Zoological Studies **62:**18 (2023)
doi:10.6620/ZS.2023.62-18

Zoological Studies

Understanding the Symbiotic Relationship between the Sea Urchin *Loxechinus albus* (Molina, 1782) and the Pea Crab *Pinnaxodes chilensis* (H. Milne Edwards, 1837): a Potential Parasitism

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Received 1 July 2022 / Accepted 27 January 2023 / Published 25 April 2023
Communicated by James D. Reimer

The echinoderm *Loxechinus albus* has a symbiotic relationship with the pinnotherid crustacean *Pinnaxodes chilensis*. Females of the crustacean develop in the terminal section of the sea urchin's digestive system, remaining there for life. This relationship has been suggested as commensalism. However, a potential negative impact on gonadal development and on the morphology of the sea urchin's digestive system suggest that it is instead parasitic. To study if there is a negative impact of the crustacean symbiont on the host, specimens of *L. albus* of all sizes were collected from a rocky shore in southern Chile. The gonadal and somatic tissues of sea urchins that were and were not harboring the pinnotherid were weighed and compared. Our results show that the presence of the pinnotherid was related to sea urchin gonads of lower biomass, decreased gonadosomatic index levels, and alterations in the morphology of the terminal portion of the host digestive system. The lower gonadal biomass suggests a negative impact on gamete production as well as a diversion of energy due to changes of the digestive system tissues and the potential consumption of algal food by the resident crustacean. These results suggest that the prolonged relationship between these two species is one of parasitism rather than one of commensalism.

Key words: Parasitism, Symbiosis, Sea urchins, Pinnotherid, Crustacean.

BACKGROUND

Marine invertebrates have a wide variety of lifestyles. Many species become adults as independent, free-living individuals, either in the water column or as members of the benthos. Other species, however, spend at least part of their lives—compulsorily or deliberately—living in close association with other organisms (Waide et al. 1999; Thébault and Loreau 2005; Thiebot and Weimerskirch 2012; Khandeparker and Anil 2013). Such "symbiotic" associations are usually long-term, can be of different types, and can occur at different stages of development (Das and Varma 2009), and can include a diverse range of interactions,

Citation: Jaramillo HN, Salas-Yanquin LP, Büchner-Miranda JA, Cubillos VM, Montory JA, Pechenik JA, Pardo LM, Chaparro OR. 2023. Understanding the symbiotic relationship between the sea urchin *Loxechinus albus* (Molina, 1782) and the pea crab *Pinnaxodes chilensis* (H. Milne Edwards, 1837): a potential parasitism. Zool Stud **62:**18. doi:10.6620/ZS.2023.62-18.

behaviors, and physical associations. Although several of these relationships favor the guest organism, that organism must adapt to the physical limitations and to the physiological and mobility characteristics of its hosts. Guest organisms can not only modify their own morphology, physiology, reproduction and behavior to fit in better with the life-style of the host, but can often impact the physical characteristics of the host to create a better living space for themselves (Patton 1965; Bell and Stancyk 1983; Weber and Apprill 2020). This lifestyle is quite common in marine invertebrates, and members of several crustacean taxa have been identified that adopt the symbiotic lifestyle (Douglas 1994; Thiel and Baeza 2001; Narvarte and Saiz 2004; de Bruyn et al. 2009). In some cases, the between-species relationship provides an advantage for the guest at the expense of the host that must be considered as parasitism (Dales 1957).

Some species of crustaceans in the Pinnotheridae family can be symbionts, while others can be freeliving (Schmitt et al. 1973). Pinnotherids belong to the little-studied cryptofauna due to their small size, their symbiotic habits, and the taxonomic problems derived from their substantial sexual dimorphism and morphological changes during their life cycle (Martínez 2014). Many members of this family associate symbiotically with their host (McDermott 2009), either optionally or compulsorily, usually as adults (Schmitt et al. 1973; Stevens 1990; Becker and Türkay 2010). They are able to colonize multiple classes of hosts of different morphologies and habitats, such as molluscs (Geiger and Martin 1999; da Cruz-Kaled et al. 2006; Ahyong 2020), annelids (Komai et al. 2014; Britayev et al. 2017), arthropods (Feldman et al. 1996; McDermott 2009), or echinoderms (Bell and Stancyk 1983; de Bruyn et al. 2009; Tresnati et al. 2021).

Associations between pinnotherids and their hosts can sometimes be either mutualistic or commensal (Reeves and Brooks 2001), but in some cases they can also have a relationship that negatively affects their hosts (Hamel et al. 1999; Bologna and Heck 2000; Ocampo et al. 2014; 2021; Becker and Türkay 2017; Gajbhiye and Khandeparker 2017). In the case of the symbiotic relationship of pinnotherids with species of echinoderms, the pinnotherids generally obtain their food through water recirculated by the host, as seen with some holothurian species (Wells and Wells 1961), or by feeding on the pieces of macroalgae accumulated in the host digestive system, as seen in some sea urchin symbionts (Gutiérrez-Martínez 1971).

Along the Chilean coastline, an interesting interaction has been identified between the sea urchin *Loxechinus albus* (the host) and the pinnotherid crustacean *Pinnaxodes chilensis* (the guest) (Baez and Martínez 1976; Lardies and Castilla 2001; Vásquez and Bay-Schmith 2010–2011; Gonzalez-Canales et al. 2018). *L. albus* is a species of high economic importance in Chilean waters. Its range extends from northern Peru (6°S) to Tierra del Fuego in southern Chile (55°S), while its bathymetric range goes from shallow coastal water down to depths of several hundred meters (Larraín 1975). It is also one of the most important consumers of macroalgae in rocky intertidal and subtidal environments (Dayton 1985; Gebauer and Moreno 1995; Vásquez 2007). The crustacean *P. chilensis* also has a large distribution, with a range extending from the Chincha Islands, Peru (approximately 13°S) to southern Chile (approximately 53°S) (Retamal and Moyano 2010). Whereas the males of this crustacean species are free-living, the females are adapted for a symbiotic life inhabiting the final part of the sea urchin's intestine, which provides it with great environmental homogeneity (Baez and Martínez 1976) and enough food to increase its likelihood of survival and reproductive success (Lardies and Castilla 2001). Males of this species are smaller than females (Gutiérrez-Martínez 1971).

During the reproductive season, the male enters the intestine of the host sea urchin, via the sea urchin's anal orifice, in search of a mate, and then leaves the sea urchin after copulation (Gutiérrez-Martínez 1971; Gonzalez-Canales et al. 2018). The embryos of this and some related species then have a free-living larval period in the plankton (Hamel et al. 1999; Hsueh 2001; Gonzalez-Canales et al. 2018). However, as soon as these larvae settle and metamorphose, they acquire the appearance of an adult juvenile (Gutiérrez-Martínez 1971). After this stage, females necessarily look for a sea urchin host to grow inside, while males either remain in the benthos or enter a host for a short time (Gutiérrez-Martínez 1971).

The symbiotic life of the pinnotherids and the wide diversity of the echinoderm species that house them (Bell 1988; Lardies and Castilla 2001; de Bruyn et al. 2009; Vásquez and Bay-Schmith 2010–2011) are very interesting subjects for understanding the relationship between species and the ecological role that their lifestyle represents. The association between the host sea urchin *L. albus* and the guest pinnotherid *P. chilensis* has sometimes been considered as commensalism (*e.g.*, Gutiérrez-Martínez 1971); however, other authors, such as Fenucci (1967), have identified this relationship as parasitism. The growth of the *P. chilensis* female in the terminal portion of the host's digestive system forces the sea urchin to gradually increase the size of that digestive region under stress; the host's gonads also become deformed (Vásquez and Bay-Schmith 2010–2011). For example, it has been observed that the presence of the pinnotherid

Pinnotheres maculatus was associated with a decline in the GSI of the scallop *Argopecten irradians*, thereby reducing its reproductive potential (Bologna and Heck 2000). An equivalent situation has been identified in specimens of *Mytilus galloprovincialis* when the bivalve hosted females of the parasite *Pinnotheres sinensis*, which negatively impacted the condition index of the host mussels (Sun et al. 2006). Similarly, Takeda et al. (1997) found that the symbiont *Pinnixa tumida* reduced the growth of its host, the holothurian *Paracaudina chilensis*, due to consumption of the mucus secreted in the host's alimentary canal. Likewise, specimens of the oyster *Crassostrea virginica* from populations with a high rate of infestation by the pinnotherid *Pinnotheres ostreum* developed a smaller than normal gonadal area, impacting the host's reproductive capacities (O'Beirn and Walker 1999).

