The Biological Bulletin, April 2022, volume 242, number 2: 000–000. https://doi.org/10.1086/719408

Monogamy in the Burrowing Shrimp *Axianassa australis* Rodrigues & Shimizu, 1992 (Decapoda, Gebiidea, Axianassidae)

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Abstract. Our knowledge of the mating systems in burrowing shrimps (infraorders Axiidea and Gebiidea) is still rather limited. Here we describe the burrow use pattern, sex ratio, and sexual dimorphism of the burrowing shrimp *Axianassa australis* to test for monogamy, considering that monogamous species live in heterosexual pairs and exhibit a low degree of sexual dimorphism. To this end, a total of 226 individuals of *A. australis* were collected from the northeast region of Brazil. Our results showed that *A. australis* inhabited its burrows mainly as pairs, most of which were male-female pairs. In agreement with the expectations, specimens of *A. australis* were found dwelling as heterosexual pairs more frequently than expected by chance alone. The presence of ovigerous females was associated with the burrow occupation; that is, brooding females were more frequently observed in male-female combinations than solitarily. Also supporting theoretical considerations, we did not observe sexual dimorphism in body size between males and females of the population and the different categories of the burrow occupation. Conversely, sexual dimorphism in cheliped size was evident in the population, with larger chelipeds in males than in females. This observation agrees with that reported for most burrowing shrimps in which male-male competition is the main evolutionary force of sexual selection. The observations above favor the hypothesis that *A. australis* is primarily monogamous, with a small fraction of the males moderately promiscuous.

Introduction

Major reviews about the reproductive behavior of crustaceans have summarized much of the existing information about functional and evolutionary relationships between mating systems of these organisms (Bauer, 2000, 2004; Correa and Thiel, 2003; Shuster, 2007; Subramoniam, 2013). Specialists recognize four basic mating systems in crustaceans: (1) monogamy, where there is an association between two individuals of the opposite sex to reproduce or share one microhabitat for a long time period exceeding one reproductive cycle; (2) polygamy, where at least some individuals have multiple mates; (3) mate guarding, where a male guards the female from other males in order to be the sole father of her offspring; and (4) pure searching, where male mating success depends primarily on males' ability to find (and mate with) as many receptive females as possible (for further details see Correa and Thiel, 2003; Bauer, 2004).

The burrowing shrimps of the infraorders Axiidea and Gebiidea (formerly treated together as Thalassinidea) construct burrows of different shapes and depths (Griffis and Suchanek, 1991) and play an important role in shaping community structure (Pillay, 2019). Because the lifestyle of burrowing shrimps is fossorial, the reproductive behavior and, consequently, the

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Received 2 November 2021; Accepted 31 January 2022; Published online 5 April 2022.

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Abbreviations: CL, carapace length; PL, propodus length.

Online enhancement: data supplement.

mating system are poorly documented in these organisms. Instead of direct behavioral observations, mating systems of fossorial organisms have been inferred by such characteristics as sexual dimorphism in body size and chelipeds, social organization within the burrow (*e.g.*, solitarily, in pairs, or in aggregations composed by more than two shrimps), and sex ratio (Candisani *et al.*, 2001; Bilodeau *et al.*, 2005; Shimoda *et al.*, 2005; Hernáez and João, 2018; Hernáez *et al.*, 2021; for an exception, see Somiya and Tamaki, 2017). It is from these observations that we now know the evolutionary consequences that mating systems have produced in these organisms.

Burrowing shrimps are best known to inhabit their gallery solitarily (Dworschak *et al.*, 2012). This is the main reason why burrowing shrimps are expected to be polygamous, since most of these species do not live in male-female pairs (see revision by Hernáez, 2018b). While a few exceptions of burrowing shrimps living in pairs have also been reported (Berrill, 1975; Dworschak and Ott, 1993), reports have not been linked to any of the known mating systems, because the samples collected in these studies have been insufficient to reach definitive conclusions.

The availability of receptive females for reproduction is one of the most influential factors in determining crustacean mating systems, because it determines the intensity and direction of competition for mates (Bauer, 2000, 2004; Correa and Thiel, 2003). In burrowing shrimps, females are usually more abundant than males, so it was expected that many axiideans and gebiideans would not be monogamous (Felder and Lovett, 1989; Pezzuto, 1998; Nates and Felder, 1999; Hernáez and Wehrtmann, 2007; Butler *et al.*, 2009; Hernáez *et al.*, 2019), especially those with solitary habits. Although monogamy is common to many other decapods (*i.e.*, Alpheidae, Hippolytidae, Palaemonidae, and Pinnotheridae) (Baeza, 1999; Correa and Thiel, 2003; McDermott, 2005; Baeza *et al.*, 2016; Alves *et al.*, 2021), it seems to never have been reported in any member of the families Axiidea or Gebiidea.

