

First record of *Tarentola substituta*
JÖGER, 1984, and genetic identification
of *Tarentola mauritanica*
(LINNAEUS, 1758), in the Azores

Invasive nonindigenous species are wreaking havoc around the world, and because they did not coevolve with indigenous species, they can profoundly disturb their interactions and ecosystem function. Typically, alien species will compete for resources, they may predate on natural species, be carriers of diseases and parasites and they can also hybridize with local individuals (BUTTERFIELD et al. 1997). In fact, introduced species are considered second in negative effects, after human-mediated impacts on native species, habitats and whole ecosystems (SIMBERLOFF 1997; WILCOVE et al. 1998; PARKER et al. 1999; KRYSKO et al. 2011). In particular, islands are quite susceptible to species introductions, not only because they are characterized by high levels of endemic species and biological diversity (WHITTAKER & FERNÁNDEZ-PALACIOS 2007), but they are also geographically isolated ecosystems, which have undergone distinct evolutionary processes over millions of years (SHINE et al. 2000). Reports of lizard introductions in Atlantic islands are an extensive list, including the Moorish Gecko, *Tarentola mauritanica* (LINNAEUS, 1758), introduced in both Madeira (BÁEZ & BISCOITO 1993) and Azores (BARREIROS et al. 2010), the House Gecko *Hemidactylus mabouia* (MOREAU DE JONNÈS, 1818), introduced in Madeira (JESUS et al. 2002), the Madeiran Lizard *Teira dugesii* (MILNE-EDWARDS, 1829), in the Azores (MALKMUS 1984; ULFSTRAND 1961), while the geckos *Hemidactylus angulatus* HALLOWELL, 1854, *H. mabouia* and the African Rainbow Lizard *Agama agama* (LINNAEUS, 1758), have all been introduced in the Cape Verde islands (FEA 1899; JESUS et al. 2001; VASCONCELOS et al. 2009). Studies from CASE & BOLGER (1991a, 1991b) have demonstrated that at least concerning reptile introductions, large species-rich islands tend to be more “resistant” to invasive lizards, such as the cases of New Guinea, New Zealand, Australia, and New Caledonia (reviewed in CASE & BOLGER 1991b).

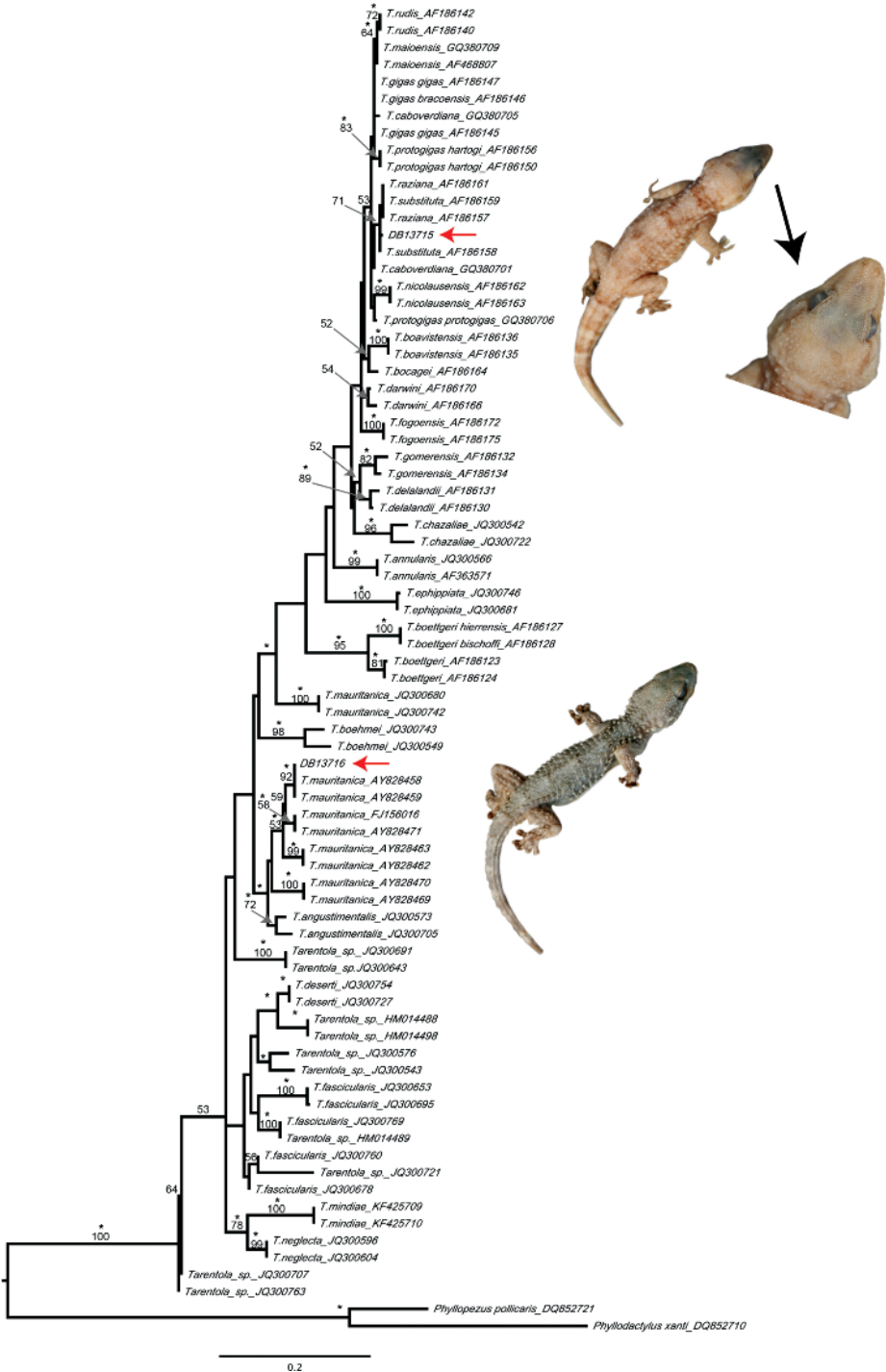
On the other hand, it has also been recently demonstrated that both in islands and continents, the establishment success of alien lizard species is higher in the presence than in the absence of congeners, increasing also with congener richness (FERREIRA et al. 2012).

