

Research Article

Contribution of Cytogenetics to the Debate on the Paraphyly of *Pachycondyla* spp. (Hymenoptera, Formicidae, Ponerinae)

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We present evidence of the paraphyly of the ant genus *Pachycondyla* resulting from our cytogenetic studies on 29 populations in 18 species from Brazil and French Guyana. It is likely that karyotypes with a large number of chromosomes and comprising mostly small acrocentric chromosomes in species within the *Pachycondyla stricto sensu* group resulted from a succession of centric fission events. On the other hand, karyotypes with a small chromosome number comprising mostly metacentric chromosomes are also interpreted as little derived and tend to undergo centric fission. The karyotypes of the group *Neoponera* are more heterogeneous and probably undergo successive cycles of rearrangements tending to increase the chromosome number by centric fission. The *apicalis* and *verena* complexes form two probable sister groups that evolved independently due to centric fissions (*verena*) and pericentric inversions (*apicalis*). Our results reveal the karyotype diversity in the genus and reinforce the hypothesis on the paraphyly of *Pachycondyla*.

1. Introduction

Among the Ponerinae, the genus *Pachycondyla* (Ponerinae, Ponerini) is one of the most ancient known genera of ants and still extant. A fossil species, *Pachycondyla rebekkae* Rust and Andersen, was found in calcareous rocks from the early Tertiary (± 55 million years) in formations in north-west Denmark [1]. The current distribution of this genus (*Pachycondyla sensu* Brown, in Bolton, [2]) is pantropical with 197 valid species [3, 4]. A recent review of the New World species of *Pachycondyla* reports 92 species alone in the Neotropics and characterizes 18 complexes of species based on morphological characters [4].

According to Kempf [5], *Pachycondyla* comprised only 10 species in the Neotropical region, whereas other Neotropical

taxa currently included in this group [3, 4] were distributed within the genera *Neoponera*, *Mesoponera*, *Pachycondyla*, *Termitopone*, and *Trachymesopus* in his catalogue. This classification was maintained until Bolton [2] proposed a synonymization based on arguments already discussed by Brown [6]. According to Schmidt's conclusions [7], and recently commented by Ward [8] who called it the "*Pachycondyla* problem," the group of ants currently denominated "*genus Pachycondyla*" is paraphyletic. Taking into account only the Neotropical taxa, this taxon would comprise six species groups (according to Schmidt's classification) not necessarily related.

Cytogenetic studies on insects not only can significantly contribute to the understanding of morphological characteristics but also can shed some light on taxonomic and

evolutionary aspects, as, for instance, on groups of species in sympatry [9] competing for the same resources or cryptic species complexes [10, 11]. In the order Hymenoptera, cytotaxonomy has been used by Baldanza et al. [12], Hoshihara and Imai [13], Gokhman [14], and Gokhman and Kuznetsova [15] as a character for taxonomic and evolutionary studies. The determination of a karyotype and the occasional observation of the occurrence of chromosome rearrangements are especially important to make inferences regarding evolutionary or speciation processes. Since gene expression is regulated at least partially by the location of neighboring genes, chromosome alterations can result in phenotype alterations [16] and drive speciation processes. Thus, the understanding of karyotype evolution is valuable for evolutionary, phylogenetic, and taxonomic studies [17, 18] and can be used as a tool to evaluate species diversity.

Regarding Formicidae, Lorite and Palomeque [19] report more than 750 morphospecies with known chromosome number, which is still a relatively small number considering the diversity of this family estimated to be about 21,000 species [20]. In the Ponerinae, cytogenetic studies have been published for 95 morphospecies in 12 genera with chromosome numbers ranging between $2n = 8$ and $2n = 120$ [19], which is a considerable variation when compared to the remaining ant families, except for Myrmeciinae. It is noteworthy that karyotype variation among populations of the same species is frequent in the genera *Myrmecia* [21] and *Pachycondyla* [22–25]. Among the Ponerinae, *Pachycondyla* has been the most studied genus (40 morphospecies) and also the one with the highest variation in chromosome number $2n = 12 - 104$ [19]. Several hypotheses have been tested to understand karyotype evolution in ants including the fusion, fission, and modal hypotheses summarized by Imai et al. ([26], see also [19, 27]). In 1988, Imai and collaborators proposed the Minimum Interaction Theory that states that the chromosome interactions in the interphasic nucleus are responsible for changes in the karyotypes [28, 29]. The same research group [21, 27] developed the karyographic method as a tool that allows to visually explain karyotype evolutionary processes based on metaphase rearrangements. Although this method has not been much used [18, 21, 30–32], it is the only way to compare a large set of karyotype data and make inferences about the studied groups, except for comparative studies using molecular cytogenetic techniques [33, 34].

