



Exploitation of reproductive barriers between *Macrobrachium* species for responsible aquaculture and biocontrol of schistosomiasis in West Africa

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ABSTRACT: *Macrobrachium* prawns are voracious predators of the freshwater snails that host the flatworms responsible for bilharzia (schistosomiasis), a health burden in many African countries. A novel strategy to decrease the disease in Africa involves the use of prawns as biocontrol agents of the snails. Although the endemic African river prawn *Macrobrachium vollenhovenii* is a natural candidate for aquaculture and biocontrol, efforts to domesticate it have been unsuccessful to date, and it is not available in the large quantities required for aquaculture and biocontrol. The Asian giant prawn *Macrobrachium rosenbergii* has been cultured worldwide for decades. Recently, novel biotechnologies were developed to create monosex (all-male) non-breeding populations for aquaculture that we suggest are also ideal for biocontrol in Africa. Since the above 2 prawn species are of the same genus, exhibit similar sizes and require a female pre-mating molt prior to egg fertilization, the potential for cross-breeding between the 2 species must be tested. To assure that all-male populations of *M. rosenbergii* will not pose such an ecological threat, we carried out cross-breeding experiments with *M. vollenhovenii*. Both interspecies encounters and attempts at artificial insemination revealed that fertilization does not occur between the 2 species. Our results demonstrate both behavioral and physiological pre-zygotic reproductive barriers between these species. We suggest that all-male *M. rosenbergii* can be used as an aquaculture species and as a biocontrol agent in areas where *M. vollenhovenii* occurs without concern for hybridization.

KEY WORDS: All-male populations · Artificial insemination · Bilharzia (schistosomiasis) · Biocontrol · Cross-breeding · *Macrobrachium* · Reproductive barrier · Aquaculture

INTRODUCTION

Freshwater prawns from the genus *Macrobrachium* are produced commercially in various countries around the world, with global production amounting to more than 500 000 tons annually (www.fao.org/

fishery/). One of the most widely cultured species, *M. rosenbergii*, attracted considerable research attention when it was shown that its yields could be markedly increased through the use of novel monosex culture technologies developed for the aquaculture industry. All-male prawns are produced through a temporal

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RNA interference manipulation that reverses the phenotypic sex of males to 'neo-females', i.e. genotypic males that function as females. Since male prawns are homogametic with respect to their sex chromosomes (ZZ), when a neo-female is crossed with a normal male, the progeny is 100% male (Ventura et al. 2012).

Recently, it was suggested that monosex *M. rosenbergii* populations can be exploited as biocontrol agents (Savaya-Alkalay et al. 2018). Support for this suggestion is found in previous studies indicating that the voracious predation abilities of *Macrobrachium* prawns could be applied in the biocontrol of a variety of snails that cause extensive economic damage and that pose significant threats to human health (Roberts & Kuris 1990, Sokolow et al. 2014, 2016, Swartz et al. 2015, Savaya-Alkalay et al. 2018) For example, some species of freshwater snails are carriers of parasites that compromise human health and impose a massive burden on health services (e.g. schistosomiasis [bilharzia]), and others cause extensive damage in the aquaculture industry (e.g. *Centrocestus* spp. as fish parasites) and in the agriculture sector (e.g. apple snails as rice paddies pests) (Mitchell et al. 2007, Cowie & Hayes 2012, Savaya-Alkalay & Sagi 2016).

A most noted concern is schistosomiasis, a parasitic disease caused by a flatworm *Schistosoma* spp. that requires 2 hosts, a snail and a vertebrate (human, for example). It is most common in Africa, where more than 200 million people are infected and nearly 400 million are at risk (Sokolow et al. 2017). The Senegal River Basin has experienced the most drastic outbreak ever recorded of schistosomiasis in the past three decades (Sokolow et al. 2017). Following the completion of the Diama dam upstream of the Senegal River Estuary, the prevalence and intensity of schistosomiasis rapidly increased in the villages and towns in the region (Southgate 1997). One reason suggested for that outbreak is the fact that the dam prevents the local prawn species *M. vollehovenii* from completing its life cycle, which requires access to the estuary. The dam prevents female prawns from migrating downstream toward the estuary where they would normally release their larvae. Similarly, their post-larvae offspring are confronted with the same problem, as their migration upstream toward the fresh water of the Senegal River is also blocked (Bauer 2011, Savaya Alkalay et al. 2014). The absence of prawns—a major predator of freshwater snails—upstream of the dam has thus created ideal growth conditions for freshwater snails, the secondary host of *Schistosoma* parasitic worms (Savaya Alkalay et al. 2014).

As a complementary measure to control schistosomiasis, there has been a preliminary attempt to re-introduce local prawns to water points in villages upstream of the Diama dam together with concomitant medical treatment provided to the children in the villages and monitoring of the prevalence and intensity of the *Schistosoma* burden in their body (Sokolow et al. 2015). Because this preliminary study yielded promising results, today a larger experiment is being conducted using prawns collected in the wild (www.theupstreamalliance.org).

However, to scale up such operations and obtain significant results regarding the effectiveness of *Macrobrachium* prawns as disease-reducing agents, there is a need for large quantities of inexpensive prawns, which is a challenge when using an undomesticated species such as *M. vollehovenii*. Due to its large size and wide distribution throughout the west coasts of Africa (Senegal in the north to Angola in the south), numerous attempts to complete its life cycle for aquaculture use under hatchery conditions have been made in the past 25 yr. Unfortunately, none have succeeded in overcoming the obstacles to breeding *M. vollehovenii* as an aquaculture product (Marioghae 1988, Willfuhr-Nast et al. 1993, Marioghae & Ayilna 1995, Makombu et al. 2014).

Since *M. rosenbergii* prawns are readily available in the aquaculture industry, we recently suggested using non-breeding all-male *M. rosenbergii* populations for responsible aquaculture and as biocontrol agents wherever snails are a major concern. The ecological safety of such a solution must be based on an environmental risk assessment that should include data on the hybridization potential between the introduced species and the native species.

