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Research Article

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New records and phylogenetic placement of two soft coral species (Octocorallia: Malacalcyonacea: Cladiellidae) from the Gulf of Kachchh, India

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Abstract

Octocorals are a highly diverse and ecologically significant faunal group within shallow coral reefs across tropical seas. The Gulf of Kachchh, on India's northwest coast, is a marginal and extreme reef system marked by heavy sedimentation, mega-tides, and significant SST variability. Although ecologically important, octocorals in this region have been poorly studied. The present study represents the first family distribution records of Cladiellidae soft corals, *Cladiella pachyclados* and *Aldersladum sodwanum* from the Gulf of Kachchh with detailed taxonomic descriptions, sclerite morphology, and molecular identification. Morphological and mitochondrial MutS gene characters support their species-level identification, but phylogenetic results showed limited resolution, grouping mixed species rather than closely related genera. This mismatch likely reflects known limitations of the mtMutS gene in octocorals and may indicate cryptic diversity and morphological similarity among related species. The present records rely on the best available morphological evidence and can be considered provisional until further taxonomic work is done, especially given the lack of molecular data from type localities. These findings underscore the urgency for comprehensive integrative taxonomic studies to accurately resolve Cladiellidae diversity in the

Kachchh. Advancing such research will not only clarify species boundaries but also inform conservation strategies essential for preserving this unique reef ecosystem.

Keywords: Octocorals, Cladiellidae, Gulf of Kachchh, taxonomic description, mtMutS gene sequencing, zoogeographical records.

1. Introduction

Octocorals represent one of the most diverse and conspicuous components of shallow water coral reef environments in tropical and subtropical regions. Members of the octocoral family Cladiellidae are mostly zooxanthellates, generally distributed in shallow waters of tropical Indo-Pacific (widespread from western Africa, the Red Sea in the west to western Pacific Islands in the east [1]. Colonies of the family are generally grow lobate to digitate but not highly branched, and their polyps are monomorphic, retractile, or non-retractile but highly contractile [2]. Family members are also characterized by distinctive forms of polyp sclerites, mainly platelets; in some cases, platelets with a central constriction or depressions resembling a 'figure-eight', rods [2, 3]. The family Cladiellidae currently comprises four genera, viz. *Aldersladum* Benayahu & McFadden, 2011; *Cladiella* Gray, 1869; *Klyxum* Alderslade, 2000; and *Ofwegenum*, McFadden, Benayahu & Samimi-Namin, 2024 [4]. Generally, colonies of Cladiellidae inhabit clearwater habitats of the Indo-Pacific. The Red Sea is the type locality for over 27 species of the genus *Cladiella*. Nevertheless, some representatives also inhabit sedimentation-influenced reef regions. Recently, Lazar et al. [3] provided a comprehensive redescription of the type material of *Cladiella* Gray, 1869, from the Red Sea, detailing colony morphology, polyp structure, and sclerite characteristics, and comparing them with related taxa.

To date, five species of *Cladiella* and one species each from *Aldersladum* and *Klyxum* of the family Cladiellidae have been reported from Indian waters [5-10]. Among India's major coral reef regions, the Gulf of Kachchh (=Kutch), located along the northwest coast of India, represents one of the northern most limits of coral distribution in the Indian Ocean [11]. The prevailing arid climate and semi-diurnal macro-tidal amplitude, heavy sediment depositions, and wide Sea Surface Temperature variability are the non-conducive factors that make the region an extreme reef environment [12-13]. Nevertheless, the Kachchh supports some residual yet highly resilient coral reef

communities [14]. The coral reef biota that reside in the Kachchh are quite distinctive in terms of their isolation and high degree of adaptation to survive in such extreme oceanographic and climatic conditions [15]. Despite being a dominant component of the reef biota, octocorals in the Gulf of Kahchh have received less attention so far. Since 1909, only 10 species have been reported [16-18], with no prior records of family Cladiellidae soft corals from these waters. Based on an integrative taxonomical approach, the present study confirms the occurrence of two previously unreported species of soft corals belonging to the family Cladiellidae, *Cladiella pachyclados* (Klunzinger, 1877) and *Aldersladum sodwanum* (Benayahu, 1993) from the Kachchh reefs. Thus, the present study confirms the first distribution record of the family Cladiellidae from the Gulf of Kachchh waters. Notably, *A. sodwanum* represents a new zoogeographical distribution record for the Indian waters. The present study also documents the *in-situ* morphological characters of the specimens, which may serve as additional taxonomic traits for species delineation.

2. Materials and Methods

Study area and sampling

The Gulf of Kachchh is an east-west-oriented, funnel-shaped indentation lying between the Kachchh mainland and the Saurashtra Peninsula along the northwest coast of India. The southern shore of the Gulf is fringed with more than 42 Islands and islets that generally harbor vast areas of mangroves and scattered coral reefs. The soft coral specimens were collected from a reef edge and sub-tidal reef sites off Narara Island in the Gulf of Kachchh Marine National Park, Gujarat, India (Fig.1). The specimens were collected during minus tide days using snorkeling/SCUBA Diving. Prior to collection, detailed in-situ morphological characters were recorded using an underwater camera. Immediately after collection, the soft corals were preserved in 90% ethanol, and sub-samples were preserved in 99% ethanol for further taxonomical and molecular analyses.