To date, it has not been investigated whether the deformations of the intestine and gonads of *L. albus* generated by the guest *P. chilensis* weaken the sea urchin or even cause its death (Vásquez and Bay-Schmith 2010–2011), but if so, then the relationship should be viewed as parasitic rather than as commensal. In the present study, we sought to characterize in detail the impacts of the symbiotic crustacean on the sea urchin host's tissues and on its gamete production to determine whether the relationship is truly commensal or in fact parasitic.

MATERIALS AND METHODS

Specimen collection

Specimens (73 individuals, with test diameters ranging from 2.6 to 9.5 cm) of the sea urchin *Loxechinus albus* were collected during January and February 2021 in the lower rocky intertidal of Calfuco Beach (39°46'50"S, 73°23'34"W), southern Chile (Fig. 1) and then maintained in the laboratory in a 30 L aquarium with circulating seawater (10°C and 33 salinity) and constant aeration. The individuals were kept under these conditions for a maximum of 2 days, until all of the specimens collected in each sampling event were processed, as described below.

Morpho-gravimetric quantifications of sea urchin

The maximum exoskeleton diameter of each sea urchin was measured using digital vernier calipers. The wet weight of each specimen was then obtained to the nearest 0.01 g using an analytical balance. Before weighing, the specimens were kept out of the seawater for 1 h to equalize water loss by aerial exposure among all individuals.

The soft tissues were then separated from each sea urchin's exoskeleton and the gonadal tissue was then separated from the rest of the soft tissues. The sex of each individual was determined using a compound microscope (Olympus BX 41) to identify the presence of eggs or sperm. These gonadal tissues were then deposited, separately, into pre-labeled and pre-weighed aluminum foil containers. The samples were then maintained at 60°C for 48 hours, until they reached a constant dry weight, and then weighed to the nearest 0.01 g to determine dry tissue weights.

The Gonadosomatic Index (GSI) was then estimated using the following equation:

GSI = (gonad dry weight / (total soft dry tissue weight)) * 100.

Pinnotherid processing

To verify the presence/absence of the pinnotherid *P. chilensis*, we dissected the digestive tissues of 73 individual sea urchins using surgical forceps. Specimens were identified following the information reported by Takeda and Masahito (2000) and Campos (2017). All of the pinnotherids were examined using a magnifying glass, to determine their sex, based on the shape of the abdomen and the presence of incubated embryos (Thatje and Calcagno 2014). In each pinnotherid, the maximum length of the carapace (Lardies and Castilla 2001; McDermott 2006) was measured using digital vernier calipers. Subsequently, the wet weight of each pinnotherid was obtained to the nearest 0.01 g. All specimens were kept for 1 h out of the water before being weighed to equalize water loss by aerial exposure among all individuals.

Impact of the pinnotherid on sea urchin digestive system morphology

To identify the impact of symbiotic pinnotherids on the morphology of the host's digestive system, we photographed the terminal portion of the digestive tract of infected and non-infected sea urchins. Each digestive system was placed in a Petri dish with seawater and photographed at 10X using a stereomicroscope coupled with a digital camera. All photographs also included a reference scale, allowing us to determine the area of the sea urchin's digestive system in which the pinnotherid symbiont was located. The images were later processed using Image J software.

Subsequently, these pinnotherids were also photographed as previously described and the area of the carapace (excluding the thoracic appendages) was measured. The pinnotherid carapace area was subsequently compared with the surface area of the host sea urchin's digestive system, in order to identify whether the host's digestive system had expanded in response to the presence of the symbiotic crustacean.

Data analyses

The normality and homoscedasticity of the data were identified using the Kolmogorov-Smirnov test and the Levene test, respectively. When these criteria were

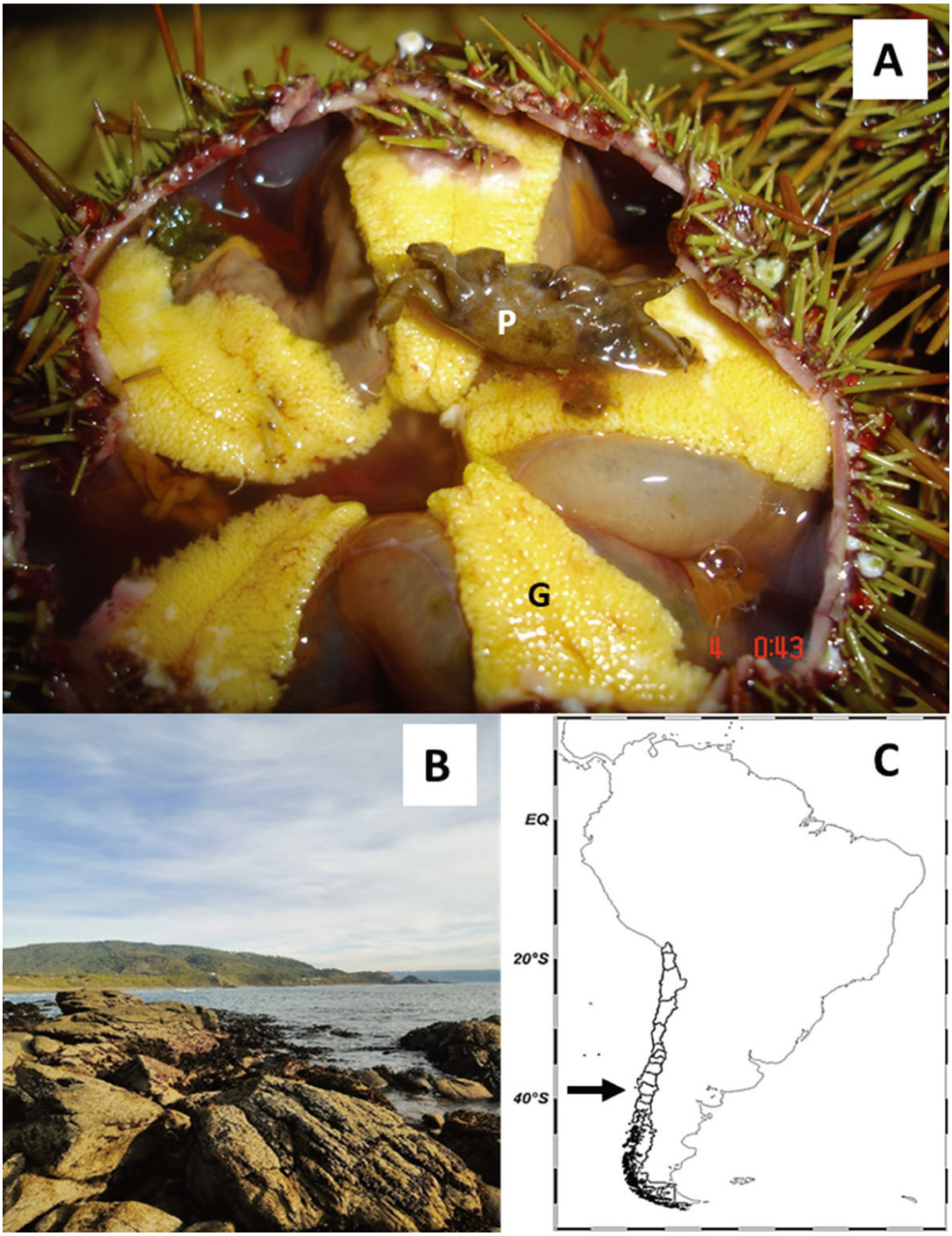

Fig. 1. A, Sea urchin without the upper part of the testa, showing the parasitic pinnotherid. P = pinnotherid, G = sea urchin gonads. B, Intertidal environment from which sea urchins were obtained. C, Map of South America, indicating (arrow) the sampling site of the sea urchin used in the present study.

not met, we used a non-parametric test (*e.g.*, ANCOVA with permutations).

