POPULATION DISTRIBUTION, SEXUAL DIMORPHISM, AND REPRODUCTIVE PARAMETERS IN THE CRAB *PINNIXA VALDIVIENSIS* RATHBUN, 1907 (DECAPODA: PINNOTHERIDAE), A SYMBIONT OF THE GHOST SHRIMP *CALLICHIRUS GARTHI* (RETAMAL, 1975) IN THE SOUTHEASTERN PACIFIC

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ABSTRACT

Pinnixa valdiviensis Rathbun, 1907 inhabits burrows of the ghost shrimp *Callichirus garthi* (Retamal, 1975) in the south-eastern Pacific. We described the host-use pattern and sexual dimorphism of *P. valdiviensis* to test for monogamy considering that the few other studied species of *Pinnixa* White inhabit their respective hosts as male-female pairs. Against expectations, *P. valdiviensis* lived either solitarily, or in small groups of up to four individuals; only a few burrows were inhabited by two crabs (either male-female or female-female pairs). The observations above argue against the notion that *P. valdiviensis* is monogamous. Furthermore, that a high frequency of solitary females were found brooding embryos and that the population sex ratio was skewed toward females suggests that males might roam among hosts in search of receptive females. We argue in favour of additional long-term (seasonal, >1 year) descriptive and experimental studies to reveal the mating tactics used by both males and females of *P. valdiviensis*. We also examined individual-level reproductive parameters in the studied species as little is known about reproduction in symbiotic crabs. Fecundity varied between 383 and 1052 eggs crab^{-1} with a mean $\pm$ SD of 774 $\pm$ 160 eggs crab^{-1} and increased significantly with female body size. Embryo volume varied between 0.0169 and 0.0443 with a mean $\pm$ SD of 0.0222 $\pm$ 0.0063 mm^3 and did not vary with female body size. Reproductive output (RO) represented a mean $\pm$ SD of 5.74% $\pm$ 0.86% of crab body dry weight and increased proportionally with crab body weight. The RO in *P. valdiviensis* is lower than that reported for other free-living and symbiotic crabs.

KEY WORDS: Chile, Crustacea, monogamy, pea crab, symbiosis

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INTRODUCTION

The adoption of a symbiotic lifestyle [symbiosis here defined *sensu* de Bary (1879) as dissimilar organisms living together] is one of the most important environmental adaptations in marine invertebrates, including brachyuran crabs, one of the most species – rich clades of crustaceans (Ross, 1983; De Grave et al., 2009). In particular, crabs from the family Pinnotheridae De Haan are recognized not only because of their predominant symbiotic lifestyle but because of their remarkable disparity in terms of morphology and behavior (Schmitt et al., 1973; Kruczynski, 1975; Bell, 1984).

Pinnotheridae, with more than 304 currently described species (De Grave et al., 2009), are collectively known as 'pea crabs' due to their smooth external appearance and rounded body shape (Ng et al., 2008; Palacios-Theil et al., 2009; Ocampo et al., 2012). Several pea crab species dwell on the mantle cavity of gastropods and bivalves, e.g., *Zaops ostreum* (Say, 1817) and *Austinotheres angelicus* (Lock-ington, 1877) living in the mantle cavity of *Crassostrea virginica* (Gmelin, 1791) and *Myrakeena angelica* (Roche-brune, 1895), respectively (for details see Schmitt et al., 1973). Other species use the gill basket of ascidians or the cloaca of holothurians and sea urchins as a refuge (Fenucci, 1967; Schmitt et al., 1973; Campos et al., 2009; Campos and Campos, 2012). Even other species inhabit galleries and burrows constructed by echiurans, annelids, and other burrowing crustaceans, e.g., ghost shrimps in the genera *Neotrypaea* and *Upogebia* (Campos and Campos, 2012).

Pea crabs also exhibit a remarkable diversity in terms of reproductive behaviors and mating strategies. For instance, the few studied species of the genus *Pinnixa* inhabit their hosts as socially monogamous pairs, e.g., *P. transversalis* (H. Milne Edwards and Lucas, 1842) and *P. chaetopterana* Stimpson, 1860 in tubes constructed by the polychaete *Chaetopterus variopedatus* (Renier, 1804) (Baeza, 1999; McDermott, 2005 and references therein). By contrast, various other pea crab species appear to be highly promiscuous.

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For instance, in the genera *Tumidotheres* and *Fabia*, male and female crabs are reported to form mating swarms in open water. Then, females (but not necessarily males) colonize host individuals for long periods of time and continuously produce embryos during the rest of their lives (Christensen and McDermott, 1958; Pearce, 1964, 1966; Campos, 1989). The fate of males after the mating swarm is not well known. Lastly, recent studies suggest a complex polygamous mating system in the ecto-symbiotic crab *Dissodactylus primitivus* Bouvier, 1917 (De Bruyn et al., 2009; Jossart et al., 2014). In this species, males and females move among hosts in search of mating partners. Parentage analyses have revealed that female polyandry is frequent and that some females produce embryo masses fertilized by up to 6 different males (Jossart et al., 2014). Although, our knowledge of the mating behavior in Pinnotheridae has increased substantially during recent years, the mating system of most pea crab species still remains unknown. Studies examining the host use pattern of pea crabs are warranted to improve our understanding of reproductive strategies in symbiotic organisms.

