

EXPLORATORY STUDIES ON THE KARYOTYPES OF SEVEN SPECIES
OF THE ANT NEOTROPICAL GENUS *Pseudomyrmex*
(HYMENOPTERA: FORMICIDAE: PSEUDOMYRMECINAE)

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ABSTRACT

The karyotypes of Neotropical Pseudomyrmecinae were analyzed for the first time. Seven species belonging to the *Pseudomyrmex* genus from four Brazilian localities had their chromosome number and morphology studied. Six of the nine species groups of *Pseudomyrmex* were sampled. Chromosome numbers ranged from $2n=24$ to $2n=70$, characterized in acrocentrics and metacentrics. Our cytogenetic studies indicate that, as in other ants, karyotype evolution in *Pseudomyrmex* may have evolved increasing chromosome number and diversifying chromosome morphology, minimizing genetics risks for deleterious mutations through centric fission and pericentric inversion. Such a karyotypic diversity is also recognized in other ants' subfamilies, especially Myrmeciinae, Ponerinae and Myrmicinae. These first cytogenetic studies in the genus *Pseudomyrmex* should contribute with other data to the evolutionary history of the Pseudomyrmecinae subfamily.

Key words: Brazilian fauna, cytogenetics, diversity, evolution, taxonomy

INTRODUCTION

The Pseudomyrmecinae subfamily is characterized by fast-moving ants, with large eyes and a well-developed sting, usually inhabiting the arboreal stratum [16]. More than 40 species out of approximately 300 described are obligate inhabitants of specialized ant-plants (myrmecophytes), apparently providing some protection to the plant [19]. Three genera of Pseudomyrmecinae are currently recognized: *Tetraponera* (Paleotropical), *Pseudomyrmex* (Neotropical, southern Nearctic) and *Myrcidris* (few localities in Brazil and Guyana) [1,16]. According to morphological and molecular data, Pseudomyrmecinae is a monophyletic subfamily that had originated in the Cretaceous and that went through most of its diversification probably in the Tertiary [19,20].

In order to focus further taxonomic studies, *Pseudomyrmex* species were morphologically defined in nine groups by Ward [15]: *ferrugineus*,

gracilis, *oculatus*, *pallens*, *pallidus*, *sericeus*, *subtilissimus*, *tenuis* and *viduus*. Some species, mainly from *ferrugineus* and *viduus* group, are ant-plant specialists, living in association with *Triplaris*, *Tachigali* and *Acacia* trees [17,18]. Moreover, this genus is well endowed with sympatric species [18], with a record of up to 18 sympatric species in a single collection site in Brazil [14].

Cytogenetic studies have supported several taxonomic issues of morphologically alike species as *Myrmecia pilosula* complex [8] and *Pachycondyla villosa* species group [11]. Also, karyotypic analyses are useful to answer questions about the evolutionary history of a group. According to the Minimum Interaction Theory [6-8], chromosomal evolution of ants tends to evolve as a whole toward increasing chromosome numbers by fission-inversion cycles [9].

Formicidae have the most impressive chromosomal variation in Hymenoptera, with chromosomal number ranging from $2n=2$, in *Myrmecia croslandi* [2], to $2n=106$, in *Dinoponera lucida* [10]. However, in spite of great karyotypic diversity, with the chromosome number published for more than 500 ant species

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[12], much has to be done for Neotropical ants' cytogenetics. For example, there is no karyotypic information of Neotropical Pseudomyrmecinae. There are records of the chromosome number in two species of *Tetraponera* from Malaysia (*Tetraponera* spp.: $2n=42$, 44) [3,5]. In this paper, we present a first series of cytogenetic studies in the genus *Pseudomyrmex*, with number and morphological chromosomal information of seven species, from Brazil.

MATERIAL AND METHODS

Chromosome observations were carried out on taxa belonging to six of nine *Pseudomyrmex* species groups, according Ward's system [15]: *gracilis* (*Pseudomyrmex gracilis* (Fabricius)); *oculatus* (*Pseudomyrmex schuppi* (Forel)); *pallens* (*Pseudomyrmex* sp. PSW-154); *pallidus* (*Pseudomyrmex* sp. PSW-156 [very close from *Pseudomyrmex simplex* (Fr. Smith), according P.S. Ward (pers. comm.)] and *Pseudomyrmex holmgreni* Wheeler); *tenuis* (*Pseudomyrmex termitarius* Smith); and *viduus* (*Pseudomyrmex penetrator* Smith). This last ant is the only species analyzed here that is a specialized inhabitant of live plant cavities (myrmecodomatia) [18], living in *Tachigali* (Fabaceae: Caesalpinoideae) plants and showing an extreme aggressive behavior.