According to Lorite and Palomeque [19], the chromosome groups reported in Formicidae suggest the occurrence of different patterns of karyotype evolution in different taxonomic groups. Aiming at contributing to the knowledge of Neotropical poneromorphs, our research group has been developing interdisciplinary studies on different species of the subfamily Ponerinae. In this study, we investigated a series of Neotropical taxa within the genus *Pachycondyla sensu* Brown (Table 1) whose monophyly has been questioned by some authors [7, 23]. We also discussed hypotheses regarding the evolution of lineages that comprise this taxon, which is so important for the conservation of forest biomes in the Neotropical region [4].

2. Material and Methods

Colonies of *Pachycondyla* spp. were collected in 13 localities (Table 2) in several states in Brazil and in French Guyana in areas of the Atlantic rainforest, cocoa plantations, Caatinga, and the Amazonian rainforest between 2000 and 2010. In order to make comparisons feasible, we used original and published information as shown in Table 2.

Species identification was carried out following the review by MacKay and MacKay [4] and the species complexes proposed by them. However, aiming at comparing the studied taxa, we also refer to the previous classification by Kempf [5], to the synonymization of different genera of Ponerinae under *Pachycondyla* by Brown in Bolton [2] and to a recent generic reclassification proposed by Schmidt [7] but still not fully formalized (Table 1).

Mitotic metaphases were obtained from cerebral ganglia and male gonads treated with 0.005% colchicine for 20–40 minutes and the chromosomes were stained with Giemsa 2% according to Imai et al. [29]. The images were captured using Image-Pro Discovery version 4.5 software under a clear field microscope. Metaphases of some of the taxa studied were used to exemplify chromosome patterns.

Our analyses were based on chromosome number and morphology. Unpublished information or available in literature [19, 35–37, Mariano et al., unpublished information] on chromosome number and their structure in the Ponerinae subfamily and for *Pachycondyla sensu* Brown is used for comparison. Chromosomes were classified according to Imai's terminology [38]. The karyotypes studied were grouped and compared mainly based on Schmidt's classification [7]. Inferences on karyotype evolution in groups within *Pachycondyla sensu* Brown were carried out based on karyographs following Imai and Crozier [27] and Imai et al. [21]. Such an analysis allows for the discussion of the direction of karyotype evolution at the taxonomic group level (Figures 1 and 6).

3. Results

A graphic comparison of the karyotype diversity among Neotropical species of Ponerinae and the taxa within the genus *Pachycondyla sensu* Brown is shown in a histogram (Figure 2). Species within the genus *Pachycondyla sensu* Brown and the Neotropical species in the same genus were discriminated from the remaining genera belonging to the subfamily Ponerinae. We found an ample chromosome variation, which had already been observed in Ponerinae, showing the extreme karyotype heterogeneity within this subfamily, especially when compared with the remaining subfamilies of Formicidae, except for the Australian Myrmeciinae [19].

The Neotropical taxa within the genus *Pachycondyla sensu* Brown studied and their respective classification according to Kempf [5], Bolton [2], Schmidt [7], and MacKay and MacKay [4] are listed in Table 1. Our results comprise four groups similar to Schmidt's proposal [7], and among these groups, *Neoponera* was the largest in this study and also the group with the most variable chromosome

TABLE 1: List of species considered here, generic classification according to Kempf, 1972 [5]; Brown [6] in Bolton, 1995 [2]; Schmidt's (2009) proposition for genera names; *Pachycondyla* species complex according to MacKay and MacKay's [4] and taxonomic unit names used in this study and based on ecological, cytogenetic, and morphological evidences.

