A Cytogenetic Approach to the Study of Neotropical *Odontomachus* and *Anochetus* Ants (Hymenoptera: Formicidae)

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ABSTRACT *Odontomachus* (Latreille) and *Anochetus* (Mayr) (Hymenoptera: Formicidae: Ponerinae) are closely related pantropical genera of ponerine ants that share morphological and behavioral characteristics. A comparative study was carried out using conventional Giemsa staining, fluorochrome staining, and fluorescent in situ hybridization. Karyotypes revealed a higher stability in chromosome number among *Odontomachus* species than among *Anochetus* species. We observed a higher frequency of metacentric chromosomes in the karyotypes of *Anochetus* compared with the more common telocentrics of *Odontomachus* species. Differences in the localization of rDNA genes on chromosomes between the two genera also were verified. rDNA genes were found on telocentric and submetacentric chromosomes in *Anochetus* and on telocentric chromosomes in *Odontomachus*. Our cytogenetic results lend support to Brown's hypothesis that *Odontomachus* has evolved from a lineage of *Anochetus*. The karyotype divergence of both genera can be explained by a model of evolution in which there is a tendency to the increase of chromosome number by centric fission. Supporting evidence for this hypothesis is discussed.

KEY WORDS Ponerinae, karyotype, fluorescence in situ hybridization, Hymenoptera

Odontomachus (Latreille) and *Anochetus* (Mayr) (Hymenoptera: Formicidae: Ponerinae) are closely related genera of ants that form the subtribe Odontomachiti *sensu* Brown (1976, 1978), very similar in morphological and behavioral features. *Odontomachus* species are usually more aggressive and larger than *Anochetus*. These genera are known since at least the Oligocene or Miocene (De Andrade 1994) and currently have a pantropical distribution, being especially abundant in the Neotropical region (Brown 1976, 1978; Ehmer and Hölldobler 1995). Brown (1976) also pointed out that *Anochetus* radiated more extensively and more radically than *Odontomachus*, a process that could have been brought about by a longer and more intense evolutionary history. *Odontomachus* comprises predator ants that are characterized by long mandibles used in a trap jaw mechanism for capturing fast prey (Gronenberg et al. 1993) that also can be used in defense against other ants and in ballistic locomotion through "bounce defense jumps" or "escape jumps" (Carlin and Gladstein 1989, Patek et al. 2006, Spagna et al. 2008). The nesting habits differ slightly between the two genera, as *Anochetus* specimens usually nest in cryptic places such as galleries in branches or rotten trunks, whereas *Odontomachus* specimens occupy cavities in rotten wood or fallen epiphytes on the ground surface (Brown 1976, 1978; Fernández 2003).

Despite the recognition of similarities between these genera, comparative studies remain scarce. Brown (1976) suggested that *Odontomachus* is probably derived from a group of *Anochetus*. This conclusion was drawn based on morphological studies and the worldwide distribution of the species. Recent studies on molecular systematics of ants using nuclear (18S, 28S, long-wavelength rhodopsin, wingless, and abdominal-a) and mitochondrial (cytochrome oxydase I) DNA sequences that included several Ponerinae genera suggest that *Odontomachus* is monophyletic and *Anochetus* is its most probable sister group (Moreau et al. 2006, Spagna et al. 2008). Cytogenetic studies have provided invaluable information for genetic diversity and taxonomy, as shown in the exemplar studies on *Myrmecia* spp. of the *pilosula* group (Hymenoptera: Formicidae: Myrmeciinae) in Australia by Crosland and Crozier (1986), Crosland et al. (1988), Imai et al. (1977, 1988a,b, 1994), Taylor (1991), Hirai et al. (1994, 1996), and Meyne et al. (1995). A large variation in chromosome number has been verified in the Hymenoptera with both extremes reported in Formicidae ranging from 2n = 2 in the Australian *Myrmecia croslandi* Taylor (Hymenoptera: Formicidae: Myrmeciinae) (Crosland and Crozier 1986) to

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Table 1. Colony code, collection information (locality and geographic coordinates), and number of individuals analyzed in this study

