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A molecular assessment and first record of *Tarentola mauritanica* (Squamata: Phyllodactylidae) on Corfu, Greece

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The Moorish wall gecko, *Tarentola mauritanica* (Linnaeus, 1758) is considered a common Mediterranean species complex of the family Phyllodactylidae. It originated in mid to late Miocene (around 8.69 Mya) (Rato et al. 2012). In the Mediterranean region (southern Europe, the Mediterranean islands and North Africa), *T. mauritanica* is classified into five distinct mtDNA haplotype groups. The European mtDNA haplotype lineage is the most geographically widespread (Harris et al. 2004a, b, Rato et al. 2010). This lineage originated probably in Morocco around 2.47 Mya and it is characterized by a single common mtDNA haplotype, distributed across a large part of southern Europe and many Mediterranean islands. Low mtDNA variability is probably the result of the combination of recent colonization events and a selective sweep process (Harris et al. 2004a, b, Rato et al. 2010, Rato et al. 2012).

The current distribution of *T. mauritanica* extends from North Africa (from Western Sahara to Tunisia), through coastal areas of Portugal, France, costal and central areas of Spain and Italy, and it is scattered along the western coast of the Balkan Peninsula (Radovanović 1941, Bons & Geniéz 1996, Martínez-Rica et al. 1997, Arnold & Ovenden 2002, Rato et al. 2012). It has also been recorded on most of the Mediterranean islands such as Sicily, Corsica, Sardinia, Malta and Crete (Martínez-Rica et al. 1997, Arnold & Ovenden 2002). This species is largely synanthropic (Arnold & Ovenden 2002). Its close coexistence with humans is responsible for anthropogenic introductions to several overseas localities, such as the Macaronesian Archipelagos, Balearic Islands, Argentina, Uruguay, and the USA (e.g., Báez & Biscoito 1993, Arnold & Ovenden 2002, Kraus 2008, Barreiros et al. 2010). In Greece, the Moorish wall gecko is distributed in the western part of the Peloponnese Peninsula, Crete (including Dia Island), and several Ionian Islands like Cephalonia, Ithaca, Strofades and Zakynthos (Chondropoulos 1986, Valakos & Mylonas 1992, Wilson 2006, Valakos et al. 2008).

The island of Corfu is the second largest (ca. 591 km²) of the Ionian Islands. With a small satellite island it forms the northwestern border of Greece, lying 2.5–20 km west off the coasts of Albania and Greece. Its Mediterranean climatic conditions and close position to the mainland are the main factors supporting the diversity of its herpetofauna with 8 amphibian and 29 reptile species (e.g., Tóth et al. 2002, Razzetti et al. 2004, Šandera et al. 2004). Several species of Corfu reptiles are probably allochthonous, e.g., *Stellagama stellio* (Linnaeus, 1758) (Tóth et al. 2002), which occurs here outside the main area of its range, and *Podarcis muralis* (Laurenti, 1768), which has recently been recorded from close to the port of Corfu Town (Hill & Mayer 2004, Šandera & Šanderová 2004). Although the island has been studied frequently by herpetologists (cf. Boettger 1889, Mertens 1961, 1968, Wütschert 1984, Keymar 1984, Chondropoulos 1986, Tóth et al. 2002, Šandera et al. 2004, Šandera & Šanderová 2004, Valakos et al. 2008), none of them have ever mentioned *T. mauritanica* to occur here.

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During a herpetological excursion on Corfu Island in August 2011, we discovered a population of *T. mauritanica* living in the central part of the island, i.e., at the football stadium of Olympos Kerkyra 1934 (39.6077872° N, 19.9029289° E; UTM 50 × 50 km 34SDJ1) at Corfu Town, quarter Kanalia near the Chalikiopulos lagoon and Corfu International Airport. During two back to back evening visits (13/14 August 2011) to the locality, we observed and photographed two adult individuals of *T. mauritanica* on the boundary wall of the stadium, two adult individuals on the substitution benches, and six adult individuals on the small houses near the football stadium. We also recorded three juvenile individuals, which might indicate a self-sustaining population of *T. mauritanica* at this locality. In July 2012, during another trip to Corfu, five adult individuals were recorded at the same spots as in 2011 (boundary wall, substitution benches and immediate vicinity), and dozens of individuals were recorded living in the tribune of the football stadium. Other recorded reptilian species in the locality were *S. stellio*, *Algyroides nigropunctatus* (Linnaeus, 1758), *Testudo hermanni* Gmelin, 1789, and *Hemidactylus turcicus* (Duméril & Bibron, 1839).

