Universidade Federal de Pernambuco
Centro de Biociências
Programa de Pós-Graduação em Genética

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Análise molecular e citogenômica de elementos de transposição no grupo *Dichotomius* (*Luederwaldtinia*) *sericeus* (Coleoptera: Scarabaeidae)

Recife
2018

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Tese apresentada ao Programa de Pós-Graduação em Genética, Área de Concentração Ciências Biológicas, da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Genética.

Orientadora: Profª. Drª. Rita de Cássia de Moura
Coorientador: Dr. Gabriel da Luz Wallau

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RESUMO

Em *Dichotomius*, o mapeamento de sequências repetitivas de DNA revelou uma dinâmica da heterocromatina constitutiva (HC), em relação à composição e localização cromossômica das sequências presentes na HC. Os objetivos deste estudo foram isolar, caracterizar e mapear elementos de transposição (TE) no grupo *D.* (*L.*) *sericeus*, visando verificar o papel desses TEs e se eles estão relacionados com a variação na composição e localização das sequências da HC. Além disso, visamos compreender a história evolutiva desses elementos. O isolamento dos elementos, sequenciamento NGS, caracterização por diferentes abordagens e Hibridização *in situ* fluorescente foram realizados. Os resultados revelaram elementos com domínios conservados de 11 superfamílias, seis retrotransposons e cinco transposons de DNA. Os mais conservados foram: *Copia*, *Gypsy*, *PiggyBac* e *Tc1-Mariner*. Também foram identificados TEs degenerados. A análise filogenética dos elementos conservados sugeriu eventos de transferência horizontal para sete TEs de três superfamílias entre diferentes táxons, incluindo cnidários, platelmintos e nematelmintos. Adicionalmente, foi observada herança vertical em diferentes elementos. O mapeamento cromossômico populacional foi realizado para os TEs *DsTc1_5*, *DsPogo_8* e *DgmarMITE*. Variação na localização e abundância foram observados, sugerindo atividade desses elementos. Devido à natureza degenerada, o *DgmarMITE* possui mobilidade *in trans*, utilizando a transposase de elementos proximamente relacionados ou não. A predominância dos elementos encontrados na eucromatina sugere que eles podem estar inseridos em regiões intergênicas, em pseudogenes e/ou relacionados a modificações na estrutura e expressão dos genes. Além disso, esses elementos não estão relacionados diretamente com a variabilidade das sequências repetitivas da HC.

Palavras-chave: Retrotransposons. Transposons de DNA. Transferência horizontal. Filogenia molecular. Hibridização *in situ* fluorescente.

ABSTRACT

In *Dichotomius*, the mapping of repetitive DNA revealed a dynamics of constitutive heterochromatin (HC), in its composition and location of the sequences of the HC. The aims of this study were to isolate, characterize and map transposable elements (TE) in the group *D.* (*L.*) *sericeus*, to verify the role of these TEs and if they are related to the variation in composition and location of the sequences of the HC. In addition, to know the evolutionary history of these elements, the isolation of elements, NGS sequencing, characterization by different approaches and fluorescent in situ hybridization were performed. The results revealed elements with conserved domains from 11 superfamilies, six retrotransposons and five DNA transposons. The most conserved were: *Copia*, *Gypsy*, *PiggyBac* and *Tc1-Mariner*. Degenerated TEs were also identified. Phylogenetic analyse of conserved elements suggested horizontal transfer events, of seven TEs of three superfamilies between different taxa, including cnidarians, platyhelminth and nematelminth. In addition, vertical transmission was observed for different elements. The population chromosomal mapping was performed for the DsTc1_5, DsPogo_8 and *Dgmar*MITE. Variation in the location and abundance, suggesting activity of these elements was observed. Due to their degenerated nature, *Dgmar*MITE has cross-mobilization, using the transposase of elements closely related or not. The predominance of these elements in euchromatin suggests that they may be inserted in intergenic regions, in pseudogenes and/or related to modifications in the structure and expression of the genes. Furthermore, these elements are not directly related to the variability of the HC repetitive sequences.

Keywords: Retrotransposons. DNA transposons. Horizontal transfer. Molecular phylogenetic. Fluorescent *in situ* hybridization.

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Artigo 1

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

Item	Definição
ºC	Graus Celcius
2n	Número diploide
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
dATP	Desoxiadenosina trifosfato
DDE	Região conservada com Asparagina-Asparagina-Glutamina
DNA	Ácido desoxirribonucleico
DNA C_0t-1	DNA enriquecido com sequências altamente e moderadamente repetitivas
dUTP	Desoxiuracila-trifosfato
FACEPE	Fundação de Amparo à Ciência e Tecnologia de Pernambuco
FISH	Hibridização *in situ* Fluorescente
H3	Histona H3
HC	Heterocromatina constitutiva
HT	Transferência horizontal
LTR	*Long term repeat*
Mar	*Mariner*
MITEs	*Miniature Inverted Repeat Transposable Elements*
Min	Minuto
NCBI	*National Center for Biotechnology Information*
Pb	Pares de base
PCR	Reação em Cadeia da Polimerase
rDNA	DNA ribossomal
S	Segundo
SOAP	Short Oligonucleotide Analysis Package
TEs	Elementos de transposição
TIR	*Terminal inverted repeat*
TSD	*Target Site Duplication*

SUMÁRIO

1 INTRODUÇÃO

1.1 PROBLEMATIZAÇÃO

O gênero *Dichotomius* (Coleoptera: Scarabaeidae) possui aproximadamente 165 espécies, sendo algumas delas pertencentes a grupos de espécies morfologicamente semelhantes, como o grupo *Dichotomius* (*Luederwaldtinia*) *sericeus*. Esse grupo foi recentemente revisado através de caracteres taxonômicos, revelando a presença de oito espécies crípticas. Nesse gênero, análises de cariótipos de 18 espécies revelaram conservação da macroestrutura cromossômica, com número diploide $2n = 18,Xy_p$ e morfologia cromossômica meta-submetacêntrica. Além de distribuição da heterocromatina constitutiva (HC) nas regiões pericentroméricas, localização do rDNA 18S predominantemente no terceiro par cromossômico e colocalização do rDNA 5S e histona H3 no segundo par.

Apesar dessa conservação, os elementos de transposição (TEs) têm sido relacionados com variações na microestrutura cromossômica. Em *Dichotomius*, o mapeamento heterólogo da fração de DNA altamente e moderadamente repetitivo (DNA C_0t-1) revelou uma dinâmica da HC, em relação a sua composição e na localização das sequências que compõem essa HC. Essa dinâmica está possivelmente relacionada à presença e atividade de elementos de transposição, uma vez que a natureza repetitiva e a capacidade de mobilidade desses elementos permitem a remodelagem e evolução de genomas.

Em *Dichotomius*, apenas um transposon não autônomo da família *Mariner*, denominado *DsMar*MITE foi caracterizado. No entanto, o mapeamento cromossômico desse transposon revelou que o *DsMar*MITE está localizado nas regiões eucromáticas e que não apresenta relação direta com a dinâmica da HC. Portanto, outros elementos que ainda não foram caracterizados podem estar relacionados com a variação da HC, em relação a sua composição e na localização das sequências que compõem essa HC.

Considerando que no gênero *Dichotomius* os elementos de transposição podem estar relacionados com variações na microestrutura cromossômica, incluindo na dinâmica da HC, propusemos analisar elementos de transposição em espécies próximas deste gênero, em particular do grupo *D.* (*L.*) *sericeus*, com a finalidade de responder as seguintes questões: (1) Quais elementos de transposição estão

estruturalmente conservados no genoma de *D*. (*L*.) *schiffleri* e/ou nas espécies do grupo *D*. (*L*.) *sericeus*?; (2) Qual a história evolutiva desses elementos? Adicionalmente, no grupo *D*. (*L*.) *sericeus* foi realizado o mapeamento cromossômico populacional de elementos degenerados e conservados estruturalmente, visando observar se os TEs contribuem para variação da HC nos cariótipos desses organimos.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Caracterizar, mapear e inferir a história evolutiva de elementos de transposição em *Dichotomius* (*Luederwaldtinia*) *schiflleri* e espécies próximas do grupo *D.* (*L.*) *sericeus*, com a finalidade de revelar a diversidade desses elementos, verificar a distribuição cromossômica e acúmulo desses elementos na heterocromatina constitutiva.

1.2.2 OBJETIVOS ESPECÍFICOS

1. Caracterizar os elementos de transposição de *D.* (*L*.) *schiffleri*, visando identificar os elementos com domínios conservados e compreender as relações filogenéticas dos elementos presentes nesse genoma.
2. Isolar e caracterizar elementos de transposição nas espécies do grupo de *D.* (*L*.) *sericeus*, com a finalidade de analisar a variabilidade interespecífica e populacional desses elementos.
3. Realizar o mapeamento de elementos transponíveis em nível populacional de espécies do grupo *D.* (*L*) *sericeus*, para verificar a presença e acúmulo desses elementos em regiões da HC.

2 REVISÃO DE LITERATURA

2.1 CONSIDERAÇÕES GERAIS DO GÊNERO *Dichotomius* (COLEOPTERA: SCARABAEIDAE: SCARABAEINAE), COM ÊNFASE NO GRUPO *Dichotomius* (*L*.) *sericeus*

Scarabaeidae, uma das famílias mais representativas da superfamília Scarabaeiodea (Coleoptera), possui cerca de 2.000 gêneros e 27.800 espécies (COSTA, 2000; RATCLIFFE; JAMESON; SMITH, 2002). Dentre suas subfamílias, Scarabaeinae se destaca devido aos serviços ambientais prestados, como a reciclagem da matéria orgânica e o controle biológico de pragas agrícolas (FLECHTMANN; RODRIGUES; SENO, 1995; NICHOLS et al., 2008). Essa subfamília apresenta cerca de 6.000 espécies, distribuídas em 200 gêneros, incluindo *Dichotomius* (HALFFTER, 1991).

O gênero *Dichotomius* Hope, 1838 pertence à tribo Coprini e é endêmico do continente Americano, com distribuição do Noroeste dos Estados Unidos à Argentina Central (MONTREUIL, 1998; VAZ-DE-MELLO et al., 2011). Esse gênero possui 165 espécies (KORASAKI et al., 2012), das quais aproximadamente 100 ocorrem no Brasil e apenas oito no nordeste brasileiro (VAZ-DE-MELLO, 2017). Esse gênero é dividido em quatro subgêneros: *Selenocopris*, *Homocanthonides*, *Dichotomius s. str*. e *Luederwaldtinia*. Dentre esses subgêneros, *Luederwaldtinia* se destaca por apresentar o maior número de espécies no Brasil (VAZ-DE-MELLO, 2017) e possuir grupos de espécies morfologicamente semelhantes, incluindo *lucasi*, *batesi* (SARMIENTO-GARCÊS; AMAT-GARCÍA, 2009) e *Dichotomius* (*L*.) *sericeus* (VAZ-DE-MELLO; LOUZADA; GAVINO, 2001).

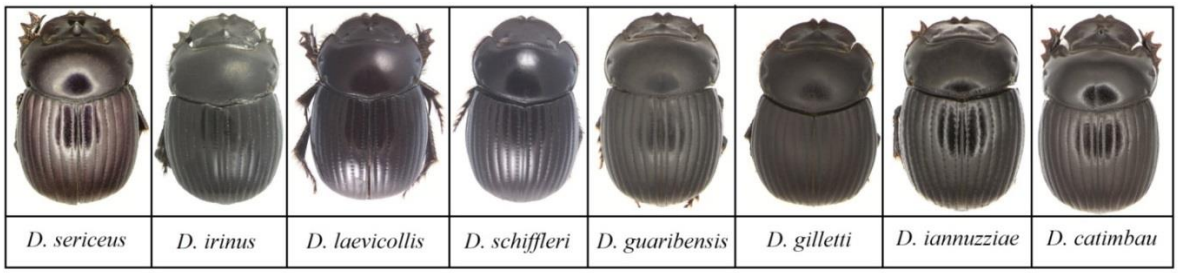
O grupo *Dichotomius* (*L*.) *sericeus* formado anteriormente por cinco espécies (*D. sericeus*, *D. irinus*, *D. laevicollis*, *D.* var. *aterrimus* e *D. schiffleri*), foi revisado recentemente por Valois *et al*. (2017) por meio de caracteres morfológicos. Neste estudo, *D.* var. *aterrimus* foi sinonimizado com *D. sericeus* e foram descritas quatro novas espécies: *D. catibaus*, *D. gilletti*, *D. guaribensis* e *D. iannuzziae* (Figura 1) (VALOIS; VAZ-DE-MELLO; SILVA, 2017).

Em relação à distribuição geográfica, as espécies do grupo *D.* (*L*.) *sericeus* são frequentemente encontradas em áreas de restinga e de Mata Atlântica ao longo da costa brasileira, embora também ocorram nas serras do Sudeste e do Sul, e na Mata

Atlântica do Paraguai e Argentina (SILVA et al., 2010; VALOIS; VAZ-DE-MELLO; SILVA, 2017). As espécies deste grupo, a exemplo de *D.* (*L.*) *schiffleri* e *D.* (*L.*) *laevicollis*, têm sido muitas vezes relacionadas à indicação de distúrbio ambiental, uma vez que a sua abundância diminui drasticamente no ecossistema com o aumento da antropização e perda de habitat (VIEIRA; LOUZADA; SPECTOR, 2008).

A espécie *D.* (*L.*) *schiffleri* Vaz-de-Mello, Louzada & Gavino, 2001 é endêmica dos ecossistemas costeiros brasileiros, com ocorrência principalmente em áreas de restinga dos estados do Espírito Santo, Bahia e Pernambuco (VIEIRA et al., 2011). Essas áreas têm sofrido uma intensa degradação devido à invasão de espécies exóticas e a expansão urbana e da agricultura nos últimos 50 anos (SCHERER; MARASCHIN-SILVA; BAPTISTA, 2005). A perda desse habitat afeta as espécies sensíveis a mudanças ambientais, como *D.* (*L.*) *schiffleri* (COSTA; BARRETTO; MOURA, 2014; VIEIRA; LOUZADA; SPECTOR, 2008) que é uma das seis espécies de Scarabaeidae ameaçadas de extinção (BRASIL, 2014).

Figura 1- Espécies do grupo *Dichotomius (L.) sericeus.* Fonte: Valois et al. 2017.



2.2 CITOGENÉTICA DO GÊNERO *Dichotomius* (COLEOPTERA: SCARABAEIDAE: SCARABAEINAE)

No gênero *Dichotomius*, as análises de citogenética clássica foram realizadas em apenas 11% das espécies descritas (CABRAL-DE-MELLO et al., 2011a; XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Esses estudos revelaram a predominância do cariótipo $2n$=18,Xy_p e morfologia cromossômica meta-submetacêntrica (Figura 2, Tabela 1). Este cariótipo é considerado derivado, resultante provavelmente de dois rearranjos cromossômicos, inversão pericêntrica e fusão cêntrica entre dois autossomos, ocasionando a redução do número diploide $2n$=20, considerado plesiomórfico para Coleoptera (CABRAL-DE-MELLO et al., 2008, 2011a; XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Nesse gênero, a configuração do bivalente

sexual na meiose do tipo Xy_p (y em forma de paraquedas) foi observada em todas as espécies analisadas, exceto *D. schiffleri* que apresentou a configuração Xy_r (y em forma de bastão) (Figura 3) (XAVIER; CABRAL-DE-MELLO; MOURA, 2014).

Figura 2 - Padrão da localização da Heterocromatina Constitutiva no gênero *Dichotomius.* Cariótipo C – bandeado em *D. iannuzziae* (= *D. laevicollis*). Fonte: Cabral-de-Mello et al. 2011.

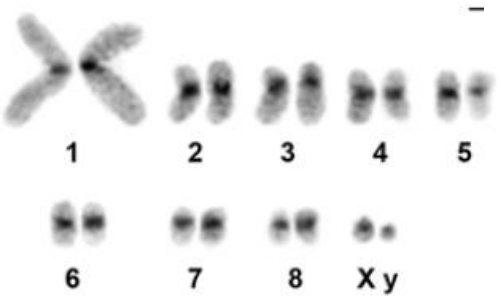
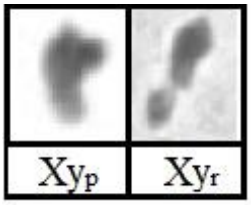


Figura 3

Figura 3 - Configuração em paraquedas (p) e em bastão (r) do bivalente sexual na meiose. Fonte: Cabral-de-Mello et al. 2010 e Xavier et al. 2014.



Quanto à heterocromatina constitutiva (HC), a análise realizada em seis espécies, revelou a sua localizada nas regiões pericentroméricas (Figura 2) e a riqueza em pares de bases GC ou neutros, nesse último caso não mostra riqueza especifica para os pares GC e AT (Tabela 1) (CABRAL-DE-MELLO et al., 2011b). Em relação à citogenética molecular, o mapeamento das famílias multigênicas rDNA e histona H3 foram realizados em 15 e nove espécies, respectivamente (CABRAL-DE-MELLO et al., 2011a). Esse estudo demonstrou uma conservação na localização dos sítios de rDNA 18S no terceiro par autossômico (apesar de ser observado também nos bivalente dois, quatro e sexuais) e a colocalização dos sítios de rDNA 5S e histona H3 na região pericentromérica do segundo par cromossômico em todas as espécies estudadas (Tabela 1).

O mapeamento da fração de DNA altamente e moderadamente repetitivo (DNA C_0t-1), realizada em sete espécies, revelou sua localização na região pericentromérica, coincidente com o padrão de bandeamento C (Tabela 1) (CABRAL-

DE-MELLO et al., 2011b; XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Entretanto, a hibridização heteróloga do DNA C_0t-1 de *D. geminatus* em cinco espécies: *D. bos*, *D. nisus*, *D. semisquamosus*, *D. laevicollis* e *D. sericeus*, evidenciou sinais teloméricos e subteloméricos. Estes resultados indicam uma dinâmica da HC no gênero *Dichotomius*, em relação a sua composição e na localização das sequências altamente e moderadamente repetitivas presentes nessa HC. Essa dinâmica pode estar relacionada à presença e movimentação de elementos de transposição (CABRAL-DE-MELLO et al., 2011b).

Tabela 1- Resultados da citogenética classica e molecular nas espécies do gênero *Dichotomius*.

Espécie	2n ♂	Banda C	Fluorocromo	45S	5S	H3	DNA C_0t-1
D. affinis	18, Xy_p			3	2		
D. bicuspi	18, Xy_p						
D. bos	18, Xy_p	Peric.	CMA^{3+}/Neutros	3	2	2	Peric.
D. bosqui	20, Xy_p						
D. carolinus	20, Xy_p						
D. crinicollis	18, Xy_p	Peric.	CMA^{3+}/Neutros	3, X	2	2	
D. depresicollis	18, Xy_p	Peric.	CMA^{3+}/Neutros	3	2	2	
D. geminatus	18, Xy_p	Peric.	CMA^{3+}/Neutros	3/4	2	2	Peric.
D. laevicollis	18, Xy_p	Peric.	CMA^{3+}/Neutros	3	2	2	Peric.
D. mormon	18, Xy_p			3/4, X	2		
D. aff. *mundus*	18, Xy_p			3	2		
D. nisus	18, Xy_p	Peric.	CMA^{3+}/Neutros	X/Y	2	2	Peric./Terminal
D. schiffleri	18, Xy_r	Peric.	CMA^{3+}/Neutros	3	2	2	Peric.
D. semiaeneus	18, Xy_p			X	2		
D. semisquamosus	18, Xy_p	Peric.	CMA^{3+}/Neutros	3, X	2	2	Peric./Terminal
D. sericeus	18, Xy_p	Peric.	CMA^{3+}/Neutros	2	2	2	Peric./Terminal
D. prox. sericeus	18, Xy_p			3	2		
D. sp	18, Xy_p			3	2		

Peric. = Pericentromerica. Fonte: Smith e Virkki 1978; Vidal 1984; Cabral-de-Mello et al., 2008; Cabral-de-Mello et al., 2011a,b,c; Xavier et al., 2014.

