

New Insights into the Male Morphotypes of the Amphidromous Shrimp *Macrobrachium olfersii* (Weigmann, 1836) (Caridea: Palaemonidae) and a Discussion on Social Dominance Hierarchies

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Male morphotypes in a population may lead to the development of social dominance and hierarchies in crustacean species. Currently, *Macrobrachium* is the decapod crustacean genus with the largest record of species that present the development of hierarchies. *Macrobrachium olfersii* has morphological characteristics that indicate the presence of male social dominance within its populations. Thus, the present study tested the hypothesis of the occurrence of male morphotypes in *M. olfersii* through morphometric and morphological analysis of the chelipeds. Sampling was carried out from March/2018 to October/2021 in seven points along the Jequitinhonha River, Northeast Brazil. A total of 264 males were collected with carapace length (CL) ranging from 4.01 to 23.70 mm. Morphological sexual maturity size was estimated at 8.95 mm CL. The morphometric and morphological analysis confirmed the presence of three adult male morphotypes: M1, M2, and M3. The characterization of the different morphotypes was mainly due to the variation in size, shape, and morphology of the largest cheliped of the second pair of pereopods. Most morphometric relationships differed significantly ($p < 0.01$) among the three morphotypes, mainly between M3 against M1 and M2. The variation in the propodus shape was also evident. This characteristic and the angulation of the spines differed significantly between morphotypes ($p < 0.01$), with the propodus of morphotype M3 being more robust and carrying a greater number of spines than the others. The occurrence of social dominance and the exaggerated development of a cheliped

(weapon) can be advantageous for dominant individuals when they need to compete for resources. This morphological trait can provide these individuals with advantages during fights and guarantee access to the best resources, whether they are shelter, food, or sexual partners. Our results add new information on the biology of *M. olfersii*, as well as on the genus *Macrobrachium*, and the occurrence of social dominance in species of this group. In addition, by describing these morphotypes in details, using a set of complementary morphological and morphometric techniques, it is possible to access the differential morphology along the *M. olfersii* males, as well as confirm a life history trait found in several *Macrobrachium* species.

Key words: Allometry, Chelipeds, Geometric morphometrics, Relative growth, Decapoda.

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BACKGROUND

Social dominance and hierarchies are mechanisms that provide access for individuals of several groups of invertebrates to better resources (i.e., shelter, food, sexual partners) (Dugatkin and Dugatkin 2007; Stewart and Tabak, 2011; Soundarapandian et al. 2013; Lord et al. 2021). When this mechanism is associated with competition between males of the same population, a polymorphism is commonly observed (Soundarapandian et al. 2013). The process of becoming a dominant individual within a population requires a high initial energy investment (López and Martín 2001; Karplus and Barki 2019; Lord et al. 2021). However, there is a compensatory return since dominant individuals are less confronted, minimizing the energy spent on agonistic events (López and Martín 2001; Lord et al. 2021).

Among invertebrates, there are numerous records about the establishment of social dominance hierarchies in species of dragonflies, cephalopods, water bugs, and spiders (Campanella 1975; Ahtiainen et al. 2006; Boal 2006; Pérez et al. 2019). However, in crustaceans, this feature also is common, especially in infraorders of decapods (Winston and Jacobson 1978; Stewart and Tabak 2011; Karplus and Barki 2019; Lord et al. 2021). In decapod crustaceans, males develop a differential morphology of their chelipeds to become dominant (Karplus and Barki 2019). These structures are used as weapons in agonistic events, influencing social hierarchies (i.e., dominant morphotypes and submissive morphotypes) within a population (Kuris et al. 1987; Mariappan et al. 2000; Correa et al. 2003; Karplus and Barki 2019; Hamasaki and Dan 2021). Among decapods,

morphotypes have been described in brachyuran (Laufer and Ahl 1995; Sal Moyano and Gavio 2012), anomurans crabs (Bueno and Shimizu 2009; Takano et al. 2016) and freshwater crayfishes (Hamasaki et al. 2020), and several genera of caridean shrimps (Thiel et al. 2010; Bauer et al. 2014; Karplus and Barki 2019).

Among caridean shrimps, *Macrobrachium* Spence Bate, 1868 currently encompasses the largest number of species that have male morphotypes. Male morphotypes have been so far described for the species *Macrobrachium acanthurus* (Weigman, 1836), by Rios et al. (2021), *M. amazonicum* (Heller, 1862) by Moraes-Riodales and Valenti (2004), *M. brasiliense* (Heller, 1862) by Nogueira et al. (2020), *M. grandimanus* (Randall, 1840) by Whortam and Maurik (2012), *M. idella* (Hilgendorf, 1898) Soundarapandian et al. (2013), *M. rosenbergii* (de Mann, 1879) Kuris et al. (1987), and *M. tenellum* (Smith, 1871) Vargas-Ceballos et al. (2021). Recently, molecular phylogeny data revealed that the genus *Cryphiops* Dana, 1852 is a junior synonym within *Macrobrachium* (Mantelatto et al. 2021). Thus, the species *Cryphiops caementarius* (Molina, 1782), which has different male morphotypes described (Rojas et al. 2012), was added to the total number of *Macrobrachium* species that show this type of social dominance.

The occurrence of male morphotypes in *Macrobrachium olfersii* (Wiegmann, 1836) is a feature that has been historically discussed. This was suggested when molecular and morphological analysis indicated that two other species of *Macrobrachium* (*M. birai* Lobão, Melo & Fernandes, 1986 and *M. holthuisi* Genofre & Lobão, 1978) were a junior synonym of *M. olfersii* (Pileggi and Mantelatto 2010, 2012). One of the main morphological characteristics used to separate these three species was the morphology of the second pair of chelipeds, precisely the structure that presents the greatest morphological variation among male morphotypes. The authors argued that this variation was not due to interspecific differences but to the possible existence of male morphotypes in this species (Pileggi and Mantelatto 2010). By then, the existence of morphotypes for *M. olfersii* still requires confirmation.

The population structure of *M. olfersii* supports the existence of morphotypes in this species. Previous studies had shown that only males reach the maximum observed sizes for this species, so males are mainly grouped in larger size classes in relation to females (Lombardi et al. 1996; Pescinelli et al. 2016). This pattern of body size difference was observed in studies that addressed populations before and after the taxonomic revision involving the *M. olfersii* species complex (Pescinelli et al. 2016). In addition to the variation in body size between males and females, there is also an evident difference in the size of the second pair of chelipeds between these groups; males have an exaggeratedly more developed cheliped than females and a more pronounced heterochelic pattern (Ammar et al. 2001; Mossolin and Bueno 2003; Pescinelli et al. 2016; Müller et al. 2018). However, none of these previous studies investigated the existence of different morphotypes in

males. Recently, a study explored the occurrence of male morphotypes in *M. olfersii* using specimens from different populations that occur along the Brazilian coast (Rossi et al. 2022), however, apparently this study did not sample all size classes of males of *M. olfersii*. Furthermore, when using specimens from different populations, possible morphological and morphometric differences could also bias the correct identification of polymorphic groups. Thus, morphological, morphometric and behavioral studies carried out with individuals from the same population are still necessary in order to corroborate the existing previous information.

Analyzing morphometric relationships between the growth of different body structures is fundamental to determining hierarchical groups in a population since differences in allometric coefficients can influence ontogenetic development, and consequently the size and shape of structures (Hartnoll 1974; Rosenberg 2002; Klingenberg 2016). The evaluation of the shape (assessed via geometric morphometrics) of the cheliped can contribute to the discrimination of male morphotypes in shrimp species, despite not being commonly used for this purpose (Nogueira et al. 2022a). In most cases, discrimination only uses the relative growth analysis (linear morphometrics) (Kuris et al. 1987; Moraes-Riodades and Valenti 2004; Rojas et al. 2012; Wortham and Maurik 2012; Pantaleão et al. 2014; Nogueira et al. 2020; Rios et al. 2021).