In order to ascertain the origin of the population, a few individuals were caught and the tips of their tails collected in ethanol for molecular analysis. All sampled specimens were released back at their localities. Genomic DNA was extracted from three samples using a commercial DNA extraction kit (DNeasy® Blood and Tissue Kit [Qiagen]). For PCR amplification of 12S rRNA and 16S rRNA fragments, we opted for the specific primers 12S rRNA 5′-ACTAGGATTAGATACCCTACTATGC-3′ and 5′-GAGGGTGACGGGCGGTGTGT-3′; 16S rRNA 5′-CGCCTGTTTACCAAAAACAC-3′ and 5′-CGGTCTGAACTCAGATCACG-3′. The temperature profile for 35 cycles of PCR amplification was 3 min at 95°C (initial denaturation step), 30 s at 95°C, 30 s at 58°C and 54°C (annealing temperature for 12S rRNA or 16S rRNA primers, respectively), 90 s at 72 °C, followed by a final extension step of 4 min at 72°C. The amplified products were purified and sequenced (3130 Genetic Analyzer by Applied Biosystems®) in both directions. For phylogenetic analyses, we aligned 57 specimens or reference sequences from GenBank (cf. Rato et al. 2010) with our sequences obtained from the species samples collected on Corfu Island. We combined 12S rRNA and 16S rRNA sequences into one sequence for each individual and used *Tarentola boettgeri* Steindachner, 1891 (AF186123, KC190930) as outgroup (following Carranza et al. 2002). Our new sequences were deposited in GenBank under accession numbers KF937795, KF937796, KF937797, KF937798, KF937799, and KF937800. A multiple sequence alignment was carried out using MEGA 5.2.2 (Tamura et al. 2011) with a MUSCLE algorithm (Edgar 2004) with manual adjustments. The data were used to construct an ML phylogenetic tree with MEGA 5.2.2 (Tamura et al. 2011). The subsequent tree search was conducted using the Tamura-Nei substitution model, using all sites for missing data treatment, the heuristic method Subtree-Pruning-Regrafting (level 5), and very weak branch swap filter. The robustness of the obtained phylogeny was evaluated by a bootstrap method with 10,000 replications.

Including the outgroup, we analysed a total of 61 combined mtDNA sequences, with an approximate length of 818 bp with 248 variable sites. ML analysis supported the tree with same topology as was reported by Rato et al. (2010) in which our three sequences (K066, K069, and K070) clustered with the European-Moroccan-Tunisian clade (Fig. 1A).

Geckos are one of the top-ten most successful introduced animal groups in the world. For example, the successful establishment rate of this group is around 76% in North America (Bomford et al. 2005). In general, the overall distribution of *T. mauritanica* in the western Balkans (scattered in the western coastal regions and absence in the eastern parts of the peninsula and on many of the Aegean Islands; cf. Martínez-Rica et al. 1997, Valakos et al. 2008) suggests that its occurrence will probably not be natural there, but rather the result of recent anthropogenic introduction from western parts of the Mediterranean or northern Africa (see Harris et al. 2004a, Rato et al. 2010). Similar conclusion were drawn in the case of *Chalcides ocellatus* (Forskål, 1775), which was probably introduced alongside a certain commercial product (plants or soil) that is exported in large volumes from Cyrenaica to destinations in the eastern Mediterranean (Kornilios et al. 2010). The widespread occurrence of *T. mauritanica* in the Peloponnese and on Crete (see Martínez-Rica et al. 1997) can be explained with the more intense commercial relationships between Greece and other important Mediterranean regions (cf. Kyriazi et al. 2013), mainly with mainland Italy and Sicily, negating a historical natural dispersion of this species in the Balkans (e.g., from its northern parts), as is also supported by the molecular results of Harris et al. (2004a) and Rato et al. (2010).

Based on our observations, we assume that *T. mauritanica* forms a newly introduced population on Corfu, and it is the 30th reptilian species of the island (cf. Tóth et al. 2002, Hill & Mayer 2004). The absence of variability among Corfu samples and their similarity with other published sequences documents that the Corfu population is a member of the mtDNA lineage that occurs in most of Europe. To us, there exist two most plausible hypotheses on the geographic origin of the *T. mauritanica* population on Corfu: (i) from the nearest population of this species on the mainland (cf. Radovanović 1941, Hunt 1957, Martínez-Rica et al. 1997, Polović & Ljubisavljević 2010) such as the Italian coast in the Apulian region (approx. 200 km distant from the port of Brindisi), coastal areas of Dalmatia and Montenegro, or Greek mainland coast (Peloponnese Peninsula, approx. 210 km); or (ii) from the Ionian Islands (Chondropoulos 1986, Valakos & Mylonas 1992, Wilson 2006, Valakos et al. 2008). Considering the very dense maritime traffic between the Italian cities of Bari or Brindisi and between the Greek Patra and Corfu, we assume that this recent introduction took place from

Italy or Greece. This corresponds with our finding the species in the vicinity of Corfu Town, the main port of the island. However, due to it's the locality's proximity to Corfu International Airport, we cannot exclude the possibility of transportation by aircraft cargo. Similar studies also support this assumption (Báez & Biscoito 1993, Jesus et al. 2002, Sciberras & Schembri 2006, Jesus et al. 2008, Vasconcelos et al. 2009, Barreiros et al. 2010, Rivera et al. 2011, Wagner et al. 2012).

