Morphological variation of the endemic reef-building genus *Mussismilia* in the Bahia State (Tropical northeastern Brazilian coast)

A. H. Silva [ID], M. M. Nogueira [ID], R. Johnsson [ID] and E. G. Neves [ID]

LABIMAR (Laboratório de Invertebrados Marinhos: Crustacea, Cnidaria e Fauna Associada), Instituto de Biologia, Universidade Federal da Bahia (UFBA), Rua Barão de Jeremoabo, 147, Ondina, Salvador, BA CEP 40170115, Brazil

Abstract

Verrill's modern *Mussismilia* (the 'brain corals') were described in the 19th century, being hitherto considered endemic reef-building species to Brazil. Contrasting with the original diagnoses, highly variable morphological patterns have been observed among the congeners. Interspecific overlapping of major taxonomical characters has resulted in quite inconclusive use of the skeleton macromorphology for the genus. Intending to corroborate the *Mussismilia* taxonomy, a comparative morphological approach was developed, combining skeleton macro- and micromorphology. A total of 132 colonies was collected between 13°S and 17°S latitude (*Mussismilia hispida* = 53, *Mussismilia harttii* = 41, and *Mussismilia braziliensis* = 38). Qualitative ($n = 9$) and quantitative characters ($n = 7$) were selected (the latter was analysed with Kruskal–Wallis and a principal component analysis). A non-parametric test was adopted due to heteroscedasticity and the irregular sampling among populations. As a result, the corallite diameter and number of septa were significantly distinct among the species ($\alpha = 0.05$). Micromorphology also differs interspecifically, being distribution and size of septal spines diagnostic for the congeners. Intraspecific variation and morphs are approached, ensuring the relevance of the skeleton for the interspecific delimitation and the species identities. Finally, field identification and/or methods based on image analyses from video transects should be adopted with caution. These practices may provide unreliable data, once the information is restricted to the view of the colony top, resulting in biased identification – majorly if the morphotypes of *M. harttii* and *M. hispida* share closely spaced corallites.

Introduction

Traditionally, identification of coral species is based on skeletal macrostructures, mainly on the characteristics attributed to the corallum and corallites, being the interspecific limits defined by the discontinuities of the diagnostic traits (Foster, 1977; Budd and Stolarski, 2009; Benzoni *et al.*, 2011; Budd *et al.*, 2012a). However, challenging the Scleractinia taxonomy, morphology may be quite inconsistent, varying intra- and interspecifically, leading to the overlapping of skeletal features and the misidentification of the morphotypes (Brown and Navin, 1992; Veron, 1995; Bruno and Edmunds, 1997; Benzoni *et al.*, 2011; Menezes *et al.*, 2013).

Incongruences on coral morphologies have been highlighted by molecular phylogenies, emphasizing the relevance of an integrative approach (Romano and Cairns, 2000; Budd and Stolarski, 2009; Benzoni *et al.*, 2011; Arrigoni *et al.*, 2012; Budd *et al.*, 2012a, 2012b). These studies have impacted the phylogeny of two important families from the Atlantic and Indo-Pacific Oceans: Faviidae and Mussidae (Fukami *et al.*, 2004, 2008; Budd *et al.*, 2012b). Using nuclear and mitochondrial markers, Nunes *et al.* (2008) have suggested a close relationship between *Favia leptophylla* Verrill, 1868 and the Brazilian endemic genus *Mussismilia* Ortmann, 1890, the species renaming as *Mussismilia leptophylla*.

Following this novelty, Budd and Stolarski (2009) presented a thorough skeletal analysis into three distinct 'scales': macromorphology, micromorphology, and microstructure, defining the family Mussidae as polyphyletic, with most members distributed into two major molecular clades, one Atlantic, including Mussidae and Faviidae, and the other represented by Pacific Mussidae and Pectiniidae.

Supported by recent literature, Mussidae is expected to comprehend two distinct subfamilies: Mussinae Ortmann, 1890 and Faviinae Gregory, 1890 – among other genera, the latter being represented by the Atlantic members of *Favia* Milne Edwards, 1857 and *Mussismilia* (Fukami *et al.*, 2004; Budd *et al.*, 2012a, 2012b), while *Scolymia* Haime, 1852 remained in Mussinae. By its turn, Faviidae has been characterized as polyphyletic within the Robust clade – although closely related to Mussidae, it has been 'lowered to the rank of subfamily' (Budd *et al.*, 2012b, p. 472). In fact, apparent similarities between Atlantic and Indo-Pacific faviids have been attributed to morphological convergence (Fukami *et al.*, 2004).

Mussismilia fossils from Mio-Pliocene have been found across the Caribbean and the Mediterranean Sea (Veron, 1995; Riegl and Piller, 2000). Currently endemic to Southwestern Atlantic, modern *Mussismilia* corals (the 'brain corals') present a wide

distribution range along the coastal Brazilian shallow water environments, occurring from Maranhão to São Paulo State (0°S/44°W to 23°S/45°W). Although represented by a few species, the genus plays an important role as reef builders. The massive *Mussismilia braziliensis* (Verrill, 1868), for instance, stands out as the major builder of the mushroom-shaped coral pinnacles, the 'Chapeirões' from Abrolhos reefs at Southern Bahia (16°S/45°W) (Castro and Pires, 2001). True coral reefs in the South Atlantic occur mostly along the northeastern section of the Brazilian coast, where the influence of the warm waters of the Guyana and Brazilian currents supports favourable conditions for the carbonate deposition, allowing reef flourishment (Maida and Ferreira, 1997).