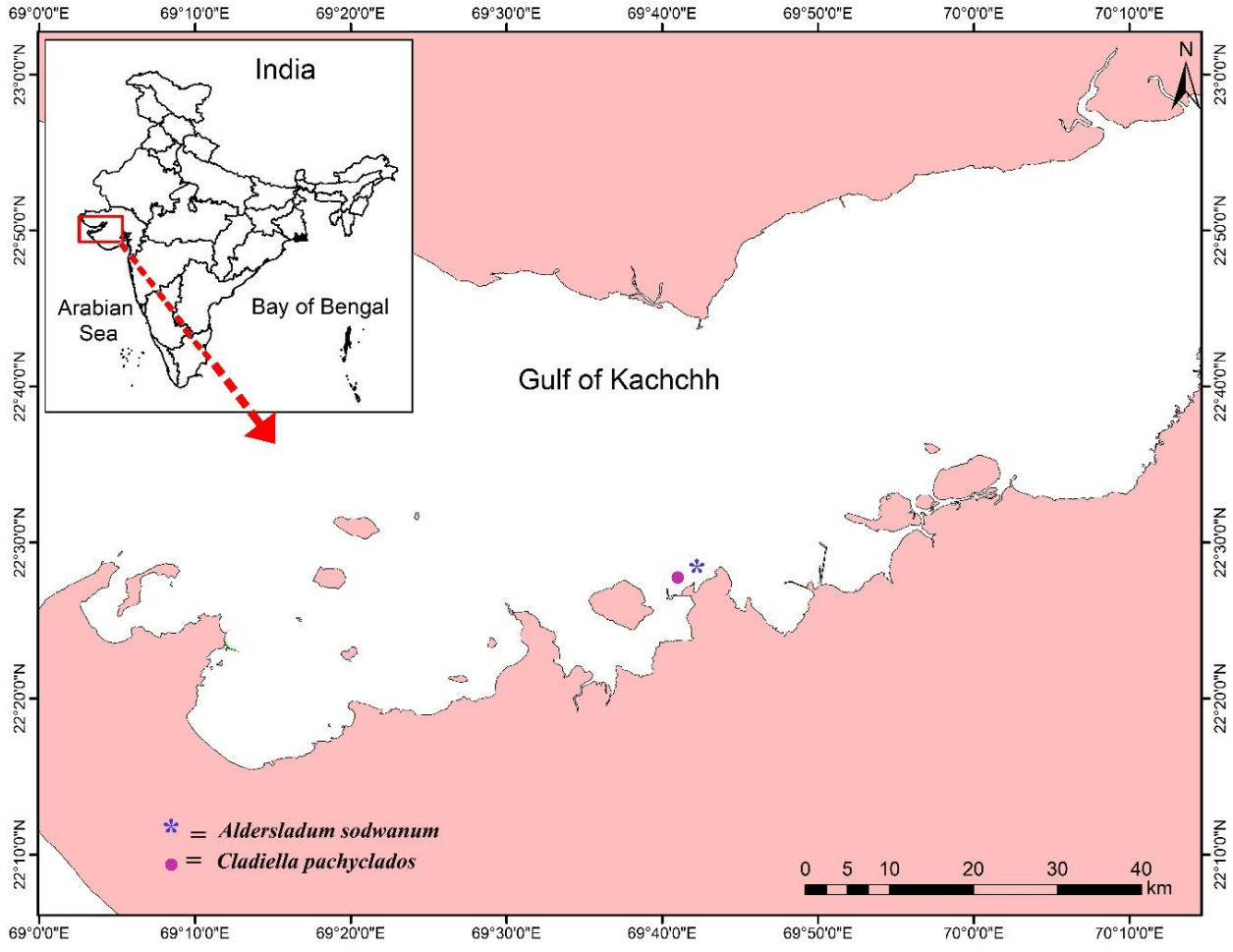


Figure 1. A map showing locations of the newly reported two soft coral species, in the Narara Reef, Gulf of Kachchh, India.

Morphological analysis

For taxonomic analysis, sclerites were extracted from different regions of the soft coral colonies by treating the tissues with 10% Sodium Hypochlorite (Hypo), followed by H_2O_2. Scanning Electron Micrograph images of the sclerites were made using a ZEISS-EVO-18 Scanning Electron Microscope. Morphometry of the sclerites was also recorded using the same. Voucher specimens were deposited at the National Zoological Collections, Zoological Survey of India, General Non-Chordata section, Indian Museum Campus - Kolkata, India, for further reference. The taxonomic status used in the present study follows McFadden et al. [2]. The specimens were identified based on the literature dealt the species [19-22].

Molecular analysis

Specimens preserved in 99% ethanol were used for DNA extraction from the soft tissues of individual specimens, employing the DNEasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. The partial MutS gene sequence was amplified via. polymerase chain reaction (PCR) following the protocols published in McFadden et al. [23]. The PCR mix, with a final volume of 25 µL, included TaKaRa Ex Taq enzyme (as supplied by the manufacturer), and the reaction was performed according to the manufacturer's instructions. The PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; 34 cycles of denaturation at 95°C for 30 seconds, annealing at 49°C for 1 minute, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. Amplified fragments of approximately 670 bp were visualized by electrophoresis on a 1% agarose gel. The PCR products were purified using ExoSAP-IT and sequenced bidirectionally using BigDye Terminator chemistry, following the manufacturer's instructions. DNA sequences were run on an ABI 3500 Genetic Analyzer. Forward and reverse sequences were assembled, trimmed, and checked for contamination using CodonCode software, followed by BLAST analysis in NCBI GenBank. The cleaned sequences were submitted to GenBank. The mtMutS gene sequences were aligned using MAFFT [24] under default settings.

To confirm the taxonomic identity of the present reported species, the aligned MutS gene dataset were compared with sequences from other species of the family Cladiellidae, downloaded from GenBank (Table 1). A molecular phylogenetic tree was constructed using the Maximum Likelihood (ML) method in RAxML v8.1.2 [25] under the GTRGAMMA model with default settings. The resulting phylogenetic tree was visualized and edited using FigTree v1.4.2 [26]. The Pairwise genetic distances were calculated using the Kimura two-parameter (K2P) model to estimate interspecific and intraspecific sequence divergence. The resulting p-distance values were used to assess genetic differentiation.

3. Results

Systematics

Phylum CNIDARIA

Class ANTHOZOA

Subclass OCTOCORALLIA

Order MALACALCYONACEA

Family CLADIELLIDAE

Genus *Aldersladum*

1. **Species *Aldersladum sodwanum* (Benayahu, 1993)** Figs. 2-3

Efflatounaria sodwanae Benayahu 1993: 11–14,

Aldersladum sodwanum Benayahu and McFadden, 2011: 84:1-11,

Specimens examined: Examined colonies (two specimens, India: Gujarat, Gulf of Kachchh: Narara reef, 22˚29'15.42"N; 069˚42'49.92"E, 3-4m depth, Coll. R. Chandran, 14.iii.2017); Reg. Nos. ZSI HQ/GNC/P4719/1 & ZSI HQ/GNC/P4816/1; National Zoological Collections, ZSI, Kolkata, India.

Description

Colony: A small patch of colonies with encrusting growth form found attached to a sub-tidal reef at a depth of 3-4 m. Zooxanthellate; total height of the colony in cross-section measured up to 11.75 mm. On the surface of the basal stalk, knob-like lobes are sparsely erected. Branches measured up to 9.40 mm in height and 3.11mm in breadth. The basal stalk is fleshy and encrusting, creamy white to brownish white in colour. Basal stalk of *in-situ* colonies covered with bushy, dark brownish polyp bundles or lobes (Figs. 2A-B). Polyps are confined mostly to the lobes, and a bunch of polyps forms polyp bundles (Figs. 2C-D). Polyps are very rarely distributed just adjacent to the basal part of the branch stalk. The neck of the anthocodia is tubular. Polyps are partially retracted, with tentacles withdrawn into the anthocodia. The size of the polyps measured up to 2.83 mm long and 0.54 mm in diameter. However, among the individual polyps that arise from the lobe base, some are lengthier and measure up to 3.64 mm X 0.59 mm. The density of polyps is reduced towards the base of the lobes. The wall of the anthocodial tube comprises longitudinal and horizontal grooves, which may facilitate the retraction and expansion of polyps. The vertical grooves on the polyps are prominent, extend as a continuation from tentacles, and run vertically along all secondary or syndytes and primary lobes. In live colonies, the aboral side of each contracted tentacle has a contrast 'fluorescent creamy white spot'. In expanded polyps of live colonies, these white spots are observed as longitudinal white markings,

196 extending up to the tentacle rachis' tip, and give a ‘white mosaic appearance to the
197 brownish in-situ colony. The oral disc of the live colony is also fluorescent white in
198 colour (Figs. 2E-G). Under a stereo-zoom microscope, it is revealed that these white
199 spots are due to the aggregation of while platelet sclerites (Fig.2E).
200
201 **Tentacles** contain two rows of pinnules on each side of the rachis. There are 5-6
202 pinnules on the outer row, and only 3-4 pinnules in the inner row (Fig. 2H). Pinnules
203 in the preserved specimen are globular-shaped. Proximal 3 pinnules are
204 comparatively bigger in size (up to 0.06 mm), and the size of the pinnules reduces
205 towards the distal rachis end. The inner pinnule row generally starts after the outer
206 row's 2nd or 3rd proximal pinnule on both sides. The pinnule arrangement pattern is not
207 uniform in all the polyps. Tentacle measured up to 0.45 mm in length and 0.31 mm in
208 breadth at the broadest central part of the pinnule rachis (Fig. 2H).
209
210 **Sclerites** are observed mostly in the polyps and are sparse in basal stalk tissues. The
211 sclerites are of platelets, mostly with median waists. Many platelets have a longitudinal
212 slit at the centre, often with a narrowed median waist, and ambiguously look like the
213 number eight. Moreover, heart-shaped sclerites with a median waist on the third side,
214 platelets with poorly developed and irregularly shaped median slits were also observed
215 (Fig. 3A). The entire shape of the platelets is from oblong to furcate ends. The sizes
216 of the platelets were measured up to 0.035 mm long and 0.021 mm in breadth. The
217 surface of the platelets is granulated or porous texture on one side and a flat surface
218 on the other side (Fig. 3B).
219
220 **Colour:** Polyps were dark brown, and the base was white in life. The colony turned
221 yellowish green immediately after being dipped in EtOH preservative, followed by
222 creamy yellow.
223
224 **Remarks:** The morphological characteristics of the presently reported specimen
225 agree with the specimen of *Aldersladum sodwanum* redescribed by Benayahu and
226 McFadden [22], namely a low, encrusting stalk base, knob-like lobes, and
227 monomorphic, partially retractile polyps that are mostly confined to the lobes. Sclerites
228 are present in the polyps and other parts of the colony, and both specimens share
229 comparable morphological and morphometric characters of sclerites. In both cases,
230 the sclerites are ‘figure-eight’- shaped platelets with a median longitudinal slit.

