



Body size variation of odorous frogs (*Odorrana grahami*) across altitudinal gradients

Shu Ting Li^{1,2}, Xue Wu^{1,2}, Da Yong Li^{1,2}, Shang Ling Lou^{1,2}, Zhi Ping Mi^{1,2} & Wen Bo Liao^{1,2}

¹Key Laboratory of Southwest China, Wildlife Resources Conservation (Ministry of Education), China West Normal University, Nanchong, 637009, P.R. China,

²Institute of Rare Animals and Plants, China West Normal University, Nanchong, 637009, P.R. China

We used skeletochronology to determine the ages of 249 (93 females, 108 males and 48 juveniles) odorous frogs (*Odorrana grahami*) from four locations covering an altitudinal span of 1030–1860 m in Sichuan Province, western China. We found distinct lines of arrested growth (LAGs) from excised toe bone for 242 individuals, and each LAG was assumed to represent one year of age. In disagreement with Bergmann's rule, body size of four *O. grahami* populations did not change clinally along the 830 m altitudinal gradient. Average adult SVL and age differed significantly among populations in females, but not in males. For both sexes, age did not predict body size. When removing the effects of age, we found significant inter-population differences in body size only for males. Post-metamorphic growth rates in males were highest from the lowest altitude, whereas growth rates of females were highest at high altitudes. Our findings suggest that, apart from age, ecological factors such as trophic resources or predation further shape body size differences between populations and sexes.

Key words: age, altitudinal gradients, body size, odorous frog, skeletochronology

INTRODUCTION

Patterns of geographical variation in body size are an important topic in evolutionary ecology. One of the most well-known generalizations for body size is Bergmann's rule, which predicts that, within species or among closely related species, body size increases with decreasing ambient temperature (Bergmann, 1847; Rensch, 1938; Mayr, 1956). This can be explained by the fact that larger surface-to-volume ratios facilitate heat loss, whereas small ratios facilitate heat retention (Bergmann, 1847). This rule attracted considerable attention over the last 160 years (Mayr, 1956; Blackburn et al., 1999; Gaston et al., 2008; Watt et al., 2010; Meiri, 2011), with the general applicability remaining vigorously debated. For ectotherms, several studies have both confirmed Bergmann's rule (Ashton, 2002; Olalla-Tárraga & Rodríguez, 2007; Ficetola et al., 2010) and provided evidence against it (Mousseau, 1997; Partridge & Coyne, 1997; Walters & Hassall, 2006).

Evidence for the prevalence of Bergmann clines in amphibians is still controversial, with a trend that most anurans follow Bergmann's rule with regard to latitude and altitude whereas urodeles tend to follow a reverse pattern (Lindsey, 1966; Ashton, 2002; Olalla-Tárraga & Rodríguez, 2007; Pincheira-Donoso et al., 2008; Ficetola et al., 2010). Adams and Church (2008) found that three *Plethodon* species displayed a significant negative correlation between body size and temperature

consistent with Bergmann's rule, whereas 37 out of 40 other species did not exhibit a pattern consistent with this prediction.

Mean air temperature related to latitude and altitude affect the geographical variation in body size of amphibians because they influence age and growth rate (Miaud et al., 1999; Lu et al., 2006; Ma et al., 2009a; Cvetković et al., 2009; Iturra-Cid et al., 2010). Most studies suggest that lower mean temperatures at higher altitude results in slower growth and later age at sexual maturity, and therefore larger body size (Lu et al., 2006; Liao and Lu 2010a, b; Liao et al., 2010), although this is not universal (e.g., Ma et al. 2009b). The odorous frog (*Odorrana grahami*) is a species inhabiting montane regions (1150–3200 m altitude) in Sichuan, Yunnan and Guizhou (southwestern China) and the Hoang Lien Son National Park in northern Vietnam (Fei et al., 2005; Frost, 2010). Egg-laying occurs in June during explosive breeding events (following Wells, 1977). Here our goal was to describe body size and age across an 830 m altitudinal gradient. We were particularly interested in testing whether the observed pattern of body size variation conforms to Bergmann's rule.

