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Cytogenetic analysis of *Pseudoponera stigma* and *Pseudoponera gilberti* (Hymenoptera: Formicidae: Ponerinae): a taxonomic approach

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Abstract

Pseudoponera stigma (F.) and *Pseudoponera gilberti* (Kempf) (Hymenoptera: Formicidae) are closely related Neotropical ants, often misidentified due to their morphological similarities. These species also share behavioral and ecological characters. In this study, we examined cytogenetic approaches as a tool to aid identification of *P. stigma* and *P. gilberti*. Both numerical and morphological karyotypic variations were identified based on different cytogenetic techniques. The karyotype formula of *P. stigma*, 2K = 10M + 4SM differs from that of *P. gilberti*, 2K = 10M + 2SM, and the CMA_3^+/$DAPI^-$ sites also differ, allowing both species to be distinguished by chromosomal characters.

Key Words: ant; cytotaxonomy; karyotype; CMA_3/DAPI

Resumen

Pseudoponera stigma (F.) y *Pseudoponera gilberti* (Kempf) (Hymenoptera: Formicidae) son hormigas neotropicales muy estrechamente relacionadas que a menudo son mal clasificadas debido a sus similitudes morfológicas. Estas especies también comparten caracteres de comportamiento y ecológicas. En este estudio, la citogenética fue utilizado como una herramienta para la caracterización y delimitación taxonómica de *P. stigma* y *P. gilberti*. Se describen variaciones cariotípicas numéricos y morfológicas en base a diferentes técnicas de citogenética. La fórmula cariotipo de *P. stigma*, 2K = 10M + 4SM difiere de la de *P. gilberti*, 2K = 10M + 2SM, así como las localizaciones de los sitios CMA_3^+/$DAPI^-$, lo que permite distinguir las especies tanto por caracteres cromosómicas.

Palabras Clave: hormigas; citotaxonomía; cariotipo; CMA_3/DAPI

Previously published cytogenetic studies of 95 ant morphospecies in the subfamily Ponerinae revealed high variation in chromosome number, ranging from 2*n* = 8 to 2*n* = 120 (Lorite & Palomeque 2010; Mariano et al. 2012). An earlier study of *Pseudoponera* Emery (Mariano et al. 2012) with conventional cytogenetics included 3 species previously placed in the genus *Pachycondyla* (Schmidt & Shattuck 2014). These species have karyotypes with both low chromosome numbers and high frequency of metacentric chromosomes. The karyotypic formula 2K = 10M + 2A was reported for *Pseudoponera gilberti* (Kempf) (Kempf 1960), 2K = 12M for *Pseudoponera stigma* (F.) (Fabricius 1804), and 2K = 14M for *Pseudoponera succedanea* (Roger) (Roger 1863).

Studies of karyotype evolution in ants suggested that karyotypes with low chromosome numbers and large chromosomes exhibit basal characteristics whereas karyotypes with larger numbers of small chromosomes represent derived states (Imai et al. 1994). The trend towards formation of smaller chromosomes by centric fission could be driven by the advantage of reducing the frequency of deleterious chromosomal translocations resulting from physical interactions. This results in an increase in the chromosome number and in the acrocentric and telocentric content. Additionally, smaller acrocentric and telocentric chromosomes could be converted into meta- and submetacentric chromosomes by pericentric inversion, and centric fusions can also occur (Imai et al. 1986, 1988). Based on these assumptions, we hypothesized that the karyotypes of *P. stigma* and *P. gilberti* would share basal characteristics (Mariano et al. 2012).

Chromosome number and morphology have been the characters most commonly used in comparative cytogenetic studies of ants, especially among closely related species that are difficult to distinguish based on morphological characters (Mariano et al. 2012). However, other cytogenetic methods have been used recently, such as CMA_3/DAPI fluorochrome staining in *Dinoponera lucida* Kempf (Mariano et al. 2008), *Wasmannia auropunctata* (Roger) (Souza et al. 2011), *Odontomachus* Latreille, *Anochetus* Mayr (Santos et al. 2010), *Mycocepurus goeldii* (Forel) (Barros et al. 2010), and *Acromyrmex striatus* (Roger) (Cristiano et al. 2013). To aid in distinguishing *P. stigma* and *P. gilberti*, we characterized the chromosomes by conventional cytogenetic technique and CMA_3/DAPI fluorochrome staining.

