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RESEARCH PAPER

Macro epibiont diversity in breeding olive ridley sea turtles *Lepidochelys olivacea* (Eschscholtz, 1829) from east coast of India

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Abstract
The epibionts associated with breeding Olive ridley sea turtle *Lepidochelys olivacea* (Eschscholtz, 1829) was studied during the offshore monitoring at Rushikulya rookery in Odisha during the breeding season 2021. Epibionts attached to different body parts of the turtles were sampled and identified to the lowest possible taxonomic level. Altogether, 324 epibionts from 14 species belonging to 12 genera and 12 families were documented. *Stomatolepas elegans* (Costa, 1838) and *Chelonibia testudinaria* (Linnaeus, 1758) were found to be most abundant species. For the first time, two species of bivalve *Pinctada margaritifera* (Linnaeus, 1758) and *Anomia sp.* were documented as epibionts attached to the carapace of olive ridley sea turtles. The species richness, abundance, diversity and distribution of epibionts in different body parts of olive ridley turtles was also studied. Shannon diversity Index was higher in case of males than females; species richness was found to be higher in males than females; dominance index was maximum in female turtles than in male turtles. This is first documentation of epibionts on olive ridley sea turtles from Rushikulya rookery in India.

Keywords Bay of Bengal · Mass nesting site · Epibiosis · Epibionts · *Lepidochelys olivacea*

Introduction

The colonization of marine living organisms is called epibiosis where a single host supports one or more epibionts (Frick and Pfaller 2013). Epibionts utilize the turtles as a substratum for attachment that provides a mobile platform which aids in suspension and filter feeding (Frick et al. 2004). A broad array of epibionts found on sea turtles including macro epibionts (cirripeds, polychaetes, poriferans, hydrozoans, bryozoans, tunicates, algae) meiofaunal epibionts (copepods and nematodes) and micro epibionts (diatoms) (Robinson et al. 2016; Ingels et al. 2020). The sea turtle and barnacle relationships are in between mutualism and parasitism, where barnacles attach to the turtles temporarily for migration and get benefitted in terms of increased survival and foraging (White et al. 2017). The distribution of epibionts on host turtles influenced by several complex factors like water flow or current patterns, recruitment dynamics, site suitability on body parts and both inter and intra specific interactions (Pfaller et al. 2008).

Sea turtles have the most diverse epibiont communities compared to other marine animals due to their wide variation in movement patterns and feeding choices among individuals. Thereby, the association between sea turtles and their epibionts can be used as biological models to study variables that influence interspecific epibiont community structure (Zardus et al. 2014; Robinson et al. 2017). The turtles occupying similar geographic location and habitats have similar epibiont communities (Frick and Pfaller 2013).

The olive ridley sea turtle *Lepidochelys olivacea* (Eschscholtz, 1829) is one of the smallest and most abundant sea turtle species distributed throughout the tropical

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regions of the World (Gatto et al. 2020). The species exhibits synchronous nesting behavior known as *arribada* (Valverde et al. 2012). In India, Gahirmatha, Rushikulya and Devi are the major mass nesting beaches for olive ridley sea turtles along the east coast (Pandav et al. 1994; Tripathy and Pandav 2008). Before, the arribada, turtles congregate in the coastal waters of Odisha. Males and females remain in nearshore waters during the winter months, after which they migrate south from the Odisha coast, reaching as far north as northern Sri Lanka and the central Bay of Bengal (Behera et al. 2018). Several organisms have been observed to be harmlessly living on the flippers, carapace, head and cloaca of these turtles (Ramos-Rivera et al. 2021) However, the study on the species diversity of epibionts on Olive ridley sea turtles in India is scanty (Robinson and Pfaller 2022). This study documents the species richness, abundance, and diversity of epibionts in *L. olivacea* turtles in the offshore waters along the east coast of India and also add knowledge on the distribution of epibionts across different body parts of both male and female turtles.

Material and methods

Study area

The Rushikulya rookery is a shallow tidal estuary formed by the river Rushikulya which flows through the Ganjam district of Odisha and opens into the Bay of Bengal near Ganjam town (19° 22′ N and 85°02′ E) (Fig. 1), Odisha along the east coast of India. This rookery is ecologically sensitive due to arribada of Olive Ridley Sea turtle. The mass nesting beach of the rookery is continuously changing with the sandspit, present along the river northern side of the river mouth which makes a difference in nesting pattern along the beach. The Olive Ridley Sea turtles congregate offshore of the rookery within an area of approx. 250 sq. km near the river mouth.

