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Mites (Arachnida, Acari) on *Astronium fraxinifolium* Schott (Anacardiaceae) from the Cerrado remnants associated with nickel mining areas

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Abstract

The Cerrado biome suffers constant impacts mainly due to agricultural activities, which can reduce food resources and habitats for many plant-dwelling mites, including important species to agriculture, such as predators. However, the mite fauna from this biome are still poorly known. Here, we present a checklist of mite species on *Astronium fraxinifolium* Schott, a Brazilian plant species threatened with extinction, from the Cerrado remnants associated with nickel mining areas, in the Niquelândia municipality, Brazil. Moreover, we performed cumulative (Mao Tau) and estimated (Jackknife 1) species accumulation curves in order to test for an effect of sampling effort and to estimate the potential number of species sheltered by the studied Cerrado remnants, and NMDS and ANOSIM analyses to test for similarity in mite species composition among samples. We sampled five *A. fraxinifolium* plants in each of six Cerrado remnants. Among these, three remnants were preserved (PR) and three were experiencing a secondary regeneration process (SR). Both PR and SR remnants were close to nickel mining areas. We recorded 1,562 mites including 17 species from 12 genera and eight families. Tetranychidae was the most diverse family, followed by Phytoseiidae and Tenuipalpidae. The most abundant species were phytophagous mites, namely *Brevipalpus* sp.1, *Oligonychus* sp., *Eotetranychus* sp.1, and the predator *Agistemus brasiliensis* Matioli, Ueckermann & Oliveira. Regarding feeding behavior, phytophagous mites were the most abundant and diverse on *A. fraxinifolium*. Both species accumulation curves (Mao Tau) for PR and SR remnants trended towards an asymptote, while estimated curves (Jackknife 1) proved to be similar to accumulation curves (Mao Tau). These results indicate that sampling was sufficient to assess mite assemblages using the methods applied in this study. No differences in mite species composition were observed between PR and SR areas. This paper is a pioneer report of mite assemblages on *A. fraxinifolium*. Furthermore, we report here two genera and one species recorded for the first time in the Cerrado biome. Our checklist can contribute to bridging the knowledge gap on the occurrence of plant mite species in Brazilian natural vegetation remnants.

Introduction

Brazilian Savannah, referred as Cerrado, is the second largest Brazilian biome in range. However, the ecosystems that comprise the Cerrado biome have been rapidly destroyed mainly due to agriculture expansion, urbanization, industrial activities and native resource exploitation. Estimates indicate that 39 to 55 % of Cerrado original coverage has been modified into agricultural fields, such as soybeans, corn, sugarcane and pasture for livestock (Machado *et al.* 2004, Nepstad *et al.* 1997, Sano *et al.* 2008). According to Dobrovolski *et al.* (2011), the agriculture area in Cerrado biome is going to increase from 38.2 to 50.4 % in 2100. These intensive modifications have caused several negative impacts on Cerrado like habitat fragmentation, biodiversity losses, and invasion by exotic species. At least 137 animal species from Cerrado are threatened to extinction (Fundação Biodiversitas 2003, Hilton-Taylor 2004). Furthermore, important ecosystem services provided by natural vegetations to agriculture could have been reduced as a consequence of these modifications; mainly pollination (Yamamoto *et al.* 2012) and natural pest control (Rezende *et al.* 2014). Some investigations suggest Cerrado remnants as important shelters in keeping natural enemies of pests, such as predatory mites and parasitoid insects, and the benefit in conserving natural vegetation in crop-yielding areas for improving natural pest control (Demite and Feres 2008, 2007a, b, 2005, Demite *et al.* 2009, Giannetti *et al.* 2011, Harterreiten-Souza *et al.* 2014, Rezende *et al.* 2014).

Although studies on Cerrado fauna and flora have been frequent and ongoing, many arthropod species are still poorly known within this biome, such as plant mites. So far, plant mites from Cerrado remnants were evaluated by Demite *et al.* (2009), Demite & Feres (2008), Lofego & Moraes (2006), Lofego *et al.* (2005) and Flechtmann (1967) at São Paulo and Mato Grosso States, Brazil. Rezende and Lofego (2011), in turn, sampled 26 phytoseiid species on 57 Cerrado native plant species from Midwest Brazil. Yet, the importance of Cerrado remnants in keeping natural enemies was verified by Rezende *et al.* (2014). According to these authors, all phytoseiid mites found in soybean plants were also recorded in Cerrado remnants suggesting a possible dispersion of these mites from natural vegetation to crops. Some phytoseiid species are predators recognized as important pest biocontrol agents in several crops (Gerson *et al.* 2003).

