

The genetic identity of the only Italian population of the genus *Macroprotodon* Guichenot, 1850 on the island of Lampedusa, Sicily

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Abstract

The only Italian population of false smooth snakes is found on Lampedusa, a small island located in the Sicilian Channel and part of the African continental shelf. The taxonomic identity of this population is currently uncertain, although it is most often attributed to *Macroprotodon cucullatus textilis* on a morphological basis. We present here the first genetic data on this population. The analysis carried out on the mitochondrial cytochrome *b* gene shows that the Lampedusan false smooth snake belongs to a clade shared with a single sample from central Tunisia. The genetic distance between this lineage and its sister group (*M. abubakeri*) is comparable to or higher than that found among many reptile species. To define the identity of this distinctive lineage, as well as the *Macroprotodon* taxonomic structure, further sampling efforts within the undersampled distribution area of this genus and more extensive analyses will be necessary.

Key words

Italy, Lampedusa, *Macroprotodon*, mtDNA, phylogeny.

Introduction

Macroprotodon Guichenot, 1850 (Colubridae, Colubrinae) is a genus with a Mediterranean (Maghrebian, S Mediterranean) chorotype (SINDACO *et al.*, 2013). At present, four species are recognized: *Macroprotodon brevis* (Günther, 1862) from the Iberian Peninsula and Morocco; *Macroprotodon abubakeri* Wade, 2001 from NE Morocco and Algeria; *Macroprotodon mauritanicus* Guichenot, 1850 from E Morocco, N Algeria, Tunisia and the Balearic Islands (introduced); and *Macroprotodon cucullatus* (Geoffroy Saint-Hilaire, 1827) widespread from Morocco to Egypt, with isolated populations in SW Israel and the Hoggar mountains (SINDACO *et al.*, 2013). However, this latter species requires a taxonomic

revision as it appears to be paraphyletic, as indicated by CARRANZA *et al.* (2004).

Lampedusa (Province of Agrigento, Sicily, Italy) is a small island located in the middle of the Sicilian Channel, and it hosts the only Italian false smooth snake population. Based on morphological characters (see WADE, 2001), most of the recent literature attributes this population to *Macroprotodon cucullatus textilis* (Duméril & Bibron, 1854) (KREINER, 2007; CAPULA *et al.*, 2011; SINDACO *et al.*, 2013; CATTANEO, 2015; DI NICOLA, 2019; DI NICOLA *et al.*, 2019). Other authors attribute it to *Macroprotodon mauritanicus* (GENIEZ, 2015) or *Macroprotodon cucullatus mauritanicus* (TURRISI & VACCARO, 2006;

Table 1. Kimura 2-parameter (lower diagonal) and uncorrected *p* (upper diagonal) genetic distances (percentages) for the *cyt b* gene between and within the four *Macroprotodon* clades recovered in Figure 1. Standard errors are reported in brackets; on the diagonal in bold within-group distances (K2P/uncorrected *p* distance).

	Lampedusa/Tunisia	<i>M. abubakeri</i>	<i>M. mauritanicus</i>	<i>M. brevis</i>
Lampedusa/Tunisia	0/0	6.72 (±1.53)	10.53 (±1.83)	10.72 (±1.76)
<i>M. abubakeri</i>	7.65 (±1.97)	0/0	12.06 (±1.98)	9.88 (±1.67)
<i>M. mauritanicus</i>	12.91 (±2.86)	15.37 (±3.28)	0.01/0.01	13.87 (±1.96)
<i>M. brevis</i>	13.44 (±2.85)	12.14 (±2.61)	18.52 (±3.63)	0.04/0.03

SPEYBROECK *et al.*, 2016; see also BUSACK & MCCOY, 1990), based on biogeographical considerations since it is the taxon reputed to be living on the Tunisian coasts in front of Lampedusa (WADE, 2001; CAPULA *et al.*, 2011).

