



SHORT NOTE

Genetic Characterization of *Melipona subnitida* Stingless Bee in Brazilian Northeast

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Abstract

The study of *M. subnitida*, a stingless bee well adapted to extreme environmental conditions, is noteworthy once the Northeastern Brazil faces climate changing predictions in which the precipitation rates are expected to decrease, and the average of temperatures to increase. The well-studied populations are limited to the Caatinga biome, where the species was considered endemic. However, the occurrence of this species has been reported in contrasting environments from arid region, such as mangrove and sandbanks in Maranhão state. Our primary goal was to characterize samples from these different environments and compare them with previously studied populations. We identified a unique mitochondrial haplotype per region. The haplotype found in Lençóis Maranhenses National Park was exclusive from this location and differed regarding the amino acid sequence when compared to the literature presented haplotypes from Caatinga, which might be related with different evolutionary processes in the distinct environments, though further studies are needed to confirm.

Melipona subnitida is highly adapted to extreme environmental conditions and can survive under long drought periods, since during water and resource scarcity, the bees reduce the colony size to a minimum number of brood and workers. Thus, the species maintains only essential tasks for the nest, being an important mechanism to save the resources stored during the bloom of rainy periods (Maia-Silva et al., 2014; 2015).

Assuming the climate change predictions for Northeastern Brazil (Marengo et al., 2011) in the semi-arid region, where average reductions in precipitation rates of up to 40–50% are expected (PBMC, 2013), the study bees resistant to these conditions, such as *M. subnitida*, is noteworthy. Considering the importance of this species regarding resistance to extreme climatic conditions, distributional modeling

studies have suggested a shift in the potential occurrence area of *M. subnitida* and its main resource plant for the near future (Maia et al., 2014; 2015; Giannini et al., 2017). Even though the impact of climate change on geographic distribution of this native bee is predictable, studies dedicated to understand how its genetic variability is distributed are still scant.

M. subnitida is usually considered endemic from the Caatinga (Zanella & Martins, 2002), but it has been observed in biomes with distinct landscapes from semi-arid region, such as in the coast of Maranhão, in Lençóis Maranhenses National Park (PNLM) (Rêgo & Albuquerque, 2006) and in Ilha Grande dos Paulinos (Rêgo et al., 2017) (Fig 1). These areas, unlike Caatinga biome, are composed of sand dunes and lakes, annual rainfall ranging from 1600 to 1800 mm (IBAMA, 2002) and surrounded by mangrove.



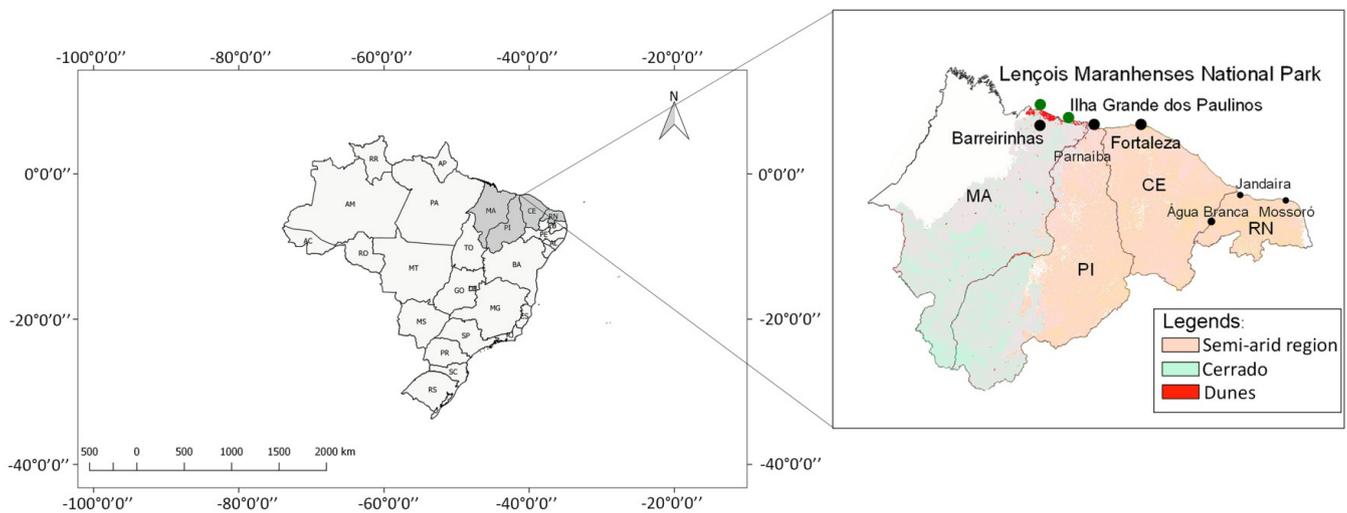


Fig 1. Map of Brazil highlighting the collecting sites of *M. subnitida* in Brazilian Northeast at different biomes. The greendots represent the locations of sampling in Maranhão (PNLM and Ilha Grande dos Paulinos) and the black dots represent the samples used from GenBank.

