RESEARCH ARTICLE

Hippolyte obliquimanus (Decapoda: Caridea: Hippolytidae): a gonochoric or hermaphroditic shrimp species?

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Abstract Several studies have reported the occurrence of hermaphroditism in caridean shrimps of the family Hippolytidae. Here we provide the first observations of population traits from the small Western Atlantic shrimp, *Hippolyte obliquimanus*, to determine its sexual system using descriptive and experimental approaches. Specimens were collected at 2-month intervals from March 2005 to May 2006 in Ubatuba Bay on the northern coast of the state of São Paulo, Brazil. The sex of individuals was checked and morphometric dimensions (carapace length, maximum pleura of second abdominal segment width, appendix masculina length, maximum propodus width, and dactyl length of the third pereopod) were also analyzed. The gonads were dissected and examined for signs of abnormalities. The possibility of sex change was experimentally evaluated under laboratory conditions. A total of 674 specimens were collected: 211 males, 339 non-ovigerous females, and 124 ovigerous females. The carapace length ranged from 0.55 to 3.20 mm, with females being significantly larger than males. *Hippolyte obliquimanus* showed sexual dimorphism in the third pereopod, absence of *cincinnuli* in the first pleopod, and no reduction of the appendix masculina in the largest males. All males examined had only testes, and all females had only ovaries. There was no sex change observed in the experiments. Together, these data indicate a population with females reaching larger sizes than males, males with a well-developed appendix masculina, and no transitional individuals. The results presented allow characterizing *H. obliquimanus* as a gonochoric species.

Introduction

Within the animal kingdom many organisms divide their reproductive output into female and male components while in many other, the sequential or simultaneous production of oocytes and sperm in the same individual occur. There exits no clear pattern of sexuality (gonochorism vs hermaphroditism) within a given taxon and both are widespread in molluscs, crustaceans, and vertebrates among other. The direction of sex change (female to male; male to female) has been explained by the size-advantage model (Ghiselin 1969; Bauer 2002 among others) but the variation in the timing of sex change and/or the factors influencing or determining the change remained unsolved. Most crustaceans are gonochoric but hermaphroditism occurs among several groups with very distant phylogenetic relationships (Juchault 1999; Bauer 2002). Genetic factors have been proposed, as well as environmental external factors, such as changes in the photoperiod duration and temperature, and factors related to the "social structure" of the population as modulators or even sex determinants or being involved in sex change in crustaceans (Ginsburger-Vogel and Charniaux-Cotton 1982; McCabe and Dunn 1997; Fielder

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2002; Parnes et al. 2003; Baeza 2006; Calado and Dinis 2007 for revision).

A remarkable feature of the reproductive biology of caridean shrimps is the wide diversity of sexual systems. Many aspects of their sophisticated reproduction patterns are related to phylogenetic (morphological and physiological), demographic (population density and distribution pattern), and environmental (habitat, refuge availability, predation pressure) factors (Correa and Thiel 2003). Caridean shrimps have an astounding array of sexual reproductive systems, including species with separate sexes (gonochorism), sequential hermaphroditism (usually protandry), and others with protandric simultaneous hermaphroditism (Bauer 2000). Of the 50 species of caridean shrimps for which the sexual system is known, 19 are gonochoric and 31 show some variation of hermaphroditism (Yaldwyn 1966; Ewald 1969; Correa and Thiel 2003; Cobos et al. 2005; Zhang and Lin 2005; Braga 2006).

In truly protandric species, all individuals of the population, mature first as males and then become females when they increase in size and age (Bauer 2000). In the male phase, individuals show a masculine external phenotype and reproductive function (Bauer 2000). In this phase, the gonads are ovotestes with a functional masculine portion and a rudimentary feminine portion, this feminine portion is already differentiated, but just very small (Bauer 2004). As the animal increases in size, the external phenotype changes to feminine, the gonad becomes completely feminine and the individual begins to reproduce as a female (Bauer 2006).

Protandric populations have size-frequency distributions with relatively little overlap between the male and the female phases, and are confirmed by observation of transitional individuals having both male and female characters and intermediate size (Bauer 2004). Besides simple protandry, there are certain variations (see Bauer 2000 for review).

Some caridean shrimps of the family Hippolytidae show protandric simultaneous hermaphroditism (Bauer 2000). As in simple protandry, individuals show a male phase and later develop into the simultaneous hermaphrodite phase. However, instead of losing all masculine features during the sex change as has been reported for other carideans (Bauer 2000), these animals retain the vas deferens, the production of sperm, and the ability to mate as males, becoming therefore simultaneous hermaphrodites (Bauer and Holt 1998). According to these authors, when these shrimps become simultaneous hermaphrodites, they can mate as females during the post-molt period and as males during the intermolt period.