Given the evidence that points to high variability in morphology among males of *M. olfersii*, the present study aims to test whether there is the presence of male morphotypes in this species using a single population. Different morphometric and morphological aspects were evaluated between adult males to determine the variation in size, relative growth, shape, and ornamentation of chelipeds. We predicted that if there are different male morphotypes, they should present evident differences between the evaluated morphometric and morphological aspects. This pattern should follow the one observed in other *Macrobrachium* species, with dominant morphotypes investing more in the development of chelipeds, therefore presenting overdeveloped claws.

MATERIAL AND METHODS

Sampling

Sampling was carried out in the Jequitinhonha River, state of Bahia, Brazil (15°58'5.941"S, 39°35'11.983"W). We conducted ten campaigns: March/2018, August/2018, June/2019, October/2019, February/2020, August/2020, November/2020, April/2021, July/2021, and October/2021. In each campaign, seven points were sampled along the river, using two different sampling methods. Those were a cylindrical trap with a rectangular mesh (mesh openings 1 mm

wide and 5 mm long, base with 36 cm in diameter and 60 cm in height) that was left for four hours; and a hand-drawn trawl net (3 m long, 1.80 m high, 10 mm mesh), which was thrown five times in each point, covering a perimeter of approximately 15 meters per point. These two methodologies were applied aiming to increase the range of individuals captured. The expected was to find differences among the sampling methods, with the cylindrical traps capturing more of the larger individuals, and the hand-drawn trawl capturing a wider range of sizes, as well as, a higher number of individuals, due the low selectivity of this sampling method (Polet 2000).

All shrimps collected at the sampling site were sorted into plastic bags (containing local water) according to the sampling point and collection method. The shrimps were then transported to the laboratory. Individuals were identified at the species level using specific literature (Melo 2003) and separated by sex through the presence (males) or absence (females) of the male appendage in the endopod of the second pair of pleopods (Valenti et al. 1987). Individuals of *M. olfersii* from the seven sampling points were considered as a single population due to reproductive characteristics of the species (amphidromous) that involve migration of individuals along the course of the river and the lack of geographic barriers.

Measurements

Males of *M. olfersii* were measured for carapace length (CL), ischium (IL), merus (ML), carpus (CaL), propodus (PrL), dactylus (DL), total length of the chelipeds (ChL) of the second pair of pereopods (Fig. 1A and B), and height of the propodus (PrH) using a digital caliper (accuracy 0.01 mm). The structures size consists in the measurement from the article basis until the posterior region of the same article, meanwhile the propodus height consist between the larger distances in the palm. The ChL corresponds to the sum of the length of all the articles (ischium, merus, carpus, propodus). Measurements were taken from both chelipeds of the second pair of pereopods. Individuals that presented any type of injury on the articles that constitute the chelipeds, that is, any missing body parts in the cheliped (major or minor) or one single article (such as the propodus) were excluded from the analyses.

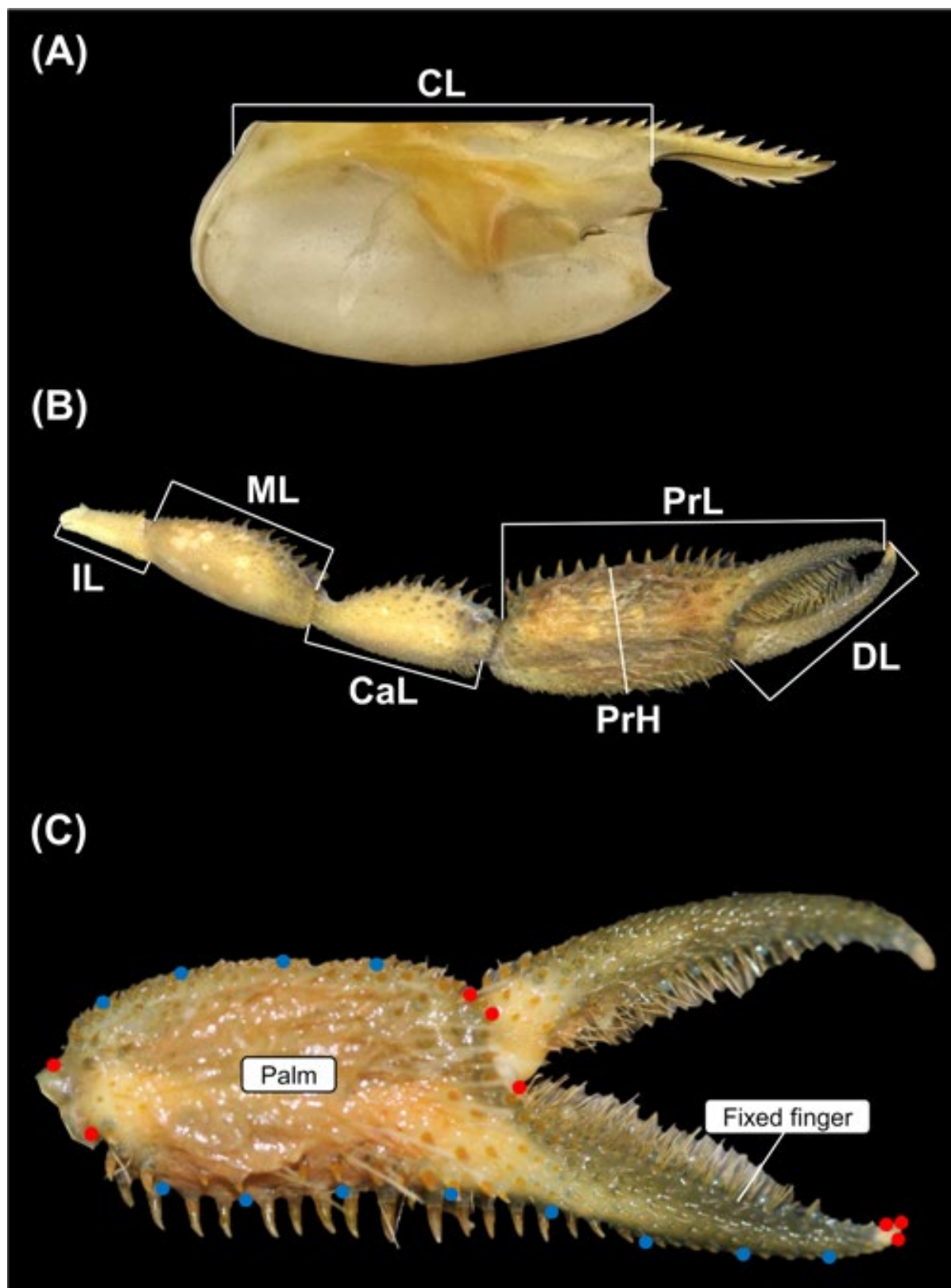


Fig. 1. (A) Carapace of *Macrobrachium olfersii* (Wiegmann, 1836). Dimension of carapace length (CL) measurements. (B) Larger cheliped of *Macrobrachium olfersii*. Exemplification of the dimensions used to measure the length and height of the articles of the larger cheliped. The same measurements were used for the smaller cheliped. (C) Propodus of the larger cheliped of *Macrobrachium olfersii* in the standard position used in the geometric morphometric analyses. Red and blue circles are the landmarks and semilandmarks, respectively. CL = Carapace length; IL = Ischium length; ML = Merus length; CaL = Carpus length; PrL = Propodus length; DL = Dactylus length; PrH = Propodus height.

