

MALE MORPHOTYPES AND MATING BEHAVIOR OF THE DANCING SHRIMP *RHYNCHOCINETES BRUCEI* (DECAPODA: CARIDEA)

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ABSTRACT

In crustaceans, the presence of large males with highly developed prehensile appendages (chelipeds or gnathopods) generally is suggestive of female monopolization during her receptive period. While mate guarding is common among some malacostracan crustaceans (brachyuran crabs and some amphipod families) it is relatively rare in caridean shrimp. Here we explored sexual dimorphism and the presence of morphotypic differences among males of the dancing shrimp *Rhynchocinetes brucei*. We furthermore quantified the behavioral events during mating interactions to examine whether mate guarding extends over the entire period of female receptivity. Males and females had similar body sizes, but males developed increasingly larger third maxillipeds and first chelipeds during ontogeny. Large males with hyperdeveloped maxillipeds and very large chelipeds featured a high degree of broken appendages and eyes, which probably results from intrasexual agonistic interactions. About 30% of the non-competitive male-female interactions with post-ovigerous females resulted in successful matings. Males usually initiated body contact with the female shortly after the female's parturial molt, and they frequently touched the female's genital region with their anterior body parts. The first (and in most cases only) spermatophore transfer event occurred at variable times (0.2-7 h) after the female's molt. Following spermatophore transfer some males guarded the female in the cage state (between their pereopods) until the female had finished spawning and was ovigerous. Post-copulatory mate guarding could last up to 70 min, but not all males guarded the female after the copulation. We suggest that mate guarding of the receptive female throughout the mating process reduces the risk of sperm competition.

KEY WORDS: dimorphism, mating behavior, mate guarding, morphotypes, *Rhynchocinetes brucei*, sexual selection

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INTRODUCTION

Defense of females, mating sites, or other resources is commonly found in brachyuran crabs (Christy, 1987) but also in some peracarid amphipods and isopods (Conlan, 1991; Jormalainen, 1998). However, in caridean shrimp mating interactions are usually very brief (Bauer, 1976, 1979; Felgenhauer and Abele, 1982; Boddeke et al., 1991), and extensive mate guarding has previously only been reported for *Macrobrachium* spp. (Choudhoury, 1971; Chow et al., 1982; Ra'anand and Sagi, 1985; Karplus et al., 2000) and *Rhynchocinetes typus* Milne Edwards, 1837 (Correa et al., 2000, 2003). In these latter species, dominant males guard females before and after copulation, preventing smaller males to access the female during her receptive period. The dominant males that engage in female mate guarding are typically characterized by one pair of strongly developed chelipeds (Kuris et al., 1987; Sagi and Ra'anand, 1988; Correa et al., 2000). These chelipeds are employed in intrasexual fights during which dominants displace subordinate males that attempt to mate with the receptive female (Correa et al., 2003). Dominant males are also often (but not always) larger than subordinate males and females, which further enhances their resource holding potential. The presence of (large) male morphotypes with strongly developed chelae in a species would thus suggest that they guard and defend females during mating interactions.

As shown experimentally for *Macrobrachium rosenbergii* De Man, 1879 and *R. typus*, guarding of receptive females by large males is a mechanism to reduce the chances that other males attach spermatophores to the female's body (Ra'anand and Sagi, 1985; Correa et al., 2003). Spermatophores attached to the female may remain intact for relatively long time periods (up to several hours), and thus the only efficient way for males to reduce the risk of sperm competition is to prevent other males from accessing the female during her receptive period. Consequently, it is expected that a large male starts guarding the female when she first becomes receptive and continues guarding until most oöcytes are fertilized.

Females of most caridean shrimp become receptive shortly (within seconds to minutes) after the parturial molt (Bauer, 2004). Thus, sperm from any spermatophore attached to the female after the molt might become available for fertilization. Whether or not sperm are still present at the moment of fertilization depends, besides spermatophore characteristics, primarily on the time interval between spermatophore attachment and spawning. While previous studies described the mating behaviors and the male-female interactions during spermatophore transfer, relatively little is known about the occurrence of events thereafter, i.e., during the time period that is preceding fertilization. The above considerations suggest a strong synchronization between mate guarding and female spawn-

ing. Quantitative mating observations for *R. typus* indicated that males guard females until shortly before spawning ends (Correa et al., 2000).

Herein we used the dancing shrimp *Rhynchocinetes brucei* Okuno, 1994 to examine the relationship between mate guarding and female receptivity. In the original description of *R. brucei*, Okuno provided information that suggested the existence of a dominant male morphotype with strongly developed chelipeds, similar to *R. typus* and many species of *Macrobrachium*. However, no information is available on different male morphotypes, sexual dimorphism, and the mating behavior in *R. brucei*. The objectives of this study thus were to: 1) confirm the presence of sexual dimorphism and different male morphotypes, 2) describe the mating behavior, and 3) examine whether mate guarding occurs.

