Morphological identification of the soft coral, *Clavularia inflata*, reveals different sclerite characters across Indonesian coral reefs

Beginer Subhan¹,*, Dietriech G Bengen¹, Sebastian Ferse²,³, Fauzan Dzulfannazhir¹, Nurlita Putri Anggraini¹, Prakas Santoso¹, Dondy Arafat¹, Lalu M. Iqbal Sani¹, Prehadi Prehadi², Hawis Madduppa¹

¹ Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, IPB University (IPB). Jl. Darmaga Raya, Bogor 16680, West Java, Indonesia
²Leibniz Centre for Tropical Marine Research (ZMT), Fahrenheitstr. 6, 28359 Bremen, Germany
³Faculty of Biology and Chemistry, University of Bremen, Leobener Str. 6, 28359 Bremen, Germany
⁴Oceanogen Environmental Biotechnology Laboklinikum, Bogor, Indonesia.
⁵Coastal and Marine Resource Management Unit of Sorong, Directorate General of Marine Spatial Management, Ministry of Marine Affairs and Fisheries, Sorong, Indonesia

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ABSTRACT

Soft corals of the genus Clavularia (Blainville 1830) have complex and difficult to distinguish morphological characteristics, making them rather difficult to identify. A paucity of information exists for Indonesian coral reefs, a complex biogeographic region at the confluence of the Pacific and Indian Ocean that covers an extensive geographic area and several marine ecoregions, is subject to several ocean current systems, and includes a high diversity of habitats. Specimens of *Clavularia inflata* were collected from eleven study sites across the Indonesian archipelago, morphologically described based on their sclerite characteristics, and compared among sites. Sclerite observations were divided into four parts for each colony, namely pinnules, crowns, calyces, and stolons. The sclerites found in *C. inflata* comprised various forms such as rods, fingers, sticks, and brackets. Morphometric measurements of *C. inflata* showed specimens from Kepulauan Seribu had the largest pinnule, calyx, and stolon sclerites compared to other locations. The maximum mean size of crown sclerites was observed for specimens from Maluku Tenggara Barat. Similarities in the shape of sclerites resulted in specimens from Kangean, Morotai, Tanjung Lesung, Bontang, Lombok and Natuna forming a cluster, while those from Maluku Tenggara Barat and Arborek were dissimilar to other sites. Smaller sclerite sizes in specimens from Arborek are likely related to strong currents at that site. In conclusion, there were variations in the size of sclerites across the eleven study sites, with several sites forming a cluster of similar sclerite dimensions. Further studies using molecular markers are suggested to further explore the possibility of cryptic species.

Introduction

More than 2,000 species of Octocorallia (Cnidaria: Anthozoa) can be found all over the world in a wide range of environments (Bayer, 1973; Fabricius and Alderslade, 2001). Octocorals have polyps with eight pinnate tentacles and sclerites, which are internal calcareous skeletal parts. Alcyonacea (soft corals) is the largest of the octocoral orders. Alcyonacea corals are abundant on coral reefs across much of the Indo-West Pacific, where they perform an essential ecological function (Tursch and Tursch, 1982; Dinesen, 1983; Dai, 1988; Riegl et al., 1995; Fabricius 1997). The current number of valid species has been estimated at approximately 3400 species of octocorals, but still needs to be verified (Williams and Cairns, 2015). For example, the World Register of Marine Species recorded as many as 3490 species of Octocorallia belonging to 370 genera, but only 3103 species were declared valid
(van Ofwegen, 2015). For Indonesian waters, Manuputty (2002) reports 28 genera of soft corals in four families, including Clavularia. C. inflata was described by Schenk (1896) based on samples from Ternate, Indonesia. After that, Utinomi (1953) described C. inflata from Taiwan and Japan. Subhan et al. (2022) discovered C. inflata across the Indonesian archipelago.

