

Evaluation of *Eunicea* species morphological diversity through sclerite analysis

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Abstract:

Candelabrum gorgonians from the genus *Eunicea* (Lamoroux, 1816) are the most diverse and abundant group of octocorals in the Caribbean reef ecosystems. With 15 valid species and over 30 more assigned to this genus, it's been proven hard to discriminate emerging species as there's an overlap of morphological traits among several specimens. In this study, colonies from Florida Keys, Panama, Cartagena, San Andrés and Curaçao, were evaluated through their sclerome to determine whether they are new morphotypes due to phenotypic plasticity or entirely different species. This was achieved by assessing morphological characters of sclerites, the most telling feature with these marine organisms. As results, 4 new morphotypes were described with their respective keys. Some morphological innovations of these new morphotypes are thought to be due to phenotypic plasticity whilst others cannot be fully explained by this phenomenon.

Keywords: *Eunicea*, sclerites, morphological traits, morphological diversity, reefs, Caribbean.

Background:

Octocorals in tropical shallow reefs are an incredibly diverse and abundant group forming “animal forest” habitats of great complexity (Sánchez, 2016). Their dominance over hard corals on certain reef systems has been related to their staggering recovering capacity to environmental disturbances and it's plausible that their abundance may increase after events of hard coral mass mortality (Sarmiento & Sánchez, 2014)(Sánchez et al., 2019). Octocorals, better known as Gorgonians, are crucial species in reef systems given that their three-dimensional nature provides structural complexity to the ecosystem, making it possible to harbor other species, acting as their habitat and refuge (van de Water et al., 2018). Apart from their importance in reefs, octocorals have gained recognition for the secondary metabolites they produce to compete for space, reproduction, avoid predation and the maintenance of an untarnished surface. Terpenoid structures produced by these marine organisms possess biomedical potential and are already in preclinical or clinical development (Gross & König, 2006). In the Caribbean, octocorals thrive and have high relative abundance in comparison to their scleractinian counterparts, making them an important target of studies in this region (Gómez et al., 2015).

Eunicea (Cnidaria: Anthozoa: Octocorallia: Plexauridae), commonly known as the ‘sea candelabrum’, is a genus of gorgonian corals endemic to the Caribbean region and distributed along the Tropical Western Atlantic Ocean (TWA) (Sánchez, 2009). *Eunicea* species have a

mutualistic symbiosis with zooxanthellae dinoflagellates restricting their distribution to the photic zone (1-70m in depth). Among the very diverse octocorals, *Eunicea* prevails as the most speciose genus with 15 species considered valid along with 30 more species assigned to it (Sánchez, 2016). There's no clear understanding about valid species in this genus because some may have been described under ecophenotypic variation (Sánchez, 2009). In addition, discriminating species proves challenging due to the occurrence of sympatric species given that there is an overlap of morphological traits among several species (Sarmiento & Sánchez, 2014).

In order to evaluate the morphologic diversity of *Eunicea*, several studies use both colony biometrics as well as morphometric traits from the sclerites found in these animals (Sánchez, 2009) (Sarmiento & Sánchez, 2014). Gorgonian sclerites are skeletal elements made of calcium carbonate found in the soft, elastic choenechyme of the organism. These microscopic calcareous pieces serve both as skeletal support as well as a defense mechanism against predators and prove to be a useful and powerful tool for species identification (Etnoyer et al., 2006). The entire sclerite composition of an octocoral, their sclerome, is comprised of spicules of different shapes and sizes that serve distinctive purposes, thus found in different parts of the animal (Lewis & Wallis, 1991) For example, in *Eunicea* species, certain spicules are only found in the tentacles and are usually smaller in size when compared to other spicules found in the rest of the animal (Bayer, 1961). Because of their distinct shapes, sclerite morphology is deemed as the single most important character for gorgonian corals identification, reason as to why there are several guides that aim to classify these structures in order to create a standardize way to evaluate species (Etnoyer et al., 2006). Although there are several different types of sclerites in the octocoral bauplan, club sclerites, the ones found in the outermost layer of the animal, seem to be most useful when discriminating species from the *Eunicea* genus (Sánchez, 2009).

