

A remarkable infestation of epibionts and endobionts of an edible chiton (Polyplacophora: Chitonidae) from the Mexican tropical Pacific

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ABSTRACT

Although epibiosis is common in polyplacophorans, we describe an unusual presence of epibionts and endobionts in a single adult specimen of *Chiton articulatus* collected in Guerrero, Mexico, from an eroded habitat of crevices with high wave activity. The epibiont and endobiont specimens covered nearly 90% of the central and lateral areas of the chiton valves while the border of mantle girdle showed no epibiosis. Crustose and filamentous algae, and crustacean arthropods from two common barnacle families, Chthamalidae and Balanidae, represent the observed epibionts. Polychaete (Annelida), bivalve mollusks from two families: Pteriidae (*Pinctada mazatlanica*) and Mytilidae (*Leiosolenus aristatus*), and crustacean arthropods from the burrowing barnacle family Cryptophialidae (*Cryptophialus wainwrighti*) represent the observed endobionts. In addition, finding of *Cryptophialus wainwrighti* represents a new geographic range extension from the type locality in Sinaloa to Guerrero. Epibiosis studies of invertebrates in the intertidal rocky shore, such as the dominant *C. articulatus*, can assist in understanding ecological relationships and patterns of diversity in coastal communities.

Additional Keywords: epibiosis, endobiosis, basibiont, Cirripedia, Chthamalus spp., Balanidae, Polychaeta, Bivalvia, *Leiosolenus aristatus*, *Pinctada mazatlanica*, Acrothoracica, *Cryptophialus wainwrighti*

INTRODUCTION

Common in aquatic habitats, epibiosis is the association between a living substrate organism (*basibiont*) and a sessile organism (*epibiont*) attached to the basibiont's outer surface without trophically depending on it (Wahl, 2010). In endobiosis, an organism (*endobiont*) lives under the external surface of its basibiont (Wahl, 1989, 1997; Wahl and Mark, 1999; Trigui El-Menif et al., 2008; Wahl,

2010; see Taylor and Wilson, 2002 for a more complex terminology). In some studies epibiosis is included generally as fouling (e.g., Mendez et al., 2014), biofouling (e.g., El Ayari et al., 2015), or without specific terminology (e.g., Buschbaum et al. 2007).

Epibiosis is found worldwide, especially in marine environments, where any exposed solid surface is likely to be colonized by organisms (Wahl, 1989). Sessile organisms are the major constituents of these communities (Canning-Clode and Wahl, 2010; Mendez et al., 2014). The basibionts more frequently studied are mollusks (Wahl and Mark, 1999; Wahl, 2010), especially those with economic importance such as gastropods and bivalves (e.g., see Table 19.2 in Dürr and Watson, 2010).

Epibiosis has been poorly documented for the class Polyplacophora, where epibionts and endobionts occur in/on the chiton valves. Arey and Crozier (1919) reported adventitious organisms on the dorsal surface of *Chiton tuberculatus* Linnaeus, 1758, including epizoic barnacles and algae, with other organisms living between the algae. Reports of chiton epibiosis have also been represented by pictures, such as in MacGinitie and MacGinitie (1968: 388, fig. 243) where *Mopalia hindsii* is pictured with its valves covered by algae and invertebrates. Bullock and Boss (1971) documented epibiotic calcareous algae, bryozoans, polychaete tubes, and the detrimental endobiont *Leiosolenus aristatus* (Dillwyn, 1817) boring into the valves of *Chiton stokesii* Broderip, 1832, in the southernmost part of the Panamic Province, and *C. tuberculatus*, from the Caribbean. Watters (1981) reported another eastern Pacific mytilid, *Leiosolenus spatiosa* Carpenter, 1857, in the valves of the chiton, *Acanthochitona hirudiniformis* (Sowerby I, 1832). Other epibionts reported on the valves of *Chiton tuberculatus* include species of the sessile barnacle genus *Tetraclita* Schumacher, 1817, calcareous tube-dwelling polychaetes, *Spirorbis* Daudin, 1800 and *Serpula* Linnaeus, 1758, and green algae including *Ulva* Linnaeus, 1753. The algae provide protection for juvenile mollusks, nematodes, archannelids, and protozoans. Bullock and Boss (1971) did not consider any of the reviewed epibionts to be

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harmful to the host. Phillips (1972) studied the biota on the intertidal chiton *Mopalia muscosa* Gould, 1846, primarily algae and mollusks, and other organisms. Dell'Angelo and Lagui (1980) mentioned an epizoic encrusting bryozoan on the valves of *Chiton olivaceus* Spengler, 1797. While most chiton epibiont and endobiont observations have been made on intertidal and subtidal species, Sigwart (2009a) documented epibiont foraminifers *Hyrrokin sarcophaga* Cedhagen 1994 on *Leptochiton arcticus* (G. O. Sars, 1878).