The populations of *M. subnitida* present in such areas differed regarding the tree species used as nesting sites often found in the Caatinga region (Câmara et al., 2004; Bruening 2006; Rêgo et al., 2017). In order to evaluate if differences among bees are restricted to behavioral characteristic or if they are reflected in the genetic signatures, we have genetically compared populations from Maranhão with mitochondrial haplotype data available in the literature.

We searched for natural nests and sampled 28 workers of *M. subnitida* in PNLM (20) and in Ilha Grande dos Paulinos (8) and the nests were approximately 1km apart from each other. The bees were stored at -20°C and DNA was extracted from antennae of one bee per colony using Chelex method (Walsh et al., 1991). For analysis of the cytochrome c oxidase I (COI) gene partial sequence were used primers mtD6 and mtD9 (Simon et al., 1994). For PCR it was used $5\mu\text{L}$ of DNA, $2.5\mu\text{L}$ of each primer, $25\mu\text{L}$ of $10\times$ PCR buffer and $15\mu\text{L}$ of nuclease-free water. Genetic material was amplified, sequenced and analyzed as described in Bonatti et al. (2014).

We amplified a 436pb of COI gene fragments from 28 individuals. These sequences were compared to 11 haplotypes from Bonatti et al. (2014), which sampled individuals from Maranhão, Piauí, Ceará and Rio Grande do Norte states of Northeastern Brazil. Haplotypes network was built using Network software v.4.6 (Polzin & Daneshmand, 2003) and MEGA software v.7.0.14 (Tamura et al., 2011) to calculate the distances among haplotypes using Tamura 3-parameter with Gamma distribution (T92+G).

Our results found one haplotype per sampled region. Nonetheless, the haplotype H4 (in Ilha Grande dos Paulinos) was shared with Mossoró-RN, Fortaleza-CE, Jandaíra-RN and Parnaíba-PI populations (Table 1) previously analyzed by Bonatti et al. (2014) and identified likely as ancestral haplotype.

On the other hand, the PNLM had its unique haplotype, H12, not shared with any other areas (Fig 2). This haplotype diverges from H7 (found in Barreirinhas-MA and Parnaíba-PI)

and H10 (found in Fortaleza-CE) by a single mutational step (Fig 2A). According to the genetic proximity between H7 and H12 haplotypes, showed by dendrogram (Fig 2B) and geographic distance between Barreirinhas and PNLM, we believe the H7 may have originated H12.

Bonatti et al. (2014) also observed one exclusive mitochondrial haplotype per locality as occurred in PNLM. This pattern may be associated with the process of colonization of new areas in Meliponini by one or few original colonies (Miranda et al., 2016). Furthermore, the low dispersal ability of daughter colonies during swarming process, the dependence upon mother colony, as well as the queen philopatry may help to explain these patterns (Nogueira-Neto, 1954; Engels & Imperatriz-Fonseca, 1990). Other researches using population genetic approaches with different stingless bee species have

Table 1. Localities where each haplotype are originally found.

Haplotypes	Localities
H1	Mossoró; Parnaíba
H2	Jandaíra
H3	Jandaíra
H4*	Mossoró; Jandaíra; Parnaíba; Fortaleza; Ilha Grande dos Paulinos
H5	Areia Branca; Jandaíra; Parnaíba
H6	Jandaíra
H7	Barreirinhas; Parnaíba
H8	Fortaleza
H9	Barreirinhas
H10	Fortaleza
H11	Areia Branca
H12*	PNLM