There exists a controversy about the evolution of sexual systems in many groups; the question is, "what is the primitive sexual system, gonochorism or hermaphroditism?".

However, to elucidate this question, it is necessary to first characterize with details, the sexual system of maximum number of species of the family Hippolytidae and after to add the morphological and molecular data to obtain a phylogeny and can determine which species are ancestors and which are descendants (Bauer 2001). After we discover the most primitive species through phylogenetic studies, we will be able to establish which is the most primitive sexual system, gonochorism or hermaphroditism? In this way, as our study proposes to determine the sexual system of one species, this study is one more step to elucidate the evolution of sexual systems in the family Hippolytidae.

Hippolyte obliquimanus Dana 1852 is endemic to the Western Atlantic coast, and its distribution is restricted to shallow waters of the Caribbean and off Brazil from Pernambuco to Santa Catarina (Udekem d′ Acoz 1996; Young 1998). It is a small shrimp that presents a total body length up to about 15 mm (Udekem d′ Acoz 1997), and along the Brazilian coast, it is found in algae beds, particularly in association with seaweed of the genus *Sargassum* (Mantelatto et al. 1999). *Hippolyte obliquimanus* has been poorly studied outside its geographical distribution and taxonomy (see Udekem d′ Acoz 1997 for revision). As far as we know, the detailed studies available deal with specific features of the redescription of the species (Udekem d′ Acoz 1997) and its fecundity (Mantelatto et al. 1999). We analyzed here the population features of *H. obliquimanus* by morphological and experimental approaches, to detect the type of sexuality (gonochorism or hermaphroditism) shown in this species.

Materials and methods

Specimens of *H. obliquimanus* were systematically sampled during the daytime by two persons using snorkeling equipment every 2 months from March 2005 to May 2006, off Itaguá Beach (23°27′414′′S, 45°3′047′′W Ubatuba, State of São Paulo, Brazil) that showed variation of temperature (23–26°C) and salinity (33–36) during the year. In this region, the specimens are found in association with seaweed of the genus *Sargassum* (Mantelatto et al. 1999). Individuals were obtained from algal turf *Sargassum* sp., always submerged to approximately 1.5 m of depth. Samples of algae were haphazardly picked from the substratum, rapidly placed in plastic bags, and examined in the laboratory. Some animals were chosen randomly and transported alive to the Laboratory of Bioecology and Crustacean Systematics in Ribeirão Preto, USP, and the remaining animals were frozen for subsequent analysis.

We measured carapace length (CL), appendix masculina length (AML), maximum pleura of the second abdominal segment width (PASW), maximum propodus width (PW),

and dactyl length (DL) of the third pereopod. The number of setae on the appendix masculina was counted and the presence or absence of coupling hooks (*cincinnuli*) on the endopod of the first pleopod was noted. Sexes were distinguished by the presence or absence of the appendix masculina on the second pleopod. Ovigerous females were identified on the basis of the presence of embryos in the abdomen. Several males and females of all sizes were dissected to examine the morphology of the gonads for signs of development and abnormalities (as ovotestes, buds of testes in females or buds of ovaries in males). All measurements and observations were done with the use of a stereomicroscope.

Voucher specimens were deposited in the Crustacean Collection of the Biology Department (CCDB) of the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), University of São Paulo, Brazil (catalogue numbers 1825–1830).

Experimental approaches

Laboratory experiments were carried out to investigate the possibility of sex change, according to modified methods of Bauer and Holt (1998). Each pair of animals was maintained in boxes of expanded polystyrene ($16 \times 14 \times 10$ cm) and containing 1.2 L of sea water (salinity 33–34, temperature 21–25°C), samples of seaweed *Ulva* sp., aeration and a 12 h light:12 h dark cycle. The sea water was collected in the same region of the samples, and it was exchanged in the boxes every 2 days. The boxes were covered with transparent plastic to enable light to enter. During the experiments, the animals were only provided with the seaweed *Ulva* sp. The alga *Ulva* sp. was used to feed the animals because the alga *Sargassum* sp. shows a quick deterioration in the laboratory. *Ulva* sp. is also found in the sampling region and it can be kept frozen until the beginning of the experiments.

Three types of mating pairs were assembled, eight male:female pairs, to verify if the conditions of experiment were adequate for mating; eight male:male pairs in order to investigate the possibility of change to a female-like protandric hermaphrodite; and 43 ovigerous female:ovigerous female pairs to test the hypothesis of protandric simultaneous hermaphroditism, as found in *Lysmata wurdemanni* (Gibbes 1850) (Bauer and Holt 1998) and *Lysmata californica* (Stimpson 1866) (Bauer and Newman 2004).

