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

João Paulo Sales Oliveira Correia¹, Cléa dos Santos Ferreira Mariano¹, Jacques Hubert Charles Delabie²,³, Sebastien Lacau⁴, and Marco Antonio Costa⁵,*

**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+/DAPI− sites also differ, allowing both species to be distinguished by chromosomal characters.

**Key Words:** ant; cytotaxonomy; karyotype; CMA/DAPI

Previously published cytogenetic studies of 95 ant morphospecies in the subfamily Ponerinae revealed high variation in chromosome number, ranging from 2n = 8 to 2n = 120 (Lorite & Palomeque 2010; Mariano et al. 2012). An earlier study of *Pseudoponera* Emery (Maria-no et al. 2012) with conventional cytogenetics included 3 species previ-ously 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 acro-centric and telocentric content. Additionally, smaller acrocentric and telocentric chromosomes could be converted into meta- and submeta-centric 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 distin-guish based on morphological characters (Mariano et al. 2012). However, other cytogenetic methods have been used recently, such as CMA/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), *Mycocaprus goeldii* (Forel) (Barros et al. 2010), and *Acro-ymnex 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/DAPI fluorochrome staining.

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¹Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, 45662-900 Ilhéus, Bahia, Brazil; E-mail: correia_jpso@yahoo.com.br (J. P. S. O. C.), csfmariano@gmail.com (C. S. F. M.), costama@uesc.br (M. A. C.)

²Laboratório de Mirmecologia, CEP/CEPLAC, Caixa Postal 7, 45600-970 Itabuna, Bahia, Brazil; E-mail: jacques.delabie@gmail.com (J. H. C. D.)

³Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, 45662-900 Ilhéus, Bahia, Brazil

⁴Laboratório de Biosistemática Animal, Universidade Estadual do Sudoeste da Bahia, Itapetinga 45700-000, Bahia, Brazil; E-mail: slacau@uesb.edu.br (S. L.)

⁵Corresponding author; E-mail: costama@uesc.br (M. A. C.)
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 A3 (CMA3) 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 a predominance of metacentric and submetacentric chromosomes, indicating a segment rich in GC base pairs, in the 1st pair of chromosomes (Fig. 2e). The karyotypes of *P. stigma* 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. gilberti* 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 submetacentric and the remaining chromosomes were metacentric.

Fluorochrome staining in *P. gilberti* revealed the presence of a single and conspicuous CMA+/DAPI interstitial marking, indicating similar taxa. In *P. stigma*, the CMA+/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 delimiting similar taxa.

The karyotypes of *P. gilberti* and *P. stigma*, with few chromosomes and a predominance of metacentric and submetacentric chromosomes,
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/DAPI markings aided in characterizing the karyotypes and distinguishing between the 2 species. The distinct CMA/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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