2.3 ASPECTOS GERAIS SOBRE ELEMENTOS DE TRANSPOSIÇÃO, COM ÊNFASE NA OCORRÊNCIA EM COLEOPTERA

Os elementos de transposição (TEs) são sequências repetitivas e dispersas de DNA, que possuem a capacidade de se moverem no genoma do hospedeiro (HARTL; LOZOVSKAYA; LAWRENCE, 1992). Devido a essa mobilidade, os TEs estão associados a diferentes efeitos, como mutações genéticas, regulação de genes

adjacentes, variação no tamanho genômico, rearranjos cromossômicos e manutenção dos telômeros (FUJIWARA et al., 2005; MAUMUS; FISTON-LAVIER; QUESNEVILLE, 2015).

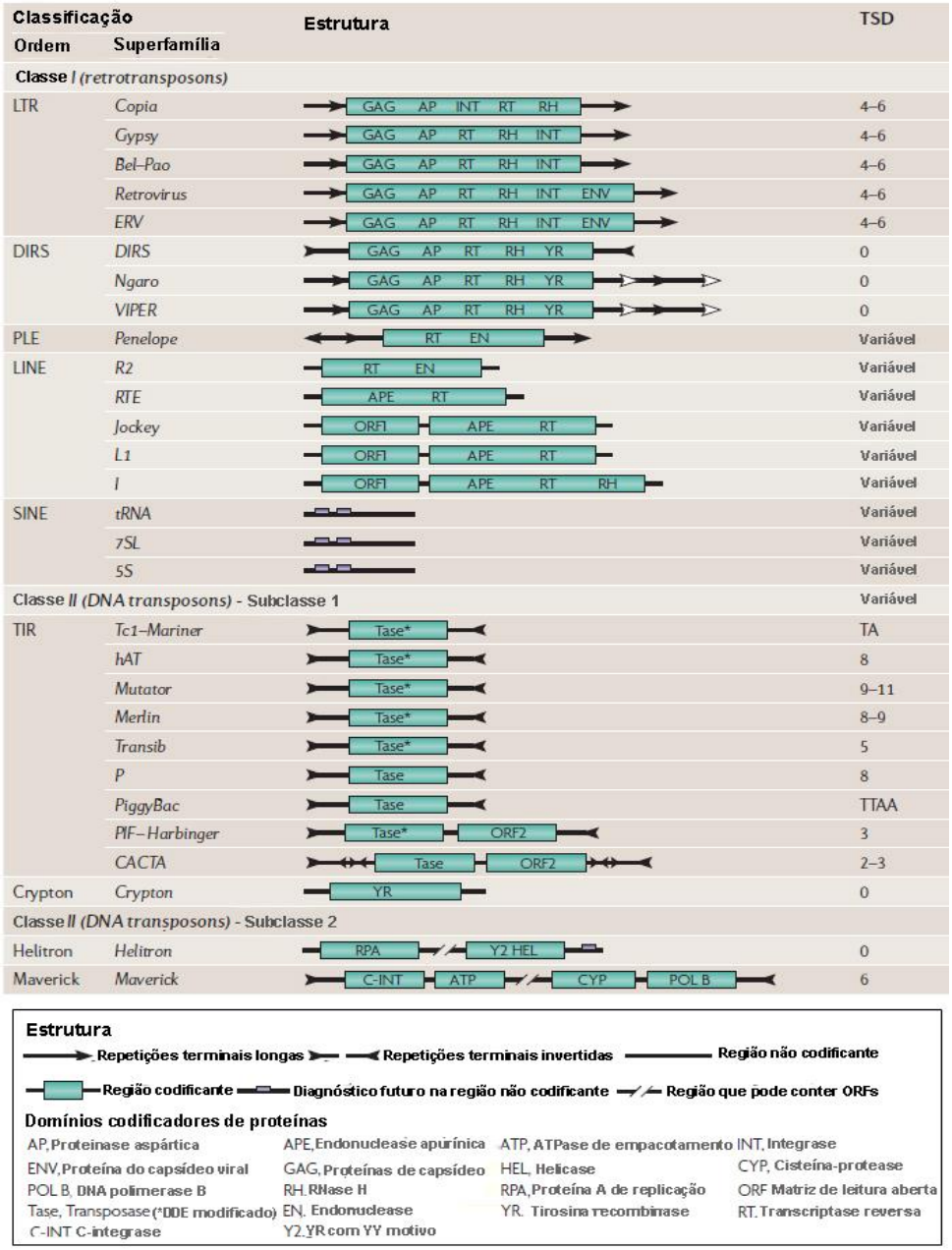
Com base no mecanismo de transposição, esses elementos são classificados em dois grupos, os de Classe I (retrotransposons) e Classe II (transposons de DNA) (WICKER et al., 2007). Nos retrotransposons, os elementos são inicialmente transcritos formando um RNA intermediário. Esse RNA serve de molde para uma nova molécula de DNA, através da transcriptase reversa, que é posteriormente inserido no sítio receptor. Devido ao fato do elemento original ser duplicado, esse mecanismo é conhecido como replicativo ou “copia e cola” (FESCHOTTE; PRITHAM, 2007; KIDWELL, 2005).

Nos transposons de DNA, os elementos se movem diretamente, sem a necessidade da formação de um RNA intermediário. Esses elementos podem ser classificados em duas subclasses. Na subclasse I, as duas cadeias do elemento são excisadas, transpostas e inseridas no sítio receptor, pela transposase (WICKER et al., 2007). Durante essa transposição, geralmente não há o aumento do número de cópias do elemento, sendo por isso, conhecido como conservativo ou “recorta e cola” (KIDWELL, 2005) Apesar disso, esses elementos podem aumentar o número de cópias pelo sistema de reparo de recombinação homologa do elemento transposto, através da transposição dos TEs durante a divisão celular para uma região não duplicada e pela recombinação ectópica (Kidwell, 2005). Na subclasse II, a única fita de DNA excisada serve de molde para uma nova cópia do elemento, em um mecanismo de replicação em círculo rolante. Nesse processo, o elemento original é duplicado, sendo por isso classificado como “copia e cola” (Figura 4) (WICKER et al., 2007).

Adicionalmente, os elementos de transposição podem ser classificados hierarquicamente em subclasse, ordem, superfamília, família e subfamília, com base em alguns critérios, como: composição gênica, estrutura das proteínas, comparação dos domínios codificantes e não codificantes e a presença e tamanho da duplicação do sítio alvo do elemento (TSD do inglês *Target Site Duplication*) (Figura 4) (WICKER et al., 2007). Considerando esses critérios, os retrotransposons são subdivididos, geralmente, em cinco ordens e 17 superfamílias e os transposons de DNA, em quatro ordens e 12 superfamílias (Figura 4) (WICKER et al., 2007). Apesar dessa

classificação, outras superfamílias são frequentemente descritas, como *PHIS*, *Transib* e *Kolobock* nos transposons de DNA (HAN et al., 2015; YUAN; WESSLER, 2011).

Figura 4- Classificação dos elementos de transposição. Fonte: Wicker et al (2007) modificado.



Os elementos podem ainda ser classificados de acordo com a sua autossuficiência funcional, como autônomos ou não autônomos. Os elementos autônomos podem codificar a maquinaria enzimática necessária para a sua mobilidade. Enquanto os não autônomos não codificam a maquinaria, necessitando assim da maquinaria dos elementos autônomos para a sua transposição, num processo de mobilização *in trans* (KIDWELL, 2005). Esses elementos não autônomos, como por ex. os MITEs (*Miniature Inverted Repeat Transposable Elements*),

geralmente, se originam pela degeneração molecular dos TEs autônomos (KIDWELL, 2005).

No genoma do hospedeiro, os elementos de transposição apresentam um “ciclo de vida” dividido em três fases: (1) invasão do genoma seguida da multiplicação do número de cópias; (2) mutação e inativação de algumas cópias, e (3) perda quantitativa dos elementos, que pode durar milhões de anos. Contudo, a eliminação completa pode ser evitada pela transferência horizontal desses elementos (KIDWELL; LISCH, 2001).

A transferência horizontal, diferente da herança vertical, se caracteriza pela passagem de material genético independente da reprodução (BROWN, 2003; WALLAU; ORTIZ; LORETO, 2012). Essa transferência, que pode ocorrer entre espécies filogeneticamente próximas ou não, é responsável pela ampla distribuição dos TEs em diferentes táxons (ROBERTSON; LAMPE, 1995a; WALLAU; ORTIZ; LORETO, 2012). Os elementos de transposição são ubíquos (Wicker, 2007), sendo frequentemente descritos em bactéria, protistas (SILVA et al., 2005), fungos (KOJIMA; JURKA, 2011), plantas (GRZEBELUS et al., 2007) invertebrados (MONTIEL et al., 2012) e vertebrados (CHALOPIN et al., 2015).

Em Coleoptera, os elementos de transposição foram analisados em aproximadamente 25 espécies pertencentes a 12 famílias (CUNNINGHAM et al., 2015; DJEBBI et al., 2017; HERNANDEZ-HERNANDEZ et al., 2017; JAKUBCZAK; BURKE; EICKBUSH, 1991; LAMPE et al., 2003; OLIVEIRA et al., 2013; PAUCHET; HECKEL, 2013; RIVERA-VEGA; MITTAPALLI, 2010; ROBERTSON et al., 2002; ROBERTSON; MACLEOD, 1993). Dessas espécies, a anotação genômica de TEs foi realizada apenas seis espécies: *Tribolium castaneum* (Tenebrionidae) (RICHARDS et al., 2008), *Phaedon cochleariae* (Chrysomelidae) (PAUCHET; HECKEL, 2013), *Dendroctonus ponderosae* (Curculionidae) (KEELING et al., 2013), *Nicrophorus vespilloides* (Silphidae) (CUNNINGHAM et al., 2015), *Hypothenemus hampei* (Curculionidae) (HERNANDEZ-HERNANDEZ et al., 2017) e *Onthophagus taurus* (Scarabaeidae) (CHOI et al., 2010). Essa anotação revelou uma predominância, geralmente, dos retrotransposons e que os TEs representam menos de 10% do genoma dessas espécies.

Na ordem Coleoptera, o mapeamento cromossômico de TEs foi realizado em apenas quatro espécies, todas da subfamília Scarabaeinae (OLIVEIRA et al., 2013;

XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Em *Coprophanaeus cyanescens*, foi mapeado um retrotransposon não-LTR do tipo *LOA*-like (223 pb), que apresentou localização predominante na heterocromatina (OLIVEIRA; MOURA; MARTINS, 2012). Adicionalmente, foram descritos e mapeados elementos da família *Mariner* em duas espécies do gênero *Coprophanaeus*, uma de *Diabroctis* (OLIVEIRA et al., 2013) e uma de *Dichotomius* (XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Em *C. ensifer*, *C. cyanescens* e *D. mimas*, o mapeamento do domínio parcial de DDE (região conservada da transposase com três aminoácidos: Asparagina-Asparagina-Glutamina) revelou a localização desses TEs nas regiões heterocromáticas dos cromossomos (OLIVEIRA et al., 2013).

Em *Dichotomius* apenas um elemento pertencente à família *Mariner* foi identificado e mapeado, o elemento denominado *DsMar*MITE. Esse elemento apresentou características de MITE, como repetições terminais invertidas perfeitas (TIRs com 21 bp), ausência de um gene codificante da transposase, uma alta riqueza de A-T (65.9%) e 267 pares de bases de tamanho. O mapeamento desse elemento revelou a localização nas regiões eucromáticas dos cromossomos, indicando que o *DsMar*MITE não está relacionado com a dinâmica de sequências da heterocromatina constitutiva no gênero *Dichotomius* (XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Dessa forma, a relação dos TEs com variações na microestrutura cromossômica do gênero *Dichotomius*, incluindo na dinâmica da HC permanece desconhecida.

Considerando a relação dos TEs na reorganização cromossômica de diferentes espécies e a frequente presença de TEs na HC de espécies de Scarabaeinae, a caracterização e mapeamento de novos elementos de tranposição no gênero *Dichotomius* poderão contribuir no entendimento da dinâmica da HC.

3 RESULTADOS

3.1 ARTIGO 1 - GENOME-WIDE CHARACTERIZATION OF TRANSPOSABLE ELEMENTS IN *Dichotomius* (*Luederwaldtinia*) *Schiffleri* (COLEOPTERA: SCARABAEIDAE): EVIDENCE OF MULTIPLE HORIZONTAL TRANSFER EVENTS

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ABSTRACT

Transposable elements (TEs) are dispersed repetitive DNA sequences found in the vast majority of organisms studied so far. TEs activity has shaped the genome of many different species. In the genus *Dichotomius* (Coleoptera) TEs have been suggested to be associated with the dispersion and dynamics of other repetitive sequences but limited molecular data for TEs exists so far. Therefore, the objective of this study was to characterize the TEs of *Dichotomius* (*Luederwaldtinia*) *schiffleri* genome to identify structurally conserved elements. Two approaches were used for characterization of the elements: RepeatExplorer for *de novo* characterization and homology search of TE databases. Phylogenetic trees of each conserved TE superfamily were constructed using the maximum likelihood method and included homologous sequences identified in NCBI and Repbase. The mobilome of *D.* (*L.*) *schiffleri* possesses elements with conserved domains that could be classified into six retrotransposon, and five DNA transposon superfamilies. The structurally conserved elements mainly belong to the *Copia*, *Gypsy*, *Tc1-Mariner* and *PiggyBac* superfamilies. Phylogenetic reconstruction revealed incongruences between the phylogeny of the elements and that of the host, except for the *Maverick* element. The characterization of *D.* (*L.*) *schiffleri* elements and phylogenetic analyses allowed the identification of putative active elements and potential horizontal transfer events between highly divergent taxa.

Keywords: DNA Transposons; Retrotransposons; Mobilome; Dung beetle.

INTRODUCTION

Transposable elements (TEs) are highly repetitive and dispersed DNA segments that are capable of moving among different regions of the host genome (Hartl et al. 1992). Because of this mobility, these elements are associated with different effects in the genome, such as direct genetic mutations, regulation of adjacent genes, chromosome rearrangements, and variation in genome size (Maumus et al. 2015). The presence of TEs in the most diverse host genomes can be explained by the process of vertical inheritance, i.e., the passage of genetic material from parents to their offspring, or by horizontal transfer, a phenomenon characterized by the movement of genetic material between organisms independent of reproduction (Robertson and Lampe 1995a; Brown 2003; Wallau et al. 2012).

Transposable elements can be classified into two major groups: Class I elements (retrotransposons) require the formation of an RNA intermediate for their transposition, and Class II elements (transposons) move directly as DNA (Finnegan 1992; Wicker et al. 2007). In addition, TEs can be divided hierarchically into subclasses, orders, superfamilies and families based on gene composition, protein structure, comparison of coding and non-coding domains, and presence and size of target site duplication (Wicker et al. 2007). Transposable elements can also be classified according to their functional self-sufficiency as autonomous and non-autonomous. Autonomous elements encode all of the machinery necessary for their transposition, whereas non-autonomous elements such as Miniature Inverted-repeat Transposable Elements (MITEs) require proteins from other elements to perform transposition (Kidwell 2005).

The presence of TEs has been described in different taxonomic groups (Elliott and Gregory 2015), and can be determined by different hybridization molecular techniques as Southern blot, chromosome mapping and by direct DNA sequencing of an element (Kidwell 2005; Lerat 2010). Previous studies investigated one or few families of TEs per species, due to

the low throughput of the techniques available at the time, however the huge development of new sequencing technologies currently allow one to have a clear idea of the entire genome of a given species including the entire mobilome (all TEs contained on it). The large amount of sequencing data available now demanded the development of several bioinformatics tools for identification and annotation of TEs in host genomes. Although several software specifically designated to mobilome characterization, exists no clear benchmarking was properly conducted to evaluate their straighten and weakness. Hence the use of complementary tools and approaches has been recommended for better characterization of the TEs repertoire of a given species (Lerat 2010).

In Coleoptera, genome annotation allowed the identification of different TEs superfamilies in *Tribolium castaneum* (Tenebrionidae) (Richards et al. 2008) (TEs constituting approximately 6% of the genome), *Onthophagus taurus* (Scarabaeidae) (1,09% of the genome) (Choi et al. 2010), *Dendroctonus ponderosae* (Curculionidae) (Keeling et al. 2013) (0,7% of portion of total length), *Nicrophorus vespilloides* (Silphidae) (Cunningham et al. 2015) (3,97% of the genome), and *Hypothenemus hampei* (Curculionidae) (8,2% of the genome) (Hernandez-Hernandez et al. 2017).

Moreover, different studies have verified the presence of elements by direct sequencing of one or few TEs families, corresponding to approximately 20 coleopteran species analyzed (Robertson and MacLeod 1993; Robertson and Lampe 1995b; Beeman et al. 1996; Braquart et al. 1999; Robertson et al. 2002; Rivera-Vega and Mittapalli 2010; Yocum et al. 2011; Oliveira et al. 2013; Xavier et al. 2014; Djebbi et al. 2017).

In the *Dichotomius* (Scarabaeidae) genus, TEs were associated with the dispersion of 18S rDNA genes and with the dynamics of moderately and highly repetitive sequences present in constitutive heterochromatin (Cabral-de-Mello et al. 2011a, b, c). Within this genus, only non-autonomous elements of the *Mariner* family have been isolated and characterized in two

species: *Dichotomius* (*Luederwaldtinia*) *schiffleri* Vaz-de-Mello, Louzada & Gavino, 2001 (Xavier et al. 2014) e *D.* (*L.*) *gilletti* Valois, Vaz-de-Mello & Silva, 2017 (Amorim et al., *in press*). Chromosome mapping of these elements revealed possible cross-mobilization events and an euchromatic and heterochromatic distribution. Furthermore, these elements are not specifically correlated with the dynamics of other repetitive sequences (Xavier et al. 2014; Amorim et al. *in press*).

The aim of this study was to perform a genome-wide TEs characterization in *Dichotomius* (*L.*) *schiffleri* focusing on the identification of potentially active TE families. Additionally, we performed phylogenetic reconstruction of the TEs recovered in order to investigate their evolutionary history. *D.* (L.) *schiffleri* mobilome data will guide future chromosome mapping studies aimed at understanding the role of these elements in the dispersion and dynamics of other repetitive DNA sequences at the populational level in this species and in other species from this genus.

MATERIALS AND METHODS

Sampling and DNA extraction

Specimens of *D.* (*L.*) *schiffleri* were collected in Maracaípe (8°31’26” S, 35°1’31” W), Pernambuco state, Brazil. The sampling was authorized by IBAMA/SISBIO, which issued a permanent license (16278-1) for the collection of zoological material of the orders Coleoptera, Orthoptera and Hemiptera and a specific license for *D.* (*L.*) *schiffleri* (50438-1). DNA was extracted from the pronotal tissue of one specimen according to the protocol of Sambrook and Russel (2001).

Sequencing and characterization of transposable elements of *Dichotomius* (*L.*) *schiffleri*

Partial genome of *D.* (*L.*) *schiffleri* was sequenced on a Solexa-Illumina HiSeq 2000 platform using a paired-end approach with an average read length of approximately 100 bp. Sequences with a quality score less than Q20 were removed from the analysis using Trimmomatic v. 0.36. The elements were characterized using two approaches, a *de novo* and homology approaches. In the first, raw read sequences were first clustered and identified in RepeatExplorer (http://repeatexplorer.umbr.cas.cz/) and the resulting clusters were reassembled usingCAP3 Sequence Assembly Program (http://doua.prabi.fr/software /cap3) to obtain final consensus sequence.

In the second approach, the genome was assembled with the SOAP program (Oligonucleotide Analysis Package) using the parameters that exhibited the best contiguity (k-mers of 75 bp and un-mask, resolve repeats and fill gaps parameters). This assembly was used for the annotation of sequences larger than 150 bp with the RepeatMasker v. 4.0.7 (http://www.repeatmasker.org/) using parameter -s (slow search) and the option of leaving low-complexity or single repeats unmasked. RMBlast tool of search and alignment were used for this analysis, as well as the Repbase library of repetitive elements employing Repbase Update REPET edition 20.05 (Flutre et al. 2011) since its classification is similar to Wicker et al. (2007).

