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Range Extension and Sexual dimorphism in *Asaccus nasrullahi* Werner, 2006 (Sauria: Phyllodactylidae), from western Iran

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Abstract
The Leaf-toed Geckos of genus *Asaccus* are distributed in Turkey, Syria, Iraq, and Iran of the Middle-East characterizing by a unique characteristic of cloacal sacs and postanal bones absence. The genus *Asaccus* includes 19 species with at least 10 species in Iran distributing along the Zagros Mountains. Sexual size dimorphism (SSD) is a body size difference between males and females of the same species, being a widespread phenomenon in reptiles. We collected additional specimens of *Asaccus nasrullahi* (Phyllodactylidae) from four new localities in Ilam and Khuzestan Provinces, western Iran. In this study, 110 adult specimens of this lizard were examined (for nine metric and four meristic characters). The analyses of morphological data revealed that the males are significantly larger than the females in eight metric characters including forelimb to hind limb length (FHL), length of forelimb (LFL), length of hind limb (LHL), head height (HH), head length (HL), head width (HW), rostrum to anus length (RAL), and tail length (TL). There is no significant difference in the rate of tail shedding between the sexes. The range of this species is extended beyond type locality in Lorestan Province into adjacent areas in Ilam and Khuzestan Provinces.

Key words: *Leaf-toed gecko, Asaccus nasrullahi, Ilam Province, Khuzestan Province, SSD*.

INTRODUCTION

The genus *Asaccus* Dixon and Anderson, 1973 (formerly included in *Phyllodactylus* Gray, 1828) is distributed in Turkey, Syria, Iraq, and Iran encompass 19 species (Uetz & Hošek, 2020) with at least 10 species in Iran distributing along the Zagros Range including *Asaccus andersoni*, *A. elisae*, *A. granularis*, *A. griseonotus*, *A. iranicus*, *A. kermanshahensis*, *Asaccus kurdistanensis*, *A. nasrullahi*, *A. tangestanensis*, and *A. zagrosica* (Nasrabadi *et al.*, 2017).

Werner (2006), based on the re-identification of a single specimen (ZMUC 3447; Zoological Museum, University of Copenhagen), which had been identified by Schmidt (1955) as *Ptyodactylus hasselquisti,* described a new species of *Asaccus*, *A. nasrullahi*. As an endemic species in some parts of the Zagros mountains in Iran, few studies have been done on this species. To our knowledge, only one study has done on the morphological and ecological aspects of this species (Torki *et al.*, 2010).

Comparative studies of sexual dimorphism should generally encompass body size as a potential determinant (Fairbairn *et al.*, 2007). The evolutionary result of selection acting differently

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on body size and other morphological traits of males and females is referred to as sexual size dimorphism (Andersson, 1994). During this study, the distribution range of *A. nasrullahi* is expanded and sexual dimorphism pattern is evaluated.

MATERIAL AND METHODS

Field study

We examined 110 adult specimens (51 males and 59 females) of *A. nasrullahi* during numerous fieldworks from June to August 2012. The specimens were collected by hand, both in day and night, on rocky substrates of the Zagros Mountains in the following localities: locality 1 or Sarab-e-Dareshahr (33° 05' N, 47° 20' E, 820 m a.s.l.), locality 2 or Bahram-e-Chobin strait (33º 05' N, 47° 27' E, 690 m a.s.l.) and locality 3 or Sheykh Makan (33° 05' N, 47° 23' E, 709 m a.s.l.), in Darr-e-Shahar township, Ilam Province.

These three localities are all along the Kabir-Kooh range and the distance from first (Sarab-e-Dareshahr) to last locality (Bahram-e-Chobin) is 10.8 Km in a northwest-southeast direction. Specimens collected from these three localities were used in statistical analyses. Some specimens were also collected in rocky areas of the Shevi or Tale-Zang Waterfall (32° 47' N, 48° 49' E, 872 m asl) in Dezful township, Khuzestan Province. These specimens were not used in statistical analyses, but identified as *A. nasrullahi* and used in the map of range extension.

