

ARTICLE

Euceramus transversilineatus (Decapoda: Porcellanidae) living in the mantle lobe of the snail *Melongena patula* (Gastropoda: Melongenidae) in the south eastern Gulf of California: Inference of a biological association

Euceramus transversilineatus (Decapoda: Porcellanidae) viviendo en el lóbulo del manto del caracol de *Melongena patula* (Gastropoda: Melongenidae) en el sureste del Golfo de California: Inferencia de una asociación biológica

Andrés Martín Góngora-Gómez¹, Manuel II García-Ulloa², Diego García-Ulloa¹, Manuel García-Ulloa^{1*}, José Salgado-Barragán³, Juan Francisco Arzola-González⁴ and Juan Antonio Hernández-Sepúlveda⁵

¹Departamento de Acuacultura, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Sinaloa, Guasave 81101, Sinaloa, México

²Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México 04510, México

³Laboratorio de Invertebrados Bentónicos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Unidad Mazatlán, Mazatlán 82040, Sinaloa, México

⁴Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Mazatlán 82000, Sinaloa, México

⁵Departamento de Medio Ambiente, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Sinaloa, Guasave 81101, Sinaloa, México

*Corresponding author: turbotuag@hotmail.com

Resumen.- Existen abundantes informes de simbiosis en los que los crustáceos viven dentro o encima de moluscos, sin embargo, hay pocos registros publicados de gasterópodos que alberguen decápodos. Se examinó la asociación biológica entre el caracol corona del Pacífico, *Melongena patula*, y el cangrejo porcelánico, *Euceramus transversilineatus*, describiendo las relaciones morfométricas de ambos invertebrados, así como algunas características sexuales del cangrejo. Se muestrearon 15 caracoles ($124,86 \pm 19,01$ mm de altura de concha) en la laguna Navachiste, en el sureste del Golfo de California, Sinaloa, México. El 86,6% de ellos albergaba al menos un cangrejo de porcelana hembra o macho ($17,82 \pm 3,27$ mm de longitud total del cuerpo) o un par (heterosexual y mismo sexo) con una proporción sexual de 2M:1H. No hubo correlaciones entre ninguna de las relaciones morfométricas analizadas entre el cangrejo y caracol. Para los cangrejos, las mediciones de ambas quelas presentaron relaciones con la longitud corporal total, longitud del caparazón y ancho del abdomen en los machos; sin embargo, no se observaron diferencias significativas entre el ancho medio y la longitud de quelas de machos, hembras o entre sexos. De las seis hembras recolectadas, cuatro eran grávidas. Las observaciones preliminares indican que el cangrejo porcelánico *E. transversilineatus* es un endosimbionte comensal que coexiste en asociación a corto plazo con *M. patula*. Su distribución poblacional sugiere que *E. transversilineatus* es polígamo con un patrón de apareamiento promiscuo.

Palabras clave: Simbiosis, cangrejo porcelánico, caracol corona del Pacífico, morfometría, Sinaloa, México

Abstract.- There are abundant reports of symbiosis wherein crustaceans live inside or on mollusks; however, there are few published records of gastropods hosting decapods. This study examines the biological association between fifteen snails (124.86 ± 19.01) from the species Pacific crown conch *Melongena patula* and the porcelain crab *Euceramus transversilineatus*. Morphometric relationships of both invertebrates, as well as some sexual characteristics of the crab were conducted. These invertebrates were sampled in the Navachiste Lagoon, in the south eastern Gulf of California, Sinaloa, Mexico. It was found 86.6% of the snails housed at least a female or a male porcelain crab (17.82 ± 3.27 mm total body length) or a pair of them (heterosexual and same sex) with a sexual proportion of 2M:1F. There were no correlations between any of the crab and snail based on their analyzed morphometric relationships. For the crab males, the measurements of both chelae presented relationships with total body length, carapace length, and abdomen width however, no significant differences were observed between the mean width and chelae length of males, females or between sexes. Of the six females collected, four were gravid. Preliminary observations indicated that the porcelain crab *E. transversilineatus* is a commensal endosymbiont that coexist in short-term association with *M. patula*. Their population distribution suggests that *E. transversilineatus* are polygamous with a promiscuous mating pattern.

Key words: Symbiosis, porcelain crab, Pacific Crown conch, morphometry, Sinaloa, Mexico

INTRODUCTION

Symbiosis is the term generally used to design biological associations between different species, and it refers to a long-term and close interaction between two different organisms or symbionts (Hoffmeister & Martin 2003). Such interactions are classified as commensalistic, parasitic or mutualistic relationships and these interactions can be intra- or inter-specific (Rizzo & Lo-Giudice 2018). Biological associations between marine invertebrates represent strategies of evolutionary adaptation in response to selective pressures exerted by environmental conditions of the ocean, interaction between members of a community and more recently by anthropogenic effects and climate change (Nagelkerken *et al.* 2016, Baker *et al.* 2018). Invertebrates such as annelids, bryozoans, crustaceans and mollusks, among others, coexist in order to acquire refuge, food, and/or reproductive niches (Baeza 2007, Beu & Zibrowius 2007, Ocampo *et al.* 2014, Tenjing 2017).

Examples of symbiotic association between mollusks which serve as hosts to crustaceans are well documented for various species throughout the world. Baeza *et al.* (2013) described the sexual and mating system of the shrimp *Paranchistus pycnodontae* Bruce, 1978, inhabiting the penguin's wing oyster *Pteria penguin* Röding, 1798, in Papua New Guinea. On the Pacific coast of Costa Rica, Mena *et al.* (2014) documented the level of infestation of the pea crab *Austinotheres angelicus* Lockington, 1877, living in the palmate oyster *Saccostrea palmula* Carpenter, 1857. Meanwhile, in the Gulf of California, García-Ulloa *et al.* (2019) detailed the sexual characteristics of the pearl oyster shrimp *Pontonia margarita* Smith in Verrill, 1869, inhabiting the mantle cavity of the rugose pen shell *Pinna rugosa* Sowerby I, 1835.

Although most porcelain crab species are free-living (Rodríguez *et al.* 2005), many of them have been found in endo or ectosymbiotic relationships (living inside or outside of the host, respectively) living as parasites, commensalistic or mutualistic relationships with a variety of organisms such as anemones (Baeza & Stotz 2003, López-Victoria *et al.* 2004), sea cucumbers (Werdning 1983), different species of molluscs (Boyko & Mikkelsen 2002) and even other crustaceans (Werdning 1977, Rocha-Duarte-Alencar *et al.* 2014). In addition, Baeza & Stotz (2001) documented host-selection by the commensal crab *Allopetrolisthes spinifrons*, Milne-Edwards, 1837, dependent on the availability of sea anemones as hosts.

The symbiotic association of anomuran crustaceans living inside marine snails is limited. It was reported that the presence of the spotted porcelain crab *Porcellana sayana*,

Leach, 1820, was living inside the shell of the queen conch, *Strombus gigas*, Linneo, 1758, in Barbados (Telford & Daxboeck 1978). These authors reported the occurrence of up to two crabs in two different snail specimens and the sexual relationship and size of the porcelain crabs. Baeza & Stotz (2001) described for the first time the presence of *A. spinifrons* between the mantle lobe and foot of the edible gastropod *Concholepas concholepas* on the north central coast of Chile.

