



## Sexual dimorphism in two species of hynobiid salamanders (*Hynobius leechii* and *Salamandrella keyserlingii*)

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Sexual dimorphism is a widespread phenomenon throughout the animal kingdom and a key topic in evolutionary biology. In this study, we quantified patterns of sexual dimorphism in two hynobiid salamanders (*Hynobius leechii* and *Salamandrella keyserlingii*) from Chinese populations. Sexual size dimorphism did not occur in either species, despite differences in body shape traits. Likely related to fecundity selection, females have relatively longer trunks in both species. Female *S. keyserlingii* have larger heads likely due to reproductive investment and ecological selection, whereas larger forelimb and hindlimb width in male *H. leechii* may be related to reproductive behaviour.

**Key words:** Asian salamander, Hynobiidae, morphology, sexual size dimorphism

### INTRODUCTION

Sexual dimorphism involves phenotypic differences between males and females within a given species, and is a widespread phenomenon throughout the animal kingdom believed to be the result of complex selective forces (Andersson, 1994; Fairbairn, 1997; Cox et al., 2007; Kupfer, 2007). Fecundity selection promotes morphological features which improve reproductive output such as higher abdominal volume of females (Hedrick & Temeles, 1989; Griffith, 1990; Jockusch, 1997). Sexual selection favours morphological traits which allow an individual to gain more or better mating partners (Darwin, 1871; Andersson, 1994). Ecological selection favours morphological characteristics which maximise survival and growth (Slatkin, 1984; Shine, 1989; Fontenot & Seigel, 2008).

In amphibians, sexual dimorphism occurs in all major groups (Kupfer, 2007), and for example Shine (1979) reported that females are larger than males in about 90% of 589 anurans and 61% of 79 salamanders. The evolution of sexual dimorphism in salamanders has attracted considerable interest (Halliday & Arano, 1991; Kalezić et al., 1992; Andersson, 1994; Malmgren & Thollesson, 1999; Serra-Cobo et al., 2000; Ivanović et al., 2008). Studies on sexual dimorphism in urodeles found sex-specific throat colouration (Hasumi, 2001), dentition (Ehmcke & Clemen, 2000; Greven et al., 2004; Clemen & Greven, 2009), volume of the vomeronasal organ (Woodley, 2007), skull geometry (Ivanović & Kalezić, 2012), dorsal body pigmentation (Pokhrel et al., 2013), number of tail vertebrae (Ficetola et al., 2013; Colleoni et

al., 2014), visceral organ mass and hematology (Finkler, 2013) and body size and shape (Romano et al., 2009; Seglie et al., 2010; Bakkegard & Rhea, 2012; Alcorn et al., 2013; Colleoni et al., 2014; Reinhard et al., 2015; Amat et al., 2015).

The family Hynobiidae, consisting of the subfamilies Hynobiinae and Onychodactylinae (Dubois & Raffaëlli, 2012), has 66 recognised species (Frost, 2016). China represents its main distribution, and is inhabited by 28 species belonging to eight genera. In the present study, we describe the sexual dimorphism of two hynobiid salamanders from Chinese populations, and discuss the results in the light of existing evolutionary concepts.

### MATERIALS AND METHODS

#### Study specimens and samples

Individuals from two species of hynobiid salamanders (*Hynobius leechii* Boulenger, 1887 and *Salamandrella keyserlingii* Dybowski, 1870) were collected from indigenous Chinese populations and closely examined. *Hynobius leechii* is distributed in Korea and northeastern China (Liaoning, Jilin, and Heilongjiang Provinces); *S. keyserlingii* has a wide distribution range, including China, Japan, Korea, Democratic People's Republic of Korea, Republic of Mongolia and the Russian Federation. Both salamanders are largely terrestrial and in areas of sympatry breed in shared lentic pools. A total of 81 adults preserved in 10% formalin were examined (*H. leechii*: 19 females, 22 males; *S. keyserlingii*: 14 females, 26 males, Appendix 1). Specimens were sexed by inspection of the

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**Table 1.** Definitions of the morphological character set and abbreviations.

