

Record clutch size in *Testudo graeca ibera* PALLAS, 1814 s. l.
from Van Gölü region (Turkey)

In astonishingly few cases clutch sizes have been exactly documented for spur-thighed tortoises (*Testudo graeca* complex). The taxonomic diversity of this group is barely understood and has long been underestimated (e. g., BUSKIRK et al. 2001; PERÄ-LÄ 2002a, 2002b; PIEH 2002; PIEH et al. 2002). It is likely that several taxa with a different natural history and reproductive mode are hidden behind what is currently known under a variety of different scientific names. Therefore, natural history data referring to a certain geographic region are highly welcome. BUSKIRK et al. (2001) reviewed clutch sizes from Europe, North Africa and the Caucasian area. In Spain, clutches contain 1-7 eggs (mean: 3.5 eggs per clutch in the Doñana reserve). In North Africa the clutch size ranges from 2-7 eggs (BUSKIRK et al. 2001). However, HIGHFIELD (1996) claimed that 12-14 eggs are the “typical clutch size” of “*Testudo whitei*” BENNETT, 1836, a dubious taxon allegedly from North Africa. This would outnumber the proven clutch sizes in the Western Mediterranean area by far. For Greece, 7 eggs are known as maximum clutch size (average 4.5 eggs), and in Romania it might be that the maximum number slightly exceeds that figure with 8 eggs. In the Caucasian area, clutches contain 2-8 but most frequently 5 or 6 eggs (BUSKIRK et al. 2001). HERZ (1999) obtained two clutches with 5 and 6 eggs from a female *T. graeca anamurensis* WEISSINGER, 1987 from the southern Turkish coast. According to LAPID & ROBINZON (2002), Israeli *T. graeca* produce as many as 6 eggs (range 1-6), with an average of 3.16 ± 0.15 eggs in the first clutch, and, when present, 3.40 ± 0.45 eggs in the second. RUDLOFF (1990) and HIGHFIELD (1990, 1996) claimed, without specifying the geographic origin of specimens and the provenance of their data, that in “*Testudo graeca*” and “*Testudo ibera*” a clutch may consist of up to 12 eggs.

Recently the Museum für Tierkunde (Museum of Zoology) Dresden (MTD) obtained a complete clutch of a female *T. graeca ibera* PALLAS, 1814 sensu lato (straight



Fig. 1: Clutch of *Testudo graeca ibera* PALLAS, 1814 sensu lato (MTD 45262-45274) originating from Van Gölü, Turkey. Scale represents 10 cm.

line carapacial length 240 mm, body mass approximately 2750 g). The tortoise was collected years ago near Van Gölü, Turkey and since then kept in captivity in Central Europe. Tortoises from the Van Gölü region are currently referred to as *T. graeca ibera* but thought to represent an undescribed taxon by PIEH et al. (2002). The clutch was laid on May 24, 2003 and comprised 13 eggs (fig. 1). All eggs were infertile, despite

Table 1: Egg dimensions of a complete clutch of 13 eggs of *Testudo graeca ibera* PALLAS, 1814 sensu lato originating from Van Gölü, Turkey. SE - Standard error of the mean, MTD - Museum für Tierkunde Dresden.

Catalogue Number	Maximum Egg Length	Maximum / Minimum Egg Diameter
MTD 45262	35.6	34.1 / 32.3
MTD 45263	35.0	32.8 / 30.9
MTD 45264	37.0	32.5 / 29.9
MTD 45265	38.5	33.0 / 28.7
MTD 45266	37.4	34.8 / 32.6
MTD 45267	37.5	34.2 / 32.3
MTD 45268	37.9	34.7 / 32.6
MTD 45269	38.2	34.0 / 32.0
MTD 45270	38.5	33.8 / 31.4
MTD 45271	33.6	32.5 / 26.7
MTD 45272	36.6	33.9 / 31.8
MTD 45273	36.9	33.0 / 31.0
MTD 45274	34.4	32.8 / 30.8
mean±SE	36.7±0.4	33.6±0.2 / 31.0±0.5
range	33.6–38.5	32.5–34.8 / 26.7–32.6

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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AUTHOR: Dr. Uwe FRITZ, Museum für Tierkunde Dresden, Staatliche Naturhistorische Sammlungen, Königsbrücker Landstraße 159, D-01109 Dresden, FR Germany

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

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Autor(en)/Author(s): Fritz Uwe

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