The incidental capture of Pacific crown conch *Melongena patula* Broderip & Sowerby, 1829, regionally known as “caracol burro” by the artisanal fishery in the southeastern Gulf of California, revealed a new biological association between this gastropod and the porcelain crab *Euceramus transversilineatus* Lockington, 1878. Therefore, the objective was to describe this association for the first time, which includes a preliminary description of the morphometric relationships of both invertebrates, as well as some sexual characteristics of the crab. Some studies have suggested that morphometric relationships between two symbiont marine invertebrates are related with their association time (Ocampo *et al.* 2012, Baeza *et al.* 2017). If our data are consistent among host-guest measurements then, the biological association will be a long-term. This information can be used for future studies related to biological association.

MATERIALS AND METHODS

Gastropods were collected at Boca de Vasiquilla, in the Navachiste coastal lagoon in September 2018. The sample site was located on the north coast of Sinaloa, Mexico (25°21'31"N-108°47'24"W) (Fig. 1).

Local fishermen engaged in the capture of fish and shrimp with the use of boats and bottom nets (15 mm between knots) had previously reported the incidental capture of snails harboring crabs either on their shell or attached to the soft tissue of the snail. The collection site was characterised by a muddy substrate and the environment conditions at the time of the sampling were 31 °C temperature, 35 salinity, strong swell, and 2 m visibility. The sampling method consisted of a fishing net which was placed at a depth of 10 m, where it remained overnight. The net was recovered early the following day (07:00 AM). The Pacific crown conch specimens were carefully untangled and placed individually in plastic bags. Then, specimens were transported to the Laboratory of Malacology at the Interdisciplinary Research Center for Regional Integral Development at the National Polytechnic Institute (CIIDIR-IPN), Sinaloa Unit, following the standard procedures (NOM)¹. This research was approved by the Ethic Committee (College of Teachers) at the CIIDIR-IPN.

¹NOM-031-SSA1-1993. Bienes y Servicios. Productos de la Pesca. Moluscos bivalvos frescos-refrigerados y congelados. Especificaciones sanitarias. <<http://www.salud.gob.mx/unidades/cdi/nom/031ssa13.html>>

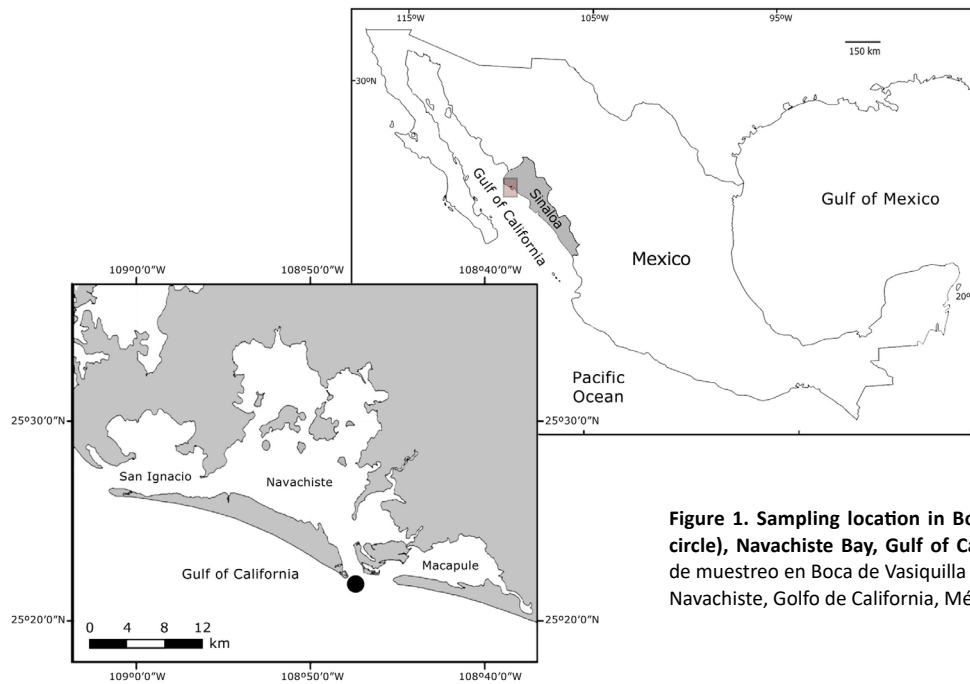


Figure 1. Sampling location in Boca de Vasiquilla (black circle), Navachiste Bay, Gulf of California, Mexico / Sitio de muestreo en Boca de Vasiquilla (círculo negro), Bahía de Navachiste, Golfo de California, México

The following measurements of the *Melongena patula* shell were obtained with a caliper (± 0.01 mm); the total height (TH) from the apex to the siphon channel; the total width (TW) from the most distal point of the opening parallel to the apex to the most distal part of the outer lip; the height of the opening (HO) from the exhalation channel to the siphonal channel; the width of the opening (WO) or the most distal distance between the inner and outer lips; and the operculum width (OW) or the distance between the points perpendicular to the maximum length of the operculum (Fig. 2).

The crabs were identified as *Euceramus transversilineatus* Lockington, 1878 following Hendrickx (1999)², and the following traits were recorded for each specimen: the total body length (TBL) from the tip of the telson to the tip of the rostrum; the cephalothorax length (LC) from the tip of the rostrum to the posterior end of the carapace; the left (LLCH) and right (RLCH) chela length of the chelae from

the end of the dactylum to the most distal edge of each chela; and left (WLCH) and right (WRCH) chelae width or the longest perpendicular distance from the propodium. The sex of the crabs was determined based on the position of the genital pore (base of the third or fifth pair of pereopods for females and males, respectively) (de Azevedo-Ferreira 2018). The abdomen width (WA), the longest distance perpendicular to the total length of the abdomen, was recorded in males and females. For each ovigerous female, the egg mass (EM) was separated from the abdomen and placed in a Petri dish for counting under microscopic observation. The *E. transversilineatus* specimens were stored in glass jars and fixed in 70% ethanol stored. The crabs and empty conchs were kept in the Laboratory of Malacology at CIIDIR-IPN, Sinaloa Unit, and labeled as: Malacology/symbiosis-porcelánido/2018(15) and Malacology/symbiosis-snail/2018(15), respectively.

²Hendrickx RME. 1999. Adenda a la colección de referencia de invertebrados de la Estación Mazatlán, UNAM y análisis de la fauna de crustáceos isópodos del Pacífico mexicano, julio 1996-julio 1997. Universidad Nacional Autónoma de México. Instituto de Ciencias del Mar y Limnología. Bases de datos SNIB-CONABIO. Proyecto No. H170. Distrito Federal. <<http://www.gbif.org/es/dataset/9254107a-b15f-42cd-9287-7adce4b4e42d>>

