

## A new finding of *Salamandra lanzai* in the Upper Sangone Valley (NW Italy) marks the species' most disjunct population (Amphibia: Urodela: Salamandridae)

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**Abstract.** The presence of *Salamandra lanzai* was confirmed for the Upper Sangone Valley (Turin Province, NW Italy), within the Parco Naturale Orsiera Rocciavré. The species attribution was further supported by morphological and genetic (16S) analysis and represents the north-eastern most limit of the species' distribution. This salamander was so far known only for a few major alpine valleys of Italy (Po, Pellice, and Germanasca Valleys), and France (Guil Valley). The new finding is especially interesting since it is separated from its closest known locality by about 15 km. For such a reason this population needs to be carefully managed.

**Keywords.** *Salamandra lanzai*, new finding, Sangone Valley, Cottian Alps, NW Italy.

*Salamandra lanzai* is a peculiar alpine urodele: recognised as a distinct species in 1988, it soon became the subject of intensive research activity (e.g., Ribéron et al., 1996; Andreone and Sindaco, 1999) aimed at unveiling its life history requirements and distribution, with important conservation applications. Following its discovery, the distribution of *S. lanzai* initially appeared limited to a few alpine valleys in Italian and French Cottian Alps, roughly around the Monviso Massif (Andreone, 2007), and until recently its known distribution was limited to about 50 observations in France (31 1 × 1 km UTM squares, of which 10 confirmed) and 60 in Italy (45 1 × 1 km UTM squares, of which 31 confirmed) (Andreone et al., 2004).

In France *S. lanzai* is currently present exclusively in the Guil Valley, at an altitude ranging from 1800-2600 m a.s.l., in sites characterised by the presence of alpine prairie (Ribéron et al., 1996). In Italy it is known from three major valleys: Germanasca, Po, and Pellice Valleys, with some further records in the Angrogna Valley (a secondary valley tributary of the Pellice Valley) at an altitude ranging 1200-2600 m a.s.l. Moreover, a recent report by Sindaco (2006) places the meridional limit of this species at Oncino (Po Valley).

Although its distribution appears restricted to the upper portions of these valleys, there are some unconfirmed reports of alpine salamanders for other north-western parts

of the Alps. Moreover, most of these reports turned out to be false and likely due to anecdotal findings and incorrect taxonomic determinations (Andreone and Sindaco, 1999; Andreone, 2006, 2007), and there is the perspective that some are indeed true, and the lack of recent confirmed records may be due to a general disappearance of the species. In fact, as pointed out by Ribéron (2003), the species was probably more widespread in the past, as corroborated by old French reports of Abriès, and the Ubaye and Agnelle Valleys (Ribéron, 2003), and by the presence of a preserved specimen from the Italian Maritime Alps, dated from the end of the nineteenth century (Andreone and Sindaco, 1999; Andreone et al., 2004).

Recently, we obtained information of the possible presence of black salamanders in the upper Sangone Valley, Turin Province (E. Giuliano, pers. comm.). Although these observations were quite circumstantial and made by local rangers they were not accompanied by any photographs or voucher specimens, and therefore remained doubtful for some years. A first visit in the Parco Naturale Orsiera Rocciavré (by P. Eusebio Bergò, V. Mercurio, and E. Giuliano) did not confirm the records. This was not the case of the summer of 2006, when on the occasion of a further visit to the same site, one of us (GT) found a salamander. The single female was found overnight on 31<sup>th</sup> July 2006 (h 00.20). The site is located at 2227 m a.s.l., and consists of an alpine meadow with herbaceous cover and rocky debris due to glacial erosion. The area lies in a typical alpine valley, with intense fog formation and heavy and frequent precipitations, due to condensation of wet atmosphere coming from the Po Plain. These are most likely to be good microclimatic conditions for the species, which is known to require high humidity levels. We do not provide the exact coordinates of this site for conservation reasons, but they will be transmitted to the Italian herpetological database to update the species' distribution pattern.

