps. Sci. Am. 274, 94–99.

Sarà, M., Vacelet, J., 1973. Ecologie des Démosponges. In: Grassé, P.P. (Ed.), Spongiaires. Masson, Paris, pp. 462–576.

Schluter, D., 2001. Ecology and the origin of species. Trends Ecol. Evol. 16, 372–380.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin ver. 2000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Solé-Cava, A.M., Boury-Esnault, N., 1999. Patterns of intra and interspecific genetic divergence in marine sponges. Mem. Qld. Mus. 44, 591–601.

Streelman, J.T., Alfaro, M., Westneat, M.W., Bellwood, D.R., Karl, S.A., 2002. Evolutionary history of the parrotfishes: biogeography, ecomorphology, and comparative diversity. Evolution 56, 961–971.

Swearingen III, D., Pawlik, J., 1998. Variability in the chemical defense of the sponge *Chondrilla nucula* against predatory fishes. Mar. Biol. 131, 619–627.

Usher, K.M., Sutton, D.C., Toze, S., Kuo, J., Fromont, J., 2004. Sexual reproduction in *Chondrilla australiensis* (Porifera: Demospongiae). Mar. Freshwater Res. 55, 123134.

Via, S., 2002. The ecological genetics of speciation. Am. Nat. 159, S1–S7.

Vicente, V.P., 1990. Overgrowth activity by the encrusting sponge *Chondrilla nucula* on a coral reef in Puerto Rico. In: Rützler, K. (Ed.), New Perspectives in Sponge Biology. Smithsonian Institution Press, Washington, DC, pp. 436–442.

Wiedenmayer, F., 1977. Shallow-water Sponges of the Western Bahamas. Birkhäuser Verlag, Basel, Stuttgart.