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Comparative analysis on Sclerite Morphometry: a useful tool in the soft corals *Lobophytum* sp. (Sarcophytidae: Octocorallia: Anthozoa) identification from Andaman and Nicobar Islands, India

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Abstract

Soft corals are sessile, marine benthic organisms that play an important role in the coral reef ecosystem. The identification of soft coral species is challenging due to their morphological deviations. Octocorals are one of least studied groups in Indian waters due to a lack of expertise. A total of 60 species under the genus *Lobophytum* have been identified across the world's oceans while India shares a total of 21 species including 18 species from the Andaman and Nicobar Islands. During the study, *Lobophytum* specimens were collected from coral reef regions of the Andaman and Nicobar Islands employing SCUBA-diving and hand-picking methods. Specimens were distinguished with lobate, plate-like, or capitate with a prominent stalk and dimorphic, fully retractile polyps into thick coenenchyme. Species were identified by extracting sclerites from various regions of the colony with 5% sodium-hypochlorite (capitulum surface and interior, base surface, and interior) examined under a stereo-zoom microscope. Sclerite structure and measurements, as well as type composition, have been examined in ten *Lobophytum* species in this study. In different regions of the same colony, the structural composition of each species sclerites varies. Sclerites of various shapes like club-shaped, rod-shaped, spindle-shaped, shuttles, capstans, dumbbells, cylindrical, barrel-shaped, irregular-shaped, and cross-shaped were observed in ten *Lobophytum* species. The colony morphology also plays a role in taxonomy and species arrangement in systematics. Aside from colony morphology, zooid arrangement in the colony is also important for species identification. The colour of the colonies does not have a proper role in species identification because they show a variety of colours due to their symbiosis with zooxanthellate microalgae. The colony colours are determined by zooxanthellate concentration, temperature, depth, and other environmental factors. However, after preservation, most colonies turn white, beige white, or cream in colour. Hence, Sclerite morphometric is a very useful characteristic for species identification in the *Lobophytum* species.

Keywords: Taxonomy, finger leather coral, lobes, octocoral, Andaman

Introduction

Octocorals are commonly called as alcyonarians belonging to the Octocorallia subclass of the Anthozoa class of Cnidaria which consist of 3597 nominal species from the world's oceans (WoRMS, 2023). The subclass Octocorallia distinguished from other subclass by the presence of eight tentacles and eight mesenteries (Daly *et al.*, 2007). They are one of the significant benthic components in coral reef ecosystem by providing microhabitats to many invertebrates and vertebrates (Lau *et al.*, 2019). They are widely distributed in tropical and subtropical waters, and from intertidal to reef slope region whereas few pennatulaceans can be found at greater depths up to 6400 m (Pérez *et al.*, 2016). Formerly,

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since 1981 the subclass Octocorallia was classified under three distinct orders named as Helioporacea (Blue corals), Pennatulacea (Sea pens) and Alcyonacea (soft corals, sea fans and sea whips) (Bayer, 1981; Fabricius and Alderslade, 2001; Daly *et al.*, 2007). Recently, the systematics of Octocorallia has updated based on the phylogenomics (McFadden *et al.*, 2022). According to the recent classification, Octocorallia classified into two orders, Malacalcyonacea (46 families) and Scleralcyonacea with (36 families) (McFadden *et al.*, 2022, WoRMS, 2023). Apart from phylogenomics, the traditional taxonomy considered several characters as important to classify octocorals such as colony morphology, presence or absence of an internal skeletal axis, axis composition i.e. whether solid calcium carbonate or proteinaceous axis, axis with little to no calcified material, colonies with or without a skeletal axis or axial-like layer formed by sclerites, polyps surrounded in a fleshy mass of coenenchyma or connected basally by stolons or membranes and solitary polyps (Studer, 1887; Grasshoff, 1999, Hickson, 1930; Bayer, 1981; Fabricius and Alderslade, 2001). Octocoral tissue embedded with small calcium carbonate tissue with mineral form small aggregates of skeletal elements called sclerites (Thomson and Simpson 1909; Fabricius and Alderslade, 2001). These sclerites play an important role for identification of octocoral species (Conci, *et al.*, 2021; Tentori and van Ofwegen, 2011).

Soft corals are among the most diverse and successful members of the subclass Octocorallia of class Anthozoa. They are without a skeletal axis, colonies lobate, plate-like or capitate with a conspicuous stalk, not highly branched. Polyps monomorphic or dimorphic, fully retractile into thick coenenchyme. Polyp has rod shaped scleties, spindles, clubs and scales, often arranged as collaret and/or points but sometimes absent. The species under the genus *Lobophytum* are commonly called as devils hand corals or devils hand leather corals because of the capitulum modified as lobes or crests seems to be finger like projections in capitulum with short wide stalk or encrusting. Sclerites of surface layer are clubs; sclerites of interior are capstans and spindles. Colour of colonies light yellow, beige, brown, greenish. Polyps often white. There are about 60 species reported from world's seas while India contributes 21 species including 18 species from Andaman and Nicobar Islands. The current paper aims to present different types of sclerites in the *Lobophytum* species with their morphometrics and their role in identification of species.

