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Article

Comparative Study of Morphological Characteristics and Chemical Component Screening of Callyspongia sp. from Samalona Island and Gondong Bali Island, Spermonde Archipelago

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ABSTRACT

Differences in habitat conditions may be associated with variations in the morphology and chemical component profiles of marine sponges. This study aimed to compare the morphology and chemical component profiles of Callyspongia sp. from Samalona Island (inner zone) and Gondong Bali Island (outer zone) in the Spermonde Islands and to examine their relationships with habitat conditions. A descriptive-comparative approach was employed, involving measurements of physicochemical water parameters, macroscopic and microscopic morphological analyses, and identification of chemical components using GC-MS. Samalona Island exhibited a total dissolved solids concentration of 3820 ppm, water transparency of 70%, underwater visibility of 7 m, and salinity of 28.6‰, whereas the corresponding values for Gondong Bali Island were 2335 ppm, 100%, 9 m, and 30.4‰, respectively. Callyspongia sp. from Samalona Island exhibited a branched growth form, bright yellow coloration, numerous small oscula, a relatively smooth surface, a denser reticulate skeletal framework, and relatively slender spicules. In contrast, Callyspongia sp. from Gondong Bali Island exhibited an erect tubular growth form, yellowish-brown coloration, a large terminal osculum, a rough surface, a more open skeletal framework, and relatively larger spicules. Both specimens possessed monaxon megascleres of the oxea, style, and strongyle types. Cyclotrisiloxane, hexamethyl- was detected at 76.43% and 34.97%, whereas N-ethyl-1,3-dithioisoindoline was detected at 8.39% and 6.98% in samples from Samalona Island and Gondong Bali Island, respectively. Descriptively, variations in habitat conditions coincided with differences in the morphology and chemical component profiles of Callyspongia sp.

1. Introduction

Infectious and non-communicable diseases remain major global health challenges, while drug resistance has driven the search for new therapeutic agents derived from natural products [1], [2]. Indonesia, as part of the Coral Triangle region, possesses high marine biodiversity and has the potential to provide sources of bioactive compounds for the development of health-related applications [3].

The Spermonde Archipelago in the Makassar Strait exhibits environmental gradients ranging from inner zones located near the mainland, middle zones, to outer zones extending toward the open ocean [4], [5]. Inner zones near the mainland tend to receive stronger nutrient, sediment, and anthropogenic pressure inputs, whereas outer zones are relatively more oligotrophic and receive weaker terrestrial influences [6], [5]. These gradients in water quality may influence microbial communities and benthic organisms, including marine sponges.

Sponges are sessile benthic and filter-feeding organisms that produce secondary metabolites as defense mechanisms and adaptations to environmental pressures. The genus Callyspongia is known to produce various metabolites with biological activities, particularly antimicrobial, antibacterial, antioxidant, and other pharmacological activities [7]. Its metabolite profiles may differ even within the same species due to variations in environmental conditions and interactions with symbiotic microorganisms [8], [9].

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Differences in aquatic environmental quality may occur concurrently with variations in sponge morphology and secondary metabolism [10]. Sponge morphology is plastic and responds to variations in environmental conditions. An erect growth form may reduce the horizontal surface area available for particle deposition, while light availability, suspended solids, and salinity may be associated with pigmentation, skeletal architecture, and spicule dimensions [11]-[15]. Environmental conditions may influence the production of sponge secondary metabolites, whereas geographic differences among locations may be associated with variations in metabolite composition [16], [17].

Previous studies have reported antioxidant activity of Callyspongia sp. extracts [18], differences in antibacterial activity of sponges among locations in the Spermonde Archipelago [19], and variations in the compound profiles of Callyspongia aerizusa under different coral cover conditions [20]. The identification of chemical components in Callyspongia diffusa using GC-MS has also been reported by Bindu [21]. However, these studies have not integrated habitat differences, macroscopic and microscopic morphology, and GC-MS-based chemical component profiles within a single comparative study. Therefore, this study focuses on chemical component screening and does not include pure compound isolation or direct bioactivity testing.

This study aimed to determine differences in the morphology of Callyspongia sp. inhabiting the inner zone (Samalona Island) and outer zone (Gondong Bali Island), determine differences in sponge extract chemical compound profiles between the two zones, and analyze the relationship between habitat conditions and sponge morphology and chemical compound profiles. The study was limited to macroscopic and microscopic morphology, methanol fractions analyzed using GC-MS, and the parameters of temperature, pH, salinity, total dissolved solids (TDS), electrical conductivity (EC), water transparency, and underwater visibility. Bioactivity was not directly tested but was reviewed based on referenced scientific literature.

