Newts in time and space: the evolutionary history of Triturus newts at different temporal and spatial scales

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CHAPTER 1

INTRODUCTION & SUMMARY

Introduction

Species are confined in all four dimensions of space and time. But while geographical borders can be defined where no more individuals of a certain species can be found, temporal borders are more difficult to define, as they can not be determined directly, but rather inferred from the fossil record, palaeogeography, and genetics. It is difficult to determine when an ancestral species ceases to be and the derived species comes into existence (see, for example, DE QUEIROZ, 2007). Darwin, for example, considered species to be part of a continuum of diversification, without any real border.

Species' distributions are continuous in areas of, for example, favourable habitat, amenable ecological conditions or lack of competitors. Closer to the border, population density starts decreasing, and the distribution will pass from continuous to patchy, until no more individuals are found. In the case of two closely related parapatric species, these empty patches can be filled by related competing species (ARNTZEN, 2006).

Through time, the range of a species contracts and expands, populations split and merge, gene flow stops and restarts. This also follows the suitability of habitat through time. Climatic changes push populations into different areas, with expansions when the climate is more favourable and retractions when conditions are worse. Distribution becomes patchy, then continuous, and then patchy again, over and over in cycles. Given enough time between contractions and expansions, the populations that meet will be different enough from the populations that had split, and reproductive isolation will have developed.

This thesis is a contribution to unravel the phylogenetic history of a genus of newts, at different scales. It starts by taking a broad picture of the history of the genus, and will then *zoom in* into higher and higher detail, going to phylogeography and further into local ecological conditions that determine species range limits together with the presence of a closely related competitor. Like with species, the limits between these approaches are somewhat arbitrary.

Newts

Newts are part of a family of salamanders (family Salamandridae; subfamily Pleurodelinae) that evolved from other amphibians around a hundred million years ago (STEINFARTZ *et al.*, 2006). The objects of this study are the large-bodied European newts: the crested and the marbled newts. They form the genus *Triturus*, a group that occurs all over Europe and western Asia. *Triturus* was once a larger genus, comprising not only the large-bodied newts, but also other medium and small sized European newts, now forming *Lissotriton*, *Mesotriton* and *Ommatotriton* (GARCÍA-PARÍS *et al.*, 2004); members of the crested newt group were once considered subspecies of *T. cristatus* (ARNTZEN and WALLIS, 1999), and the pygmy marbled newt, *Triturus pygmaeus*, was until just recently considered a subspecies of *Triturus marmoratus* (GARCÍA-PARÍS *et al.*, 2001).

The distribution of the *Triturus* species is essentially parapatric, their ranges only slightly overlap (Figure 1). This pattern repeats itself in every area where two or more members of this genus meet (ARNTZEN and WALLIS, 1991; see also CHAPTERS 2, 8 and 9). The largest area of overlap is between the great crested newt (*T. cristatus*) and

