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First record of *Thysanozoon nigrum* Girard, 1851 and *Tytthosoceros nocturnus* Newman and Cannon, 1996 (Platyhelminthes: Polycladida: Pseudocerotidae) from the Gujarat coast, India

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Short communication

Abstract

Polyclad flatworms are a diverse group of colourful marine invertebrates with over 4,000 known species. Four species were encountered during the survey of the intertidal zone of the Okha coast. The present study provides the first occurrence record and description of two species of Polyclads: *Thysanozoon nigrum* Girard, 1851 and *Tytthosoceros nocturnus* Newman & Cannon, 1996 from the Gujarat coast. Before this study, *T. nocturnus* had only been observed in Australia. At the same time, *T. nigrum* had been previously recorded in the sub-tropical western Atlantic along the coast of Florida, Texas, Bermuda, and the Bahamas, as well as in the Indo-Pacific from the region around Singapore.

Keywords: Polyclad, marine flatworm, new geographical range, Indian Ocean, Gujarat

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 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 feed on small polychaetes, crustaceans, and molluscs. They capture prey by using mucus and engulf it with their everted pharynx, sometimes even preying on other polyclads (Jennings, 1957; Kate, 2010). Polyclads are also scavengers, consuming dead or injured animals, with a primary diet that includes 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.

The study of polyclads has been overlooked in India, leaving us with little understanding of their distribution on the eastern and western shores (Dixit *et al.*, 2021) and they compiled an annotated checklist of 68 species of Polyclads belonging to 16 genera in seven families from the Indian Ocean. In the present study, we are reporting new distribution records of two species of polyclads, *T. nigrum* Girard, 1851, and *T. nocturnus* Newman and Cannon, 1996, for the first time from Indian waters.

Material and methods

The specimens were encountered during routine surveys of the seagrass beds of Mithapur (22° 26' 5.064" N, 68° 59' 36.924" E), Paga Reef (22° 28' 2.9" N, 69° 12' 42.4" E) and at the intertidal zone of the Okha coast (22° 28' 43.77" N, 69° 3' 59.34" E) (Fig. 1). Mithapur encompasses a significant stretch of the Arabian Sea coast, offering a rich tapestry of marine and coastal ecosystems. This region boasts a diverse array of coastal habitats, including sandy beaches and rocky tidepools, while offshore areas feature expansive coral reefs, seagrass beds, and extensive sand flats. Water depths in the study area range from 3 to 12 meters. Mithapur's coast is renowned for its exceptional biodiversity, teeming with a multitude of invertebrates and vertebrates. Among the rich array of marine life found here are various species of fishes, crustaceans, nudibranchs, polyclads, and polychaetes, further enhancing the ecological significance of this coastal region. Paga Reef boasts an impressive array of reef biodiversity, encompassing a diverse range of both fauna and flora attributed to its diverse marine habitats like seagrass beds, coral reefs and sand flats. At its western edge during low tide, is an exposed sand patch which reveals lush seagrass meadows predominantly comprised of the *Halophila* and

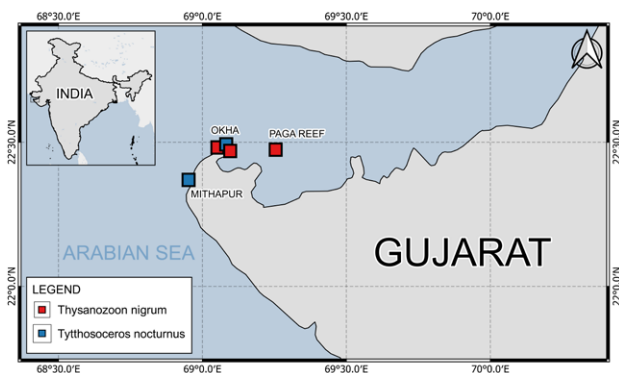


Fig. 1. Geographical distribution of *Thysanozoon nigrum* and *Tythosoceros nocturnus* in Gujarat coastal waters

Halodule genera. Notably, there is a discernible decrease in coral density from the periphery towards the centre of the reef, although certain tidal pools exhibit exceptions to this pattern. The coral assemblages within the reef exhibit remarkable diversity, showcasing various forms including sponges, sea anemones, zoantharians, tube worms, crabs, gastropods, bivalves, and echinoderms. In total, this vibrant ecosystem hosts over 200 species of marine invertebrates, contributing to the richness and complexity of Paga Reef's marine life (Singh *et al.*, 2004). The Okha Reef has a flat rocky intertidal belt with many tide pools and crevices. The substratum here is made of limestone rock. The sandy beach gradually slopes into the rocky intertidal belt from the landward side. Despite the damage to the reef due to anthropogenic influences, there is a rich diversity of flora and fauna.

