Scientific note: southernmost record of *Mourella caerulea* and evidence of old and gradual colonization to the south

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Abstract – Little is known about the native bees of Uruguay and no scientific research has been carried out until now. This study aimed to contribute to the knowledge of the native bees, still greatly undermined in Uruguay. To achieve that, workers from the potential species were collected from different locations of the country. Firstly, they were morphologically identified through a taxonomic key and complemented with the Cytochrome Oxidase subunit I sequence, used to infer phylogenetic trees and a haplotype network together with others available in Genbank. Nest characteristics and both morphological and molecular analyses confirm the presence of *Mourella caerulea* in Uruguay, which constitutes the southernmost formal record of the species. Population analyses suggest that *M. caerulea* could have colonized the region from the north, slowly and gradually, through the upper regions of the Pampa's plain.

meliponini / chromatic dimorphism / COI / native bee

There are thousands of native bees species in America responsible for maintaining plant diversity of the continent (Michener 2000). The Meliponini tribe (Hymenoptera: Apidae), commonly known as “stingless bees”, are essential pollinators of neotropical flora (Roubik 1992), and have been raised by humans long before the Europeans arrival to America, being Mayas the oldest example (Álvarez 2016). Uruguay has a high diversity of flora for a temperate region dominated by grasslands. It is considered a transitional phytogeographical zone and included in the different biogeographical regions depending on the author (revised by Pérez-Quesada and Brazeiro 2013). Fauna associated with this flora, such as pollinators, has not been detail studied. This study aimed to contribute to the knowledge of the native bees, still greatly undermined in Uruguay. The hypothesis of this study is that the species present in Uruguay is one of those previously reported for the region. Workers were

collected manually between spring and summer of 2018 on several locations of Uruguay (Figure 1a): 2 from Maldonado (nest, – 34.568850, – 55.085439), 1 from Rocha (nest, – 34.3825053, – 54.4933653), 3 from Tacuarembó (nest, – 31.7097802, – 56.0072956) and 3 caught in flight in Lavalleya (– 34.60117, – 55.46377 and – 34.424794, – 55.10193039). FCE-HY 1917, FCE-HY 1918 and FCE-HY 1919 were added to the entomology collection (Facultad de Ciencias, Uruguay).

The topography of the nest's surroundings consists of a highlander landscape with granite rocks, in areas varying from 164 to 227 m of elevation. Nests were located underground, where the entrance had a diameter of about 5 mm and more than 20 cm deep. The ground around the nest's entrance was clean, without any vegetation except a few grasses. Workers are approximately 7-mm long metallic extremely dark green body with yellow lines on the scutellum and labrum. The anterior wing is approximately 4 mm long, extending beyond the apex of the metasoma, with the marginal cell wider than the submarginal area and it has 7 hamuli. Interestingly, a chromatic dimorphism was found on some workers from Maldonado, which have orange

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Figure 1. Regional map indicating localities used in this study (**a**) and main molecular results (**b**, **c**). Samples from Uruguay are shown in grey and in black are those studied previously. **a** Samples taken for this study (Tacuarembó 1, Maldonado 2, Lavalleja 3, 4, Rocha 5). **b** Phylogenetic estimation by Maximum Likelihood of COI gene. Numbers at nodes are bootstrap values by Maximum Likelihood, Neighbour Joining and Maximum Parsimony methods, in that order. **c** Haplotype network. Branch lengths are proportional to the amount of changes, each change shown as a perpendicular bar.

abdomen. All these characteristics agree with the description made by Camargo and Wittmann (1989) for *Mourella caerulea* (Friese 1990). According to Michener (2007) key, morphological characteristics match those belonging to the *Mourella* genus. The same idea was supported by three specialized taxonomists, i.e.: Dres. Leopoldo Alvarez (Universidad Nacional de La Plata, Argentina), Juliana Galaschi-Teixeira (Instituto tecnológico VALE, Brazil) and Favizia Freitas de Oliveira (Universidade Federal da Bahia, Brazil).