MATERIALS AND METHODS

Study area and sampling methods

The study area was located in Ningnan County in western China. The annual average precipitation in the county

Correspondence: Wen Bo Liao (Liaobo_0_0@126.com)

Table 1. Resources, locations, mean annual temperatures and altitudes of *Odorrana grahami* sampled from four localities in western China. Mean annual temperature data results from Ningnan County Weather Office.

Sites	Latitude (north)	Longitude (east)	Altitude (m)	Mean annual temperature (°C)	No. of females	No. of males	No. of juveniles
Wuyi	27°11.24'	102°38.50'	1030	19.1	28	28	1
Tianwan	27°10.13'	102°36.40'	1590	17.2	15	28	4
Xiyao	26°52.73'	102°49.73'	1630	16.7	23	17	4
Masang	27°15.49'	102°37.82'	1860	15.7	27	35	39

is 960 mm, and mean annual temperatures show a systematic decline with increasing altitude (Table 1; data obtained from the Ningnan County Weather Office).

We caught 249 frogs by hand during several nights between June and August in 2010 and 2011. Specimens were collected at four sites (towns) with altitudes ranging from 1030 to 1860 m (Table 1). We sexed each individual by direct observation of the secondary sexual characteristics (i.e. the vocal sacs in adult males and the ova in adult females) and measured body size (the snout-vent length: SVL) using a vernier calliper with an accuracy of 0.1 mm. We removed the first two phalanges of the longest toe and stored them in 10% neutral buffered formalin for skeletochronology.

Age determination

We estimated individual age by skeletochronological methods (see Castanet & Smirina, 1990; Li et al., 2010). The skin and muscle tissues of each digit were removed and the remaining bones were decalcified in 5% nitric acid for 48 hours. We then washed the decalcified digits in running tap water for 24 hours and stained them for 180 min in Harris's haematoxylin. Subsequently, we dehydrated the stained bones through successive ethanol stages for 1 hour. Cross-sections (13 µm in thickness) of the phalanx with the smallest medullar cavity and the thickest cortical bone were selected and mounted on glass slides. Mid-diaphysis sections were chosen for observation of the number of lines of arrested growth (LAG) under a microscope, and we photographed the best sections using a Motic BA300 digital camera mounted on a Moticam2006 light microscope at ×400 magnification. LAGs were assessed by two people, assuming that each LAG corresponds to an annual arrest of individual growth during overwintering (November–February). All fingers were collected from 16 June to 10 August, i.e. after five months of emergence from hibernation, and we therefore added 0.5 years to the age determined through LAGs. False and double lines were defined following the suggestion of Sinsch et al. (2007). Endosteal resorption of long bones starts from the inner surface of the bone,

enlarging the marrow cavities and eroding a portion of LAGs when frogs have completed their hibernation (Rozenblut & Ogielska, 2005). We compared the diameter of the smallest juvenile cross-section (one year old without resorption) with the diameter of the resorption line of adults to confirm endosteal resorption following Castanet and Smirina (1990).

Growth estimate

Growth was estimated according to von Bertalanffy's (1957) model regularly used for amphibians (e.g., Lu et al., 2006; Ma et al., 2009a; Liao et al., 2011). We adopted the equation $S_t = S_{\max} (1 - e^{-kt+b})$, where S_t is body size at age t , S_{\max} is the estimated asymptotic maximum size, k is a growth coefficient and b is a constant. The growth rate can then be calculated as $R = dS/dt = k(S_{\max} - S_t)$.

Statistical analysis

All statistical analyses were performed using SPSS v.15.0. We used Kolmogorov-Smirnov tests to test whether variables within each group were normally distributed. We applied Student's t -tests to test for differences in body size and age between males and females within each population when applicable. We used one-way ANOVA to test differences in age across the populations. For each sex, differences in average body size among populations were tested using general linear models (GLMs), treating SVL as a dependent variable and population as factor. To investigate whether population differences in mean SVL could be explained by age differences, age was included as a covariate in a separate model. The correlation between body size and age was evaluated using linear regression. All values are shown as mean±SD, and statistical tests were two-tailed.

