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The evolution of the adult body form of the crested newt (*Triturus cristatus* superspecies, Caudata, Salamandridae)

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Abstract

We characterized the adult body form of the crested newt (*Triturus cristatus* superspecies) and explored its evolution. From seven morphometric traits, we determined that body size, interlimb distance and head width define the body form. None of the morphometric traits showed a phylogenetic signal. Three body-shape morphotypes (*Triturus dobrogicus* + *T. cristatus*, *Triturus carnifex* + *Triturus macedonicus* and *Triturus karelinii* + *Triturus arntzeni*) and three body-size morphotypes (*T. dobrogicus*, *T. cristatus* and all other crested newts) could be recognized. The ancestral phenotype (a large body with a short trunk and a wide head) characterized *T. karelinii* and *T. arntzeni*. *Triturus carnifex* and *T. macedonicus* had a somewhat different phenotype (large body and wide head, accompanied by mild body elongation). The most derived phenotype included body size reduction and more pronounced body elongation in *T. cristatus* and, especially, in *T. dobrogicus*. Body elongation occurred by trunk lengthening but not head and tail lengthening. Additionally, contrary to other tetrapods, evolutionary axis elongation in crested newts was followed by a decrease in body size. We advocate the hypothesis that ecology drives the evolution of body form in crested newts.

Key words: Morphotype – body elongation – modularity – phylogenetic signal

Introduction

The term 'body form' refers to the robust morphological features of an organism's external morphology and encompasses both size- and shape-related characteristics. Body form can differ between species but also between groups of species. As such, traits that define body form are important from the perspective of evolutionary morphology.

Among tailed amphibians, the genus *Triturus* (Rafinesque, 1815) is recognized as a suitable model organism for addressing various evolutionary aspects, including body form evolution (e.g. Arntzen et al. 2007). *Triturus,* the 'big-bodied group' of European newts, comprises two monophyletic groups: the crested (*Triturus cristatus* superspecies) and the marbled newts (*Triturus marmoratus* (Latreille, 1800) and *Triturus pygmaeus* (Wolterstoff, 1905)). Since Wolterstorff's (1923) landmark paper, three groups of crested newts, which differ in trunk and appendage size, have been acknowledged. In general, the species *Triturus karelinii* (Strauch, 1870), *Triturus arntzeni* (Litvinchuk, Borkin, Džukić, Kalezić, Khalturin and Rosanov, 1999), *Triturus carnifex* (Laurenti, 1768) and *Triturus macedonicus* (Karaman, 1922) have a relatively large body, with a stocky trunk, a wide and long head, long limbs and a relatively short tail (e.g. Arntzen and Wallis 1999). In contrast, *Triturus dobrogicus* (Kiritzescu, 1903) is characterized by a relatively small body size, a slender and elongated trunk, short limbs, a small and elongated head and a long tail. The body type of *T. cristatus* (Laurenti, 1768) is intermediate. These groups differ in their ecological demands (Arntzen 2003). *Triturus dobrogicus* inhabits permanent or long-lasting large water bodies and exhibits a prolonged aquatic period (Arntzen and Wallis 1999; Arntzen 2003; Litvinchuk et al. 2007). In contrast, *T. karelinii* and *T. arntzeni,* and to a lesser extent *T. macedonicus* and *T. carnifex*, are generally confined to smaller bodies of water, exhibiting shorter aquatic periods. *Triturus cristatus* is again intermediate in this respect.

It is well known that different ecomorphological patterns exist in the crested newts. It can be anticipated that the relative size of limbs, trunk and tail is important in locomotion and habitat usage. Some of these expectations (e.g. the association of a longer tail with increased swimming speed and a shorter interlimb distance with increased running speed) have been experimentally demonstrated to be adaptations to locomotion in aquatic and terrestrial habitats. Contrary to expectations, body elongation in crested newts has been connected with a reduction in swimming speed (Gvoždik and Van Damme 2006). Additionally, the most prominent evolutionary change in the body form of European newts is body elongation, which suggests that the issue of axial patterning of the crested newts should be addressed.

None of the previous studies dealing with crested newt size and shape variation described body form in a strict statistical manner nor did they evaluate the morphometric trait variance decomposition at different hierarchical levels i.e. the levels of: (1) presumed body form, (2) species, (3) population and (4) intrapopulation. Furthermore, it is important to statistically determine those morphometric traits that define body form and to examine their evolution in a phylogenetic framework.

In comparative studies of phenotypic variation, comprehending phylogenetic relationships is pertinent because similarity due to a shared evolutionary history could be a confounding factor. As far as the crested newts are concerned, recent phylogenetic studies have confirmed that crested newts show a rapid diversification early in their evolutionary history.

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Nuclear and mitochondrial genetic characteristics suggested a hard polytomy (Arntzen et al. 2007; Espregueira Themudo et al. 2009). However, a newly available, highly supported mitogenomic phylogeny (Wielstra and Arntzen 2011) provides a solid basis for studying crested newt body form variation in an evolutionary context.

The goals of this study are as follows: (1) to identify discrete adult crested newt body forms, (2) to place adult crested newt body form variation in a phylogenetic framework and (3) to address axial patterning in crested newts.

Materials and Methods

Samples

Our data were obtained from both previously analysed populations (22 populations, Kalezić et al. 1997; Crnobrnja-Isailović et al. 1997; Litvinchuk et al. 1999) and new populations (17 populations). As the crested newt species often hybridize along their parapatric species boundaries, we only included populations located away from the contact zones. At present, the taxonomy of the crested newts is in a state of flux. Here, we have used recent taxonomical recommendations that proposed that *T. carnifex* and *T. macedonicus* (Arntzen et al. 2007), as well as *T. karelinii* and *T. arntzeni* (Espregueira Themudo et al. 2009), are different species. Sampled individuals were deposited in the Batrachological Collections housed in the Institute for Biological Research 'Siniša Stanković', Belgrade, and at the University of Athens.

For details on localities and sample sizes, see Table 1. The marbled newt *T. marmoratus* was used as an outgroup (12 populations, 336 individuals).

The following seven measurements, which have been suggested to characterize body morphs in crested newts (e.g. Wolterstorff 1923), were used to quantify the adult body form in crested newts: snout-vent length (SVL), interlimb distance (D), head width (HW), head length (HL), forelimb length (FLL), hindlimb length (HLL) and tail length (TL). All measurements were taken with a digital calliper to a precision of 0.01 mm. Bilaterally symmetric traits (D, FLL and HLL) were measured from the right side of the body.

Measurements were taken from specimens that were preserved in 70–75% ethanol for more than 5 years. Therefore, biases that could be made by measuring and comparing fresh and ethanol-preserved specimens have been avoided.

