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## STUDIES ON THE KARYOTYPE OF THE ANT *PACHYCONDYLA HARPAX* (FORMICIDAE: PONERINAE: PONERINI) IN SOUTHERN BAHIA, BRAZIL

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### ABSTRACT

Cytogenetic analyzes were carried out on populations of *Pachycondyla harpax* (Fabricius, 1804) (Hymenoptera: Formicidae: Ponerini) in southern Bahia, Brazil. The chromosome number was variable with  $2n = 90-92$ , and the chromosomes, predominantly acrocentric, were characterized by being small in size. The karyotype formulas of the studied populations were: Ilhéus  $2K = 16M + 76A$ , Una  $2K = 16M + 74A$  and Belmonte  $2K = 16M + 76A$ . The karyotype of a fourth population (Igrapiúna) was undetermined. Fluorochrome banding shows the presence of heterochromatic blocks rich in GC in the pericentromeric region of one of the metacentric pairs, indicating the nucleolus organizer regions. The first case of a diploid male in the genus *Pachycondyla* is reported.

Key Words: chromosomes, fluorochrome banding metaphase, Ponerini, predatory ant, Neotropical Region

### RESUMÉN

Estudios citogenéticos fueron realizados en poblaciones de *Pachycondyla harpax* (Fabricius, 1804) (Hymenoptera: Formicidae: Ponerini) al sur de la Bahía, Brasil. El número cromosómico es variable, con  $2n = 90-92$ , cromosomas predominantemente acrocentricos caracterizados por ser de pequeño tamaño. Las formulas cariotípicas de las poblaciones estudiadas fueron: Ilhéus  $2K=16M + 76A$ , Una  $2K = 16M + 74A$  y Belmonte  $2K = 16M + 76A$ . El cariotipo de una cuarta población (Igrapiúna) no se determinó. Los resultados de coloración con fluorocromos muestran la presencia de bloques heterocromaticos ricos en GC en la región pericentromérica de uno de los pares metacéntricos, indicando la región organizadora de nucleótidos. Es reportado el primer caso de diploidia en machos del genero *Pachycondyla*.

Palabras Clave: cromosomos, bandas fluorocromo, metafase, Ponerini, hormiga cazadora, Región Neotropical

As currently defined (Mackay & Mackay 2010), *Pachycondyla* (Hymenoptera: Formicidae: Ponerini) has a pantropical distribution with 264 valid species (Bolton 2014). For the Neotropics, a recent revision of the genus (Mackay & Mackay 2010) increased the number of known species from 60, as recorded by Fernández & Sendoya (2004), to 91 species grouped into 18 complexes suggested from morphological characters. This diversity puts *Pachycondyla* amongst the most species-rich within the Ponerines. Studies exploring the genus' molecular genetics (Schmidt 2013)

and cytogenetics (Mariano et al. 2012) strongly suggest its paraphyly, and this is under review in several research groups. Currently *Pachycondyla harpax* (Fabricius, 1804) is included either in the true *Pachycondyla* genus (*sensu* Kempf 1972 & Schmidt 2013) or in the *crassinoda* group (*sensu* Mackay & Mackay 2010).

*Pachycondyla harpax* is considered the most common species of the genus in the New World with a widespread distribution (Mackay & Mackay 2010). It has been the subject of research evaluating morphological (Smith 1858; Forel

1901; Wheeler 1925; Norton 1968), behavioral (García-Perez et al. 1997) and genetic characters, sometimes within the context of biogeographical studies.

A range of cytogenetic studies have been conducted in several insect orders (Hoshiba & Imai 1993; Van Wilgenburg et al. 2006; Poggio et al. 2007; Tavares et al. 2010; Mariano et al. 2012), providing information independent from morphological characters, commonly used for taxonomic studies, that may reveal differences and/or similarities between organisms with a priori indistinguishable characters (Sessions 1996). In the Formicidae, Lorite & Palomeque (2010) reported chromosomal variation in over 750 morphospecies of ants, rendering the family the highest in chromosomal variability amongst the insects. Contrasting extremes within the ants are exemplified by *Myrmecia pilosula* ( $2n = 2$ ) (Crosland & Crozier 1986) and *Dinoponera lucida* ( $2n = 120$ ) (Mariano et al. 2004, 2008) as species with the lowest and highest numbers of chromosomes in the Formicidae, respectively.

