UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

YHANNDRA KARINE DIAS SILVA

EVOLUÇÃO E ESTRUTURA CROMOSSÔMICA EM ESPÉCIES DO CLADO CYPERID

Recife

2022

YHANNDRA KARINE DIAS SILVA

EVOLUÇÃO E ESTRUTURA CROMOSSÔMICA EM ESPÉCIES DO CLADO CYPERID

Tese apresentada ao Programa de Pós-graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como um requisito parcial para obtenção do título de Doutora em Biologia Vegetal.

Orientadora: Profa. Dra. Andrea Pedrosa Harand

Recife

2022

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD

Silva, Yhanndra Karine Dias
Evolução e estrutura cromossômica em espécies do clado Cyperid/ Yhanndra Karine Dias Silva– 2022.

123 f. : il., fig., tab.

Orientadora: Andrea Pedrosa Harand

Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Biologia Vegetal, Recife, 2022.
Inclui referências e apêndice.

1. Cyperaceae 2. Cromossomos vegetais 3. DNA I. Harand, Andrea Pedrosa (orient.) II. Título

584.84 CDD (22.ed.) UFPE/CB – 2023 -182

Elaborado por Elaine C Barroso - CRB-4/1728

YHANNDRA KARINE DIAS SILVA

TÍTULO DO TRABALHO

Dissertação/Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Doutora em Biologia Vegetal.

Aprovada em 22/08/2022.

BANCA EXAMINADORA

_____
Profa. Dra. Profa. Dra. Andrea Pedrosa Harand

(Orientadora)

Universidade Federal de Pernambuco

_____
Profa. Dr. Prof. Dr. Luiz Gustavo Rodrigues Souza

(Examinador Interna)
Universidade Federal de Pernambuco

_____
Prof. Dr. Reginaldo de Carvalho (Examinadora Externa)
Universidade Federal Rural de Pernambuco

_____
Dr. Juan Urdampilleta (Titular externo)

Instituto Multidiciplinario de Biologia Vegetal (IMBIVCONICET)

_____
Prof. Dr. Tiago Ribeiro Barros dos Santos (Titular externo)

Universidade Federal de Mato Grosso

Santana

Que direi de ti?
Certa que te verei d'outro lado
vou juntar meus retalhos
e viver o amor que aprendi.

Aquele amor sem medo,
sem mágoa, desespero
Sincero e sem preconceito
Causando em todos desassossego

Um amor profundo
Quase intransigente
que mexe com a vida da gente

Tu me mostraste esse amor
Com muita dor, o viverei
Certa que d'outro lado de encontrarei

Para minha querida vovó Ilza Santana Dias,
In memorian

AGRADECIMENTOS

Vou tentar ser sucinta, mas é fato que falo muito, e escrevo proporcionalmente. Outro fato é que é difícil resumir minha gratidão para com as pessoas que listarei abaixo. Segurem aí. Primeiramente, sou muito grata a Deus por todo sustento nesses últimos anos tão difíceis. Foram muitas perdas, muitas lutas travadas dentro e fora da mente. Perder minha avó foi o maior golpe que já tive, e se sobrevivi e lutei tanto, foi para honrar quem ela foi. Se alguém me ensinou um amor genuíno, a ser doce nas adversidades, que não olha com suspeita ou maus olhos para o objeto desse amor, esse alguém foi Dona Ilza. Essa tese é para você, vó!

Os meus pais não me ajudaram "normal" não. Eles me carregaram muitas vezes. É duro não acreditar em si, não conseguir olhar para suas conquistas com afeto e meus pais foram maravilhosos sempre. Todas as vezes que ouço-os falando a meu respeito com orgulho, um pouquinho da minha falta de amor próprio se esvai. Eu nunca vou saber agradecê-los corretamente por tanto apoio, seja financeiro, emocional, ou todas as vezes que precisei de cuidados médicos ou um colo. E fazem tudo com muito amor. Amo-os demais. Essa tese é também para vocês! À minha irmã caçula, com todo afeto do mundo, de quem se inspirava na sua "danda", mas hoje eu que me inspiro em você, que é a pessoa mais dedicada que conheço, te amo! Tenho vários anjos na família: meu avô muito fofo e doce, com um olhar de admiração e orgulho sempre, te amo demais, vô! Aos meus primos maravilhosos: Bruna, Álvaro, Igor e Miss, minha tia maravilinda Marilza <3 obrigada sempre, amo vocês infinito! Um agradecimento especial aos meus sogros, que cuidaram muito de mim todas as vezes que precisei dormir na Várzea. Sempre tinha um café quentinho e boas conversas. Amo vocês, e não imaginam o tanto!

Aos amigos e colegas, vocês são demais! Eu temo em fazer um agradecimento nominal e esquecer alguém, mas é necessário aqui. Obrigada Vivi, Yuri, Nath, Gil. Vocês torcem comigo, oram comigo, são minha segunda família e não tem tempo que diminua o que temos. Obrigada! O laboratório de citogenética e evolução vegetal, vulgo "o melhor lab do Brasil" meu deu amigos e colegas maravilhosos! Sério, eu não tenho necessidade de falar assim, mas preciso dizer a verdade: melhor lab! Primeiramente, minha amiga e madrinha Brena. Como sou feliz por tu nas nossas vidas! Te amo, galega! <3 Amália e Amandão são peças muito importantes na minha caminhada, obrigada, meus amores! Amandinha, você faz falta no lab! Jéssica DIVA que tanto me ajuda, ouve e compartilha algumas peculiaridades comigo. Aos queridos Ana, Bruna, Claudio, Erton, Géssica, Gustavinho, GG, Mariana, Mariela. Nath, Paulinho meu xodó,

Thiaguito e Yennifer, meu muito obrigada por tornarem os dias mais leves e ouvirem tantas reclamações e piadas auto depreciativas. E parabéns por terem tirado leite de pedra nesses anos pandêmicos. Vocês são demais!!

E agora preciso respirar fundo. Afinal, como agradecer devidamente à minha orientadora? Com certeza eu representei um desafio para Andrea, pois atrapalhada que sou, fiz muitas besteiras nos últimos anos. Ao contrário dela, que todos admiram por ser incrivelmente organizada e com as ideias no lugar, com muita certeza do que quer. E isso foi o que salvou minha tese (e acho que o trabalho de todos), bem como sua prontidão para ajudar e generosidade. Obrigada também por chorar comigo em alguns momentos, e por ouvir, entender, pedir mais de mim, e também por todos os momentos divertidos. Obrigada também pela oportunidade que me deu para estudar na Alemanha, melhorar meu trabalho e conhecer tanta gente boa por lá. Obrigada pela amizade, por cada desafio proposto e por me guiar neles. Ps: Espero que você tenha alunos mais organizados no futuro! :P

Ao querido Prof e amigo Luiz Gustavo, muita gratidão por todas as trocas, incentivos profissionais e o afeto com minha família. Tenho um carinho enorme por você e sua família também. Nunca saberei como agradecer a você. Mas vai aqui o meu MUITO obrigada!

Um agradecimento muito importante ao Professor Marcelo Guerra, que além de ter emprestado uma planta para a realização deste trabalho, sempre pergunta como está o andamento dele, se interessa e troca ideias sobre tudo. Obrigada, meu querido!

E como fazer pesquisa sem colaboradores, não é? Por isso, agradeço imensamente a cada querido colaborador, em especial ao Andreas Houben, por tamanha generosidade em ensinar seus conhecimentos, ouvir, dar outra perspectiva sobre o assunto. E por todos os momentos hilários, que me fizeram enxerga-lo de uma forma diferente. Uma das melhores pessoas que convivi. Obrigada! Mari, Yennifer, André Marques, Lucas, muito obrigada por cada contribuição, conversa, pelos dados, vocês são generosos demais comigo.

E agora, o mais importante. SIM, mais importante. Meu amor, minha família, meu ajudador, marido etc. Obrigada, amor, por tanto cuidado e por acreditar mais em mim do que eu. Você olha para além do que consigo ver aqui, e isso não tem preço. Casar contigo foi a melhor coisa que fiz. Há quase onze anos você dispensa tanto amor e me dá forças para continuar, seja inspirando com sua dedicação, ou simplesmente dizendo que eu consigo, que eu não estou sozinha, e que tem jeito para minhas causas impossíveis :p te amo para sempre!

E por fim, preciso tecer um agradecimento importante a UFPE e ao PPGBV, em especial a Felipe e Soraya, pois resolveram tantas burocracias, sempre muito solícitos e gentis. E também aos órgãos de fomento, que lutam às duras penas nesse país sem governo. Obrigada CNPq e CAPES!

RESUMO

Em organismos eucariotos, o centrômero é a região dos cromossomos onde há ligação dos microtúbulos do fuso, sendo o mesmo essencial para a estabilidade do genoma ao mediar a segregação mitótica e meiótica. Todavia, existem espécies que possuem cromossomos cujos microtúbulos se ligam ao longo de quase todo o seu comprimento, os chamados cromossomos holocêntricos. Espécies do clado Cyperid, que compreende as famílias Cyperaceae, Juncaceae e Thurniaceae, são genericamente consideradas como holocêntricas. No entanto, recentemente foi descoberto que algumas possuem cromossomos monocêntricos, ou seja, centrômero localizado. Com base nisso, buscamos entender se as linhagens que divergiram primeiro em Cyperaceae são de fato holocêntricas, quais sequências repetitivas compõem o genoma das mesmas, e se as espécies monocêntricas do clado Cyperid possuem sequências centroméricas específicas. No primeiro capítulo, foram realizadas contagens cromossômicas inéditas para espécies de famílias Cyperaceae e Thurniaceae, detectando relativa conservação numérica apesar de variação para a espécie *Hypolytrum schraderianum*. No segundo capítulo, as frações repetitivas de *H. schraderianum* e outras duas espécies de Mapanioideae (*Diplasia karatifolia* e *Mapania sylvatica*, Cyperaceae) foram caracterizadas. As espécies apresentaram entre 0,275 e 0,335pg/1C e a fração repetitiva correspondeu a valores entre 4.74 – 23% do genoma, com predomínio de DNAs satélites. A fim de identificar o tipo de organização centromérica em *H. schraderianum*, realizamos imunocoloração com a RpCENH3 e investigamos a distribuição cromossômica dos principais *repeats*, os quais mostraram padrões característicos para cromossomos monocêntricos, exceto pela ausência de um satélite centromérico. No terceiro capítulo, é apresentado um panorama do DNA repetitivo da espécie monocêntrica *Juncus effusus* (Juncaceae), assim como da estrutura e composição dos seus centrômeros, os quais foram montados e anotados. O DNA repetitivo de *J. effusus* é composto principalmente por retroelementos, seguido por DNAs satélites. Através das análises *in silico*, propomos que três principais satélites compõem os centrômeros e pericentrômeros de *J. effusus*, em arranjos de diferentes tamanhos intercalados com retroelementos, apesar de não serem exclusivos dessas regiões. Além disso, foi possível confirmar a co-localização do JefSAT1-155 com a CENH3 em parte dos cromossomos. Sendo assim, caracterizamos os cromossomos de outros representantes de Cyperids também como monocêntricos, demonstrando uma organização atípica para espécies com essa organização centromérica.

Palavras-chave: Centrômeros; Cyperaceae; Juncaceae; Holocêntricos; DNA repetitivo.

ABSTRACT

In eukaryotic organisms, centromeres are the chromosome regions where spindle microtubules attach, being essential for genome stability by mediating mitotic and meiotic segregation. However, there are species whose microtubules attach along almost the entire chromosome length, the so-called holocentric chromosomes. The Cyperid clade, which comprises the families Cyperaceae, Juncaceae and Thurniaceae, is generically identified as being formed only by holocentric species. However, it was recently discovered that some species have monocentric chromosomes, with a localized centromere. Based on this, we seek to understand if the lineages that diverged first in Cyperaceae are in fact holocentric, which repetitive sequences make up their genome, and if the monocentric species from Cyperids have specific centromeric sequences. In the first chapter, new chromosome counts were reported for species of Cyperaceae and Thurniaceae, detecting relative numerical conservation despite variation for the species *Hypolytrum schraderianum*. In the second chapter, the repetitive fractions of *H. schraderianum* and two other Mapanioideae species (*Diplasia karatifolia* and *Mapania sylvatica*, Cyperaceae) were characterized. The species presented between 0.275 and 0.335 pg/1C and the repetitive fraction corresponded to 4.74 – 23% of the genomes, with a predominance of satellite DNAs. In order to identify the type of centromeric organization in *H. schraderianum*, we performed immunostaining with RpCENH3 and investigated the chromosomal distribution of the main repeats, which showed characteristic patterns for monocentric chromosomes, except for the absence of a specific centromeric satellite. In the third chapter, an overview of the repetitive DNA of the monocentric species *Juncus effusus* (Juncaceae) is presented, as well as the structure and composition of its centromeres, which were assembled and annotated. The repetitive DNA of *J. effusus* is composed mainly of retroelements, followed by satellite DNA. Through *in silico* analyses, we propose that three main satellites compose the centromeres and pericentromeres of *J. effusus*, although they are not exclusive to these regions, and in arrays of different sizes interspersed with retroelements. Furthermore, it was possible to confirm the co-localization of JefSAT1-155 with JeCENH3 in part of the chromosomes. Therefore, we characterize monocentric chromosomes in representatives of Cyperids, demonstrating an atypical chromosomal organization for species with this centromeric organization.

Keywords: Centromeres; Cyperaceae; Juncaceae; Holocentrics; Repetitive DNA.

SUMÁRIO

APRESENTAÇÃO

O clado Cyperid é bem suportado filogeneticamente, sendo formado pelas famílias Cyperaceae, Juncaceae e Thurniaceae (CHASE *et al.*, 2006). Citogeneticamente, a família Cyperaceae apresenta uma grande variação nos números cromossômicos diploides ($2n = 4 – 224$) e sítios de DNAr (DA SILVA; QUINTAS; VANZELA, 2010; LIPNEROVA *et al.*, 2013; RIBEIRO *et al.*, 2018; ROALSON, 2008; SOUSA *et al.*, 2011). A maioria dos trabalhos foi realizado na subfamília Cyperoideae, com a subfamília Mapanioideae ainda pouco representada, possuindo apenas análises de contagens cromossômicas (MÁRQUEZ-CORRO *et al.*, 2018). Na família Juncaceae, *Juncus* L. é o maior gênero, e apresenta cerca de 315 espécies, seguido por *Luzula* DC, gênero irmão com cerca de 115 espécies e cromossomos holocêntricos bem caracterizados (HECKMANN *et al.*, 2013). A menor família deste clado, Thurniaceae, é composta por dois gêneros, *Thurnia* Hook.f. e *Prionium* E.Mey., e quatro espécies, *T. jenmanii* Hook.f., *T. polycephala* Schnee, *T. sphaerocephala* (Rudge) Hook.f., que são endêmicas da parte nordeste da Bacia Amazônica, e *Prionium serratum* (Lf) Drège ex E.Mey, endêmico da África do Sul (Kubitzki, 1998; Mucina & Rutherford, 2006).

A presença de cromossomos holocêntricos foi considerada por muito tempo uma sinapomorfia para o clado Cyperid (GREILHUBER, 1995). Isso se deve, em parte, às detalhadas análises realizadas em algumas espécies das famílias Cyperaceae e Juncaceae, cujos achados foram também extrapolados a todas as espécies do clado, bem como aos pequenos cromossomos que dificultam a observação de possíveis constrições primárias (DAVIES, 1956; HECKMANN *et al.*, 2013; MARQUES *et al.*, 2015; NAGAKI; KASHIHARA; MURATA, 2005). Análises recentes, entretanto, confirmaram a existência de cromossomos monocêntricos em espécies de Juncaceae (GUERRA; RIBEIRO; FELIX, 2019) e no gênero monotípico *Prionium serratum* (L.f.) Drège (Thurniaceae, BAEZ *et al.*, 2020). Por meio de análises citogenéticas, incluindo imunolocalização de histonas modificadas e enriquecidas na região centromérica, bem como do comportamento meiótico, foi possível demonstrar que ao menos quatro espécies de *Juncus* são, de fato, monocêntricas (GUERRA; RIBEIRO; FELIX, 2019). Para *Prionium serratum*, foi desenvolvido um anticorpo contra a proteína centromérica CENH3, além da observação do padrão para outras marcas epigenéticas do pericentrômero (H3S10-ph e H2AT120) e uma detalhada análise do DNA repetitivo, incluindo *repeats* centroméricos (BAEZ *et al.*, 2020).

Com isso, estes trabalhos recentes mostram a necessidade de análises mais detalhadas sobre os centrômeros e a organização do genoma em espécies de Cyperid. No primeiro capítulo dessa tese, a fim de ampliar o banco de dados citogenéticos dessas famílias, foram realizadas as primeiras contagens cromossômicas para Thurniaceae, que possui apenas quatro espécies, das quais duas espécies foram analisadas: *Thurnia sphaerocephala* e *Prionium serratum.* Também foram feitas contagens inéditas para Cyperaceae com ênfase na subfamília Mapanioideae, que possuía apenas uma contagem anterior (MÁRQUEZ-CORRO *et al.*, 2018). No segundo capítulo, com foco na subfamília Mapanioideae (Cyperaceae), as principais questões foram: 1) as espécies que divergiram primeiro em Cyperaceae são holocêntricas? 2) quais DNAs repetitivos compõem os genomas dessas espécies e como estes estão organizados? 3) existem *repeats* potencialmente centroméricos em alguma dessas espécies? Por fim, o terceiro capítulo buscou aprofundar as análises no gênero *Juncus,* utilizando a espécie monocêntrica *Juncus effusus,* a fim de responder as seguintes questões: 1) quais DNAs repetitivos formam o genoma de *J. effusus*? 2) o que compõe o centrômero dessa espécie? 3) *J. effusus* compartilha sequências com a espécie holocêntrica *L. elegans* e, se sim, em que classes de *repeats*? Em conjunto, os dados foram utilizados para uma melhor compreensão da estrutura e a evolução cromossômica, particularmente dos centrêmeros, de espécies do clado Cyperid.

1 INTRODUÇÃO

1.1 CENTRÔMEROS

O centrômero, em geral visível como a constrição primária dos cromossomos, é essencial para a estabilidade do genoma. Isso se dá pelo papel exercido pelo mesmo na segregação cromossômica durante a mitose e meiose, de forma que uma falha no evento pode levar a ganho ou perda de material genético (CUACOS; H. FRANKLIN; HECKMANN, 2015). Em organismos eucariotos, um complexo de proteínas, denominado cinetócoro, se forma na região centromérica com a finalidade de unir os cromossomos aos microtúbulos do fuso, mediando a correta segregação dos mesmos (MELTERS *et al.*, 2012). Além disso, o centrômero também é responsável pela coesão entre as cromátides-irmãs (LERMONTOVA; SANDMANN; DEMIDOV, 2014).

O DNA centromérico pode variar em composição de sequência entre os organismos. Sendo assim, o que define a maioria dos centrômeros dos eucariotos é a presença da variante histônica centromérica, a CENH3 (CUACOS; H. FRANKLIN; HECKMANN, 2015), importante na formação e manutenção de centrômeros ativos (EARNSHAW *et al.*, 2013) e cuja formação é determinada por mecanismos epigenéticos (EKWALL, 2007). Entretanto, a aparente perda de CENH3 em algumas espécies ou a presença de regiões do centrômero livres desta proteína, desafiam a ideia de que a CENH3 é essencial para a formação do cinetócoro e sua função (DRINNENBERG *et al.*, 2014; OLIVEIRA *et al.*, 2020). Embora possua tanto função como posição conservadas, a sequência da CENH3 tem rápida evolução. A conservação da função das proteínas centroméricas, apesar de sua rápida evolução, representa um paradoxo (HENIKOFF; AHMAD; MALIK, 2001). Isso pode ser explicado como resultado de uma evolução adaptativa (MAHESHWARI *et al.*, 2017), sendo essa proteína o resultado da evolução convergente de várias linhagens de histonas H3 para uma função centromérica comum (MALIK; HENIKOFF, 2003). Para averiguar como se dá a especificidade do centrômero foi realizado um experimento em cevada, no qual dois telossomos foram gerados sem as sequências centroméricas, e, apesar de não terem essas sequências, várias proteínas do centrômero funcional puderam ser identificadas por imunocoloração, indicando a formação de novos centrômeros (NASUDA *et al.*, 2005).

Além de proteínas, a região do centrômero é geralmente composta por DNA repetitivo, que varia em composição de sequência entre os organismos. E apesar de ser determinado

epigeneticamente e as repetições centroméricas não serem suficientes ou necessárias para a formação do centrômero (MCKINLEY; CHEESEMAN, 2016), essas sequências são importantes constituintes dessa região (CHENG *et al.*, 2002; MARQUES *et al.*, 2015; RIBEIRO *et al.*, 2017), com DNAs satélites e elementos transponíveis sendo os tipos mais abundantes.

Para entender a estrutura e função do centrômero, portanto, é necessário conhecer (1) a correta distribuição da CENH3 nos cromossomos, (2) os mecanismos de acumulação dos elementos repetitivos no genoma, (3) como estes se ligam às proteínas centroméricas, bem como (4) os mecanismos de diversificação desses elementos (PLOHL; MEŠTROVIĆ; MRAVINAC, 2014). Em uma ampla análise com diferentes organismos, foi assumido que a sequência repetida em tandem mais abundante encontrada em um dado genoma pertenceria ao centrômero, confirmando o que já tinha sido observado em trabalhos prévios de caracterização do centrômero. Sabe-se, no entanto, que nem sempre a repetição em tandem mais abundante em um dado genoma é centromérica, como observado em *Citrus* (BARROS E SILVA *et al.*, 2010). Embora essas sequências estejam pouco conservadas, elas mostraram um modo similar de evolução (MELTERS *et al.*, 2013).

Tipos de centrômeros

Do ponto de vista estrutural os cromossomos podem ser monocêntricos, metapolicêntricos e holocêntricos. A maioria das espécies de plantas apresentam cromossomos do primeiro tipo, conhecidos por sua visível constrição primária, na qual se ligam os microtúbulos do fuso durante a divisão celular. Durante a anáfase, os cromossomos migram em forma de “V”, devido ao tamanho restrito da região centromérica (CUACOS; H. FRANKLIN; HECKMANN, 2015). Rearranjos cromossômicos podem levar à fusão de dois cromossomos, gerando um cromossomo dicêntrico. Embora esses cromossomos possam existir de forma natural, são tipicamente consequência de rearranjos profundos no genoma (MCCLINTOCK, 1939; STIMPSON; MATHENY; SULLIVAN, 2012). A ideia de que esses cromossomos são instáveis não se aplica a todos os organismos, como os humanos e outros mamíferos que possuem cromossomos dicêntricos e que são herdados através da meiose. Essa estabilidade se deve ao fenômeno de inativação de um dos centrômeros (SULLIVAN; SCHWARTZ, 1995).

Em *Pisum sativum* L. foi observado o primeiro exemplo de um tipo possivelmente intermediário de centrômero, o centrômero metapolicêntrico, que possue entre três e cinco domínios de CENH3 numa constrição primária estendida (NEUMANN *et al.*, 2012).

Posteriormente, organização similar foi observada no gênero irmão *Lathyrus* L. Essas espécies expressam duas variantes distintas de CENH3 que são co-localizadas nos diferentes domínios (NEUMANN *et al.*, 2016). O terceiro tipo de organização é observado nos cromossomos holocêntricos, os quais apresentam atividade centromérica ao longo de praticamente toda a extensão cromossômica. Entretanto, em *Cuscuta europaea*, foram observados cromossomos holocêntricos que apresentam regiões livres de CENH3, mas com os microtúbulos ligados em toda extensão dos cromossomos, o que levanta outras questões sobre a organização dos holocentrômeros (OLIVEIRA *et al.*, 2020).

1.1.1 Cromossomos holocêntricos: comportamento no ciclo celular e formas deidentificação

Os cromossomos que não possuem uma constrição primária visível em metáfase são chamados de holocêntricos, sendo esse um dos critérios usados para sugerir a holocentricidade em uma espécie. Holocêntricos são caracterizados por ter inúmeros loci da proteína CENH3 dispersos ao longo de praticamente todo cromossomo, organizados em aglomerados ou em bandas (OLIVEIRA *et al.*, 2020; SCHUBERT, Veit *et al.*, 2020). Com relação à divisão celular, tanto em mitose como em meiose são observadas diferenças substanciais em comparação com os cromossomos monocêntricos. Em intérfase, o DNA se encontra disperso no núcleo e para iniciar a divisão é necessário um alto nível de condensação, realizado pelas proteínas condensina I e II, sendo esta última especialmente enriquecida em centrômeros de holocêntricos, tornando-o mais rígido (MADDOX *et al.*, 2004). Em anáfase mitótica, as cromátides irmãs de holocêntricos migram em paralelo, não em forma de “V”, devido à presença difusa do centrômero em quase todo o comprimento cromossômico. A extensa coesão ao longo do holocentrômero assegura sua orientação paralela antes da fixação do fuso (MADDOX *et al.*, 2004; MELTERS *et al.*, 2012). Outra característica importante apresentada por algumas espécies holocêntricas, especialmente plantas, é a meiose invertida, ou seja, quando as cromátides irmãs exibem uma ligação anfitélica ao fuso. Nesse caso, são subsequentemente separadas durante a meiose I e por último, as cromátides homólogas não irmãs apresentam disjunção mais comum na anáfase II (CABRAL *et al.*, 2014).

O primeiro cientista a reconhecer um cromossomo holocêntrico e suas peculiaridades foi o zoológo Franz Schrader, observando o fato em cochonilhas (*Hemiptera*). Experimentalmente, a presença do cromossomo holocêntrico foi confirmada a partir da técnica de raio-X. Por meio dela foi possível gerar fragmentos cromossômicos que se comportaram

como cromossomos individuais e foram corretamente transmitidos para a próxima geração de células (HUGHES-SCHRADER; RIS, 1941; SCHRADER, 1935). Outra maneira de detectar holocêntricos a partir de técnica com irradiação foi recentemente proposta. Combinando-a com citometria de fluxo, foram testados critérios para diferenciar espécies monocêntricas e holocêntricas. As primeiras deveriam apresentar o conteúdo de DNA nuclear reduzido e um aumento do coeficiente de variação (CV) do pico G1 após a fragmentação induzida por radiação gama. Parte do DNA, os fragmentos gerados pelo processo de irradiação, seria potencialmente eliminado na forma de micronúcleo ao final do ciclo de divisão pós radiação. Todavia, esses parâmetros não foram suficientes para permitir a correta diferenciação dos dois tipos cromossômicos, mas os autores observaram inesperadamente diferenças na proporção de núcleos em G2 (ZEDEK *et al.*, 2016). Em meiose, também há características que podem auxiliar na identificação de holocêntricos, tais como a estrutura em caixa formada pelos bivalentes (VANZELA; GUERRA, 2000) bem como a meiose invertida (CABRAL *et al.*, 2014). Os cromossomos holocêntricos podem ser identificados também por métodos de imunocoloração, no qual é usado um anticorpo marcado que se liga em uma das proteínas centroméricas, tais como a CENH3. No caso de uma organização holocêntrica, deverá ser observada a marcação da proteína ao longo de todo quase toda a extensão do cromossomo, enquanto em monocêntricos, será visto um sinal localizado e que está delimitará à constrição primária que o caracteriza (BAEZ *et al.*, 2020; MARQUES *et al.*, 2015; MARQUES; PEDROSA-HARAND, Andrea, 2016). Além disso, outras proteínas, como a tubulina, podem ser muito importantes na identificação dos centrômeros, como em *Cuscuta europaea,* em que a tubulina está presente em todo comprimento cromossômico (holocêntrico) e com regiões livres de CENH3 (OLIVEIRA *et al.*, 2020).

1.1.2 Evolução de cromossomos holocêntricos

Os cromossomos holocêntricos têm sido amplamente reportados em animais, como os nemátodas, como *Caenorhabditis elegans,* organismo modelo para esses estudos em animais, os insetos e aracnídeos (ALBERTSON; THOMSON, 1993; DRINNENBERG *et al.*, 2014; SCHNEIDER *et al.*, 2015). Em plantas, esses cromossomos são características tanto de famílias ou gêneros de monocotiledôneas, como as Cyperaceae Juss. (LUCEÑO *et al.*, 1998) e *Luzula* DC. (NAGAKI; KASHIHARA; MURATA, 2005). E também em eudicotiledôneas, como *Cuscuta* L., subgenero *Cuscuta* (PAZY; PLITMANN, 1994) e *Drosera* L., um gênero que

possui espécies holocêntricas e monocêntricas (SHEIKH; KONDO, 1995; SHIRAKAWA; NAGANO; HOSHI, 2011). Em Myristicaceae R.Br, apenas *Myristica fragrans* Houtt. foi identificada como holocêntrica, sem mais informações citológicas para os demais membros da família (FLACH, 1966). Na família Melanthiaceae Batsch, a atividade cinetocórica foi relatada, sendo a família considerada holocêntrica (TANAKA; TANAKA, 1977). No geral, o número de espécies com esse tipo cromossômico é provavelmente subestimado, devido ao pequeno tamanho e alto número cromossômico observado em muitas espécies, dificultando a interpretação do fenômeno com base apenas em análise do comportamento de divisão celular ou da morfologia dos cromossomos (CUACOS; H. FRANKLIN; HECKMANN, 2015).

Enquanto a cromatina contendo CENH3 está localizada dentro da constrição primária dos cromossomos monocêntricos e em quase todo comprimento dos holocêntricos, ela está contida entre 3 a 5 regiões do centrômero polimetacêntrico (NEUMANN *et al.*, 2012; 2015*).* Os cromossomos polimetacêntricos contêm ambos CENH3/CENP-A e embora seja considerado um tipo intermediário entre mono- e holocêntricos, eles funcionam de forma similar aos monocêntricos (SCHUBERT *et al.,* 2020). O mecanismo de transição de cromossomos monocêntricos para holocêntricos ainda é desconhecido. Estima-se que os holocentrômeros tenham surgido cerca de 14 vezes de forma independente ao longo da história evolutiva, sendo 9 vezes em animais e 5 em plantas. Embora não se tenha certeza se o último ancestral comum de todos os eucariotos era holocêntrico ou monocêntrico, o estado monocêntrico é considerado ancestral (MELTERS *et al.*, 2012). Outro trabalho propõe que a taxa de transição de holocêntricos para monocêntricos é duas ordens de magnitude maior do que o inverso, apesar de não concluir qual o estado ancestral, pela baixa representatividade de espécies confirmadas como holocêntricas (ESCUDERO; MÁRQUEZ-CORRO; HIPP, 2016). Outros autores ainda sugerem que o estado holocêntrico é ancestral ou que os monocêntricos derivam dos holocêntricos (MOORE *et al.*, 1997; SYBENGA, 1981). Em insetos, essa transição de monocêntricos para holocêntricos ocorreu quatro vezes em linhagens distintas, onde houveram perdas independentes da proteína centromérica CENH3 e, diferente de outros eucariotos, os grupos holocêntricos têm um centrômero independente da CENH3 (DRINNENBERG *et al.*, 2014).

Os cromossomos holocêntricos possuem a vantagem de não sofrerem perdas de DNA, sendo mais tolerantes a ocorrência de fissões e fusões, pois geram fragmentos com sítios de fixação para as fibras do fuso (MÁRQUEZ-CORRO; ESCUDERO; LUCEÑO, 2018; SCHUBERT, Veit *et al.*, 2020). O reflexo disso é que grupos de plantas que possuem cromossomos holocêntricos apresentam uma grande variabilidade de números cromossômicos,

que vão desde 2*n* = 4 em *Rhynchospora tenuis* Link a 2*n* = 224 em *Cyperus cyperoides* (L.) Kuntze (ROALSON, 2008). E algumas espécies de *Eleocharis, Carex* e *Cyperus* apresentam de 4 a 6 citótipos, devido aos fenômenos de fusão e fissão, comuns nesses grupos (ROALSON, 2008). Essa alta variabilidade sugere que esse fenômeno é mais relevante do que a poliploidia para a variação numérica em espécies holocêntricas (HIPP; ROTHROCK; ROALSON, 2009). Todavia, ao comparar as linhagens holocêntricas conhecidas com suas linhagens monocêntricas mais próximas, foi observado que as diferentes taxas de especiação entre monocêntricos e holocêntricos não estão relacionadas a diferentes tipos de centrômeros (MÁRQUEZ-CORRO *et al.*, 2019).

2. DNA REPETITIVO

O genoma dos organismos eucariotos é composto por tipos distintos de sequências de DNA, cópia única e DNA repetitivo, sendo este último, junto aos eventos de poliploidia, o maior responsável pela variação no tamanho do genoma (LEE; KIM, 2014). A fração repetitiva é em grande parte composta por elementos transponíveis, assim chamados pela sua capacidade de se transpor dentro do genoma, através de diferentes mecanismos, dispersos de forma mais ou menos uniforme nos genomas, e pelos DNA satélites, com repetições em tandem (BOURQUE *et al.*, 2018). Os avanços em técnicas citogenômicas e bioinformáticas vêm permitindo maior compreensão da estrutura e composição dos genomas. Uma dessas tecnologias é o sequenciamento de nova geração (NGS), pelo qual é possível realizar sequenciamento de baixa cobertura (*genome skimming*) a fim de identificar sequências repetitivas no genoma. Uma plataforma que vem revelando grande potencial na identificação dessas sequências repetitivas, como DNA satélites e elementos transponíveis é o RepeatExplorer (NOVAK *et al.*, 2013; NOVÁK *et al.*, 2017; NOVÁK *et al.*, 2020).

2.1 DNA satélites

Os DNA satélites são uma classe de sequências repetitivas que formam longas matrizes de unidades similares repetidas em tandem, compondo porções significativas de muitos genomas de plantas (ÁVILA ROBLEDILLO *et al.*, 2018). As sequências em tandem podem ser classificadas em microssatélites com repetições de 2-5 pb, minissatélites de 6 a 100 pb e os DNAs satélites (satDNA), com uma unidade repetitiva maiores que 100 pb e que formam

arranjos de até 100 Mb (MEHROTRA; GOYAL, 2014). Os satélites podem ser específicos de um grupo de espécies de um mesmo gênero ou ser compartilhado entre vários gêneros em uma família (DE FELICE *et al.*, 2004; NEUMANN *et al.*, 2021), ou seja, essas sequências evoluem rapidamente, mas podem ao mesmo tempo se mostrar conservadas. Em *Brassica* L., por exemplo, o satélite CentBr foi observado em várias espécies do gênero por técnicas de hibridização *in situ* fluorescente e também no gênero relacionado *Raphanus* L. Entretanto, algumas espécies do próprio gênero *Brassica* não apresentaram sinais desse satélite, sugerindo que esse *repeat* divergiu durante a evolução entre essas linhagens (KOO *et al.*, 2011).

Os DNAs satélites têm sido amplamente reportados como componente centromérico em espécies de plantas, tais como em *Arabidopsis thaliana* L. (MARTINEZ-ZAPATER; ESTELLE; SOMERVILLE, 1986), *Triticum aestivum* L. (CHENG; MURATA, 2003), *Oryza sativa* L.(CHENG *et al.*, 2002) e *Zea mays* L. (NAGAKI *et al.*, 2003). Uma das características desses satélites é sua rápida evolução, como no caso de feijão, onde duas sequências centroméricas distintas compõem o seu centrômero e provavelmente divergiram de forma independente em um curto tempo (IWATA *et al.*, 2013). A rápida evolução desses satélites envolve o processo de homogeneização, gerando satélites espécie-específicos, como em *Oryza sativa.* No entanto, alguns satélites também possam ser detectados em espécies pouco relacionadas, como arroz e milho (CHENG *et al.*, 2002). Uma das hipóteses para a variabilidade dos satélites é que estes competem para serem transmitidos para as gerações seguintes, como uma corrida, onde evoluem para se ligar com mais eficiência ao cinetócoro, aumentando suas chances de serem transmitidos à progênie (JIANG *et al.*, 2003). Por muito tempo foi aceito que os centrômeros de holocêntricos não apresentariam satélites em sua composição devido a sua conformação genômica. Entretanto, a partir da análise da fração repetitiva de *Rhynchospora pubera* Vahl foi descoberto o primeiro satélite específico do centrômero de holocêntricos (MARQUES *et al.*, 2015), compartilhado com outras duas espécies do gênero (RIBEIRO *et al.*, 2017), mas não com todas até o momento, o que mostra a variabilidade da fração repetitiva dentro do próprio gênero.

1.1.3 Elementos transponíveis

Os elementos transponíveis possuem capacidade de se mover dentro do genoma e, baseado em seus mecanismos de transposição, podem ser divididos em Classe I, retrotransposons, e Classe II, transposons de DNA (LEE; KIM, 2014). Os transposons se

replicam no genoma pelo modo “corta e cola” e não utilizam um intermediário de RNA, podendo ser divididos em três subclasses principais: os que são “cortados” como DNA de fita dupla e reinseridos no genoma, ou seja, os clássicos transposons “cut-and-paste”; os que se replicam em um mecanismo circular, como os Helitrons; e os que utilizam no mecanismo de transposição uma polimerase auto-codificada, como os Mavericks (BOURQUE *et al.*, 2018; KAPITONOV; JURKA, 2001; PRITHAM; PUTLIWALA; FESCHOTTE, 2007).

Os retrotransposons são especialmente abundantes em plantas. Em milho, por exemplo, estima-se que esses elementos compõem cerca de 85% do genoma (SCHNABLE *et al.*, 2009). Eles se mobilizam por um mecanismo de "copiar e colar", através do qual um intermediário de RNA é transcrito reversamente em uma cópia de cDNA que é inserida em outra parte do genoma (BOURQUE *et al.*, 2018). Os retrotransposons são divididos em cinco ordens: retrotransposons do tipo LTR, elementos DIRS-like, elementos Penelope-like (PLEs), LINEs (*long interspersed elements*) e SINEs (*short interspersed elements*). Essa classificação é baseada na organização estrutural e filogenia da transcriptase reversa destes retroelementos (MAKAŁOWSKI *et al.*, 2019). No caso dos retrotransposons LTR, a classificação se dá pela similaridade do domínio da transcriptase reversa e da distribuição espacial das subunidades do gene Pol ao longo da estrutura do elemento. O grupo dos *Ty1*-copia apresenta as seguinte ordem para as subunidades: protease (PR), integrase (IN), transciptase reversa (RT) e RNase H (RH). No caso dos elementos *Ty3*-Gypsy as subunidades estão distribuídas da seguinte maneira: PR, RT, RH e IN (ZHANG *et al.*, 2014). Alguns exemplos notáveis são as linhagens Angela (trigo), BARE1 (cevada) e Opie (maize) (SANMIGUEL *et al.*, 1998; VICIENT *et al.*, 1999; WICKER *et al.*, 2001).

Os retrotransposons do tipo LTR são altamente abundantes nos genomas das plantas e já foram chamados de “DNA lixo”, pois pensava-se serem inertes, em contraste com os genes. Entretanto, foi observado que devido ao seu modo replicação do tipo “copia e cola”, com moléculas de RNA servindo como moldes tanto para tradução quanto para transcrição reversa, esses elementos requerem atividade transcricional. Isso permite que essas sequências alcancem um número altíssimo de cópias no genoma (QIU; UNGERER, 2018; SCHULMAN, 2013). Há diversas evidências de atividade transcricional, tais como observadas em espécies em divergência climática e estresse ambiental (GRANDBASTIEN, 1998; KALENDAR *et al.*, 2000).

Estudos de distribuição cromossômica desses elementos têm sido muito importantes para compreender a dinâmica de evolução dos mesmos, o que permite comparar a composição genômica entre espécies de um mesmo gênero (DE SOUZA *et al.*, 2018) ou de grupos não

diretamente relacionados. Alguns desses elementos são especialmente enriquecidos na região do centrômero, como os Ty3/gypsy, especificamente do clado CRM, encontrado em diversas espécies (NEUMANN *et al.*, 2021; SU *et al.*, 2018; TRAN *et al.*, 2015). Uma das hipóteses é que esse CRM é direcionado para o centrômero por um motivo específico, um cromodomínio, que se localiza no C-terminal de sua integrase. O fato de estar presente em muitas espécies, aliado aos estudos de atividade transcricional deste retroelemento, sugere que o CRM pode desempenhar um papel importante para os centrômeros (NEUMANN *et al.*, 2011).

1.1.4 Elementos repetitivos e evolução do genoma

Antes de serem descobertos e posteriormente descritos em 1940-50 por Bárbara (MCCLINTOCK, 1950) os elementos transponíveis eram chamados de DNA lixo, pois se tinha uma visão de que esse DNA não codificava proteínas. Entretanto, após décadas de estudos foi visto que os TEs desempenham um papel fundamental na função do genoma, na diversidade e evolução dos cromossomos, e na especiação (KLEIN; O'NEILL, 2018). A ausência de correlação entre tamanho do genoma e sua complexidade, denominada como paradoxo do valor C, é em parte causada pelos DNAs repetitivos, sendo um fenômeno comum observado em plantas superiores (FEDOROFF, 2012). O acúmulo (amplificação) ou perda (remoção/deleção) de elementos repetitivos, em conjunto com os eventos de poliploidia, são os principais responsáveis pela variação no tamanho do genoma entre espécies (DE SOUZA *et al.*, 2018). A proporção de Ty1/copia e Ty3/gypsy não obedece a um padrão entre as famílias de plantas, variando amplamente. Por exemplo, Ty1/copia é mais abundante em *Rhynchospora pubera* (MARQUES *et al.*, 2015) e *Musa* L. (D'HONT *et al.*, 2012) enquanto Ty3/gypsy é mais abundante em algumas espécies de *Helianthus* L. (QIU; UNGERER, 2018) e *Solanum lycopersicum* L. (PAZ *et al.*, 2017).

Raramente os elementos transponíveis estão distribuídos aleatoriamente no genoma. Em alguns casos, eles podem ter se amplificado em determinado genoma e estarem envolvidos na diferença entre os conteúdos de DNA total de algumas espécies, como o Ty3/gypsy em *Helianthus* e o retrotransposon BARE-1 em *Hordeum* L. (KALENDAR *et al.*, 2000; QIU; UNGERER, 2018). Os elementos transponíveis desempenham um papel importante na resposta de seus hospedeiros diante dos estresses ambientais, podendo afetar, por exemplo a função de genes individuais, bem como influenciar na regulação gênica (MAKAREVITCH *et al.*, 2015). Embora seja difícil identificar essas mutações no genoma e fazer inferências sobre a relação

entre os elementos transponíveis e das mudanças ambientais, alguns exemplos podem ajudar a elucidar a correlação entre as inserções dos elementos em genes e suas consequências para o hospedeiro. A reinserção de um retrotransposon pode levar a uma duplicação gênica, como ocorreu no tomateiro, no qual o gene regulador da forma alongada da planta (SUN) teve sua expressão aumentada pelo efeito dessa duplicação (XIAO *et al.*, 2008). Em Brassicaceae, o retrotransposon ONSEN é ativado em estresse térmico ambiental e foi observado uma preferência por regiões gênicas, o que sugere um possível papel regulatório (ITO *et al.*, 2013). Outro exemplo é o melanismo industrial em *Biston betularia*, onde houve uma substituição da forma típica pálida para a forma negra. Um retrotransposon grande se inseriu no gene chamado *Cortex*, provocando o melanismo nessa espécie (HOF *et al.*, 2016). Além disso, os retrotransposons do tipo LTR podem também desempenhar um papel na diversidade cariotípica entre grupos relacionados, como em *Eleocharis niederleinii* Boeckeler, representante da família Cyperaceae, onde houve acúmulo significativo de LTR em alguns cromossomos, explicando também o aumento do tamanho do genoma dessa espécie em comparação com as demais do gênero (DE SOUZA *et al.*, 2018). Em espécies de *Brachiaria*, retroelementos da mostraram localização proximal, sugerindo ser essa região um hotspot para inserção desses elementos, especialmente da superfamília Ty3/Gypsy (SANTOS *et al.*, 2015). Esses resultados mostram o importante papel desses elementos na evolução dos genomas e das espécies.

