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Original article

Mitochondrial discrimination of stingless bees *Tetragonisca angustula* (Apidae: Meliponini) from Santa Catarina state, Brazil*

Diego Fagundes KOLING[1], Geraldo MORETTO[2]

[1] Genolab, Rua Floriano Peixoto 425, Centro, CEP 89010 500 – Blumenau – SC, Brazil
[2] Departamento de Ciência Naturais, Universidade Regional de Blumenau, CEP 89012-900, Blumenau-SC, Brazil

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Abstract – The stingless bee *Tetragonisca angustula* is a meliponini bee naturally distributed from Argentina to Mexico. Morphologically, based on the mesepisternum color, it is separated into *T. angustula angustula* and *T. angustula fiebrigi* subspecies. The objective of this study was to characterize the restriction site variation in the mitochondrial DNA of each subspecies. Samples of worker bees were collected from two regions of Santa Catarina state, known as having a natural distribution of each subspecies. Each of the 138 colonies collected in the distribution region of *T. a. angustula* and of 72 colonies from the region of *T. a. fiebrigi* was individually analyzed. The ATPases 8, 6 and COIII mitochondrial genes were amplified and digested with seven restriction enzymes (PCR + RFLP). Large genetic differences were observed among bees collected in both geographical areas of natural distribution for the *T. angustula* subspecies. Four enzymes showed different restriction patterns that allowed separation of the subspecies.

***Tetragonisca angustula* / stingless bees / Meliponini / PCR+RFLP / mtDNA**

1. INTRODUCTION

The meliponini is a group of approximately 400 species of stingless bees that exhibit highly social behavior (Kerr et al., 1996; Velthuis, 1997; Michener, 2000). The stingless bees have a wide geographical distribution, the neotropical region is where most of the species occur (Camargo and Pedro, 1992). In Brazil, approximately 300 species were recorded (Kerr et al., 1999), being found in various ecosystems, such as the Atlantic Forest (Ramalho, 2003), Amazon Forest (Brown, 2001; Brown and Albrecht, 2001), Pantanal and Cerrado areas (Mittermeier et al., 2005).

The stingless bees play an important role in maintaining the ecological balance among several species of angiosperms and fauna of various ecosystems. (Janzen, 1980). According to the ecosystem, it is estimated that approximately 40 to 90% of angiosperms are dependent on this group of bees (Kerr et al., 1996). Some species of small size are responsible for the pollination of many species of angiosperms with small flowers (Nogueira-Neto et al., 1986; Nogueira-Neto, 1997; Coletto-Silva, 2005).

Tetragonisca angustula, popularly known in Brazil as "Jataí" is a meliponini bee that is characterized by its rusticity and important ecological role. It has been maintained in meliponiculture due to the excellent quality of honey and propolis produced (Miorin et al., 2003) and is also used in pollination of many cultivars (Heard, 1999).

Morphologically, based on the mesepisternum color (thoracic region), *T. angustula* is

Corresponding author: G. Moretto,
gmoretto@furb.br
* Manuscript editor: Marina Meixner

divided into two subspecies: *T. angustula angustula* and *T. angustula fiebrigi* characterized by the black and yellow mesepisternum, respectively (Schwarz, 1938). Both subspecies have different geographical distribution. While *T. a. angustula* is widely distributed in the Americas, ranging from Mexico to Argentina, *T. a. fiebrigi* is restricted to certain regions of Brazil (São Paulo, Paraná, western state of Santa Catarina), part of Argentina and Paraguay (Nogueira-Neto, 1970).

On the other hand, Castanheira and Contel (1995) found variation in the mesepisternum color of *T. angustula* samples collected in the states of Paraná and São Paulo. This variation suggested that the variability in the mesepisternum coloration is controlled by several genes and that the cross between individuals of basic color (black and yellow) may result in offspring with some degree of variation in their colors.

Few molecular studies have been conducted in this species of bees. Castanheira and Contel (2005) found significant correlation between the mesepisternum color, with the hexokinase allele HK^{88} being found with great frequency in more yellow bees, indicating that it is an allele that has its origin in *T. a. fiebrigi*. Oliveira et al. (2004) using RAPD molecular markers, distinguished two *T. angustula* groups based in its natural geographic distribution. However, although this molecular marker showed great genetic variability among populations, it was not able to separate *T. a. angustula* and *T. a. fiebrigi* subspecies (Baitala et al., 2006). Therefore, this study was an effort to obtain genetic markers that could differentiate those two subspecies. Characterization of *T. a. angustula* and *T. a. fiebrigi* is here reported through the analysis of a mitochondrial DNA fragment (mtDNA) using the PCR + RFLP method.

