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SHORT NOTE

First Cytogenetic Study Through Conventional Staining of The Ant Genus *Blepharidatta* Wheeler, 1915 (Hymenoptera: Formicidae: Attini)

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Abstract

Blepharidatta is a rare Neotropical ant genus in the Attini tribe of the subfamily Myrmicinae. It has only four valid species and among them *Blepharidatta delabiei* was recently described and there is little knowledge on its biology. This study is the first cytogenetic characterization for the genus *Blepharidatta* and also presents the biology of *B. delabiei*. Cytogenetic analyses revealed a karyotype 2n = 28 with acrocentric and metacentric chromosomes and a karyotypic formula (m: metacentric, a: acrocentric): 2K = 16m + 12a. We observed that ants of this species have diurnal habits with higher foraging activity in the afternoon and are possibly omnivorous as they accepted the baits used. The distance between colonies varied from 5 to 7 meters.

Introduction

Blepharidatta Wheeler (1915) is a Neotropical ant genus in the Attini tribe represented by only four species that nest in the soil and leaf litter and are distributed in the central and western Amazon basin, in different localities in the Cerrado, in the Caatinga, and in the Atlantic Forest in Brazil (Brandão et al., 2015). Wheeler (1915) described the genus and stated that these ants "evidently" belonged to the Attini tribe. The Blepharidattini tribe was proposed by Wheeler and Wheeler (1991) comprising the genera *Blepharidatta* and *Wasmannia*. However, this grouping is no longer valid since Ward et al. (2015) placed it together with other tribes within the Attini in its current conception. It is worthwhile to mention that *Blepharidatta* specimens have the habit of keeping the remains of prey in the main chamber of the nest for a while. The observation that these carcasses are often covered by fungi led to the hypothesis that this behavior could represent one of the possible initial steps that led to the close symbiosis between ants and fungi observed today in the Attina (Diniz & Brandão, 1997; Diniz et al., 1998; Rabeling et al., 2006). This hypothesis is further reinforced by the existence of ancestral groups (e.g. *Cyphomyrmex* and *Myrmicocrypta*) that use arthropod corpses to cultivate fungi as a source of food (Hölldobler & Wilson, 1990).

Out of the four valid species in this genus, *Blepharidatta brasiliensis* (Wheeler 1915), *Blepharidatta conops* (Kempf 1967), *Blepharidatta delabiei* (Brandão et al., 2015), and *Blepharidatta fernandezi* (Brandão et al., 2015), so far there is published data on the biology of only *B. conops* (Diniz et al., 1998; Brandão et al., 2008; Pereira et al., 2014). Some studies suggested that populations of *B. conops* found in the

Brazilian Caatinga and Cerrado were actually two distinct species based mainly on the distinct morphology of the phragmotic queens (Pereira et al., 2014; Brandão et al., 2015). *Blepharidatta delabiei* is the most recently described species within the genus (Brandão et al., 2015). There is no published information on its biology so far, mainly due to the difficulty in locating colonies. This species is distributed in the leaf litter of Atlantic Forest remnants in the states of Bahia and Minas Gerais (Cassano et al., 2009, where *Blepharidatta* sp. = *B. delabiei*; Brandão et al., 2015). The analysis of mitotic chromosomes has been widely used for cytotaxonomic studies in some groups of eukaryotes (Imai, 1983; Lorite & Palomeque, 2010; Kretschmer et al., 2018; Magalhães et al., 2020). The study of karyotypes allows inferences on species differentiation, degree of kinship, evolutionary processes, and the phylogenetic position of the taxa studied (Guerra, 1988; Mariano et al., 2019).

An important aspect of chromosome evolution in ants is the haplodiploid nature of their reproductive system. As males are haploid, there is no meiosis to produce gametes (Palomeque et al., 1990), which leads to the possibility that in Formicidae as in the entire order Hymenoptera, chromosomal alterations are much more tolerated than in other groups of organisms (Gokhman, 2009). Several chromosomal rearrangements can be tolerated by hymenopterans because aneuploids within this group are often viable and fertile and even though aberrations in meiosis are apparently more deleterious in males than in females, hymenopteran males lack this important checkpoint (Gokhman, 2009).

In Formicidae, more than 800 species have been cytogenetically studied (Lorite & Palomeque, 2010, Mariano et al., 2019, Aguiar et al., 2020). The huge variation in chromosome number ranging from 2n=2 to 2n=120 (Lorite & Palomeque, 2010) may provide good models to investigate the role of chromosomal variation in speciation. The Attini tribe alone currently comprises 48 genera with a total of 2.703 valid species, but there is cytogenetic information for only 21 genera (Cardoso et al., 2018) with karyotypes ranging from 2n = 4 to 2n = 64 (Alves-Silva et al., 2014; Murakami et al., 1998; Barros et al., 2013; Mariano et al., 2019; Cardoso & Cristiano, 2021). This means that the karyotypes of 56.25% of the Attini genera, such as *Blepharidatta,* are unknown.