Before morphometric analysis, the presence of outliers was verified using the interquartile range method. When identified, outliers were removed from the dataset (Hawkins 1980; Knorr and Ng 1998). The normality of the data was tested using the Shapiro-Wilk test ($\alpha = 0.05$) and the appropriate analyses were applied according to the parametricity of the data. To verify if the second pair of pereopods presented heterochely and handedness, a Mann-Whitney tests were applied ($\alpha = 0.05$), since these two characteristics can influence the morphometric relationships. We applied for the heterochely the size of the cheliped (larger and smaller) as independent variable and for the handedness the side of the larger cheliped (right and left). For both analyses, measurements of the total length of the chelipeds were used as dependent variables, then the analyses were performed using Statistica Statsoft 7.0 software.

Morphometric analysis

Individuals were separated and grouped into possible morphotypes based on the observation of cheliped morphology, according to variations that configure a polymorphism among males (cheliped size, number, size and angle of spines, presence of setae, and pubescence in the propodus, and degree of heterochely) (Kuris et al. 1987; Moraes-Riodades and Valenti 2004; Nogueira et al. 2020; Rios et al. 2021). The previously established morphological categories were submitted to a principal component analysis (PCA), an exploratory analysis that delimits the formation of groups based on a matrix containing the morphological variables measured to determine which variables are the most significant in the definition of groups. A non-hierarchical K-means cluster analysis (Sokal and Rohlf 1979) was applied to the morphometric data to initially separate juveniles from adults and then the possible morphotypes within adults. K-means is based on previously established groups through an iterative process in which it aims to minimize the variance within the groups and maximize the variance between the different groups. The results of the K-means age groups were refined by discriminant analysis ($\alpha = 0.05$). Discriminant analysis (DA) was then applied to verify significance, refine data, and assess the classification of the previously defined groups (by initial morphological analysis), establishing the final division of the morphological groups (Sampedro et al. 1999). Both analyses were performed using the PAST 4.05 software.

The validated groups (juveniles and morphotypes) were compared by analyzing the relative growth of the articles that constitute the chelipeds. This analysis was performed by linear regression ($\alpha = 0.05$). Then, an analysis of covariance (ANCOVA, $\alpha = 0.05$) was applied to verify if there were differences between the angular or linear coefficients of the morphometric variables between groups, as well as if the data of each morphological group were better adjusted to a single line or if

they must be represented by different linear equations (Pantaleão et al. 2014). Relative growth is a method that assesses the relationship between different body dimensions (dependent variables) with an independent variable (CL) (Moraes-Riodades and Valenti 2002). This analysis is based on the allometric equation $y = a \cdot x^b$ (Hartnoll 1978), which was linearized by the logarithmic equation $\ln y = \ln a + b \cdot \ln x$, where y = the measured dependent variable, x = the independent variable (CL), a = the point at which the line fixes on the coordinate axis (intercept), and b = the curve representing the allometric coefficient of the structure (slope). The allometric constant values were evaluated using Student's t-test ($\alpha = 0.05$), using the Statistica Statsoft 7.0 software. The null hypothesis $H_0: b = 1$, would indicate allometric status as positive allometry ($b > 1$), negative allometry ($b < 1$) and isometry ($b = 1$) (Hartnoll 1978).

Size at the onset of sexual maturity

The result of the most explanatory variables of PCA used to separate age categories (juveniles and adults) was applied to estimate the size at the onset maturity (SOM). SOM was estimated using the CL50% method (Sampedro et al. 1999). To estimate the maturity value, individuals were separated into size classes, based on carapace length (CL; independent variable) using the Sturges formula, and according to the relative frequency of each class (dependent variable). Then, the data were fitted to the logistic curve ($y = \frac{1}{1 + e^{r(CL - CL_{50})}}$), with CL_{50} being the carapace length at which 50% of the population is mature and r the slope of the curve.

Morphological analysis

Morphological analysis was performed to describe the morphology of adult male morphotypes confirmed by morphometric analyses. To this end, the ornamentations (the same used for the initial separation of the groups), and the variation in the size and shape of the larger cheliped were described. The angulation of the spines present in the propodus of the male morphotypes was evaluated to observe if there is a significant variation in the angle of projection of these structures between the morphotypes since these are considered important morphological characteristics in the determination of male morphotypes in some species of *Macrobrachium*. Dominant morphotypes are expected to have spines distributed along the cheliped, with wider angulations in comparison to the subordinate morphotypes, speculating that the spines work as defensive corporal features to the dominant morphotypes (Kuris et al. 1987; Moraes-Riodades and Valenti 2004; Nogueira et al. 2020; Rios et al. 2021). We randomly analyzed 10 spines present on the propodus of the largest cheliped from 10 individuals of each determined morphotype (adults only). Photographs of the

cheliped spines were taken using a stereomicroscope trinocular Zeiss Stemi 2000C. The angulation projection was measured using the angle tool from the software Zeiss AxioVision. After the measurements, the values of the spine angles were compared between the groups using a Kruskal-Wallis analysis ($\alpha = 0.05$) to verify if there is a difference between them, followed by a posteriori Dunn test ($\alpha = 0.05$).

Geometric morphometrics (General procedures)

To confirm the presence of morphotypes, a geometric morphometrics tool was used to assess if there was a statistical difference in the observed variation in the shape of the propodus of the largest cheliped of the morphotypes of *M. olfersii*. The images used in the geometric morphometric analysis were captured using a professional camera (Canon EOS Rebel T100) with an attached macro photography lens (100 mm). The same person photographed all specimens, and images were taken at the maximum resolution with a camera attached to a tripod. The distance between the lens and the structure was standardized in all photographs (40 cm). The constancy of the zoom and the position of the body structure were also standardized.

In this step, only one structure was analyzed between each morphotype, being the propodus of the largest cheliped of the second pair of pereopods. The positioning of the structures for photography was defined based on the handedness pattern between chelipeds. If there was no laterality, the largest propodus on both the right and left sides of the body can be considered analogous (i.e., symmetrically corresponding). Therefore, the photographs of the propodus were mirrored during the analysis so that they were oriented and standardized in the same anatomical position (Klingenberg et al. 2002). The propodus was analyzed because it is the main structure among the articles that constitute the second pair of chelipeds and the structure where the most notable morphological changes occur regarding sexual dimorphism, ontogenetic variation, or social dominance (Mariappan et al. 2000; Dennenmoser and Christy 2013; Lezcano et al. 2015; Karplus and Barki 2019).

Landmarks and semilandmarks were digitized on the structures to acquire the propodus shape. Eight landmarks were used to characterize the propodus shape and another 12 semilandmarks to capture the variation in the contour of the same structure (Fig. 1). All landmarks and semilandmarks were digitized using the tpsDig2 software (Rohlf 2005). A Generalized Procrustes Analysis (GPA) was then performed to superimpose, scale and rotate all landmarks and semilandmarks that were digitized in each structure (Rohlf and Slice 1990; Rohlf 2015). Errors related to measurements, capture of photographs, and digitization of landmarks was evaluated

following the protocol proposed by Viscosi and Cardini (2011), which means performing the entire process twice.

The weight matrix (partial warps + uniform components) that describes the shape of the structures was obtained using the software tpsRelw v.1.49 (Rohlf 2010), while the variation of the shape of the structures was obtained using the software tpsRegr v.1.31 (Rohlf 2009). The size of the structures was estimated by the centroid size. This variable is defined by the square root of the sum of the squared distances of each landmark and semilandmark and the central mass of the structure (Bookstein 1997). It was also obtained by the tpsRelw v.1.49 software. Multivariate regression was performed between centroid size and shape variables to verify the presence of an allometric effect in the dataset. Residues generated by this regression were used in subsequent statistical analyses between male morphotypes of *M. olfersii* to remove the allometric effect from the dataset (Klingenberg 1998 2016).