Statistical analyses

Analyses were performed for each sex separately due to the existence of considerable sexual dimorphism in crested newts (Ivanović et al. 2008).

The morphometric data were partitioned in size and shape sets. Individual size was taken as the geometric mean of all body measures for each specimen, and Mosimann's ratio method (Darroch and Mosimann 1985) was used to obtain shape variables. To derive shape variables, the population samples were treated as operational taxonomic units (OTUs). For each OTU, each morphometric measure was divided by the geometric mean of all of the measures. This adjustment removed isometric size but not size-related (allometric) shape. Preliminary analyses removing allometric size (analyses of residuals from regression of shape variables on size) showed similar results (results were not shown). We assumed that in our further analyses, shape was maximally deprived of size influences.

As a phylogenetic framework in which to place body size and shape characteristics, we used a mitogenomic phylogeny (Wielstra and Arntzen 2011; see Fig. 1). To characterize the phylogenetic dependence of traits, we used a canonical procedure that allowed decomposition of quantitative trait variance along the phylogenetic tree (Ollier et al. 2006). The first step was to define an orthonormal vectorial basis, which represented the topology of the phylogenetic tree, and the second step was to determine the variance decomposition of traits on a vectorial basis, which yielded the structure functions called orthograms.

Four tests computed from the variance decomposition were used to test the null hypothesis, which was the complete absence of phylogenetic dependence: (1) R2Max statistics were used to test the alternative hypothesis that the unique vector of the orthonormal basis explained the significant part of the trait variance (punctual effect), (2) S2R2k statistics were used to test the alternative hypothesis that the vectors near the tips or the root (high or low value of S2R2k, respectively) explained the significant part of the trait variance, (3) $D_{\max}$ statistics were used to test the alternative hypothesis that some successive vectors explained a significant part of the trait variance and (4) SCE, which measures the average local variation of the orthogram values, was used to test the alternative hypothesis that there are significant differences in variance explained by vectors and their neighbours (precedent or subsequent). The distribution of the statistics under the null hypothesis and the confidence limits of the cumulative orthograms were built using 999 random permutations of the trait values. The analyses were conducted using the adephylo package (Jombart et al. 2010) in R 2.10.1 (R Development Core Team 2008).

Table 1. The geographical locations and sample sizes of males (m) and females (f) for the analysed crested newt populations

Locality	Country	Latitude	Longitude	m	f
Triturus dobrogicus				99	91
Ivanovo	Serbia	44°44′N	20°42′E	31	20
Novi Kneževac	Serbia	46°03′N	20°06′E	10	12
Ravenica	Serbia	44°43′N	19°58′E	10	16
Svetozar Miletić	Serbia	45°51′N	19°13′E	9	14
Slavonski Brod	Croatia	45°10′N	18°01′E	12	9
Orle	Croatia	45°41′N	16°14′E	15	14
Batevo	Ukraine	48°22′N	22°24′E	12	6
Triturus cristatus				80	108
Štubik	Serbia	44°17′N	22°21′E	14	15
Miroč	Serbia	44°29′N	22°20′E	18	17
Vorniceni	Moldova	47°09′N	28°27′E	18	29
Elizavetino	Russia	59°28′N	29°52′E	6	10
Kamenskoe	Ukraine	47°32′N	35°22′E	18	27
Syanki	Ukraine	49°01′N	22°54′E	6	10
Triturus carnifex				135	124
Žumberak	Croatia	45°47′N	15°29′E	14	20
Istarske Toplice	Croatia	45°22′N	13°51′E	24	16
Lički Osik	Croatia	44°36′N	15°25′E	10	9
Salakovci	Croatia	45°03′N	14°05′E	20	15
Svetvinčenat	Croatia	45°05′N	13°53′E	19	16
Štanjel	Slovenia	45°49′N	13°50′E	12	18
Podstrmec	Slovenia	45°48′N	14°34′E	25	17
Turjanci	Slovenia	46°37′N	16°04′E	11	13
Triturus macedonicus				145	184
Bjeloši	Montenegro	42°22′N	18°54′E	14	20
Donji Štoj	Montenegro	42°23′N	19°06′E	15	13
Donji Lokanj	Montenegro	41°54′N	19°16′E	19	20
Jezero	Montenegro	42°24′N	18°50′E	12	18
Gornji Ceklin	Montenegro	42°21′N	18°59′E	19	29
Rid	Montenegro	42°23′N	18°58′E	15	18
Kostobačilo	FYR Macedonia	41°03′N	20°48′E	29	32
Kerkyra	Greece	39°41′N	19°49′E	6	13
Lake Prespa	Greece	40°51′N	21°08′E	5	12
Seli	Greece	40°34′N	21°57′E	5	4
Zygos	Greece	39°53′N	21°17′E	6	5
Triturus karelinii				31	30
Maikop	Rusia	44°37′N	40°07′E	19	26
Demerdzhi	Ukraine	44°45′N	34°24′E	12	4
Triturus arntzeni				32	34
Berovo	FYR Macedonia	41°43′N	22°52′E	9	13
Agios Kosmas	Greece	41°06′N	24°40′E	8	6
Alistrati	Greece	41°05′N	24°00′E	5	5
Dadia	Greece	41°08′N	26°09′E	6	6
Kentriko	Greece	41°10′N	22°54′E	4	4
Grand total				522	571

To test the phylogenetic dependence of traits, the final variable data set included: (1) one body-size variable, the geometric mean of all body measures for each specimen, and (2) five body-shape variables (Mosimann transformed): SVL, HW, FLL, HLL and interlimb distance.

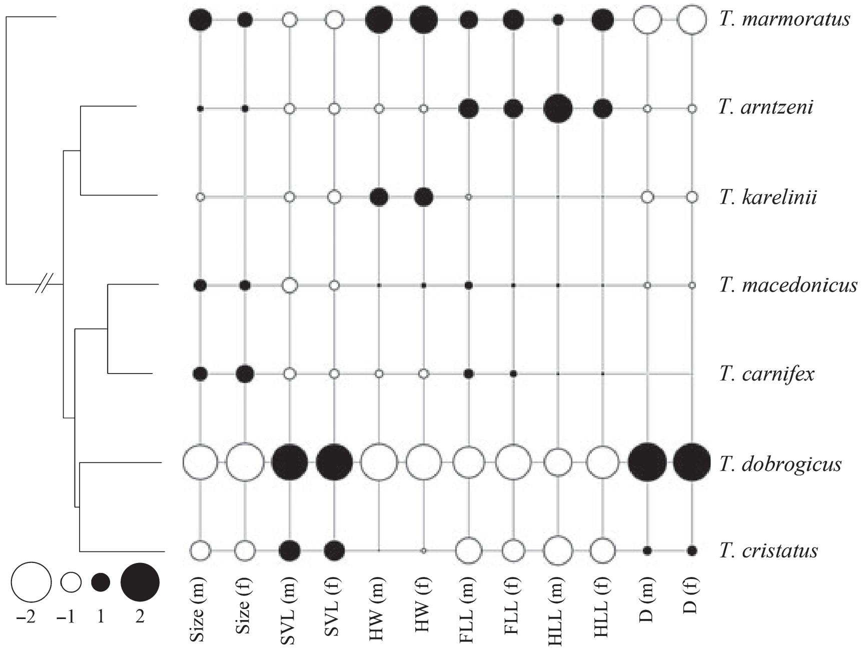

Fig. 1. Analysis of the male (m) and female (f) adult body size and shape traits in a phylogenetic framework. The scale shows circles with sizes proportional to the values of the orthonormal vectors (white and black for negative and positive values, respectively)

