# Epibionts Associated with the Nesting Marine Turtles Lepidochelys olivacea and Chelonia mydas in Jalisco, Mexico: A Review and Field Guide

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#### Abstract

The diversity and frequency of epibiota collected over three years (2001, 2002, 2008) from sea turtles (*Lepidochelys olivacea* and *Chelonia mydas*) nesting on Teopa Beach in Jalisco State, Mexico, are described. This diversity is compared to epibiotic assemblages procured from these same turtle species nesting on other Mexican beaches, and the role these turtles play in the conservation and dispersal of these epibiota is discussed. Given the increased awareness of epibionts and the desire of many researchers to make positive identifications, specific diagnoses, photographs and a collecting protocol will serve as a basic aid to the collection and accurate identification of epibionts found on turtles living along the Pacific coast of Mexico.

#### Resumen

Se describe la diversidad y la frecuencia de los epibiota colectados durante tres años de las tortugas marinas (*Lepidochelys olivacea y Chelonia mydas*) que anidan en la playa de Teopa en el estado de Jalisco, México. Esta diversidad es comparada a los conjuntos epibionticos adquiridas por estas mismas especies de tortugas que anidan en otras playas Méxicanas, y se discute el papel que estas tortugas desempeñan en la conservación y en la dispersión de estos epibiotas. Dado el conocimiento creciente de epibiontes y el deseo de muchos investigadores de hacer identificaciones positivas, diagnosis específicas, fotografías y un protocolo de colecta servirán como ayuda básica a la colección y la identificación de epibiontes encontrados en las tortugas que viven a lo largo de la Costa del Pacífico de México.

## Introduction

Conservation of sea turtles includes management of nesting beaches, nest translocation to protect hatcheries, head starting, reduction or elimination of natural predators, and protection against poaching and fishing (Bjorndal 1995). What has only recently received attention are the coevolutionary interrelationships that exist between turtles and their epibionts, and how these relationships can affect turtle health and ecology. For example, Greenblatt et al. (2004) proposed the parasitic leech *Ozobranchus branchiatus* as a vector candidate implicated in transmitting turtle herpes virus; subsequently, Lazo-Wasem et al. (2007) suggested a potentially similar role for the copepod *Balaenophilus*. There are several studies on the distribution and abundance of epibionts on Atlantic Ocean turtles, in particular on nesting loggerheads (*Caretta caretta*). Previous work on these turtles showed variation of epizoic diversity dependent in part on relatively localized geography (Caine 1986; Frick et al. 1998). Both these studies documented large numbers of epibionts, with Frick et al. (1998) reporting nearly 90 species on loggerheads from Georgia, USA.

Turtles inhabiting the Pacific Ocean have come under scrutiny for epibiont diversity (Hernández-Vázquez and Valadez-González 1998; Gámez Vivaldo et al. 2006; Angulo-Lozano et al. 2007). These studies focused on nesting olive ridley turtles along the Pacific coast of Mexico. More recently, epibiont diversity was documented on free-swimming turtles sampled in their foraging habitats adjacent to Palmyra Atoll (Gomez et al. 2011) and detailed data has been provided for grapsoid crab epibionts (Planes and Plagusia) found on central and eastern Pacific (Hawaii, USA, and Mexico) populations of olive ridley turtles (Frick, Kopitsky et al. 2011). Although these and several gray literature technical reports document Pacific Ocean marine turtle epibionts, most accounts of diversity are only relatively brief checklists and in some cases the organisms depicted in the accompanying photographs are misidentified. To address this situation, the present study provides a detailed account of epibionts found on the nesting Olive Ridley Sea Turtle (Lepidochelys olivacea) and Green Sea Turtle (Chelonia mydas) from ongoing conservation work on both these species along the Mexican coast. In addition to the general frequency patterns of epibionts, we provide photographs of most of the taxa encountered, a review of their general natural history and, when possible, their current taxonomic status. Included in this account is a general overview of the diagnostic characters that allow for the identification of each epibiont. This information should provide workers in the field with a basic field guide to most of the epibiont taxa likely to be encountered on nesting Pacific coast turtles.

#### Materials and Methods

Teopa Beach (locally Playa Teopa) is on the western Pacific coast of Mexico in Jalisco State, part of an area commonly referred to as the Costa Alegre. The turtles sampled were found nesting along a beach 6 km south of Costa Careyes and Chamela (approximately lat 19.5°N, long 105.1°W) during a parallel nest monitoring program where *Lepidochelys olivacea* (Olive Ridley Sea Turtle) and *Chelonia mydas* (Green Sea Turtle, known locally as *prieta negra*, or black turtle) are known to nest (Pinou et al. 2009). Coastal conservation programs (such as that of the Centro Para la Proteccion y Conservación de Tortugas Marinas, Playa Teopa, and Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras, Universidad de Guadalajara) scan the beach nightly for nesting turtles during the nesting season, from mid-July to May.

Epibionts were collected according to the method described by Frick et al. (1998) and standardized according to Caine (1986) during monitoring of the beach for sea turtle nests (Pinou et al. 2009). Project manager Alejandro Peña de Niz of the Centro Para la Protección y Conservación de Tortugas Marinas, Playa Teopa, was responsible for training teams of three workers to locate and collect epibionts, which were collected only after a turtle nested. Epibionts were collected from the carapace, flippers and neck region of the turtles. All collected specimens were placed into tubes with 70% alcohol and labeled with a host number (corresponding to the turtle nest number on nesting data sheets). These samples were taken to the Peabody Museum of Natural History at Yale University, where they were sorted and identified to the lowest taxon possible. Voucher specimens are catalogued in the collections of the Yale Peabody Museum Division of Invertebrate Zoology, with data available from the Yale Peabody Museum online database (YPM IZ Catalog 2011).

#### Results

The occurrence of 16 different epibiont taxa collected from nesting *Lepidochelys olivacea* and *Chelonia mydas* at Teopa Beach, Jalisco, Mexico, during the 2001, 2002 and 2008 nesting seasons are reported here (Table 1). The general taxon "algae" is included, mainly for comparison to other epibiont studies that have also included this epibiont component. Algae can exist as a nearmicroscopic epibiont, easily overlooked, and are difficult to quantify.

