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KARYOTYPE DIFFERENTIATION AMONG FOUR *DINOPONERA* (FORMICIDAE: PONERINAE) SPECIES

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Abstract

Ants in the genus *Dinoponera* (Hymenoptera: Formicidae: Ponerinae) are among the largest sized Formicidae of the World. In Brazil *Dinoponera* has an allopatric distribution, and several species occur in threatened biomes. We characterized karyotypes of the following 4 species*: Dinoponera australis* Emery, *Dinoponera gigantea* Perty, *Dinoponera lucida* Emery, and *Dinoponera quadriceps* Santschi. Karyotype analysis found that all 4 species have high numbers of small-sized chromosomes (*D. australis*, 2n = 114; *D. gigantea*, 2n = 82; *D. lucida*, 2n = 118/120; *D. quadriceps*, 2n = 92). A moderate variation in chromosome number was observed among the 4 species, which suggests the occurrence of chromosome rearrangements during karyotype evolution in *Dinoponera*. An exclusive A^{MT} chromosome pair was found to occur in all *Dinoponera* species studied thus far, which we conclude is a probable synapomorphy in *Dinoponera*.

Key Words: Ponerinae, cytogenetics, Hymenoptera, Formicidae

Resumo

As formigas do gênero *Dinoponera* (Hymenoptera: Formicidae: Ponerinae) estão entre as maiores Formicidae do Mundo. *Dinoponera* é alopatricamente distribuído no Brasil com várias espécies ocorrendo em biomas ameaçados. No presente trabalho foi feita a caracterização do cariótipo de quatro espécies de *Dinoponera*: *Dinoponera australis* Emery, *Dinoponera gigantea* Perty, *Dinoponera lucida* Emery, e *Dinoponera quadriceps* Santschi. Um grande número de cromossomos pequenos foi encontrado no cariótipo de todas as espécies analisadas (*D. australis*, 2n = 114; *D. gigantea*, 2n = 82; *D. lucida*, 2n = 118/120; *D. quadriceps*, 2n = 92). Uma moderada variação no número de cromossomos foi observada entre as espécies estudadas, o que sugere a ocorrência de rearranjos cromossômicos durante a evolução cariotípica neste gênero. O presente estudo também confirma a presença de um par cromossômico A^{MT} em todas as espécies de *Dinoponera* estudadas até o momento, o que provavelmente representa uma sinapomorfia para este gênero.

Palavras chave: Ponerinae, citogenética, Hymenoptera, Formicidae

The South American endemic genus *Dinoponera* (Hymenoptera: Formicidae) Roger ranks among the largest ants worldwide and includes 6 valid species: *Dinoponera australis* Emery, *Dinoponera gigantea* Perty, *Dinoponera longipes* Emery, *Dinoponera lucida* Emery, *Dinoponera mutica* Kempf, and *Dinoponera quadriceps* Santschi (Bolton et al. 2006). The genus ranges from northern Brazil and Peru, to northern Argentina. The species have a mostly allopatric distribution (Fig. 1), which is likely related to their adaptive evolution to the distinct biome each species occupies. *Dinoponera lucida* is listed as endangered in Brazil (Ministério Do Meio Ambiente 2003) and occurs along the eastern coastline in the