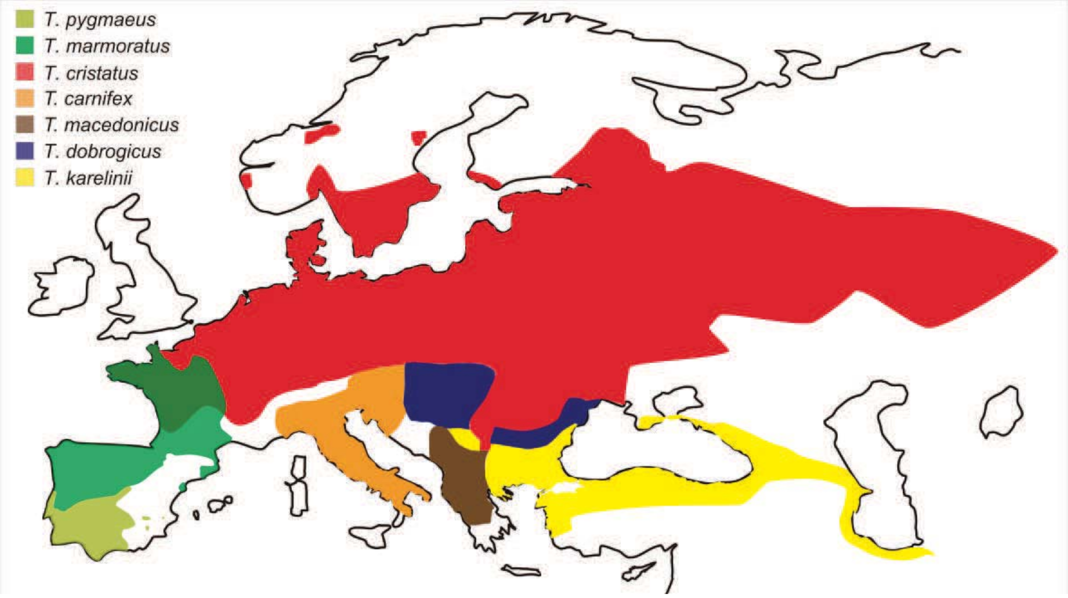

Figure 1 - Distribution of *Triturus* newts in Eurasia. Notice the area of overlap between the marbled newt (*Triturus marmoratus*) and the great crested newt (*T. cristatus*), in France.

the marbled newt (*T. marmoratus*), but in finer spatial detail, the two forms are well separated (ARNTZEN and WALLIS, 1991). The two species have different ecological requirements: marbled newts occur predominantly at forests and hilly terrain with scrub and hedges, while crested newts prefer flat and open areas (ARNTZEN and WALLIS, 1991; JEHLE and ARNTZEN, 2000).

Crested newts

Crested newts are present in most of Europe and western Asia. Their taxonomy has changed considerably over the last two centuries. All of the species now recognized used to be considered subspecies of the great crested newt, *Triturus cristatus* (Laurenti, 1768). Five species are currently recognized: the northern crested newt, *Triturus cristatus* (Laurenti, 1768), the Italian crested newt, *Triturus carnifex* (Laurenti, 1768), the Danube crested newt, *Triturus dobrogicus* (Kiritzescu, 1903), the southern crested newt, *Triturus karelinii* (Strauch, 1870), and the Macedonian crested newt, *Triturus macedonicus* (Karaman, 1922). The Macedonian crested newt was just recently raised to species level given its allopatric distribution to its sister species, the Italian crested newt, and the level of genetic differentiation (see CHAPTER 2). The two known subspecies of the southern crested newt, *T. k. karelinii* and *T. k. arntzenii* may someday be raised to species level as well, given their substantial genetic differentiation (see CHAPTER 4).

Morphologically the species are very similar. They are all large newts with heavily build, and warty skin. Their dorsal side is usually dark brown to black, while their sides are sometimes punctuated with small white spots. Males present a serrated

Figure 2 - *Triturus carnifex* from an introduced population in the region Veluwe, The Netherlands.

dorsal crest and a white band in their tale during the breeding season. Their belly is yellow to orange, with variable number of white and black spots. The Danube crested newt is the most slender and elongated, which might be an adaptation to its more aquatic habitat (ARNTZEN and WALLIS, 1999). The species can be distinguished by the number of rib-bearing vertebra: *T. karelinii* has 14, *T. carnifex* and *T. macedonicus* have 15; *T. cristatus* has 16; and *T. dobrogicus* has 17 or 18 (ARNTZEN and WALLIS, 1999).

Distribution

Triturus carnifex and T. macedonicus

The Italian crested newt, *Triturus carnifex* (Laurenti, 1768), is present south of the Alps and occupies Italy, Slovenia, Croatia, and Austria. It has been introduced in several places, like the Azores in Portugal (where it is the only newt present; MALKMUS, 1995), Geneva in the French-Swiss border (ARNTZEN, 2001), Birmingham and Surry in England (BEEBEE and GRIFFITHS, 2000), Veluwe in The Netherlands (BOGAERTS, 2002), and Bavaria, Germany (FRANZEN *et al.*, 2002). The Macedonian crested newt, *Triturus macedonicus* (Karaman, 1922) was until recently considered a subspecies of *T. carnifex*, but as a result of this thesis, this taxon has been raised to species level (see CHAPTER 2). It occurs in Macedonia, Greece, Serbia, Montenegro, Albania and southern Bosnia and Herzegovina.

Figure 3 *Triturus carnifex* female.

Figure 4 Larva of *Triturus cristatus*. Notice the dark spots on the dorsal tail fin and the thin long fingers, characteristic of larval stages of *Triturus*.