After checking for potential phylogenetic dependence in the data set, we used the morphometric data to test for the existence of two or more distinct body forms in crested newts. We assumed the existence of a hierarchy of morphological variation at the following levels (in decreasing order): (1) among presumed body forms, (2) among species, (3) among populations and (4) within populations. If the majority of character variation was captured at the level of body form, we accepted the presence of body forms. The three main steps to test for body form existence were (1) defining the body form models, (2) estimating the variance components in each model and (3) determining the most probable model among a set of potential models. We conducted a principal components analysis (PCA), with the species as the basic units, to define body form models, summarize overall patterns of shape variables and visualize the pattern of species grouping. Utilizing these results, we raised and tested seven models of species clustering (Table 2).

Table 2. Seven models of crested newts species clustering. Numbers represent species affiliation to species clustering

	I	II	III	IV	V	VI	VII
Triturus dobrogicus	1	1	1	1	1	1	1
Triturus cristatus	2	2	2	2	2	1	1
Triturus carnifex	3	3	3	2	2	2	2
Triturus macedonicus	3	3	3	2	2	2	2
Triturus arntzeni	3	4	3	2	2	3	2
Triturus karelinii	3	4	4	3	2	3	2

Estimation of the variance components in each model was performed using a PROC MIXED procedure (SAS 9.1 for Windows, SAS Institute Inc., Cary, NC, USA), with partitioned phenotypic variances in size and shape between body forms, among species nested within body forms and among populations nested within species. A nested ANOVA (PROC GLM with type III sums of squares, SAS 9.1) was

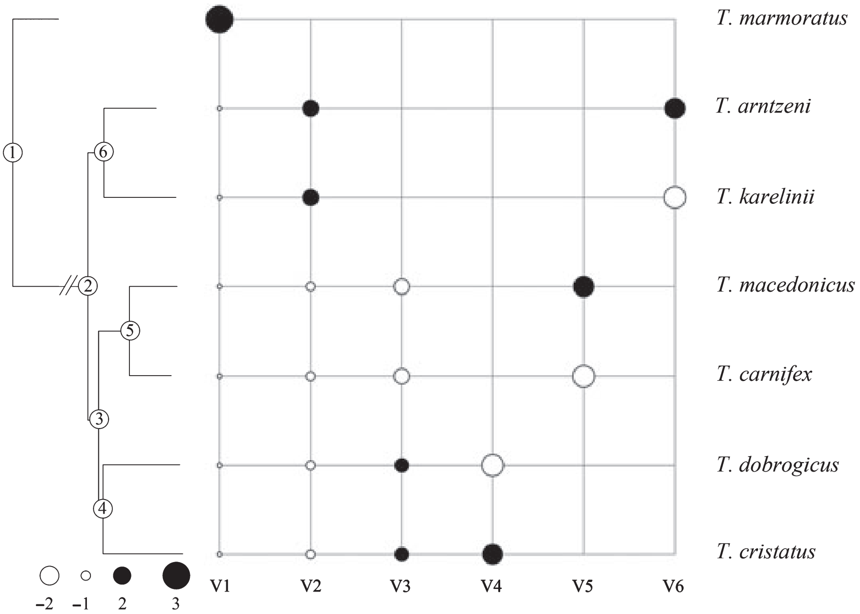

Fig. 2. Variance decomposition of six quantitative morphological traits in crested newts across the orthonormal basis defined by the phylogenetic tree topology. The scale shows circles with sizes proportional to the values of the orthonormal vectors (white and black for negative and positive values, respectively)

used to test for differences in the size and shape at different hierarchical levels (between body forms, among species and among populations). To account for multiple testing, we used the Bonferroni correction at a significance level of 0.05. To determine the most probable model among a set of potential models, the second-order Akaike's information criterion (ΔAIC_c) and Akaike weights (w_i) (Burnham and Anderson 2002) were used. Akaike weight represents the probability that a model is the best among those being considered for the data set (Burnham and Anderson 2002).

To test for the existence of two or more distinct body forms, the variables for the subsequent analyses were selected according to criteria of redundancy ($r > 0.8$). The final data set included: (1) one