The Archipelago of the Azores is located 1,500 km west of mainland Portugal, harboring low levels of herpetofaunal richness (three amphibians and two terrestrial reptiles) and no endemics (LOUREIRO et al. 2008). Because of the potential ecological effects these alien species can cause, it is essential to provide data on all known non-indigenous herpetofaunal species via vouchers, accurate identification of the species, documentation of the place and time when it was found and assessment of the possible invasion pathway and stages of the species (KRYSKO et al. 2011). In this study, the authors identify two specimens of *Tarentola* found in the Azores using molecular tools.

Two individuals of the genus *Tarentola* were collected at Ramalho, São Miguel, Azores (37°44'16.69" N, 25°41'31.69" W) in 2010. Both specimens were euthanized and preserved in 96 % ethanol. Muscle tissue was taken from their tails to perform the genomic extraction, following the standard high-salt protocol (SAMBROOK et al. 1989). Amplification of the mitochondrial fragment of the 12S rRNA gene was carried out by Polymerase Chain Reaction (PCR), using the primers 12Sa and 12Sb developed by KOCHER et al. (1989), following the conditions described in HARRIS et al. (1998). All amplified fragments were sequenced using the automated sequencer ABI Prism® 310 Genetic Analyzer (Applied Biosystems), following the manufacturer's protocols. All newly generated sequences were submitted to GenBank with accession numbers KJ814252 and KJ814253. For the specific identification of the above two species of *Tarentola*, previously published sequences of 12S rRNA from the genus (CARRANZA et al. 2000, 2002; HARRIS et al. 2004a; PERERA & HARRIS 2008; RATO et al. 2010; VASCONCELOS et al. 2010; RATO et al. 2012; BSHAENA & JOGER 2013) were included in the analysis with *Phyllodactylus xanti* COPE, 1863, and *Phyllopezus pollicaris* (SPIX, 1825) representing outgroup sister taxa to