Please update paragraph to: The specimens were observed during extensive surveys conducted from November 2022 to February 2023 in the seagrass meadows of Mithapur and Paga Reef, as well as in the intertidal zone along the Okha coast. The surveys were performed during low tides. The physiochemical factors, including salinity, pH, and temperature were measured using Hanna portable meters, while the dissolved oxygen was measured using a Lutron portable Dissolved Oxygen meter. Due to the low density of animals observed and the ease of identifying these animals via external morphology, no attempts were made to collect reference specimens. Macro and super macro modes on the Olympus TG-6 camera were used to capture pictures showcasing the key identifying traits. Identification was confirmed by photographic evidence along with morphological analysis.

Results

Thysanozoon nigrum Girard, 1851

Material examined

Three specimens were observed during the study period. The first specimen was observed on 22nd December 2022 at the Paga Reef seagrass meadows (22° 28' 2.9" N; 69° 12' 42.4" E), the second specimen was observed on 16th February 2023 along the Okha reef (22° 28' 45.12" N; 69° 4' 40.2816" E) and the third specimen was also observed along the Okha reef on 18th February 2023 (22° 28' 43.068" N; 69° 4' 25.014"E).

Systematics

Kingdom : Animalia
Phylum : Platyhelminthes
Subphylum : Rhabditophora

Order : Polycladida
 Suborder : Cotylea
 Family : Pseudocerotidae
 Genus : *Thysanozoon*
 Species : *nigrum*

Distribution and habitat

Thysanozoon nigrum had been previously recorded in the sub-tropical western Atlantic along the coast of Florida, Texas, Bermuda, and the Bahamas, as well as in the Indo-Pacific from the region around Singapore (Ong *et al.*, 2018). The present study is being reported from the Indian mainland for the first time. The first specimen was observed in the *Halophila decipiens* seagrass beds of Western Paga Reef; while the other two specimens were observed at the Okha coast, which encompasses a variety of habitats, such as coral reefs, algal reefs, sandy and rocky areas, providing an ideal niche for Polyclads. All specimens were found under rubble and along rocky slopes in tidepools with sandy substrate located in the upper and middle intertidal zone of the Okha coast at night.

The water quality and environmental conditions of the habitat where *T. nigrum* was found were determined by the physiochemical parameters of the tidepools. The salinity of the tidepools in this area was found to range between 37.3 - 37.5 ppt. The pH values recorded were slightly alkaline, ranging from 8.10 - 8.21. Dissolved oxygen levels were measured between 4.9 and 5.2, which falls within the healthy range for most marine organisms. The temperature of the tidepools was between 22.2 °C - 23.7 °C.

External morphology

All three specimens were roughly 50-75 mm long and 25-30 mm wide; their morphological plasticity made exact morphometrics challenging. The *Thysanozoon* genus is likely the most straightforward among polyclad genera to identify. This is because its dorsal surface is covered with many noticeable papillae, a defining characteristic of the genus. *T. nigrum* is the only species in the genus *Thysanozoon* that is characterized by an entirely black body and covered dorsally in black papillae (Ong *et al.*, 2018) (Fig. 2A). While some individuals may exhibit multiple white speckles, others have little to no white pigmentation. The ventral side is lighter in colour and devoid of any white spots, unlike the dorsal side. The pseudo-tentacles are ruffled and square-shaped with white tips formed by a cluster of dots on the lateral sides (Fig. 2B). Cerebral eyespots are horseshoe-shaped and in a clear oval area with a short line projecting anteriorly and posteriorly (Newman and Cannon,

2003). Differentiating the species within the genus can be challenging due to their similar internal structure. Therefore, colouration plays a significant role in distinguishing the species (Dixit *et al.*, 2017).

Tytthosoceros nocturnus Newman and Cannon, 1996

Material examined

Two specimens were observed during the study period. The first specimen was observed in the seagrass meadows of Mithapur on 13th November 2022 (22° 26' 5.064" N; 68° 59' 36.924" E), and the second specimen was observed along the Okha coast on 16th December 2022 (22° 28' 42.8592" N; 69° 4' 25.8096" E).

