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SPONGE RESEARCH DEVELOPMENTS

Phenotypic variability in the Caribbean Orange Icing sponge *Mycale laevis* (Demospongiae: Poecilosclerida)

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Abstract Sponge species may present several morphotypes, but sponges that are morphologically similar can be separate species. We investigated morphological variation in *Mycale laevis*, a common Caribbean reef sponge. Four morphotypes of *M. laevis* have been observed (1) orange, semi-cryptic, (2) orange, massive, (3) white, semi-cryptic, and (4) white, massive. Samples of *M. laevis* were collected from Key Largo, Florida, the Bahamas Islands, and Bocas del Toro, Panama. Fragments of the 18S and 28S rRNA ribosomal genes were sequenced and subjected to phylogentic analyses together with sequences obtained for 11 other *Mycale* species and additional sequences retrieved from GenBank. Phylogenetic analyses confirmed that the genus *Mycale* is monophyletic within the Order Poecilosclerida, although the subgenus *Aegogropila* is polyphyletic and the subgenus *Mycale* is paraphyletic. All 4 morphotypes formed a monophyletic group within *Mycale*, and no genetic differences were observed among them. Spicule lengths did not differ among the 4 morphotypes, but the dominant megasclere in samples collected from Florida and the Bahamas was the strongyle, while those from Panama had subtylostyles. Our data suggest that the 4 morphotypes constitute a single species, but further studies would be necessary to determine whether skeletal variability is due to phentotypic or genotypic plasticity.

Keywords Porifera · 18S rRNA · 28S rRNA · Ribosomal DNA · Caribbean · Morphotype · Genotype

Introduction

Ecological studies conducted in areas of high biodiversity may sacrifice precision in lower-level taxon sampling in order to gain a broader understanding of large-scale ecosystem processes. In community surveys, marine invertebrates are usually not completely identified to the species-level due to difficulties in field identification or lack of resolution in available taxonomic keys (e.g., Chou et al., 2004; Micheli et al., 2005; Chapman et al., 2010). Sponges (Phylum Porifera) are a good example of a taxon sampled at low resolution in many community surveys. In the widely used benthic survey methodologies employed

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by the Global Coral Reef Monitoring Network (GCRMN, http://www.gcrmn.org) and Reef Check (http://www.reefcheck.org), coral reef sponges are classified into a single group (Wilkinson, 2008). However, this single phylum is comprised of ~7000 species (Hooper & Van Soest, 2002). Furthermore, sponges are important members of the aquatic ecosystems they inhabit, both in terms of abundance and ecosystem function, they compete for space with other benthic organisms, they are dominant suspension feeders, and they provide habitat for a large and diverse number of other invertebrates (Corredor et al., 1988; Pile et al., 1996; Diaz & Rutzler, 2001; Henkel & Pawlik, 2005; Southwell et al., 2008). Given their ecological importance, why are sponges seldom identified to the level of species in the field?

The process of identifying sponge species can be difficult. Sponge identification is based on morphological attributes such as color, size and shape, the presence, and arrangement of the spiculate and fibrous skeleton, and most particularly, spicule type and size. This presents a challenge as sponges are morphologically plastic, often have different color morphs, and can change in shape and size due to environmental conditions (Palumbi, 1986) or biotic factors such as predation (Loh & Pawlik, 2009). Spicule morphology, by far the most important character state, can also vary among different habitats and growing conditions (Hooper, 1985; McDonald et al., 2002). Furthermore, sponges can incorporate abiotic materials from the environment or spicules from other sponges into their skeletons (Sollas, 1908; Teragawa, 1986; Hooper & Van Soest, 2002). Conversely, sponges that look almost identical in the field may be separate species (Klautau et al., 1999; Miller et al., 2001; Duran & Rützler, 2006; Wulff, 2006a; Blanquer & Uriz, 2007; Blanquer et al., 2008).

The Orange Icing Sponge *Mycale laevis* (Carter 1882, Order Poecilosclerida, subgenus *Mycale*) ranks as one of the 10 most common sponges on Caribbean coral reefs (Pawlik et al., 1995), and has been reported as a common associate of scleractinian corals (Goreau & Hartman, 1966; Hill, 1998). As is often observed for other sponge species, *M. laevis* has more than one growth form. It has been described as both semi-cryptic, a thinly encrusting form with most of the biomass growing under coral or other hard substrata (Fig. 1a; Wulff, 1997), and massive, a fleshy, apparent form that grows on the upper surface of substrata (Fig. 1b; Randall & Hartman, 1968; Wulff, 2006b). On reefs off Bocas del Toro, Panama and the Bahamas Islands, a white morph of *M. laevis* has also been described (Fig. 1c, d; Collin et al., 2005). This white morphotype is more common than the orange on some reefs at Bocas del Toro, exhibits both encrusting and fleshy forms (observed at the Bahamas Islands and Panama, respectively), and frequently grows in association with corals in the same manner as the orange morphotype (Loh personal observation). The white morphotype is superficially similar to the orange, having a compressible texture, a rough external surface, and osculae ringed by thin membranous collars with vertical white lines (Collin et al., 2005).