2. Research and Methodology

2.1. Research Design and Sampling

This study employed a non-experimental descriptive-comparative design integrating field surveys, laboratory analyses, and literature reviews. The preliminary survey included a review of the taxonomy and morphology of Callyspongia sp., acquisition of research permits, and snorkeling observations across several islands. Samalona Island was designated as the inner zone (IZ), whereas Gondong Bali Island was designated as the outer zone (OZ), based purposively on sponge occurrence, habitat representativeness, and differences in environmental pressures. Samples were collected in July 2025, and the entire study was conducted from July to September 2025.

The population comprised all Callyspongia sp. specimens at both study locations. Several fragments from one or more individuals at each location were pooled to form one composite sample per location. Sampling was conducted using the SCUBA diving method, with a motorized boat used as transportation support and for access to and from the diving sites. Diving activities were performed at depths of 1-7 m in the inner zone (IZ) and 7-15 m in the outer zone (OZ), following the underwater substrate contours systematically. At each sampling point, geographic coordinates were recorded using a Garmin eTrex 32x handheld GPS. Intact specimens were documented in situ and carefully detached from the substrate as fragments measuring approximately ±5-10 cm in size, then stored in bags containing seawater. Samples intended for microscopic morphological analysis were preserved in 70% ethanol to maintain the internal sponge structure, whereas samples for chemical component analysis were stored without preservatives under cold conditions using a cool box and subsequently refrigerated until the extraction process. The observation sites are presented in Figure 1, and the sampling design is summarized in Table 1.

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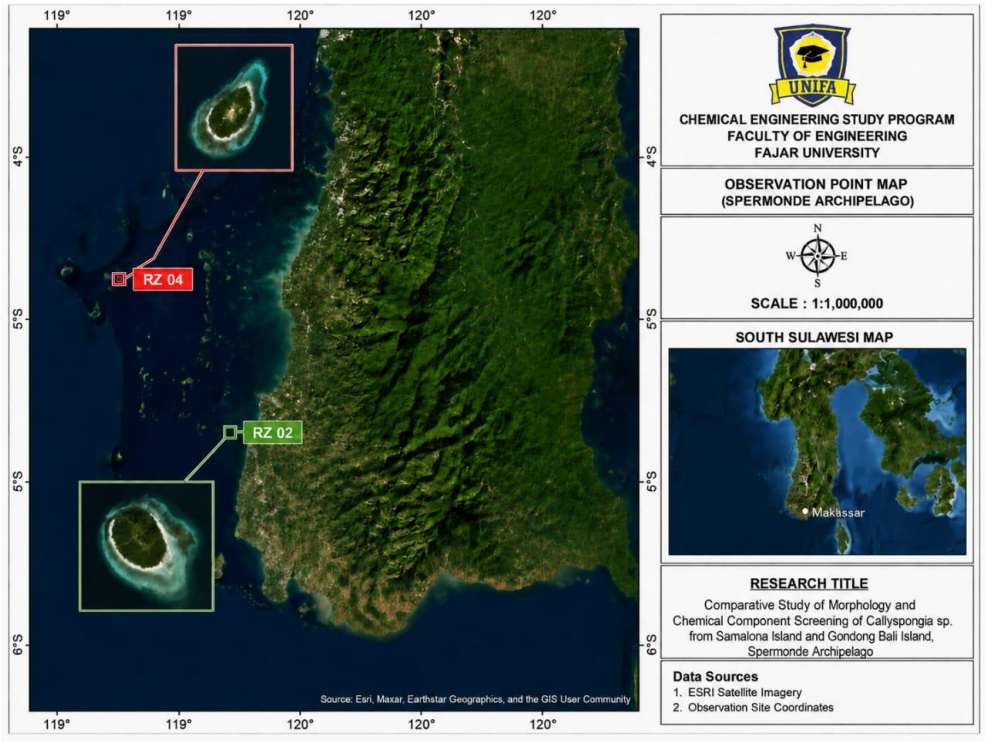


Figure 1. Locations of the study sites in the Spermonde Islands: Samalona Island (RZ 02; inner zone, IZ) and Gondong Bali Island (RZ 04; outer zone, OZ), South Sulawesi, Indonesia