Propodus shape variation between male morphotypes was investigated by a multivariate analysis of variance (MANOVA) and a canonical variance analysis (CVA) using the residuals of the weight matrix (partial warps + uniform components). CVA was performed to explore the separation of morphotypes according to the propodus shape variation. Before performing these analyzes (MANOVA and CVA), a principal component analysis (PCA) was performed using the residuals of the weight matrix to check how many components represent more than 99% of the shape variation, after identifying the components, these were used to run MANOVA and CVA. This method is commonly used to reduce the dimensionality of the data matrix and increase the power of the statistical test without affecting the representation of the variation in the shape of the structures (Mitteroecker and Gunz 2009). PAST v.1.8 software was used to perform MANOVA, CVA, and PCA.

RESULTS

A total of 264 males of *M. olfersii* were collected. The carapace size ranged from 4.01 to 23.70 mm. Chelipeds size of larger and smaller chelipeds was statistically different ($U = 20347,50$ $p < 0.01$), indicating the occurrence of heterochely. No pattern of handedness was observed ($U = 20391,50$ $p = 0.85$). Only the largest cheliped of each individual was used for morphology and morphometric analyses.

PCA results corroborate the separation of *M. olfersii* males into juvenile and three different adult morphotypes (Fig. 2), designated as morphotypes 1, 2, and 3 (M1, M2, and M3, respectively). Principal components 1 and 2 (PC1 and PC2) explain 97.94% and 1.18% of the morphometric data

matrix, respectively, accounting for 99.12% of the total explanation (Table 1). ChL followed by PrL were the structures with the greatest contribution to PC1, indicating these structures can be used to separate the groups.

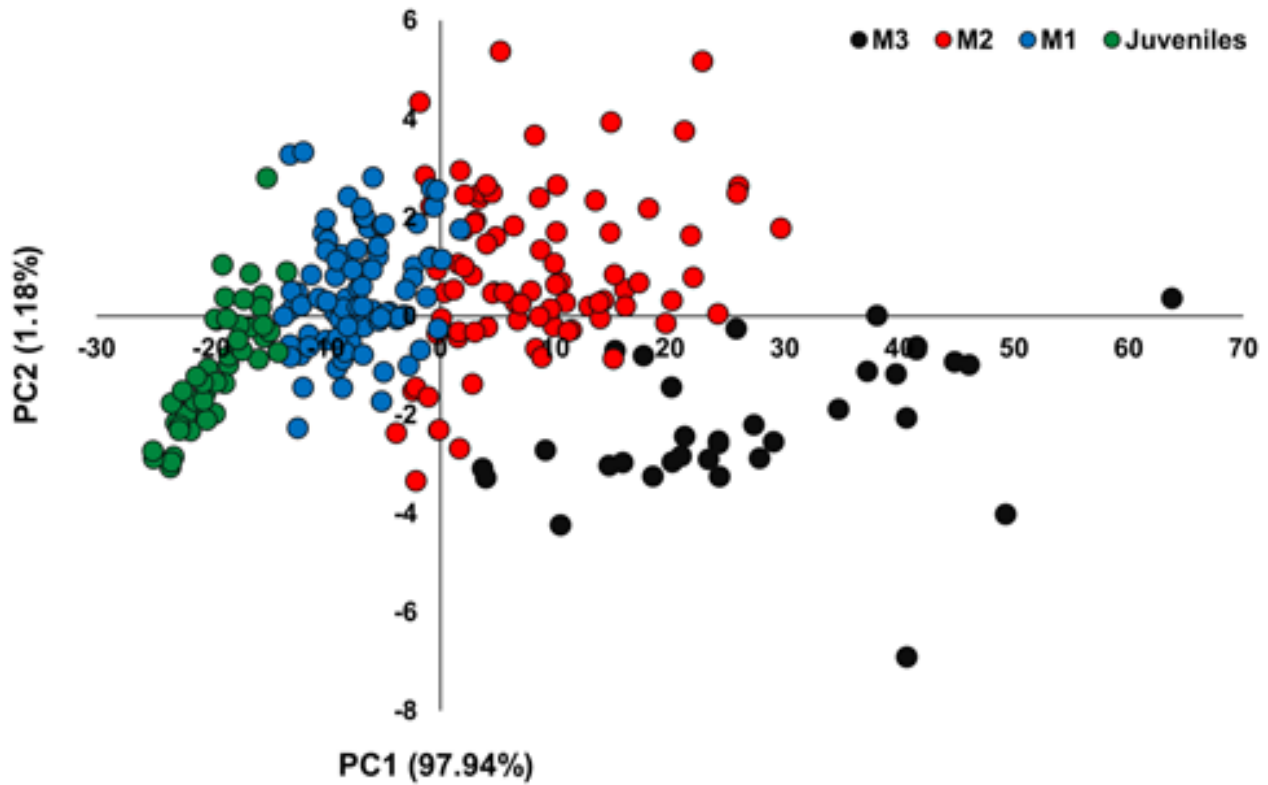


Fig. 2. *Macrobrachium olfersii* (Wiegmann, 1836). Principal Component Analysis (PCA) of morphometric variables. Values indicate the projection of components 1 and 2 (PC1 and PC2).

Table 1. *Macrobrachium olfersii* (Wiegmann, 1836). Correlation (Cor.) and contribution (Con.) values of the morphometric variables resulting from the Principal Component Analysis

Variable	PC1		PC2		PC3		PC4	
	Cor.	Con.	Cor.	Con.	Cor.	Con.	Cor.	Con.
CL	0.875	0.183	-0.480	-0.915	0.040	0.121	-0.011	-0.049
IL	0.910	0.077	-0.295	-0.227	-0.100	-0.123	0.037	0.069
ML	0.991	0.162	0.024	0.036	-0.025	-0.059	0.098	0.353
CaL	0.988	0.172	0.007	0.011	-0.074	-0.188	0.098	0.374
PrL	0.993	0.414	0.068	0.257	0.071	0.432	-0.060	-0.554
DL	0.960	0.197	-0.040	-0.075	-0.243	-0.730	-0.126	-0.571
PrH	0.933	0.126	0.149	0.183	-0.231	-0.456	0.065	0.192
ChL	1.000	0.824	0.010	0.076	0.005	0.063	0.013	0.243
Eigenvalue	255.78		3.08		1.19		0.52	
% variation	97.94		1.18		0.45		0.20	

CL = carapace length; IL = ischium length; ML = merus length; CaL = carpus length; PrL = propodus length; DL = dactylus length; PrH = propodus height; ChL = major cheliped length. Note: The numbers in bold correspond to the extremes of weighting for individuals in PC1.

Morphometric analysis

The separation of male morphotypes was confirmed by the discriminant analysis ($p < 0.05$), with more than 90% of the individuals correctly classified (J vs M1 = 99.17%; M1 vs M2 = 92.26%; M2 vs M3 = 97.71%). Among the 264 males of *M. olfersii*, 50 were identified as juveniles, 83 as M1, 98 as M2, and 33 as M3. The presence of outliers was not detected in the morphometric analysis. The results of the covariance analysis demonstrate that the relative growth of all morphometric relationships of the largest cheliped is statistically different between groups (Table 2), with all groups differing in the intercept of the line on the axis. There was an exception for the relationship PrH vs CL between juveniles and M1, which differed in the slope of the line. Despite the significant difference, relative growth demonstrates overlap in the size classes between groups. Some individuals that have similar carapace length, however, differ in the cheliped length (Fig. 3). Linear regression analysis showed that all morphometric relationships differed between groups ($p < 0.05$). None of the morphometric relationships showed positive allometry. Negative allometries were found for all morphometric relationships referring to individuals from M1 and for the majority regarding juveniles and M2. There were exceptions for the PrH vs CL relationship in juveniles and the IL vs CL, DL vs CL, and PrH vs CL relationships for M2, which showed isometry. For all morphometric relationships of M3, isometries were found (Table 3). Morphological sexual maturity was measured from the ratio of juveniles and individuals of the M1 morphotype. The size at which 50% of the population reaches sexual maturity was 8.95 mm (Fig. 4), with the smallest adult male having 7.03 mm CL and the largest juvenile male having 11.47 mm CL.