RESULTS

Of the 249 individuals (93 females, 108 males and 48 juveniles), a series of narrow concentric hematoxylinophilic lines separated by wider layers of paler background with sparsely distributed osteocytes

Table 2. Differences in mean body size (mm) and age (yrs) between males and females within each *Odorrana grahami* population at different altitudes using Student's t - tests. Values in descending order are mean±SD.

Altitude (m)	Males	Females	t	p	Males	Females	t	p
1030	70.8±4.2	85.0± 3.2	5.42	<0.001	3.5±1.1	3.6±0.9	0.40	0.69
1590	69.2±9.5	67.8±15.4	0.90	0.37	3.3±1.1	3.6±1.4	0.36	0.72
1630	69.4±6.7	82.8±15.4	3.43	0.001	3.3±1.3	3.6±1.1	0.68	0.50
1860	67.2±5.8	69.5±8.7	1.41	0.17	3.1±1.0	2.8±1.0	0.21	0.23

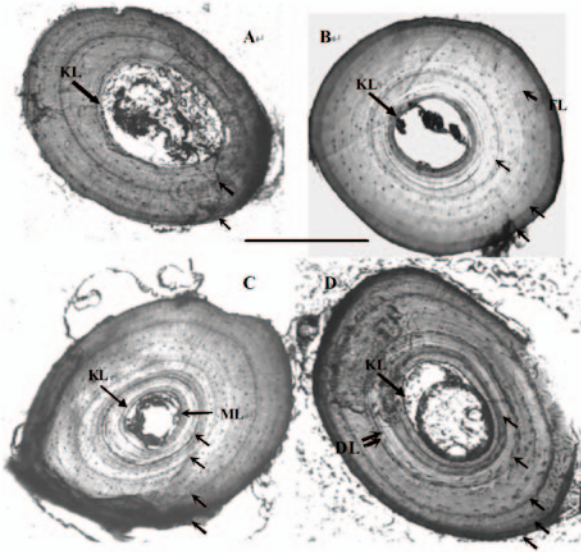


Fig. 1. Four selected examples of hematoxylin-stained cross-sections of the phalangeal bone in *Odorrana grahami*. A) a 1.5-yr old male; B) a 2.5-yr old female; C) a 3.5-yr old female; D) a 4.5-yr old male) Arrows indicate the lines of arrested growth (LAG). KL, ML, FL and DL represent Resorption Line, Metamorphosis Line, False Line and Double Line. Scale bar: 200 µm.

in cross-sections of phalanges were observed in 242 individuals (Fig. 1). False and double lines were rarely observed. Based on the comparison between the diameters, we did not find complete resorption of the innermost LAG for any individual. Incomplete resorption, observed in 6.5% (6 of 93) of females and in 8.3% (9 of 108) of males, did not affect age determination.

Average age did not differ between males and females within each population (Table 2; Student's *t*-test: $p > 0.05$). Adult average age was significantly different among the four populations in females (one-way ANOVA: $F_{3,89} = 3.33, p = 0.02$), but not in males ($F_{3,103} = 0.69, p = 0.60$). Differences between pairs of populations based on post hoc multiple comparisons (Fisher's LSD) are shown in Table 3. When breeding for the first time, frogs were 2.5 years old for both sexes in all the four populations. The highest attained age was 5.5 years for males and females in all populations with the exception of 7.5 years for males at 1590 m altitude.

Average adult SVL differed between males and females for populations at 1030 m and 1630 m altitude, but not between 1590 m and 1860 m (Table 2). Average SVL differed significantly among the four populations in females ($F_{3,90} = 10.51, p < 0.001$), but was unrelated with altitude. For males, SVL did not differ among populations ($F_{3,105} = 1.53, p = 0.21$). Multiple comparisons indicated that body size was similar between any two populations