A series of studies has examined the relationship between different mating systems and sexual dimorphism of decapod crustaceans (Correa and Thiel, 2003; Bauer, 2004). Overall, experts agree that in polygamous species, where competition for receptive females is intense, males are often larger in body size than females and invest heavily in structures, such as chelipeds, that are used as armament against other potential competitors (Hartnoll, 1974; Baeza and Asorey, 2012). On the contrary, in monogamous species sexual dimorphism of both body size and weaponry is reduced or absent because sexual selection is weak, given that monogamy evolved from fidelity between heterosexual pairs (Bauer, 2004). In burrowing shrimps, females usually are larger than males (*i.e.*, reverse sexual dimorphism: *e.g.*, Devine, 1966; Botter-Carvalho *et al.*, 2007; Rosa-Filho *et al.*, 2013), whereas males develop substantially larger chelipeds than females, used to defend the gallery against invasion of other males, especially during intrasexual competition for receptive females (Shimoda *et al.*, 2005; Hernáez and João, 2018).

In this study, we were particularly interested in examining the mating system of *Axianassa australis* Rodrigues and Shimizu, 1992, the only representative of Axianassidae along the Brazilian coast (Hernáez, 2018a). This species inhabits the coastal mangroves and mud flats near the low-tide level in the Gulf of Mexico, including Florida, to Parana, Brazil (Felder, 2001; Botter-Carvalho *et al.*, 2015). Different studies have examined the gallery morphology (Dworschak and Rodrigues, 1997), larval development (Rodrigues and Shimizu, 1992; Strasser and Felder, 2005), feeding behavior (Coelho and Rodrigues, 2001), and reproductive biology (Botter-Carvalho *et al.*, 2015) of *A. australis*. Furthermore, previous studies have anecdotally reported the presence of some heterosexual pairs in *A. australis* (one pair: Dworschak and Rodrigues, 1997; four pairs: Botter-Carvalho *et al.*, 2015), which may be an indicator of monogamy.

Given the above, we tested the hypothesis that the pairing of *A. australis* is associated with a monogamous mating system. We examined the burrow-use pattern, sex ratio, and sexual dimorphism of *A. australis* from the northeast region of Brazil. If *A. australis* is monogamous, then it is expected that the sex distribution of shrimps in pairs is non-random, with male-female pairs being found more often than expected by chance alone. In agreement with theory, we also expected that the population would exhibit an unbiased sex ratio and reduced sexual dimorphism in both body and cheliped size. Finally, we also tested for the presence of a sex-specific relative growth pattern in species as a way to contribute to the knowledge of the evolutionary consequences of mating systems for sexual dimorphism in crustaceans.

Materials and Methods

Study area and shrimp sampling

Specimens of *Axianassa australis* Rodrigues and Shimizu, 1992 were collected during July 2017 in the intertidal zone at Lago da Santana (02°55′03″ S, 41°22′28″ W), Piauí, northeastern region of Brazil (Fig. 1). The study site is a marine lagoon characterized by fine sediment in which mangrove plants appear as the main biotic component of the habitat. *Axianassa australis* is the dominant macroinvertebrate in the intertidal zone of this area. The entrances to the burrows constructed by *A. australis* are easy to identify at the surface of the sediment because of their typical volcano shape: 1–3 cm high and 6–20 cm in diameter at the base, with one opening in the surface.

Samples were randomly collected at low tide during periods of lower daily temperature, when individuals are located near the surface, which facilitates shrimp capture (Hernáez and João, 2018). Shrimps were collected from the burrows by using a handmade yabby pump (for details see Dworschak, 2015).