The endemic Mexican chiton *Chiton articulatus* Sowerby in Broderip and Sowerby, 1832, is the largest, most abundant, and dominant chiton of the intertidal rocky shore (Galeana-Rebolledo et al., 2014) found along the tropical Pacific coast. It occurs between the states of Sinaloa and Oaxaca, 23°N to 15°N (Ferreira, 1983; Reyes-Gómez and Salcedo-Vargas, 2002; Kaas et al., 2006; Reyes-Gómez et al., 2010). *Chiton articulatus* is used as food, for fish bait, and targeted as an artisanal fishery (García-Ibáñez et al., 2013; Flores-Garza et al., 2012a). It has gained regional importance and economic interest in the southern Mexican Pacific, where restaurants offer it as a gourmet and aphrodisiac item (Ríos-Jara et al., 2006; Avila-Poveda and Abadia-Chanona, pers. observ.). However, it is not currently cultivated and is unregulated by the government.

The aim of this work is to describe the epibionts and endobionts found outside and inside of the valves of a single adult specimen of *Chiton articulatus*, collected in the southern portion of its known area of distribution.

MATERIALS AND METHODS

During one of several campaigns to evaluate the biodiversity of mollusks in the intertidal rocky shores of Guerrero State, Mexico (Galeana-Rebolledo, 2011; Flores-Garza et al., 2012b; Galeana-Rebolledo et al., 2012, 2014), an unusual adult specimen of *Chiton articulatus* was observed to be heavily infested with epibionts and endobionts. The chiton with epibiosis was collected at Ojo de Agua, Guerrero, Mexico (17.300°N, 101.0526°W) from exposed rocks facing the open ocean, where human harvesting would be difficult. The specimen and its epibionts were relaxed following protocols described by Avila-Poveda (2013), fixed with 90% ethanol, and preserved in 70% ethanol. The specimen measured 43.4 mm in length and 32.1 mm in width including the mantle girdle. This corresponds to the adult stage in the species, according to Avila-Poveda and Abadia-Chanona (2013). This specimen was deposited at the Santa Barbara Museum of Natural History (SBMNH), Santa Barbara, California, USA (SBMNH 235597). The epi- and endobionts observed were recorded according to chiton valve number (I–VIII), identified, and deposited at the SBMNH and the Colección Nacional de Crustáceos (CNCR) at the Instituto de Biología of the Universidad Nacional Autónoma de México (IB-UNAM).

RESULTS

The epibionts and endobionts specimens cover nearly 90% of the central and lateral areas of the chiton valves, while the border where the valve had contact with the mantle girdle did not display epibiosis (Figure 1).

EPIBIONTS

The epibionts included two algal morphotypes, one filamentous and the other crustose. Both types were distributed on every chiton valve. Other epibionts were crustaceans, two distinct barnacles, chthamalines and balanids (Figures 1, 12–15), with 26 epibionts in total. All specimens were <4 mm in diameter. The chthamalines (Chthamalidae) were *Chthamalus* Ranzani, 1817 species (Figures 13–14, SBMNH 235604). Also found were six tubiferous, calcareous balanid bases with pores (Balanidae) (Figures 16–17, SBMNH 235609).