It's imperative to better understand the morphological diversity of *Eunicea* species as gorgonians not only represent the greater biomass on various Caribbean reefs but also because its forecasted that under the changing climate this group of corals may become the dominant fauna on reefs as they are seemingly undisturbed by acidification and temperature anomalies (Gómez et al., 2015). Generating knowledge about reefs is pressing because in the Caribbean alone they provide goods and services estimated at 4.6 billion USD. If conservation and management strategies aren't set, national economies and coastal communities will suffer from substantial economic losses in the future (Burke & Maidens, n.d.). In addition, further studying octocorals is a promise to medicine as these organisms produce potential compounds to treat human diseases. Therefore, the aim of this study is to evaluate and describe the morphological diversity of *Eunicea* species through their sclerites and determine whether they could be assigned to morphotypes or potential new species.

Methods:

Sampling

Colonies of *Eunicea* were collected at five locations in the Caribbean: Florida Keys, USA; Bocas del Toro, Panama; Cartagena, Colombia; San Andres, Colombia and Curaçao, Brazil within a depth range between 5 and 45 m. Morphotype 1 was collected at 10m, Morphotype 2 between 15-20m, Morphotype 3 at 16m and Morphotype 4 between 15-26m.

At least three colonies of each morphotype by locality were sampled. A piece of tissue was preserved in DMSO for DNA extraction, and a piece of dry tissue of each colony was preserved for the posterior morphological identification and sclerites analyses. In total 43 samples from different morphotypes of the *Eunicea* genus were collected.

All samples collected used for morphological analyses can be found at the Natural History Museum of Universidad de Los Andes under the following IDs: ANDES-O 7561, ANDES-O 7585, ANDES-O 7586, ANDES-O 7602.

Sclerome extraction

For sclerome analysis the protocol proposed by Janes was followed (Janes, 2008). Isolation of sclerites is achieved by placing the tissue sample in a test tube and adding 20mL of 5.25% sodium hypochlorite. A cloth is placed on top of the test tube preventing dust to enter the sample and allowing gases to escape. Samples were to rest until tissue was completely dissolved and no more bubbles formed at the surface. Every few hours the test tubes were gently swirled. Once the tissue was dissolved, sodium hypochlorite was siphoned with a pipette. Sclerites were left undisturbed in the test tube. Later, 0,5mL of Hydrogen peroxide (buffered to a neutral pH) were added. Sclerites were rinsed with 20mL of distilled water to the test tube while stirring it gently. Sclerites were allowed to settle at the bottom and water was siphoned with a pipette. The rinsing process was repeated around 6 times (Janes, 2008). Sclerites where isolated for both the entire animal and just the polyp to better identify which sclerites were found in which part of the bauplan of the animal.

Morphology description and analyses

Once sclerites were isolated, these were visualized under the microscope on different focal distances (4X & 10X). Photographs were taken under the same focal distances to later create a key for each morphotype. All morphotypes had around 130 photographs that were analyzed. Such photographs were taken with the SWIFTCAM camera and the SwiftImaging application. Sclerites where classified using the Illustrated Key by Bayer (1961) and the species descriptions made by Sanchez (2009). After the sclerite classification, keys were created with the program Adobe Photoshop 2021. All morphotypes were compared to *Eunicea clavigera* because in

preliminary phylogenies using these individuals, they are nearest to this pre-existing species. For such comparisons the taxonomic keys proposed by Bayer (1961) and Sánchez (2009) were used.

Results:

