

PROTANDRIC SIMULTANEOUS HERMAPHRODITISM IN *PARHIPPOLYTE MISTICIA* (CLARK, 1989) (CARIDEA: HIPPOLYTIDAE): IMPLICATIONS FOR THE EVOLUTION OF MIXED SEXUAL SYSTEMS IN SHRIMP

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A B S T R A C T

The sexual system of the shrimp *Parhippolyte misticia* (Clark, 1989), inhabiting the rocky subtidal at Okinawa, Japan and Kimbe Bay, Papua New Guinea, was examined. Dissections suggested that the population consisted of male phase (MP) and functional simultaneous euhermaphrodite (EH) individuals. MPs have cincinulli and appendices masculinae on the first and second pair of pleopods, respectively, gonopores located at the coxae of the third pair of walking legs, and ovotestes with a well-developed male portion containing sperm, but an undeveloped female portion. EHs lacked appendices masculinae and cincinulli. However, they have male gonopores and ovotestes with well-developed ovaries containing mature oocytes and testes with sperm. When EHs were maintained in pairs, both shrimp molted and spawned eggs which attached below the pleon and developed as embryos, demonstrating that EHs can reproduce as males and inseminate other EHs acting as females. These results demonstrate that *P. misticia* is a protandric simultaneous hermaphrodite, as reported before for other shrimp of the genera *Lysmata* and *Exhippolysmata*. Also, these results suggest that protandric simultaneous hermaphroditism might have evolved more than once independently in shrimp from the diverse and species-rich Infraorder Caridea. Future research aimed at disentangling the phylogenetic relationship of *Parhippolyte*, *Lysmata*, *Exhippolysmata* and other closely related genera (*Calliasmata*, *Lysmatella*, *Barbouria*) and describing the sociobiology of additional representatives from the genera above is needed to understand the evolutionary history of sexual systems in caridean shrimp.

KEY WORDS: Barbouridae, Lysmatidae, mating system, *Parhippolyte*, sex allocation, sex ratio

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INTRODUCTION

The infraorder Caridea is one of the most species-rich clades of decapod crustaceans – 3268 species have been described so far (De Grave et al., 2009). Caridea is also the most diverse in terms of sexual systems among decapod crustaceans. Most commonly, caridean shrimp are assumed to be gonochoric, with individuals reproducing exclusively as male or female during their lifetime. Nonetheless, the actual number of detailed studies demonstrating gonochorism in species from this clade are limited (*Thor dobkinsi* Chace, 1972 and *T. floridanus* Kingsley, 1878 – Bauer and VanHoy, 1996; *Pontonia margarita* Verrill, 1869 – Baeza, 2008; *Pontonia mexicana* Guérin-Méneville, 1855 – Baeza et al., 2011; *Hippolyte williamsi* Schmitt, 1924 – Espinoza-Fuenzalida et al., 2008; *Processa riveroi* Manning and Chace, 1971 and *P. bermudensis* (Rankin, 1900) – Bauer and Laporte, 2011). Other shrimp are reported to be protandric hermaphrodites, where individuals change from male to female at some point in time during their life (Bauer, 2006). Examples of pure or mixed protandry (populations in which individuals that undergo sex change coexist with other conspecifics that mature either as pure males or females and that does not change sex)

include *Pandalus* spp. (Charnov, 1982; Charnov and Bull, 1989; Bergström, 2000 and references therein), *Processa edulis* (Risso, 1816) (Noël, 1976; but see Bauer and Laporte, 2011), *Crangon crangon* (Linnaeus, 1758) (Boddeke et al., 1991; Schatte and Saborowski, 2006; but see Hufnagl et al., 2010), *Thor manningi* Chace, 1972 (Bauer, 1986), *Mer-guia rizophorae* (Rathbun, 1900) (Baeza, 2010a) and *Thor amboinensis* (De Man, 1888) (Baeza and Piantoni, 2010). Lastly, protandric simultaneous hermaphroditism (PSH) has been demonstrated in at least 19 out of 40 recognized species of shrimp from the genus *Lysmata* and *Exhippolysmata* (see Baeza, 2009 and references therein). In these sequentially simultaneous hermaphroditic species, juveniles invariably mature as functional males (or male phase [MP] individuals) first, and later they become functional simultaneous hermaphrodites (or euhermaphrodite phase [EH] individuals) capable of reproducing both in the male and female role (Bauer and Holt, 1998; Fiedler, 1998; Baeza, 2006, 2009).

Shrimp from the Infraorder Caridea are also recognized for their outstanding diversity in terms of habitat, lifestyles, body sizes, and color (De Grave et al., 2009). For instance, various species have been described from chemoautotrophic

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environments in the deep sea (*Alvinocaris alexander* Ah-yong, 2009 and *Lebbeus wera* Ah-yong, 2009 – Ah-yong, 2009). Many other species have established symbiotic relationships with sessile marine invertebrates in shallow tropical seas (*Pontonia margarita* and *P. mexicana* – Baeza, 2008; Baeza et al., 2011) and some of these symbiotic species have bright colors and provide cleaning services to diverse fishes (*Lysmata grabhami* (Gordon 1935) – Limbaugh et al., 1961; *Urocaridella* sp. C – Becker and Grutter, 2005). Even others species have conquered semi-terrestrial environments inhabiting mangrove forests and intertidal fossilized coral terraces where they walk out of water and leap into the air when in the presence of potential predators (*Merguia rhizophorae* – Baeza, 2010a). The ecological diversity of the carideans suggests that shrimp in this clade are ideal model systems to explore the role of environmental conditions in favoring or constraining evolutionary innovations, including gender expression, in the marine environment. Unfortunately, the scarcity of studies on the natural history of many species and the paucity of molecular phylogenies in this group has hampered such studies so far.

In this study, we are particularly interested in understanding the sexual system of shrimp from the genus *Parhippolyte*. Phylogenetic analyses suggest that this genus might be the sister group to the genera *Lysmata* and *Exhippolysmata* (Christoffersen, 1987; Fiedler et al., 2010; but see Baeza et al., 2009; Baeza, 2010b). Studies on sex allocation both at the individual and population level and on the behavioral ecology of the species from this genus may aid in understanding the evolutionary origin of protandric simultaneous hermaphroditism, the most remarkable sex expression pattern yet reported in Caridea (Bauer and Holt, 1998; Fiedler, 1998; Baeza, 2009). Bauer (2000) proposed that PSH evolved in the tropics from an ancestral symbiotic protandric species of *Lysmata* that became a specialized fish cleaner. Restricted mobility of individuals resulting from their association with anemone hosts and, hence, reduced probability of encountering mating partners (due to low density) would have favored PSH (also see Bauer, 2006 and Baeza, 2009). This hypothesis was partially supported by a recent study that used Bayesian inference to reconstruct ancestral character states in shrimp from the genus *Lysmata* and *Exhippolysmata* (Baeza, 2009). In agreement with Bauer's hypothesis, ancestral character states reconstruction indicated that the most common recent ancestor of *Lysmata* and *Exhippolysmata* was most likely a species that lived at low density, e.g., socially monogamous). However, the ancestral lifestyle was equally likely to be free-living or symbiotic (Baeza, 2009). Baeza (2009, 2010b) and later Fiedler et al. (2010) have recognized that the information so far available on the reproductive biology of shrimp from other genera related to *Lysmata* and *Exhippolysmata*, including *Parhippolyte*, is too scarce to accurately resolve the character state of the ancestral species of *Lysmata* and *Exhippolysmata*. New studies testing hypotheses on the evolutionary origin of PSH require new descriptions of the natural history and sexual behavior of members of other closely related genera, including *Lysmatella*, *Calliasmata*, *Merguia*, and *Parhippolyte* (Baeza, 2009, 2010b; Fiedler et al., 2010).

The aim of this study was first to determine the sexual system of the shrimp *Parhippolyte misticia* (Clark, 1989) by examination of internal anatomy, size-frequency distribution analysis, and behavioral observations in the laboratory. *Parhippolyte misticia* is native to the Indo Pacific and has been recorded in Palau (Caroline Islands), Okinawa (Japan) and West New Britain in Papua New Guinea (Chace, 1997; CF and JAB, unpublished data). Little is known of the life history of this species and details of sex allocation such as sex ratio have seldom been reported in shrimp, regardless of their sexual system. Thus, our second goal was to describe the population dynamics and sex ratio of this species in the field, to gain a better understanding of its overall biology and mating system.

MATERIALS AND METHODS

Specimen Collection

Specimens of *P. misticia* were collected from Cape Maeda (26°26'92"N, 127°43'48"E), Mizugama (26°21'27"N, 127°44'21"E), and Odo (26°5'6"N, 127°42'54"E) coastal locations in Okinawa, Japan during 2003-2004 and 2005-2006 and in Hanging Gardens reef (5°25'24"S, 150°5'33"E), Kimbe Bay, Papua New Guinea during 2009. This species can be found nocturnally in underwater caves, overhangs and crevasses, often in large numbers. These shrimp avoid light, and will retreat into holes and cracks when approached with an underwater light. In Papua New Guinea collections were conducted during SCUBA diving using small aquarium nets and flashlights. In Okinawa, collections were carried out using fish traps (24 × 24 × 38 cm, 5-mm diameter entry, 2-mm mesh size) baited with Sauri, *Cololabis* spp. Traps were placed at night time, using SCUBA, in underwater caves and crevasses in the reef substrate, and then retrieved approximately half an hour later. Most specimens were taken to the laboratory and fixed in a 10% formalin neutral solution. Ten individuals, collected at Mizugama, were taken live back to the laboratory for breeding experiments.