The pea crab *Pinnixa valdiviensis* Rathbun, 1907 is one out of four species from the genus *Pinnixa* reported for the coast of the south eastern Pacific, i.e., Chile and Peru (Garth, 1957). *Pinnixa valdiviensis* has been reported from Chincha Islands, Peru (approximately 13°S) to southern Chile (approximately 53°S) (Garth, 1957; Retamal and Moyano, 2010). Also, a recent study has reported this species in San Julián Bay, Argentina, south western Atlantic (Torres, 2006). *Pinnixa valdiviensis* can be found inhabiting tubes of the polychaete *Chaetopterus variopedatus variopedatus* and the innkeeper worm *Urechis chilensis* (Müller, 1852) (Garth, 1957; Torres, 2006). Although, *P. valdiviensis* is common along the south eastern Pacific, information about the population biology and reproductive behavior of this pea crab is absent.

The aim of this study was to investigate the host use pattern and sexual dimorphism in *P. valdiviensis*, a symbiotic crab of the intertidal burrowing shrimp *Callichirus garthi* (Retamal, 1975). We expected a socially monogamous mating system in *P. valdiviensis* considering that this reproductive behavior has been reported before in other congeneric species (Baeza, 1999; McDermott, 2005). We also examined individual-level reproductive parameters as little is known about reproduction in this and other symbiotic crabs.

MATERIALS AND METHODS

Taxonomic Statement

Pinnixa valdiviensis has been previously recorded to live in association with the polychaete worm *Chaetopterus variopedatus* and the innkeeper worm *Urechis chilensis* (Garth, 1957; Torres, 2006). Our study reports for the first time *P. valdiviensis* living in association with *C. garthi*. We confirmed that our specimens belonged to *P. valdiviensis* after conducting a detailed study of diagnostic characters in the genus *Pinnixa*. In all studied specimens, the carapace exhibited two short cardiac ridges and a transverse hepatic ridge. The chelae were robust with two teeth on the prehensile edge and a dactyl bearing a median tooth. Lastly, the second segment of the palpus in the third maxilliped was long and narrow (Garth, 1957; Torres, 2006). The morphology of our specimens fits remarkably well with diagnostic features in *P. valdiviensis* (Garth, 1957; Torres, 2006). Part of the material analyzed in the present study was deposited in the Museo de Zoología, Universidad de Costa Rica (a lot of 15 males and 12 females, catalog number MZUCR-3334-01).

Study Area

The host-use pattern of the crab *P. valdiviensis*, living within intertidal burrows of the intertidal ghost shrimp *C. garthi*, was studied from August to September 2011 at Las Machas beach, northern Chile (18°25′S, 70°19′W) (Fig. 1a, b). This beach is composed of fine sediments originating, in great part, from the nearby rivers Lluta and San José (Soto et al., 2002). The studied beach is exposed and influenced by high energy waves, especially during the austral autumn (April-June) and winter (July-September) (Hernáez and Wehrtmann, 2007). The ghost shrimp *C. garthi* is restricted to the area located between the Lluta and San José Rivers (approximately 8 km), with high burrow densities in the intertidal zone near the river Lluta (Hernáez and Wehrtmann, 2007). The entrances to the burrows constructed by *C. garthi* are easy to identify at the surface of the beach due to their relatively large diameter (0.8 ± 0.03 mm P. Hernáez, unpubl. data) (Fig. 1a). Each burrow is occupied by at least one ghost shrimp of *C. garthi* (Hernáez and Wehrtmann, 2007).

Sampling of Pinnixa valdiviensis

Pea crabs were collected from *C. garthi* burrows with a hand-made "yabbie" pump (diameter = 77 mm, length = 100 cm). Each burrow was pumped up to three times during crab sampling. Usually, the sediment around the burrows collapsed after the second or third pumping; this collapsing of the sediment eliminated the risk of sampling the same burrow (sampling unit) more than once (Alves and Pezzuto, 1998; Peiró et al., 2013). During sampling, the number of crabs per burrow was recorded. Individuals of *P. valdiviensis* collected from each burrow were separated from sand grains using a fine-mesh (0.5 mm) sieve, rinsed with seawater, placed into individual plastic bags, immediately preserved in alcohol (70%), and transported to the Universidade de Sao Paulo, Brazil.

In the laboratory (about 1 month after sampling), each crab was sexed using pleon and pleopod morphology. Male crabs have a thin and narrow pleon and a pair of long and thin pleopods (gonopods) on the ventral surface of the first pleomere, whereas female crabs have large and wide pleons and four pairs of long setose pleopods from the second to the fifth pleomeres (Garth, 1957). Next, the following measurements were taken from all collected crabs under a stereomicroscope (Motic® FMZ 168 series): carapace width (CW, the widest measure between the lateral margins of carapace), carapace length (CL, from the anterior margin of the rostrum to the posterior margin of the carapace) and length of the propodus of the left and right cheliped (PL). Then, each female crab was classified according to the presence or absence of embryos carried beneath the pleon (brooding or non-brooding). Lastly, embryos carried by brooding females were classified according to three different categories (Wehrtmann, 1990); stage I: rounded embryos with yolk uniformly distributed, no eye pigments visible, stage II: ovoid embryos, embryos with eye pigments elongated, stage III: ovoid embryos, with eyes well developed and the pleon free.

