



## Sexual dimorphism in a freshwater atyid shrimp (Decapoda: Caridea) with direct development: a geometric morphometrics approach

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### Abstract

*Neocaridina davidi* is a caridean shrimp that has gained popularity in recent years as an ornamental species. Using geometric morphometrics, we investigated sexual dimorphism in carapace and second abdominal segment shape of *N. davidi*. Adult females displayed a more elongated carapace and a longer rostrum than males. However, male carapace shape was similar to that of juvenile females. The second abdominal pleura was more elongated and wider in adult females than in males. Significant differences were found in centroid size for the carapace and the second abdominal segment between sexes, which is consistent with sexual size dimorphism. These results support the hypothesis of a “pure search” mating system in *N. davidi*, where small males search actively for receptive females, and after insemination they continue searching.

**Key words:** *Neocaridina davidi*, carapace, second abdominal segment, geometric morphometrics, sexual dimorphism

### Introduction

Sexual dimorphism is a widespread phenomenon among animal species (Andersson 1994; Wyman *et al.* 2013). The evolution of sexually dimorphic characters has been widely studied, and it has been explained mainly based upon mechanisms of sexual selection, intrinsic differences between the reproductive roles of males and females, and ecological causes (Hedrick & Temeles 1989; Herler *et al.* 2010). But at the same time a certain concern has arisen regarding the methods that are used for measuring the magnitude of these dimorphisms. The first approaches were based on traditional morphometrics, which consist on the analysis of measured distances on individuals by multivariate statistical methods, yet they were not able to recover the overall shape of the studied anatomical structure (see Rohlf & Marcus 1993 for revision). However, on the past two decades geometrical methods have been developed in the attempt to overcome these limitations. Geometric morphometrics is the statistical analysis of shape variation based on landmark coordinates (Mitteroecker & Gunz 2009). Unlike common metrical approaches, this method considers the whole geometry of the studied structures and thus is able to provide an accurate measure of the shape even when analysing complex morphological forms (Bertin *et al.* 2002). In landmark-based geometric morphometric methods two- or three-dimensional landmarks coordinates are collected. These landmarks define the locations of particular anatomical traits representing discrete biological attributes. After the effects of non-shape variation (i.e. variation in position, orientation and scale of the specimens) are mathematically held constant, the resulting shape variables are then analysed (Adams *et al.* 2004). The presence of a hard skeleton with spines and sutures in decapod crustaceans, simplifies the task of defining homologous landmarks. Hence, geometric morphometric techniques constitute a valid approach for evaluating shape variability (Trevisan *et al.* 2012;

Zimmermann *et al.* 2012). Many studies have been conducted applying geometric morphometric techniques in decapod crustaceans to assess sexual dimorphism (Accioly *et al.* 2013; Barria *et al.* 2011; Giri & Loy 2008; Marochi *et al.* 2016; Responde *et al.* 2015; Rufino *et al.* 2004; Scalici & Gibertini 2009; Trevisan *et al.* 2012), allowing in some cases to find subtle but significant differences in structures where traditional morphometrics were not capable of doing so (Accioly *et al.* 2013; Barria *et al.* 2011).

*Neocaridina davidi* (Bouvier, 1904) (Caridea, Atyidae) is a freshwater species native from Asia. Over the past several years, it has become one of the most popular ornamental shrimp species in the aquarium industry due to its attractive colouration, its tolerance of varying water qualities and its brooding ecology (Heerbrandt & Lin 2006; Klotz *et al.* 2013; Patoka *et al.* 2015). Furthermore, *N. davidi* has morphological features that are associated with highly abbreviated development. It deposits relatively large eggs which females carry attached to their pleopods during embryonic development, and hatching gives rise directly to decapodids adapted to a benthic life habit. These decapodids have well-developed appendages, eyes and sensory setae on the antennae, but no free uropod (Barbier 2010; Pantaleão *et al.* 2015).

The aim of the current study is to apply a novel quantitative technique to investigate potential sexual dimorphism in carapace and second abdominal segment shape of the red-cherry shrimp *Neocaridina davidi* based on geometric morphometrics analysis.