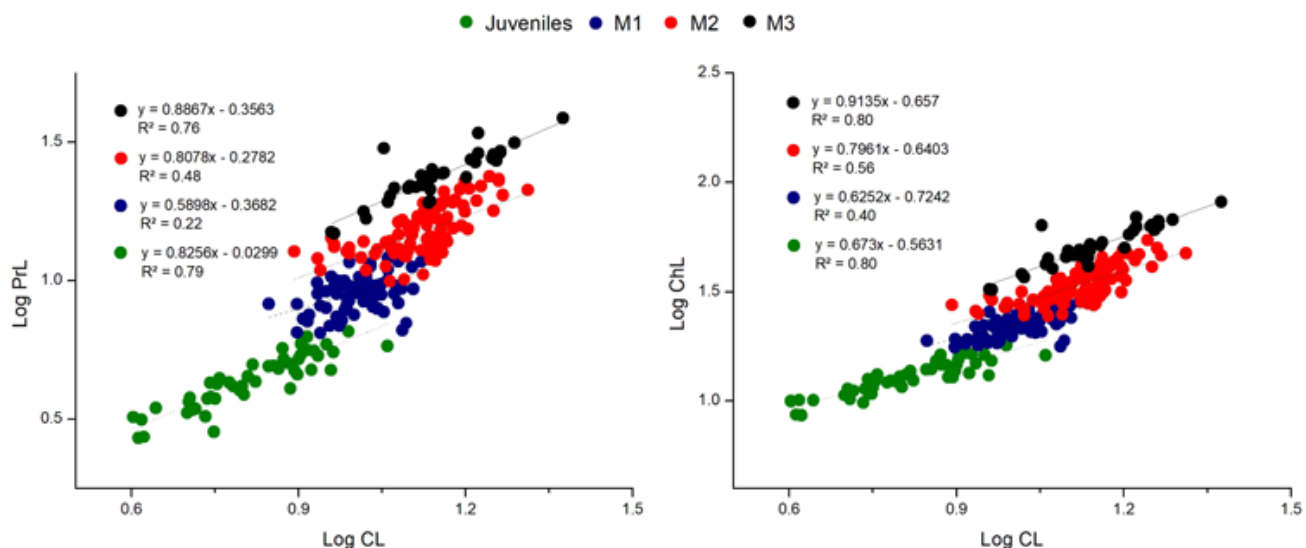


Fig. 3. *Macrobrachium olfersii* (Wiegmann, 1836). Discrimination of juveniles and adult morphotypes (M1, M2, and M3) according to the most explanatory morphometric variables from the principal component analysis, propodus length (PrL), and largest cheliped length (ChL).

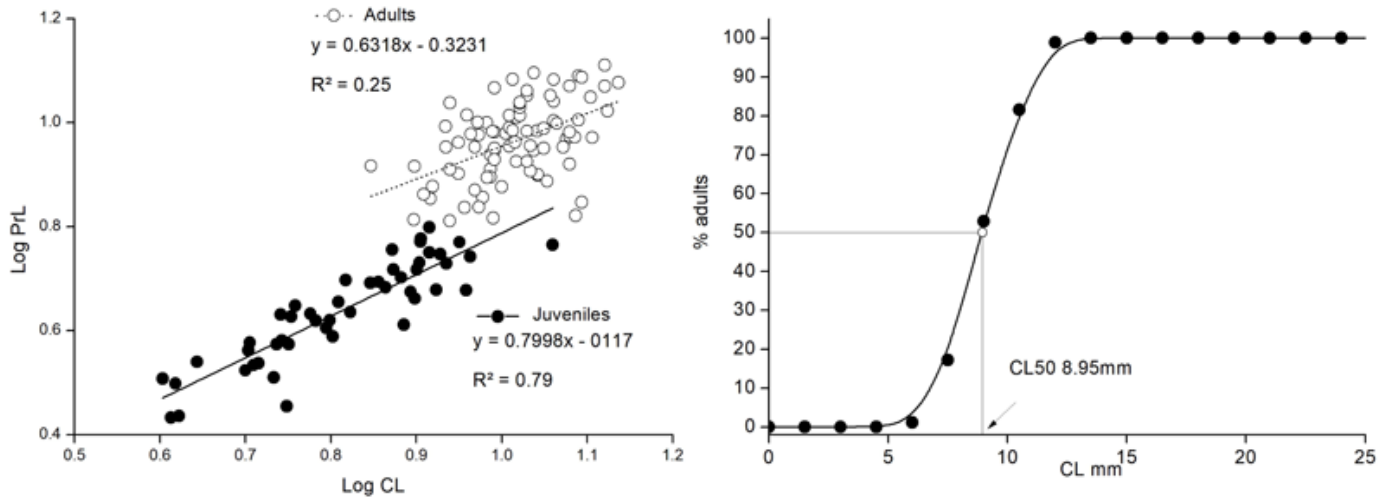


Fig. 4. *Macrobrachium olfersii* (Wiegmann, 1836). (A) Regression of the morphometric relationship of the propodus length (PrL) Vs. carapace length (CL) demonstrates the separation between juvenile and adult males. (B) A logistic curve shows the size at which 50% of males reach sexual maturity (CL₅₀).

Table 2. *Macrobrachium olfersii* (Wiegmann, 1836). Results of analysis of covariance (ANCOVA) of logarithmized morphometric variables

Relation	Groups	Parameters (log)	F	p
IL Vs. CL	J Vs. M1	a	21.23	< 0.05
		b	2.21	0.13
	M1 Vs. M2	a	3.96	< 0.05
		b	3.14	0.07
	M2 Vs. M3	a	18.25	< 0.05
		b	0.38	0.53
ML Vs. CL	J Vs. M1	a	67.06	< 0.05
		b	0.14	0.70
	M1 Vs. M2	a	83.55	< 0.05
		b	1.75	0.18
	M2 Vs. M3	a	242.60	< 0.05
		b	2.69	0.10
CaL Vs. CL	J Vs. M1	a	47.83	< 0.05
		b	0.05	0.81
	M1 Vs. M2	a	83.31	< 0.05
		b	3.53	0.06
	M2 Vs. M3	a	212.62	< 0.05
		b	1.87	0.17
PrL Vs. CL	J Vs. M1	a	113.51	< 0.05
		b	3.14	0.07
	M1 Vs. M2	a	89.31	< 0.05
		b	0.74	0.38
	M2 Vs. M3	a	121.86	< 0.05
		b	0.66	0.41
DL Vs. CL	J Vs. M1	a	84.50	< 0.05
		b	0.92	0.33
	M1 Vs. M2	a	95.94	< 0.05
		b	0.92	0.33

	M2 Vs. M3	<i>a</i>	98.88	< 0.05
		<i>b</i>	0.29	0.58
PrH Vs. CL	J Vs. M1	<i>a</i>	–	–
		<i>b</i>	0.30	< 0.05
	M1 Vs. M2	<i>a</i>	102.44	< 0.05
		<i>b</i>	1.05	0.30
	M2 Vs. M3	<i>a</i>	248.54	< 0.05
		<i>b</i>	0.001	0.97
ChL Vs. CL	J Vs. M1	<i>a</i>	96.40	< 0.01
		<i>b</i>	0.24	0.62
	M1 Vs. M2	<i>a</i>	104.51	< 0.05
		<i>b</i>	2.25	0.13
	M2 Vs. M3	<i>a</i>	206.69	< 0.05
		<i>b</i>	1.01	0.31

CL = carapace length; IL = ischium length; ML = merus length; CaL = carpus length; PrL = propodus length; DL = dactylus length; PrH = propodus height; ChL = major cheliped length.

