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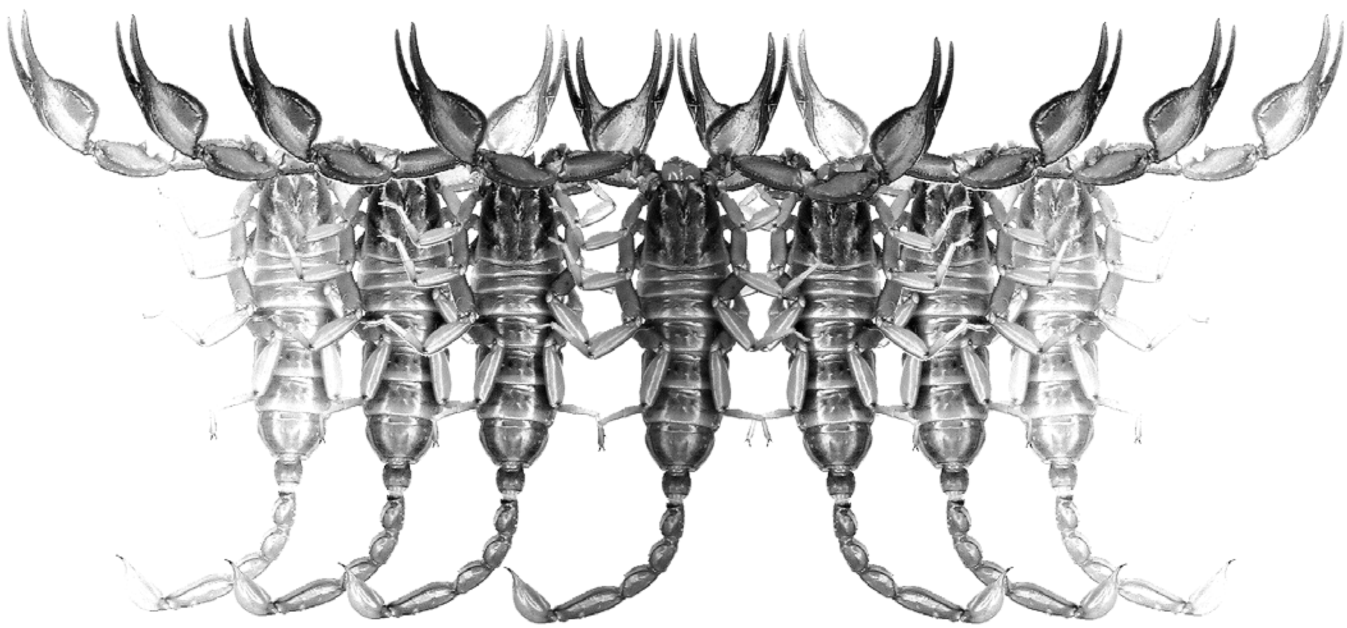

Morphological Variation of *Mesobuthus martensii* (Karsch, 1879) (Scorpiones: Buthidae) in Northern China

Lu Zhang & Ming-Sheng Zhu

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Morphological variation of *Mesobuthus martensii* (Karsch, 1879) (Scorpiones: Buthidae) in Northern China

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Summary

Comparative statistical analysis of morphology of *Mesobuthus martensii* and *Mesobuthus eupeus* (Scorpiones: Buthidae) indicated highly significant differences between these two species. Mann-Whitney U test showed that: except Ca_L/AW, Ca_AW/PW and Met-V_L/H in female, Ca_AW/PW and Met-V_L/H in male, all morphometric ratios demonstrated significant differences between *M. martensii* and *M. eupeus*. Principal component analysis (PCA) showed that *M. martensii* and *M. eupeus* are clearly different species in morphology. Three statistical analyses (Kruskal-Wallis one-way ANOVA, PCA and cluster analysis) were applied to *M.martensii* from 27 confirmed localities in five provinces of northern China. The results suggested that: (a) Although *M. martensii* is common and widespread in northern China, its morphology does not vary significantly, but there is more variation in females than males, and the variation both in males and females is below species level; (b) the populations from Hebei, Ningxia, Inner Mongolia, and Liaoning Provinces were similar to each other while the populations from Qinghai were separated from the others; (c) except Met-I_L/W and Met-V_L/W, each ratio of metasoma in both females and males of Qinghai populations was smaller and the ratio of Ca_L/AW was larger than those from other provinces.

Introduction

Mesobuthus martensii was originally described by Karsch (1879) as *Buthus martensii*, based on a single male specimen, presumably collected in Singapore by Prof. de Martens. However, Kishida (1939) thought that the primary specimen on which the original description of *B. martensii* was based and examined by Karsch (1879) had been wrongly labelled "from Singapore". Then, Simon (1880) described a new species from China, *Buthus confucius*, which was later found to be a synonym of *Buthus martensii* (Karsch, 1881). Later, Wu (1936) reported on two families, four genera and four species of scorpions from China, based mainly on the material deposited in the Chinese Academy of Sciences. Among these species, he identified *B. martensii*. Kishida (1939) redescribed *B. martensii* in a more detailed manner. Qi et al. (2004) redescribed this species again using modern standard characters and added data on ecology and geographical distribution for this species in China. Although *M. martensii* has been listed several times from China, morphological information about this species has been insufficient.

Because *M. martensii* is one of the most widely used scorpion species in traditional Chinese medicine (Zhang et al., 2007), the venom properties of this species have been thoroughly studied. Over the past decade, more than 70 different peptides or toxins have been isolated from venom of this species (Goudet et al., 2002). Toxin researchers record the source populations of *M. martensii*, but usually do not investigate geographic variation among toxins.

M. martensii is a very common and widespread scorpion species and has been recorded in China from Inner Mongolia, Beijing, Liaoning, Shanxi, Hebei, Henan, Shandong, Anhui, Jiangsu, Fujian, and Hubei Provinces (Zhu et al., 2004). It is usually found on the hills with open vegetation formations. It can also be present near human habitations, in gardens, cracks in walls and burrows in the ground (Song et al., 1982; Song, 1998).

Since the species is so widespread, we decided to test the existence of the intra- and interspecific geographic variation in two *Mesobuthus* species found in China, *M. martensii* and *M. eupeus*, and to clarify possible geographic variation in *M. martensii*. The results we obtained may also help monitor the source populations of animals for toxin researchers. *M. eupeus* specimens were used in this study as comparative material to make comparison homogeneous, because this is the only scorpion species found within the same geographic range, Ningxia and Inner Mongolia, as *M. martensii.*

All studied populations belong to the formally recognized subspecies *M. martensii martensii*; another subspecies, *M. martensii hainanensis* (Birula), has only been recorded from southern China, Hainan Province (Zhu et al., 2004). A survey by Shi et al. (2007) of *M. martensii* in China did not include Hainan Province, and

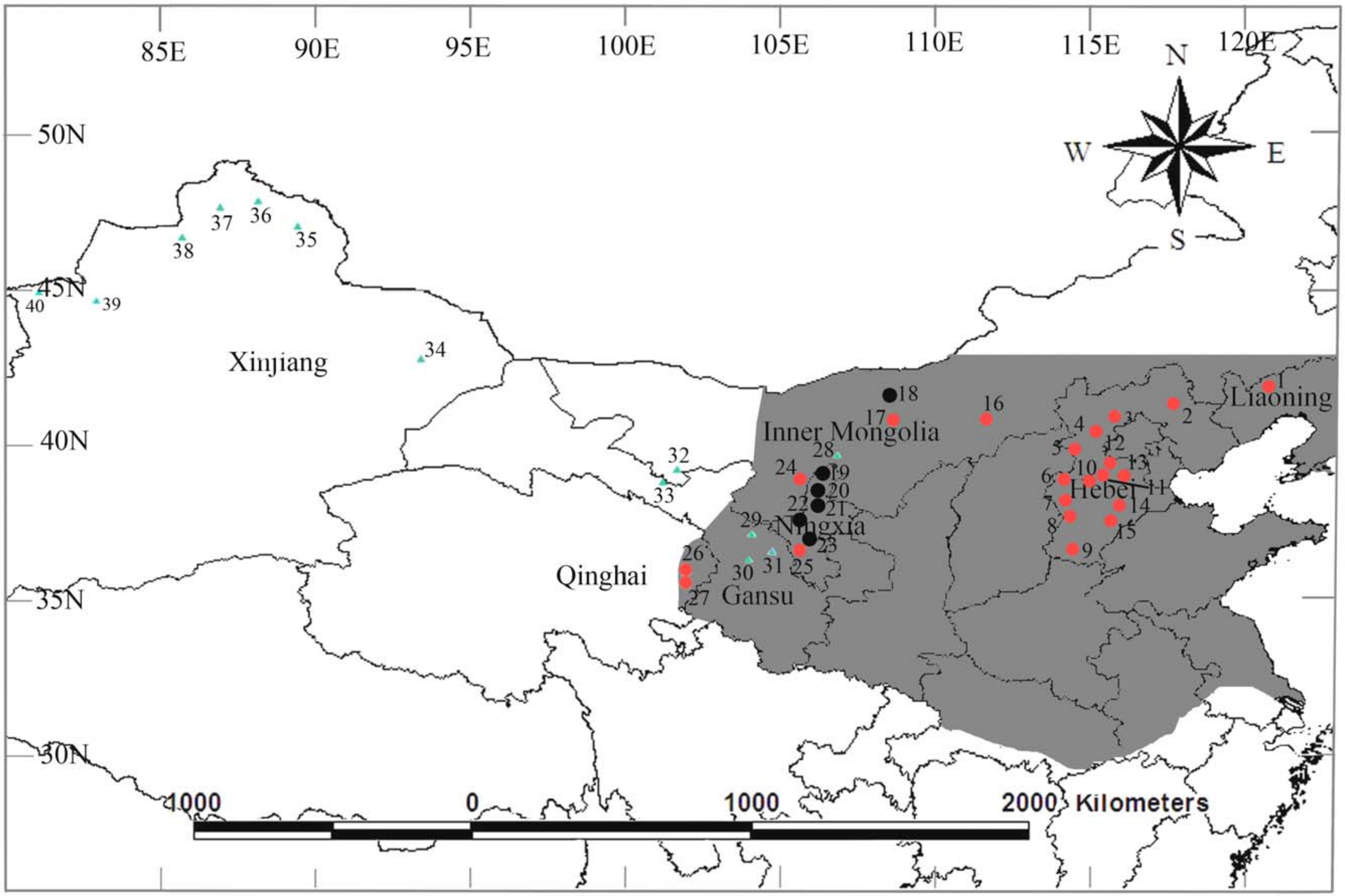


Figure 1: Sampling localities of *Mesobuthus martensii*(●), *M. eupeus*(▲), and sympatric sites(●). The gray region shows the range of *M. martensii* in China. Locality numbers are shown in the text.

therefore only included the range of *M. martensii martensii*.