Phenotypic plasticity is a strategy by living beings to improve their probabilities of survival in different locations and to utilize new niches as they become available (Pigliucci, 2005). Variation in morphometric characteristics might be the result of adaptation to different food items or environmental conditions across different locations. Environmental factors such as light intensity and water flow can affect variations in sclerite composition (Prada et al., 2008; Prada and Hellberg, 2013). Indonesian coral reefs vary widely in their environmental characteristics such as currents, sediment load and turbidity, or nutrients and phytoplankton abundance (Gordon, 2005; Tang et al., 2018), likely driving differences in the morphometrics of soft corals across this range of environments. Soft coral morphological investigations have been carried out in a variety of octocoral species (Sebens and Johnson, 1991; Fabricius and Alderslade, 2001), but are still scarce for Indonesia.

Previous studies have shown that there is variation or plasticity in the morphology of octocorallia affected by differences in environmental conditions such as depth (West et al., 1993), water flow (Velimirov, 1976; Sebens, 1984) and habitat (Kim et al., 2004). Morphometric investigations can be utilized to uncover soft coral behavior, such as preferences for particular food particle sizes (Vogel, 1981), and provide information on feeding capacities, exploitation of accessible food items (Kinzie, 1973; Sponaugle and LaBarbera, 1991; Lin and Dai, 1996), feeding performance, and food niche (Dai and Lin, 1993).

Despite the original type specimen of C. inflata being collected from Indonesia, there remains a paucity of information on the genus Clavularia for Indonesian coral reefs, which are a particularly extensive and complex biogeographic area that has given rise to a multitude of species and morphotypes. Therefore, this study aimed to morphometrically describe Clavularia inflata samples collected from a range of Indonesian coral reefs by using sclerite morphological features.

Materials and Methods

Study sites, specimen and tissue sampling

Samples for morphometric analyses were collected from eleven locations (Anambas, Natuna, Tanjung Lesung, Kepulauan Seribu, Kangean, Bontang, Lombok, Wakatobi, Morotai, Maluku Tenggara Barat, and Arborek) (Figure 1). Before being taken, the sample colonies were documented from various angles, namely close-up and wide-angle photos of the colony and its surroundings using a Canon G16 camera in an underwater housing.

Sampling was carried out using a purposive sampling approach to avoid the risk of the number of samples not meeting the needs of the analysis. Purposive sampling is one of the methods used by researchers following the principle of nonprobability sampling, namely by taking samples at locations where it is certain that the species to be sampled is present (Etikan et al., 2015). Samples were collected from between 4 - 7 colonies at each site at a depth of about 2-5 meters. A colony, referring to an individual, was defined as a discrete patch of polyps connected by stolons, and only colonies more than 3 m apart were sampled (Bastidas et al., 2002). Upon collection of the sample underwater, it was placed into a sample bottle with a volume of either 5 or 10 ml filled with seawater. Furthermore, when returning to the boat, the sample was transferred into a sterile sample bottle filled with 97% ethanol. When the sample arrived at the laboratory, it was stored in a freezer at 4º Celcius until further analysis.

Specimens collected for morphological identification were analyzed in the laboratory. Observation of soft coral morphology comprised the outside of the sample and sclerites contained within the tissue. Sclerites are calcareous structures in the tissue of soft corals. Sclerites were sampled from four particular parts of the colony, namely pinnules (finger-like extensions along the tentacles’ sides), crowns (tentacles covering the upper side of polyps, located between tentacles and calyx), calyces (the middle part of polyps), and stolons (branching between polyps) (Figure 2). The determined portion of the polyp was taken and 2–4 drops of sodium hypochlorite solution added directly onto the sample, resulting in dissolution of the tissue. Morphological species identification was carried out using Fabricius and Alderslade (2001), Utinomi (1953), Bayer et al. (1983) and Schenk (1896).
Figure 1. Sampling sites of *Clavularia inflata* at eleven coral reef locations across Indonesia.

Figure 2. Morphology of *Clavularia inflata*: (A) colony within the coral reef habitat; (B) colony before sampling; (C) preserved colony; (1) upper polyp; (2) calyx; (3) part of stolon.

