Analysis of the morphological characteristics and direction of morphology-based selective breeding of Procambarus clarkii

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Research Article

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Abstract

In order to explore the breeding direction of morphological selection of *Procambarus clarkii*, the morphological characteristics of five *P. clarkii* basic populations from different regions in China were comprehensively analyzed by multivariate statistical analyses. The results showed that there were significant differences in most morphological parameters among populations and between sexes. In the discriminatory analysis, the most discriminant characteristics for distinguishing females among populations were body weight (BW), first abdominal segment width (FASW), third abdominal segment width (TASW) and third abdominal segment height (TASH), whereas for males, the characteristics were body weight (BW), carapace length (CL), carapace width (CW) and third abdominal segment width (TASW). The most significant variables of the differences between sexes were body weight (BW), third abdominal segment width (TASW) and double cheliped weight (DCW). This study would be beneficial to understanding the main morphological characteristics of *P. clarkii*, which could provide basic data of the collected germplasm resources and some reference for indicating the direction of *P. clarkii* morphology-based breeding. The germplasm resources with stronger abdomen, smaller carapace and smaller cheliped would be the selection targets, and all-female breeding would also be one of important breeding directions of for *P. clarkii*.

1. Introduction

*Procambarus clarkii*, is a freshwater crayfish, which is native to central and southern United States and northeastern Mexico (Henttonen and Huner 1999). It is one of the most notorious invasive species worldwide (Barbaresi and Gherardi 2000; Cruz and Rebelo 2005; Zhu and Yue 2008; Yue et al. 2010). It was introduced into China from Japan in 1930s (Kawai and Kobayashi 2005). Because of its high adaptability to environment and high reproduction ability (Fernández-Cisnal et al. 2018; An et al. 2020), it has been widely distributed throughout China (Li et al. 2012; Pan et al. 2020), and the natural distribution and main producing areas are the middle and lower reaches of the Yangtze River (Yan et al. 2021). *P. clarkii* is favored by consumers because of its delicious meat and rich nutrition. In recent years, *P. clarkii* has become one of the most economically important aquaculture species in China (Wang et al. 2005; Li et al. 2012). The production of *P. clarkii* reached nearly 2,633,600 tons in 2021 according to the Crayfish Industry Report 2022 in China reported by China Society of Fisheries (http://www.nftec.agri.cn/). However, due to high intensity fishing and deterioration of the living environment, the wild resources of *P. clarkii* have been declining (Liu et al. 2020). It is insufficient attention paid to the germplasm and breeding of *P. clarkii* under the background of vigorous development of the aquaculture industry. Such led to the increasingly prominent problems such as smaller individual size, frequent disease occurrence, larger carapace percentage, and serious germplasm degradation of *P. clarkii* (Yi et al. 2017; Wang et al. 2019; Peng et al. 2021). The study on the morphological differences of the crayfish in different geographical regions will be beneficial to the protection of germplasm resources and breeding of fine varieties.

In general, morphological differentiation can appear as a consequence of genetic differences or environmental factors or their interaction (Begg and Waldman 1999; Pakkasmaa and Piironen 2001).
Genetic diversity is investigated through assessing the differences between and within populations by using various genetic markers from the perspective of genetics (Moore et al. 2018; Li et al. 2021). Multivariate statistical analysis of morphological characters has also proven to be a powerful technique in population differences and stock discrimination (Palma and Andrade 2002; Turan et al. 2006; Maguire and Dakić 2011; Chen et al. 2015; Porrini et al. 2015). The analyses of morphological difference can reflect the differences among different populations or even individuals simply, quickly and effectively, and complementary to genetic study (Fevolden and Hessen 1989; Sint et al. 2007; Bertocchi et al. 2008).

Thus, in this study, morphological characteristics of five basic populations of *P. clarkii* from different regions were analyzed and compared through applying morphometry and multivariate analysis methods. It was expected to evaluate the morphological differences and sexual dimorphism to provide some reference for its germplasm resources utilization and the morphology-based selective breeding direction of *P. clarkii*.