The salamander was measured for its basic parameters: its size of 89 mm snout-vent length and 165 mm total length fell within the typical range of *S. lanzai*; in addition, we also ascertained the specific identity by comparing the photograph to that of other individuals from the Italian and French Alps. This showed the species' distinctive and diagnostic characters, such as the absence of paravertebral glands on the trunk, a massive body with a flattened head, a rounded tail tip, and interdigital webbing (Fig. 1).

Moreover, the tip of the third toe was clipped and stored in pure ethanol for subsequent genetic analysis. The species attribution was then confirmed by the comparison of a fragment of 535 bp of the 16S rRNA gene, the suggested standard DNA barcoding marker for vertebrate, and hence therefore amphibians (Vences et al., 2005). Fourteen specimens of *S. lanzai* sampled in nine different localities (one from Sangone Valley, six from Po Valley, three from Germanasca Valley, two from Pellice Valley and two from Angrogna Valley) were also analysed to have a good comparative series. Total genomic DNA was extracted from the tissue samples using proteinase K digestion (1 mg/ml concentration) followed by a standard salt extraction protocol (Bruford et al., 1992). We used the primers 16SA-L 5'-CGCCTGTTTATCAAAAACAT-3' and 16SB-H 5'-CCGGTCTGAACTCAGATCACGT-3', modified from Kocher et al. (1989) and Palumbi et al. (1991). PCRs were performed in 25µl reactions using 3µl of genomic DNA, 1µl of each 10 pmol primer, 0.5µl of total dNTP 10mM in water (Promega), 0.1µl of 5U/µl GoTaq®, 5µl 5x Green GoTaq® Reaction Buffer (Promega) and 14.4 µl of water. PCRs were performed using the following conditions: an initial denaturation step at 94 °C for 90 s, 33 cycles of denaturation



**Fig. 1.** The female of *Salamandra lanzai*, photographed in the Upper Sangone Valley.

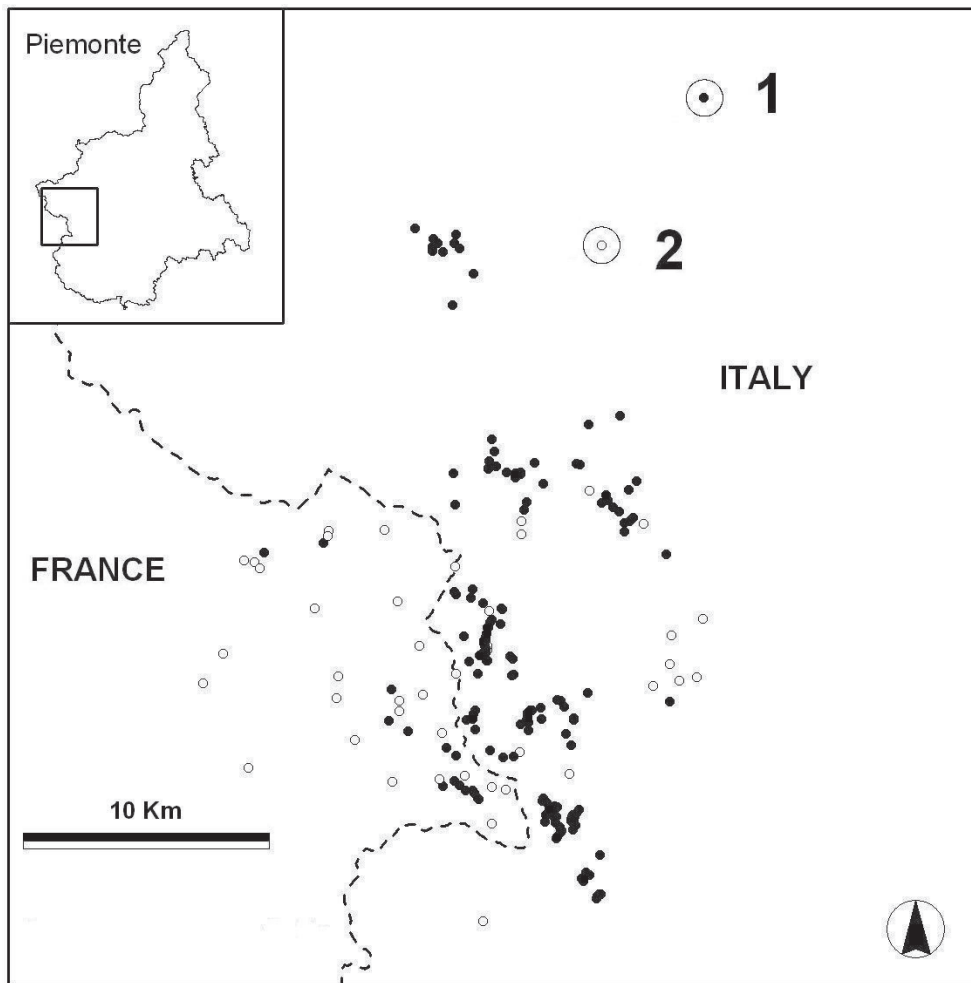
at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 90 s, and a final extension of 300 s at 72 °C. PCR products were loaded onto 1% agarose gels, stained with ethidium bromide, and visualised on a “Gel Doc” system (PeqLab). If results were satisfying, products were purified using QIAquick spin columns (Qiagen). The light strands were sequenced using an ABI3730XL by Macrogen Inc. (sequences GenBank accession numbers: EF191028- EF191041). Sequences were manually edited and aligned using the BioEdit sequence alignment editor (version 7.0.5, Hall, 1999).

