

The first record of the genus *Lamellibrachia* (Siboglinidae) tube worm along with associated organisms in a chemosynthetic ecosystem from the Indian Ocean: A report from the Cauvery-Mannar Basin

A.Mazumdar*¹, P. Dewangan¹, A. Peketi¹, Firoz Badesab¹, Mohd Sadique¹, Kalyani Sivan¹, Jittu Mathai¹, Ankita Ghosh¹, A. Zatale¹, S.P.K Pillutla¹, Uma C², C. K. Mishra¹, Walsh Fernandes¹, Asha Tyagi³, Tanojit Paul⁴

¹Gas Hydrate Research Group, CSIR-National Institute of Oceanography
Dona Paula, Goa-403004, India

²Kerala University of Fisheries and Ocean Studies, Kochi, Kerala-682506

³K.J. Somaiya College of Science and Commerce, University of Mumbai,
Maharashtra- 400077

⁴Manipal Institute of Technology, Manipal, Karnataka-576104
Corresponding author email: maninda@nio.org

Abstract

Here we report for the first time of the genus *Lamellibrachia* tubeworm and associated chemosynthetic ecosystem from a cold-seep site in the Indian Ocean. The discovery of cold-seep was made off the Cauvery-Mannar basin onboard *ORV Sindhu Sadhana* (SSD-070; 13th to 22nd February 2020). The chemosymbiont bearing polychaete worm is also associated with squat lobsters (*Munidopsis* sp.) and Gastropoda belonging to the family Buccinidae. Relict shells of chemosynthetic *Calyptogena* clams are ubiquitous at the seep sites. The *Lamellibrachia* tubes were found to be firmly anchored into the authigenic carbonate crusts. The authigenic carbonate crusts (chemoherm) are packed with large *Calyptogena* shells (whole shell and fragments). Very high concentrations (3800-12900 μM) of hydrogen sulfide (H_2S) in the interstitial waters (40 cmbsf) is responsible for the sustenance of chemosymbiont bearing tubeworms. The posterior end of the tube penetrates downwards into the H_2S -rich zone. The high concentration of H_2S at ~ 40 cmbsf is attributed to sulfate reduction via anaerobic oxidation of methane (AOM) pathway. Methane hydrate was observed within the faults/fractures in the sediments. The presence of ethane and propane along with methane in the headspace gases and $\delta^{13}\text{C}_{\text{CH}_4}$ values (-28.4 to -79.5 ‰ VPDB) suggest a contribution of deep-seated thermogenic methane.

Keywords: cold-seep, gas flares, methane hydrate, methane, hydrogen sulfide, chemosymbiont, tubeworm

1. Introduction

Marine cold-seep ecosystems are characterized by the build-up and/ or emission of methane across the sediment-water interface and accumulation of very high interstitial hydrogen sulfide concentrations near the sediment-water interface at low-temperature sea-bed conditions (Levin, 2005; Levin et al., 2016). The cold-seep ecosystem occurs as patches of variable shape and size across the ocean floor depending upon the areal extent of hydrocarbon conduits like fractures and faults (Panieri et al., 2017). The biotic communities thriving at the cold-seeps are characterized by an endemic ecosystem comprised of chemosynthetic and heterotrophic fauna. The flux of H₂S and CH₄ fluxes at the sediment-water interface (Portail et al., 2015) primarily controls the faunal diversity and spatial distribution of cold-seep ecosystems. On the other hand, a steady flux of methane and hydrogen sulfide gases control the growth and sustenance of cold-seep ecosystems.

Cold-seeps and associated ecosystems are recorded from numerous sites across the world ocean (Sibuet and Olu-Le Roy, 2002; Levin, 2005; Vanreusel et al., 2009; Olu et al., 2010; Mazumdar et al., 2019; Feng et al., 2020), including Hikurangi basin (off New Zealand); Congo-Angola and Nigeria margins (off West Africa), Nankai Trough (Japan); upper, middle and lower Louisiana slope/ Florida escarpment (Gulf of Mexico); Gulf of Cádiz and areas of Nile deep Sea site (Eastern Mediterranean); Barbados Trench; Nordic margin; Makran coast (off Pakistan); off Papua New-Guinea; South China Sea; Queen Charlotte Basin (off the Pacific north coast of British Columbia); Krishna-Godavari basin (Mazumdar et al., 2019); Arctic sediment (Astrom et al., 2020) and below the Larsen Ice Shelf off the Antarctic (Niemann et al., 2009). The Global attention to the genesis and sustenance of methane cold-seeps is attributed to interest in evolutionary biology, bioprospecting of extreme ecosystems, the contribution of methane emissions to global warming, and application in methane hydrate exploration (Le Bris et al., 2016).

Here we report for the first time association of *Lamellibrachia* tubeworm with methane seep related chemosynthetic ecosystem from the Indian Ocean. The seep site is identified off Mannar Basin (within the EEZ of India). The observed ecosystem developed on a carbonate chemoherm without active gas flares. However, methane flares were observed at other locations (Peketi et al., *under rev.*). The sites are also characterized by chemosymbiont bearing and associated heterotrophic biotic assemblages and shallow methane hydrates (2-3 mbsf). The seep site is located at 1644 m water depths (Pressure: 13.9 MPa and Temperature: 4.95 °C).

2. Geology

The Mannar basin (Fig. 1A), located between India and Srilanka, is a pull-apart sub-basin of the Cauvery-Mannar Basin along the eastern continental margin of India. The basin came into existence in the Late Jurassic/ Early Cretaceous due to fragmentation of the Gondwanaland and drifting of the India-Srilanka landmass system away from Antarctica/Australia continental plate (Yoshida et al., 1992; Premarathne et al., 2016). Numerous deep extensional faults developed in the NE-SW direction during rifting and had initiated active subsidence that resulted in the formation of graben and horst blocks (Bastia and Radhakrishna, 2012; Yanqun et al., 2017). Differential subsidence resulted in the formation of sub-basins within the Cauvery basin. The Gulf of Mannar sub-basin is further divided into western and eastern segments by the NE-SW-aligned Mandapam-Delft ridge (Rao et al 2010). Curray (1984) opined that the first rifting between India, Sri Lanka, and Antarctica occurred through the Cauvery - Palk Strait - Gulf of Mannar zone but this rift did not progress into the seafloor spreading stage.

Sediment thickness of more than 6 km has been reported from the deep-waters of the Mannar basin (Ratnayake et al., 2017). Seismic and drill well studies indicate six sedimentary sequences including a thick Early Cretaceous rift fill sequence (principal source horizon) overlain by a complete succession of Tertiary sequence (Rao, 2006; Biswas, 2012).

