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BARNACLES CLASSIFICATION OF SEA TURTLES IN BAJA CALIFORNIA SUR (MEXICO)

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1. ABSTRACT

The assemblage study of epibiont fauna and flora on sea turtles, specially, ciripeds recruitment, is yet not studied enought. This work proposes to identify and determine the anatomical distribution, incidence and frequency of presentation of the different species of cirripeds in *Chelonia mydas* and *Caretta caretta* sea turtles in the Peninsula of Southern California, Mexico. During 2017, seven field trips were carried out in the Ojo de Liebre and Guerrero Negro lagoons and in the Gulf of Ulloa. In these monitoring a total of 127 sea turtles were captured, 117 *C. mydas*, 9 *C. caretta* and 1 *L. olivacea* (without cirripeds). A total of 1,064 cirripeds were collected from the captured sea turtles. The collected cirripeds were identified into six different species: *Chelonibia testudinaria*, *Platylepas hexastylos*, *Stephanolepas muricata*, *Stephanolepas praegustator*, *Lepas anatifera* and *Balanus trigonus*. Then, the incidence and anatomical frequency of presentation of the six species of studied barnacles was done. Thus, the anatomical distribution, incidence and frequency baseline of cirripeds presentation on three important feeding areas for *C. mydas* and *C. caretta* sea turtles was generated. This baseline information will help future national and international studies, using it as a reference.

2. INTRODUCTION

There are seven species of sea turtles in the world, they belong to the Testudines Order and both Cheloniidae and Dermochelyidae families. Within the Cheloniidae family are the *Chelonia mydas* (Eastern pacific green turtle), *Caretta caretta* (loggerhead turtle), *Eretmochelys imbricata* (hawksbill turtle), *Lepidochelys olivacea* (olive ridley turtle), *Lepidochelys kempii* (kemp’s Ridley turtle) and *Natator depressus* (flatback turtle); and in Dermochelyidae family, the leatherback turtle, *Dermochelys coriacea*. In Mexico six of the seven species of sea turtles are found and in Baja California Sur five: *C. mydas*, *C. caretta*, *E. imbricata*, *L. olivacea* and *D. coriacea* (Márquez, 1996).

2.1 Sea turtles morphology

Sea turtles have a carapace that protects their body. This carapace is attached to the spine by a dorsal part (Márquez, 1996). The Chelonidae family sea turtles bones are covered with calcified dermal tissue that form keratin scutes or scales that depending on the species are different in size and number. This characteristic, allows to differentiate the species from each other (Ripple, 1996, Wynejen, 2001). The marginal, lateral or costal, central or vertebral, nuchal and inframarginal scutes are used for the species identification.Scutes of the head are also used for the differentiation of species, being the prefrontals the most used (Wyneken, 2001) (see annex 1).

2. 2 Epibionts

There is a wide variety of organisms that can colonize an emeriging superface in marine ecosystems (Schämer, 2005). These organisms are called epibiosis if they are placed on a living organism and biofouling if their disposition is on an artificial surface with an anthropogenic origin (Wahl, 1989).

An epibiont or barnacle is known as the organism that grows and lives attached to another; however, a basibiont is the organism that acts as host or substrate (Wahl, 1989). Sea turtles constitute a substrate for a large variety of organisms and provide a shelter or protection against predation (Epilion, 1986, Young, 1986, Frick & Pfaller, 2013).

It is common to find epibiont fauna and flora on sea turtles, such as cirripeds, macroalgae, bryozoans, cnidarians, polychaetes, amphipods, etc. (Frazier et al., 1985; Caine, 1986; Gramentz, 1988, Frick & Pfaller, 2013). Within this group of epibionts, cirripeds are the most abundant (Caine, 1986, Kitsos et al., 2005) and are considered the first ones to colonize as well as provide refuge and substrate for other organisms (Frick et al., 2002, Frick & Pfaller, 2013)

2.3 Cirripeds life cycle

The complete cirripeds life cycles are only known in a few cirriped species, and there are few studies of them (Moyse, 1961, Molenock and Gomez, 1972, Lang, 1979). In cirripeds the larval development comprises phases of larval development combining nektonic and planktonic stages. First, six nauplii stages which feed and develop successively and allowe larva development changing their exoskeletons (Pochai, et al., 2017). Finally, the last cyprid stage that it does not feed and its goal its specialize in the substrate selection and in the posterior fixation (Zardus & Hadfield, 2004 a). (Fig. 1).

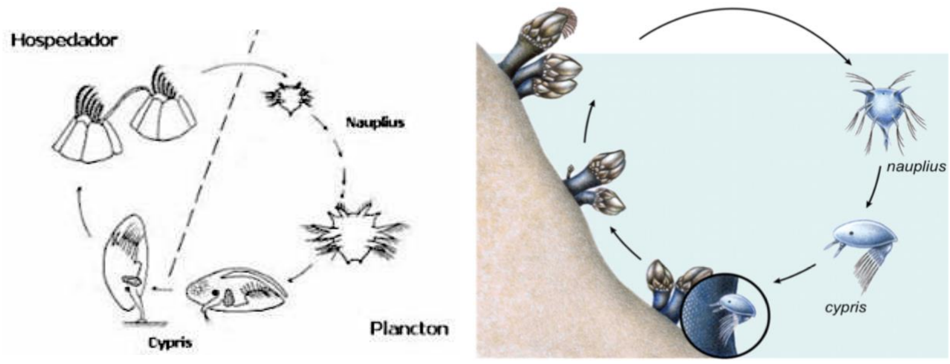

Figure 1. Generalized life cycle of a cirriped. (Modified from Spivak, 2005) (Left). Pedunculated cirriped life cycle, *Pollicipes pollicipes* barnacle. Drawing by Telma Costa. (Cruz, et al 2015) (Right).

2.4 Objetives

- **General objetive:**

Identify and classify the different species of barnacles on sea turtles of Ojo de Liebre and Guerrero Negro lagoons and the Gulf of Ulloa.

- **Specific objetives:**

Determine the highest incidence and frequency cirriped species on sea turtles.

Determine the highest barnacle incidence anatomical area of sea turtles.

Compare the number of barnacles between turtle species and determine the anatomical area that has the highest incidence on the turtles species.

Determine the possible reasons for the above objectives.

3. DATA AND METHODS

3.1 Geographic situation

The field work was carried out during the months of May to September 2017 in:

1) Gulf of Ulloa (GU) which is located approximately between 25º and 27º of North latitude and between 112º and 114º of West longitude, from the south of Punta Abreojos to Cabo San Lázaro.

2) Ojo de Liebre lagoon (OLL).

3) Guerrero Negro lagoon (GNL). These lagoons are a part of the Biosphere Reserve of "El Vizcaino" which extends between the 27 ° 35'W - 27 ° 55'N and 113 ° 58'W - 114 ° 10'W 27 ° 35 'N and 27 ° 55 'N; 113 ° 58 'W and 114 ° 10' W geographical coordinates in the state of Baja California Sur (Mexico). (Fig. 2).

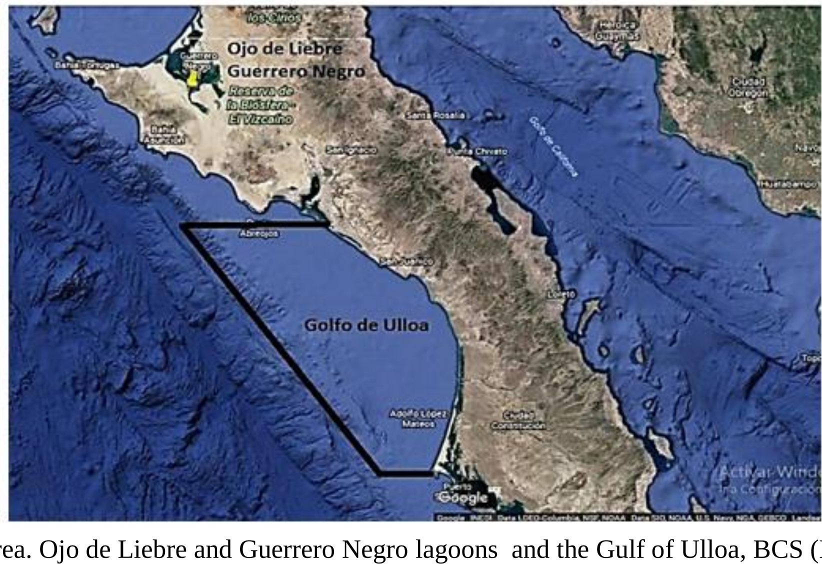

Figure 2. Study area. Ojo de Liebre and Guerrero Negro lagoons and the Gulf of Ulloa, BCS (Mexico).