Material and methods

Collection and Preservation of specimens

Soft corals, *Lobophytum* specimens were collected from intertidal regions to subtidal regions of the Andaman and Nicobar Islands. The surveys of subtidal regions were attained through employing snorkelling and self-contained underwater breathing apparatus (SCUBA) from intertidal to shallow water subtidal depth up to 25m. The *in-situ* photographs were taken by a Canon G15 camera with underwater housing. All the collected specimens were fixed in 4% buffered-formalin in seawater, rinsed with freshwater, and, after 24 hours, placed in 70% alcohol for long-term preservation. *Ex-situ* photographs were taken in the lab by a Canon G15.

Preparation of sclerites

Sclerites were extracted by removing a thin layer of tissue from the surface and interior of the capitulum or lobes and stalk or base and placed in a boiling tube or watch glass. Few drops of 5% sodium hypochlorite solution were added and allowed for 10 to 15 minutes to dissolve the tissue sometimes it may take an hour and leave the sclerites intact. The sclerites were rinsed thoroughly with distilled water and once with a 70% ethanol and transferred by dropper to an ordinary slide for study by following Fabricius and Alderslade (2001). These sclerites are examined under stereo-zoom microscope (LEICA M 205A, DFC 5000) for taxonomic identification. The identification was done by using literature of von Marenzeller (1886), Moser (1919), Tixier-Durivault (1956, 1957, 1966, 1970a &b), Verseveldt (1971, 1983), Verseveldt and Tursch, (1979), Verseveldt and Benayahu (1983), and Alderslade and Shirwaiker (1991) while the classification was adopted according to Bayer (1981), and McFadden *et al.* (2022) and records of valid names based on World Record of Marine Species (2023).

Results and Discussion

A total of ten species of *Lobophytum* species are used to study the comparative morphometrics of both colony and sclerites viz., *Lobophytum altum* Tixier-Durivault, 1956, *L. catalai* Tixier-Durivault, 1957, *L. crassum* von Marenzeller, 1886, *L. crebriplicaltum* von Marenzeller, 1886, *L. hirsutum* Tixer-Durivault, 1956, *L. pauciflorum* (Ehrenberg, 1834), *L. pusillum* Tixier-Durivault, 1970, *L. sarcophytoides* Moser, 1919, *L. schoedei* Moser, 1919 and *L. variatum* Tixier-Durivault, 1957.

Colony morphology: Generally common types of morphology were observed in all ten collected species. However, because this group lacks a skeletal axis, colonies are spread and supported by a hydroskeleton and sclerites which made of calcium carbonate and other minerals. Colony shape is primarily influenced by substrate depth and type, including rock, sand, live coral, dead coral, and others. However, the size and structure of most underwater colonies vary among species or even within species. These colonies have a low stalk, which is considered the base, and a wide capitulum. Some species have a plain capitulum, and in some species, the capitulum is modified into fingerlike lobes, crest-like lobes that are present throughout the colony, crests shapes originate from the edges of the colony, and the center might be occupied with lobes or free of lobes. Because colony morphology varies within species, it is extremely difficult to identify the species in situ. As a result, species identification is limited to sclerite morphology and length.

The colony of *Lobophytum altum* has a distinct stalk; the wide capitulum contains crested lobes that form at the periphery of the colony, and hollow center devoid of lobes (Figure 1: a). The colonies of *Lobophytum catalai*, *L. crassum*, and *L. sarcophytoides* have an encrusted capitulum that is large plate shaped with the edges rising upward and forming thick or thin crests on the edges; the basal portion of the colony is low and lacks a distinct stalk (Fig 1: b-f; Fig 2: e). *L. crassum* has encrusted, low, distinct stalk. Sometimes, the colonies of *L. crassum* are large, and the capitulum is broad, forming crest-like lobes that are distributed throughout the capitulum. Some colonies have crests that eventually spread from the colony's periphery to the centre. But the *L. sarcophytoides* colony is encrusted, has a low stalk with a capitulum that is bowl-shaped, and the peripheral region of the capitulum rose upwards and formed crests on the edges. In *L. crebriplicatum*, the colony is encrusting with a low stalk; the polyparium is the same width as the stalk, and thick lobes rise above the edges of the capitulum and form a hollow or bowl-shaped structure (Fig 1: g). in *Lobophytum hirsutum*, *L. pauciflorum*, and *L. pusillum*, there are finger-like lobes on the surface of the capitulum (Figure 1: h; Figure2: a-d). The formation of lobes, the width of the lobes, and the zooids arrangements at the summit of the lobe and on its sides vary from each other. *L. hirsutum* contains a low stalk and more erect or fingerlike lobes; *Lobophytum pauciflorum* is broadly encrusted, and the capitulum is rather flat with upright lobes and densely arranged lobes. In some colonies, the summits of the lobes are pointed or rounded, and in a few colonies, the erected lobes are disposed far from each other, and sometimes the lobes are present only on the peripheral region of the colony. Sometimes individual lobes are fused at their bases, forming crests like compound lobes. Contrarily, colonies of *Lobophytum pusillum* with a low stalk, a wide capitulum, and spherical or finger-like lobes are not found in all areas of the capitulum. The polyparium's centre is flat, has zooids that are easily seen, and lacks any lobes. Except for the center, the edges of the polyparium are formed as erected finger-like lobes. Small ridges or nearly flat capitulum are characteristics of *Lobophytum schoedei* and *L. variatum* (Fig 2: f-h). The *Lobphytum variatum* colony, on the other hand, is low-encrusting and spreads across the substrate. The colony is encrusting, has a short stalk, and a thin capitulum that resembles a plate or bowl with a few marginal lobes.