Abbreviations	Variable definition
SVL	snout-vent length, from the tip of snout to the posterior margin of the cloaca
HL	head length, from the tip of the snout to the gular fold
HW	head width, width of the head at its widest point
HH	head height, height of the head at its highest point
TL	tail length, from the posterior margin of the cloaca to the tip of the tail
TH	tail height, height of the tail at its highest point
FLL	forelimb length, from the base of the forelimb to the tip of the longest finger
HLL	hindlimb length, from the base of the hindlimb to the tip of the longest toe
AGS	distance between axilla and groin, the space between the posterior base of the forelimb and the anterior base of the hindlimb
FLW	forelimb width, the maximum width of the forelimb
HLW	hindlimb width, the maximum width of the hindlimb

gonads through a small ventro-lateral incision. Vouchers are deposited at the Henan University of Science and Technology Museum (HNUSTM) and the Museum of Chengdu Institute of Biology (CIB) at the Chinese Academy of Sciences (CAS). To quantify intersexual morphological differences, eleven morphometric characters were analysed (Table 1). All measurements were acquired to the nearest 0.01 mm by Jianli Xiong using digital callipers.

### Statistical analysis

All characters measured were tested for normality (Kolmogorov-Smirnov test) before analyses. Sexual dimorphism in SVL was analysed with *t*-tests for independent samples, and SVL was then excluded from subsequent analyses due to a high collinearity with other considered variables (e.g. Romano et al., 2009). To minimise deviations from normality and distortion caused by allometric relationships, all data were log<sub>10</sub>-transformed, and tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's

**Table 2.** Descriptive statistics of original morphometric characters (mm) in females and males of *Hynobius leechii* and *Salamandrella keyserlingii*.

Variables	<i>Hynobius leechii</i>				<i>Salamandrella keyserlingii</i>			
	Female(n=19)		Male(n=22)		Female(n=14)		Male(n=26)	
	mean±S.D	range	mean±S.D	range	mean±S.D	Range	mean±S.D	range
Snout-vent length	64.66±5.81	54.76-73.49	62.61±3.68	55.87-68.41	59.63±3.26	53.78-65.41	59.13±4.06	52.20-65.77
Head length	14.46±1.09	11.19-15.61	14.77±1.13	13.12-17.16	14.04±0.80	12.56-15.17	14.77±1.38	12.97-19.66
Head width	10.61±1.14	8.7-13.0	10.85±1.17	9.40-13.40	10.04±0.68	8.88-11.58	11.68±1.59	9.93-16.47
Head height	6.69±1.16	4.60-8.59	6.78±0.87	5.73-8.86	5.61±0.43	4.71-6.08	6.54±1.39	5.36-11.22
Tail length	42.93±5.03	33.64-52.53	44.60±5.49	37.46-55.31	49.09±6.75	39.83-63.06	56.44±6.84	44.44-69.17
Tail height	5.43±0.93	3.84-7.04	6.01±1.21	3.96-8.02	6.84±0.74	6.03-8.88	8.35±0.99	6.58-11.85
Length of forelimb	14.06±1.64	11.46-16.19	12.09±1.59	9.09-15.25	13.76±1.29	12.02-16.60	15.14±0.98	12.45-17.70
Length of hindlimb	17.23±1.38	15.2-19.90	16.60±1.25	14.90-19.20	13.30±1.06	10.29-14.64	15.21±1.06	12.41-16.58
Space between axilla and groin	30.79±2.83	25.83-37.95	28.27±1.71	24.48-31.29	30.78±4.08	25.94-39.34	28.92±3.15	23.95-38.38
Forelimb width	2.86±0.58	1.60-3.74	3.58±0.64	2.42-4.96	3.11±0.34	2.54-3.84	3.54±0.35	2.93-4.18
Hindlimb width	4.18±0.47	3.44-5.07	4.68±0.66	3.34-6.34	4.15±0.49	3.09-4.98	4.46±0.57	3.37-5.55

test). Since the variances were homogeneous, a principal component analysis (PCA) was performed. The first principal component (PC1) is generally interpreted as an axis of body size variation (Reyment et al., 1984; Bookstein, 1985), and relative shape differences are expressed in subsequent axes (Schäuble, 2004). Next, a univariate analysis of covariance (ANCOVA) was conducted with sex as a factor and the PC1 score as a covariate for each morphological variable independently (following Guillaumet et al., 2005; Romano et al., 2009). This allowed for the determination of the variables that differed between males and females. Data analysis was conducted using SPSS version 17.0 (SPSS Inc., Chicago). Values were presented as mean±standard deviation, and the significance level was set at  $\alpha=0.05$ .

