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The relative growth of walking legs of adult Chinese mitten crabs, *Eriocheir sinensis* in the Odra estuary (Poland). The major axis statistical modeling

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Key words: Chinese mitten crab, merus, propodus & carpus, walking leg, relative growth, major axis, slope test, elevation test, isometry test, allometry

Abstract

We have quantified the relative growth of the merus and the propodus & carpus of walking legs of an adult of *Eriocheir sinensis* against the carapace width by fitting the major axis lines. Different lines have been fitted to data relative to male and female individuals. Slope and elevation tests have been performed to investigate sexual dimorphism. Isometry tests have been used to classify the relative growth of each part of the legs against the carapace width as isometric or allometric.

All the slope tests have not rejected the null hypothesis of a common slope for the major axes fitted to male data and to female data. However, all the elevation tests have rejected the null hypothesis of a common elevation; so sexual dimorphism was detected for all measured leg parts. The isometry tests have rejected the null hypothesis of isometry only for the merus of the first (left and right) legs of male individuals. All the other isometry tests have not rejected the null hypothesis of isometry. So the relative growth of all the measured leg parts, but the merus of the first (left and right) legs of males, against the carapace width was isometric both for males and for females.

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INTRODUCTION

The Chinese mitten crab, *Eriocheir sinensis* is an invasive species, known to have negative impact on local fisheries (Veldhuizen, Stanish 1999), river banks (Rudnick et al. 2000) and native fauna (Clark et al. 1998). In Europe, the crab was recorded for the first time in the early 20th century in the River Aller in Germany. It has been found from Portugal and Great Britain in the west, through France, The Netherlands, Denmark and Poland, to Finland and Estonia in the east (Panning 1939, Rudnick et al. 2000, Czerniejewski et al. 2003). In Poland, *Eriocheir sinensis* has been observed by fishermen as by-catch in the Southern Baltic since at least 1928 (Grabda 1973). Actually in Poland this species is most numerous in Szczecin Lagoon and in Lake Dabie (North-Western Poland) as by-catch, where its count in some seasons of the year reaches 20 specimens a day (Czerniejewski et al. 2003).

Crustaceans are particularly suitable for studies on the relative growth due to their hard exoskeleton that facilitates precise body measurements. Frequently, there are large differences in the growth rates between males and females (Hartnoll 1978). However, walking legs have rarely been measured, and their relative growth had almost never been investigated. In Kobayashi (2002), the relative growth of the third walking leg merus against carapace width was quantified for the Japanese mitten crab, *Eriocheir japonica*. To our knowledge, there are no publications addressing the relative growth of walking legs of the Chinese mitten crab.

In this paper the relative growth of the merus and the propodus & carpus of walking legs of *Eriocheir* *sinensis* against the carapace width is quantified using the major axis fitting line procedures. The main objective was to investigate whether statistically significant differences between males and females (sexual dimorphism) were present and to establish whether the growth of leg parts with respect to CW was isometric or allometric.

MATERIALS AND METHODS

The study involved a total of 233 Chinese mitten crab individuals trapped as by- catch in fyke nets, in spring and autumn 2001-2002 in the Szczecin Lagoon. All crabs were adult. Live crabs were delivered to the laboratory and their sex was determined based on the abdomen shape and the presence of gonopods (Schäferna 1935).

Subsequently, each crab was placed in a double-wall plastic bag and frozen (-20°C). The crabs were stored frozen until further analyses. For those, the crabs were thawed and their maximum carapace width (CW) was measured. Measurements were also taken on each walking legs. The merus length and the propodus & carpus length (Fig. 1) were measured on four right walking legs and on four left walking legs. The measurements were recorded to 0.01 mm, with computer-interfaced Helios RS 232 electronic callipers (Helios, Poland). The measurements of the considered leg parts have been coded as follows:

MjR	the length of the merus of the jth leg on the right side of the crab
MjL	the length of the merus of the jth leg on the left side of the crab
PCjR	the length of the propodus & carpus of the jth leg on the right side of the crab
PCjL	the length of the propodus & carpus of the jth leg on the left side of the crab
J	1,2,3,4

Statistical Methods

We used the carapace width (CW) as an overall scalar measure of the size. The relative growth of a leg part against CW may be expressed by using the log-Huxley equation (Huxley 1932):

$$\log(Y) = a + b \times \log(CW) \quad (1)$$

where Y is the linear measure of the leg part considered. Remember that if b=1, then the relationship expressed by model (1) is called isometric. If b≠1, the relationship is called allometric. Parameters a and b in model (1) are called