Sclerite morphometrics: Colonies of each species have various kinds of sclerites viz., poorly formed club shaped sclerites in the surface layer of the capitulum and base, spindle shaped sclerites in the interior of the lobe, crests or capitulum, and capstans, dumbbell shaped, cylinder shaped and spindles in the interior of the base. The detailed type of sclerites and their measurements are given in Table 1.

Surface layer of lobe or capitulum: The sclerites of the surface layer of *Lobophytum* are predominant with club-shaped sclerites rather than rod-shaped, shuttle-shaped, or fusiform-shaped. The structure of club-shaped sclerites varies from one species to another. In *L. altum*, club-shaped sclerites have warty heads and handles; in *L. catalai*, *L. crebriplicatum*, *L. hirsutum*, and *L. pauciflorum*, club-shaped sclerites have low developed heads; club shaped sclerites of *L. crassum* have tiny spines at the top of the club head that are directed upwards; clubs are weakly developed heads in *L. irregulari*, but clubs have softly developed heads, smaller rod shaped, few clubs have strong heads; and handle cone shaped prominences in *L. pusillum; L. sarcophytoides* has clubs with irregularly placed warts on the heads of the club, *L. schoedei* has clubs are the heads with a centre wart, and few have thick warty heads without central wart, and *L. variatum* contain smaller clubs have a middle wart and lower handle a girdle of wart and larger clubs often have warty heads (Fig 3: a-j).

Interior of lobe or capitulum: Sclerites of interior layer of *Lobophytum* contain spindle shaped, oval shaped, fusiform shaped, and in some species four rayed crosses. The structure of lobe interior sclerites are also vary from one species another. In *L. altum* contain spindles contain irregular shaped prominences and cylinder shaped sclerites

with medium warts and covered with warts; *L. catalai* has cylinders contain two to four distinct sets of warts, the longer pointed spindles, prominences are irregularly distributed. The structure of capstans of *L. crassum*, two whorls of large spiny warts, and two terminal clusters of warts and spindles are covered with numerous whorls. In, *L. crebriplicatum*, pointed or blunt ended spindles with irregular distributed warts or distinct girdles present on their surface. But in *L. hirsutum* contain spindles have middle zoned bigger warts and smaller warts are dispersed or form girdles towards both ends of the spindles. Spindles with irregularly distributed prominences or warts present in *L. irregulari.* Whereas in *L. pauciflorum* contain pointed or blunt ended spindles with zones of warts. In, *L. pusillum*, spindles are with high warts, at centre of sclerite may be zoned. Sometimes often irregular in shaped which curved. The spindles of *L. sarcophytoides* are slender, pointed with small spined warts prominenses which may sometimes be zoned and some spindles have antler like warts. But in spindles of *L. schoedei* covered with covered with girdles of warts. Whereas in *L. variatum* contains shuttle shaped blunt ended spindles have four to six girdles of warts which are unevenly distributed on sclerites (Figure 3: k-t).

Surface layer of the base or stalk: *L. altum* contains small, club-shaped and rod-shaped sclerites; these clubs have warty heads and handles of low cone prominence. *L. catalai*, *L. crebriplicatum*, and *L. irregulari* contain club-shaped sclerites with poorly developed heads and warty handles. *L. crassum* clubs have a cone shape with large warts on the surface. *L. hirsutum* contains clubs with a wide head and one to two girdles of warts on the handle. In *L. pauciflorum*, there are warty clubs and short, wider spindles. In *L. pusillum*, clubs have softly developed heads and handle cone-shaped prominences and rod shaped sclerites. In *L. sarcophytoides,* it has a big, warty head with a small, thin handle and one or two girdle-like prominences. *L. schoedei* has clubs, which are the heads with a centre wart, and few have thick, warty heads, while *L. variatum* clubs are dense and comprise clearly whorls of warts with one central wart on the handle (Figure 4: a-j).