Daily visual observations were made of the condition of the gonads, molting, the presence of new embryos under the abdomen, the stage of development of the eggs and hatched larvae. Classification of embryo stages followed the methods of Wehrtmann (1990): stage I, eggs with uniform yolk; stage II, eye pigment visible; and stage III, eyes

clearly visible, free abdomen. This classification was made under a stereomicroscope to verify if the eggs were viable. When one of the individuals died, the other remained alone and the observations continued.

Statistical analyses

All samples had similar proportion of males:non-ovigerous females:ovigerous females, and for this reason the bimonthly data were pooled for further analyses. The following statistical analyses were performed by means of the Statistica 7.0 program: Kolmogorov–Smirnov to test the normality of samples (variables: CL, AML, PASW, PW, and DL), Kruskal–Wallis test with Mann–Whitney test to, a posteriori, compare the measures of animals (variables: CL, AML, PASW, PW, and DL) between males and females (ovigerous and non-ovigerous together). To evaluate the relationship between carapace length and the other variables, a regression analysis was performed (Sokal and Rohlf 1979; Zar 1996). For the analysis of relative growth, the function $y = ax^b$ was used where y = dependent variable (AML, PASW, PW, and DL), x = independent variable (CL), a = intercept, and b = slope of the regression equation (Hartnoll 1982). The growth pattern was established for each parameter by the constant b, where $b = 1$ denoted isometric growth, $b < 1$ denoted negative allometric growth, and $b > 1$ denoted positive allometric growth. Student's t-tests were used to test the value of b (H_0: $b = 1$; H_1: $b \neq 1$). For all tests $\alpha = 0.05$ was used.

Results

A total of 674 individuals was collected, of which 211 (31.3%) were males, 339 (50.3%) were non-ovigerous females, and 124 (18.4%) were ovigerous females. The size-frequency distribution of individuals showed a unimodal pattern with a non-normal distribution skewed to smaller size classes (K-S $d = 0.085$; $p < 0.01$) (Fig. 1). Females (ovigerous and non-ovigerous; median = 1.56 mm) reached larger sizes than males (median = 1.25 mm) ($U = 34898.50$; $p < 0.001$). The smallest and largest individuals captured were females (Fig. 1), and this was the pattern in all sampled months.

All measures of secondary sexual characters showed non-normal distribution ($p < 0.001$). Males had a prehensile third pereopod (Fig. 2a), and females had a non-prehensile third pereopod (Fig. 2b), evidencing sexual dimorphism. The maximum propodus width and the dactyl length of the third pereopod were larger in males than in females (PW: $U = 10533.00$; DL: $U = 26198.50$; $p < 0.001$). However, the maximum pleura of second abdominal segment width
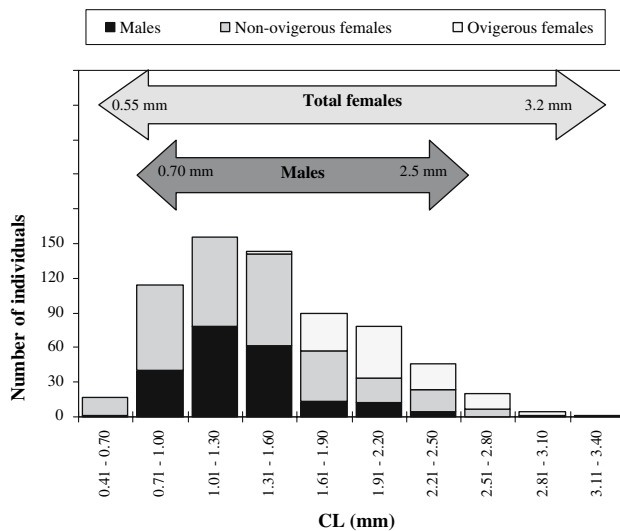
Fig. 1 *Hippolyte obliquimanus*. Size frequency distribution for the total of individuals collected (*CL* carapace length)

was larger in females than in males (PASW: $U = 29507.50$; $p < 0.001$). All male individuals analyzed lacked *cincinnuli* on the endopod of the first pleopod. The size of the appendix masculina (Fig. 2c) ranged from 0.04 to 0.18 mm; the number of setae on the appendix masculina ranged from two to six, with 75% of males having five setae.

