

Morphometric Analysis of Characters in Three Subspecies of Leaf-Cutting ants, *Acromyrmex subterraneus* (Hymenoptera: Formicidae)

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Abstract – Identifications of leaf-cutting ants, species and subspecies of *Acromyrmex*, in particular, more precisely regarding the three subspecies of *A. subterraneus* (*A. s. subterraneus*, *A. s. brunneus* and *A. s. molestans*), which are based on a characteristic of great risk for differentiation, such as the coloration of the largest worker. Seeking to provide subsidies to elucidate the systematic problem among subspecies of *A. s. brunneus*, *A. s. subterraneus* and *A. s. molestans*, a morphometric analysis of characters in polymorphic leaf-cutting ant workers was carried out. Through the identification of subspecies by means of the workers' morphology, it is possible to consider the hypothesis that *A. s. molestans* is a different species. However, there is also a taxonomic problem between subspecies *A. s. brunneus* and *A. s. subterraneus*. On the other hand, in most comparisons made between subspecies *A. s. subterraneus* and *A. s. molestans*, they were not significant or were inversely-proportional significant. Based on the morphometric analysis of characters, it is concluded that there is a problem in the systematization of subspecies *A. s. subterraneus brunneus* and *A. s. subterraneus*, with *A. s. molestans* likely being another species.

Keywords – *Acromyrmex subterraneus brunneus*, *Acromyrmex subterraneus subterraneus*, *Acromyrmex subterraneus molestans*, Leaf-cutting ants.

I. INTRODUCTION

Fungi-cultivating ants (Formicidae: Myrmicinae: Attini) can be found exclusively in the New World, indicating that their origin and subsequent radiation throughout the Neotropical region happened after the separation of South America from Africa [1]. Species are distributed from the United States of America (Latitude 40° N) [2] to Argentina (Latitude 44° S) [2]-[3]. In South America, they are not present in the Trans-Andean regions [1]-[4], and in Central America they do not occur in some islands of the Antilles [1], [5]-[6]. The Attini Tribe is currently composed of 17 genera and 246 described species [7], with some genera requiring taxonomic revisions due to the existence of many subspecies [8].

Brazil has 21 species and 11 subspecies of the genus *Acromyrmex* [9]-[13]. However, for Della-Lucia et al. (1993), there are 20 species and 9 taxonomic subspecies, accepted as *Acromyrmex*. The genus *Acromyrmex*, from a

taxonomic point of view, is one of the most difficult of the family Formicidae, for the species' marked polymorphism and great individual variability. Although the head and thorax spines are important systematic characters, their proportions vary from colony to colony and between individuals from the same colony, and, at a certain frequency, bilateral head asymmetry can be found [9], [14]. Thus, the need for morphological studies is essential for the understanding of subspecies of the *A. subterraneus* complex.

The identification keys of species and subspecies of *Acromyrmex* [9], [15]-[16], more specifically with respect to two subspecies of *A. subterraneus* (*A. s. subterraneus* and *A. s. brunneus*), were based on a characteristic of great risk for differentiation, such as the coloration of the largest worker. The differentiation of the subspecies *A. s. molestans*, in turn, is facilitated because the largest workers have peculiar characteristics: their heads usually have a darker forehead, and their lower pronotal spines are strongly bent forward. Several authors have addressed the difficulties in differentiating the three subspecies by morphological characters [9], [17]-[18].

In the description of the main identification key developed by Gonçalves (1961), the largest workers of the subspecies *A. s. brunneus* differed only from *A. s. subterraneus*, as the former had a black or dark black color, unlike the latter, which can be light brown or sometimes yellowish.

In the light of the above, the present research carried out a morphometric analysis of characters in polymorphic leaf-cutting ant workers of the *Acromyrmex subterraneus* complex. This study seeks to provide subsidies to elucidate the systematic problem between subspecies *A. s. brunneus*, *A. s. subterraneus* and *A. s. molestans*.

II. MATERIAL AND METHODS

1- Collection and Maintenance of Colonies

Sixty nests of subspecies of *Acromyrmex subterraneus* were collected in Piracicaba, Jacareí and Eldorado, municipalities of São Paulo state, Brazil, as well as in Viçosa, Minas Gerais, Brazil; and stored in the Laboratory

of Social Insects-Pests (LIDP), Vegetal Protection Department, Phytosanitary Defense Sector, at the College of Agricultural Sciences, UNESP, Botucatu, SP. However, for the morphometric study of workers, 5 nests of each subspecies were used, adopting the following nomenclature:

Colony Initials	Subspecies	Origin
P1, P2, P3, E1, E5;	<i>A. subterraneus brunneus</i>	Jacarei, Piracicaba and Eldorado, São Paulo State, Brazil
V2, V5, V6, V7, V13	<i>A. subterraneus molestans</i> .	Viçosa, Minas Gerais State, Brazil
V10, V22, V23, V24, V25	<i>A. subterraneus subterraneus</i>	Viçosa, Minas Gerais State, Brazil

The nests were kept in a closed system, using 6-liter transparent cylindrical acrylic containers, with a 2-cm layer of gypsum at the bottom to keep the fungus garden moist. Two equidistant holes were drilled in the containers, to which transparent plastic hoses measuring 3 cm in diameter and 5 cm in length were connected. To one of the hoses, a 1-liter cylindrical acrylic container was attached for leaf storage. The other hose was attached to a container with the same capacity for deposition of depleted vegetal material (garbage).

For fungi growth maintenance, the ants were provided leaves of the vegetal species *Lygustrum* sp., *Phyllanthus* sp. and *Acalypha* sp.

2- *Subspecies Identification through Workers' Morphology*

The identification of subspecies was performed through the study of workers' general morphology, adopting the procedures described in the subsequent items. Specimens from the largest workers of the nests were collected for identification under stereomicroscope, using the identification key developed by Gonçalves (1961) and comparing with the keys by Andrade (1991) and Mayhé-Nunes (1991).

3- *Workers' Polymorphism*

In this experiment, the size variation of workers, and five nests of each subspecies were studied.

Workers of different sizes were visually separated into six size categories: very small (VS), small (S), medium I (MI), medium II (MII), large I (LI) and large II (LII). Fifty workers of each size category were measured in each nest.

The measures adopted were HL - head length (from the cephalic vertex to the forehead base); HW - head width (longest distance through the occipitals); ID - interocular distance; BL - body length (not including head); and PTL - posterior tibial length (third pair of legs). These measurements were done under stereomicroscope with micrometer eyepiece and converted in millimeters.

4- *Statistical Analyses*

Morphometric data were subjected to analysis of variance by Snedecor's F test for comparison between means, with 1% probability levels, as well as Student's t test, with a 5% probability level, for comparison between mean differences

in relation to the pre-established size categories. Pearson's simple correlation analyses were performed between morphometric variables, and significance was tested at 1% and 5% probability levels.

III. RESULTS AND DISCUSSIONS

1- *Subspecies Identification through Workers' Morphology*

The nests from Jacarei, Piracicaba and Eldorado, Sao Paulo State, Brazil, were identified as belonging to subspecies *Acromyrmex subterraneus brunneus*. Of the 36 nests collected in Viçosa, MG, 17 were identified as belonging to subspecies *A. subterraneus subterraneus*, and 19 to subspecies *A. subterraneus molestans*.

The identification keys of species and subspecies of *Acromyrmex* [9], [15]-[16], more specifically with respect to two subspecies of *A. subterraneus* (*A.s. subterraneus* and *A.s. brunneus*), were based on a characteristic of great risk for differentiation, such as the coloration of the largest worker. The differentiation of subspecies *A. subterraneus molestans*, in turn, is facilitated because the largest workers have peculiar characteristics: their heads usually have a darker forehead, and their lower pronotal spines are strongly bent forward.

Several authors have addressed the difficulties in distinguishing the three subspecies by morphological characters [9], [17].

In the description of the main identification key developed by Gonçalves (1961), the largest workers of subspecies *A. subterraneus brunneus* differed only from *A. subterraneus subterraneus*, as the former had a black or dark brown coloration, unlike the latter, which can be light brown or sometimes yellowish.

The present study found subspecies *A. subterraneus brunneus* in the city of Piracicaba - SP, which matches the subspecies found by Andrade (1991) in that municipality. As for the collection done in Eldorado, SP, the five nests collected in the Atlantic Forest area were identified as belonging to subspecies *A. subterraneus brunneus*, with no other subspecies being found, as verified by Andrade (1991). The largest workers from the nests collected in Viçosa, MG, presented predominantly a light brown or brown color, but in some colonies black or dark brown specimens were found as well. Gonçalves (1961) also observed, in one same nest, specimens with very different tegument hues. This observation, once again, shows some confusion in the differentiation of subspecies *A. subterraneus brunneus* and *A. subterraneus subterraneus*.

About spine arrangement, an important character of differentiation in Gonçalves's (1961), Andrade's (1991) and Mayhé-Nunes's (1991) keys, no difference was found mainly in the lower pronotal spine, which was straight and faced forward in all workers observed and grouped as belonging to subspecies *Acromyrmex subterraneus subterraneus* and *A. subterraneus brunneus*. Thus, to identify which species occurs in Viçosa, only the color character was used, which led to *A.s. subterraneus*.