Morphological Observations of Reproductive Characters

To determine the sexual system of *P. misticia*, observations on the reproductive morphology were conducted on a total of 90 specimens collected in Okinawa during 2003-2004, and 41 specimens collected in Papua New Guinea during 2009. The carapace length (CL; distance from the posterior margin of the orbit to the mid-caudo-dorsal margin of carapace) of all shrimp was measured under the stereomicroscope to the nearest 0.01 mm. Also, we recorded in all shrimp the presence/absence of cincinulli (coupling hooks) on the endopods of the first pleopods, of appendices masculinae on the endopods of the second pleopods, of brooded embryos on the pleopods, and of male and female gonads underneath the carapace. Next, we recorded the stage of the vitellogenic oöcytes in shrimp with female gonads. Lastly, we measured the length of one appendix masculina (excluding spines) under the stereomicroscope to the nearest 0.01 mm in shrimp bearing this structure.

The characters above permitted the identification of MPs and EHs according to the presence/absence of a particular set of traits. MPs are individuals with coupling hooks on the endopods of first pleopods, appendices masculinae

on the endopods of the second pleopods, and ovotestes with paired posterior testes and poorly developed anterior ovaries underneath the pleon. EHs are individuals without coupling hooks on the endopod of first pleopods and without appendices masculinae on the endopods of the second pleopods but with paired ovotestes (with a well developed ovarian portion) underneath the pleon (see e.g., Bauer and Holt, 1998; Baeza et al., 2008; Baeza, 2010a, b).

Our initial observations suggested that *P. misticia* might be a protandric simultaneous hermaphroditic shrimp due to the existence of small individuals with appendices masculinae and an ovotestes in large individuals brooding embryos, as reported before for shrimp from the genus *Lysmata* and *Exhippolysmata* (Bauer and Holt, 1998; Fiedler, 1998; Braga et al., 2008; Baeza, 2009). Thus, during dissections and measurements of the different studied traits, we also focused on recognizing individuals with a combination of male and female traits, e.g. shrimp with appendices masculinae on the second pleopods and mature ovaries, indicating an ontogenetic shift from MP to EH. These "transitional" individuals have been reported before for various other species of shrimp that undergo strong shifts in sex allocation during their lifetime (strictly protandric and protandric-simultaneous hermaphrodites – Bauer, 1986; Bauer and Holt, 1998; Gavio et al., 2006; Baeza and Anker, 2008; Baeza et al., 2008; Baeza, 2009) and represent a reliable indication of sex change or sex phase change in a wide variety of marine organisms, including caridean shrimp (Bauer, 1986; Bauer and Holt, 1998; Baeza et al., 2008; Baeza, 2009).

Lastly, we examined the development of the gonads in shrimp of various body sizes. We selected a total 34 individuals with body sizes that described the complete size range of *P. misticia* for histological examination of their gonads. Entire cephalothoraxes or gonads were dissected, dehydrated in an alcohol series, and embedded in paraffin (melting point, 56 to 58°C; Nacalai Tesque). Serial sections (7 μ m) were made using a microtome (Pika Seiko, Ltd), stained with Mayer's acid hematoxylin and eosin, and mounted with MGK medium (Matsunami Glass Industries).

Functional Determination of Reproductive System

To investigate whether egg-brooding *P. misticia* individuals can also function as males, and thus to demonstrate that shrimp are functional simultaneous hermaphrodites, we conducted mating observations. Six aquaria (18 \times 30 \times 24 cm) were used to maintain shrimp during the experiment, each with an under-gravel filter, coralline gravel, and covered on all sides and top with opaque black plastic to restrict light to a minimum. Water temperature was maintained at 24–28°C. Shrimp were fed commercially available frozen prawn fillets (*Marsupenaeus japonicus* [Spence Bate, 1888]) once per day. The experimental group consisted of four pairs of egg-brooding individuals in four separate aquaria. Additionally, two egg-brooding individuals were kept in separate aquaria and served as the control group. All individuals were assessed daily for egg and embryo development, as well as reproductive status. It was not possible to make direct observations of copulation in these dark conditions. Thus, the presence or absence of exuviae in the aquarium was used as indirect evidence of copulation and spawning. Female-role individuals within the carideans invariably molt

prior to copulation and subsequently spawn eggs into their brood chamber (Bauer and Holt, 1998; Fiedler, 1998; Bauer and Newman, 2004; but see Lacoursière-Roussel and Sainte-Marie, 2009 for an exception). We also assessed and recorded the condition of vitellogenic oocytes under the carapace, the presence and developmental stage of brooded embryos, as well as any newly hatched larvae. All such observations were done at night under low light to minimize any potential stress.

Following the rationale of previous experiments (Fiedler, 1998; Baeza and Anker, 2008; Baeza et al., 2008; Baeza, 2009), if paired brooding shrimp produced normally developing broods, it was inferred that either the other brooding shrimp in the aquarium acted as a male to inseminate its partner, that the shrimp was capable of self-fertilization, or that sperm from previous copulations was used for fertilization. The former two cases demonstrate functional hermaphroditism. Isolated brooding shrimp served as controls. If brooding shrimp in isolation failed to successfully produce and brood normally developing eggs, both the possibilities of self-fertilization and exogenous sperm storage, can be eliminated.

Seasonal Reproductive Dynamics in Okinawa, Japan

To investigate the seasonal dynamics of *P. misticia* reproduction, shrimp were collected monthly at the Odo coast, Okinawa between January 2005 and January 2006. Collections were not possible in May and September 2005, because of poor weather conditions. Shrimp were captured using fish traps (see above) at each site. We attempted to collect a minimum of 50 individuals per month. However, less than 50 shrimp were collected in June, August and December, 2005. All shrimp were fixed in 10% formalin neutral solution. As above, we measured CL and classified individuals as MPs, EHs, and transitionals (see results).

Three different population parameters were calculated for each month: sex ratio, size at sex phase change, and proportion of brooding EHs. Sex ratio was estimated as the quotient between the number of MPs and the number of MPs plus EHs in the population (Baeza et al., 2010). For each sampling date, we tested for deviations from a 1 : 1 sex ratio using the binomial test (Wilson and Hardy, 2002). Size at sex phase change (L_{50}) for the different months was estimated as the size at which the probability to be EH is 0.5 using logistic regression as in Baeza et al. (2008).

RESULTS

Morphological Observations of Reproductive Characters

A total of 28 and 14 out of the 90 and 41 shrimp captured in Okinawa and Papua New Guinea, respectively, were classified as MPs by external morphology (Figs. 1, 2, 3). All of these MP shrimp had gonopores on the coxae of the last pair of pereopods. Also, all these MPs had cincinulli (coupling hooks [CH or c]) on the endopods of the first pleopods (Fig. 1A, B). Appendices masculinae on the endopods of the second pair of pleopods were observed in 26 out of 28 MPs examined from Okinawa (Figs. 1C-E, 2B). Relatively long spines on the distal portion of the appendices masculinae were observed in 24 out of 28 MPs examined from Okinawa (Figs. 1C-E, 2C). MPs lacking appendices

