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The influence of altitude on the size and shape of two newt species *Triturus cristatus* (Laurenti, 1768) and *Triturus dobrogicus* (Kiritzescu, 1903) in *Slovakia*

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Abstract: We analyze the relationships between the altitude as an ecological factor and the length of limbs in two closely related species of newts, *Triturus cristatus* and *Triturus dobrogicus*. We mainly focused on changes in the length of limbs that are important variables in classification using Wolterstorff index. Finding that the values of Wolterstorff index in individuals at extreme altitudes frequently overlap, put forward the idea of underlying dependence in their morphological characters. We found that in *T. dobrogicus* body size of males and females is inversely related to altitude, i.e. the individuals from sites in higher altitudes are smaller. There was no significant difference between sexes in this case. In *T. cristatus* body size increases with altitude, but only in males, not in females. There were no significant differences in body size between sexes of this species. Both species show significant sexual dimorphism in body shape measured by the length of both their limbs and trunks. Males have longer limbs and shorter trunks than conspecific females. Females of *T. dobrogicus* exhibit shorter and narrower heads than males. There were no significant differences related to altitude in *T. dobrogicus*. Females of *T. cristatus* have longer limbs and shorter trunks at higher altitudes, but this trend was not found in conspecific males. Though there is a trend in change of female body shape in *T. cristatus*, neither *T. cristatus* nor *T. dobrogicus* were significantly different in body shape. Principal Component Analysis revealed also correlation between limb length and interlimb distance. In spite of apparent trend in the change of the limb length in relation to the altitude this correlation was not significant. However, comparing more morphological (head morphology) and environmental variables and analyzing larger samples in the future may bring new knowledge on ecology, relationships and evolution of these two species.

Key words: Triturus cristatus, T. dobrogicus, head, limbs, morphometry, altitude, Slovakia

Introduction

One of the most reliable morphological character in determining the species identity of the newts *Triturus cristatus* and *T. dobrogicus* is the ratio of the limb length to the interlimb distance - the Wolterstorff index (Arntzen, Wallis 1994; Piálek *et al.* 1999). Wolterstorff index (Wolterstorff 1923) is calculated as the ratio of the forelimb length (P.a.) to the interlimb distance (L.i.e.) multiplied by one hundred (P.a. : L.i.e. × 100). Values of Wolterstorff index increase from *T. dobrogicus* to *T. cristatus*, which holds for males as well as females. The values for males and females in *T. dobrogicus* are 45-52 % and 34-45 %, respectively, for males and females in *T. cristatus* 60-65 % and 49-54 %, respectively (Wolterstorff 1923). Kalezič *et al.* (1990) report that various species of the genus *Triturus* differ in the ratio of head width to snout-vent length (L.t.c. : L. × 100). This so-called Kalezič index is not used so often by now as Wolterstorff index.

The use of Wolterstorff index is also one of the most often discussed issues. Although Arntzen, Wallis (1999) consider it demonstrative, they point out some potential problems involved in it: 1) Statistical representation. Values of Wolterstorff index are presented as an interval with minimum and maximum, which hides information on frequency distribution within the interval and the input of new data is likely to increase the interval width. More data thus may lead to less reliable determination. 2) Nonbiological variation. Change in snout-vent length of stored individuals after fixation, as well as differences in measuring methods (Baruš, Oliva 1992) can bias the values of Wolterstorff index. 3) Sexual dimorphism. Both parameters that enter the Wolterstorff index can be statistically significant as characteristics of sexual dimorphism (Kalezič *et al.* 1990), therefore the index must be calculated separately for males and females. Males have on average longer limbs than females. Freytag (1988) confirmed that the interlimb distance is greater in females than in males. In this way he revealed an error in Wolterstorff's statement that the interlimb distance in males is greater than in females. 4) Allometric variation. Allometry in various species of the genus *Triturus* was studied in detail (Higginbottom, 1853; Hagström 1977, 1980; Herre 1932; Lác 1957; Rehák 1983). A tendency to more rapid growth of interlimb distance vs limb length indicates that Wolterstorff index will attain lower average values in larger or longer individuals than in smaller or shorter ones. 5) Hybridization. The possibility of hybridization of *T. cristatus* × *T. dobrogicus* was confirmed already by Lác (1957), who regarded morphologically intermediate forms as „var. intermedia", and later also by other authors (Bucci-Innocenti *et al.* 1983; Brede *et al.* 2000; Sova 1973; Lantz, Callan 1954; Arntzen, Hedlung 1990; Morozov-Leonov *et al.* 2003; Horák 2000). Hybrid individuals in contact zones of these two species show intermediate characteristics, which can lead to erroneous identification. 6) Geographic variation. Neighbouring populations show more similar values of Wolterstorff index than distant populations