2. Materials And Methods

2.1 Sampling and data collection

Five basic *P. clarkii* populations for breeding were collected from different regions in China (Table S1). Collection locations of Chaohu, Yangxin, Honghu and Hanchuan were located in the middle and lower reaches of the Yangtze River, while Gaoyou is in the Huaihe River. The five regions belong to natural distribution and main producing areas of *P. clarkii*, where the crayfish had high genetic diversity relatively (Li et al. 2012; Li et al. 2016; Yi et al. 2018; Yi et al. 2020). A total of 527 individuals (239 females and 288 males) with an intact body were randomly sampled from the five populations. Eleven characters were measured sequentially on each specimen. The dimension characters included total length (TL), body length (BL), carapace length (CL), carapace width (CW), first abdominal segment length (FASL), first abdominal segment width (FASW), third abdominal segment length (TASL), third abdominal segment width (TASW) (What distance the previous characters refer to respectively was shown in Fig. 1) and third abdominal segment height (TASH, refers to the distance between the midpoint of the dorsal and ventral margins of the third abdominal segment). The weight characters included body weight (BW) and double cheliped weight (DCW). The dimensions were measured using a digital calliper (± 0.01 mm), while the body weight and double cheliped weight were measured using an electronic balance (± 0.01 g). In order to reduce the error caused by measurement, all characters were measured by the same person.

2.2 Data analysis

In order to eliminate the influence of size on morphological characteristics of *P. clarkii*, the ratio of each morphological character to body length (BL) was standardized for further analyses. In this study, a total of 10 morphological proportion parameters were selected, and all the morphological parameters were expressed as means and standard deviations.
In order to remove bias caused by sexual dimorphism (Sint et al. 2007; Hamasaki et al. 2020), the specimens were separated by sex and analyzed respectively with multivariate analysis. The Kolmogorov-Smirnov test was carried out on each morphological parameter in order to test whether the parameter obey a normal distribution or not. The parameters consistent with normal distribution were subjected to one-way ANOVA. For the parameters that were not normally distributed, difference in means between populations were assessed using ANOVA after testing for non-parametric Kruskal-Wallis ANOVA on ranks (Dashinov et al. 2020). Similarly, T-test and non-parametric Mann-Whitney U test were used to compare whether the two sexes significantly different in the morphological parameters. For the discriminant analysis, all variables were entered one by one using a stepwise procedure where only variables contributing in the lowering of Wilk’s lambda are retained for analysis (Poulet et al. 2005; Dashinov et al. 2020). And in the discriminant functions, standardized coefficients with large absolute values correspond to parameters with greater discriminating ability (Jónsdóttir et al. 2016; Duretanović et al. 2017; Maguire et al. 2017). A cross-validation test was computed in order to evaluate the effectiveness of discriminant analysis (Marques et al. 2006; Konan et al. 2010). For the cluster analysis, after calculating the average of 10 morphological proportion parameters of each population, the nearest neighbor clustering method was carried out with the Euclidean distances (Konan et al. 2010; Mavule et al. 2016).

Microsoft Excel 2019 and SPSS 23.0 were used for descriptive statistics, ANOVA and discriminant analysis. OriginPro 2021 was used for the cluster analysis.

3. Results

3.1 Analysis of variance

There was a significant difference on the majority of the morphological parameters among different populations of males and females by the one-way ANOVA and K-W test (Table S2). For 10 morphological parameters, there was no significant difference on the parameter of TASL ($P > 0.05$) among the female populations, while there was no significant difference on the two parameters of FASW and TASL ($P > 0.05$) among the male populations. There was no significant difference on the two parameters of TL and TASH ($P > 0.05$) between the males and females.