Species	Colony code	Locality	Kppen's climatic classification	Coordinates	Specimens analyzed (*N*)
Odontomachus meinerti	Od-01, Od-02	Itambé (SAF), Bahia, Brazil	Am	15° 11′15″ S, 40° 33′45″ W	7
	Od-03	São José da Vitória (CP), Bahia, Brazil	Am	15° 03′ 45″ S, 39° 18′ 45″ W	2
	Od-04	Itororó (CP), Bahia, Brazil	Am	15° 03′45″ S, 40° 03′45″ W	1
Odontomachus chelifer	Od-05	Serra Bonita (PAF), Camacã, Bahia, Brazil	Am	15° 26′15″ S, 39° 26′15″ W	5
	Od-06	Boa Nova (SAF), Bahia, Brazil	Am	14° 18′45″ S, 40° 11′15″ W	2
Anochetus horridus	An-03	Petit Saut–French Guyana (SAmF)	Af	5° 20′ N, 53° 41′W	5
Anochetus altisquamis	An-01, An-02	Serra Bonita (PAF), Camacã, Bahia, Brazil	Am	15° 26′15″ S, 39° 26′15″ W	9

2n = 120 in the Neotropical *Dinoponera lucida* Emery (Hymenoptera: Formicidae: Ponerinae) (Mariano et al. 2008). Regarding the tribe Ponerini, the karyotypes of only a few species within 10 genera have been succinctly described so far, including 10 *Anochetus* species and eight *Odontomachus* species of the Oriental and Australian regions (for review, see Mariano and Delabie 2004). However, the aforementioned studies mainly discussed the chromosome number for these species without any reference to chromosome morphology.

Previous studies revealed that *Anochetus* is more variable cytogenetically than *Ondontomachus.* Its chromosome numbers ranged from 2n = 24–2n = 30, whereas *Odontomachus* frequently showed higher chromosome numbers with modal number 2n = 44 (Imai et al. 1977, 1984b). Whenever the karyograms were available, it was possible to verify a lack of metacentric chromosomes in *Odontomachus* and its relatively common occurrence in *Anochetus.* Despite the success of molecular genetics and a few other disciplines in recent comparative analysis, cytogenetics seem to be paramount in answering some prominent questions about biodiversity, such as species delimitation, cryptic species analysis or evolutionary biology, being an important approach in the so-called integrative taxonomy (Schlick-Steiner et al. 2010). Its potential remains unexplored to address evolutionary questions regarding the karyotype evolution in *Anochetus* and *Odontomachus.*

The Neotropical region is regarded as having the richest ant fauna worldwide with the Ponerinae as one of the predominant subfamilies (Fernández 2003). In Brazil, the occurrence of 12 species of *Odontomachus* and eight species of *Anochetus* has been reported (Kempf 1974; Brown 1976, 1978; Brandão 1991; Agosti and Johnson 2003). We carried out a comparative study on the karyotypes of species of both genera and investigated whether chromosomal rearrangements may be involved in their divergence. For this comparison, we analyzed *Anochetus altisquamis* Mayr (Hymenoptera: Formicidae), *Anochetus horridus* Kempf (Hymenoptera: Formicidae), and *Odontomachus meinerti* Forel (Hymenoptera: Formicidae), all species living on the forest floor or in the litter. We also included in the analysis *Odontomachus chelifer* (Latreille), the most basal *Odontomachus* species according to the phylogenetic tree presented by Spagna et al. (2008). We analyzed the aforementioned species using conventional Giemsa staining, sequential fluorochrome staining, and fluorescence in situ hybridization (FISH).

Materials and Methods

Samples. The nests of *Odontomachus* and *Anochetus* analyzed in this study were collected in localities of the state of Bahia, Brazil, and French Guyana shown in Table 1. The sampled areas are in the Atlantic rain forest and Amazon forest domains. Climate of these localities is defined according to Köppen's classification (Table 1). Serra Bonita is a private reserve with a preserved primary Atlantic rain forest area at 800 m above sea level. The other areas correspond to secondary Atlantic rainforest (SAF), secondary Amazon forest (SAmF), and cocoa plantations (CP). The number of nests analyzed for each species in this study varied according to the species abundance in the collection areas. Voucher specimens of each colony were deposited at the Laboratório de Mirmecologia, Centro de Pesquisas do Cacau (CEPEC), Ilhéus, Bahia, Brazil.