Comparison of morphometric and mtDNA analyses within the genus *Triturus* revealed higher reliability when using molecular data (14 % of errors in species classification) than morphometric data (31 % of errors in species classification; Arntzen, Wallis 1999). Arntzen, Wallis (1994) mentioned Principal Component Analysis (PCA) as an alternative to Wolterstorff index. PCA was used by Kalezič *et al.* (1990), who examined 4 newt species. Principal component PC1 represented the total body length, second principal component the variability of interlimb distance, considered as an important parameter in species classification.

Malmgren, Thollesson (1999) reported, on the basis of PCA results, the limb length as one of the most important measures of sexual dimorphism in *T. cristatus* and *T. (Lissotriton) vulgaris*.

Problems in identification of *T. cristatus* and *T. dobrogicus* in contact zones of their distribution ranges caused by overlapping values of Wolterstorff index are reported by Kautman, Zavadil (2001). The overlapping values of this index in extreme altitudes bring forward an idea to track the dependence of newt morphological characteristics on altitude along the altitudinal gradient from lowland, aquatic forms of *T. dobrogicus* toward mountain, more terrestric forms of *T. cristatus* (Kautman, Zavadil 2001). Detecting such dependence would put to trial the reliability of Wolterstorff index as an identification tool for the species of the genus *Triturus*.

The shift of Wolterstorff index was mentioned also by Zavadil (1996), who pointed out possibly higher morphological variation in *T. cristatus* and *T. dobrogicus* than that reported by Wolterstorff. Average value of Wolterstorff index for *T. dobrogicus* from southern Moravia was lower in both sexes, in females being near the lower limit estimated by Wolterstorff and in males even below this limit. The upper limit in males was shifted upwards, the lower limit was shifted downwards. In females both upper and lower limit was shifted downwards.

Both endothermic and ectothermic animals are influenced by numerous external factors. Various adaptations evolved as a response to these factors (Losos 1984). One of such adaptations is trunk elongation and limb reduction together with typical winding body movement, known in aquatic animals and/or implying transition from terrestrial to more (or entirely) aquatic way of life. Conversely, limb elongation together with shortened and more robust body indicates more terrestrial way of life (Young 1950; Lande 1978).

Besides other factors the limb length may relate to the time spent in aquatic phase (Arntzen, Wallis 1999). From this point of view it might be interesting to confront the values of Wolterstorff index with body proportions of species from the genus *Triturus*. The index values grow from *T. dobrogicus* to *T. cristatus*, indicating longer limbs and shorter aquatic phase in *T. cristatus* when comparing to *T. dobrogicus*. The duration of aquatic phase in *T. cristatus* is roughly 5 months (Bouton 1986; Griffiths, Mylotte 1987; Andreone, Giacoma 1989), whereas in *T. dobrogicus* it reaches about 6 months (Karaman 1948; Jehle *et al.* 1997).

Variation in body size of adults in relation to altitude is known in various amphibian species. Growth of body proportions, elongation and delayed maturity in higher altitudes were described, e.g. in *Desmognathus ocoee*, *T. (Mesotriton) alpestris*, and *T. marmoratus* (Tilley 1980; Miaud *et al.* 2000). Local climatic conditions in different altitudes can influence the morphology of individuals (Miaud *et al.* 2001).

The aim of this study was to analyse body size variation among populations in the two mentioned newt species with wide altitudinal distribution in Slovakia. Body size is a complex attribute and it is unrealistic to expect a single factor underlying its variation, although the relationship with temperature has always been in the focus (Van Voorhies 1996; Angilletta *et al.* 2004). In amphibians, growth rates can decrease dramatically after the attainment of sexual maturity (e.g. Hemelaar 1988; Miaud *et al.* 1999), and delayed reproduction can, thus, allow a prolonged growth period and the attainment of larger adult size. As a consequence, interpopulational variation in adult body size can be explained by difference in the age structure.