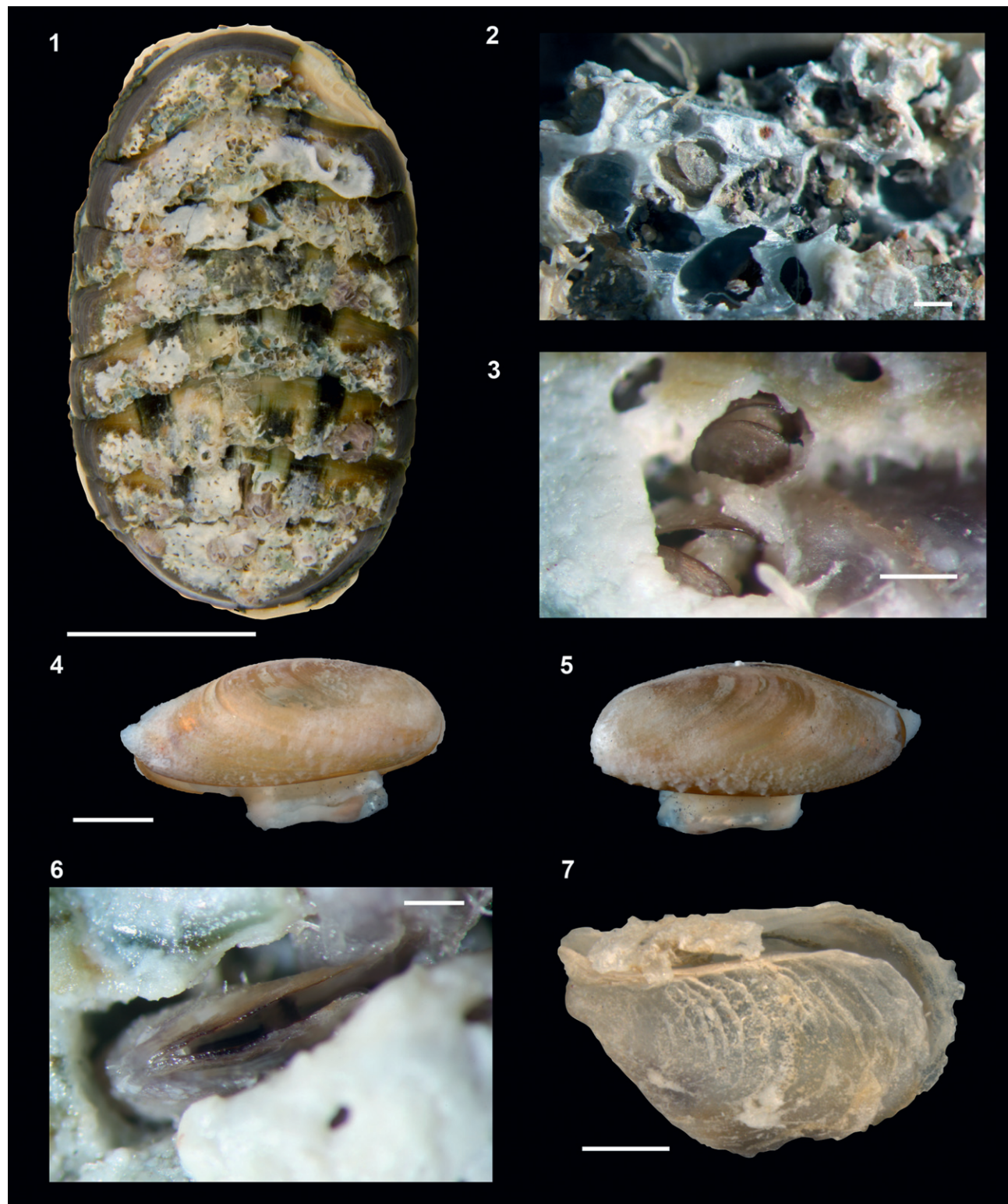
ENDOBIANTS

One individual of a free-living polychaete (Annelida) was found in chiton valve VIII. The polychaete could not be identified due to its small size (< 2 mm length) and damage during dissection. Mytilidae endobionts were recorded with 68 individuals of *Leiosolenus aristatus* (Dillwyn, 1817) (Figures 2–5, SBMNH 235588–235594); one *L. aristatus* specimen had perforated the chiton valve, ending just 1–2 mm short of the dorsal musculature. One *Pinctada mazatlanica* (Hanley, 1856) specimen was inside the valve and byssally attached to the chiton valve surface; whereas two *P. mazatlanica* (Figures 6–7, SBMNH 235595–235596) specimens were found deep inside the valves in abandoned boreholes.

