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Vertebral shape and body elongation in *Triturus* newts

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ABSTRACT

Body elongation in vertebrates is often related to a lengthening of the vertebrae and an increase in their number. Changes in the number and shape of vertebrae are not necessarily linked. In tailed amphibians, a change in body shape is mostly associated with an increase in the number of trunk and tail vertebrae. Body elongation without a numerical change of vertebrae is rare. In *Triturus* aquatic salamanders body elongation is achieved by trunk elongation through an increase in the number of trunk vertebrae. We used computed microtomography and three-dimensional geometric morphometrics to document the size, shape and number of trunk vertebrae in seven *Triturus* species. The data suggest that body elongation has occurred more frequently than body shortening, possibly related to a more aquatic versus a more terrestrial locomotor style. Our results show that body elongation is achieved through an increase in the number of trunk vertebrae, and that interspecific differences in vertebral shape are correlated with this pattern of elongation. More gracile trunk vertebrae were found in the more elongated species. The shape differences are such that single trunk vertebrae can be used for the identification of species with a possible application in the identification of subfossil and fossil material.

1. Introduction

Evolution of the vertebrate body form is largely achieved by changes in the axial skeleton. In many instances body proportions are reflected by the number of vertebrae per region and/or the morphology of the vertebrae (Wake, 1966; Lindsey, 1975; Ward and Brainerd, 2007; Woltering, 2012). For example, among the ray-finned fishes, an eel-like body form evolved multiple times from the dorsoventrally deeper body form. Such body elongation is achieved by an increase in the number of vertebrae, the elongation of vertebrae or a combination of both (Reece and Mehta, 2013). In sauropod dinosaurs, the evolution of their extremely long neck involved an increase in the number and length of cervical vertebrae (Taylor and Wedel, 2013). In birds, short-necked swifts have 13 cervical vertebrae (Starck, 1979) while the long-necked swans have 22–25 cervical vertebrae (Woolfenden, 1961). Conversely, the number of vertebrae may be fixed and be independent of body shape. A striking example is the number of cervical vertebrae in mammals, which is 7 in the whale as well as in the giraffe (Galis, 1999), suggesting some evolutionary constraint on the number, but not on the size and shape of the cervical vertebrae (Narita and Kuratani, 2005).

The vertebral column of tailed amphibians (Amphibia, Urodela) consists of the following five regions (Mivart, 1870): (i) the cervical region, consisting of a single cervical vertebra, namely the atlas; (ii) the trunk region with all presacral vertebrae to which the ribs are attached; (iii) the sacral region with a single vertebra bearing stout transverse processes that serve for the attachment of the sacral ribs and the pelvic girdle; (iv) the caudosacral region with typically two or three stout vertebrae; and (v) the caudal region with all remaining vertebrae. In salamanders, the numbers of trunk and caudal vertebrae vary markedly among species, giving rise to a wide range of phenotypes, from short-bodied and long-tailed to long-bodied and short-tailed (Arntzen et al., 2015). Trunk elongation by a lengthening of the vertebrae, without a change in their number, is only known for the plethodonthid *Pseudoeurycea lineola* (Wake, 1991; Parra-Olea and Wake, 2001). Trunk vertebrae are morphologically more or less uniform, but some variation in their size along the vertebral column was found (Worthington and Wake, 1972; Wake and Lawson, 1973). Vertebral shape in tailed amphibians has been subjected to several comparative analyses, mostly in the context of taxonomy (Ratnikov and Litvinchuk, 2007, 2009) and in the study of subfossil material (Estes and Darevsky, 1977; Estes,

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1981), but there is a paucity of information on the evolutionary relationship in the number and shape of vertebrae.

Among salamandrid salamanders, marked changes in body shape and vertebral number characterize the group of large-bodied *Triturus* newts (Arntzen et al., 2015), which make this group suitable for exploring the relationship between changes in the number and shape of vertebrae. Body elongation in *Triturus* newts was first recognized by Wolterstorff (1923), who proposed a morphological index (Wolterstorff index, WI = the ratio between forelimb length and interlimb distance) as a discriminator for *Triturus* species. *Triturus* morphotypes range from the stout and long-legged *Triturus marmoratus* and *T. pygmaeus*, with the smallest values of the WI, to the slender and short-legged *T. dobrogicus* with the highest WI (Arntzen and Wallis, 1999). In *Triturus* newts, the numbers of trunk vertebrae directly correspond to the trunk length. Eight currently recognized species can be classified into a series of five morphotypes (Arntzen and Wallis, 1999; Arntzen, 2003). The number of trunk vertebrae in *Triturus* varies from 12 in *T. marmoratus* and *T. pygmaeus*, to 13 in *T. karelinii* and *T. ivanbureschi*, 14 in *T. macedonicus* and *T. carnifex*, 15 in *T. cristatus* and 16 or 17 in *T. dobrogicus* (Arntzen, 2003; Arntzen et al., 2015). There is some intraspecific variation in the number of trunk vertebrae in *Triturus* newts, but the large majority of individuals do not deviate from the modal number of their species (Slijepčević et al., 2015). *Triturus* species also differ in their ecological preferences. The more terrestrial species *T. marmoratus* that annually spends only two months in the water has a short and stout body with 12 trunk vertebrae whereas, at the other extreme, *T. dobrogicus* is the most aquatic species that spends six months in the water and has an elongated and slender body with 16 or 17 trunk vertebrae (Arntzen, 2003). Body elongation in *Triturus* has been regarded as an adaptation for locomotor performance in the aquatic environment (Arntzen and Wallis, 1999; Ivanović and Arntzen, 2014) although no clear association between body shape and locomotor performance was found (Gvoždík and Van Damme, 2006).

