

New distribution record of *Prosthiosomum trilineatum* Yeri & Kaburaki, 1920 (Platyhelminthes: Polycladida) from Great Nicobar Island, India with insights into its ecology

Satya Swaroop Nanda¹ and Chandrakasan Sivaperuman¹

¹Zoological Survey of India, ANRC, Sri Vijaya Puram, Andaman and Nicobar Islands, India - 744 102

*Corresponding author's E-mail:- satya.s.nanda@gmail.com, <https://orcid.org/0009-0007-8499-0839>
sivaperuman1975@gmail.com

Abstract

The present study documents the first confirmed record of *Prosthiosomum trilineatum* from Great Nicobar Island, India, thereby extending the known distribution range of the species to the Southernmost Island of the Indian coastline. Two specimens were recorded during nocturnal intertidal surveys conducted in the Breakwater Jetty and Gandhi Nagar regions, where they were encountered beneath rocks within shallow reef-associated habitats. Detailed observations on external morphology, habitat characteristics, and spawning ecology were carried out under laboratory conditions. The specimens measured approximately 10-11 mm in length and exhibited the characteristic dorsal colouration and eyespot arrangement diagnostic of the species. Habitat assessment revealed slightly alkaline and moderately oxygenated tidepool conditions with salinity ranging between 37.3–37.5 ppt. Spawning behaviour was documented for one individual maintained in captivity. Egg deposition occurred three days after collection, during which the specimen adopted a contracted posture and deposited 147 golden-coloured eggs individually onto the glass surface through the female gonopore. Each egg was enclosed within a protective capsule containing a single embryo. No parental care or copulatory behaviour was observed during the study period. The present record contributes to the understanding of the diversity, distribution, and reproductive biology of polyclad flatworms from the Andaman and Nicobar archipelago, a region that remains poorly explored with respect to marine flatworm fauna.

Key words: *Polyclad*; *Marine Flatworm*; *New geographical range*; *Andaman Sea*; *Great Nicobar Island*

Introduction

Polyclad flatworms, also known as free-living flatworms, are a diverse group of marine invertebrates of the phylum Platyhelminthes, found in oceans worldwide, with over 4,000 known species (Faubel, 1984). They can be found in a wide range of marine environments, from intertidal tidepools to the depths of the ocean trenches (Ang *et al.*, 1998). Their size can vary significantly depending on the species, ranging from just a few millimetres to several inches in length. They have a flattened body shape ideal for life on the ocean floor. Their flattened, ribbon-like bodies enable them to navigate through the water effortlessly and rapidly. One of the most striking features of polyclad flatworms is their colouration. Many species have bright, vivid colours, often with complex and intricate patterns. These colours serve several purposes. Sometimes, they warn potential predators, indicating that the flatworms are toxic or distasteful (Rodríguez *et al.*,

2021). In other cases, they are used to attract mates or to camouflage against their surroundings. Colours and patterns have traditionally served to distinguish different species of Polyclad flatworms over an extended period.

Polyclads are carnivorous animals that consume small-sized polychaetes, crustaceans, and molluscs. They capture their prey using mucus and engulf it with their everted pharynx. They even prey on other polyclads (Jenning, 1957; Kate, 2010). They also scavenge, readily feeding on dead or injured animals. They primarily feed on oysters, bivalves, and ascidians (Newman and Cannon, 1995). Hermaphroditic in nature, polyclads have both male and female reproductive organs (Janine *et al.*, 2013). They reproduce sexually, with two individuals exchanging sperm to fertilize each other's eggs. Afterwards, the fertilized eggs are discharged into the water and metamorphose into free-swimming larvae, eventually settling on the seafloor and maturing into

adult polyclads (Kate, 2010). Polyclad flatworms play an essential role in marine ecosystems. The predatory nature of polyclads allows them to regulate populations of other invertebrates, contributing to ecosystem stability (McNab *et al.*, 2021). Additionally, their colourful appearance and distinct patterns make them a popular focus for ecotourism and scientific research.

Materials and Methods

Study Area

The study was conducted in Great Nicobar Island, India, with surveys carried out across a range of coastal and intertidal and reef habitats (Fig. 1). Sampling was

undertaken at Break Water Jetty (07.001506°N, 93.946993°E); Laxman Beach (07.022847°N, 93.920634°E); Joginder Nagar (06.940545°N, 93.916248°E); Vijay Nagar (06.919974°N, 93.910083°E); Laxmi Nagar (06.8960963°N, 93.8994262°E); Dayankar Beach (06.8862617°N, 93.8989893°E); Gandhi Nagar (06.839923°N, 93.897422°E); Shastri Nagar (06.800751°N, 93.887639°E) and Galathea Bay (06.8016268°N, 93.8803807°E). These sites encompass diverse coastal environments, including rocky intertidal patches dominated by seaweeds and algal growth, coral reef areas, and extensive sand flats, all of which provide suitable habitats for Polyclads and facilitate assessments of their distribution across Great Nicobar Island.