Because *M. martensii* is mainly distributed in northern China, we chose five northern provinces: Liaoning, Hebei, Inner Mongolia, Ningxia, and Qinghai. The sampling scheme includes almost the easternmost and westernmost edges of the range of this species in China. The distance between them is about 2300km, and the elevation of sample sites range from 10m (Xiongxian County [13] in Hebei province) to 2300m (Jianzha County [26] and Tongren County [27] in Qinghai Province). See Figure 1 for a map of sampling localities.

Material and Methods

Scorpions were collected under stones or clods of dirt during the daytime and placed into 75% alcohol. Specimens were deposited in the scorpion collection at the Museum of the College of Life Sciences, Hebei University (MHBU), Baoding, China. Measurements were taken with >0.001mm accurate micrometric ocular with the XTL-II stereomicroscope (Beijing Electronic Optical Equipment Factory). All measurements are in mm. Terminology follows Hjelle (1990). The methods of measurement are after Sissom et al. (1990). Statistical analyses were conducted using SPSS13.0 for Windows. Measurements of 15 characters in each group of males and females of *M. martensii* and *M. eupeus* were used in the morphometric analysis. The univariance analysis of *M. martensii* included descriptive statistics (means and standard deviations [SD]) for each variable (Table 5 and Table 6). The Mann-Whitney U test was used to determine significant differences between *M. martensii* and *M. eupeus* (Table 1 and Table 2). Principal component analysis (PCA) was applied between *M. martensii* and *M. eupeus*, and within *M. martensii*, to limit over parameterization by reducing the data set to several principle components, and to determine whether any of the geographic populations are morphologically distinct. Kruskal-Wallis one-way ANOVA was applied to *M. martensii* to determine statistical significance of differences wholly ($p<0.05$). Cluster analysis was conducted in order to investigate the relationships between the morphologies of the five provinces. The hierarchical clustering scheme was applied to the mean ratios of each province. The squared Euclidean distance was used to measure the interval and two different cluster methods were applied (Between-groups linkage, Within-groups linkage). Of the seven possible cluster methods, Between Groups linkage, Within Groups linkage, Nearest Neighbor, Furthest Neighbor, Centroid Clustering, Median Clustering, and Ward's method, some are too concentrated while others are too sensitive. We choose Between Groups linkage and Within Groups linkage to do our cluster analysis (He, 2004).

Abbreviations of morphometric ratios

Ca_L/AW: carapace length to anterior width; Ca_AW/PW: carapace anterior width to posterior width; Fem_L/W: pedipalp femur length to width; Pat_L/W: pedipalp patella length to width; Ch_L/W: pedipalp chela length to width; Met-I_L/W: metasomal segment I length to width; Met-I_L/H: metasomal segment I length to height; Met-II_L/W: metasomal segment II length to width; Met-II_L/H: metasomal segment II length to height; Met-III_L/W: metasomal segment III length to width; Met-III_L/H: metasomal segment III length to height; Met-IV_L/W: metasomal segment IV length to width; Met-IV_L/H: metasomal segment IV length to height; Met-V_L/W: metasomal segment V length to width; Met-V_L/H: metasomal segment V length to height; n = sample size.

Specimens examined

Using the traditional criteria of morphological taxonomy, we identified *M. martensii* and *M. eupeus* from the following localities:

***Mesobuthus martensii* (Karsch, 1879)** (total 161 specimens, 93 ♀♀, 68 ♂♂): ***Hebei Province*:** 1♀, Pingshan County [7], 12 August 2008, Jian-Ying Fu leg. (Ar.-MHBU-HBPS0801); 1♀, Wuqiang County [14], 3 May 2005, Hui-Qin Ma leg. (Ar.-MHBU-HBWQ0501); 1♀, 1♂, Fuping County [6], 2 October 2005, Hui-Qin Ma leg. (Ar.-MHBU-HBFP0501-0502); 2♀♀, 2♂♂, Longhua County [2], 27 August 2004, Wei-Guang Lian leg. (Ar.-MHBU-HBLH0401-0404); 1♀, Mancheng County [11], 6 May 2005, Zi-Zhong Yang leg. (Ar.-MHBU-HBMC0501); 1♂, Mancheng County [11], 2 May 2008, Li-Min Zhu leg. (Ar.-MHBU-HBMC0801); 5♀♀, 5♂♂, Zhuolu County [4], 5 July 2004, Feng Zhang leg. (Ar.-MHBU-HBZL0401-0410); 2♀♀, Chicheng County [3], 2 October 2002, Zhi-Sheng Zhang leg. (Ar.-MHBU-HBCC0201-0202); 1♀, 2♂♂, Xiongxian County, [13] 20 July 2004, Chao-Ying Fan leg. (Ar.-MHBU-HBXX0401-0403); 5♀♀, 1♂, Zanhuang County [8], 4 May 2008, Yan-Hai Zhao leg. (Ar.-MHBU-HBZH0801-0806); 5♀♀, 2♂♂, Laishui County [12], 28 June 2004, Jian Song leg. (Ar.-MHBU-HBLS0401-0407); 4♀♀, 2♂♂, Tangxian County [10], March 2007, Shi-Ning Liu & Zhi-Yong Di leg. (Ar.-MHBU-HBTX0701-0706); 1♂, Handan County [9], 26 August 2004, Tie Wang leg. (Ar.-MHBU-HBHD0401); 1♂, Yuxian County [5], July 2006, Li-Min Zhu leg. (Ar.-MHBU-HBYX0601); 1♂, Zaoqiang County [15], July 2005, Feng Zhang leg. (Ar.-MHBU-HBZQ0501). ***Ningxia Province*:** 3♀♀, 1♂, Zhongning County [22], 15 August 2008, Ming-Sheng Zhu & Feng Zhang leg. (Ar.-MHBU-NXZN0801-0804); 5♀♀, 5♂♂, Shizuishan [19], 16 August 2008, Guang-Xin Han, Dong Sun &

Character	*M. martensii* N=93	*M. eupeus* N=247	Mann-Whitney U test	
Ca_L/AW	1.479±.047	1.470±.044	.129	ns
Ca_AW/PW	.608±.019	.612±.016	.068	ns
Fem_L/W	3.180±.160	2.992±.146	.000	*
Pat_L/W	2.624±.164	2.411±.097	.000	*
Ch_L/W	4.757±.246	3.718±.211	.000	*
Met-I_L/W	1.019±.048	.869±.035	.000	*
Met-I_L/H	1.211±.063	1.026±.044	.000	*
Met-II_L/W	1.265±.056	1.028±.036	.000	*
Met-II_L/H	1.414±.068	1.081±.042	.000	*
Met-III_L/W	1.374±.050	1.070±.038	.000	*
Met-III_L/H	1.494±.064	1.093±.044	.000	*
Met-IV_L/W	1.665±.060	1.358±.053	.000	*
Met-IV_L/H	1.823±.066	1.599±.068	.000	*
Met-V_L/W	2.114±.074	1.853±.065	.000	*
Met-V_L/H	2.397±.089	2.395±.120	.869	ns

Table 1: Mann-Whitney U test analysis of morphometric measurements of the ***females*** of *M. martensii* and *M. eupeus*.
*, $P<0.05$; ns, no significant difference.

Character	*M. martensii* N=68	*M. eupeus* N=234	Mann-Whitney U test	
Ca_L/AW	1.472±.054	1.491±.050	.005	*
Ca_AW/PW	.625±.025	.625±.019	.909	ns
Fem_L/W	3.379±.242	3.188±.164	.000	*
Pat_L/W	2.743±.157	2.520±.095	.000	*
Ch_L/W	4.194±.409	3.526±.228	.000	*
Met-I_L/W	1.062±.061	.897±.034	.000	*
Met-I_L/H	1.235±.082	1.051±.040	.000	*
Met-II_L/W	1.305±.073	1.045±.036	.000	*
Met-II_L/H	1.419±.077	1.135±.049	.000	*
Met-III_L/W	1.424±.078	1.092±.044	.000	*
Met-III_L/H	1.499±.061	1.145±.043	.000	*
Met-IV_L/W	1.711±.080	1.391±.048	.000	*
Met-IV_L/H	1.796±.084	1.589±.063	.000	*
Met-V_L/W	2.174±.139	1.860±.070	.000	*
Met-V_L/H	2.416±.127	2.376±.103	.080	ns

Table 2: Mann-Whitney U test analysis of morphometric measurements of the ***males*** of *M. martensii* and *M. eupeus*.
*, $P<0.05$; ns, no significant difference.

Cheng-Li Zhang leg. (Ar.-MHBU-NXSZS0801-0810); 1♀, Yinchuan [20], 10 August 2007, Zhi-Yong Di leg. (Ar.-MHBU-NXYC0701); 2♀♀, 1♂, Yinchuan [20], August 2008, Qi-Qi Wu leg. (Ar.-MHBU-NXYC0801-0803); 3♀♀, 3♂♂, Tongxin County [23], 6 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXTX0701-0706); 3♀♀, 3♂♂, Wuzhong [21], 7 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXWZ0701-0706); 3♀♀, 3♂♂, Haiyuan County [25], 6 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXHY0701-0706). ***Inner Mongolia*:** 1♀, Hohhot [16], July 1983, Li-Ta Wu leg. (Ar.-MHBU-IMHT8301); 4♀♀, 3♂♂, Urat Middle Banner [18], 16 July 2008, Dong Sun & Cheng-Li Zhang leg. (Ar.-MHBU-IMUMB0801-0807); 13♀♀, 11♂♂, Alxa Left Banner [24], 20 July 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-IMALB0701-0724); 2♀♀, 1♂,

Component		**1**	**2**	**3**	**4**
Initial Eigenvalues	**Total**	9.793	1.147	1.024	1.008
	% of Variance	65.286	7.648	6.829	6.721
Ca_L/AW		.091	-.453	.566	.632
Ca_AW/PW		-.100	.865	.214	.080
Fem_L/W		.573	.336	.357	.271
Pat_L/W		.716	.213	.213	.012
Ch_L/W		.912	-.010	-.066	.041
Met-I_L/W		.932	.022	-.006	.041
Met-I_L/H		.904	-.006	-.104	.007
Met-II_L/W		.968	-.006	-.059	.008
Met-II_L/H		.962	-.039	-.134	.012
Met-III_L/W		.979	-.015	-.084	.016
Met-III_L/H		.966	-.032	-.143	.011
Met-IV_L/W		.973	-.040	-.025	-.021
Met-IV_L/H		.910	-.045	.019	-.121
Met-V_L/W		.924	-.022	.066	-.078
Met-V_L/H		.194	-.164	.645	-.710

Table 3: PCA of ***females*** between *M. martensii* and *M. eupeus.*

Urat Front Banner [17], 10 September 1986, Li-Ta Wu leg. (Ar.-MHBU-IMUFB8601-8603). ***Liaoning Province***: 20♀♀, 15♂♂, Beipiao [1], 30 July 2003, Feng Zhang, Wei-Guang Lian & Shao-Jie Dong leg. (Ar.-MHBU-LNBP0301-0335). ***Qinghai Province***: 3♀♀, 3♂♂, Jianzha County [26], 30 June 2008, Zhi-Yong Di leg. (Ar.-MHBU-QHJZ0801-0806); 2♀♀, Tongren County [27], 30 June 2008, Zhi-Yong Di leg. (Ar.-MHBU-QHTR0801-0802).