3.2 Discriminant analysis of different populations

There were significant differences on morphological characteristics among populations, for both males and females of *P. clarkii*. Multivariate discriminant analysis could be used to distinguish which morphological characteristics contributed most to the differences among populations. After the stepwise procedure in the discriminant analysis, the females retained six variables (BW, CW, FASL, FASW, TASW, TASH) that most distinguished different populations, while the males retained eight variables (BW, TL, CL, CW, FASL, TASW, TASH, DCW). There were all highly significant in the discrimination model for the females ($\text{Wilks'}\lambda = 0.311$; $F = 13.268$; $P < 0.001$) and males ($\text{Wilks'}\lambda = 0.249$; $F = 14.596$; $P < 0.001$).
Summary statistics demonstrated that for the female populations, the first and second discriminant functions contributed to 88.2% of the total variance (74.2% and 14.0%, respectively) indicating that the greatest proportion of the total variance was due to the first two discriminant functions. And the canonical R for those functions were 0.738 and 0.429, respectively (Table 1). In discriminant function 1, the parameters with larger absolute values of standardized coefficients were BW (0.826) and TASW (0.733). In discriminant function 2, the parameters with larger absolute values of standardized coefficients were FASW (-0.644), TASW (0.892) and TASH (0.656). The parameters may account for most of the variation in the first two discriminant functions. Correspondingly, for the male populations, the canonical R for the first two discriminant functions were 0.734 and 0.540, respectively (Table 1). The first discriminant function accounted for 62.3% of the explained variance and was weighed mostly by BW (1.232), CL (0.626) and CW (-0.767). The second discriminant function accounted for 22.0%, and the parameter with the largest absolute value of the standardized coefficient was TASW (0.790). The larger the absolute value was, the greater the ability to discriminate the populations was.

The separation and overlap of male and female populations of *P. clarkii* could be better demonstrated by making scatter plots of the first two discriminant functions (Fig. 2). The scatter plots for female and male populations showed a similar result, that is, the population Ch significantly deviated from the other four geographic populations. For the females, as the first discriminant function was marked by high positive loading for BW and TASW, the higher the value of BW and TASW are, the more likely the females belong to the populations from Gaoyou, Yangxin, Honghu or Hanchuan. Similarly, the second discriminant function was marked by high positive loadings for TASW and TASH and high negative loading for FASW. It seems to provide some discrimination between the Gy population, Hc population and Yx population, Hh population. For the males, as well as the females, the first discriminant function was marked by high positive loading for BW and CL and high negative loading for CW. So the lower the value of CW is, the more likely it was that the males belong to the Ch population, and the higher for the value of BW and CL are, the more likely it was that the males belong to the other four populations. The second discriminant function was marked by high positive loading for TASW. And the higher the value of TASW is, the more likely it was that the males belong to the Hc population.
Table 1
Standardized canonical discriminant function coefficients, eigenvalue, percentage of explained variance, and canonical correlations from the discriminant analysis for female and male *P. clarkii* populations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Function 1</th>
<th>Function 2</th>
<th>Function 3</th>
<th>Function 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>0.826</td>
<td>0.227</td>
<td>0.547</td>
<td>-0.439</td>
</tr>
<tr>
<td>CW</td>
<td>-0.227</td>
<td>0.273</td>
<td>-0.726</td>
<td>0.459</td>
</tr>
<tr>
<td>FASL</td>
<td>-0.296</td>
<td>0.113</td>
<td>0.438</td>
<td>0.809</td>
</tr>
<tr>
<td>FASW</td>
<td>-0.294</td>
<td>-0.644</td>
<td>0.662</td>
<td>0.035</td>
</tr>
<tr>
<td>TASW</td>
<td>0.733</td>
<td>0.892</td>
<td>-0.216</td>
<td>0.222</td>
</tr>
<tr>
<td>TASH</td>
<td>-0.571</td>
<td>0.656</td>
<td>0.170</td>
<td>-0.547</td>
</tr>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>1.193</td>
<td>0.225</td>
<td>0.114</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>Variance (%)</strong></td>
<td>74.2</td>
<td>14.0</td>
<td>7.1</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Canonical R</strong></td>
<td>0.738</td>
<td>0.429</td>
<td>0.320</td>
<td>0.265</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>1.232</td>
<td>0.164</td>
<td>0.365</td>
<td>-0.319</td>
</tr>
<tr>
<td>TL</td>
<td>0.064</td>
<td>-0.542</td>
<td>-0.017</td>
<td>-0.139</td>
</tr>
<tr>
<td>CL</td>
<td>0.626</td>
<td>0.204</td>
<td>0.708</td>
<td>-0.117</td>
</tr>
<tr>
<td>CW</td>
<td>-0.767</td>
<td>0.065</td>
<td>-0.400</td>
<td>-1.266</td>
</tr>
<tr>
<td>FASL</td>
<td>-0.288</td>
<td>0.317</td>
<td>-0.043</td>
<td>0.316</td>
</tr>
<tr>
<td>TASW</td>
<td>0.435</td>
<td>0.790</td>
<td>-0.375</td>
<td>0.326</td>
</tr>
<tr>
<td>TASH</td>
<td>-0.521</td>
<td>0.114</td>
<td>0.803</td>
<td>0.087</td>
</tr>
<tr>
<td>DCW</td>
<td>-0.578</td>
<td>-0.604</td>
<td>-0.134</td>
<td>1.118</td>
</tr>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>1.166</td>
<td>0.411</td>
<td>0.191</td>
<td>0.104</td>
</tr>
<tr>
<td><strong>Variance (%)</strong></td>
<td>62.3</td>
<td>22.0</td>
<td>10.2</td>
<td>5.6</td>
</tr>
<tr>
<td>** Canonical R**</td>
<td>0.734</td>
<td>0.540</td>
<td>0.400</td>
<td>0.307</td>
</tr>
</tbody>
</table>

