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RESEARCH PAPER

First Record of Sea Urchin *Salmaciella oligopora* (H.L. Clark, 1916) (Echinoidea; Camarodonta; Temnopleuridae) from India : its Structural and Molecular Analysis

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Abstract

Salmaciella oligopora (Clark,1916), a rare sea urchin species which was initially described as Genus *Salmacis* from Tasmanian coast, Australia in 1916 was later recorded in the Philippines Archipelago in 2015. Till date there is no report on occurrence of *S.oligopora* in Indian waters. This paper reports the distribution of temnopleurid sea urchin *S.oligopora* for the first time between Chennai and Pondicherry Coasts, South East Coast of India. Echinoides were collected at depths of 20-30 m by gill nets during April 2016 to December 2016. 4 specimens were identified as *Salmaciella oligopora* based on morphological characteristics and molecular analysis using 18S rRNA sequencing. *Salmaciella oligopora* collected from India was redescribed with photographs and compared with other species from GenBank based on molecular data through nucleotide analysis in BLASTn (Basic Local Alignment Search Tool of nucleotide) and the result showed 99% similarity in Genbank sequences. Phylogenetic tree analysis showed close relationship to the Temnopleuridae family. This record extends the northern range of this species and indicates a wider but patchy distribution.

Keywords: *Salmaciella oligopora,* taxonomy, molecular phylogeny, South East Coast of India.

Introduction

The sea urchin genus *Salmaciella* (Mortensen *et al*.,1943) (Echinoidea: Temnopleurida) includes 3 valid extant species: *S.dussumeiri* L. Agassiz (Agassiz & Desor 1846) (type species), *S. erythracis* (H.L.Clark 1912) and *S.oligopora* (H.L.Clarke 1916). *S. dussiemeiri* is geographically distributed in the Indo-west pacific region comprising the Bay of Bengal, Red sea (Clark & Rowe *et al*., 1971), Seychelles (Clarke *et al*.,1984) and Australia, in the benthic continental shelf between a depth of 10 -180m (Rowe & Gates 1995) while *S.erythracis* is distributed in the southern African coast along Mosambique and Tansania (Clark Courtman 1976). The third species *S. oligopora* after its discovery from Sandon Bluffs, New South Wales, Australia and description by (Clark 1916) has been considered endemic to the East Pacific and showed strong resemblance to *S. dussumieri* and *S.erythriacis* except for the differences in the relative number of ambulacral plates in each column, form of pedicellariae valve base and colouration. The base of the valves of the globiferous pedicellariae was rather higher than wide and the upper lateral corners are elongated to a much greater degree than in any other species of *Salmacis* and flare outwards slightly. Clark (1967, 1968) made comments on the echinoderms precise measurements and the ratios while describing thespecies. Work of Clark and Rowe (1971) on the shallow water echinoderms of Indo-West Pacific is said to be an important landmark in the taxonomy of echinoderms. They have also commented on the identity of some of the echinoderms from India. In many asian and mediterranenan countries the sea urchins are higly priced marine resource due to the high demand and economically important role as they have high nutritional value (Salon, 1985; Yokota, Matranga & Smolenicka, 2002). Roe consists of water, protein, lipid, carbohydrate, vitamins, and minerals (Kat & Schroeter, 1985). The Indian coastal waters harbours over 50 species of sea urchins of which 14 have been found to be edible. (James, 1985; Kaliaperumal & James 1993). Development of sea urchin aquaculture has been characterized by enhancement of wild populations followed by research on their growth, nutrition, reproduction, and suitable culture systems. In this paper we made the first report on the occurrence of *S.oligopora* along the continental slopes of Chennai and Marakkanam coasts, Tamilnadu, South India. This species has never been reported

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previously from the Indian waters. We identified and examined the morphological features of *S.oligopora* and conducted molecular identification (18S ribosomal gene sequence) Molecular differences between the obtained specimens and Genbank data of *S.oligopora* and other species were also compared using phylogenetic tree analysis is presented in this paper.