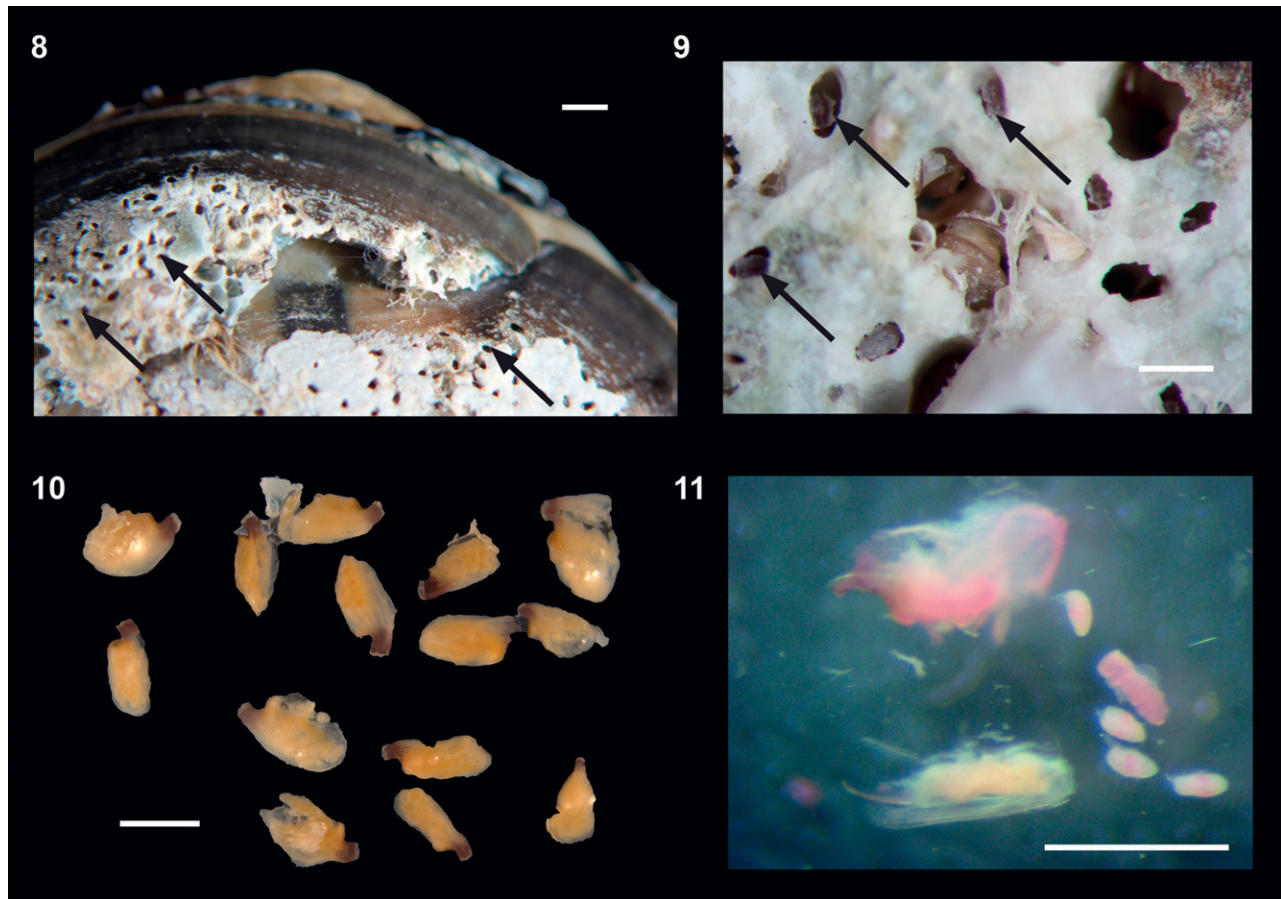
Burrowing acrothoracicans (Cryptophialidae) included 391 *Cryptophialus wainwrighti* Tomlinson, 1969 (Figures 8–11, SBMNH 235599 and 235605 and CNCR 29987). The chiton valves had many small, more or less circular holes on the surface, after dissection of the valves, each hole yielded one *Cryptophialus* female. No minute males were observed. Females, about of 1 mm in length, were apparently brooding embryos, as an opened specimen released four ovoid embryos of cyprids with immature antennules (Figure 11). During dissections (n=4) eggs were observed. The first female had 23 eggs with no eyes, in the second female had 10 eggs with eyes, the third 15 eggs with eyes (Fig. 11), and the last female had no eggs. Eggs with more marked eyes represent the cyprid stage, and during dissections earliest stages with eyes forming were observed, but not any earlier naupliar stages.

ABUNDANCE BY CHITON VALVE

Epibionts and endobionts were present on all eight chiton valves, with 495 individual organisms in total, 6% were epibionts (n=32) and 94% endobionts (n=463) (Table 1). The anterior region, valve I to III, had fewer



Figures 1–7. *Chiton articulatus*. **1.** Dorsal view with numerous juvenile barnacles, largely chthamaline barnacles, plus a few balanid barnacle bases, generally on the eroded valves encrusted and riddled with smaller epibionts. Scale bar = 1 cm. SBMNH 235597. **2.** Mytilid bivalve *Leiosolenus aristatus* boring into valves. Scale bar = 1 mm. **3.** Close up of posterior end of *L. aristatus* in valves. Scale bar = 500 μ m. **4, 5.** Right and left lateral views of *L. aristatus*, specimen length 1 mm. SBMNH 235588. **6, 7.** Pteriid bivalve *Pinctada mazatlanica* nestling into old boreholes in valves of *C. articulatus*. Scale bar = 500 μ m.



Figures 8–11. *Chiton articulatus*. **8.** Close up of valves showing boreholes (arrows indicating some) of the acrothoracican barnacle, *Cryptophialus wainwrighti*. Scale bar = 1 mm. **9.** Close up of some boreholes showing the opercular bars of the female barnacles (arrows). Scale bar = 500 μ m. **10.** Fourteen *C. wainwrighti* females with eggs and developing embryos in their mantle cavities (dark “neck” of sac supporting opercular bars seen in Figure 9, extending toward opening of the burrow). Scale bar = 1 mm. **11.** Partially dissected female with four immature cyprid larvae. Scale bar = 1 mm.

epibionts compared with the central and posterior regions; valve III had the fewest epibiosis (n=34 organisms) in contrast, valve VIII had the greatest (n=117 organisms) (Figure 18).