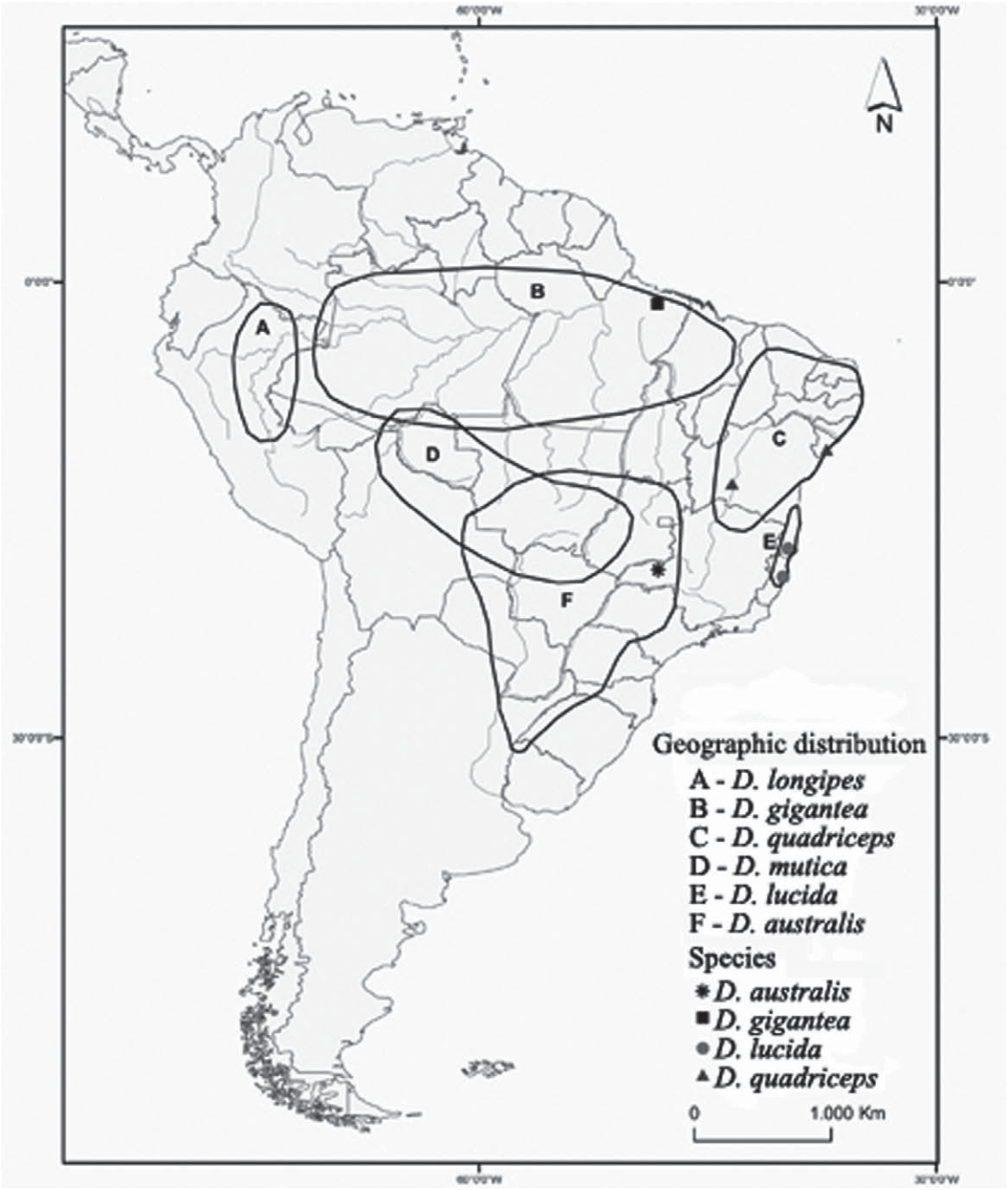

Fig. 1. Distribution map of the genus *Dinoponera* adapted from Paiva & Brandão (1995). Lines delimit the geographic distribution of each species. Symbols correspond to collection localities listed in Table 1.

central region of the Atlantic rainforest; *D. gigantea* and *D. longipes* have been reported in distinct regions of the Brazilian and Peruvian Amazon; *D. quadriceps* is restricted to the Caatinga in northeastern Brazil, whereas *D. mutica* and *D. australis* both occur in the Cerrado close to Bolivia (*D. mutica*) and northern Argentina (*D. australis*) (Kempf 1971; Paiva & Brandão 1995; Mariano et al. 2008). It is noteworthy that the Atlantic rainforest, a large part of the Amazon forest, the Caatinga, and the Cerrado are all subject to strong deforestation due to the expansion of human population and activities (Coimbra-Filho & Câmara 1996; Myers et al. 2000; Laurence et al. 2001; Leal et al. 2003).

All *Dinoponera* species are morphologically very similar in body size and color and are distinguished by only a few relatively discrete morphological characters such as the cuticular ornamentation, pilosity, and petiole shape. Furthermore, there is a series of clear synapomorphies that distinguish this genus from other Ponerinae (Schmidt 2009).

Previous studies on some species of *Dinoponera* have mostly focused on population ecology, reproductive biology, and behavior. Information is available only on *D. australis* (Paiva & Brandão 1995), *D. gigantea* (Fourcassié & Oliveira 2002), *D. lucida* (Peixoto et al. 2008, 2010), and *D. quadriceps* (Monnin & Peeters 1999; Vasconcellos et al. 2004).