All copies were extracted with “One code to find them all” tool (Bailly-Bechet et al. 2014) to obtain better reconstruction of the elements. The fragments were filtered according to their coverage relative to the reference element, maintaining only TEs with a coverage higher than 40%. Additionally, consensus sequences of the elements were obtained by clustering all copies using the CD-HIT-EST algorithm (Li and Godzik 2006) with thresholds of 80% identity and 80% coverage.

The sequences obtained with the two approaches, that showed similarity to already described elements, were analyzed in ORFfinder (https://www.ncbi.nlm.nih.gov/ orffinder/) to

identify open reading frames (ORFs). Based on these ORFs, the presence of conserved domains was verified in the Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Phylogenetic analysis of transposable elements

Phylogenetic analysis was performed for each superfamily of identified elements. Protein sequences larger than 100 amino acids were used for these analyses. In addition, the sequences of other species obtained with the Blastp tool (default parameters) of the NCBI platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Censor tool of Repbase (http://www.girinst.org/censor/index.php) were used. The sequences were aligned using MAFFT on a web server (http://mafft.cbrc.jp/alignment/server/) and edited manually with the Aliview software to remove redundant sequences derived from different copies of the same elements and non-informative sites.

Phylogenetic trees were reconstructed by the maximum likelihood method using the PhyML 3.0 server (http://www.atgc-montpellier.fr/phyml/). The evolutionary model for each tree was chosen on this server considering Akaike's information criterion. Branch support was evaluated by aLRT statistics (Guindon et al. 2010) and the trees were visualized and personalized in Figtree v. 1.4.0. Outgroups were determined based on the superfamilies of the closest elements. However, midpoint rooting was used when the sequence of a close element was not satisfactory.

RESULTS

Transposable elements of *Dichotomius (L.) schiffleri*

RepeatExplorer results indicated that TEs accounted for 9.45% of the partial genome of *D.* (*L.*) *schiffleri*, with 6.2% retrotransposons and 3.25% DNA transposons. Comparison of the

methods showed the presence of different elements with conserved domains, identified by either approach but never by both (Table 1). These elements with conserved domains belong to six superfamilies of retrotransposons (*Gypsy*, *Copia*, *I*, *Jockey*, *Penelope*, and *RTE*) and five superfamilies of DNA transposons (*Tc1-Mariner*, *hAT*, *PiggyBac*, *PHIS*, and *Maverick*). The *Tc1-Mariner* (18 TEs), *Gypsy* (11 TEs) and *PiggyBac* (7 TEs) superfamilies were the most representative (Table 1).

De novo characterization revealed approximately 50 elements highly degenerated, exhibiting no characteristic domain and low identity to described elements. Therefore, it was not possible to infer their accurate classification. Additionally, the homology approach identified elements without conserved domains of the *hAT* (8 TEs), *Tc1-Mariner* (6 TEs), *Gypsy* (5 TEs), *Kolobok* (5 TEs) and *Transib* (1 TE) superfamilies, as well as 10 short interspersed nuclear element (SINEs) (Supplementary Table 1).

Considering both approaches, different partial or intact domains were identified. Reverse transcriptase and DDE (asparagine-asparagine-glutamine) domains were the most common in retrotransposons and DNA transposons, respectively (Table 1). In retrotransposons, different intact domains were observed for *Copia* and *Gypsy* superfamilies, such as reverse transcriptase, integrase and RNase H, in addition to the *gag* gene in *Copia* elements (Table 1). Different *Tc1-Mariner* and *PiggyBac* elements with intact domains were identified amongst DNA transposons, as well as ORFs larger than 350 amino acids (Table 1). However, we found no terminal inverted repeats.

TE Superfamilies phylogenetic trees

We were able to reconstruct eight superfamilies trees out of 11 superfamilies characterized. *D.* (*L.*) *schiffleri* TEs showed low identity with elements of other species and sometimes long branches, particularly in *Penelope* and LINE I trees, indicating the

accumulation of several amino acid differences in comparison with previous characterized TEs from other species (Supplementary Fig. 1-7). However, three trees from *PiggyBac*, *Pogo* (which belongs to the *Tc1-Mariner* superfamily) and *ISL2EU* (*PHIS* superfamily) showed major incongruence compared with the host tree. Besides that, elements of these superfamilies showed high identity with elements of phylogenetically distant species (Fig 1, 2 and 3; Supplementary Table 2).

In *PiggyBac* superfamily tree, *D.* (*L.*) *schiffleri elements* were distributed among six different clades, including two elements in the first clade (clade I) and one element in each of the remaining clades (Fig. 1, clades II, III, IV, V and VI). Elements isolated from Hymenoptera predominated in this phylogeny, including clades V and VI which contained TEs exclusively found in this order clustered with *D.* (*L.*) *schiffleri* elements (Fig. 1). The presence of elements isolated from other species from the Coleoptera order was only observed in clades I and IV, with amino acid identity of 74% to *Anoplophora glabripennis* and 68% to *Aethina tumida* elements (Fig. 1). In clades I, III and IV, the *D.* (*L.*) *schiffleri* elements formed groups with those of the platyhelminth *Schmidtea mediterranea*, with identity ranging from 73% to 98% (Fig. 1).

Pogo phylogenetic tree showed elements of *D.* (*L.*) *schiffleri* distributed in seven clades, with two elements in the fourth clade (Fig. 2 clade IV) and one element in each of the remaining clades (I, II, III and V). Elements isolated from chordates predominated in clades I, II and VI, with identity values to *D.* (*L.*) *schiffleri* TEs ranging from 59% to 76% (Fig. 2). In clades IV and VII, the *D.* (*L.*) *schiffleri* TEs were grouped with elements exclusively found in the orders Diptera and Hymenoptera, respectively (Fig. 2). Additionally, elements of the platyhelminth *S. mediterranea* (75% identity), the nematode *Trichuris suis* (79% identity) and the cnidarian *Hydra vulgaris* (81% identity) were observed in clades I, II and V, respectively (Fig. 2).

Regarding the phylogeny of *ISL2EU*, the element isolated from *D.* (*L.*) *schiffleri* formed an exclusive group with TEs from insects of the family Formicidae (Hymenoptera) (Fig. 3). When compared to *D.* (*L.*) *schiffleri*, these elements exhibited high identity, reaching 95% identity with an element from *Solenopsis invicta* (Fig. 3).

DISCUSSION

Characterization of transposable elements of *Dichotomius* (*L.*) *schiffleri*

Analysis of the partial genome of *Dichotomius* (*L.*) *schiffleri* using RepeatExplorer and RepeatMasker permitted the identification of different TEs. These tools have previously shown good performance in characterizing repetitive sequences in different species (Lerat 2010; Barghini et al. 2014; García et al. 2015; Gebre et al. 2016). However, we observed that neither method alone was able to identify all elements present in the *D.* (*L.*) *schiffleri* genome. It supports others findings showing the need to concomitantly use different methods for TE detection (Lerat 2010; Platt et al. 2016; Sotero-Caio et al. 2017), when dealing with low coverage genome assemblies/ sequencing. In addition, as far as we are concerned, it was the first attempt to compare the performance of a *de novo* approach focusing on raw reads TE characterization and a homology approach focusing on TE characterization after genome assembly. All other studies so far only used one or other approach the characterize the TE content of a giving species.

The different TE profile found by each methodology is probably related to methodological differences between these programs. RepeatExplorer identifies repetitive sequences using a *de novo* approach based on the reasoning that transposable elements are highly repetitive and should be sequenced more frequently than single copy genes, while RepeatMasker uses a homology-based approach based on similarity of sequences with a curated TE library (Lerat 2010; Novák et al. 2013). Such strategies are then expected to recover a

different set of elements: *de novo* based on highly repetitive reads can characterize TEs with high copy number regardless their similarity with a known TE library but miss low copy number TE families. While homology based approach using a known TE library can characterize TE families with both low and high copy number as long as they present enough similarity based on the threshold established, hence this approach can be biased to the detection of previous known TEs and heavily affected by the contiguity of the assembly generated. Although such differences were expected it was surprising to find the complete absence of over position in the TEs found by each approach.

An abundance of retrotransposons in relation to DNA transposons, as observed in *D.* (*L.*) *schiffleri*, has been reported for different Coleoptera species (Richards et al. 2008; Choi et al. 2010; Keeling et al. 2013). Exceptions are the genome of *Nicrophorus vespilloides*, containing 3.35% DNA transposons and 0.6% retrotransposons (Cunningham et al. 2015), and *Hypothenemus hampei* with 6.02% DNA transposons and 2.25% retrotransposons (Hernandez-Hernandez et al. 2017). These results can be explained by the genomic size of the Coleoptera species, which varies from 154 Mb to 2,578 Mb (Hanrahan and Johnston 2011). The superfamilies of elements identified in *D.* (*L.*) *schiffleri* have been reported previously in other Coleoptera genomes, particularly *Tribolium castaneum* (Richards et al. 2008), *Dendroctonus ponderosae* (Keeling et al. 2013), and *H. hampei* (Hernandez-Hernandez et al. 2017).

When we compared *Copia*, *Gypsy*, *Tc1-Mariner* and *PiggyBac* elements of *D.* (*L.*) *schiffleri* to the complete structure described by Wicker et al. (2007), these elements are structurally conserved and contain most the necessary components to be active, except for the *gag* gene of *Gypsy* elements, long terminal repeats of retrotransposons, and terminal inverted repeats of DNA transposons. These domains could not be characterized with our data.

Evidence of horizontal transfer events

The different transposons of *D. schiffleri* showed instances of highly divergent sequences. Divergences found in the sequences of different elements of *PiggyBac* and *Pogo* may be related to the accumulation of modifications or to an independent origin by horizontal transfer, as previously suggested for *Drosophila* species (Bargues and Lerat 2017). However, in *D.* (*L.*) *schiffleri*, the accumulation of modifications appears to be responsible only for the divergence among elements of the same clade. The identity between elements of phylogenetically distant species and the incongruence between the phylogeny of the element and that of the host suggest the occurrence of horizontal transfer events along the evolutionary history of *D.* (*L.*) *schiffleri* elements, since these characteristics are considered evidences for this type of transfer (Silva et al. 2004; Wallau et al. 2012).

D. (*L.*) *schiffleri PiggyBac*, *Pogo* and *ISL2EU* clustered closely with elements from the platyhelminth *Schmidtea mediterranea*, the cnidarian *Hydra vulgaris*, the nematode *Trichuris suis* and the ant *Solenopsis invicta*. Amino acid sequence comparisons among these sequences were much more similar than expected by vertical transfer (Supplementary Table 2). Conservation of these elements by vertical inheritance seems unlikely, when we compare the divergence time of the Coleoptera with cnidarians (640 Mya), platyhelminths and nematodes (580 Mya), and Hymenoptera (350 Mya) (Peterson et al. 2004; Parfrey et al. 2011; Misof et al. 2014; Reis et al. 2015).suggesting that those elements were exchanged by horizontal transfer between these species or through intermediate species. Considering the first hypothesis (direct transfer between species) geographic and temporal contact and niche sharing are essential prerequisites for horizontal transfer take place (Silva et al. 2004; Carareto 2011).

In the case of the Pogo element from *Trichuris suis* and *D.* (*L.*) *schiffleri*, which presented amino acid identity of 78,95 %, this prerequisite is fullfilled. *T. suis* is a nematode found in the gastrointestinal system of pigs and its eggs can be found in the feces of this animal (Nejsum et al. 2009), and *D. (L.) schiffleri* coprophagous feeding behavior (Costa et al. 2014)

could enable the HT of Pogo ele*ment*s directly among these species. Regarding the ISL2EU element found shared by the ant *Solenopsis invicta* and *D. (L.) schiffleri*, whith amino acid identity of 95,68 %, both species can be found in the Pernambuco state, Brazil (Silva et al. 2010), and the fact that *S. invicta* is an active predator of arthropod eggs, including those of Coleoptera (Zenger and Gibb 2001; Barden et al. 2011), may have facilitated the horizontal transfer of the ISL2EU e*lement* among these species.

In the case of *Hydra vulgaris* cnidarian (shared one Pogo element with *D. (L.) schiffleri* with 81,46 % of amino acid identity) and the platyhelminth *S. mediterranea* (shared 3 different PiggyBac elements with amino acid identity ranging from 73,47 to 98,31) these species have current differen species range. *H. vulgaris* have a wide distribution across different continents, including the Americas (Jankowski et al. 2008), having feeding habit involving different arthropods, such as dipteran larvae (Deserti et al. 2017). While *S. mediterranea* is endemic in the western Mediterranean region (Lázaro et al. 2011), this species presents feding habit associated with different insect larvae (Harrath et al. 2004). Therefore, the similarity between different *PiggyBac* and Pogo elements of these species of *D.* (*L.*) *schiffleri* suggests that horizontal transfer events have occurred through an intermediate species.

Several HT events involving DNA transposon TEs found inplatyhelminth and insect species has been described, including insects found in South America, *Heliconius melpomene (Lavoie et al. 2013)* and *Rhodnius prolixus (Filée et al. 2015)*. Our data gives further support that DNA transposons from the *Tc1-mariner* superfamily are the TEs most involved in HT as shown by other large scale studies while a lower frequency is found for *PiggyBac* elements (Wallau et al. 2012; Dotto et al. 2015; Peccoud et al. 2017).

CONCLUSIONS

In genome-wide characterization of the transposable elements of a species it is necessary to use different approaches. In *Dichotomius* (*L.*) *schiffleri*, structurally conserved elements of

the *Copia, Gypsy, Tc1-Mariner* and *PiggyBac* superfamilies are potentially active. This fact renders them candidates associated with the dispersion and dynamics of other repetitive DNA sequences in the genus *Dichotomius*, however, chromosomal mapping of these sequences is necessary to evaluate such hypothesis. The majority of the *D.* (*L.*) *schiffleri* TEs are probably vertically inherited. Nevertheless, geographic contact allowed a recent horizontal transfer of *ISL2EU* element between *D.* (*L.*) *schiffleri* and *S. invicta* and a more ancient transfer between *T. suis* and *D.* (*L.*) *schiffleri.* While at least other HT events probably mediated by unsampled intermediate species can also be suggested.

REFERENCES

Bailly-Bechet M, Haudry A, Lerat E (2014) "One code to find them all": a perl tool to conveniently parse RepeatMasker output files. Mob DNA 5:1–15. doi: 10.1186/1759-8753-5-13

Barden SA, Held DW, "Fudd" Graham LC (2011) Lack of Interactions Between Fire Ant Control Products and White Grubs (Coleoptera: Scarabaeidae) in Turfgrass. J Econ Entomol 104:2009–2016. doi: 10.1603/EC11131

Barghini E, Natali L, Cossu RM, et al (2014) The peculiar landscape of repetitive sequences in the olive (*Olea europaea* L.) genome. Genome Biol Evol 6:776–791. doi: 10.1093/gbe/evu058

Bargues N, Lerat E (2017) Evolutionary history of LTR-retrotransposons among 20 *Drosophila* species. Mob DNA 8:1–15. doi: 10.1186/s13100-017-0090-3

Beeman RW, Thomson MS, Clark JM, et al (1996) Woot, an active gypsy-class retrotransposon in the flour beetle, *Tribolium castaneum*, is associated with a recent mutation. Genetics 143:417–426

Blaser M, Schmid-Hempel P (2005) Determinants of virulence for the parasite *Nosema whitei* in its host *Tribolium castaneum*. J Invertebr Pathol 89:251–257. doi: 10.1016/j.jip.2005.04.004

Braquart C, Royer V, Bouhin H (1999) DEC: A new miniature inverted-repeat transposable element from the genome of the beetle *Tenebrio molitor*. Insect Mol Biol 8:571–574. doi: 10.1046/j.1365-2583.1999.00144.x

Brown JR (2003) Ancient horizontal gene transfer. Nat Rev Genet 4:121–132. doi: 10.1038/nrg1000

Cabral-de-Mello DC, de Moura R de C, de Melo AS, Martins C (2011a) Evolutionary dynamics of heterochromatin in the genome of *Dichotomius* beetles based on chromosomal analysis. Genetica 139:315–325. doi: 10.1007/s10709-011-9551-7

Cabral-de-Mello DC, Moura RC, Martins C (2011b) Cytogenetic mapping of rRNAs and histone H3 genes in 14 species of *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae) beetles. Cytogenet Genome Res 134:127–135. doi: 10.1159/000326803

Cabral-de-Mello DC, Oliveira SG, de Moura RC, Martins C (2011c) Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: insights into the evolutionary dynamics of multigene families and heterochromatin. BMC Genet 12:1–12. doi: 10.1186/1471-2156-12-88

Carareto CM (2011) Tropical Africa as a cradle for horizontal transfers of transposable elements between species of the genera *Drosophila* and *Zaprionus*. Mob Genet Elements 1:179–186. doi: 10.4161/mge.1.3.18052

Chen Y ping, Pettis JS, Zhao Y, et al (2013) Genome sequencing and comparative genomics of honey bee microsporidia, *Nosema apis* reveal novel insights into host-parasite interactions. BMC Genomics 14:1–16. doi: 10.1186/1471-2164-14-451

Choi J-H, Kijimoto T, Snell-Rood E, et al (2010) Gene discovery in the horned beetle *Onthophagus taurus*. BMC Genomics 11:1–19. doi: 10.1186/1471-2164-11-703

Costa CMQ, Barretto JW, Moura R de C (2014) Changes in the dung beetle community in response to restinga forest degradation. J Insect Conserv 18:895–902. doi: 10.1007/s10841-014-9697-6

Cunningham CB, Ji L, Wiberg RAW, et al (2015) The genome and methylome of a beetle with complex social behavior, *Nicrophorus vespilloides* (coleoptera: Silphidae). Genome Biol Evol 7:3383–3396. doi: 10.1093/gbe/evv194

Deserti MI, Esquius KS, Escalante AH, Acuña FH (2017) Trophic ecology and diet of *Hydra vulgaris* (Cnidaria; Hydrozoa). Anim Biol 67:287–300.

Djebbi S, Amara W Ben, Makni H, et al (2017) Molecular characterization of mariner-like elements in *Bruchus pisorum* and *Bruchus rufimanus* (Coleoptera: Bruchidae). Arch Biol Sci 69:353–360. doi: 10.2298/ABS160407111D

Dotto BR, Carvalho EL, Silva AF, et al (2015) HTT-DB: Horizontally transferred transposable elements database. Bioinformatics 31:2915–2917. doi: 10.1093/bioinformatics/btv281

Elliott TA, Gregory TR (2015) Do larger genomes contain more diverse transposable elements? BMC Evol Biol 15:1–10. doi: 10.1186/s12862-015-0339-8

Ellis JD, Munn PA (2005) The worldwide health status of honey bees. Bee World 86:88–101. doi: 10.1080/0005772X.2005.11417323

Filée J, Rouault J-D, Harry M, Hua-Van A (2015) Mariner transposons are sailing in the genome of the blood-sucking bug *Rhodnius prolixus*. BMC Genomics 16:1–17. doi: 10.1186/s12864-015-2060-9

Finnegan DJ (1992) Transposable elements. Curr Opin Genet Dev 2:861–867. doi: 10.1016/S0959-437X(05)80108-X

Flutre T, Duprat E, Feuillet C, Quesneville H (2011) Considering transposable element diversification in de novo annotation approaches. PLoS One 6:1–15. doi: 10.1371/journal.pone.0016526

García G, Ríos N, Gutiérrez University V (2015) Next-generation sequencing detects repetitive

elements expansion in giant genomes of annual killifish genus *Austrolebias* (Cyprinodontiformes, Rivulidae). Genetica 143:353–360.