## RESULTS

Mean SVL of females was higher than SVL for males in both species, at however non-significant differences (*H. leechii*:  $t=1.326$ ,  $df=29.580$ ,  $p>0.05$ ; *S. keyserlingii*:  $t=0.389$ ,  $df=38$ ,  $p>0.05$ ); measurements of the other traits are listed in Table 2. In both species, three principal components were extracted (Table 3). PC1 described the largest proportion of overall variation (50.96% in *H. leechii* and 43.23% in *S. keyserlingii*), and all original variables loaded positively onto this component. Therefore, the individual scores on PC1 were used to estimate the differences in overall body size. Other axes explained 49.04% and 56.77% of the variances in *H. leechii* and *S. keyserlingii*, respectively, and the factor scores for these components were retained as being representative of body shape.

Differences in shape between the sexes were further revealed by the applied ANCOVA (Table 3). In both species, the interaction term of sex and PC1 was not significant and removed from the model for all variables. In *H. leechii*, FLL and AGS was significantly higher for females, and FLW and HLW were higher in males. In *S. keyserlingii*,

HL and AGS were significantly larger in females than in males (see Table 1 for trait abbreviations).

## DISCUSSION

For both hynobiid salamanders, we revealed sexual size dimorphism in body shape without marked differences in body size. Selection can drive the evolution of differences in a wide variety of characteristics, and the differential sexual shape dimorphism among the studied species is likely caused by different selective forces (Alcorn et al., 2013; Colleoni et al., 2014). Differences in trunk length were found for both species, in addition to head shape dimorphism in *S. keyserlingii* and limb shape dimorphism in *H. leechii*.

A female-biased dimorphism in trunk length has been previously reported in other salamanders (e.g., *Plethodon kentucki*, Marvin, 2009; *S. salamandra*, Labus et al., 2013; *Salamandrina perspicillata*, Romano et al., 2009; *Neurergus microspilotus*, Rastegar-Pouyani, 2013), and is likely linked to fecundity selection (Hedrick & Temeles, 1989; Jockusch, 1997). Trunk length is directly correlated with the length of the pleuroperitoneal cavity which provides space for eggs or offspring; a longer trunk can also increase the ability for fat storage to enhance reproductive success (Shine, 1979; Kalezic et al., 1992; Marvin, 2009).

The observed female-biased sexual dimorphism in head size for *S. keyserlingii* may be due to a combination of fecundity and ecological selection (Zhang et al., 2014). Sexual selection often favours males with larger heads during male-male competition (e.g., Fauth & Resetarits, 1999; Bovero et al., 2003; Marvin, 2009). However, a larger head can also facilitate an increased food intake advantageous for egg production (Selander, 1972; Shine, 1979, 1989; Malmgren & Thollensen, 1999; Romano et al., 2012). Ecological selection also favours sexual differences in diet (Cooper & Vitt, 1989; Fauth & Resetarits, 1999; Serra-Cobo et al., 2000; Bovero et al., 2003; Fontenot

**Table 3.** Factor loadings for the principal components (PC; eigenvectors), eigenvalues and proportion of total variance described by the first three components obtained from PCA on a correlation matrix, and results of ANCOVA with PC1 scores as covariate tests for differences in morphological variables. All variables are log-transformed. Significant values in *italic*.