For long unchangeable, and represented by three congeners, *Mussismilia* genus was modified in the last decade by a new addition: *M. leptophylla* (Nunes *et al.*, 2008; Budd *et al.*, 2012a, 2012b). Originally, the subplocoid *M. braziliensis* (Verrill, 1868) and *Mussismilia hispida* (Verrill, 1902), and the phaceloid *Mussismilia harttii* (Verrill, 1868) were described as *Acanthastrea* Milne Edwards & Haime, 1848 (*M. braziliensis*) and *Mussa* (Oken, 1815) (*M. harttii* and *M. hispida*) (Verrill, 1868, 1901, 1902). Being distinguishable from other genera because of exclusive micromorphological characteristics of the septa teeth, *Mussismilia* species have been separated by the colony form (and pattern of corallite development), corallite wall, calice size, septal thickness, and number of septa per cycle (Budd *et al.*, 2012a, 2012b). According to Budd and Stolarski (2009) and Budd *et al.* (2012a), major micromorphological aspects (e.g. septa marginal teeth, ornamentation of the septal face) have also shed light on the controversies related to the skeleton interspecific variation, and have become an important tool for consolidating the genus taxonomy.

Regarding the species currently accepted as *Mussismilia*, *M. harttii* singularly displays a phaceloid development ('corallites walls of adjacent corallites separated by void space; each corallite forms a branch', *sensu* Budd *et al.*, 2012a, p. 481, 2012b). Without tissue lying between the corallites, 'true' phaceloids may be promptly recognized by the widely spaced units, lacking costa, exotheca, coenosteum, and/or coenosarc. However, even pointed out as a strong diagnostical character, a few colonies may show some degree of fusion between adjacent polyps, forming closely spaced corallites with deposition of exothecal elements. Considering Verrill's morphotypes, phaceloid variants were described as follows: (1) *conferta* with corallites joined by a vesicular exotheca (very similar to *M. hispida*); (2) *laxa* with dichotomous corallum without exotheca (typical phaceloid) (3); *intermedia* with partially free corallites joined by an exotheca at the base, and finally (4) *confertifolia* with corallites separated by deep grooves at short distances (Table 1).

Most *M. harttii* morphotypes may be observed across their entire range of geographical distribution. However, studies by Laborel (1969/70) have not supported 'conferta', probably because the huge development of the exothecal elements – an unusual pattern, which bias the recognition of the species in the field, leading *M. harttii* be misidentified as *M. hispida*.

Two geographic subspecies have been described for *M. hispida* (Laborel, 1969/70): *M. hispida tenuisepta* and *M. hispida hispida*. The former is distributed northwards São Francisco River (10°S), and the latter occurring southwards (Laborel, 1969/70). Contrasting with *M. hispida hispida*, Verrill (1901) has originally classified *Mussa* (*Symphyllia*) *tenuisepta* as those colonies with moderately broad, irregular calyces, polystomodeal, with numerous and thinner septa. As similarly proposed by Amaral *et al.* (2009), colonies with meandroid corallites fit in the 'hispida tenuisepta' pattern, while colonies with regular corallites are 'hispida hispida' (Table 1).

Anthropogenic impacts on the *Mussismilia* corals have been the focus of intense debate, due to the overall risks of the productivity impoverishment of the coastal seas, and the biodiversity losses along the Tropical South Atlantic (Dutra *et al.*, 2006; Francini-Filho *et al.*, 2008; Miranda *et al.*, 2013). Despite major worldwide concern on global climate changes, several other factors (e.g. chemical, biological and solid pollution, deforestation, urbanization, unsustainable exploration of the natural resources) are rapidly depleting local coral communities (Leão *et al.*, 2016; Kubicek *et al.*, 2019). Thus, baseline surveys represent an unprecedented strategy to support reef resilience and its conservation. Alternatively, studies dealing with natural morphological variation within and among populations may also provide answers to how a changing world is affecting the species adaptation and the ecological interactions as well.

Therefore, intending to corroborate the definition of the interspecific limits and the species identities as well, the present study aims to analyse qualitatively and quantitatively the macro- and microstructures of the endemic brain corals *M. hispida*, *M. harttii*, and *M. braziliensis* through a population approach.

Materials and methods

The Bahia State has one of the longest coastlines of Brazil (~1100 km), located between 11°27′26.70″S and 18°20′9.35″S of latitude, being characterized by pristine natural environments, including rocky shores, coral reefs, sandy beaches, mangroves, and estuaries (Tessler and Goya, 2005). The study area comprises two distinct geographical sections: the Todos-os-Santos Bay and the South Littoral (SL), including the following true reefs: Caramuanas, Boa Viagem, Moreré, and Abrolhos Archipelago (Figure 1). Sampling was carried out by snorkeling and scuba diving at 1.0–5.0 m depth. Colonies of *Mussismilia hispida*, *Mussismilia braziliensis*, and *Mussismilia harttii* ranging from 10.0 to 30.0 cm were randomly selected and removed using a hammer and a chisel. A minimum distance of 3.0 m between neighbouring colonies was adopted to cover a greater morphological diversity. In the laboratory, the corals were bleached in a solution of sodium hypochlorite (2%), washed, and dried at ambient temperature. Samples from the Abrolhos Archipelago were donated by the 'Laboratório de Recifes de Corais e Mudanças Globais' (RECOR/IGEO/UFBA). Testimonies previously deposited in the Cnidaria Collection of the Museu de História Natural da Universidade Federal da Bahia (acronym: UFBA) were also examined. All collected samples were deposited in the UFBA.

Taxonomy and morphometric analysis

Skeleton structures, including corallum and corallites, were qualitatively and quantitatively evaluated (Table 1). Species identification and morphometric variables were supported by the specialized literature (Verrill, 1868, 1901, 1902; Laborel, 1969/70; Foster, 1977, 1979; Neves, 2004; Budd *et al.*, 2012a). Morphometric measurements were developed under a Nikon SMZ 1000 stereomicroscope with an eyepiece micrometre and a Nikon Coolpix 995 digital camera attached. Skeleton fragments were also mounted with aluminium pin stubs for septal teeth and spine analysis, being previously covered with a double-sided sticky tape, sputter-coated with 35 nm of gold in a Denton Vacuum Desk V ion coater, and examined through a Jeol JSAA-6610LV. Scanning electron microscope (SEM) images supported the microstructure analysis.