In this study, we tested for association of body size and shape with positional environmental variables and with sex difference in the species The analysis of altiitude that might directly influence body size and demography (e.g. temperature and length of the activity period) allowed us to not only to explore the variation patterns in this species, but also to identify the possible causes of this variation.

Materials and methods

Animals and sites

The individuals measured were (i) obtained from the museum collections (fixed in formaldehyde) and (ii) captured at selected sites of Slovakia. Fixed individuals come from following institutions: Faculty of Natural Sciences, Comenius University in Bratislava (11 individuals), the Natural History Museum in Bratislava (27 individuals), the East Slovakian Museum in Košice (61 individuals), the Šariš Regional Museum in Bardejov (35 individuals). We also used data from already measured material, provided by MUDr. Vít Zavadil (54 individuals). The living individuals were captured during their sexual activity in aquatic phase in the day-time using a landing net. Captured individuals were narcotized by phenoxietanol (1 : 2 500) to facilitate measuring. After the measuring animals were put on solid surface until the narcotization ceased. Individuals were then released back into the water.

The individuals of *T. dobrogicus* were collected in the altitudes from 102 m (Borša, eastern Slovakia) to 290 m (Barca, eastern Slovakia). The individuals of *T. cristatus* come from altitudes from 109 m (Ruské, eastern Slovakia) to 813 m (Torysky, eastern Slovakia).

We excluded from statistical analyses all immature, damaged and hybrid individuals or those showing great morphological similarity and thus unidentifiable without biochemical or molecular analysis. Living individuals of *T. dobrogicus* were captured at following sites: 1) Trenčianska Teplá (221 m a.s.l., western Slovakia). Two pools close to each other, smaller of them partially filled with various garbage. Newts were abundant in the larger pool (c. 10 × 5 m, maximum depth 2 m), overgrown with submerged macrophytes and branches of nearby trees. 2) Moravský Sv. Ján (150 m). The site (a pool c. 12 × 8 m) is located in the floodplain of Morava River. Submerged vegetation and tree roots make a suitable habitat for the newts to hide and lay eggs.

Measurement methods

We measured following morphological variables (Opatrný et al. 1973): 1) snout-vent length (L.): from the tip of the snout to the posterior tip of cloaca. 2) tail length (L.cd.): from the posterior tip of cloaca to the tip of the tail. Total length (L.tot. = L. + L.cd.): from the tip of the snout to the tip of the tail. 3) head length (L.c.): from the tip of the snout to the jaw angle (several methods of measuring the head length exist). Head width (Lt.c.): measured at the widest part of the head. 5) forelimb length (P.a.): from the limb base to the tip of the longest finger. When measured, the straightened limb is positioned at the right angle to the trunk axis. Each forelimb was measured separately. 6) hindlimb length (P.p.): measuring is analogous to the forelimb length. 7) interlimb distance (L.i.e.): from the forelimb base to the hindlimb base, measured on each body side.

Statistical methods

All statistical tests were done using a PC running Stagraphics 5 statistical software. Shape and size pattern variables were tested for significant differences in the within/between group variance using an ANOVA (type III, Strauss 1987).

Each variable was tested for normality. In multivariate analyses the variables were submitted to principal component analysis (PCA; Malmgren, Tholesson 1999). Principal components of mensural data were computed from the covariance matrix of logarithmically transformed data. The logarithmic transformation preserves allometries, standardizes variances, and produces a scale-invariant covariance matrix (Jolicoeur 1963,Strauss 1985). PCA is a multivariate technique that may be employed to summarize data sets combining large number of variables. We worked with 10 variables (L., L.cd., L.c., Lt.c., P.a.1, P.a.2, P.p.1, P.p.2, L.i.e.1, L.i.e.2). The first pricipal component (PC1) of a set of linear measurements provides an appropriate structural size measure (Rising, Somers 1989). The importance of the remaining components in this study can be judged from the amount of variance associated with them. The first four components are used These variations are independent of general size vector (PC1) and they can be most likely attributed to the shape of newts. A measure of the size or shape equivalence or difference between two selected groups of newts was specified by ANOVA of the component scores (Somers 1986). The component scores (shapes or size) were regressed on altitude to examine the potential influence of habitats on morphometric characters of newts.