Sclerites extracted from the samples were inspected using a ZEISS light microscope with an Axio CAM ERC5 camera and AxioVision 4.8 application. Sclerites in the pinnule section were
inspected with a magnification of 0.5 x 40, sclerites from stolons and crowns were observed with a magnification of 0.5 x 10, and those from the calyx section were observed with a magnification of 0.5 x 4. Each of these parts was photographed and images stored as .zvi (Zeiss Video Images) files.

**Data analysis**

Data were analyzed based on individuals or populations as OTU (Operational Taxonomic Units). This analysis illustrates morphometric variations in nature by taking the average value of each population for each morphometric character (Koutecký, 2015). Population analysis on the basis of OTUs makes it easy to analyze the relationship of population proximity to morphometric characters (Koutecký, 2015). The relationship between the location of *C. inflata* populations and their morphometric characters was assessed using principal component analysis (PCA) (Rao, 1964), assigning scores based on population groups and morphometric characters (Koutecký, 2015). Analysis of the main components was done with the R 4.0 software using the ade4 package (Dray and Dufour, 2007).

**Results**

Polyps of *C. inflata* were generally similar to polyps in other cnidarian animals, especially in Clavulariidae (Figure 3 – A1). Each top polyp has 8 tentacles. On the tentacles there are pinnules like fine hairs that cover the inner surface of each tentacle. Sclerites in polyps have a variety of different shapes and distinct sizes in each part, namely in the pinnules, crowns, calyces, and stolons.

The sclerites that have the shortest size are found in the pinnule. These sclerites comprise a variety of forms such as grains with various geometric shapes, such as platelet or finger biscuit. These tiny sclerites allow the tentacles to move more freely when coming out of the polyp. Sclerites in the crown are generally smooth, but not as smooth as those in the pinnule; in this section there are teeth that cover the sclerite surface (Figure 3-B1).

The form of sclerites found in the crown is more diverse. This shows that this part of the polyp is the most complex, as the crown has to be able to contract and extend. The form of sclerites found in the crown are curved, spindle-shaped sclerites (oval with a shaft in the middle). This curved type of sclerites is located closest to the calyx section; the sclerites are arranged horizontally on each side of the crown so that it can form cylindrical cavities. The sclerites observed in this section also comprise straight spindles, located at the bottom of the tentacles and above the curved spindle sclerites. These sclerites are arranged vertically with facing angles that have narrow distances and are arranged to form eight chevrons (“V” shapes arranged vertically stacked), and between each chevron there are no sclerites. In the middle part of the polyp there is the calyx, and the sclerites in this section have the longest size compared to those in other parts. The calyx is mostly covered by a type of rod-shaped sclerites (Figure 3-C1). This form is found starting from the lower border of the tentacle on the aboral surface part of the mouth of the polyp, and is not found on the surface of the mouth (oral surface). This shape and size plays a role in strengthening and elevating the cylindrical plane in the middle of the *C. inflata* polyp.

Calyx sclerites generally have a rougher surface texture compared to sclerites located at the top of the polyp. The surfaces of the sclerites in this section feature small teeth. These serrations increase the adhesion to the tissue so as to maintain the position of the sclerites.