Gebre YG, Bertolini E, Pè ME, Zuccolo A (2016) Identification and characterization of abundant repetitive sequences in Eragrostis tef cv. Enatite genome. BMC Plant Biol 16:1–13. doi: 10.1186/s12870-016-0725-4

Guindon S, Dufayard JF, Lefort V, et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst Biol 59:307–321. doi: 10.1093/sysbio/syq010

Hanrahan SJ, Johnston JS (2011) New genome size estimates of 134 species of arthropods. Chromosome Research 19: 809. doi: 10.1007/s10577-011-9231-6

Harrath AH, Charni M, Sluys R, et al (2004) Ecology and distribution of the freshwater planarian *Schmidtea mediterranea* in tunisia. Ital J Zool 71:233–236. doi: 10.1080/11250000409356577

Hartl DL, Lozovskaya ER, Lawrence JG (1992) Nonautonomous transposable elements in prokaryotes and eukaryotes. Genetica 86:47–53. doi: 10.1007/BF00133710

Hernandez-Hernandez EM, Fernández-Medina RD, Navarro-Escalante L, et al (2017) Genome-wide analysis of transposable elements in the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): description of novel families. Mol Genet Genomics 292:565–583. doi: 10.1007/s00438-017-1291-7

Jankowski T, Collins AG, Campbell R (2008) Global diversity of inland water cnidarians. Hydrobiologia 595:35–40. doi: 10.1007/s10750-007-9001-9

Keeling CI, Yuen MM, Liao NY, et al (2013) Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. Genome Biol 14:1–19. doi: 10.1186/gb-2013-14-3-r27

Kidwell MG (2005) Transposable Elements. In: Gregory TR (ed) The Evolution of the Genome, 1st edn. Elsevier ed., San Diego, pp 165–221

Kotnova AP, Glukhov IA, Karpova NN, et al (2007) Evidence for recent horizontal transfer of gypsy-homologous LTR-retrotransposon gtwin into *Drosophila erecta* followed by its amplification with multiple aberrations. Gene 396:39–45. doi: 10.1016/j.gene.2007.02.019

Lavoie CA, Platt RN, Novick PA, et al (2013) Transposable element evolution in *Heliconius* suggests genome diversity within Lepidoptera. Mob DNA 4:1–10. doi: 10.1186/1759-8753-4-21

Lázaro EM, Harrath AH, Stocchino GA, et al (2011) The freshwater planarian *Dugesia sicula* Lepori from Sardinia (Platyhelminthes, Tricladida). BMC Evol Biol 11:1–15. doi:

10.1007/BF00015533

Lerat E (2010) Identifying repeats and transposable elements in sequenced genomes: how to find your way through the dense forest of programs. Heredity (Edinb) 104:520–533. doi: 10.1038/hdy.2009.165

Li W, Godzik A (2006) Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659. doi: 10.1093/bioinformatics/btl158

Maumus F, Fiston-Lavier AS, Quesneville H (2015) Impact of transposable elements on insect genomes and biology. Curr Opin Insect Sci 7:30–36. doi: 10.1016/j.cois.2015.01.001

Misof B, Liu S, Meusemann K, et al (2014) Phylogenomics resolves the timing and pattern of insect evolution. Science 346:763–767. doi: 10.1126/science.1257570

Nejsum P, Thamborg SM, Petersen HH, et al (2009) Population dynamics of *Trichuris suis* in trickle-infected pigs. Parasitology 136:691–697. doi: 10.1017/S0031182009005976

Novák P, Neumann P, Pech J, et al (2013) RepeatExplorer: A Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. Bioinformatics 29:792–793. doi: 10.1093/bioinformatics/btt054

Oliveira SG, Cabral-de-Mello DC, Moura RC, Martins C (2013) Chromosomal organization and evolutionary history of *Mariner* transposable elements in Scarabaeinae coleopterans. Mol Cytogenet 6:1–9. doi: 10.1186/1755-8166-6-54

Parfrey LW, Lahr DJG, Knoll AH, Katz LA (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc Natl Acad Sci 108:13624–13629. doi: 10.1073/pnas.1110633108

Pauchet Y, Heckel DG (2013) The genome of the mustard leaf beetle encodes two active xylanases originally acquired from bacteria through horizontal gene transfer. Proc Biol Sci 280:1–8. doi: 10.1098/rspb.2013.1021

Peccoud J, Loiseau V, Cordaux R, Gilbert C (2017) Massive horizontal transfer of transposable elements in insects. Proc Natl Acad Sci 114:4721–4726. doi: 10.1073/pnas.1621178114

Peterson KJ, Lyons JB, Nowak KS, et al (2004) Estimating metazoan divergence times with a molecular clock. Proc Natl Acad Sci 101: 6536–6541. doi: 10.1073/pnas.0401670101

Platt RN, Blanco-Berdugo L, Ray DA (2016) Accurate transposable element annotation is vital when analyzing new genome assemblies. Genome biology and evolution 8: 403–410. doi: 10.1093/gbe/evw009

Reis M, Thawornwattana Y, Angelis K, et al (2015) Uncertainty in the Timing of Origin of Animals and the Limits of Precision in Molecular Timescales. Curr Biol 25:2939–2950. doi: 10.1016/j.cub.2015.09.066

Richards S, Gibbs RA, Weinstock GM, et al (2008) The genome of the model beetle and pest *Tribolium castaneum*. Nature 452:949–955. doi: 10.1038/nature06784

Rivera-Vega L, Mittapalli O (2010) Molecular characterization of mariner-like elements in emerald ash borer, *Agrilus planipennis* (Coleoptera, Polyphaga). Arch Insect Biochem Physiol 74:205–216. doi: 10.1002/arch.20357

Robertson HM, Lampe DJ (1995a) Distribution of transposable elements in arthropods. Annu Rev Entomol 40:333–357. doi: 10.1146/annurev.ento.40.1.333

Robertson HM, Lampe DJ (1995b) Recent horizontal transfer of a mariner transposable element among and between Diptera and Neuroptera. Mol Biol Evol 12:850–862

Robertson HM, MacLeod EG (1993) Five major subfamilies of mariner transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. Insect Mol Biol 2:125–139. doi: 10.1111/j.1365-2583.1993.tb00132.x

Robertson HM, Soto-Adames FN, Walden KK, et al (2002) The mariner transposons of animals: horizontally jumping genes. In: Syvanen M, Kado CI (eds) Horizontal gene transfer, 2nd. Academic Press. A Division of Harcourt, Inc., San Diego, California, pp 173–185

Sambrook J, Rusel WD (2001) Molecular Cloning. A Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York

Silva GL, Santos EM dos, Gomes JP (2010) Predação de ovos de *Corythomantis greeningi* Boulenger, 1896 (Anura, Hylidae) por *Solenopsis invicta* Buren, 1972 (Formicidae: Myrmicinae). Biote. Biotemas 23:153–156 . doi: 10.5007/2175-7925.2010v23n4p153

Silva JC, Loreto EL, Clark JB (2004) Factors that affect the horizontal transfer of transposable elements. Curr issues Mol Biol 6: 57-72.

Sotero-Caio CG, Platt RN, Suh A, Ray DA (2017) Evolution and diversity of transposable elements in vertebrate genomes. Genome biology and evolution 9: 161–177. doi: 10.1093/gbe/evw264

Wallau GL, Ortiz MF, Loreto ELS (2012) Horizontal transposon transfer in eukarya: Detection, bias, and perspectives. Genome Biol Evol 4:689–699. doi: 10.1093/gbe/evs055

Wicker T, Sabot F, Hua-Van A, et al (2007) A unified classification system for eukaryotic transposable elements. Nat Rev Genet 8:973–982 . doi: 10.1038/nrg2165

Xavier C, Cabral-de-Mello DC, Moura RC (2014) Heterochromatin and molecular characterization of *Dsmar*MITE transposable element in the beetle *Dichotomius schiffleri* (Coleoptera: Scarabaeidae). Genetica 142:575–581. doi: 10.1007/s10709-014-9805-2

Yocum GD, Toutges MJ, Roehrdanz RL, Dihle PJ (2011) Insertion of miniature subterminal

inverted repeat-like elements in diapause-regulated genes in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Eur J Entomol 108:197–203 . doi: 10.14411/eje.2011.027

Zenger JT, Gibb TJ (2001) Identification and impact of egg predators of *Cyclocephala lurida* and *Popillia japonica* (Coleoptera: Scarabaeidae) in turfgrass. Environ Entomol 30:425–430 . doi: http://dx.doi.org/10.1603/0046-225X-30.2.425

Table

Table 1 - Classification, approach, size and domains of the conserved elements of *D. (L.) schiffleri.*

Transposable elements	Number of TEs consensus	Approach	Number of TEs by approach	ORF size (aa)	Domains
Retrotransposon					
Copia	5	RepeatExplorer-CAP3	-	-	-
		SOAP-RepeatMasking	5	656 - 177 aa	RT/rve/RNaseH/gag
Gypsy	11	RepeatExplorer-CAP3	6	513 - 37 aa	RT/rve/RNaseH
		SOAP-RepeatMasking	5	280 - 160 aa	RT/rve/RNaseH
I	5	RepeatExplorer-CAP3	3	755 - 249 aa	RT
		SOAP-RepeatMasking	2	490 e 259 aa	RT
Jockey	2	RepeatExplorer-CAP3	1	192 aa	RT
		SOAP-RepeatMasking	1	220 aa	RT
Penelope	5	RepeatExplorer-CAP3	4	264 - 102 aa	RT
		SOAP-RepeatMasking	1	221 aa	RT
RTE	2	RepeatExplorer-CAP3	2	62 e 30 aa	RT
		SOAP-RepeatMasking	-	-	-
DNA Transposon					
Tc1-Mariner	18	RepeatExplorer-CAP3	6	375 - 108 aa	DDE/ HTH
		SOAP-RepeatMasking	12	468 – 95 aa	DDE/ HTH
hAT	2	RepeatExplorer-CAP3	2	589 e 113 aa	C-terminal of transposase
		SOAP-RepeatMasking	-	-	-
PiggyBac	7	RepeatExplorer-CAP3	1	492 aa	DDE
		SOAP-RepeatMasking	6	553 – 115 aa	DDE
PHIS	2	RepeatExplorer-CAP3	-	-	-
		SOAP-RepeatMasking	2	305 e 200 aa	DDE
Maverick	2	RepeatExplorer-CAP3	2	230 e 234 aa	Integrase/Pol B
		SOAP-RepeatMasking	-	-	-

Abbreviations: RT - Reverse Transcriptase; rve - Integrase; DDE - region with three amino acids, Asparagine-Asparagine-Glutamine; HTH - Helix-turn-Helix; Pol B – DNA Polymerase B

Figures

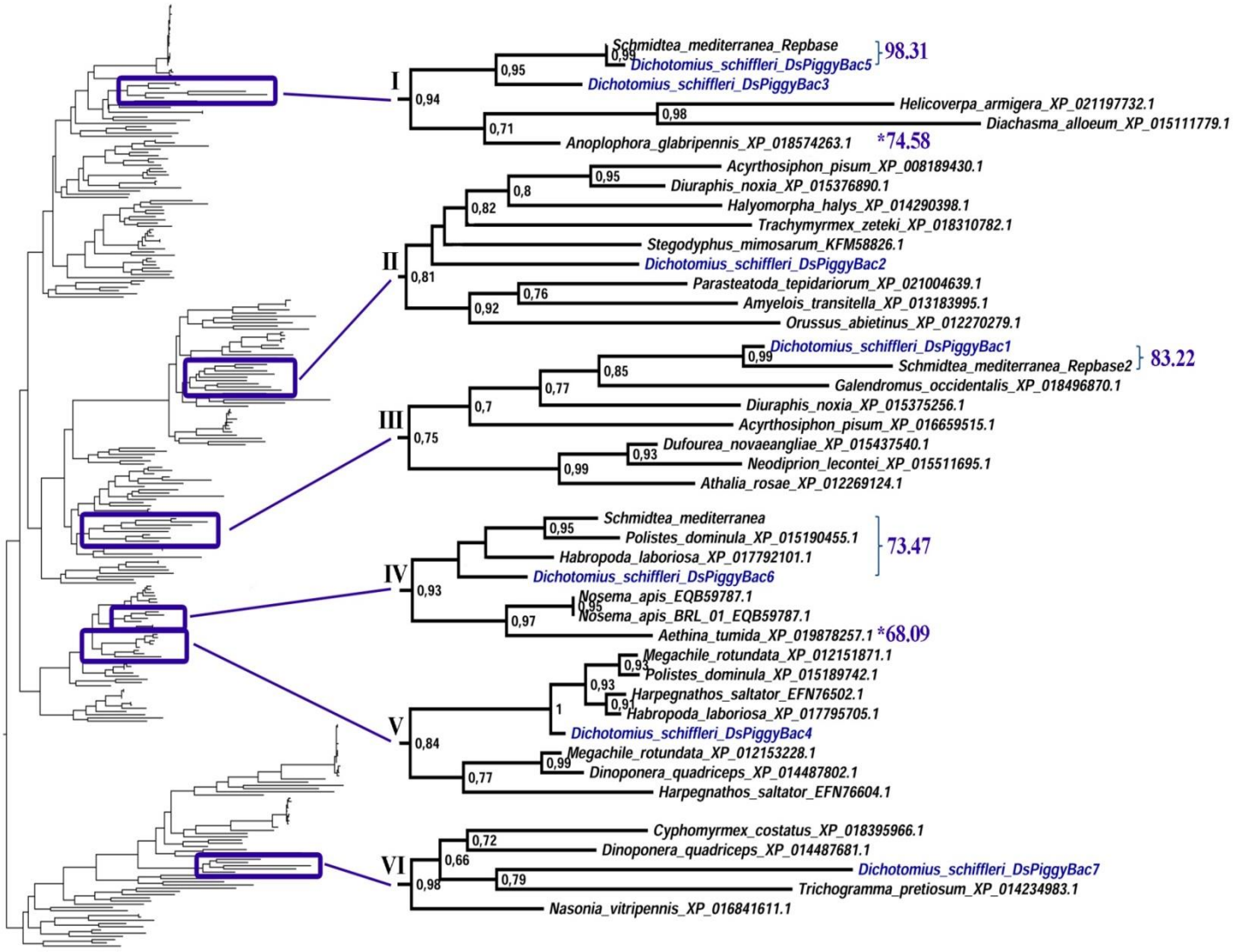

Figure 1 - Phylogenetic tree of *PiggyBac* elements estimated with the maximum likelihood method. The evolutionary model selected and used was LG +G +F. The highlighted clades (I, II, III, IV, V and VI) contain elements of *Dichotomius (L.) schiffleri.* Sequences obtained in this study are shown in blue. When compared to the *D. (L.) schiffleri* elements, the identity with Coleoptera species is indicated by an asterisk and possible horizontal transfer events by curly brackets. Branch supports correspond to aLRT values.

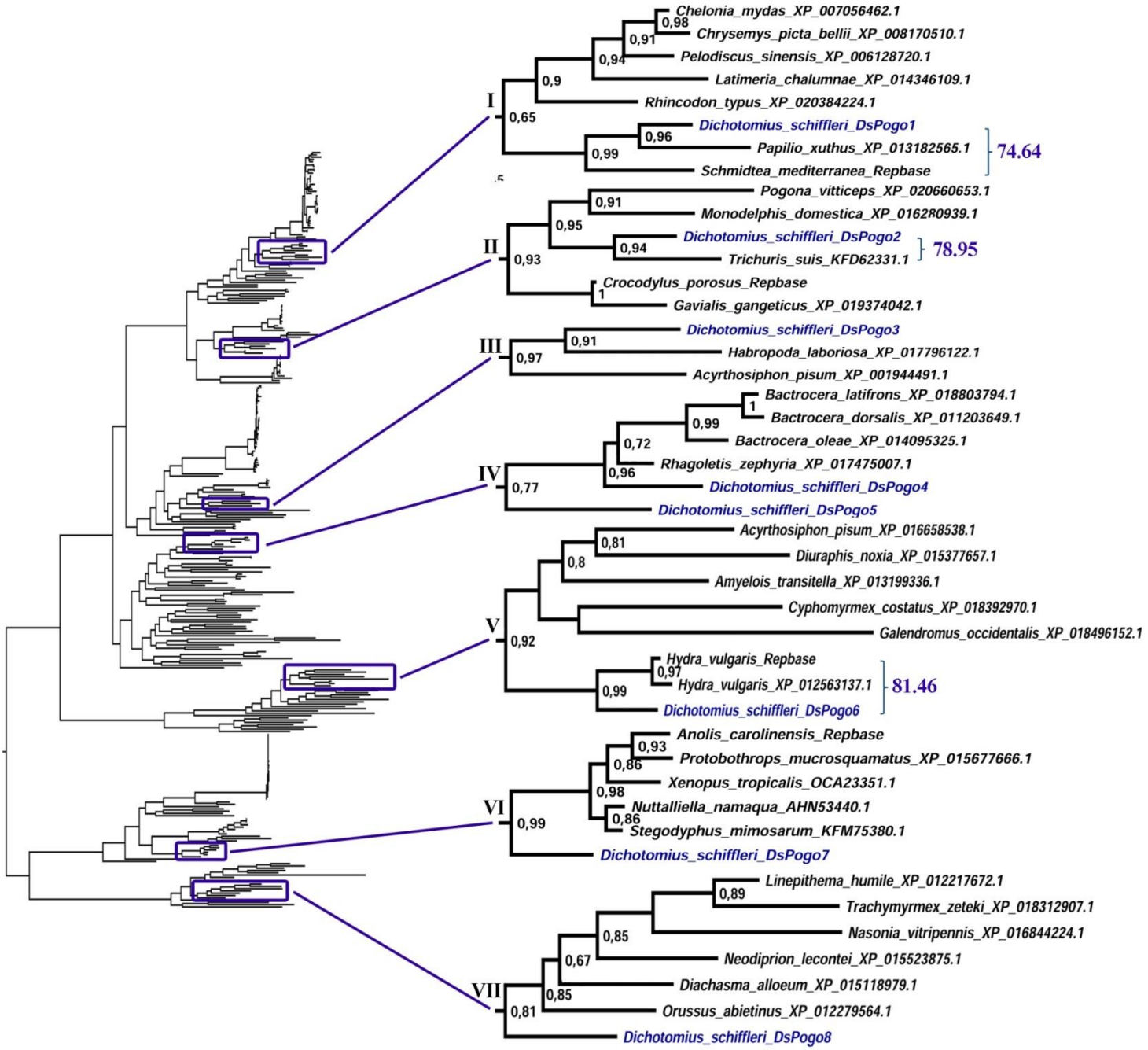

Figure 2 - Phylogenetic tree of *Pogo* elements estimated with the maximum likelihood method. The evolutionary model selected and used was LG +G +F. The highlighted clades (I, II, III, IV, V, VI and VII) contain elements of *Dichotomius (L.) schiffleri.* Sequences obtained in this study are shown in blue. When compared to the *D. (L.) schiffleri* elements, the identity of species with possible horizontal transfer events is indicated by curly brackets. Branch supports correspond to aLRT values.