3.2 Method of capture

A total of 127 sea turtles were captured. In the Ojo de Liebre and Guerrero Negro lagoons 117 *Chelonia mydas* were captured and in the Gulf of Ulloa, 9 *Caretta caretta* and 1 *Lepidochelys olivacea*.

On the one hand, in OLL and GNL, the turtles were captured with “Castillo nets" (100 m long, 5 m deep on sea turtle flow channels. Moreover, another capture technique was used. This other technique consits in enclosing the turtle using monifilament nets of 150 m length by 6 depth.

On the other hand, in GU turtles were captured by rodeo technique adapting the technique of Limpus (1971, 1978 and 1980).

After the turtle was captured, morphometric measurements of each individual were recorded following the Bolten methodology (1999): curved carapace length (CCL), curved carapace width (CCW), straight carapace length (SCL), straight carapace width (SCW), body depth (BD), plastron length (PL), total tail length (TTL) and weight. Then, according to the Balazs methodology of (2000), the turtles were marked on the rear flippers with monel / inconel plates.

3.3 Samples collection

To determine the presence of barnacles, a quick and detailed visual inspection was carried out. The samples collection was done following these steps:

a) First, the turtle was placed in a dorsal position. Then, in a systematic order and with a cranio-caudal and dorsal-ventral orientation, the presence of barnacles on the skin, head, neck, front fins, carapace, rear fins and tail was observed. After that, it was carried out the same process in a ventral position.
b) Once the presence of balanos was identified, the anatomical area was recorded and photographs were taken.
c) Then, in the anatomical area that the barnacle was found, pictures were taken. It is important to account the disposition of the cirriped, that is, if the organism is alone or forming aggregations.
d) After the pictures were taken, the anatomical area where the organism was found was registered and named (for example, central scute 1)
e) Finally, depending on the species, barnacle size and the anatomical area the cirriped was pulled of from the turtle using metal tweezers or spatula (Fig. 13).

Afterwards, in 500 ml plastic flasks, the collected organisms were kept and fixed with 70% ethanol immersion. After the process all the animals were released and all of the samples were taken to the Oceanography Laboratory in the Autonomous University of Baja California Sur (UABCS). There, one by one, the samples were cleaned, measured and pictures of them were taken. The samples were classified and identified at the lowest possible taxonomic level by Monroe and Limpus (1979), Badillo (2007), Frick et al. (2010, 2011).

4. RESULTS

According to their CCL, *C.mydas* captured in OOL, GNL and GU, were: juveniles (30 to 50 cm), subadults (51 to 77 cm) and adults (>77 cm). In GU there were also captured *9 C. caretta*, 4 subadults and 5 adults (<90 cm) as well as an adult *L. olivácea* (<65 cm) (Peckham et al. 2007).

Table 1. Number of sea turtle captured in the three diferent areas attending to the

Monitoring (duration)	Specie	Number of juveniles	Number of subadults	Number of adults	Total number of turtles
OLL (may-sep)	*C.mydas*	2	61	35	98
GNL (ago & sep)	*C.mydas*	2	15	25	19
GU (ago)	*C. caretta*	-	4		9
GU (ago)	*L. olivacea*	-	-	1	1

From the 127 sea turtles, the colected barnacles were from 49 C. mydas, from OLL and GNL and 9 *C. caretta* from the GU (the olive ridley turtle captured did not present barnacles). In total, 1,064 samples of ciripeds were obtained of 6 species and 5 different genus (Table 2).

Table 2. Found and collected cirripeds species in Eastern Pacific green turtles (*C. mydas*) in OLL and GNL (May-Sep 2017) and in loggerhead turtles (*C.caretta*) in GU (August 2017) (n = 57 turtles)

Specie	*C. mydas*	%	Turtles (%)	*C. caretta*	%	Turtles (%)
	N=48			N=9		
CRUSTACEA CIRRIPEDIA	369	(47.3%)	34 (70.8%)	144	(50.5%)	9 (100%)
Chelonibia testudinaria	340	(43.6%)	19 (39.5%)	7	(2.5%)	2 (22.2%)
Platylepas hexastylos	52	(6.6%)	14 (29.1%)	120	(42.1%)	9 (100%)
Stephanolepas muricata	18	(2.3%)	3 (6.25%)	3	(1.05%)	2 (22.2%)
Stephanolepas praegustator				6	(2.1%)	1 (11.1%)
Balanus trigonus				5	(1.75%)	1 (11.1%)

Total	779			285		

The most abundant cirriped was ***Chelonibia testudinaria*** (Linnaeus, 1758) (Fig. 3). This barnacle was present in every turtles and was the most abundant on the carapace (Fig. 4A), followed by the plastron (Fig. 4B), skin, flippers (anterior and rear) (Fig. 4C) and head (Fig. 4D). Furthermore, in some organisms was also found the presence of complementary males (Zardus, & Hadfield, 2004). (Fig. 5).

Figure 3*: C.testudinaria* specimens after clean them. Dorsal view (left), ventral view (center) and front view (right).

Figure 4. *C.testudinaria.* A) Location on the carapace, plastron (B), flippers(C) and head (D).

Figure 5. *C. testudinaria* on the rhamphotheca of C. mydas (left). Complementary males presence on a *C. testudinaria* specimen (red arrows) (right).

The second most abundant cirriped was was ***Platylepas hexastylos*** (Fabricius, 1798) (Fig.6). It was found in the hard parts of the turtle (carapace and plastron) (Fig. 7 and 8) as well as in soft parts (skin and flippers).

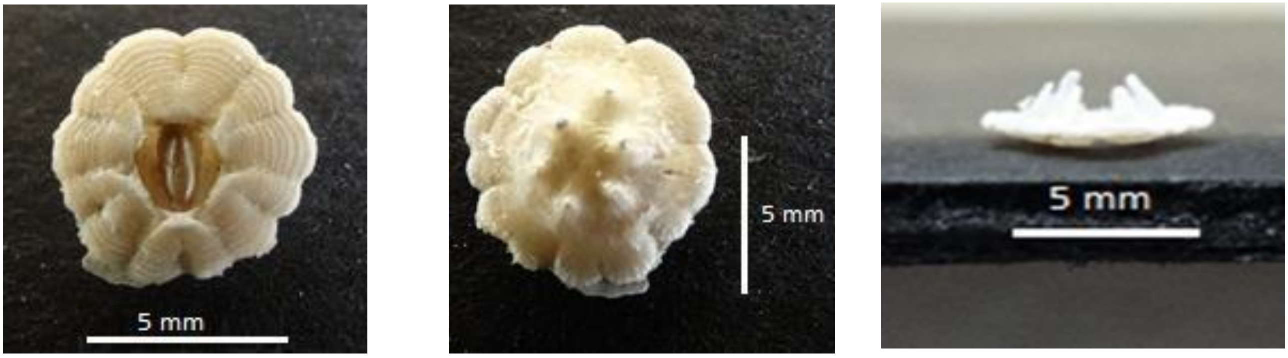

Figure 6*:* *P.hexastylos* after cleaning them. Dorsal view (left), ventral view (center) and front view (right).

Figura 7. *P*. *hexastylos* on intermarginal scutes (left). Injury caused by this barnacle (right).

