

Satellite DNA dynamics following the neo-Y chromosome formation in the electric fish *Eigenmannia* sp. 2 (Teleostei, Gymnotiformes)

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Abstract

Fish sex chromosomes undergo frequent turnover, and the genus *Eigenmannia* illustrates this dynamism through multiple, apparently independent sex chromosome systems described across closely related taxa. In *Eigenmannia* sp. 2, a fusion between an ancestral XY sex chromosome pair and an autosome produced a neo-Y chromosome within an X1X1X2X2/X1X2Y multiple sex chromosome system, but the repetitive DNA content and molecular differentiation of this recently derived chromosome have remained uncharacterized. Here, we characterized the satellite DNA (satDNA) repertoire of *Eigenmannia* sp. 2 using male and female genomic short-read libraries, identifying 166 satDNA families, 91% of which grouped into 23 superfamilies, supporting diversification from a shared ancestral pool under the satellite library model. Comparison of genomic abundance between sexes identified 17 families with at least a two-fold sex bias. Fluorescence in situ hybridization of eight representative families showed that four occupy multiple pericentromeric loci across the karyotype, including X1 and/or X2, yet none produced a detectable signal on the neo-Y. Repeat landscape analysis further showed that three of these families remain largely homogeneous and show low sequence divergence elsewhere in the genome, arguing against a simple failure to detect diverged copies on the neo-Y. Together, these results indicate that neo-Y formation in *Eigenmannia* sp. 2 involved remodeling of its satDNA content, consistent with loss or rapid divergence of repetitive sequences at or shortly after the fusion event, and provide the first satDNA-level view of sex chromosome differentiation in this genus.

Introduction

Fish sex chromosomes undergo frequent turnover, switching between systems and even between the chromosome pairs that carry sex determination (Wang et al. 2024). This evolutionary dynamism is especially evident in *Eigenmannia* (Teleostei: Gymnotiformes: Sternopygidae), where multiple, apparently independent sex chromosome systems have been described across closely related taxa. Even populations assigned to the same species complex may exhibit distinct configurations, including male heterogamety, female heterogamety, and multiple sex chromosome systems (Almeida-Toledo et al. 2000; Henning et al. 2008, 2011; Silva et al. 2009; Araya-Jaime et al. 2017; Fernandes et al. 2020; de Araújo et al. 2023). The extent of sex chromosome diversity within *Eigenmannia* is likely still underestimated, given the unresolved taxonomy of the genus and evidence that several nominal species comprise complexes of cryptic lineages (Cardoso and Dutra 2023). Together, these patterns highlight *Eigenmannia* as a particularly informative system for investigating the chromosomal processes underlying the origin and diversification of sex chromosome systems.

The taxonomy of *Eigenmannia* remains incompletely resolved, and several karyotypically distinct lineages cannot yet be confidently assigned to a described species. To allow consistent comparison across studies, researchers have used informal, sequentially numbered designations (e.g., *Eigenmannia* sp. 1, sp. 2, sp. 3) for these lineages based on diploid number, karyotype structure, and sex chromosome system, independently of their formal taxonomic status (Almeida-Toledo et al. 1984, 2000; Henning et al. 2008, 2011). *Eigenmannia* sp. 2 refers to the lineage with $2n = 31$ (X_1X_2Y , males) and $2n = 32$ ($X_1X_1X_2X_2$,

females), first described by (Almeida-Toledo et al. 1984) and used consistently under this name ever since; we retain it here for comparability with this literature, as formal taxonomic revision of the lineage is still pending (Dutra et al. 2024).

Within this context, the $X_1X_1X_2X_2/X_1X_2Y$ system of *Eigenmannia* sp. 2 represents a particularly informative example of sex chromosome turnover. This system originated through a chromosome fusion event in which an ancestral XY sex chromosome fused with an autosome, giving rise to a neo-Y chromosome (Almeida-Toledo et al. 1984; Henning et al. 2008). Although previous studies established the chromosomal origin of the multiple sex chromosome system, the molecular evolution of the sequences carried by the fused chromosomes remains largely unexplored. Consequently, the repetitive DNA landscape and molecular differentiation of the neo-Y chromosome of *Eigenmannia* sp. 2 remain essentially uncharacterized.

Satellite DNAs (satDNAs) provide an informative framework for investigating this process. After recombination is suppressed or reduced during sex chromosome differentiation, repetitive sequences may show independent patterns of amplification, contraction, divergence, and loss between homologous chromosomes, contributing to progressive sex chromosome differentiation (Charlesworth et al. 2005). SatDNAs consist of tandemly repeated sequences that are frequently enriched in centromeric, pericentromeric, and other heterochromatic regions (Garrido-Ramos 2017), and their abundance and sequence composition are shaped by mechanisms including unequal crossing-over, gene conversion, and transposition (Dover 1986; Plohl et al. 2012). Accordingly, comparisons of satDNA composition between sexes, or between chromosomes involved in a fusion and their unfused homologs, can provide insight into the repetitive sequences that have been retained, amplified, reduced, or lost during sex chromosome differentiation.