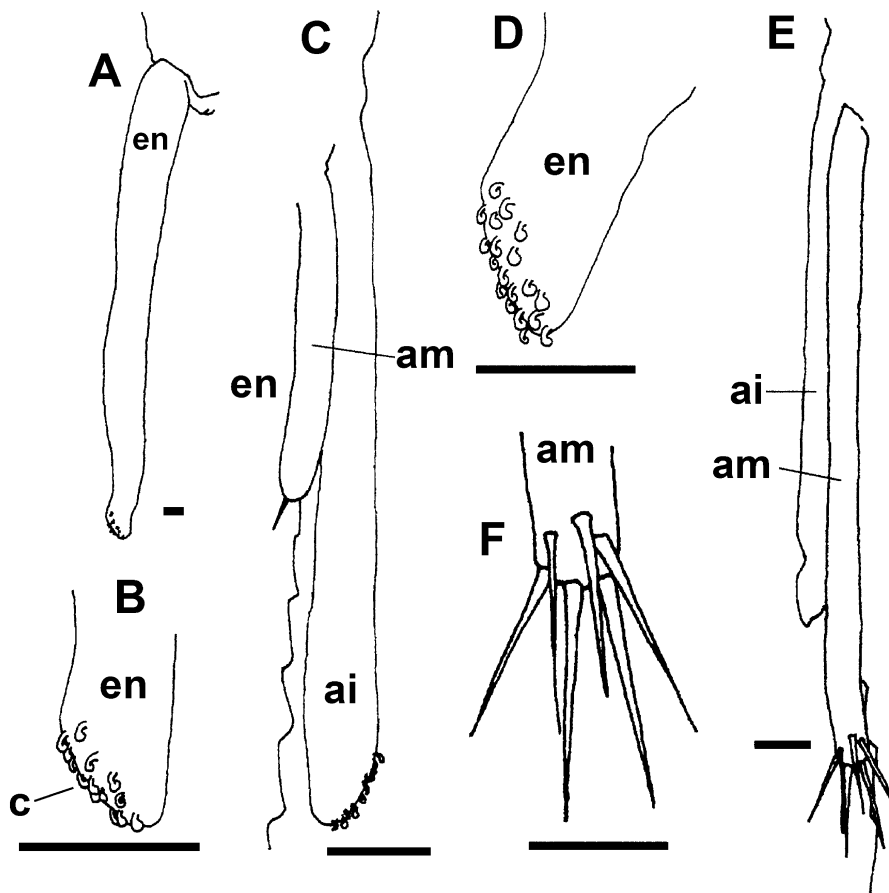


Fig. 1. MP pleopods of *Parhippolyte misticia*. A, Endopod (en) of first pleopod; B, Distal portion of the endopod (en) of the first pleopod bearing coupling hooks (c); C, Inner margin of the second pleopod showing appendix masculina (am) and appendix interna (ai); D, Endopod (en) of first pleopod [note the greater number of coupling hooks (c) present at the distal end of the endopod compared to those present in A]; E, Inner margin of the second pleopod showing appendix masculina (am) and appendix interna (ai); F, Distal portion of the appendix masculina bearing large number of distal spines. Pleopods in A, B, and C pertain to a male of 4.63 mm CL. Pleopods in D, E, and F pertain to a male of 8.28 mm CL. All scale bars, 0.1 mm.

masculinae and/or spines on this appendage's distal portion were among the smallest observed in our sample (Fig. 2B, C). The number of cincinnuli on the endopod of the first pleopods, the length of the appendices masculinae and the number of spines on the distal portion of the appendices masculinae, all increased linearly with the carapace length of MPs ($r^2 > 0.6$ and $P < 0.01$ in all cases, Fig. 2A-C). From 5 MPs (samples from PNG), sperm was retrieved from the gonopores after dissection of their vas deferens. Sperm cells were shaped like inverted umbrellas (Fig. 3A). Lastly, dissections of the gonads from MP shrimp (samples from Okinawa) demonstrated the presence of ovotestes with an undeveloped anterior female portion full of immature oöcytes that lacked coloration and a posterior male gonad containing sperm cells with the same morphology as sperm retrieved from the vas deferens (Fig. 3B, C). Oviducts and sperm ducts were observed in all these MPs.

Sixty-one and 14 out of the 90 and 41 shrimp captured in Okinawa and Papua New Guinea, respectively, were classified as EHs by external morphology. All these 75 shrimp had gonopores on the coxae of the fifth pereopods. Cincinnuli on the endopod of the first pleopods were absent in all these EHs (Fig. 4A, B). Appendices masculinae on the

endopods of the second pleopods were observed in 9 of these EHs, but their size was considerably reduced (Fig. 4C). The remaining EHs in our sample lacked appendices masculinae (Fig. 4D). Of the 75 EHs, 54 (72%) carried embryos in different stages of development. Dissections demonstrated the existence of paired ovotestes in both brooding and non-brooding EHs (Fig. 3D-F). Ovotestes were located above the hepatopancreas and featured a relatively large anterior female portion full of vitellogenic oöcytes (Fig. 3D, E) and a relatively small posterior male gonad portion with sperm that projected posterior into the first pleomere (Fig. 3D, F).

In one out of the 131 shrimp examined, both male external traits (cincinnuli in the first pleopods and well developed appendices masculinae in the endopods of the second pleopods as in Fig. 1A, B and C-E, respectively) and EHs internal characters (ovotestes with oöcytes and sperm) (Fig. 3G-I) were noticed after dissection. This "transitional" shrimp was slightly smaller (10.7 mm CL) than the largest males in the sample (Figs. 2, 5). The number of cincinnuli in the first pleopods and the number and length of the appendices masculinae in the second pleopods of this shrimp (10.7 mm CL) was alike to that of males of similar body size in our sample (Fig. 2).

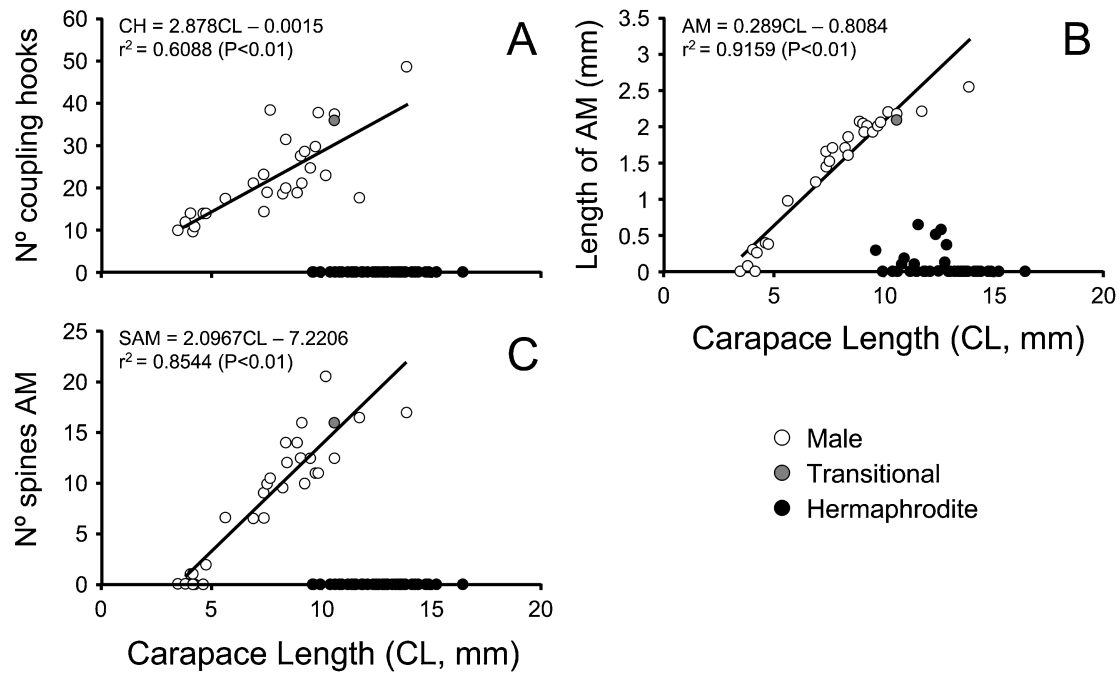


Fig. 2. The relationship between body size and MP characters in *Parhippolyte misticia*. A, Relationship between carapace length (CL) and the number of coupling hooks (CH) on the endopod of the first pleopod; B, Relationship between carapace length and the length of the appendix masculina (AM); C, Relationship between carapace length and the number of spines on appendix masculina (SAM).

The carapace length of MPs varied between 2.09–5.72 mm (mean $3.8 \text{ mm} \pm 0.9 \text{ SD}$); EHs ranged in carapace length from 4.2–6.5 mm (5.4 ± 0.7) (Fig. 6).

Functional Determination of Reproductive System

When embryo-brooding EH individuals ($n = 8$ in total) were paired together to determine whether they could also function as males, all these shrimp molted and spawned a new batch of oocytes to the brooding chamber. The oocytes remained attached to the pleopods, and signs of their embryonic development, i.e., early blastulae formation, were recorded after five days. All these shrimp successfully hatched their embryos as larvae. In contrast, all brooding shrimp isolated from conspecifics failed to successfully produce and brood normally developing eggs. These solitary shrimp molted and spawned oocytes beneath the pleon. However, their oocytes invariably disappeared from the pleopods within a few days after spawning.

Seasonal Reproductive Dynamics in Okinawa, Japan

A total of 787 shrimp was measured and sexed in Okinawa to examine population dynamics. Individuals in each sexual phase were found year-round at the study site (Fig. 6, 7). Throughout the sampling period, sex ratios varied between 0.48 and 0.87 with a mean ($\pm \text{SD}$) of $0.70 (\pm 0.12)$ (Fig. 7A). Monthly samples with sex ratios skewed in favor of MPs were observed more frequently than expected by chance alone (10 : 1 vs 10.5 : 10.5, binomial test, $P < 0.0037$; Fig. 7A). Thus, the population at Okinawa is characterized by a predominance of MPs throughout the year.

The percentage of brooding EHs during the sampling period varied between 0.19 and 0.94 with a mean ($\pm \text{SD}$) of $0.45 (\pm 0.29)$. Brooding EHs were more common between

June and August than during the rest of the year (Fig. 7B). Thus, *P. misticia* reproduces continuously but with varying intensity during the year, with more intense reproduction during warmer than colder months. Small males ($< 5.0 \text{ mm CL}$) were intermittently found during the year at Okinawa (Fig. 6). However, they were more frequently observed during October and November. Thus, recruitment in *P. misticia* is intermittent but appears to be mostly restricted to the boreal fall.