The identity of Lampedusan false smooth snakes therefore appears uncertain and has not yet been genetically verified. Here we analyze this population with a genetic approach for the first time, with the aim of shedding light on its taxonomical status and phylogenetic relationships. This could be a starting point to clarify the origin of this population, which may have been introduced (CORTI *et al.*, 2009), and to address the issue of a specific conservation policy if it should it prove to be native.

Materials and methods

Politically part of the Italian Republic, from a geological point of view Lampedusa is a limestone ‘horst’ with a surface area of approximately 20.2 km² belonging to the North African continental shelf. It is part of the Pelagic Islands and is located 113 km east of Tunisia and 205 km south of Sicily. The island is almost entirely characterised by rocky outcrops, grasslands and uncultivated land.

In order to infer the phylogenetic relationships between the Lampedusan samples and those from other populations, we analysed the mitochondrial DNA cytochrome *b* (*cyt b*) sequence, a fragment often used in this type of analysis (CARRANZA *et al.*, 2004; VASCONCELOS & HARRIS, 2006; SALVI *et al.*, 2017). Fragments of tissue ranging from 2 to 20 mg from six Lampedusan specimens, roadkills found between 2016 and 2019, were collected and stored in absolute ethanol. DNA was extracted as described in TAGLIAVIA *et al.* (2016). Crude lysate was used as template for PCR amplification with primers CB1F and CB2R (KOCHER *et al.*, 1989). PCR products were then sequenced (BMR Genomics) with primer CB1F.

The sequences, each about 330 nucleotides long, were analysed and manually proofread with the DNA sequencing software CHROMAS v. 2.6.6 (Technelysium Pty. Ltd. 1998, Queensland, Australia). The coding gene fragments of *cyt b* were translated into amino acids to assess the lack of stop codons. Later, using CLUSTAL W (LARKIN *et al.*, 2007) with default parameters, the sequences generated in this study were aligned with data downloaded

from GenBank (see NAGY *et al.*, 2003; CARRANZA *et al.*, 2004; VASCONCELOS & HARRIS, 2006; SILVA-ROCHA *et al.*, 2015) corresponding to all the available sequences (between 291–310 bp) of the *Macroprotodon* genus (Fig. 1). Six species belonging to the Colubridae and Psammophiidae families were used as outgroups (see CARRANZA *et al.*, 2004).

The phylogenetic analysis was performed with Maximum Likelihood (ML) under the Akaike Information Criterion, by using ‘Smart Model Selection’ (SMS) (LEFORT *et al.*, 2017), implemented in PHYML v. 3 (GUINDON *et al.*, 2010). Node support was estimated using bootstrap values (FELSENSTEIN, 1985) with 1,000 replicates. Genetic distances were calculated with the Kimura 2-parameter (K2P) model (cf. CARRANZA *et al.*, 2004) and with the uncorrected *p* distances (NEI & KUMAR, 2000) using MEGA X software (KUMAR *et al.*, 2018). The most appropriate evolutionary model was the GTR+G+I model (-Log likelihood value 1593.36), with a 0.45 estimate of invariable sites and a 1.21 discrete approximation of the gamma distribution. In addition, Bayesian analyses were performed in BEAST 1.10.4 (DRUMMOND *et al.*, 2012; SUCHARD *et al.*, 2018). For Bayesian analyses, four independent chains for 10 million generations were run, sampling every 500 generations. Parameter convergence was verified by examining the effective sample sizes (ESS above 2543.4) using TRACER v1.7 (RAMBAUT *et al.*, 2018).

Results

Including the outgroups, 58 sequences of 255 bp total length were analysed. Both ML and BI trees showed a congruent topology and the resultant tree identified four major clades (Fig. 1). All the Lampedusan samples show the same private haplotype (GenBank accession numbers MT031797–MT031802), new for the genus *Macroprotodon*, and they differ in a single fixed substitution from the Central Tunisian Bou Hedma sample (GenBank accession number AY188026), reported as *M. cucullatus* by NAGY *et al.* (2003) and as *M. mauritanicus* by VASCONCELOS & HARRIS (2006) (see JOGER, 2003). These samples form a well-defined clade with a high bootstrap value (96%).