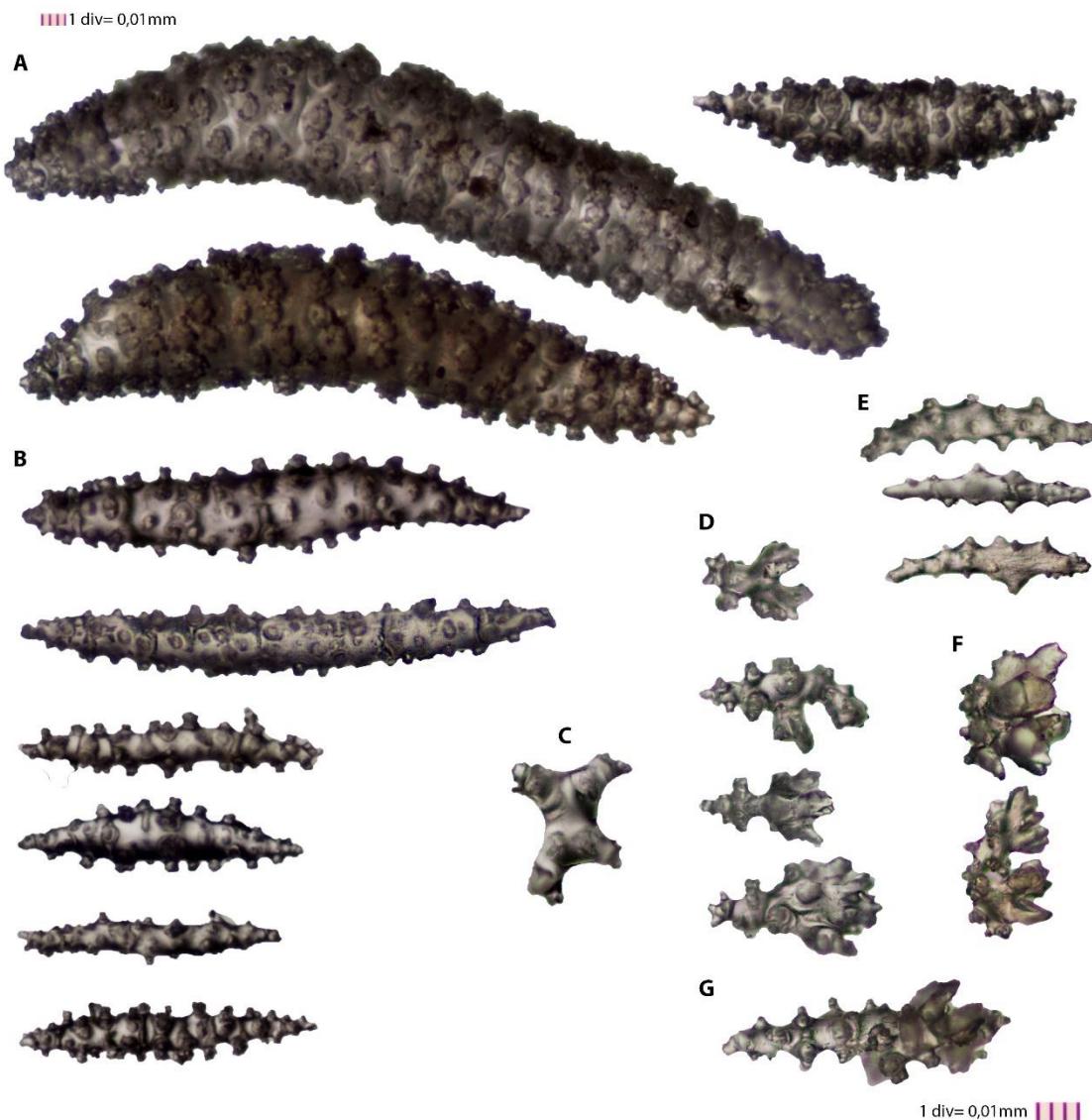


Figure 1. Morphotype 1 (MI): A. Spindles B. Axial sheath spicules C. Butterfly spicules D. Club sclerites under different shapes including common and tetrahedral E. Anthocodial spicules F. Foliate spheroids G. Torches. A & B are at 4X magnification. C, D E F & G are at 10X.