Statistics

A total of six corallites per colony were randomly selected and examined using a grid of numbers (Foster, 1985). Colony diameter (D_{col} = mean of the two major axes of the colony [mm]), which is influenced by the age, and meandroid and/or

Table 1. Characterization of the *Mussismilia* species, including morphotypes, macro- and microstructures with special inclusion of *Mussismilia leptophylla*

Species	Mussismilia hispida	Mussismilia braziliensis	Mussismilia harttii	M. leptophylla
Morphotypes	**hispida tenuisepta** meandroid corallites **hispida hispida** regular corallites		*conferta* corallites joined by a vesicular exotheca *laxa* dichotomous corallum without exotheca *intermedia* partially free corallites joined by an exotheca at the base *confertifolia* corallites separated by deep grooves at short distances	
Fossil	Panama, in the Miocene and Quaternary, Florida in the Pliocene	Brazil, from Pleistocene to Holocene	Panama, in the Miocene and Quaternary, and Costa Rica from the Pliocene to Pleistocene	Unknown
Distribution	From Maranhão (01°01′00″S, 41°48′30″W) to Santa Catarina (29°23′55″S, 48°19′37″W) (except for Sergipe and Paraná)	Bahia (11°27′85″S, 037° 22.07W) and Espírito Santo (21°21′12.2″S, 40° 06′51.0″W)	From Rio Grande do Norte (4° 48′22.6″S, 36°58′32.6″W) to Espírito Santo (21°21′12.2″S, 40°06′51.0″W), including Fernando de Noronha and Atol das Rocas (except for Sergipe)	Bahia (11°27′85″S, 037° 22.07W) to Martin Vaz (20°31′29″S, 29° 19′29″W)
Colony	Massive, hemispherical	Massive, forming large domes	Phaceloid	Massive, moderately flattened
Corallite	Subplocoid, irregular, oval, or elliptical	Subplocoid, polygonal, cerioid, oval, or elliptical (submeandroid)	Phaceloid, oval, or elliptical	Subplocoid, rounded, triangled, elongated
Columella	Well-developed	Small	Well-developed	Little, of a loose, open texture or well-developed, and spongy
Corallite diameter (mm)	10–15 (*16–26 in the present study*)	8–10 (*6–18 in the present study*)	15–30 (*7–31 in the present study*)	6–8
Number of septa cycles	5 (the last one incomplete)	4 (the last one incomplete)	5 (the last one incomplete)	3–4 (when 4, the last one may be complete or incomplete)
Septo-costae	Present with rows of spines	Small or absent	Present with rows of spines	Present, uniformly spaced
Distance among corallites (mm)	3–4 (corallites separated by double walls)	Fused walls (no coenosteum)	>10 (no common walls, corallites separated by deep grooves)	2–3 (walls well-developed)
Septal microstructures	Elongated and sharp teeth	Short and sharp teeth	Short and sharp teeth	Numerous slender septal teeth
Epitheca	Reduced	Reduced	Conspicuous and well-marked	Well-developed and concentrically wrinkled
Budding	Intra and extracalicinal, bidirectional or multidirectional	Intracalicinal	Intra and extracalicinal, bidirectional or multidirectional	Intracalicinal
Centres per series	2–4	2–6	2–3	None

Based on Amaral (1992), Budd *et al.* (2012a), Verrill (1868, 1901, 1902), and data from the present study.

polystomodeal corallites (irregular patterns) were not considered; for the statistical analysis we included: N_{cor} = corallite number in an area of 5 cm^2; D_{ica} = inner diameter of callice, mean of the two major inner axes of theca cavity margins (mm), D_{icor} = corallite diameter, mean of the two major axes of the outer margins of corallite (mm); N_{sep} = number of septal elements; C_{sep} = mean length from septa first cycle to columella (mm); P_{col} = mean columellar fossa depth (mm), D_{cor} = mean distance between calice, based on the inner theca margin between two close corallites (mm). Due to heteroscedasticity and the distinct number of samples per population, a non-parametric Kruskal–Wallis test (Theodorsson-Norheim, 1986) was developed as well to evaluate which characteristics differed significantly among the populations and species. To visualize all traits together per locality and per species, a principal component analysis (PCA) was adopted (Jolliffe, 2002). For interspecific analysis, only populations where the three species occurred sympatrically were used (i.e. Caramuanas and Moreré reefs). Packages of Vegan (Oksanen *et al.*, 2017) and ggplot2 (Wickham, 2009) in R environment, and Excel 2010 (Microsoft©) were used for the statistical analysis.