Population Distribution of Pinnixa valdiviensis

We explored whether or not pea crabs were found solitarily, in pairs, or in aggregations in burrows of the ghost shrimp *C. garthi*. For this purpose, we examined whether the distribution of *P. valdiviensis* in burrows of *C. garthi* differed significantly from a random distribution by comparing the observed distribution with the Poisson distribution (Elliott, 1983). Significant differences between the distributions were examined using a Chi-square goodness of fit test (Sokal and Rohlf, 1995).

The sex ratio of the population was estimated as the number of males divided by the total number of males and females collected. The observed sex ratio was tested for deviations from an expected 1:1 sex ratio using a binomial test (Wilson and Hardy, 2002).

Sexual Dimorphism and Allometric Growth in Pinnixa valdiviensis

We tested whether or not *P. valdiviensis* exhibited sexual dimorphism in body size by comparing the average CW of male and female crabs using a *t*-test or Mann-Whitney *U* test depending on whether or not the variances between the compared datasets were homogenous.

In brachyuran crabs, including members of the genus *Pinnixa*, the first pair of thoracic appendages bear large chelipeds that serve as weapons during intra-sexual interactions (Hartnoll, 1978, 1982). We examined whether or not the length of the propodus of the cheliped increased linearly with body size in males and females of *P. valdiviensis*. The relationship between cheliped propodus length and crab CW was examined using the allometric model $y = a \times x^b$ (Hartnoll, 1978, 1982). The slope (*b*) of the
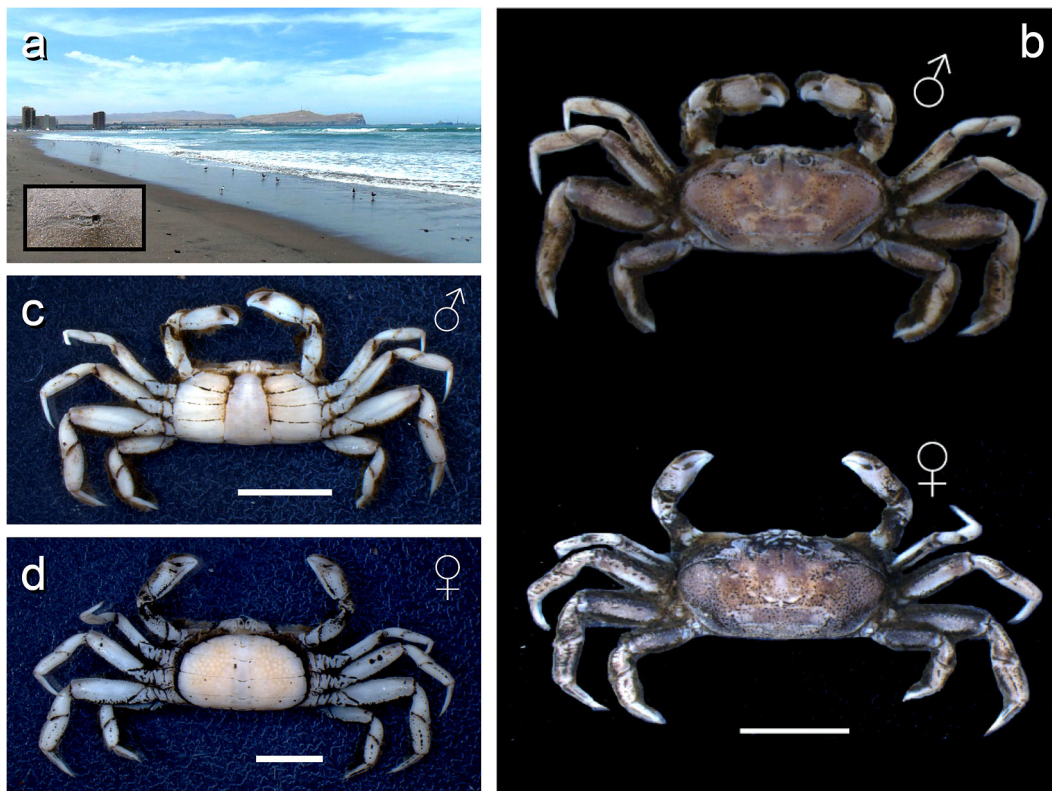
Fig. 1. a, intertidal at 'Las Machas' beach, northern Chile; the inset shows a burrow opening of the ghost shrimp *Callichirus garthi*; b, dorsal view of male and female individuals of the pea crab *Pinnixa valdiviensis*, scale bar = 6 mm; c, ventral view of a male pea crab *Pinnixa valdiviensis*, scale bar = 5 mm; d, ventral view of a female pea crab *Pinnixa valdiviensis*, scale bar = 5 mm. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

log-log least-squares linear regression represents the rate of power increase ($b > 1$) or decrease ($b < 1$) of the propodus with a unit of increase in body size (CW) of crabs. To determine if the studied relationship deviated from linearity (isometry), a *t*-test was used to examine if the estimated slope *b* of each studied pair of variables deviated from the expected slope of unity (Sokal and Rohlf, 1995). If the cheliped grows more or less than proportionally with a unit increase in body size of *P. valdiviensis*, then the slope will be >1 or <1, respectively (Hartnoll, 1978). Pea crabs with missing claws or limbs were excluded from the allometric analyses above.

