South Western Journal of Horticulture, Biology and Environment *Vol.9, No.1, 2018*
pp.1-13
P-Issn: 2067- 9874, E-Issn: 2068-7958 Art.no. e18101

SEXUAL DIMORPHISM AND GEOGRAPHIC VARIATION OF BODY SIZE IN THE MOUNTAIN BROWN FROG (*Rana macrocnemis* BOULENGER, 1885) IN IRAN

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ABSTRACT. *The current study investigates patterns in sexual size dimorphism (SSD) and geographic variation in body size of the Mountain brown frog (Rana macrocnemis), collected from 8 locations in mountainous regions of western Iran. In this study we examine 13 morphometric characters to test the pattern of sexual dimorphism in size and shape using bivariate and multivariate methods. Pearson's correlation analysis was used to evaluate the relationship between body size and altitude (test the Bergmann's rule in mountain frogs), and we used the log-scaled size of one sex regressing against the log-scaled size of the other sex to test for allometry vs. isometry (Rensch's rule). Our results indicate that sizes of female characters are significantly larger than male characters and sexual dimorphism occurs not only in body size, but also in body shape. Furthermore, the results implied that females and males tended to be larger in higher altitude (follow the Bergmann's rule) and variations in SSD of R. macrocnemis do not support the Rensch's rule and its inverse.*

KEY WORDS: *Sexual size dimorphism, Rana macrocnemis, Bivariate and multivariate methods, Body size, Body shape.*

INTRODUCTION

Sexual dimorphism describes existence of phenotypic differences between males and females, and also involves a series of different traits such as coloration, size, shape, behavior and etc… within species (Lowe & Hero

2012, Rastegar-Pouyani et al. 2013). Study of sexual dimorphism may be lead to ecological, physiological and evolutional insights within a population that can be measured (Morrison et al. 2004, Campbell & Echternacht 2003). Generally, three patterns have been proposed for sexual size dimorphism in amphibians: Female-biased, i.e. females larger than males; male-biased, i.e. males larger than females and unbiased i.e. males equal to females (Zhang et al. 2016). In most invertebrates and ectothermic vertebrates, females are larger than males, but in some species of amphibians, the males are larger than female in moderate mountain climates (Serra-Cobo et al. 2000, Zhang et al. 2016). Among anurans, analyses of intraspecific geographical variability in morphology have often revealed extensive variation in body size (Lee 1993; Mendelson 1998; Castellano et al. 2000). Geographical variation in morphology variation may be related to mixed factors that are not always well understood (Monnet & Cherry 2002, Lowe & Hero 2012). Several hypotheses have been proposed to explain the potential forces producing geographical variability in morphology including: predation pressure (Schneider et al. 1999), effects of environmental parameters on growth rates (Castellano et al. 2000), variation in the level and nature of sexual selection and sexual size dimorphism and drift and founder effects or other non-selective genetic factors (Demetrius 2000). The formulation of general ecological and evolutionary rules has a long and contentious history. One such rule, originally proposed by Bergmann (1847) and later redefined by Rensch (1936). Bergmann's rule predicts that body size increases with latitude, and also organism tend to be larger in colder climates (such as high-altitude populations) than in warmer climates. Several authors have hypothesized that ectotherms follow Bergmann's rule (Lindsey 1966, Atkinson & Sibly 1997), whereas others have contested the converse is true (Cowles 1945; Allee et al. 1949, Mousseau 1997). Rensch's rule proposed that SSD increases with increasing body size in species when males are larger than females, and decreases with body size in species where females are the larger sex (Liao et al. 2013, Feng et al. 2015).

The mountain brown frog is often found across the forest and sub-alpine belts from Asia Minor and the Caucasus Mountains to Iran (Afsar et al. 2015, Tarkhnishvili et al. 2001). In Iran, *Rana macrocnemis* distributed in the western and north western edges of the Iranian Plateaue (Safaei-Mahroo et al. 2015, Najibzadeh et al. 2017). In this study, we aimed to: 1) explore the sexual size dimorphism of the mountain brown frog, *Rana* *macrocnemis* in northwest and west of Iran 2) determine how do altitudinal differences affect body size of this species.

MATERIAL AND METHODS

We studied 59 adult specimens (females: 28; males: 31) of *Rana macrocnemis* found from 8 localities in mountainous regions of western Iran. (Table 1, Fig. 1).

Table 1. Details for the 8 sites occupied by *Rana macrocnemis* that we visited during this study.