Comparative satellitome analyses have revealed that sex chromosome differentiation is frequently accompanied by substantial changes in the abundance and chromosomal distribution of satDNAs. In Neotropical fishes, several studies have identified satDNA families showing marked sex-biased abundance or preferential accumulation on heteromorphic sex chromosomes. Such patterns have been reported in the multiple sex chromosomes of *Arhinolemur elongatus* (Crepaldi and Parise-Maltempi 2020), *Pyrrhulina semifasciata* (de Moraes et al. 2023), *Hoplias malabaricus* (Toma et al. 2024), and in five *Harttia* species (Deon et al. 2024; Sassi et al. 2025). Remarkably, despite the homologous sex chromosome pairs in Amazonian *Harttia* species, each lineage accumulated a distinct repertoire of sex-linked satDNAs, highlighting the rapid and lineage-specific dynamics of these sequences during sex chromosome differentiation (Sassi et al. 2025). Whether similar changes in satDNA composition accompanied the origin and subsequent differentiation of the multiple sex chromosome system of *Eigenmannia* sp. 2 remains unknown.

Here, we investigated the evolutionary dynamics of repetitive DNAs associated with the X_1X_2Y sex chromosome system in *Eigenmannia* sp. 2. By comparing male and female satellitomes and mapping sex-biased satDNA families, we aimed to determine whether the formation of the neo-Y was

accompanied by specific changes in its repetitive DNA landscape. We discuss whether satDNA families retained on the ancestral X chromosomes but absent from the neo-Y provide evidence of sequence loss or remodeling associated with the fusion event that originated the neo-Y.

Our analyses reveal several satDNA families that occur on the X_1 and/or X_2 chromosomes but are undetectable on the neo-Y. This distribution points to a remodeling of the repetitive DNA landscape following the formation of the neo-Y and suggests that these sequences were either lost or underwent sufficient sequence divergence to become no longer recognizable during the subsequent differentiation of the fused chromosome.

Materials and Methods

Sample collection

Ten male and ten female specimens of *Eigenmannia* sp. 2 were collected from Rio Batalha, Bauru, São Paulo (20°22'10.4"S 51°20'16.0"W). All animals were collected with the authorization of the Brazilian Environmental Agency ICMBIO/SISBIO license (License number 97277-1). The procedures used for the sampling, maintenance and analysis of the fishes were performed in compliance with the standards followed by the Ethics Committee on the Use of Animals of the Universidade Estadual Paulista (CEUA 716/2023).

Mitotic chromosomal obtainment and DNA extraction

Cell suspensions containing mitotic metaphases were obtained following the protocol of Foresti et al. (1981). Briefly, this method consists of inhibiting microtubule polymerization with colchicine, subjecting cells to hypotonic treatment, and fixing them in methanol:acetic acid solution. Twenty microliter drops of the resulting cell suspension were placed onto clean, dry glass slides and air dried. Slides were stained with 5% Giemsa solution in phosphate buffer (pH 6.7) for 10 minutes.

Following the initial cytogenetic analyses, genomic DNA samples from males and females of *Eigenmannia* sp. 2 specimens were extracted from muscle tissue fragments using the NucleoSpin® Tissue Kit (Columns and 2-mL Collection Tubes). The extracted DNA was treated with RNase A (20 mg/mL; Invitrogen) to remove residual RNA, following the manufacturer's instructions.

DNA sequencing

Genomic DNA from one male and one female individual was sequenced by BGI using the BGISEQ-500 platform with 150-bp paired-end reads. Approximately 2.4 Gb of sequencing data were generated per individual. The raw sequencing data are available in the NCBI Sequence Read Archive (SRA) under accession numbers SRR40283535 (male) and SRR40283534 (female).

Bioinformatic analyses and characterization of *Eigenmannia* sp. 2 satellitome

Raw sequencing reads were quality filtered and trimmed to remove adapters and low-quality sequences using Trimmomatic (Bolger et al. 2014). The satellitome of *Eigenmannia* sp. 2 was characterized using an iterative approach combining TAREAN (Tandem Repeat Analyzer; Novák et al. (2017) and the satMiner pipeline (Ruiz-Ruano et al. 2016). For each iteration, $2 \times 500,000$ male reads were randomly selected and analyzed with TAREAN using default parameters. Consensus sequences classified as high- or low-confidence satellite DNAs were retained and used to construct a custom satDNA database. Reads showing similarity to the identified satDNAs were subsequently removed from the original libraries using DeconSeq (Schmieder and Edwards 2011), and the remaining reads were subjected to a new round of TAREAN analysis. This procedure was repeated until no additional satDNA families were detected.