2. MATERIAL AND METHODS

2.1. Worker samples

Worker bees from 210 *T. angustula* feral colonies, were collected from tree-trunks, stone walls and in rustic hives from two regions of Santa Catarina state. Individuals were collected from 138 colonies distributed among 18 municipalities from the Vale do Itajaí region (central to northern part of the state), and 72 colonies in 19 municipalities from the middle and far west of the state (Fig. 1 and Tab. I). Each sample was placed in 70% ethanol and stored at –20 °C.

2.2. Morphological analysis

Each worker bee from every colony sampled, was morphologically separated into subspecies using the mesepisternum coloration classes from Castanheira and Contel (1995). Two to ten individuals were analyzed for each colony, resulting in 1368 bees from the Vale do Itajaí region and 482 bees from he Western region of Santa Catarina. Each collected bee was individually observed under a stereomicroscope and, according to a graded color scale ranging from black to yellow mesepisternum, were classified into following five classes: black (1), black to intermediate (2), intermediate (3), intermediate to yellow (4) and yellow (5). Individuals belonging to classes 1 and 2 and classes (4 and 5) were separated into *T. a. angustula,* and *T. a. fiebrigi*, respectively, while workers in class 3 were considered as intermediate and not included in either subspecies.

2.3. Molecular analysis

2.3.1. DNA extractions

The mtDNA analysis (PCR+RFLP) was conducted with one individual per colony. Total DNA was extracted from a leg of each *T. angustula*, using the rapid extraction method adapted by Anderson and Fuchs (1998). Each *T. angustula* worker bee, after being washed in 70% ethanol, was placed in a Petri dish and an anterior leg was removed with the aid of forceps. After dissection, the leg was transferred into a microcentrifuge tube containing 40 μL of 2× lysis buffer (120 mg/mL Proteinase K, 0.1M KCl, 0.02M Tris-HCl pH 8.3, 5 mM $MgCl_2$, 0.9% Tween 20, 0.9% NP40 and 0.02% of gelatin). The tube and contents were incubated first at 65 °C for 30 min, then at 97 °C for 10 minutes and then diluted into 20 μL of dH2O. The extracted DNA was stored at 20 °C for later use.