Table 1. Sampling locations and sampling design

Code	Zone	Location	Coordinates
RZ 02	Inner Zone (IZ)	Samalona Island	5°7’22.678”S; 119°20’20.537”E
RZ 04	Outer Zone (OZ)	Gondong Bali Island	4°42’46.037”S; 119°3’16.643”E

2.2. Equipment and Materials

The equipment used in this study included an Olympus Tough TG-6 underwater digital camera, Neubauer haemocytometer, 120 mm Buchner funnel filtration set, Phase Contrast Microscope (PCM), EZ-9909 5-in-1 water quality tester, Secchi disk, rotary vacuum evaporator (rotavap) system, snorkeling equipment (snorkeling mask, snorkel, fins, wetsuit, buoy, and reef boots/booties), Self-Contained Underwater Breathing Apparatus (SCUBA) diving equipment consisting of a compressed air cylinder (scuba tank), regulator (first and second stages), Buoyancy Control Device (BCD), submersible pressure gauge, Garmin eTrex 32x handheld Global Positioning System (GPS), motorized boat (boat/speedboat), and gas chromatography-mass spectrometry (GC-MS) instrumentation.

The materials used included Callyspongia sp. sponge specimens, seawater, 0.2 µm filtration membranes, methanol (analytical grade, 96% purity), ethanol (analytical grade, 70% purity), n-hexane (analytical grade, 96% purity), and distilled water. Other supporting equipment and materials were used according to the stages of sample collection, preparation, extraction, fractionation, and analysis.

2.3. Measurement of Physicochemical Water Parameters

Water quality parameters were measured in situ at each sampling point. Temperature, pH, salinity, total dissolved solids (TDS), and electrical conductivity (EC) were measured using an EZ-9909 5-in-1 water quality tester. Water column visibility was measured using a Secchi disk, which was slowly lowered vertically into the water column until the disk was no longer visible for the first time. Subsequently, water transparency was determined

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based on the average depth at which the Secchi disk disappeared (D1) and reappeared (D2), using the equation D = (D1 + D2)/2. All measurements were performed in triplicate under relatively calm water conditions to minimize the effects of wave action and light reflection, and the measurement results were subsequently averaged.

2.4. Macroscopic and Microscopic Morphological Analysis

The macroscopic morphology of the sponge was observed under living conditions (in situ) and after sample collection (ex situ) based on growth form, coloration, oscula, texture, and surface ornamentation characteristics. Documentation was performed using an underwater camera and a digital camera after sample coding. Microscopic morphology was analyzed through observations of the internal sponge structures, including skeletal arrangement and spicule characteristics. Samples were preserved in 70% ethanol and subsequently prepared through sequential washing with sterile filtered seawater (0.2 µm), rinsing with 96% methanol, and sectioning of approximately ±2 cm of the mesohyl tissue. Transverse sections were observed using a phase-contrast microscope with the aid of a haemocytometer at 100× magnification. All observations were documented as morphological character data of the sponge [22], [23]. Microscopic analyses were conducted at the Aquatic Microbiology Laboratory and Aquatic Animal Physiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Airlangga.

2.5. Extraction, Evaporation, and Fractionation

Sponge extraction was adapted from the method used for marine sponge extracts by [24]. The samples were sorted and cleaned of debris, sand, and attached organisms using sterile seawater previously filtered through a 0.2 µm membrane, followed by rinsing with 96% methanol (PA). The samples were cut into smaller pieces to increase the contact surface area and weighed to obtain 500 g of material. The samples were transferred into sterile 2000 mL glass jars and extracted with 1000 mL of 96% methanol (PA) at a sample-to-solvent ratio of 1:2. Maceration was performed at room temperature for seven days under closed and light-protected conditions, with daily agitation. The mixture was subsequently filtered using a Buchner funnel, and the filtrate was collected for the concentration process.

The methanolic filtrate was concentrated using a rotary vacuum evaporator based on pressure reduction principles, allowing solvent evaporation at a lower temperature [24], [25]. The operational parameters were set at approximately 100 mbar pressure, 55°C temperature, and 64 rpm rotational speed. Evaporation was continued until a viscous crude extract was obtained.

Liquid-liquid fractionation was adapted from an extract partitioning method using solvents with different polarities [26]. A total of 15 mL of concentrated methanolic extract was gradually added to 96% n-hexane (PA), consisting of 10 mL in the first partition, 10 mL in the second partition, and 5 mL in the subsequent stage. The mixture was separated using a separating funnel until methanol and n-hexane phases were formed; fractions with the same solvent phase were then combined. The methanol fraction was selected for GC-MS analysis. The extraction, evaporation, and fractionation procedures were conducted at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin.