1.2 CLADO CYPERID

O Clado Cyperid é composto pelas famílias Thurniaceae, Juncaceae e Cyperaceae, sendo bem suportado filogeneticamente (CHASE *et al.*, 2006) e também incluiria Rapataceae e Mayacaceae, porém com um baixo suporte filogenético (BOUCHENAK-KHELLADI; MUASYA; LINDER, 2014). Cyperid foi considerado por muito tempo como um clado holocêntrico e esta característica seria uma sinapomorfia do grupo (GREILHUBER, 1995). Em algumas espécies, a holocentricidade foi confirmada por meio de detalhadas análises (HECKMANN *et al.*, 2013; MARQUES *et al.*, 2015). Já para a grande maioria, os pequenos cromossomos dificultam a correta identificação dos centrômeros e a presença de holocentrômeros é atribuída apenas pela proximidade evolutiva (BAEZ *et al.*, 2020; CUACOS; H. FRANKLIN; HECKMANN, 2015). Descobertas recentes, todavia, mostram que o clado possui representantes com cromossomos monocêntricos, como algumas espécies de *Juncus* (Juncaceae) e a espécie do gênero monotípico *Prionium serratum* (Thurniaceae) (BAEZ *et al.*, 2020; GUERRA; RIBEIRO; FELIX, 2019). Através de uma análise detalhada da meiose e da

observação do padrão de histonas centroméricas em duas espécies de *Juncus*, foi confirmado a monocentricidade desses representantes do gênero, levantando a hipótese de que outros gêneros de Juncaceae, ou mesmo do clado Cyperid, poderiam ter espécies com cromossomos monocêntricos (GUERRA; RIBEIRO; FELIX, 2019). Além disso, uma análise meticulosa em *Prionium serratum* (Juncaceae) demonstrou que essa espécie também é monocêntrica. Com o uso de CENH3 desenvolvida para a espécie, além de outras histonas, como H2AT120 e H3S10ph, foi possível observar o padrão de distribuição cromossomômico *dot-like* característica dos monocentrômeros, bem como a tubulina ligada a regiões discretas de CENH3. O trabalho também revelou que *P. serratum* possui satélites centroméricos, confirmados pela colocalização com a CENH3 (BAEZ *et al.*, 2020).

1.2.1 Família Cyperaceae Juss.

A família Cyperaceae Juss. tem uma distribuição cosmopolita, principalmente nos trópicos. Tem cerca de 5.500 espécies distribuídas em 2 subfamílias (Mapanioideae e Cyperoideae), 98 gêneros e 15 tribos, constituindo a terceira maior família de monocotiledôneas em diversidade de espécies, depois de Orchidaceae Juss. e Poaceae Barnhart (GOVAERTS *et al.*, 2018; SEMMOURI *et al.*, 2019). Algumas ciperáceas possuem importância econômica, sendo relevantes para a etnobotânica e horticultura (SIMPSON; INGLIS, 2001). A família é originária do hemisfério sul, tendo dispersado depois para o resto do mundo, com duas migrações no Cretáceo para a Austrália da América do Sul, primeiro da subfamília Mapanioideae e segundo da tribo Schoeneae. Mapanioideae teve maior diversificação na Austrália e América do Sul, e algumas tribos como Trilepideae, Sclerieae, Rhynchosporeae também se originaram na América do Sul, com ocasionais inserções no hemisfério norte, sendo a primeira no Paleoceno (SPALINK *et al.*, 2016). Apesar de a diversidade ser maior nos trópicos, o gênero *Carex* L. é um dos gêneros mais ricos em espécies e se distribui em regiões temperadas (GLOBAL CAREX GROUP, 2015).

Devido às suas inflorescências complexas e morfologia floral reduzida, a família apresenta dificuldades de delimitação. Uma das características do grupo mais informativos filogeneticamente são os embriões, e junto aos dados moleculares, suportam sua monofilia e a divisão em duas subfamílias (Mapanioideae e Cyperoideae). O estudo também suporta a estreita relação com Juncaceae (MUASYA *et al.*, 2009; SEMMOURI *et al.*, 2019).

Cyperaceae constitui um especializado grupo de plantas, especialmente com relação a sua estrutura reprodutiva (KUKKONEN, 1994). A polinização das espécies da família é feita principalmente pelo vento, ou seja, são anemófilas (JUDD, 2008), com suas espécies possuindo traços anemófilos, tais como as flores esverdeadas, inodoras e sem nectários (ENDRESS, 1998). A maioria das Cyperaceae tem pólen em forma de pera, de paredes finas, denominado "pseudomonads", para polinização pelo vento, porém a tribo Hypolytreae tem pólen do “tipo Mapania”, revestido com lipídios, sugerindo polinização por animais (SIMPSON *et al.*, 2003). O pólen do tipo pseudomonads parece ser uma sinapomorfia para Cyperaceae (FURNESS; RUDALL, 2011) e pode ter contribuído para o grande sucesso reprodutivo do grupo (ROCHA; VANZELA; MARIATH, 2018).

1.2.2 Citogenética de Cyperaceae

Diversos gêneros de Cyperaceae, como *Rhynchospora, Eleocharis, Bulbostylis* e *Fimbristylis* têm dados citogenéticos que mostram a ocorrência de cromossomos holocêntricos no grupo (DE SOUZA *et al.*, 2018; LÓPEZ; AVELIANO; FLORENCIA, 2017; MARQUES *et al.*, 2015; ZUMSTEIN; WANGWASIT; DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCE, MAHASARAKHAM UNIVERSITY, MAHASARAKHAM 44150, THAILAND, 2019). Em *Eleocharis*, análises da meiose mostram com clareza a natureza holocêntrica desse gênero. Todavia, espécies dêe *Rhynchospora* possuem dados são mais detalhados, pois foi realizada a imunocoloração com a proteína CENH3 para confirmar a distribuição desta ao longo de toda extensão cromossômica (MARQUES *et al.*, 2015; SANTOS *et al.*, 2015). A maioria dos estudos citogenéticos da família Cyperaceae foi realizado na subfamília Cyperoideae, com alguns dados de contagem cromossômica para a subfamília Mapanioideae (DIAS *et al.*, 2020; MÁRQUEZ-CORRO *et al.*, 2018). A subfamília Cyperoideae apresenta variabilidade intraespecífica, intra- e intergenérica de números cromossômicos (ARGUELHO *et al.*, 2012; HIPP; ROTHROCK; ROALSON, 2009; LÓPEZ; AVELIANO; FLORENCIA, 2017).

As espécies de Cyperaceae apresentam uma dinâmica evolutiva envolvendo fenômenos de fissões e fusões cromossômicas devido aos cromossomos holocêntricos, meiose invertida em alguns representantes, e variação no tamanho do genoma (CABRAL *et al.*, 2014; DE SOUZA *et al.*, 2018; HIPP; ROTHROCK; ROALSON, 2009). Os eventos de fissão e fusão dificultam o estabelecimento de um número básico para a família, todavia, a ocorrência de várias espécies

com números cromossômicos múltiplos de 5, sugere que esse seja o número básico de Cyperaceae, com outros números possíveis, tais como *x* = 2, 4, 6 e 12 (LUCEÑO *et al.*, 1998).

Com relação à distribuição de sítios de DNAr 5S e 35S, Cyperaceae possui ampla variabilidade, com espécies variando entre 2 a 10 sítios de 5S e entre 2 e 45 sítios de 35S. A posição do DNAr 35S foi sempre terminal para todas as espécies analisadas, já a posição do DNAr 5S é terminal ou intersticial (DA SILVA; QUINTAS; VANZELA, 2010; SOUSA *et al.*, 2011; RIBEIRO *et al.*, 2017). Essa distribuição predominantemente terminal do DNAr também foi observada em outras espécies com cromossomos holocêntricos, tais como *Drosera* e *Cuscuta* (FURUTA; KONDO, 1999; GUERRA; GARCÍA, 2004). No caso do 35S, a variabilidade em número e posição dos sítios pode estar relacionada ao seu potencial de recombinação ectópica (PEDROSA-HARAND *et al.*, 2006).

As bandas de heterocromatina constitutivas observadas em *Rhynchospora* apresentam um padrão similar ao observado em monocêntricos, com bandas mais fortes ou fracamente marcadas na região terminal e intersticial (VANZELA; GUERRA, 2000). Além disso, foi observada também a predominância de bandas heterocromáticas terminais em espécies do gênero *Eleocharis,* frequentemente associada às NORs, constituindo bons marcadores para diferenciar os seus subgêneros (DA SILVA; QUINTAS; VANZELA, 2010; MAXIMIANO DA SILVA *et al.*, 2008).

Em cromossomos monocêntricos é comum a presença de DNAs satélites específicos do centrômero, já em holocêntricos, devido a diferenças na organização centromérica, acreditava-se na ausência dessas sequências no centrômero A espécie *Luzula elegans*, pertencente à família irmã de Cyperaceae, por exemplo, teve seu genoma amplamente analisado, porém os satélites encontrados não têm distribuição centromérica (HECKMANN *et al.*, 2013). Entretanto, ao analisar *Rhynchospora pubera* foi descoberto a primeira sequência de DNA satélite centrômero-específico, denominado *Tyba*. Além disso, foi encontrado um retrotransposon centromérico do tipo LTR, linhagem CRM (Ty3/gypsy, CRRh) (MARQUES *et al.*, 2015). O satélite *Tyba* foi encontrado em apenas três das quatro espécies estudadas do gênero *Rhynchospora* (RIBEIRO *et al.*, 2017), o que deixa em aberto se outras espécies da mesma família teriam algum satélite centrômero-específico ou se CRRh seria mais conservado em outras espécies do gênero e família. Além disso, a subfamília Mapanioidae não possui dados citogenômicos que auxiliem na compreensão da composição genômica e organização centromérica de espécies de divergência precoce na família Cyperaceae.

O objetivo desta tese foi compreender, através de diferentes técnicas citogenômicas e bioinformáticas, a composição, distribuição e estrutura cromossômica de espécies pertencentes

ao Clado Cyperid. A tese está dividida em três capítulos, os quais tratam de diferentes famílias e gêneros dentro deste clado, com maior ênfase em análises centroméricas, composição do DNA repetitivo e sua distribuição ao longo dos cromossomos.

3. CAPÍTULOS

3. CYTOGENOMIC ANALYSIS IN SOME Mapanioideae SPECIES SUGGESTS A LATER TRANSITION TO HOLOCENTRICITY IN Cyperaceae

Yhanndra Dias, Mariana Baéz, Yi-Tzu Kuo, Lucas Costa, Veit Schubert, André Vanzela, Marcus Alves, Andreas Houben, Andrea Pedrosa-Harand

Artigo a ser submetido à revista Botanical Journal of the Linnean Society

Link: https://academic.oup.com/botlinnean

AN OVERVIEW OF THE REPETITIVE FRACTION IN MAPANIOIDEAE SPECIES REVEALS LEAN GENOMES

Yhanndra Dias, Mariela Analía Sader, Yi-Tzu Kuo, Lucas Costa, Mariana Baéz, Veit Schubert, André Vanzela, Marcus Alves, Andreas Houben, Andrea Pedrosa-Harand

Artigo a ser submetido à revista Botanical Journal of the Linnean Society

Link: https://academic.oup.com/botlinnean

AN OVERVIEW OF THE REPETITIVE FRACTION IN MAPANIOIDEAE SPECIES REVEALS LEAN GENOMES

Yhanndra Dias[1], Mariela Analía Sader, Yi-Tzu Kuo[3], Lucas Costa[1], Mariana Baéz[2], Veit Schubert[3], André Vanzela[5], Marcus Alves[4], Andreas Houben[3], Andrea Pedrosa-Harand[1]

[1]*Laboratório de Citogenética e Evolução Vegetal, Departamento de Botânica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil*

[2]*Plant breeding Department, University of Bonn, Bonn, Germany*

[3]*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany*

[4]*Laboratório de Morfo-Taxonomia Vegetal, Departamento de Botânica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Brasil*

[5]*Laboratório de Citogenética e diversidade de plantas, Departmento de Biologia geral, Universidade Estadual de Londrina, Londrina, Paraná, Brasil*

ORCIDs:

Yhanndra Dias, 0000-0002-6488-4736

Mariela Analía Sader, 0000-0001-8188-2217

Yi-Tzu Kuo, 0000-0001-5893-3784

Mariana Baéz, 0000-0002-7874-6385

Veit Schubert, 0000-0002-3072-0485

André Vanzela, 0000-0002-2442-2211

Lucas Costa, 0000-0002-4798-097X

Marcus Alves, 0000-0001-9281-2257

Andreas Houben, 0000-0003-3419-239X

Andrea Pedrosa-Harand, 0000-0001-5213-4770

Corresponding author:

Andrea Pedrosa-Harand

Universidade Federal de Pernambuco

Centro de Biociências

Departamento de Botânica

Laboratório de Citogenética e Evolução Vegetal

R. Prof. Moraes Rego, s/n, CDU

50670-901 Recife PE Brazil

Tel: + 55 81 2126 8846 or 8352

Fax: + 55 81 2126 8348

ABSTRACT

Although most species have chromosomes with a localized centromere (monocentric) visible as a primary constriction, some group of organisms have developed holocentric chromosomes, where centromeres appear dispersed along most of their length. Until recently, the Cyperid clade (Cyperaceae, Juncaceae and Thurniaceae), was believed to be entirely composed of species with holocentric chromosomes. However, the existence of monocentric species in both latter families has been recently demonstrated, making the investigation of early diverging lineages in Cyperaceae crucial for understanding when the transition from mono- to holocentricity evolved within this family. To better understand the centromere organization in this family, we analysed the localization of the centromeric protein CENH3 and the repeat composition and distribution in the early divergent species of subfamily Mapanioideae, *Hypolytrum schraderianum*. Repeat abundance and similarity was then compared to *Diplasia karatifolia* and *Mapania sylvatica*, two other species from the same subfamily. A considerable abundance of satellite DNAs was found in all three species, despite a small proportion of repetitive elements in these small-sized genomes. The most abundant satDNA of *H. schraderianum* was a non-centromeric element, rather subtelomeric, while Ty3-gypsy elements showed a proximal distribution. Our data show the importance of further investigations within the Mapanioideae clade, in order to analyze the evolution of repeats and centromeric composition.

KEYWORDS: Sedges, Holocentricity, Immunostaining, satellite DNA, LTR retrotransposons

INTRODUCTION

Centromeres are essential for genome stability, mediating chromosome segregation in mitosis and meiosis. An active centromere is defined by the presence of the centromeric histone variant CENH3 (also known as CENPA), present in most eukaryote centromeres. The majority of plant species have monocentric chromosomes, known for their usually visible primary constriction and the restricted location of CENH3 (Cuacos *et al.*, 2015). However, holocentric chromosomes do not show a primary constriction and their centromeric activity is dispersed over almost the entire chromosome length (Melters *et al.*, 2012). It is estimated that holocentricity has appeared at least 14 times independently over the course of evolutionary history, five times in plants and nine in animals. Although it is uncertain whether the last common ancestor of all eukaryotes was holocentric or monocentric, the monocentric state is considered ancestral (Melters *et al.*, 2012; Schubert *et al.*, 2020; Senaratne *et al*., 2022).

In plants, holocentric chromosomes were described in members of the families Cyperaceae Juss. (Maximiano da Silva *et al.*, 2008; Marques *et al.*, 2015; Chung *et al.*, 2018) and Juncaceae Juss. (Heckmann *et al.*, 2013), as well as in Melanthiaceae Batsch (Tanaka & Tanaka, 1977), *Cuscuta* L. (Convolvulaceae) and *Drosera* L. (Droseraceae) (Pazy & Plitmann, 1994; Sheikh & Kondo, 1995; Shirakawa *et al.*, 2011). The Cyperid clade, formed by the families Thurniaceae, Juncaceae and Cyperaceae, has a well-supported phylogenetic relationship and the holocentricity has long been attributed as a synapomorphy of the group (Greilhuber, 1995; Chase *et al.*, 2006; Givnish *et al.*, 2010). However, the presence of both holocentric and monocentric organization in certain taxa previously considered to be composed of only holocentrics shows that this extrapolation might be incorrect.. Recent findings of monocentric species in species of *Juncus* L. (Juncaceae) and in the monotypic *Prionium* (Thurniaceae) are examples (Guerra *et al.*, 2019; Baez *et al.*, 2020).

The family Cyperaceae has about 5,500 species divided into two subfamilies (Mapanioideae, c. 180 species, and CyperoideaeCyperoideae, c. 5,350 species) with a wide range of chromosome numbers, especially in the subfamily Cyperoideae, ranging from $2n = 4$ to 224 (Roalson, 2008; Semmouri *et al.*, 2019). The subfamily Mapanioideae, still underrepresented, has less variability in chromosome numbers (Márquez-Corro *et al*., 2018; Dias *et al*., 2020). The Cyperoideae genera such as *Bulbostylis* Kunth, *Carex* L., *Eleocharis* R.Br., *Fimbristylis* Vahl and *Rhynchospora* Vahl contain species with holocentric chromosomes (Maximiano da Silva *et al.*, 2008; Marques *et al.*, 2015; López *et al.*, 2017; Chung *et al.*, 2018; Zumstein *et al.*, 2019). However, demonstration of holocentricity by means

of location of the specific centromeric CENH3 or meiotic behavior are only available for *Rhynchospora* and *Eleocharis* (Maximiano da Silva *et al.*, 2008; Marques *et al.*, 2015; Ribeiro *et al.*, 2017).

Repetitive elements are important components of centromeres of most species (Plohl *et al.*, 2014) and one of the main causes for the differences in genome sizes between species, leading to genomic abundance variation such as 3% in the small genome of *Utricularia gibba* L., up to 85% in larger genomes such as maize (Schnable *et al.*, 2009; Ibarra-Laclette *et al.*, 2013). In an analysis with 50 plants, the increase in genome size was positively correlated with the abundance of repetitive elements (Michael, 2014). In contrast, some species with small genomes, such as in carnivorous plants, a drastic reduction in the repetitive fraction was observed (Leushkin *et al.*, 2013; Ibarra-Laclette *et al.*, 2013). The transposable elements are the main constituents of most plant genomes (Schnable *et al.*, 2009; Ibarra-Laclette *et al.*, 2013), except for few species in which the satellite DNA fraction is the most abundant (Han *et al.*, 2008; He *et al.*, 2015).

Both transposable elements and satellites DNA can assist in the identification of the centromere location/type, especially in karyotypes with small chromosomes, wherethe identification of the primary constriction is difficult, such as in species of *Phaseolus* (Ribeiro *et al.*, 2020), *Arabidopsis thaliana* (Shibata, 2004) and *Prionium serratum*. In this latter species, a combined approach showed colocalization of centromeric satellites and CENH3 at the primary constriction of its small chromosomes, confirming yet another monocentric representative within Cyperid, in addition to *Juncus* (Guerra *et al.*, 2019; Baez *et al*., 2020).

In this work, aiming to investigate the repeats composition in the Cyperaceae family, we analyzed three species of the early divergent subfamily Mapanioideae (*Diplasia karatifolia* Rich., *Hypolytrum schraderianum* Nees. and *Mapania sylvatica* Aubl.). The repetitive fraction of these three genomes was comparatively analysed and a possible correlation with the centromeric organization was tested by means of the chromosomal location of some repeats.

METHODS

Plant material

Adult individuals of *Diplasia karatifolia* Rich., *Hypolytrum schraderianum* Nees and *Mapania sylvatica* Aubl., used here for Next-Generation Sequencing (NGS) and flow cytometry, were

collected in the Amazon rainforest. Two additional individuals of *H. schraderianum* were collected in the Atlantic Forest. Details about collection sites are given in Table 1.

Genome size measurements

The DNA content of *the three species* was estimated by flow cytometry according to Loureiro *et al*. (2007). Briefly, young leaves were chopped simultaneously with the reference standard [*Raphanus sativus* cv. saxa (2C = 1.11 pg) or *Lycopersicon esculentum* cv. Stupicke (2C = 1.96 pg)] in a Petri dish kept on ice, containing 2 mL of Woody Plant Buffer (WPB). The samples were filtered through a 30 µm disposable mesh (CellTrics, SYSMEX, Norderstedt, Germany) followed by addition of 50 µg/mL propidium iodide (from a stock of 1 mg/mL; Sigma-Aldrich) and 50 µg/mL RNase. Up to three individuals of each species were measured, with three replicates per individual. The samples were measured in a CyFlow Space flow cytometer (SYSMEX, Norderstedt, Germany) equipped with a green laser (532 nm). Alternatively, leaves from one *H. schraderianum* individual were chopped with the reference standard in a petri dish using the reagent kit 'CyStain PI Absolute P' (Sysmex) following the manufacturer's instructions. The resulting nuclei suspension was filtered through a 50 µm mesh (CellTrics, Sysmex) and measured on a BD Influx cell sorter (BD Biosciences).

The histograms of relative fluorescence were obtained using the software Flomax v.2.3.0. (SYSMEX, Norderstedt, Germany). Mean fluorescence and coefficient of variation were assessed at half of the fluorescence peak. The DNA content (2C) of the target species was calculated using the equation "2C target (pg) = (G1 target/G1 standard) x 2C standard (pg)". "G1" refers to the mean fluorescence value emitted by nuclei in the G1 stage of interphase and "2C standard" refers to the DNA content from somatic cell nuclei of the reference standard used in the measurement.

Genomic DNA extraction, genome sequencing and repeat characterization

Young leaves from *D. karatifolia, H. schraderianum* and *M. sylvatica* were used for genomic DNA extraction using the CTAB protocol (Ferreira & Gratapaglia, 1998). The samples were quantified on 1% agarose gel and Nanodrop, and then, sequenced using the HiSeq 2500 platform (Illumina) by BGI for generating 250-bp paired-end reads with a low genome coverage (1 Gbp per sample).

Satellite DNAs (satDNAs) and transposable elements (TEs) were characterized individually and comparatively by graphic-based clustering using the RepeatExplorer pipeline (Novak *et al.,* 2017). A total of 500,000 reads were analysed for each species, corresponding to approximately c. 0.3-0.4× genome coverage each. A custom database including previously identified tandem repeats (Marques *et al.,* 2015; Ribeiro *et al.,* 2017). was included to aid annotation (Neumann *et al.,* 2019). Clusters with a percentage above 0.01% of the genome were manually checked in order to identify the most abundant repeat families. SatDNAs were identified using the TAREAN tool (Tandem Repeat Analyzer) also implanted in RepeatExplorer pipeline (Novák *et al.,* 2017). A dot-plot was performed to compare and identify the satellite families for each species and between species by command line using DOTTER (Sonnhammer & Durbin, 1995). The similarity identified between clusters with DOTTER was used to classify families. Satellite DNA families were then named starting with the abbreviation of the species (for example, Hsc for *Hypolytrum schraderianum*) followed by the term "SAT", the order of abundance and length of the monomer after the hyphen.

Probe generation and fluorescence *in situ* hybridization (FISH)

Cytogenomic analyzes were performed using a sample of *H. schraderianum* from Paraná. We selected two variants of satDNA sequences from the same family (HscSat1-182), 5S and 35S rDNA, as well as a LTR retrotransposon from the Ty3/gypsy Chromovirus Reina lineage for FISH. For PCR amplification of HsSat1-182 and Reina, primers were designed in Primer 3 (Untergasser *et al.,* 2012) implemented in Geneious version 9.1 (http://www.geneious.com). Primers were designed facing outwards in the case of HscSat1-182, while for Reina, the integrase domain was used after confirmation through NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi; Marchler-Bauer *et al*., 2017). For 35S rDNA probe, p*Ta*71 clone of wheat (25-28S, 5.8S and 18S rDNA; Gerlach, Bedbrook, 1979) was amplified by miniprep and labelled by nick translation with Alexa488-dUTP, while for the 5S rDNA probe, a combination of four Cy3-labelled oligonucleotides were used (Waminal *et al*., 2018).

PCR were performed in 50 μL reactions containing 20 ng of genomic DNA from *H. schraderianum*, 0.1 mM dNTP, 2 mM $MgCl_2$, 1× PCR buffer, 0.4 μM of each primer (Suppl Table 1), 0.4× TBT and a homemade Taq DNA polymerase. The program involved 30 cycles of amplification (1 min at 95°C, 1 min at the annealing temperature, see supp Table 1, and 1 min at 72°C). Sequence identity for each element (HscSat1-182 and Reina) were confirmed by sequencing PCR products in ABI 3500 sequencer (Applied Biosystems®) at the Sequencing

Platform of the Bioscience Centre at Federal University of Pernambuco.For probes, DNA was labelled by nick translation with Atto488 or Atto550 using a labelling kit following manufacturer's instructions (Jena Bioscience).

For slide preparations, roots were pretreated with 2mM 8-hydroxyquinoline for 24 h at 4°C, fixed in ethanol:acetic acid 3/1, v/v) for 2 h and stored at −20°C. After enzymatic digestion with 2% cellulose, 2% pectinase, 2% pectolyase in citrate buffer (0.01 M sodium citrate dihydrate and 0.01 M citric acid) for 2 h and 30 min at 37°C, mitotic slides were prepared by air-drying (Carvalho & Saraiva, 1993). The best slides were selected for Fluorescence *in situ* hybridization (FISH) and destained using Carnoy for 30 min and 100% ethanol for 1 h. FISH was perfomed as described by Aliyeva-Schnorr *et al*. (2015) and the slides were counterstained with DAPI (4′,6-diamidino-2-phenylindole) (Sigma) in antifade (Vectashield, Vector). Images were captured using an epifluorescence microscope BX61 (Olympus) and processed for brightness and contrast using Adobe Photoshop version CS5.

Indirect immunostaining

For chromosome preparations, young roots from the same individual used before were fixed in 4% paraformaldehyde in Tris buffer (10 mM Tris, 10 mM EDTA, 100 mM NaCl, 0.1% Triton, pH 7.5) for 5 minutes on ice in a vacuum and for another 25 minutes on ice only. The slides were prepared using a Cytospin (Shandon, Germany) as described by Jasencakova *et al* (2001). Immunostaining was performed as described by Houben et al. (2007). The *Rhynchospora pubera* CENH3 (rabbit anti-RpCENH3, Marques *et al*., 2015) was used as a primary antibody (diluted 1:200) and a Cy3-conjugated anti-rabbit IgG (Dianova) (diluted 1:500) was used as a second antibody. After incubating overnight at 4ºC, the slides were washed 3 times with 1× PBS and the secondary antibody was applied for 1 h at 37ºC. Slides were then washed 3 times with 1 ×PBS and counterstained and captured as described above.

RESULTS

Small genomes of Mapanioideae showed low repeat abundance but high diversity of elements

In order to investigate the genomic organization in species of Mapanioideae, we characterized the repetitive DNA fraction of three species from different genera. First, we

estimated the genome sizes of *D. karatifolia, H. schraderianum* and *M. sylvatica* by flow cytometry to calculate sequencing coverage. All three species showed small DNA content: *D. karatifolia* and *M. sylvatica* showed 1C = 0.335 pg and 0.305 pg, respectively, while individuals from *H. schraderianum* showed 2C = 0.275, 0.285 and 0.375 pg. Despite this intraspecific variation, no correlation with chromosome number was observed (Table 1, Dias *et al*., 2020).

Cluster-based analysis of repeat composition in *D. karatifolia, H. schraderianum* (from Amazonia) and *M. sylvatica* revealed a total low abundance of repeats (17.68,23 and 4.74%, respectively), as expected for their small genome sizes (Table 1, Table 2). Satellite DNAs were the major components in all three species, followed by the LTR retrotransposons from different lineages (Fig. 1, Table 2). Comparative analysis of all clusters (Fig. 1) did not suggest closer proximity between these species considering sequence similarity, as expected, because they are from different genus. While LTR-retrotransposons were always shared between at least two species, the satDNA clusters were usually exclusive from one or other, indicating higher sequence divergence for these latter elements.

In *M. sylvatica*, the satellite DNAs constitutes 1.75% of genome. We identified two satellite DNAs, the MsySAT1-181 formed by six clusters and MsySAT2-475 formed by one cluster (Suppl. Fig. 1B). In *H. schraderianum*, satellite DNAs represented 6.954% of the genome and were grouped into 11 clusters. After sequence comparison, they were divided into two families composed by the most abundant family, the HscSAT1-182 and HscSAT2-1422 (Suppl. Fig. 1C). Nevertheless, although TAREAN has identified this large satellite (HscSAT2-1422) with high confidence, it was not possible to amplify it by PCR using different primer combinations (data not shown), being excluded from further analyses. The satellites DNAs of *D. karatifolia* were grouped into 30 clusters, which represent three families, named DkaSAT1-437-1730, DkaSAT2-28-2350 and DkaSAT3-260 with 17, 12 and 1 cluster respectively (Suppl. Fig. 1D) and together they represented 5.387% of the genome of this species. Despite their relative abundance, satDNAs showed low sequence divergence within species, with two or three families each (Suppl. Fig. 1).

Transposable element composition diverged among the three species. *Diplasia karatifolia* is composed by 11 lineages of LTR retrotransposons, five Ty1/copia and six Ty3/gypsy. From the nine lineages that comprise *H. schraderianum* genome, four were Ty1/copia and five Ty3/gypsy. The Ty1/copia superfamily has a higher abundance in the genome of this species (92% of LTR elements). The species with the lowest proportion of transposable elements is *M. sylvatica*, with only six lineages of retroelements, three Ty1/copia

and three Ty3/gypsy, being the Ty1/copia superfamily representing the vast majority (84%) of LTR elements.

The Ty1/copia Angela lineage is present in all three species, being the most abundant retroelement in *H. schraderianum* and *M. sylvatica*, while Ty3/gypsy Retand lineage is more abundant in *D. karatifolia* (Suppl. Table 2). The Chromovirus clade was observed in low abundance, with only one cluster with at least 0,01% abundance (Galadriel) in *M. sylvatica*, and three lineages (CRM, Reina and Tekay) in *D. karatifolia* and *H. schraderianum* (Suppl. Table 2). Other lower abundant elements were also identified, such as LINEs in *H. schraderianum*, pararetrovirus in *D. karatifolia*, as well as DNA transposons and ribosomal DNAs (Fig. 1, Suppl. Table 2).

Chromosomal distribution of repetitive elements in *H. schraderianum*

In order to investigate the chromosomal distribution in *H. schraderianum* we selected two variants of the HscSAT1-182 satDNA family (HsSAT1/1-182, HsSAT1/2-182) due their abundance, and the most abundant Chromovirus element (Reina), besides rDNA, for *in situ* hybridization on mitotic chromosomes. The HscSAT1/1-182 and HscSAT1/2-182 belong to the same family and have the same monomer size, however probes from unique regions for each subfamily were used and hybridized together on the same slide. Both subfamilies showed distinct signals at interstitial and terminal regions, with only a few partially colocalized signals on some chromosome pairs (Fig. 2A). The Chromovirus Reina probe showed mostly dot-like signals at insterstitial regions of all chromosomes, perhaps due to the condensation of the small chromosomes of this species, which needs a better investigation about the distribution of this element (Fig. 2B). *H. schraderianum* has only one 5S rDNA and one 35S cluster per haploid complement (Fig. 2C).

Monocentricity in *Hypolytrum schraderianum* is evidenced by RpCENH3 immunostaining

To test whether Mapanioideae in Cyperaceae has holocentric or monocentric chromosomes, we performed immunostaining in *H. schraderianum* using an antibody developed against the centromere protein CENH3 from *Rhynchospora* (subfamily Cyperoideae; Marques *et al*., 2015). The small chromosomes of *H. schraderianum* hindered the observation of clear primary

constrictions, typical for monocentric chromosomes. We did not observe the line-like pattern expected for holocentrics, but the signals for RpCENH3 were restricted to a small region in most chromosomes, suggesting the presence of monocentric chromosomes within this species (Fig. 3A). In interphase nuclei, similar CENH3 clusters were observed, not associated with the heterochromatic chromocentres that were frequenly observed at the nuclear periphery (Fig. 3B).

DISCUSSION

Diversity of transposable elements in species with a small genome

The plants studied here did not have considerable variation neither in chromosome number (Dias *et al*., 2020) or genome size. The subfamily Mapanoideae is thus so far characterized by small genome sizes (between 269 – 367 Mbp), and a shorter range of chromosomes numbers, from 2*n* = 46 to 2*n* = 76 (Márquez-Corro *et al*., 2018; Dias *et al*., 2020) when compared to the Cyperoideae. In this later subfamily, there is a wide variation in DNA content and chromosome number, from 2*n* = 4 to 2*n* = 224, generaly attributed to the holocentricity nature of the best studied genera of this subfamily (Lipnerova *et al.*, 2013; Ribeiro *et al.*, 2018; de Souza *et al.*, 2018). Although recently challenged in insects (Senaratne *et al.*, 2022), this higher variation in chromosome number in holocentric organisms is probably caused during cell division, because chromosome breaks generate fragments with centromeric activity (Jankowska *et al.*, 2015), which could potentially give rise to new chromosomes (Kandul, Lukhtanov, & Pierce, 2007; Roalson, 2008). Identifying the point of transition from monocentric to holocentric in Cyperaceae is also important for investigating correlations between holocentricity and diversification in the family and in the Cyperid clade (Márquez-Corro *et al*., 2018).

Here we present the first characterization of repetitive DNA in the subfamily Mapanioideae. A low proportion of repeats (4.74 to 23%) was observed in the analyzed species, consistent with their small genomes and small chromosome sizes (Dias *et al*., 2020). The repetitive fraction of these genomes was composed of a diversity of transposable elements lineages, mainly in *D. karatifolia* and *H. schraderianum*, with 11 and nine lineages, respectively. The same occurs with other plants with compact genomes, but highly diverse repetitive fractions, such as in *Prionium serratum, Utricularia gibba, Phaseolus*, *Cajanus* and *Vigna* genus and *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000; Ibarra-Laclette *et al.*, 2013; Ribeiro *et al.*, 2020; Baez *et al.*, 2020). Indeed, it was argued that there is

no relationship between diversity of transposable elements and genome size (Elliott & Gregory, 2015).

Regarding the abundance of the LTRs, they represent 5.116%, 5.025%, 1.16% in *D. karatifolia*, *H. schraderianum* and *M. sylvatica*, respectively. About the superfamilies, Ty3/gypsy is more abundant in *D. karatifolia* (3.093% of the genome) especially due to the presence of the Retand lineage. And Ty3/copia is more abundant in H. schraderianum (3.136% of the genome). The abundances between these superfamilies varied between genera, although all Cyperids analysed so far, except for *D. karatifolia*, they showed a predominance of the Ty1/copia elements over Ty3/gypsy, irrespectively of chromosome type. In Cyperaceae this predominance was observed for species of *Eleocharis* (de Souza *et al*., 2018) and *Rhynchospora* (Marques et al., 2015); in Juncaceae, *Luzula elegans* (Heckmann *et al*., 2013); and in Thurniaceae, *Prionium serratum* (Baez *et al*., 2020). The lineage Angela seems to be the main responsible for the high abundance of Ty1/copia in the three genomes analyzed here, similar to *Luzula elegans*, in which Angela represented about 33% of its genome (Heckmann *et al*., 2013).

Satellite DNAs: big families, despite small diversity

Although the transposable elements are mainly responsible for the pronounced difference in genome sizes in several species (Schnable *et al.*, 2009; Macas *et al.*, 2015; McCann *et al.*, 2020), satellite DNAs can also account for a considerable fraction of some genomes (Heckmann *et al.*, 2013; Ávila Robledillo *et al.*, 2018; Ribeiro *et al.*, 2020). The satellites identified in the species analysed here comprised between 1.750-6.954% of the genome of each genome. None of the identified Mapanioideae satDNAs showed similarity with the holocentromere-specific Tyba (Marques *et al*., 2015). In the family Cyperaceae a lower diversity of satellites (ranging from one to four in the genus *Rhynchospora*) was observed when compared to the other Cyperid families: 19 families in *Prionium serratum* and 37 families in *Luzula elegans* (Heckmann *et al*., 2013; Ribeiro *et al*., 2017; Baez *et al*., 2020). Outside the Cyperid clade, the diversity of satellites may be also high, as in the genus *Vicia*, where the majority of the analyzed species has more than 10 families and the amount of putative satellites reaches 31 and 51 in *V. faba* and *V. peregrina*, respectively (Macas *et al*., 2015). Some species, however, are composed by a low diversity and proportion of satellite repeats (Liu *et al.*, 2019).

Distribution of repeats in *Hypolytrum schraderianum*

We investigated the distribution of the major satDNA familiy of *H. schraderianum*. Satellite DNAs accumulate in different parts of the chromosomes, frequently in the (peri)centromeric or subtelomeric regions (Garrido-Ramos, 2015) and the most abundant satDNA is frequently the centromeric repeat in many species. Satellites HscSAT1/1-182 and HscSAT1/2-182 belong to the same family and have the same monomer size, but showed different localization patterns, with only partial colocalization of signals on some chromosomes. Within Cyperids, a similar pattern occurs in the monocentric *Prionium*, where satellite subfamilies differ in their chromosomes locations, such as PsSat156a and b in *P. serratum* (Baez *et al*., 2020). Although the very small size of the chromosomes makes a precise localization difficult, most signals of both subfamilies were present at terminal and interstitial chromosome regions, suggesting a mostly subtelomeric localization.

The Ty3/gypsy Chromovirus Reina was selected for FISH, because elements of the Chromovirus clade are often associated with the centromeres and pericentromeres in several species and can be useful in identifying these regions, especially in species with small genomes (Van-Lume *et al.*, 2019; Baez *et al.*, 2020). Furthermore, in Cyperids, one element of the Chromovirus clade was identified in the genus *Rhynchospora* distributed throughout the holocentromere (Marques *et al*., 2015). Although Reina is not a typical centromeric repeat such as the CRM (Neumann *et al.*, 2011), it was the most abundant Chromovirus in *Hypolytrum* genome, and was reported in *Beta* as a pericentromeric repeat (Weber *et al.*, 2013). Therefore, making it an interesting target in the investigation of the centromere in *Hypolytrum.* Indeed, in *H. schraderianum* we observed a dot-like pattern for the Reina element, perhaps due to the condensation of the chromosomes, which are already small.

The number of rDNA sites observed in *H. schraderianum* is within the expected range for species of the family. The family Cyperaceae has great rDNA variability, ranging from 2 to 10 5S sites and 2 to 45 35S sites. The 35S rDNA position was always terminal for all species analyzed, whereas the 5S rDNA position is terminal or interstitial in some species (Da Silva *et al.*, 2010; Sousa *et al*., 2011; Ribeiro *et al.*, 2017).

In this work, we analysed the distribution of a CENH3 in *H. schraderianum* to understand the centromere organization in this group, sister to the holocentric Cyperoideae lineage (Semmouri *et al*., 2019). The RpCENH3 antibody generated for *R. pubera* (Marques *et al*., 2015) on *H. schraderianum* chromosomes evidenced signals in a restricted location, arguing for a typical monocentric organization on that species. A similar distribution was also observed for a retrotransposon lineage (Chromovirus Clade), reinforcing that the monocentric

type may be the ancestral centromeric organization in Cyperaceae and might be maintained in the subfamily Mapanioideae.

Monocentricity is likely the ancestral condition for the whole Cyperid clade as well, since this centromere organization was also tracked to two other families within this clade, *Prionium serratum*, from Thurniaceae, and four species from *Juncus*, Juncaceae (Guerra *et al*., 2019; Baez *et al*., 2020). Thus, Holocentric representatives within the family have most likely been originated from monocentric chromosomes, possibly in the Cyperoideae subfamily.

Although the CENH3 antibody used here was designed for *R. pubera* (Marques *et al*., 2015) and also applied to other species of the genus (Ribeiro *et al*., 2017), we could successfully use it for a species from the other subfamily. The fact that *H. schraderianum* CENH3 could be detected with a *R. pubera* antibody suggests some degree of sequence conservation between both centromeric proteins, even when showing different centromere organization. CENH3 function is typically conserved across species, but the protein sequences may vary enough to be specific for a genus (Kowar *et al.*, 2016; Oliveira *et al.*, 2020). In *Cuscuta*, for example, CENH3 structure varied significantly between monocentric species and their holocentric counterparts in a extant that variants could be detected and specific antibodies have to be generated for cytogenetics purporses (Oliveira *et al*., 2020).

CONCLUSION

Despite the low proportion of repetitive elements, compatible with their small genomes, we identified a significant diversity of transposable elements in the three species analyzed in this work. The satellites in *H. schraderianum* have the classic distribution in the subtelomeric region, very common for this type of repeat, and although the two satellites are from the same subfamily, they are not always overlapped in the same region. More efforts are then needed to elucidate centromere composition evolution in this group.

ACKNOWLEDGEMENTS

The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) PROBRAL CAPES/DAAD project number 88881.144086/2017-01) (YD), and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnologico; grant number

141725/2018-4 to Y.D.) for the grants awarded. We are also grateful to Thomas Wayt (The New York Botanical Garden) for the collection of *Hypolytrum schraderianum* from Bahia.

REFERENCES

Albertson DG & Thomson JN. **1993**. Segregation of holocentric chromosomes at meiosis in the nematode,*Caenorhabditis elegans*. *Chromosome Research* **1**: 15–26.

Aldrup-MacDonald M & Sullivan B. **2014**. The past, present, and future of human centromere genomics. *Genes* **5**: 33–50.

Aliyeva-Schnorr L, Beier S, Karafiátová M, Schmutzer T, Scholz U, Doležel J, Stein N & Houben A. **2015**. Cytogenetic mapping with centromeric bacterial artificial chromosomes contigs shows that this recombination-poor region comprises more than half of barley chromosome 3H. *The Plant Journal* **84**: 385–394.

Ambrožová K, Mandáková T, Bureš P, Neumann P, Leitch IJ, Koblížková A, Macas J & Lysak MA. **2011**. Diverse retrotransposon families and an AT-rich satellite DNA revealed in giant genomes of Fritillaria lilies. *Annals of Botany* **107**: 255–268.

Arguelho EG, Michelan VS, Nogueira FM, Da Silva CRM, Rodriguez C, Trevisan R & Vanzela ALL. **2012**. New chromosome counts in Brazilian species of *Rhynchospora* (Cyperaceae). *Caryologia* **65**: 140–146.

Arslan M, Devisetty UK, Porsch M, Große I, Müller JA & Michalski SG. **2019**. RNA-Seq analysis of soft rush (*Juncus effusus*): transcriptome sequencing, de novo assembly, annotation, and polymorphism identification. *BMC Genomics* **20**: 489.

Ávila Robledillo L, Koblížková A, Novák P, Böttinger K, Vrbová I, Neumann P, Schubert I & Macas J. **2018**. Satellite DNA in *Vicia faba* is characterized by remarkable diversity in its sequence composition, association with centromeres, and replication timing. *Scientific Reports* **8**: 5838.

Baez M, Kuo YT, Dias Y, Souza T, Boudichevskaia A, Fuchs J, Schubert V, Vanzela ALL, Pedrosa-Harand A & Houben A. **2020**. Analysis of the small chromosomal Prionium serratum (Cyperid) demonstrates the importance of a reliable method to differentiate between mono- and holocentricity. *Evolutionary Biology*.

Barros e Silva AE, Marques A, dos Santos KGB & Guerra M. **2010**. The evolution of CMA bands in *Citrus* and related genera. *Chromosome Research* **18**: 503–514.

Bouchenak-Khelladi Y, Muasya AM & Linder HP. **2014**. A revised evolutionary history of Poales: origins and diversification: Evolutionary History of Poales. *Botanical Journal of the Linnean Society* **175**: 4–16.

Bourque G, Burns KH, Gehring M, Gorbunova V, Seluanov A, Hammell M, Imbeault M, Izsvák Z, Levin HL, Macfarlan TS, Mager DL & Feschotte C. **2018**. Ten things you should know about transposable elements. *Genome Biology* **19**: 199.

Bureš P, Zedek F & Marková M. **2013**. Holocentric Chromosomes. In: Greilhuber J, Dolezel J, Wendel JF, eds. *Plant Genome Diversity Volume 2*. Vienna: Springer Vienna, 187–208.

Cabral G, Marques A, Schubert V, Pedrosa-Harand A & Schlögelhofer P. **2014**. Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. *Nature Communications* **5**: 5070.

Carvalho CR & Saraiva LS. **1993**. An air drying technique for Maize chromosomes without enzymatic maceration. *Biotechnic & Histochemistry* **68**: 142–145.

Chase MW, Fay MF, Dewey DS, Maurin O, Rønsted N, Davies J, Pillon Y, Petersen G, Seberg O, Tamura MN, Asmussen CB, Hilu K, Borsch T, Davies JI, Stevenson DW, Pires JC, Givinish TJ, Sytsma KJ, McPherson MA, Graham SW & Rai HS. **2006**. Multigene Analyses of Monocot Relationships: a summary. *Aliso* **22**: 63–75.

Cheng Z, Dong F, Langdon T, Ouyang S, Buell CR, Gu M, Blattner FR & Jiang J. **2002**. Functional Rice centromeres are marked by a satellite repeat and a centromere-specific retrotransposon. *The Plant Cell* **14**: 1691–1704.

Cheng ZJ & Murata M. **2003**. A centromeric tandem repeat family originating from a part of Ty3/ *gypsy* -retroelement in wheat and its relatives. *Genetics* **164**: 665–672.

Chung KS, Hoshino T, Masaki T, Im HT & Ji SJ. **2018**. Chromosome counts of six Korean *Carex* species (Cyperaceae). *Cytologia* **83**: 229–233.

Cuacos M, H. Franklin FC & Heckmann S. **2015**. Atypical centromeres in plants—what they can tell us. *Frontiers in Plant Science* **6**.

Davies EW. **1956**. Cytology, evolution and origin of the aneuploid series in the genus *Carex*. *Hereditas* **42**: 349–365.

De Felice B, Wilson R R, Ciarmiello L & Conicella C. **2004**. A novel repetitive DNA sequence in lemon [*Citrus limon* (L.) Burm.] and related species Bruna. *Journal of Applied Genetics* **45**: 315–320.

