

## Out of Africa: did *Emys orbicularis occidentalis* cross the Strait of Gibraltar twice?

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**Abstract.** The narrow Strait of Gibraltar has separated the African and European continents since the Miocene (5.3 Mya), with a different degree of permeability for Mediterranean taxa. Southern and northern regions of the Iberian Peninsula and Morocco, respectively, are key areas to evaluate the colonization dynamics and biogeographic history of taxa occurring at both sides of this strait. The Ibero-Maghrebian subspecies of the European pond turtle, *Emys orbicularis occidentalis*, is patchily distributed and threatened throughout most of the Iberian Peninsula and northern Morocco and its origin is thought to be in North Africa. Here we expand the geographic sampling across the Iberian Peninsula and Morocco, with special emphasis in the southern tip of the peninsula and northern Morocco, and analyze mtDNA sequences of 183 *E. o. occidentalis* to better understand the complex biogeographic history of this subspecies. We provide for the first time evidence for shared haplotypes of Iberian and North African pond turtles, with an additional haplotype in the southern Iberian Peninsula derived from Moroccan haplotypes. This supports the hypothesis that the Strait of Gibraltar constitutes no significant biogeographic barrier for *E. orbicularis*. However, the newly discovered shared, or extremely similar, haplotypes of European pond turtles from the southern Iberian Peninsula and Morocco suggest either that at least two independent natural colonization waves from Morocco have reached the Iberian Peninsula or that Moroccan turtles were accidentally or deliberately introduced there.

**Keywords:** colonization, Iberian Peninsula, Morocco, phylogeography.

### Introduction

The Ibero-Maghrebian subspecies of the European pond turtle, *Emys orbicularis occidentalis*, is patchily distributed throughout most of the Iberian Peninsula and northern Morocco (Velo-Antón et al., 2008, 2015), i.e. on both sides of the Strait of Gibraltar, which has separated the African and European continents since the Miocene (5.3 Mya). Recent phylogenetic

and population genetic analyses of African and Iberian pond turtles suggest a complex biogeographic scenario (Stuckas et al., 2014), with a pre-Pleistocene invasion of North Africa from an unknown European source region and a later colonization of the Iberian Peninsula from Morocco. Moroccan populations show higher levels of genetic diversity compared to Iberian populations and are structured in two main groups, the Rif Mountains and the Middle Atlas Mountains plus the Atlantic coast. Regarding mtDNA, these Moroccan groups are paraphyletic with respect to Iberian samples. Furthermore, in the Iberian Peninsula genetic diversity decreases northwards (Velo-Antón et al., 2008), both supporting the hypothesis of a colonization from Morocco and rapid population expansion across the Iberian Peninsula.

For some species distributed on both sides of the Strait of Gibraltar, recent phylogeographic studies have identified high levels of genetic structure in southern Iberian populations (e.g. Kaliontzopoulou et al., 2011; Miraldo et al., 2011; Santos et al., 2012; Velo-Antón et al., 2012) and Morocco (Beukema et al.,

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2010), unveiling cryptic lineages and suggestive of complex biogeographic processes since the Pliocene. Despite the comprehensive sampling accumulated for *E. o. occidentalis* over the last decade (Fritz et al., 2007; Velo-Antón et al., 2008, 2011a; Pedall et al., 2011; Stuckas et al., 2014), isolated populations in both the Iberian Peninsula and mainly Morocco remained undetected and unstudied for a long time. In particular, recent studies discovered highly fragmented populations in the southern Iberian Peninsula (Jiménez-Cazalla and Castro-Rodríguez, 2014; Romero et al., 2014) and Morocco (Velo-Antón et al., 2015), which are imperilled by massive habitat loss. The current situation of *E. o. occidentalis* in southern Cádiz (Jiménez-Cazalla and Castro-Rodríguez, 2014) and the Sierra de Grazalema, Málaga (Romero et al., 2014), is precarious, with only a few individuals persisting in isolated localities. These turtles probably represent the last survivors of formerly widely distributed populations in the southern Iberian Peninsula.

In the present study we significantly expand the previous geographical sampling (Velo-Antón et al., 2008; Stuckas et al., 2014) of *E. o. occidentalis* throughout the Iberian Peninsula and Morocco to achieve a better understanding of the complex biogeography and evolutionary history of European pond turtles in this region. We study the genetic diversity and structure of Iberian and Moroccan populations using 82 newly collected samples from previously unknown localities and the mitochondrial cytochrome *b* gene as a phylogeographic marker.