Interior of the base or stalk: The structural composition of stalk interior sclerites plays a significant role in the confirmation of species because it varies from one species to another. The stalk interior sclerites of *Lobophytum altum* contain capstans, barrels, dumbbells, and cylinders, with a middle waist and spindles also present. *L. catalai* contains capstans, oblong or cylinder-shaped, shuttle-shaped, and spindle-shaped sclerites; *L. crassum* has capstans, which are two girdles of spiny warts with clusters at the end and pointed spindles. In the stalk interior of *L. crebriplicatum*, numerous cylindrical forms, and pointed spindles. *L. hirsutum* contains oblong to cylindrical capstans and a few spindle-shaped sclerites. *L. irregulari* oval-shaped and pointed spindle-shaped sclerites are present. *L. pauciflorum* contains cylindrical and capstan-shaped sclerites with two to four girdles of compound warts and four rayed crosses. Whereas only pointed spindles with warty zones are present in the *L. sarcophytoides*, spindles that are distinctly broader and with a few irregularly distributed warts are structured in the girdles present in the *L. schoedei*. The sclerites structure varies in *L. variatum*, with cylinder-shaped, blunt-ended, fusiform shaped sclerites; numerous shuttles and prominences that are cones, simple warts, and compound warts (Figure: 4: k-t).

Andaman and Nicobar Islands are known as the most ecologically significant region in the Indo-Pacific region, with abundant coastal and marine biodiversity. Despite being one of the most important components of the reef ecosystem, comprehensive soft coral inventories are sporadic and scarce. The current study aims to provide colony morphological and sclerite comparative analysis of ten species of the genus *Lobophytum* from the Andaman and Nicobar Islands, which could be useful for easy identification.

Table 1: Comparative sclerite morphometry of soft corals, ten *Lobophytum* species (Sarcophytidae) from the Andaman and Nicobar Islands

Attributes		*Lobophytum altum* Tixier-Durivault, 1956	*Lobophytum catalai* Tixier-Durivault, 1957	*Lobophytum crassum* von Marenzeller, 1886	*Lobophytum crebriplicaltum* von Marenzeller, 1886	*Lobophytum hirsutum* Tixier-Durivault, 1956	*Lobophytum pauciflorum* (Ehrenberg, 1834)	*Lobophytum pusillum* Tixier-Durivault, 1970	*Lobophytum sarcophytoides* Moser, 1919	*Lobophytum schoedei* Moser, 1919	*Lobophytum variatum* Tixier-Durivault, 1957
Distance between two auto zooids	At the peripheral region or on the lobes (mm)	0.8 to 1.2	0.5 to 1.5	0.7-1.5	1.0 to 1.5	1.1-2.2	1.5 to 2.0	1.7 to 3.7	0.4 to 0.7	0.3 to 0.7	0.5 to 0.8
	At the base of lobe or centre of the capitulum (mm)	up to 2.5 mm	up to 3.0	1-4	3 to 4	2-3	Up to 7.0	2.5 to 4.7	Up to 1	0.5 to 1.5	Up to 1
No. of Siphonozooids present between two autozooids	At the peripheral region	1-3	1-2	1-2	1-2	1-3	3-4	Up to 6	1-2	1	1-2
	At the centre of the capitulum	Up to 5	Up to 8	2-6	Up to 7	2-5	Up to 8	Up to 10	2	1-2	3
Surface layer of the capitulum	Type of sclerites	Clubs	Rod and club shaped sclerites	Club and shuttles	Rod shaped or club shaped	Club shaped	Club, fusiform shaped and spindle-shaped	Shuttle or Volcano shaped and rod shaped	Club shaped	Rods and club shaped	Club shaped
	Length of sclerites (mm)	0.11 to 0.27	0.06 to 0.20	Clubs (0.10-0.17) few are up to 0.24 Shuttles (0.09-0.16)	0.16 to 0.33	0.12 to 0.28	0.10 to 0.25	0.08 to 0.26	0.06 to 0.23	0.08 to 0.22	0.07 to 0.19

Attributes		*Lobophytum altum* Tixier-Durivault, 1956	*Lobophytum catalai* Tixier-Durivault, 1957	*Lobophytum crassum* von Marenzeller, 1886	*Lobophytum crebriplicaltum* von Marenzeller, 1886	*Lobophytum hirsutum* Tixier-Durivault, 1956	*Lobophytum pauciflorum* (Ehrenberg, 1834)	*Lobophytum pusillum* Tixier-Durivault, 1970	*Lobophytum sarcophytoides* Moser, 1919	*Lobophytum schoedei* Moser, 1919	*Lobophytum variatum* Tixier-Durivault, 1957
Interior of the capitulum	Type of sclerites	Spindles, cylinder shaped and four rayed crosses	Oval shaped, capstans, cylinders and spindles	Oblong, subcylindrical or fusiform and spindle shape	Spindle shaped	Straight, curved pointed spindles.	Spindle-shaped	cylinder and spindle	Slender, and pointed spindles	Spindle shaped	Rods, cylinders, spindles, fusiform and shuttles
	Length (mm)	Up to 0.37	Up to 0.34	0.16-0.20 and spindles (Up to 0.36)	Up to 0.34	Up to 0.49	Up to 0.45	0.21 to 0.36	Up to 0.40	Up to 0.40	Up to 0.36
Surface layer of the stalk	Type of sclerites	club shaped and cylinder shaped	Small rods and club shaped	Clubs more or like surface layer of the capitulum	Club shaped	Club shaped	Clubs	Rod and club shaped	Club shaped	Club shaped sclerites	Club shaped
	Length (mm)	0.08 to 0.18	0.07 to 0.20	0.09-0.16	0.10 to 0.18	0.10 to 0.18	0.08 to 0.20	0.07 to 0.18	0.09 to 0.21	0.08 to 0.20	0.08 to 0.25
Interior of the stalk	Type of sclerites	Capstans, barrel shaped, dumbbell, cylinder shaped and spindles	Capstans, oblong or cylinders, shuttle and spindles	Capstans and spindles	Oblong, sub cylinders and pointed spindles	Oblong capstans to cylindrical shaped and spindle shaped	Capstans and cylindrical or fusiform-shaped sclerites	Dumbbells and capstans	Pointed spindles	Spindles	Cylinder, blunt ended fusiform shaped sclerites, shuttles
	Length (mm)	Capstans Up to 0.29 mm; spindles, up to 0.38	Capstans up to 0.28 mm shuttles measure up to 0.26 spindles up to 0.38	Capstans (0.17-0.20), and spindles (0.33)	Up to 0.39	0.15 to 0.24 Spindles up to 0.33	0.17 to 0.24	0.16 to 0.20	0.19 to 0.33	Up to 0.39	Up to 0.39