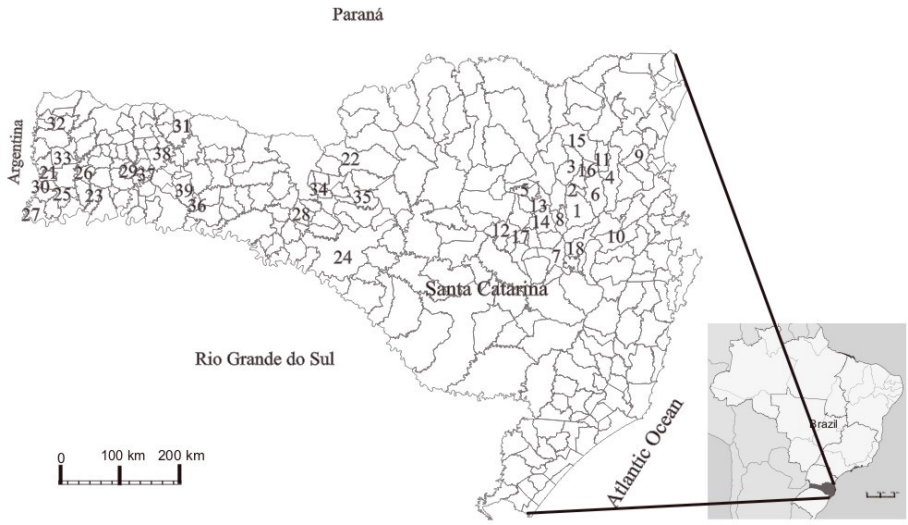

Figure 1. Map of part South America showing Santa Catarina state (Brazil) with the municipalities where *Tetragonisca angustula* subspecies were sampled: Apiúna (1) Ascurra (2) Benedito Novo (3) Blumenau (4) Dona Emma (5) Indaial (6) Ituporaga (7) Lontras (8) Luiz Alves (9) Nova Trento (10) Pomerode (11) Pouso Redendo (12) Presidente Getúlio (13) Rio do Sul (14) Rio dos Cedros (15) Timbó (16) Trombudo Central (17) Vidal Ramos (18) Belmonte (21) Caçador (22) Caibi (23) Campos Novos (24) Iporã do Oeste (25) Iraceminha (26) Itapiranga (27) Joaçaba (28) Pinhalzinho (29) Santa Helena (30) São Domingos (31) São José do Cedro (32) São Miguel do Oeste (33) Treze Tilhas (34) Videira (35) Arvoredo (36) Águas Frias (37) Quilombo (38) Xaxim (39). Samples 1 to 18 = Vale do Itajaí, Samples 21 to 39 = Western.

2.3.2. PCR+RFLP analysis

Mitochondrial ATPases 8, 6 and COIII genes were amplified using PCR and the following primers: mtD19 5′-GAA ATT AAT TGT GGA GCA CAT AG-3′ and mtD22 5′-AAG TGT TCA ACA CAG TAT CA -3′ (Simon et al., 1994). The PCR reactions were performed in a total volume of 25 μL for each sample, containing 1× of standard buffer solution recommended by the manufacturer (Invitrogen), 2.5 mM $MgCl_2$, 250 μM of dNTP, 1 μM of each primer (mtD19 and mtD22), 2.5 units of Taq DNA polymerase (Invitrogen) and 1 μL of total DNA. Each PCR reaction was submitted to initial denaturation at 94 °C/5 min, followed by 35 cycles of denaturation at 94 °C/1, annealing for 1 min and 20 s at 44 °C, elongation at 64 °C/2 min, and finally, an extension to the end at 64 °C for 10 minutes. The PCR products were analyzed in 0.8% agarose gel, stained with ethidium bromide and visualized under UV light, with the image being captured through a photodocumentation system.

To determine the presence of restriction sites, the ATPases 8, 6 and COIII fragment was digested for a minimum period of 6 h with the following restriction enzymes: *Ase* I, *Dra* I, *Eco*R I, *Eco*R V, *Hae* III, *Hind* III , and *Hinf* I. Each digestion was performed in a 20 μL reaction mixture using 2 μL of the PCR product and 2 units of the enzymes with appropriate buffer. The results of the digestion with restriction enzymes *Eco*R I, *Eco*RI, *Hae* III, *Hinf*I, and Hind III, were analyzed in 1.5% agarose gel, then stained and visualized in the same manner as that used in the amplification process. The results obtained with the digestion performed with endonucleases *Dra* I and *Ase* I were analyzed in 12% polyacrylamide gel and visualized through silver staining.

3. RESULTS

3.1. Morphological analysis

The morphological analysis based on the mesepisternum coloration are shown in the table1. Some level of intracolonial variation was verified, especially among the bees collected

Table I. Number of colonies (N) of *Tetragonisca angustula* sampled, their geographic coordinates origin in the Santa Catarina state and individual subspecies identification based on morphological and molecular analysis.

