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Preliminary assessment of mitochondrial variation in the insular endemic, biogeographically enigmatic Noronha skink, *Trachylepis atlantica* (Squamata: Scincidae)

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Islands are important model systems to understand the evolution and diversification of many organisms, including lizards. On one hand, representatives of many groups of lizards have managed to disperse over the sea to islands, and evolved into endemic insular species. Some lizards have undertaken almost incredible voyages, crossing the Indian Ocean from Australasia to the Malagasy region in the genus *Cryptoblepharus* (ROCHA et al. 2006), and multiple times throughout the Atlantic Ocean from Africa to South America as in geckos (GAMBLE et al. 2011) and skinks. Sometimes lizards underwent spectacular radiations on these isolated archipelagos (VENCES 2009), with the Caribbean anoles probably being the most prominent example (LOSOS 2009). In most such cases, each island harbours a single representative of a radiation, but cases of intra-island divergence processes, with distinct genetic lineages or subspecies are known (e.g., BROWN et al. 2016), sometimes even on very small islands (e.g., MACLEOD et al. 2015).

The Noronha skink, *Trachylepis atlantica* (SCHMIDT, 1945) is one of the most enigmatic examples of long-distance overseas dispersal in lizards. It only occurs on the Fernando de Noronha archipelago in the tropical Atlantic Ocean, three degrees of latitude south of the Equator and 354 km offshore from the Brazilian coast (Cabo de São Roque, Maxaranguape, state of Rio Grande do Norte), consisting of one main island of ca. 17 km², and of 20 satellite islands and islets. Of these only the main island is human-inhabited, and the archipelago is a National Park of Brazil, and a World Heritage Site. The extant terrestrial vertebrate fauna of Noronha contains four endemic species, two birds (*Vireo gracilirostris* and *Elaenia ridleyana*)

and two reptiles – besides *T. atlantica*, also the amphisbaenid *Amphisbaena ridleyi* is considered an endemic of the archipelago (SERAFINI et al. 2010). The Noronha skink was originally described in the genus *Mabuya* considered at the time to be cosmopolitan, but which has been split into several genera since. The Neotropical species form a monophyletic group, except for *T. atlantica* which several independent molecular phylogenetic studies have instead firmly embedded within the African-Malagasy genus *Trachylepis*, suggesting its ancestors have colonized Noronha directly from Africa (MAUSFELD et al. 2002, CARRANZA & ARNOLD 2003, WHITING et al. 2006). Besides this phylogenetic assessment, very little is known on this skink which has been reported to be very abundant on Fernando de Noronha. SAZIMA et al. (2007) reported on nectar-feeding and probable pollination by this lizard, and GASPARINI et al. (2007) provided additional information on unusual diet and threats by new and introduced predators. In-depth baseline data on the trophic ecology, thermal biology, activity patterns and habitat use of *T. atlantica* were published by ROCHA et al. (2009). DNA sequences from five and three individuals were studied by CARRANZA & ARNOLD (2003) and WHITING et al. (2006), respectively, without indications of relevant genetic divergence among them. Yet, a more comprehensive assessment of genetic variation of this skink across the Noronha archipelago is still lacking.

To obtain first insights into the molecular differentiation within and among populations of *T. atlantica*, we sampled individuals from five sites between 11–14 April 2017. Our sampling sites roughly covered a transect from the southwestern to the northeastern part of the main is-

land, and included the largest satellite island, the Ilha Rata (Table 1). Active lizards were captured during the day by hand or with a noose, their tail tips taken as tissue samples and preserved in 99.9% ethanol, and lizards immediately released at the site of capture. We extracted DNA using a standard salt extraction method and amplified and sequenced a fragment of mitochondrial DNA (mtDNA) of the gene for cytochrome c oxidase subunit I (COI) with

primers AnF1 (ACHAAYCAYAAAGAYATYGG) and AnR1 (CCRAARAATCARAADARRTGTTG) of LYRA et al. (2017). Sequences were quality-checked and trimmed in Geneious R6 (<http://www.geneious.com>, KEARSE et al. 2012) and submitted to GenBank (accession numbers MH206487–MH206535). From the obtained sequences we then reconstructed a Maximum Likelihood tree in MEGA7 (KUMAR et al. 2016) from an alignment obtained with the



Figure 1. *Trachylepis atlantica* on the Fernando de Noronha archipelago. (A) Specimen from Ilha Rata; (B) specimen from Praia do Porto hidden in a rock fissure (note syntopic crab in the background); (C) specimen on a tree trunk, far from the coast near Fernando de Noronha Airport; (D–E) pre mating and mating behaviour of a pair of *T. atlantica* within Noronha village (Vila do Remedios).

Table 1. Geographical coordinates of sampling sites of *Trachylepis atlantica* on the Fernando de Noronha Archipelago, and sample numbers per site. All samples were taken within 100 m of the coordinates given except locality PL where three samples were taken at Mirante Praia do Leão (-3.869688°, -32.433867°).

Code	Locality	N samples	Latitude	Longitude
RA	Ilha Rata	13	-3.813280°	-32.390172°
PO	Praia do Porto	13	-3.835573°	-32.401905°
BO	Praia do Bode	12	-3.847759°	-32.433881°
BP	Mirante Baía dos Porcos	1	-3.851721°	-32.440863°
PL	Praia do Leão	10	-3.869809°	-32.438623°

Muscle algorithm, and entered this tree together with the alignment in the software Haploviewer, written by G. B. EWING (<http://www.cibiv.at/~greg/haploviewer>) which implements the methodological approach of SALZBURGER et al. (2011), to obtain a haplotype network.