2.6. Identification of Chemical Components Using GC-MS

The methanol fraction was transferred into a sterile 10 mL glass vial and analyzed using gas chromatography-mass spectrometry [Agilent 7890] at the Forensic Laboratory of the South Sulawesi Regional Police. The analysis was performed using an [Agilent 19019S-436HP-5 ms] capillary column with dimensions of 60 m × 250 µm × 0.25 µm, helium as the carrier gas, a flow rate of 150 mL/min, a pressure of 18,406 psi, a separation velocity of 26,128 cm/s, a maximum temperature of 350°C, a temperature increase of 10°C every 5 min, and a maximum retention time of 30 min. This technique separates volatile components by gas chromatography and generates mass spectra to aid compound identification. Tentative identification was performed by matching the mass spectra against a library using the GC-MS software. Only candidate compounds with a library match quality (Qual) score of ≥ 70 that were dominant chemical components and had potential bioactivity were reported.

2.7. Data Analysis

Descriptive analysis was conducted to characterize the morphological features of the sponge, chemical components of the extracts, and physicochemical conditions of the waters. Meanwhile, comparative analysis was performed to compare the results between the inner and outer zones. The analytical results were subsequently

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interpreted to explain the relationship between habitat conditions and variations in the morphological characteristics and chemical components of Callyspongia sp.

4. Results and Discussion

3.1. Habitat Conditions and Physicochemical Parameters of the Waters

Samalona Island (IZ) represents the inner zone of the Spermonde Archipelago, located close to the mainland of Makassar City. Its waters are relatively shallow and utilized for various activities, including marine tourism, boat transportation, and local community activities. The proximity to coastal areas causes the waters around Samalona Island (IZ) to be more influenced by terrestrial inputs, turbidity, and sedimentation. In addition, anthropogenic influences from the coastal area of Makassar, including the discharge of drainage channels into the waters surrounding Losari Beach, potentially contribute to changes in the physicochemical characteristics of the waters at this site.

Gondong Bali Island (OZ) represents the outer zone of the Spermonde Archipelago and is located farther from the mainland compared with Samalona Island (IZ). Its waters are more exposed to the effects of currents, waves, and water mass exchange with the open ocean. Although terrestrial influence is relatively lower, the waters around Gondong Bali Island (OZ) remain potentially affected by human activities, such as shipping, fisheries, and marine debris transported by currents. The measured physicochemical parameters of both habitats are presented in Table 2.

Table 2. Comparison of physicochemical water parameters between sampling locations

Parameter	Unit	Samalona Island (IZ)	Gondong Bali Island (OZ)	Marine Biota Quality Standard*
Temperature	°C	29.51	30.11	28-30
Water Transparency	%	70	100	NA
Underwater Visibility	m	7	9	NA
Salinity	‰	28.6	30.4	33-34
Potential of Hydrogen (pH)	-	8.32	8.10	7-8.5
Electrical Conductivity (EC)	mS/cm	14.87	16.91	NA
Total Dissolved Solids (TDS)	ppm	3820	2335	NA

**Note: Decree of the State Minister for the Environment No. 51/2004, Appendix III (marine biota); NA = reference value not available. Source: Primary data, 2025*

Based on Table 2, the differences in water characteristics between Samalona Island (IZ) and Gondong Bali Island (OZ) were primarily observed in TDS, water transparency, underwater visibility, salinity, and electrical conductivity (EC). Meanwhile, temperature and pH showed relatively small differences of 0.60°C and 0.22, respectively. Temperature, salinity, and pH were compared with marine biota water quality standards, whereas EC, TDS, percentage of water clarity, and visibility were used as supporting parameters because specific quality standards for these parameters were not available in the applied reference.

The water temperature at Samalona Island (IZ) was 29.51°C, while Gondong Bali Island (OZ) recorded a temperature of 30.11°C. The temperature at Gondong Bali Island (OZ) slightly exceeded the upper limit of the marine biota water quality standard (28-30°C), whereas the temperature at Samalona Island (IZ) remained within this range. Nevertheless, both temperature values are still within the biological tolerance range of tropical sponges. [27] reported that sponges can tolerate temperatures of up to 36-40°C, while [28] found that sponges exhibit optimal growth at 30°C. Therefore, the temperature variation between the two locations was relatively small compared with the variations observed in other measured parameters.

