

# Sexual System, Sex Ratio, and Group Living in the Shrimp *Thor amboinensis* (De Man): Relevance to Resource-Monopolization and Sex-Allocation Theories

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**Abstract.** The sexual system of the symbiotic shrimp *Thor amboinensis* is described, along with observations on sex ratio and host-use pattern of different populations. We used a comprehensive approach to elucidate the previously unknown sexual system of this shrimp. Dissections, scanning electron microscopy, size-frequency distribution analysis, and laboratory observations demonstrated that *T. amboinensis* is a protandric hermaphrodite: shrimp first mature as males and change into females later in life. *Thor amboinensis* inhabited the large and structurally heterogeneous sea anemone *Stichodactyla helianthus* in large groups (up to 11 individuals) more frequently than expected by chance alone. Groups exhibited no particularly complex social structure and showed male-biased sex ratios more frequently than expected by chance alone. The adult sex ratio was male-biased in the four separate populations studied, one of them being thousands of kilometers apart from the others. This study supports predictions central to theories of resource monopolization and sex allocation. Dissections demonstrated that unusually large males were parasitized by an undescribed species of isopod (family Entoniscidae). Infestation rates were similarly low in both sexes ( $\approx 11\%$ – $12\%$ ). The available information suggests that *T. amboinensis* uses pure search promiscuity as a mating system. This hypothesis needs to be formally tested with mating behavior observations and field measurements on the movement pat-

tern of both sexes of the species. Further detailed studies on the lifestyle and sexual system of all the species within this genus and the development of a molecular phylogeny are necessary to elucidate the evolutionary history of gender expression in the genus *Thor*.

## Introduction

Sexual systems vary considerably in shrimps from the infraorder Caridea. Many species are gonochoric, with individuals in a population expressing only one sex during their lifetime (e.g., *Hippolyte williamsi*: Espinoza *et al.*, 2008; *Pontonia margarita*: Baeza, 2008). In other species, each individual in the population reproduces first as a male and changes sex to female later in life. Examples of strict protandry include *Pandalus platyceros* and *P. goniurus* (Butler, 1964, 1980; Berström, 1997, 2000). Departures from the classic example of strict protandry have been documented in various species. For instance, in *Processa edulis* (Noël, 1976) and *Crangon crangon* (Boddeke *et al.*, 1991; Schatte and Saborowski, 2006), protandric males (*i.e.*, shrimps that turn to females later in life) coexist with primary females (*i.e.*, shrimps that remain females for their entire lives) in the same population. Most recently, protandric simultaneous hermaphroditism *sensu* Bauer and Holt (1998), where individuals initially mature and reproduce as males and later become simultaneous hermaphrodites, has been confirmed in several species of *Lysmata* and two species of *Exhippolysmata* (Baeza, 2009).

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Gender expression within the infraorder Caridea might vary noticeably at the generic level. For instance, in the genus *Thor*, a clade that includes 12 species worldwide (De Grave *et al.*, 2009), two species have been confirmed as gonochoric (*T. dobkini* and *T. floridanus*: Bauer and VanHoy, 1996). In contrast, a third species, *T. manningi*, exhibits partial protandry with populations composed of primary males (*i.e.*, individuals born as males that do not change sex), primary females, and protandric individuals (Bauer, 1986; Bauer and VanHoy, 1996). Shrimp from the genus *Thor* also demonstrate a considerable diversity of lifestyles: some species are symbiotic with sea anemones (*Thor amboinensis*: Khan *et al.*, 2004) or *Porites* sp. corals (*T. marguitae*: Chace, 1997), others live in algal mats (*T. algicola*: Wicksten, 1987), and still others perch on blades in seagrass meadows (*T. manningi*: Bauer, 1986). This ecological and behavioral diversity suggests that shrimp from the genus *Thor* might be used as a model to explore the importance of environmental conditions in explaining the evolution of sex allocation in crustaceans. However, the lack of studies on the natural history and sexual systems of most species of this genus are hampering comparative studies.

In this study, we examined the sexual system of the shrimp *Thor amboinensis*. The “squat anemone shrimp” or “sexy shrimp” *Thor amboinensis* is a circumtropical “ornamental” species symbiotic with several sessile macroinvertebrates, including sea anemones (Fransen, 1989; Guo *et al.*, 1996; Wirtz, 1997; Khan *et al.*, 2004). Importantly, its symbiotic lifestyle—symbiosis here defined *sensu* de Bary (1865) quoted by Vermeij (1983) as dissimilar organisms living together—can be helpful in improving our understanding of principles central to theories of sex allocation and resource monopolization as applied to marine organisms.

Sex-allocation theory is one of the most robust branches in evolutionary biology (West *et al.*, 2000; Munday *et al.*, 2006; Schärer, 2009). One of its main goals is to predict the optimal sex ratio in species featuring a wide variety of sexual systems (Charnov, 1982). For instance, for sequential hermaphrodites, sex ratio is expected to be biased toward the first sex. Mathematical modeling suggests that sex ratio favors the sex that experiences lower fertility values (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). During recent decades, experiments and field observations have provided partial support for the prediction above. Sex-ratio biases toward the first sex have been reported for several sequential hermaphrodites (Charnov and Bull, 1989; Collin, 1995). On the other hand, a recent review of sex ratios in 40 species of protandric fish and invertebrates from five phyla demonstrated a large range in sex ratios (male/male+female:

≈0.11–0.89; Allsop and West, 2004). Clearly, more experimental studies and detailed field data are necessary to improve our understanding of sex-ratio evolution.

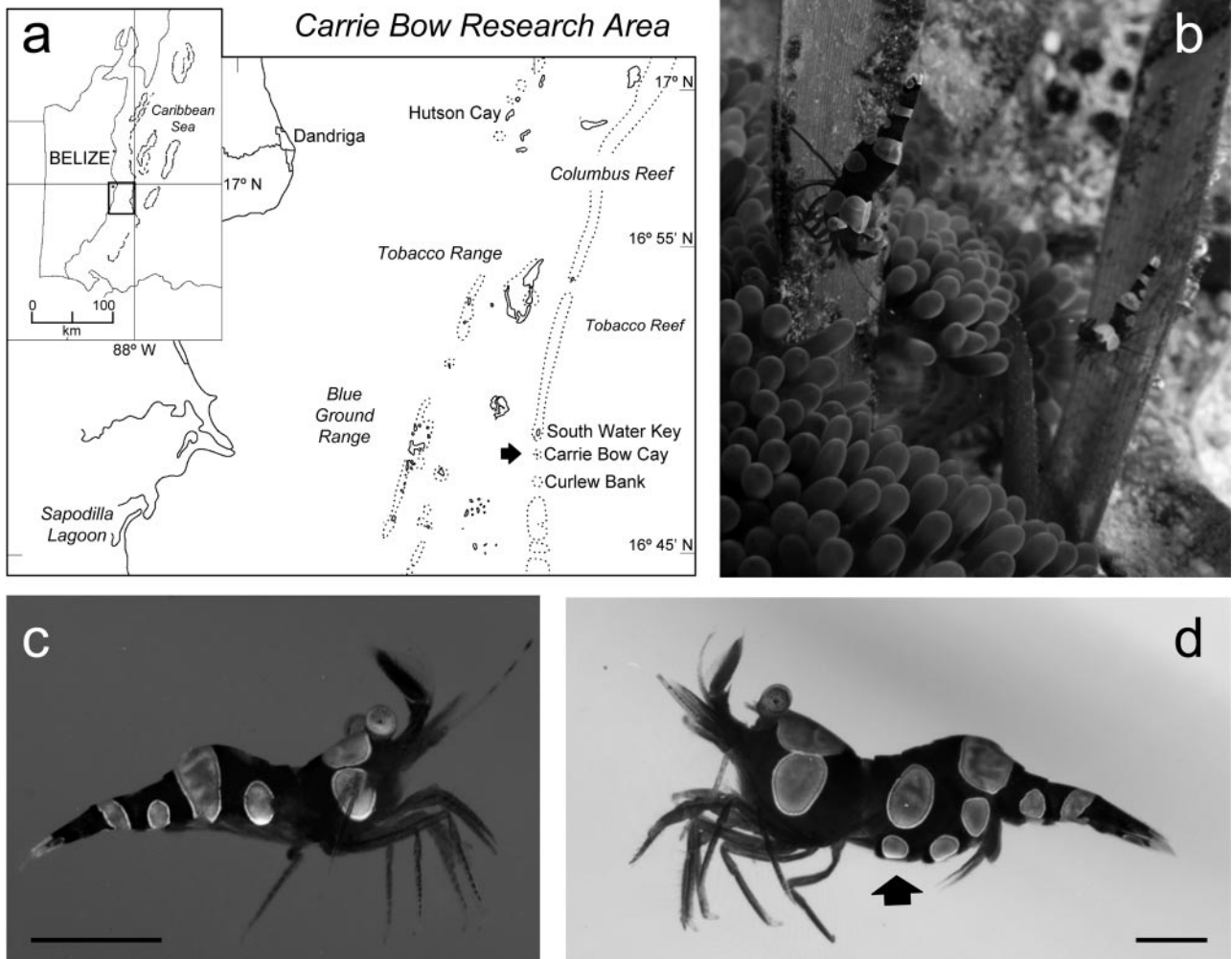
Resource monopolization is a process that affects either directly or indirectly the lifetime reproductive success of an individual (Wilson, 1975; Emlen and Oring, 1977; Shuster and Wade, 2003; Baeza and Thiel, 2007). In symbiotic species, theory predicts that territoriality (shelter defense) or guarding of resources evolves when monopolization is “economic”; that is, when the benefits of monopolization exceed the costs of defense (Brown, 1964; Emlen and Oring, 1977). Recent theoretical considerations suggest that host structural complexity and host size (relative to the species using a host) are the most relevant parameters modulating the cost and benefit of refuge monopolization and, therefore, the distribution of symbiotic crustaceans (Baeza and Thiel, 2003, 2007; Baeza, 2008). In relatively small and structurally simple hosts, defense against intruders might be energetically economical, whereas relatively large hosts with considerable structural complexity should be relatively difficult to defend (Baeza *et al.*, 2001; Thiel and Baeza, 2001; Baeza and Thiel, 2007; Baeza, 2008). Thus, relatively large hosts should shelter large groups because guarding behaviors become energetically expensive, while small hosts are expected to harbor single or pairs of conspecifics (Baeza and Thiel, 2003, 2007). A few manipulative and observational experiments in some symbiotic crustaceans support the predictions above (Baeza and Thiel, 2003, 2007; Baeza, 2008). However, more information is needed to determine the generality of these predictions.