Region	Municipality	Altitude (m)	Geographical coordinates		Colonies (N)	Subspecies identification based on analysis:	
			Latitude(S)	Longitude(W)		Morphological	Molecular
Vale do Itajaí	Apiúna	87	27° 02′ 08″	49° 23′ 23″	6	*T.a.angustula*	*T.a.angustula*
	Ascurra	87	26° 57′ 19″	49° 22′ 32″	6	*T.a.angustula*	*T.a.angustula*
	Benedito Novo	130	26° 46′ 58″	49° 21′ 52"	2	*T.a.angustula*	*T.a.angustula*
	Blumenau	21	26° 55′ 10″	49° 03′ 58″	48	*T.a.angustula/T.a.fiebrigi*[a]	*T.a.angustula*
	Dona Emma	370	26° 59′ 05″	49° 43′ 32″	2	*T.a.angustula*	*T.a.angustula*
	Indaial	64	26° 53′ 52″	49° 13′ 54″	5	*T.a.angustula*	*T.a.angustula*
	Ituporanga	360	27° 24′ 52″	49° 36′ 09″	16	*T.a.angustula*	*T.a.angustula*
	Lontras	330	27° 09′ 58″	49° 32′ 31″	3	*T.a.angustula*	*T.a.angustula*
	Luiz alves	70	26° 43′ 14″	48° 55′ 58″	9	*T.a.angustula*	*T.a.angustula*
	Nova Trento	30	27° 17′ 09″	48° 55′ 47″	3	*T.a.angustula*	*T.a.angustula*
	Pomerode	85	26° 44′ 26″	49° 10′ 37″	2	*T.a.angustula*	*T.a.angustula*
	Pouso Redendo	354	27° 15′ 29″	49° 56′ 02″	8	*T.a.angustula*	*T.a.angustula*
	Presidente Getúlio	255	27° 03′ 02″	49° 37′ 22″	10	*T.a.angustula*	*T.a.angustula*
	Rio do Sul	341	27° 12′ 51″	49° 38′ 35″	7	*T.a.angustula*	*T.a.angustula*
	Rio dos Cedros	85	26° 44′ 18″	49° 16′ 27″	3	*T.a.angustula*	*T.a.angustula*
	Timbó	68	26° 49′ 24″	49° 16′ 18″	4	*T.a.angustula*	*T.a.angustula*
	Trombudo Central	350	27° 17′ 57″	49° 47′ 25″	2	*T.a.angustula*	*T.a.angustula*
	Vidal Ramos	370	27° 23′ 31″	49° 21′ 21″	2	*T.a.angustula*	*T.a.angustula*
Western	Águas Frias	345	26° 52′ 48″	52° 51′ 33’	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Arvoredo	362	27° 04′ 28″	52° 27′ 21’	1	*T.a.fiebrigi*	*T.a.fiebrigi*
	Belmonte	612	26° 50′ 29″	53° 34′ 32’	3	*T.a.fiebrigi*	*T.a.fiebrigi*
	Caçador	920	26° 46′ 31″	51° 00′ 54’	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Caibi	337	27° 04′ 18″	53° 14′ 52″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Campos Novos	934	27° 24′ 06″	51° 13′ 30″	3	*T.a.fiebrigi/T.a.angustula*[b]	*T.a.fiebrigi*
	Iporã do Oeste	557	26° 49′ 34″	53° 30′ 00″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Iraceminha	445	26° 49′ 21″	53° 16′ 28″	2	*T.a.fiebrigi/T.a.*angustula[c]	*T.a.fiebrigi*
	Itapiranga	206	27° 10′ 10″	53° 42′ 44″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Joaçaba	522	27° 10′ 41″	51° 30′ 17″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Pinhalzinho	515	26° 50′ 53″	52° 59′ 31″	3	*T.a.fiebrigi/intermediate/T.a.angustula*[d]	*T.a.fiebrigi*
	Quilombo	425	26° 43′ 34″	52° 43′ 10″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	Santa Helena	530	26° 56′ 15″	53° 37′ 09″	2	*T.a.fiebrigi/T.a.*angustula[e]	*T.a.fiebrigi*
	São Domingos	635	26° 33′ 29″	52° 31′ 54″	13	*T.a.fiebrigi*	*T.a.fiebrigi*
	S. José do Cedro	731	26° 27′ 18″	53° 29′ 39″	2	*T.a.fiebrigi*	*T.a.fiebrigi*
	S. Miguel d'Oeste	645	26° 43′ 31″	53° 31′ 05″	21	*T.a.fiebrigi*	*T.a.fiebrigi*
	Treze Tilias	796	27° 00′ 06″	51° 24′ 23″	3	*T.a.fiebrigi/intermediate/T.a.angustula*	*T.a.fiebrigi*
	Videira	750	27° 00′ 30″	51° 09′ 06’	4	*T.a.fiebrigi*	*T.a.fiebrigi*
	Xaxim	770	26° 57′ 42″	52° 32′ 05’	2	*T.a.fiebrigi*	*T.a.fiebrigi*

[a] *T.a.angustula* (98.7%), *T.a.fiebrigi* (1.3%)
[b] *T.a.angustula* (8.3%), *T.a.fiebrigi* (91.7%)
[c] *T.a.angustula* (20%), *T.a.fiebrigi* (80%)
[d] *T.a.angustula* (15%), *T.a.fiebrigi* (75%), Intermediate (10%)
[e] *T.a.angustula* (10), *T.a.fiebrigi* (90%)
[f] *T.a.angustula* (5.26%), *T.a.fiebrigi* (84.4%), Intermediate (10.5%)

in the western region, where 85% were classified as *T. a. fiebrigi* for belonging to classes 4 and 5 and 12% were considered as *T. a. angustula*, with almost all belonging to class 1. The other bees collected in this region (3%) were not included in any of the two subspecies, they were classified in class 3, which includes the group of bees with mesepisternum of intermediate coloration. In the Vale do Itajaí region, 98.7% of the samples belonged to classes 1 (black mesepisternum) and 2 (black to intermediate mesepisternum), which were

classified as *T. a. angustula*. The others belonged to classes 4 and 5, being therefore morphologically classified as *T. a. fiebrigi*.