MATERIALS AND METHODS

Collection and Maintenance of Shrimp for Experiments

Dancing shrimp, *R. brucei*, are common in subtidal waters of the Philippines, Hong Kong and the Great Barrier Reef (~ 5-30 m depth) (Okuno, 1994). All shrimp used in the present study were collected during the period July through September 2009 in Hong Kong subtidal shallow waters at Ninepin Group, Trio and Waglan Islands (22°15'N, 114°21'E; 22°11'N, 114°18'E and 22°18'N, 114°19'E, respectively). The areas sampled were on gently sloped sandy bottoms with large boulders, mainly covered with encrusting coralline algae and occasional patches of corals at a depth of 7-10 m. Preliminary surveys indicated that during daytime, shrimp frequently hide in crevices, under the sea anemone *Entacmaea quadricolor* or among the spines of the sea urchin *Diadema setosum*. Therefore, divers focused on this habitat to catch shrimp by gently driving individuals into hand-held transparent plastic bags (Ziploc). On the boat the shrimp were immediately placed in a large plastic cooler with water from the sampling site, and thereafter transferred to flowing seawater tanks in the laboratory where they were maintained with ad libitum food supply, e.g., crushed mussels, barnacles and sea urchins.

Ovigerous females captured in the field were kept without males and monitored twice daily (~ 8:00 and ~ 20:00 each day) to identify the time (day or night) when a female had released its larvae. These post-ovigerous females were then used for the mating experiment with males that had been maintained in tanks without access to females. The water temperature in the laboratory fluctuated between 27°C and 29°C from August to September.

Morphometric Measurements of Shrimp

To determine whether males feature distinct traits and different ontogenetic stages, as previously reported for *R. typus* (Correa et al., 2000), we conducted morphometric measurements of 70 shrimp, excluding those poorly preserved or with broken body parts. Thirty-three of the measured shrimp (from Trio Island) accidentally had died shortly after capture due to extraordinarily high water temperatures (> 30°C) in the seawater laboratory; 11 individuals died from various causes after being held for certain time periods (days to weeks) in the laboratory. Due to the rarity of males among those 44 dead shrimp, we additionally took morphometric measurements of 26 live males used in the mating experiments.

For the measurements, pictures of the shrimp were taken with a digital camera mounted on a Leica dissecting microscope and traits were measured with the software *ImageJ* (<http://rsb.info.nih.gov/ij/>, last accessed 13 March 2010). We measured the carapace length (CL; measured from the ocular lobe to the posterior edge of the carapace), the length of the last article of the third maxilliped (ML), the maximum length of the propodus on the first cheliped (PL), the carpus height of the first cheliped (CH) and the length of the spine on the carpus of the first cheliped (SL) (Fig. 1A, B). We performed principal component analysis (PCA) using *PRIMER 6* to detect morphological characters that are suitable for distinguishing males of different ontogenetic stages and identifying individuals with similar morphological characters. We standardized ML, PL, and CH with CL, and SL with CH, because these measures were

correlated. Input variables were square-root transformed and normalized before performing PCA.

To determine the size distribution of female and male *R. brucei*, we analyzed the CL of 108 individuals, including the shrimp used for the morphometric analysis (70, see above), females used in the mating experiments (24), females that became ovigerous outside of mating experiments (10), and shrimp that had the CL measured but were excluded from morphometric analyses due to broken body parts (4).

Mating Experiments

All mating experiments were conducted in a non-competitive environment to avoid interference between the mating male and secondary males. To ensure sexual maturity of males, only individuals with CL > 5 mm were chosen for the mating experiments. For each replicate we recorded the male ontogenetic stage and male and female CL. Preliminary observations had suggested that mating usually happened shortly after female molting. Therefore, post-ovigerous females (5.4-8.3 mm CL) were isolated immediately after having released their larvae (before the parturial molt). Each post-ovigerous female was then placed with a male (6.5-8.6 mm CL) in a small tank (13 cm × 23 cm or 8 cm × 15 cm) held at room temperature (23°C-27°C). The standing seawater in the observation tanks was changed every 24 h.

We used indoor video filming for the mating observations. Lighting was provided with overhead white fluorescent lights from 6:00 to 20:00, while red light was used for night filming. Video recordings were ended if the female did not molt and/or mate within 48 h. Observations were extended for another 24 h when females molted on the second day, i.e., adding to a total of 72 observation hours. At the end of mating observations, all females that became ovigerous were isolated in individual containers with flowing seawater and monitored daily to determine the duration of embryo incubation.

We classified the mating interactions as successful matings, failed-brood matings, no-brood matings, and no-molt interactions. In the successful matings, the female carried eggs at the end of the recording period and released larvae after the embryo incubation period. In the failed-brood matings, the female was ovigerous at the end of the recording period, but aborted the clutch within 1-3 days after first being identified as ovigerous. In the no-brood interactions, the female mated but was not ovigerous at the end of the recording period. In the no-molt interactions, the female did neither molt nor mate. Observed matings involved all three ontogenetic stages of male *R. brucei*. We show all successful and failed-brood matings but analyzed only the mating behaviors of the successful matings in detail (see below).

A first rapid video analysis revealed that spermatophore transfer events (STE) often happened within 60 min after female molting. Therefore, according to these two key events (molt and STE), we divided the mating periods into 3 phases: pre-molt, molt-to-STE, and post-STE. During these phases intensive observations were conducted and all behaviors were quantified. The pre-molt phase was defined as the 60 min before the female's molt, the phase "molt-to-STE" was the time interval between the molt and the STE, and for the interval "post-STE" we considered 180 min after the STE. In the case of multiple STEs, the "molt-to-STE" phase was delimited by the first STE; the "post-STE" phase began after the last STE.