The aim of this study was first to determine the sexual system of the symbiotic shrimp *T. amboinensis* by examination of internal/external anatomy, size-frequency distribution analysis, and laboratory observations. Our second goal was to observe the ecology of *T. amboinensis* in the field. We focused on describing the sex ratio and host-use pattern of various populations because such information contributes to a deeper understanding of sex allocation and resource monopolization theories as applied to marine organisms.

## Materials and Methods

### *The model species Thor amboinensis*

The circumtropical shrimp *Thor amboinensis* is not a “cleaner shrimp” (Guo *et al.*, 1996), although it is brightly colored, having a reddish brown body with iridescent yellowish white spots bordered by thin white and blue bands (Herrnkind *et al.*, 1976; Stanton, 1977; Calado, 2008; pers. obs.) (Fig. 1). *Thor amboinensis* has been reported to live with a wide variety of coelenterate (Sarver, 1979; Guo *et al.*, 1996; Wirtz, 1997, and references therein) and non-coelenterate hosts (crinoids: Criales, 1984; cerianthids: Guo *et al.*, 1996). It has occasionally been observed in small cracks in



**Figure 1.** (a) Study site in Belize (arrow points to the three sampling areas in the barrier reef) (illustration by Molly Ryan, Smithsonian Institution; used with permission from Klaus Reutzler). (b) Close-up of the sea anemone *Stichodactyla helianthus* showing a female (on left) and male (on right) of *Thor amboinensis* perched on sea grass blades. (c) Male of *T. amboinensis* (scale bar = 1 mm). (d) Female of *T. amboinensis* showing two spots at the flange of the second and third pleopods (arrow points at spot in second pleopod) (scale bar = 1 mm).

the rock face without a host (Wirtz, 1997). In Hawaii, this species completes its larval development and undergoes metamorphosis without induction from its host species *Antheopsis papillosa* (Sarver, 1979). Adult specimens from insular China (Taiwan and Pescadore Islands) show no strong preference for the anemones in which they are most commonly found (*Entacmea quadricolor* and *Stichodactyla tapetum*: Guo *et al.*, 1996). However, adults locate anemones using chemical and visual cues (Guo *et al.*, 1996). Given its weak host preference, *T. amboinensis* is considered a symbiotic generalist (Guo *et al.*, 1996).

In Madeira and the Canary Islands, *T. amboinensis* is the most common associate of the anemone *Telmatactis cricoides* (67% and 54%, respectively), with a mean of 2.3 shrimp per host (Wirtz, 1997). However, in the northern Caribbean, the occurrence of the shrimp on anemones is low

(20% and 13% in *Lebrunia danae* and *Bartholomea annulata*, respectively), where usually one, and no more than two, shrimps per anemone have been reported (Herrnkind *et al.*, 1976; Stanton, 1977). Similarly, an average of two shrimp per host of *Stichodactyla haddoni* is reported for Moreton Bay, Australia (Khan *et al.*, 2004). In contrast, in Taiwan and the Pescadore Islands, as many as 11 *T. amboinensis* individuals have been observed in a single host individual (in *Stichodactyla tapetum*: Guo *et al.*, 1996). In the latter case, the largest shrimp within each group tends to be near the center of the oral disc and smaller individuals at the periphery (Guo *et al.*, 1996).

*Thor amboinensis* either maintains close contact with its anemone hosts (near the center of the oral disc in *Stichodactyla tapetum* [Guo *et al.*, 1996], on tentacles in *Antheopsis papillosa* [Sarver, 1979], and climbing down among

tentacles in *Lebrunia danae* [Herrnkind *et al.*, 1976, Stanton, 1977]), or remains on the substrate close to the periphery of the host's tentacles (in *Bartholomea annulata* and *Condylactis gigantea* [Nizinski, 1989], *Entacmea quadricolor* [Guo *et al.*, 1996], and *Stichodactyla haddoni* [Khan *et al.*, 2004]). Shrimp can also be found at the base of the column beneath the crown of tentacles (Guo *et al.*, 1996; Khan *et al.*, 2004; pers. obs.). The posture and resting behavior of this shrimp is unique: the abdomen is kept flexed upward dorsally and is almost constantly waved upward/downward or circularly. (Herrnkind *et al.*, 1976; Calado, 2008; pers. obs.). In the laboratory, shrimps consume the tentacles and mucus of its host, *S. haddoni* (Khan *et al.*, 2004).

#### Study sites

The sexual system, host-use pattern, and population sex ratio of *T.amboinensis* were studied during 2008 and 2009 in the shallow subtidal (1.2–3 m) of three reefs in Belize—South Water Cay (SWK: 16°48'43½N, 88°04'54½W); Carrie Bow Cay (CBC: 16°48'14½N, 88°04'54½W); and Curlew Bank (CB: 16°47'24½N, 88°04'53½W). In 2009, an additional site was sampled at Sesoko Island (26°38'N, 127°51'E), Okinawa, Japan (Fig. 1a).

In Belize, all study sites were dominated by patches either of coral rubble partially covered by algae (*i.e.*, *Padina* spp.) and undetermined cyanobacteria, the sea grass *Thalassia testudinum*, the coral *Porites porites*, or a partial mixture of the above. Small schools of juveniles or juveniles plus adults of yellowhead wrasse *Halicoeres garnoti*, bluehead wrasse *Thalassoma bifasciatum*, clown wrasse *H. maculipinna*, and slippery dick *H. bivittatus* were frequently observed patrolling the reef in search of prey. More sedentary predators such as the harlequin bass *Serranum tigrinus* were also common at the study site. Shrimps were sampled from the sun anemone *Stichodactyla helianthus*.

In Okinawa, the study site was dominated by coral rubble, beds of the boring giant clam *Tridacnia crocia*, small patches of table corals *Acropora* spp., coral heads of *Pocillopora* spp., or a mixture of these. Sea snakes (*Hydrophis* spp.) and various species of lionfish (most commonly *Pterois volitans* and *P. antennata*) were found either patrolling the reef or finding refuge among corals during the day. Shrimps were sampled from the giant sea anemone *S. cf. tapetum*.

#### Sexual system of *Thor amboinensis*

**Dissections and scanning electron microscopy.** To reveal the sexual system of the studied shrimp, observations on reproductive anatomy were made on a total of 145 specimens collected during 2008 and 2009 near the Smithsonian Marine Station at Carrie Bow Cay (CBC), Belize. Shrimp were collected from the sun sea anemone *Stichodactyla*

*helianthus* by scuba diving, placed in resealable plastic bags, and transported live to CBC. In the laboratory, the carapace length (CL), the length of the largest of the first pair of chelipeds, and the second abdominal segment (maximum lateral length of the pleuron) of all shrimp were measured under the stereomicroscope to the nearest 0.025 mm. The presence or absence of gonopores on the coxae of the fifth pereopods was recorded for each individual. Then, the first and second pleopods were dissected and the presence or absence of appendices internae and masculinae were recorded. These characters permitted the identification of males and females according to the presence (males) or absence (females) of gonopores on the fifth pereopods and of appendices masculinae (males) on the endopods of the second pleopods (see Bauer, 1986; Baeza, 2008; Espinoza *et al.*, 2008). Finally, the gonads of 25 males (CL = 1.38–2.95 mm) and 25 females (CL = 1.45–2.9 mm) were selected on the basis of the external characters previously mentioned and dissected to confirm the shrimps' sex.