<i>Pachycondyla</i> species	Genera according to Kempf, 1972 [5]	Genus according to Brown in Bolton, 1995 [2]	Schmidt's (2009) genera name proposition	MacKay and MacKay's [4] <i>Pachycondyla</i> species complex	Name of the taxonomic unit used in this study
<i>Pachycondyla apicalis</i> (Latreille, 1802)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>apicalis</i>	<i>Neoponera-apicalis</i>
<i>Pachycondyla arhuaca</i> (Forel, 1901)	<i>Mesoponera</i>	<i>Pachycondyla</i>	<i>Pachycondyla (Incertae Sedis)</i>	<i>arhuaca</i>	—
<i>Pachycondyla carinulata</i> (Roger, 1861)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>crenata</i>	<i>Neoponera-crenata</i>
<i>Pachycondyla concava</i> (Mackay and Mackay, 2010)	—	<i>Pachycondyla</i>	—	<i>emiliae</i>	<i>Neoponera-emiliae</i>
<i>Pachycondyla constricta</i> (Mayr, 1884)	<i>Mesoponera</i>	<i>Pachycondyla</i>	<i>Mayaponera</i>	<i>constricta</i>	—
<i>Pachycondyla crassinoda</i> (Latreille, 1802)	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>crassinoda</i>	<i>Pachycondyla</i>
<i>Pachycondyla crenata</i> (F Smith, 1858)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>crenata</i>	<i>Neoponera-crenata</i>
<i>Pachycondyla curvinodis</i> (Forel, 1899)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>foetida</i>	<i>Neoponera-foetida</i>
<i>Pachycondyla gilberti</i> (Kempf, 1960)	<i>Trachymesopus</i>	<i>Pachycondyla</i>	<i>Pseudoponera</i>	<i>stigma</i>	<i>Pseudoponera</i>
<i>Pachycondyla goeldii</i> (Forel, 1912)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>crenata</i>	<i>Neoponera-crenata</i>
<i>Pachycondyla harpax</i> (Fabricius, 1804)	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>crassinoda</i>	<i>Pachycondyla</i>
<i>Pachycondyla impressa</i> (Roger, 1861)	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>crassinoda</i>	<i>Pachycondyla</i>
<i>Pachycondyla inversa</i> (F Smith, 1858)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>foetida</i>	<i>Neoponera-foetida</i>
<i>Pachycondyla marginata</i> (Roger, 1861)	<i>Termitopone</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>laevigata</i>	<i>Neoponera</i>
<i>Pachycondyla metanotalis</i> (Luederwaldt, 1918)	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>emiliae</i>	<i>Pachycondyla</i>
<i>Pachycondyla moesta</i> (Mayr, 1870)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>crenata</i>	<i>Neoponera-crenata</i>
<i>Pachycondyla stigma</i> (Fabricius, 1804)	<i>Trachymesopus</i>	<i>Pachycondyla</i>	<i>Pseudoponera</i>	<i>stigma</i>	<i>Pseudoponera</i>
<i>Pachycondyla striata</i> (Smith, 1858)	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>Pachycondyla</i>	<i>crassinoda</i>	<i>Pachycondyla</i>
<i>Pachycondyla succedanea</i> (Roger, 1863)	<i>Trachymesopus</i>	<i>Pachycondyla</i>	<i>Pseudoponera</i>	<i>stigma</i>	<i>Pseudoponera</i>
<i>Pachycondyla unidentata</i> Mayr, 1862	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>crenata</i>	<i>Neoponera-crenata</i>
<i>Pachycondyla venusta</i> (Forel, 1912)	<i>Neoponera</i>	<i>Pachycondyla</i>	—	<i>emiliae</i>	<i>Neoponera-emiliae</i>
<i>Pachycondyla verенаe</i> (Forel, 1922)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>apicalis</i>	<i>Neoponera-verenaе</i>
<i>Pachycondyla villosa</i> (Fabricius, 1804)	<i>Neoponera</i>	<i>Pachycondyla</i>	<i>Neoponera</i>	<i>foetida</i>	<i>Neoponera-foetida</i>

TABLE 2: Chromosome number and karyotypes of 29 Neotropical *Pachycondyla* populations/species. FG: French Guyana, others: Brazil: BA: state of Bahia, MG: state of Minas Gerais, SP: state of São Paulo. *Referred to as *P. gp. venusta* in the original publication.