Thirteen invertebrate epibionts were identified to species: the crustaceans Balaenophilus manatorum, Lepas anserifera, L. hilli, Chelonibia testudinaria, Conchoderma virgatum, Platylepas decorata, P. hexastylos, Stomatolepas cf. elegans, Planes major and Podocerus chelonophilus; the annelid (leech) Ozobranchus branchiatus; and the gastropod mollusk Stramonita biserialis. Two cnidarians were also collected, one tentatively

	Systematic group			Frequency			% Frequency		
Epibiont taxon			<i>L. olivacea</i> (n = 124)	<i>C. mydas</i> (n = 6)	Combined hosts (n = 130)	L. olivacea	C. mydas	Combined hosts	
Actiniaria	Cnidaria: Actiniaria	2	2	0	2	16	_	2	
Algae (filamentous)	Chlorophyta	a	a				_		
Balaenophilus manatorum	Crustacea: Copepoda	432	22	1	23	18	17	18	
Chelonibia testudinaria	Crustacea: Cirripedia	127 <sup>b</sup>	30	5	35	24	83	27	
Conchoderma virgatum	Crustacea: Cirripedia	720	36	3	39	29	50	30	
Lepas anserifera	Crustacea: Cirripedia	1	0	1	1	_	17	<1	
Lepas hilli	Crustacea: Cirripedia	425	13	6	19	10	100	15	
Obelia?	Cnidaria: Hydrozoa	1 <sup>c</sup>	0	1	1	_	17	<1	
Ozobranchus branchiatus	Annelida: Hirudinea	176	32	0	32	26	_	25	
Planes major	Crustacea: Brachyura	4	4	0	4	3	_	3	
Platylepas decorata	Crustacea: Cirripedia	4	0	1	1	_	17	<1	
Platylepas hexastylos	Crustacea: Cirripedia	75	26	1	27	21	17	21	
Podocerus chelonophilus	Crustacea: Amphipoda	48	5	0	5	4	—	4	
Remora remora	Vertebrata: Perciformes	1	1	0	1	<1	_	<1	
Stomatolepas cf. elegans	Crustacea: Cirripedia	499	68	2	70	55	33	54	
Stramonita biserialis	Mollusca: Gastropoda	1	0	1	1	—	17	<1	

TABLE 1. Epibiont frequency on Lepidochelys olivacea and Chelonia mydas from Jalisco, Mexico.

<sup>a</sup> Not counted, only noted in general.

<sup>b</sup> No complemental males counted, only "adults."

<sup>c</sup> Taxon is represented by a single colony, rather than an individual.

identified as the hydroid *Obelia* sp. and the other an unknown actiniarian; these latter are essentially not resolvable given their condition (the specimens were damaged or not preserved in a way that permitted detailed study of key diagnostic characters). One vertebrate species, the sharksucker *Remora remora*, was collected. Crustaceans are the dominant epibionts of both olive ridley and green turtles. More than 40% of the observed epibiont taxa were crustaceans, and most of these were barnacles. Of these, the large balanomorph barnacle *Chelonibia testudinaria* is the most conspicuous epibiont, typically attached prominently to the carapace. Numerically, however, the stalked barnacle *Conchoderma virgatum* dominated, representing 30% of all epibionts identified and with a host occurrence frequency of 30%. With respect to host occurrence, the balanomorph barnacle *Stomatolepas elegans* was dominant; it was on more than 50% of all turtles sampled. Of the non-Crustacea epibionts, the most important was the leech *Ozobranchus branchiatus*, which was found on 26% of all olive ridleys. Although none of the six green turtles we sampled at Teopa Beach had *O. branchiatus* epibionts, notably several of those we encountered later at the Universidad de Guadalajara field station Campamento La Gloria (Jalisco, Mexico) had specimens of this same parasitic leech.

Several epibionts occurred at very low density (less than 5% occurrence), but are nonetheless important for a variety of reasons (see commentary and discussion below). These were the barnacles *Lepas anserifera* and *Platylepas decorata*, the crab *Planes major*, the snail *Stramonita biserialis*, hydroids (cf. *Obelia*) and the sharksucker *Remora remora*.

The relative diversity of epibionts we observed in this study, as compared to other studies published previously on *Lepidochelys olivacea* (Table 2), clearly shows that the diversity we report is much greater than that reported earlier and takes into account the expansion of the epibiont host in our study to include both *L. olivacea* and *Chelonia mydas* at the Teopa Beach site.

We observed no strong trends distinguishing the two epibiont turtle host species; unique observations are probably relative to sampling effort.

#### **Taxonomic Commentary**

CRUSTACEA Copepoda: Harpacticoida: Balaenophilidae *Balaenophilus manatorum* (Ortiz, Lalana and Torres) Figure 6D

These copepods were reported previously (Lazo-Wasem et al. 2007) as *Balaenophilus umigamecolous* Ogawa, Matsuzaki and Misoki. Recently, Suárez-Morales (2007) examined this material and from it has concluded the species *B. umigamecolous* to be a junior synonym of the *B. manatorum*, an epibiont first reported from a manatee (Ortiz et al. 1992). To establish this synonymy, Suárez-Morales (2007) also had to transfer *B. manatorum* from its original name combination *Harpetichechus manatorum* to *Balaenophilus*.

These harpacticoids occur in an unknown association with the barnacle Stomatolepas (Lazo-Wasem et al. 2007). Careful examination of all barnacle samples has not uncovered an association between this copepod and any barnacle other than Stomatolepas (e.g., Chelonibia, Platylepas). Balaenophilus are not obvious when the turtles are inspected for epibionts and are only found when samples containing Stomatolepas are examined carefully under a dissecting microscope. Adults of these copepods are typically less than 3 mm long, but are easily recognized by the distinctive form of their first pair of legs, which bear strong apical claws on the endopod and exopod (see Suárez-Morales and Lazo-Wasem 2009). That Balaenophilus are truly ectoparasitic on turtles (Ogawa et al. 1997) has yet to be adequately shown, although in a recent redescription of B. manatorum these barnacles are again referred to as ectoparasites (Suárez-Morales and Lazo-Wasem 2009).

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## Lepadomorpha Family Lepadidae *Conchoderma virgatum* Spengler Figure 1F,G

There is no mistaking the striped appearance of this lepadomorph, which Pilsbry (1916) went so far as to describe as "handsome." The species is distributed worldwide and attaches to a variety of animate and inanimate substrates. Typically *Conchoderma virgatum* occurs as a secondary epibiont of turtles, almost always attached in small aggregations to the commensal barnacle *Chelonibia testudinaria*, which is the primary epibiotic substrate. Rarely it is found attached directly to the turtle's skin.

## *Lepas hilli* Leach Figure 1A–C *Lepas anserifera* Linnaeus Figure 1D

The stalked barnacles of the genus Lepas are widely distributed throughout the world's oceans and are commonly attached to flotsam, the underside of almost all kinds of watercraft, and wharves and dock pilings. Given their attachment to drifting or otherwise mobile structures, we can expect the distribution of all species in this genus to be cosmopolitan. Several of the common forms are similar in appearance, but we have referred most of the identifiable Lepas to L. hilli Leach, the first known record occurring on olive ridley turtles. We could possibly be mistaking these barnacles for L. pacifica Henry, but the validity of that species is certainly questionable. In fact, L. pacifica was originally described as a subspecies, L. pectinata pacifica (Henry 1940), and although the full species attribution has been in occasional use in nontaxonomic treatments (Cheng and Lewin 1976; Bernstein and Jung 1979; Celis et al. 2008), it was never formally elevated to specific status. A careful reanalysis of the taxonomy is needed to ascertain its validity.