Results

A total of 132 corallums was examined (*Mussismilia hispida* $n = 53$; *Mussismilia harttii* $n = 41$; *Mussismilia braziliensis* $n = 38$), comprising 792 corallites, and resulting in 5544 data analysed; all means, standard deviation, and total range are summarized in Table 2. In *M. hispida* colonies, only the number of septa showed statistical difference among populations with higher values in Moreré population (Tables 2 and 3). On the other hand, the marginal teeth and the spines did not show interpopulational variation. Septal ornamentation is composed by conical and elongated spines. Bi- or trifurcated conical spines are regularly distributed along the septal faces (Figure 2A, E, I), while those elongate, with regular tips (not bifurcated), are distributed linearly along the septa (Figure 2B, F).
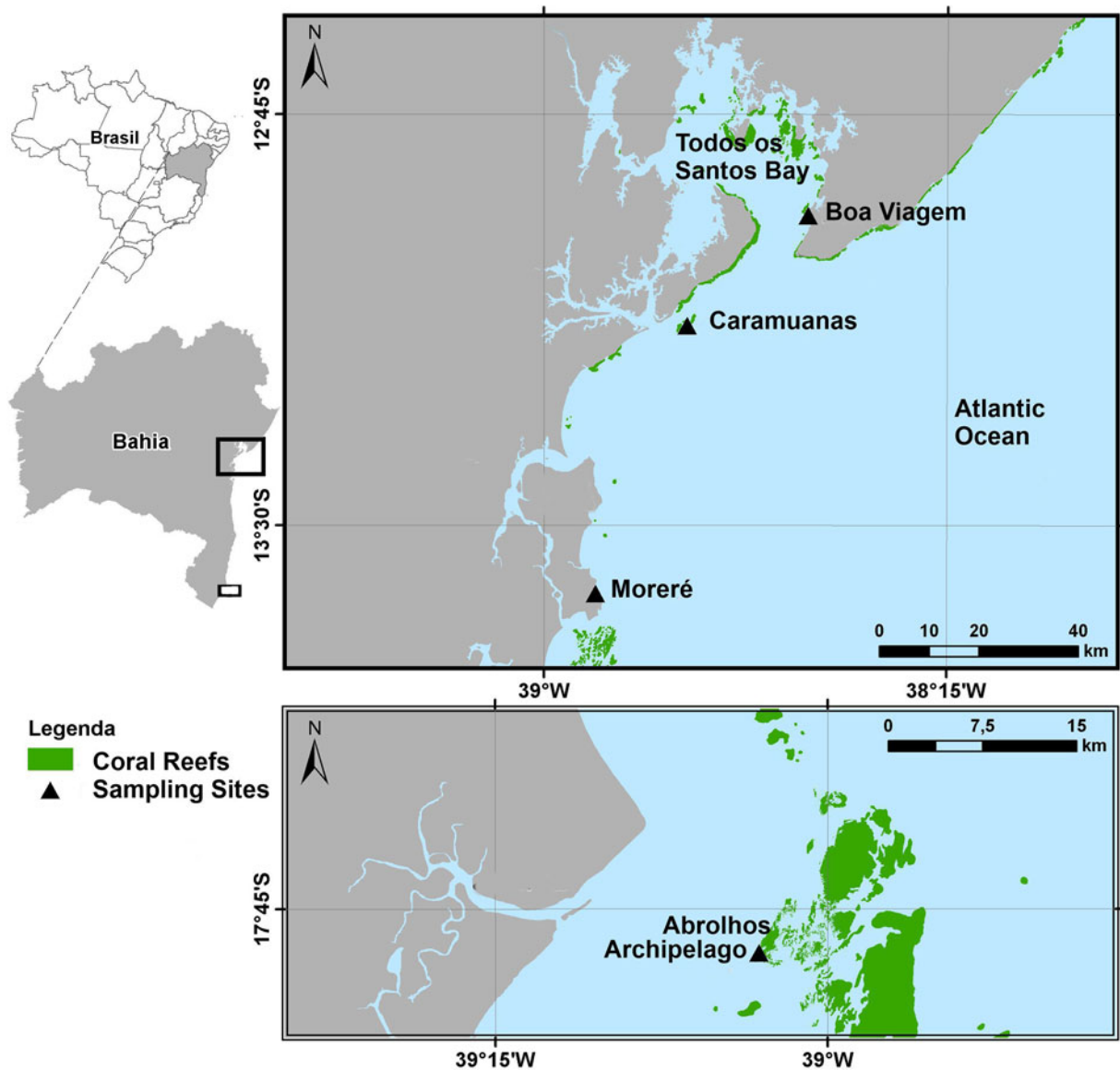
Figure 1. Map of the study area and sampling sites, including Boa Viagem reef in the Todos-os-Santos Bay, and reefs from the South Littoral: Caramuanas, Moreré (Boipeba Island), and Abrolhos Archipelago.

For *M. harttii* colonies, corallite diameter, number of septal elements, mean length from septa first cycle to columella, and mean distance between calice were statistically different among populations with higher values for colonies from Moreré; corallite number and mean distance between calice showed higher values in the Caramuanas population (Tables 2 and 3). Contrasting with *M. hispida*, teeth and septal spines were highly inconspicuous among the populations (Figure 3). On the other hand, *M. braziliensis* showed significant difference among the populations for the number of septal elements (higher in the Moreré population) and the mean columellar fossa depth (mm) (higher values in the Abrolhos population) (Table 3). As supported by SEM images, septal spines vary among the populations (Figure 4). In the Moreré population, spines are conical with rounded edges, forming a perpendicular line along the septal teeth (Figure 4A, B). This line is a continuous deposition of aragonite fibres composed by two larger granules separated by a smaller one (Figure 4E). The spines from Abrolhos colonies are conic and wide at the base, being distally bi- or trifurcated (Figure 4B, E). This population has an exquisite sequence of three spines forming a slight curvature at an obtuse angle below the septal tooth. In the Caramuanas population, bi- or trifurcated conical spines are intercalated and

sparsely distributed along the lateral septum surface (Figure 4D, F). Similarly, the spines of septal teeth are multidirectional and bifurcated, being irregularly arranged (Figure 4G, H).

