

Sexual size dimorphism in *Neurergus kaiseri* (Caudata: Salamandridae) in south-western Zagros Mountains, Iran

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Abstract.—Using bivariate and multivariate techniques we evaluated sexual size dimorphism in 13 body-related and six head-related metrics from 99 live specimens of the Lorestan newt, *Neurergus kaiseri*. Analyses of variance of 12 metrics showed that average sizes for all these characters in females are significantly ($P < 0.05$) greater than in males. However, one character (vent length) is larger in males than females ($P < 0.001$). Evaluation of 13 metrics showed that average size dimorphism is apparent in 10 characters with nine characters showing these differences at $P \leq 0.01$ and one character at $P < 0.05$ confidence levels. Principal Components Analysis of external characters provided a good separation of males and females. Although body measurements gave a clear pattern of differences between the sexes in *N. kaiseri*, head measurements showed no such distinctions.

Key words. *Neurergus kaiseri*, Principal Component Analysis (PCA), sexual size dimorphism, southwestern Iran, cloaca

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Introduction

Sexual dimorphism shows widespread and recognizable patterns in many species and has been studied for more than a century (reviewed in Fairbairn et al. 2007). Sexual size dimorphism is common in animal taxa, but is highly variable in magnitude and direction (Andersson 1994; Fairbairn 1997; Brandt and Andrade 2007). Sexually dimorphic traits have been surveyed in different classes of vertebrates, including birds (Temeles 1985; Temeles et al. 2000), primates (Crook 1972), amphibians (Schäuble 2004; Vargas-Salinas 2006; McGarrity and Johnson 2008; Malmgren and Thollesson 1999; Kalezić et al. 1992), lizards (Bruner et al. 2005; Kaliontzopoulou et al. 2007), and snakes (Feriche et al. 1993; Shine et al. 1999). Amphibian females generally grow larger than males and female body size is often correlated to clutch size (Duellman and Trueb 1986; Rafinski and Pecio 1989; Kalezić et al. 1992). In amphibians, the most strikingly dimorphic sexual characteristics are seasonal; however, most species also show permanent sexual differences in morphometrics and morphology (Malacarne and Cortassa 1983).

Although mature female amphibians are generally larger than males, and female body size is often correlated to clutch size, there are examples where males are the larger. This can be attributed to high degrees of agonistic male behavior such as combat during the reproduc-

tive season. Several theories have been developed to explain ecological and evolutionary significance for sexual size dimorphism (e.g., Slatkin 1984; Andersson 1994). As stated by Darwin (1871) sexual selection is likely the most important single cause that generates dimorphism, but other factors such as female reproductive strategy and competition for food resources have been considered to be significant (Duellman and Trueb 1986).

In the present paper, we explore and discuss sexual size dimorphism in the Lorestan newt *Neurergus kaiseri* in the southwestern mountains of Iran. The aim is to describe the expression of intersexual differences in this species to reveal sexually dimorphic traits that can be important in systematic and evolutionary research.

Material and methods

We measured 99 live specimens of *Neurergus kaiseri* found in the southern Zagros ranges. The average annual precipitation in the southern Zagros ranges from 400 to 800 mm per year. The dominant vegetation cover around streams is oak tree (*Quercus brantii*) open woodlands. The active period of *N. kaiseri* in its aquatic environment starts in March and ends in July, a period when temperature enables breeding and feeding. The *N. kaiseri* used in the present study (58 males, 41 females) were all caught in the daytime between the 7th and 13th April 2012. The

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Figure 1. Male *Neurergus kaiseri* have a fleshy protuberance at the base of the tail (A), whereas female has a prominent cloaca but without the protuberance (B). Photos by Mozafar Sharifi.

method of capture was by hand, with individuals taken from among or under stones in the shallow water at the side of the stream. After measuring, the *N. kaiseri* were released unharmed at the location of their capture. The sex of each individual was assessed according to external secondary sexual characters: males have a fleshy protuberance at the base of the tail, whereas females have a prominent cloaca, but without the protuberance (Fig 1). Juveniles are differentiated from mature adults in having a smaller body length and lacking both the protuberance and the prominent cloaca (Baran and Atatür 1998).

Thirteen variables (Table 1) for all specimens were measured with calipers to the nearest 0.1 mm. To test significant differences of sexually dimorphic metrics, Independent Sample *t*-test (2-tailed) as well as Principal Component Analysis (PCA: correlation matrix) at the significance level of 0.01 were used. In multivariate analyses the variables were carried out into two sets (body- and head-related measurements) and were analyzed separately using PCA based on Pearson's correlation coefficients. SPSS software version 16, Excel, and Past software were used for running the statistical analyses.