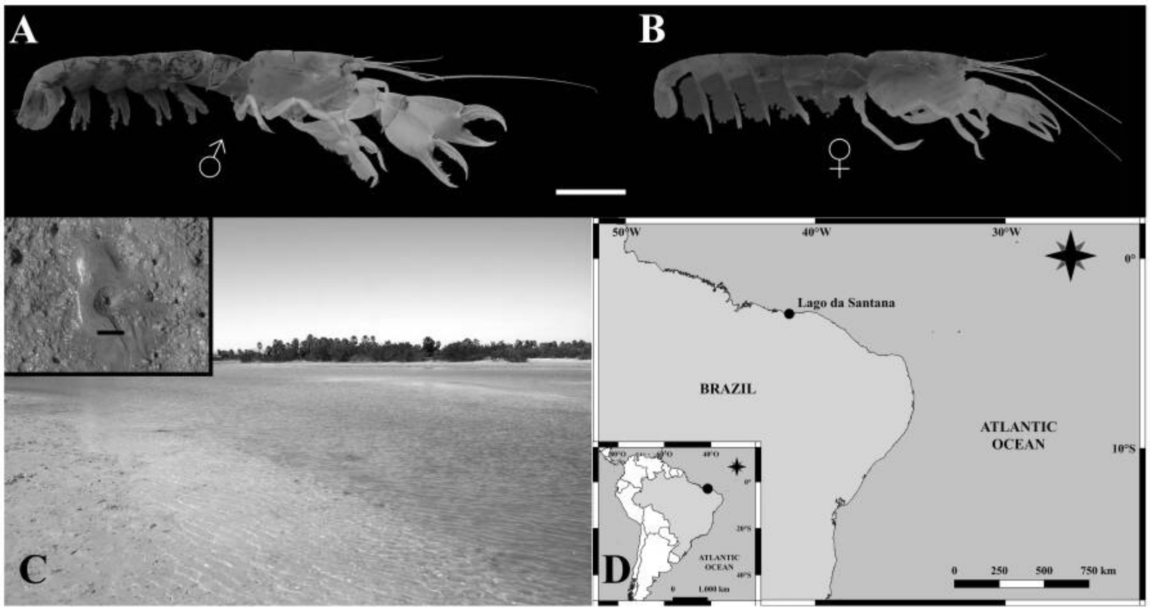

Figure 1. Lateral view of the burrowing shrimp *Axianassa australis*. (A) Male. (B) Female. Scale bar = 5 mm. (C) Intertidal zone at Lago de Santana, coast of Piauí, northeast region of Brazil; inset shows a burrow opening of *A. australis*. Scale bar = 1 cm. (D) Geographical position of the study area (dot). Photos of *A. australis* by Paulo Pachelle, used with permission.

This device consists of a tube (5 cm in diameter, 100 cm in length), a plunger with a handle on the upper end, and a washer that can be adjusted with a thumb screw on the lower end. The end of the pump is stuck into the sand at the same time the handle is pulled, sucking water, sand, and organisms into the tube. Each burrow was pumped up to three times, recording the pairing status, or not, of each shrimp collected (see Hernáez *et al.*, 2021). 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. Suction pumping of burrows with a yabby pump, like the one used during this study, is an efficient method for sampling organisms living in intertidal burrows (Rodrigues, 1966; see also Hernáez, 2018a). After shrimp collection, the specimen or specimens from each burrow were carefully rinsed with seawater, placed in individual plastic bags, and preserved in 70% ethanol until further examination in the laboratory. Part of the material analyzed in this study was deposited in the Museu de Zoologia of the Universidade de São Paulo, Brazil (MZUSP 39004).

In the laboratory, each shrimp was sexed based on the presence or absence of the first pleopod (absent in males, present in females; Hernáez, 2018a). We also classified females as brooding or non-brooding, according to the presence or absence of embryos carried beneath the abdomen, respectively. Developing embryos of ovigerous females were classified into two stages: initial stage, characterized by rounded eggs with uniform yolk and no visible eye pigments of the embryos, and final stage, characterized by ovoid eggs with elongated and barely visible eye pigments or fully developed eyes and free abdomen of the embryos. We used a stereomicroscope (Zeiss Stemi SV-6, to the nearest 0.1 mm; Oberkochen, Germany) equipped with a digital analysis image system (Zeiss AxioCam MRc5) to measure the carapace length (CL), defined as the distance measured from the postorbital margin to the posterior margin of the carapace, and the major cheliped propodus length (PL), defined as the distance between both propodal articulations, excluding the fixed finger (for details, see the dataset in the supplementary material, available online).

Social structure and sex ratio

To test for monogamy in *A. australis*, we examined the social structure (here defined as the number of shrimps occupying the same burrow) and male-female association pattern (*i.e.*, the different combinations of males and/or females occupying the same burrow). First, we explored whether burrowing shrimps occurred alone, in pairs, or in aggregations within burrows. Therefore, we examined whether the distribution of *A. australis* in burrows (*i.e.*, the frequency of occurrence of burrows without shrimps and with different numbers of shrimps) differed significantly from a random distribution by comparing the observed distribution with the Poisson distribution (Elliott, 1983). Significant differences between the distributions were examined by using a chi-square test of goodness of fit (Sokal and Rohlf, 2011).

Since a relatively large proportion of burrows were found to contain pairs of shrimps (see *Results*), we asked whether the sexes were randomly distributed between shrimp pairs inhabiting the same burrow. We compared the observed distribution with the binomial distribution. The expected random frequencies of distribution of the different sexes were calculated based on the proportion of males and females recorded in the population. A chi-square test of goodness of fit was used to inspect for significant differences between the distributions as indicated above (Elliott, 1983). In parallel, we conducted a chi-square test of independence ($P > 0.05$) by comparing the frequency of ovigerous females and non-brooding females in burrows with one and two shrimps (Sokal and Rohlf, 2011) to answer the question of whether the reproductive status of females is independent of the social structure. Also, we explored whether the presence of males in heterosexual pairs was determined by the reproductive status of females. A chi-square test of independence ($P > 0.05$) was also conducted to detect significant differences between the frequencies of males with brooding and non-brooding females (Sokal and Rohlf, 2011). Last, the sex ratio of the population was analyzed 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

The occurrence of sexual dimorphism (*i.e.*, distinct relative difference in body size and chelipeds between the sexes) in *A. australis* was evaluated by comparing the average of body sizes (CL) and chelipeds (PL) between male and female shrimps, using a *t*-test or Mann-Whitney *U* test, depending on whether the variances between the compared datasets were homogenous.

