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Evidence for Gradients of *Melipona rufiventris* (Hymenoptera: Apidae) Genetic Diversity within the Brazilian Semiarid

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ABSTRACT

The species *Melipona rufiventris* Lepeletier is part of the group of stingless bees native to Brazil, *commonly known as uruçu-amarela*, whose populations have suffered high losses in recent years by the increasing destruction of native semi-arid vegetation and the predatory collection of honey. This study provides an assessment of the *genetic* diversity and structure of *M. rufiventris* populations in the Brazilian semiarid using microsatellite markers, a starting point for assessing spatial and temporal genetic changes of bee populations in Brazil. After PCR testing of 37 potential microsatellite primer pairs, only nine markers (24.3%) revealed polymorphism in *M. rufiventris*. When the data set was divided into 3 collection sites (Campo Maior, Castelo do Piauí, and Guadalupe) it was observed that Campo Maior presented the highest average in relation to the number of alleles/population (3.0), ranging from 1 to 7, when compared to Castelo do Piauí and Guadalupe. PCoA and Bayesian analysis (Structure) showed a separation of the individuals into two distinct groups with some degree of intersection, thus confirming a significant genetic differentiation among populations. This information is essential to aid in the conservation of the species, since both groups identified in this study, (1) Campo Maior and (2) Castelo do Piauí + Guadalupe, should be managed as distinct units for conservation purposes. In this sense, conservation strategies should focus on minimizing habitat degradation within each region, and on avoiding the translocation between colonies as the species is exploited in meliponiculture.

Keywords: *Uruçu-amarela*, Stingless bee, *Rufiventris* group, Microsatellites markers.

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INTRODUCTION

Stingless bees belong to the family Apidae in the order Hymenoptera and are members of the tribe Meliponini, which includes all genera of extant stingless bees [1, 2]. Meliponines, in turn, are pollinators of the local native flora and cultivated crops [3-7], and therefore, of great ecological and economic significance, and adapted to survive in a variety of habitats, such as different natural forest environments, savanna, wetlands, protected areas, farmlands, wooden houses, among others [8]. The intensification of agriculture aiming solely at increasing food/forage production could lead to habitat loss and fragmentation, which have been recognized as crucial factors for the decline of stingless bee populations, mainly due to the inappropriate use of fertilizers and pesticides [9-12].

The species *Melipona rufiventris* Lepeletier, 1836 is part of the group of stingless bees native to Brazil, commonly known as *uruçu-amarela*, whose populations have suffered high losses in recent years by the increasing destruction of

native semi-arid vegetation and the predatory collection of honey. It is currently included on the red list of threatened Brazilian fauna as an Endangered Apidae [13].

In addition to these constraints imposed by anthropogenic disturbances of the natural habitat, drastically reducing many populations, studies have shown that the virgin queens of *M. rufiventris* mate with only one male (monandry) during a nuptial flight [14]. So, all females will share the same father, inheriting the male's chromosomes. Genetic variability among their offspring could be, therefore, low. Although single mating is an ancestral condition in this group, low colony-level genetic diversity can not be taken for granted, since alternative mechanisms to increase intra-colonial genetic diversity can be developed to surpass natural biological constraints [15, 16].

Knowledge of the genetic variability and diversity in *M. rufiventris* and its populations, and how they are structured, is essential to a correct interpretation of population dynamics. Information on the population structure of the species can also be used to predict the potential impact of disturbances on the habitat and to formulate appropriate management strategies for species conservation [17]. Molecular markers can be used to measure genetic diversity and variability within and among populations of a single species from different geographical origins [18]. Hence, important questions of conservation relevance can be addressed to this threatened stingless bee species.

This study provides a snapshot of the genetic diversity and structure of *Melipona rufiventris* populations in the Brazilian semiarid using microsatellite markers, a starting point to understand the spatial and temporal genetic changes of *M. rufiventris* populations in Brazil.

MATERIALS AND METHODS

Bee materials and genomic DNA isolation

Worker bees were randomly collected from natural colonies distributed in 3 locations as follows: 25 nests in Campo Maior (*CAM*; coordinates: 4°49'19"S, 42°09'52"W), 7 nests in Castelo do Piauí (*CAP*; coordinates: 5°23'15"S, 41°31'17"W), and 6 nests in Guadalupe (*GUA*; coordinates: 6°47'30"S, 43°34'14"W), all in the states of Piauí-Brazil. All samples were taken to the laboratory and stored at -20°C until further use. Genomic DNA was extracted from each adult worker's thorax using the standard HotSHOT protocol [19]. Alkaline lysis buffer was heated to 95°C for 60 min; then, samples were cooled to 4°C and pH adjusted to 5 with 40 mM Tris-HCl. DNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific) and quality was checked using electrophoresis on 0.8% agarose gels.