Table 1. Definitions of the morphometric character set and abbreviations used for body- and head-related metrics.

Abbreviations	Variable definition
Body measurements	
W	Weight
SVL	Snout to vent length, tip of snout to anterior margin of cloacal lips
TL	Tail length, posterior margin of cloacal lips to tip of tail
LFL	Length of fore limb, anterior margin of front leg to tip of the longest finger
LHL	Length of hind limb, anterior margin of hind leg to tip of the longest toe
FHL	Forelimb to hindlimb length, posterior margin of front leg (axilla) to anterior margin of hind leg (groin)
VL	Vent length, anterior margin of cloacal lips to posterior margin of cloacal lips
TLL	Total length, tip of snout to tip of tail (SVL + VL + TL)
Head measurements	
HL	Head length, tip of snout to posterior region of neck
HW	Head width, largest width of head, in line with the corner of the mouth
HH	Height of head, margin of lower jaw to upper of eye, in line with the eyes
IOD	Interorbital distance, shortest distance between eyes
DN	Distance of nostrils, from one nostril to the other
LW	Length of wrinkles under throat, tip of snout to posterior margin of wrinkles under throat

Results

The results of the Independent Sample *t*-test (2-tailed) show that in *N. kaiseri* most characters differed significantly between sexes ($P \leq 0.01$), so that in each variable females are larger than males, excluding vent length (Table 2). In *N. kaiseri*, 89% of body related metrics were significantly sexually dimorphic (Table 2). In contrast, there were fewer such sex related differences (50%) in measurements related to head morphology. The mean male to female Snout to Vent Length (SVL) ratio was 0.86 for *N. kaiseri* (Table 2). In Bivariate Analyses, SVL in relation to the Total Length (TL; measured from tip-of-snout to tip-of-tail), was dimorphic in *N. kaiseri* (males $46.59\% \pm 0.004$ SE, females $49.36\% \pm 0.004$ SE; $P < 0.001$). The relation between Tail Length (TL) to the Total Length and Forelimb to Hindlimb Length (FHL) to SVL were not significantly sexually dimorphic in *N. kaiseri*. Males of *N. kaiseri* had the shorter tail (males 56.76 ± 0.93 SE, females 63.20 ± 0.92 ; $P < 0.001$).

Analysis of metrics indicated general trends in variation. Loadings for the first two components are given in Table 2, and the individual specimens are projected onto these components in Figure 3. In each PCA there were high positive loadings for all characters on PC1. This axis is therefore interpreted as a general size measure. Contrasting positive and negative loadings were found

on PC2, indicating general shape measures as important for this separation.

Body variables gave a clear pattern of differences between the sexes in *N. kaiseri*, while head measurements showed no such distinctions (Figs. 1 and 2). Factor loadings for principal components (Table 3) revealed that a total of 75.9% and 58.3% of the variability for *N. kaiseri* could be explained by the first two components (PC1 and PC2) for body- and head-related traits, respectively. The first component, which explained 62.1% and the second component, which explained 13.7% of the total character variation for body characters, provided complete separation between males and females (Figs. 3, 4, and 5). The first component, which explained 39% and the second component, which explained 19.3% of the total character variation for head measurements, do not prove complete separation between males and females (Figs. 3, 4, and 5). The remaining components (PC3 \pm PC9) individually explained $< 12\%$ of the total variation for this species on body related traits, and did not reveal any readily interpretable patterns. Factor loadings for discriminant Hotelling's T^2 revealed that with using body measurements, males and females were well separated (Hotelling's T^2 : 1307.9, F: 175.28, $P < 0.001$), but with using head variables the sexes were not separated (Hotelling's T^2 : 29.351, F: 5.63, $P < 0.001$), and revealed these measurements unsuitable for determination of sexual dimorphism in *N. kaiseri* (Fig. 2).

Table 2. Descriptive statistics (mean, standard error of mean, and range) of 13 external characteristics (mm) in males and females of *Neurergus kaiseri*; *n*: number; SE: standard error of mean. Morphometric Abbreviations: W (Weight), SVL (Snout-Vent Length), TL (Tail Length), HH (Height of Head), LFL (Length of Forelimb), LHL (Length of Hindlimb), FHL (Forelimb to Hindlimb Length), VL (Cloacal Length), IOD (Interorbital Distance), DN (Distance of Nostrils).