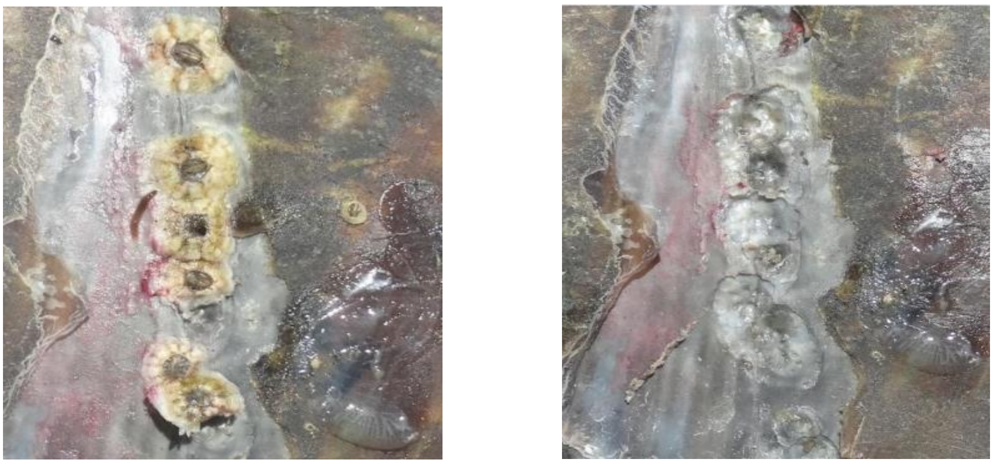

Figura 8. *P.hexastylos* on the carapace (left). Injury caused by this barnacle on the turtle scute (right).

Another classified barnacle was ***Stephanolepas muricata*** (Fischer, 1886) (Fig. 9).

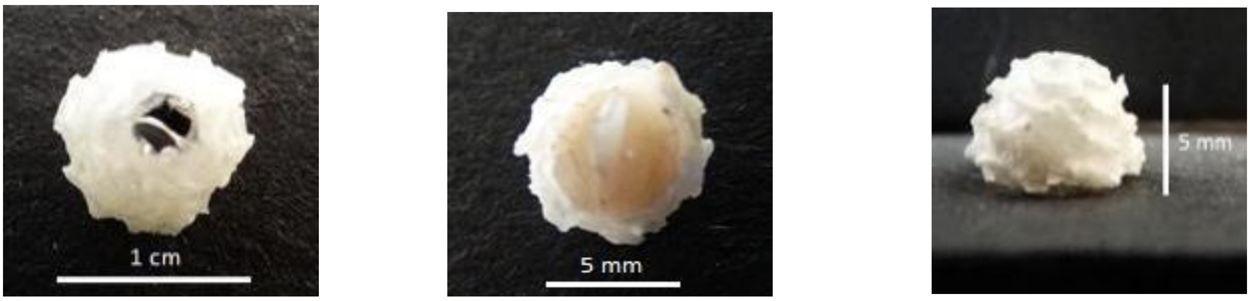

Figure 9. *S, muricata* specimens. Dorsal view (left), ventral view (center) and front view (right).

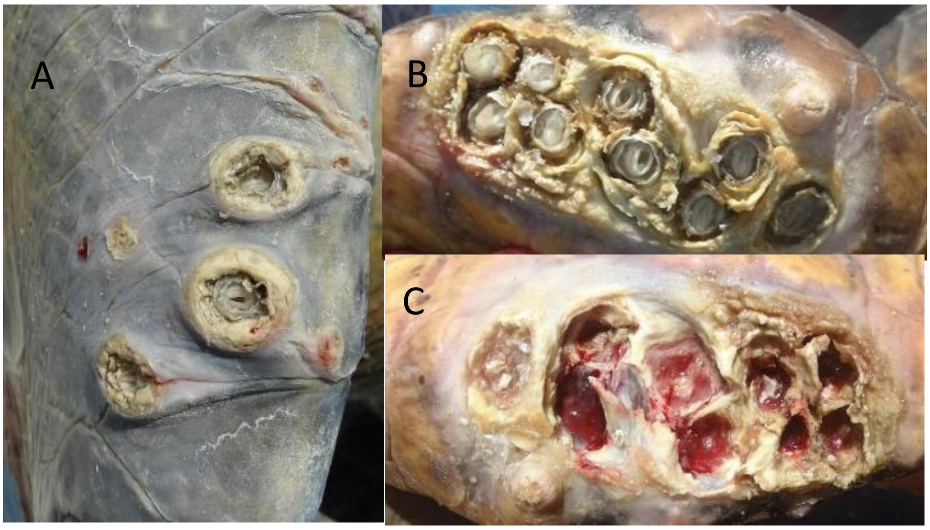

Figure 10. *S. muricata* on the anterior flipper of a loggerhead turtle (A). *S.muricata* aggregations on a flipper of a Eastern Pacific green turtle (B). Injuries caused on the skin (C).

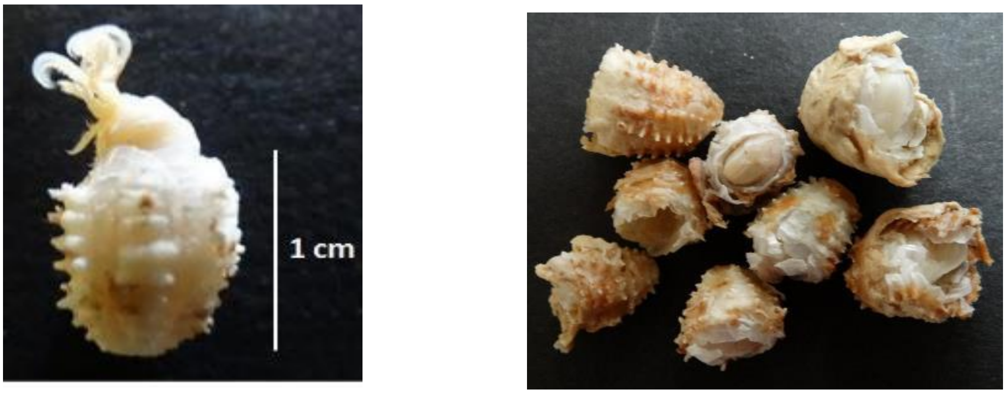

Figure 11. *S. muricata* specimens

Another identified barnacle of the Stephanolepas genus was ***Stephanolepas praegustator*** (Pilsbry, 1910) (Fig. 12). This banacle was found on a loggerhead turtle specimen on the area of the skin, the neck area and the tongue, near to the throat (Fig. 13).

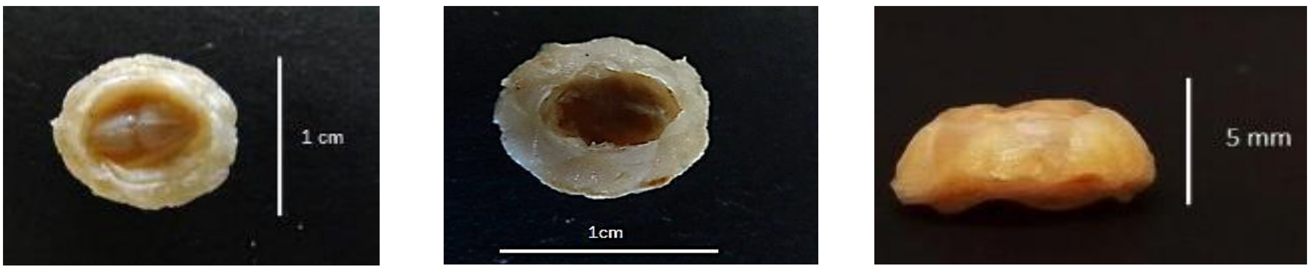

Figure 12. *S.praegustator* specimens. Dorsal view (left), ventral view (center) and front view (right).

Figure 13. *S.praegustator* on the loggerhead turtle dorsal and lateral area of the neck (left). *S.praegustator* specimens fallen off after their collection (center). *Stephanolepas praegustator* on the tongue, near to the throat, of a loggerhead turtle specimen from the Gulf of Ulloa (right).

Figure 14. *S.praegustator* specimens on the soft zone between the head and the anterior right flipper (left) of a Eastern Pacific green turtle. *S.praegustator* on the ventral area of the anterior right flipper of a Eastern pacific green turtle (right).

Lepas anatifera (Clark et al., 1975) (Fig. 15) order Pedunculata (Lamarck, 1818), was identified. *L. anatifera* was found on the loggerhead turtle *C. testudinaria* shell and on the loggerhead turtle right rear flipper (Fig. 16).

Figura 15. *L.anatifera* specimens after cleaning them. Dorsal view (left), ventral view (center) and front view (right).

Figura 16. *L.anatifera* specimens on the loggerhead turtle right rear flipper (left). *L. anatifera* specimens on the loggerhead turtle *C. testudinaria* shell on the carapace and plastron (center and right).