3.2. Molecular analysis

The amplified mtDNA segment containing the genes of ATPases 8, 6 and COIII enzymes was 1800 bp. No length polymorphism was observed in all individuals sampled in this study.

In the RFLP analysis endonucleases *Hind* III and *Hae* III showed no digestion product in any of the worker bees examined. The digestion of the PCR product with endonuclease *Eco*R V, resulted in two fragments of approximately 1200 and 600 bp, although it showed no polymorphism among bees collected. However, several polymorphic sites among bees from both geographic regions were identified with the other four restriction enzymes. The restriction enzyme *Eco*R I did not generate digestion product in *T. angustula* from the Vale do Itajaí region, but two fragments were observed in all samples from Western state of Santa Catarina (Fig. 2a). Digestion with *Hinf* I resulted in two and one specific fragments in bees from Western and Vale do Itajaí regions, respectively (Fig. 2b).

Endonucleases *Ase* I and *Dra* I generated several fragments, although in some of them, the size could not be accurately estimated. However, several bands were specific in bees from the Vale do Itajaí and others for samples collected in the Western region. With the *Ase* I enzyme, we observed the presence of two bands (approximately 86 and 130 bp), exclusive to *T. angustula* from the Western region and one band of approximately 98 bp exclusive of bees from the Vale do Itajaí. With the use of *Dra* I, two bands were obtained (102 and 125 bp) exclusively in bees from the Vale do Itajaí. In the samples from the western region, part of the bees presented two bands sized 100 and 110 bp, while two bands (105 and 112 bp) were exclusively found in the other bees this geographic region. This enzyme also generated two major bands of 300 bp, both with specific sizes on bees from each geographic region.

4. DISCUSSION

The PCR+RFLP technique applied in this study showed that the mtDNA 19 and mtDNA 22 *primers*, although being designed to amplify the ATPases 8, 6 and COIII region of the mitochondrial genome of *Apis mellifera* (Simon et al., 1994), produced excellent amplification products in *T. angustula* when used at the annealing temperature of 44 °C. This methodology also made it possible to determine the size of the mtDNA fragment containing the genes of ATPases 8, 6 and COIII enzymes and several specific restriction sites in *T. angustula,* sampled in different geographical regions of Santa Catarina state. The size of 1800 bp of the amplified mtDNA fragment in this species of bees is approximately equal to that found for *M. quadrifasciata* (Moretto and Arias, 2005) *M. mondury* and *M. rufiventris* (Barni et al., 2007) and is approximately 100 bp larger than *Plebeia* species (Francisco et al., 2001).

The *Eco*R I restriction site in the ATPases 8, 6 and COIII region present in several *Plebeia* and *Melipona* species, (Francisco et al., 2001; Weinlich et al., 2004), has been reported as absent in the restriction map of *T. angustula* (Arias et al., 2006). In our study, it was absent from all bees collected in the Vale do Itajaí, but was present in all samples from the western region. The Vale do Itajaí region, where the *EcoR* I site was not verified, is part of the Atlantic forest ecosystem, where the natural dispersion of *T. a. angustula* occurs and the western region of Santa Catarina, characterized by the presence of the *EcoR* I site, is part of the distribution of *T. a. fiebrigi*.