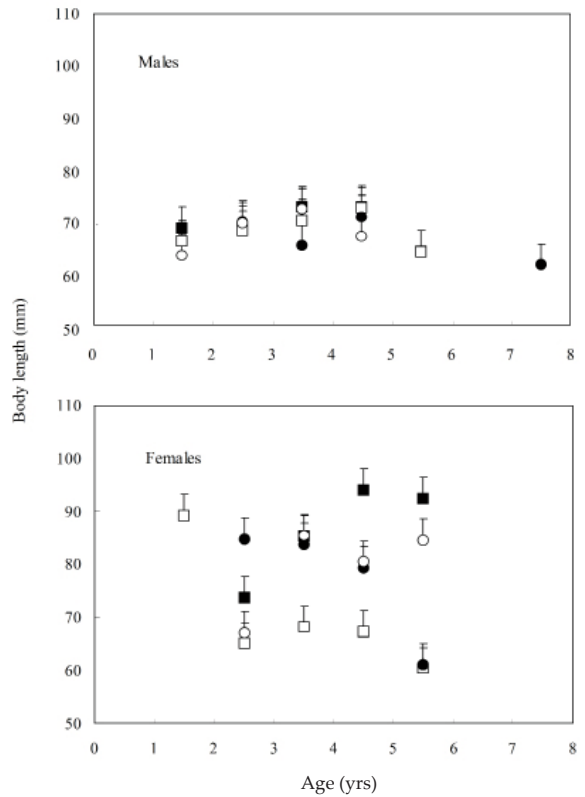


Fig. 2. Body length by age of *Odorrana grahami* from four study sites. The 1030 m, 1590 m, 1630 m and 1830 m sites are represented by close squares, open squares, close circles and open circles, respectively. Vertical bars are standard errors. Sample size is given beside the average.

in males (post hoc test, all $p > 0.19$). For females, body size at the 1030 m and 1630 m sites were similar ($p = 0.55$) and significantly larger than at 1590 m and 1830 m ($p < 0.001$). After removing the influences of age, differences in SVL still remained in females (ANCOVA: $F_{3,105} = 8.51, p < 0.001$) but not in males ($F_{3,89} = 1.45, p = 0.23$). Age did not affect SVL among the populations in both sexes (males, $F_{3,105} = 1.56, p = 0.22$; females, $F_{3,89} = 2.35, p = 0.13$). Within each age class, males did not differ in size among populations (Fig. 2; 2.5 year class, $F_{3,51} = 2.97, p = 0.06$; 3.5 year class, $F_{3,18} = 0.62, p = 0.61$; 4.5 year class, $F_{3,15} = 0.11, p = 0.95$; 5.5 year class, $F_{2,4} = 0.35, p = 0.73$), but females were different (2.5 year class, $F_{3,37} = 5.68, p = 0.003$; 3.5 year class, $F_{3,15} = 0.90, p = 0.46$; 4.5 year class, $F_{3,16} = 4.80, p = 0.02$; 5.5 year class, $F_{1,3} = 14.20, p = 0.03$).

For both sexes, we found no correlation between adult body size and age for the 1030 m and the 1860 m sites, and a positive correlation for the 1590 m and the 1630 m sites (Table 4). The von Bertalanffy model demonstrated that females were generally larger than

Table 3. Age differences between pairs of populations based on post hoc multiple comparisons (Fisher's LSD).

Altitude (m)	1590		1630		1860	
	Males	Females	Males	Females	Males	Females
1030	0.372	0.977	0.519	0.850	0.156	0.007
1590			0.890	0.894	0.016	0.673
1630					0.578	0.023

Table 4. Linear regression analyses of body size (mm) on age (years) within each *Odorrana grahami* population sampled from four different altitudes.

Altitude (m)	Males					Females				
	Regression equation	F	df	r ²	p	Regression equation	F	df	r ²	p
1030	Y=65.0+1.63X	5.6	27	0.18	0.026	Y=55.4+8.13X	12.7	27	0.53	0.001
1590	Y=69.0+0.08X	0.02	26	0.00	0.960	Y=81.6.5-3.79X	1.8	14	0.12	0.205
1630	Y=73.2-1.16X	1.9	16	0.07	0.292	Y=86.4-1.01X	0.1	22	0.01	0.739
1860	Y=53.2+3.71X	14.5	32	0.32	0.001	Y=50.7+6.71X	12.7	23	0.37	0.002

males throughout the life cycle for all populations. Males had a higher growth rate than females within each population except at 1590 m altitude (Table 5).