No	Populations	Females	Males	Total Samples	Range of altitude (m)
1	Lorestan_Nurabad	5	2	7	1840-1850
2	Ardebil_Neor	10	0	10	2510-2534
3	Tabriz_Bostanabad	6	0	6	2219-2230
4	Kermanshah_Charkhalan	3	4	7	2117-2180
5	Urmia_Zive	1	11	12	2368-2381
6	Hamedan_Takht Nader	1	5	6	2798-2818
7	Arak_Ture	2	4	6	2049-2063
8	Esfahan_Chadegan	0	5	5	2126-2138

The specimens were caught during March and July 2016. The method capture was by hand and the sex of the each specimen identified according to external secondary sexual characters such as nuptial pad development. Thirteen morphometric characters for all specimens according to Crochet et al. (1995) were measured with Calipers to the nearest 0.1mm: snout-vent length (SVL), head width (HW), femur length (FL) and tibia length (TL), Digitus primus length (DP), Callus internus length (C.int), Snout-eye distance (DRO), Nostril-eye distance (from the nostril to the anterior edge of eye) (EN), Head length (Head length from mandibular articulation to tip of snout) (HL), internarial distance (ID), Eye length (LO), Fourth toe length from base of outer metatarsal tubercle (LF) and Webbing between third and fourth toe from base of outer metatarsal tubercle (W). The examined frogs were subsequently released at the habitat of their capture.

The obtained data were normally distributed according to the Kolmogorov-Smirnov test. The statistical significance for a hypothesis of sexual dimorphism in the studied frogs was tested using analysis of variances (ANOVA) to compare difference of means of each character between the sexes. As well, principal component analysis (PCA) to explore the sexual dimorphism in *R. macrocnemis* was run with significant characters.

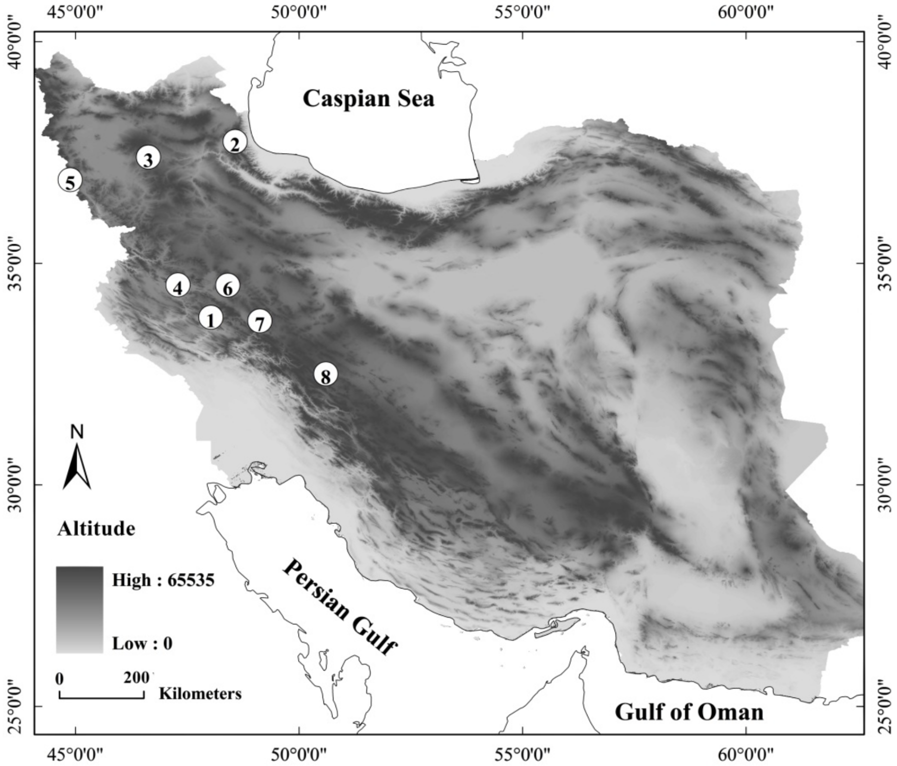

Figure 1, Map showing the localities of *Rana macrocnemis* (Boulenger, 1885) samples from Iran. For identification localities numbers, refer to table 1.

Most studies only used snout-vent length (SVL) as the measure of body size (Feng et al. 2015, Mcgarrity & Johnson 2009, Khonsue et al. 2001, Liao et al. 2013). As previously mentioned in introduction, body size is often vary geographically (Serra-Cobo et al. 2000, Zhang et al. 2016), and there are relationship between body size (SVL) and altitude (Feng et al. 2015).

