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Research Article

Intraspecific and Intracolonial Variation in the Profile of Venom Alkaloids and Cuticular Hydrocarbons of the Fire Ant *Solenopsis saevissima* Smith (Hymenoptera: Formicidae)

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Fire ants are aggressive Neotropical ants that are extensively similar in general biology and morphology, making species identification difficult. Some fire ant species are top-rated pests spreading throughout the world by trade vessels. Many researchers attempted to sort between invasive and native species by using chemical characters, including patterns of venom alkaloids. The present study is the first to report intraspecific variation in some chemical characters, namely, cuticular hydrocarbons and venom alkaloids, within the Brazilian fire ant species *Solenopsis saevissima* and also reports on within-nest variations among members of different castes. Two different haplotypes (cryptic species) of *S. saevissima* were clearly identified, one presenting a predominant combination of the venom alkaloids *cis*- and *trans*-2-methyl-6-undecylpiperidine with the cuticular hydrocarbons C_{23}, 3-Me-C_{23}, 10-$C_{25:1}$, C_{25}, and 3-Me-C_{25}, and the other a predominant combination of *cis*- and *trans*-2-methyl-6-tridecenylpiperidine with predominance of 12-$C_{25:1}$, C_{25}, 11-Me-C_{25}, 3-Me-C_{25}, 13-$C_{27:1}$, C_{27}, and 13-Me-C_{27}. Intranest variations revealed that the proportions among these compounds varied sensibly among workers of different sizes, gynes, and males (no alkaloids were detected in the latter). Larva contained vestiges of the same compounds. The recorded chemical profiles are quite different from previous reports with *S. saevissima* samples from São Paulo. The finds thus support other recent claims that *S. saevissima* includes cryptic species; the study, moreover, adds the find that they can occur in the same geographical location.

1. Introduction

The fire ants of the genus *Solenopsis* Westwood include species considered pests of worldwide importance, especially *Solenopsis invicta* Buren, which were accidentally transported to other countries outside their native range in South America. To date, fire ant invasion is a major concern in the USA. These ants, particularly those belonging to the *Solenopsis saevissima* species group, react aggressively when their earthen nests are disturbed. Their stings, in addition to pain, can cause serious anaphylactic reactions to sensitive subjects (e.g., [1]).

The species *Solenopsis saevissima* Smith is native to South America and common in Brazil [2], wherein it is potentially responsible for over 35% of the registered accidents with insects (personal communication of Mário Sérgio Palma).

This fire ant was not as extensively studied as other species officially considered pests, like *S. invicta* and *Solenopsis richteri* Forel.

The fire ants are unique among arthropods for their venom composition—alkaloids combined with trace amounts of protein [3, 4]—besides being of special interest to taxonomists because of the historical difficulty of distinguishing between the different species, particularly in South America [5–7]. Cuticular hydrocarbons proved useful in separating between similar species in other difficult groups of ants [8, 9], and the profiles of cuticular hydrocarbons of some *Solenopsis* species have already been determined [10–13]. The use of relative amounts of venom alkaloids—currently credited to be species specific—has also been proposed [8, 14]. In fact, these chemical characters were also used in attempts to build a solid phylogeny among fire ant species [4, 8, 15]. Yet, considering the great number of extant species of *Solenopsis*, there are few comparative or qualitative studies of cuticular hydrocarbons and venom alkaloids available so far [14, 16, 17].

The venom alkaloids and cuticular hydrocarbons of *S. saevissima* were determined by [18] and recently confirmed by [14]; both studies were based on field-obtained samples from São Paulo State, Southeastern Brazil. Yet, another study [19] from the French Guyana presented another chemical profile for *S. saevissima* completely different from these other studies. However, a recent study [7] challenged the present classification of the Brazilian fire ant *S. saevissima* as a single species, having observed that it apparently embraces several distinct genetic lineages with similar morphology (i.e., cryptic species); these authors, for example, detected a distinct lineage of *S. saevissima* that occurs along the littoral of Brazil that apparently hybridizes with *Solenopsis geminata* F. It thus remained to be investigated if *S. saevissima* from different geographical localities in Brazil would have the same venom alkaloid composition.

The present investigation described the cuticular hydrocarbons and venom alkaloids of *S. saevissima* from different regions in Brazil and the intraspecific variations from analyzing individuals of different castes and sex within a same locality. The results are compared with finds from other authors.

2. Materials and Methods

2.1. Chemicals. *n*-Alkane standards (range C_{16}–C_{31}) were purchased from Sigma Aldrich (Aldrich, Germany, 98%), and anhydrous sodium thiosulphate (Merck, Brazil, 99%) was used as provided. Distilled solvents used were hexane (98.5%), ethanol, and ethyl acetate (99.5%), all from Merck, Brazil.

2.2. Collection of Samples. Samples of workers of mixed sizes were obtained upon disturbing nests of *S. saevissima* in the field at Brasilia, Distrito Federal (15°48′00”S 47°51′50′′W), Palmas, Paraná State (26°13′44′′S 52°40′15′′W), and Rio de Janeiro (22°51′45”S 43°13′26′′W), Brazil (Figure 1). Additionally, samples of each individual caste were obtained from five whole fire ant nests collected from a house garden at the municipality of Pedro do Rio, Rio de Janeiro, Brazil (22°20′30′′S 43°07′44′′W) following the methods for handling and rearing these insects in the laboratory described in [20].

