

First distribution record of regular echinoids (Echinodermata; Echinoidea) from Chennai Coast, South India

Radhika Rajasree S.R.^{1*}; Gobala krishnan M.¹; Karthih M.G.¹;
Aranganathan L.¹

Received: May 2017

Accepted: July 2017

1-Centre for Ocean Research, Sathyabama Institute of Science & Technology, Chennai-600119, Tamilnadu, India.

*Corresponding author's Email: radhiin@gmail.com

Keywords: Sea urchin, Taxonomy, Molecular phylogeny, South India

Introduction

Sea urchins have recently been attracting considerable research interest as sources of a number of highly valuable bioactive compounds that possess antitumour, antiviral, antimicrobial and anticoagulant properties that hold great promise for use in pharmacological applications (Ryoyama, 1974; Wardlaw and Unkles, 1978; Okigbo *et al.*, 2008). This necessitated a thorough stock assessment study of regular echinoids in Indian coastal waters to promote viable culture practices. The Indian coastal waters harbour over 50 species of sea urchins of which 14 have been found to be edible (James, 1983; Kaliaperumal and James, 1993). In this paper, an attempt has been made to provide an updated systematic account and distribution status which includes the first record in Indian seas of a

number of regular sea urchin species such as *Salmaciella oligopora*, *Astropyga radiata* and *Prinocidaris baculosa* along the continental shelf of Chennai, Kovalam and Marakkanam coasts, Tamilnadu, South India. Among these *S. oligopora* species has been reported for the first time from Indian waters. We identified and examined the morphological features of *S. oligopora* and conducted molecular identification (18S ribosomal gene sequence).

Materials and methods

Sample collection and identification

The live specimens of sea urchin species *S. oligopora*, *A. radiata* and *P. baculosa* were collected using mini trawl nets with mesh size of 50mm at depths of 10–45m 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) (Fig. 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 Institute of Science and Technology, 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 10L 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 (1916). The important morphological characters such as oral test and aboral view, morphometric measurements of test and spine, colouration, genital plates etc., 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 the Centre for Nano Science and Nanotechnology, Sathyabama Institute of Science and Technology, India.

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 *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 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 analyses

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 1000 trees was obtained. Similarities were calculated from partial 18S rDNA sequences of Temnopleuridae family, with the exclusion of ambiguous nucleotides using MEGA ver 7.0 (Tamura and Stecher, 2016).

Results and discussion

Taxonomy

Phylum Echinodermata

Class Echinoidea Leske, 1778

Subclass Euechinoidea Bronn, 1860

Order Camarodonta Jackson, 1912

Infraorder Echinidea Kroh and Smith, 2010

Family Temnopleuridae A. Agassiz, 1872

Genus *Salmaciella* Mortensen, 1942

Salmaciella oligopora (H.L. Clark, 1916; Mortensen, 1942)

Key features of family Temnopleuridae
A. Agassiz, 1872

Test not sculptured (or rarely so) but with pits on horizontal sutures or at angles of coronal plates; latter united by dowelling. Tubercles usually crenulated; test with distinct pits, troughs or pores the angles of the sutures are extensively sculptured.

Key to the genus of Salmaciella
Mortensen, 1942

Sub conical test: Anal opening eccentrically arranged towards periproctal edge, ambulacral plates reduced on alternate plates aborally, very distinctive angular plates on the oral side. The primary spines are banded in dark and light greenish tints. Test greyish brown
..... *S. erythraxis*

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*

(Clark and Courtman-Stock, 1976; Schultz, 2010)

S. oligopora (H.L. Clark, 1916)

Description

Test is small in size, slightly pale brown with olive green tinge (Fig. 2A) strong, hemispherical or discoid in shape and a distinctly flattened ventral side (Fig. 2B). The margin of oral side slightly sunken towards the peristome (Fig. 3C). Five genital plates have developed as madreporites sites. Ambulacrum almost half as broad as interambulacrum (Fig. 3F). The margin of Interambulacral plates was formed as a zig-zag line on the interracial suture (Fig. 3E). The number of ambulacral plates in a column is from 45 to 56.

Size

Horizontal test diameter, 23.4- 40.1 mm; test height, 10.1-13.2 mm; peristome, 7.1-11.2 mm.

Spine and colour

In *S. oligopora* the primary spines reach up to a length of 0.3–1.3cm. The long ambital spines were widened distally with distinct shovel like ends (Fig. 3A & 3B). The miliary spines and pedicellariae are white, the secondary spines have alternate white and maroon bands.

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 42cm in one minute (Miskelly, 2002).

Distribution

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

Taxonomy

Phylum Echinodermata

Class Echinoidea Leske, 1778

Order Diadematoida

Family Diadematidae Gray, 185

Genus *Astropyga* Gray, 1825

Astropyga radiata Leske, 1778

Key features of family Diadematidae
peters, 1855

Spine usually hollow, long, cylindrical, thin and breakable, with a finely thorny or ridged surface. Peristome is without plates and with soft skin. Test often somewhat flattened, rather flexible, sometimes rigid.

Genus Astropyga Gray, 1825

Description

Genital plates conspicuously elongated into the interambulacra between the aborally bulging ambulacra, test very much flexible.

Astropyga radiata Leske, 1778

Taxonomic description

Test diameter is between 110mm-125mm, flattened or slightly concave on the aboral side. Test with intense red bands bordered by fluorescent blue spots (Fig. 2C); anus red-brown with intermittent whitish red spots. The spines are up to 20mm-40mm, long and are grouped in five vertical clusters in between which are V-shaped areas with no spines corresponding to

the interambulacral plates (Fig. 2E). Periproct flat or low conical.

