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Phylogeography and fine-scale population structure of the Spectacled Salamander (*Salamandrina perspicillata* and *S. terdigitata*).

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Salamanders of the genus *Salamandrina* are endemic to Italy and exclusively occur along the Apennine Mountain chain (Lanza, 1988). They are the only salamanders with four toes on the front- and hind-limb and represent the most basal split within the family Salamandridae which took place approximately 90 my ago (Steinfartz *et al.*, 2007). Until a few years ago, *Salamandrina* was considered a monotypic genus. However, both mitochondrial genes (Mattocchia *et al.*, 2005) as well as nuclear coded allozymes (Nascetti *et al.*, 2005; Canestrelli *et al.*, 2006a), provided strong evidence for the existence of two deeply differentiated congeneric species with independent evolutionary histories. While *S. perspicillata* (Savi, 1821) is distributed in the northern part (from Liguria to Campania), *S. terdigitata* (Lacépède, 1788) occurs in the south of Italy from Campania to Calabria (Fig. 1). Various scenarios have been proposed for the divergence of the *taxa*, which has been proposed to have begun 6-11 mya (Mattocchia *et al.*, 2005; Nascetti *et al.*, 2005).

Salamandrina species are in need of strict protection (EU habitat and species directive Annexes II and IV) as they are threatened by changes in the water level, caused by aqueducts and irrigation plants, by pollution of the aquatic habitat, and probably also by removal for the pet trade (Zuffi, 1999). Relatively little is known about the biology of these elusive salamanders, but some aspects of their life history, in particular the reproductive biology, have been well studied in

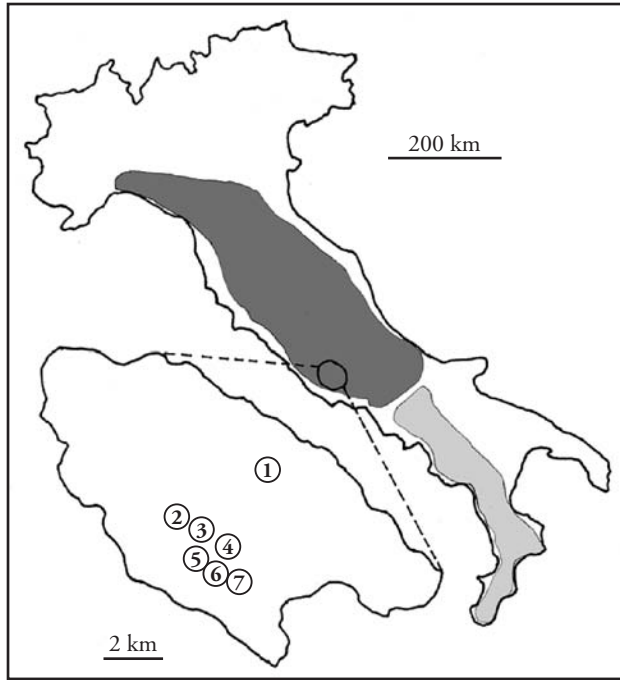


Figure 1. Distribution of *Salamandrina terdigitata* (dark grey) and *S. perspicillata* (light grey). The enlarged area depicts the location of seven sampling sites in Frosinone and Latina that are the focus of the population genetic analysis on a small geographic scale.

populations of the northern species (e.g., Della Rocca *et al.*, 2005; Angelini *et al.*, 2006, 2008).

Some interesting patterns have already become apparent in the molecular studies conducted so far (Mattocchia *et al.*, 2005; Nascetti *et al.*, 2005): in both species genetic diversity, based on the number of mitochondrial haplotypes, was very low despite their evolutionary age, compared to other amphibian species with similar phylogeographic histories (e.g., *Bombina pachypus*: Canestrelli *et al.*, 2006b). We are currently conducting a comprehensive molecular phylogeographic analysis of this genus to elucidate this interesting pattern in more detail. We also aim to better understand the genetic relationships of the extant populations within each species. Therefore, we have developed a set of ten fast evolving nuclear markers (microsatellite DNA) to use in combination with mitochondrial markers (cytochrome b and cytochrome oxidase I) to analyse the genetic structure among and within *Salamandrina* populations on a small and large geographic scale, covering the entire ranges of both species. As expected, individuals of *S.*

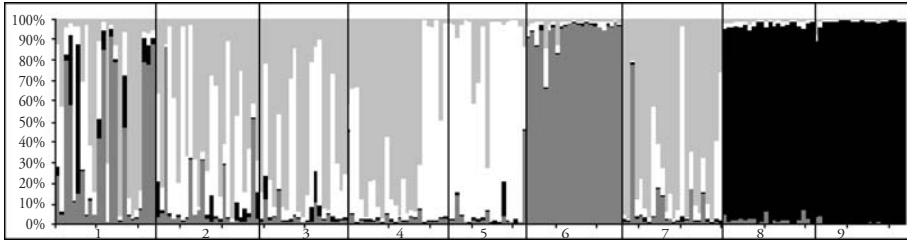


Figure 2. Bayesian analysis of population genetic structure using the software STRUCTURE of seven populations of *Salamandrina perspicillata* from Latium and two populations of *S. terdigitata* from Calabria (8 and 9) using a preliminary set of seven microsatellite markers. The four colours indicate the best supported number of clusters into which all 204 individuals were grouped and show the proportion of membership into each cluster of every animal.

terdigitata and *S. perspicillata* could be unambiguously assigned to the respective species using either type of marker. The number of haplotypes and the haplotype diversity were extremely low within each species. For example, among 144 individuals from eight populations of *S. perspicillata* (between 1 and 24 individuals per population) we only found five haplotypes of cytochrome b (630 bp), and among 42 individuals from two populations of *S. terdigitata*, we also found five haplotypes. Polymorphic microsatellite markers, however, allow resolution on a much finer scale, and allow us to determine the level of gene flow among neighbouring populations of *S. perspicillata* in Latium. Even at this small geographic scale (maximal distance between two sites was 6 km), we have already found distinct genetic clusters (Fig. 2) with significant levels of genetic distance.

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