We suggest that *T. mauritanica* was introduced to Corfu probably after 2000. In 2002, a few individuals of this species were supposedly observed in the north of the island in Agios Stefanos, which is about 30 km from Corfu Town (J. Všetička pers. comm.). Our observations suggest that the colonization of *T. mauritanica* on Corfu Island started around 2000, because studies conducted before this point of time (see citations above) did not indicate the presence of this species on the island in spite of intensive field research.

Introduced reptiles can have negative impacts on native species (predation, competition for food, basking sites and other resources, spread of diseases and parasites, over-

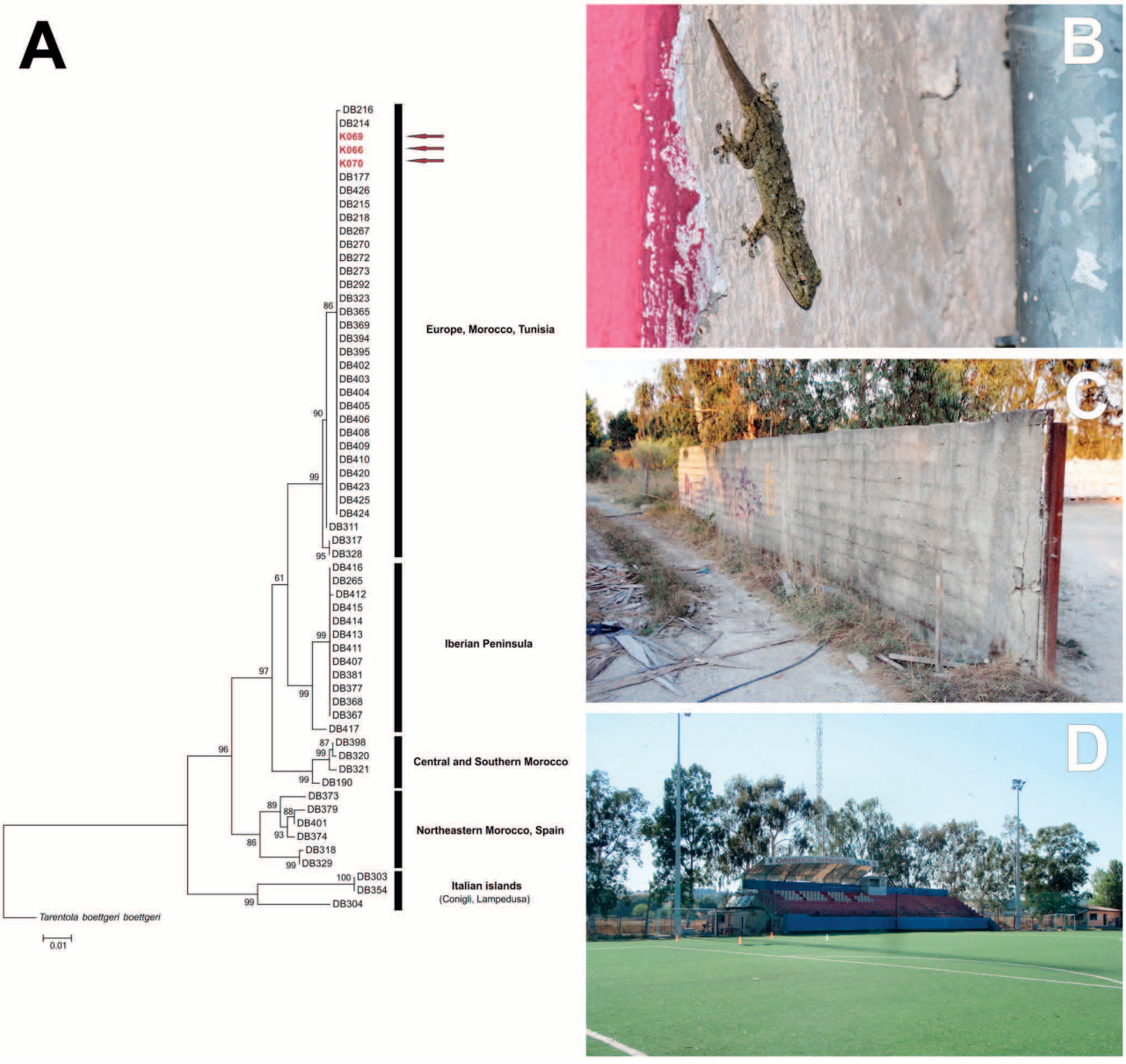

Figure 1. (A) Phylogenetic position of three specimens of *Tarentola mauritanica* from Corfu (red arrow) in a mitochondrial phylogeny of this species complex as inferred from Maximum Likelihood Analyses (12S rRNA and 16S rRNA) of 57 specimens from GenBank. (B) Adult individual found on the wall of the substitution benches. (C) Boundary wall of the stadium. (D) Football stadium of Olympos Kerkyra 1934.

population etc.). These processes are especially harming on islands (see, e.g., Powell et al. 1990, Butterfield et al. 1997). Therefore, future mapping of the occurrence of *T. mauritanica* in the whole of Corfu is necessary in order to obtain more information on its potential impacts on the native fauna.

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