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Mandibles of Leaf-Cutting Ants: Morphology Related to Food Preference

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Abstract

Feeding adaptation is crucial for the ecological success of animals, which explore specific or varied resources according to the suitability of the selected morphological feature. We hypothesized that the mandibles of leaf-cutting ants exhibit a specialization because of the specific food preference of some species. The objective of this study was to highlight possible morphological differences between the mandibles of leaf-cutting ant species of the genus *Atta* related to food preference, i.e., to investigate the morphofunctionality. A detailed description of the mandibles of workers of the grass-cutting ant *Atta bisphaerica* and of the leaf-cutting ant *Atta sexdens rubropilosa* was provided. For morphometric analysis, 50 individuals of four size classes were dissected for removal of the right mandible and one sample of each caste was processed for scanning electron microscopy. It was observed differences between species and among castes, demonstrating the clear specialization of workers. Although the results of mandibular morphometry accept the hypothesis of morphofunctionality, further investigation is needed taking into consideration the body size of foragers, robustness and factors that confer greater resistance to the mandibles such as zinc content, the force employed during cutting, and mandibular biomechanics.

Introduction

The mandibular morphology reflects the species' adaptations to particular foraging habits and social life (Gronenberg et al., 1997). Leaf-cutting ants (*Atta* and *Acromyrmex*), in particular, use different parts of plants for cultivation of their symbiotic fungus, with some species preferring dicotyledons and others grasses (monocotyledons) (Fernandez et al., 2015). Grass-cutting ants tend to have more massive and shorter mandibles, while the mandibles of leaf-cutting ants are longer and less massive (Fowler et al., 1986). Actually, comparing *Atta* species with these different plant preferences has demonstrated they had similar mandible morphology, primarily differing in distal tooth length, while *Acromyrmex* exhibited a higher variation in the mandible format as a whole (Camargo et al., 2015).

In general, the leaf blade of grasses contains abundant cells with lignified walls (Scheffer-Basso et al., 2002; Pelegrine et al., 2009), which renders them more resistant to cutting, as well as solid particles of hydrated silica (SiO_2nH_2O) found in the epidermal cells (Motomura et al., 2002). The accumulation of silicon has been associated with a variety of functions, including protection against fungal attacks, resistance to water loss, stimulation of photosynthesis, and structural support (Lux et al., 2003; Müller, 2003; Hattori et al., 2005). Moreover, silica plays an important role in the interaction between herbivores and plants. Massey et al. (2006a, 2006b, 2007, 2008, 2009) showed that silica accumulation in grasses increases leaf abrasiveness, causing the wear and deterioration of mandibles and impairing insect feeding.

In view of this previous knowledge, we hypothesized that the mandibular morphology of workers differs according to the plants preferentially cut by them. Therefore, this study compared the morphology of worker mandibles between castes (soldier, forager, generalist and gardener according to Wilson, 1980). It is expected that the mandible of grass-

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cutting ants is more massive and shorter, while the mandible of leaf-cutting ants would be longer and less massive.

Material and methods

Two leaf-cutting ant species, *A. s. rubropilosa* and *A. bisphaerica*, were used in this study. Field and laboratory colonies were used. For the collection of *A. bisphaerica* workers, a single fungus chamber of an adult nest was excavated on the Santana farm located near the Lageado Experimental Farm of UNESP, Botucatu, São Paulo, Brazil (geographic coordinates: 22º 50’ 46” S and 48º26’2” W). In this farm, the pasture was predominantly covered with *Paspalum* sp. with some patches of *Brachiaria* here and there. *A. s. rubropilosa* workers were obtained from an adult laboratory colony (10 years old, 50 liters of fungus garden). For morphometric analysis, 50 individuals of each caste were dissected for removal of the right mandible under a stereoscope: gardener (head width 0.8-1.2 mm; n=50), generalist worker (head width 1.3-1.6 mm; n=50), forager (head width 1.7-2.2 mm; n=50), and soldier (head width ≥ 3.3 mm; n=50). This caste determination was proposed by Wilson (1980, 1983). The observations were made on the ventral side of right mandibles.

The following measurements were made using a micrometer eyepiece coupled to a stereomicroscope: 1) length of the mandible; 2) width of the mandible; 3) length of the first tooth; 4) length of the second tooth; 5) number of teeth and 6) head width (mm).