Examined characters

A total of 13 morphometric characters (nine metrics and four meristics) were recorded. The metric characters comprise RAL (rostrum to anus length, according to Werner (1971)), HW (head width; in its widest part), HL (head length; from the tip of rostrum to the anterior border of ear, according to Goren & Werner (1993)), HH (head height; at the level of eyes), LFL (length of forelimb; in an extended position from axilla to tip of the longest finger), LHL (length of hind limb; in an extended position from groin to tip of the longest toe), FHL (forelimb to hind limb length; from axilla to groin), TL (tail length; from posterior margin of the anus to tip of tail, in just original tails) and AW (anus width). The meristic characters include BT (the number of tubercle rows on dorsum), CT (number of crossbars on the dorsal side of tail), IL (number of infralabials), and SL (number of supralabials) (Fathinia *et al.*, 2011).

The metric characters were measured by a digital caliper to the nearest 0.01 mm accuracy. The meristic ones were recorded using a stereomicroscope. To determine the sex of specimens, we noticed the presence or absence of two swellings at the base of the tail just behind the anus. The swellings accommodate hemipenes in males (Fig. 1). Some specimens were fixed and deposited in Razi University Zoological Museum (RUZM) as vouchers and the remaining specimens were released into their natural habitat after measuring the specifications. Each location was surveyed just once to avoid repeated catching of the same specimens.

Statistical analyses

Both univariate and multivariate analyses were done on the dataset. The Shapiro-Wilk test was employed to determine the normality of variables. Before conducting statistical analyses, our data were divided into four datasets: datasets 1 to 3 conform to localities 1 to 3 and dataset 4 coincides with datasets 1 to 3 combined. Each of these four datasets was analyzed separately for sexual dimorphism. Independent sample t-test as well as Mann-Whitney U test was used for univariate analysis of normally and non-normally distributed characters, respectively. To determine the sexual dimorphism of *A. nasrullahi* at the multivariate level, we used Principal Component Analysis (PCA: correlation matrix). TL and CT were not included in the PCA analysis because a total number of 75 individuals had missed values (i.e. regenerated tails). The Chi-square test was used to determine if the rate of broken tails is significant between males and females. All the statistical analyses were done using SPSS 16.