Nine colonies of *Pseudomyrmex* spp. were collected from four localities in Brazil (Table 1), and about nine specimens from each colony were studied. Vouchers were deposited at Laboratório de Mirmecologia, CEPEC/CEPLAC, Ilhéus, Bahia, Brazil, under the collective number #5444, and in the WARD's collection at the Department of Entomology and Center for Population Biology, University of California at Davis, California, USA.

Chromosome metaphases were obtained using cerebral ganglia or testis from prepupae, taken soon after the meconium rejection. Slides were prepared using the air-drying technique of Imai *et al.* [7], and then stained with Giemsa. The metaphases were photographed using an Olympus BX60 microscope equipped with camera.

Chromosome morphology was defined in two groups: Acrocentric (A) and Metacentric (M) [4]. The karyotypes formulas given in Table 1 follow Imai *et al.* [8].

RESULTS

Chromosome number of the *Pseudomyrmex* species ranged from $2n=24$ to $2n=70$ (see Table 1), with both acrocentric and metacentric chromosomes. For karyotypes, see Figure 1 (*pallidus* group) and Figure 2 (other groups).

In the *pallidus* group, two different colonies of *P. holmgreni*, from distinct collected sites at Ilhéus, had the same karyotype number of $2n=50$ chromosomes, but with two different constitutions: 2 metacentrics and 48 acrocentrics, and 6 metacentrics and 44 acrocentrics chromosomes. *Pseudomyrmex* sp. nr. *simplex* PSW156 had two very distinct karyotypes. One of them had a remarkable karyotype of $2n=43$ chromosomes, with a heteromorphic pair comprising one metacentric and two acrocentric chromosomes, with a total of 7 metacentrics and 36 acrocentrics. The other one had 50 chromosomes, being 2 metacentric and 48 acrocentrics.

P. gracilis (*gracilis* group) had $2n=70$ chromosomes, all acrocentrics, with the highest chromosome number recorded in the subfamily. *Pseudomyrmex schuppi* (*oculatus* group) had its cytogenetics analysis only carried out with males. Its karyotype had $n=24$

Table 1. Data of *Pseudomyrmex* species collections, used in this study. *Pseudomyrmex* groups: (1) *gracilis*; (2) *oculatus*; (3) *pallens*; (4) *pallidus*; (5) *tenuis*; (6) *viduus*; Brazil: BA: Bahia State, ES: Espírito Santo State, MG: Minas Gerais State; n =haploid karyotype (males), $2n$ =diploid karyotypes (females), K =number of chromosome arms in haploid karyotypes, $2K$ = number of chromosome arms in diploid karyotypes.

Species/ <i>Pseudomyrmex</i> group	Colony collection	Number of individuals	Chromosome numbers	Karyotype formula
<i>P. gracilis</i> (1)	Castelo - ES	6	$2n=70$	$2K=70A$
<i>P. schuppi</i> (2)	Ilhéus - BA	5	$n=24$	$K=5M + 19A$
<i>Pseudomyrmex</i> sp. PSW154 (3)	Viçosa - MG	8	$2n=44$	$2K=6M + 38A$
<i>P. holmgreni</i> (4)	Ilhéus - BA	6	$2n=50$	$2K=6M + 44A$
<i>P. holmgreni</i> (4)	Ilhéus - BA	6	$2n=50$	$2K=2M + 48A$
<i>Pseudomyrmex</i> sp. nr. <i>simplex</i> PSW156 (4)	Viçosa - MG	8	$2n=43$	$2K=7M + 36A$
<i>Pseudomyrmex</i> sp. nr. <i>simplex</i> PSW156 (4)	Viçosa - MG	6	$2n=50$	$2K=2M + 48A$
<i>P. termitarius</i> (5)	Ilhéus - BA	5	$2n=48$	$2K=48A$
<i>P. penetrator</i> (6)	Marituba - PA	4	$2n=24$	$2K=24M$