Linear or non-linear regressions were performed as required in each case. Potential adjustment regressions were used for the ratios of total wet weight, gonadal dry weight and dry weight of the other sea urchins' tissues with its diameter as an independent variable. Linear regressions were used to assess the relationships of the pinnotherid carapace length and the dry weight of the sea urchin gonads, with the sea urchin diameter serving as the independent variable in both cases. The same analysis was used for the data of wet weight, dry weight of other tissues and the area of the sea urchin intestine, with the length of the pinnotherid carapace serving as the independent variable. Logistic regression was used to determine the relationship between the length of the carapace and the pinnotherid tissue weight, with the reproductive condition of the female serving as the dichotomous variable (ovigerous – non ovigerous).

Comparisons of total wet weight between sea urchins with and without pinnotherid symbionts, as well as between male and female sea urchins were made using ANCOVA analysis. The same analysis was used for comparisons of the terminal area of the intestine in parasitized and non-parasitized sea urchins, the carapace area of the pinnotherid with the expansion of the sea urchin's digestive system, and the gonadal weight with the "other tissues" of male and female sea urchins. The covariate for the first two cases was the diameter of the sea urchin test and, for the last two, the length of the pinnotherid carapace.

ANCOVA with permutations (5000 permutations) was used to analyze the sea urchin gonadal dry weight and gonad somatic index, according to the presence or absence of pinnotherids. In addition, GSI was analyzed in relation to the sea urchin sex (male and female); in each of these cases, the test diameter of the sea urchin was used as the covariate.

The same test was used to analyze by sex (males and female sea urchins), the wet weight data and dry weight of other sea urchin tissues according to the presence or absence of the pinnotherids, with the sea urchin diameter serving as the covariate.

Statistical analyses were carried out using Sigmaplot, STATISTICA 7.0 software and R statistical package (Team RC 2021).

RESULTS

Size and frequency distribution of sea urchins with symbiont pinnotherids

The test diameters of the sea urchins collected from the population at Calfuco ranged between 2.6 and 9.5 cm, with most of the tests being between 6.0 and 8.0 cm (Fig. 2A). Of the 73 sea urchins that were collected, 85.7% were infested with pinnotherids (Fig. 2B); 47% of the infected sea urchins were females and 53% were males.

Gravimetry of sea urchin and the impact of infesting crab

Sea urchin total wet weight

Sea urchin total wet weight was strongly related to the sea urchin's test diameter (Nonlinear regression: $F_{(1,72)}$ 1429.5; $P < 0.001$, Fig. 3A). Sea urchins harboring pinnotherids had significantly greater total wet weights than those without pinnotherids (ANCOVA: $F_{(1,70)}$ 5.222; $P = 0.002$, Fig. 3B), and the impact was similar for infested sea urchins of both sexes (ANCOVA with permutations: $F_{(1,58)}$ 5000; $P = 0.078$, Fig. S1A).

Larger parasitized sea urchins typically harbored larger pinnotherid symbionts (Linear Regression: $F_{(1,60)}$ 77.43; $P < 0.001$, Fig. 3C), and the relationship was similar regardless of the sex of the sea urchin host (ANCOVA: $F_{(1,59)}$ 1.361; $P = 0.267$, Fig. S1B).

Sea urchin gonads

Larger sea urchins tended to have heavier gonads (Non linear Regression: $F_{(1,71)}$ 85.64; $P < 0.001$, Fig. 4A).

The relationship between sea urchin dry gonadal weight and the sea urchin's test diameter was significantly altered by the presence of the pinnotherid, with parasitized individuals generally having smaller gonads (ANCOVA with permutations: $F_{(1,69)}$ 5000; $P < 0.001$, Fig. 4B). A significant interaction between the sex and diameter of the sea urchin was also identified for the dry weight of the gonad of parasitized males and females (ANOVA with permutations: $F_{(1,58)}$ 5000; $P = 0.022$, Fig. S2A). On the other hand, sea urchins with larger gonads tended to harbor significantly larger pinnotherids (Linear Regression: $F_{(1, 61)}$ 32.911; $P < 0.001$, Fig. 4C). Finally, no significant differences were identified in the gonadal dry weight of sea urchin between males and females depending on the size of the hosted pinnotherid (ANCOVA: $F_{(1,59)}$ 2.262; $P = 0.138$, Fig. S2B).

"Other soft tissues" of the sea urchin

Larger sea urchins had more non-gonadal soft tissues than smaller sea urchins (Nonlinear Regressión: $F_{(1, 64)}$ 105.1; $P < 0.001$, Fig. 5A). The presence of the pinnotherid was not associated with the weight of non-gonadal sea urchin tissues (ANCOVA with permutations: $F_{(1,62)}$ 5000; $P = 0.5$, Fig. 5B), for both male and female sea urchins (ANCOVA with permutations: $F_{(1,62)}$ 5000; $P = 1$, Fig. S3A). On the other hand, sea urchins with the largest weight of the 'other tissues' tended to harbor significantly larger pea crabs (Linear Regression: $_{F(1,55)}$ 47.00; $P < 0.05$, Fig. 5C), but no differences between sexes were identified regarding the size of the pinnotherid (ANCOVA: $F_{(1,63)}$ 3.169; $P = 0.07$, Fig. S3B).

Sea urchin gonadosomatic index

There was no significant relationship between sea urchin size and the gonadosomatic index (GSI) (ANCOVA with permutations: $F_{(1,62)}$ 5000; $P = 0.103$). However, higher values were seen in sea urchins that were not hosting pinnotherids (ANCOVA with permutations: $F_{(1,62)}$ 5000; $P = 0.011$), with

