UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM
EM CIÊNCIAS BIOLÓGICAS
(Biologia Celular, Molecular e Microbiologia)

ANÁLISE DOS DNAS REPETITIVOS DE *Ceraeochrysa claveri* (NEUROPTERA), UMA ABORDAGEM CITOGENÔMICA

VITÓRIA LOUREJAN FERREIRA

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Orientador: Dr. Diogo Cavalcanti Cabral de Mello
Coorientador: Dra. Vanessa Bellini Bardella

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AUTORA: VITÓRIA LOUREJAN FERREIRA

ORIENTADOR: DIOGO CAVALCANTI CABRAL DE MELLO

COORIENTADORA: VANESSA BELLINI BARDELLA

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Profa. Dra. VANESSA BELLINI BARDELLA (Participaçao Virtual)
Departamento de Biologia Geral e Aplicada / Unesp IB Rio Claro

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Profa. Dra. DENILCE MENESES LOPES (Participaçao Virtual)
Departamento de Biologia Geral / Universidade Federal de Viçosa

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RESUMO

As DNAs satélites (satDNAs), conhecidos por seus papéis significativos na estruturação e evolução do genoma, foram caracterizados em oito espécies de Chrysopidae utilizando abordagens genômicas e bioinformáticas. Além disso, o cariótipo de *Ceraeochrysa claveri* foi caracterizado em detalhes, revelando a constituição comum observada em Neuroptera, 2n=12,XY. Para o grupo, é a primeira vez que a distribuição da heterocromatina é descrita, com ocorrência de blocos pericentroméricos. O mapeamento do DNA ribossômico maior (rDNA) foi divergente das poucas outras espécies de neurópteros estudadas, evidenciando o dinamismo deste agrupamento no grupo. Quanto aos satelitomas, mapeamos as satDNAs por meio do mapeamento cromossômico usando Hibridização *in situ* Fluorescente (FISH) e CHRISMAPP em *Ceraeochrysa claveri* e *Chrysopa pallens*, respectivamente. O impacto das satDNAs na organização do genoma dos neurópteros é variável, já que diferentes números de satDNAs foram identificados, variando de uma única satDNA até 23 famílias de satDNA. Além disso, a quantidade total de satDNAs também foi bastante variável, representando de cerca de 2,5% a 21,55% do genoma. Curiosamente, observamos uma alta renovação da biblioteca de satDNA entre as espécies, sugerindo o surgimento frequente e a eliminação total de repetições de forma independente, contrariando a hipótese da biblioteca. O mapeamento físico desses repetições nos cromossomos de *C. claveri* e *C. pallens* forneceu informações detalhadas sobre sua distribuição genômica, revelando a acumulação das principais satDNAs na eucromatina. Nossos dados fornecem insights iniciais sobre a organização do genoma de DNAs satélites (satDNAs) entre os Neurópteros, contribuindo para o entendimento dessas repetições nos genomas de insetos. Esses achados auxiliarão pesquisas futuras sobre montagem de genomas e a evolução geral dessas importantes espécies.

Palavras-chave: controle biológico, citogenética, evolução, RepeatExplorer, DNA repetitivo

ABSTRACT

Satellite DNAs (satDNAs), known for their significant roles in genome structuration and evolution, were characterized in eight Chrysopidae species using genomic and bioinformatic approaches. Additionally, the karyotype of *Ceraeochrysa claveri* was characterized in details, revealing the common constitution observed in Neuroptera, 2n=12,XY. For the group it is the first time that the heterochromatin distribution is described, with occurrence of pericentromeric blocks. The mapping of major ribosomal DNA (rDNA) was divergent from the other few neuropteran species studied, evidencing dynamism for this cluster in the group. Concerning the satellitomes, additionally for *Ceraeochrysa claveri* and *Chrysopa pallens* we mapped the satDNAs using chromosomal mapping through Fluorescence *in situ* Hybridization (FISH) and CHRISMAPP, respectively. The impact of satDNAs on the genome organization of neuropterans is variable, as distinct number of satDNAs were identified from a single satDNA to 23 satDNA families. Moreover, the total amount of satDNAs was also quite variable representing from about 2.5% to 21.55% of the genome. Interestingly, we observed a high turnover of the satDNA library between species, suggesting the frequent emergence and total elimination of repeats independently, contrary to the library hypothesis. The physical chromosome mapping of these repeats in *C. claveri* and *C. pallens* provided detailed insights into their genomic distribution, revealing accumulation of main satDNAs on euchromatin. Our data provide initial insights into the genome organization of satellite DNAs (satDNAs) among Neuropterans, contributing to our understanding of theserepeats in insect genomes. These findings will aid future research on genome assembly and the overall evolution of these important species.

Key-words: biological control, cytogenetics, evolution, RepeatExplorer, repetitive DNA

SUMÁRIO

Introdução

1.1. DNAs repetitivos: estrutura e função

Os DNAs repetitivos fazem uma parte importante do genoma e integram a maior parte da heterocromatina e são DNAs repetidos em tandem (um após o outro) ou dispersos pelo genoma, podendo ser representados por elementos de transposição ou DNAs satélites. Os DNAs repetitivos podem variar em sequência de nucleotídeos, tamanho da sequência, número de cópias e organização cromossômica. Essas sequências podem ter cópias de centenas a milhares e variam de 5 a 1000 pares de bases (CHARLESWORTH *et al*. 1994; GARRIDO-RAMOS 2017). Diversos estudos apontam que os DNAs repetitivos podem estar envolvidos na reparação gênica, reparo e replicação de DNA, organização e atividade dos centrômeros, telômeros, segregação cromossômica (ANLEITNER e HAYMER 1992; KRAEMER e SCHMID 1993).

Os elementos de transposição (TE) são sequências de DNA que se movimentam pelo genoma e podem se encaixar em locais de genes e regiões reguladoras. Eles compreendem uma grande parte do genoma e são sequências de número alto até milhares de pares de bases e ocorrem também em repetições de até milhares (FERRETI 2020). Devido a essa capacidade eles podem influenciar na evolução do genoma ao criar adaptações biológicas, e também podem ter efeito deletério de genes ou inserção de sequências impedindo a função do gene causando mudança no fenótipo do indivíduo. Os TE são divididos em duas classes de acordo com os transposons. A classe I constitui nos retrotransposons em que uma cópia de RNA é reversamente transcrito em um cDNA e inserido no genoma e a classe II constitui nos transposon em que ocorre a transposição de uma cópia de DNA diretamente no genoma (SILVA 2021).