Pleura of second abdominal segment width, propodus width, dactyl length, and appendix masculina length increased together with the increase of the carapace (Table 1). The number of setae showed a positive and significant correlation with appendix masculina length

($r = 0.38$; $p < 0.05$). Although these characters increased with greater carapace length, their growth patterns were different (Table 1; Fig. 3). Males showed isometric growth for the appendix masculina and propodus, and negative allometric growth for both the dactyl and pleura. Females showed negative allometric growth for the propodus and dactyl, and positive allometric growth for the pleura.

A total of 63 males (sizes ranging from 0.70 to 2.40 mm CL) and 78 females (0.80–3.18 mm CL) from samples of July and January were dissected and examined internally. All males had paired testes, with each testis possessing a large duct, the vas deferens. This duct opened through the gonopore, located on the coxa of the fifth pereopod. All females had paired ovaries. Each ovary possessed an oviduct that ended in a gonopore, which opened externally on the coxa of the third pereopod. We did not observe any abnormalities in the gonads of either sex.

In the experiments with male:female pairs, all females became ovigerous and the eggs remained incubating in the abdomen until stage II, when they were aborted. Individuals remained alive 17 days on average (from 11 to 26 days). Six females with mature ovaries at the beginning of the experiment underwent a molt and spawned eggs 2 days after the beginning of the experiment. After 4 more days, the eggs reached stage II. However, these females then aborted the eggs from 1- to 3-day period. Furthermore, two other females with immature ovaries at the beginning of the experiment, underwent a molt and spawned eggs 5 days after the beginning of the experiment and after 4 more days the eggs reached stage II. However, after 3 more days, both

Fig. 2 *Hippolyte obliquimanus*. Secondary sexual characters. **a** third pereopod, prehensile in males (2.1 mm CL) and **b** non-prehensile in females (2.6 mm CL). **c** Second pleopod, presence of appendix masculina in males (1.9 mm CL) and **d** absence in females (2.3 mm CL) (*P* propodus, *D* dactyl, *AM* appendix masculina, *AI* appendix interna, *scale* 0.1 mm)
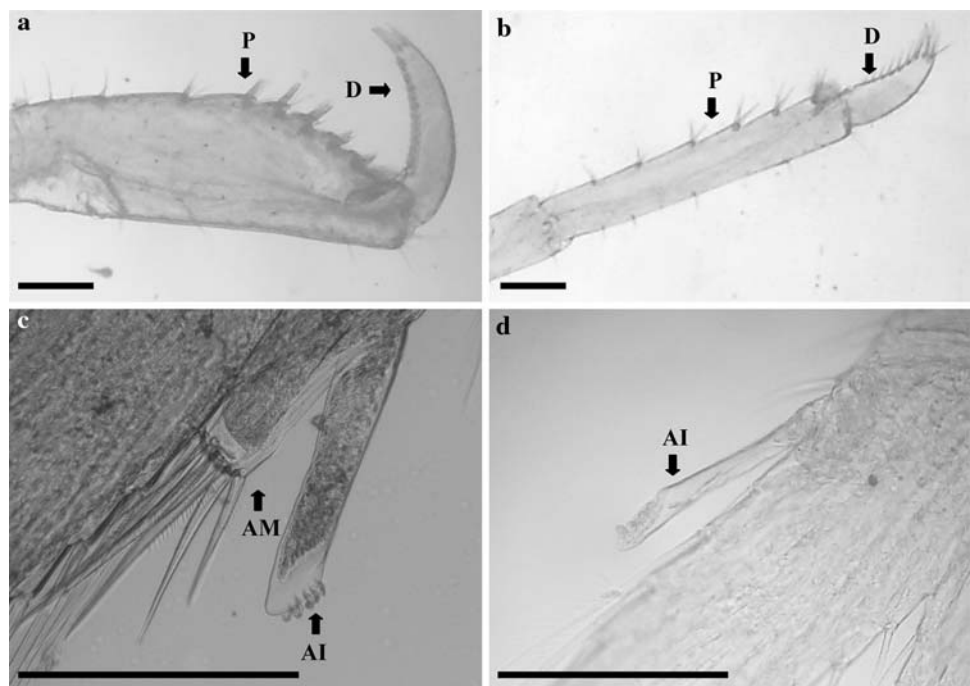
Table 1 *Hippolyte obliquimanus,* regression equations of the total individuals