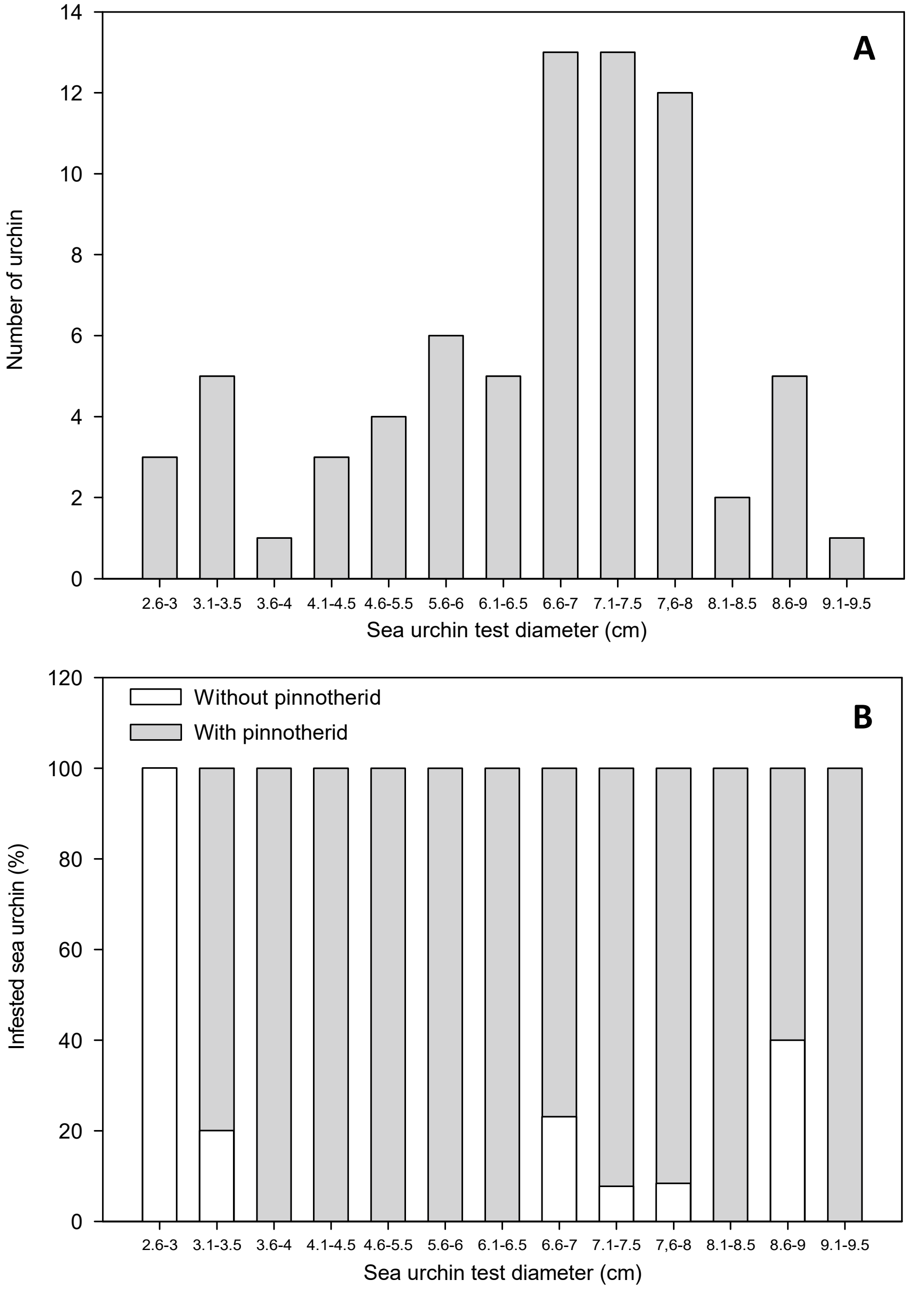

Fig. 2. A, Frequency distribution of sea urchin test diameter for the specimens sampled from Calfuco beach in January and February 2021. B, Relationship between sea urchin test diameter and the proportion of infested and non-infested sea urchins. N = 73.

parasitized sea urchins presenting the lowest values of gonadosomatic index (Fig. 6).

Sea urchin sizes and pinnotherid size

Larger sea urchins tended to harbor larger pinnotherids (Linear Regression: $F_{(1,60)}$ 87.97; $P < 0.001$). The smallest infested sea urchin identified during this research had a test diameter of 3.1 cm, while the largest had a test diameter of 9.5 cm (Fig. 7A).

All pinnotherids collected from sampled sea urchins were females. The most extreme carapace

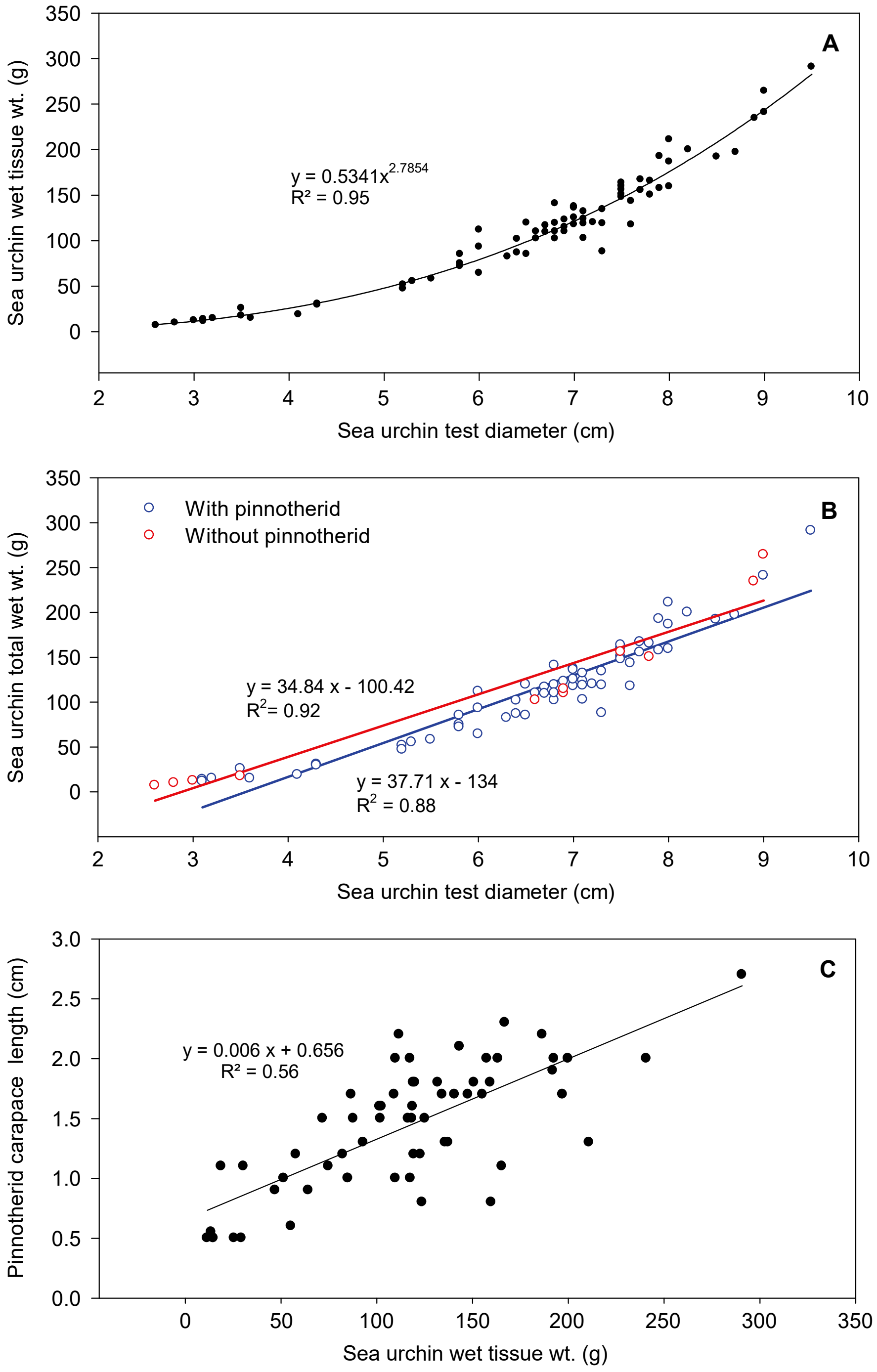

Fig. 3. A, Relationship between sea urchin size and its total wet weight for the specimens collected from Calfuco Beach. N = 73. B, Sea urchin wet weight with pinnotherid and without pinnotherids, and C, Relationship between total wet weight of the sea urchin host and the carapace length of the harbored pinnotherid. N = 62.

lengths for the pinnotherid females found inside the sea urchin hosts were 0.5 and 2.7 cm (Fig. 7B). Whether the pinnotherid females were ovigerous or non-ovigerous was significantly related to their wet weight (Logistic regression: Wald = 9.139; *d.f.* = 1; *P* = 0.002) and size of the female host crab (Logistic regression: Wald = 4.360; *d.f.* = 1; *P* = 0.004). In the study population, the smallest gravid female recorded was 1.2 cm (Fig. 7B).