Nevertheless, whether Cerrado areas conversion to agriculture persist, as estimated by Dobrovolski *et al.* (2011), many mite species could disappear, including unknown taxa and species with potential economic value like predator mites. Therefore, studies aiming to assess mite assemblages on native plants from Cerrado are necessary in order to reduce knowledge gap concerning these arthropods in their natural environment. Here, we sampled mites on *Astronium fraxinifolium* Schott (Anacardiaceae), a threatened plant species (IBAMA 1992), from Cerrado vegetation remnants associated with nickel mining areas. Furthermore, we compared mite fauna composition between more conserved vegetation remnants and remnants previously exploited for nickel mining in order to assess whether the impact of these activities alters plant mite assemblage structure. We expected that areas previously exploited for mining present distinct species composition relative to more conserved remnants.

Material and methods

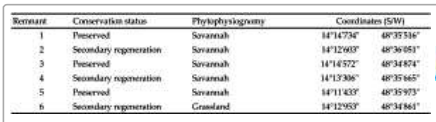
Target plant species

Astronium fraxinifolium (Anacardiaceae) is a native plant species from Cerrado biome and popularly known as "gonçalo-alves". This is a medium size arboreous species (Lorenzi 1992), being usually found in human altered environments, such as highway edges or forestry

remnants (Aguiar *et al.* 2001). This is a pioneer, heliophytic species which presents great economic value since its wood can be used for furniture manufacturing; besides its use for programs to recovering natural degraded areas. This plant species was selected as target plant due to its abundance and common occurrence in all Cerrado remnants evaluated. Although it showed high abundance in Cerrado remnants studied, *A. fraxinifolium* was classified as a threatened species according to IBAMA ordinance n. 37-N, April 03 1992 (IBAMA 1992).

Field samples

Six Cerrado remnants belonging to the mining Enterprise Anglo-American Brazil, Niquelândia unit, Goiás State, Brazil, were sampled during May 2012 (Table 1). Among these remnants, three were more preserved (PR) and the other three experienced secondary regeneration process (SR). The SR remnants were previously explored for nickel mining in the past as opposed to the most preserved PR remnants. The nickel mines were closed for SR areas about 30 years ago. Moreover, the PR phytophysiognomy are savannah formations while the SR remnants presented more sparse trees and grassland formation with transition to savannah (Table 1). Both SR and PR remnants were located close to mining areas, at most, one kilometer away from these sites.



Remnant	Conservation status	Phytophysiognomy	Coordinates (S/W)	
1	Preserved	Savannah	14°14'734"	48°35'536"
2	Secondary regeneration	Savannah	14°12'603"	48°36'051"
3	Preserved	Savannah	14°14'572"	48°34'874"
4	Secondary regeneration	Savannah	14°13'306"	48°35'665"
5	Preserved	Savannah	14°11'420"	48°35'973"
6	Secondary regeneration	Grassland	14°12'953"	48°34'861"

Table 1. Geographic coordinates, phytophysiognomy and conservation status of six Cerrado remnants belonging to Anglo-American Enterprise, Niquelândia, Goiás State, Brazil.

Mite sampling

We selected five *A. fraxinifolium* individuals from each remnant, totaling 30 plants evaluated (n = 15 for PR and RS in each). For mite sampling, we extracted 10 leaves around the canopy from each selected plant using pruning shears. The extracted leaves were immediately inserted into 1 L vials (one vial separately per plant) containing about 200 mL of 70 % alcohol. Then, the vials were vigorously shaken for 30 seconds in order to wash the leaves thoroughly and, after this, they were left to rest during five minutes. Next, the leaves were carefully removed and the vials were labeled. In this way, each vial represented mite assemblage for each selected plant.

In the lab, each sample was transferred to a sedimentation glass and left to rest during 15 minutes. After this period, we discarded the superficial liquid (about 50 mL) and the sample was transferred slowly to Petri dishes to be observed under dissecting microscope. All mites found were mounted on microscope slides with Hoyers medium (Krantz and Walter 2009). The mites were identified and counted under phase contrast microscope.