To detect morphological differences between mandibles of the different ant species, samples of the right mandible of workers of all size classes were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, critical point dried, sputtered with gold (Robards, 1978), and examined by scanning electron microscopy (SEM) (FEI Quanta™ 200) at the Center of Electron Microscopy, Institute of Biosciences (Centro de Microscopia Eletrônica do Instituto de Biociências – CME-IBB), UNESP, Botucatu, SP. For determination of the occurrence of wear, the mandible of an *A. s. rubropilosa* forager was collected 1 day after emergence and submitted to the same procedures for SEM analysis.

We analyzed the data and verified that all variables weren’t normally distributed (Shapiro-Wilk normality test), being thus log-transformed. Principal Component Analysis (PCA) was used to examine variation in the morphometric data among species using the free software PC-ORD 5.15 (McCune & Mefford, 2011), with exception of the number of teeth. The number of principal components (PCs) was determined considering eigenvalues higher than those generated by the Brokenstick method. The morphometric measurements that most contributed to the axes, were selected by Pearson ($r > 0.7$). After that, we performed an ANOVA comparing the PCA scores with Tukey HSD post-test (α=0.05), to point out the differences among the variables.

Results

We found differences between species and among castes (ANOVA, $F_{7;391}=455.2$, $P<0.001$). All castes differed among them, except between soldiers from *A. s. rubropilosa* and *A. bisphaerica* (P=0.31) and *A. bisphaerica* generalist workers with *A. s. rubropilosa* foragers (P=0.11) (Table 2).

PCA analysis found that the first principal component explains 91.5% of the variation. Also it is strongly correlated with the four morphometric variables. The first principal component increases with decreasing length mandible (r= -0.96), width mandible (r= -0.96), length of the first teeth (r= -0.98) and length of the second teeth (r=-0.93). This suggests that these four morphometric measures vary together. If one decreases, then the remaining also decreases. It would follow that species and castes would tend to be grouped due to their mandible features, in terms of the morphometric measurements (Figure 1).

Differences were also detected within the different castes of each species. Soldiers of the two species had larger and more robust mandibles compared to the other castes. Mandibular morphology of foragers although similar to that of soldiers, were smaller mandibles and with teeth more worn (Figure 6-7). The mean number of teeth was 7 or 8 for *A. bisphaerica* and 7 to 9 for *A. s. rubropilosa* (Table 1). Tooth

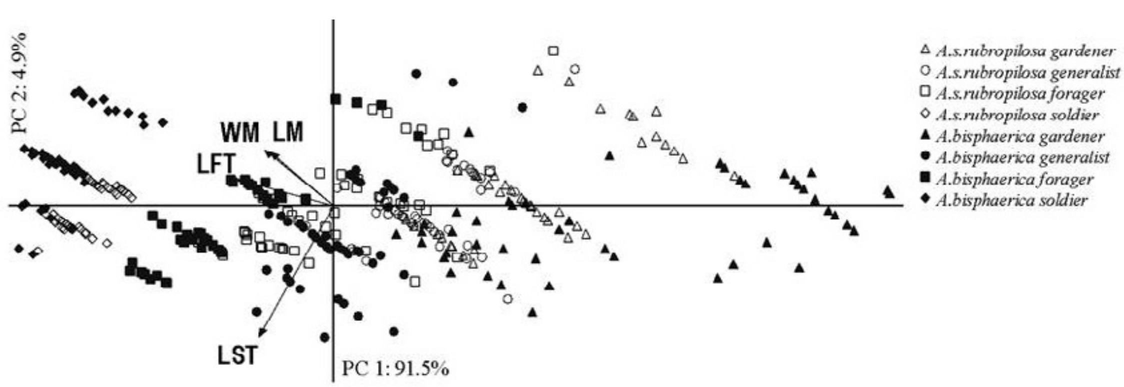


Fig 1. Principal components analysis of mandible morphometric data of *Atta sexdensrubropilosa* and *Atta bisphaerica* in each worker caste for the variables width mandible (WM), Length mandible (LM) Length of the fisrt tooth (LFT) and length of the second tooth (LST) First two principal components (PCs) are plotted with the proportion of variance explained by each component printed next to the axes labels.

Table 1. Morphometric measures (mean and standard deviation) (mm) of the mandibles and teeth of *Atta bisphaerica* and *Atta sexdens rubropilosa*.

