Iranian Journal of Animal Biosystematics (IJAB)
Vol.16, No.1, 21-35, 2020
DOI: 10.22067/ijab.v16i1.85063

IJAB
ISSN: 1735-434X (print); 2423-4222 (online)

RESEARCH ARTICLE Open access

Sexual dimorphism in the scorpions of the genus *Odontobuthus* Vachon, 1950 (Scorpiones: Buthidae)

Barahoei, H.[1], Navidpour, Sh.[2], Aliabadian, M.[1,3], Siahsarvie, R.[1,4] and Mirshamsi, O.[1,3*]

[1]*Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran*
[2]*Razi Reference Laboratory of Scorpion Research, Razi Vaccine and Serum Research Institute, P.O. Box 31975/148, Department of Venomous Animals and Toxins, Hesarak, Karaj, Iran*
[3]*Research Department of Zoological Innovations (RDZI), Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*
[4]*Rodentology Research Department, Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*

(Received: *13 November 2019*; Accepted: *13 January 2020*)

Abstract
The Sexual dimorphism (SD) in body size is very common among the scorpions. In this study, the SD was investigated in two aspects of size and shape in the genus *Odontobuthus* Vachon, 1950 as a small genus of the family Buthidae. This genus has six fossorial species of which four are distributed in Iran. For this purpose, 43 morphometric variables, consisting of 38 metric measurements and five meristic characters were digitized in the six species, *O. bidentatus*, *O. doriae*, *O. tavighiae*, *O. tirgari*, *O.* sp.1 and *O.* sp.2. The results show that Sexual Size Dimorphism (SSD) were significant in three species: *O. doriae*, *O. tavighiae* and *O.* sp.2. While *O.* sp.1 represented a SD for more aspects of shape, *O. bidentatus* did not show a significant SD for all studied traits. The amount of SD in size and shape were not the same in different species. The results showed that males have larger metasoma than females even when they are pulled to the same size. Having larger metasoma may correspond to a more efficient performance during mating, predation or combat with other males, so it should be under a high sexual selection. Type II ANOVA showed a significant interaction between species and sex for shape, but not for size. It suggests that the evolution of SD for size has been in parallel for all studied species, while it has been in different directions for shape. Among meristic variables, only the number of pectin denticles were sexually dimorphic, with males having more denticles than females in all studied species.

Key words: *morphometric, meristic, sexual dimorphism, size, shape, sexual selection.*

INTRODUCTION

Sexual dimorphism (SD) is a common feature throughout the animal kingdom and involves differences in morphology, physiology, and behavior between males and females of the same species. It can be forced by a variety of different factors, including niche partitioning between sexes, natural selection for fecundity or parental care, sexual selection through courtship rituals or intrasexual competitions (Shine, 1989; Andersson, 1994; Zhang *et al.*, 2014). Natural and sexual selections initiated different costs and benefits in males and females that ultimately lead to achieving SD in shape and size (Booncham *et al.*, 2007). Differences in measured values of certain morphological traits known as sexual size dimorphism (SSD). The evolutionary reasons for SSD are still subject to controversies (Kuo *et al.*, 2009; Palen-Pietri *et al.*, 2019). To comprehend the

*Corresponding Author: mirshams@um.ac.ir

operators of SD it is often essential to investigate both shape and size differences between males and females. However, in some arachnid groups, investigation of sexual shape dimorphism has been ignored in favor of absolute size differences between sexes (McLean *et al*., 2019).

SD can be explained by both proximate and ultimate causations. Much understanding can be achieved by the integrative study of sexual size and shape dimorphism at both proximate and ultimate approaches. The proximate causes of size and shape dimorphism might correspond to the factors that control the intersexual growth rate and the difference in growth trajectories of each body part, respectively (Butler & Losos 2002; John-Alder *et al*. 2007).

Furthermore, from the perspective of ultimate causations, fecundity, niche partitioning and sexual selection have been proposed as leading causes for the evolution of SD (Cooper & Vitt 1993; Censky, 1997; Katsikaros & Shine, 1997; Monnet & Cherry, 2002; Tague, 2005; Schwarzkopf, 2005; Thompson & Withers, 2005).

The SD have been investigated in many scorpions (Haradon, 1984; Kovarik, 2004; Ozkan *et al*., 2006; Booncham *et al*., 2007). The SD in body size is very common among the scorpions. Generally, females are larger than males (*Liocheles australasiae* and *Tityus trimtatis* are exceptions). Males of several scorpion species possess bigger telsons than females, in some species (*Centruroides, Isometrus*, *Hadogenes*, and *Urodacus* spp.), males have an elongated metasoma (Carlson *et al*., 2014; Sentenská *et al*., 2017). Some morphological characters such as carapace length which are not affected by allometry are suitable for showing sexual size dimorphism (SSD). The shape of body structures may also be vary between sexes. Sexual shape dimorphism in scorpions is normally expressed by the elongation of the pedipalp or the metasoma on males (the latter is more general). The presence of apophyses, genital papillae or stronger cuticular carination of pedipalp and metasomal segments (e.g. *Centruroides*, *Rhopalurus*, and *Tityus*) in males are examples of shape dimorphism in scorpions. The SD also may affect the shape of other structures, such as the form of pedipalp manus and telson. The best-known example of SD in scorpions has occurred in the pectinal teeth number. In most cases, females have shorter pectins and fewer pectinal teeth than males (Polis, 1990).

In general, SD in scorpions can be classified into as following six types: difference in body size; the difference in the shape of structures; the presence of a feature in one sex; stronger development of features in one sex; difference in the texture of the body surface and, the intersexual difference in meristic characteristics.

Here, we apply the statistical analysis of morphometric characters to investigate the sexual size and shape dimorphism in the genus *Odontobuthus* Vachon, 1950. This taxon is a small genus belongs to the family Buthidae with six decribed fossorial species distributed in India, Pakistan, Iran, Iraq, United Arab Emirates, and Oman (Lowe, 2010). Four species are distributed in Iran consisting *O. bidentatus*, *O. doriae*, *O. tavighiae* and *O. tirgari* (Lourenço & Pézier, 2002; Lowe, 2010; Mirshamsi *et al*., 2013; Navidpour *et al*., 2013). This study aims to open a new window on SD in the genus *Odontobuthus* to elucidate some possible evolutionary causes the intersexual size and shape differences in this taxon.

MATERIAL AND METHODS

A total of 102 individuals belonging to four *Odontobuthus* species including *O. bidentatus*, *O. doriae*, *O. tavighiae* and *O. tirgari* were studied. Also, two undescribed populations from Sistan and Baluchistan (*O.* sp.1) and Kerman (*O.* sp.2) provinces were added to the staticstical analyses (Table 1). For each species, the specimens from geographically close localities were selected, and wherever the sample size was too small, some specimens from farther localities were included. For this purpose, a Fisher F-test was performed on intera- and inter-locality variance to avoid the inter-locality morphological differences among specimens considered in each species. Only adult specimens were entered in the statistical analyses.

