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Phylogeny, biogeography, and evolution of two Mediterranean snakes, *Malpolon monspessulanus* and *Hemorrhois hippocrepis* (Squamata, Colubridae), using mtDNA sequences

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Abstract

Variation in 815 bp of mitochondrial DNA from two gene fragments (300 bp of cytochrome *b* and 395–515 bp of 12S rRNA) for 26 *Malpolon monspessulanus*, and cytochrome *b* for a further 21 individuals, indicates that this species originated in the Maghreb area of Northwest Africa. Here, an estimated 3.5–6 Mya, it divided into the western *M. m. monspessulanus*, and an eastern clade including *M. m. insignitus* and *M. m. fuscus*. The very limited genetic differentiation between Maghreb and Southwest European populations of this form suggests that it arrived in the Iberian Peninsula only recently. Population genetics and demographic tests indicate subsequent expansion in this area around 83,000–168,000 year ago. Because present populations of *Malpolon* arrived recently, mid-Pliocene and at least some Pleistocene fossils of the genus *Malpolon* in Southwest Europe are probably derived from an earlier invasion from the Maghreb, possibly as early as the end of the Miocene period, 5.3–5.9 Mya, when there was a temporary land bridge across the site of the Strait of Gibraltar and the Mediterranean Sea desiccated. The descendants of this earlier invasion must have eventually become extinct, perhaps during one of the Pleistocene glaciations. In contrast to the western *M. m. monspessulanus*, the greater genetic divergence found in the eastern clade of *M. monspessulanus* suggests that it dispersed at an earlier date and probably over a longer period, spreading eastwards through northern Libya and Egypt to Syria, Iraq, and Iran, and around the Mediterranean Sea through Turkey into the Aegean archipelagos and the Balkan peninsula. The western and eastern units of *M. monspessulanus* have different dorsal color pattern, differences in skull structure and exhibit an 8.4% uncorrected genetic divergence in the combined gene fragments investigated here. It is consequently recommended that they should be treated as separate species: *M. monspessulanus* (sensu stricto) and *Malpolon insignitus* **stat. nov.**, the latter including the subspecies *Malpolon insignitus fuscus* **comb. nov.** The same combined mitochondrial gene fragments used in *Malpolon* were investigated in 20 individuals of *Hemorrhois hippocrepis*, and of cytochrome *b* alone in a further 17. They indicate that this species also originated in the Maghreb and again invaded the Iberian Peninsula quite recently. Some of the most recent invasions of the Iberian Peninsula by reptiles and amphibian taxa could probably be anthropogenic in origin. Some other species including *M. monspessulanus* and *H. hippocrepis*, may have crossed naturally, by 'hopping' across the Strait of Gibraltar via temporary islands on the shallowest parts that were exposed during sea-level fall associated with Pleistocene glaciations.

Keywords: *Malpolon*; *Hemorrhois*; Phylogeography; Continental invasion; Strait of Gibraltar; Camarinal Sill; Cytochrome *b*; 12S rRNA

1. Introduction

At the western end of the Mediterranean, there has been considerable movement of reptile and amphibian lineages between the Iberian Peninsula of Southwest Europe and the Maghreb area of Northwest Africa, which comprises

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Morocco, northern Algeria, and Tunisia. Much of this migration has been southwards into the Maghreb, with several instances probably occurring during the Messinian period, 5.3–5.9 Mya, when a land connection briefly developed between the two areas at the site of the present Strait of Gibraltar, causing the Mediterranean Sea to desiccate (Duggen et al., 2003; Hsü et al., 1977, 1973; Krijgsman et al., 1999). Such cases involve considerable genetic differentiation between related Iberian and Maghreb taxa and include sharp-ribbed newts (*Pleurodeles*) (Carranza and Arnold, 2004; Carranza and Wade, 2004), discoglossid frogs (*Discoglossus* and *Alytes*) (Arntzen and Garcia-Paris, 1995; Fromhage et al., 2004; García-París and Jockusch, 1999; Martínez-Solano, 2004; Martínez-Solano et al., 2004), spadefoot toads (*Pelobates*) (García-París et al., 2003), typical frogs (*Rana*) (Plötner, 1998), fire-salamanders (*Salamandra*) (Escoriza et al., in press), and possibly one lineage of the *Podarcis hispanica* group of lacertid lizards (Pinho et al., 2006). A lesser though still marked genetic differentiation suggests that some southward migrations were more recent, occurring after the collapse of the Gibraltar land bridge and the subsequent refilling of the Mediterranean Sea from the Atlantic. They are consequently likely to have been across water rather than land and include at least one lineage of *P. hispanica* (Harris et al., 2002) and the lacertid lizard *Psammodromus algirus* (Carranza et al., in press). Very recent southward migrations (resulting in no overt differentiation in the mitochondrial gene fragments investigated so far) include the terrapins *Emys orbicularis* (Lenk et al., 1999) and *Mauremys caspica* (Busack, 1986; Fritz et al., 2005), and a second colonization by the newt *Pleurodeles* (Batista et al., 2004; Carranza and Arnold, 2004). Northward migration associated with marked genetic differentiation was less common but appears to have occurred in the Messinian in at least one lineage of *Chalcides* skinks (Carranza and Arnold, unpublished data) and in the lacertid lizard *Acanthodactylus erythrurus* (Harris et al., 2004). Virtual absence of differentiation in mitochondrial DNA suggests that other northward colonizations of the Iberian Peninsula by Maghreb species were much more recent. They include a tortoise, *Testudo graeca* (Álvarez et al., 2000), a false smooth snake, *Macroprotodon brevis ibericus* (Carranza et al., 2004), a tree frog, *Hyla meridionalis* (Busack, 1986), and a chameleon, *Chamaeleo chamaeleon*, which may have arrived twice (Paulo et al., 2002).

