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ORIGINAL ARTICLE

Cytogenetic variability in four species of *Gnamptogenys* Roger, 1863 (Formicidae: Ectatomminae) showing chromosomal polymorphisms, species complex, and cryptic species

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Abstract

Gnamptogenys includes 138 described species that are widely distributed, with high diversity, in the Neotropics. Some Neotropical species have taxonomic issues, as is the case with *Gnamptogenys striatula*, for which morphological variations have been observed between different populations. For the ant species with taxonomic issues, classical and molecular cytogenetic studies have assisted in the resolution of these issues. Cytogenetic studies of *Gnamptogenys* are scarce and have only been reported for 14 taxa. These reports have rarely presented chromosomal morphology. Considering the importance of the taxonomic revision of some species, such as *G. striatula*, the present study cytogenetically characterized four species of *Gnamptogenys*: *G. striatula*, *G. moelleri*, *G. regularis*, and *G. triangularis*, discussing their phylogenetic and biogeographic characteristics. The number of chromosomes ranged from 2n = 26 to 2n = 44, with distinct karyotypes at both species and population levels. All four species presented a pair of 18S rDNA gene markers that coincided with GC-rich regions. In the case of *G. striatula* from the Atlantic rainforest, a chromosomal polymorphism was observed, with chromosomal translocations being the likely origin of this polymorphism. Two populations of *G. striatula* showed karyotype differences, thus corroborating previous morphological data indicating the existence of a species complex in this taxon. In addition, *G. regularis* showed a polymorphism involving a chromosome pair bearing ribosomal genes, possibly caused by unequal crossing-over. Although *G. moelleri* has a well-defined taxonomy, a population from the eastern Amazon rainforest presented a divergent karyotype from the Atlantic rainforest populations, suggesting the existence of a cryptic species in this taxon.

Keywords Ant · Biodiversity · Chromosome number · 18S rDNA gene · Taxonomy · Evolution

Introduction

The genus *Gnamptogenys* Roger, 1863 contains 138 described species that are widely distributed in Neotropical, Nearctic, Indo-Malayan, and Australian regions (Lattke 1995; Lattke et al. 2008). The greatest diversity of *Gnamptogenys* spp. occurs in the Neotropical region, with 90 valid species, corresponding to approximately 64% of all species in this genus and including 33 species in Brazil (Feitosa 2015; Camacho and Feitosa 2015). *Gnamptogenys* has been divided into 10 groups, of which four groups (*taivanensis*, *coxalis*, *epinotalis*, and *laevior*) include Old World species (Lattke et al. 2004) and the remaining six groups (*striatula*, *minuta*, *rastrata*, *sulcata*, *mordax*, and *concinna*) belong to the Neotropical region (Lattke 1995). The available phylogenetic data, based on morphological characteristics, suggests two main clades for the Neotropical

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Gnamptogenys spp.: the *striatula* group, which is the least derived and the sister group of the other Neotropical groups (Lattke 1995).

Lattke et al. (2008) have emphasized the complexities of the taxonomic resolution of some Neotropical species, such as *Gnamptogenys striatula* Mayr, 1884, because of their remarkable morphological variability in different populations, which blurs their delimitation and suggests that it is a species complex. The authors have pointed out some possible reasons for these morphological variations, such as phenotypic plasticity for different environments, the existence of cryptic species and hybrid zones, and character displacement (Lattke et al. 2008). Cytogenetics has been a useful tool for understanding the complex taxonomic groups in the family Formicidae, using the principles of integrative taxonomy (Cristiano et al. 2013; Correia et al. 2016; Santos et al. 2018; Aguiar et al. 2017; Silva-Rocha et al. 2017; Santos et al. 2018). These cytogenetic studies have helped delimit ant taxa by identifying karyotypic differences, including variations in the number, morphology, and pattern of chromosomal banding (classical cytogenetics) and/or the distribution of ribosomal gene regions (molecular cytogenetics).

The number of chromosomes in Neotropical ants has been shown to range from 2n = 4 in *Strumygenys louisianae* (Alves-Silva et al. 2014) to 2n = 120 in *Dinoponera lucida* (Mariano et al. 2008). The minimum interaction theory seeks to explain this chromosomal variation (Imai et al. 1994). According to this theory, there is a tendency towards an increase in the number of chromosomes, with a consequent reduction in size, by means of centric fissions. Through in-tandem growth of heterochromatin, these smaller chromosomes acquire telomeric stability. Thus, according this theory, the greater physical distance between chromosomes in the interphasic nucleus, through their reduction of size, results in a decreased chance of deleterious chromosomal rearrangements.

Cytogenetic studies of *Gnamptogenys* are scarce and have only been reported for 13 taxa. Only a few of these studies have provided information about chromosomal morphology (Table 1). The number of chromosomes ranges from 2n = 16 in *Gnamptogenys* sp. 2 (Mariano et al. 2015) to 2n = 68 in *G. annulata* (Borges et al. 2004). The groups *rastrata*, *striatula*, and *mordax* of the Neotropical region and *coxalis* of the Oriental region have species with cytogenetic information available. However, there is no previous information regarding chromosomal banding or the mapping of ribosomal genes for this genus. Based on the available cytogenetic data for *Gnamptogenys* spp., Mariano et al. (2015) proposed the evolution of karyotypes according to the assumptions of the minimum interaction theory.

Considering the complex taxonomic resolution of some species of *Gnamptogenys*, such as *G. striatula*, the present study aimed to characterize four species of *Gnamptogenys*: *G. striatula* and *G. moelleri* (Forel, 1912), both belonging to the *striatula* group; *G. triangularis* (Mayr, 1887) from the *rastrata* group; and *G. regularis* Mayr, 1870 from the *mordax* group, by utilizing classical and molecular cytogenetic techniques. The information presented herein will assist in the taxonomic resolution of these species.