D'Hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O, Noel B, Bocs S, Droc G, Rouard M, Da Silva C, Jabbari K, Cardi C, Poulain J, Souquet M, Labadie K, Jourda C, Lengellé J, Rodier-Goud M, Alberti A, Bernard M, Correa M, Ayyampalayam S, Mckain MR, Leebens-Mack J, Burgess D, Freeling M, Mbéguié-A-Mbéguié D, Chabannes M, Wicker T, Panaud O, Barbosa J, Hribova E, Heslop-Harrison P, Habas R, Rivallan R, Francois P, Poiron C, Kilian A, Burthia D, Jenny C, Bakry F, Brown S, Guignon V, Kema G, Dita M, Waalwijk C, Joseph S, Dievart A, Jaillon O, Leclercq J, Argout X, Lyons E, Almeida A, Jeridi M, Dolezel J, Roux N, Risterucci AM, Weissenbach J, Ruiz M, Glaszmann JC, Quétier F, Yahiaoui N & Wincker P. **2012**. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature* **488**: 213–217.

Dias YK, Baez M, Alves MV, Thomas WW, Vanzela ALL, Almeida EM, Elliott TL, Amorim BS, Houben A, Pedrosa-Harand A, Marhold K, Kučera J, Acuña CA, Akopian JA, An'kova TV, Arora J, Aytaç Z, Cavalcanti TB, Calvente A, Catalan P, Chernyagina OA, Chernysheva OA, Cordeiro JMP, Daviña JR, Deanna R, Delgado L, Erst AS, Felix LP, Forni-Martins ER, Gallego F, Facco MG, Gianini Aquino AC, Gomes de Andrade MJ, Graham SA, Hojsgaard DH, Honfi AI, Ikeda H, Khalbekova KU, Krivenko DA, Lakhanpaul S, Peñas MLL, Lavia GI, Leonova TV, Lian L, Lomonosova MN, López-González B, Martínez EJ, Matos SS, Melo AL, Torres EM, Mitrenina EYu, El Mokni R, Valls JFM, Morero RE, Moura CO, Oliveira RC, Oliveira Maekawa V, Olonova MV, Pandit MK, Peruzzi L, Pinzani L, Potseluev OM, Probatova NS, Pulkina SV, Reutemann AV, Rico E, Rua GH, Santos FJ, Santos GS, Santos Sousa MW, Silva MC, Silva RC, Silva Santos AM, Silvestri MC, Singh J, Singhal VK, Souza PCSS, Tashev A, Pozzobon MT, Turginov OT, Berto ACV, Veklich TN & Wang W. **2020**. IAPT chromosome data 33/4. *TAXON* **69**: 1394–1405.

Dolezel J, Sgorbati S & Lucretti S. **1992**. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* **85**: 625–631.

Dong F, Miller JT, Jackson SA, Wang GL, Ronald PC & Jiang J. **1998**. Rice (*Oryza sativa*) centromeric regions consist of complex DNA. *Proceedings of the National Academy of Sciences* **95**: 8135–8140.

Drinnenberg IA, deYoung D, Henikoff S & Malik HS. **2014**. Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. *eLife* **3**: e03676.

Earnshaw WC, Allshire RC, Black BE, Bloom K, Brinkley BR, Brown W, Cheeseman IM, Choo KHA, Copenhaver GP, DeLuca JG, Desai A, Diekmann S, Erhardt S, Fitzgerald-Hayes M, Foltz D, Fukagawa T, Gassmann R, Gerlich DW, Glover DM, Gorbsky GJ, Harrison SC, Heun P, Hirota T, Jansen LET, Karpen G, Kops GJPL, Lampson MA, Lens SM, Losada A, Luger K, Maiato H, Maddox PS, Margolis RL, Masumoto H, McAinsh AD, Mellone BG, Meraldi P, Musacchio A, Oegema K, O'Neill RJ, Salmon ED, Scott KC, Straight AF, Stukenberg PT, Sullivan BA, Sullivan KF, Sunkel CE, Swedlow JR, Walczak CE, Warburton PE, Westermann S, Willard HF, Wordeman L, Yanagida M, Yen TJ, Yoda K & Cleveland DW. **2013**. Esperanto for histones: CENP-A, not CenH3, is the centromeric histone H3 variant. *Chromosome Research* **21**: 101–106.

Ekwall K. **2007**. Epigenetic Control of Centromere Behavior. *Annual Review of Genetics* **41**: 63–81.

Elliott TA & Gregory TR. **2015**. Do larger genomes contain more diverse transposable elements? *BMC Evolutionary Biology* **15**: 69.

Endress PK. **1998**. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge Univ. Press.

Escudero M, Hipp AL, Waterway MJ & Valente LM. **2012**. Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (*Carex*, Cyperaceae). *Molecular Phylogenetics and Evolution* **63**: 650–655.

Escudero M, Márquez-Corro JI & Hipp AL. **2016**. The Phylogenetic Origins and Evolutionary History of Holocentric Chromosomes. *Systematic Botany* **41**: 580–585.

Fedoroff NV. **2012**. Transposable elements, epigenetics, and genome evolution. *Science* **338**: 758–767.

Flach M. **1966**. Diffuse centromeres in a dicotyledonous plant. *Nature* **209**: 1369–1370.

Furness CA & Rudall PJ. **2011**. Selective microspore abortion correlated with aneuploidy: an indication of meiotic drive. *Sexual Plant Reproduction* **24**: 1–8.

Garrido-Ramos MA. **2015**. Satellite DNA in plants: more than just rubbish. *Cytogenetic and genome research* **146**: 153–170.

Gerlach, WL, Bedbrook JR. 1979. Cloning and characterization of ribosomal DNA from wheat and barley. Nucleic Acid Research 7:1869-1885.

Givnish TJ, Ames M, McNeal JR, McKain MR, Steele PR, dePamphilis CW, Graham SW, Pires JC, Stevenson DW, Zomlefer WB, Briggs BG, Duvall MR, Moore MJ, Heaney JM, Soltis DE, Soltis PS, Thiele K & Leebens-Mack JH. **2010**. Assembling the tree of the monocotyledons: plastome sequence phylogeny and evolution of Poales [1]. *Annals of the Missouri Botanical Garden* **97**: 584–616.

Global Carex Group. **2015**. Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): a new broader circumscription: Making *Carex* Monophyletic. *Botanical Journal of the Linnean Society* **179**: 1–42.

Govaerts R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T & Bruhl JJ. **2018**. World checklist of Cyperaceae. Kew: The Board of Trustees of the Royal Botanic Gardens, Kew. *https://wcsp.science.kew.org/*.

Grandbastien M. **1998**. Activation of plant retrotransposons under stress conditions. *Trends in Plant Science* **3**: 181–187.

Greilhuber J. **1995**. Chromosomes of the monocotyledons (general aspects). In: *Monocotyledons: systematics and evolution*. Royal Botanic Gardens, Kew, 379–414.

Guerra M & García MA. **2004**. Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). *Genome* **47**: 134–140.

Guerra M, Ribeiro T & Felix LP. **2019**. Monocentric chromosomes in Juncus (Juncaceae) and implications for the chromosome evolution of the family. *Botanical Journal of the Linnean Society* **191**: 475–483.

Håkansson A. **1954**. Meiosis and pollen mitosis in x-rayed and untreated spikelets of *Eleocharis palustris*. *Hereditas* **40**: 325–345.

Han YH, Zhang ZH, Liu JH, Lu JY, Huang SW & Jin WW. **2008**. Distribution of the tandem repeat sequences and karyotyping in cucumber (*Cucumis sativus* L.) by fluorescence in situ hybridization. *Cytogenetic and Genome Research* **122**: 80–88.

He Q, Cai Z, Hu T, Liu H, Bao C, Mao W & Jin W. **2015**. Repetitive sequence analysis and karyotyping reveals centromere-associated DNA sequences in radish (*Raphanus sativus* L.). *BMC Plant Biology* **15**: 105.

Heckmann S, Macas J, Kumke K, Fuchs J, Schubert V, Ma L, Novák P, Neumann P, Taudien S, Platzer M & Houben A. **2013**. The holocentric species *Luzula elegans* shows interplay between centromere and large-scale genome organization. *The Plant Journal* **73**: 555–565.

Henikoff S, Ahmad K & Malik HS. **2001**. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**: 1098–1102.

Hipp AL, Rothrock PE & Roalson EH. **2009**. The evolution of chromosome arrangements in *Carex* (Cyperaceae). *The Botanical Review* **75**: 96–109.

Hof AE van't, Campagne P, Rigden DJ, Yung CJ, Lingley J, Quail MA, Hall N, Darby AC & Saccheri IJ. **2016**. The industrial melanism mutation in British peppered moths is a transposable element. *Nature* **534**: 102–105.

Houben A, Schroeder-Reiter E, Nagaki K, Nasuda S, Wanner G, Murata M & Endo TR. **2007**. CENH3 interacts with the centromeric retrotransposon cereba and GC-rich satellites and locates to centromeric substructures in barley. *Chromosoma* **116**: 275–283.

Hughes-Schrader S & Ris H. **1941**. The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. *Journal of Experimental Zoology* **87**: 429–456.

Ibarra-Laclette E, Lyons E, Hernández-Guzmán G, Pérez-Torres CA, Carretero-Paulet L, Chang TH, Lan T, Welch AJ, Juárez MJA, Simpson J, Fernández-Cortés A, Arteaga-Vázquez M, Góngora-Castillo E, Acevedo-Hernández G, Schuster SC, Himmelbauer H, Minoche AE, Xu S, Lynch M, Oropeza-Aburto A, Cervantes-Pérez SA, de Jesús Ortega-Estrada M, Cervantes-Luevano JI, Michael TP, Mockler T, Bryant D, Herrera-Estrella A, Albert VA & Herrera-Estrella L. **2013**. Architecture and evolution of a minute plant genome. *Nature* **498**: 94–98.

International Wheat Genome Sequencing Consortium, Wicker T, Gundlach H, Spannagl M, Uauy C, Borrill P, Ramírez-González RH, De Oliveira R, Mayer KFX, Paux E & Choulet F. **2018**. Impact of transposable elements on genome structure and evolution in bread wheat. *Genome Biology* **19**: 103.

Ito H, Yoshida T, Tsukahara S & Kawabe A. **2013**. Evolution of the ONSEN retrotransposon family activated upon heat stress in Brassicaceae. *Gene* **518**: 256–261.

Iwata A, Tek AL, Richard MMS, Abernathy B, Fonsêca A, Schmutz J, Chen NWG, Thareau V, Magdelenat G, Li Y, Murata M, Pedrosa-Harand A, Geffroy V, Nagaki K & Jackson SA. **2013**. Identification and characterization of functional centromeres of the common bean. *The Plant Journal*: **76**:47–60.

Jankowska M, Fuchs J, Klocke E, Fojtová M, Polanská P, Fajkus J, Schubert V & Houben A. **2015**. Holokinetic centromeres and efficient telomere healing enable rapid karyotype evolution. *Chromosoma* **124**: 519–528.

Jasencakova Z, Meister A & Schubert I. **2001**. Chromatin organization and its relation to replication and histone acetylation during the cell cycle in barley. *Chromosoma* **110**: 83–92.

Jiang J, Birchler JA, Parrott WA & Kelly Dawe R. **2003**. A molecular view of plant centromeres. *Trends in Plant Science* **8**: 570–575.

Jin W, Melo JR, Nagaki K, Talbert PB, Henikoff S, Dawe RK & Jiang J. **2004**. Maize centromeres: organization and functional adaptation in the genetic background of Oat. *The Plant Cell* **16**: 571–581.

Judd WS (Ed.). **2008**. *Plant systematics: a phylogenetic approach*. Sunderland, Mass: Sinauer Associates.

Kalendar R, Tanskanen J, Immonen S, Nevo E & Schulman AH. **2000**. Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences* **97**: 6603–6607.

Kandul NP, Lukhtanov VA & Pierce NE. **2007**. Karyotypic diversity and speciation in Agrodiaetus butterflies. *Evolution* **61**: 546–559.

Kapitonov VV & Jurka J. **2001**. Rolling-circle transposons in eukaryotes. *Proceedings of the National Academy of Sciences* **98**: 8714–8719.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P & Drummond A. **2012**. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Klein SJ & O'Neill RJ. **2018**. Transposable elements: genome innovation, chromosome diversity, and centromere conflict. *Chromosome Research* **26**: 5–23.

Koo DH, Hong CP, Batley J, Chung YS, Edwards D, Bang JW, Hur Y & Lim YP. **2011**. Rapid divergence of repetitive DNAs in Brassica relatives. *Genomics* **97**: 173–185.

Kowar T, Zakrzewski F, Macas J, Kobližková A, Viehoever P, Weisshaar B & Schmidt T. **2016**. Repeat Composition of CenH3-chromatin and H3K9me2-marked heterochromatin in Sugar Beet (*Beta vulgaris*). *BMC Plant Biology* **16**: 120.

Lee SI & Kim NS. **2014**. Transposable elements and genome size variations in plants. *Genomics & Informatics* **12**: 87.

Lermontova I, Sandmann M & Demidov D. **2014**. Centromeres and kinetochores of Brassicaceae. *Chromosome Research* **22**: 135–152.

Leushkin EV, Sutormin RA, Nabieva ER, Penin AA, Kondrashov AS & Logacheva MD. **2013**. The miniature genome of a carnivorous plant *Genlisea aurea* contains a low number of genes and short non-coding sequences. *BMC Genomics* **14**: 476.

Liao YJ, Zhai HF, Zhang B, Duan TX & Huang JM. **2011**. Anxiolytic and sedative effects of dehydroeffusol from *Juncus effusus* in mice. *Planta Medica* **77**: 416–420.

Lipnerova I, Bures P, Horova L & Smarda P. **2013**. Evolution of genome size in *Carex* (Cyperaceae) in relation to chromosome number and genomic base composition. *Annals of Botany* **111**: 79–94.

Liu Q, Li X, Zhou X, Li M, Zhang F, Schwarzacher T & Heslop-Harrison JS. **2019**. The repetitive DNA landscape in *Avena* (Poaceae): chromosome and genome evolution defined by major repeat classes in whole-genome sequence reads. *BMC Plant Biology* **19**: 226.

López MG, Aveliano F & Florencia G. **2017**. Cytogenetics, genome size and anther anatomy in *Bulbostylis* (Cyperaceae). *Caryologia* **70**: 238–247.

Loureiro J, Rodriguez E, Dolezel J & Santos C. **2007**. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany* **100**: 875–888.

Luceño M, Mendes AP, Vanzela ALL & Alves MV. **1998**. Agmatoploidy and Symploidy in *Rhynchospora cephalotes* (L.) Vahl (Cyperaceae). *Cytologia* **63**: 79–81.

Macas J, Novák P, Pellicer J, Čížková J, Koblížková A, Neumann P, Fuková I, Doležel J, Kelly LJ & Leitch IJ. **2015**. In Depth Characterization of Repetitive DNA in 23 Plant Genomes Reveals Sources of Genome Size Variation in the Legume Tribe Fabeae (A Houben, Ed.). *PLOS ONE* **10**: e0143424.

Maddox PS, Oegema K, Desai A & Cheeseman IM. **2004**. 'Holo'er than thou: Chromosome segregation and kinetochore function in *C. elegans*. *Chromosome Research* **12**: 641–653.

Maheshwari S, Ishii T, Brown CT, Houben A & Comai L. **2017**. Centromere location in *Arabidopsis* is unaltered by extreme divergence in CENH3 protein sequence. *Genome Research* **27**: 471–478.

Makałowski W, Gotea V, Pande A & Makałowska I. **2019**. Transposable elements: classification, identification, and their use as a tool for comparative genomics. In: Anisimova M, ed. *Evolutionary Genomics*. New York, NY: Springer New York, 177–207.

Makarevitch I, Waters AJ, West PT, Stitzer M, Hirsch CN, Ross-Ibarra J & Springer NM. **2015**. Transposable elements contribute to activation of maize genes in response to abiotic stress (M Freeling, Ed.). *PLoS Genetics* **11**: e1004915.

Malik HS & Henikoff S. **2003**. Phylogenomics of the nucleosome. *Nature Structural & Molecular Biology* **10**: 882–891.

Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY & Bryant SH. **2017**. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research* **45**: D200–D203.

Marques A, Ribeiro T, Neumann P, Macas J, Novák P, Schubert V, Pellino M, Fuchs J, Ma W, Kuhlmann M, Brandt R, Vanzela ALL, Beseda T, Šimková H, Pedrosa-Harand A & Houben A. **2015**. Holocentromeres in *Rhynchospora* are associated with genome-wide centromere-specific repeat

arrays interspersed among euchromatin. *Proceedings of the National Academy of Sciences* **112**: 13633–13638.

Márquez-Corro JI, Luceño M, Jiménez-Mejías P, Escudero M, Martín-Bravo S, Hipp AL, Chung KS, Muasya AM, Rothrock PE, Weber JA & Naczi R. **2018a**. IAPT chromosome data 28. *Taxon* **67**: 1235–1245.

Márquez-Corro JI, Escudero M & Luceño M. **2018b**. Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives. *Chromosome Research* **26**: 139–152.

Martinez-Zapater JM, Estelle MA & Somerville CR. **1986**. A highly repeated DNA sequence in Arabidopsis thaliana. *Molecular and General Genetics MGG* **204**: 417–423.

Maximiano da Silva CR, González-Elizondo MS, Rego L do NA de A, Torezan JMD & Vanzela ALL. **2008**. Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae). *Australian Journal of Botany* **56**: 82.

McCann J, Macas J, Novák P, Stuessy TF, Villaseñor JL & Weiss-Schneeweiss H. **2020**. Differential genome size and repetitive dna evolution in diploid species of *Melampodium* sect. *Melampodium* (asteraceae). *Frontiers in Plant Science* **11**: 362.

McClintock B. **1939**. The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences* **25**: 405–416.

McClintock B. **1950**. The origin and behavior of mutable loci in maize. *Proceedings of the National Academy of Sciences* **36**: 344–355.

Mehrotra S & Goyal V. **2014**. Repetitive sequences in plant nuclear dna: types, distribution, evolution and function. *Genomics, Proteomics & Bioinformatics* **12**: 164–171.

Melters DP, Paliulis LV, Korf IF & Chan SWL. **2012**. Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Research* **20**: 579–593.

Melters DP, Bradnam KR, Young HA, Telis N, May MR, Ruby J, Sebra R, Peluso P, Eid J, Rank D, Garcia J, DeRisi JL, Smith T, Tobias C, Ross-Ibarra J, Korf I & Chan SW. **2013**. Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. *Genome Biology* **14**: R10.

Michael TP. **2014**. Plant genome size variation: bloating and purging DNA. *Briefings in Functional Genomics* **13**: 308–317.

Moore G, Aragón-Alcaide L, Roberts M, Reader S, Miller T & Foote T. **1997**. Are rice chromosomes components of a holocentric chromosome ancestor? *Plant Molecular Biology* **35**: 17–23.

Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW & Smets E. **2009**. Phylogeny of cyperaceae based on DNA sequence data: current progress and future prospects. *The Botanical Review* **75**: 2–21.

Nagaki K, Song J, Stupar RM, Parokonny AS, Yuan Q, Ouyang S, Liu J, Hsiao J, Jones KM, Dawe RK, Buell CR & Jiang J. **2003**. Molecular and cytological analyses of large tracks of centromeric DNA reveal the structure and evolutionary dynamics of maize centromeres. *Genetics* **163**: 759–770.

Nagaki K, Kashihara K & Murata M. **2005**. Visualization of diffuse centromeres with centromere-specific histone h3 in the holocentric plant *Luzula nivea*. *The Plant Cell* **17**: 1886–1893.

Naish M, Alonge M, Wlodzimierz P, Tock AJ, Abramson BW, Schmücker A, Mandáková T, Jamge B, Lambing C, Kuo P, Yelina N, Hartwick N, Colt K, Smith LM, Ton J, Kakutani T, Martienssen RA, Schneeberger K, Lysak MA, Berger F, Bousios A, Michael TP, Schatz MC & Henderson IR. **2021**. The genetic and epigenetic landscape of the *Arabidopsis* centromeres. *Science* **374**: eabi7489.

Nasuda S, Hudakova S, Schubert I, Houben A & Endo TR. **2005**. Stable barley chromosomes without centromeric repeats. *Proceedings of the National Academy of Sciences* **102**: 9842–9847.

Neumann P, Navrátilová A, Koblížková A, Kejnovský E, Hřibová E, Hobza R, Widmer A, Doležel J & Macas J. **2011**. Plant centromeric retrotransposons: a structural and cytogenetic perspective. *Mobile DNA* **2**: 4.

Neumann P, Navrátilová A, Schroeder-Reiter E, Koblížková A, Steinbauerová V, Chocholová E, Novák P, Wanner G & Macas J. **2012**. Stretching the rules: monocentric chromosomes with multiple centromere domains (BA Sullivan, Ed.). *PLoS Genetics* **8**: e1002777.

Neumann P, Schubert V, Fuková I, Manning JE, Houben A & Macas J. **2016**. Epigenetic histone marks of extended meta-polycentric centromeres of lathyrus and pisum chromosomes. *Frontiers in Plant Science* **7**.

Neumann P, Oliveira L, Čížková J, Jang T, Klemme S, Novák P, Stelmach K, Koblížková A, Doležel J & Macas J. **2021**. Impact of parasitic lifestyle and different types of centromere organization on chromosome and genome evolution in the plant genus *Cuscuta*. *New Phytologist* **229**: 2365–2377.

Novak P, Neumann P, Pech J, Steinhaisl J & Macas J. **2013**. RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. *Bioinformatics* **29**: 792–793.

Novák P, Ávila Robledillo L, Koblížková A, Vrbová I, Neumann P & Macas J. **2017**. TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads. *Nucleic Acids Research* **45**: e111–e111.

Oliveira L, Neumann P, Jang TS, Klemme S, Schubert V, Koblížková A, Houben A & Macas J. **2020**. Mitotic Spindle Attachment to the Holocentric Chromosomes of Cuscuta europaea Does Not Correlate With the Distribution of CENH3 Chromatin. *Frontiers in Plant Science* **10**: 1799.

Ourari M, Coriton O, Martin G, Huteau V, Keller J, Ainouche ML, Amirouche R & Ainouche A. **2020**. Screening diversity and distribution of Copia retrotransposons reveals a specific amplification of BARE1 elements in genomes of the polyploid Hordeum murinum complex. *Genetica* **148**: 109–123.

Paz RC, Kozaczek ME, Rosli HG, Andino NP & Sanchez-Puerta MV. **2017**. Diversity, distribution and dynamics of full-length Copia and Gypsy LTR retroelements in Solanum lycopersicum. *Genetica* **145**: 417–430.

Pazy B & Plitmann U. **1994**. Holocentric chromosome behaviour in *Cuscuta* (Cuscutaceae). *Plant Systematics and Evolution* **191**: 105–109.

Pedrosa A, Sandal N, Stougaard J, Schweizer D & Bachmair A. **2002**. Chromosomal map of the model legume *Lotus japonicus*. *Genetics* **161**: 1661–1672.

Pedrosa-Harand A, de Almeida CCS, Mosiolek M, Blair MW, Schweizer D & Guerra M. **2006**. Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris* L.) evolution. *Theoretical and Applied Genetics* **112**: 924–933.

Plohl M, Meštrović N & Mravinac B. **2014**. Centromere identity from the DNA point of view. *Chromosoma* **123**: 313–325.

Pritham EJ, Putliwala T & Feschotte C. **2007**. Mavericks, a novel class of giant transposable elements widespread in eukaryotes and related to DNA viruses. *Gene* **390**: 3–17.

Qiu F & Ungerer MC. **2018**. Genomic abundance and transcriptional activity of diverse gypsy and copia long terminal repeat retrotransposons in three wild sunflower species. *BMC Plant Biology* **18**: 6.

Ribeiro T, Marques A, Novák P, Schubert V, Vanzela ALL, Macas J, Houben A & Pedrosa-Harand A. **2017**. Centromeric and non-centromeric satellite DNA organisation differs in holocentric *Rhynchospora* species. *Chromosoma* **126**: 325–335.

Ribeiro T, Buddenhagen CE, Thomas WW, Souza G & Pedrosa-Harand A. **2018**. Are holocentrics doomed to change? Limited chromosome number variation in Rhynchospora Vahl (Cyperaceae). *Protoplasma* **255**: 263–272.

Ribeiro T, Vasconcelos E, dos Santos KGB, Vaio M, Brasileiro-Vidal AC & Pedrosa-Harand A. **2020**. Diversity of repetitive sequences within compact genomes of *Phaseolus* L. beans and allied genera Cajanus L. and Vigna Savi. *Chromosome Research* **28**: 139–153.

Roalson EH. **2005**. Phylogenetic relationships in the Juncaceae inferred from nuclear ribosomal DNA internal transcribed spacer sequence data. *International Journal of Plant Sciences* **166**: 397–413.

Roalson EH. **2008**. A synopsis of chromosome number variation in the Cyperaceae. *The Botanical Review* **74**: 209–393.

Robillard É, Le Rouzic A, Zhang Z, Capy P & Hua-Van A. **2016**. Experimental evolution reveals hyperparasitic interactions among transposable elements. *Proceedings of the National Academy of Sciences* **113**: 14763–14768.

Rocha DM, Vanzela ALL & Mariath JEA. **2018**. Comparative study of microgametogenesis in members of Cyperaceae and Juncaceae: a shift from permanent pollen tetrads to pseudomonads. *Botanical Journal of the Linnean Society* **188**: 59–73.

Ruiz-Ruano FJ, Castillo-Martínez J, Cabrero J, Gómez R, Camacho JPM & López-León MD. **2018**. High-throughput analysis of satellite DNA in the grasshopper *Pyrgomorpha conica* reveals abundance of homologous and heterologous higher-order repeats. *Chromosoma* **127**: 323–340.

Sader M, Vaio M, Cauz-Santos LA, Dornelas MC, Vieira MLC, Melo N & Pedrosa-Harand A. **2021**. Large vs small genomes in Passiflora: the influence of the mobilome and the satellitome. *Planta* **253**: 86.

SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y & Bennetzen JL. **1998**. The paleontology of intergene retrotransposons of maize. *Nature Genetics* **20**: 43–45.

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du F, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen W, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B,

Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn MW, Ying K, Yeh CT, Emrich SJ, Jia Y, Kalyanaraman A, Hsia AP, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia JM, Deragon JM, Estill JC, Fu Y, Jeddeloh JA, Han Y, Lee H, Li P, Lisch DR, Liu S, Liu Z, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Bennetzen JL, Dawe RK, Jiang J, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA & Wilson RK. **2009**. The B73 Maize Genome: Complexity, Diversity, and Dynamics. *Science* **326**: 1112–1115.

Schneider MC, Mattos VF, Carvalho LS & Cella DM. **2015**. Organization and behavior of the synaptonemal complex during achiasmatic meiosis of four buthid scorpions. *Cytogenetic and Genome Research* **144**: 341–347.

Schrader F. **1935**. Notes an the mitotic behavior of long chromosomes. *CYTOLOGIA* **6**: 422–430.

Schubert V, Neumann P, Marques A, Heckmann S, Macas J, Pedrosa-Harand A, Schubert I, Jang TS & Houben A. **2020**. Super-resolution microscopy reveals diversity of plant centromere architecture. *International Journal of Molecular Sciences* **21**: 3488.

Schulman AH. **2013**. Retrotransposon replication in plants. *Current Opinion in Virology* **3**: 604–614.

Semmouri I, Bauters K, Léveillé-Bourret É, Starr JR, Goetghebeur P & Larridon I. **2019**. Phylogeny and systematics of cyperaceae, the evolution and importance of embryo morphology. *The Botanical Review* **85**: 1–39.

Senaratne AP, Cortes-Silva N, Drinnenberg IA. 2022. Evolution of holocentric chromosomes: Drivers, diversity, and deterrents. *Seminars in Cell & Developmental Biology* 127:90-99.

Sheikh SA & Kondo K. **1995**. Differential staining with orcein, giemsa, CMA, and DAPI for comparative chromosome study of 12 species of Australian *drosera* (Droseraceae). *American Journal of Botany* **82**: 1278–1286.

Shibata F. **2004**. Differential localization of the centromere-specific proteins in the major centromeric satellite of Arabidopsis thaliana. *Journal of Cell Science* **117**: 2963–2970.

Shirakawa J, Nagano K & Hoshi Y. **2011**. A chromosome study of two centromere differentiating Drosera species, D. arcturi and D. regia. : 453–463.

da Silva CRM, Quintas CC & Vanzela ALL. **2010**. Distribution of 45S and 5S rDNA sites in 23 species of *Eleocharis* (Cyperaceae). *Genetica* **138**: 951–957.

Simpson DA, Furness CA, Hodkinson TR, Muasya AM & Chase MW. **2003**. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany* **90**: 1071–1086.

Simpson DA & Inglis CA. **2001**. Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *Kew Bulletin* **56**: 257.

Šmarda P, Bureš P, Horová L, Leitch IJ, Mucina L, Pacini E, Tichý L, Grulich V & Rotreklová O. **2014**. Ecological and evolutionary significance of genomic GC content diversity in monocots. *Proceedings of the National Academy of Sciences* **111**: E4096–E4102.

Sonnhammer ELL & Durbin R. **1995**. A dot-matrix program with dynamic threshold control suited for genomic DNA and protein sequence analysis. *Gene* **167**: GC1–GC10.

Sousa A, Barros e Silva AE, Cuadrado A, Loarce Y, Alves MV & Guerra M. **2011**. Distribution of 5S and 45S rDNA sites in plants with holokinetic chromosomes and the "chromosome field" hypothesis. *Micron* **42**: 625–631.

de Souza TB, Chaluvadi SR, Johnen L, Marques A, González-Elizondo MS, Bennetzen JL & Vanzela ALL. **2018**. Analysis of retrotransposon abundance, diversity and distribution in holocentric Eleocharis (Cyperaceae) genomes. *Annals of Botany* **122**: 279–290.

Spalink D, Drew BT, Pace MC, Zaborsky JG, Starr JR, Cameron KM, Givnish TJ & Sytsma KJ. **2016**. Biogeography of the cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. *Journal of Biogeography* **43**: 1893–1904.

Stimpson KM, Matheny JE & Sullivan BA. **2012**. Dicentric chromosomes: unique models to study centromere function and inactivation. *Chromosome Research* **20**: 595–605.

Su H, Liu Y, Liu Y, Birchler J & Han F. **2018**. The behavior of the maize b chromosome and centromere. *Genes* **9**: 476.

Sullivan BA & Schwartz S. **1995**. Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. *Human Molecular Genetics* **4**: 2189–2197.

Sullivan LL & Sullivan BA. **2020**. Genomic and functional variation of human centromeres. *Experimental Cell Research* **389**: 111896.

Tanaka N & Tanaka N. **1977**. Chromosome studies in *Chionographis* (Liliaceae). *CYTOLOGIA* **42**: 753–763.

Tek AL, Song J, Macas J & Jiang J. **2005**. Sobo, a recently amplified satellite repeat of potato, and its implications for the origin of tandemly repeated sequences. *Genetics* **170**: 1231–1238.

The Arabidopsis Genome Initiative. **2000**. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **408**: 796–815.

Tran TD, Cao HX, Jovtchev G, Neumann P, Novák P, Fojtová M, Vu GTH, Macas J, Fajkus J, Schubert I & Fuchs J. **2015**. Centromere and telomere sequence alterations reflect the rapid genome evolution within the carnivorous plant genus *Genlisea*. *The Plant Journal* **84**: 1087–1099.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M & Rozen SG. **2012**. Primer3—new capabilities and interfaces. *Nucleic Acids Research* **40**: e115–e115.

Van-Lume B, Mata-Sucre Y, Báez M, Ribeiro T, Huettel B, Gagnon E, Leitch IJ, Pedrosa-Harand A, Lewis GP & Souza G. **2019**. Evolutionary convergence or homology? Comparative cytogenomics of Caesalpinia group species (Leguminosae) reveals diversification in the pericentromeric heterochromatic composition. *Planta* **250**: 2173–2186.

Vanzela ALL & Guerra M. **2000**. Heterochromatin differentiation in holocentric chromosomes of Rhynchospora (Cyperaceae). *Genetics and Molecular Biology* **23**: 453–456.

Vicient CM, Kalendar R, Anamthawat-Jónsson K, Suoniemi A & Schulman AH. **1999**. [No title found]. *Genetica* **107**: 53–63.

Vondrak T, Ávila Robledillo L, Novák P, Koblížková A, Neumann P & Macas J. **2020**. Characterization of repeat arrays in ultra-long nanopore reads reveals frequent origin of satellite DNA from retrotransposon-derived tandem repeats. *The Plant Journal* **101**: 484–500.

Vymazal J. **2014**. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological Engineering* **73**: 724–751.

Wang B, Yang X, Jia Y, Xu Y, Jia P, Dang N, Wang S, Xu T, Zhao X, Gao S, Dong Q & Ye K. **2021**. High-quality *Arabidopsis thaliana* genome assembly with nanopore and hifi long reads. *Genomics, Proteomics & Bioinformatics*: S1672022921001741.

Waminal NE, Pellerin RJ, Kim NS. et al. 2018. Rapid and efficient fish using pre-labeled oligomer probes. *Scientific Reports* **8**:8224.

Weber B, Heitkam T, Holtgräwe D, Weisshaar B, Minoche AE, Dohm JC, Himmelbauer H & Schmidt T. **2013**. Highly diverse chromoviruses of *Beta vulgaris* are classified by chromodomains and chromosomal integration. *Mobile DNA* **4**: 8.

Wicker T, Stein N, Albar L, Feuillet C, Schlagenhauf E & Keller B. **2001**. Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution: Genome organisation in 211 kb DNA of diploid wheat. *The Plant Journal* **26**: 307–316.

Xiao H, Jiang N, Schaffner E, Stockinger EJ & van der Knaap E. **2008**. A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* **319**: 1527–1530.

Zedek F, Veselý P, Horová L & Bureš P. **2016**. Flow cytometry may allow microscope-independent detection of holocentric chromosomes in plants. *Scientific Reports* **6**: 27161.

Zhang L, Yan L, Jiang J, Wang Y, Jiang Y, Yan T & Cao Y. **2014**. The structure and retrotransposition mechanism of LTR-retrotransposons in the asexual yeast *Candida albicans*. *Virulence* **5**: 655–664.

Zumstein W, Wangwasit K, & Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand. **2019**. Chromosome counts and karyotype reports from Fimbristylis (Cyperaceae) in Thailand. *Thai Forest Bulletin (Botany)* **47**: 123–132.

Table 1. Species of Mapanioideae used for genome sequencing and genome size estimations, collection sites and vouchers

Species	1C (pg)	$2n^a$	CV	Collection sites and vouchers
Diplasia karatifolia Rich. ex Pers	0,335	60	5,09	Amazonas, Manaus, Adolpho Ducke Reserve, near the camp at Acará stream, 26 September 2018, M. Alves & Y. Dias 04-2018 (UFP).
Hypolytrum schraderianum Nees	0,275	60	4,66	Amazonas, Manaus, Adolpho Ducke Reserve, Tower Access Trail, 26 September 2018, M. Alves & Y. Dias 05-2018 (UFP).
Hypolytrum schraderianum	0,375	60	4,96	Bahia, Mun. Camacan. Serra Bonita, Private Reserve (RPPN), 15°25'7"S, 39°32'58"W, 300-400 m. 20 September 2018, W. W. Thomas, P.J.S. Silva Filho & L.H. Daneu 16811 (JPB, NYBG).
Hypolytrum schraderianum	0,285	76		Paraná, Paranaguá, Ilha do Mel, near Bica do Norinho, -25°33'59.9", -48°18'25.7". 02 July 1995, M.C. Dias & E. Rocha s.n. (FUEL 29197).
Mapania sylvatica Aubl.	0,305	-	5,07	Amazonas, Manaus, Adolpho Ducke Reserve, Tower Access Trail, 26 September 2018, M. Alves & Y. Dias 0X-2018 (UFP).

A Chromosome number were retrieved from Dias et al. 2020.

Table 2. Comparative Repeat content in in the genomes of selected Mapanioideae species (*Diplasia karatifolia, Hypolytrum schraderianum* and *Mapania sylvatica).*

Repetitive sequence	Superfamily	Lineage	*Diplasia karatifolia* (%)	*Hypolytrum schraderianum* (%)	*Mapania sylvatica* (%)
LTR Retrotransposons	Ty1/Copia	Ale	0.618	0.668	-
		Angela	1.105	2.053	0.781
		Ikeros	0.184	0.285	0.129
		Tork	-	0.130	0.070
		Ivana	0.039	-	-
		Sire	0.078	-	-
	Total Ty1/Copia		2.023	3.136	0.98
	Ty3/Gypsy	Chromovirus/Tekay	0.208	0.073	-
		Chromovirus/CRM	0.246	0.057	-
		Chromovirus/Galadriel	-	-	0.011
		Chromovirus/Reina	0.125	0.206	
		Non-chromovirus/Retand	1.835	0.068	0.027
		Non-chromovirus/ OTA/Athila	0.078	1.486	0.140
		Non-chromovirus/ OTA/ Tat/Ogre	0.601	-	-
	Total Ty3/Gypsy		3.093	1.889	0.18
	Total LTR		5.116	5.025	1.16
Non-LTR Retrotransposons		LINE	-	0.078	-
DNA Transposon			0.381	0.023	0.088
Pararetrovirus			0.041	-	-
rDNA			0.535	0.574	0.380
Satellite DNA			5.387	6.954	1.750
Unclassified			6.219	10.334	1.360
Total			17.68%	23%	4.74%

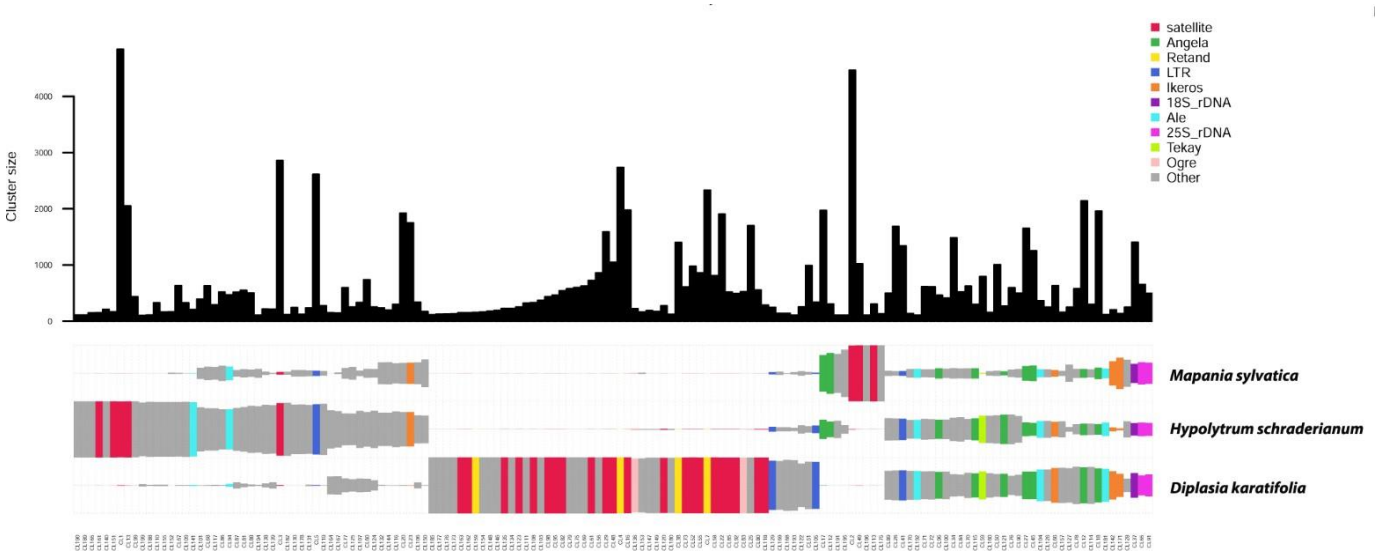


Figure 1. Comparative analysis of repeats between Mapanioideae species performed with RepeatExplorer,. Divergence of repeat composition between these different genera is more pronounced for satellite DNA clusters and between *Diplasia* and the other two genera, as expected from phylogenetic analysis (Semmouri *et al*., 2018).

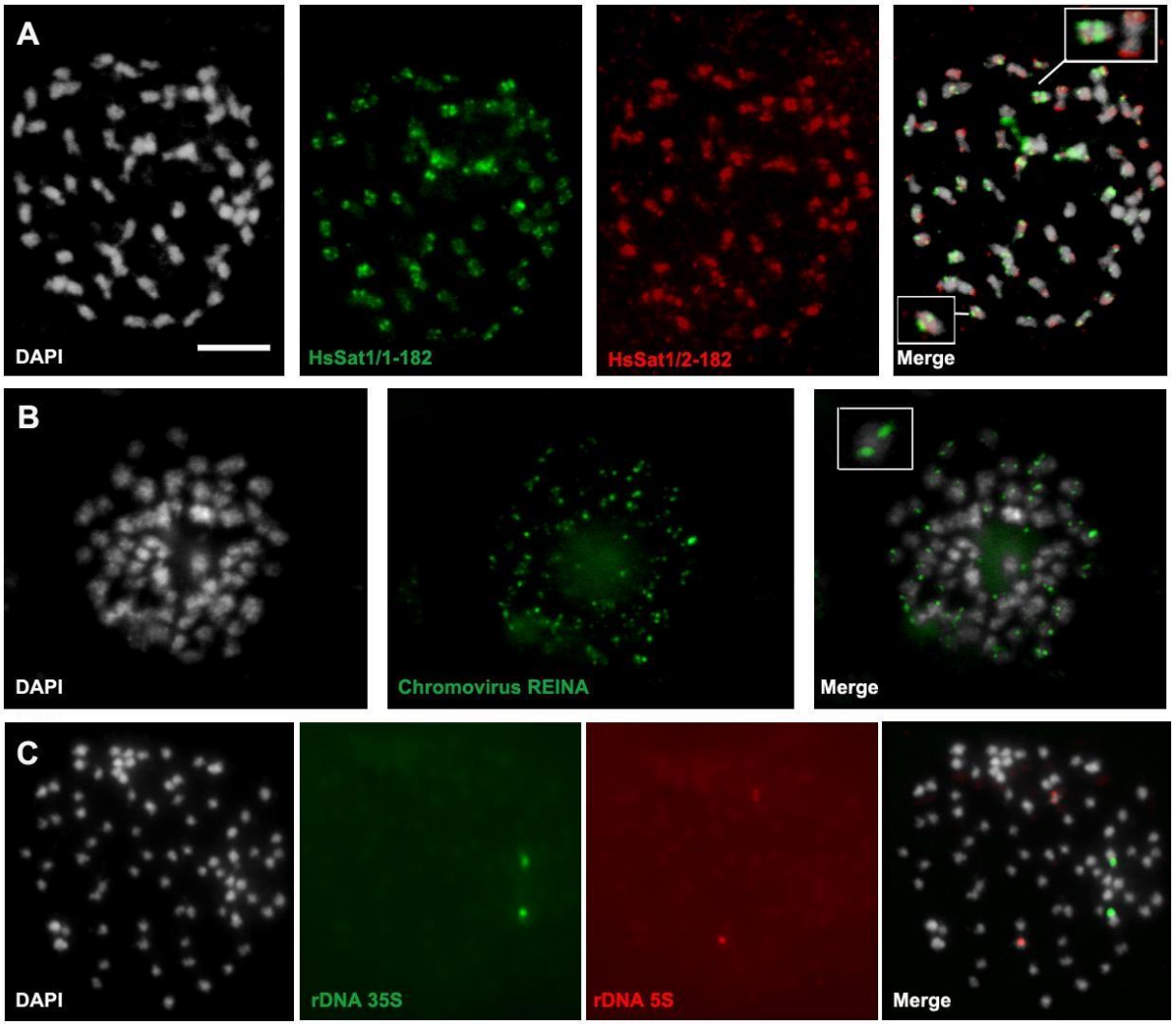


Figure 2. Mitotic metaphases of *Hypollytrum schraderianum* showing the distribution of two variants of the major satellite family, one retrotransposon and the ribosomal DNA 35S and 5S. A) HscSAT1/1-182 (green) and HscSAT1/2-182 (red), B) Ty3-Gyspsy Chromovirus Reina (green), C) Ribosomal DNA 35S (green) and 5S (red). Bar = 5 µm.

Figure 3. Immunodetection of the centromeric protein RpCENH3 (green) in *Hypolytrum schraderianum* mitotic chromosomes (A) and interphase nuclei (B).Bar = 2µm (A) and 1µm (B).