All the groups studied so far that have moved between the Maghreb and Iberia have relatively small body sizes. Here, we investigate two large colubrid snakes, the Montpellier snake, *Malpolon monspessulanus* (Hermann, 1804), and the Horseshoe whip-snake, *H. hippocrepis* (Linnaeus, 1758). *M. monspessulanus* may grow to 200 cm and a weight of 1.5 kg, and is distributed in Mediterranean habitats almost right around this sea, being absent only from most of Italy. Morphology indicates that its closest relative is the only other species assigned to *Malpolon*: *M. moilensis*.

Three subspecies are currently recognised (De Haan, 1999). *Malpolon m. monspessulanus* occurs in Southwest Europe and the western Maghreb, where it is found in Morocco and coastal Algeria as far east as Algiers. It usually has 19 dorsal scale rows at mid-body, and males have a dark 'saddle' on the foreparts. *Malpolon monspessulanus insignitus* ranges from east Morocco through Algeria and Tunisia eastwards around the Mediterranean Sea to western Syria. It again usually has 19 dorsal scale rows at mid-body but no 'saddle' is present in males, and colouring often includes narrow longitudinal pale stripes. In east Morocco and west Algeria it replaces *M. m. monspessulanus* away from the coast, on the high plateau, so the two subspecies overlap longitudinally but not latitudinally for several 100 km. *M. monspessulanus fuscus*, which occurs in Turkey and Southeast Europe and eastwards through north Iraq and western Iran, differs from *M. m. insignitus* in regularly having 17 dorsal scale rows at mid-body. Populations in some areas in the east of the range of *M. monspessulanus* cannot be clearly assigned to either *M. m. insignitus* or *M. m. fuscus*, those from the more arid parts of Syria, Jordan, and Iraq having either 17 or 19 scale rows. Differences in skull structure have also been reported between western and eastern populations of *M. monspessulanus* (Szyndlar, 1988).

Hemorrhois hippocrepis may grow to 185 cm and also occurs in Mediterranean habitats, but it has a much more restricted distribution than *M. monspessulanus*, being confined to the Maghreb, the southern two-thirds of the Iberian Peninsula and the Mediterranean islands of Sardinia, Zembreta, and Pantellaria (see Fig. 1). The population on the last island is regarded as a separate subspecies, *H. h. nigrescens* (Cattaneo, 1985). The closest relative of *H. hippocrepis* is *H. algirus*, which occurs in drier areas of the Maghreb (Utiger, 2002).

Malpolon monspessulanus and *H. hippocrepis* are mainly diurnal snakes (although, *H. hippocrepis* may sometimes be found active at dusk and at night in warm weather. They play important roles in Iberian and Maghreb reptile communities (Feriche, 1998; Pleguezuelos, 1998a,b; Pleguezuelos and Fahd, 2004). They are both significant prey items for many vertebrate predators including raptors, and they eat a wide range of smaller vertebrates, especially lizards in the case of *M. monspessulanus* and mammals in *H. hippocrepis* (Pleguezuelos, 1998a,b). *Malpolon* has an extensive fossil record in Southwest Europe dating back to the mid-Pliocene (Bailón, 1991; Balin, 2005; Barroso and Bailón, 2003; Gleed-Owen, 2001; Szyndlar, 1988, 1991). Much of the fossil material consists of vertebrae, which are difficult to assign to species, but a partial brain case from a mid-Pliocene site at Layna, northeast Spain has been assigned to a new taxon, *M. mlynarskii* (Szyndlar, 1988). A basiparasphenoid bone of about the same age from Sète, Mediterranean France agrees with that of *M. monspessulanus* itself (Bailón, 1991). Relatively long presence of *Malpolon* in North Africa is also supported by fossils, from the uppermost Pliocene of Casa Blanca, Morocco (Bailón,