Material and Methods

Sample Collection and Identification

Eight live specimens of *S.oligopora* were collected using fishing nets from depths of 20 – 30 m from three areas along the Chennai-Pondichery Coast, South East Coast of India viz., Kovalam (12.°48’37.61’N, 80°53’48.57.′E), Marakkanam (12°5'16.25"N 80°15'20.80"E) and Pondicherry (11°46'23.38’N 79°57'26.86’E) (Figure 1). The live animals were stocked in plastic containers filled with aerated seawater and were transported to the Marine Biotechnology Laboratory, Centre for Ocean Research, Sathyabama University, Chennai. In the laboratory, the specimens were photographed using Nikon (D5300) and the specimens were washed with 0.5% *Ampicillin* solution to remove pathogenic bacteria before immersing in UV- sterilized filtered sea water stocked in closed glass aquarium tanks of 10 L capacity. The animals were fed *ad libitum* with green macro algae *Chaetomorpha antennina, Ulva rigida and Enteromorpha sp.* The sea urchin specimens were identified based on the descriptions of Mortensen (1943) and Clark, H. L. (1916). The important morphological characters were photographed with a fully automated Inverted Fluorescence microscope (Leica DM16000B) and automated stereo zoom microscope (Leica M205A). The skeleton samples were later dried in open air and zooidal measurements as well as skeletal parts were photographed using Field Emission Scanning Electron Microscope (FESEM-SUPRA 55- CARL ZEISS, GERMANY) available in Centre for Nanoscience and Nanotechnology, Sathyabama University, India. Finally, the specimens were taken into the collections of Centre for Ocean Research Museum.

DNA Amplification and Sequencing

Genomic DNA was extracted from the gonadal tissue of the specimens by the Phenol Chloroform method (CAGL standardized protocol for fish tissue extraction and Quiagen kit (Anup Mandal et al., 2014). Polymerase chain reaction (PCR) for amplification of the partial 18S rRNA gene was performed using a test sample along with positive and negative control (Forward primer: 5'-CAGCAGCCGCGGTAATTCC-3' and Reverse

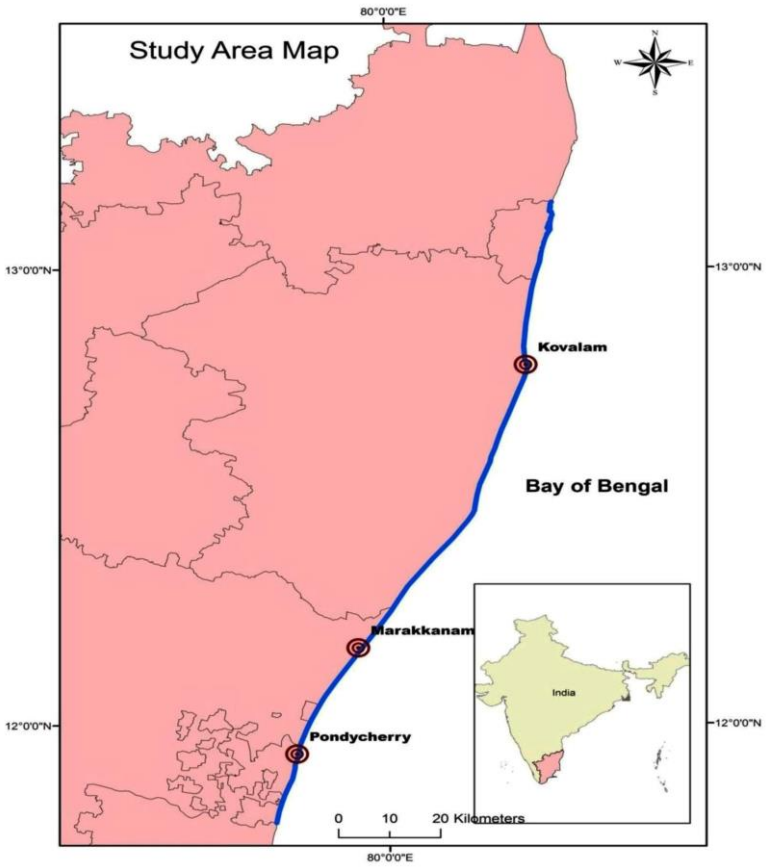

Figure 1. *Salmaciella oligopora.* The map shows the collection site of Chennai coast.

primer: 5'-CCCGTGTTGAGTCAAATTAAGC-3'). Sequencing of the amplified product was done (both forward & reverse direction) following the CAGL-standardized protocol using Genetic Analyzer ABI-3500. Additionally, another combination of 18S rRNA primers was tested and comparative sequence analysis was done for probable species identification.