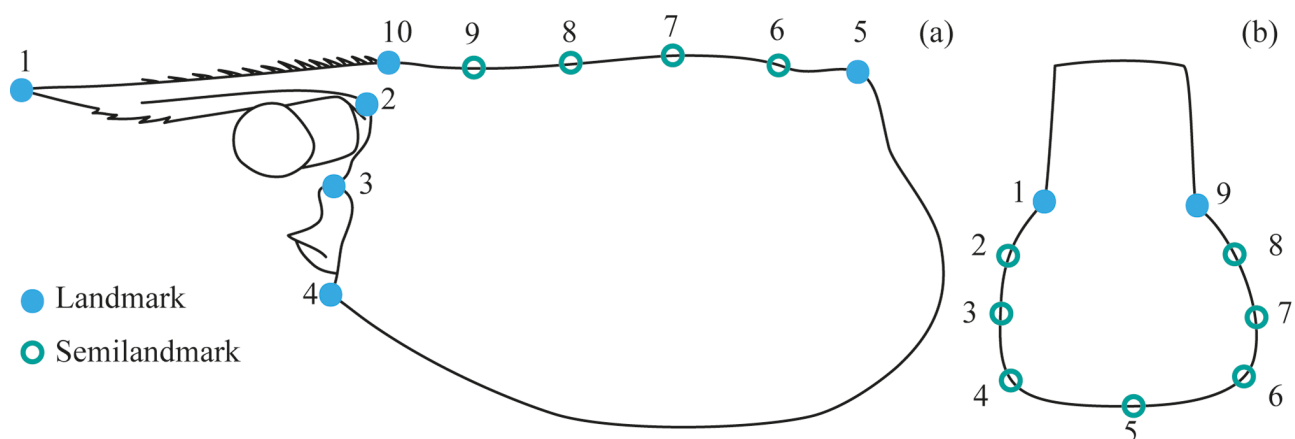
## Materials and methods

The study was carried out on two samples of *Neocaridina davidi* obtained from a stock (commercial supplier: Acuamanus Aquarium, Buenos Aires, Argentina) maintained in the laboratory under controlled conditions (water temperature: 27±1 °C; pH 7.5; photoperiod: 14L: 10D). Specimens were kept alive but out of the water during the image acquisition. Two samples were used to photograph the two rigid body structures of interest, one for the carapace and one for the second abdominal pleura. 92 individuals (57 females and 35 males) were sampled to obtain carapace images, and 61 individuals (29 females and 32 males) for the second abdominal pleura. Specimens were sexed based on the morphology of the first and second male pleopods (Barbier 2010; Pantaleão *et al.* 2015). Although juveniles may be useful for ontogenetic studies, they were excluded of the analysis as sexual dimorphism in male pleopods is not evident during the early stages (Pantaleão *et al.* 2015), and thus sex classification was not possible. Sexual maturity in females was assessed by observation of the ovary through the semi-translucent carapace.

All specimens were photographed in lateral view with a Sony DSC-TF1 digital camera (16.1 megapixels) under a stereoscopic microscope (Carl Zeiss Stemi 2000-C). The alignment of the two teeth on the pterygostomial angle of the carapace with the optical axis was used as criteria for standardizing the specimen position under the microscope.

Six landmarks were digitized for the carapace using the tpsDig 2.22 and tpsUtil 1.6 software (Rohlf 2004a; 2005), and two for the second abdominal pleura (Table 1, Fig 1). The selection of the landmarks was based on Bissaro *et al.* (2013) and Torres *et al.* (2014). In addition, four semilandmarks were digitized for the carapace and seven for the second abdominal pleura. Semilandmarks are used to represent homologous curves and surfaces by sets of points, establishing a geometric homology between corresponding semilandmarks across the sample (Gunz & Mitteroecker 2013). The coordinates of semilandmarks were aligned with the tpsRelw 1.54 software (Rohlf 2004b) using the sliding semilandmark algorithm, i.e. the semilandmarks are allowed to slide along their curve or surface in order to remove the effects of arbitrary spacing of semilandmarks. The bending energy between all specimens and the average shape is minimized by the iterative sliding (Gunz & Mitteroecker 2013). Landmarks coordinates were submitted to a Generalized Procrustes analysis (GPA) in PAST software (Hammer *et al.* 2001). Since raw landmark coordinates do not only comprise information on size and shape of the landmark configurations, but also on their position and orientation, landmark coordinates are not directly suitable for statistical analysis (Mitteroecker *et al.* 2013). GPA translates all specimens to the origin, scales them, and rotates them to minimize the total sums-of-squares deviations of the landmark coordinates from all specimens to the average configuration (Adams *et al.* 2013), and thus removes non-shape variability. Centroid size (CS) was used as measure of size; it was computed as the square root of the summed squared distances between all landmarks and their centroid with the tpsRelw software. Differences in CS for the carapace and second abdominal segment

between males and females were analysed with a Mann-Whitney-Wilcoxon Test performed in R 3.2.3 software (R Core Team 2015).