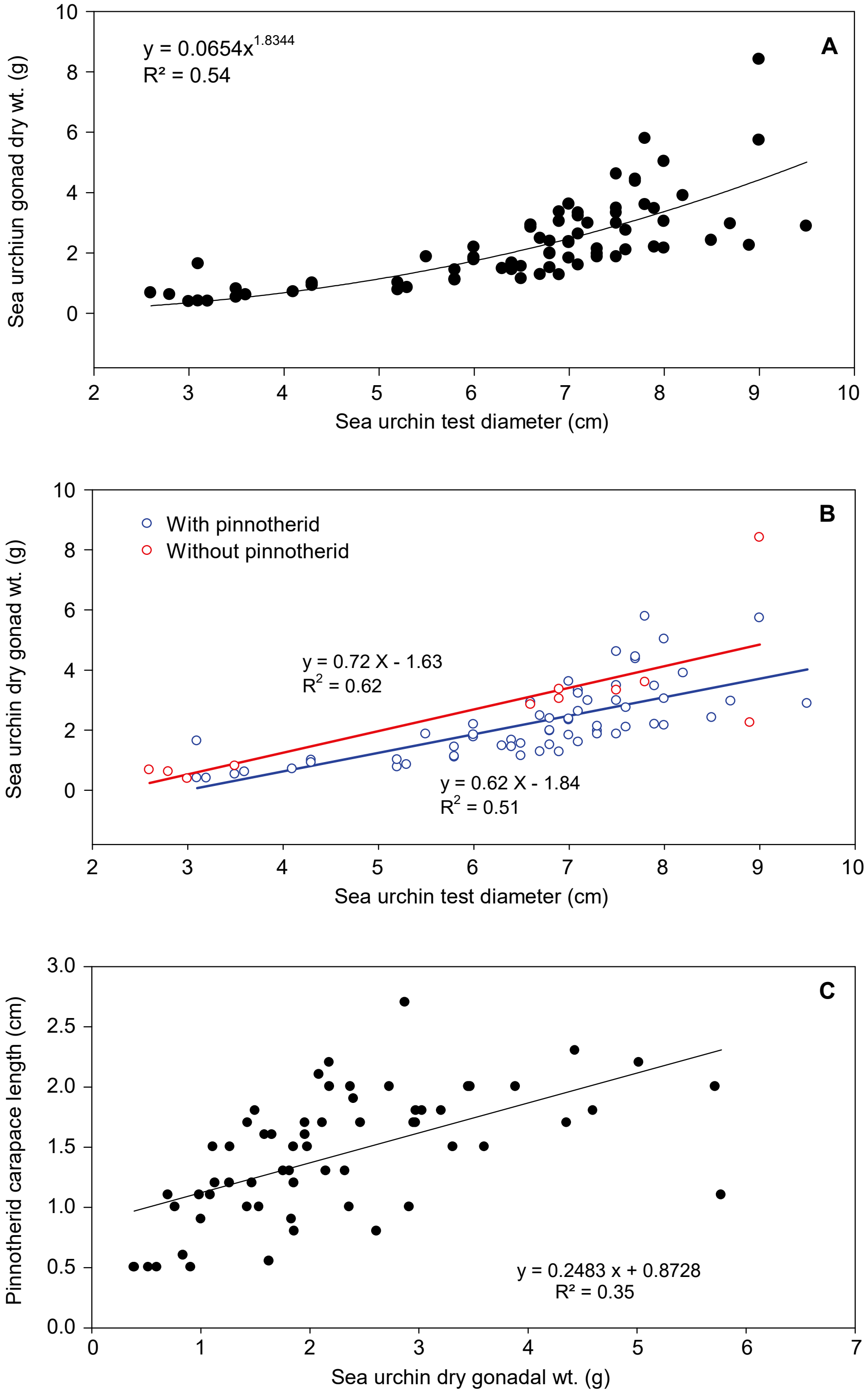

Fig. 4. A, Relationship between the dry gonadal weight and the test diameter of the sea urchins collected on Calfuco Beach in 2021. N = 73. B, sea urchin test diameter and dry gonadal weight in sea urchins with pinnotherid and without pinnotherid symbionts, and C, Relationship between symbiont-harboring sea urchin dry gonadal weight and the length of the pinnotherid carapace. N = 62.

Presence of pinnotherid and morphology of the sea urchin's digestive system

The diameter of the terminal section of the sea urchin's intestine was significantly larger for sea urchins that were harboring pinnotherids than for sea urchins that were not hosting pinnotherids (ANCOVA: $F_{(1,11)}$ 7.387; P = 0.02, Fig. 8A). The expanded area of an

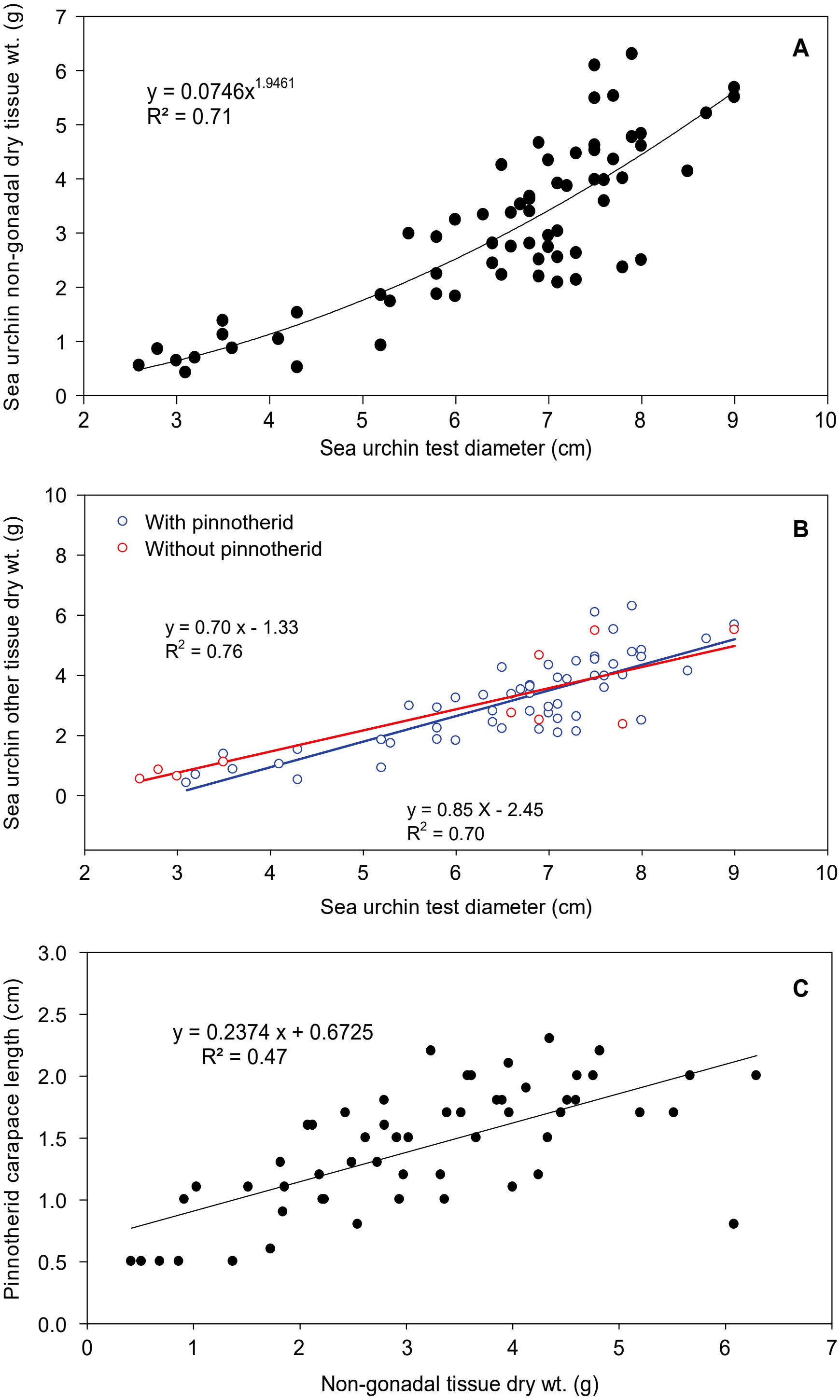

Fig. 5. A, Relationship between the test diameter and dry weight of the non-gonadal tissues of the sea urchins collected at Calfuco Beach. N = 73. B, Relationship between presence or absence of pinnotherid symbionts and the weight of non-gonadal sea urchin tissues. C, Influence of the size of the hosted pinnotherid on the dry non-gonadal tissue weight of the sea urchin specimens collected in the Calfuco Beach. N = 62.

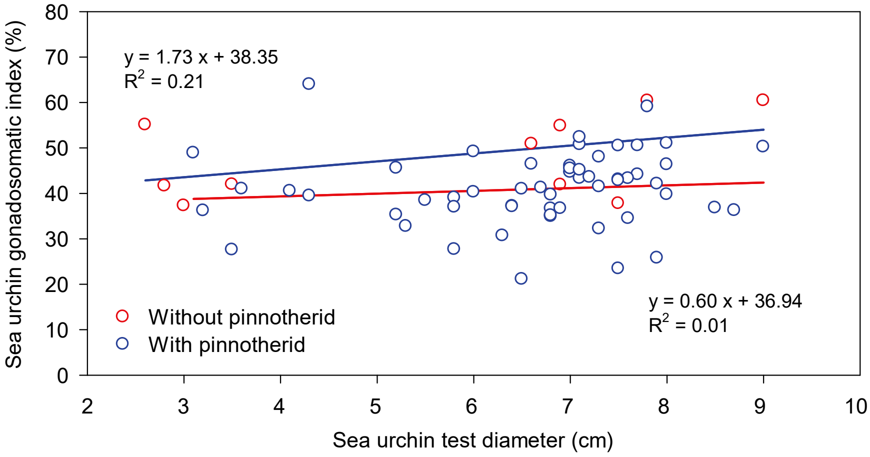

Fig. 6. GSI for sea urchins with and without pinnotherids inside the host digestive system, as a function of host test diameter.