Methods

As a part of flipper tagging of Olive ridley sea turtles programme conducted by the Zoological Survey of India, the mating pairs of Olive Ridley Sea turtles were sampled along the off shore of Rushikulya rookery (Fig. 1). Male and female olive ridley sea turtles were captured using a trammel net (locally designed triangular scoop net) in the offshore waters. All the captured turtles were tagged on both their front flippers using Inconel tags (National Band and Tag Co., USA) marked with a serial number on the top and return address – "ZSI, NAPO, KOL-53, IN; director@zsi.gov.in" on the reverse side. Epibionts collected from different body parts of turtles were preserved in 70% of ethanol and labelled with a host number (corresponding to the tag number attached) for identification following the standardized protocol (Frick et al. 1998). The collected samples were sorted and identified to the lowest possible using standard keys and descriptions (Heemstra 1986; Williams 1986; Chan and Prabowo 2009; McGowin et al. 2011; Behera 2017). All the collected samples were deposited at the repository of Estuarine Biology Regional Centre, Zoological Survey of India, Gopalpur, Odisha, India.

Data analysis

The Kolmogorov- Smirnov test was used to check the normal distribution of data by computing the maximum distance between the cumulative distributions of the two samples. The Kruskal- Wallis non-parametric test was performed to compare barnacle abundance between male and female turtles. The Shannon diversity index, Index of dominance, Margalef's richness index and equitability index between male and female turtles were computed. PERMANOVA test had been used to compare assemblage structure and species composition of epibionts on different body parts. To determine the differences in the structure of the epibiont community between different samples with the presence and absence of epibionts, analysis of similarity (ANOSIM) was performed using log $x + 1$ transferred abundance data and Bray- Curti's similarity measure. A similarity percentage test (SIMPER) was performed to identify the relative contribution of each taxon to any dissimilarity values between the epibiont assemblages of Olive ridley turtles on different body parts. The graphic representation of neighbour joining clustering was done using Euclidean similarity index, which shows the difference in epibiont community composition among different body parts. All the analysis were performed and graphs were generated using Past 4.03 (Hammer et al. 2001) software and MS Excel 2019.

Results

A total 324 specimens belonging to 14 species were collected from 27 (09 female and 18 male) Olive Ridley Sea turtles. Among all the epibionts, two species each from Cnidarians and Annelids, seven species of Arthropods, two species of Molluscs, and only one species was from Pisces (Teleostei) (Table 1 and Fig. 2). The eight identified invertebrate species of epibionts are *Ozobranchus branchiatus* (Menzies, 1798) (Ozobranchidae: Rhynchobdellida); *Amphibalanus amphitrite* (Darwin, 1854) (Balanomorpha: Balanidae); *Amphibalanus reticulatus* (Utinomi, 1967) (Balanomorpha: Balanidae); *Stomatolepas elegans* (Costa, 1838) (Balanomorpha: Coronulidae), *Chelonibia testudinaria* (Linnaeus, 1758) (Balanomorpha: Chelunibiidae);

