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Notes on Epibionts and Ectoparasites of marine turtles on the coast of Brazil

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Abstract: The study focused on records of epibionts and ectoparasites of marine turtles on the northeastern coast of Brazil, specifically *Chelonia mydas* and *Lepidochelys olivacea*. A total of 117 Ozobranchidae leeches were identified in 10/19 sea turtles, predominantly *Ozobranchus branchiatus*, with six specimens remaining classified as *Ozobranchus* sp. due to the absence of gill filaments for precise identification. Among the affected sea turtles, 52.6% presented leeches. Epibionts, comprising 14 specimens, included *Chelonibia testudinaria*, *Platylepas hexastylos*, and *Lepas (Lepas) anatifera*. The study also reported the first occurrence of *Ozobranchus margoi* as an ectoparasite of *L. olivacea* in Brazilian waters. While *O. branchiatus* has been extensively documented, records of *O. margoi* in Brazilian waters are limited, highlighting the need for further research in the northeast Brazil region. Additionally, the study emphasizes the importance of standardized collection methods to ensure accurate identification of parasites. The findings contribute to understanding the health threats to marine turtles and underscore the significance of ongoing conservation efforts.
Keywords: Conservation, Epibionts, Leeches, Sea Turtles

1. Introduction

Sea turtles are chelonians that have adapted to the aquatic environment, as evidenced by their development of a hydrodynamic carapace and modification of their limbs into flippers. Although cosmopolitan, these animals also migrate long distances between feeding and nesting areas. However, they also maintain a vital connection with the terrestrial environment, primarily for oviposition, which occurs once per year, especially in tropical and subtropical climates (Pritchard, 1997; Carvalho, 2022; Simantiris, 2024).

Five species occur and reproduce on the Brazilian coast, namely: green turtle (*Chelonia mydas*), loggerhead turtle (*Caretta caretta*), hawksbill turtle (*Eretmochelys imbricata*), olive ridley turtle (*Lepidochelys olivacea*), and leatherback turtle (*Dermochelys coriacea*), which face threats mainly related to anthropogenic action (Baptistotte, 1994; ICMBIO, 2022; Carvalho, 2022; IUCN, 2022). In addition to anthropogenic actions, sea turtles are susceptible to various diseases and pathogens, including infections triggered by viruses, fungi, bacteria, and parasites, which affect the health and survival of the affected specimens (Baptistotte, 2014).

Although there is extensive literature on epibionts and other associated organisms (see Robinson and Pfaller, 2022), in many situations, knowledge about the parasites affecting sea turtles is limited due to restrictions on access to relevant biological material. This reduces the availability of helpful information on the fauna of ectoparasites, endoparasites, epibionts, and other associated organisms, consequently compromising the understanding of these animals' health. Global studies reveal that the parasitic fauna of sea turtles is still not fully known, with most known species being digenetic trematodes, highlighted by a study in Brazil that reinforced this trend (Veloso Ramos *et al*., 2021).

Although having a limited amount of information, through a literature search, it is possible to perceive that Brazil stands out in research on the parasitic fauna of sea turtles, especially in the southern region, except the species: *D. coriacea*, which does not have studies of this nature in the country, with *C. mydas*, *L. olivacea*, *E. imbricata* and *C. caretta*, which presents studies more related to the presence of parasitic helminths (Werneck *et al*., 2008a, 2008b; Goldberg *et al*., 2013; Werneck *et al*., 2014; Werneck *et al*., 2015a, 2015b; Binoti *et al*., 2016; Gomes *et al*., 2017; Meira Filho *et al*., 2017; Werneck *et al*., 2019a, 2019b; Veloso Ramos *et al*., 2021; Cavaco *et al*., 2021; Gomes *et al*., 2022; Cavaco *et al*., 2023; Silva *et al*., 2023, Alves *et al*., 2025).