Table 3. *Macrobrachium olfersii* (Wiegmann, 1836). Regression analysis of the morphometric data of the largest cheliped. Carapace length was used as the independent variable

Relation	Group	N	Intercept (log)	Inclin.	r ²	T(b=1)	Regression <i>p</i> -value	State of allometry
IL Vs CL	J	50	0.0537	0.6523	0.749	6.46	< 0.01	-
	M1	84	0.0541	0.7670	0.706	4.30	< 0.01	-
	M2	98	0.0658	0.9349	0.674	0.98	< 0.01	=
	M3	33	0.0889	0.8652	0.745	1.51	< 0.01	=
ML Vs CL	J	50	0.0464	0.5667	0.751	9.33	< 0.01	-
	M1	84	0.0820	0.6027	0.389	4.83	< 0.01	-
	M2	98	0.0660	0.7457	0.566	3.85	< 0.01	-
	M3	33	0.0878	0.9300	0.776	0.79	< 0.01	=
CaL Vs CL	J	50	0.0635	0.5813	0.628	6.59	< 0.01	-
	M1	84	0.0889	0.6075	0.355	4.41	< 0.01	-
	M2	98	0.0690	0.8227	0.592	2.56	< 0.01	-
	M3	33	0.0956	0.9850	0.766	0.15	< 0.01	=
PrL Vs CL	J	50	0.0612	0.8255	0.786	2.84	< 0.01	-
	M1	84	0.1193	0.5897	0.220	3.43	< 0.01	-
	M2	96	0.0866	0.8077	0.474	2.21	< 0.01	-
	M3	33	0.0876	0.8866	0.759	1.29	< 0.01	=
DL Vs CL	J	50	0.0723	0.8737	0.747	1.74	< 0.01	-
	M1	84	0.1000	0.7547	0.402	2.45	< 0.01	-
	M2	98	0.0931	0.8935	0.484	1.14	< 0.01	=
	M3	33	0.1498	0.9836	0.567	0.10	< 0.01	=
PrH Vs CL	J	50	0.0623	0.9833	0.834	0.26	< 0.01	=
	M1	84	0.1523	0.5938	0.145	2.63	< 0.01	-
	M2	98	0.1355	0.8130	0.265	1.37	< 0.01	=
	M3	33	0.1327	0.8055	0.528	1.46	< 0.01	=
ChL Vs CL	J	50	0.0484	0.6729	0.796	6.74	< 0.01	-
	M1	84	0.0845	0.6252	0.393	4.43	< 0.01	-
	M2	98	0.0707	0.7960	0.564	2.88	< 0.01	-
	M3	33	0.0796	0.9134	0.803	1.08	< 0.01	=

CL = carapace length; IL = ischium length; ML = merus length; CaL = carpus length; PrL = propodus length; DL = dactylus length; PrH = propodus height; ChL = largest cheliped length.

Morphological analysis

The carapace length of juvenile individuals ranged from 4.01–11.47 mm CL, with the largest cheliped length ranging from 8.60–18.04 mm ChL. The three morphotypes had a carapace length range of 7.03–13.70 mm for M1, 7.80–20.50mm for M2, and 9.10–23.70 mm for M3. Regarding the largest cheliped length, the variation among the three morphotypes was 17.62–30.50 mm for M1, 24.51–54.50 mm for M2, and 32.30–81.60 mm for M3. This indicates that the cheliped size presents greater variability between morphotypes than CL. Table 4 shows the size variation of all structures referring to the largest cheliped among the male groups.

Table 4. *Macrobrachium olfersii* (Wiegmann, 1836). Mean and size variation (mm) of the morphometric variables evaluated in juveniles and adults (morphotypes). Carapace length was used as the independent variable

Group	Variable	Mean ± S.d.	Range
Juvenile	CL	6.67 ± 1.65	4.01-11.47
	IL	2.53 ± 0.44	1.41-3.29
	ML	3.06 ± 0.49	2.04-4.20
	CaL	3.08 ± 0.56	2.11-4.45
	PrL	4.53 ± 1.01	2.71-6.56
	DL	2.41 ± 0.57	1.18-3.57
	PrH	0.79 ± 0.20	0.41-1.22
	ChL	13.21 ± 2.43	8.60-18.04
M1	CL	10.45 ± 1.41	7.03-13.70
	IL	3.80 ± 0.47	2.56-5.00
	ML	4.91 ± 0.64	3.78-6.46
	CaL	4.94 ± 0.68	3.69-6.49
	PrL	9.40 ± 1.57	6.47-12.90
	DL	4.76 ± 0.77	3.30-6.76
	PrH	1.90 ± 0.38	1.12-2.84
	ChL	23.06 ± 3.13	17.62-30.50
M2	CL	13.34 ± 2.30	7.80-20.50
	IL	4.93 ± 1.08	3.26-10.10
	ML	7.04 ± 1.27	5.13-10.50
	CaL	7.27 ± 1.40	4.91-10.40
	PrL	15.33 ± 3.50	5.30-23.80
	DL	7.72 ± 2.00	4.95-18.80
	PrH	3.48 ± 1.10	1.93-10.00
	ChL	34.57 ± 6.73	24.51-54.50
M3	CL	14.42 ± 3.21	9.10-23.70
	IL	5.80 ± 1.26	3.70-8.80
	ML	10.82 ± 2.48	6.63-16.30
	CaL	11.18 ± 2.71	6.78-17.80
	PrL	24.29 ± 5.37	14.83-38.70
	DL	11.68 ± 3.01	4.48-18.10
	PrH	7.38 ± 1.73	4.12-12.00

ChL

52.11 ± 11.57

32.30-81.60

CL = carapace length; IL = ischium length; ML = merus length; CaL = carpus length; PrL = propodus length; DL = dactylus length; PrH = propodus height; ChL = major cheliped length.

Through the morphological differences observed in the largest cheliped, it was possible to separate three morphotypes of adult males (M1, M2, and M3). They varied in shape, size, presence of spines, and pubescence in the palm of the propodus. M1 individuals have a cheliped similar to juveniles, being relatively small, with short spines and slightly projecting on the upper surface of the propodus and carpus; there is no gap formation between the fixed finger and the movable finger. Although they do not show pubescence in the palm of the propodus, they present evident heterochely (Fig. 5D). M2 males have larger and wider chelipeds than M1 males, with a greater number of spines on the articles containing a more obtuse angulation. There is no gap between the fixed finger and the movable finger, and it is possible to observe a scarce pubescence in the palm of the propodus. Heterochely is more pronounced than in M1 (Fig. 5F). M3 males have notably more robust chelipeds, with the propodus, carpus, and merus having a rounded shape, especially in the propodus. Robust spines are present along the entire cheliped with an approximately orthogonal angulation, mainly in the propodus. There is an evident formation of a gap between the fixed finger and the movable finger, in addition to the presence of tufts of setae on the entire inner surface of the fingers. The palm region of the propodus presents a thick layer of pubescence (Fig. 5H). Heterochely in M3 is the most evident among morphotypes of *M. olfersii*.

Kruskal-Wallis analysis showed that there are differences in the angulation of cheliped spines among morphotypes (M1, M2, and M3) ($H = 45.82$; $p < 0.01$), with all morphotypes differing from each other (Dunn, $p < 0.01$) (Table 5).