DISCUSSION

We found that skeletochronology allowed us to assess individual ages of the subtropical frog *O. grahami*. Food availability in winter may affect the formation of lines of arrested growth (Morrison et al., 2004). For *O. grahami*, the hibernation begins in early November and lasts until later February (Fei et al., 2005). The clear LAG formation likely resulted from the fact that physiological functions of the frogs were depressed by low environmental temperature during the hibernation period.

For most anurans, females are larger than males (Monnet & Cherry, 2002; Kupfer, 2007; Liao et al., 2010; Liao & Lu, 2011a, b; Liu et al., 2011; Mao et al., 2012; Liu et al., 2012; Lou et al., 2012). Sexual size dimorphism may be a consequence of differences in age and/or growth rate (Wells, 2007). For four *O. grahami* populations, age differences did not result in sexual size dimorphism. At the 1030 m and the 1630 m sites, females had significantly larger body size than males, suggesting that sexual size dimorphism results from the differential growth rates. At 1590 m and 1830 m altitude, a non-significant sexual size dimorphism was the result of similar growth rates for both sexes.

Patterns for geographical body size variation in amphibians remain controversial (Olalla-Tárraga & Rodríguez, 2007; Adams & Church, 2008), and both Bergmann's rule as well as the opposite of it have been reported (Acker et al., 1986; Cvetković et al., 2009; Ma et al., 2009b). In contrast with Bergmann's rule, we found no clinal variation of body size of *O. grahami* with altitude. A similar longevity observed in the four populations did not result in larger body size in higher altitudes for both sexes, inconsistent with evidence to support that indeterminate growth and increased longevity leads to larger body size in cold climates (Cvetković et al., 2009). Significant differences in body size among populations

were observed in females, but not in males, providing evidence that sexes may respond differently to climatic variation or maternal investment (Ficetola et al., 2010). Romano and Ficetola (2010) also found influences of genetic structure and local factors (i.e. type of oviposition site) on the ecogeographic variation of body size.

Body size of adult amphibians is determined by size at metamorphosis, growth rate and growth duration (Lu et al., 2006; Liao & Lu, 2011b). In most anuran species, large eggs often lead to large metamorphs and in turn large adults (Crump, 1984; Dziminski & Roberts, 2006). Anurans living in colder conditions often produce larger eggs, in order to allocate the limited resources to fewer individual offspring (Roff, 2002; Morrison et al., 2004). For *O. grahami*, data on altitudinal variation in egg size and size at metamorphosis are unavailable.

Average age in anurans tends to increase with altitude (Lu et al., 2006; Ma et al., 2009b; Liao & Lu, 2011b; Liao & Lu, 2012). For *O. grahami*, individuals from high altitudes were smaller than those at low altitudes, but did not differ in average age. Life-history theory predicts that delayed maturation allows devoting more energy to somatic growth (Kozłowski et al., 2004), and in anurans often contributes to average age and thus body size in colder environments (Miaud et al., 1999; Lu et al., 2006; Ma et al., 2009a). However, this does not seem to be the case for *O. grahami*, for which the proportion of individuals at minimal maturation age was similar at all sites.

In males, growth rates at 1030 m were highest, a fact which should be linked to higher temperature at lower altitudes, as observed elsewhere (Duellman & Trueb, 1986; Hemelaar, 1988; Miaud et al., 1999; Eaton et al., 2005). However, body size of females at given ages differed between populations. The populations at 1590 m and 1860 m altitude are subject to occasional harvesting for food, and the fact that harvesting is likely selective towards large individuals might have influence on both the age structure as well as the selective pressures imposed upon these populations.

Table 5. Growth parameters of von Bertalanffy's model ($S_t = S_{\max} (1 - e^{-kt+b})$) for *Odorrana grahami* populations sampled from four different altitudes. Definitions of the parameters are given in the text.