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Fig. 1. Map of collection sites. The circles represent the collection points.

Materials and Methods

Colonies of *P. stigma* and *P. gilberti* were collected in forest areas or cocoa plantations in the states of Pernambuco, Bahia, and Espírito Santo, Brazil (Fig. 1; Table 1), from Oct 2011 to Aug 2013. Specimens were identified based on Mackay & Mackay (2010), Schmidt (2013), and Schmidt & Shattuck (2014) in addition to the original descriptions of each species. Vouchers from each sampled nest were deposited in the CPDC collection of the Laboratório de Mirmecologia CEPEC/CEPLAC at Ilhéus, Bahia, Brazil.

Metaphase plates were obtained from cerebral ganglion cells of prepupae by following the methods of Imai et al. (1988). Prepared slides were stained with Giemsa solution in 0.06 M phosphate buffer, pH 6.8, at a ratio of 1:30 for 30 min. Metaphase slides of high quality were photographed with an Olympus BX-41 photomicroscope with a digital camera attached. Karyograms were organized with the use of Adobe Photoshop CS6 software 13.0x 64, arranged according to Levan et al. (1964), and karyotypic formulas were determined from the karyograms.

Base-specific fluorochrome double staining with chromomycin A_3 (CMA_3) and 4,6-diamidino-2-phenilindole (DAPI) followed the method of Schweizer (1976), with modifications proposed by Guerra & Souza (2002). Slides were mounted with Vectashield mounting medium and covered with a coverslip. Slides were analyzed in a DMRA2 Leica epifluorescence photomicroscope and images captured with the Leica IM50 software (Leica Microsystems Imaging Solutions Ltd., Cambridge, United Kingdom).

Results

Thirteen colonies and 182 specimens of both species were sampled, although *P. gilberti* was the most frequently collected (Table 1). Cytogenetic analysis based on multiple samples of *P. gilberti* and *P. stigma* consistently showed a distinct karyotype for each species. Chromosome numbers and karyotypic formulas for each nest sampled and analyzed are given in Table 1.

The karyotype of *P. gilberti* showed $2n = 12$ (females) and $n = 6$ (males), with the 1st pair larger than the remaining chromosomes. With the exception of the 4th chromosome pair that was submetacentric, the remaining chromosomes were metacentric (Figs. 2a, b, and e). The karyotypes of *P. stigma* had $2n = 14$ (females) and $n = 7$ (males) chromosomes (Figs. 2c, d, and f). In this species, the 1st and 2nd pairs were larger and differed in size whereas the remaining chromosomes were very similar in length. The 3rd and 4th pairs were submetacentrics and the remaining chromosomes were metacentric.

Fluorochrome staining in *P. gilberti* revealed the presence of a single and conspicuous $CMA_3^+/DAPI^-$ interstitial marking, indicating a segment rich in GC base pairs, in the 1st pair of chromosomes (Fig. 2e). In *P. stigma*, the $CMA_3^+/DAPI^-$ stained segment was located on the short arm of the 4th chromosome pair (Fig. 2f).

Discussion

Both *P. stigma* and *P. gilberti* have very similar external morphology (Kempf 1960; Mackay & Mackay 2010). They are distributed sympatrically and mate at the same time of year (Mackay & Mackay 2010). These species differ mainly in the shape and sculpturing of clypeus and mandibles (Kempf 1960; Mackay & Mackay 2010).

