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Kicking *Triturus arntzeni* when it's down: large-scale nuclear genetic data confirm that newts from the type locality are genetically admixed

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Abstract

We collected nuclear DNA data (52 markers) with next-generation sequencing for nine *Triturus* newt specimens, including the holotype and two of the paratypes of *T. arntzeni*, from the type locality at Vrtovač in eastern Serbia. We compare these data to a reference set composed of the four crested newt species distributed in eastern Serbia namely *T. cristatus*, *T. dobrogicus*, *T. ivanbureschi* and *T. macedonicus* to determine to which of these species the newts from the type locality of *T. arntzeni* should be attributed. The majority of alleles in individuals from Vrtovač is derived from *T. macedonicus*, but a considerable number of *T. ivanbureschi* alleles is also present; alleles typical for *T. cristatus* and *T. dobrogicus* are found at low frequency. Accordingly, we interpret Vrtovač as a *T. macedonicus* – *T. ivanbureschi* hybrid population, albeit not composed of F1 hybrids but of genetically admixed individuals derived through multiple generations of backcrossing. The data support the notion that the name *T. arntzeni* should not be applied to a species newly distinguished in *T. karelinii sensu lato* (to which the name *T. ivanbureschi* has been given). We conclude that because of the hybrid nature of the individuals from Vrtovač, the name *T. arntzeni* should be placed not only in the synonymy of *T. macedonicus* but also in the synonymy of *T. ivanbureschi*. In this study we demonstrate that next-generation sequencing can provide high quality data for type material with degraded DNA and therefore can play an important role in taxonomy.

Key words: DNA degradation, Ion Torrent, Next-generation sequencing, *Triturus cristatus* superspecies, *Triturus ivanbureschi*, *Triturus macedonicus*

Introduction

In a recent paper in this journal, we showed that the crested newt species *Triturus karelinii* (Strauch, 1870) comprises (at least) one more species: *T. ivanbureschi* Arntzen and Wielstra 2013 (in Wielstra *et al.*, 2013c). The first hint of *T. ivanbureschi* representing a distinct species came from deep genetic divergence of mitochondrial DNA (Wielstra & Arntzen, 2011; Wielstra *et al.*, 2010). These lineages were subsequently found to differ in environmental space (Wielstra *et al.*, 2012) and were eventually confirmed to also represent discrete nuclear gene pools (Wielstra *et al.*, 2013a).

The name *T. arntzeni* Litvinchuk, Borkin, Džukić and Kalezić, 1999 (in Litvinchuk *et al.*, 1999), with Vrtovač, Serbia as type locality, has been applied to a species newly distinguished in *T. karelinii sensu lato* distributed on the Balkan Peninsula and western Asiatic Turkey (e.g. Arntzen & Wielstra, 2010; Espregueira Themudo *et al.*, 2009). However, the supposed differences of *T. arntzeni* from other newts (genome size, protein variation and morphological characteristics) have been put into question (Arntzen & Wielstra, 2010; Stoyanov *et al.*, 2011) and in a review of the species identity of crested newts from Vrtovač, the bulk of evidence (particularly genome size and three nuclear DNA markers) pointed towards *T. macedonicus* being the crested newt species occurring at this site (Wielstra *et al.*, 2013c). Wielstra *et al.* (2013c) concluded that the name *T. arntzeni* is a junior synonym of *T. macedonicus* (Karaman, 1922) and should not be applied to the newly distinguished species.

Wielstra *et al.* (2013c) failed to obtain genetic data for the type material of *T. arntzeni*, presumably because of DNA degradation. Nuclear data available for other individuals from Vrtovač concerned just three markers, which

perspective for taxonomy as well as population genetics (Wandeler *et al.*, 2007). Several next-generation sequencing protocols actually involve a step in which high quality DNA is fragmented for library preparation, perhaps not dissimilar to what happens naturally when DNA breaks down.

Data accessibility

Data associated with this paper have been deposited in the Dryad online data repository under doi:10.5061/dryad.g3375 as follows. For the nine individuals from Vrtovač: 1) raw Ion Torrent reads in FASTQ format; 2) BWA alignments in SAM format; 3) raw SNP reports in VCF format; 4) filtered SNP report used to construct consensus sequences; and 5) number of reads per individual per marker. For the comparison with all *Triturus* species in the reference set: 6) table containing data in genotypic format; 7) allelic variants per marker; 8) FASTA files of reconstructed sequences; 9) BAPS input file (GENEPOP format); 10) Structure input file; and 11) the BAPS and Structure output. For the comparison with the species *T. ivanbureschi* and *T. macedonicus*: 12) NewHybrids input file; and 13) table with distribution of *T. ivanbureschi* and *T. macedonicus* alleles in newts from the *T. arntzeni* type locality.

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