Altitude (m)	Males				Females			
	S _{max}	k	b	Growth rate	S _{max}	k	b	Growth rate
1030	73.1	44.74	65.51	79.92	98.9	0.68	0.32	12.81
1590	69.9	1.39	-0.85	4.53	88.7	0.38	-0.43	6.97
1630	69.4	6.22	-9.64	41.85	69.4	6.22	-9.64	24.32
1860	72.0	2.61	1.84	30.47	72.8	3.45	24.0	29.49

Ecological factors such as trophic regimes and predation or competition may further explain differences in body size between populations. Indeed, environmental factors such as precipitation and humidity (Ashton, 2002) or food availability have been shown to affect geographic variation in body size (Miaud et al., 2001; Chown & Klok, 2003; Meiri et al., 2007). In our study, high-altitude populations are subjected to lower temperatures, and may further be exposed to factors such as reduced food availability, slower metabolic properties and lower competition and predator risk. That they were not characterized by larger body size might be due to shorter activity periods which reduce their net growth (Sinsch et al., 2010; Oromi et al., 2012).

ACKNOWLEDGEMENTS

We thank two anonymous reviewers and Dr. Robert Jehle for comments on an earlier draft of this manuscript. We thank the financial support received, including the National Natural Science Foundation of China (31101633), the Innovative Team Foundation of China West Normal University and the Foundation of Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), China West Normal University, P.R. China (XNYB01-3). We declare that all animals used in the study were treated humanely and ethically following all applicable institutional animal care guidelines in China.

REFERENCES

- Acker, P.M., Kruse, K.C. & Krehbiel, E.B. (1986). Aging *Bufo americanus* by skeletochronology. *Journal of Herpetology* 20, 570–574.
- Adams, D.C. & Church, J.O. (2008). Amphibians do not follow Bergmann's rule. *Evolution* 62, 413–420.
- Ashton, K.G. (2002). Do amphibians follow Bergmann's rule? *Canadian Journal of Zoology* 80, 708–716.
- Bergmann, C. (1847). Über die verhältnisse der warmeökonomie der thiere zu ihrer grosse. *Göttinger Studien* 1, 595–708.
- Blackburn, T.M., Gaston, K.J. & Loder, N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions* 5, 165–174.
- Castanet, J. & Smirina, E. (1990). Introduction to the skeletochronological method in amphibians and reptiles. *Annales des Sciences Naturelles Zoologie (Paris)* 11, 191–196.
- Chown, S.L. & Klok, C.J. (2003). Altitudinal body size clines: Latitudinal effects associated with changing seasonality. *Ecography* 26, 445–455.
- Crump, M.L. (1984). Intraclutch egg size variability in *Hyla crucifer* (Anura: Hylidae). *Copeia* 1984, 302–308.
- Cvetković, D., Tomašević, N., Ficetola, G.F., Crnobrnja-Isailović, J. & Miaud, C. (2009). Bergmann's rule in amphibians: combining demographic and ecological parameters to explain body size variation among populations in the common toad *Bufo bufo*. *Journal of Zoological Systematics and Evolution Research* 47, 171–180.
- Duellman, W.E. & Trueb, L. (1986). *Biology of Amphibians*. McGraw-Hill, New York.
