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Morphometric approaches reveal sexual differences in the carapace shape of the horsehair crab, *Erimacrus isenbeckii* (Brandt, 1848)

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Abstract

Animal shape has become sophisticated through evolution or phenotypic plasticity to adapt and survive changing environmental conditions. Sexual differences, which are sex-specific characteristics, form an important research topic that has also long fascinated biologists. Morphometrics is a traditional approach that enables the quantitative description and interpretation of variation in shape based on morphological features. In this study, we attempted to apply two morphometrics approaches (landmark-based or measurement-based) to identify sexual dimorphic traits in the carapace of the horsehair crab, *Erimacrus isenbeckii* (Brandt, 1848). Both approaches revealed that its carapace showed apparent sexual dimorphism, enabling the discrimination of the male and female carapace with more than 90 % accuracy. Moreover, the landmark-based approach found that carapace shape showed fluctuating asymmetry as well as directional asymmetry in both males and females. Fluctuating asymmetry consists of small random deviations from perfect bilateral symmetry and may be tightly linked to environmental changes. Our data suggest that responsiveness to environmental stressors during carapace formation might be different in female and male horsehair crabs. This study indicates that landmark-based geometric and traditional morphometrics can serve as a strong tool to distinguish female from male horsehair crabs using carapace shape. This is the first report to detail the use of morphological features to show sexual differences in horsehair crab. Morphometric parameters obtained from this study will be a useful reference dataset that can be applied for geographic comparisons of carapace shape of horsehair crab.

Key words: *Erimacrus isenbeckii*; geometric morphometrics; carapace shape; sexual dimorphism

Introduction

Morphometrics is a fundamental biological approach that enables the quantitative description and interpretation of variation in shape of morphological features (Rohlf 1990). It can be classified into two approaches, traditional and geometric. The traditional approach is based on absolute measurements of focal measurable traits (e.g., scales, segments, number of specific traits) and their relation to each other. In contrast, the geometric approach is based on multivariate space and size of biological shapes and is widely used with the collection of two- or three-dimensional coordinates of biologically definable outlines and landmarks (Adams et al. 2004). Additionally, geometric morphometrics has allowed the symmetry or asymmetry of shape to be investigated. Biological asymmetry can be described by the frequency distribution of the difference between the right and left sides of individuals within or among a population (Palmer and Strobeck 1986). Generally speaking, there are three types of bilateral asymmetry: fluctuating asymmetry, directional asymmetry, and antisymmetry. Fluctuating asymmetry refers to small random deviations from perfect bilateral symmetry. Directional asymmetry is normally a greater development of a focal trait on one side of the plane. Antisymmetry is characterized whenever one side is usually greater than the other, although the position of the larger side varies randomly in a population, leading to a bimodal distribution of frequencies between the right and left sides of individuals (Palmer and Strobeck 1992). Although directional symmetry and antisymmetry are developmentally regulated and therefore likely to have adaptive significance, fluctuating asymmetry is not likely to be adaptive. Those approaches have been successfully used to discriminate variation in shape within and among species and populations in a number of animal taxa, including crabs (Grinang et al. 2019; Kalate et al. 2017; Long et al. 2013; Marchiori et al. 2014).

The horsehair crab, *Erimacrus isenbeckii* (Bandt, 1848), is widely distributed in the North Pacific ranging from the Alaskan coast via Kamuchatka to the northern part of Japan, mainly along the Hokkaido coast (Sasaki 2003). The horsehair crab is a large edible crab and therefore is an important commercial resource in Hokkaido. Although annual catch of horsehair crab in Hokkaido was approximately 27,000 tons during 1950s, that has declined drastically approximately 2,000 tons due to overfishing (Sasaki 2003). To recover available resources, a total allowable catch has been enforced in Hokkaido, including a complete ban of catches of all females and a part of males that have a carapace length less than 8.0 cm (Sasaki 2003). Several studies focusing on larval survival and development were conducted with the purpose of establishing an aquaculture system for horsehair crab (Ichikawa et al. 2013, 2014a, 2014b, 2018; Jinbo et al. 2005, 2007; Nagano et al. 1999). However, there are no studies that focused on sexual differences in morphology and/or ethology, even though males are larger than females. This confers higher commercial value to males. In terms of morphological features, the shape of the pleon, which is an abdominal trait, shows apparent sexual dimorphism in horsehair crab: whereas in males it is sharper, it is roundish in females. On the other hand, there are no clear differences in the carapace and chela between males and females. The present study attempted to identify the best morphological trait on the carapace to diagnose male and female horsehair crabs by landmark-based geometric morphometrics, and to clarify sex-based differences of carapace shape by traditional morphometrics.