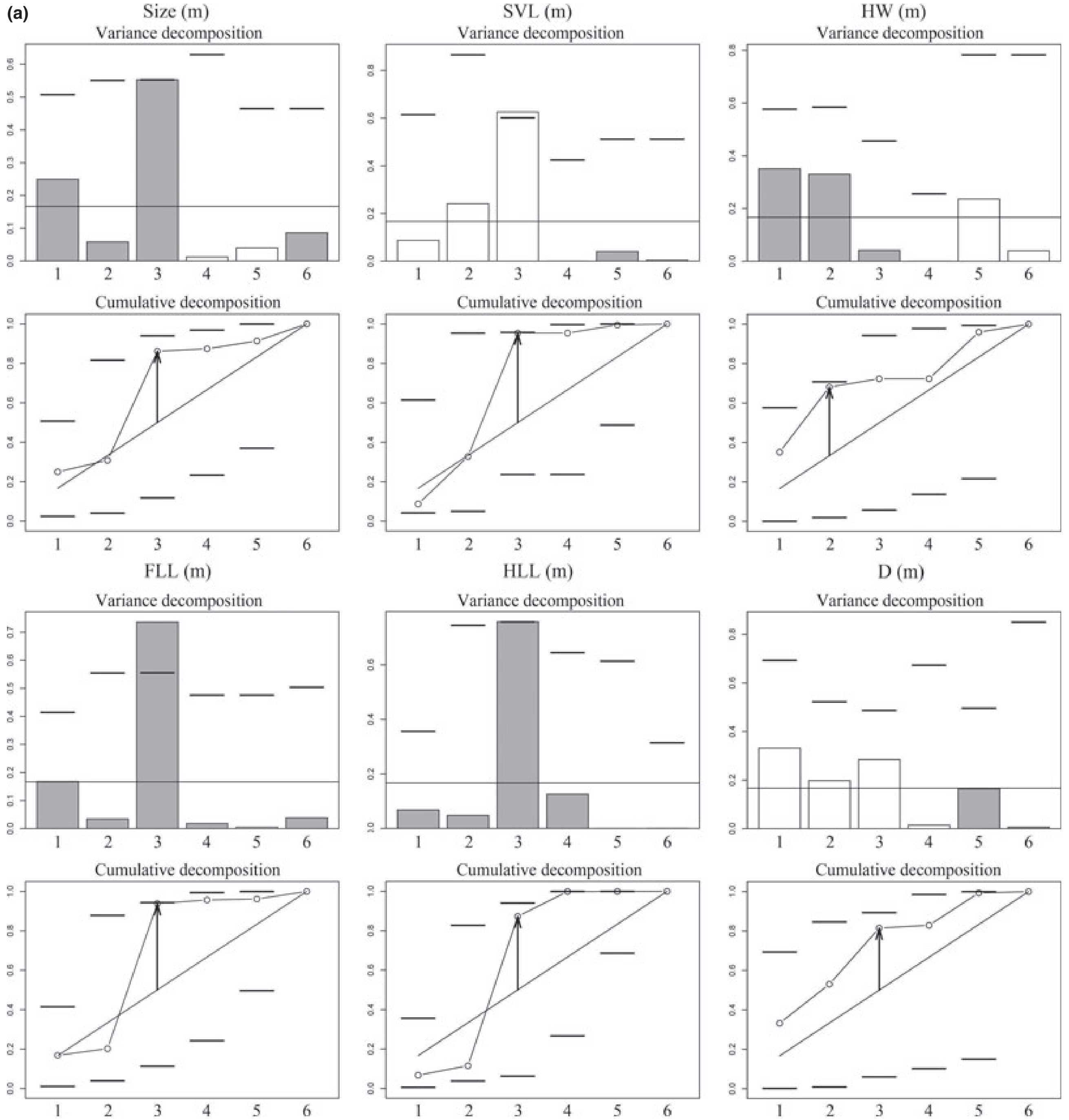

Fig. 3. Variance decomposition of six quantitative morphological traits in males (a) and females (b) using the orthogram plot (upper panel) and the cumulative orthogram plot (lower panel). In the orthogram plots, the abscise gives the number of the vectors associated with nodes, while the ordinate shows the contribution of the vector to the variance of the trait given by the squared regression coefficient (white and grey for positive and negative coefficients, respectively); dashes correspond to the upper confidence limit at 5% deduced from 999 Monte Carlo permutations, and the solid line represents the mean value. In the cumulative orthogram plots, the ordinate shows the cumulated contribution of successive vectors to the variance; circles represent the observed value of cumulated squared regression coefficients; the solid diagonal line represents the expected value under the absence of phylogenetic dependence; and dashes correspond to the bilateral 95% confidence interval. The vertical arrow indicates the position of maximum deviation from the expected value (diagonal line)

body-size variable, the geometric mean of all body measures for each specimen, and (2) five body-shape variables (Mosimann transformed): HW, HL, FLL, interlimb distance and TL.

Results

Phylogenetic signal in morphometric traits

Tree topology, together with vectorial basis (Fig. 2), allowed us to identify which vector was accounted to which node and, therefore, obtain a ranking of the nodes. The orthograms for males and females had similar patterns of decomposition of trait variance for morphometric traits (Fig. 3a,b). The orthograms showed that, for both sexes, vector 3 explained the greatest part of the variance for body size, SVL, FLL and HLL. For these four traits, the third vector departed from the expected value under the hypothesis of absence of phylogenetic dependence (given by the solid line in Fig. 3a,b, variance decomposition) and peaked outside of the confidence limit (given by the dashes in Fig. 3a,b, variance decomposition). The cumulative orthogram (Fig. 3a,b, cumulative decomposition) confirmed the predominance of the third vector in the variance distribution and its maximal departure from the expected value under the absence of phylogenetic dependence. However, of the four statistics tested (R2Max, SkR2k, $D_{\max}$