Variable	Males (<i>n</i> = 58)		Females (<i>n</i> = 41)		<i>P</i>
	Mean \pm SE	Range	Mean \pm SE	Range	
Body measurements					
W	6.2 \pm 0.2	3.2 – 9.8	7.2 \pm 0.3	3.8 – 11.4	0.0
SVL	54.8 \pm 0.5	47.9 – 61.8	63.6 \pm 0.8	54.1 – 78.9	0.0
TL	56.8 \pm 0.9	33.5 – 72.8	63.3 \pm 0.9	52.6 – 75.9	0.0
LFL	20.1 \pm 0.2	16.3 – 22.3	20.5 \pm 0.2	16.9 – 23.1	0.05
LHL	21.6 \pm 0.2	17.2 – 24.6	22.4 \pm 0.3	19.2 – 26.0	0.0
FHL	29.4 \pm 0.4	18.0 – 35.8	35.0 \pm 0.6	26.9 – 41.6	0.0
LW	13.8 \pm 0.2	11.1 – 16.7	14.4 \pm 0.4	6.2 – 18.9	0.1
VL	6.2 \pm 0.2	4.5 – 7.9	2.1 \pm 0.4	1.4 – 2.5	0.0
TLL	117.8 \pm 1.4	100.1 – 137.3	128.9 \pm 1.4	111.8 – 146.5	0.0
Head measurements					
HL	13.5 \pm 0.1	10.7 – 16.0	13.6 \pm 0.1	12.1 – 15.0	0.2
HW	11.0 \pm 0.1	9.5 – 13.4	11.1 \pm 0.2	5.7 – 12.5	0.7
HH	5.6 \pm 0.1	4.9 – 7.4	6.0 \pm 0.1	5.3 – 7.2	0.0
IOD	7.1 \pm 0.1	5.9 – 8.0	7.4 \pm 0.1	6.2 – 8.8	0.0
DN	3.8 \pm 0.1	1.2 – 4.6	4.0 \pm 0.0	3.5 – 4.7	0.01

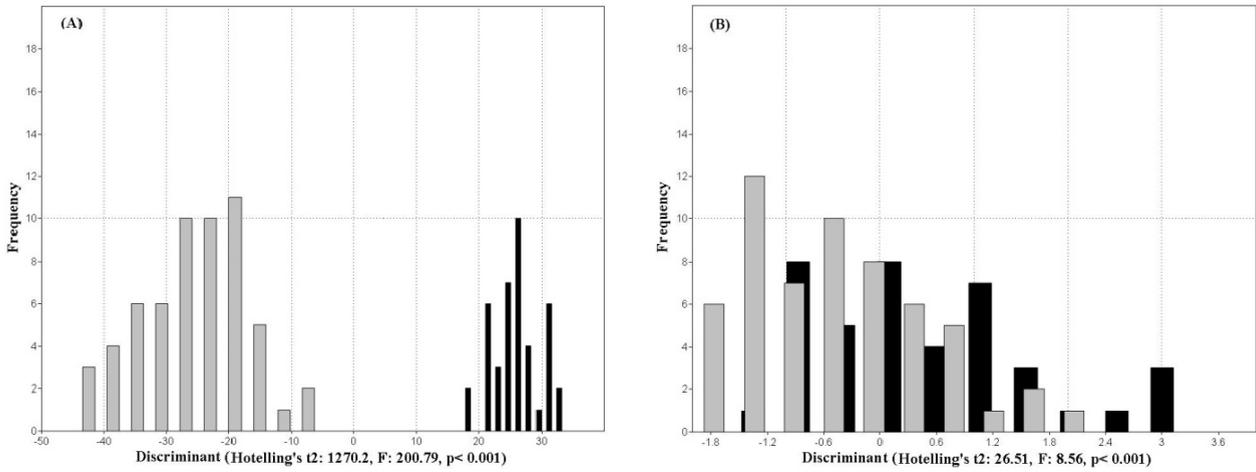


Figure 2. Discriminant Hotelling’s T^2 for separation of male and female *Neurergus kaiseri*. (A) Body-related characters; (B) Head-related variables. Note that on base head-related variables male and female aren’t well separated.