However, in addition to these typical platelets, the Gulf of Kachchh specimen also exhibited heart-shaped sclerites with a median waist on the third side (Fig. 3A), which were not reported by Benayahu and McFadden [22]. Furthermore, the present study provides new in-situ morphological observations supported by clear photographs, whereas the earlier description by Benayahu and McFadden [22] was based on an old museum specimen, precluding the documentation of live characters. Notably, pinnule characters were not reported in the earlier study but are described here for the first time. Thus, the present study contributes additional diagnostic characters that enhance the precise identification of *A. sodwanum*.

Geographical distribution: Distribution of the species has been confirmed previously from Sodwana Bay, Africa; Mombasa, Wasini Is, Kilifi (Mooring) of the Kenya coast; Aoti, the North-east coast of Taiwan; and the Gulf of Oman, Iran [1]. The present study confirms its extended distribution to the Gulf of Kachchh waters, India.

Ecological notes: Colonies are found growing on dead coral boulders in a reef flat that is exposed only during minus low tides. This species distribution has also been observed from Laku point, Gugad Reef, Boriya Reef, and Pirotan Island in the Gulf of Kachchh. The species is found to co-exist with hard coral species having extended polyps, such as *Goniopora* spp. and Hydrozoans. Under stressed conditions, colonies shed off their polyp bundles on the substrates, and each of them grows as an individual colony by developing an enlarged holdfast-like structure at the detached end. This character hints at their potential to propagate in the aquaria.

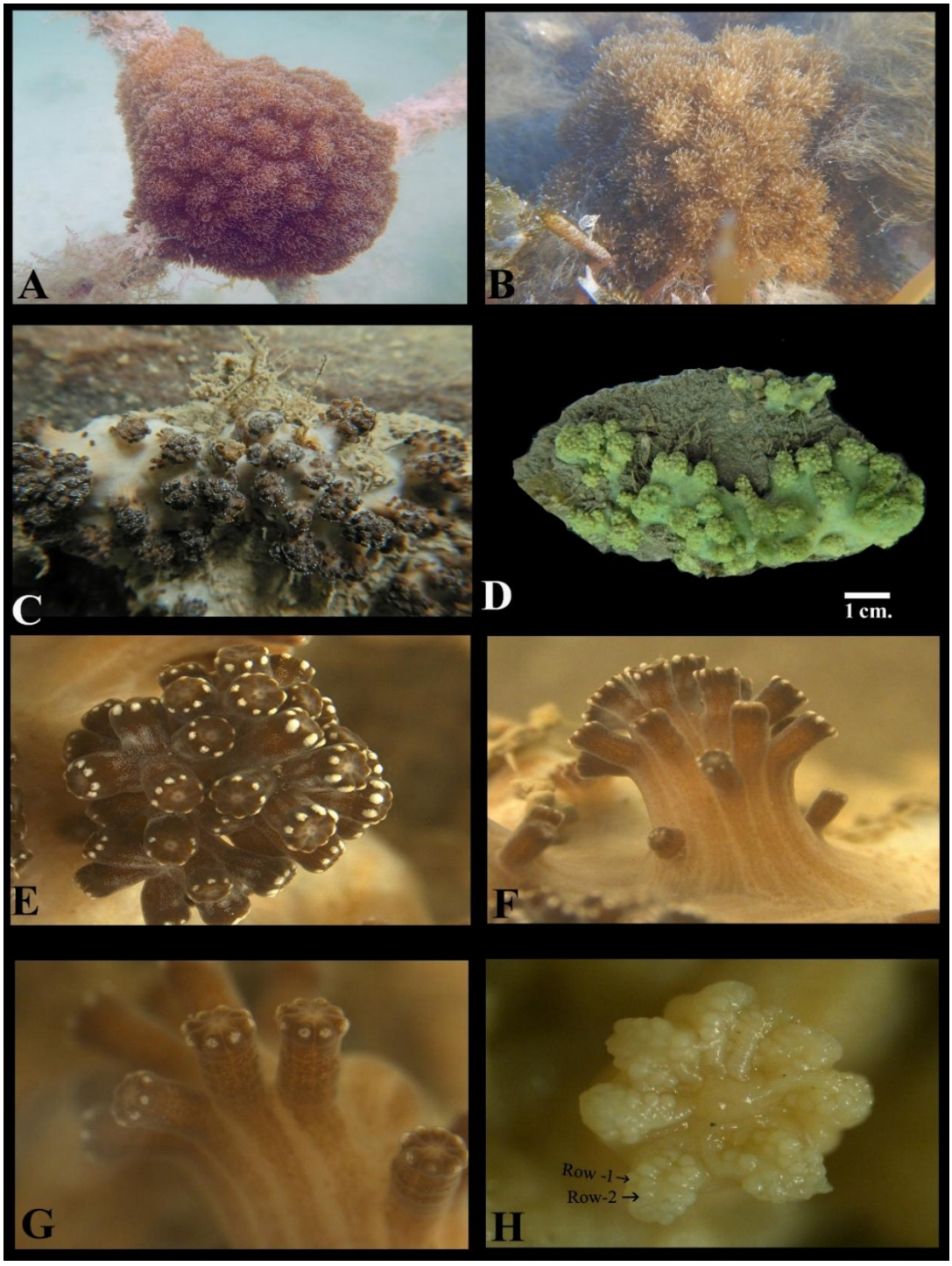


Figure 2. Colonies of *Aldersladum sodwanum* (ZSI HQ/GNC/P4719/1). A-B) Completely expanded in-situ colonies; C) Live colony with retracted polyp bundles; D) Preserved colony; E-G) A partially retracted polyp bundle, showing tubular neck zone of the anthocodia; H) Close-up image of an expanded polyp, showing mouth, and arrangement of pinnules in the tentacles.