Materials and Methods

Sample collection

A total of 84 crabs consisting of 38 females and 46 males were collected in Funka Bay, Pacific Ocean, Southern Hokkaido, Japan in April 2019 (Fig. 1). Average body weight was 305.8 ± 111.5 (standard deviation) g and 173.0 ± 46.1 g, and average carapace length was 85.3 ± 11.9 mm and

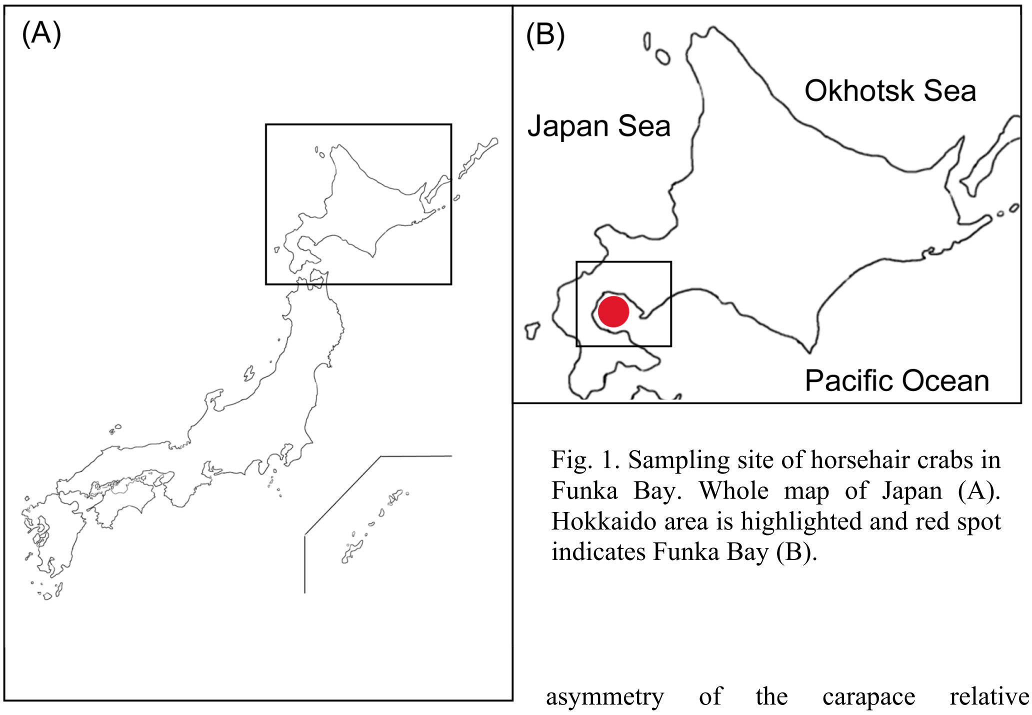

Fig. 1. Sampling site of horsehair crabs in Funka Bay. Whole map of Japan (A). Hokkaido area is highlighted and red spot indicates Funka Bay (B).

71.3 ± 7.6 mm in males and females, respectively. Fishing permission of horsehair crab for this study was granted by the Hokkaido Governor.

Landmark-based geometric morphometrics

For morphometric analysis, the carapace of 38 females and 46 males was used. All carapaces were photographed twice with a digital camera (Olympus TOUGH TG-5) under a stationary and fixed condition. Nine, 12, 15, and 20 landmarks were selected along with the margin of the carapace and defined as models 1 to 4, respectively (Fig. 2). Each landmark was digitized by tpsDig2 software version 2.31 (Rohlf 2010). Initially, the symmetric and asymmetric components of carapace shape were calculated from the means of each image. Measurement error is of critical importance when judging symmetry or asymmetry. To investigate the significance of directional asymmetry of the carapace relative to measurement error, all carapaces were digitized twice in landmark models 1 to 4.

To analyze the shape variables and validate the amount of measurement error, Procrustes analysis of variance (ANOVA) was performed. The following factors were considered: “Individual”, which presumes the overall variation in the dataset; “Side”, which estimates the effects related to directional asymmetry; and “Individual*Side” interaction, which estimates fluctuating asymmetry. In the ANOVA, the mean squares (MS) was involved in the individual effect and was used to estimate variation in an individual, whereas the MS related to the interaction (Individual*Side) between left and right side was used to estimate directional asymmetry. The existence of directional asymmetry was clarified statistically through the main effect of “Side”. “Sex” was considered as an additional main effect. The variation in shape of the carapace between males