Identification of *Lepas hilli* is based on two diagnostic characters that distinguish this species from the equally common and well-distributed *L. anatifera* Linnaeus: *L. hilli* has a very smooth shell and lacks internal umbonal teeth on the base of

		Turtles sampled and localities						
Epibiont taxon	Systematic group	C. mydas <sup>a</sup> Jalisco, Teopa Beach (n = 6)	L. olivacea <sup>a</sup> Jalisco, Teopa Beach (n = 124)	L. olivacea, C. mydas Combined <sup>a</sup> Jalisco, Teopa Beach (n = 130)	<i>L. olivacea</i> <sup>b</sup> Jalisco, La Gloria (n = 46)	L. olivacea <sup>c</sup> Michoacan and Oaxaca (n = 28)	<i>L. olivacea</i> <sup>d</sup> Sinaloa, Playa Ceuta (n = 12)	
Algae (filamentous)	Chlorophyta	×	×	×	×			
Actiniaria	Cnidaria: Hydrozoa		×	×				
Obelia?	Cnidaria: Hydrozoa	×		×				
Ozobranchus branchiatus	Annelida: Hirudinea	×	×	×	×		×	
Conchoderma virgatum	Crustacea: Cirripedia	×	×	×	×	×	×	
Lepas anatifera	Crustacea: Cirripedia				×		×	
Lepas anserifera	Crustacea: Cirripedia	×		×			×	
Lepas hilli	Crustacea: Cirripedia	×	×	×				
Chelonibia testudinaria	Crustacea: Cirripedia	×	×	×	×		×	
Platylepas hexastylos	Crustacea: Cirripedia	×	×	×	×	×		
"Platylepas" decorata	Crustacea: Cirripedia	×		×				
Stomatolepas cf. elegans	Crustacea: Cirripedia	×	×	×		×		
Balaenophilus manatorum	Crustacea: Copepoda	×	×	×				
Podocerus chelonophilus	Crustacea: Amphipoda		×	×		$\times^{\mathrm{f}}$		
Planes spp. <sup>e</sup>	Crustacea: Brachyura		×	×	×		×	
Stramonita biserialis	Mollusca: Gastropoda	×		×				
Remora remora	Vertebrata: Perciformes		×	×	×	×		

TABLE 2. Comparison of epibionts encountered in the current study and three previous studies of Mexican Lepi-dochelys olivacea. The sample from Teopa Beach, in Jalisco, Mexico, combined both L. olivacea and Chelonia mydas.

<sup>a</sup> Present study.

<sup>b</sup>Hernández-Vázquez and Valadez-González 1998.

<sup>c</sup>Gámez Vivaldo et al. 2006.

<sup>d</sup> Angulo-Lozano et al. 2007.

<sup>e</sup>Range of possible *Planes* taxa combined for comparison to accommodate new taxonomic changes.

<sup>f</sup>Misidentified as the amphipod Caprella sp.

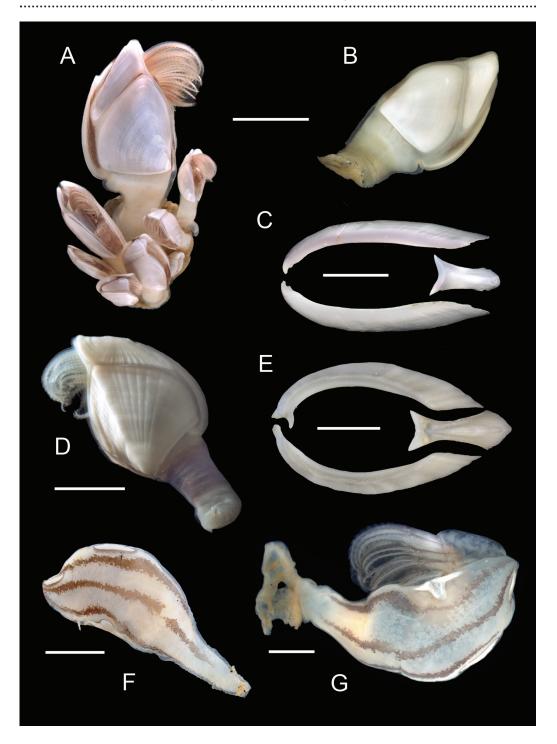


FIGURE 1. **A.** Lepas hilli, YPM IZ 028439. **B.** L. hilli, YPM IZ 048180. Scale bar 5 mm (A, B). **C.** L. hilli, YPM IZ 048180. Basal view of scuta and carina. Scale bar 2 mm. **D.** L. anserifera, YPM IZ 048179. Scale bar 3 mm. **E.** L. anatifera, YPM IZ 048176. Basal view of scuta and carina. Scale bar 5 mm. **F.** Conchoderma virgatum, YPM IZ 041641. Scale bar 5 mm. **G.** C. virgatum, YPM IZ 035724. Scale bar 2 mm.

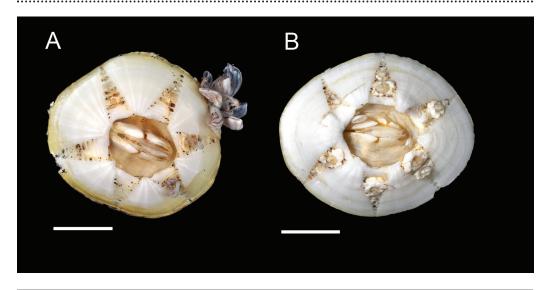


FIGURE 2. *Chelonibia testudinaria*. **A.** YPM IZ 048135. This specimen is the substrate for *Conchoderma virgatum* and *Lepas hilli*. Scale bar 10 mm. **B.** YPM IZ 028450. Complemental males adhering to parietal junctions (radii). Scale bar 15 mm.

the right scuta, whereas *L. anatifera* has an umbonal tooth (see Figure 1E) and the shell is striate (Pilsbry 1916), although the latter feature can be relatively weak and almost indiscernible. Undoubtedly, when large aggregations of small lepadomorphs are encountered on these turtles there is a high probability that more than one species is present.

## Balanomorpha Family Chelonibiidae *Chelonibia testudinaria* Linnaeus Figure 2

*Chelonibia testudinaria* is the largest and most conspicuous barnacle epibiont of the Jalisco turtles. It is usually found on the carapace and is easily recognized by its large size (which can exceed 75 mm), domed appearance and conspicuous starlike radii of the shell. Complemental males often adhere to the shell radii (Figure 2B; see discussion by Zardus and Hadfield 2004) and are typically not counted in epibiont tallies. These barnacles can be a substrate for other barnacles, such as *Conchoderma virgatum*, and it has been suggested (Rawson et al. 2003) that *C. testudinaria* plays a role as a pioneer species in determining the structure of epizoan communities in loggerhead turtles.

Although this barnacle has been recognized for hundreds of years, the taxonomy of this species has recently been called into question (Frick and Ross 2001). Genetic work supports the idea that there might be cryptic species of *Chelonibia*, particularly in the Pacific Ocean (Rawson et al. 2003).