In addition to the *Eco*R I site, *Ase* I, *Dra* I, and *Hinf* I also established different restriction patterns among *T. angustula* from both regions studied. *Hinf* I, which has been used to investigate the diversity of *Apis cerana* subspecies (Sihanuntavong et al., 1999), when used for the cleavage of the ATPases 8, 6 and COIII region, cut once in the bees from the Vale do Itajaí region but twice in samples from the Western region. A band of 1000 bp found in bees from both regions indicates the presence of a common site among them and the bands of 500 bp and 300 pb shown in bees from the

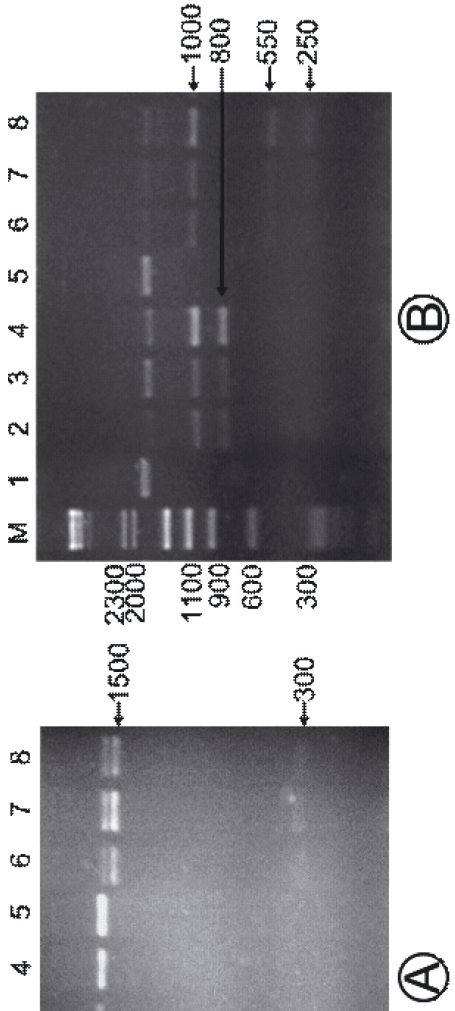

Figure 2. Agarose (1.5%) gels showing different banding patterns observed between *T. angustula* bees from Santa Catarina state for the ATPases 8, 6 and COIII region after digestion with endonuclease *EcoR* I (A) and *Hinf* I (B). Columns 1and 5 = no digested fragment; 2, 3, 4 = Vale do Itajai; 6, 7, 8 = West region. M: molecular weight markers λ/*Hind* III and φx174/*Hae* III (in Kb).

western region is the result of another site restriction, Therefore, the fact that the last site has been found only in the region of natural distribution of *T. a. fiebrigi* may be considered as a specific marker of this subspecies.

The endonucleases *Dra* I and *Ase* I were used to assess the variability of the mitochondrial genome of *Apis mellifera* subspecies (Garnery et al., 1993; Franck et al., 1998) and *Melipona quadrifaciasta* subspecies (Moretto and Arias, 2005; Souza et al., 2008). In this study, although the bees sampled in both regions of Santa Catarina belong to the *Tetragosnisca* genus, we verified the occurrence of several restriction sites of *Dra* I and *Ase* I. This evidence suggests that the enzymes that recognize A + T restriction sites can also be used to detect polymorphism in *T. angustula* subspecies. Among all the bands that resulted from digestion with these two enzymes, some were specific for each of the geographical regions of natural distribution where *T. a. angustula* and *T. a. fiebrigi* subspecies were described.

Based on the mesepisternum color study of Schwarz (1938), it was considered that both *T.angustula* subspecies naturally occur in the state of Santa Catarina. *T. a. fiebrigi* would be dispersed throughout the western region of the state and *T. a. angustula* would be found exclusively in the Atlantic forest. All bees sampled in the Vale do Itajaí region showed molecular markers not found in bees colleted in the western region and almost 99% were morphologically identified as *T. a. angustula* and only around 1% were classified as *T. a. fiebrigi*. However, samples from western Santa Catarina also revealed several molecular markers specific of bees from that region, but great discrepancy was observed when compared with morphological makers. The morphological patterns typical of the *fiebrigi* subspecies (classes 4 and 5) was found in only 85% of the samples, with 12% exhibiting the morphological pattern (class 1) typical of *T. a. angustula* and 3% (with intermediate color), could not be included in either subspecies.