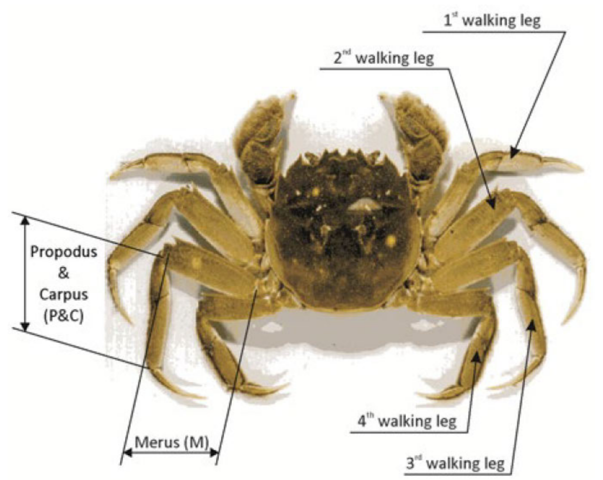

Fig. 1. Chinese mitten crab (according to Veldhuizen & Stanish 1999) and the measured leg parts: Merus and Propodus & Carpus.

respectively "elevation" and "slope". The model (1) was fitted several times for the different characteristics considered using the Major Axis (MA) Regression, also called "model II regression" (Sokal, Rohlf 1995; Smith 2009; Warton, Weber 2002).

Regarding the parameters estimation, MA is, as linear regression, a least squares method, which means that parameters are estimated by minimizing the residual sum of squares. In MA regression, the residuals are measured perpendicularly to the fitted line. In linear regression residuals are measured in the direction parallel to the Y axis. The MA method should be preferred over linear regression in morphometric studies for several reasons (Sokal, Rohlf 1995; Smith 2009; Warton, Weber 2002).

In the context of MA line fitting, several statistical hypothesis tests may be carried out. We have fitted the models

$$\log(Y) = a_m + b_m \log(CW)$$

$$\log(Y) = a_f + b_f \log(CW)$$

respectively to male and female data. The slope test is used to investigate if the slope parameters of the MA lines may be considered equal (H0: $b_m = b_f$) or must be considered significantly different (H0: $b_m \neq b_f$) at 5%. The elevation test is used to investigate if the elevation parameters of the MA lines may be considered equal (H0: $a_m = a_f$) or must be considered significantly different (H0: $a_m \neq a_f$) at 5%, given that the slope parameters are considered equal. The isometry test is used to investigate if the relationship considered in model (1) may be considered isometric (H0: $b = 1$) or must be considered significantly allometric (H1: $b \neq 1$) at 5%.

RESULTS

A total of 233 individuals (106 males and 127 females) of *Eriocheir sinensis* were captured. The carapace width and the length of 16 walking leg parts (merus and propodus & carpus of all walking legs) were measured on each crab. So, the original data set consisted of 3961 data.

Some graphical tools of data exploration (Cleveland dotplots, boxplots and bivariate plots) have been used to detect outliers and seasonal or annual effect of inspection. Several outliers were detected. Data relative to 21 individuals (12 males and 9 females) were removed from the data set. The final sample consisted of 212 individuals (94 males and 118 females). No seasonal or annual effect was detected.

Summary statistics (minimum, the 1st quartile, mean, the 3rd quartile, maximum) conditional on sex of all measured characteristics are presented in Table 1. The length of merus and propodus & carpus in males were significantly longer than in females. There are no differences in the mean length of merus and propodus & carpus between left and right of particular pairs of crabs (Table 1).

Model (1) was fitted to male data and to female data for all measured leg parts. All the obtained results are summarized in Table 2.

For example, looking at the first row of Table 2, we may see that for M1R the estimated parameters were $a_m = -0.776$, $b_m = 1.074$, $a_f = -0.800$ and $b_f = 1.054$. The slope test statistic assumed the value of 0.106, with the p-value of 0.744; so the null hypothesis was not rejected (at 5%) and we concluded that the major axes fitted to male and female M1R data shared a common slope. The elevation test statistic assumed the value of 322.421, with the p-value<0.000; the null hypothesis was rejected and we concluded that the elevation parameters of the major axes fitted to male and female M1R data were significantly different at 5%. Looking at Table 2, we may see that all the slopes were not significantly different for males and females. However, in all cases the elevation parameters were significantly different, showing the presence of sexual dimorphism in the relative growth of all considered parts of walking legs.