## Materials and methods

### Sample collection and mitochondrial DNA sequencing

We collected 55 and 27 new samples from Iberian and Moroccan populations, respectively, considerably expanding the number of sampled populations, in particular with respect to their spatial distribution. We collected blood (either preserved in ethanol or as dried blood spots) or tissue samples (buccal swabs) and extracted genomic DNA using the EasySpin commercial kits, following the manufacturer's

protocols, with an extended lysis time to maximize the yield. We PCR-amplified a fragment of ca 1100 base pairs of the mitochondrial cytochrome *b* (cyt *b*) gene and sequenced PCR products according to the procedures described in previous publications (Velo-Antón et al., 2008; Stuckas et al., 2014). Below, we follow the haplotype nomenclature system of Lenk et al. (1999) in that a Roman numeral identifies the mitochondrial lineages of *E. orbicularis* and *E. trinacris* and consecutive letters, individual haplotypes of each lineage.

### Genetic analyses

Using GENEIOUS (<http://www.geneious.com/>), we manually aligned and merged our new sequences with a previously published comprehensive data set (Stuckas et al., 2014), consisting of cyt *b* sequences for *E. orbicularis* and *E. trinacris* that represent virtually the entire distribution ranges of these species. The resulting data set of 1513 sequences contained a total of 183 sequences from *E. o. occidentalis*, including our 82 new samples (see online supplementary table S1).

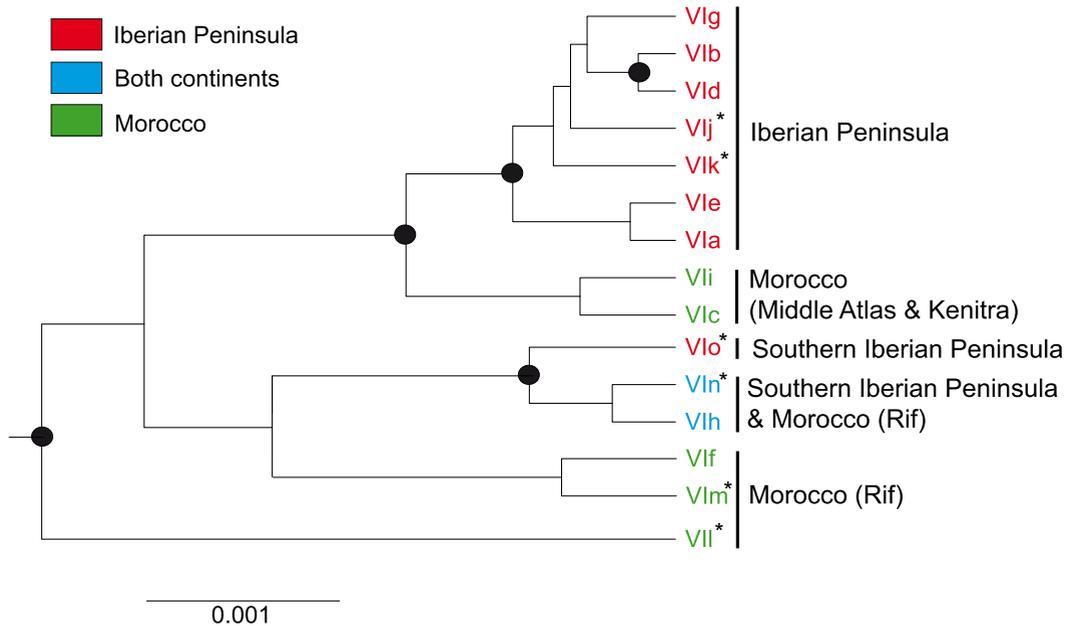
We first collapsed all sequences into haplotypes and assessed phylogenetic relationships using a Bayesian inference (BI) approach. We obtained the best fitting model of nucleotide substitution for our dataset using jMODELTEST v.2.1.4 (Darriba et al., 2012) and the Bayesian information criteria correction (BICc; HKY+G). Then we conducted three independent runs of four Markov chains using BEAST v1.7.5 (Drummond et al., 2012). We used 10 million generations, with a sampling frequency of 100 generations, and discarded 25% of the trees as burn-in. To verify that the analyses were stationary, we examined the effective sample sizes (ESSs) using TRACER v1.6 (all parameter values of ESS were above 300), and confirmed chain convergence by comparing the three runs. We used the remaining trees to obtain a maximum clade credibility summary tree with posterior probabilities for each node in TREEANNOTATOR v1.7.5 (distributed with the BEAST package).

