

## First record of a tunnel breeding population of *Pleurodeles waltl* and two other records of Iberian cave dwelling urodeles

David Herrero & Arlo Hinckley

Cl. Embajadores, 161. 3º C. 28045 Madrid. C.e.:cuanmida@hotmail.com

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**RESUMEN:** Los anfibios pueden utilizar cuevas y otros hábitats subterráneos como refugios por su características térmicas, para evitar la depredación, como lugares de alimentación y como sitios de reproducción. Algunos urodelos son troglobios (especies cavernícolas obligadas, incapaces de sobrevivir en el exterior, fuera de ambientes de poca luz) restringidos a los hábitats cavernícolas y muestran características troglomórficas, como ojos degenerados y despigmentación. Otros anfibios utilizan las cuevas para completar alguna etapa de su ciclo de vida y pueden o no mostrar hasta cierto punto caracteres de especies troglomórficas. Muchos anfibios europeos se han encontrado ocasionalmente o regularmente dentro de las cuevas durante parte de su ciclo de vida. Este artículo constata dos nuevas especies de urodelos cavernícolas: *Pleurodeles waltl* y *Lissotriton boscai*, y confirma nuevamente el uso de cuevas para reproducirse por un tercer urodelo (*Salamandra salamandra*). Para acabar, confirma la importancia de túneles abandonados para los anfibios, aportando nuevos datos sobre su ecología en estos hábitats.

Caves and other subterranean habitats contain a biodiversity that has long intrigued biologists, such as Darwin and Lamarck. Often limited in light, these places have revealed a great number of diverse and unique species. The occurrence of amphibians in caves and associated subterranean habitats is not novel. Amphibians can use caves and other subterranean habitats for thermal refugia, to avoid predation, as feeding habitats and as breeding sites. A number of salamanders are troglobitic (obligate cavernicole species unable to survive outside) restricted to cave habitats and exhibiting special characters adapted to these environments, such as degenerated eyes and depigmentation (Hoffmann, 2002). Other amphibians, majority, use caves to complete some aspect of their life cycles and may or may not exhibit some level of troglomorphy. Anurans are occasionally observed

in subterranean habitats, but no species are known to be obligate cavernicoles.

In the Palearctic region, amphibians are relatively common in subterranean habitats. However, only one species is an obligate cavernicola (*Proteus anguinus*) (Rosa & Penado, 2013). Despite this, many European amphibian species have been occasionally or regularly found inside caves during part of their life cycles including: *Salamandra salamandra*, *Triturus marmoratus*, *Discoglossus pictus*, *Pelodytes punctatus*, *Bufo spinosus* and *Bufo calamita* in Spain (Giménez-Lopéz & Guarner Deu, 1982); *Chioglossa lusitanica* in Portugal (Gilbert & Malkmus, 1989); *Pelophylax ridibundus*, *Bufo viridis* and *Bombina bombina* in the Republic of Moldova (Andreev *et al.*, 1997); *S. salamandra*, *Triturus vulgaris*, *Triturus cristatus*, *Bufo bufo*, *B. viridis*, *Hyla arborea*, *Rana temporaria*, *Rana dalmatina* in Slovakia (Uhrin & Lesinsky, 1997); *R. tempora-*

ria in Slovenia (Poboljsaj *et al.*, 1997); *B. bufo* has been reported to be found in caves also in Greece and Italy (Boudou *et al.*, 1977; Bologna, 1982). Lanza (1983) reports *Euproctus platycephalus*, *S. salamandra*, *Salamandrina terdigitata*, and all the Italian newts belonging to the genus *Triturus*, *P. punctatus*, *B. bufo*, *Hyla intermedia* and *Rana italica* (Dolce & Bressi, 1998).