The parameters of water transparency and underwater visibility revealed differences in water transparency conditions between the two locations. Samalona Island (IZ) exhibited a water transparency value of 70% with an underwater visibility of 7 m, whereas Gondong Bali Island (OZ) showed 100% water transparency with an underwater visibility of 9 m. The lower water transparency at Samalona Island (IZ) was associated with inputs of suspended sediments from drainage channels around Losari Beach and urban waste carrying suspended particles

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into coastal waters, as well as sediment resuspension caused by snorkeling activities [29]. Furthermore, increased nutrient inputs from domestic waste and tourism activities may stimulate phytoplankton blooms, which subsequently reduce light penetration within the water column [30]. In contrast, Gondong Bali Island (OZ) exhibited clearer waters with relatively lower terrestrial influence.

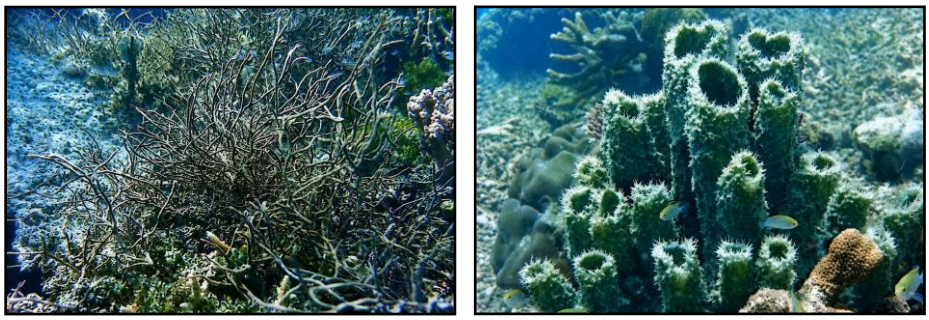
Water transparency and underwater visibility are parameters related to the ability of light to penetrate the water column. Lower water transparency indicates reduced light penetration, which may limit sponge distribution at certain depths, particularly for sponge species associated with photosynthetic symbionts [31], [32]. Nevertheless, the visibility values at both locations indicate that the water conditions remain suitable for supporting sponge presence.

The salinity of Samalona Island (IZ) waters was 28.6‰, whereas Gondong Bali Island (OZ) recorded a salinity value of 30.4‰. Both values were below the marine biota seawater quality standard for salinity (33-34‰). The lower salinity at Samalona Island (IZ) was presumably related to freshwater inputs from the mainland through drainage channels in Makassar City, resulting in seawater dilution [29]. Meanwhile, Gondong Bali Island (OZ) exhibited higher salinity due to its location in the outer zone, where terrestrial influences are relatively limited. The optimal salinity range for sponges is generally 30-35‰; therefore, the lower salinity recorded at Samalona Island (IZ) may potentially induce osmotic stress in sponges [28]. In contrast, pH values at Samalona Island (IZ) (8.32) and Gondong Bali Island (OZ) (8.10) remained within the marine biota quality standard range (7-8.5) and were within conditions that support sponge survival [28].

The EC value at Samalona Island (IZ) was 14.87 mS/cm, while Gondong Bali Island (OZ) recorded 16.91 mS/cm. The lower EC value at Samalona Island (IZ) was presumably associated with the dilution of dissolved ion concentrations due to freshwater inputs from the mainland, whereas the higher value at Gondong Bali Island (OZ) indicates more stable water conditions with better-maintained concentrations of dissolved salt ions [33]. Although EC does not yet have a specific quality standard for sponge habitats, the optimal EC range in seawater with salinity levels of 33-35‰ is approximately 40-55 mS/cm [33].

The TDS values at Samalona Island (IZ) and Gondong Bali Island (OZ) were 3820 ppm and 2335 ppm, respectively. The higher TDS concentration at Samalona Island (IZ) was presumably associated with inputs of organic matter from tourism activities, domestic waste, terrestrial runoff, and organism decomposition, whereas Gondong Bali Island received lower anthropogenic influences [34], [29], [30]. Although no specific TDS standard has been established for sponge habitats, elevated TDS caused by excessive organic matter accumulation may potentially promote eutrophication, reduce dissolved oxygen levels, and interfere with sponge filtration processes [33].