Still in the municipality of Viçosa, the other part of the collected material clearly had the characteristics of

subspecies *A. subterraneus molestans*, described by Gonçalves (1961), such as the pair of lower pronotal spines strongly bent forward and the head with darker forehead. Thus, two coexisting subspecies were found in the same location. Andrade (1991) also observed more than one subspecies in the same location, which refutes the characteristics of allopatric population.

In view of the above, it could be hypothesized that *A. s. molestans* is another species. There is also a systematic problem between subspecies *A. subterraneus brunneus* and *A. s. subterraneus*.

2- Workers' Polymorphism

As per statistical analysis ("t" Test) of morphology data, it was possible to confirm the existence of six size categories in the studied subspecies, due to significant differences between the means of assessed parameters (Tables 1-3).

The size categories were separated using the Head Width (HW) parameter. Said parameter has also been used in other investigations related to size distribution of *Atta cephalotes*, *A. sexdens*, *A. texana*, *Acromyrmex coronatus*, *A. octospinosus* and *A. volcans* workers [19], [4], [20]-[21].

The mean head widths of size categories in *A. s. subterraneus*, *A. s. brunneus* and *A. s. molestans* were, respectively, very small (VS) 0.8 mm, 0.8 mm and 0.75 mm; small (S) 1.10, 1.58 and 1.36 mm; large I (LI) 2.02, 1.89 and 1.58 mm, large II (LII) 2.32, 2.18 and 1.85 mm (Tables 1-3).

Comparing the overall means of assessed morphometric parameters in the five nests of *A. s. brunneus*, there was no difference between data obtained in nest P3, from Piracicaba, and nest E1, from Eldorado, both São Paulo state's municipalities. As for data on the other three nests, P1, P2 (Piracicaba) and E5 (Eldorado), there was significant difference between assessed parameters, with the exception of P2 and P3 nests, whose means were not different from E1 as to the body length (BL) parameter (Table 1).

In *A.s. subterraneus*, when it comes to the overall means of the head length (HL), head width (HW) and body length (BL) parameters, workers from different nests were significantly different when nest V5 was compared with the others. Nests V2 and V7, V5 and V13 did not differ from each other in relation to the posterior tibial length (PTL) parameter, but nest V6 differed from all of them. The latter did not differ from the others in relation to the interocular distance (ID) parameter, but there were differences between nests V2, V13 and V5, V7 (Table2).

Table 1. Analysis of variance components (F Test) and "t" test of morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories in *Acromyrmex subterraneus brunneus*. Total of 1500 measures.

	Assessed Parameters (mm)				
	HL	HW	BL	PTL	ID
Nests					
P ₁	1.27 d	1.38 c	3.99 c	1.27 d	1.24 d
P ₂	1.39 b	1.47 b	4.27 a	1.40 b	1.34 b

	Assessed Parameters (mm)				
	HL	HW	BL	PTL	ID
P ₃	1.42 a	1.51 a	4.21 b	1.44 a	1.37 a
E ₅	1.33 c	1.39 c	3.99 c	1.33 c	1.26 c
E ₁	1.43 a	1.52 a	4.25 ab	1.44 a	1.38 a
Size Category					
VS	0.82 f	0.80 f	2.39 f	0.68 f	0.71 f
S	1.00 e	1.00 e	2.93 e	0.87 e	0.90 e
MI	1.21 d	1.27 d	3.71 d	1.18 d	1.17 d
MII	1.46 c	1.58 c	4.43 c	1.53 c	1.44 c
LI	1.75 b	1.89 b	5.35 b	1.83 b	1.73 b
LII	1.96 a	2.18 a	6.04 a	2.16 a	1.96 a
V. C. (%)	7.09	8.01	6.97	7.11	7.88
F Test					
Nests	143.7**	97.5**	70.1**	169.6**	112.8**
Sizes	5143.4**	5215.6**	5942.9**	8413.0**	5477.0**

Mean values followed by the same letter do not differ from each other by the "t" test at a 5% probability. ** Significant at 1% level. (1) Size category: VS - very small; S - small; MI - medium I; MII - medium II; LI - large I; LII - large II.

Table 2. Analysis of variance components (F Test) and "t" test of morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories in *Acromyrmex subterraneus subterraneus*. Total of 1500 measures.