DISCUSSION

EPIBIONTS

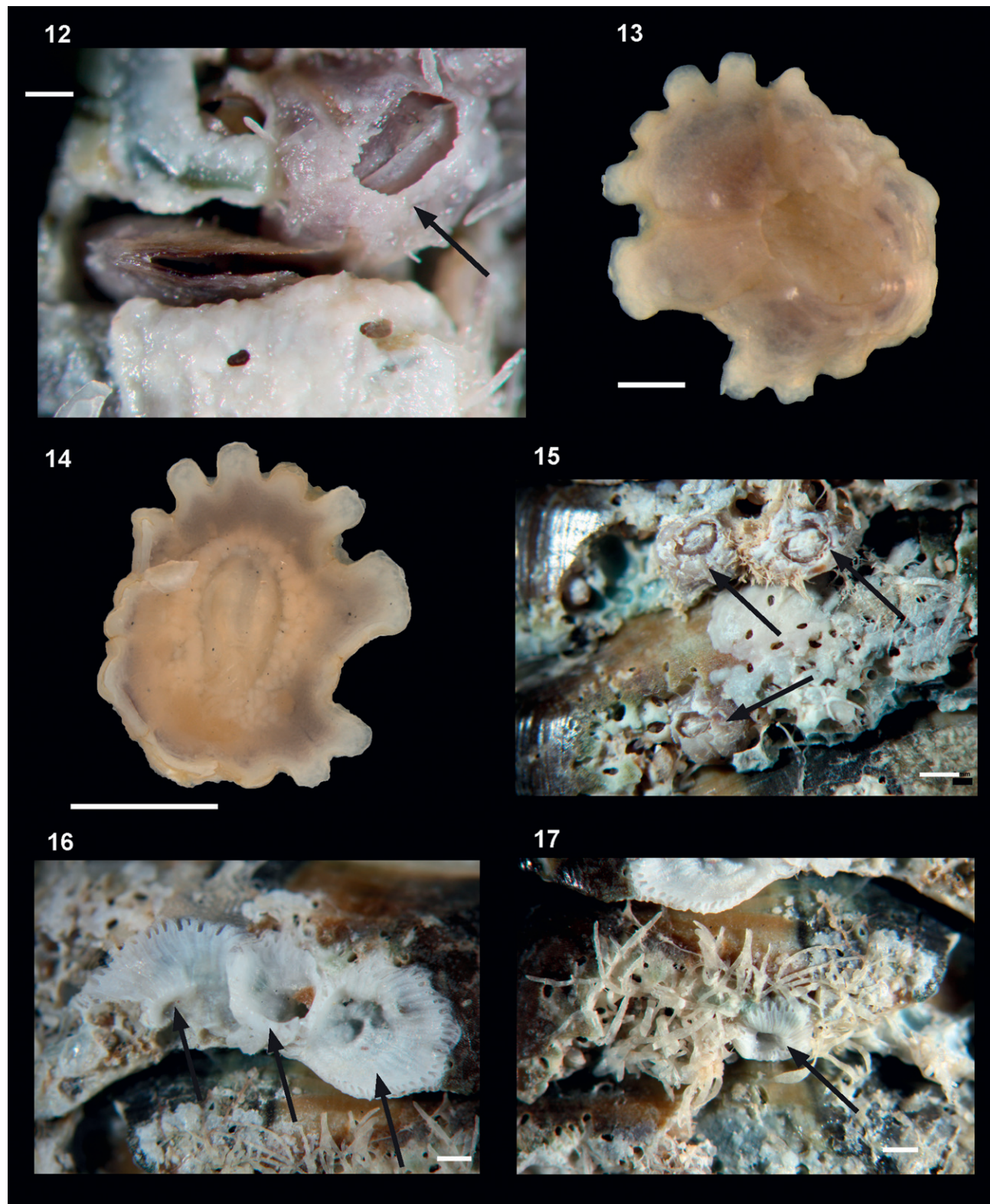
Chthamalines and Balanids: There is uncertainty about the identification of the chthamaline acorn-barnacle epibionts. According to Meyers et al. (2013), there are potentially three species of *Chthamalus* at this latitude. One is a northern species that is more typical of sheltered habitats, *C. southwardorum* Pitombo and Burton, 2007 (according to Newman et al. [2016] proposed name change). The other two are found in wave-exposed habitats, the northern *C. hedgecocki* Pitombo and Burton, 2007 and the southern *C. panamensis* Pilsbry, 1916. However, Chan et al. (2016) restricted the latter to south of 15°N (Tehuantepec), whereby there would be but two

species, *C. hedgecocki* from exposed environments and *C. southwardorum* relatively protected ones. While chances are that the juveniles on this chiton were likely the former, the later cannot be ruled out.

Balanid Bases: Likewise, the balanid bases observed could not be specifically identified. The tubiferous calcareous bases with pores found are typical of balanids (Newman and Ross, 1976). However, the bases alone cannot be identified to subfamily, much less generic level, as the specimens were incomplete and some were likely immature. Considering the balanids that are recorded for this area and their characteristics, the bases could be from any one of three of the four subfamilies present in the region: Amphibalaninae Pitombo, 2004, Concavinae Zullo, 1992 and Megabalaninae Newman, 1979.