Microsatellite markers testing and genotyping

Initially, cross transferability of heterologous microsatellite primers was tested in the *M. rufiventris* genome to find polymorphic loci **(Table 1)**. PCR amplification was carried out using 19 microsatellite primer pairs developed from *M. subnitida* [20] and 18 pairs from *M. fasciculata* [8].

PCR reactions were carried out in a total volume of 10 µL containing approximately 10-50 ng of DNA, 1× PCR buffer (40 mM Tris-HCl; 100 mM KCl), 0.2-0.25 µM of primers, 2.5-3.0 mM $MgCl_2$, 0.5-1.0 µM of each dNTP, 0.25-0.75 U of Invitrogen *Taq* DNA polymerase. All amplifications were carried out using a VERITI™ Gradient Thermal cycler (Life Technologies). PCR conditions (PCR_1) were as follows: 94°C for 5 min, 30 × (94°C for 40 s, Ta (50-60)°C for 30 s, 72°C for 40 s) and 72°C for 7 min. An alternate PCR profile (PCR_2) was used to optimize primers *Mfsc*11, based on the following conditions: 94 °C for 1 min, 40 × (94 °C for 30 s, Ta°C for 30 s, 72 °C for 30 s) and finally 72 °C for 3 min. SSR markers were screened by silver nitrate detection on denatured 6% polyacrylamide gels. A 10-bp ladder (Life Technologies) was used as a size marker. Amplifications of microsatellite loci were considered successful when the gel presented one or two clear and consistent bands, with product sizes close to those of the original species.

Table 1. Markers, primer sequence, experimental parameters used for the amplification of microsatellite loci in *Melipona rufiventris*, and their GenBank accession number.

Loci	Primers (5' → 3')	Repeat motif	Ta (°C)	Allele size range (bp)	PCR profile	GenBank Accession number
*Msub*2	F:GCCCAAAGATGGTATGCCG R: ACGAGGCGGATTCAACGAG	$(ACG)_{14}$	60	172-177	PCR_1	KM494946

*Msub*3	F: CTCGGCGCACAATTCGAG R: GGTTATTTCGCCGGCAAGC	$(CGTT)_{11}$	60	132-136	PCR_1	KM494947
*Msub*18	F: TCCCGATTTCCACCGATCC R: GCCGACCTCTTCGACGG	$(ACG)_{18}$	60	142-160	PCR_1	KM494953
*Msub*31	F: TTACCGTCTGTGCTACTGATCC R:TGTCTGTCTGTCTGTCTATCTTTCTG	$(AGAT)_{14}$	60	134-150	PCR_1	KM494956
*Msub*38	F: AATACTCTGTTTCTTCCAGGGG R: CTGAAATTGCTTTCGTGCC	$(AAAG)_{15}$	60	110-135	PCR_1	KM494958
*Msub*46	F: CACTGTTTCTCCAGTTGCTGTC R: GTTTCGTTCGCGTGATTTC	$(AAAG)_{12}$	60	113-132	PCR_1	KM494960
*Msub*48	F: AAAGAGCGTAGGACTTCCACAG R: CATCCATCTATCCGTACATCCA	$(GGAT)_{10}$	58	115-119	PCR_1	KM494961
*Msub*51	F: GGCGTTACAAAGGGGAGAA R: AGTTGACAGCGTTTCCTACCTC	$(AGAA)_9$	60	148-152	PCR_1	KM494962
*Mfsc*11	F: GGAAGGACGAGAGAATTCAAGA R: ATAGTCGTTTGTCGCGAGTGTA	$(CTT)_{13}$	50	142-168	PCR_2	KT730153
*Mfsc*13	F: GCAGTAACGGTAGCAGTGGTG R: ACTCCTTTCTCCTTCTCGGTCT	$(ACG)_{16}$	52	157	PCR_1	KT730154

Ta, Annealing temperature; PCR profiles: (PCR_1 = [94°C-5 min; 30 cycles × (94°C-40 seg; Ta-30 seg; 72°C-40 seg); 72°C-7 min], PCR_2 = [94°C-1 min; 40 cycles × (94°C-30 seg; Ta-30 seg; 72°C-30 seg); 72°C-3 min].