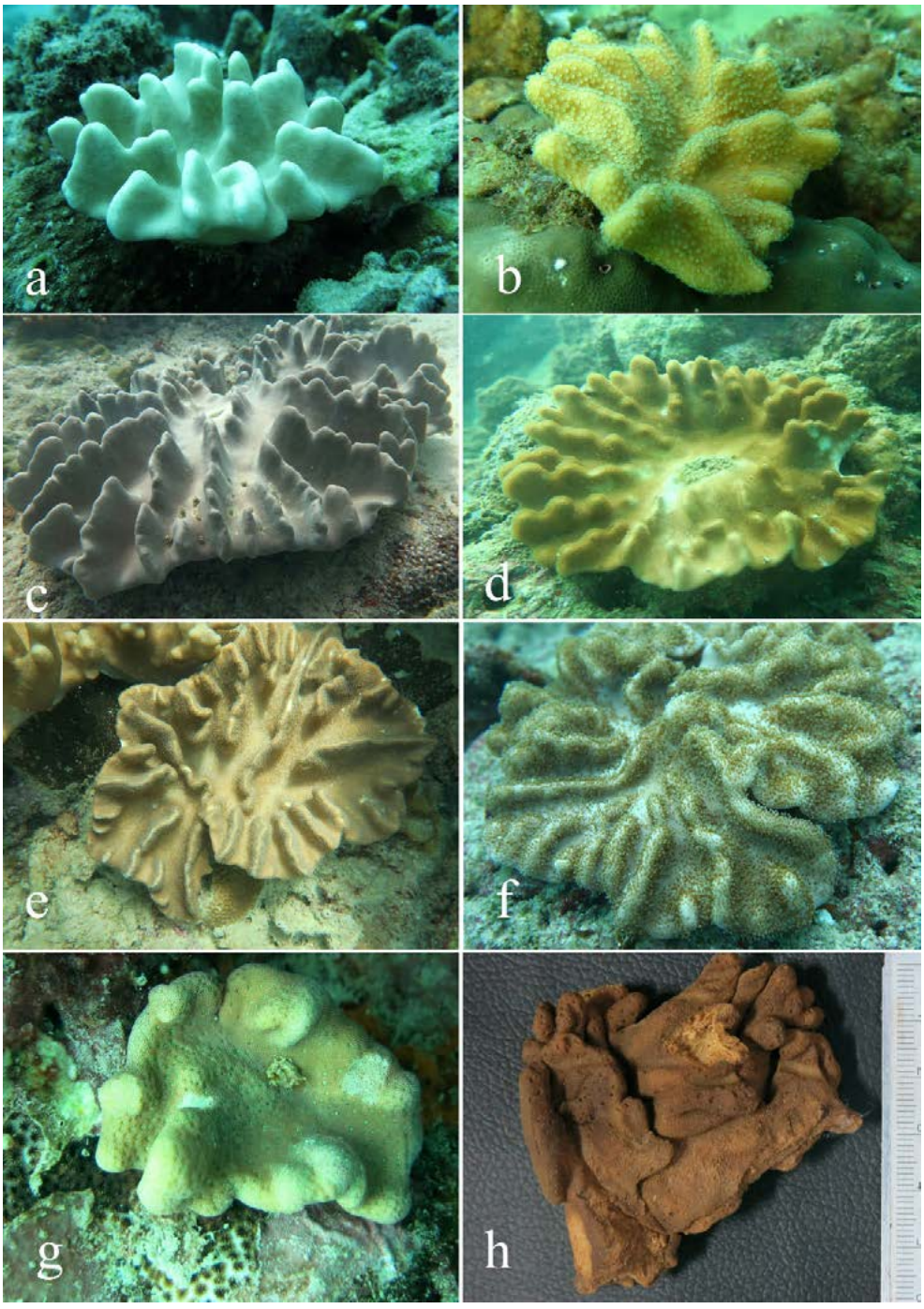

Figure 1: a-h examples of *Lobophytum* colonies; a. *Lobophytum altum* Tixier-Durivault, 1956 has bifurcated or low crest like lobes raised from edges of the colony, stalk is low; b. *Lobophytum catalai* Tixier-Durivault, 1957 contain thick crest like forms distributed throughout capitulum; c-f. *Lobophytum crassum* von Marenzeller, 1886 crest like lobes raised from colony edge and continuous towards the center, stalk or base is low almost as much as width of capitulum; g. *Lobophytum crebriplicaltum* von Marenzeller, 1886 thick crest raised at edges of the colony; h. *Lobophytum hirsutum* Tixer-Durivault, 1956 low stalk and more erect or fingerlike lobes