Table 1 Summary of available cytogenetic data in the literature of *Gnamptogenys* spp. Species, groups according to Lattke classification (1995) and (2004), chromosome number diploid/haploid, karyotypic formula, locality, and bibliographical references. Classification of chromosomes according to Imai (1991): M: metacentric, A: acrocentric. Brazilian States: MG: Minas Gerais and BA: Bahia

Specie	Group	2n/n	Karyotypic formula	Locality	Reference
Gnamptogenys annulata	*mordax*	68	6M + 62A	Brazil-BA	Borges et al. 2004
Gnamptogenys binghami	*coxalis*	–/22	–	Malaysia	Imai et al. 1983
Gnamptogenys menadensis	*coxalis*	42	–	Malaysia	Imai et al. 1983
Gnamptogenys moelleri	*striatula*	34/17	20M + 14A	Brazil-BA	Mariano et al. 2015
Gnamptogenys pleurodon	*striatula*	32/16	–	French Guiana	Mariano et al. 2015
Gnamptogenys sp. nv.	–	46/23	16M + 30A	Brazil-BA	Borges et al. 2004
Gnamptogenys sp. 1	–	36/18	–	Brazil-BA	Mariano et al. 2015
Gnamptogenys sp. 2	–	16/8	–	Brazil-BA	Mariano et al. 2015
Gnamptogenys sp. 1	–	26/13	14M + 12A	Costa Rica	Mariano et al. 2015
Gnamptogenys sp. 2	–	32/16	16M + 16A	Costa Rica	Mariano et al. 2015
Gnamptogenys sp. 2	–	36	–	Malaysia, Singapore	Goni et al. 1982, Imai et al. 1983
Gnamptogenys striatula	*striatula*	34	24M + 10A	Brazil-BA, MG	Borges et al. 2004
Gnamptogenys triangularis	*rastrata*	20/10	–	French Guiana	Mariano et al. 2015

Materials and methods

Four *Gnamptogenys* species were cytogenetically studied (Table 2). Sample collection was performed under the authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for the collection of biological material issued to Luísa Antônia Campos Barros (SISBIO accession number 32459). Ant vouchers (workers) were identified by Jacques Hubert Charles Delabie and deposited in the reference collection at the Laboratório de Mirmecologia, Centro de Pesquisas do Cacau (CPDC/Brazil). All colonies of *Gnamptogenys* spp. studied in the present work were collected from decaying trunks lying on the ground, which is characteristic nesting of most species of the genus (Lattke et al. 1995).

The metaphases were obtained from cerebral ganglia of the larvae after meconium elimination, according to Imai et al. (1988). To determine the number and chromosome morphology of the *Gnamptogenys* spp., at least 10 metaphases of each specimen were analyzed for chromosome number determination and 10 for species for karyotypic formula. For subsequent techniques, at least 30 metaphases were analyzed for each species (Table 2). The karyotypes of the *Gnamptogenys* spp. were arranged in order of decreasing size of the chromosomes of the long arm base, based on the morphology according to the measurement of Levan et al. (1964). Adobe® Photoshop® CS6 and Image Pro Plus® were the software used for mounting the chromosomal karyotype and measurements, respectively.

Specific GC- and AT-rich regions were detected using sequential staining with the fluorochrome Chromomycin A_3 (CMA_3) and 4′6-diamidino-2-phenylindole (DAPI) following the technique reported by Schweizer (1980), for all the species, with modifications that consisted in the removal of Distamycin.

Ribosomal 18S gene clusters of the four species were detected by fluorescence *in situ* hybridization (FISH), according to Pinkel et al. (1986), with the use of the 18S rDNA probe by amplification via PCR (polymerase chain reaction) employing the primers rDNA 18SF1 (5′-GTC ATA GCT TTG TCT CAA AGA-3′) and 18SR1.1 (5′-CGC AAA TGA AAC TTT TTT AAT CT-3′) designed for the bee *Melipona quinquefasciata* (Pereira 2006) and isolated from total DNA of the ant *Camponotus rufipes*. 18S rDNA probes were labeled by an indirect method using digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany), and the FISH signals were detected with anti-digoxigenin-rhodamine (Roche Applied Science), following the manufacturer's protocol.

The metaphases were observed and documented using a fluorescence microscope Olympus BX 60, coupled with capture system Q-Color3 Olympus® images, using the software Q capture® with the filters WB (450–480 nm), WU (330–385 nm), and WG (510–550 nm) for analyzing CMA_3, DAPI, and rhodamine, respectively.

Table 2 Species of *Gnamptogenys* cytogenetically analyzed in the present study. Colonies, species, Lattke classification (1995), locality (geographical coordinates), total number of individuals and metaphases analyzed by colony, diploid chromosome number, and karyotypic formula. Classification of chromosomes according to Levan et al. (1964): m: metacentric, sm: submetacentric, st: subtelocentric. Brazilian States: MG: Minas Gerais, RJ: Rio de Janeiro, and MA: Maranhão

Colonies	*Gnamptogenys* spp.	Groups	Locality (coordinates)	Ind/ Met	2n	Karyotypic formula
CI 61	*G. triangularis*	*rastrata*	Araponga–MG (20°67′S, 42°51′W)	6/48	24	18m + 6sm
Lu 62	*G. regularis*	*mordax*	Ponte Nova–MG (20°38′S, 42°92′W)	4/56	26	25m + 1sm[a]
Lu 62	*G. regularis*	*mordax*	Ponte Nova–MG (20°38′S, 42°92′W)	3/46	26	24m + 2sm
CI 104	*G. striatula*	*striatula*	Rio de Janeiro–RJ (23°02′S, 43°47′W)	10/122	32	20m + 10sm + 2st
CI 105	*G. striatula*	*striatula*	Rio de Janeiro–RJ (23°02′S, 43°47′W)	4/35	32	20m + 10sm + 2st
CI 14	*G. striatula*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	1/10	34	18m + 7sm + 9st[b]
CI 18	*G. striatula*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	2/31	34	18m + 7sm + 9st[b]
CI 19	*G. striatula*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	1/8	34	18m + 7sm + 9st[b]
CI 20	*G. striatula*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	5/98	34	18m + 7sm + 9 st[b]
CI 20	*G. striatula*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	2/24	34	18m + 8sm + 8st
CI 26	*G. moelleri*	*striatula*	Petrópolis–RJ (22°53′S, 43°18′W)	5/134	34	18m + 8sm + 8st
CI 75	*G. moelleri*	*striatula*	Viçosa–MG (20°80′S, 42°85′W)	6/87	34	18m + 8sm + 8st
CI 349	*G. moelleri*	*striatula*	Viçosa–MG (20°75′S, 42°88′W)	4/36	34	18m + 8sm + 8st
CI 350	*G. moelleri*	*striatula*	Viçosa–MG (20°75′S, 42°88′W)	1/19	34	18m + 8sm + 8st
CI 389	*G. moelleri*	*striatula*	Viçosa–MG (20°75′S, 42°88′W)	4/79	34	18m + 8sm + 8st
CI 180	*G. moelleri*	*striatula*	Açailândia–MA (4°83′S, 47°52′W)	9/109	44	22m + 14sm + 8st

