Short Communication

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A Simple Non-destructive Method for the Study of the Palatine Teeth of *Triturus* Newts (Amphibia: Salamandridae)

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Abstract: In order to overcome the restrictions and difficulties in using the palatine teeth as a taxonomic trait of crested newts (*Triturus cristatus* superspecies), a new method for documenting the palatine teeth in live individuals has been developed. The protocol is applicable in the field work as well. It makes possible the morphometric characterization of the palatine teeth and the further statistical analysis of the data obtained. For its purpose, a teeth impress is taken, which is then photographed. The selected traits are measured on the digital image by means of graphical software.

Key words: *Triturus*, methodology, palatine teeth, dental impressions

Introduction

According to the modern concept, genus *Triturus* Rafinesque, 1820, includes 8 species and one undescribed taxon of the species rank (Wielstra *et al*. 2013a, b). The species are very similar in their exterior morphological characteristics and species differentiation based only on this is often not reliable enough.

The arrangement of the teeth rows on the palate was used as a diagnostic characteristic in family Salamandridae for the first time by Schreiber (1875). Later, Nikolsky (1918) pointed out that the two rows of palatine teeth were parallel in *Triturus cristatus* (Laurenti, 1768), and their proximal ends were drawn apart in *Triturus karelinii* (Strauch, 1870). This difference between the two taxa was cited repeatedly (e.g. Bannikov *et al*. 1977, Kuzmin 1999), but there were only a few tangible investigations of this trait. Vallée (1959) ascertained differences among the palatine teeth of *Triturus marmoratus* (Latreille, 1800), *T. cristatus* and their natural hybrid (so-called *T. blasii*). The work of Viertel (1981) is the only detailed study of palatine teeth in the European representatives of the family Salamandridae, but it did not treat the particular species of the *Triturus cristatus* group (regarded them as subspecies of *T. cristatus*). In all cases, the studies carried out up to now were descriptive and contained no metric characteristics and statistical analyses of this trait. This was mainly due to the limited methodological possibilities for characterization of the palatine teeth.

The investigation of palatine teeth on bone preparations (e.g. Vallée 1959) requires the animals to be killed, which is unacceptable, especially with species of high conservation status, such as the crested newts. The palatine teeth arrangement can also be studied in X-ray photos (e.g. Tzankov, Stoyanov 2008), but X-raying is undesirable for living specimens, particularly because it takes high numbers to meet the excerpt volume requirements of the statistical analyses. Above all, the palatine teeth investigation of X-ray photos and bone preparations is impossible in field studies. Furthermore, the X-ray photos are relatively expensive, and the bone preparations are laborious to make. The study of palatine teeth by direct observations (e.g. Viertel 1981) yielded satisfactory results only when performed on fixed material. The direct observations of palatine teeth rows in living newts (e.g. Naumov, Tzankov 2008) are prob-

lematic, because these teeth are almost transparent and difficult to discern (Fig. 1D). On the other hand, the associated blood vessels are clearly visible and often produce a distorted picture of the teeth rows' form, although resembling it to a certain extent. In addition, the proximal ends of the teeth rows cannot be observed and photographed at right angles even when the lower jaw is gaped at the maximum. The lower jaw can be pulled down beyond the maximum functional angle in fixed specimens, but this is inadmissible *in vivo*.

In this work we describe a new method for documenting the palatine teeth on living newts, which is developed with the aim of overcoming the restrictions and difficulties of the methods applied up to now. The method is a modification of the idea of Carranza, Wade (2004) for the use of dental impressions: the authors obtained impressions of the teeth of *Pleurodeles poireti* (Gervais 1835) by filling the mouths of the animals (fixed material) with warm plasticine.

Description of the Method

The main points of the method are: taking a tooth impression, documenting it by digital photography, and measuring the traits in the digital image. The method consists of the following steps:

1. Preparation of matrix. A 40 x 4 mm plate is cut from a flexible plastic sheet ca. 1 mm thick. A uniform layer of soft plasticine is spread onto one of the surfaces of one of the ends of the plate. The area of this layer measures 10 x 4 mm, its thickness is 0.5 mm (Fig. 1A).