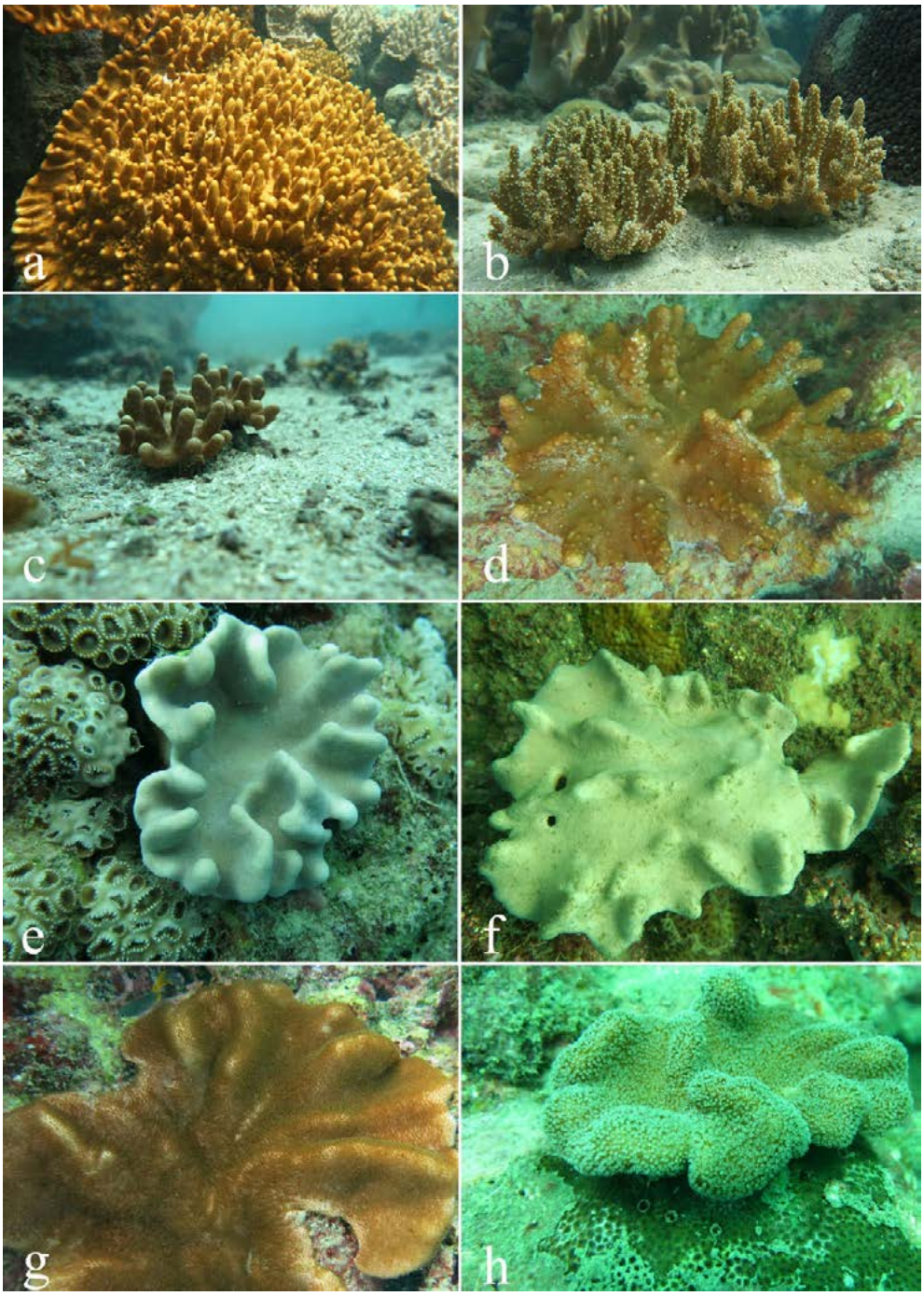

Figure 2: a-h examples of *Lobophytum* colonies; a-c, *Lobophytum pauciflorum* (Ehrenberg, 1834), *a-capitulum* has finger like lobes entire colony from edges to centre of the colony, low stalk; *b- finger* like lobes are erected highly and divided lobes at edges of the colony; *c- finger* like lobes with round upper portion raised from edges of the colony;d. *Lobophytum pusillum* Tixier-Durivault, 1970, capitulum has finger like lobes and crests from edges to center of the colony, autozooids visible in small tubercles exist throughout capitulum, and low stalk; e. *Lobophytum sarcophytoides* Moser, 1919 capitulum bowl-shaped, and the peripheral region of the capitulum rose upwards and formed