[a] Heterozygous individuals for the polymorphism involving regions of ribosomal genes in *G. regularis*

[b] Heterozygous individuals for the chromosomal polymorphism observed in *G. striatula* from Petrópolis-RJ

Results

The sampled localities of *Gnamptogenys* spp. in Brazil are indicated in Fig. 1. The chromosome number and karyotypic data obtained for the *Gnamptogenys* species under investigation in the present study are shown in Table 2. The chromosome number observed in *G. triangularis* was 2n = 24 (2n = 18m + 6sm; Fig. 2a). 18S rDNA gene clusters and CMA_3^+ markers were observed in the interstitial region of the long arm of the largest metacentric pair in *G. triangularis* (Fig. 2b; Fig. S1a).

The species *G. regularis*, from an agricultural area in the Atlantic rainforest in the locality of Ponte Nova, showed 2n = 26 chromosomes, with the existence of chromosomal polymorphism among individuals within the same colony (Fig. 3). In the heterozygous condition, there was only one submetacentric chromosome in diploid cells (2n = 25m + 1sm), whereas in the homozygous condition, a submetacentric pair was found (2n = 24m + 2sm, Fig. 3a, b). Homozygous individuals with two metacentric chromosomes were not observed. The 18S rDNA and CMA_3^+ regions were located on the long arm of the polymorphic chromosomal pair of *G. regularis*. The difference in size of the clusters between the homologs was so large that it changed the chromosomal morphology (Fig. 3c; Fig. S1c). Homozygous individuals with this chromosome pair, having the same size as the larger 18S rDNA clusters (Fig. 3d; Fig. S1e), were also detected. However, homozygous individuals with smaller 18S rDNA clusters were not observed.

G. striatula also showed variations in karyotype among the studied populations. In a restinga area of the Atlantic rainforest in Rio de Janeiro (RJ), *G. striatula* showed 2n = 32 chromosomes (2n = 20m + 10sm + 2st) (Fig. 4a), but in the highland areas of the Atlantic rainforest in Petrópolis (RJ), *G. striatula* showed 2n = 34, with the presence of another polymorphism. At this locality, heterozygous individuals had an extra subtelocentric chromosome (2n = 18m + 7sm + 9st), whereas in the homozygous condition, a submetacentric pair (2n = 18m + 8sm + 8st) was observed (Fig. 4b, c). Homozygous individuals with two subtelocentric chromosomes were not observed.

More evident chromosomal variation was observed between populations of *G. moelleri.* In both highland and agricultural areas of the Atlantic rainforest in Petrópolis (RJ) and Viçosa (MG), *G. moelleri* presented 2n = 34 (2n = 18m + 8sm + 8st; Fig. 4d, e). However, the Amazon population of *G. moelleri* in Açailândia (MA) showed 2n = 44 (2n = 22m + 14sm + 8st; Fig. 4f).

The 18S rDNA clusters and GC-rich regions were located in the interstitial region of the short arm of the sixth metacentric pair in *G. striatula* from a restinga area of the Atlantic rainforest (Fig. 5a; Fig. S2a) and in the interstitial region of the short arm of the fifth metacentric pair in *G. striatula* from a highland area of the Atlantic rainforest (Fig. 5b; Fig. S2c). *G. moelleri* from both the Atlantic (Fig. 5c; Figs. S3a and S3c) and Amazon rainforests (Fig. 5d; Fig. S3e) presented 18S rDNA clusters and GC-rich chromatin in the interstitial region of the short arm of the fifth metacentric pair. Secondary constrictions were observed in these regions containing 18S rDNA genes and CMA_3^+ (Fig. 4, asterisks).

All four species investigated in the present study showed a single chromosomal region with GC-rich chromatin (CMA_3^+), which coincided with clusters of 18S ribosomal genes. None of the studied species exhibited AT-rich chromatin (Figs. S1, S2, and S3s) or traces of mosaicism for polymorphic conditions.

Discussion

Intra- and interspecies karyotypic variability in *Gnamptogenys*

All the species investigated here shared cytogenetic characteristics, to some extent. However, large variations were observed in several chromosomal features, such as chromosome number and morphology and polymorphic conditions, thus highlighting the karyotypic diversity within *Gnamptogenys*, at both the species and population levels. The observed chromosomal variation was not restricted to distant populations belonging to different biomes, such as the Atlantic and Amazon rainforests. The highland and restinga areas of the Atlantic rainforest studied here were only 57 km apart, with almost 1000 m in altitude variation.

All studied species and their populations had a single pair of chromosomes carrying clusters of 18S ribosomal genes, which were GC-rich regions. This trait is common among Neotropical ants (Santos et al. 2016; Barros et al. 2016; Aguiar et al. 2017; Teixeira et al. 2017). *G. striatula* and *G. moelleri* had secondary constriction regions, corresponding to the location of the 18S rDNA genes. Similar constrictions have previously been observed in other ants, such as *Mycocepurus goeldii* (Barros et al. 2010, 2012) and *Atta* spp. (Barros et al. 2014a). Nucleolus organizer regions (NORs), which include rRNA genes (18S, 5.8S, and 28S), are located in regions that may form secondary constrictions in metaphasic chromosomes (Sumner 2003).

The species *G. triangularis*, of the *rastrata* group from the Atlantic rainforest, showed a different chromosome number (2n = 24) in the present study, compared to a previous study of specimens from the Amazon rainforest (2n = 20, Mariano et al. 2015), which did not report any information regarding chromosome morphology. Taking into account the distance between the two populations and their different biogeographic characteristics, these two populations may not correspond to the same species.