In this study, we investigated genetic differences among 4 morphotypes of *M. laevis* using partial 18S and 28S rRNA ribosomal gene sequences. Samples

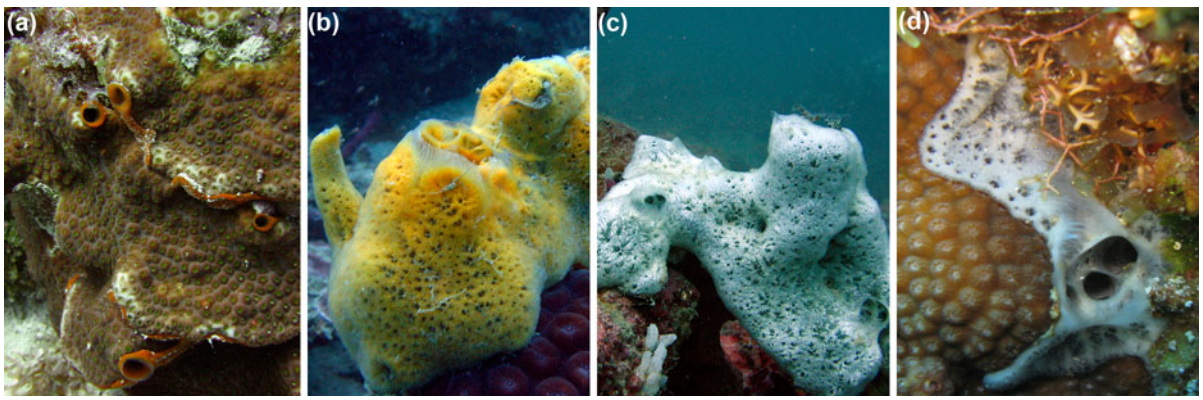

Fig. 1 The four morphotypes of *Mycale laevis*: **a** orange, semi-cryptic, **b** orange, massive, **c** white, massive, **d** white semi-cryptic

were collected from the coral reefs off Key Largo, Florida, Bocas del Toro, Panama, and the Bahamas Islands. In addition, we sequenced 11 species of *Mycale*, and retrieved 5 other sequences from Genbank to perform phylogenetic analyses and determine the taxonomic status of the 4 morphotypes of *M. laevis*. We also examined spicule morphology and diversity and calculated spicule dimensions to compare among the 4 morphotypes of *M. laevis*.

Materials and methods

Sample collection

When possible, three samples of each morphotype of *M. laevis* were collected from each of the study sites where they occurred (Table 1). The orange semi-cryptic morphotype was present at all the sites, while the orange massive morphotype was found only at Bocas del Toro. The white semi-cryptic morphotype was collected at Tuna Alley reef, Bahamas (2 samples), while the white massive morphotype was found only at Bocas del Toro. For our 3 sampling locations, the massive morphotypes (both orange and white), were only found at Bocas del Toro.

One to three samples of the following 11 *Mycale* species were collected and added to the analysis of samples of *M. laevis* to enhance phylogenetic tree resolution: *M. lingua* (Bowerbank 1866, collected in Norway by P. Cardenas) and *M. grandis* (Gray 1867, Singapore, S. C. Lim) from the subgenus *Mycale*; *M. sulevoidea* (Sollas 1902, Singapore, S. C. Lim), *M. adhaerens* (Lambe 1893, Hong Kong, Y. H. Wong and P. Y. Qian), and *M. carmigropila* (Hajdu & Rutzler, 1998, Panama, T. L. Loh) from the subgenus *Aegogropila*; *M. parishi* (Bowerbank 1875, Singapore, S. C. Lim) from the subgenus *Zygomycale*; *M. microsigmatosa* (Arndt 1927, Panama, T. L. Loh) and *M. fistulifera* (Row 1911, Israel, M. Ilan) from the subgenus *Carmia*; *M. laxissima* (Duchassaing & Michelotti 1864, Florida Keys, T. L. Loh) from the subgenus *Arenochalina*, and two unidentified species-*Mycale* cf. *lingua* from Norway and *Mycale* sp. J57 from Italy, both from the subgenus *Aegogropila* (subgenus identified by and collected by P. Cardenas). As an additional genus within the Poecilosclerida, *Desmapsamma anchorata* was collected by W. Leong from Key Largo, Florida. Samples were collected between 2007 and 2010 and immediately preserved in 95–100% ethanol, frozen at −20°C, or freeze-dried. All samples were then stored at −20°C until they were extracted.

Caribbean species were identified by comparing morphological and spicule characters with Hajdu & Rutzler (1998) and with the sponge voucher collection at the Bocas Research Station of the Smithsonian Tropical Research Institute (specimens submitted by C. Diaz and R. Thacker). Other *Mycale* species were identified by their respective collectors.

DNA extraction and sequencing

DNA was extracted using the Puregene kit (Gentra Systems). The most commonly genetic markers used to investigate taxonomic and phylogenetic issues in sponges are the 18S and 28s rRNA genes, and the mitochondrial gene cytochrome oxidase I (COI). Here, we designed the primer set 18sMycale01F 5′-ATAACTGCTCGAACCGTATGGCCT-3′ and 18SMycale01R 5′-AAACGCTAACATCCACCGATCCCT-3′ based on an 18S rRNA sequence of *M. fibrexilis* available from GenBank (AF100946) to amplify a fragment of 786 bp from the 18S rRNA gene. However, no amplification could be obtained for the species *M. lingua*, *M. parishi*, *Mycale* cf. *lingua*, *Mycale* sp. J57, 1 sample of the orange massive morphotype, and all white morphotypes of *M. laevis*, with the 18S rRNA primers described above. Amplification was finally obtained with the primer set 18sMycale02F 5′-CAACGGGTGACGGAGAATTA-3′ and 18sMycale02R 5′-TTTCAGCCTTGCGACCATACTC-3′, which was designed based on the consensus sequence of the poecilosclerid library available at GenBank. Amplification of a fragment from the 28S rRNA gene was performed using the forward primer 28sCallyF 5′-TGCGACCCGAAAGATGGTGAACTA-3′ and reverse primer 28sCallyR 5′-ACCAACACCTTTCCTGGTATCTGC-3′ (López-Legentil et al., 2010). No consistent amplification could be obtained for a fragment of the mitochondrial gene COI, although we used several universal primers and designed new ones based on poecilosclerid sequences retrieved from GenBank.