Os DNAs satélites são sequências compostos por monômeros que se repetem em tandem. Sabe-se que essas regiões apresentam uma evolução rápida, por meio da evolução en concerto. Os DNAsat se concentram na heterocromatina e nas áreas teloméricas e centroméricas (SILVA 2021). São conhecidos por ter função na atividade dos centrômeros e telômeros (incluindo alongamento e replicação), manutenção da heterocromatina e também são partes fundamentais de cromossomos sexuais em diversos organismos como planta (*Rumex acetosa*), platelminto (*Schistosoma mansoni*), grilo (*Eneoptera surinamensis*), mosca da fruta (*Drosofila melanogaster*) (BROSSEAU 1960; CHARLESWORTH *et al*. 1994; MARIOTTI *et al*. 2008; LEPESANT *et al*. 2012; PALACIOS-GIMENEZ *et al*. 2017; KHOST *et al*. 2017; FERRETI 2020).

O genoma contém esses DNAsat em locais com uma grande abundância de sequencias ricas em AT ou em GC. Antes das técnicas de sequenciamento avançarem e a genética molecular permitir análise mais avançadas de bioinformática como o TAREAN e o *Repeatexplorer* (NOVAK et al. 2020), a análise de DNAsat era feita utilizando fragmentos dos DNAs recuperados de eletroforeses e seus géis de agarose e utilizando primer de DNAs feitos pela hibridização do Southern Blot, uma técnica mais antiga, para clonar e caracterizar microssatélites (THAKUR *et al*. 2021).

1.2. A família Chrysopidae: estudos cromossômicos

A família Chrysopidae é uma das famílias mais importantes da ordem Neuroptera e possui mais de 1200 espécies divididas em 86 gêneros. A maioria delas se alimentam

de larvas e ovos de outros insetos, podendo ter uma significância para controle biológico em lavouras (BROOKS e BARNARD 1990). Chrysopidae é dividida em três subfamilias sendo elas Notochrysinae, Apochrysinae e Chrysopinae. As duas primeiras correspondem apenas 3% das espécies, sendo Chrysopinae a mais abundante em número de espécies e também dividida em quatro tribos: Ankylopterigini, Belonopterigini, Chrysopini e Leucochrysini (BROOKS e BARNARD 1990).

As larvas e adultos de crisopídeos possuem formas alimentares e aparência diferentes, sendo assim holometábolos, ajudando na sobrevivência de ambos já que não interferem no nicho ecológico um do outro. Os adultos são pequenos e possuem asas hialinas e seu corpo é da cor verde. Já as larvas possuem três instares e possuem pernas ambulatórias, fazendo que eles sejam rápidos e consigam capturar suas presas. Após o terceiro instar, elas empupam e saem indivíduos adultos (FREITAS 2001, 2002)

A identificação das espécies nesta família tem dificuldades devido a morfologia muito parecida, e a identificação correta é muito importante para o sucesso das operações de controle biológico, já que podem ser liberadas em locais com as pragas não correspondentes ou com clima em que a espécie pode não se adaptar (ALMEIDA e STOWTHAMER 2003). Por esse motivo se faz necessário o uso de outras técnicas para identificar os indivíduos como a biologia molecular e a citogenética.

Em Chrysopidae, não existem muitos estudos citogenéticos tendo apenas 24 espécies caracterizadas das 1200 espécies existentes no grupo. Por esse motivo é necessário a caracterização das demais espécies que podem ter potencial para controle biológico e para ajudar na identificação mais precisa das espécies de Chrysopidae (NEW 1984).

Para as análises citogenéticas foram usados ambos os sexos para a comparação dos cromossomos sexuais, mas as gônadas do macho foram essenciais para a visualização

das divisões já que os espermatozoides estão em constante produção. Na maioria dos indivíduos foram encontrados o cromossomo Y menor comparado ao cromossomo X (NEW 1984).

Nos estudos das espécies *C. externa* e *C. argentina* foi constatado que a taxa de divisão das células é muito baixa e o momento que apresenta mais células mitóticas é na hora 48, quando o embrião começa a se formar. Dessa forma é difícil utilizar indivíduos adultos para conseguir suas células meióticas (ACEVEDO 2024).

Dos estudos das 24 espécies caracterizadas, foram encontradas fórmulas cariotípicas diferentes para as espécies da subfamília Chrysopinae. No trabalho de NAVILLE e DE BEAUMONT (1933; 1936) foram analisadas 12 espécies e foram encontrados diferentes fórmulas como 2n=12, 2n=10 e 2n=14. Sendo assim é difícil estabelecer qual seria a fórmula cariotípica para Chrysopidae sabendo que não foram analisados os dados em comparação com outros grupos de Neuroptera e que essas análises foram feitas antes da descoberta do gênero Ceraeochrysa. Por isso é necessário mais estudo com foco na citogenética geral dos cromossomos e cromossomos individuais, assim como a estrutura de seus DNAsat para entender como foi sua evolução em relação ao grupo.

1.3. Ceraeochrysa claveri

A espécie *Ceraeochrysa claveri* pertence à família Chrysopidae e é um pequeno inseto predador de ovos e outros insetos de tegumento mole, eles possuem alta taxa reprodutiva e buscam presas com alto nível e intensidade, podendo ser usado como controle biológico em lavouras e em cultivos que as pragas se instalam em locais de difícil acesso para outros tipos de controle biológico (ALMEIDA *et al*. 2009). Existem registros

que essa espécie se alimenta de vários insetos como *whiteflys* (Hemiptera), *trips*, lepidópteras, pulgões e ácaros (SCUDELER *et al*. 2013).

Essa espécie é uma das mais importantes em ecossistemas de agricultura por ser um ávido predador sendo já utilizado no *Programa Integrated Pest Management*. Devido ao seu alto fator de reprodução, estudos de monitoramento genético e ecotoxicidade podem ser feitos com essa espécie para analisar como inseticidas se comportam com esta espécie, visando deixá-la viva, já que ela também faz parte do controle de pragas (GASTELBONDO-PASTRANA *et al*. 2019)

2. Objetivos

2.1. Geral

Caracterizar o cariótipo e a fração repetitiva do genoma de *Ceraeochrysa claveri* por análises cromossômicas e genômicas, visando entender o padrão de organização e evolução destas sequências e do cariótipo da espécie.

2.2. Específicos

• Analisar os cromossomos da espécie *Ceraeochrysa claveri* por técnicas de coloração convencional, buscando caracterizar o cariótipo da espécie e identificar possíveis polimorfismos;

• Prospectar a partir de dados de sequenciamento genômico DNAs satélites e elementos de transposição mais abundantes no genoma de *Ceraeochrysa claveri* e localizar os mesmos cromossomicamente, visando entender os padrões de organização e evolução destas sequências;

• Auxiliar na montagem da fração repetitiva do genoma da espécie do ponto de vista cromossômico.

• Realizar a comparação dos DNAs satélites em espécies relacionadas.