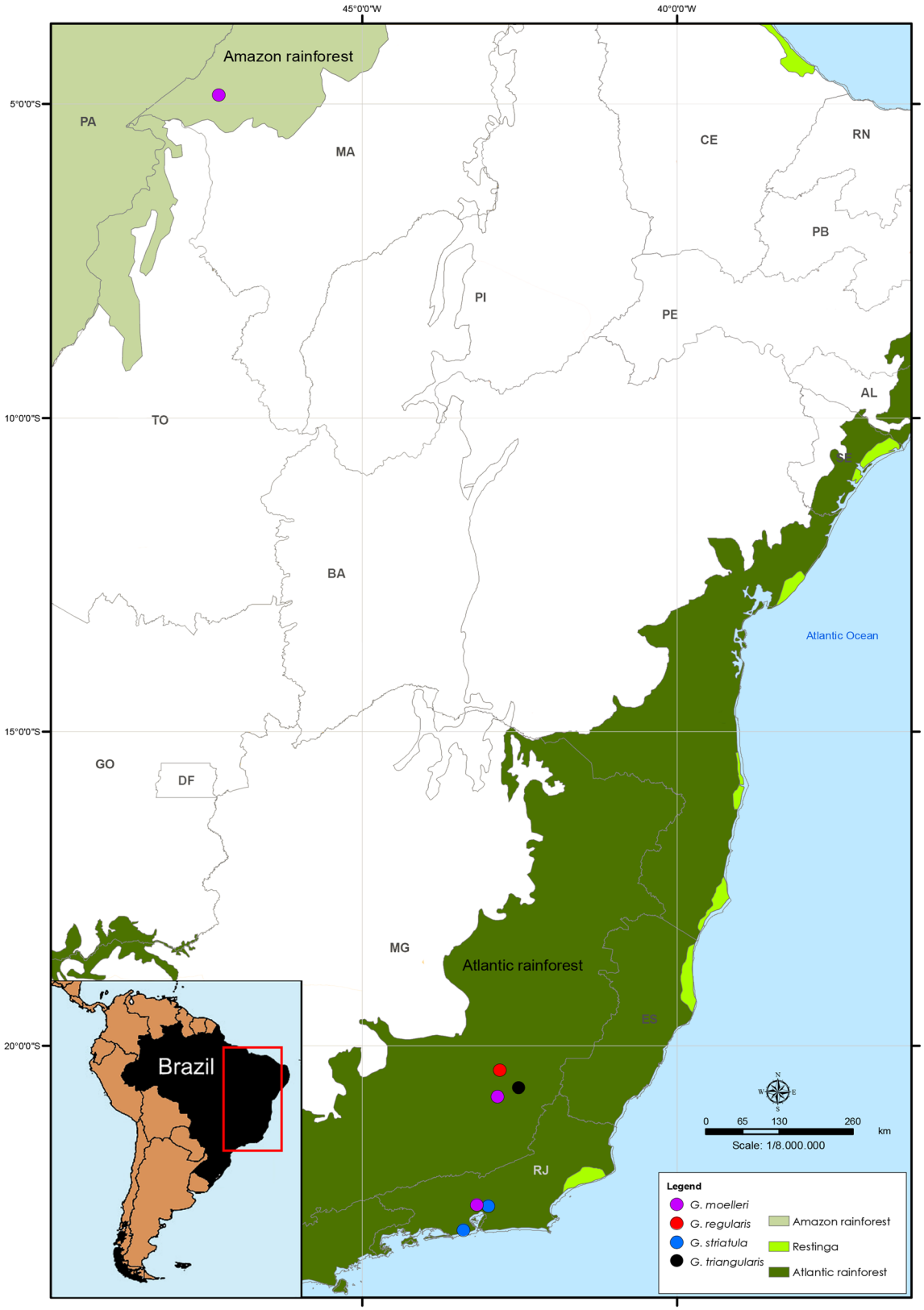

Fig. 1 Sampled localities of *Gnamptogenys* spp. in Brazil. The colored dots represent the collection sites in the Amazon rainforest and Atlantic rainforest

Among the *Gnamptogenys* spp. from the *mordax* group, *G. regularis* from the Atlantic rainforest at Ponte Nova showed the lowest number of chromosomes for this group (2n = 26), whereas *G. annulata* from a cocoa plantation in

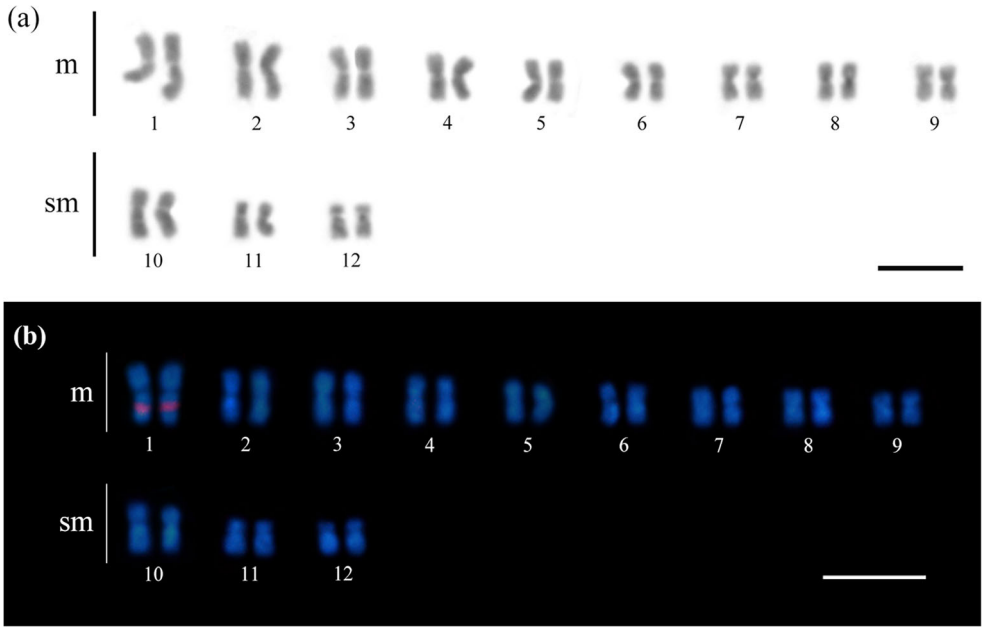

Fig. 2 Conventional and molecular cytogenetics of *Gnamptogenys triangularis* (*rastrata* group): **a** female karyotype (2n = 24, 18m + 6sm), **b** fluorescence *in situ* hybridization analysis for 18S rDNA (red blocks). Bar = 5 μm

the Atlantic rainforest, presented 2n = 68 chromosomes, which is the largest number reported for the *Gnamptogenys* genus (Borges et al. 2004). Thus far, the *mordax* group appears to have the greatest amount of chromosome variability