Mesobuthus eupeus **(C.L. Koch, 1839)** (total 481 specimens, 247 ♀♀, 234 ♂♂): ***Inner Mongolia***: 9♀♀, 9♂♂, Alxa Right Banner [32], 30 July 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-IMARB0701-0718); 9♀♀, 9♂♂, Wuhai [28], 23 July 2008, Dong Sun leg. (Ar.-MHBU-IMWH0801-0818) ; 9♀♀, 9♂♂, Urat Middle Banner [18], 16 July 2008, Dong Sun & Cheng-Li Zhang leg. (Ar.-MHBU-IMUMB0808-0825); ***Gansu Province***: 26♀♀, 43♂♂, Gaolan County [30], 3 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-GSGL0701-0769); 26♀♀, 20♂♂, Jingyuan County [31], 5 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-GSJY0701-0746); 13♀♀, 21♂♂, Jingtai County [29], 1 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-GSJT0701-0734); 13♀♀, 20♂♂, Jingtai County [29], 17 August 2008, Ming-Sheng Zhu & Feng Zhang leg. (Ar.-MHBU-GSJT0801-0833); 9♀♀, 9♂♂, Shandan County [33], 29 July 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-GSSD0701-0718); ***Ningxia Province***: 26♀♀, 21♂♂, Wuzhong [21], 7 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXWZ0707-0753); 9♀♀, 9♂♂, Tongxin County [23], 6 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXTX0707-0724); 9♀♀, 9♂♂, Yinchuan [20], 10 August 2007, Man-Chao Xie, Zhi-Yong Di & Yan-Na Fu leg. (Ar.-MHBU-NXYC0702-0719); 9♀♀, 9♂♂, Shizuishan [19], 16 August 2008, Guang-Xin Han Dong Sun & Ming-Sheng Zhu leg. (Ar.-MHBU-NXSZS0811-0828); 9♀♀, 9♂♂, Zhongning County [22], 15 August 2008, Ming-Sheng Zhu & Feng Zhang leg. (Ar.-MHBU-NXZN0805-0822); ***Xinjiang Province***: 6♀♀, 3♂♂, Jinghe County [39], 7 August 2008, Ming-Sheng Zhu, Feng Zhang, Guang-Xin Han & Dong Sun leg. (Ar.-MHBU-XJJH0801-0809); 5♀♀, 7♂♂, Wenquan County [40], 6 August 2008, Ming-Sheng Zhu, Feng Zhang, Guang-Xin Han & Dong Sun leg. (Ar.-MHBU-XJWQ0801-0812); 5♀♀, 6♂♂, Hoboksar Mongol Autonomous County [38], 22 August 2006, Feng Zhang, Hui-Qin Ma & Shi-Ning Liu leg. (Ar.-MHBU-XJHF0601-0611); 6♀♀, 6♂♂, Buerjin County [37], 23 July 2007, Dong Sun & Lu Zhang leg. (Ar.-MHBU-XJBEJ0701-0712); 24♀♀, 6♂♂, Hami [34], 2 August 2008, Ming-Sheng Zhu, Feng Zhang, Guang-Xin Han & Dong Sun leg. (Ar.-MHBU-XJHM0801-0830); 11♀♀, 6♂♂, Aletai [36], 25 July 2007, Dong Sun & Lu Zhang leg. (Ar.-MHBU-XJALT0701-0717); 14♀♀, 3♂♂, Fuyun County [35], 28 August 2006, Feng Zhang, Hui-Qin Ma & Shi-Ning Liu leg. (Ar.-MHBU-XJFY0601-0617).

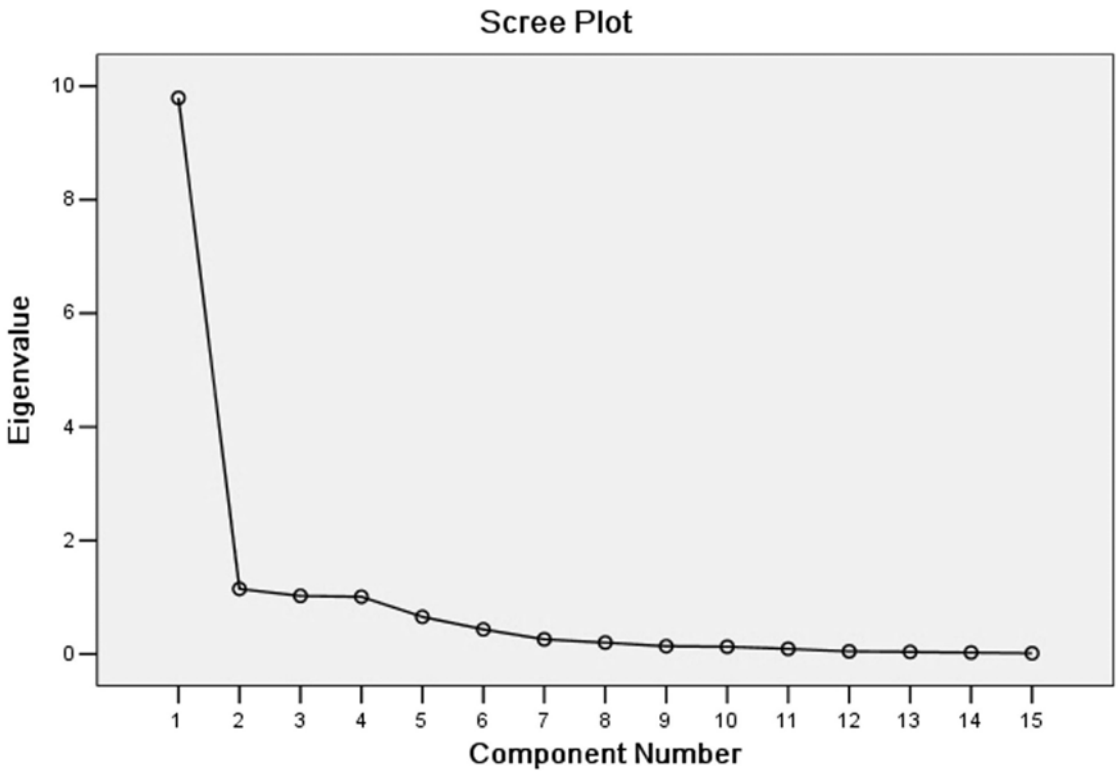


Figure 2: The scree plot of PCA for ***females*** of *M. martensii* and *M. eupeus*.

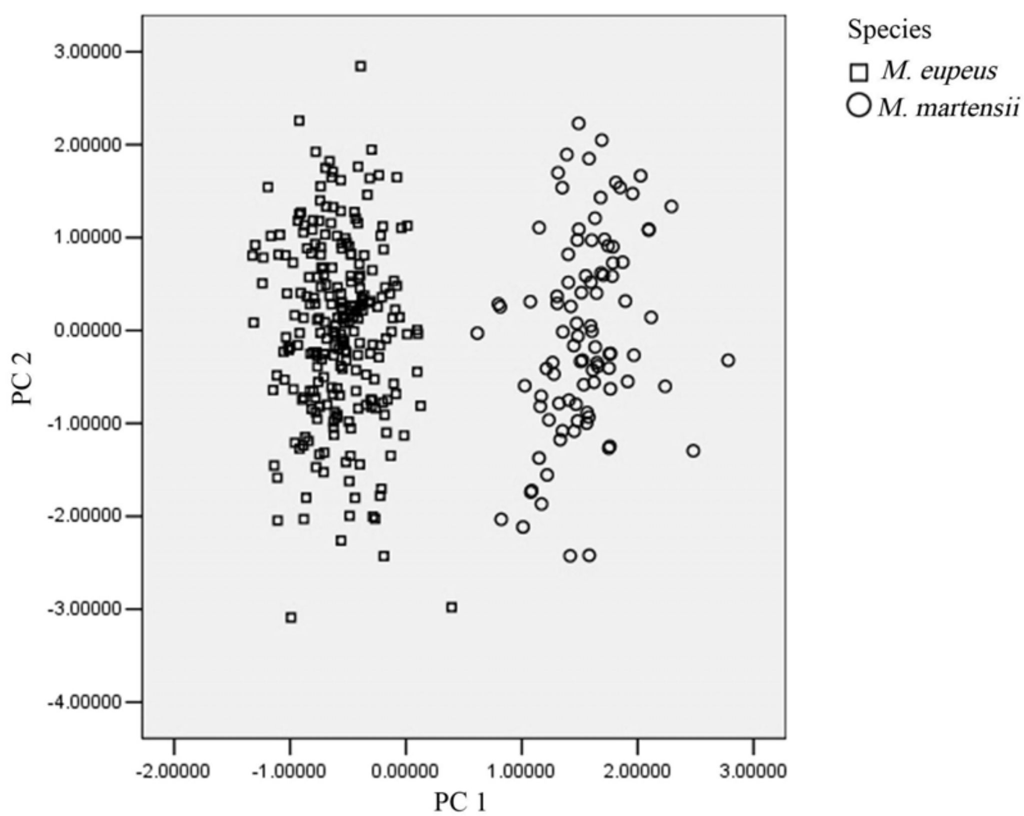


Figure 3: ***Females*** of *M. martensii* and *M. eupeus* for PC 1 and PC 2.