Reproductive Parameters in *Pinnixa valdiviensis*

A total of 17 female crabs brooding early (stage I) embryos that were collected from within the burrows of *C. garthi* were used for estimating three different reproductive parameters: fecundity, reproductive output, and egg size. First, the CW of each female was measured under a stereomicroscope to the nearest 0.1 mm. The embryos were then gently collected with forceps from underneath the pleon of each female, measured under a microscope equipped with a calibrated ocular micrometer (widest and longest axis for each of twenty embryos per crab), and then directly counted under a stereoscope. Egg volume was estimated with the formula for the volume of an ellipsoid (Turner and Lawrence, 1979), $EV = 1/6(LS2\pi)$, where $L =$ long axis and $S =$ short axis. Fecundity was estimated as the total number of embryos per female crab. Next, each female crab and respective embryo mass was dried for 48 h at 70°C and weighed to the nearest 0.01 mg with an analytical balance (Sartorius; ±0.01 mg). Reproductive output was estimated as the ratio between dry weight of embryos and dry weight of the females carrying the same embryos. This latter parameter represents the amount of resources (biomass) that females invest in reproduction (see Baeza, 2006).

We tested whether reproductive output increased linearly (isometrically) with female body size. The relationship between reproductive output and female body dry mass was examined using the allometric model $y = a \times x^b$ (Hartnoll, 1982). The slope b of the log-log least-squares linear regression represents the rate of power increase ($b > 1$) or decrease ($b < 1$) of the estimate of reproductive allocation with a unit of increase in crab dry

mass. To determine whether the relationship deviated from linearity, a *t*-test was used to test if the estimated slope *b* deviated from the expected slope of unity. Before conducting the test above, assumptions of normality and homogeneity of variances were checked and found to be satisfactory (Zar, 1996).

RESULTS

Population Distribution of *Pinnixa valdiviensis*

A total of 64 crabs of *P. valdiviensis* were collected from within 43 (44.8%) out of 96 sampled burrows of the ghost shrimp *C. garthi* during this study. A total of 24 males (37.5%) and 40 females (62.5%) were retrieved from these burrows and a total of 35 (87.5%) out of the 40 sampled females were ovigerous. The sex ratio was biased towards females in the population (sex ratio = 0.375; Chi-square test of goodness of fit: $\chi^2 = 4.0$, df = 1, $P < 0.05$).

The number of *P. valdiviensis* per host burrow varied between 0 and 4 (average ± SD = 0.67 ± 0.96 crabs burrow^{-1}). The population distribution of *P. valdiviensis* within burrows of *C. garthi* did not differ significantly from a Poisson random distribution (Chi-square test of goodness of fit: $\chi^2 = 0.67$, df = 2, $P = 0.7153$; Fig. 2a).

Thirty (69.77%) out of the 43 burrows harboring *P. valdiviensis* were inhabited by a single crab. The population of solitary crabs exhibited a sex ratio that did not differ significantly from the overall population sex ratio (sex ratio = 0.3 (solitary population) versus 0.375 (overall population sex ratio); Chi-square test of goodness of fit: $\chi^2 = 0.43$, df = 1, $P = 0.512$). Eighteen (85.71%) solitary
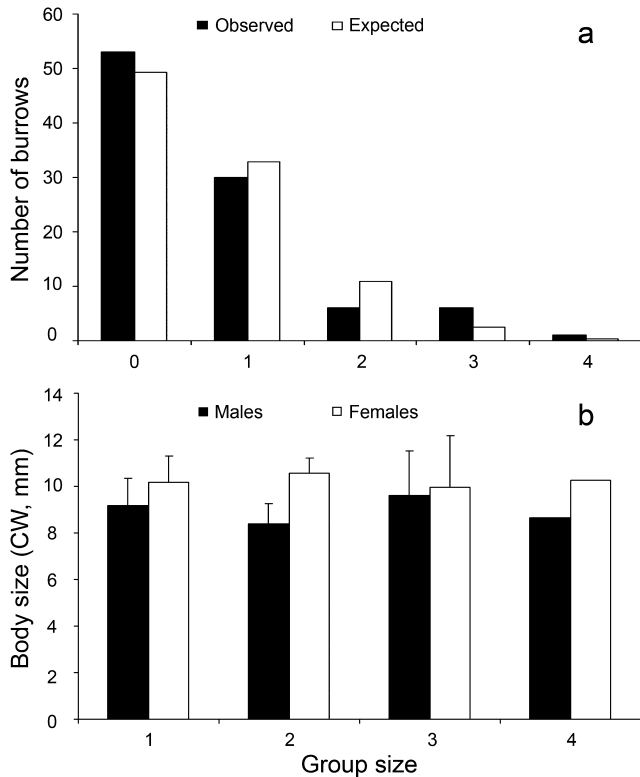
Fig. 2.   a, population distribution (number of crabs per burrow) of the pea crab *Pinnixa valdiviensis*, symbiotic with the ghost shrimp *Callichirus garthi* at Las Machas beach, northern Chile; b, body size (CW, mean ± standard deviation [vertical bars]) of male and female pea crabs *Pinnixa valdiviensis* inhabiting burrows of the ghost shrimp *Callichirus garthi* in different group sizes.