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### Table 1. Sclerite lengths of *Clavularia inflata* samples grouped by region of sample (pinnule, crown, calyx and stolon) across sampling location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Pinnule (tentacle)</th>
<th>Crown</th>
<th>Calyx</th>
<th>Stolon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Min Mean (+SE)</td>
<td>n</td>
<td>Max</td>
</tr>
<tr>
<td>Anambas (ANB)</td>
<td>476.8 ± 24.95</td>
<td>100</td>
<td>100</td>
<td>594.4 ± 19.64</td>
</tr>
<tr>
<td>Natuna (NAT)</td>
<td>1164.5 ± 54.49</td>
<td>100</td>
<td>100</td>
<td>596.6 ± 54.49</td>
</tr>
<tr>
<td>Tanjung Lesung (TL)</td>
<td>660.5 ± 20.56</td>
<td>100</td>
<td>100</td>
<td>540.1 ± 27.64</td>
</tr>
<tr>
<td>Bontang (BON)</td>
<td>927,35</td>
<td>100</td>
<td>100</td>
<td>544.8 ± 20.96</td>
</tr>
<tr>
<td>Lombok (LMB)</td>
<td>1277,94</td>
<td>100</td>
<td>100</td>
<td>546.7 ± 13.91</td>
</tr>
<tr>
<td>Kangean (KGN)</td>
<td>1221.6 ± 22.71</td>
<td>100</td>
<td>100</td>
<td>546.2 ± 18.97</td>
</tr>
<tr>
<td>Morotai (MOR)</td>
<td>1211,63</td>
<td>100</td>
<td>100</td>
<td>576.8 ± 18.07</td>
</tr>
<tr>
<td>Arborek (ARB)</td>
<td>1326,4 ± 22.19</td>
<td>100</td>
<td>100</td>
<td>576.1 ± 20.84</td>
</tr>
<tr>
<td>Kepulauan Seribu (SRB)</td>
<td>1305,87</td>
<td>100</td>
<td>100</td>
<td>544.8 ± 20.96</td>
</tr>
<tr>
<td>Maluku Tenggara Barat (MTB)</td>
<td>1229.1 ± 38.34</td>
<td>100</td>
<td>100</td>
<td>544.8 ± 20.96</td>
</tr>
<tr>
<td>Wakatobi (WKT)</td>
<td>1118,43</td>
<td>100</td>
<td>100</td>
<td>18.94</td>
</tr>
<tr>
<td>Natuna (NAT)</td>
<td>1034,38</td>
<td>100</td>
<td>100</td>
<td>546.7 ± 18.97</td>
</tr>
</tbody>
</table>

Dimensions are given in µm.

The last part assessed was the lower part of the *C. inflata* polyp below the anthostele, the stolon (Figure 3-D). This section is the hardest part of the polyp. Sclerites found and observed in this section have a shorter length than those from the calyx but have a sturdier and more rounded shape. This sturdy part of the stolon also connects polyps to each other so that it contributes to the development of the *C. inflata* colony. Stolon sclerites are located at the bottom of the polyp; this part is closest to the substrate on which the colony grows. This part of the polyp, besides having a harder and denser texture, also has a darker color. In this section the sclerites have a variety of shapes but have the same general characteristics, namely a wider and longer serrated diameter and a shape that tends to be rounded (Figure 3-D1). The surface of the sclerites observed in both parts also has the same characteristics.

The length of the sclerites varied across the study area, as well as the length of the sclerites found in the different parts of the polyp (Table 1). The maximum mean sclerite size in the pinnule part was found in the Kepulauan Seribu area with a size of 145.5 ± 19.27 µm, while the smallest mean size (35.5 ± 0.7 µm) was found in Arborek. The maximum sclerite size in the calyx (1605.6 ± 39.58 µm) was found in the Kepulauan Seribu area, while the lowest (858.1 ± 27.22 µm) was found in Arborek. The maximum sclerite size in the stolons (749.50 ± 52.80 µm) was again observed in Kepulauan Seribu, while the lowest was found in Morotai with a size of 437.4 ± 16.75 µm.

**Figure 4.** Principle Component Analysis (PCA) based on four morphometric measurements (size of sclerites from four different parts of the polyp) of *Clavularia inflata* samples collected from eleven locations throughout Indonesia.
Tenggara Barat and Arborek were distinct, while those from Kangean, Morotai, Tanjung Lesung, Bontang, Lombok and Natuna formed a cluster. Arborek displayed a negative correlation with ‘crown’, reflecting the smaller mean size of crown sclerites in specimens collected there, while Wakatobi was characterized mostly by large crown and pinnule sclerites. Specimens from Maluku Tenggara Barat were associated with large sclerites found in the pinnule and calyx, while the cluster Lombok, Bontang, Tanjung Lesung, Natuna Kangean and Morotai was characterized by smaller sclerites in stolon and calyx.