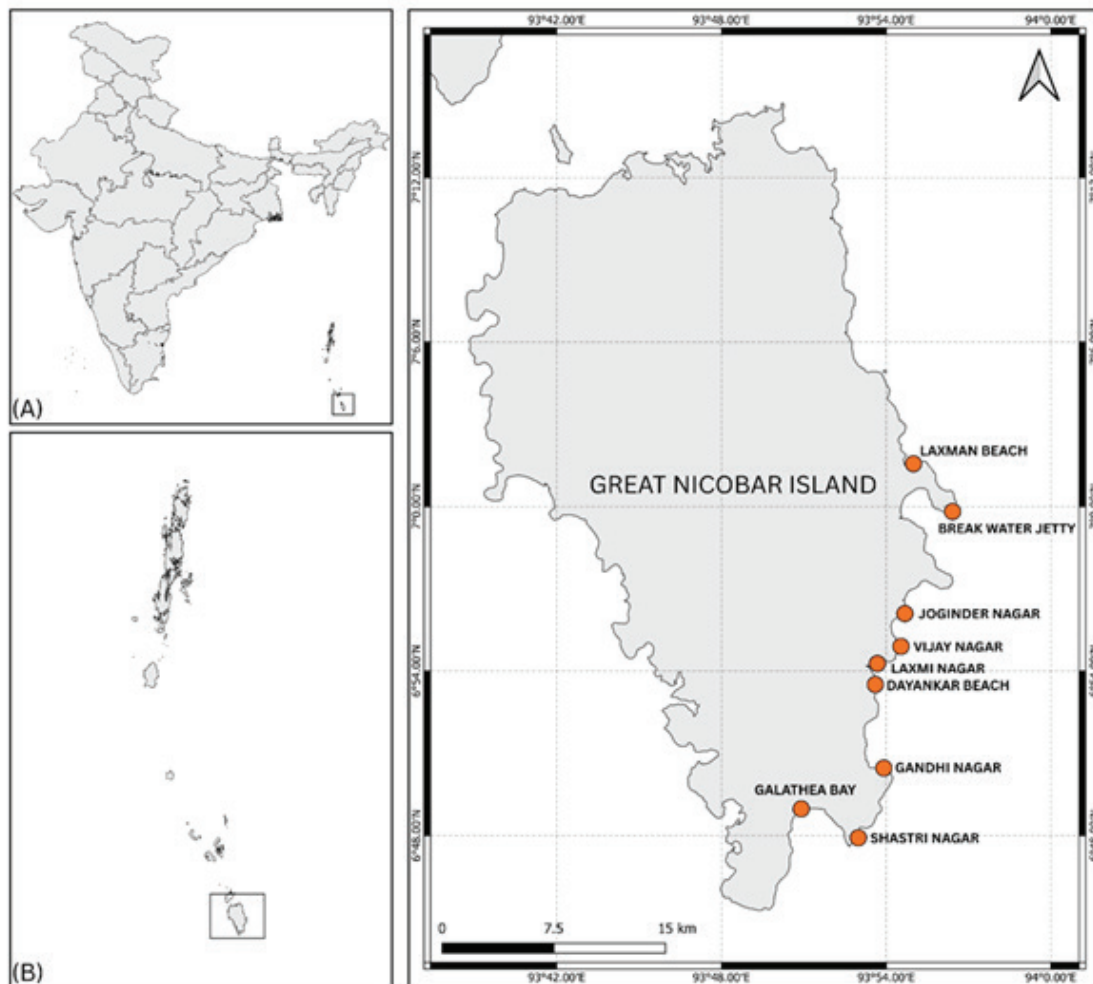


Fig.1. Location map of Great Nicobar Island showing the surveyed sites along the eastern coastline. Insets illustrate (A) the position of Great Nicobar Island within the Indian subcontinent and (B) its location within the Andaman and Nicobar archipelago. The main map highlights the nine surveyed coastal sites.