All amplifications were performed in a 25 µl total-reaction volume with: 1.25 µl of each primer (10 µmol), 12.5 µl GoTaq Colorless or Green Master Mix (Promega), and 0.5 µl DNA. A single soak at 94°C

Table 1 List of (a) 18S rRNA and (b) 28S rRNA sequences obtained from *M. laevis*, with replicate number for each morphotype-location, collection locations and sites, haplotype code, haplotype frequency, and GenBank accession numbers (Acc. No)

Morphotype	Replicate no.	Collection location	Collection site	Haplotype code	Haplotype frequency	Acc. No.
(a)						
Orange, semi-cryptic	1	Key Largo, Florida	Dixie Shoals	A03	0.0667	GU208832
Orange, semi-cryptic	2	Key Largo	Conch Wall	A15	0.6	HQ709340
	3		North Dry Rocks			HQ709341
Orange, semi-cryptic	2	Bahamas	Sweetings Cay			HQ709343
	3					HQ709344
Orange, semi-cryptic	2	Bocas del Toro, Panama	Adriana's Reef			HQ709346
	3					HQ709347
White, semi-cryptic	2	Bahamas	Tuna Alley			HQ709352
White, massive	2	Bocas del Toro	Old Point			HQ709350
Orange, massive	1		Punta Caracol			GU208833
Orange, semi-cryptic	1	Bahamas	Sweetings Cay	A06	0.0667	HQ709342
Orange, semi-cryptic	1	Bocas del Toro	Adriana's Reef	A09	0.0667	HQ709345
White, massive	1	Bocas del Toro	Hospital Point	A13	0.0667	HQ709349
Orange, massive	2	Bocas del Toro	Hospital Point	A16	0.0667	HQ709348
White, semi-cryptic	1	Bahamas	Tuna Alley	A26	0.0667	HQ709351
(b)						
White, massive	1	Bocas del Toro, Panama	Hospital Point	C01	0.882	HQ709333
	2		Old Point			HQ709335
	3					HQ709334
White, semi-cryptic	1	Bahamas	Tuna Alley			HQ709336
	2					HQ709337
Orange, semi-cryptic	2	Key Largo, Florida	Conch Wall			HQ709325
	3		North Dry Rocks			HQ709326
Orange, semi-cryptic	1	Bocas del Toro	Adriana's Reef			HQ709327
	2					HQ709328
	3					HQ709329
Orange, semi-cryptic	1	Bahamas	Sweetings Cay			HQ709330
	2					HQ709331
	3					HQ709332
Orange, massive	1	Bocas del Toro	Punta Caracol			GU324493
	3		Juan Point			HQ709339
Orange, semi-cryptic	1	Key Largo	Dixie Shoals	C06	0.0588	GU324492
Orange, massive	2	Bocas del Toro	Old Point	C16	0.0588	HQ709338

The replicate number refers to the replicate of each morphotype found at each location, and matches the labels in Fig. 2

for 5 min was followed by 40 amplification cycles (denaturation at 95°C for 30 s; annealing at 45°C for 28sCally and 18SMycale01 primer sets, and 50°C for 18SMycale02 primers, for 30 s; and extension at 68°C for 2 min), and a final extension at 72°C for 5 min for 28sCally and 18SMycale01 primer sets, and 10 min for 18SMycale02 primers in a Peltier PTC-200 gradient PCR.

PCR products were run in a 1% agarose gel to check for amplification results. The BigDye TM terminator v. 3.1 was used to carry out sequencing reactions with the same primers used in the

amplification step. Sequences were obtained in an ABI Prism 3100 automated sequencer. Nucleotide diversity for each gene fragment was estimated with DnaSP v. 4 (Rozas et al., 2003), and haplotype frequencies with Arlequin v. 2000 (Schneider et al., 2000).

Phylogenetic analyses

Sequences were aligned using BioEdit Sequence Alignment Editor v. 7.0.9 (Hall, 1999) and ClustalW (Larkin et al., 2007). Partial 18S and 28S rRNA sequences for *Mycale fibrexilis* (Wilson 1894; 18S: AF100946, 28S: AY026376) and two poecilosclerid species- *Iotrochota birotulata* (Higgin 1877; 18S: EU702421, 28S: AY561884) and *Tedania ignis* (Duchassaing & Michelotti 1864; 18S: AY737642, 28S: AY561878) were obtained from GenBank, as well as the 18S sequence for *Mycale* sp. 16 (AY737643). 18S and 28S rRNA sequences from the haplosclerid *Callyspongia plicifera* (Lamarck 1814; 18S: EU702412, 28S: AF441343) were used as outgroup sequences. The program jMODELTEST 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to select the best model of DNA substitution. The transitional model with unequal base frequencies (Posada, 2003) was selected for the 18S rRNA region, with substitution rates varying among sites according to a invariant and gamma distribution (TIM1+I+G), while the variable-frequency Tamura-Nei evolution model (Tamura & Nei, 1993) was selected for the 28S rRNA region, with substitution rates varying among sites according to a gamma distribution (TrN+G).

