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New record of *Salamandra algira* isolated on the north-western Tingitana peninsula, with some notes on the reproductive modes within the species

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RESUMEN: Las poblaciones de *Salamandra algira tingitana* (norte de Marruecos) son especialmente interesantes desde el punto de vista evolutivo debido a la existencia de poblaciones vivíparas y ovovivíparas en el norte de la península Tingitana. En esta nota identificamos una nueva población de *S. a. tingitana* en una montaña aislada al noroeste de esta península, y

situada entre dos sublinajes que se corresponderían a poblaciones vivíparas y ovovivíparas. Resumimos la información hasta ahora publicada sobre el viviparismo en *S. algira*, y resaltamos la necesidad de estudios más detallados que permitan conocer mejor la distribución de los sublinajes presentes en esta subespecie, y la distribución y requerimientos ecológicos de las poblaciones vivíparas.

Viviparity, the delivery of fully developed terrestrial juveniles, rarely occurs among salamanders (Wake, 2003; Buckley, 2012). Two sister salamander species, the fire salamander (*Salamandra salamandra*) and the North African fire salamander (*Salamandra algira*) have independently evolved viviparity and represent the only two cases of intraspecific variation in reproductive modes within salamanders.

Two independent origins of viviparity have been identified in *S. salamandra*: 1) a Pleistocene transition among north Iberian populations of *S. s. bernardezi* that later introgressed into *S. s. fastuosa* (García-París *et al.*, 2003), and 2) a Holocene origin in two insular populations of *S. s. gallaica* on the northwestern

Iberian coast (Velo-Antón *et al.*, 2007, 2012). In *S. algira*, viviparity is thought to have evolved during the last 600 000 years in a sublineage of *S. a. tingitana* (Beukema *et al.*, 2010). These viviparous populations were suggested to be restricted within the northern range of *S. algira*, north of the Oued Martil (Figure 1), while ovoviviparous populations of *S. a. tingitana* occur between Oued Laou and Oued Martil, representing two additional sublineages. The distribution of these sublineages, however, is still unclear. Only a few individuals from a low number of localities were analyzed within each sublineage (Beukema *et al.*, 2010) and thus hampering a more accurate identification of their present distributions. On the other hand, it is

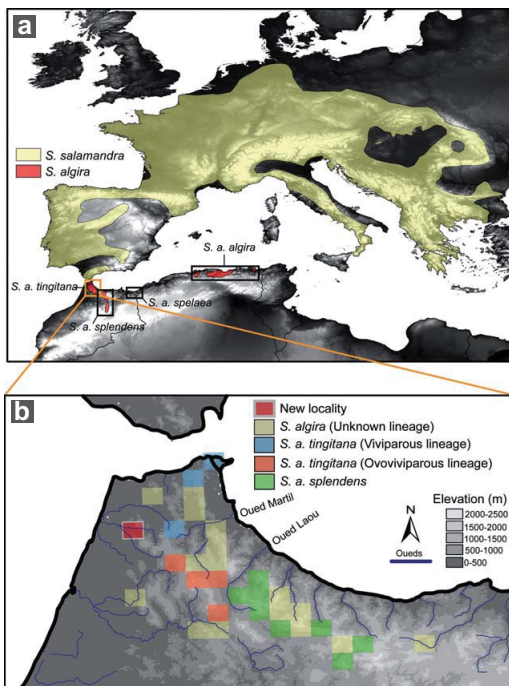


Figure 1. (a) Distribution of *S. salamandra* and *S. algira*. Subspecies range for *S. algira* are also depicted. (b) Distribution of *S. algira tingitana* in the Tingitana peninsula based on records from Beukema *et al.* (2013). UTM squares (10 km) in brownish represent *S. algira* populations that have not been genetically studied (see Beukema *et al.*, 2010), while blue and orange squares indicate “viviparous” and “ovoviviparous” lineages of *S. a. tingitana* respectively. Green squares represent identified *S. a. splendens* populations.

Figura 1. (a) Distribución de *S. salamandra* y *S. algira*. También se representa la distribución de las tres subespecies de *S. algira*. (b) Distribución de *S. algira tingitana* en la península Tingitana basado en los registros de Beukema *et al.* (2013). Los cuadrados UTM (10 km) en color marrón representan poblaciones de *S. algira* que no han sido estudiadas genéticamente (véase Beukema *et al.*, 2010), mientras que los cuadrados azules y anaranjados indican linajes de *S. a. tingitana* “vivípara” y “ovovivípara” respectivamente. Los cuadrados verdes representan poblaciones pertenecientes a *S. a. splendens*.



Photo G. Velo-Antón

Figure 2. Some examples of the colour pattern of the studied juveniles (a, b, c) and larvae (d). Red coloration was absent in the observed juveniles, although the coloration pattern was quite variable.

Figura 2. Algunos ejemplos del patrón de color de individuos juveniles estudiados (a, b, c) y larvas (d). La coloración roja estuvo ausente en los juveniles observados, aunque el patrón de coloración era bastante variable.

assumed that populations north of the Oued Laou group in the same sublineage, and thus salamander populations north of Oued Martil are thought to be viviparous.