3. Methodology

The expedition (SSD-070) was carried out in the Mannar basin onboard *ORV Sindhu Sadhana* from 13th to 22nd February 2020. The multi-beam bathymetry shows several pockmark-like features in the Mannar basin; one such pockmark was investigated in the expedition SSD-070 (Fig. 1A). The water-column (WC) images produced by the multibeam echosounder (Atlas-Hydrosweep DS) did not detect any gas flares over the pockmark. Seabird CTD profiler (Fig. 1B) and sound velocity profiling (SVP) data were used to generate water column images. Salinity, temperature, and SVP data were used to carry out the depth corrections for multibeam data. We collected seabed samples at the center of the pockmark (Lat: 7°51.37086'N Long: 78°36.45660' E; Water depth: 1644 m) using a spade-corer (48cm (L) x 48cm (B) x 44cm (H)) and a gravity corer (PVC liner; inner diameter: 10 cm). Handpicked organisms from the spade cores were stored in either isopropyl alcohol or in -20°C refrigerator for the shore-based analysis. Authigenic carbonates and hard shells were cleaned, dried at room temperature for petrographic/ chemical analyses, and taxonomic identification. Sediment pore-fluid/ gas-extraction and preservation for onshore analyses of concentrations and isotope ratios

(Mazumdar et al., *in prep.*) were carried out on board. We recovered gas hydrate samples from the gravity core, which were stored in gas-tight tubes for on-shore carbon isotope ratio measurements.

4. Results and Discussion

4.1 Biotic Association

4.1a Polychaete Tubeworm: *Lamellibrachia* sp. and ecological significance

Two whitish polychaete tubeworms (*Lamellibrachia* sp.) were recovered in a spade core from the pockmark (SSD-070-5). The tubes were firmly anchored into the authigenic carbonate crust (Fig. 2A). The length of tube-1 and tube-2 are 93.5 and 57 cm respectively and are found to lie 50 and 26 cm above the carbonate crust. The posterior part of the tube is translucent and sinuous (Fig.2B). The external surface of the tube is relatively smooth due to a lack of sharply projecting collars (figure-2C) and resembles (Supplementary fig.1) the appearance of *Lamellibrachia columna* (Southward 1991) and *Lamellibrachia anaximandri* (Southward et al., 2011). The growth rings are discernible in the top 32 mm. The maximum diameter of tubes- 1 and 2 are 13.4 and 15 mm respectively. The vestimentiferan tubes are composed of giant β -chitin crystallites embedded in a protein matrix (Gaill et al., 1992) and the thickness of the chitin wall is \sim 2.5 mm at the anterior end. The diameter of the tubes decreases downwards and tapers at the posterior end. Goose barnacles (*Neolepus* sp.; Mazumdar et al., 2019) and byssus threads are attached to the outer surface of the tubes (Fig. 2C).

Tube-1 was dissected to observe the soft body of the worm. The different segments of the polychaete worm body like branchial plume (l = 0.84 cm), vestimentum (l = 4.7cm), trophosome (l = 2.89 cm), and trunk (l = 9.9 cm) are marked in the Figure-2D and E. The width of the branchial plume and trophosome are 1 and 0.8 cm respectively, whereas, the trunk part tapers from 0.8 to $<$ 0.1 cm over a length of 9.9 cm. The red colour of the worm is attributed to the presence of hemoglobin within the vascular blood and coelomic fluid which can carry both oxygen and hydrogen sulfide essential for the survival of the chemosynthetic organisms (Arp et al., 1987; Fisher et al., 1988). Unlike hydrothermal vent associated tubeworms, where the H₂S enters the worm's body through the branchial plume (a set of highly vascularized gills), in the cold-seep tubeworms, H₂S is transported into the cold-seep tubeworms through the root-like posterior extension of the body (Julian et al., 1999) which is buried deep inside the sediment. H₂S is produced in the sediment via the sulfate reduction-AOM pathway (Vossmeyer et al., 2012). The tubeworms lack a mouth or gut and host an endosymbiotic chemolithoautotrophic γ -Proteobacterium (S- bacteria) inside the bacteriocyte cells hosted in the vascularized trophosome sac (Duperron et al., 2009). The polychaete worms acquire the symbionts from the ambient environment at the larval stage (Harmer et al., 2008). Through H₂S

oxidation, the symbionts in the bacteriocyte cells fix CO₂ into organic molecules thus fulfilling the nutritional requirements of the polychaete worm. Sulfate ion, a by-product product of HS⁻ oxidation, and H⁺ produced via other metabolic processes are released from the coelomic fluid across the root into the sediment interstitial waters (Dattagupta et al., 2006). The SO₄²⁻ elimination is accompanied by anion (HCO₃⁻) uptake across the root epithelium. The sulfate-sulfide recycling process is energetically economical and plays an important role in the long-term sustenance of the organisms (Dattagupta et al., 2006). Additionally, Thiel et al (2012) reported two different carbon fixation pathways for the endosymbiont namely, the Calvin-Benson-Bassham (CBB) and reductive tricarboxylic acid (rTCA) cycles.

Vestimentiferan tubeworms associated with methane seeps are slow-growing organisms and individuals may be older than 200 years (Cordes et al., 2005; 2007), which may be attributed to stable environments without resource limitation and lack of lethal predation. Assuming a conservative growth rate of 0.77 cm y⁻¹ (Fisher et al., 1997) the estimated age of the longer tube (tube A) in our study is 121 years.

The genus *Lamellibrachia* Webb, 1969 is one of the few Siboglinidae worms with a broader geographic and habitat distribution such as seeps, vents, and whale falls in the Pacific, Atlantic, Caribbean, and Gulf of Mexico (Bright and Lallier 2010; Feldman et al. 1998; Kobayashi et al. 2015; Nishijima et al. 2010; Watanabe et al. 2010). Before this report, there was no record of this genus from the Indian Ocean. Figure-3 shows the global distribution of eight species of the genus *Lamellibrachia* Webb polychaete tubeworms (modified after Mccowin and Rouse, 2018). The present discovery (marked as a star in figure-3) is the first report of *Lamellibrachia* sp. from the Indian Ocean. Earlier Mazumdar et al. (2019) reported polychaete tubeworm belonging to *Sclerolinum* sp from the Krishna-Godavari methane seepage sites.

4.1b Squat lobsters, gastropod and relict Calyptogena shells

Two morphotypes (I and II) of squat lobster (Figs. 4A to D) belonging to the genus *Munidopsis* were recovered from the pockmark site SSD-070-5. The morphotypes reported here are distinct from those reported earlier from the K-G basin (Supplementary fig. 2) cold-seep sites (Mazumdar et al., 2019). Numerous species (~260) of the genus *Munidopsis* have been reported across the globe from cold-seeps, hydrothermal vents, deep-sea decomposing natural sunken woods, and whale carcasses (Martin and Haney, 2005; Marin 2020). The morphotype I (Fig. 4 A and B) and morphotype II (Fig. 4C and D) show distinctly different carapace, abdominal, cheliped, telson, and sternum structures. Although the lengths of carapace and abdomen segments in morphotype I (11 mm & 5.5 mm) is

comparable to that of morphotype II (12 mm & 4 mm), the rostrum in morphotype I (6 mm) is significantly bigger than that of morphotype II (3.5 mm). Morphotype I shows prominent transverse grooves and serrate granules on the carapace which is missing in morphotype II, whereas later has setae on the carapace. The triangular abdomen part with broad ridges and groves in morphotype I is distinct from the rounded form in morphotype II. The segmentation patterns of the telson, sternum, and chelipeds are also different in the two morphotypes. The morphotype I and II are heterotrophs and their food sources include chemosynthetic bacteria, meiobenthos, and soft bodies of chemosymbiont bearing mussels (Thurber et al., 2011; Tsuchida et al., 2011; Macavoy et al., 2008).