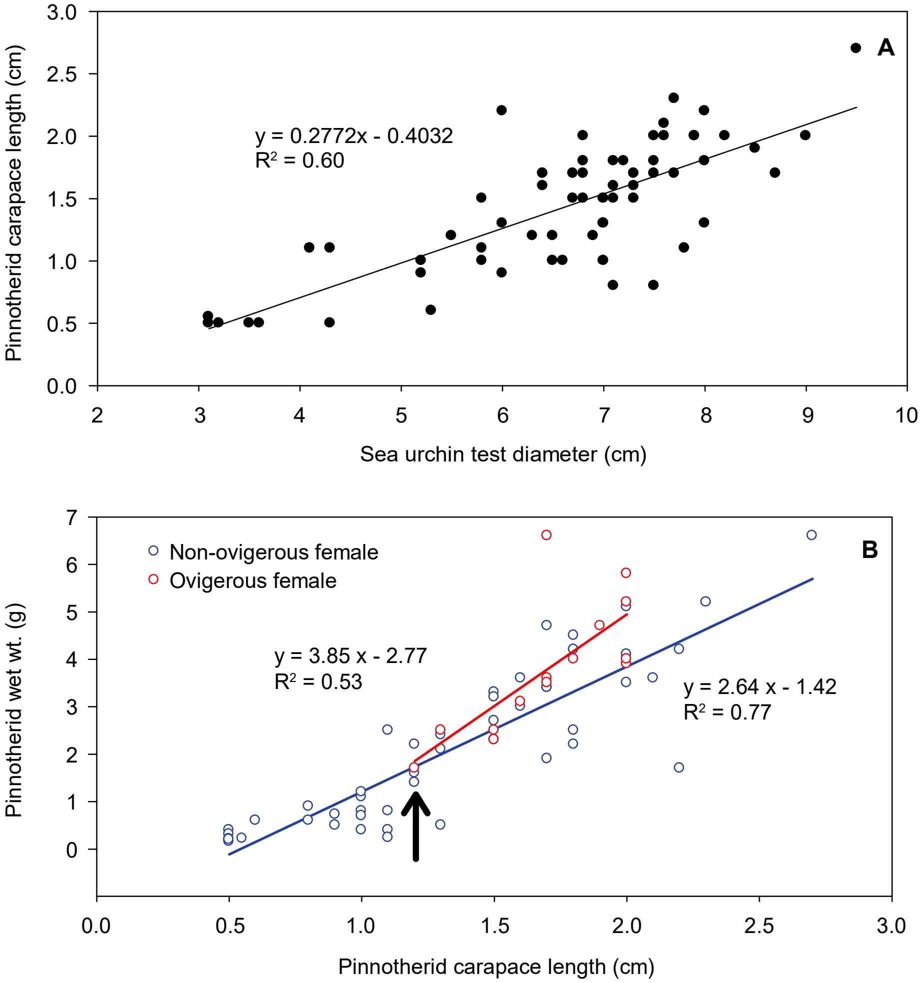

Fig. 7. A, Diameter of the sea urchin host and its relationship with the size of the pinnotherid guest. N = 62. B) Size and wet weight of pinnotherid females according to their reproductive condition. Arrow shows minimum incubation size (1.2 cm). N = 62.

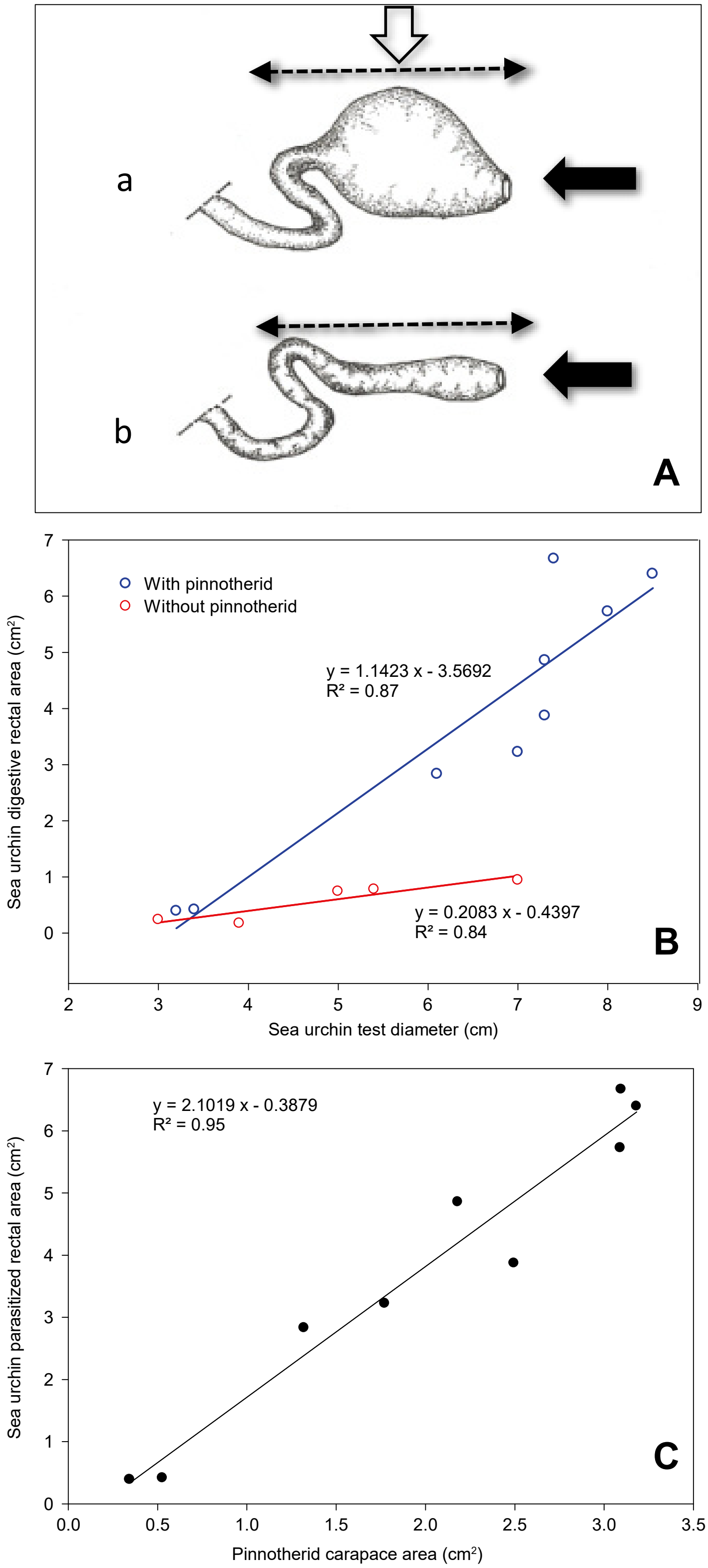

Fig. 8. A, Diagram of the final section of a sea urchin intestine for a) infested and b) non-infected individuals. Solid arrows indicate the terminal portion of the digestive tract (*i.e.*, the anus). The clear, vertical arrow indicates where the hosted pinnotherid was located. Broken arrows show the area of the digestive tract used for comparison between parasitized and non-parasitized sea urchins. B, Impact of the pinnotherid symbiont on the area of the host sea urchin intestine (rectal section). C, Relationship of the widening of the intestine (rectal section) with the diameter of the pinnotherid guest.

infested sea urchin intestine was up to 400% larger than that of a non-infested individual.

The area of the rectal intestinal section of the sea urchin host was strongly related to the presence of pinnotherids (ANCOVA: $F_{(1,11)}$ 7.492; $P < 0.01$, Fig. 8B) as well as to the sea urchin's diameter (ANCOVA: $F_{(1,11)}$ 29.286; $P < 0.01$, Fig. 8B). In addition, the size of the gut area of infested sea urchins was significantly related to the carapace area of the infesting pinnotherids (Linear Regression: $F_{(1,8)}$ 135.021; $P < 0.001$, Fig. 8C).