Relation	Sex	n	Power function $y = ax^b$	Linear equation Ln $y = \ln a + b \ln x$	$r*$	$b \neq 1$	GP
CL × AML	M	208	AML = 0.07 CL$^{0.89}$	Ln AML = –2.66 + 0.89 Ln CL	0.67	No	=
CL × PASW	M	146	PASW = 0.47 CL$^{0.77}$	Ln PASW = –0.75 + 0.77 Ln CL	0.68	Yes	–
	F	309	PASW = 0.45 CL$^{1.11}$	Ln PASW = –0.80 + 1.11 Ln CL	0.84	Yes	+
CL × PW	M	197	PW = 0.13 CL$^{1.03}$	Ln PW = –2.04 + 1.03 Ln CL	0.76	No	=
	F	404	PW = 0.08 CL$^{0.51}$	Ln PW = –2.52 + 0.51 Ln CL	0.63	Yes	–
CL × DL	M	197	DL = 0.22 CL$^{0.79}$	Ln DL = –1.51 + 0.79 Ln CL	0.73	Yes	–
	F	404	DL = 0.19 CL$^{0.57}$	Ln DL = –1.69 + 0.57 Ln CL	0.75	Yes	–

CL carapace length, *AML* appendix masculina length, *PASW* pleura abdominal segment width, *PW* propodus width, *DL* dactyl length, *M* males, *F* females, *n* number of individuals, *r* correlation coefficient, *GP* growth pattern, + positive allometry, – negative allometry, = isometry, * significant correlation ($p < 0.05$)
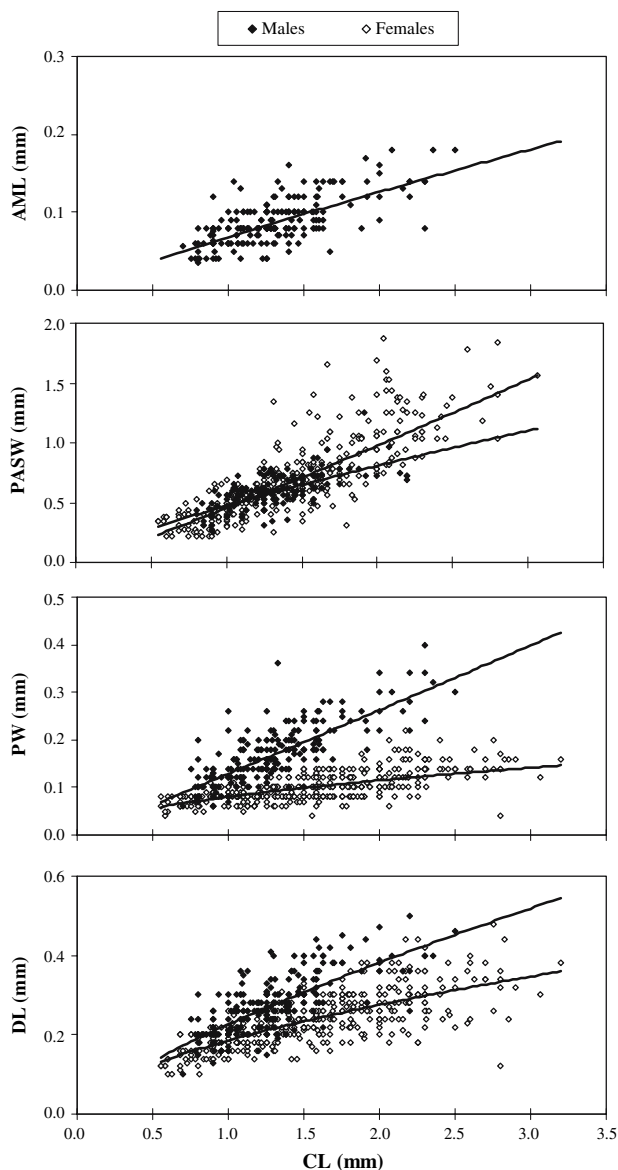

Fig. 3 *Hippolyte obliquimanus.* Dispersion diagrams for males and females (*CL* carapace length, *AML* appendix masculina length, *PASW* pleura abdominal segment width, *PW* propodus width, *DL* dactyl length)

females aborted the eggs. When these females aborted, their ovaries were mature, indicating that the females were ready to spawn again.

In the experiments with male:male pairs, individuals remained alive for 44 days on average (from 21 to 59 days). At the end of the experiment, no mating behavior and/or change of the initial morphological condition were observed. In one pair, one male survived 59 days and the other 67 days. Moreover, these individuals underwent nine molts and achieved 2.30 and 2.35 mm carapace length, respectively, at the end of the experiment.

In the experiments with ovigerous female:ovigerous female pairs, individuals remained alive for 25 days on average (from 2 to 98 days). All 86 observed females underwent molts, spawned unfertilized eggs, aborted these eggs after several days and underwent another molt after losing their eggs. The period for which females kept unfertilized eggs in the abdomen ranged from 1 to 10 days, averaging 2.9 days. When a female died and the other female remained alone, spawning of unfertilized eggs was also observed. Consecutive spawnings were also observed, and the period between the molt after discarding eggs and the next spawning ranged from 1 to 22 days, averaging 8 days.