Following completion of the iterative searches, the identified satDNA sequences were compared and grouped into families according to the criteria proposed by Ruiz-Ruano et al. (2016), generating the satellitome catalog of *Eigenmannia* sp. 2. SatDNA families were named according to decreasing genomic abundance following the nomenclature proposed by Ruiz-Ruano et al. (2016) and then proceeded to a filtering step to remove multigene families. The abundance and sequence divergence of each satDNA family were subsequently estimated by masking male and female genomic libraries against the satDNA catalog using RepeatMasker, based on subsamples of $2 \times 10,000,000$ reads from each sex. Abundance estimates were compared between males and females to identify satDNA families showing sex-biased representation. For satDNA families subsequently mapped to the sex chromosomes by FISH, individual repeat landscapes were generated from the RepeatMasker outputs to compare their abundance and sequence divergence profiles between males and females.

Primer Design, PCR amplification, and fluorescence *in situ* hybridization (FISH)

Eight satDNA families were selected for cytogenetic mapping using two complementary criteria (Table S1). First, the six most abundant satDNA families with repeat units ≥ 40 bp were selected to characterize the chromosomal distribution of the major components of the *Eigenmannia* sp. 2 satellitome while allowing suitable primer design. In addition, to investigate whether marked differences in genomic representation between the male and female libraries could be associated with the X_1X_2Y sex chromosome system, two further candidates were included. Eigsp2Sat015-162 represented the most abundant female-enriched family showing an approximately twofold difference between libraries ($F/M = 2.02$), whereas Eigsp2Sat030-480 represented the most abundant male-enriched family showing an approximately two-fold difference ($F/M = 0.46$) among repeats suitable for primer design. Three additional female-biased families with markedly higher F/M ratios (Eigsp2Sat161-62, $F/M = 13.7$; Eigsp2Sat165-58, $F/M = 15.4$; Eigsp2Sat166-102, $F/M = 49.3$) were not selected for cytogenetic mapping, as their extremely low absolute genomic abundance ($\leq 0.04\%$ of the genome, and as low as 0.0002% in males for Eigsp2Sat166-102) precluded reliable primer design and made their F/M estimates more susceptible to sampling noise

The PCR amplification protocol consisted of an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of 95°C for 20 seconds, annealing at 56°C for 15-30 seconds, and extension at 72°C for

15-50 seconds, with a final extension step at 72 °C for 10 minutes. Amplification was confirmed by 2% agarose gel electrophoresis (Figure S1), and PCR products were quantified using a NanoDrop spectrophotometer (ThermoFisher Scientific, Branchburg, NJ, USA). For probe construction, amplicons were labeled via nick translation using Atto488-dUTP (green) and Atto550-dUTP (red) nucleotides (Jena Biosciences, Jena, Germany) following the manufacturer's instructions. The resulting labeled probes were subsequently applied for *in situ* hybridization following the protocol described by (Sassi and Cioffi 2026)

Statistics and reproducibility

At least 20 metaphases were examined to confirm the diploid number (2n) and the FISH results. The best metaphases were captured using an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan) coupled with the CoolSNAP capture system and Image Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA).

Results

Satellitome characterization

The satellitome of *Eigenmannia* sp. 2 comprised 166 satDNA families identified after eight iterations of the satMiner protocol, which were further grouped into 23 superfamilies (Table S2). Repeat unit lengths ranged from 16 to 4,580 bp, with a median of 46.5 bp, reflecting a strong predominance of short monomers and a smaller number of families with considerably longer repeat units. The consensus sequences showed a mean A+T content of 56.7%. The mean sequence divergence across male and female libraries was 5.53%.

Of the 166 satDNA families identified, 151 (91%) grouped into 23 superfamilies, while 15 (9%) showed no detectable relationship to other families in the catalog. Monomer size was highly conserved within superfamilies (median coefficient of variation = 7.8%) compared to the unrelated singleton families (CV = 177.3%), suggesting diversification from a shared ancestral monomer under the satellite library model. Among the four satDNA families physically mapped to the sex chromosomes, three (Eigsp2Sat001-90, Eigsp2Sat005-354, Eigsp2Sat015-162) belonged to structured superfamilies (SF9, SF22, and SF8, respectively), whereas Eigsp2Sat003-1023, the family mapped exclusively to the X₂ centromere, showed no detectable relatives, suggesting an independent origin within the satellitome.

Comparison of satDNA abundance between male and female libraries revealed marked sex-biased differences for a subset of families. Using a twofold difference as the threshold, 17 of the 166 satDNA families showed sex-biased abundance. Fifteen were more abundant in females, whereas only two, Eigsp2Sat030-480 and Eigsp2Sat077-29, were more abundant in males, with female-to-male abundance ratios of 0.46 and 0.49, respectively. Female-biased families showed substantially more significant differences between the sexes, with female-to-male ratios ranging from 2.02 to 49.32. However, some of the highest ratios were associated with very low absolute abundance in one of the libraries and should

therefore be interpreted cautiously, as small differences in read representation can disproportionately affect ratio estimates for low abundance satDNAs. Abundance estimates, sequence divergence, and female-to-male ratios for all satDNA families are provided in Table S2.