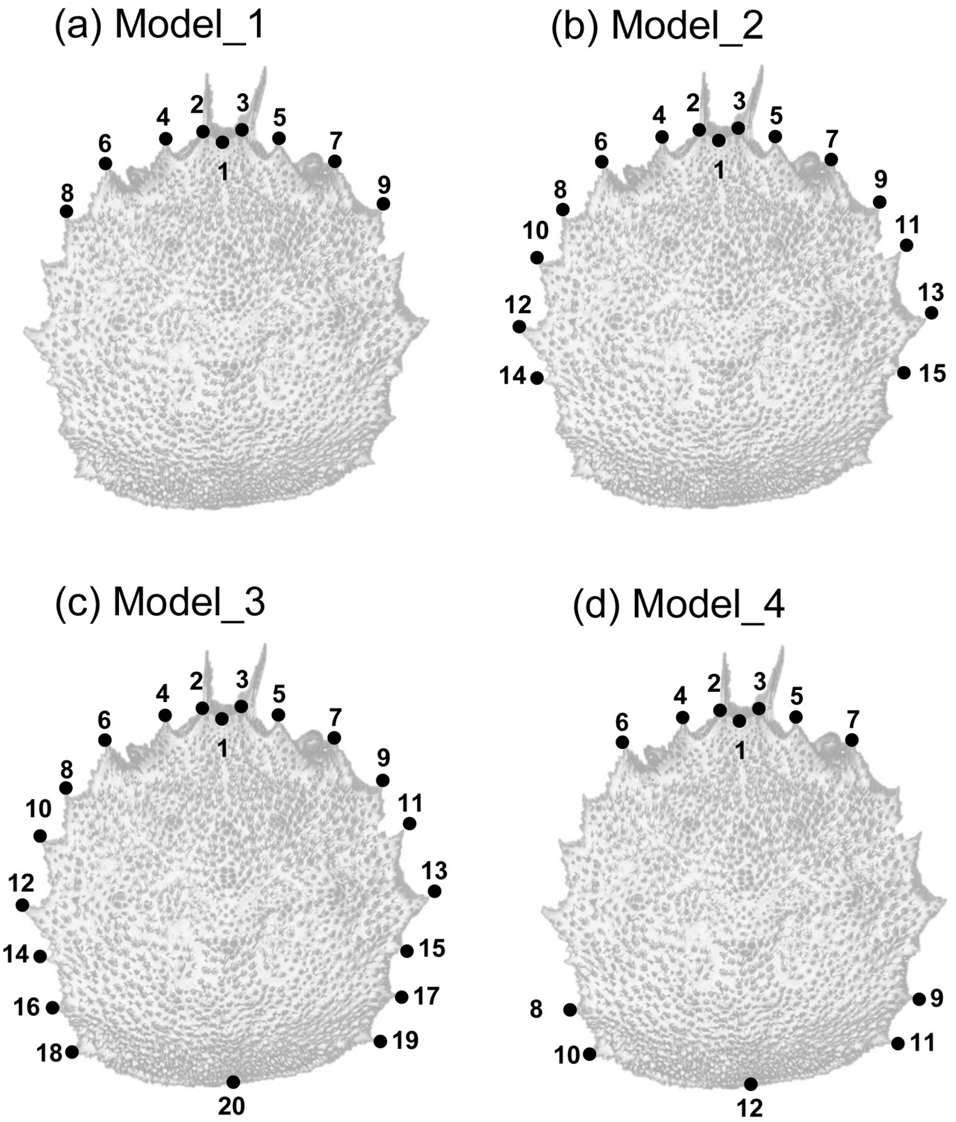

Fig. 2. Models of landmark on the carapace of horsehair crab.

and females in the entire dataset was analyzed by principal component analysis (PCA) based upon the covariance matrix of symmetric and asymmetric components. The sexual differences were assessed using Procrustes distances and Hotelling's T-square, which were the products of a canonical variates analysis (CVA) and discriminant function analysis (DFA), respectively. The results of the Procrustes distance and T-square between groups were tested for significance using 1,000 permutations. DFA was also used to determine whether the two groups (male or female) could be reliably distinguished. All landmark-based morphometric and statistical analyses were conducted by MORPHO J, version 1.07a (Klingenberg 2011).

Traditional morphometrics

Seven traits (see Fig. 3) were measured with Fiji software (Schindelin et al. 2012) to the nearest 0.01

mm: carapace width (CW), carapace length (CL), orbital spine width (OW), upper carapace width (UCW), lower carapace width (LCW), orbital cavity length (OCL), and carapace length to the eye orbit (CLO). OCL and CLO were measured on both left and right sides (Fig. 3). To estimate the sexual differences between carapace measurable traits, analysis of covariance (ANCOVA) was performed with CW as a covariate using R software version 3.5.3 (R Core Team 2019). The homogeneity of the slopes of the regression lines was tested.