Over 100 species of Ponerines have been karyotyped so far, and they are now considered as the subfamily with the greatest heterogeneity in their number of chromosomes (Lorite & Palomeque 2010). Mariano et al. (2012) reported for the genus *Pachycondyla* a variation ranging from  $2n = 12$  [*Pachycondyla unidentata* Mayr 1862] to  $2n = 104$  [*Pachycondyla striata* (Smith 1858)]. These results strengthen the idea of paraphyly of the genus proposed by Schmidt (2013).

Imai et al. (1986, 1988, 1994), in the course of studying more than 500 species of eukaryotes, stated that the chromosome interactions in the interphase nucleus are responsible for changes in the karyotypes, and that the outcomes of these interaction are fundamental in explanations of evolutionary processes. This idea has dominated studies of karyotype evolution in ants although other hypotheses have been discussed (Lorite & Palomeque 2010). In many cases, chromosomal rearrangements are directly engaged in the processes of speciation, where the relationship between these processes and evolution of the karyotype can be observed in some ant genera (see review by Lorite & Palomeque 2010).

Mariano et al. (2006) defined the chromosome number in *Pachycondyla harpax* individuals from a single colony in southern Bahia. However, the frequent karyotype variation in species of this genus, and its wide morphological variation, leads to suspect this "species" may be more than one (Mackay & Mackay 2010; Longino 2010). Thus, a study of cytogenetics, ecological and morphological features of *P. harpax* was carried out in order to verify if such information can be useful in recognizing sibling species (Mariano et al. 2006; Delabie et al. 2008; Mariano et al. 2012).

## MATERIAL AND METHODS

Colonies from different localities in the southern state of Bahia, Brazil, located in the Central Corridor of the Atlantic Forest, were collected from Apr, 2011 to May, 2012: Experimental fields of the Cocoa Research Center (CEPEC/CEPLAC), county of Ilhéus; Vera Cruz Farm, southwest of Una Biological Reserve (REBIO), county of Una; Experimental Station Gregory Bondar (CEPEC/CEPLAC) Barrolândia district, county of Belmonte; and Michelin Ecological Reserve, county of Igrapiúna.

A minimum of 6 larvae or pre-pupae were analyzed per colony, with slides prepared from cerebral ganglia of larvae or male gonads. Once extracted, samples were fixed for obtaining mitotic metaphase chromosomes following Imai et al. (1988). The slides were stained with Giemsa diluted in Sörensen buffer at 3%, pH 6.8. To determine chromosomal number and karyotype structure, we followed the nomenclature of Levan et al. (1964), which considers chromosome arm size ratios and arranges them based on the centromere position. Six slides from each colony were subjected to sequential fluorochrome staining Chromomicina A3 (CMA3) and 4'-diamidino-2-phenylindole (DAPI), which fluoresces when excited by light of a specific wavelength, following Schweizer (1980). Voucher specimens were deposited in the collection of the Laboratory of Myrmecology (CP-DC), Cocoa Research Center, CEPLAC, Itabuna, Bahia, Brazil. Metaphases were observed by an Olympus BX51 photomicroscope and by an epifluorescent Leica DMRA2 photomicroscope with a 1.6X magnifier. Images were captured using Leica IM50 software (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). Karyograms were organized using Adobe Photoshop 7.0 image editing software.

## RESULTS

Variation in chromosome number observed in the different colonies of *P. harpax* was  $2n = 90$  or  $2n = 92$ , and chromosomes were divided in 2 groups, acrocentric (A) and metacentric (M) (Table 1). All observed karyotypes were characterized by a large number of small chromosomes and composed of 8 pairs of metacentric chromosomes, common to the populations of Ilhéus, Belmonte and Una, plus a variable number of acrocentric chromosomes ranging between 37 and 38 pairs (Fig. 1).

Several male pupae were found only in one opportunity due to the seasonality (Sep to Nov) of the production of this sex, but the cytogenetic analysis was possible only on a single slide where 9 between 15 metaphases were in good conditions for study. It was found in the colony collected at Barrolândia (Belmonte), and presented a diploid

TABLE 1. COLLECTING LOCALITIES AND RESULTS OF CYTOGENETIC ANALYSES IN *PACHYCONDYLA HARPAX*, BAHIA, BRAZIL, MAY 2011-JUN 2012.