In the successful matings, the duration of various types of contact positions between mating pairs were recorded from each video sequence. We also recorded the exact moment of female molting, STE, and when the female could first be recognized as ovigerous (FOV), in order to calculate the interval STE-FOV. The egg mass under the pleon of ovigerous females could be clearly identified as a dark mass (Fig. 1C), which was in stark contrast to the other semi-transparent body parts. Often the female pleon was covered by the male during the mating interactions – in these cases we took the first moment when the male moved away from the female and "uncovered" the pleon with the egg mass as the moment when she was first ovigerous (FOV). Our estimates are thus conservative and the interval STE-FOV might actually have been shorter.

RESULTS

Male Morphotypes and Sexual Dimorphism

Three male morphotypes can be distinguished in *R. brucei* (Fig. 1D-F), which are herein termed *typus*, *intermedius*,

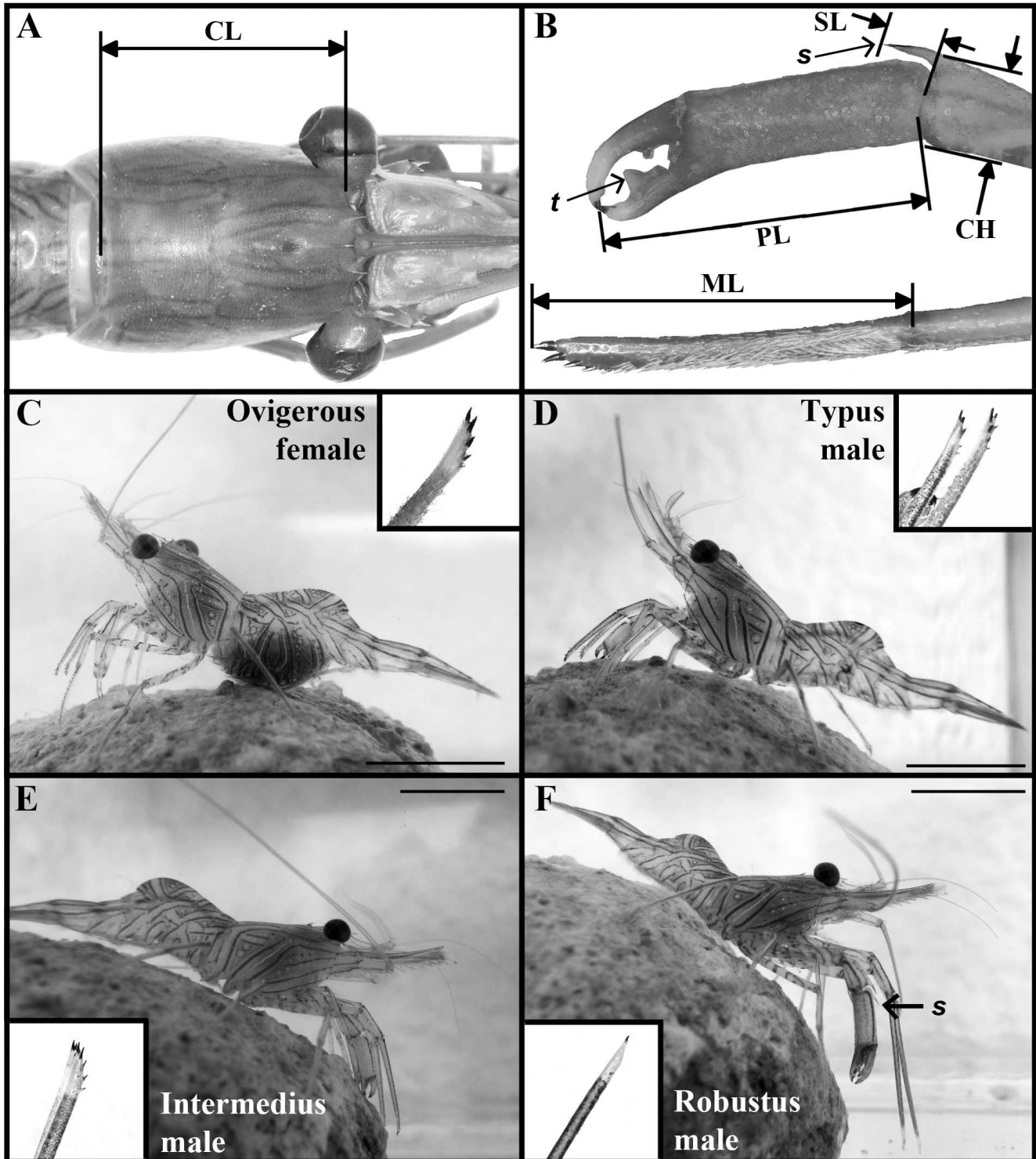


Fig. 1. Photographs of *Rhynchocinetes brucei*. A, carapace; B, cheliped, and maxilliped; C-F, female and the three ontogenetic male stages. In (A) and (B), morphometric measurements are shown; CL: carapace length, ML: length of the last article of the third maxilliped, PL: maximum length of the propodus on the first cheliped, CH: height of the carpus on the first cheliped, and SL: length of the spine on the carpus of the first cheliped. In (C-F), insert images on the full-body pictures show the tip of the third maxilliped; females, typus and intermedius males have 5-9 spines whereas robustus males have only one spine (note that the tip is a three-dimensional structure and thus some spines might not be visible on the images). In (B) and (F), "s" indicates the spine at the distal edge of the carpus of the first cheliped; "t" indicates the prominent tooth in the propodus extension that forms the chela together with the dactylus. Scale bars in (C-F) represent 10 mm.