Triturus cristatus

The great or northern crested newt is the most widespread species of crested newt. It occurs all the way from Great Britain to northern France, central and eastern Europe north of the Alps and the Carpathians, southern Scandinavia, and southwestern Siberia.

Triturus dobrogicus

The Danube crested newt is present along the Danube river basin, encompassing Slovakia, Hungary, Romania, Bulgaria and Moldova. Its distribution is divided into two regions: the Dobrogean and the Pannonian. The two populations are separated by the Carpathian Mountains, but seem to be connected through the Iron Gate in the Danube (ARNTZEN, 2003).

Triturus karelinii

T. karelinii occurs along the southern shore of the Iranian Caspian Sea, Georgia, Azerbeijan, the Russian Black Sea coast, Crimea (Ukraine), Turkey, Bulgaria, northern Greece, and Serbia. The southern crested newt has two recognized subspecies: *T. k. karelinii* from Iran, Azerbeijan, Georgia, Russia and Ukraine, and *T. k. arntzenii*, from the Balkans. The populations from Turkey have an unknown status (see CHAPTERS 2 and 4). There is a known enclave of *T. k. karelinii* in eastern Serbia that is completely surrounded by populations of three other crested newts (ARNTZEN and WALLIS, 1999).

Figure 5 - Male *Triturus karelinii* from Bozdag, Turkey. The dorsal crest, typical during the breeding season, is folded to the right, and so is not clearly visible.

Conservation and threats

T. dobrogicus is considered a near threatened species by the International Union for Conservation of Nature (IUCN), due to the rate of population decline caused by habitat loss throughout its distribution range. It is also threatened by hybridization with its neighbouring crested newt species, given its central position and limited distributional range (ARNTZEN *et al.*, 2006b).

T. macedonicus is not listed in the IUCN red list, as it was considered a subspecies of *T. carnifex* in the latest assessment. However, the entry for *T. carnifex* already mentions major decline of the Balkan populations due to decrease in spring precipitation, possibly a consequence of global climate change (ARNTZEN *et al.*, 2006a).

Although the trend in the other species is for population decrease due to deforestation and pollution of wetlands, the IUCN red list lists them as least concern, as they consider that given their wide distribution, the speed of the decline is not fast enough to include it in a more threatened category (ARNTZEN *et al.*, 2006c).

Figure 6 Typical breeding site for newts in Turkey. Nets seen in the left and right are used to capture larvae and breeding adults.

Marbled newts

This section was adapted from Espregueira Themudo & Arntzen (2009)

The marbled newts are two species of *Triturus*: the northern marbled newt, *Triturus marmoratus* (Latreille, 1800); and the pygmy marbled newt, *Triturus pygmaeus* (Wolterstorff, 1905). The pygmy marbled newt was just recently recognized as a full species, while it used to be considered a subspecies of *Triturus marmoratus* (GARCÍA-PARÍS *et al.*, 2001). Arguments in favour of this position include the level of genetic differentiation between the two, diagnostic morphological characters and the lack of hybrids in Spain. However, the situation in Portugal is spatially more complex, as the two species are in contact, and some hybrids have been detected (see CHAPTERS 6 and 9). *Triturus marmoratus* is clearly larger, with a strong build, rough skin, a more or less uniform dark ventral colouration with white stipples and a hard-green dorsal and lateral coloration in a course network. Characteristic features of *T. pygmaeus* are a small body size, elegant built, smooth skin, greyish and spotted ventral colouration and an olive-green dorsal and lateral coloration in a fine network.

Distribution

Triturus marmoratus

The range of *T. marmoratus* covers major parts of France, Spain and Portugal. In France, *T. marmoratus* is found in the southwestern part of the country, northwards to Normandy and Paris. In central France the range of *T. marmoratus* overlaps with that of *T. cristatus*, with interspecific hybridisation taking place (ARNTZEN and WALLIS, 1991). In Spain, *T. marmoratus* is found all over the northern part of the country, in the east southwards to the valley of the Ebro, in the centre as far south as the Sierra de Guadarrama and in the west as far south as the Sierra de Gata (ALBERT and GARCIA-PARIS, 2004). In Portugal, *T. marmoratus* is found all across the northern part of the country, excluding the coastal zone south of Aveiro. There is also evidence of an isolate around Caldas da Rainha surrounded by populations of *T. pygmaeus* (see CHAPTER 9). The southern border runs from the Serra de Gata at the Spanish border, southwards to reach but not cross the river Tejo in central Portugal, approximately following the line Castelo Branco - Abrantes - Leiria.