Color

Colour varies from red to black, but the red cross is always clearly visible.

Spine

Red, orange band with spine (Fig. 2D), Primary spines black, finely ridged and long, easily breakable, filled with loose mesh work.

Biology

A. radiata has been observed in shallow sea grass beds, sandy shelves (De Beer, 1990). In the coral region just below the low tide level on the shallow reef (Herring, 1972).

Distribution

Well-known tropical Indo west central pacific (including the Red Sea) species (Rowe and Gates, 1995; Vanden Berghe, 2000), south east coast of India.

Taxonomy

Phylum Echinodermata

Class Echinoidea Leske, 1778

Subclass Cidaroida

Order Cidaroida

Family Cidaridae Gray, 1825

Genus *Prionocidaris* A. Agassiz, 1863

Prionocidaris baculosa (Lamarck, 1816)

Key features of family Cidaridae Gray, 1825

High interambulacral plates with massive, blunt primary spines, widely separated from the each other, usually

surrounded by much smaller, often spatulate, secondary spines. Ambulacral and interambulacral plates cover the peristome up to the mouth.

Key characters of genus of Prionocidaris A. Agassiz, 1863

Ocular plate in contact with the periproct; large globiferous pedicellariae with numerous fringes on stalk; primary spines with serrated edges. The genus has spots and stripes on the collar and neck of the primary spines and its transverse oval and confluent areoles.

Prionocidaris baculosa (Lamarck, 1816)

Taxonomic description

Test

Slightly flattened above and below, ambitus round or slightly pentagonal, flattend ventral side (Fig. 2F). Horizontal diameter of the test may reach more than 50mm. Oral side slightly sunken towards peristome (Fig. 3H).

Ambulacra

Marginal series of tubercles very regular, small and continuous; inside the marginal series, as a rule, two double series of much smaller tubercles. Plate conjugate pore pairs in single almost vertical series. (Fig. 3G). Primary tubercles crenulated but not perforated (Fig. 3I).

Interambulacral

Scrobicular tubercles prominent, outside the circular a few tubercles about half that size, median space

closely covered with small tubercles, naked zigzag line along.

Spine

Brown with green band, Length up to 110mm-120mm (Fig. 2G), Primary spine are Very variable; shape from almost cylindrical to spindle – like, tapering distally or to point more or less widened, banded, oral primaries simple, mostly straight cut.

Color

The denuded test has dark purple or brown apical plates and ambulacra (Fig. 2I), irregularly banded, collar always with purple spots (Fig. 2H).

Distribution

This species was common in the Indian Ocean from the Red Sea to Mauritius and Ceylon, also in the Philippines and Indonesian waters and from Japan to the west coast of Australia from the shore to a depth of about 200m. Also distributed in SE Arabia, West India, Pakistan, Maldives area, Bay of Bengal, East Indies, China and south Japan (Clark and Rowe, 1971).

Biology

P.baculosa can cover itself in sea-grass and algal debris or lagoon areas with rocky outcrops and some isolated coral heads.

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 *S. oligopora*. This sequence data have been submitted to the Gen bank account of ID (KX838956). The study reported by Jeffery, 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 phylogenetic branches (Fig. 4). We confirmed the identification of the specimen as *S. oligopora* after comparison and analysis with 18 closely related species of the Temnopleuridae family obtained from the Genbank. Totally nine different genus groups of Temnopleuridae family were analysed. The phylogenetic tree construction suggests that our specimen has a very close resemblance to *S. sphaeroides*. The results were compared to groups of deuterostomes and 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 DNAm1 program (Felsenstein, 1985).

Discussion

Identification of echinoids species from the south east coast of India has been

identified under taxonomical key guides: 1971 monographic work of Clark and Rowe, Echinoderms From the Kiunga Marine National Reserve, Kenya and Echinoidea: with pentameral symmetry. Schultz (2015) based on the collection and field observations, the present study of the first distributional records for the regular echinoids species from the south coast of India, Chennai, resulted in recognitions of 3 species belonging to 3 families and 3 genera. *S. oligopora*, which had not been previously reported from India, has shown new distributional records from the Indian waters. The previous description of *S. oligopora* species distributed exclusively along New South Wales, Australian coast (Clarke, 1916). Miskelly (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 centimeters per minute on its oral spines. Though Mortensen (1942) described the species *Salmaciella* and described in detail about 409 echinoid Taxa in his article (1943), there are no descriptive records of *S. oligopora* other than Clark's monography (1916) and we located the animals between 10m to 30m depths along the continental shelf. Recently Schultz (2015) reported the occurrence of *S. oligopora* in the Philippines coast. According to him, the animals live in sheltered bays in the open bottom. *A. radiata*, and *P. baculosa* species recorded as first distribution echinoids species from the Chennai coastal waters, occur in depths of more than 30m of the continental shelf region

from Kovalam and Marakkanam coasts, Chennai, Tamil Nadu South India. *A. radiata* is highly venomous and can pierce through a wet-suit. Very sensitive to light and have the ability to shoot venom loaded spines at a short distance and sometimes hitch lift on the back of crabs (www.whatsthatfish.com/fish/red-urchin/330). *Prionocidaris baculosa* can be found hiding in the sea grass beds *Thalassodendron ciliatum* (Forskål, 1757).

Conclusions

This is the first record of the regular echinoid species *A. radiata*, *P. baculosa* and *S. oligopora* recorded for the first time from Indian waters, filling a gap of its distribution in the East Coast of India. It is clear that despite the efforts, knowledge and basic ecological information about echinoderms and infaunal communities in the Indian Seas and the region is still incomplete.

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