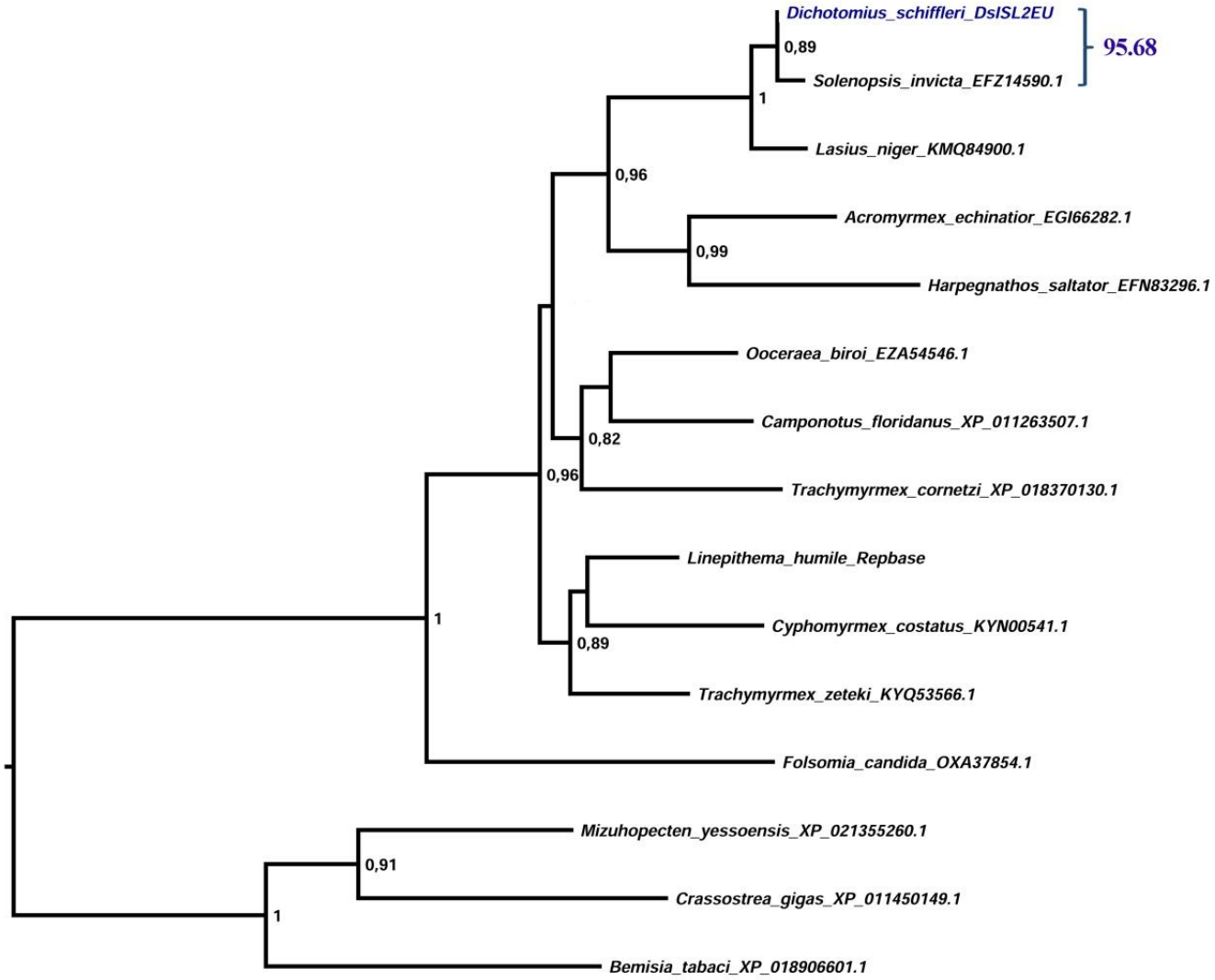

Figure 3 - Phylogenetic tree of *ISL2EU* elements estimated with the maximum likelihood method. The evolutionary model selected and used was LG +G +I +F. Sequences obtained in this study are shown in blue. When compared to the *D.* (*L.*) *schiffleri* elements, the identity of species with possible horizontal transfer events is indicated by curly brackets. Branch supports correspond to aLRT values.

Supplementary material **Molecular Genetics and Genomics**

Genome-wide characterization of transposable elements in *Dichotomius* (*Luederwaldtinia*) *schiffleri* (Coleoptera: Scarabaeidae): evidence of multiple horizontal transfer events

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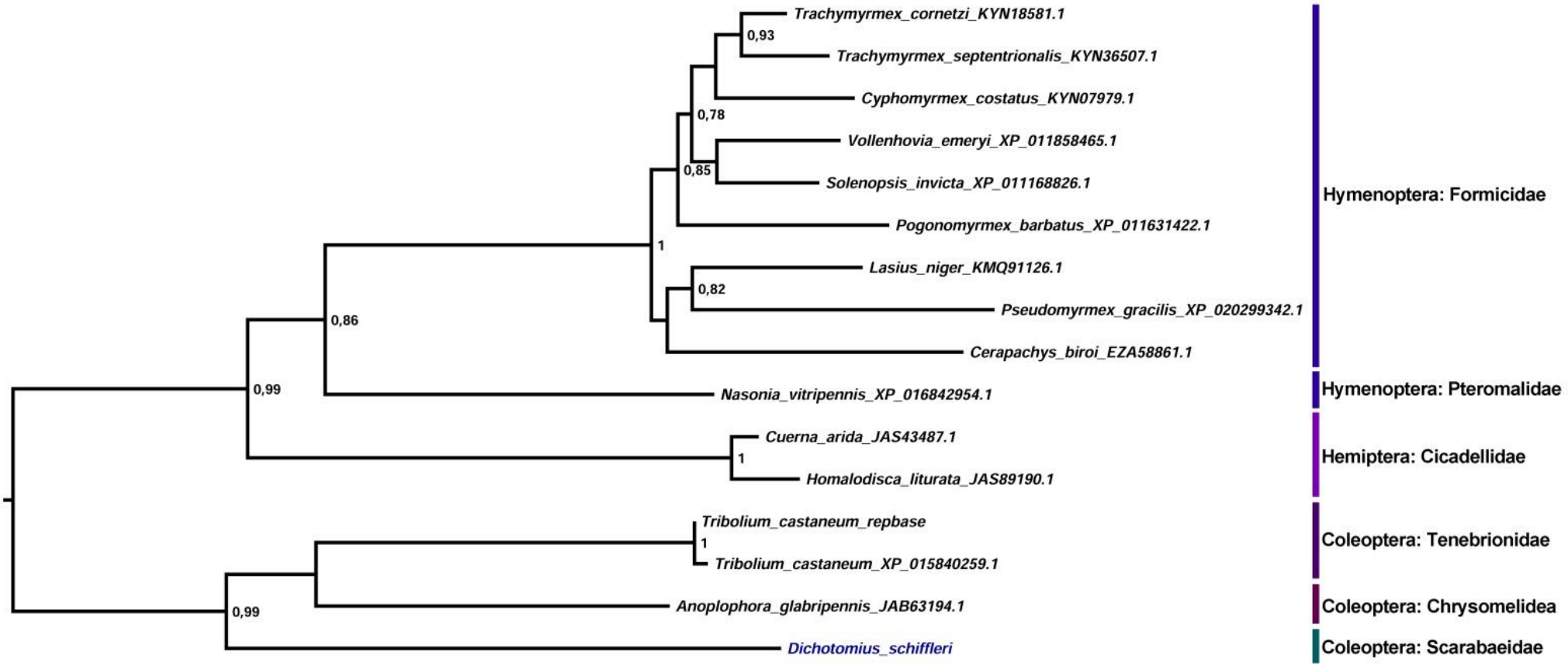
Supplementary Table 1 - Classification, number and size of the elements without conserved domains.

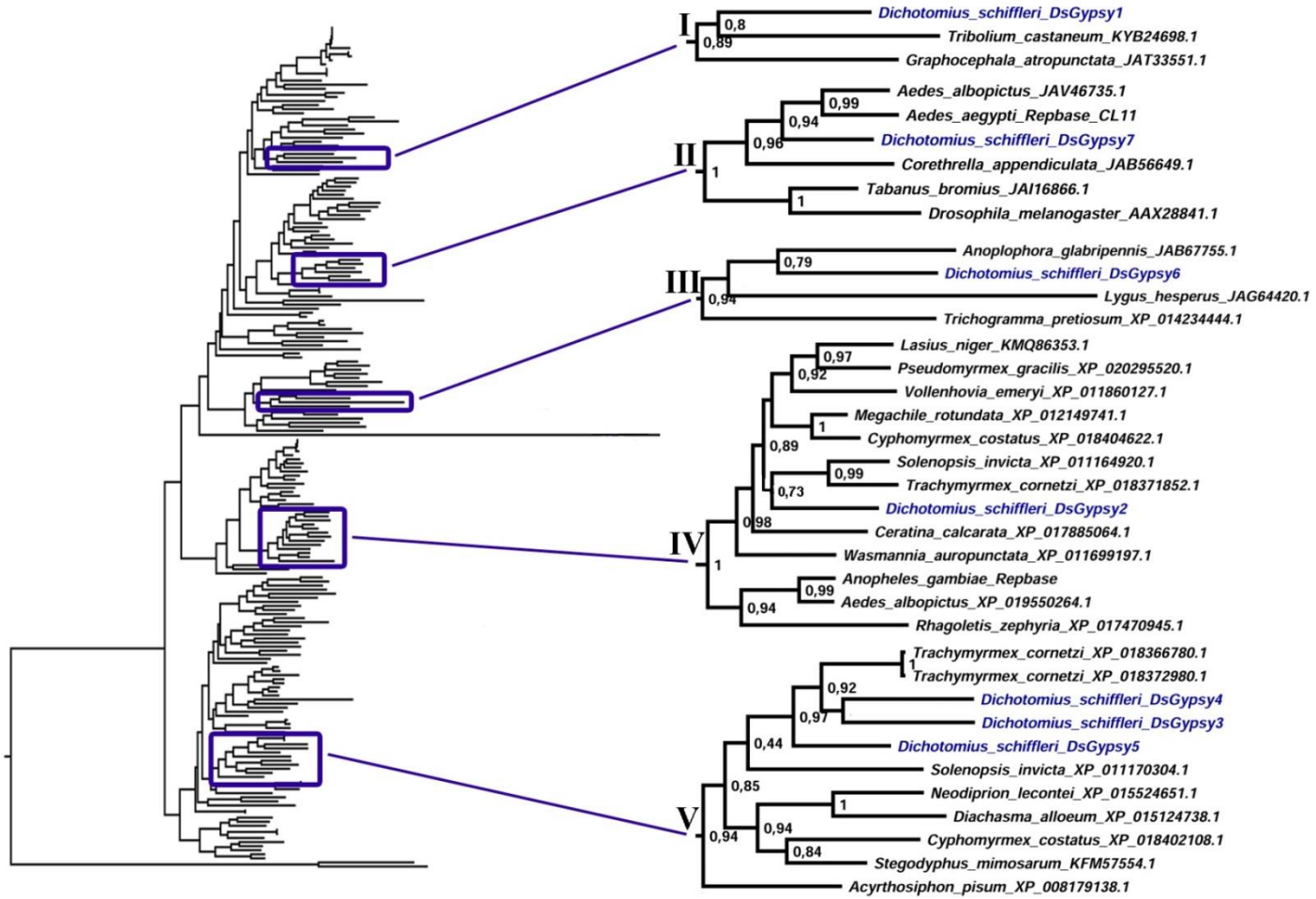
Transposable elements	N° of Tes consensus	Smaller and bigger consensus size
SINEs	10	42 - 101 pb
Gypsy	5	182 - 495 pb
hAT	8	267 – 1654 pb
Tc1-Mariner	6	307 – 974 pb
Kolobok	5	363 – 1625 pb
Transib	1	1406 pb

Supplementary Table 2 - Potencial horizontal transfer events

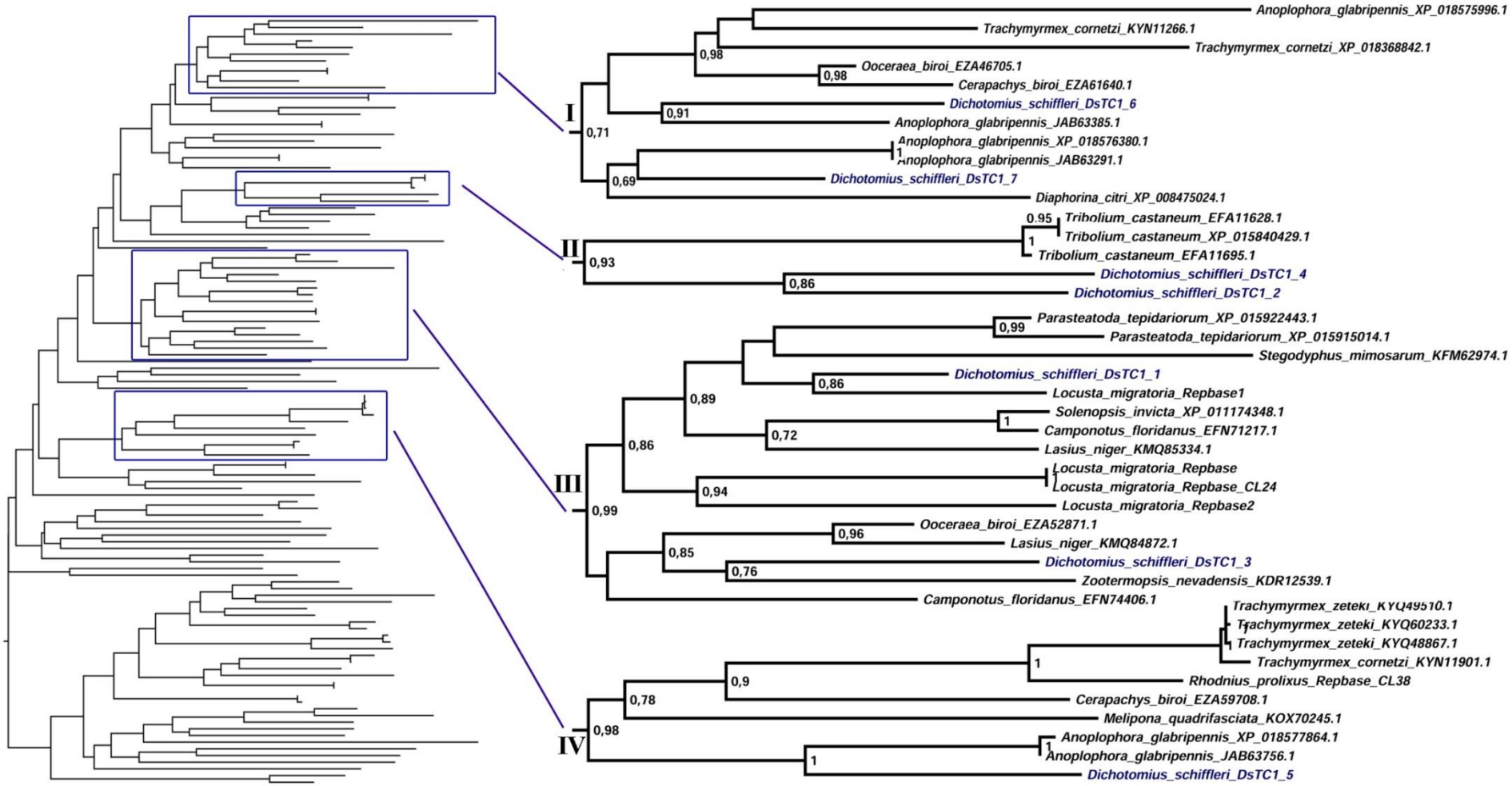
Classification of the elements	Name of the element of *D. schiffleri*	Horizontal transfer events		
		Figures: Clades	Species	Identity
PiggyBac	*DsPiggyBac5*	Fig.1, clade I	*Schmidtea mediterranea*	98,31
	DsPiggyBac1	Fig.1, clade III	*Schmidtea mediterranea*	83,22
	DsPiggyBac6	Fig. 1, clade IV	*Schmidtea mediterranea*	73,47
Pogo	*DsPogo1*	Fig. 2, clade I	*Schmidtea mediterranea*	74,66
	DsPogo2	Fig. 2, clade II	*Trichuris suis*	78,95
	DsPogo7	Fig. 2, clade V	*Hydra vulgaris*	81,46
ISL2EU	*DsISL2EU*	Fig. 3	*Solenopsis invicta*	95,68



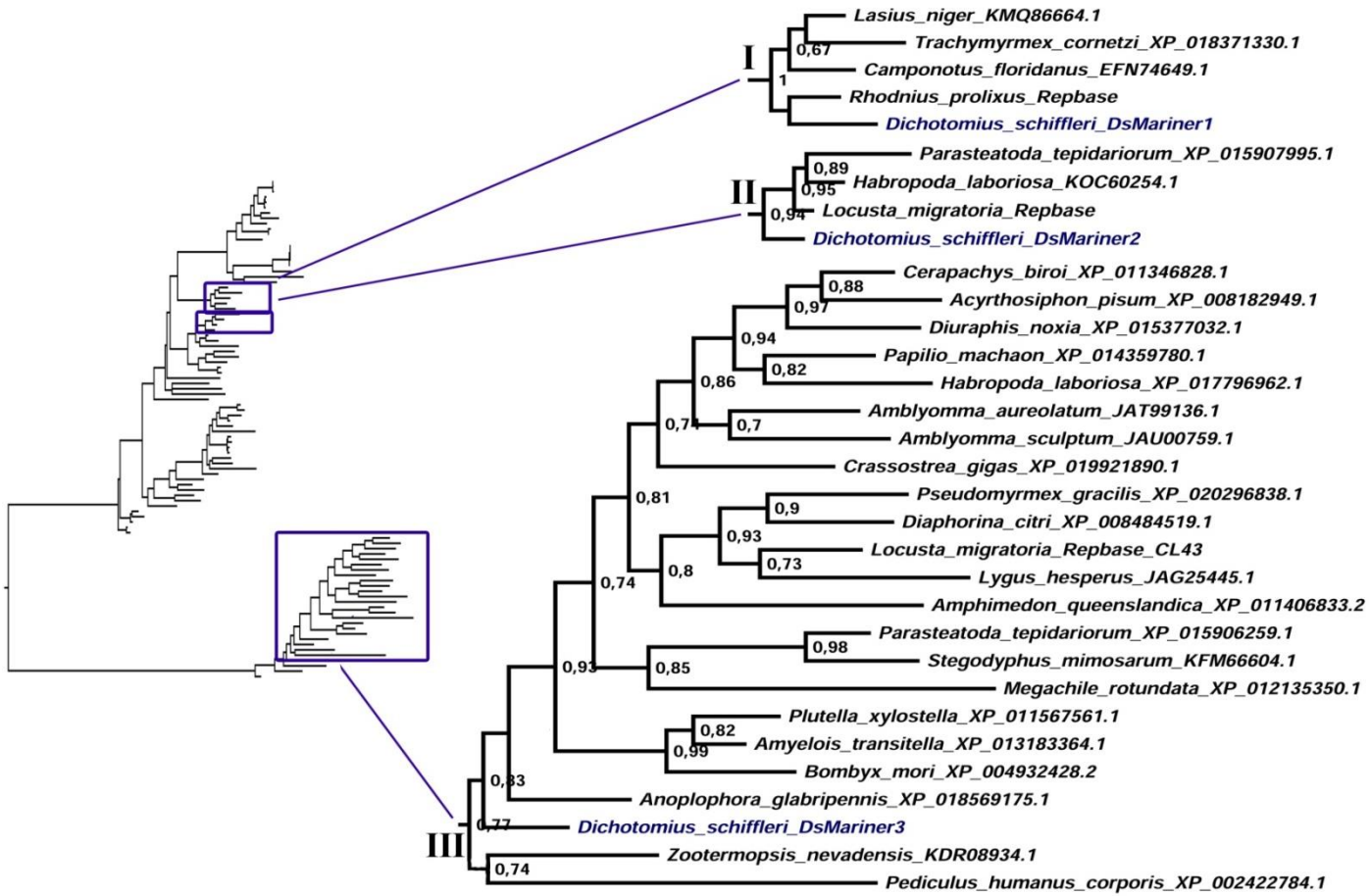
Supplementary Figure 1- Phylogenetic tree of *Maverick* elements estimated with the maximum likelihood method. The evolutionary model selected and used was LG +G +F. The bars represent the different orders and families of insects. Sequence obtained in this study is shown in blue. Branch supports correspond to aLRT values.