In addition, we evaluated the evolutionary relationships of sequences with a statistical parsimony network as implemented in TCS 1.21 (Clement et al., 2000), using the default 95% probability threshold.

To examine the geographic distribution of genetic diversity in *E. o. occidentalis*, we grouped the sequences as belonging either to the Iberian Peninsula or Morocco. However, we explored genetic diversity of the Iberian group also without the southern Iberian haplotypes VIh, VIIn and VIo, which are shared with Morocco or closely related to Moroccan haplotypes (table 1). As North Africa has been identified as the source region for *E. o. occidentalis* and Moroccan populations are genetically more variable than Iberian populations, we divided Moroccan samples in two subgroups, according to the differentiation revealed by microsatellite analyses (Stuckas et al., 2014): the Rif and the Middle Atlas Mountains. The latter subgroup included also the two samples of Stuckas et al. (2014) from Kenitra province (Atlantic coast). Then, we assessed the number of segregating sites (*S*), the nucleotide ( $\pi$ ) and haplotype diversity (*Hd*) for each group and subgroup using DnaSP v5.10 (Librado

**Table 1.** Summary table of all genetic diversity and demographic parameters measured for *Emys orbicularis occidentalis* and geographical subgroups. *N*, sample size; *S*, polymorphic sites; *H*, number of haplotypes; *Hd*, haplotype diversity;  $\pi$ , nucleotide diversity; Tajima's *D*; Fu's *F<sub>S</sub>*. Significant results for *F<sub>S</sub>* shown in bold ( $P < 0.02$ ). We do not represent demographic measures for Moroccan subgroups (NA = not available).

Group	<i>N</i>	<i>S</i>	<i>H</i>	<i>Hd</i>	$\pi$	Tajima's <i>D</i>	<i>F<sub>S</sub></i>
<i>E. o. occidentalis</i>	183	22	15	0.71	0.0025	-1.26	-3.02
Iberian Peninsula	129	16	10	0.52	0.0017	-1.36	-1.07
Iberian Peninsula (excluding VIh, VIIn, VIo)	124	13	7	0.48	0.0009	-1.5	-1.93
Morocco	54	14	7	0.64	0.0018	-1.38	-0.22
Rif Mountains	31	8	5	0.39	0.0016	NA	NA
Middle Atlas (and Kenitra)	23	6	2	0.24	0.0006	NA	NA



**Figure 1.** Bayesian consensus tree based on 1030 bp of mtDNA (*cyt b*) of *Emys orbicularis occidentalis* (outgroups removed for clarity). Black dots at nodes represent posterior probabilities  $> 0.98$ . Asterisks indicate new haplotypes. Colours indicate Iberian (red), Moroccan (green) and shared haplotypes between continents (light blue).

and Rozas, 2009). We also calculated two tests of selective neutrality (Tajima's *D* and Fu's *F<sub>S</sub>*) to examine *E. o. occidentalis* as a whole and the Iberian and Moroccan groups for signatures of demographic expansion.

## Results

In addition to the nine previously reported haplotypes for *E. o. occidentalis* (Stuckas et al., 2014), we discovered six new haplotypes (fig. 1; table S1). Haplotypes VIj, VIk and VIo are endemic to the Iberian Peninsula; haplotypes VII and VIIm are endemic to Morocco (Rif Moun-

tains); and haplotype VIIn is occurring both in the southern Iberian Peninsula (Cádiz province) and Morocco (Rif). We also discovered haplotype VIh, previously only known from Morocco (Rif), in southern Spain (Málaga).

Bayesian phylogenetic analyses resulted in a well-supported clade corresponding to *E. o. occidentalis* (posterior probability: 1.0). However, there was weak phylogenetic structure within the subspecies (fig. 1). Three haplotypes (VIf, VIIm, VII) from the Rif Mountains were not monophyletic. The remaining haplotypes clustered in two well-supported subclades: one sub-

clade was comprised of haplotypes shared between the Iberian Peninsula and Morocco (VIh and VIh) plus another haplotype endemic to the southern Iberian Peninsula (VIo) and the second subclade consisted of all remaining Iberian and Moroccan haplotypes.