TABLE 1. Data of 102 specimens of six species of *Odontobuthus* were used for morphometric analyses.

Species	Locality	Sex	Museum code (Zoology Museum of Ferdowsi University of Mashhad)
Odontobuthus sp.1	Sistan and Baluchistan province	4♀, 3♂	ZMFUM-scr-1301-1306, 1308
Odontobuthus bidentatus	Khuzestan province	9♀, 2♂	ZMFUM-scr-2061-2064, 2066, 2067, 2069-2073
Odontobuthus doriae	Tehran province	16♀, 3♂	ZMFUM-scr-2001-2010, 2012-2019, 2038
Odontobuthus sp.2	Fars and Kerman provinces	9♀, 5♂	ZMFUM-scr-1330-1342, 2039
Odontobuthus tavighiae	Hormozgan province	13♀, 10♂	ZMFUM-scr-1345-1359, 1362-1367, 2074-2075
Odontobuthus tirgari	Sistan and Baluchistan province	13♀, 15♂	ZMFUM-scr-1369, 1371-1372, 1374-1378, 1399, 1402, 1405-1407, 1410, 1412-1413, 1416-1418, 1421, 1423-1424, 1427-1429, 1432

Morphological measuremenets (Fig. 1)

In total, 43 morphometric variables consisting of 38 metric and five meristic characters were measured as below:

Metric measurements: MFL: movable finger length; ChL (MFL+ML): chela length; ML: manus length; MW: manus width; MD: manus depth; PaL: patella length; PaW: patella width; FL: femur length; FW: femur width; PeL (ChL+FL+PL): pedipalp length; CAW: carapace anterior width; CPW: carapace posterior width; CL: carapace length; X: distance between anterior margin of carapace and anterior edge of median eyes; Y: distance between anterior edge of median eyes and posterior margin of carapace; MsL: mesosoma length; T3L: tergite III length; T3W: tergite III width; Mt(1-5)L: length of metasomal segments I-V; Mt(1-5)W: width of metasomal segments I-V; Mt(1-5)D: depth of metasomal segments I-V; TL: telson length; TW: telson width; TD: telson depth; MtTL: metasoma and telson length; BL: body length.

Meristic characters: MFDR: number of movable finger denticle rows; PDN: number of pectin denticles; NLLAA: number of lateral lobes of anal arch; Mt3VC: number of pairs of denticles on ventral carinae of third metasomal segment; NVDAA: number of ventral denticles of anal arch.

Measurements of morphometric characters of adult *Odontobuthus* specimens were taken with a >0.02 mm Olympus micrometer ocular lens (OSM-4) applied to an optical Olympus SZ40 stereomicroscope base on Lamoral (1979) and Stahnke (1970). Sissom (1990) used for nomenclature.

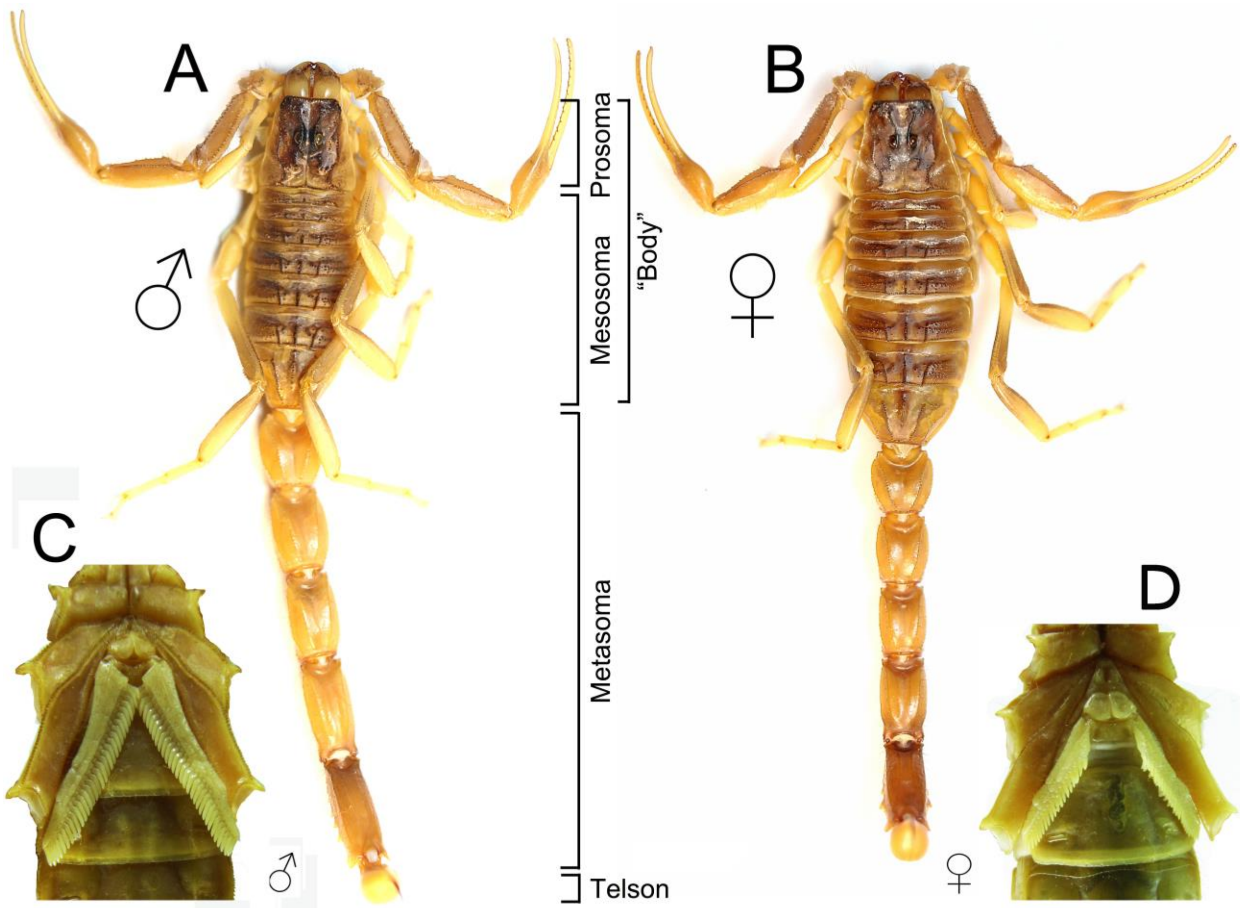

FIGURE 1. Morphology of representative male and female scorpions (*Odontobuthus* sp.1). A) male; B) Female; C) Male pectines; D) Female pectines. The image of the male was pulled to the same size as the female to better show the differences in shape.