Molecular Analyzes

The phylogenetic tree was drawn using the maximum likelihood (ML) method. For the resulting tree and random resampling of the sequences, bootstrapping method was performed. The phylogenetic tree representing a consensus of 500 trees was obtained. Similarties was calculated from partial 18S rDNA sequences of Temnopleuridae family, with the exclusion of ambiguous nucleotides using MEGA ver 7.0 (Tamura *et al*., 2016).

Results and DiscussionS

Systematics

Phylum Echinodermata
Class Echinoidea Leske, 1778
Subclass Euechinoidea Bronn, 1860
Order Camarodonta Jackson, 1912
Infraorder Temnopleuridea Kroh and Smith, 2010
Family Temnopleuridae A. Agassiz, 1872
Genus *Salmaciella* Mortensen, 1942
Species *Salmaciella oligopora* (H.L. Clark, 1916; Mortensen, 1942)

Key to the genus of *Salmaciella* Mortensen, & 1942

Subconical test: anal opening eccentrically arranged towards periproctal edge, ambulacral plates reduced on alternate plates aborally, very distinctive angular plates on the oral side.

1. Long equatorial spines facing downwards forming fringed appearance, the primary spines are banded in dark and light greenish tints .Test greyish brown ***S.erythracis***

Equatorial spines short in size, widened distally with distinct ends. Spine have bands of white with maroon, brown and light olive. Test: color pale fawn to light olive green ***S.oligopora (***H.L.Clark *et al*.,1924:16; Clark & Courtman-Stock *et al*.,1976 231; Schultz *et al*., 2010:156; Venkataraman & Padmanaban *et al*., 2013:99; James D. B *et al*.,1983 1: 403-406).

Description

Test: Small in size, slightly pale brownish with olive green tinge (Figure 2A to 2B). strong, hemispherical or discoid shape and distinctly flattend ventral side (Figure 2C to 2D). Five genital plates have developed as madreporites sites (Figure 3D to 4C). Margin of oral side slightly sunken towards

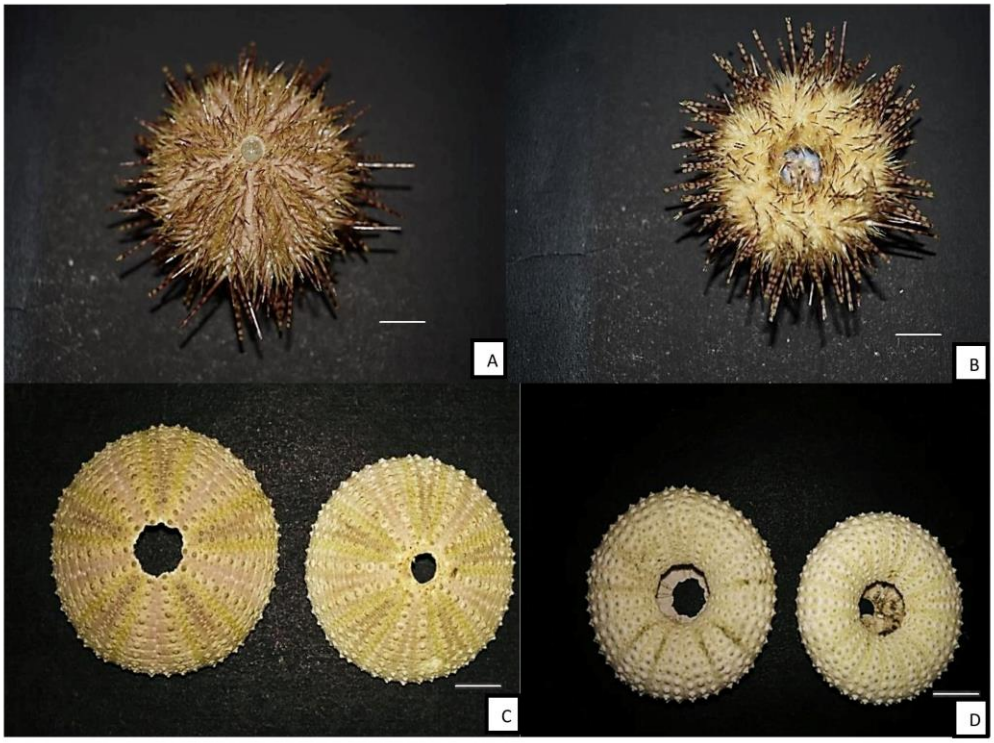

Figure 2. *Salmaciella oligopora*. A. Full aboral view; B. Full Oral view; C. Dorsal side of denuded test; D. Venral side of denuded test.