Locality	Coordinates	Col./ind*	2n	2K
Ilhéus	S 14°45.3' W 39°13.9'	2/11	92	2K = 16M+76A
Una	S 15°18.3' W 39°09.3'	1/12	90	2K = 16M + 74A
Belmonte	S 16°05' S W 39°12'	1/17	92	2K = 16M + 76A
Igrapiúna	S 13°47' W 39°0.9' W	1/6	92	not defined

\*Number of colonies and analyzed individuals.

karyotype similar to that of worker pre-pupae from the same colony (Fig. 1a) ( $2n = 92$ ), with 8 pairs of metacentric (M) and 38 pairs of acrocentric chromosomes (a), and with karyotype formula  $2K = 16M + 76A$  (Fig. 1b).

In metaphases submitted to fluorochrome staining 2 common  $CMA_3$  markings were observed in the pericentromeric regions of a metacentric pairs, in each of the observed karyotypes. DAPI staining these chromosome regions showed negative markings which reveal richness in GC base pairs (Figs. 2a, 2b, 2c and 2d). However, no specific pattern for regions rich in AT base pairs was observed in the studied populations.

#### DISCUSSION

Taking into account a previous analysis (Mariano et al. 2006), we currently know 3 different diploid karyotypes for *P. harpax* in southern Bahia, with  $2n = 90, 92, 96$  chromosomes. These are characterized by a large number of small chromosomes. Compared with other species of Ponerinae, these karyotypes exhibit a chromosomal pattern found in *Pachycondyla crassinoda*, *Pachycondyla impressa* and *Pachycondyla striata* (Mariano et al. 2006, 2012), as well as in 4 species of *Dinoponera* (Santos et al. 2012). This pattern seems characteristic to some basal genera and subfamilies (Mariano et al. 2004), in which chromosomes show a tendency for centric fissions and their consequent increase in number, while they decrease in size. This pattern is considered a rarity in ants (Santos et al. 2012).

Similar karyotype variability has been found in other species and/or complexes of the same genus in the same region of Bahia. Some species of the *Pachycondyla foetida* complex [*P. villosa*, *P. bactronica* (erroneously named *curvinodis*, see Fernandes et al. 2014), and *P. inversa*] (Mariano et al. 2012), with very similar external morphology have different karyotypes, agonistic behavior, and biochemical characters (Lucas et al. 2002). Similarly, such variability has been observed in karyotypes of the *Pachycondyla apicalis* species complex, which exhibit a very large variability, with  $2n = 36, 40, 68$  (Mariano 2004; Delabie et al. 2008), or *D. lucida* with  $2n = 106, 118$  or  $120$  (Mariano et al. 2008).

The fluorochrome  $CMA_3$  and DAPI-marking pattern is common to all populations studied. Marking was observed in a single metacentric chromosome pair (not individually identified on the karyotypes). According with Mariano & Delabie (2013) these complementary regions may indicate nucleolar organizer regions, as has already been observed in other ants, such as *Tapinoma nigerrimum* (Lorite et al. 1997) and *D. lucida* (Mariano et al. 2008). Generally the GC-rich chromatin (marked by  $CMA_3 +$ ) is associated with the location of nucleolar organizer region (NOR) (Sumner 2003), which is an important tool for evolutionary studies. The  $CMA_3 +$  -NOR association is common in many zoological groups such as in insects like bees (Rocha et al. 2002; Brito et al. 2003) or in vertebrates (Vicari et al. 2006), as well in chromosomes as in interphasic nuclei.

The diploid male karyotyped in *P. harpax* must be highlighted due to the small number of records of this phenomenon in Formicidae, besides the low number of males found in nests of genus *Pachycondyla* and the seasonality of the male production. Crozier & Pamilo (1996) recorded 17 cases of diploid males in ants, and although they are generally considered sterile (Cournault & Aron 2009), they do appear to be fertile in some species (Crozier & Pamilo 1996; Krieger et al. 1999; Yamauchi et al. 2001). The occurrence of diploid males has also been reported in more than 60 species of Hymenoptera, including several species of bees (solitary and social), wasps, and ants (Van Wilgenburg et al. 2006; Heimpel & Boer 2008; Tavares et al. 2010). All this suggests the occurrence of diploid males is a normal occurrence in the haplodiploid system, but it remains a rare event (within the context of the life span of a species). Simply put, diploid males are rarely studied because they are rarely sampled (Ross 1988; Mariano 2004).