Statistical analyses

Univariate and multivariate statistical analyses were applied to evaluate the inter- and intra-species morphological SD. The SD was first studied on the metric measurements (hereafter called main data) and then the analyses were performed on size and shape separately. For the latter purpose, the overall size of each specimen was calculated as the square root of the sum of all the squared variables (Navarro *et al*., 2004). Shape variables were calculated as the log shape ratio of the metric variables (Eldredge 1972; Navarro *et al*., 2004). To identify sexually dimorphic characters in each species, a Welch two-sample t-test was performed on each metric character per species and its non-parametric equivalent, Mann-Whitney U test, for the meristic data. To visualize the size SD in each species, the box plot was graphed on the overall size of different sexes for each species. A type II two-way analysis of variance (ANOVA) was performed on the overall size to evaluate whether different sexes are significantly different in size and whether the SD in size is parallel in different species of *Odonthobotus.* The same analysis was carried out on each single metric character. SD in metric characters may mostly reflect the pure differences in size. We, therefore, performed both type II ANOVA and Welch two-sample t-test analyses on shape data. The latter analysis shows, whether SD in each character is still distinguishable when their overall size pulled to the same scale. For any significant sexually dimorphic meristic data, a box plot for the two sexes of different species was graphed (it was also performed for metric data but not shown here to avoid lengthening the article).

For multivariate analyses, the pairwise correlation among shape characters was calculated and in the cases of high correlation (R > 0.9), only one of the characters was kept in the subsequent analyses. The multivariate SD in shape was tested through a multivariate analysis of variance on shape data, using factors of species, sex, and their interaction, and considering individuals as random factor. The ordination of the different sexes of the six species was plotted on overall size and the first axis (PC 1) of the principal component analysis (PCA) on shape data, to see whether the same orientation in different species can be seen.

All the morphometric analyses were performed using the R language (R Development Core Team 2019). The car Package (Fox & Weisberg, 2019) was used for type II ANOVA, ggplot2 Package (Wickham 2016) for graphing the plots, and picante Package (Kembel *et al*., 2010) for pairwise correlation among characters.

RESULTS

ANOVA analyses

Welch two-sample t-test on overall size between males and females of the studied species represented a SD for size with a high degree of significance for *O. doriae*, *O. tavighiae*, and *O.* sp.2, while the overall size of two sexes were not significantly different for the other three species, *O. bidentatus*, *O. tirgari* and *O.* sp.1 (Table 2). The box plot on the same characters for the males and females of the six species points out the same results (Fig. 2).

TABLE 2. Sexual dimorphism of the overall size in each the *Odontobuthus* species studied using Welch two-sample t-test. Bold numbers show where the sexual dimorphism is significant.

Species / Character	*O. bidentatus*	*O. doriae*	*O. tavighiae*	*O. tirgari*	*O.* sp.1	*O.* sp.2
***P*- value**	0.2178	**0.0131**	**0.0316**	0.2536	0.0822	**0.0231**

The Welch two-sample t-test analysis on the 38 metric measurements (main data), indicates that each variable shows SD in at least one of the studied species. T3W showed a significant differences in five species (all except *O.* sp.1), while carapace characters (CPW, CL, X, and Y) only showed this differences for four (Table 3). Accorrding to the results, *O.* sp.2 showed SD for all variables except in CAW and T3L, *O. tirgari* in 26 variables, *O. tavighiae* in 21 variables, *O. bidentatus* in eigth variables, *O. doriae* in five variables, and *O.* sp.1 in one variable (T3L) (Table 3). As for overall size, in all cases, females showed a higher value compared to males.

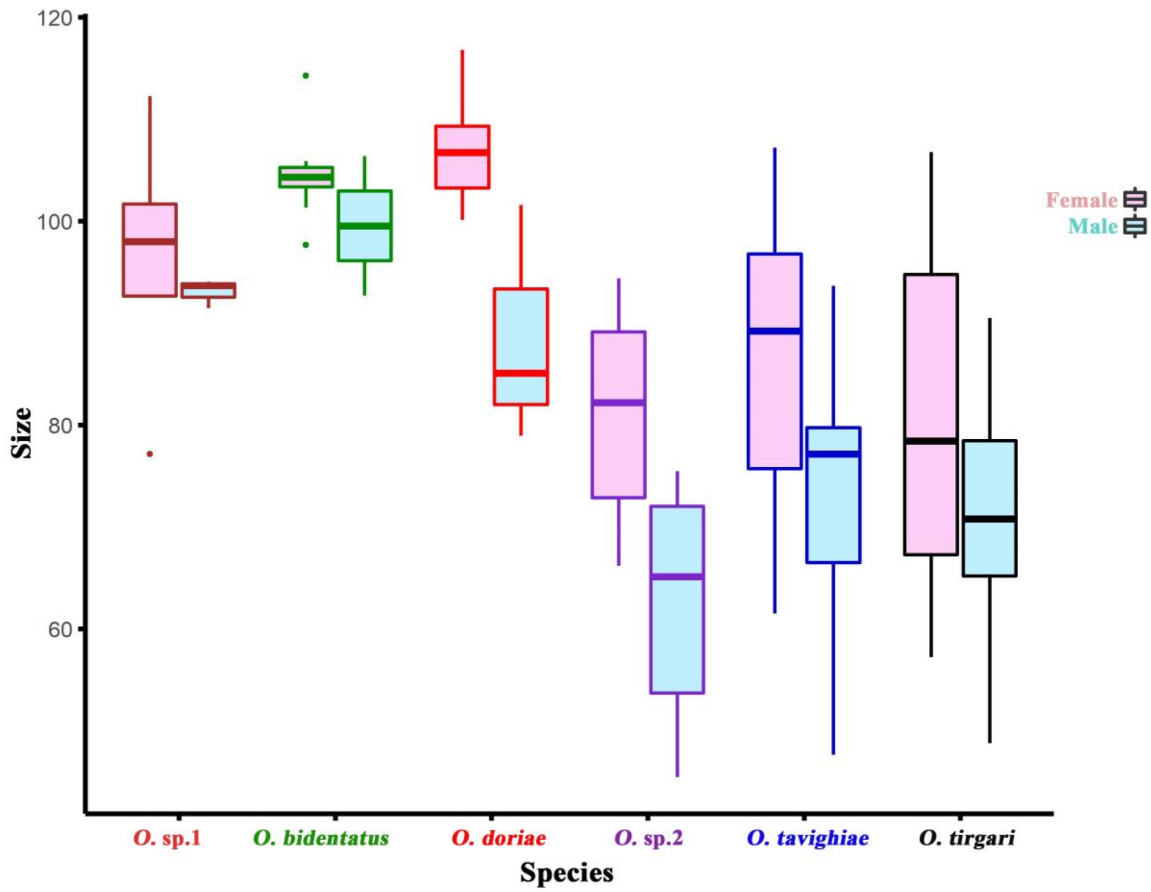

FIGURE 2. Boxplots on overall size for the males (in blue) and females (in pink) of the six species of *Odontobuthus*.