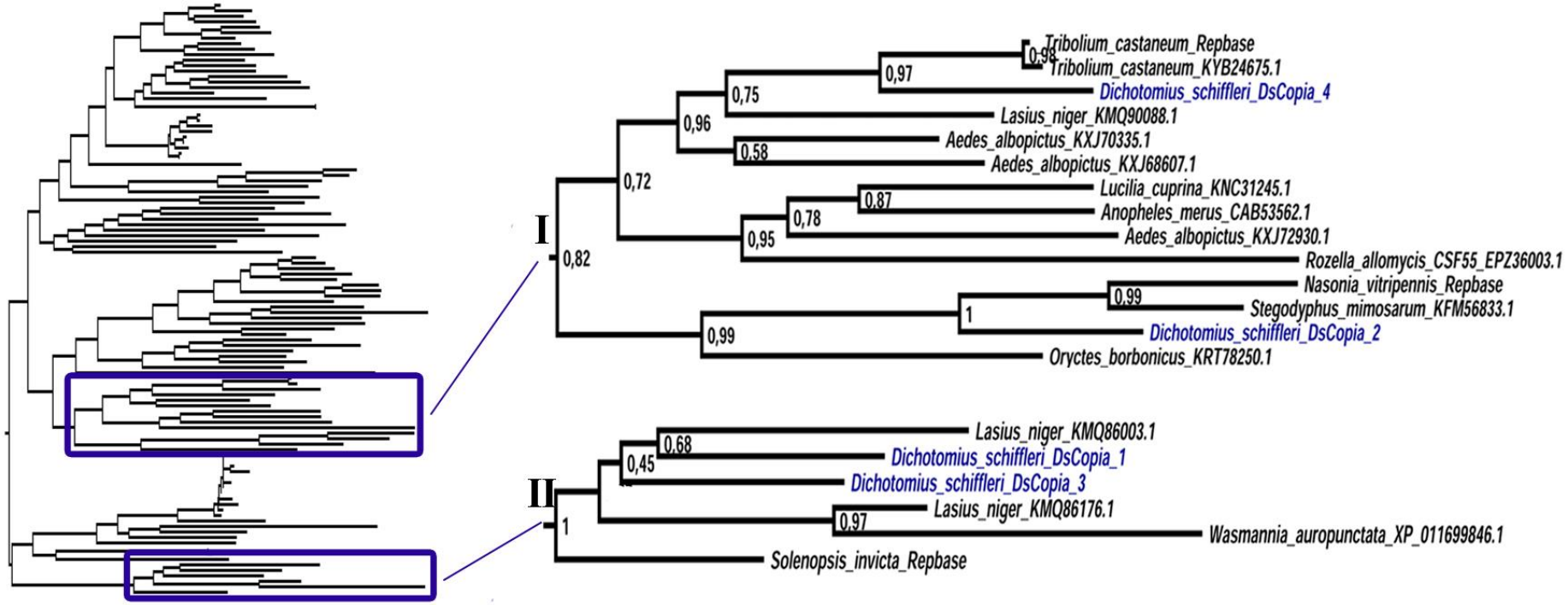
Supplementary Figure 2 - Phylogenetic tree of *Gypsy* elements estimated with the maximum likelihood. The evolutionary model selected and used was LG +G +F The highlighted clades (I, II, III, IV and V) contain elements of *D. (L.) schiffleri.* Sequence obtained in this study is show in blue. Branch supports correspond to aLRT values.



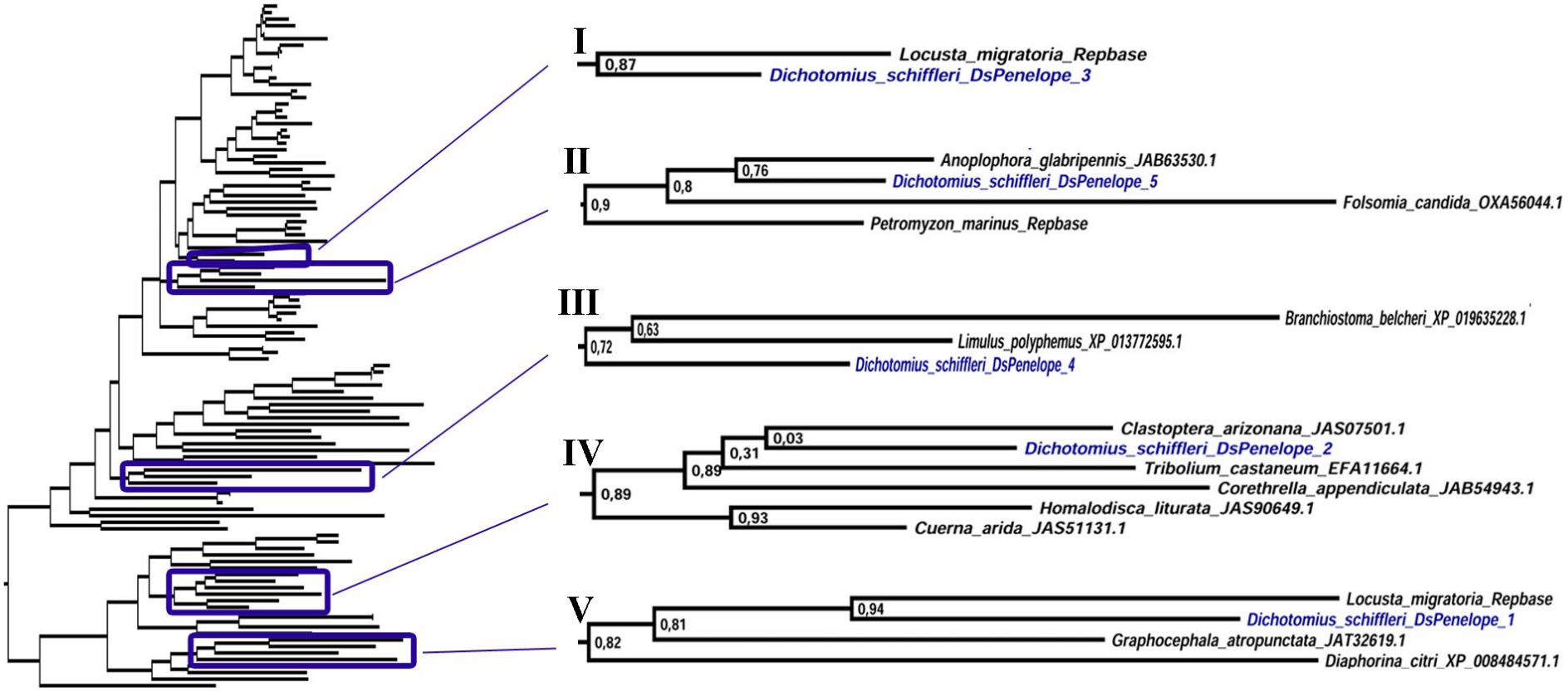
Supplementary Figure 3 - Phylogenetic tree of *Tc1* elements estimated with the maximum likelihood. The evolutionary model selected and used was LG +G +I +F. The highlighted clades (I, II, III and IV) contain elements of *D. (L.) schiffleri.* Sequences obtained in this study are shown in blue. Branch supports correspond to aLRT values.



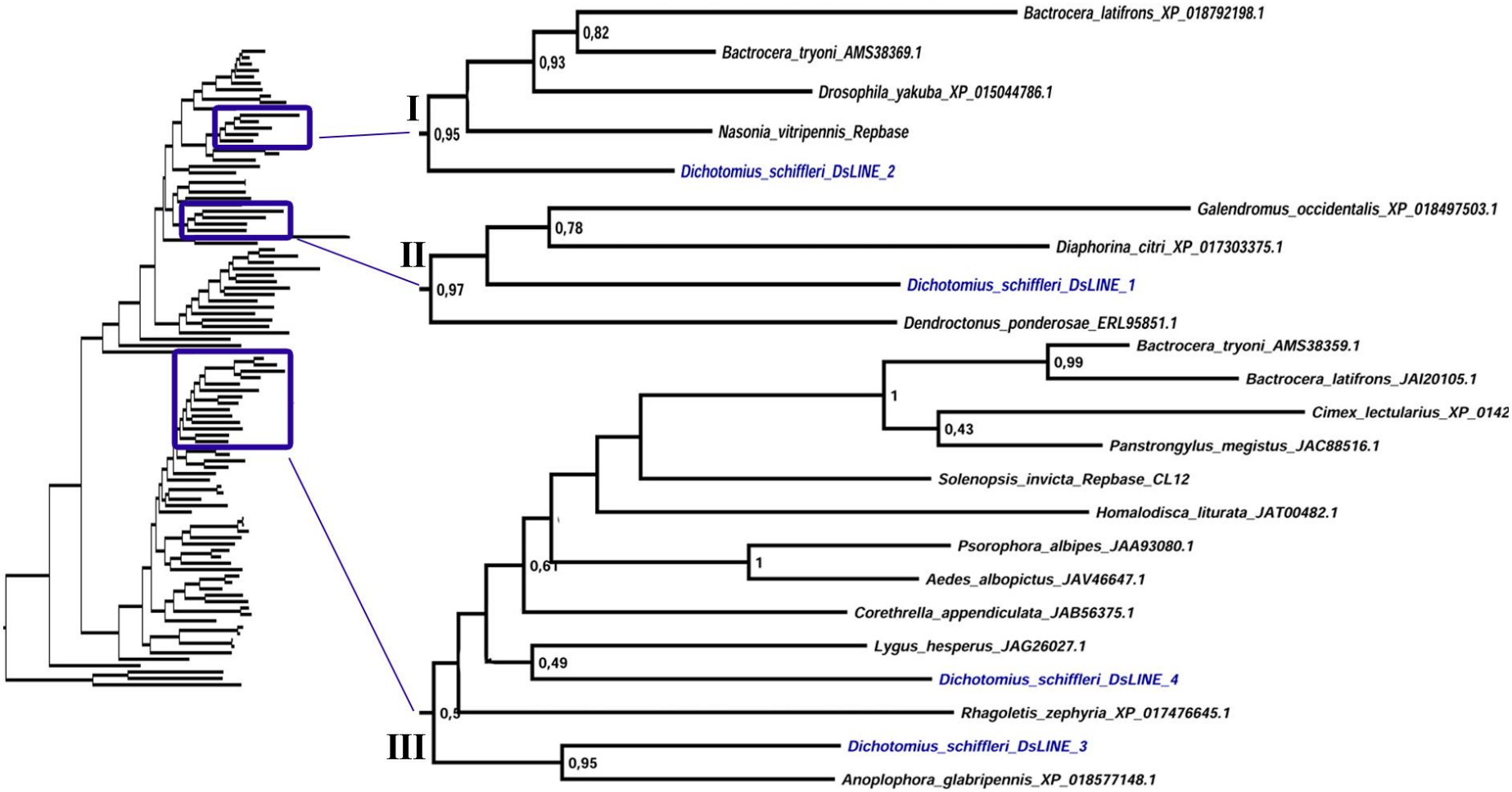
Supplementary Figure 4 - Phylogenetic tree of *Mariner* elements estimated with the maximum likelihood. The evolutionary model selected and used was LG +G. The highlighted clades (I, II and III) contain elements of *D. (L.) schiffleri.* Sequences obtained in this study are shown in blue. Branch supports correspond to aLRT values.



Supplementary Figure 5 -Phylogenetic tree of *Copia* elements estimated with the maximum likelihood method. The evolutionary model selected and used was LG +G +I. The highlighted clades (I and II) contain elements of *D. (L.) schiffleri*. Sequences obtained in this study are in blue. Branch supports correspond to aLRT values.



Supplementary Figure 6 - Phylogenetic tree of *Penelope* elements estimated with the maximum likelihood. The highlighted clades (I, II, III, IV and V) contain elements of *D. (L.) schiffleri.* The evolutionary model selected and used was LG +G +F. Sequences obtained in this study are shown in blue. Branch supports correspond to aLRT values



Supplementary Figure 7 - Phylogenetic tree of *LINE I* elements estimated with the maximum likelihood. The evolutionary model selected and used was LG +G +F. The highlighted clades (I, II and III) contain elements of *D. (L.) schiffleri.* Sequences obtained in this study are show in blue. Branch supports correspond to aLRT values.

3.2 ARTIGO 2 - ATIVIDADE E CONSERVAÇÃO DE ELEMENTOS *TC1-MARINER* NO GÊNERO *Dichotomius* (COLEOPTERA)

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RESUMO

No gênero *Dichotomius*, os elementos de transposição (TE) têm sido relacionados com a variação na composição da heterocromatina constitutiva e na localização das sequências de DNAs moderadamente e altamente repetitivos. Dessa forma, os objetivos deste trabalho foram verificar a presença de dois transposons de DNA conservados estruturalmente em espécies do gênero *Dichotomius*, bem como realizar o mapeamento cromossômico populacional desses elementos em espécies do grupo *D.* (*L.*) *sericeus*, visando compreender o papel dessas sequências repetitivas na evolução genômica do grupo. Nesse trabalho, a presença dos TEs foi verificada por PCR, utilizando *primers* de elementos caracterizados em *D.* (*L.*) *schiffleri*. O mapeamento cromossômico dos elementos *DsTC1_5* e *DsPogo_8*, que pertencem a superfamília *Tc1-Mariner*, foi realizado pela hibridização *in situ* fluorescente em espécies do grupo *D.* (*L.*) *sericeus* provenientes de três populações cada. O mapeamento cromossômico revelou sinais dispersos nas regiões eucromáticas dos autossomos e ausência nos cromossomos sexuais. Adicionalmente, foi observada uma diferença populacional na localização cromossômica e abundância desses elementos. Esses resultados sugereriram uma herança vertical, com a presença desses elementos no ancestral comum as espécies de *Dichotomius*. Além disso, apesar desses elementos apresentarem mobilidade e atividade, os mesmos possivelmente não estão relacionados à variação das outras sequências repetitivas presentes no genoma de espécies de *Dichotomius*.

Palavras-chaves: Citogenômica comparativa; FISH; Transposon de DNA; *DsTC1_5* e *DsPogo_8*.

INTRODUÇÃO

Os elementos de transposição (TEs) são sequências repetitivas de DNA com capacidade de mobilidade (Hartl et al. 1992), que podem estar relacionadas com modificações na expressão e estrutura dos genes (Maumus et al. 2015; Warren et al. 2015). Esses elementos desempenham um papel fundamental na organização cromossômica, possibilitando a ocorrência de diferentes rearranjos cromossômicos, como deleções, duplicações, inversões e translocações (Delprat et al. 2009; Fonseca et al. 2012; Maumus et al. 2015). A origem desses rearranjos pode estar relacionada a um efeito indireto dos elementos, através da recombinação ectópica entre diferentes cópias ou um efeito direto dependente da transposição, sendo esse mecanismo exclusivo para os transposons de DNA (Kidwell 2005). Assim, os elementos contribuem na diversificação gênica e cromossômica entre espécies e populações (Maumus et al. 2015; Warren et al. 2015).

Os elementos de transposição têm sido analisados por diferentes abordagens (Kidwell 2005; Lerat 2010; Biscotti et al. 2015). Dentre elas, o mapeamento cromossômico têm se mostrado eficiente para compreender a distribuição e evolução dessas sequências repetitivas (Biscotti et al. 2015). Além disso, quando a análise citogenética molecular é comparativa, populacional ou entre espécies filogeneticamente próximas, permite também compreender os efeitos dos elementos de transposição na evolução dos genomas hospedeiros (Biscotti et al. 2015).

O mapeamento cromossômico de elementos de transposição foi realizado em poucas espécies de Coleoptera, sendo todas pertencentes à subfamília Scarabaeinae (Oliveira et al., 2013; Xavier et al., 2014, Amorim et al., 2018). Nessas espécies foram mapeados elementos não autônomos ou parte de um domínio especifico de um elemento autônomo. No gênero *Dichotomius*, o mapeamento de elementos degenerados da família *Mariner*, revelou padrões

cromossômicos populacionais e interespecíficos distintos (Xavier et al. 2014; Amorim et al., 2018).

Recentemente o mobiloma de *Dichotomius* (*Luederwaldtinia*) *schiffleri* Vaz-de-Mello, Louzada e Gavino, 2001 foi caracterizado, revelando a presença de elementos pertencentes a seis superfamílias de retrotransposons e cinco de transposons de DNA (Amorim et al., *in prep.*). Nesse estudo, foram indentificados elementos estruturalmente conservados e potencialmente ativos, pertecentes às superfamilias *Copia*, *Gypsy*, *PiggyBac* e *Tc1-Mariner*, que podem estar relacionados a remodelação e evolução do genoma em *Dichotomius*.

Considerando a possível relação dos elementos de transposição com a variação de outras sequências repetitivas no gênero *Dichotomius* e o papel dos transposons de DNA nos microrearranjos cromossômicos, realizamos o mapeamento cromossômico de elementos estruturalmente conservados da superfamília *Tc1-Mariner*, nas espécies do grupo *D.* (*L.*) *sericeus* presentes em três populações do Nordeste brasileiro. Além disso, verificamos a presença a desses elementos em todas as espécies de *Dichotomius* das localidades amostradas, visando observar a evolução, distribuição e dispersão dessas sequências repetitivas no gênero.

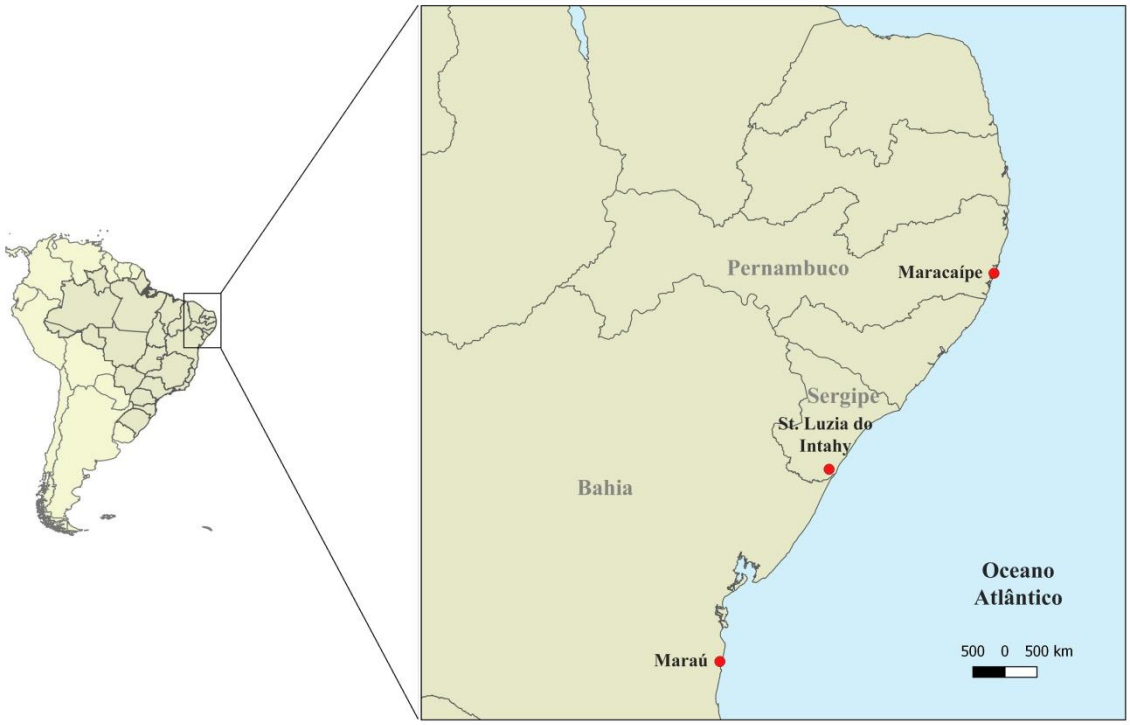
MATÉRIAIS E MÉTODOS

Coletas e amostragem

Nesse trabalho foram analisadas sete espécies do gênero *Dichotomius* coletados em remanescentes de restinga de Maracaípe – Pernambuco, Brasil (MAC) (8°31’26”S 35°01’31”W) e de Maraú – Bahia, Brasil (MAR) (14°09’38”S 39°00’23”W) e de Mata Atlântica em Santa Luzia do Intahy – Sergipe, Brasil (SLI) (11°22’11”S 37°25’12”W) (Quadro 1 e Figura 1). As espécies estudadas estão distribuídas em populações separadas por pelo menos 500 km. As coletas foram realizadas com armadilhas do tipo *pitfall* iscadas com fezes humanas e, autorizadas pelos administradores das áreas de estudo e pelo IBAMA/SISBIO, licença

permanente de material zoológico da Classe Insecta (16278-1) e licença especifica para *D.* (*L.*) *schiffleri* (50438-1).

Figura 1 - Mapa da região nordeste do Brasil destacando os pontos de coletas.