One sample per nest was genetically analysed except for the sample from Tacuarembó, which was not included. A 663-nucleotide sequence of the gene COI was recovered following the Galaschi-Teixeira et al. (2018) laboratory recommendation. After corroborating each nucleotide substitutions and the absence of insertions/

deletions, results show that all samples have the same haplotype (GenBank MW145008), including the chromatic dimorphism. Phylogenetic reconstruction was carried out by different criteria and 1000 Bootstrap pseudoreplicates, including all known haplotypes of the potential species from Brazil available in GenBank (KY274882 from Bagé; KY274873 from Alegrete; KY274859 from Viamão; KY274858 from Turuçu (a); KY274853 from Turuçu (b); KY274852 from Candiotá) and *Tetragonisca weyrauchi* (KC853324); *T. fiebrigi* (KC853322); *T. angustula* (KC853323); *Frieseomelitta* sp. (MK397275); *Lestrimelitta niitkib* (MH917100); *Melipona bicolor* (AF370439; AF466146); *M. scutellaris* (KP202303). Uruguayan haplotype is within a monophyletic group of *M. caerulea* (Figure 1b), with high bootstrap value and independent of

Table 1. Pairwise F_{ST} values between populations. S. Brazil: Southern Brazil (Turuçu-Bagé-Candiota). * p value < 0.05

	Uruguay	S. Brazil	Viamão
S. Brazil	0.51*		
Viamão	1.00	0.32	
Alegrete	1.00*	0.63*	1.00

method tried, with twenty-six synonymous polymorphic sites. More closely related to the Uruguayan haplotype are those from Southern Brazil (i.e.: Turuçu-Bagé-Candiota), and Northern Brazilian populations are more ancestral (i.e.: Alegrete-Viamão), suggesting a progressive invasion from north to south (Figure 1b). This could reflect the natural colonization of these bees, since their nests are underground and therefore are not a possible target for commercial applications, discarding the anthropic transport of hives (Galaschi-Teixeira et al. 2018).

For populational analyses, the Uruguayan haplotype and those reported by Galaschi-Teixeira et al. (2018) were analysed together and every nest was taken as an individual (39 individuals). Molecular analyses indicate that populations are differentiated. Exact tests of population differentiation based on haplotypes frequencies show statistically significant differences among populations (p value < 0.0001). However, there were significant differences in pairwise comparisons between Uruguay and Southern Brazil (p value = 0.0004), Uruguay and Alegrete (p value = 0.00025), and Southern Brazil and Alegrete (p value = 0.0001). Global F_{ST} value was 0.67, and pairwise F_{ST} values between populations are mostly high (> 0.5), except for the pair Southern Brazil-Viamão (F_{ST} = 0.32), evidencing its closer relationship (Table 1). This differentiation could be due to the low vagility of these bees (Nogueira-Neto 1954). Consistent with the above, populations are in balance between differentiation by genetic drift and with gene flow, showing a pattern of isolation by distance although with marginal p value (R = 0.87, p value = 0.07), reflecting a gradual and neutral differentiation, and Tajima's and Fu's neutrality tests are not significant (data not shown). Thus *M. caerulea* could have colonized the region from the north, slowly and gradually, through the upper regions of the Pampas plain.

AUTHORS' CONTRIBUTIONS

SP, IT and ES collected data. SP and ES did the morphological analyses. SP and IT did the molecular and bioinformatic analyses. SP wrote the draft, and IT and ES commented on manuscript previous versions.

Note scientifique : présence de *Mourella caerulea* la plus méridionale et preuves d'une ancienne et progressive colonisation vers le sud.

Meliponini / dimorphisme chromatique / COI / abeille indigène.

Eine Wissenschaftliche Notiz über den südlichsten Fund von *Mourella caerulea* und Hinweise auf deren alte und graduelle Ausbreitung nach Süden.

Meliponini / Farbdimorphismus / COI / einheimische Bienen.

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