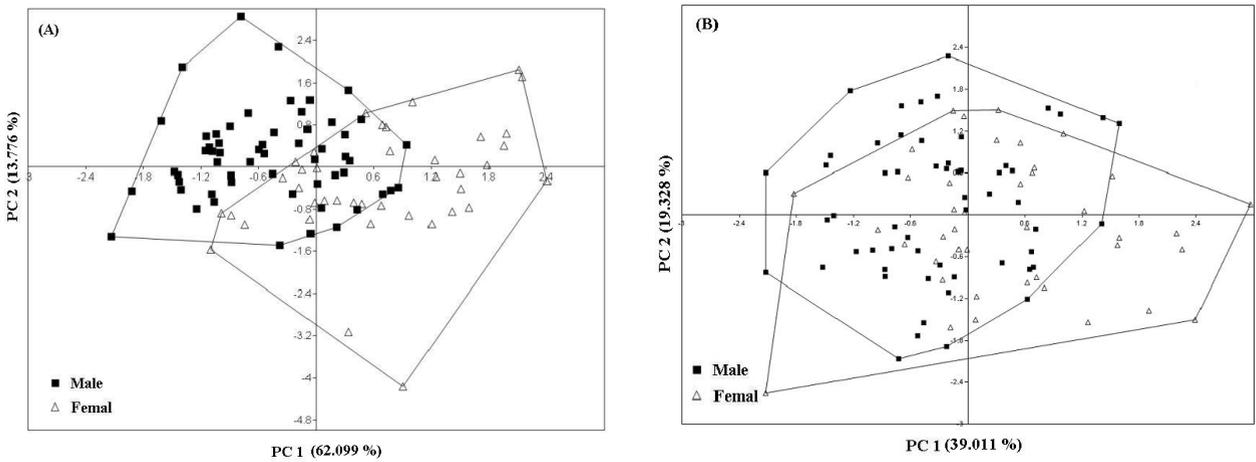


Figure 3. Principal Component Analysis (PCA) on sexual dimorphism in *Neurergus kaiseri*. Scatter plots of principal component scores for the first two principal axes, with convex polygons for males and females. Loadings are shown in Table 3. (A) Body-related characters; (B) Head-related variables, significant sex differences are noted on neither PC1, nor PC2.

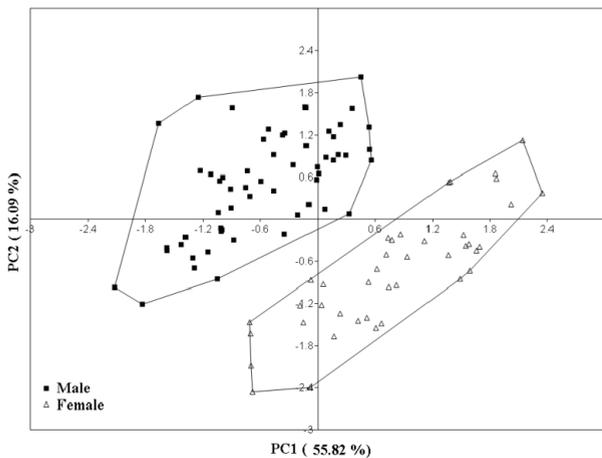


Figure 4. Principal Component Analysis (PCA) on seasonal sexual dimorphism in *Neurergus kaiseri*. Scatter plots of principal component scores for the first two principal axes, with convex polygons for males and females. Loadings are shown in Table 3.

Discussion

The Lorestan newt *Neurergus kaiseri* was shown not to exhibit sexually dimorphism in head-related metrics. This is in agreement with other studies on head morphology in newts (Malmgren and Tholleson 1999; Rafinski and Pecio 1989; Kalezic et al. 1992). These results do not support the ecological model that *N. kaiseri* has developed intersexual differences in feeding strategies along a niche divergence process (Slatkin 1984; Andersson 1994) driven by the two factors, the rate of feeding and type of food consumed (Shine 1989). The first factor considers that substantial intersexual difference in body size lead to differences in feeding rates between the sexes. The second factor comprises species where the sexes diverge in trophic morphology as a result of intersexual differences in dietary preferences. Both male and female newts experience high energetic costs during the reproductive season (Halliday and Arano 1991; Griffiths