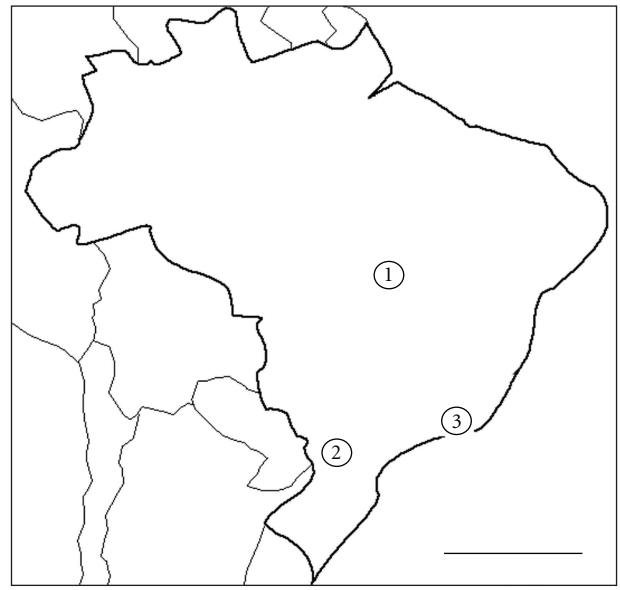

Figure 1: Sites of sample collections within Brazil. (1)-Brasilia, Distrito Federal; (2)-Palmas, Parana; (3)-Rio de Janeiro, RJ. These localities are quite different in terms of climate and vegetation. Scale bar = 800 km.

Species identification was based on the series of characters given in [5] and additional useful traits from [14]; the following diagnostic characters of major workers of *S. saevissima* were confirmed: no postpetiolar process, complete mandibular costulae, absence of a frontal medial streak or ocellus, and a poorly developed median clypeal tooth. Voucher specimens are deposited in the Adolph Hempel Entomological Collection of Instituto Biológico de São Paulo, SP, Brazil.

2.3. Sample Preparation. Random worker samples obtained in the field were directly immersed in 100 μL of hexane. Workers from the whole nests were separated in the laboratory into different size classes (size interval, mean weight ± SD) as follows: minor workers (1-2 mm; 0.40 ± 0.08 mg), medium workers (3-4 mm; 0.9 ± 0.18 mg), and major workers (5-6 mm; 2.0 ± 0.52 mg). This division was merely analytical, as most authors only recognize a continuous size range from minors to majors. Males (1.9 ± 0.4 mg) and gynes (unmated queens; 3.1 ± 0.2 mg) were separately analyzed.

Venom Alkaloids. Females of different size classes and castes were cold-anesthetized and had their venom sacs dissected with a fine forceps and macerated in bidistilled ethyl acetate. The venom extracts were adjusted to a final extract concentration of 1 mg/mL.

Cuticular hydrocarbons from body wash: the bodies without venom glands were washed with distilled water