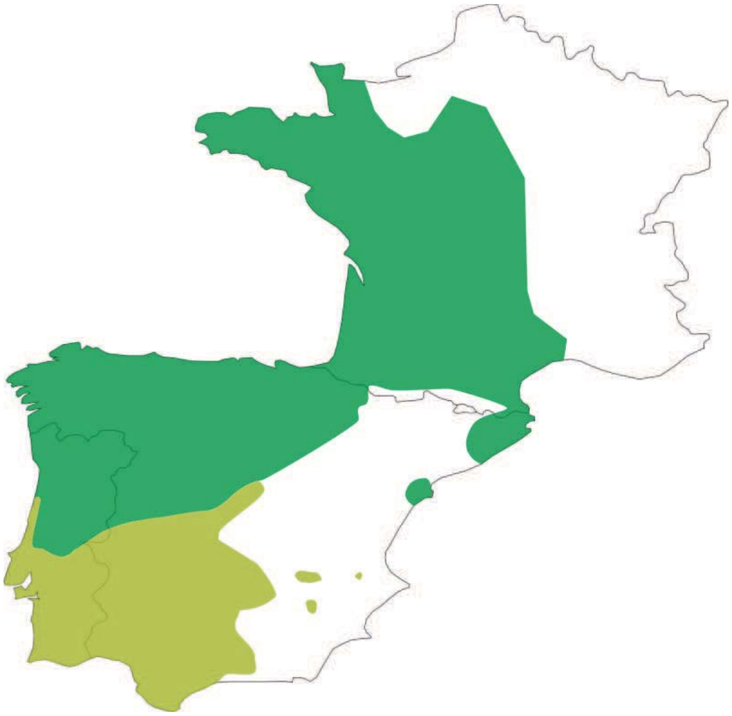

Figure 7 Distribution of *Triturus marmoratus* and *T. pygmaeus* in Western Europe. *Triturus marmoratus* is depicted in the darker shade of green, and *T. pygmaeus* in olive green.

Figure 8 *Triturus marmoratus* from Dordogne, France.

Triturus pygmaeus

The range of *T. pygmaeus* covers the southern part of the Iberian Peninsula, with the exception of the eastern and southeastern parts of Spain. The northernmost localities of *T. pygmaeus* are situated in the Portuguese coastal zone, as far north as Aveiro. In central Portugal the range of *T. pygmaeus* is contiguous with that of *T. marmoratus*. In Spain the range follows the southern slopes of the Sistema Central, including the Sierra de Gata, Sierra de Gredos and the Sierra de Guadarrama (GARCÍA-PARÍS, 2004). The shortest documented distance between populations of both species in the Madrid area is c. 6 km (GARCÍA-PARÍS *et al.*, 2001).

Conservation and threats

The significant loss of habitat in the south of the Iberian Peninsula, specially by the decrease in the number of temporary ponds caused by desertification led IUCN to list the pygmy marbled newt as 'Near Threatened' (STUART *et al.*, 2008). In Gerês National Park (northwest of Portugal), several amphibian species, including *T. marmoratus* are infected by an iridovirus that causes high mortality (ALVES DE MATOS *et al.*, 2002). Other more general causes of decline include the draining of temporary ponds as a consequence of the intensification of agriculture; increase urbanization; and predation by invasive species, such as the

Figure 9 Female *Triturus marmoratus* from Porto, Portugal. Notice the orange dorsal strip, warty skin, and tissue regeneration in the tailtip, a few weeks after the tailtip was cut for sampling.

Louisiana crayfish (*Procambarus clarkii*) and the sunfish (*Lepomis gibbosus*). These combined factors are causing the disappearance of several populations in southern Spain, contributing to the fragmentation of its distribution (García-París et al., 2001). The situation in *T. marmoratus* is more stable than in *T. pygmaeus*, despite its regression in the western coast of Portugal, as it was replaced by *T. m. pygmaeus* (CHAPTER 9).

Scope of the thesis

This thesis is roughly divided in two sections. The first concerns the phylogenetic history of the genus *Triturus*, how species are related to each other, and the effect of some evolutionary processes on the inferred phylogeny. The last chapter of this section, on the phylogeography of the marbled newt group, links this to the second section. The second half of the thesis concerns the relationship between the distribution ranges of species and the environmental conditions. The main focus was on the effect that the local environment has on the relative competitiveness of sibling species, and ultimately on their distribution limits.