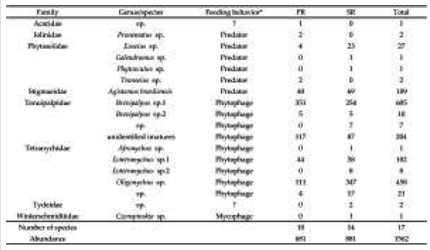
Data Analysis

We elaborated species accumulation curves using Mao Tau to determine the chance of recording new species with increased sampling effort. Also, we performed Jackknife 1 to estimate species richness for both PR and SR remnants. Jackknife 1 is employed to estimate the theoretical species richness in a habitat (Santos 2003, Heltshe and Forrester 1983). Both Mao Tau and Jackknife were calculated with software EstimateS v. 7,51, using 500 randomizations (Colwell 2006). Estimate species richness (Jackknife 1) was compared to accumulation curves (Mao Tau) by graphical analysis, checking the overlap of error bars to the mean (Cumming *et al.* 2007).

Also, we applied non-metric multidimensional scaling (NMDS), using Simpson matrix, in order to compare mite fauna composition on *A. fraxinifolium* between PR and SR remnants. The significance of NMDS groups was tested through Analysis of Similarity (ANOSIM) adopting alpha < 0,05 (Clarke 1993).

Results

We recorded 1,562 mites from 17 species in 12 genus and eight families on *A. fraxinifolium* plants from Cerrado remnants belonging to Anglo-American Enterprise. Among the mites sampled, only one species was determined up to species level, namely *Agistemus brasiliensis* Matioli, Ueckermann & Oliveira (Stigmaeidae), 12 up to genus level and four were unidentified species (Table 2). Tetranychidae was the most diverse family with five species, followed by Phytoseiidae and Tenuipalpidae with four and three species, respectively. Only one species was recorded in the other families. The families with highest number of individuals collected were Tenuipalpidae, Tetranychidae and Stigmaeidae (Table 2).



Family	Genus/species	Feeding behavior*	PR	SR	Total
Acarida	sp.	?	1	0	1
Ichnidae	*Pronematus* sp.	Predator	2	0	2
Phytoseiidae	*Iphiseius* sp.	Predator	4	23	27
	Galendromus sp.	Predator	0	1	1
	Phytoseius sp.	Predator	0	1	1
	Typhlodromus sp.	Predator	2	0	2
Stigmaeidae	*Agistemus brasiliensis*	Predator	40	69	109
Tenuipalpidae	*Brevipalpus* sp.1	Phytophage	351	294	645
	Brevipalpus sp.2	Phytophage	5	5	10
	sp.	Phytophage	0	7	7
	unidentified immatures	Phytophage	117	87	204
Tetranychidae	*Afonychus* sp.	Phytophage	0	1	1
	Eotetranychus sp.1	Phytophage	44	98	142
	Eotetranychus sp.2	Phytophage	0	9	9
	Oligonychus sp.	Phytophage	111	347	458
	sp.	Phytophage	4	17	21
Tydeidae	sp.	?	0	2	2
Hybozoberoididae	*Czenspinskia* sp.	Mycophage	0	1	1
Number of species			10	14	17
Abundance			891	881	1562

Table 2. Mites sampled on *A. fraxinifolium* plants from preserved (PR) and secondary regeneration (SR) Cerrado remnants belonging to Anglo-American Enterprise, Niquelândia municipality, Goiás State, Brazil. * Feeding behavior: Predator (Krantz and Walter 2009, Ferla &

Moraes 2003, Gerson *et al.* 2003, Moraes and Fletchamnn 2008; McMurtry and Croft 1997), Phytophage (Krantz and Walter 2009, Moraes and Flechtmann 2008) and Mycophage (Krantz and Walter 2009, Baker and Wharton 1952).

The most abundant species were the phytophagous mites *Brevipalpus* sp.1 (Tenuipalpidae), *Oligonychus* sp., *Eotetranychus* sp.1 (Tetranychidae), and the predator *A. brasiliensis*. Other less abundant species were predators *Pronematus* sp. (Iolinidae), *Euseius* sp., *Galendromus* sp., *Phytoscutus* sp. and *Transeius* sp. (Phytoseiidae), phytophages *Brevipalpus* sp.2 (Tenuipalpidae), *Afronychus* sp., and *Eotetranychus* sp.2 (Tetranychidae), mycophage *Czenspinskia* sp. (Winterschmidtiidae) and one unidentified species for each Acaridae, Tenuipalpidae, Tetranychidae and Tydeidae families (Table 2).