Results

Size (PC1)

Component loadings for both species are shown in Table 1 and 2 including percentage of the total variance associated with each component. The size (PC1) in each species explains more than a half of total variability (Table 1 and 2). Sexes of both species did not differ significantly in size but both differ in shape (Table 3). The size appears to be sensitive to the altitude of occurrence. While the size of *T. cristatus* tends to increase with altitude (in males significantly, in females insignificantly - Table 4, Fig. 4), size of both males and females in *T. dobrogicus* significantly decrease with altitude (Fig. 6 and 7, Table 5). It seems that at the contact zone (Fig. 1) the size in both species is smaller.

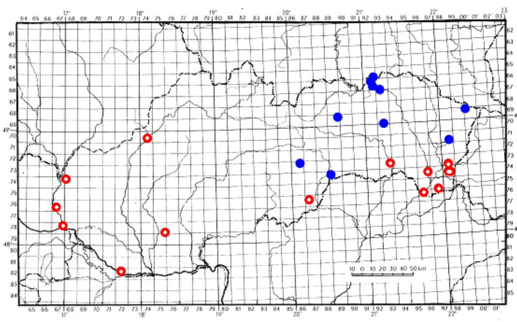

Fig. 1. Map of Slovakia with sites where the examined newts were obtained. solid circles - *T. cristatus*, empty circles - *T. dobrogicus*.

Shape variation - limbs (PC2)

In *T. cristatus*, there is most pronounced trend of variation from individuals with relatively shorter limbs and longer interlimb distance to individuals with longer limbs and shorter interlimb distance (PC2 - Table 1). In *T. dobrogicus* (PC2 - Table 2) there is also an inverse relationship between limb length and interlimb distance, though somewhat weaker. Moreover, individuals with relatively shorter limbs and longer interlimb distance have shorter and narrower head, while individuals with relatively longer limbs and shorter interlimb distance have longer and broader head.

Triturus cristatus	PC1	PC2	PC3	PC4
forelimb length 1	0.28791	-0.43131	-0.05118	-0.06189
forelimb length 2	0.29202	-0.40071	-0.25181	-0.03329
hind limb length 1	0.33664	-0.30772	0.06613	-0.18172
hind limb length 2	0.33822	-0.31662	0.03472	-0.08539
interlimb distance 1	0.29875	0.38911	-0.17776	-0.36085
interlimb distance 2	0.29711	0.38657	-0.20899	-0.37322
head width	0.34261	0.02565	0.46180	0.43316
head length	0.28168	0.17430	-0.65560	0.63023
tail length	0.30429	0.29726	0.41972	0.25803
snout-vent length	0.37051	0.19549	0.18407	-0.18396
explained variance [%]	**60.8**	**24.3**	**6**	**4.5**

Table 1. Principal Component Analysis, component loadings of first four principal components (PC1 - PC4) and percentage of the total variance of variables in *Triturus cristatus* explained by individual principal components. PC1 - principal component PC1 (size and shape changing with size), PC2 - principal component PC2 (shape), PC3 - principal component PC3 (shape), PC4 - principal component PC4 (shape).

Triturus dobrogicus	PC1	PC2	PC3	PC4
forelimb length 1	0.35427	-0.24034	-0.27460	-0.09083
forelimb length 2	0.34752	-0.23843	-0.30633	-0.09552
hind limb length 1	0.35857	-0.22359	-0.22520	0.04314
hind limb length 2	0.36655	-0.21534	-0.21893	0.02515
interlimb distance 1	0.34271	0.33230	0.18828	0.32744
interlimb distance 2	0.34810	0.32192	0.18575	0.30940
head width	0.16502	-0.38235	0.55871	-0.42033
head length	0.04582	-0.51687	0.49834	0.49831
tail length	0.29255	0.29055	0.30811	-0.58441
snout-vent length	0.37048	0.27787	0.08199	0.10329
explained variance [%]	**58.4**	**18.6**	**13.5**	**4.1**

Table 2. Principal Component Analysis, component loadings of first four principal components (PC1 - PC4) and percentage of the total variance of variables in *Triturus dobrogicus* explained by individual principal components. PC1 - principal component PC1 (size and shape changing with size), PC2 - principal component PC2 (shape), PC3 - principal component PC3 (shape), PC4 - principal component PC4 (shape).