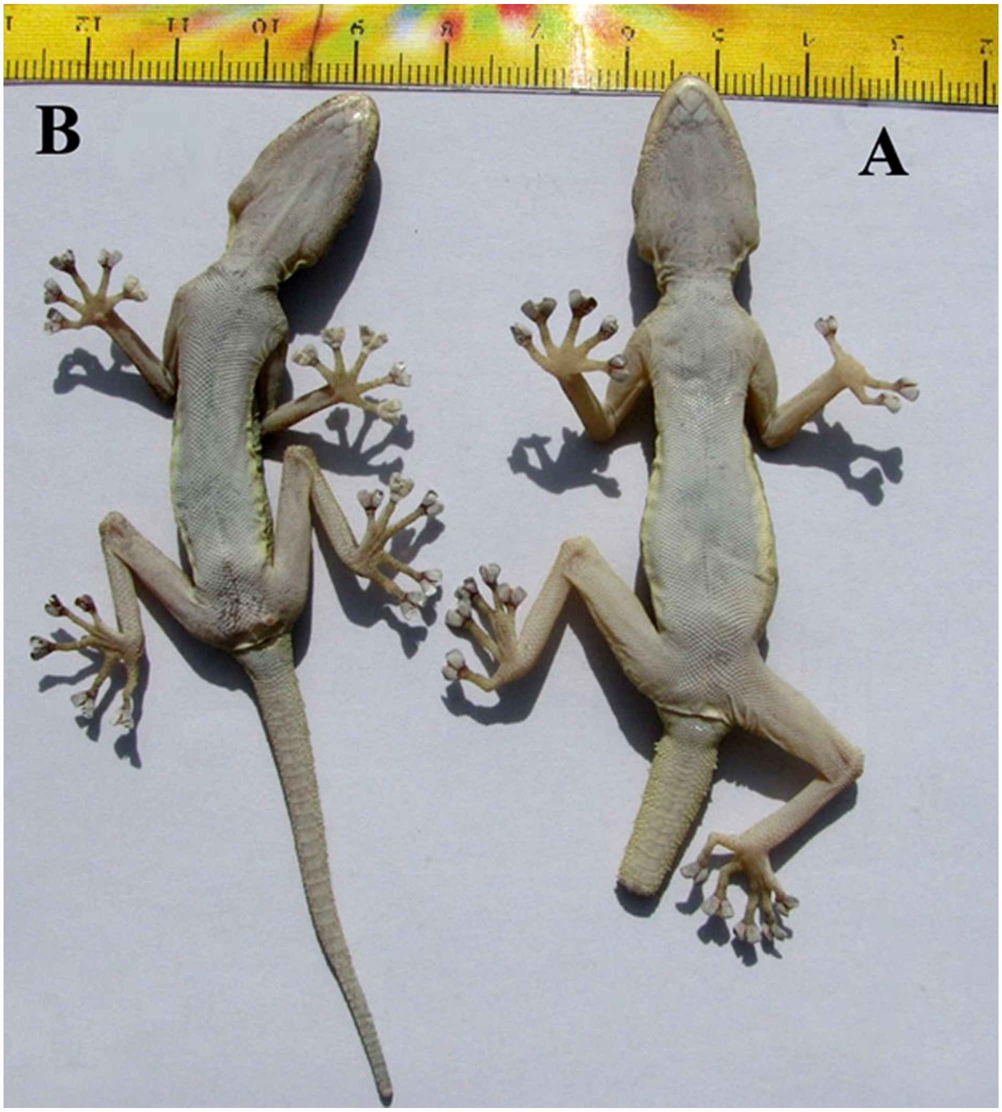

FIGURE 1. Ventral view of adult male (A) and female (B) specimens of *Asaccus nasrullahi*.

RESULTS

New localities for *Asaccus nasrullahi* are reported in Ilam (Darr-e-Shahr township) and Khuzestan (Dezful township) Province. These new localities extend the range of *Asaccus nasrullahi* beyond previous records of this species in Lorestan Province (Tang-e-Gavshomar and Shahbazan regions) (Fig. 2). A total of 13 morphological characters (nine metrics and four meristics) were included in this study. Univariate analyses using Mann-Whitney U-test and Independent-sample t-test were done for each locality and pooled data. These results show that there is no significant difference in characters between two sexes in dataset 1 (Locality 1; Sarab-e-Dareshahr) ($P > 0.05$; Table 1).

The population in dataset 2 (Locality 2, Bahram-e-Choobin) showed a significant male-biased difference between the two sexes only in the character FHL ($P = 0.028$; Table 2). In contrast, the population in dataset 3 (Locality 3, Sheikh-Makan) showed significant male-biased differences in four characters HW, LFL, RAL, and TL ($P < 0.05$; Table 3). The results of univariate analyses for dataset 4 (localities 1 to 3) showed male-biased significant differences between sexes in eight out of 13 characters FHL, LFL, LHL, HH, HL, HW, RAL, and TL ($p \leq 0.05$). All meristic characters are not significantly different between the sexes ($P > 0.05$) (Table 4).