When the latter analysis is performed on shape data (Table 3), out of 48 characters, only 26 characters showed a significant SD for at least one of the species and the other 12 characters did not show sexually dimorphic in any of the studied species. Whitin the 26 sexually dimorphic characters, Paw and CL showed a significant difference in three species, and the other in one or two. *O.* sp.1, with 18, and *O. tirgari*, with 11 characters, represented the most range of SD for shape. *O.* sp.2 showed SD only for MD and *O. bidentatus* did not show any SD for the shape data studied. The same as in main data, whenever the SD were significant, and females showed a larger value compared to males. This is not, however, the case for the five metasoma segments length (MT1-5L) in *O.* sp.1, in which the males are larger than females when they have an equal overall size.

Type II two-way ANOVA on overall size, using species and sex factors (Table 4), showed that size was significantly different both among species and between sexes. The interaction between species and sex was, however, not significant, indicating that in the studied species SD had a parallel effect, *i.e.,* females are always bigger than males. The same pattern was seen when looking at each part of the metric variables (Table 5). In other words, all the 38 variables of main data were inter-specifically and inter-sexually significant, with no significant interaction between species and sex, implying that SD in all the six species evolved in the same way, resulting in the bigger females compared to the males. Type II two-way ANOVA on shape data shows the significant inter-species difference for 23 characters and significant SD for 12. The species*sex interaction was significant for 13 shape variables (Table 5).

TABLE 3. The *P*- values between sexes in six species of *Odontobuthus*, for metric characters using Welch two sample t-test.

Species	***O.* sp.1**		***O. bidentatus***		***O. doriae***		***O.* sp.2**		***O. tavighiae***		***O. tirgari***	
Character	Main data	Shape data	Main data	Shape data	Main data	Shape data	Main data	Shape data	Main data	Shape data	Main data	Shape data
MFL	>0.05	>0.13	>0.44	>0.12	*	>0.87	*	>0.70	*	>0.43	*	>0.09
ChL	>0.57	>0.35	>0.55	>0.28	>0.05	>0.74	*	>0.76	*	>0.31	*	>0.08
ML	>0.78	>0.38	>0.74	>0.89	>0.17	>0.69	*	>0.46	>0.05	>0.42	*	>0.24
MW	>0.28	*	>0.73	>0.79	*	*	*	>0.18	>0.69	>0.42	*	>0.29
MD	>0.74	>0.87	>0.68	>0.74	>0.07	>0.10	*	**	>0.08	>0.37	*	*
PaL	>0.67	>0.99	>0.58	>0.53	>0.20	>0.94	*	>0.92	>0.05	>0.42	>0.06	>0.94
PaW	>0.12	**	*	>0.47	>0.12	>0.89	*	>0.77	*	**	*	**
FL	>0.84	>0.34	>0.83	>0.76	>0.08	*	*	>0.33	>0.05	>0.83	*	>0.21
FW	>0.35	>0.19	>0.65	>0.73	>0.09	>0.23	*	>0.33	**	>0.10	**	>0.31
PeL	>0.66	>0.90	>0.63	>0.74	>0.09	>0.53	*	>0.62	*	>0.74	*	>0.21
CAW	>0.10	*	>0.22	>0.27	>0.06	>0.74	>0.09	>0.22	*	>0.95	**	>0.05
CPW	>0.16	*	***	>0.29	>0.05	>0.14	**	>0.27	*	>0.47	*	*
CL	>0.28	**	>0.32	>0.24	*	*	*	>0.95	*	>0.33	*	***
X	>0.08	**	*	>0.60	>0.09	>0.08	**	>0.49	**	*	**	>0.07
Y	>0.10	*	**	>0.63	>0.09	>0.79	*	>0.50	**	>0.21	**	>0.07
MsL	>0.09	**	*	>0.85	**	>0.74	*	>0.31	*	>0.57	>0.43	>0.10
T3L	*	*	*	>0.56	>0.13	>0.92	>0.08	>0.17	**	>0.12	>0.13	>0.92
T3W	>0.12	*	*	>0.18	*	>0.31	*	>0.58	*	>0.47	*	*
MT1L	>0.37	*	>0.90	>0.33	>0.24	>0.93	*	>0.35	>0.06	>0.55	>0.13	>0.89
MT1W	>0.67	>0.07	>0.64	>0.81	>0.24	>0.89	*	>0.30	>0.07	>0.97	*	*
MT1D	>0.43	>0.06	>0.41	>0.29	>0.17	>0.97	*	>0.92	>0.05	>0.88	>0.05	>0.07
MT2L	>0.18	**	>0.99	>0.53	>0.31	>0.92	*	>0.21	>0.09	>0.65	>0.12	>0.99
MT2W	>0.48	>0.11	>0.48	>0.13	>0.22	>0.69	*	>0.71	>0.07	>0.57	*	*
MT2D	>0.87	>0.52	>0.06	>0.54	>0.16	>0.82	*	>0.56	>0.05	>0.67	>0.05	>0.16
MT3L	>0.31	**	>0.84	>0.07	>0.20	>0.65	*	>0.10	>0.09	>0.28	>0.12	>0.96
MT3W	>0.56	>0.15	>0.67	>0.38	>0.22	>0.64	*	>0.79	*	>0.81	*	**
MT3D	>0.82	>0.61	>0.37	>0.43	>0.18	>0.88	*	>0.67	*	>0.74	*	*
MT4L	>0.33	**	>0.86	>0.89	>0.22	>0.97	*	>0.96	>0.10	>0.10	>0.14	>0.77
MT4W	>0.95	>0.52	>0.22	>0.61	>0.26	>0.95	*	>0.46	>0.06	>0.16	*	>0.16
MT4D	>0.38	>0.45	>0.50	>0.65	>0.18	>0.70	*	>0.38	*	>0.92	*	*
MT5L	>0.57	**	>0.72	>0.49	>0.17	>0.77	*	>0.99	>0.07	>0.40	>0.15	>0.24
MT5W	>0.32	>0.16	**	>0.59	>0.19	>0.81	*	>0.42	*	>0.82	**	*
MT5D	>0.0	>0.37	>0.25	>0.87	>0.17	>0.84	**	>0.76	>0.05	>0.49	*	>0.15
TLL	>0.48	>0.55	>0.56	>0.32	>0.13	>0.82	*	>0.79	*	>0.97	*	>0.45
TLW	>0.16	*	>0.24	>0.18	>0.06	>0.20	*	>0.17	*	**	*	>0.27
TLD	>0.49	>0.47	>0.28	>0.16	>0.07	>0.19	**	>0.81	*	>0.20	*	>0.53
MTTL	>0.50	*	>0.81	>0.60	>0.20	>0.89	*	>0.18	>0.07	>0.36	>0.11	>0.93
BL	>0.59	*	>0.57	>0.77	>0.09	>0.58	*	>0.78	*	>0.99	>0.13	>0.08

Stars show significant *P*- values (*: 0.05-0.01; **: 0.01-0.001; ***: <0.001).