Macrobrachium prawns exhibit a specific reproductive behavior: following ovarian development, females undergo a pre-mating molt (PMM) that signals their reproductive receptivity. Normally, a receptive female is guarded by a fertilizing male (Mallikarjuna Rao 1965). This guarding behavior is crucial for a successful insemination since the PMM renders the female highly vulnerable to cannibalism (Sandifer & Smith 1985). As mentioned, the reproductive behavior of *M. rosenbergii* has been extensively studied (Chow et al. 1982, Barki et al. 1991), but this is not the case for *M. vollehovenii*, and clearly, interactions between the 2 species have not been studied to date. Insofar as both species exhibit similar sizes and include large individuals (exceeding 200 g) (Kuris et al. 1987, Savaya Alkalay et al. 2014), they are presumably 2 of the largest of all the *Macrobrachium* species.

Although the body shapes of various *Macrobrachium* species are similar, clear differences are apparent with respect to the last segment of the second chelae (i.e. propodus) in terms of shape, size and texture (Short 2004, Sarkar et al. 2012, Soundarapandian et al. 2014). However, the morphologies and behaviors of only a few *Macrobrachium* species have been rigorously studied. For example, the claws of *M. rosenbergii* males have been exhaustively detailed and are now used to distinguish between different male morphotypes (Kuris et al. 1987). The blue-claw male morphotype, the largest in the population, is dominant and territorial and exhibits high reproductive success in the context of the social structure of *M. rosenbergii* populations. Because male morphotypes—and hence social structure—in *M. vollehovenii* have not been described yet, in this study we only included similarly large-sized males of both species with a specific focus on the hybridization potential of *M. rosenbergii* males and *M. vollehovenii* females. We recorded both cross-species encounters and artificial inseminations (resulting in sperm–egg interactions), to examine the potential for *M. rosenbergii* male and *M. vollehovenii* female hybridization, from behavioral and physiological perspectives.

MATERIALS AND METHODS

Animals and morphological comparisons

All prawns used in this study were kept in freshwater recirculated aquaculture system (RAS) tanks outfitted with biomechanical filters. All-male *Macrobrachium rosenbergii* prawns were supplied by the Tiran group through Colors (Hatzeva) and were held in 100 l tanks in facilities at Ben-Gurion University of the Negev (BGU), Beer-Sheva, Israel. *M. rosenbergii* prawns were fed 3 times a week with shrimp pellets (Raanan Fish Feed, I.Z Milout, 40% protein) and once a week with frozen food (*Artemia* and bloodworms, Ocean Nutrition). Water temperature ranged between 25 and 29°C.

M. vollehovenii prawns for the cross-breeding experiments were collected during 2016 around the Diama dam in the Saint-Louis region of Senegal (16° 12' 52.65" N, 25° 20' 16.07" W) and were held at the hatchery of Espoir Pour La Santé (EPLS) (16° 03' 05.0" N, 16° 25' 58.9" W) in 500 l RAS tanks. *M. vollehovenii* prawns were fed 3 times a week with fresh shrimp from the local market. Water temperature ranged between 24 and 29°C. The sex and weight of each animal were recorded.

To conduct our study, we looked for comparable males of the 2 species in terms of body size and claw morphometry. The morphologies of individuals of both species were compared using a published dataset of 781 single-aged *M. rosenbergii* males from aquaculture ponds in India (Aflalo et al. 2012) compared with our dataset of 475 multi-aged wild *M. vollehovenii* males that we collected during 2013 and 2016 from the Senegal River. In preparation for the study, *M. rosenbergii* individuals were differentiated according to the 3 morphotypes defined by Kuris et al. (1987), namely small males (SM), orange claw (OC) and blue claw (BC), based on the propodus-to-carapace length ratio. Insofar as *M. vollehovenii* morphotypes could not be defined from the present study, we focused in this study only on large *M. vollehovenii* males (>100 g), which are similar in size to *M. rosenbergii* BC males (Aflalo et al. 2012) (Table 1). To explore the differences in the propodus length (PL) to carapace length (CL) ratio among the 4 groups (SM, OC and BC *M. rosenbergii*, and large *M. vollehovenii*), a 1-way ANOVA test was conducted using Statistica 12 (StatSoft 2013). The assumptions of normality and homogeneity of variances were tested through Shapiro-Wilk and Levene's test, respectively. The assumptions were not met, therefore the data were log-transformed. The post-hoc test was Tukey HSD and the level of significance used was 0.05. For the study, comparably sized males with similar PL/CL ratios were selected from both species.

Pre-cross-breeding

To verify that the *M. rosenbergii* males selected for shipment from Israel to Senegal were indeed reproductively viable, a representative sample of 10 BC males were stocked individually in a RAS, each comprising a 100 l tank, and 3 reproductive females were added to each tank. The tanks were monitored daily to identify PMM females and successful fertilizations.

Table 1. Morphological data of *Macrobrachium rosenbergii* males of 3 morphotypes (SM: small males; OC: orange claw; BC: blue claw) and large *M. vollehovenii* (Mv) males. PL/CL: propodus length divided by carapace length. Data are mean \pm SE. *M. rosenbergii* data were acquired from Aflalo et al. (2012)

	SM (n = 190)	OC (n = 528)	BC (n = 63)	Large Mv (n = 78)
PL/CL	0.96 \pm 0.01	1.26 \pm 0.01	2.09 \pm 0.03	2.20 \pm 0.3
Weight (g)	30.6 \pm 0.8	99.2 \pm 1.4	141.6 \pm 2.2	183.9 \pm 3.8

Each BC male that successfully fertilized at least 1 female was marked and then isolated in a cage until its shipment to Senegal. Six of the 10 representative *M. rosenbergii* males fertilized *M. rosenbergii* females and were found to be reproductively viable. In total, 50 BC males of the same population were shipped to Senegal and placed in a tank with a receptive *M. vollehovenii* female for the cross-breeding experiments.