In parsimony network analysis, all *E. o. occidentalis* haplotypes grouped in the same cluster (fig. 2; table S1). Haplotype VIh is found across the Rif Mountains ( $n = 27$ ). Other haplotypes from the Rif (VII, VIIm) and the southern Iberian Peninsula (VIh, VIIn, VIo) are connected by 1–5 mutational steps to haplotype VIh. The most divergent haplotype, VIo, was found in Málaga ( $n = 1$ ) and differs in two steps from VIh, which was found in Cádiz and in the Rif. The two haplotypes from the Middle Atlas and Kenitra province (VIc, VIi) differ in just two and one mutational steps from VIh and the Iberian group, respectively. This “Iberian group” (VIa, VIb, VIc, VIe, VIg, VIj, VIk) shows a clear star-like network pattern as frequently found in demographic expansion scenarios, with one widespread common haplotype (VIa;  $n = 99$ ).

Compared to Iberian *E. o. occidentalis*, Moroccan pond turtles show a higher genetic diversity ( $Hd$ ,  $\pi$ ; table 1), even though the number of Iberian samples almost doubled the Moroccan samples. Within Morocco, the turtles from the Rif Mountains had a higher genetic diversity than the populations from the Middle Atlas and Kenitra (table 1). Fu’s  $F_S$  values were significantly negative both in *E. o. occidentalis* as a whole and in the Iberian Peninsula (including or excluding the southern Iberian haplotypes; table 1), suggestive of population expansion.