ENDOBIONTS

Polychaeta: Galleries of annelids have also been observed on other chiton species collected along the



Figures 12–17. Balanomorph cirripeds from *Chiton articulatus*. **12.** Chthamaline barnacle, *Chthamalus* sp. (arrow) attached to valve. Scale bar = 500 μ m. **13, 14.** Juvenile of *Chthamalus* sp. removed from one valve and photographed from above and below. Scale bars = 500 μ m and 1 mm respectively. **14.** Juvenile *Chthamalus* sp. in ventral view. Scale bar = 1 mm. **15.** Balanid barnacles (arrows) attached to valve. Scale bar = 1 mm. **16.** Tubiferous balanid barnacle bases (arrows) on valve II. Scale bar = 500 μ m. **17.** Balanid basis (arrow) amongst the algal fronds, between valves II and III. Scale bar = 5 mm.

Table 1. Summary of epibionts and endobionts found, on and in the valves respectively, of a single specimen of *Chiton articulatus*. Valve I (anterior) to VIII (posterior); btb: balanine tuberous bases. All of *Chthamalus* were juvenile of at least two if not three species, *C. hedgecocki*, *C. southwardorum* and *C. panamensis* according to Meyers et al. (2013) or *C. hedgecocki* and *C. southwardorum* according to Chan et al. (2016).

N° of valve	Epibionts		Endobionts	
	N° of organisms	Species	N° of organisms	Species
I	1	<i>Chthamalus</i> spp.	4	<i>Leisolenus aristatus</i>
II	3	Balanidae: btb	34	<i>Cryptophialus wainwrighti</i>
			2	<i>L. aristatus</i>
III	1	Balanidae: btb	34	<i>C. wainwrighti</i>
			13	<i>L. aristatus</i>
IV	2	<i>Chthamalus</i> spp.	18	<i>C. wainwrighti</i>
			1	<i>L. aristatus</i>
V	1	Balanidae: btb	7	<i>L. aristatus</i>
			4	<i>Chthamalus</i> spp.
VI	9	<i>Chthamalus</i> spp.	1	<i>Pinctada mazatlanica</i>
			77	<i>C. wainwrighti</i>
VII	5	<i>Chthamalus</i> spp.	9	<i>L. aristatus</i>
			1	<i>P. mazatlanica</i>
VIII	5	<i>Chthamalus</i> spp.	57	<i>C. wainwrighti</i>
			12	<i>L. aristatus</i>
Total	32		34	<i>C. wainwrighti</i>
			11	<i>L. aristatus</i>
			1	<i>P. mazatlanica</i>
			36	<i>C. wainwrighti</i>
			1	Polychaeta
				<i>L. aristatus</i>
				<i>C. wainwrighti</i>
			101	
			463	

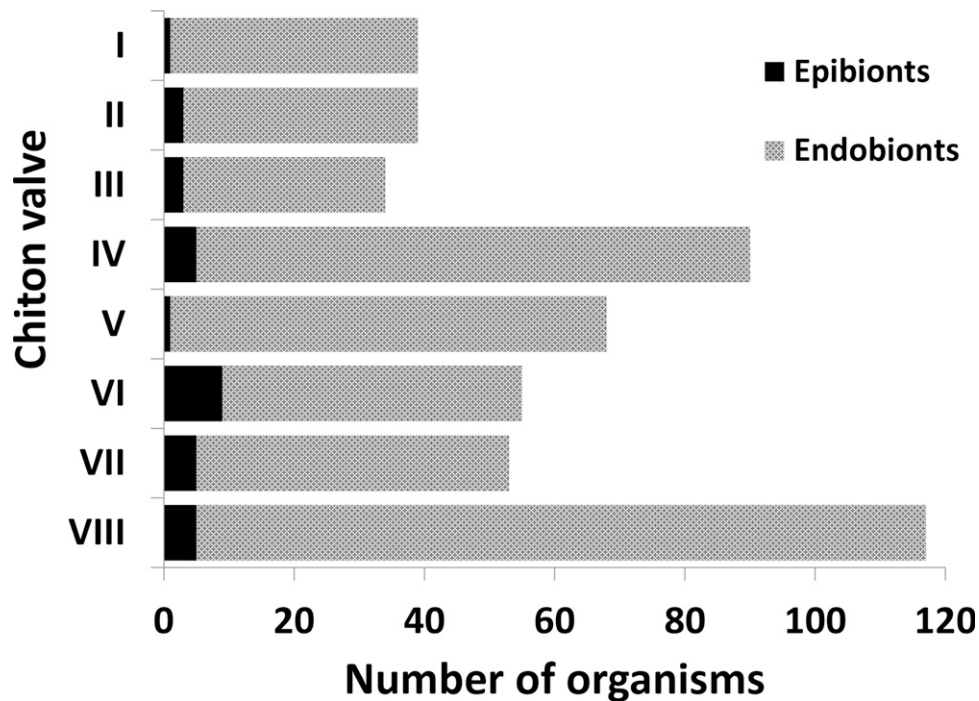


Figure 18. Abundance distribution of epibionts and endobionts by each valve of a single specimen of *Chiton articulatus*. Chiton valves: I, anterior; II–VII, intermediates; VIII, posterior.

coast of Guerrero, including *Chiton albolineatus* Broderip and Sowerby, 1829, *Lepidochitona* sp., *Chaetopleura unilineata* Leloup, 1954, and *Chaetopleura lurida* (Sowerby, 1832). While they have not been studied here, representative specimens are deposited at the Colección Nacional de Moluscos (CNMO) at IB-UNAM.