Reproductive behavior of *M. vollehovenii*

Aquarium encounters of *M. vollehovenii* males and females

To compare the reproductive behavior of *M. vollehovenii* to that of *M. rosenbergii*, which is well known (Chow et al. 1982), we used a Go-Pro Hero 3+ camera to document 35 h of interaction between a large *M. vollehovenii* male (>100 g) and spent (mature female that had laid eggs in the past, i.e. reproductively capable) *M. vollehovenii* females. While *M. vollehovenii* mating behavior has not been well documented, that of *M. rosenbergii* has been described to include 4 stages, all of which occur after the PMM (Chow et al. 1982):

1. Contact: initial contact is with the antennules, and later, the male grasps the female's pleopods, uropods and pereopods with his first pereopods.
2. Seizure of female: the male holds the female between his long second chelipeds.
3. Mounting: the male mounts the female while searching for her sternum with his first pereopods. The male starts to turn the female upside down in preparation of the actual copulation.
4. Mating: the male completes turning the female over and then angles his first 2 pleopods strongly backwards, after which he presses them firmly on the female's sternum. At this point, he attaches the spermatophore.

In contrast to the study of *M. rosenbergii* mating behavior (Chow et al. 1982), which used females after their PMM, in the current study of *M. vollehovenii* mating behavior we used females with developed ovaries (visible through the dorsal carapace) that had not yet undergone PMM. This approach was taken to ensure that we would observe mate selection behavior prior to the female's PMM. The animals were kept in a 120 l aquarium (100 × 40 × 40 cm) with aeration. During the documentation, the sex ratio was maintained at 4 females to 1 male. Each female was marked on its carapace to facilitate

later detection of pre- and post-molt individuals. The aquarium was monitored for male guarding behavior, female PMM and spermatophore attachment.

Cross-species encounters

Six RAS setups, each comprising two 500 l tanks connected to a 200 l biomechanical filter, were used for the cross-species encounter experiments at the EPLS hatchery in the Saint-Louis region of Senegal. This experiment was designed as a pairwise setup. In each RAS system, 1 tank served as the positive control and the other as the treatment group. This was set to eliminate the effects of any differences in the water conditions between the treatments and controls. Each positive control tank was stocked with 1 mature *M. vollehovenii* male and 3 spent *M. vollehovenii* females. Similarly, each treatment tank contained 1 *M. rosenbergii* BC male and 3 spent *M. vollehovenii* females. All control and treatment tanks were monitored each morning to record molting events, gonad development, reproductive behavior and fertilization. Water temperature was maintained between 24 and 29°C. Each female that laid eggs was removed to a different tank where it was housed separately in a marked cage that was monitored daily for embryo development. To maintain the 1:3 sex ratio throughout the experimental period, each removed female that laid eggs was replaced with a new spent female. A total of 23 h of underwater video (Go-Pro Hero 3+ camera) was recorded and further used to analyze behavioral patterns. To compare the reproductive performance of the treatment and control groups, the following events were quantified: (1) the number of females that performed PMM, (2) the number of females that spawned, (3) the number of cannibalized females, (4) the numbers of viable spawns (i.e. egg hatched and subsequent embryonic development was observed) and (5) the number of egg batches that were disposed.

Artificial insemination

Artificial insemination must be done within a short time-window of a few hours following the female's PMM, in which the female is reproductively receptive. In addition, the female should be voided of a spermatophore attached to her sternum. To acquire receptive *M. vollehovenii* females without attached spermatophores, *M. vollehovenii* 'detector' males