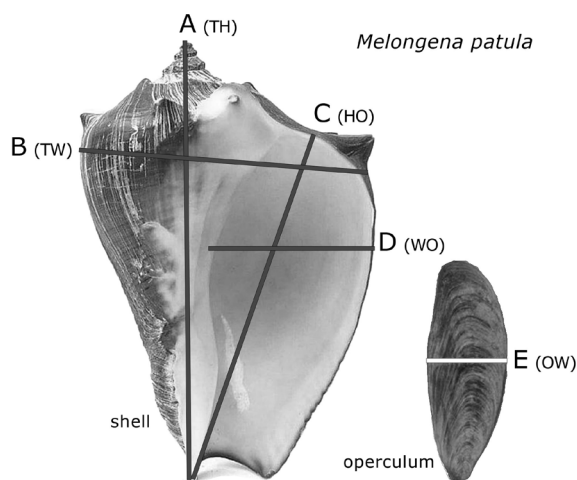


Figure 2. Body measurements of *Melongena patula*. A= total height (TH), B= total width (TW), C= height of the opening (HO), D= width of the opening (WO), E= operculum width (OW) / Medidas corporales de *Melongena patula*. A= altura total (TH), B= anchura total (TW), C= altura de la abertura (HO), D= anchura de la abertura (WO), E= anchura del opérculo (OW)

A sample size of fifteen individuals, considering an alpha significance criterion of 0.05, maintains a sufficient statistical power of 0.8, according to Cohen (1988, 1992) in correlations of $r \geq 0.65$ (and $r \leq -0.65$). Therefore, although in this study it is not possible to validate subtle correlations, the strong significant correlations, that is, when $r \geq 0.65$ and $r \leq -0.65$, are statistically valid (Fig. 3). Calculations were made with the `pwr.r.test` function from the `pwr v1.3-0` R package (Champely 2020). Command used: `pwr.r.test (n= NULL, r= values from 0.1 to 0.95 in increments of 0.05, sig. level= 0.05, power= 0.8)`. Vignette of `pwr v1.3-0` in Champely (2020)³.

The normality and homoscedasticity (Lilliefors & Bartlett's tests, respectively) of data were evaluated before applying the appropriate statistical tests (Zar 1996). Pearson's correlation coefficient (r_p) was calculated between all measurements of male and female *E. transversilineatus* against each other, and separately against all measurements of *M. patula* using *R*-base. *R*-base was used to perform *t*-tests on the LLCH, LRCH, WLCH, WRCH of males, females, and between sexes of *E. transversilineatus*. The correlation heatmap was generated using *R* libraries `reshape2` (Wickham 2007) and `ggplot2` (Wickham 2016). Statgraphics Centurion (ver. 14.0, Softonic International S.A., Spain) was used to perform the statistical tests ($P \leq 0.05$).

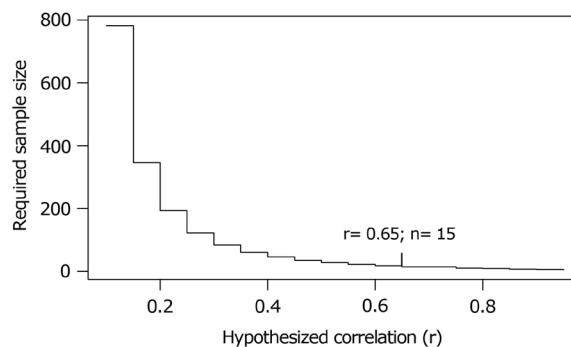


Figure 3. Sample size required (Y axis) to obtain a statistical power of 0.8, considering an alpha of 0.05, given the hypothetical correlation between 2 variables (X axis) / Tamaño de muestra requerido (eje Y) para obtener un poder estadístico de 0,8, considerando un alfa de 0,05, dada la hipotética correlación entre 2 variables (eje X)

³<<https://cran.r-project.org/web/packages/pwr/vignettes/pwr-vignette.html>>

RESULTS

Fifteen snails were collected from the Navachiste Lagoon. The TH fluctuated from 101.55 to 174.23 mm. The coefficients of variation for all measurements (TH, TW, HO, WO, and OW) were <23% (Table 1). All snail measurements were correlated with each other (Pearson's correlation $r_p = 0.93$, $P = 0.0001$, mean value).

Thirteen of the gastropods (86.6%) housed at least one porcelain crab (female or male) or pair (heterosexual and same sex) distributed as follows: four snails with a solitary male, one snail with a solitary non-gravid female, three snails with a solitary gravid female, one snail with a heterosexual pair (non-gravid female), one snail with a

heterosexual pair (gravid female), and three snails with one male pair each. The crabs had a pearly white color and were strongly attached to the mantle lobe with the propodium and dactyl of each cephalothorax appendage (Fig. 4). Of the eighteen crabs observed, twelve (66%) were male and six (33%) were female for a sex ratio of 2M:1F.

The correlations between the crab TBL and LC with the metric characteristics (TH, TW, HO, WO and OW) of *Melongena patula* are respectively shown in Figures 5 and 6.

Table 1. Body measurements (mm) of *Melongena patula* (n= 15) / Medidas corporales de *Melongena patula* (n= 15)

	<i>Melongena patula</i>				
	TH	TW	HO*	WO	OW
Mean	124.86	90.70	109.01	45.82	26.75
Standard deviation (±)	19.01	13.98	21.02	10.45	4.53
Minimum and maximum limits	101.55-174.23	79.87-114.91	91.86-141.45	34.60-60.02	21.18-37.09
Coefficient of variation (%)	15.22	15.41	19.28	22.80	16.93

*HO, height of the opening, OW, operculum width; TH, total height; TW, total width; WO, width of the opening

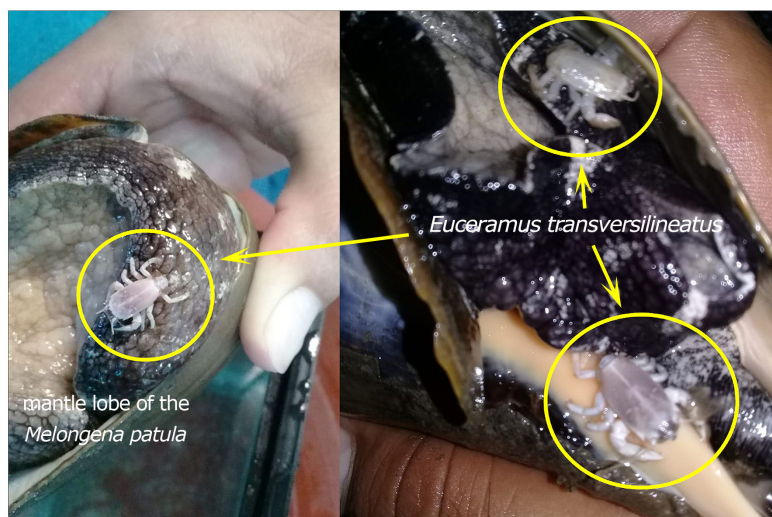


Figure 4. Porcelainid crabs *Euceramus transversilineatus* (yellow circles) living in the mantle lobe of the Pacific crown conch *Melongena patula* / Cangrejos porcelánidos *Euceramus transversilineatus* (círculos amarillos) viviendo en el lóbulo del manto del caracol concha del Pacífico *Melongena patula*