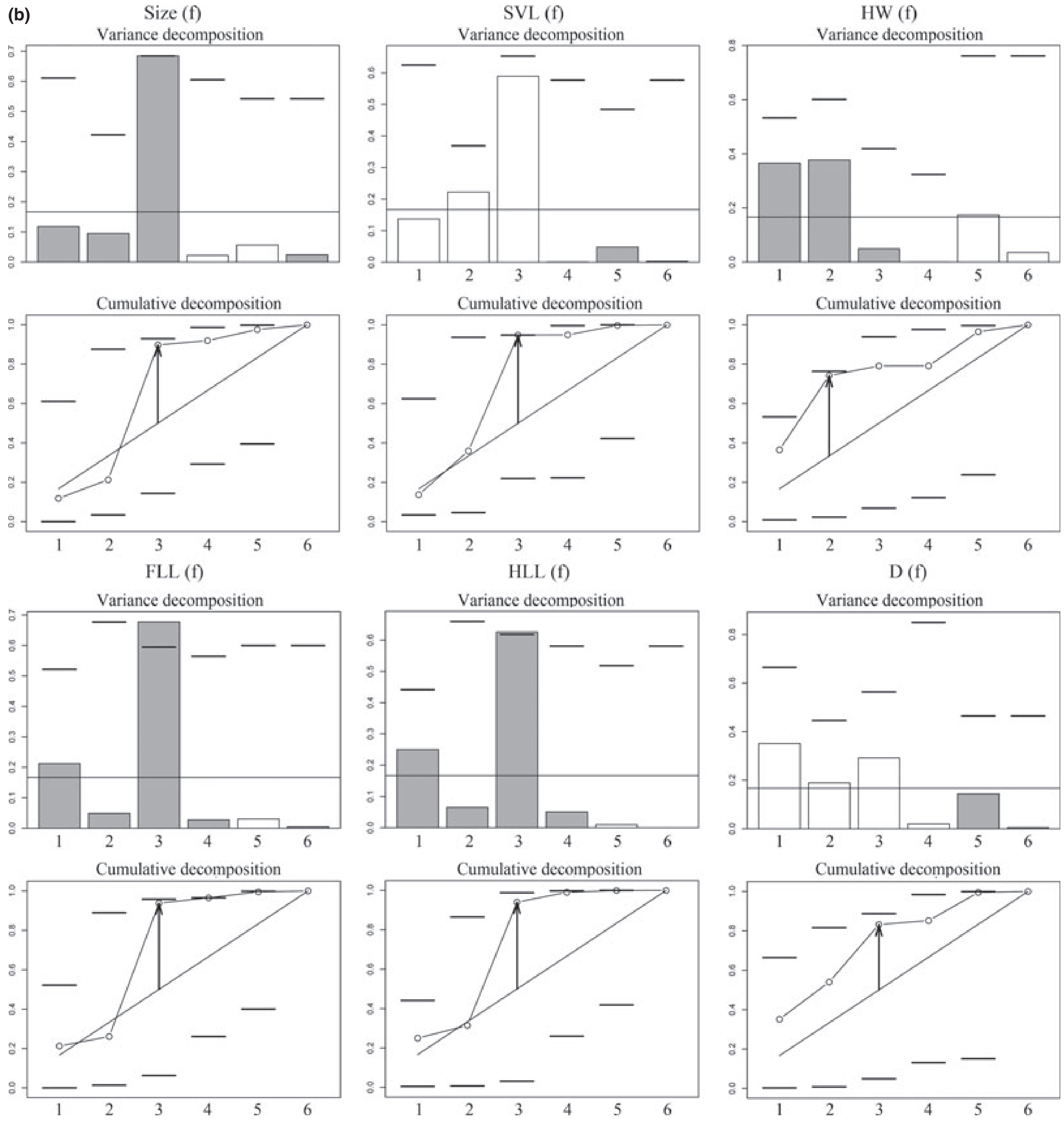

Fig. 3. (Continued)

Table 3. Testing against phylogenetic dependence, as defined by Ollier et al. 2006

Test	Body size		Snout-vent length		Head width		Forelimb length		Hindlimb length		Interlimb distance	
	Obs	p	Obs	p	Obs	p	Obs	p	Obs	p	Obs	p
R2Max												
m	0.55	0.25	0.63	0.12	0.35	0.79	0.74	**0.03**	0.76	0.10	0.33	0.96
f	0.68	0.09	0.59	0.31	0.38	0.68	0.68	0.08	0.63	0.09	0.35	0.95
SkR2k												
m	2.79	0.75	2.68	0.74	2.56	0.84	2.77	0.81	2.95	0.70	2.50	0.82
f	2.88	0.73	2.61	0.77	2.35	0.93	2.63	0.79	2.50	0.88	2.43	0.84
$D_{\max}$												
m	0.36	0.14	0.45	0.13	0.35	0.18	0.44	0.09	0.37	0.09	0.31	0.25
f	0.40	0.26	0.45	0.23	0.41	0.05	0.44	0.10	0.44	0.08	0.33	0.19
SCE												
m	0.19	0.47	0.32	0.37	0.22	0.47	0.31	0.19	0.33	0.13	0.22	0.40
f	0.26	0.36	0.31	0.38	0.32	0.30	0.31	0.29	0.33	0.22	0.25	0.36

Significant p values in bold. m, males; f, females.

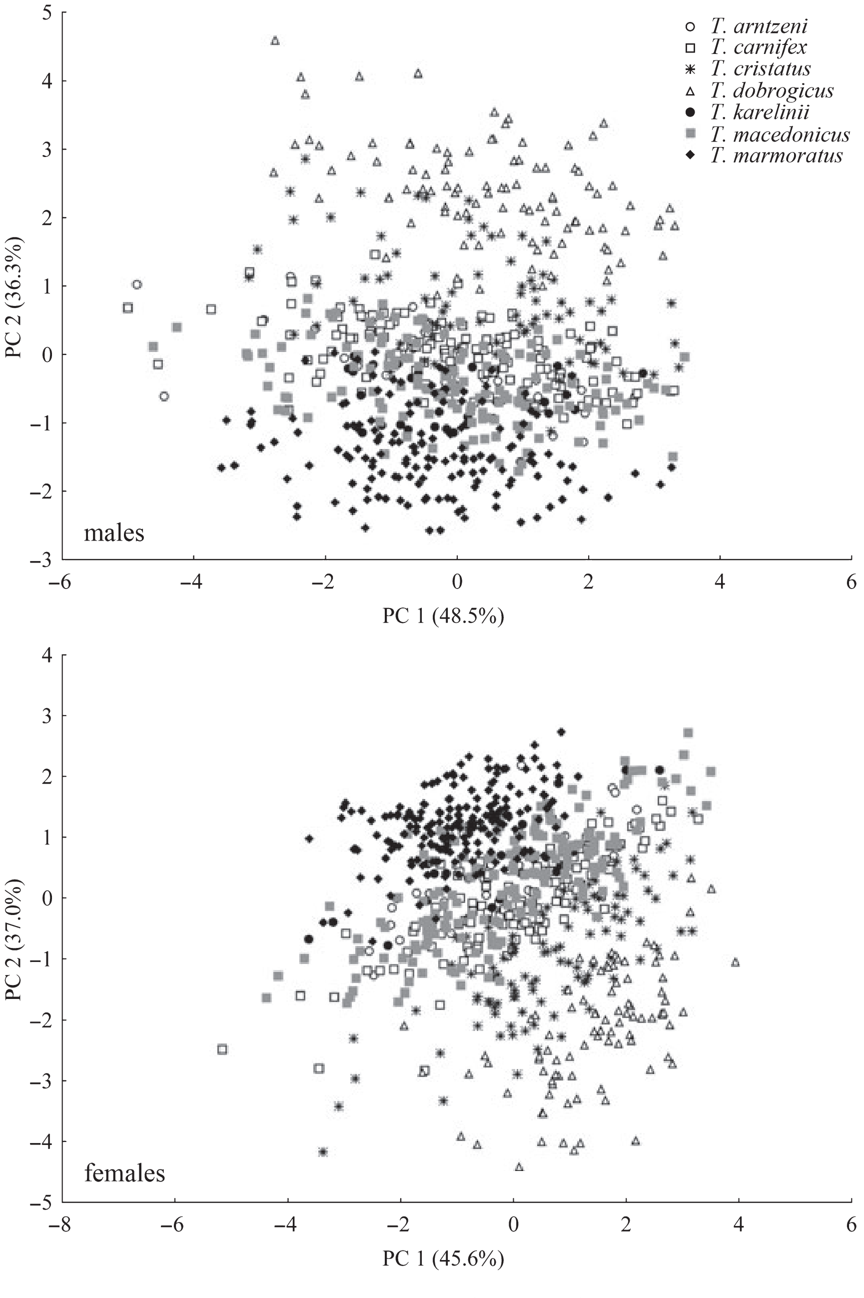

Fig. 4. Principal component analysis of shape variables for *Triturus* newts

and SCE), only R2Max was significant for FLL in males (Table 3), indicating that a single punctual modification of the trait occurred at a particular node. In summary, a single punctual event occurred at node 3, between the *T. dobrogicus* + *T. cristatus* and *T. macedonicus* + *T. carnifex* lineages, with shortening of the forelimbs in *T. dobrogicus* and *T. cristatus*.