Discussion and conclusion

Population and morphological features

Females of *H. obliquimanus* reached a larger size than males. This size dimorphism is not uncommon for the species of *Hippolyte* that have been studied (Udekem d′ Acoz 1996), such as *H. inermis* Leach 1815 (Cobos et al. 2005) and *H. zostericola* (Smith 1873) (Román-Contreras and Romero-Rodríguez 2005). Many species of Hippolytidae have populations composed of smaller males and larger females (Bauer 2004), and some of these species show protandry, such as *Chorismus antarcticus* (Pfeffer 1887) (Clarke 1985). However, others are gonochoric, such as

Tozeuma carolinense Kingsley 1878 (Ewald 1969), *Thor dobkini* Chace 1972 and *Thor floridanus* Kingsley 1878 (Bauer and VanHoy 1996).

Larger body size has advantages for caridean females, because egg production is positively correlated with the size of the female (Carpenter 1983; Bauer 1991; Gherardi and Calloni 1993), as observed previously in *H. obliquimanus* (Mantelatto et al. 1999). Small-sized males have the advantage of avoiding detection by predators and spend little energy on growth (Bauer 2004), and furthermore, in some species, the agility of small males is an advantage in searching for mates (Baeza 2007). In some species, e.g., *Lysmata wurdemanni* Gibbes 1850, eggs are more costly than sperm, not only in terms of energy but also of time (Baeza 2006).

Despite the smaller size reached by males of *H. obliquimanus*, they had a larger propodus and dactyl of the third pereopod than females, and also had a prehensile pereopod, characterizing the sexual dimorphism in the species. The prehensile pereopod in males may be an adaptation for holding the female during mating (Bauer 2004). Such dimorphism has been previously reported in other carideans, e.g., *Paratya curvirostris* (Heller 1862) (Carpenter 1983) and *Pandalus danae* Stimpson 1857 (Butler 1980), and in a few genera of Hippolytidae: *Hippolyte clarki* Chace 1951 (Butler 1980), *Hippolyte* spp. (Chace 1972), in the partially protandric *Thor manningi* Chace, 1972 (Bauer 1986), and in the gonochoric *T. dobkini* and *T. floridanus* (Bauer and VanHoy 1996). The difference in the third pereopod was one of the characteristics utilized to separate true males of hermaphroditic individuals in the male phase of the protandric *Thor manningi* (Bauer 1986).

The growth pattern of the third pereopod propodus differed between males and females. Males showed isometric growth, indicating that the propodus grows at the same rate as the body. However, females showed negative allometric growth, i.e., the propodus grows more slowly than the body. The growth pattern of the third pereopod dactyl did not differ between the sexes, with both males and females showing negative allometric growth, i.e., the dactyl grows more slowly than the body. In some species, females have special characters associated with spawning and incubation of embryos beneath the abdomen (Bauer 2004). The pleura of the second abdominal segment of *H. obliquimanus* females showed positive allometric growth, indicating that the pleura grows more quickly than the body in this caridean. This increase of abdominal segment size enlarges the space below the abdomen, where the egg mass is deposited during spawning and protected during the incubation (Bauer 2004). If this species were a protandric hermaphrodite, males could show isometric growth of the pleura, because larger males would become females and they would show an increase in the pleura width; however,

H. obliquimanus males showed negative allometric growth of the pleura.

Many caridean species have *cincinnuli* on the first pleopod. The presence of *cincinnuli* was one of characters used to differentiate male-phase of female-phase in the protandric simultaneous hermaphroditic *Lysmata wurdemanni* (Bauer and Holt 1998), then if *H. obliquimanus* were protandric simultaneous hermaphrodite, this character could also be used. However, there are many variations in the form and size of these structures in different species (Bauer 2004). Furthermore, there are species in which, the males do not possess such a structure on the first pleopod, as in *T. manningi* (Bauer 1986) and *H. obliquimanus* (present study).

As in gonochoric shrimps, the appendix masculina of this species increased together with the size of the body, and there was no indication of appendix reduction in the larger male, as occurs in many protandric shrimps (Bauer 2000). A similar relationship between the size of the carapace and of the appendix masculina was reported for *H. inermis* (Cobos et al. 2005), which is recognized as gonochoric by those authors.