Lastly, throughout the sampling period, size at sex phase change varied between 10.31 and 13.56 mm CL with a mean ($\pm \text{SD}$) of $11.76 (\pm 0.96)$. Visual examination of the data indicated that size at sex phase change was smaller during the warmer months compared to the colder months of the year in the study population (Fig. 7C).

DISCUSSION

Sexual System of *Parhippolyte misticia*

Evidence on the population's structure, external sexual morphology and internal anatomy of *P. misticia* strongly indicates that this species is a protandric simultaneous hermaphrodite. First, the population is composed of MPs and EHs (and not pure females) and MPs were, on average, smaller than EHs. This size-frequency distribution of the sex phases agrees with expectations for protandric simultaneous hermaphrodites (Bauer and Holt, 1998; Fiedler, 1998; Bauer, 2004; Baeza, 2009). Second, no shrimp with characteristics of pure females were observed among the smallest body size classes of *P. misticia*. The absence of small juvenile females in the study populations rules out gonochorism (separate sexes) as the sexual system of *P. misticia*. Third, at least one transitional shrimp having traits of both MPs

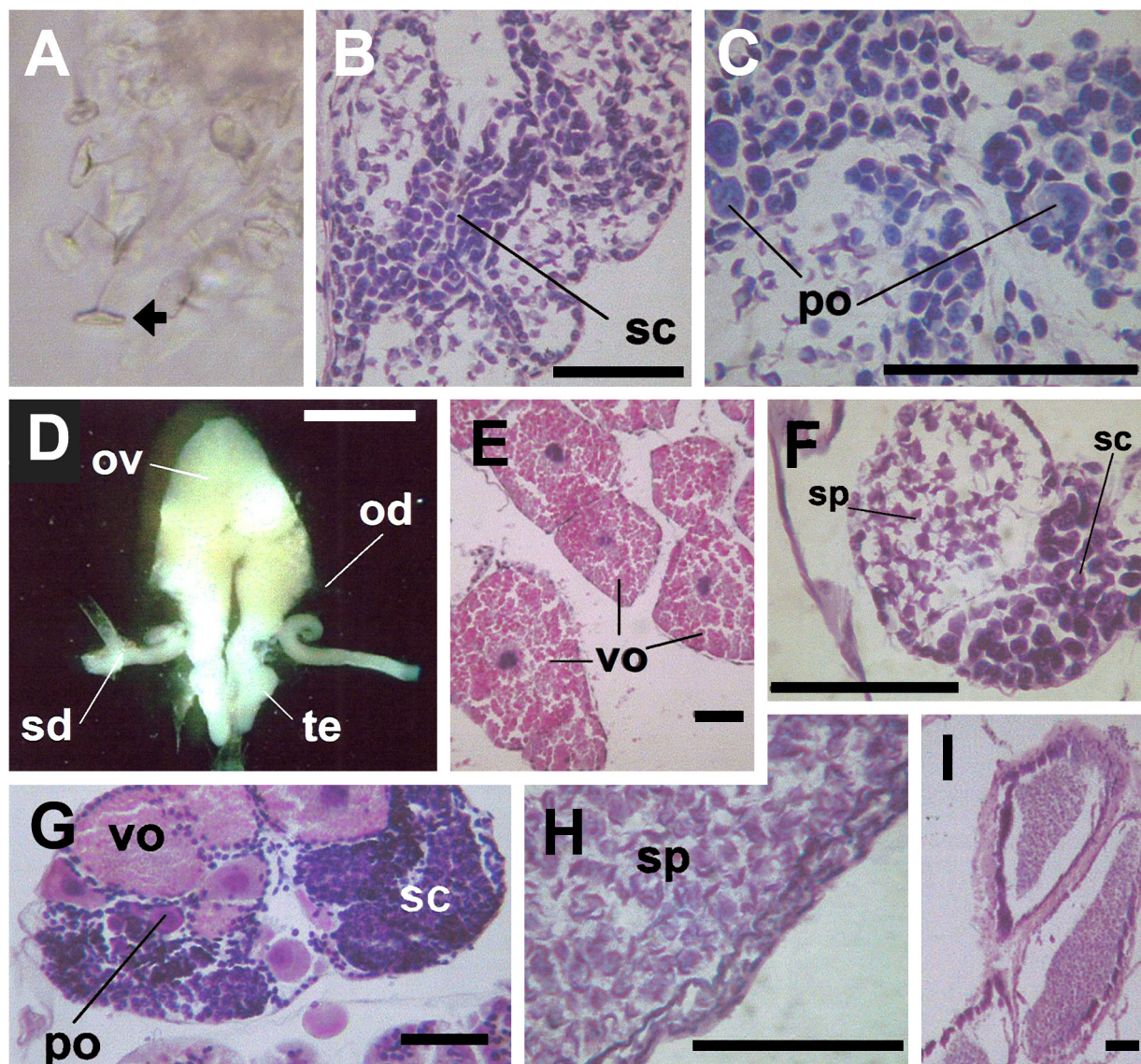


Fig. 3. Representative micrographs of histological sections of MP, transitional, and EH individuals of *Parhippolyte misticia*. A, Sperm retrieved from the vas deferens of an EH individual. Sperm retrieved from the vas deferens of MP individuals featured the same morphology, arrow points at one of the sperm cells in the micrograph; B, Testicular portion of the gonad of a MP individual with spermatocytes (sc) (9.43 mm CL); C, Ovarian portion of the gonad of the same MP individual in B with previtellogenic oocytes (po) (scale bar, 0.1 mm); D, Gonad of EH individual showing anterior ovarian (ov) and posterior testicular (te) portions [note the oviducts (od) and seminal ducts (sd) developing from the ovarian and testicular portions of the gonad, respectively]; E, Vitellogenic oocyte (vo) in the ovarian portion of the gonad of an EH individual (15.39 mm CL); F, Spermatocytes (sc) and sperm cells (sp) in the testicular portion of the gonad of the same EH individual in F; G, Mixed ovarian and testicular portion of the gonad of a transitional individual (9.94 mm CL) [note the presence of previtellogenic oocytes (po), vitellogenic oocytes (vo), and spermatocytes (sc)]; H, Close-up showing sperm (sp) cells at the testicular portion of the gonad of the same transitional individual in G; I, Sperm duct full of sperm of the same transitional individual in G and H. Scale bars = 0.1 mm in B, C, E, F, G, H and I. Scale bar = 1 mm in D.

and EHs (male gonopores with sperm and ovotestes with mature oocytes, respectively) was observed in one of the study populations (Okinawa). Furthermore, several other transitional individuals were observed during our study of the population dynamics of *P. misticia* in Okinawa. All this information demonstrates that *P. misticia* is a protandric simultaneous hermaphrodite; shrimp invariably start their benthic life as MPs. Then, these MPs become “transitional” individuals later in life. During this transitional stage that might last a single or a few inter-molt periods, shrimp lose the appendices masculinae and develop female characters,

e.g., maturing ovaries. During the next molt, shrimp become EHs (functional simultaneous hermaphrodites), capable of reproducing both as male and female at the same time, and remain so for the rest of their lives.

The sexual system of *P. misticia* is quite similar to that observed in shrimp in the genera *Lysmata* and *Exhippolysmata*. Indeed, PSH appears to represent a fixed trait in shrimp from the genus *Lysmata*, which contains 36 valid species (Baeza, 2009; De Grave et al., 2009). Similarly, two out of the four species from the genus *Exhippolysmata* in which sex expression has been studied are reported to initiate their life as MPs