Species	2n	Karyotype	Locality/coordinates	Reference
<i>P. apicalis</i>	2n = 36	28M + 8A	Ilhéus-BA; 14°45'S 39°13'W	[25]
<i>P. apicalis</i>	2n = 40	30M + 10A	Belmonte-BA; 16°05'S 39°12'W	[25]
<i>P. apicalis</i>	2n = 68	48M + 20A	Uruçuça-BA; 14°34'S 39°16'W	[25]
<i>P. arhuaca</i>	2n = 36	36A	FG: Chutes Voltaire 05°27'N 54°03'W	this study
<i>P. concava</i> *	2n = 54	6M + 48A	Itoioró-BA; 15°7'S 40°5'W	[35]
<i>P. carinulata</i>	2n = 24	4M + 20A	Ilhéus-BA; 14°45'S 39°13'W	[35]
<i>P. constricta</i>	2n = 30	30A	Ilhéus-BA; 14°45'S 39°13'W	[35]
<i>P. crassinoda</i>	2n = 62	22M + 40A	Ilhéus-BA; 14°45'S 39°13'W	[14]
<i>P. crenata</i>	2n = 26	2M + 24A	Viçosa-MG; 20°45'S 45°52'W	[23]
<i>P. curvinodis</i>	2n = 26	4M + 22A	Ilhéus-BA; 14°45'S 39°13'W	[35]
<i>P. curvinodis</i>	2n = 28	22M + 6A	Una-BA; 15°16'S 39°05'W	[35]
<i>P. gilberti</i>	2n = 12	10M + 2A	Arataca-BA; 15°15'S 39°24'W	this study
<i>P. goeldii</i>	2n = 24	24A	FG: Petit Saut; 05°20'N 53°41'W	[35]
<i>P. harpax</i>	2n = 96	12M + 84A	Ilhéus-BA; 14°45'S 39°13'W	[24]
<i>P. impressa</i>	2n = 94	8M + 86A	Ibicuí-BA; 14°53'S 40°02'W	this study
<i>P. inversa</i>	2n = 30	20M + 10A	Ilhéus-BA; 14°45'S 39°13'W	[35]
<i>P. marginata</i>	2n = 46	28M + 18A	Viçosa-MG; 20°45'S 45°52'W	[35]
<i>P. moesta</i>	2n = 26	26A	Viçosa-MG; 20°45'S 45°52'W	[23]
<i>P. metanotalis</i>	2n = 70	16M + 54A	Camacã-BA; 15°23'S 39°33'W	this study
<i>P. stigma</i>	2n = 12	12M	Porto Seguro-BA; 16°23'S 39°10'W	this study
<i>P. striata</i>	2n = 104	4M + 100A	Camacã-BA; 15°23'S 39°33'W	[24]
<i>P. succedanea</i>	2n = 14	14M	FG: Chutes Voltaire 05°27'N 54°03'W	this study
<i>P. unidentata</i>	2n = 12	12M	Ilhéus-BA; 14°45'S 39°13'W	[35]
<i>P. venusta</i>	2n = 48	26M + 22A	Viçosa-MG; 20°45'S 45°52'W	[35]
<i>P. verenae</i>	2n = 42	30M + 12A	Ilhéus-BA; 14°45'S 39°13'W	[25]
<i>P. verenae</i>	2n = 62	14M + 48A	Ilhéus-BA; 14°45'S 39°13'W	[25]
<i>P. verenae</i>	2n = 58 – 60	14M + 44A	Viçosa-MG; 20°45'S 45°52'W	[25]
<i>P. verenae</i>	2n = 64	12M + 52A	Rio Claro-SP; 22°23'S 47°32'W	[25]
<i>P. villosa</i>	2n = 34	12M + 22A	Ilhéus-BA; 14°45'S 39°13'W	[35]

number and karyotypes (Table 2). We present information on taxa within nine of the 18 species complexes defined by MacKay and MacKay [4] (Table 2). When comparing these classifications, there is unanimity among the authors solely on the *Pachycondyla stricto sensu* group. There is a certain consensus regarding the group *Neoponera* according to Kempf's catalogue [5] and Schmidt's proposal [7] (Table 1). Although Schmidt [7, page 197] has placed *Pachycondyla metanotalis* Luederwaldt, 1918 in his clade *Neoponera*, we followed Kempf's classification [5] for the aforementioned species since Schmidt's proposal is not backed by any new data, for the fact that *P. metanotalis* is a soil-dwelling species as most species in the clade *Pachycondyla* [5, 7], for morphological criteria not detailed herein, and because its karyotype is much closer to other species in the *Pachycondyla stricto sensu* group than to the *Neoponera* in this study.

A total of 29 populations was studied (Table 2), and several different populations were sampled for some taxa, therefore each line in this table represents one of these

such populations as they can have different karyotypes with distinct characteristics. The chromosomes are classified [38] according to if they are acrocentric (A) or metacentric (M). The chromosome complements found are extremely variable showing from a few metacentric chromosomes ($2n = 12$) of large size (*Pachycondyla unidentata* Mayr, 1862) to a large number of minute acrocentric chromosomes ($2n = 104$ in *Pachycondyla striata* (Smith, 1858) (Table 2) and confirming the tendency shown in Figure 1: karyotypes with a few chromosomes have large chromosomes whereas karyotypes with a large number of chromosomes have small chromosomes (see examples in Figure 3).

The simple observation of chromosome morphology reveals great similarity among karyotypes of *Pachycondyla crassinoda* (Latreille, 1802), *Pachycondyla harpax* (Fabricius, 1804), *Pachycondyla impressa* Roger, 1861, *P. metanotalis*, and *P. striata* (group *Pachycondyla sensu stricto*) and also in the karyotypes of *Pachycondyla gilberti* (Kempf, 1960), *Pachycondyla succedanea* (Roger, 1863), and *Pachycondyla*