Chromosomal mapping by FISH

FISH mapping revealed distinct chromosomal distribution patterns among the eight selected satDNA families (Figure 2). Seven families were organized into discrete chromosomal clusters, predominantly located in pericentromeric regions, whereas Eigsp2Sat030-480 showed a dispersed distribution across the chromosome complement. Eigsp2Sat030-480, the only family selected based on male-biased genomic abundance ($F/M = 0.46$), did not produce the discrete clustered signals observed for the other seven families, suggesting a predominantly scattered genomic organization at the cytogenetic level.

Among the clustered satDNAs, three families showed broad, multi-locus pericentromeric distributions. Eigsp2Sat001-90 hybridized to the pericentromeric regions of eight chromosome pairs, including X_2 . Eigsp2Sat003-1023 produced pericentromeric signals on nine chromosome pairs, also including X_2 , whereas Eigsp2Sat005-354 showed a similar distribution across nine chromosome pairs, including both X_1 and X_2 , and colocalized with Eigsp2Sat003-1023 on six of these pairs.

Three other families displayed more restricted distributions. Eigsp2Sat002-42 was detected at the interstitial region of a single chromosome pair, which also carried an Eigsp2Sat001-90 cluster. Eigsp2Sat006-109 was restricted to the paracentromeric region of chromosome pair 10, whereas Eigsp2Sat007-40 was detected exclusively at the paracentromeric region of chromosome pair 7. Finally, Eigsp2Sat015-162 hybridized to the pericentromeric regions of three chromosome pairs, including both X_1 and X_2 .

Repeat landscape analysis of sex-chromosome-associated satDNAs

To further investigate the genomic organization of the satDNA families associated with the sex chromosomes, individual repeat landscapes were generated for Eigsp2Sat001-90, Eigsp2Sat003-1023, Eigsp2Sat005-354, and Eigsp2Sat015-162 using male and female genomic reads. These analyses allowed comparisons of both relative abundance and sequence divergence between sexes, providing additional information on the evolutionary dynamics of these repeats in the X_1X_2Y sex chromosome system.

Except for Eigsp2Sat001-90, the three other families displayed landscape profiles characterized by a single narrow peak at low Kimura divergence (below $\sim 5\%$), with a decline toward higher divergence values and little to no representation beyond 20% (Figure 3). This profile indicates that these families are currently composed of largely homogeneous repeat copies, maintaining low sequence divergence across their genomic copies. These families also showed a modest excess of female-derived reads across the divergence spectrum ($F/M = 1.29, 1.38, \text{ and } 2.02$, respectively), consistent with the overall genomic abundance estimates reported above. Conversely, Eigsp2Sat001-90 showed a broader

multimodal distribution, with its main peak at ~5% divergence followed by secondary peaks extending to higher divergence values (up to ~30%), suggesting weaker or more heterogeneous homogenization across its multiple genomic loci.

Discussion

Our results reveal a highly diverse satDNA repertoire in *Eigenmannia* sp. 2 and show that much of this diversity is organized into groups of evolutionarily related repeats. Together with their non-random chromosomal distribution, these features provide a framework for understanding how satDNA diversification has contributed to genome organization and, particularly, to the differentiation of the multiple sex chromosome system in this species.

The satellitome of *Eigenmannia* sp. 2 is composed of 166 satDNA families, one of the largest satellitomes described so far, representing 5.81% and 6.55% of the male and female genomes, respectively. Repeat units vary considerably in size, from 16 to 4,580 bp, although most families (73%) have monomers shorter than 100 bp, following the general pattern reported for other fish satellitomes (Silva et al. 2017). Despite the large number of satDNA families, most occur at low genomic abundance: only 11 individually represent more than 0.1% of the genome, and the ten most abundant families account for 43.5% of the total satellite fraction. Thus, *Eigenmannia* sp. 2 presents a particularly diverse satDNA repertoire, but without a correspondingly large proportion of its genome being occupied by these repeats.

An interesting feature of this satellitome is the high proportion of related satDNA families. Of the 166 families, 151 were assigned to 23 superfamilies, whereas only 15 showed no detectable relationship with other repeats in the catalogue. Superfamilies contained between two and 21 families of satDNAs, and monomer length was generally conserved among their members. A similar pattern was previously reported in *Megaleporinus macrocephalus*, in which a large number of satDNA families were also organized into superfamilies, indicating that diversification of related repeats can represent an important component of satellitome evolution in fishes (Utsunomia et al. 2019). In *Eigenmannia* sp. 2, however, the proportion of families assigned to superfamilies is even higher, with more than 90% of the catalogue showing detectable relationships with other satDNAs. This suggests that much of the observed diversity arose through the diversification of pre-existing repeat lineages rather than through the independent origin of numerous unrelated sequences. Such a pattern is compatible with the library model of satDNA evolution, in which related variants coexist within genomes and undergo independent changes in sequence and abundance over evolutionary time (Fry and Salser 1977; Ruiz-Ruano et al. 2016).