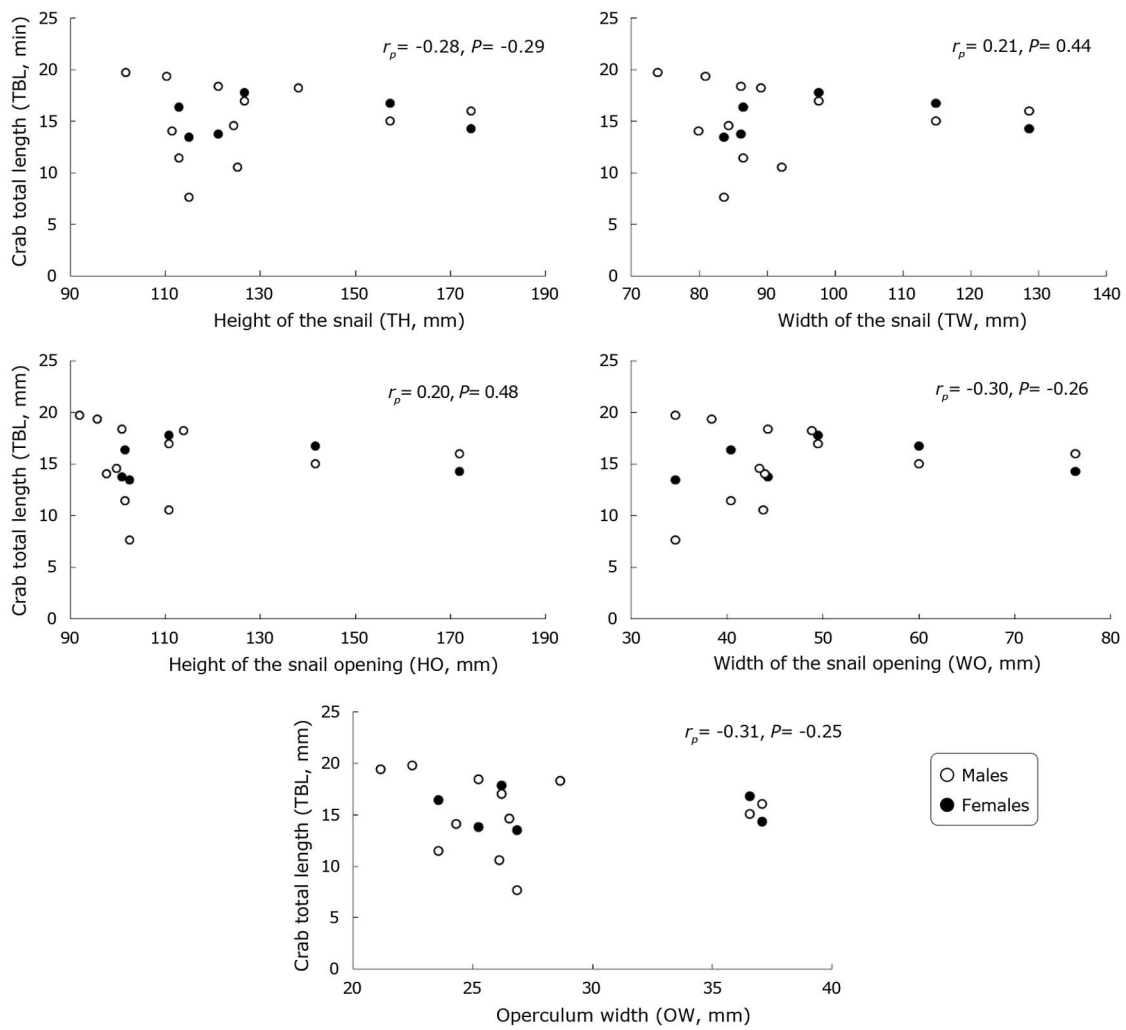


Figure 5. Relationships between *Euceramus transversilineatus* (TBL, mm) and *Melongena patula* (TH, TW, HO, WO, and OW, mm) measurements. Males= empty circles, females= full circles / Relaciones entre las medidas de *Euceramus transversilineatus* (TBL, mm) y *Melongena patula* (TH, TW, HO, WO y OW, mm). Machos= círculos vacíos, hembras= círculos llenos

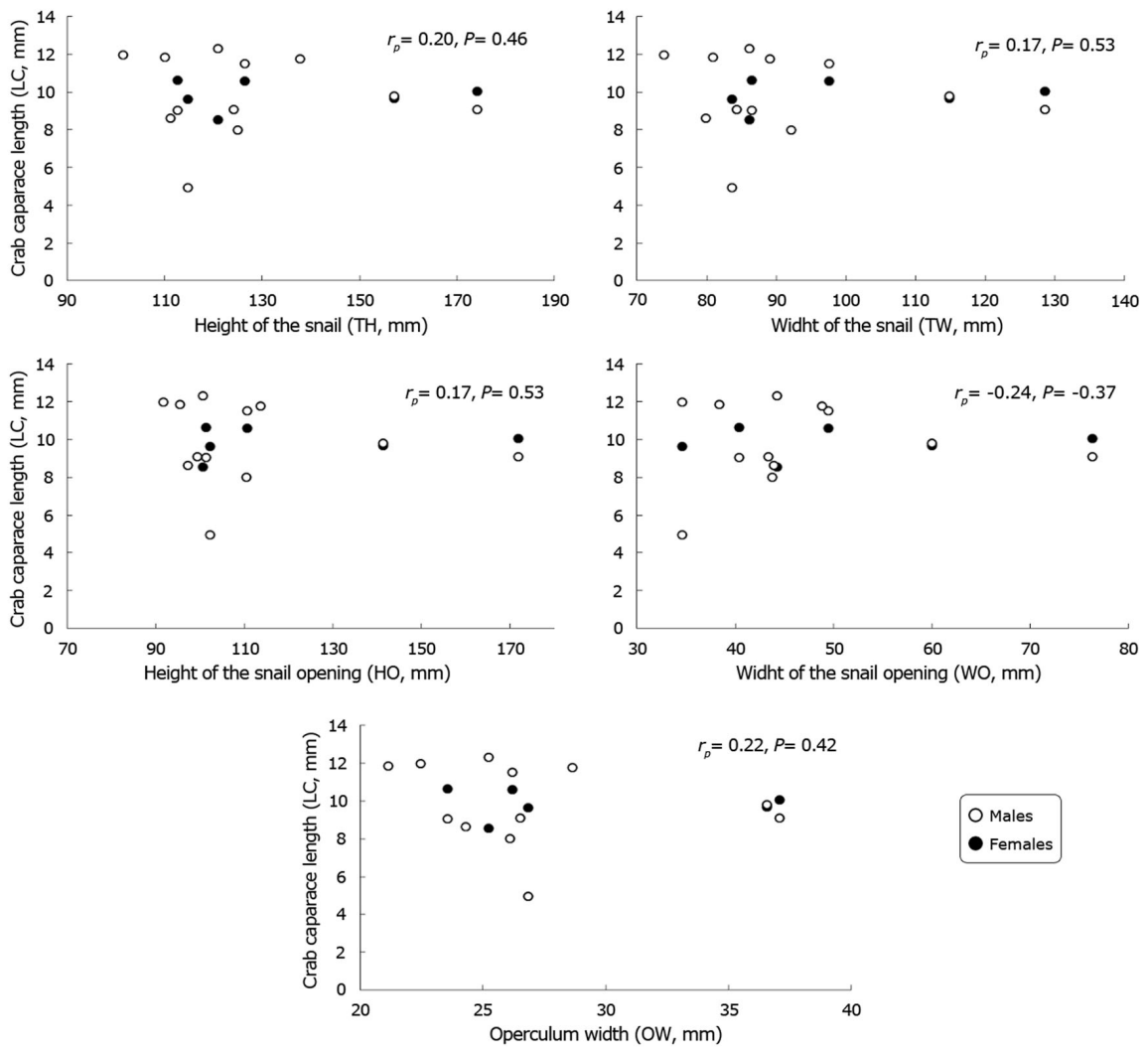


Figure 6. Relationships between *Euceramus trasversilineatus* (LC, mm) and *Melongena patula* (TH, TW, HO, WO, and OW, mm) measurements. Males= empty circles, females= full circles / Relaciones entre las medidas de *Euceramus transversilineatus* (LC, mm) y *Melongena patula* (TH, TW, HO, WO y OW, mm). Machos= círculos vacíos, hembras= círculos llenos

There was no correlation between any of the crab and snail measurements analyzed; r_p varied from 0.17 to 0.30 (Pearson's correlation). Negative and positive r_p values were obtained when comparing the relationships between snails and crabs by sex (Table 2).