peristome (Figure 3C). Ambulacral and Interambulacral pore pairs usually five in number, arranged in a slightly horizontal row closer to the apical system on the dorsal side. The margin of Interambulacral plates was formed as a zig– zag line on the interracial suture (Figure 3F to 4H). Ambulacrum almost half as broad as interambulacrum (Figure 3E). The number of ambulacral plates in a column is from 45 to 56. Large and small secondary tubercles in ambulacral and adambulacral plates are crenulated (Figure 3E to 3F and 4E to 4F). with scattered miliary tubercles. The base of the valves of the globiferous pedicellariae is rather higher than wide and the upper lateral corners are elongated to a much greater degree than in any other species of *Salmaciella* and flare outwards slightly. Genital plates covered with slightly primary tubercles.(Figure 4D).

Size

Horizontal test diameter, 23.4- 40.1 mm; test height, 10.1-13.2 mm; peristome, 7.1–11.2mm. The height therefore just less than half the horizontal diameter.

Spine & Color

In *S.oligopora* the primary spines reaches upto a length of 0.3 – 1.3cm. The long ambital spines were widened distally with a distinct shovel like ends (Figure 3A to 3B). The miliary spines and pedicellariae are white, the secondary spines have alteranate white and maroon bands. They may be broadly tipped with olive-green; sometimes there was also a band of olive green near the middle of the spine. Shows the microstructural characteristics of base spine, Aboral spine spines and Gonopore view of *S.oligopora* taken in Scanning Electron Microscope. (Figure 4A to 4B and 4G).

Biology

This species occurs on the continental shelf along the upper limit of the intertidal zone. This specimen has been selected as the holotype. This species walks rapidly on its oral spines and can travel 42 cm in one minute (Miskelly *et al*., 2002) .

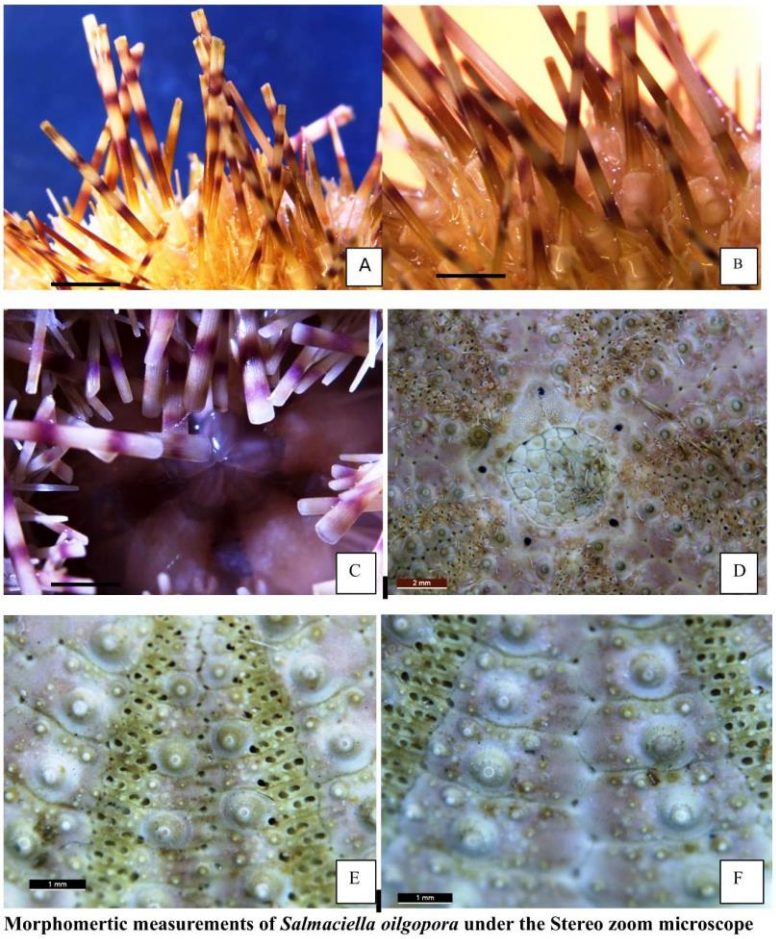

Figure 3. (A-B) White band with maroon color spine; C Oral side (Aristotle's lantern phase); D, Aboral side (Five gonopore phase); E, Ambulacral column pairs; F, Interambulacral column pairs. Scale Bars: (A-B) = 2mm, C = 1mm Morphometric measurements of *Salmaciella oilgopora* under the Stereo zoom microscope