The crayfish that best represents the morphological characteristics of the populations were shown in Fig. 3, and the main morphological characteristics to distinguish the populations of *P. clarkii* were compared. For female populations, the BW/BL ratio of the Ch population was significantly lower than that of the other four populations, and the Yx population was significantly lower than that of the Gy, Hh and Hc populations. Only two populations (Yx and Hc) exhibited significant difference in the value of the
FASW/BL ratio. The TASW/BL ratio of the Hc population was significantly larger than that of the Ch, Yx and Hh populations, and the Gy population was also significantly larger than that of the Ch and Yx populations. The Ch and Yx populations exhibited a significant larger TASH/BL ratio than other three populations. Likewise, for male populations, the BW/BL ratio of the Ch population was significantly lower than that of the other four populations, and the Yx population was also significantly larger than that of the Hh population. The Ch and Hh populations exhibited a significant lower CL/BL ratio than other three populations. The CW/BL ratio of the Hh population was significantly lower than that of the Yx and Hc populations. The highest value of the TASW/BL ratio was observed in the Hc population.

The cross-validation procedures revealed that 310 of 527 crayfish (58.82%) were correctly classified based on their external morphology (Table 2). Specifically, there was little difference between the percentage of the males (62.50%) and females (54.39%) correctly classified. The accuracy rate of classification among different geographic populations of the same sex was low, indicating that the morphological differences among different geographic populations of the same sex were small. Yet the Ch population had obtained the best proportion classification of the males (78.33%) and females (80.43%), which were much higher than the other four populations. The results indicated that the morphological characteristics of the Ch population had low similarity with other populations.
Table 2
Cross-validated classification (count and percentage) matrix for the male and female *P. clarkii* populations.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Population</th>
<th>Predicted group membership (count and percentage)</th>
<th>Total (count and percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ch</td>
<td>Gy</td>
</tr>
<tr>
<td>Female</td>
<td>Ch</td>
<td>37 (80.4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gy</td>
<td>5 (10.0)</td>
<td>21 (42.0)</td>
</tr>
<tr>
<td></td>
<td>Yx</td>
<td>3 (6.0)</td>
<td>7 (14.0)</td>
</tr>
<tr>
<td></td>
<td>Hh</td>
<td>0</td>
<td>5 (10.2)</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>0</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Male</td>
<td>Ch</td>
<td>47 (78.3)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td></td>
<td>Gy</td>
<td>14 (19.2)</td>
<td>41 (56.2)</td>
</tr>
<tr>
<td></td>
<td>Yx</td>
<td>2 (4.1)</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td></td>
<td>Hh</td>
<td>3 (6.1)</td>
<td>7 (14.3)</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>3 (5.3)</td>
<td>8 (14.0)</td>
</tr>
</tbody>
</table>

### 3.3 Cluster analysis

The male and female populations showed a similar grouping in the hierarchical cluster analysis (Fig. 4). Five populations were clustered into two well-defined clusters in the two models (males and females). The first branch included only the Ch population, the Gy, Hc, Hh and Yx populations were clustered in the second branch. The results showed that the samples from Chaohu were phenotypically gathered into one cluster, while the morphological similarity between the Gy, Hc, Hh and Yx populations was great and were grouped into another cluster.