Dinoponera have queenless colonies, where reproduction is taken over by a fertile dominant worker known as a "gamergate". Gamergate physiology and behavior are modified according to dominance status relative to the other workers (Monnin & Peeters 1999). The occurrence of gamergates has been reported in 8 genera of Ponerinae (Peeters 1993, 2012).

In the last decade, different research groups have examined aspects of ant phylogeny using molecular data (e.g. Brady 2003; Ward & Brady 2003; Saux et al. 2004; Ward & Downie 2005; Moreau et al. 2006; Brady et al. 2006; Schmidt 2009). These studies have contributed important information on basic aspects of ant evolution in different subfamilies, even though there are limitations regarding taxon sampling and resolution (Schmidt 2009). In his recent molecular phylogenetic study of Ponerinae, Schmidt (2009) suggested that *Dinoponera* and *Pachycondyla sensu* Kempf (1972) are sister genera, however, only a single *Dinoponera* species, *D. australis,* was included in this analysis.

Important information regarding Formicidae genetics and evolution has been also achieved by cytogenetic studies on a range of ants. Currently, karyotypes or chromosome numbers for approximately 700 ant populations or species are available in the literature (Lorite & Palomeque 2010; Santos et al. 2010; Mariano et al. 2012).

The knowledge on *Dinoponera* cytogenetics is still very fragmentary, since there is cytogenetic information for only 2 species: *D. lucida* (Mariano et al. 2004, 2008; Barros et al. 2009) and *D. gigantea* (Aguiar et al. 2011). These studies have revealed that *D. lucida* has the largest chromosome number reported in Hymenoptera, which ranges from 106 to 120, and *D. gigantea* has 2n = 82 (Mariano et al. 2008). Although only *D. lucida* is officially listed as endangered (Ministério Do Meio Ambiente 2003), we may reasonably expect that other species in this genus might also be prone to similar population decline because of their shared peculiar reproductive strategy, small population size, and populations that are becoming increasingly more isolated due to native habitat loss.

Our multidisciplinary collaborative network is engaged in a series of studies regarding the genus *Dinoponera,* as well as several other Ponerinae genera in Brazil (i.e., *Anochetus*, *Pachycondyla*, *Thaumatomyrmex*), which include biogeographical, chemical, cytogenetic, ecological, ethological, and molecular aspects. Herein, we report the results of a comparative cytogenetic study carried out on *D. australis*, *D. gigantea*, *D. lucida,* and *D. quadriceps* collected in different biomes in Brazil.

Material and Methods

Nine colonies were collected in 6 different localities in Brazil (Table 1) and at least 2 colonies per species were analyzed. The ant nests were taken from aggregated populations in native vegetation remnants. Some of the colonies were kept at the Laboratório de Mirmecologia (Centro de Pesquisas do Cacau - CEPEC/Comissão Executiva para o Plano da Lavoura Cacaueira - CEPLAC) at an average temperature of 27 °C. Other colonies were processed at the collection site or at the Laboratório de Citogenética de Insetos (Universidade Federal de Viçosa - UFV).

The metaphases were obtained from cerebral ganglia and male gonads of prepupae according to Imai et al. (1988). Metaphases were stained with Giemsa, photographed with an Olympus

Table 1. Collection data, number of individuals analyzed, chromosome number, and Fluorochrome staining pattern for 4 species of *Dinoponera*.

Species	Locality*	Geographic coordinates	Number of colonies/ sample size	2n (n)
D. australis	Uberlândia-MG	18°55'S 48°16'W	2/3	114
D. gigantea	Marituba-PA	01°22'S 48°20'W	2/16	82
D. lucida	Teixeira de Freitas-BA	17°32'S 39°44'W	1/8	(60)
	Linhares-ES	19°23'S 40°04'W	1/12	118 (59)
D. quadriceps	Bom Jesus da Lapa-BA	13°15'S 43°25'W	1/8	92
	Sambaíba–SE	11°00'S 37°12'W	2/10	

*All states in Brazil: BA: Bahia; ES: Espírito Santo; MG: Minas Gerais; PA: Pará; SE: Sergipe.

