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**PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DE BIOMAS TROPICAIS**

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**Reconstrução filogenética do número ancestral cromossômico dos gêneros *Anochetus* Mayr, 1861 e *Odontomachus* Latreille, 1804 (Hymenoptera: Formicidae: Ponerinae)**

Ouro Preto  
Minas Gerais/Brasil  
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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ecologia de Biomas Tropicais do Instituto de Ciências Exatas e Biológicas da Universidade Federal de Ouro Preto, como requisito parcial para a obtenção do título de Mestre.

Orientador: Prof. Dr. Maykon Passos Cristiano

Coorientador: Prof. Dr. Danon Cleme Cardoso

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<b>Sumário</b>	
<b>Resumo .....</b>	<b>10</b>
<b>Introdução geral .....</b>	<b>11</b>
<b>Referências .....</b>	<b>13</b>
<b>Abstract .....</b>	<b>17</b>
<b>Introduction .....</b>	<b>18</b>
<b>Material and methods .....</b>	<b>21</b>
1.1 Data Assembling .....	21
1.2 Phylogenetics Inferences .....	24
1.3 Chromosome ancestral estimation.....	24
<b>Results.....</b>	<b>25</b>
2.1 Phylogenetics.....	25
2.2 Chromosome Ancestral Estimation .....	28
<b>Discussion .....</b>	<b>30</b>
<b>References.....</b>	<b>33</b>
<b>Supporting Information .....</b>	<b>40</b>
<b>Considerações Finais .....</b>	<b>59</b>

## Lista de Figuras

**Figura 1.** Filogenia de formigas trap-jaw reconstruída a partir do programa MrBayes. Os cladogramas A-G são destacados em cores diferentes. A probabilidade posterior é indicada em cada nó. Grupos morfológicos propostos por Brown et al. 1978, são mostrados no lado direito da árvore. O grupo externo pode ser encontrado no topo da figura, em cor cinza.

..... 27

**Figura 2.** Árvore filogenética gerada através de inferências de Máximo Verossimilhança, para os gêneros *Anochetus* e *Odontomachus*, pelo conjunto de dados dos cinco genes concatenados.

Valores de Bootstrap são indicados em cada nó. .... 52

**Figura 3.** Árvore podada obtida da análise bayesiana de dados concatenados com base em sequências parciais dos genes 28S, CAD, COI, LWR e Wg na subfamília Ponerinae, incluindo reconstrução do estado cromossômico haplóide ancestral inferida usando o software Chromevol 2.0. As cores indicam o número do cromossomo haplóide, conforme visto na legenda do lado esquerdo. Os nós ancestrais contendo uma probabilidade posterior igual ou superior a 95% para a estimativa do número cromossômico são destacados por um círculo preto..... 29

**Figura 4.** Árvore gerada pelo programa ChromeSSE, a partir de inferência bayesiana, para espécies dos gêneros *Anochetus* e *Odontomachus*, que possuem o número cromossômico descrito. As probabilidades posteriores e o número cromossômico ancestral, estão indicados em cada nó. .... 53



**Figura 5.** Árvore indicando o provável número cromossômico ancestral para os grupos de *Anochetus* e *Odontomachus*, resultantes de inferência bayesiana e posterior análise pelo programa ChromEvol, incluindo espécies que não possuem o número cromossômico descrito. Os números cromossômicos são indicados pelas cores vermelho ( $n = 15$ ) e verde ( $n = 22$ ). As probabilidades são indicadas em cada nó, variando de 0-100. .... **55**

**Figura 6.** Árvore indicando o provável número cromossômico ancestral para os grupos de *Anochetus* e *Odontomachus*, resultantes de inferência bayesiana e posterior análise pelo programa ChromSSE, incluindo espécies que não possuem o número cromossômico descrito. A legenda para o número cromossômico haploide está inclusa no lado direito da figura. As probabilidades são indicadas em cada nó, variando de 0-100. .... **57**

## Lista de Tabelas

- Tabela 1.** Espécies usadas para a construção da Filogenia Molecular. Cada gene está referenciado pelo seu acesso no site GenBank..... **40**
- Tabela 2.** Número cromossomo haploide e fórmula cariotípica para espécies de *Anochetus* e *Odontomachus*, descritos na literatura. Abreviações nas colunas indicam: *n* número cromossômico haploide, M cromossomos metacêntricos, SM cromossomos submetacêntricos, ST cromossomos subtelocêntricos, T cromossomos telocêntricos e A cromossomos acrocêntricos..... **22**
- Tabela 3.** Esquema de partição usado na análise filogenética. Esquemas de partição e modelos de substituição de nucleotídeos foram identificados pelo PartitionFinder e usados no alinhamento concatenado de todas sequências. .... **54**
- Tabela 4.** Valores de suporte para inferência Beysiana (PP) e Máximo Verossimilhança (BS) com os respectivos grupos para os gêneros *Anochetus* e *Odontomachus*..... **25**

## Resumo

Dados filogenéticos e moleculares recentes estão mudando nosso conhecimento sobre as relações entre as espécies e os processos evolutivos que conduzem os padrões de variação cromossômica, observados em formigas (Hymenoptera: Formicidae). Compreender a origem desses animais e sua história evolutiva é um trabalho intrigante e desafiador. As formigas exibem grande variações referentes a morfologia, comportamento e nas estruturas do cariótipo. Reunindo informações genéticas sobre as formigas trap-jaw pertencentes a subfamília Ponerinae, reconstruímos as relações filogenéticas que inferem a condição monofilética entre os gêneros *Anochetus* e *Odontomachus*. Conseqüentemente a evolução cromossômica foi analisada a partir da inferência do número cromossômico ancestral, sendo este  $n = 15$  para ambos os gêneros. No gênero *Anochetus* observamos uma manutenção do número cromossômico ancestral na maioria das espécies indicando a ocorrência de inversões pericêntricas, a diminuição deste número na espécie *Anochetus emarginatus* Fabricius 1804, e *Anochetus cf masdaraszi* que provavelmente ocorreu pelo processo de fusão centromérica e um aumento no número do cariótipo ancestral em *Anochetus horridus* Brown, 1978 sugerindo fissões centroméricas. Em relação ao gênero *Odontomachus*, o grupo *rixosos* manteve o número cromossômico ancestral em todas as espécies, enquanto um alto ganho no número cromossômico nas espécies do grupo *haematodos* foi observado. Nossos resultados recorrem à Teoria da Interação Mínima para explicar os processos evolutivos em formigas trap-jaw, embora as fissões centroméricas não se apresentam como os principais eventos de rearranjo cromossômico em todos os clados dos gêneros *Anochetus* e *Odontomachus*.

**Palavras chave:** análise filogenética, evolução cromossômica, Formicidae, Ponerinae, .

## Introdução geral

Formigas (Hymenoptera: Formicidae) apresentam-se como um grupo altamente diverso e capaz de ocupar variados nichos no espectro dos ambientes em que se encontram, estando presentes em todos os biomas exceto nos polos (Hölldobler & Wilson, 1990). A diversificação morfológica destes animais, além dos variados estilos de vida podem ser apontados como a chave para o sucesso ecológico neste grupo (Brady *et al.*, 2006). Considerando esta diversificação, os estudos evolutivos na família Formicidae tornam-se consideravelmente importantes para melhor compreendermos os múltiplos processos capazes de conduzir às variações observadas nas espécies e às relações estabelecidas entre elas (Crosland, Crozier, & Jefferson, 1988; Hirai *et al.*, 1996; Wilson & Holldobler, 2005; Smith & Fisher, 2009; Cristiano, Cardoso, & Fernandes-Salomão, 2013; Micolino, Cristiano, & Cardoso, 2020).

Pertencente à grande família Formicidae, a subfamília Ponerinae agrupa um total de 1.266 espécies distribuídas em 47 gêneros, ocupando o quarto lugar em relação a riqueza entre as subfamílias de formigas (Bolton, 2020). Majoritariamente distribuídas na região neotropical, estima-se que este grupo tenha se originado no período Cretáceo, há aproximadamente 99 milhões de anos (Schmidt & Shattuck, 2014; Larabee *et al.*, 2016). Os integrantes da subfamília Ponerinae geralmente se organizam em pequenas colônias, castas monomórficas, com pequena diferenciação entre rainhas e operárias, que em geral são forrageadoras solitárias (Wilson & Holldobler, 2005; Santos *et al.*, 2010).

*Anochetus* e *Odontomachus*, caracterizados por mandíbulas longas e retas inseridas em ambos os lados da linha cefálica com dois ou três dentes dispostos em uma série vertical (Brown Jr., 1978). Graças a essas mandíbulas, os integrantes destes gêneros são conhecidos como formigas *trap-jaw*. O dente único no ápice do pecíolo separa as espécies de *Odontomachus* das

espécies de *Anochetus*, que têm dois dentes. Em uma vista anterior da cabeça, *Odontomachus* apresentam linhas apofisárias em forma de V de invertido e escuro, que convergem para formar um sulco. Em *Anochetus* estas linhas estão ausentes, e no mesmo lugar se observa uma linha em forma de U (Fisher & Smith, 2008).

Realizando a reconstrução das relações filogenéticas Spagna *et al.*, 2008, propõe o monofiletismo para o grupo *Odontomachus*, tendo *Anochetus* como provável grupo irmão. Porém estudos posteriores assumiram que os gêneros possuíam uma condição parafilética (Santos *et al.*, 2010; Schmidt, 2013). A fim de elucidar esta questão Larabee *et al.*, 2016 reuniram 49 espécies de *Anochetus* e 43 espécies de *Odontomachus*, analisando as sequências de 5 genes (mitocondrial citocromo oxidase I (COI); wingless (WNG); long-wave rodopsina (LWR), rudimentar (CAD) e subunidade ribossomal 28S), através de inferências Baeyesianas e de Máxima Verossimilhança verificou-se o monofiletismo recíproco entres estes gêneros.

Usando as espécies reunidas no trabalho de Larabee *et al.*, 2016 e atualizando dados depositados nos site GenBank (Benson *et al.*, 2000), inferimos novamente a filogenia das formigas trap-jaw para compreender os processos envolvidos na evolução cariotípica dos gêneros *Anochetus* e *Odontomachus*, os programas ChromEvol (Mayrose, Barker, & Otto, 2010) e ChromSSE (Goldberg & Igić, 2012) foram utilizados para a inferência do número cromossômico ancestral.

Estudos prévios observaram que rearranjos cromossômicos podem induzir o isolamento reprodutivo e posterior processo de especiação em organismos eucariotos (Ayala & Coluzzi, 2005; Faria & Navarro, 2010). Hoffmann & Rieseberg, 2008 analisando diversos táxons como dípteros, ortópteros e fungos, sugerem que processos de inversão cromossômica, são capazes de acumular variações que contribuíram substancialmente para o processo de isolamento reprodutivo. A evolução de formas, tamanho e composição dos cromossomos, resulta em uma

imensa variedade de cariótipos agindo como barreira pré zigótica capaz de impedir o fluxo gênico entre as linhagens (Schubert, 2007).