Ant species	Caste	Head width	Mandibular length	Mandibular width	Length of 1st tooth	Length of 2nd tooth	Number of teeth
Atta bisphaerica	Gardener	0.76±0.20	0.40±0.10	0.25±0.09	0.08±0.06	0.04±0.02	10.14±2.12
	Generalist	1.29±0.10	0.67±0.06	0.46±0.04	0.20±0.04	0.09±0.03	7.92±0.73
	Forager	2.13±0.19	0.99±0.08	0.70±0.06	0.26±0.05	0.13±0.04	7.78±0.79
	Soldier	4.79±0.43	1.58±0.08	1.11±0.07	0.49±0.07	0.14±0.03	7.78±0.68
Atta sexdens Rubropilosa	Gardener	0.89±0.13	0.51±0.10	0.30±0.06	0.10±0.03	0.05±0.02	9.46±1.15
	Generalist	1.32±0.08	0.65±0.06	0.40±0.05	0.13±0.03	0.07±0.02	8.84±1.08
	Forager	2.02±0.18	0.88±0.11	0.52±0.07	0.17±0.05	0.09±0.03	8.36±1.05
	Soldier	3.79±0.23	1.54±0.07	0.94±0.05	0.41±0.04	0.17±0.03	8.64±0.80

wear was observed when adult foragers of undetermined age (Figure 10) were compared with an adult on day 1 after hatching (Figure 11). As can be clearly seen in Figure 10, the teeth of probably older foragers were slightly rounder, probably due to wear out, when compared to the naive forager worker (1-day old) (Figure 11), in which a larger number of teeth and more pointed teeth can be seen.

Generalist workers of *A. bisphaerica* had more elongated mandibles when compared to *A. s. rubropilosa* (Figure 4-5) and presented no tooth wear. However, Figure 4 shows wear out of the mandible of generalists *A. bisphaerica* workers.

The elongated mandibles of *A. bisphaerica* were also observed in gardeners of this species (Figure 2-3). No tooth wear was observed in this caste of the both species. Gardeners had a larger number of teeth and more pointed teeth than the other castes analyzed (Table 1).

In all castes of the two species the teeth had a serrated appearance characterized by a progressive reduction in their size. The main difference between species was the larger size of the distal tooth in *Atta bisphaerica*.

These results demonstrate that the mandible morphology is different between species and within castes. However, a difference within castes seems to be found at the same features in both species.