TABLE 4. Two-way ANOVA on overall size considering two factors of species and sex in the six species of *Odontobuthus*.

	Df	Sum Sq	Mean Sq	F- value	Pr (>F)
Species	5	15114	3022.9	21.465	<4.4e-14
sex	1	3035	3034.7	21.549	1.2e-05
Species*Sex interaction	5	546	109.2	0.775	0.57
Residuals	90	12674	140.8		

TABLE 5. Type II two-way ANOVA on two factors of species and sex for each metric charcters studied. Based on shape data, analysis done only for characters showing sexual dimorphism. Bold numbers are the significant values.

Character	**Species**		**Sex**		**Species * Sex**	
	Main data	**Shape data**	**Main data**	**Shape data**	**Main data**	**Shape data**
MFL	F = 17.94 **P < e-11**	-	F = 29.14 **P < e-06**	-	F = 0.53 P = 0.75	-
ChL	F = 15.42 **P < e-10**	-	F = 27.29 **P < e-05**	-	F = 0.66 P = 0.65	-
ML	F = 17.16 **P < e-11**	-	F = 20.93 **P < e-04**	-	F = 0.79 P = 0.55	-
MW	F = 36.33 **P < e-15**	F = 92.77 **P < e-15**	F = 26.58 **P < e-05**	F = 11.98 **P < e-04**	F = 1.23 P = 0.30	F = 1.28 P = 0.28
MD	F = 32.94 **P <e-15**	F = 48.43 **P < e-15**	F = 29.35 **P < e-06**	F = 11.76 **P < e-04**	F = 1.23 P = 0.29	F = 1.11 P = 0.35
PaL	F = 15.60 **P < e-10**	-	F = 21.78 **P < e-04**	-	F = 0.70 P = 0.62	-
PaW	F = 19.99 **P < e-12**	F = 6.36 **P < e-04**	F = 47.57 **P < e-09**	F = 17.85 **P < e-04**	F = 0.12 P = 0.98	F = 2.05 P = 0.07
FL	F = 16.62 **P < e-10**	F = 36.22 **P <e-15**	F = 22.22 **P < e-05**	F = 0.62 P = 0.43	F = 0.90 P = 0.48	F = 0.88 P = 0.49
FW	F = 21.85 **P < e-13**	-	F = 34.42 **P < e-07**	-	F = 0.69 P = 0.63	-
PeL	F = 16.13 **P < e-10**	-	F = 25.29 **P < e-05**	-	F = 0.73 P = 0.59	-
CAW	F = 20.33 **P < e-12**	F = 9.85 **P < e-06**	F = 33.07 **P < e-06**	F = 24.22 **P < e-05**	F = 0.26 P = 0.93	F = 2.11 P = 0.07
CPW	F = 17.82 **P < e-11**	-	F = 38.27 P < e-07	-	F = 0.49 P = 0.78	-
CL	F = 19.51 **P < e-12**	F = 18.78 **P < e-12**	F = 34.90 **P < e-07**	F = 36.10 **P < e-07**	F = 0.58 P = 0.70	F = 4.19 **P < e-03**
X	F = 21 **P < e-13**	F = 3.54 **P < e-03**	F = 48.82 **P < e-09**	F = 23.73 **P < e-05**	F = 0.69 P = 0.62	F = 1.40 P = 0.22
Y	F = 20.62 **P < e-12**	F = 20.65 **P < e-12**	F = 43.06 **P < e-08**	F = 12.99 **P < e-04**	F = 0.29 P = 0.91	F = 3.32 **P < e-03**
MsL	F = 9.73 **P < e-06**	F = 8.46 **P < e-05**	F = 18.97 **P < e-04**	F = 0.07 P = 0.78	F = 0.97 P = 0.43	F = 4.20 **P < e-03**

TABLE 5. Continued

T3L	F = 8.64 ***P* < e-05**	F = 19.21 ***P* < e-12**	F = 28.63 ***P* < e-06**	F = 2.33 *P* = 0.13	F = 0.96 *P* = 0.44	F = 3.36 ***P* < e-03**
T3W	F = 16.43 ***P* < e-10**	F = 7.07 ***P* < e-04**	F = 35.63 ***P* < e-07**	F = 15.41 ***P* < e-04**	F = 0.39 *P* = 0.84	F = 3.31 ***P* < e-03**
MT1L	F = 15.44 ***P* < e-10**	F = 7.36 ***P* < e-05**	F = 13.32 ***P* < e-03**	F = 0.32 *P* = 0.57	F = 1.27 *P* = 0.28	F = 3.58 ***P* < e-03**
MT1W	F = 15.75 ***P* < e-10**	F = 2.60 ***P* = 0.03**	F = 21.61 ***P* < e-04**	F = 4.51 ***P* = 0.03**	F = 0.91 *P* = 0.47	F = 2.66 ***P* = 0.02**
MT1D	F = 17.75 ***P* < e-11**	-	F = 18.72 ***P* < e-04**	-	F = 0.97 *P* = 0.43	-
MT2L	F = 15.79 ***P* < e-10**	F = 3.93 ***P* < e-03**	F = 12.04 ***P* < e-03**	F = 2.67 *P* = 0.10	F = 1.60 *P* = 0.16	F = 5.18 ***P* < e-04**
MT2W	F = 21.36 ***P* < e-13**	F = 14.95 ***P* < e-09**	F = 19.26 ***P* < e-04**	F = 1.15 *P* = 0.28	F = 0.94 *P* = 0.45	F = 2.94 ***P* = 0.01**
MT2D	F = 19.04 ***P* < e-12**	-	F = 22.11 ***P* < e-05**	-	F = 0.71 *P* = 0.61	-
MT3L	F = 18.02 ***P* < e-11**	F = 6.90 ***P* < e-04**	F = 14.14 ***P* < e-03**	F = 1.64 *P* = 0.20	F = 0.72 *P* = 0.13	F = 5.71 ***P* < e-04**
MT3W	F = 22.09 ***P* < e-13**	F 18.91 ***P* < e-12**	F = 22.58 ***P* < e-05**	F = 2.57 *P* = 0.11	F = 1.14 *P* = 0.34	F = 3.36 ***P* < e-03**
MT3D	F = 16.55 ***P* < e-10**	F = 4.25 ***P* < e-03**	F = 22.67 ***P* < e-05**	F = 1.74 *P* = 0.18	F = 0.56 *P* = 0.72	F = 0.85 *P* = 0.51
MT4L	F = 19.92 ***P* < e-12**	F = 9.23 ***P* < e-06**	F = 13.59 ***P* < e-03**	F = 6.38 ***P* = 0.01**	F = 1.37 *P* = 0.24	F = 4.28 ***P* < e-03**
MT4W	F = 21.74 ***P* < e-13**	-	F = 20.58 ***P* < e-04**	-	F = 0.70 *P* = 0.62	-
MT4D	F = 19.19 ***P* < e-12**	-	F = 24.72 ***P* < e-05**	-	F = 0.62 *P* = 0.68	-
MT5L	F = 17.96 ***P* < e-11**	F = 1.04 *P* = 0.39	F = 16.19 ***P* < e-03**	F = 2.88 *P* = 0.09	F = 1.24 *P* = 0.29	F = 1.11 *P* = 0.35
MT5W	F = 21.57 ***P* < e-14**	F = 14.30 ***P* < e-09**	F = 31.90 ***P* < e-06**	F = 7.24 ***P* < e-03**	F = 0.39 *P* = 0.84	F = 2.09 *P* = 0.07
MT5D	F = 15.04 ***P* < e-09**	-	F = 24.01 ***P* < e-05**	-	F = 0.88 *P* = 0.49	-
TLL	F = 15.57 ***P* < e-12**	-	F = 25.40 ***P* < e-06**	-	F = 0.70 *P* = 0.62	-
TLW	F = 24.97 ***P* < e-14**	F = 14.2 ***P* < e-09**	F = 35.38 ***P* < e-07**	F = 10.73 ***P* < e-03**	F = 0.57 *P* = 0.72	F = 0.184 P = 0.96
TLD	F = 24.62 ***P* < e-14**	-	F = 30.37 ***P* < e-06**	-	F = 0.77 *P* = 0.57	-
MTTL	F = 18.47 ***P* < e-11**	F = 7.53 ***P* < e-05**	F = 16.46 ***P* < e-03**	F = 3.42 *P* = 0.06	F = 1.28 *P* = 0.27	F = 6.46 ***P* < e-04**
BL	F = 16.42 ***P* < e-10**	F = 16.80 ***P* < e-10**	F = 20.53 ***P* < e-04**	F = 1.61 *P* = 0.20	F = 0.74 *P* = 0.59	F = 1.48 *P* = 0.20