During measurements and dissections, we focused primarily on recognizing individuals with a combination of male and female traits (*e.g.*, shrimp with appendices masculinae on the second pleopods [male character] and mature ovaries). 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, 2008, 2009) and represent a reliable indication of sex change in a wide variety of marine organisms (gastropods: Collin *et al.*, 2005; shrimps: Bauer, 1986; Bauer and Holt, 1998; Baeza, 2008, 2009; fish: Devlin and Nagahama, 2002, and references therein).

Lastly, 21 samples (including the thoracic sterna of 4 males, 2 females, and 2 transitionals) from shrimps collected in 2009 at CBC were prepared for scanning electron microscopy (SEM). Samples were fixed in 3% glutaraldehyde on the island, stored at 6 °C, and processed for SEM in the laboratory. They were rinsed in water three times and dehydrated using a graded series of ethanol of 50%, 75%, 96%, and 100%. Then, the samples were immersed into two changes of hexamethyldisilazane (HMDS) and air-dried to be sputter-coated with gold and analyzed on a Philips XL-30 ESEM at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

**Sexual dimorphism.** A subsample of 75 males and 42 females was used for the analysis of sexual dimorphism. We examined whether the chelae on the first pair of pereopods and the pleuron of the second abdominal segment increase linearly with body size in males and females of *T. amboinensis*. These two structures were selected because in the Hippolytidae, including shrimps from the genus *Thor*, the first pair of thoracic appendages bears the larger of the two

pairs of chelae. These structures are used for inter-sexual communication or as weapons during intra-sexual interactions (Shuster and Wade, 2003). In turn, the pleuron of the second abdominal segment is greatly enlarged and helps protect (*e.g.*, from physical abrasion) the embryos carried by females beneath the abdomen (Bauer, 2004).

The relationship between the length of the propodus of the first cheliped or the length of the pleuron of the second abdominal segment and body size (CL) of shrimps was examined using the allometric model  $y = ax^b$  (Hartnoll, 1978, 1982). The slope  $b$  of the log-log least-squares linear regression represents positive allometric ( $b > 1$ ) or negative allometric ( $b < 1$ ) growth of the cheliped and abdominal segment. To determine if the relationship deviated from linearity, a Student's *t*-test was used to test whether the estimated slope  $b$  deviates from the expected slope of unity.

*Sex change: Laboratory observations.* Shrimps collected during the summer of 2009 in the vicinity of CBC were observed to determine whether males change sex to females with time. Immediately after collection, 12 males (with visible appendices masculinae) were maintained separately in sets of three ( $n = 4$ ) in 19-l buckets with running seawater (29–31 °C, 34‰) for a total of 23 days. Males selected for these observations were among the largest observed in the field (1.53–2.0 mm CL). Large males were selected and maintained in sets of three to maximize the chance of observing sex change (Baeza and Bauer, 2004; Baeza, 2007). In each bucket, one sea anemone *Stichodactyla helianthus* and small pieces of coral rubble with fouling organisms (crustose coralline red algae) were provided as a perch and food for the shrimp. At the end of 23 days, each individual was examined externally for the presence of embryos beneath the abdomen, the absence of appendices masculinae in the second pleopods, and the presence of white spots at the base of the second and third pleopods. These spots were a reliable characteristic of females and transitional individuals and were invariably absent in male shrimps (see Results) (Fig. 1). The gonads of individuals with any of the characteristics above were dissected and examined for the presence of oocytes.

#### *Sex ratio in T. amboinensis*

The sex ratio of *T. amboinensis* was studied at South Water Cay (SWK), Carrie Bow Cay (CBC), and Curlew Bank (CB). We examined sex ratio separately at CBC for samples collected during 2008 and 2009. At each site, the first 40 sea anemones found during free-diving were examined for the presence or absence of shrimp. Male and female individuals were counted when shrimp were found in anemones. During shrimp counts, we took special care to examine small crevices on the coral rubble, seagrass blades, and coral branches in the immediate vicinity of the target sea

anemone, to record all associated shrimp. Then, the diameter of each sea anemone was measured with a plastic ruler to the nearest 1 mm at CBC and CB. Logistic problems impeded measuring sea anemones at SWK. Lastly, we examined the sex ratio of *T. amboinensis* during 2009 at Sesoko Island, Okinawa, Japan, where the shrimp sampling, counting, and sexing were conducted in the same way as in Belize.

For each study site and sampling date, we used the binomial test (Wilson and Hardy, 2002) to test for deviations from a 1:1 sex ratio. We discarded the smallest, most probably immature, males from each sample for the analyses of sex ratio, although all males, regardless of their size, had well-developed appendices masculinae (see Results). The rationale for discarding the very small males was to describe sex ratios for only the sexually mature individuals in each population.

Importantly, we were not able to distinguish female from transitional individuals in the field. However, samples from Belize (2009) demonstrated that the frequency of these transitional forms in the population was remarkably low (1.72% out of 116 shrimp, see Results). Thus, to correct for the presence of the transitional shrimp during our sampling, we recalculated sex ratio for each population, considering that 1.72% of the studied population were not functional females. This analysis demonstrated that any bias in the sex ratio reported by the first analysis was not affected by this error in our calculations (analysis not shown here).

#### *Population distribution of Thor amboinensis*

The population distribution of *T. amboinensis* was examined at the three study sites in Belize by analyzing the data from the same samples used for the description of the sex ratio. We investigated whether the distribution of shrimps in sea anemones differed significantly from a random distribution by comparing the observed distribution with the Poisson distribution (Elliot, 1983). A chi-square test of goodness of fit was used to determine significant differences between the distributions (SAS Institute, 2004). A Poisson regression was used to test for a significant effect of sea anemone size on shrimp number (SAS Institute, 2004). Before the test, the assumption of over-dispersion was examined and found to be satisfactory.

#### *Group size composition in Thor amboinensis*

To reveal group size composition of *T. amboinensis*, we performed a second sampling at CBC, in an unperturbed area located adjacent to that previously used for collecting shrimps for dissections and experiments. The first 38 sea anemones observed were examined for the presence or absence of shrimps at this adjacent area. Once shrimps were spotted in a sea anemone, all of the individuals in that particular host were collected using aquarium nets, placed in

small plastic resealable bags, and transported live to the field laboratory on CBC. The CL and sex of each shrimp were determined (see above).

## Results

### *Sexual system of Thor amboinensis*

**Dissections and SEM.** Of 116 shrimps examined, 75 and 39 were classified as males and females, respectively, considering only their external morphology. Dissections demonstrated that all males sexed according to external features (male gonopores on the coxae of the fifth pair of pereopods and appendices masculinae on the second pair of pereopods) had paired testes with lateral sperm ducts that contained sperm cells (Fig. 2a, b). SEM of one of these males confirmed the absence of female gonopores on the coxae of its third pair of legs. Appendices masculinae with a large number of relatively long spines were well developed in all males, including those among the smallest size classes (4 males between 0.75 and 1.0 mm CL). Appendices masculinae usually reached the distal end of the endopod in the second pair of pleopods (Fig. 2a) or, on occasion, extended past it.

All shrimps classified as females were characterized by the absence of appendices masculinae in the endopods of the second pereopods (Fig. 2c, d). SEM of two of these females confirmed the presence of female gonopores on the coxae of the third pereopods and the absence of male gonopores on the coxae of the fifth pair of legs. Thirty of these 39 females (76.9%) carried embryos. The smallest brooding female was 1.72 mm CL. The paired ovaries of both ovigerous and nonovigerous females were located above the hepatopancreas and projected backward into the first abdominal segments (Fig. 2e). Importantly, we noticed that females invariably had two pairs of spots, one pair on the flange of the second pleopods and another on the flange of the third pleopods. The coloration of these spots was the same as that of spots in the carapace and abdomen: iridescent yellowish white bordered by two thin bands (an inner white and an outer blue) (Figs. 1d, 2d). None of the males examined had spots on the pleopods (Fig. 1c).