**FIGURE 1.** Location of landmarks and semilandmarks on each anatomic structure in *Neocaridina davidi*. (a) carapace; (b) second abdominal pleura; left side, lateral view.

Allometry was tested by multivariate regression of the Procrustes coordinates on CS for the whole data set and for females and males separately using R 3.2.3 software. The linear model used is:  $CS_i = PR_i' b + e_i$ , where  $PR_i$  is the vector of Procrustes coordinates for each specimen, and  $e_i$  is the error term (not correlated with PR).

The statistical significance of the allometric regressions was tested by a permutation test against the null hypothesis of complete independence between the dependent (Procrustes coordinates) and independent variables (CS). Royston's Normality Test (Royston 1983) was applied to each population sample to check for normality. Shape variation of the carapace and second abdominal pleura was first analysed performing a principal component analysis (PCA) on shape data (Procrustes coordinates). Sexual dimorphism was tested with a multivariate Cramer test (Baringhaus & Franz 2004) on shape data. When allometry was detected, differences between sexes were also evaluated on the residuals of the regression, as these are considered allometry-free shape variables. For the carapace, differences between males, adult females and juvenile females were analysed, whereas for the second abdominal pleura only males and adult females were considered.

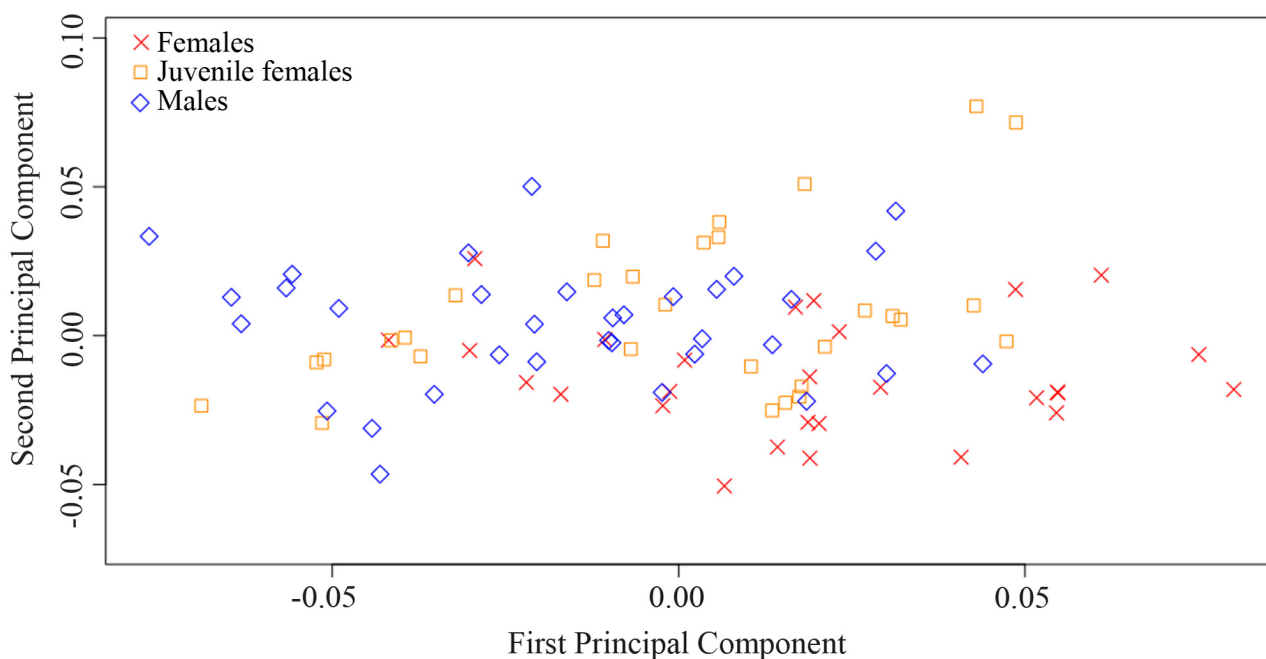
**TABLE 1.** Identification of landmarks used in both sexes of *Neocaridina davidi*.