3.2. Macroscopic Morphology



(a) (b)

Figure 2. Macroscopic morphology of Callyspongia sp.: (a) Samalona Island (IZ) and (b) Gondong Bali Island (OZ)

Macroscopic morphological observations revealed clear differences between Callyspongia sp. from Samalona Island (IZ) and Gondong Bali Island (OZ), as shown in Figure 2. The specimen from Samalona Island/IZ (Figure 2a) exhibited a branching (ramose) growth form, characterized by thin, slender cylindrical branches growing irregularly to form a clustered structure. The observed body coloration was bright yellow, with small oscula distributed over the body surface and along the branches. The surface was relatively smooth, with an undulating relief consisting of shallow depressions and subtle elevations. In contrast, the specimen from Gondong Bali Island/OZ (Figure 2b)

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exhibited an upright tubular growth form, consisting of clusters of cylindrical tubes arising from a common colony base. Each tube possessed a single, relatively large terminal osculum with a well-defined margin. The body surface was rougher, with hispid to conulose ornamentation, and the observed body coloration was yellowish brown.

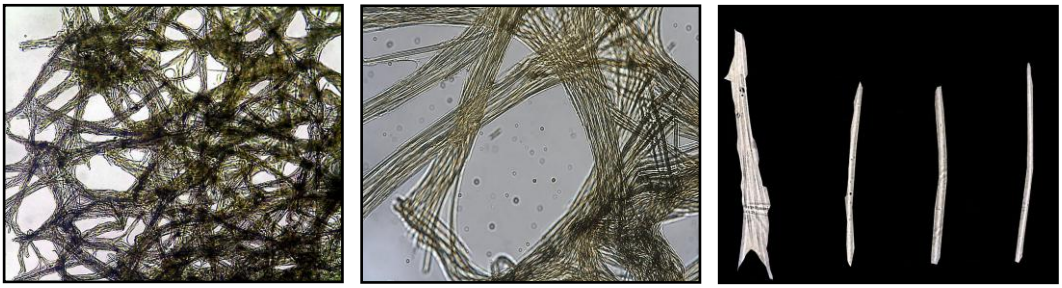
The differences in growth form were associated with the environmental conditions at the two sampling locations. Samalona Island (IZ) had a water clarity of 70% and an underwater visibility of 7 m; under these conditions, a branching morphology with relatively few horizontal surfaces may reduce areas available for particle deposition. Upright morphologies are known to accumulate less sediment than growth forms with larger horizontal surface areas [11], [12]. The relatively smooth surface of the Samalona Island (IZ) specimen may also help reduce sediment accumulation, as deposited sediment can cover the sponge surface and interfere with its aquiferous system [13]. Some sponges are known to possess surface-cleaning mechanisms, including mucus production [35], however, this characteristic cannot be regarded as a self-cleaning mechanism in the specimens examined in the present study because it was not directly tested.

In contrast, the upright tubular growth form observed at Gondong Bali Island (OZ) occurred under clearer water conditions, with 100% water clarity and an underwater visibility of 9 m. These conditions are consistent with the observations of [36] regarding associations between sponges and environmental conditions in the Spermonde Archipelago, as well as those of [11], indicating that tubular morphologies are commonly encountered in clear waters and are characterized by a predominance of vertical surfaces that relatively reduce sediment accumulation. Also demonstrated that tubular sponge growth can increase under high-light conditions when sediment deposition is eliminated [37] . The higher salinity and electrical conductivity at Gondong Bali Island (OZ) further indicate more oceanic water characteristics. Meanwhile, the hispid–conulose surface is primarily associated with skeletal architecture, particularly the projection of spicule tracts or bundles toward the body surface; environmental conditions are therefore more appropriately regarded as factors that may modulate this characteristic rather than as its direct cause.

Differences in body coloration between the two specimens were also observed in conjunction with differences in light conditions. Callyspongia sp. from Samalona Island (IZ) was bright yellow under conditions of lower light penetration, whereas Callyspongia sp. from Gondong Bali Island (OZ) was yellowish brown under higher-light conditions. Light intensity is known to influence symbiotic cyanobacteria and sponge pigmentation [38], [12], while symbiotic bacteria associated with Callyspongia have been reported to produce carotenoids [39]. Therefore, the bright yellow coloration observed at Samalona Island (IZ) is hypothesized to be associated with the contribution of photosymbiont pigments under lower-light conditions, whereas the yellowish-brown coloration at Gondong Bali Island (OZ) is hypothesized to be associated with the contribution of photosymbiont pigments under higher-light conditions. However, because neither pigments nor symbiont communities were analyzed in this study, the proposed relationships among body coloration, light conditions, and photosymbiont contributions remain interpretative.