*Haplotypes found in this present work

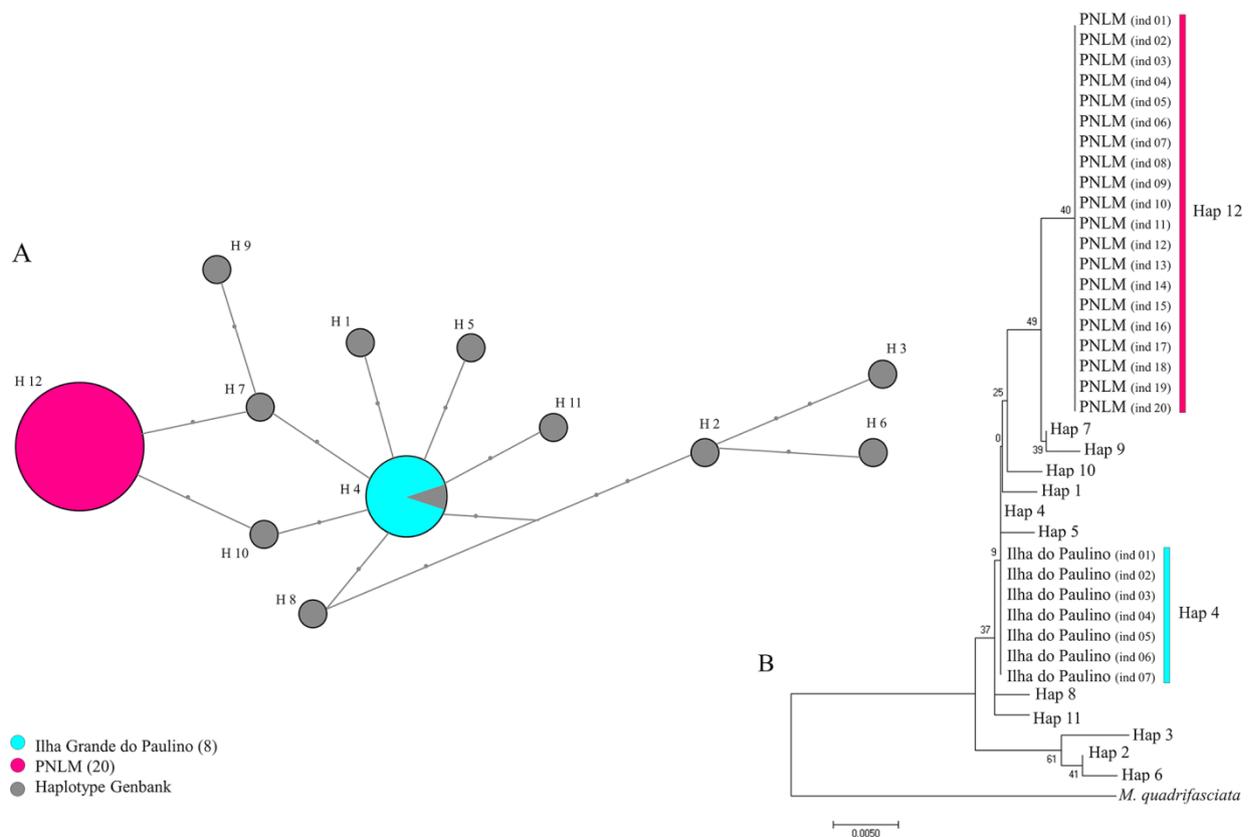


Fig 2. Haplotypes from different regions: A) Haplotype network showing shared haplotypes from PNLM, Ilha Grande dos Paulinos populations and GenBank obtained through Median Joining algorithm; B) Likelihood dendrogram of genetic proximity amongst *Melipona subnitida* locations, haplotypes from GenBank (KC879031 to KC879041) and the outgroup *M. quadrifasciata* (access number EU163150). The numbers shown next the branches represent the bootstrap support after 10.000 replications.

also found similar results (Tavares et al., 2007; Francisco et al., 2008; Batalha-Filho et al., 2010; Brito et al., 2013; Bonatti et al., 2014, Miranda et al. 2016; Galaschi-Teixeira et al., 2018).

When we analyzed and compared the DNA sequence of the two haplotypes identified here (H4 and H12), we observed that they differed from each other in two mutational steps. The replacement of a guanine by an adenine at 128 position translates into changes in one amino acid (valine-isoleucine). This non-synonymous change at the amino acid level was not observed in Bonatti et al. (2014) where all mutations found in the 11 haplotypes were neutral, but has been observed in Brito et al. (2013) for *Partomana mulata*.

It is noteworthy that, albeit it has been observed that *M. subnitida* has a wide flight range (Silva et al., 2014), the dunes might act as geographic barriers, hindering the gene flow among these populations. These dunes are surrounded by lakes, and altogether they form a large complex of landscape without any vegetation or any other substrate, which could allow bee dispersion to long distances.

The change in the amino acid sequence of COI protein from PNLM may reflect a random factor or it may be the result of selective pressures driven by the environment where these bees live. These areas differ in the type of trees where they build their nests, climatic conditions, and vegetation structures.

Notwithstanding, the natural process of desertification may play a role limiting gene flow among populations, since the movement of dunes ends up covering green areas, limiting the few chances of nesting sites. Genomic studies with a more thorough sampling will be necessary to better understand the evolutionary history of bees in this region.

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Authors Contributions

The authors provided performance data and genetic analysis, including laboratories procedures and availability of laboratory from University of São Paulo, Ribeirão Preto.

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