3. Resultados e discussão

Os métodos e resultados obtidos durante o mestrado serão apresentados e discutidos no manuscrito intitulado “Enhancing genomic understanding of the natural predator *Ceraeochrysa claveri* through cytogenomic analysis and first insighs about satellitomes in Neuroptera insects” a ser submetido para a revista Insect Molecular Biology, INSS: 1365-2583.

4. Manuscrito

Enhancing genomic understanding of the natural predator *Ceraeochrysa claveri* through cytogenomic analysis and first insighs about satellitomes in Neuroptera insects

Abstract

Satellite DNAs (satDNAs), known for their significant roles in genome structuration and evolution, were characterized in eight Chrysopidae species using genomic and bioinformatic approaches. Additionally, the karyotype of *Ceraeochrysa claveri* was characterized in details, revealing the common constitution observed in Neuroptera, 2n=12,XY. For the group it is the first time that the heterochromatin distribution is described, with occurrence of pericentromeric blocks. The mapping of major ribosomal DNA (rDNA) was divergent from the other few neuropteran species studied, evidencing dynamism for this cluster in the group. Concerning the satellitomes, additionally for *Ceraeochrysa claveri* and *Chrysopa pallens* we mapped the satDNAs using chromosomal mapping through Fluorescence *in situ* Hybridization (FISH) and CHRISMAPP, respectively. The impact of satDNAs on the genome organization of neuropterans is variable, as distinct number of satDNAs were identified from a single satDNA to 23 satDNA families. Moreover, the total amount of satDNAs was also quite variable representing from about 2.5% to 21.55% of the genome. Interestingly, we observed a high turnover of the satDNA library between species, suggesting the frequent emergence and total elimination of repeats independently, contrary to the library hypothesis. The physical chromosome mapping of these repeats in *C. claveri* and *C. pallens* provided detailed insights into their genomic distribution, revealing accumulation of main satDNAs on euchromatin. Our data provide initial insights into the genome organization of satellite DNAs (satDNAs) among Neuropterans, contributing to our understanding of theserepeats in insect genomes. These findings will aid future research on genome assembly and the overall evolution of these important species.

Key-words: biological control, cytogenetics, evolution, RepeatExplorer, repetitive DNA

Introduction

Satellite DNAs (satDNAs) are tandemly repeated sequences that play significant roles in the structuration of eukaryotic genomes, affecting their functioning and influencing evolution (Garrido-Ramos, 2017; Lower et al., 2018; Thakur et al., 2021). For example, satDNAs are major components of pericentromeric heterochromatin, impacting centromeric evolution and function (Melters et al., 2013; Sullivan et al., 2017). They can be actively transcribed, modulating gene expression (Pezer et al., 2012; Feliciello et al., 2015), and they play roles in karyotype evolution (Adega et al., 2009; Vieira-da-Silva et al., 2015), hybridization between species (Ferree and Barbash, 2009; Jagannathan and Yamashita, 2021), and the speciation process (Ugarković, 2013; Louzada et al., 2020). These repeats occupy variable portions of the genome, sometimes exceeding 50%, typically forming long arrays. In contrast, transposable elements (TEs) are generally dispersed throughout the genome, constituting the main class of dispersed repetitive DNA (Charlesworth et al., 1994; Biscotti et al., 2015; Garrido-Ramos, 2017).

For a long time, satDNAs have been studied using classical molecular techniques and cytogenetic tools. More recently, advances in genome sequencing and the development of computational tools for repeat analysis have allowed deeper characterization of this genomic fraction (Garrido-Ramos, 2017; Lower et al., 2018). Through low-coverage sequencing of short reads and analysis using RepeatExplorer2/TAREAN (Novák et al. 2020), it is possible to identify nearly all satDNAs in a genome (Novák et al., 2010, 2013, 2017), collectively referred to as the "Satellitome" (Ruiz- Ruano et al., 2016). This type of analysis, in combination with physical chromosome mapping, has facilitated advancements in understanding the structure and evolution of satDNA repeats across a broad spectrum, including multiple species. Using this strategy,significant progress has been made in understanding the

structural and evolutionary roles of satDNAs in several insect orders, such as Orthoptera, Coleoptera, Lepidoptera, Hymenoptera, and Diptera (e.g., Palacios-Gimenez et al., 2017; Cabral-de-Mello et al., 2021; Pereira et al., 2021; de Lima and Ruiz-Ruano, 2022; Rico-Porras et al., 2024). Conversely, in other insect orders, such as Neuroptera, satDNAs have been studied usingclassical methods and remain poorly understood or even unknown, such as in Neuroptera(Palomeque and Lorite, 2008).

Neuroptera, an order of insects found worldwide, encompasses a diverse group with about 5,800 described species grouped in 15 families, ranking as the sixth largest order among the 11 Holometabolous insects (Oswald and Machado, 2018). Among these, the family Chrysopidae, commonly known as green lacewings, is a cosmopolitan and extensive family comprising 1,415 described species across 81 genera. Due to their predation on eggs and various soft-bodied arthropods, different green lacewing species have been utilized in biological pest control within various agroecosystems (Freitas and Penny, 2001; McEwen et al., 2001; Pappas et al., 2011). One notable species is *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae), a polyphagous predator commonly found in agroecosystems in the Neotropical region (Albuquerque et al., 2001; Freitas and Penny, 2001).

Although highly important for their role in preying on pest arthropods in agricultural systems (Stelzl and Devetak, 1999), neuropteran insects have been largely ignored at the chromosomal and genomic levels. Advances in these areas could be beneficial in multiple aspects, as understanding genomic structure can contribute to crop protection (Hodjat, 2022; King et al., 2023). Chromosomal studies have primarily described the number and sex chromosomes of only a few species. For example, in the most diverse family, Myrmeleontidae (1,659 species), only 38 representatives have been karyotyped (Kuznetsova et al., 2015). In Chrysopidae (1,415 species), about 30 species

have been studied (Asana and Kichijo, 1936; Kichijo, 1934; Hirai, 1957; New, 1984; Páez et al., 2020; Acevedo et al., 2024), and in Ascalaphidae (431 species), 8 species have been karyotyped (Kuznetsova et al., 2015). Mapping of repetitive DNAs is limited to four species in the families Myrmeleontidae and Ascalaphidae, where 18S rDNA and telomeric repeats were mapped (Kuznetsova et al., 2016). The first genome of a neuropteran, *Chrysopa pallens*, was assembled at the chromosome level in 2021 (Wang et al., 2021), revealing that repetitive sequences comprise 38.31% of its genome. However, most of these sequences remained unclassified due to a lack of repetitive sequence studies in Neuropterida. For other sequenced genomes, the repetitive fraction has not been studied, with only the genome repeat length described (Wang et al., 2021).