Conventional Cytogenetics. Mitotic metaphases where obtained from cerebral ganglia treated with 0.005% colchicine for 20 min according to Imai et al. (1988a). Chromosomes were stained with Giemsa (2% stock solution in phosphate buffer, pH 6.8) and photographed using an CX-41 microscope equipped with a C-7070 digital camera (Olympus, Tokyo, Japan). A minimum of five methaphases per individual was analyzed. Chromosomes were described according to Levan's terminology (Levan et al. 1964).

Sequential Fluorochrome Staining. Fluorochrome staining (chromomycin A3 [CMA_3]/4,6-diamidino-2-phenylindole [DAPI]) followed Schweizer's method (Schweizer 1976). A CMA_3 (0.34 mg/ml) solution was added to each slide, which was covered with a coverslip and incubated at room temperature (RT) for 1 h. The slides were then briefly rinsed in alcohol series and dried. Subsequently, a DAPI solution (2 μg/ml) was added to each slide, which was covered

Table 2. Chromosome no. and karyotype formula of the species studied

Species	2n(n)	Karyotype formula	Country: reference
Anochetus altisquamis	30	2K = 12M + 6SM + 2ST + 10T	Brazil: present study
Anochetus graeffei Mayr	30	2K = 18 M + 2 SM + 10 T	India: Imai et al. (1984b)
	38	Not reported	Indonesia: Imai et al. (1984a)
Anochetus horridus	46	2K = 8M + 4SM + 34T	French Guyana: present study
Anochetus madaraszi Mayr	28	2K = 8M + 6 SM + 4 ST + 10 T	India: Imai et al. (1984b)
Anochetus modicus Brown	30	Not reported	Indonesia: Imai et al. (1984a)
Anochetus sp.	(17), 34	Not reported	Malaysia: Tjan et al. (1985)
Anochetus sp. 1	24	Not reported	Malaysia: Goñi et al. (1982)
Anochetus sp. 2	(19)	Not reported	Malaysia: Goñi et al. (1982)
Anochetus sp. 4	30	2K = 10 SM + 6M + 6 ST + 8 T	India: Imai et al. (1984b)
Anochetus sp. 5	34	2K = 6 M + 4 SM + 4 ST + 20 T	India: Imai et al. (1984b)
Anochetus yerburyi Forel	30	2K = 16 M + 14 T	India: Imai et al. (1984b)
Odontomachus chelifer	44	2K = 4SM + 40T	Brazil: present study
Odontomachus latidens Mayr	(15)	Not reported	Malaysia: Imai et al. (1983)
	32	Not reported	Indonesia: Imai et al. (1984a)
Odontomachus meinerti	44	2K = 4SM + 6ST + 34T	Brazil: present study
Odontomachus rixosus Smith	(15), 30	30, 30 + 1 B	Malaysia: Goñi et al. (1982); Imai et al. (1983)
Odontomachus simillimus Smith	(22), 44	Not reported	Malaysia: Goñi et al. (1982); Imai et al. (1983); Tjan et al. (1985)
	(22), 44	Not reported	Indonesia: Imai et al. (1984a)
Odontomachus sp.	44	2K = 2 SM + 4 ST + 38 T	Australia: Imai et al. (1977)
Odontomachus sp. 3	(22), 44	Not reported	Malaysia: Goñi et al. (1982)

Chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and telocentric (T) following Levan's nomenclature.

with a coverslip and incubated at RT for 30 min. After incubation, the slides were mounted in Vectashield solution (Vector Laboratories, Burlingame, CA). All procedures were performed in a dark room. After 3 d, the slides were observed using an epifluorescence microscope (DMRA2, Leica Microsystems Imaging Solutions Ltd., Cambridge, United Kingdom), and the images were captured using IM50 software (Leica Microsystems Imaging Solutions Ltd.).