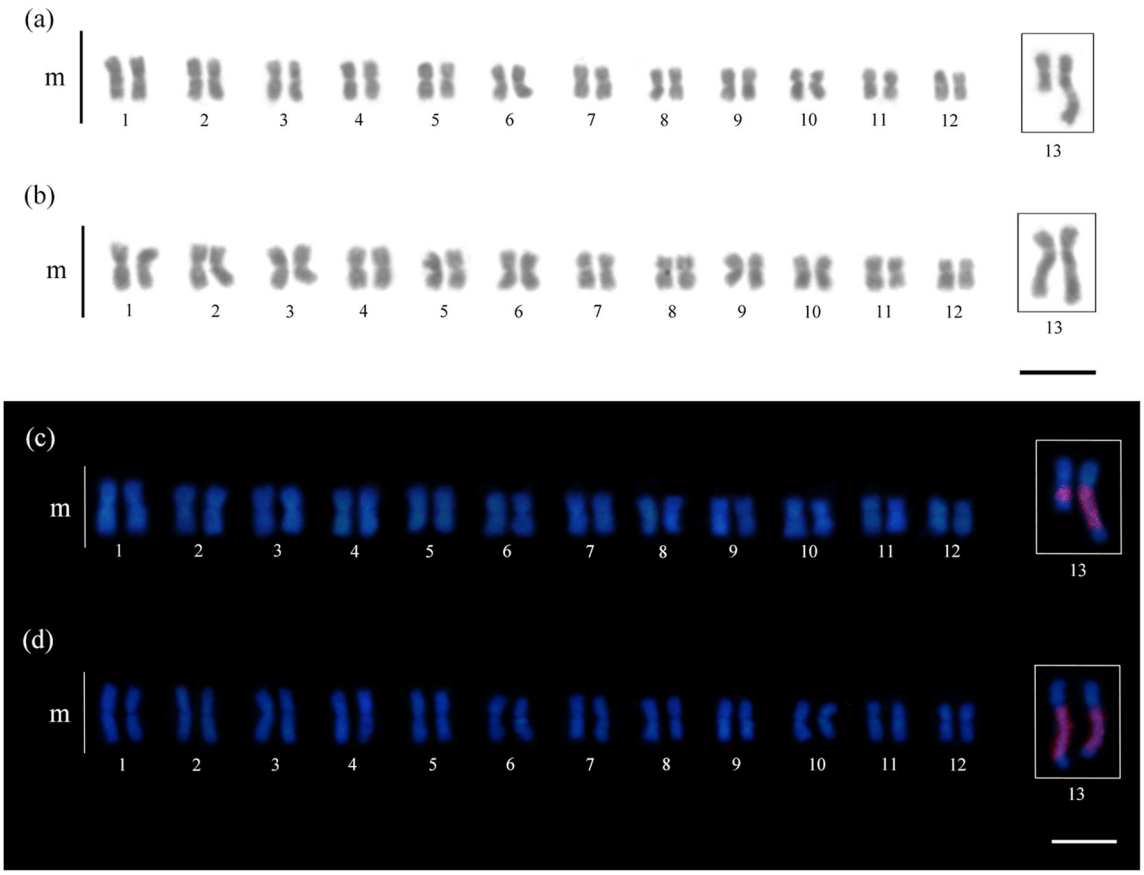

Fig. 3 Conventional and molecular cytogenetics of *Gnamptogenys regularis* (*mordax* group). Female karyotypes with **a** 2n = 26, 25m + 1sm and **b** 2n = 26, 24m + 2sm. **c**, **d** Metaphases after fluorescence *in situ* hybridization with 18S rDNA probe (red blocks). The 18S rDNA regions were located on the long arm of the polymorphic chromosomal pair. The boxes show a polymorphic chromosomal pair in **a**, **c** the heterozygous state and **b**, **d** one of the homozygous states. Bar = 5 μm