Neighbor-joining (NJ) and maximum parsimony (MP) analyses were conducted using MEGA 4 (Tamura et al., 2007). Neighbor-joining analysis was conducted using the Maximum Composite Likelihood model, and data were re-sampled using 5,000 bootstrap replicates. For the MP analysis, the search method used was the close neighbor interchange with random addition trees at a replication level of 10, and data were re-sampled using 5,000 bootstrap replicates. A maximum likelihood analysis was performed in PAUP 4.0b10 (Swofford, 1998) for both 18S rRNA and for 28S rRNA sequences using the evolution models determined from jMODELTEST. Data were re-sampled using 100 bootstrap replicates. MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used to calculate the Bayesian posterior probabilities of branch nodes. The Monte Carlo Markov Chain length was initially set to 1 million generations with sampling every 100th generation and with a burn-in value of 2,500. The average standard deviation of split frequencies between two independent chains reached a value of less than 0.01 after 2,178,000 and 1,655,000 generations for 18S rRNA and 28S rRNA sequences, respectively.

Spicule morphology

For each morphotype of *M. laevis* present at each geographic location, spicules were analyzed from 2–3 individuals. From each sampled individual, a small piece of tissue that included both the ectosome and choanosome was immersed in a 50% solution of chlorine bleach (2.5% sodium hypochlorite in water) and left to oxidize overnight. The spicule mass was then rinsed twice with deionized water and stored in 100% ethanol. Spicules were mounted on a slide and viewed using a compound light microscope under 200–400× magnification. For each sponge sample, the lengths of up to twenty spicules for each spicule type observed were measured using a calibrated ocular micrometer. The lengths of the head and foot portions of the anisochelae were measured as well. Statistical analyses comparing the spicule lengths of the 4 morphotypes of *M. laevis* using one-way ANOVA nested by location-morphotype, and the subsequent Tukey's post-hoc analysis were conducted with JMP 7.0 (SAS Institute Inc.).

Results

Genetic data and phylogenetic analysis

Amplification using 18S and 28S rRNA primers resulted in consensus sequences of 786 and 439 bp, respectively. All sequences were deposited in GenBank (accession numbers are listed in Table 1). Partial 18S rRNA gene sequences obtained for all *Mycale* species sequenced in this study revealed 17 unique haplotypes, and an overall nucleotide diversity of 0.0126. The 15 samples of *M. laevis* had 7 haplotypes for 18S rRNA, with a nucleotide diversity

of 0.0054. The most common haplotype (A15) of *M. laevis* was recovered from 9 samples (relative frequency = 0.6), 2 orange semi-cryptic morphotypes from each of the 3 sampling locations, 1 for the orange massive morphotype from Bocas, 1 for the white massive morphotype from Bocas, and 1 for the white semi-cryptic morphotype from the Bahamas. The other 6 haplotypes of *M. laevis* were each represented by 1 sample only for the orange semi-cryptic and massive, and white massive morphotypes sampled from different locations (Table 1a). Partial 28S rRNA gene sequences revealed a total of 13 haplotypes, and an overall nucleotide diversity of 0.014 among all *Mycale* species analyzed. The 17 samples of *M. laevis* were represented by 3 haplotypes and a nucleotide diversity of 0.0045. The most common haplotype (C01) was obtained for 15 samples of *M. laevis* (relative frequency = 0.882), corresponding to all morphotypes analyzed, and from all sampled locations. The other 2 haplotypes obtained for *M. laevis* belonged to the orange semi-cryptic morphotype from Key Largo and the orange massive morphotype from Bocas del Toro (Table 1b). Haplotypes of *M. laevis* for both 18S and 28S rRNA did not appear to be grouped by geographic location or morphotype.

Regardless of geographic location or morphotype, the 4 phylogenetic analyses performed with 18S rRNA (Fig. 2a) and 28S rRNA (Fig. 2b) indicated that all individuals of *M. laevis* formed a monophyletic clade within the genus *Mycale* with bootstrap support values >50 for all analyses. Correspondingly, all the *Mycale* species analyzed except one formed a well-supported clade (Fig. 2). The sole exception was a sequence retrieved from GenBank for *M. fibrexilis*, which grouped most closely with the poecilosclerid *D. anchorata* for 18S rRNA and *T. ignis* for 28S rRNA.

Members of the subgenus *Aegogropila* represented in this study did not group in the same clade, but were interspersed with members from other *Mycale* subgenera, including *Mycale*, *Arenochalina*, *Zygomycale*, and *Carmia* (Fig. 2). *Mycale carmigropila* grouped most closely with *M. laxissima* from the subgenus *Arenochalina*. The subgenus *Mycale* appeared to be paraphyletic, with some species of the subgenus grouping with members from the subgenera *Arenochalina* and *Aegogropila* (Fig. 2).

Spicule morphology

All 4 morphotypes of *M. laevis* had the palmate anisochelae typical of the genus *Mycale*. Individuals either had only large anisochelae, or both large and small anisochelae, and the dominant megasclere for all samples was either the subtylostyle or the strongyle, depending on collection location (Fig. 3). Megascleres for *M. laevis* from Key Largo and the Bahamas were strongyles, while megascleres for individuals from Bocas del Toro were subtylostyles, regardless of morphotype. Other common microscleres found included raphides and sigmas (Table 2). Across all samples, the smaller anisochelae II (22.59 ± 0.67 μm, $n = 163$) were rarer, only observed in 11 of 17 samples, while the larger anisochelae I were found in all samples of *M. laevis* (Table 2), with a mean length of 80.27 ± 0.45 μm ($n = 295$). Mean lengths of subtylostyles and strongyles were 517.32 ± 2.76 μm ($n = 180$) and 456.62 ± 3.76 μm ($n = 160$), respectively. For the anisochelae I, significant differences in spicule length were observed between samples from different locations and morphotype ($P < 0.0001$), but the differences were not grouped by morphotype. The anisochelae I of all morphotypes of *M. laevis* from Bocas del Toro were significantly larger than those from the orange semi-cryptic morphotypes of *M. laevis* from Key Largo and the Bahamas, but no differences were found among the 3 morphotypes at Bocas del Toro. Anisochelae I from the Bahamian white morphotypes were intermediate in length between those from morphotypes from Key Largo and Bocas del Toro. No significant differences were found between strongyle lengths from Key Largo and Bahamas samples ($P = 0.4429$), and subtylostyle lengths among the 3 morphotypes found at Bocas del Toro ($P = 0.0766$).