A search for body forms

As the testing for phylogenetic dependence demonstrated an absence of phylogenetic signal in the analysed phenotypic traits, there was no need for phylogenetic correction of the morphological data. To identify morphotypes, an exploratory analysis of body size and body shape was performed. A PCA of body shape variables clearly showed the complete segregation of *T. dobrogicus* and *T. marmoratus* individuals for both sexes (Fig. 4). Specimens of four species (*T. carnifex*, *T. macedonicus*, *T. arntzeni* and *T. karelinii*) grouped together in terms of body shape variables, while *T. cristatus* individuals occupied an intermediate position between *T. dobrogicus* and the other crested newt species.

To statistically discriminate species groups in terms of multivariate body size and shape, we created seven models of species clustering. On each model, a nested ANOVA was applied to evaluate the differences in size and shape at different hierarchical levels (between body forms, among species and among populations). This analysis revealed highly significant differences ($p < 0.0001$) between body forms, among species nested within body forms and among populations nested within species for all models, with the exception of FLL and TL (Tables 4 and 5). We found no significant differences among populations in FLL of males in all tested models and no differences between species for three of seven models. In five of seven tested models for females, non-significant differences were found between species. In addition, we found no significant differences between species in TL in three and five models of seven tested in males and females, respectively (Tables 4 and 5).

For all models, the body size and shape variables showed a consistent patterns of variance partitioning on different hierarchical levels but not for different variables within each model (e.g. for the body size, D and HW, most of the variance was observed between body forms, for the HL among populations and for the FLL and TL within populations) (Tables 4 and 5).

Most of the variance was detected between body forms for size, D and HW. Other variables that showed the highest variance within populations (FLL and TL) and between populations (HL) for both sexes (Tables 4 and 5) did not contribute at the same level to body form differentiation. As most of the partitioned variance for body forms in the analysed models was found for body size, D and HW, we extracted these three traits as the traits defining body form in crested newts.

The best fitting model describing variation in body size for both sexes was found to be model I: (1) *T. dobrogicus*, (2) *T. cristatus* and (3) *T. macedonicus* + *T. carnifex* + *T. arntzeni* + *T. karelinii*. For the D and HW, the best model for both sexes appeared to be model VI: (1) *T. dobrogicus* + *T. cristatus*; (2) *T. macedonicus* + *T. carnifex*; and (3) *T. arntzeni* + *T. karelinii* (Table 4). However, several other models were close to the best model ($\Delta AIC_c < 2$), both for size and shape. Females appeared to differ less among the models than did males (see models with $\Delta AIC_c < 2$, and Akaike weight for the size, D and HW, Table 6).

Discussion

A key insight of this study is that in crested newts, body size, interlimb distance and head width have the main impacts on body form. This finding is contrary to previous statements that the ratio of interlimb distance and forelimb length was the critical factor describing interspecific differentiation in body form (e.g. Wolterstorff 1923; Arntzen and Wallis 1999). Furthermore, three body-shape morphotypes (*T. dobrogicus* + *T. cristatus*, *T. carnifex* + *T. macedonicus* and *T. karelinii* + *T. arntzeni*) and three body-size morphotypes (*T. dobrogicus*, *T. cristatus* and all other crested newts) were recognized as the most likely models that describe body forms in these newts.

The mapping of body form traits over crested newt phylogeny strongly suggests that the ancestral crested newt body form is characterized by a robust body with a short trunk and a wide head. This ancestral pattern is shown by the species *T. karelinii* and *T. arntzeni*. An outgroup species in our analyses, *T. marmoratus*, is even more robust. Evolutionary changes are observed in the other crested newts. Both *T. carnifex* and *T. macedonicus* show a preservation of size, but mild elongation compared to the ancestral body form. *Triturus cristatus* shows size reduction and body elongation. *Triturus dobrogicus* has the most derived pattern of size reduction and body elongation.

The most prominent evolutionary changes in body form of the European newts in general are related to body elongation (Gvoždik and Van Damme 2006). Vertebral column elongation is not the only possible contributor to this process; both an increase in head length and a decrease in the depth of the body can similarly result in body form elongation (e.g. Caldwell 2003). Here, we confirm that head length does not contribute to body elongation in crested newts. Therefore, crested newts follow the general rule that axial elongation is associated with vertebral column elongation. Crested newts accomplish vertebral column elongation by increasing the vertebral number (e.g. Arntzen and Wallis 1999), as is the case in many vertebrate groups (e.g. Polly et al. 2001; Ward and Brainerd 2007). Another way to elongate the vertebral column is by elongating the vertebrae themselves (Ward and Brainerd 2007), and a few cases are known in caudate amphibians (*Lineatriton* spp., Plethodontidae) (Parra-Olea and Wake 2001). Whether vertebrae elongation further reinforced the body form elongation in crested newts has not been explored yet.

With regard to body elongation, the developmental modularity of axial patterning is critical from an evolutionary and ontogenetic point of view (e.g. Caldwell 2003). Our study provides insight into axial patterning in crested newts and evaluates the influence of traits (i.e. head, trunk and tail lengths) that potentially act on body elongation. Only trunk length influences the pattern of body elongation in crested newts, indicating that the trunk and tail represent at least partially separated developmental modules. Such regional changes along the axial skeleton influencing elongation are common in amphibians and reptiles (e.g. Wake 1966; Polly et al. 2001). Further evaluation of the modularity of axial patterning in crested newts should be the subject of future studies.

Table 4. Results of nested ANOVAs and variance partitioning for six variables for seven potential body form models in males. Non-significant values in bold. Each variance component of each morphometric trait is expressed as the percentage of the total variance (%)