Comparison between the male and female libraries identified 17 families showing at least a two-fold difference in abundance, including 15 female-biased and two male-biased satDNAs. Sex-biased abundance of satellite repeats has frequently been reported in fishes with differentiated sex chromosomes, particularly when specific families accumulate on the W or Y chromosomes (Utsunomia et al. 2019; Crepaldi and Parise-Maltempi 2020; Kretschmer et al. 2022; Deon et al. 2024; Toma et al.

2024; Sassi et al. 2025). In *Eigenmannia* sp. 2, however, sex-biased abundance does not necessarily imply sex-specific accumulation. Females possess two copies of both X_1 and X_2 ($X_1X_1X_2X_2$) whereas males possess one copy of each together with the neo-Y (X_1X_2Y). Consequently, repeats enriched on X_1 or X_2 may show greater abundance in females simply because of chromosome dosage.

This system also differs from the classical scenario of highly differentiated sex chromosomes. Because the neo-Y originated through a chromosome fusion and still participates in a meiotic trivalent with X_1 and X_2 (Almeida-Toledo et al. 1984) its evolutionary trajectory may not involve the same degree of recombination suppression and repetitive DNA accumulation typically associated with older, highly differentiated Y or W chromosomes. Thus, the absence of a general enrichment of satDNAs on the neo-Y is not unexpected and may reflect the relatively recent and structurally distinct origin of this multiple sex chromosome system. Eigsp2Sat015-162 provides an example of such pattern, as this satDNA shows an F/M ratio of 2.02 and occurs at pericentromeric regions of three chromosome pairs, including X_1 and X_2 . Its approximately two-fold female enrichment is therefore compatible, at least in part, with the higher copy number of these chromosomes in females.

Chromosomal mapping revealed a clear difference between X_1 and X_2 and the neo-Y. The mapped satDNA families were distributed across several chromosome pairs and were therefore not specific to the sex chromosomes. However, several of these families were detected on X_1 and/or X_2 , whereas no visible FISH signal was observed on the neo-Y. Importantly, these satDNAs are not all closely related to each other, as they belong to different superfamilies, and Eigsp2Sat003-1023 was not assigned to any superfamily. Therefore, the absence of FISH signals on the neo-Y is observed for multiple, evolutionarily distinct satDNA families rather than for a single group of related repeats. This suggests that the repetitive DNA composition of the neo-Y differs from that of X_1 and X_2 .

This pattern is concordant with previous cytogenetic observations reporting loss of heterochromatin on the neo-Y of *Eigenmannia* sp. 2 (Almeida-Toledo et al. 2000). A similar pattern has also been described in independently evolved X_1X_2Y systems of other fishes, including *Hoplias malabaricus* (Toma et al. 2024) and *Gymnotus pantanal* (Utsunomia et al. 2014). Together, these observations reinforce the idea that changes in repetitive DNA accompany the structural differentiation of recently derived sex chromosomes.

One possible explanation is that part of the repetitive DNA associated with the ancestral chromosomes was removed or reorganized during, or after, the chromosome rearrangement that originated the neo-Y. Repetitive and centromeric regions are frequently involved in chromosome rearrangements, and recent long-read studies have shown that satellite arrays can be altered at fusion breakpoints (Flynn et al. 2023; de Lima et al. 2025). In *Eigenmannia* sp. 2, the absence of detectable FISH signals on the neo-Y matches this pattern of remodeling, although the timing and extent of these changes remain unclear.

Overall, our results show that *Eigenmannia* sp. 2 possesses a diverse satellitome largely structured into evolutionarily related repeat families. Although most satDNAs occur at low genomic abundance and

many are distributed across multiple chromosomes, their representation differs between X_1 and X_2 chromosomes and the neo-Y. These data provide an initial view of satDNA organization in the multiple sex chromosome system of *Eigenmannia* sp. 2 and suggest that changes in repetitive DNA accompanied its differentiation.

Conclusions

Our study extends the knowledge of sex chromosome evolution in *Eigenmannia* beyond chromosomal morphology by showing that the neo-Y is characterized by a remodeled satDNA landscape when compared to X_1 and X_2 . The distribution of the repetitive sequences observed supports the hypothesis that the satDNA turnover is closely connected with the structural origin and the early molecular differentiation of the derived X_1X_2Y system. Together with cytogenetic and satellitome approaches, our results offer a framework for the study of the interaction between chromosomal rearrangements and repetitive DNA dynamics during the evolution of multiple sex chromosome systems in Neotropical fishes.