	Assessed Parameters (mm)				
	HL	HW	BL	PTL	ID
Nest					
V ₂	1.44 a	1.54 a	4.51 a	1.46 a	1.39 a
V ₅	1.50 b	1.60 b	4.62 b	1.49 b	1.42 b
V ₆	1.45 a	1.57 c	4.47 ac	1.51 c	1.41 ab
V ₇	1.45 a	1.54 ac	4.45 c	1.46 a	1.39 a
V ₁₃	1.46 a	1.57 c	4.49 ac	1.50 b	1.43 b
Size Category					
VS	0.80 a	0.80 a	2.39 a	0.67 a	0.71 a
S	1.07 b	1.10 b	3.32 b	1.02 b	1.00 b
MI	1.33 c	1.41 c	4.07 c	1.34 c	1.29 c
MII	1.60 d	1.73 d	4.91 d	1.66 d	1.56 d
LI	1.85 e	2.02 e	5.70 e	1.93 e	1.82 e
LII	2.10 f	2.32 f	6.62 f	2.28 f	2.08 f
V. C. (%)	7.2	5.3	4.3	4.4	5.5
F Test					
Nests	12.6**	29.2**	35.1**	37.2**	17.1**
Size	5410.6**	11830.4**	16159.4**	20771.4**	10744.4**

Mean values followed by the same letter do not differ from each other by the "t" test at a 5% probability. ** Significant at 1% level. (1) Size category: VS - very small; S - small; MI - medium I; MII - medium II; LI - large I; LII - large II.

Table 3. Analysis of variance components (F Test) and “t” test of morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories in *Acromyrmex subterraneus molestans*. Total of 1500 measures.

	Assessed Parameters (mm)				
	HL	HW	Mean BL	PTL	ID
Nest					
V ₁₀	1.33 a	1.39 a	4.37 a	1.36 a	1.26 a
V ₂₂	1.19 b	1.22 b	3.83 b	1.23 b	1.10 b
V ₂₃	1.25 c	1.33 c	3.91 c	1.28 c	1.20 c
V ₂₄	1.12 d	1.19 d	3.69 d	1.18 d	1.08 b
V ₂₅	1.17 b	1.21bd	3.77 c	1.19 d	1.08 b
Size Category					
VS	0.78 a	0.75 a	2.30 a	0.61 a	0.66 a
S	0.94 b	0.93 b	2.89 b	0.81 b	0.83 b
MI	1.07 c	1.12 c	3.50 c	1.06 c	1.01 c
MII	1.29 d	1.36 d	4.20 d	1.38 d	1.24 d
LI	1.47 e	1.58 e	4.91 e	1.64 e	1.44 e
LII	1.72 f	1.85 f	5.70 f	2.00 f	1.70 f
V.C. (%)	5.7	5.7	3.9	4.8	5.7
F Test					
Nests	411.9**	428.1**	927.3**	460.5**	533.7**
Sizes	6374.9**	8044.6*	17238.4*	18794.6*	8575.9**

Mean values followed by the same letter do not differ from each other by the “t” test at 5% probability. ** Significant at 1% level. (1) Size category: VS - very small; S - small; MI -medium I; MII - medium II; LI - large I; LII - large II.

Nests V₂₂ and V₂₅ of *A.s. molestans* did not differ statistically as to the head length parameter, but the others differed from each other. There was statistical difference in the means of the head width parameter in nests V₁₀ and V₂₃. Body length did not differ only in nests V₂₃ and V₂₅. Tibial length and interocular distance did not differ only between nests V₂₄, V₂₅ and V₁₀, V₂₃, respectively (Table3).

The differences in workers’ sizes between the studied nests may have been due to intra - and inter-specific variation, already reported by other authors [1], [22]-[23].

In the analysis of variance (F Test), all differences were significant in all assessed parameters, both in the comparison by nests and by size category in the three studied ants (Tables 1-3).

When it comes to the correlation analysis between the morphometric parameters assessed in all nests, separated by species and subspecies, there was a significant correlation between them, and Pearson’s correlation coefficients were high (Tables 4-6).

Comparing data separately by species, subspecies and size category, the morphometric parameters are directly related. The correlations were significant and the coefficients were high, except for the very small (VS) category, in which a low correlation was found. The correlations were higher when the interocular distance (ID) parameter was compared with the others (Tables 7-25).

Table 4. Pearson’s correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.99**	0.98**	0.98**	0.99**
HW	-	-	0.98**	0.98**	0.99**
BL	-	-	-	0.98**	0.98**
PTL	-	-	-	-	0.98**
ID	-	-	-	-	-

**Significant correlation at 1%, N = 1500

Table 5. Pearson’s correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.98**	0.98**	0.97**	0.98**
HW	-	-	0.99**	0.99**	0.99**
BL	-	-	-	0.99**	0.99**
PTL	-	-	-	-	0.99**
ID	-	-	-	-	-

**Significant correlation at 1%, N = 1500

Table 6. Pearson’s correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers of different size categories from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.99**	0.98**	0.98**	0.98**
HW	-	-	0.98**	0.98**	0.99**
BL	-	-	-	0.99**	0.98**
PTL	-	-	-	-	0.98**
ID	-	-	-	-	-

