

the fact that the female was kept together with three males. Table 1 summarizes the dimensions of the eggs which fall within the range reported by BUSKIRK et al. (2001) for *T. graeca*. The eggs are nearly spherical, slightly depressed and bear now the collection numbers MTD 45262-45274.

It is unknown whether captive conditions influence clutch size in *Testudo*. However, it has to be pointed out that the reported record clutch might be the result of an optimal maintenance.

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Genetic variation in *Pleurodeles waltl* MICHAELLES, 1830 across the Strait of Gibraltar derived from mitochondrial DNA sequences

The Strait of Gibraltar is known as a natural barrier to gene flow for many amphibian and reptile species. Around 5.3 – 5.5 Mya, water from the Atlantic Ocean filled the Mediterranean basin – which had

dried up during the Messinian salinity crisis some 5.6 Mya (Hsü et al. 1977) – separating the terrestrial fauna into allopatric units. Variation in levels of genetic differentiation estimated using protein electrophoretic data, however, implied that not all herpetofauna were equally affected by this event (BUSACK 1986), as some species showed evidence of gene flow after the opening of the Strait. For the wall lizards of the *Podarcis hispanica* (STEINDACHNER, 1870) species complex, for example, the opening of the Strait of Gibraltar was not directly related to genetic divergences between Iberian and Moroccan populations (HARRIS et al. 2002), while it may have been for *Acanthodactylus erythrus* (SCHINZ, 1833) (HARRIS et al. 2004).

Our aim was to examine the genetic diversity within part of the 12S rRNA mtDNA gene of Spanish Ribbed Newts, *Pleurodeles waltl* MICHAELLES, 1830, across the Strait of Gibraltar and compare this with published data from other species. We compared a molecular clock previously applied to *Pleurodeles* and *Euproctus* (CACCONI et al. 1994), in order to try to assess how long the populations have been separated, and if divergences were concordant with the opening of the Strait. Finally, we also compared our results to two recent studies of *Pleurodeles* phylogeography (CARRANZA & ARNOLD 2004; VEITH et al. 2004) to more precisely assess the distribution of genetic variation across the species range.

Genomic DNA was extracted following standard high-salt protocols. The 12S fragment was amplified by PCR using the primers published in KOCHER et al. (1989) and conditions described in HARRIS (2001). Sampling localities are given in table 1. The amplified products were sequenced on an automated sequencer (ABI 310). New sequences were deposited on Genbank, accession numbers AY522558 to AY522566. Sequences were aligned against one previously published *P. waltl* (CACCONI et al. 1994). In total ten sequences of 374 base pairs were included. Alignment was facile, as only one single base pair insertion was needed. Including the indel, six sites were parsimoniously informative. There was no homoplasy, so the sequences were joined into a median network (BANDELT et al. 2000). The previously published sequence

of unknown locality (CACCONI et al. 1994) is identical to two of our sequences from individuals from Morocco.

According to our results, two distinct groups of haplotypes can be identified, shown in fig. 1. One group is made up of specimens of *P. waltl* from Portugal and Spain, and the second one includes the Moroccan samples and one individual from Cadiz. The level of genetic variation between these two groups is approximately 1.1%. If we use the evolution rate proposed by CACCONI et al. (1994), this level of divergence implies a separation of approximately 3.5 My, which is after the formation of the Strait of Gibraltar and similar to 3.2 My estimated by CARRANZA & ARNOLD (2004). After the Strait opened, the Cadiz region was an island and remained separated from the rest of the Iberian Peninsula by the Guadalquivir Rift for some million years (JONG 1998). *Pleurodeles* in this region of South-West Iberia are apparently genetically more closely related to North African populations than to Iberian populations. The same is also observed in *Podarcis* lizards (HARRIS et al. 2002), but levels of genetic divergence between the two clades are higher in *Podarcis*. The existence of multiple haplotypes in Morocco, and its wide distribution, argues against an anthropogenic introduction as suggested by CARRANZA & ARNOLD (2004), but rather natural colonization in an extremely recent evolutionary time frame.

Table 1: Specimens of *Pleurodeles waltl* MICHAELLES, 1830, sequenced for this analysis with locality and specimen code number. A photo of the specimens Pw1 and Pw2 is provided in CARRETERO et al. (2004).