Mann-Whitney U test on meristic variables showed that PDN is strongly sexually dimorphic in all species. LLAA and Mt3VC show a significant difference in *O. tirgari* and MFDR and VAAD are not sexually dimorphic in any of the species studied (Table 6). Figure 3 shows a box plot graphed on PDN variation in different sexes of the six species, representing that the number of pectin denticles in males is always more than in females.

TABLE 6. Sexual dimorphism in six species of *Odontobuthus* based on meristic variables by Mann-Whitney U test.

Species / Character	*O.* sp.1	*O. bidentatus*	*O. doriae*	*O.* sp.2	*O. tavighiae*	*O. tirgari*
MFDR	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
PDN	*	*	**	**	***	***
LLAA	>0.05	-	-	-	-	*
Mt3VC	>0.05	>0.05	-	>0.05	>0.05	*
VAAD	-	>0.05	>0.05	>0.05	>0.05	>0.05

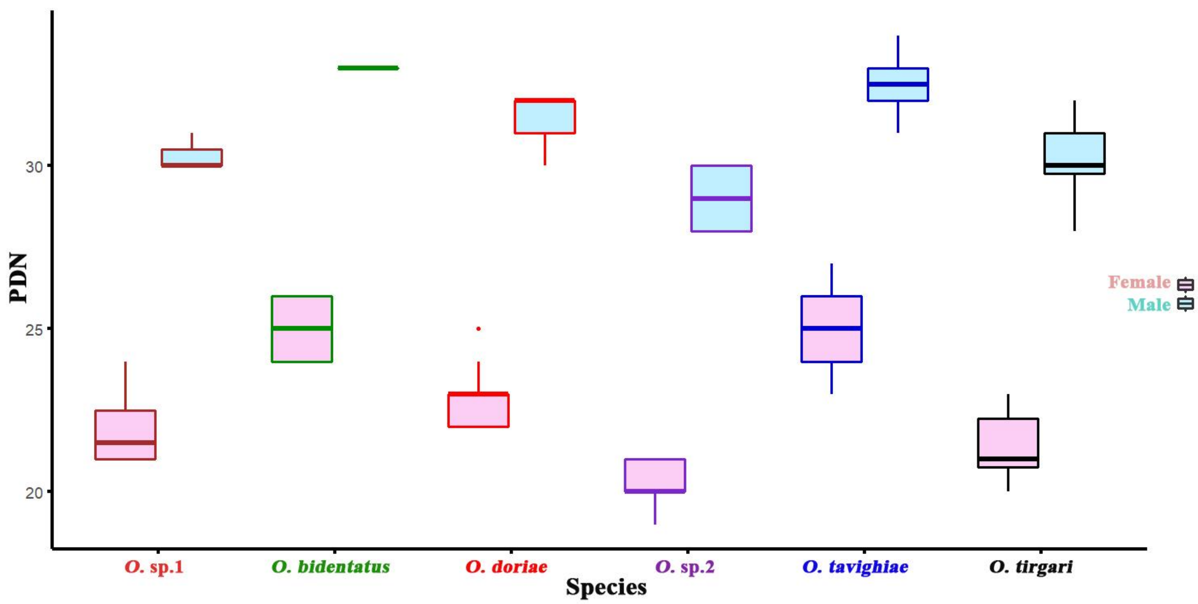

FIGURE 3. Boxplots based on PDN for the males (in blue) and females (in pink) of the six species of *Odontobuthus*. All specimens show strong sexual dimorphism.

Multivariate analyses

Type II two-way MANOVA on shape variables, using the factors of species and sex and considering the individuals as random effect showed significant difference between the studied species, sexes and the interaction between species and sex (Table 7). When the analysis is performed on the shape data and overall size together, the same results were seen, but the sex effect and the species*sex interaction were, respectively, more (F=15.58, P < 10-12) and less (F= 1.68, P < 0.02) significant compared to using shape data. It implies that considering the size into accounts increases the inter-sexual morphometric difference, but decreases the interaction of species*sex.

TABLE 7. Type-II two-way MANOVA on shape variables in the six species of *Odontobuthus*, using the two factors of species and sex.

	Df	Pillai	approximated F	num Df	den Df	Pr (>F)
Species	5	2.46856	12.2591	35	440	< 2.2e-16
Sex	1	0.33738	6.1101	7	84	8.8e-06
Species*Sex interaction	5	0.59776	2.7070	35	440	0.008
Residuals	90					

A biplot was graphed on the first principal component of the shape data (PC 1) and the overall size (Fig. 4). The PC1 includes more than 42% of the overall variance, mainly composed of the variance of MD, MW, TLW, MT5W, MT4W, and MT3W. All the species, and also the different sexes of each species, were ordinated separately on the plot. The SD in size and shape was not, however, the same in different species. For three species, *O. tirgari*, *O.* sp.1 and *O. bidentatus*, the SD is more seen in shape rather than in size, wherease *O.* sp.2 and *O. tavighiae*, did show an opposite trend, *i.e.* their two sexes were more different in size than in shape. *O. doriae* is sexually highly dimorphic in both shape and size. The males and females of the latter species were more similar, in both shape and size, to their respective sex of *O. bidentatus* than to each other.