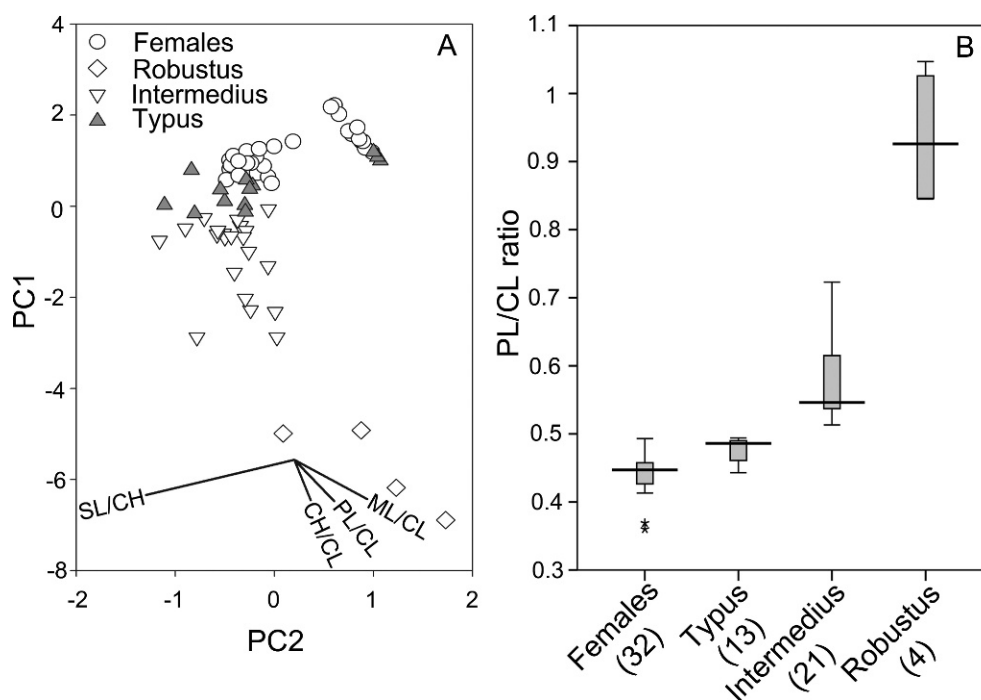


Fig. 2. A, principal component analysis of morphometric measurements of *Rhynchocinetes brucei*. Composition of the principal components (PC1 and PC2) was overlaid on the figure. Assignment of male ontogenetic stages was based on PL/CL ratio (length of the propodus of the first cheliped to carapace length). B, distribution of PL/CL ratio in the three ontogenetic male stages of *R. brucei*. The boundaries of the boxes represent 25 and 75 percentiles; the line passing through the box is the median. Whiskers above and below the boxes are maximum and minimum values within 1.5 times the interquartile range. Asterisks are outliers beyond 1.5 times the interquartile range.

and robustus, following the nomenclature introduced for the male morphotypes of *R. typus* (Correa et al., 2000). Males in the final ontogenetic stage, termed robustus, are most easily recognized. They have elongated third maxillipeds with one pointed tip and one large spine on the carpus of the first cheliped (Fig. 1F). In contrast, the other two ontogenetic male stages, typus and intermedius, are characterized by 6-9 dark spines on the tips of the maxillipeds and the lack of a pronounced spine on the carpus of the first cheliped (Fig. 1D, E). The chelipeds of the robustus males are very large, but they have no particular setation fields as found in the cheliped of *R. typus* (Correa et al., 2000). Robustus males of *R. brucei* furthermore have a prominent tooth in the propodus extension that forms the chela together with the dactylus (Fig. 1B).

The PCA allowed distinguishing the three morphotypes (Fig. 2A). The ratios ML/CL, PL/CL, and CH/CL were similar in eigenvector directions, thus performed similarly (Fig. 2A). Since the PL/CL ratio can be easily measured, we used it here to differentiate the three ontogenetic male stages of *R. brucei*. In typus males, the PL/CL ratio was < 0.5 , in intermedius males it was 0.5-0.8, and in robustus males > 0.8 (Fig. 2B). Adult females had a similar PL/CL ratio as typus males, ranging from 0.3 to 0.5. Females were of similar sizes as males, but the robustus males were among the largest males (Fig. 3). The average CL of females was 7.09 ± 1.26 (mean \pm SD) mm, typus males had 7.16 ± 0.75 , intermedius males 7.84 ± 0.50 , and robustus males 8.16 ± 0.79 .

We observed various types of body damage, epibionts, and parasites in *R. brucei*. On one robustus, the propodus had a punchmark, which was probably caused by the chela grip of a contender. Of the six robustus males collected, only one was physically intact; all others (83%) had one or more appendages damaged or eye broken (2 of the robustus males had at least one maxilliped broken, 1 robustus had both maxillipeds and both eyes broken, and two of them had at least one eye broken). The eyes were completely broken and those individuals were effectively blind on the broken eyes. In the robustus males, the maxillipeds were broken substantially, suggesting that they could not anymore be used efficiently as weapons during escalated fights. Among all the other analyzed shrimp (21 intermedius, 13 typus males, and 32 females), no intermedius male and only one typus male (8%) and one female (3%) had one eye broken each.

One male in the robustus stage had epibionts (serpulid polychaetes) growing on the cheliped and eye, suggesting that this is the terminal molt stage. However, the robustus male shown in Fig. 1F, which clearly had only one spine on the last article of the third maxilliped, molted later in the laboratory. Two shrimp (a female and an intermedius male) had bopyrid isopods under the carapace.