Here we advance in the understanding of genomic structure and evolution for the satDNAs in Neuroptera, using as source related species of Chrysopidae. Based on an integrative analysis of genomic and bioinformatic approaches we prospected and characterized the satellitomes of eight species and analyzed them in a comparative manner and in a phylogenetic context. Moreover, we advance on the characterization of the genome structure of *C. claveri* using a complementary analysis by physical chromosome mapping of the prospected repeats. Our data revealed distinct number of satDNA families, depending of the species, contributing to advance in the understanding of satellitomes as insects a whole.

Material and Methods

Genomes database and analysis of satellitomes in Chrysopidae species

Adult insects and eggs of *C. claveri* (Chrysopinae) were obtained from a laboratory colony of the Institute of Bioscience of Rio Claro at UNESP, Brazil. This species was our start point of analysis due the availability of alive material for chromosome obtaining. In

this way, head and pronotum of five adult males were used for genomic DNA (gDNA) extraction using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacture´s protocol. Through Illumina HiSeq platformusing the service of Novogene (Sacramento, CA, USA), paired-end reads from a male genome of *C. claveri* were obtained. It was provided a total of 1 Gb of sequencing data of 150 bp. Additionally, in order to have a broader scenario about satDNAs onthe genomes of Neuroptera, seven other species Chrysopidae were selected for the analysis, considering the phylogenetic hypothesis for the family (Wang et al., 2021). This included other representatives of Chrysopinae, *Chrysopa pallens*, *Chrysoperla frurcifera*, *C. zastriwi sillemi*, *C. carnea*, and *Italochrysa pardalina*; one Apochrysinae, *Apochrysa matsumurae*; and one Nothochrysinae, *Nothochrysa sinica*. The accession numbers for the studied genomes are given on **Table 1**.

The raw reads were uploaded, processed, filtered, and interlaced using the tools available on Galaxy/RepeatExplorer2 (https://repeatexplorer-elixir.cerit-sc.cz/) (pre-processing) and after the RepeatExplorer2/TAREAN (Novák et al. 2017; 2020) was run. The 0.001% parameter for cluster size was selected, while for the other parameters the default option was set. Besides the satDNAs identified by TAREAN we additionally checked the fifty more abundant clusters checking the occurrence of spherical or star-like graphs in the searching of satDNAs, in the output of RepeatExplorer2.

All identified satDNAs were analyzed through BLAST against each other (blast all-to-all) to check possible homologous. The all satDNA monomers were analyzed though BLAST in NCBI and masked in Repbase/CENSOR (Bao et al., 2015) to verify similarity with previously described sequences, mainly focused on TEs. The RepeatMasker software was run to assess the abundance and divergence of satDNAs repeats, utilizing a total of 1,000,000 randomly

selected reads and using as query dimers of each satDNA or at least 200 bp for short monomers. The abundance of each satDNAswas normalized based on the number of base pairs used as input (150,000,000 bp). For divergence estimation based in Kimura 2-parameter (K2P) distances, the script 'calcDivergenceFromAlign.pl' from the RepeatMasker suite (Smit et al., 2017) was run.

Chromosome obtaining of *Ceraeochrysa claveri*

Adult insects and eggs of *C. claveri* were obtained from a laboratory colony of the Institute of Bioscience of Rio Claro at UNESP, Brazil. Head and pronotum of five adult males were used for genomic DNA (gDNA) extraction using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacture´s protocol. While the eggs were used to obtain chromosomal preparations from embryos with about 30-35 of development, following the protocol described by Webb et al. (1978) with modifications. Embryos were transferred to a microtube containing 100 µL of colchicine solution (0.02% in NaCl 0.9%) and homogenized by pipetting. After 30 minutes 600 µL of hypotonic solution (KCl 0.75 M) was added for 5 minutes. The next step consisted of fixation using 600 µL Carnoy´s modified solution (3:1, 100% ethanol:glacial acetic acid) being the material resuspended by pipetting. The tube was centrifugated at 5,000 rpm for 3 minutes and the supernatant was discarded. Finally, the material was resuspended by pipetting in 100 µL of 50% glacial acetic acid. The slides were prepared by adding drops of this final solution in heated slides at 42 ºC. Slides for conventional analysis were stained with Giemsa 5% or with DAPI (4',6-diamidino-2-phenylindole). For heterochromatin location we used the protocol for C-banding from Sumner et al. (1972).

Chromosomal location of repeats

The ten most abundant satDNAs were amplified through PCR from the genome of *C. claveri* using specific primers, while the 18S rDNA was amplified from *Diatraea saccharalis* (Lepidoptera) using the primers Sca18SF (5' CCC CGT AAT CGGAAT GAG TA) and Sca18SR (5' GAG GTT TCC CGT GTTGAG TC) (Cabral-de-Mello et al., 2011). The insect telomeric repeat $(TTAGG)_n$ was amplified through a non-template PCR using the primers $(TTAGG)_5$ and $(CCTAA)_5$, following the protocol of Ijdo et al. (1991). For chromosomal mapping of satDNAs, telomeric probe and 18S rDNA we followed the protocol outlined by Cabral-de-Mello and Marec (2021). The probed were labeled with digoxigenin-11-dUTP (Roche, Mannheim, Germany) biotin-16-dUTP (Sigma, St Louis, MO, USA) through Nick-Translation, using using the DNA Polymerase I/DNase I mix (Invitrogen, San Diego, CA). The digoxigenin labelled probes were detected using anti-digoxigenin-rhodamine (Roche), while the biotin labelled probes were detected using Alexa Fluor 488-conjugated streptavidin (Invitrogen). The chromosomes were counterstained with DAPI and mounted in VECTASHIELD (Vector, Burlingame, CA, USA). Images of the hybridized chromosomes were captured using a CCD digital camera (Olympus DP71) coupled to the Olympus BX51 fluorescence microscope equipped with appropriate filter sets (Olympus, Hamburg, Germany). The images were pseudocoloured, merged to combine chromosomes and probe signals and processed using Adobe Photoshop® CS6 (Adobe Systems, San Jose, CA, USA).

Additionally, acknowledged to the availability of the chromosome level assembled genome for *C. pallens*, we mapped the satDNAs identified for this species on its pseudochromosomes using the CHRISSMAPP approach, following Rico-Porras et al., (2024).

Results

General structure of *Ceraeochrysa claveri* karyotype

The karyotype of *C. claveri* was constituted of five pairs of autosomes and XY sex system, 2n=12,XY with all chromosomes telocentric, but being possible to identify a extremely small short arm (**Figure 1a**). The general analysis of repetitive DNAs distribution revealed the heterochromatin occupying the pericentromeric regions of all chromosomes (**Figure 1b**). The mapping of 18S rDNA revealed occurrence of two largeclusters on the X chromosome, one located on the short arm and one on the proximal region of long arm. Additionally, small clusters were observed on the short arm of the Ychromosome and the third chromosome pair of autosomes (**Figure 1c**).