### Family Platylepadidae Platylepas hexastylos (Fabricius) Figure 3

This flattened turtle barnacle is instantly recognizable by the distinctive concentric ridges and striations of the shell and the

six sculptured buttressing pillars visible on the underside with the basis removed. The junction of the buttressing pillars extends to a distinctive suture on the outside wall of the plate, giving each a bi-lobed appearance. *Platylepas hexastylos* is widespread and frequently encountered on various marine turtles.

#### Platylepas decorata Darwin Figure 4

A few specimens of *Platylepas decorata* were collected from a single individual green turtle. Recently, Frick and Zardus (2010) discussed the difficulty surrounding the historical taxonomy of this species and the confusion in the literature as to how it has been reported. In doing so they discuss the distinction between this and the barnacle it has been confused with, *Cryptolepas darwiniana* Pilsbry, Furthermore, Zardus and Balazs (2007) have discussed the presence of this barnacle in green turtles from Hawaii and describe the characteristics of its attachment to turtle skin.

Recognition of this species is relatively easy and it is not likely to be confused with the much more common *Platylepas hexastylos*. Although both species have diagnostic buttressing pillars, *P. decorata* lacks the concentric horizontal striae seen in *P. hexastylos*. Furthermore, *P. decorata* has a parallel beaded ridge along the outside center of each plate and several projections along the base of the shell.

#### Stomatolepas cf. elegans (Costa) Figure 5

The barnacle *Stomatolepas* cf. *elegans* (Costa) is the most common balanomorph living as an epibiont on the Jalisco population of nesting turtles. Typically small (approximately 4 to 8 mm), this barnacle is often found in the inner creases of the neck, forelegs, hind legs and tail. It is easily recognized by the appearance of the

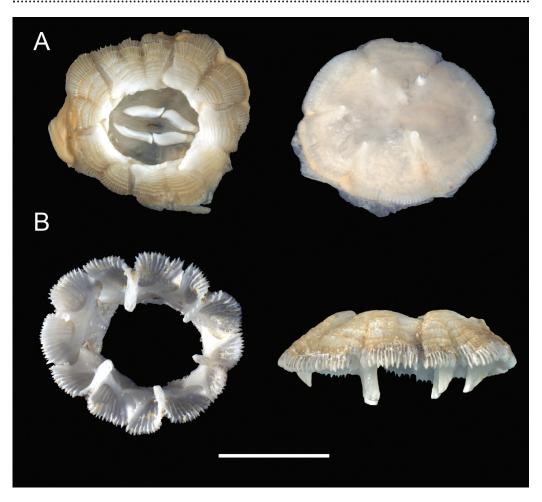
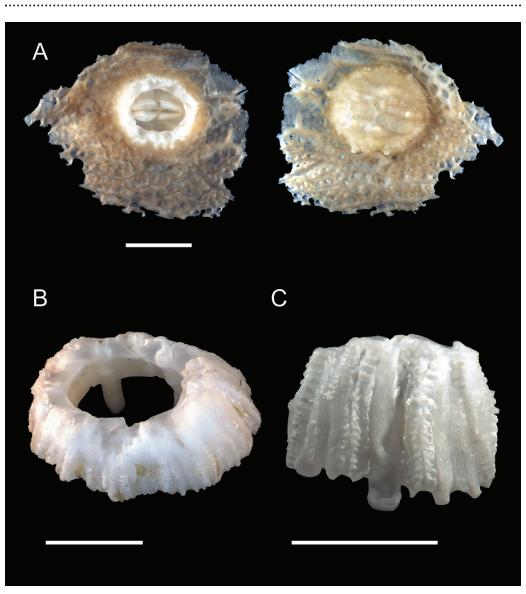


FIGURE 3. *Platylepas hexastylos*. **A.** YPM IZ 039094. Oral and obverse view of preserved specimen. **B.** YPM IZ 052356. Ventral and lateral view of dried shell. Scale bar 5 mm.

outer plates, which are ornamented with small calcareous scales arranged in a chevron pattern (Pilsbry 1916). The genus Stomatolepas has a long and somewhat confused history and a revision is currently being attempted (E.A. Lazo-Wasem, unpublished data). Recently, in describing the new species S. pilsbryi, Frick et al. (2010) made a compelling argument clarifying the distinction between S. praegustator and S. elegans, with which the former has often been confused or placed in synonymy, each one having been used as the valid name for the same taxon at different times (Newman and Ross 1976; Monroe and Limpus 1979; Monroe 1981). This work, which established the taxonomic status of both species, designates a neotype for S. elegans, for which no definitive type material was known. Nonetheless, the study was focused primarily on Atlantic Ocean specimens and occurrences (Frick et al. 2010). Although it cited a cosmopolitan distribution for S. elegans, no rigorous analysis of large numbers of Pacific coast Stomatolepas has ever been done. We have found in our analysis of hundreds of specimens evidence of great morphological variation in the Jalisco Stomatolepas population. For the most part, the species we see is closest to S. elegans sensu

*lato*, primarily because our specimens are found on the animal corpus and not within the gullet as is typical of most *S. praegustator* (Pilsbry 1910; J.D. Zardus, personal communication).

The variation we see in our specimens is broad enough to straddle the two species boundaries as outlined by Pilsbry (1916). Furthermore, the Jalisco Stomatolepas are never found deeply imbedded in the skin, but instead are attached superficially. This is fundamentally different from the typical Stomatolepas, which usually have to be dug out of the skin of dead turtles. Although quite possibly endpoints of a variability continuum, there are two distinct morphs in the Jalisco material. One form is squat and flattened, with large irregular scales. The other is tall with several rows of very regular scales. Of the latter there are many examples both small and large, so the differences are apparently not allometric. Genetic analyses (T. Pinou et al., in preparation) suggest that the Jalisco population fits the parameters of a single species, in spite of the notable morphological differences, and reveals no species level variability within this population. A morphometric analysis (E.A. Lazo-Wasem et al., in preparation) of Stomatolepas



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FIGURE 4. "*Platylepas*" *decorata*. **A.** YPM IZ 048220. Oral and obverse view of preserved specimen with sheath of host tissue. Scale bar 2 mm **B.** YPM IZ 048219. Oblique view of intact dried shell. Scale bar approximately 1 mm. **C.** YPM IZ 048219. Rostral plate of shell. Scale bar 1 mm.

samples may help unravel the details of the observed morphological variability.