Data analysis

The genotyped data were analyzed using Micro-Checker 2.2.3 [21] to test for the presence of null alleles or possible scoring inconsistencies. The number of alleles (A), observed and expected heterozygosities (H_O and H_E), and the polymorphic information content (PIC) were determined using CERVUS 3.0.3 [22]. Allelic richness (A_R) was calculated by FSTAT version 2.9.3.2 [23]. Tests for Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium were conducted using the web-based software GENEPOP [24]. Bonferroni-corrected P-values were used to assess the significance ($P<0.05$). A Bayesian grouping admixture model was used to infer possible population structuring using the software STRUCTURE v2.3.3 [25]. The program was set up for 1,000,000 Markov chain Monte Carlo repetitions after an initial burn-in of 100,000 steps. The estimate of the best K was calculated based on 10 replications for each K (from 1 to 4) as described by [26] using STRUCTURE HARVESTER v.0.6.92 [27]. The program CLUMPP v.1.1.2 [28] was used to align the five repetitions of the best K. The program DISTRUCT v.1.1 [29] was used to graphically display the results produced by CLUMPP. Population structure was also analyzed using principal coordinate analysis (PCoA) as implemented in GENALEX v.6.5 [30].

RESULTS AND DISCUSSION

Several genetic diversity studies have been conducted on bee populations using transferred microsatellite markers [31-35].

After PCR testing of 37 potential heterologous microsatellite primer pairs, only nine markers (24.3%) revealed polymorphism in *M. rufiventris*. A monomorphic primer (*Mfsc*13) amplified clear and consistent bands, however, only one band was detected within the studied populations. All the other primer pairs (73%) produced unsatisfactory results with strong non-specific banding patterns or showed no amplification, even though *M. subnitida* and *M. fasciculata* are congeners of *M. rufiventris*. According to Silva *et al*. [8] amplification success declines according to the genetic distance between the taxa, that is, the phylogenetic proximity is the main success factor in the transferability of heterologous primers. Other factors such as the size and complexity of the genome and the location of the microsatellite, whether in the coding region or not, may also influence the transferability of microsatellite primers.

Four loci, *Mfsc*11, *Msub*31, *Msub*38, and *Msub*51, significantly ($P < 0.05$) departed from HWE when the data set was examined as a whole, generally due to the presence of null alleles or missing data. These loci showed heterozygote excess and possible causes are the artifactual scoring of nonspecific and stuttering bands [21], as well as the small effective population size [36, 37]. Allelic richness (A_R) ranged from 2 to 6.6. with a mean of 3.2, while PIC values vary between 0.12 and 0.67, with an average of 0.37, therefore, moderately informative. PIC values above 0.5 are considered very informative; moderately informative between 0.25 and 0.5 and less informative lower than 0.25 [38]. The observed

heterozygosity (Ho) varied from 0.00 to 0.85 with a mean of 0.47, whereas the expected heterozygosity (He) presented values between 0.14 and 0.72, with a mean of 0.43. The *Msub*31 locus was the most polymorphic, and *Msub*51 was the only locus that presented some evidence of null alleles, but at a lower frequency than 0.200 **(Table 2)**. Null allele frequencies below 0.200 are acceptable in most microsatellite data sets [39].

The mean He was the same as that found by Lopes *et al*. [40] for *M. rufiventris* (He = 0.43) and higher than those found in studies with other meliponine species such as 0.38 for *M. mondury* and 0.35 for *M. mandacaia* [41], and 0.105 for *M. mondury* and 0.189 for *M. quadrifasciata* [31]. The genetic diversity found in *M. rufiventris* although superior to most of the aforementioned studies is considered low, and this reduced value is associated with genetic, biological, and environmental aspects, as is the case of anthropogenic interferences. These interferences contribute to the reduction of genetic variability through habitat fragmentation and predatory actions of honey collection, which reduce the number of colonies in a given locality [40-44].