Hence, to expand the knowledge on the genus *Blepharidatta*, we investigated the karyotype of *B. delabiei* using classical cytogenetics and some aspects of its biology.

Material and Methods

Colonies of *B. delabiei* were collected along the Ilhéus/Una highway near Águas de Olivença, Ilhéus, Bahia, Brazil (15º00'13.3"S 39º00'53.3"W). The collecting permit (SISBio number 63623-1) was issued to Laís Leal Lopes by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). *B. delabiei* colonies were found near an indigenous reserve. Nests were located with the help of bait distribution (bread and sardines) offered to foragers.

Three colonies were taken to the Laboratory of Social Arthropods at the Universidade Estadual de Santa Cruz, Ilhéus, where they were kept in artificial nests (Delabie et al., 2021). The ants were identified by Dr. J. H. C. Delabie. Mitotic metaphases were obtained following the protocol of Imai et al. (1988) using prepupal cerebral ganglia. We analyzed 10 larvae from each of the 3 sampled colonies, totaling 30 samples and 117 metaphases. The best quality metaphases were captured and analyzed using an image capture system (Olympus BX-51, a Q-Capture Pro image capture camera, and the Image Pro Plus software).

The karyograms were assembled using the Adobe Photoshop® CS3 Extended software following the nomenclature by Levan et al. (1964).

Results

The colonies of *B. delabiei* were found close to an indigenous reserve with well-preserved vegetation. Considering the rarity of this species, we believe that this ant can be used as a biological indicator of environmental quality and a low degree of anthropic disturbance, which confirmed the findings by Cassano et al. (2009).

We observed that this species has diurnal habits with higher foraging activity in the afternoon. The distance between colonies varied from 5 to 7 meters. All colonies of *B. delabiei* were found in the leaf litter and were extremely discrete. The ants of this species do not recruit high numbers of workers and forage very slowly. Thus, it was necessary to wait for the workers to carry the bait into the nest in order to locate the colony accurately. These ants are possibly omnivorous as they accepted the bait of both bread and sardines.

We collected a total of 236 adult individuals from the three colonies (Table 1) and immatures were present in all colonies. The number and morphology of chromosomes and the karyotypic formula were described using conventional staining (Figure 1). The mitotic index was low (<10 metaphases/slide) and in a large percentage of the prepared slides, no mitotic metaphases were observed. We analyzed 30 individuals (all workers, 10 individuals per colony) and a total of 117 metaphases.

Table 1: Demographics of *Blepharidatta delabiei* colonies including immatures.

	Queens	workers	males	immatures
Colony 1	-	95	1	>15 larvae
Colony 2	1	97	-	>20 larvae
Colony 3	1	28	-	>10 larvae

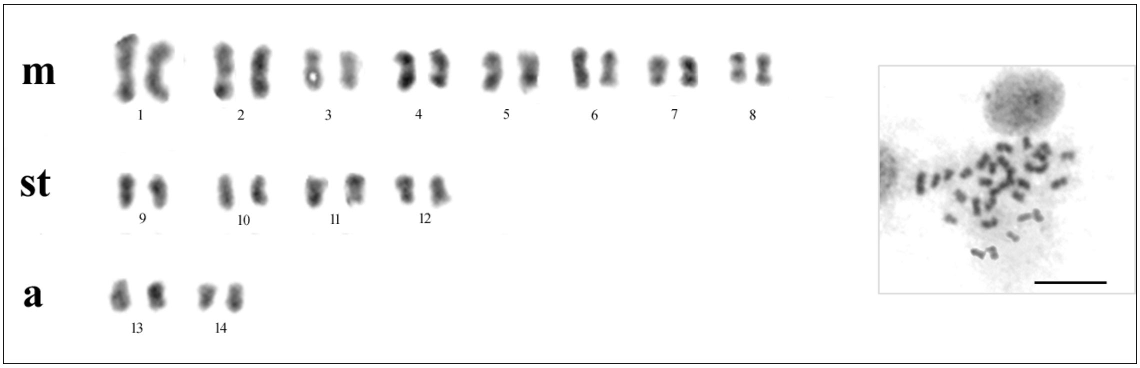

Fig 1. *Blepharidatta delabiei* karyotype 2n = 28 with chromosomes classified according to Levan et al. (1964), where m: metacentric, st: subtelocentric, a: acrocentric. Bar: 5 µm.