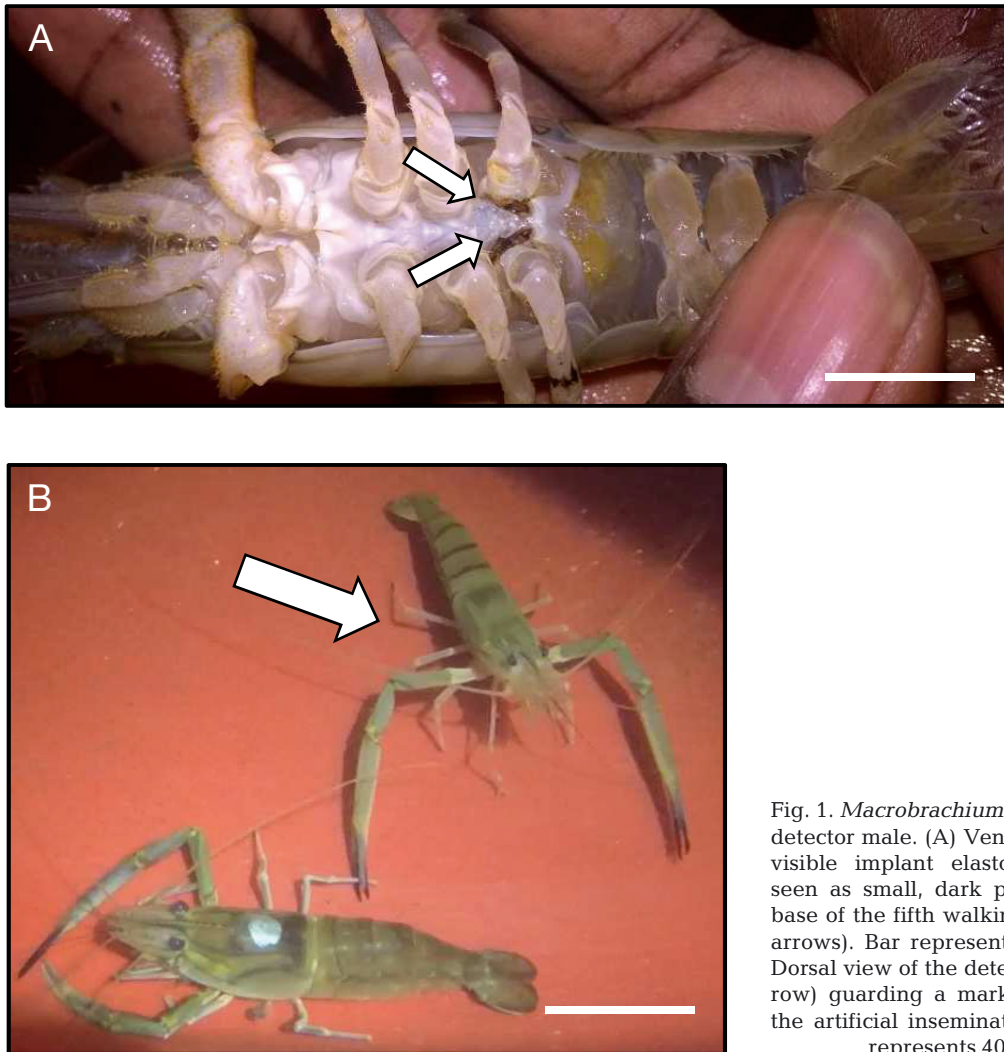


Fig. 1. *Macrobrachium vollenhovenii* detector male. (A) Ventral view. The visible implant elastomer can be seen as small, dark patches at the base of the fifth walking legs (white arrows). Bar represents 15 mm. (B) Dorsal view of the detector male (arrow) guarding a marked female in the artificial insemination tank. Bar represents 40 mm

that were unable to eject their spermatophores were used (Sandifer & Smith 1979, 1985, Ra'anani & Sagi 1985). Detector *M. vollenhovenii* males were created by effectively sealing their gonophores by injecting a visible implant elastomer (Fig. 1A). To ensure that the sealing was successful, a small drop of 'Super Glue' was applied to the top of each gonopore (Fig. 1A). The resulting detector males exhibited normal reproductive behavior but were unable to eject spermatophores. Two detector males and 12 reproductive (spent) *M. vollenhovenii* females were stocked in each of two 3000 l tanks attached to a 400 l biomechanical filter. All females were marked with a waterproof marker on the dorsal side of their carapaces to facilitate the identification of freshly molted females. Every morning the tanks were monitored for receptive females; the absence of the mark on their

dorsal carapace, their soft cuticle and being guarded by the detector male indicated they were ready for artificial insemination (Fig. 1B). Artificial insemination was performed using the method described by Sandifer et al. (1984). In brief, electrodes or an electroejaculator was placed on the base of the fifth walking legs of a large, mature male where the gonopore is located. To cause the males to eject spermatophores, the animal was stimulated with a voltage of 4–6 V for 1–2 s, and spermatophores were collected by forceps and deposited on the receptive females' sperm receptacle area (ventral side, between the base of the third and fifth pereopods). Each female that was artificially inseminated with spermatophores, whether of *M. vollenhovenii* (control, 8 attempts) or *M. rosenbergii* (treatment, 12 attempts) origin, was kept in a separate, marked, floating basket to facilitate daily

monitoring. Females were monitored until eggs were laid. In the event that the eggs were retained on the female's pleopods, embryonic development was periodically monitored under a microscope.

Phylogenetic analyses

The analysis involved 24 nucleotide sequences of the 16S ribosomal RNA gene that were found in the NCBI database and their accession numbers are presented in Supplement 1 at www.int-res.com/articles/suppl/q010p487_supp/. The crayfish *Cherax quadricarinatus* serves as an outgroup. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). The evolutionary history was inferred using the neighbor-joining method (Saitou & Nei 1987).

RESULTS

Morphological comparisons

Adult *Macrobrachium vollenhovenii* males (475) and females (292) were collected. The males averaged 64.2 ± 2.6 g (mean \pm SE) with a range of 4 to 235 g and the females averaged 45.3 ± 1.4 g with a range of 6 to 129 g. The large-sized, mature *M. vollenhovenii* males found in the present study and in the samples we observed in our previous work in the Senegal River Basin (Savaya Alkalay et al. 2014) were comparable to large *M. rosenbergii* BC males in terms of body size and claw morphometry (Table 1) (Kuris et al. 1987, Aflalo et al. 2012). The PL/CL ratios of the 4 groups of males were significantly different (1-way ANOVA: $F_{1,3} = 365.42$, $p < 0.001$) except for the *M. rosenbergii* BC male and the large *M. vollenhovenii* male groups (Tukey's HSD, $p = 0.07$). The body and second cheliped morphology of a large *M. vollenhovenii* male (196 g) and a large *M. rosenbergii* BC male is depicted in Fig. 2. These males exhibited similar features in terms of body shape and size, cheliped shape and spination, and PL/CL ratio (Fig. 2A,C). However, a close-up on the tip of the propodus revealed that the teeth on the internal part of the chela are much smaller in size in *M. rosenbergii* (Fig. 2D) than in *M. vollenhovenii* (Fig. 2B). Clear differences were also observed on the exterior side of the chela: whereas the dactylus in *M. rosenbergii* is covered with velvet-like setae (Fig. 2D), the texture of that area in *M. vollenhovenii* is the same as the rest of the chela.

Behavioral comparisons

Aquarium encounters

Successful *M. vollenhovenii* copulation between a male and a female of the species was documented (Video S1 at www.int-res.com/articles/suppl/q010p487_supp/) from the beginning of the male's guarding behavior, which commenced prior to PMM, through the molting event and until copulation. The continuation of the guarding behavior the following day was also recorded. We observed all 4 stages described for *M. rosenbergii* during *M. vollenhovenii* mating behavior. However, because we used females prior to their PMM, the first stage (contact), as well as many brief 'seizure of female' attempts indicating guarding, were already evident 8 h before PMM and involved different females in the tank. As the time approached to the PMM of the receptive female, that female spent increasingly longer time in proximity to the guarding male, which protected her from the other females by using his large claws in the seizure posture (see Video S2).

As seen in Video S1, once the female started the actual PMM (1:07), the *M. vollenhovenii* male's behavior was similar to that described for *M. rosenbergii* males, including the 'mounting' and 'mating' stages as described above. Due to the angle of the camera, the actual spermatophore attachment cannot be seen in the video; however, the following day we verified visually the spermatophore attachment in a similar location as in *M. rosenbergii*.