The r_p values (Pearson's correlation) for females and males ranged from -0.02 to 0.18 and -0.19 to 0.15, respectively. No correlations were found between the snail measurements and the TBL and LC of *Euceramus transversilineatus* by sex.

Porcelain crabs had a minimum and maximum TBL of 7.66 and 19.79 mm, respectively, meanwhile the LC ranged from 4.95 to 12 mm (Table 3). The LLCH and WLCH presented the highest coefficients of variation (28.68 and 33.54%, respectively).

Table 2. Regression equations, correlation coefficients (r_p), standard error of slope and P -value by sexes between *Euceramus transversilineatus* and *Melongena patula* measurements taken as indicated in Figure 2 / Ecuaciones de regresión, coeficientes de correlación (r_p), error estándar de la pendiente y valor de P por sexos entre *Euceramus transversilineatus* y *Melongena patula* tomadas como se indica en la Figura 2

	Regression	r_p	SE_s	P -value
Females	TBL= 15.161+0.001 TH	0.02	0.03	0.96
	TBL= 14.034+0.013 TW	0.14	0.05	0.78
	TBL= 15.534-0.001 HO*	-0.02	0.03	0.97
	TBL= 14.866+0.011 WO	0.09	0.06	0.86
	TBL= 15.507-0.003 OW	-0.01	0.15	0.98
	LC= 9.720+0.001 TH	0.04	0.15	0.94
	LC= 9.111+0.007 TW	0.18	0.02	0.73
	LC= 9.438+0.004 HO	0.13	0.01	0.80
	LC= 9.570+0.005 WO	0.11	0.02	0.82
	LC= 9.957-0.003 OW	-0.02	0.06	0.96
Males	TBL= 14.257+0.007 TH	0.04	0.06	0.89
	TBL= 15.804-0.006 TW	-0.02	0.07	0.93
	TBL= 15.287-0.001 HO	-0.01	0.05	0.98
	TBL= 13.015+0.046 WO	0.15	0.10	0.65
	TBL= 16.987-0.066 OW	-0.08	0.23	0.78
	LC= 10.467-0.005 TH	-0.05	0.03	0.88
	LC= 11.016-0.013 TW	-0.09	0.04	0.77
	LC= 11.057-0.011 HO	-0.11	0.03	0.72
	LC= 9.441+0.009 WO	0.05	0.05	0.88
	LC= 12.118-0.084 OW	-0.19	0.13	0.54

*HO= snail opening height; LC= length of crab carapace; OW= snail operculum width; TBL= Total body length of the crab, TH= snail total height, TW= snail total width, WO= snail opening width

Table 3. Body measurements (mm) of *Euceramus transversilineatus* (n= 18) / Medidas corporales de *Euceramus transversilineatus* (n= 18)

	<i>Euceramus transversilineatus</i>					
	TBL	LC*	LLCH	WLCH	LRCH	WRCH
Mean	17.82	10.6	7.81	1.55	8.44	1.45
Standard deviation (\pm)	3.27	1.79	2.24	0.52	1.67	0.41
Minimum and maximum limits	7.66-19.79	4.95-12.31	2.55-11.15	0.41-2.82	3.08-10.46	0.41-2.03
Coefficient of variation (%)	18.35	16.88	28.68	33.54	19.78	28.27

*LC, length of crab carapace; LLCH, length of the left chela; LRCH, length of the right chela; TBL, total body length of the crab; WLCH, width of the left chela; WRCH, width of the right chela

There were significant correlations among several measurements of males *E. transversilineatus* (Fig. 7). Notably, the measurements of both chelae presented relationships (Pearson's correlation) with TBL, LC, and WA in males; their carapace length was highly correlated with the length of both chelae (LC vs. LLCH: $r_p=0.99, P=0.01$ and LC vs. RLCH: $r_p=0.95, P=0.05$) and the WRCH ($r_p=0.99, P=0.01$). For females, LC was correlated with the LLCH ($r_p=1, P=0.001$).

There were no significant differences (Unpaired *t*-test) between the mean LLCH, RLCH, WLCH, and RLCH of males, females, or between sexes (Table 4).

Of the six females collected, four were gravid (three solitary and one in a pair). The number of eggs per female ranged from 181 (13.78 mm TBL) to 565 (17.82 mm TBL) with an average of 304 ± 177.15 . No correlation (Pearson's correlation) was observed between the number of eggs and the abdomen width ($r_p=-0.68, P=0.32$); however, larger females contained more eggs ($r_p=0.9, P=0.10$).

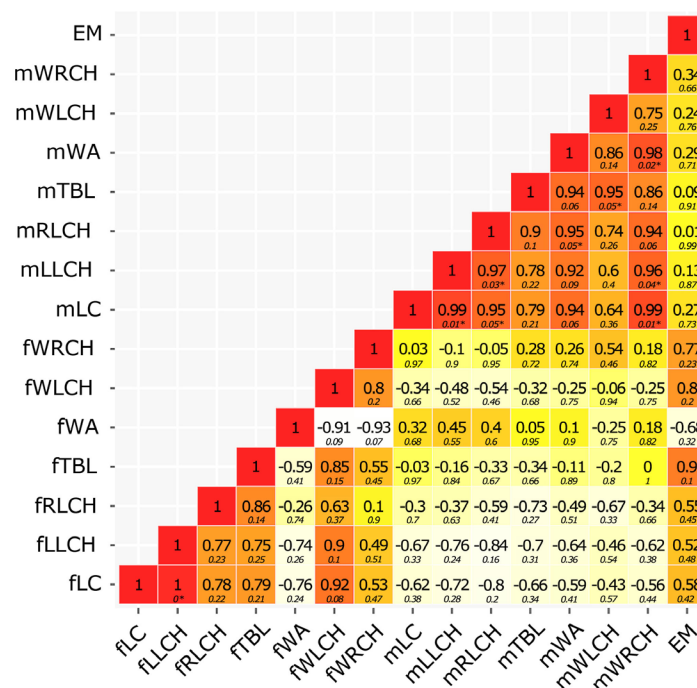


Figure 7. Correlation heatmap comparing *Euceramus transversilineatus* measurements between sexes. Measurements with initial “m” or “f” belong to males and females, respectively. EM, egg mass; LC, carapace length; LLCH, length of left chela; RLCH, length of right chela; TBL, total body length; WA, width of abdomen; WLCH, width of left chela; WRCH, width of right chela. Upper value= r_p value; lower value= P - value. *Significant correlation at 95% / Correlación (heatmap) comparando las medidas de *Euceramus transversilineatus* entre sexos. Las medidas con letra inicial “m” o “f” pertenecen a machos y hembras, respectivamente. EM, masa de huevos; LC, longitud de caparazón; LLCH, longitud de quela izquierda; RLCH, longitud de quela derecha; TBL, longitud corporal total; WA, anchura del abdomen; WLCH, anchura de la quela izquierda; WRCH, anchura de la quela derecha. Valor superior= valor de r_p ; valor inferior= valor de P . *Correlación significativa a 95%