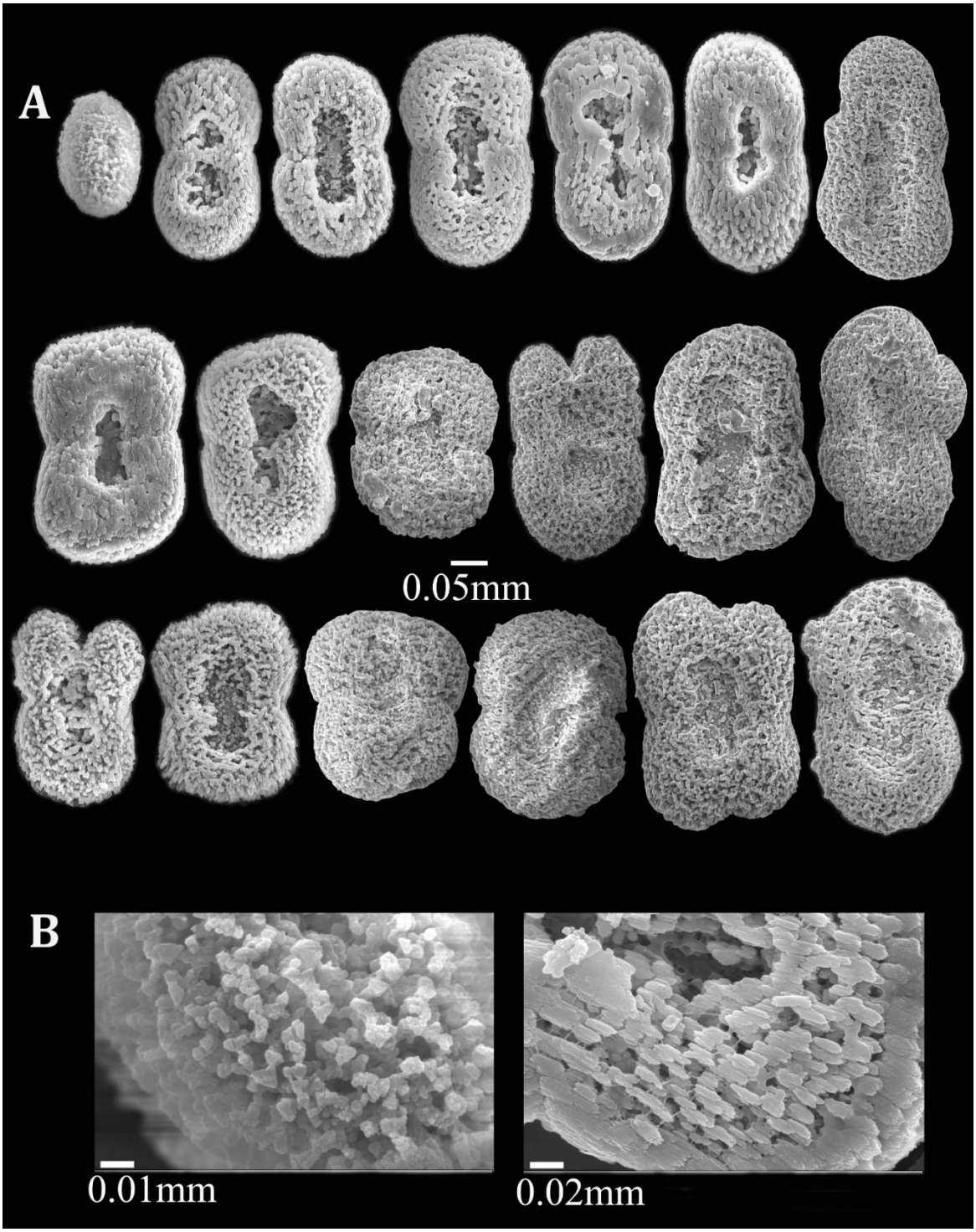


Figure 3. Sclerites from the colony *A. sodwanum* (ZSI HQ/GNC/P4719/1). A) Figure-eight sclerites from the polyps and coenenchyme of the colony**;** B) Surface ornamentation on the front & back sides of the sclerites.

Genus *Cladiella*

2. Species *Cladiella pachyclados* (Klunzinger, 1877) Figs. 4 -7

Alcyonium pachyclados Klunzinger, 1877: 24, Fig. 5.

? *Alcyonium pachyclados*; May 1899: 106 (Zanzibar; Philippines); Hickson 1900: 503.

? *Alcyonium pachyclados*; Hickson 1902: 72; Pratt 1903: 534; Pratt 1905: 258; Thomson & Henderson 1906: 416, (Zanzibar); Cohn 1908: 235 (Madagascar); Kükenthal 1910: 34; Thomson 1910: 570, pl. 2, Fig. 14; pl. 4, Fig. 33 & 34 (South Africa); Lüttschwager 1914: 20–22 (Indo-Pacific); Lüttschwager 1922: 522, pl. 1, Fig. 1 (Philippines); Hickson 1930: 219 (Annam, Vietnam); Thomson & Dean 1931: 40 (Indonesia); Roxas 1933: 357 (Philippines).

Microspicularia pachyclados; Macfadyen 1936: 29.

? *Lobularia pachyclados*; Tixier-Durivault 1944: 340–341 (Red Sea); Tixier-Durivault 1948: 219–225, Figs. 215–220.

? *Cladiella pachyclados*; Utinomi 1959: 308 (Taiwan); Tixier-Durivault, 1966: 50, Fig. 30 (Tongatapu, Tonga); Tixier-Durivault, 1970a: 202 (New Caledonia); Verseveldt 1971: 9–10, Fig. 4 (Madagascar); Benayahu, 1997: 210–212 (Guam); Rao & K. Devi 2003: 13 & 14, Fig. 7 (Little Andaman, India); Vibha et al. 2015: 1787–1789: Figs. 2–8 (Port Blair, Andaman, Nicobar Islands);

? *Cladiella pachyclados*; Tixier-Durivault, 1972: 58 (Madagascar listed only); Verseveldt 1974: 95 (New Caledonia listed only); Benayahu & Loya 1977: 372 (Eilat, Red Sea listed only); Verseveldt, 1977: 3 (American Samoa listed only); 1978: 49 (Guam listed only); van Ofwegen, & Vennam 1994: 136 (Ambon, Indonesia listed only); Benayahu 1995: 106 (Ryukyu Islands, Japan listed only); Fabricius 1995: 151 (Heron Island, Australia listed only); Imahara 1996: (Ryukyu Islands, Japan listed only); van Ofwegen 1996: 207 (Laing Island, Bismarck Sea listed only); Vennam & van Ofwegen 1996: 438 (Laccadive listed only); Iha & Yoshino 1997: 93 (Ryukyu Islands, Japan listed only); Benayahu 2002: 13–14 (Ryukyu Islands, Japan listed only); Benayahu et al. 2003: 55 (Mozambique listed only); Benayahu, et.al. 2004: 550 (Taiwan listed only); Fabricius & McCorry 2006: 29 (Hong Kong listed only); van Ofwegen 2007: 141 (New Caledonia listed only); Latypov & Selin 2008: 147 (Vietnam listed only); McFadden et al. 2011: 25

(Israel, Eilat listed only), Benayahu et al. 2012: 1532 (Penghu Archipelago, Taiwan listed only); Padmakumar & Chandran, 2012: 62 (Little Andaman listed only); Seah et al. 2015: 302 (Singapore listed only); Benayahu et al. 2018: 4 (Dongsha Atoll, Taiwan listed only); Schleyer et al. 2019: 2487 (Europa Is., France listed only).

Not *Alcyonium pachyclados*; Stiasny 1937: 392.

Cladiella pachyclados Lazar et al. 2025 Figs. 41B, 44 & 45.

Specimens examined: Examined colony (one specimen, India: Gujarat, Gulf of Kachchh: Narara reef, 22˚28'00.11"N; 069˚40'37.83"E; 0.5-1.5 m depth; Coll. R. Chandran, 12.iv.2018); Reg. No. ZSI/HQ/GNC/P4660/1; National Zoological Collections, Kolkata, India.

Description:

Colony: Zooxanthellate, monomorphic with autozooids in the polypary. Polyps are mainly distributed on the lobes and the capitulum, with fewer polyps (Figs. 4A-B). A completely expanded in situ colony appeared arborescent (Fig. 4C). Lobes of the retracted in-situ colony are short, digitate-like, but the lobes of the preserved colony look short and knob-shaped (Fig.4A-B & 4D-F). The knob-shaped, retracted lobes measured up to 7.6mm in diameter. Subdivided branches were observed occasionally. The live colony showed remarkable retraction capacity in reducing its size multifold upon disturbance.

The examined (preserved) colony is creamy white in colour in EtOH, 1.7 cm in height, and 2.35 mm in breadth. Stalk short, void of any polyps. The margin of the capitulum extends beyond the stalk (Fig. 4F). Polyp pits are densely arranged on the lobes. The distance between polyp pits was measured from 0.4 mm to 0.54 mm in polypary.