Genetic distances were computed with MEGA (version 3.1, Kumar et al., 2004) using as reference a homologous sequence of *S. atra atra* (without a specific locality from “Austria”) and a sequence of *S. a. aurorae* (from Bosco del Dosso, Italy) (sequences kindly provided by M. Veith). The uncorrected p-distance, and hence the lowest genetic distance, between *S. lanzai* and both *S. atra* subspecies were around 3.2-3.4%.

The finding of *S. lanzai* in the Upper Sangone Valley marks a remarkable extension of the species’ distribution, and it shows that this salamander is not limited to the main alpine valleys (Guil Valley in France and Germanasca, Pellice and Po Valleys in Italy), and also gives new light on the species’ conservation, since *S. lanzai* is considered a “Vulnerable” species according to the IUCN Red List (IUCN 2006). In particular, the factors threatening this salamander are limited in their geographical extent, and are mainly due to habitat alteration, tourist pressure, and associated vehicular traffic (Andreone et al.,

2004). In light of these considerations, the new site of the Sangone Valley appears quite secure, due to its distance from any close roads, and the fact that it does not host any tourist facilities. Furthermore, it is included in a protected area (Parco Naturale Orsiera Rocciavré), which potentially represents an advantage in terms of the species' conservation.

The species' narrow distribution and the fragmented status of its populations are also important conservation factors (Andreone et al., 2004). The new finding is particular worth of attention also because it is separate from the closest population (Massello Valley) by about 14.5 km and by the xerothermic Chisone Valley, that represents therefore a major



**Fig. 2.** The distribution of *Salamandra lanzai* based upon current data. The black circles represent confirmed sites, while the open circles represent unconfirmed sites. (1) indicates the new (confirmed) location in the Upper Sangone Valley, and (2) indicates the (unconfirmed) “Fontanette” finding.

physical barrier (Fig. 2). A closest finding was given in the map provided by Andreone et al. (2004). Anyhow, we do not take into consideration this locality since its coordinates were not clearly ascertained and the toponym ("Fontanette") was not found on cartography. Thus we consider this finding as doubtful, pending for further confirm.