Component		1	2	3
Initial Eigenvalues	Total	9.443	1.329	1.038
	% of Variance	62.956	8.861	6.920
Ca_L/AW		-.162	.799	.058
Ca_AW/PW		.004	-.802	.135
Fem_L/W		.536	.080	.386
Pat_L/W		.776	.152	.173
Ch_L/W		.749	-.078	.011
Met-I_L/W		.916	.058	-.132
Met-I_L/H		.892	.059	-.123
Met-II_L/W		.955	-.002	-.138
Met-II_L/H		.937	-.002	-.134
Met-III_L/W		.962	-.006	-.141
Met-III_L/H		.961	-.013	-.157
Met-IV_L/W		.969	-.002	-.076
Met-IV_L/H		.892	-.065	.091
Met-V_L/W		.904	-.005	.126
Met-V_L/H		.376	.028	.833

Table 4: PCA of ***males*** between *M. martensii* and *M. eupeus*

Results

Mann-Whitney U test

In females, the mean ratios of all measurements are larger in *M. martensii* than those of *M. eupeus* with the exception of Ca_AW/PW. A "larger" ratio in this context implies a thinner segment. According to Mann-Whitney U test analysis, except Ca_L/AW, Ca_AW/PW and Met-V_L/H, all measured characters show significant differences between females of *M. martensii* and *M. eupeus* (Table 1).

In males, the mean ratios of all measurements are larger in *M. martensii* than those of *M. eupeus* with the exception of Ca_L/AW and Ca_AW/PW. According to Mann-Whitney U test analysis, except Ca_AW/PW and Met-V_L/H, all measured characters show significant differences between males of *M. martensii* and *M. eupeus* (Table 2).

The results of Mann-Whitney U test on variation between sexes of *M. martensii* and *M. eupeus* show that: except Ca_AW/PW and Met-V_L/H, all measured characters show significant differences between *M. martensii* and *M. eupeus*, and almost each segment of *M. martensii* is thinner than *M. eupeus*.

Principal component analysis (PCA) for M. martensii and M. eupeus

In Table 3, four principal components are associated with eigenvalues greater than 1, while the total eigenvalue is 12.972, and the total of the four components reaches 86.484% of the accumulated variance. The matrix of component loadings is given in Table 3. The scree plot is in Fig. 2.

The score plots of the first and second principal components (PCs), *PC*1 *vs.* *PC*2 (Fig. 3), it was observed that the females of *M. martensii* and *M. eupeus* were separated and formed two groups, which indicated for females, at least, *M. martensii* and *M. eupeus* are different species.

Table 4 shows the matrix of component loadings for the males and the three principal components which are associated with eigenvalues greater than 1. The total eigenvalue is 11.81, and the total of the three components reaches 78.737% of the accumulated variance. The scree plot is in Fig. 4.

In the score plots of the first and second principal components (PCs), *PC*1 *vs.* *PC*2 (Fig. 5), it was observed that the males of *M. martensii* and *M. eupeus* were separated and formed two groups from each other, which indicated for males, *M. martensii* and *M. eupeus* are different species.

The results of PCA between sexes of *M. martensii* and *M. eupeus* indicated that *M. martensii* is clearly different from *M. eupeus*; they are therefore supported as different species.

Kruskal-Wallis one-way ANOVA

The results of our Kruskal-Wallis one-way ANOVA on variation between sexes (Tables 5 and 6) suggest that the variation in females is larger than in males, evidenced by wholly significant statistical differences in

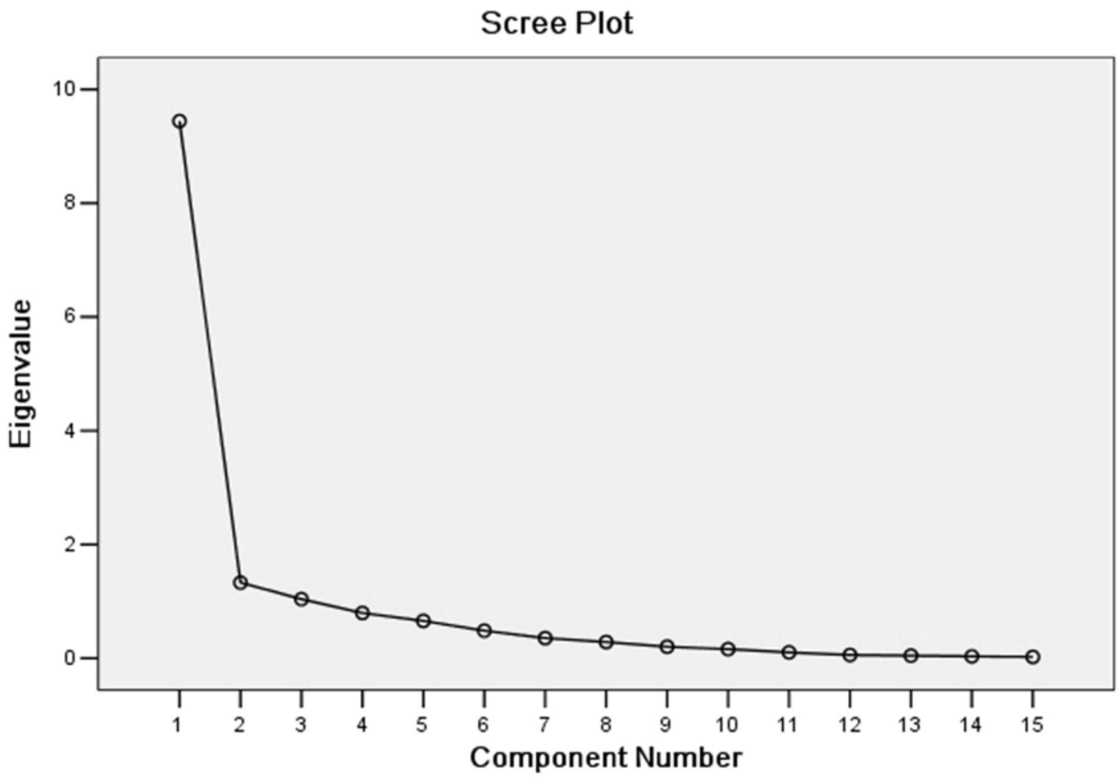


Figure 4: The scree plot of PCA for ***males*** of *M. martensii* and *M. eupeus*.

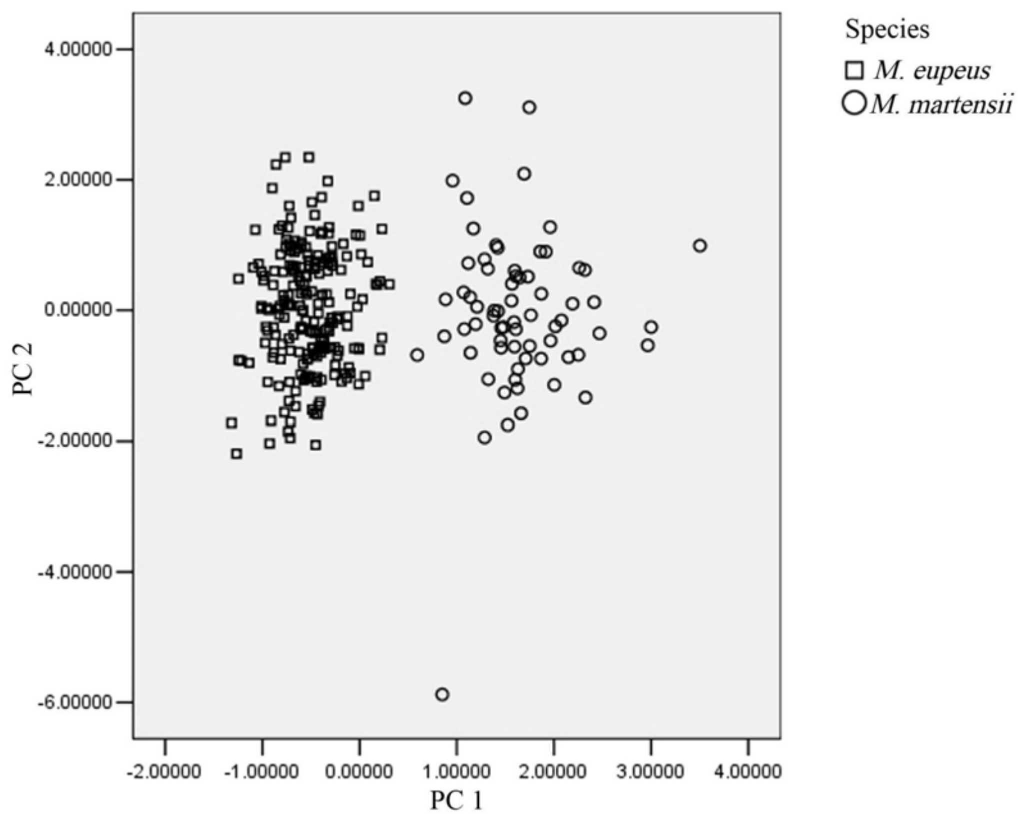


Figure 5: ***Males*** of *M. martensii* and *M. eupeus* for PC 1 and PC 2.

Province	Hebei	Ningxia	Inner Mongolia	Liaoning	Qinghai	Kruskal-Wallis	
N	28	20	20	20	5	P	
Ca_L/AW	1.465±.047	1.494±.044	1.501±.040	1.449±.030	1.534±.049	.000	*
Ca_AW/PW	.602±.026	.611±.017	.611±.015	.609±.014	.607±.013	.420	ns
Fem_L/W	3.075±.180	3.224±.148	3.182±.100	3.298±.104	3.121±.084	.000	*
Pat_L/W	2.569±.161	2.572±.217	2.664±.098	2.719±.127	2.595±.119	.002	*
Ch_L/W	4.744±.261	4.689±.342	4.824±.138	4.774±.163	4.765±.330	.217	ns
Met-I_L/W	.998±.051	1.061±.052	1.033±.025	.995±.024	1.015±.031	.000	*
Met-I_L/H	1.219±.076	1.238±.062	1.207±.048	1.189±.051	1.166±.042	.008	*
Met-II_L/W	1.246±.070	1.300±.052	1.273±.042	1.254±.032	1.246±.039	.004	*
Met-II_L/H	1.423±.096	1.418±.052	1.409±.046	1.423±.052	1.344±.040	.075	ns
Met-III_L/W	1.369±.056	1.404±.051	1.383±.040	1.355±.028	1.321±.031	.001	*
Met-III_L/H	1.513±.081	1.494±.069	1.477±.034	1.505±.041	1.415±.029	.011	*
Met-IV_L/W	1.647±.076	1.688±.060	1.683±.036	1.664±.039	1.605±.038	.005	*
Met-IV_L/H	1.817±.078	1.825±.069	1.812±.062	1.854±.042	1.769±.021	.006	*
Met-V_L/W	2.080±.077	2.155±.059	2.156±.064	2.075±.056	2.128±.044	.000	*
Met-V_L/H	2.371±.105	2.407±.086	2.418±.093	2.412±.063	2.360±.050	.248	ns

Table 5: Descriptive statistics for ***females*** of *M. martensii* (means±SD). *, P<0.05; ns, no significant difference.