Thus, karyotypic variation observed in *P. harpax* has been accompanied by little morphological variation, but with a clearly diverging number of chromosomes, suggesting the possible occurrence of cryptic speciation. Such a pattern has been observed in other species of the same genus (Lucas et al. 2002; Delabie et al. 2008; Mariano et al. 2012; Fernandes et al. 2014) as in other genera such as *Myrmecia pilosula* (Imai et al.

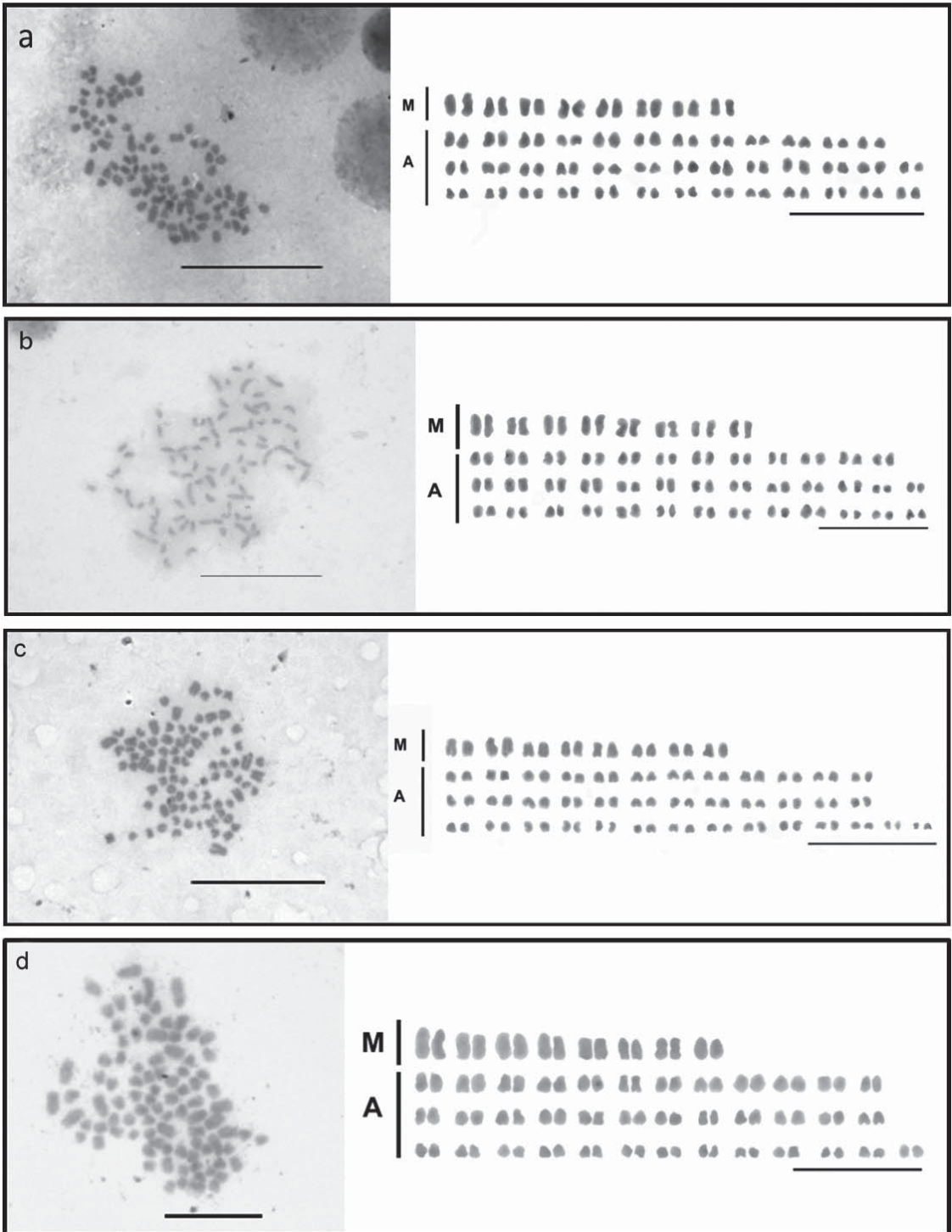


Fig. 1. Metaphases and karyotypes of *Pachycondyla harpax*, conventional staining using Giemsa. a) female worker, Ilhéus, Bahia, 2n = 92; b) diploid male, Barrolândia, Belmonte, Bahia, 2n = 92. c) female worker, Barrolândia, Belmonte, Bahia 2n = 92. d) Vera Cruz district, southwest of Una Biological Reserve, 2n = 90. Bar: 5  $\mu$ m. A = acrocentric and M = metacentric.