females carried eggs underneath the pleon (12, 3, and 3 females carried eggs in stages I, II, and III of embryonic development, respectively).

Only six out of 43 burrows (13.95%) inhabited by crabs contained pairs. Three of these pairs comprised two ovigerous females. The remaining three pairs were comprised by one male and one ovigerous female. No male-male pairs were observed. The small percentage of burrows harboring pairs of crabs precluded conducting statistical analyses to test whether or not the sexes were randomly distributed within these same burrows. All nine females found in pairs carried eggs underneath the pleon (6, 2, and 1 female carried eggs in stages I, II, and III of embryonic development, respectively).

Six (13.95%) out of 43 burrows inhabited by crabs contained trios. Three of these trios comprised two males and one ovigerous female. One trio comprised three males and another trio comprised three females (two of these females were ovigerous). The last trio comprised two females (one ovigerous) and one male crab. Lastly, the single burrow inhabited by four crabs contained two males and two ovigerous females.

A comparison between burrows inhabited by one, two, and three + four crabs, revealed no significant differences in the body size of both males and females (two-way ANOVA: group size effect: $F = 0.098$, df = 2, 63, $P > 0.05$). The ANOVA demonstrated an effect of sex on body size ($F = 8.43$, df = 1, 63, $P < 0.05$) and the interaction

term was not significant ($F = 4.06$, df = 2, 63, $P > 0.05$) (Fig. 2b).

Sexual Dimorphism and Allometric Growth in *Pinnixa valdiviensis*

The CW of male and female crabs varied between 5.8 and 12.6 mm (mean ± SD, 9.2 ± 1.65 mm) and between 4.6 and 11.8 mm (10.2 ± 1.29 mm), respectively. The majority of males (79.2%) and females (90.0%) were observed in the size range of 7.1 to 11.0 mm CW and 9.1 and 12.0 mm CW, respectively (Fig. 3a, b). The mean CW of females was larger than that of males (Mann-Whitney U-test: $U = 269.00$, $P < 0.01$). Nonetheless, males attained larger final body sizes than females. A high percentage of ovigerous
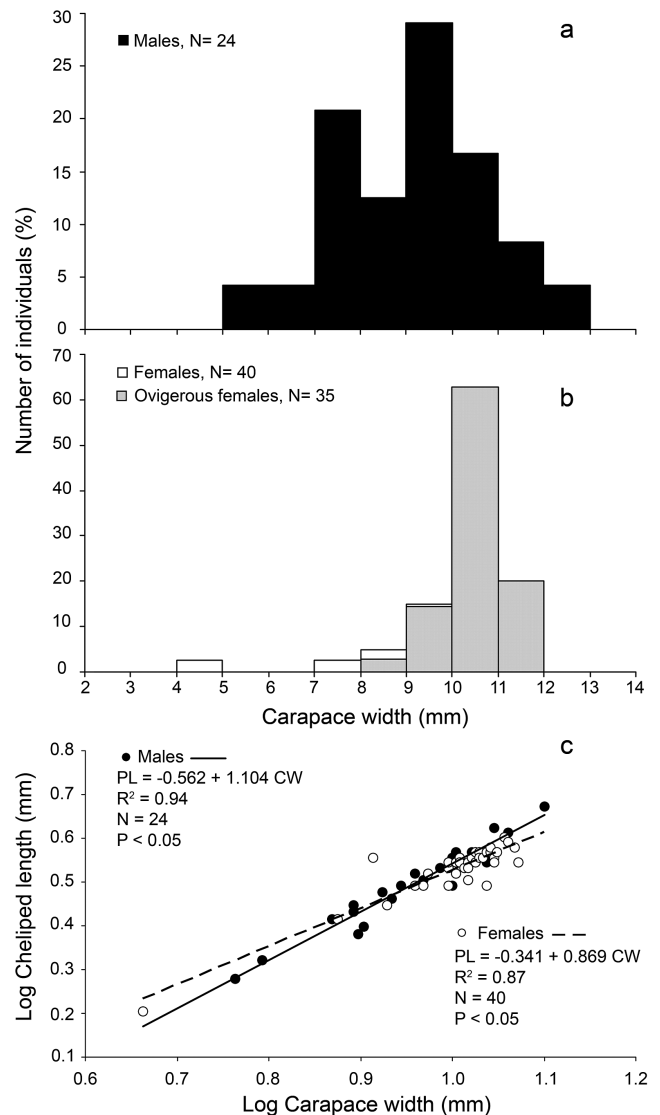