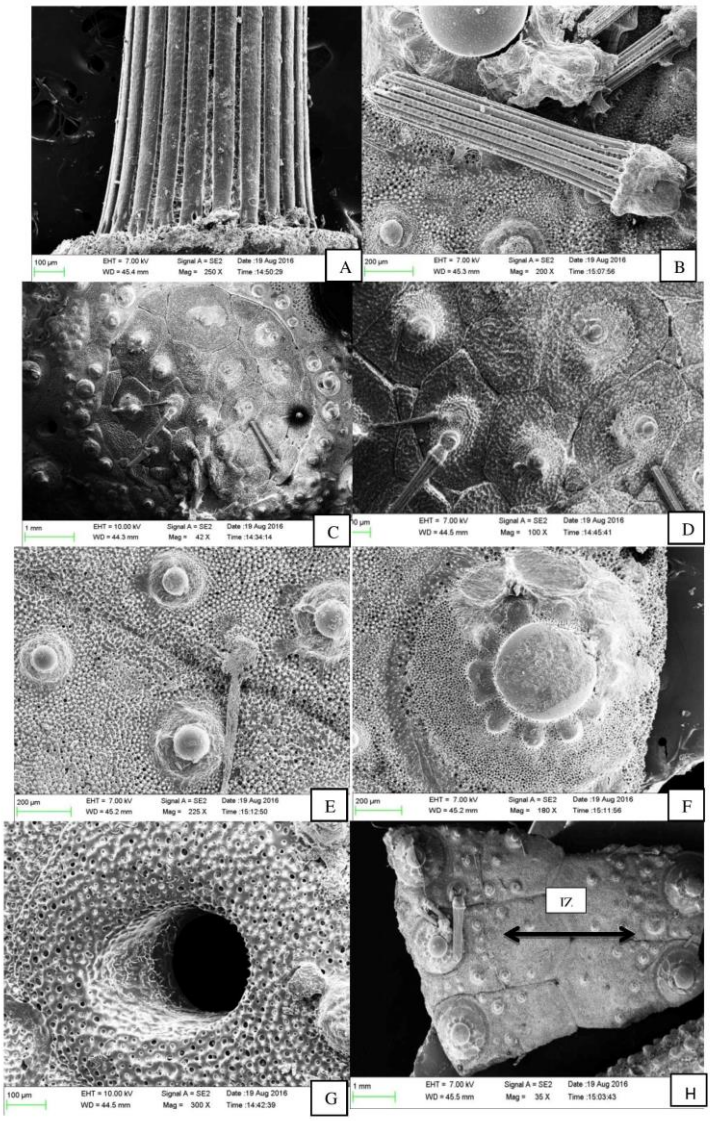

Figure 4. A, Base of spine; B, Aboral spine; C, Full aboral view; D, Five genital plates; E, Secondary tubercles; F, Primary tubercle; G, Gonopore view; H, Interambulacral zone (IZ).
Morphometric measurements of *Salmaciella oligopora* under the Field Emission Scanning Electron Microscope (FE-SEM)

Distribution

Australia (Queensland), New South Wales, Tasmania, Victoria, South Australia, and Western Australia and Philippines. *Southeast coast of India (Chennai coast)* .

DNA Sequence Features

The sequences of our Indian *S.oligopora* specimens were initially compared with GenBank data of 3 *Salmaciella* species such as Genbank id AF279211, AF279189, AF279163 and showed 99% similarity with *S.oligopora* species. In total, 636 base pairs (bp) of the 18S rRNA gene sequence were obtained from the *Salmaciella oligopora*. This sequence data have been submitted to the Gen bank account of ID (KX838956). The study reported by (Jeffery *et al*., 2003) reveals that the partial gene and 1773-bp 18S rRNA gene identities always agreed at the genus level, and 99% of assignments were the *Salmacis belli* species assignments by the partial gene method.