Sexual size dimorphism in *Neurergus kaiseri*

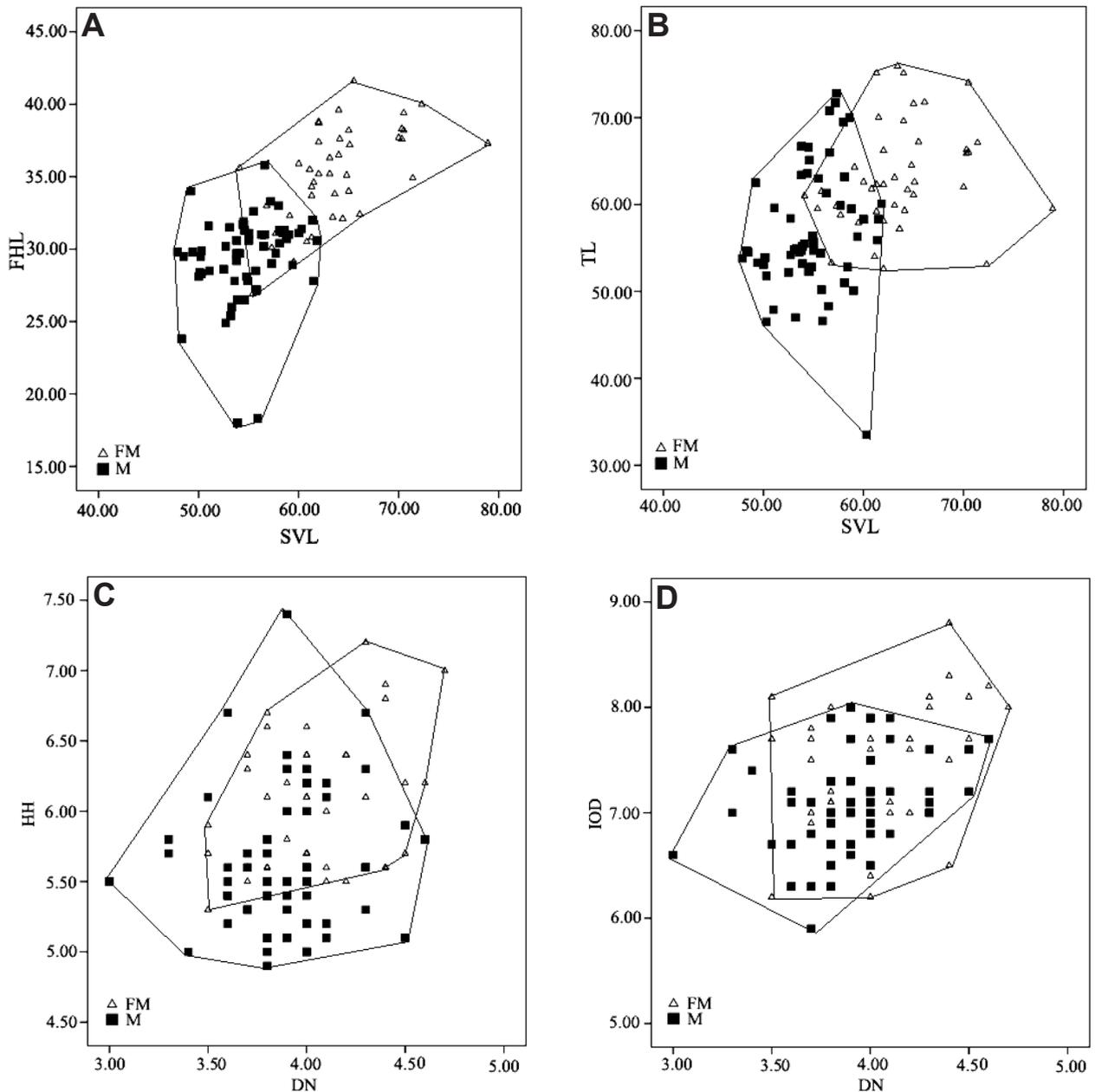


Figure 5. Bivariate scatterplots of variables on sexual dimorphism with convex polygons for males and females in *Neurergus kaiseri*. (A and B) Body-related characters (C and D) Head-related variables. Body measurements gave a clear pattern of differences between the sexes in *N. kaiseri*, while head measurements showed no such distinctions. All values in mm.

1996) and both spend considerable time feeding when not involved in courtship. This indicates that feeding rates between males and females might be similar within species, even during the breeding period, suggesting that sexual dimorphism resulting from feeding rates and diet may be negligible.

The separation of sexes in statistical analyses was high in *N. kaiseri*. Sexual dimorphism was attributed to females showing large values for dimensions related to fecundity, such as SVL and distance of FHL, contrasted with large values for cloaca in males. In all analyses the female SVL and FHL metrics were highly significant in the observed patterns, contrasting against the male CL.