Analisando os processos microevolutivos em formigas Hirotsami *et al.*, 1988 sugeriram que a variação cromossômica nestes organismos ocorreu por sequentes eventos de fissões centroméricas. Estes seriam responsáveis pelo aumento no número de cromossomos, reduzindo o tamanho dos cromossomos metacêntricos de braços longos em cromossomos menores, telocêntricos ou acrocêntricos. Desta maneira, estruturas menores se tornariam mais estáveis, e as possíveis interações entre os braços cromossômicos seriam reduzidas, diminuindo a possibilidade de mutações. Posteriormente, analisando outros organismos eucariotos, foi proposta a Teoria da Interação Mínima (MIT), expandindo a ideia de que as fissões centroméricas seriam a mais importante força a guiar os processos evolutivos em todos os eucariotos (Imai, Taylor, & Crozier, 1994; Imai, Satta, & Takahata, 2001; Imai *et al.*, 2002).

Em nossas análises podemos inferir que os processos que guiaram a evolução cromossômica nestes grupos foram ocasionados por eventos de fissão e fusão centromérica, bem como foi possível perceber ocorrência de eventos de inversão cromossômica, como previamente foi proposto para o gênero *Mycetophylax* (Cardoso *et al.*, 2014) e para a tribo de abelhas Meliponini (Travenzoli *et al.*, 2019).

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**Phylogenetic reconstruction of the ancestral chromosomal number of the genera *Anochetus* Mayr, 1861 and *Odontomachus* Latreille, 1804 (Hymenoptera: Formicidae: Ponerinae)**

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**Abstract**

Recent phylogenetic and molecular data are changing our knowledge about the relations between species and evolutionary processes that conducted the chromosome variation, observed in ants (Hymenoptera: Formicidae). Ants exhibit tremendous variations in morphology, behaviour and karyotype structures. By assembling genetic and chromosome information about the trap-jaw ants from the subfamily Ponerinae, we reconstructed the phylogenetic relationships that infer the monophyletic condition between the *Anochetus* and *Odontomachus* genera and estimated their haploid chromosome ancestral number. According to our new inferences these clades have a haploid ancestral chromosome number  $n = 15$ . In the *Anochetus* genus we observed a maintenance of the karyotype chromosomal number in most species indicating the occurrence of pericentric inversions, a loss of this number in *Anochetus emarginatus* Fabricius, 1804 and *Anochetus cf. madaraszi* which most likely occurred by centromeric fusion, and an increase in the ancestral karyotype number in *Anochetus horridus* Brown, 1978 suggesting centromeric fissions. The *Odontomachus* genus showed a maintenance of the ancestral karyotype number in the *rixosos* group and several gains in all species from the *haematodos* group. Our findings suggest that the centric fissions was not the principal events that led the karyotype evolution in the-trap jaw ants, since the maintenance of the chromosome ancestral number, fusion and fission are equally involved on the variation of chromosomal structure in those genera.

**Key words:** chromosome evolution, Formicidae, phylogenetic analyse, Ponerinae.

## **Introduction**

Chromosome number and structure are fundamental aspects of genome organization that can be related to interspecific divergences. By this means, morphological and numeric chromosomal traits are relevant to understanding the genomic organization of eukaryotes, often being key components to comprehend evolutionary pathways and karyotypic differentiation (Imai *et al.*, 1986, 1994, 2001; Hirotsami *et al.*, 1988; Hirai *et al.*, 1996; Goodisman, Kovacs, & Hunt, 2008; Lorite & Palomeque, 2010; Menezes *et al.*, 2014; Cardoso *et al.*, 2014; Velasco *et al.*, 2014; de Aguiar *et al.*, 2020; Micolino, Cristiano, & Cardoso, 2020). Chromosomes differ in size, shape and genetic information, as in their number and redundancy. These variations can be observed in individual organisms of a population and between different species. Considering all their characteristics, they play a very important role in evolutionary studies (Schubert, 2007). Analysing plants and vertebrates Rieseberg, 2001 assumed that chromosome rearrangements could act reducing gene flow, more effectively than by other mechanisms. These thoughts are concordant with what has been postulated some decades before, when it was proposed that changes in chromosome patterns were very important in isolated lineages, promoting a rapid speciation in vertebrates (Bush *et al.*, 1977).

In the Formicidae family, previous studies have been done to understand the evolution of chromosomal changes at the species level. As an example Cardoso *et al.*, 2014 reconstructed the ancestral chromosome number of *Mycetophylax* genus, and by matching these analyses with karyotype and chromosome banding inferences proposed that centromeric fusion and fission were responsible for the chromosomal variation in this genus. Also, their findings suggested that the lineages of *Mycetophylax morschi* Emery, 1888; should be treated as two phylogenetically isolated species by the differences in their karyotype structure. In a subsequent

work, cytogenetic and molecular techniques revealed differences between lineages of *M. morschi*, besides the morphology and number of the chromosomes, and also including the localization of the 45S rDNA cluster, reinforcing the hypotheses that these lineages should be treated as a cryptical or even distinct species (Micolino *et al.*, 2019). Cristiano *et al.* (2013) performed a study combining molecular and cytogenetic data which suggested that *Acromyrmex striatus* Roger, 1863; should be allocated to a new genus. In a brand-new study, based in morphological characters, size of colony, behaviour, chromosome inferences and phylogenetic analyses, the *Acromyrmex striatus*, *Acromyrmex silvestrii* Emery, 1905 and the subspecies *Acromyrmex silvestrii bruchi* Forel, 1902 were allocated in a new genus *Amoimyrmex* Cristiano, Cardoso & Sandoval, 2020. These species were redescribed as *Amoimyrmex striatus* (Emery, 1905) **comb. nov.**, *Amoimyrmex silvestrii* (Forel, 1902) **comb. nov.** and *Amoimyrmex bruchi* (Forel, 1912) **comb. nov. et estat. rev.** (Cristiano *et al.*, 2020).

As exposed above, ants (Hymenoptera: Formicidae) are an interesting group to investigate evolutionary changes on chromosomes since they present a huge karyotype variation. For instance, they bear the lowest possible chromosome number as  $n = 1$  in *Myrmecia croslandi*, reaching up to the impressive number of  $n = 60$  in the neotropical species *Dinoponera lucida*, Emery (Crosland & Crozier, 1986; Mariano *et al.*, 2008, Cardoso *et al.*, 2018). All ants belong to the Formicidae family, with 17 extant subfamilies, with most known species concentrated in four big groups: Myrmicinae, Formicinae, Dolichoderinae and Ponerinae (Bolton, 2020).

Species from the Ponerinae subfamily exhibit plenty characteristics in common, like solitary foraging, small colonies, small differentiation between workers and the queen and monomorphic worker caste (Wilson & Holldobler, 2005; Schmidt, 2013). In their first phylogeny description, by morphological analyses, the monophyletic condition in the subfamily Ponerinae was supported, and *Odontomachus* was considered as a genus that probably derivate

from an *Anochetus* ancestor (Brown Jr., 1978). The Ponerinae monophyly was corroborated by molecular phylogenetics studies, as was proposed by Brown years ago (Moreau *et al.*, 2006; Brady *et al.*, 2006).

In a molecular approach, a phylogenetic hypothesis Ponerinae was inferred using a multiple sequence alignments involving four core genes (*wingless* (WG), *long-wavelength rhodopsin* (LW Rh), *rudimentary* (CAD), the nuclear large subunit (28S) ribosomal RNA gene). The results concluded that the *Odontomachus* and *Anochetus* genera form a clade with an uncertain reciprocal monophyly, since relationships within the group are very poorly resolved (Schmidt, 2013).

The reciprocal monophyly of the genera *Anochetus* and *Odontomachus* was recently supported by phylogenetic inferences including 49 specimens of *Anochetus* and 43 specimens of *Odontomachus*, besides 42 members of Ponerinae as outgroups. To reconstruct the phylogenetic tree the same genes of the Schmidt's work were used, plus the cytochrome oxidase I (COI) gene (Larabee *et al.*, 2016).

*Anochetus* chromosome numbers vary from  $n = 14$  to  $n = 23$ , while in *Odontomachus*, most species that have their karyotype described, present  $n = 22$ , except for *Odontomachus latidens* and *Odontomachus rixosus* that exhibit  $n = 15$ , as can be seen in the Ant Chromosome dataBase (ACdB) (Cardoso, Santos, & Cristiano, 2018). Moreover, in *Anochetus* we observed a high variability at the karyotype structure level, while in *Odontomachus* the karyotype structure presents less variation in all cytogenetically analysed species, notable by the absence of metacentric and high presence of telocentric chromosomes. The chromosome variation in eukaryotes, especially in ants, is often explained by the "Minimum Interaction Theory", which proposes that chromosomal evolution occurs by increases in number of chromosomes, following Robertsonian fissions (Imai *et al.*, 1986; Hirotsami *et al.*, 1988; Imai *et al.*, 1994).

Despite the plethora of phylogenetic inference publications that can currently be found in the literature, few studies utilize these inferences to reconstruct the possible ancestral chromosome states. In the present study besides reconstructing a phylogenetic inference for the *Anochetus* and *Odontomachus* genera, our framework combined these analyses to infer their ancestral chromosome number, relating them with karyotypical structure. Our results revealed that in the Ponerinae subfamily, fusions and pericentric rearrangement events can be seen as equally important as Robertsonian fissions. As such, this approach allows a better comprehension of the processes that would have possibly led the microevolutionary patterns in the trap-jaw ants.

## **Material and methods**

### **1.1 Data Assembling**

Gene sequence data were obtained from GenBank (Benson *et al.*, 2000) for five gene fragments: the mitochondrial gene cytochrome oxidase I (COI); the three nuclear protein-coding genes: wingless (Wg), long-wavelength rhodopsin (LWR) and rudimentary (CAD); and the nuclear large subunit ribosomal RNA gene (28S) (Supporting information Tab. 1).

Sequences were assembled and edited in MEGA X program (Kumar *et al.*, 2018) analigned using the ClustalW for the 28 S gene, and Prank (Löytynoja & Goldman, 2010) for the other four protein coding genes whose amino acid sequence was downloaded for these genes alongside their nucleotide sequence from GenBank. To ensure codon positions were correct, protein alignments were constructed and mapped to nucleotide alignments using RevTrans (Wernersson & Pedersen, 2003). Ambiguously aligned regions of the genes and introns from LWR and CAD genes were removed before further analyses.

A Python script was developed for pruning trees when only clades whose chromosome numbers have been previously determined were needed from the full tree (See Chromosome ancestral Estimation below). This script was deployed in an iPython notebook (available on <https://bit.ly/2W0vI3w> and annexed in the Supporting information) including detailed usage instructions for code reusability and transparency. The ETE 3 library (Huerta-Cepas, Serra, & Bork, 2016) functions were used for the pruning method. A tree in the Newick format is used as an input and clade selections can be done by a dropdown menu or text input widgets. The output is a pruned tree in the Newick format including only the selected clades optionally summing branch lengths when required by the user for proper tree scaling.