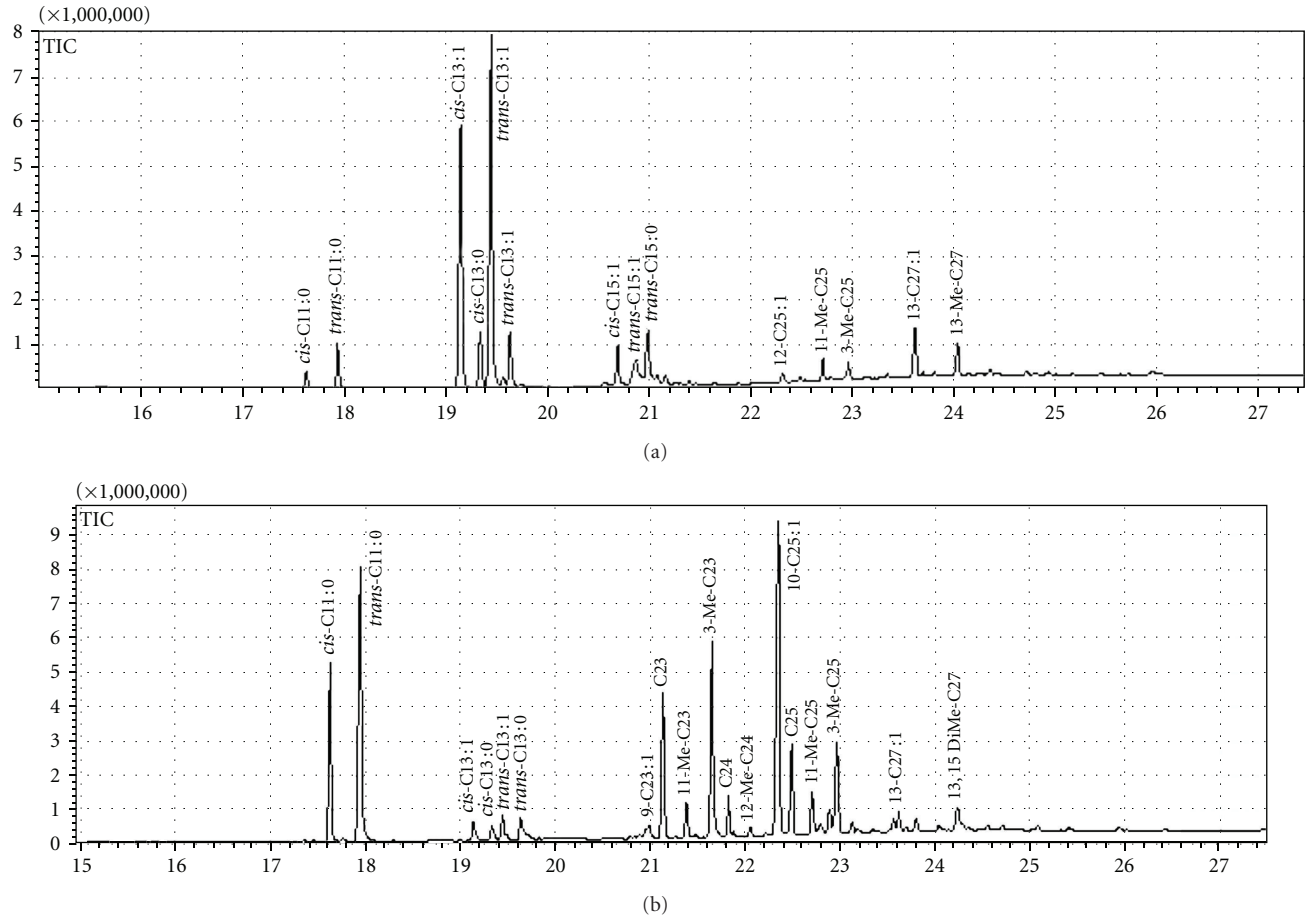

Figure 2: Total ion current chromatograms of (top) field worker sample of *Solenopsis saevissima* variety A from Brasília, Distrito Federal, Brazil; (below) field worker sample of *S. saevissima* variety B from Rio de Janeiro, Brazil. For further information on the chemical fingerprints, refer to Table 1 and narrative.

(in 5 mL for 10 min) three times, dried with a piece of filter paper, and then dipped into 2 mL of hexane for 5 min. The obtained body wash extracts were then adjusted to 1 mg/mL in hexane.

Cuticular Hydrocarbons from Crushed Heads. Several workers of different size classes, gynes, and males were cold-anesthetised and decapitated. The excised heads were crushed in 2 mL of bidistilled hexane, filtered, and dried, and the resulting wash was adjusted to 1 mg/mL in hexane.

For quantification of venom alkaloids, certain amounts of individuals (workers or gynes) were pooled, immersed in hexane for 10 min, and the final yield of dry extract weighed with an analytical scale, being divided by the number of individuals used. The same method for measuring cuticular hydrocarbons proved impracticable as the extracted amounts were too small to be accurately measured; thus, the relative amounts of cuticular hydrocarbons could be only estimated from comparing the peak area of *n*-alkane standards with obtained peaks from GC-MS chromatograms with known numbers of individuals and solvent volume.

2.4. GC-MS Analyses. The obtained extracts—venom alkaloids and body and head hydrocarbons—were analyzed by gas chromatography and mass spectrometry (GC-MS) by injecting 1 μL of each extract in a GCMS-QP2010 (SHIMADZU) system equipped with a RTX-5MS silica capillary column (30 m × 0.25 mm × 0.25 μm). Helium was the carrier gas, used at a flow rate of 1.0 mL/min on split mode. The MS were taken at 70 eV, and scanning speed was set to 1228, from *m/z* 50 to 700. The interface temperature was maintained at 280°C. The injector temperature was 250°C. Oven temperature was programmed to increase at 12°C/min from 50°C to 330°C, with a final hold time of 1 min.

2.5. Retention Indexes. Resulting alkaloids and cuticular hydrocarbons were identified by matching their retention indices and acquired mass spectra with entries in the internal mass spectra library (Wiley 275) and published literature [21, 23, 26, 30], while the *n*-alkane external standards were used to bracket the retention indexes as whole numbers: 1600–3100 [26].

Table 1: Venom alkaloids identified from hexane extracts of *Solenopsis saevissima* from different regions within Brazil.

Compounds	Short name	Diagnostic ions, *m/z* (relative abundance)	RT (min)	RI (calc.)
2-Me-6-undecenyl piperidine	C11:1	251 (M^+), 236, 180, 124, 111, 98	17.40	1819
cis-2-Me-6-undecyl piperidine	*cis*-C11	253 (M^+), 252, 238, 98	17.61	1841
trans-2-Me-6-undecyl piperidine	*trans*-C11	253 (M^+), 252, 238, 98	17.94	1884
cis-2-Me-6-tridecenyl piperidine	*cis*-C13:1	279 (M^+), 278, 124, 111, 98	19.11	2021
cis-2-Me-6-tridecyl piperidine	*cis*-C13	280 (M^+), 266, 98	19.30	2052
trans-2-Me-6-tridecenyl piperidine	*trans*-C13:1	279 (M^+), 264, 180, 124, 111, 98	19.41	2066
trans-2-Me-6-tridecyl piperidine	*trans*-C13	280 (M^+), 266, 98	19.60	2091

Notes: RT (min): retention times in minutes; RI (calc.): calculated retention indexes based on [21] using external standards as in Methods. Compounds are identified by comparing with mass spectra from [22, 23].

Table 2: Cuticular hydrocarbons from hexane extracts of fire ants *Solenopsis saevissima* obtained from different regions of Brazil.