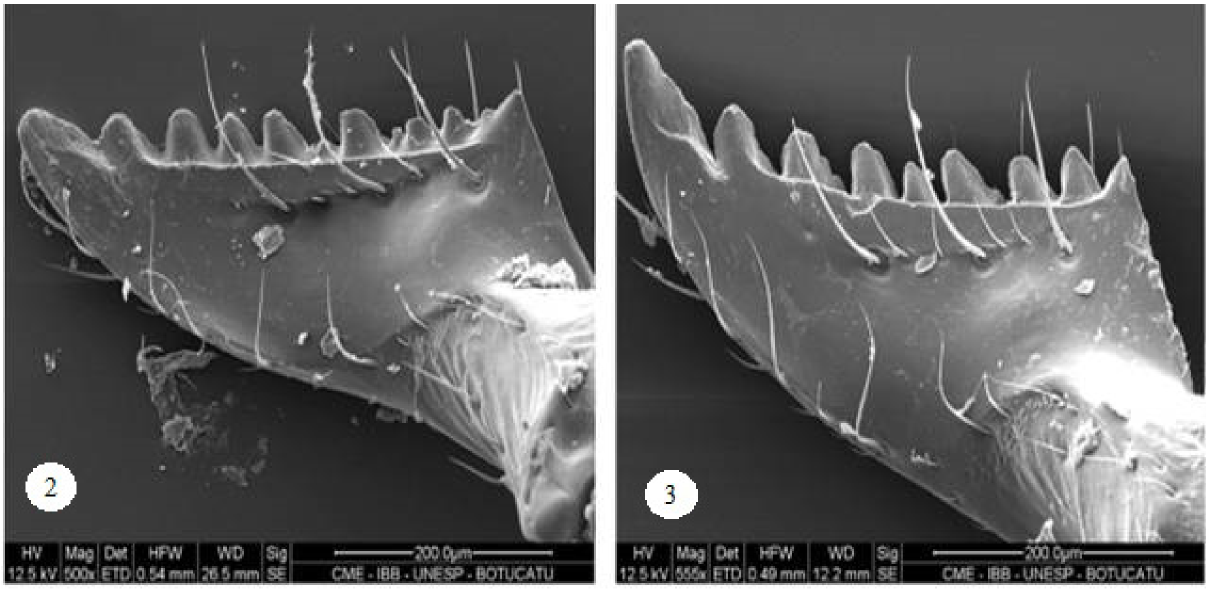
Discussion

Leaf-cutting ants in general (*Atta* and *Acromyrmex*) use different parts of plants for cultivation of the symbiotic fungus. Some species prefer dicotyledons, while others prefer grasses (monocotyledons) (Fernandez et al., 2015), a fact raising the hypothesis of a greater degree of specialization in view of the specific food preferences of these species. The results of the present study accept the hypothesis of morphofunctionality of leaf-cutting ant (*A. s. rubropilosa*) and grass-cutting ant mandibles (*A. bisphaerica*) is related to food preference. This hypothesis was raised by Fowler et al. (1986) based on the morphological differences observed in these groups. In this respect, in species that preferentially cut grasses the foragers tend to have shorter mandibles compared to foragers of leaves

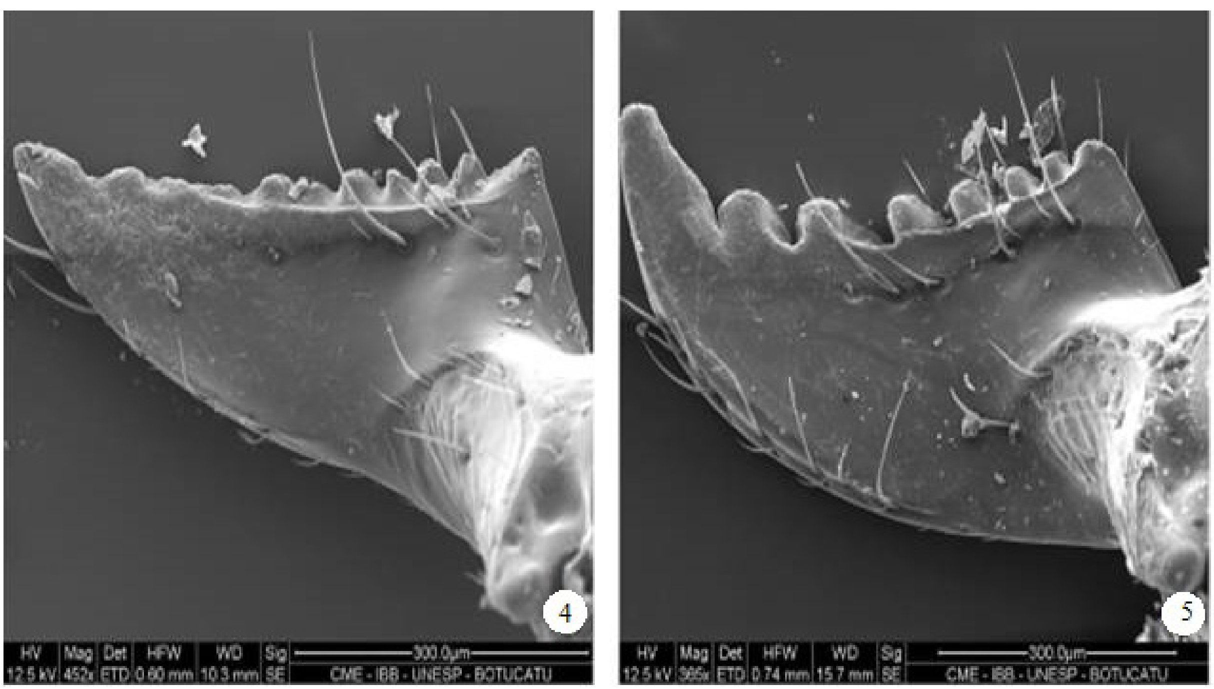
Table 2. Statistical summary of multiple comparison by Tukey HSD (α=0.05). SS: *A. s. rubropilosa* soldiers; FS: *A. s. rubropilosa* foragers; GS: *A. s. rubropilosa* generalist workers; GS: *A. s. rubropilosa* gardeners; SB: *A. bisphaerica* soldiers; FB: *A. bisphaerica* foragers; GB: *Atta bisphaerica* generalist workers; GB: *A. bisphaerica* gardeners.