Gastropod (Fig. 4E) belonging to the family Buccinidae (Kantor et al 2013) known to be associated with cold-seeps and hydrothermal vents was recovered from the study sites. The 6 cm long gastropod sample has a 3 cm high spire while the last whorl is 2.5 cm long. Individual whorls (5 in number) have rounded margins with smooth axial ribs. The siphoncal canal is elongated with a broad inner lip. The foot part (soft body) is also visible. Cold-seep gastropods show multiple feeding habits like grazing, filter-feeding, predation, and scavenging. Additionally, Sasaki et al. (2010) reported the presence of symbiotic bacteria intracellularly in the ctenidium, on the exterior of the gill, and fine-grained pyrite grains in the diminished digestive system indicating possible chemosynthetic feeding habit as well.

Large-sized (up to 15 cm long) relict shells of *Calyptogena* sp. (Fig. 4F) are recorded from the study site. *Calyptogena* shells are also present as part of carbonate chemoherm in association with the tubeworms. In contrast, bathymodiolus shell fragments were scarce. *Calyptogena* sp. are chemosynthetic organisms and use H₂S for the production of energy and body mass following the hemoglobin-H₂S-O₂-CO₂ biochemical pathway commonly observed in thiotrophic chemosynthesis as discussed for the tubeworms (Dupéron et al., 2013). More than 60 species of *Calyptogena* are reported from the cold-seep and vent regions (Sibuet & Olu 1998; Barry et al., 2007). *Calyptogena* sp. may grow at a rate >4 cm year⁻¹ at some locations (Turekian & Cochran 1981; Turekian et al. 1983). Earlier Mazumdar et al (2019) reported the presence of large *Calyptogena* shells from cold-seeps off the K-G basin.

4.2 Occurrence of methane hydrate, Interstitial H₂S, and role of AOM

Methane hydrate was observed as fracture fillings within the sediments (Fig. 4G and H). The thawing of methane hydrate in the network of cracks results in the brittle and fragmented nature of the muddy sediment. The presence of ethane and propane along with methane in the hydrate phase and $\delta^{13}\text{C}_{\text{CH}_4}$ values (-28.4 to -79.5; Mazumdar et al., *in prep.*) suggest a contribution of deep-seated

thermogenic methane likely transmitted through fault-fracture systems. Thermogenic methane in the surface sediments was earlier reported by Rasheed et al. (2014) from the S-W end of the Mannar basin. The remarkable range of the $\delta^{13}\text{C}_{\text{CH}_4}$ values of the methane-hydrates shows a complex system characterized by micro and macro fractures responsible for advection of thermogenic gases ($\delta^{13}\text{C} > -50\text{‰}$ VPDB: Whiticar et al 1999) and production of biogenic methane ($< -50\text{‰}$) below the SMTZ (~30-35 cmbsf). The bottom water P-T conditions based on the CTD data supports the stability of methane hydrate within the sediments.

High concentrations of hydrogen sulfide (3800 to 12932 μM : Mazumdar et al., *in prep.*) are reported below the sediment-water interface. The production of hydrogen sulfide in the sediment can be explained by microbially mediated sulfate reduction processes dominated by anaerobic oxidation of methane pathway (Knittel and Boetius, 2009) represented by equation-1:



The HCO_3^- ion is consumed by the precipitation of CaCO_3 (Eq. 2) and forms the chemoherm (Fig.5A and B).



The interstitial HS^- (H_2S) is consumed via multiple pathways e.g., (i) precipitation of Fe-sulfide or sulfurized organic matter in the sediments, (ii) consumption by chemosynthetic organism including microbial mats, and (iii) reoxidation at or above the sediment-water interface via oxidative recycling to $\text{S}^0/\text{SO}_4^{2-}$ using oxidants like O_2 and NO_3 (Fernandes et al., 2020; Volvoikar et al 2020)

4.3 Association of tubeworm and carbonate crust

The association of the tubeworm with hard substratum (carbonate crust: Fig. 5 Aand B) may be attributed to the settling of larval vestimentiferans gregariously on exposed rocks which eventually form dense colonies (Fisher et al., 1997). The roots of the tubeworms extend deeper below the hard substratum where H_2S concentrations are enough for their sustenance. The posterior end of the tubes is reported to be thin (~70 μm) and quite permeable (permeability coefficient at 20 °C of $0.41 \times 10^{-3} \text{cm s}^{-1}$) to H_2S (Julian et al., 1999). It is interesting to note that although plenty of large shells could be recovered (Fig. 5 A and B), no live *Calypptogena* sp. could be found along with the tubeworm. The lack of live *Calypptogen* sp. and the absence of active gas flare suggest low H_2S concentration near the sediment-water interface which is essential for sustenance of the *Calypptogena* to survive and grow. However, the tubeworm's posterior part could penetrate to the depth below the seafloor (~30-40 cmbsf) where H_2S is in sufficient concentrations for growth and proliferation of the

tubeworms. Association of tubeworm, carbonate bioherm and *Calyptogena/Bathymodiolus* have also been reported globally (Teichert et al., 2005; Bayon et al., 2009; Bowden et al., 2013; Feng et al., 2013).

5. Conclusion

We report for the first time presence of *Lamellibrachia* sp. and associated chemosynthetic ecosystem from the Indian Ocean. The chemosynthetic fauna associated with the methane cold-seeps and shallow hydrate were discovered from the Mannar basin located between India and Srilanka. The tubeworms are attached to the authigenic carbonate crust enriched with large relict shells of *Calyptogena* sp., a chemosymbiont bearing bivalve. Squat lobsters and gastropod belonging to the *Munidopsis* sp. and Buccinidae family respectively are integral components of the chemosynthesis-based ecosystem. The *Lamellibrachia* polychaete also bears sulfide oxidizing endosymbionts in their trophosome which is responsible for the energy and body mass synthesis. High concentrations of H₂S at some depths below the sediment-water interface are responsible for the sustenance of the tubeworms based on the chemosynthesis based ecosystem. Anaerobic oxidation of methane coupled with sulfate reduction in the sediment is responsible for the interstitial H₂S generation. Carbon isotope ratios of methane and the presence of ethane and propane in the headspace gas suggests a mixing of thermogenic and biogenic methane at the study site. A detailed RoV- based survey would be essential to understand the distribution of the chemosynthetic communities and their relationships to geological and chemical properties of the cold-seep sites.

Acknowledgment

We acknowledge Director, CSIR-NIO, and Secretary, MoES for supporting the gas hydrate program. The background information for the Cauvery-Mannar basin was generated through the CSIR-funded GEOSCAPE program. We thank CSIR-NIO's research vessel management team (Mr. Siddharth Vernekar and Mr. Harish Kumar) for their useful contributions during the SSD-070 cruise. Thanks to Drs. Mandar Nanajkar and Sabyasachi Sautya for useful suggestions and insightful discussion.

Author statement: A. Mazumdar carried out result interpretation, manuscript preparation; P. Dewangan interpreted geophysical results and contributed to manuscript preparation; A. Peketi, Firoz Badesab, Mohd Sadique, Kalyani Sivan, Jittu Mathai, Ankita Ghosh, A. Zatale, S.P.K Pilllutra, Uma C, C. K. Mishra, Walsh Fernande, Asha Tyag, Tanojit Paul participated in onboard sampling and onshore analyses.