Fig. 3.   a, size-frequency distribution of male and female individuals of the pea crab *Pinnixa valdiviensis* collected from burrows of the ghost shrimp *Callichirus garthi* at Las Machas beach, northern Chile; b, size-frequency distribution of female individuals of the pea crab *Pinnixa valdiviensis* collected from burrows of the ghost shrimp *Callichirus garthi* at Las Machas beach, northern Chile. c, Relative growth of cheliped (propodus length) as a function of carapace width in males and females of *Pinnixa valdiviensis*. Regression line: continuous in males and dashed in females.

females (87.5%) were observed during the study period and their body size varied between 8.2 and 11.8 mm CW (10.5 ± 0.73 mm) (Fig. 3b).

We explored if the males and females of *P. valdiviensis* exhibited heterochely; whether or not one of the chelipeds was invariably larger than the second in the pair. In four (16.7%) male and seven (17.5%) female crabs, respectively, the size of the right and left cheliped was exactly the same. In the remaining 20 males and 33 females, one of the chelipeds was larger than the second in the pair. The largest of the chelipeds in the pair was 7.22 ± 4.95% and 9.28 ± 6.91% larger than the smallest cheliped in the pair in males and females, respectively (paired *t*-test: males: $t = 6.09$, df $= 19$, $P < 0.0001$; females: $t = 7.96$, df $= 32$, $P < 0.0001$). The largest of the chelipeds was observed, respectively, on the right side of the body 75% of the times in males ($n = 20$) and 51.5% of the times in females ($n = 33$). These frequencies did not differ significantly from an expected random distribution of 1:1 in females but were marginally significant in males (Chi-square of goodness of fit, $\chi_1^2 = 0.03$, $P > 0.05$ for females, and $\chi_1^2 = 5.00$, $P < 0.05$ for males). Overall, males and females of *P. valdiviensis* do exhibit heterochely. However, in the two sexes, this condition is not well developed because: 1) some individuals in the population exhibited chelipeds of exactly the same size, and 2) the difference was not considerable (<10%) in males and females with chelipeds of dissimilar size.

The size of the largest cheliped (propodus length) differed with respect to the status and degree of allometry depending on the sex of the crabs. In males, the growth of the cheliped was isometric with respect to body size; the slope of the relationship between male CW and propodus length did not differ significantly from unity ($b = 1.1$, $P > 0.05$, Fig. 3c). In females, the cheliped presented negative allometry; the slope of the relationship between female CW and propodus length was significantly smaller than unity ($b = 0.87$, $P < 0.05$, Fig. 3c).

Reproductive Parameters in *Pinnixa valdiviensis*

Fecundity varied between 383 and 1052 eggs crab⁻¹ with a mean ± SD of 774 ± 160 eggs crab⁻¹. Embryo number increased significantly with female body size (*t*-test: $t = 3.96$, df $= 1, 16$, $P < 0.01$; Fig. 4a). Embryo volume varied between 0.0169 and 0.0443 with a mean ± SD of 0.0222 ± 0.0063 mm³. No correlation between embryo size and crab body size was found (*t*-test: $t = 0.54$, df $= 1.16$, $P > 0.05$; Fig. 4b). Reproductive output varied between 4.43 and 7.27% and represented a mean ± SD of 5.74 ± 0.86% of crab body dry weight. Reproductive output increased proportionally with crab body weight, i.e., RO exhibited isometry, as the slope of the line describing the relationship between these two variables (after log-log transformation) was not significantly different from unity (*t*-test: $t = 1.48$, df $= 1, 14$, $P > 0.05$; Fig. 4c).

DISCUSSION

Population Distribution of *Pinnixa valdiviensis*

Pinnixa valdiviensis inhabits burrows of the ghost shrimp *C. garthi* either solitarily or in small groups of up to four
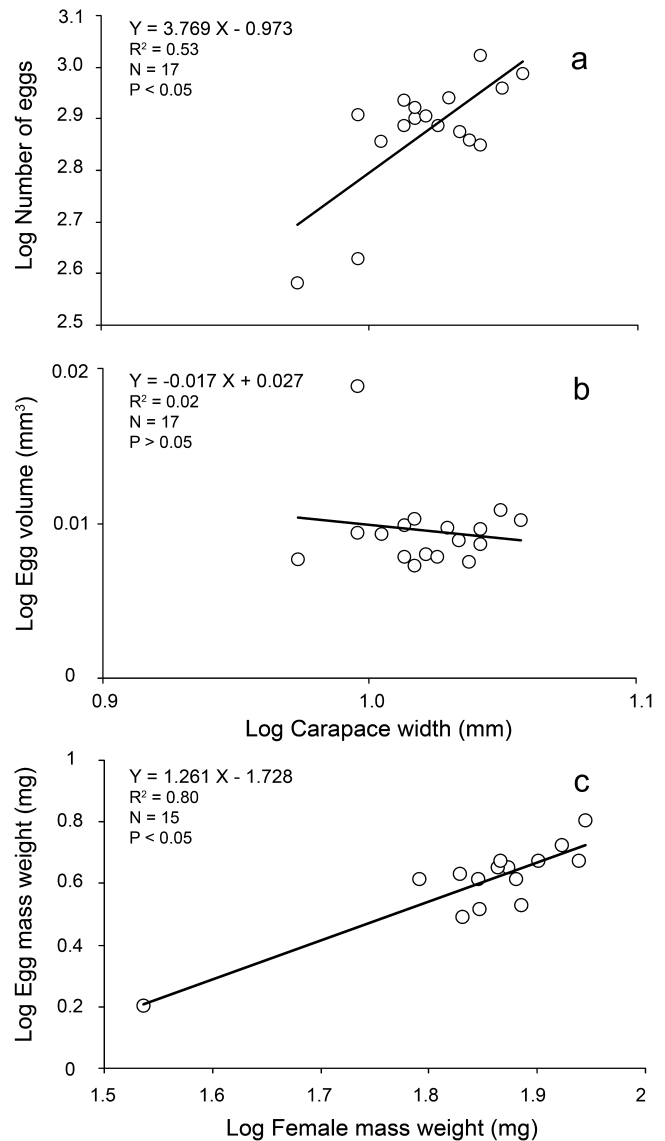