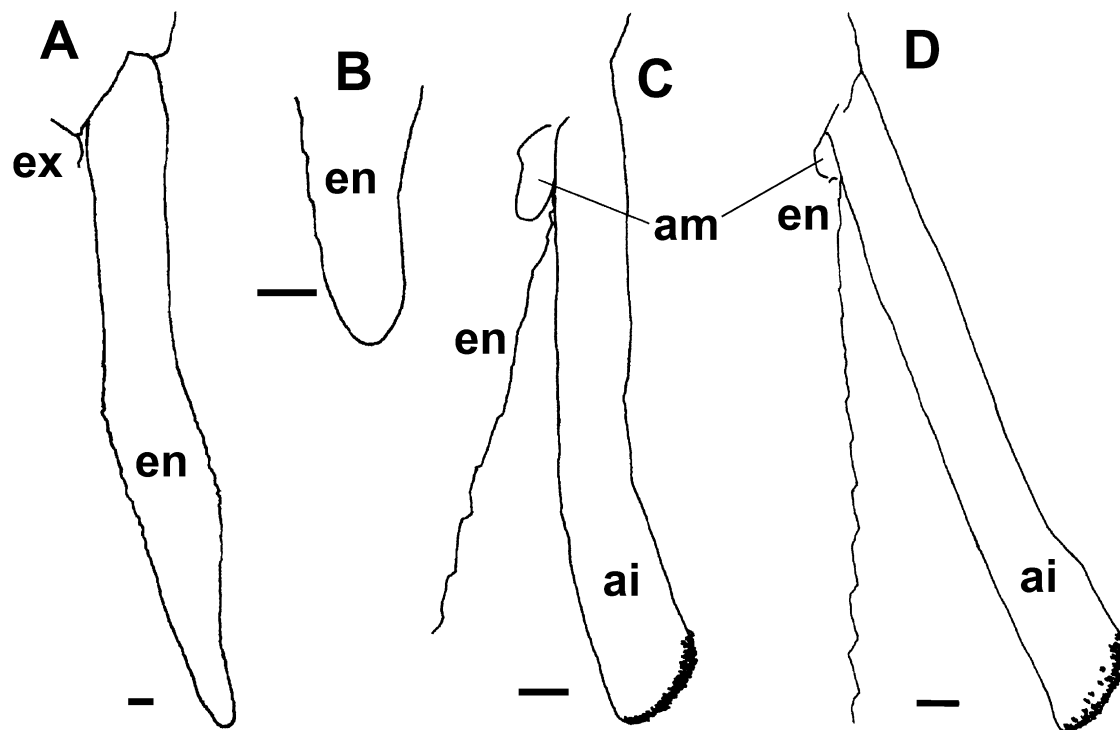


Fig. 4. EH pleopods of *Parhippolyte misticia*. A, Endopod (en) of first pleopod in individual of 11.2 mm CL; B, Distal portion of the endopod (en) of the first pleopod of the same individual in A; C, Inner margin of the second pleopod showing vestigial appendix masculina (am) and appendix interna (ai) of the same individual in A and B; D, Inner margin of the second pleopod showing vestigial appendix masculina (am) and appendix interna (ai) in an individual of 13.64 mm CL. All scale bars, 0.1 mm.

and to turn into EHs later in life (Braga et al., 2009 and references therein). The discovery of PSH in *P. misticia* raises the issue of this sexual system evolving more than once during the phylogenetic history of crustaceans within the diverse and species-rich Caridea.

At present, the available information on the phylogenetic affinities of *Lysmata*, *Exhippolysmata*, *Parhippolyte*, and other closely related genera does not allow us to infer with certainty the actual number of times that PSH has evolved in carideans. Recent studies have shown that *Lysmata*, *Exhippolysmata*, *Parhippolyte*, and a fourth genus comprised of semi-terrestrial shrimp with a strictly protandric sexual system (*Merguia rhizophorae* – Baeza, 2010a), are all closely related each one to another (Baeza et al., 2009; Baeza, 2010b; Fiedler et al., 2010). Importantly, all phylogenetic reconstructions (using different molecular markers, i.e., 16S and/or 28S, and analyses, e.g., maximum parsimony, minimum evolution, maximum likelihood, Bayesian inference) have retrieved *Lysmata* and *Exhippolysmata* as a single monophyletic clade (Baeza et al., 2009; Baeza, 2010b; Fiedler et al., 2010). On the other hand, the position of the genus *Parhippolyte* with respect to *Lysmata* + *Exhippolysmata* and *Merguia* is less clear. Some phylogenetic analyses place *Parhippolyte* in a more basal position than *Lysmata* + *Exhippolysmata* but in a more derived position than *Merguia* (scenario 1). In contrast, other reconstructions suggest that the genus *Merguia* is sister to a clade comprised of *Lysmata* + *Exhippolysmata* and additionally indicate that *Parhippolyte* has a more basal position than *Lysmata* + *Exhippolysmata* and *Merguia* (Fiedler et al., 2010) (scenario 2).

Thus, the phylogenetic relationships inferred for the genera above so far suggest two plausible scenarios for the evolution of PSH in Caridea: 1) a single origin of PSH in an ancestral species featuring a strictly protandric sexual system (under scenario 1), or 2) a single origin of PSH followed by the replacement of PSH by strict protandry in *Merguia* (under scenario 2). At present, it is not possible to distinguish which one of these scenarios is true. Additional multi-locus phylogenetic studies are needed to reveal the number of times and ecological conditions that has favored PSH in shrimp from the Infraorder Caridea. Also, further detailed studies on the lifestyle and sexual system of representatives from the genera *Barbouria*, *Calliasmata*, and *Lysmatella* are warranted, as they are relevant for elucidating the fascinating evolutionary history of gender expression in shrimp.

Population Dynamics of *Parhippolyte misticia*

Reproductive biology of *P. misticia* in subtropical Okinawa is similar to that observed in warm temperate rather than in tropical crustaceans. For instance, although, EHs brooded continuously during the year, a large proportion of shrimp restricted reproductive activity to the spring/summer boreal months. Embryo development last approximately 13–18 days in *P. misticia* (Onaga and Fiedler, unpublished data). Thus, EHs most probably reproduce repeatedly during the year, especially during spring and summer months. Reproductive activity increasing during the warmer months of the year is the rule rather than the exception in crustaceans, including shrimp, from boreal and austral temperate latitudes (e.g., boreal: Allen, 1966; Oh and Hartnoll, 2004; austral:

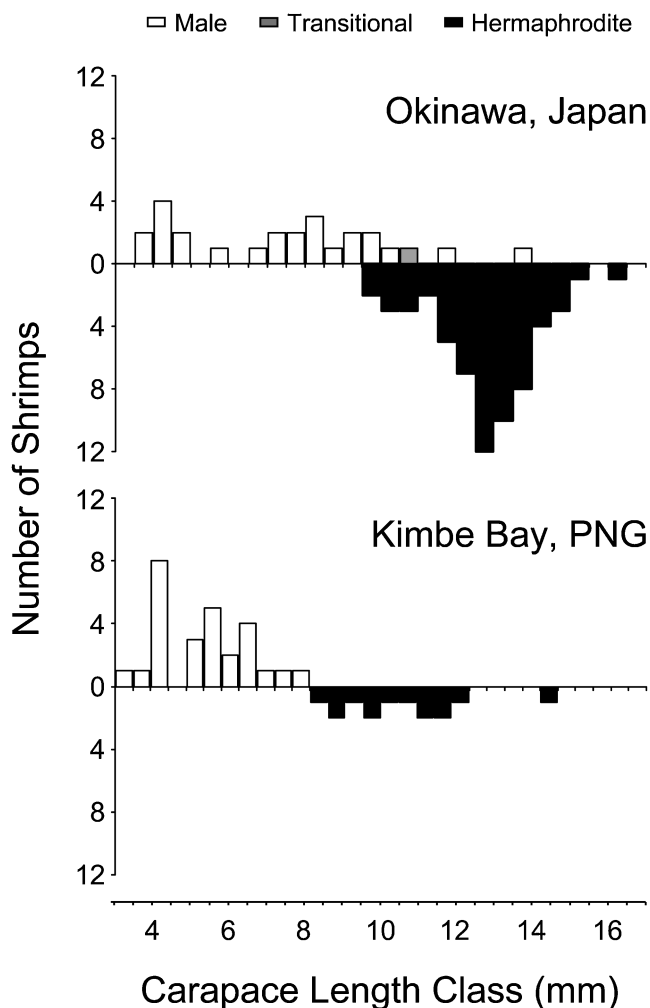


Fig. 5. Size frequency distribution of *Parhippolyte misticia* collected at Okinawa, Ryukyu Islands, Japan ($n = 90$) and Kimbe Bay, West New Britain, Papua New Guinea ($n = 41$). Only individuals initially examined to determine the sexual system of the species are shown. On average, the body size of MPs is smaller than that of EHs (see results for further details).

Antezana et al., 1965; Baeza and Thiel, 2000). In contrast, marine shrimp from tropical/subtropical localities usually reproduce continuously and with similar intensity throughout the year (Felder, 1982; Bauer, 1985, 1989; but see Baeza et al., 2010). Also in agreement to that reported for other temperate species (Antezana et al., 1965; Allen, 1966; Baeza and Thiel, 2000), recruitment in *P. misticia* occurred mostly in the boreal fall, albeit the presence of small males in the population suggest that recruitment is somewhat intermittent during the remaining of the year. In contrast, recruitment in tropical shrimp is rather continuous throughout the year but episodic (Bauer, 1985, 1989; Bauer and Laporte, 2011). Temperature, photoperiod and primary productivity, among others, are considered important conditions driving both reproductive periodicity and recruitment in decapod crustaceans (Sastry, 1983; Bauer, 1985, 1989, 1992). Although Okinawa is a subtropical locality characterized by abundant tropical marine life, e.g., diverse and abundant species of corals, water temperatures vary considerably at the study site (ranging between 25.5°C and 28°C during spring and summer and between 21°C and 23.5°C during

fall and winter during the study period – Onaga and Fiedler, unpublished data). This variability in abiotic conditions (unusual for subtropical localities) resembles that of warm temperate environments and might be explaining reproductive seasonality in *P. misticia*. Increased reproductive periodicity is known in many marine invertebrates, including crustaceans inhabiting localities closer to edge of their range of distribution (Olive, 1992). Overall, *P. misticia* represents an example of a subtropical species with considerable reproductive seasonality. Future studies might reveal decreasing reproductive seasonality in localities closer to the Equator, e.g., in Papua New Guinea.