A study in Rio Grande do Sul analyzed commensal barnacles associated with *C. mydas* and *C. caretta*, identifying different species of these turtles (Bugoni *et al*., 2001). In northern Brazil, epibionts associated with green turtles (*C. mydas*) were investigated, identifying arthropods and leeches (Pereira *et al*., 2006). Another study in Rio Grande do Sul addressed the presence of *Ozobranchus margoi* parasitizing loggerhead turtles, highlighting the association of these leeches with tumors and fibropapillomatosis (Rodenbusch *et al*., 2012). Vanstreels *et al*. (2023) investigated the relationship between environmental factors and the manifestation of fibropapillomatosis in green turtles (*Chelonia mydas*) in Espírito Santo, Brazil, highlighting the presence of leeches on the animals as a potential factor associated with the disease. Recently, studies have shown that *Ozobranchus* spp. may vary in their ability to transmit alphaherpesvirus 5 (ChHV5), the etiological agent associated with fibropapillomatosis, to turtles (Rittenburg *et al*., 2021; Kane *et al.,* 2021; Farrell *et al*., 2021; Vanstreels *et al*., 2023). These data reinforce the importance of constant monitoring of the occurrence of these leeches on turtles.

Studying the parasitic fauna of sea turtles in Brazil is crucial for understanding the ecological interactions and their impacts on the health of these animals. Future research should address knowledge gaps, particularly in less-explored regions such as the northeastern coast, and incorporate innovative methods for parasite identification and monitoring. In this regard, the present study aimed to conduct the first survey of epibionts and ectoparasites in sea turtles on the northeastern coast of Brazil, describing their intensity and frequency to assist future research efforts.

2. Materials and Methods

The study was conducted along the coast of the Sergipe-Alagoas Basin, northeastern Brazil, spanning 254 km from Piaçabuçu, south of Alagoas state, to the city of Conde, north of Bahia state. Beach monitoring activities were systematically conducted from 2010 to 2019 through the Programa Regional de Monitoramento de Encalhes e Anormalidades (PRMEA)/Regional Stranding and Anomalies Monitoring Program (Reis *et al*., 2019). During this period, stranded dead sea turtles were rescued and referred to the Rehabilitation and Marine Fauna Depetrolization Center of the Fundação Mamíferos Aquáticos (FMA) in Aracaju/SE. The animals were found dead and underwent post-mortem evaluation (Geraci and Lounsbury, 2005; Reis *et al*., 2019). The animals were found in five locations: Estância - Abaís/SE, Aracaju/SE, Barra dos Coqueiros/SE, Conde/BA, and Mangue Seco/BA.

During the necropsy, the following information was recorded: species (Márquez, 1990), age class, sex (Wyneken, 2001), date, stranding location, and overall body condition (Poor, Fair, Good) (Thompson *et al*., 2009). During post-mortem evaluation procedures, in addition to collecting samples from internal organs, ectoparasites, when present, were collected and stored in the curation sector of the FMA.

During this period, 19 sea turtles from the northeast coast of Brazil, treated as part of the study, were sampled by convenience sampling. Epibionts and ectoparasites were collected during the analysis of these animals. The samples were preserved in 70% ethanol and kept in the curation sector of the FMA, located in Aracaju, Sergipe (-11.12005, -37.13713). The samples were sent for identification to the Universidade Federal de Sergipe, situated on the São Cristóvão campus. The epibionts and ectoparasites were placed on Petri dishes and observed under a stereomicroscope to evaluate their external characteristics.

The illustrations were made according to Alves *et al*. (2022) and assembled using GIMP 2.10.34 software. We have provided detailed illustrations to assist other workers and researchers in identifying materials similar to those reported in this study. The leeches were determined according to Davies (1978) and De León González *et al*. (2021). The epibionts were determined using the work of Lazo-Wasem *et al*. (2011). The prevalence, mean intensity, and mean abundance of infection were assessed for ectoparasite and epibiont species, as described by Bush *et al*. (1997).

The data used were derived from previously collected samples and existing databases, which contained all the necessary information for the research. All research was conducted with ethical considerations in collaboration with the FMA. The animal material related to the turtle carcasses was previously collected by the FMA, which has legal authorization SISBIO n° 21570-16 to carry out activities to collect and rescue stranded animals.

3. Results

The turtles belonged to the following species: (10) Green Turtle - *Chelonia mydas* (Linnaeus, 1758) and (8) Olive Ridley Turtle - *Lepidochelys olivacea* (Eschscholtz, 1829). One of the turtles was unidentified because it lacked a carapace.