References:

- Arp A J, Childress, J J and Vetter R D 1987 The sulphide binding protein in the blood of the vestimentiferan tube-worm *Riftia pachytila*, is the extracellular haemoglobin; *J. Exp.Biol.* **128** 139–158.
- Åström E K, Sen A, Carroll M L and Carroll J 2020 Cold-seeps in a warming Arctic: Insights for benthic ecology; *Frontiers in Marine Science.***7** 244.
- Barry J P, Whaling P J, and Kochevar R K 2007 Growth, production, and mortality of the chemosynthetic vesicomyid bivalve, *Calyptogena kilmeri* from cold-seeps off central California; *Marine Ecology.* **28(1)** 169-182.
- Bastia R and Radhakrishna M 2012 Basin Evolution and Petroleum Prospectivity of the Continental Margins of India; Vol. 59, 1st Edition, Page Count: 432, Elsevier.
- Bayon G, Henderson G M and Bohn M 2009 U–Th stratigraphy of a cold-seep carbonate crust; *Chemical Geology.* **260(1-2)** 47-56.
- Biswas 2012 Status of petroleum exploration in India; PINSA . **475-494** 78 475-494.
- Bowden DA, Rowden AA, Thurber AR, Baco AR, Levin LA, Smith C R 2013 Cold-seep epifaunal communities on the Hikurangi Margin, New Zealand: composition, succession, and vulnerability to human activities; *PLoS One.* **8(10)** e76869.
- Bright M and Lallier F 2010 The biology of Vestimentiferan tubeworms. *Oceanogr Mar Biol An Annu Rev.* **48** 213–65
- Cordes E E, Arthur M A, Shea K, Arvidson R S, & Fisher C R 2005 Modeling the mutualistic interactions between tubeworms and microbial consortia; *PLoS.Biol.* **3(3)** e77.
- Cordes E E, Bergquist D C, Redding M L and Fisher C R 2007 Patterns of growth in cold-seep vestimentiferans including *Seepiophila jonesi*: a second species of long-lived tubeworm; *Marine Ecology.* **28(1)** 160-168.
- Curray J R, 1984 *Sri Lanka: is it a mid-plate platelet?: Journal of the National Aquatic Resources Agency.* **31** 30-51.
- Dattagupta S, Miles L L, Barnabei M S and Fisher C R 2006 The hydrocarbon seep tubeworm *Lamellibrachia luymesii* primarily eliminates sulfate and hydrogen ions across its roots to conserve energy and ensure sulfide supply; *Journal of Experimental Biology.* **209(19)** 3795-3805.
- Duperron S, Gaudron S M, Rodrigues C F, Cunha M R, Decker C and Olu K 2013 An overview of chemosynthetic symbiosis in bivalves from the North Atlantic and Mediterranean Sea; *Biogeosci.* **10** 3241-3267.
- Duperron S, De Beer D, Zbinden M, Boetius A, Schipani V, Kahil N and Gaill F 2009 Molecular characterization of bacteria associated with the trophosome and the tube of *Lamellibrachia* sp., a siboglinid annelid from cold-seeps in the eastern Mediterranean; *FEMS microbiology ecology.* **69(3)** 395-409.

- Feldman R A, Shank T M, Black M B, Baco A R, Smith C R, and Vrijenhoek R C 1998 Vestimentiferan on a whale fall. *Biol Bull.***194** 116–9
- Feng D, Cordes E E, Roberts H H and Fisher C R 2013 A comparative study of authigenic carbonates from mussel and tubeworm environments: Implications for discriminating the effects of tubeworms; *Deep Sea Research Part I: Oceanographic Research Papers.***75** 110-118.
- Feng J, Li N, Luo M, Liang J, Yang S, Wang H and Chen D 2020 A Quantitative Assessment of Methane-Derived Carbon Cycling at the Cold-seeps in the Northwestern South China Sea; *Minerals.***10(3)** 256.
- Fernandes S, Mazumdar A, Peketi A, Anand S S, Rengarajan R, Jose A, Manaskanya A, Carvalho M A and Shetty D 2020 Sulfidization processes in seasonally hypoxic shelf sediments: a study off the West coast of India; *Marine and Petroleum Geology.* **117** 104353.
- Fisher C R, Childress J J and Sanders N K 1988 The role of vestimentiferan hemoglobin in providing an environment suitable for chemoautotrophic sulfide-oxidizing endosymbionts; *Symbiosis.* **5** 229–246.
- Fisher C, Urcuyo I, Simpkins M and Nix E 1997 Life in the slow lane: growth and longevity of cold-seep vestimentiferans; *Mar. Ecol.* **18** 83–94.
- Gaill F, Persson J, Sugiyama J, Vuong R and Chanzy H 1992a The chitin system in the tubes of deep-sea hydrothermal vent worms; *J. Struct. Biol.* **109** 116–128.
- Harmer T L, Rotjan R D, Nussbaumer A D, Bright M, Ng A W, DeChaine E G and Cavanaugh C M 2008 Free-living tube worm endosymbionts found at deep-sea vents; *Applied and Environmental Microbiology.***74(12)** 3895-3898.
- Julian D, Gaill F, Wood E R I C, Arp A J, and Fisher C R 1999 Roots as a site of hydrogen sulfide uptake in the hydrocarbon seep vestimentiferan *Lamellibrachia* sp.; *Journal of Experimental Biology.***202(17)** 2245-2257.
- Kantor Y I, Puillandre N, Fraussen K, Fedosov A E and Bouchet P 2013 Deep-water Buccinidae (Gastropoda: Neogastropoda) from sunken wood, vents and seeps: molecular phylogeny and taxonomy; *Journal of the Marine Biological Association of the UK.***93(8)** 2177-2195.
- Knittel K and Boetius A 2009 Anaerobic oxidation of methane: progress with an unknown process; *Ann. rev. microb.* **63**, 311-334.
- Kobayashi G, Miura T, Kojima S. 2015 *Lamellibrachia sagami* sp. Nov., a new vestimentiferan tubeworm (Annelida: Siboglinidae) from Sagami Bay and several sites in the northwestern Pacific Ocean. *Zootaxa.* **4018** 97–108.
- Le Bris N, Arnaud-Haond S, Beaulieu S, Cordes E, Hilario A, Rogers A, van de Gaever S, Watanabe H 2016 Hydrothermal Vents and Cold-seeps; *In First Global Integrated Marine Assessmented.* United Nations (Cambridge University Press) 853-862.
- Levin L 2005 Ecology of cold-seep sediments: interactions of fauna with flow, chemistry and microbes; *In Oceanography and Marine Biology: an annual review* (eds.) Gibson R N, Atkinson R J A and Gordon J D M, 431-46 (Taylor and Francis).