BRACHYURA Family Grapsidae *Planes major* McLeay Figure 6A, B

Specimens of the oceanic crab *Planes major* McLeay were encountered only rarely in our samples and were totally absent from the 2008 collections. According to published accounts, which cite *P. major* as its junior synonym, *P. cyaneus* Dana (see Ng and Ahyong 2001) has a long but infrequently reported association with various marine turtles and is broadly distributed in the Atlantic and the Pacific (Chace 1951; Spivak and Bas 1999). Several papers have reported *P. cyaneus* from marine turtles, including loggerhead turtles harvested as bycatch in the South Atlantic Ocean (Carranza et al. 2003) and nesting olive ridleys from the Pacific coast of Mexico (Hernández-Vázquez and Valadez-González 1998; Angulo-Lozano et al. 2007). Although it has been suggested that there is a cleaning relationship between epibi-

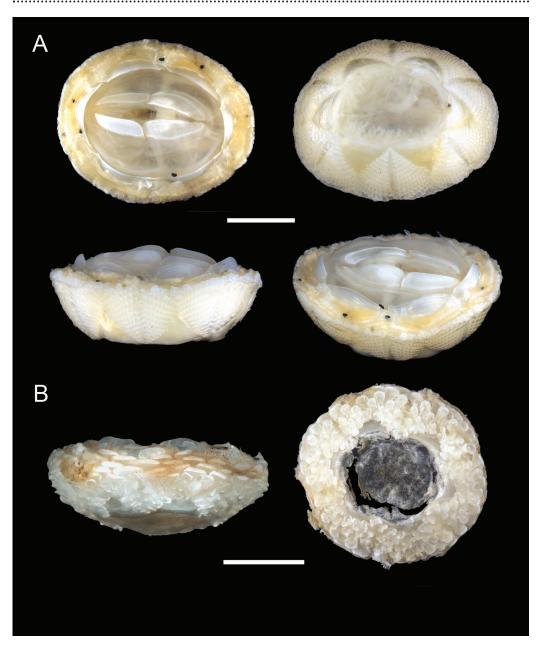


FIGURE 5. *Stomatolepas* cf. *elegans*. **A.** YPM IZ 041655. Typical form. Scale bar 3 mm. **B.** YPM IZ 039162. Low form showing irregular pattern of lateral projections. Scale bar 2 mm.

otic crabs such as *P. minutus* and their hosts (Frick et al. 2004), this symbiosis is apparently of little significance in the Jalisco turtles because of the rarity of *P. cyaneus*. Frick, Zardus et al. (2011) found *P. major* and its congener *P. marinus* Rathbun to be common on olive ridleys in foraging areas adjacent to the Jalisco nesting site. It is possible that differences in behavior between foraging turtles and nesting turtles influence the occurrence of *P. major* on olive ridleys.

AMPHIPODA: GAMMARIDEA Family Podoceridae *Podocerus chelonophilus* (Chevreux and De Guerne) Figure 6C

Podocerid amphipods reported previously from loggerhead turtles have been assigned to one of two species: *Podocerus* 

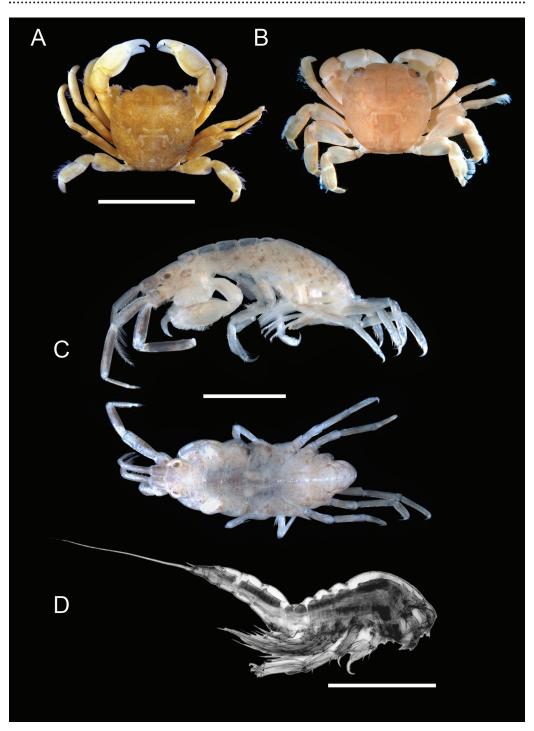


FIGURE 6. **A.** *Planes major*, YPM IZ 035430. **B.** *P. major*, YPM IZ 035523. Scale bar 15 mm (A, B). **C.** *Podocerus chelonophilus*. Lateral and dorsal views. Scale bar 2 mm. **D.** *Balaenophilus manatorum*, YPM IZ 028506. Lateral view. Scale bar 0.5 mm (from Lazo-Wasem et al. 2007).

*chelonophilus* (Chevreux and De Guerne) and the more recently described *P. umigame* Yamato. In particular, *P. chelonophilus* has been reported several times, although occasionally under other names or species (such as *P. cheloniae* Stebbing). Baldinger (2000) examined specimens collected from around the world and showed that the morphology of *P. chelonophilus* is highly variable; therefore, all the supposed features distinguishing *P. chelonophilus* from *P. umigame* are not enough to justify the retention of both species and synonymized them.

Although we encountered relatively few specimens of Podocerus chelonophilus, in all likelihood it is numerically more abundant than is observed when collecting them from nesting turtles. Many gammaridean amphipods species are nestlers (cryptic), keeping well hidden amongst tufts of algae or in small spaces of whatever substrate they inhabit. Rarely are they conspicuous unless they are somehow dislodged from the substrate. Generally speaking, they will not leave their substrate unless they sense that conditions are no longer favorable or are otherwise disturbed, whereupon they abandon their nestling place (E.A. Lazo-Wasem, personal observation). For example, dislodged algae washing ashore is usually devoid of amphipods, which have abandoned the algae long before. This presumed ability to sense the looming beach as the turtle migrates inshore probably induces the amphipods to abandon the turtle and spend some time in the water column living freely before finding another turtle. Examination of pelagic olive ridleys would probably yield higher population densities of P. chelonophilus.

Angulo-Lozano et al. (2007) noted the incorrect assignment of "*Caprella* sp." to amphipods in a photograph of olive ridley epibionts (Gámez Vivaldo et al. 2006), pointing out that they were instead probably *P. chelonophilus*. The photograph, however, was not detailed enough or clear enough to make a real identification possible. In fact, all that can be said for certain is that these were definitely not *Caprella* and that they had podocerid facies. Hernández-Vázquez and Valadez-Gonzáles (1998) reported gammaridean amphipods from olive ridleys nesting at La Gloria, Jalisco, Mexico, but did not identify these any further. Our specimens, therefore, represent the first authenticated records of *P. chelonophilus* living epibotically on olive ridleys.

Although amphipod identification is often difficult for nonspecialists, *Podocerus* amphipods are easy to recognize by their distinctive mottled coloration and dorsoventrally flattened appearance, the latter not a typical feature of most gammaridean amphipods. In dorsal view, their eyes bulge distinctively. Furthermore, their narrow abdomen (pleon and urosome) is typically curled completely under the front of the abdomen, thereby emphasizing the length of the rear sets of legs. For several new species described in recent years, virtually nothing is known about their biology (there is no identification key), therefore the existence of potential associations between other *Podocerus* species and other hosts is unknown.

## ANNELIDA: HIRUDINEA Family Ozobranchidae Ozobranchus branchiatus (Menzies) Figure 7B, C

Ozobranchus branchiatus (Menzies) was apparently first reported occurring on olive ridleys by Gámez Vivaldo et al. (2006). Previously, most other occurrences were recorded from green turtles (McDonald and Dutton 1990). Bunkley-Williams et al. (2008) have reported the first occurrence of this leech from a Hawksbill Sea Turtle, *Eretmochelys imbricata* Linnaeus (note that of the two different spellings—"*brachiatus*" and "*branchiatus*" — the authors use throughout their text, the latter is correct).