factor	**n**	**PC1 score (SE) arithmetic mean**	**PC2 score (SE) arithmetic mean**
sex TC			
- *males*	33	-0.25 (0.46)	-1.14 (0.21)
- *females*	42	0.19 (0.36)	0.90 (0.18)
		F=0.58 p=0.4563 (n.s.)	F=54.94 p=0.0000 (h.s.)
sex TD			
- *males*	40	0.12 (0.36)	-0.71 (0.20)
- *females*	44	-0.11 (0.38)	0.65 (0.17)
		F=0.18 p=0.6816 (n.s.)	F=27.56 p=0.0000 (h.s.)

Table 3. One-way ANOVA: differences in size (PC1 score) and shape (PC2 score) of *T. cristatus* and *T. dobrogicus* between sexes. TC - *Triturus cristatus*, TD - *Triturus dobrogicus*, SE - standard error of the arithmetic mean, n - sample size (number of individuals), F - F-statistic of the ANOVA F test, p - p-value of the ANOVA F test (the significance level is set to 0.05), n.s. - result not significant ($p > 0.05$), s. - significant ($0.05 > p > 0.01$), h.s. - highly significant ($p \leq 0.01$).

	n	PC1	PC2
		F = 6.87	F = 2.85
males	33	p = 0.0135 (s.)	p = 0.1013 (n.s.)
		r = 0.43	r = 0.29
		F = 3.48	F = 15.27
females	42	p = 0.0694 (n.s.)	p = 0.0004 (h.s.)
		r = 0.28	r = 0.53

Table 4. Regression analysis in *Triturus cristatus*: dependence of size (PC1 score) and shape (PC2 score) on altitude in both sexes. n - sample size (number of individuals), PC1 -principal component PC1 (size) in *Triturus cristatus*, PC2 -principal component PC2 (shape) in *Triturus cristatus*, F - F-statistic of the ANOVA F-test, p – p-value of the ANOVA F-test (the significance level is set to 0.05), n.s. - result not significant (p > 0.05), s. - significant (0.05 > p > 0.01), h.s. - highly significant (p≤0.01), r - Pearson product-moment correlation coefficient.

	n	PC1	PC2
		F = 10.93	F = 2.13
males	40	p = 0.0021 (h.s.)	p = 0.1529 (n.s.)
		r = -0.47	r =-0.23
		F = 5.13	F = 2.01
females	44	p = 0.0262 (s.)	p = 0.1638 (n.s.)
		r = -0.34	r =-0.21

Table 5. Regression analysis in *Triturus dobrogicus*: dependence of size (PC1 score) and shape (PC2 score) on altitude in both sexes. n - sample size (number of individuals), PC1 - principal component PC1 (size) in *Triturus dobrogicus*, PC2 - principal component PC2 (shape) in *Triturus dobrogicus*, F - F-statistic of the ANOVA F-test, p – p-value of the ANOVA F-test (the significance level is set to 0.05), n.s. - result not significant (p > 0.05), s. - significant (0.05 > p > 0.01), h.s. - highly significant (p≤0.01), r - Pearson product-moment correlation coefficient.

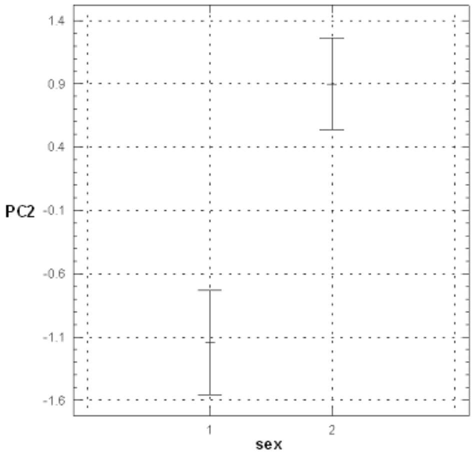

Fig. 2. One-way ANOVA, difference in shape (PC2) between sexes in *T. cristatus*. 1 - males, 2 - females.