| Landmark                       | Description  |
|--------------------------------|--|
| <b>Carapace</b>                |  |
| 1                              | Tip of rostrum   |
| 2                              | Maxima of orbital cavity curvature                     |
| 3                              | Inferior orbital angle of carapace/ postrostral carina |
| 4                              | Tip of tooth on pterygostomial angle                   |
| 5                              | Posterodorsal vertices of carapace                     |
| 6–9                            | Semilandmarks  |
| 10                             | First postorbital teeth                                |
| <b>Second abdominal pleura</b> |  |
| 1                              | Anterior base of pleura                                |
| 2–8                            | Semilandmarks  |
| 9                              | Posterior base of pleura                               |

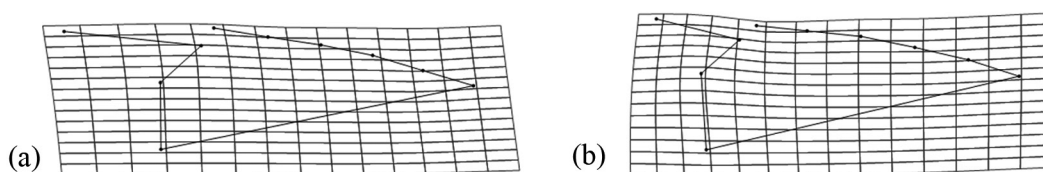
## Results

**Centroid size.** Mean and standard deviation values of the CS were obtained for the carapace:  $4.87 \pm 0.86$  in females and  $4.32 \pm 0.35$  in males. As for the second abdominal pleura:  $3.77 \pm 0.72$  in females, and  $2.59 \pm 0.34$  in males. Significant differences were found between sexes for the carapace CS ( $W = 1311$ ,  $P = 0.008$ ), and for the second abdominal segment CS ( $W = 858$ ,  $P < 0.001$ ).

**Shape. Carapace.** A statistically significant relationship between shape and size across all specimens ( $R^2 = 0.6582$ ,  $F = 6,838$ ,  $P < 0.001$ ) illustrated significant allometry. However, when analysed separately, females exhibited significant allometry ( $R^2 = 0.9343$ ,  $F = 4.98$ ,  $P < 0.0017$ ) whereas for males no statistically significant relationship between shape and size was found ( $R^2 = 0.076$ ,  $F = 2.148$ ,  $P = 0.08$ ). Principal component analysis (PCA) provided a graphic display of the overall shape variation (Figure 2). The first 3 principal components accounted for 83.37 % of total variance. Males and juvenile females are grouped together, while adult females are separated mainly by the first axis. Adult females displayed a longer rostrum than males (landmarks 1 and 10). Carapace was longer in adult females (landmarks 3 to 5) than in males (Figure 3).



**FIGURE 2.** Scatter plot of first *versus* second principal component axes for the total variation of the carapace shape for females, juvenile females and males of *Neocaridina davidi*.



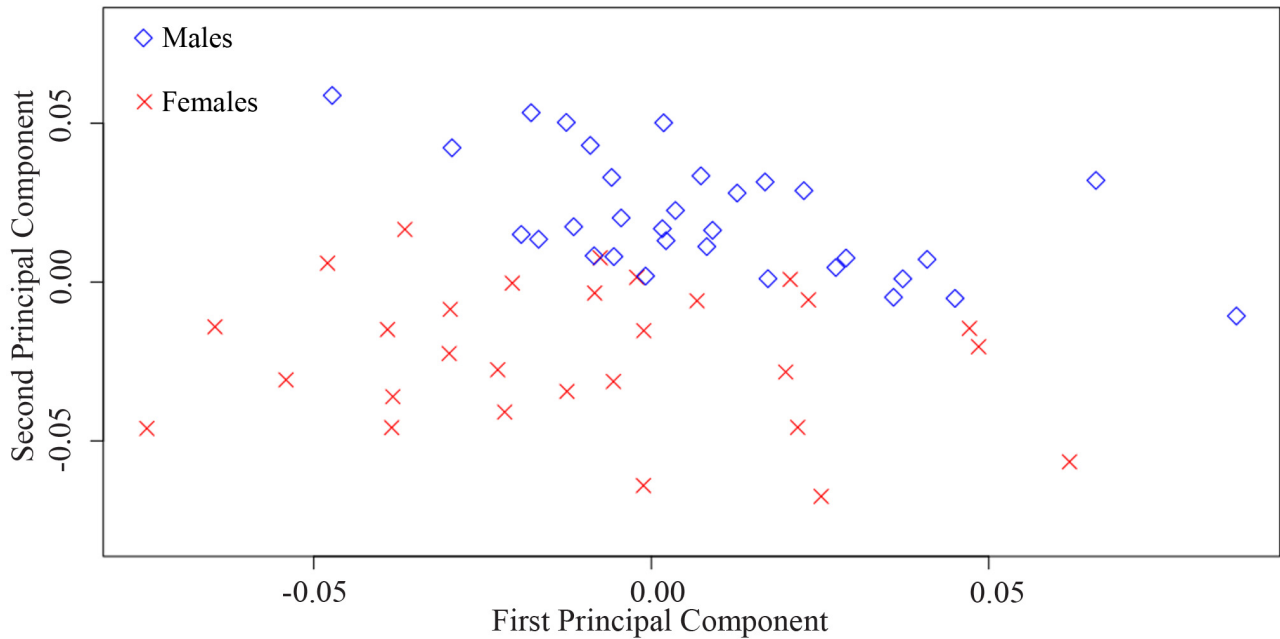
**FIGURE 3.** Relative deformations grids illustrating the variation in the mean shape of the carapace for (a) females and (b) males.