Variables	<i>Hynobius leechii</i>					<i>Salamandrella keyserlingii</i>				
	PC1	PC2	PC3	F	<i>p</i> -value	PC1	PC2	PC3	F	<i>p</i> -value
Head length	0.646	-0.025	0.486	0.129	0.722	0.670	-0.008	0.545	5.609	<i>0.023</i>
Head width	0.918	0.033	-0.136	0.205	0.654	0.757	0.450	0.178	0.025	0.875
Head height	0.874	0.058	-0.131	0.918	0.344	0.627	0.607	0.270	0.190	0.666
Tail length	0.898	-0.009	-0.218	0.008	0.928	0.628	0.014	-0.632	0.145	0.705
Tail height	0.849	-0.261	0.108	1.132	0.294	0.787	-0.125	0.034	2.351	0.134
Length of forelimb	0.312	0.804	-0.079	23.163	<i>0.000</i>	0.751	-0.235	-0.405	0.001	0.980
Length of hindlimb	0.323	0.396	0.771	3.969	0.054	0.745	0.100	-0.359	2.694	0.109
Space between axilla and groin	0.470	0.733	-0.298	26.489	<i>0.000</i>	0.092	0.840	0.041	9.282	<i>0.004</i>
Forelimb width	0.746	-0.372	-0.070	24.020	<i>0.000</i>	0.736	-0.388	0.085	0.014	0.907
Hindlimb width	0.752	-0.372	-0.003	9.900	<i>0.003</i>	0.476	-0.663	0.433	0.469	0.498
Eigenvalue	5.096	1.691	1.025			4.323	1.948	1.292		
Proportion	50.961	67.871	78.126			43.226	62.706	75.626		

& Seigel, 2008; Seglie et al., 2010), and a female-biased sexual dimorphism in head size has previously been observed in other salamander species (*S. perspicillata*, Romano et al., 2009; *Tylostotriton verrucosus*, Seglie et al., 2010; *N. microspilotus*, Rastegar-Pouyani et al., 2013).

Sexual dimorphism in limb size is widespread in salamanders, in most cases involving longer and more robust limbs for males compared to females (*E. platycephalus*, Bovero et al., 2003; *Triturus cristatus* and *Lissotriton vulgaris*, Malmgren & Tholleson, 1999; *E. asper*, Seglie et al., 2010; *S. salamandra*, Labus et al., 2013). The mating sequence of *H. leechii* involves the use of forelimbs as well as hindlimbs by males, and more robust limbs likely enhance the process of insemination (Park et al., 1996; Park and Park, 2000). Reinhard et al. (2015) revealed that enlarged male forelimbs can be attributed to courtship behaviour, whereas in other cases its functional significance is unknown (Romano et al., 2009; Rastegar-Pouyani et al., 2013).

Geographic differences in sexual dimorphism can be caused by differential ecological constraints on sexual selection or competition (Selander, 1966; Dobson and Wigginton, 1996; Frafjord and Stevy, 1998). Park et al. (1996) found sexual dimorphism in SVL for *H. leechii* from South Korea, however without noticeable differences in head width and tail depth. Hasumi (2010) reported that female *S. keyserlingii* from northern Japan possessed wider heads, and males possessed longer tails. The results reported here are partially consistent with those of Park et al. (1996) and differ from Hasumi (2010), providing evidence for geographical variation in sexual dimorphism in both studied species (see also Serra-Cobo et al., 2000; Angelini et al., 2015).

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## APPENDIX

### Appendix 1. Specimens examined.

#### *Hynobius leechii* N=41

HNUSTM1005003, HNUSTM1005046 - HNUSTM1005050, HNUSTM1005052 - HNUSTM1005053, HNUSTM1005062 - HNUSTM1005069, HNUSTM1005081 - HNUSTM1005083, HNUSTM1005086 - HNUSTM1005093, HNUSTM1005101 - HNUSTM1005102, HNUSTM1005104 - HNUSTM1005111, CIB-XM2072 - CIB-XM2073, CIB-XM2098, CIB-XM2136, Lushuihe, Fusong County, Jilin Province, China in May 2010.

#### *Salamandrella keyserlingii* N=40

CIB18372 – CIB18382, CIB18384 - CIB18396, CIB18398 - CIB18407, CIB18409 - CIB18414, Baihe, Antu County, Jilin Province, China in April 1994.