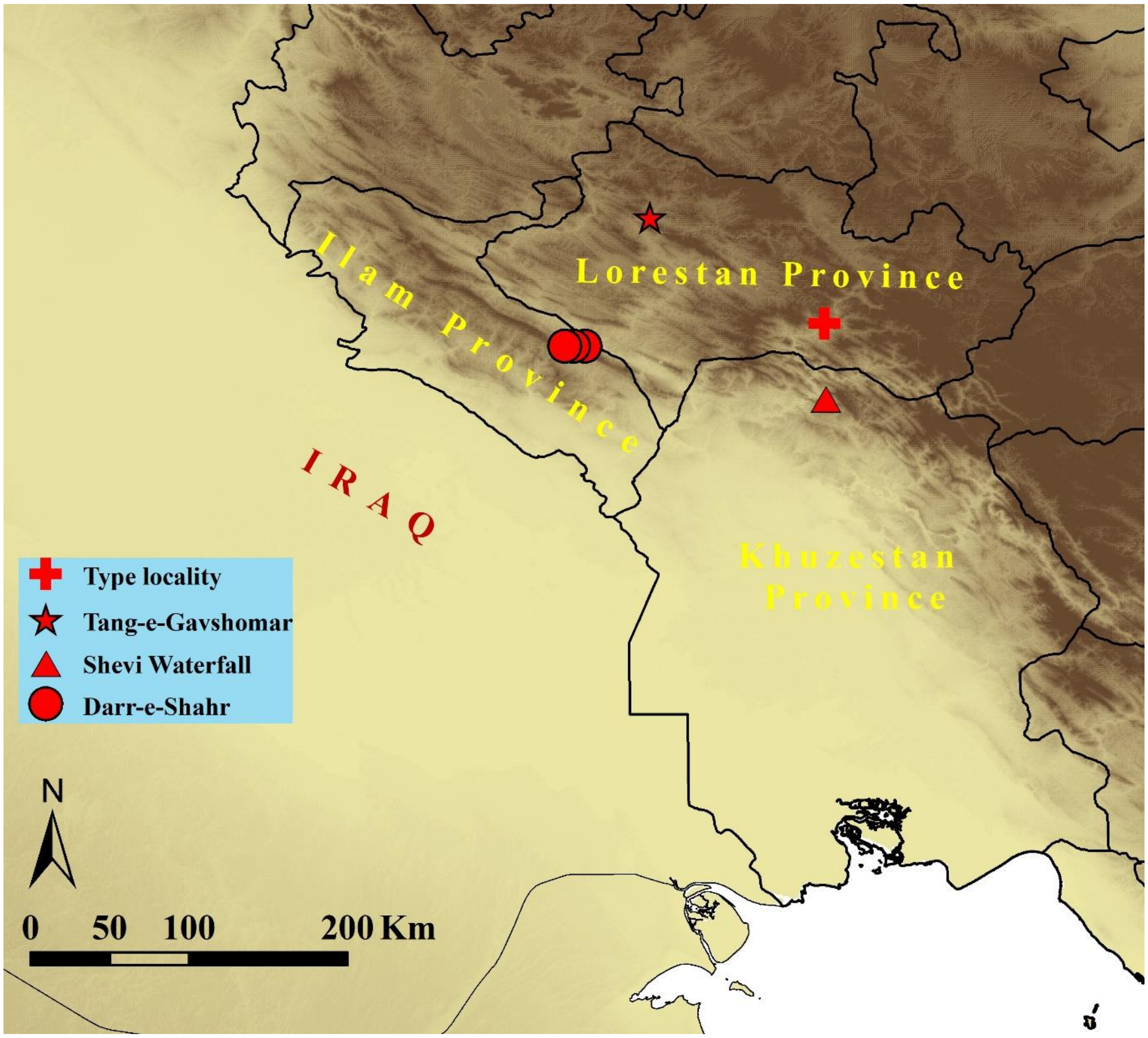

FIGURE 2. A map shows the previous and new localities of *Asaccus nasrullahi* in western Iran. Previous records; green Plus sign, holotype locality in Shahbazan region and green star, Tang-e-Gavshomar region. New localities; green triangle, Shevi Waterfall and green circles, Sarab-e-Darreshahr (left), Sheikh-Makan (middle), and Bahram-e-Choobin (right) along the Kabirkooh range in Ilam province.

A total number of 75 individuals (35 males and 40 females) have broken tails. The result of the chi-square test (X^2 = 0.009, P = 0.926) shows that the rate of broken tails does not show a significant difference between the sexes. Principal Component Analysis (PCA) was carried out on the significantly different characters between sexes in dataset 4. The character TL was excluded from this analysis because of having missed values.

The results of this analysis show that the first four axes collectively represent 95.18% of the total variation (Table 5). Of this, 82.3 is explained by PC1, with all characters (i.e., FHL, LFL, LHL, HH, HL, HW, RAL) mainly responsible for the observed variation, and 5.28% is explained by PC2, in which FHL and HH have the highest values. This analysis shows that the PC1 is chiefly responsible for the separation of males and females of *Asaccus nasrullahi* (Fig. 3).

TABLE 1. The results of univariate analysis using the Mann-Whitney U and Independent Sample t-test on the standardized metric and meristic characters of *Asaccus nasrullahi* in locality 1 (Sarab-e-Darreshahr). Abbreviations: SD, standard deviation; D. of d., the direction of difference; Sexes, 1 = male and 2 = female; T-test, Independent Sample t-test; U-test, Mann-Whitney U. All measurements in millimeter.