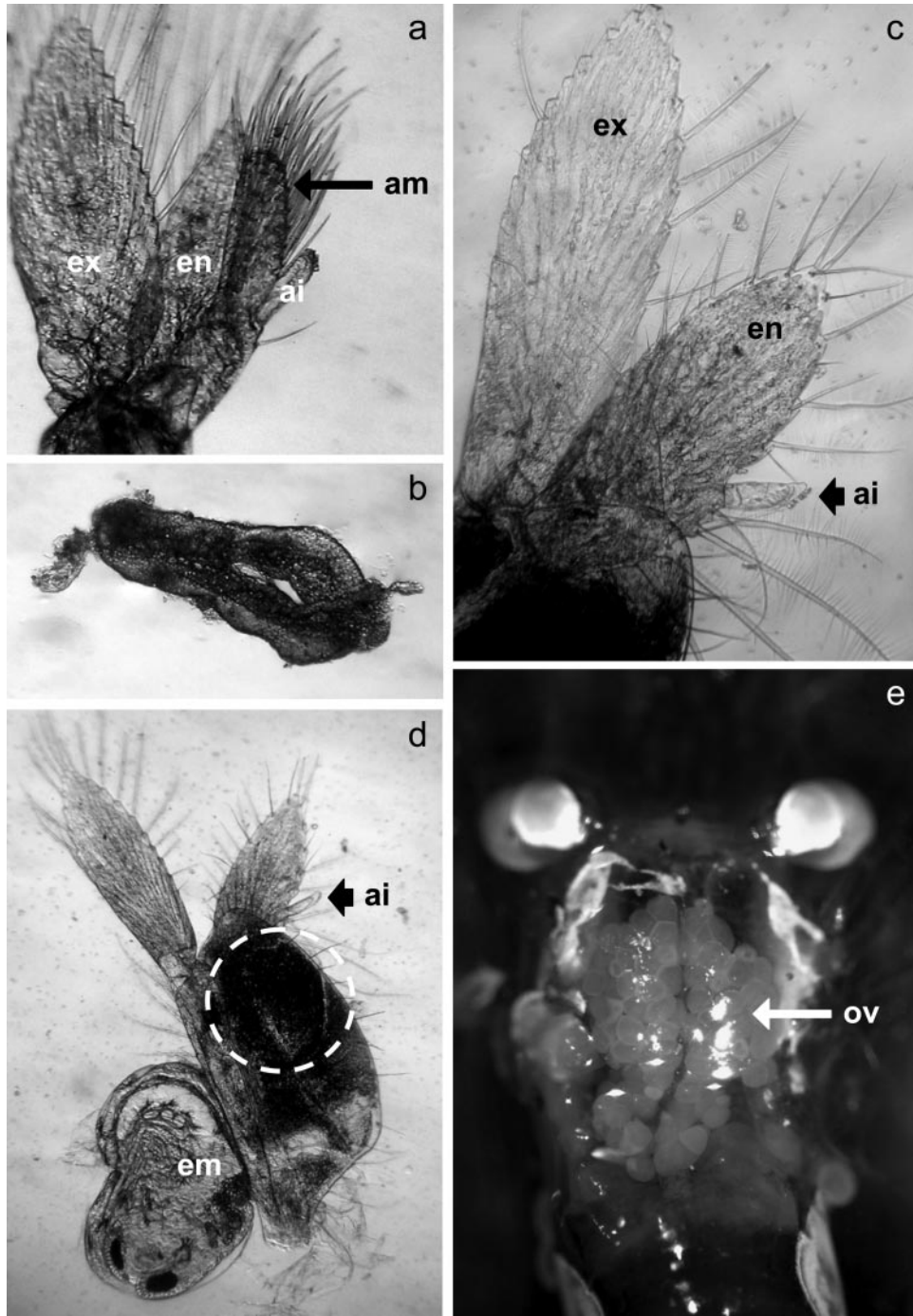
In 2 out of 116 shrimp examined we noticed both male external traits (appendices masculinae as in Fig. 2a) and female internal characters (ovary with green yolky oocytes as in Fig. 2e) after dissection. SEM of these two transitional individuals confirmed the presence of both female and male gonopores on the coxae of the third and fifth pereopods, respectively (Fig. 3a–c). The body size of these two transitional individuals (1.9 and 2.18 mm CL) was similar to that of the largest males in our sample (Fig. 4). As in females, these two transitional specimens displayed white spots on the second and third pleopods.

**Sexual dimorphism in *Thor amboinensis*.** The CL of males and females varied respectively between 0.83 and 3.0

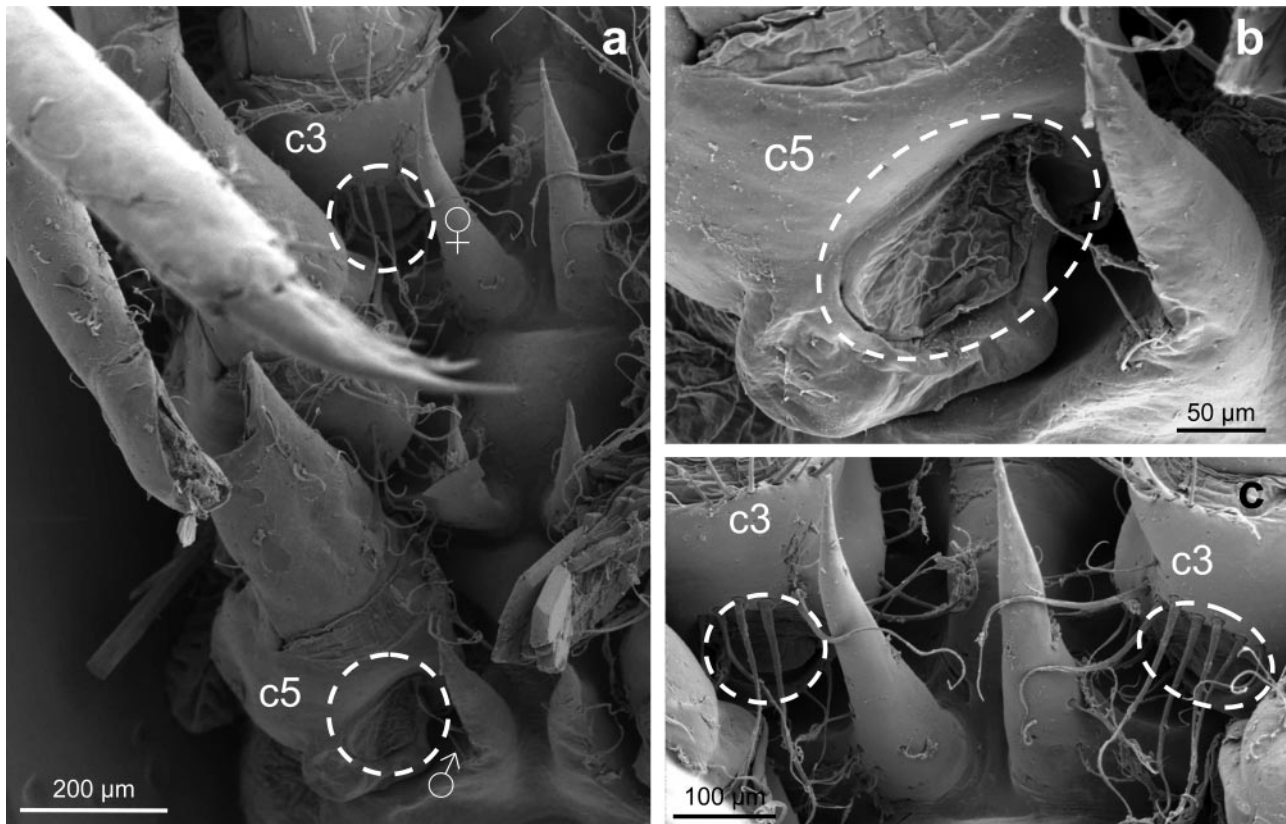
mm (mean  $\pm$  s.d.:  $1.47 \pm 0.31$ ) and between 1.15 and 2.93 mm ( $2.14 \pm 0.38$ ). On average, males were smaller than females (one-way ANOVA;  $F = 103.8$ ,  $df = 1, 116$ ,  $P << 0.0001$ ). Interestingly, the largest individual in our sample was a male (3.0 mm CL) considerably larger than most males in the population (Fig. 4). Dissection of this and the second largest male in our sample (2.5 mm CL) demonstrated the presence of a parasitic isopod (family Entoniscidae) instead of testes on top of the hepatopancreas (Fig. 5). SEM of these two parasitized males demonstrated the presence of female gonopores on the third coxae in addition to the male gonopores on the fifth coxae (as in Fig. 3a–c), suggesting male feminization by the parasite. Dissection of 25 other randomly selected male and female shrimps detected one other male and three other non-brooding females parasitized by the same isopod. The frequency of occurrence for this parasite in males and females was relatively low (11% and 12%, respectively) and did not differ between the sexes (chi-square test,  $P > 0.05$ ). Regardless of the unusually large (parasitized) males in the population, there is marked sexual dimorphism with respect to body size in *T. amboinensis*, as expected for a sequential hermaphrodite (Fig. 4).