In monogamous species, disproportionate sexual dimorphism of chelipeds is not observed in males because sexual selection is weak, given that monogamy evolved from fidelity between heterosexual pairs (Andersson, 1994). In contrast, males in polygamous species invest heavily in structures, such as chelipeds, that are used as armament during male-male competition for sexual partners (Hernáez and João, 2018 and Hernáez *et al.*, 2021 and references therein). Herein we conducted an analysis of relative growth and analysis of covariance (ANCOVA) to test whether the growth pattern of males and females has an indirect effect on the mating system in *A. australis*. We examined the relationship of PL × CL of shrimps by using the allometric model $Y = a \times X^b$ (Hartnoll, 1978, 1982). The slope (b) of the log-log least squares linear regression represents the rate of exponential increase ($b > 1$) or decrease ($b < 1$) of the propodus with a unit of increase in body size (CL) of shrimps. We used separate *t*-tests to examine whether the estimated slope of the relationship between PL and CL for males and females deviated from the expected isometric ratio ($b = 1$) (Zar, 2010). Next, we conducted an ANCOVA to test whether PL differed between sexes, including CL as a covariate to control for individual body size. In the ANCOVA, we assumed homogeneity of slopes if there was no significant interaction between the main factor (sex) and the covariate (CL) (Sokal and Rohlf, 2011). If the ANCOVA detected a significant effect of sex in the different growth pattern studied, then we concluded that *A. australis* is not monogamous. Burrowing shrimps with missing claws or limbs were excluded from the allometric analysis.

Results

Social structure and sex ratio

A total of 106 males and 120 females (96 of which were brooding females with embryos in different developmental stages) were retrieved from 260 sampled burrows during the study period. The overall sex ratio did not differ significantly from evenness (chi-square test of goodness of fit: males:females = 0.88:1.00; $\chi^2_1 = 0.87$, $P = 0.352$). The number of shrimps found in inhabited burrows ($n = 133$ burrows) varied between 1 and 2 individuals (1.82 ± 0.38 shrimp burrow^{-1}). Because of the presence of empty burrows and those inhabited by a pair of shrimps, the distribution of *Axianassa australis* within burrows differed significantly from a Poisson random distribution with a frequency greater than expected by chance (chi-square test of goodness of fit: $\chi^2_4 = 58.96$, $P < 0.001$; Fig. 2A; Table 1). In particular, the number of burrows harboring a pair of shrimps expected by chance was substantially lower than the observed frequency (41 *vs.* 93 burrows).

A total of 40 (15%) burrows harbored a single shrimp out of 260 sampled burrows: 11 males and 29 females (20 of which were brooding females). A total of 93 (36%) burrows harbored 2 shrimps ($n = 204$ shrimps) out of all sampled burrows (see also Table 1). From these burrows, we found two shrimps of *A. australis*, in all possible combinations (male + female; male + male; female + female), within the same gallery (Fig. 2B). However, most of these combinations were between a male shrimp and a female shrimp. Indeed, a total of 86 (92%) of these pairs were heterosexual couples; in 72 of all heterosexual pairs there was an ovigerous female with embryos in initial ($n = 35$) and late ($n = 37$) developmental stages. Taking into consideration the binomial distribution, the number of burrows harboring heterosexual pairs expected by chance would have been 72. Therefore, shrimps were found as heterosexual pairs more frequently than expected by chance. In addition, the frequency of brooding and non-brooding females in burrows with one and two shrimps was independent of social structure (chi-square test of independence: $\chi^2_1 = 1.60$, $P = 0.207$). On the other hand, males were