Compounds	Short name	Diagnostic ions, *m/z*	RT (min)	RI (calc.)	RI (lit.)
docosane	C22	310 (M^+), 113, 99, 85, 71, 57	20.43	2200	2200
tricosane	C23	324 (M^+), 113, 99, 85, 71, 57	21.14	2300	2300
11-Methyl-tricosane	11-Me-C23	338 (M^+), 323, 196, 168, 140	21.40	2336	2336
3-Methyl-tricosane	3-Me-C23	338 (M^+), 323, 309, 281	21.65	2372	2375
tetracosane	C24	338 (M^+), 113, 99, 85, 71, 57	21.84	2400	2400
12-Methyl-tetracosane + 11-methyl-tetracosane	12-Me-C24 + 11-Me-C24	352 (M^+), 337, 323, 210, 196, 182, 168	22.14	2434	2435
12-Pentacosene	12-C25:1	350 (M^+), 111, 97, 83, 69, 55	22.34	2477	—
10-Pentacosene	10-C25:1	350 (M^+), 111, 97, 83, 69, 55	22.36	2483	—
Pentacosane	C25	352 (M^+), 113, 99, 85, 71, 57	22.49	2500	2500
11-Methyl-pentacosane	11-Me-C25	366 (M^+), 351, 224, 196, 168	22.70	2532	2534
3-Methyl-pentacosane	3-Me-C25	366 (M^+), 351, 337, 309	22.95	2570	2574
Hexacosane	C26	366 (M^+), 113, 99, 85, 71, 57	23.15	2600	2600
13-Methyl-hexacosane	13-Me-C26	365 (M^+), 351, 210, 196, 182, 168	23.34	2630	2633
12-Heptacosene	12-C27:1	378 (M^+), 111, 97, 83, 69, 55	23.64	2670	—
13-Heptacosene	13-C27:1[c]	378 (M^+), 111, 97, 83, 69, 55	23.67	2671	—
Heptacosane	C27	380 (M^+), 113, 99, 85, 71, 57	23.80	2700	2700
13-Methyl-heptacosane	13-Me-C27	394 (M^+), 379, 224, 196, 168	24.08	2736	2733
n-Octacosane	C28:1	392 (M^+), 111, 97, 83, 69, 55	24.18	2747	—
13,15-Dimethyl-heptacosane	13,15-DiMe-C27	408 (M^+), 239, 197	24.25	2756	2756
3-Methyl-heptacosane	3-Me-C27	394 (M^+), 379, 365, 337	24.38	2772	2774
Octacosane	C28	394 (M^+), 295, 267, 239, 196, 168	24.60	2800	2800
3,7,11-Trimethyl-heptacosane	3,7,11-TriMe-C27	422 (M^+), 393, 323, 253, 197, 127	24.82	2830	2833
tridecane	C30	422 (M^+), 113, 99, 85, 71, 57	26.01	3000	3000

Notes: Compounds identified by comparison with mass spectra from [12]/[24, 25]. RT: retention times; RI (calc.): calculated retention indexes (based on Van den Dool and e Kratz, 1963 [21]); RI (lit.): retention indexes as recorded in [26–28]. For further information on the collection sites, refer to narrative.

2.6. Derivatization by Dimethyl Disulfide/Iodine. Alkenes were derivatized according with the methodology described in [31].

3. Results

3.1. Samples from Different Geographical Localities. The samples with workers of mixed sizes revealed the existence of two radically different chemical profiles (see Figure 2) within *S. saevissima*, which shall be referred heretofore as *variety A* and *variety B*. The venom alkaloids of both varieties were composed by isomers of 2-methyl-6-undecylpiperidine (C11) and 2-methyl-6-tridecenylpiperidines (C13), but isomers of C13 prevailed in the venom profile of variety A (with only trace amounts of 2-methyl-6-pentadecylpiperidines), while isomers of C11 prevailed in variety B. Cuticular

hydrocarbons were also different to the same extent (see Figure 2). A complete list of identified venom alkaloids is given in Table 1, and a complete list of the cuticular hydrocarbons observed is given in Table 2 (shorter names for each compound are given in these tables). Relative proportions of the main cuticular hydrocarbons identified in field samples of *S. saevissima* varieties A and B are given in Table 3. Variety A was found in Brasilia and Paraná, while variety B was retrieved from Rio de Janeiro. Molecular evidence (not shown) confirmed that the obtained varieties correspond to two consistent and distinct mtDNA haplotypes within the *S. saevissima* clade—that is, they are cryptic species. These molecular results will be dealt with in a separate publication.

Preliminary tests (not shown) with 100% ethanol demonstrated that this solvent is also useful for recovering hydrocarbons and alkaloids from fire ants, as long as the mixture is injected in the GC-MS no later than within 2 weeks of collection.

3.2. Whole-Nest Samples. Further field inspections (not shown) revealed that *S. saevissima* varieties A and B were abundant at the mountains of Rio de Janeiro; thus, a strategic collection point at the city of Pedro do Rio was elected for obtaining whole nests. *Solenopsis saevissima* was the only fire ant species found around that area, and nests of the two cryptic varieties were present. For sorting in the field between the varieties, we devised the following method: (i) a 15 cm wide carton circle (the "arena") was placed in a plastic or glass bowl; (ii) a sample of one nest was collected with a spoon and placed on one side of the paper arena; (iii) another spoonful sample, from the other nest, was placed on the opposite side of the paper arena; (iv) the degree of aggressiveness of the ants was observed for a few moments. Fighters invariably proved being of different varieties when later analysed by GC-MS. An example of the test can be seen online at http://archive.org/details/AggressivenessTestsFireAnts.

3.3. Intraspecific Variation Range

3.3.1. Venom Alkaloids. Minor workers yielded ~7 μg per venom sac ($N = 2$ groups of 1,000 ants), while media workers yielded ~12 μg per venom sac ($N = 1$ group of 1,000 ants), and major workers yielded ~25 μg per venom sac ($N = 2$ groups of 400 ants). Gynes yielded ~90 μg of venom alkaloids per venom sac ($N = 15$). Table 4 illustrates the relative amounts of each venom alkaloid among workers of different sizes and gynes. Relative proportions of alkaloid isomers of workers varied with size: minor workers always had higher proportions of *trans* isomers (Table 4), while greater size classes had increased amounts of *cis* isomers; *cis* isomers were always predominant in the venom of gynes. Venom alkaloids were not detected in neither body wash nor head extracts of males. Larvae of both varieties contained detectable amounts of the same venom alkaloids of workers (not shown).