The Kimura 2-parameter (K2P) sequence divergence of the *cyt b* gene fragment used in this study between all four main *Macroprotodon* groups is, on average, 13.3%

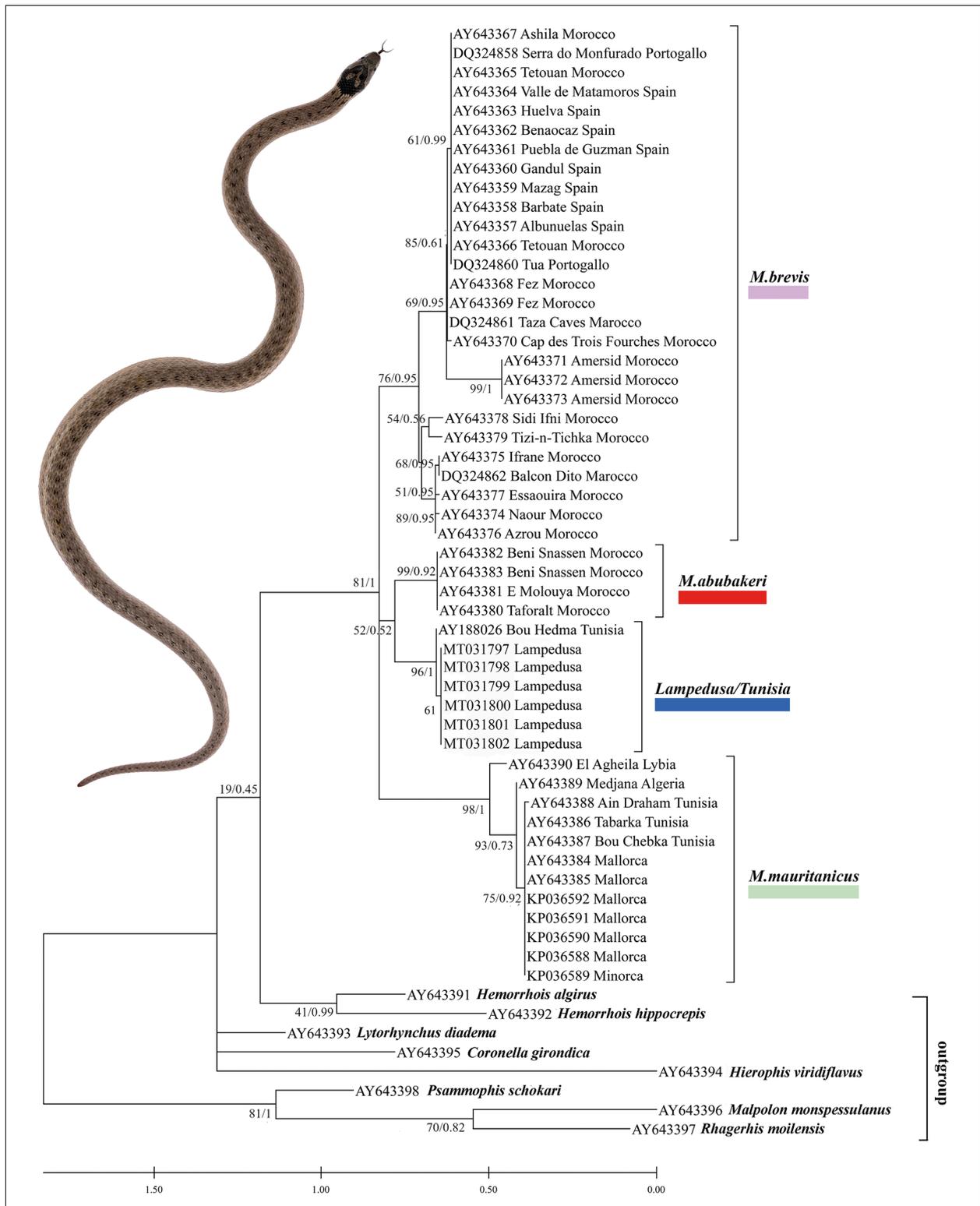


Fig. 1. Maximum Likelihood (ML) tree of *Macroprotodon* inferred from the mitochondrial cytochrome *b* gene. The numbers at nodes are ML bootstrap values and BI posterior probabilities. Except for the samples from Lampedusa, the remaining *cyt b* GenBank accession numbers are from NAGY *et al.* (2003), CARRANZA *et al.* (2004), VASCONCELOS & HARRIS (2006) and SILVA-ROCHA *et al.* (2015).

(Table 1), close to the value (12%) obtained with *cyt b* by CARRANZA *et al.* (2004) for three *Macroprotodon* lineages.