This report adds two new species to the upper list of amphibian species found inside caves: *Pleurodeles waltl* and *Lissotriton boscai*, and states again the use of caves to breed by *S. salamandra*. All of them were found during a bat survey made in January 2014 in the municipalities of Villanueva de San Carlos, Calzada de Calatrava, Mestanza and San Lorenzo de Calatrava (Ciudad Real, Spain).

The observation area consists of at least five railroad tunnels, currently unused and located near the river Ojailén, which are declared LIC microreserve because of its importance as refuge for different bat species. The surrounding landscape is quartzite outcrops and slopes of well-preserved Mediterranean forest oaks, cork oaks and gall-oaks with riparian vegetation. The tunnels have different length, the longest, where the larval and metamorphic *S. salamandra* were observed, having about 850m (38° 35' 10,03" N / 3° 52' 19,10" W), while the tunnel where the presence of *P. waltl* and *L. boscai* was recorded has around 400m long (38° 31' 49,33" N / 3° 53' 22,04" W). The tunnels had a medium temperature around 22°C while outside the temperature was around 8°C during the morning and under 0°C at night.

*P. waltl* was found in the deepest part of the tunnel in a small stream that poured from a source of water. A large sized (18 and 21 cm) couple was observed (Figure 1a). Both

newts were active (at midday) and seemed to have a good condition. Near them, evidence of breeding was reported when a few larvae were found in the stream. The nearest source of water outside the cave is more than 200 m away. This aquatic environment has neither plants nor stones in the water, so eggs must have been laid in the ground or in some holes at the sides of the tunnel where a female was found. The water was full of bat faeces and the density of sighted invertebrate preys was very low and probably different to that one outside the cave so it would be interesting to verify if the larvae would be able to complete their metamorphosis. The larvae we found had a small size, so we suspect that they may have been born recently but the fact is that we cannot proof the time since they hatched because larvae development can be modified depending on the water temperature (Figure 1b). Outside the cave, reproduction has not been reported in this zone at this time of the year by the authors. We suspect that the advanced breeding period observed inside the cave could be supported by the optimal temperatures found inside (around 22°C) respect to the 8°C outside the cave while the survey was made, between 11 a.m. - 13 p.m.

*L. boscai* was seen in the same spot that *P. waltl*. At least eight individuals were found, being of both sexes (Figure 1c). No presence of breeding was reported in this species. Although it usually lays their eggs in aquatic plants, it is also capable of using leaves and other sinked materials to lay their eggs (Garcia-París, 1985), so further visits along the breeding period should be carried out to verify if cave reproduction exists.

Fire salamander larvae (*S. salamandra*) were seen in all the tunnels and we suspect that these act as important breeding points



**Figure 1.** (a) an adult of *P. waltl*, (b) *P. waltl* larvae, (c) an adult of *L. boscai* and (d) a metamorphic *S. salamandra*.  
**Figura 1.** (a) un adulto de *P. waltl*, (b) larvas de *P. waltl*, (c) adulto de *L. boscai* y (d) un metamórfico de *S. salamandra*.

given the scarce sources of water around this winter. In the largest tunnel, there were high densities of larvae and several metamorphs were found (Figure 1d). Most of these metamorphs were in bad condition, specially the ones found further away from the entry. Bad condition can be related to the low prey availability. Some invertebrates were found in the water but none were found out of it. Anyways, it would be audacious to make any statements with this data, and further studies should be done to know if this tunnel could act as a sink for the population or as an opportunistic breeding point and population source of metamorphs (Manenti *et al.*, 2010).

Abandoned tunnels have shown to be important places for many organisms like bats, amphibians and invertebrates. This new observation highlights again the importance

of these environments for amphibians and brings new facts about their ecology in these habitats. But this new information also comes with a lot of questions to resolve in the future, like: Is the presence of the species in this habitat temporal and accidental, or permanent? Can some of the species (like the case of our fire salamander population) depend in some places totally on these bodies of water to breed? Can these habitats function as recruitment points in dry years? Can some of these species (e.g., *L. boscai*) complete the whole cycle in the cave/tunnel?

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