The sample covariance matrix was ill conditioned i.e. it is not possible to calculate accurately the inverse matrix by a computational algorithm. Thus parametric tests were not suitable for the analysis of this data set, since it could lead to a numerical error and statistic misinterpretation. The best approach to analyse this dataset is a non-parametric test, particularly one that does not require the inverse of the covariance matrix.

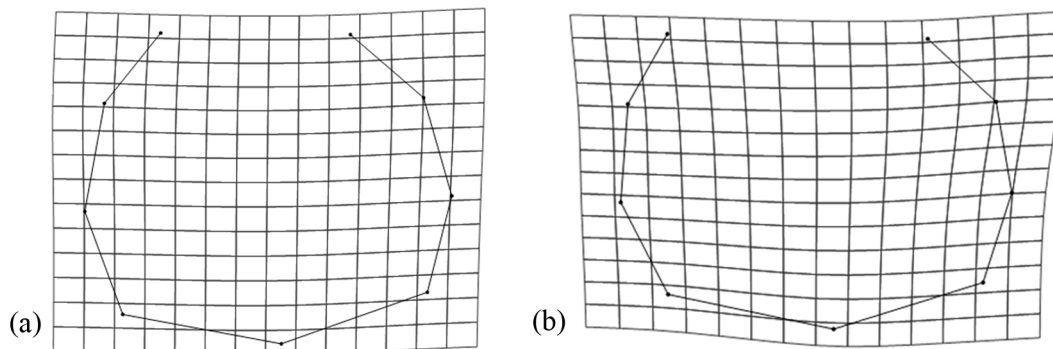
The multivariate Cramer test performed on shape variables and allometry-free shape variables, yielded significant differences between females (adult and juvenile) and males ( $P = 0.0009$ ;  $P = 0.01149$ ). When adult females and juvenile females were analysed separately, significant differences were found between them ( $P = 0.0015$ ;  $P < 0.0001$ ), and between adult females and males ( $P = 0.0005$ ;  $P < 0.0001$ ). However, no differences were found between juvenile females and males ( $P = 0.1044$ ;  $P = 0.8326$ ).

**Second abdominal pleura.** Data was not normally distributed. Royston's Normality Test was applied and the null hypothesis was rejected ( $P= 0.0184$ ) in the case of the male second abdominal pleura. For that reason a non-parametric test was used to assess the significance of the multivariate regression. A statistically significant relationship between shape and size across all specimens ( $R^2 = 0.8556$ ,  $F = 13.83$ ,  $P= 0.001$ ) illustrated significant allometry.

Sexual dimorphism was found for the second abdominal pleura. PCA assessed on Procrustes coordinates illustrated the overall morphological variation (Figure 4). The first 2 principal components accounted for 77.36 % of total variance. Males and females were clearly separated by the second principal axis. Cramer test performed on Procrustes coordinates size showed significant differences between sexes ( $P<0.001$ ). Females had a wider (landmarks 3, 4, 5 and 7) and taller (landmarks 1, 5 and 9) second abdominal pleura, than males (Figure 5).



**FIGURE 4.** Scatter plot of first *versus* second principal component axes for the total variation of the second abdominal pleura shape for females and males in *Neocaridina davidi*.



**FIGURE 5.** Relative deformations grids show the variation in the mean shape of the second abdominal segment for (a) females and (b) males.