### 3.4 Discriminant analysis of sex

In order to distinguish which morphological characteristics contributed most to the differences between the females and males, a stepwise discriminant analysis was performed. Among all the variables
analyzed, eight morphological characteristics were considered relevant and remained in the model, and the discriminant model established is remarkably effective (Wilks’ $\lambda = 0.373; F = 109.037; P < 0.001$). In the unique discriminant function, the parameters with larger absolute values of standardized coefficients were BW (1.229), TASW (0.803) and DCW (-1.068) (Table S3). The three most discriminant characteristics of males and females were compared in Fig. 5. The results showed that the BW/BL ratio and the DCW/BL ratio of the males were significantly larger than the females, while the TASW/BL ratio of the females was significantly larger than that of the males. Similarly, after the cross-validation procedure, 92.05% of the females were correctly classified, and 88.19% of the males were correctly classified (Table S4). The classification accuracy was high, indicating that the morphological differences between male and female individuals were great.

4. Discussion

Generally, crustaceans have high morphological plasticity (Maguire et al. 2017). And the variation in the external morphology of a species is generally interpreted as an adaptation to the habitat environment (Dimmock et al. 2004; Brian et al. 2006; Ferrito et al. 2007; Demchenko and Tkachenko 2017). Geographic isolation, which blocks gene exchange among different populations, is generally considered to be a prerequisite for population differentiation (Gould and Woodruff 1978; Trizio et al. 2005). Yet the eventually formed morphological differences could be a consequence of the environmental (Haddaway et al. 2012) and genetic factors (Cataudella et al. 2010; Maguire et al. 2014).

The results of variance analysis showed that there were significant differences in most morphological parameters among the five basic populations of *P. clarkii*. Multivariate stepwise discriminant analysis was used to identify the best combination of variables to distinguish populations, and according to the absolute value of the standardized coefficient of the discriminant function, the variables with higher discriminant ability can be found (Anastasiadou et al. 2009; Mavule et al. 2016; Duretanović et al. 2017; Melesse et al. 2022). In the present study, the standardized morphological parameters of BW, FASW, TASW and TASH contributed significantly to distinguishing female populations, and the most discriminant characteristics for distinguishing the male populations were focused on BW, CL, CW and TASW. That is, in addition to body weight, the combination of abdomen related variables played an important role in distinguishing *P. clarkii* female populations, and the carapace related variables significantly contributed to distinguishing the male populations. Recently *P. clarkii* is highly loved by consumers and has become an important aquatic economic animal in China (Wang et al. 2005; Yi et al. 2017). The tail meat of *P. clarkii* is the most edible part for people to consume presently (Devesa et al. 2002). The tail meat content is related with some morphological traits to some extent, including body weight and those that are mainly concentrated in the abdomen of *P. clarkii* (Wang et al. 2020). At present, the research on morphological traits has been widely used in the breeding of some other various aquatic animals (Wang et al. 2016; Zou et al. 2017; Li et al. 2018). Generally, for *P. clarkii*, the greater the proportion of abdomen and the smaller the proportion of carapace, the higher the meat content (Wang et al. 2020). Therefore, the selection conducted around meat content will be the one direction of *P. clarkii*
breeding, and the germplasm resources with stronger abdomen and smaller carapace are the selection targets for genetic breeding of *P. clarkii*.

A cross-validation test was computed to assess the ability of variables to discriminate *P. clarkii* populations (Konan et al. 2010; Freire et al. 2017; Dashinov et al. 2020). After the cross-validation procedure, there was 58.82% of both sexes of *P. clarkii* were correctly classified in their groups. The accuracy of classification was low, indicating that the more individuals with similar morphological characteristics among populations (Konan et al. 2010; Mavule et al. 2016). Meanwhile, the higher the percentage of populations correctly classified, the greater the morphological differences among populations (Duretanović et al. 2017). It showed that the greatest difference appeared between the Ch population and the other four populations. The results of cross-validation for male and female *P. clarkii* populations were also supported by the hierarchical cluster analysis. Cluster analysis can classify different populations, and the clustering results reflect the distance of the kinship between populations (Yang et al. 2020; Prasetyo et al. 2022). In this study, the male and female populations showed same grouping in the hierarchical cluster analysis that got the Ch population into a cluster and grouped the Gy, Yx, Hh and Hc populations into another cluster. Obviously, the clustering results did not conform to the general rule that the closer the geographical distance, the more similar the morphological characteristics (Cheng et al. 2005). It is worth noting that *P. clarkii* is an economically important species farmed in China, and its seedlings or adults may exist in geographically distant water areas through other unnatural factors such as trade. Therefore, the hopping dispersal path of *P. clarkii* may be the result of natural migration and human factors (Barbaresi et al. 2004; Yi et al. 2020). Such may also be an important reason for the small morphological differences among different populations.