Supplementary Data

Supplementary Table 1. List of probes with their respective primer sequences used for repeat amplification in *Hypolytrum schraderianum*

Target repeat	Primer name	Primer sequence (5'-3')	Fluorochrome	Annealing temperature
HscSAT1/1-182	HscSAT1/1-182F	ACAGCCCAAATTGAGCTGAA	ATTO 488	60ºC
	HscSAT1/1-182R	TTTTGCGCAATTCAAGCTCA		
HscSAT1/2-182	HscSAT1/2-182F	TTGCATCAATTTTGCGCAAATGT	ATTO 550	60ºC
	HscSAT1/2-182R	TTGTATGCGCTGCAATTGTGT		
REINA	REINAF	GTGGTGGACCGCCTAACAA	ATTO 488	55ºC
	REINAR	AGTGAACGGGTGTGACAAGG		

Supplementary table 2. List of consensus sequences of the satellite DNAs from species of Mapanioideae

Specie	Satellite	Cluster	Monomere size (bp)	Consensus sequence
D. karatifolia	DkaSAT1	1	472	TAATAATAATATATGTACATATTAATTTCAATAAAAATAACTAATGAACTTGA ATTCCTTAGCCGCTAAGCAACCTCGGATTTTGAAACATTTTGCAAACCAATGT CTGTCTAAACTTGGGACTCTTCATAAATTCGTACTGCTATTCCATTTCTGTTAT TTTTTTGTTTATCCTTTAATATGATTTTGATTTTTGAATGAAAATCTGGGAGTA TATTATATTTCCATTGATTTCATTCCTGAAATTAAAACAATTTTAGTATCTCGC TTTATCCAACGTCGTTTTTTAATAATAATAGGAGTGCGAAGGCAATAATGAG TATAAAAGCAATAGTAATAATAGCAATAATAATATTAATAAAAGTATGAATA AAAAAATTAATAATAATTACAACACTAATATAACAAATAATGATGATGGTGA CGATCACCATCATAACAACAACAGCAACACAAAAAAAATATTATTAT
	DkaSAT1	6	437	CTAATAAAACTTATAATGATGATGGTGATAATCATCATCTTAACAATAACAAC ACGACAACGAAAATATTATTATTGGTAATTATAAATATAAATATTCATTGTAGT AAAAACAACTGATGCACTTGAATTACTTAGCCGCTAAGCAACCGTGGATTTTG ATGATTTTGTAAATCAAGATATGTCCAAACTTGGGACTCATCGTATATTCATAC GCTATTCCATTTTTGTTATTCTTTTTGCTTGTCCTTTAATATGATTTTCATTTTTG ATTAAAATCAGGGAATATATTCTATTTTCGTTGATTTCATTAACGAAATTAAAA ACTTTTAGCGTTTCGCTTTATGAATCATTGTTATTTACAAATAATTGAAATGAG AAGAAACAATAAGTATCAAAGCTGCGGTGATAAGAGCAATAATAATATTAAC
	DkaSAT1	7	864	ACTGAAATTGTAATGTAGGAAATGACAAATACAGCTGAGATACAGCAACAAT ACGATGTTAAAGATTCTTTAATTCGGGTCTTCTTTATATTGATGAAATTTTGCA AAATCTGGGGTTGCTTAGCGGCTAAGGAATCTCCGTGCGTCAGCTGTTGTGAC ACATTAGTATGAATATTAGCATTGCTGTTGTTGTATTCATTACGATTATCACTC TCACAGTTAATGTTGTTATTATCTGTTTATTATTGTTTTTTAAATTATTATTGTT ATTGTTAATATTATTATTACTTGTTATTATTATCACTGTACGAGCTGTTTGTAA AAGTCAACTCAAATTGTCTTTCAATTTAATTAAATAAATCATTGAAAATTTCC TGTCTTCCAATATTTTATTTCAGAAACTAAAATAGTAATGTAGAAAATAAAAA AAGAAGCAAATAAACAATCGCAATATGAATATCTGAACTGTCCTTAATTTGTA TTTCTATTGATCGACCAAACCGTTCGAAATCCGCTGCTACATAGCGCTAATGG AATTCCAGTGCCAATTGTTATTATTGCAACTAATATGGATATTGGGATTGTTCT TGTCAAAATCGATATTGTTATTTCAATTACTCTTACTATTATCAAGTGGTTACG

GTCTTTTTAATTAACATTATTATTCAAATTTCTTATAATTTGGAATTCCTATTA
ACAAATTGTTGTTATTATTATAATTTTTACAATTGGTGTTGTTATTTTATCACT
GATAATCTTGTAAATATACAACGACTTCATAAAACTCAGCACTTAAAGTGTTT
CAATAAAATTAATTAAGCCAGTGAAAATTCGAGGTAATCCAATATTTATTTAT
ATAAAAAA

DkaSAT1 12 858

CGTGCGTGGTGTTTATAAAAGTCAACTCACAATGCGTTTCTGTTTCATTAATTA
AATCATTGAAATTTCAATATAATGTGCTATTTTATTTCAGAACCTAAAATTAT
AATGTAGAAAAAGAAAAGAAGCGACGAATATACAATCGCAGTACGAATATG
TGAAGAGAGTCCTTAATTTCAATCTCCCTGATGGACGTAACCGTTTCAAATCC
ACGGTTACTTAGCGGAAAAGGAATTCCAGTGCCAATTGTCATTATTGCCACTA
ATATGAATATTAGCTTTGTTGTTATCACAATCATAGCGCGACTATTACATTTAT
TCTTAGTATTATCAAGTTGTTATGATTTTTTTTAACAACAACTATTATTTTAAA
TTCATATTATGAACAAAATGTTACAATTATTATAATTCTTACTATTAGTGACGT
TATTTTATAGTTGATATTGTTGCAATTGTACATAGGGTTGATAAAAGTGAACT
CTTAAAGAGTTTAAATATAATTAATTGAGCCAGAGAATATTCGACATAATCGA
ATTTTTCTCTATTTCAGAAAATAAATTATACCTTACAAAATGGCTAATGCAAT
ATAAGGATCCGTAGCAATATGATGTTAAAGTGTCTTTAATTTTGTTTTTTTAGG
TTGTCGAAAATTTTCAAAATAAGCAGTTGCTTAGCGGCTAAGGAATCTCCGTG
CATCAACTATTTCAAGGCAATTAGTATGAATATTAGCATTTTTCATATTATAAT
ATTTACGGTTATTACTATTGCTATTAATGTTCTTATTTTCATTTCATTAATGGTT
TATAAATTAATATTGTTATAATTTATATTAGTGTTCATTTTATTGTTATTGTTAT
CAC

DkaSAT1 17 870

TATAATTATTTTAATGATTTTTTATTGATATTATTATCAATGTACAATGTGTTG
ACAAAAGTCAACTCAAAAGGTGTTTCAATTTCATTAATTAAATCAATGAAAAT
TCAACATATTCCGATATTTTACTCCAAAAACTAAAATTATAATGTAGAAAATA
AAAGAAGTGTCGAATATACAATCGCAATATGAATATCTCGAGAGTCCTCAAT
TTGTATTTTTCTTGATGCACCTCTTTCGAAATCCGTGGTTACTTAGCGGTAAAT
GAATCCTAGTGCCAGTTGCTATTATTGCATCTAATACGAATATTAACATTATT
GTTATCATAATCATTGCGATTATTCCAATTATTCTTACTATTATCACGTTGTTA
TGGTTTTTTTTAATGAGTATTTTTATTTCTATTTTGAATTCTTATTATTATCAAAT
ACCGATTATCAAATTGTTATTATTATTAAAATTCCTAATTTTATCATTGTTATT
TTGTCATTGTTACTCTTGTAAATATACAAAGAGTTGATAACACTTGACTCTTA
ATGTGTTTAAATTTAATTAGTTATGCAATGAAAATTCGACATAATCCTACATT
TCTTTGCTTCGAAAACAAGAATTATGATGTCGAAAGTAGCAAATGCAATTAA
GGTAGAGCGGCAATATGATGCGCAAAGAGTCCTTAATTTGTTTTTTTTTATAT
TGAACAAATTTTTCAAAATTTGTAGTTGCTTAGCGGCTAAGTAATCTCCATGC
ATCAACTGTTATAATGCAATTAATATGAAAATTAACAGTGCTGTTATTATAAT
CATTACAATTATTACTGTAACTATTAACTTTGTTATTATCATTTTATTACTGCTT
TTTAAATTATTATTAT

DkaSAT1 22 869

GTTATCGTCACCGTACGAGGTGTTGATAAAATTCAACTCAAAATGTGTTTCAA
TTTCACTAATTAAATGAATGAAAATTGAATATTTTGCAATATTTTATTTCAGA
AACTAAAATAACAATGTACAAAATAAAAGAAGAACAAATATAAAACCGCAA
TATTTTTAACTGAGGTAACCTTTATTTATAATTTTGCTGATGGACCAAATCGTT
CGGAATCTGCAGTTACTTAGCAGTGAAGGAAATCCGGTGCCAATTGTTCTTAT
TGCGGCTAGTGTGGACGTTCGCATTATTGTTAACATAATCATTAAGATTATCA
CATTTATTCTTACTAATATCAAGTTGTTATGGATTTCTTAACTAATATTATTAT
CTTAAATTCTTATTATTATCAAATTCATATTATCAAGTAGTCATCATTATTATT
GTTTTTTCCATTGTTGTGTTTATTTTATCATTGATATTGTTCTGGACAAACAAA
GAGTAGATAAAATTCAACACTTAAAGTGTTTAAATATAATTAATTAAGCCAAT
GAAAATTCAACAAAATCCAATATTACGCTATTTCAAAGAAGAAAATTAAAAT
GGATAAAATGGCAAATACAATTAAGACACAGTAGCAATATTATGTCAAAGGG
TCCTTATTCCGTTTTTTTTATACGGACGAAAATTTTCAAAATTCTGCACTTACT
TAGCGGCTAAGGAACCTCCGTGCATCAATAATTCCAATGCAATTGATATGAAT
AATAGCATTGTTGTTATTATAATCATTAAGATTATTACTATTACTATTAATGTT
GTTATTATCATTTTATTATTGCTTTTTAAATAATTATTATAGTTATAATTATTAT
TAACATTTCTTATTGTT

DkaSAT1 47 900

AATCGTCGTAATCAAATAATAATAGAAATAAGAAGGCGAACATAATAAGAAC
ACCAATAGTTATAACAACAAAAATAATACTAGTAAAATGAAGAACAACAAAA
TAATAATAATAACGAAAATAACAACACAAATAACGACGGTAACGATGATAAC
CATCATTAAAAAAGCAACAAGGACAATTATAATATTATTGTTAATAGTAATA
AAACATATACTAATTGCAATAAAAACAACTGATGCATTTCAATTCCTTAGCTC
CTAAGCAACTGCGGATTTTGAAAGATTTGATCATTAGAGAAAATTGCAAATTA
TGGACGCGATCGTGTTTTTATTACTTTTACGTATTTTGTATATTATTAATTTGG
TTTCTTAATAAAAATCTGGGAAGAGATGGTATTTTAATGGACAATTATTTAAA
TCGAAAAACATTTCGTATTTTGTTTCCTCAATCGTAGCTATTCAATAATAACG

GAAATGAGAAAGCGATAATAAGAATAACAGTAATAATAAAAATAACACAAT
AATATTAGTAAAACTACGAATAACAATATAATAAAAATAACAACAATAATAA
CACTAATAATGATGACAGCAATGATAAACGTCGCAAAAATAACAACGGTGAC
CACAAATATATTATAATTCATTGTAACAAAAATACACATCAGTCGCAACAAA
AACAACTGATGAATTTGGTTACTTAGCCGCTAAGCAATTGCGGATTTTGAACC
ATTTCATCAATATACAAAAATGCAAATTAAGGGCGCGATTGTTTTTTTATGAC
TTTATGCATTTCCTACATTTTAATTTTGGTTTTTGATGAAAAATCAAGGAATAT
ATTGCACTTTAATTAGCATAATTATTGAAGTTAAAACACTTTTAGGATTTTTTT
CCCTC

DkaSAT1	48	470	GTAATTCAAGTGCATCAGTTGTTTTTGCTACAATGAATGTGTACATTTATAACT ACCAATAATAATATTTTTGCTGTCGTCGTTGTTATTGTTGTGATGATGATCATC ACCATCATCCTTATTAGTTTTATTAGTGTCGTTATTATTATTAATTTTTTTATTC TTGCATTTATTAATATTATTTTGGCAATTATTGCTGTTGGTTTTGTACTTATTAT TGCTTTCACATTTCTGTAATTATTAAATAACAACGATTGATAATGCGATTCTA AAAGTGTTTTAATTTCGTTCATGAAATCAATGAAAATAGAATATATTCCTTGA TTTTAATTCAAAAGCCAAAATCATATTGAAGGATGAGCAAAAAAAAATAATAA AAATAGAATAGTGGTATGAATA
			TATGATGAGTCCCAAGATTAGACATATCTTTATTTACAAAATCTTTCAAATTC CGCCGTTGCTTAGCGGCTAA
DkaSAT1	50	463	TAATTATCGAATTCCAATTACGAAATTGTCCTAGTTATTATAATTCTTGCAATT ATTATGGTTATTTTAGCATTGATATTGTCGTAAATTTATGAATTGTTCACAAAA CACAACTCTTAAGTGGTTTAATTTTAATTCATTAAGCCAATGAAAATTCGACA TAATCCTTTATTTCTTTATTTCAGATACTAAAATAATAATGTAGAAAATAGCA AATGCAATTGAGATGCAGCCGCAAAATGATGTTAAAGGGTCTTTAATTTGGTT TACTTTTATATTCGTCAAAATTTTCAAAATCAGCAGTTGCTTAGCGGCTAAGT AATCTCCGAGCATCAGCTGTTGTGTTGCAATTAATACGAATATTAGCATTATT GTTATCATATTCATTCCTTTATTTCAATTATTCTTACATTTGTCATGTTATTATT GCTTTTTAAATTAATATTATTATTTTAAATTATTA
DkaSAT1	55	451	ATAATATTATTACTTGTAGTAAAAAAATATATATTAATTGCAGTAAAAACAAC TGATCCATTTGGATTTCTTAGCCGCTAAGCAACTGCTGATTTTGAAAGATTTG ATCAATATAGAAAACTGAAGATTGAGGACGCGATCGTATTTTTATTACTTTTA TATCTATCCAATATTATAATATTGGTTTGTGAAGCAAAATCTCGGAATATATC ATATTTTAATTGACATAATTAATGAAATTAAAACACTTTTAGTGTTTTGTTTCC TCAATCGTTGTTATTTAGTAATAATGGATGCAATAAAGCAAAAATAAGAATA ACCATAATAGTATTAATCATGACAATAGTATTGGTAAATACAAGAATAACAA AATAATAACCATAACAACAATAATAACTCTAATAATGACGGTAGCGATATGA CCATCATAAAAATAACAACAGCGACAAAA
DkaSAT1	56	474	TAAAACTAATAATGATGATGGTGATGATCATCATCATAACAATAACAACAAC GACAACAAAAATATTATTATTGGTAATTATAAATATACATATTCATTGTAGCA AAAACTACTGATGCACTTGAATTCCTTAGCCGCTAAGCAACCGCGGATATTGA AAGATTTTTGTAAATCAAGATTTGTCTAAACTTCCGACTCATCATATATTCAC ACTACTATTCCATTTTTGTTATTTTTTATGCTTATCCTTTAATATGATCATGATT TTTGATTTAAAATCAGGGAGTATATTATATTTTCATTGATTTCACGAGTGAAA TTAAAATACTTTTAGCATTTCGCTTTGTCAACCGTTGCTATTTAATAACTATAG AAATGAGAAAGAAATAATAAGTATAAAATCAACAGTAATAATTGCAATAATA TTATTAATAAATGTAAGAATATTAAAATTAATAATAAAAACAACACTAA
DkaSAT1	57	472	TTTAATTCAAAAATCAAAATTATACTAAAGGGTAAGCAAAAAAATGACAAAA ATTGTACAGGAGTACGATTATGTGATGAGTCCCAAGTATAGACATATCTTGAT TTGCAAAATCTTTCAAAATCCGTGGTTGCTTAGCGGCTAAGCAATTCAAGTGC ATCAGTTGTTTTTGCGGCAACTAATATGCATATTTACTATTACCAATAACAAT ATTTTTGTTGTCGCTGTTGTTGTTGTTTTGATGATTATCATCACCACCATCTTTA TTAGATTTGTTTGTGTTGTTATTATTATTAATTTCACTATTCTTACTGCTGTTAA TATTATTATTTCTATTACTACTGTTGCTTTTATAATTATTATTGCAATCTCAATT CTTTTATCATCAAATAACAGAGGTTGATAAAGCAAAATGCTAAAAGTGTGTTA ATTTCGATGATGAAATCAATGAAAATATGATACACTCCCTTAT
DkaSAT1	59	868	TAGTAAGAATAATTGTAATTGCCCCAGTGATTAGGATAACAATAAAGCTAAT ATTCATATTAGTTGCAGTAATAACAAAGGGAACGGAAATTCTTTTTCCGCTAT GTAACGGTCGATTTTTAAAGATGTGGTCCATCAAGAAGAATTCAAATTAAGG ACTCTTTGGAAGCTCATATTTCGATTGTATATATGTGGCTTTTTTAATTTTCAG CATTGTAATTTTAGTTTTCGCATTGAAATATCGGAATATAATGAATTTTTAAA GATTTAATTTGTAAAATTGAAACGCTTTTTGTGTTCACTTTTATCAACTCCTCA CGCATTCATAATAATAACAATATAAAAGACATTAGTAGTAATTATAACAATA AAAATTTGAAAGCGGTAATAAAATGATAATAACAACATTTACAGTAGCAGTA ATGGTCGTAGTGATTGTTATAAGAATAATACTATTATTCACATTAATTGCAGC

			ATAATAGTTGATGCACGGAGATTACTTAGCCGCTAAGCAACTACAAATTTTGT AAAATTTATTCAACATAAAAAAACAAAATTAAATGCTCTTTAGACATCATGCC GCCGCTGTGTCTTAAGTGCACATGAAATTTTCAACGTTATAATTTGAGTTGTT GAAATAAACAAATGTGGGTTTTTGTTGAAATTTGATTTGCTCAATTAAATAAA TTCAAACACTACAAGAACTGAGTTTCTTCAATTGTTTTTATATTAACGACAAT AACAACGTCAAAATAGCAATAATAATAGTAAGAGTTATAATAATAATCGTAA TTTGCACATTGGAAAATTGCAACAATAGGAATTTAAAATAATTATATTAGTTC GAGAAGCCTTAACCACTTGATAA
	DkaSAT1	69	1730	TATGTCGAATGTGCATTGGCTTAGATAATTAAATTGAACCCTTTATCAGTTTA GTATTATCAACTCTTTGTTTGTTCACAATAATAACAGAGGCAAAATAACAGCA GTAGTATTAAGAATTGTAATAATAATAGCAATTTGATAGTCGAAATTTGATAA TAATTAGAAATTACAATACTAATACTAATTAAAAAAACCATAACAACATGAT AATAGTTTGAATATCTGTAATAATCGCAATGATTATGATAACAATATTGCTAT AACATTCATATTAGTTGCAAAAATTACAGTTGGCATTGTAATTTCTTTACCGCT AAGAAACCGTCGAATTCGAGAGATTTGGTCTACCAAAGAATATACAAATTAA GGGCTCTCCGCGTAGCCACACTGCAATTGCATATTCGACGCTTTTTGTACTTTC TACATTATAATTTTAGTTTTACAAATAAAATATCGCAACACATTGAATTTTCGT TGATTTAATTAAAGGAAATGGGTCGCCTTTTGAGTTGACTTTTGCCAACACAT CATACGCTGAGAGTAATAGCAACAAAAGACATTAGAAATGATGATAATGGTA ATAATATGATAAGCAACAATAAAATCACAGTAACGACATTAGTAGTCACTAT ATTAACCGTAATGATTATAGCAACAACAACGCTAATATTCATATTAATTCCAA TATAACAGTTGGTGCTCTGAGATTCCTTAGCCGCTAAGCAACTGCCGATTTTG AAAAAATTCGTCAATATAGAAAAAACAAAATTAAACACTCTTTACACATCAT ATTGCCCCTCTATCTTAGTTCCATTTGCAATTTTCTACAATATAATTTTAGTTTT CGAAATAAAAAAGTATATGATTATGTCGAATTTTTCATTAGCTAAGTTGATTA AATTTAAACCCATAAGGAGTTGAGCGTTACCAACTCTTTGTTTATTTACAGCA ATAACAAGGACAAAATAACAATAATAGTTTTAAAAATTATAATAATAATAAC AATTTGACTGTCGGAATTTGACAGTAATAAGAAATTAAAAGAATAATACTAA TTAAAAAAAACCACAATAACTGGATGATAGTGAGAATAACTGCAATAAATGC AACGCTAACTATAATAATAATGCTAATTTTTTTTTAGTTGCAAAAATAACAAT TTATGCAGGAATTTCCTTATCGCGAGATAACTGCCGATTTCGAAAGATTTGGT CCATCAAGAAAAATACATATTACGGACTCTCCGGATATCAAAATTGCGATTGT ATATTCACAGGTTTTTTGCTTTCTGCATTATAATTTTTGTTTTTAAAATAAAAT ATCGCAATACATTGAATTTTCATTGATTTAATTGAAGAAATTGGATGTCCTTCT GAGTTGACTTTTGTCAACATATCGTACACCGACAATAATAAAAATAAAATAC ATTGTAAATAATAATAACAATAATTCAAAAAGCAGCAATGAAATGTTAATAA CAACTTTAACAGTAACAGCATTAATCGTAATTATTATAACGACAACGATGCCA TTATTCATATTAATTGCATCATAACAGCTGAAGCATGGAGATTCCTTAGCCGC TAAGCAACTTCCGATTTAGAAAAATTTCGTCAATATAAAGAAAACCAAATTA CATGTTCTCTACACATCATATTGCCGCTCCAAATTAATTGCATTTCTTATTTTC TTCAGTACAATTTTAGTTTTCGAAATAAAGAAATATAGGAT
	DkaSAT1	99	863	TTTATTTTTTGAAACACAATATAGTAAAATATCAAATTTTGAATGACTTCTTCC TTGAAATTAAAATCCTTTAAGAGTAGAATTTTATCAACTCCACAAACTAGATA ATAATAGCAACAAGAAAATTACATTAGTAATAGCAATAACAATAATAATTTG GAAAACAACAATATAATGATAAATTACGACATTAATTATTACTACAATAATC GCAATGATTATAATAACTGCAACCCTAATATTCATATTAATTGCATCATAACC GTTGTTGCACGGAGATTACTTAGCCGCTAAGCAACTACATATTTTGAAAAATT TCATCAATATGGAAAGTCCAAATGAGAGACTCCTTTGTCGAGTCATCTTGCGG CCATATGTTTGTTGACTTGCTATTTTCTAGATTATAATTTTAGTTATTTAAATA AACAAACATGGGAACATGTTGAATTCTTATTGACTTGATAAACTAAATTTAAT CCTTTAAAGAGACGAGTTTTATCAGCTCTTTATATATTTATAACAGTAAAAAA AGAAAAATAGCAATAATTATATTATGAATTATCATAGTAATAATAATTTGATA ATAACAATATGAATTTAAAATAATAATATTAATTTAAAAAACCATATTGACTT CACAATAGGAAGAATAATTATAACGATGGCAATGATTATGATAATATAGTGC TAATTTTCATATATCAGGAGCAATAATAAAATTTGGCACCAGAATTCCTTTAC CGCAAAGTAGCCACGAATTTCGAGAAATTTGGTCCATCAGAGAAAAAAATAC ACATTAAGAACTCCACATATATATATATTTGGAATGCACCGTAATTGCCTCCT TTTCTCTACATTACAAT
	DkaSAT1	135	841	TAATGGGAACAATAATTGTAATAATTGCAATGATTATGACAAAATAGTGCTA ATTTTCATATATCGGTAGGGATAATAAAATTTGGAACCGGAATTCTTTTACAG CAAAGCAGCCACGAATTTCGAAAGATTTGGTCCTTCAAGGAAAAAATACAAA TTAAGAACTCTACATACATACATATTTCGATTGCATCGTCATTGCTTCCTTTTT TCCATATTACGAATCTATTTTTTGATGCACAATACAGTAAAAATTTGAGTTTTT AATGACTTCTTTCCTGAAATTAGAATCCTTTAAGAGTAGAATTTTATCAACGA CACAAATCTTGGTAATAACTGCAAAAAGAAAATTACATTAATAATAGTACTA ACAATAATAATTTGGAAAACAACAATATAGTGATAAATTACGACATTAATTG TTATAATAACTGCAATGCTAGTATTCGTATGAATGGCATTATAACAATTGTTG CACGGAGATTACTTAACCGCTAAGCAACTACTGATTTTGTGAAATTTGATCAA

			TATAGAAAGTCCAAAGGAGAGACTCCACAGTCGAGTCGTGCTGCGGCCACAT TTTGATTGACTAGCTATTGTCTAGGTTGTAATTTTAGTTTTTCAAACAAAGAAA CATAGGAATATGTTGCATTTTTTTTTGAATTGATTAATTAAACTTAATCCCTTCA AGTGACGAGTTTTGTCAACACTTTACACATTTATAACAACAAAAACCATAAAA TAACAATAATTATGTTATGAATTATGATAGTAATAATAATTTGACAATAATTA TAAGATTTTAAATTTATAATATTAATTTAAAAAACCGTAAGACTTCA
	DkaSAT1	142	466	TGATGGTGATCGTCACCTTCATCATTTTTTGTTATATTAGTGTTGTAATTACTA TTAATTTTTTTATTCTAACTTTTTTTAATATTATTATTGCTATTATTACTATTGC TTTTATACTCGTTATTGTCTTCGCAATTCTGTTATTATTAAATAACAACGACGG ATAAAGCGAAATGCTAAAAGTGTTTTAATTTCAGCACTGAAATCAATGAAAA TATAATATACTCCCAGATTTTCATATAAAAATCAAAATAATATTAAAGGATAA ACACAAAAATAACAAAAATGGCATAGCAGTACGAATTTATGAAGAGTCCCGA GTTTAGACATATATGGATTTACAATATTTATCAAAATCCGCGGTTGCTTAGCG GCTAAGTAATTCGAGTGCATCGGTTGTTTTTATTGAAATAAATTTAGATATTT ATTATTATTGTTATTATTAATATTTTTGTTGTTGTTGTTA
	DkaSAT2	32	2350	CAAAAATGTCGCAAACATGCCTCTTAATATGTATTTTGAGCTATATATGCGCT ATGCACGAATTTTTCAACCATTTCATTGCCTAAGTTACTCTAGAAAATTAACT AATTGCACAAATGTGCTCGAGGTGTCATCATCTACTCGCTTTAATACCTGATT TGTTCAAATTTATACCCACTTGAGTAAAATCGGGGCAAAAATCAGTGTAAAAT GCATTTTGTGCTATGTATAATGTGTAATTGTGTGCTCTAATCATTTCGTTGTGT GATTCATGCCTTAAAATTAATAAATTGCACTGATATGATCGAGACGTCATCAT CATTCTGCTTTATTACCATTCACGCAAATTTAGAAAAGATCGCGGAAAATTGT AGCAAACAGGCTGCTTACGGCGCATTGTGTGCACCATAAATGCTGTAAAGGT GTTTCTCAATCAATTTAACGCGTAATTCATGCTTCAAAGTCAATTACTTCTGCT GGTGAGCTTGAAGTATCATTATATATTTGCTTTACTATCGGATTCACACAGAT TTGTGCACATTTATTCAAAACTAATGCAAACATGCTGTTAGAAGGCATTTTGA TCTTCAGACACGCTTTAATTTAGTTTTTCAACAATTTATTTGCGTAATTAATTT CATATAATTAATTAATTGCTTTGACGTACTTGAGACACCTTTGTGTATGTGCTT TAGTGCCTTATTTGTGCTAATATATACAAAATTGGTGCAAACAAGCTGCTCTC AACGCATTCTGAGCACTGTGTGTGCCGTAATTGTGTTTTCTGATCAATTCAAG TCGCAATTCACGCCTGAAAATCAATTAATTACGCTGATATTCTTGAAATGTCA CTACCTATTTGGTTTGCTACCTGATTCACACTAAGTTGTGCACATTAAATCGA AATTGAAGCGATCATGTTGCTTAAAGTGCTTTCCGAGCACTATGTACGCTGTA ATTGTGCTTTTCAATCATTTCCGTGAGTAATTCATGCCCGAAAATCAGTTAATT GTGCTGATATGATTGAGACATCACTACCTATCAGCTTTAATAACTTATTCACG CAATTTTATGCACAATTGAACGAAATTGAGGCTGACATGCTTTTAAAAATACA TTCTGAGCACTATGTATGCCTTGAGTGCGTTCATAAACAATTTAGTGCGTTATT CGAGCGTGAAAATCAATTATTTGCGCAGATGTGCTAGAATAAAAATTATCTAT TTGTTTCAACACCTGATTGATCCTGATTTATACACATTTAATAAAAAATGTTGC AAACAAGCTTCTCAATATGCATTTTGAGCACTCCATGTGCTTTAATTGAGTTTT CAACCATTGCTTTGCGCATGTTTTGCGACAGAATCAGTTAATTGCTCTATGTG CTCGTGGCATCGTTATCTATTCGCTTTAATATCTGATTTCAGCTATTTTATACA CAATTGAGCAAAATCGACGCAGAAAAACTGCATAAAATGCTCTTTGAGCAAT GTACAATCAGTAATTGTGTGCCTCAATCATTTCATTGCTTAATTAATGCTTTAA AATTAAATAACTGCAACGACGTGCTTGAGACATTATAATCAGTTTGCTTTGCT CCCTCATTTACTCTGTTTATGCAAGATTGAGGAAAATTGATTCAAGCAAACTG CTTAAAATGCATTCTGCGCACAGTACATGCTGTAATTGTGTTTCTCAATCGATT TAAAGCTTAATTCGTGCTTGAAAACCAGTTAATTACGCTGATGAGATTGAAAT ATCAATATCTGCCTGCATTTTCTGGGATTCACACCAATTTGGGTGCATTAAAT CAAAACTAAAGCAAACACGCAGCTTAAAATGTGTTTTGAGGACTACATATGC CTTAATTTAGCATTTCGACCTTTTAGGTGCAACGTAATTAATTAATTGCACTGA CGTGCTTTAAACACCTTTTTCCATGTGCTTCAATGCCTTATTTATGCCAATTGG TACAAGATTGAACAAATCTGAAACAAAAATATTGCTTACAGTGAATTCCGTG CGCTATACACGATGTAATTGTGTTTTCGAGTCAATTTATTATGCAATTAGTCCT TGAAAATAAATTAATTGCGCCCATGTACTTGAGACATCATTATCTATCTGCTT TAATACTTTATTTATGCAATTTCATACACAATAGAACGAAATTGAAGCAAAAA TGCGGCTGAAAATGCATTCTGAGCACTCCGTAAATGGCAATTCTGTTCATAAT CACTTCAATGTGTAATTCAACCATGAAAATCAATTAATTGCACTGATGTGCCC GAGGCAAAATTATCTATTTGTTTTAATACTTGATTTGTGCTTATTTATACTCAT TTTAAT
	DkaSAT2	51	1420	ATATAAGCTTTACTTGTGTTCTTCAATCATTTCATTGAGCAATTTATGTTTGAA AATCCATTAATTGTACTACTGTGCTTGACACATCATCATCTCTATGTGTTTAAT ACCTGATTTACGCAAATGTATGCACAATTGAGAGAAATCGATGCACACATGC TGCTAAAAATGCATTTAGAGCACCACATACACTGTAAGCGTGCTCTTAATCAA TTTAATGCGTAATACATGCTTGAAAATCAATTAATTGCAATGATGTGCAGGAG ACAAAGTTATCTATTTATTTTAATAACTGATTCATGCTAATTTATGCACATTAA ATCAAAAATTATGTAAACAAGCTGTTTAATATGCATTTTGAGCACTATAGACT CTCTAATTGAGTTTTTCAAACATTTATTTGGATAATTTATTCTAGAAAATCAAC

			TAATTGCACTAATGTGCTTGAGACTTCAATATCTATTTGGTTTAATACCTTATT TACTCTAATTTTCATACAATTGAGCAAAATCGATGCACTTAAAACTGTTTAAA ATTCATTTTGAGCGCTGTATAATCTCTAATTGTGTTTTTCAATCATTTCAAGTG CAATTCAGGCTTTCAGATAAATTAATTATGCTGATGTGCTTGAGACATCGTTA TATGTTTGTTTTATTACACTTATTTGCTATATATAAGCTTTAATTGGGTTCTCC AATTAATTGCAACAATGTTCTGGAAACATAATTGTCAATTTGCTTTATGACCT GATTCACGCTAATTTATACACATTTAATCAAAATTGATGCAAGCAAGCAGCAT AAAATGCATTTTGAGCACTACATAAGCATTAGTTGTTTTTTCAATCATTTTTTT TTTTTTGCTCAATTCACGCAAGAAAATTCATTTAATTGTACTACTCTGCTTGAG ACTCCTTTATATCTATGCTTTAATATCTGATTTACACAAATTTATGCCAAATTG AGCGAAATTGATGGCAACGTGCTGCTTAAAATGCATTCTGAACACTGTATGA ACTGTAATTGCATTCTTAATGAATTCAATGAATAATTCATGTATGAAAATTAA TTAAGTGTTGCAATCATATCATTGCGTAATTCGTGCTTTAAAATCGACTGATT GCACTGATGTGCTTGAGACATAGCTATTTGTTTGCTTTATTATCTTATTGACCC TAATTTAGTCAAGATTGAGGAAAATTGTGGCAAACAAGTTGCTTAAAATGCA TTTTGAGCACTATATAATCTTTAATTGAGTTCATCAATCGTTTCATTCCATAAA TCATGCATTACAATCAATTAATTGCAACAATGTGCTGGAAACAAAATTATCTT TTTGCTTTAACACCTGATTCATGCTAATTTATTCACATTAAACAACAATTGATG CAAACAAGCTGCTTAAAATGCATGTTGAGCACT
DkaSAT2	87	28	TGGGGTCTAGGGTCTAGGGTTTAGGGTT
DkaSAT2	104	2040	AGCACATATGTACAATTAATTGATTTTCGTGCCCAAATTCCTCTGAGAAATGC TTGAAAGATGCAATTCCTGCGAATGTAGTGCTCATAGTGCATTTTGAGCAGTT TGTTTGGAGCAATTTTGAGAAATTGAGGATAAATTGGCAGGAATAAAAAATG AAAGCAGACAGAAAATGATGTGTAAAGCACATCAATGCAATTAATCGATATT CAATCGTAAATTATGCCGTTAAACGATCGACAAATACAATTAGGGCATATAT GGTGCCAGATTTGCAGTCTAAACGGCCTGTTTCCTCCAATTTTGATTTAGTGTG CAGAAATCTTAGTGAATCACGTATTACGGCGAATAGATGATGACATTTTGAG AACAATGGCGCAATTAATTGATTTTGAAGCATGATTGAGGGATTAAATTGATT GGAAAACCCCATTAGAGCGTATGTTGTGCACAAAGTGCATTGCTTGCGTCGCG AGTGCTTCGATTTTGATCAATTTCATATAAATTTGTGTAAATAGGGTAATAAA GGAAATAGATTATGGTGTCTCACGCACATCAGTGCGATTAATAGATTTTAAAG TGTGAATTACGCAGAGAATTGATTGTAGAGTAAAACAAATGTCTTTGACGAG CTGGAAATGCATTTCATGCAGCCTTTTCAAATCAATTTTGATTTGGAGATGTG CAGAAATTAGTGAATCAGGTAGTAAAGCAAAAAGATAACGATGGTTCAAATG CCTGGGTGTAATTAATTATTTTACGAGCTTGAGATGGGGATTAAACATTTTGA AGAACACAATTACACGCACATAGCGCACACATTGCGTTGTAAGCATCATGCTT GCTCGAATTGCTGTCAATTTTGTTTAAATTAGTATAAATAAGTTATTAAATGA GCCAGATAATGGTGCCAAAAGCACATCCGTGCAGTTAATTGATTTTAATACTT CAATTACACGACATACTCTATGAAAAGTACGACTGGAGCGTATATAGGGCTA AAATTGCGTTTTATGCCGATTGTTTGCTTCAAGTTTGACAATTGTATACAAATT AGTGATAATATGGTATTAAAACCAGCAGATAATTACGTCACAAGCACATGAG TGTAATTGATTGATTTTCATGCCTGAATTACGCATTAAATTGATTAGAAATGC AATTACAGAGCATGTGCAAATCGAAACATATTTTAAGCTATTCGTTTAGGGTC AGTTATGCTAATGTTTCCTAATTGACATGAATCAAGTATTAATGCAAGTAGAT AATAATGACTCAATTGCATTAGCGCAATTAATGAATTTTCTAGCACGAATTTC GCATTGAATTAATTGAAGAACACGGTCAATGCTGATATTTTTCCGGAAAGGCA TTTTGAGCGGCTTGTTTGCCTCAATTGGATTGTAAGCATCATGTTCGTTTGAAT TGCTATCAATTTCTTTTAAATTGGCACAAATAAGGTATTAAAGGAGCCTTATA GTGGCGCCAAAAGCACATCCGTGCAATTAATTGATTTTAACGCATCAATTACG CAACAAACTTATCGAAAAATACTACTGGAGCATACATTGTGCTCAAAATGCG TTTTATGCGGATTGTTTGCATCAATTTTGACTATTGCGCACAAATTAGTGCGA ATCAGGTTGCAAAGCCAGTAGACTGTTACGTCTCAAGCGCATGAGTGTAATTG GTTGATTTTCTTGCCTGAATTATGCATTAAATTGGTTACAAAATTAACTGCAG AGCATGTACATATCAAATATATTCTAATCTGCTTCTTTTGCGGCAACTATGCA AAATGCTTGTCAATTCAAGTGACTCGAGTATTAATGCAAATAGATGAAGATGT CTCAATTGTATCAATGCAATTAATTGATTGTCTAGTATGAATTGTGCAATGAA ATAATTGAAGAACACCATCACTTCTGATATTGTTCTGCAAATGCATTTTGATC GGCTCGTTTGCCTCAATTTCTCAAAAATGATTTAAATTAGCACGAATCAGGTA TTAAAGCCAATAAATAATTATGTTCCC
DkaSAT2	114	1330	TAGACATCCTTATCTGTTTGTTTTAATACCTGATTTACTCTTATTTGTACATAA TTGAGCAAAATCGGTGCAAACGTACTGCTTAGAATGCAATTTGATCGCTGTAT AATCTGTAATTGTGTGTTTCGATCATTTCTTTGCGTAATTCAAGCTTCGAAATC AATTAATTGCATTCAAGTCCTTGAGACATCATCATTAGTTTGCTTTATTATCTT ATTTACAAAAATTTAGAGAGCACAGAGGAAAATTAACTCAAACAGGCTGCCT GAAATGTATTTCGAGCACTATATTAGCATTAATTGCGTCCTTCAATCGTTTCGT TGCACAGTTCGTGCTTTACAATTAATTAATTGCAACGACGTGCCGGAAACAAA ATTGCTGTTTTCCTTTAATACTTGATTCACTCTAATTTATCCACATTTAATTAA CTAAAATTGACGCAAACAAGGTGCTTAAAATGCATTTTTGGGCACCATGTACG

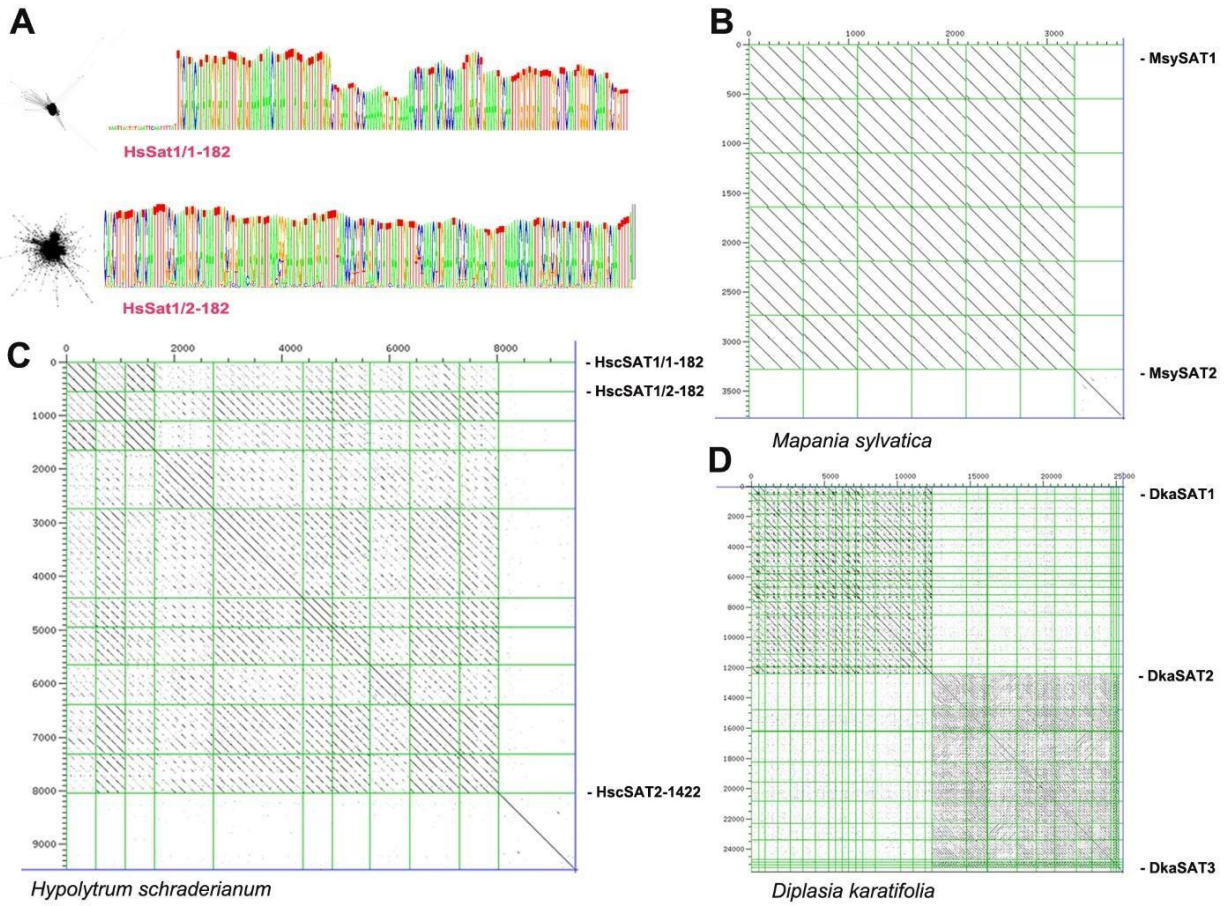
			CTTTAATTGTGTTCTTCAATTATTTGATCTTGCAATTCATGCTTTAAAATCAAT TAATTGCACTACTATGATTGAGACATCTTTATCTCTGGGCCTTAATTAATGTCT TATTTATGCAAATTTAGACACAATTGAATGAAACCGACGCAAACATGCATCC AAAAATGCATTCAGAGCACTATGTATGGAGTAATTGAGTTCTTAATATATTTA AAGCCATTCGTGCTTGAAAATCAATTAATTGCAGTAATGTGCTGGAGACAAA ATTATCTATTTGTTTTAATACCAGATTTATGCTAATTTATGCACATTAAATCTA AAATTTTGCAAACAAGCTGCTTAGTAAGCATTTTGAGCACTCTAGGCTCTATA ATTGAGTTTTCTGAACCATTTCTTTGCATAATTTTTGCCAGAAAAACAAACCA ATTGCGCTGATGTGCTTGAGACGACATTATCTATTTGTTTGAAACCTGATTGC ACTAATTTGCACTCAATTGATGCAAACAAACTGCTGAAAATGCATTTTGAGCA CTGTACAATCTTTGATTATGTGTTTAAATCATTTTACGGTGTAATTCGAGCTTC CAAATCAATTAATTGCACTGATGTGCTAGAGACCTCTTTATATATTTGCTTTGT AACACTTAATTGCGCTATATAAGCTTCAATTAAATTTTCCAATTAATTGCAAC TTTGCTCTGGAAACATAATTATCTACTTGCATTAATAACTAATTCACCCCAATT TATACACATGCAATCTTAATTGATGCAATCACGCTGCAAAAATGGATTTTGTG CACCATATGAGTTTTAATTGAGTTCTTCAACTATTTCTCTGGTGCGCTT
	DkaSAT2	130	1240	AAAGAAAACGTATGTAGTGTTGAAAATGCATTTTAAGCCGCTTGTTTGCTTCA ATTTTGACAAAATGTGCATAAATTAATGTGAATCTGGTATTAAAATAAATAGA TAATAATGTTTCTAGCATATTAATGCAATTAACTATTTTTATGCATGAATTACA CAATAAATTGACTGGAAACACAATTATAGCGTTTATAGTACTCATAGTGCATT TTTAAGCAGCATGTTTGCGTCAATTTTGGTCAATTATGTATAAATTTGCATAA ATAAGGTATTAAAACAAATAGATAAAGACGCACCAAGCACCCTAATGTAATT AATTAACTTTAAAGCATGAATTACGCAAAGATATAATTAAAAAAGCACAACT AGCAGCGTATCCAGTGCTCAGAAAGCATCTACGTAGCTTATTTAAATCAATTT TGCTAAATTGAATACAAATTAGTGTAAATCAGGTATTCAAGCAAATAGATAA TGATGTCTCAAGAATATTAGAACAATTAATTGATTTTCAAGCATAAATTACAT GAAGAAAAGCTTGAAAAACACAATTAGAACATATATAATGCTCAAAATACAT TTTAAACCACTTGTTTGCATTAATTTTGATGAAATGTGTATAAATTAGCTTGAA ATAGGTATTGAAATAAATAAATAAATATGTTTCTAGCACATCAATACAATTAA CTGATTTTCAAGCATAATGTACGCATTAAATTCATTAAAAACACAATTATAGC ATATACAGTGCTCAGAATGCATTTTAAGCAACTTGTTTGTGTCAATTTTGCCTA GTTGTCTATGAACTAGCTTGAATCAGGTTTCAAAGCATAATGATCTCTCGAGC ACATCATGCAGTTAATTGATTGTTAAGCATGAATTATGCAAAGAAATGATAGT TGGTTTTCAAGAGTGAATTACGCAAACAAATAATTGGAAAACACAATTAAAG TATACAAAGTGCTCAATCAATTAGCATTTTAAGCATACGAAGCACATCGGTGC ATTTTAAGCCACTTGTTTAGCATGAATTACGCAATACAATGATTGAAAAACAC CATTACAGTGTATATAGTACTCAAAATATATTTTAAGCAGTTTCTTTGCATCG ATTTTGCAAATTCTGCATAAATTAACAAAAATCAAATATGAAAGCAAATGGA TAATGATATCTCAAGTAATTAGTGTGGTTAATTGATTGTCAAGCATGAATTAT CCAGAGAAATATTTGAAAAAAAAAAC
	DkaSAT2	137	1480	CAACGATTTCCATGCGTAATTTGTGCTAGAAAATCCACTAATTGCACTAATTT GCTTGGGACTTCGTTATCAATTTGTTCTAACGTCTCATTTACGCTAATTTGTAC ACAATTGAGCAAAATTGATGCAAACAAACTGCTTAAAATGCATTTTGAACGC TGTATAATCTCCAATTGTGTGTTTCAATCAATCTTGCGTAATTCATGCTTAAGA ACCAATTAATTGCATTGATGTGCTGGAGACATCGCCATTTGTTTGCTTTATTTT CTTATTTACCCCAATATAGACAAGATTGAGTTAAATTGAGGCAAACAAGCTGC TTACAATGCATTTGCAGCACTATATAGGCTTTAATTGTGTTCCTCAATCATTTC ATTGCATAATTCACGCATTGCAATCAATTAATTACAACGATGTGCCGAAAACA AAATTATCCTTTTTCTCAGACGCCAGATTCGAACTAAGTTAGACACATTTAAA AGAAATTGAAGCCAAAAAGCTGCTTTGAATGCATTTCAAGCACTATGTAACCT TTGATTGTGTTCTTCAATTATTTCATGGCACAATCATTCTTAAAAATCAGTTAA TTGCACTACTACATAGGGGACACCATTATCTCTATGCTTTAATATCTGATTTAC GCAAATTTGTTCAGGGCTGAGCGAAATTGATGAAAACATGGTGCTTAAAATG CATTCTGAACACTATATGAACTGTAATTGTGTTCTTAATCAATGCAACGCTTA ATTCATGCCAGAAAATCAATTAATTGCACTGTTCTACAGGAAAAAAAATATCT GGTTATTTAAATACCTGATATATGCTAAATTATGCAAATTAAATCGACATTGT TGCAAACGGACGGCTCAAACATACATTTTGATCACCATATGCACTTTAGTTGA GTTTTTCAACCATTTCTATGCTTAATTGATGCAGGAAAATCAACTAATTGTACT AGTGTGCTTGCGACTTCATTATCTGCTTGTTTTAATGCCTGATTTACGCAAATT TGTACACAATTGAGCAAATTGTTGAAAACAAACTGCTTAAATTCATTTTGAGT GCCGTATTATCTGTAATAGAGTGTTTCAATCATGAATTGTGTTTTTCAATGAAT TCTTTGCCCAATTGAGGTACTAAAATTAATTAATTGAACTACTATGCTTGAGA CACCTTTATCTCCATGATTTAATATTTGATTTATGCAAATTTAGACACAATTGA GTGGAATTGCCGCAAACATGCCGCTTAAAATGCATTCTGAACACCATATGAA CTGCAATTGTCTTCTTAATCAATTTAAGACGTAATTCATGCTTGAAAATCAATT TACCGCACTTATGTGCTGGAAACAAAATTATCTAGTTGTTTTAATACGTGATT TATGCTGATTTATACAAATTAAATTAGCATTATTTCAAACGGGTGGCTCAATA TGAATTTCGATAACTGTTTGTGCTCCAAAATTGGGATTTT