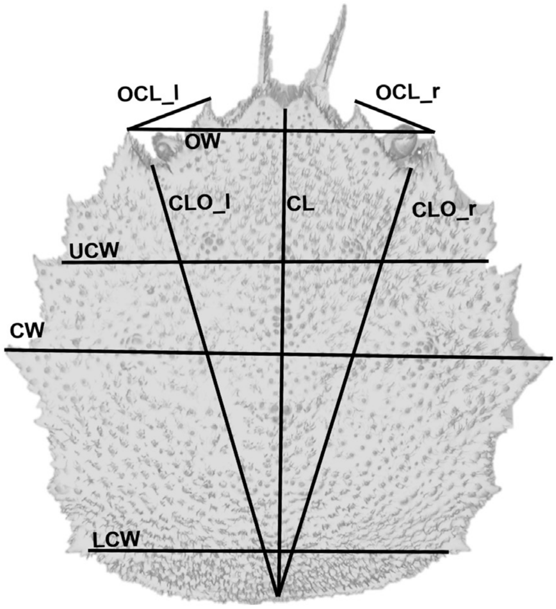

Fig. 3. Measurable traits on horsehair crabs including carapace width (CW), carapace length (CL), orbital spine width (OW), upper carapace width (UCW), lower carapace width (LCW), orbital cavity length (OCL), and carapace length to eye orbit (CLO). "r" and "l" indicate right and left side, respectively.

Results

Comparison between sexes by landmark-based geometric morphometrics

The size of male and female horsehair crabs used in this study was obviously different (Fig. 4). The Procrustes ANOVA in all models showed that the shape of the carapace differed significantly between males and females ($p < 0.001$). Moreover, the measurement error did not exceed the values of other factors (Table 1). The carapace showed both directional asymmetry ("Side" factor) and fluctuating asymmetry ("Individual * Side" factor) between males and females. The distribution of variance obtained from the Procrustes ANOVA implied that the effect of the side of the carapace and the interaction between the side and carapace identity were significant. This suggests not only that the carapace has both directional and fluctuating asymmetry, but also that the level of fluctuating asymmetry is higher than the measurement error (Table 1).

PCAs of variation in carapace shape showed that the first two PCs accounted for 49.2 % (PC1 = 30.3 %; PC2 = 18.9 %) in model 1, 49.8 % (PC1 = 31.2 %; PC2 = 18.6 %) in model 2, 38.9 % (PC1 = 23.7 %; PC2 = 15.2 %) in model 3, and 51.1 % (PC1 = 29.9 %; PC2 = 21.2 %) in model 4 of the

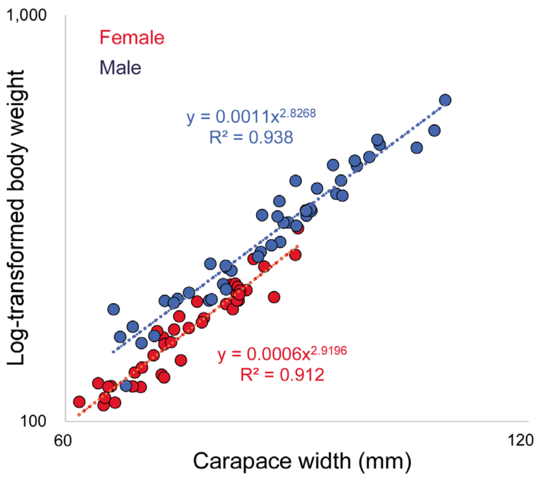

Fig. 4. Relationship between body weight and carapace width for female and male horsehair crabs. Values of carapace width (x-axis) were converted to natural logarithm values. Both red and blue dotted lines indicate the regression curves of males and females.

Table 1. Comparison of Procrustes ANOVA among four models for sex with "Individual" as the random effect, and "Sex" as an additional main effect.