3.4. Cuticular Hydrocarbons from Head and Body. Workers always yielded 12–14 μg of head hydrocarbons and ~32 μg of body cuticular hydrocarbons ($N = 10$). Males yielded ~83 μg (head) and ~135 μg (body) ($N = 3$) of cuticular hydrocarbons, and queens yielded ~84 μg (head) and ~ 238 μg (body) ($N = 3$).

Table 3: Relative abundance (%) of cuticle hydrocarbons from random worker samples of *Solenopsis saevissima* varieties A ($n = 5$) and B ($n = 13$) obtained from different nests from three different states in Brazil (see Figure 1). Values given in bold were tested for each species variety using Students' t-test, and differing means were attributed to different letters.

Cuticular hydrocarbon	*S. saevissima* variety A	*S. saevissima* variety B
C23	—	12.16 ± 2.48
11-Me-C23	—	2.10 ± 0.69
3-Me-C23	—	21.42 ± 1.94
C24	—	1.75 ± 0.51
12-Me-C24 + 11-Me-C24	—	0.77 ± 2.21
C25:1	**8.52 ± 3.32 a**	**50.74 ± 6.69 b**
C25	**4.06 ± 2.47 a**	**4.38 ± 0.95 a**
11-Me-C25	**9.73 ± 2.19 a**	**2.198 ± 0.89 b**
3-Me-C25	**12.91 ± 1.60 a**	**5.66 ± 0.84 b**
C26	3.24 ± 1.70 a	tr
C27:1	25.49 ± 8.47	tr
C27	4.04 ± 2.30	tr
13-Me-C27	24.19 ± 7.98	tr
11,15-DiMe-C27	2.00 ± 1.27	—
3-Me-C27	3.23 ± 0.62	—
13,15-DiMe-C27	4.48 ± 1.65	—
C30	2.01 ± 0.24	—
Total	100.00	100.00

Notes: (—): not found; (tr): trace amounts. For information on the compounds, collection sites, and species varieties, refer to narrative.

Based on relative abundance within chromatograms, workers had 1-2 μg of total hydrocarbons, while males had about 5 μg, and queens had about 6 μg. Relative abundance between total alkaloids and total hydrocarbons proved quite variable between different individuals. Hydrocarbons from head yielded alkaloid-free clear chromatograms.

Hydrocarbons obtained from the hexane extracts of head and body extracts were always the same (not shown), thus these results were qualitatively equivalent. A comparison of the head extracts of major workers of *S. saevissima* varieties A and B is shown in Figure 3. Main cuticular hydrocarbons of *S. saevissima* variety A were 13-heptacosene, 13-methyl-heptacosane, and 3-methyl-pentacosane, while the main cuticular hydrocarbons of *S. saevissima* variety B were tricosane, 3-methyl-tricosane, 10-pentacosene, pentacosane, and 3-methyl-pentacosane (see Figures 2 and 3).

The relative proportions of cuticular hydrocarbon obtained from body extracts of workers of different sizes and castes of *S. saevissima* varieties A and B are shown in Table 5. The relative amounts of cuticular hydrocarbons varied sensibly among workers of different sizes and castes. A full chromatogram illustrating the differences between cuticular

Table 4: Relative abundance (%) of piperidinic alkaloids obtained from hexane extracts of small workers (SWs), medium workers (MWs), and major workers (LWs) and gynes (Gs) from different nests of *Solenopsis saevissima* varieties A ($n = 3$) and B ($n = 4$) from Pedro do Rio, Rio de Janeiro state, Brazil.

Piperidine	*S. saevissima* variety A				*S. saevissima* variety B			
	SW	MW	LW	G	SW	MW	LW	G
C11:1	—	—	—	—	—	—	—	0.35 ± 1.15
cis-C11	tr	—	—	33.22	4.16 ± 2.16	7.51 ± 4.27	12.21 ± 5.70	61.79 ± 12.99
trans-C11	tr	tr	tr	35.15	95.84 ± 2.16	98.48 ± 4.28	87.79 ± 5.70	tr
cis-C13:1	tr	31.52–35.76	49.69–55.00	17.68	tr	tr	tr	33.15 ± 15.92
cis-C13	tr	tr	tr	4.04	—	—	tr	1.37 ± 0.94
trans-C13:1	97.80–98.00	60.02–64.24	45.53–50.31	7.76	tr	tr	tr	tr
trans-C13	—	—	—	2.15	—	—	—	tr
Unknown	—	—	—	—	—	—	—	tr
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Notes: Compounds identified by comparing with mass spectra from Brand et al. 1972 [29] and Leclercq et al. 1994 [23]. (—) = not found; (tr) = trace amounts. No venom alkaloids were found on males. For further information on the extraction and species varieties, refer to narrative.