Sex Ratio and Size at Sex Phase Change in *Parhippolyte misticia*

In *P. misticia*, sex ratio was biased towards MPs during most of the study period. Sex ratio varies considerably in protandric simultaneous hermaphrodites. For instance, in some species of *Lyssmata*, sex ratio is extremely biased toward MPs, e.g., more than 90% of the population is comprised by MPs in *L. galapagensis* Schmitt, 1924 – Baeza, 2009. In others, EHs outnumber MPs in the population, e.g., *Exhippolyata oplophoroides* (Holthuis, 1948) – Baeza et al. (2010). Low population density has been considered relevant in explaining sex ratios skewed toward EHs in protandric simultaneous hermaphrodites. Young MPs facing infrequent male mating opportunities (due to low population density) are expected to turn into EHs early in life so to assure reproduction via the male and female function whenever mating opportunities arise (Baeza et al., 2010). Such an early sex phase change is thought to determine the dominance of EHs in “low abundance” species. The MP skewed sex ratio observed in *P. misticia*, as well as in various other species of *Lyssmata*, might rather be explained by sex allocation theory as developed for strictly sequential (protandric) hermaphrodites.

Sex allocation theory predicts male skewed sex ratios in species exhibiting strict protandry (Charnov and Bull, 1989). This prediction rests on the assumption that male and female fertility increases with size and that low levels of mortality occur during sexual transition (Charnov and Bull, 1989). The same MP bias could be expected in protandric simultaneous hermaphrodites given their considerable shift of reproductive resources towards the female function after sex phase change, e.g., nearly 120 and 35 units of mass are invested in ovaries per unit mass invested in sperm in EHs of *Lyssmata wurdemannii* (Gibbes, 1850) and *L. pedersenii* Rhyne and Lin 2006, respectively – Baeza, 2007a, 2010c. Visual examination of the ovotestes in *P. misticia* (during dissections) suggests that allocation to ovary mass is considerably greater than that to sperm mass. Studies on size-specific fertility, sex-specific resource allocation and on the cost of sex phase change are needed to explain the observed male skewed sex ratio in *P. misticia* and other protandric simultaneous hermaphrodites. Overall, the remarkable variability in sex ratio reported for protandric simultaneous hermaphrodites suggests that different sets of environmental conditions drive sex phase ratio in these species. The future development of mathematical models that specifically predict sex phase ratio in protandric simultaneous hermaphrodites are needed to improve our understanding of sex ratios in species with

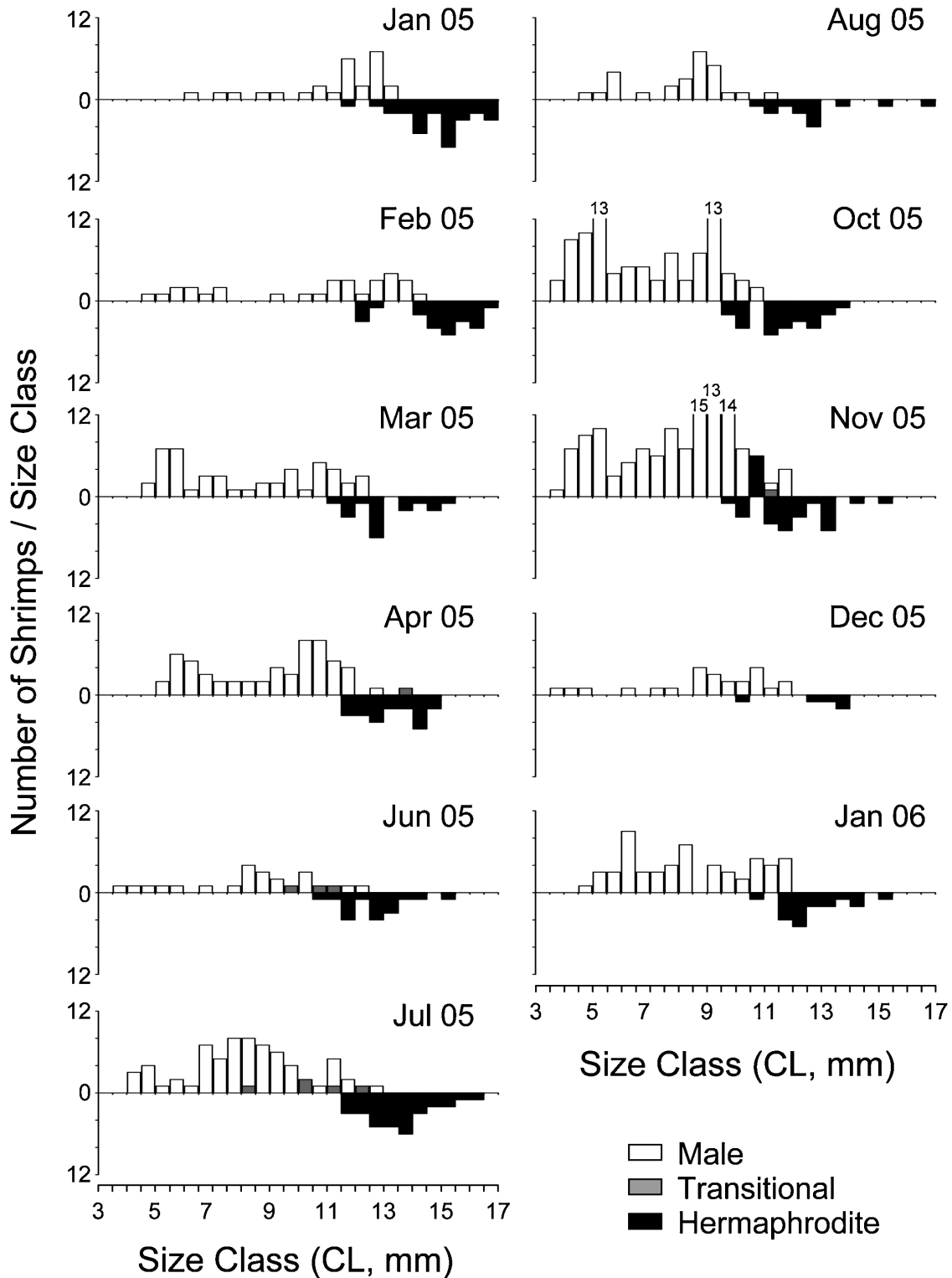


Fig. 6. Population dynamics of *Parhippolyte misticia* at Odo Coast, Okinawa, Japan, between January 2005 and January 2006.

“mixed” sexual systems (shrimp from the genera *Parhippolyte*, *Exhippolysmata*, and *Lysmata*).

Size at sex phase change varied throughout the year in the studied population of *P. misticia*. During warmer months, MPs turn into EHS earlier in life while the opposite occurred

during colder months when males delayed sex phase change. Laboratory experiments have demonstrated that temperature and photoperiod affect the timing of sex phase change in other protandric simultaneous hermaphrodites (Bauer, 2002; Baldwin and Bauer, 2003). For instance, in *L. wurdemanni*,

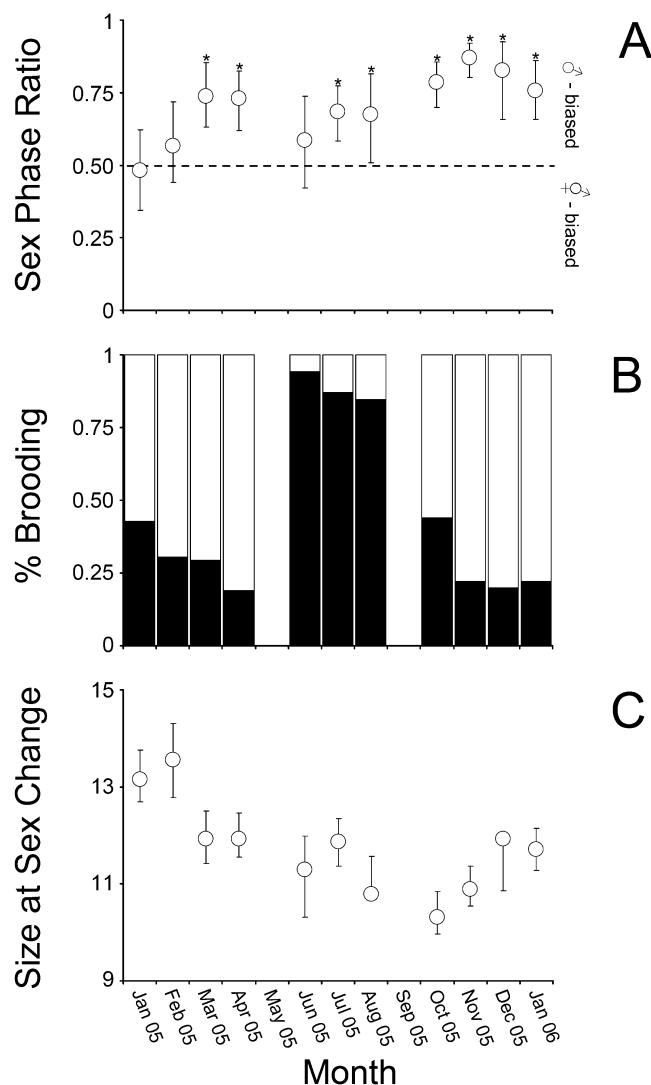


Fig. 7. Sex ratio (A), proportion of brooding EHs (B), and size at sex phase change (C) of *Parhippolyte misticia* at Odo Coast, Okinawa, Japan, between January 2005 and January 2006.