Systematics

Kingdom : Animalia
 Phylum : Platyhelminthes
 Subphylum : Rhabditophora
 Order : Polycladida
 Suborder : Cotylea
 Family : Pseudocerotidae
 Genus : *Tytthosoceros*
 Species : *nocturnus*

Distribution and habitat

Not much information is available regarding the global distribution of this species. *T. nocturnus* had only been observed in Australia (Newman and Cannon, 1996). The present study is being reported from the Indian coastline for the first time. Two specimens were observed during the study. One of the specimens was observed on the Okha coast, which encompasses a variety of habitats, such as coral reefs, algal reefs, and sandy and rocky areas, providing an ideal niche for Polyclads. The specimen was found out in the open next to rubble and along rocky slopes in tidepools with sandy substrate located in the upper and middle intertidal zone of the Okha coast at dusk and the other specimen was observed in the *Halodule sp.* seagrass meadows of Mithapur.

The water quality and environmental conditions of the habitat where *T. nocturnus* was found were determined by the physiochemical parameters of the tidepools. The salinity of the tidepools in this area was found to range between 37.3 - 37.5 ppt. The pH values recorded were slightly alkaline, ranging from 8.10 - 8.21. Dissolved oxygen levels were measured between 4.9 and 5.2, which falls within the healthy range

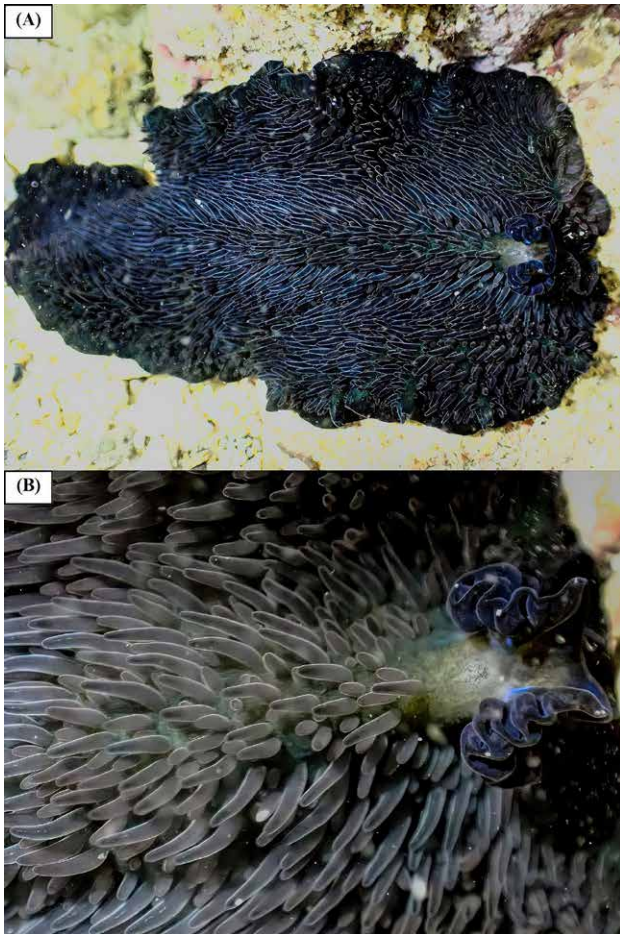


Fig. 2. A) Dorsal section of *Thysanozoon nigrum*, B) Close-up of pseudo-tentacles and cerebral eyespots of *Thysanozoon nigrum*

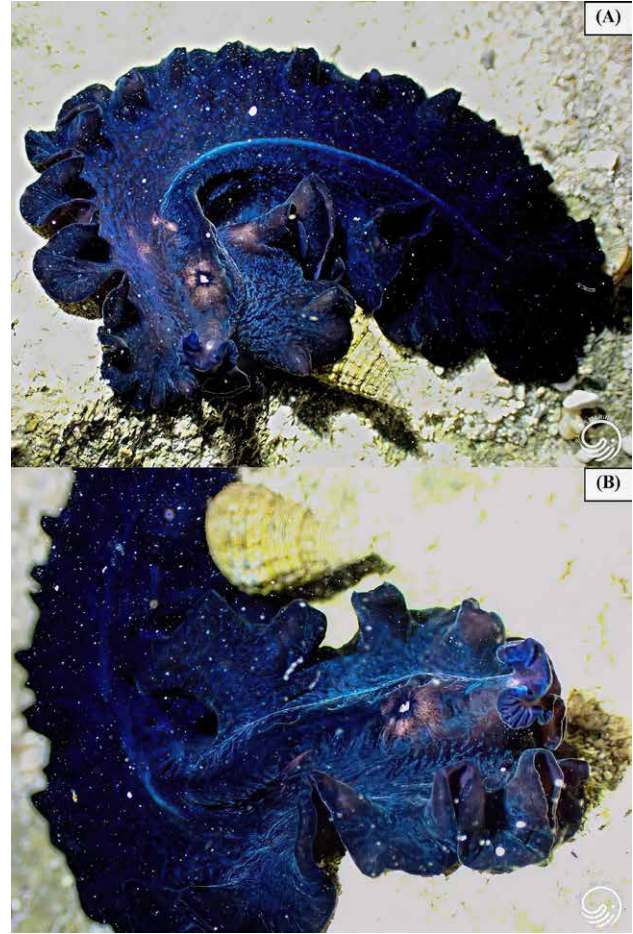


Fig. 3. A) Dorsal section of *Tyththosoceros nocturnus*, B) Close-up of pseudo-tentacles and cerebral eyespots of *Tyththosoceros nocturnus*