- Dziminski, M.A. & Roberts, J.D. (2006). Fitness consequences of variable maternal provisioning in quacking frogs (*Crinia georgiana*). *Journal of Evolutionary Biology* 19, 144–155.
- Eaton, B.R., Paszkowski, C.A., Kristensen, K. & Hiltz, M. (2005). Life-history variation among populations of Canadian toads in Alberta, Canada. *Canadian Journal of Zoology* 83, 1421–1430.
- Fei, L., Ye, C.Y., Jiang, J.P., Xie, F. & Huang, Y.Z. (2005). *An Illustrated Key to Chinese Amphibians*. Sichuan Publishing Group. Sichuan Publishing House of Science and Technology, Chengdu, China.
- Ficetola, G.F., Scali, S., Denoël, M., Montanaro, G., et al. (2010). Ecogeographical variation of body size in amphibians: comparing the hypotheses using the newt *Triturus carnifex*. *Global Ecology and Biogeography* 19, 485–495.
- Frost, D.R. (2010). *Amphibian Species of The World: An Online Reference. Version 5.4 (8 April, 2010)*. Electronic Database accessible at The American Museum of Natural History, New York, USA.
- Gaston, K.J., Chown, S.L. & Evans, K.L. (2008). Ecogeographical rules: elements of a synthesis. *Journal of Biogeography* 35, 483–500.
- Hemelaar, A.S.M. (1988). Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology* 22, 369–388.
- Iturra-Cid, M., Ortiz, J.C. & Ibargüengoytia, N.R. (2010). Age, Size, and Growth of the Chilean Frog *Pleurodema thaul* (Anura: Leiuperidae): Latitudinal and Altitudinal Effects. *Copeia* 2010, 609–617.
- Kozłowski, J., Czarnofeski, M. & Dańko, M. (2004). Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integrative and Comparative Biology* 44, 480–493.
- Kupfer, A. (2007). Sexual size dimorphism in Amphibians: an overview. In: D.J. Fairbairn, W.U. Blanckenhorn, T. & Szekely (Eds.). *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*, pp. 50–60. Oxford University Press, New York.
- Li, C., Liao, W.B., Yang, Z.S. & Zhou, C.Q. (2010). A skeletochronological estimation of age structure in a population of the Guenther's frog, *Hylarana guentheri*, from western China. *Acta Herpetologica* 5, 1–11.
- Liao, W.B. & Lu, X. (2010a). Age structure and body size of the Chuanxi tree toad *Hyla annectans chuanxiensis* from two different elevations (China). *Zoologischer Anzeiger* 248, 255–263.
- Liao, W.B. & Lu, X. (2010b). A skeletochronological estimation of age and body size by the Sichuan torrent frog (*Amolops mantzorum*) between two populations at different altitudes. *Animal Biology* 60, 479–489.
- Liao, W.B. & Lu, X. (2011a). Variation in body size, age and growth in the Omei Treefrog (*Rhacophorus omeimontis*) along an altitudinal gradient in western China. *Ethology Ecology & Evolution* 23, 248–261.
- Liao, W.B. & Lu, X. (2011b). A comparison of reproductive output of the Omei Treefrog (*Rhacophorus omeimontis*) between high and low elevations. *Animal Biology* 61, 263–276.
- Liao, W.B., Lu, X., Shen, Y.W. & Hu, J.C. (2011). Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology* 78, 215–228.