Tentacles are completely retractable within the lobe wall, leaving open polyp pits with a 'ring-wall' protruding calyx. Maximum outer diameter of the calyx measured up to 1.38 mm. Sterile stalks observed with vertical and horizontal shrink lines.

Colour: *In-situ* colony milky white in colour with dark brownish polyps.

Sclerites from the lobe surface are of 'dumb-bells' with definite median waists in a size (l x b) range from 0.06 X 0.04 mm to 0.104 X 0.061 mm (Fig. 5A). But, some

different dumb-bells with short median waists were also observed, and their size was measured up to 0.107 X 0.049 mm. Sclerites of the sterile stalk surface appeared similar to those of the lobe surface. Their size also measured from 0.059 X 0.028 mm to 0.113 X 0.066 mm (Fig.5B). Lobe interior sclerites are also of dumb-bells and comparatively stouter than the lobe surface sclerites. The size of the lobe interior sclerites measured from 0.081 X 0.058 mm to 0.117 X 0.064 mm (Fig. 6A). Dumb-bells of stalk interior measured up to a size similar to the lobe interior sclerites (i.e., measured up to 0.118 X 0.066) (Fig. 6B**)**. Most dumb-bell sclerites have two whorls of warts on both sides and a definite median waist. But rarely dumb-bells with irregular whorls of warts with shorter median waists or without median waists were also observed. Crosses were also observed very rarely. Polyps' scleromes are small platelets, figure-eight shaped and rod-like, mostly with median waist-constrictions and some with one or two central translucent central pits in each lobe of the platelets. In rare scleromes, central translucent pits are connected and form a central long translucent pit (Fig. 6C). The size of the polyp platelets measured up to 0.065 mm X 0.0321mm. Sclerites from all parts of the colony are transparent or white in colour. All examined taxonomical characters confirm that the examined specimen is *Cladiella pachyclados* (Klunzinger, 1877) [27].

Remarks. *Cladiella pachyclados* shows some similarities to *C. krempfi*. Several studies have compared the taxonomic characters of these two species. Hickson (1930) [27] regarded *Alcyonium* (=*Cladiella*) krempfi as identical with *C. pachyclados*, based on a re-examination of the type specimens from Annam and material of *A*. (=*C*.) *pachyclados* from different parts of the world. Verseveldt [20] noted that *C. krempfi* differs from *C. pachyclados* in two respects: (1) the sclerites in *C. pachyclados* reach up to 0.12 mm long, while in *C. krempfi* they reach only 0.10 mm; and (2) in *C. pachyclados*, the retracted zooids are surrounded by a distinctly protruding calyx (ring-wall), whereas in *C. krempfi* the calyx is absent and the surface between zooids is flat. The specimen examined in the present study possesses ring-wall calyces. The sclerites measured up to 0.118 mm, consistent with *C. pachyclados*. In this specimen, calyces are mainly distributed on the lobes of the colony, while in *C. krempfi* the calicular pits occur on both the capitulum surface and lobes [28-29]. Tixier-Durivault (1966) [18] also reported elliptical or figure-eight cortical sclerites with two distinct centers in the tentacles of *C. pachyclados*, which correspond with the present

specimen (Fig. 4c). In a recent redescription of Red Sea type specimens, Lazar et al. [3] provided detailed SEM images and morphometric features of *C. pachyclados*. In the holotype (ZMB CNI 1874), polyp scleromes are various platelets of figure-eight and rod-like shapes, measuring 0.04–0.08 mm (Lazar et al. [3] fig. 44a). Similarly, polyps of the present Kachchh specimen (ZSI/HQ/GNC/P4660/1) have platelets of comparable shapes, measuring up to 0.065 × 0.027 mm (Fig. 6C).

Geographical distribution: The species has been recorded previously from numerous localities in the Red Sea, Pacific Ocean, and Indian Ocean, including Madagascar off the East African coast [20], as well as Luzon, Zanzibar, New Britain, the Maldives, the China Strait, and the Sri Lankan side of the Gulf of Mannar. Within India, it has previously been reported from the Andaman and Nicobar Islands [30] and the Lakshadweep Islands [31]. The present study confirms the new distribution of this species to the Gulf of Kachchh reefs.

Ecological notes: Colonies were infrequently observed on the subtidal edges of the Narara Island, Gulf of Kachchh. The species exhibits a remarkable capacity for polyp retraction. Zooxanthellae distribute densely in the polyps and tentacles, whereas the coenenchyme remains creamy white, giving the colony a bleached appearance.

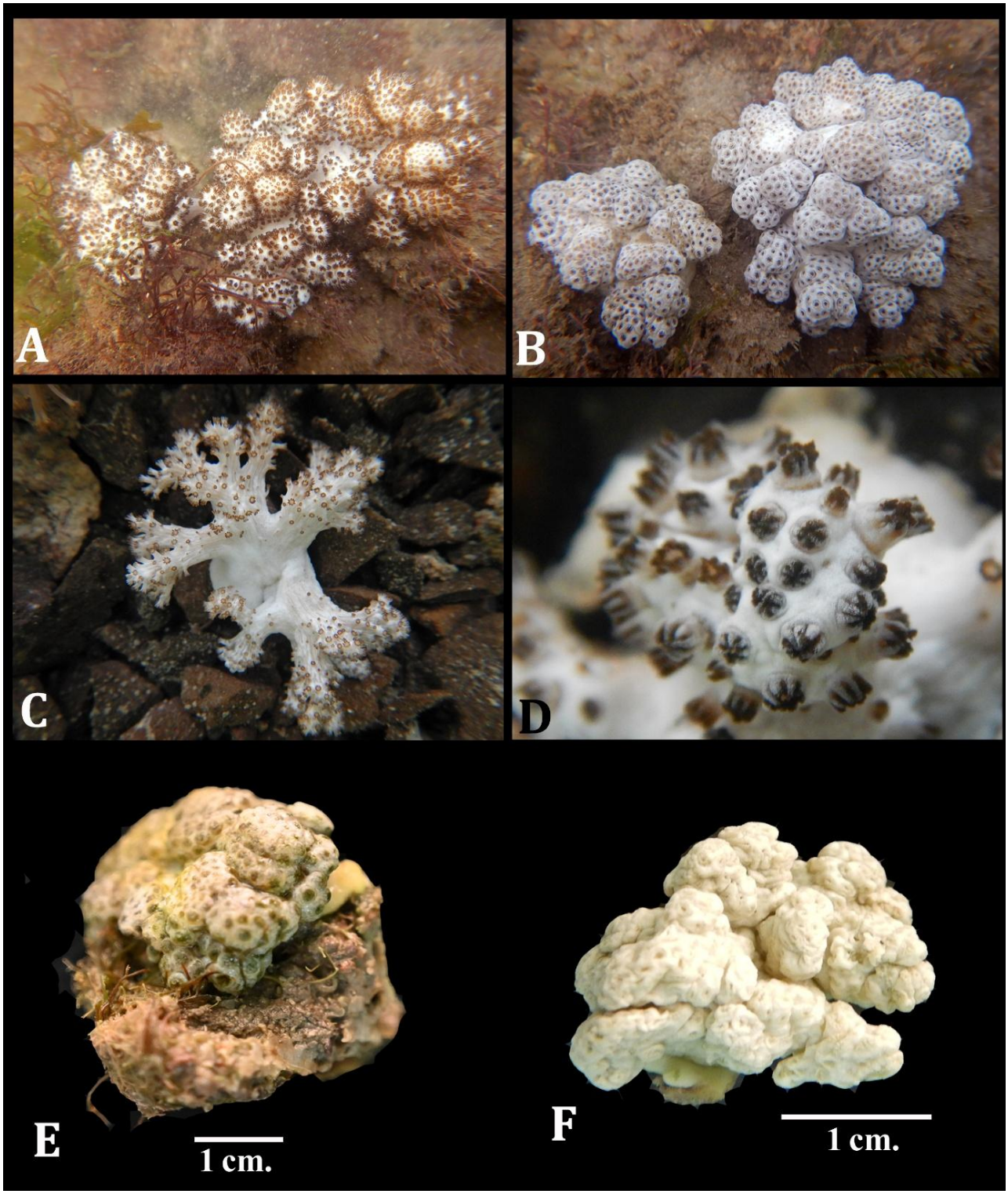


Figure 4. Colony of the species *Cladiella pachyclados* (ZSI/HQ/GNC/P4660/1). A-B) In-situ colonies with expanded polyps and retracted polyps; C) Completely expanded colony; D)Partially retracted polyps in a live colony. E) Colony, immediately after soaking in EtOH; F) Preserved colony.