crests on the edges; f. *Lobophytum schoedei* Moser, 1919 Small ridges or nearly flat capitulum; h. *Lobophytum variatum* Tixier-Durivault, 1957, broad capitulum with low crests and low stalk

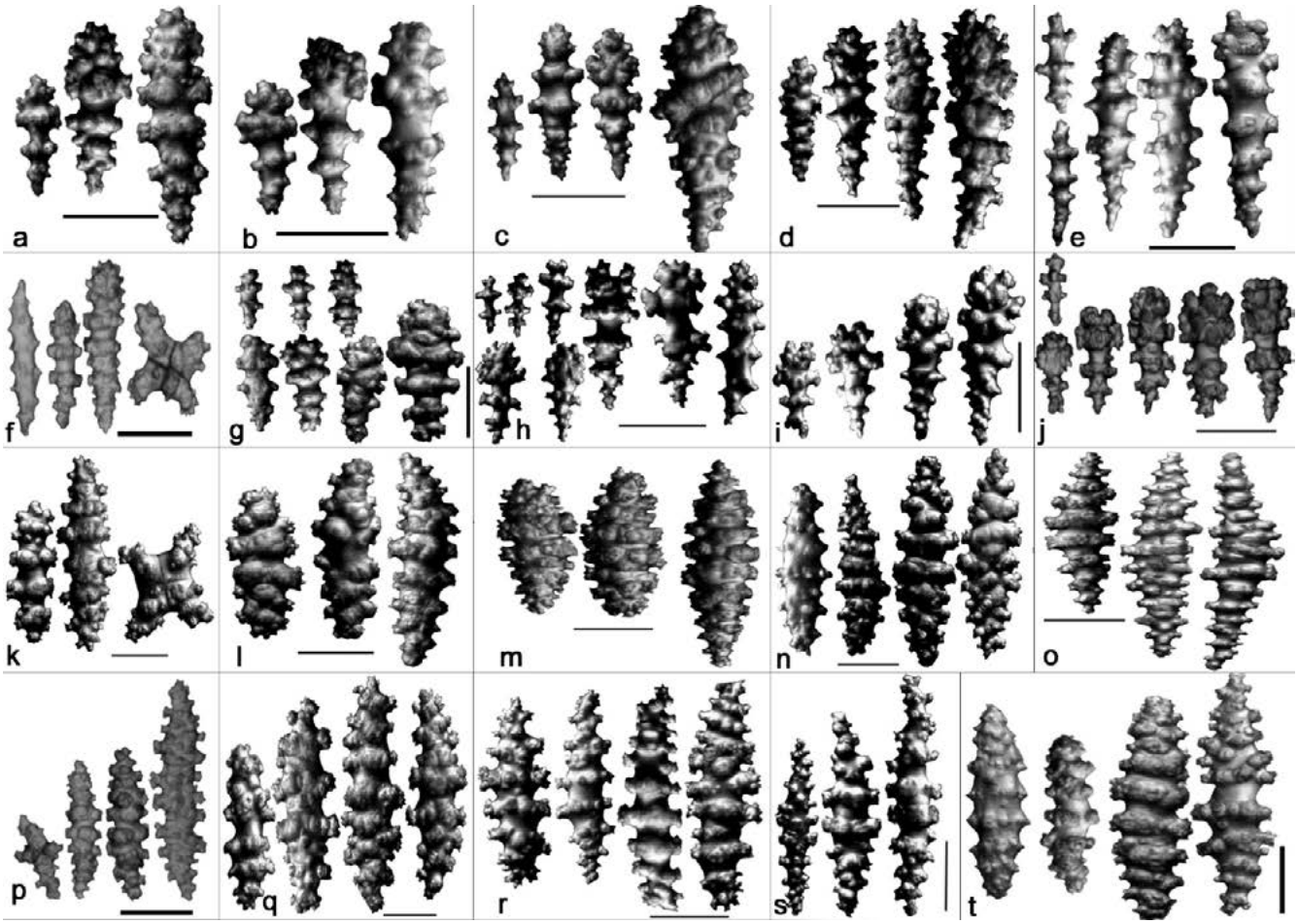

Figure 3: a-j. Sclerites of surface layer of lobe of *Lobophytum* species, a. *L. altum;* b, *L. catalai;* c. *L.crassum*; d. *L.crebriplicaturm*; e. *L. hirsutum*; f. *L. pauciflorum*; g. *L. pusillum*; h. *L.sarcophytoides*; i. *L. schoedei*; j. *L.variatum*; k-t. Sclerites of interior layer of lobe of *Lobophytum* species, k. *L. altum;* l, *L. catalai;* m. *L.crassum*; n. *L.crebriplicaturm*; o. *L.hirsutum*; p. *L. pauciflorum*; q. *L. pusillum*; r. *L.sarcophytoides*; s. *L. schoedei*; t. *L.variatum*