Evaluation of potential interactive effects of body size (SVL) and altitudes were examined using the linear regression. Then, Pearson's correlation analysis was conducted for testing the relationship between body size and altitude.

We also used the log-scaled size of one sex regressing against the log-scaled size of the other sex to test for allometry vs. isometry (Rensch's rule). The reduced major axis (RMA) slope can be estimated as the ratio of male to female standard deviations (Sokal & Rohlf 1995).

When log10 (female size) was plotted on the x-axis and log10 (male size) plotted on the y-axis, a slope larger than 1.0 provided evidence for Rensch's rule

(Fairbairn 1997). SPSS software (IBM SPSS Statistics for Windows, Version 22.0., Armonk, NY: IBM Corp.) was used for running the statistical analyses.

RESULTS

According to the morphometric measurements taken from 59 adult specimens the snout-vent length of the individuals of *Rana macrocnemis* ranged between 26.68 - 64.33 mm (mean: 45.65). The ANOVA analysis of our material showed that five characters were sexually dimorphic (P< 0.05) (Table 2).

Table 2. The ANOVA-based intrasexual comparison of morphometric character states of *Rana macrocnemis*. Degrees of freedom = 1. Significant characters are emphasised in bold. Abbreviations: F = F value, Sig. = Significance as P-value.

Character	F	Sig.
SVL	129.658	0.001
I.D	1.339	0.252
HL	157.963	0.002
HW	148.987	0.004
D.R.O	0.658	0.421
EN	2.888	0.095
L.O	3.528	0.065
FL	151.937	0.001
C.INT	0.002	0.965
TL	150.948	0.001
D.P	3.611	0.062
L.F	2.748	0.103
W	2.561	0.115

Furthermore, we compared the sexual dimorphism of the mountain brown frog by PCA. Principal-component analysis at the level of female-male of *R. macrocnemis* showed that the first three principal components explained 97.56% of the total variation. The first PC (body size) had an eigenvalue of 38.87 and in itself explained 89.46% of the total variation. PC2 (Eigenvalue 2.43) and PC3 (Eigenvalue 1.08) accounted for 5.60%

and 2.50% of the total variance, respectively. Indeed, Most of the morphological variability (89.46%) between male-female within the data set of 59 the mountain brown frogs was caused by PCI (Table 3).

Table 3. Factor loading on first three Principal components elicited from a correlation matrix of four morphological characters of 59 *Rana macrocnemis* from northwest, and west of Iran. Morphological measurements: snout-vent length (SVL), head length (HL), head width (HW), femur length, (FL) and tibia length (TL).

Character	PC 1	PC 2	PC 3
SVL	0.697	-0.558	-0.424
HL	0.187	-0.196	0.255
HW	0.246	-0.199	0.705
FL	0.460	0.709	-0.264
TL	0.453	0.327	-0.432
Eigenvalue	38.87	2.43	1.08
% variance	89.46	5.60	2.50

Our results show that PC1 was correlated positively and most strongly with SVL (indicating that body length accounts for the dominant source of size variation) and PC2 was correlated positively and most strongly with FL (indicating that femur length accounts for the dominant source of shape variation) in *R. macrocnemis* (Fig. 2).

Moreover, correlation analysis to evaluate the relationship between SVL and altitude (females: $r = 0.65$, $P < 0.001$; males: $r = 0.42$, $P < 0.029$), show that altitude had significant relationship on all five morphometric characters. Regression plots drawn show a fairly marked positive relationship between increased altitude and character expression. Indeed, females and males tended to be larger in higher altitude (Fig 3).

The slope of the RMA regression of log10 (male size) on log10 (female size) across populations was not significantly different from 1 (slope = 0.757, $R^2 = 0.15$, $P = 0.44$) (Fig. 4).

DISCUSSION

Our data, ontained in the current study confirms the general patterns of body size variation in anurans (Monnet & Cherry 2002, Liao et al. 2013,