Fig. 4. Individual reproductive parameters in *Pinnixa valdiviensis*. a, fecundity, relationship between female body size (CW) and egg number after log-log transformation of the variables; b, egg size, relationship between female body size (CW) and egg volume after log-log transformation of the variables; c, reproductive output, relationship between female body weight (dry) and egg mass weight (dry) after log-log transformation of the variables.

individuals. The population distribution of *P. valdiviensis* did not differ significantly from a random distribution, only a few burrows were inhabited by two crabs, and these burrows inhabited by couples harbored either two females or one male and one female crab. Our observations in *P. valdiviensis* disagree with that reported for other pea crabs from the same genus. *Pinnixa transversalis* and *P. chaetopterana* inhabit their respective host species as male-female, i.e., socially monogamous, pairs with greater frequency than expected by chance alone (Pearse, 1966; Grove and Woodin, 1996; Baeza, 1999).

The information above and the presence of a relatively high frequency of host burrows with solitarily females carrying fertilized eggs (in different stages of development)

suggests that *P. valdiviensis* is not socially monogamous. If males of *P. valdiviensis* were monogamous, all or a large proportion of the solitary females should have not been observed carrying fertilized eggs beneath the pleon. Indeed, females from other monogamous crustaceans that exhibit a symbiotic lifestyle do not brood embryos when found solitarily in/on their hosts, i.e., the caridean shrimp *Paranchistus pycnodontae* Bruce, 1978 symbiotic with the winged pearl oyster in the Indo-Pacific (Baeza et al., 2013). In *P. valdiviensis*, the existence of solitary females brooding embryos in different stages of development additionally suggests that the males might be roaming among host burrows in search of mates and then abandoning females (once found) soon after insemination (see below).

Lastly, the female-skewed sex ratio in the studied population of *P. valdiviensis* represents another line of reasoning indicating that this pea crab is not monogamous and suggests that males, but not females, might be leaving hosts (at least temporarily) in search of mating partners. Males and females are found in similar proportions in populations of symbiotic crustaceans that exhibit a monogamous mating system (Baeza, 1999, 2008 and references therein). In *P. valdiviensis*, sex specific differences in mortality rates by predators, driven by a greater propensity of males (compared to females) to switch among host individuals, could lead to the observed sex ratio skew towards females in the population. Female-skewed sex ratios have been reported before in other symbiotic and free-living crustaceans in which males frequently roam among hosts in search of receptive females (Nakashima, 1987; Baeza and Díaz-Valdés, 2011).

Unfortunately, our observations do not allow further conclusions about the mating system of *P. valdiviensis*. We argue in favour of additional long-term (seasonal, >1 year) descriptive and experimental studies in this species to reveal the mating strategies displayed by both male and female individuals of *P. valdiviensis*.

Sexual Dimorphism and Allometric Growth in *Pinnixa valdiviensis*

Males of *P. valdiviensis* were, on average, smaller than females, in agreement with that reported for other congeneric species, e.g., *P. transversalis* (Baeza, 1999). Nonetheless, males attained larger final body sizes than females, and the overlap in CW between males and females was substantial. Altogether, the above data suggest that *P. valdiviensis* is sexually monomorphic with regards to body size.

Males and females of *P. valdiviensis* differed with respect to the status of allometry of the major cheliped. In males, the growth of the cheliped was isometric with respect to body size while in females, the cheliped exhibited negative allometry. Considerable sexual dimorphism in cheliped size (males ≫ females) and positive allometric growth of the chelipeds in males, but not in females, is often reported in both symbiotic and free-living species in which males compete for receptive females via overt aggression (Correa and Thiel, 2003; Bauer, 2004; Baeza and Thiel, 2007). The absence of positive allometric growth herein reported for the major cheliped of *P. valdiviensis* suggests that aggression when competing for access to receptive females might not be prominent in males of the species. This same observation also suggests that males of *P. valdiviensis* might be competing against each other for access to females using exploitative tactics, e.g., roaming, rather than interference, i.e., overt aggression. Whether or not males of *P. valdiviensis* do move among different burrows of *C. garthi* actively searching for sexual partners remains to be experimentally addressed.