Model 1	Effect	SS	MS	df	*F*	p-value
	Sex	0.0157473	0.002249615	7	23.63	<.0001
	Individual	0.05464062	9.51927×10^{-5}	574	2.2	<.0001
	Side	0.00227658	0.000325226	7	7.5	<.0001
	Individual * Side	0.02518856	4.33538×10^{-5}	581	5.89	<.0001
	Measurement error	0.00865236	$7.3575\text{E} \times 10^{-6}$	1176		
Model 2	**Effect**	**SS**	**MS**	**df**	***F***	**p-value**
	Sex	0.00746672	0.000574363	13	14.47	<.0001
	Individual	0.04231803	0.000039698	1066	3.12	<.0001
	Side	0.00153858	0.000118352	13	9.29	<.0001
	Individual * Side	0.0137421	0.000012736	1079	7.69	<.0001
	Measurement error	0.00361495	1.6552×10^{-6}	2184		
Model 3	**Effect**	**SS**	**MS**	**df**	***F***	**p-value**
	Sex	0.00939168	0.00052176	18	18.07	<.0001
	Individual	0.04105046	2.88681×10^{-5}	1422	2.98	<.0001
	Side	0.00180381	0.000100212	18	10.34	<.0001
	Individual * Side	0.01395685	9.6923×10^{-6}	1440	6.01	<.0001
	Measurement error	0.0046988	1.6114×10^{-6}	2916		
Model 4	**Effect**	**SS**	**MS**	**df**	***F***	**p-value**
	Sex	0.00193782	0.000193782	10	4.74	<.0001
	Individual	0.03270909	4.08864×10^{-5}	800	2.09	<.0001
	Side	0.00314517	0.000314517	10	16.1	<.0001
	Individual * Side	0.01582598	1.95383×10^{-5}	810	6.66	<.0001
	Measurement error	0.00481373	2.9352×10^{-6}	1640		

SS: sums of squares, MS: mean squares, df: degrees of freedom.

Table 2. Canonical variate analysis and discriminant function analysis indicating morphological differences in the shape of the carapace between male and female horsehair crabs.

Model	Number of landmarks	Procrustes distance	p-value	T-square	p-value
1	9	0.0195	< 0.0001	174.5881	< 0.0001
2	15	0.0134	< 0.0001	323.9445	< 0.0001
3	20	0.0153	< 0.0001	504.6917	< 0.0001
4	12	0.0069	< 0.0001	171.4272	< 0.0001

total variation in shape (Fig. 5). Although an 80 % confidence ellipse area in PCA of model 4 showed a total overlap between males and female, the other three models clearly separated the sexes. Results of PCA were well supported by both CVA and DFA (Table 2). In terms of CVA, the Procrustes distances of the male and female carapace were 0.0195, 0.0134, 0.0153, and 0.0069 ($p < 0.0001$) in models 1, 2, 3 and 4, respectively, providing statistical evidence for sexual differences in