Satellitome analysis of eight Chrysopidae species

Using the RepeatExplorer2/TAREAN analysis it was possible the identification of the most abundant satDNAs of each species. The general characteristics of the satellitomes were highly variable between the species. Depending on the species variable number of reads were analyzed by RepeatExplorer2/TAREAN. The number of satDNAs found in the species varied from 1 to 23, observed in *C. z. sillemi* and *Nothochrysa sinica*, respectively. For *C. furcifera* and *A. matsumurae* no satDNAs were identified. Analysis of homology revealed no homologous satDNAs between the species. The monomer size of satDNAs varied from only 07 bp in *C. claveri* to 5,796 bp (*I. pardalina*), while the mean size between the species ranged from 165 bp to 1,870, both species belonging to Chrysopinae subfamily, *C. z. sillemi* and *I. pardalina*. In all species the satDNAs were A+T-rich, ranging from 50% to 81.8%. The lowest mean enrichment of A+T was observed in *C. pallens* with 67.93% and the highest was observed in *C. z. sillemi* with 73.9%.

Concerning the abundance, quite divergent numbers were observed, with variation from solely about 2.5% of the genome in *C. pallens* and *N. sinica* to 21.55% in *I. pardalina*. In all species the lowest abundant satDNA represent less than 0.005% while the most abundant was variable from 0.62% in *N. sicica* to more than 10% of the genomes in *C. z. sillemi* and 17% in *I. pardalina*. Coincidently these two species correspond to representatives with highest amount of satDNAs among the studied species. Generally, the satDNAs presented low divergence K2P values, around 10% in all species. The lowest mean divergence is from *N. sinica* (K2P 7.33%) and the highest in in *C. z. sillemi* (K2P 13.69%). Individually, the less divergent satDNA was noticed in *C. pallens* with K2P of 1.71% and the highest divergent was evidenced in *I. pardalina* with K2P of 28.06%. The specific characteristics for each satDNA could be seen in **Table 1**.

The mapping of satellite DNAs on the chromosome of *Ceraeochrysa claveri* and *Chrysopa pallens*

The satellite repeats were mapped on the chromosomes through two distinct strategies. The FISH mapping was done in *C. claveri* as we had chromosome preparations available. While in *C. pallens* the satDNAs were mapped through CHRISMAPP on assembled chromosomes.

In *C. claveri* contrasting patterns were observed for chromosomal distribution of the satDNAs, as follows: The CclaSat01-744, CclaSat02-315, and CclaSat09-206 were spread along all chromosomes mostly on euchromatin of all chromosomes, being the signals highly abundant for CclaSat01-744. The CclaSat02-315 presented signals along the entire chromosomes and for CclaSat09-206 only few signals were noticed in all chromosomes. The other satDNAs presented discrete blocks, varying in number and positions. Three satDNA families, including CclaSat03-, CclaSat04-, and CclaSat06-

were present in all chromosomes (**Figure 2c,d,f**), being the last two placed on the short chromosomal arms (**Figure 2d,f**), while the CclaSat03- places on the pericentromeric blocks, coinciding with the heterochromatin. Additionally, CclaSat03- presented a terminal signal on the long arm of chromosome X (**Figure 2c**). The CclaSat05- was exclusive for the sex chromosomes, located on pericentromeric area of the Y chromosome and on the short arm of X chromosome (**Figure 2e**). For CclaSat10-, the signals were restricted for the three largest autosomal pairs, placed on the short chromosomal arms (**Figure 2i**). Small located and discrete dots were noticed for the satDNA Ccla07- in all autosomes, with variable positions, including proximal, interstitial, and terminal (**Figure 2g**). Finally, for the satDNA CclaSat08- no signals were visualized (data not shown).

For *C.pallens* through CHRISMAPP on assembled genome we were able to map the satDNAs in the species. Among the satDNAs successfully mapped the CpalSat03- revealed extensive distribution along all chromosomes, except the Y chromosome. Although we do not have information about the location of heterochromatic blocks in the species the distribution of this repeat covered full chromosomes, including large blocks, evidencing accumulation, resembling heterochromatic areas. The other satDNAs presented only scattered mapping, being the CpalSat02-, CpalSat04-, CpalSat05-, CpalSat06-, CpalSat07-, CpalSat11- more abundantly mapped, than the others. Remarkably it was observed for these satDNAs more enrichment on the chromosomes 1 and 2. The other satDNAs revealed only few areas of mapping, even the CpalSat01- that is the most abundant in the species. It could be due the collapsing on the genome assembly of these repeats (**Figure 3**).

Discussion

General structure of karyotype and repetitive DNAs in *Ceraeochrysa claveri* is very similar to other neuropterans

In Neuroptera, the karyotypes were described only for few species, including representatives of the family Chrysopidae, revealing in most of them the same diploid number observed for *C. claveri*, that is the modal for the family and could be the ancestral number for the subfamily Chrysopinae in which *C. claveri* belongs (Asana and Kichijo, 1936; Kichijo, 1934; Hirai, 1957; New, 1984; Páez et al., 2020; Acevedo et al., 2024). From a karyotype composed of 2n=12 distinct chromosome numbers arose by chromosomal rearrangements, including fusions and fissions, explaining the chromosomal variability observed in the group, with diploid number ranging from 10 to 26 (Blackmon et al. 2017). Concerning the morphology of the chromosomes, most Neuroptera, including *C. claveri* also presents exclusively telocentric chromosomes, but some species with biarmed chromosomes were also reported, putatively as result of inversions or due to occurrence of fusions. The sex system formed by XY chromosomes as noticed for *C. claveri* is highly predominant in neuropterans, studied to date, the only exceptions are two species with X0 and one species with multiple sex system (Asana and Kichijo, 1936; Hirai, 1957; Kuznetsova et al., 2015; Blackmon et al. 2017). This suggests extreme stability for the general organization of the sex system in the group, compared to autosomes, with uncommon involvement in large chromosomal rearrangements, as reported to autosomes. It is important to highlight that the study of more species belonging to Neuroptera family are important to shed more precise light on the patterns of chromosomal evolution, that is poorly know in comparison to other insect groups.