		I			II			III			IV			V			VI			VII		
Variable	Source of variation	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.
Head width (HW)	Body form	2	114.62	35.5	3	82.85	4.1	3	62.72	2.3	2	121.80	55.6	3	81.32	38.9	1	226.41	53.9	1	109.63	20.9
	Species (body form)	3	16.07	14.9	2	23.48	38.8	2	47.78	40.5	3	9.81	5.7	2	13.38	11.7	4	13.40	8.5	4	35.90	27.1
	Population (species)	33	2.70	6.0	33	2.70	6.9	33	2.70	6.9	33	2.70	4.7	33	2.70	6.0	33	2.70	4.6	33	2.70	6.3
	Error			43.6			50.3			50.3			34.0			43.4			33.0			45.7
Head length (HL)	Body form	2	66.28	0.0	3	85.27	27.3	3	82.33	19.7	2	76.33	0.0	3	53.56	0.0	1	130.34	11.5	1	33.05	0.0
	Species (body form)	3	66.37	23.0	2	15.19	0.0	2	39.03	8.7	3	54.59	23.0	2	65.06	23.0	4	55.20	17.4	4	71.45	23.0
	Population (species)	33	15.19	45.9	33	15.19	42.8	33	15.19	42.5	33	15.19	45.9	33	15.19	45.9	33	15.19	42.5	33	15.19	45.9
	Error			31.0			29.8			29.1			31.0			31.0			28.7			31.0
Forelimb length (FLL)	Body form	2	46.56	22.4	3	42.81	20.2	3	64.18	24.3	2	21.26	0.0	3	39.40	24.9	1	34.56	1.9	1	93.13	31.4
	Species (body form)	3	5.43	8.1	2	**4.55**	8.3	2	**3.36**	4.7	3	28.21	26.5	2	**0.25**	0.0	4	22.96	25.5	4	4.32	5.4
	Population (species)	33	**1.03**	0.0	33	**1.03**	0.0	33	**1.03**	0.0	33	**1.03**	0.0	33	**1.03**	0.0	33	**1.03**	0.0	33	**1.03**	0.0
	Error			69.5			71.5			71.0			73.5			75.1			72.6			63.2
Interlimb distance (D)	Body form	2	235.92	57.5	3	157.81	56.5	3	129.14	27.5	2	232.36	67.8	3	158.43	58.0	1	456.83	69.9	1	257.78	39.8
	Species (body form)	3	19.25	5.9	2	15.66	3.7	2	65.48	28.8	3	14.56	3.5	2	18.24	4.7	4	17.29	4.5	4	55.72	22.3
	Population (species)	33	3.97	6.7	33	3.97	7.2	33	3.97	8.0	33	3.97	5.2	33	3.97	6.8	33	3.97	4.7	33	3.97	6.9
	Error			29.9			32.7			35.8			23.5			30.5			20.9			31.0
Tail length (TL)	Body form	2	46.86	19.4	3	31.39	20.7	3	37.29	18.6	2	38.60	22.3	3	31.24	19.3	1	76.26	27.6	1	74.32	22.1
	Species (body form)	3	**2.38**	0.0	2	**0.60**	0.0	2	4.40	1.2	3	6.37	2.8	2	**2.65**	0.0	4	5.69	2.1	4	4.79	1.4
	Population (species)	33	3.39	12.7	33	3.39	12.1	33	3.39	13.1	33	3.39	12.4	33	3.39	13.0	33	3.39	11.7	33	3.39	12.6
	Error			67.8			67.1			67.1			62.5			67.7			58.7			63.9
Body size	Body form	2	188.31	47.9	3	145.30	44.1	3	172.05	34.9	2	159.15	37.6	3	131.47	41.9	1	314.31	53.9	1	290.20	48.4
	Species (body form)	3	4.25	0.0	2	5.28	0.0	2	21.97	7.0	3	31.92	10.1	2	**3.88**	0.0	4	24.14	5.8	4	17.03	3.6
	Population (species)	33	10.90	20.3	33	10.90	22.2	33	10.90	23.4	33	10.90	21.2	33	10.90	22.9	33	10.90	16.2	33	10.90	19.3
	Error			31.8			33.7			34.7			31.2			35.2			24.1			28.7
Variables with maximal variance at the body form level		D, body size			D, body size			Body size			HW, D, body size			D, body size			HW, D, body size			Body size		

Table 5. Results of nested ANOVAs and variance partitioning for six variables for seven potential body form models in females. Non-significant values in bold. Each variance component of each morphometric trait is expressed as the percentage of the total variance (%)

		I			II			III			IV			V			VI			VII		
Variable	Source of variation	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.	df	*F*	var.
Head width (HW)	Body form	2	55.02	24.0	3	43.02	0.0	3	41.29	3.0	2	55.52	45.9	3	37.02	29.7	1	109.69	45.7	1	61.8	16
	Species (body form)	3	12.55	15.0	2	17.97	33.3	2	30.00	30.7	3	11.04	5.8	2	14.43	11.0	4	10.86	9.7	4	22.92	21.9
	Population (species)	33	2.08	4.3	33	2.08	4.8	33	2.08	4.7	33	2.08	3.4	33	2.08	4.2	33	2.08	3.5	33	2.08	4.3
	Error			56.7			61.9			61.6			44.8			55.1			41.0			57.7
Head length (HL)	Body form	2	45.81	0.0	3	56.21	16.3	3	49.68	11.0	2	46.50	0.0	3	34.45	0.0	1	87.13	8.3	1	26.44	0
	Species (body form)	3	48.75	16.4	2	23.02	2.9	2	38.49	8.9	3	46.84	16.4	2	54.93	16.4	4	42.57	12.6	4	53.92	20.3
	Population (species)	33	14.37	47.0	33	14.37	45.1	33	14.37	44.9	33	14.37	47.0	33	14.37	47.0	33	14.37	44.5	33	14.37	43.8
	Error			36.6			35.7			35.3			36.6			36.6			34.6			35.8
Forelimb length (FLL)	Body form	2	56.11	32.2	3	51.55	30.0	3	77.17	30.8	2	34.33	5.8	3	45.50	27.1	1	64.35	22.1	1	111.69	40.6
	Species (body form)	3	**1.06**	0.0	2	**1.50**	0.9	2	**1.55**	0.3	3	29.31	22.6	2	**0.96**	0.0	4	21.99	15.4	4	**1.21**	0
	Population (species)	33	1.85	4.5	33	1.85	4.4	33	1.85	4.4	33	1.85	4.3	33	1.85	4.2	33	1.85	3.7	33	1.85	3.9
	Error			63.4			64.8			64.5			67.3			68.7			58.7			55.5
Interlimb distance (D)	Body form	2	185.44	55.4	3	129.47	52.0	3	136.69	29.2	2	180.57	62.9	3	126.68	54.9	1	358.73	66.7	1	253.13	43.4
	Species (body form)	3	**13.96**	4.4	2	14.68	4.5	2	55.23	24.0	3	20.63	4.9	2	17.46	4.3	4	18.08	5.0	4	44.55	17.4
	Population (species)	33	3.52	6.1	33	3.52	6.6	33	3.52	7.2	33	3.52	4.9	33	3.52	6.2	33	3.52	4.3	33	3.52	5.6
	Error			34.1			36.8			39.6			27.3			34.6			24.0			33.7
Tail length (TL)	Body form	2	10.20	4.2	3	7.35	5.5	3	8.51	4.6	2	11.57	26.1	3	7.80	16.6	1	18.94	8.3	1	11.23	3.7
	Species (body form)	3	3.59	0.0	2	**2.12**	0.0	2	**3.35**	2.5	3	**0.44**	0.0	2	**0.59**	0.0	4	**2.81**	0.0	4	8.17	3.4
	Population (species)	33	2.33	10.3	33	2.33	9.5	33	2.33	8.8	33	2.33	5.4	33	2.33	6.6	33	2.33	9.6	33	2.33	9.1
	Error			85.5			85.0			84.1			68.5			76.8			82.2			83.7
Body size	Body form	2	168.56	48.1	3	137.82	43.6	3	181.89	35.7	2	155.20	35.8	3	129.24	41.4	1	292.71	54.2	1	283.77	48.1
	Species (body form)	3	11.84	3.0	2	17.54	5.0	2	34.02	11.4	3	46.14	15.7	2	17.25	4.3	4	34.60	9.0	4	25.62	6.2
	Population (species)	33	9.78	19.0	33	9.78	20.1	33	9.78	20.7	33	9.78	19.1	33	9.78	21.3	33	9.78	14.4	33	9.78	17.1
	Error			29.9			31.3			32.2			29.4			33.0			22.4			28.6
Variables with maximal variance at the body form level		D, body size			D, body size			Body size			HW, D, body size			D, body size			HW, D, body size			D, body size		