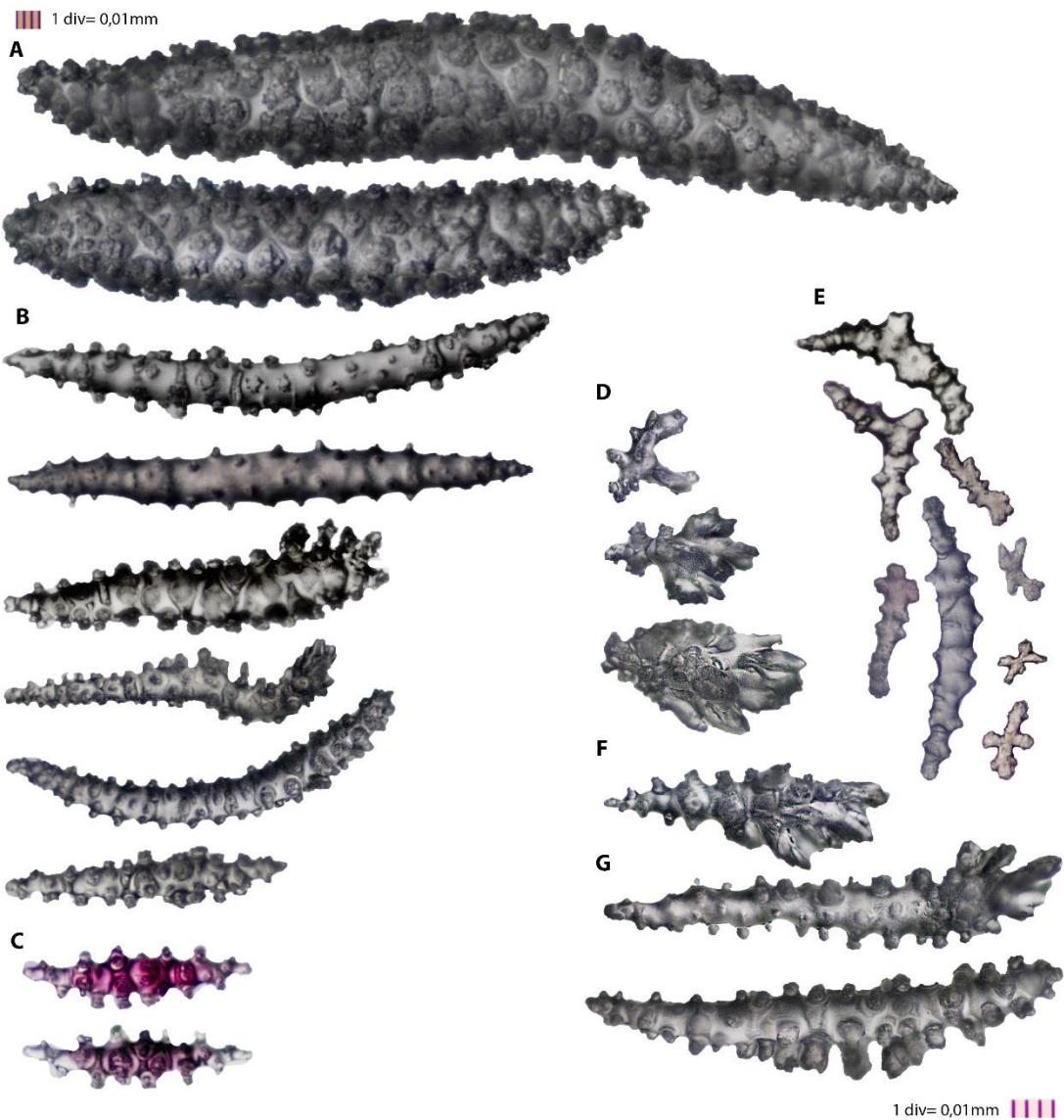


Figure 2. Morphotype 2 (M2): A. Spindles B. Axial sheath spicules C. Axial sheath spicules with pink coloration D. Club sclerites under different shapes including leafy, common and tetrahedral E. Anthocodial spicules (Some have a slight orange tint to them) F. Torch under greater magnification G. Axial sheath spicules with ornamentation at the tip (top) and unilaterally spinned forms (bottom) under greater magnification. A, B & C are under 4X. D, E, F & G are under 10X.