Comparisons	Mean difference	Confidence Intervals Lower Bound	Confidence Intervals Upper Bound	P- value adjusted
FS-JS	0.606	0.491	0.720	< 0.0001
FS-SB	-0.756	-0.870	-0.641	< 0.0001
FS-SS	-0.670	-0.785	-0.555	< 0.0001
GS-GB	0.173	0.058	0.288	0.0001
JB-GB	0.742	0.627	0.857	< 0.0001
JS-GB	0.502	0.386	0.617	< 0.0001
SB-GB	-0.860	-0.975	-0.745	< 0.0001
SS-GB	-0.774	-0.889	-0.659	< 0.0001
JB-GS	0.569	0.454	0.684	< 0.0001
JS-GS	0.328	0.214	0.443	< 0.0001
SB-GS	-1.033	-1.148	-0.918	< 0.0001
SS-GS	-0.947	-1.062	-0.833	< 0.0001
JS-JB	-0.240	-0.355	-0.125	< 0.0001
SB-JB	-1.603	-1.717	-1.488	< 0.0001
SS-JB	-1.517	-1.632	-1.402	< 0.0001
SB-JS	-1.362	-1.477	-1.247	< 0.0001
SS-JS	-1.276	-1.391	-1.162	< 0.0001
SS-SB	**0.085**	**-0.029**	**0.200**	**0.3097**
FS-FB	0.253	0.139	0.368	< 0.0001
GB-FB	0.357	0.242	0.473	< 0.0001
GS-FB	0.531	0.416	0.645	< 0.0001
JB-FB	1.100	0.985	1.215	< 0.0001
JS-FB	0.859	0.745	0.974	< 0.0001
SB-FB	-0.502	-0.617	-0.387	< 0.0001
SS-FB	-0.416	-0.531	-0.302	< 0.0001
GB-FS	**0.104**	**-0.011**	**0.219**	**0.1096**
GS-FS	0.277	0.162	0.392	< 0.0001
JB-FS	0.846	0.732	0.961	< 0.0001

*Significant difference at 95% family-wise confidence level by Tukey HSD test.



Figs 2-3. ventral side of right mandibles of *Atta bisphaerica* (2) and *Atta sexdens rubropilosa* (3) gardeners.



Figs 4-5. Ventral side of right mandibles of *Atta bisphaerica* (4) and *Atta sexdens rubropilosa* (5) generalist workers.