Province	Hebei	Ningxia	Inner Mongolia	Liaoning	Qinghai	Kruskal-Wallis	
N	19	16	15	15	3	P	
Ca_L/AW	1.465±.064	1.479±.052	1.486±.045	1.451±.041	1.512±.076	.095	ns
Ca_AW/PW	.623±.040	.623±.017	.630±.017	.628±.016	.614±.011	.600	ns
Fem_L/W	3.199±.202	3.454±.287	3.443±.210	3.511±.083	3.131±.125	.000	*
Pat_L/W	2.671±.165	2.750±.129	2.806±.192	2.755±.112	2.778±.151	.156	ns
Ch_L/W	4.252±.485	3.969±.227	4.383±.419	4.080±.178	4.656±.732	.017	*
Met-I_L/W	1.063±.059	1.101±.055	1.040±.078	1.054±.025	.997±.037	.006	*
Met-I_L/H	1.237±.070	1.291±.082	1.195±.101	1.231±.035	1.152±.033	.002	*
Met-II_L/W	1.287±.075	1.330±.095	1.312±.077	1.302±.023	1.257±.067	.108	ns
Met-II_L/H	1.413±.078	1.431±.096	1.418±.084	1.425±.043	1.379±.088	.618	ns
Met-III_L/W	1.404±.045	1.479±.123	1.418±.061	1.410±.033	1.350±.038	.009	*
Met-III_L/H	1.505±.063	1.505±.067	1.484±.073	1.512±.035	1.448±.045	.342	ns
Met-IV_L/W	1.675±.053	1.734±.092	1.736±.081	1.723±.082	1.637±.034	.009	*
Met-IV_L/H	1.775±.082	1.783±.101	1.830±.091	1.813±.050	1.755±.024	.205	ns
Met-V_L/W	2.071±.090	2.225±.088	2.246±.171	2.181±.143	2.150±.062	.000	*
Met-V_L/H	2.359±.109	2.430±.109	2.488±.169	2.422±.091	2.317±.027	.040	*

Table 6: Descriptive statistics for ***males*** of *M. martensii* (means±SD). *, P<0.05; ns, no significant difference.

Ca_L/AW, Pat_L/W, Met-II_L/W, Met-III_L/H and Met-IV_L/H of females, but not in males, although Ch_L/W and Met-V_L/H are significant statistically different only in males; Fem_L/W, Met-I_L/W, Met-I_L/H, Met-III_L/W, Met-IV_L/W and Met-V_L/W in both males and females show differences that are statistically significant, but Ca_AW/PW and Met-II_L/H show no statistically significant difference.

PCA for M. martensii

In Table 7 five principal components are associated with eigenvalues greater than 1, while the total eigenvalue is 10.411, and the total of the five components reaches 69.411% of the accumulated variance. The matrix of component loadings is given in Table 7. The scree plot is in Fig. 6.

Component		1	2	3	4	5
Initial Eigenvalues	Total	4.855	1.861	1.493	1.139	1.063
	% of Variance	32.369	12.404	9.957	7.592	7.089
Ca_L/AW		.077	.622	.020	-.223	.407
Ca_AW/PW		.237	.021	.333	.640	.416
Fem_L/W		.495	.092	.340	.189	-.488
Pat_L/W		.376	-.193	.664	.115	-.307
Ch_L/W		.210	.152	.646	-.044	.404
Met-I_L/W		.659	.280	-.268	.462	-.020
Met-I_L/H		.557	-.343	-.347	.296	.299
Met-II_L/W		.737	.176	-.239	.074	-.178
Met-II_L/H		.676	-.397	-.084	-.201	.143
Met-III_L/W		.809	.130	-.200	.000	-.117
Met-III_L/H		.661	-.492	-.072	-.289	.187
Met-IV_L/W		.725	.114	-.065	-.071	-.138
Met-IV_L/H		.576	-.494	.164	-.158	.120
Met-V_L/W		.536	.666	-.066	-.154	.068
Met-V_L/H		.620	.246	.269	-.379	-.017

Table 7: PCA for ***females*** of *M. martensii.*

In the score plots of the first and second principal components (PCs), *PC*1 *vs. PC*2 (Fig. 7), it was observed that the females of *M. martensii* were not separated to form independent groups. Likewise in Fig. 8, *PC*1 *vs. PC*3, Fig. 9, *PC*1 *vs. PC*4 and Fig. 10, *PC*1 *vs. PC*5. All results indicate for females, the variation of *M. martensii* was below the species level.

In Table 8, four principal components are associated with eigenvalues greater than 1, while the total eigenvalue is 10.471, and the total of the four components reaches 69.814% of the accumulated variance. The matrix of component loadings is shown, and the scree plot is in Fig. 11.

In the score plots of the first and second principal components (PCs), *PC*1 *vs. PC*2 (Fig. 12), it was observed that the males of *M. martensii* were not separated to form independent groups. Likewise in Fig. 13, *PC*1 *vs. PC*3, and Fig. 14, *PC*1 *vs. PC*4. All results indicate for males, the variation of *M. martensii* was below the species level.

The PCA results for both sexes of *M. martensii* indicated that the variation of *M. martensii* was below the species level.

Cluster analysis

The hierarchical clustering scheme was applied to the mean ratios for each province and dendrograms were obtained as follows: the horizontal scale represents the distance or levels of mergers between clusters, so the longer the distance on the horizontal axis, the greater the degree of dissimilarity between clusters tends to become.

Using a 'Between-groups' linkage method to the five geographic clusters of females (Fig. 15), the last merger was between {Hebei and Qinghai} and the other clusters at the distance of 25, the largest grouping was divided into two clusters: {Hebei and Qinghai} and others. Here, the relative distance (the merger level) between {Hebei and Qinghai} and others was the largest, so the difference between these two clusters was the largest in obtained data set. Next, {Liaoning} and others were also divided at the distance of around 13.5. The first merger was between {Ningxia} and {Inner Mongolia}.

Using a 'Within-groups' linkage method to the five geographic clusters of females (Fig. 16), the last merger was between {Hebei and Qinghai} and the other clusters at the distance of 25. Next, {Liaoning} and others were also divided at the distance of around 12.5. The first merger was between {Ningxia} and {Inner Mongolia}.

Using a 'Between-groups' linkage method to the five geographic clusters of males (Fig. 17), the last merger was between {Qinghai} and the other clusters at the distance of 25. Next, {Hebei}, {Inner Mongolia} and others were also divided at the distance of around 9. The first merger was between {Ningxia} and {Liaoning}.

Using a 'Within-groups' linkage method to the five geographic clusters of males (Fig. 18), the last merger was between {Qinghai} and the other clusters at the distance of 25. Next, {Hebei} and others were also divided at the distance of around 13.5. Then, {Inner Mongolia} and others were also divided at the distance

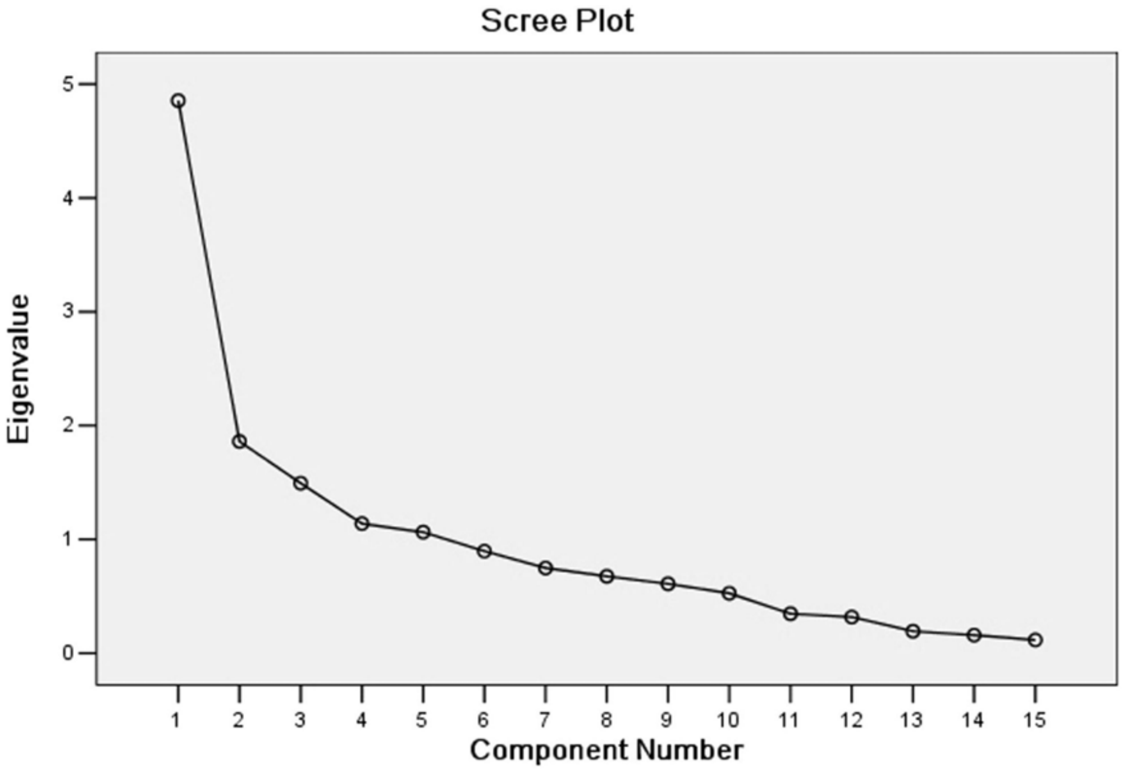


Figure 6: The scree plot of PCA for ***females*** of *M. martensii*.