Fluorescent In Situ Hybridization. For *A. altisquamis* and *A. horridus*, rDNA sites were detected using FISH and by using rDNA 45S *Arabidopsis thaliana* probes labeled with cyanine 3 (Cy3) by nick translation according to Moscone et al. (1996), with the following modifications. After aging for three days at RT, the slides were incubated at 60°C for 30 min. After incubation, 100 μl of denaturation mix (100% formamide, 2× standard saline citrate [SSC], and alcohol series) were added to each slide, which was covered with a plastic coverslip. The slides were then heated at 70°C for 7 min and rinsed in distilled water to remove the denaturation mix. After being dried for 1 h at room temperature, 10 μl of hybridization mix (rDNA 45S probes [5 ng/μl, labeled with Cy3], 100% formamide, 50% dextran, 20× SSC, and distilled water) previously heated at 75°C for 10 min was added to each slide. The slides were then covered with a coverslip and denatured again at 75°C for 10 min. After denaturation, the slides were sealed with a rubber solution and incubated at 37°C in a humid chamber for a minimum of 18 h. After hybridization, the slides were washed in 2× SSC and 0.1× SSC (72% of stringency) and mounted in DAPI/Vectashield medium. The adopted procedures for capturing images were the same as described for the fluorochrome staining (CMA_3/DAPI).

Results

Conventional Cytogenetics. The chromosome number and karyotype formula for each species are shown in Table 2. Conventional Giemsa staining in metaphases of *O. meinerti* (2n = 44), *O. chelifer* (2n = 44), and *A. altisquamis* (2n = 46) allowed us to observe and distinguish chromosome morphology used to construct the ideograms shown in Fig. 1.

Sequential Fluorochrome Staining. One CMA_3-positive band was localized on a telocentric chromosome pair in both *O. meinerti* (15th pair) and *O. chelifer* (11th pair). Despite the differences in the karyotypes of both species, the chromosome pairs with CMA_3-positive bands are highly similar in morphology (Figs. 1 and 2).

Fluorescence In Situ Hybridization. Two sites of rDNA 45S genes were identified in *A. altisquamis* and *A. horridus*. These sites showed a size heteromorphism detected by the difference in the strength of the hybridization signal on the chromosomes. This pattern was observed in at least five metaphases of the two analyzed individuals of each species. The rDNA genes were localized on the ninth chromosome pair in *A. altisquamis* and on the 10th chromosome pair in *A. horridus* (Figs. 1 and 2).

Discussion

Despite morphological similarities between *Odontomachus* and *Anochetus* species, their karyotypes displayed remarkable intergeneric and interspecific differences. Our investigations corroborate previous studies (Imai et al. 1977; Goñi et al. 1982; Imai et al. 1984a,b; Tjan et al. 1985), which revealed that *Anochetus* shows higher karyotype diversity than *Odontoma-*