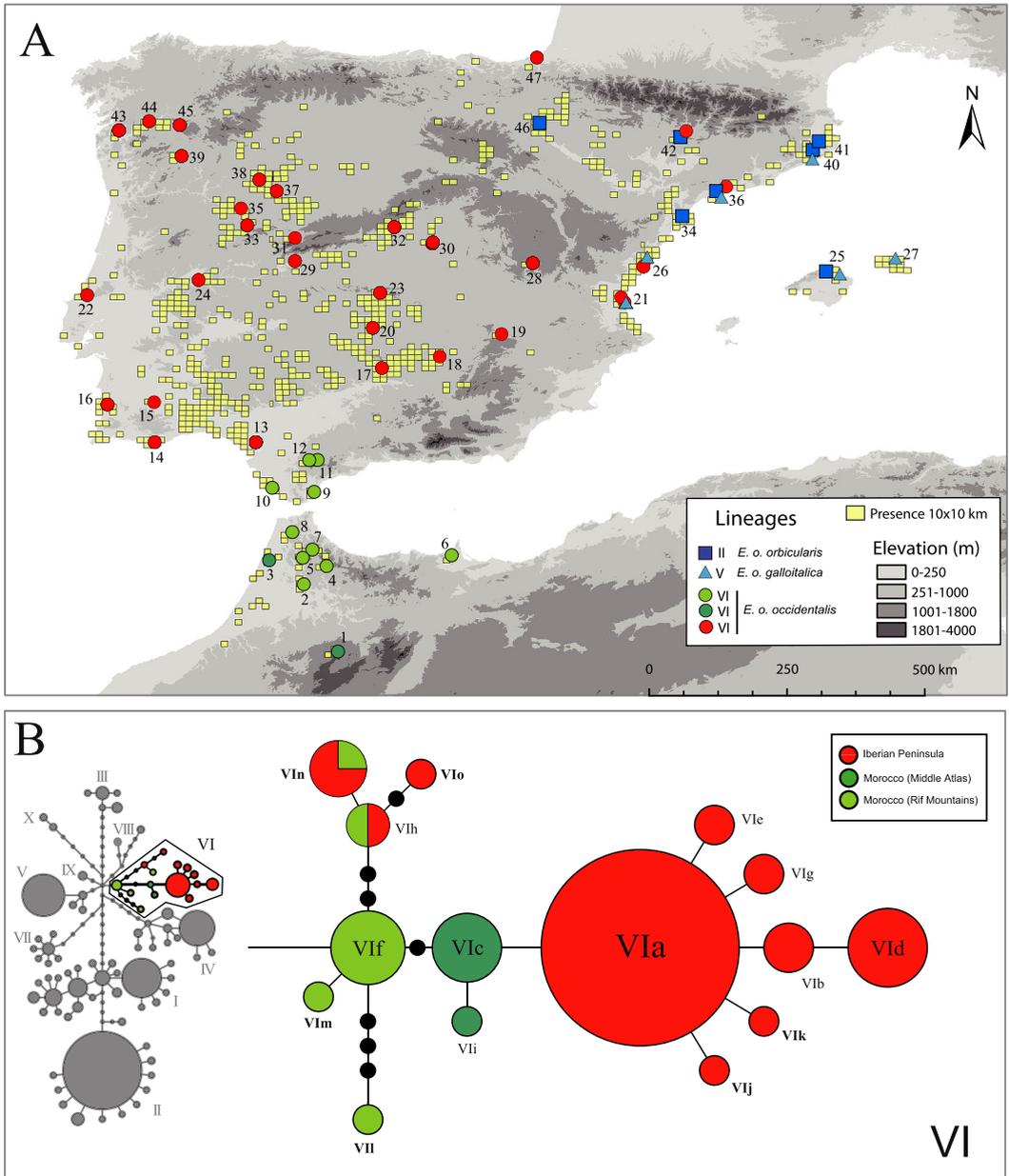
## Discussion

Our study significantly expanded the previous sampling for the Iberian Peninsula (Pedall et al., 2011; Velo-Antón et al., 2011a; Stuckas et al., 2014) and includes data for 15 new localities, some of which represent isolated remnant populations consisting of a few turtles only (e.g. Albacete, Cádiz, Cuenca, Málaga, Jaén; ta-

ble S1; fig. 2A). If the secondary contact zone in the northeastern Iberian Peninsula (Fritz et al., 2007; Pedall et al., 2011) is disregarded, all Iberian pond turtles represent the genetic lineage (VI) corresponding to *E. o. occidentalis*. This subspecies is also distributed in Morocco and has there its greatest genetic diversity (Stuckas et al., 2014). The low genetic diversity, the star-like haplotype network of most Iberian samples (fig. 2B), and demographic results (Velo-Antón et al., 2008, 2011b) suggest recent arrival from Morocco (Fritz et al., 2007; Stuckas et al., 2014) and population expansion throughout the Iberian Peninsula.

In the present study we provide for the first time evidence for haplotype sharing between Moroccan and Iberian populations (VIh, VIIn). Moreover, three new haplotypes (VIh, VIIn, VIo) from southern Iberia are derived from haplotype VIh from the Rif Mountains (Morocco) and not from Iberian haplotypes.

These shared or extremely similar haplotypes from the southern Iberian Peninsula and Morocco argue for very recent genetic exchange. It is well known that turtles and tortoises have been extensively used for food in (pre)historical times and chelonians frequently served as live provisions during sea voyages (e.g. Vamberger et al., 2011; Fritz et al., 2013). In recent times, turtles and tortoises were also sold in large numbers as pets. *Emys orbicularis* is not an exception, and the extant populations from Corsica, Sardinia and the Balearic Islands are thought to be naturalized (Fritz et al., 1998, 2007; Lenk et al., 1999; Pedall et al., 2011; Vamberger et al., 2015). Moreover, there is firm genetic evidence for long-distance translocations of pond turtles in many parts of Europe as a result of massive trade (Fritz et al., 2004; Velo-Antón et al., 2011a; Vamberger et al., 2015). Thus, it cannot be excluded that the ‘Moroccan haplotypes’ from southern Spain result from introduced turtles from Morocco. There are also two other well-known cases where Moroccan reptiles have evidently been introduced to the Iberian Peninsula (*Chameleo chameleon*: Paulo



**Figure 2.** (A) Present distribution of *Emys orbicularis* in the Iberian Peninsula and Morocco (yellow cells) and sampling locations identifying each mitochondrial lineage (symbols) and population group (colours) in *E. o. occidentalis*. (B) On the left, haplotype network for ca 1500 samples of *E. orbicularis* and *E. trinacris* sampled throughout their entire distribution ranges showing main mitochondrial lineages; on the right, haplotype relationships and geographic distribution within *E. o. occidentalis*. Lines connecting haplotypes indicate mutational steps and black dots represent missing intermediate haplotypes that were not found in the analyzed individuals.

et al., 2002; *Testudo graeca* in the Doñana National Park: Graciá Martínez et al., 2015).

Alternatively, if the turtles with ‘Moroccan haplotypes’ were not introduced, at least two independent natural colonization waves from Morocco must have reached the Iberian Peninsula. This implies transoceanic dispersal across the Strait of Gibraltar. During Pleistocene low sea level stands, the Strait of Gibraltar was only 5 km wide (Zazo, 1999), and even today the sea strait has a width of only approximately 15 km. Transoceanic dispersal, even across much larger distances, has been used to explain the shallow genetic divergence of other freshwater turtle species (Parham et al., 2008; Vamberger et al., 2014, 2015). Under this scenario, a first colonization wave (probably during the Pleistocene) would have given rise to the endemic Iberian haplotypes (fig. 1). Then, a second very recent colonization event from Morocco would have resulted in the arrival of the ‘Moroccan haplotypes’ in southern Spain.