Although in insects the use of rDNA genes has been extensive applied as chromosomal marker to understand the evolution of karyotypes and genomic organization (Cabrero and Camacho 2008; Nguyen et al., 2010; Cabral-de-Mello et al., 2011; Souza-Firmino et al., 2020; Cunha et al., 2023), in Neuroptera the location of major rDNA clusters was reported only in three species, with occurrence of exclusively autosomal sites or on autosomes plus the X chromosome (Kuznetsova et al., 2016). These patterns are contrasting to the observed in *C. claveri* as the rDNA clusters are on both sex chromosomes and in one autosomal pair. These preliminary data suggests that at least the rDNA location could be highly dynamics in Neuroptera, as observed in other insects, instead of macro-chromosomal structure conservation. Kuznetsova et al. (2016) suggested that in antlions a single chromosome pair carrying the major rDNA could be the ancestral state. Concerning the mapping of "insect type" telome repeat, i.e. $(TTAGG)_n$, no signals on chromosomes were observed, suggesting that this repeat is absent in this species, or in low, hampering the detection through FISH. The loss of "insect type" is common among insects (Frydrychová et al., 2004) and it was also reported in other two Neuroptera species, including the Chrysopidae *Chrysoperla carnea* (Frydrychová et al., 2004), but in other species belonging to Ascalaphidae and Myrmeleontidae this motif was identified (Kuznetsova et al., 2016; 2019), evidencing distinct ways for telomere organization in the group.

Satellitome analysis reveals first insights about organization of satellite DNAs in Neuroptera

The satellitome analysis using bioinformatic approach have been conducted in some groups of insects, as Orthoptera, Coleoptera, Hymenoptera, Hemiptera, Diptera, and Lepidoptera (e.g. Palacios-Gimenez et al., 2017; Mora et al., 2020; Cabral-de-Mello et al., 2021; Pereira et al., 2021; 2023; Camacho et al., 2022; de Lima et al., 2022;

Gasparotto et al., 2022; Montiel et al., 2022; Cabral-de-Mello et al. 2023; Mora et al., 2023; Rico-Porras et al., 2024). Although, for Neuroptera or closely sister orders, as Raphidioptera, Megaloptera, and Strepsiptera (Misof et al., 2014), no descriptions were reported, even for single satDNAs. In this way, our data adds new insights about satDNA organization in insects.

Some of the general characteristics of satDNAs the eight species of Neuroptera studied here are similar to other insects, including enrichment of the A+T content (Palomeque and Lorite, 2008) and high variability of the monomer sizes (Pereira et al., 2021; Anjos et al., 2023; Šatović-Vukšić and Plohl, 2023; Rico-Porras et al., 2024). Specifically for *C. pallens* the completely assembled genome identified that 38.31% of its genome is composed of repeats. But as annotated here, only a small portion this repetitive fraction is composed by satDNAs (2.5%), suggesting that other repeats, putatively dispersed ones are main component of *C. pallens* genome. Although we do not have the total amount of all repeats in the genomes of the other species, it is evident that the satDNAs contribute differentially for genome organization between species, as high different abundances were noticed, from 2.5% to 21.55%. Great divergence for amount of satDNAs were reported for example in sister species of mammals (Sena et al., 2020), fish (Utsunomia et al., 2019; Crepaldi et al., 2021), and bees from the genus *Melipona* (Pereira et al., 2021). This is in accordance with the library hypothesis that predicts mainly quantitative changes of the satDNA families between related species (Fry and Salser, 1977). On the other hand, contracting the Library Hypothesis the species did not share the satDNA repeats, an unexpected pattern. The species presented exclusive satDNAs, including the species belonging to the same genus. This indicates high turnover of satDNA library in Neuroptera, by rapid modification of satDNAs among species, evidencing also the possibility of birth of new satDNAs after speciation process.

Divergently to other most insects in which most abundant satDNAs are enriched on heterochromatin areas, mainly centromeres of monocentric species, the two most abundant satDNAs in *C. claveri* are accumulated on euchromatin. Similarly, for *C. palllens* euchromatic distribution was also probable, as indicated the CHRISMAPP. In general, the euchromatic satDNAs are less in abundance in comparison to heterochromatic ones. Although more common on heterochromatin, the occurrence of satDNAs on euchromatin have been identified in multiple insects, as in the beetle *Chrysolina americana* in which most part of satDNA families are euchromatic (Rico-Porras et al., 2024) and in the hemipteran *Oxycarenus hyalinipennis* with the all 26 satDNA families spread virtually on the entire extension of euchromatin, irrespective of their abundance (Cabral-de-Mello et al., 2023). In *Drosophila melanogaster* the Rsp-like and 1.688 g/cm^3 satDNA families (Sproul et al., 2020) are also euchromatic, as well as in the beetle *T. castaneum* nine satDNAs predominantly reside on euchromatin, and some satDNA families are distributed on euchromatin of grasshopper (Milani et al., 2021), other beetles (Montiel et al. 2021), and bugs (Pita et al., 2017). These data challenge the traditional idea that euchromatin is depleted in satDNAs. *Ceraeochrysa claveri* is an exceptional case as even the two most abundant satDNA families, that corresponds to about 41% of the satDNA content, are laced on euchromatin.

Conclusions

Overall, our data gives the first insights about the genome organization of satDNAs among Neuropterans, contributing to the understanding of organization and evolution of these repeats on insect genomes. Although some characteristics of the Neuroptera satellitome falls on the common placement among insects, the euchromatic satDNAs is less common and challenges the idea of enrichment of this type of sequence only in

heterochromatin. The impact of satDNAs on the composition and organization of Neuroptera genomes is quite variable, even in related species. Additionally, we observed high turnover of satDNA library, by extensive emergence or elimination of repeats independently, on the contrary of the library hypothesis ideas. This data also contributes to the future works dealing the genome assembly and understanding of global genome evolution of these important species.

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Table

Table 1. General characteristics of satellite DNAs prospected in Neuroptera species genomes.

Species	Number of satDNA	Mean monomer size	Monomer size variation	Mean A+T	A+T variation	Total abundace	Abundance range	Mean divergence	Divergence range
Chrysopinae									
Ceraeochrysa claveri	22	200 bp	07 - 744 bp	70.59%	54.9% - 77.7%	5.90%	0.0048% - 1.47%	8.73%	2.60% - 16.73%
Chrysopa pallens	21	728 bp	18 – 4,742 bp	67.93%	50.0% - 81.8%	2.50%	0.0027% - 0.79%	8.23%	1.71% - 22.76%
Chrysoperla carnea	10	301 bp	21 - 697 bp	68.62%	60.0% - 76.1%	3.71%	0.0047% - 2.68%	9.88%	3.60% - 22.68%
Chrysoperla furcifera	-	-	-	-	-	-	-	-	-
Chrysoperla zastrowii sillemi	01	165 bp	-	73.9%	-	10.08%	-	13.69%	-
Italochrysa pardalina	09	1.870 bp	122 – 5,796 bp	66.01%	55.5% - 76.6%	21.55%	0.0047% - 17%	12.38%	3.94% - 28.06%
Apochrysinae									
Apochrysa matsumarae	-		-	-	-	-	-	-	-
Nothochrysinae									
Nothochrysa sinica	23	352 bp	33 – 3,090 bp	69.40%	57.5% - 81.3%	2.54%	0.0025% - 0.62%	7.33%	2.11% - 22.33%