To conclude, the last identified barncle was ***Balanus trigonus***. This barnacle was found on the loggerhead turtle carapace.

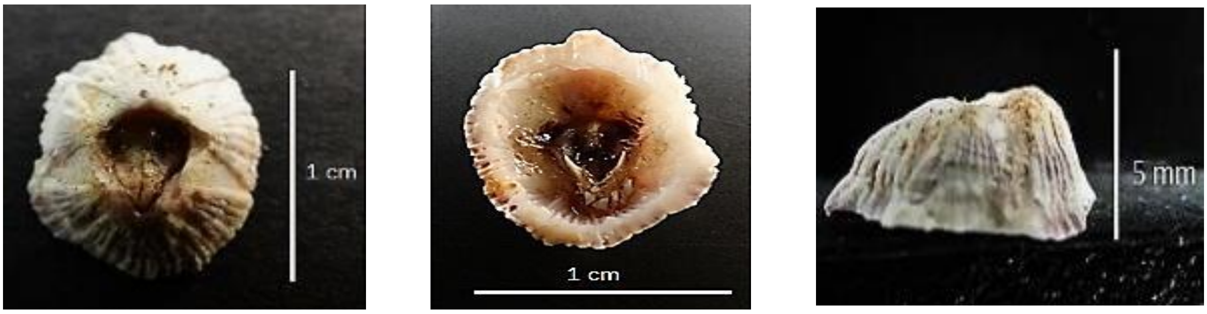

Figura 17. B.trigonus specimens after cleaning them. Dorsal view (left), ventral view (center) and front view (right).

Table 3 shows the *C. testudinaria* and *S.muricata* size and weight from the GU loggerhead turtles. The rest of the barnacles species were not abundant enought and theie size and weight could not be measured.

Table 3. *C. testudinaria* and *S. muricata* values of *C. caretta*.

Cirriped specie	Large (cm). Median and E.D.	Intervale	Width (cm). Median and E.D.	Intervale	Weight (g). Median and E.D.	Intervale
C. testudinaria	4.85 ± 1.79	0.7-8.35	4 ± 1.45	0.7-6.5	23.04 ± 19.76	0.9-70
S.muricata	1.17 ± 1.41	0.6-6.15	1.34 ±1.08	0.75-5.3	3.38 ± 8.66	0.8-19.5

Table 4 shows the *C. testudinaria*, *S.muricata* and *P.hexastylos* size and weight from the OLL and GNL Eastern Pacific green turtles. The rest of the barnacles species were not abundant enought and theie size and weight could not be measured.

Table 4. *C. testudinaria*, *S. muricata* and *P. hexastylos* values of *C. mydas*.

Cirriped specie	Large (cm). Median and E.D,	Intervale	Width (cm). Median and E.D.	Intervale	Weight (g). Median and E.D.	Intervale
C. testudinaria	3.25 ± 1.4	0.9-4.65	2.86 ± 1.23	0.9-4.8	7.69 ± 6.82	0.7-29.4
S.muricata	0.45 ± 0.49	0.1- 0.6	0.37 ± 0.42	0.1-2.5	0.6 ± 0.33	0.1-1
P.hexastylos	1.01 ± 1.26	0.3-1.25	0.83 ± 0.64	0.5-1.1	4.22 ± 10.25	0.3-29.6

3. DISCUSSION.

The main characteristic of sea turtles life cycle is the fact that in their juvenile, subadult and adult stages (not including nesting) they are far from their birth place. Depending on the species, during these different stages they travel very long distances through different seas and oceans, so in these moments a different fauna and flora epibiont assemblage will be produced. *C.caretta* of GU are turtles that were born on Japan and travelled through the Pacific Ocean in their migration route. It is believed that the juvenile *C. mydas* that arrive at OLL and GNL are coming from long migratory journeys, as Seminoff et al. (2003) reported (Michoacan, Galapagos Islands, Revillagigedo Islands...). In addition, it supons that the subadult and adults specimens that spend around 15 to 20 years and 20 to 40 years in these lagoons could also come from other places. Thus, the difference in number and diversity of barnacle species between the GU *C. caretta* turtles and the LOL and LGN *C. mydas* turtles can be estimated.

Most epibionts and barnacles studies are focused on *C. testudinaria* and *C. caretta* barnacle of *C. caretta*, mainly because *C.caretta* is the specie with the highest number of barnacles reported studies among the 7 species of sea turtles (Frick et al., 2000).This cirriped and the rest of identified barnacle species classified, have been previously reported in the region in which this study has been carried out (Table 5).

Table 5. Identified cirripeds species in the Eastern Pacific area.

Barnacle species	Autors	Area
C. testudinaria	Angulo-Lozano *et al.*, 2007	Sinaloa, Mexico
C. testudinaria	Henry, 1941	La Paz, Mexico
C. testudinaria	Ross and Newman, 1967	Bahia Magdalena, Mexico
C. testudinaria	Vivaldo *et al.*, 2006	Michoacan and Oaxaca, Mexico

P. hexastylos	Hernández-Vázquez and Valádez-González, 1998)	Galapagos Islands
P. hexastylos	Vivaldo *et al.*, 2006	Michoacan and Oaxaca, Mexico
S. muricata	Balazs, 1980	Galapagos Islands
L. anatifera	Monroe and Limpus, 1979	Pacific Ocean
B. trigonus	Monroe and Limpus, 1979	Pacific Ocean

Nowadays, there are no records of the *S. praegustator* presence in the Pacific Eastern, but in the Pacific Indo-West (Jones et al., 1990, Monroe and Limpus, 1979: 203) and in the Caribbean Sea and Atlantic Ocean (Lutcavage and Musick, 1985; Pilsbry, 1910; Wells, 1966; Young, 1991). Therefore, it is established that *C.mydas* hosts this cirriped (as shown in Fig. 22) which was previously reported in only 3 of the 7 sea turtles species: *C. caretta* from the Atlantic (Pilsbry, 1910; Wells, 1966) and the Pacific (Monroe and Limpus, 1979), *L. kempii* (Lutcavage and Musik, 1985) and *N. depressus* (Limpus *et al.*,1983).

<u>Cirripeds size</u>

C.testudinaria, *P. hexastylos* and *S. muricata* size are bigger in *C. caretta* than in *C. mydas* (Hayashi and Tsuji 2008, Fuller *et al.*, 2010). *C.testudinaria* of GU *C. caretta* turtles were larger (maximum basal diameter) than those present in OLL and GNL *C. mydas* turtles (Fuller *et al.*, 2010) (Table 1). This difference could be due to the difference feeding habits of the two turtles species. *C. caretta* turtles often feed on subbenthic organisms (Bjorndal 1997) that provide barnacles (indirectly) a larger number of organisms and particles to feed on (Fuller *et al.*, 2010). Green turtles are considered herbivores (Bjorndal 1980) and although this statement is not all true considering as opportunistic omnivores (Reséndiz *et al.*, 2018), this food searching pattern in coastal lagoons would cause relatively sediments disturbance, thus affecting the barnacles size. (Fuller *et al.*, 2010). An another factor that can affect the size is carapace texture which is also different between species. *C.caretta* turtles have more rugose or scaly carapace than *C.mydas*, therefore, it could facilitates the larvae colonization (Fuller *et al.*, 2010) providing a larger fixation area, which gets a more fixation secure. This will be more difficult to the barnacle cleaning during turtle self-cleaning (Heithaus *et al.*, 2002; Schofield *et al.*, 2006) or when a fish symbiotic cleaning behavior is carried out (Balazs *et al.*, 1994; Schofield *et al.*, 2006). Since there are no studies comparing *C. caretta* and *C. mydas*, *P. hexastylos* and *S. muricata* size, it is difficult to conclude why they are larger in one species than in others. Only 1 of the 9 *C.caretta* presented *P. hexastylos*, and the size of these specimens was larger than those of *C. mydas* (Tables 3 and 4).The size of *S. muricata* in *C. caretta* was much bigger than in *C. mydas* (Tables 3 and 4). The reasons for this size difference could be several and be the same as *C. testudinaria* (each turtle species feeding habits or the carapace).

Cirriped anatomic location

Each cirriped species was found on different anatomical areas. Table 6 shows barnacle species anatomical areas where they were found

Table 6. Anatomical areas of *Chelonia mydas* and *Caretta caretta* where the six species of cirripeds were found.