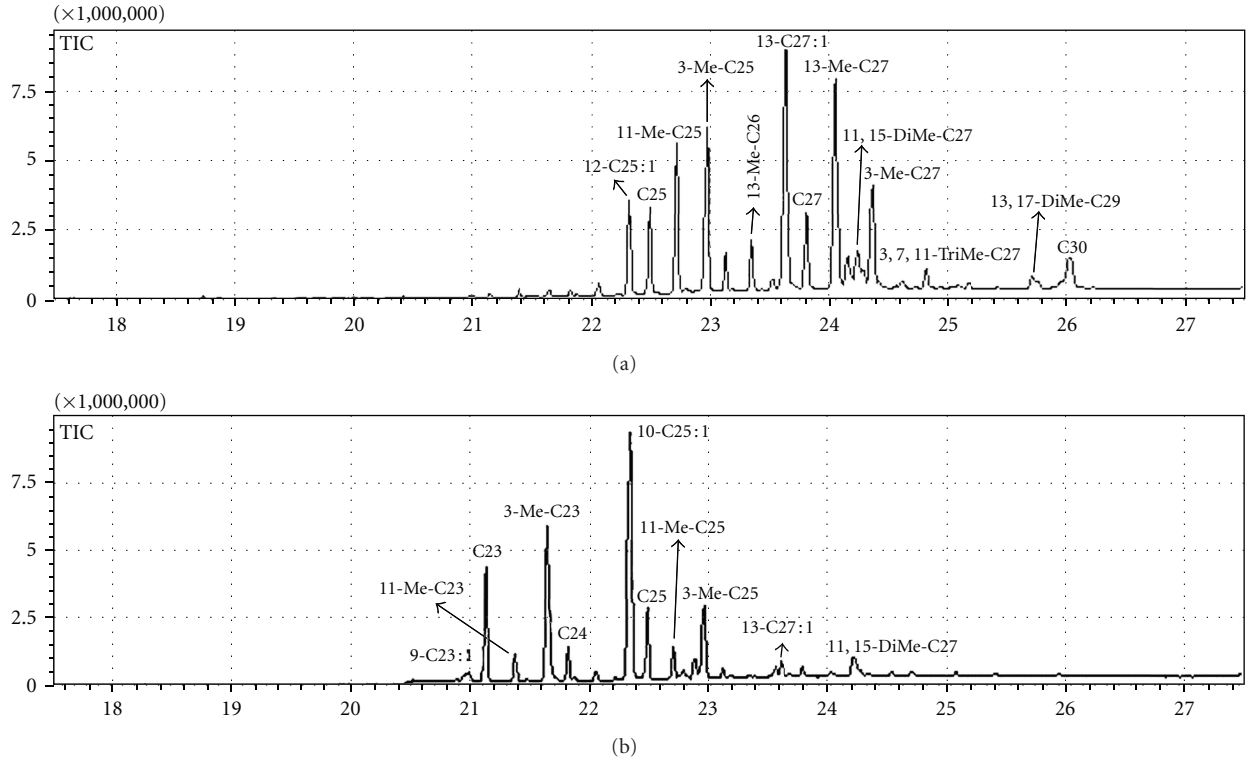

Figure 3: Total ion current chromatograms of hydrocarbons from head extracts of major workers of *Solenopsis saevissima* varieties A (top) and B (below) from Rio de Janeiro, Brazil.

hydrocarbons and venom alkaloids of major and minor workers of *S. saevissima* variety B is given in Figure 4.

4. Discussion

4.1. Samples of Different Geographical Localities. Random samples of mixed worker sizes suggested that the described cryptic varieties are widespread and common over Brazil, in agreement with the scenario predicted by [7] using other tools. It remains to be investigated if the cryptic species appointed by [7] would correspond to similar chemical profiles. A complete scenario can be easily obtained by further sampling from other localities. Other varieties within nominal *S. saevissima* are probably also present. The practice of testing workers for aggressiveness was very helpful in locating a nest of the less prevalent *S. saevissima* variety A within the study area, as it dispensed the need for returning several samples back to the laboratory for GC/MS analyses.

Table 5: Relative abundance (%) of cuticle hydrocarbons from small workers (SWs), medium workers (MWs), and major workers (LW), gynes (Gs) and males (Ms) of *Solenopsis saevissima* varieties A and B, collected in Pedro do Rio, Rio de Janeiro, Brazil.

Cuticular hydrocarbon	*Solenopsis saevissima* variety A (minimum–maximum interval)					*Solenopsis saevissima* variety B ($n = 5$; mean ± SD)				
	SW ($n = 4$)	MW ($n = 4$)	LW ($n = 4$)	G ($n = 2$)	M ($n = 3$)	SW	MW	LW	G	M
C22	—	—	—	—	—	—	—	—	tr	—
C23	1.52–1.87	—	—	tr	—	12.45 ± 4.6	12.02 ± 7.13	9.77 ± 3.06	13.92 ± 1.93	21.81 ± 1.86
11-Me-C23	—	tr	—	tr	—	—	—	—	—	—
3-Me-C23	1.87–2.22	—	—	tr	—	17.37 ± 1.22	16.87 ± 1.18	18.77 ± 2.56	19.27 ± 2.56	18.33 ± 3.11
C24	—	—	—	tr	—	2.62 ± 1.10	2.34 ± 1.75	2.00 ± 1.00	3.40 ± 0.23	4.70 ± 0.60
12-Me-C24 + 11-Me-C24	—	tr	tr	tr	—	—	—	—	—	—
12-C25:1	6.31–7.48	3.91–4.20	4.99–5.21	3.75–45.38	17.06–31.77	—	—	—	—	—
10-C25:1	—	—	—	—	—	43.23 ± 10.86	42.15 ± 13.12	43.77 ± 6.14	34.92 ± 0.92	28.63 ± 5.89
C25	8.17–9.00	4.47–5.31	6.20–9.29	1.18–16.77	3.51–15.33	9.97 ± 5.16	9.20 ± 6.70	8.02 ± 4.34	10.45 ± 0.72	19.57 ± 5.62
13-Me-C25	—	—	—	—	—	1.37 ± 0.69	1.68 ± 0.84	1.88 ± 0.94	1.75 ± 0.88	—
11-Me-C25	6.05–9.23	11.58–13.18	10.8–13.01	7.53–9.82	2.24–7.76	—	—	—	—	—
3-Me-C25	12.22–13.37	10.04–11.08	9.02–9.58	13.27–16.83	10.10–20.73	7.40 ± 2.50	7.34 ± 2.18	6.85 ± 0.62	7.46 ± 0.47	6.48 ± 0.68
C26	—	—	—	1.08–3.02	—	—	—	—	—	—
13-Me-C26	1.45–2.10	1.93–3.28	1.72–2.57	0.00–1.30	10.29–10.63	—	—	—	—	—
12-C27:1	—	—	—	—	—	1.00 ± 1.00	1.10 ± 0.55	1.17 ± 0.59	1.00 ± 1.00	—
13-C27:1	20.53–22.30	26.88–28.83	19.27–22.20	16.28–30.44	—	—	—	—	—	—
C27	15.06–16.08	2.33–3.1	4.60–5.00	0.00–6.56	—	tr	tr	tr	tr	tr
13-Me-C27	15.12–17.8	25.93–28.69	25.30–28.05	0.00–14.36	—	—	—	—	—	—
n-C28:1	—	1.00–1.39	1.08–2.43	tr	—	1.57 ± 0.79	1.97 ± 0.99	2.27 ± 1.14	1.75 ± 0.87	—
13-15-DiMe-C27	3.17–4.00	3.98–4.08	4.26–5.06	0.00–6.85	2.96–12.11	tr	tr	tr	tr	—
3-Me-C27	—	—	—	—	—	tr	tr	tr	tr	tr
C28	—	tr	1.84–2.08	tr	—	—	—	—	—	—
C29	—	—	—	tr	—	—	—	—	—	—
3,7,11-TriMe-C27	—	tr	tr	tr	—	—	—	—	—	—
C30	—	1.32–2.21	tr	tr	—	—	—	—	—	—
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Notes: (—): not found; tr: trace amounts; *n*: number of nests evaluated.