Ectoparasites - Regarding ectoparasites, 117 leeches were collected and identified, belonging to the family Ozobranchidae: (12) *Ozobranchus margoi* (Apathy, 1890) and (99) *Ozobranchus branchiatus* (Menzies, 1791). Six leeches remained classified as *Ozobranchus* sp., as they lacked gill filaments, which made it challenging to identify these specimens. The intensity of *O. branchiatus* was higher. It was observed that both *C. mydas* and *L. olivacea* were parasitized by both leech species, although coinfection by an individual was not observed. Among the affected sea turtles, 52.6% presented leeches. Epibionts - 14 epibionts were also collected and identified as belonging to three species: (4) *Chelonibia testudinaria*, (5) *Platylepas hexastylos*, and (5) *Lepas* (*Lepas*) *anatifera*. Among the sampled turtles, 31.5% presented epibionts. Prevalence information is depicted in Fig. 1.

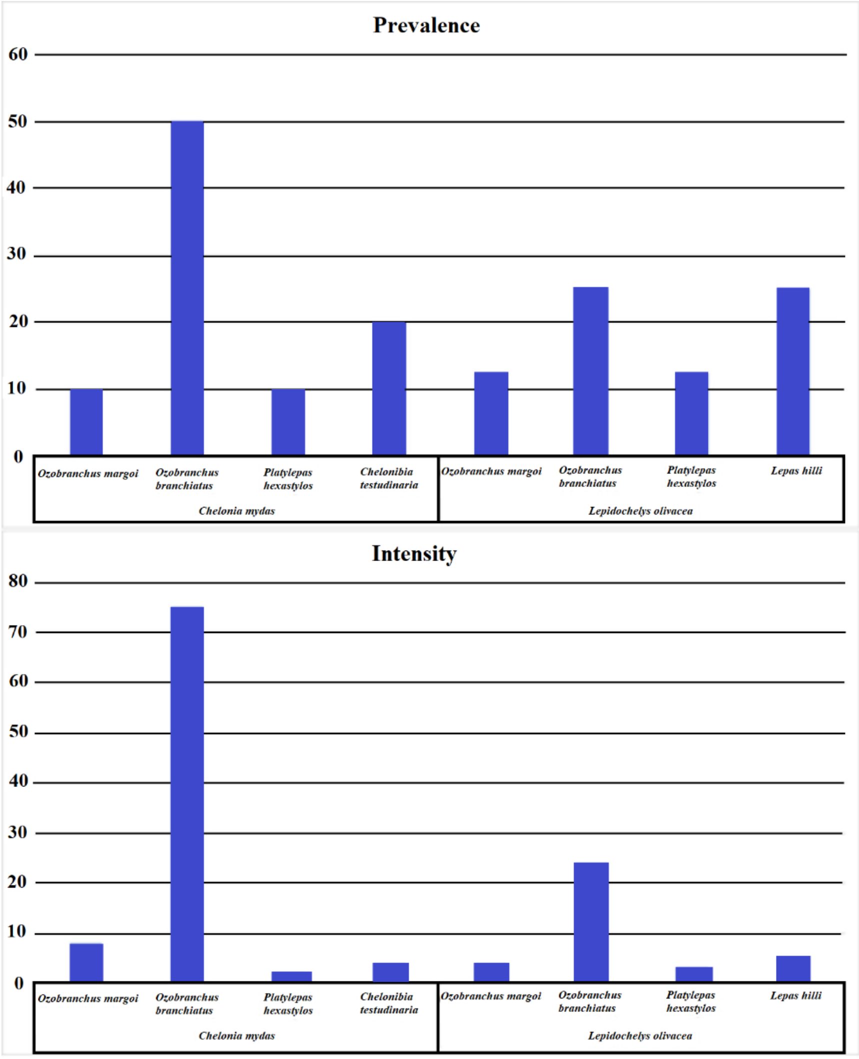

Figure 1 - Prevalence of ectoparasites and epibionts in sea turtles attended on the Brazilian coast.

The classification and morphological description of the findings, along with their respective prevalence and intensity indices, are organized and presented below. Illustrations were created to assist other researchers with similar findings.

Phylum Annelida
Order Rhynchobdellida
Suborder Oceanobdelliformes Tessler & de Carle, 2018
Family Ozobranchidae Pinto, 1921
Genus *Ozobranchus* Quatrefages, 1852
Species *Ozobranchus margoi* (Apáthy, 1890) (Fig. 2).