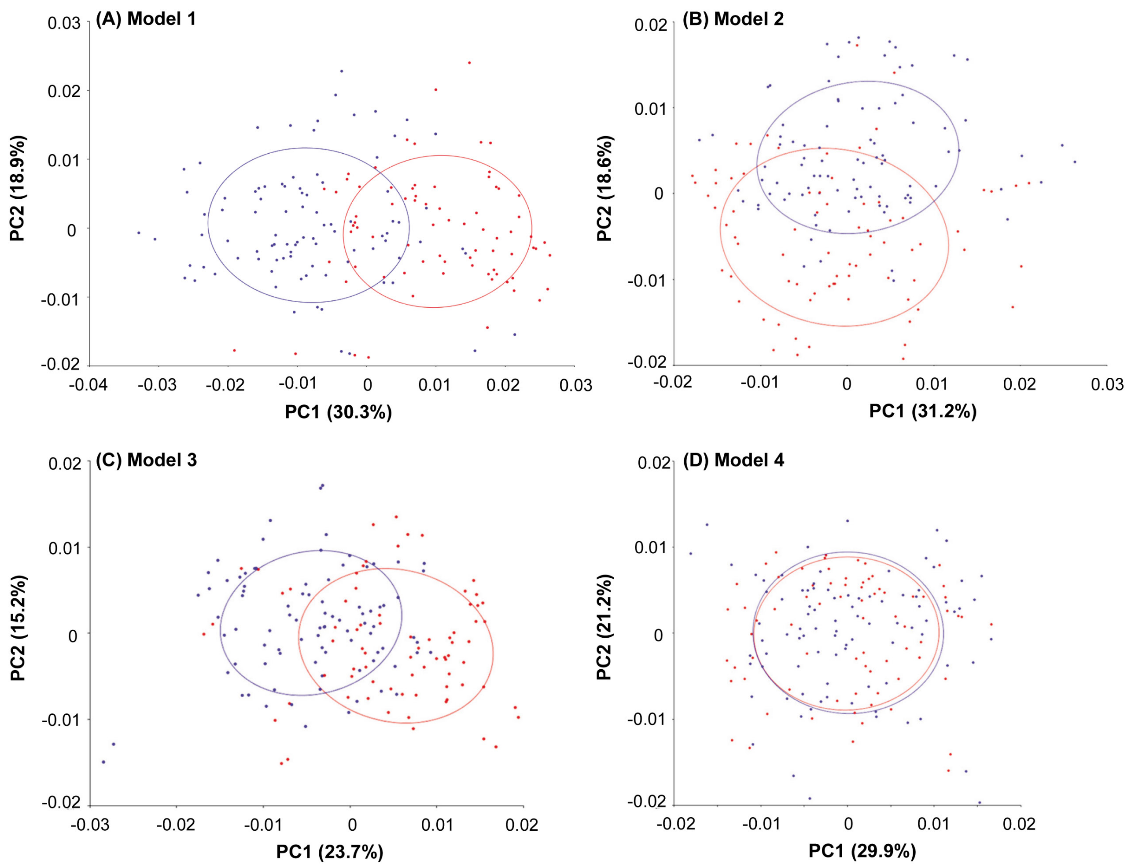

Fig. 5. Biplot of PCA of each landmark model showing variations in the carapace shape of the female (red dots) and male (blue dots) horsehair crabs. Confidence ellipses show a probability of 0.8.

carapace shape. Likewise, DFA suggested obvious differences in carapace shape between sexes. Moreover, cross-validation of DFA showed that model 3 had the highest score (93.2 % correct classification) enabling the distinction of either males or females (Table 3).

Comparison between sexes by measurement-based morphometrics

To estimate the sexual differences in focal measurable traits, we selected and analyzed six traits. All traits between males and females showed homogeneity of the slopes of the regression line against CW, and four traits (OW, UCW, LCW, and CL) indicated statistically significant differences between sexes ($p < 0.05$, Fig. 6; Table 4). On the other hand, both right and left sides of OCL and CLO showed no significant differences between sexes.

Discussion

Generally, several morphological features such as the chela, pleon, and carapace are known to show apparent sexual dimorphism in various crab species. The size of the chelae is a famous sexually dimorphic trait in many decapods (Mariappan et al. 2000). The larger chela of the male crab can be used for fighting to attain females, for defense against other males, or for holding the female during courtship. However, there are no apparent

Table 3. Model assignment after Discriminant Function Analysis cross-validation.

Model_1	Female	Male	Total	Correct classification (%)
Female	60	16	76	78.9%
Male	14	78	92	84.8%
Model_2	**Female**	**Male**	**Total**	**Correct classification (%)**
Female	64	12	76	84.2%
Male	12	80	92	87.0%
Model_3	**Female**	**Male**	**Total**	**Correct classification (%)**
Female	69	5	74	93.2%
Male	6	82	88	93.2%
Model_4	**Female**	**Male**	**Total**	**Correct classification (%)**
Female	57	17	74	77.0%
Male	17	73	90	81.1%