	Character	Sex	No.	Mean±SD	*P-value*	Test used	D. of d.
Meristics	BT	1	17	7.45±0.79	0.949	T-test	F = M
		2	35	7.43±0.9			
	CT	1	6	13±0.89	1.000	U-test	F = M
		2	13	12.85±1.21			
	IL	1	17	7.88±0.70	0.882	U-test	F = M
		2	35	7.86±0.73			
	SL	1	17	11.18±0.95	0.728	U-test	F = M
		2	35	11.23±0.97			
Metrics	AW	1	17	6.89±0.65	0.707	T-test	F = M
		2	35	6.98±0.82			
	FHL	1	17	30.17±3.45	0.920	T-test	F = M
		2	35	30.27±3.39			
	LFL	1	17	29.28±2.07	0.447	T-test	F = M
		2	35	28.82±2.07			
	LHL	1	17	38.9±3.01	0.208	U-test	F = M
		2	35	37.88±2.51			
	HH	1	17	7.08±0.63	0.294	T-test	F = M
		2	35	6.91±0.51			
	HL	1	17	16.85±1.31	0.243	T-test	F = M
		2	35	16.43±1.16			
	HW	1	17	13.35±1.21	0.064	T-test	F = M
		2	35	12.77±0.94			
	RAL	1	17	65.93±5.06	0.344	T-test	F = M
		2	35	64.41±5.51			
	TL	1	6	78.02±6.57	0.051	T-test	F = M
		2	13	71.54±6.10			

DISCUSSION

Asaccus nasrullahi presented marked sexual dimorphism in general body size and several body parts, with males being significantly larger than females in eight out of 13 studied characters. Our results show that there is considerable variation in sexual dimorphism among different populations of *A. nasrullahi*. For instance, in dataset 1 (Sarab-e-Dareshahr) there is no difference between the sexes. In dataset 2 (Bahram-e-Choobin) males are larger than females in only one character while in dataset 3 (Sheikh-Makan) males are larger than females in four metric characters. But the pooled data show that males are larger than females in eight metric characters. These incongruences in SSD results among subsets and pooled datasets may be the result of insufficient sampling in the three sampled locations.

TABLE 2. The results of univariate analysis of *Asaccus nasrullahi* in locality 2 (Bahram-e-Choobin). Abbreviations as in Table 1. Abbreviations: SD, standard deviation; D. of d., the direction of difference; Sexes, 1 = male and 2 = female; T-test, Independent Sample t-test; U-test, Mann-Whitney U. All measurements in millimeter.

Character		Sex	No.	Mean±SD	*P-value*	Used test	D. of. D.
Meristics	BT	1	8	7.71±0.87	0.111	T-test	M = F
		2	13	7.17±0.62			
	CT	1	3	13.33±0.58	0.116	T-test	M = F
		2	3	12±1.00			
	IL	1	8	7.88±0.64	0.224	U-test	M = F
		2	13	8.15±0.38			
	SL	1	8	11.75±0.71	0.736	T-test	M = F
		2	13	11.62±0.96			
Metrics	AW	1	8	6.92±0.69	0.596	T-test	M = F
		2	13	6.76±0.63			
	FHL	1	8	31.95±2.99	**0.028**	T-test	M > F
		2	13	28.61±3.21			
	LFL	1	8	29.35±2.78	0.757	T-test	M = F
		2	13	28.89±3.53			
	LHL	1	8	39.22±3.43	0.302	T-test	M = F
		2	13	37.82±2.62			
	HH	1	8	7.24±0.83	0.293	T-test	M = F
		2	13	6.9±0.62			
	HL	1	8	16.96±1.39	0.176	T-test	M = F
		2	13	16.17±1.14			
	HW	1	8	13.11±1.31	0.128	T-test	M = F
		2	13	12.39±0.79			
	RAL	1	8	65.81±6.17	0.281	T-test	M = F
		2	13	62.77±6.07			
	TL	1	3	69.03±1.52	0.52	T-test	M = F
		2	3	70.56±3.46			

Many taxa show geographic variation in SSD with moderate differences in proportional sizes between the sexes (Harvey & Ralls, 1985; Schwaner & Sarre, 1988). But, at least in one case (*Morelia spilota*), proportional sizes between the sexes show dramatic differences among different populations. Different causes have been proposed to explain such variations in SSD. For example, selective pressures on male body size may vary among different localities, resulting in geographic variation in SSD among distinct populations of the same species (Shine & Fitzgerald, 1995). Another explanation for this phenomenon is the genetic control of SSD in different populations (Pearson *et al.*, 2002).