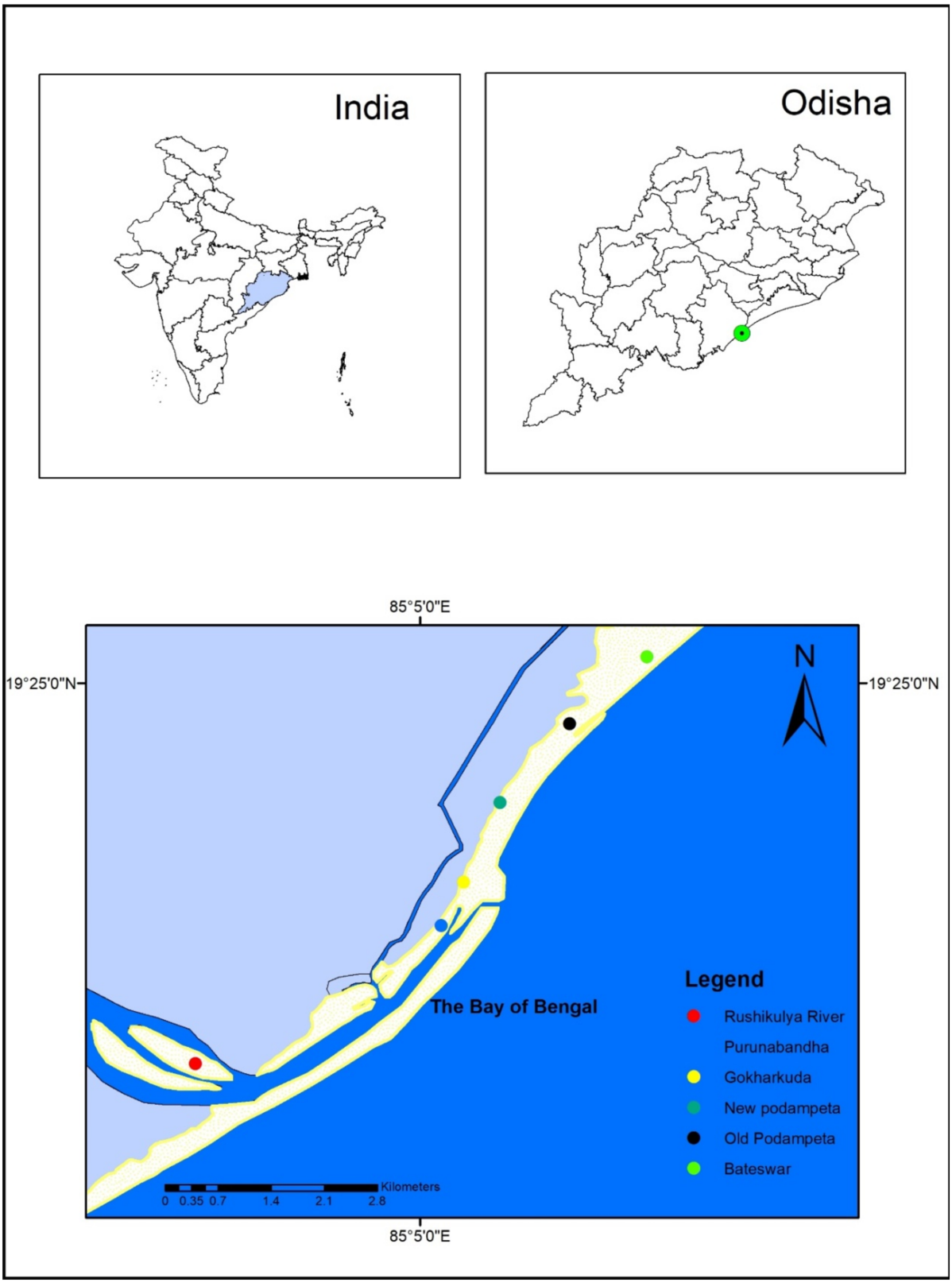


Fig. 1 The location of Rushikulya rookery, Odisha, India

Table 1 Checklist of epibionts collected from Olive Ridley Sea turtle at Rushikulya Mass Nesting Site

Sl no.	Epibionts	Phylum	Attachment site
1	*Alcyonium* sp.	Cnidaria	Carapace
2	*Calliactis* sp	Cnidaria	Carapace, Plastron
3	*O. branchiatus* (Menzies, 1798)	Annelida	Cloaca, abdomen, Hind flippers
4	*Ozobranchus* sp.	Annelida	Cloaca, abdomen, Hind flippers
5	*A. amphitrite* (Darwin, 1854)	Arthropoda	Carapace
6	*S. elegans* (Costa, 1838)	Arthropoda	Neck region, flippers, Abdomen
7	*C. testudinaria* (Linnaeus, 1758)	Arthropoda	Carapace, Plastron
8	A. *reticulatus* (Utinomi, 1967)	Arthropoda	Carapace
9	*O. warwicki* Gray, 1825	Arthropoda	Carapace
10	*Smilium* sp.	Arthropoda	Carapace
11	*L. anatifera* Linnaeus, 1758	Arthropoda	Carapace, Plastron, Flippers
12	*P. margaritifera* (Linnaeus, 1758)	Mollusca	Carapace
13	*Anomia* sp.	Mollusca	Carapace
14	*R. remora* (Linnaeus, 1758)	Chordata	Carapace, Plastron

Lepas anatifera Linnaeus, 1758 (Scalpellomorpha: Lepadidae) *Octolasmis warwicki* Gray, 1825 (Scalpellomorpha: Poecilasmatidae); *Pinctada margaritifera* (Linnaeus, 1758) (Ostreida: Margaritidae). Among all epibionts *Remora remora* (Linnaeus, 1758) (Carangiformes: Echeneidae) was only one vertebrate recorded. Two species of Cnidarians viz. *Alcyonium* sp., *Calliactis* sp., one species of Arthropod viz. *Smilium* sp. and one species of Mollusc viz. *Anomia* sp. could be identified up to genus level only (Fig. 2a–o). Overall, among all the epibionts 50% were crustaceans. It is also observed that the arthropods are most dominant as epibionts of Olive ridley sea turtles (Fig. 3). The relative abundance of *S. elegans* was found to be maximum followed by *C. testudinaria* in both the male and female turtles. Comparisons between the relative abundance of different epibiont species in both male and female turtles is depicted in Fig. 4. Shannon diversity Index was higher in case of males (1.283) than females (1.003); species richness was found to be higher in males (2.041) than females (1.292); dominance index was maximum in female turtles (0.526) than in male turtles (0.449) and the equitability index showed that the species were evenly distributed in male (0.52) than in female turtles (0.51) (Fig. 5). The Kolmogorov–Smirnov test ($D = 0.35714$, $P = 0.267$) was used for comparison of distributions of the two samples. The Kruskal-Walli's test ($H = 2.769$, $P = 0.096$) showed no statistically significant difference ($P > 0.05$ at 95%CI).