Table 1

Summary statistics (minimum, the 1st quartile, mean, the 3rd quartile, maximum) of all measured characteristics, conditional on sex.

Leg part	Sex	Min.	1st qu.	Mean	3rd qu.	Max.
M1R	Females	28.45	34.53	36.89	39.25	45.21
	Males	30.20	41.09	43.90	47.50	53.16
M2R	Females	35.17	43.84	47.13	50.39	57.42
	Males	39.39	51.34	54.70	58.60	64.73
M3R	Females	37.40	44.88	48.18	51.14	59.16
	Males	39.52	51.23	55.23	69.57	65.50
M4R	Females	31.91	37.51	40.30	42.88	47.82
	Males	31.18	43.08	45.93	49.93	58.03
M1L	Females	28.86	35.01	37.05	39.46	44.98
	Males	29.07	41.51	44.08	47.80	55.40
M2L	Females	37.12	43.79	47.14	50.20	56.74
	Males	37.44	51.94	54.93	58.55	66.54
M3L	Females	38.79	45.09	48.44	51.58	61.27
	Males	40.12	52.23	55.53	60.08	68.11
M4L	Females	30.40	37.68	40.37	42.91	49.11
	Males	30.88	44.16	46.06	49.21	54.53
PC1R	Females	24.99	30.46	32.77	35.12	40.18
	Males	28.44	35.37	38.43	41.49	45.95
PC2R	Females	31.52	40.11	42.76	45.41	51.92
	Males	36.47	45.40	48.44	51.43	58.33
PC3R	Females	34.68	40.43	43.94	47.42	53.92
	Males	37.05	45.46	49.13	53.15	59.22
PC4R	Females	25.45	31.16	33.53	36.00	40.78
	Males	27.51	35.94	38.09	41.08	47.34
PC1L	Females	25.31	30.14	32.06	34.70	38.82
	Males	27.71	35.01	37.37	40.45	45.15
PC2L	Females	32.05	38.65	41.94	44.98	53.16
	Males	34.76	44.47	48.01	51.61	57.96
PC3L	Females	32.65	41.12	43.68	46.88	52.93
	Males	36.38	45.94	49.13	52.54	60.31
PC4L	Females	25.87	30.81	32.83	35.39	41.08
	Males	26.91	34.80	37.26	40.47	44.41
CW	Females	50.37	62.05	65.57	69.74	83.85
	Males	51.93	65.42	69.78	75.00	83.53

The isometry tests were also performed for all the measured leg parts. The results are summarized in Table 3. For example, looking at the first row of Table 3, we may see that for the merus of the first right leg (M1R) of males, the isometry test statistic was 0.213, with the p-value of 0.039. So the null hypothesis of isometry was rejected, that is the relative growth of the merus of the first right leg against the carapace width was significantly allometric at 5%. Note that the p-values in Table 3 are less than the significance level of 5% only for variables M1R and M1L and only for the male data. We deduced that only the relative growth of the merus of the first left and right leg of males was

Table 2

Estimated elevations (a) and slopes (b) for all measured variables, for males and females. Slope test statistics and p-values. Elevation test statistics and p-values.

Leg part	Estimated parameters				Hypothesis test			
	Males		Females		Slope test		Elevation test	
	a_m	b_m	a_f	b_f	stat	p-value	stat	p-value
M1R	-0.776	1.074	-0.800	1.054	0.106	0.744	322.421	<0.000
M2R	-0.179	0.985	-0.431	1.024	0.466	0.495	230.964	<0.000
M3R	-0.130	0.975	-0.365	1.013	0.275	0.600	112.100	<0.000
M4R	-0.712	1.069	-0.549	1.015	0.784	0.376	111.441	<0.000
M1L	-0.952	1.116	-0.649	1.019	2.063	0.151	250.364	<0.000
M2L	-0.354	1.027	-0.459	1.031	0.004	0.949	224.836	<0.000
M3L	-0.479	1.059	-0.522	1.052	0.008	0.929	94.958	<0.000
M4L	-0.512	1.023	-0.726	1.058	0.211	0.646	84.738	<0.000
PC1R	-0.741	1.034	-0.840	1.035	0.000	0.989	186.433	<0.000
PC2R	-0.192	0.959	-0.303	0.970	0.021	0.885	79.023	<0.000
PC3R	-0.303	0.989	-0.521	1.029	0.199	0.655	33.363	<0.000
PC4R	-0.964	1.084	-0.939	1.064	0.065	0.798	61.818	<0.000
PC1L	-0.723	1.023	-0.983	1.064	0.391	0.532	185.901	<0.000
PC2L	-0.591	1.051	-0.732	1.068	0.053	0.818	93.291	<0.000
PC3L	-0.421	1.016	-0.646	1.057	0.217	0.641	41.796	<0.000
PC4L	-0.720	1.022	-0.911	1.052	0.120	0.729	56.207	<0.000

Table 3

Isometry test results: estimated slopes, test statistics and p-values, for males and females.