Figures

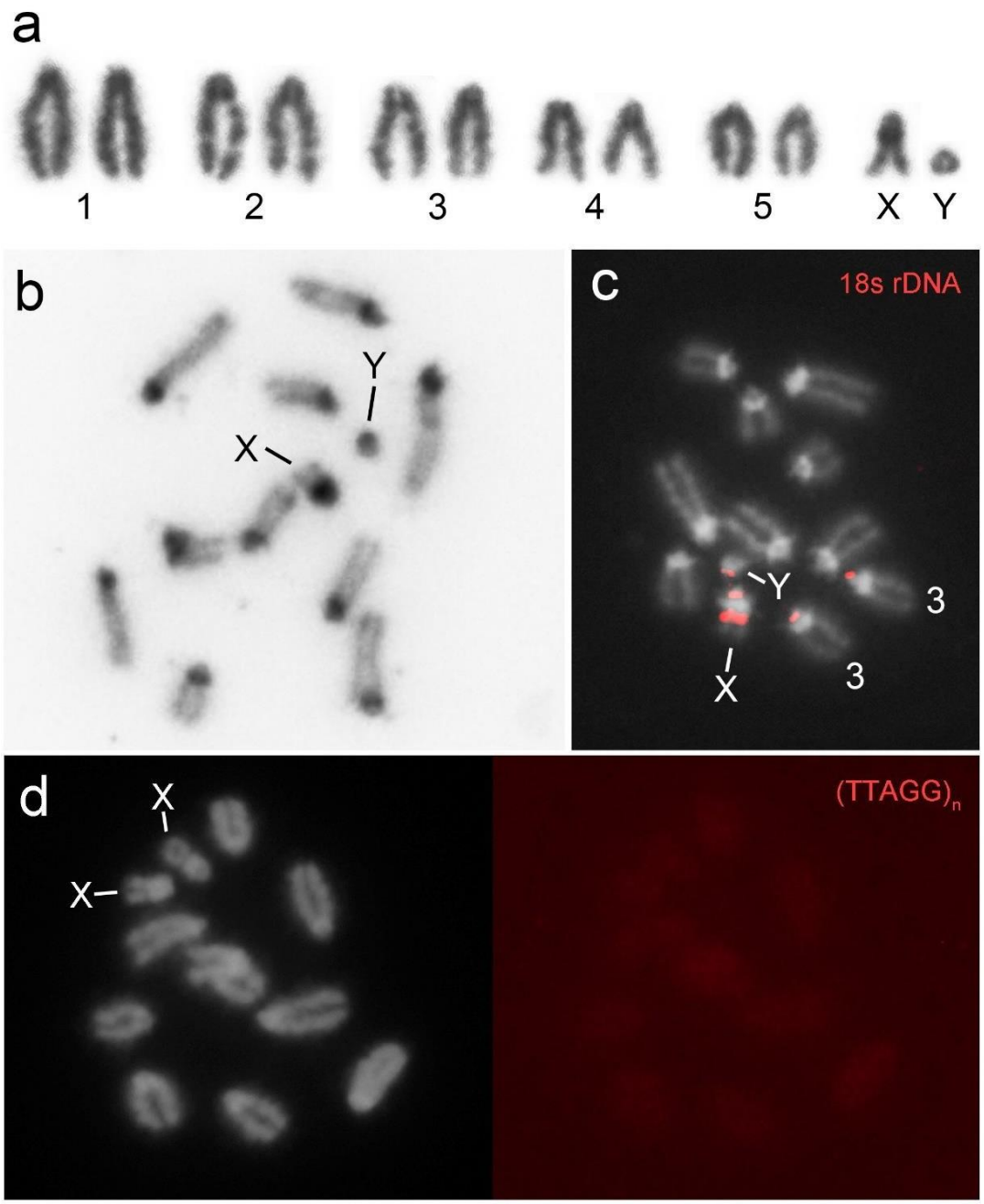

Figure 1. General characteristics of the karyotype of *C. claveri*. (a) Karyotype from a male mitotic metaphase stained with Giemsa 5%, 2n=12,XY. (b) C-banding showing the occurrence of pericentromeric heterochromatin in a mitotic metaphase from a male. (c) FISH mapping of 18S rDNA in a mitotic metaphase from a male, showing rDNA clusters in the chromosome 3 and also in the X and Y chromosomes. (d) FISH experiment using as probe the telomeric repeat (TTAGG)n, revealing absence of signals.