A positive correlation between body size of shrimps and the length of the propodus of the cheliped and the length of the pleuron of the second abdominal segment was detected for shrimps of both sexes ( $P < 0.001$  in all cases, Fig. 4). Interestingly, neither the abdominal pleuron nor the cheliped differed with respect to the status and degree of allometry, depending on the sex of the shrimps. In males and females, the cheliped had negative allometry; the slope of the relationship between shrimp body size and cheliped size was significantly lower than 1 (Table 1; Fig. 4b). Also, cheliped relative growth was similar in males and in females ( $P = 0.9807$ ). Similarly, the second abdominal pleuron had negative allometry both in males and in females, and its relative growth did not differ between the sexes ( $P = 0.8105$ ). Nevertheless, this pleuron was wider in females than in males at any given body size ( $F = 280.16$ ,  $df = 1, 113$ ,  $P << 0.001$ ; Fig. 4c; Table 1). The pleuron of transitional shrimp had a width similar to that observed in males of the same body size (Fig. 4c).

**Sex change: Laboratory observations.** In the test to determine whether males change sex to females later in life, the largest three out of the 12 observed individuals shifted sex to females before the 23 days. All three shrimp that matured as females lost the appendices masculinae in the second pair of pleopods and developed white spots at the flange of the second and third pleopods. Two of the three new females were brooding embryos (one had an intermediate- and the other a late-stage embryos). The non-brooding and the two brooding newly developed females had ovaries with



**Figure 2.** Anatomical and morphological differences between males and females of *Thor amboinensis*. (a) Exopod (ex) and endopod (en) of second pleopod in male with appendix interna (ai) and appendix masculina (am, arrow points at appendix masculina). (b) Testes from male. (c) Exopod (ex) and endopod (en) of second pleopod in female (arrow points at appendix interna [ai]). Notice the absence of appendix masculina. (d) Second pleopod in female with attached embryo (em) in advanced stage and spot at the flange (circled). The arrow points at appendix interna (ai). (e) Dorsal view of female after dissection with ovaries (ov) full of vitellogenic oocytes.



**Figure 3.** Scanning electron microscopy (SEM) view of transitional individuals of *Thor amboinensis*. (a) Ventral view of transitional shrimp; male and female gonopores on the coxae of the fifth (c5) and third (c3) pereopods, respectively. The male (♂) and female (♀) gonopores are encircled. (b) Ventral view of transitional shrimp; detail of the male gonopore on coxae of fifth pereopod (encircled). (c) Ventral view of transitional shrimp; detail of the female gonopores on coxae of third pereopods (encircled).

developing green (vitellogenic) oocytes. Two males died before the end of the 23-day observation period.

#### Sex ratio in *Thor amboinensis*

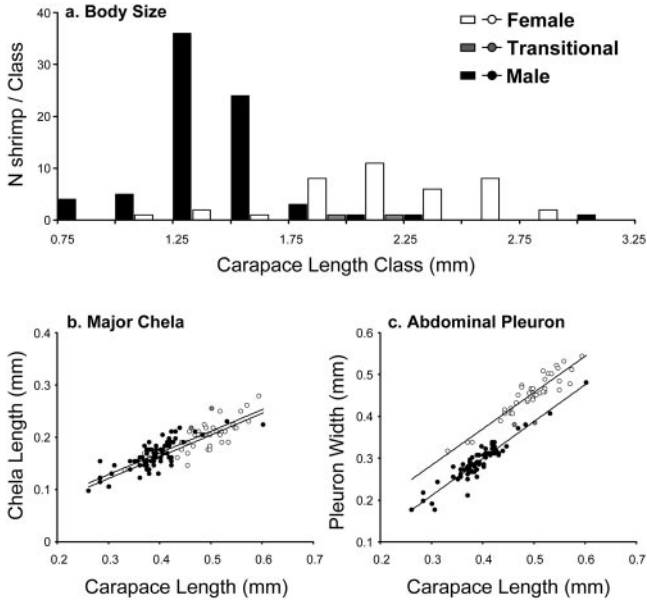
Sex ratio ([males]/[males + females]) deviated significantly from a random distribution of equal numbers of males and females in all but one sampling site (CBC 2008:  $P = 0.0002$ ; CBC 2009: first sampling,  $P < 0.0001$ ; CBC 2009: second sampling,  $P = 0.0063$ ; SWK:  $P = 0.0003$ ; CB:  $P = 0.0076$ ). Only at Okinawa did the observed proportion of males in the population not deviate significantly from the expected binomial frequency ( $P = 0.0593$ ). However, this was explained by the low power of the test determined by the small sample size ( $n = 18$ ). Overall, males dominated the population in proportions of about 0.66 to 0.90 (Fig. 6).

#### Population distribution of *Thor amboinensis*

Occurrence frequency of *T. amboinensis* on host sea anemones was 65.0%, 72.5%, and 67.5% at SWK, CBC, and CB,

respectively. Considering only sea anemones in which shrimp were found, the number of shrimps per host varied between 1 and 5 (mean  $\pm$  s.d. =  $2.58 \pm 1.33$ ,  $n = 26$ ), 1 and 7 ( $2.62 \pm 1.68$ ,  $n = 29$ ), and 1 and 7 ( $2.74 \pm 1.72$ ,  $n = 27$ ) at SWK, CBC, and CB, respectively. The population distribution of *T. amboinensis* on its host sea anemone did not display a random pattern at 2 out of 3 of the study sites (chi-square test of goodness of fit, SWK:  $\chi^2 = 15.52$ ,  $df = 4$ ,  $P = 0.0037$ , CB:  $\chi^2 = 12.21$ ,  $df = 4$ ,  $P = 0.0159$ ) (Fig. 7a, c), and at CBC (Fig. 7b) the  $P$  value for nonrandomness was only marginally non-significant ( $\chi^2 = 9.16$ ,  $df = 4$ ,  $P = 0.0572$ ) (Fig. 7b). Overall, the discrepancy between the observed and expected distribution was explained by the number of hosts harboring no shrimp (greater than expected by chance alone, decomposition of the chi-square test of goodness of fit,  $\chi^2 = 18.37$ ,  $df = 1$ ,  $P < 0.0001$ ), the frequency of hosts with 2 or 3 shrimp (lower than expected,  $\chi^2 = 5.32$ ,  $df = 1$ ,  $P < 0.0211$ ), and the observed number of hosts harboring 4 or more shrimp (greater than expected,  $\chi^2 = 4.86$ ,  $df = 1$ ,  $P < 0.0275$ ) (Fig. 7d). Thus, *T. amboinensis* occurred in moderate or large aggregations on sea anemones.





**Figure 4.** Sexual dimorphism in body size of *Thor amboinensis* shown by the size-frequency distribution of males and females (a) and relative growth of the major chela (b) and pleuron of the second abdominal segment (c) as a function of carapace length. Measurements are in millimeters. Linear regression equation obtained after log-log transformation of the data are shown for each sex in Table 1. Two transitional individuals detected in the sample are shown.

At CBC, no statistically significant relationship was found between sea anemone diameter and shrimp number ( $n = 40$  hosts, Poisson regression;  $P = 0.4799$ ). However, larger hosts harbored larger shrimp aggregations in CB ( $n = 40$  hosts, Poisson regression;  $P < 0.0001$ ) (Fig. 8). The removal of influential data points did not change the results from these analyses at the two sites (5 influential data points detected at CBC and 4 at CB).

The distribution of sexes among groups with different numbers of shrimp was analyzed. Data from the three sampling sites in Belize were pooled because of their similarities in host occupancy and population distribution. In the 23

instances that shrimp were found solitarily on sea anemones, they were all males. When hosts harboring only two shrimps were considered, male-male and male-female pairs were recorded in 8 and 14 out of 22 sea anemones. No female-female pairs were observed. The number of males and females in the sampled population was 157 and 60, respectively. As seen from the binomial distribution, the number of male-male and male-female pairs expected by chance alone in the population would have been slightly larger ( $n = 9$ ) and slightly smaller ( $n = 11$ ), respectively, than those observed, but the deviation was not significant ( $P > 0.05$  in both cases).