Specie	***Chelonia. myda*** **anatomical area**	***Caretta caretta*** **anatomical area**
C. testudinaria	Head, carapace, plastron and ramphothecha.	Head, carapace and plastron
P. hexastylos	Neck dorsal surface, carapace, plastron, anterior flippers dorsal surface and rear flippers ventral surface (left flipper).	Tail dorsal surface and neck dorsal surface.
S. muricata	Anterior flippers dorsal and ventral surface.	Anterior and rear flippers surface (dorsal and ventral)
S. praegustator	Soft area between head and right anterior flipper, right anterior flipper ventral area and right rear flipper ventral area	Neck dorsal and lateral area and mouth (tongue)
L. anatifera	No present	Right rear flipper dorsal area

Cirripeds incidence and frecuency

Cirripeds incidence (Fig. 18) and frequency (Fig. 19) of the dorsal and the ventral part were elaborated by pictures and field work recording. The 6 different barnacle species incidence, was represented in a single image. *C. testudinaria* was the most abundant cirriped in both species and according to its presentation its incidence was classified as low, moderate and high. Due to the large number of banacle species and the percentage of them, the different anatomical areas were represented in different images. As not all the ciripeds species were present in the turtles, only the most representative frequency on each turtle species was made. Thus, in *C. mydas* were represented *C. testudinaria*, *P.hexastylos* and *S.muricata* and in *C. caretta* were represented: *C.testudinaria* and *S. muricata.*

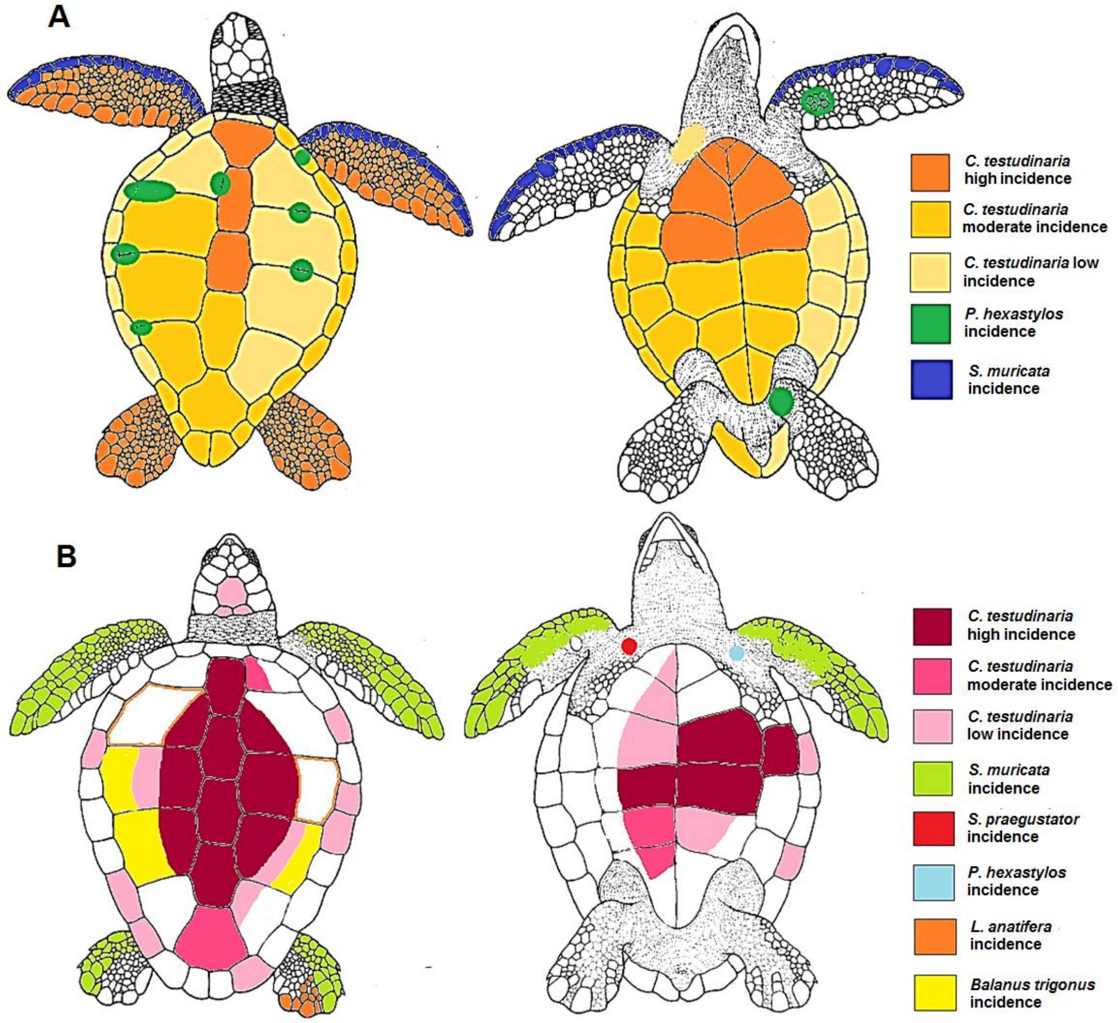

Figure 18. *Chelonibia testudinaria, Platylepas hexastylos, Stephanolepas muricata, Stephanolepas praegustator, Lepas anatifera* and *Balanus trigonus* incidence on A) *Chelonia mydas* dorsal and ventral areas and B) *Caretta Caretta* dorsal and ventral areas.