Leg part	Males			Females		
	est. b	stat	p-value	est. b	stat	p-value
M1R	1.074	0.213	0.039	1.054	0.105	0.256
M2R	0.985	-0.044	0.677	1.024	0.050	0.588
M3R	0.975	-0.048	0.647	1.013	0.026	0.783
M4R	1.069	0.165	0.113	1.015	0.034	0.718
M1L	1.116	0.238	0.021	1.019	0.041	0.663
M2L	1.027	0.070	0.501	1.031	0.067	0.469
M3L	1.059	0.126	0.225	1.052	0.089	0.338
M4L	1.023	0.049	0.642	1.058	0.094	0.309
PC1R	1.034	0.085	0.413	1.035	0.055	0.552
PC2R	0.959	-0.080	0.446	0.970	-0.050	0.591
PC3R	0.989	-0.017	0.871	1.029	0.048	0.604
PC4R	1.084	0.140	0.178	1.064	0.133	0.151
PC1L	1.023	0.052	0.621	1.064	0.136	0.143
PC2L	1.051	0.110	0.290	1.068	0.119	0.199
PC3L	1.016	0.029	0.778	1.057	0.084	0.366
PC4L	1.022	0.039	0.706	1.052	0.075	0.422

significantly allometric. The growth of all other leg parts against the carapace width was isometric.

In Figure 2 and 3 the scatterplots of the logs of the length of all the measured leg parts against log (CW) are presented with the estimated major axes.

Discussion

Crabs belonging to the genus of *Eriocheir* are known for their active life style related to their catadromous habit, especially for *Eriocheir sinensis* migrating from the coastal areas of China and Korea to new areas (Panning 1939). Once sexually mature (after 1-5 years), they return to their spawning ground (Panning 1939; Veldhuizen, Stanish 1999). The migration usually begins in the late summer (Panning 1939; Veldhuizen, Stanish 1999; Czerniejewski, Wawrzyniak 2006). Hence the largest catches are obtained in the autumn. In the Odra estuary, most of the crabs (as much as 85.4%) were caught in autumn (Czerniejewski, Wawrzyniak 2006). High locomotor activity in this species is considered to be necessary during this life stage. However, walking legs in similar species of crabs, *Eriocheir japonica* are relatively longer in juveniles than in adults (Kobayashi 2002). As Kobayashi (2002) noted for adults, it may be much easier to migrate over a long distance than it is for juveniles migrating upstream. Adults have increased locomotor activity after growing to a larger size and they only need to flow downstream when the river is rising. In adults of the Chinese mitten crab, the length of each leg segment versus carapace width varied a lot between legs (see Table 1). The merus and propodus & carpus segments would be advantageous as longer segments to dig in and grasp firmly to resist dislodging, wave action and for long distance migrations.

A clear sexual dimorphism, with males reaching larger leg sizes than females, is well known in brachyuran crabs (Abello 1990; De Giosa, Czerniejewski 2011). The most frequent analyses are related to the first pair of legs – chelipeds, which are markedly larger in males than females. These differences are usually explained by their additional function - in more aggressive crab males, chelipeds are not only used for feeding, but also in agonistic behavior or copulation (Sarda et al. 1995, Spooner et al. 2007). In our study, walking legs of adult males of *Eriocheir sinensis* were relatively longer than those of adult females. Males showed a greater length of merus, and propodus & carpus than females. For example, the percentage M1R difference was 11.35% and the percentage PC1R difference was 10.30%, for a crab of medium CW. Kobayashi (1999) and Kobayashi and Matsuura (1993) showed that for *Eriocheir japonica* long legs and large chelae in males are useful during mating. After crabs migrate downstream from the freshwater area to the tidal