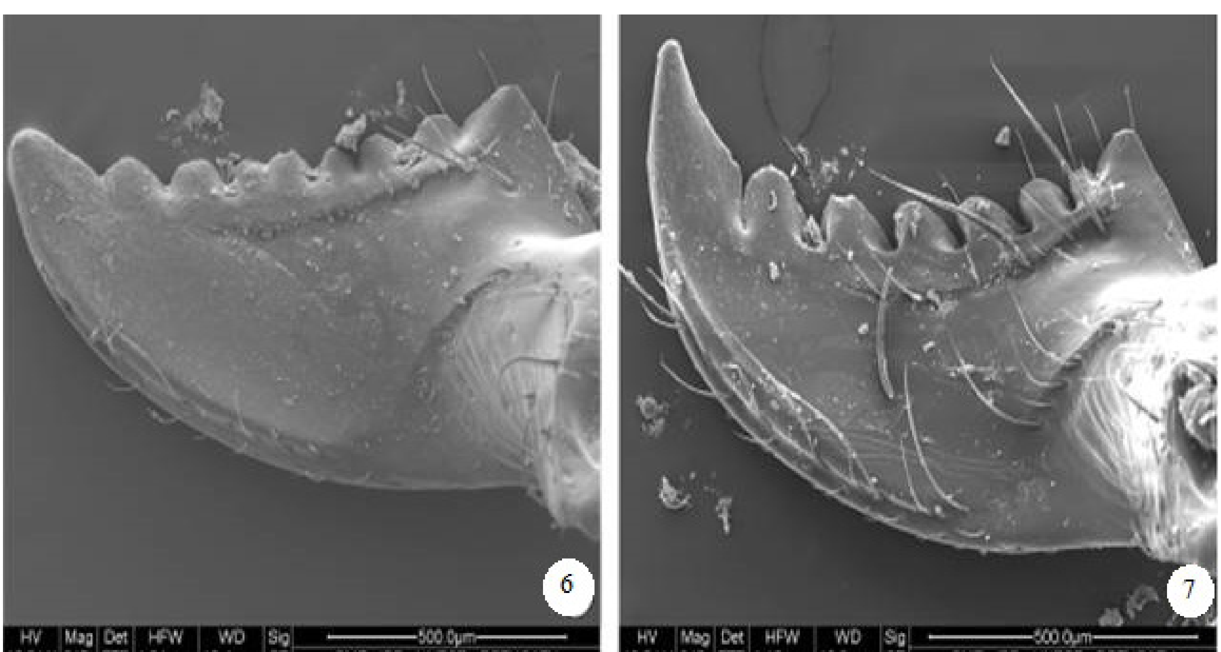
which tend to have less massive mandibles. As a consequence, these foragers cut plants differently. For example, during foraging *Atta capiguara* assumes a downward position while cutting the leaf blade of grasses, performing the cut and immediately returning to the nest. In contrast, in leaf-cutting ants the metathoracic legs serve as a pivot during cutting and their mandibles slide across the leaf blade. Behavioral differences between grass-cutting and leaf-cutting ants are also observed during cultivation of the symbiotic fungus (Lopes, 2004). Leaf-cutting ants exhibit a higher degree of processing of the foraged material that resembles grinding before incorporation of the symbiotic fungus. In contrast, in the case of grass-cutting ants the degree of processing is lower, i.e., the leaf blades are just arranged on the fungus garden and the symbiotic fungus is subsequently incorporated (Lopes, 2004). This behavior is probably related to the greater difficulty in processing the material due to the higher proportion of lignified tissues in grass leaves (Lopes, 2004).

In the present study, mandible morphometry was useful to discriminate between species and castes (Figure 1). These results are possibly related to the recent evolutionary history of the two species. The genus *Atta* is monophyletic (Bacci et al., 2009), with relatively recent adaptive radiation about 8 to 13 million years ago (Brady et al., 2006). However, the identification of adaptive radiation is based on the phylogenetic relationships of species and not on the dating of known fossils of *Atta*, which are scarce for the genus. There is only one trace fossil of an *Atta* nest from the Miocene located in Patagonia, Argentina (Laza, 1982).