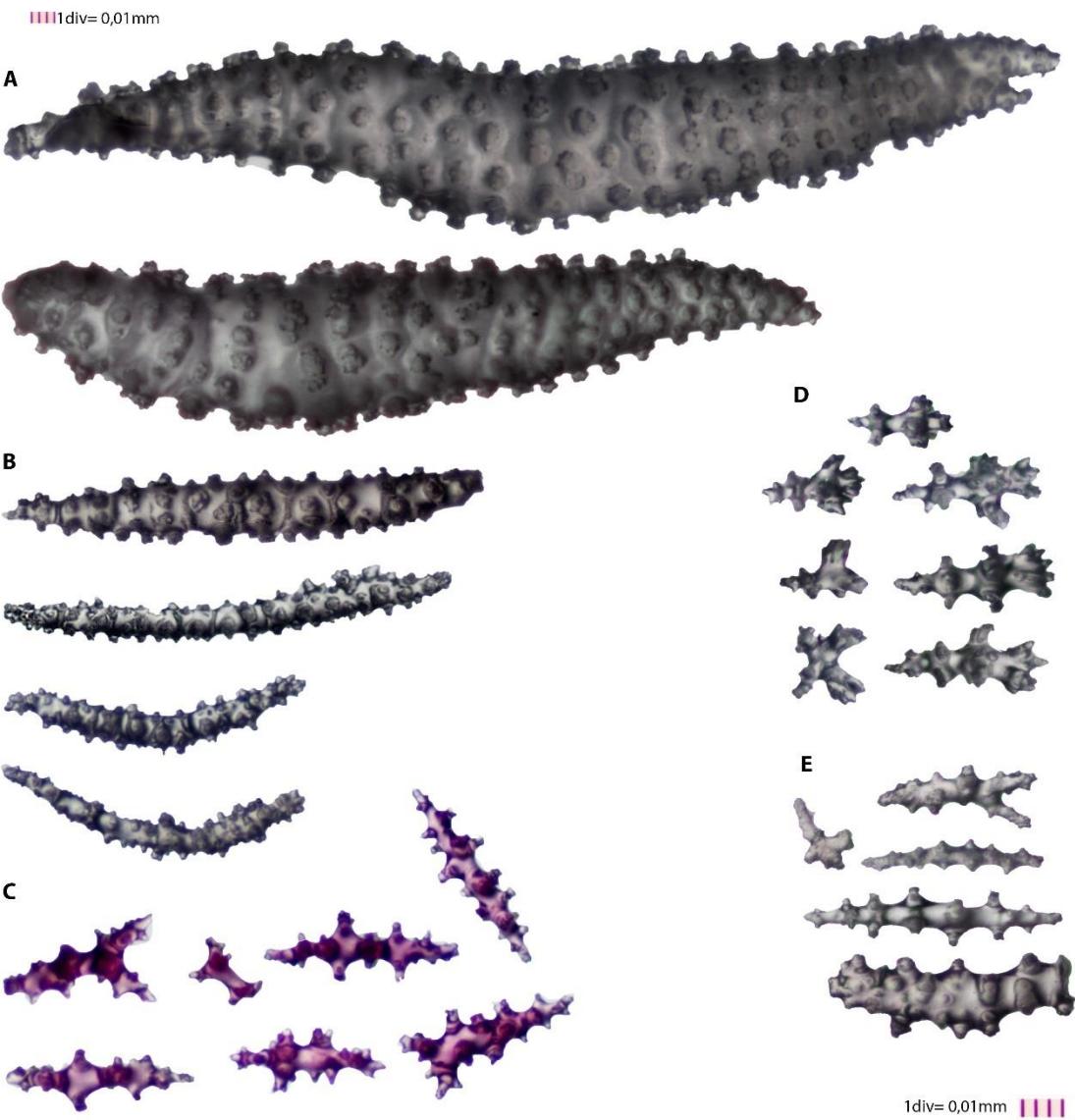


Figure 3. Morphotype 3 (M3): A. Spindles B. Axial sheath spicules C. Axial sheath spicules with pink coloration D. Club sclerites and torches E. Anthocodial spicules. A, B & C are under 4X. D & E are under 10X.