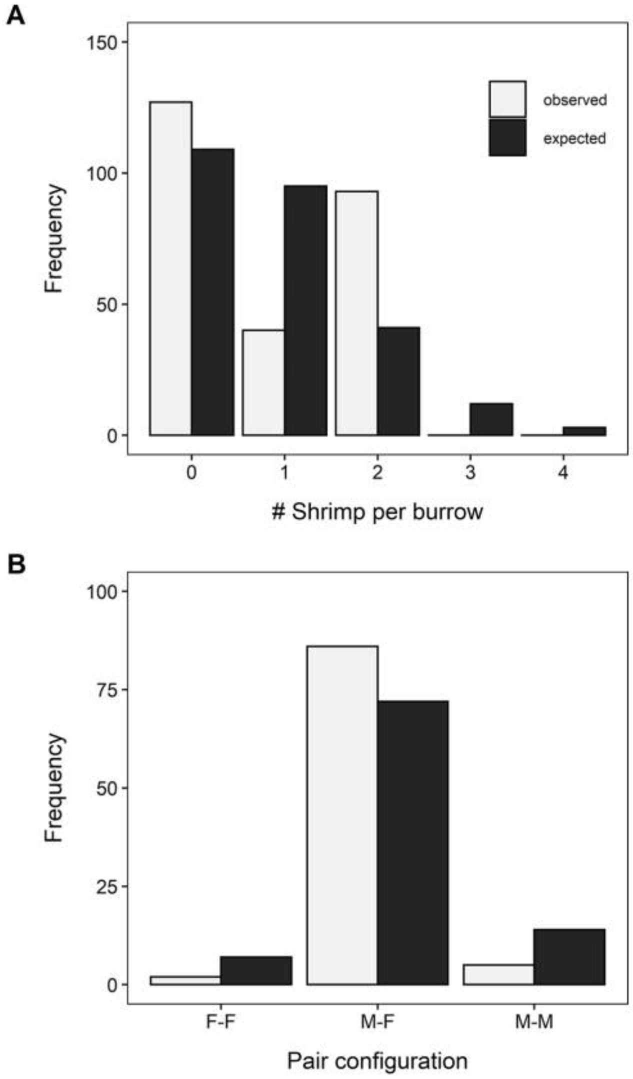

Figure 2. Burrow use pattern of *Axianassa australis* at Lago de Santana, Piauí, northeast region of Brazil. (A) Social organization of *A. australis*; observed frequency of shrimps within burrows differed significantly from an expected Poisson random distribution. (B) Male-female association pattern of *A. australis* found as pairs inside the burrows; observed frequency of heterosexual pairs differed significantly from the expected binomial random distribution.

more likely to share a burrow with a female if she was reproductive ($\chi^2_1 = 35.59$, $P < 0.001$).

Sexual dimorphism and allometric growth

The overall body size (CL) of male and female shrimps ranged, respectively, from 3.5 to 12.4 mm (mean ± SD: 8.0 ± 2.3 mm) and from 3.2 to 11.8 mm (7.8 ± 2.1 mm). The average CL of the population did not differ statistically between males and females (*t*-test, variances were homogeneous: $t_{224} = 0.61$, $P = 0.544$), indicating the absence of sexual dimorphism with respect to body size in *A. australis*. A similar tendency was observed comparing the CL of males and females in homosexual (*t*-test, variances were homogeneous: $t_{14} = -1.17$, $P = 0.262$) and heterosexual (*t*-test, variances were homogeneous: $t_{168} = 0.39$, $P = 0.696$) pairs (Fig. 3A; Table 1). Conversely, male chelipeds reached, on average, a larger size than female chelipeds, denoting sexual dimorphism with respect to chelipeds in *A. australis* (males >> females; $t_{173} = 8.00$, $P < 0.001$). Similarly, male chelipeds were significantly larger than those of females in solitary shrimps and heterosexual pairs ($t_{127} = 7.68$, $P < 0.001$) but not in homosexual pairs ($t_{13} = 0.69$, $P = 0.501$) (Fig. 3B; Table 1).

A positive correlation was detected between the size (CL) of males and females found as heterosexual pairs ($t_{83} = 20.46$, $P < 0.001$); 83.5% of the variation in female size was explained by male size in shrimps living in heterosexual pairs (Fig. 4A). In 48 (56%) of the 85 burrows harboring heterosexual pairs, the male was larger than the female (average ± SD = 8.4 ± 2.1 mm CL *vs.* 7.6 ± 2.0 mm CL), whereas in 36 (42%) the male was smaller than the female (average ± SD = 7.6 ± 2.2 mm CL *vs.* 8.3 ± 2.3 mm CL).

The ANCOVA showed a significant effect of sex and CL body size on cheliped PL (Table 2A). However, when regression lines were compared between males and females, the interaction was not significant, which demonstrated that the covariate had the same effect for all levels of the categorical factor (Table 2A). A positive correlation was detected between cheliped (PL) and body size (CL) in males and females of the total population (Fig. 4B, C; Table 2). Analysis of allometric growth between cheliped PL and CL revealed an isometric relationship in shrimps of both sexes, that is, the slope was = 1 (Table 2B).

Discussion

We predicted that the burrowing shrimp *Axianassa australis* was monogamous. Thus, we expected that both the population distribution of this shrimp in galleries and the sex distribution of shrimp in pairs would be non-random, with paired shrimp and male-female pairs, respectively, found more often

Table 1

Distribution of sexes (males and females) by social category in the burrowing shrimp Axianassa australis

Social category	No. of males/ females	Body size (CL, mm; mean ± SD) of males/females	Cheliped size (PL, mm;mean ± SD) of males/females
Solitary	11/29	8.3 ± 1.3/7.2 ± 2.0	9.9 ± 1.7/7.4 ± 1.8
Homosexual pair	10/6	6.8 ± 3.4/8.7 ± 2.3	8.9 ± 4.0/7.7 ± 2.1
Heterosexual pair	85/85	8.1 ± 2.2/8.0 ± 2.1	9.9 ± 2.7/6.7 ± 2.0

CL, carapace length; PL, propodus length; SD, standard deviation.