3.3. Microscopic Morphology

Microscopic observations revealed that both Callyspongia sp. specimens exhibited a reticulate skeletal pattern with monaxon megascleres consisting of oxea, style, and strongyle types. Although both specimens shared the same spicule composition, differences were observed in skeletal architecture and spicule dimensions between the two locations.



(a) (b) (c)

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Figure 3. Microscopic morphology of Callyspongia sp. from Samalona Island (IZ): (a) skeletal structure at relatively low magnification, (b) skeletal structure at relatively high magnification, and (c) isolated spicules under dark-field microscopy. Source: Primary data, 2025.

In Figure 3a, Callyspongia sp. from Samalona Island (ZD) exhibited a reticulate skeletal architecture consisting of golden-brown to dark-brown spongin fibres that formed a three-dimensional network with anastomosing connections at the nodes. The intersection of multiple fibres formed relatively thick nodes and triangular to polygonal meshes of variable sizes that were densely arranged. Primary fibres were thicker, darker, spicule-cored, and contained parallel multispicular tracts following the fibre axis. Secondary fibres were thinner and lighter in colour, with paucispicular arrangements, connecting the primary fibres in a transverse orientation. Figure 3b further illustrates the dense spicule tracts, exfoliated spicules, and oval to polygonal meshes that were partially elongated following the orientation of primary fibres. In Figure 3c, monaxon megascleres appeared elongated, relatively slender, straight to slightly curved, smooth-surfaced, and without spines.

The relatively dense arrangement of primary fibres and meshes in Callyspongia sp. from Samalona Island (IZ) was observed concurrently with water transparency of 70% and underwater visibility of 7 m. This denser skeletal structure may potentially provide greater structural reinforcement under water-column conditions with relatively higher particle loads. However, this interpretation remains descriptive because total suspended solids (TSS), sedimentation rates, and skeletal mechanical strength were not directly measured.



(a) (b) (c)

Figure 4. Microscopic morphology of Callyspongia sp. from Gondong Bali Island (OZ): (a) skeletal structure at relatively low magnification, (b) skeletal structure at relatively high magnification, and (c) isolated spicules under dark-field microscopy. Source: Primary data, 2025.

In Figure 4a, Callyspongia sp. from Gondong Bali Island (OZ) displayed a complex reticulate pattern characterized by numerous intersection points, polygonal openings ranging from narrow to wide, relatively uniform fibre density, and more slender and regularly arranged nodes. This pattern was identified as isodictyal reticulation. Figure 4b shows a reticulate skeleton with cored fibres; primary fibres were paucispicular, thinner, and more transparent, whereas secondary fibres were even thinner, ranging from unispicular to paucispicular. The relatively long distances between nodes resulted in more open and wider meshes. Figure 4c demonstrates elongated monaxon megascleres that were straight to slightly curved, smooth-surfaced, and without spines, but relatively larger, thicker, and more robust than those of Callyspongia sp. from Samalona Island (IZ).

The 100% water transparency and 9 m underwater visibility recorded at Gondong Bali Island (OZ) indicated lower concentrations of suspended particles. Suspended particles can reduce light penetration and may clog the aquiferous system or cause abrasion of sponge tissues [40], [12], [13]. This condition reduces the functional pressure for compacting spicule bundles or reinforcing the skeleton, allowing sponges to maintain more loosely arranged spicules, slender fibre nodes, and open skeletal cavities. These characteristics reflect skeletal plasticity in response to local environmental conditions and are consistent with variations in spicule density and size among Callyspongia populations from different geographical locations [41], [42].