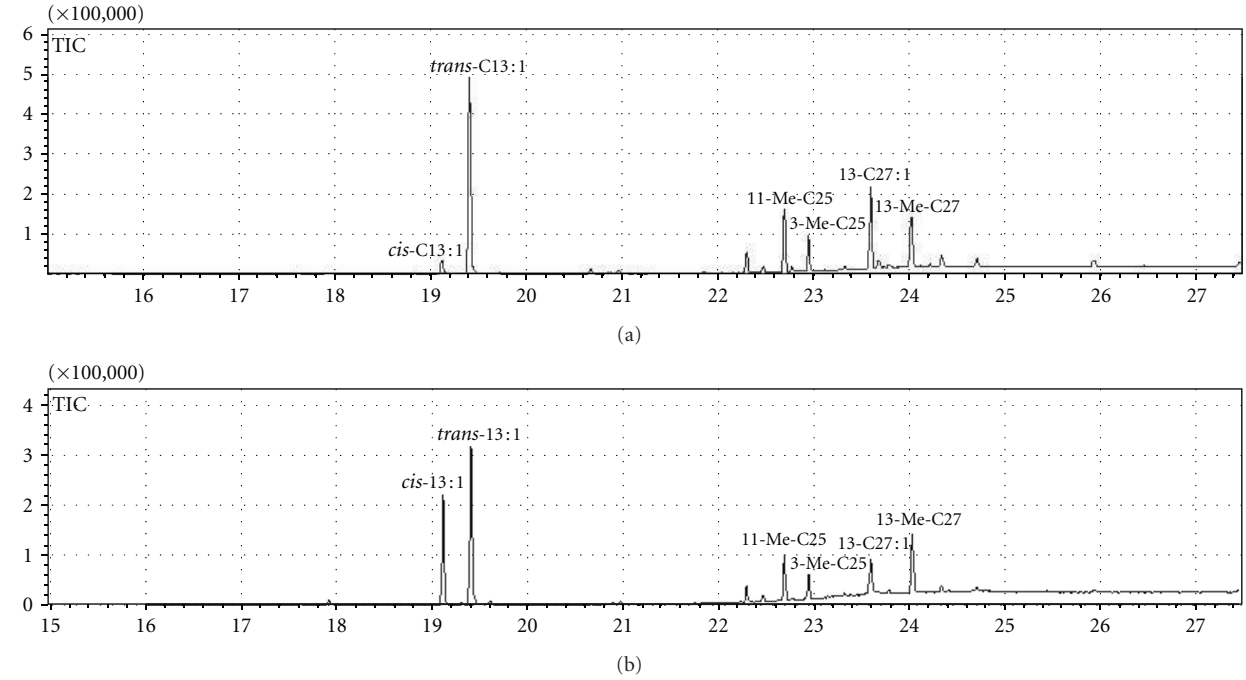

Figure 4: Total ion current chromatograms of body washes of workers of *Solenopsis saevissima* variety A from Rio de Janeiro, Brazil: (top) Minor workers; (below) Major workers.

The fact that ethanol provided good chemical profiles of samples collected in the field is interesting, as it is a more common, cheaper, and less volatile solvent. This opens the possibility of checking the alkaloids present in long-standing fire ant samples deposited in wet collections. It should be noted, however, that care should be taken in such cases as polar solvents can be harmful to some analytical systems, for they can interact with the column matrix.