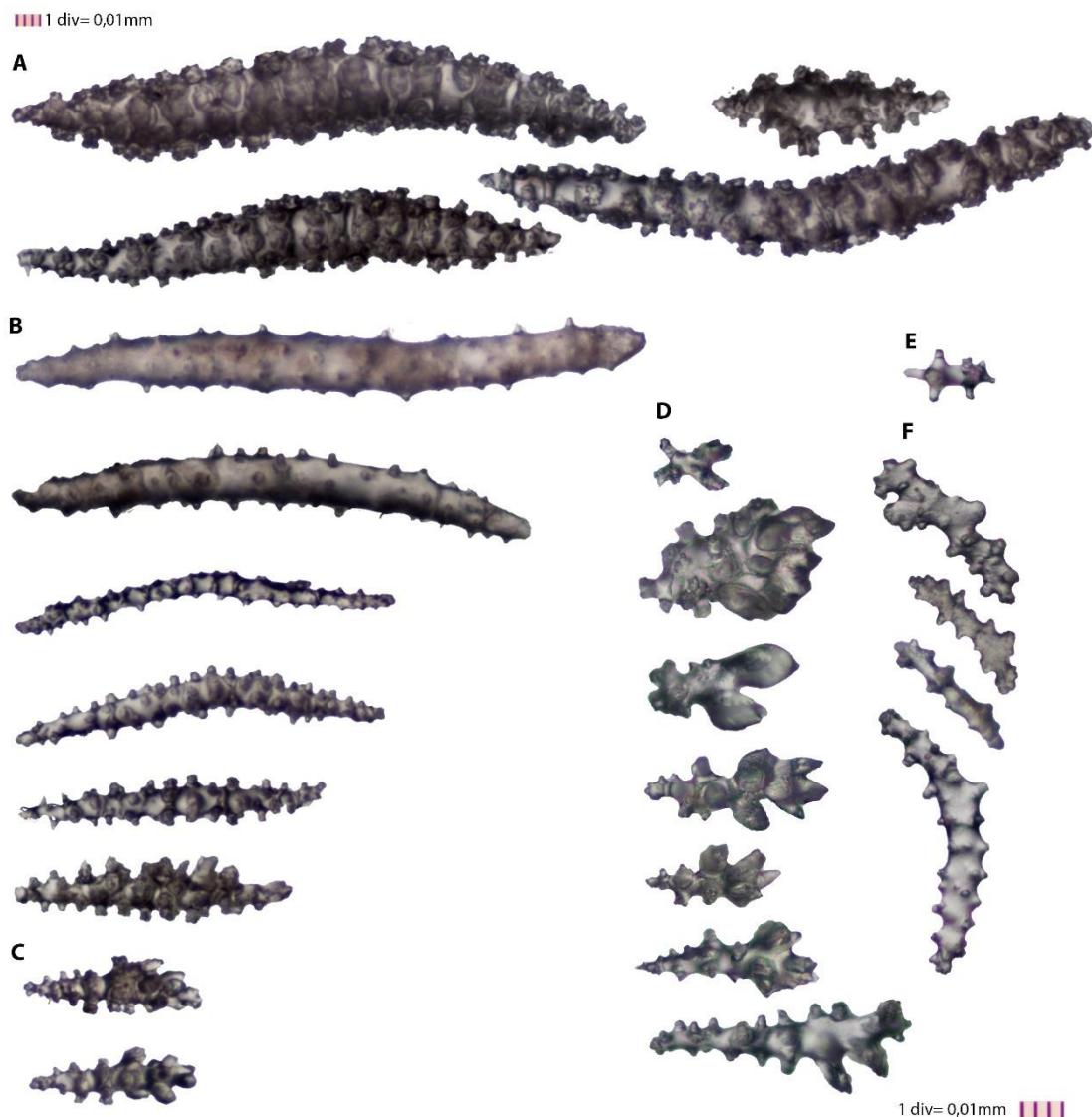


Figure 4. Morphotype 4 (M4): A. Spindles B. Axial sheath spicules C. Torches D Club sclerites under different shapes including leafy, common and tetrahedral E. Octoradiate spicules F. Anthocodial spicules. A, B & C are under 4X. D, E & F are under 10X.

Discussion and conclusion:

All morphotypes show some type of innovation when compared to *E. clavigera*. Morphotype 1 (M1) possess spicules that haven't been recorded in *E. clavigera* such as the tetrahedral club sclerites (Figure 1. D. top), foliate spheroids and butterfly spicules. Foliate spheroids are common in the genus *Eunicea* and are present in three species, according to Bayer, *Eunicea asperula*, *E. knighti* and *E. calyculata* forma *calyculata* (Bayer, 1961). Butterfly spicules, although not as common in *Eunicea* appear on *Eunicea laxispica* on the axial sheath layer

(Bayer, 1961). However, tetrahedral clubs are entirely new to the genus. In addition to the innovations mentioned above, M1 also presents the loss of the purplish/pinkish colored axial sheath spicules that is common in *E. clavigera*. Although the appearance of foliate spheroids and butterfly spicules are shared characteristics with the four species mentioned above, the new club sclerites and the loss of the colored spicules represents an innovation both in the genus level and the individual's level.