Discussion

From the analyses of the 18S and 28S rRNA gene fragments, the morphological differences observed among the 4 morphotypes of *M. laevis* lack a genetic basis. In addition, no differences in spicule morphology were observed among the 4 morphotypes within a location. However, there were differences in spicule type between sampling locations. Populations of

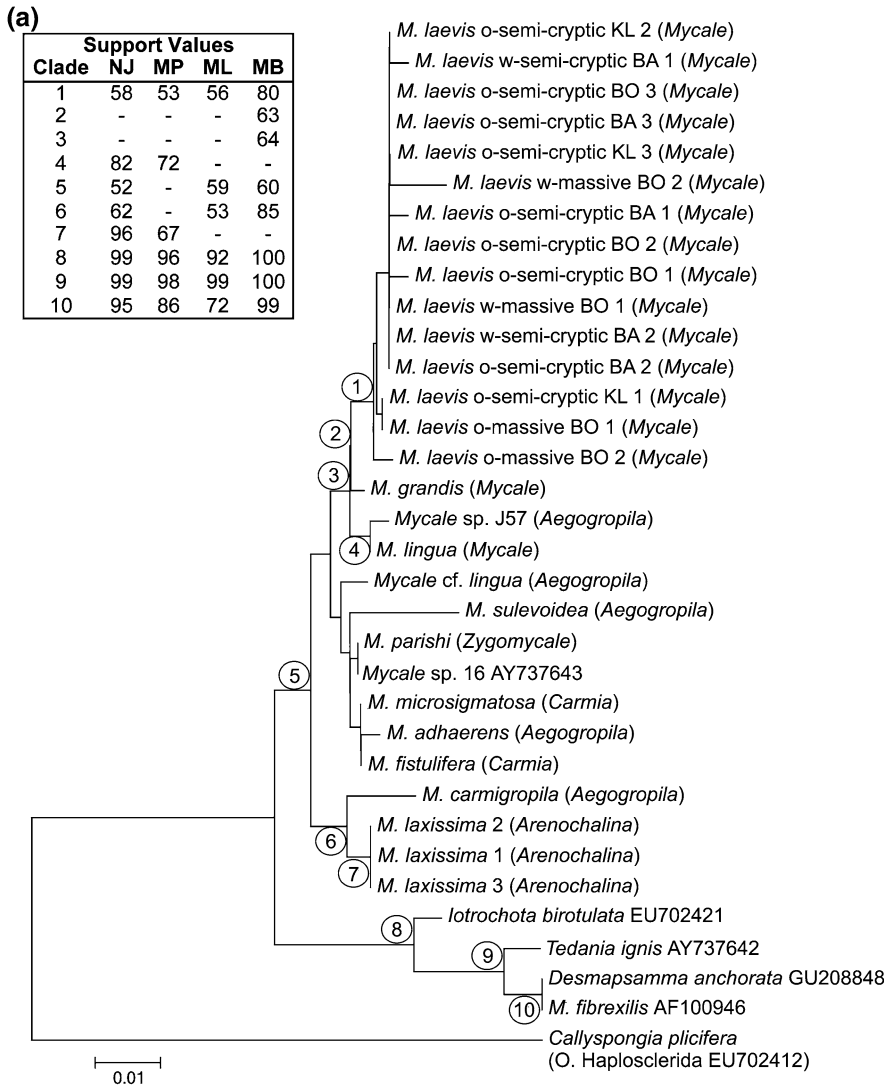

Fig. 2 Phylogenetic tree of fragments of **a** 18S and **b** 28S rRNA gene sequences of sponges from the order Poecilosclerida denoting the phylogenetic position of morphotypes of *M. laevis*. *Callyspongia plicifera* from the Order Haplosclerida was used as outgroup. Italicized labels in parentheses denote the subgenus of the *Mycale* species. Labels for *M. laevis* (o/w-semi-cryptic, o/w-massive) denote the morphotype of the sample analyzed with o: orange, w: white. Collection site is indicated by *KL* Key Largo, Florida; *BA* Bahamas; *BO* Bocas del Toro, Panama. The GenBank accession numbers for the 18S and 28S fragments, respectively, are provided for reference sequences. Tree topology was obtained from neighbor-joining (NJ) analysis. Well-supported clades are labeled, with the corresponding individual bootstrap values from NJ, maximum parsimony (MP), maximum likelihood (ML) analyses and the posterior probabilities from the MrBayes analysis (MB) listed in the *table inset*. The *scale bar* represents 0.01 and 0.02 substitutions for 18S and 28S, respectively

M. laevis in Key Largo and the Bahamas had strongyles as their dominant megasclere, while those from Bocas del Toro, Panama had substylostyles. Variation in the megascleres of *M. laevis* has been reported previously in the literature. Subtylostyles were the only megasclere observed in *M. laevis* from Belize (Hajdu & Rutzler, 1998), while styles and strongylostyles were reported for specimens from Curaçao and Puerto Rico (Van Soest, 1984). Although spicules, especially microscleres, can be present or absent in sponge individuals depending on the collection location (Zea, 1987), it is rare for

(b)

Support Values				
Clade	NJ	MP	ML	MB
1	86	68	78	94
2	-	-	-	87
3	76	67	-	74
4	-	-	-	67
5	54	-	-	90
6	71	59	84	99
7	57	-	52	52
8	87	77	66	76
9	92	-	-	-
10	61	-	-	-
11	88	-	64	90