The ANOSIM analysis had been performed to test for differences in epibiont community structure between body parts and was found to be significant ($R = 0.39$, $P < 0.05$). The PERMANOVA analysis showed statistically significant site-based differences in the epibiont species composition and community structure of both male and female turtles ($F = 2.46$, $P < 0.05$). Similarly, the neighbour joining clustering plot revealed the species composition to be different among different body parts (Fig. 6). The SIMPER analysis conveyed overall average dissimilarity of 85.16% between the male and female turtle body parts (Table 2). *S. elegans* (49.65%), *C. testudinaria* (23.75%), *O. branchiatus* (5.12%) contributed to more than 50% of the difference. The total abundance of epibionts across various body regions of turtles is illustrated in Fig. 7. In male turtles, the groin region exhibits the highest species abundance, while the flippers show the lowest abundance of species. Conversely, in female turtles, the majority of species are observed on the head and neck, with the plastron exhibiting the least abundance of epibionts where in flippers and cloaca with no epibionts were recorded.

Discussion

The epibiont communities in many marine megafauna populations worldwide have been well studied (Robinson and Pfaller 2022; Fuller et al. 2010; Violante-Huerta et al. 2017). On the Mexican beaches, 21 species of noticeable epibiont fauna had been recorded for *L. olivacea* (Violante-Huerta 2018), while epibionts in *L. olivacea* from the Mexican Pacific had recorded (Gámez et al. 2009). The presence of 16 nominal species of barnacles from all 7 extant sea turtle species were recorded in Pacific Ocean and the barnacle *Stomatolepas elegans* was found to be the most common barnacle followed by *Chelonibia testidinaria* (Zardus 2021). The lack of information regarding Indian Ocean Sea turtle epibionts was emphasised (Robinson and Pfaller 2022). In total 42 species of Cirripedia of 26 genera and 12 families were documented from Odisha (Trivedi et al. 2021). Seven invertebrate commensals were recorded from nesting female turtles at Gahirmatha, five of which were cnidarians, one Porifera, and one Arthropoda highlighting the abundance of