M2 also has tetrahedral club sclerites (Figure 2. D. top) and retains the colored axial sheath spicules. However, the anthocodial spicules present different forms than those typical in *E. clavigera*. These spicules with four discernible tips seem to be present in *Eunicea mammosa* and *E. palmeri* as tentacular rods (Bayer, 1961), making them a shared characteristic between these morphotype and preexisting species. The slight coloration of some of these anthocodial spicules appears to be new when compared to *E. clavigera* and the two species that carry similarly shaped rods. Furthermore, this morphotype has spicules with different ornamentations on the axial sheath (Figure 2. G) with denser tips, that can be considered torches, and unilaterally spinned forms. Torches are very common in the *Eunicea* genus, but unilaterally spinned forms occur on some species. These spicules often occur in the outer rind of *E. palmeri*, *E. calyculata* and *E. knighti* and the outer cortex on *E. laciniata* (Bayer, 1961). In similar fashion to M1, M2 shares certain characteristics with other *Eunicea* species that *E. clavigera* lacks. In that sense, these characteristics are innovative when compared to this sister species but aren't innovative for the entire genus. Again, the only characteristic that appears to be new for the entire genus are the tetrahedral clubs.

Morphotype 3 maintains various characteristics present in *E. clavigera*. However, in M3, the colored axial sheath spicules present different forms than the typical ones observed in *E. clavigera*, as they have forked ends to produce tripods (Figure 3. C top left & bottom right). This tripod-colored spicules are rare in *Eunicea* as they have only been recorded to be on *E. succinea*, *E. laxispica* and now on this morphotype (Bayer, 1961). On the other hand, this morphotype, along to M1 and M2, also possesses tetrahedral clubs. Finally, the shape of the anthocodial spicules varies greatly as it they are tripod-shaped, needle-like and some have four tips. Tripod-shaped anthocodial spicules have not yet been described in any species, making it a possible innovation for this morphotype and the entire genus.

Regarding Morphotype 4, this individual no longer has colored axial sheath spicules. However, like the other individuals here analyzed, this morphotype has tetrahedral clubs. In addition, this morphotype possesses the octoradiate spicule (Figure 4.E) which has been recorded to be on *E. asperula* and *E. clavigera* (Sánchez, 2009). Similarly, to M2, M4 has unilaterally spinned spicules (Figure 4. B 6th from top to bottom). Given that the octoradiate and the unilaterally spinned spicules are shared with at least one described species, the only innovation in the genus

level would be the tetrahedral club. However, the loss of pigmentation on the axial sheath spicules should be noted as an innovation at the individual's level.

In sum, all morphotypes here analyzed share a new characteristic that has never been reported on any *Eunicea* species, which is the tetrahedral club. However, all morphotypes share innovations when compared to their sister species *Eunicea clavigera*. Whether the innovations are at a genus level or at an individual's level, these morphotypes here analyzed appear to be different to *E. clavigera*. Such differences can be accounted to phenotypic plasticity, genetic differentiation, or an effect of both variables (Prada et al., 2008).

Cases of phenotypic plasticity have been recorded in the *Eunicea* genus, where it was proven that colonies of *E. flexuosa* varied in their plastic response according to whether they were deep-water or shallow-water colonies. The plastic response of this genus of octocorals extends to the sclerome and has been correlated with differences in water motion, light, etc (Prada et al., 2008). Given that the samples analyzed here were taken under varying bathymetric conditions, which in turns means a variation in water motion and light, it's possible that the innovations presented in each morphotype could be a plastic response to their specific environmental conditions. However, cases recording phenotypic plasticity in octocorals tend to report changes in the size rather than the form and color of the sclerites, meaning that phenotypic plasticity alone couldn't probably explain all the innovations seen in this study (Clavico et al., 2007)(Rowley et al., 2015). Considering that these morphotypes are showing novelties in shapes and coloring, its plausible that genetic differentiation could account to those changes that can't be solely explained by plasticity. This aligns with the preliminary phylogeny obtained by Sarmiento in which these 4 morphotypes were located as sister clades to *Eunicea clavigera*. However, it's necessary to do robust molecular analyses, such a population structure analysis, to better determine if this morphotypes are genetically different to *E. clavigera* and can be considered new morphotypes or species (Prada et al., 2008).

Studies like this are necessary to better understand the structure of octocoral communities in the Caribbean, a zone that depends highly on the wellbeing of coral reefs. Better understanding the diversity of this genus is imperative under the future forecasts for our changing oceans, where octocorals might become the dominant fauna in this region of the Atlantic. Furthermore, given the pressures that our oceans are facing, knowing the different morphotypes of this genus is key to design strategic and efficient conservation projects in the region.