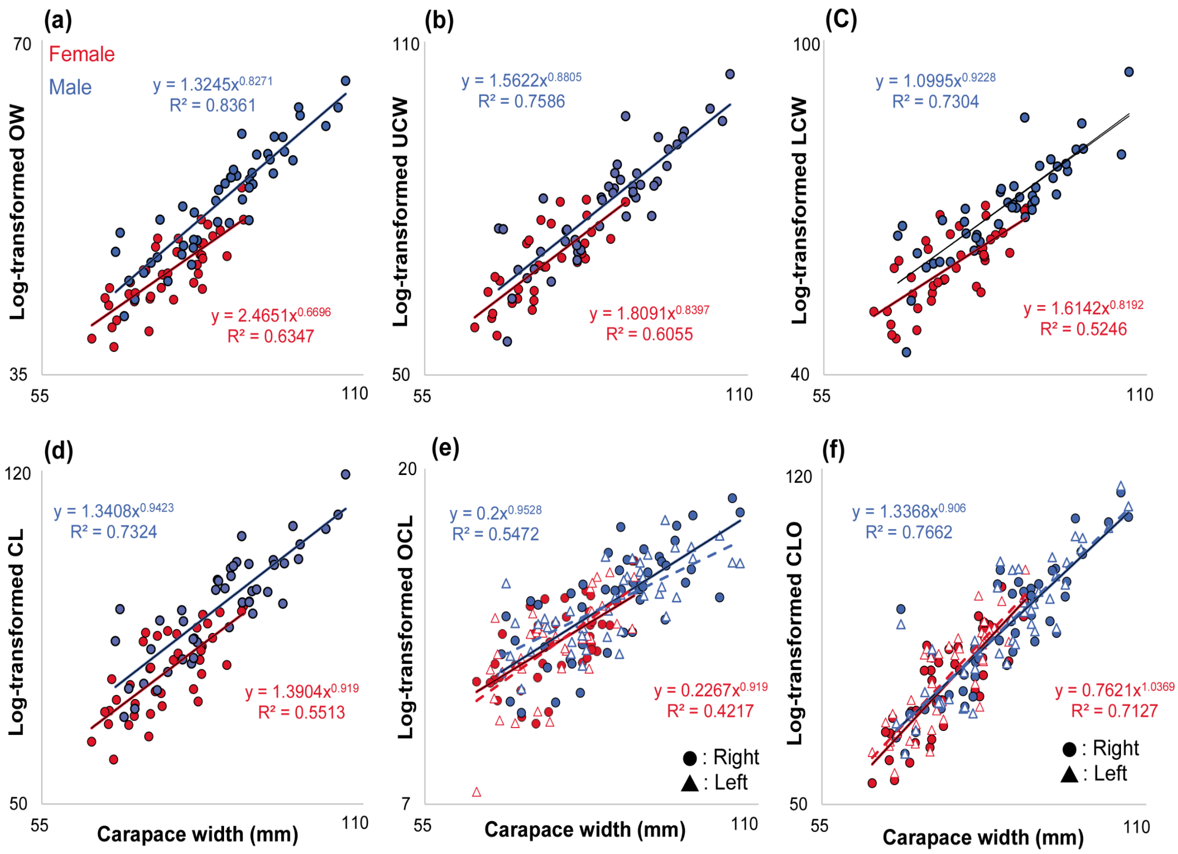

Fig. 6. Relationships between carapace width and each distinct trait between female and male horsehair crabs. (a) Orbital spine width (OW), (b) upper carapace width (UCW), (c) lower carapace width (LCW), (d) carapace length (CL), (e) orbital cavity length (OCL), and (f) carapace length to eye orbit (CLO). Values of carapace width (x-axis) were converted to natural logarithm values. Both red and blue lines indicate the regression curves of males and females, respectively. In (e) and (f), dotted lines indicate left side parameters, and mathematical formula and R^2 values indicate data from the right side only.

Table 4. ANCOVA comparisons of linear regression parameters between female and male horsehair crabs.

Traits versus CW	Homogeneity of the slopes of the regression lines		Sex	
	F value	p-value	F value	p-value
OW	3.67	0.06	26.89	< 0.01
UCW	1.31	0.26	6.829	0.011
LCW	1.04	0.31	11.97	< 0.01
CL	0.19	0.67	13.77	< 0.01
OCL_right	0.01	0.93	0.70	0.41
OCL_left	2.20	0.14	1.65	0.20
CLO_right	0.80	0.37	0.04	0.84
CLO_left	0.71	0.40	0.71	0.40

differences between the chelae of female and male of horsehair crabs. On the other hand, the shape of the pleon is the most general feature for distinguishing males and females in crab species, including horsehair crab. In terms of sexual dimorphism of the carapace, a few studies have been conducted using morphometric approaches with freshwater crabs *Potamon elbursi* (Kalate et al. 2018) and *Isolapotamon nimboni* (Grinang et al. 2019), implying that sexual dimorphism is less likely to appear in the carapace than in the chela or pleon. Our landmark-based geometric and traditional morphometrics with several multivariate analyses (PCA, CVA, and DFA) demonstrated that the carapace of horsehair crab has apparent sexual dimorphic characteristics, suggesting that males or females can be distinguished by the shape of the carapace.

Our results also indicate that the horsehair crab carapace showed both fluctuating asymmetry and directional asymmetry between females and males. Fluctuating asymmetry is considered an indicator of developmental instability, reflecting the ability of individuals to buffer themselves during developmental stages in response to various environmental stresses. This implies that fluctuating asymmetry is tightly linked to geographic distribution, possibly representing environmental alterations along the distribution of a species (Kark 2001). Unlike fluctuating asymmetry, directional asymmetry (and antisymmetry) have not been considered as reliable indicators of developmental instability, because these types of asymmetry are caused in part by either environmental or genetic factors and are therefore difficult to associate with developmental instability (Palmer and Strobeck 2003). To date, unlike geographic differences, little attention has been paid to sexual differences in these types of asymmetry. All horsehair crabs used in this study were collected at the same time and location, implying that there is no geographic difference between sexes although fluctuating asymmetry showed significant differences. This result suggests that responsiveness to environmental stressors during carapace formation might be different between female and male horsehair crabs. The present study indicates that landmark-based geometric and traditional morphometrics can be a strong tool to distinguish females and males in horsehair crab using carapace shape. This is the first report in horsehair crab to detail morphological features from the point of view of sexual differences, suggesting that traditional and geometric morphometric parameters obtained from this study will be a useful reference dataset that can be applied for geographic comparisons of carapace shape of horsehair crab.

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References

Adams, D. C., Rohlf, F. J., Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. Ital. J. Zool. 71: 5–16.