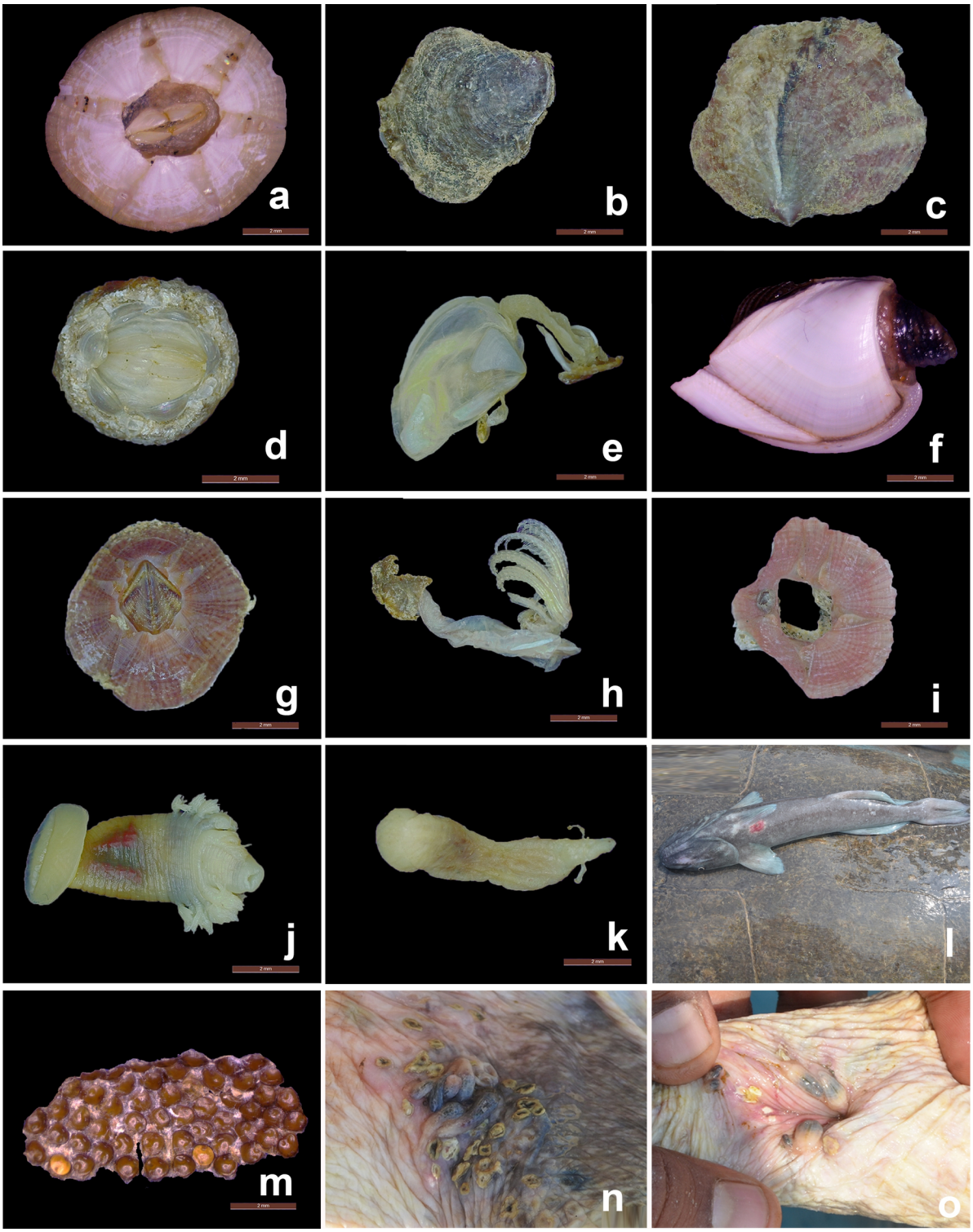


Fig. 2 **a** *Chelonibia testudinaria* (Linnaeus, 1758); **b** *Pinctada margaritifera* (Linnaeus, 1758); **c** *Anomia* sp.; **d** *Stomatolepas elegans* (Costa, 1838); **e** *Octolasmis warwicki* Gray, 1825; **f** *Lepas anatifera* Linnaeus, 1758; **g** *Amphibalanus amphitrite* (Darwin, 1854); **h** *Smilium* sp.; **i** *Amphibalanus reticulatus* (Utinomi, 1967); **j** *Ozobranchus branchiatus* (Menzies, 1798); **k** *Ozobranchus* sp.; **l** *Remora remora* (Linnaeus, 1758); **m** *Alcyonium* sp.; **n** *Ozobranchus* sp. perforates into groin of turtle; **o** *Ozobranchus branchiatus* attaches to cloaca of a male turtle

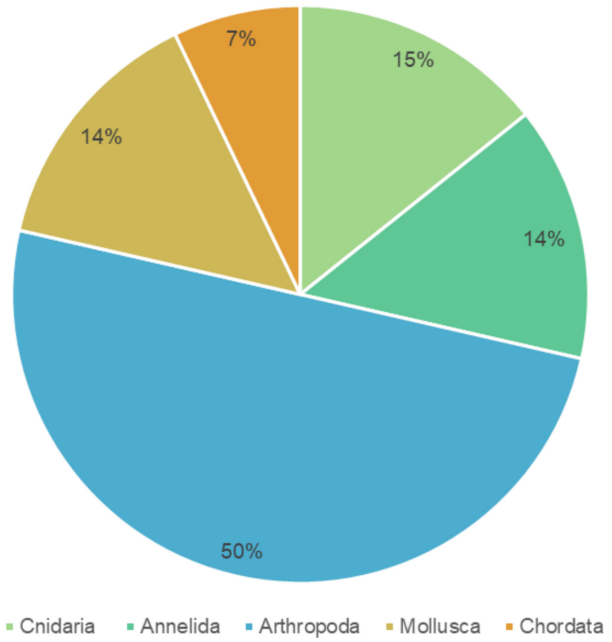
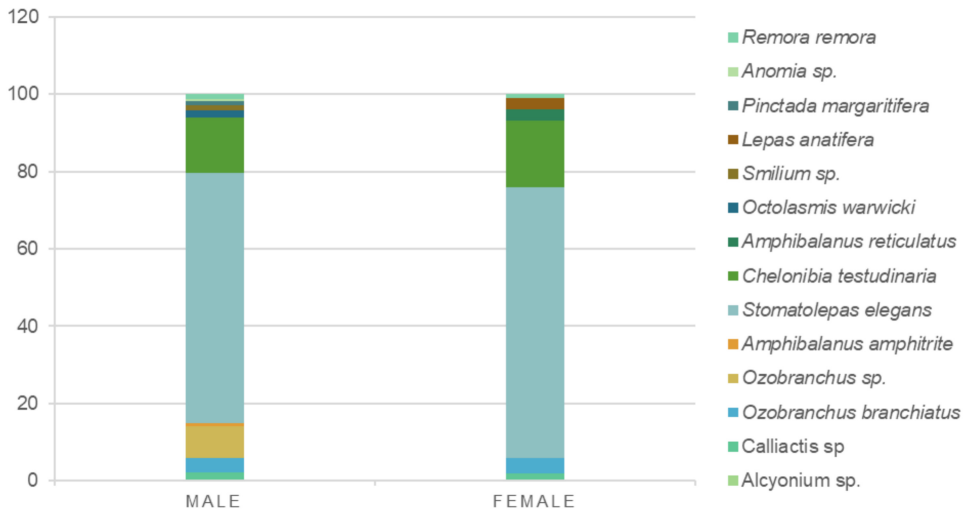