These results can be interpreted as primarily concordant with the fecundity model. Previous studies on amphibians have shown that females are generally larger than males in body size (Duellman and Trueb 1986), possibly because fecundity increases with increasing female body size. Males, however, can often increase their lifetime reproductive success through other life history traits in species with little or no agonistic behavior; for example by maturing at an early age. Moreover, Kalezic et al. (1992), showed that the trunk length (corresponding to FHL) is directly correlated to the length of the pleuroperitoneal cavity in *Triturus newts* to which *Neurergus* is a closely related.

Table 3. Factor loadings for the first two principal components (eigenvectors) for *Neurergus kaiseri* from multivariate analyses (Principal Components Analysis, PCA) on body- and head-related variables.

Variable	<i>Neurergus kaiseri</i>		
	PC1	PC2	PC3
Body measurements			
SVL	0.848	0.124	-0.304
TL	0.755	-0.570	0.293
LFL	0.668	0.470	0.447
LHL	0.734	0.420	0.041
FHL	0.762	-0.025	-0.534
TLL	0.934	-0.298	0.123
Eigenvalue	3.726	0.827	0.680
% of variability	62.099	13.776	11.328
Cumulated %	62.099	75.874	87.202
Head measurements			
HH	0.484	0.794	0.049
DN	0.631	-0.540	-0.003
IOD	0.654	0.140	-0.482
Eigenvalue	1.699	0.985	0.887
% of variability	39.011	19.328	17.216
Cumulated %	39.011	58.339	75.555

Male *N. kaiseri* could be distinguished from females in having a larger cloaca (Fig. 1). The cloacal swelling in male newts is most notable laterally and ventrally compared to females, and may be an important factor in male mating success. Most of the cloacal volume is occupied by glands secreting substances forming the spermatophore, although tubules emanating from the pheromone-producing dorsal gland are present—especially in the caudal region of the cloaca (Sever et al. 1990). The dorsal gland itself, which is known to be greatly enlarged during the breeding season in some newts, lies anterior to the pelvic girdle. As proposed by Sever et al. (1990), it is quite likely that both the rate of spermatophore production and the synthesis of courtship pheromones—factors contributing to male mating success—are under the influence of sexual selection, thus increasing the size and volume of structures in the cloacal region.

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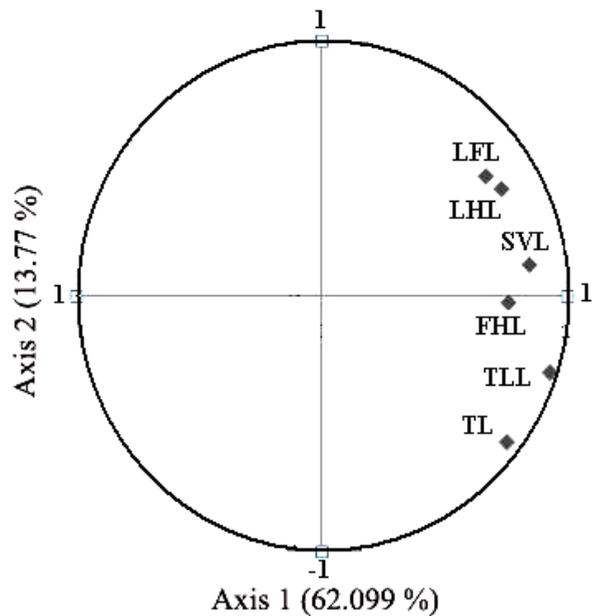


Figure 6. Factor loadings for the first two principal components in analyses of body-related characters. Loadings on PC1 are all close to one and positive, and this component is interpreted as a general size measure. On PC2 positive loadings (characters above the abscissa) are contrasted with negative loadings (below), and the component is interpreted as a measure of shape that discriminates between males and females (Figs. 4 and 5). Abbreviations as in Table 1.

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Somaye Vaissi is a M.Sc. student in systematic zoology at Department of Biology, Razi University, Iran. She has earned her B.Sc. in animal biology from the same department. She is currently the curator of a Captive Breeding Facility for *Neurergus microspilotus* at Razi University funded by the Mohamed bin Zayed Species Conservation Fund. Her current research activities with two species of *Neurergus* involve several topics associated with husbandry and health of the newts in captivity. These include nutrition, growth, development and their health. She has contribution in detecting chytrid fungus and other diseases such as red-leg syndrome and rickettsial inclusions in the newts.