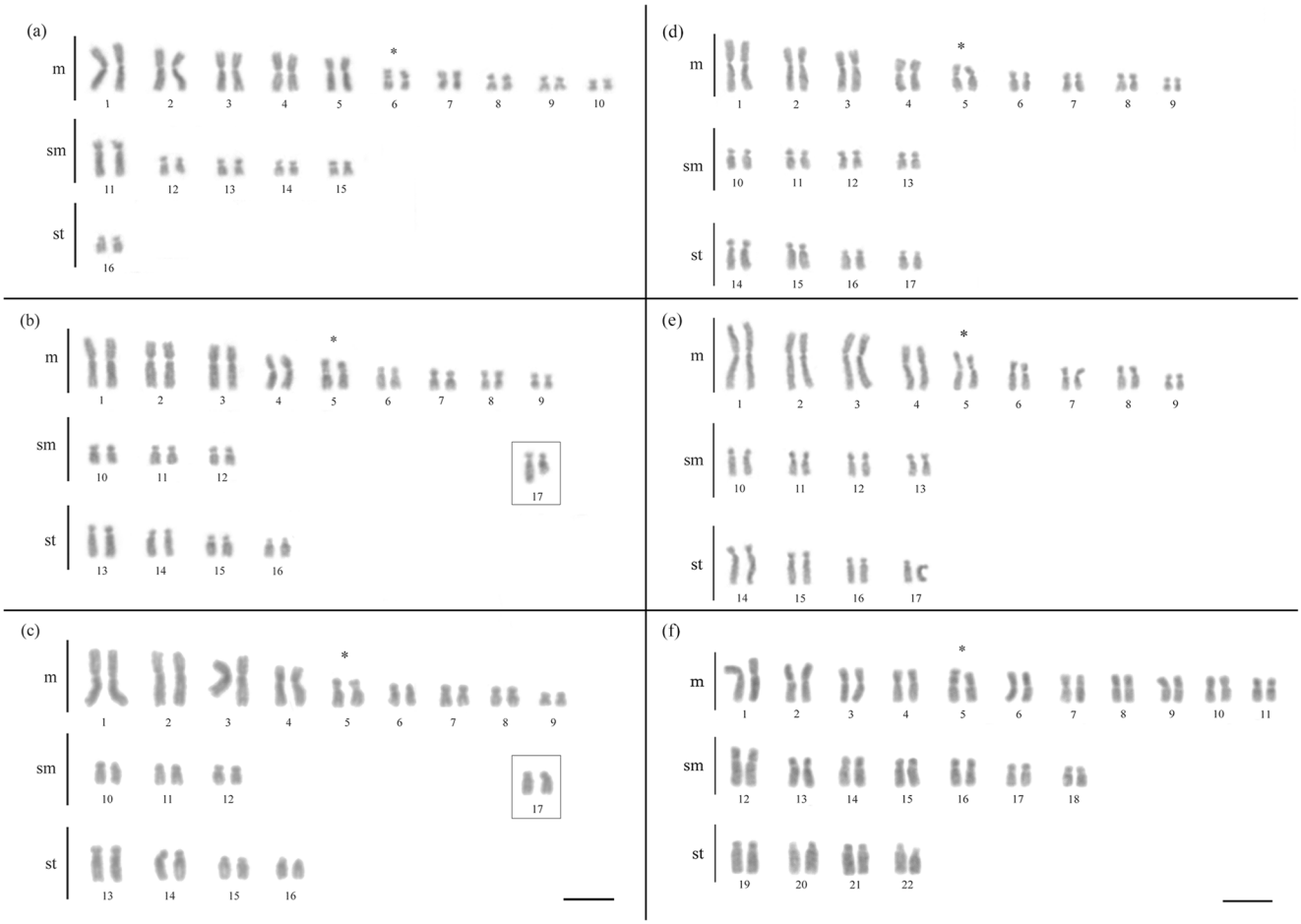

Fig. 4 Female karyotypes of *Gnamptogenys* spp. (*striatula* group) and its localities: **a** *G. striatula* from Rio de Janeiro (2n = 32, 20m + 10sm + 2st), **b** *G. striatula* from Petrópolis (2n = 34, 18m + 7sm + 9st), **c** *G. striatula* from Petrópolis (2n = 34, 18m + 8sm + 8st), **d** *G. moelleri* from Petrópolis (2n = 34, 18m + 8sm + 8st), **e** *G. moelleri* from Viçosa (2n = 34, 18m + 8sm + 8st), and **f** *G. moelleri* from Açailândia (2n = 44, 22m + 14sm + 8m). The boxes show polymorphic chromosomal pair in *G. striatula* from Petrópolis, **b** in the heterozygous state, and **c** in one of the homozygous states. Asterisks indicate metacentric chromosome pairs with secondary constriction. Bar = 5 μm

within *Gnamptogenys*, and in this locality, *G. regularis* showed a polymorphism involving the chromosome pair bearing 18S rDNA clusters. Since both homozygous and heterozygous conditions were observed within the same colony, this polymorphic trait may have originated from the queen or the male. However, it was not possible to harvest any male larvae from the nests and polygyny has been reported in nests of *G. regularis* (Lattke 1990; Lattke et al. 2008), which makes it impossible to establish the origin of the polymorphic chromosome.

It is probable that a chromosomal polymorphism of such highly duplicated chromatin as the rDNA gene clusters of *G. regularis* may have originated by duplications/deletions of these sequences, due to unequal crossing-over between homologous chromosomes (Kasahara 2009; Schubert and Lysac 2011). In fishes, NOR polymorphisms are common (Vicari et al. 2003, 2006; Phimpan et al. 2013) and usually originate from unequal crossing-over or the exchange of chromatin between sister chromatids. In ants, this is the first report of a polymorphism involving rRNA gene loci. An increase in the number of rRNA gene clusters in the grasshopper, *Oedipoda fuscocincta*, has been shown to lead to an increase in NOR activity due to gene duplication (Camacho et al. 1986). A polymorphism has been reported in the fungus farming ant, *Trachymyrmex fuscus*, involving euchromatic regions (Barros et al. 2014). Duplication of gene loci allows the accumulation of independent mutations and the acquisition of new functions, eventually contributing to speciation (Palomeque et al. 1993).

Two types of chromosomal variation were observed in the species *G. striatula*, which belongs to the *striatula* group. The first type of variation was in the number and morphology of chromosomes between individuals from Rio de Janeiro (2n = 32) and Petrópolis (2n = 34) localities. This is consistent with the findings of Borges et al. (2004), who reported that specimens from Viçosa and Ilhéus also had 2n = 34 chromosomes. The second type of variation was polymorphism in a pair of chromosomes within colonies from Petrópolis. A comparison