small MPs speed up maturation as hermaphrodites under conditions of high water temperature and long day length, which in turn, emulate natural late spring and summer conditions (Bauer, 2002). Similarly, the social environment is most relevant in determining the timing of sex phase change in protandric simultaneous hermaphrodites (Baeza and Bauer, 2004; Baeza, 2007b). In *L. wurdemanni*, small MPs appear to speed up maturation as EHs with increasing number of recruits and decreasing number of larger EHs in the surroundings (Zhang and Lin, 2007). In general, the observed shifts in size at sex phase change observed in *P. misticia* agree with the effect of both biotic (including social) and abiotic conditions in the timing of sex phase change in protandric simultaneous hermaphrodites. The variability in size at sex phase change in *P. misticia* is also consistent with the notion that variation in sex ratio in natural populations of protandric simultaneous hermaphrodites is expected whenever population structure is not stable due to e.g., changes on recruitment intensity and availability of larger EHs (see Baeza et al., 2010).

Outlook

Overall, this study used a varied approach to characterize the sexual system of a marine shrimp. A combination of size measurements, examination of external morphology and internal anatomy, and behavioral observations in the laboratory demonstrated PSH in *P. misticia*. The study species is closely related to other peculiar shrimp either recognized because of their unusual sexual system (*Lysmata* and *Exhippolysmata* are also protandric simultaneous hermaphrodites – Bauer and Holt, 1998; Fiedler, 1998; Baeza, 2009; Braga et al., 2009) or unique lifestyle (strictly protandric shrimp from the genus *Merguia* are semi-terrestrial – Baeza, 2010a). Future research aimed at disentangling the phylogenetic relationship of these and other closely related genera (*Callinectes*, *Lysmatella*, *Barbouria*) and describing the sociobiology of additional representatives from these same genera is warranted as will probe most useful in understanding the evolutionary history of sex expression in carideans and the historical origin and environmental conditions favoring outstanding sex allocation patterns in animals.

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REFERENCES

- Ahyong, S. T. 2009. New species and new records of hydrothermal vent shrimps from New Zealand (Caridea: Alvinocarididae, Hippolytidae). *Crustaceana* 82: 775-794.
- Allen, J. A. 1966. The dynamics and interrelation of mixed population of Caridea found off the North-East coast of England, pp. 45-66. In: H. B. Barnes (ed.), *Some Contemporary Studies of Marine Science*. Allen and Unwin, London, England.
- Antezana, T., E. Fagetti, and M. T. Lopez. 1965. Observaciones bioecológicas en decápodos comunes de Valparaíso. *Revista de Biología Marina, Valparaíso* 12: 1-60.
- Baeza, J. A. 2006. Testing three models on the adaptive significance of protandric simultaneous hermaphroditism in a marine shrimp. *Evolution* 60: 1840-1850.
- . 2007a. Male mating opportunities affect sex allocation in a protandric simultaneous hermaphroditic marine shrimp. *Behavioral Ecology and Sociobiology* 61: 365-370.
- . 2007b. Sex allocation in a simultaneously hermaphroditic marine shrimp. *Evolution* 62: 2360-2373.
- . 2008. Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlanica*, in the tropical eastern Pacific coast. *Marine Biology* 153: 387-395.
- . 2009. Protandric simultaneous hermaphroditism is a conserved trait in *Lysmata* (Caridea: Lysmatidae): implications for the evolution of hermaphroditism in the genus. *Smithsonian Contributions to Marine Science* 38: 95-110.
- . 2010a. Sexual system and natural history observations on semi-terrestrial shrimp *Merguia rhizophorae*. *Invertebrate Biology* 129: 266-276.
- . 2010b. Molecular systematics of peppermint and cleaner shrimps: phylogeny and taxonomy of the genera *Lysmata* and *Exhippolysmata* (Crustacea: Caridea: Hippolytidae). *Zoological Journal of the Linnean Society* 160: 254-265.