An assemble of 25 species from the Ponerinae subfamily was selected with chromosome numbers previously described in the literature, including 10 *Anochetus* and 10 *Odontomachus* species, and 5 other species as outgroup. This dataset was used to infer the chromosome ancestral condition of trap-jaw ants.

**Table 2:** Chromosome haploid numbers and karyotypical formula for *Anochetus* and *Odontomachus* species found in the literature. Column names meanings: N haploid chromosome number, M metacentric chromosome, SM submetacebtric chromosome, ST subtelocentric chromosome, T telocentric chromosome and A acrocentric chromosome.

<u>Species</u>	<u>n</u>	<u>M</u>	<u>SM</u>	<u>ST</u>	<u>T</u>	<u>A</u>	<u>Reference</u>
<i>Anochetus altisquamis</i> Mayr, 1887	15	6	3	1	5		Santos <i>et al.</i> , 2010
<i>Anochetus emarginatus</i> Fabricius, 1804	14			–			Santos <i>et al.</i> , 2010, Mariano <i>et al.</i> , 2015
<i>Anochetus cf madaraszi</i> Mayr 1937	14			–			Imai <i>et al.</i> , 1984
<i>Anochetus cf graeffei</i> Mayr, 1937	15; 19	9	1		5		Imai <i>et al.</i> , 1984 Imai <i>et al.</i> , 1985
<i>Anochetus graeffei1</i>	15; 19			–			Imai <i>et al.</i> , 1984 Imai <i>et al.</i> , 1985
<i>Anochetus graeffei2</i>	15; 19			–			Imai <i>et al.</i> , 1984

						Imai <i>et al.</i> , 1985
<i>Anochetus horridus</i> Brown, 1978	23	4	2		17	Mariano <i>et al.</i> 2011
<i>Anochetus modicus</i> Kempf, 1954	15			–		Imai <i>et al.</i> , 1985
<i>Anochetus targionii</i> Emery, 1894	15	8	1	1	5	Aguiar <i>et al.</i> , 2020
<i>Brachyponera chinensis</i> Emery	11			–		Imai & Kubota 1972
<i>Leptogenys diminuta</i> Smith, 1857	16			–		Goni <i>et al.</i> , 1982; Imai <i>et al.</i> , 1983; Imai <i>et al.</i> , 1984
<i>Leptogenys iridescens</i> Smith, 1857	23			–		Imai <i>et al.</i> , 1983 Imai <i>et al.</i> , 1985
<i>Odontoponera transversa</i> Smith, 1857	23; 21	7			16	Imai <i>et al.</i> , 1984
<i>Neoponera villosa</i> Fabrius, 1804	17	6			11	Mariano <i>et al.</i> , 2000 Mariano <i>et al.</i> , 2007
<i>Odontomachus bauri</i> Emery, 1852	22			–		Teixeira 2018
<i>Odontomachus chelifer</i> Latreille, 1802	22		2		20	Santos <i>et al.</i> , 2010
<i>Odontomachus haematodus</i> Linnaeus, 1758	22		4	9	9	Aguiar <i>et al.</i> 2020
<i>Odontomachus hastatus</i> Fabricius	22		2	1	19	Mariano <i>et al.</i> 2011
<i>Odontomachus latiden</i> Mayr, 1867	15; 16			–		Imai <i>et al.</i> 1983
<i>Odontomachus meinerti</i> Forel, 1905	22		2	3	17	Santos <i>et al.</i> 2010
<i>Odontomachus rixosus</i> , Smith	15			–		Goni <i>et al.</i> 1982 Imai <i>et al.</i> 1983



<i>Odontomachus rixosus</i> <sup>2</sup>	15	–			Goni <i>et al.</i> , 1982 Imai <i>et al.</i> 1983
<i>Odontomachus scalptus</i> Brown, 1978	22	1	8	13	Mariano <i>et al.</i> 2011
<i>Odontomachus simillimus</i> Smith, 1858	22	–			Imai <i>et al.</i> 1985

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## 1.2 Phylogenetics Inferences

For the phylogenetic inference the following methods were applied: Partition Finder 2 (Lanfear *et al.*, 2017) for choosing partitioning schemes and models of molecular evolution using the concatenated alignment of five genes. The concatenated alignment was divided in 13 subsets (one for 28S and three for each protein coding gene) and the best model for each partition was determined (Supporting information Tab. 3). After that, the concatenated alignment was used as input in Mr. Bayes 3.2.2 (Ronquist & Huelsenbeck, 2003) with a compound Dirichlet prior for more accurate branch lengths estimation (command line: “*brlenspr = Unconstrained:GammaDir (1.0, 0.10, 1.0, 1.0)*”), and each partition set according to the results of the Partition Finder 2 analyses. Base frequencies, substitution rates, and gamma shaped parameters were unlinked across partitions. Each analysis consisted of two simultaneous Markov Chain Monte Carlo (MCMC) runs with four chains per run for 40 million generations. The first 25% of the sampled trees were discarded as burn-in. A Maximum likelihood tree was recovered using the rapid bootstrapping algorithm (1000 replicates), in the RAxML NG program (Kozlov *et al.*, 2019). The same partition scheme used in Mr. Bayes analyses was used for Maximum likelihood inferences.

## 1.3 Chromosome ancestral estimation

The softwares chromEvol 1.3 (Mayrose, Barker, & Otto, 2010) and chromoSSE (Goldberg & Igić, 2012) were used to infer the best chromosome evolutionary model and the

haploid ancestral number. The Bayesian tree exposed in this study was pruned for taxa containing chromosome number descriptions and used to infer the ancestral state. For our analyses the constant rate model presented itself as the best chromosome evolution hypotheses between the options displayed in chromEvol 1.3, since other models included polyploid and demi-polyploid duplications which are very rare in metazoa. In the same way, the polyploid and demi-polyploid events were not set for chromoSSE.

## Results

### 2.1 Phylogenetics

Analyses resulted in trees that basically display the same phylogenetic relationships, particularly within the genes *Anochetus* and *Odontomachus*. The topological structures of these trees were retained across Bayesian inference (Fig.1) and the Maximum Likelihood inference methods (Fig. 2, Supporting Information).

The support values of Bayesian and maximum Likelihood inferences for each clade are available in the Table 4.

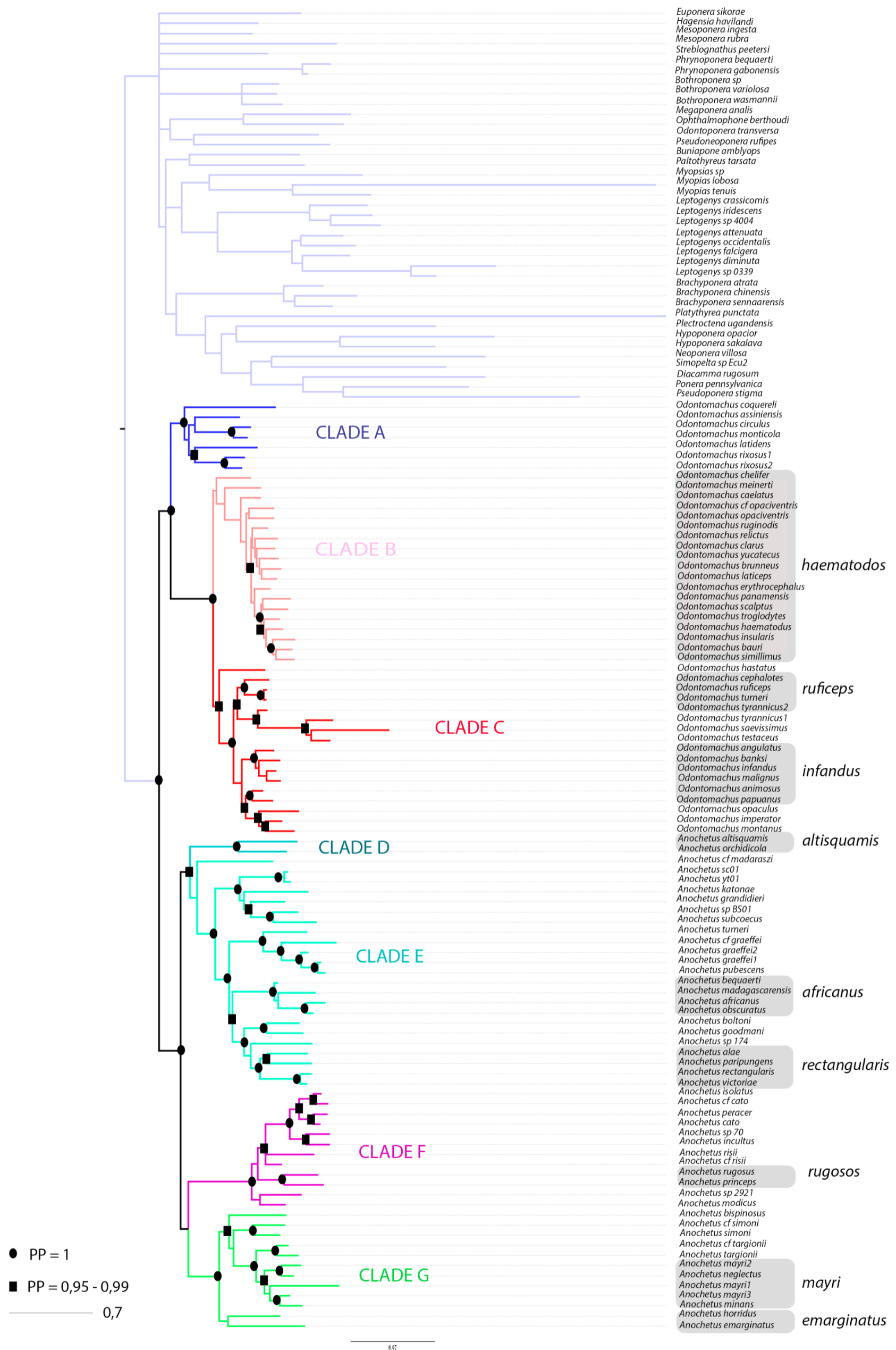
**Table 4.** Support values for Bayesian Inference Posterior Probability (PP) and Maximum Likelihood (BS) with the respective groups of the genus *Anochetus* and *Odontomachus*.

Clade	MrBayes (PP)	RAxML (rBS)
<i>Anochetus</i> + <i>Odontomachus</i>	1.0	81
<i>Odontomachus</i>	1.0	75
<i>Anochetus</i>	1.0	100
Clade A	1.0	99
Clade B	0.99	52
Clade C	0.75	43
Clade D	1.0	100
Clade E	1.0	100

Clade F	1.0	100
Clade G	0.93	64

The clades A, B and C are formed by species of *Odontomachus* genus. Clade A displays high support values in both trees PP: 1.00 and BS: 99. Clade B is represented by the *hastatus* group and shows PP: 0.99 and a low value of BS: 52. Clade C included the *ruficeps* and *infandus* groups with PP: 0.75 and BS: 43.