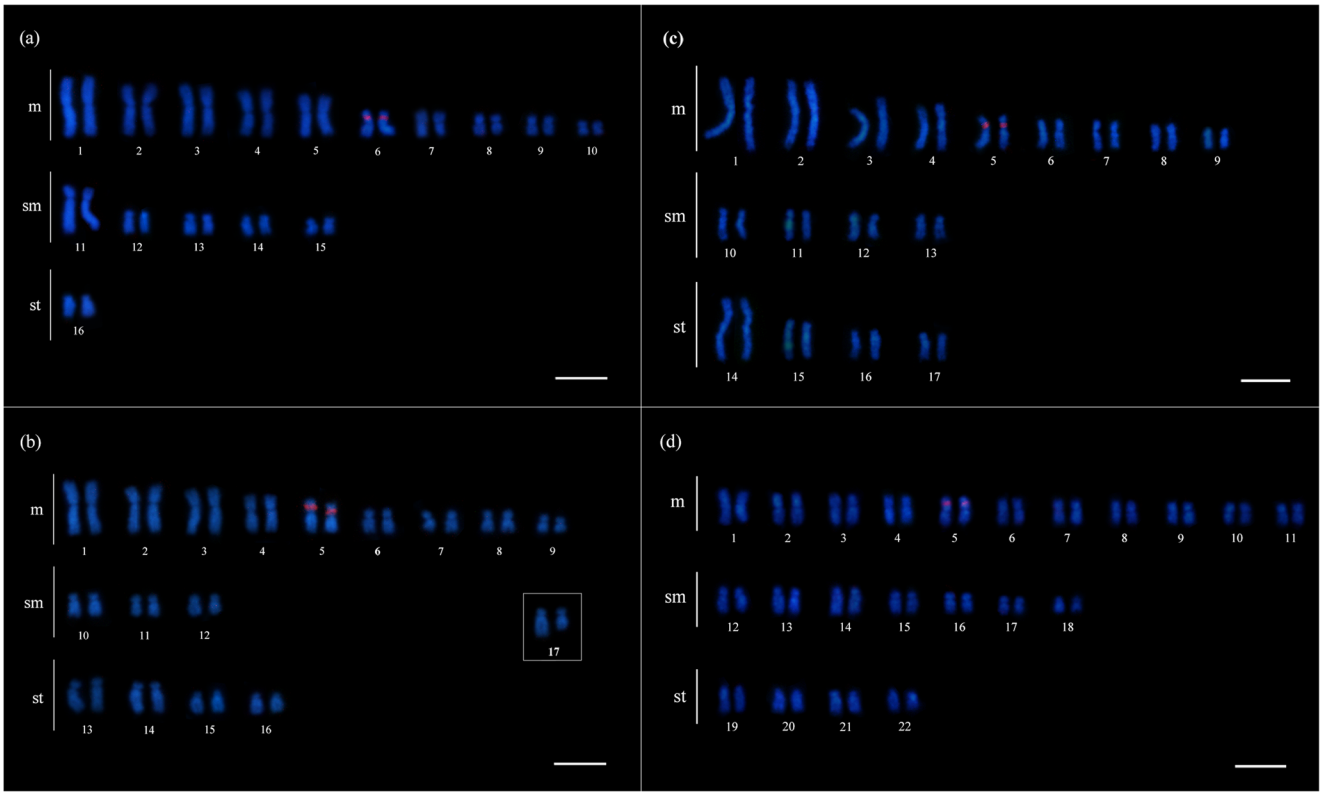

Fig. 5 Female karyotypes of *Gnamptogenys* spp. (*striatula* group) after fluorescence *in situ* hybridization with 18S rDNA probe (red blocks): **a** *G. striatula* from Rio de Janeiro, **b** *G. striatula* from Petrópolis, **c** *G. moelleri* from Petrópolis, and **d** *G. moelleri* from Açailândia. Box in **b** shows non-carrier heterozygous polymorphic chromosomal pair of 18S rDNA genes clusters. Bar = 5 μm

of the karyotypes of ants from these three distant localities (Petrópolis, Viçosa, and Ilhéus) from the Atlantic rainforest showed great similarity in both chromosome number and morphology, with the exception of the chromosome polymorphism observed in ants from Petrópolis.

Furthermore, comparisons made between populations of the restinga and highland regions of the Atlantic rainforest corroborated these similarities and showed that differences were not limited to variation in a single chromosomal pair. Both populations had four pairs of small metacentric chromosomes, assuming that the chromosome pair that carries the NOR clusters is intermediate and potentially variable in size because of late condensation in prophase (Sumner 2003). The polymorphic chromosome pair does not bear NOR clusters and therefore, is not subject to such size variations, as postulated by Sumner (2003). In the homozygous state, the polymorphic chromosome pair was considered to be small and submetacentric, and therefore, the two populations had four smaller submetacentric chromosomes. With the similarities fixed, an investigation of the differences becomes possible. The restinga population of Rio de Janeiro had a larger metacentric chromosome pair (pair 4; Fig. 4a) and a larger submetacentric pair (par 11; Fig. 4a) than the population from Petrópolis. Additionally, the restinga population had only one subtelocentric chromosome pair (par 16; Fig. 4a), whereas the Petrópolis population had four subtelocentric pairs, of which two were large.

Because of the high intraspecific variation in several morphological traits of *G. striatula*, it is considered a species complex (Camacho 2013; Lattke et al. 2008). The individuals vary significantly in size, sculpture, hairiness, the position of propodeal spiracles, and petiole shape. Camacho (2013) also reported high ecological tolerance and variations in behavior and morphology, only in *G. striatula* and not in other species of the genus. However, due to the absence of a pattern in these variations, many species, subspecies, and varieties have been described in the course of time and posteriorly synonymized in subsequent studies (Camacho 2013), thus reinforcing the hypothesis of species complex in *G. striatula*.

The Rio de Janeiro and Petrópolis field localities used in this study are both Atlantic rainforest regions; however, they have different phytophysiognomies, since the former is considered a restinga (sandy coastal plain) and the latter, a highland area (dense montane forest). Individuals from these two populations did not show clear morphological divergence from each other, but they had different chromosomal traits and belonged to different environments. Thus, these two populations may actually be two different cryptic species, which may hybridize (according to the possibility raised by Lattke et al. 2008), producing variant chromosomes. However, the

karyotype configuration of the polymorphic individuals from Petrópolis was not consistent with hybridization, since the heterozygous condition had a subtelocentric chromosome, which is not present in specimens from Rio de Janeiro or in homozygous individuals from Petrópolis. It is not yet possible to conclude that such cytogenetic differences observed between these two populations represent a speciation pattern for *Gnamptogenys* from the *striatula* group. Further investigations using different tools are necessary to clarify this issue.

The second type of variation observed was chromosomal polymorphism in the *G. striatula* population from the Atlantic rainforest at Petrópolis (RJ, 2n = 34). This variation was characterized by a difference in the size of the long arm of a chromosome pair. In all four colonies from this population, heterozygous individuals were observed and only one of the colonies showed homozygous individuals with a submetacentric minor chromosome. *G. striatula* colonies may have several queens or gamergates (mated fertile workers) that share reproduction tasks and are, therefore, functionally polygynous (Blatrix and Jaisson 2000; Giraud et al. 2000). Thus, the queens/gamergates, or some of them, may be carriers of the large subtelocentric chromosome. The independent occurrence of the polymorphism in all four colonies was implausible. Instead, a stock of males carrying the subtelocentric chromosome was found to be responsible for fecundating the gamergates/queens in these nests. However, as the males in these colonies were not analyzed, additional studies with larger sampling sizes are required to answer this question.

The cytogenetic techniques used in the present study, involving fluorochromes (CMA_3, DAPI) and FISH with an 18S rRNA gene probe, did not identify markers for the polymorphic chromosome of the *G. striatula* (2n = 34) population from Petrópolis. Thus, one can rule out the possible presence of these repeating regions in the chromosome involved in the rearrangement, thereby suggesting translocation as a possible cause of the structural modification of the chromosome (Imai et al. 1977; Schubert and Lysac 2011). The hypothesis that pericentric inversions occurred, followed by unequal crossing-over (Kasahara 2009), may be deemed unlikely, since the short arms of the polymorphic chromosome pair are identical in size.