- Levin LA, Baco A R, Bowden D A, Colaco A, Cordes E E, Cunha M R, Demopoulos AWJ, Gobin J, Grupe B M and Le J 2016 Hydrothermal vents and methane seeps: rethinking the sphere of influence; *Front. Mar. Sci.* **3** 72.
- MacAvoy S E, Carney R S, Morgan E and Macko S A 2008 Stable isotope variation among the mussel *Bathymodiolus childressi* and associated heterotrophic fauna at four cold-seep communities in the Gulf of Mexico; *Journal of Shellfish Research.* **27**(1) 147-151.
- Marin I 2020 Northern unicorns of the depths: diversity of the genus *Munidopsis* Whiteaves, 1874 (Decapoda: Anomura: Munidopsidae) in the northwestern Pacific Ocean, with descriptions of three new species along the Russian coast; *Progress in Oceanography.* **183** 102263.
- Martin J W and Haney T A 2005 Decapod crustaceans from hydrothermal vents and cold-seeps: a review through 2005; *Zoological Journal of the Linnean Society.* **145**(4) 445-522.
- Mazumdar A, Dewangan P, Peketi A, Gullapalli S, Kalpana M S, Naik G P, Shetty D, Pujari S, Pillutla S P K, Gaikwad V V and Nazareth D 2019 The first record of active methane (cold) seep ecosystem associated with shallow methane hydrate from the Indian EEZ; *Journal of Earth System Science.* **128**(1) p.18.
- Mccowin M F & Rouse, G W 2018 A new Lamellibrachia species and confirmed range extension for *Lamellibrachia barhami* (Siboglinidae, Annelida) from Costa Rica methane seeps; *Zootaxa.* **4504**(1) 1-22.
- Niemann H, Fischer D, Graffe D, Knittel K, Montiel A, Heilmeyer O, Nathen K, Pape T, Kasten S and Bohrmann G 2009 Biogeochemistry of a low-activity cold-seep in the Larsen B area, western Weddell Sea, Antarctica; *Biogeosci.* **6** 2383-2395.
- Nishijima M, Lindsay DJ, Hata J, Nakamura A, Kasai H, Ise Y, et al. 2010 Association of thioautotrophic bacteria with deep-sea sponges. *Mar Biotechnol.* **12** 253–60.
- Olu K., Cordes E E, Fisher C R, Brooks J M, Sibuet M, & Desbruyères D (2010). Biogeography and potential exchanges among the Atlantic equatorial belt cold-seep faunas. *PloS one*, **5**(8), e11967.
- Panieri G, Bünz S, Fornari, D J, Escartin J, Serov P, Jansson P, Torres M E, Johnson J E, Hong W, Sauer S, and Garcia R 2017 An integrated view of the methane system in the pockmarks at Vestnesa Ridge, 79 N; *Marine Geology.* **390** 282-300.
- Portail M, Olu K, Escobar-Briones E, Caprais J C, Menot L, Waeles M, Cruaud P, Sarradin PM, Godfroy A and Sarrazin J 2015 Comparative study of vent and seep macrofaunal communities in the Guaymas Basin; *Biogeosci.* **12** 5455-79.
- Premarathne, U., Suzuki, N., Ratnayake, N., & Kularathne, C. 2016 Burial and thermal history modelling of the the Mannar Basin, offshore Sri Lanka. *Jr of Petroleum Geol.* **39** 193–213.
- Rao M V, Chidambaram L, Bharktya D, and Janardhanan M 2010 Integrated analysis of Late Albian to Middle Miocene sediments in Gulf of Mannar shallow waters of the Cauvery Basin, India: A sequence stratigraphic approach; *In Proceedings of 8th biennial international conference and exposition on petroleum geophysics, Hyderabad.*
- Ratnayake A S, Y k Sampei, and C W Kularathne 2017. Current status of hydrocarbon exploration in Sri Lanka; *International Journal of Oil, Gas and Coal Technology.* **16**(4) 377-389.

- Sasaki T, Warén A, Kano Y, Okutani T, and Fujikura K 2010 Gastropods from recent hot vents and cold-seeps: systematics, diversity and life strategies; In *The vent and seep biota*. Springer, Dordrecht, pp. 169-254.
- Rao S, V 2006 Discovering Medium-Giant Fields- Perspectives and Challenges; *6th International Conference and Exposition on Petroleum Geophysics, Kolkata*.
- Sibuet M and Olu-Le Roy K 2002 Cold-seep communities on continental margins: structure and quantitative distribution relative to geological and fluid venting patterns; *Ocean margin systems*. Springer, pp. 235-251.
- Sibuet M, and Olu K 1998 Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins; *Deep-Sea Research(Part II, Topical Studies in Oceanography)*. **45(1)** 517-567.
- Southward E C, Andersen A C, and Hourdez S 2011 *Lamellibrachia anaximandri* n. sp., a new vestimentiferan tubeworm (Annelida) from the Mediterranean, with notes on frenulate tubeworms from the same habitat; *Zoosystema*. **33(3)** 245-279.
- Southward EC 1991 Three new species of Pogonophora, including two vestimentiferans, from hydrothermal sites in the Lau Back-arc Basin (Southwest Pacific Ocean); *Journal of Natural History*. **25(4)** 859-881
- Teichert B M, Bohrmann G, and Suess E 2005 Chemoherms on Hydrate Ridge unique microbially-mediated carbonate build-ups growing into the water column; *Palaeogeography, Palaeoclimatology, Palaeoecology*. **227(1-3)** 67-85.
- Thiel V, Hügler M, Blümel M, Baumann H I, Gärtner A, Schmaljohann R, Strauss H, Garbe-Schönberg D, Petersen S, Cowart D A and Fisher C R 2012 Widespread occurrence of two carbon fixation pathways in tubeworm endosymbionts: lessons from hydrothermal vent associated tubeworms from the Mediterranean Sea; *Frontiers in Microbiology*. **3** 1-23.
- Thurber A R, Jones W J, and Schnabel K 2011 Dancing for food in the deep sea: bacterial farming by a new species of yeti crab. *PLoS One*. **6(11)** e26243.
- Tsuchida S, Suzuki Y, Fujiwara Y, Kawato M, Uematsu K, Yamanaka T, Mizota C and Yamamoto H 2011 Epibiotic association between filamentous bacteria and the vent-associated galatheid crab, *Shinkaia crosnieri* (Decapoda: Anomura) *Marine Biological Association of the United Kingdom; Journal of the Marine Biological Association of the United Kingdom*. **91(1)** p.23.
- Turekian K K, and Cochran J K 1981 Growth rate of a vesicomid clam from the Galapagos spreading center; *Science*. **214** 909-911.
- Turekian K, K Cochran J, and Bennett J 1983 Growth rate of a vesicomid clam from the 21 N East Pacific Rise hydrothermal area; *Nature*. **303** 55-56.
- Vanreusel A, Andersen A C, Boetius A, Connelly D, Cunha M R, Decker C, Hilario A, Kormas K A, Maignien L, Olu K 2009 Biodiversity of cold-seep ecosystems along the European margins; *Oceanography*. **22** 110-127.