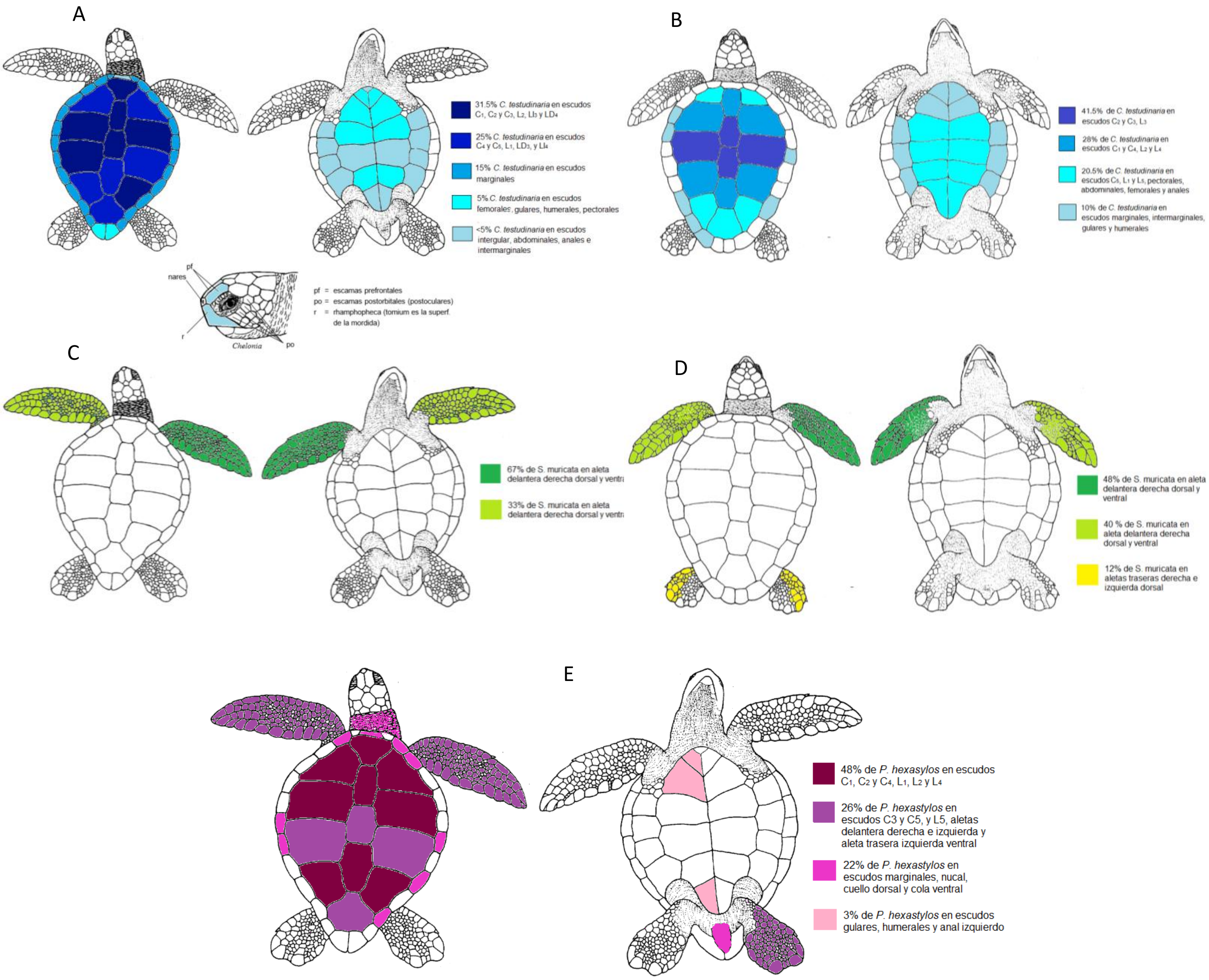

Figure 19. A) *Chelonibia testudinaria* frecuence on *Chelonia mydas* dorsal and ventral view; B) *Chelonibia testudinaria* frecuence on *Caretta caretta* dorsal and ventral view; C) *Stephanolepas muricata* frecuence on *Chelonia mydas* dorsal and ventral view; D) *Stephanolepas muricata* frecuence on *Caretta caretta* dorsal and ventral view and E) *Platylepas hexastylos* frecuence on *Chelonia mydas* dorsal and ventral view.

At the moment *only C. testudinaria* abundance studies have been carried out. Therefore, this comparative study of the present cirripeds abundance of *C.caretta* and *C.mydas* has been developed. The barnacle species incidence of the two sea turtles species was different (Fig. 22). *C.testudinaria* had the high abundance in both species. Caine (1986); Matsuura and Nakamura (1993); Frick *et al.*, (1998) and Casale (2004) mentioned this barnacle as the most abundant epibiont. However, *P. hexastylos* and *S. praegustator* incidence was higher in *C.mydas* than in *C. caretta* and *S.muricata* had a higher incidence in *C. caretta* than in *C.mydas*. *L. anatifera* and *B. trigonus* were only found in GU *C.caretta* turtles and their incidence was low.

Besides, not many studies of *C. testudinaria* frequency have been carried out. In the present study, the frequency and the anatomical area of the identified barnacles different species differ from one species to another. In *C. caretta*, *C. testudinaria* presented the highest frequency. In general, its distribution was not uniform (Matsuura and Nakamura 1993) and was higher in the carapace than in the plastron and higher in the central 2 and central 3 and lateral 3 (41.5%) scutes (Fig. 23.) The central scutes 1 and central 4 represent 28% and with less frequently (20.5%) on the central 5 and lateral 1 and 5 scutes. This shows that *C. testudinaria* is hghly present in the central and elevated parts of the carapace. This result is similar to Matsuura and Nakamura (1993) work where the *C. testudinaria* distribution was higher in the central areas compared to lateral areas and similar to Pfaller *et al.*, (2008) where *C. testudinaria* was more abundant in the carapace central areas. Kitsos *et al.*, (2005) and Frick and Slay (2000) observed that *C.testudinaria* preferred to settle in marginal scutes. In the present study, the marginal scutes represented, with the inframarginal, gular and humeral scutes of the plastron, the *C. testudinaria* lowest percentage (10%), so this is why these anatomical areas are not the suitable to the this cirriped establishment. However, the plastron rest scutes (pectoral, abdominal, femoral and anal scutes) presented double frequency (20.5%).

The non-uniform *C. testudinaria* distribution could be due to the carapace water flow patterns (Logan and Morreale, 1994). Frick and Slay 2000 mention that this barnacle is on those body parts that are more displayed to the hydrodynamic current caused by the turtle movement. Kitsos *et al.*, (2005) found *C. testudinaria* on the marginal, this could be because in that anatomical zone there are lower flow rates and so the settlement of sediments patterns and small particles would be higher (Schärer, 2007) what would be an optimal area for the this cirriped growth. Moriarty *et al.*, (2008) discovered that *C. testudinaria* is able to move after its initial settlement, to locate more desirable places for feeding, moving from a relatively low current flow pattern (marginal region) to a higher current flow (carapace anterior and central region). Other factors such as the hydrodynamics and the scute surface, turtles behaviour patterns (placement of the anterior flippers in the resting moments, scraping against hard surfaces with a deleting possible purpose or mating behavior), epibionts interactions (for instance, predation, competition) and even their desiccation tolerance when turtle floats on the surface with part of the carapace on above the water (Pfaller *et al.*, 2008) would create regions with different settlement and feeding conditions for *C. testudinaria* (Bjorndal, 2003).

Pfaller *et al.*, (2008) mencion *C. testudinaria*, could alternate water flow patterns developing micro-eddies, which could create additional favorable settlement sites for other barnacle and epibiont species. The *C. testudinaria* distribution in ventral area is higher on pectoral, abdominal, femoral and anal scutes than on the gular and humeral scutes and could be due to the previous mentioned factors. However, there are not studies about plastron as a assemblaje area in order to compare results.

Nájera-Hillman *et al.,* (2012) characterized the *C. testudinaria* distribution on *C.mydas* juveniles of Magdalena Bay, Mexico. In the present study, there is no pattern in the *C. testudinaria* spatial distribution of *C. mydas*. Nevertheless, it is presented in aggregate forms (Hayashi and Tsuji 2008; Nájera-Hillman *et al.,* 2012) and its distribution frequency is more abundant in the carapace region mostly in the central scutes 1, 2 and 3 and lateral 2 and 3 (left) and lateral 4 (right). This is similar to Nájera-Hillman *et al.,* (2012) observed, who mention that the central scutes 2 and 3 and lateral scutes 2 and 3 are the ones with the highest percentage of distribution; contrary to Fuller *et al.,* (2010) who observed that *C.mydas* centrals 1 and 2 scutes had a specimens small aggregations and that the *C. testudinaria* highest percentage was in the marginal scutes. This fact differs from the present study where the distribution frequencywas 15%. In the plastron, as happens with *C. caretta*, only one previous study has been done showing the anatomical area frequency and abundance. Nájera-Hillman *et al.,* (2012) differentiated 6 *C. mydas* ventral part zones of the anterior and lateral zones with higger frequency than those in the central and posterior areas. The captured *C.mydas* in the present study had the highest frequency also in the anterior zone of the plastron (without including the pectoral scutes). However, intermarginal scutes did not present the same frequency that Nájera-Hillman *et al.,* (2012) reported. *C. testudinaria* plastron distribution pattern has not been previously investigated. Nonethelless, *C.mydas* physical contact when they scrape sea floor while looking for seaweed and seagrass (López-Mendilaharsu *et al.,* 2005) can produce the detachment of some barnacle; especially those on the central regions of the plastron. The plastron marginal regions have less contact with the sea bottom due to their raised position. In addition, it can be assumed that turtles have to lift their necks base to feeding from the bottom, so that is why the anterior plastron region would suffer less friction and would contributing to a higger barnacles abundance (Najera-Hillman *et al.,* 2012).

P. hexastylos distribution in *C. caretta* was very scarce and only one specimen was found on the soft area between the plastron and the anterior left flipper. Chelonibia differs from Platylepas in their latter; it is smaller and has six calcareous plates instead of eight as *C. testudinaria*. Generally, they widely colonize the skin of its host and embed depeer than other barnacles, originating great lesions in turtles softest areas. *P. hexastylos*, will settle in anatomical areas where *C. testudinaria* and other cirripeds do not settle. This will allow it to find areas with less settlement competition, feeding search and reproduction.