DISCUSSION

While some symbiotic interactions can be beneficial to both the host and the symbiont or can benefit one species without affecting the other species (*e.g.*, Silliman et al. 2003; Buhl-Mortensen and Mortensen 2004), others can be detrimental to the host (Buhl-Mortensen and Mortensen 2004; Ďuriš et al. 2011). Pinnotherid crustaceans are commonly found in association with a variety of invertebrate hosts (de Gier and Becker 2020); however, the relationships with those hosts are not always well understood. Many cases are known not to be parasitic (da Cruz-Kaled et al. 2006; Burukovsky and Marin 2018) but some others clearly are parasitic (Zaixso et al. 2009; Watts et al. 2018; Trottier and Jeffs 2015; Ocampo et al. 2021). Our results show that the symbiotic relationship between *Pinnaxodes chilensis* and the sea urchin *Loxechinus albus* is associated with reduced gonadal production and lower GSI for both male and female sea urchins, and also impacts the sea urchin's digestive system, which is where the symbiotic pinnotherid resides. Such negative impacts on hosts have been defined as parasitic (O'Beirn and Walker 1999). Negative impacts on the host due to the presence of pinnotherid symbionts have been previously described for a variety of aquatic invertebrate hosts. Pinnotherid symbionts have been found to damage to their hosts' gills, reduce filtration efficiency, steal the collected food, decrease the hosts' tissue weight, and even inhibit reproduction (Sun et al. 2006; Miller et al. 2008; Mena et al. 2014; Ocampo et al. 2014 2021; Becker and Türkay 2017; Yasuoka and Yusa 2017). Parasitism involving pinnotherids has also been identified in species of holothurians from the Indo-West Pacific, such as *Holothuria fursocinerea* (Ng and Manning 2003). In some host species there was an even greater physical impact on the host than was found in the present research. For example, the pinnotherid *Pinnotheres halingi* lives in the respiratory tree of sea cucumbers, and can prey—under adverse conditions of feeding deficiencies—on the host's internal organs, killing it in the process (Tresnati et al. 2021).

More than 85% of the Calfuco Beach sea urchins used in the present research were found harboring pinnotherid crustaceans in the digestive tract. In this species, the prevalence of the infestation is very variable and seems to follow a latitudinal trend along the Chilean coast, as indicated by Runil (2014). That author found an especially high prevalence of the parasite at low latitudes, with between 90 and 100% of the sea urchins being infested, while the prevalence decreased substantially to between 6 and 43% towards higher latitudes. There were also some populations in which pea crab infestations were not recorded at all (*e.g.*, Bahia Mansa, Caleta Larenas, Punta San Pedro, Cabo Phillips). Likewise, Gutiérrez-Martínez (1971) found that 99% of sea urchins were infected in a population in Northern Chile (*e.g.*, Hornitos beach, 22°54'00"S, 70°17'00"W), and between 91 and 100% of the sea urchins sampled from a population near Valparaíso (33°02'46"S, 71°37'11"W) harbored pinnotherids (Baez and Martínez 1976).

In our study population, pinnotherids were only found in sea urchins that had test diameters larger than 3.0 cm, suggesting that these pinnotherids do not infect smaller sea urchins. The smallest pinnotherid found inside any of the sea urchins sampled in our study was 0.5 cm in carapace length, larger than the pinnotherid's megalopa stage (0.13 cm, Gonzalez-Canales et al. 2018). However, Baez and Martínez (1976) recorded pinnotherids inside sea urchins with a minimum carapace length of 0.19 cm, suggesting that the pinnotherid may have invaded the host at a later stage of development. There is no available information on the habitat where the specimens of the early post-metamorphic stages of *P. chilensis* are found. It is still unclear, however, whether the host is initially colonized by the megalopa larval stage, or instead by the metamorphosed juvenile.

The impact of the symbiont on host gonadal development is interesting. Although gonadal dry weight increased with the size of the infected sea urchin, infected individuals had smaller gonads than non-parasitized individuals of similar sizes. However, although the presence of a pinnotherid does not seem to prevent gonadal development in the host, it does negatively impact the host's reproductive capacities, suggesting a reduction in fitness (de Bruyn et al. 2009). This process seems to be associated with a reduced number of gametes generated rather than by alterations in the synchrony of the gametogenic cycle in the gonads of parasitized sea urchin (Vásquez and Bay-Schmith 2010–2011). One of the direct factors that could be affecting the gonadal weight of parasitized sea urchins, and its association with reduced gamete production seems to be related to the diet of the pinnotherid. Most species of pinnotherids that inhabit the host's digestive tract feed on the host's fecal pellets (Miyake 1939; Fenucci 1967; Jangoux 1987). Reduced reproductive output of the host has been recorded in the symbiotic relationship between the calyptraeid gastropod *Crepidula cachimilla* and the pinnotherid *Calyptraeotheres garthi*, with the parasite causing a temporary castration of the host (Ocampo et al. 2014). Future research should address this issue to reliably identify the origin of the food consumed by the parasitic pea crab and the energy impact of that consumption on the sea urchin host.

The pinnotherids in our study caused an expansion of the rectal section of the host's digestive system, increasing the parasitized area by up to 400% with respect to the intestine of a non-parasitized sea urchin of equivalent size, as previously noted by Vásquez and Bay-Schmith (2010–2011). This expansion of the intestine basically involves the "stretching of the intestinal wall in the rectal region, displacement of the aboral end of the gonad with respect to the genital pore, compensatory elongation of its gonoduct and obliteration of the digitiform lobes of the gonadal wall in direct contact with the expanded rectum" (Vásquez and Bay-Schmith 2010–2011), or produces a small deformation in the gonads, that could eventually become very harmful (Fenucci 1967). Anatomical modifications in the digestive tract have also been identified in invertebrates that host the pinnotherids *Echinoecus convictor*, *E. pentagonus* or *E. rathbunae* (see Jangoux 1987). In expanding the digestive system of *L. albus*, a real physical or functional damage on the sea urchin has not been identified, although it is known that the thickness of the intestinal walls decreases progressively with expansion, the epithelial foldings are lost, and the villi of the digestive wall are reduced, likely impacting the absorption of nutrients there (Vásquez and Bay-Schmith 2010–2011). The existence of potential injuries in this tissue due to the presence of pea crab cannot be ruled out, which would imply an extra energy cost for the regeneration of lesions (de Bruyn et al. 2009). The expansion of the parasitized area of the digestive could certainly involve an extra energy investment, but it could also have implications for the digestive processes of the sea urchin host. This issue is likely to decrease gamete production, identified in this research as a lower gonadal biomass seen in parasitized individuals.

P. chilensis females are significantly larger than the males (*e.g.*, Gutiérrez-Martínez 1971; Baez and Martínez 1976; Runil 2014), as also noted in other members of this genus (Thatje and Calcagno 2014). On the other hand, the smallest carapace length of the 57 females of *P. chilensis* sampled in our study that were found to be carrying eggs, was 1.2 cm, while in the population of Mehuín beach (a place near Calfuco Beach) it was 1.74 cm (Lardies and Castilla 2001). However, in northern Chile, an initial incubation size in *P. chilensis* of only 0.82 cm has been identified, suggesting that populations that inhabit in different locations may have different maturation sizes (Lardies and Castilla 2001).