For these reasons, special attention should be paid to provide effective conservation for this urodele in the Upper Sangone Valley. While the other sites are likely to be in contact and thus still experiencing significant genetic and population exchange, the new site is, at least in the light of current knowledge, almost isolated from the major population nuclei. Anyhow, the genetic analyses conducted on the basis of the mitochondrial DNA did not exhibit any differences compared with the 13 samples from the other Italian sites, suggesting that genetic flow occurred until recently. Data formerly presented for Cyt b analysis also showed that there is no significant genetic variation between the known populations of *S. lanzai*, thus suggesting founder effect due to repeated bottlenecks or isolation of small population nuclei due to climatic oscillations during the Pleistocene glaciation (Ribéron et al., 2002).

The new finding considerably widens the species' known distribution. However, seen that a single individual only was found after a considerable time lapse, the species appears to be quite elusive at this site. This leads us to consider that the distribution of this salamander might be wider than formerly believed, and that therefore the unconfirmed reports from other areas might be valid. For these reasons, further research is urgently needed to clarify whether some of the old and unconfirmed reports are incorrect or simply due to research deficiencies.

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#### REFERENCES

- Andreone, F. (2006): *Salamandra di Lanza* [Lanza's salamander]. In: Atlante degli Anfibi e dei Rettili d'Italia, Sindaco, R., Doria, G., Razzetti, E., Bernini, F., Eds, p. 196-201. Firenze, Edizioni Polistampa.
- Andreone, F. (2007): *Salamandra lanzai*. *Salamandra di Lanza*. In: Fauna d'Italia. Amphibia, Lanza, B., Andreone, F., Bologna, M.A., Corti, C., Razzetti, E., Eds. Bologna, Calderini. (in press)
- Andreone, F., Miaud, C., Eusebio Bergò, P., Doglio, S., Stocco, P., Ribéron, A., Gautier, P. (2004): Living at high altitude: testing the effects of life history traits upon the conservation of *Salamandra lanzai* (Amphibian, Salamandridae). *Ital. J. Zool.* **71**: 35-43.



- Andreone, F., Sindaco, R. (1999): Erpetologia del Piemonte e della Val d'Aosta. Atlante degli Anfibi e dei Rettili. Monografie **26**. Torino, Museo Regionale di Scienze Naturali.
- Bruford, M.W., Hanotte, O., Brookfield, J.F.Y., Burke, T. (1992): Single-locus and multi-locus DNA fingerprint. In: Molecular Genetic Analysis of Populations: A Practical Approach, Hoelzel, A.R., Ed., p. 225-270. Oxford, IRL Press.
- Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. **41**: 95-98, Version 7.0.5 (Biological Sequence Alignment Editor for Windows 95/98/NT/XP. Downloaded on 5 September 2006.
- IUCN (2006): 2006 IUCN Red List of Threatened Species. Downloaded on 5 September 2006.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. **86**: 6196-6200.
- Kumar, S., Tamura, K., Nei, M. (2004): MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Briefings in Bioinformatics **5**: 150-163.
- Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The Simple Fools Guide to PCR. Version 2.0, Dept. Zool. and Kewalo Marine Lab., Univ. of Hawai'i.
- Ribéron, A. (2003): La Salamandre de Lanza. In: Les Amphibiens de France, Belgique et Luxembourg, ACEMAV coll., Duguet, R., Melki, F. Eds, p. 286-289. Mèze, Parthénope Collection.
- Ribéron, A., Miaud, C., Guyétant, R. (1996): Taille, sex-ratio et structure d'âge d'une population de *Salamandra lanzai* (Caudata, Salamandridae) dans les Alpes du sud-est de la France. Bull. Soc. Herp. France **77**: 35-45.
- Ribéron, A., Sotiriou, E., Miaud, C., Andreone, F., Taberlet, P. (2002): Lack of genetic diversity in *Salamandra lanzai* revealed by cytochrome *b* gene sequences. Copeia **2002**: 229-232.
- Sindaco, R. (Ed.) (2006): Segnalazioni faunistiche piemontesi e valdostane. Riv. Piem. St. Nat. **27**: 443-459.
- Vences, M., Thomas, M., Bonett, R. M., Vieites, D.R. (2005): Deciphering amphibian diversity through DNA barcoding: chances and challenges. Phil. Trans. R. Soc. London, Ser. B **360**: 1859-1868.