Declarations

Author contributions

Conceptualization: Manuela Cândido de Mello Ferro, Fabio Porto-Foresti, Ricardo Utsunomia; Methodology: Manuela Cândido de Mello Ferro, Henrique Tadaka Uehara, Maria Eduarda Gislotti Ribeiro, Michel O. F. Capana, Alan M. de Oliveira, Rodrigo Zeni dos Santos, Caio Augusto Gomes Goes, Francisco de Menezes Cavalcante Sassi, Ricardo Utsunomia; Formal analysis and investigation: Manuela Cândido de Mello Ferro, Henrique Tadaka Uehara, Maria Eduarda Gislotti Ribeiro, Michel O. F. Capana, Alan M. de Oliveira, Rodrigo Zeni dos Santos, Caio Augusto Gomes Goes, Francisco de Menezes Cavalcante Sassi, Marcelo de Bello Cioffi, Fabio Porto-Foresti, Ricardo Utsunomia; Writing – original draft preparation: Manuela Cândido de Mello Ferro, Ricardo Utsunomia; Writing – review and editing: all authors; Funding acquisition: Marcelo de Bello Cioffi, Fabio Porto-Foresti, Ricardo Utsunomia; Supervision: Ricardo Utsunomia. All authors read and approved the final manuscript.

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

All procedures involving live animals were approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Estadual Paulista (UNESP) (protocol CEUA 716/2023) and were conducted under collection authorization from the Brazilian Environmental Agency (ICMBio/SISBIO, license no. 97277-1). All methods were carried out in accordance with institutional and national guidelines and regulations for the use of animals in research.

Data availability

The raw sequencing reads generated in this study are available in the NCBI Sequence Read Archive (SRA) under accession numbers SRR40283535 (male) and SRR40283534 (female). The consensus sequences of the 166 satDNA families identified are deposited at Figshare (<https://doi.org/10.6084/m9.figshare.33889327>). GenBank accession numbers will be added upon deposit completion. Supplementary Tables S1 and S2 accompanying this article contain the primer sequences and the complete satDNA family catalog, respectively.