In the sea anemones with two or more shrimps ( $n = 59$ ), 2, 46, and 11 of these host individuals harbored shrimp aggregations with a female-biased, male-biased, or equal sex ratio. Taking into consideration the hosts harboring two or more shrimps, aggregations had male-biased sex ratios more frequently than expected by chance alone (46:13 vs. 29.5:29.5, binomial test,  $P < 0.0001$ ). Also, visual examination of the data suggested that small and moderate aggregations (greater than 2 but smaller than 5 individuals per host) have a sex ratio more male-biased than that of hosts harboring relatively larger numbers of shrimps (6 and 7 shrimp host<sup>-1</sup>) (Fig. 9). However, logistic regression indicated no effect of shrimp group size on sex ratio ( $P = 0.215$ ).

*Group structure in Thor amboinensis*

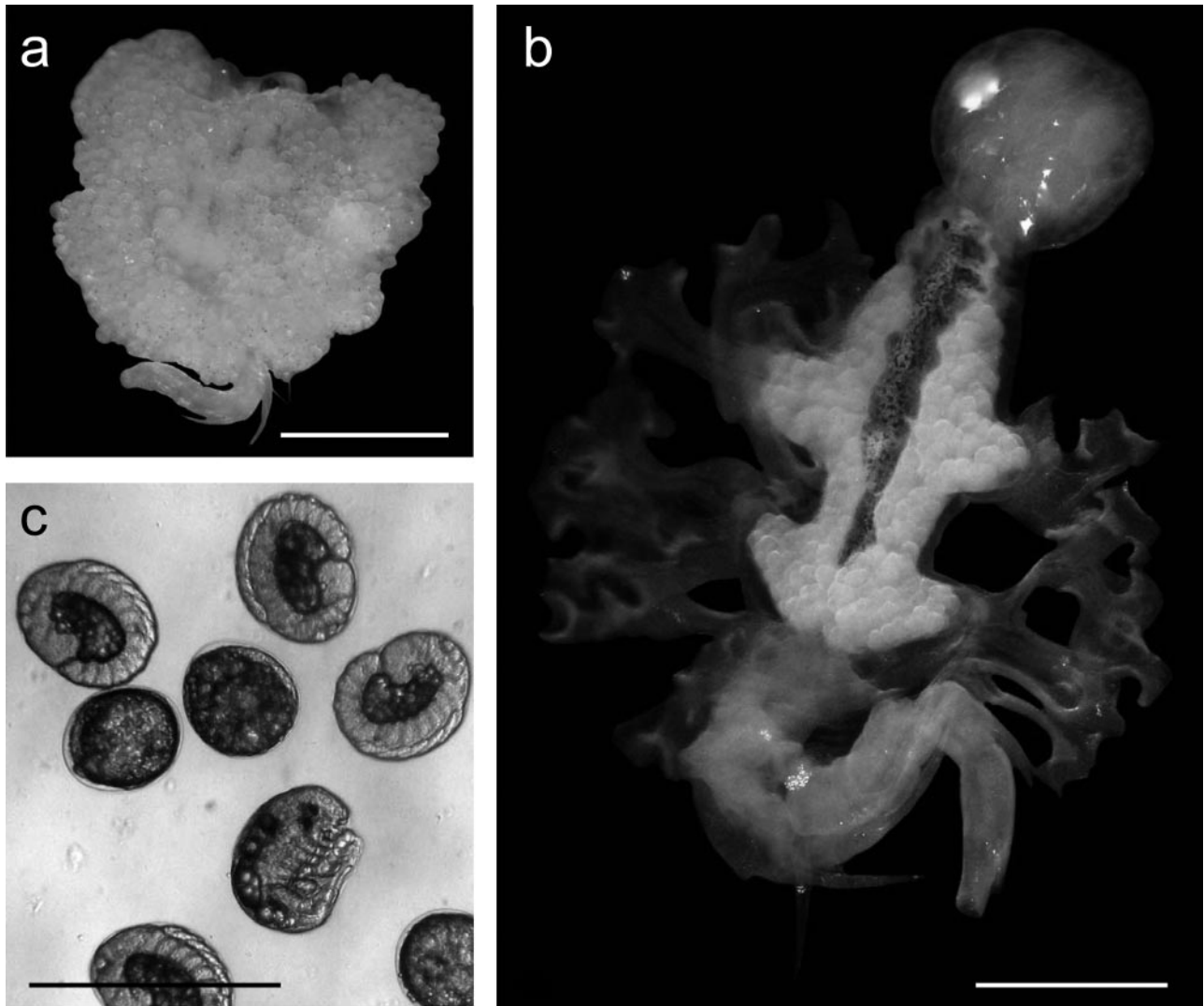
No major differences in population distribution and frequency of occurrence of *T. amboinensis* on host anemones were observed between the first and second samplings at CBC. In the second sampling, the number of shrimp per individual host varied between 1 and 11, with an average ( $\pm$  s.d) of 3.82 ( $\pm$  2.76). Three out of 5 shrimp living solitarily on sea anemones were males. Male-male and male-female pairs were recorded in 2 and 8 out of 10 host individuals harboring only two shrimps. Again, no female-female pairs were observed. In sea

**Table 1**

*Relative growth of selected structures in males and females of Thor amboinensis*

y	x	Regression	$r^2$	$SE_s$	$t_s$	P
<b>Males</b>						
CheL	CL	$y = 0.4146x + 0.004$	0.6133	0.017	15.36	<0.001
AbdL	CL	$y = 0.885x - 0.055$	0.8861	0.016	3.14	0.0025
<b>Females</b>						
CheL	CL	$y = 0.4162x + 0.004$	0.5915	0.018	10.89	<0.001
AbdL	CL	$y = 0.869x - 0.022$	0.8697	0.019	2.39	0.0216

The regression equations, correlation coefficients, and standard errors of the slopes ( $SE_s$ ), of each studied variable are shown. CL, CheL, and AbdL = carapace length, length of the propodus of the major cheliped, and length of the second abdominal pleuron, respectively. Allometry was negative for all structures.

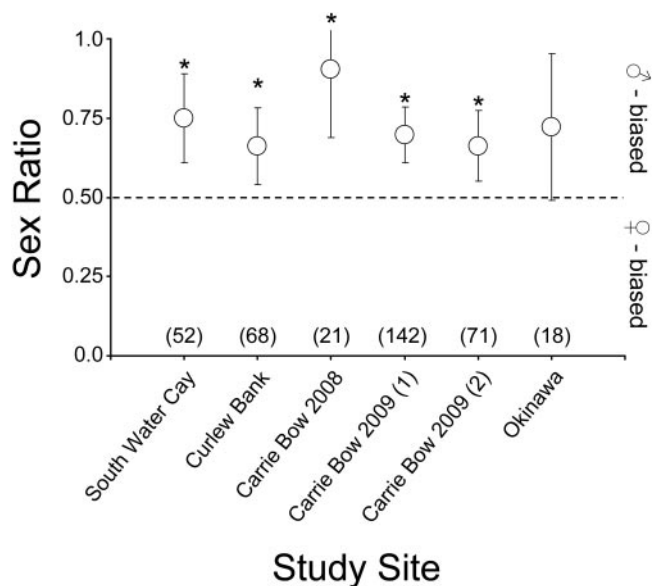


**Figure 5.** Undescribed species of parasitic isopod from the family Entoniscidae. (a) Parasite brooding embryos (scale bar = 1 mm). (b) Parasite general body morphology after embryo extraction (scale bar = 1 mm). (c) Embryos from the same brood showing different stages of development (scale bar = 100  $\mu$ m).

anemones harboring two or more shrimps ( $n = 31$ ), 5, 15, and 11 aggregations had a female-biased, male-biased, or equal sex ratio, respectively. The effect of shrimp group size on sex ratio detected with Poisson regression was barely nonsignificant (considering only hosts with two or more shrimp;  $P = 0.0508$ ).

The examination of the distribution of sizes in each group did not reveal any indication of female or male monopolization by males or females, respectively (Fig. 10). Most commonly, females were the largest shrimp within a group, as expected for a sequential hermaphrodite. A male was the largest individual in only three groups, and in two of these groups that male was parasitized by the entoniscid isopod (see above) (groups #30 and #33 in Fig. 10). When more than one male or female cohabited in the same sea anemone,

the difference in body size between the largest and second largest individual of the same sex was either minimal (males: groups #3, 9, 13, 16, and 17; females: groups #22, 25, and 28 in Fig. 10), moderate or large (males: groups #3, 16, 22, and 25; females: groups #23, 27, and 29 in Fig. 10). The size of the largest male and female within a group was not correlated ( $r^2 = 0.013$ ,  $F = 0.34$ ,  $df = 1,27$ ,  $n = 28$  groups with males and females cohabiting,  $P = 0.5644$ ). However, the size of the largest male and female within a group increased with group size (males:  $F = 25.35$ ,  $df = 1,30$ ,  $n = 31$ ,  $P < 0.0001$ ; females:  $F = 6.18$ ,  $df = 1,29$ ,  $n = 30$ ,  $P = 0.0168$ ). Group size explained 45.8% and 18.9% of the variation in male and female body size, respectively. The information above suggests that shrimp aggregations do not have a complex social structure.