Comparing *M. hispida*, *M. harttii*, and *M. braziliensis*, significant differences were observed in the non-parametric analysis for all the analysed characters (N_{cor}: $P = 0.0000$; D_{ica}: $P = 0.0000$; D_{icor}: $P = 0.0000$; N_{sep}: $P = 0.0000$; C_{sep}: $P = 0.0000$; P_{col}: $P = 0.0000$; D_{cor}: $P = 0.0000$). The PCA shows 76.10% in axis 1 and 12.21% in axis 2 (Figure 5). The results showed differentiation between the species; colonies of *M. braziliensis* from both areas showed a distinct group from other species populations. *Mussismilia harttii* populations from Caramuanas and Moreré showed a trend in cluster in different groups, suggesting that for this species, the environment may play an important role in the morphological pattern of colonies. There is an intersection between individuals of *M. harttii* from Moreré with *M. hispida*; the overlapping of the characteristics in *M. harttii* is strongly marked in the variant 'conferta' in which the phaceloid development is feebly distinguishable (Figure 5). In the PCA, colonies of *M. harttii* from Moreré showed high convergence with *M. hispida* morphology. In contrast, the microstructures are conspicuously different, being a valuable tool for defining the interspecific limits

Table 2. Mean, standard deviation (SD), and range of *M. hispida*, *M. harttii*, and *M. braziliensis* morphological characters measured in the present study

Morphological traits	Sample site	M. hispida			M. harttii			M. braziliensis		
		Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
N_{cor}	Boa Viagem	8.50	2.84	6–15						
	Caramuanas	8.59	2.76	4–16	6.64	2.09	4–11	24.31	3.90	19–32
	Moreré	8.15	2.51	4–14	5.31	1.77	2–8	25.29	4.04	14–31
	Abrolhos	–	–	–	–	–	–	22.13	2.15	19–26
D_{ica}	Boa Viagem	18.36	1.83	16–22						
	Caramuanas	17.13	2.48	13–23	14.49	3.28	9–20	10.43	1.20	8–13
	Moreré	18.57	1.95	14–23	18.57	1.95	12–28	10.32	1.29	9–13
	Abrolhos	–	–	–	–	–		9.14	2.25	3–11
D_{icor}	Boa Viagem	22.25	2.25	17–26						
	Caramuanas	20.94	2.90	16–26	17.48	5.17	7–29	12.52	2.16	6–15
	Moreré	21.36	2.19	18–27	23.16	3.16	19–31	13.29	1.69	11–18
	Abrolhos	–	–	–	–	–		12.84	0.93	11–14
N_{sep}	Boa Viagem	45.24	3.98	38–51						
	Caramuanas	58.65	8.05	43 74	56.01	8.90	41–77	37.72	4.77	29–44
	Moreré	65.40	8.57	55–84	70.87	17.05	46–107	42.86	5.52	35–52
	Abrolhos	–	–	–				35.10	5.14	27–45
C_{sep}	Boa Viagem	11.92	1.37	9–14						
	Caramuanas	11.02	1.75	7–14	9.89	3.25	6–19	5.75	0.68	4–7
	Moreré	10.66	1.53	8–14	12.09	1.77	8–15	6.56	1.07	5–9
	Abrolhos	–	–	–				5.67	0.77	4–7
P_{col}	Boa Viagem	12.59	1.79	11–17						
	Caramuanas	9.19	2.23	5–13	10.63	2.83	6–16	4.49	1.21	2–7
	Moreré	9.18	1.98	6–13	10.29	2.30	5–15	5.17	0.78	4–6
	Abrolhos	–	–	–				5.85	0.84	5–8
D_{cor}	Boa Viagem	3.37	1.16	2–6						
	Caramuanas	3.67	1.64	1–9	9.78	3.12	4–19	1.41	0.27	1–2
	Moreré	3.46	1.19	2–6	8.03	2.64	4–13	1.62	0.87	1–5
	Abrolhos	–	–	–				1.74	0.48	1–3

N_{cor}, corallite number in an area of 5 cm^2; D_{ica}, inner diameter of callice, mean of the two major inner axes of theca cavity margins (mm), D_{icor}, corallite diameter, mean of the two major axes of the outer margins of corallite (mm); N_{sep}, number of septal elements; C_{sep}, mean length from septa first cycle to columella (mm); P_{col}, mean columellar fossa depth (mm); D_{cor}, mean distance between calice, based on the inner theca margin between two close corallites (mm).

(Figure 3). The septal spines in *M. hispida* and *M. harttii* occur in greater densities than those in *M. braziliensis*. The spines are slender in *M. hispida*; curved on the top in *M. harttii*; and thicker, bi- and trifurcated, with a grainy appearance in *M. braziliensis*.

Discussion

The results obtained in this study corroborate previous studies regarding the establishment of morphotypes within the *Mussismilia* genus, with strong evidence of macro- and micro-morphological variation, majorly in *Mussismilia hispida* and *Mussismilia harttii* (Verrill, 1868, 1901; Laborel, 1967, 1969/70). As observed, *Mussismilia* species are well-defined, and the morphological patterns may be attributed to natural variation and the influence of fine-scale environmental elements, as well. Moreover, Nogueira *et al.* (2015) demonstrate that variable morphologies of *Mussismilia* related to the structural complexity of the colony may act on the richness and abundance of the associated fauna. This makes the approach also important for

understanding the composition and structure of the local benthic community – indicating that studies dealing with coral morphologies may have strong implications on the knowledge of the biodiversity.

Before the present study, data dealing with morphological variation among Brazilian corals were restricted to four species (all of them also distributed in the Caribbean): *Siderastrea stellata*, *Siderastrea radians*, *Favia gravida*, and *Montastraea cavernosa* (Amaral, 1994; Neves, 2004; Santos *et al.*, 2004; Amaral and Ramos, 2007; Menezes *et al.*, 2013). The results obtained with the endemic *Mussismilia* support the relevance of the corallite analysis and the micromorphology for the scleractinian taxonomy.