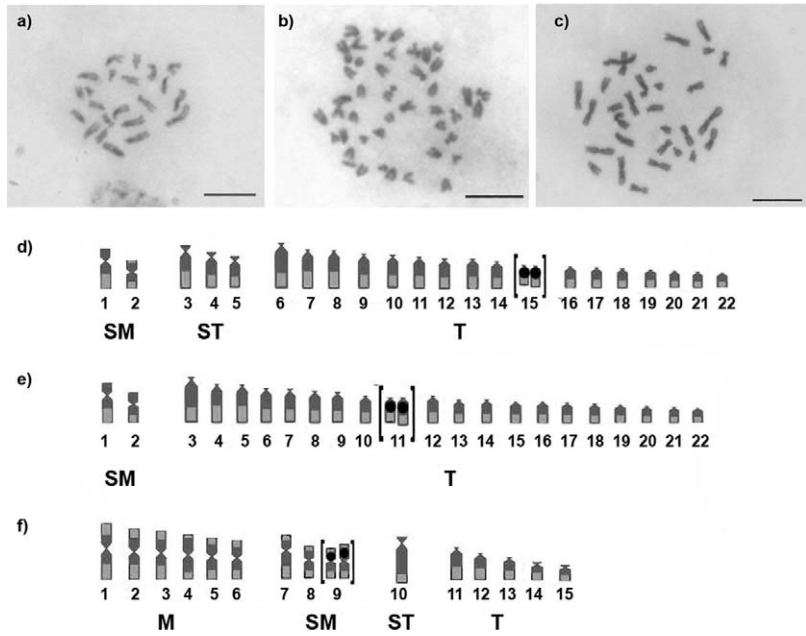

Fig. 1. Metaphases of haploid male of *O. meinerit* (a), female of *O. chelifer* (b) and female of *A. altisquamis* (c), and ideograms representing the haploid chromosome complement of *O. meinerti* (d), *O. chelifer* (e), and *A. altisquamis* (f). In the ideograms, CMA3-positive chromosomes are shown in brackets for *O. meinerti* and *O. chelifer*. For *A. altisquamis*, the chromosome pair in brackets represents sites of rDNA genes localized by FISH. Bars = 5 μm.

chus. The species of *Anochetus* and *Odontomachus* analyzed so far show that the karyotypes in the former include metacentric chromosomes that differ from the latter, whose karyotypes are composed by telocentric, subtelocentric, or submetacentric chromosomes (Imai et al. 1977, 1984b). It is noteworthy that neither *O. chelifer*, the basal-most species in Spagna's phylogeny, nor *O. meinerti* have metacentric chromosomes following the same karyotypic pattern previously reported in this genus. Considering these assumptions,

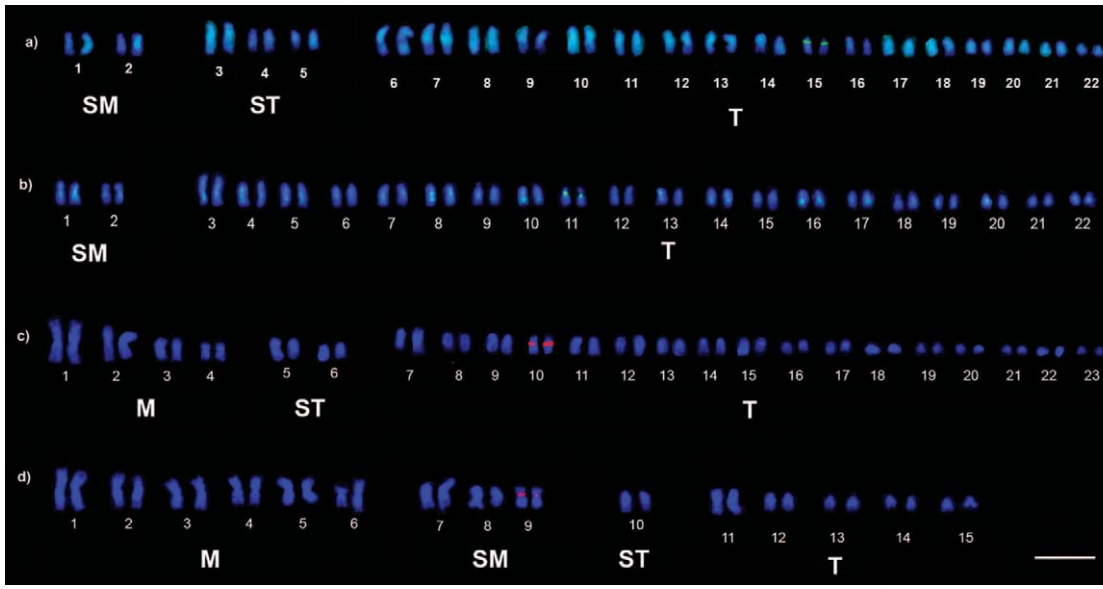

Fig. 2. Karyotypes of *O. meinerti* (a), *O. chelifer* (b), *A. horridus* (c), and *A. altisquamis* (d). CMA_3-positive bands (green bands) are shown on the 15th chromosome pair of *O. meinerti* and on the 11th chromosome pair of *O. chelifer*. rDNA 45S genes (red bands) localized by FISH are shown on the 10th chromosome pair of *A. horridus* and on the ninth chromosome pair of *A. altisquamis*. Bars = 5 μm. (Online figure in color.)

changes in chromosome morphology may have played an important role in the divergence between these genera. According to Imai et al. (2001), the most probable event responsible for chromosome evolution and karyotype differentiation in insects, and particularly in ants, is centric fission.