**Significant correlation at 1%, N = 1500

Table 7. Pearson’s correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the very small size category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.59**	0.52**	0.40**	0.57**
HW	-	-	0.55**	0.40**	0.69**
BL	-	-	-	0.37**	0.48**
PTL	-	-	-	-	0.45**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 8. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the small size category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.89**	0.79**	0.66**	0.84**
HW	-	-	0.81**	0.70**	0.91**
BL	-	-	-	0.64**	0.79**
PTL	-	-	-	-	0.66**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 9. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium I size category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.90**	0.82**	0.85**	0.89**
HW	-	-	0.87**	0.88**	0.91**
BL	-	-	-	0.84**	0.88**
PTL	-	-	-	-	0.90**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 7. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium II size category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.93**	0.82**	0.83**	0.94**
HW	-	-	0.83**	0.83**	0.94**
BL	-	-	-	0.77**	0.83**
PTL	-	-	-	-	0.83**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 11. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large I category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.79**	0.62**	0.67**	0.89**
HW	-	-	0.56**	0.74**	0.87**
BL	-	-	-	0.57**	0.69**
PTL	-	-	-	-	0.75**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 12. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large II size category from five nests of *Acromyrmex subterraneus brunneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.86**	0.76**	0.76**	0.88**
HW	-	-	0.80**	0.76**	0.86**
BL	-	-	-	0.71**	0.76**
PTL	-	-	-	-	0.75**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 13. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the very small size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.34**	0.20**	0.16**	0.24**
HW	-	-	0.53**	0.42**	0.69**
BL	-	-	-	0.37**	0.53**
PTL	-	-	-	-	0.43**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 14. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the small size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HWC	BL	PTL	ID
HL	-	0.74**	0.42**	0.50**	0.68**
HW	-	-	0.54**	0.55**	0.78**
BL	-	-	-	0.64**	0.55**
PTL	-	-	-	-	0.61**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 15. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium I size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.80**	0.70**	0.57**	0.77**
HW	-	-	0.71**	0.61**	0.75**
BL	-	-	-	0.67**	0.61**
PTL	-	-	-	-	0.60**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 16. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium II size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.81**	0.63**	0.51**	0.78**
HW	-	-	0.63**	0.60**	0.80**
BL	-	-	-	0.52**	0.58**
PTL	-	-	-	-	0.57**
ID	-	-	-	-	-

**Significant correlation at 1%; N = 250

Table 17. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large I size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.82**	0.69**	0.68**	0.83**
HW	-	-	0.74**	0.67**	0.84**
BL	-	-	-	0.63**	0.70**
PTL	-	-	-	-	0.69**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 18. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large II size category from five nests of *Acromyrmex subterraneus subterraneus*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.35**	0.31**	0.21**	0.32**
HW	-	-	0.74**	0.55**	0.73**
BL	-	-	-	0.62**	0.76**
PTL	-	-	-	-	0.57**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 19. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in different size categories from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.99**	0.98**	0.98**	0.98**
HW	-	-	0.98**	0.98**	0.99**
BL	-	-	-	0.99**	0.98**
PTL	-	-	-	-	0.98**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 1500

Table 20. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the very small size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.45**	0.36**	0.15**	0.38**
HW	-	-	0.34**	0.25**	0.54**
BL	-	-	-	0.25**	0.36**
PTL	-	-	-	-	0.24**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 21. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the small size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.66**	0.72**	0.67**	0.64**
HW	-	-	0.68**	0.71**	0.79**
BL	-	-	-	0.87**	0.72**
PTL	-	-	-	-	0.79**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 22. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium I size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.68**	0.56**	0.57**	0.66**
HW	-	-	0.58**	0.64**	0.71**
BL	-	-	-	0.74**	0.57**
PTL	-	-	-	-	0.63**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 23. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium II size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.90**	0.86**	0.86**	0.90**
HW	-	-	0.87**	0.89**	0.94**
BL	-	-	-	0.90**	0.88**
PTL	-	-	-	-	0.87**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 24. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large I size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.91**	0.84**	0.85**	0.91**
HW	-	-	0.83**	0.86**	0.94**
BL	-	-	-	0.89**	0.83**
PTL	-	-	-	-	0.84**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 25. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large II size category from five nests of *Acromyrmex subterraneus molestans*.