The occurrence of chromosomal polymorphisms due to duplications, translocations, and inversions has previously been reported for many other ant species (reviewed by Lorite and Palomeque 2010; Barros et al. 2014b; Aguiar et al. 2017). Translocations are more common in ant species with lower chromosome numbers (n < 12; Imai et al. 1977; Barros et al. 2014b). However, in the present study, the chromosomal polymorphism was observed in *G. striatula*, which has 17 chromosomes. Nevertheless, there are reports of chromosomal polymorphisms due to translocations in ants with a high chromosome number (n > 12), such as in *Camponotus rufipes* (n = 20; Mariano et al. 2001; Aguiar et al. 2017). The chromosomal polymorphism in *G. striatula* (2n = 34) clearly altered the morphology of one chromosome in the pair of homologues and was easily detected. However, small alterations involving short chromosomal fragments may not change the chromosome's morphology, making identification difficult (Kasahara 2009). This kind of "invisible" rearrangement emphasizes the importance of using more general cytogenetic markers, such as FISH with microsatellite probes.

The species *G. moelleri*, which is also included in the *striatula* group, showed variation in chromosome number among the populations studied. The Atlantic rainforest populations from Viçosa, Petrópolis (present study), and Bahia (Mariano et al. 2015) showed the same chromosome number (2n = 34). However, the karyotype of the same species from the Amazon rainforest population in Açailândia showed 2n = 44 chromosomes. Despite this broad difference, it is important to observe some patterns between these karyotypes, such as the number of subtelocentric chromosomes and the number of metacentric chromosomes larger than the NOR-bearing chromosome pair (chromosome pairs 1-4).

The taxonomic revisions available for the Neotropical *Gnamptogenys* spp. do not raise any questions regarding the taxonomic status of *G. moelleri* (Lattke 1995; Lattke et al. 2008). Nevertheless, the extent of karyotype divergence of the Amazon population (2n = 44), compared to populations from the Atlantic rainforest (2n = 34) indicated the existence of a cryptic species within this taxon or a complex of species gradually varying with intermediate karyotypes between these two distant localities. It is possible that stabilizing selection influenced the maintenance of external morphology in the species (Moen et al. 2013).

The cytogenetic data obtained for *G. striatula* corroborated previous morphological information concerning the existence of a species complex in this taxon. Additionally, these data highlighted the existence of a cryptic species within *G. moelleri*, a taxon characterized by the absence of morphological variation. Further cytogenetic data, combined with perspectives from ecological, behavioral, chemical, and molecular biological studies, may be the best approach to consolidate the delimitation of this species, according to the concept of integrative taxonomy.

Hypothesis of chromosome evolution for the karyotypes of *Gnamptogenys* spp. observed in the present study

In the present study, the cytogenetic relationships between three groups of *Gnamptogenys* spp. were investigated, by taking into consideration the phylogenetic hypothesis proposed by Lattke (1995). According to this phylogeny, the *striatula* group, represented by the species *G. striatula* and *G. moelleri*, is the sister group of the clade that includes the *rastrata* and

mordax groups, represented by *G. triangularis* and *G. regularis*, respectively.

Populations of *G. striatula* and *G. moelleri* from the Atlantic rainforest presented similar karyotypes, with 2n = 34 (18m + 8sm + 8st) and both populations presented 18S rDNA genes on the fifth metacentric pair, suggesting a plesiomorphic configuration within the *striatula* subgroup.

To explain the karyotype observed in the *G. striatula* population from the restinga region (2n = 32), we propose a hypothesis that assumes two events: (i) the occurrence of Robertsonian translocation between a large subtelocentric and a smaller subtelocentric chromosome, resulting in the formation of a metacentric chromosome, and (ii) minor modifications, such as tandem heterochromatin growth in the telomeric region or duplications of small repetitive regions of other large subtelocentric chromosomes that are sufficient to change the morphology of the chromosome to submetacentric, without drastically altering its size. The proposed translocation is supported by the presence of a chromosomal polymorphism observed in the Petrópolis population, which may be due to an independent translocation event.

The karyotype of *G. moelleri* from Açailândia (2n = 44) may be considered more derivative, but the extent of the divergence makes it difficult to suggest a specific mechanism for the chromosome evolution among different populations of this species. Investigating the karyotypes of other populations of *G. moelleri* will be important to better understand the mechanisms involved in the substantial modification of the karyotype of this Amazon rainforest population.

The karyotypes observed in *G. regularis* (2n = 26, *mordax* group) and *G. triangularis* (2n = 24, *rastrata* group) not only differed from each other, but also significantly differed from those observed in *G. moelleri* (2n = 34, 44) and *G. striatula* (2n = 32, 34), thereby indicating a high degree of karyotypic diversification in *Gnamptogenys* spp. This suggests that chromosomal rearrangements are part of the evolutionary history of this genus.

Final considerations

Variation in chromosome number among *Gnamptogenys* spp. was 2n = 16, 20, 24, 26, 32, 34, 36, 42, 44, 46, and 68 chromosomes (Table 1, present study) and belongs to *striatula*, *mordax*, and *rastrata* groups from the Neotropical region and the *coxalis* group from the Oriental region. Further analysis presented here at the population level revealed that two species in the current study presented structural chromosome polymorphisms that involved 18S rDNA regions in the case of *G. regularis* (2n = 26). Furthermore, *G. striatula* (2n = 32, 34) and *G. moelleri* (2n = 34, 44) showed inter-population karyotypic divergence. Thus, the data presented herein highlight the significance of understanding karyotype evolution in the genus *Gnamptogenys*, taking into account the phylogenetic suborganization (morphological groups according to Lattke 1995). Generally, the comparison of karyotypes between two phylogenetically related species provides insights into the evolution of the karyotype for a given group. The limited cytogenetic data available for *Gnamptogenys* show great complexity and karyotypic diversity for the genus, probably reflecting its long evolutionary history (Lattke 1995; Lattke et al. 2008) and wide geographic distribution.

In the present study, the analysis of *G. striatula* and *G. moelleri* karyotypes revealed a distinction between their populations, thus highlighting the importance of population studies. Additionally, identification of the patterns of chromosomal polymorphisms was also feasible, which in turn, provided insights into the evolution of karyotypes in *G. regularis* and *G. striatula*. Moreover, performing additional studies in different populations of *G. moelleri* will be useful in testing the hypothesis of the cryptic species. Thus, the chromosomal data presented here for *G. striatula* indicate that cytogenetics is a promising tool for delimiting the species within this species complex.

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