DkaSAT2	152	1100	GTGTTTTTCAAACAGTTTAATCCATAACTGAAGCATGAATATATATTCATTAC GCTCAGGTATTTGAACCATGGTTATCTACTTGCTTTCCTGCTTGATTCACAAAT TTGGGCACATTTCCTAATCAAAATGGATGCGAACGTGCTGCATTAAATGCATT TTTAGCACGATAAAATCTTTAGTTGGATTCTTCAACCAATTCTCTGCGTAATTG ACACATTAAAATCAATTAATCGCACTGGTGAGATCGAGACACTATTATCTATT TTCTTTAATATCCTATTTACACTAATTTATACGAAATTGATCAAAATTGAAGC ATAAATGATGCATACAATGCACTGTGCGCACTATATGCGCTGAGTGGAGTTTT CCAATCAGGTTAATCCCTCGATCACGCTTGAAAATCAATTAATTGAGCCAGCG TCCTCAAAACATCGTCATCTACTTGCAGTAATACCTGATTAGCTCAGATTTAC GCACATGCAATCAAAATTGATGTAAACAGGCCGTTTGAAATGCTATTCTGGCA CCATGTGTGCTTTAATTGTTTTTGTCAATCGTTTAACTGCATAATTCATACTTG AAAATCAATTAATTGCACTGATGCAATTGAGGCGTTGTTATCTATTTGCCTTA ATCCTTGATTCTTGTTAATTGATAAACATTTAGCATAATTGATGCAATCTAGC AGCTTAAAATACATTTTAATTTCCACATGCTCTGCAGTTGTCTTTCTAATTAAT TTAAGGTGTAATTCAGGCAAGAAAATCAATCAATTGCACTAATGTTCTTGAGA CGTAGTTATCTACTGGCTTCACTACCAGATTCTCACTAATTTGTGCATATGTAA TCAAAACTGATGCACACAAATTGCATAAGATGCATTTCGTGCACAATATATGC TCCAGTTGTATTTTTCAATCATTTTGTTGTGTAATTGGTGCAGTAAAATCAATT AATTGCAGGGATGTGCTTTTGGCACCATCATCTGGTTCCTTTAATACGTTATTT ACGCTAATTTAAACGAAATTGATGAAAATTGAAGCAAACATGATGCTTATAA TGCATTGTGCGTGTTATATACGTTGTAATT
DkaSAT2	161	1280	TAGTGCCCAGAATGCATTTCAAGCTGCTTCTTCGCAGCAGTTTTGCTCAATTGT GCATAACATAGCTTGAAGAAATAATCAAAGCAAACAGATAATGATGTCTCAA GCGCACGTGCGCAATTCATTGATTCTCAAGCACGAATATGCAAAGAAATGAT TGGAAAAACCAACTAAAGCATATATATTTCTCAAAATGCACTTGAGACAGCT GGTTGGCATCAATTTTCCTCAATATTGTCCAAACTGATTTTAAGCATGCCAAT AAAGCGAATGGATAATGATGTCTCAAGCACATCATGCAATTGATTGACTGTC AAGCACGATTACGCAAAGAAATGTTTGGAAAAAAATAATTGAAGCATATATG GTGCTCAGAATGCTTTTTAAGCTGCTTGTTTGCGTCAATTTTGATGAAATGTGT TTAAATTAGCGTGGATAAGGCTTTAAAGCGAATACATAATTCTGTTTCTAGCA CATCAGTGCAATTGACTAATTTTAAAGCATGAATTACGCATTATATTTGTTGA AAACACAAACAGAGCTTCTATAGTATTCGTAATGATTTCTAAGCAGAATGTCT GCCTCAATTTTGGTCAATTGTGTATGAATTAGTGTGAATTAGGCATCAATGCA GACAGATAACGTCGTCTCGAGCACATTAGTGCAATTAATTAATTATCAAGCAT GAACTACACATAGAAATGATCGGAAAACACAACAAAAGCGTATATGGCGCTC GAAATGCATTTCAAGAAGATTCTTTGTGTCAATTTTCCTCTATATTGCCCAAAT TTTTCGTAAACGAGGTAATAAATCAAATGCATAACAGTGTCTTATGCACATCA GATCAATTAATTGATGTTAAAGCCGGAATATTGGAAAGAAGTGATTGAAGCA CGCAATTGCCGATTATATATTGCTCGAATTGCAATTTAAGCAGTTTGTTTCAG ACAATTTTGGTCCGTCGTGCGTAAATTATCACAAATCAGGTATTAAAGCAAAT GGATAGCGATGTCTCCGGAATATCAGCGCCCTCAGTTAATTTTCAGGAGTGAA TTACTCGTGGAAATGATCCGAAATCACAATTACAGCGTATATGGTATTCAATA TGCATTTTAAGTAGCTCGTGAGCATAAATCTTTAAAAAATTAGCAAAAAATTA GCATCAATCCGGTATTAAAACAAGTAGATAATTTTGTTTCTTGCACACAAGTG CAATTAATTTAATTTTAAAACACGAATTACAGCACCAAATTGTTTAAAAACAC AATTACAGCTTA
DkaSAT2	167	181	TCATCACTTTTTTGCGCAATTCAAGCTCAATAATCAATAAATTATGTTTATTTA CATCAAATATCACTATATGTATGCAATAAAATTGAATTCACACTAATTAAACA GCATTTGAACCCAATTGAAGCCAAAATACACACAAATTGCATTTTCAGCTCAA TTTGGGCTGTAATTGAGTTTT
DkaSAT2	4	183	TTTAAAGCATGAATTACGCAAAGAAATGATTGAAGAACACAATTAAAGCTTA TATAGTGCTCAAAATGCATTTTAAGCAGCTTGTTTGCATCAATTTTGCTCAATT GTGTATAAATTAGCGTAAATCAGGTATTAAAGCAAATAGATAATTATGTTTCC AGCACATCAGTGCAATTAATTGAT
DkaSAT2	18	183	TGAAGCAAACATGCTGCTTAAAATGCATTTTGAGCACTATATAAGCTGTAATT GTATTTTTCAATCAGTTCAATGCCTAA TTGATGCTTGAAAATCAATTAATTGCACTGATGCGCTTGAAACATCATTATCT ATTTGCTTTAATACCTTATTCACACTAATTTGTGCACATTTGATCAAAAT
DkaSAT3	52	260	GAGCCCGCAGGGGAGCGTGGGGGTCCGCACGAAGCGTAAATTACGTTTTTGC CCACACAAATAACAGCGCCCTCTGCCGCCGTACGGCGCCGTAAGCGGGGCAC AGGGCAGGGACAACAGCCCACGCCCCCCCAGCCAAGGTGCTCCCCCTCAATA TGCAAAACCCATTTTTTGCATCCAAACAGCGTACTTTTAACTCTCATCGCTGTT TCAGCCACCGCCAAGCCATGACTCGGTCGGCGCTTGGGCGCCGCCGAGCG

M. sylvatica	MsySAT1	1	182	GCTCAGAATGCAATTTTGCAGCTTGTTTGCATCAATTTTGCGCAAATGTGCAT AAATTAGCGTGAATCAGGTATTAAAGGGAATAGATAATGATATTTTAAGCAC GTCAGTACAATTAATTGATTTTCAAACTTGAAATACAGAAGGAACTGATTTAA AAATCGAATTGCACCGTATACAAT
	MsySAT1	2	182	ACGCACTACAATTGTGTTTTTAAATCAGTTCTTTGCGTATTTCATGTTTCACAA TCAATTAATAGTACTGACGTGATGTAAATATCATTATCTATTCCCTTTAATACC TGATTCACGCTAATTTATGCACATTTGCGCAAAATTGATGCAAACAAGCTGTC AAATTGCATTCTGAGCATTGT
	MsySAT1	5	182	GCGCAAATGTGCATAAATTAGCGTGAATCAGGTATTAAAGGGAATAGATAAT GATATTTTAAGCACGTCAGTACAATTAATTAATTGTCAAATATGAAATACGCA AAGAACTGATTTAAAAACACAATTGTAGCGCATACAATGCTCAGAATGCAAT TTGACAGCTTGTTTGCATCAATTTT
	MsySAT1	9	182	TTGATGCAAACAAGCTGCAAAATTGCATTCTGGGCATTCTATACGTTGCAATT CGGTTTTTAAATCAGTTCCTTGTGTATTTCAAGTTTGAAAACTAATTAATTATA CTGACGTGCTTAAAATATCATTATCTATTCTCTTTAATACTTGATTCACGCTAA TTTATGCACATTTGCGCAAAA
	MsySAT1	19	182	TGCTCAGAGTGCAATTTGACAGCTTGTTTGCATCAATTTTGCGGAAATGTGCA TAAATTGGCATGAATCAGATATTAAAGGGAATAGATAATGATATTTTAAGCA CGTCAGTACAATTAATTGATTTTCAAACTTGAAGTACGGAAGGAACTGATTTA AAAATAGAATTGCACCGTATACAA
	MsySAT1	49	182	CAATTTTACAGCTTGTTCGCATCAATTTTGCTCAAATGTGCATAAATTAGCAT GAATTAGATATTAAAGGGAATAGATAATGATATTTGAACCACGTCAGTACTA TTAATTGATTGCCAAACATGAAATACGCAAAGAATTGATTTAAAATCACAATT GCAGAGCATACAATGCTCAGAATG
	MsySAT2	3	475	CTCTCGCCGCAGTGGGGGGTTCGGATGGCATTGGAATGCTGCTGCCGGCCCAT AATGAAGCCTCGGATGACCCTCTCACTTGCGCCTTGCTTTCCATGTCGTATGA TGTTGGATTGCTGGCGGTGGCCCCTCTCGTTGCCTCGGATGACCCACGTATCT TGTGCCTCGGTAAAAACCCCATGCGTGCTTGCATCGCTACGGGGTTGTGGTTG CCTGCGGGTTTAGTTTGCCGGATGATGTTTTCGGTCATTCGCGTACGGCGGTC CCTTGCTGCTGCCTGTTCTTGTTGACCTTCTCGTCGCCAGCTTCTCGGCTATTG AGGTCGCGGGGTTGGTGCTTGGGGGCGCTTGCGGCAGCTGGCATTGGACTGT GGCGACGCGTGAGCGGTCCTGGACTAGCTCTGCATGGCGGGCTCGCCGCACT TTGGTGCGGCCAACCGTCATGGCGCTGTCTTTTCACATGCCCACCCGTGCAG
H.schraderianum	HscSAT1	1	181	GTTTAATTAGTGTGAATTCAATTTTATTGCATACATATAGTGATATTTGATGTA AATAAACATAATTTATTGATTATTGAGCTTGAATTGCGCAAAAAAGTGATGAA AAACTCAATTACAGCCCAAATTGAGCTGAAAATGCAATTTGTGTGTATTTTGG CTTCAATTGGGTTCAAATGCT
	HscSAT1	2	182	ATTGTCAAACATGAAATACGCAAAGAATTGATTTAAAAACACAATTGCAGCG CATACAATGCTCAGAATGCAATTTTACAGCTTGTTTGCATCAATTTTGCGCAA ATGTGCATAAATTAGCGTGAATTAGATATTAAAGGGAATAGATAATGATATTT GAACCACGTCAGTACTATTAATTG
	HscSAT1	4	181	ATGTATATTTACATCAAATATCATTATATTTATGCATTAAAATTGAATTCACGC TAATTAAACAGCATTCGAACCCAATTGAAGCCAAAATACACATAAATTGCAT TTTGAGCTCAATTTGGGCTGTAATTGAGTTTTTCATCACTTTTTTGCGCAATTC AAGCTCAATAATCAATTAATT
	HscSAT1	42	1400	CCGAACTTAGTACTTAATATGTGTGTTTTCTAGAAATAATATCTTCTATACTGA ATAACAACTATAGTGTTTCCTACATGAGAAGCCCAACCCCCCCTCCGGAGCGA GGGTGGTTGGCACCGAACTTATCTAGTATAATATATATATGTATTATTAATGT TAACTAGAATATGAGTTTCCACTTCTAGTTAACATTGCCGGATGTCGGCCTTC AGGCCGATACTATGGGCCAACACCCTTTGGCCCATAGGTTAGGTACGTCACA GGTCCGGAAACATTCCCCTTCGGGTTAGCGAGGAGGGAACCGACGACCTATC CCGAAGAAATCACAGTTTTTCTTGATCCAAAGTTTATTCCTAGGCTCGTTCGC GCCAGCGAATGAGTGTTTATACCTTGGTTATAGGAAAACCCCTTCTAGTACAG AAATACAGGAGTTATACGACTGTTTTTACTGACTAGAAGTACCGCTTCTCAAA CATTGAAAAATAGAGCTAGTCCCCACTATTAAATCCCCACCGGGGTGTCCTAG TGCAGATGAGCTGAACAAATTCAATGTCCTAGCGATAGCGATCTTATAAAGG GATGTTAACCTAAAACCTTACCCTGAGCGCAGCGAGTATAAAGCCCACCGGC GCCTGAGGTCACCGCTCCTTATATATAAGTACTAGATAAGTTCTGGTTTTGCC AAACCGAGGGTCTCCAAAAACCACACCTTAATGGTGGTCTCTACCTCGGCCCA CTACCAGGCATGACCCTGGGGTGTTCTCTGGATGGATGCCGTTAATGACCGAG CCACCAAGGAGGTCTATTCTTTAAGCACCTCTTATAGGGGAGTTAACTAGTTA GGTCTGCTTCTCAATCCTTAGGAGTAGGTCATAGGCTCTAAGTGTGTGTCCGG

			CTGTTCCCTACCAGACTTCTTAGATAGTAAGTCCGTGCCCCTTAAACCCGTAG GTCCCCAGGGCTTTCGTATCCTGTATCGGCTGTCCACGTTCCTAACTGAGCTA ACTTCCCGTTTACTGAGTTGCCTCCAGTTTTTTCTCCCTCAAATCTCGGGGTAG ATCTCTCTACCATTTAGGGAGTTGAGTCCCACATCCAGATATTGAACACCAGT TGAAGCCGTCTCGTCTTATCTGTTAGTCTCCTTGGTACTCTCCCAGAGAGAAA TAAGGGATCAGACAGCATCTATCGAGTATTTATCCAATTCGATAGTTCTAACA GATAAGTTTCTTCAACCATCTACGATACCATTTTCGAAACACAATACCAGGAA GAAAAGATTAGTGAAACTGTTTTTTTCTGTTTTGTAACTGAGCGAAGCGAGTA TAAGCCCACCGGCGTTTGAGGTAACCTATTTGTATTCCTCTAGTTCTTACAACT AAGTACTAAGTTCCTGACTCCA
	HscSAT1	71	1090	TTAATTAGTGCGAATCAGGTATAAAAGCTTATAGTTAGTGACGTTTCAAGCAT ATAAGTTGAAGTTTTTGACTTCCAGGCATGAATTGAGGAAATAAATGTTAAAT AACAGATAATTAAAAGCAAGTATTGTGCTGAAAATTCGTTATAAACATCCTGT ATGCGTCAATTTTGTCAGATAAGAATTAATTAGCGAGTGTCAGGTATCAAATC GCATTGTTAAGGTAATTTCAAGCATATAAGTTTACGTTTGTGATTTTTGGGAA TGAAAAGAACTGAATTAAATCATAGTATTAAAGCATTTGTAATGCTGAAAAT GTGTTTTTGGCTGCTTGTATGCATCAATGTAGTTGAAATGTGAGTTAATTAGC AAGAATCATGCACCAAATCGTATTGTTAATGTAGTTTCAAGCATATTATTAAC ATTTGTGATTTTTAGGCATGAATTGAGCAAAGAACTGGACTAAAACATACAAT TAAATTAATTATAGCGCTGAAAATGTATTTTAAGCAACCTGTTTGCCTCCATTT TGTTCAAATGTGAATTAATTAGCGAGAATCAGATATCACATCGTATTGGCAAT TTAACTTCAAGCTTCTAAGTTAATGTCTGTGATTTCTAGGCATGAATTAAATA ACCGATAAATAACATACAATTAAAGCATGGATTGTGCAGAAAATGCGTTTAA TGCAGTCTGTTTGAGTCAATTTTGTTCAAATTTGACTTAAATATTGCTAATCAG GTATTAAATCTTTTTGTCAATGAAGTTTGAAGAATATAAGTTAAAGTTTGTGT TTTCCACTCATCAATCGAGCAAGGAACTGAGTTAAATCATACTATTAAAGCAT TTGTAGTGCTGAAAATGTGTTTCAAGCATCCTGTATGCTTCAATTTTAACCAA ATATGAATTAACTGGCGAGAATCATGTATCAAATTGTATTGTTAATGTAATTT CATGCACATAAGTTAACGTTTGTGATTTCCAAGCATGAATTGAGCAAAGAACT GAATTAAAACATATAATTAAAGTATTTATAGTGCTCAAAATATATTTTAAGCA AACTGTTTCAATTCATATTGTTCAAATGAAAA
	HscSAT1	78	1660	ATTAGCGAGGAAAAGTTATTGAAGCATACAGATGATGATGTCTCAAGCACAA AAGAAAAATTGATTGAATGTCAAGCATGAATTATGCAAAGAACTGATTGAAA AACACAAATACAGCGTATATAGTGCTAAGAATGCATTTTACCAGCTTGTTTGC ATCAATTTTGTATGAATGTGTATCAATTAGCGAGAATAAGGTAATAAAACATA TAGATATTGATGTTTCAAGAACATATAGTGTAATTAATTGACAGTCAAGCATG AATTAAGCCTAGAACTGATTGAAATAAGAAATCACTGCTTATTTAGTGCTCAG AATGCATTTTTAGTAGTTTATTTGCATCAATTTTGTTCAAGTGTGTATAAATTA GGGTGAATCAGGTAATAAAGCACTTCGATAATAATTTTTCAAGGGCAAAAGT CAAATTGATTGGCAGCCAAGCATGAATTACACAATGAACTGATTGAACTACT AAATTACAGCGTATATAGTGCTCGCAATGCCTTTTAAACAGCATATTTACATC AATTTCATTGAAATGTGTATGAATTAGCATGAATAAGGTATTAAAGTTTATAG ATAATGATCTTTCAAGTGCATCAGAGTAATTAATTGATTTTCAAACGTGCGTT ACGTTAAGAACTGAATGAAAAATACTATTTGAGCGTAAATAATTTTCTGAATG CATTCTAAGATGCATGTATGCATCATTTTTGTTCAAATGTGTATGAATTAGCGT GAATAAGGTAATAAAGCATATAGCTAACGTTGGTTCAAGCGTATCAGAGTAA TTAATTAATTGATTGCCGAGCATGAGTTACATTAAAAACTGATGGAAAAACAT AATTAAGCATATATAGTGCTCAGAATGGATTTCAAGGAGCTCGTTTGCATAAA TTTTATTCAAATATGTATTAATTAGCGAGATTCAAGTAATAGTGCATATAGAT AACAATGTTTCAAGCACATATCGTGTGATTAGTTGATTTTCAAGTAGGAATTA CGAATAAAACTGATTGATAGAAAAACTTACAGTGTATATAGTGCTCACGATG AATTTTAGCAGGCTATTTGCTTCACTTTTATCGAAATGTTTATCAATTAGCGTG AATCTGGTAATAATGCACTTTGATAATAATTTTTCGAGAGCAAAAGTCTGATG GAATGATTTTTCAAGCTTGAATTATGCAAGGAACTGACGAAAATACATATTTA TAATATGTACAGTGCTCATAATGCATTTTAAGCAACATGGATGCACCAATTTT GTCCAAATGCATATAAATCACCCTGAATCAGGTATTAAAGCAAATAGATAAT GATGTTTCCGGCGCATTAATCTATTTTATTTATTTTCATGCTGGAATTATGCAA ATAATAGAATGAAAAACACCAATTACAGCCTATATAGTGCTCAGTATGCAGT TTAGGCAGCCCTTTTGCATCAATTTTGTTTAAATGTGTACTAATTTGCCAGAAT CATGTAATAAAGCATATAGATAATTATTATATTTAAAGCAAGTATACTGCAAT TAACTGATTATCATGCATGAATTACGCTATCAACAGATTGAAAAATCTAATTG AAGAGTAAATAGAGCTCAGAATTCTTATTAAAAAGCCAATTCTGTATCACGTT TGATAAATATACATTA
	HscSAT1	104	184	TGCTTGTTTGCATCAATTTTGTTCAAATGTGTATTAATTAGCGAGAATCAGGT AATAAAGCATATAGATAATGATGTTTCAAGCATATATAGTGTAATTAACTGAT TGTCAAGCATGAATTACGCAAAGAACTGATTAAAAAACACAATTAGAGCGTA AATAGTGCTCAGAATGCATTTTAAGC

HscSAT1	118	709	CAAGCACGAATTACGCAAAGAACTGATTGAATAACACAATTACAGCGTATAT AGTGCCTCAAAATGCGTTTTAAGCAGCTTGTTCGCTTCAATTTTGCTCAAATA ATTATAAATCAGCATGAATCAGGTATTGAAGCATAGATATTTCAAGCACATCA GTATAATTAATTTATTCACAAGCATGAATTACACAAAGAACTGATTGAAAAA CACAATTACAGCATATATAGTGCTCAGAATGCATTTCAAGAAGTATGTTTGCA TCAATTTTGCTCAAATGTGTATAAACTAGCATGCGTCTCGTTTGAAAGCATAT ATATAATGACAATTTAGGCATACCAGTATAATTAATTGACTGTCAAGCATGAA TTATGAAAAAAAAAACTGATTGAAGAACACAATTACAGTTTTGAGCACCTTT GTTTGTGTCAATTTTGCTCAAACGTGCTTAAATTAGCATGAATAAGGTATTCA AACATGTAGATAATAATGTTTTAAGCACATCAGTGTAATTAATTGATTGTCAA GGATGACCTGTCCAAAGAACTGATTGAAAACACCATTACAGCATATATATAG TGCTCAGAATGCATTTTAAGTTGTATTTTTGCATCAATTTTACTTAAATGTGCA TAAATTAACTTCAATCGGGTATTAAAAGCATACAGATAATTATGATTCAAGCA CATCAGTGCAATTAATTTATTTA
HscSAT1	140	739	AATTAATTTTTGGCTCGATTCATGCATTACAATCATAATTGTACTGATGTGATT GAAGCATCATTAACTATATGATTTATAATCTGATTTTTACTAATTAATTTAGAT TTTTAACTAAATTAATACAAATAAATGGCATAAAATGCATGTTGAGCACTATA CATGCAGTAACTGTATTTTTTTTTCATGAATTCTTGACTCAATTCAAGCATTAC AATCACAAAATTACACCGATGTAATTGAAACATCATTAAAAATATAGTGTAG TATCCGATTTTCTAGCTAATTCACATTTGAAGAAAATTGACGCAATCAGATGG CATAAAATGCATTTTCAGCACTATACGTACAGTAATTGTATTTTTCAAAAATA GATATTGGCTTAATTAATGCATTACAATCACAAAAATGCACAGATGTAGTTGA AGCGTCGTTAACTATATTGTTTAAAACATGTTTCTCGCTAATTTAATTCACATT TAAATAAACTGATGCAAAACAAAAGGCATAAAATACATTTTCAGCTTTTACAT ACTATAATTGTATTTTTTTTTAATATGTTCTTGGCTCAATTCGTGCAATGCAAT CACAAAATTTCATTGATACGCTTTAATCATCATTTAACTACATGATTTATAGCC TGATTCTCACTGATTAATATACATTTGAAAAAATAGATGCAAACAAGTCGTAT AAAATACATTTTGAATGTTATATGGGCTGTAATTATATTTTTTTT
HscSAT1	144	918	ACAAATTAGCATGAATCTGATATTAAAGATTATAGATAATGATGTTTCAAGAA CATCAGTGCGATTAATTGACTGTCAAGAATAAATTAGGTAAAGAACTGATTG AAAAACACGATTATAGCGTATCCAGTGCTCACAACGCATTTTAAGCAGCTTCT TTTCATGAATTTTCCTCCAATGTGTATAAATTATCCTGAATAAGGTATTAAAGT ATATGGATAATAATTTTTCAAGCACATCAGTGTAATTAATTAATTGTCAAGCA CGAATTACGCAAAGAACCGATCAAAAAACACAATTAAGGCGTATATAGTGCT CGGAATGCGTTTAAAGCAGCTTTATAGCATTAATTTCCCACAAATGTGTAAAA ACTAGCGAGAATCATGCATTAAAGCAAATAGGTAATGATGTTTCATGCATATT AGTGTAATTAATTGATTTTCAAGTATGGATTATACATTGCAATGATGAGAAAC ACAATAACAGCGTATATAGTGCGCAAAATACATTTTAAGCAGCTTGTTTGCAT CATTTTTGCTCAAATGTGTATAAATTAGCTTGAATAAAGTATTAAAGATTATA GATAATTATATTTCAAGTACATCGGGATAATTAATTGATTGTCAAGCCAGAAT TACACAAAAAAACTAATTGAAAATCAGAATTACAGCGTACATAGTGTGCTCA GAATTCATTTTAAGCAGTTTGTTGGTATCAATTTTGCTCGAACGTACATACATT AGCATTAATCAGATATTAAAGCATATAGATAATGATTTGTCATGCATATTAGC ATAATTTATTGATTGCCAAGTATGAATTATGCAAAGAAATGATTGGAAAAATT CAATTACAGCGTATATAGTGCTCAGAATGCATCTTAAGCTGCTTGTTTGCGTA ACTTTTGCTCAAATGTGT
HscSAT1	162	732	ATTGAAGCATATAGATAATGATGTTTTAATTACATCAGTTTAATTAATTTATTT TTCAAGCATGAATTACGCAATGAACTGATTGAAAAACACAATTACACATTAT ATTGTGCTTAGATTGCATTTTGAGCAGCTCTCTTGGATCAATTTAGCTCAAATG TGCATAAATTAGCACGAATCAGGTATTAAAGCATGTAGATAATAATTTTTTAA CCATGTCAGTATAATTAATAGTTTGTCAAGCGTGAATTATGCAATGAACAGAT TGCAAAACTCACTTGCAGCGTATATAGTGCTCAGAATGTATTTAAGCAGCTTG TTTGCATCAATTTTGCTAAAATGCATATATAAGTTAGCATGAATCTGGCCCCA AAGATTTACACACAGTATGATGCACATCGGTGCAATTAATTGATTTTCGAGCT TCCATTGCGCAGATTGAACTAATTGAAAAACACAATTACGTTGTTGGTAGTGC TCGGAATGCATTTTAAGCAGATTGTTTGCATCAATTTTGCTCAAATGCGCACA AATTTGCGCGAATCTGGTATTAAATATTATAGGTAATAATATTTCGAGCACAT CGGTGTAATTCATTAATTTCCAAGCATCAATTATGCAAAGAGCCGGCTGAAAA ACACAATTACATTGTATATAGTGGTGAGAATGAGTTTTAAGCAGCTTTCTTGC ATCAATTTAGCTCAACTGTGTGTAAATTAGCACGAATCAGGC



Suplementary figure 1. Structural organization of satDNA Hsc… and all to all comparison of satDNAs identified in Cyperid species. Sequence logos of the two satellites subfamilies from HscSAT1-182 from *H. schraderianum* analysed in this work (A). Dot-plot showing all satellite repeats identified by TAREAN in analysed *Mapania sylvatica* (B), *Hypolytrum schraderianum* (C) and *Diplasia karatifolia* (D).

4. AN OVERVIEW OF THE REPEATOME OF THE MONOCENTRIC *Juncus effusus* (Juncaceae) AND ITS CENTROMERIC ORGANIZATION

Yhanndra Dias[1], André Marques[2], Yennifer Mata-Sucre[1], Lucas Costa[1], Mariana Baéz[3], Andreas Houben[4], Andrea Pedrosa-Harand[1]

Artigo a ser submetido à revista Botanical Journal of the Linnean Society

Link: https://academic.oup.com/botlinnean

An overview of the repeatome of the monocentric *Juncus effusus* (Juncaceae) and its centromeric organization

Yhanndra Dias[1], André Marques[2], Yennifer Mata-Sucre[1,2], Lucas Costa[1], Mariana Báez[3], Andreas Houben[4], Andrea Pedrosa-Harand[1]

[1]*Laboratory of Plant Cytogenetics and Evolution, Department of Botany, Federal University of Pernambuco, Recife, Pernambuco, Brazil*

[2]*Max Planck Institute for Plant Breeding Research, Cologne, Germany*

[3]*Plant Breeding Department, University of Bonn, Bonn, Germany*

[4]*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany*

ORCIDs:

André Marques 0000-0002-9567-2576

Andrea Pedrosa-Harand, 0000-0001-5213-4770

Andreas Houben, 0000-0003-3419-239X

Lucas Costa, 0000-0002-4798-097X

Mariana Baéz, 0000-0002-7874-6385

Yennifer Mata-Sucre, 0000-0001-7905-0630

Yhanndra Dias, 0000-0002-6488-4736

Corresponding author:

Andrea Pedrosa-Harand

Universidade Federal de Pernambuco

Centro de Biociências, Departamento de Botânica

Laboratório de Citogenética e Evolução Vegetal

R. Prof. Moraes Rego, s/n, CDU

50670-901 Recife PE Brazil

Tel: + 55 81 2126 8846 or 8352, Fax: + 55 81 2126 8348

ABSTRACT

Juncus is the largest genus of the family Juncaceae and for a long time was considered as holocentric. Recents findings, however, pointed four species of the genus as having monocentric chromosomes. Thus, *Juncus* centromere organization and evolution need to be reassessed. Here, we characterized the repetitive fraction of *Juncus effusus* (2*n* = 42), analysing the composition and distribution of the main repetitive elements cytogenetically and along its assembled genome. In addition, we compared *J. effusus* and *Luzula elegans* repeatomes to investigate if these species share any repetitive DNA sequences despite different centromeric organization. We showed that the small *J. effusus* genome (0.275 pg/1C) is mainly composed of retroelements, especially the Ty1/copia superfamily, followed by satellite DNA. The most abundant elements, Angela and Athila, showed non-uniform scattered patterns, with distinct abundances at different chromosome pairs. The three satellite DNA families identified were mainly (peri)centromeric. However, immuno-FISH revealed that JefSat1-155, although present at the centromere, is not strictly centromeric. The assembly and annotation of centromeres confirmed that these repeats are interspersed in different ways at different centromeres. Comparison to *Luzula* showed that repeat families are largely exclusive to one or other species, with the sharing of only two classes of LTR-retrotransposons (Angela and Athila), besides rDNAs. The analyses confirmed the monocentric organization of *J. effusus* centromere and revealed a diversity of different satellites and retroelements..

Keywords: Centromere organization – Centromeric sequences - –Repetitive elements – Retroelement –Satellite DNAs - Chromosome evolution - Rushes

INTRODUCTION

Centromeres are essential for genome stability through the mediation of chromosome segregation in mitosis and meiosis (Cuacos *et al.*, 2015). Chromosomes that have a localized centromere, visible as a primary constriction, are called monocentric, while holocentric chromosomes have centromeric activity distributed along almost the entire chromosome axis (Melters *et al.*, 2012). Holocentric chromosomes also differ in mitotic and meiotic behaviour (Schubert *et al.*, 2020). Most organisms have monocentric chromosomes, but holocentricity originated independently at least 14 times throughout the evolution of eukaryotes, nine of them in animals and at least five times in plants (Melters *et al.*, 2012). However, the identification of centromere type is not always trivial and may be incorrect (Guerra *et al.*, 2019; Baez *et al.*, 2020), and when a chromosome type is defined, it may be incorrectly extrapolated to the rest of the family/group (Melters *et al.*, 2012; Heckmann *et al.*, 2013; Šmarda *et al.*, 2014).

The transition mechanisms behind the trasition between mono and holocentric are unknown, but monocentricity is usually considered the ancestral state (Nagaki *et al.*, 2005; Neumann *et al.*, 2012; Melters *et al.*, 2012; Schubert *et al.*, 2020), although some authors propose the opposite or do not rule out the possibility of a holocentric ancestor (Moore *et al.*, 1997; Escudero *et al.*, 2016). The differences in genomic organization between these two centromeric types show how complex and impactful the transition from mono- to holocentric was. In insects, the transition to holocentricity was associated with loss of the CENH3 genes, the most important centromeric protein, which is used for its epigenetic definition (Drinnenberg *et al.*, 2014; Cuacos *et al.*, 2015). In the genus *Cuscuta*, the transition had a significant impact on the genomes of these species, such as changes in epigenetic marks and in the composition of repetitive sequences. One of the most striking differences is the presence of satellite DNAs in monocentric species of the genus, and their absence , together with the lack of histone H2AT120, in the centromere of holocentric allied (Neumann *et al.*, 2021).

Repetitive DNAs are the major components of plant genomes, being classified into tandem (mainly satellite DNAs) and dispersed (mainly transposable elements) repeats (Neumann *et al.*, 2011). Satellite DNAs (satDNAs) are usually arranged in long, and frequently, head-to-tail arrays. They are a substantial part of many genomes and have been widely reported as a core component of plant and animal centromeres (Dong *et al.*, 1998; Aldrup-MacDonald & Sullivan, 2014; Sullivan & Sullivan, 2020; Wang *et al.*, 2021). Centromeric satDNAs are useful in the delimitation and characterization of this chromosomal region, as seen in rice

(Cheng *et al.*, 2002), beans (Iwata *et al.*, 2013), *Vicia faba* (Ávila Robledillo *et al.*, 2018), *Prionium serratum* (Baez *et al.*, 2020), and many other monocentric species. In holocentrics, centromeric satellite DNA was identified only in the genus *Rhynchospora* from Cyperaceae (Marques *et al.*, 2015; Ribeiro *et al.*, 2017), while in other groups, such as *L. elegans* and holocentric *Cuscuta* species, these sequences were preferentially found in the terminal region of chromosomes (Heckmann *et al.*, 2013; Neumann *et al.*, 2021).

Transposable elements are, in general, the most abundant repeat type in plant genomes and largely responsible for genome variation in many groups (Schnable *et al.*, 2009; Macas *et al.*, 2015; Sader *et al.*, 2021). They are dispersed and usually show uniform distribution in larger genomes or chromosomes and a more restricted pericentromeric distribution in small genome or chromosomes (de Souza *et al.*, 2018; Báez *et al.*, 2019, 2020; Ribeiro *et al.*, 2020; Sader *et al.*, 2021; Ibiapino *et al*., 2022). In *Cuscuta*, the variation in genome size is driven by the differential accumulation of LTR retrotransposons and satellite DNA, especially in monocentric species, which showed greater variation than in holocentric counterparts (Neumann *et al.*, 2021; Ibiapino *et al*., 2022). In contrast, small genomes tend to have a smaller fraction of transposable elements (Ribeiro *et al.*, 2017; Baez *et al.*, 2020; Sader *et al.*, 2021).

In plants, Class I LTR-retroelements are the most abundant transposable elements, but the prevalence of Ty1/copia and Ty3/gypsy varies in a given species or even among related species (Marques *et al.*, 2015; Van-Lume *et al.*, 2019; Ribeiro *et al.*, 2020; Baez *et al.*, 2020; Sader *et al.*, 2021). Ty1/copia elements often show an uniformly dispersed distribution (Heckmann *et al.*, 2013; de Souza *et al.*, 2018), while the Ty3/gypsy often show preferential locations, such as some lineages from the Clade Chromovirus found at pericentromeres or centromeres (Marques *et al.*, 2015; Van-Lume *et al.*, 2019; Neumann *et al.*, 2021). In the holocentric species *Luzula elegans* (Juncaceae), the Ty1/copia lineage Angela is the most abundant and makes up about 33% of the genome. It is dispersed uniformly along the chromosomes, but not associated with the centromere. Indeed, no centromeric repeat was identified in *Luzula elegans* genome (Heckmann et al., 2013).

Few groups of plants are known to have both monocentric and holocentric types of centromeres among their representatives, as *Drosera* and *Cuscuta* (Pazy & Plitmann, 1994; Shirakawa *et al.*, 2011; Neumann *et al.*, 2021). The monocot families Juncaceae, Cyperaceae and Thurniaceae form the Cyperid clade, which was considered, until recently, a clade of species possessing only holocentric chromosomes (Greilhuber, 1995). This was largely due to the detailed studies carried out in *Luzula* (Heckmann *et al.*, 2013) and some genera of

Cyperaceae, such as *Eleocharis*, *Carex* and *Rhynchospora* (Da Silva *et al.*, 2008; Marques *et al.*, 2015; Więcław *et al.*, 2020), as well as the difficulty in analysing karyotypes with very small chromosomes. This make data from particular taxa being extrapolated to the whole group. However, recent findings have challenged this view and showed that *Prionium serratum* (Thurniaceae), some species of *Juncus* and *Hypolytrum schraderianum* (Cyperaceae) are, in fact, monocentric (Guerra *et al.*, 2019; Baez *et al.*, 2020; Dias *et al.*, unpublished data), indicating independent transitions events to holocentricity within Cyperaceae and Juncaceae. *Juncus* L. is the largest genus in Juncaceae Juss., with about 315 species, followed by *Luzula* DC, the holocentric sister genus, with about 115 species (Roalson, 2005). Both show a wide range of chromosome numbers, with *Juncus* varying from $2n = 18$ to $2n = 170$, with $2n = 40$ being the most common count (Drábková, 2013).

Monocentricity in *Juncus* species from different clades (*Juncus effusus* L., *J. marginatus* Rostk., *J. microcephalus* Kunth and *J. tenuis* Willd.) was revealed by immunodetection of histones H3S10ph and H2AT133 (*J. marginatus* and *J. microcephalus*), meiotic behaviour (*J. microcephalus*), as well as based on chromosome morphology (Guerra *et al.*, 2019). Phosphorylation signals for both H3S10ph and H3T133ph appeared mostly as two dots at the median region of chromosomes and a clear monocentromere-like shape was visualized on mitosis metaphase chromosomes. Moreover, a typical reductional meiosis was observed (Guerra *et al.*, 2019). Genomic assembly of *J. effusus* was also confirmed the monocentric organization (Hofstatter *et al.*, 2022).

Juncus effusus (soft rush) is a species with an almost cosmopolitan distribution, having biotechnological importance for phytoremediation of contaminated water and for its medicinal properties (Liao *et al.*, 2011; Vymazal, 2014) and, therefore, its transcriptome was characterized (Arslan *et al.*, 2019). In this work, we performed a detailed analysis of the main repetitive DNA elements of this species by means of genome skimming, bioinformatics and chromosomal mapping of repeats, aiming to understand the genomic organization of these monocentric chromosomes. Additionally, the previously assembled genome (Hofstatter *et al.*, 2022 was investigated in terms of the distribution and organization of the identified repeats. We seek to answer the following questions: Which are the main repetitive sequences that make up the *Juncus effusus* genome? How are these elements distributed along *Juncus effusus* genome and centromeres? Do the monocentric *Juncus* and the holocentric *Luzula* share repetitive DNA sequences?

METHODS

Plant material

Samples of *Juncus effusus* L. were collected in Biritiba Mirim, SP, Brazil and grown in the Experimental Garden of the Department of Botany at the Federal University of Pernambuco and Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). A sample (voucher LPF 16950) was deposited in the EAN herbarium (Prof. Jayme Coelho de Moraes, Federal University of Paraíba, Areia, Paraíba, Brazil). The genome was assembled from the ornamental accession *Juncus effusus* var. spiralis, commercially available.

Estimation of genome size

The genome size of *J. effusus* was estimated by flow cytometry. Sample preparation was done according to Loureiro *et al*. (2007). Young leaves of *J. effusus* were chopped simultaneously with leaves of *Raphanus sativus* L. cv. Saxa (2C = 1.11 pg, Dolezel *et al*., 1992) in a Petri dish (kept on ice) containing 2 mL of Woody Plant Buffer (WPB). The sample was filtered through a 30-µm disposable mesh filter (CellTrics, SYSMEX, Norderstedt, Germany) and 50 µg/mL propidium iodide (from a stock of 1 mg/mL; Sigma-Aldrich) was added to the final mixture. Seven replicates were made and samples were measured in a CyFlow Space flow cytometer (SYSMEX) equipped with a green laser (532 nm). Histograms of relative fluorescence were obtained using the software Flomax v.2.3.0. (SYSMEX, Norderstedt, Germany). Mean fluorescence and coefficient of variation were assessed at half of the fluorescence peak. The absolute DNA content (pg/2C) was calculated multiplying the ratio of the G1 peaks by the genome size of the internal standard.