Incubation Period and Mating Success

All ovigerous females released their larvae overnight ($N = 45$), i.e., they were first found without embryos during the morning surveys at 8:00 A.M. Most of the females that

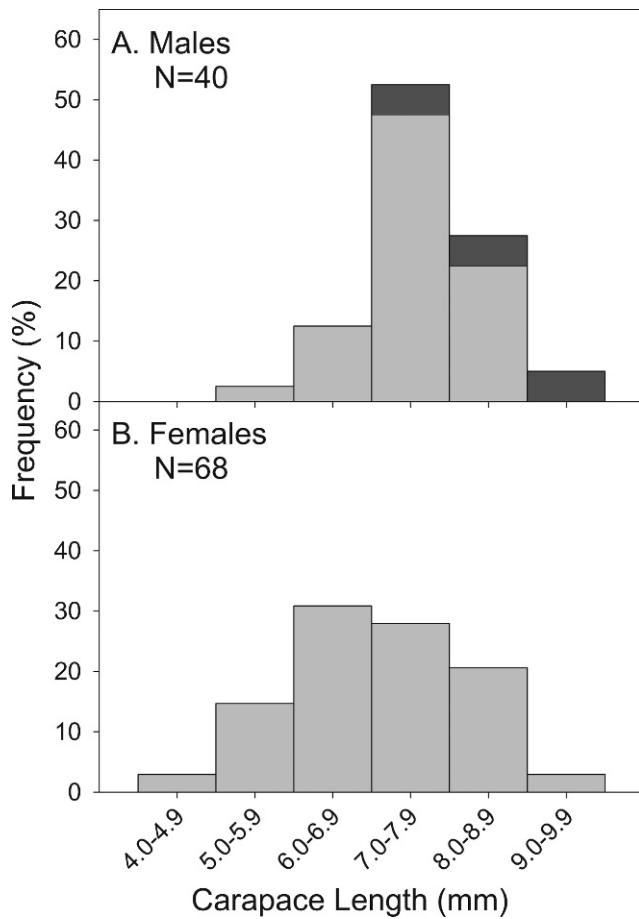


Fig. 3. Frequency of the carapace length of male (A) and female (B) *Rhynchocinetes brucei* collected at three subtidal sites in Hong Kong between July and September 2009; dark columns in (A) represent robustus males.

became ovigerous in the laboratory (> 80%) incubated their embryos for a full 9 days and released them on day 10 (Fig. 4). Also, the last females from the field were still ovigerous after 9 days and released their embryos on day 10 (Fig. 4). There was no evidence that embryo release by field-collected females was clustered in time, and every day some of these field females released their larvae (Fig. 4). Female molting in the laboratory occurred asynchronously, but mostly from late afternoon to dawn, throughout the study period.

The mating success in the 19 non-competitive mating interactions could be classified according to female reproductive status (ovigerous or non-ovigerous) at the end of the observation period and the subsequent incubation time (< 3 or 9-10 days) of the ovigerous females. There were five successful matings (one robustus, three intermedius, and one typus, Table 1A), two failed-brood matings (one intermedius and one typus, see * in Table 1B), one no-brood mating, and 10 no-molt interactions (seven intermedius and 3 typus). In one additional case, the ovigerous female died before completing 3 days of incubation, but the mating interaction was inferred to be successful because the mating behaviors and STE duration were similar to those in successful matings (see # in

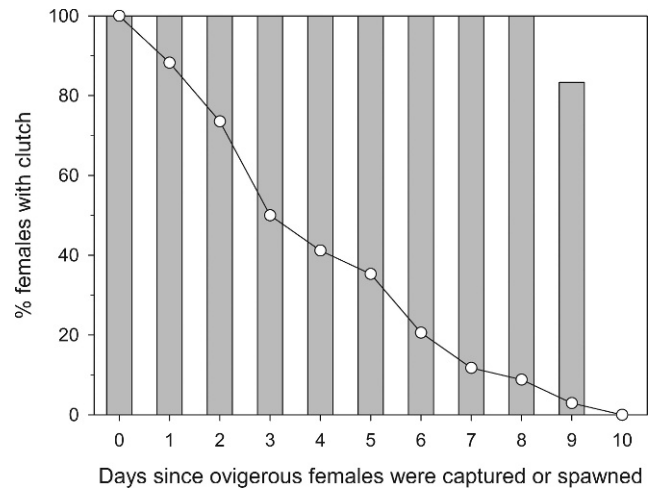


Fig. 4. Duration of the brooding period of ovigerous females *Rhynchocinetes brucei* collected in the field (open dots, 34 individuals) and females that became ovigerous after successful matings in the laboratory (columns, 11 individuals, including females from mating experiments and females that became ovigerous after the video recording period).

Table 1B). In the no-brood interaction, the female molted during the 48h observation period; the male performed an STE, but that female never released eggs, and thus did not become ovigerous. The intermedius male involved in this no-brood interaction had the bopyrid isopod, which are known as parasitic castrators (Calado et al., 2008). A detailed quantitative analysis was only conducted for the five successful matings, but for comparative reasons we also show the behavior of the two failed-brood and the inferred successful matings (see below).