During our visit to the Noronha Archipelago, we found *T. atlantica* being a very common lizard and occurring in a variety of habitats of the whole island (Fig. 1), including tree trunks at perch heights over 2 m and within the island's main village, where we recorded a mating pair (Figs 1D–E). However, the species was decisively most common at the coast where it mostly inhabited, sometimes in high densities, volcanic rocks partly very close to the water, and sometimes sharing its habitat with marine crabs (Fig. 1).

We obtained sequences of the COI fragment (586 bp) for 49 individual skinks, collected in five localities (Table 1, Fig. 2). The sequences grouped into 18 distinct haplotypes, differing by a maximum of 6 mutations from each other, which corresponds to a maximum uncorrected pairwise

distance of 1.0%. The haplotype network constructed from these sequences displays a star-like structure, with a central haplotype found in four of the five populations (all except BP from where only a single individual was sequenced), 12 singletons (haplotypes found in single individuals only), and 13 haplotypes that differed by just a single mutation from the central haplotype (Fig. 2). No phylogeographic structure was apparent, and the slightly more divergent haplotypes (differing by 2–3 mutational steps) came from various populations of the main Noronha Island but not from Ilha Rata.

The star-like network, haplotype sharing between almost all populations and lack of phylogeographic structure are in agreement with a single, large panmictic population of *T. atlantica*, at least from a mitochondrial perspective. The amount of genetic variation is much below the value typically characterizing sister species of squamates (uncorrected COI pairwise distances on average 6.1% for squamates in Madagascar; NAGY et al. 2012) and also distinctly below the values found between deep intraspecific lineages in other *Trachylepis* for this same gene fragment (>6%; VENCES et al. 2014). Therefore, our results do not provide any indication for a differentiation of populations of *T. atlantica* at the species or subspecies level, or for recognizing distinct management units for conservation. We hypothesize this is due to its basically continuous distribution and very small range, whereas other Brazilian lizards with distinct phylogeographic structure have wider distributions. This applies even to range-restricted species such as the coastal *Liolaemus lutzi* where three distinct population clusters are recognizable both in mtDNA and microsatellite data (ARIANI et al. 2013), but over a range of about 200 km and thus extending more than ten-fold the distribution area of *T. atlantica*. Given its isolated phylogenetic position and morphological distinctness, the status

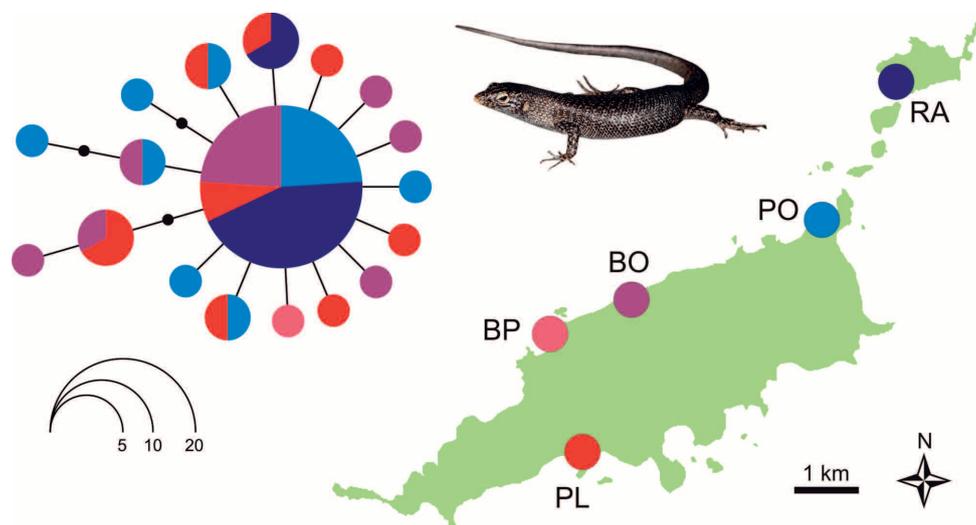


Figure 2. Haplotype network based on 591 bp of the mitochondrial COI gene in 49 individuals of *Trachylepis atlantica* from five localities on the Fernando de Noronha Archipelago as shown in the map. Colors of localities in the map match those in the network. Small black dots in the network represent additional mutational steps. See Table 1 for a summary of samples and geographic coordinates.

of *T. atlantica* as distinct species endemic to the Noronha archipelago has never been in doubt. Yet, it is worth mentioning that our study confirms this status and rejects a human-mediated introduction (e.g., from a yet undiscovered African *Trachylepis* population) because it is highly unlikely that the large number of haplotypes detected have evolved in the short time span since the discovery of the island by humans.

Our study cannot give but a first and preliminary assessment of the genetic differentiation of *T. atlantica*. The DNA barcoding marker chosen is often not sufficiently informative to assess fine-scale variation at the population level or current demographic variation. We anticipate that more variable nuclear markers, such as microsatellites, might well reveal a differentiation between some of the populations, in particular between Ilha Rata and the mainland, and characterize these as distinct conservation management units. Also, it needs to be emphasized that *T. atlantica* certainly occurs on numerous additional small satellite islands which we have not sampled and thus not assessed genetically. However, with the current evidence at hand, we conclude that there is no evidence for any taxonomic subdivision or substantial intraspecific differentiation of *T. atlantica*.

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