2. Taking the impression. It is desirable that the animal is pre-anesthetized, as handling of an active animal is much more difficult, requires experience and special attention not to traumatize the newt. The mouth of the animal is carefully opened using a flexible plastic spatula. The end of the plate coated in plasticine is pressed against the palatine teeth. In order to get a distinct impression, the newt's mouth is cautiously closed and gently pressed with a finger for 2–3 s to the lower wall of the mouth cavity in the sublingual area. The matrix is detached from the palate by pulling it down, while the mouth of the animal is opening. The matrix is then taken out of the mouth, taking care not to touch the palatine or other teeth again. The impression obtained that way is an exact negative copy of the palatine teeth.

3. Documenting. The matrix bearing the teeth impression is placed onto a measuring tape and then photographed by a digital camera with an appropriate focal distance for documenting small objects, or by a stereo-microscope equipped with a digital camera (Fig. 1A).

4. Image processing. The photos are sized and contrasted using graphic software to acquire the desired quality (Fig. 1B). The scale of the software is calibrated after the measuring tape in the photo, and the measures of interest are taken of the polygon formed by the two palatine teeth rows (Fig. 1C).

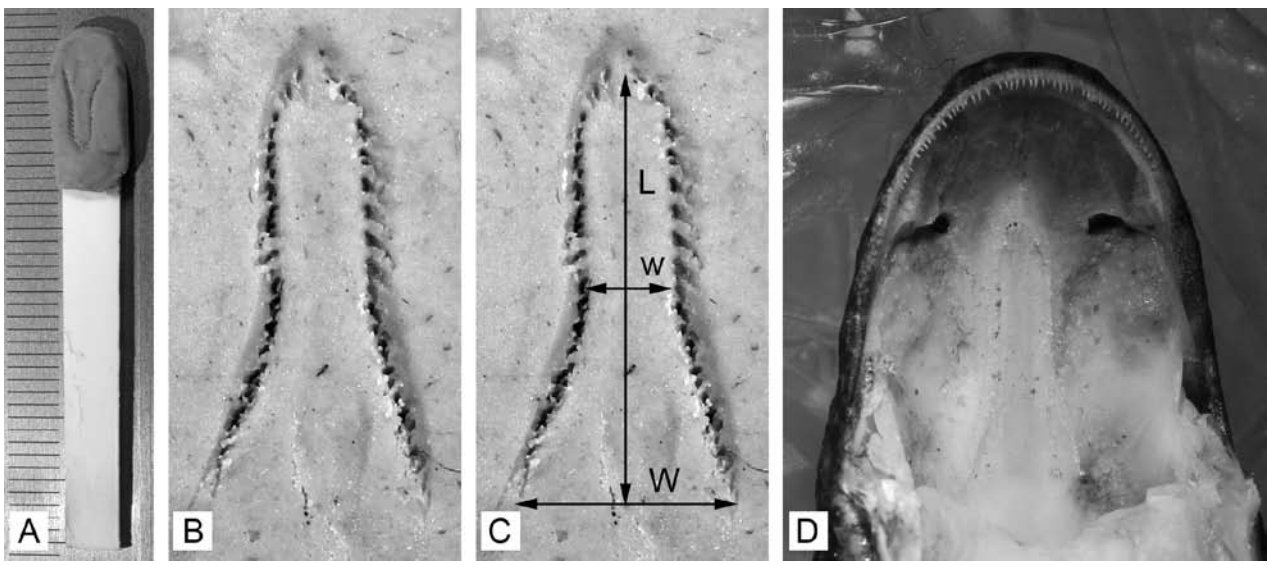

Fig. 1. A: Matrix with a teeth impression on a measuring tape. B: Processed image of the impression. C: The measuring setup of the polygon outlined by the teeth rows (L – polygon length; W – polygon width at the proximal ends of the teeth rows; w – middle width (the distance between the teeth rows at the middle of L)). D: The real view of the palatine teeth of the same individual (lower jaw fixed opened at over 90 degrees)