## Discussion

The two anatomic areas that were analysed: carapace and second abdominal segment, displayed shape variation related to sexual dimorphism in *Neocaridina davidi*. Adult females displayed a more elongated carapace and a longer rostrum than males. However, when analysing carapace shape in juvenile females, they were

indistinguishable from males. Similar results were found by Scalici & Gibertini (2009) in the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) where females and males only showed significant differences in carapace shape after reaching sexual maturity. The authors attributed it to similar selective pressures in juvenile females and males (related to the environment), *versus* different needs in reproduction and sexual selection in adult females. So, body shape evolution would not solely be driven by different evolutionary pressures on each sex, but also on different stages.

The selection pressure due to female fecundity may be reflected in sex-specific phenotypes, particularly in the region that contains reproductive organs (Cheng & Kuntner 2015). Thus, reproductive features of females might be modulating the intraspecific variation found in the cephalothorax shape in *Neocaridina davidi*, a species with high volume eggs, direct development with lecithotrophic first stage (Pantaleão *et al.* 2015), and a quick ovarian development with short interspawning periods (Tropea & López Greco 2015). Fully developed female gonads extend dorsally in the cephalothorax and reach the first portion of the pleon. Hence, the differences in carapace shape between adult females and juvenile females, as between adult females and males might likewise be related to changes in the onset of sexual maturity. The second abdominal pleura were more elongated and wider in females than in males. Similar results were found in *Macrobrachium rosenbergii* (de Man, 1879) (Nagamine & Knight 1980), *Macrobrachium tenellum* (Smith, 1871) (Cabrera Peña 1982), *Macrobrachium hainanense* (Parisi, 1919) (Mantel & Dudgeon 2005), *Palaemonetes antennarius* (Milne Edwards, 1837) (Anastasiadou *et al.* 2009) and *Litopenaeus vannamei* (Boone, 1931) (Accioly *et al.* 2013). The expansion of the second abdominal pleura in females may be related to the formation of the brood chamber, since *Neocaridina davidi*, as well as most species of the genus *Neocaridina*, shows complete suppression of larval development, and females carry the eggs in the brood chamber during embryonic development (Barbier 2010; Vogt 2013). Thus, the space below the abdomen into which the embryo mass is deposited during spawning, is enlarged as the pleura of the first three abdominal segments increase in size and the sterna of this segments widen (Bauer 2004).

Size is sexually dimorphic in this species as well, with females on average larger than males (Barbier 2010), as verified by the significant differences found in CS between sexes both for carapace and second abdominal segment. In decapods, sexual size dimorphism is considered to be a good predictor of mating systems (Rasch & Bauer 2016). In a “pure search” (promiscuous) mating system, as described by Wickler & Seibt (1981), males search until they find a receptive female, and after the spermatophore is transferred mating partners separate. The only form of competition between males for the receptive females is a rapid and efficient search (Bauer *et al.* 2014). Therefore, small cryptic and agile males would have an advantage in searching compared to bigger ones (Correa & Thiel 2003; Rasch & Bauer 2016). Rasch & Bauer (2016) studied the mating system of the processid caridean *Ambidexter symmetricus* Manning & Chace, 1971. This species has high-density populations of mobile individuals and sexual size dimorphism, with females larger than males, and little or no sexual dimorphism in weaponry, similarly to *N. davidi*. Their observations of mating behaviour supported the hypothesis of a pure-search mating system, as males showed little interest before the female parturial molt, and copulated with females only after it.

Many studies have been conducted concerning female and male differences in *Neocaridina davidi*. For example, Liang (2002) listed the third and fourth pair of pereopods as sexually dimorphic, being the third pereopod of the male longer than that of the female. The author also indicated that the propodus of the male is curved, bow shaped and expanded distally, and the posterior margin is covered with spinules; whilst the dactylus presents hook-shaped spinules distally. Later, Klotz *et al.* (2013) reported the number and disposition of teeth on the rostrum of adult red-cherry shrimps as a slightly sexually dimorphic character (most males show smaller and more appressed dorsal teeth on rostrum, compared to females), and the third pair of pereopods as a distinct sexually dimorphic character (male propodus slightly curved, dactylus shorter, with stronger and more curved spines at posterior margin than in female). However, to our knowledge this is the first work in which sexual dimorphism in *N. davidi* is addressed using a quantitative method instead of the classical qualitative approach. Differences in the overall shape of the carapace and second abdominal segment between adult females and males were evidenced for the first time. In this regard geometric morphometrics demonstrates to be an effective tool for unravelling subtle morphometric differences.

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