**Figure 6.** Sex ratio (male/total individuals) of the adult population of *Thor amboinensis* at different localities in Belize and in Okinawa, Japan.

**Discussion**

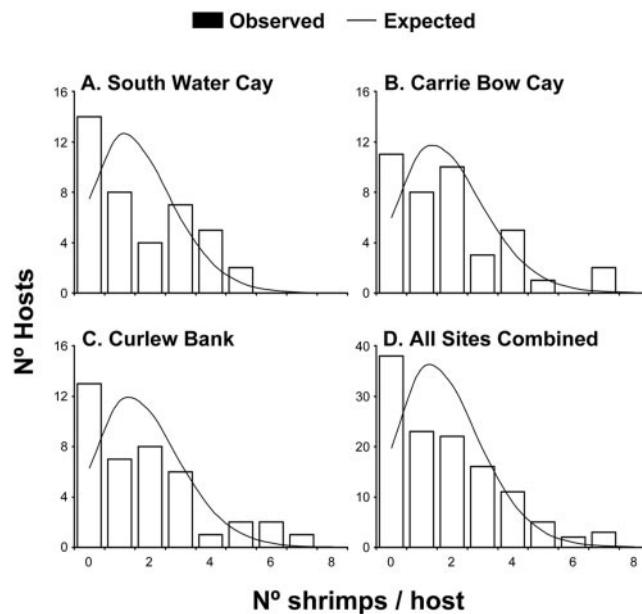
*Sexual system of Thor amboinensis*

In *Thor amboinensis*, the population is composed of males that were, on average, smaller than females. Only two parasitized males that appeared to be feminized reached unusually large body sizes and broke this pattern. The identity of the isopod parasite and its detrimental effect on males and females of the studied shrimp species deserves more attention. The size-frequency distribution of the sexes agreed with expectations for protandric hermaphrodites (Bauer, 2004). Importantly, transitional individuals having characteristics of both males and females (appendices masculinae, ovaries, and spots at the pleopods) were occasionally found in the studied population, and laboratory observations demonstrated that large males can and do undergo sex changes to females in short periods of time (< 23 days). All this information demonstrates that the studied species is a protandric sequential hermaphrodite. Shrimps are born and reproduce as males first, then become “transitional” individuals later in life—apparently after a single molt. In this transitional stage, shrimps lose the appendices masculinae and develop female characters (e.g., white spots on pleopods, maturing ovaries). During the next molt, shrimps turn into females and remain so for the rest of their lives.

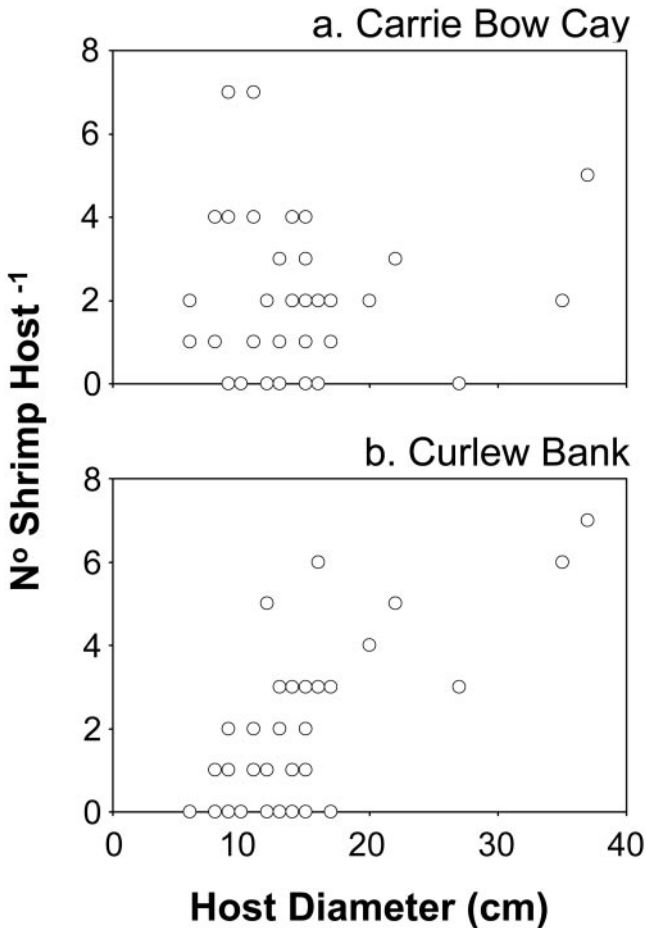
The sexual system of *T. amboinensis* differs from that of other members in the genus. *Thor dobkini* and *T. floridanus* are gonochorist (separate sexes: Bauer and VanHoy, 1996). In the partial protandric hermaphrodite *T. manningi*, the population is composed of 50% primary males (individuals that are born male with prehensile third pereopods and do

not change sex), 49% protandrous hermaphrodites (males that are born without prehensile pereopods and change sex to females later in life), and 1% primary females (shrimps that are born female and remain female) (Bauer, 1986). Given this report of partial protandry within the genus, it would be difficult with our data to differentiate between pure and partial protandry in *T. amboinensis*. However, in view of the relatively high abundance of primary males reported for *T. manningi* (50%), if primary males were part of the studied population, then we should have been able to detect them. Nonetheless, different male morphotypes (with or without prehensile pereopods) were never observed in our samples, and the largest size classes consisted primarily of females (plus some unusually large parasitized males). The absence of different male morphotypes is inconsistent with the notion of partial protandry in *T. amboinensis*.

If the studied population included a very small number of primary females (as in *T. manningi*), then our relatively small sample size ( $n = 145$ ) could lead to primary females going undetected. However, small individuals (CL < 2.00 mm) in our studied populations were almost invariably male, and all of them had prehensile pereopods. Although we observed a few small females in our samples (e.g., 1.15 mm CL), these females were never as small as the smallest males in the population (0.82 mm CL). Such small females might represent males that changed sex early in life to cope with exceptional social conditions such as low availability of females in the surroundings (Baeza, 2007). We believe



**Figure 7.** Population distribution of the shrimp *Thor amboinensis* inhabiting sea anemones in shallow subtidal patch reefs at different localities in the Caribbean. (a) South Water Cay. (b) Carrie Bow Cay. (c) Curlew Bank. (d) All sites combined. Observed frequency of shrimps on hosts differs significantly from the expected Poisson random distribution.



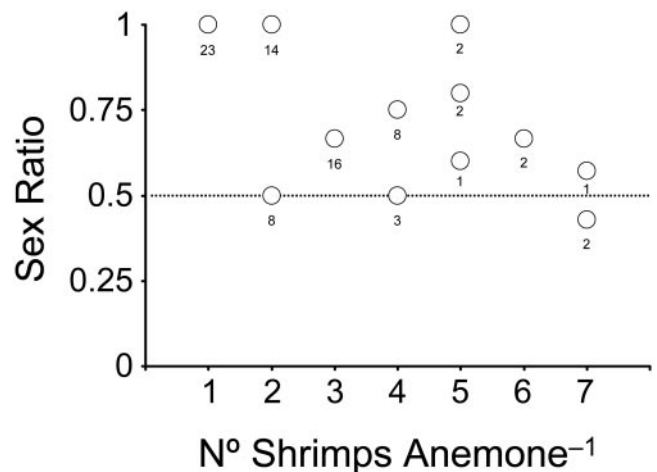
**Figure 8.** Correlation between host diameter (cm) and number of shrimp harbored. (a) Curlew Bank. (b) Carrie Bow Cay.

that the likelihood of partial protandry in *T. amboinensis* is low. Nonetheless, the existence of primary females in *T. amboinensis* cannot be ruled out given our limited data set.