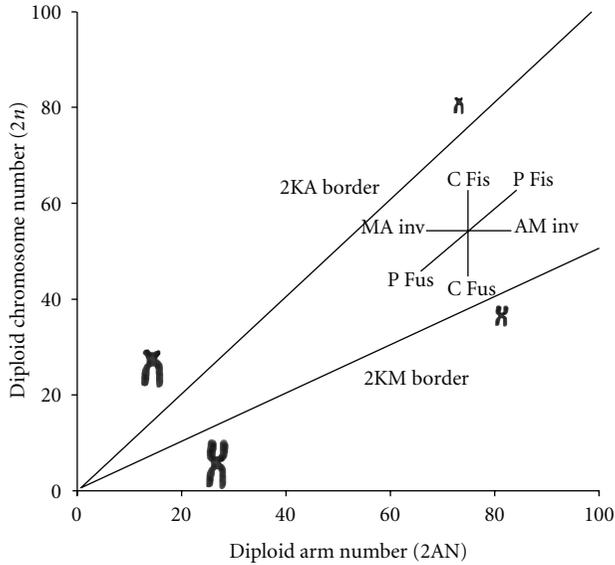


FIGURE 1: Karyograph adapted from Imai et al. [21] for ants. Since the genome is approximately constant for the whole Formicidae family, a proportional smaller chromosome size corresponds to the increase in the chromosome number. 2KA border: upper limit to the number of acrocentric chromosomes in diploid cells; 2KM border: lower limit to the number of metacentric chromosomes in diploid cells. C Fis: centric fission, C Fus: centric fusion, P Fis: pericentric fission, P Fus: pericentric fusion, AM inv: acrocentric-metacentric inversion, MA inv: metacentric-acrocentric inversion.

stigma (Fabricius, 1804) (group *Pseudoponera*), which coincidentally also form such groups according to Kempf [40], Schmidt [7], and MacKay and MacKay [4] (Table 2).

The study of the several clusters of *Pachycondyla sensu* Brown using the karyograph method (Figure 4) shows the clustering of species within the *Pachycondyla sensu stricto* group, all with a large number of acrocentric chromosomes, of species of *Pseudoponera*, with predominately metacentric chromosomes, and the great variation found in the karyotypes of species classified within the group *Neoponera*. The point distribution suggests that the most frequent rearrangements in these karyotypes were centric fissions and pericentric inversions (A-M type), and these rearrangements favor an increase in the number of chromosomes. Except for an isolated point on the right close to the 2KM limit in Figures 4 and 5 (which represents the population of *Pachycondyla apicalis* (Latreille, 1802) from Uruçuca), the karyotypes with larger numbers of chromosomes also tend to have mostly acrocentric chromosomes.

Some species have the same chromosome number but their morphology can be quite variable as a result of the aforementioned rearrangements. Six species have karyotypes that comprise only one morphological type of chromosome; in three of these species the karyotype is comprised of acrocentric chromosomes exclusively, and the other three species have karyotypes with only metacentric chromosomes (Table 2, Figures 4 and 5). In the karyograph, which shows taxa within the group *Neoponera* (11 species, 16 karyotypes, Figure 5),

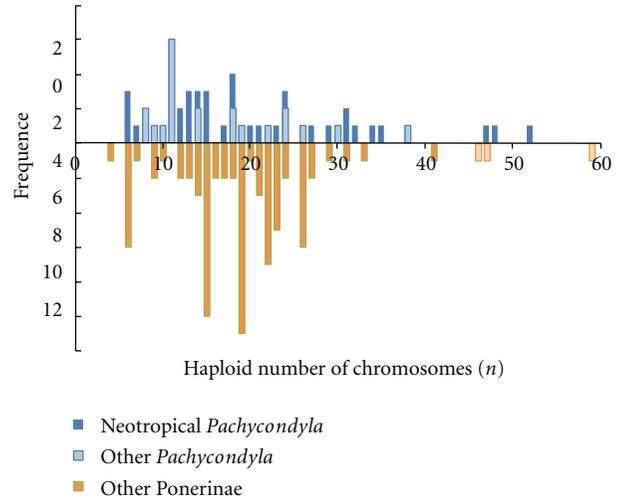


FIGURE 2: Distribution and frequency of haploid chromosome numbers in the Ponerinae subfamily, up to the X-axis: *Pachycondyla* spp.; down to the X-axis: other Ponerinae. The bars in lighter color in the range of “other Ponerinae” represent the known karyotypes in the *Dinoponera* genus (ref: as in Table 1 for Neotropical *Pachycondyla* spp.; for the others (genera *Anochetus*, *Centromyrmex*, *Cryptopone*, *Diacamma*, *Dinoponera*, *Hypoponera*, *Leptogenys*, *Odontomachus*, *Odontoponera*, *Platythyrea*, *Ponera*): [19, 36, 37, 39], *Hypoponera* spp.: $n = 6, 19$; *Leptogenys* spp.: $n = 15, 21$; *Platythyrea* spp.: $n = 20, 22$; *Thaumatomyrmex* spp.: $n = 10, 21, 31$ [Mariano et al., unpublished information]).

we highlighted the clusters of species within the *apicalis*, *crenata*, *emiliae*, *foetida*, and *verenae* groups.