The ‘Lampedusa/Tunisia’ clade is the sister group of *M. abubakeri* with weak bootstrap support (52%) and

their genetic distances are 7.65% (Kimura 2-parameter) and 6.72% (uncorrected *p*), comparable to or higher than that found between other colubrid species (MEZZASALMA *et al.*, 2015; SALVI *et al.*, 2017; KINDLER *et al.*, 2017).

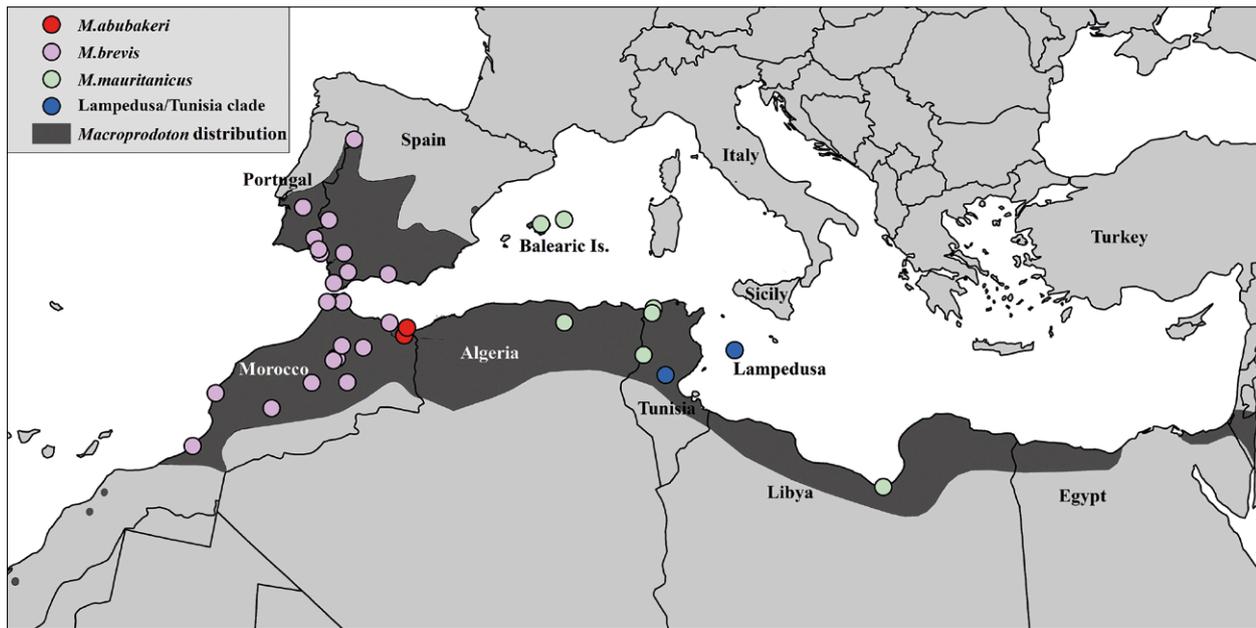


Fig. 2. Geographic range of the *Macroprotodon* genus and distribution of all the samples included in the analysis, distinguished on the basis of the lineages recognized by CARRANZA *et al.* (2004), except for the Lampedusa/Tunisia clade which has been identified in the present work.