The results of conventional staining revealed the karyotype 2n = 28 in all analyzed individuals with a constant karyotypic formula 2K = 16m + 8st + 4a (m: metacentric, st: subtelocentric, a: acrocentric) (Figure 1). Although morphometric studies were not performed, the analyzed metaphases showed chromosomes with a discrete size variation.

Discussion

This is the first study carried out to cytogenetically characterize a population of *B. delabiei.* A total of 21 genera in the Attini tribe were cytogenetically described. The range of recorded diploid chromosome numbers shows a remarkable variation from 2n = 4 in *Strumigenys louisianae* (Roger, 1863) (2n = 4) to 2n = 64 in *Mycetophylax lectus* (Forel, 1911) (= *Cyphomyrmex lectus*) (Alves-Silva et al., 2014; Murakami et al., 1998; Barros et al., 2013; Mariano et al., 2019; Cardoso & Cristiano, 2021).

In Attini, only the genera *Atta* and *Acromyrmex* have a karyotype considered constant of 2n = 22 and 2n = 38, respectively (Cristiano et al., 2013; Barros et al., 2014; Barros et al., 2021), whereas there is intrageneric variation in the karyotype in the remaining genera within the clade (Murakami et al., 1998, Cardoso, et al., 2021; Texeira et al., 2022). The closest genera to *Blepharidatta* based on molecular analyses are *Wasmannia* and *Allomerus* (Ward et al., 2015). The only species in the genus with a known karyotype, *Wasmannia auropunctata* (Roger, 1863), has a karyotype of 2n = 32 and the karyotypic formulae are 2K = 20m + 12a (Souza et al., 2011) and 2K =16m + 10sm + 6st (Aguiar et al., 2020) with acrocentric, metacentric, and telocentric chromosomes. *Allomerus* is also considered a genus close to *Blepharidatta* with only two species with a known karyotype, *Allomerus decemarticulatus* (Mayr, 1878) with 2n = 28 and karyotypic formula of 2K = 18m+6sm+2st+2a and *Allomerus octoarticulatus* (Mayr, 1878) with 2n = 44 and a karyotypic formula of 2K = 4sm+ 40a (Aguiar et al., 2020).

Future discussions regarding karyotype evolution in this clade should include information on *Allomerus* species since they cluster with *Blepharidatta* and *Wasmannia* in the study on the phylogeny and biogeography of the subfamily Myrmicinae by Ward et al. (2015). These authors obtained a grouping that places *Wasmannia* closer to *Allomerus* than to *Blepharidatta*. However, regarding morphology and taxonomy, *Blepharidatta* and *Wasmannia* are closer (Brown, 1953; Wheeler; Wheeler, 1991; Longino; Fernández, 2007). The point here is that karyotypic information on closely related taxa can provide additional evidence of their taxonomic affinities (Sumner, 2003) and our results on *B. delabiei* reinforce the greater proximity between *Blepharidatta* and *Wasmannia*, both in chromosome morphology and karyotype.

Regarding its natural history, *B. delabiei* shares more characteristics with *B. brasiliensis* than with *B. conops*, both in colony structure and morphology. In a study on the behavioral ecology and natural history of *B. brasiliensis*, Rabeling et al. (2006) noticed that this species is also omnivorous and nests in the leaf litter, but they did not find any queens with phragmotic cephalic discs as is seen in colonies of *B. conops* (Brandão et al., 2001). The foraging schedule of *B. delabiei* is like that of *B. conops*, since *B. delabiei* workers forage preferentially during the day, with greater activity in the afternoon. The workers of *B. conops* also forage outside the nest during the day, avoiding the hottest period, even though some workers stay out of the nest all day long (Brandão et al., 2001). However, *B. brasiliensis* foragers concentrate their activities predominantly at night, and at midday, this activity ceases and no other worker is observed until the late afternoon (Rabeling et al., 2006).

Our results help to expand the knowledge on the genus *Blepharidatta* regarding its natural history and karyotype. The variation in chromosome number and morphology between species with low and high numbers of chromosomes within the Attini tribe are some of the examples that demonstrate the importance of cytogenetic studies since they can contribute to the systematics of ants.

Authors' Contribution

LLL: Conceptualization, methodology, investigation, formal analysis, writing (original draft/Review and editing)

CSFM: Conceptualization, methodology, investigation, formal analysis, writing (original draft/Review and editing)
JHCD: Conceptualization, writing (original draft/Review and editing)
JGS: Conceptualization, methodology, investigation, writing (original draft/Review and editing)

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