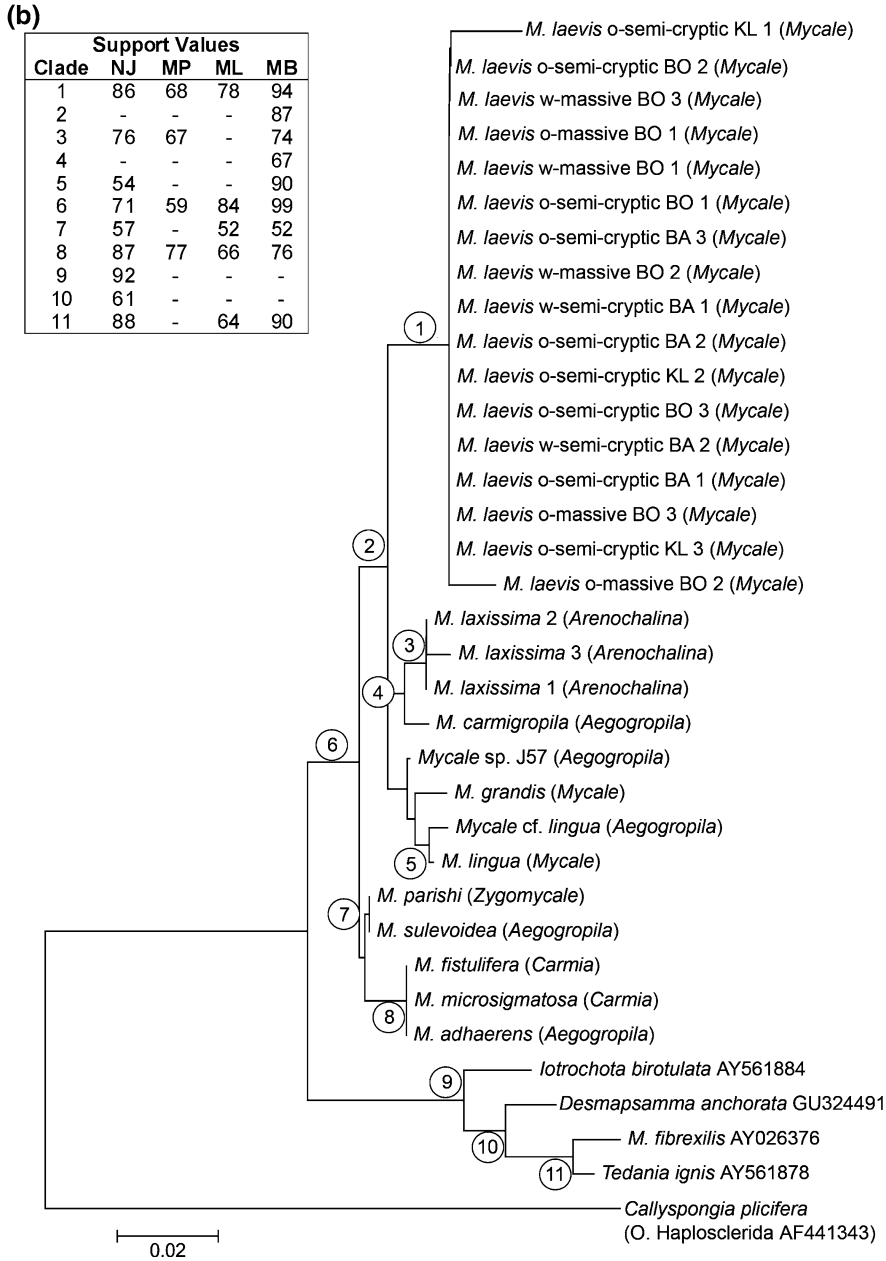

Fig. 2 continued

megasclere types to vary. Hartman (1967) argued for the designation of subspecies of *Neofibularia nolitangere* based on geographical variation in megascleres. Variation in spicule type and shape can be explained by silicon limitation (Maldonado et al., 1999), but seawater chemistry is unlikely to be the determining factor for the different spicule types found in *M. laevis*, as samples were all collected from shallow Caribbean coral reefs. Alternatively, for this species, megasclere type may not be a stable or valid taxonomic character. Further research, including samples from more specimens and populations, is needed to investigate these apparent geographic differences in megascleres among samples of *M. laevis*.