Unfortunately, we were able to obtain only a few samples from Cádiz and Málaga provinces, and the rareness of turtles from there makes it difficult to decide whether they are native or introduced. These local populations have now low densities and are probably relicts from formerly more abundant and widely distributed populations that collapsed due to habitat loss (Jiménez-Cazalla and Castro-Rodríguez, 2014). In any case, the high level of genetic diversity of Moroccan populations compared to the Iberian ones, and the position of Moroccan haplotypes in both the phylogenetic tree and haplotype network (figs 1 and 2B), support the North African origin for *E. o. occidentalis* as hypothesized by Stuckas et al. (2014).

The general phylogeographic pattern of *E. o. occidentalis* fits the paradigm of North Africa acting as a refuge in the western Mediterranean Basin during the Pleistocene (see Husemann et al., 2014 for a review). Several other Ibero-Maghrebian amphibians and reptiles show similar phylogeographies, suggestive of recent colonizations from Morocco (e.g. *Pleurodeles waltl*,

Veith et al., 2004; *Macroprotodon*, Carranza et al., 2004; *Malpolon monspessulanus* and *Hemorrhois hippocrepis*, Carranza et al., 2006; *Mauremys leprosa*, Fritz et al., 2006; *Hyla meridionalis*, Recuero et al., 2007; *Tarentola mauritanica*, Rato et al., 2012; *Testudo graeca*, Graciá et al., 2013).

Moroccan populations of *E. o. occidentalis* are genetically structured, both with respect to mitochondrial (fig. 1B) and microsatellite data (Stuckas et al., 2014), and this pattern allows some inferences. The southernmost populations from the Middle Atlas, and most probably also the few surviving individuals along the Atlantic coast (cf. Velo-Antón et al., 2015), represent a distinct genetic group (Stuckas et al., 2014) that is in haplotype network analysis intermediate between the genetic clusters from Iberia and the Rif Mountains (haplotypes VIc and VII; fig. 2B). We found in the Middle Atlas populations weak genetic differentiation and low diversity. In contrast, the populations from the Rif show a distinctly higher genetic divergence and diversity. The common haplotype VIc is widespread across the Rif and some additional, in part well-differentiated, haplotypes (VIh, VII, VIi, VIj, VIk, VIl, VIm, VIo) are found in the western Rif (fig. 2B; table S1). This suggests that haplotype lineage VI originated in the Rif. Other examples of a similarly pronounced genetic divergence of wide-spread amphibian and reptile species in the Rif Mountains include *Salamandra algira* (Beukema et al., 2010) and *Tarentola mauritanica* (Rato et al., 2012).

In conclusion, our study provides for the first time evidence for shared haplotypes of Iberian and North African pond turtles, reinforcing the hypothesis that the Strait of Gibraltar constitutes no significant biogeographic barrier for *E. orbicularis*. Pond turtle populations in Morocco are ancestral to Iberian populations and contain much of the genetic diversity of *E. o. occidentalis*. However, the Moroccan populations are also highly fragmented and endangered (Velo-Antón et al., 2015), so that a conservation strategy should be designed to preserve their genetic

diversity. Although extensive fieldwork campaigns have been conducted in southern Spain (Jiménez-Cazalla and Castro-Rodríguez, 2014; Romero et al., 2014), future efforts are needed to obtain a better understanding of the origin of the southernmost Iberian populations.