Contact Postures During Mating Interactions

Three main types of contact postures were observed during the mating interactions: overlap, touching, and cage positions (Fig. 5). During overlap, mating partners mutually overlapped each other with some parts of their bodies, but not their heads. In most overlap postures, the males were above the females, covering the female with either the thorax or pleon (Fig. 5B). During touching, the male frequently faced towards the female touching the female's various body regions with its head and mouth appendages (Fig. 5C). In the five successful matings, the female body region mostly touched by the male was the anterior pleon (Fig. 5C). During cage, the male positioned himself directly above the female, with both individuals facing in the same direction, and the females being guarded between the pereopods of the male. In most cage positions, the male positioned his head in contact with the female's thorax and occasionally he vigorously tapped the female's thorax with his thoracic appendages.

Key Events and Phases During Mating Interactions

Mating interactions occurred during both day light and night hours (Table 1). The phase "molt-to-STE" varied greatly in length between the mating pairs, ranging from 5 to 401 min. However, in 3 out of 5 successful matings, the

Table 1. Timing of the four main events (female molting, spermatophore transfers, female spawning, and larvae release) and carapace length (CL) and proportion (m/f CL) of mating pairs *Rhynchocinetes brucei* in the laboratory. In addition to (A) successful matings, (B) data of one inferred successful mating (#), and two failed-brood matings (*) are also mentioned. STE – Spermatophore Transfer Event, FOV – moment when female was first identified as fully ovigerous, NA – data not available.

Males	Date	Molt (time)	STE (time)	Molt-to-STE (min)	STE-FOV (min)	Day larval release	male CL	female CL	m/f CL
A. Successful matings									
Robustus	29-Aug	20:16	20:42	26	45	8-Sep	7.18	6.41	1.12
Intermedius	25-Aug	1:45	2:37	52	60	3-Sep	7.69	7.06	1.09
Intermedius	26-Aug	4:57	5:02	5	70	4-Sep	6.54	6.37	1.03
Intermedius	7-Sep	5:19	12:00	401	52	17-Sep	8.14	5.80	1.40
Typus	24-Aug	17:48	20:08	140	21	3-Sep	7.35	6.22	1.18
Average (\pm SD)				125 (163)	50 (19)				
B. Other matings									
Intermedius (#)	26-Aug	3:02	3:38	35	44	NA	7.72	6.87	1.12
Intermedius (*)	6-Sep	4:19	13:29	550	16	NA	8.65	6.48	1.34
Typus (*)	2-Sep	8:38	13:10	272	11	NA	7.43	7.17	1.04

STE occurred < 60 min after the female had molted (Table 1). During the last phase, post-STE, the female became ovigerous (FOV) within 21-70 min after the STE (Table 1).

The STEs were similar in all successful matings, and lasted only one second or less. In two matings, we observed two STEs; in the remaining three matings, only one STE occurred. During the STE the male positioned itself at an angle ($\sim 45^\circ$) above the female, and then bent its pleon underneath the female's body so that their genital regions were in contact for a short moment, during which the spermatophore was transferred. The STE was ended either

by the male retracting its pleon from underneath the female (4 out of the 7 STE observed) or by the female escaping by abdominal flexing.

In most of the mating observations, we could not observe the initiation of the spawning process and the progression of egg deposition because the male covered the pleon of the female. In these cases the female could be first identified as ovigerous (FOV) once the pair ended the cage position (see below) (Table 1). One female was not concealed by the male and we directly observed the entire process of egg mass deposition from the gonads to the pleon, which took ~ 15 min.

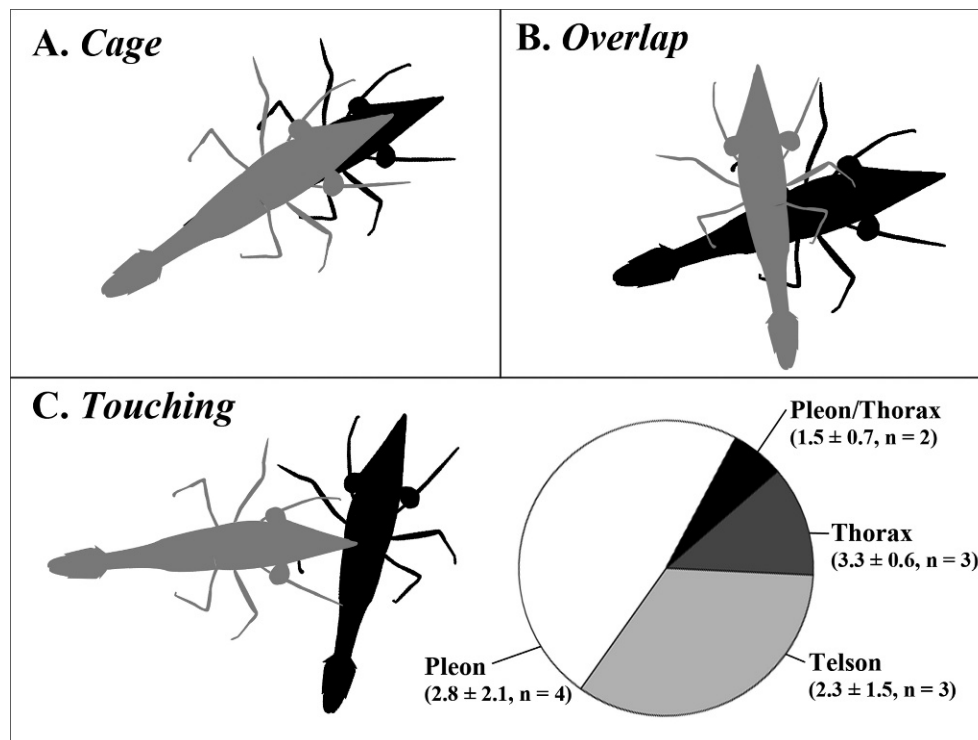


Fig. 5. Diagram of the positions overlap, touching and cage observed during the pair mating of *Rhynchocinetes brucei* in laboratory. Black and gray shrimp represent the female and the male, respectively. Pie-chart shows the frequency percent duration of time the males spent touching of the different female body parts contacted by the male during the touching position of the five successful matings; numbers show the average number of contacts (mean \pm standard deviation; n of the males touching the respective body parts).