- Volvoikar S, Mazumdar, A, Peketi, A, Dewangan, P, Sawant, B, Manaskanya, A, Goswami, H, Das, D and Pujari, S 2020 Contrasting sulfidization in the turbidite and hemipelagic sediments of Bengal Fan; *Marine and Petroleum Geology*. P.104408.
- Vossmeier A, Deusner C, Kato C, Inagaki F, and Ferdelman T 2012 Substrate-specific pressure-dependence of microbial sulfate reduction in deep-sea cold-seep sediments of the Japan Trench; *Frontiers in microbiology*.**3** 253.
- Watanabe H, Fujikura K, Kojima S, Miyazaki JI, Fujiwara Y 2010 Japan: vents and seeps in close proximity. In: Vent seep biota. Top. Geobiol. **33** Dordrecht: Springer. p. 379–401.
- Whiticar M J 1999 Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane; *Chemical Geology*. **161(1-3)** 291-314.
- Yanqun Q I N, Zhang G, Zhifeng J I, Zhi L I, Yiping W U, Xinglong W A N G, and Liang X 2017 Geological features, hydrocarbon accumulation and deep water potential of East Indian basins; *Petroleum Exploration and Development*. **44(5)** 731-744.
- Yoshida M., Funaki M., and Vitanage P W 1992. Proterozoic to Mesozoic east Gondwana: the juxtaposition of India, Sri Lanka, and Antarctica. *Tectonics*. **11(2)** 381-391.

Figure and plate captions

Figure 1. (a) Location of cold-seep sites in the Cauvery-Mannar basin (image from Google earth); (b) A doughnut-shaped pockmark observed in the Mannar basin. The inner and outer diameters of the pockmark are 50 m and 300 m, respectively.

Figure 2. (a) chitin tubes of *Lamellibrachia* sp. associated with carbonate crust recovered in a spade core from the study site; (b) anterior and posterior end of the *Lamellibrachia* tubes. (c) a magnified view of the anterior end show growth rings or collars; (d) a magnified view of the anterior tube opening showing branchial plume and sheath lamellae. Goose-barnacles (*Neolepas* sp.) are attached to the exterior surface of the tube; (e) soft body of the polychaete extracted from the tube.

Figure 3. Global distribution of *Lamellibrachia* polychaete tubeworm (modified after . Mccowin and Rouse, 2018). The discovery from the Cauvery-Mannar basin is marked by a star.

Figure 4. (a) Dorsal and (b) ventral view of squat lobster morphotype I belonging to the genus *Munidopsis*; (c) Dorsal and (d) ventral view of squat lobster morphotype II belonging to the genus *Munidopsis*; a Gastropoda shell belonging to the family Buccinidae with the soft body (foot part); (f) whole shell of the genus *Calyptogena* showing growth layers; (g) fracture filling methane hydrate shown by arrows; (h) methane hydrate recovered from the fractures.

Figure 5. (a) and (b) Authigenic carbonate crust (chemoherm) with embedded *Calyptogena* shells (CL).

Supplementary Figure 1. Comparison of the outer texture of chitinous tubes of *Lamellibrachia anaximandri* and *Lamellibrachia columna* with the *Lamellibrachia* sp reported in the present work. The absence of prominent collars and contorted Posterior ends are the notable similarity of the tubes. Detailed analyses of the soft body morphology and DNA analyses are required for the identification of the species (ongoing).

Supplementary Figure 2. Comparison of squat lobsters morphotypes belonging to the genus *Munidopsis* recorded in the present work with the morphotypes reported in Mazumdar et al. (2019). Note the distinct carapace features of the different forms. Species-level identification would require DNA based studies (ongoing).

Figure 1:

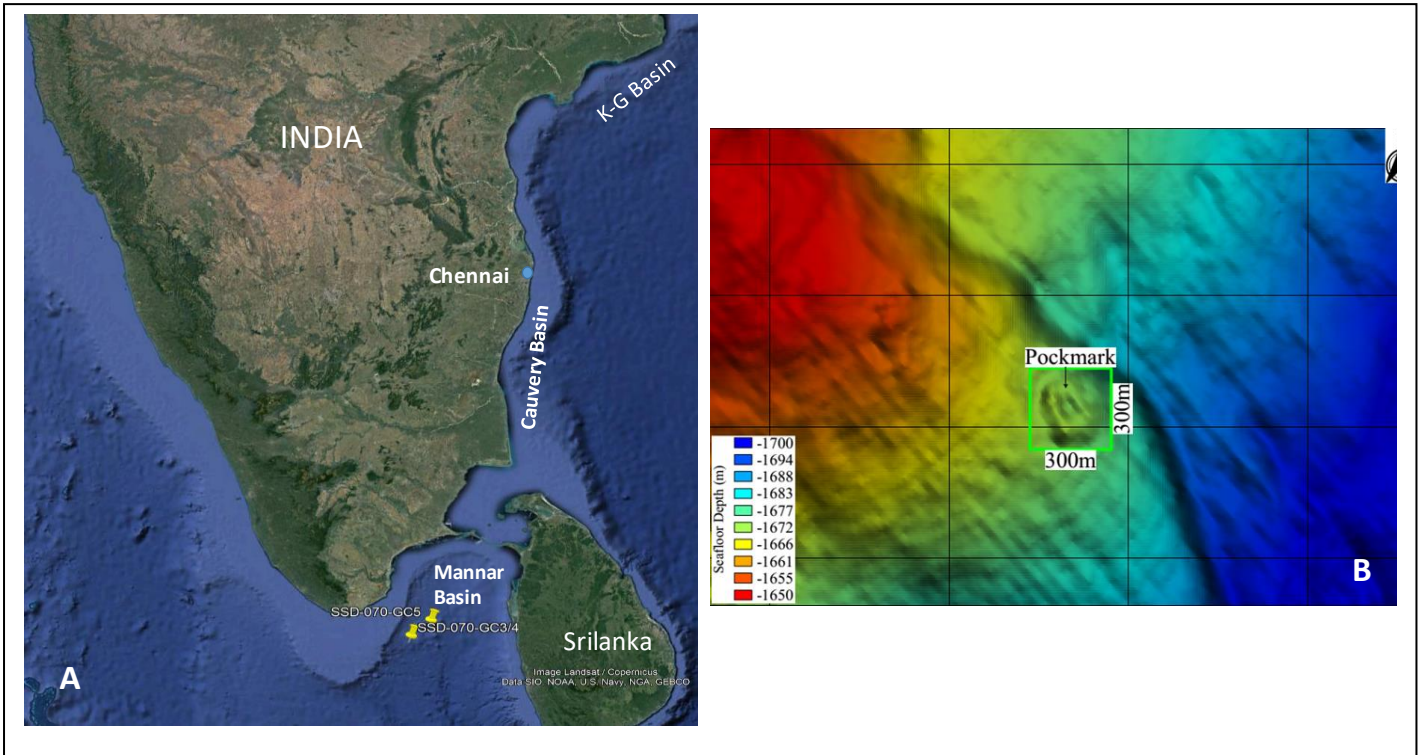


Figure 2:

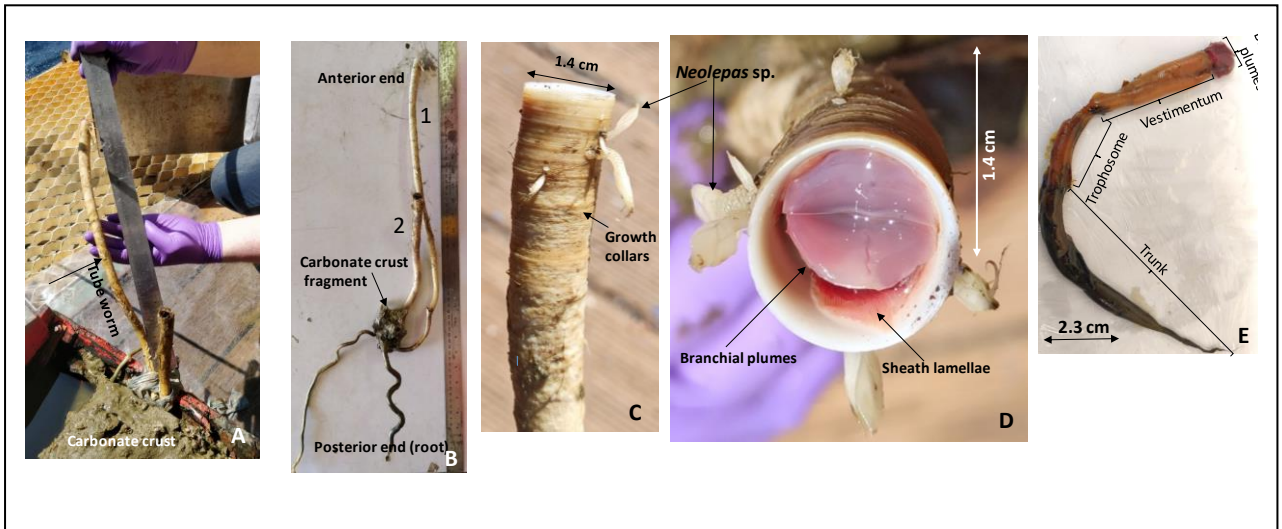


Figure 3:

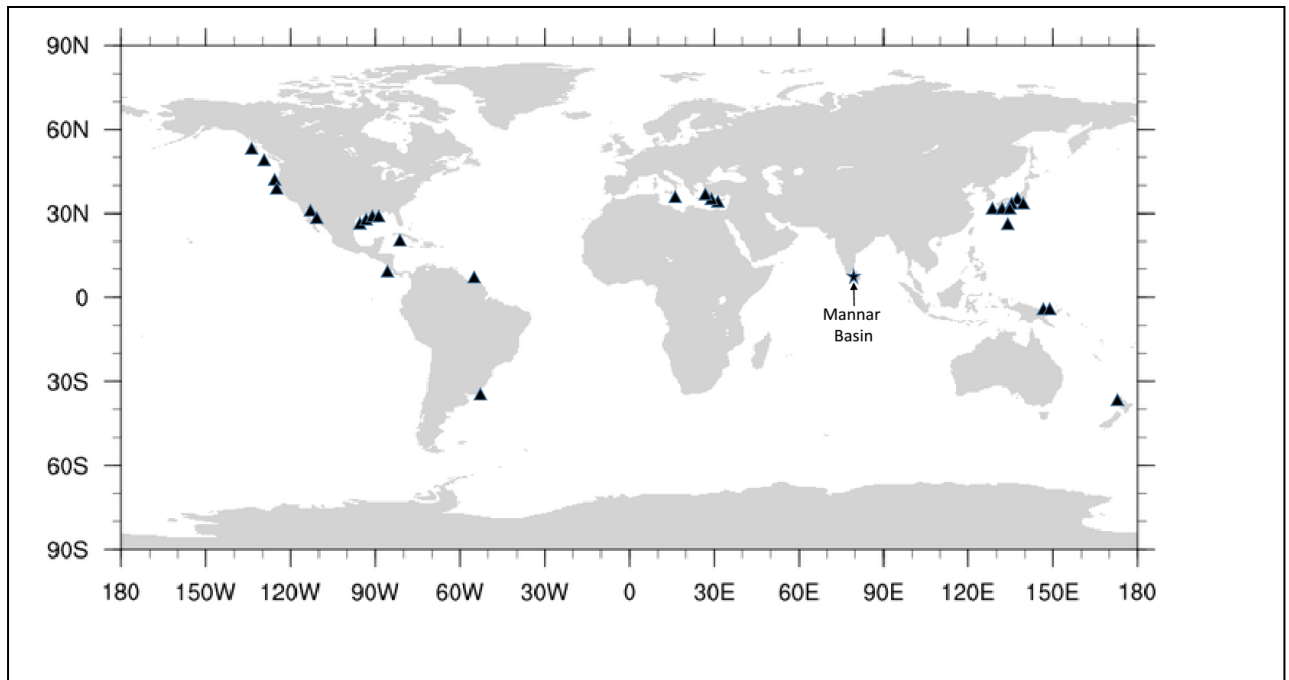


Figure 4

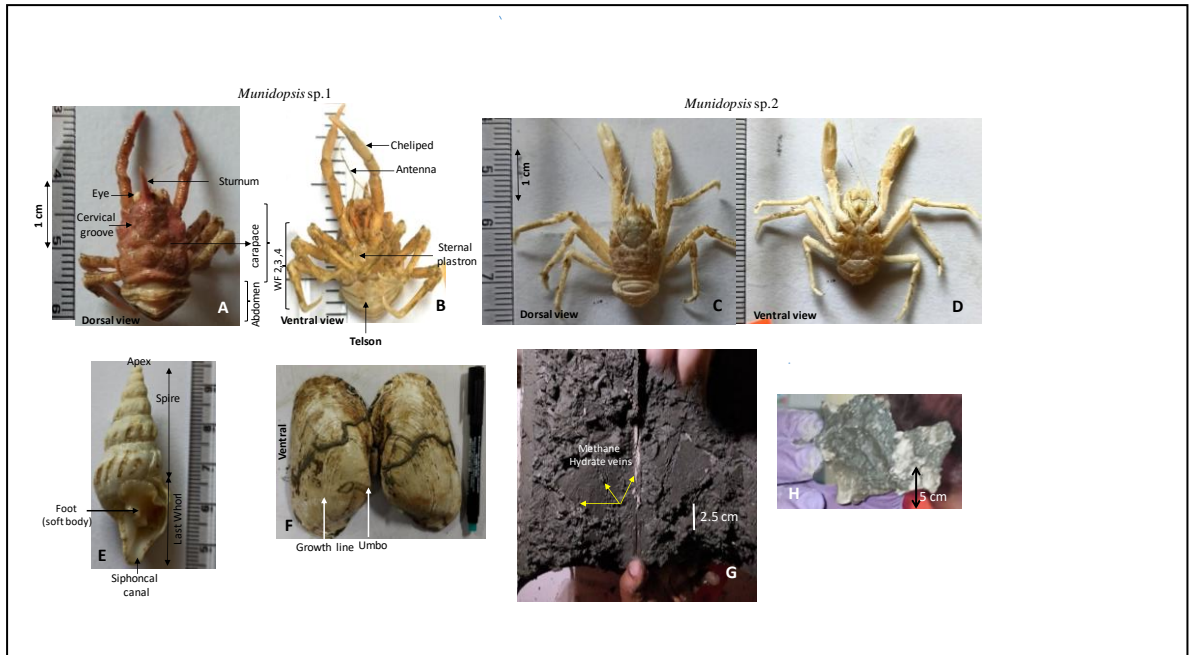
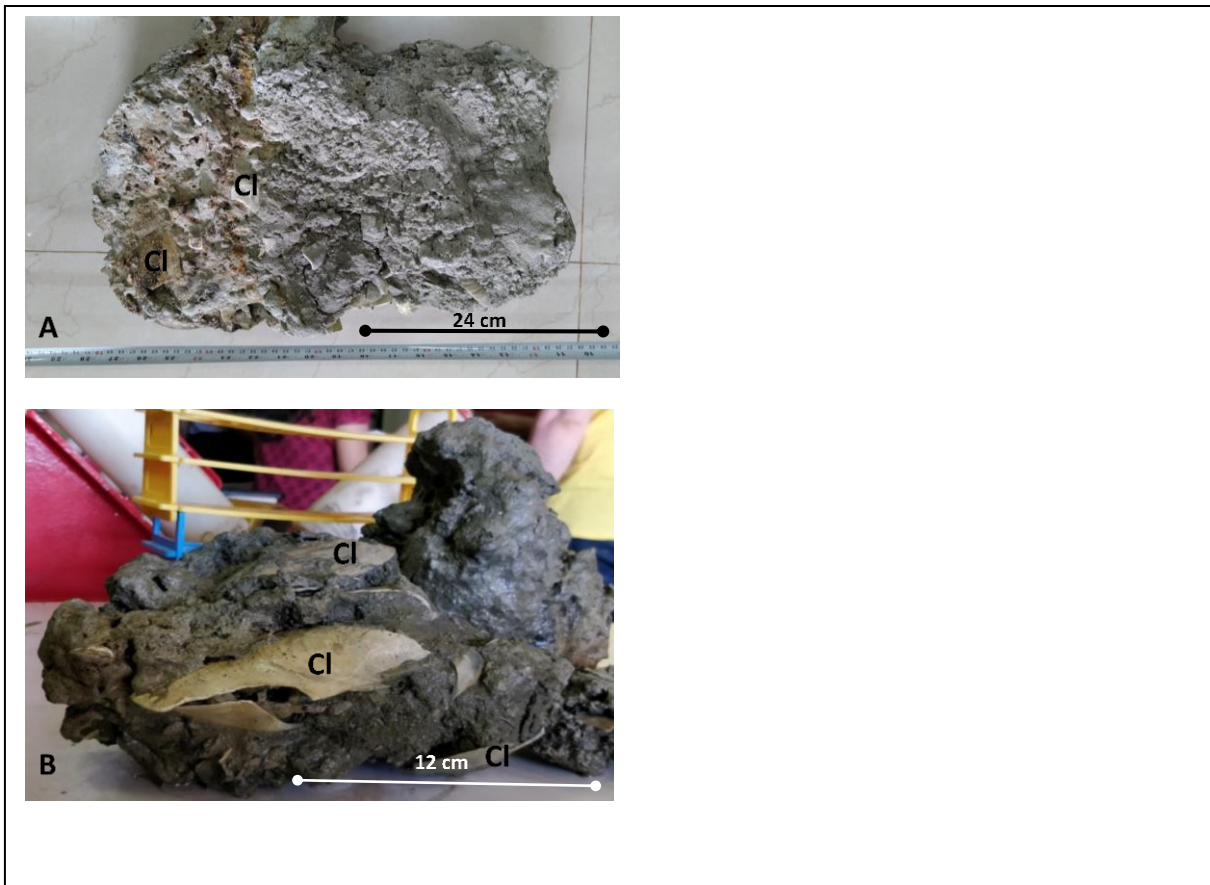
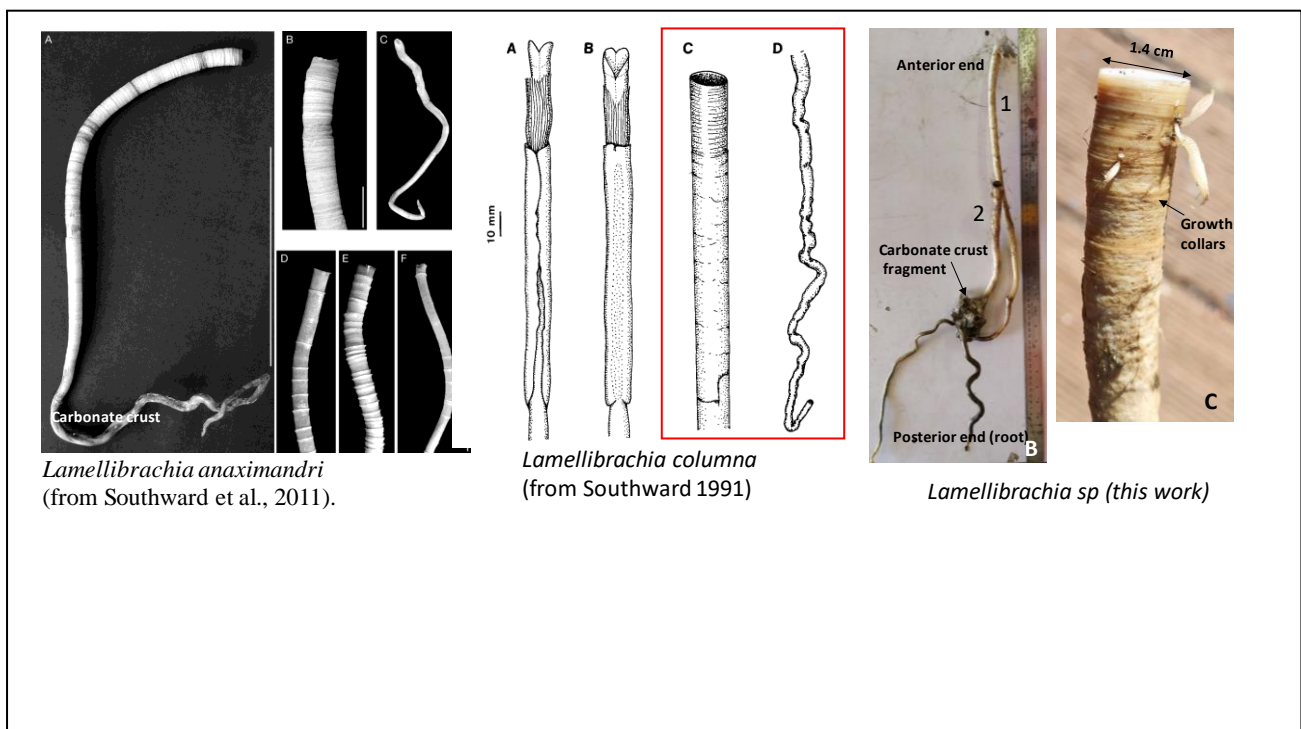


Figure 5



Supplementary Figure 1

Supplementary Figure 1. Comparison of the outer texture of chitinous tubes of *Lamellibrachia anaximandri* and *Lamellibrachia columna* with the *Lamellibrachia* sp reported in the present work. The absence of prominent collars and contorted Posterior ends are the notable similarity of the tubes. Detailed analyses of the soft body morphology and DNA analyses are required for the identification of the species (ongoing).



Supplementary Figure 2

Supplementary Figure 2. Comparison of squat lobsters morphotypes belonging to the genus *Munidopsis* recorded in the present work with the morphotypes reported in Mazumdar et al. (2019). Note the distinct carapace features of the different forms. Species-level identification would require DNA based studies (ongoing).