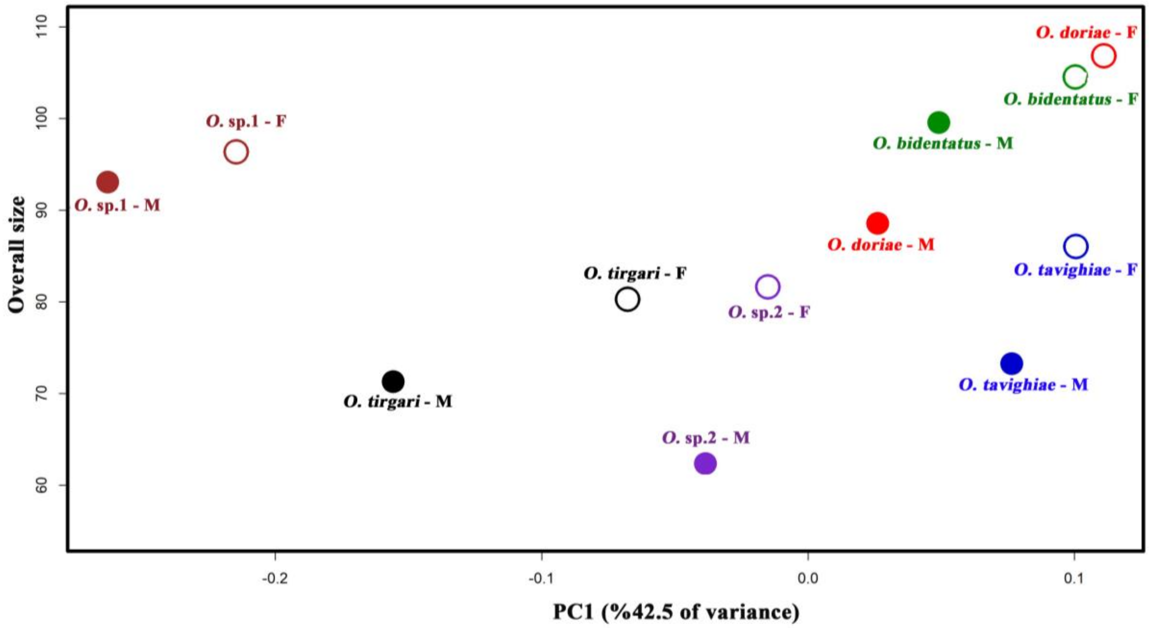

FIGURE 4. The biplot of the mean value of the males (solid circles) and females (open circles) of the six species of *Odontobuthus*, on the first principal component of the shape data (PC1) against the overall size.

DISSCUSSION

In most invertebrates, females are larger than males. Selection represents a direct relationship between the size of the female, fertility and egg production. This relationship is suggested as a strong and effective force in creating SD (Mori *et al*., 2017). In arachnids, sexual size dimorphism is very common in which males are smaller than females. Selection for smaller males is affected by cannibalism and mortality (Polis, 1990; Mori *et al*., 2017).

In scorpions, usually, males matured as early as females, and their rate of mortality is much higher because of the behavioral difference. In the breeding season, the males are very active which caused an increase in the rate of mortality due to cannibalism or predation. These movements also reduce the feeding activity of males. Also, males usually construct more shelters compared to females and young males (Polis, 1990).

Generally, the average size of females is larger than the average size of males. However, univariate statistical analysis based on metric variables, without removing the size effect, only shows SD in *O. tavighiae*, *O. doriae* and, *O.* sp.2. In these species, all body parts in females are larger than males. Univariate analysis did not show SD in *O.* sp.1 (P= 0.0822) (Table 2). However, after removing the effect of size the difference between males and females of *O.* sp.1 is evident. Unlike Mori *et al*. (2017), the size does not affect SD alone as it is characterized after the removal of the size effect. The shape of body structures might be different between males and females. In scorpions, SD in shape is characterized by elongation of pedipalp and metasoma in males (latter is more common). SD might affect the shape of pedipalp manus and telson (Polis, 1990).

According to Table 3, several characters such as PaW and CL showed both size and shape dimorphism. Based on univariate statistical analyses some variables such as FL, CAW, Y, MsL, MT1-5L and, BL showed size dimorphism in one species and shape dimorphism in another species. Therefore, not only size but also shape affects the differences between sexes**.** Based on statistical analysis, *O. tavighiae* shows a remarkable SSD in chela, carapace, mesosoma, and telson which are larger than males. Although, after removing the effect of size the SSD was not significant for this species (Table 3).

Scorpion pectins are special anatomical structures which functionally played an important role in finding mate location, courtship behavior, and reproduction. This part shows a remarkable SD in scorpions (Polis, 1990; Booncham *et al*., 2007). As predicted in the study of meristic characters the pectins (PDN; Table 6) shows a significant difference between sexes. Pectins are larger in males in terms of length and number of teeth and the angle between two pectins in males is less than females.

The interaction between species and sex in the analysis of variance on the overall size is not statistically significant, suggesting that in all of the examined species the SSD is parallel (Table 4) and has always resulted in increasing the female's overall size relative to males (Mori *et al*., 2017). While the shape SD in different species of the genus *Odontobuthus* has not been parallel (Table 7, Fig. 4). In terms of size, *O. tavighiae*, and *O.* sp.2, and regarding the shape, *O. bidentatus*, *O. tirgari*, and, *O.* sp.1 shows SD. *O. doriae* shows remarkable size and shape dimorphism (Table 2, Fig. 4). The close affinity of *O. doriae* with *O. bidentatus* is congruent with the phylogenetic topologies presented by Azghadi *et al*. (2014). These two species have been separted geographically by Zagros Mountains (Lowe, 2010). Furthermore, SD in *O.* sp.1 is not parallel, *i.e.* the length of its metasomal segments in males is relatively larger than females (Fig. 1). This species lives on sandy substrates and longer metasoma might correspond to more efficient performance during mating, predation or combat with other males and, hence, to have arisen through sexual selection (Carlson *et al*., 2014).

Based on the data presented here, it seems that selection, either sexual or sex-specific, has a parallel effect on size but non- parallel on shape. It favors the larger female size which directly affects the fecundity and egg-producing capability of females. But in some cases, selective pressures have caused a different effect. As size and shape may have different effects on fitness, the study of sexual size and shape dimorphism using geometric morphometric methods might be promising and could help in unraveling different aspects of sexual shape dimorphism in scorpions.

ACKNOWLEDGMENTS

This study was supported by a grant from the Office of Research Affairs, Ferdowsi University of Mashhad, Iran to H.B. (Project 3/45941).