DNA extraction, genome sequencing and repeat characterization

Young leaves from *J. effusus* were used for genomic DNA extraction, using the DNeasy Plant Mini Kit (Qiagen). The DNA was quantified on 1% agarose gel and Nanodrop and sequenced using the HiSeq 2500 platform (Illumina) at low coverage by BGI Americas Corporation – Cambridge, generating 150-bp paired-end reads.

The repetitive DNA characterization was performed by a graphic-based clustering using the RepeatExplorer2 pipeline (Novak *et al.*, 2020). A total of 9,401,618 reads were

uploaded to the platform and 395472 from these reads were analysed, corresponding to a coverage of ca. 0.1×, filtered by quality with default settings (95% of bases equal to or above the quality cut-off value of 10) and interlaced. Clustering was performed with default settings of 90% similarity over a 55% minimum sequence overlap. The *Find RT Domains* tool and additional database searches (BLASTx) were used to identify protein domains for repeat annotation. Graph layouts of individual clusters were examined interactively using the SeqGrapheR tool (Novak *et al.* 2013). All clusters with at least 0.01% of the genome were annotated and manually checked to identify the most abundant families in the genome. The proportion of the main types of repeats was calculated from the amount of reads in the individual annotated clusters, in relation to the total reads, after excluding those of putative contamination (similarity to mitochondrial or plastid DNA).

For the identification of satDNAs, the TAREAN tool (Tandem Repeat Analyzer), also implanted in RepeatExplorer (Novák *et al.*, 2017), was used. To assess the degree of similarity between and classify the satellites , a Geneious alignment using the software Geneious version 9.1 (https://www.geneious.com; Kearse *et al.*, 2012) was performed using default parameters. We classify families and superfamilies based on the percentage of identity between the satellites. Sequences with similarities between 50-80% were considered to belong to the same superfamily, while similarities between 80-95% were considered subfamilies of the same family. Clusters with similarities greater than 95% were considered variants of the same family (Ruiz-Ruano et al. 2016). Moreover, a dot-plot using the consensus sequences of all identified repeats was performed in DOTTER to grasp the all-to-all similarity (Sonnhammer & Durbin, 1995)..

To analyse comparatively the genomes of *J. effusus* and *Luzula elegans*, sequences from *L. elegans* (ERX1349704) were downloaded through the NCBI and edited was previously described. The sequences were then uploaded RepeatExplorer2 and a comparative analysis were carried out with a random data set of 1,500,000 reads for *L. elegans* and 358,600 reads for *J. effusus*, corresponding to 0.1× the genome of each species.

Probe generation and fluorescence *in situ* hybridization (FISH)

For chromosome analyses, three satellites were selected: JefSAT1-155, JefSAT2-180 and JefSAT3-364, representing the most abundant superfamilies identified, as well as two LTR-retrotransposon: Angela and Athila, corresponding the most abundant element of the Ty1/copia

and Ty3/gypsy superfamilies, respectively. Primers were designed with Primer 3 (Untergasser *et al.*, 2012) implemented in Geneious version 9.1. In the case of satDNAs, the consensus sequences were used as target. When a satDNAs was represented by more than one cluster, the most abundant was selected for this purpose. For the LTR-retrotransposons, the integrase domain was confirmed in the NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi; Marchler-Bauer *et al.*, 2017) and used for the same purpose. Primers were designed facing outwards (satDNAs), while for the retrotransposons, primers faced inwards and should amplify fragments around 300 pb (Suppl. Table 1).

PCR reactions were performed in 50 μL reactions containing 10 ng of genomic DNA from *Juncus effusus*, 0.1 mM dNTP, 2 mM $MgCl_2$, 1× PCR buffer, 0.4 μM of each primer (Suppl. Table 1), 0.4× TBT and a Taq DNA polymerase. The PCR program involved 30 cycles of amplification (1 min at 95°C, 1 min at the annealing temperature and 1 min at 72°C; Supp. Table 1). All PCR products were checked by sequencing and labelled by nick translation using Alexa-488 (Jena Bioscience) or Cy3-dUTP (Thermo Scientific; Suppl. Table 1) with DNase I (Thermo Scientific) and DNA polymerase I (Invitrogen) or a nick translation kit following manufacturer's instructions (Jena Bioscience).

Mitotic chromosomes were prepared using the air-drying method with modifications (two hours of enzymatic digestion) with 2% cellulase Onozuka and 20% pectinase Sigma (Ribeiro *et al.*, 2017) using pretreated roots with 2mM 8-hydroxyquinoline for 24 h at 4°C, fixed in Carnoy ethanol:acetic acid 3:1 (v/v) for 2 h and stored at −20°C. The best slides were selected for Fluorescence *in situ* hybridization (FISH) after screening with 1 μg/mL DAPI in glicerol and destained using Carnoy for 30 min and 100% ethanol for 1 h. FISH was performed as described by Pedrosa *et al.* (2002), except for JefSAT2-180, for which we did the FISH also at lower stringency, washing six times with 6× SSC at room temperature. The slides were counterstained with 2 μg/mL DAPI in Vectashield (Vector) mounting medium. The images of the best mitotic metaphases were captured using an BX61 epifluorescence microscope (Olympus) equipped with a cooled CCD camera (Orca ER, Hamamatsu) or an DM5500 epifluorescence microscope (Leica) and the Leica Las AF software. Overlapping, brightness and contrast adjustments were performed in Adobe Photoshop® CS3.

Sequential detection of CENH3 and DNA satellite repeats

The JeCENH3: VRTKHFSSRPAGSGRPRKR-C peptide was used to produce polyclonal antibodies in rabbits Using the services of LifeTein (www.lifetein.com). Mitotic preparations were made from root meristems fixed in paraformaldehyde and Tris buffer (10 mM Tris, 10 mM EDTA, 100 mM NaCl, 0.1% Triton, pH 7.5) for 40 min on ice in a vacuum and for another 20 min only on ice. After washing twice in 1× PBS for 10 min, the roots were digested in a cellulase-pectinase solution containing 1x PBS buffer and squashed in 1x PBS. The coverslips were removed in liquid nitrogen and the slides were air dried and stained in DAPI:Vectashield for slide selection under the epifluorescence microscope. The best slides were incubated in 3% (w/v) bovine serum albumin (BSA) containing 0.1% Triton X-100 in PBS. Immunostaining was performed using the primary antibodies: rabbit anti-JeCENH3 (diluted 1:300) and mouse anti-alpha-tubulin (clone DM 1A, Sigma, diluted 1:200). As secondary antibodies, a Cy3-conjugated anti-rabbit IgG (Dianova) and a FITC-conjugated anti-mouse Alexa488 antibody (Molecular Probes) were used in a 1:500 dilution each. Slides were incubated overnight at 4 °C, washed 3 times in 1 × PBS and then the secondary antibodies were applied. Immuno-FISH was performed following Houben et al. (2007), the slides were washed with PBS for 15 min, postfixed in 4% paraformaldehyde in PBS for 5 min, and then probed with the satellite JefSAT1-155. Labelling and detection of DNA repeats were performed as described above.

RESULTS

The repetitive DNA composition of *Juncus effusus*

The DNA content of *J. effusus* was estimated to be 0.275 pg (1C), corresponding to 268.95 Mbp. A total of 9,401,618 reads were uploaded and a random sampling of 395,472 reads was analysed using the graphic-based clustering of RepeatExplorer2 to characterize the repetitive DNA fraction, which corresponded to a final 0.1× genome coverage. A total of 211 clusters with abundance above 0.01% was generated, indicating that the repetitive DNA sequences of *J. effusus* comprises about 24.035% of its genome (Table 1). Most of the repeats remained unclassified (9.022%), and among those annotated, the majority was LTR-retrotransposons(6.4%), with six lineages of Ty1/copia and two of Ty3/gypsy (Fig. 1). The Ty1/copia superfamily was dominant, making up about 4.81% of the genome and Angela was the most abundant lineage (3.45%). Ty3/gypsy contributed to 1.6% of this genome, where 1.56% was from the non-Chromovirus Athila lineage (Table 1). The CRM lineage from the

Chromovirus clade, usually associated with centromeres, was the second most abundant Ty3/gypsy lineage, but represented only 0.028% of *J. effusus* genome. Class II elements, DNA transposons, made up a small fraction (0.695%), with TIR/MuDR_ MUTATOR being the most abundant.

Satellite DNA was the second most representative repetitive DNA type, making up 4.92% of the genome (Table 1). Ten clusters corresponded to this type of sequence, but only the most abundant one represented a high-confidence repeat (CL 1). CL10 (5,575 bp) and CL18 (1,595 bp) were not detected in a tandem orientation in the genome assembly, and thus not further considered. Based on the percentage of similarity, the satDNA clusters were first grouped into six families, five of them distributed into two superfamilies, SF1 and SF2 (Table 2). The most abundant satellite in the genome (JefSAT1, 1.75%) showed monomers of 155 (CL1) and 154 (CL30) bp, with 91% identity along its length. Two others SatDNA families (JefSAT3 and JefSAT4, both 364 bp long) were grouped into superfamily SF1 (1.57% abundance), with 75% identity between them. The remaining three satDNA families (JefSAT2, 5 and 6), with monomers varying between 122 and 180 bp, were grouped into SF2 (1.55%), with identities between 51-80%. Other tandem repeats, such as 5S and 35S rDNA represented together 2.99% of the genome.

Chromosome mapping and immuno-FISH indicate a diverse distribution of repeats

To investigate the chromosomal organization of *J. effusus* repeats, the three most abundant satellite families, as well as the most abundant Ty1/copia (Angela) and Ty3/gyspy (Athila) lineages, were selected for fluorescent *in situ* hybridization. Despite the small chromosomes, which difficult the correct assignment of repeats to particular chromosomal domains, a dot-like pattern covering the region of primary constriction was visualized for satDNA probes in several chromosomes, and suggests a centromeric or pericentromeric location. In some cases, the satellite signals covered the entire pericentromere or proximal regions of chromosome, as observed for JefSAT1-155 and JefSAT2-180 (Fig. 2A, B). JefSAT1-155 labelled large blocks of decreasing intensity in three chromosome pairs, and weaker signals on eighteen chromosomes pairs, which represented most of the chromosome complement (Fig. 2A). Low stringency hybridization showed the JefSAT2-180 signals in fifteen chromosome pairs (probably representing the distribution of all satellite families of SF1), with strong signals in one pair (Fig. 2B). JefSAT3-365 showed weaker signals in most chromosomes of the complement (Fig. 2C).

The Ty1/copia/Angela probe, in contrast to the pattern observed for the satellites, showed scattered signals along the chromosomes, but in a non-uniform distribution (Fig. 2D). The Ty3/gyspy Athila retroelement was amplified in one pair of chromosomes without a clear preferential distribution on other chromosomes (Fig. 2E). The mapping of CENH3 showed clear dot-like signals on chromosomes and a partial overlapping with signals of JefSAT1-155. JefSAT1-155 is, thereforepresent in the centromeres of *J. effusus*, but is not restricted to tha domain. Its signals expand beyond the CENH3 chromatin, and some centromeres, defined by CENH3 signals, do not have this satellite (Fig. 3).

Annotation of *Juncus effusus* centromeres

To further investigate J. effusus centromeric organization, we performed a detailed comparison of repeat organization among the 21 centromeres of the assembled chromosomes. We found JefSAT1-155, JefSAT2-180 and JefSAT3-365 making up the centromeres and pericentromeres of *J. effusus* in different combinations (Fig. 4). JefSAT1-155 and JefSAT2-180 were identified in thirteen and fifteen chromosome pairs, respectively, overlapping in some chromosome pairs. JefSAT3-365, on the other hand, was present in variable amounts on all *J. effusus* chromosomes, and therefore, overlapped with the two other centromeric satellites, but was usually less abundant than JefSAT1-155 or JefSAT2-180, except for Chr. 14 (Fig. 4).

The satellites do not form equal-sized arrays, but varied between large and small blocks, which occupied a considerable part of the centromere and eventually beyond, comprising a putative (peri) centromeric region from 1 Mbp in Chr. 21 up to 8 Mbp in Chr. 3 (Fig. 4). When present on a given chromosome, JefSAT1-155 is the predominant satDNA, forming large arrays followed by JefSAT2-180. Neither Athila nor Angela elements were enriched in pericentromeric regions, rather extended over most of the chromosome lengths, less enriched at terminal regions (Fig. 4). Moreover, they were interspersed between arrays of the three satellite DNAs of *J. effusus*, but no particular pattern of arrangement or preferential distribution between retroelements and satellites was observed in centromeres or pericentromeres. Thus, the centromeric regions of *J. effusus* chromosomes are arranged in a not uniform and complex structure.

DISCUSSION

Repetitive DNA composition in a relatively small genome

Here we characterized the repetitive genome fraction of *J. effusus*. The amount of repetitive DNA in *J. effusus* (24% for a 1C = 268.95 Mbp) is comparable to some monocentric species of the same phylogenetic clade (Cyperids) with similar genome size: *Prionium serratum* (26.9% for a 1C = 335 Mbp; Baez *et al.*, 2020) and *Hypolytrum schraderianum* (23% for a 1C = 268.95 Mbp; Dias *et al*., unpublished data). On the other hand, the large holocentric genome species from this clade possess higher repetitive proportions: *Luzula elegans* (61% for a 1C = 3.81 Gbp genome; Heckmann *et al.*, 2013) and *Rhynchospora pubera* (41,16% for a 1C = 1.61 Gbp; Marques *et al.*, 2015). Only 7.1% of all repeats comprised transposable elements, of which 6.4% were LTR retrotransposons. Transposable elements are the most abundant repetitive DNA in plant genomes, and can make up to 83% of these genomes, with LTR retrotransposons being the most representative (International Wheat Genome Sequencing Consortium *et al.*, 2018). In large genomes, genome size is often associated with large proportions of transposable elements, as found in *Cuscuta*, *Fritillaria*, *Luzula* and *Passiflora* (Ambrožová *et al.*, 2011; Heckmann *et al.*, 2013; Neumann *et al.*, 2021; Sader *et al.*, 2021). The most abundant element of *J. effusus* is from the Angela lineage, comprising 3.45% of the genome and more than half of all annotated LTR retrotransposons. Our comparative analysis also pointed this element as one of the few repeats shared between *J. effusus* an *L. elegans*. In this holocentric species, rich in repetitive elements, Angela comprised more than half of all repeats (33% of the genome). Angela is also associated with genome increase in palm species, in response to stress generated by aridity (Schley *et al.*, 2021), and significantly present in other species of Cyperids, such as *Rhynchospora pubera* (Marques *et al.*, 2015), indicating that this element has probably an important role in the evolution of monocentric and holocentric Cyperids.

Generally, species with small genomes show a tendency for repeats to be preferentially accumulated at (peri)centromeric or terminal chromosome regions (Ribeiro *et al.*, 2020; Baez *et al.*, 2020; Sader *et al.*, 2021). In terms of the chromosomal distribution, the most abundant element, Angela, was uniformly dispersed in the *L. elegans* chromosomes (Heckmann *et al.*, 2013) contrasting to the scattered pattern observed in the smaller chromosomes of *J. effusus*. This pattern was consistent with the distribution of Ty1/copia elements along the pseudomolecules, more than 70% of which represented by Angela, which was less enriched in gene- or satellite-rich regions. Outside Cyperids, in the monocentric *Hordeum murinum*, a pool of Ty1/copia probes, including different lineages from the Angela clade, showed a uniform

distribution in the chromosomes, except in the centromeric region and in the NOR (Ourari *et al.*, 2020).

An evident predominance of Ty1/copia over Ty3/gypsy elements (three-fold variation) was observed in *J. effuses* genome, and only a small fraction of the centromeric-like CRM clade was detected. This situation was similar to *Luzula elegans*, where the total absence of CRM and a small amount of Ty3/gypsy elements were observed (Heckmann *et al.*, 2013). The CRM clade is present in the centromeres of several monocentric as well as holocentric species (Jin *et al.*, 2004; Marques *et al.*, 2015; Neumann *et al.*, 2021), although a centromeric distribution is not always observed, as seen in *Eleocharis* (de Souza *et al.*, 2018). *Juncus effusus* showed a very low abundance of DNA transposons (0.695%) with only two lineages above 0.01% of the genome. Transposon DNAs were much less abundant than LTR retrotransposons, as observed in several plants species, such as from *Phaseolus* and *Cajanus* (Ribeiro *et al.*, 2020), *Luzula elegans* (Heckmann *et al.*, 2013) and Mapanioideae species (Dias et al., unpublished data). The difference in sequence between *J. effusus* and *L. elegans* repeats can be explained by the time of divergence between these two genera (44.3 Mya; Escudero *et al.*, 2012). Only a few highly conserved elements were shared, such as Angela. Angela has also been shown to be the most conserved LTR retrotransposon within the *Fabeae* tribe, with an origin estimated around 23–16 Mya (Macas *et al.*, 2015). Although increased genomes correlate to higher proportions of repetitive DNA, species with small genomes can show greater diversity of satellite DNA (Baez *et al.*, 2020; Sader *et al.*, 2021). Furthermore, satellite DNA may compose a considerable percentage of the repetitive DNA of some species, such as present in the legumes of the tribe *Fabeae*, ranging from 6.97 – 12.3% in genomes from 1C = 1.77 – 13.41 Gbp (Macas *et al.*, 2015; Ávila Robledillo *et al.*, 2018).

The dynamic sequence organization on centromeres/pericentromere of *Juncus effusus*

Satellite DNAs, together with transposable elements, are the major components of the centromeres of *J. effusus* as most monocentric species (Ruiz-Ruano *et al.*, 2018). In *J. effusus*, three satellite DNAs are associated with the centromeric composition . However, the overall sequence composition differs for each centromere, with some being highly enriched for one of those satDNAs, while others have all three repeats present in different? proportions.

Furthermore, at least for JefSAT1-155, a non centromere exclusive postion was observed being the sequence also present in CENH3-free regions.

Structural centromeres variants has been observed in some plants, with different centromeric satellite repeats compound these regions or even being absent (repeat-free centromeres) (Gong et al., 2012). In *Phaseolus vulgaris*, CentPv1 and CentPv2 satellites are present in two distinct chromosomal subsets that evolved independently within the common bean centromeres (Iwata *et al.*, 2013). On the other hand, the monocentric *Prionium serratum* showed two different subfamilies of centromeric repeats interspersed in different proportions in all centromeres (Baez *et al.*, 2020). A complex centromeric composition was also observed in *Beta* species, where chromosome-specific sets of repeated DNA elements, including satellite DNAs, were present at Beta centromeres, with a satDNA present in only one centromere, but exhibiting signals in other chromosomal regions (Gindullis *et al*., 2001).

The chromosome organization in *J. effusus* was clearly influenced by its monocentric nature and its compact genome (Hofstatter *et al.*, 2022). Nevertheless, centromeric and pericentromeric regions could not be clearly delimited for each chromosome based on satellite DNA, retrotransposon and methylation distribution. Although Ty3/gypsy elements tend to extend less towards chromosome ends than Ty1/copia, they still being abundant along most of the chromosome length. Furthermore, satellite DNAs are distributed in numerous clusters along each chromosome, also interrupted by TEs, and may extend several Mbp towards chromosome arms in some cases. ChIPseq data will be necessary for further centromere characterization, but the present results reinforce the diversity of *J. effusus* centromere organization, in contrast to the typical monocentric organization, with a centromere core composed of long satellite DNA arrays, surrounded by an enrichment of retroelements in pericentromeric regions, as observed in for *Arabidopsis thaliana* (Wang *et al.*, 2021). When additional telomere-to-telomere plant genome assemblies become available, it will be possible to evaluate if *J. effusus* centromere organization maybe common among other monocentrics.

CONCLUSIONS

In the present study, we showed an overview of the repetitive fraction and centromeric organization of the monocentric species *Juncus effusus*. SatDNAs represent a significant component of this genome and the three most abundant families were intermingled and

unevenly distributed in its (peri)centromeric regions, as confirmed by the centromere annotation. Monocentricity impacted genome organization of *J. effusus*, but its telomere-to-telomere assembly revealed a complex (peri)centromeric structure and composition, different to the typical monocentromere organization from *Arabidopsis thaliana*.

ACKNOWLEDGEMENTS

The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; PROBRAL CAPES/DAAD project number 88881.144086/2017-01 to A.P.H) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnologico; grant number 141725/2018-4 to Y.D.) for the financial support. We are also grateful to Prof. Dr. Marcelo Guerra (UFPE) and Prof. Dr. Leonardo Pessoa Felix (UFPB) for kindly providing the plant material and for the previous study that instigated us to start this work.

AUTHOR CONTRIBUTIONS

YD performed low-coverage repeat analyses, FISH experiments and drafted the first version of the manuscript; AM, LC and YM-S performed *in silico* analysis of repeats in the assembled genome; YM-S performed immuno-FISH experiments; MB analysed low-coverage repeat data; AH, AM and AP-H discussed the data, provided resources and laboratory structure and supervised the work; AP-H designed the study. All authors read and approved the final version of the manuscript.

REFERENCES

Albertson DG & Thomson JN. **1993**. Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. *Chromosome Research* **1**: 15–26.

Aldrup-MacDonald M & Sullivan B. **2014**. The past, present, and future of human centromere genomics. *Genes* **5**: 33–50.

Aliyeva-Schnorr L, Beier S, Karafiátová M, Schmutzer T, Scholz U, Doležel J, Stein N & Houben A. **2015**. Cytogenetic mapping with centromeric bacterial artificial chromosomes contigs shows that this recombination-poor region comprises more than half of barley chromosome 3H. *The Plant Journal* **84**: 385–394.

Ambrožová K, Mandáková T, Bureš P, Neumann P, Leitch IJ, Koblížková A, Macas J & Lysak MA. **2011**. Diverse retrotransposon families and an AT-rich satellite DNA revealed in giant genomes of Fritillaria lilies. *Annals of Botany* **107**: 255–268.

Arguelho EG, Michelan VS, Nogueira FM, Da Silva CRM, Rodriguez C, Trevisan R & Vanzela ALL. **2012**. New chromosome counts in Brazilian species of *Rhynchospora* (Cyperaceae). *Caryologia* **65**: 140–146.

Arslan M, Devisetty UK, Porsch M, Große I, Müller JA & Michalski SG. **2019**. RNA-Seq analysis of soft rush (*Juncus effusus*): transcriptome sequencing, de novo assembly, annotation, and polymorphism identification. *BMC Genomics* **20**: 489.

Ávila Robledillo L, Koblížková A, Novák P, Böttinger K, Vrbová I, Neumann P, Schubert I & Macas J. **2018**. Satellite DNA in Vicia faba is characterized by remarkable diversity in its sequence composition, association with centromeres, and replication timing. *Scientific Reports* **8**: 5838.

Báez M, Vaio M, Dreissig S, Schubert V, Houben A, Pedrosa-Harand A. 2019. Together but different: the subgenomes of the bimodal *Eleutherine* karyotypes are differentially organized. *Frontiers in Plant Science* **10**:1170

Baez M, Kuo YT, Dias Y, Souza T, Boudichevskaia A, Fuchs J, Schubert V, Vanzela ALL, Pedrosa-Harand A & Houben A. **2020**. Analysis of the small chromosomal Prionium serratum (Cyperid) demonstrates the importance of a reliable method to differentiate between mono- and holocentricity. *Evolutionary Biology*.

Barros e Silva AE, Marques A, dos Santos KGB & Guerra M. **2010**. The evolution of CMA bands in *Citrus* and related genera. *Chromosome Research* **18**: 503–514.

Bouchenak-Khelladi Y, Muasya AM & Linder HP. **2014**. A revised evolutionary history of Poales: origins and diversification: Evolutionary History of Poales. *Botanical Journal of the Linnean Society* **175**: 4–16.

Bourque G, Burns KH, Gehring M, Gorbunova V, Seluanov A, Hammell M, Imbeault M, Izsvák Z, Levin HL, Macfarlan TS, Mager DL & Feschotte C. **2018**. Ten things you should know about transposable elements. *Genome Biology* **19**: 199.

Bureš P, Zedek F & Marková M. **2013**. Holocentric Chromosomes. In: Greilhuber J, Dolezel J, Wendel JF, eds. *Plant Genome Diversity Volume 2*. Vienna: Springer Vienna, 187–208.

Cabral G, Marques A, Schubert V, Pedrosa-Harand A & Schlögelhofer P. **2014**. Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. *Nature Communications* **5**: 5070.

Carvalho CR & Saraiva LS. **1993**. An air drying technique for maize chromosomes without enzymatic maceration. *Biotechnic & Histochemistry* **68**: 142–145.

Chase MW, Fay MF, Dewey DS, Maurin O, Rønsted N, Davies J, Pillon Y, Petersen G, Seberg O, Tamura MN, Asmussen CB, Hilu K, Borsch T, Davies JI, Stevenson DW, Pires JC, Givinish TJ, Sytsma KJ, McPherson MA, Graham SW & Rai HS. **2006**. Multigene analyses of monocot relationships: a summary. *Aliso* **22**: 63–75.

Cheng Z, Dong F, Langdon T, Ouyang S, Buell CR, Gu M, Blattner FR & Jiang J. **2002**. Functional rice centromeres are marked by a satellite repeat and a centromere-specific retrotransposon. *The Plant Cell* **14**: 1691–1704.

Cheng ZJ & Murata M. **2003**. A centromeric tandem repeat family originating from a part of ty3/ *gypsy* -retroelement in wheat and its relatives. *Genetics* **164**: 665–672.

Chung KS, Hoshino T, Masaki T, Im HT & Ji SJ. **2018**. Chromosome counts of six Korean *Carex* species (Cyperaceae). *Cytologia* **83**: 229–233.

Cuacos M, H. Franklin FC & Heckmann S. **2015**. Atypical centromeres in plants—what they can tell us. *Frontiers in Plant Science* **6**.

Davies EW. **1956**. Cytology, evolution and origin of the aneuploid series in the genus *Carex*. *Hereditas* **42**: 349–365.

De Felice B, Wilson R R, Ciarmiello L & Conicella C. **2004**. A novel repetitive DNA sequence in lemon [Citrus limon (L.) Burm.] and related species Bruna. *Journal of Applied Genetics* **45**: 315–320.

D'Hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O, Noel B, Bocs S, Droc G, Rouard M, Da Silva C, Jabbari K, Cardi C, Poulain J, Souquet M, Labadie K, Jourda C, Lengellé J, Rodier-Goud M, Alberti A, Bernard M, Correa M, Ayyampalayam S, Mckain MR, Leebens-Mack J, Burgess D, Freeling M, Mbéguié-A-Mbéguié D, Chabannes M, Wicker T, Panaud O, Barbosa J, Hribova E, Heslop-Harrison P, Habas R, Rivallan R, Francois P, Poiron C, Kilian A, Burthia D, Jenny C, Bakry F, Brown S, Guignon V, Kema G, Dita M, Waalwijk C, Joseph S, Dievart A, Jaillon O, Leclercq J, Argout X, Lyons E, Almeida A, Jeridi M, Dolezel J, Roux N, Risterucci AM, Weissenbach J, Ruiz M, Glaszmann JC, Quétier F, Yahiaoui N & Wincker P. **2012**. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature* **488**: 213–217.

Dias YK, Baez M, Alves MV, Thomas WW, Vanzela ALL, Almeida EM, Elliott TL, Amorim BS, Houben A, Pedrosa-Harand A, Marhold K, Kučera J, Acuña CA, Akopian JA, An'kova TV, Arora J, Aytaç Z, Cavalcanti TB, Calvente A, Catalan P, Chernyagina OA, Chernysheva OA, Cordeiro JMP, Daviña JR, Deanna R, Delgado L, Erst AS, Felix LP, Forni-Martins ER, Gallego F, Facco MG, Gianini Aquino AC, Gomes de Andrade MJ, Graham SA, Hojsgaard DH, Honfi AI, Ikeda H, Khalbekova KU, Krivenko DA, Lakhanpaul S, Peñas MLL, Lavia GI, Leonova TV, Lian L, Lomonosova MN, López-González B, Martínez EJ, Matos SS, Melo AL, Torres EM, Mitrenina EYu, El Mokni R, Valls JFM, Morero RE, Moura CO, Oliveira RC, Oliveira Maekawa V, Olonova MV, Pandit MK, Peruzzi L, Pinzani L, Potseluev OM, Probatova NS, Pulkina SV, Reutemann AV, Rico E, Rua GH, Santos FJ, Santos GS, Santos Sousa MW, Silva MC, Silva RC, Silva Santos AM, Silvestri MC, Singh J, Singhal VK, Souza PCSS, Tashev A, Pozzobon MT, Turginov OT, Berto ACV, Veklich TN & Wang W. **2020**. IAPT chromosome data 33/4. *TAXON* **69**: 1394–1405.

Dolezel J, Sgorbati S & Lucretti S. **1992**. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* **85**: 625–631.

Dong F, Miller JT, Jackson SA, Wang GL, Ronald PC & Jiang J. **1998**. Rice (*Oryza sativa*) centromeric regions consist of complex DNA. *Proceedings of the National Academy of Sciences* **95**: 8135–8140.

Drábková, ZL. 2013. A survey of karyological phenomena in the juncaceae with emphasis on chromosome number variation and evolution. *The Botanical Review*. 79, 401–446.

Drinnenberg IA, deYoung D, Henikoff S & Malik HS. **2014**. Recurrent loss of CENH3 is associated with independent transitions to holocentricity in insects. *eLife* **3**: e03676.

Earnshaw WC, Allshire RC, Black BE, Bloom K, Brinkley BR, Brown W, Cheeseman IM, Choo KHA, Copenhaver GP, DeLuca JG, Desai A, Diekmann S, Erhardt S, Fitzgerald-Hayes M, Foltz D, Fukagawa T, Gassmann R, Gerlich DW, Glover DM, Gorbsky GJ, Harrison SC, Heun P, Hirota T, Jansen LET, Karpen G, Kops GJPL, Lampson MA, Lens SM, Losada A, Luger K, Maiato H, Maddox PS, Margolis RL, Masumoto H, McAinsh AD, Mellone BG, Meraldi P, Musacchio A, Oegema K, O'Neill RJ, Salmon ED, Scott KC, Straight AF, Stukenberg PT, Sullivan BA, Sullivan KF, Sunkel CE, Swedlow JR, Walczak CE, Warburton PE, Westermann S, Willard

HF, Wordeman L, Yanagida M, Yen TJ, Yoda K & Cleveland DW. **2013**. Esperanto for histones: CENP-A, not CENH3, is the centromeric histone H3 variant. *Chromosome Research* **21**: 101–106.

Ekwall K. **2007**. Epigenetic control of centromere behavior. *Annual Review of Genetics* **41**: 63–81.

Elliott TA & Gregory TR. **2015**. Do larger genomes contain more diverse transposable elements? *BMC Evolutionary Biology* **15**: 69.

Endress PK. **1998**. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge Univ. Press.

Escudero M, Hipp AL, Waterway MJ & Valente LM. **2012**. Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (*Carex*, Cyperaceae). *Molecular Phylogenetics and Evolution* **63**: 650–655.

Escudero M, Márquez-Corro JI & Hipp AL. **2016**. The phylogenetic origins and evolutionary history of holocentric chromosomes. *Systematic Botany* **41**: 580–585.

Fedoroff NV. **2012**. Transposable Elements, Epigenetics, and Genome Evolution. *Science* **338**: 758–767.

Flach M. **1966**. Diffuse Centromeres in a Dicotyledonous Plant. *Nature* **209**: 1369–1370.

Furness CA & Rudall PJ. **2011**. Selective microspore abortion correlated with aneuploidy: an indication of meiotic drive. *Sexual Plant Reproduction* **24**: 1–8.

Garrido-Ramos MA. **2015**. Satellite DNA in Plants: More than Just Rubbish. *Cytogenetic and Genome Research* **146**: 153–170.

Gindullis F, Desel C, Galasso I, Schmidt T. 2001. The large-scale organization of the centromeric region in Beta species. *Genome Research* Feb;11(2):253-65.

Givnish TJ, Ames M, McNeal JR, McKain MR, Steele PR, dePamphilis CW, Graham SW, Pires JC, Stevenson DW, Zomlefer WB, Briggs BG, Duvall MR, Moore MJ, Heaney JM, Soltis DE, Soltis PS, Thiele K & Leebens-Mack JH. **2010**. Assembling the tree of the monocotyledons: plastome sequence phylogeny and evolution of Poales [1]. *Annals of the Missouri Botanical Garden* **97**: 584–616.

Global Carex Group. **2015**. Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): a new broader circumscription: Making *Carex* Monophyletic. *Botanical Journal of the Linnean Society* **179**: 1–42.

Govaerts R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T & Bruhl JJ. **2018**. World checklist of Cyperaceae. Kew: The Board of Trustees of the Royal Botanic Gardens, Kew. *https://wcsp.science.kew.org/*.

Grandbastien M. **1998**. Activation of plant retrotransposons under stress conditions. *Trends in Plant Science* **3**: 181–187.

Greilhuber J. **1995**. Chromosomes of the monocotyledons (general aspects). In: *Monocotyledons: systematics and evolution*. Royal Botanic Gardens, Kew, 379–414.

Guerra M & García MA. **2004**. Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). *Genome* **47**: 134–140.

Guerra M, Ribeiro T & Felix LP. **2019**. Monocentric chromosomes in *Juncus* (Juncaceae) and implications for the chromosome evolution of the family. *Botanical Journal of the Linnean Society* **191**: 475–483.

Han YH, Zhang ZH, Liu JH, Lu JY, Huang SW & Jin WW. **2008**. Distribution of the tandem repeat sequences and karyotyping in cucumber (*Cucumis sativus* L.) by fluorescence in situ hybridization. *Cytogenetic and Genome Research* **122**: 80–88.

He Q, Cai Z, Hu T, Liu H, Bao C, Mao W & Jin W. **2015**. Repetitive sequence analysis and karyotyping reveals centromere-associated DNA sequences in radish (*Raphanus sativus* L.). *BMC Plant Biology* **15**: 105.

Heckmann S, Macas J, Kumke K, Fuchs J, Schubert V, Ma L, Novák P, Neumann P, Taudien S, Platzer M & Houben A. **2013**. The holocentric species *Luzula elegans* shows interplay between centromere and large-scale genome organization. *The Plant Journal* **73**: 555–565.

Henikoff S, Ahmad K & Malik HS. **2001**. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**: 1098–1102.

Hipp AL, Rothrock PE & Roalson EH. **2009**. The evolution of chromosome arrangements in Carex (Cyperaceae). *The Botanical Review* **75**: 96–109.

Hof AE van't, Campagne P, Rigden DJ, Yung CJ, Lingley J, Quail MA, Hall N, Darby AC & Saccheri IJ. **2016**. The industrial melanism mutation in British peppered moths is a transposable element. *Nature* **534**: 102–105.

Hofstatter PG, Thangavel G, Lux T, Neumann P, Vondrak T, Novak P, Zhang M, Costa L, Castellani M, Scott A, Toegelova H, Fuchs J, Mata-Sucre Y, Dias Y, Vanzela ALL, Huettel B, Almeida CCS, Simkova H, Souza G, Pedrosa-Harand A, Macas J, Mayer KFX, Houben A, Marques A (2022) Repeat-based holocentromeres influence genome architecture and karyotype evolution. *Cell*, in press.

Houben A, Schroeder-Reiter E, Nagaki K, Nasuda S, Wanner G, Murata M & Endo TR. **2007**. CENH3 interacts with the centromeric retrotransposon cereba and GC-rich satellites and locates to centromeric substructures in barley. *Chromosoma* **116**: 275–283.

Hughes-Schrader S & Ris H. **1941**. The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. *Journal of Experimental Zoology* **87**: 429–456.

Ibarra-Laclette E, Lyons E, Hernández-Guzmán G, Pérez-Torres CA, Carretero-Paulet L, Chang TH, Lan T, Welch AJ, Juárez MJA, Simpson J, Fernández-Cortés A, Arteaga-Vázquez M, Góngora-Castillo E, Acevedo-Hernández G, Schuster SC, Himmelbauer H, Minoche AE, Xu S, Lynch M, Oropeza-Aburto A, Cervantes-Pérez SA, de Jesús Ortega-Estrada M, Cervantes-Luevano JI, Michael TP, Mockler T, Bryant D, Herrera-Estrella A, Albert VA & Herrera-Estrella L. **2013**. Architecture and evolution of a minute plant genome. *Nature* **498**: 94–98.

Ibiapino A, García MA, Amorim B, Baez M, Costea M, Stefanović S, Pedrosa-Harand A. 2022. The evolution of cytogenetic traits in *Cuscuta* (Convolvulaceae), the genus with the most diverse chromosomes in angiosperms. *Frontiers in Plant Science* **13**:842260.

International Wheat Genome Sequencing Consortium, Wicker T, Gundlach H, Spannagl M, Uauy C, Borrill P, Ramírez-González RH, De Oliveira R, Mayer KFX, Paux E & Choulet F. **2018**. Impact of transposable elements on genome structure and evolution in bread wheat. *Genome Biology* **19**: 103.

Ito H, Yoshida T, Tsukahara S & Kawabe A. **2013**. Evolution of the ONSEN retrotransposon family activated upon heat stress in Brassicaceae. *Gene* **518**: 256–261.

Iwata A, Tek AL, Richard MMS, Abernathy B, Fonsêca A, Schmutz J, Chen NWG, Thareau V, Magdelenat G, Li Y, Murata M, Pedrosa-Harand A, Geffroy V, Nagaki K & Jackson SA. **2013**.

Identification and characterization of functional centromeres of the common bean. *The Plant Journal*: n/a-n/a.

Jankowska M, Fuchs J, Klocke E, Fojtová M, Polanská P, Fajkus J, Schubert V & Houben A. **2015**. Holokinetic centromeres and efficient telomere healing enable rapid karyotype evolution. *Chromosoma* **124**: 519–528.

Jasencakova Z, Meister A & Schubert I. **2001**. Chromatin organization and its relation to replication and histone acetylation during the cell cycle in barley. *Chromosoma* **110**: 83–92.

Jiang J, Birchler JA, Parrott WA & Kelly Dawe R. **2003**. A molecular view of plant centromeres. *Trends in Plant Science* **8**: 570–575.

Jin W, Melo JR, Nagaki K, Talbert PB, Henikoff S, Dawe RK & Jiang J. **2004**. Maize centromeres: organization and functional adaptation in the genetic background of Oat. *The Plant Cell* **16**: 571–581.

Judd WS (Ed.). **2008**. *Plant systematics: a phylogenetic approach*. Sunderland, Mass: Sinauer Associates.

Kalendar R, Tanskanen J, Immonen S, Nevo E & Schulman AH. **2000**. Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences* **97**: 6603–6607.

Kandul NP, Lukhtanov VA & Pierce NE. **2007**. Karyotypic diversity and speciation in *Agrodiaetus butterflies*. *Evolution* **61**: 546–559.

Kapitonov VV & Jurka J. **2001**. Rolling-circle transposons in eukaryotes. *Proceedings of the National Academy of Sciences* **98**: 8714–8719.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P & Drummond A. **2012**. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Klein SJ & O'Neill RJ. **2018**. Transposable elements: genome innovation, chromosome diversity, and centromere conflict. *Chromosome Research* **26**: 5–23.

Koo DH, Hong CP, Batley J, Chung YS, Edwards D, Bang JW, Hur Y & Lim YP. **2011**. Rapid divergence of repetitive DNAs in Brassica relatives. *Genomics* **97**: 173–185.

Kowar T, Zakrzewski F, Macas J, Kobližková A, Viehoever P, Weisshaar B & Schmidt T. **2016**. Repeat Composition of CenH3-chromatin and H3K9me2-marked heterochromatin in Sugar Beet (Beta vulgaris). *BMC Plant Biology* **16**: 120.

Lee SI & Kim NS. **2014**. Transposable Elements and Genome Size Variations in Plants. *Genomics & Informatics* **12**: 87.

Lermontova I, Sandmann M & Demidov D. **2014**. Centromeres and kinetochores of Brassicaceae. *Chromosome Research* **22**: 135–152.

Leushkin EV, Sutormin RA, Nabieva ER, Penin AA, Kondrashov AS & Logacheva MD. **2013**. The miniature genome of a carnivorous plant *Genlisea aurea* contains a low number of genes and short non-coding sequences. *BMC Genomics* **14**: 476.

Liao YJ, Zhai HF, Zhang B, Duan TX & Huang JM. **2011**. Anxiolytic and sedative effects of dehydroeffusol from *Juncus effusus* in Mice. *Planta Medica* **77**: 416–420.

Lipnerova I, Bures P, Horova L & Smarda P. **2013**. Evolution of genome size in *Carex* (Cyperaceae) in relation to chromosome number and genomic base composition. *Annals of Botany* **111**: 79–94.

Liu Q, Li X, Zhou X, Li M, Zhang F, Schwarzacher T & Heslop-Harrison JS. **2019**. The repetitive DNA landscape in Avena (Poaceae): chromosome and genome evolution defined by major repeat classes in whole-genome sequence reads. *BMC Plant Biology* **19**: 226.

López MG, Aveliano F & Florencia G. **2017**. Cytogenetics, genome size and anther anatomy in *Bulbostylis* (Cyperaceae). *Caryologia* **70**: 238–247.

Loureiro J, Rodriguez E, Dolezel J & Santos C. **2007**. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany* **100**: 875–888.

Luceño M, Mendes AP, Vanzela ALL & Alves MV. **1998**. Agmatoploidy and symploidy in *Rhynchospora cephalotes* (L.) Vahl (Cyperaceae). *Cytologia* **63**: 79–81.

Macas J, Novák P, Pellicer J, Čížková J, Koblížková A, Neumann P, Fuková I, Doležel J, Kelly LJ & Leitch IJ. **2015**. In Depth Characterization of Repetitive DNA in 23 Plant Genomes Reveals Sources of Genome Size Variation in the Legume Tribe Fabeae (A Houben, Ed.). *PLOS ONE* **10**: e0143424.

Maddox PS, Oegema K, Desai A & Cheeseman IM. **2004**. 'Holo'er than thou: Chromosome segregation and kinetochore function in *C. elegans*. *Chromosome Research* **12**: 641–653.

Maheshwari S, Ishii T, Brown CT, Houben A & Comai L. **2017**. Centromere location in *Arabidopsis* is unaltered by extreme divergence in CENH3 protein sequence. *Genome Research* **27**: 471–478.

Makałowski W, Gotea V, Pande A & Makałowska I. **2019**. Transposable elements: classification, identification, and their use as a tool for comparative genomics. In: Anisimova M, ed. *Evolutionary Genomics*. New York, NY: Springer New York, 177–207.

Makarevitch I, Waters AJ, West PT, Stitzer M, Hirsch CN, Ross-Ibarra J & Springer NM. **2015**. Transposable elements contribute to activation of maize genes in response to abiotic stress (M Freeling, Ed.). *PLoS Genetics* **11**: e1004915.

Malik HS & Henikoff S. **2003**. Phylogenomics of the nucleosome. *Nature Structural & Molecular Biology* **10**: 882–891.

Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY & Bryant SH. **2017**. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research* **45**: D200–D203.

Marques A, Ribeiro T, Neumann P, Macas J, Novák P, Schubert V, Pellino M, Fuchs J, Ma W, Kuhlmann M, Brandt R, Vanzela ALL, Beseda T, Šimková H, Pedrosa-Harand A & Houben A. **2015**. Holocentromeres in *Rhynchospora* are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. *Proceedings of the National Academy of Sciences* **112**: 13633–13638.

Márquez-Corro JI, Luceño M, Jiménez-Mejías P, Escudero M, Martín-Bravo S, Hipp AL, Chung KS, Muasya AM, Rothrock PE, Weber JA & Naczi R. **2018a**. IAPT chromosome data 28. *Taxon* **67**: 1235–1245.

Márquez-Corro JI, Escudero M & Luceño M. **2018b**. Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives. *Chromosome Research* **26**: 139–152.

Martinez-Zapater JM, Estelle MA & Somerville CR. **1986**. A highly repeated DNA sequence in *Arabidopsis thaliana*. *Molecular and General Genetics MGG* **204**: 417–423.

Maximiano da Silva CR, González-Elizondo MS, Rego L do NA de A, Torezan JMD & Vanzela ALL. **2008**. Cytogenetical and cytotaxonomical analysis of some Brazilian species of *Eleocharis* (Cyperaceae). *Australian Journal of Botany* **56**: 82.

McCann J, Macas J, Novák P, Stuessy TF, Villaseñor JL & Weiss-Schneeweiss H. **2020**. Differential genome size and repetitive DNA evolution in diploid species of *Melampodium* sect. *Melampodium* (Asteraceae). *Frontiers in Plant Science* **11**: 362.

McClintock B. **1939**. The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences* **25**: 405–416.

McClintock B. **1950**. The origin and behavior of mutable loci in maize. *Proceedings of the National Academy of Sciences* **36**: 344–355.

Mehrotra S & Goyal V. **2014**. Repetitive sequences in plant nuclear DNA: types, distribution, evolution and function. *Genomics, Proteomics & Bioinformatics* **12**: 164–171.

Melters DP, Paliulis LV, Korf IF & Chan SWL. **2012**. Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Research* **20**: 579–593.