Odontomachus species most often have a higher chromosome number than *Anochetus* species and a higher number of telocentric chromosomes (Table 2). Remarkably, most *Odontomachus* species analyzed to date showed 2n = 44, except for *Odontomachus latidens* Mayr and *Odontomachus rixosus* (Smith) (Hymenoptera: Formicidae) (Table 2). Another important result from our study is that *A. horridus* showed 2n = 46, an exception in this genus whose modal chromosome number is 2n = 30. To the best of our knowledge, this is the highest chromosome number yet reported in the subtribe Odontomachiti. *Anochetus* is a more diverse genus with six different karyotypes known, including those in this study, and whose chromosome numbers range from 2n = 24–2n = 46.

The minimum interaction theory proposed by Imai et al. (1988a) states that chromosome numbers tend to increase by centric fission and this process could be evolutionarily favored in ants, as it reduces contact between non homologous chromosomes and therefore reduces the genetic risks of deleterious translocations during meiosis. Based on our data, we conclude that centric fission may have played an important role in the divergence between these genera, thus explaining the higher number of telocentric chromosomes in *Odontomachus.* Furthermore, the apparent more stable karyotypes in *Odontomachus* species, as well as the higher modal number of chromosomes (2n = 44), suggest their more recent divergence relative to *Anochetus.*

Our results can further support a basal position of *Anochetus* regarding the localization of the ribosomal genes and the variation in chromosome number in the species studied. In *A. altisquamis*, ribosomal genes were identified on the short arm of the ninth chromosome pair, which is submetacentric. In contrast, *A. horridus* ribosomal genes were located on telocentric chromosomes that correspond to chromosome pairs showing CMA_3 bands in *O. chelifer* and *O. meinerti.* Several previous studies have shown that the rDNA sites are GC-rich regions and therefore coincide with CMA_3 bands (Schmid 1978, Manicardi and Gautam 1994, Grozeva et al. 2004, Almeida et al. 2006).

The localization of rDNA genes on telocentric chromosomes may be a derived character that is highly likely to have been present in the ancestral lineage of *Anochetus* from which *Odontomachus* might have evolved. It is therefore reasonable to speculate that *A. altisquamis* most likely does not form a single line of direct ancestry with the other species compared here, and it may represent a more ancestral lineage in the subtribe Odontomachiti. If this hypothesis is correct, the NOR-bearing chromosome pair of *Odontomachus* may have resulted from fissioning of submetacentric chromosomes in its ancestral lineage. However, the karyotype of *A. horridus* represents a derived condition that has also arisen in this genus. According to Brown (1976), *Anochetus* represents the primitive stock of the subtribe and *Odontomachus* arose from some group of *Anochetus.* This conclusion is supported by several morphological and behavioral characters pointed out by this author. Our cytogenetic results lend support to this idea especially considering the presence of both ancestral and derived "*Odontomachus* like" karyotypes among *Anochetus* species.

Spagna's molecular phylogeny is the most complete phylogeny available to date for the subtribe Odontomachiti. Only two species of *Anochetus* and 12 species of *Odontomachus* were included in the analysis, a number that comprises respectively only 2 and 19% of the known species of each genus. A great deal of additional information is necessary to more precisely assess the phylogenetic relationships and, consequently an unambiguous establishment of the directions of karyotype evolution in *Odontomachus* and *Anochetus.* A comparative karyotype study that includes more species of both genera and a well-resolved phylogeny will reduce bias in the reconstruction of the evolutionary events involved in their divergence.

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References Cited

Agosti, D., and N. F. Johnson. 2003. Antbase: described species by country. (http://atbi.biosci.ohio-state.edu:210/hymenoptera/tpelox?ctry_name=Brazil).