Cross-species encounters

Thirty successful fertilizations of *M. vollenhovenii* females by conspecific males (positive control) were recorded (Table 2). One such representative case is depicted in Fig. 3. In the treatment tanks, in contrast, 8 receptive females laid eggs in the presence of *M. rosenbergii* males, but in all 8 cases, no spermatophore was observed prior to egg laying. These eggs were therefore not fertilized and were aborted after a few days (Table 2). In addition, we observed 2 cases of a PMM *M. vollenhovenii* female in the presence of a *M. rosenbergii* male that did not guard the female, which was eventually cannibalized by the other females in the tank or by the male itself (Table 2). A representative case of cannibalism associated with a PMM is depicted in Fig. 4A, which is a still image from a 1 h recording showing that the *M. rosenbergii* male does not exhibit its typical repro-

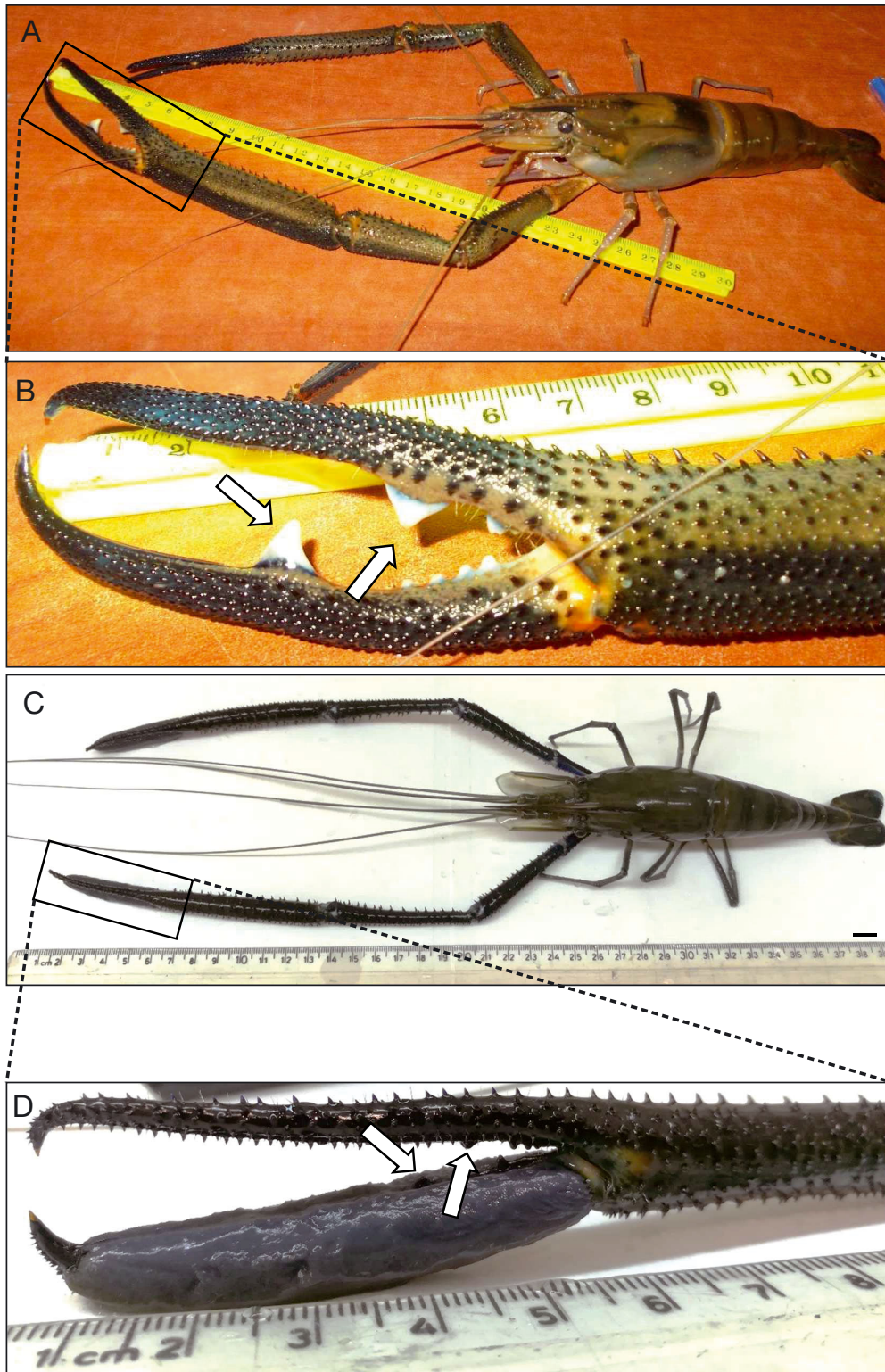


Fig. 2. Dorsal view of large males. (A) Overall view of a *Macrobrachium vollenhovenii* male. The yellow ruler is 30 cm long. (B) Enlargement of the tip of the propropodus of a *M. vollenhovenii* male. White arrows indicate the locations of the 2 large propropodus teeth. Ruler shows 1 cm intervals. (C) Overall view of a *M. rosenbergii* BC male. The ruler is 40 cm long. Bar represents 1 cm. (D) Enlargement of the tip of the propropodus of a *M. rosenbergii* BC male. White arrows indicate the locations of the teeth inside the tip of the propropodus

Table 2. Intra- and cross-species reproductive performance of 3 *Macrobrachium vollenhovenii* (Mv) females housed with a *M. vollenhovenii* male (Mv × Mv) or a *Macrobrachium rosenbergii* (Mr) male (Mv × Mr). PMM: pre-mating molt events; eggs: number of females that laid eggs; dead: death of the female; hatch: number of egg clutches that yielded live larvae; disposed: number of clutches that were aborted

	PMM	Eggs	Dead	Hatch	Disposed
Mv × Mv control	30	30	2	27	1
Mv × Mr treatment	10	8	2	0	8

ductive behavior (i.e. guarding), while other *M. vollenhovenii* females continuously irritate the freshly molted, receptive female. In an additional case of cannibalism (Fig. 4B), the *M. rosenbergii* male itself preyed upon a receptive *M. vollenhovenii* female instead of exhibiting guarding behavior.