Specimen collection

The specimen examined in the present study was recorded during nocturnal low-tide surveys conducted in the intertidal zones of Breakwater Jetty and Gandhi Nagar, Great Nicobar Island, India. The individual was encountered beneath intertidal rocks and was carefully collected using a fine paintbrush to avoid external damage. Following collection, the specimen was temporarily maintained in a 5-L container containing ambient seawater for detailed behavioural and morphological observations. Photographic documentation was carried out both in situ and under ex-situ conditions to accurately record live colouration and dorsal patternation, which are important diagnostic features in polyclad taxonomy. After examination, the specimen was preserved in absolute ethanol and deposited for future molecular analyses. Species identification was confirmed based on external morphological characters and comparison with previously published descriptions.

Results

Material examined

Two specimens of the polyclad flatworm were recorded from the intertidal zones of the Breakwater Jetty and Gandhi Nagar regions during nocturnal low-tide surveys. Both individuals were encountered beneath overturned rocks within shallow intertidal reef habitats, suggesting a cryptic microhabitat preference. Surveys were conducted during night hours to improve detection efficiency, as polyclads are predominantly nocturnal and remain concealed under rocks and coral rubble during daytime low tides.

Repository specimens:

One specimen (ZSI/ANRC/M/32753) has been deposited at the National Zoological Collection, Zoological Survey of India, Andaman and Nicobar Regional Centre (ZSI/ANRC), Sri Vijaya Puram, Andaman and Nicobar Islands, India.

Systematics

Kingdom : Animalia
Phylum : Platyhelminthes

Subphylum : Rhabditophora
Order : Polycladida
Suborder : Cotylea
Family : Prosthlostomidae
Genus : *Prosthlostomum*
Species : *Prosthlostomum trilineatum*

External morphology

Both the specimens were roughly 10-11 mm long and 3.2-4.1 mm wide; their morphological plasticity made exact morphometrics challenging. The body was broadly rounded anteriorly and gradually tapered towards the posterior end. The dorsal surface appeared smooth and exhibited a milky white coloration. Along the anterior margin, 26 marginal eyespots were arranged in two irregular rows. Posterior to these rows, three distinct, arc-shaped transference bands were observed: an outermost yellow-orange band, followed by a blackish-brown band, and finally a white band. The blackish-brown band featured a central interruption forming a colourless notch, within which two clusters of eyespots were located. A median yellow-orange longitudinal stripe, bordered on either side by thin black bands, extended from the colourless notch to the posterior tip of the body. The ventral surface was semi-transparent, revealing key anatomical features: the mouth opening was situated medially just posterior to the cerebral region, followed sequentially by the pharynx, male gonopore, female gonopore, and terminal sucker.

Distribution and habitat

Prosthlostomum trilineatum was originally described by Yeri and Kaburaki (1920) from Hatakejima on the Kii Peninsula, Japan. Since its original description, the species has been reported from several regions across the Indo-Pacific, including Japan, Micronesia, Papua New Guinea, the Red Sea, and Singapore (Newman and Cannon, 2005). Within India, the species was previously mentioned from the Andaman Islands (ZSI-e-News, 2012) and later documented from the Ratnagiri coast of Maharashtra by Pitale *et al.*, (2014). The present study represents the first confirmed record of *Prosthlostomum trilineatum* from Great Nicobar Island, India, thereby extending its known distribution range to the Southernmost Island of the Indian coastline.

The specimens were encountered in the rocky intertidal zone, with both the individuals being observed exposed near coral rubble. The surrounding tidepools featured a sandy substratum interspersed with rocky outcrops overgrown with algae and seaweeds. The habitat characteristics of *Prosthiostomum trilineatum* were assessed based on the physicochemical properties of the tidepool environment. Salinity levels ranged between 37.3 and 37.5 ppt, while pH values were slightly alkaline, varying from 8.10 to 8.21. Dissolved oxygen concentrations were measured between 4.9 and 5.2 mg/L, indicating moderately oxygenated conditions suitable for intertidal fauna.