Fig. 5. *Macrobrachium olfersii* (Wiegmann, 1836). Specimens and chelipeds of the male morphotypes, (A and B) Juvenile, (C and D) Morphotype 1, (E and F) Morphotype 2, and (G and H) Morphotype 3. All scale bars correspond to 10 mm, except for the scale bar present in 6B, which corresponds to 5 mm.

Table 5. *Macrobrachium olfersii* (Wiegmann, 1836). Mean, maximum and minimum values of spine angulation measured at different points of the largest cheliped of morphotypes M1, M2, and M3

Morphotype	Spine Angle (°)	
	Mean	Range
M1	27.82 ± 7.09	14.07-39.45
M2	38.71 ± 7.45	28.82-55.27
M3	82.79 ± 7.91	66.27-98.53

Geometric morphometrics

A total of 194 shrimp were analyzed for the geometric morphometric analysis: 66 individuals from M1, 95 individuals from M2, and 33 individuals from M3.

Propodus shape variation

Statistical differences in propodus shape were observed among all male morphotypes (MANOVA; Wilk's lambda = 0.2345; $F = 15.98$; $p < 0.001$). The analysis accurately separated M1, M2, and M3 individuals with efficacy of 80%, 73%, and 93%, respectively.

The CVA also showed differences in the propodus shape between morphotypes, with an overlap between morphotypes 1 and 2 higher than the morphotypes 2 and 3. The canonical variable 1 (CV1) explained 89.17% of the variation while the canonical variable 2 (CV2) explained 10.83% of the variation (Fig. 6A).

The variation in propodus shape among morphotypes was more evident in M3 than in relation to the other two male morphotypes (M1 and M2). The main differences were located in the anterior and posterior regions of the palm and the fixed finger (Fig. 6B). The palm in M3 has a more robust shape than the other two morphotypes, so this region is wider in M3. In addition, the fixed finger of M3 is shorter and more robust than the fixed finger of the other two male morphotypes (M1 and M2; Fig. 6B).



Fig. 6. *Macrobrachium olfersii* (Wiegmann, 1836). (A) Scatter plot of canonical variation analysis (CVA) performed with the coordinates of variation in the propodus shape of the male morphotypes. (B) Variation in the propodus shape of each male morphotype. M3 presents evident differences in the shape of the palm region and also in the fixed finger in relation to the other morphotypes.

DISCUSSION

The morphometric and morphological analyses applied confirmed the occurrence of different morphotypes. The main differences found were related to the size, development, shape, and ornamentation of the largest cheliped of the second pair of pereopods. Such differences confirm our initial predictions. Allometric relationships pointed out differences in structures that determine the separation of hierarchical groups in this species, an aspect that is generally found in other congener species that present social dominance (Karplus and Barki 2019; Nogueira et al. 2020).

The number of morphotypes that are described is a variable characteristic among *Macrobrachium* species. Some species present two morphotypes, such as *M. grandimanus*, *M. brasiliense*, and *M. caementarius* (Wortham and Maurik 2012; Rojas et al. 2012; Nogueira et al. 2020), while others present up to 5 morphotypes, such as *M. tenellum* (Vargas-Ceballos et al. 2021). The latter is the only species to present such a high number of morphotypes. Three morphotypes were found for *M. olfersii* (M1, M2, and M3), just as previously observed for *M. rosenbergii*, *M. idella*, and *M. acanthurus* (Kuris et al. 1987; Soundarapandian et al. 2013; Rios et al. 2021). Thus, the hierarchical structure is similar in these species.

***M. olfersii* morphotypes descriptions**

In the recent study by Rossi et al. (2022), the same amount of male morphotypes was found for *M. olfersii* compared to the present study, which highlights the importance of analyzing the variation of different populations of the same species. Nevertheless, the application of the morphotypes studies in a single population may help to improve the knowledge about this feature in *M. olfersii*, since it will not only confirm the presence of distinct males morphotypes, but also avoid any bias that could be present by possible distinct local variations of different populations. It is important to highlight that a minute description for each morphotype is necessary, to prevent that possible doubts in the morphotypes separation arise. By carrying out this detailed description, it is possible to reduce, or even suppress the description of subtypes (transitional morphotypes), such as the once observed for *M. rosenbergii*, which previously 5 morphotypes were described, but only three remains due two of them were rectify as subtypes (Kuris et al. 1987). In addition, the use of a robust description, in details, will help in future studies that aim to approach some biological and ecological features in the three morphotypes of *M. olfersii*.

Another relevant aspect is the presence of juveniles, since they are the previous morphological distinction before the M1. By using these demographic categories, and separating them from the adults using the sexual maturity, we were able to access the observed overlap that occurs in the carapace length (CL) among the juveniles and the morphotypes. Thus, this demonstrate the importance that the morphometry of the cheliped and the morphology of the propodus, as well as, the detailed ornamentation description in the separation of the distinct morphotypes of *M. olfersii*. We emphasize the importance of conducting behavioral studies to truly understand the dominance that exist between these male morphotypes and the size variations that occurs among them, that will bring more support to the described here and by Rossi et al. (2022).

Population structure studies of *M. olfersii* support the occurrence of representatives of the morphotypes described in the present study. These studies have reported significant variations in the

size of chelipeds, mainly among males of different size classes (Lombardi et al. 1996; Ammar et al. 2001; Mossolin and Bueno 2002 2003; Pescinelli et al. 2016). Our result reinforces that the presence of morphotypes is a common feature in some species of this genus as mentioned by Moraes-Riodades and Valenti (2004). In addition to the eight species with different morphotypes already described, at least 19 other species are also expected to present this characteristic according to behavioral observations carried out on the interaction of males (Karplus and Barki 2019).

Some males of *Macrobrachium* have an exaggerated development of chelipeds. Generally, they have a mating system called "mate guarding", in which males are larger than females and have overdeveloped chelipeds that are used as weapons. All caridean species that have described male morphotypes presumably have this type of mating system (Correa and Thiel 2003; Thiel et al. 2010; Bauer and Thiel 2011; Bauer et al. 2014; Karplus and Barki 2019). Alternatively, some species of the same genus also present a mating system called "pure search", in which females are larger than males. These species do not have overdeveloped chelipeds and do not present any type of agonistic behavior to gain access to sexual partners (Correa and Thiel, 2003; Bauer and Thiel 2011). Therefore, some species of *Macrobrachium* do not have male morphotypes, specifically in the case of species that have the "pure search" mating system. In these species, there is no variation in total length and morphology of chelipeds, as happens in *M. jelskii* (Miers, 1878) and *M. pantanalense* Dos Santos, Hayd & Anger, 2013 (Nascimento et al. 2020; Nogueira et al. 2022a).

On the other hand, species that have morphological and behavioral characteristics that indicate the presence of morphotypes may still not have the exaggerated development of chelipeds. This is the case for *M. iheringi* (Ortmann, 1897) (Nogueira et al. 2019 2022b). In the same way, there may be a variation in populations of species whose attribute has already been reported, demonstrating the absence of morphotypes in specific populations, such as in *M. amazonicum* (Pantaleão et al. 2012; Paschoal et al. 2019; Silva et al. 2019). This variation between the non-occurrence of morphotypes in some species or only in specific populations may be related to different factors, such as regulations in the production of peptides due to the small size of the androgenic gland (Rocha and Barbosa 2017; Paschoal and Zara 2019), or by environmental factors (Maciel and Valenti 2009), e.g., nutrients available in the environment, availability of shelters, temperature, and social growth control. Another possibility is related to selective sampling (Santos et al. 2016; Rios et al. 2021), by excluding the variety of size classes, i.e. picking only smaller individuals.