	Assessed Parameters				
	HL	HW	BL	PTL	ID
HL	-	0.94**	0.87**	0.82**	0.94**
HW	-	-	0.88**	0.83**	0.94**

Table 27. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in different size categories from five nests of two subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

	Assessed Parameters									
	<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
	HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
<i>A. s. brunneus</i>										
HL	0.93**	0.95**	0.94**	0.95**	0.94**	0.88**	0.90**	0.90**	0.89**	0.89**
HW	0.93**	0.95**	0.95**	0.95**	0.94**	0.89**	0.90**	0.91**	0.90**	0.89**
BL	0.94**	0.95**	0.95**	0.96**	0.95**	0.90**	0.91**	0.92**	0.91**	0.90**
PTL	0.94**	0.95**	0.95**	0.96**	0.95**	0.92**	0.93**	0.94**	0.93**	0.91**
ID	0.94**	0.95**	0.95**	0.95**	0.95**	0.88**	0.90**	0.90**	0.90**	0.89**

**Significant correlation at 5%; N = 1500

Table 28. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the very small (VS) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

	Assessed Parameters									
	<i>A. subterraneus subterraneus</i>					<i>B. subterraneus molestans</i>				
	HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
<i>A. s. brunneus</i>										
HL	0.05 ns	0.04 ns	-0.05 ns	-0.11 ns	0.02 ns	-0.01 ns	0.04 ns	0.00 ns	0.04 ns	0.00 ns
HW	0.05 ns	0.09 ns	0.06 ns	0.05 ns	0.07 ns	-0.18**	-0.09 ns	0.03 ns	0.04 ns	-0.06 ns
BL	0.03 ns	-0.04 ns	-0.07 ns	0.11 ns	0.07 ns	-0.06 ns	0.03 ns	0.11 ns	0.04 ns	-0.09 ns
PTL	0.02 ns	0.03 ns	0.03 ns	0.08 ns	0.04 ns	-0.08 ns	-0.02 ns	0.01 ns	0.13**	-0.02 ns
ID	-0.06 ns	-0.02 ns	-0.01 ns	-0.01 ns	0.03 ns	-0.13**	-0.05 ns	-0.05 ns	0.11 ns	-0.08 ns

**Significant correlation at 5%; N = 250

	Assessed Parameters				
	HL	HW	BL	PTL	ID
BL	-	-	-	0.82**	0.88**
PTL	-	-	-	-	0.83**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 250

Table 26. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in different size categories from five nests of two subspecies of *Acromyrmex subterraneus*.

	Assessed Parameters				
	<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>	HL	HW	BL	PTL	ID
HL	-	0.94**	0.87**	0.82**	0.94**
HW	-	-	0.88**	0.83**	0.94**
BL	-	-	-	0.82**	0.88**
PTL	-	-	-	-	0.83**
ID	-	-	-	-	-

**Significant correlation at 5%; N = 1500

Table 29. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the very small (VS) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
	HL	0.05 ns	-0.06 ns	-0.05 ns	-0.09 ns	-0.08 ns
	HW	0.05 ns	0.03 ns	0.04 ns	0.02 ns	0.07 ns
	BL	-0.17**	0.08 ns	0.07 ns	-0.14**	-0.03 ns
	PTL	-0.01 ns	-0.03 ns	0.03 ns	0.09 ns	0.04 ns
	ID	-0.16**	-0.01 ns	0.07 ns	0.09 ns	-0.02 ns

**Significant correlation at 5%; N = 250

Table 30. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the small (S) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters									
		<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
<i>A. s. brunneus</i>		HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
	HL	0.05 ns	0.07 ns	0.09 ns	0.08 ns	0.08 ns	0.13**	0.14**	0.12 ns	0.17**	0.12 ns
	HW	0.04 ns	0.00 ns	0.00 ns	0.03 ns	-0.03 ns	0.16**	0.15**	0.09 ns	0.21**	0.16**
	BL	-0.12 ns	-0.07 ns	-0.11 ns	-0.04 ns	-0.09 ns	0.07 ns	0.13**	0.05 ns	0.09 ns	0.13**
	PTL	0.03 ns	0.03 ns	0.00 ns	0.10 ns	0.04 ns	0.12 ns	0.15**	0.07 ns	0.13**	0.15**
	ID	0.00 ns	-0.03 ns	-0.03 ns	0.02 ns	-0.04 ns	0.07 ns	0.10 ns	0.00 ns	0.12 ns	0.09 ns

**Significant correlation at 5%; N = 250

Table 31. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the small (S) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
	HL	-0.18**	-0.13**	-0.16**	-0.02 ns	-0.13**
	HW	-0.20**	-0.13**	-0.20**	0.03 ns	-0.14**
	BL	-0.24**	-0.20**	-0.07 ns	-0.06 ns	-0.16**
	PTL	-0.12**	-0.11 ns	-0.02 ns	0.00 ns	-0.09 ns
	ID	-0.23**	-0.13**	-0.03 ns	0.04 ns	-0.11 ns