Melters DP, Bradnam KR, Young HA, Telis N, May MR, Ruby J, Sebra R, Peluso P, Eid J, Rank D, Garcia J, DeRisi JL, Smith T, Tobias C, Ross-Ibarra J, Korf I & Chan SW. **2013**. Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. *Genome Biology* **14**: R10.

Michael TP. **2014**. Plant genome size variation: bloating and purging DNA. *Briefings in Functional Genomics* **13**: 308–317.

Moore G, Aragón-Alcaide L, Roberts M, Reader S, Miller T & Foote T. **1997**. Are rice chromosomes components of a holocentric chromosome ancestor? *Plant Molecular Biology* **35**: 17–23.

Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW & Smets E. **2009**. Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *The Botanical Review* **75**: 2–21.

Nagaki K, Song J, Stupar RM, Parokonny AS, Yuan Q, Ouyang S, Liu J, Hsiao J, Jones KM, Dawe RK, Buell CR & Jiang J. **2003**. Molecular and cytological analyses of large tracks of centromeric DNA reveal the structure and evolutionary dynamics of maize centromeres. *Genetics* **163**: 759–770.

Nagaki K, Kashihara K & Murata M. **2005**. Visualization of diffuse centromeres with centromere-specific histone h3 in the holocentric plant *Luzula nivea*. *The Plant Cell* **17**: 1886–1893.

Naish M, Alonge M, Wlodzimierz P, Tock AJ, Abramson BW, Schmücker A, Mandáková T, Jamge B, Lambing C, Kuo P, Yelina N, Hartwick N, Colt K, Smith LM, Ton J, Kakutani T, Martienssen RA, Schneeberger K, Lysak MA, Berger F, Bousios A, Michael TP, Schatz MC & Henderson IR. **2021**. The genetic and epigenetic landscape of the *Arabidopsis* centromeres. *Science* **374**: eabi7489.

Nasuda S, Hudakova S, Schubert I, Houben A & Endo TR. **2005**. Stable barley chromosomes without centromeric repeats. *Proceedings of the National Academy of Sciences* **102**: 9842–9847.

Neumann P, Navrátilová A, Koblížková A, Kejnovský E, Hřibová E, Hobza R, Widmer A, Doležel J & Macas J. **2011**. Plant centromeric retrotransposons: a structural and cytogenetic perspective. *Mobile DNA* **2**: 4.

Neumann P, Navrátilová A, Schroeder-Reiter E, Koblížková A, Steinbauerová V, Chocholová E, Novák P, Wanner G & Macas J. **2012**. Stretching the rules: monocentric chromosomes with multiple centromere domains (BA Sullivan, Ed.). *PLoS Genetics* **8**: e1002777.

Neumann P., Novak P, Hostakova N, Macas J. 2019. Systematic survey of plant LTR-retrotransposons elucidates phylogenetic relationships of their polyprotein domains and provides a reference for element classification. *Mobile DNA* 10:1.

Neumann P, Schubert V, Fuková I, Manning JE, Houben A & Macas J. **2016**. Epigenetic histone marks of extended meta-polycentric centromeres of *Lathyrus* and *Pisum* chromosomes. *Frontiers in Plant Science* **7**.

Neumann P, Oliveira L, Čížková J, Jang T, Klemme S, Novák P, Stelmach K, Koblížková A, Doležel J & Macas J. **2021**. Impact of parasitic lifestyle and different types of centromere organization on chromosome and genome evolution in the plant genus *Cuscuta*. *New Phytologist* **229**: 2365–2377.

Novak P, Neumann P, Pech J, Steinhaisl J & Macas J. **2013**. RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. *Bioinformatics* **29**: 792–793.

Novák P, Ávila Robledillo L, Koblížková A, Vrbová I, Neumann P & Macas J. **2017**. TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads. *Nucleic Acids Research* **45**: e111–e111.

Novák P, Neumann P. & Macas, J. 2020. Global analysis of repetitive DNA from unassembled sequence reads using RepeatExplorer2. *Nature Protocols* 15:3745–3776.

Oliveira L, Neumann P, Jang TS, Klemme S, Schubert V, Koblížková A, Houben A & Macas J. **2020**. Mitotic spindle attachment to the holocentric chromosomes of *Cuscuta europaea* does not correlate with the distribution of CENH3 chromatin. *Frontiers in Plant Science* **10**: 1799.

Ourari M, Coriton O, Martin G, Huteau V, Keller J, Ainouche ML, Amirouche R & Ainouche A. **2020**. Screening diversity and distribution of Copia retrotransposons reveals a specific amplification of BARE1 elements in genomes of the polyploid *Hordeum murinum* complex. *Genetica* **148**: 109–123.

Paz RC, Kozaczek ME, Rosli HG, Andino NP & Sanchez-Puerta MV. **2017**. Diversity, distribution and dynamics of full-length Copia and Gypsy LTR retroelements in *Solanum lycopersicum*. *Genetica* **145**: 417–430.

Pazy B & Plitmann U. **1994**. Holocentric chromosome behaviour in *Cuscuta* (Cuscutaceae). *Plant Systematics and Evolution* **191**: 105–109.

Pedrosa A, Sandal N, Stougaard J, Schweizer D & Bachmair A. **2002**. Chromosomal map of the model legume *Lotus japonicus*. *Genetics* **161**: 1661–1672.

Pedrosa-Harand A, de Almeida CCS, Mosiolek M, Blair MW, Schweizer D & Guerra M. **2006**. Extensive ribosomal DNA amplification during Andean common bean (Phaseolus vulgaris L.) evolution. *Theoretical and Applied Genetics* **112**: 924–933.

Plohl M, Meštrović N & Mravinac B. **2014**. Centromere identity from the DNA point of view. *Chromosoma* **123**: 313–325.

Pritham EJ, Putliwala T & Feschotte C. **2007**. Mavericks, a novel class of giant transposable elements widespread in eukaryotes and related to DNA viruses. *Gene* **390**: 3–17.

Qiu F & Ungerer MC. **2018**. Genomic abundance and transcriptional activity of diverse gypsy and copia long terminal repeat retrotransposons in three wild sunflower species. *BMC Plant Biology* **18**: 6.

Ribeiro T, Marques A, Novák P, Schubert V, Vanzela ALL, Macas J, Houben A & Pedrosa-Harand A. **2017**. Centromeric and non-centromeric satellite DNA organisation differs in holocentric Rhynchospora species. *Chromosoma* **126**: 325–335.

Ribeiro T, Buddenhagen CE, Thomas WW, Souza G & Pedrosa-Harand A. **2018**. Are holocentrics doomed to change? Limited chromosome number variation in *Rhynchospora* Vahl (Cyperaceae). *Protoplasma* **255**: 263–272.

Ribeiro T, Vasconcelos E, dos Santos KGB, Vaio M, Brasileiro-Vidal AC & Pedrosa-Harand A. **2020**. Diversity of repetitive sequences within compact genomes of *Phaseolus* L. beans and allied genera Cajanus L. and Vigna Savi. *Chromosome Research* **28**: 139–153.

Roalson EH. **2005**. Phylogenetic relationships in the Juncaceae inferred from nuclear ribosomal dna internal transcribed spacer sequence data. *International Journal of Plant Sciences* **166**: 397–413.

Roalson EH. **2008**. A synopsis of chromosome number variation in the Cyperaceae. *The Botanical Review* **74**: 209–393.

Robillard É, Le Rouzic A, Zhang Z, Capy P & Hua-Van A. **2016**. Experimental evolution reveals hyperparasitic interactions among transposable elements. *Proceedings of the National Academy of Sciences* **113**: 14763–14768.

Rocha DM, Vanzela ALL & Mariath JEA. **2018**. Comparative study of microgametogenesis in members of Cyperaceae and Juncaceae: a shift from permanent pollen tetrads to pseudomonads. *Botanical Journal of the Linnean Society* **188**: 59–73.

Ruiz-Ruano FJ, Castillo-Martínez J, Cabrero J, Gómez R, Camacho JPM & López-León MD. **2018**. High-throughput analysis of satellite DNA in the grasshopper *Pyrgomorpha conica* reveals abundance of homologous and heterologous higher-order repeats. *Chromosoma* **127**: 323–340.

Sader M, Vaio M, Cauz-Santos LA, Dornelas MC, Vieira MLC, Melo N & Pedrosa-Harand A. **2021**. Large vs small genomes in *Passiflora*: the influence of the mobilome and the satellitome. *Planta* **253**: 86.

SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y & Bennetzen JL. **1998**. The paleontology of intergene retrotransposons of maize. *Nature Genetics* **20**: 43–45.

Schley RJ, Pellicer J, Ge XJ, Barrett C, Bellot S, Guignard MS, Novák P, Suda J, Fraser D, Baker WJ, Dodsworth S, Macas J, Leitch AR, Leitch IJ. (2022) The ecology of palm genomes: repeat-associated genome size expansion is constrained by aridity. New Phytologisty.

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du F, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen W, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J,

Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn MW, Ying K, Yeh CT, Emrich SJ, Jia Y, Kalyanaraman A, Hsia AP, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia JM, Deragon JM, Estill JC, Fu Y, Jeddeloh JA, Han Y, Lee H, Li P, Lisch DR, Liu S, Liu Z, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Bennetzen JL, Dawe RK, Jiang J, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA & Wilson RK. **2009**. The B73 Maize Genome: Complexity, Diversity, and Dynamics. *Science* **326**: 1112–1115.

Schneider MC, Mattos VF, Carvalho LS & Cella DM. **2015**. Organization and behavior of the synaptonemal complex during achiasmatic meiosis of four buthid scorpions. *Cytogenetic and Genome Research* **144**: 341–347.

Schrader F. **1935**. Notes an the mitotic behavior of long chromosomes. *CYTOLOGIA* **6**: 422–430.

Schubert V, Neumann P, Marques A, Heckmann S, Macas J, Pedrosa-Harand A, Schubert I, Jang TS & Houben A. **2020**. Super-resolution microscopy reveals diversity of plant centromere Architecture. *International Journal of Molecular Sciences* **21**: 3488.

Schulman AH. **2013**. Retrotransposon replication in plants. *Current Opinion in Virology* **3**: 604–614.

Semmouri I, Bauters K, Léveillé-Bourret É, Starr JR, Goetghebeur P & Larridon I. **2019**. Phylogeny and systematics of Cyperaceae, the evolution and importance of embryo morphology. *The Botanical Review* **85**: 1–39.

Sheikh SA & Kondo K. **1995**. Differential staining with orcein, giemsa, CMA, and DAPI for comparative chromosome study of 12 species of australian *Drosera* (Droseraceae). *American Journal of Botany* **82**: 1278–1286.

Shibata F. **2004**. Differential localization of the centromere-specific proteins in the major centromeric satellite of *Arabidopsis thaliana*. *Journal of Cell Science* **117**: 2963–2970.

Shirakawa J, Nagano K & Hoshi Y. **2011**. A chromosome study of two centromere differentiating *Drosera* species, *D. arcturi* and *D. regia*. : 453–463.

da Silva CRM, Quintas CC & Vanzela ALL. **2010**. Distribution of 45S and 5S rDNA sites in 23 species of *Eleocharis* (Cyperaceae). *Genetica* **138**: 951–957.

Simpson DA, Furness CA, Hodkinson TR, Muasya AM & Chase MW. **2003**. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany* **90**: 1071–1086.

Simpson DA & Inglis CA. **2001**. Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *Kew Bulletin* **56**: 257.

Šmarda P, Bureš P, Horová L, Leitch IJ, Mucina L, Pacini E, Tichý L, Grulich V & Rotreklová O. **2014**. Ecological and evolutionary significance of genomic GC content diversity in monocots. *Proceedings of the National Academy of Sciences* **111**: E4096–E4102.

Sonnhammer ELL & Durbin R. **1995**. A dot-matrix program with dynamic threshold control suited for genomic DNA and protein sequence analysis. *Gene* **167**: GC1–GC10.

Sousa A, Barros e Silva AE, Cuadrado A, Loarce Y, Alves MV & Guerra M. **2011**. Distribution of 5S and 45S rDNA sites in plants with holokinetic chromosomes and the "chromosome field" hypothesis. *Micron* **42**: 625–631.

de Souza TB, Chaluvadi SR, Johnen L, Marques A, González-Elizondo MS, Bennetzen JL & Vanzela ALL. **2018**. Analysis of retrotransposon abundance, diversity and distribution in holocentric *Eleocharis* (Cyperaceae) genomes. *Annals of Botany* **122**: 279–290.

Spalink D, Drew BT, Pace MC, Zaborsky JG, Starr JR, Cameron KM, Givnish TJ & Sytsma KJ. **2016**. Biogeography of the cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. *Journal of Biogeography* **43**: 1893–1904.

Stimpson KM, Matheny JE & Sullivan BA. **2012**. Dicentric chromosomes: unique models to study centromere function and inactivation. *Chromosome Research* **20**: 595–605.

Su H, Liu Y, Liu Y, Birchler J & Han F. **2018**. The behavior of the maize b chromosome and centromere. *Genes* **9**: 476.

Sullivan BA & Schwartz S. **1995**. Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. *Human Molecular Genetics* **4**: 2189–2197.

Sullivan LL & Sullivan BA. **2020**. Genomic and functional variation of human centromeres. *Experimental Cell Research* **389**: 111896.

Tanaka N & Tanaka N. **1977**. Chromosome Studies in Chionographis (Liliaceae). *Cytologia* **42**: 753–763.

Tek AL, Song J, Macas J & Jiang J. **2005**. Sobo, a recently amplified satellite repeat of potato, and its implications for the origin of tandemly repeated sequences. *Genetics* **170**: 1231–1238.

The Arabidopsis Genome Initiative. **2000**. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **408**: 796–815.

Tran TD, Cao HX, Jovtchev G, Neumann P, Novák P, Fojtová M, Vu GTH, Macas J, Fajkus J, Schubert I & Fuchs J. **2015**. Centromere and telomere sequence alterations reflect the rapid genome evolution within the carnivorous plant genus *Genlisea*. *The Plant Journal* **84**: 1087–1099.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M & Rozen SG. **2012**. Primer3—new capabilities and interfaces. *Nucleic Acids Research* **40**: e115–e115.

Van-Lume B, Mata-Sucre Y, Báez M, Ribeiro T, Huettel B, Gagnon E, Leitch IJ, Pedrosa-Harand A, Lewis GP & Souza G. **2019**. Evolutionary convergence or homology? Comparative cytogenomics of Caesalpinia group species (Leguminosae) reveals diversification in the pericentromeric heterochromatic composition. *Planta* **250**: 2173–2186.

Vanzela ALL & Guerra M. **2000**. Heterochromatin differentiation in holocentric chromosomes of Rhynchospora (Cyperaceae). *Genetics and Molecular Biology* **23**: 453–456.

Vicient CM, Kalendar R, Anamthawat-Jónsson K, Suoniemi A & Schulman AH. **1999**. [No title found]. *Genetica* **107**: 53–63.

Vondrak T, Ávila Robledillo L, Novák P, Koblížková A, Neumann P & Macas J. **2020**. Characterization of repeat arrays in ultra-long nanopore reads reveals frequent origin of satellite DNA from retrotransposon-derived tandem repeats. *The Plant Journal* **101**: 484–500.

Vymazal J. **2014**. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological Engineering* **73**: 724–751.

Wang B, Yang X, Jia Y, Xu Y, Jia P, Dang N, Wang S, Xu T, Zhao X, Gao S, Dong Q & Ye K. **2021**. High-quality *Arabidopsis thaliana* genome assembly with nanopore and hifi long reads. *Genomics, Proteomics & Bioinformatics*: S1672022921001741.

Weber B, Heitkam T, Holtgräwe D, Weisshaar B, Minoche AE, Dohm JC, Himmelbauer H & Schmidt T. **2013**. Highly diverse chromoviruses of *Beta vulgaris* are classified by chromodomains and chromosomal integration. *Mobile DNA* **4**: 8.

Wicker T, Stein N, Albar L, Feuillet C, Schlagenhauf E & Keller B. **2001**. Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution: Genome organisation in 211 kb DNA of diploid wheat. *The Plant Journal* **26**: 307–316.

Xiao H, Jiang N, Schaffner E, Stockinger EJ & van der Knaap E. **2008**. A Retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* **319**: 1527–1530.

Zedek F, Veselý P, Horová L & Bureš P. **2016**. Flow cytometry may allow microscope-independent detection of holocentric chromosomes in plants. *Scientific Reports* **6**: 27161.

Zhang L, Yan L, Jiang J, Wang Y, Jiang Y, Yan T & Cao Y. **2014**. The structure and retrotransposition mechanism of LTR-retrotransposons in the asexual yeast *Candida albicans*. *Virulence* **5**: 655–664.

Zumstein W, Wangwasit K, & Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand. **2019**. Chromosome counts and karyotype reports from *Fimbristylis* (Cyperaceae) in Thailand. *Thai Forest Bulletin (Botany)* **47**: 123–132.

Table 1. Composition and relative abundance of repetitive DNA in the *Juncus effusus* genome

Repetitive sequence	**Superfamily**	**Lineage**	(%)
LTR Retrotransposons			6.408
	Ty1/Copia		4.814
		Angela	3.455
		Sire	0.559
		Alesia	0.380
		Tork	0.326
		Ale	0.051
		Ivana	0.042
	Ty3/Gypsy		1.594
		Non-chromovirus/ OTA/Athila	1.566
		Chromovirus/CRM	0.028
DNA Transposon			0.695
35S rDNA			2.935
5S rDNA			0.063
Satellite DNA			4.920
Unclassified			9.022
Total			24.035%

Table 2. Features of satellite DNA identified in the *Juncus effusus* genome

Satellite family	Superfamily	Cluster	Monomere size (bp)	Abundance (%)	Confidence TAREAN
JefSAT1	-	1, 30	154, 155	1.75	High, Low
JefSAT2	SF-2	6, 21	122, 180	1.53	Low
JefSAT3	SF-1	5	364	1.48	Low
JefSAT4	SF-1	44	364	0.09	Low
JefSAT5	SF-2	66	174	0.04	Low
JefSAT6	SF-2	78	179	0.03	Low
Total				4.92	

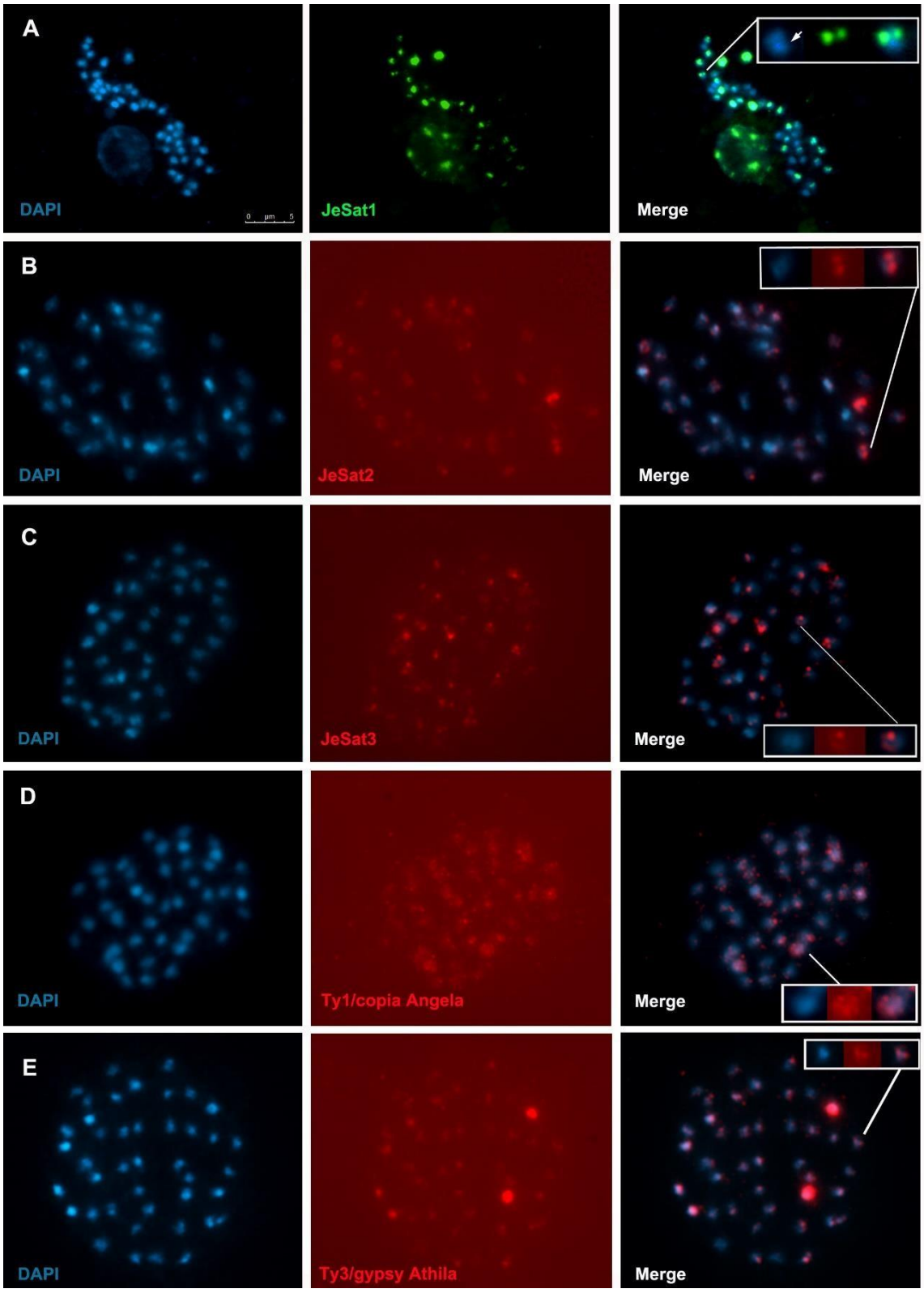


Figure 1. Mitotic metaphases of *Juncus effusus* showing the (peri)centromeric distribution of three satellites and a scattered, non-uniform patterns of two retroelements. **A)** JefSAT1-155; **(B)** JefSAT2-180; **C)** JefSAT3-365; **D)** Ty1/copia Angela; **E)** Ty3/gypsy Athila. Arrow indicates primary constrictions, while insets show dot-like signals (or scattered, in D) in amplified chromosomes. Bar size = 5µm.

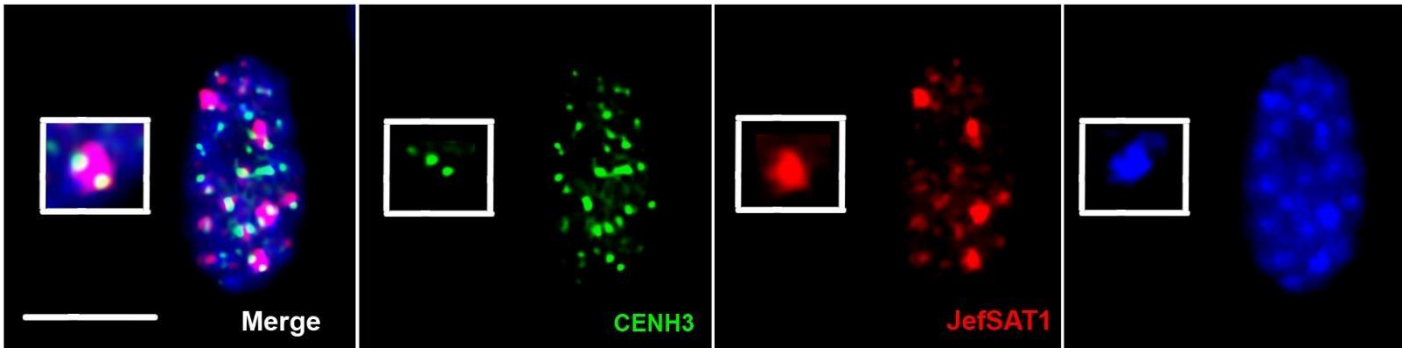


Figure 2. Immunostaining using the CENH3 antibody developed for *Juncus effusus* in an interphase nucleus of the same species followed by FISH with JefSAT1-155. ,A partial colocalization of the centromeric protein with the satellite is observed in som centromeres. Inset shows that JefSAT1-155 signal label the whole chromocentre, while CENH3 signal is more restricted. Bar =5µm

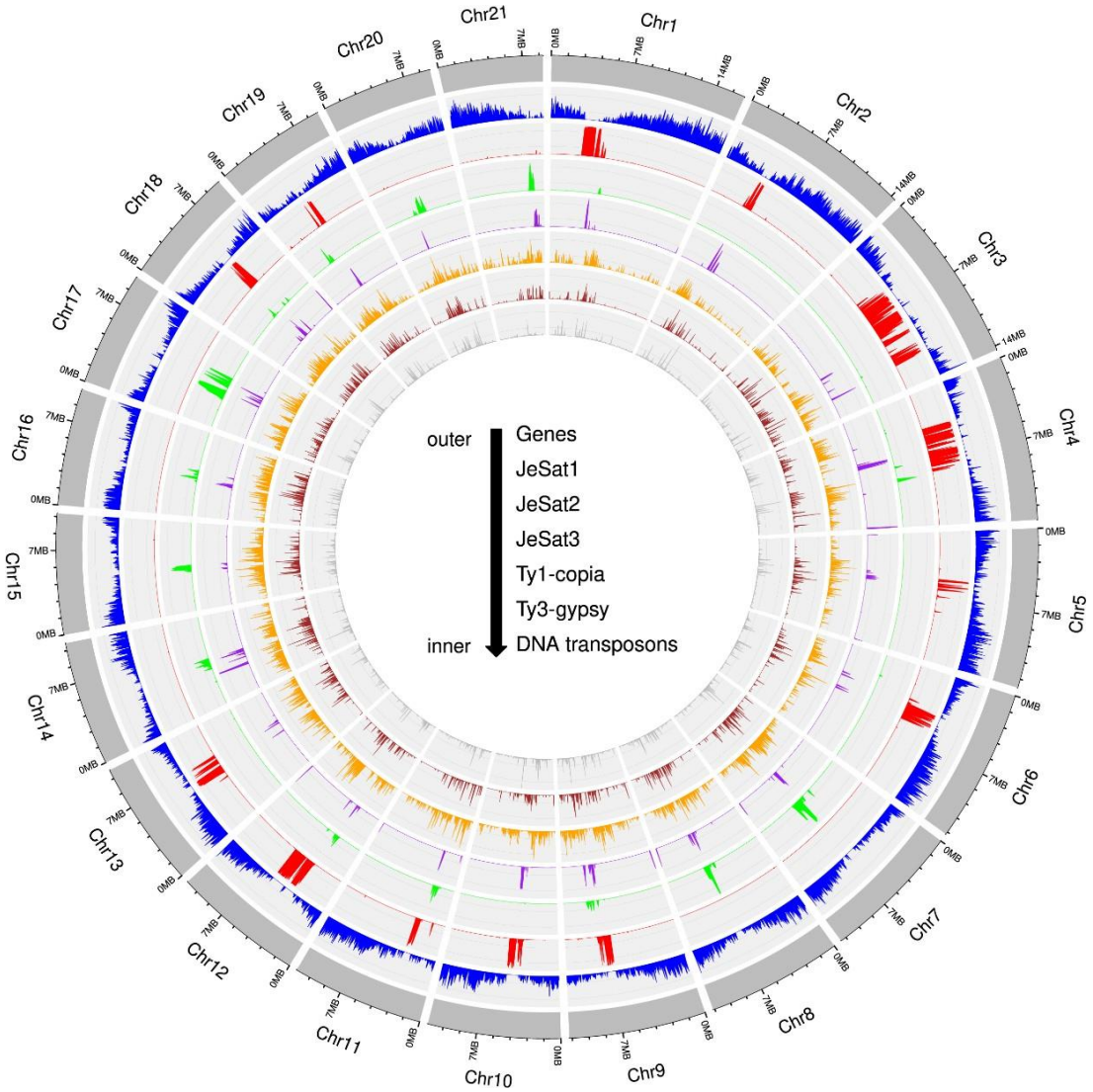


Figure 3. *In silico* distribution of the major tandem DNA repeats in *Juncus effusus* pseudomolecules (gray bars in the outer circle). JefSAT1 (purple), JefSAT2 (dark green) and JefSAT2 (lilac) are interspersed in different proportions at different centromeres. The graph also shows the distribution of genes, Ty3/gypsy, Ty1/copia and DNA transposons along the chromosomes. The height of the peaks represents the relative frequency of a sequence in a given position.

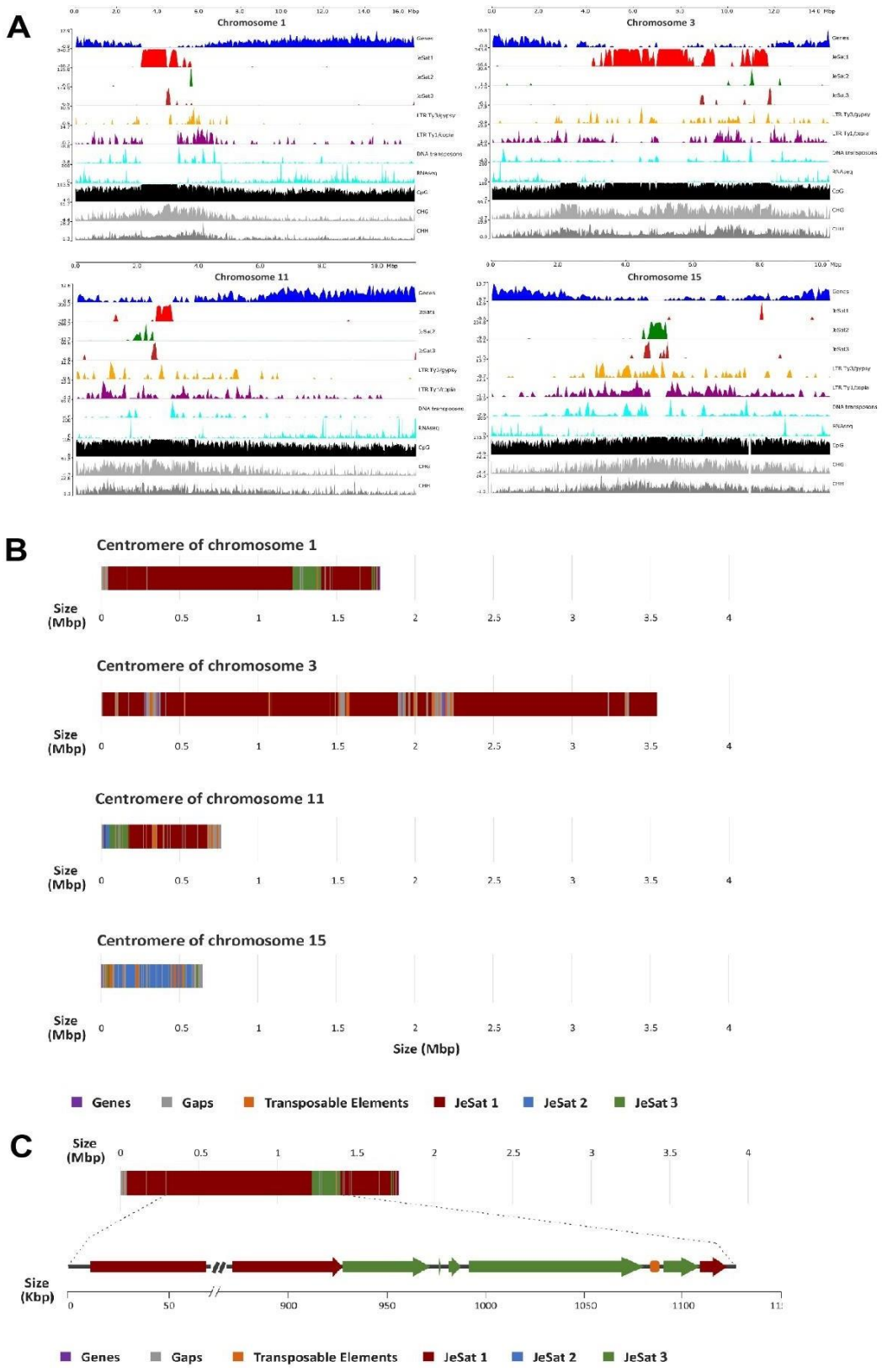


Figure 4. A) Centromere annotation of different *Juncus effusus* chromosomes. Note that proximal chromosome regions are enriched in repetitive elements and gene-poor. B) Four different configurations of the centromere of *J. effusus*, where satellites are combined in different ways. JefSAT1-155 is the most abundant in several chromosomes, followed by JefSAT2-180. C) Repeats interspersed with satellites arranged in different array sizes.

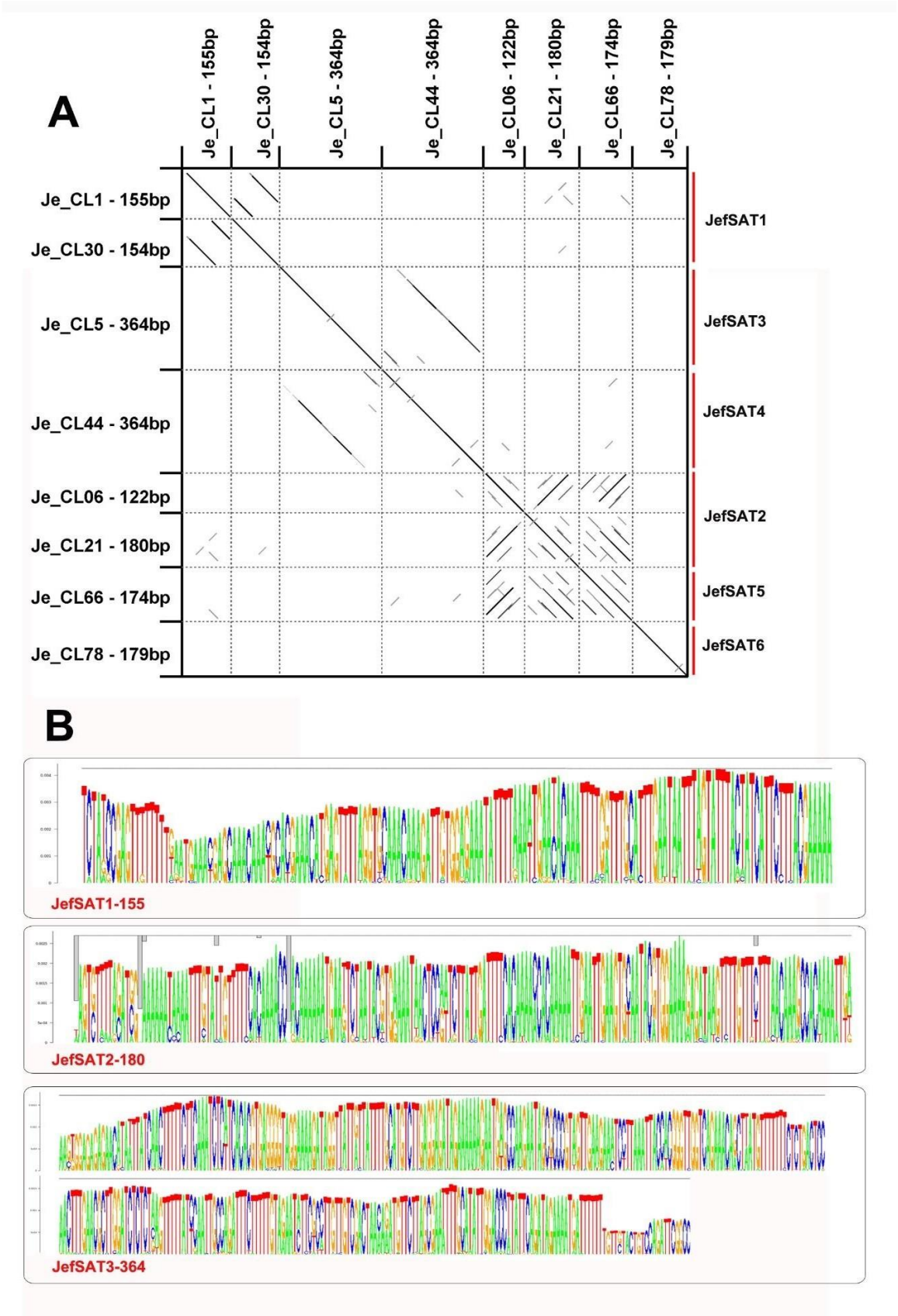
Supplementary data

Supplementary Table 1. List of probes and primer sequences used for repeat amplification in *Juncus effusus*

Target repeat	Primer name	Primer sequence	Annealing temperature	Fluorescence
JefSAT1	JefSAT1F	TGAACACAAATTTTGGTTTGCATTTG	60ºC	ATTO 488
	JefSAT1R	TGTTTCGTGCGTTTGTTTGC		
JefSAT2	JefSAT2F	TCAATAAGATTGGAGTTATTTTCACGT	60ºC	Cy3
	JefSAT2R	AGTGCAATACGTGGAAAAGAGA		
JefSAT3	JefSAT3F	TCTACAAAAAGAAATCTGGCAATTGGA	60ºC	Cy3
	JefSAT3R	AGCGAATTTTGCATATTGGACCC		
Ty1/Copia Angela	AngF	TGTGGACCAATGAGTACGCC	55ºC	Cy3
	AngR	TCCTCCTTTCGGACACTCCA		
Ty3/Gypsy Athila	AthF	GGGGTATCGACTTTATGGGACC	55ºC	Cy3
	AthR	TCTCCCTATTCGAAAGCTCTGC		

Supplementary Table 2. List of consensus sequences of the satellites DNA from *Juncus effusus*

Satellite	Cluster	Monomere size (bp)	Consensus sequence
JefSAT1	1	155	AAAATCTATCCGAGTTTTTTTTGAATGAAACGAGCAAACAAACGC ACGAAACATGAGTTTTAGGTGCAAACAAAGATTGTGGAGAAATAT TTTAAAATGAACACAAATTTTGGTTTGCATTTGAATAATTTAGTAT TTACTATCATTCTTTACGA
JefSAT1	30	154	GATACACATTTTGATATGCATTTGAATTATTTAGTATTTACTATCA TTCTTTACGAAAAATCTATCCGAGTTTTTTTATATGAAATGTGCAA ACAAACGCATGAAACATGAGTTTTAGGTCCAAAAGAAGATTGTGG AGAAATATTTTAAAATG
JefSAT2	6	122	TCACAAAACCGCAAAAATTAAAATCTAATACATGGAAAAAACTCC ATTGTTATTGATTTTCACAATACCAAAAAAATCGAATTGAGGTAC AATACATGGAAAGAAATAATATTTTGTTATTT
JefSAT2	21	180	CTTCAATTTTGTGGCTTTGTTAAAGAAACAAAACTTATCTCTTTTC CACGTATTGCACTTCAATTCAATTTTTGTGTTTTTGTGAAAATCAA TAAGATTGGAGTTATTTTCACGTATTAGAATTCATTTTTTGTGGTT TTGTGAAAAACAATACAAATTTTATTTTTTCGATCTAAAACA
JefSAT3	5	364	TCTGAATAAGGGTCCAATATGCAAAATTCGCTAAGAACATCTGTT AGTCGACAACAAGGCGAATATTCTACAAAAAGAAATCTGGCAATT GGATAAAAACTTAGAGAGATCCAAGCGTTCAAAGTTGGAGTCAG AGAAAAAAACATAAGCTGTTTCTGACCAACCCGTGGTAATTTAAG GATTTCTTCAACTACGGGCGCTATTCTTGGCTCATTCTTTTTTCTAT CTCCTCAAGACATGCAAAATAACAATAACCTATCTTTTCCTACCCC TCACGTGTGATGAGATTTGAAGAAAAAGTTGATAAATATGTTCTC TCTCCACTTGGCCGAATCTGGACAGTAGAACAAAAAATCATTTTC TA
JefSAT4	44	364	CTCTCCCCTTGGCTGAATCTGGACAGCAAAAAAATTTATTTTCTAT CTGAATGCGGGCACAATATTTTAAATTAGTCGCATTGATCTAGTA GATGACAACTAGGAGATTATTTTGCAAAAAGAATCAGGGCAATTG GATAAATAATGAGGGAGATCTAAGCGTTCAAAGTTGGAGTCACA ACACCAAAACATAAGCTGTTTCAGAACATGCCCGTTGTAATTTAA GGAGTTCTTCCACTACGGGCGCTCTTCTTGCCTCCATTTTCTTTCG ATCTACTCAAGACAAATAAAATAACAATTAACTACTGATTCATAT CCCTCTTGTGTGATTAGATTTGAAGAAAATGTCGATTAAAGATCTT CT
JefSAT5	66	174	TTTTCAATGTATTAGATATAAATTTTTGCGGTTTTGTGGAAGCAAC AAAAACTGGCTTTTTTCCGTGAATTAATTCGATTTTTATGGTTTTG TGAATATCTATAAAAAATGAGTTTTTTCGATGTATTAGACTTCAAT TTTTGTGGTTTTGTGAAAATCAATACAAATTATGTT
JefSAT6	78	179	TTCATTTAAGACACGCACCGCTATAAATAACTTATTATTCGAAATT CTTTACTCAAGACACTATATATATCCTCCCTCTATAATATCATACA AAAATTTCTCAGTTTTACATCAACACTTGCCCGGAATGCGAAACC CTAGTACTCAAGCACCATTTCATGATCAAAAAATGTTTAATC



Supplementary Figure 1. A) Dot plot of all DNA satellites found of the *Juncus effusus* species showing the families and superfamilies formed by these repeats. **B)** Logo of the main *Juncus effusus* satellites.

4. CONSIDERAÇÕES FINAIS

1. As espécies investigadas de Mapanioideae, Cyperoideae e Thurniaceae possuem de 40 a 76 cromossomos pequenos (em torno de 1 µm), com variação intraespecífica entre indivíduos de *H. schraderianum* oriundos de diferentes localidades. Para Thurniaceae essas foram as primeiras contagens números cromossômicos, assim como para os gêneros *Becquerelia*, *Hypolytrum* e *Diplasia.*

2. As três espécies de Mapanioideae analisadas apresentam pequeno tamanho do genoma compatível com uma baixa proporção de DNA repetitivo e maior abundância de DNAs satélites.

3. A fração repetitiva de *J. effusus* compreende cerca de 24% do genoma da espécie, sendo proporcional ao tamanho do seu genoma (1C = 268.95 Mbp). Os principais elementos desse genoma são retroelementos LTR e DNA satélites.

4. Os centrômeros e regiões pericentroméricas de *J. effusus* apresentam estrutura complexa e diversificada, sendo formados por três principais DNAS satélites distribuídos de forma alternada entre cromossomos e intercalados com retroelementos.

5. O ancestral do clado Cyperid era formado por representantes com cromossomos monocêntricos, mantidos em gêneros das três famílias. A holocentricidade se originou de forma independente dentro das famílias Cyperaceae, possivelmente na base de Cyperoideae e no gênero *Luzula*.

6. Os monocentrômeros investigado de Cyperids (*J. effusus*) não apresentam organização típica. Expansão dessas análises para outros representantes do grupo é necessária.

REFERÊNCIAS

ALBERTSON, Donna G.; THOMSON, J. Nichol. Segregation of holocentric chromosomes at meiosis in the nematode,Caenorhabditis elegans. **Chromosome Research**, [*s. l.*], v. 1, n. 1, p. 15–26, 1993.

ARGUELHO, Edihanne G. *et al.* New chromosome counts in Brazilian species of *Rhynchospora* (Cyperaceae). **Caryologia**, [*s. l.*], v. 65, n. 2, p. 140–146, 2012.

ÁVILA ROBLEDILLO, Laura *et al.* Satellite DNA in Vicia faba is characterized by remarkable diversity in its sequence composition, association with centromeres, and replication timing. **Scientific Reports**, [*s. l.*], v. 8, n. 1, p. 5838, 2018.

BAEZ, M. *et al.* **Analysis of the small chromosomal *Prionium serratum* (Cyperid) demonstrates the importance of a reliable method to differentiate between mono- and holocentricity**. [*S. l.*]: Evolutionary Biology, 2020. preprint. Disponível em: http://biorxiv.org/lookup/doi/10.1101/2020.07.08.193714. Acesso em: 3 ago. 2020.

BARROS E SILVA, Ana Emília *et al.* The evolution of CMA bands in Citrus and related genera. **Chromosome Research**, [*s. l.*], v. 18, n. 4, p. 503–514, 2010.

BOUCHENAK-KHELLADI, Yanis; MUASYA, A. Muthama; LINDER, H. Peter. A revised evolutionary history of Poales: origins and diversification: Evolutionary History of Poales. **Botanical Journal of the Linnean Society**, [*s. l.*], v. 175, n. 1, p. 4–16, 2014.

BOURQUE, Guillaume *et al.* Ten things you should know about transposable elements. **Genome Biology**, [*s. l.*], v. 19, n. 1, p. 199, 2018.

CABRAL, Gabriela *et al.* Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. **Nature Communications**, [*s. l.*], v. 5, n. 1, p. 5070, 2014.

CHASE, M. W. *et al.* Multigene Analyses of Monocot Relationships: a summary. **Aliso**, [*s. l.*], v. 22, p. 63–75, 2006.