Table 4. Unpaired *t*-tests of width and length for the right and left chelae of *Euceramus transversilineatus* males, females and between sexes / Pruebas de *t* no pareadas de la anchura y longitudes de las quelas derecho e izquierda de machos, hembras y entre sexos de *Euceramus transversilineatus*

	Chelae	Chelae trait	Mean 1	Mean 2	<i>t</i> -value	Degrees of freedom	<i>P</i> -value
Males vs. Males	Right vs. Left	Width	1.56*	1.31	1.06	21.19	0.3003
		Length	7.37	6.89	0.49	19.80	0.6226
Females vs. Females	Right vs. Left	Width	1.45	1.27	1.28	7.63	0.2375
		Length	7.09	6.76	0.59	8.96	0.5663
Males vs. Females	Right vs. Right	Width	1.31	1.27	0.23	14.19	0.8175
		Length	6.89	6.76	0.17	15.51	0.8669
	Left vs. Left	Width	1.56	1.45	0.55	15.96	0.5890
		Length	7.38	7.09	0.32	14.02	0.7486

*Means are in centimeters

DISCUSSION

Compared with other marine invertebrates such as bivalves (Salas-Moya *et al.* 2014) and ascidians (Levitt-Barmats & Shenkar 2018) that host endosymbiotic crustaceans, the snail *M. patula* is a large and morphologically complex host which is capable of housing up to two porcelain crabs in the mantle cavity. Our observations coincide with those of Telford and Daxboeck (1978) who found up to two specimens of the crab *Porcellana gayana* on the inner surface of the operculum of the snail *Strombus gigas*. They reported that the porcellanids move inside the snail mantle cavity when these are disturbed by external factors (*e.g.*, predators) and, when the foot is retracted, the crabs remain immovable and hidden between the foot and operculum. This behavior also was observed in this study (Fig. 4). The movement of the crabs inside *M. patula* would be subject to the displacement or retraction of the structures inside the snail (mantle, foot, and operculum; Telford & Daxboeck 1978), which could explain the state of apparent immobility of crabs when they were discovered at the edge of the foot and operculum.

Different endosymbiont porcellanids receive protection from their hosts in various ways. For example, *P. sayana* has been found living on the shell of the brachyuran crab *Stratiolibinia bellicosa* Oliveira, 1934, which offer protection in the form of camouflage provided by its shell and displaces the guest without exposing the crab to the marine substratum (Rocha-Duarte-Alencar *et al.* 2014). The snail shell represents a difficult barrier to be breached by a variety of predators (Němec & Horsák 2019); thus, the size, thickness, and color of the *M. patula* shell guarantees the protection of *E. transversilineatus* against small predators such as fish and other crustaceans.

Although there is evidence that some porcelain crabs parasitize their host (Bierbaum & Shumway 1988, Sun *et al.* 2006), the data collected in our study are not sufficient to explore a possible symbiotic effect the crab may confer on *M. patula*; further studies should address this question. For now, considering exclusively the protection granted by the snail, *E. transversilineatus* may be defined as an endocommensal. In fact, *E. transversilineatus* exhibits body characteristics typical of an endosymbiotic species, such as a flattened but corpulent body, with a small face, strong pereopods adapted for climbing and thick abdomen (Ross 1983, Bauer 2004). Its pearly white color would represent a disadvantage since it contrasts sharply against the dark muddy bottom (Carbajal Enzian *et al.* 2018), which highlights the importance of a protective service acquired when this porcelain crab is living associated with *M. patula*.

Corey & Reid (1991), Góngora-Gómez *et al.* (2015) and García-Ulloa *et al.* (2019) correlated the volume of bivalve shells with morphometric measurements of endosymbiotic crustaceans in terms of the space available for their accommodation. However, in the present study, no correlation was found between snail and crab measurements by crab pool and sex. Specimens of *M. patula* with HO and WO ranging from 91.86 to 141.45 and from 34.60 to 60.02 mm, respectively, accommodated up to two crabs measuring between 7.66 and 19.79 mm TBL. However, considering their mean size (124.86 ± 19.01 mm), a given snail could accommodate more than two crabs; a hypothesis that warrants further investigation. Baeza *et al.* (2017) found up to four palemonid shrimp *Periclimenes paivai* Costa, 1844, located in the inner region of the umbrella and tentacles of the *Lychnorhiza lucerna* Haeckel, 1880, jellyfish, a morphologically complex and large host (89-220 mm in diameter). In our study, the lack of correlation between snail and crab measurements suggests that these invertebrates do not grow together. Thus, their symbiotic association could be considered a short-term commensal relationship benefitting the guest *E. transversilineatus*, specifically, ensuring the survival and reproduction of the porcellanid crab in the shelter provided by the morphology of the snail. Similar observations were noted by Baeza *et al.* (2013) and García-Ulloa *et al.* (2019) for pontonid shrimps reproducing inside mollusks.

The reproductive advantage conferred on the guest by the host is strongly related to the concept of protection. Grafen & Ridley (1983) and Thiel *et al.* (2003a) concluded that monogamy between symbiont hosts is related to a sexual proportion without bias toward either sex, so that it would be common to find heterosexual pairs of symbionts in each host (Baeza *et al.* 2015). Our study does not support this hypothesis for at least two reasons: (1) the presence of twice the number of males as females suggests that the mating and reproduction system of *E. transversilineatus* is polygamous, as reported by Thiel *et al.* (2003b) for *A. spinifrons* inhabiting the anemone *Phymanthea pluvia*, Drayton in Dana, 1846; and (2) the sexual distribution of the crab in *M. patula* (solitary males, solitary females, heterosexual pairs with gravid or non-gravid females and pairs of males) indicates that both males and females move between hosts in search of reproductive partners, reflecting polygamous and promiscuous reproductive behavior (Baeza 2008, Baeza *et al.* 2016). This would result in the crab having low fidelity to the host snail, where it would remain exclusively for reproductive purposes (Ocampo *et al.* 2012). The presence of *E. transversilineatus* in 86.6% of the snails captured indicates a high degree of symbiotic occupation; however, the results obtained are

based on data from a single sample (September, summer) and patterns may vary during other seasons or at other sites with different environmental conditions. This hypothesis should be verified through ongoing research (Puente-Tapia *et al.* 2018).

Unlike pontonid shrimp in which the male is smaller than the female (Baeza 2008, Aucoin & Himmelman 2010, Góngora-Gómez *et al.* 2015), *E. transversilineatus* showed no sexual dimorphism ($r_p = -0.34$, $P = 0.66$). Moreover, the chelae dimensions among males, females, and between the two sexes were not significantly different. This pattern suggests that pregnant females do not need the protection of a male during the egg incubation. Proportionally, larger chela in males indicates that this appendix is used as a weapon for defense, attack, or protection in monogamous species (Baeza & Thiel 2007); this was not the case in our study.