Regarding feeding behavior, phytophages were the most abundant and diverse on *A. fraxinifolium*, since they presented 1,212 individuals and eight species. For predator mites, we sampled 142 individuals and six species, while only one individual of a mycophage species (*Czenspinskia* sp.) was found. Three individuals from two species had unknown behavior.

Both accumulation curves (Mao Tau) determined for PR and SR remnants tend to an asymptote, indicating that enough sample efforts to assess mite assemblage on *A. fraxinifolium* were performed. Moreover, the estimated richness (Jackknife 1) proved to be similar to the accumulation curve, since their confidence interval bars overlap with Mao Tau mean for both PR and SR remnants (Figure 1A and B). According to ANOSIM, no differences in mite species composition between PR and SR remnants were observed (ANOSIM, r = -0.015; p = 0.64), suggesting a high overlap in fauna composition on *A. fraxinifolium* (Figure 2).

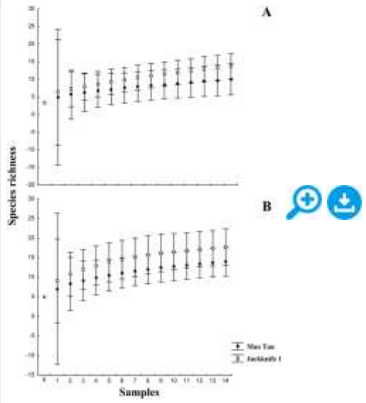


Figure 1. Accumulation (Mao Tau) and estimated richness (Jackknife 1) curves determined for Cerrado remnants belonging to Anglo-America Enterprise, Niquelândia, Goiás State, Brazil. (A) preserved (PR) and (B) secondary regeneration (SR) remnants.

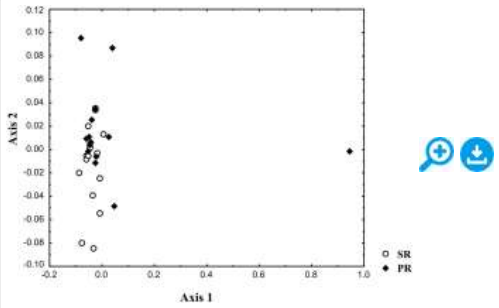


Figure 2. Results of Non-metric multidimensional scaling (NMDS), using Simpson matrix, applied to test similarity in mite fauna composition on *A. fraxinifolium* between preserved (PR) and secondary regeneration (SR) remnants.

Discussion

Despite being directly influenced by nickel mining activities, Cerrado remnants shelters substantial mite richness and abundance since we collected 17 mite species on a single host plant in a unique sample event (in March 2012). Both PR and SR remnants showed similar patterns in mite species occurrence, as suggested by NMDS and ANOSIM.

The mite genera recorded in this paper had been already sampled in Cerrado remnants from São Paulo, Goiás and Mato Grosso States by previous authors (Rezende *et al.* 2014, Rezende and Lofego 2011, Demite *et al.* 2009, Demite and Feres 2008, Lofego and Moraes 2006, Lofego *et al.* 2005 and Aranda 1974), except for genera *Phytoscutus* (Phytoseiidae) and *Afronychus* (Tetranychidae), which were recorded here for first time in Brazil Midwest Cerrado. Some of the genera sampled were also recorded in other Brazilian natural vegetations, such semi-deciduous forest remnants from São Paulo State, namely *Agistemus*, *Brevipalpus*, *Euseius*, *Eotetranychus*, *Galendromus*, *Pronematus* and *Transeius* (Demite *et al.* 2013, Buosi *et al.* 2006 and Demite and Feres 2005).

Agistemus brasiliensis was the only taxa identified up to species level and it is reported here for first time to Cerrado Biome on a Brazilian native plant. This species was only previously reported in agroecosystems, like citrus (Matioli *et al.* 2002) and vineyards (Johann *et al.* 2013) crops, in Brazil. Only Rezende *et al.* (2014) recorded another species from the same genus on native plants from Cerrado remnants, namely *Agistemus floridanus* Gonzalez.