Two other marine leech species are known to parasitize marine turtles: *Ozobranchus margoi* (Apathy) and *O. jantseanus* Oka. These are easily distinguished from *O. branchiatus* by the number of paired branchiae: *O. margoi* has five pairs, *O. branchiatus* has seven pairs and *O. jantseanus* has eleven pairs.

## MOLLUSCA: GASTROPODA Family Muricidae Stramonita biserialis (Blainville) Figure 7A

Stramonita biserialis was represented by a single specimen collected on an olive ridley turtle in 2001 and is the first record of this species living on olive ridley turtles. S. biserialis is a Pacific coast predatory drill that uses the radula to drill holes in the shell of its prey. It can reach a length of 75 mm (Keen 1971). This recently metamorphosed juvenile was probably present because of the epibiotic barnacles that are a potential prey item. Muricids are well adapted to preying on sessile crustaceans (Paine 1966; Palmer 1982; Vermeij 2001; Gregory 2004). In recovering tissue for an unpublished study of the molecular genetics of Stomatolepas barnacles (T. Pinou et al., in preparation), one of us (Lazo-Wasem) noticed that a significant percentage of these barnacles essentially lacked tissue and were seemingly degraded (no useable DNA could be recovered). Possibly these were actually barnacles that had been fed on by drills such as S. biserialis, a plausible supposition given the ease a drill would have in attacking a barnacle that lacks a tightly fitting operculum. Nonshell access to barnacles is an evolutionary strategy to reduce the time it takes a drill to attack its prey (Vermeij 2001), which makes turtle barnacles such as Stomatolepas likely candidates for predation by this and other muricid mollusks.

#### VERTEBRATA: OSTEICHTHES Family Echineidae *Remora remora* (Linnaeus) Figure 8

Remora have been infrequently reported associated with marine turtles. Total occurrences are few and the nature of the association beyond being mere "hitchhikers" (phoresis) is not well understood (Sazima and Grossman 2006). Historically, however, a practical side to this association was well known apparently as far back at the late 15th century in the Americas: a fisherman would attach a line to a living remora that was allowed to swim freely, search for and attach to a marine turtle, and was then pulled back to the fishing vessel (Gudger 1919; Weiner 1921). *Remora remora* has been recorded on olive ridleys in Mexico (Diaz et al. 1992; Hernández-Vázquez and Valadez-González 1998), so the present observation is not surprising. Although this common remora is an expected epibiont, another species of sharksucker, *Echeneis naucrates*, is also

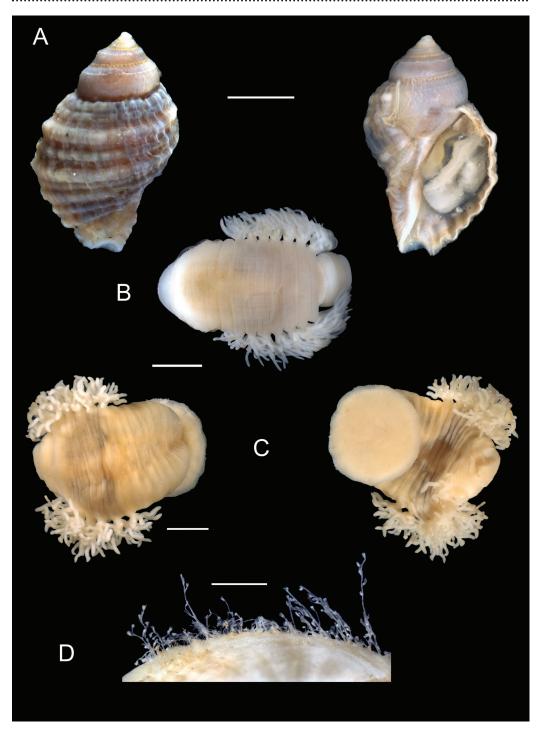


FIGURE 7. **A.** *Stramonita biserialis*, YPM IZ 028490. Outer and aperture view. Scale bar 5 mm. **B.** *Ozobranchus branchiatus*, YPM IZ 028413. Dorsal view. Scale bar 2 mm. **C.** *O. branchiatus*, YPM IZ 041631. Dorsal and ventral view. Scale bar 1 mm. **D.** *Obelia*? YPM IZ 028446. Detail of colony growing attached to edge of the barnacle Chelonibia testudinaria (YPM IZ 028491). Scale bar 2 mm.



FIGURE 8. Remora (*Remora remora*) attached to the carapace of an Olive Ridley Sea Turtle (*Lepidochelys olivacea*). Photograph by A. Peña de Niz.

known to occasionally attach to marine turtles (Sazima and Grossman 2006). The two are relatively easy to identify, but misidentification is possible in the field if specimens are not retained as vouchers for confirmation (Sazima 2006). *R. remora* is stout and uniformly brown or gray (some speckling possible) compared to the more slender *E. naucrates*, which typically has a black mid-body stripe bordered with white stripes along the body in small individuals, or only in the head region for larger individuals (I. Sazima, personal communication, 2009).

#### Discussion

The relatively many turtles sampled for epibionts during an extended period (the 2001, 2002 and 2008 nesting seasons) provides sufficient epibiont data to reveal generalized trends beyond what was seen in previous, briefer studies (for a single nesting season only). This and other studies of Pacific marine turtles make clear that overall epibiont diversity is much lower than that observed in Atlantic Ocean turtles and that the relative percentage of obligate turtle epibionts (those living exclusively on marine turtles and sometimes other vertebrates; that is, never free-living) is also much higher. For example, both Caine (1986) and Frick et al. (1998) observed more than 50 epibiont species for southwestern Atlantic turtles (86 different epibiont species in the latter study). Similarly, high epibiont diversity was also reported recently for loggerhead turtles nesting in the Gulf of Mexico (Lener 2010). Much of the diversity reported in those studies are nonobligate epibiont species and represent a subset of the local benthic diversity, given the high proportion of cryptic (that is, nestling) invertebrates such as amphipods and polychaete worms. In the Jalisco epibionts,

however, the only amphipod found, Podocerus chelonophilus, is a known obligate epibiont of turtles. No polychaetes were found; that is, the local benthic fauna contributes neglibly to the epibiont fauna. A similar pattern of low overall diversity combined with a high proportion of obligate epibionts was also reported for green turtles feeding in the waters surrounding Palmyra Atoll in the North Pacific (Gomez et al. 2011). Thus, clearly epibiont patterns of diversity are geographically influenced and with enough data might be predictable. We can only speculate on why epibiont diversity is low on Pacific turtles. However, notably, the general invertebrate diversity available for epibiont recruitment is remarkably different at Mexican coastal localities as compared to the North Pacific Palmyra Atoll, given the very different ecological settings. Despite the carbonate environment of the atoll and its rich benthic fauna, the proportion of nonobligate epibionts was low, a pattern that mirrors what we observed at Jalisco. This suggests a general trend of overall low diversity in Pacific epibionts regardless of geography or ecological setting.