Almeida, C. M., C. Campener, and D. M. Cella. 2006. Karyotype characterization, constitutive heterochromatin and nucleolus organizer regions of *Paranaita opima* (Coleoptera, Chrysomelidae, Alticinae). Genet. Mol. Biol. 29: 475–481.

Brandão, C.R.F. 1991. Adendos ao catálogo abreviado das formigas da Região Neotropical (Hymenoptera: Formicidae). Rev. Bras. Entomol. 35: 319–412.

Brown, W. L., Jr. 1976. Contributions toward a reclassification of the Formicidae. Part VI. Ponerinae, tribe Ponerini, subtribe Odontomachiti. Section A. Introduction, subtribal characters. Genus *Odontomachus.* Stud. Entomol. 19: 167–171.

Brown, W. L., Jr. 1978. A supplement to the world revision of *Odontomachus* (Hymenoptera, Formicidae). Psyche 84: 281–285.

Carlin, N. F., and D. S. Gladstein. 1989. The "bouncer" defense of *Odontomachus* ruginodis and other odontomachine ants (Hymenoptera: Formicidae). Psyche 96: 1–19.

Crosland, M.W.J., and R. H. Crozier. 1986. Myrmecia pilosula, an ant with only one pair of chromosomes. Science (Wash., D.C.) 231: 1278.

Crosland, M.W.J., R. H. Crozier, and H. T. Imai. 1988. Evidence for several sibling biological species centred on *Myrmecia pilosula* (F. Smith) (Hymenoptera: Formicidae). J. Aust. Entomol. Soc. 27: 13–14.

De Andrade, M. L. 1994. Fossil Odontomachiti ants from the Dominican Republic (Amber Collection Stuttgart: Hymenoptera, Formicidae. VII: Odontomachiti). Stuttg. Beitr. Naturkunde Ser. B 199: 1–28.

Ehmer, B., and B. Hölldobler. 1995. Foraging behavior of *Odontomachus bauri* on Barro Colorado Island, Panama. Psyche 102: 215–224.

Fernández, F. 2003. Introdución a las hormigas de la región Neotropical. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia.

Goñi, B., H. T. Imai, M. Kubota, M. Kondo, H. Yong, and Y. P. Tho. 1982. Chromosome observation in tropical ants in western Malaysia. Ann. Rep. Natl. Inst. Genet. (Jpn.) 32: 71.

Gronenberg, W., J. Tautz, and B. Hölldobler. 1993. Fast trap jaws and giant neurons in the ant *Odontomachus.* Science (Wash., D.C.) 262: 561–563.

Grozeva, S., V. G. Kuznetsova, and S. Nokkala. 2004. Patterns of chromosome banding in four nabid species (Heteroptera, Cimicomorpha, Nabidae). Hereditas 140: 99–104.

Hirai, H., M. T. Yamamoto, K. Ogura, Y. Satta, M. Yamada, R. W. Taylor, and H. T. Imai. 1994. Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the *Myrmecia pilosula* species complex. Chromosoma 103: 171–178.

Hirai, H., M. T. Yamamoto, R. W. Taylor, and H. T. Imai. 1996. Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus *Myrmecia* (Formicidae). Chromosoma 105: 190–196.

Imai, H. T., R. H. Crozier, and R. W. Taylor. 1977. Karyotype evolution in Australian ants. Chromosoma 59: 341–393.

Imai, H. T., W. L. Brown, Jr., M. Kubota, H. Yong, and Y. P. Tho. 1983. Chromosome observations on tropical ants from western Malaysia II. Ann. Rep. Natl. Inst. Genet. (Jpn.) 34: 66–69.

Imai, H. T., M. Kubota, W. L. Brown, Jr., M. Ihara, M. Tohari, and R. I. Pranata. 1984a. Chromosome observations on tropical ants from Indonesia. Ann. Rep. Natl. Inst. Genet. (Jpn.) 35: 46–48.

Imai, H. T., C. Baroni Urbani, M. Kubota, G. P. Sharma, M. N. Narasimhanna, B. C. Das, A. K. Sharma, A. Sharma, G. B. Deodikar, V. G. Vaidya, and M. R. Rajasekarasetty. 1984b. Karyological survey of Indian ants. Jpn. J. Genet. 59: 1–32.