Artificial insemination

Four successful cases of artificial insemination (out of 8 attempts) were recorded in the control group, where *M. vollenhovenii* spermatophores were deposited on receptive *M. vollenhovenii* females. The treatment was deemed successful when the berried females held their broods until embryonic development. In contrast, none of the 12 attempts at artificial insemination in the treatment group, in which *M. rosenbergii* spermatophores were deposited on receptive *M. vollenhovenii* females, was successful (Table 3). These females lost their eggs within 3 to 7 d post laying. Representative clutches from both the control (Fig. 5A) and treatment (Fig. 5B) groups

were photographed under a microscope. Eggs from the control group exhibited normal embryonic development for Day 6 of growth, as evidenced by the clearly visible head and tail of the embryo, while the eggs from the treatment group were devoid of embryos and were aborted.

Phylogenetic analyses

The optimal tree with the sum of branch length = 1.00970234 is shown in Fig. 6. All positions containing gaps and missing data were eliminated. There was a total of 408 positions in the final dataset. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances, computed using the maximum composite likelihood method (Tamura et al. 2004), are expressed in units of the number of base substitutions per site.

DISCUSSION

Accounting for 17% of the global population, Africa produces only 2% of global aquaculture biomass, with crustacean culture at a negligible scale (www.fao.org/fishery/). One of the most promising candidates for crustacean aquaculture in Africa is the freshwater prawn *Macrobrachium rosenbergii*, which is not a native species. Monosex culture ensures that even escapees from aquaculture operations cannot reproduce and become an invasive species (Silva-Oliveira et al. 2011); however, the possibility of cross-

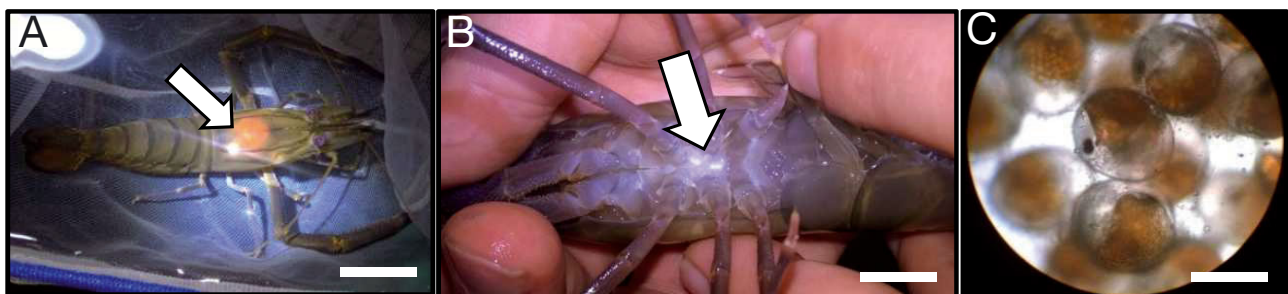


Fig. 3. Outcomes of encounters between *Macrobrachium vollenhovenii* males and receptive *M. vollenhovenii* females. (A) Female dorsal view showing a developed ovary (arrow). Bar represents 2 cm. (B) Female ventral view — spermatophores are deposited on the female's receptaculum (arrow). Bar represents 1 cm. (C) Examination of fertilized eggs under a microscope. Eyed eggs indicate normal embryonic development. Bar represents 0.2 mm