High morphological similarity and the complex taxonomy of this group, especially prior to the revision of *Pachycondyla* (Mackay & Mackay 2010), made identification of these species difficult, and may have contributed to conflicting results in previous studies (e.g., Mariano et al. 2012). In the present study, which included a large sample size, the karyotypes with $2n = 12$ (2K = 10M + 2SM) for *P. gilberti* and $2n = 14$ (2K = 10M + 4SM) for *P. stigma* were consistently verified in different localities, a result that reinforces the importance of integrated studies using both morphological and genetic data to aid in delimitating similar taxa.

The karyotypes of *P. gilberti* and *P. stigma*, with few chromosomes and a predominance of metacentric and submetacentric chromosomes,

Table 1. Collection localities, species, geographic coordinates, number of nests, and specimens sampled.

Collection locality (municipality)–Brazilian state	Species	Geographic coordinates		No. of nests (No. of specimens)	2*n* (*n*)	Karyotype formula (2K)
		Latitude	Longitude			
Igrapiúna (Res. Michelin)–BA	*P. gilberti*	13.6458°S	39.1706°W	1 (12)	12 (6)	10M + 2SM
Itajuípe (CEPLAC)–BA	*P. gilberti*	14.6850°S	39.3669°W	1 (18)	12 (6)	10M + 2SM
Moreno–PE	*P. gilberti*	8.1400°S	35.1494°W	2 (38)	12	10M + 2SM
Porto Seguro (ESPAB)–BA	*P. gilberti*	16.4192°S	39.1611°W	2 (23)	12 (6)	10M + 2SM
Sooretama–ES	*P. gilberti*	19.1472°S	40.0706°W	1 (7)	12	10M + 2SM
Una (Faz. Ararauna)–BA	*P. gilberti*	15.2111°S	39.1847°W	2 (15)	12 (6)	10M + 2SM
Ilhéus (CEPLAC)–BA	*P. stigma*	14.7856°S	39.2222°W	3 (59)	14 (7)	10M + 4SM
Valença (Faz. Expedito)–BA	*P. stigma*	13.3361°S	39.1706°W	1 (10)	14 (7)	10M + 4SM