Imai, H. T., R. W. Taylor, M.W.L. Crosland, and R. H. Crozier. 1988a. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. Jpn. J. Genet. 63: 159–185.

Imai, H. T., R. W. Taylor, R. H. Crozier, M.W.L. Crosland, and G. P. Browning. 1988b. Chromosomal polymorphism in the ant *Myrmecia* (*pilosula*) n=1. Ann. Rep. Nat. Inst. Genet. (Jpn.) 38: 82–84.

Imai, H. T., R. W. Taylor, and R. H. Crozier. 1994. Experimental bases for the minimum interaction theory. I. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (Hymenoptera: Formicidae: Myrmeciinae). Jpn. J. Genet. 69: 137–182.

Imai, H. T., Y. Satta, and N. Takahata. 2001. Integrative study on chromosome evolution of mammals, ants and wasps based on the minimum interaction theory. J. Theor. Biol. 210: 475–497.

Kempf, W. W. 1974. A remarkable new Neotropical species in the ant genus *Odontomachus* Latreille (Hymenoptera: Formicidae). Stud. Entomol. 17: 551–553.

Levan, A., K. Fredga, and A. Sonberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Manicardi, G. C., and D. C. Gautam. 1994. Cytogenetic investigation on the holokinetic chromosomes of *Tetraneura akinire* (Sasaki) (Homoptera. Pemphigidae). Caryologia 47: 159–165.

Mariano, C.S.F., and J.H.C. Delabie. 2004. Dinoponera lucida Emery (Formicidae: Ponerinae): the highest number of chromosome known in Hymenoptera. Naturwissenschaften 91: 182–185.

Mariano, C.S.F., S. G. Pompolo, L.A.C. Barros, E. Mariano-Neto, S. Campiolo, and J.H.C. Delabie. 2008. A biogeographical study of the threatened ant *Dinoponera lucida* Emery (Hymenoptera: Formicidae: Ponerinae) using cytogenetic approach. Insect Conserv. Divers. 1: 161–168.

Meyne, J., H. Hirai, and H. T. Imai. 1995. FISH analysis of the telomere sequences of bulldog ants (*Myrmecia:* Formicidae). Chromosoma 104: 14–18.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: diversification in the age of Angiosperms. Science (Wash., D.C.) 312: 101–104.

Moscone, E. A., M. A. Matzke, and A.J.M. Matzke. 1996. The use of combined FISH/GISH in conjunction with DAPI counterstaining to identify chromosomes containing transgenes inserts in amphidiploid tobacco. Chromosoma 105: 231–236.

Patek, S. N., J. E. Baio, B. L. Fisher, and A. V. Suarez. 2006. Multifunctionality and mechanical origins: ballistic jaw propulsion in trap-jaw ants. Proc. Natl. Acad. Sci. USA 103: 12787–12792.

Schweizer, V. 1976. Reverse fluorescent chromosome banding. Chromosoma 58: 317–324.

Schlick-Steiner, B. C., F. M. Steiner, B. Seifert, C. Stauffer, E. Christian, and R. H. Crozier. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entomol. 55: 421–438.

Schmid, M. 1978. Chromosome banding in Amphibia I: constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla.* Chromosoma 66: 361–388.

Spagna, J. C., A. I. Vakis, C. A. Schmidt, S. N. Patek, X. Zhang, N. D. Tsutsui, and A. V. Suarez. 2008. Phylogeny, scaling, and the generation of extreme forces in trap-jaw ants. J. Exp. Biol. 211: 2358–2368.

Taylor, R. W. 1991. *Myrmecia croslandi* sp. n. a karyologically remarkable new Australian jack-jumper ant (Hymenoptera: Formicidae: Myrmeciinae). J. Aust. Entomol. Soc. 30: 288.

Tjan, K. N., H. T. Imai, M. Kubota, W. L. Brown, Jr., W. H. Gotwald, Jr., H. Yong, and C. Leh. 1985. Chromosome observations of Sarawak ants. Ann. Rep. Natl. Inst. Genet. (Jpn.) 36: 51.

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