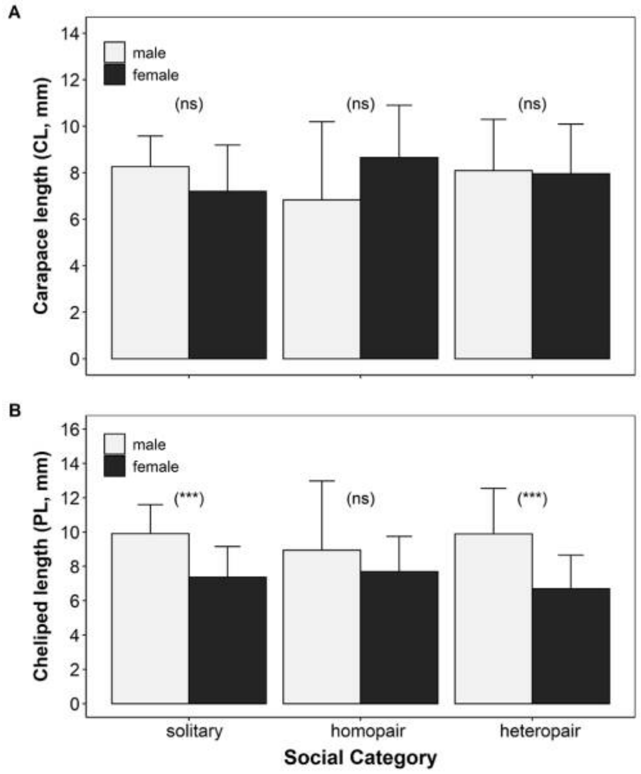

Figure 3. Sexual dimorphism in *Axianassa australis*. Average (± standard deviation) of body size (carapace length [CL]; A) and cheliped size (propodus length [PL]; B) in male and female shrimps of *A. australis* present in different social category groups (solitary, homopair [homosexual pair], heteropair [heterosexual pair]). Significant (***$P < 0.001$) and non-significant (ns, $P > 0.05$) *P*-values are indicated.

than expected by chance. In agreement with the two expectations above, *A. australis* inhabited its burrows mainly as pairs (70% of the sampled burrows harboring 2 shrimps), which were mostly composed of a male and a female (91% of the pairs). This finding constitutes what seems to be the first documented case in which a burrowing shrimp species was found dwelling as heterosexual pairs. This form of social organization is unusual in burrowing shrimps, a group in which the burrows are normally inhabited by only one individual (*e.g.*, *Callichirus major* (Say, 1818 [in Say, 1817-1818]): Rodrigues, 1976; *Callichirus seilacheri* (Bott, 1955): Hernáez and João, 2018; *Neotrypaea harmandi* (Bouvier, 1901): Somiya and Tamaki, 2017; *Audacallichirus mirim* (Rodrigues, 1966), *Lepidophthalmus siriboia* Felder & Rodrigues, 1993, *Neocallichirus guara* (Rodrigues, 1971), *Neocallichirus maryae* Karasawa, 2004, *Neocallichirus pinheiroi* Hernáez, Windsor, Paula & Santana, 2020: Hernáez, 2018a). Although a few exceptions of burrowing shrimps living in pairs have also been reported (*e.g.*, *Axiopsis serratifrons* (A. Milne-Edwards, 1873): Dworschak and Ott, 1993; *Neaxius vivesi* (Bouvier, 1895): Berrill, 1975; *Lepidophthalmus bocourti* (Milne-Edwards, 1870): Hernáez *et al.*, 2021), our study provides the necessary evidence to conclude that *A. australis* lives mainly in heterosexual pairs, as has been reported in other clades of decapod crustaceans in which monogamy is the most common mating system (*e.g.*, Alpheidae: *Synalpheus brevicarpus* (Herrick, 1891): Alves *et al.*, 2021; Lysmatidae: *Lysmata grabhami* (Gordon, 1935): Wirtz, 1997; Palaemonidae: *Pontonia manningi* Fransen, 2000: Baeza *et al.*, 2016; Pinnotheridae: *Tubicolixa chaetopterana* (Stimpson, 1860): Baeza, 1999; see also Baeza *et al.*, 2009 for monogamy in the genus *Lysmata*).

Another interesting question that we asked is whether the male in *A. australis* abandons the female soon after mating. Although this question is difficult to answer without the use of direct observations, our data suggest that heterosexual pairing in this species is for a long time period, that is, it exceeds one reproductive cycle. If the association between the sexes in *A. australis* were restricted to a short period, we would expect that males would not pair with females, regardless of their reproductive state. This was not the case since, according to our data, males shared burrows with brooding and non-brooding