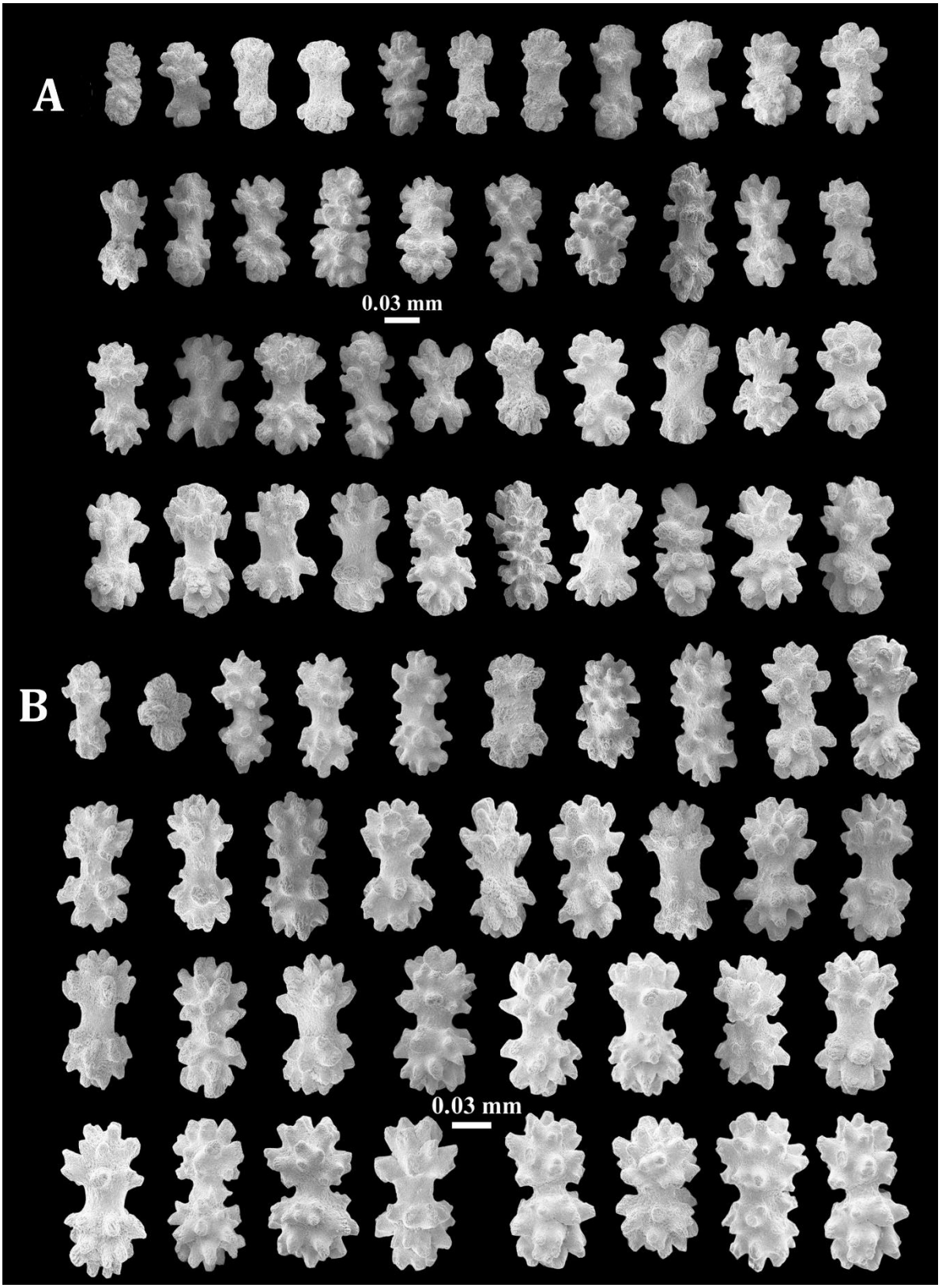


Figure 5. Sclerites from the surface of the colony *C. pachyclados* (ZSI/HQ/GNC/P4660/1). A) Sclerites from the lobe surface B) Sclerites from the stalk surface.