In *H. obliquimanus*, isometric growth for the appendix masculina was observed, which means that the growth of the appendix was proportional to the growth of the body. The gonochoric *T. floridanus* also shows isometric growth of the appendix masculina (Bauer and VanHoy 1996). The protandric simultaneous hermaphrodite *Lysmata californica* (Stimpson 1866) shows negative allometric growth, in that, the appendix masculina becomes relatively smaller as the carapace grows (Bauer and Newman 2004). Negative allometric growth of the appendix masculina has also been reported for the hermaphrodites in the male phase of *T. manningi* (Bauer 1986). However, the primary males of the same species showed isometric growth (Bauer 1986). Therefore, isometric growth of the appendix masculina can be considered as the pattern of gonochoric species, and negative allometric growth can be considered as the pattern of protandric species.

No reduction in the number of setae in the larger male of *H. obliquimanus* was observed. The appendix masculina of *H. obliquimanus* bore 2–6 setae, with 5 setae in the majority of the males, and a wider variation in smaller individuals. Other species show a similar variation: males of *L. wurdemanni* have 1–9 setae on the appendix masculina (Bauer and Holt 1998), and males of *L. californica* have 3–8 setae (Bauer and Newman 2004). The setae of the appendix masculina may help in the transfer of spermatophores to the females (Bauer 2004). This variation in the number of setae of *H. obliquimanus* is common, because younger individuals may have fewer setae than adults, as a function of body growth. Unfortunately, existing studies on sexual systems in shrimps of the genus *Hippolyte* (Zupo 1994, 2000,

2001; Cobos et al. 2005; Zupo and Messina 2007) do not provide information on this aspect, making comparisons or inferences impossible.

All males of *H. obliquimanus* had only testes and all females had only ovaries, and no evidence of ovotestes was observed. In contrast, the majority of protandric hermaphrodites in the Caridea have ovotestes during one phase of their life (Bauer 2004).

Mating experiments

In the male:female pairs, consecutive mating was not observed, probably because of the short time that the animals remained alive. Although mating was not observed, the presence of eggs in stage II of development constitutes direct evidence that mating did occur. The occurrence of mating in all male:female pairs would demonstrate that the experimental conditions were appropriate. On the other hand, the short survival and the high rate of abortion of females in the stage II might indicate that some factors were inadequate, despite all efforts to resemble the natural conditions in the experiments. This seems to have affected primarily the male:female pairs where mating did occur, since the animals in the male:male pairs and in the female:female pairs survived for considerably longer time periods. Possibly the reduced survival of the individuals in the male:female pairs is caused by stress during the mating interactions, but this requires further study. Regardless of the reasons for the reduced survival and the high rate of abortion in the male:female treatment, the most relevant result is that no indication for sex change was found in any of the replicates from this treatment. And we believe that the problems found (reduced survival and high rate of abortion) did not influence the results in the experiments with male:male pairs and in the female:female pairs.

In one of the male:male pairs, one of the animals survived 59 days and another 67 days; these individuals reached 2.30 and 2.35 mm carapace length at the end of the experiment, and both together underwent nine molts in total. These individuals were among the largest males found in the population, the median size of males was 1.25 mm. Thus, if this species were protandric, these males reached the size to become females but after several molts they showed no morphological evidence of sex change. Nonetheless, one could argue that the laboratory conditions caused stress in the animals, and that these males might have refrained from changing to female, because it was more economical energetically to continue as male than changing to female. However, the fact that females continued to produce eggs during our experiments suggests that energy constraints had no or only minor effects on the experimental shrimp. Since no indication for sex change was found in any of the treatments, and since adult females were unable to reciprocally fertilize other females, the results from the experiments clearly support our conclusion that *H. obliquimanus* is a gonochoric species.

Sexual system

Protandric populations, in general, are usually characterized by the occurrence of only males in the smaller size classes and only females in the larger ones, and by the presence of transitional individuals (individuals of intermediate size that lose male sexual characters and develop female sexual characters) in the population (Bauer 2000). The males of *H. obliquimanus* are smaller than the majority of the females, as occurs in protandric shrimps. However, there is a large overlap between the sexes and some (nonovigerous) females are smaller than the smallest male. If this species were protandric, there would be many primary females in the population. However, no transitional individuals were detected.

In protandric shrimps, the transition from male to female generally is a gradual process, which in *L. wurdemanni* and *Lysmata rathbunae* Chace 1970 involves five molts, during which the appendix masculina regresses, and the number of setae decreases (Zhang and Lin 2005). *Hippolyte obliquimanus* did not show transitional individuals, and males showed a positive correlation between the carapace length, and the appendix masculina and between the size of the carapace and the number of setae. These relationships can indicate the gonochoric condition of this species. Nevertheless, *Hippolyte inermis* did show neither transitional individuals nor ovotestes (Zupo 1994, 2000, 2001; Zupo and Messina 2007), but it is recognized as protandric hermaphroditic by these authors.