Among crustaceans, females of greater body length have a larger abdomen to contain more eggs (Salas-Moya *et al.* 2014); however, in our study no significant correlation was observed between female body dimensions and number of eggs. This pattern has been reported by García-Ulloa *et al.* (2019) for females of the pearl oyster shrimp *Pontonia margarita* in symbiosis with the rugose pen shell *Pinna rugosa*.

In our study, (1) the porcelanid crab *E. transversilineatus* was found in the mantle cavity and edge of the operculum of the snail *M. patula*, and thus can be considered an endosymbiont; (2) the measurements of both invertebrates were not correlated, suggesting a short-term association; (3) *E. transversilineatus* is a commensal endosymbiont that is favored by snail protection without offering any apparent benefit in return; (4) the lack of correlation between the morphometry of female and male crabs suggests that females can fend for themselves regardless of their reproductive status; (5) the population distribution of *E. transversilineatus* presumes that both females and males leave their gastropod hosts to look for reproductive partners in other snails, reflecting a polygamous and promiscuous mating pattern; (6) at the same time, suggesting low fidelity to the host snail by porcellanids, as found by Thiel *et al.* (2003b); and finally, (7) the mantle cavity of *M. patula* is a comfortable and safe environment that favors the reproduction and temporary housing of ovigerous females of *E. transversilineatus*.

Our results are in accordance with reports of other species of symbiotic crustaceans. However, further research is needed to develop generalizations regarding the mechanisms of social and reproductive behavior of *E. transversilineatus* in symbiotic association with *M. patula*. Future studies should also focus on the conditions that favor or force the association between these invertebrates as an evolutionary strategy.

ACKNOWLEDGEMENTS

The authors thank the Instituto Politécnico Nacional for the logistical and economic support provided for the development of this research through the projects: “Morfometría y fisiología de la almeja venus *Chione undatella* de Bahía La Paz, y el puerto minero de Santa Rosalía, Baja California Sur, México” (IPNSIP 20195442), and “Incidencia estacional de nematodos y *Perkinsus marinus* de la almeja venus *Chione undatella* de bahía de La Paz y del puerto minero de Santa Rosalía, Baja California Sur, México” (IPNSIP 20190071). We thank Biol. Javier Quiñónez Gutiérrez for his invaluable help in the work of collecting organisms. Thanks to Dr. Kristin Sullivan for the English edition.

LITERATURE CITED

- Aucoin S & JH Himmelman. 2010. A first report on the shrimp *Pontonia* sp. and other potential symbionts in the mantle cavity of the penshell *Pinna carnea* in the Dominican Republic. *Symbiosis* 50: 135-141.
- Baeza JA. 2007. The origins of symbiosis as a lifestyle in marine crabs (genus *Petrolisthes*) from the Eastern Pacific: Does interspecific competition play a role? *Revista de Biología Marina y Oceanografía* 42: 7-21.
- Baeza JA. 2008. Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlanica*, in the tropical Eastern Pacific coast. *Marine Biology* 153: 387-395.
- Baeza JA & WB Stotz. 2001. Host-use pattern and host-selection during ontogeny of the commensal crab *Allopetrolisthes spinifrons* (H. Milne Edwards, 1837) (Decapoda: Anomura: Porcellanidae). *Journal of Natural History* 35: 341-355.
- Baeza JA & WB Stotz. 2003. Host-use and selection of differently-colored sea anemones by the symbiotic crab *Allopetrolisthes spinifrons*. *Journal of Experimental Marine Biology and Ecology* 284: 25-39.
- Baeza JA & M Thiel. 2007. The mating system of symbiotic crustaceans. A conceptual model base on optimality and ecological constrains. In: Duffy JE & M Thiel (eds). *Reproductive and social behavior: Crustaceans as Model Systems*, pp. 245-255. Oxford University Press, Oxford.
- Baeza JA, M Furlan, AC Almeida, SP Barros-Alves, DFR Alves & V Fransozo. 2013. Population dynamics and reproductive traits of the ornamental crab *Porcellana sayana*: implications for fishery management and aquaculture. *Sexuality and Early Development of Aquatic Organisms* 1: 1-12.
- Baeza JA, CA Hemphill & R Ritson-Williams. 2015. The sexual and mating system of the shrimp *Odontonia katoi* (Palemonidae, Pontoniidae), a symbiotic guest of the ascidian *Polycarpa aurata* in the Coral Triangle. *PLoS ONE* 10: e0121120. <<https://doi.org/10.1371/journal.pone.0121120>>
- Baeza JA, L Simpson, LJ Ambrosio, R Guéron & N Mora. 2016. Monogamy in a hyper-symbiotic shrimp. *PLoS ONE* 11: e0149797. <<https://doi.org/10.1371/journal.pone.0149797>>