The close congeners, *S. stellata* and *S. radians*, are brooding corals with panmictic populations that occur sympatrically along the Brazilian coast – the gene flow across long geographical distances has been also attributed to levels of intraspecific variation in the species (Neves *et al.*, 2008). Indeed, the morphotypes of *S. stellata* and *S. radians* show a high overlap of diagnostic characteristics, being a source of misinterpretations, and thus challenging
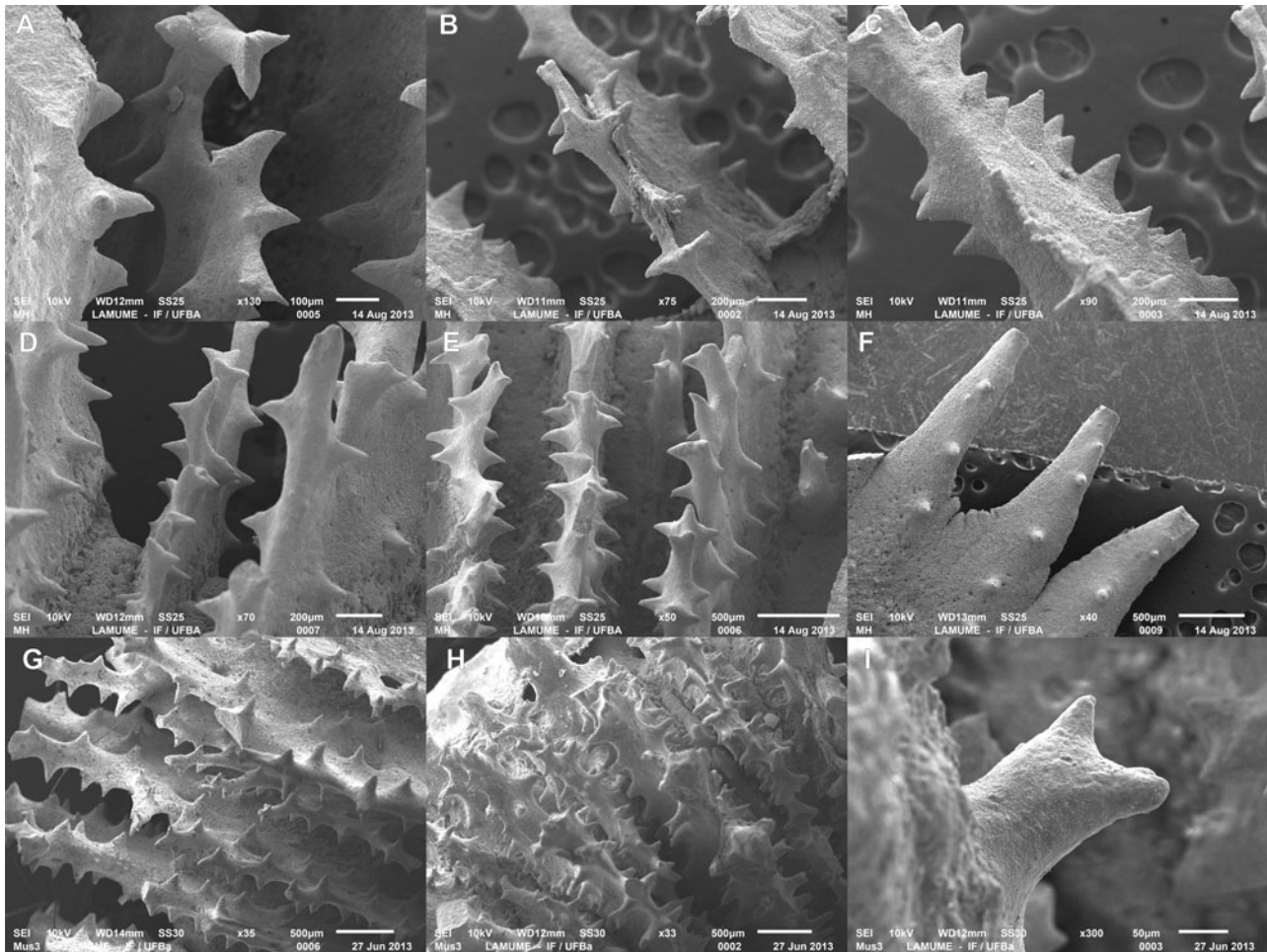
Figure 2. SEM images of micromorphological characters of *Mussismilia hispida*. Variation of septal teeth and spines in colonies from three reefs: Caramuanas (A–C), Moreré (D, E), and Boa Viagem (F). Septal teeth, bi/trifurcated, and distribution of spines along the septal face and margins (G, H). Details of septal spine (I).

researchers worldwide. However, as seen to *Mussismilia*, micromorphology of the theca and radial structures has also proved to be an essential tool for *Siderastrea* taxonomy (Neves, 2004).