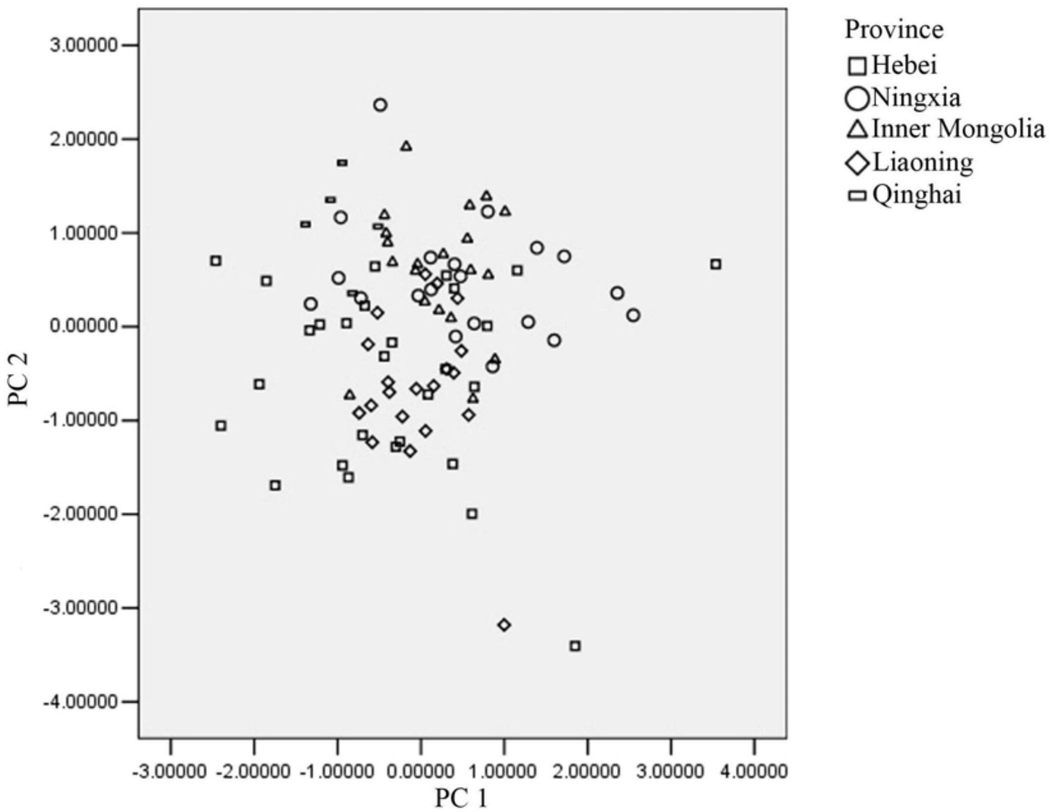


Figure 7: ***Females*** of *M. martensii* for PC 1 and PC 2.

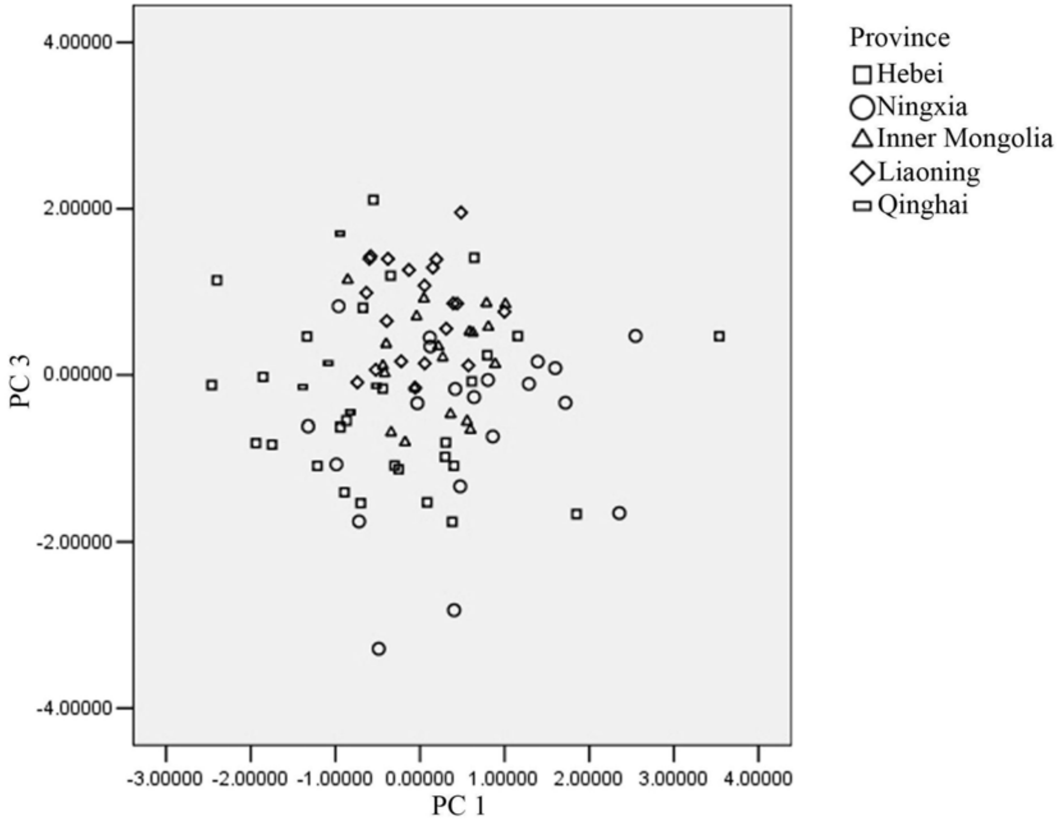


Figure 8: ***Females*** of *M. martensii* for PC 1 and PC 3.

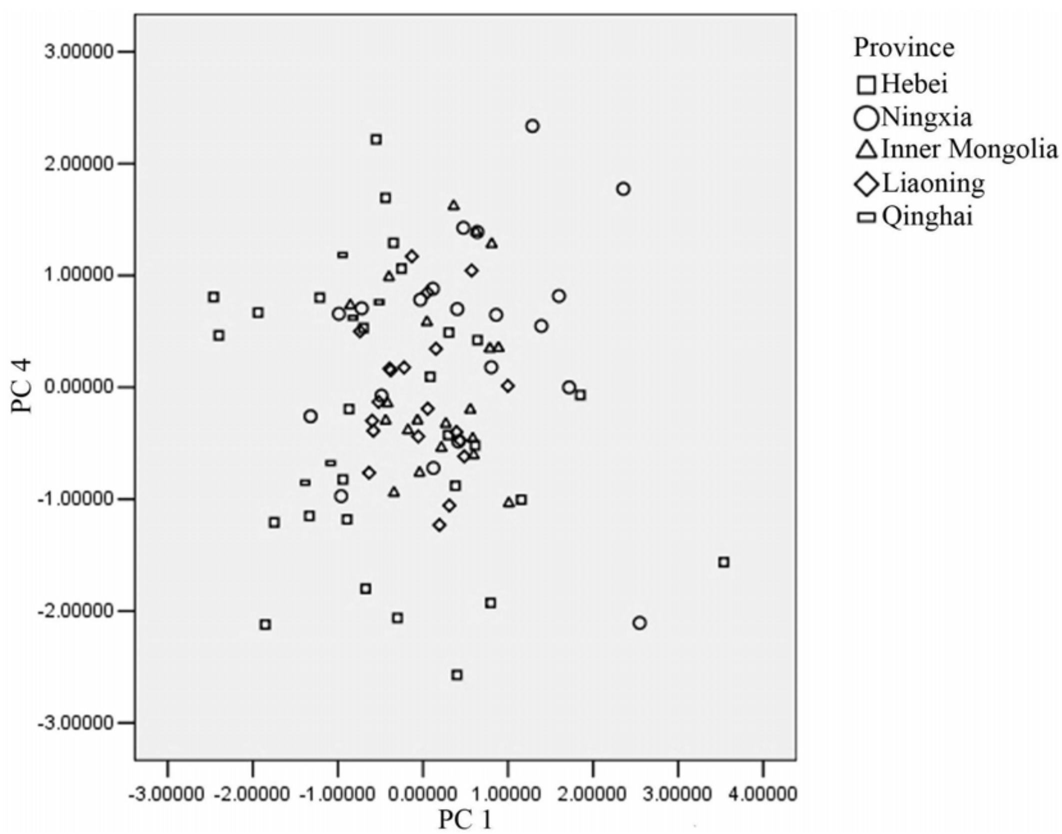


Figure 9: ***Females*** of *M. martensii* for PC 1 and PC 4.

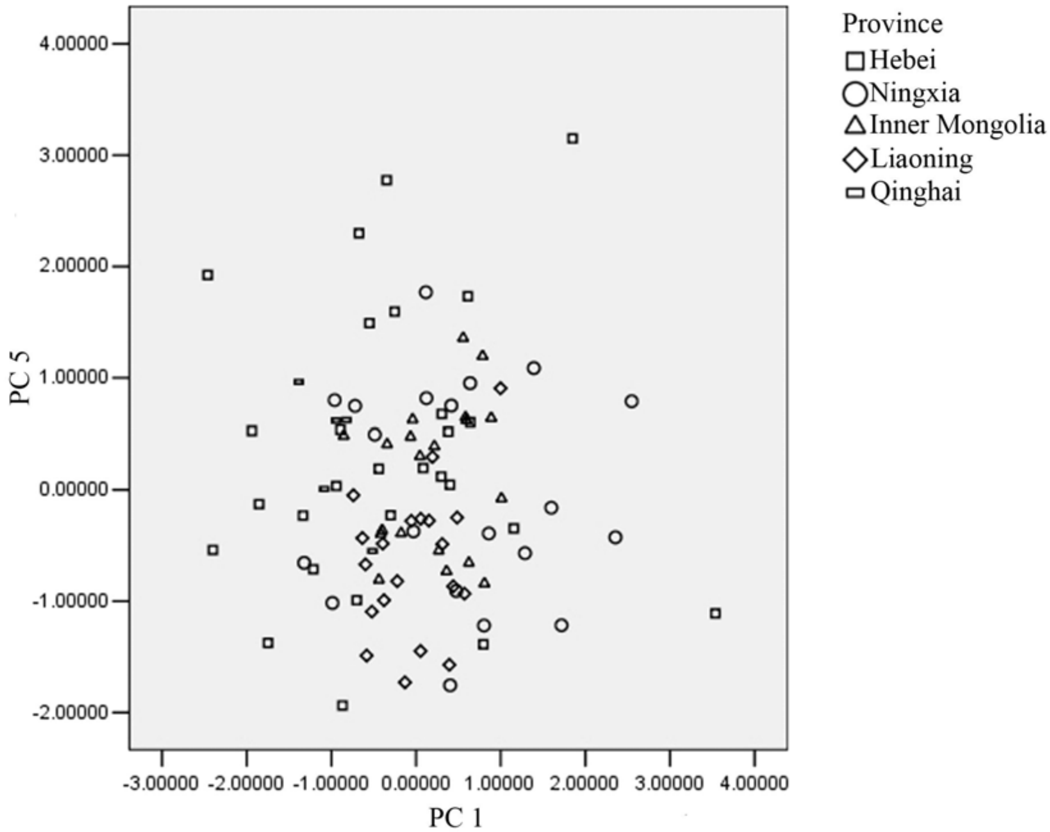


Figure 10: ***Females*** of *M. martensii* for PC 1 and PC 5.