In addition, *H. obliquimanus* showed an appendix masculina growth pattern similar to gonochoric species, and in the male:male pairs they did not show any evidence of a reduction in the size of the appendix masculina. All of the males of *H. obliquimanus* showed the prehensile pereopod. During the experiments, morphological changes or behavior in the individuals that might indicate sex change were not observed. Nevertheless, the animals were fed only with alga *Ulva* sp., so maybe the lack of some feeding had influenced growth and/or sex reversal of the animals. According to Zupo (2000, 2001), the sex reversal of *Hippolyte inermis* is determined by microalgan food. Recently, Calado and Dinis (2007) have demonstrated that sex change in *Lysmata seticaudata* (Risso, 1816) (a protandric simultaneous hermaphroditic species) is triggered if visual and/or tactile interactions are allowed and that this process is affected by temperature.

Except for the present study on the sexual system of *H. obliquimanus*, *Hippolyte inermis* is the only species of the genus thoroughly studied with regard to this aspect. Reverberi (1950; as *Hippolyte viridis*) suggested the occurrence

Table 2 Sexual systems described in some species of the family Hippolytidae

Species	Sexual system	Locality	Reference
Heptacarpus pictus (Stimpson, 1871)	Gonochoric	United States	Bauer ([1976](#))
Hippolyte inermis Leach, 1815		Spain	Cobos et al. ([2005](#))
Hippolyte obliquimanus Dana, 1852		Brazil	Present study
Nauticaris marionis Bate, 1888		New Zealand	Yaldwyn ([1966](#))
Thor dobkini Chace, 1972		United States	Bauer and VanHoy ([1996](#))
Thor floridanus Kingsley, 1878			
Tozeuma carolinense Kingsley, 1878		United States	Ewald ([1969](#))
Chorismus antarcticus (Pfeffer, 1887)	Protandric	United States	Clarke ([1985](#))
Hippolyte inermis Leach, 1815		Italy	Reverberi ([1950](#)); Zupo ([1994](#), [2000](#), [2001](#)); Zupo and Messina ([2007](#))
Thor manningi Chace, 1972	Protandric with primary males	Puerto Rico	Bauer ([1986](#))
Exhippolysmata ensirostris Kemp, 1914	Protandric simultaneous hermaphroditism	India	Kagwade ([1982](#))
Exhippolysmata oplophoroides (Holthuis, 1948)		Brazil	Braga ([2006](#))
Lysmata amboinensis (De Man, 1888)		Hawaii	Fiedler ([1998](#))
Lysmata californica (Stimpson, 1866)		United States	Bauer and Newman ([2004](#))
Lysmata grabhami (Gordon, 1935)		Spain	Wirtz ([1997](#))
Lysmata nilita Dohrn and Holthuis, 1950		Italy	Udekem d' Acoz ([2003](#))
Lysmata rathbunae Chace, 1970		United States	Zhang and Lin ([2005](#))
Lysmata seticaudata (Risso, 1816)		Spain	Udekem d' Acoz ([2003](#))
Lysmata wurdemanni (Gibbes, 1850)		United States	Bauer and Holt ([1998](#))

of protandry in *H. inermis*; other authors (Zupo [1994](#), [2000](#), [2001](#); Zupo and Messina [2007](#)) agreed with this proposed sexual system and studied the influence of diet on sex differentiation of this species. However, *H. inermis* was characterized as gonochoric by means of population studies, appendix masculina growth and gonadal histology (Cobos et al. [2005](#)). Therefore, the controversy persists and careful observations of the living animals, as well as adequate laboratory experiments, are necessary. Nevertheless, the sexual system does not follow the same pattern in all species of the same genus (see Table 2 for review).

In conclusion, we can infer that *H. obliquimanus* in the subtropical southwestern Atlantic region shares reproductive traits with other gonochoric hippolytid species, especially in that the females reached larger sizes than the males, the males had the appendix masculina well developed, there were no transitional individuals in size, males had only testes, and females had only ovaries. Our results allow characterizing *H. obliquimanus* as a gonochoric species. We consider our study as the first step within a more complex framework of resolving the sexual system of this and other species in this genus using a multi-disciplinary approach involving population dynamics, size-frequency, growth experiments at different conditions (temperature, density, food), behavioral studies, and phylogeny. Such an integrative view could be the key to understand the evolution of gonochorism and hermaphroditism in this and in many other taxa.

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