LITERATURE CITED

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovačić, M., Zardoya, R., Rüber, L., 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. Molecular Phylogenetic Evolution 69(3), 619-633.

Andersson, M., 1994. *Sexual Selection*. Princeton University Press, Princeton, New Jersey. 624 p.

Booncham, U., Sitthicharoenchai, D., Pradatsundarasar, A., Prasarnpun, S., Thirakhupt, K., 2007. Sexual Dimorphism in the Asian Giant Forest Scorpion, *Heterometrus laoticus* Couzijn, 1981, *NU* Science Journal 4(1), 42-52.

Butler, M.A., Losos, J.B., 2002. Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. Ecological Monographs 72, 541-559.

Carlson, B.E., McGinley, S., Rowe, M.P., 2014. Meek Males and Fighting Females: Sexually-Dimorphic Antipredator Behavior and Locomotor Performance Is Explained by Morphology in Bark Scorpions (*Centruroides vittatus*). PLoS ONE 9(5), e97648. DOI: 10.1371/ journal.pone.0097648

Censky, E.J., 1997. Female mate choice in the non-territorial lizard *Ameiva plei* (Teiidae). Behavioral Ecology and Sociobiology 40, 221-225.

Cooper, W.E.J., Vitt, L.J., 1993. Female mate choice of large male broad-headed skinks. Animal Behaviour 45, 683-693.

Eldredge, N., 1972. Systematics and evolution of *Phacops rana* (Green, 1832) and *Phacops iowensis* Delo, 1935 (Trilobita) from the middle Devonian of North America. Bulletin of the American Museum of Natural History 147, 45-114.

Fathinia, B., Rastegar-Pouyani, N., 2011. Sexual dimorphism in *Trapelus ruderatus ruderatus* (Sauria: Agamidae) with notes on the natural history. Amphibian and Reptile Conservation 5(1), 15-22(e22).

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Haradon, R.M., 1984. New and redefined species belonging to the Paruroctonus baergi group (Scorpiones, Vaejovidae). The Journal of Arachnology 12, 205-221

Katsikaros, K., Shine, R., 1997. Sexual dimorphism in the tusked frog, *Adelotus brevis* (Anura: Myobatrachidae): the roles of natural and sexual selection. Biological Journal of the Linnean Society 60, 39-51.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W. K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463-1464.

Kovarik, F., 2004. A review of the Genus Heterometrus Ehrenberg, 1828, with Descriptions of Seven New Species (Scorpiones, Scorpionidae). Euscorpius 15, 1-60.

Kuo, C.Y., Lin, Y.T., Lin, Y.S., 2009. Sexual Size and Shape Dimorphism in an Agamid Lizard, *Japalura swinhonis* (Squamata: Lacertilia: Agamidae). Zoological Studies 48(3), 351-361.

Lamoral, B.H., 1979. The scorpions of Namibia. Annals of the Natal Museum 23, 497-784.

Lourenço, W.R., Pézier, A., 2002. Taxonomic consideration of the genus *Odontobuthus* Vachon (Scorpiones, Buthidae), with description of a new species. Revue suisse de Zoologie 109(1), 115-125.

Lowe, G., 2010. A new species of *Odontobuthus* (Scorpiones: Buthidae) from northern Oman. Euscorpius 96, 1-22.

McLean, C.J., Garwood, R.J., Brassey, C.A., 2019. Sexual dimorphism in the size and shape of the raptorial pedipalps of Giant Whip Spiders (Arachnida: Amblypygi). Journal of Zoology 1-10. DOI: 10.1111/jzo.12726

Mirshamsi, O., Azghadi, S., Navidpour, Sh., Aliabadian, M., Kovařík, F., 2013. *Odontobuthus tirgari* sp. nov. (Scorpiones, Buthidae) from the eastern region of the Iranian Plateau. Zootaxa 3731(1), 153-170.

Monnet, J.M., Cherry, M.I., 2002. Sexual size dimorphism in anurans. Proceedings of the Royal Society of London: Series B - Biological Sciences 269, 2301-2307.

Mori, E., Mazza, G., Lovari, S., 2017. Sexual Dimorphism. Encyclopedia of Animal Cognition and Behavior 433, 1-7. DOI: 10.1007/978-3-319-47829-6.

Navarro, N., Zatarain, X., Montuire, S., 2004. Effects of morphometric descriptor changes on statistical classification and morphospaces. Biological Journal of the Linnean Society 83, 243-260.

Navidpour, Sh., Soleglad, M.E., Fet, V., Kovařík, F., 2013. Scorpions of Iran (Arachnida, Scorpiones). Part IX. Hormozgan province, with a description of *Odontobuthus tavighiae* sp. n. (Buthidae). Euscorpius 170, 1-29.

Olsson, M., Shine, R., Wapstra, E., Ujvari, B., Madsen, T., 2002. Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. Evolution 56(7), 1538-1542.

Ozkan, O, Adiguzel, S., Kar, S., 2006. Parametric values of *Androctonus crassicauda* (Oliver, 1807) (Scorpiones: Buthidae) from Turkey. Journal of Venomous Animals and Toxins including Tropical Diseases 12, 549-559.

Palen-Pietri, R., Ceballos, A., Peretti, A.V., 2019. Sexual dimorphism and patterns of sexual behavior in Lustrochernes argentines (Pseudoscorpiones: Chernetidae). Journal of Arachnology 47, 344-350.

Polis, G.A., 1990. The biology of scorpions. Stanford University Press. 233 Pp.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schwarzkopf, L., 2005. Sexual dimorphism in body shape without sexual dimorphism in body size in water skinks (*Eulamprus quoyii*). Herpetologica 61, 116-123.

Sentenská, L., Franziska, G., Richard, M., Kropf, C., 2017. Sexual dimorphism in venom gland morphology in a sexually stinging scorpion. Biological Journal of the Linnean Society 122(2), 429-443. DOI: 10.1093/biolinnean/blx067.

Sissom, W.D., 1990. Systematics, biogeography and paleontology. In: Polis, G.A. (Ed.), Biology of Scorpions. Stanford University Press, Stanford, California, 64–160.

Shine, R., 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. The Quarterly Review of Biology 64(4), 419-461.

Stahnke, H.L., 1970. Scorpion nomenclature and mensuration. Entomological News 81, 297-316.

Tague, R.G., 2005. Big-bodied males help us recognize that females have big pelvis. American Journal of Physical Anthropology 127, 392-405.

Thompson, G.G., Withers, P.C., 2005. Size-free shape differences between male and female Western Australian dragon lizards (Agamidae). Amphibia-Reptilia 26, 55-63.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Zhang, X., Xiong, J.L., Lv, Y.Y., Zhang, L., Sun, Y.Y., 2014. Sexual size and shape dimorphism in the Wushan salamander, *Liua shihi* (Liu, 1950) (Urodela: Hynobiidae). Italian Journal of Zoology, 81(3), 368-373. DOI: 10.1080/11250003.2014.920927.