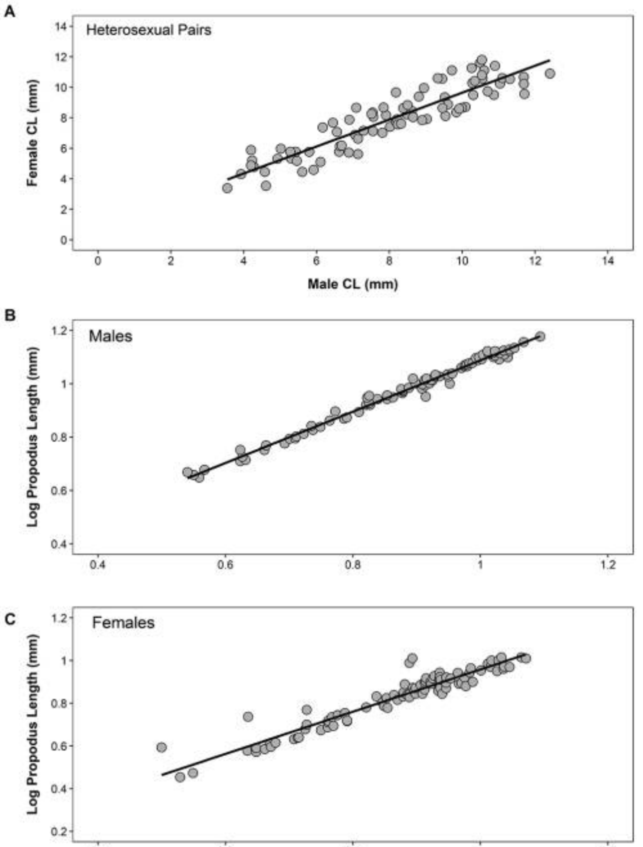

Figure 4. The size-assortative pairing and relative growth pattern in *Axianassa australis*. Relationship between carapace length (CL) of males and females of the burrowing shrimp *A. australis* found as pairs inside the same burrow (A). Relative growth of the cheliped (propodus length [PL]) as a function of CL in males (B) and females (C) of *A. australis*. Measurements are in millimeters. The numbers of males and females used for the analysis of major claw allometry are 86 and 89, respectively.

Table 2

Relative growth of the burrowing shrimp Axianassa australis *at Lago de Santana, Piauí, northeast region of Brazil*

(A) Source of variation	ANCOVA: cheliped PL df	SS	MS	F	P
CL)	1	3.23	3.23	3342.06	$<0.001^{***}$
Sex	1	0.77	0.77	798.00	$<0.001^{***}$
CL × sex	1	0.00	0.00	0.60	0.439^{ns}
Residuals	171	0.17	0.00		

(B) Sex	Regression: PL × CL Equation	r^2	SE_t	t_{df}	P
Males	PL = 0.96CL + 0.13	0.99	0.01	84.72_{84}	$<0.001^{***}$
Females	PL = 0.99CL −0.03	0.91	0.03	29.48_{87}	$<0.001^{***}$

(A) Summary results of the analysis of covariance (ANCOVA) testing the effects of shrimp sex and carapace length (CL) on the propodus length (PL) of *A. australis* individuals. (B) Relationship between the major cheliped (PL) and CL of male and female shrimp. The regression equations (in the $\log^{10}$ form), correlation coefficients (r^2), standard errors of the slopes (SE), t-value plus degrees of freedom (t_{df}) and corresponding P-values of each studied variable are shown. Data were log transformed ($\log^{10}$) to attend ANCOVA assumptions. Significant ($^{***}P < 0.001$) and non-significant ($P > 0.05$; ns) P-values are indicated. MS, mean square; SS, sum of squares.

females and, when brooding embryos, carried different embryo developmental stages. These observations reinforce the idea that once copulation occurs, the male remains with the female throughout the embryos' incubation process until the hatching of the embryos, as is expected to occur in monogamous species. In strictly faithful monogamous decapods, individuals share a specific microhabitat or refuge as male-female pairs for a long time period exceeding one reproductive cycle, and males pair with females regardless of their reproductive state (Grove and Woodin, 1996; Baeza, 1999, 2008; Baeza *et al.*, 2016). This latter pattern agrees with the one observed in *A. australis*.

A small proportion of the total shrimps (18%) were found inhabiting individually within their respective burrows. This included males (n = 11), brooding females (n = 20), and non-ovigerous females (n = 9). Although these combinations were not predictable, that is, they were the result of chance, we believe that it is totally plausible that a small fraction of the population does not behave like the majority, generating a certain behavioral plasticity. This could help explain why some ovigerous females were found living alone within their burrows. In other words, these females would have been abandoned by the males soon after mating. This is the case in *Alpheus armatus* and *Pontonia margarita*, two monogamous species that inhabit their respective host species as male-female, in which some males usually do switch between hosts in search of extra-pair copulations (Knowlton, 1980; Knowlton and Keller, 1983; Baeza, 2008). The presence of solitary individuals may also be associated with the inefficiency of suction sampling. We mention this because the difficulty of collecting specimens of burrowing shrimps is usually associated with the behavior of these organisms, because some individuals may be situated preferably deeper in the burrow and, thus, out of reach of the yabby pump (Hernáez *et al.*, 2008). This does not seem to be the case in *A. australis*, since this species constructs galleries whose depth does not exceed 130 cm (Dworschak and Rodrigues, 1997).