Astronium fraxinifolium plants showed great abundance of phytophages, sheltering eight species of these mites. Among them, Tenuipalpidae and Tetranychidae were the most representative, highlighting species from *Brevipalpus* and *Oligonychus* genera. Some species of these genera presents agricultural interest since they can act as pest on crops (Moraes and Flechtmann 2008). Some *Brevipalpus* species can transfer viruses to their host plants. In Brazil, *Brevipalpus phoenicis* (Geijskes) is considered an important pest because it can infect citrus plants with leprosis viruses as well as coffee plants with ring spot viruses (Moraes and Flechtmann 2008, Reis *et al.* 2004). *Oligonychus* species can affect the photosynthesis rate on their host plant during feeding which hinders yield, depending on the species (Moraes and Flechtmann 2008). However, the phytophagous mites sampled in this work, including *Brevipalpus* and *Oligonychus* species, probably do not threaten neighboring agriculture to vegetation remnants. We provide three arguments that substantiate this hypothesis: (i) *Brevipalpus* species were confirmed by taxonomists and both *Brevipalpus* sp.1 and sp.2 are probably undescribed species (RJF Feres, pers. com.). (ii) Phytophages from Cerrado plants presumably do not disperse from natural vegetation to the crops as suggested by field experiment performed by Rezende *et al.* (2014). According to these authors, no phytophagous mite species from Cerrado remnants were sampled in soybean plants from Brazilian Midwest crops neighboring natural vegetation. In contrast, some predator mites collected in Cerrado remnants were also sampled in soybean crops which suggest possible dispersion of these beneficial arthropods from natural vegetation to the nearby monoculture (Rezende *et al.* 2014). (iii) Native plants probably cannot support pest mite development and reproduction. In a review concerning plant mite species from Brazil, Araújo and Daud (submitted) verified that some important pest mites were sampled on native plants from Cerrado and Atlantic forest remnants, such as *Tetranychus urticae* Koch and *B. phoenicis*, for example. However, these phytophagous species were always collected in low numbers on native plants, both in the Cerrado and the Atlantic forest, which suggest low suitability of native plants as food for these pests. Nevertheless, other experiments must be performed to test phytophagous mite dispersion from natural remnants to nearby crops in order to verify whether Cerrado remnants threaten or not agriculture yield.

Additionally, *A. fraxinifolium* plants showed considerable richness and abundance of predator mites. *Agistemus brasiliensis* was the most abundant predator; however, Phytoseiidae exhibited the most species richness among them. Species from Stigmaeidae and Phytoseiidae families demonstrate potential to be used as pest biocontrol agents since many previous experimental assays suggested the great capacity of these mites in preying on phytophagous mites and insects (e.g. Ferla & Moraes 2003, Gerson *et al.* 2003, Nomikou *et al.* 2001, Furtado & Moraes, 1998). Matioli and Oliveira (2007) evaluated *A. brasiliensis* biological cycle at different temperatures and observed acceptableness of the predator in feeding on *B. phoenicis*, which suggest the importance of *A. brasiliensis* as a natural enemy on this relevant citrus pest.

The results of accumulation (Mao Tau) and estimated richness (Jackknife 1) curves indicated enough effort to assess mite assemblage on *A. fraxinifolium* through sample methods applied in this study. Furthermore, the probability of sampling new mite species according to sample effort increase could be low for both SR and PR remnants. These curve patterns differ from other studies performed in natural vegetation remnants (Feres *et al.* 2007 and Walter and Proctor 1998) where the authors did not find asymptote for accumulation species curve, suggesting high probability of natural vegetation in harboring higher mite diversity. However, both PR and SR Cerrado remnants receive similar and frequent impact from nickel mining activities given their proximity to the mining enterprise. Therefore, it is expected that these vegetation remnants are significantly more altered and, consequently, harbor less plant mite species. As a result of these similar impacts, mite fauna composition did not show differences between PR and SR remnants.

This paper is a pioneer report of mite assemblage on *A. fraxinifolium*, an extinction threatened plant species from Cerrado remnants. Yet, *Phytoscutus* and *Afronychus* genera and *A. brasiliensis* species were recorded for Cerrado biome for the first time. At least, two species sampled here are potentially new taxa to be described in future taxonomic studies. Moreover, we observed similar impacts from mining activities on PR and SR remnants since their mite fauna did not show differences in composition species. This pattern was probably due to the proximity of both PR and SR remnants to the mining sites. Future studies comparing close and more distant areas (preferentially pristine areas) from mining enterprises must be conducted to elucidate the effect of this impact on mite occurrence and abundance. The present study highlights the importance of keeping natural vegetation in order to preserve mite diversity on native plants rendering its database useful to future conservation programs for natural ecosystems.

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