Spawning ecology

As in all the polyclads, these creatures are hermaphroditic and produce offspring by internal fertilization. Generally, when both the mates are receptive, polyclads exhibit a unique precopulatory activity, penis fencing. In this, the anterior end of each flatworm's body is bent up, and the penis is everted. The animals then try to thrust their everted penises against each other while at the same time trying not to get inseminated themselves. This is both competitive and reciprocal in nature since each of the flatworms wants to impregnate the other but avoid being fertilized. Penis fencing may end in one of three ways: sperm bundles can be delivered to both organisms, to one organism, or none at all (Tong and Ong, 2020). No copulatory behavior was observed, even when the specimens were kept together in the same tank under direct supervision. When not being monitored, the specimens were housed separately in individual tanks. Three days after capture, one specimen was observed laying eggs on the inner surface of the glass tank. The flatworm was seen in a contracted posture on the glass wall during this process. Egg deposition occurred through the female gonopore, with each egg laid individually onto the surface. The specimen remained in this position for over nine minutes before it began to move away. A total of 147 eggs were laid in a single layer, each enclosed within a protective capsule and arranged in an irregular pattern. The eggs had a distinct golden hue, and each capsule contained a single developing embryo. No signs of parental care were noted during the course of this study.

Discussion

Polycladida represents one of the most diverse and morphologically heterogeneous groups of marine flatworms in Indian waters, a diversity likely promoted by the extensive range of coastal and estuarine habitats present along the Indian coastline. Ecosystems such as mangrove-fringed estuaries, mudflats, sandy shores, seagrass meadows, coral reefs, rocky intertidal zones, and coral rubble habitats provide a wide variety of ecological niches and feeding opportunities that support a rich polyclad fauna. Despite this habitat diversity, the polyclad fauna of India remains comparatively underexplored.

The earliest contribution to the study of Indian polyclads was made by Laidlaw (1902), who described seven species from Minicoy Island in the Lakshadweep Archipelago. Among these were *Pseudobiceros gardinieri*, *Planocera armata*, *Prosthiostomum cooperi*, and *Prosthiostomum elegans*; however, these specimens were originally collected from the Maldives rather than Indian waters. Subsequent advances in Indian polyclad taxonomy remained sporadic until the work of Apte and Pitale (2011), who described two species from Lakshadweep and reported additional distributional records. Later, Dixit and Raghunathan (2013) documented a new record and described *Bulaceros newcannorum* from the Lakshadweep Islands. Further contributions were made by Shrinivaasu *et al.* (2018), who reported four species from the Gulf of Mannar and compiled a checklist of 55 species belonging to seven families from Indian waters. However, they clarified that several species previously included in Indian records, including *Pseudobiceros gardinieri*, *Planocera armata*, *Prosthiostomum cooperi*, and *Prosthiostomum elegans*, were actually based on Maldivian material. Pitale and Apte (2017) subsequently expanded knowledge of intertidal polyclads from the Maharashtra coast by reporting four new records and describing *Stylostomum mixtomaculatum*. Research on the Andaman and Nicobar fauna gained momentum with Dixit *et al.* (2019), who described *Pseudoceros galatheensis* and *Pseudoceros nigropunctatus* from the archipelago. Later, Dixit *et al.* (2021) described *Pseudoceros bipurpurea* and *Pseudoceros galaxea* from Agatti Island, Lakshadweep, and provided an updated checklist comprising 68 species

of polyclad flatworms from Indian waters. More recently, Dixit and Sreeraj (2024) described two additional species from Lakshadweep, namely *Paraplanocera kalpeniense* and *Pseudoceros bifascia*. Subsequently, Nanda *et al.*

(2024) reported two new distributional records for India, *Thysanozoon nigrum* and *Typhlosoceros nocturnus*, increasing the known diversity of Indian polyclads to 72 species.



Fig. 2. (A) Dorsal view of *Prosthiostomum trilineatum* showing characteristic colour pattern and marginal eyespots; (B) ventral view displaying the semi-transparent body and arrangement of ventral anatomical structures; (C) egg capsules of *Prosthiostomum trilineatum* deposited on the glass surface under captive conditions.

Collectively, these studies underscore the remarkable yet still insufficiently explored diversity of polyclad flatworms inhabiting the coastal and marine ecosystems of India.

Declarations

Ethical statement

The Polyclad species sampled during the present study are not protected under The Wildlife Protection Act, 1972 (Last amended in 2017) of the Ministry of Environment and Forest, Government of India.

Author contribution statement

The authors confirm their contributions as follows: Funding acquisition, project administration, supervision,

resources, investigation, and validation: Chandrakasan Sivaperuman; Conceptualization, writing original draft, visualization, methodology, and data analysis: Satya Swaroop Nanda and Chandrakasan Sivaperuman; Field surveys and data collection: Satya Swaroop Nanda; Writing-review & editing: Satya Swaroop Nanda and Chandrakasan Sivaperuman. All authors reviewed the results and approved the final version of the manuscript.

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Competing Interest

Authors do not have any competing interest to declare.

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