When the data set was divided into 3 collection sites (Campo Maior, Castelo do Piauí, and Guadalupe) it was observed that Campo Maior presented the highest average in relation to the number of alleles/population (3.0), ranging from 1 to 7, when compared with Castelo do Piauí and Guadalupe. Departure from Hardy-Weinberg equilibrium was observed at *Mfsc*11, *Msub*18, *Msub*31, and *Msub*38 loci in the same collection site, after Bonferroni correction. No loci in samples from Castelo do Piauí and Guadalupe deviated significantly from HWE, P > 0.05 **(Table 2)**.

Table 2. Variability of 9 microsatellite loci and estimates of genetic diversity in *Melipona rufiventris* within the Brazilian semiarid.

Loci	Campo Maior-PI (*n=25*)					Castelo do Piauí-PI (*n=7*)					Guadalupe-PI (*n=6*)				
	A	**H_O**	**H_E**	**PIC**	**pHWE**	**A**	**H_O**	**H_E**	**PIC**	**pHWE**	**A**	**H_O**	**H_E**	**PIC**	**pHWE**
*Msub*2	2	0.360	0.301	0.252	0.556	2	0.250	0.250	0.195	1.000	1	0.000	0.000	0.000	-
*Msub*3	2	0.640	0.444	0.341	0.057	2	0.333	0.333	0.239	1.000	2	0.333	0.333	0.239	1.000
*Mfsc*11	2	0.818	0.495	0.367	0.0017*	1	0.000	0.000	0.000	-	3	0.667	0.667	0.535	0.309
*Msub*18	4	0.750	0.557	0.466	0.0005*	1	0.000	0.000	0.000	-	1	0.000	0.000	0.000	-
*Msub*31	7	1.000	0.79	0.741	0.000*	2	0.500	0.409	0.305	1.000	2	0.500	0.429	0.239	1.000
*Msub*38	5	0.421	0.679	0.606	0.0003*	2	1.000	0.600	0.375	0.398	2	1.000	0.571	0.535	0.314
*Msub*46	2	0.571	0.455	0.346	0.344	1	0.000	0.000	0.000	-	0	0.000	0.000	0.000	-
*Msub*48	2	0.240	0.216	0.189	1.000	2	0.400	0.356	0.269	1.000	1	0.000	0.000	0.000	-
*Msub*51	1	0.000	0	0	0	2	0.000	0.485	0.346	0.030	1	0.000	0.000	0.000	-
Mean	3	0.533	0.437	0.367	–	1.6	0.275	0.270	0.192	–	1.4	0.277	0.222	0.172	–

A: Number of alleles in the population; Ho: Observed heterozygosity; He: Expected heterozygosity; PIC: Polymorphic information content; pHWE: Hardy-Weinberg equilibrium probability; *: 5% significance (Bonferroni correction < 0.005).

When estimating the values of the F statistic, based on these molecular markers, the Fis was -0.177, which indicates a low level of inbreeding, whereas, the overall Fst and Rst were 0.151 and 0.288, respectively. The value found for Fst was of similar magnitude compared to that estimated in a previous report for *Melipona asilvai* populations using microsatellite markers (Fst = 0.166) [45]. Although the FST value (0.151) obtained in this study, based on heterologous markers, is not very high, it indicates some level of structure in the populations, which becomes particularly relevant for a threatened species such as *M. rufiventris*. Also according to Nei [46], Fst values < 0.05 are considered low, between 0.05 and 0.15 moderate, and Fst values > 0.15 are high, thus indicating a high population structure within the study area.

The principal coordinate analysis (PCoA) showed a separation of the species into two main groups

with some degree of intersection, thus confirming a significant genetic differentiation among the three populations **(Figure 1a)**. In the Bayesian analysis, the optimal K-value was determined to be 2, where the analysis revealed two distinct groupings **(Figures 1b-1c)**.