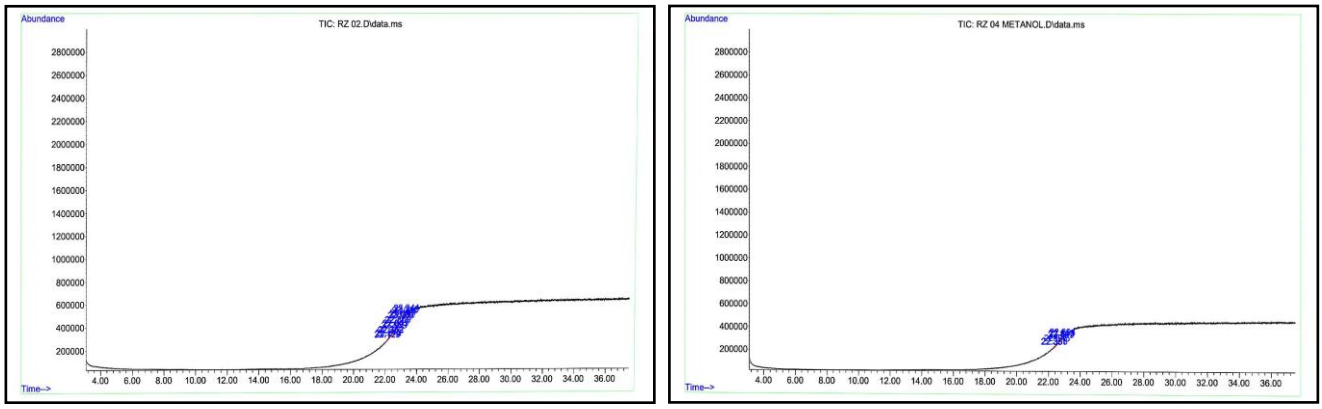
Based on the shape of the spicule ends, both specimens possessed the same megasclere types, namely oxea with two pointed ends, style with one pointed end and one blunt end, and strongyle with both ends blunt or rounded. The spicules of Callyspongia sp. from Samalona Island (IZ) were relatively slender under a salinity condition of 28.6‰, whereas the spicules of Callyspongia sp. from Gondong Bali Island (OZ) were larger and more robust under a salinity condition of 30.4‰. This pattern is consistent with previous reports describing relationships between skeletal development, spicule width, and salinity; however, salinity is not considered the sole determining

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factor because spicule characteristics are also regulated by biological controls and other environmental factors [14], [15].

3.4. Chemical Component Profile of Callyspongia sp. Based on GC-MS Analysis

The GC-MS chromatograms of the methanolic extracts of Callyspongia sp. are presented side by side in Figure 5. Callyspongia sp. from Samalona Island (IZ) exhibited several peaks within a retention time range of 22.129–23.344 min (Figure 5a), whereas Callyspongia sp. from Gondong Bali Island (OZ) showed peaks within the range of 22.351–22.894 min (Figure 5b). Tentative compound identification was obtained through spectral matching against the Wiley Library database and filtered based on Qual values ≥70.



(a) (b)

Figure 5. GC-MS chromatograms of methanolic extracts of Callyspongia sp.: (a) Samalona Island (IZ) and (b) Gondong Bali Island (OZ). Source: Primary data, 2025.

The GC-MS chromatograms of the methanolic extracts of Callyspongia sp. from Samalona Island (IZ) and Gondong Bali Island (OZ) are presented in Figure 5. Chemical constituents were tentatively identified by matching their mass spectra against the Wiley library using a Qual score threshold of ≥70. One candidate compound detected in both samples was N-ethyl-1,3-dithioisoindoline ($C_{10}H_{13}NS_2$; 211.4 g/mol). This compound was detected at a retention time of 23.109 min in the Samalona Island sample, with a relative peak area of 8.39%, and at 22.388 min in the Gondong Bali Island sample, with a relative peak area of 6.98%.

N-ethyl-1,3-dithioisoindoline is a candidate secondary metabolite tentatively classified as an alkaloid and is of interest for further investigation as part of the metabolite diversity of sponge extracts. This compound has been reported in the hydrosol of Thymbra capitata based on GC-MS analysis [43]. It has also been identified in an extract of the fungal species Trichoderma afroharzianum in a study investigating antibacterial activity against Bacillus subtilis and Klebsiella pneumoniae [44]. These findings suggest that the occurrence of this compound in microbial extracts warrants further investigation in relation to its potential bioactivity, particularly its antibacterial activity.

4. Conclusion

Callyspongia sp. from Samalona Island (IZ) and Gondong Bali Island (OZ) exhibited differences in morphological characteristics between locations, whereas both samples shared the same spicule characteristics, comprising oxea, style, and strongyle monaxon megascleres. GC-MS analysis tentatively identified N-ethyl-1,3-dithioisoindoline in both samples as a candidate secondary metabolite putatively belonging to the alkaloid class, with differing relative abundances. Differences in habitat conditions coincided with variations in the morphology and chemical component profiles of Callyspongia sp., although the relationships among these variables remain descriptive. These findings provide a basis for further investigation into the relationships between habitat characteristics and variations in sponge morphology and chemical component profiles. Future studies are recommended to include additional water quality parameters, increase the number of samples and sampling sites, incorporate more replicates, and extend the observation period to enable more robust analyses of relationships among variables. In addition, compound isolation, analyses of symbiotic microorganisms, and bioactivity assays are needed to confirm the sources and biological potential of the chemical components of Callyspongia sp.

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