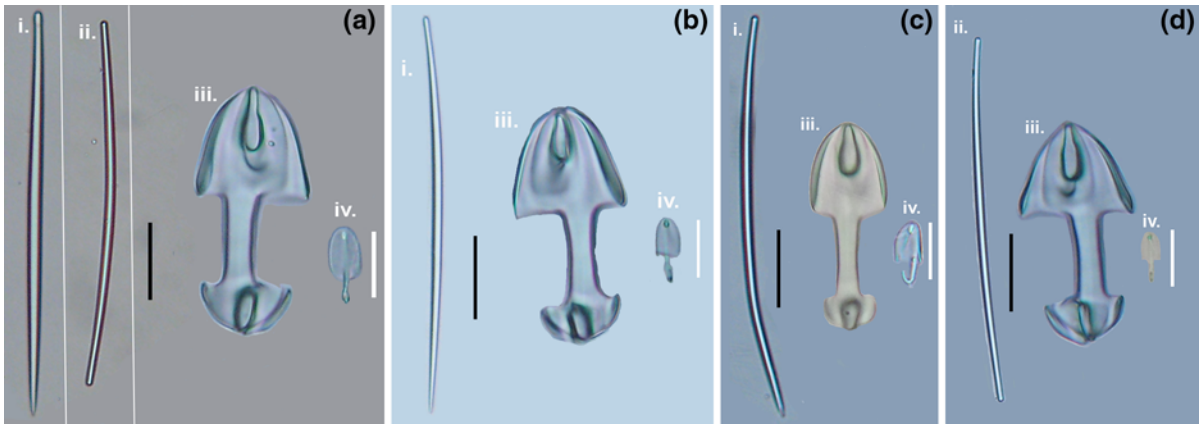

Fig. 3 The characteristic spicules of the morphotypes of *M. laevis* combining spicule types found in all geographic locations studied: **a** orange, semi-cryptic (Key Largo, Bahamas, Bocas del Toro), **b** orange, massive (Bocas del Toro), **c** white, massive (Bocas del Toro), **d** white, semi-cryptic (Bahamas). Samples from Key Largo and the Bahamas have strongyles as their dominant megasclere, while the dominant megasclere in samples from Bocas del Toro is the subtylostyle. *i* subtylostyle, *ii* strongyle, *iii* anisochela I, *iv* anisochela II. *Black scale bar* = 100 μm, *white scale bar* = 20 μm

Since this manuscript was accepted for publication, we visited islands in the southeastern Caribbean (April 2011), and observed both the orange and white forms of *M. laevis* growing on the same reefs, with an intermediate white-orange form on some reefs as well. Variation between the orange and white forms was also noted by Williams & Bunkley-Williams (1990), and attributed to sponge bleaching in response to temperature changes. Megascleres of *M. laevis* collected from Curaçao (orange, semi-cryptic) and Martinique (orange, white and intermediate, all massive) were all subtylostyles ~500 μm in length.

The different phylogenetic analyses using 18S and 28S rRNA gene sequences revealed the same basic genetic structure, and grouped all morphotypes of *Mycale laevis* in the same clade with the other sequenced species of *Mycale*. Thus, our results support the validity of using the presence of anisochelae, in combination with a monoaxonic megasclere, as a taxonomic character to distinguish the genus *Mycale*. The presence of chelae appears to be an autapomorphy for the poecilosclerids, as they have a complex structure and are unique to the order (Erpenbeck et al., 2007). The same study of the Poecilosclerida showed that species with chelae were monophyletic while those lacking chelae were polyphyletic.

Phylogenetic analyses with 18S and 28S rRNA also indicated that sponges of the subgenus *Aegogropila* were polyphyletic as they grouped with sponges from 4 other subgenera- *Arenochalina*, *Mycale*, *Zygomycale*, and *Carmia*. The sponge *M. carmigropila* has ectosomal features related to both subgenera *Aegogropila* and *Carmia*, and is provisionally assigned to *Aegogropila* (Hajdu & Rutzler, 1998). Despite its name, *M. carmigropila* was more closely related to *M. laxissima* (subgenus *Arenochalina*) than to either *Aegogropila* or *Carmia*. Finally, the subgenus *Mycale* appeared to by paraphyletic. Analysis of 18S rRNA sequences grouped *M. grandis*, *M. lingua* and all sequences of *M. laevis* in a clade with *Mycale* sp. J57 (*Aegogropila*). In the 28S rRNA analyses, the clade representing the subgenus *Mycale* included species from *Arenochalina* and *Aegogropila*. Our results suggest that a revision of the subgenus classification within *Mycale* combining both molecular and morphological data may be needed.

Intra-specific morphological diversity is often associated with genetic divergence or with differences in local environmental conditions. In our study, the observed differences in shape and color among the 4 morphotypes of *M. laevis* did not correlate with genetic data. Field observations have revealed that the massive growth form of *M. laevis* dominates on overfished, predator-scarce reefs like Bocas del Toro, but is largely absent on reefs protected from fishing and with high predator densities like those off Key Largo (Loh & Pawlik, 2009). As all the morphotypes of *M. laevis* are palatable to spongivorous fish, the

Table 2 Spicule types and lengths from the four morphotypes of *M. laevis*

Morphotype	Collection location	Replicate	Spicules			
			Megasclere	Length (μm)	Microsclere	Length (μm)
Orange and semi-cryptic	Key Largo, Florida	1	Strongyle	399.63–485.44–554.88 (20)	Anisochela I	55.00–78.31–147.50 (20), (40.94%; 20.75%)
					Anisochela II	37.38–55.70–71.88 (8), (52.90%; 21.94%)
					Sigma	30.00–41.00–50.00 (20)
					Raphide	39.9–54.72–68.4 (20)
		2	Strongyle	250.00–416.25–480.00 (20)	Anisochela I	68.75–75.19–81.25 (20), (41.90%; 18.37%)
					Anisochela II	20.00–23.75–27.50 (2), (63.16%; 23.68%)
					Sigma	18.75–24.84–30.00 (8)
					Raphide	40.00–52.13–80.00 (20)
		3	Strongyle	395.00–461.25–510.00 (20)	Anisochela I	67.50–73.33–77.50 (3), (40.91%; 20.45%)
					Sigma	25.00–35.83–45.00 (12)
					Raphide	38.75–52.88–90.00 (20)
	Bahamas	1	Strongyle	370.00–466.75–525.00 (20)	Anisochela I	67.50–78.81–85.00 (20), (44.57%; 20.94%)
					Raphide	37.50–52.31–75.00 (20)
		2	Strongyle	330.00–466.25–530.00 (20)	Anisochela I	66.25–74.69–82.50 (20), (40.67%; 19.41%)
					Raphide	45.00–59.50–67.50 (20)
		3	Strongyle	375.00–443.75–505.00 (20)	Anisochela I	65.00–75.38–85.00 (20), (41.96%; 17.74%)
					Anisochela II	17.50–19.17–20.00 (3), (60.87%; 13.04%)
					Sigma	20.00–29.75–35.00 (5)
					Raphide	45.00–55.75–67.50 (20)
	Bocas del Toro, Panama	1	Subtylostyle	450.00–527.75–585.00 (20)	Anisochela I	67.50–83.31–92.50 (20), (43.51%; 22.21%)
					Sigma	30.00–41.75–55.00 (20)
					Raphide	57.50–70.38–76.25 (20)
		2	Subtylostyle	450.00–528.25–565.00 (20)	Anisochela I	65.00–80.38–87.50 (20), (44.63%; 19.28%)
					Sigma	25.00–39.00–50.00 (20)
					Raphide	50.00–68.38–100.00 (20)
		3	Subtylostyle	470.00–521.50–565.00 (20)	Anisochela I	80.00–84.81–90.00 (20), (45.10%; 18.57%)
					Anisochela II	15.00–20.25–22.50 (20), (66.05%; 18.52%)
					Sigma	30.00–44.75–55.00 (20)
					Raphide	60.00–73.94–80.00 (20)