In the present research, a positive and significant relationship was documented between the size of the host and the size of the pinnotherid guest, both between the total wet weight of the sea urchin and the size of the pinnotherid, and between the degree of expansion of the sea urchin's digestive system and the size of the parasite's exoskeleton. All of these relationships show that both the host and the guest likely grow simultaneously. This idea of simultaneous growth between parasitic females and the host sea urchin has also been suggested by Vásquez and Bay-Smith (2010–2011). Thus, it seems feasible to deduce that sea urchins are colonized by the symbiont at small sizes and that it is the same guest pinnotherid that continues to develop in union with the infested sea urchin. In the pea crab *Dissodactylus primitivus*, it has been suggested that infestation would occur at the larval stage or immediately after the pea crab's metamorphosis (de Bruyn et al. 2009). In *P. chilensis* there is usually just a single female found living inside the host, although two pea crab individuals (one male, one female) have occasionally been found within a single host, but only for temporary mating purposes (Fenucci 1967; Baez and Martínez 1976). This normal condition of a single female pinnotherid in each host makes it possible to identify that the relationship of sizes between host and guest would be continuous with the advance of the symbiosis. That is, once the pinnotherid infests the sea urchin, both would continue to grow, as has been indicated for the relationship between the oyster *Crassostrea angulata* and the pinnotherid *Arcotheres sinensis* (Kuo et al. 2018). Notwithstanding the above, in our research it was possible to identify that for hosts of the same size, pinnotherid females of different sizes were sometimes found. This situation suggests that the colonization of the sea urchin does not always occur in the early juvenile stage. It is also possible that a non-previously parasitized sea urchin, larger than 3.0 cm (minimum size recorded in our research), can be colonized by a pinnotherid, or a previously parasitized sea urchin can be colonized again after the death of the original parasite pea crab female. A third possibility is that the guest could grow at different rates in different hosts. This is a topic that merits future research.

In our study population, the largest pinnotherids were found to be living inside the largest sea urchins. The available information on the age and size of the sea urchin for the region closest to our sampling beach (Calfuco) indicates that a sea urchin with a test diameter of 9.0 cm would have an estimated age of approximately 6.5 years (Gebauer and Moreno 1995). Considering that the largest pinnotherid females identified in our study population (2.7 cm) were found in sea urchins with diameters of 9.0 cm, this would imply that the approximate age of the largest pinnotherid would be 6.5 years, assuming that the infestation had occurred very early in the post-settlement life of the sea urchins. However, in our research, the smallest sea urchin harboring a pinnotherid symbiont had a test size of 3.0 cm, with an estimated age of 1.5 years (Gebauer and Moreno 1995); this suggests that the maximum endosymbiotic lifespan for a pinnotherid female (the largest recorded in this research) would be approximately 5 years (Fig. 9). Information on the longevity of pea crab species is very scarce, but the life cycle for the ectosymbiont pinnotherid *Dissodactylus mellitae* is estimated to be between 12 and 15 months (Bell and Stancyk 1983). Future research should address this issue in order to establish the veracity of the estimates made in this research on the maximum parasite age of the pinnotherid *P. chilensis*.

In summary, our results show that approximately 82% of the sea urchins found along Calfuco beach had pinnotherids living in their digestive tract, and that the sea urchin-pinnotherid relationship generates negative effects on the host, reducing both gonadosomatic index and gonadal production, potentially reducing its reproductive capacities substantially. Likewise, the presence of the crustacean produces a distention of the sea urchin's digestive tract in the area in which the pinnotherid lodges, which in turn can create energy demands and interfere with energy acquisition of the sea urchin hosts. These likely negative effects on the host make it clear that the relationship between both species is a parasitic rather than commensal one.

CONCLUSIONS

This study provides a comprehensive assessment of the symbiotic relationship between the sea urchin *Loxechinus albus* and the pea crab *Pinnaxodes chilensis*. Only the crustacean females used the terminal portion of the host's digestive tract as a place of residence. This relationship was evidenced in a high percentage of the sea urchin population studied, in which the pinnotherid generated negative impacts on the host, as evidenced

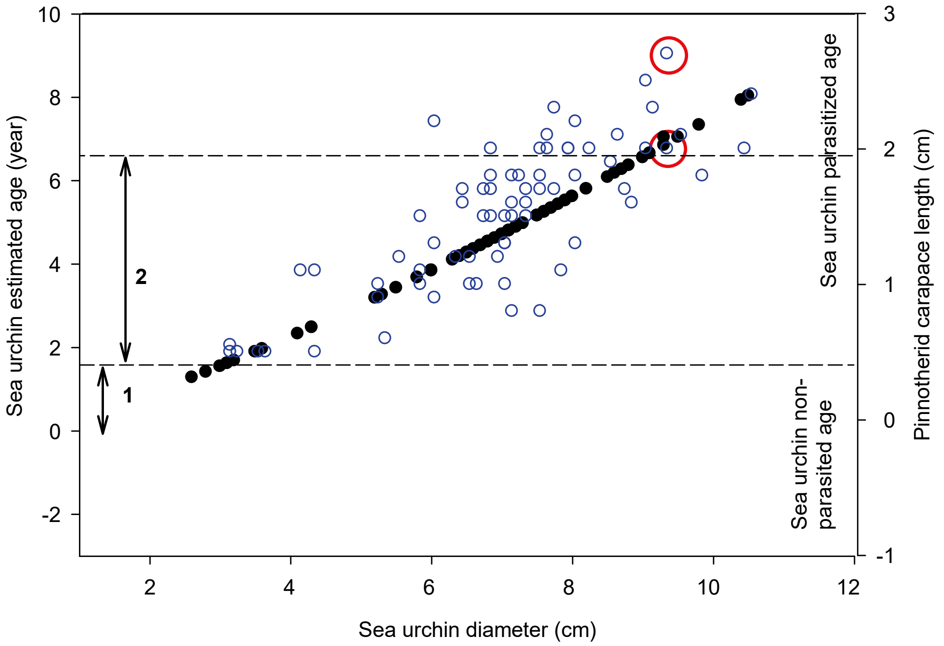

Fig. 9. Sea urchin age as estimated from the sea urchin's test diameter (black circles) based on Gebauer and Moreno (1995). Blue circles correspond to the pinnotherid sizes. Arrow 1 corresponds to the period before the guest arrived inside the sea urchin. Arrow 2 corresponds to the range of sea urchin sizes containing the smallest and largest pinnotherids (approx. 3 and 9 cm diameter of test) found in this study. This size range corresponds to a growth period of 5 years for the sea urchin (estimated according to Gebauer and Moreno 1995) and for the guest crab as well, assuming that infestation by the pinnotherid occurred as a very early juvenile, shortly after metamorphosis. The two circled points (red circles) show the sea urchin that harbored the largest pinnotherid symbionts. Data below the lower dash line shows sizes of sea urchins that were never parasitized by pea crabs. The upper dash line indicates the age of the sea urchins in which the largest parasitic pinnotherid was found.

by a lower gonadal weight, a reduced condition index and alterations in the morphology of the digestive tract where the pinnotherid resided. This evidence suggests that the relationship is parasitic rather commensal with potentially substantial impacts on the reproductive capabilities of the host.

Acknowledgments: The authors gratefully acknowledge the financial support of the Fondo Nacional de Investigación Científica y Tecnológica de Chile (FONDECYT) through grant number 1180643 and ANID-Chile (REDES 190027 UACh) to ORC and FONDECYT grant number 1190875 to VMC.

Authors' contributions: HNJ, LPS, JAB, and ORC designed the study. HNJ, LPS, and JAB performed the samplings collections. HNJ, LPS, JAB, VMC, JAM, and LMP, collected and analysed the data and prepared illustrations. OCR, HNJ, LPS, and JAB prepared the initial version of the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript. All authors cooperated on the experimental design and worked on the manuscript.

Competing interests: The authors declare that they have no conflicts of interest.

Availability of data and materials: All data are provided within the manuscript.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Supplementary materials

Fig. S1. A) Relationship between sea urchin test diameter and total wet weight for parasitized sea urchin males (red circles) and females (blue circles). B) Relationship between the total wet weight of male (red circles) and females (blue circles) host sea urchins and the carapace length of the pinnotherid. N = 62. (download)

Fig. S2. A) Relationship between sea urchin test diameter and dry gonadal weight in males (red circles) and females (blue circles) of parasitized sea urchins. B) Relationship between the gonad dry weight of male (blue circles) and female (red circles) sea urchin and the carapace length of the hosted pinnotherid. N = 62. (download)

Fig. S3. A) Relationship between sex (red circles: male, blue circles: female) of the sea urchin host on non-gonadal tissue weight. B) Influence of the size of the hosted pinnotherid on the dry non-gonadal tissue weight of the male (read circles) and females (blue circles) of sea urchin specimens collected in the Calfuco Becah. N = 62. (download)