Finally, we hypothesized the possible pathways of karyotype evolution in several groups of the Neotropical *Pachycondyla sensu* Brown for which we have enough data (nomenclature according to the last column of Table 1): *Neoponera apicalis*, *Neoponera crenata*, *Neoponera foetida*, *Neoponera verenae*, *Pseudoponera* and *Pachycondyla sensu stricto*. The representation (Figure 6) follows the model suggested by Imai and Crozier [27] developed for the interpretation of mammal karyotype evolution and is based on a hypothesis of karyotype variation essentially driven by fission.

4. Discussion

We can observe groups associated to the taxonomic position of species (Table 1) and some coincided with Schmidt's proposal [7], which splits *Pachycondyla* into 13 clades, with *Mayaponera* and *Neoponera* (both endemic), *Pseudoponera*, and *Pachycondyla* for the Neotropical Region.

Contrary to what has been reported for genera such as *Atta*, *Acromyrmex*, and *Pheidole*, in which the species already studied have a constant or not so variable karyotype [19], the karyotype groupings are extremely variable in species of *Pachycondyla* as well as in some distinct populations of the nominal species. Chromosome morphology is also variable, and it is noteworthy that, in most karyotypes with large chromosome numbers ($n > 11$, according to

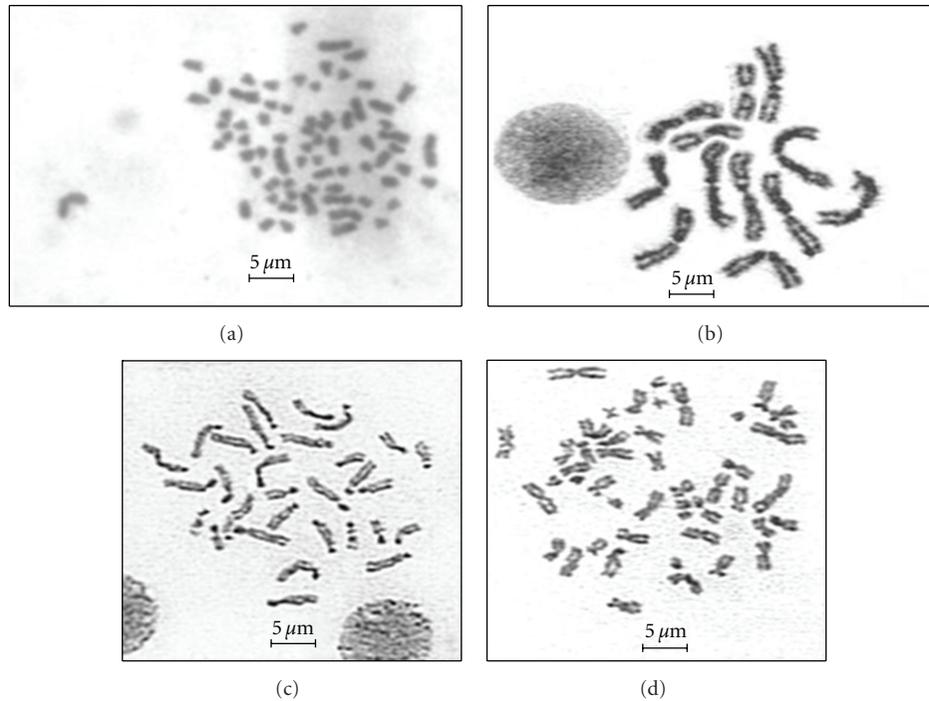


FIGURE 3: Metaphasic plates representing the different chromosome patterns found in *Pachycondyla* species. (a) *Pachycondyla impressa*, $2n = 70$, karyotype with a high number of chromosomes, mostly acrocentric. (b) *Pachycondyla unidentata*, $2n = 12$, karyotype with a low number of chromosomes, comprised exclusively by type M chromosomes of large size. (c) *Pachycondyla arhuaca*, $2n = 36$, Karyotype comprised exclusively by type A chromosomes. (d) *Pachycondyla venusta*, $2n = 54$, Karyotype comprised by types A and M chromosomes, a pattern found in many species and very common in the *Neoponera* group.