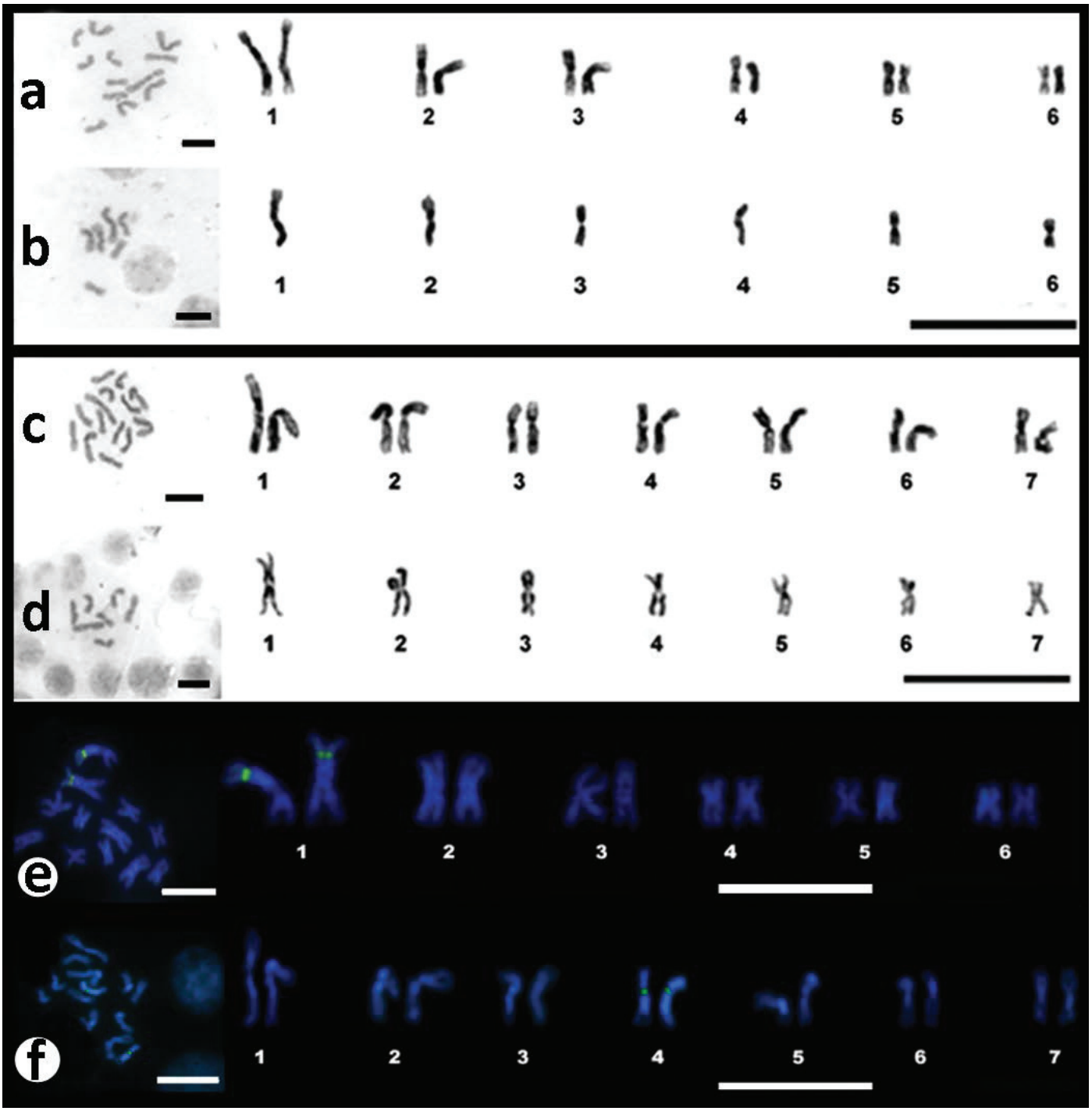

Fig. 2 Metaphases, (a–d) karyograms, and (e and f) karyograms with fluorochrome staining CMA_3/DAPI: (a) female and (b) male of *P. gilberti*; (c) female and (d) male of *P. stigma*; (e) CMA_3^+ band on the 1st pair, *P. gilberti*; (f) CMA_3^+ band on the 4th pair, *P. stigma*. Bar = 10 µm.

are in contrast to those of other species of Ponerini, which have up to *n* = 60 chromosomes. Low chromosome number is thought to be plesiomorphic (Imai et al. 1994; Lorite & Palomeque 2010; Mariano et al. 2012).

Other *Ponera*-group genera, such as *Diacamma* Mayr (Imai et al. 1984; Karnik et al. 2010), *Ponera* Latreille (Imai & Kubota 1972; Imai et al. 1988; Lorite & Palomeque 2010), and *Cryptopone* Emery (Imai & Kubota 1972; Imai et al. 1977, 1983), also have species with low chromosome numbers. Schmidt (2013) delimited a monophyletic clade of *Ponera*-group genera based on molecular data, but no morphological synapomorphies have been identified that support the clade (Schmidt & Shattuck 2014).

The CMA_3^+/$DAPI^-$ markings aided in characterizing the karyotypes and distinguishing between the 2 species. The distinct CMA_3^+/$DAPI^-$ sites, which are chromosomal segments rich in GC base pairs, in the karyotypes of *P. gilberti* (1st pair) and *P. stigma* (4th pair) may correspond to their Nucleolus Organizer Regions, as observed in other insects (Manicardi et al. 1996; Kuznetsova et al. 2001; Grozeva et al. 2004; Almeida et al. 2006; Santos et al. 2010). This correlation, however, must be further confirmed with the Nucleolus Organizer Regions banding technique.

Cytogenetic information combined with morphological data was effective in distinguishing *P. stigma* and *P. gilberti.* The original descrip-

tion of *P. stigma* was little detailed (Fabricius 1804; Mackay & Mackay, 2010). Individuals of this species are identified through comparison of morphological, biological, and ecological characters, which may cause errors in identification. A more detailed morphological analysis of *P. stigma*, with a new description of this species is currently in preparation.

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