Table 6. The second-order Akaike's information criterion differences (ΔAIC_c) and Akaike weights (w_i) for the seven proposed body form models

	Body size		Head width		Forelimb length		Interlimb distance		Head length		Tail length	
Hypothesis	m	f	m	f	m	f	m	f	m	f	m	f
Delta AICc (ΔAIC_c)												
I	**0**	**0**	1.5	1.3	8.1	2.7	1.3	0.4	**0**	**0**	**0**	4.6
II	3.2	2.9	2.7	0.3	9.2	8.1	2.2	1.7	**0**	0.6	1.2	4.2
III	6.3	2.9	2.7	2	7.5	5.3	4.3	3.4	1.2	1.3	3.9	6.5
IV	7.1	3.6	0.4	0.3	8	10.6	1.1	1.7	**0**	**0**	5.4	**0**
V	1.6	1.8	2.1	1.8	**0**	2.8	2.7	2.3	**0**	**0**	1.9	2.5
VI	5.4	1.9	**0**	**0**	10.1	10	**0**	**0**	1.9	1.9	3.9	3.4
VII	4.1	0.8	2.2	1.5	6.6	**0**	3.4	2.3	**0**	**0**	2.8	6.7
Akaike weight (w_i)												
I	**52.1**	**32.3**	13.5	11.6	1.6	16.1	17.5	23.5	**16.8**	**17.7**	**39.5**	5.7
II	10.5	7.6	7.4	19.2	0.9	1.1	11.1	12.2	**16.8**	13.1	21.7	6.9
III	2.2	7.6	7.4	8.2	2.1	4.4	3.9	5.2	9.2	9.2	5.6	2.2
IV	1.5	5.3	23.4	19.2	1.6	0.3	19.3	12.2	**16.8**	**17.7**	2.7	**56.6**
V	23.4	13.1	10.0	9.1	**89.9**	15.4	8.7	9.1	**16.8**	**17.7**	15.3	16.2
VI	3.5	12.5	**28.6**	**22.3**	0.6	0.4	**33.4**	**28.7**	6.5	6.8	5.6	10.3
VII	6.7	21.6	9.5	10.5	3.3	**62.3**	6.1	9.1	**16.8**	**17.7**	9.7	2.0

Highest ranking models in bold. m, males; f, females.

Contrary to other groups with evolutionary axis elongation, in which elongation is followed by an increase in body size, body size actually decreased in *T. cristatus* and *T. dobrogicus*. The smaller body size might be related to a different life history strategy compared to the other crested newt species, such as a slower growth rate and/or the reaching of maturity at an earlier age. The scarcely available data do not rule out such a possibility (e.g. Cogălniceanu and Miaud 2002). Additionally, body elongation could be a necessary consequence of evolutionary size reduction to maintain fecundity (e.g. Griffith 1990). The adaptive significance of elongation and its role in crested newt lifestyles still remains to be elucidated.

The divergence of *T. dobrogicus* seems to have been accompanied by a niche shift, corresponding to the invasion of a new ecospace (e.g. Arntzen 2003; Arntzen et al. 2007). At the moment, the most compelling argument for this scenario is that patterns of parallel evolution for various phenotypic traits point to an overall influence of the environment on the evolution of crested newts (Cvijanović et al. 2009; Wielstra and Arntzen 2011). A scenario in which phylogenetic constraints shape body form evolution seems less likely, as key characters that define body form (body size, interlimb distance and head width) do not reflect phylogenetic relatedness.

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Zusammenfassung

Die Evolution der erwachsenen Körperform vom nördlichen Kammmolch (Triturus cristatus Superspecies)

In dieser Arbeit untersuchen wir die Evolution der erwachsenen Körperform vom nördlichen Kammmolch (*Triturus cristatus* superspecies). Aufgrund der Analyse von sieben morphometrischen Eigenschaften haben wir festgestellt, dass die Körpergröße, Abstand zwischen den Extremitäten und die Kopfbreite die Körperform definieren. Keine der analysierten morphometrischen Eigenschaften weist die Anwesenheit des phylogenetischen Signals auf. Es sind drei Morphotypen in Bezug auf die Körperform (*T. dobrogicus* + *T. cristatus*, *T. carnifex* + *T. macedonicus*, und *T. karelinii* + *T. arntzeni*) und drei Morphotypen in Bezug auf die Körpergröße (*T. dobrogicus*, *T. cristatus* und alle übrigen Kammmolche) zu unterscheiden. Der Vorfahrtypus (großer Körper mit kurzem Rumpf und breitem Kopf) zeichnet *T. karelinii und T. arntzeni* aus. *Triturus carnifex* und *T. macedonicus* haben einen einigermaßen veränderten Phänotyp (großer Körper und breitem Kopf, wobei der Körper leicht verlängert ist). Der abgeleitete Phänotyp zeichnet sich durch die Reduktion der Körpergröße und ausgeprägte Verlängerung des Körpers bei *T. cristatus* und insbesondere bei *T. dobrogicus* aus. Die Verlängerung des Körpers bei nördlichen Kammmolchen erfolgt durch die Verlängerung des Rumpfes, jedoch nicht des Schwanzes. Im Gegensatz zu anderen Tetrapoden wurde die evolutive Verlängerung der Hauptachse bei den nördlichen Kammmolchen von der Körperverkleinerung begleitet. Wir vertreten die Hypothese, dass die Evolution des Körpers bei den Kammmolchen von der Ökologie geführt wird.

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