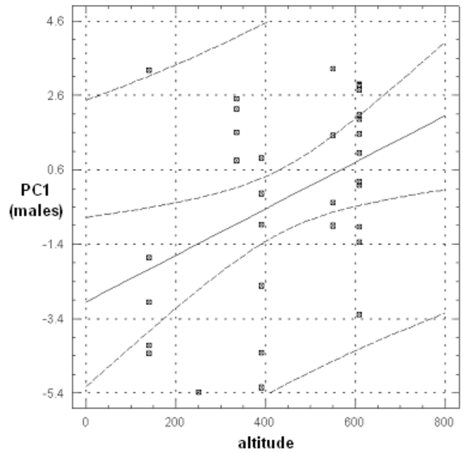

Fig. 4. Regression analysis, size (PC1) of *T. cristatus* males in relation to altitude. PC1 - principal component PC1 (size) for *Triturus cristatus*.

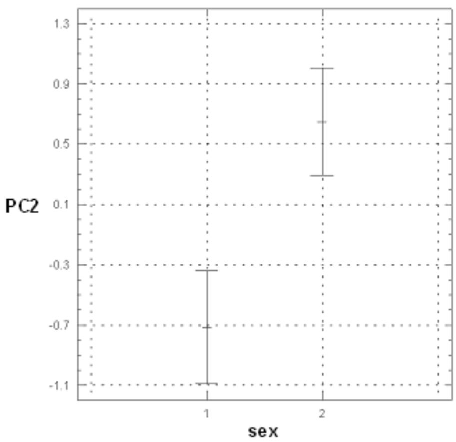

Fig. 3. One-way ANOVA, difference in shape (PC2) between sexes in *T. dobrogicus*. 1 - males, 2 - females.

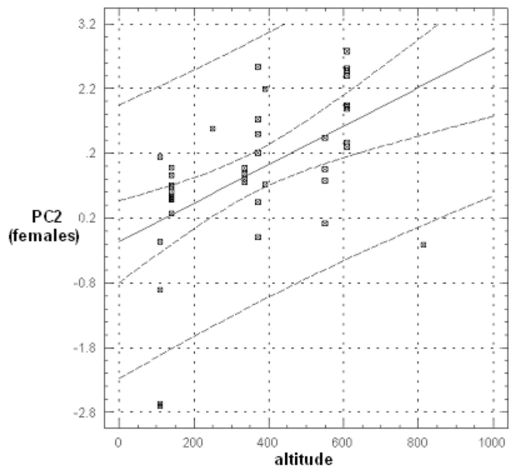

Fig. 5. Regression analysis, limb length and interlimb distance (PC2) in relation to altitude in *Triturus cristatus* females. PC2 - principal component PC2 (change of shape) for *Triturus cristatus*.

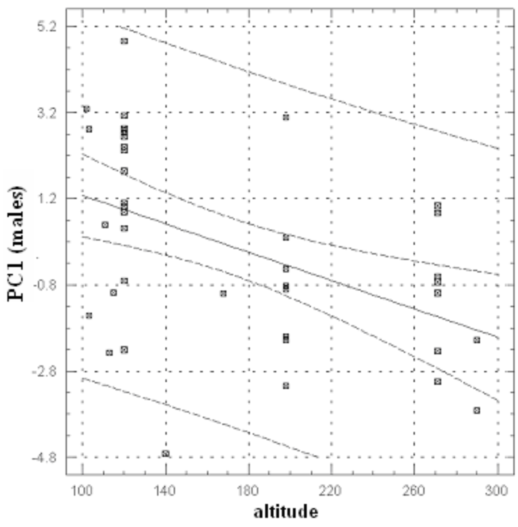

Fig. 6. Regression analysis, size (PC1) of *T. dobrogicus* males in relation to altitude. PC1 - principal component PC1 (size) for *Triturus dobrogicus*.