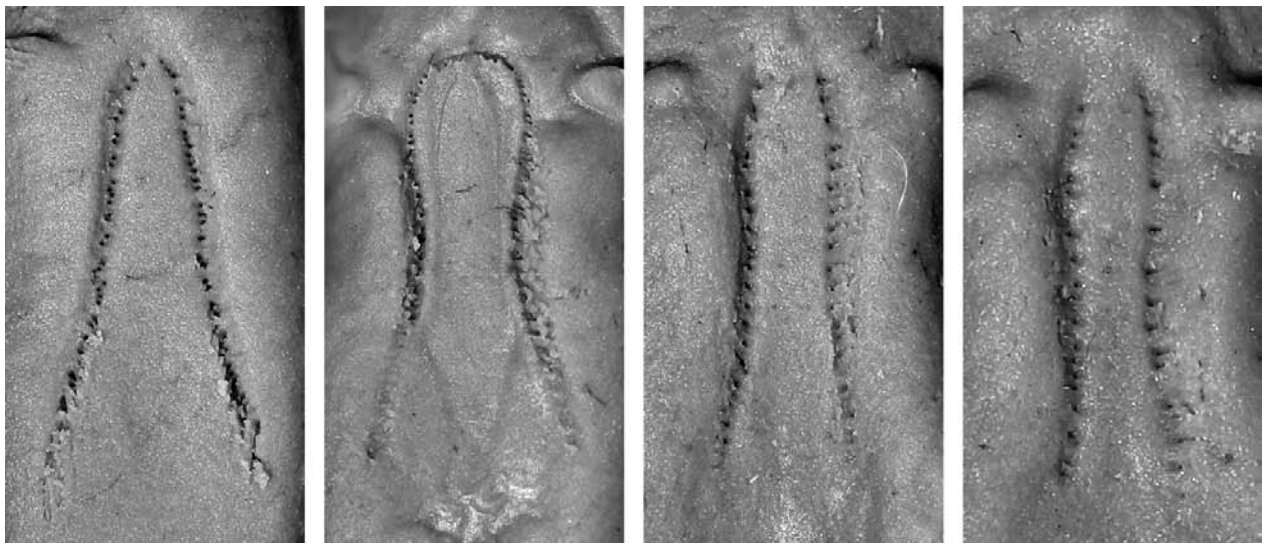

Fig. 2. Form of the teeth rows typical for the particular species, based on the studied material from Bulgaria. Left to right: *T. ivanbureschi, T. macedonicus*, *T. cristatus,T. dobrogicus*

Preliminary Results and Conclusion

Applying the method described above, the form of the palatine teeth rows was documented on 301 individuals of the genus *Triturus* collected from different areas in Bulgaria. The investigated animals belonged to 4 species: *Triturus dobrogicus* (Kiritzescu, 1903), *T. macedonicus* (Karaman, 1922), *T. cristatus*, and *T. ivanbureschi* Arntzen et Wielstra, 2013. Alongside the metric traits listed above (Fig. 1C), we also calculated some indices, which give an idea about the form of the polygon outlined by the two palatine teeth rows: polygon relative width (W/L); polygon relative middle width (w/L); and ratio of basal and middle widths (w/W).

Some exemplary images are presented in Fig. 2, corresponding to the most frequently occurring in the material studies types of teeth rows for the particular species. The greatest difference in the form of the teeth rows is observed between *T. ivanbureschi* and *T. dobrogicus* (the teeth rows form respectively a V-shaped or U-shaped figure). The other two species represent borderline cases: by the teeth rows form, *T. cristatus* is nearer to *T. dobrogicus*, and *T. macedonicus* is nearer to *T. ivanbureschi*. The results of the statistical analyses of the studied traits and indices will be published in a separate paper. The preliminary tests proved the traits involved to be efficient in distinguishing the studied species.

In our opinion, the described method avoids to a large extent the limitations of the other methods for the study of the palatine teeth in genus *Triturus*. The method is easy to apply and provides an objective study on live and uninjured animals. It does not require special equipment and can be applied in field conditions, as well as for studying museum materials.

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