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## References

- Beukema, W., de Pous, P., Donaire, D., Escoriza, D., Boggaerts, S., Toxopeus, A.G., Biem, C.A.J.M., Roca, J., Carranza, S. (2010): Biogeography and contemporary climatic differentiation among Moroccan *Salamandra algira*. *Biol. J. Linn. Soc.* **101**: 626-641.
- Carranza, S., Arnold, E.N., Wade, E., Fahd, S. (2004): Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from North-west Africa. *Mol. Phylogenet. Evol.* **33**: 523-532.
- Carranza, S., Arnold, E.N., Pleguezuelos, J.M. (2006): Phylogeny, biogeography, and evolution of two Mediterranean snakes, *Malpolon monspessulanus* and *Hemorrhois hippocrepis* (Squamata, Colubridae), using mtDNA sequences. *Mol. Phylogenet. Evol.* **40**: 532-546.
- Clement, M., Posada, D., Crandall, K. (2000): TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657-1659.
- Darriba, D., Taboada, G., Doallo, R., Posada, D. (2012): jModelTest 2: more models, new heuristics and parallel computing. *Nature Meth.* **9**: 772.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. (2012): Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**: 1969-1973.
- Fritz, U., Peh, A., Lenk, P., Mayol, J., Sättele, B., Wink, M. (1998): Is *Emys orbicularis* introduced on Majorca? *Mertensiella* **10**: 122-133.
- Fritz, U., Guicking, D., Lenk, P., Joger, U., Wink, M. (2004): When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain. *Biologia* **59** (Suppl. 14): 19-25.
- Fritz, U., Barata, M., Busack, S.D., Fritzsich, G., Castilho, R. (2006): Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geoemydidae). *Zool. Scr.* **35**: 97-108.
- Fritz, U., Guicking, D., Kami, H., Arakelyan, M., Auer, M., Ayaz, D., Ayres Fernández, C., Bakiev, A.G., Celani, A., Džukić, G., Fahd, S., Havaš, P., Joger, U., Khabibullin, V.F., Mazanaeva, L.F., Široký, P., Tripepi, S., Valdeón Vélez, A., Velo Antón, G., Wink, M. (2007): Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*) – an update. *Amphibia-Reptilia* **28**: 418-426.
- Fritz, U., Branch, W.R., Gehring, P.S., Harvey, J., Kindler, C., Meyer, L., Du Preez, L., Široký, P., Vieites, D.R., Vences, M. (2013): Weak divergence among African, Malagasy and Seychellois hinged terrapins (*Pelusios castanoides*, *P. subniger*) and evidence for human-mediated oversea dispersal. *Org. Diver. Evol.* **13**: 215-224.
- Graciá, E., Giménez, A., Anadón, J.D., Harris, D.J., Fritz, U., Botella, F. (2013): The uncertainty of Late Pleistocene range expansions in the western Mediterranean: a case study of the colonization of south-eastern Spain by the spur-thighed tortoise, *Testudo graeca*. *J. Biogeogr.* **40**: 323-334.
- Graciá Martínez, E., Botella Robles, F., Giménez Casaldueiro, A., Rodríguez-Caro, R.C., Anadón Herrera, J., Fritz, U. (2015): ¿Autóctona o introducida? Origen de la población de tortuga mora en el sureste ibérico. *Quercus* **347**: 32-40.
- Husemann, M., Schmitt, T., Zachos, F.E., Ulrich, W., Habel, J.C. (2014): Palaeartic biogeography revisited: evidence for the existence of a North African refugium for Western Palaeartic biota. *J. Biogeogr.* **41**: 81-94.
- Jiménez-Cazalla, F., Castro-Rodríguez, M. (2014): Sobre el galápagos europeo *Emys orbicularis* en el sur de Cádiz (SO de la Península Ibérica). *Bol. Asoc. Herpetol. Esp.* **25**: 82-85.
- Kaliontzopoulou, A., Pinho, C., Harris, D.J., Carretero, M.A. (2011): When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biol. J. Linn. Soc.* **103**: 779-800.
- Lenk, P., Fritz, U., Joger, U., Wink, M. (1999): Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Mol. Ecol.* **8**: 1911-1922.
- Librado, P., Rozas, J. (2009): DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452.
- Miraldo, A., Hewitt, G.M., Paulo, O.S., Emerson, B.C. (2011): Phylogeography and demographic history of *Lacerta lepida* in the Iberian Peninsula: multiple refugia, range expansions and secondary contact zones. *BMC Evol. Biol.* **11**: 170.
- Parham, J.F., Outerbridge, M.E., Stuart, B.L., Wingate, D.B., Erlenkeuser, H., Papenfuss, T.J. (2008): Introduced delicacy or native species? A natural origin of Bermudian terrapins supported by fossil and genetic data. *Biol. Lett.* **4**: 216-219.