***Leiosolenus aristatus*:** Bullock and Boss (1971) only found the mytilid bivalve *Leiosolenus aristatus* in “large specimens” of *Chiton stokesii* Broderip in Broderip and Sowerby, 1832 and *C. tuberculatus* (Linnaeus, 1758); these authors did not report the size of chitons. Watters (1981) found *Leiosolenus spatiosus* (Carpenter, 1857) in three chitons of different sizes, all of them seemingly adults. Some reports found chiton epibionts only on larger specimens (Bullock and Boss, 1971; Watters, 1981). In the western Atlantic chiton *Ceratozona squalida* (C.B. Adams, 1845), body size was unrelated to percent cover of epibiotic algae on the girdle (Connelly and Turner, 2009).

Leiosolenus aristatus occurs in warm-temperate to tropical waters in the eastern Pacific, western Atlantic, and eastern Atlantic regions (Valentich-Scott and Dinesen, 2004; Coan and Valentich-Scott, 2012). The species was reported boring in the valves of *Chiton stokesii* and *Chiton tuberculatus*. *Leiosolenus aristatus* bores into calcareous substrates, including the shells of large bivalves (e.g., *Spondylus* Linnaeus, 1758, *Chama* Linnaeus, 1758, *Ostrea* Linnaeus, 1758) and gastropods (e.g., *Haliotis* Linnaeus, 1758, *Patella* Linnaeus, 1758, *Strombus* Linnaeus, 1758, and *Pleuroploca* (P. Fischer, 1884), as well as corals and rocks (Coan and Valentich-Scott, 2012). In the collections of the Santa Barbara Museum of Natural History (SBMNH), *L. aristatus* is present in specimens of *Astraea* Röding, 1798, *Calyptraea* Lamarck, 1799, *Chama*, and *Lottia* Gray, 1833, as well as dead coral (Valentich-Scott, pers. obs. November 2016). It is usually found in shallow water, although Coan and Valentich-Scott (2012) reported shells collected as deep as 300 m. It has recently been reported from the Mediterranean Sea, boring into shells of the muricid gastropod *Stramonita haemastoma* (Linnaeus, 1767) (El Ayari et al., 2015).

Compared to *Chiton stokesii* and *C. tuberculatus* (data in Bullock and Boss, 1971), the single specimen of *C. articulatus* presented here had more *Leiosolenus aristatus* individuals boring into its valves. It is possible that this could be due to differences in shell hardness and susceptibility for fouling and boring among *C. articulatus* and its congeners. Alternatively, the valve erosion experienced by this chiton specimen might have played a significant role in allowing epibionts to settle. Watters (1981) observed chiton valve erosion was a prerequisite to mytilid boring, and that the boreholes involved the destruction of large portions of both the tegmentum and articulamentum.

***Pinctada mazatlanica*:** This pteriid bivalve is not a borer, but likely uses empty *Leiosolenus* holes as a refuge.

Pinctada mazatlanica is a large species, reaching a length of 150 mm (Coan and Valentich-Scott, 2012). The bivalves are likely only using the chiton valves as a temporary refuge during a juvenile stage. It is unknown what damage might occur to the chiton, or to the bivalves themselves, as the pteriids continue to grow.

Acrothoracican Barnacles: *Cryptophialus wainwrighti* has been reported from western Mexico (Tomlinson, 1969), found in the marine gastropods *Vasula speciosa* (Valenciennes, 1832) and *Stramonita biserialis* (Blainville, 1832). The only other eastern Pacific species in the genus is its Southern Hemisphere (mostly Chilean) counterpart, *Cryptophialus minutus* Darwin, 1854, which is known to occur within the shells of several mollusks, including *Chiton magnificus* Deshayes, 1827 (Castilla, 2009; Kolbasov, 2009; Pitombo 2010). *Chiton magnificus* is reported to range from Isla San Lorenzo, Peru (12° S) to Tierra del Fuego (55° S), but how much of this remarkably wide range the barnacle occupies is unknown. Another cryptophialid, *Australophialus utinomii* Tomlinson, 1969, attacks the giant chiton, *Dinoplax gigas* Gmelin, 1791 (Chaetopleuridae), from South Africa. Not only are these the only cryptophialid species known to attack chitons, two of them are attacking species of the same genus, *Chiton*. While the known occurrences were noted in Kolbasov (2009), he did not mention chitons in his extended discussion of interactions between acrothoracicans and their hosts. Furthermore, while Yeh et al. (2005) listed 18 chiton species known from Taiwan and nearby islands, one of which is a species of *Chiton*, none of the 18 acrothoracicans from Taiwan reported by Chan et al. (2014), including two species of *Cryptophialus*, are known to attack chitons.