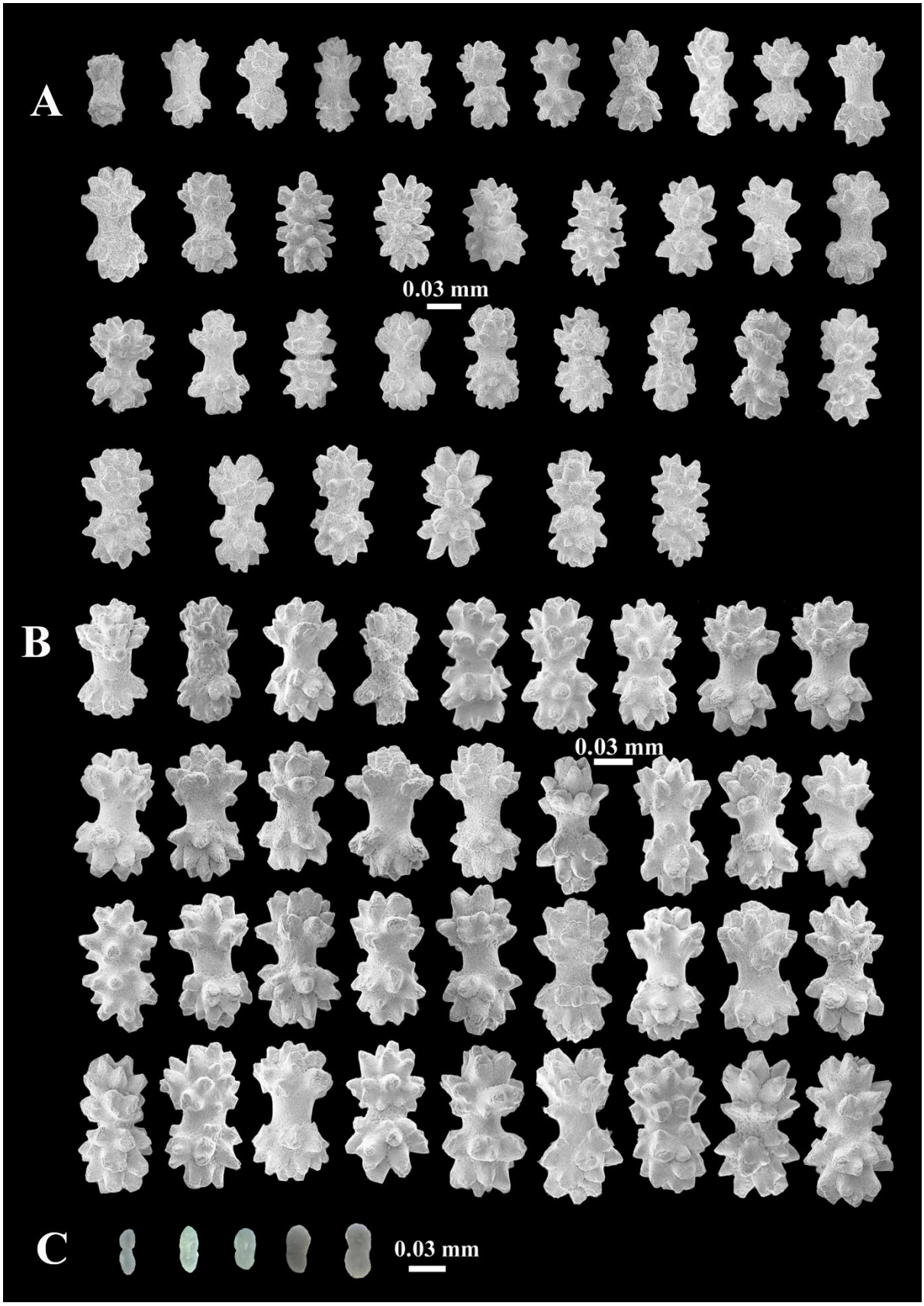


Figure 6. Sclerites from the coenenchyme of the colony *C. pachyclados* ZSI/HQ/GNC/P4660/1). A) Sclerites from the lobe interior B) Sclerites from stalk interior; C. Platelets from polyps.

Phylogenetic analysis

The mtMutS gene sequences confirmed the evolutionary placement of *C. pachyclados* and *A. sodwanum* collected from Kachchh, India. The maximum likelihood (ML) tree indicated that *C. pachyclados* (PV158295) clustered within the *Cladiella* clade, although it did not form a true monophyletic group. Similarly, *A. sodwanum* (OR752865) was grouped within the *Aldersladum* genus, supporting its identification. The similarity of mtMutS gene sequences and distinct phylogenetic clustering support assigning these species to the family Cladiellidae (Fig. 7). Similarly, the molecular gene for *A. sodwanum* (OR752865) from Kachchh, India, shows a close relationship with *A. sodwanum* MTAU CO31520 (JX991193) of Kenya, and differed from the Taiwanese specimen (JX991193) by a p-distance of 0.001, indicating very close sequence similarity. The *C. pachyclados* (PV158295) specimen from Kachchh, India, is closely related to *C. pachyclados* (ZMTAU CO33604: JX991146 and ZMTAU CO35507: JX991197) and *C. sphaerophora* ZMTAU CO34132 (GQ342471), with modest genetic divergence from conspecifics from Taiwan, with p-distance values of 0.003–0.004 based on available NCBI gene sequence data (Table 1). However, in terms of morphological characteristics, *C. pachyclados* resembles *C. krempfi* (Hickson, 1919), as noted in the remarks section.

Phylogenetic reconstructions revealed that the genera *Cladiella* and *Aldersladum* are not monophyletic in the mtMutS tree (Fig. 7). Although species cluster near their respective conspecifics, they do not form clear monophyletic clades. At the genus level, relationships within the family Cladiellidae remain unresolved, as *Cladiella*, *Aldersladum*, and *Klyxum* appear intermixed. Furthermore, the mitochondrial gene mtMutS is widely used in octocoral systematics due to its conserved nature and effectiveness in resolving higher-level relationships. Nevertheless, its low sequence variation at the species level poses a major limitation, as highlighted in this study of the Cladiellidae family (soft corals).

438 **Table 1**. GenBank accession numbers for specimens from different genera of the family
439 Cladiellidae.
440

Species	**Museum**	**Locality**	***mtMutS***
Ofwegenum coronalucis	SMNHTAU_Co_39048	Oman	OR487121
	UF 17263	Oman	OR487122
	UF 15877	Oman	OR487123
	BOMAN-09174	Oman	OR487124
	UF 15882	Oman	OR487125
Ofwegenum aff. *coronalucis*	SMNHTAU_Co_38223	Aquarium trade, USA	OR487121
Ofwegenum verseveldti	SMNHTAU_Co_33097	Israel	GU356012
Ofwegenum kloogi	SMNHTAU_Co_34426	Reunion	OR487117
	SMNHTAU_Co_38229	Reunion	OR487118
Ofwegenum colli	NTM C13089	Australia	OR487120
Aldersladum jengi	SMNHTAU_Co_33607	Taiwan	JX991144
Aldersladum sodwanum	SMNHTAU_Co_31520	Kenya	JX991193
	ZSI HQ/GNC/P4719/1	**India**	**OR752865**
Cladiella australis	SMNHTAU_Co_36313	Taiwan	MH516863
	SMNHTAU_CO_36912	Taiwan	MH516570
	SMNHTAU_Co_36987	Taiwan	MH516571
	SMNHTAU_Co_36042	Mad agascar	OR487126
Cladiella bottae	SMNHTAU_Co_34648	Taiwan	JX991145
Cladiella kashmani	SMNHTAU_Co_32334	Kenya	JX991195
	SMNHTAU_Co_32246	Kenya	JX991194
Cladiella pachyclados	SMNHTAU_Co_33604	Taiwan	JX991146
	SMNHTAU_Co_35507	Palau	JX991197
	ZSI/HQ/GNC/P4660/1	**India**	**PV158295**
Cladiella sphaerophora	SMNHTAU_Co_34132	Israel	GQ342471
Cladiella tuberculoides	SMNHTAU_Co_34686	Taiwan	JX991227
	SMNHTAU_Co_34642	Taiwan	JX991226
Cladiella tuberosa	SMNHTAU_Co_34669	Taiwan	JX991149
Klyxum sp.	UF2684	N. Marianas	OR487127
Klyxum adii	SMNHTAU_Co_32636	Kenya	JX991199
Klyxum flaccidum	SMNHTAU_Co_32221	Kenya	JX991200
Klyxum utinomii	SMNHTAU_Co_34639	Taiwan	JX991151
	SMNHTAU_Co_34127	Israel	GQ342476

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Table. 2. Pairwise p-distances (K2P model) of *Aldersladum sodwanum* and *Cladiella pachyclados* MutS gene sequences in bold are from the present study; other sequences originate from the Penghu Archipelago, Taiwan (Benayahu et al. 2012).

S.No	Species	Specimen_voucher	Locality	accession no	1	2	3	4	5	6	7	8	9
1		**P4719/1**	**India**	**OR752865**									
2	*A. sodwanum*	ZMTAU: CO31520	Taiwan	JX991193	0.001								
3		ZMTAU: CO35392	South China Sea	MH516555	0.004	0.005							
4	*A. jengi*	ZMTAU: CO33607	Taiwan	JX991144	0.004	0.005	0.000						
5		**P4660/1**	**India**	**PV158295**	0.003	0.004	0.004	0.004					
6		ZMTAU: CO35507	Taiwan	JX991197	0.003	0.004	0.004	0.004	0.003				
7	*C. pachyclados*	ZMTAU: CO33604		JX991146	0.004	0.005	0.005	0.005	0.004	0.004			
8	*C. bottai*	ZMTAU: CO34648		JX991145	0.002	0.003	0.002	0.002	0.002	0.000	0.003		
9	*C. tuberosa*	ZMTAU: CO34669		JX991149	0.002	0.003	0.005	0.005	0.005	0.003	0.007	0.003	
10	*C. sphaerophora*	ZMTAU: CO34132		GQ342471	0.001	0.003	0.003	0.003	0.001	0.001	0.003	0.000	0.003