Component		1	2	3	4
Initial Eigenvalues	Total	5.671	1.919	1.633	1.248
	% of Variance	37.808	12.796	10.889	8.321
Ca_L/AW		-.089	.534	.232	-.614
Ca_AW/PW		.079	-.672	-.198	.492
Fem_L/W		.578	.212	.203	.206
Pat_L/W		.553	.225	.440	.085
Ch_L/W		-.019	-.282	.599	.123
Met-I_L/W		.597	.493	-.447	.240
Met-I_L/H		.578	.557	-.389	.307
Met-II_L/W		.729	-.297	-.136	-.452
Met-II_L/H		.745	-.331	-.178	-.317
Met-III_L/W		.778	.021	-.243	-.073
Met-III_L/H		.788	-.257	-.177	-.072
Met-IV_L/W		.836	-.054	.040	-.114
Met-IV_L/H		.760	-.356	.204	-.056
Met-V_L/W		.608	.222	.415	.154
Met-V_L/H		.607	.102	.491	.240

Table 8: PCA for ***males*** of *M. martensii.*

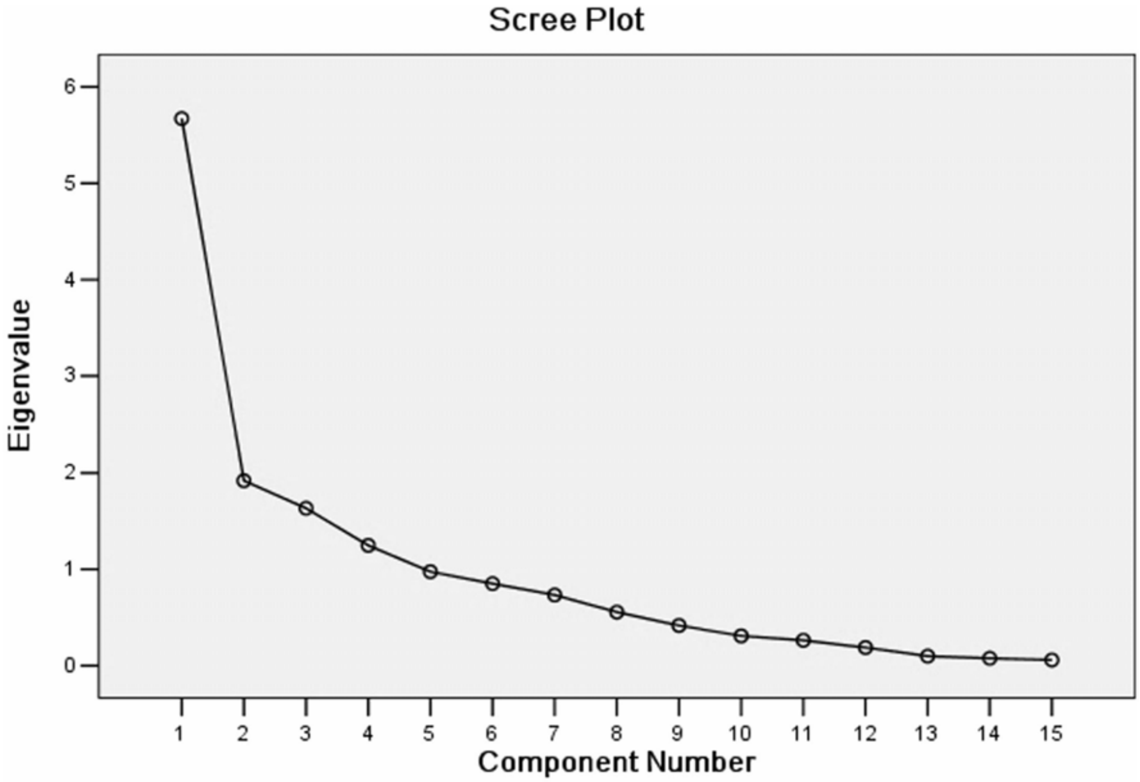


Figure 11: The scree plot of PCA for ***males*** of *M. martensii*.

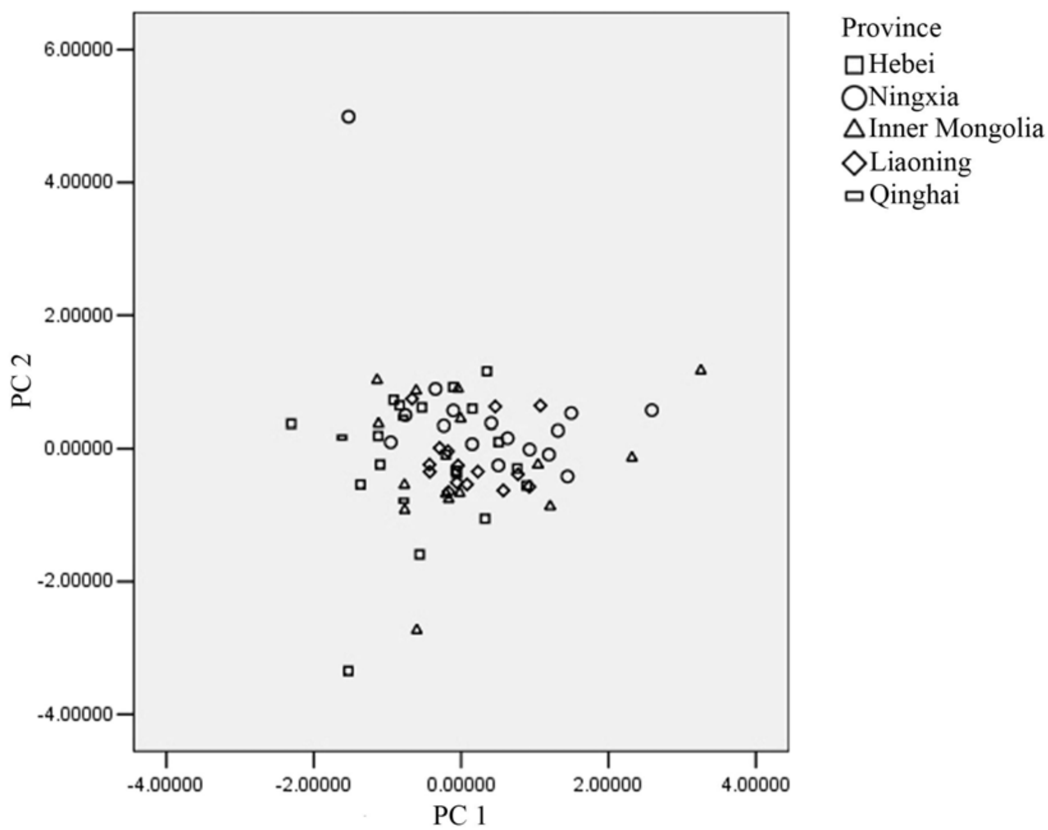


Figure 12: ***Males*** of *M. martensii* for PC 1 and PC 2.

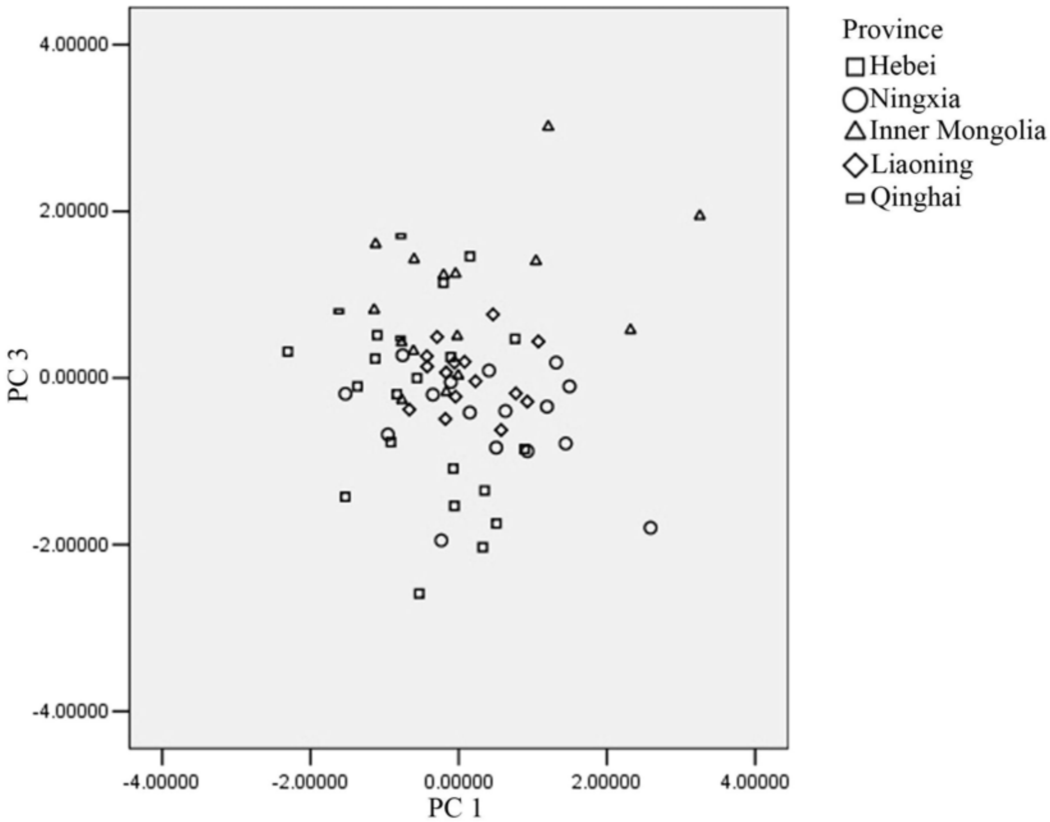


Figure 13: ***Males*** of *M. martensii* for PC 1 and PC 3.