The only other acrothoracican barnacle known from the west coast of Mexico is the lithoglyptid *Kochlorine hamata* Noll, 1872. While previously known from elsewhere in the world, Tomlinson (1969) reports it from Acapulco, Guerrero, Mexico, and in the Gulf of Panama. The burrow opening of this genus differs from that of *Cryptophialus* in being slit-like rather than round or oval and the opercular bars are correspondingly relatively long and fusiform with the sac rather than being supported by an elongate neck. While *K. hamata* is known to attack a wide variety of gastropods as well as coral and at least one balanomorph barnacle, but like most acrothoracicans, it is not known to attack chitons.

Although brooding females of *Cryptophialus wainwrighti* were found, their age is unknown. Utinomi (1961) reported on the development one acrothoracican species, *Berndtia purpurea* Utinomi, 1957. Based on his studies, and that most of the females examined were sexually mature, it could be assumed that the ones in this study were at least a year old. It is possible that the minute males were not observed because they were dislodged during removal of the females from the chiton valves or were left attached to the burrow (Tomlinson, 1969). It is possible that earlier nauplius stages occurred

before hatching while the embryos were still retained within the mantle cavity (Tomlinson, 1969).

Acrothoracican barnacles can be found in large numbers in limestone as well as in basibionts (Kolbasov, 2009). Pitombo (2010) provides good images of the Chilean gastropod *Concholepas concholepas* Bruguière, 1789 riddled with the burrows of *Cryptophialus minutus*. As an example another cryptophialid, *Australophialus melampygos* (Berndt, 1907), is often found infesting the New Zealand abalone *Haliotis iris* Gmelin, 1791. In one case, up to 3350 boring epibionts were recorded in a single shell. *Australophialus melampygos* has also been reported boring into the mussel *Perna canaliculus* (Gmelin, 1791). *Haliotis iris* and *P. canaliculus* are extensively harvested as food sources and the aquacultural environment does not appear to provide a suitable habitat for the recruitment of *A. melampygos*, perhaps because of the poor larval mobility of this species (Batham and Tomlinson, 1965; Webber et al., 2010).

These findings for the distribution of epibionts and endobionts on their basibiont are similar to those of Bullock and Boss (1971), who reported that the posterior edge of the intermediate valves of chitons is usually more eroded in large individuals and thus provide a better substrate for newly settling *Leiosolenus*. Sigwart (2009a) showed that parasitic forams preferentially settled on the posterior valve, apparently because the forams are filter-feeding when they first settle and then transition to a true parasitic lifestyle later in life. In Sigwart (2009b), bryozoan parasites on *Nierstraszella* Sirenko, 1992, had posterior distribution, but among the gills, in the ventral side of the chiton. More epibiosis was recorded on central and posterior region of the chiton (Figure 18).

The bivalves and sessile barnacles on the chiton valves were juveniles. It is not known if they can reach their reproductive state in the limited space on the chiton valve (Bullock and Boss 1971; Watters, 1981). On the other hand, the epibiotic relationship may have potential benefits for barnacles, since their reproductive success relies on the proximity of the mating individuals (Wahl, 1989); the chiton thus may provide a suitable substratum for mating to happen in a suboptimal environment. Although epibionts in other cases may compete with their host for food resources (Wahl, 1989), this does not seem likely to be happening between *Chiton articulatus* and the epibionts and endobionts observed. This species of chiton is a rock-scraping grazer, whereas the barnacles and the bivalves feed on plankton (Celis et al., 2007; Coan and Valentich-Scott, 2012).

Epibiosis in this case not only is likely to result in a loss of functional aesthetes (dorsal chiton valve sensory organs that could have multiple sensory functions, reviewed in Vendrasco et al., 2008) but the action of burrowers (principally *L. aristatus* and *C. wainwrighti*) likely leads to greatly weakened valves (Watters, 1981). Valves also function as an important dorsal armor (Vendrasco et al., 2008). The effects of valve weakening on the behavior of chitons are unknown although it may affect the movement as well as strength of their valves, impairing their resistance

to physiological stress during high wave exposure. Chiton defense mechanisms also could be potentially negatively affected, as has been reported for burrowing crabs (Mendez et al., 2014). The epibiosis on chiton valves could be potentially highly detrimental to its normal lifestyle.

While the results presented are from a single specimen, these findings are likely not an isolated case (e.g., Alvarez-Cerrillo et al. 2014; 2016), at least in this chiton species. Epibiosis studies in invertebrates that are dominant and keystone in the intertidal rocky shores as *Chiton articulatus*, could help to understand ecological relationships and patterns of diversity of the coastal community. Finally, this chiton species could serve as a model in quest for answers to different biological, ecological, and fisheries problems involving epi- and endosymbiosis.

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