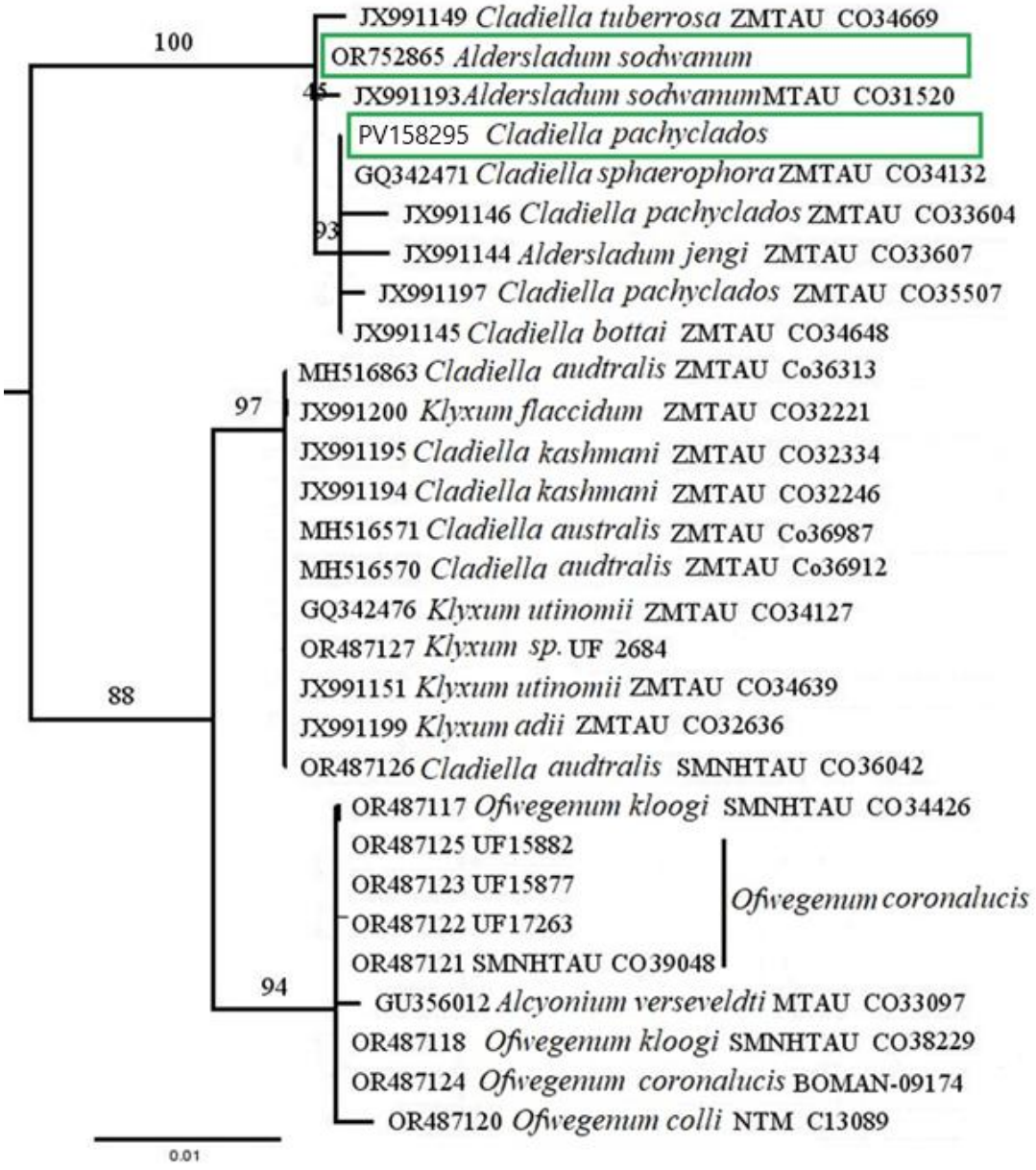


Figure 7. Maximum Likelihood Phylogenetic tree showing the Phylogenetic position of *Aldersladum sodwanum* and *Cladiella pachyclados* within the family Cladiellidae, based on Maximum Likelihood (ML) analysis of concatenated mtMutS sequences.

4. Discussion

The present study confirms the first occurrence of two representatives of the family Cladiellidae from murky, sedimentation-influenced marginal reefs of the Gulf of Kachchh. The new findings of *Aldersladum sodwanum,* and *Cladiella pachyclados* in the Gulf of Kachchh mark the first molecular confirmation in Indian waters, expanding their known geographic distribution beyond their type localities. Integrating morphological and molecular data enhances the reliability of species identification and distributional records, especially in poorly studied and environmentally challenging reef habitats. Given the potential cryptic diversity within *C. pachyclados*, the molecular findings from this study establish a valuable reference point for future comparative research. Soft corals belonging to the genus *Cladiella* are widely distributed, ranging from the African coast and the Red Sea in the west to Hawaii in the east [32], with 61 valid species [4]. Despite their broad distribution across various habitat types, their occurrence in Indian waters has so far been largely restricted to clear-water reef regions such as Lakshadweep and the Andaman & Nicobar Islands, and with only rare records from the Gulf of Mannar [7, 33, 30]. Earlier descriptions by Verseveldt [20] and Lazar et al. [3] relied on museum specimens, whereas we provide clearer morphological and molecular details observed in this study, documented with high-quality photographs. This approach addresses taxonomic gaps by combining field observations with preserved specimens. Rajesh et al. [33] reported *C. pachyclados* only from the Tuticorin coast in the Gulf of Mannar; whereas our study confirms its presence on the Indian mainland with both morphological and molecular data. Similarly, Benayahu et al. [34] suggested cryptic diversity exists within *C. pachyclados*, meaning visually similar populations may be genetically related. This shows the need for more integrative taxonomic research, discussed in the next section with molecular findings. Moreover, this newly distributed data indicates notable ecological resilience in the Gulf of Kachchh to its harsh conditions, including high turbidity and elevated summer sea surface temperatures (SSTs).

Phylogenetic analysis of the mtMutS gene, a mitochondrial gene commonly used to distinguish species, shows that the genera *Cladiella* and *Aldersladum* are not monophyletic (i.e., do not form groups containing all descendants of a common ancestor). The specimens from this study cluster with their respective species.

However, they do not form distinct, well-supported monophyletic groups. At the genus level, relationships within the family Cladiellidae remain unclear, with *Cladiella*, *Aldersladum*, and *Klyxum* intermixed. This observation aligns with previous research. Single-gene phylogenies (evolutionary trees based on a single gene sequence) in Anthozoa often lack resolution due to slow mitochondrial DNA evolution and limited marker variability (e.g., cytochrome oxidase subunit I (COI) and mtMutS). As a result, phylogenetic signals (genetic evidence of relatedness) are weak and node support (statistical confidence in branching points) is low, especially among closely related taxa. Recent perspectives emphasize that single-gene datasets are insufficient for resolving higher-level relationships in Octocorallia (a group of soft corals). In contrast, recent phylogenomic research using ultraconserved elements (UCEs, highly similar DNA regions across species) has provided much greater resolution. This offers a more robust framework for anthozoan systematics and overcomes the limitations of single-gene analyses [35-36] (McFadden et al. 2006; Erickson et al. 2020).

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Author contributions:

RC: Collection of specimens, taxonomical identification, writing the original draft and revision; RP: Molecular sequencing of specimens, processing the molecular data; manuscript preparation and completion of manuscript; RS: Correction of manuscript, SEM imagings of sclerites; CS: Manuscript improvement, plates and figures preparation; DB: Review the manuscript, validation and supervision. All authros read and approved the manuscript.

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Data Availability Statement

The authors confirm that the molecular datasets generated and/or analysed during the current study are available in the NCBI repository, ttps://www.ncbi.nlm.nih.gov/nuccore/OR752865 and https://www.ncbi.nlm.nih.gov/nuccore/PV158295]

Permissions to collect specimens

The specimens were collected by Marine National Park authority, Jamnagar (Gujarat Forest Dept.), as part of their annual Bio-physical monitoring surveys, conducted during 2017-2018. Collected specimens were doposited at National Zoological Collections, ZSI-Kolkata with voucher numbers, P4660/1 and P4719/1 for further reference.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial and non-financial interests in relation to the work.

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