for most marine organisms. The temperature of the tidepools was between 22.2 °C - 23.7 °C.

Morphology

Both specimens were roughly 60 - 80 mm long and 25 - 30 mm wide. Due to their morphological plasticity, exact morphometrics were difficult to obtain. Upon initial observation, the specimens appear velvety black. Still, upon closer inspection, the specimen appears to be sapphire blue with a teal hue (Fig. 3a). Pseudotentacles are earlobe-shaped with greyish-blue tips (Fig. 3b). The ventral side is brownish and medially dark blue with a single male pore being a key identification feature.

Remarks

Upon initial observation, this species can be confused with *Pseudoceros bolool* (Newman and Cannon, 1994). However, unlike *T. nocturnus*, *P. bolool* is evenly black. Also, *T. nocturnus* is raised medially, while *P. bolool* is flat (Newman and Cannon, 1996).

Discussion

Polycladida might be the most extensive and heterogeneous category of flatworms present in the waters of India. This greater diversity is probably due to India's wide range of estuarine and marine habitats. The Indian coast offers a mix of environments, including estuarine areas with mangroves and mudflats, sandbars, seagrasses, coral reefs, rocky and coral rubble areas, and a wide range of food sources that may have contributed to the high species diversity. India's polyclad fauna is poorly understood (Laidlaw, 1902). Described seven species from Minicoy Island (Lakshadweep Islands), which helped establish the polyclad research field in India addition to providing a checklist of polyclads known from India that included 55 species in seven families (Shrinivaasu *et al.*, 2018), four species from reef areas in the Gulf of Mannar Islands. Four species, namely *Pseudobiceros gardineri* Laidlaw, 1902, *Planocera armata* Laidlaw, 1902, *Prosthiosomum cooperi* Laidlaw, 1902, and *Prosthiosomum elegans* Laidlaw, 1902 are included in that list. Moreover, two previously unknown species were identified and characterized in the Andaman

and Nicobar Islands (Dixit *et al.*, 2019) *i.e.*, *Pseudoceros galatheensis* sp. nov. and *Pseudoceros nigropunctatus* sp. nov., and two more new species and one new record from Lakshadweep (Apte and Pitale, 2011), *i.e.*, *Pseudoceros agattiensis* and *Pseudoceros stellans*. Pitale and Apte 2017, added to the research of intertidal polyclads off the coast of Maharashtra, reporting four new records and one new species, *i.e.*, *Stylostomum mixtomaculatum* sp. nov. This was followed by one new record and one new species from Lakshadweep (Dixit and Raghunathan, 2013), *i.e.*, *Bulaceros newcannorum* sp. nov. The present study presents two new distribution records from India, *i.e.*, *Thysanozoon nigrum* Girard, 1851 and *Tythosoceros nocturnus* Newman and Cannon, 1996. As a result, 70 polyclad species are currently known from India (Hyman, 1952; Apte and Pitale, 2011; Sreeraj and Raghunathan, 2011; Dixit and Raghunathan, 2013; Sreeraj and Raghunathan, 2013, 2015; Venkataraman *et al.*, 2015; Pitale and Apte, 2017; Shrinivaasu *et al.*, 2018; Dixit *et al.*, 2019; Dixit *et al.*, 2021). Besides external characters, molecular data techniques will benefit when employed in phylogenetic analysis. Further surveys are crucial to gather additional information regarding these animals' worldwide distribution and ecology.

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Author contributions

Conceptualization: SSN, AK; Methodology: SSN, AK; Data Collection: SSN, AK; Data Analysis: SSN, AK; Writing Original Draft: SSN, AK; Writing Review and Editing: SSN, AK, KV; Supervision: KV.

Conflict of interests

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

This study was conducted following the ethical standards and guidelines established by the ethical committee at the Marine National Park, Jamnagar and the Gujarat Forest Department. All procedures involving animals/humans/ environment, etc. were approved by the appropriate institutional review boards/ ethics committees (No. WLP/RTC/28/C/717-19/2022-23).

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