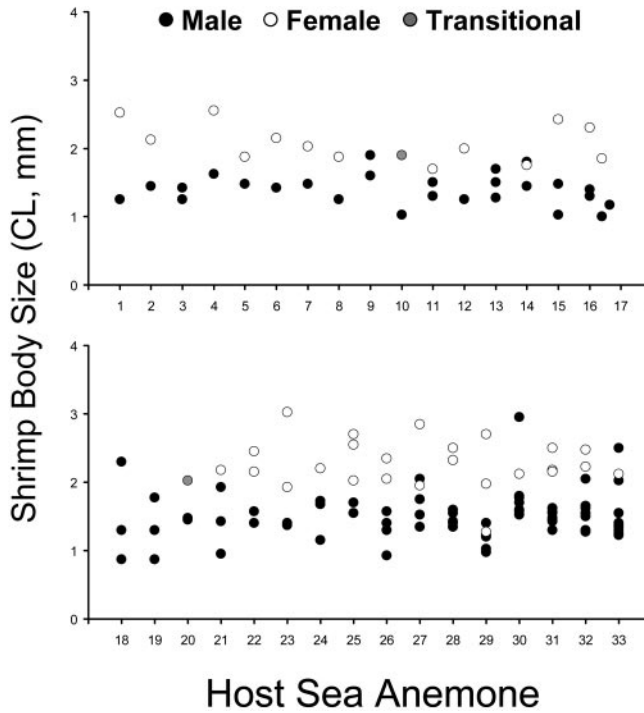
Protandry in *T. amboinensis* is quite different from that in *T. manningi*, in which three morphotypes (primary males, primary females, and sequential hermaphrodites) coexist in the same population. Bauer (1986) hypothesized that protandry in *T. manningi* originated in primary females and not in males, because it will pay (in term of fitness) for primary females to develop male characteristics during their juvenile (non-reproductive) stage. Such primary females, once turned into protandric hermaphrodites, would have increased their lifetime reproductive output compared to that of primary females (Bauer, 1986). We believe that a similar mechanism is responsible for the evolution of protandry in *T. amboinensis*. Also, Bauer (1986) hypothesized that primary males persist in populations of *T. manningi* thanks to an evolutionary innovation—the third prehensile pereopod that gives males an advantage in female insemination over

hermaphroditic males (Bauer, 1986; Bauer and VanHoy, 1996). Nonetheless, the scenario proposed by Bauer (1986) about the origin of this structure no longer seemed plausible after males with prehensile third pereopods were documented in the gonochoric species *T. dobkini* and *T. floridanus* (Bauer and VanHoy, 1996). Assuming that gonochorism is the ancestral condition in *Thor*, the origin of prehensile structures predates the evolution of protandry (Bauer, 1986; Bauer and VanHoy, 1996). We believe that prehensile third pereopods evolved in response to intra-sexual selection (male-male competition) and not inter-sexual selection in an ancestral gonochoric species. This view is supported by the existence of two types of primary males in *T. floridanus*, one without prehensile third pereopods, which might represent male morphs with the primitive genotype for the third pereopods (Bauer and VanHoy, 1996), and another with prehensile third pereopods (Bauer and VanHoy, 1996). In protandric species, such a secondary sexual character would have allowed primary males to persist in a population once it was invaded by sequential hermaphrodites, as proposed later by Bauer and VanHoy (1996).

Overall, the existence of different types of protandry in *Thor* might be the result of (1) two independent evolutionary origins from different ancestral gonochoric species, or (2) a single origin of protandry (as in *T. manningi*) followed by elimination of some morphotypes in more derived species (as in *T. amboinensis*) due to the action of sexual selection (Shuster and Wade, 2003). Further detailed studies on the lifestyle and sexual system of the species in this genus and the development of a molecular phylogeny are necessary to elucidate the fascinating evolutionary history of gender expression in the genus *Thor*.



**Figure 9.** Relationship between sex ratio within groups and group size in *Thor amboinensis*.



**Figure 10.** Carapace length of shrimp within groups for shrimp ( $n \geq 2$ ) inhabiting different sea anemone hosts at Carrie Bow Cay, Belize.

*Sex ratio in Thor amboinensis*

Sex ratio in the protandric hermaphrodite *T. amboinensis* was female-biased in the various studied populations, one of them thousands of kilometers distant from the others. These results agree with one of the main predictions of sex allocation theory developed for sex-changing species, which states that sex ratio in sequential hermaphrodites is biased toward the first sex (Charnov, 1982). In *T. amboinensis*, males dominated the population with proportions of 0.66 to 0.90. This range in sex ratio variation is similar to that reported for other populations of sequential and sequential-simultaneous hermaphrodites (molluscs: Collin, 2006; shrimps: Anker *et al.*, 2006; JAB, unpubl. data).

The prediction of female-biased sex ratios in protandric species rests on the assumption that male and female fertility increases with size and that levels of mortality are low during sexual transition (Charnov and Bull, 1989). Information is lacking to explore these assumptions for *T. amboinensis*. To the best of our knowledge, in females of caridean shrimp, a positive relationship between fecundity and body size has been reported for every species in which these variables have been examined (see Corey and Reid, 1991, and references therein). In turn, male mating ability either increases (*Pandalus latirostris*: Chiba *et al.*, 2003) or decreases (*Lysmata wurdemanni*: Bauer, 2002) with body size. No formal experiments exploring the costs (in terms of mortality) of sex change have been conducted in shrimp.

However, various studies examining the effect of social conditions on sex change of strictly protandric, bidirectional sex changers and protandric simultaneous hermaphrodites have not reported death of individuals immediately before, during, or after change of sex or sex phase (shrimp: Baeza and Bauer, 2004; Baeza, 2007; gastropods: Collin *et al.*, 2005; polychaetes: Berglund, 1986).

*Group living in Thor amboinensis*

*T. amboinensis* inhabited the large and structurally heterogeneous sea anemone *Stichodactyla helianthus* in large groups (up to 11 individuals) more frequently than expected by chance alone. This sea anemone might represent a large and heterogeneous shelter that is too expensive (in terms of energy and time) for monopolization by single or paired shrimps. In agreement with the idea that sun sea anemones are difficult to monopolize, the quotient between host diameter and shrimp length was  $\approx 45$  (calculation based on the average-size anemone [13.3-cm diameter] and the largest shrimp [3.0-mm CL] observed in the field). Although the above ratio is a raw measure of space availability within a host (Baeza and Thiel, 2003), this number clearly suggests that a single large sea anemone should be able to host a considerable number of shrimp at the same time.

It must be highlighted that, together with host characteristics, other processes such as predation pressure might be driving group living in *T. amboinensis*. Mortality risk associated with attacks by predators or parasites is predicted to spread evenly among individuals living in groups (Hamilton, 1971). Predation might be particularly important in *T. amboinensis* because individuals spend considerable time centimeters away from the anemone’s tentacles. During sampling, we observed various fish species both in the Caribbean and Indo-Pacific that might be predators of *T. amboinensis*, including sit-and-wait (*e.g.*, the lionfish *Pterois antennata* in Okinawa) and search-and-catch (*e.g.*, *Hali-choeres* spp. wrasses in both oceans) predators. Interestingly, when perturbed during sampling, shrimp promptly escaped using the “escape response” (backward tail flipping) that propelled them toward the tentacles or near the column of the anemone. This behavior might be interpreted as an indication that predator avoidance in the field is relevant for *T. amboinensis*. Furthermore, although we did not observe any instance of predation on this shrimp at our study sites, Stanton (1977) reported heavy predation by wrasses and the harlequin bass *Serranum tigrinus*. All these fish were frequently observed in Belize. Overall, the information available so far does not allow determining whether or not predation pressure (together with refuge characteristics) is relevant in favoring group living in the studied species.

### Implications for the mating system of *Thor amboinensis*

Our study suggests that host structural complexity might constrain the evolution of territoriality in males of *T. amboinensis*. Male mating strategies strongly depend on the environmental potential for monopolization of females or resources (hosts) to attract females (Wilson, 1975; Shuster and Wade, 2003; Baeza and Thiel, 2007). Thus, given the impossibility of host monopolization, males should attempt to increase mating opportunities by using exploitative rather than interference mating strategies (Bauer, 2004; Baeza and Thiel, 2007). In “pure-search” exploitative strategies, males are continuously searching for females. Once a receptive female is found, there is no evident courtship, insemination takes place rapidly, and males depart immediately after copulation in search of other receptive females (Bauer and Abdalla, 2001). This behavior is expected to favor small body size and reduced or no weapons (*e.g.*, chelipeds) in males because that leads to an increase in agility and encounter rates with receptive females (Shuster and Wade, 2003; Baeza and Thiel, 2007). Supporting the idea of a pure-search mating system in *T. amboinensis*, males were smaller than females on average, and these males did not have well-developed chelipeds or any other structure that could be used to fight for females (*e.g.*, maxillipeds as in *Rhynchocinetes typus*: Correa *et al.*, 2003). Our results agree with the idea that *T. amboinensis* uses a pure-search mating system. Unfortunately, our data do not reveal to us the most important details about the species’ reproductive behavior. Future studies should explore (1) if males switch hosts in search of females, (2) if predation risk impacts male mating strategies (host swapping), (3) if males guard females before and/or after copulation, and (4) if females exhibit passive or active mate choice.

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