This thesis consists of eight scientific chapters, apart from this introduction, most of which have been published in (or, at least, submitted to) international peer reviewed scientific journals.

Summary

The interspecific relationships in the genus *Triturus* are incompletely known. In **CHAPTER 2**, we attempt to resolve them by using allozyme and mtDNA data. Despite the large number of markers used, relationships continue to elude us. The results suggest that speciation in the group occurred during a short time period (the end of the Middle Miocene). Paleogeographic reconstructions of the presumed centre of origin (the Balkans) support this hypothesis. We proposed here that *T. macedonicus* should be raised to full species given its allopatric distribution and high genetic divergence with *T. carnifex*.

The best way to test the scenario presented in CHAPTER 2 is to look at multiple independent markers that, unfortunately, were not readily available. **CHAPTER 3** describes the process through which dozens of markers were designed and tested for the genus *Triturus*. Out of more than fifty markers tested, five provided promising results with enough variability to study the phylogeny and phylogeography of the genus. This opened the door not only for CHAPTER 4, but also for 5 and 6.

Taking CHAPTER 2 as the starting point and with the tools developed in CHAPTER 3, **CHAPTER 4** attempts to decipher the history of the genus *Triturus*. The study includes samples from 15 individuals of the seven species of the group. Locations were selected to cover most of the variability in the group, with the exception of areas close to other species. Hybridization is known to occur in these areas, and could bias the inferences made. Results show that all the genes, except the two mtDNA ones, have incongruent phylogenetic signals. We used phylogenetic networks to visualize the alternative phylogenetic signals and have built a phylogenetic tree based on a Bayesian hierarchical method that obtains the species tree based on individual gene trees. This approach successfully resolved the branching order of the newts, although time intervals are very narrow, confirming the near simultaneous speciation scenario of CHAPTER 2. We also found a high genetic differentiation between the two forms of the southern crested newt (*T. karelinii*) and proposed that they should be raised to full species (*T. karelinii* and *T. arntzeni*).

As described in CHAPTER 4, hybridization can have a confounding effect on phylogenetic inferences. Incomplete lineage sorting can also produce similar patterns as gene flow, further complicating matters. **CHAPTER 5** takes a new approach in distinguishing between gene flow and incomplete lineage sorting, only possible in species with very well defined species borders and limited dispersal capability as the newts, which limits gene flow to a narrow geographical region.

As can be seen in the previous chapters, the crested newts show complex (although interesting) patterns resulting from compound interactions, especially in the Balkans. The marbled newts, on the other hand, are only two species, and therefore we can cover their evolutionary history in more detail, as it is simpler. **CHAPTER 6** studies the phylogeography of the two species of marbled newts. Being just a pair and not a group of species, relationships are not problematic. Their sibling relationship is well established (see CHAPTER 2). This chapter also explores how differences in ecological conditions (see CHAPTER 8) along a hybrid zone affect its structure and the amount of gene flow between species.

The morphological distinction of adults of the two marbled species is relatively straightforward, even though variation is present. Eggs and larvae, on the contrary, are easy to spot and collect, but impossible to distinguish. **CHAPTER 7** describes a cheap and fast molecular technique that allowed the identification of the large number of samples used in CHAPTERS 8 and 9.

Based on a morphological and genetic identification of individuals of the two species of marbled newts and published distributional data, **CHAPTER 8** identified ecological factors associated with the range border of the two species of marbled newt. Ecological models defining the distribution of the two species differed in four main areas: the northern most region of contact close to Aveiro, the rest of the coastal area (see also CHAPTER 9), the region coinciding with the Tejo river, and the remainder contact zone going until Madrid.

In the coastal area of Portugal, close to Caldas da Rainha, the northern marbled newt was found where only pygmy marbled newts were thought to occur. This prompted a detailed study on the distribution of the two species in this area described in **CHAPTER 9**. This study revealed a small pocket of populations of the northern species surrounded by populations of its sister southern species. Given the distance of this pocket to the main distribution, we believe that the enclave was created by *T. pygmaeus* moving north, superseding *T. marmoratus*, rather than the latter species expanding southwards.

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