Variation in the measures of morphometric characters were found among *T. angustula* samples collected in several parts of Brazil. The study of morphological changes in fifteen characters in samples of bees from eight local populations conducted by Diniz-Filho et al. (1998), showed significant differences, with north-south clinal variation in body size. An east-west clinal variation in the size of the wing, explained as a possible racial mixing, was also found in *T. angustula* samples collected in the southeastern region of the country (Castanheira and Contel, 2005). However, despite the variation in mesepisternum color found in this study, mainly in bees from the western region, several markers obtained through the PCR+RFLP technique were specific to bees from each geographic region. Oliveira et al. (2004) also found similar results, 22% of the population of bees, initially identified as *T. a. angustula* based on the mesepisternum color, were *T. a. fiebrigi*, when analyzed with RAPD molecular markers. Thus, the presence of specific mtDNA markers, in each natural distribution regions for *T. a. angustula* and *T. a. fiebrigi*, suggests restricted gene flow between the two groups of bees analyzed in this work. According to Castanheira and Contel (2005), the polygenic inheritance of the mesepisternum color must be a cause of variability found in local *T. angustula* populations.

Traditionally, the identification of *T. angustula* subspecies was based on the mesepisternum color (Schwarz, 1938). However, the natural variability within each subspecies and the origin of hybrid colonies that may occur through contact of the two subspecies may lead to false identifications. Thus, the data from mtDNA verified in this study combined with the RAPD data reported in Oliveira et al. (2004), can be used as molecular markers for the analysis of *T. a. angustula* and *T. a. fiebrigi* populations.

According to Nogueira-Neto (1970), *T. angustula* is a bee widely used in meliponiculture and this encourages farmers and hobbyists to exchange bee colonies. This practice may promote contact and establish gene flow between the two subspecies and develop hybrid populations. Therefore, the mtDNA markers found in this work can be used to determine the maternal origin of commercial and natural populations.

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Caractérisation des abeilles sans aiguillon (*Tetragonisca angustula*) de l'Etat de Santa Catarina (Brésil) par l'ADN mitochondrial.

***Tetragonisca angustula* / abeilles sans aiguillon / Meliponini / PCR+RFLP / ADN mitochondrial**

Zusammenfassung – Mitochondriale Unterscheidung von stachellosen Bienen (*Tetragonisca angustula)* aus dem Staat Santa Catarina, Brasilien. Die in Brasilien allgemein als "Jataí" bekannte Melipone *Tetragonisca angustula* wird in die Unterarten *T. a. angustula* und *T. a. fiebrigi* unterteilt, die durch ein schwarzes, bzw. gelbes Mesepisternum gekennzeichnet sind. Die beiden Unterarten haben eine unterschiedliche Verbreitung. Während *T. a. angustula* auf dem amerikanischen Doppelkontinent, von Mexiko bis Argentinien, weit verbreitet ist, ist *T. a. fiebrigi* auf bestimmte Gegenden von Brasilien (São Paulo, Paraná, Westen von Santa Catarina), einen Teil von Argentinien und Paraguay beschränkt. In dieser Untersuchung sollten genetische Marker zur Unterscheidung dieser beiden Unterarten gefunden werden. Hier berichten wir über die Charakterisierung von *T. a. angustula* und *T. a. fiebrigi* durch die Analyse eines mitochondrialen DNA (mtDNA) Fragments mit der PCR + RFLP Methode.
Die morphologischen und molekularen Analysen wurden an Arbeitsbienen aus natürlichen Nestern von *T. angustula* durchgeführt, die aus zwei verschiedenen Regionen in Santa Catarina stammten. Die gesammelten Arbeiterinnen wurden mit Hilfe einer morphometrischen Untersuchung (Pigmentierung des Mesepisternums) in die Unterarten *T. angustula angustula* und *T. a. fiebrigi* klassifiziert. Dabei wurden zwischen 2 und 10 Individuen pro Volk untersucht. Die Analyse der mtDNA (PCR+RFLP) wurde an einem Individuum pro Volk durchgeführt. Die mitochondriale Region mit den Genen für die ATPasen 8 und 6, sowie COIII wurde mittels PCR amplifiziert und für mindestens 6 Stunden mit den folgenden Restriktionsenzymen verdaut: *Ase* I, *Dra* I, *Eco*R I, *Eco*R V, *Hae* III, *Hind* III, und *Hinf* I. Große genetische Unterschiede wurden zwischen den Bienen gefunden, die in den verschiedenen Verbreitungsgebieten gesammelt wurden. Die Ergebnisse von vier Enzymen (*Ase* I, *Dra* I, *Eco*R I and *Hinf* I) zeigten verschiedene Restriktionsmuster, die die Unterscheidung der Unterarten erlaubten.

***Tetragonisca angustula* / Stachellose Bienen / Meliponini / PCR+RFLP / mtDNA**

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