BX60 microscope equipped with a digital camera, and analyzed with Image Pro Plus® version 4.1 analysis software (Media Cybernetics). Due to the large number and small size of the chromosomes, several metaphases were incomplete, so the chromosome number was determined after evaluation of all metaphases available on the slides. The classification of the chromosomes followed Imai's (1991) nomenclature. Voucher specimens of each colony were deposited at the Laboratório de Mirmecologia, Centro de Pesquisas do Cacau (CEPEC), Ilhéus, Bahia, Brazil.

Results and Discussion

Karyotype analysis revealed a well-defined pattern of a large number of small chromosomes, mostly acrocentric, in the 4 *Dinoponera* species analyzed. The chromosome number ranged from 2n = 82 (*D. gigantea*) to 2n = 120 (*D. lucida*) (Table 1).

Dinoponera lucida showed variation in chromosome number among the 2 populations that were sampled in different localities. In a colony collected in Teixeira de Freitas, Bahia, the haploid chromosome number was n = 60, whereas in a colony collected more to the south in Linhares, Espírito Santo, the chromosome number was 2n = 118 (n = 59). This karyotype variation in *D. lucida* was first reported by Mariano et al. (2008), who found 4 different karyotypes for this species, ranging from 2n = 106 to 2n = 120. According to these authors, the fragmentation of the Atlantic rainforest due to climatic variation in the early Quaternary and modern anthropogenic effects contributed to population isolation, and when taken together, these help to explain the observed differences. Numerical variation in the karyotype of different colonies within a species has been reported for *Rhytidoponera metallica* (Smith) [5 karyotypes ranging from 2n = 22 to 46 (Imai et al.