We used micro CT-scanning to document the size and shape of the first and the second trunk vertebrae in the genus *Triturus*. Three-dimensional (3D) geometric morphometrics was used to analyze the data in a phylogenetically based comparative analysis. We chose to work on the first and second trunk vertebrae because these two vertebrae appear to be less subject to evolutionary change than the posterior part of the vertebral column (Worthington and Wake, 1972; Wake and Lawson, 1973) and to ensure that homologous structures were compared among species with different numbers of trunk vertebrae in the vertebral column. Specifically we investigated:

- changes in the number, size and shape of the trunk vertebrae across species;
- allometric variation in vertebral shape;
- whether individual vertebrae can be assigned to particular species.

2. Materials and methods

2.1. Species and samples

3D models of the first and second trunk vertebrae were constructed for 146 alcohol-preserved newts from the herpetological collection of Naturalis Biodiversity Center, Leiden, representing seven out of eight currently recognized *Triturus* species plus *Calotriton asper*, the sister genus of *Triturus* (Steinfartz et al., 2007; Arntzen et al., 2015). Information on sampling localities, sample sizes and museum collection records are given in Table S1 in the supplementary online Appendix. A time-calibrated phylogeny (Fig. 1) for the group of interest was taken from Arntzen et al. (2015).

2.2. Data collection and variables

All materials were scanned with a SkyScan 1171 micro computed tomography (CT)-scanner (SkyScan, Artselaar, Belgium)

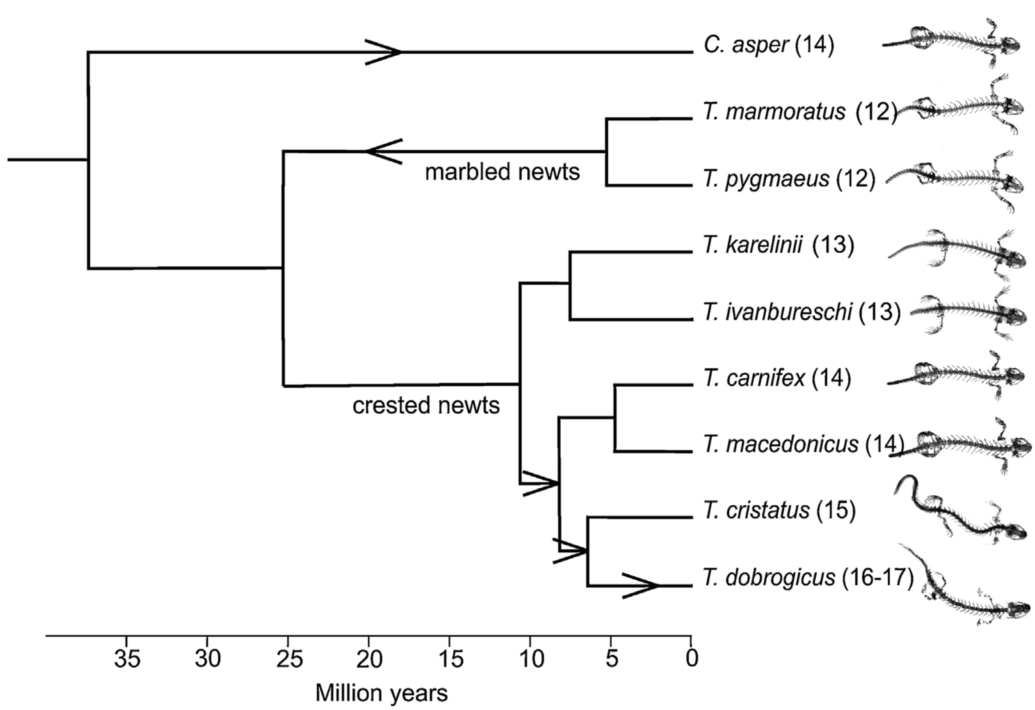

Fig. 1. Time-calibrated phylogeny of the genus *Triturus* and *Calotriton asper* with inferred changes in the modal number of trunk vertebrae (from Arntzen et al., 2015). Evolutionary increase in the number of trunk vertebrae is indicated by arrows pointing to the right, while decrease is indicated by arrows pointing to the left. Modal number of trunk vertebrae and thumbnail images of the skeletons are given next to the species names.