The clades D, E, F and G are composed by species of *Anochetus* genus. Clade D is formed by the *altisquamis* group with PP: 1.0 and BS: 100. Clade E which the *rectangularis* group belongs, has BP: 1.0 and BS: 100. The clade F contains high support values are also observed: BP: 1.0 and BS: 100. The clade G includes the groups *mayri* and *emarginatus* with BP.: 0.93 and rBS: 64.

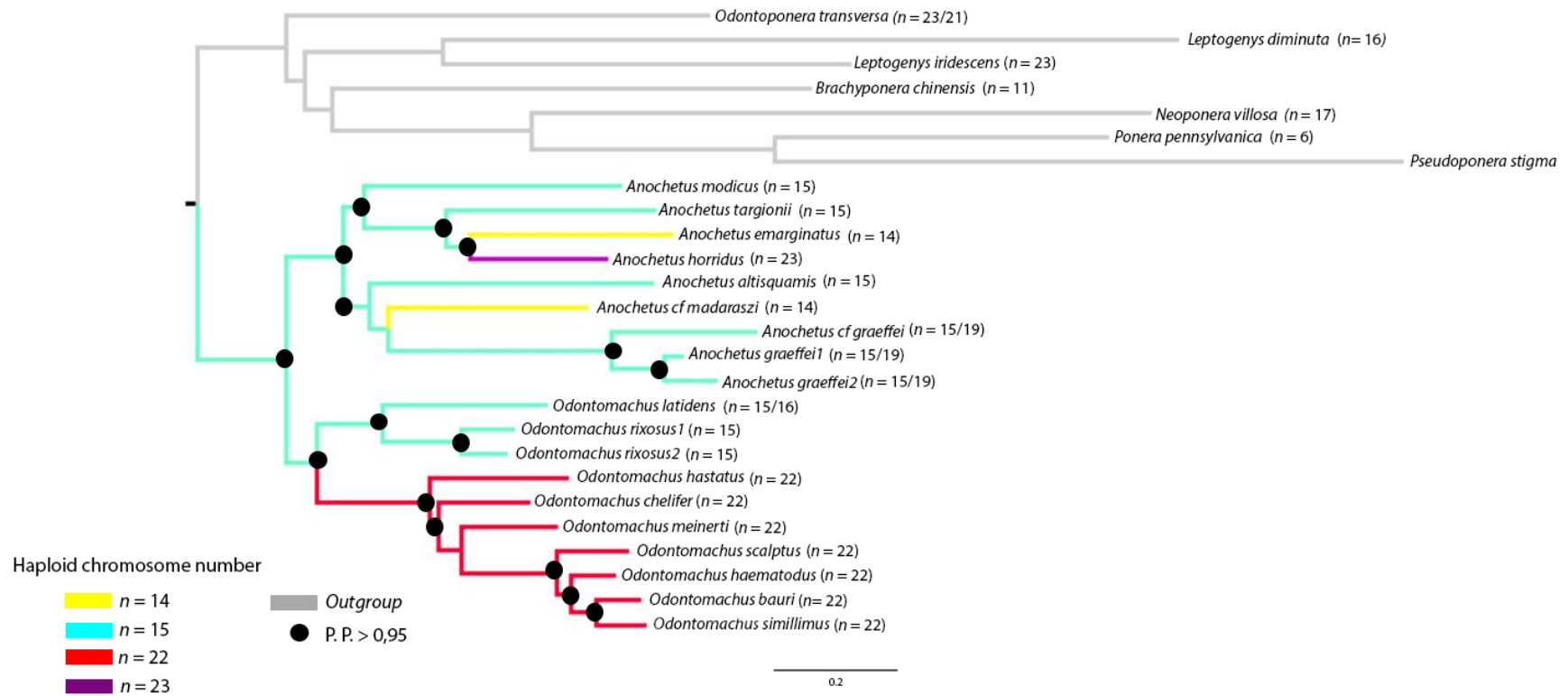


**Figure 1.** Phylogeny of trap-jaw ants reconstructed from MrBayes. Clades A-G are highlighted in different colours. Morphological groups proposed by Brown et al., 1978, are shown in the right side of the tree. The outgroup can be found in the top of the figure, in grey colouring.

## 2.2 Chromosome Ancestral Estimation

Our results indicated the chromosomal ancestral number of *Odontomachus* and *Anochetus* genera was  $n = 15$  (PP = 0.98, Fig. 3). In *Odontomachus* we observed a maintenance of  $n = 15$  as ancestral chromosome number for *Odontomachus latidens* and *Odontomachus rixosos* species. In *haematodos* group this number evolved to  $n = 22$ . Most ancestral chromosome numbers were inferred with high posterior probabilities (above 95%), with some notable exceptions. This was the case for two ancestors representing the transition between groups D and E in *Anochetus*. The reason for such is probably due to the existence of descriptions for both chromosome numbers  $n = 15$  and  $n = 19$  in *Anochetus cf graeffei* and *Anochetus graeffei*.

For the ChromEvol best model the final rate parameters values were 5.93,  $1.87 \times 10^{-5}$  and 39.37 for chromosomal duplication, gain and loss events, respectively. This model was selected over models including missing data and all ChromoSSE models due to higher posterior probability values found. Both ChromoSSE and ChromEvol pruned ancestral chromosome number trees and the full data ChromEvol tree proposed similar ancestral haploid chromosome numbers and can be regarded as equivalent. We used Tracer 1.5 (Rambaut & Drummond, 2009) to determine that our sampling of the posterior distribution had reached a effective sample size (ESS) and to ascertain that the number of generations required to reach stationarity of the posterior distribution had been reached. For all models including the full phylogenies for the full data see the Supporting Information (Fig.4, 5 and 6).



**Figure 3:** Pruned tree obtained from the Bayesian analysis of concatenated data based on partial sequences of the 28S, CAD, COI, LWR and Wg genes in the Ponerinae subfamily, including ancestral haploid chromosome state reconstruction inferred using the Chromevol 2.0 software. Colors indicate the haploid chromosome number as seen in the left-side legend. Ancestral nodes containing a posterior probability above 95% for the chromosome number estimation are highlighted by a black circle.

## Discussion

According to previous studies (Imai *et al.*, 1984; Santos *et al.*, 2010; Mariano *et al.*, 2011) *Anochetus* displays a higher karyotypic difference than *Odontomachus*, whose common characteristics include the total absence of metacentric chromosomes and high number of telocentric chromosomes (See Table 2). The chromosome evolutionary patterns in ants and in other Hymenoptera is usually explained by the Minimal Interaction Theory (MIT) (Imai *et al.*, 1986, 1994). The MIT proposes that the chromosome number of eukaryotes tends to increase by centric fissions, those events being essential to reducing the interaction between the long arms of chromosomes, minimizing genetic risks occasioned by reciprocal translocations. In that way, increases in chromosome number are followed by a decrease in the size of these chromosomes (Hirotsami *et al.*, 1988; Hirai *et al.*, 1996; Schubert, 2007). Recent analyses with wasps, ants and mammals, reinforced the hypothesis that centric fissions were the main force in the chromosome evolution of all eukaryotes (Imai *et al.*, 2001, 2002).

Under our chromosome ancestral analyses, the common ancestor of *Anochetus* and *Odontomachus* had  $n = 15$  chromosomes. The reciprocal monophyly from *Odontomachus* and *Anochetus* was seen with high support values (PP=1.0, BS=81), which is not assumed by previously studies that infer the paraphyletic condition between the *Odontomachus* and *Anochetus* genus (Moreau *et al.*, 2006; Santos *et al.*, 2010; Schmidt, 2013), although being coincident with the most recent molecular results (Larabee *et al.*, 2016).

The maintenance of  $n = 15$  in several *Anochetus* species was observed, excepted by an increase in *Anochetus horridus* ( $n = 23$ ), and a reduction of the chromosome number in *Anochetus emarginatus* and *Anochetus cf. masdaraszi*. A different pattern was observed in *Odontomachus*, in which an increase of chromosome number from  $n = 15$  to  $n = 22$  was found

in all the *haematodos* group, whilst the ancestral chromosome number was maintained in the lineages of the other groups.

The species *A. modicus*, *A. targionii*, *A. altisquamis* and *A. graeffei* maintain their chromosome number of  $n = 15$  (Imai *et al.*, 1986; Santos *et al.*, 2010; Mariano *et al.*, 2011, 2015; de Aguiar *et al.*, 2020). During the evolutionary course of the lineages in which these species arose alterations in the number of metacentric chromosomes can be observed, which encourage us to assume that pericentric inversions drive the evolutionary process in this case. This pattern of maintenance in the haploid chromosome number across the evolutionary lineages was also observed in some bees of Meliponini tribe (Travenzoli *et al.*, 2019), corroborating our ideas that this could be a noteworthy event observed in Hymenoptera.

The variation in *A. emarginatus* and *A. cf. masdaraszi* indicated a reduction from the haploid ancestral condition  $n = 15$  to  $n = 14$  (Imai *et al.*, 1984; Santos *et al.*, 2010). This reduction in the chromosome number could be explained by centric fusion. Imai *et al.*, 1986, assumed that acrocentric chromosomes could be fused generating metacentric, submetacentric or subtelocentric new chromosomes. In more recent studies with chromosome evolutionary reconstruction in the *Mycetophylax* genus, and cytogenetic banding information, it was suggested that centromeric fusions, more than fissions, guided the evolutionary patterns of changes in the chromosome structure of this genus (Cardoso *et al.*, 2014). In the Meliponini tribe, analyses based on molecular and cytogenetic data, indicated that the chromosomal number in the Neotropical species, decreased from an ancestral  $n = 18$  to  $n = 17$  in current species, by a centromeric fusion (Travenzoli *et al.*, 2019). A reduction in chromosome numbers could also be observed in a mammalian group (Muntiacinae, Cervidae) which was proposed to be explained by multiple tandem and centromeric fusions of small ancestral acrocentric chromosomes (Wang & Lan, 2000). In another example species from the



*Pholidoptera* Wesmaël, 1838 genera, displayed a reduction of an ancestral common number from  $2n = 32$  to  $2n = 29$  in 10 out of 16 current species (Warchałowska-Śliwa et al., 2017).

The species *A. horridus* exhibits a high number of telomeric chromosomes ( $n = 4 M + 2 SM + 17 T$ ) (Mariano *et al.*, 2015). In our phylogenetic inference this species presented itself as a sister group of *A. emarginatus*. Where in *A. emarginatus* species we see a reduction of a chromosome number, in *A. horridus* we observe a massive gain in chromosome number. *A. horridus* demonstrated a high presence of telocentric chromosomes, when compared to the species of the *Anochetus* genus. In that way, it is tempting to assume that centromeric fission was responsible for the evolutionary process that led to the current karyotypical structure in this species.