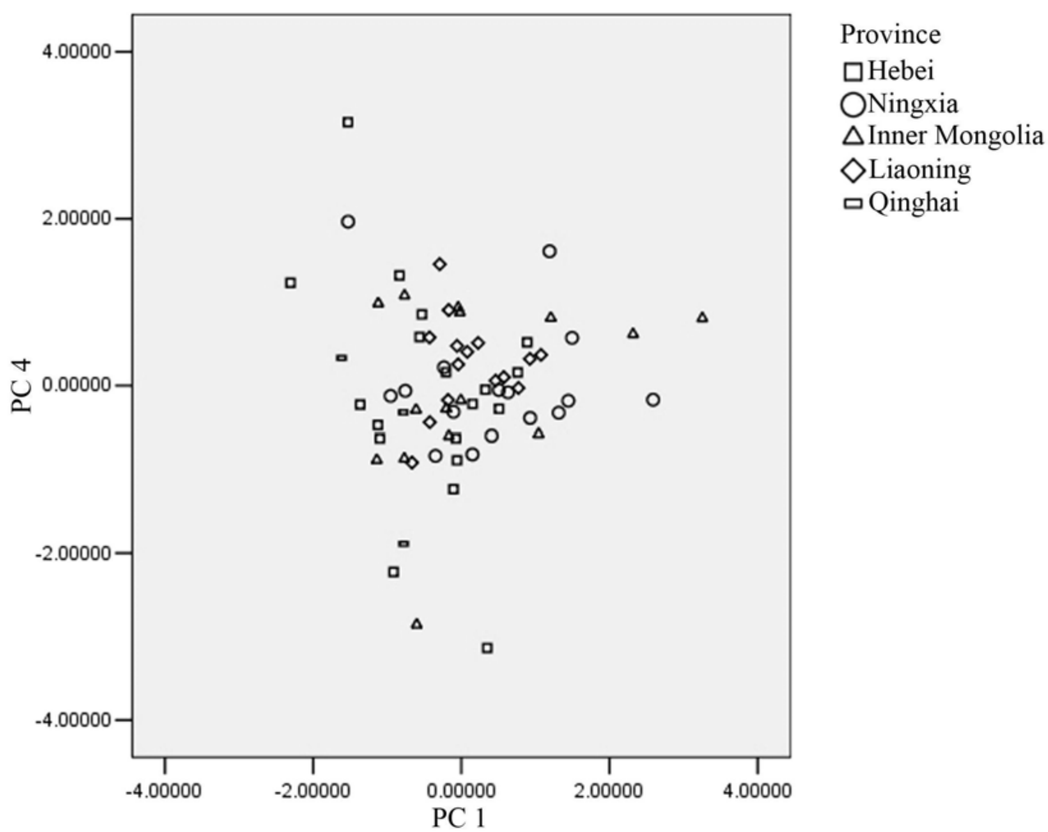
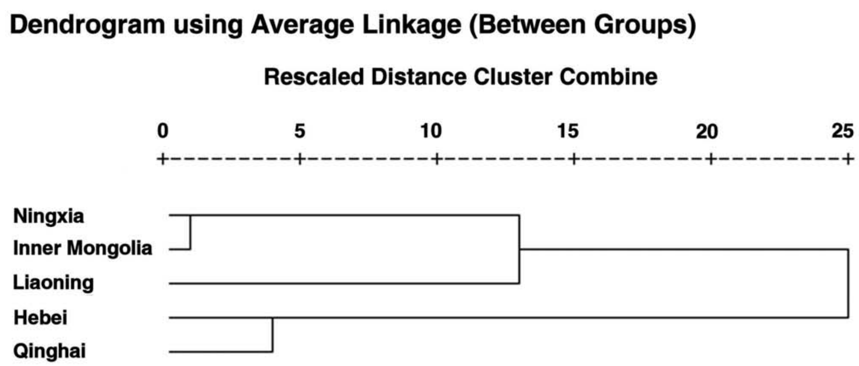


Figure 14: ***Males*** of *M. martensii* for PC 1 and PC 4.



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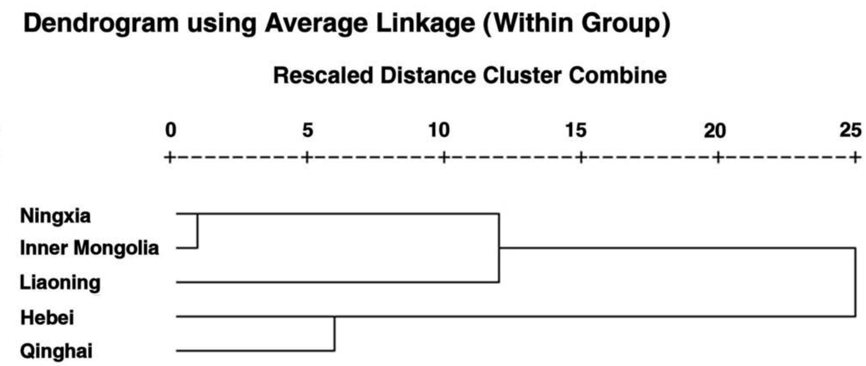

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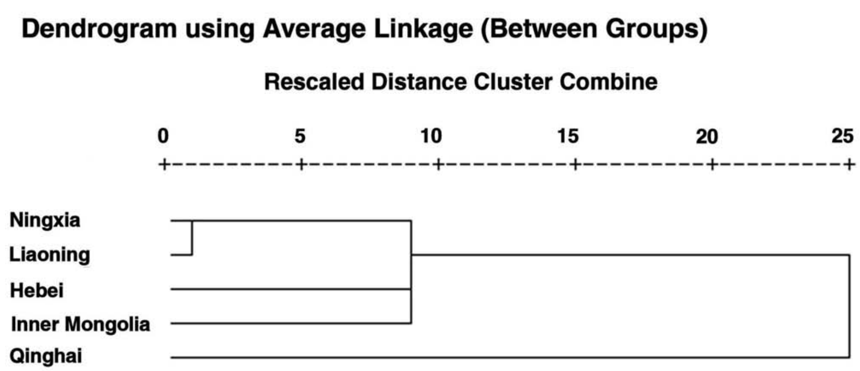

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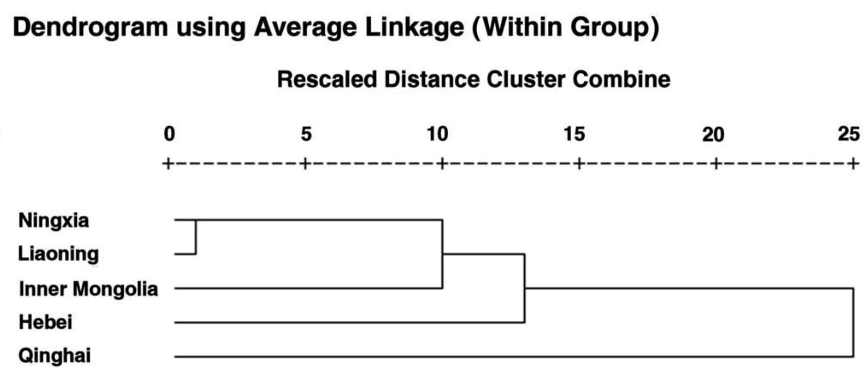

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Figures 15-18: Cluster analysis dendrograms. **15.** Between-groups linkage of ***females***. **16.** Within-groups linkage of ***females***. **17.** Between-groups linkage of ***males***. **18.** Within-groups linkage of ***males***.

of 10. The first merger was between {Ningxia} and {Liaoning}.

The results of cluster analyses of both sexes suggest that the morphology of Qinghai populations differs substantially from other provinces.

Discussion

According to Mann-Whitney U test and principal component analysis (PCA), *M. martensii* and *M. eupeus* are morphologically distinct, although their geographic ranges can partially overlap. Therefore we confirm that *M. martensii* and *M. eupeus* are two different species. *M. eupeus* is mainly found in arid regions in temperate China, while *M. martensii* is usually found in semiarid, subhumid and humid regions in temperate zones; their habitats are different.

Based on field surveys and GIS-based ecological niche modeling (Shi et al., 2007), *M. martensii* appears to be restricted to latitudes south of 43°N and north of the Yangtze River, bordered by the Helan Mountains and the Tengger and Mo Us sand desert in the west, and limited by the sea in the east (Fig. 1). The sampling scheme in our analyses included nearly the easternmost (Beipiao, 41.8°N, 120.8°E [1]) and westernmost (Jianzha County [26], 35.92°N, 102°E; Tongren County [27], 35.55°N, 102°E) edges of the range of this species in China; the distance between these localities is about 2300km.

The environment of the five provinces is different. Liaoning is a subhumid region with vegetation mainly represented by forest steppe and meadow steppe; Hebei is also subhumid with vegetation mainly represented by deciduous broad-leaved forest and forest steppe; Ningxia and Inner Mongolia are semiarid zones with vegetation mainly represented by steppe; Qinghai is a subhumid, semiarid region with vegetation mainly represented by forest, meadow and meadow steppe. Besides the environmental differences named above, all of the Qinghai area is located above 2300m a.s.l. and is often known as the ‘Qinghai Plateau’. Animal populations, including scorpions, may be influenced by such an altitude. All specimens collected in other provinces were found below 1500m, indeed most were collected below 1000m. Jin et al. (2006) measured morphological characters associated with altitude variation in lizards, *Phrynocephalus vlangalii* (Agamidae) in Qinghai and Gansu, and found that body size decreased with increasing altitude; arm and leg length were negatively correlated with altitude: the relative arm and leg length increased with increasing altitude (but at the same time, relative tail length decreased).

Compared with specimens collected from other provinces, the ratio of Ca_L/AW for Qinghai populations is larger, while the ratios of Met-I_L/H, Met-II_L/W, Met-II_L/H, Met-III_L/W, Met-III_L/H, Met-IV_L/W, Met-IV_L/H and Met-V_L/H are smaller (Table 5 and Table 6), which indicates *M. martensii* in Qinghai has a narrower prosoma and a broader metasoma.

According to statistical analyses, the morphological variation of *Mesobuthus martensii* in northern China is not large but populations from Qinghai are morphologically less similar to those collected from other provinces. *Mesobuthus martensii* is mainly distributed in northeastern China where the land is generally flat, ever since the Permian period. Through most of this geographic range, once the land had separated from the sea, there were no large environmental differences until the Pleistocene, when the Qinghai-Tibetan Plateau began to rise. During the middle and late Pliocene, somewhat before the Pleistocene, the altitude of the Qinghai-Tibetan Plateau was only about 1000m. Towards the end of the Pliocene, the plateau started to rise strongly and through the early, middle and late Pleistocene it rose about 1000m in each period. Through the Holocene Epoch, and today, the plateau stands at the altitude of 4700m (Zhang, 1999; Du et al., 1998). Therefore, the chances for geographic species isolation were few before the Pleistocene. Since the time of the rise of the Qinghai-Tibetan Plateau was only about two million years ago, and it is possible that this has been inadequate time for a new species to evolve.

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