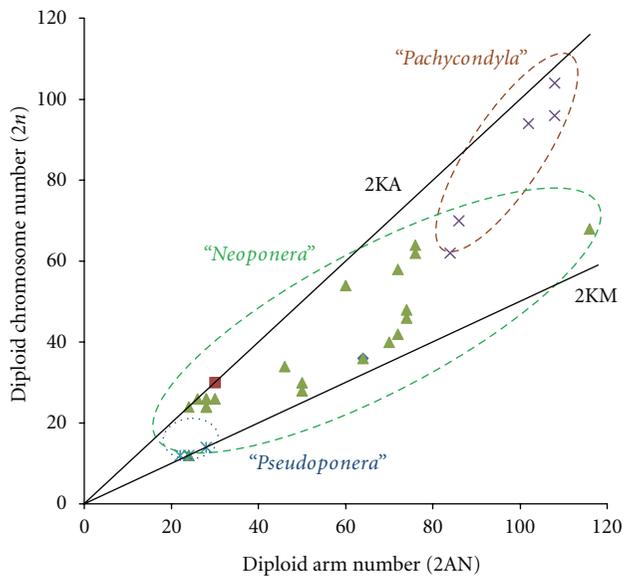


FIGURE 4: Karyograph of the Neotropical *Pachycondyla sensu* Brown. The ellipses circle the more representative species groups of our sampling according Schmidt [7] (see Table 1); two isolated points (square, diamond) represent single taxa not incorporated in a group (*P. arhuaca*, *P. constricta*).

the criteria of Imai et al., [41]), the chromosomes are submetacentric and acrocentric, which allows us to infer that fission and pericentric inversions (A-M ou M-A) are the most frequent chromosome rearrangements in the evolution

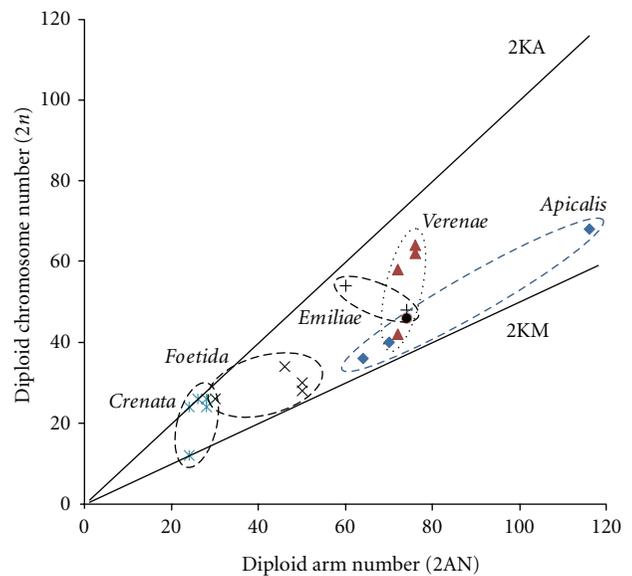


FIGURE 5: Karyograph of the Neotropical *Neoponera* according the Schmidt's proposal. The ellipses circle the more representative species groups (last column of Table 1); an isolated black circle represents a single species (*P. marginata*) not incorporated in a group.

of these karyotypes. These rearrangements can be either responsible or coadjutant in speciation processes, especially in the complexes of cryptic species sampled in this study (*apicalis*, *verenae*, and *foetida* groups).

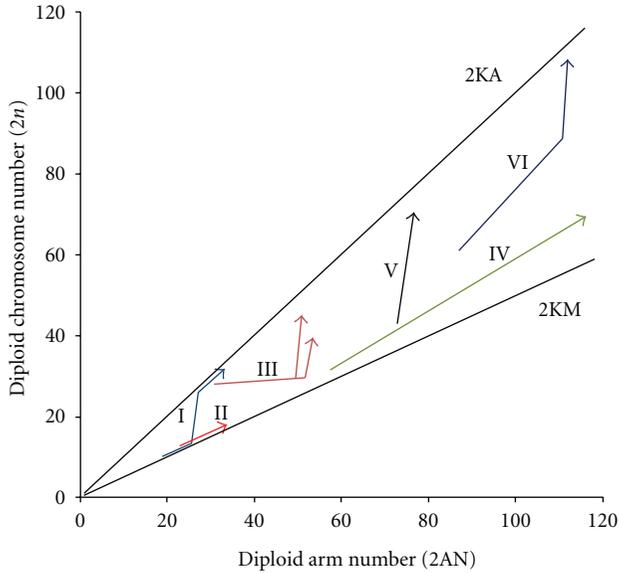


FIGURE 6: Possible pathways of karyotype evolution in several groups of Neotropical *Pachycondyla sensu* Brown. The Roman numbers correspond to the more representative species groups studied here (nomenclature according the last column of Table 1): I: *Neoponera-crenata*; II: *Pseudoponera*; III: *Neoponera-foetida*; IV: *Neoponera-apicalis*; V: *Neoponera-verenae*; VI: *Pachycondyla sensu stricto*. This schematic representation follows the suggested model of Imai and Crozier [27] for karyotypical evolution in mammals and is based on a hypothesis of a karyotype variation essentially driven by fission.