Although it is tempting to speculate, there is nothing to suggest that Pacific olive ridley and green turtles can somehow regulate their epibiota to limit nonobligate forms (thereby contributing to lower diversity). However, a mechanism involving a turtle immune response to epibiont resistance by Lepidochelys olivacea or Chelonia mydas would be an interesting avenue of research. Frick, Kopitsky et al. (2011) have inferred that chemical mediation could have some role in regulating the embedding of coronuloid barnacles to certain hosts, but this scenario cannot be extrapolated to attachment by other groups of epibionts, especially nonobligate forms. Instead, a more plausible explanation of low epibiont diversity on Pacific green and olive ridley turtles might be the relative absence of algae and hydroids on these hosts; although present, the densities of these epibiotic elements are low. Both algae and hydroids are not only epibionts themselves, they also function as potential primary substrates for cryptic invertebrates such as amphipods (Bousfield 1973; Johnson 1986). The lack of this potential habitat undoubtedly affects nonobligate cryptic epibionts. More detailed work into the ecological structure of epibiont populations and how the nonobligate species are recruited should prove insightful in this regard.

Documentation of the biodiversity of marine turtle epibionts has gained much attention as invertebrate and sea turtle biologists try to understand the interrelationship between sea turtles and the vast biodiversity of marine organisms that live on turtle carapaces, plastrons and soft tissues. Most epibiont studies in the literature lack any discussion of collection protocol and often list organisms haphazardly collected from a particular sea turtle species nesting on a particular beach. Some papers give the body landscape location where the epibiont was collected (Pfaller et al. 2008; Fuller et al. 2010) and, although their conclusions about spatial distribution patterns were not compelling, obviously research along these lines requires that epibionts must be collected carefully. A haphazard approach that does not indicate body landscape location will result in lost information that may be significant for the life history of both turtle and epibiont. We therefore, in an effort to standardize the procurement of turtle epibionts, provide here protocol for collecting and documenting these from sea turtles (Appendices 1 and 2).

#### Acknowledgments

We thank Melissa Salgado, Damon Nakamura and Laura Smowle for their help with fieldwork; these students were supported by the Yale College Studies in the Environment Program. Further support for M. Salgado was received from the Yale Class of 1954, and L. Smowle received additional support from the Environmental Engineering Yale Summer Program. Fieldwork by A. Feuerstein was supported by the Yale Peabody Museum Summer Internship Program (2008). Additional support was provided by Connecticut State University-AAUP to T. Pinou. The Brignone family of Costa Careyes, Jalisco, Mexico, generously provided field and logistical support, and access to beaches where the study was conducted, and helped arrange lodging for two of us (E.A. Lazo-Wasem and A. Feuerstein) during the 2008 field season. Also, the family of A. Peña de Niz, especially his wife Dulce, provided considerable general assistance to A. Feuerstein while she lived in Jalisco during the summer of 2008. We thank our colleagues at the University of Guadalajara for their assistant at Campamento La Gloria. Ivan

Sazima (Universidade Estadual de Campinas) generously supplied the diagnostic characters to distinguish remoras. Suggestions by reviewers Adam Baldinger (Museum of Comparative Zoology, Harvard University), Christopher Boyko (Dowling College), Michael Frick (Gainesville, Florida) and John Zardus (The Citadel) greatly improved the manuscript. Lourdes Rojas (Yale Peabody Museum) helped with many iterations of the data tables and Daniel Drew (Yale Peabody Museum) pointed out valuable references for historical accounts of remora.

Received 2 June 2011; revised and accepted 15 July 2011.

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#### Appendix 1: Standardized Collecting Protocol for Marine Turtle Epibionts

Most epibiont reports are derived from sampling conducted during the monitoring of sea turtle nesting. Scientists working on beaches with sea turtle monitoring programs are those currently providing the most epibiont information. The bilingual collection data sheet provided here was developed from a combination of the turtle monitoring data sheets currently used by the Riverhead Foundation for Marine Research and Preservation (Long Island, New York, USA) and the Programa Universitario para la Protección y Conservación de la Tortuga Marina, Universidad de Guadalajara, Centro Universitario de Ciencias Biologicas y Agropecuario (CUBCA, Guadalajara, Mexico).

These standardized data collecting sheets are general enough to be used for any sea turtle species by any sea turtle research program. Included is a diagram (dorsal and ventral) of a generic marine turtle that can be used to indicate host locations sampled for epibionts. Letter labels can be used to designate body regions. These lettered regions should correspond to appropriately labeled specimen tubes. An editable version of the data collecting sheet provided here is available for download at http://peabody.yale.edu/collections/invertebrate-zoology/turtle-epibiont-project.

#### Field Sampling

Whenever possible preserve specimens collected from different areas on the turtles separately, according to the following body landscape regions:

Head (A) and neck (B) Forelimbs (C) and shoulders (D) Hind limbs (E) and cloacal area (F) Carapace (G) Plastron (H)

Place epibiont specimens in containers labeled according to the turtle and its body landscape region where collected. Do not put in more than the volume of the container can hold. Immediately fill the container to the top with 75% alcohol and seal the lid. Invert the jar a few times to disperse the alcohol throughout the samples. *Note:* Use 75% alcohol to achieve the target of 70% alcohol after wet (waterretaining) specimens are added.

If any DNA extraction is to be done, the alcohol used to fix specimens must be consumable-grade ethyl alcohol. DNA will not be properly preserved with methyl ("denatured") alcohol or isopropyl ("rubbing") alcohol.

It is crucial to mark the date on the data sheets in the format "dd month yyyy" (for example, 05 Nov 2007). This ensures that there can be no confusion as to the precise date. Along with a unique turtle number (specific for a particular year) and the body landscape region, this will uniquely identify the collecting event. Although informally the nesting season can be noted, the day, month and year the sample is collected must always be clear.

#### Labeling Specimens for

#### Research and Museum Curation

Sort each specimen container by grouping and counting identical taxa. Write the necessary sample identification in pencil or alcohol-resistant ink on a wet-strength paper label placed inside the container. Wet-strength label stock marketed as Resistall can be obtained from University Products, Inc., Holyoke, Massachusetts, USA (http://www.universityproducts.com/). Similar prod-ucts can be substituted. Standard paper stock disinte-grates rapidly in fluid preservative and should not be used. Secondary labeling on the outside of the jar can be done for sample identification only, but must not be relied on as the only label. Labels on a plastic jar can become smudged and illegible, and should be avoided when possible.

When sorted, the samples must be distinguishable immediately by a unique identifier, therefore a simple number and letter combination is not sufficient. There have been many instances in which two samples from different years bear the same number, leading to much confusion. A sample identification label should include the year (not the season) when the sample was taken as a prefix, as follows: 2011-1 through 2011-XXX.