Table 2 continued

Morphotype	Collection location	Replicate	Spicules			
			Megasclere	Length (μm)	Microsclere	Length (μm)
Orange and massive	Bocas del Toro, Panama	1	Subtylostyle	442.75–501.40–529.00 (20)	Anisochela I	15.68–21.02–22.8 (20) (63.73%; 21.02%)
					Anisochela II	69–82.37–86.25 (20) (45.38%; 18%)
					Sigma	23–51.75–71.88 (20)
					Raphide	34.2–58.21–65.55 (20)
		2	Subtylostyle	425.00–507.00–560.00 (20)	Anisochela I	67.50–78.38–82.50 (20), (46.01%; 18.66%)
					Anisochela II	18.75–21.00–23.75 (20), (62.80%; 7.38%)
					Sigma	35.00–48.30–60.00 (20)
					Raphide	52.50–65.75–75.00 (20)
		3	Subtylostyle	475.00–525.00–660.00 (20)	Anisochela I	77.50–87.75–97.50 (20), (43.87%; 19.94%)
					Anisochela II	20.00–23.25–30.00 (20), (63.17%; 19.09%)
					Sigma	25.00–47.30–60.00 (20)
					Raphide	70.00–83.94–105.00 (20)
White and massive	Bocas del Toro, Panama	1	Subtylostyle	430.00–505.00–570.00 (20)	Anisochela I	77.50–83.13–92.50 (8), (38.72%; 16.73%)
					Anisochela II	15.00–19.5–23.75 (20) (66.03%; 18.59%)
					Sigma	25.00–36.31–47.50 (20)
					Raphide	70.00–79.00–97.50 (20)
		2	Subtylostyle	410.00–521.75–585.00 (20)	Anisochela I	67.50–86.25–97.50 (20), (43.19%; 19.28%)
					Anisochela II	20.00–22.38–25.00 (20), (64.80%; 19.55%)
					Sigma	30.00–44.50–60.00 (20)
					Raphide	50.00–68.56–120.00 (20)
		3	Subtylostyle	445.00–518.25–570.00 (20)	Anisochela I	65.00–80.44–90.00 (20), (42.35%; 18.10%)
					Anisochela II	15.00–18.81–22.50 (20), (63.79%; 21.93%)
					Sigma	20.00–37.75–50.00 (20)
					Raphide	62.50–68.44–77.50 (20)
White and semi-cryptic	Bahamas	1	Strongyle	405.00–485.75–555.00 (20)	Anisochela I	75.00–83.13–87.50 (4), (41.35%; 16.92%)
					Anisochela II	17.50–20.75–22.50 (9), (60.24%; 18.67%)
					Sigma	20.00–32.06–40.00 (17)
					Raphide	47.50–55.38–63.75 (20)
		2	Strongyle	375.00–445.50–510.00 (20)	Anisochela I	67.50–77.06–87.50 (20), (42.25%; 19.71%)
					Sigma	20.00–29.75–45.00 (20)
					Raphide	38.75–48.25–53.75 (20)

The spicule lengths are presented in the 'shortest-mean length-longest' format, with the number of spicule replicates in parentheses. The second set of parentheses for the anisochelae denote the proportional length of the anisochela head and foot, respectively

sponge is likely grazed down when predators are abundant, and thus restricted to a semi-cryptic growth form in refugia under coral colonies and other hard reef substrata (Loh & Pawlik, 2009). In the south-eastern Caribbean, massive forms of *M. laevis* dominated where spongivore density is low, such as on the island of Martinique, and overfished reefs off St Lucia (personal observation).

The results of this study suggest that, like other sponge species, *M. laevis* exhibits morphological plasticity under different environmental conditions. Sympatric color morphotypes of another common Caribbean sponge, *Callyspongia vaginalis*, also did not exhibit any significant differences in gene sequences despite clear differences in surface architecture (López-Legentil et al., 2010). A note of caution bears repeating, however, as the genetic analyses presented here were based on a single genetic marker (ribosomal RNA), and further studies with more specimens and additional genetic markers are necessary to assess whether morphological variability of *M. laevis* is due to phentotypic plasticity or genotypic variation.

Conclusion

Sponges are morphologically plastic, may have different color morphotypes, and can change in shape and size due to environmental conditions. In this study, we analyzed the variation in two fragments of the ribosomal genes 18S and 28S rRNA to assess the taxonomic status of 4 morphotypes of the Orange Icing sponge, *M. laevis*. Analysis of the gene fragments provided no evidence for differentiation among the morphotypes. Based on this study, the most parsimonious explanation for the observed morphological variability in *M. laevis* is differences in local environmental conditions, such as the abundance of sponge-eating predators.

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