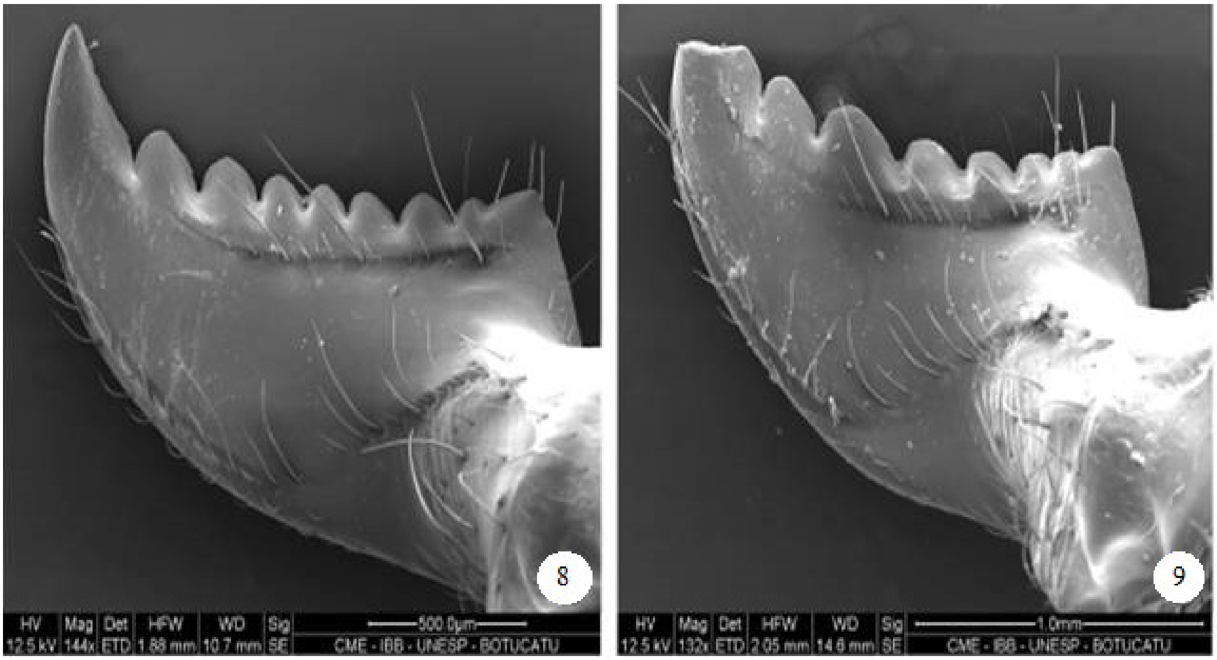
This intimate phylogenetic relationship between the species studied (*A. s. rubropilosa* and *A. bisphaerica*) and their recent evolutionary history explain the morphological differences of the mandibles, at least in terms of the features evaluated (Table 1). These species are widely distributed in the Neotropical region and differ in nesting site, food preference and behavior. The grass-cutting ant *A. bisphaerica* is only found in Brazil (Wilson, 1986), particularly in the states of São Paulo, Minas Gerais, Rio de Janeiro and Mato Grosso do Sul (Forti & Boaretto, 1997). In contrast, *Atta sexdens* and its subspecies are distributed from Costa Rica to Argentina and Paraguay (Wilson, 1986), with *A. s. rubropilosa* occurring in the midwestern and southeastern regions of Brazil (Forti & Boaretto, 1997). The nesting site is a result of the biome. In this respect, *A. bisphaerica* nests are shallow and are generally found in open areas such as pastures characterized by a high incidence of insolation, while *A. s. rubropilosa* nests are relatively deep and are only found in shadow areas, such as native and eucalyptus forests (Forti et al., 2011).

Morphological differentiation between castes was observed in both species studied (Figures 2-3, 4-5, 6-7, 8-9, and 10-11) and was supported by the separation of species and grouping of the same castes by PCA analysis, using the mandible morphometric data. Gardeners are the smallest workers which are always found inside the nest and in larger quantities. The typical tasks attributed to this set of workers occur exclusively inside the nest, including brood care, final treatment of the substrate and hyphal inoculation into the fragments incorporated into the fungus garden (Wilson, 1983). Their mandibles are small in *A. bisphaerica* they are more elongated compared to the other castes since the tasks of gardeners are the most laborious and precise, with their teeth showing no wear (Figure 2-3). Generalist workers perform a relatively larger number of tasks that range from the elimination of residues from the colony and caring for the brood and queen to tasks related to cultivation of the symbiotic fungus (Wilson, 1983). The mandibles of *A. bisphaerica* generalists are visibly more elongated than those of *A. s. rubropilosa*, probably to facilitate the grass blades manipulation (Figure 4-5). Gardeners and generalists are the predominant size classes in the population of a leaf-cutting ant colony and are responsible for a large number of tasks related to preparation of the plant material during the process of incorporation (Peregrine & Cherrett, 1974, 1976; Littledyke & Cherrett, 1976). The elongated mandibular morphology observed only in *A. bisphaerica* gardeners and generalists might be related to the way grasses are processed. The leaf blades of grasses are not chopped or processed before incorporation as observed for dicotyledon leaf fragments which have a pulp-like appearance after processing by workers. According to Lopes et al. (2004), the latter processing facilitates the implantation of hyphal tufts.