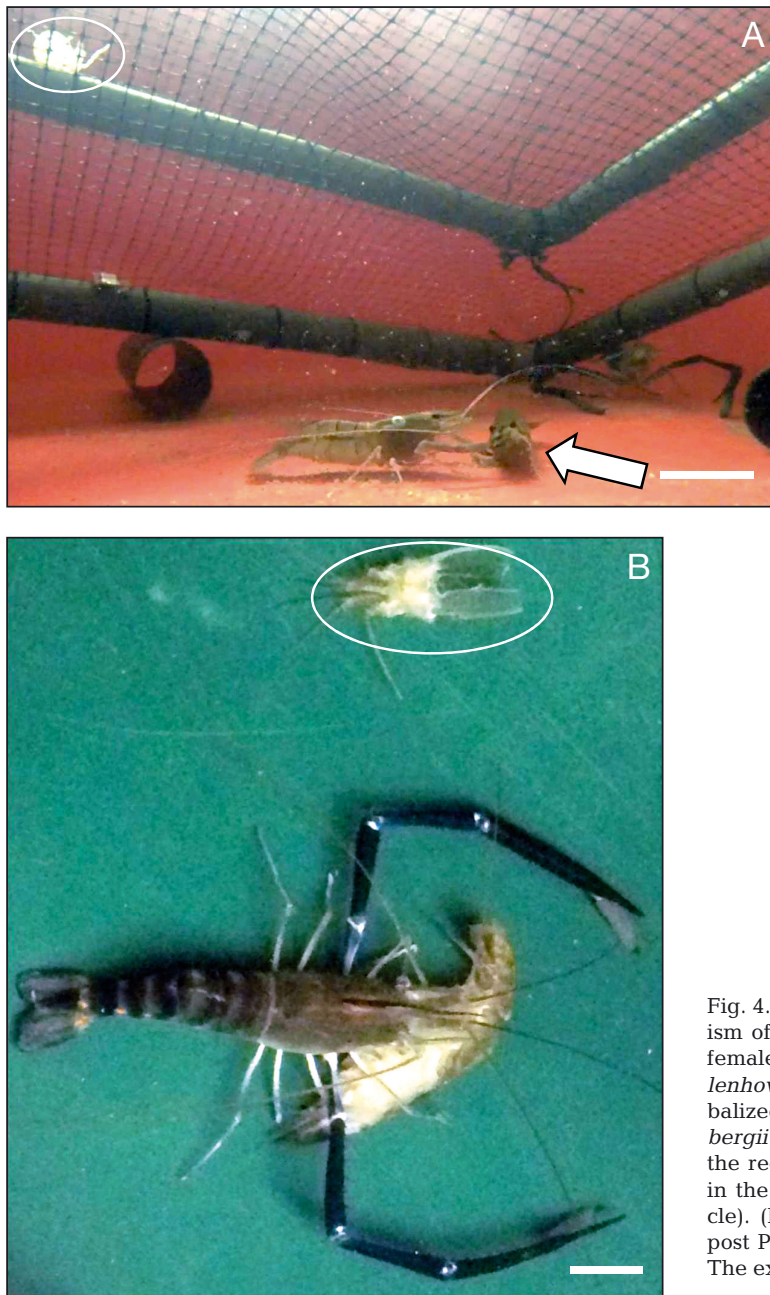


Fig. 4. Cross-species encounters and cannibalism of post pre-mating molt (PMM) receptive females. (A) A receptive *Macrobrachium vollehovenii* female (white arrow) being cannibalized by another female, while the *M. rosenbergii* male (seen at the back) is not guarding the receptive female. The exuvia can be seen in the top left corner of the image (white circle). (B) *M. rosenbergii* male cannibalizing a post PMM receptive *M. vollehovenii* female. The exuvia can be seen at the top of the image (white circle). Bar represents 4 cm

Table 3. Artificial insemination of *Macrobrachium vollehovenii* (Mv) females using *M. vollehovenii* spermatophores (Mv × Mv) or *M. rosenbergii* (Mr) spermatophores (Mv × Mr). Insemination attempts: number of artificial insemination procedures that were conducted; eggs laid: number of females that laid eggs following the artificial insemination; eggs not laid: number of females that did not lay eggs following the artificial insemination attempt; eggs hatched: number of egg clutches that successfully hatched following the artificial insemination; eggs discarded: number of egg clutches that were laid following the artificial insemination, but were discarded; dead female: number of females that died following the artificial insemination attempt

	Insemination attempts	Eggs laid	Eggs not laid	Eggs hatched	Eggs discarded	Dead female
Mv × Mv control	8	7	1	4	3	0
Mv × Mr treatment	12	10	2	0	9	1

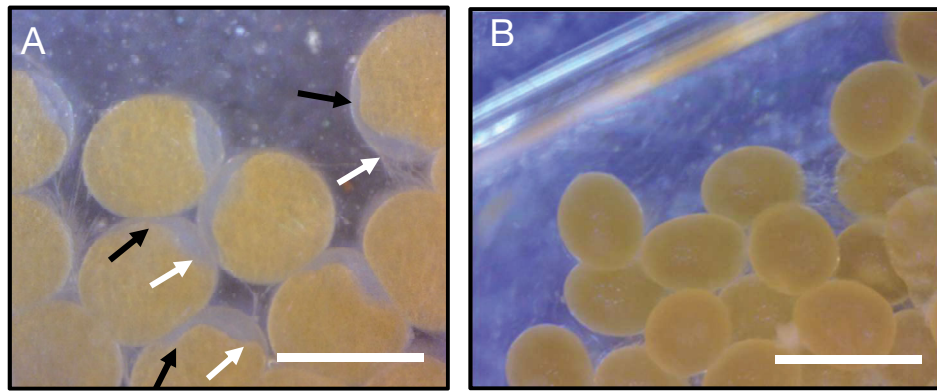


Fig. 5. Egg development following artificial insemination. (A) Positive control: embryonic development 6 d after insemination of *Macrobrachium vollenhovenii* eggs with conspecific spermatophore. The embryos are visible and their heads and tails are marked with white and black arrows, respectively. Bar represents 0.2 mm. (B) Treatment group: unfertilized *M. vollenhovenii* eggs, 4 d after insemination with *M. rosenbergii* spermatophore. No embryo development is apparent. Bar represents 0.2 mm