chromosomes, with 5 metacentrics and 19 acrocentrics. *Pseudomyrmex* sp. PSW154 (*pallens* group) has its karyotype with $2n=44$ chromosomes, 6 metacentrics and 38 acrocentrics. All species of the *pallidus* and *pallens* groups had karyotypes with high chromosome number ($n>12$), with a prevalence of acrocentric chromosomes. *Pseudomyrmex termitarius* (*tenuis* group) had $2n=48$ chromosomes. This species, together with *P. gracilis*, were the only ones with karyotypes exclusively constituted of acrocentric chromosomes. The chromosome number of *P. penetrator* (*viduus* group) was $2n=24$ chromosomes, all metacentric. This karyotype had the lowest chromosome number of all the Pseudomyrmecinae we studied.

Except for *P. penetrator*, all the studied species had high-number karyotypes ($n>12$). They were

constituted either by both metacentric and acrocentric chromosomes, with prevalence of the last or only by acrocentric ones. The only low-number karyotype ($n\leq 12$) had all metacentric chromosomes, as commonly observed in such karyotypes [6].

DISCUSSION

Chromosome diversity has long been raising questions of what mechanisms would be involved in chromosome evolution. After extensive karyological studies, Imai *et al.* [7] proposed the minimum interaction theory to discuss karyotype evolution in eukaryotes. This theory points out that karyotypes evolve toward minimizing genetic risks due to deleterious reciprocal translocations, and predicts that increase in chromosome number by centric fission is