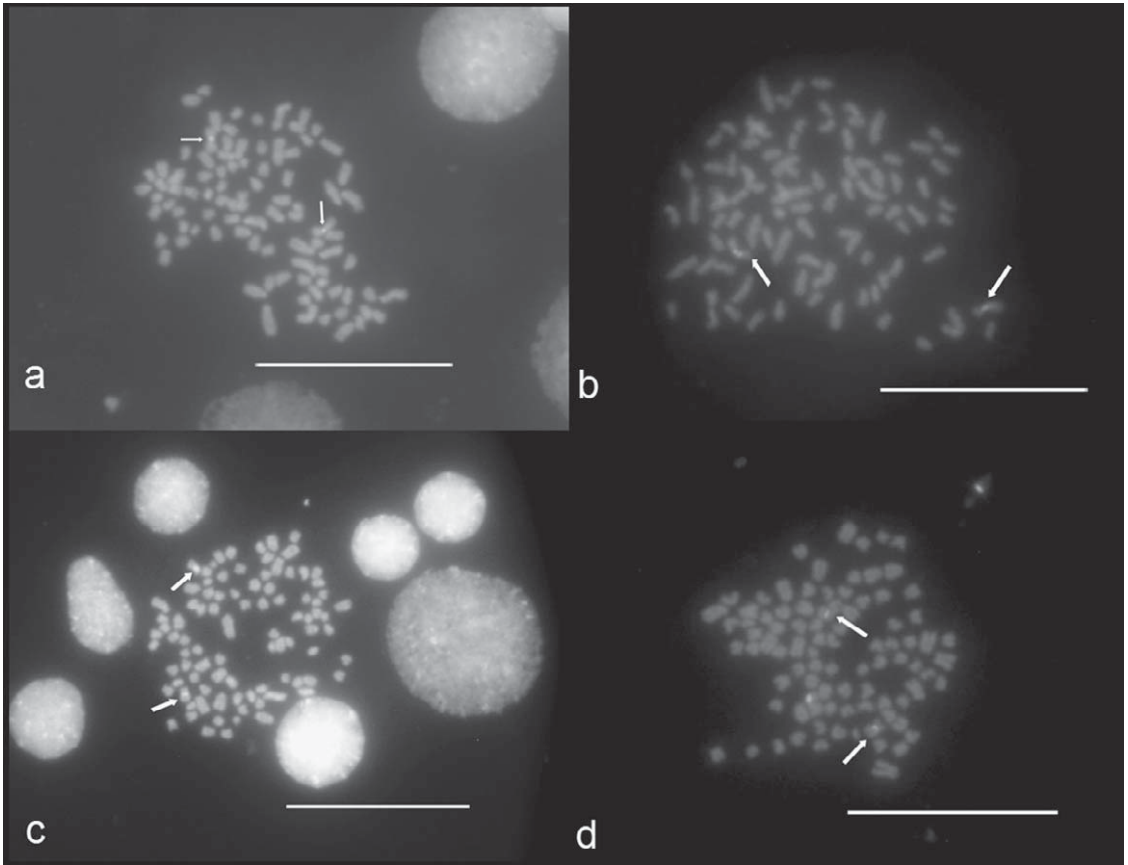


Fig. 2. Diploid metaphases of *Pachycondyla harpax* submitted to sequential staining with fluorochromes CMA3/DAPI a) Ilhéus, Bahia; b) Barrolândia, Belmonte, Bahia; c, d) Vera Cruz district, southwest of Una Biological Reserve. Arrows indicate the same chromosome pair with negative AT-rich regions. Bar: 5  $\mu$ m.

1994), all suggesting that a single morphospecies may correspond to 2 or more genetically isolated taxa (Delabie et al. 2008). Additionally, morphological analyses of individuals of *P. harpax* collected in other locations, where cytogenetic analysis was not possible, revealed obvious differences, suggesting genetic divergence and possible karyotype variability. Another study is currently being carried out to search for valuable morphological characters that might allow discriminating eventual cryptic species close from *P. harpax*.

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