Fig. 3 Phylum wise distribution of epibionts on olive ridley sea turtles

Metridium, *Adamsia*, and *Bougainvillia*, the most frequent commensal, which is adhered to the entire surface of the turtle's carapace, while the cirriped *Balanus* is only found in the turtle's neck and carapace (Hejmadi et al.1989).

The present work analysed the differences in the epibiont communities recorded from different body parts of male and female Olive ridley sea turtles. During the present study, *S. elegans* was recorded to be the most prevailing epibiont species, adhering to the soft body parts like neck, flippers and abdominal region. The species was found to form colony in both male and female turtles. *C. testudinaria* was also recorded to be a common species found in both male and female on the hard surfaces like carapace, head, and plastron region. Molluscs and Cnidarians were found to be attached to the carapace covered with algae. Two species of Bivalves *P. margaritifera* and *Anomia sp.* were reported for the first time as epibionts attached to the carapace of olive ridley sea turtles. The Sucker fish *R. remora*, was found attached to the plastron and carapace of the turtles in the offshore waters while turtles are in mating position. The groin area of male and head and neck area of female was found to have maximum number of epibionts. *S. elegans* is dominantly found in the groin, head and neck region of both turtles. Also, *Ozobranchus sp*. and *O. branchiatus* were found in the groin area and in cloacal area of male turtles, which is a vector of Fibropapiloma-associated herpes virus (Greenblatt et al. 2004). No epibionts were collected from the cloaca of female turtles during the study period. It can be hypothesized that the distinct variations in epibiont load and community structure between different body parts of sea turtles, such as the head or tail and the carapace or plastron, can be attributed to the contrasting surface properties of these areas. Specifically, the 'soft-tissue' skin of the head and tail is believed to offer a different substrate for settlement compared to the keratinized scutes of the carapace, potentially influencing the abundance of key species like *S. elegans* and *C. testudinaria*. For instance, *S. elegans* was notably abundant on the neck, flipper and abdomen but was notably absent on the carapace and plastron, while *C. testudinaria* demonstrated unique adaptations for adhering solely to the carapace or plastron without penetrating the surface, unlike other coronuloids that reside in the skin (Robinson et al. 2019). A detailed checklist of the epibionts found associated with Olive ridleys is provided (Table 2). The species diversity and richness of epibionts on the sea turtles was found to be influenced by factors like the geographic range, habitat use, behaviour, and body surface characteristics of the turtles (Robinson and Pfaller 2022).