TABLE 3. The results of univariate analysis of *Asaccus nasrullahi* in locality 3 (Sheikh-Makan). Abbreviations as in Table 1. Abbreviations: SD, standard deviation; D. of d., the direction of difference; Sexes, 1 = male and 2 = female; T-test, Independent Sample t-test; U-test, Mann-Whitney U. All measurements in millimeter.

Character		Sex	No.	Mean±SD	*P-value*	Used test	D. of. D.
Meristics	BT	1.0	26.0	7.52±0.56	0.176	T-test	M = F
		2.0	11.0	7.8±0.55			
	CT	1.0	6.00	12.67±1.21	0.395	U-test	M = F
		2.0	3.00	12±0.00			
	IL	1.0	26.0	8.27±0.53	0.646	U-test	M = F
		2.0	11.0	8.36±0.50			
	SL	1.0	26.0	11.88±0.95	0.054	U-test	M = F
		2.0	11.0	11.27±0.65			
Metrics	AW	1.0	26.0	7.03±0.39	0.055	T-test	M = F
		2.0	11.0	7.36±0.61			
	FHL	1.0	26.0	31.75±1.65	0.13	T-test	M = F
		2.0	11.0	30.48±2.40			
	LFL	1.0	26.0	30.46±1.38	**0.001**	T-test	M > F
		2.0	11.0	28.52±1.37			
	LHL	1.0	26.0	40.17±1.38	0.061	T-test	M = F
		2.0	11.0	38.33±2.38			
	HH	1.0	26.0	7.34±0.64	0.056	T-test	M = F
		2.0	11.0	6.9±0.58			
	HL	1.0	26.0	17.14±0.65	0.084	T-test	M = F
		2.0	11.0	16.65±1.00			
	HW	1.0	26.0	13.68±0.63	**0.006**	T-test	M > F
		2.0	11.0	12.99±0.70			
	RAL	1.0	26.0	68.86±2.46	**0.027**	T-test	M > F
		2.0	11.0	65.22±4.54			
	TL	1.0	7.00	77.92±3.54	0.157	T-test	M = F
		2.0	3.00	67.95±8.05			

Several hypotheses have been proposed to explain the intraspecific variation in SSD including 1) fecundity selection in which females have larger body size than males (Andersson, 1994; Sandercock, 2001; Rastegar Pouyani *et al.*, 2015), 2) niche partitioning through which males and females are different in the size of some parts of the body (such as head size) to use different niche dimensions (Smith & Nickel, 2002), 3) Sexual selection acting on either sex may select for SSD (Raihani *et al.*, 2006). These selection processes may be reinforced via female choice (Thornhill & Alcock, 1983; Choe & Crespi, 1997).

TABLE 4. The results of univariate analysis of *Asaccus nasrullahi* in all three localities. Abbreviations as in Table 1. Abbreviations: SD, standard deviation; D. of d., the direction of difference; Sexes, 1 = male and 2 = female; T-test, Independent Sample t-test; U-test, Mann-Whitney U. All measurements in millimeter.