- . 2010c. The symbiotic lifestyle and its evolutionary consequences: social monogamy and sex allocation in the hermaphroditic shrimp *Lysmata pederseni*. *Naturwissenschaften* 97: 729-741.
- , and A. Anker. 2008. *Lysmata hochi* n. sp., a new species of hermaphroditic shrimp from the southern Caribbean. *Journal of Crustacean Biology* 28: 148-155.
- , and R. T. Bauer. 2004. Experimental test of social mediation of sex change in a protandric sequential hermaphrodite: the marine shrimp *Lysmata wurdemanni* (Crustacea: Caridea). *Behavioral Ecology and Sociobiology* 55: 544-550.
- , and C. Piantoni. 2010. Sexual system, sex ratio and group living in the shrimp *Thor amboinensis* (De Man): relevance to resource-monopolization and sex-allocation theories. *Biological Bulletin* 219: 151-165.
- , and M. Thiel. 2000. Host use pattern and life history of *Liopetrolisthes mitra*, an associate of the black sea urchin *Tetrapygus niger*. *Journal of the Marine Biological Association of the United Kingdom* 80: 39-45.
- , J. Reitz, and R. Collin. 2008. Protandric simultaneous hermaphroditism and sex ratio in the shrimp *Lysmata nayaritensis*. *Journal of Natural History* 41: 2843-2850.
- , J. A. Bolaños, J. E. Hernandez, C. Lira, and R. López. 2011. Monogamy does not last long in *Pontonia mexicana*, a symbiotic shrimp of the Amber pen-shell *Pinna carnea* from the southeastern Caribbean Sea. *Journal of Experimental Marine Biology and Ecology* 407: 41-47.
- , C. D. Schubart, P. Zillner, S. Fuentes, and R. T. Bauer. 2009. Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Lysmatidae): the evolutionary origins of pair-living, protandric simultaneous hermaphroditism and species status. *Biological Journal of the Linnean Society* 96: 415-424.
- , A. A. Braga, L. S. López-Greco, E. Perez, M. L. Negreiros-Fransozo, and A. Fransozo. 2010. Population dynamics, sex ratio and size at sex change in a protandric-simultaneous hermaphrodite, the spiny shrimp *Exhippolysmata oplophoroides*. *Marine Biology* 157: 2643-2653.
- Baldwin, A. P., and R. T. Bauer. 2003. Growth, survivorship, life span, and sex change in the hermaphroditic shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Marine Biology* 143: 157-166.
- Bauer, R. T. 1985. Diel and seasonal variation in species composition and abundance of the caridean shrimps (Crustacea, Decapoda) from seagrass meadows in northern Puerto Rico. *Bulletin of Marine Science* 36: 150-162.
- . 1986. Sex change and life history pattern in the shrimp *Thor mannigi* (Decapoda: Caridea): a novel case of partial protandric hermaphroditism. *Biological Bulletin* 170: 11-31.
- . 1989. Continuous reproduction and episodic recruitment in nine shrimp species inhabiting a tropical seagrass meadow. *Journal of Experimental Marine Biology and Ecology* 127: 175-187.
- . 1992. Testing generalizations about latitudinal variation in reproduction and recruitment patterns with scyoniid and caridean shrimp species. *Invertebrate Reproduction and Development* 22: 193-202.
- . 2000. Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. *Journal of Crustacean Biology* 20(Spec. No. 2): 116-128.
- . 2002. Reproductive ecology of a protandric simultaneous hermaphrodite, the shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Journal of Crustacean Biology* 22: 742-749.
- . 2004. Remarkable Shrimps. Oklahoma University Press, Norman, USA.
- . 2006. Same sexual system but variable sociobiology: evolution of protandric simultaneous hermaphroditism in *Lysmata* shrimps. *Integrative and Comparative Biology* 46: 430-438.
- , and G. J. Holt. 1998. Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. *Marine Biology* 132: 223-235.
- , and S. Laporte. 2011. Gonochoric sexual system in the caridean shrimps *Processa riveroi* and *P. bermudensis* (Decapoda: Processidae) inhabiting a tropical seagrass meadow. *Journal of the Marine Biological Association of the United Kingdom*. doi:10.1017/S0025315411000622.
- , and W. A. Newman. 2004. Protandric simultaneous hermaphroditism in the marine shrimp *Lysmata californica* (Caridea: Hippolytidae). *Journal of Crustacean Biology* 24: 131-139.
- , and R. VanHoy. 1996. Variation in sexual systems (protandry, gonochorism) and reproductive biology among three species of the shrimp genus *Thor* (Decapoda: Caridea). *Bulletin of Marine Science* 59: 53-73.
- Becker, J. H. A., and A. S. Grutter. 2005. Client-fish ectoparasite loads and cleaner-shrimp hunger levels affect the cleaning behaviour of the cleaner-shrimp *Urocaridella* sp. C. *Animal Behavior* 70: 991-996.
- Bergström, B. I. 2000. The biology of *Pandalus*. *Advances in Marine Biology* 38: 55-245.
- Boddeke, R., J. R. Bosschier, and P. C. Goudswaard. 1991. Sex change, mating, and sperm transfer in *Crangon crangon* (L.), pp. 164-182. In: R. T. Bauer and J. W. Martin (eds.), *Crustacean Sexual Biology*. Columbia University Press, New York, USA.
- Braga, A. A., L. S. López-Greco, D. C. Santos, and A. Fransozo. 2009. Morphological evidence for protandric simultaneous hermaphroditism in the caridean *Exhippolysmata oplophoroides*. *Journal of Crustacean Biology* 29: 34-41.
- Chace, F. A. Jr. 1972. The shrimps of the Smithsonian-Bredin Expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology* 98: 1-179.
- . 1997. The caridean shrimps (Crustacea: Decapoda) of the Albattross Philippine Expedition, 1907-1910, Part 7: families Atyidae, Eugonatonotidae, Rhynchocinetidae, Bathypalaemonellidae, Processidae, and Hippolytidae. *Smithsonian Contributions to Zoology* 587: 1-106.
- Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, USA.
- , and J. J. Bull. 1989. Non-Fisherian sex ratios with sex change and environmental sex determination. *Nature* 338: 148-150.
- Christoffersen, M. L. 1987. Phylogenetic relationships of hippolytid genera, with an assignment of new families for the Crangonoidea and Alpheoidea (Crustacea, Decapoda, Caridea). *Cladistics* 3: 348-362.
- Clark, J. 1989. *Koror misticus*, new genus, new species (Decapoda: Hippolytidae), a cave shrimp from Palau. *Journal of Crustacean Biology* 9: 445-452.
- De Grave, S. D., N. D. Pentcheff, S. T. Ahyong, T.-Y. Chan, K. A. Crandall, P. C. Dworschak, D. L. Felder, R. M. Feldmann, C. H. J. M. Fransen, L. Y. D. Goulding, R. Lemaitre, M. E. Y. Low, J. M. Martin, P. K. L. Ng, C. E. Schweitzer, S. H. Tan, D. Tshudy, and R. Wetzler. 2009. A classification of living and fossil genera of decapod crustaceans. *Raffles Bulletin of Zoology*, Supplement 21: 1-109.
- De Man, J. G. 1888. Bericht über die von Herrn Dr. J. Brock im indischen Archipel gesammelten Decapoden und Stomatopoden. *Archiv für Naturgeschichte* 53: 289-600.
- Espinoza-Fuenzalida, N. L., M. Thiel, E. Dupre, and J. A. Baeza. 2008. Is *Hippolyte williamsi* gonochoric or hermaphroditic? A multi-approach study and a review of sexual systems in *Hippolyte* shrimps. *Marine Biology* 155: 623-635.
- Felder, D. L. 1982. Reproduction of the snapping shrimps *Synalpheus fritzmulleri* and *S. apiceros* (Crustacea: Decapoda: Alpheidae) on a sublittoral reef off Texas. *Journal of Crustacean Biology* 2: 535-543.
- Fiedler, G. C. 1998. Functional, simultaneous hermaphroditism in female-phase *Lysmata amboinensis* (Decapoda: Caridea: Hippolytidae). *Pacific Science* 52: 161-169.
- , A. L. Rhyne, R. Segawa, T. Aotsuka, and N. V. Schizas. 2010. The evolution of euhermaphroditism in caridean shrimps: a molecular perspective of sexual systems and systematics. *BMC Evolutionary Biology* 10: 297. doi:10.1186/1471-2148-10-297.
- Gavio, M. A., J. M. Orensanz, and D. Armstrong. 2006. Evaluation of alternative life history hypotheses for the sand shrimp *Crangon franciscorum* (Decapoda: Caridea). *Journal of Crustacean Biology* 26: 295-307.
- Gibbes, L. R. 1850. On the carcinological collections of the cabinets of natural history in the United States: with an enumeration of the species contained therein and descriptions of new species. *Proceedings of the American Association for the Advancement of Science* 3: 167-201.
- Gordon, I. 1935. On new or imperfectly known Species of Crustacea Macrura. *Journal of the Linnean Society, Zoology* 39: 307-351.
- Guérin-Méneville, F. E. 1855. Animaux articulés avec pieds articulés. In: R. de la Sagra (ed.), *Historia física política y natural de la isla de Cuba*. Segunda Parte. *Historia Natural*. Tomo VIII (Atlas de Zoología): 1-371.
- Holthuis, L. B. 1948. Note on some Crustacea Decapod Natantia from Surinam. *Proceedings van de Koninklijke Nederlandsche Akademie van Wetenschappen* 51: 1104-1113.
- Hufnagel, M., A. Temming, and A. Dänhardt. 2010. Hermaphroditism in brown shrimp: lessons from field data and modelling. *Marine Biology* 157: 2097-2108.

- Kingsley, J. S. 1878. Notes on the North American Caridea in the Museum of the Peabody Academy of Science at Salem, Mass. Proceedings of the Academy of Natural Sciences of Philadelphia 1878: 89-98.
- Lacoursière-Roussel, A., and B. Sainte-Marie. 2009. Sexual system and female spawning frequency in the sculptured shrimp *Sclerocrangon boreas* (Decapoda: Caridea: Crangonidae). Journal of Crustacean Biology 29: 192-200.
- Limbaugh, C., H. Pederson, and F. A. Chace Jr. 1961. Shrimps that clean fishes. Bulletin of Marine Science 11: 237-257.
- Linnaeus, C. 1758. Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis. Edition 10. Vol. 1. Laurentii Salvii, Holmiae.
- Manning, R. B., and F. A. Chace Jr. 1971. Shrimps of the family Processidae from the Northwestern Atlantic Ocean (Crustacea: Decapoda: Caridea). Smithsonian Contributions to Zoology 89: 1-41.
- Noël, P. 1976. L'évolution des caractères sexuels chez *Processa edulis* Risso (Décapode, Natantia). Vie Milieu 26: 65-104.
- Oh, C. W., and R. G. Hartnoll. 2004. Reproductive biology of the common shrimp *Crangon crangon* (Decapoda: Crangonidae) in the central Irish Sea. Marine Biology 144: 303-316.
- Olive, P. J. W. 1992. The adaptive significance of seasonal reproduction in marine invertebrates: the importance of distinguishing between models. Invertebrate Reproduction and Development 22: 165-174.
- Rankin, W. M. 1900. The Crustacea of the Bermuda Islands, with notes on the collections made by the New York University Expeditions in 1897 and 1898. Annals of the New York Academy of Sciences 12: 521-548.
- Rathbun, M. J. 1900. Results of the Branner-Agassiz expedition to Brazil. I. The decapod and stomatopod Crustacea. Proceedings of the Washington Academy of Sciences 2: 133-156.
- Rhyne, A. L., and J. Lin. 2006. A western Atlantic peppermint shrimp complex: redescription of *Lysmata wurdemanni*, description of four new species, and remarks on *Lysmata rathbunae* (Crustacea: Decapoda: Hippolytidae). Bulletin of Marine Sciences 79: 165-204.
- Risso, A. 1816. Histoire naturelle des Crustacés des environs de Nice. La Librairie Greque-Latine-Allemande, Paris, France.
- Sastry, A. N. 1983. Ecological aspects of reproduction, pp. 179-270. In, J. J. Vernberg and W. B. Vernberg (eds.), The Biology of Crustacea. Vol. 8. Academic Press, New York, USA.
- Schatte, J., and R. Saborowski. 2006. Change of external sexual characteristics during consecutive moults in *Crangon crangon* L. Helgoland Marine Research 60: 70-73.
- Schmitt, W. L. 1924. The Macrura and Anomura collected by the Williams Galapagos Expedition, 1923. Zoologica, New York 4: 161-171.
- Spence Bate, C. 1888. Report on the Crustacea Macrura collected by the Challenger during the years 1873-76. Report on the Scientific Results of the Voyage of H.M.S. "Challenger" during the years 1873-76 24: 1-942.
- Verrill, A. E. 1869. On the parasitic habits of Crustacea. The American Naturalist 3: 239-250.
- Wilson, K., and I. C. W. Hardy. 2002. Statistical analysis of sex ratios: an introduction, pp. 48-92. In, I. C. W. Hardy (ed.), Sex Ratios: Concepts and Research Methods. Cambridge University Press, Cambridge, England.
- Zhang, D., and J. Lin. 2007. Effects of density and simulated recruitment and mortality on sex change in a protandric simultaneous hermaphroditic shrimp, *Lysmata wurdemanni*. Marine Biology 150: 639-645.

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