Phylogenetic Tree

In the phylogenetic tree, Maximum likelihood (ML) method demonstrated the presence of non-discriminatory phylogentic branches (Figure 5). We confirmed the identification of the specimen as *Salmaciella oligopora* after comparison and analyzis with 18 closely related species of Temnopleuridae family viz. *Amblypneustes formosus, Amblypneustes ovum, Holopneustes inflatus, Holopneustes*

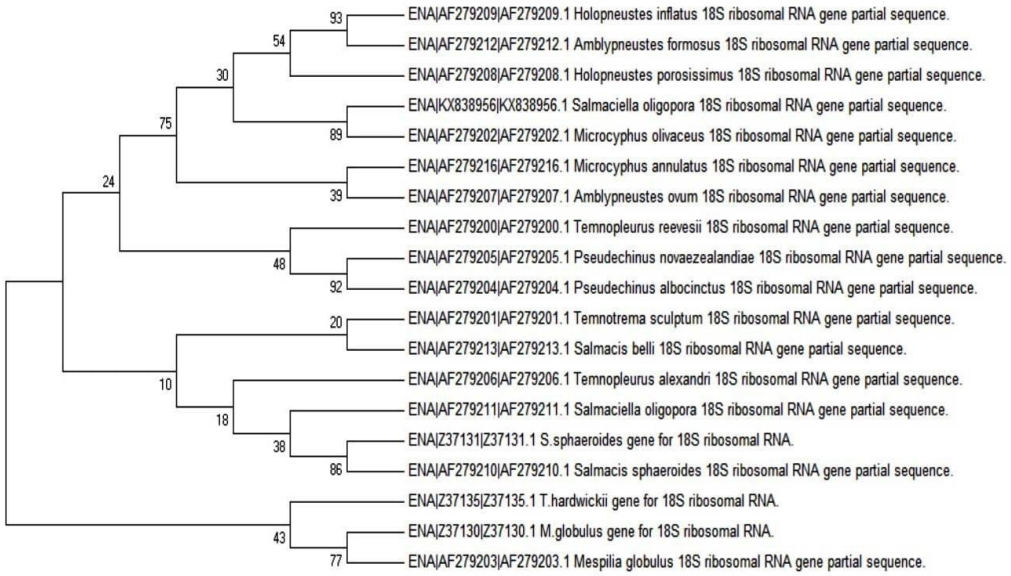

Figure 5. Maximum-likelihood (ML) trees of the *Salmaciella oligopora* inferred from the 18S ribosomal gene sequence.

porosissimus, Mespilia globules, Microcyphus annulatus, Microcyphus olivaceus, Pseudechinus albocinctus, Pseudechinus novalzealandiae, Salmaciella oligopora, Salmacis belli, Salmacis sphaeroides, Temnopleurus alexandri, Temnopleurus reevesii,Temnopleurus hardwickii, Temnotrema sculptum obtained from Genbank. Totally nine different genus groups of Temnopleuridae family were analysed. The phylogenetic tree construction suggest that our specimen has very close resemblance with *Salmacis sphaeroides.* The result compared to grouping of deuterostomes was supported by a high value obtained by bootstrapping (71.2%). Next, using the same alignment, we constructed another phylogenetic tree by the maximum-likelihood method using the fast DNAml program (Felsenstein *et al*., 1985; Olsen *et al*., 1993).

Discussions

Our specimens were identified as *S.oligopora* which has not been previously reported from India. Our species description coincides with previous description of *S.oligopora* species distributed exclusively along New South Wales, Australian coast (Clarke *et al*.,1916). (Miskelly *et al*.,2002) described the species as endemic to Tasmania and described *S.oligopora* as a rapid mover in comparison with other species which can travel 42 centimeter per minute on its oral spines. Though (Mortensen *et al*.,1943) has described about the species *Salmaciella*, and described in detail about 409 echnoid *Taxa* in his article (1943), there is no descriptive records of *S.oligopora* other than (Clark's monogrpahy *et al*.,1916) and we located the animals between 10m to 30m depth along continental shelf. Recently (Heinke Schultz, 2015) reported the occurrence of *S.oligopora* in Philippines coast .According to him, the animals live in sheltered bays in open bottom. Guido Poppe (2007) in Marine Iconography of Philippines Archipelago (http://www.poppe-images.com/?t=17&photoid=943837) recorded the described species at 7m depth and photographed *S.oligopora*. With this report the distribution of *S.oligopora* is shown to be continues from Austrian coast, Malaysia and East coast of India. Further surveying is required in order to establish the true extend of this species which has not yet been shown any occurrence in Peninsular and southwest coast of India.

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