Fig. 4 Species wise relative abundance of epibionts on olive ridley sea turtles



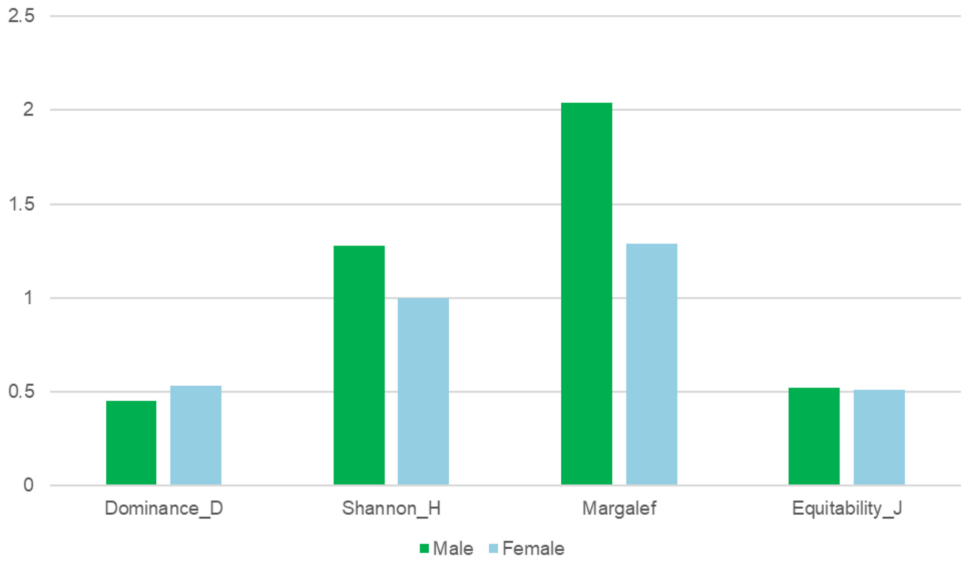


Fig. 5 Comparison of diversity indices of male and female olive ridley sea turtles

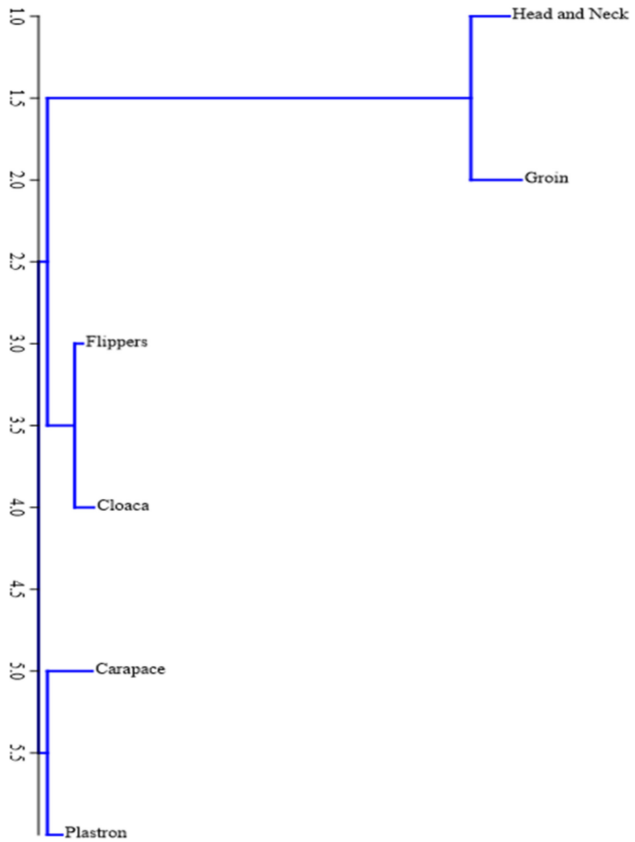


Fig. 6 The neighbour joining clustering plot depicts the species composition to be different among different body parts

Table 2 Relative contribution of each taxon to any dissimilarity values between the epibiont assemblages of Olive ridley turtles on different body parts determined by SIMPER test

Taxon	Average dissimilarity	Contribution (%)	Cumulative (%)
S. elegans	42.28	49.65	49.65
C. testudinaria	20.22	23.75	73.4
Ozobranchus sp.	5.32	6.25	79.65
O. branchiatus	4.36	5.12	84.77
Callictis sp.	2.53	2.97	87.75
R. remora	2.17	2.55	90.29
A. reticulatus	2.08	2.45	92.74
L. anatifera	2.02	2.37	95.11
O. warwicki	1.28	1.50	96.61
Similium sp.	0.96	1.13	97.74
P. margaritifera	0.64	0.75	98.49
A. amphitrite	0.64	0.75	99.25
Anomea sp.	0.32	0.38	99.62
Alcyonidium sp.	0.32	0.38	100

Male and female sea turtles exhibit distinct epibiont communities as a result of various factors. It has been suggested that female turtles may harbour a more diverse array of epibionts in comparison to their male counterparts. The disparity in epibiont communities between male and female sea turtles could potentially be influenced by a multitude of factors such as their habitat utilization, feeding behaviour, and reproductive activities. One plausible explanation for the divergence in epibiont communities between male and female sea turtles lies in their specific habitat preferences and foraging habits. Research indicates that the epibiont communities found on sea turtles are indicative of their feeding grounds rather than their nesting sites (Lim et al. 2020). For example, olive ridley turtles, which share foraging areas with both leatherback and green turtles, are anticipated to possess intermediary epibiont communities between the two