Grinang, J., Das, I., Ng, P. K. L. (2019). Geometric morphometric analysis in female freshwater crabs of Sarawak (Borneo) permits addressing taxonomy-related problems. PeerJ 7: e6205.

Ichikawa, T., Iwamoto, A., Tsuzaki, T., Ohta, K., Hamasaki, K., Takeuchi, T. (2013). Effects of supplementation of rearing water with diatom *Thalassiosira* sp. And dietary n-3 highly unsaturated fatty acids on the survival and development period of horsehair crab *Erimacrus isenbeckii* Zoeae. Aquaculture Sci. 61: 293–302.

Ichikawa, T., Murakami, N., Morioka, T., Murakami, K., Hamasaki, K. (2014a). Effects of light environment on the sinking phenomenon and the development of larvae of the horsehair crab *Erimacrus isenbeckii* in rearing tank. Aquaculture Sci. 62: 65–73 (In Japanese with English abstract).

Ichikawa, T., Nawa, Y., Hamasaki, K., Murakami, K. (2014b). Ontogenetic change of phototaxis, vertical distribution, body density and external morphology during larval development in the horsehair crab *Erimacrus isenbeckii* reared in the laboratory. Nippon Suisan Gakkaishi 80: 349–359 (In Japanese with English abstract).

Ichikawa, T., Hamasaki, K., Murakami, K. (2018). Larval survival, development and growth in the horsehair crab, *Erimacrus isenbeckii*, cultured under different photoperiod conditions. Aquaculture Res. 49: 2511–2517.

Jinbo, T., Hamasaki, K., Ashidate, M. (2005). Effects of Artemia nauplii density on survival, development and feeding of larvae of the horsehair crab *Erimacrus isenbeckii* (Crustacea, Decapoda, Brachyura) reared in the laboratory. Nippon Suisan Gakkaishi 71: 563–570 (In Japanese with English abstract).

Jinbo, T., Hamasaki, K., Ashidate, M. (2007). Effects of water temperature on survival, development and feeding of larvae of the horsehair crab *Erimacrus isenbeckii* (Crustacea, Decapoda, Brachyura) reared in the laboratory. Nippon Suisan Gakkaishi 73: 1081–1089 (In Japanese with English abstract).

Kalate, A., Keikhosravi, A., Naderloo, R., Hajjar, T., Schubart, C. D. (2018). Morphometric characterization of the freshwater crab *Potamon elbursi* Pretzmann, 1962 in the Caspian Sea and Namak Lake hydrographic systems. J. Crustacean Biol. 38: 91–100.

Kark, S. (2001). Shifts in bilateral asymmetry within a distribution range: the case of the chukar partridge. Evolution 55: 2088–2096.

Klingenberg, C. P. (2011). MORPHOJ: an integrated software package for geometric morphometrics. Mol. Eco. Res. 11: 353–357.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., Foy, R. J. (2013). Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. PLoS ONE 8: e60959.

Mariappan, R., Balasundaram, C., Schmitz, B. (2000). Decapod crustacean chelipeds: an overview. J. Biosci. 25: 301–313.

Marchiori, A. B., Bartholomei-Santos, M. L., Santos, S. (2014). Intraspecific variation in *Aegla longirostri* (Crustacea: Decapoda: Anomura) revealed by geometric morphometrics: evidence for ongoing speciation? Biol. J. Linn. Soc. 112: 31–39.

Nagano, J., Munehara, H., Shimazaki, K. (1999). Embryonic development of the hair crab *Erimacrus isenbeckii*. J. Crustacean Biol. 19: 77–83.

Palmer, A. R., Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. Ann. Rev. Ecol. Syst. 17: 391–421.

Palmer, A. R., Strobeck, C. (1992). Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. Acta Zool. Fennica 191: 57–52.

Palmer, A. R., Strobeck, C. (2003). Fluctuating asymmetry analysis revisited. In: Polak, M. (Ed.) Developmental instability (DI): cause and consequences. Oxford University Press, Oxford, p. 279–319.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/ (last accessed 7 February 2020).

Rohlf, F. J. (1990). Morphometrics. Ann. Rev. Ecol. Syst. 21: 299–316.

Rohlf, F. J. (2010). TPSDig2, version 2.16. Stony Brook, NY: Department of Ecology and Evolution, State University of New York. Available at: https://life.bio.sunysb. edu/ morph/ (last accessed 7 February 2020).

Sasaki, J. (2003). Horsehair crab in Hokkaido. In J. Ootomi, S. Watanabe (Eds.) Crustacean resources and their diversity. p. 31–35 (in Japanese).

Schindelin, J., Arganda-Carreras, I., Frise, E., Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods 9: 676–682.

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