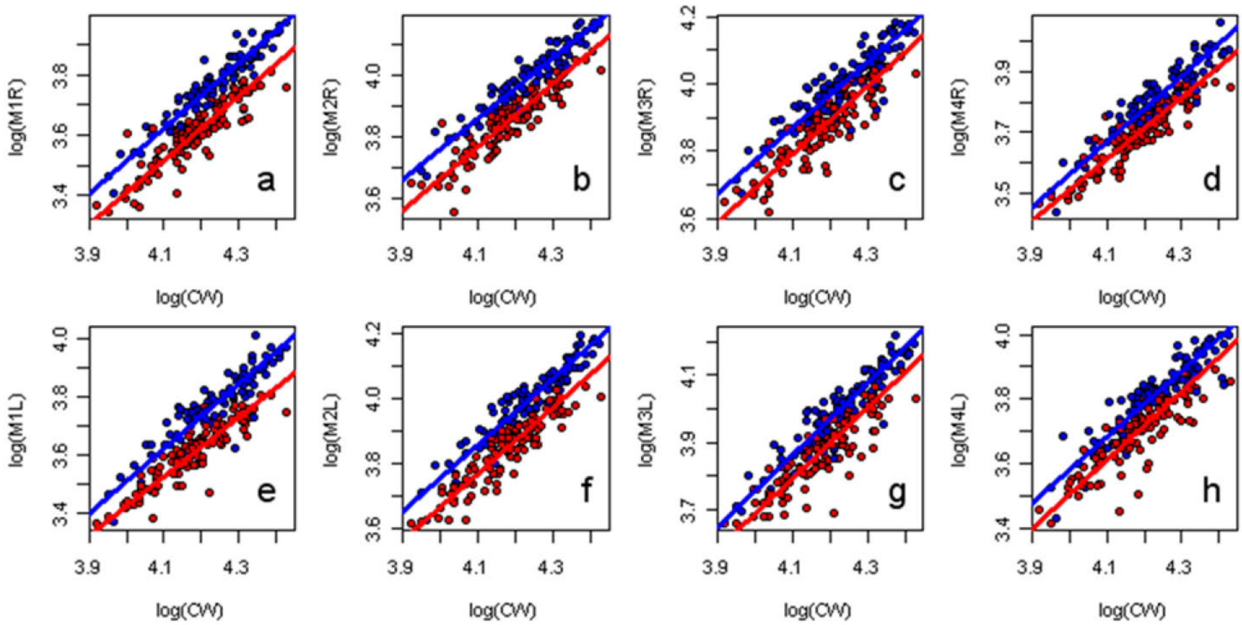

Fig. 2. Scatterplots of a) log(M1R), b) log(M2R), c) log(M3R), d) log(M4R), e) log(M1L), f) log(M2L), g) log(M3L), h) log(M4L), against log(CW) with estimated MA lines. Blue=males, Red=females.