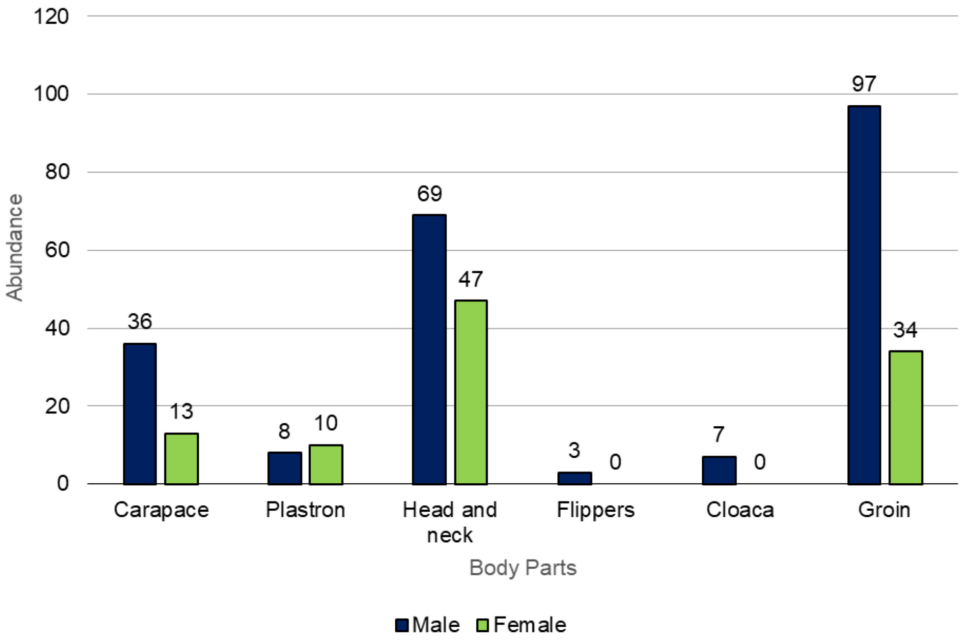


Fig. 7 Comparison of abundance of Epibionts on different body parts of olive ridley turtles

species (Robinson et al. 2017). This implies that the varying foraging behaviours of male and female turtles could result in differences in the epibiont communities they support. Furthermore, the reproductive behaviours of female turtles, such as nesting, might also play a role in the higher diversity of epibionts observed in females. Female turtles are recognized for their distinct nesting habits and may visit multiple nesting sites (Wright et al. 2012). This behaviour could expose female turtles to a wider range of environments and potentially distinct epibiont communities when compared to male turtles, which might not demonstrate the same nesting patterns. Additionally, the physical condition of turtles has been associated with the richness and abundance of epibionts, with a decrease in turtle condition corresponding to an increase in epibiont species diversity and abundance (Nolte et al. 2020). Given that female turtles invest substantial energy in reproduction, including nesting and egg-laying, they may undergo fluctuations in body condition that could impact their epibiont communities. In summary, the variations in epibiont communities between male and female sea turtles can be ascribed to a combination of factors encompassing habitat utilization, feeding behaviour, nesting practices, and physical condition. Female turtles, with their distinctive reproductive behaviours, may accommodate a wider range of epibionts in comparison to male turtles.

In the present study, the highest percentage to epibionts were recorded in the groin (40%) followed by head and neck region (35%) and carapace (15%). We found that the species richness is highest in neck (11) followed by carapace (03) and groin (03). We also found there is little difference in community composition of epibionts in male and female turtles which may be because of the mating activity. During mating, some epibionts may have dislodged from carapace of the female as a result of the mounting of male. Similarly, the cloacal region of the female gets affected by the mating process, which might have damaged the epibionts on the cloaca. As we found the diversity of epibionts was low in females, which led to higher dominance value. These discrepancies in epibiont settlement patterns among sea turtle species could be influenced by the habitat preferences and behaviours exhibited by each host, potentially exposing them to larval epibionts from diverse habitats (Robinson et al. 2019).

The present study forms a baseline data on the epibiont communities found on the body surface of the olive ridley sea turtles along the coasts of Odisha, India. Documentation of the richness of marine turtle epibionts is of much interest for invertebrate and sea turtle scientists in order to figure out how sea turtles interact with the enormous species of marine animals that reside on turtle carapaces, plastrons, and soft tissues. This precedent data on turtle epibionts might be highly beneficial for future directions in applying suitable management strategies and making effective conservation choices for these vulnerable species as companion research.

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Data availability The data availability statement is not required.

Declarations

Conflict of interest The authors declare no conflict of interest.

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