The results indicate that skeleton variation occurs on two scales in *Mussismilia*: macro and micromorphological. According to the literature, teeth and septal spines are diagnostic
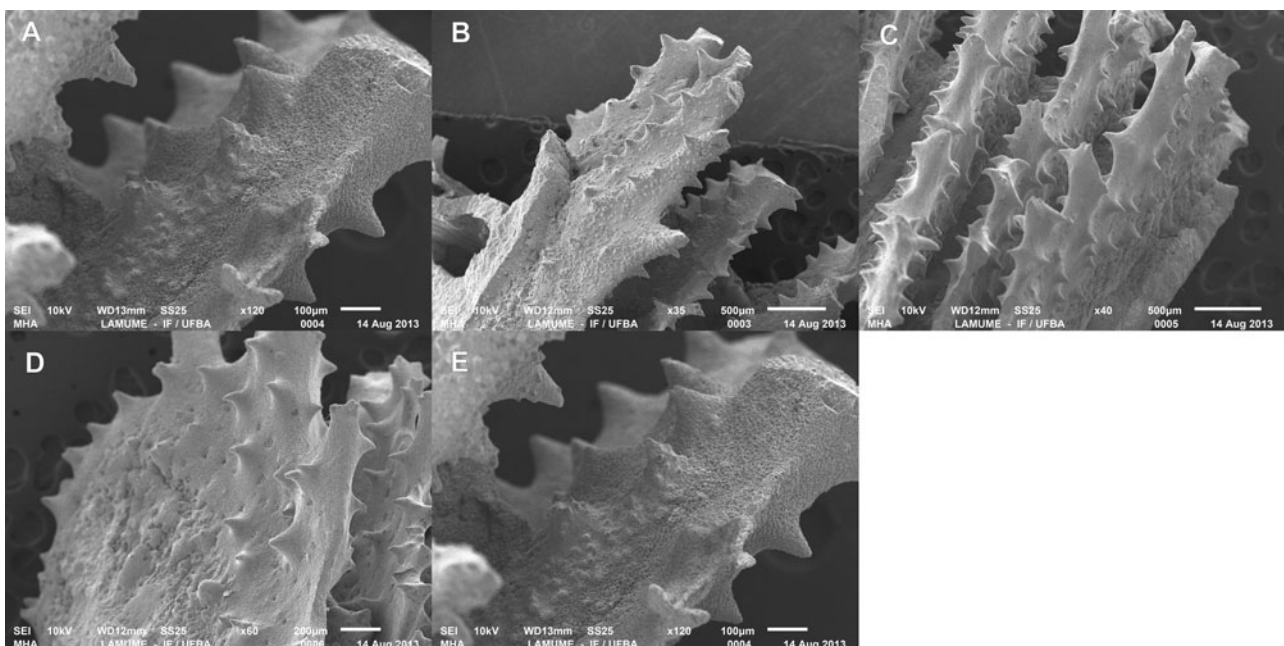

Figure 3. SEM images of micromorphological characters of *Mussismilia harttii*. Variation of septal teeth and spines in colonies from two reefs. Moreré (A, B) and Caramuanas (C, D). Distribution of spines on the septa face and margins (E).

Table 3. Results of Kruskal–Wallis test among the populations of each *Mussismilia* species

Morphological traits	M. hispida		M. harttii		M. braziliensis	
	KW–H (3;52)	P	KW–H (2;42)	P	KW–H (2;83)	P
N_{cor}	0.8676	0.8332	6.5925	0.0370*	5.36	0.0686
D_{ica}	3.0276	0.3874	8.4774	0.0144	1.6552	0.4371
D_{icor}	1.9848	0.5756	12.8014	0.0017*	0.5552	0.7576
N_{sep}	27.333	0.0000*	12.0259	0.0024*	9.7955	0.0075*
C_{sep}	3.8234	0.2812	7.3713	0.0251*	4.7795	0.0917
P_{col}	18.321	0.0815	0.5748	0.7502	6.8161	0.0331*
D_{cor}	0.4038	0.9395	9.2299	0.0099*	2.5567	0.2785

N_{cor}, corallite number in an area of 5 cm^2; D_{ica}, inner diameter of callice, mean of the two major inner axes of theca cavity margins (mm), D_{icor}, corallite diameter, mean of the two major axes of the outer margins of corallite (mm); N_{sep}, number of septal elements; C_{sep}, mean length from septa first cycle to columella (mm); P_{col}, mean columellar fossa depth (mm); D_{cor}, mean distance between calice, based on the inner theca margin between two close corallites (mm).

characters for Mussidae corals (Neves *et al.*, 2006; Budd and Stolarski, 2009; Budd *et al.*, 2012a, 2012b). Indeed, our SEM images reveal that the characteristics and arrangement of microstructures differ interspecifically, supporting the species' identities.