	Character	Sex	No.	Mean±SD	*P-value*	Used test	D. of. D.
Meristics	BT	1.0	51.0	7.53±0.96	0.554	T-test	M = F
		2.0	59.0	7.44±0.81			
	CT	1.0	15.0	12.93±0.96	0.385	U-test	M = F
		2.0	19.0	12.58±1.12			
	IL	1.0	51.0	8.08±0.63	0.622	U-test	M = F
		2.0	59.0	8.02±0.66			
	SL	1.0	51.0	11.63±0.96	0.123	U-test	M = F
		2.0	59.0	11.32±0.92			
Metrics	AW	1.0	51.0	6.97±0.53	0.783	T-test	M = F
		2.0	59.0	7.00±0.76			
	FHL	1.0	51.0	31.26±2.65	**0.023**	T-test	M > F
		2.0	59.0	29.94±3.22			
	LFL	1.0	51.0	29.89±1.93	**0.002**	U-test	M > F
		2.0	59.0	28.78±2.33			
	LHL	1.0	51.0	39.6±2.42	**0.000**	U-test	M > F
		2.0	59.0	37.95±2.55			
	HH	1.0	51.0	7.24±0.66	**0.004**	T-test	M > F
		2.0	59.0	6.9±0.54			
	HL	1.0	51.0	17.01±1.02	**0.004**	T-test	M > F
		2.0	59.0	16.41±1.12			
	HW	1.0	51.0	13.48±0.98	**0.000**	T-test	M > F
		2.0	59.0	12.73±0.88			
	RAL	1.0	51.0	67.4±4.34	**0.001**	T-test	M > F
		2.0	59.0	64.2±5.45			
	TL	1.0	16.0	76.29±5.72	**0.009**	T-test	M > F
		2.0	19.0	70.82±5.92			

Competition between males may favor large body size in species in which males compete intensely for females (Mitani *et al.*, 1996; Dunn *et al.*, 2001; Lindenfors *et al.*, 2003; Raihani *et al.*, 2006). In this case, larger males are more likely to survive or mate with females (Clutton-Brock & Harvey, 1977; Anderson & Vitt, 1990). The positive correlation between combat success and body size has been studied and proved in lizards (Olsson, 1992; Zucker & Murray, 1996). However, the phenomenon of male combat suggests sexual selection for larger male size, predicting a phylogenetic correlation between male-biased SSD and male aggressive behavior. Thus, sexual selection for large males will not occur unless success in combat also translates into greater reproductive success (Cox *et al.*, 2003; Kratochvíl & Frynta, 2006). The “rostrum to anus length” (RAL) and forelimb to hind limb length (FHL) in males of *A. nasrullahi* are greater than those in females. These two male-biased characters (RAL and FHL) can be explained by the sexual selection theory, stating combat among males occur for females.

TABLE 5. Loadings from a principal component analysis of seven significant metric characters of *Asaccus nasrullahi*. Variables loading strongly on each principal component are shown in bold.

Variables	**PC1**	**PC2**	**PC3**	**PC4**
FHL	**0.853**	**-0.358**	**0.368**	0.060
LFL	**0.885**	-0.129	**-0.304**	**0.288**
LHL	**0.920**	0.049	-0.108	0.030
HH	**0.842**	**0.468**	0.218	0.131
HL	**0.965**	0.020	-0.048	-0.078
HW	**0.916**	0.018	-0.111	**-0.336**
RAL	**0.963**	-0.057	0.020	-0.063
Eigenvalues	5.763	0.370	0.302	0.228
% of Variance	82.322	5.285	4.317	3.257
Cumulative%	82.322	87.607	91.925	95.182