Yet, in the *Odontomachus* genus, we observed the maintenance of the haploid chromosome number ( $n=15$ ) in *Odontomachus rixosos* and *Odontomachus latidens*, but the lack of chromosomal formula does not allow us to infer about evolutionary events that could have happened in these clades.

In species from the *haematodos* group (*O. bauri*, *O. chelifer*, *O. haematodos*, *O. hastatus*, *O. meinerti*, *O. scalptus* and *O. simillimus*) the total absence of metacentric, and high presence of telocentric chromosomes, indicate that centric fissions were responsible for changes in the chromosome structure in this group, promoting an increase in the number of chromosomes from  $n=15$ , to  $n = 22$ . In other groups such as mammals, wasps and especially in ants, the centric fissions were strongly suggested as the principal force that drives the chromosome evolutionary patterns (Imai *et al.*, 2001).

Our results suggested that in *Odontomachus*, particularly in the *haematodos* group, centric fissions were the main force that conducted the evolutionary events. Although, in *Anochetus* the maintenance of the ancestral chromosome number in most of the analysed

species and a loss of chromosome number in two species (*A. emarginatus*; *A. madaraszi*) suggested that pericentric rearrangements and centric fusions led the chromosome changes in this genus.

Our phylogenetic inferences reinforce that *Odontomachus* and *Anochetus* genus indeed display a monophyletical relationship, and by chromosome evolution analyses we suggest that the ancestral chromosome number shared by the two genera was  $n = 15$ . Furthermore, our framework indicated that the centric fissions was not the major force that led the karyotype evolution in the-trap jaw ants, since the maintenance of the chromosome ancestral number, fusion and fission are equally involved on the variation of chromosomal structure in this group.

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## Supporting Information

**Table 1.** Species used in Molecular Phylogeny. Sequence numbers refer to GenBank accession numbers.

Species	Voucher	28S	CAD	COI	LWR	Wg
<i>Anochetus africanus</i>	USNM ENT 01124337	KU504996	KU504739	KU504829	KU504918	KU254466
<i>Anochetus alae</i>	USNM ENT 01124338	-	KU504740	KU504830	KU504919	KU254467
<i>Anochetus altisquamis</i>	USNM ENT 01124339	KU504997	KU504741	KU504831	KU504920	KU254468
<i>Anochetus bequaerti</i>	CASENT0763843	-	KU504821	-	-	KU254469
<i>Anochetus bispinosus</i>	USNM ENT 01124340	KU504998	KU504742	KU504832	KU504921	KU254470
<i>Anochetus boltoni</i>	CASENT0487895	KU504999	KU504743	KU504833	KU504922	KU254471
<i>Anochetus cato</i>	USNM ENT 01124341	KU505029	KU504744	-	-	KU254472
<i>Anochetus cf cato</i>	USNM ENT 01124342	KU505000	KU504745	KU504834	KU504923	KU254473
<i>Anochetus emarginatus</i>	CASENT0763844	JQ023505	-	EU155425	EU155443	EU155462
<i>Anochetus goodmani</i>	CASENT0053884	KU505003	KU504747	KU504836	KU504925	KU254474
<i>Anochetus graeffei1</i>	CASENT0763845	-	KU504822	-	KU504926	KU254475
<i>Anochetus graeffei2</i>	USNM ENT 01124343	KU505004	KU504748	KU504837	KU504927	KU254476
<i>Anochetus cf graeffei</i>	USNM ENT 01124344	-	KU504756	KU504844	KU504932	KU254477
<i>Anochetus grandidieri</i>	CASENT0157523	KU505005	KU504749	KU504838	KU504928	KU254478
<i>Anochetus horridus</i>	USNM ENT 01124400	KU505006	KU504750	KU504839	KU504929	KU254479
<i>Anochetus incultus</i>	USNM ENT 01124345	KU505007	KU504751	-	-	KU254480
<i>Anochetus isolatus</i>	USNM ENT 01124346	-	KU504752	KU504840	KU504952	KU254481
<i>Anochetus katonae</i>	USNM ENT 01124347	KU505008	KU504753	KU504841	KU504930	KU254482
<i>Anochetus madagascarensis</i>	CASENT0136590	KU505009	KU504754	KU504842	KU504931	KU254483
<i>Anochetus cf madaraszi</i>	USNM ENT 01124348	KU505010	KU504755	-	-	KU254484
<i>Anochetus mayri1</i>	CASENT0763846	JQ023506	JX310574	-	JN675409	JN419126
<i>Anochetus mayri2</i>	USNM ENT 01124349	KU505011	KU504757	KU504845	KU504933	KU254485
<i>Anochetus mayri3</i>	USNM ENT 01124350	KU505012	KU504758	KU504846	KU504934	KU254486
<i>Anochetus minans</i>	USNM ENT 01124351	KU505013	KU504759	KU504847	KU504936	KU254487

<i>Anochetus modicus</i>	CASENT0763847	KU505014	KU504823	-	KU504937	KU254488
<i>Anochetus neglectus</i>	USNM ENT 01124352	KU505015	KU504760	KU504848	KU504938	KU254489
<i>Anochetus obscuratus</i>	USNM ENT 01124353	KU505016	KU504761	KU504849	-	KU254490
<i>Anochetus orchidicola</i>	USNM ENT 01124354	KU505018	KU504763	KU504851	KU504941	KU254491
<i>Anochetus paripungens</i>	USNM ENT 01124355	KU505019	KU504764	KU504852	KU504940	KU254492
<i>Anochetus peracer</i>	USNM ENT 01124356	KU505020	KU504746	KU504835	KU504924	KU254493
<i>Anochetus princeps</i>	CASENT0763848	EU155409	KU504824	EU155426	EU155444	EU155463
<i>Anochetus pubescens</i>	CASENT0135206	KU505022	KU504766	KU504854	KU504943	KU254494
<i>Anochetus rectangularis</i>	USNM ENT 01124357	-	KU504767	-	KU504944	KU254495
<i>Anochetus risii</i>	USNM ENT 01124358	KU505028	KU504768	KU504863	KU504951	KU254496
<i>Anochetus cf risii</i>	USNM ENT 01124359	KU505001	KU504765	-	KU504942	KU254497
<i>Anochetus rugosus</i>	USNM ENT 01124360	KU505023	KU504769	KU504855	KU504945	KU254498
<i>Anochetus sc01</i>	CASENT0159871	-	KU504775	KU504856	KU504946	KU254499
<i>Anochetus simoni</i>	USNM ENT 01124361	KU505024	KU504770	KU504857	KU504947	KU254501
<i>Anochetus cf simoni</i>	USNM ENT 01124362	KU505002	KU504771	KU504858	KU504948	KU254500
<i>Anochetus sp 70</i>	USNM ENT 01124363	-	KU504772	KU504859	-	KU254502
<i>Anochetus sp 174</i>	USNM ENT 01124364	KU505017	KU504762	KU504850	KU504939	KU254503
<i>Anochetus sp 2921</i>	USNM ENT 01124365	-	KU504773	KU504860	KU504949	KU254504
<i>Anochetus sp BS01</i>	USNM ENT 01124366	KU505025	KU504774	KU504861	KU504950	KU254505
<i>Anochetus subcoecus</i>	USNM ENT 01124367	-	KU504777	KU504864	KU504953	KU254506
<i>Anochetus targionii</i>	USNM ENT 01124401	KU505030	KU504778	KU504865	KU504954	KU254507
<i>Anochetus cf targionii</i>	USNM ENT 01124368	KU505026	KU504776	KU504862	KU504935	KU254508
<i>Anochetus turneri</i>	USNM ENT 01124369	-	KU504779	KU504866	KU504955	KU254509
<i>Anochetus victoriae</i>	USNM ENT 01124370	-	KU504780	KU504867	KU504956	KU254510
<i>Anochetus yt01</i>	CASENT0147171	KU505027	KU504781	KU504868	KU504957	KU254511
<i>Odontomachus angulatus</i>	USNM ENT 01124374	KU505033	KU504784	-	KU504959	KU254514
<i>Odontomachus animosus</i>	USNM ENT 01124375	KU505034	KU504785	KU504877	KU504960	KU254515
<i>Odontomachus assiniensis</i>	USNM ENT 01124376	KU505035	KU504786	KU504878	KU504961	KU254516

<i>Odontomachus banksi</i>	USNM ENT 01124377	KU505036	KU504787	KU504879	KU504984	KU254517
<i>Odontomachus bauri</i>	CASENT0763849	KU505037	KU504825	EU155429	EU155447	EU155466
<i>Odontomachus brunneus</i>	USNM ENT 01124378	KU505038	KU504788	KU504880	KU504962	KU254518
<i>Odontomachus caelatus</i>	USNM ENT 01124402	KU505039	KU504789	KU504881	KU504963	KU254519
<i>Odontomachus cephalotes</i>	CASENT0200850	KU505040	KU504790	KU504882	KU504964	KU254520
<i>Odontomachus chelifer</i>	USNM ENT 01124379	KU505042	KU504791	KU504883	KU504965	KU254521
<i>Odontomachus circulus</i>	CASENT0340431	KU505043	KU504792	KU504884	KU504966	KU254522
<i>Odontomachus clarus</i>	USNM ENT 01124380	KU505044	KU504793	KU504885	KU504967	KU254523
<i>Odontomachus coquereli</i>	CASENT0134224	KU505045	KU504794	KU504886	KU504968	KU254524
<i>Odontomachus erythrocephalus</i>	USNM ENT 01124381	KU505046	KU504795	KU504887	KU504969	KU254525
<i>Odontomachus haematodus</i>	USNM ENT 01124382	KU505047	KU504796	KU504888	KU504970	KU254526
<i>Odontomachus hastatus</i>	USNM ENT 01124383	KU505071	KU504797	KU504889	KU504971	KU254527
<i>Odontomachus imperator</i>	CASENT0200151	KU505064	KU504813	KU504907	KU504989	KU254528
<i>Odontomachus infandus</i>	CASENT0763850	KU505048	KU504826	KU504890	KU504972	KU254529
<i>Odontomachus insularis</i>	USNM ENT 01124384	KU505049	KU504798	KU504891	KU504973	KU254530
<i>Odontomachus laticeps</i>	USNM ENT 01124385	KU505050	KU504799	KU504892	KU504974	KU254531
<i>Odontomachus latidens</i>	USNM ENT 01124386	KU505051	KU504800	KU504893	KU504975	KU254532
<i>Odontomachus malignus</i>	USNM ENT 01124387	KU505052	KU504801	KU504894	KU504976	KU254533
<i>Odontomachus meinerti</i>	USNM ENT 01124403	KU505053	KU504802	KU504895	KU504977	KU254534
<i>Odontomachus montanus</i>	USNM ENT 01124396	KU505054	KU504803	KU504897	KU504978	KU254535
<i>Odontomachus monticola</i>	USNM ENT 01124388	KU505055	KU504804	KU504898	KU504979	KU254536
<i>Odontomachus cf opaciventris</i>	USNM ENT 01124389	KU505041	KU504806	KU504896	KU504991	KU254538
<i>Odontomachus opaciventris</i>	USNM ENT 01124390	KU505056	KU504805	KU504899	KU504980	KU254537
<i>Odontomachus opaculus</i>	USNM ENT 01124391	KU505057	KU504807	KU504900	KU504981	KU254539
<i>Odontomachus panamensis</i>	USNM ENT 01124392	KU505058	KU504808	KU504901	KU504982	KU254540
<i>Odontomachus papuanus</i>	CASENT0215929	KU505059	KU504809	KU504902	KU504983	KU254541
<i>Odontomachus relictus</i>	USNM ENT 01124393	KU505060	KU504810	KU504903	KU504985	KU254542
<i>Odontomachus rixosus</i> <sup>1</sup>	CASENT0763851	KU505061	KU504828	KU504904	KU504986	KU254543