According to MacKay and MacKay [4], even though *Pachycondyla sensu* Brown is morphologically heterogeneous, the morphological characters were not consistent enough to justify splitting the group into distinct genera. However, similarly to Schmidt's [7], our results question the monophyly of *Pachycondyla* in its current acceptance. Thus, our results suggest the cooccurrence of multiple genera once there are totally independent patterns of karyotype evolution that strongly converge with Schmidt's conclusions [7]. We found groups with distinct patterns of karyotype evolution thus organized.

- (a) Karyotypes with a large number of chromosomes and comprising mostly small acrocentric chromosomes in species within the *Pachycondyla stricto sensu* group (*P. crassinoda*, *P. harpax*, *P. impressa*, *P. metanotalis*, and *P. striata*) which most likely resulted from a succession of centric fission events (Figures 3(a), 6). These karyotypes follow the same pattern of those found in the three species of *Dinoponera* with available cytogenetic information [42], which is the sister genus of the clade *Pachycondyla* according to Schmidt [7].
- (b) Karyotypes with a small chromosome number ($n \leq 11$ according to criteria in Imai et al. [41]) and comprising mostly metacentric chromosomes correspond to the pattern found in species within the group *Pseudoponera* (*P. cauta*, *P. gilbertii*, and *P. stigma*) and

can be interpreted as little derived karyotype patterns which tend to undergo centric fission (Figure 3(b)).

- (c) The karyotypes of *Neoponera* exemplify the karyotype evolution according to the model proposed by Imai et al. [21]: the karyotypes undergo successive cycles of rearrangements tending to increase the chromosome number by centric fission. The species included in *Neoponera* are considered the most diverse morphologically and behaviorally among the ponerine [7] and this diversity translates into the variety of karyotypes (Figure 3(c)).
- (d) The case of populations within the taxa *P. apicalis* and *P. verenae* studied herein exemplifies an interesting evolutionary model based on biogeography. The two forms coexist along their range, which comprises practically only tropical and subtropical terrestrial environments in the Neotropical Region [25, 43]. A more refined analysis of the morphological criteria suggests that each nominal taxon is a complex of cryptic species of allopatric distribution [25], which is corroborated by the cytogenetic study: the *apicalis* and *verenae* complexes form two probable sister groups that probably evolved independently due mainly to centric fissions (*verenae*) and pericentric inversions (*apicalis*) (Figures 5 and 6).

All these examples adequately illustrate the karyotype heterogeneity in *Pachycondyla* and reinforce the argument of the cooccurrence of several genera, at least in the Neotropical region. The cytogenetic studies indicate groupings that do not seem to have recent ancestry and also strongly suggest the paraphyly of the "*Pachycondyla* problem," according to Ward [8], as each group follows a distinct evolutionary pattern (Figure 4).

Some of these patterns are not exclusive of the species represented herein; they have been reported in known ant karyotypes such as in species of the Australian genus *Myrmecia* [21] and corroborate the occurrence of different evolutionary patterns in insects.

The diversity of karyotypes found in the known species of *Pachycondyla* in the Neotropics is supported by the antiquity of this group of ants and reinforces a tendency observed in karyotypes of Formicidae: the increase and diversification of chromosome number and morphology in a basal subfamily such as Ponerinae contrasting with the low variation and relative stability in some genera of more derived subfamilies such as Dolichoderinae, Formicinae, and Myrmicinae [22, 44]. A similar phenomenon was observed in the Australian Myrmeciinae [21, 26], but the idea formerly well accepted that these ants are basal and ancestral is no longer supported by recent molecular phylogenies [45]. This situation leads to the very intriguing question of what is shared by the Myrmeciinae and Ponerinae to be so variable with respect to their karyotypes whereas karyotypes seem to be rather uniform in related subfamilies?

Among the ants, it is noteworthy the occurrence of cryptic species complexes and sibling species: morphologically indistinguishable species recently diverged (sibling-species) or that maintain strongly convergent characters

(cryptic species), and not separable using the traditional methods of identification [10, 11], in which characters such as behavior, chemical signature, and karyotype composition act as mechanisms of reproductive isolation (in Neotropical *Pachycondyla*, see, for instance, Lucas et al. [46]). Such a phenomenon has been reported for ants and many other organisms, and there are likely to be different speciation processes as there are multiple species concepts [47]. Thus, the use of different criteria for the description of species (alpha level taxonomy) is justified, and these criteria have been tested using the integrated taxonomy approach, which consists of using complementary areas such as molecular genetics, ecology, behavior, cytogenetics, and chemistry among others [48]. This approach strengthens the necessity of interdisciplinary studies and emphasizes the importance of multiple tools for taxonomic studies, a consensus among several authors [48, 49]. Therefore, besides confirming the validity of a species recognized by other methods, cytogenetics can contribute to the study of the origin and definition of species limits, as well as to the understanding of the evolution of organisms [49]. We hope our study will shed some light on the classification of the genus *Pachycondyla*, which still needs further disentangling.

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