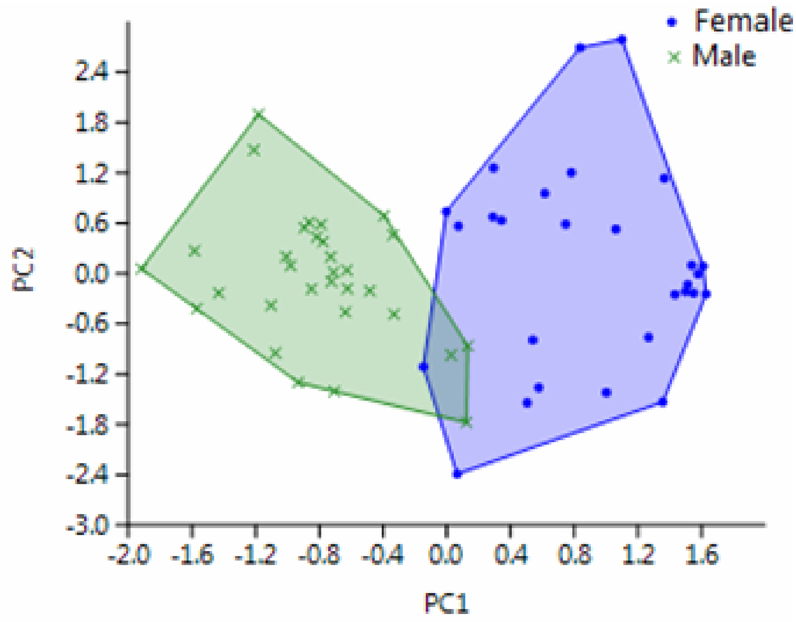

Figure 2, Scatter plot of specimens based on the first and the second principle components. Morphometric variation between sexes in *Rana macrocnemis*.

Shine 1979), showing that size of female characters is significantly larger than male characters. Furthermore, our analysis demonstrated that, sexual dimorphism extent not only in body size (SVL was significantly and positively correlated with sexual dimorphism), but also in body shape (FL) in *R. macrocnemis*, indicating that femur length accounts for the dominant source of shape variation in this species. According to previous studies, there are several reasons that could explain female- biased SSD in amphibians including *R. macrocnemis*:

First. Sexual selection is generally thought to favor larger males (male-biased SSD), where males strongly compete for mates (Székely et al. 2004). Then, male competition is the major factor in male body size (Shine 1979). In most anurans males did not defend territories, did not offer resources to females, did not care for offspring, and all males, small or large, were able to fertilize all eggs of any female (Vargas-Salinas 2006). Then, intersexual selection is not expected to favor the reproductive success of larger males (Vargas-Salinas 2006). It seems to that, large body

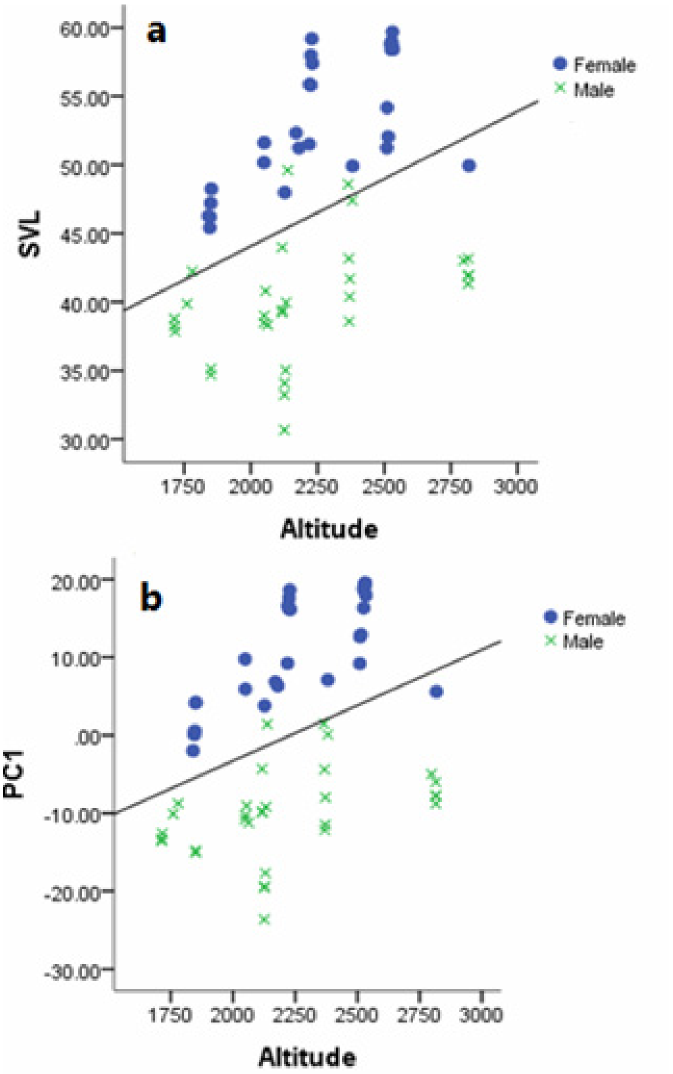

Figure 3, Linear regression of snout vent length (SVL; a) and the first principle component (PC1; b) on altitude for *Rana macrocnemis*.

size in males may not effect to success ampelxus for males in the cases, but this possibility needs to be studied in this species.