Morphology: Based on six specimens. Conspicuous body, divided into a narrow trachelosome and a broad urosome. Specimen length averaging up to 10.5mm. Body pigmentation is faint and milky, without spots or markings on the body. Tubercles absent. The oral sucker is tiny, not more expansive than the width of the trachelosome. One pair of ocular spots on the oral sucker. The caudal sucker is large, almost as wide as the maximum body width. Five pairs of gills with branched and digitiform lateral projections, one pair per segment (Figure 2a, b). The first pair is more anterior, prominent, developed, and complex than the other pairs, which are progressively smaller and less complex towards the posterior end of the body. The two or three anterior pairs of gills are typically bifurcated. None of the specimens had the proboscis externalized.

Hosts, prevalence, and intensity: *Chelonia mydas* [Prevalence: 10%; mean intensity: 8.00 (±0.00); mean abundance: 0.8 (±2.52)] *Lepidochelys olivacea* [Prevalence: 12.5%; mean intensity: 4.00 (±0.00); mean abundance: 0.5 (±1.41)]

Location: Aracaju, Sergipe, Northeastern coast of Brazil.
Infection Site: Skin

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Distribution in Brazil: Varies according to the host.

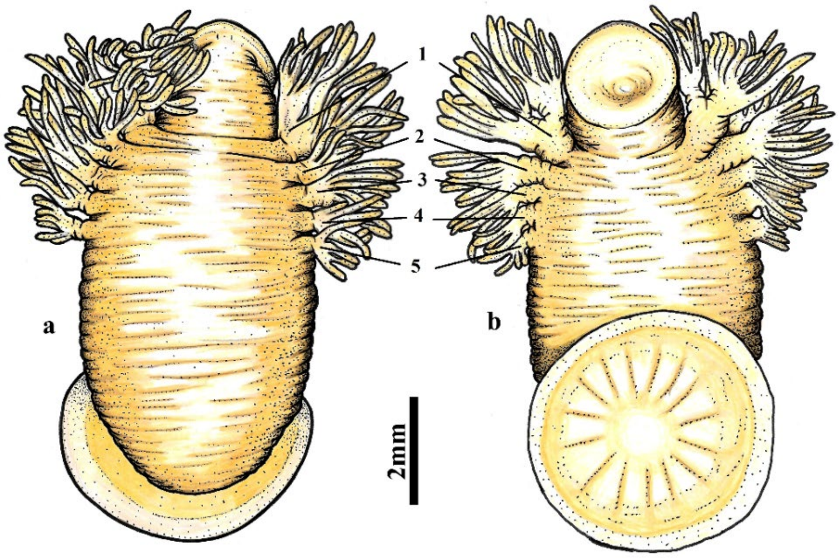

Figure 2 - *Ozobranchus margoi* collected from the skin of *Chelonia mydas* on the northeastern coast of Brazil; (a) - dorsal view and (b) - ventral view. The numbers highlight the quantity of pairs of lateral gills of the species.

Species *Ozobranchus branchiatus* (Menzies, 1791) (Fig. 3)

Morphology: Based on four specimens. Flattened body, divided into a narrow trachelosome and a broad urosome. Specimen length averaging up to 9mm. No pigmentation or milky appearance. There are no spots or markings on the body. Tubercles absent. The oral sucker is scarcely distinguishable from the neck and not wider than the width of the trachelosome. The caudal sucker is large, the same width, or slightly wider than the maximum body width. Seven pairs of gills with branched and digitiform lateral projections. The first pair, more anterior, is larger and more complex than the other pairs, which are progressively smaller towards the posterior end of the body. None of the specimens had the proboscis externalized.

Hosts: *Chelonia mydas* [Prevalence: 50%; mean intensity: 21.33 (±18.03); mean abundance: 6.4 (±13.35)]; *Lepidochelys olivacea* [Prevalence: 25%; mean intensity: 12.00 (±11.31); mean abundance: 3.0 (±7.01)]

Location: Aracaju, Sergipe, Northeastern coast of Brazil.

Infection Site: Skin

Distribution in Brazil: Varies according to the host.