The combination of a year and turtle number permits any number to be re-used without confusion. Include an abbreviation for the body landscape region (instead of the letters A to H) on the paper label with the collection number inside the container, to indicate where on the turtle the epibiont came from. This descriptive labeling is more reliable for long-term data preservation because it is not easily confused. Suggested standardized abbreviations include:

> Head (H) and neck (N) Forelimbs (FOR) and shoulders (SHD) Hind limbs (HIN) and cloacal area (CLO) Carapace (CAR) Plastron (PLS)

For example, the label "2011–68 CAR" indicates that the specimens in the jar belong to the 68th turtle sampled in 2011 and that the epibionts were removed from the carapace. More detailed notes can be provided on the data sheet.

#### Shipment of Specimens

Be sure to travel with a copy of the permit authorizing the collection of the epibionts and a letter or permit indicating that the specimens can legally be transported out of the originating country. Samples to be transported by aircraft (in personal baggage or as air cargo) must not be in ethyl alcohol, which is a violation of International Air Transport Association (IATA) regulations. Instead, samples should be carefully decanted through a small screen, the screen rinsed with shipping fluid (see below for a description) back into the container, and additional shipping fluid added to fill the jar.

To seal the jar, wrap a small amount of Teflon<sup>\*</sup> tape (available from hardware and plumbing supply stores) around the threads and close the jar tightly. Seal the outside of the lid by wrapping it with white or black electrical tape pulled tight on itself and cut cleanly. These steps will insure no leakage.

The shipping fluid to use is Carosafe<sup>\*</sup>, a proprietary product marketed by Carolina Biological Supply Co., Burlington, North Carolina, USA (http://www. carolina.com/). This fluid is safe for shipping specimens that may ultimately be the subject of molecular work. Dilute the concentrated fluid (9 parts water to 1 part concentrate). Because it is not a hazardous material, there is no fear that material will be confiscated. It is imperative, however, to have a copy of the proprietary information in the box with the specimens, along with note to Transportation Security Administration (TSA) inspectors that reads as follows:

#### To: TSA Inspectors

These preserved scientific specimens are being shipped in Carosafe\* liquid, a nonhazardous material as defined by current IATA regulations, and thereby is allowable as a shipping solution. Its primary ingredient is propylene glycol, a US FDA-approved food additive. If specimen containers are opened for inspection, they must be resealed tightly and placed upright to prevent leakage; if specimens dry out they will lose their scientific value.

Thank you,	
Name:	
Title:	

Be sure to include a technical description, or Material Safety Data Sheet (MSDS) of the Carosafe\* liquid (or other acceptable shipping fluid) with the samples.

# Specimen Deposition and

## US Reporting (Importation)

As soon as possible after arrival at the receiving institution, the shipping fluid must be decanted from the sample jar through a 0.5 mm mesh standard screen (to catch tiny epibionts) and the screen rinsed with 75% nondenatured alcohol. Add more alcohol to make sure that the sample container is filled to the top, sufficient to dilute any remaining shipping fluid.

Within 180 days a US Fish and Wildlife Service 3-177 Import/Export Permit must be filed with the US FWS regional office or through the US FWS Electronic Declarations (eDecs) website (https://edecs.fws.gov/); forward a printed copy once electronic approval is received.

This filing, although mandatory, does not need to take place during import and export. Researchers should be prepared to remind inspectors at the time of import of the 180-day reporting period, and it is important to carry a copy of the relevant page of the US Code of Federal Regulations (Title 50, Part 14, "Importation, Exportation, and Transportation of Wildlife" [50 CFR 14.64(b)(3)]) that describes the reporting rules. .....

# Appendix 2: Epibiont Data Collecting Sheet

Field number (Número de campamento)			Date and time (Fecha y hora)			
Beach number (Núm	iero de la play	ra)				
City/Town(Ciudad/Pueblo)			_ County (Municipal)			
Latitude and longitue	de (Latitud y l	ongitud)				
				ees (grados)/decimals (decimales)		
Host species (Especie						
🗆 Positive (Po	sitivo)	🗆 Probable	(Probable)	□ Unsure (Inseguro)		
Nearest body of wate	er (Cuerpo de	agua mas cerc	cana)			
□ Offshore (Fi	uera de la cost	a)		□ Inshore (Costa)		
Water temperature (	Temperatura c	lel agua)	Air tempera	ture (Temperatura del aire)		
Cloacal temperatura	(Temperatura	cloacal)				
Ни	man Interacti	on (Informaci	ón sobre la inte	eracción humana)		
Fishery interaction (I	nteracción pes	cadera):				
$\Box$ Yes (Sí) $\Box$	No (No)	🗆 Cannot b	e determined (No se puede determinar)			
Evidence of boat colli	sion (Evidenci	a de golpes de				
🗆 Yes (Sí) 🛛	No (No)	e determined (	l (No se puede determinar)			
[all	measurement	Measurements and weights	<i>nts (Medidas)</i> in metric units	(todos métricos)]		
Straight carapace leng	gth (Largo rect	o del caparazó	n)			
Straight notch to notc	h (Recto marc	ca a marca)				
Curved carapace leng	th (Largo curv	vo del caparazó	ón)			
Curved notch to notc	h (Curvo mare	ca a marca)				
Straight maximum ca	rapace width (	Ancho máxin	no recto del cap	parazón)		
Curved maximum ca	rapace width (	Ancho curvo	del caparazon)			
Plastron length across	s mid-line (La	rgo del plastró	n, a travéz de la	línea media)		
				o de la cola extendida		
	,			Tags present (¿Etiquetas presente?) □ Yes (Sí) □ No (No)		
		$\sim$	$\sum$	If yes, enter identification number for both PIT and metal tags		
	$\mathcal{P}($		Y	(Si sí, el Número de identificación para PIT y "metal tags"):		
		t C	$\mathcal{A}$	Left fore flipper (Aleta izquierda)		
$\bigcirc$	)		$\left( \right)$	Right fore flipper (Aleta derecha)		

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Appendix 2 cont	inued						
Blood drawn? (¿Sacaron sangre?) Date (Fecha)		□ Yes (Sí) □ No (No) Time (Hora)		Initials (Iniciales	s)		
Photos taken? (¿Tomaron fotos?)		$\Box$ Yes (Sí) $\Box$ No (No)					
	Turtle (Tortuga)						
	Nest (Nido) Track (Rastro)						
Number of eggs c Egg translocation Date (Fecha)	ollected (Número notes (Ficha de se Time	de huevos co embrado) (Hora)	electados) Nest n	umber (Número de	e nido)		
Sul	bnest Co	blumn lumna)	Row (Hilera)	Number of eggs (Huevos semb	s buried		
Eggs damaged? (¿Huevos dañados?) Eggs damaged during transportation (Huevos dañados en el transporte) Eggs damaged while buried (Huevos dañados en el sembrado) Observer (Observador)							
Epibionts collecte	d? (¿Epibióticas co	olectados?)	□ Yes (Sí)	🗆 No (No)			
Notes (Notas):	_						