Second, Age differences (Young 2005, Liao et al. 2011, Monnet & Cherry 2002) and survival (Vargas-Salinas 2006) between males and females: Females are larger than males in frogs because there are high levels of mortality in males (Meshaka 2001). Wells (2001) argues that

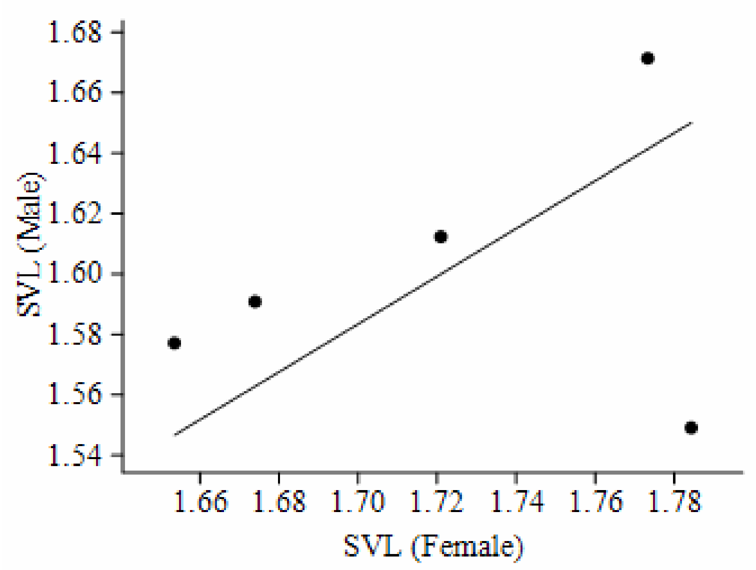

Figure 4, RMA regression of $\log_{10}$ (male size) against log10 (female size) across populations of *Rana macrocnemis* [Slope: 0.757, t: 0.84, R^2: 0.15, P: 0.44]. Each dot represents a single population based on the mean snout vent length (SVL) of females and males (n = 5).

acoustic competition during breeding season in frogs might explain lower local survival in males. Indeed, lower survival in males means that females have more time to grow than males (Vargas-Salinas 2006). Although for *R. macrocnemis* supplemental study are necessary for support all these points that mentioned above.

Furthermore, body size in the mountain brown frog may be influenced by foraging completion (Shine 1989), altitude (Ashton & Feldman 2003), local climate (Zhang et al. 2016), latitude and longitude (Schäuble 2004), and etc.

Among anurans, analyses of intraspecific geographical variability in morphology have often revealed extensive variation in body size (Lee 1993; Mendelson 1998; Castellano et al. 2000). Bergmann's rule predicts that body size increases with latitude, and also organism tend to be larger in colder climates than in warmer climates. Altitude can have important environmental and climatic consequences, and is often used as a general environmental index in much the same way as latitude (Schäuble 2004). Ashton and Feldman (2003) found strong support for Bergmann's rule in some nonavian reptiles (increasing in size with increasing elevation or decreasing temperature).

Also our results show that altitude had significant relationship on body size and supported the Bergmann's rule. Females and males tended to be larger in higher altitude.

Our statistical analysis demonstrated that, females are bigger than males, in other words SSD on the mountain brown frog relationships were inconsistent with Rensch's rule and the inverse of it (slope = 0.757, R^2= 0.15, P = 0.44). In many examples, allometry in male-biased SSD follows Rensch's rule at the interspecific and intraspecific level (Fairbairn 1997, Smith & Cheverud 2002, Stephens & Wiens 2009, Fairbairn et al. 2007). However, the trend is questionable in species with female-biased SSD (Webb & Freckleton 2007). In anurans, sexual size dimorphism is generally female-biased (Arak 1988). Rensch's rule is nearly universal among taxa with male-biased SSD (Liao et al. 2013).

Acknowledgments. We would like to thank the Iranian Department of Environment for sampling authorization and support during fieldwork, especially West Azerbaijan, East Azerbaijan and Kohgiluyeh and Boyer-Ahmad Department of Environment. We would also like to thank the following persons contributing greatly in different parts of this study during field work: Mr. Amiri, Ghaedrahmati, Fatahi, Farahmand, Hosseinpour and Azizi.

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