4.2. Venom Alkaloids and Cuticular Hydrocarbons. The reported amount of cuticular hydrocarbons extracted from fire ants is dramatically superior—sometimes >200 times—to other much larger ants such as *Ectatomma ruidum* Roger [32] and *Ectatomma brunneum* Smith F. and *Atta laevigata* Smith F. (unpublished results of E.G.P.F.). This disparate abundance of waxes on the body of fire ants must have some biological implication, which probably explains why they can conveniently be separated by nest debris by flotation [20]. In comparison with previous studies with fire ants, in the present report we found they were in agreement with cuticular hydrocarbons reported by [13] from similar extractions with *S. invicta* and *S. richteri*, yet another study [33] found as little as 300 ng per ant. We think that such difference could be explained by either populational or specific differences, but the fact that the reported values proved also quite variable between different studies with the same species indicates that the matter would merit further comparative investigation. We ought however to emphasize that our chromatograms with different samples within the same nest suggest that there is considerable intraspecific variation in the alkaloids/hydrocarbons ratio.

The abundance of venom alkaloids in the body wash extracts is also elevated, and it should be noted that these ants apparently discharge the contents of their venom glands upon immersion in organic solvent. However, we herein recovered about one-third of the amount reported by [22] with *S. invicta*. Again, it is possible that such difference be due to specific variations, but it should be minded that these authors employed different methods for their venom estimates. The fact that venom alkaloids were also recovered from bodies with excised abdomens indicates that they accumulate for some time on the exoskeleton of the insects. This alkaloidal covering might serve as armor against entomopathogenic fungi and predators, or even have some important role in nestmate recognition, given their abundance.

4.3. Venom Alkaloids and Cuticular Hydrocarbons as Taxonomic Tools. The sampled regions of the present study, from being distant from each other, present marked differences in climate, soil, and vegetation. The different patterns of venom alkaloids and cuticular hydrocarbons of the collected samples indicate they belong to two different cryptic species (compare Tables 1, 2, and 4). These patterns were yet markedly different from reports for *S. saevissima* by [14] based on samples from São Paulo, thus indicating the existence of other cryptic species in Brazil. The additional profile published by [19] further indicates the existence of another cryptic species in French Guyana.

The existence of cryptic species in this clade was solidly demonstrated in the broad study of [7], using other methods. Our finds with chemical characters thus add evidence to the conclusions of Ross et al. (2009) that different evolutionary

entities were grouped in the nominative species *S. saevissima* because of morphological similarity. The actual number of cryptic species and the range of their variation could only be established by investigations employing further different approaches (e.g., isoenzymes, larval characters) based on extensive sampling from other regions of South America. This is an issue of major interest as it pertains the biological boundaries of individual species, how many actually exist, and how they are set in nature and thus warrants immediate attention.

Moreover, the present report also imposes exceptions to the belief (e.g., [10, 34]) that venom alkaloidal composition in fire ant venoms is species specific. Based solely on the pattern of venom alkaloids and following the results in these studies, *S. saevissima* variety B would have been assumed to be *S. geminata* or some close relative, like *Solenopsis xyloni* McCook; variety A would have been likewise mistaken for *S. richteri*.

It seems unlikely that venom alkaloid composition of the different species could be interpreted as remissive of the phylogenetic relationships among fire ants, as first proposed by [29] and discussed in [18]. These authors suggested that the chemical structure of venom alkaloids from different species might reflect evolutionary relationships within fire ants; species with most diverse venom alkaloids would stand a step higher in the taxonomic history of the group. As remarked by [18], this assumption was based on studies of the few North American species. From regarding the herein described patterns of *S. saevissima* in face of preterit reports, it follows that there are at least three completely different alkaloidal patterns recorded within this nominal taxon and that such patterns are strikingly similar to the venom patterns of other species considered distant from each other as, for example, *S. geminata* versus *S. invicta*, which belong to different groups of fire ants species.

4.4. Caste Variations. As mentioned, workers of fire ants range in size (polymorphism) over a broad continuum [35], and thus we decided to adopt an arbitrary division into minor, medium, and major workers. Moreover, worker size also depends on the physiological status and age of the nest [35, 36]. This implies that the arbitrary size class of media workers includes specimens of minor and major workers. Considering this aspect, intermediary workers were predominant in the collected samples. The obtained results confirm previous observations that the worker venom alkaloid *trans/cis* isomeric ratio is dependent on the ant size, with minor workers having higher *trans/cis* isomeric ratios (Tables 1 and 2), and are suggestive that the trans/cis ratio gradually changes at some point within the intermediary size range. A clear trend of ever-increasing relative amounts of venom *cis*-piperidinic alkaloids is perceived towards larger females, queens. Similar trend was observed with other species of fire ants, including *S. invicta* and *S. geminata* [4, 22, 29, 37]. Venom alkaloids were not detected in the male body nor head extracts.

The pattern of cuticular hydrocarbons of *S. saevissima* variety A was quite different from the one obtained for *S. saevissima* variety B. Moreover, intercaste differences in the patterns of cuticular hydrocarbons of *S. saevissima* were detected in both varieties, illustrated by small variations in the relative amounts of the main compounds. For instance, there was a clear tendency for reduction in the relative amounts of C_{23} from minor workers towards major workers (Table 2). Gynes presented a wider range of different cuticular hydrocarbons, whist males presented marked proportion alterations on the same compounds (Table 2).

The present study generally depicts the knowledge gap about fire ant populations in their native South American range. Most of what is currently assumed about the group was extrapolated from laboratory experimentation with North American *S. invicta* and based on a few scattered observations with other species in South America. The validity of currently accepted fire ant species must be revisited, as should their diagnostic features. Chemical characters are indeed valuable parameters that aid species identification, but given the similarity between the obtained chromatograms and those of other distinct species (*S. saevissima* versus *S. geminata* or *S. xyloni*) and the considerable dissimilarity among nominal *S. saevissima* populations, at present chemical characters have to be employed with great caution and also in association with other characters and techniques (e.g., mtDNA). We also hope to have contributed with practical methods and guidance to expand the survey of these characters with other distant fire ant populations.

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