<i>Odontomachus rixosus</i> 2	USNM ENT 01124394	KU505062	KU504811	KU504905	KU504987	KU254544
<i>Odontomachus ruficeps</i>	CASENT0763852	EU155420	KU504827	EU155438	EU155457	EU155476
<i>Odontomachus ruginodis</i>	USNM ENT 01124395	KU505063	KU504812	KU504906	KU504988	KU254545
<i>Odontomachus saevissimus</i>	MCZ0093	KU145860	-	KU146027	KU146316	KU146405
<i>Odontomachus scalptus</i>	USNM ENT 01124404	KU505065	KU504814	KU504908	-	KU254546
<i>Odontomachus simillimus</i>	CASENT0159290	KU505066	KU504815	KU504909	KU504990	KU254547
<i>Odontomachus testaceus</i>	USNM ENT 1124405	KU145847	KU145928	KU146080	KU146303	KU146392
<i>Odontomachus troglodytes</i>	CASENT0116074	KU505067	KU504816	KU504910	KU504992	KU254548
<i>Odontomachus turneri</i>	USNM ENT 01124397	-	-	-	KU504993	-
<i>Odontomachus tyrannicus</i> 1	USNM ENT 1124406	KU145850	KU145931	KU146083	KU146306	KU254549
<i>Odontomachus tyrannicus</i> 2	CASENT020024	KU505068	KU504817	KU504911	KU504994	KU254549
<i>Odontomachus yucatecus</i>	USNM ENT 01124398	KU505069	KU504818	KU504912	KU504995	KU254550

<b>Species</b>	<b>Voucher</b>	<b>28S</b>	<b>CAD</b>	<b>COI</b>	<b>LWR</b>	<b>Wg</b>
<i>Bothroponera variolosa</i>	CASENT0260241	JQ023530	JX310638	-	JN675459	JN419154
<i>Bothroponera wasmannii</i>	CASENT0260242	JQ023529	JX310640	DQ176299	JN675461	JN419153
<i>Bothroponera</i> sp	USNM ENT 01124371	-	KU504819	-	-	KU254551
<i>Brachyponera atrata</i>	CASENT0260243	JQ023531	JX310612	KU504869	JN675435	JN419155
<i>Brachyponera chinensis</i>	CASENT0260244	AB126802	JX310616	GQ264572	JN675439	JN419156
<i>Brachyponera sennaarensis</i>	CASENT0260245	JQ023532	JX310628	-	JN675450	JN419157
<i>Buniapone amblyops</i>	CASENT0260263	-	JX310609	-	JN675432	JN419178
<i>Diacamma rugosum</i>	CASENT0260213	-	JX310580	HQ853328	JN675414	JN419131
<i>Euponera sikorae</i>	CASENT0260249	EF013032	JX310629	DQ176229	EF013612	JN419150
<i>Hagensia havilandi</i>	CASENT0260250	JQ023537	JX310622	KU504870	-	JN419162
<i>Hypoponera opacior</i>	CASENT0260218	EU155410	JX310587	KU504871	EU155445	EU155464
<i>Hypoponera sakalava</i>	CASENT0260219	JQ023515	JX310588	-	EF013577	JN419137
<i>Leptogenys attenuata</i>	CASENT0260223	EU155411	JX310592	KU504872	EU155446	EU155465

<i>Leptogenys crassicornis</i>	CASENT0260224	JQ023522	JX310593	-	JN675423	JN419145
<i>Leptogenys diminuta</i>	CASENT0106010	EF013000	-	-	EF013580	EF013708
<i>Leptogenys falcigera</i>	CASENT0260225	JQ023519	JX310594	-	JN675424	JN419142
<i>Leptogenys iridescens</i>	CASENT0260226	-	JX310595	-	KC006064	JN419141
<i>Leptogenys occidentalis</i>	CASENT0260227	JQ023520	JX310596	-	JN675425	JN419143
<i>Leptogenys</i> sp 4004	USNM ENT 01124372	KU505031	KU504782	-	-	KU254513
<i>Leptogenys</i> sp 0339	USNM ENT 01124373	KU505032	KU504783	KU504873	KU504958	KU254512
<i>Megaponera analis</i>	CASENT0260251	JQ023540	JX310610	KU504874	JN675433	JN419165
<i>Mesoponera ingesta</i>	CASENT0260255	-	JX310624	KU504875	JN675446	JN419168
<i>Myopias lobosa</i>	RA026	DQ353650	-	-	DQ353246	DQ353130
<i>Myopias</i> sp	CASENT0260230	JQ023524	JX310599	-	JN675428	JN419147
<i>Myopias tenuis</i>	CASENT0260231	JQ023525	JX310600	KU504876	JN675429	JN419148
<i>Odontoponera transversa</i>	CASENT260235	EU155422	JX310607	DQ353399	EU155459	EU155478
<i>Ophthalmophone berthoudi</i>	CASENT0260261	-	JX310613	-	JN675436	JN419175
<i>Mesoponera rubra</i>	CASENT0260256	-	JX310626	-	JN675448	JN419169
<i>Neoponera villosa</i>	CASENT0260260	JQ023548	JX310639	-	JN675460	JN419174
<i>Paltothyreus tarsata</i>	CASENT0260262	-	JX310636	KU504913	JN675457	JN419176
<i>Phrynoponera bequaerti</i>	CASENT0401941	DQ401009	-	-	-	KU254552
<i>Phrynoponera gabonensis</i>	USNM ENT 01124399	-	KU504820	KU504914	-	KU254553
<i>Platythyrea lamellosa</i>	CASENT0260200					
<i>Platythyrea punctata</i>	CASENT0260201	JQ023504	JX310643	HQ440172	EF013620	JN419122
<i>Platythyrea quadridentata</i>	CASENT0260202					
<i>Plectroctena ugandensis</i>	CASENT0260270	EU155424	JX310647	KU504915	EU155461	EU155480
<i>Ponera pennsylvanica</i>	CASENT0260271	JQ023553	-	KJ445354	JN675464	JN419185
<i>Ponera</i> sp MAL01	CASENT0260272					
<i>Pseudoneoponera rufipes</i>	CASENT0260239	JQ023527	JX310627	KU504916	JN675449	JN419151
<i>Pseudoponera stigma</i>	CASENT0260265	DQ353617	JX310635	DQ353336	JN675456	JN419179
<i>Simopelta</i> sp Ecu2	CASENT0260276	JQ023558	JX310651	-	JN675468	JN419190

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<i>Streblognathus peetersi</i>	CASENT0260277	JQ023559	JX310653	KU504917	JN675470	JN419191
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## Script

Python script developed for pruning trees:

```
from ete3 import Tree
```

```
#variable t is original tree
```

```
t=Tree("(((((((Odontomachus_angulatus:0.01567999999999972,(Odontomachus_banksi:0.01816799999999962,(Odontomachus_infandus:0.00855799999999955,Odontomachus_malignus:0.01219799999999987):0.006360000000000032):0.00256999999999961):0.011765000000000025,((Odontomachus_animosus:0.009294000000000024,Odontomachus_papuanus:0.017401):0.00584799999999964,((Odontomachus_imperator:0.01461699999999991,Odontomachus_montanus:0.024430000000000007):0.00554699999999969,Odontomachus_opaculus:0.033663):0.011221000000000037):0.0030930000000000124):0.006873000000000018,((Odontomachus_cephalotes:0.020547000000000037,(Odontomachus_ruficeps:0.0024960000000000537,Odontomachus_turneri:0.002319999999999887):0.01624399999999998):0.005949000000000038,(((Odontomachus_saevisimus:0.06587899999999997,Odontomachus_tectaceus:0.01623599999999973):0.00425399999999998,Odontomachus_tyrannicus1:0.022558000000000022):0.040920999999999985,Odontomachus_tyrannicus2:0.008753999999999984):0.016778000000000015):0.003346999999999989):0.012268999999999974,Odontomachus_hastatus:0.03903699999999999):0.004975000000000007,((((((((Odontomachus_bauri:0.01398499999999997,Odontomachus_simillimus:0.015996000000000001):0.0026780000000000137,Odontomachus_insularis:0.019172000000000022):0.005475999999999981,Odontomachus_haematodus:0.014230000000000002):0.0017369999999999886,Odontomachus_troglytes:0.013280999999999987):0.0027290000000000037,(Odontomachus_panamensis:0.023618999999999946,Odontomachus_scalptus:0.021069999999999978):0.0017390000000000046):0.005390000000000006,Odontomachus_erythrocephalus:0.013441000000000036):0.0027149999999999674,((((Odontomachus_brunneus:0.016530000000000045,Odontomachus_laticeps:0.012952000000000019):0.0018319999999999448,Odontomachus_yucatecus:0.016197999999999935):0.002238999999999991,Odontomachus_clarus:0.01582399999999995):0.0012800000000000589,Odontomachus_relictus:0.019306000000000045):0.0024729999999999475,Odontomachus_ruginodis:0.013589999999999991):8.6800000000000354E-4):0.004280000000000006,(Odontomachus_cf_opaciventris:0.021424,Odontomachus_opaciventris:0.02130999999999994):0.0023560000000000247):0.004525000000000001,Odontomachus_caelatus:0.017309000000000002):0.013371999999999995,Odontomachus_meinerti:0.03
```