Code	Locality
Pw1	Al-Jadida, Morocco
Pw2	Al-Jadida, Morocco
Pw3	Al-Jadida, Morocco
Pw4	Torreira, Portugal
Pw5	Torreira, Portugal
Pw6	Sagres, Portugal
Pw7	Sagres, Portugal
Pw8	Badajoz, Spain
Pw9	Cadiz, Spain

To conclude, our results suggest that there are two genetically distinct groups within *P. waltl*, but that these are not separated by the Strait of Gibraltar. By combining our data with that from two recent studies (fig. 2) it seems that the Guadalquivir river approximately separates the two groups, although detailed sampling in this area is still needed. A similar distribution pattern is seen in *Discoglossus* (GARCIA-PARIS & JOCKUSCH 1999). Our results further highlight the diverse phylogenetic patterns of herpetofauna observed from this geologically complex region.

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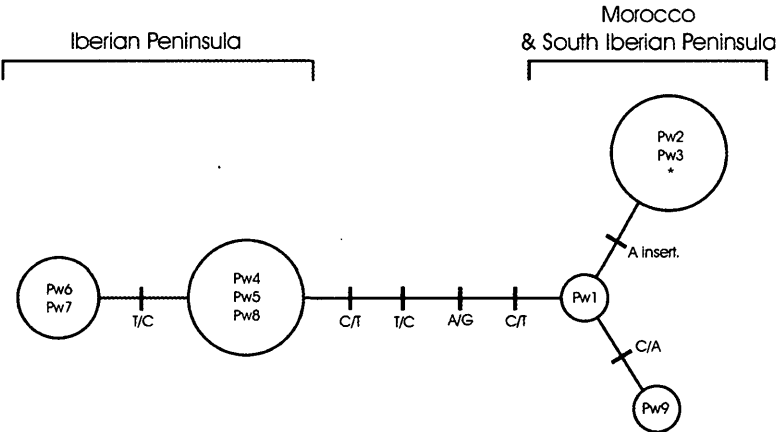


Fig. 1: Network showing relationship between *Pleurodeles waltl* sequences. * indicates the previously published sequence (CACCONI et al. 1994). All inferred mutations are shown.

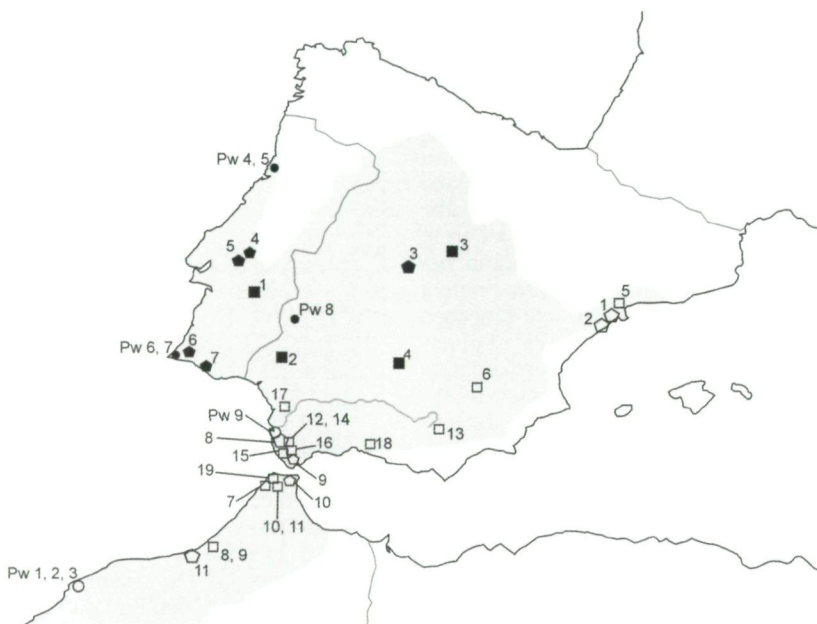


Fig. 2: Distribution of *Pleurodeles waltl* MICHAËLLES, 1830 (grey shaded area) and sampling localities (from this study, CARRANZA & ARNOLD 2004, VEITH et al. 2004). Filled symbols represent one genetic clade relative to the other (unfilled symbols). The Guadalquivir river is shown, and approximately separates the two clades.

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