The unbiased sex ratio in the studied population of *A. australis* represents another line of reasoning indicating that this burrowing shrimp is primarily monogamous. Males and females are found in similar proportions in populations of decapods that exhibit a monogamous mating system (Correa and Thiel, 2003; Bauer, 2004 and references therein). In axiidean and gebiidean populations, females are in general more abundant than males, so it is expected that many of these species are not monogamous (Felder and Lovett, 1989; Pezzuto, 1998; Nates and Felder, 1999; Hernáez and Wehrtmann, 2007; Butler *et al.*, 2009; Hernáez *et al.*, 2019, 2021), especially those species with solitary habits. In *A. axianassa*, the overall sex ratio, biased toward females, reinforces the idea that a small fraction of the males, but not females, might be leaving their burrows (at least temporarily) in search of new sexual partners. This is because in a female-biased sex ratio population, the risk of encountering other males while searching is minimized by the high probability of finding a female rather than a male (Mathews, 2002). In the same way, the propensity of some males in *A. axianassa* to leave their gallery in search of receptive females could be driving sex-specific mortality rates caused by antagonistic interactions between males or by predation.

We hypothesized that the burrowing shrimp *A. australis* is primarily monogamous; and, thus, we expected that the population exhibited a reduced sexual dimorphism in both body size and cheliped size. Our results partially agree with the expectations above: sexual dimorphism in terms of body size was not detected in the population, but males invested considerably more in chelipeds than females. In *A. australis*, one male is paired with one female of similar size, both living in a protected habitat, that is, the burrow. Once paired, the male will need to protect the pair's space; and, thus, cheliped size should play a crucial role in the defense of the burrow against invasion from other shrimps from the same or opposite sex. Interestingly, sexual dimorphism in terms of chelipeds was only important between males and females that lived solitarily or in heterosexual pairs, which reinforces one main notion: a larger cheliped can confer comparative advantages both to solitary males in search of extra-pair mating opportunities and to paired males in defense of their pair's space.

As mentioned, male-female pairs of shrimps were found occupying the same gallery during the study period, between which there was a strong correlation of body size of males and females (Fig. 4A). The size-assortative pairing is usually observed in monogamous free-living and symbiotic crustaceans with an unbiased sex ratio (see revision by Correa and Thiel, 2003 and references therein). This pattern agrees with the one observed in *A. australis* in which there was a tight correlation between male and female size and no biased sex ratio. In the same way, male-female pairs are found assorted by size, and mates usually are size matched in monogamous crustaceans (Knowlton, 1980; Boltaña and Thiel, 2001). According to Andersson (1994), the expectation in monogamous species is the absence of disproportionate sexual dimorphism both in body size and in chelipeds, because these kinds of sexual selection in monogamous species are weak. In *A. australis*, there was no sexual dimorphism in terms of body size (CL), but males developed substantially larger chelipeds than their mates (Fig. 3). Thus, the sexual selection in this species is likely influenced by male behavior during the search for new sexual partners and/or the defense of the pair's space, that is, the burrow. The result above suggests that pair formation in *A. australis* is mostly driven by male-male competition, as reported in monogamous caridean shrimp where both mates have similar sizes as a consequence of a certain need to control the mate and defend it against extra-pair matings (Correa and Thiel, 2003).

All aspects studied here support the idea that *A. australis* exhibits a primarily monogamous mating system but in which a small fraction of the males exhibit promiscuous behavior. These aspects are as follows: most individuals live as heterosexual pairs (Fig. 2), the sex ratio is unbiased in the population, and there is a lack of sexual dimorphism in body size but considerable difference in weaponry between the sexes (Fig. 3). All of this evidence suggests that a small fraction of the males in *A. australis* are not strictly faithful. The mating system in this species is similar to that previously reported for the alpheid *Alpheus armatus* Rathbun, 1901 and the palaemonid *Pontonia manningi* Fransen, 2000, two symbiotic shrimp species also found in male-female pairs but in which some males do occasionally switch between host individuals in search of extra-pair copulations (Knowlton, 1980; Baeza *et al.*, 2016). The mechanisms of intrasexual selection that give shape to the differences in morphology between the sexes in *A. australis* appear to be an evolutionary consequence of the social organization and intense male sexual competition for receptive females. Future studies should answer the question of whether the deduced mating system of *A. australis* depends on phylogenetic (morphological and physiological), demographic (population density, distribution pattern), or environmental (habitat, refuge availability, predation pressure) factors or some combination of all of these.

Acknowledgments

We are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing funding in the form of a postdoctoral research fellowship for PH (2015/09020-0) and to Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial aid through a Researcher Fixation Scholarship for PH (process BFP-0196-1.08/20). MAAP is also grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowship grant awarded (305957/2019-8). The biological material studied herein was collected with official collecting permission (51578-1, 58845-1) given by Sistema de Autorização e Informação em Biodiversidade (SISBIO) of the Brazilian Ministry of Environment (MMA). Finally, we deeply thank to two anonymous referees, who helped to improve the content and format of the manuscript.

Data Availability

All data related to this article will be included in the Supporting Information openly available in the Figshare Repository https://figshare.com/s/213a970b0f8c2f155c47.

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