049600000000023):0.00724799999999977,Odontomachus\_chelifer:0.02944499999999994  
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96):0.0038020000000000276):0.008093999999999999,(Anochetus\_emarginatus:0.064680999  
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us\_incultus:0.019955999999999974,Anochetus\_sp\_70:0.02002699999999996):0.013925999  
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isii:0.04131299999999999):0.006398000000000015,(Anochetus\_princeps:0.0332639999999  
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999):0.007037999999999989):0.05327100000000001):0.006592000000000042,((Anochetus\_  
altisquamis:0.049709999999999976,Anochetus\_orchidicola:0.041032999999999986):0.0405  
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i:0.028985000000000004,(Anochetus\_sp\_BS01:0.021328000000000014,Anochetus\_subcoecu  
s:0.036482000000000014):0.018837000000000048):0.00585099999999994,Anochetus\_kato  
nae:0.054174):0.003359000000000001,(Anochetus\_sc01:0.0033560000000000256,Anochetu  
s\_yt01:0.005510999999999988):0.03731099999999998):0.020457999999999976,(((Anochet  
us\_cf\_graeffei:0.046154000000000003,(Anochetus\_graeffei1:0.0062049999999999605,(Anoc  
hetus\_graeffei2:0.003011999999999959,Anochetus\_pubescens:0.006554000000000004):0.01  
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3671600000000008):0.02895399999999924,((((Anochetus\_alae:0.03370199999999954,A  
nochetus\_pariungens:0.03723799999999999):0.006454000000000071,(Anochetus\_rectangu  
laris:0.010084000000000037,Anochetus\_victoriae:0.006300000000000028):0.033658999999  
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0.105841000000000002,Pseudoponera\_stigma:0.198836):0.033633000000000024):0.0436659  
999999998,(Neoponera\_villosa:0.179764000000000003,Simopelta\_sp\_Ecu2:0.147086):0.01  
6973000000000016):0.025139000000000022,((Hypoconerops\_opacior:0.129699,Hypoconerops\_  
sakalava:0.080400000000000003):0.08714,Plectroctena\_ugandensis:0.167622):0.0124210000  
00000015):0.013115999999999961,Platythyrea\_punctata:0.386739):0.024627000000000001):  
0.008797999999999973,(Buniapone\_amblyops:0.092990000000000002,Paltothyreus\_tarsata:0  
.09691899999999998):0.01964699999999997,((((Leptogenys\_attenuata:0.0356790000000000  
016,Leptogenys\_occidentalis:0.046576000000000006):0.006967999999999974,((Leptogenys  
\_diminuta:0.071572000000000002,Leptogenys\_sp\_0339:0.021376000000000006):0.09119,Le  
ptogenys\_falcigera:0.03997299999999998):0.008450000000000013):0.062609000000000003,  
(Leptogenys\_crassicornis:0.048516000000000004,(Leptogenys\_iridescens:0.03490799999999  
99995,Leptogenys\_sp\_4004:0.04197899999999999):0.017471999999999988):0.0774139999  
999998):0.0300890000000000032,((Myopias\_lobosa:0.305259000000000006,Myopias\_tenuis  
:0.06615899999999997):0.06975999999999999,Myopias\_sp:0.128405):0.023187000000000  
013):0.013441999999999954):0.0057540000000000037,Euconerops\_sikorae:0.119786,Hagensia  
\_havilandi:0.08323599999999998,((Megaponera\_analis:0.09046599999999999,Ophthalmoph  
one\_berthoudi:0.08444099999999999):0.061531,(Odontoponera\_transversa:0.105111999999  
99998,Pseudoneoponera\_rufipes:0.11426799999999998):0.019896000000000025):0.009341  
999999999961,Mesoponera\_ingesta:0.078656,Mesoponera\_rubra:0.149602,(Phrynoponera\_b

```
equaerti:0.023990000000000001,Phrynoponera_gabonensis:0.0047760000000000025):0.1204
9199999999999999,Streblognathus_peetersi:0.091598999999999999):0.028502499999999972);;"
)
```

```
#variable t2 is original tree copy
```

```
t2 = t.copy()
```

```
#clades which are not well resolved in the original tree
```

```
deletethis = ["Anochetus_cf_risii", "Paltothyreus_tarsata", "Buniapone_amblyops",
"Streblognathus_peetersi", "Mesoponera_rubra", "Mesoponera_ingesta", "Hagensia_havilandi",
"Euponera_sikorae", "Bothroponera_variolosa", "Bothroponera_sp",
"Bothroponera_wasmannii", "Phrynoponera_gabonensis", "Phrynoponera_bequaerti"]
```

```
#clades which have chromosome count
```

```
chromosome_clades = ["Odontomachus_rixosus1", "Odontomachus_rixosus2",
"Odontomachus_latidens", "Odontomachus_chelifer", "Odontomachus_meinerti",
"Odontomachus_scalptus", "Odontomachus_haematodus", "Odontomachus_simillimus",
"Odontomachus_bauri", "Odontomachus_hastatus", "Anochetus_altisquamis",
"Anochetus_modicus", "Anochetus_horridus", "Anochetus_emarginatus",
"Anochetus_targionii", "Anochetus_cf_madaraszi", "Anochetus_cf_graeffei",
"Anochetus_graeffei1", "Anochetus_graeffei2", "Brachyponera_chinensis",
"Leptogenys_diminuta", "Leptogenys_iridescens", "Odontoponera_transversa",
"Neoponera_villosa", "Ponera_pennsylvanica", "Pseudoponera_stigma"]
```

```
#code to get nodes which are not the outgroup
```

```
pruneme = []
```

```
for node in t.traverse("postorder"):
```

```
    # node has a name and name is well resolved
```

```

if node.name and node.name not in deletethis:

    print(node.name)

    #add name to list of interest

    pruneme.append(node.name)

pruneme = tuple(pruneme)

#variable t is now pruned to retain only well resolved clades. Be sure to set the
preserve_branch_length accordingly

t.prune(pruneme, preserve_branch_length=True)

#list of well resolved clades is printed

print(pruneme)

#pruned tree is printed

print(t)

#pruned tree is saved to disk without poorly resolved clades

t.write(outfile="new_tree.nw")

#variable t2 is pruned to retain only clades with chromossome counts

t2.prune(tuple(chromossome_clades))

#chromossome-only clades are printed

print(chromossome_clades)

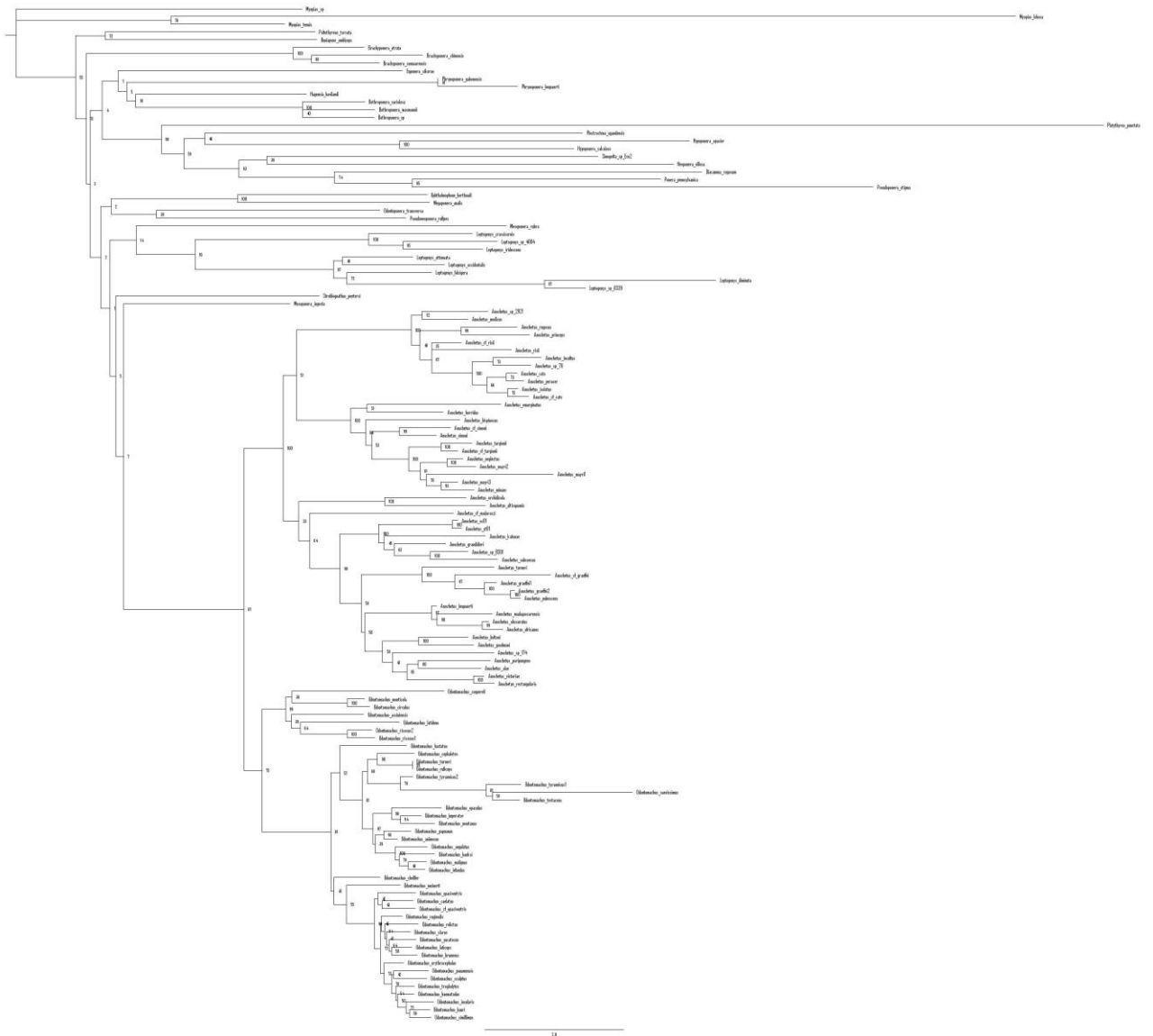
```

```
#chromosome-only tree is printed
```