Reproductive Parameters in *Pinnixa valdiviensis*

Fecundity, but not egg size, increased with body size in *P. valdiviensis* as reported before for most species of brachyuran crabs in which fecundity and egg size has been studied (Hines, 1991; Reid and Corey, 1991). Also, average fecundity reported herein for *P. valdiviensis* is smaller than that reported for other species from the same genus. For instance, in *P. chaetopterana*, an average-sized female will produce between 1191 and 8826 eggs per clutch, depending upon the host species from which it is retrieved (Grove and Woodin, 1996). To the best of our knowledge, there are no reports on egg size in other species of the genus *Pinnixa*. Table 1 summarizes the available information about egg production in pea crabs and illustrates a considerable variability in both fecundity and egg size among species. Also, fecundity in *P. valdiviensis* is considerably smaller than that reported for other crabs from the same family that inhabit the mantle cavity of mollusks (Ocampo et al., 2012 and references in Ta-

Table 1. Fecundity and egg size in pea crabs from the family Pinnotheridae. The range in carapace width (CW) of ovigerous females, the number of eggs (NE), and egg volume are shown for the different species. CW, carapace width; NE, number of eggs; EV, egg volume; RO, reproductive output; n.a., information not available. *Eggs recently produced (stage I).

Species	CW (mm)	NE	EV (mm^3)	RO	Life style	References
Austinixa patagoniensis (Rathbun, 1918)	8.0-n.a.	830-2472	n.a.	n.a.	Cohabitant	Alves and Pezzuto (1998)
Austinotheres angelicus (Lockington, 1877)	4.0-7.6	680-3300	n.a.	n.a.	Entosymbiotic	Cabrera-Peña et al. (2001); Campos and Vargas-Castillo (2014)
Calyptraeotheres garthi	3.1-7.1	201-3838	0.010-0.019	0.28-0.89	Entosymbiotic	Ocampo et al. (2012)
Dissodactylus mellitae	2.8-4.7	64-372	0.012 ± 0.003	n.a.	Ectosymbiont	Bell and Stancyk (1983)
Pinnaxodes chilensis	8.2-20.3	2134-15,898	0.048-0.072*	0.65-0.85	Entosymbiotic	Lardies and Castilla (2001)
Pinnixa valdiviensis	8.2-11.8*	383-1052*	0.017-0.044*	0.04-0.07	Cohabitant	This study
Zaops ostreum	9.4-10.8	7957-9456	n.a.	n.a.	Entosymbiotic	Christensen and McDermott (1958)

ble 1). Unlike pea crabs from *Pinnixa*, calcification of the exoskeleton in the entosymbiotic *Calyptraeotheres*, *Fabia*, *Pinnaxodes*, *Tunicotheres*, and *Zaops* is greatly reduced and, in most of the species above, their ovaries extend out of the cephalothorax into the pleon (Schmitt et al., 1973; Hines, 1992). The poor carapace calcification most likely allows distension of the body and the subsequent increase in gonad size and fecundity in these mollusk- and sea urchin-dwelling species (Hines, 1992). We argue in favor of additional studies examining fecundity, habitat (hosts), and body shape to improve our understanding of those intrinsic (growth pattern) and extrinsic (ecological) conditions favoring and constraining fecundity and egg size in marine, including symbiotic, organisms.

The mean reproductive output (RO) for *P. valdiviensis* was much smaller than that reported for the slipper limpet-dwelling *Calyptraeotheres garthi* (Fenucci, 1975) (44-55% of body weight; Ocampo et al., 2012), the sea urchin-dwelling *Pinnaxodes chilensis* (H. Milne Edwards, 1837) (70-81% of body weight; Lardies and Castilla, 2001) and the oyster-dwelling crabs *Zaops ostreum* (Say, 1817) (66% of body weight; Hines, 1992) and *Fabia subquadrata* Dana, 1851 (97% of body weight; Hines, 1992). The above are the only four other species of pea crabs for which this parameter has been measured; there are no reports of RO in other species of *Pinnixa*. Also, RO in *P. valdiviensis* scaled isometrically with female body weight; female crabs increased allocation to brood mass proportionally with a unit of increase in body weight. As in *P. valdiviensis*, brood weight exhibits an isometric or nearly isometric constraint with increasing female body size in most free-living and symbiotic brachyuran crabs (Hines, 1992; Ocampo et al., 2012; for an exception, see Bolaños et al., 2012). Nonetheless, in most of these free-living crabs, RO is limited to about 10% of body weight, while in symbiotic crabs, RO is more variable and limited to 50-97% of body weight. Limitations on space available for yolk accumulation in the body cavity appear to be the main factor constraining brood size both in free-living and symbiotic crabs (Hines, 1992; for an exception, see Bolaños et al., 2012). Comparative studies that will help us to understand those environmental conditions (e.g., host ecology and biology) driving life history traits in pea crabs are underway.

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