In *C. mydas P. hexastylos* abundance was higher in the dorsal compared to the one in the ventral area which presented a low percentage. In the plastron, this barnacle was present in the gular, humeral and left anal scutess. However, the soft areas (neck and tail) and the anterior and rear flippers sheltered a significant amount of this cirriped, being the left rear flipper where they were represented a bigger number (44 individuals) and in tail (11 individuals) and neck (14 individuals) both in the dorsal and ventral area; this is why it is difficult to being able to specify if they present higher affinity for an specific area, it can be assume that this barnacle colonizes the turtle soft areas more than *C. testudinaria*. It is important to mention that central scute 1 (109 individuals) and in central scute 2 (47 individuals) was where more *P. hexastylos* were found. Lateral scutes also presented a high percentage being the marginal scutes *P. hexastylos* percentage scarce and being the left scutes the most abundant. In the carapace region, *P. hexastylos* was in an added way, placing itself in rows on the limit between one scute and another (Fig. 8).

Some authors mention *P. hexastylos* but without specifying in what area is present. Zakhama-Sraieb *et al.,* (2010) found specimens fixed to skin flippers, neck and plastron. Badillo (2007) on the head, plastron, carapace and skin and Kitsos *et al.,* (2005) in the carapace. Hernandez-Vazquez and Valadez-González (1998) in the flippers. Only Limpus *et al.,* (1983) reported the presence on the flippers ventral surface, without specifying which and Balazs *et al.,* (1987) on the neck and on the pelvic area.

S. muricata was found forming aggregations or individualy in the anterior and rear flippers of the the two turtle species. This cirriped differs from *P. hexastylos* because it completely encapsulates into turtles skin. Its shell is fragile and presents different sutural structures that radiate outwards to be anchored inside the epidermis of the turtle. This barnacle penetrates the skin and sometimes bones causing damage (Fig. 10). Instead of causing infection, the turtle reacts by creating a fibrous connective tissue envelope (Monroe and Limpus, 1979; Monroe, 1981). In *C. caretta*, aggregations presence, number and size were higher than in *C. mydas*. Badillo (2007) initially reported this barnacle and Frick *et al.*, 2011 tested 19 *C. mydas* in San Ignacio lagoon (Mexico) identifiying this barnacle on the front and rear flippers front edges as well as in the skin and on 5 *C. caretta* front fins. Within the GU 9 captured *C.caretta turtles*, 100% all of them presented *S. muricata* and within the 48 *C. mydas* only 14 (29.1%) presented this barnacle. One of the reasons for this difference species could be the great amount of epibiont fauna and flora that *C.caretta* hosts.

S. praegustator (Pilsbry, 1910) (Fig. 13) has similar characteristics to *S. muricata* and *Stephanolepas elegans*. It can be differentiated from these because both barnacles penetrates inside the skin causing deep lesions in the tissue of its host. However, *S. praegustator* does not affect the tissue. It also presents an apico-basal form when observing the barnacle from the top. Pilsbry (1910) and Wells (1966) found this barnacle in the esophagus and flippers of *C.caretta* (Wells, 1966; Monroe and Limpus, 1979) and skin (without specifying where). Sosa-Cornejo *et al.,* (2012) identified this cirriped in nesting olive ridleys in Sinaloa (Mexico) but without specifying the anatomical zone where the specimens were found, however, they provided photographs of *S. praegustator* useful for identification.

In the present study, two specimens of this cirriped were found in the ventral area of the right anterior flipper of a *C. mydas*, 15 individuals in the ventral area of the anterior flipper of *C. mydas* and one specimen in the soft zone between the head and the right front fin of *C. mydas* (Fig.14). In *C. caretta, S. praegustator* were found in the dorsal and lateral area of the neck and in the mouth (Fig.13) which coincides with that reported by Pilsbry (1910) and Wells (1966). Lazo-Wasem *et al.,* (2011) found *Stomatolepas cf. elegans* in the body of two *C. mydas* (not specifying where). Nowadays, there is discussion between *S. elegans* and *S. praegustator* as well as the entire genus Stephanolepas; Lazo-Wasem *et al.,* (2011) could have misidentified these barnacles since the photographs they providef were very similar to those that Frick *et al.,* (2010) presented of *S. praegustator*. The same could happen in the study of Badillo (2007) of *C. caretta* where they identifiyed 20 specimens of *S.elegans* in the throat, lateral parts of the neck, base of the anterior flippers, base of the tail and palate possibly mistakenly identified as *S. praegustator*.

L. anatifera (Clark *et al.,* 1975) (Figure 16) belongs to the family Lepadidae (Darwin, 1852). These barnacles are attached to inert floating objects in shallow water, although some species are found on a wide variety of marine vertebrates (Foster, 1987). *L. anatifera* has a cosmopolitan distribution, being the most observed within the order Pedunculta (Caine, 1986, Fick *et al.,* 1998). It is one of the first species cited as sea turtle epibionts by Tukey (1818) and Gruvel (1920). *L. anatifera* was found in the dorsal area of the right rear flipper (Table 4) forming aggregations in a *C.caretta* specimen. This cirriped was also found on individuals of *C. testudinaria* in the carapace and plastron of individuals of *C. caretta*. *L. anatifera* prefers to settle on the hard parts of the turtle because this barnacle is a fixed filter feeder and does not present a movement after its settlement as *C. testudinaria* (Moriarty *et al.,* 2008) and therefore they can be found in areas with moderate flow where food availability is higher (Pfaller *et al.,* 2008). Domènech *et al.,* (2015) reported on carapace and plastron of *C.caretta* but without specifying the specific place. Relini (1980), Badillo (2007) and Karaa *et al.*, (2012) found specimens of *L. anatifera* on the marginal scutes and on the intermarginal scutes of the plastron and in smaller numbers on the head *C.caretta* (Karaa *et al.*, 2012).

There are only two studies about the incidence of *L. anatifera*. Alonso (2007) reported this barnacle in the carapace of juvenile *C.mydas* specimens in feeding and development areas in Uruguay, but once again without specifying details, such as the anatomical area and Fuller *et al.,* (2010) established that *L. anatifera* in the Mediterranean was more abundant in *C.caretta* than in *C.mydas*.

B. trigonus (Darwin, 1854) (fig.19) is a cosmopolitan specie. It is considered invasive due to its capacity to colonize the hulls of boats, thus increasing its distribution (Zullo, 1992). *B. trigonus* unlike the other cirripeds is a generalist species that, in addition to being fixed to inanimate substrate, has been reported on Posidonia oceanic rhizomes and on gastropod and lamelibranch shells (Relini, 1980). *B. trigonus* was found in the carapace of a GU *C.caretta*. Generally, this cirriped is located in the hard parts of the turtle as the carapace (Badillo, 2007, Domènech *et al.,* 2015, Karaa *et al.*, 2012), in the head (Karaa *et al.*, 2012) and less often in the plastron (Karaa *et al.*, 2012; Kitsos *et al.*, 2005).

CONCLUSIONS

Six different species of barnacles, belonging to five different genus, were identified.

Chelonibia testudinaria was the highest incidence cirriped on the two studied turtles.

Cirriped frequency was different depending on the sea turtle species *(C. mydas* and *C. caretta*) and also depending on the cirriped species.

It is important to study the possible consequences produced on turtle by the assembly of the different barnacle species identified in the study.