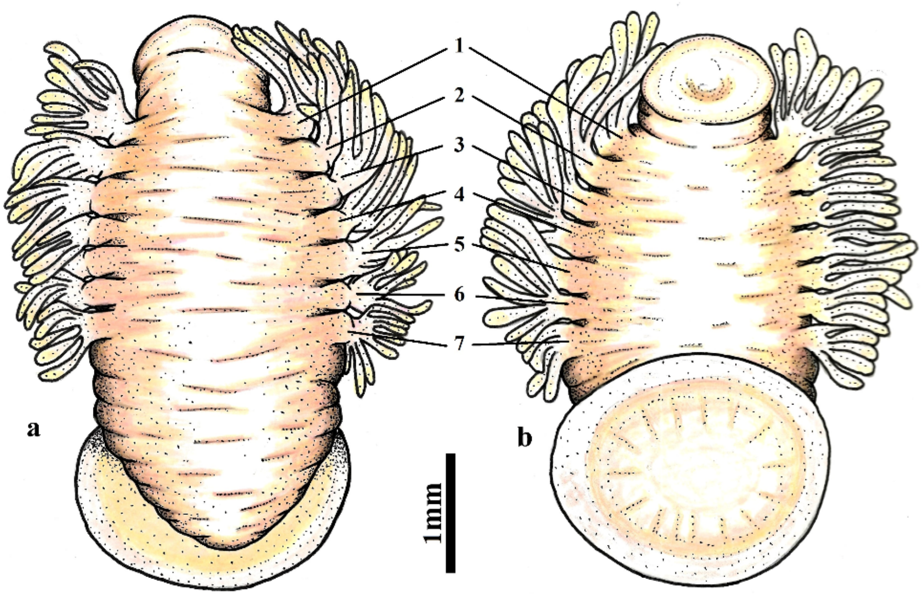

Figure 3 - *Ozobranchus branchiatus* collected from the skin of *Chelonia mydas* on the northeastern coast of Brazil; (a) - dorsal view and (b) - ventral view. The numbers highlight the quantity of pairs of lateral gills of the species.

Phylum Arthropoda
Class Thecostraca
Order Balanomorpha
Family Chelonibiidae Pilsbry, 1916
Genus *Chelonibia* Leach, 1817
Species *Chelonibia testudinaria* (Linnaeus, 1758) (Fig. 4a)

Morphology: Based on two specimens. Large and conspicuous epibiont commonly found on the carapace of marine turtles. It is easily recognizable due to its size, dome-shaped appearance, and conspicuous star-shaped rays on the characteristic shell.

Substrate: *Chelonia mydas* [Prevalence: 20%; mean intensity: 2.00 (±0.00); mean abundance: 0.4 (±0.84)]

Location: Aracaju, Sergipe, Northeastern coast of Brazil.

Attachment site: Carapace.

Distribution in Brazil: Throughout the Brazilian coastline.

Family Coronulidae Leach, 1817
Genus *Platylepas* Gray, 1825
Species *Platylepas hexastylos* (Fabricius, 1798) (Fig. 4b, c)

Morphology: Based on three specimens. Flattened and with distinct ridges and concentric grooves on the upper shell (Fig. 4 b). It has six visible sculpted support pillars on the underside (Fig. 4c).

Substrate: *Chelonia mydas* [Prevalence: 10%; mean intensity: 2.00 (±0.00); mean abundance: 0.2 (±0.63)]; *Lepidochelys olivacea* [Prevalence: 12.5%; mean intensity: 3.00 (±0.00); mean abundance: 0.37 (±1.06)]

Location: Aracaju, Sergipe, Northeastern coast of Brazil.

Attachment site: Carapace.

Distribution in Brazil: Throughout the Brazilian coastline.

Order Scalpellomorpha
Family Lepadidae Darwin, 1852
Genus *Lepas* Linnaeus, 1758
Species *Lepas* (*Lepas*) *anatifera* Linnaeus, 1758 (Fig. 4d)

Morphology: Based on an extensive collection of specimens. The external shell is striated with five lateral plates and has an umbonal tooth. Long cirrus extending outward from the shell along with the legs. Short peduncle (likely cut during specimen removal).

Substrate: *Lepidochelys olivacea* [Prevalence: 25%; mean intensity: 2.5 (±2.12); mean abundance: 0.62 (±1.40)]

Location: Aracaju, Sergipe, Northeastern coast of Brazil.

Attachment site: Carapace.

Distribution in Brazil: Throughout the Brazilian coastline.