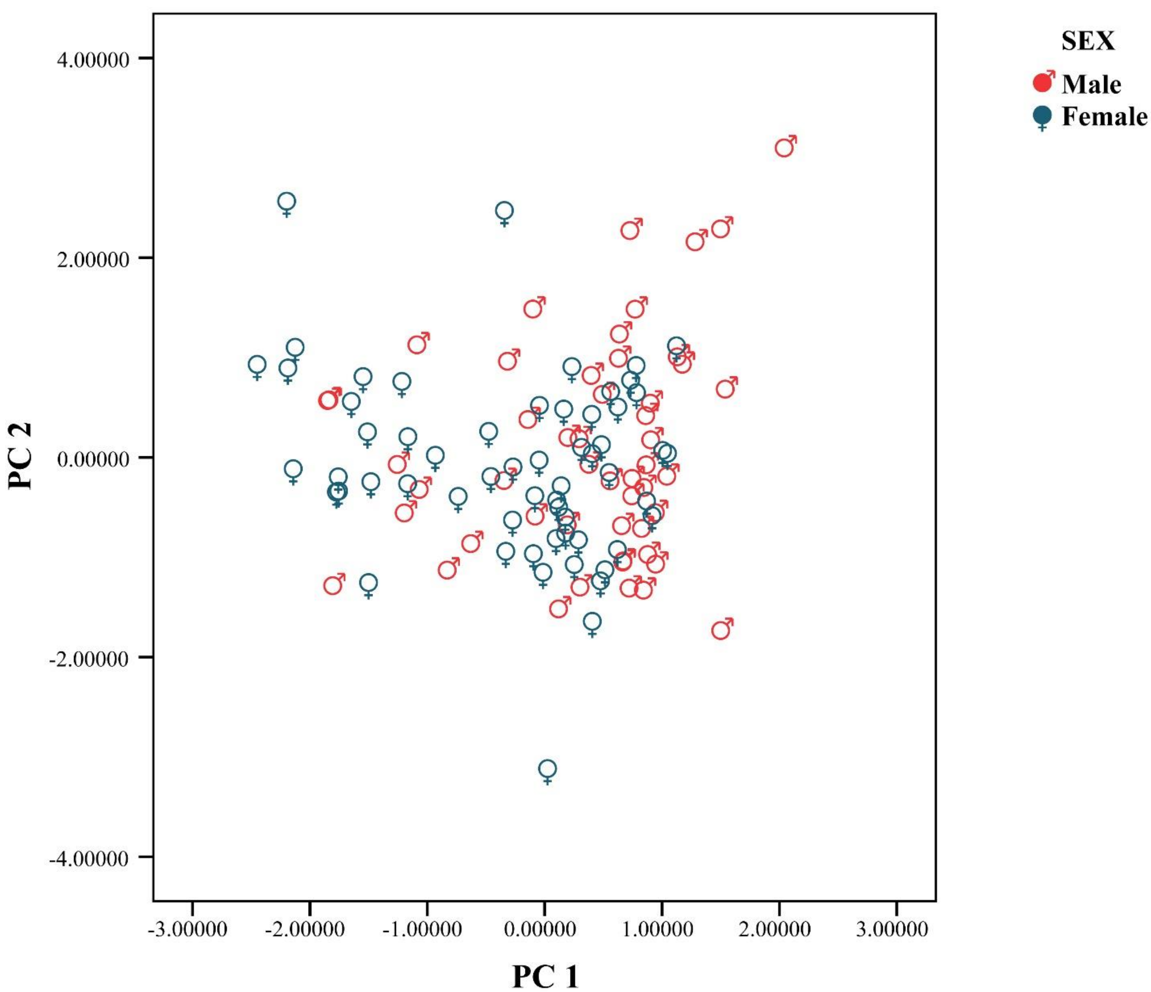

FIGURE 3. Ordination of the individual males and females of *Asaccus nasrullahi* on the first two principal components, showing poor separation.

In the current study, the significant male-biased differences in head dimensions (i.e. HL, HH, and HW) between sexes of *A. nasrullahi* were proved. Many studies have shown male-biased differences in the size of head dimensions in a variety of lizards (Barbadillo *et al.*, 1995; Hews, 1996; Smith *et al.*, 1997; Smith & Nickel, 2002; Verrastro, 2004; Fathinia *et al.*, 2011; Karamiani *et al.*, 2013). Head size is a character that can support the niche divergence hypothesis in other vertebrates (Selander, 1972; Shine, 1989). There is a direct relation between head size and bite force which may have a direct effect on dominance. These traits (head dimensions) may have been evolved under both sexual and natural selections (Huyghe *et al.*, 2005). These ideas may explain the presence of larger heads in males of *A. nasrullahi*.

The values of LFL and LHL in males of *A. nasrullahi* are significantly greater than those in females. Males can take advantage of having long limbs by increasing maximum sprint speed, allowing them to catch prey, escape predators (Calsbeek & Smith, 2003), chase females for mating (Fathinia *et al.*, 2011), and defense territory (Peterson & Husak, 2006) more effectively. A combination of these causes may explain male-biased differences in the limbs of *A. nasrullahi*.

Shedding a portion or most of the tail followed by regeneration is an ability that one can see in a majority of lizards. Tail shedding can take place as the result of a predator attack or intraspecific conflicts. This behavior allows a lizard to have an efficient escape (Cott, 1957). As the main purposes of this research were investigating sexual dimorphism and distribution of *Asaccus nasrullahi*, additional studies are needed to shed light on the causes and consequences of tail shedding in this gecko. In summary, a combination of three different causes may have driven the SSD in *A. nasrullahi*: sexual selection, niche divergence, and natural selection.

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