Tarentola (GAMBLE et al. 2011). The obtained sequences were aligned using MAFFT v.7.017 (KATO & STANDLEY 2013) with default parameters (gap opening penalty = 1.53; gap extension penalty = 0.123; progressive method = FFT-NS-2), as implemented in the software Geneious Pro 5.5.9 (created by Biomatters; available from < <http://www.geneious.com/> >). Calculation of the likelihood scores and choice of the best model of sequence evolution was carried out using Modeltest v.2.1.4 (DARRIBA et al. 2012), under the Akaike Information Criterion (following POSADA & BUCKLEY 2004). The phylogenetic tree was built under Maximum Likelihood (ML) and Bayesian Inference (BI) assumptions. In the ML analysis, the program GARLI v.2.0 (ZWICKL 2006) and the GTR+G model of substitution, were used. Tree search was conducted using between 1,000 and 15,000 generations (parameter genthreshfortopterm) considering a stochastic algorithm, each resulting in a single best tree. The resulting likelihood values were compared, and the best value was obtained under 8,000 generations. Bootstrap support was calculated from 1,000 bootstrap pseudo-replicates (FELSENSTEIN 1985), using genthreshfortopterm setting 8,000. A majority rule consensus tree was generated using the software Phyutility (SMITH & DUNN 2008). The BI analysis was conducted using MrBayes v.3.2.2 (HUELSENBECK & RONQUIST 2001) and the obtained model of substitution. Both runs began with a random starting tree and ran for 2*10⁶ generations, saving one tree per 100 generations. Convergence and appropriate sampling were confirmed by examining the standard deviation of the split frequencies between the two simultaneous runs and the Potential Scale Reduction Factor diagnostic. A burn-in was performed by discarding the first 5,000 trees (= 25 %) of each run, and a majority-rule consensus tree was generated from the remaining trees.

In total, the authors analyzed 78 sequences of 12S rRNA consisting of 308 bp each. Both ML and BI phylogenetic analyses generated the same tree topology. According to the genealogy obtained, specimen DB13716 belongs to the species *T. mauritanica* and individual DB13715 to *T. substituta* (Fig.1). Specifically, DB13716



clustered with the widespread European and North African clade of the *T. mauritanica* species-complex (RATO et al. 2010, 2012). The finding of *T. mauritanica* specimens in the Azores was already reported earlier by BARREIROS et al. (2010). However, these authors did not carry out a genetic study to determine the lineage (or lineages) which these geckos belonged to. Nevertheless, at least one of their specimens was captured in a lettuce box coming from mainland Portugal, suggesting that they either belonged to the common European/North African or the Iberian clade (PERERA & HARRIS 2008; RATO et al. 2010, 2012). Since this European/North African clade is the most widespread lineage of the complex (HARRIS et al. 2004a, 2004b; PERERA & HARRIS 2008; HARRIS et al. 2009; RATO et al. 2010, 2012), covering almost the entire Mediterranean Basin, it is not surprising that this is also the lineage found here in the Azores. A real novelty is the finding in the Azores of an individual of *T. substituta*, an endemic species from São Vicente, Cape Verde Islands. By examining the picture of the specimen (Fig. 1), there are also some morphological traits that correspond to the genetic species diagnosis (VASCONCELOS et al. 2012), such as the four symmetrical butterfly or X-shaped dorsal crossbands and more than 21 interorbital scales. That *Tarentola* geckos do not avoid living in close vicinity to human settlements, can lead to accidental anthropogenic introductions of these geckos into new areas (PERERA et al. 2008).

According to the STATISTICS REGIONAL SERVICE OF THE AZORES (2014), there is mainly exportation of goods from the Azores to Cape Verde, and not the other way around. However, the geckos may have been accidentally transported on the way back. According to MEDEIROS et al. (2010), *T. mauritanica* seems to be well adapted to

coastal and urban environments in the Azores, as demonstrated by the recent reports of live specimens in both Faial and São Miguel islands, and also by the finding of juveniles. If that is the case, it is advisable to periodically make an evaluation of the state of this and other introduced populations to avoid negative impacts on the environmental dynamics of the region.

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Fig. 1 (opposite page): Maximum Likelihood (ML) phylogenetic tree for the 12S rRNA mitochondrial gene of *Tarentola*, using both *Phyllodactylus xanti* COPE, 1863, and *Phyllopezus pollicaris* (SPIX, 1825), as outgroups. Previously published sequences of 12S rRNA from the genus *Tarentola* (CARRANZA et al. 2000; CARRANZA et al. 2002; HARRIS et al. 2004a; PERERA & HARRIS 2008; RATO et al. 2010; VASCONCELOS et al. 2010; RATO et al. 2012; BSHAENA & JOGER 2013) were included in the analysis.

Values above the nodes represent the bootstrap score for the ML analysis; stars point to Bayesian Inference posterior probabilities above 95 %. The red arrows identify the specimens analyzed in this study (corresponding pictures on the right) and their position within the genealogy.

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