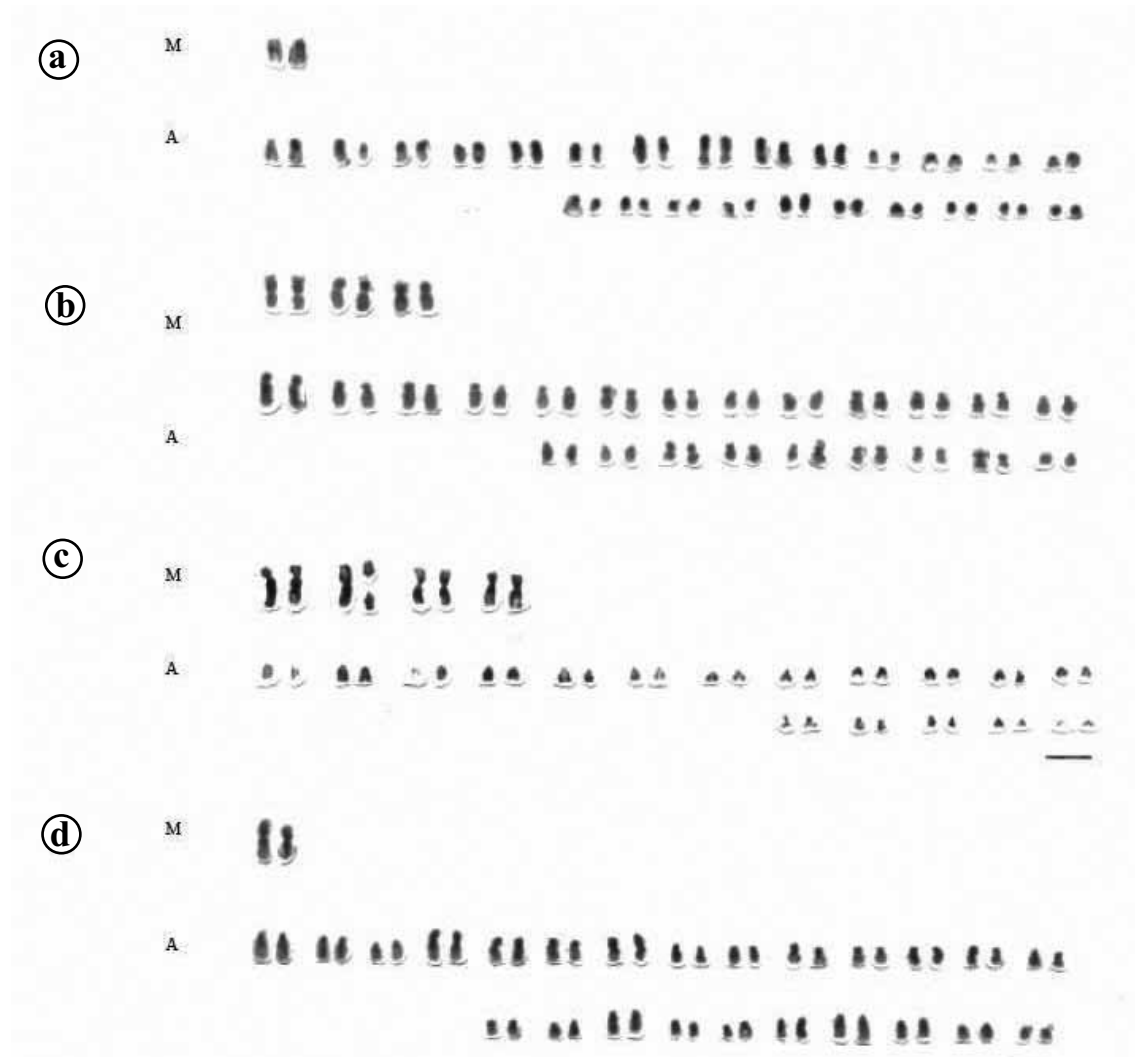


Figure 1. Karyotypes of *Pseudomyrmex pallidus* group: a) *P. holmgreni* $2n=50$; b) *P. holmgreni* $2n=50$; c) *Pseudomyrmex* sp. nr. *simplex* PSW156 $2n=43$; d) *Pseudomyrmex* sp. nr. *simplex* PSW156 $2n=50$. M- metacentric chromosomes line and A- acrocentric chromosomes line. Bar = 5 μ m.