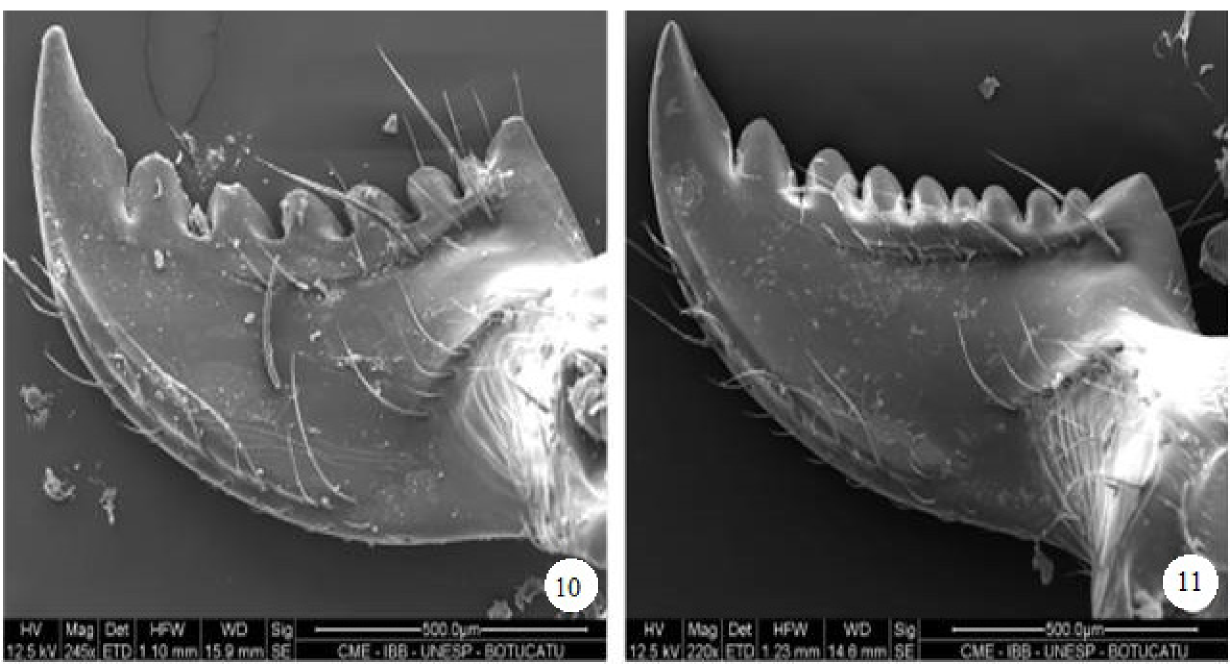
On the other hand, the function of foragers is to explore, to excavate, to recruit other ants, and mainly to cut new plant material at field (Wilson, 1983), i.e., in both species studied these workers are not involved in the more laborious activities of plant processing, but rather in the exploration and cutting of plant material. As a consequence, the mandibles of these workers exhibited more marked tooth wear (Figure 6-7) reflecting the repetitive cutting effort. This assumption is mandibles of different age workers (Figure 10-11). Finally, the task attributed to soldiers is the defense of the nest (Wilson, 1983), an activity that requires larger and more robust mandibles (Figure 8-9) and is not related



Figs 6-7. ventral side of right mandiblesof *Atta bisphaerica* (6) and *Atta sexdensrubropilosa* (7) foragers.



Figs 8-9. ventral side of right mandibles of *Atta bisphaerica* (8) and *Atta sexdens rubropilosa* (9) soldiers.



Figs 10-11. ventral side of right mandibles of *Atta sexdens rubropilosa* foragers at different ages, showing natural wear due to foraging activity. Adult of undetermined age (10) and adult one day after hatching (11).

with plant manipulation, explaining why soldier mandibles were not different between species. Like foragers, this group is not involved in the processing of plant material and no morphological differentiation between species is therefore observed. Also, *A. bisphaerica* generalist mandibles did not differ from *A. s. rubropilosa* forager mandibles suggesting that task allocation between species must be different. Actually, task allocation is different between species in function of which abilities the task require.

The mandibles of ants have many functions, such as catching prey, fight, cutting leaves, brood care, and communication (Paul, 2001). Although an important tool for many tasks (Hölldobler & Wilson, 1990), there is little information on the morphology of ant mandibles and no quantitative data regarding mandibular differences are available. The specialization of mandibles does not only depend on their morphology, but is also related to the velocity of movement and the force generated by them. For example, catching prey obviously requires movement characteristics that differ from those necessary for cracking seeds (Paul, 2001). Thus, the veins patterns of grasses and dicotyledons and the greater abundance of lignified cells in grasses should also influence the specialization of mandibles. Although the results of mandibular morphometry accept the hypothesis of morphofunctionality, further investigation is needed taking into consideration the body size of foragers, robustness and factors that confer greater resistance to the mandibles such as zinc content, the force employed during cutting, and mandibular biomechanics.

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