**Discussion**

Coral colonies grow and develop by the emergence of new polyps. This process is also known as budding, namely vegetative reproduction of polyps, which in Octocorals grow out of the stolons of branching polyps ([Iha and Yoshino, 1997](#)). The growth of polyps from stolons will form a typical colony growth patterns in C. *inflata* that is known as stolonate. Generally this type of colony form is also found in other species of the genera Clavularia and Cornularia.

The hardest part of the polyps are the stolons and the softest parts are the pinnules. This reflects the fact that the upper part of the polyp is a part that is regularly moving (flexible), e.g. during feeding, while the bottom section is a rigid part that provides structure (anthostele). The stolon with its harder tissue properties plays an important role in the expansion of C. *inflata* colonies. Stolons interconnect between polyps and propagate on the substrate they occupy, and the sturdy stolon character functions as the strongest part to maintain the base for the colony ([Alino et al., 1992](#)).

The stems of polyps of soft corals with rigid colonies such as C. *inflata* have two main parts, namely anthocodia and anthostele ([Bayer et al., 1983](#)). The anthocodia in Clavularia is a soft section of the stem below the head (tentacles and crown). The anthostele (=calyx) is the part that is located under the anthocodia into which it can be withdrawn, and attaches the polyp to the stolon. It is completely stiffened by rows of densely packed sclerites, which form a rigid and hard network ([Weinberg, 1986](#)).

Based on morphometric analysis, there were variations in size in the four assessed polyp parts across the eleven study sites. Based on the size of sclerites, specimens collected from Kangean, Morotai, Tanjung Lesung, Bontang, Lombok and Natuna formed a cluster. These locations were characterized by pinnule and crown sclerites of nearly the same size. Specimens from other locations (Arborek and Maluku Tenggara Barat) were more distinct and dissimilar from specimens collected elsewhere.

The variation in morphometric characteristics might be the result of adaptation to different food items or environmental conditions across the different locations sampled in this study. Indonesian coral reefs vary widely in their environmental characteristics such as currents, sediment load and turbidity, or nutrients and phytoplankton abundance ([Gordon, 2005; Pratiwi et al., 2017; Ayu et al., 2017; Tang et al., 2018](#)). Sclerite composition can vary with light intensity and/or water motion ([Prada et al., 2008; Prada and Hellberg, 2013](#)). Sclerites of specimens collected at Arborek were smaller in three parts of the polyp than for specimens collected at the other locations. The status of coral reefs in Arborek is described as good, with environmental conditions that are very conducive to the growth of coral reefs ([Sutono et al., 2020](#)). However, studies of the octocoral *Isis hippuris* in Wakatobi found that the size of sclerites in sites with conditions generally considered less optimal for reef growth (turbid water and high nutrient concentrations) was smaller than in areas with better water conditions ([Rowley et al., 2015](#)). Smaller sclerite sizes in the Arborek specimens thus may be related to strong currents at that location. Smaller size of sclerites has previously been linked to increased water flow ([Kim et al., 2004](#)).

Polyps of the soft corals *Dendronephthya hemprichi* and *D. sinaiensis* display significant variation in morphological features, which may have resulted from differences in the size of the phytoplankton items upon which they feed ([Grossowicz and Benayahu, 2012](#)). The size of pinnules indicates their suitability for filtering a certain particle size ([Rubenstein and Koehl, 1977](#)). Morphological differences between polyp characteristics, including sclerites, may indicate different feeding habits and feeding rates ([Grossowicz and Benayahu, 2012](#)).

**Conclusion**

In conclusion, there were variations in the size structure of sclerites collected from four regions of C. *inflata* polyps sampled at eleven study sites. Differences in sclerite shapes and sizes are important distinguishing features among corals of the genus Clavularia, but can also arise due to differences in environmental conditions. The distinct morphometric features of specimens from two of the sample sites may thus reflect local environmental features, or indicate the presence of cryptic species. Further studies using additional molecular markers
are suggested to further explore the possibility of cryptic species.

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