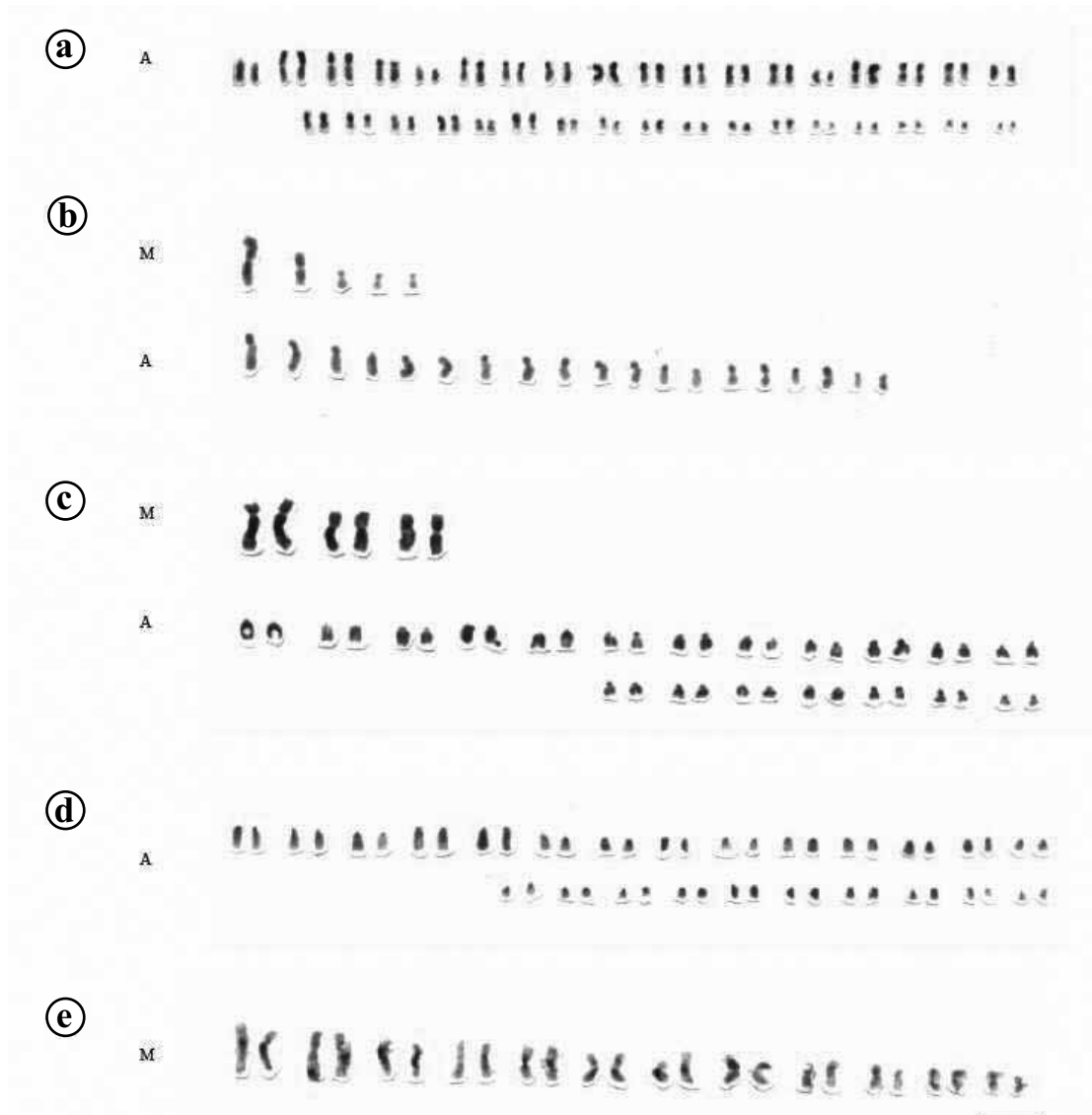


Figure 2. Karyotypes of *Pseudomyrmex* species from *gracilis*, *oculatus*, *pallens*, *tenuis* and *viduus* groups, respectively: a) *P. gracilis* $2n=70$; b) *P. schuppi* $n=24$; c) *Pseudomyrmex* sp. PSW154 $2n=44$; d) *P. termitarius* $2n=48$; e) *P. penetrator* $2n=24$. M- metacentric chromosomes line and A- acrocentric chromosomes line. Bar = 5 μ m.

one way to mitigate such risks. A study of chromosome diversity observed in mammals, ants and wasps [9] supported that chromosome evolution tends to evolve as a whole toward increasing chromosome number by centric fission and that centric fusion plays a biological role in eliminating heterochromatin.

Considering cytogenetic information of distinct groups of Formicidae [reviewed by Mariano CSF, PhD thesis, Federal University of Viçosa, Viçosa, Brazil], great chromosome diversity is recognized for different ant subfamilies as Myrmicinae ($2n=8-70$), Myrmeciinae ($2n=2-94$) and Ponerinae ($2n=8-106$). Those studies also pointed out that subfamilies considered as primitive have shown karyotypes

with high chromosome number, generally with diversification of chromosome morphology and reduction in chromosome sizes. In the light of the minimum interaction theory, we can expect that once those groups suffered an early radiation, a high number of chromosome events were required to evolve to modern karyotype structure.

A recent study on the phylogeny and evolution of Pseudomyrmecinae [19] with molecular and morphological data, confirms the monophyly of the subfamily, as well as most of its groups (exceptions for *pallens* and *viduus* groups). Also these data support the hypothesis, recently confirmed by a molecular phylogeny of the whole Formicidae

family [13] of a sister-group relationship between Pseudomyrmecinae and Myrmeciinae, which implies a Cretaceous origin of the pseudomyrmecines stem-group in the southern hemisphere.

In this Pseudomyrmecinae phylogeny study [19], the clade arrangement allowed to recover seven of the nine species groups [15]. In this analysis, *P. gracilis* and *P. termitarius* belong to the two most basal clades of the genus, while the other taxa (or the ones closely related to those studied here) are distributed in several groups where the species of the *pallidus* group are neighbor on the consensus tree to the ant-plant specialists that live in association with *Tachigalia*, while those belonging to the *oculatus* and *pallens* groups are distributed in independent clades.

Based on cytogenetic information, considering that nine (seven *Pseudomyrmex* plus two *Tetraponera*) species are currently known at least for their chromosome number, we can ask about the chromosome rearrangements involved in karyotype evolution reaching currently karyotype diversity on *Pseudomyrmex* groups and the Pseudomyrmecinae subfamily as a whole. Unfortunately, there is no karyotypical information congruent with the Ward and Downie's study [19], neither among our observations nor on the *Tetraponera* genus [3,5]. Thus, we are unable to make any inference about the contribution of chromosome rearrangements to the speciation processes suffered by the genus *Pseudomyrmex* due to the lack of cytogenetic information. To supplement these studies, the cytogenetics of much more taxa in this subfamily should be necessary.

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