- Liao, W.B., Zhou, C.Q., Yang, Z.S., Hu, J.C. & Lu, X. (2010). Age, size and growth in two populations of the dark-spotted frog *Rana nigromaculata* at different altitudes in southwestern China. *The Herpetological Journal* 20, 77–82.
- Liao, W.B. & Lu, X. (2012). Adult body size = $f(\text{initial size} + \text{growth rate} \times \text{age})$: explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evolutionary Ecology* 26, 579–590.
- Lindsey, C.C. (1966). Body sizes of poikilotherm vertebrates at different latitudes. *Evolution* 20, 456–465.
- Liu, W.C., Liu, Y.H., Huang, Y., Mi, Z.P. & Li, C. (2012). Skeletochronological Study on Age Structure of a Chinese Endemic Frog (*Rana omeimontis*). *Asian Herpetological Research* 3, 252–257.
- Liu, Y.H., Liao, W.B., Zhou, C.Q., Mi, Z.P. & Mao, M. (2011). Asymmetry of testes in Guenther's Frog, *Hylarana guentheri* (Anura: Ranidae). *Asian Herpetological Research* 2, 234–239.
- Lou, S.L., Jin, L., Liu, Y.H., Mi, Z.P., et al. (2012). Altitudinal variation in age and body size in Yunnan Pond Frog (*Pelophylax pleuraden*). *Zoological Science* 29, 493–498.
- Lu, X., Li, B. & Liang, J.J. (2006). Comparative demography of a temperate anuran, *Rana chensinensis*, along a relatively fine altitudinal gradient. *Canadian Journal of Zoology* 84, 1–7.
- Ma, X.Y., Tong, L.N. & Lu, X. (2009a). Variation of body size, age structure and growth of a temperate frog, *Rana chensinensis*, over an elevational gradient in northern China. *Amphibia-Reptilia* 30, 111–117.
- Ma, X.Y., Lu, X. & Merilä, J. (2009b). Altitudinal decline of body size in a Tibetan frog *Nanorana parkeri*. *Journal of Zoology* 279, 364–371.
- Mao, M., Huang, Y., Mi, Z.P., Liu, Y.H. & Zhou, C.Q. (2012). Skeletochronological study of age, longevity and growth in a population of *Rana nigromaculata* (Amphibia: Anura) in Sichuan, China. *Asian Herpetological Research* 3, 258–264.
- Mayr, E. (1956). Geographical character gradients and climatic adaptation. *Evolution* 10, 105–108.
- Meiri, S. (2011). Bergmann's Rule – what's in a name? *Global Ecology and Biogeography* 20, 203–207.
- Meiri, S., Yom-Tov, Y. & Geffen, E. (2007). What determines conformity to Bergmann's rule? *Global Ecology and Biogeography* 16, 788–794.
- Miaud, C., Andreone, F., Ribéron, A., De Michelis, S., et al. (2001). Variations in age, size at maturity and gestation duration among two neighbouring populations of the alpine salamander *Salamandra lanzai*. *Journal of Zoology* 251, 251–260.
- Miaud, C., Guyétant, R. & Elmberg, J. (1999). Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology* 249, 61–73.
- Monnet, J.M. & Cherry, M.I. (2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society B* 269, 2301–2307.
- Morrison, C., Hero, J.M. & Browning, J. (2004). Altitudinal variation in the age at maturity, longevity, and reproductive lifespan anurans in subtropical Queensland. *Herpetologica* 60, 34–44.
- Mousseau, T.A. (1997). Ectotherms follow the converse to Bergmann's rule. *Evolution* 51, 630–632.
- Olalla-Tárraga, M.A. & Rodríguez, M.A. (2007). Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. *Global Ecology and Biogeography* 16, 606–617.
- Oromi, N., Sanuy, D. & Sinsch U. (2012) Altitudinal variation of demographic lifehistory traits does not mimic latitudinal variation in natterjack toads (*Bufo calamita*). *Zoology* 115, 30–37.
- Partridge, L. & Coyne, J.A. (1997). Bergmann's rule in ectotherms: Is it adaptive? *Evolution* 51, 632–635.
- Pincheira-Donoso, D., Hodgson, D.J. & Tregenza, T. (2008). The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizards? *BMC Evolutionary Biology* 8, 68–81.
- Rensch, B. (1938). Some problems of geographical variation and species-formation. *Proceedings of the Linnean Society of London* 150, 275–285.
- Romano, A. & Ficetola, G.F. (2010). Ecogeographic variation of body size in the spectacled salamanders (*Salamandrina*): influence of genetic structure and local factors. *Journal of Biogeography* 37, 2358–2370.
- Roff, D.A. (2002). *Life History Evolution*. Sinauer Associates, Sunderland, MA.
- Rozenblut, B. & Ogielska, M. (2005). Development and growth of long bones in European water frogs (Amphibia: Anura: Ranidae), with remarks on age determination. *Journal of Morphology* 265, 304–317.
- Sinsch, U., Marangoni, F., Oromi, N., Leskovar, C., et al. (2010) Proximate mechanisms determining size variability in natterjack toads. *Journal of Zoology* 281, 272–281.
- Sinsch, U., Oromi, N. & Sanuy, D. (2007). Growth marks in Natterjack Toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. *Herpetological Journal* 17, 129–137.
- von Bertalanffy, L. (1957). Quantitative laws in metabolism and growth. *Quarterly Review of Biology* 32, 217–231.
- Walters, R.J. & Hassall, M. (2006). The temperature-size rule in ectotherms: May a general explanation exist after all? *The American Naturalist* 167, 510–523.
- Watt, C., Mitchell, S. & Salewski, V. (2010). Bergmann's rule: a concept cluster? *Oikos* 119, 89–100.
- Wells, K.D. (1977). The social behaviour of anuran amphibians. *Animal Behavior* 25, 666–693.
- Wells, K.D. (2007). *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago, USA.

Accepted: 24 May 2013