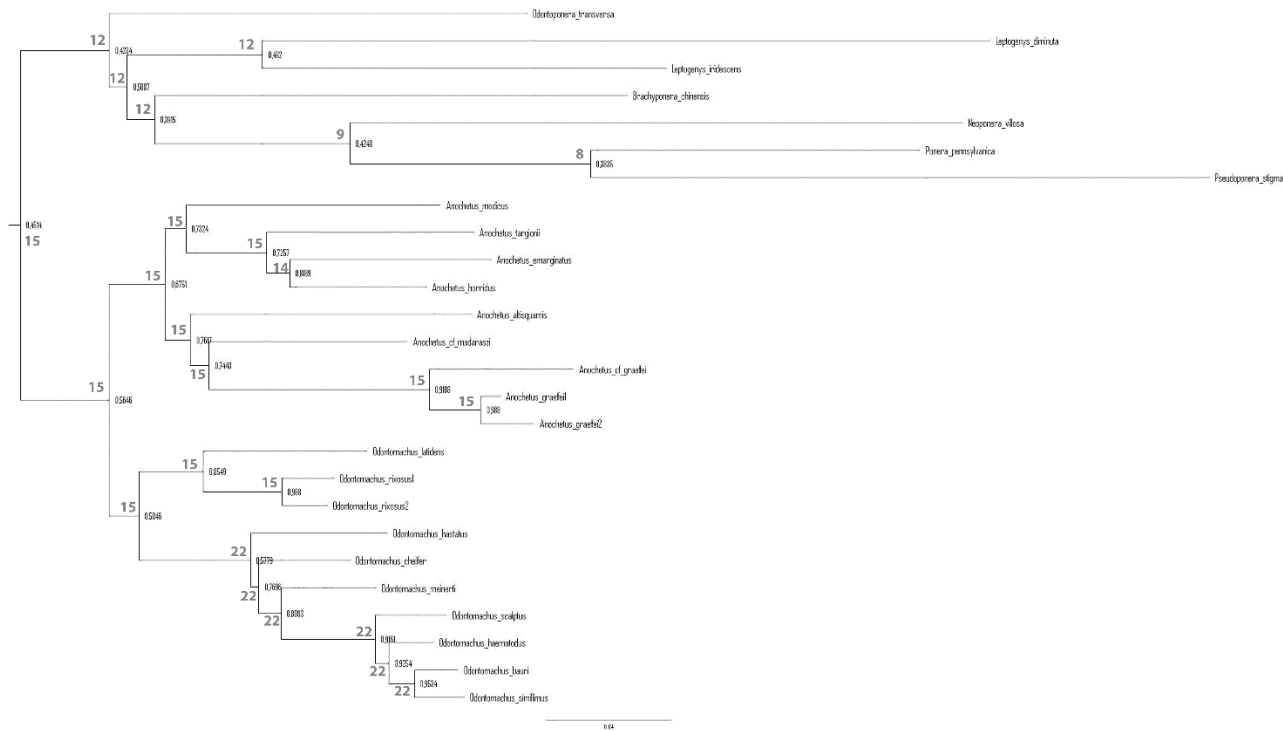
```
print(t2)
```

```
#chromosome-only tree is saved to disk
```

```
t2.write(outfile="new_tree_chromo.nw")
```



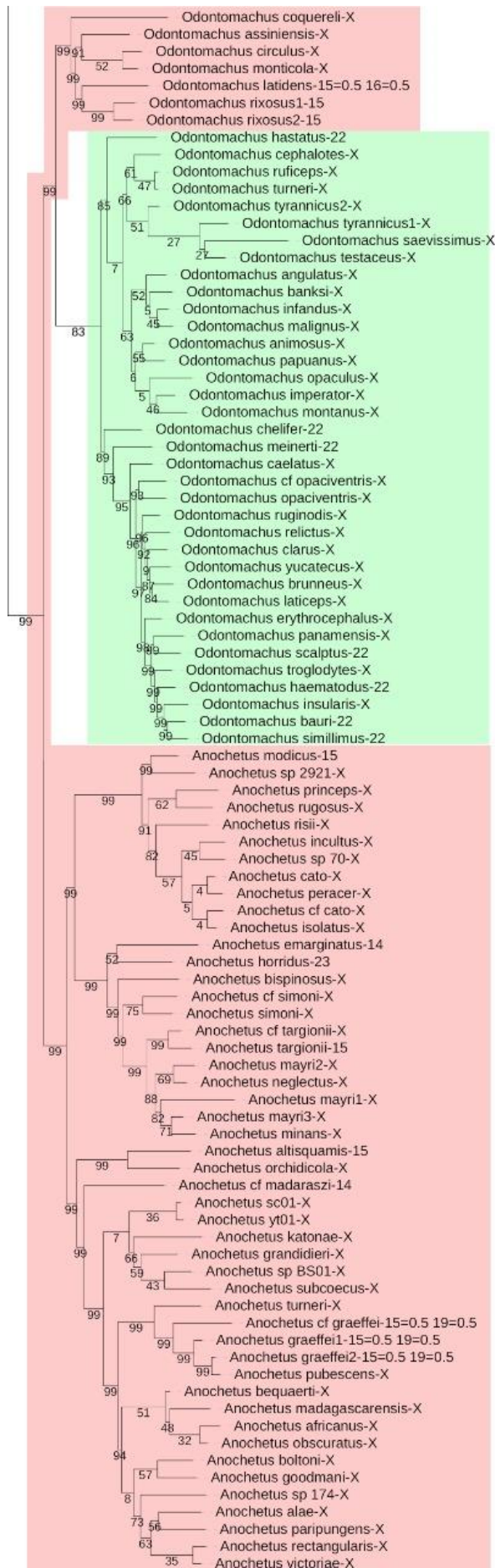
**Figure 2.** Maximum Likelihood phylogenetic tree for the *Anochetus*, *Odontomachus* and outgroup five genes data. Bootstrap values are indicated at each node label.



**Figure 4** – ChromoSSE Bayesian Inference for the Chromosome annotated species in the *Anochetus*, *Odontomachus* and outgroup five gene pruned tree. Chromosome numbers of the highest probabilities are indicated for each ancestral node.

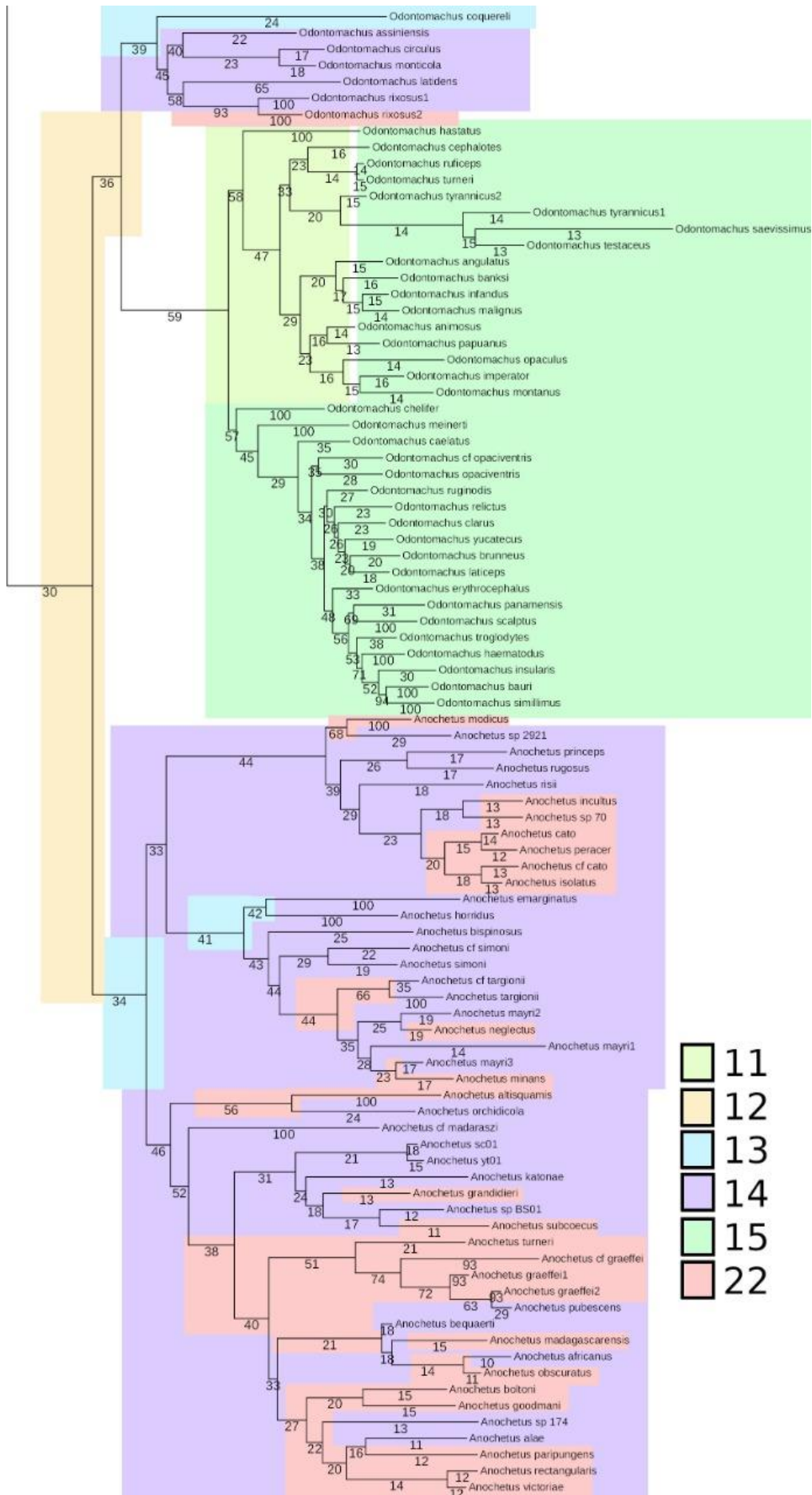
**Table 3.** Partitioning scheme used in phylogenetic analyses. Partition schemes and models of nucleotide substitutions were identified by PartitionFinder and used for analyses on full concatenated sequence alignments.

Partition	Block	Models
p1	28S	GTR+I+G
p2	CAD pos 1, WNG pos 2	GTR+I+G
p3	WNG pos 1, CAD pos 2	GTR+I+G
p4	CAD pos 3	SYM+G
p5	COI pos 1	GTR+I+G
p6	COI pos 2	GTR+I+G
p7	COI pos 3	GTR+I+G
p8	ROD pos 1	SYM+I+G
p9	ROD pos 2	GTR+I+G
p10	ROD pos 3	SYM+I+G
p11	WNG pos 3	GTR+G





**Figure 5.** *Anochetus* and *Odontomachus* ancestral chromosome Bayesian Inference results for the ChromEvol unpruned tree including missing data for clades without Chromosome number descriptions. Red equals to 15 chromosomes and green to 22 chromosomes. Probabilities are indicated at each node label as number ranging from 0-100.



**Figure 6.** *Anochetus* and *Odontomachus* ancestral chromosome Bayesian Inference results for the ChromoSSE unpruned tree including missing data for clades without Chromosome number descriptions. A color legend is included in the right side of the figure for haploid chromosome number. Probabilities are indicated at each node label as number ranging from 0-100.

## Considerações Finais

Neste estudo objetivamos melhor compreender os processos evolutivos que guiaram a evolução cromossômica nos gêneros de formigas *Anochetus* e *Odontomachus*, pertencentes a subfamília Ponerinae, reconstruindo suas relações filogenéticas para então inferir a condição do número cromossômico ancestral.

Os resultados apresentados sugerem que inversões pericêntricas, fissões e fusões centroméricas teriam ocorrido ao longo da história evolutiva destes organismos. Alternativamente ao que foi proposto pela Teoria da Interação Mínima, não se observa um protagonismo dos processos de fissões centroméricas como sendo a principal força que liderou os padrões evolutivos nos gêneros *Anochetus* e *Odontomachos*.

Desta forma, tencionamos acrescentar conhecimento sobre os processos estruturadores das variadas formas cariotípicas encontradas na família Formicidae, bem como atentamos a necessidade de futuros estudos necessários para a melhor elucidação destes processos.