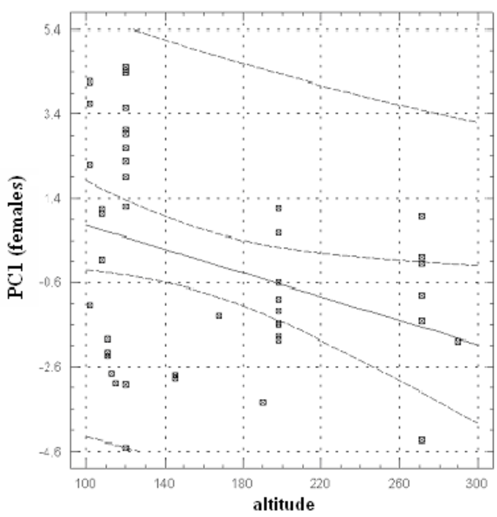

Fig. 7. Regression analysis, size (PC1) of *T. dobrogicus* females in relation to altitude. PC2 - principal component PC2 (change of shape) for *Triturus dobrogicus*.

The differences in body shape (PC2) related to sex are statistically highly significant (Table 3). Males of *T. cristatus* tend to have longer limbs and shorter interlimb distance, while females have relatively shorter limbs and longer interlimb distance (Fig. 2). Similar highly significant differences in body shape (PC2) occur in *T. dobrogicus*. Males tend to have relatively longer limbs and longer, broader head, while females have longer interlimb distance and smaller head (Fig. 3). Shape variables in *T. dobrogicus* showed weaker response to altitude than in *T. cristatus*. Both sexes of *T. dobrogicus* did not change the shape with increasing altitude while in females of *T. cristatus* changes are highly significant (Tables 4–5). With increasing altitude in females there is a trend of increasing limb length and decreasing interlimb distance.

Shape variation - tail/head (PC3)

T. cristatus exhibits negative relationships between head length and both head width and tail length. There are individuals with long, narrow head and short tail, and individuals with short, broader head and longer tail (Table 1).

In *T. dobrogicus* there are also the strongest relationships between head length, head width and tail length, but all these parameters correlate positively. There are individuals with longer and broader head and longer tail, and individuals with shorter, narrower head and shorter tail (Table 2).

Shape variation - head (PC4)

In *T. cristatus* a trend towards negative relationships between head shape and interlimb distance accounts for 4,5 % of the total variability. Individuals with longer and broader head tend to have shorter interlimb distance whereas individuals with shorter and narrower head have longer interlimb distance.

T. dobrogicus shows a trend towards negative relationships between head length and both head width and tail length, explaining 4,1 % of the total variability. There are individuals with tendency for longer, narrower head and shorter tail, and individuals with shorter, broader head and longer tail.

Discussion

We tried to test the hypothesis that in *T. cristatus* and *T. dobrogicus* limb length is significantly correlated with altitude as a proxy for several important ecological factors. Any single factor may not affect the trait individually, but as a complex they may induce morphological, physiological and behavioural changes in the ontogenetic process of individual species (Losos *et al.* 1984). Different traits influenced by the external factors during a long time period can be traced also in the process of phylogeny.

Kalezič *et al.* (1990) used PCA to distinguish 4 species of newts. Arntzen, Wallis (1994) regarded this analysis as an alternative to the Wolterstorff index (Wolterstorff 1923), by now the standard in species identification. Our PCA results support the hypothesis about limb length change related to altitude. Kalezič *et al.* (1990) considered the interlimb distance as the most important parameter, which well describes the taxonomic and geographic distribution of populations. Results of our research are consistent with this hypothesis, since the interlimb distance in correlation with limb length account for relatively high percentage of total variance of input variables (24,3 % in *T. cristatus* and 18,6 % in *T. dobrogicus*).

Comparison of sexes in *T. cristatus* as well as in *T. dobrogicus* showed that morphological variation related to sexual dimorphism is manifested more markedly in shape than in size, as state also Butler, Losos (2002) in their study of sexual dimorphism in lizards of the genus Anolis from Greater Antiles.

Results of One-Way ANOVA show differences in trends of limb length change vs interlimb distance between sexes in both species. In *T. dobrogicus* the analyses of shape dimorphism related to sex yielded results that correspond with the morphological description given by other authors, who describe *T. dobrogicus* as a species with slender body, narrow head and shorter limbs, which is apparent particularly in females (Wolterstorff 1923; Lác 1957, 1968; Zavadil 1995). Analyses in both species confirm shorter limbs and longer interlimb distance in females and longer limbs in males. One possible explanation of this difference in shape may be the fecundity model (Malmgren, Thollesson 1999), which assumes that sexual dimorphism is related to different roles of males and females in reproduction process. While the elongated abdomen of females may promote production of larger amount of eggs (Shine 1988), elongated limbs may promote transfer of spermatophor.