**Significant correlation at 5%; N = 250

Table 32. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium I (MI) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters									
		<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
<i>A. s. brunneus</i>		HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
	HL	-0.13**	-0.16**	-0.16**	-0.15**	-0.15**	0.19**	0.25**	0.20**	0.24**	0.22**
	HW	-0.11 ns	-0.13**	0.17**	-0.13**	-0.13**	0.16**	0.22**	0.18**	0.20**	0.21**
	BL	0.00 ns	0.02 ns	-0.14 ns	0.00 ns	0.00 ns	-0.17**	-0.06 ns	-0.06 ns	-0.10 ns	-0.04 ns
	PTL	-0.08 ns	-0.09 ns	0.13**	-0.04 ns	-0.10 ns	0.01 ns	0.08 ns	0.05 ns	0.05 ns	0.09 ns
	ID	0.12 ns	0.16**	0.19**	0.14**	0.15**	0.08 ns	0.13**	0.05 ns	0.12 ns	0.13**

**Significant correlation at 5%; N = 250

Table 33. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium I (MI) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
	HL	-0.21**	-0.19**	-0.04**	-0.08 ns	-0.20**
	HW	0.16**	0.16**	0.01 ns	0.05 ns	0.17**
	BL	-0.19**	-0.21**	0.07 ns	-0.10 ns	-0.21**
	PTL	-0.16 ns	-0.07 ns	0.10 ns	-0.01 ns	-0.08 ns
	ID	-0.13**	-0.10 ns	-0.04 ns	0.01 ns	-0.11 ns

**Significant correlation at 5%; N = 250

Table 34. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium II (MII) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters									
		<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
<i>A. s. brunneus</i>		HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
	HL	-0.21**	0.27**	0.22**	0.28**	0.24**	-0.29**	-0.19**	-0.25**	-0.37**	-0.25**
	HW	-0.18**	0.23**	-0.15**	0.25**	0.22**	-0.32**	-0.23**	-0.29**	-0.42**	-0.27**
	BL	-0.01 ns	0.08 ns	0.04 ns	0.06 ns	0.04 ns	-0.38**	-0.28**	-0.31**	-0.50**	-0.34**
	PTL	0.20**	0.27**	0.18**	0.28**	0.24**	-0.35**	-0.27**	-0.30**	-0.46**	-0.30**
	ID	0.23**	0.28**	0.17**	0.27**	0.27**	-0.37**	-0.28**	-0.34**	-0.49**	-0.34**

**Significant correlation at 5%; N = 250

Table 35. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the medium II size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
	HL	-0.06 ns	-0.04 ns	0.09 ns	0.10 ns	0.01 ns
	HW	-0.13**	-0.12 ns	0.00 ns	0.07 ns	-0.07 ns
	BL	-0.19**	0.18**	0.04 ns	-0.12 ns	-0.13**
	PTL	-0.18**	-0.16**	0.00 ns	-0.02 ns	-0.10 ns
	ID	-0.19**	-0.17**	0.02 ns	0.02 ns	-0.10 ns

**Significant correlation at 5%; N = 250

Table 36. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large I size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters									
		<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
<i>A. s. brunneus</i>		HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
	HL	0.05 ns	0.04(ns)	0.08 ns	0.07 ns	0.10 ns	-0.18**	0.03 ns	0.18**	-0.06 ns	-0.11 ns
	HW	0.10 ns	0.04 ns	0.16**	0.07 ns	0.16**	-0.22**	0.01 ns	0.12 ns	-0.14**	-0.17**
	BL	-0.05 ns	-0.13 ns	0.02 ns	-0.06 ns	0.01 ns	-0.35**	0.12 ns	-0.02 ns	-0.23**	-0.28**
	PTL	0.11 ns	0.10 ns	0.08 ns	0.12 ns	0.15**	-0.28**	0.05**	-0.11 ns	-0.18**	-0.20**
	ID	0.12 ns	0.12 ns	0.18**	0.16**	0.19**	-0.22**	0.03**	0.11**	-0.09 ns	-0.16**

**Significant correlation at 5%; N = 250

Table 37. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large I (LI) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
HL		0.03 ns	-0.06 ns	-0.09 ns	-0.01 ns	0.08 ns
HW		-0.03 ns	-0.11 ns	-0.18**	-0.06 ns	0.01 ns
BL		0.00 ns	0.07 ns	0.04 ns	0.13**	0.01 ns
PTL		0.06 ns	-0.02 ns	-0.05 ns	-0.05 ns	0.07 ns
ID		-0.07ns	-0.14**	-0.21**	-0.11 ns	-0.01ns