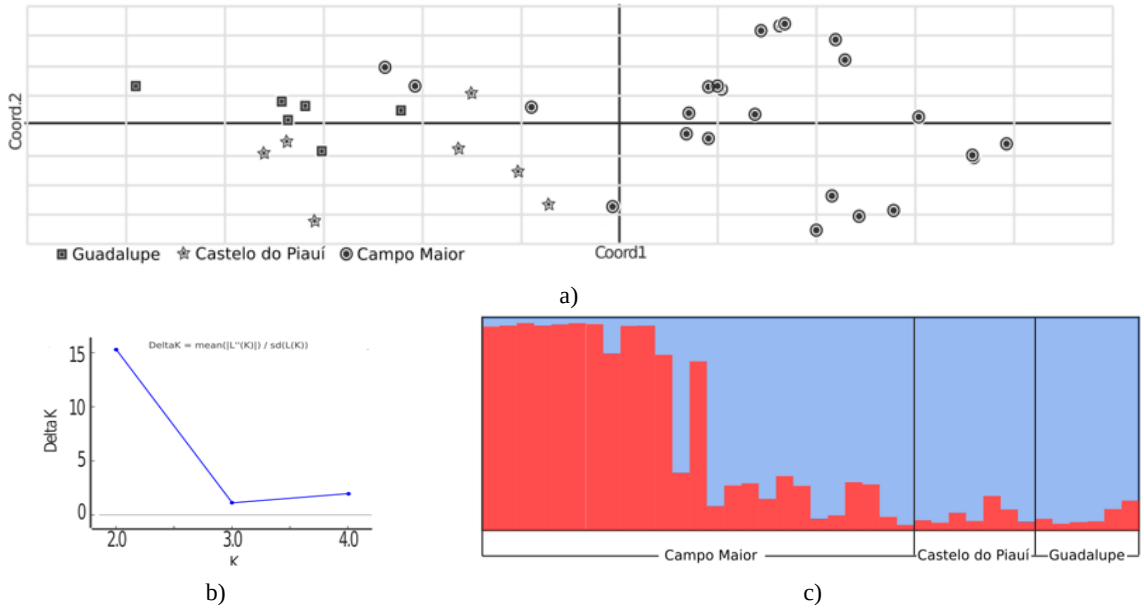

Figure 1. a) Scatter-plot of the principal coordinate analysis (PCoA) using *Melipona* microsatellite loci; b) Determination of the best number of clusters from STRUCTURE analysis; c) Bar plot from inferred population structure of *Melipona rufiventris* using the Bayesian grouping admixture model-based program STRUCTURE (K = 2). Individuals are represented by each bar.

Although the data generated by this study indicate some level of structure in the populations collected from the semiarid, the extent of this genetic differentiation in the '*rufiventris* group' should be further investigated by analyzing additional samples from Castelo do Piauí and Guadalupe, and with a more widespread sampling of the study landscape. Even if the sample size within these study sites has been sufficient to generate well-supported information and to carry out an exploratory assessment of the population structuring of *Melipona rufiventris* in this semiarid region, on the other hand, it could also be a source of fluctuations due to small sample sizes [47]. Therefore, our results should not be over-interpreted. Nevertheless, this information is still paramount to aid in the conservation of the species, since both groups identified in this study, (1) Campo Maior and (2) Castelo do Piauí + Guadalupe, should be managed as distinct units for conservation purposes.

CONCLUSION

Our analyses showed a separation of the individuals into two distinct groups with some degree of intersection, thus confirming a significant genetic differentiation among populations. This information is important to aid in the conservation of the species since both groups identified in this study should be managed as distinct units for conservation purposes, and they can probably be defined as Evolutionary Significant Units (ESUs). In this sense, conservation strategies should focus on minimizing habitat degradation within each region, and on avoiding the translocation between colonies as the species is exploited in meliponiculture.