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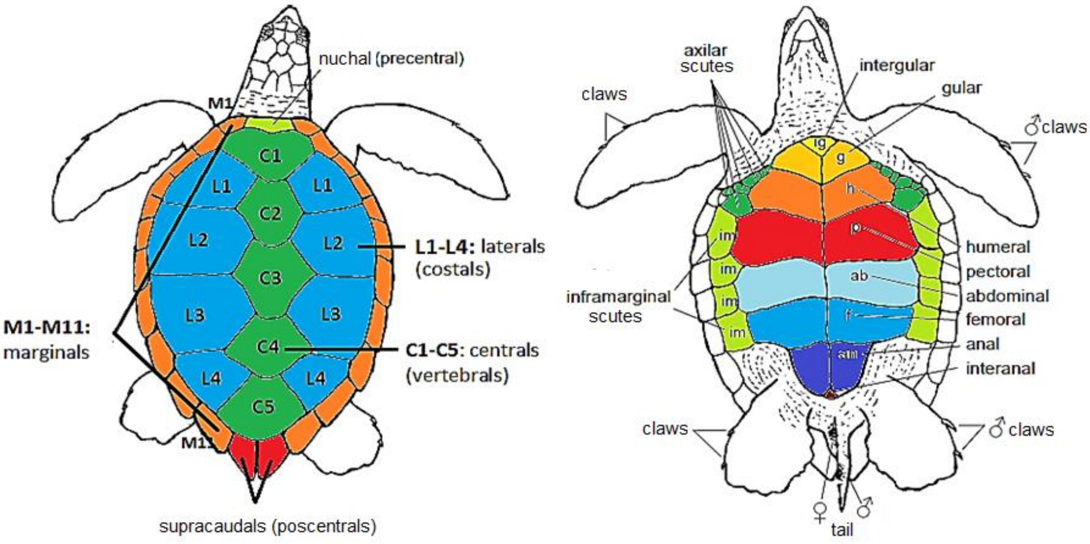
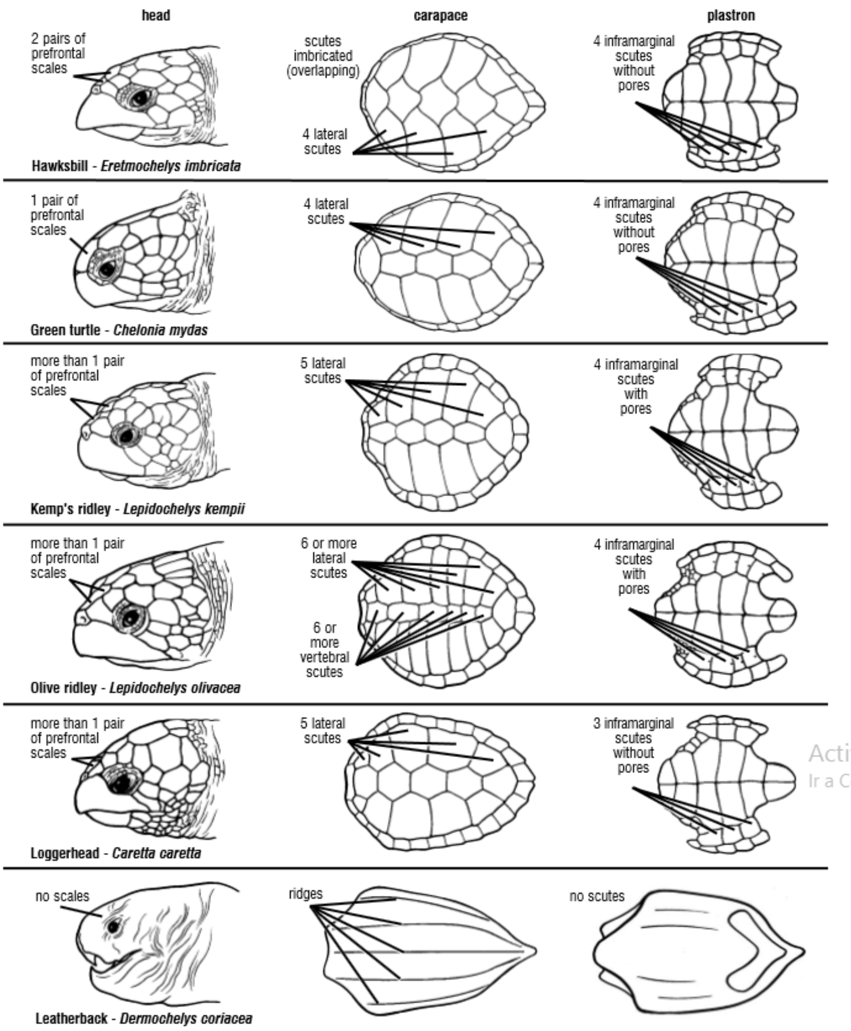
ADITIONAL INFORMATION

ANNEX 1

Schematic representation of the marginal, central and lateral carapace scutes and the plastron scutes. Image by by Eckert, K. L et al., 2000, painted Ibon García Gallego and taken and modified.



Sea turtles carapace, head and plastron scales representation (Wyneken, 2001).



ANNEX 2

Identified barnacle species taxonomic information

Taxonomy
Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Sessilia
Suborder: Balanomorpha
Family: Chelonibiidae (Pilsbri, 1916)
Genud: Chelonibia (Leach, 1817)
Specie: *Chelonibia testudinaria* (Linnaeus, 1758)

Taxonomy
Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Sessilia
Suborder: Balanomorpha
Family: Platylepadidae (Newman y Ross, 1976)
Genus: Platylepas (Gray, 1825)
Specie: Platylepas hexastylos (Fabricius, 1798)

Taxonomy
Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Sessilia
Suborder: Balanomorpha
Family: Platylepadidae (Newman y Ross, 1976)
Genus: Stephanolepas (Fischer, 1886)
Specie: *Stephanolepas muricata* (Fischer, 1886)

Taxonomy

Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Sessilia
Suborder: Balanomorpha
Family: Platylepadidae (Newman y Ross, 1976)
Genus: Stephanolepas (Fischer, 1886)
Specie: *Stephanolepas praegustator* (Pilsbry, 1910)

Taxonomy

Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Pedunculata
Suborder: Lepadomorpha
Family: Lepadidae (Darwin, 1852)
Genus: Lepas (Linnaeus, 1758)
Specie*: Lepas anatifera* (Linnaeus, 1758)

Taxonomy

Subphylum: Crustacea
Class: Maxillopoda
Infraclass: Cirripedia
Superorder: Thoracica
Order: Sessilia
Suborder: Balanomorpha
Family: Balanidae (Leach, 1917)
Genus: Balanus (Da Costa, 1788)
Specie: *Balanus trigonus* (Darwin, 1854)

OPINIÓN PERSONAL

1. Descripción detallada de las actividades desarrolladas durante la realización del TFT

Durante mi programa de movilidad MUNDUS en la Universidad Autónoma de Baja California Sur (UABCS) en el segundo semestre de 2017 (febrero-junio 2017) realicé las prácticas institucionales para poder realizar el TFG en base a ellas. Además para que pudieran convalidarse estas prácticas tuve que presentar unas estancias de investigación con un contenido similar al TFG. Después durante este año he estado terminando las partes del TFG que no realicé en la UABCS.

2. Formación recibida (cursos, programas informáticos, etc.)

No he recibido formación durante el TFG. Adicionalmente, durante la realización de las estancias de investigación en la UABCS, participé como ponente gracias al Proyecto Salud de Tortugas Marinas en la primera Reunión Internacional de Tortugas Marinas del Pacífico Oriental y cuarta Reunión Nacional sobre Tortugas Marinas en México del 11 al 14 de octubre de 2017 en Morelia, Michoacán. Esta ponencia fue de un póster titulado "CLASIFICACION DE BALANOS EN TORTUGAS MARINAS DE BAJA CALIFORNIA SUR. BARNACLES CLASSIFICATION OF MARINE TURTLES IN BAJA CALIFORNIA SUR". Este póster reflejaba el trabajo realizado durante los meses previos a la finalización del periodo de prácticas y con el objetivo de buscar las bases para la elaboración posterior del TFG. Así mismo, fue registrado en la página web "Researchgate" donde se puede consultar en el siguiente enlace:https://www.researchgate.net/publication/320456960_CLASIFICACION_DE_BALANOS_EN_TORTUGAS_MARINAS_DE_BAJA_CALIFORNIA_SUR_BARNACLES_CLASSIFICATION_OF_MARINE_TURTLES_IN_BAJA_CALIFORNIA_SUR

3. Nivel de integración e implicación dentro del departamento y relaciones con el personal

El nivel de integración e implicación dentro del departamento y relaciones con el personal en la UABCS tanto como en la ULPGC fueron excelentes.

4. Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

Dentro de los aspectos positivos durante el desarrollo del TFG el poder haber estudiado los balanos que presentan las tortugas marinas de la region ha sido interesante ya que por una parte, es importante saber qué especies de cirrípedos podrían albergar estos animales y en segundo lugar debido a que a nivel global existen pocos trabajos que estudien estos organismos y mucho menos a nivel regional y local, por lo que sería la primera vez que se realizaría un trabajo de esta índole

Respecto a los negativos como anteriormente se ha mencionado que a nivel global existen pocos trabajos que estudien estos organismos y mucho menos a nivel regional y local ha dificultado poder realizar el TFG. De la misma manera, tener que redactarlo en inglés también fue una dificultad.

5. Valoración personal del aprendizaje conseguido a lo largo del TFT.

Ha sido gratificante poder haber logrado un buen aprendizaje durante la realización del TFG.