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Figures

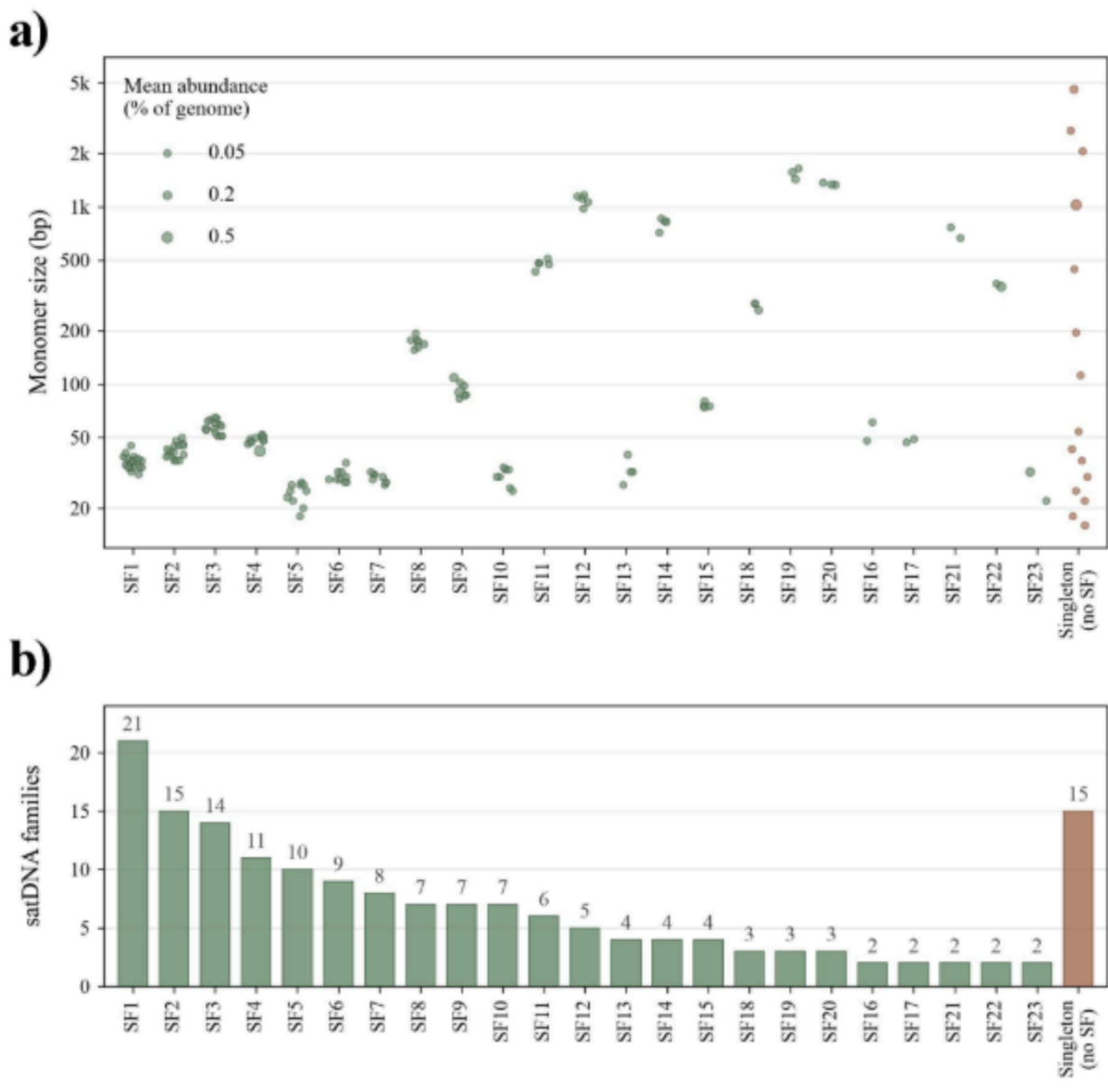


Figure 1

Superfamily structure of the *Eigenmannia* sp. 2 satellitome. (a) Monomer size of all 166 satDNA families by superfamily (log scale). Bubble area is proportional to mean genomic abundance (male and female averaged). (b) Families per superfamily: 151 of 166 (91 %) fall into 23 superfamilies of 2–21 members, and 15 are unassigned. Monomer size varies 1.24-fold within a superfamily in the median, against 16–4,580 bp across the satellitome