Rehák (1983) suggests that elongated limbs in *T. cristatus* males relate to their sexual behaviour in a way of improved ability to manoeuvre during the courtship.

The percentage of variability explained by the first principal component (size) is high and similar in both species (*T. cristatus* 60,8 %; *T. dobrogicus* 58,4 %), indicating approximately the same magnitude of the body size response to external factors (Hay *et al.* 1995; Feller, Hedges 1998; Carroll 1999). Percentage of variability due to the change of shape in relation to the interlimb distance is lower (*T. cristatus* 24,3 %; *T. dobrogicus* 18,6 %). This variability relates mostly to the change of limb length vs interlimb distance. These are the morphological measures that Wolterstorff (1923) used to calculate his index for species identification. Various authors (Arntzen, Wallis 1999; Zavadil 1996; Kautman, Zavadil 2001) recorded a shift in the values of Wolterstorff index with the change of altitude and/or in the contact zone of range of both species that brings an idea of clinal dependence of the limb length change on altitude. Our results corroborate this hypothesis in case of *T. cristatus* females. In case of *T. cristatus* males, as well as in both sexes of *T. dobrogicus*, this hypothesis was not supported. Despite the observable trend of limb elongation with increasing altitude the results of statistical analyses appear insignificant. One possible cause might be the restricted altitudinal range of *T. dobrogicus* sites in our dataset (188 m), since data from extreme altitudes were excluded from the analyses due to possible occurrence of hybrids and potential bias in the results. According to various authors (Arntzen, Wallis 1994; Piálek *et al.* 1999) the Wolterstorff index is a reliable method of distinguishing the two newt species. However, both the hypothesis about overlapping values of this index at sites of extreme altitudes in the species' range (Kautman, Zavadil 2001) and our results suggest that the Wolterstorff index should be considered as a variable contingent on altitude. This is supported by the trend in the change of shape in *T. cristatus* females, whose interlimb distance increases with increasing altitude. This intraspecific geographic variation (Lác 1957) indicates that the method introduced by Wolterstorff (1923) should be supplemented by molecular or genetic investigations so as to identify the species more reliably (Arntzen, Wallis 1999).

Analyses of the pattern in size variation (PC1) show that the size of *T. cristatus* males increases with increasing altitude. However, in *T. cristatus* females positive correlation between body size and altitude does not reach the significance level. Assuming that temperature decreases with increasing altitude, it seems likely that *T. cristatus* males follow the Bergmann's rule (Ashton 2002). Positive correlation between altitude and body size was found also in *Triturus (Lissotriton) montandoni* (Janiga, Mlichová 2004). Individuals of this species from higher altitudes were more robust (heavier) than individuals from lower altitudes.

The size of *T. dobrogicus* decreases with increasing altitude in both sexes. To test this hypothesis, another potential explanatory factors, not just the altitude, should be examined.

According to our investigation, both *T. dobrogicus* and *T. cristatus* develop larger body size in allopatric conditions (*T. dobrogicus* in lower altitudes, *T. cristatus* in higher altitudes). In areas of sympatry (relatively high altitudes for *T. dobrogicus* and relatively low altitudes for *T. cristatus*), both species develop smaller body size. While *T. cristatus* tends to follow the Bergmann's rule (Ashton 2002), *T. dobrogicus* seems to follow the inverse of this rule.

Less prominent trends in change of shape (PC3, PC4) are also interesting. Results of PCA show variability mainly in head parameters (length, width) or in the tail length. Though we did not submitted these trend to further analyses, some hypotheses indicate that the change of head shape or the tail length can be an important measure of intraspecific as well as interspecific variability. This is the case of the hypothesis about Kalezič's index, which distinguishes species of the genus *Triturus* using the ratio of head width to tail length multiplied by one hundred (Kalezič *et al.* 1990). That trend corresponds also with the ecological model (Shine 1989) assuming that changes of morphological parameters (e.g. head morphology) are related to different ecological adaptations, in this case probably to foraging strategies (size and amount of the prey). Despite of negative results in some studies of the changes in head shape (Malmgren, Thollesson 1999; Rafinski, Pecio 1989), future studies in this field can throw more light on the taxonomy of these two species.

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