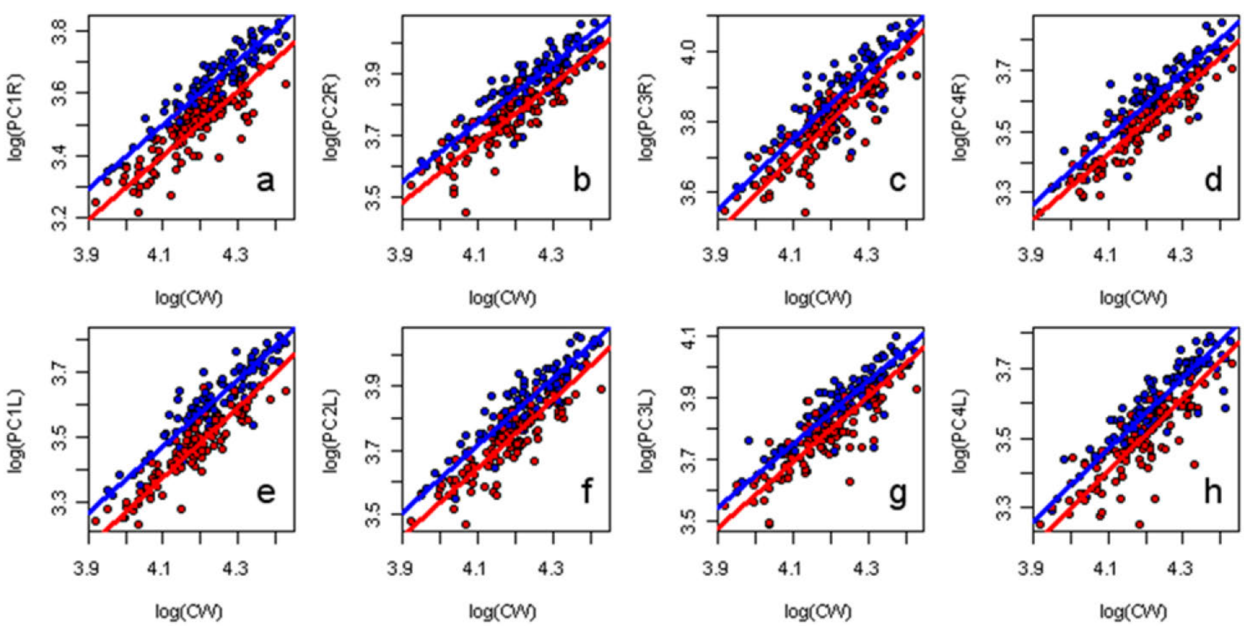

Fig. 3. Scatterplots of a) log(PC1R), b) log(PC2R), c) log(PC3R), d) log(PC4R), e) log(PC1L), f) log(PC2L), g) log(PC3L), h) log(PC4L), against log(CW) with estimated MA lines. Blue=males, Red=females.

area, males actively wander in the coastal area and search for mates (Kobayashi, Matsuura 1994). Mating of crabs is initiated by the active behavior of males grasping and mounting the females. According to Kobayashi (2002) longer legs in males are likely useful for successful guarding, as well as they give them much greater mobility (Kobayashi, Matsuura 1994). This suggests that males' ability to move faster gives them better opportunities to catch food, as well as in agonistic behavior or copulation, and in consequence, to achieve a higher rate of growth and a better condition. The significantly better condition of males than females in the Chinese mitten crab in the Odra estuary is supporting this hypothesis (Czerniejewski 2010). Better condition of males may result not only from the nutritional state, but also from having larger chelipeds and walking legs compared to females of the same carapace width.

In *Eriocheir sinensis* segments of the 2nd and 3rd pair of walking legs are the longest, and the 1st and 4th pair of walking legs are the shortest. Klärner and Barnes (1986) showed that each leg had a different function for the crayfish when walking: the 2nd walking leg lifts and stabilize the body and the 3rd leg shares these functions and produces the largest proportion of the propulsive force. In *Procambarus clarkii* each leg has its own dynamic pattern, possibly due to the angle of the insertion of the leg into the thorax (Jamon, Clarac 1995).

In our study some crabs have regenerated legs, smaller length of merus and propodus & carpus than others with the same carapace width (e. g. especially red dots on scatterplots in Fig. 2 and 3). These results are not surprising, because crabs sometimes lose their legs. Although decapod crustaceans possess the ability to regenerate the lost appendages, they cannot immediately regenerate these appendages to normal sizes. Bennett (1973) reported that a regenerated claw of the brachyuran, *Cancer pagurus* is functionally approximately half of the size of a similar not regenerated claw. Skinner and Graham (1972) found that regenerated walking legs of *Gecarcinus lateralis* are approximately two-thirds of the size of their corresponding limbs of the opposite side of the animal.

A number of investigators have studied the role of the relative growth of crabs in determining the size at the sexual maturity, an important tool in characterizing the sexual dimorphism, systematics, physiological and behavioral patterns (Clayton 1990, Abelló et al. 1990, Sarda et al. 1995). The studies undertaken during the last decades, however, revealed important features of the growth in different families and/or genera. The growth processes have also been studied in the Japanese mitten crab (Kobayashi 2002) and in the Chinese mitten crab (De Giosa, Czerniejewski 2011). De Giosa and Czerniejewski (2011) noted that for females, the relative growth of the carapace length and carapace height against carapace width were isometric, and for all other linear measures (claw length, height, width), the relationship with carapace width was positively allometric. For males, only the relative growth of the carapace length was isometric, but all the others were positively allometric. In this paper we have showed that for the Chinese mitten crab the growth pattern for the merus of the first left and right legs are allometric only for males. The growth of all other leg parts against the carapace width was isometric. It was unexpected, because in *Eriocheir japonica* only in small crabs (20 mm<CW<40 mm), the growth of a segment of walking legs was isometric, but in adults it was negative allometric (Kobayashi 2002). However, Kobayashi (2002) used the classical linear regression method instead of the major axis approach. In some other species of decapods, the segments of the walking legs were isometric or positively allometric, making the total allometry for a member as a whole positive (Sarda et al. 1995).

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