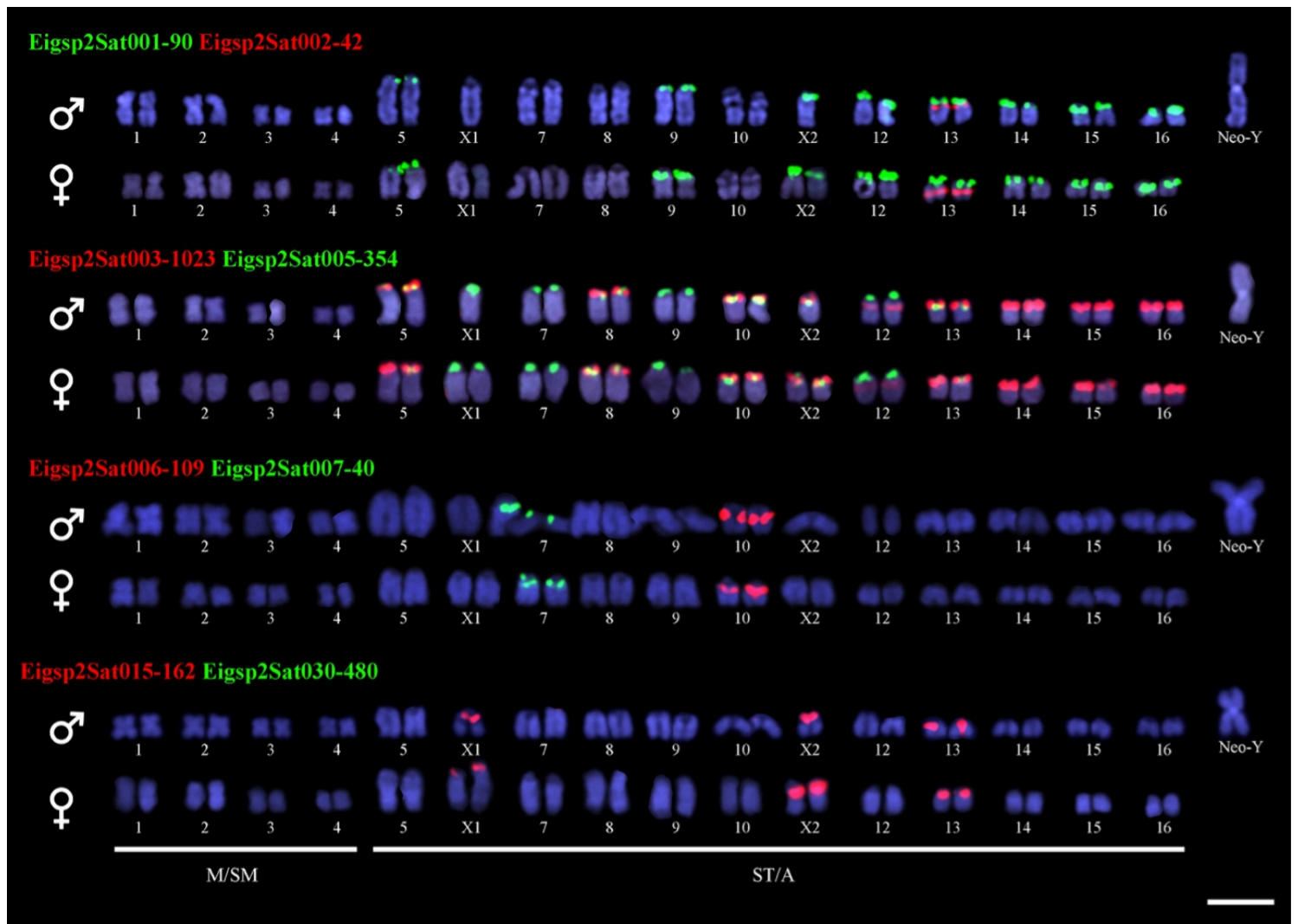


Figure 2

FISH karyotypes of satDNAs in mitotic chromosomes of *Eigenmannia* sp 2. The signals detected are organized by double FISH, male and female karyotypes respectively. Bar = 5 μm

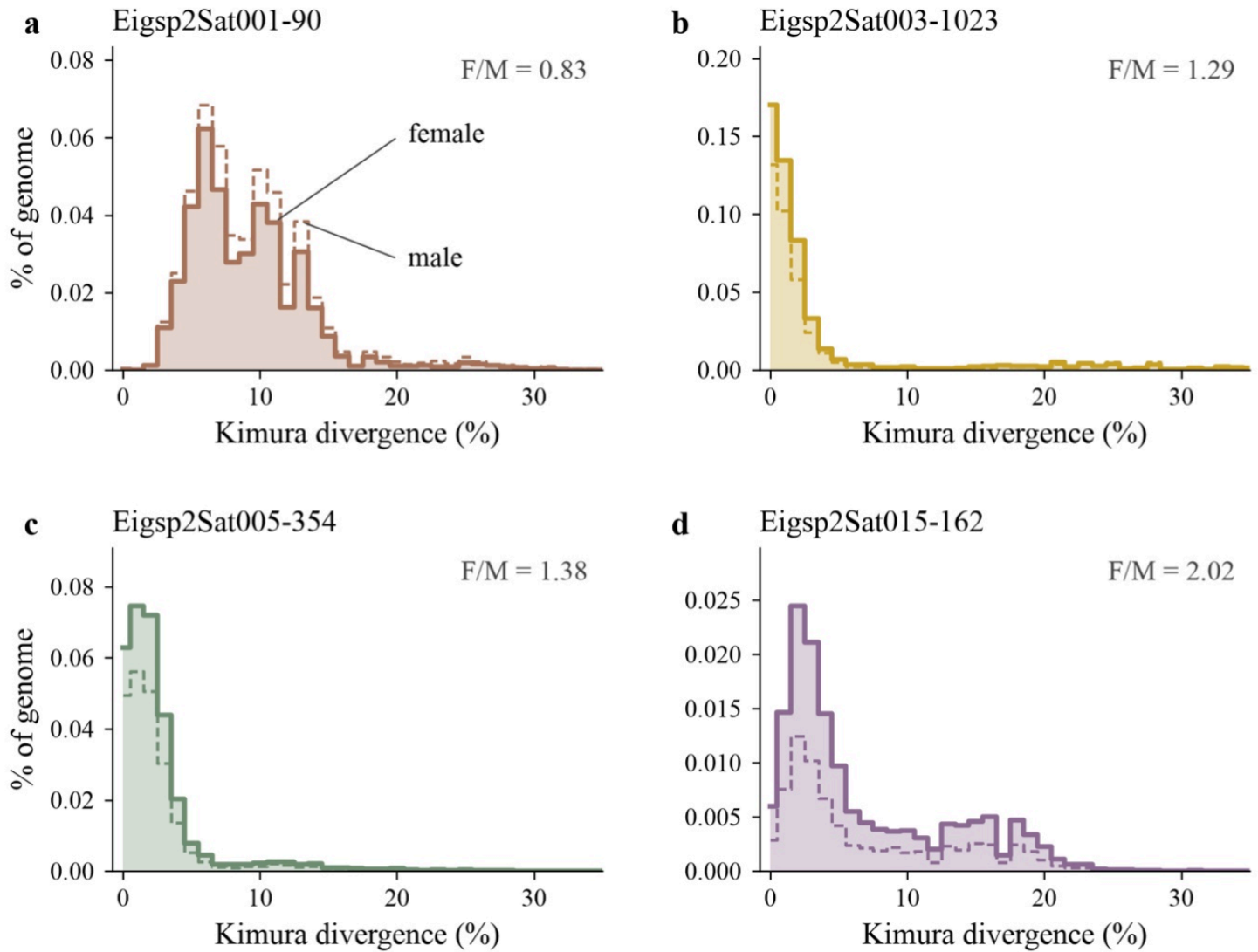


Figure 3

Individual repeat landscapes for Eigsp2Sat001-90, Eigsp2Sat003-1023, Eigsp2Sat005-354, and Eigsp2Sat015-162 using male and female genomic reads

Supplementary Files

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