CHENG, Zhukuan *et al.* Functional Rice Centromeres Are Marked by a Satellite Repeat and a Centromere-Specific Retrotransposon. **The Plant Cell**, [*s. l.*], v. 14, n. 8, p. 1691–1704, 2002.

CHENG, Zhi-Jun; MURATA, Minoru. A Centromeric Tandem Repeat Family Originating From a Part of Ty3/ *gypsy* -Retroelement in Wheat and Its Relatives. **Genetics**, [*s. l.*], v. 164, n. 2, p. 665–672, 2003.

CUACOS, Maria; H. FRANKLIN, F. Chris; HECKMANN, Stefan. Atypical centromeres in plants—what they can tell us. **Frontiers in Plant Science**, [*s. l.*], v. 6, 2015. Disponível em: http://journal.frontiersin.org/Article/10.3389/fpls.2015.00913/abstract. Acesso em: 3 ago. 2020.

DA SILVA, Carlos Roberto Maximiano; QUINTAS, Carolina Cristina; VANZELA, André Luís Laforga. Distribution of 45S and 5S rDNA sites in 23 species of Eleocharis (Cyperaceae). **Genetica**, [*s. l.*], v. 138, n. 9–10, p. 951–957, 2010.

DE FELICE, Bruna *et al.* A novel repetitive DNA sequence in lemon [Citrus limon (L.) Burm.] and related species Bruna. **Journal of Applied Genetics**, [*s. l.*], v. 45, n. 3, p. 315–320, 2004.

DE SOUZA, Thaíssa B *et al.* Analysis of retrotransposon abundance, diversity and distribution in holocentric Eleocharis (Cyperaceae) genomes. **Annals of Botany**, [*s. l.*], v. 122, n. 2, p. 279–290, 2018.

D'HONT, Angélique *et al.* The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. **Nature**, [*s. l.*], v. 488, n. 7410, p. 213–217, 2012.

DIAS, Yhanndra K. *et al.* IAPT chromosome data 33/4. **TAXON**, [*s. l.*], v. 69, n. 6, p. 1394–1405, 2020.

DRINNENBERG, Ines A *et al.* Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. **eLife**, [*s. l.*], v. 3, p. e03676, 2014.

EARNSHAW, W. C. *et al.* Esperanto for histones: CENP-A, not CenH3, is the centromeric histone H3 variant. **Chromosome Research**, [*s. l.*], v. 21, n. 2, p. 101–106, 2013.

EKWALL, Karl. Epigenetic Control of Centromere Behavior. **Annual Review of Genetics**, [*s. l.*], v. 41, n. 1, p. 63–81, 2007.

ENDRESS, Peter K. **Diversity and evolutionary biology of tropical flowers**. Repred. Cambridge: Cambridge Univ. Press, 1998. (Cambridge tropical biology series).

ESCUDERO, Marcial *et al.* Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (Carex, Cyperaceae). **Molecular Phylogenetics and Evolution**, [*s. l.*], v. 63, n. 3, p. 650–655, 2012.

ESCUDERO, Marcial; MÁRQUEZ-CORRO, J. Ignacio; HIPP, Andrew L. The Phylogenetic Origins and Evolutionary History of Holocentric Chromosomes. **Systematic Botany**, [*s. l.*], v. 41, n. 3, p. 580–585, 2016.

FEDOROFF, Nina V. Transposable Elements, Epigenetics, and Genome Evolution. **Science**, [*s. l.*], v. 338, n. 6108, p. 758–767, 2012.

FLACH, M. Diffuse Centromeres in a Dicotyledonous Plant. **Nature**, [*s. l.*], v. 209, n. 5030, p. 1369–1370, 1966.

FURNESS, Carol A.; RUDALL, Paula J. Selective microspore abortion correlated with aneuploidy: an indication of meiotic drive. **Sexual Plant Reproduction**, [*s. l.*], v. 24, n. 1, p. 1–8, 2011.

GLOBAL CAREX GROUP. Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): a new broader circumscription: Making *Carex* Monophyletic. **Botanical Journal of the Linnean Society**, [*s. l.*], v. 179, n. 1, p. 1–42, 2015.

GOVAERTS, R. *et al.* **World checklist of Cyperaceae. Kew: The Board of Trustees of the Royal Botanic Gardens, Kew.** [*S. l.*], 2018. Disponível em: Acesso em: 8 abr. 2020.

GRANDBASTIEN, M. Activation of plant retrotransposons under stress conditions. **Trends in Plant Science**, [*s. l.*], v. 3, n. 5, p. 181–187, 1998.

GREILHUBER, J. Chromosomes of the monocotyledons (general aspects). *In*: MONOCOTYLEDONS: SYSTEMATICS AND EVOLUTION. [*S. l.*]: Royal Botanic Gardens, Kew, 1995. p. 379–414.

GUERRA, Marcelo; GARCÍA, Miguel A. Heterochromatin and rDNA sites distribution in the holocentric chromosomes of *Cuscuta approximata* Bab. (Convolvulaceae). **Genome**, [*s. l.*], v. 47, n. 1, p. 134–140, 2004.

GUERRA, Marcelo; RIBEIRO, Tiago; FELIX, Leonardo P. Monocentric chromosomes in Juncus (Juncaceae) and implications for the chromosome evolution of the family. **Botanical Journal of the Linnean Society**, [*s. l.*], v. 191, n. 4, p. 475–483, 2019.

HECKMANN, Stefan *et al.* The holocentric species *Luzula elegans* shows interplay between centromere and large-scale genome organization. **The Plant Journal**, [*s. l.*], v. 73, n. 4, p. 555–565, 2013.

HENIKOFF, Steven; AHMAD, Kami; MALIK, Harmit S. The Centromere Paradox: Stable Inheritance with Rapidly Evolving DNA. **Science**, [*s. l.*], v. 293, n. 5532, p. 1098–1102, 2001.

HIPP, Andrew L.; ROTHROCK, Paul E.; ROALSON, Eric H. The Evolution of Chromosome Arrangements in Carex (Cyperaceae). **The Botanical Review**, [*s. l.*], v. 75, n. 1, p. 96–109, 2009.

HOF, Arjen E. van't *et al.* The industrial melanism mutation in British peppered moths is a transposable element. **Nature**, [*s. l.*], v. 534, n. 7605, p. 102–105, 2016.

HUGHES-SCHRADER, Sally; RIS, Hans. The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. **Journal of Experimental Zoology**, [*s. l.*], v. 87, n. 3, p. 429–456, 1941.

ITO, Hidetaka *et al.* Evolution of the ONSEN retrotransposon family activated upon heat stress in Brassicaceae. **Gene**, [*s. l.*], v. 518, n. 2, p. 256–261, 2013.

IWATA, Aiko *et al.* Identification and characterization of functional centromeres of the common bean. **The Plant Journal**, [*s. l.*], p. n/a-n/a, 2013.

JIANG, Jiming *et al.* A molecular view of plant centromeres. **Trends in Plant Science**, [*s. l.*], v. 8, n. 12, p. 570–575, 2003.

JUDD, Walter S. (org.). **Plant systematics: a phylogenetic approach**. 3rd eded. Sunderland, Mass: Sinauer Associates, 2008.

KALENDAR, R. *et al.* Genome evolution of wild barley (Hordeum spontaneum) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 97, n. 12, p. 6603–6607, 2000.

KAPITONOV, V. V.; JURKA, J. Rolling-circle transposons in eukaryotes. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 98, n. 15, p. 8714–8719, 2001.

KLEIN, Savannah J.; O'NEILL, Rachel J. Transposable elements: genome innovation, chromosome diversity, and centromere conflict. **Chromosome Research**, [*s. l.*], v. 26, n. 1–2, p. 5–23, 2018.

KOO, Dal-Hoe *et al.* Rapid divergence of repetitive DNAs in Brassica relatives. **Genomics**, [*s. l.*], v. 97, n. 3, p. 173–185, 2011.

LEE, Sung-Il; KIM, Nam-Soo. Transposable Elements and Genome Size Variations in Plants. **Genomics & Informatics**, [*s. l.*], v. 12, n. 3, p. 87, 2014.

LERMONTOVA, Inna; SANDMANN, Michael; DEMIDOV, Dmitri. Centromeres and kinetochores of Brassicaceae. **Chromosome Research**, [*s. l.*], v. 22, n. 2, p. 135–152, 2014.

LIPNEROVA, I. *et al.* Evolution of genome size in Carex (Cyperaceae) in relation to chromosome number and genomic base composition. **Annals of Botany**, [*s. l.*], v. 111, n. 1, p. 79–94, 2013.

LÓPEZ, Maria Gabriela; AVELIANO, Fernández; FLORENCIA, Galdeano. Cytogenetics, genome size and anther anatomy in *Bulbostylis* (Cyperaceae). **Caryologia**, [*s. l.*], v. 70, n. 3, p. 238–247, 2017.

LUCEÑO, M. *et al.* Agmatoploidy and Symploidy in Rhynchospora cephalotes (L.) Vahl (Cyperaceae). **CYTOLOGIA**, [*s. l.*], v. 63, n. 1, p. 79–81, 1998.

MADDOX, Paul S. *et al.* "Holo"er than thou: Chromosome segregation and kinetochore function in C. elegans. **Chromosome Research**, [*s. l.*], v. 12, n. 6, p. 641–653, 2004.

MAHESHWARI, Shamoni *et al.* Centromere location in *Arabidopsis* is unaltered by extreme divergence in CENH3 protein sequence. **Genome Research**, [*s. l.*], v. 27, n. 3, p. 471–478, 2017.

MAKAŁOWSKI, Wojciech *et al.* Transposable Elements: Classification, Identification, and Their Use As a Tool For Comparative Genomics. *In*: ANISIMOVA, Maria (org.). **Evolutionary Genomics**. New York, NY: Springer New York, 2019. (Methods in Molecular Biology). v. 1910, p. 177–207. *E-book*. Disponível em: http://link.springer.com/10.1007/978-1-4939-9074-0_6. Acesso em: 15 nov. 2021.

MAKAREVITCH, Irina *et al.* Transposable Elements Contribute to Activation of Maize Genes in Response to Abiotic Stress. **PLoS Genetics**, [*s. l.*], v. 11, n. 1, p. e1004915, 2015.

MALIK, Harmit S; HENIKOFF, Steven. Phylogenomics of the nucleosome. **Nature Structural & Molecular Biology**, [*s. l.*], v. 10, n. 11, p. 882–891, 2003.

MARQUES, André *et al.* Holocentromeres in *Rhynchospora* are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 112, n. 44, p. 13633–13638, 2015.

MÁRQUEZ-CORRO, J.I. *et al.* IAPT chromosome data 28. **Taxon**, [*s. l.*], v. 67, n. 6, p. 1235–1245, 2018.

MÁRQUEZ-CORRO, José Ignacio; ESCUDERO, Marcial; LUCEÑO, Modesto. Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives. **Chromosome Research**, [*s. l.*], v. 26, n. 3, p. 139–152, 2018.

MARTINEZ-ZAPATER, Jose M.; ESTELLE, Mark A.; SOMERVILLE, Chris R. A highly repeated DNA sequence in Arabidopsis thaliana. **Molecular and General Genetics MGG**, [*s. l.*], v. 204, n. 3, p. 417–423, 1986.

MAXIMIANO DA SILVA, Carlos Roberto *et al.* Cytogenetical and cytotaxonomical analysis of some Brazilian species of Eleocharis (Cyperaceae). **Australian Journal of Botany**, [*s. l.*], v. 56, n. 1, p. 82, 2008.

MCCLINTOCK, B. The Behavior in Successive Nuclear Divisions of a Chromosome Broken at Meiosis. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 25, n. 8, p. 405–416, 1939.

MCCLINTOCK, B. The origin and behavior of mutable loci in maize. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 36, n. 6, p. 344–355, 1950.

MEHROTRA, Shweta; GOYAL, Vinod. Repetitive Sequences in Plant Nuclear DNA: Types, Distribution, Evolution and Function. **Genomics, Proteomics & Bioinformatics**, [*s. l.*], v. 12, n. 4, p. 164–171, 2014.

MELTERS, Daniël P *et al.* Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. **Genome Biology**, [*s. l.*], v. 14, n. 1, p. R10, 2013.

MELTERS, Daniël P. *et al.* Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. **Chromosome Research**, [*s. l.*], v. 20, n. 5, p. 579–593, 2012.

MUASYA, A. Muthama *et al.* Phylogeny of Cyperaceae Based on DNA Sequence Data: Current Progress and Future Prospects. **The Botanical Review**, [*s. l.*], v. 75, n. 1, p. 2–21, 2009.

NAGAKI, Kiyotaka *et al.* Molecular and cytological analyses of large tracks of centromeric DNA reveal the structure and evolutionary dynamics of maize centromeres. **Genetics**, [*s. l.*], v. 163, n. 2, p. 759–770, 2003.

NAGAKI, Kiyotaka; KASHIHARA, Kazunari; MURATA, Minoru. Visualization of Diffuse Centromeres with Centromere-Specific Histone H3 in the Holocentric Plant *Luzula nivea*. **The Plant Cell**, [*s. l.*], v. 17, n. 7, p. 1886–1893, 2005.

NASUDA, S. *et al.* Stable barley chromosomes without centromeric repeats. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 102, n. 28, p. 9842–9847, 2005.

NEUMANN, Pavel *et al.* Epigenetic Histone Marks of Extended Meta-Polycentric Centromeres of Lathyrus and Pisum Chromosomes. **Frontiers in Plant Science**, [*s. l.*], v. 7, 2016. Disponível em: http://journal.frontiersin.org/article/10.3389/fpls.2016.00234. Acesso em: 15 nov. 2021.

NEUMANN, Pavel *et al.* Impact of parasitic lifestyle and different types of centromere organization on chromosome and genome evolution in the plant genus *Cuscuta*. **New Phytologist**, [*s. l.*], v. 229, n. 4, p. 2365–2377, 2021.

NEUMANN, Pavel *et al.* Plant centromeric retrotransposons: a structural and cytogenetic perspective. **Mobile DNA**, [*s. l.*], v. 2, n. 1, p. 4, 2011.

NEUMANN, Pavel *et al.* Stretching the Rules: Monocentric Chromosomes with Multiple Centromere Domains. **PLoS Genetics**, [*s. l.*], v. 8, n. 6, p. e1002777, 2012.

NOVAK, P. *et al.* RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. **Bioinformatics**, [*s. l.*], v. 29, n. 6, p. 792–793, 2013.

NOVÁK, Petr *et al.* TAREAN: a computational tool for identification and characterization of satellite DNA from unassembled short reads. **Nucleic Acids Research**, [*s. l.*], v. 45, n. 12, p. e111–e111, 2017.

BAEZ, M. *et al.* Analysis of the small chromosomal *Prionium serratum* (Cyperid) demonstrates the importance of a reliable method to differentiate between mono- and holocentricity. [S.l.]: **Evolutionary Biology**, 2020. Disponível em: <http://biorxiv.org/lookup/doi/10.1101/2020.07.08.193714>. Acesso em: 3 ago. 2020.

CHENG, Z. *et al.* Functional Rice Centromeres Are Marked by a Satellite Repeat and a Centromere-Specific Retrotransposon. **The Plant Cell,** ago. 2002. v. 14, n. 8, p. 1691–1704.

DRINNENBERG, I. A. *et al.* Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. **eLife**, 23 set. 2014. v. 3, p. e03676.

EKWALL, K. Epigenetic Control of Centromere Behavior. **Annual Review of Genetics**, 1 dez. 2007. v. 41, n. 1, p. 63–81.

MARQUES, A. *et al.* Holocentromeres in *Rhynchospora* are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. **Proceedings of the National Academy of Sciences**, 3 nov. 2015. v. 112, n. 44, p. 13633–13638.

PEDROSA-HARAND, Andrea. Holocentromere identity: from the typical mitotic linear structure to the great plasticity of meiotic holocentromeres. **Chromosoma**, set. 2016. v. 125, n. 4, p. 669–681.

MÁRQUEZ-CORRO, J. I. *et al.* Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution in sedges (Cyperaceae). **Molecular Phylogenetics and Evolution**, jun. 2019. v. 135, p. 203–209.

ESCUDERO, M.; LUCEÑO, M. Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives. **Chromosome Research**, set. 2018. v. 26, n. 3, p. 139–152.

MCKINLEY, K. L.; CHEESEMAN, I. M. The molecular basis for centromere identity and function. **Nature Reviews Molecular Cell Biology**, jan. 2016. v. 17, n. 1, p. 16–29.

MOORE, G. *et al.* Are rice chromosomes components of a holocentric chromosome ancestor? Plant **Molecular Biology**, 1997. v. 35, n. 1/2, p. 17–23.

OLIVEIRA, L. *et al.* Mitotic Spindle Attachment to the Holocentric Chromosomes of Cuscuta europaea Does Not Correlate With the Distribution of CENH3 Chromatin. **Frontiers in Plant Science**, 24 jan. 2020. v. 10, p. 1799.

RIBEIRO, T. *et al.* Centromeric and non-centromeric satellite DNA organisation differs in holocentric Rhynchospora species. **Chromosoma**, mar. 2017. v. 126, n. 2, p. 325–335.

ROALSON, E. H. A Synopsis of Chromosome Number Variation in the Cyperaceae. **The Botanical Review**, jun. 2008. v. 74, n. 2, p. 209–393.

SCHUBERT, Veit *et al.* Super-Resolution Microscopy Reveals Diversity of Plant Centromere Architecture. **International Journal of Molecular Sciences**, 15 maio. 2020. v. 21, n. 10, p. 3488.

SYBENGA, J. Specialization in the behaviour of chromosomes on the meiotic spindle. **Genetica**, dez. 1981. v. 57, n. 2, p. 143–151.

PAZ, Rosalía Cristina *et al.* Diversity, distribution and dynamics of full-length Copia and Gypsy LTR retroelements in Solanum lycopersicum. **Genetica**, [*s. l.*], v. 145, n. 4–5, p. 417–430, 2017.

PAZY, Batia; PLITMANN, Uzi. Holocentric chromosome behaviour inCuscuta (Cuscutaceae). **Plant Systematics and Evolution**, [*s. l.*], v. 191, n. 1–2, p. 105–109, 1994.

PEDROSA-HARAND, Andrea *et al.* Extensive ribosomal DNA amplification during Andean common bean (Phaseolus vulgaris L.) evolution. **Theoretical and Applied Genetics**, [*s. l.*], v. 112, n. 5, p. 924–933, 2006.

PLOHL, Miroslav; MEŠTROVIĆ, Nevenka; MRAVINAC, Brankica. Centromere identity from the DNA point of view. **Chromosoma**, [*s. l.*], v. 123, n. 4, p. 313–325, 2014.

PRITHAM, Ellen J.; PUTLIWALA, Tasneem; FESCHOTTE, Cédric. Mavericks, a novel class of giant transposable elements widespread in eukaryotes and related to DNA viruses. **Gene**, [*s. l.*], v. 390, n. 1–2, p. 3–17, 2007.

QIU, Fan; UNGERER, Mark C. Genomic abundance and transcriptional activity of diverse gypsy and copia long terminal repeat retrotransposons in three wild sunflower species. **BMC Plant Biology**, [*s. l.*], v. 18, n. 1, p. 6, 2018.

RIBEIRO, Tiago *et al.* Centromeric and non-centromeric satellite DNA organisation differs in holocentric Rhynchospora species. **Chromosoma**, [*s. l.*], v. 126, n. 2, p. 325–335, 2017.

ROBILLARD, Émilie *et al.* Experimental evolution reveals hyperparasitic interactions among transposable elements. **Proceedings of the National Academy of Sciences**, [*s. l.*], v. 113, n. 51, p. 14763–14768, 2016.

ROCHA, Danilo M; VANZELA, André L L; MARIATH, Jorge E A. Comparative study of microgametogenesis in members of Cyperaceae and Juncaceae: a shift from permanent pollen tetrads to pseudomonads. **Botanical Journal of the Linnean Society**, [*s. l.*], v. 188, n. 1, p. 59–73, 2018.

SANMIGUEL, Phillip *et al.* The paleontology of intergene retrotransposons of maize. **Nature Genetics**, [*s. l.*], v. 20, n. 1, p. 43–45, 1998.

SCHNABLE, P. S. *et al.* The B73 Maize Genome: Complexity, Diversity, and Dynamics. **Science**, [*s. l.*], v. 326, n. 5956, p. 1112–1115, 2009.

SCHNEIDER, Marielle C. *et al.* Organization and Behavior of the Synaptonemal Complex during Achiasmatic Meiosis of Four Buthid Scorpions. **Cytogenetic and Genome Research**, [*s. l.*], v. 144, n. 4, p. 341–347, 2015.

SCHRADER, Franz. Notes an the Mitotic Behavior of Long Chromosomes. **CYTOLOGIA**, [*s. l.*], v. 6, n. 4, p. 422–430, 1935.

SCHULMAN, Alan H. Retrotransposon replication in plants. **Current Opinion in Virology**, [*s. l.*], v. 3, n. 6, p. 604–614, 2013.

SEMMOURI, Ilias *et al.* Phylogeny and Systematics of Cyperaceae, the Evolution and Importance of Embryo Morphology. **The Botanical Review**, [*s. l.*], v. 85, n. 1, p. 1–39, 2019.

SHEIKH, Shamimul Alam; KONDO, Katsuhiko. DIFFERENTIAL STAINING WITH ORCEIN, GIEMSA, CMA, AND DAPI FOR COMPARATIVE CHROMOSOME STUDY OF 12 SPECIES OF AUSTRALIAN *DROSERA* (DROSERACEAE). **American Journal of Botany**, [*s. l.*], v. 82, n. 10, p. 1278–1286, 1995.

SHIRAKAWA, Jun'ichi; NAGANO, Katsuya; HOSHI, Yoshikazu. A chromosome study of two centromere differentiating Drosera species, D. arcturi and D. regia. [*s. l.*], n. 64, p. 453–463, 2011.

SCHUBERT, V. *et al.* Super-Resolution Microscopy Reveals Diversity of Plant Centromere Architecture. **International Journal of Molecular Sciences**, 15 maio. 2020. v. 21, n. 10, p. 3488.

SIMPSON, D. A. *et al.* Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. **American Journal of Botany**, [*s. l.*], v. 90, n. 7, p. 1071–1086, 2003.

SIMPSON, David A.; INGLIS, Cecilia A. Cyperaceae of Economic, Ethnobotanical and Horticultural Importance: A Checklist. **Kew Bulletin**, [*s. l.*], v. 56, n. 2, p. 257, 2001.

SOUSA, A. *et al.* Distribution of 5S and 45S rDNA sites in plants with holokinetic chromosomes and the "chromosome field" hypothesis. **Micron**, [*s. l.*], v. 42, n. 6, p. 625–631, 2011.

SPALINK, Daniel *et al.* Biogeography of the cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. **Journal of Biogeography**, [*s. l.*], v. 43, n. 10, p. 1893–1904, 2016.

STIMPSON, Kaitlin M.; MATHENY, Justyne E.; SULLIVAN, Beth A. Dicentric chromosomes: unique models to study centromere function and inactivation. **Chromosome Research**, [*s. l.*], v. 20, n. 5, p. 595–605, 2012.

SU, Handong *et al.* The Behavior of the Maize B Chromosome and Centromere. **Genes**, [*s. l.*], v. 9, n. 10, p. 476, 2018.

SULLIVAN, Beth A.; SCHWARTZ, Stuart. Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. **Human Molecular Genetics**, [*s. l.*], v. 4, n. 12, p. 2189–2197, 1995.

TANAKA, Noriyuki; TANAKA, Nobunori. Chromosome Studies in Chionographis (Liliaceae). **CYTOLOGIA**, [*s. l.*], v. 42, n. 3–4, p. 753–763, 1977.

TRAN, Trung D. *et al.* Centromere and telomere sequence alterations reflect the rapid genome evolution within the carnivorous plant genus *Genlisea*. **The Plant Journal**, [*s. l.*], v. 84, n. 6, p. 1087–1099, 2015.

VANZELA, André L.L.; GUERRA, Marcelo. Heterochromatin differentiation in holocentric chromosomes of Rhynchospora (Cyperaceae). **Genetics and Molecular Biology**, [*s. l.*], v. 23, n. 2, p. 453–456, 2000.

VICIENT, Carlos M. *et al.* [No title found]. **Genetica**, [*s. l.*], v. 107, n. 1/3, p. 53–63, 1999.

WICKER, Thomas *et al.* Analysis of a contiguous 211 kb sequence in diploid wheat (Triticum monococcum L.) reveals multiple mechanisms of genome evolution: Genome organisation in 211 kb DNA of diploid wheat. **The Plant Journal**, [*s. l.*], v. 26, n. 3, p. 307–316, 2001.

XIAO, Han *et al.* A Retrotransposon-Mediated Gene Duplication Underlies Morphological Variation of Tomato Fruit. **Science**, [*s. l.*], v. 319, n. 5869, p. 1527–1530, 2008.

ZEDEK, František *et al.* Flow cytometry may allow microscope-independent detection of holocentric chromosomes in plants. **Scientific Reports**, [*s. l.*], v. 6, n. 1, p. 27161, 2016.

ZHANG, Lulu *et al.* The structure and retrotransposition mechanism of LTR-retrotransposons in the asexual yeast *Candida albicans*. **Virulence**, [*s. l.*], v. 5, n. 6, p. 655–664, 2014.

ZUMSTEIN, Wilailux; WANGWASIT, Kamolhathai; DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCE, MAHASARAKHAM UNIVERSITY, MAHASARAKHAM 44150, THAILAND. Chromosome counts and karyotype reports from Fimbristylis (Cyperaceae) in Thailand. **Thai Forest Bulletin (Botany)**, [*s. l.*], v. 47, p. 123–132, 2019.

APÊNDICE A: IAPT CHROMOSOME DATA 33/4

Yhanndra K. Dias Silva, Mariana Baez, Marccus V. Alves, Wayt W. Thomas, André L.L. Vanzela, Erton M. de Almeida, Tammy L. Elliott, Bruno S. Amorim, Andreas Houben & Andrea Pedrosa-Harand

Publicado em:

Artigo publicado na revista TAXON IAPT CHROMOSOME DATA 33/4 (A1)

Em dezembro de 2020

Yhanndra K. Dias Silva, Mariana Baez, Marccus V. Alves, Wayt W. Thomas, André L.L. Vanzela, Erton M. de Almeida, Tammy L. Elliott, Bruno S. Amorim, Andreas Houben & Andrea Pedrosa-Harand*

* Address for correspondence: andrea.harand@ufpe.br

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; PROBRAL CAPES/DAAD project number 88881.144086/2017-01) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant number 141725/2018-4 to Y.D.)

All materials CHN.

CYPERACEAE

Subfamily Cyperoideae

Becquerelia cymosa Brongn., 2*n* = 40; Brazil, Paraíba, E.M. Almeida 2856 & M. Fernandes (EAN 29294).

Subfamily Mapanioideae

Diplasia karatifolia Rich., 2*n* = 60; Brazil, Amazonas, M. Alves & Y. Dias 04-2018 (UFP).

Hypolytrum schraderianum Nees, 2*n* = 60; Brazil, Amazonas, M. Alves & Y. Dias 05-2018 (UFP); Brazil, Bahia, W. W. Thomas, P.J.S. Silva Filho & L.H. Daneu 16811 (JPB, NYBG); 2*n* = 76; Brazil, Paraná, M.C. Dias & E. Rocha s.n. (FUEL 29197, HCF 9642, VIES 26685).

THURNIACEAE

Prionium serratum (L.f.) Drège ex E.Mey., 2*n* = 46; South Africa, Western Cape, T.L. Elliott TE2016_413 (BOL).

Thurnia sphaerocephala (Rudge) Hook.f., 2*n* = 46; Brazil, Amazonas, M. Alves & Y. Dias 10-2018 (UFP).

IAPT chromosome data 33/4

Yhanndra K. Dias Silva, Mariana Baez, Marccus V. Alves, Wayt W. Thomas, André L.L. Vanzela, Erton M. de Almeida, Tammy L. Elliott, Bruno S. Amorim, Andreas Houben & Andrea Pedrosa-Harand*

* Address for correspondence: andrea.harand@ufpe.br

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; PROBRAL CAPES/DAAD project number 88881.144086/2017-01) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant number 141725/2018-4 to Y.D.)

* First chromosome count for the family.

** First chromosome count for the genus.

CYPERACEAE

Subfamily Cyperoideae

** *Becquerelia cymosa* Brongn.

2*n* = 40, CHN. Brazil, Paraíba, Jacaraú, 06°38′13″S, 35°14′49″W; 148 m, 11 Aug 2019, E.M. Almeida 2856 & M. Fernandes (EAN 29294) [Fig. 7A].

Subfamily Mapanioideae

** *Diplasia karatifolia* Rich.

2*n* = 60, CHN. Brazil, Amazonas, Manaus, Reserva Adolpho Ducke, near the field camp facilities at the Acará stream, 26 Sep 2018, M. Alves & Y. Dias 04-2018 (UFP) [Fig. 7B].

** *Hypolytrum schraderianum* Nees

2*n* = 60, CHN. Brazil, Amazonas, Manaus, Reserva Adolpho Ducke, access to the observation tower trail, 26 Sep 2018, M. Alves & Y. Dias 05-2018 (UFP) [Fig. 7C]. Brazil, Bahia, Mun. Camacan, Serra Bonita Private Reserve (RPPN), 15°25′07″S, 39°32′58″W, 300–400 m, 20 Sep 2018, W.W. Thomas, P.J.S. Silva Filho & L.H. Daneu 16811 (JPB, NYBG) [Fig. 7D].

2*n* = 76, CHN. Brazil, Paraná, Paranaguá, Ilha do Mel, near Bica do Norinho at 25°33′59.9″S, 48°18′25.7″W, 2 Jul 1995, M.C. Dias & E. Rocha s.n. (FUEL 29197, HCF 9642, VIES 26685), recollected in 2019 for the present analysis [Fig. 7E].

THURNIACEAE

* *Prionium serratum* (L.f.) Drège ex E.Mey.

2*n* = 46, CHN. South Africa, Western Cape, Groot Winterhoek Wilderness Area, on edge of trail near Disa Pool, approximately 1 km from parking lot, 32°59′54.132″S, 19°04′13.9368″W, 924 m, 20 Oct 2018, T.L. Elliott TE2016_413 (BOL) [Fig. 7F].

* *Thurnia sphaerocephala* (Rudge) Hook.f.

2*n* = 46, CHN. Brazil, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, stream near communal kitchen for the local lodging, 27 Sep 2018, M. Alves & Y. Dias 10-2018 (UFP) [Fig. 7G].

The families Cyperaceae, Juncaceae and Thurniaceae form the well-supported Cyperid clade (Chase & al., 2006), which also includes Rapateaceae and Mayacaceae, although with low support (Bouchenak-Khelladi & al., 2014). Cyperaceae has a cosmopolitan distribution, but it originated in South America and diversified in the Northern Hemisphere (Spalink & al., 2016). It comprises 90 genera and about 5500 species distributed in two subfamilies (Cyperioideae, Mapanioideae), constituting the third-largest family of monocotyledons in species diversity, after Orchidaceae and Poaceae (Semmouri & al., 2019; Govaerts & al., 2020). Some Cyperaceae species, such as *Cyperus papyrus* L., *C. rotundus* L. and *C. esculentus* L., are economically important and relevant for ethnobotany and horticulture (Simpson & Inglis, 2001). Sister to Cyperaceae-Juncaceae, Thurniaceae is composed of two genera, *Thurnia* Hook.f. and *Prionium* E.Mey., and four species, *T. jenmanii* Hook.f., *Thurnia polycephala* Schnee, *T. sphaerocephala* (Rudge) Hook.f., which are endemic to the northestern part of the Amazon Basin, and *Prionium serratum* (L.f.) Drège ex E.Mey, endemic to South Africa (Kubitzki, 1998; Mucina & Rutherford, 2006).

Several cytogenetic studies have been performed in Cyperaceae, especially addressing the high diversity of chromosome numbers, which range from 2*n* = 4 to 224, with probable ancestral number *x* = 5, and the variability of rDNA sites (Roalson, 2008; da Silva & al., 2010; Sousa & al., 2011; Lipnerová & al., 2013; Ribeiro & al., 2018; Carta & al., 2020). However, the subfamily Mapanioideae is still underrepresented and has only one chromosome count, for *Chrysitrix capensis* L., with 2*n* = 46 (Márquez-Corro & al., 2018).

Holocentricity is one of the most striking cytogenetic characteristics of the family, being a synapomorphy for Cyperaceae and the sister group, Juncaceae (Greilhuber, 1995).

Chromosomal fusions and fissions leading to dysploidy, polyploidy, inverted meiosis and variation in genome size are part of the evolutionary dynamics of Cyperaceae, and some of these features are related to holocentricity (Vanzela & al., 2000; Hipp & al., 2009; Cabral & al., 2014; Ribeiro & al., 2018, Souza & al., 2018). Although the Cyperid clade is recognized as holocentric, there is no evidence for it outside Cyperaceae and Juncaceae. In fact, our work provides the first chromosome counts for the family Thurniaceae.

For slide preparations, root tips were pretreated with 2 mM 8-hydroxyquinoline for 24 h at 10°C, fixed in ethanol : acetic acid (3 : 1, v : v) for 2 h and stored at −20°C. After enzymatic digestion (2% cellulase Onozuka, 20% pectinase Sigma, or 2% cellulase, 2% pectolyase, 20% pectinase, Sigma, both in 0.01 M citric acid–sodium citrate buffer, pH 4.8) for 90 min at 37°C, mitotic preparations were performed by air-drying (Carvalho & Saraiva, 1993) or by squashing in a drop of 45% acetic acid. Slides were selected after staining with 2 μg/ml of DAPI (4′,6-diamidino-2-phenylindole, Sigma) in glycerol (1 : 1, v : v).

For all analysed species, which showed small chromosomes (around 1 μm) of decreasing sizes, chromosomal numbers were counted for the first time. *Becquerelia cymosa* showed 2*n* = 40 (Fig. 7A). *Diplasia karatifolia* and *H. schraderianum* from the states of Amazonas and Bahia had 2*n* = 60 (Fig. 7B–D), while *H. schraderianum* from Paraná showed 2*n* = 76 (Fig. 7E). *Prionium serratum* and *T. sphaerocephala* had 2*n* = 46 (Fig. 7F,G). Primary constrictions have not been clearly observed in any sample, but this may be due to their small chromosome sizes. Thus, holocentricity should be evaluated by other approaches. Although intraspecific variability was observed for *H. schraderianum*, the same chromosome number was observed in the two studied genera of subfamily Mapanioideae, *Diplasia* Rich. and *Hypolytrum* Pers., which belong to different phylogenetic subclades (Semmouri & al., 2019). Similarly, the same chromosome number was observed for both Thurniaceae genera. This study provides information on early-diverging lineages of Cyperids and suggests that dysploidy and polyploidy are relevant in the evolution of this clade.

LITERATURE CITED

Bouchenak-Khelladi, Y., Muasya, A.M. & Linder, H.P. 2014. A revised evolutionary history of Poales: Origins and diversification. Bot. J. Linn. Soc. 175: 4–16. https://doi.org/10.1111/boj.12160

Cabral, G., Marques, A., Schubert, V., Pedrosa-Harand, A. & Schlögelhofer, P. 2014. Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. Nature, Commun. 5: 5070. https://doi.org/10.1038/ncomms6070

Carta, A., Bedini, G. & Peruzzi, L. 2020. A deep dive into the ancestral chromosome number and genome size of flowering plants. New Phytol. 228: 1097–1106. https://doi.org/10.1111/nph.16668

Carvalho, C.R. & Saraiva, L.S. 1993. An air drying technique for maize chromosomes without enzymatic maceration. Biotechnol. Histochem. 68: 142–145. https://doi.org/10.3109/10520299309104684

Chase, M.W., Fay, M.F., Dewey, D.S., Maurin, O., Rønsted, N., Davies, J., Pillon, Y., Petersen, G., Seberg, O., Tamura, M.N., Asmussen, C.B., Hilu, K., Borsch, T., Davies, J.I., Stevenson, D.W., Pires, J.C., Givinish, T.J., Sytsma, K.J., McPherson, M.A., Graham, S.W. & Rai, H. S. 2006. Multigene analyses of monocot relationships: A summary. Aliso 22: 63–75.

Da Silva, C.R., Quintas, C.C. & Vanzela, A.L. 2010. Distribution of 45S and 5S rDNA sites in 23 species of Eleocharis (Cyperaceae). Genetica 138: 951–957. https://doi.org/10.1007/s10709-010-9477-5

Govaerts, R., Simpson, D.A., Goetghebeur, P., Wilson, K.L., Egorova, T. & Bruhl, J.J. 2020. World checklist of Cyperaceae. Richmond, U.K.: The Board of Trustees of the Royal Botanic Gardens, Kew. http://apps.kew.org/wcsp/ (accessed 8 Apr 2020).

Greilhuber, J. 1995. Chromosomes of the Monocotyledons (general aspects). Pp. 379 – 414 in: Rudall, P.J., Cribb, P.J., Cluter, D.F., Humpries, C.J. Monocotyledons: Systematics and evolution. Richmond, U.K.: Royal Botanic Gardens, Kew.

Hipp, A.L., Rothrock, P.E. & Roalson, E.H. 2009. The evolution of chromosome arrangements in Carex (Cyperaceae). Bot. Rev. (Lancaster) 75: 96–109. https://doi.org/10.1007/s12229-008-9022-8

Kubitzki K 1998. Thurniaceae. In: Kubitzki K (ed) The families and genera of vascular plants. Flowering plants – Monocotyledons. Alismatanae and Commelinanae. (except Gramineae). Pp. 455–457, *Springer*, Berlin.

Lipnerová, I., Bureš, P., Horová, L. & Šmarda, P. 2013. Evolution of genome size in Carex (Cyperaceae) in relation to chromosome number and genomic base composition. Ann. Bot. (Oxford) 111: 79–94. https://doi.org/10.1093/aob/mcs239

Márquez-Corro, J.I., Luceño, M., Jiménez-Mejías, P., Escudero, M., Martín-Bravo, S., Hipp, A.L., Chung, K.-S., Muasya, A.M., Rothrock, P.E., Weber, J.A. & Naczi, R. 2018. [Report]. In: Marhold, K. & Kučera, J. (eds.), IAPT chromosome data 28. Taxon 67: 1235–1245. https://doi.org/10.12705/676.39

Mucina, L. & Rutherford, M. C. (eds.) 2006. The vegetation of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute.

Ribeiro, T., Buddenhagen, C.E., Thomas, W.W., Souza, G. & Pedrosa-Harand, A. 2018. Are holocentrics doomed to change? Limited chromosome number variation in Rhynchospora Vahl (Cyperaceae). Protoplasma 255: 263–272. https://doi.org/10.1007/s00709-017-1154-4

Roalson, E.H. 2008. A synopsis of chromosome number variation in the Cyperaceae. Bot. Rev. (Lancaster) 74: 209–393. https://doi.org/10.1007/s12229-008-9011-y

Semmouri, I., Bauters, K., Léveillé-Bourret, É., Starr, J.R., Goetghebeur, P. & Larridon, I. 2019. Phylogeny and systematics of Cyperaceae, the evolution and importance of embryo morphology. Bot. Rev. (Lancaster) 85: 1–39. https://doi.org/10.1007/s12229-018-9202-0

Simpson, D.A. & Inglis, C.A. 2001. Cyperaceae of economic, ethnobotanical and horticultural importance: A checklist. Kew Bull. 56: 257–360. https://doi.org/10.2307/4110962

Sousa, A., Barros e Silva, A.E., Cuadrado, A., Loarce, Y., Alves, M.V. & Guerra, M. 2011. Distribution of 5S and 45S rDNA sites in plants with holokinetic chromosomes and the "chromosome field" hypothesis. Micron 42: 625–631. https://doi.org/10.1016/j.micron.2011.03.002

Souza, T.B., Chaluvadi, S.R., Johnen, L., Marques, A., González-Elizondo, M.S., Bennetzen, J.L. & Vanzela, A.L.L. 2018. Analysis of retrotransposon abundance, diversity and distribution in holocentric Eleocharis (Cyperaceae) genomes. Ann. Bot. (Oxford) 122: 279–290. https://doi.org/10.1093/aob/mcy066

Spalink, D., Drew, B.T., Pace, M.C., Zaborsky, J.G., Starr, J.R., Cameron, K.M., Givnish, T.J. & Sytsma, K.J. 2016. Biogeography of the cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. J. Biogeogr. 43: 1893–1904. https://doi.org/10.1111/jbi.12802

Vanzela, A.L.L., Luceño, M. & Guerra, M. 2000. Karyotype evolution and cytotaxonomy in Brazilian species of Rhynchospora Vahl (Cyperaceae). Bot. J. Linn. Soc. 134: 557–566. https://doi.org/10.1111/j.1095-8339.2000.tb00551.x

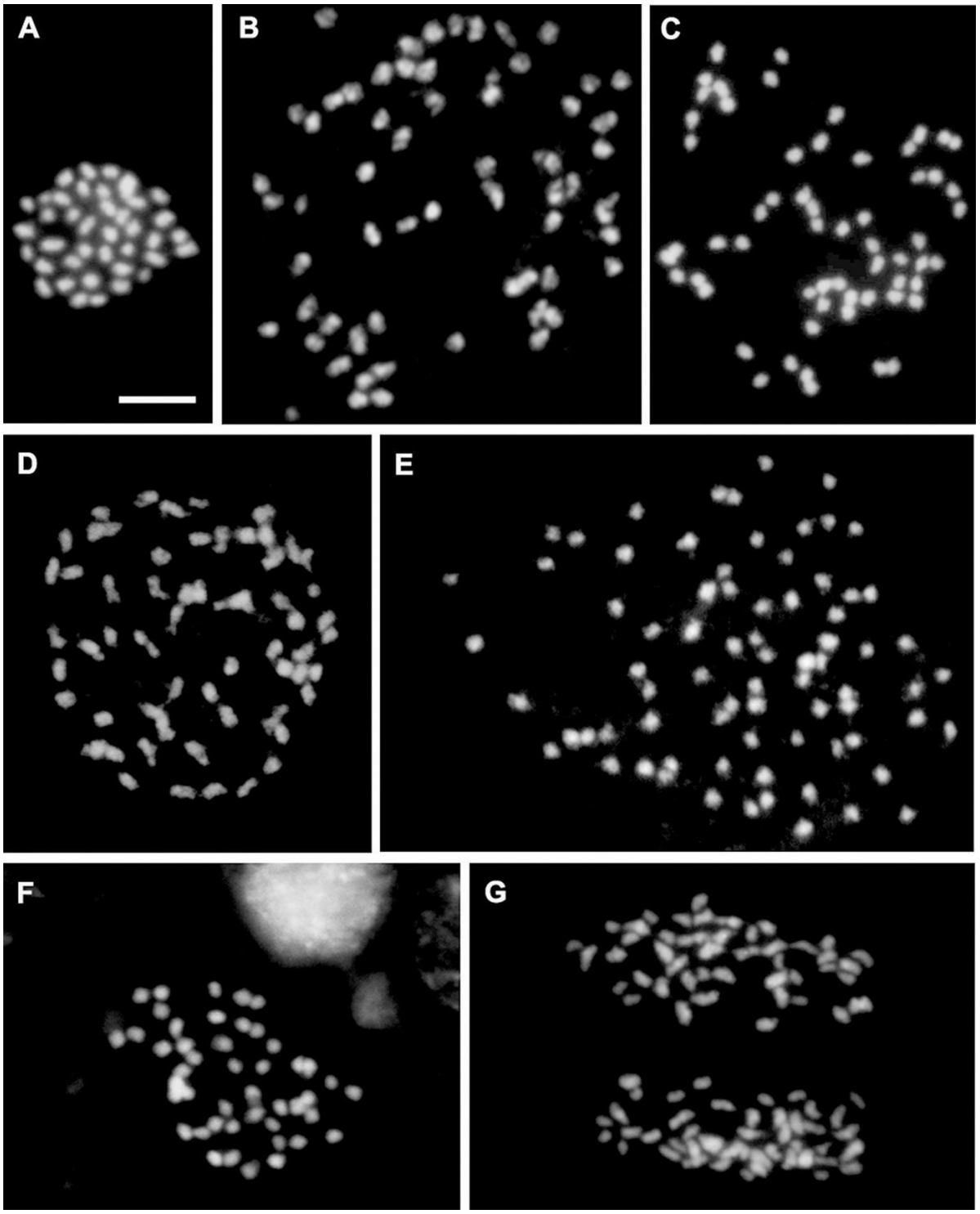


Fig. 1. Mitotic metaphase (A–F) and anaphase (G) chromosomes of: A, Becquerelia cymosa, 2n = 40; B, Diplasia karatifolia, 2n = 60; C, Hypolytrum schraderianum from Amazonas State, 2n = 60; D, Hypolytrum schraderianum from Bahia State, 2n = 60; E, Hypolytrum schraderianum from Paraná State, 2n = 76; F, Prionium serratum, 2n = 46; G, Thurnia sphaerocephala, 2n = 46. — Scale bar in A = 5 µm.