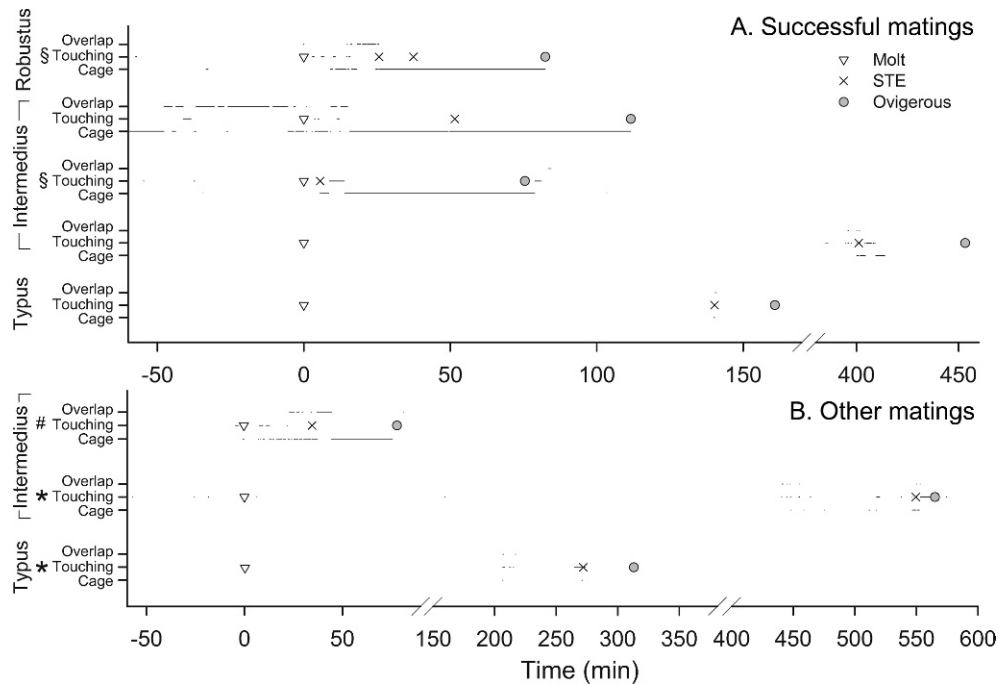


Fig. 6. Ethogram of the behavioral positions overlap, touching and cage exhibited by (A) successful mating pairs, and (B) other mating pairs of *Rhynchocinetes brucei* in laboratory. In (A), successful matings with two STEs are indicated with (\$); the interval between the two STEs with the intermedius male was too short to be distinguished. In (B), other matings, including one inferred successful mating (#), and two failed-brood matings (*) of an intermedius male and a typus male, respectively. The occurrence of the events molt, spermatophore transfer (STE), and female first ovigerous (FOV) are indicated.

General Sequence of Mating Interactions

The occurrence of the different body postures (touching, overlap, cage) during the mating interactions was highly variable (Fig. 6). During the pre-molt phase, we observed a few short-term touching positions, mainly targeting the female's thorax and pleon, and some very brief cage postures in 3 of the 5 matings while no contact occurred in the other two matings (Fig. 6A). During the "molt-to-STE" phase, the proportion of contacts increased, especially the cage posture, which preceded most STEs. In the two matings where two STEs occurred, there was no contact or only the overlap posture before the first STE; both males started the cage position after the first STE and continued until the second STE (Fig. 6A).

After the STE, we observed a strong contrast in the mating behavior of the five pairs. Three of the five males immediately returned to guard the female in the cage position and kept that position (with occasional breaks or short periods of overlap) during almost the entire interval STE-FOV (range: 99.9-100.0%); the contact ended by males moving away from the females. In the other two pairs, the males performed cage posture for only 0 and 8% of the interval STE-FOV. However, these two males remained within one body length of the female for variable time periods after the STE (the typus male for 90% and the intermedius for 2% of the interval STE-FOV).

The mating behavior of the inferred successful mating (Fig. 6B) was also similar to the matings with mate guarding. In the two failed-brood matings (where females aborted the eggs within 3 days after the mating interactions), the males performed a prolonged STE, which lasted

5 seconds and 14 seconds for the typus and intermedius, respectively (Fig. 6B). Compared to the successful matings, in these failed-brood matings the males had less contact with the females, longer "molt-to-STE" phases, more frequent overlap postures, and less frequent cage postures.

DISCUSSION

Sexual Dimorphism and Male Morphotypes in *Rhynchocinetes brucei*

Our results confirmed the presence of a more robust (and possibly dominant) male morphotype in *R. brucei*. These males with particularly long maxillipeds and chelipeds were also relatively large compared to the other males (Fig. 3). However, they were of similar sizes as the largest females, suggesting that there is no or only a minor sexual size dimorphism in *R. brucei*. This is in contrast to *Macrobrachium* spp. and *R. typus* where the large (and dominant) males are usually substantially larger than females (Nagamine and Knight, 1980; Kuris et al., 1987; Correa and Thiel, 2003; Mariappan and Balasundaram, 2004).