- Paulo, O.S., Pinto, I., Bruford, M.W., Jordan, W.C., Nichols, R.A. (2002): The double origin of Iberian peninsular chameleons. *Biol. J. Linn. Soc.* **75**: 1-7.
- Pedall, I., Fritz, U., Stuckas, H., Valdeón, A., Wink, M. (2011): Gene flow across secondary contact zones of the *Emys orbicularis* complex in the Western Mediterranean and evidence for extinction and re-introduction of pond turtles on Corsica and Sardinia (Testudines: Emydidae). *J. Zool. Syst. Evol. Res.* **49**: 44-57.
- Rato, C., Carranza, S., Harris, D.J. (2012): Evolutionary history of the genus *Tarentola* (Gekkota: Phyllodactylidae) from the Mediterranean Basin, estimated using multilocus sequence data. *BMC Evol. Biol.* **12**: 14.
- Recuero, E., Iraola, A., Rubio, X., Machordom, A., García-París, M. (2007): Mitochondrial differentiation and biogeography of *Hyla meridionalis* (Anura: Hylidae): an unusual phylogeographical pattern. *J. Biogeogr.* **34**: 1207-1219.
- Romero, D., Farfán, M.A., Duarte, J., Narváez, L., Real, R. (2014): El galápagos europeo requiere atención urgente en Málaga. *Quercus* **339**: 56-57.
- Santos, X., Rato, C., Carranza, S., Carretero, M.A., Pleguezuelos, J.M. (2012): Complex phylogeography in the southern smooth snake (*Coronella girondica*) supported by mtDNA sequences. *J. Zool. Syst. Evol. Res.* **50**: 210-219.
- Stuckas, H., Velo-Antón, G., Fahd, S., Kalboussi, M., Rouag, R., Arculeo, M., Marrone, F., Sacco, F., Vamberger, M., Fritz, U. (2014): Where are you from, stranger? The enigmatic biogeography of North African pond turtles (*Emys orbicularis*). *Org. Divers. Evol.* **14**: 295-306.
- Vamberger, M., Corti, C., Stuckas, H., Fritz, U. (2011): Is the imperilled spur-thighed tortoise (*Testudo graeca*) native in Sardinia? Implications from population genetics and for conservation. *Amphibia-Reptilia* **32**: 9-25.
- Vamberger, M., Stuckas, H., Ayaz, D., Lymberakis, P., Šíroký, P., Fritz, U. (2014): Massive transoceanic gene flow in a freshwater turtle (Testudines: Geoemydidae: *Mauremys rivulata*). *Zool. Scr.* **43**: 313-322.
- Vamberger, M., Stuckas, H., Sacco, F., D'Angelo, S., Arculeo, M., Cheylan, M., Corti, C., Lo Valvo, M., Marrone, F., Wink, M., Fritz, U. (2015): Differences in gene flow in a twofold secondary contact zone of pond turtles in southern Italy (Testudines: Emydidae: *Emys orbicularis galloitalica*, *E. o. hellenica*, *E. trinacris*). *Zool. Scr.* **44**: 233-249.
- Veith, M., Mayer, C., Samraoui, B., Barroso, D.D., Bogaerts, S. (2004): From Europe to Africa and vice versa: evidence for multiple intercontinental dispersal in ribbed salamanders (genus *Pleurodeles*). *J. Biogeogr.* **31**: 159-171.
- Velo-Antón, G., García-París, M., Cordero Rivera, A. (2008): Patterns of nuclear and mitochondrial DNA variation in Iberian populations of *Emys orbicularis* (Emydidae): conservation implications. *Conserv. Genet.* **9**: 1263-1274.
- Velo-Antón, G., Wink, M., Schneeweiß, N., Fritz, U. (2011a): Native or not? Tracing the origin of wild-caught and captive freshwater turtles in a threatened and widely distributed species (*Emys orbicularis*). *Conserv. Genet.* **12**: 583-588.
- Velo-Antón, G., Becker, C.G., Cordero-Rivera, A. (2011b): Turtle carapace anomalies: the roles of genetic diversity and environment. *PloS ONE* **6**: e18714.
- Velo-Antón, G., Godinho, R., Harris, D.J., Santos, X., Martínez-Freiria, F., Fahd, S., Larbes, S., Pleguezuelos, J.M., Brito, J.C. (2012): Deep evolutionary lineages in a Western Mediterranean snake (*Vipera latastei/monticola* group) and high genetic structuring in Southern Iberian populations. *Mol. Phylogenet. Evol.* **65**: 965-973.
- Velo-Antón, G., El Marsini, B., Fritz, U., Fahd, S. (2015): Distribution and conservation status of *Emys orbicularis* in Morocco. *Vertebr. Zool.* **65**: 131-134.
- Zazo, C. (1999): Interglacial sea levels. *Quat. Intern.* **55**: 101-113.

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