Quadro 1- Espécies de *Dichotomius* analisadas e populações amostradas no nordeste do Brasil

Espécies	Subgênero/grupo	População	Nº tombamento
D. bos (Blanchard, 1846)	*Dichotomius s. str.*	SLI: Santa Luzia do Intahy – Sergipe	M10485 e M10487
D. geminatus (Arrow, 1913)	*Luederwaldtinia*	MAC: Maracaípe – Pernambuco SLI: Santa Luzia do Intahy – Sergipe	M9046, M9058, M10480 e M10481
D. guaribensis Valois, Vaz-de-Mello & Silva, 2017	*Luederwaldtinia/* grupo *D.* (*L.*) *sericeus*	MAC: Maracaípe – Pernambuco	M9037 e M9042
D. iannuzziae Valois Vaz-de-Mello & Silva, 2017	*Luederwaldtinia/* grupo *D.* (*L.*) *sericeus*	SLI: Santa Luzia do Intahy – Sergipe	M10451 e 10452
D. nisus (Olivier, 1789)	*Luederwaldtinia*	MAC: Maracaípe – Pernambuco	M9078 e 9080
D. schiffleri Vaz-de-Mello, Louzada & Gavino, 2001	*Luederwaldtinia/* grupo *D.* (*L.*) *sericeus*	MAC: Maracaípe – Pernambuco SLI: Santa Luzia do Intahy – Sergipe MAR: Maraú – Bahia	M9012, M9031, M8999,M10659, M10664 e M10669
D. semisquamosus (Curtis, 1845)	*Luederwaldtinia*	MAC: Maracaípe – Pernambuco	M9099

Extração de DNA, isolamento e sequenciamento de DNA

Para extração de DNA foi utilizado o tecido do pronoto e o protocolo descrito por Sambrook e Russel (2001). Nas diferentes espécies, a presença de elementos de transposição foi verificada através da reação em cadeia da polimerase (PCR) em três indivíduos de cada espécie por cada população. Nessas reações foram utilizados *primers* desenhados na região da transposase dos elementos estruturalmente conservados *DsTc1_5F* e *DsPogo_8F* de *D.* (*L.*) *schiffleri*, que pertence a superfamília *Tc1-Mariner* e caracterizados por Amorim et al. (*in prep*). Esses *primers* foram: *DsTc1_5F* (5'ATGGATTCTGCGACGGTAAC) e *DsTc1_5R* (5'CAAATCGGGT AAGCTGGTGT); *DsPogo_8F* (5'CACACGTTGCTTCTCACTC) e *DsPogo_8R* (5' GGCATCAAAATCTG GAAAGC).

A presença dos elementos foi confirmada através do sequenciamento e pelo tamanho do fragmento amplificado e esperado, sendo 400pb para o *DsTc1_5* e de 600pb para o *DsPogo_8*. Para o sequenciamento, os produtos amplificados por PCR foram purificados com ExoSAP-IT (Affymetrix/USB) e sequenciados pela Macrogen Inc, através do sequenciador automático ABI3730XL (ABI, CA, USA). As sequências obtidas foram processadas no pacote Staden, visando obter a sequência consenso e excluir da análise as bases com qualidade de Phred menor que 20. Para a confirmação dos elementos foi realizado o alinhamento no MAFFT (https://mafft.cbrc.jp/alignment/server/) das sequências obtidas com os elementos descritos em *D.* (*L.*) *schiffleri* por Amorim et al. (*in prep.*).

Preparação cromossômica e Hibridização *in situ* Fluorescente (FISH)

As preparações cromossômicas foram obtidas através da técnica clássica de esmagamento dos folículos testiculares em ácido acético a 50%. A hibridização *in situ* fluorescente (FISH) seguiu o protocolo de Pinkel et al. (1986) e as modificações propostas por Cabral-de-Mello et al. (2010) para as espécies de Scarabaeinae. As sondas foram hibridizadas

simultaneamente, sendo os elementos *DsTc1_5* e *DsPogo_8* marcados com dUTP-digoxigenin (Roche) e biotin-14-dATP (Invitogen), respectivamente. Essas sondas foram detectadas com anti-digoxigenin-rhodamine (Roche) e FITC-avidin (Invitogen).

A FISH foi realizada em *D.* (*L.*) *schiffleri*, *D.* (*L.*) *guaribensis* e *D.* (*L.*) *iannuzziae* (que pertencem ao grupo *D.* (*L.*) *sericeus*), sendo essa análise realizada em diferentes populações com base na distribuição dessas espécies. As imagens foram capturadas no microscópio de epifluorescência Leica DM 2500 e o brilho e contraste das imagens foram otimizados no Photoshop CS5.

RESULTADOS

A amplificação por PCR revelou a presença dos elementos com domínios conservados *DsTc1_5* e *DsPogo_8*, pertencentes a superfamília *Tc1-Mariner*, nas diferentes espécies analisadas. Contudo, não houve amplificação do transposon *DsTc1_5* em *D. iannuzziae* e em *D. geminatus* da população de Maracaípe, Pernambuco (Quadro 2).

Quadro 2 - Presença ou ausência de amplificação dos *elementos DsTc1_5 e DsPogo_8* nas espécies do gênero *Dichotomius* analisadas.

Espécies	***D. bos***	***D. geminatus***		***D. guaribensis***	***D. iannuzziae***	***D. nisus***	***D. schiffleri***			***D. semisquamosus***
População / TE	SLI	MAC	SLI	MAC	SLI	MAC	MAC	MAR	SLI	MAC
DsTc1_5	P	A	P	P	A	P	P	P	P	P
DsPogo_8	P	P	P	P	P	P	P	P	P	P

MAC = Maracaípe; MAR = Maraú; SLI = Santa Luzia do Itanhy; P = Presença; A = Ausência.

O mapeamento cromossômico de *DsTc1_5* e *DsPogo_8* revelou sinais dispersos nas regiões eucromáticas dos cromossomos em todas as espécies e populações analisadas (Figura 2). Em *D. schiffleri* (2n=18, Xy_r), foram observados sinais na maioria dos autossomos e ausência nos cromossomos sexuais (Figura 2a, b, c). Na população Maracaípe – PE, os dois elementos estão presentes em todos os autossomos, exceto no par quatro. O *DsPogo_8* também

está ausente no par oito (Figura 2a). Em Maraú – BA, esses transposons estão presentes em todos os autossomos (Figura 2b). Na população de Santa Luzia do Itanhy – SE, o elemento *DsPogo_8* está disperso em todos os autossomos, exceto no par seis (Figura 2c). Nessa população, o *DsTc1_5* está presente apenas nos bivalentes um, quatro, cinco e sete (Figura 2c).

Na espécie *D. guaribensis* (2n=18, Xy_r), o elemento *DsPogo_8* foi observado nos cinco primeiros pares autossômicos, enquanto que *DsTc1_5* está presente em todos os cromossomos, exceto no par seis (Figura 2d). Em *D. iannuzziae* (2n=18, Xy_p), apenas o transposon *DsPogo_8* foi observado, estando presente em todos os cromossomos, exceto no par seis (Figura 2e). A análise comparativa mostrou uma maior abundância dos elementos *DsTc1_5* e *DsPogo_8* em *D. schiffleri*, nas populações de Maracaípe – PE e Maraú – BA, respectivamente (Figura 2). Foi observada também uma grande abundância do *DsPogo_8* em *D. guaribensis* (Figura 2d).

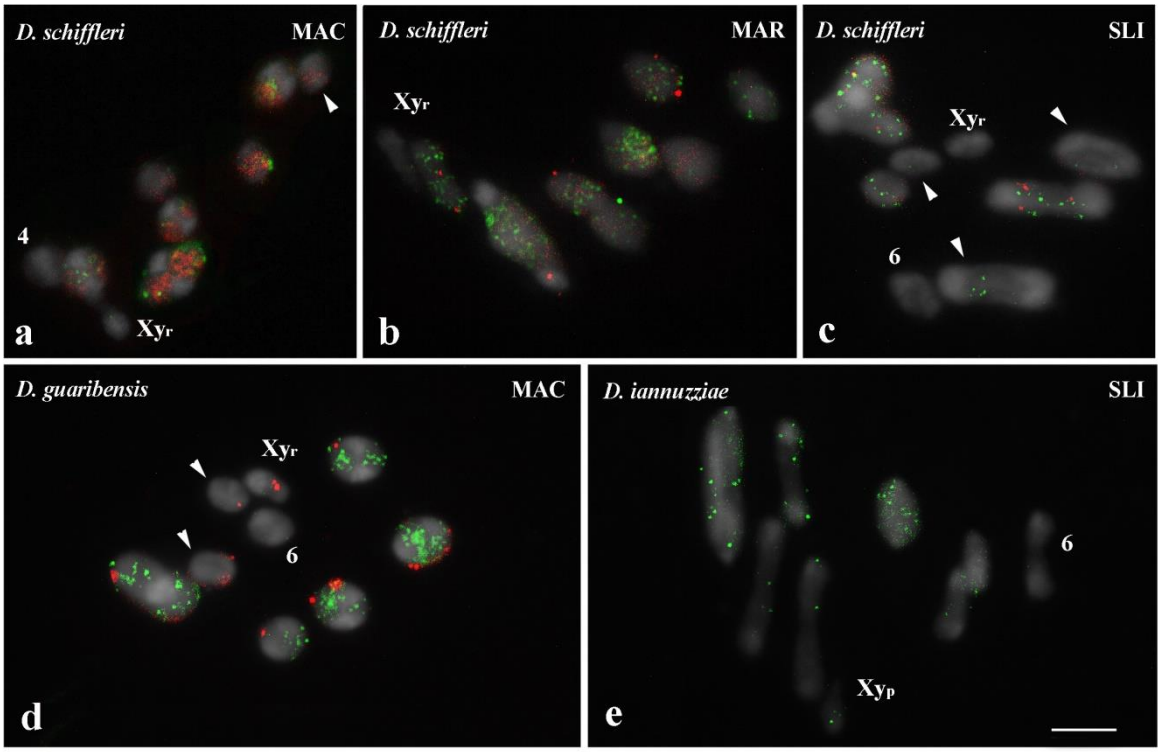


Figura 2 - Mapeamento cromossômico dos elementos *DsTc1_5* (Vermelho) *e DsPogo_8 (*verde) em células meióticas de espécies do grupo *Dichotomius* (*L.*) *sericeus.* Em (a,b,c) *D. schiffleri* das populações de Maracaípe, Pernambuco (MAC), Maraú – Bahia (MAR) e Santa Luzia de Itanhy – Sergipe (SLI). Em (d) *D. guaribensis* de MAC. Em (e) *D. iannuzziae* de SLI. Os números indicam os pares cromossômicos sem marcação, enquanto que as cabeças de seta os pares marcados apenas por um elemento. Barra = 5 µm.

DISCUSSÃO

A presença de transposons de DNA estruturalmente conservados em diferentes espécies do grupo *D.* (*L.*) *sericeus*, do subgênero *Luederwaldtinia* e de subgêneros diferentes, sugere uma conservação desses transposons em *Dichotomius.* No gênero *Dichotomius*, esses elementos colonizaram possivelmente o ancestral comum dessas espécies e sua distribuição pode estar relacionada à herança vertical, como relatado para diferentes elementos de transposição em espécies de *Drosophila* (Bargues and Lerat 2017) e em insetos da família Aphididae (Bouallègue et al. 2017).

A ausência de *DsTc1_5* em *D.* (*L.*) *iannuzziae* e numa população de *D.* (*L.*) *geminatus* revela uma distribuição desigual desse elemento. Esse tipo de distribuição pode ser explicado pela perda estocástica de elementos nos genomas de algumas espécies (Silva et al. 2004; Wallau et al. 2012), como proposto no gênero *Drosophila* (Bargues and Lerat 2017). Essa perda pode ser evidenciada pela variação populacional do elemento (Ortiz and Wallau 2015), como observado neste trabalho em *D. geminatus*. Nessa espécie, a ausência do *DsTc1_5* em Maracaípe pode estar relacionada diretamente ao tamanho do fragmento (que segundo dados do ICMBIO e UC Brasil, possui 76 hectares e menos que 0,15% dos outros fragmentos), uma vez que em pequenas populações as perdas aleatórias de sequências gênicas, incluindo os elementos de transposição são mais frequentes (Ortiz and Wallau 2015). Contudo, não podemos descartar que o elemento *DsTc1_5* apresente uma grande variação na sua sequência, o que impediria a sua detecção.

A localização dos transposons *DsTC1_5* e *DsPogo_8* em regiões eucromáticas é semelhante aos elementos MITE mapeados em espécies do grupo *D.* (*L.*) *sericeus*, como *D.* (*L.*) *schiffleri* (Xavier et al. 2014), *D.* (*L.*) *gilletti* e *D.* (*L.*) *guaribensis* (Amorim et al. *in press*). Nas outras espécies de Scarabaeinae (*Coprophanaeus cyanescens*, *C. ensifer* e *Diabroctis mimas*)

em que foram mapeados elementos *Tc1-Mariner*, foram observados sinais predominantemente nas regiões heterocromáticas (Oliveira et al. 2013).

A presença dos elementos *DsTC1_5* e *DsPogo_8* nas regiões eucromáticas, pode estar relacionada a modificações diretas na estrutura e expressão dos genes, como descrito na literatura (Maumus et al. 2015; Warren et al. 2015). Entretanto, os elementos *DsTC1_5* e *DsPogo_8* podem estar inseridos em regiões intergênicas ou em pseudogenes, como proposto anteriormente no gafanhoto *Eyprepocnemis plorans* (Montiel et al. 2012) e para o elemento *DgMarMITE* em espécies do grupo *D.* (*L*). *sericeus* (Amorim et al. *in press*). Adicionalmente, nessa região a riqueza de elementos pode estar relacionada com microrrearranjos cromossômicos, os quais foram descritos anteriormente na literatura (Maumus et al. 2015).

Quando o mapeamento populacional e interespecífico dos elementos *DsTC1_5* e *DsPogo_8* foram comparados, foi verificada uma variação na localização e abundância desses transposons, indicando mobilidade e atividade desses elementos. Nos *Tc1-Mariner*, essa atividade foi proposta em poucas espécies (Rivera-Vega and Mittapalli 2010), como *Drosophila mauritiana* (Jacobson et al. 1986) e a formiga *Messor bouvieri* (Muñoz-López et al. 2008). Em *Dichotomius* evidência de atividade *in trans* foi recentemente encontrada para o elemento não autônomo *DgMarMITE*, em espécies do grupo *D.* (*L.*) *sericeus* (Amorim et al. *in press*).

A integridade molecular, possível atividade e a presença dos transposons analisados em diferentes espécies de *Dichotomius*, tornam esses elementos candidatos para a remodelagem e evolução genômica desse gênero. Por outro lado, a dispersão desses elementos nas regiões eucromáticas sugere que eles não estão relacionados diretamente com a variação na composição da heterocromatina e na localização da fração C_0t*-1* DNA, identificada por Cabral-de-Mello et al. (2011a). Dessa forma, para esclarecer mais sobre essa variação é necessário mapear outros

transposons de DNA estruturalmente conservados no gênero *Dichotomius*, como por exemplo, os elementos *PiggyBac*.

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REFERÊNCIAS

Amorim IC, Costa RGC, Xavier C, Moura R de C (*in press*) Characterization and chromosomal mapping of the *Dgmar*MITE transposon in populations of *Dichotomius* (Luederwaldtinia) sericeus species complex (Coleoptera: Scarabaeidae). IN PRESS

Bargues N, Lerat E (2017) Evolutionary history of LTR-retrotransposons among 20 *Drosophila* species. Mob DNA 8:7 . doi: 10.1186/s13100-017-0090-3

Biscotti MA, Olmo E, Heslop-Harrison JS (Pat) (2015) Repetitive DNA in eukaryotic genomes. Chromosom Res 23:415–420. doi: 10.1007/s10577-015-9499-z

Bouallègue M, Filée J, Kharrat I, et al (2017) Diversity and evolution of mariner-like elements in aphid genomes. BMC Genomics 18:1–12. doi: 10.1186/s12864-017-3856-6

Cabral-de-Mello DC, de Moura R de C, de Melo AS, Martins C (2011a) Evolutionary dynamics of heterochromatin in the genome of *Dichotomius* beetles based on chromosomal analysis. Genetica 139:315–325. doi: 10.1007/s10709-011-9551-7

Cabral-de-Mello DC, Moura RC, Martins C (2010) Chromosomal mapping of repetitive DNAs in the beetle *Dichotomius geminatus* provides the first evidence for an association of 5S rRNA and histone H3 genes in insects, and repetitive DNA similarity between the B chromosome and A complement. Heredity (Edinb) 104:393–400. doi: 10.1038/hdy.2009.126

Cabral-de-Mello DC, Moura RC, Martins C (2011b) Cytogenetic mapping of rRNAs and histone H3 genes in 14 species of *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae) beetles. Cytogenet Genome Res 134:127–135. doi: 10.1159/000326803

Cabral-de-Mello DC, Oliveira SG, de Moura RC, Martins C (2011c) Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: insights into the evolutionary dynamics of multigene families and heterochromatin. BMC Genet 12:1–12. doi: 10.1186/1471-2156-12-88

Delprat A, Negre B, Puig M, Ruiz A (2009) The transposon Galileo generates natural chromosomal inversions in *Drosophila* by ectopic recombination. PLoS One 4:1–13. doi: 10.1371/journal.pone.0007883

Dotto BR, Carvalho EL, Silva AF, et al (2015) HTT-DB: Horizontally transferred transposable elements database. Bioinformatics 31:2915–2917. doi: 10.1093/bioinformatics/btv281

Fonseca NA, Vieira CP, Schlötterer C, Vieira J (2012) The DAIBAM MITE element is involved in the origin of one fixed and two polymorphic *Drosophila virilis* phylad inversions. Fly (Austin) 6:71–74. doi: 10.4161/fly.19423

Hartl DL, Lozovskaya ER, Lawrence JG (1992) Nonautonomous transposable elements in prokaryotes and eukaryotes. Genetica 86:47–53. doi: 10.1007/BF00133710

Jacobson JW, Medhora MM, Hartl DL (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. Proc Natl Acad Sci USA 83:8684–8688. doi: 10.1073/pnas.83.22.8684

Kidwell MG (2005) Transposable Elements. In: Gregory TR (ed) The Evolution of the Genome, 1st edn. Elsevier ed., San Diego, pp 165–221

Lerat E (2010) Identifying repeats and transposable elements in sequenced genomes: how to find your way through the dense forest of programs. Heredity (Edinb) 104:520–533. doi: 10.1038/hdy.2009.165

Maumus F, Fiston-Lavier AS, Quesneville H (2015) Impact of transposable elements on insect genomes and biology. Curr Opin Insect Sci 7:30–36. doi: 10.1016/j.cois.2015.01.001

Montiel EE, Cabrero J, Camacho JPM, López-León MD (2012) Gypsy, RTE and Mariner transposable elements populate *Eyprepocnemis plorans* genome. Genetica 140:365–374.

doi: 10.1007/s10709-012-9686-1

Muñoz-López M, Siddique A, Bischerour J, et al (2008) Transposition of Mboumar-9: Identification of a New Naturally Active mariner-Family Transposon. J Mol Biol 382:567–572. doi: 10.1016/j.jmb.2008.07.044

Oliveira SG, Cabral-de-Mello DC, Moura RC, Martins C (2013) Chromosomal organization and evolutionary history of *Mariner* transposable elements in Scarabaeinae coleopterans. Mol Cytogenet 6:1–9. doi: 10.1186/1755-8166-6-54

Ortiz M de F, Wallau GL (2015) Transferência Horizontal de Elementos de Transposição. In: Carareto CMA, Monteiro-Vitorello CB, Sluys M-A Van (eds) Elementos de transposição. Editora Fiocruz, Rio de Janeiro, pp 115–140

Peccoud J, Loiseau V, Cordaux R, Gilbert C (2017) Massive horizontal transfer of transposable elements in insects. Proc Natl Acad Sci 114:4721–4726. doi: 10.1073/pnas.1621178114

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938. doi: 10.1073/pnas.83.9.2934

Rivera-Vega L, Mittapalli O (2010) Molecular characterization of mariner-like elements in emerald ash borer, agrilus planipennis (Coleoptera, Polyphaga). Arch Insect Biochem Physiol 74:205–216. doi: 10.1002/arch.20357

Sambrook J, Russel WD (2001) Molecular Cloning. A Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York

Silva JC, Loreto EL, Clark JB (2004) Factors that affect the horizontal transfer of transposable elements. Curr issues Mol Biol 6: 57-71.

Smith S, Virkki N (1978) Coleoptera. In: John B (ed) Animal Cytogenetics. Berlin, Stuttgart, p 366

Vidal R (1984) Chromosome numbers of Coleopteru from Argentina. Genetica 65: 235-239.