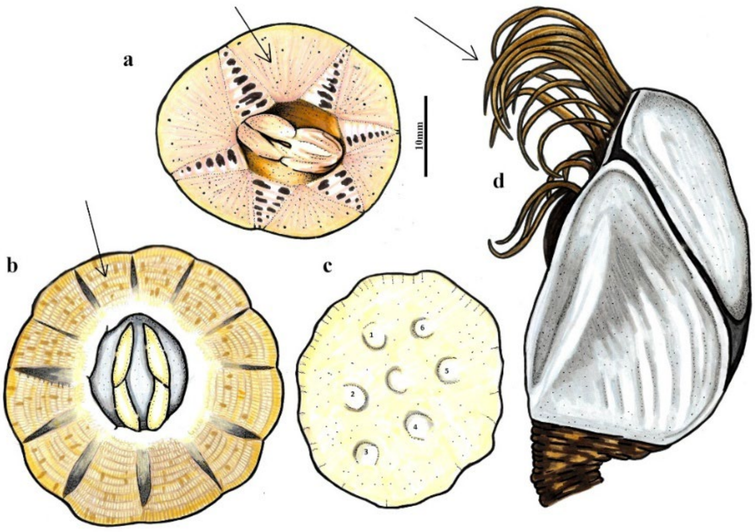

Figure 4 - Figure 4. Epibionts of sea turtles on the northeastern coast of Brazil; (a) - Dorsal view of *Chelonibia testudinaria*, collected from Chelonia mydas, arrow indicates the characteristic star-shaped rays; (b) and (c) - dorsal view, arrow indicates the concentric grooves on the upper shell, and ventral view, numbers represent the support pillars of *Platylepas hexastylos*, respectively; (d) - lateral view of *Lepas anatifera*, arrow indicates the long cirrus externalized from the shell.

Three of the 19 animal samples collected for the study contained 19 lice, including one *L. olivacea*, one *C. mydas*, and one unidentified carcass (due to the absence of the carapace), whose species was not identified.

4. Discussion

Ectoparasites and Epibionts: The documentation of the biodiversity of epibionts and ectoparasites of sea turtles has gained considerable attention, reflecting a growing interest in understanding the complex interaction between these aquatic reptiles and the vast diversity of marine organisms that colonize their carapaces, plastrons, and soft tissues (Lazo-Wasem *et al*., 2011). Since the presence of these organisms is closely linked to the body condition and health of turtles, factors such as stress, predation, and diseases can result in variations in the composition of this fauna, indicating reduced mobility, fragility of the animal, and potential interference with the normal development of the carapace in younger individuals (Alves, 2016; Basílio *et al*., 2020).

The family Ozobranchidae comprises leeches found in both marine and freshwater environments, which are known to parasitize aquatic chelonians, crocodilians, cetaceans, and occasionally some seabirds (Richardson, 1969). Currently, six species are recognized: *Bogabdella diversa* Richardson, 1969; *Ozobranchus branchiatus* (Menzies, 1791); *Ozobranchus jantseanus* Oka, 1912; *Ozobranchus margoi* (Apáthy, 1890); *Ozobranchus papillatus* Kaburaki, 1921; and *Ozobranchus polybranchus* Sanjeeva Raj, 1951. Among these, only *O. margoi* and *O. branchiatus* are marine species with a wide geographical distribution, especially in tropical regions (Tseng *et al*., 2018). Furthermore, they stand out as the most widely documented species in the literature compared to those of the family occurring in freshwater environments, which have a more limited distribution. The distribution of *O. margoi* and *O. branchiatus* is directly related to the dispersal and migratory habits of their hosts, sea turtles, which occupy a diversity of niches and undergo periodic migrations from feeding habitats to breeding habitats (Bowen and Karl, 2007; Cheng-Tsung and I-Jiunn, 2013). However, despite being known species, there are few records of their occurrence along the Brazilian coastline (Fig. 5).

Figure 5 - Figure 5. The map shows the regions where studies on ectoparasites and epibionts of sea turtles have been conducted along the Brazilian coastline so far, including the presence of *Ozobranchus* spp. The dark green, yellow, and blue dots mark regions that previously recorded ectoparasites and epibionts in *Chelonia mydas* and *C. caretta*. The light green marks the new location for recording in *C. mydas* and *L. olivacea*.

Greenblatt *et al*. (2004) investigated the relationship of these leeches with the spread of herpesvirus associated with fibropapillomatosis in Hawaiian green turtles (*C. mydas*). Fibropapillomatosis (FP) is a pathology that affects turtles, causing the growth of tumor masses on the skin, shell, and internal organs, impacting the locomotion and survival of the animals (Garcês and Pires, 2022).