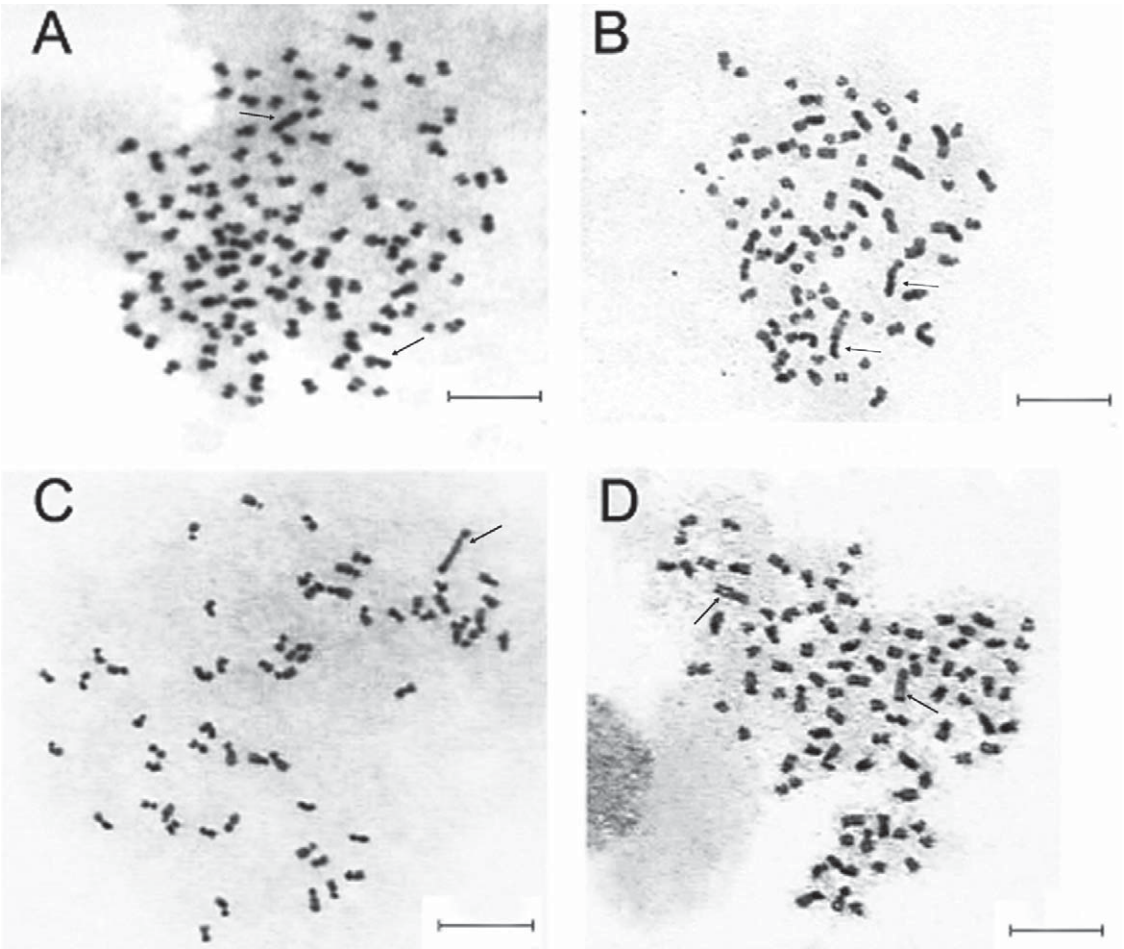

Fig. 2. Metaphases of the 4 species analyzed in the present study. (A) *Dinoponera australis*, 2n = 114, (B) *D. gigantea*, 2n = 82, (C) *D. lucida,* n = 59 , and (D) *D. quadriceps*, 2n = 92. Bars = 5µm. Arrows indicate A^{MT} chromosomes.

1977)], *Myrmecia pilosula* (F. Smith) [karyotypes ranging from 2n = 21 to 30 (Imai et al. 1994)], and in the complex *Pachycondyla apicalis-verenae* [karyotypes ranging from 2n = 42 to 64 (Delabie et al. 2008)].

Dinoponera is considered to be closely related to *Pachycondyla* based on morphological, molecular, and ecological data (Schmidt 2009). This strongly suggests that these 2 groups share a recent common ancestor. The first molecular phylogenetic studies including species of both *Dinoponera* and *Pachycondyla* did not elucidate the relationship between these 2 genera (Moreau et al. 2006; Ouellette et al. 2006). However, a recent molecular phylogeny of the Ponerinae by Schmidt (2009) suggested a close relationship between one *Dinoponera* species (*D. australis*) and different species of *Pachycondyla* [*Pachycondyla harpax* (Fabricius), *Pachycondyla crassinoda* (Latreille), *Pachycondyla striata* Fr. Smith, and *Pachycondyla impressa* (Roger)], all included in *Pachycondyla stricto sensu*. There are many similarities between these *Pachycondyla* species and *Dinoponera*, such as a relatively large sized body, general habitus, and ground living habits. Regarding cytogenetics, *Dinoponera* and *Pachycondyla stricto sensu* (*P. crassinoda*, *P. harpax, P. impressa, Pachycondyla metanotalis* Luederwaldt, and *P. striata*) also share a high chromosome number (2n = 62, 2n = 96, 2n = 94, 2n = 70, and 2n = 104, respectively) (Mariano et al. 2006, 2012).

Similarities were also observed among these species regarding chromosome morphology and size, as most chromosomes are small and acrocentric. It is noteworthy that this pattern of a large number of small acrocentric chromosomes has rarely been observed in ants. The aforementioned similarities thus lend support to the close phylogenetic relationship between these 2 genera.

According to Imai et al. (1988), acrocentric chromosomes predominate in ants and are derived from metacentric chromosomes that have undergone centric fissions. Such a process could lead to the increase of chromosome number with a reduction in size as a strategy to prevent the occurrence of deleterious translocations during meiosis.

A conspicuous chromosome pair [pseudo-acrocentric (A^{MT})], already identified in *D. lucida* karyotype (Mariano et al. 2004, 2008), was detected in the 4 *Dinoponera* species studied herein (Fig. 2). A^{MT} chromosomes, according to Imai (1991), arise from a relatively fast growth of heterochromatin on the smaller chromosome arms after a centric fission. The karyotype of the remaining species in this genus, *D. mutica* and *D. longipes*, are still to be analyzed. The presence of A^{MT} chromosomes in the remaining 2 *Dinoponera* species should be verified in order to confirm whether this character is synapomorphic for the genus.

Our data revealed high karyotype differentiation in *Dinoponera* and indicate that chromosome number and morphology can be suitable for the cytotaxonomy of this genus. Further analyses regarding chromosome banding and specific gene location will provide the necessary information for a better understanding of the karyotype evolution within the genus.

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