Discussion

Three out of four clades confirm the taxonomic arrangement previously reported by CARRANZA *et al.* (2004), in which the putative species *M. brevis*, *M. abubakeri* and *M. mauritanicus* are provisionally assigned by the authors pending more comprehensive studies. Another well differentiated lineage groups the Lampedusa and Bou Hedma samples together. The distinctiveness of the Bou Hedma *cyt b* sequence had previously been detected by VASCONCELOS & HARRIS (2006). False smooth snakes from Lampedusa and Bou Hedma should however share the same morphotype assigned to *M. c. textilis* by WADE (2001); both localities in fact fall within the geographic range of this morphologically defined taxon (see CARRANZA *et al.*, 2004).

The origin of most of the herpetofauna of Lampedusa and its satellite island, Conigli Islet, has long been attributed to the land connection between the island and the nearby Tunisian coasts, which existed until the Last Glacial Maximum (LANZA & BRUZZONE, 1960; LANZA, 1973; MASSETI, 2002). However, biomolecular approaches have recently shed light on a more complex pattern, driven by both human-mediated and natural dispersal (CARRETERO *et al.*, 2009; HARRIS *et al.*, 2009; STÖCK *et al.*, 2015). In the case of the false smooth snake, a single fixed substitution was found among the Lampedusan and Central Tunisian *cyt b* sequences. This would support the idea of natural dispersion from Africa to Lampedusa during the last glaciation (see AVELLA *et al.*, 2017; SENCZUK *et al.*, 2017). However, at present it is not possible to properly assess the absence of the Lampedusan haplotype in Tunisia, due to the lack of mainland samples. It will therefore

be necessary to increase the sample size and expand the research in the coastal areas in front of Lampedusa (e.g. governorates of Sfax, Mahadia and Monastir) in order to draw a clearer picture.

The absence of genetic variability is a recurring pattern in island reptiles. It may be due to bottleneck processes such as the founder effect, which may explain, for example, the lack of variability in non-native *Macroprotodon* populations (SILVA-ROCHA *et al.*, 2015). But it can also be attributed to population size fluctuations, as is often observed in strongly declining species (CAPULA, 2004). Furthermore, a scarce genetic variability of the source population cannot be excluded, as is probably the case in *Macroprotodon abubakeri*, of which only one haplotype is currently known (CARRANZA *et al.*, 2004; VASCONCELOS & HARRIS, 2006). In any case, the lack of genetic diversity in the Lampedusan sample is difficult to explain, also due to the inadequacy of the mainland sample.

Our results do not allow any taxonomic conclusions on Lampedusan false smooth snakes, given their distinctiveness compared to the previously taxonomically defined clades and the confirmed paraphyletic status of *Macroprotodon cucullatus textilis*, to which they have been assigned (CARRANZA *et al.*, 2004). Similarly, the taxonomy of the *Macroprotodon* genus is still far from being resolved (see CARRANZA *et al.*, 2004; VASCONCELOS & HARRIS, 2006). To shed light on this issue it will be necessary to obtain a representative sample of the entire geographic range of this genus. Furthermore, due to their exclusively maternal inheritance, mitochondrial data alone are not enough to fully define the identified lineages as evolutionary entities (BONNET *et al.*, 2017), and therefore it will be necessary to expand the investigation

to include nuclear DNA, genomic markers (SNPs), species distribution modelling and morphological characters (e.g. landmark based morphometry).

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