Although consistent differences in the macromorphology were not statically supported among localities (except for two characters: diameter of corallites and the number of septa), all the four morphs described by Verrill (1901) for *M. harttii* were found and analysed in Bahia State – namely, 'conferta', 'confertifolia', 'laxa', and 'intermedia'. It is irrefutable that spaced corallites with phaceloid development represent a natural tendency of this species. However, some degree of morphological overlapping may occur between the morph 'conferta' and *M. hispida*, particularly because of the fusion of the adjacent exotheca (joining the nearby corallites superficially). Only by checking the lower development zone of the corallites, the phaceloid development of *M. harttii* is evident. Hence, the morph 'conferta' is not easily distinguishable from *M. hispida in situ* through visual identification of the colony top (which makes the method of identification based on images from video transects unreliable).
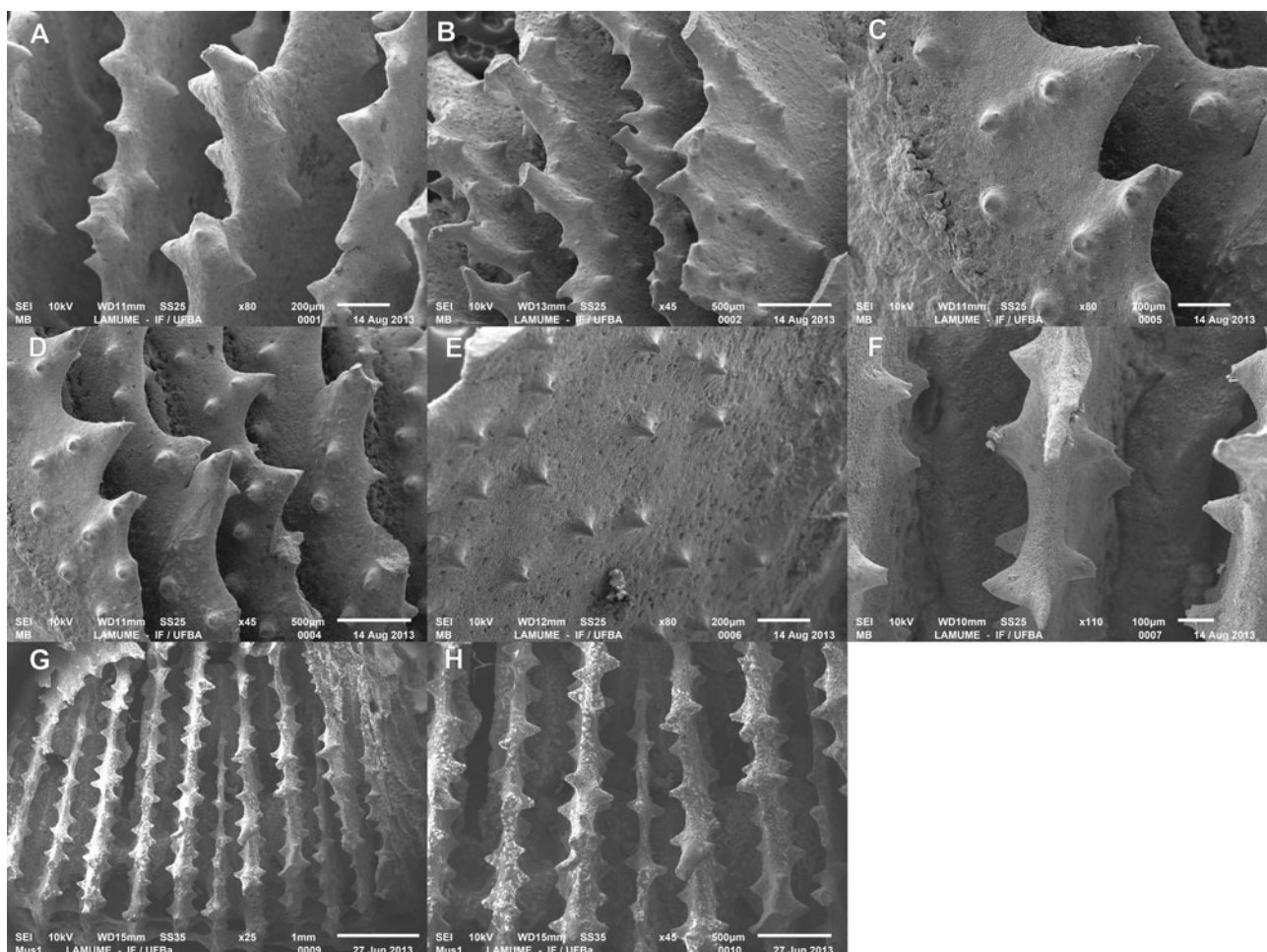

Figure 4. SEM images of micromorphological characters of *Mussismilia braziliensis*. Variation of septal teeth and spines in colonies from three reefs. Moreré (A, B), Abrolhos Archipelago (C, D), and Caramuanas (E, F). Septal teeth and distribution of spines on the septa face and margins (G, H).
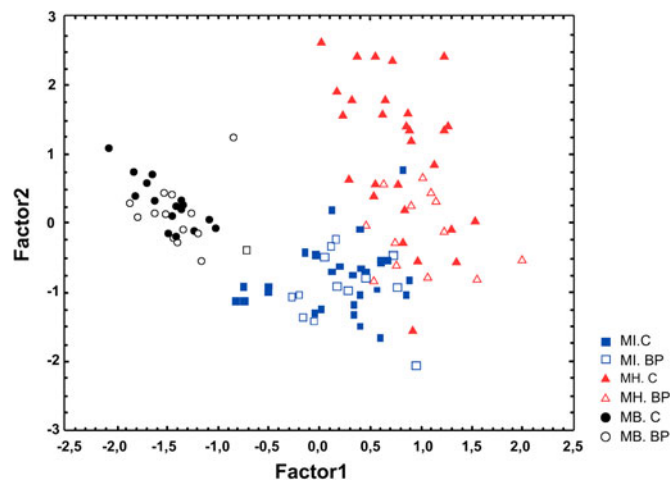
Figure 5. Graph and result of PCA with morphological variables of the species of *M. hispida* (MI.C; MI.BP), *M. harttii* (MH.C; MH.BP), and *M. braziliensis* (MB.C; MB.BP). Distribution of characters in the axes and factors that most influenced the analysis (MI.C, Caramuanas; MI.BP, Moreré; MH.C, Caramuanas; MH.BP, Moreré; MB.C, Caramuanas; MB.BP, Moreré).

In this study, the morphotypes of *M. harttii* and *M. hispida*, occurred sympatrically in three reefs Caramuanas, Moreré, and Boa Viagem, and biotic and abiotic factors are expected to vary in some scale at each locality. Similarly, Amaral and Ramos (2007) found distinguishable morphological patterns in *Favia* corals at two different spatial scales, within different environments of a single reef and between distinct reefs. Additionally, budding may also act as a trigger for morphological divergences, as observed in the morphs 'hispida hispida' (with regular corallites) and 'hispida tenui-septa' (with irregular corallites) (Amaral *et al.*, 2009).

The development of new polyps in coral colonies (extracalicinal *vs* intracalicinal budding) is an important taxonomical attribute, being the intracalicinal budding advantageous for colonial growth, once adding new mature elements it may improve the success of reproductive activity. Mixed budding patterns in a single species have been observed in *S. stellata* and *F. gravida* (Neves, 2004, authors' personal communication). However, the development of *Mussismilia* colonies has been strictly attributed to the intracalicinal budding (Laborel, 1969/70), with irregular polyps (or 'lobed') of *M. hispida* evidence of the poliestomodeal pattern. Indeed, mixed budding is uncommon among most scleractinian corals, and extracalicinal budding is unknown for *M. hispida* and *M. harttii*. Therefore, a new colonial development strategy is here reported for the genus.

Finally, the characters selected in this study were consistent with the definition of the interspecific limits of *Mussismilia* corals, ensuring Verrill's morphotypes, and the relevance of the traditional skeleton analysis for the taxonomy of reef-building genera.

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