The large first cheliped and the long third maxilliped of the robustus male suggest a strong intrasexual selection among male *R. brucei*, which is usually accompanied by a selective pressure towards large male size. The high incidence of body damage observed in the robustus males of *R. brucei* supports the suggestion that these males aggressively fight for access to receptive females. We also observed one fight between two robustus males, in which

the loser was found dead a day later with a broken eye and broken tips on both maxillipeds.

Why did sexual selection result in the evolution of large chelipeds and maxillipeds in male *R. brucei* but not in large male body size? Possibly sexual selection is opposed by viability selection, suppressing the evolution of large male body size (see Wikelski and Trillmich, 1997; Blanckenhorn, 2000). In many crustaceans (Wellborn, 1994) and in other aquatic species (Quinn et al., 2001; Johnson and Zúñiga-Vega, 2009), strong predation pressure selects against large male body size. Predatory fish are common in the environment of *R. brucei* (S. Chak and C. P. Dumont, personal observations) but future studies need to show whether predation or other factors, e.g., female preferences, influence body size selection in this shrimp species.

Mating Behavior in *Rhynchocinetes brucei*

Male *R. brucei* often contacted the female shortly before and after her parturial molt, as seen in other shrimp species (Bauer and Abdallah, 2001). Especially after the molt some males touched the females in their posterior thoracic region, which probably is part of the courtship behavior of *R. brucei*. Male attention to the posterior ventral region of the female's thorax has also been reported for several species of *Macrobrachium* (Ling, 1969; Chow et al., 1982; Ching and Velez, 1985), another freshwater shrimp (Felgenhauer and Abele, 1982), and for penaeid shrimp (Misamore and Browdy, 1996). This touching behavior could be interpreted as inspection behavior, i.e., to identify the reproductive status of the female. However, it might also be analogous to male *R. typus* rubbing the female's gonopore region with their fourth pereopods, which was interpreted as stimulatory behavior (Correa et al., 2000). We suggest that males touching the ventral parts of the female might either test her receptivity status or stimulate the female to initiate ovulation, or both. These behaviors, clearly identified as courtship, vary in duration between species. During this phase, the males attending the females usually also chase away other males (Choudhoury, 1971; Ching and Velez, 1985; Karplus et al., 2000), which will also reduce the risk of sperm competition.

Most males of *R. brucei* only performed a single spermatophore transfer event (STE) during the copula. In two matings we observed two STEs, but we cannot ascertain that a spermatophore was transferred during each event. Regardless, the number of STEs is substantially lower than in the congener *R. typus*, where all males transfer several spermatophores, both in non-competitive and in competitive situations (Correa et al., 2000, 2003). In most other shrimp species, individual males usually transfer only one single spermatophore to the female (Seibt and Wickler, 1979; Chow et al., 1982; Berg and Sandifer, 1984; Zhang et al., 1998; Bauer, 2004), but females may receive multiple spermatophores from several males (Höglund, 1943; Hoffmann, 1973; Bauer and Abdallah, 2001).

Postcopulatory Mate Guarding in *Rhynchocinetes brucei*

In several cases, the male *R. brucei* guarded the female after spermatophore transfer in the so-called cage posture

until the female had completed the spawning process. Similar postcopulatory mate guarding is known from species of *Macrobrachium* and from *R. typus* (Ra'anan and Sagi, 1985; Correa et al., 2000), but it is uncommon in other shrimp species (Bauer, 2004). Postcopulatory mate guarding in *Macrobrachium* does not cover the entire period from STE until completion of female spawning (Chow et al., 1982; Ra'anan and Sagi, 1985). However, female *M. rosenbergii* remain in the territory of the dominant males and retreat towards their mating partner if threatened (Nagamine and Knight, 1980). Choudhoury (1971) also had mentioned that male *M. acanthurus*, Wiegmann 1836, defend their female mating partners against other males until the female's new exoskeleton has completely hardened.

Postcopulatory mate guarding lasted throughout female spawning in 3 out of 5 successful males, but was only very brief or absent in the other 2 males. This differs from *R. typus* where all male morphotypes show postcopulatory mate guarding in a non-competitive situation, i.e., when alone with the female (Correa et al., 2003). These results suggest that male *R. brucei*, which guarded females only briefly (or not at all) after spermatophore transfer, may have engaged in alternative mating behavior.

Outlook

The lack of mate guarding in most shrimp species could potentially increase the risk of multiple matings and sperm competition. Multiple matings are common in *Macrobrachium* spp. and in *R. typus* (Ra'anan and Sagi, 1985; Thiel and Correa, 2004). The risk of sperm competition may have enhanced intrasexual selection in males of these species leading to the evolution of large males with powerfully developed chelipeds, which can defend receptive females against other males. The present study showed sexual dimorphism in cheliped and maxilliped length in *R. brucei*, but no size dimorphism as also suggested by similar carapace lengths in males and females reported in a previous study (Okuno, 1994). Our study also revealed that some male *R. brucei* guarded receptive females throughout the entire spawning process, but future studies are needed to reveal whether these males are capable to fend off male competitors during postcopulatory mate guarding.

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