Furthermore, since there is sexual dimorphism in the external morphology of *P. clarkii* (Shen et al. 2022). A stepwise discriminant analysis was performed to identify the variables that contributed most to gender differences. The result showed that the most discriminant characteristics for distinguishing sex were BW, TASW and DCW. The reason for the significant difference on body weight between males and females is that the males have larger double cheliped weight than the females. The sexual dimorphism of the cheliped of *P. clarkii* may be the result of their widespread use by the male in fighting, display, and courtship (Shimoda et al. 2005; Yasuda et al. 2017). The smaller the cheliped of *P. clarkii*, the higher the meat content (Craig and Wolters 1988), which is one of the important reasons why the meat content of female crayfish is higher than that of male crayfish (Peng et al. 2021). Moreover, under the condition of the same body length, the female *P. clarkii* individuals usually have larger the third abdominal segment width than the males. This may be more conducive to the escape movement of female *P. clarkii*, and may be related to its reproductive behavior, especially the behavior of the egg holding (Mariappan and Balasundaram 2004; Bauer and Delahoussaye 2008). So, all-female breeding or the directional breeding of smaller cheliped would be one of important breeding directions for *P. clarkii* in the future.

5. Conclusion
The main morphological characteristics to distinguish the populations and sexes of *P. clarkii* were investigated by morphological characteristics in combination with discriminant analysis for basic populations of *P. clarkii*. For the female populations of *P. clarkii*, in addition to body weight, the differences focused on the combination of abdomen related variables. Of male populations, the carapace related variables showed significantly different. The most discriminant characteristics for distinguishing sex were abdominal width and cheliped weight. The results of this study could provide some reference for the protection of germplasm resources and the selection direction of elite varieties of *P. clarkii*.

**Declarations**

**Competing interests**

The authors have no competing interests to declare that are relevant to the content of this article.

**Ethics Statement**

This study has been approved by the Institutional Animal Care and Use Committee (IACUC) of Huazhong Agricultural University (Wuhan, China) and conducted in accordance with ethical standards and according to the national and international guidelines.

**Data Availability Statement**

All data generated or analysed during this study are included in this published article and its supplementary information files.

**Acknowledgments**

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**Author contributions**

**Qishuai Wang:** Conceptualization, Methodology, Investigation, Formal analysis, Writing (original draft).  
**Siqi Yang, Ruixue Shi** and **Feifei Zheng:** Investigation. **Yanhe Li:** Resources, Investigation, Project administration, Supervision, Writing (review and editing). All authors read and approved the final manuscript.

**References**


Figures

![Figure 1](image-url)
The morphological measurement indexes of *P. clarkii*. TL, total length; BL, body length; CL, carapace length; CW, carapace width; FASL, first abdominal segment length; FASW, first abdominal segment width; TASL, third abdominal segment width and TASW, third abdominal segment width.

Figure 2
Figure 3

Box plots comparing the most discriminant characteristics of the female (A) and male (B) *P. clarkii* populations. The box is interquartile range and whiskers are minima and maxima of morphometric
values. Outliers are the values that are more than $1.5 \times$ inter-quartile range. The same letters above the plots denote statistically significant differences between populations ($P < 0.05$).

Figure 4

Diagram of cluster analyses of five populations of female (A) and male (B) *P. clarkii*.
Figure 5

Box plots comparing the most discriminant characteristics of the male and female *P. clarkii*.

**Supplementary Files**

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- Supplementalinformation.docx