The molecular results of Greenblatt *et al*. (2004) indicated that leeches could act as mechanical vectors by transporting Herpesvirus type 5 (ChHV 5) between turtles, as they frequently feed on tumors. Santoro and Mattiucci (2009) complemented this research by reporting 34 species of parasites in sea turtles in Costa Rica, including the leech *O. branchiatus*. Parasitic diversity in this region may be higher than reported due to low host sampling. Recently, *Ozobranchus* spp. removed from healthy sea turtles did

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not test positively for the presence of the ChHV5 virus (Farrel *et al*., 2021). In this regard, the transmission mode of the virus in wild sea turtles remains unknown (Dujon *et al*., 2021).

The presence of Ozobranchidae in *L. olivacea* was mainly associated with *O. branchiatus*, which has been extensively documented over the years, especially in Mexico (Frazier, 1989; Angulo *et al*., 2007; Lazo-Wasem *et al*., 2011; Sosa *et al*., 2012). Although authors such as Hernández-Vásquez and Valadez-González (1998) have reported the presence of *Ozobranchus* sp. in *L. olivacea* in Mexico, the lack of species specification keeps this finding uncertain. The present study identified *O. branchiatus* in both *L. olivacea* and *C. mydas*, which has recently been reported spanning Mexico, Australia, and New Zealand (Burreson, 2020; Bahena *et al*., 2020; Ramos-Rivera *et al*., 2021). This pattern highlights the dispersal capability of these leeches through their host turtles' migrations. Interestingly, these studies observed the coexistence of the barnacle *C. testudinaria* with *O. branchiatus* in practically all records. This suggests a possible relationship worthy of analysis in future research with more individuals being investigated.

In Brazil, Pereira *et al*. (2006) contributed to understanding epibionts associated with green turtles in the northern region, identifying arthropods and annelids, such as *O. branchiatus* and the barnacle *C. testudinaria*. However, the precise identification of this leech was also uncertain for the authors, highlighting the need for a more careful analysis of this material. The barnacle *P. hexastylos,* identified in *C. mydas* and *L. olivacea*, and the barnacle *L. anatifera,* identified in *L. olivacea*, comprise the first findings in these two turtles in Brazilian waters.

Melo *et al*. (2020) described the structure of fibropapillomas in *C. mydas*, reporting the presence of *Ozobranchus* spp. in the pathology, which connects to the previous work of Greenblatt *et al*. (2004). Zamana *et al*. (2017) explored the possible association of other epibionts, such as barnacles, with fibropapillomatosis in green turtles, indicating the lack of specific studies on this relationship and emphasizing the need for future research. These studies together provide a comprehensive view of the health threats to sea turtles, including parasites and the spread of ChHV5, factors that can impact essential conservation procedures for these animals. Although typical, leeches pose a risk factor as they can cause direct damage and act as vectors for infectious agents, thereby interfering with turtle population numbers (Rodenbusch *et al*., 2012).

Rodenbusch *et al*. (2012) investigated the presence of *O. margoi* parasitizing loggerhead turtles (*C. caretta*) in Rio Grande do Sul, Brazil, highlighting the potential impact of parasitic diseases on the health of these animals, as these leeches are considered possible vectors of ChHV5, reinforcing the relevance of studies for professionals from various fields. The species *O. margoi* mentioned in this study was previously recorded in *L. olivacea* along the Pacific coast of Costa Rica (Majewska *et al*., 2015). Therefore, this study presents the first documented occurrence of *O. margoi* in *L. olivacea* on the Brazilian coast. Although the presence of this leech is familiar, the data show that its incidence in Brazilian waters appears to be less frequent compared to its counterpart, *O. branchiatus*. Additional studies in various regions are needed to map the distribution along the Brazilian coast accurately.

Six leeches from *L. olivacea* were identified only to the genus *Ozobranchus* due to the lack of gill filaments in the specimens for a more precise diagnosis. This may have occurred due to handling during specimen collection, reinforcing the need for standardization in collecting these organisms from their hosts. Lice on sea turtles are uncommon; however, this finding was not considered a case of ectoparasite infection, as there is suspicion that these particular carcasses may have interacted with other animals while exposed, thus contracting these lice. Our study reports the first record of *O. margoi* and *O. branchiatus* in stranded turtles (*C. mydas* and *L. olivacea*) in northeastern Brazil. This is also the first report of *O. margoi* as an ectoparasite of *L. olivacea* in Brazilian jurisdictional waters.

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