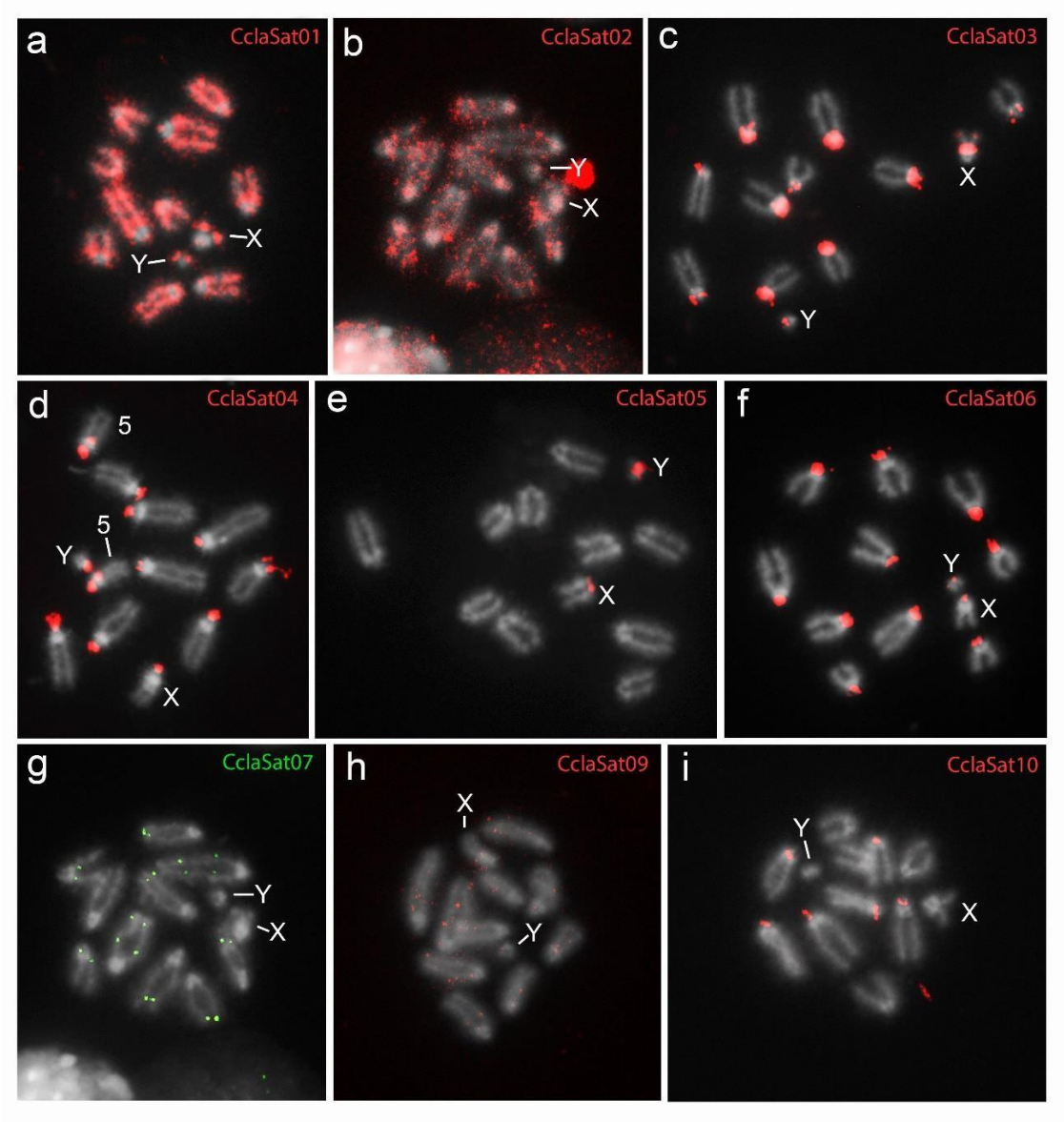

Figure 2. FISH mapping of the abundant satDNAs in the mitotic metaphase of males of *C. claveri*. The probes are directly indicated in the images.

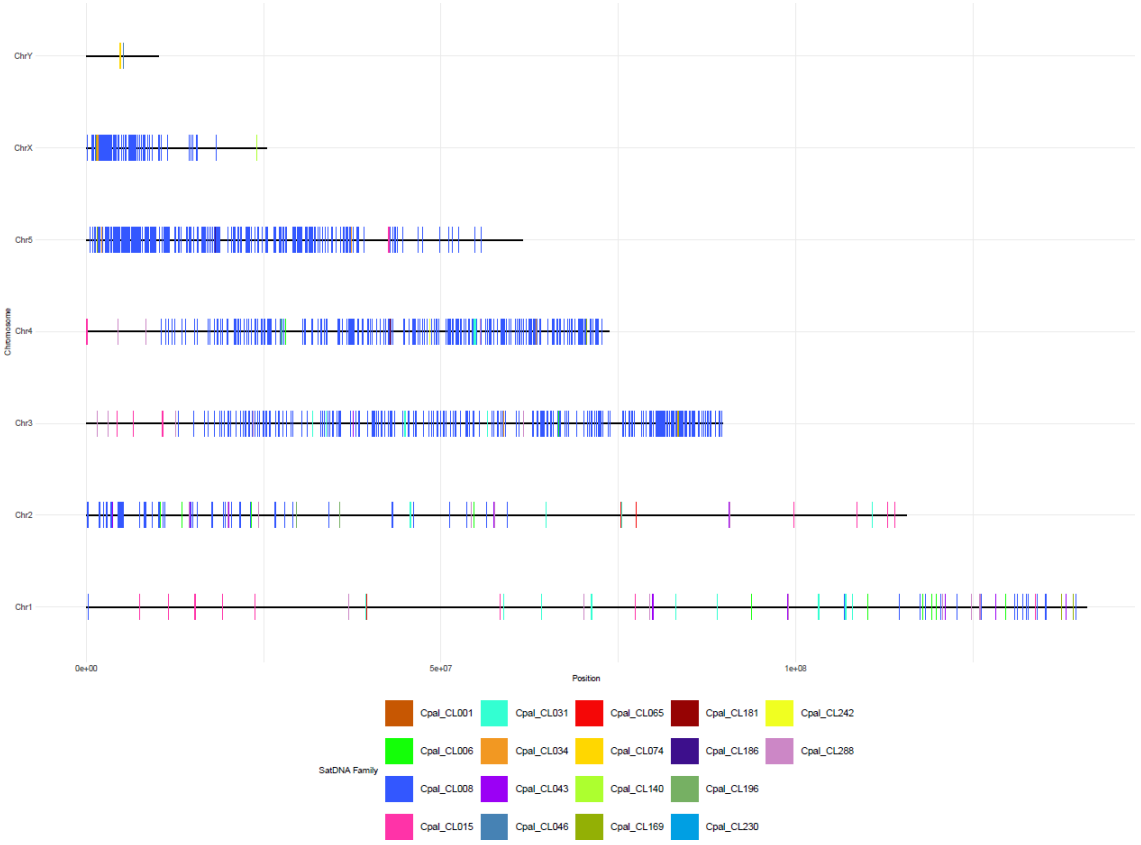

Figure 3. Chromosomal distribution of satellite DNAs in *C. pallens* revealed by CHRISSMAP. Note the accumulation in putative euchromatic areas.

5. Conclusões Gerais

Os estudos citogenéticos em Ceraeochrysa claveri evidenciaram um número cromossômica similar ao relatado para a maioria das espécies do grupo. Entretanto, a localização diferencial do sítio de 18S DNAr exibiu a plasticidade genômica de C. Claveri. Um cenário similar foi observado quanto a prospecção dos DNAs satélites em C. Claveri em comparação com outras espécies de Chrysopinae, *Chrysopa pallens*, *Chrysoperla frurcifera*, *C. zastriwi sillemi*, *C. carnea*, and *Italochrysa pardalina*; bem como com representantes de Apochrysinae, *Apochrysa matsumurae*; e Nothochrysinae, *Nothochrysa sinica*. Tais variações foram observadas tanto no número de famílias de DNA satélite quanto na abundância dos DNAs repetitivos dentro de cada grupo, mostrando uma acentuada diferenciação no grupo, sendo esse devido aos eventos de amplificação ou redução característicos aos DNAs satélites. Apesar disso, os diferentes DNA satélites encontrados em foram recorrentes em regiões eucromáticas tanto em C. claveri quanto em C. pallens, reforçando assim, a distribuição não restrita dessas repetições apenas na região heterocromática. De modo geral, os neurópteros mostraram-se como um grupo propício à exploração dos DNAs satélites, visto a riqueza de diversidade observados na primeira prospecção dessas regiões repetitivas no grupo.

6. Referências bibliográficas (texto principal)

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