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REFERENCES

1. Michener CD. The Meliponini. In: Vit P, Pedro SRM, Roubik DW (eds) Pot-Honey: A legacy of stingless bees. New York: Springer; 2013. pp. 3-17.
2. Chakuya J, Gandiwa E, Muboko N, Muposhi VK. A review of habitat and distribution of common stingless bees and honeybee species in African savanna ecosystems. Trop Conserv Sci. 2022;15(1):1-12.
3. Ramírez VM, Ayala R, González HD. Crop Pollination by Stingless Bees. In: Vit P, Pedro SRM, Roubik DW (eds) Pot-Pollen in Stingless Bee Melittology. New York: Springer; 2018. pp. 139-53.
4. Pushpalatha S. The role of stingless bees in crop pollination and their various nesting behaviour. Int J Recent Sci Res. 2019;10(09):34701-2.
5. Bernauer OM, Tierney SM, Cook JM. Efficiency and effectiveness of native bees and honey bees as pollinators of apples in New South Wales orchards. Agric Ecosyst Environ. 2022;337:108063.
6. Real-Luna N, Rivera-Hernández JE, Alcántara-Salinas G, Rojas-Malavasi G, Morales-Vargas AP, Pérez-Sato JA. Stingless bees (Tribe Meliponini) in Latin American agroecosystems. Rev Mexicana Cienc Agric. 2022;13(2):331-44.
7. Layek U, Das A, Karmakar P. Supplemental stingless bee pollination in Fennel (Foeniculum vulgare Mill.): An assessment of impacts on native pollinators and crop yield. Front Sustain Food Syst. 2022;6:820264. doi:10.3389/fsufs.2022.820264
8. Silva GR, Souza IGB, Pereira FM, Souza BA, Lopes MTR, Bentzen P, et al. Genome-wide discovery and characterization of microsatellite markers from Melipona fasciculata (Hymenoptera: Apidae), cross-amplification and a snapshot assessment of the genetic diversity in two stingless bee populations. Eur J Entomol. 2018;115:614-9.
9. Rhodes CJ. Pollinator decline - an ecological calamity in the making? Sci Prog. 2018;101(2):121-60.
10. Vázquez DE, Balbuena MS, Chaves F, Gora J, Menzel R, Farina WM. Sleep in honey bees is affected by the herbicide glyphosate. Sci Rep. 2020;10:10516. doi:10.1038/s41598-020-67477-6
11. Chmiel JA, Daisley BA, Pitek AP, Thompson GJ, Reid G. Understanding the effects of sublethal pesticide exposure on honey bees: a role for probiotics as mediators of environmental stress. Front Ecol Evol. 2020;8:22.
12. Toledo-Hernández E, Peña-Chora G, Hernández-Velázquez VM, Lormendez CC, Toribio-Jiménez J, Romero-Ramírez Y, et al. The stingless bees (Hymenoptera: Apidae: Meliponini): a review of the current threats to their survival. Apidologie. 2022;53(1):8.
13. ICMBIO-MMA (Instituto Chico Mendes, Ministério do Meio Ambiemte). Executive summary of Brazil's red book of threatened species of fauna. Instituto Chico Mendes de Conservação da Biodiversidade; 2016. Available from: http://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoesdiversas/dcom_sumario_executivo_livro_vermelho_ed_2016.pdf Last accessed 19 Dec. 2021.
14. Lopes DM, Silva FO, Fernandes-Salomão TM, Campos LADO, Tavares MG. Microsatellite loci for the stingless bee Melipona rufiventris (Hymenoptera: Apidae). Mol Ecol Resour. 2009;9(3):923-5.
15. Jaffé R, Pioker-Hara FC, Santos CF, Santiago LR, Alves DA, Kleinert AMP, et al. Monogamy in large bee societies: A stingless paradox. Naturwissenschaften. 2014;101(3):261-4.
16. Veiga JC, Ruiz GRS, Carvalho-Zilse GA, Menezes C, Contrera FAL. Queens remate despite traumatic mating in stingless bees. Curr Zool. 2022;68(1):81-92.
17. Drossart M, Gérard M. Beyond the decline of wild bees: optimizing conservation measures and bringing together the actors. Insects. 2020;11(9):649.
18. Oi CA, Oliveira-Silva RA, Francoy TM, Imperatriz-Fonseca VL, Del Lama MA. Genetic and morphometric variation in Schwarziana quadripunctata and

Schwarziana mourei (Hymenoptera: Apidae: Meliponini). Insect Soc. 2022;69(4):345-54. doi:10.1007/s00040-022-00878-0

19. Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). BioTechniques. 2000;29(1):52-4.
20. Souza IGB, Paterson I, Mcbride MC, Souza BA, Pereira FM, Lopes MTR, et al. Isolation and characterization of 23 microsatellite loci in the stingless bee Melipona subnitida using next-generation sequencing. Conserv Genet Resour. 2014;7(1):239-41.
21. Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P. Micro-Checker: software for identification and correcting genotyping errors in microsatellites data. Mol Ecol. 2004;4(3):535-8.
22. Kalinowski ST, Taper ML, Marshal TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignments. Mol Ecol. 2007;16(5):1099-106.
23. Goudet J. FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. 2002. Available from: http://www.unil.ch/izea/softwares/fstat.html. Last accessed 19 Dec. 2021.
24. Raymond M, Rousset F. An exact test for population differentiation. Evolution. 1995;49:1280-3.
25. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155(2):945-59.
26. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. 2005;14(8):2611-20.
27. Earl DA, Vonholdt BM. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012;4(2):359-61.
28. Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics. 2007;23(14):1801-6.
29. Noah AR. Distruct: a program for the graphical display of population structure. Mol Ecol Notes. 2004;4(1):137-8.
30. Peakall R, Smouse PE. GenAlEx 65: Genetic analysis in Excel population genetic software for teaching and research: An update. Bioinformatics. 2012;28:2537-9.
31. Koser JR, Francisco FO, Moretto G. Genetic variability of stingless bees Melipona mondury Smith and Melipona quadrifasciata Lepeletier (Hymenoptera: Apidae) from a meliponary. Sociobiology. 2014;61(3):313-4.
32. Landaverde-González P, Enríquez E, Ariza MA, Murray T, Paxton RJ, Husemann M. Fragmentation in the clouds? The population genetics of the native bee Partamona bilineata (Hymenoptera: Apidae: Meliponini) in the cloud forests of Guatemala. Conserv Genet. 2017;18(3):631-43.
33. Pfeiffer V, Silbernagel J, Guédot C, Zalapa J. Woodland and floral richness boost bumble bee density in cranberry resource pulse landscapes. Landsc Ecol. 2019;34(5):979-96.
34. Yu Y, Zhou S, Zhu X, Xu X, Wang W, Zha L, et al. Genetic differentiation of eastern honey bee (Apis cerana) populations across Qinghai-Tibet plateau-valley landforms. Front Genet. 2019;10:483.
35. Frantine-Silva W, Augusto SC, Tosta THA, Pacheco AS, Kotelok-Diniz T, Silva CA, et al. Genetic diversity and population structure of orchid bees from the Brazilian savanna. J Apic Res. 2021;60(3):385-95.
36. Waples RS. Testing for Hardy-Weinberg proportions: have we lost the plot? J Hered. 2015;106(1):1-9.
37. Kidner J, Theodorou P, Engler JO, Taubert M, Husemann M. A brief history and popularity of methods and tools used to estimate micro-evolutionary forces. Ecol Evol. 2021;11(20):13723-43. doi:10.1002/ece3.8076
38. Botstein D, White RL, Skolnick M. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32(3):314-31.

39. Dakin EE, Avise JC. Microsatellite null alleles in parentage analysis. Heredity. 2004;93(5):504-9.
40. Lopes DM, Campos LAO, Salomão TMF, Tavares MG. Comparative study on the use of specific and heterologous microsatellite primers in the stingless bees Melipona rufiventris and M. mondury (Hymenoptera, Apidae). Gen Mol Biol. 2010;33:390-3.
41. Miranda EA, Batalha-Filho H, Oliveira PS, Alves RMO, Campos LAO, Waldschmidt AM. Genetic diversity of Melipona mandacaia SMITH 1863 (Hymenoptera, Apidae), an endemic bee species from Brazilian caatinga, using ISSR. Psyche. 2012:2012:372138.
42. Jaffé R, Veiga JC, Pope NS, Lanes ÉCM, Carvalho CS, Alves R, et al. Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change. Evol Appl. 2019;12(6):1164-77.
43. Kelemen EP, Rehan SM. Conservation insights from wild bee genetic studies: Geographic differences, susceptibility to inbreeding, and signs of local adaptation. Evol Appl. 2021;14(6):1485-96.
44. Patenković A, Tanasković M, Erić P, Erić K, Mihajlović M, Stanisavljević L, et al. Urban ecosystem drives genetic diversity in feral honey bee. Sci Rep. 2022;12(1):17692. doi:10.1038/s41598-022-21413-y
45. Lima-Junior CA. Melipona asilvai Moure, 1971 (Hymenoptera: Apidae): distribuição potencial, variação morfométrica e diversidade genética. Doctoral thesis in Agricultural Sciences, Universidade Federal do Recôncavo da Bahia, Cruz das Almas. 2015; 72 pp.
46. Nei M. Estimation of average heterozygosity and genetic distance from small number of individuals. Genetics. 1978;89(3):583-90.
47. Bounas A, Catsadorakis G, Logotheti A, Voslamber B, Magoulas A, Tsaparis D. Conservation genetics of a resident population of Greylag Goose (Anser anser) at the southernmost limit of the species' distribution in Europe. Avian Res. 2018;9(1):47.