**Significant correlation at 5%; N = 250

Table 38. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large II size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameter									
		<i>A. subterraneus subterraneus</i>					<i>A. subterraneus molestans</i>				
<i>A. s. brunneus</i>		HL	HW	BL	PTL	ID	HL	HW	BL	PTL	ID
HL		0.00 ns	0.02 ns	0.02 ns	-0.05 ns	0.00 ns	-0.38**	-0.28**	-0.26**	-0.49**	-0.37**
HW		0.18**	0.19**	0.15**	0.11 ns	0.17**	-0.38**	-0.29**	-0.27**	-0.46**	-0.36**
BL		0.05 ns	0.08 ns	0.05 ns	-0.01 ns	0.05 ns	-0.48**	-0.38**	-0.33**	-0.59**	-0.42**
PTL		0.24**	0.21**	0.20 ns	0.20**	0.24**	-0.35**	-0.25**	-0.24**	-0.47**	-0.33**
ID		0.15**	0.15**	0.14**	0.05 ns	0.13**	-0.41**	-0.32**	-0.29**	-0.53**	-0.39**

**Significant correlation at 5%; N = 250

Table 39. Pearson's correlation coefficient between morphometric parameters, head length (HL), head width (HW), body length (BL), posterior tibial length (PTL) and interocular distance (ID), measured in workers in the large II (LII) size category from five nests of subspecies of *Acromyrmex subterraneus subterraneus* and *A. s. molestans*.

		Assessed Parameters				
		<i>A. subterraneus subterraneus</i>				
<i>A. s. molestans</i>		HL	HW	BL	PTL	ID
H		0.12 ns	-0.05 ns	0.03 ns	0.00 ns	-0.01 ns
LC		0.09 ns	-0.09 ns	-0.02 ns	-0.02 ns	-0.03 ns
BL		0.06 ns	0.12**	0.00 ns	-0.05 ns	-0.03 ns
PTL		0.13 ns	-0.02 ns	0.06 ns	-0.05 ns	-0.07 ns
ID		0.12 ns	-0.06 ns	0.04 ns	-0.08 ns	0.00 ns

**Significant correlation at 5%; N = 250

The comparison between all the morphometric parameters measured in the three studied taxa also resulted in high correlation coefficients (Tables 26-27). However, when comparisons were made by size category, most of the coefficients were little significant (between *A. s. subterraneus* and *A. s. brunneus*), not significant, or inversely-proportional significant, as in most comparisons made between the subspecies of *A.s. subterraneus* and *A.s. molestans* (Tables 26-39). These results show another indication that the subspecies are different indeed.

It has been stated that the size variation of a species of *Atta* is continuous and comprises very distinct castes morphologically – minimum, medium and large [24]. The same authors addressed castes of the genus *Acromyrmex* only once, fitting the latter into an intermediate category between *Trachymyrmex* and *Atta*, as having at least one polymorphic species, with two subcastes of workers. The size distribution of workers of subspecies *A. subterraneus*

was continuous as well, but the morphological differences of the categories were not so pronounced compared to the difference found in *Atta*; in any case, they suggest three subspecies, rather than two.

For the subspecies of *Acromyrmex subterraneus* and *A. s. molestans*, three different subcastes were observed – small, medium and large, different from the findings by Wetterer (1999) for species *A. coronatus*, *A. octospinosus* and *A. volcanus*, having found a narrow size distribution in the first, and bimodal in the other two, which corresponds to two subcastes of workers (small and large). This author believes that the intermediate (medium) subcaste is an extension of the size distribution of the smallest workers, not a distinct caste.

Other authors have also observed the existence of three polymorphic castes in a subspecies of *A. subterraneus*. The reference authors [25] verified anatomical differences in the venom glands of workers with three sizes of cephalic

capsules in *A. s. subterraneus* and believe that this means the existence of three polymorphic castes in this subspecies – minimum, medium and maximum.

3- Main Remarks

- 1) Through the identification of subspecies by means of workers' morphology, it can be hypothesized that *A.s. molestans* is a different species. However, there is also a systematic problem between subspecies *A.s. brunneus* and *A. s. subterraneus*.
- 2) The morphometric analysis of workers' polymorphism showed that most of the coefficients were little significant between *A.s. subterraneus* and *A.s. brunneus*. On the other hand, in most comparisons made between subspecies of *A.s. subterraneus* and *A.s. molestans*, they were not significant or were inversely-proportional significant.

IV. CONCLUSION

Based on the morphometric analysis of characters, it is concluded that there is a systematic problem between subspecies *A.s. brunneus* and *A.s. subterraneus*, with *A.s. molestans* likely being another species.

ACKNOWLEDGMENT

L.C. Forti gratefully acknowledges the support of the National Council for Scientific and Technological Development [*Conselho Nacional de Desenvolvimento Científico e Tecnológico*] (CNPq) (Grant No. 301167/2003-6). We would like to thank the Coordination for the Improvement of Higher Education Personnel [*Coordenadoria de Aperfeiçoamento de Nivel Superior*] (CAPES) for the scholarship granted to R.S. Camargo.

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