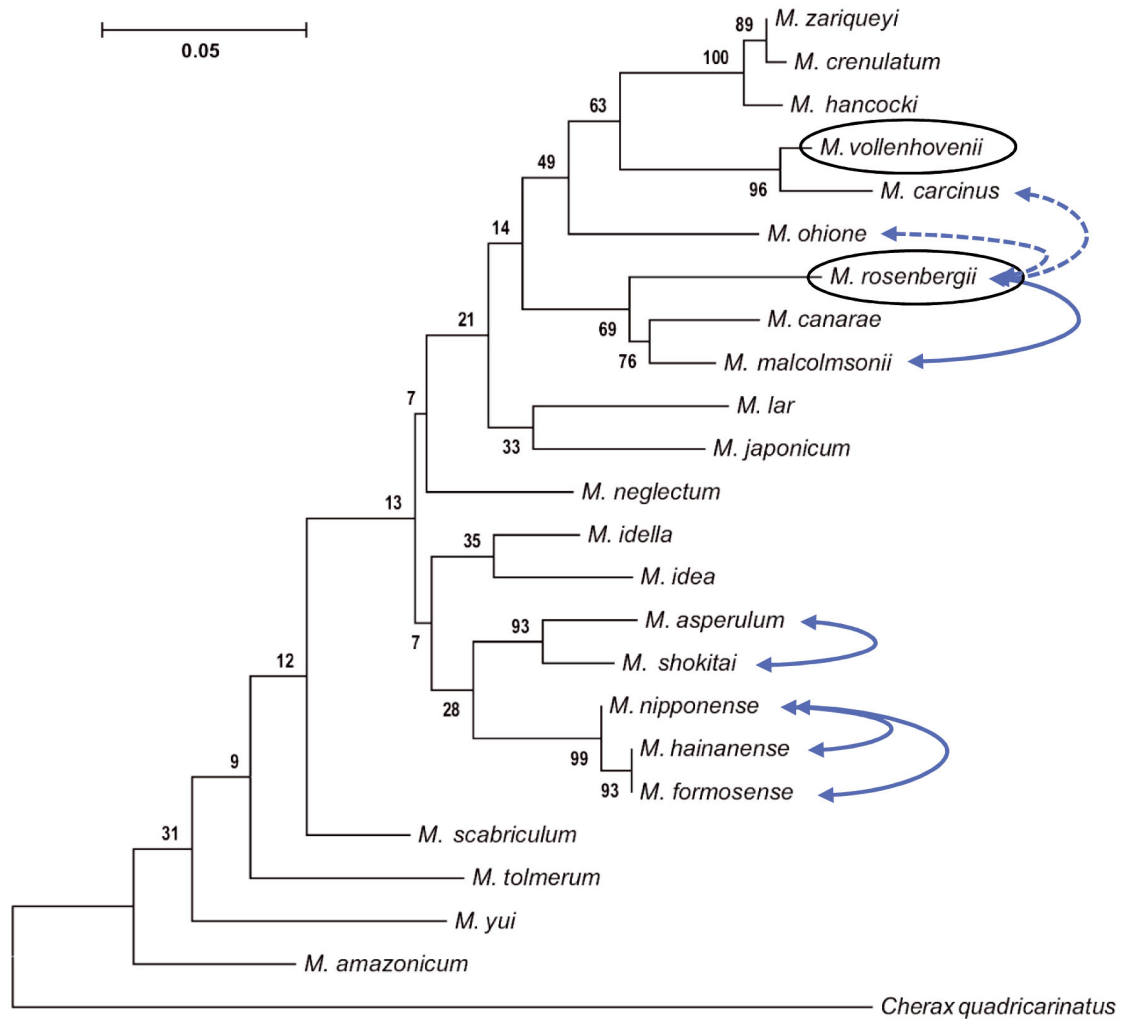


Fig. 6. Evolutionary relationships of 23 *Macrobrachium* species according to mitochondrial 16S gene. *M. rosenbergii* and *M. vollenhovenii* are shown in black circles. Solid and dashed arrows between different species represent successful and unsuccessful cross-species hybridization attempts, respectively

breeding with local species should also be considered to ensure that *M. rosenbergii* aquaculture is indeed responsible. Our study addressed this question with respect to the African river prawn *M. vollehovenii*, and interbreeding between these species was found to be unlikely due to significant reproductive barriers. This opens the path for the introduction of all-male *M. rosenbergii* for aquaculture purposes in Africa.

In addition to their aquaculture potential, all-male *M. rosenbergii* could be exploited as biocontrol agents against schistosomiasis (Roberts & Kuris 1990, Sokolow et al. 2014, 2015). The observed feasibility of biological control, together with the limited success of drug treatment, strongly indicate that viable schistosomiasis control may be achieved by adopting a multi-pronged strategy. While drug treatment will reduce disease intensity and prevalence, biocontrol will lower its reinfection rate. To achieve these goals, large quantities of the biocontrol agent will be required, and we suggest exploiting the readily available non-breeding monosex populations of *M. rosenbergii* for this purpose (Savaya-Alkalay & Sagi 2016, Savaya-Alkalay et al. 2018). Prior to its practical application, however, rigorous environmental assessments, including, but not limited to, ruling out the possibility of interbreeding between *M. rosenbergii* and the local prawn *M. vollehovenii*, are needed to ensure the safety of this novel approach.

While the mating behavior and claw appearance of *M. rosenbergii* males are well described (Chow et al. 1982, Kuris et al. 1987), similar information about *M. vollehovenii* is lacking. A comparative study performed with *M. vollehovenii* specimens and adult *M. rosenbergii* animals (Aflalo et al. 2012) found that the large males of the 2 species were similarly sized, which was also reflected in their claw morphometric data as determined by the PL/CL ratio. Other than the relative sizes and teeth occurrence on the claws, the morphologies of the propodus segments of the 2 species differed in terms of texture. The similarities we found in the morphological parameters of the large males of the 2 species led to their selection for the comparative reproductive study. The dearth of information about *M. vollehovenii*, however, particularly the lack of clear morphotype definitions in the context of the social structure of *M. vollehovenii* males, dictates the need for further study of this species.

As reported above, we did not find any major differences between pre- and post-PMM mating behaviors of the 2 species. However, when *M. rosenbergii* males and *M. vollehovenii* females were stocked in the same tank, neither fertilization nor typical repro-

ductive guarding behavior occurred, and in some cases, we observed female mortality due to cannibalism. These results and the failure of all attempts at artificial insemination suggest that *M. rosenbergii* males and *M. vollehovenii* females cannot cross-breed under any conditions and that 2 levels of pre-zygotic barriers (i.e. behavioral and physiological) exist.

Successful breeding between *Macrobrachium* species has been reported only in those species that are naturally present in the same habitat, that are not geographically isolated from one another and that are closely related phylogenetically. To the best of our knowledge, only 4 hybridization attempts have resulted in viable offspring: *M. nipponense* × *M. formosense* (Uno & Fujita 1972), *M. asperulum* × *M. shokitai* (Shokita 1978), *M. nipponense* × *M. hainanense* (Fu et al. 2004) and *M. rosenbergii* × *M. malcolmsonii* (Sankolli et al. 1980, Soundarapandian & Kannupandi 2000). All 4 of these pairings meet the 3 criteria stated above for successful hybridization (Fig. 6). In contrast, hybridization attempts between geographically isolated or phylogenetically distant species have not succeeded to date (Sandifer et al. 1980, Graziani et al. 2003). *M. rosenbergii* and *M. vollehovenii* that originate from geographically isolated areas (South-East Asia and West Africa, respectively) are also phylogenetically remote, as can be seen in the phylogenetic tree (Fig. 6).

In conclusion, we call for responsible field experiments to investigate *M. rosenbergii* all-males for aquaculture and biocontrol in Africa. Our study is the first step towards the introduction of non-native and non-invasive *M. rosenbergii* prawns into West Africa. Additional risk assessment is warranted prior to such introduction, including additional cross-breeding attempts with local *Macrobrachium* spp. (if present) and a comprehensive environmental survey to be followed by a contained field experiment. Once environmental concerns have been addressed appropriately, commercial aquaculture operations and large-scale biocontrol efforts could be permitted, to the benefit of local communities in West Africa.

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