- Baeza JA, SP Barros-Alvez, R Amorin-Lucerna, SF Barbosa-Lima & D Fernandes-Rodrigues-Alves. 2017.** Host-use pattern of the shrimp *Periclimenes paivai* on the scyphozoan jellyfish *Lychnorhiza lucerna*: probing for territoriality and inferring its mating system. *Helgoland Marine Research* 71: 17. <<https://doi.org/10.1186/s10152-017-0497-8>>
- Baker DM, CJ Freeman, JCJ Wong, ML Fogel & N Knowlton. 2018.** Climate change promotes parasitism in a coral symbiosis. *The ISME Journal* 12: 921-930.
- Bauer RT. 2004.** Remarkable shrimps, 282 pp. Oklahoma University Press, Norman.
- Beu AG & H Zibrowius. 2007.** *Cymatium* (Gastropoda: Ranellidae) living inside the mantle cavity of the pterioidean bivalves *Atrina*, *Pinna* and *Pecten*. *Journal of Molluscs Studies* 73: 113-115.
- Bierbaum RM & SE Shumway. 1988.** Filtration and oxygen consumption in mussels, *Mytilus edulis*, with and without pea crab, *Pinnotheres maculatus*. *Estuaries* 11: 264-271.
- Boyko CB & PM Mikkelsen. 2002.** Anatomy and biology of *Mysella pedroana* (Mollusca: Bivalvia: Galeommatoidea), and its commensal relationship with *Blepharipoda occidentalis* (Crustacea: Anomura: Albuneidae). *Zoology Anzeiger* 241: 149-160.
- Carbajal-Enzian P, J Santamaría & D Baldárrago. 2018.** Guía ilustrada para el reconocimiento de poliplacóforos, gasterópodos y cefalópodos con valor comercial en el Perú, 34 pp. Instituto del Mar del Perú, Callao. <https://www.biblioimarpe.imarpe.gob.pe/bitstream/123456789/3314/1/GUIA_GASTEROPODOS.pdf>
- Champely S. 2020.** pwr: Basic Functions for Power Analysis. R package version 1.3-0. <<https://CRAN.R-project.org/package=pwr>>
- Cohen J. 1988.** Statistical power analysis for the behavioral sciences, 567 pp. Lawrence Erlbaum Associates, New York.
- Cohen J. 1992.** Statistical power analysis. *Current Directions in Psychological Science* 1(3): 98-101.
- Corey S & DM Reid. 1991.** Comparative fecundity of decapod crustaceans: The fecundity of decapod crustaceans: The fecundity of thirty-three species of nine families of caridean shrimp. *Crustaceana* 60: 270-294.
- de Azevedo-Ferreira LA. 2018.** New records of intersexuality in porcelain crabs (Crustacea: Decapoda; Anomura: Porcellanidae). *Nauplius* 26: e2018009. <<https://doi.org/10.1590/2358-2936e2018009>>
- García-Ulloa D, V Landa-Jaime, AM Góngora-Gómez, M García-Ulloa & JA Hernández-Sepúlveda. 2019.** Sexual and reproductive traits of the pearl oyster shrimp *Pontonia margarita* (Decapoda: Palemonidae), symbiotically inhabiting the mantle cavity of the rugose pen shell *Pinna rugosa* (Bivalvia: Pinnidae). *Zoologia* 36: e29774. <<https://doi.org/10.3897/zoologia.36.e29774>>
- Góngora-Gómez AM, NP Muñoz-Sevilla, JA Hernández-Sepúlveda, M García-Ulloa II & M García-Ulloa. 2015.** Association between the pen shell *Atrina tuberculosa* and the shrimp *Pontonia margarita*. *Symbiosis* 66: 107-110.
- Grafen A & M Ridley. 1983.** A model of mate guarding. *Journal of Theoretical Biology* 102: 549-567.
- Hoffmeister M & W Martin. 2003.** Interspecific evolution: Microbial symbiosis, endosymbiosis and gene transfer. *Environmental Microbiology* 5: 641-649.
- Levitt-Barmats Y & N Shenkar. 2018.** Observations on the symbiotic relationships between the caridean shrimp *Odontonia sibogae* (Bruce, 1972) and its ascidian host *Herdmania momus* (Savigny, 1816). *PLoS ONE* 13: e0192045. <<https://doi.org/10.1371/journal.pone.0192045>>
- López-Victoria M, LM Barrios, H Kraus & LA Osorio. 2004.** New aspects on the symbiotic relationships between *Dardanus fucosus* (Crustacea: Paguridae), *Callactis tricolor* (Cnidaria: Hormathiidae) and *Porcellana sayana* (Crustacea: Porcellanidae). *Boletín de Investigaciones Marinas y Costeras* 33: 261-264.
- Mena S, C Salas-Moya & IS Wehrtmann. 2014.** Living with a crab: effect of *Austinotheres angelicus* (Brachyura, Pinnotheridae) infestation on the condition of *Saccostrea palmula* (Ostreoida, Ostreidae). *Nauplius* 22: 151-158.
- Nagelkerken I, KA Pitt, MD Rutte & RC Geertsma. 2016.** Ocean acidification alters fish-jellyfish symbiosis. *Proceedings of the Royal Society B* 283(1833): 20161146. <<https://doi.org/10.1098/rspb.2016.1146>>
- Němec T & M Horsák. 2019.** Specific damage recognized on land snail shells as a tool for studying predation intensity: differences related to habitat and predator types. *Contributions to Zoology* 88: 277-296.
- Ocampo EH, JD Nuñez, M Cledón & JA Baeza. 2012.** Host-specific reproductive benefits, host selection behavior and host use pattern of the pinnotherid crab *Calyptraeotheres garthi*. *Journal of Experimental Marine Biology and Ecology* 429: 36-46.
- Ocampo EH, JD Nuñez, M Cledón & JA Baeza. 2014.** Parasitic castration in slipper limpets infested by the symbiotic crab *Calyptraeotheres garthi*. *Marine Biology* 161: 2107-2120.
- Puente-Tapia FA, R Gasca, G Genzano, A Schiariti & A Carrara-Morandini. 2018.** New records of association between *Brachyscelus cf. Rapacoides* (Arthropoda: Amphipoda) and medusae (Cnidaria: Scyphozoa and Hydrozoa) from São Sebastião Channel, southeast Brazil. *Brazilian Journal of Oceanography* 66: 301-306. <<http://doi.org/10.1590/s1679-87592018017806603>>
- Rizzo C & A Lo-Giudice. 2018.** Marine invertebrates: Underexplored sources of bacteria producing biologically active molecules. *Diversity* 10: 52. <<https://doi.org/10.3390/d10030052>>
- Rocha-Duarte-Alencar CE, A Barbosa-Moraes, SAS Nascimento-Moraes, PV Nascimento-Araújo & FA Morais-Freire. 2014.** First record of the association between the porcellanid crab *Porcellana sayana* and the brachyuran crab *Stratiolinia bellicosa*. *Marine Biodiversity Records* 7: e82. <<https://doi.org/10.1017/S175526721400061X>>
- Rodríguez IT, G Hernández & DL Felder. 2005.** Review of the western Atlantic Porcellanidae (Crustacea: Decapoda: Anomura) with new records, systematic observations, and comments on biogeography. *Caribbean Journal of Sciences* 41: 544-582.

- Ross DM. 1983.** Symbiotic relations. In: Vernberg SJ & WB Vernberg (eds). *The biology of Crustacea 7*: 163-212. Academic Press, New York.
- Salas-Moya C, S Mena & IS Wehrtmann. 2014.** Reproductive traits of the symbiotic pea crab *Austinotheres angelicus* (Crustacea, Pinnotheridae) living in *Sacossatrea palmula* (Bivalvia, Ostreidae), Pacific coast of Costa Rica. *Zookeys* 457: 239-252.
- Sun W, S Sun, W Yuqi, Y Baowen & S Weibo. 2006.** The prevalence of the pea crab, *Pinnotheres sinensis*, and its impact of the condition of the cultured mussel, *Mytilus galloprovincialis*, in Jiaonan waters (Shandong Province, China). *Aquaculture* 253: 57-63.
- Telford M & C Daxboeck. 1978.** *Porcellana sayana* Leach (Crustacea; Anomura) symbiotic with *Strombus gigas* (Linnaeus) (Gastropoda: Strombidae) and with three species of hermit crabs (Anomura: Diogenidae) in Barbados. *Bulletin of Marine Science* 28: 202-205.
- Tenjing SY. 2017.** Pea-crab (*Pinnotheres* sp.) as commensal inside the shells of edible wedge clams and their predators along southwest coast of India. *Journal of the Marine Biology Association of India* 59: 59-66.
- Thiel M, A Zander & JA Baeza. 2003a.** Movements of the symbiotic crab *Liopetrolisthes mitra* between its host sea urchin *Tetrapygyus niger*. *Bulletin of Marine Science* 72(1): 89-101.
- Thiel M, A Zander, N Valdivia, JA Baeza & C Rueffer. 2003b.** Host fidelity of a symbiotic porcellanid crab: the importance of host characteristics. *Journal of Zoology* 261: 353-362.
- Werding B. 1977.** Los porcellánidos (Crustacea: Anomura: Porcellanidae) de la región de Santa Marta, Colombia. *Anales del Instituto de Investigaciones Marinas de Punta Betín* 9: 173-214.
- Werding B. 1983.** Kommensalische porcellaniden aus der Karibik (Decapoda, Anomura). *Crustaceana* 45: 1-14.
- Wickham H. 2007.** Reshaping Data with the reshape Package. *Journal of Statistical Software* 21: 1-20.
- Wickham H. 2016.** ggplot2: Elegant Graphics for Data Analysis, 258 pp. Springer-Verlag, New York.
- Zar H. 1996.** Biostatistical analysis, 718 pp. Prentice-Hall, Englewood Cliffs.

Received 10 December 2019 and accepted 28 October 2020

Associated editor: Dr. Rodrigo Román P.