In a recent fieldwork trip to Morocco (April 2014) we found an unknown population of *S. algira* on an isolated mountain on the north-western Tingitana peninsula (JbelHaouch Ben Kre'aa, Figure 1). We found 12 recently metamorphosed juveniles (with reabsorbed gills and a typically black and yellow coloration pattern; Figure 2), active along the edge of a small pond (35.56 /-5.78; Figures 1 and 3), and four larvae within the pond using a dip net. In a previous fieldwork trip in the same area, four larvae were observed

in a small stream at a distance of 1km from this pond (G.Velo-Antón, unpublished data). We also searched for adults under bark and stones, although we could not detect any salamander likely due to the dry weather conditions in that day. The presence of this ovoviviparous population in this region has important implications to understand the evolutionary history and distribution of both reproductive modes within *S. a. tingitana*. Given the location of the new population (north of Oued Martil) we might assume that these individuals could belong to the viviparous lineage of *S. a. tinginata*. To confirm this hypothesis we sequenced a fragment of the cytochrome b gene (cytb) of three larvae and three juveniles

collected in this locality. These data were pooled with a previously published genetic dataset (Beukema *et al.*, 2010) to identify to which sublineage of *S. a. tingitana* (viviparous vs. ovoviviparous) our samples belonged. We obtained a cytb fragment over 1000 base pairs, which was trimmed to 351 bp for comparison with the available sequences. All analyzed sequences yielded a single haplotype, which corresponds to an haplotype (Genbank number: HQ190881) found in BeniMaharone, north of Oued Laou and 50 km far from the new population, which belongs to one of the two ovoviviparous clades of *S. a. tingitana*.

The presence of both recently metamorphosed juveniles and larvae in this pond, and the lineage identification of the samples, confirms the ovoviviparous reproduction mode north of the Oued Martil. Interestingly, the isolated occurrence of this salamander population in JbelHaouch Ben Kre'aa confirms the predictive modeling of the "ovoviviparous" lineage of *S. a. tingitana* (Beukema *et al.*, 2010). The combination of climatic (influenced by the Atlantic rather than the Mediterranean)

and geologic conditions in this isolated mountains are thus similar to *S. a. tingitana* populations south of Oued Martil. Moreover, the coloration patterns found in the studied juveniles are typical of *S. a. tingitana* (lack of red discolorations and tendency for melanism, Bogaerts & Donaire-Barroso, 2003; Beukema *et al.*, 2013). This trait, however, varies widely within and among populations and does not constitute a diagnostic character to separate viviparous and ovoviviparous lineages (especially at young age) and, thus, it should be regarded as local adaptive divergence.

The geographical extent of viviparity in *S. a. tingitana* is still poorly understood, since identification of this reproduction mode relies on scattered observations of females depositing metamorphosed juveniles along northern populations in the Tingitana peninsula (Donaire-Barroso & Bogaerts, 2001; Donaire-Barroso *et al.*, 2001; Martínez-Medina, 2007), and the absence of aquatic larvae during fieldwork surveys. However, small numbers of larvae have been observed during periods of abundant rainfall in JbelHaouch, north of the

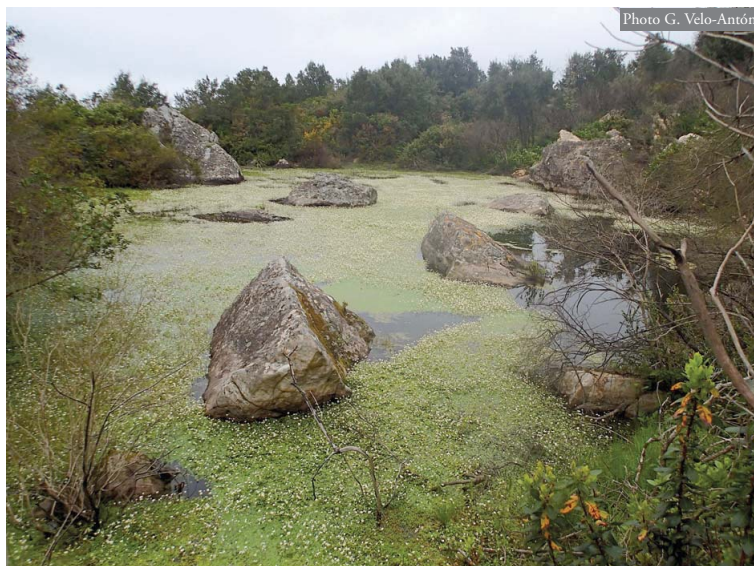


Figure 3. Pond where metamorphosed juveniles and larvae were found.

Figura 3. Laguna donde se encontraron juveniles y larvas metamorfoseadas.

Tingitana peninsula (Martínez-Medina, 2001, 2007). Identification of truly viviparous and ovoviviparous populations is critical to understand the evolution of viviparity in *S. algira*. For instance, further characterization of female clutches and the study of embryonic development should be conducted in *S. a. tingitana*, as it has been done in its sister species *S. salamandra* across northern Iberian populations (Buckley *et al.*, 2007; Velo-Antón *et al.*, 2014). At one hand, more genetic data should be gathered across the range of *S. a. tingitana* to better identify the distribution of the sublineages within this subspecies, and to confirm that viviparous populations are in fact monophyletic. On the other hand, niche modelling analysis has detected environmental differences between the distributions of the viviparous and ovoviviparous *S. a. tingitana*

(Beukema *et al.*, 2010); being the “ovoviviparous” lineage characterized by a higher dependence on precipitation, while the “viviparous” lineage occurs in more climatically stable areas, lower altitudes and karstic-limestone surfaces. Thus, special attention should be paid in karstic-limestone areas in the Tingitana peninsula to support this hypothesis.

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