The Amazon River shrimp (*M. amazonicum*) is the only species so far that may or may not have populations with distinct male morphotypes. Most populations that have morphotypes have been reported in rivers with access to the estuary (Nogueira et al. 2020), while those that do not have distinct male morphotypes are in exclusively freshwater environments (Pantaleão et al. 2012;

Paschoal et al. 2019; Silva et al. 2019). In populations of this species without distinct male morphotypes, females are larger than males and present characteristics of the “pure search” mating system. Therefore, these populations need to be analyzed with caution. A study by Anger (2013) reported the possibility that specific populations of *M. amazonicum* from isolated estuarine regions, or that do not present morphotypes, may belong to another species. These populations of *M. amazonicum* with such characteristics may be *M. pantanalense*, especially because they are found in environments outside its type locality (Vergamini et al. 2011; Calixto-Cunha et al. 2021), and present high morphological similarity and genetic proximity to *M. amazonicum* (Vergamini et al. 2011; Robe et al. 2012). Thus, we recommend that future studies that address populations of *M. amazonicum* that are located in the central-west, southeast, and south regions of Brazil and that present only small males use molecular methods to assist in species identification.

Most *Macrobrachium* species with the presence of distinct male morphotypes present characteristics decisive for their identification (Kuris et al. 1987; Moraes-Riodades and Valenti 2004; Rojas et al. 2012; Nogueira et al. 2020; Rios et al. 2021; Vargas-Ceballos et al. 2021). These characteristics are mostly represented by the size, color, and morphology of the cheliped, and in addition, body size can also vary, but this characteristic is not always observed (Holthuis 1950 1952). Although there is no marked difference in carapace length between the three described morphotypes of *M. olfersii*, cheliped length varies significantly, mainly between M3 and the other morphotypes. This pattern was also found for *M. acanthurus*, *M. amazonicum*, and *M. rosenbergii* (Kuris et al. 1987; Moraes-Riodades and Valenti 2004; Rios et al. 2021). This shows that body size does not establish a hierarchical pattern between morphotypes, since in many cases there is an overlap in carapace length among groups. This reinforces the importance of chelipeds in the discrimination of male morphotypes and in the establishment of dominance among morphotypes since this structure is essential for the success of dominant individuals during fights (Barki et al. 1992 1997; Karplus 2005).

Morphometry of the major cheliped

The allometric coefficients observed for the three male morphotypes and in juveniles of *M. olfersii* indicate a difference between M3 and the other groups. M3 is the only group that presents an isometric development of all the articles that constitute the largest cheliped of the second pair of pereopods. The other groups have mostly shown negative allometry in the development of these structures. Furthermore, the slope values showed an increase from M1 to M3, demonstrating the importance of chelipeds in the categorization of social dominance hierarchies in *Macrobrachium*. This change in the larger morphotype configures a greater investment in the development of the

larger cheliped. This structure is more frequently used to strongly squeeze opponents during fights, so dominant males have advantages over submissive males, facilitating access to better resources, such as territories, food, and sexual partners (Conover and Milner 1978; Mariappan et al. 2000).

There was a high overlap in carapace length between male morphotypes and even with juveniles. There were small dominant individuals with CL similar to large juveniles. However, when we compare the cheliped length of these same individuals (smallest M3 and largest juvenile), we observed that this structure in M3 males was twice the size than in juveniles. This indicates differential investment in the development of chelipeds by the dominant individuals, even in cases where animals are small (CL). Due to this high overlap in CL among the male morphotypes, we can assume that the transition from one morphotype to another in *M. olfersii* occurs in just one molt, as was suggested for *M. amazonicum* (Pantaleão et al. 2014). Many factors can influence the transition between morphotypes, the main one being the presence or absence of a dominant individual at the site. These individuals are known to occupy a specific area in the environment, and when the dominant morphotype dies or loses its weapon, another individual usually starts to grow at a higher rate and becomes the dominant one (Karplus and Barki 2019; Ibrahim et al. 2021; Vargas-Ceballos et al. 2021).

Chelipeds morphology and propodus shape

The morphology of chelipeds varies in *M. olfersii*, mainly regarding spines, presence of pubescence, and propodus shape. The amount and arrangement of spines varying among morphotypes are expected in *Macrobrachium* (Moraes-Riodades and Valenti 2004; Nogueira et al. 2020; Rios et al. 2021), since these structures are used in agonistic behavior, being considered defensive ornamentation (Moraes-Riodades and Valenti 2004). In *M. olfersii*, the number of spines, as well as their angulation, is evidently higher in M3, another diagnostic feature for the identification of this morphotype. These characteristics (amount and angle of spines) may be related to the frequency of disputes that occur between dominant individuals of these shrimps, which may increase the damage caused to opponents during fights (Thiel et al. 2010; Rojas et al. 2012; Bauer et al. 2014; Nogueira et al. 2021).

The presence of pubescence in chelipeds is also a feature used to separate the morphotypes of *M. olfersii*. This ornamentation, despite being common in dominant morphotypes of some *Macrobrachium* species, was included as a comparative parameter only among the morphotypes of *M. acanthurus* (Rios et al. 2021), as the authors suggested there is a relationship between this structure and reproductive fitness. In *M. grandimanus*, a characteristic similar to pubescence in the claws was also recorded, being the presence of a patch of setae on the propodus of dominant

morphotypes (Wortham and Maurik 2012). As in *M. acanthurus*, this pubescence of *M. olfersii* morphotypes may play chemoreceptor functions for the perception of mating-receptive females (Altner et al. 1983). Another hypothesis is that these structures can serve as a visual signal from dominant to submissive individuals to establish dominance without a direct fight. The presence of pubescent setae on the cheliped lets this structure appear larger than it actually is, inhibiting potential competitors (Wortham and Maurik 2012). In *M. olfersii*, this can be represented not only by the presence of pubescence but also by the difference found in the propodus shape between the male morphotypes, a structure that is more robust in M3.

The geometric morphometric analysis demonstrated that this tool can be effective in identifying and separating male morphotypes in shrimp species by evaluating whether the differences observed between the propodus shape are significant. A variation in the shape of this structure was observed among morphotypes of *M. olfersii*, showing an increase in the palm region of the propodus of M3, which is more robust and wider, while the fixed finger is shorter and more robust, in comparison to M1 and M2, being a pattern similar to that observed between the propodus shape of the morphotypes *Açu* and *Mirim* of *M. brasiliense* (Nogueira et al. 2022). The more robust shape of the propodus in M3 means that this structure can generate more force than the propodus of subordinate morphotypes since a larger propodus can accommodate more muscle mass (Levinton et al. 1995; Palaoro et al. 2020). Therefore, the dominant morphotypes are likely to be able to squeeze their opponents more tightly than the other morphotypes, helping these organisms to maintain dominance over submissive males.

CONCLUSIONS

The results presented here confirmed the existence of three morphotypes among adult males of *M. olfersii*, named M1, M2, and M3. The separation of these groups is mainly given by the differences in the morphology and morphology of the largest cheliped of the second pair of pereopods, following the pattern that is observed for other species with morphotypes in *Macrobrachium*. Although the individual's body characteristics confirm the existence of a social hierarchy within this species, studies on reproductive behavior as well as competitive interactions between males still need to be carried out to determine the role of each morphotype in the population. In addition, the confirmation of a description of morphotypes in one more species of *Macrobrachium*, by using a single population, reinforces that the occurrence of social dominance hierarchies may be a characteristic of this genus, being exclusive to species that have a mating

system that is built on "mate guarding", where the largest and strongest morphotypes monopolized the receptive females, and smaller males use alternative mating tactics to gain access to females.

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