References:

Bayer, F. M. (1961). *STUDIES ON THE FAUNA OF CURAÇAO AND OTHER CARIBBEAN ISLANDS: No. 55. The shallow-water octocorallia of the West Indian Region.*

Washington, DC, EEUU: Smithsonian Institution.

Burke, L., & Maidens, J. (n.d.). *Reefs at Risk in the Caribbean*. 84.

Clavico, E. E. G., De Souza, A. T., Da Gama, B. A. P., & Pereira, R. C. (2007). Antipredator Defense and Phenotypic Plasticity of Sclerites From *Renilla muelleri*, a Tropical Sea Pansy. *The Biological Bulletin*, 213(2), 135–140. <https://doi.org/10.2307/25066629>

Etnoyer, P., Cairns, S. D., Sánchez, J. A., Reed, J. K., Lopez, J. V., Schroeder, W. W., Brooke, S. D., Watling, L., Baco-Taylor, A., Williams, G. C., Lindner, A., France, S. C., & Bruckner, A. W. (2006). *Deep-Sea Coral Collection Protocols. NOAA Technical Memorandum NMFS-OPR-28*. 56.

Gómez, C. E., Paul, V. J., Ritson-Williams, R., Muehllehner, N., Langdon, C., & Sánchez, J. A. (2015). Responses of the tropical gorgonian coral *Eunicea fusca* to ocean acidification conditions. *Coral Reefs*, 34(2), 451–460. <https://doi.org/10.1007/s00338-014-1241-3>

Gross, H., & König, G. M. (2006). Terpenoids from Marine Organisms: Unique Structures and their Pharmacological Potential. *Phytochemistry Reviews*, 5(1), 115–141.

<https://doi.org/10.1007/s11101-005-5464-3>

Janes, M. P. (2008). *Laboratory methods for the identification of soft corals (Octocorallia: Alcyonacea)*. 14.

Lewis, J. C., & Wallis, E. V. (1991). The Function of Surface Sclerites in Gorgonians (Coelenterata, Octocorallia). *The Biological Bulletin*, 181(2), 275–288.

<https://doi.org/10.2307/1542099>

Prada, C., Schizas, N. V., & Yoshioka, P. M. (2008). Phenotypic plasticity or speciation? A case from a clonal marine organism. *BMC Evolutionary Biology*, 8(1), 47.

<https://doi.org/10.1186/1471-2148-8-47>

Rowley, S. J., Pochon, X., & Watling, L. (2015). Environmental influences on the Indo-Pacific octocoral *Isis hippuris* Linnaeus 1758 (Alcyonacea: Isididae): genetic fixation or phenotypic plasticity? *PeerJ*, 3, e1128. <https://doi.org/10.7717/peerj.1128>

Sánchez, J. A. (2009). Systematics of the candelabrum gorgonian corals (*Eunicea* Lamouroux; *Plexauridae*; Octocorallia; Cnidaria). *Zoological Journal of the Linnean Society*, 157(2), 237–263. <https://doi.org/10.1111/j.1096-3642.2008.00515.x>

Sánchez, J. A. (2016). Diversity and Evolution of Octocoral Animal Forests at Both Sides of Tropical America. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas Saco del Valle (Eds.), *Marine Animal Forests* (pp. 1–33). Springer International Publishing.

https://doi.org/10.1007/978-3-319-17001-5_39-1

Sánchez, J. A., Gómez-Corrales, M., Gutierrez-Cala, L., Vergara, D. C., Roa, P., González-Zapata, F. L., Gnecco, M., Puerto, N., Neira, L., & Sarmiento, A. (2019). Steady Decline of Corals and Other Benthic Organisms in the SeaFlower Biosphere Reserve (Southwestern Caribbean). *Frontiers in Marine Science*, 6, 73.

<https://doi.org/10.3389/fmars.2019.00073>

Sarmiento, A., & Sánchez, J. A. (2014). Evaluating the diversity of the Caribbean candelabrum corals (*Eunicea*: *Plexauridae*: *Octocorallia*). 35.

van de Water, J. A. J. M., Allemand, D., & Ferrier-Pagès, C. (2018). Host-microbe interactions in octocoral holobionts—Recent advances and perspectives. *Microbiome*, 6(1), 64.

<https://doi.org/10.1186/s40168-018-0431-6>