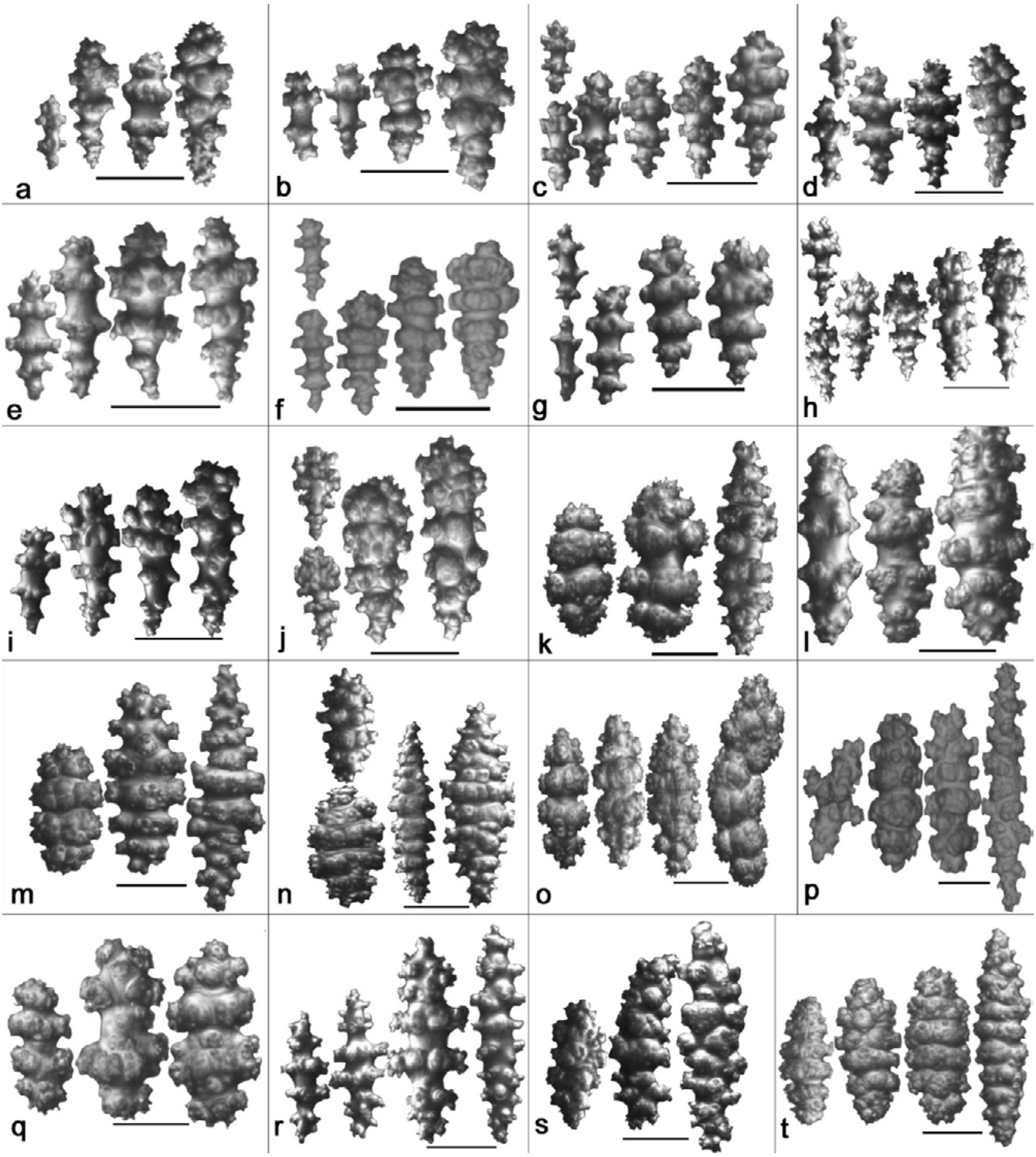

Figure 4: a-j. Sclerites of surface layer of base of *Lobophytum* species, a. *L. altum;* b, *L. catalai;* c. *L.crassum*; d. *L.crebriplicaturm*; e. *L.hirsutum*; f. *L. pauciflorum*; g. *L. pusillum*; h. *L.sarcophytoides*; i. *L. schoedei*; j. *L.variatum*; k-t. Sclerites of interior layer of base of *Lobophytum* species, k. *L. altum;* l, *L. catalai;* m. *L.crassum*; n. *L.crebriplicaturm*; o. *L.hirsutum*; p. *L. pauciflorum*; q. *L. pusillum*; r. *L.sarcophytoides*; s. *L. schoedei*; t. *L.variatum*

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