Wallau GL, Ortiz MF, Loreto ELS (2012) Horizontal transposon transfer in eukarya: Detection, bias, and perspectives. Genome Biol Evol 4:689–699. doi: 10.1093/gbe/evs055

Warren IA, Naville M, Chalopin D, et al (2015) Evolutionary impact of transposable elements on genomic diversity and lineage-specific innovation in vertebrates. Chromosom Res 23:505–531. doi: 10.1007/s10577-015-9493-5

Xavier C, Cabral-de-Mello DC, de Moura RC (2014) Heterochromatin and molecular characterization of *Dsmar*MITE transposable element in the beetle *Dichotomius schiffleri* (Coleoptera: Scarabaeidae). Genetica 142:575–581. doi: 10.1007/s10709-014-9805-2

4 DISCUSSÃO GERAL

4.1 CARACTERIZAÇÃO DE ELEMENTOS DE TRANSPOSIÇÃO NO GRUPO *D.* (*L.*) *sericeus*

No genoma parcial de *Dichotomius* (*L.*) *schiffleri* foram identificados elementos de transposição pertencentes a 11 superfamílias. Esses elementos foram caracterizados por ferramentas bioinformáticas com abordagens *de novo* e de homologia e que se demonstram eficientes apenas quando combinadas. Esse resultado demonstrou a necessidade de utilizar metodologias conjuntas para caracterizar os elementos de uma espécie, como sugerido por Lerat (2010). A analise estrutural revelou TEs conservados do tipo *Copia*, *Gypsy*, *PiggyBac* e *Tc1-Mariner*. Esses elementos apresentaram estrutura e/ou tamanho dos elementos autônomos, descritas por Wicker et al. (2007) e Feschotte e Pritham, (2007), exceto as repetições terminais e o gene *gag* nos *Gypsy*.

Em *D.* (*L.*) *gilletti*, a amplificação por PCR possibilitou o isolamento do *Dgmar*MITE, através de *primers* de elementos de outros insetos. Esse elemento apresentou características de elementos degenerados MITEs, incluindo o grande número de cópias, riqueza em AT, repetições terminais invertidas (TIRs) perfeitas e ausência de transposase (KUANG et al., 2009). Quando analisada, a TIR desse elemento apresentou 100% de similaridade com o TE *AfMar*2 de *Abracris flavolineata* (PALACIOS-GIMENEZ; BUENO; CABRAL-DE-MELO, 2014). Esse resultado indica que o elemento isolado em *D.* (*L.*) *gilletti* pertence a família *Mariner*.

4.2 EVOLUÇÃO DOS ELEMENTOS DE TRANSPOSIÇÃO

Em *D.* (*L.*) *schiffleri* foi observada a similaridade de elementos com TEs de espécies filogeneticamente distantes, além da incongruência nas filogenias desses elementos e dos hospedeiros. Essas características são consideradas uma das principais evidências de transferência horizontal (HT) (SILVA; LORETO; CLARK, 2004; WALLAU; ORTIZ; LORETO, 2012). Considerando esses resultados, foi sugerido a ocorrência de transferência horizontal nos TEs *PiggyBac*, *Pogo* e *ISL2EU* entre diferentes táxons, como espécies de platelminto, fungo, cnidário, nematódeo, formiga e cordata.

Na transferência horizontal o compartilhamento geográfico e de nichos são pré-requisitos essenciais (CARARETO, 2011; SILVA; LORETO; CLARK, 2004). Quando

comparamos com *D.* (*L.*) *schiffleri*, a maioria das espécies com os possíveis HT apresentaram distribuição geográfica semelhantes, como o cnidário *Hydra vulgaris* (JANKOWSKI; COLLINS; CAMPBELL, 2008) e o fungo *Nosema apis* (ELLIS; MUNN, 2005). Além disso, as espécies da HT apresentam interações ecológicas bem definidas com os coleópteros e/ou escarabeíneos, como parasitismo intracelular (BLASER; SCHMID-HEMPEL, 2005), hábito alimentar (BARDEN; HELD; “FUDD” GRAHAM, 2011; ZENGER; GIBB, 2001) e recurso alimentar (NEJSUM et al., 2009). Adicionalmente, essa transferência pode ter ocorrido por espécies intermediarias, como os vetores (SILVA; LORETO; CLARK, 2004).

Apesar desses eventos de HT, a maioria dos elementos caracterizados possuem provavelmente uma herança vertical, como o *DsTc1_5* e *DsPogo_8*. Nesses transposons, a distribuição em diferentes espécies de *Dichotomius* sugere fortemente uma colonização antiga e uma herança vertical, assim como em TEs de *Drosophila* (BARGUES; LERAT, 2017). Essa distribuição desigual deve estar relacionada à perda estocástica do elemento, como descrito na literatura (SILVA; LORETO; CLARK, 2004; WALLAU; ORTIZ; LORETO, 2012) e relatado em *Drosophila* (BARGUES; LERAT, 2017).

Em relação ao *Dgmar*MITE, a presença desse transposon apenas nas espécies relacionadas, *D.* (*L.*) *gilletti* e *D.* (*L.*) *guaribensis*, sugere origem no ancestral dessas espécies e uma herança vertical para elas. Alternativamente, nessas espécies a origem do *Dgmar*MITE pode ser independente, através da transferência horizontal, uma vez que essa transferência é frequentemente relatada nos TEs *Mariner* (ROBERTSON, 1995; ROBERTSON; LAMPE, 1995a), incluindo os MITE (MINAYA et al., 2013). Nesse sentido, a origem do *Dgmar*MITE parece ser mais recente em *D.* (*L.*) *gilletti*, uma vez que o mapeamento cromossômico revelou uma localização predominante na eucromatina e pares heteromórficos, os quais podem ser característicos para elementos novos (JUNAKOVIC et al., 1998). Contudo, essa diferença pode estar relacionada com uma amplificação diferencial dessas sequências ou com a eliminação desse elemento na região heterocromática de *D.* (*L.*) *guaribensis*.

4.3 MAPEAMENTO CROMOSSÔMICO DOS ELEMENTOS DE TRANSPOSIÇÃO

Nas espécies do grupo *D.* (*L.*) *sericeus*, o mapeamento cromossômico do *Dgmar*MITE, *DsTC1_5* e *DsPogo_8*, que pertencem a superfamília *Tc1-Mariner*, revelou uma dispersão nas regiões eucromáticas. Esse resultado é semelhante a

outros transposons de DNA mapeados, incluindo o *DsMarMITE* em *D.* (*L.*) *schiffleri* (XAVIER; CABRAL-DE-MELLO; MOURA, 2014). Nessas regiões, esses elementos podem estar relacionados a modificações na estrutura e expressão dos genes (MAUMUS; FISTON-LAVIER; QUESNEVILLE, 2015; WARREN et al., 2015), inseridos em regiões intergênicas ou em pseudogenes, como proposto no gafanhoto *Eyprepocnemis plorans* (MONTIEL et al., 2012). Além disso, foi observado o *Dgmar*MITE nas regiões heterocromáticas de *D.* (*L.*) *guaribensis*, semelhante a outros TEs *Mariner* mapeados em Scarabaeinae (OLIVEIRA et al., 2013). Esses elementos podem constituir uma importante fração da heterocromatina constitutiva dessas espécies.

Adicionalmente, o mapeamento cromossômico revelou uma variação na localização e abundância entre as espécies e/ou populações. Esse resultado indica atividade dos transposons estruturalmente conservados (*DsTC1_5* e *DsPogo_8*) e atividade *in trans* do elemento não autônomo (*Dgmar*MITE). Nesse último caso, os elementos podem utilizar a transposase de TEs proximamente relacionados ou não (GARZA et al., 1991; YANG et al., 2009). Nos insetos, a maioria dos elementos *Tc1-Mariner* não está ativo (RIVERA-VEGA; MITTAPALLI, 2010; ROBERTSON; LAMPE, 1995b), sendo a atividade descrita em poucas espécies, como na formiga *Messor bouvieri* (MUÑOZ-LÓPEZ et al., 2008). Alternativamente, nas regiões eucromáticas e heterocromáticas, a variação na abundância do *Dgmar*MITE pode estar relacionada a recombinação ectópica e/ou outros mecanismos de evolução em concerto, como proposto em TEs de *Drosophila erecta* (LOHE et al., 1995).

Quando comparado com a dinâmica das sequências repetitivas no gênero *Dichotomius*, proposta por Cabral-de-Mello et al. (2011b), o mapeamento dos elementos *Dgmar*MITE, *DsTC1_5* e *DsPogo_8* revelou padrões cromossômicos distintos, indicando que esses transposons não estão relacionados diretamente com essa dinâmica. Contudo, outros elementos que ainda não foram mapeados podem estar associados a essa dinâmica. Dessa forma, é necessário mapear outros elementos potencialmente ativos identificados nesse estudo, como os TEs da superfamília *PiggyBac*.

5 CONCLUSÕES

1. Os elementos de transposição estruturalmente conservados do tipo *Copia*, *Gypsy*, *PiggyBac* e Tc1-*Mariner* caracterizados em *Dichotomius* (*L.*) *schiffleri,* são provavelmente ativos e podem estar relacionados com a evolução do genoma dessa espécie.
2. No gênero *Dichotomius*, os transposons *DsTC1_5* e *DsPogo_8* apresentam possivelmente uma origem antiga e distribuição pela herança vertical, enquanto que *DgMar*MITE origem recente e múltipla, através de transferência horizontal.
3. A variação interespecífica e populacional dos elementos *DsTC1_5*, *DsPogo_8* e *DgMar*MITE, referente a localização e abundância, está relacionada a sua atividade e/ou recombinação ectópica.
4. A presença dos elementos *DsTC1_5*, *DsPogo_8* e *DgMar*MITE em regiões eucromáticas, sugere que esses transposons podem afetar a expressão e estrutura de genes e que possivelmente não estão relacionados à dinâmica das sequências repetitivas no gênero *Dichotomius*.

REFERÊNCIAS

BARDEN, S. A.; HELD, D. W.; “FUDD” GRAHAM, L. C. Lack of Interactions Between Fire Ant Control Products and White Grubs (Coleoptera: Scarabaeidae) in Turfgrass. **Journal of Economic Entomology**, [s. l.], v. 104, n. 6, p. 2009–2016, 2011.

BARGUES, N.; LERAT, E. Evolutionary history of LTR-retrotransposons among 20 *Drosophila* species. **Mobile DNA**, [s. l.], v. 8, n. 1, p. 7, 2017.

BLASER, M.; SCHMID-HEMPEL, P. Determinants of virulence for the parasite Nosema whitei in its host *Tribolium castaneum*. **Journal of Invertebrate Pathology**, [s. l.], v. 89, n. 3, p. 251–257, 2005.

BRASIL. Lista Nacional Oficial de Espécies da Fauna Ameaçada de Extinção. Portaria nº 444, de 17 de dezembro de 2014. Ministério do Meio Ambiente. **Diário Oficial da União**, [s. l.], v. 245, n. 1, p. 121–126, 2014.

BROWN, J. R. Ancient horizontal gene transfer. **Nature Reviews Genetics**, [s. l.], v. 4, n. 2, p. 121–132, 2003.

CABRAL-DE-MELLO, D. C. et al. Karyotype differentiation patterns in species of the subfamily Scarabaeinae (Scarabaeidae, Coleoptera). **Micron**, [s. l.], v. 39, n. 8, p. 1243–1250, 2008.

CABRAL-DE-MELLO, D. C. et al. Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: insights into the evolutionary dynamics of multigene families and heterochromatin. **BMC Genetics**, [s. l.], v. 12, n. 1, p. 88, 2011. a.

CABRAL-DE-MELLO, D. C. et al. Evolutionary dynamics of heterochromatin in the genome of *Dichotomius* beetles based on chromosomal analysis. **Genetica**, [s. l.], v. 139, n. 3, p. 315–325, 2011. b.

CARARETO, C. M. Tropical Africa as a cradle for horizontal transfers of transposable elements between species of the genera *Drosophila* and *Zaprionus*. **Mobile genetic elements**, [s. l.], v. 1, n. 3, p. 179–186, 2011.

CHALOPIN, D. et al. Comparative analysis of transposable elements highlights mobilome diversity and evolution in vertebrates. **Genome Biology and Evolution**, [s. l.], v. 7, n. 2, p. 567–580, 2015.

CHOI, J.H. et al. Gene discovery in the horned beetle *Onthophagus taurus*. **BMC Genomics**, [s. l.], v. 11, n. 1, p. 703, 2010.

COSTA, C. Estado de conocimiento de los Coleoptera Neotropicales. **Knowledge Creation Diffusion Utilization**, [s. l.], 2000.

COSTA, C. M. Q.; BARRETTO, J. W.; MOURA, R. de C. Changes in the dung beetle community in response to restinga forest degradation. **Journal of Insect Conservation**, [s. l.], v. 18, n. 5, p. 895–902, 2014.

CUNNINGHAM, C. B. et al. The genome and methylome of a beetle with complex social behavior, *Nicrophorus vespilloides* (coleoptera: Silphidae). **Genome Biology and Evolution**, [s. l.], v. 7, n. 12, p. 3383–3396, 2015.

DJEBBI, S. et al. Molecular characterization of mariner-like elements in *Bruchus pisorum* and *Bruchus rufimanus* (Coleoptera: Bruchidae). **Archives of Biological Sciences**, [s. l.], v. 69, n. 2, p. 353–360, 2017.

ELLIS, J. D.; MUNN, P. A. The worldwide health status of honey bees. **Bee World**, [s. l.], v. 86, n. 4, p. 88–101, 2005.

FESCHOTTE, C.; PRITHAM, E. J. DNA Transposons and the Evolution of Eukaryotic Genomes. **Annual Review of Genetics**, [s. l.], v. 41, n. 1, p. 331–368, 2007.

FLECHTMANN, C. A. H.; RODRIGUES, S. R.; SENO, M. C. Z. Controle biológico da mosca-dos-chifres (*Haematobia irritans irritans*) em Selvíria, Mato Grosso do Sul. 3. Levantamento de espécies fimícolas associadas à mosca. **Revista Brasileira de Entomologia**, [s. l.], v. 39, p. 249–258, 1995.

FUJIWARA, H. et al. Telomere-specific non-LTR retrotransposons and telomere maintenance in the silkworm, *Bombyx mori*. **Chromosome Research**, [s. l.], v. 13, n. 5, p. 455–467, 2005.

GARZA, D. et al. Introduction of the transposable element mariner into the germline of *Drosophila melanogaster*. **Genetics**, [s. l.], v. 128, n. 2, p. 303–310, 1991.

GRZEBELUS, D. et al. Diversity and structure of PIF/Harbinger-like elements in the genome of *Medicago truncatula*. **BMC Genomics**, [s. l.], v. 8, p. 1–14, 2007.

HALFFTER, G. **Historical and ecological factors determining the geographical distribution of beetles (Coleoptera: Scarabaeidae: Scarabaeinae)**, 1991.

HAN, M. J. et al. The diversification of PHIS transposon superfamily in eukaryotes. **Mobile DNA**, [s. l.], v. 6, n. 1, p. 1–11, 2015.

HARTL, D. L.; LOZOVSKAYA, E. R.; LAWRENCE, J. G. Nonautonomous transposable elements in prokaryotes and eukaryotes. **Genetica**, [s. l.], v. 86, n. 1–3, p. 47–53, 1992.

HERNANDEZ-HERNANDEZ, E. M. et al. Genome-wide analysis of transposable elements in the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): description of novel families. **Molecular Genetics and Genomics**, [s. l.], v. 292, n. 3, p. 565–583, 2017.

JAKUBCZAK, J. L.; BURKE, W. D.; EICKBUSH, T. H. Retrotransposable elements R1 and R2 interrupt the rRNA genes of most insects. **Proceedings of the National Academy of Sciences of the United States of America**, [s. l.], v. 88, n. 8, p. 3295–3299, 1991.

JANKOWSKI, T.; COLLINS, A. G.; CAMPBELL, R. Global diversity of inland water cnidarians. **Hydrobiologia**, [s. l.], v. 595, n. 1, p. 35–40, 2008.

JUNAKOVIC, N. et al. Accumulation of transposable elements in the heterochromatin and on the Y chromosome of *Drosophila simulans* and *Drosophila melanogaster*. **Journal of Molecular Evolution**, [s. l.], v. 46, n. 6, p. 661–668, 1998.

KEELING, C. I. et al. Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. **Genome Biology**, [s. l.], v. 14, n. 3, p. R27, 2013.

KIDWELL, M. G. Transposable Elements. In: GREGORY, T. R. (Ed.). **The Evolution of the Genome**. 1. ed. San Diego: Elsevier ed., 2005. p. 165–221.

KIDWELL, M. G.; LISCH, D. R. Perspective: Transposable Elements, Parasitic Dna, and Genome Evolution. **Evolution**, [s. l.], v. 55, n. 1, p. 1–24, 2001.

KOJIMA, K. K.; JURKA, J. Crypton transposons: Identification of new diverse families and ancient domestication events. **Mobile DNA**, [s. l.], v. 2, n. 1, p. 1–17, 2011.

KORASAKI, V. et al. Taxocenose de Scarabaeinae (Coleoptera: Scarabaeidae) em Benjamin Constant, AM. **Acta Amazonica**, [s. l.], v. 42, n. 3, p. 423–432, 2012.

KUANG, H. et al. Identification of miniature inverted-repeat transposable elements (MITEs) and biogenesis of their siRNAs in the Solanaceae: New functional implications for MITEs. **Genome Research**, [s. l.], v. 19, n. 1, p. 42–56, 2009.

LAMPE, D. J. et al. Recent horizontal transfer of mellifera subfamily mariner transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer. **Molecular Biology and Evolution**, [s. l.], v. 20, n. 4, p. 554–562, 2003.

LOHE, A. R. et al. Horizontal transmission, vertical inactivation, and stochastic loss of

Mariner-like transposable elements. **Molecular biology and evolution**, [s. l.], v. 12, n. 1, p. 62–72, 1995.

MAUMUS, F.; FISTON-LAVIER, A. S.; QUESNEVILLE, H. Impact of transposable elements on insect genomes and biology. **Current Opinion in Insect Science**, [s. l.], v. 7, p. 30–36, 2015.

MINAYA, M. et al. Distribution and evolutionary dynamics of Stowaway Miniature Inverted repeat Transposable Elements (MITEs) in grasses. **Molecular Phylogenetics and Evolution**, [s. l.], v. 68, n. 1, p. 106–118, 2013.

MONTIEL, E. E. et al. Gypsy, RTE and Mariner transposable elements populate Eyprepocnemis plorans genome. **Genetica**, [s. l.], v. 140, n. 7–9, p. 365–374, 2012.

MONTREUIL, O. Analyse phylogénétique et paraphylie des Coprini et Dichotomiini (coleoptera: Scarabaeidae). Scénario Biogéographique. **In Annales de la Société Entomologique de France**, [s. l.], v. 34, p. 135–148, 1998.

MUÑOZ-LÓPEZ, M. et al. Transposition of Mboumar-9: Identification of a New Naturally Active *Mariner*-Family Transposon. **Journal of Molecular Biology**, [s. l.], v. 382, n. 3, p. 567–572, 2008.

NEJSUM, P. et al. Population dynamics of *Trichuris suis* in trickle-infected pigs. **Parasitology**, [s. l.], v. 136, n. 6, p. 691, 2009.

NICHOLS, E. et al. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. **Biological Conservation**, [s. l.], v. 141, n. 6, p. 1461–1474, 2008.

OLIVEIRA, S. G. et al. Chromosomal organization and evolutionary history of *Mariner* transposable elements in Scarabaeinae coleopterans. **Molecular cytogenetics**, [s. l.], v. 6, n. 1, p. 54, 2013.

OLIVEIRA, S. G.; MOURA, R. C.; MARTINS, C. B chromosome in the beetle Coprophanaeus cyanescens (Scarabaeidae): emphasis in the organization of repetitive DNA sequences. **BMC Genetics**, [s. l.], v. 13, n. 1, p. 1, 2012.

PALACIOS-GIMENEZ, O. M.; BUENO, D.; CABRAL-DE-MELO, D. C. Chromosomal mapping of two *Mariner*-like elements in the grasshopper *Abracris flavolineata* (Orthoptera: Acrididae) reveals enrichment in euchromatin. **European Journal of Entomology**, [s. l.], v. 111, n. 3, p. 329–334, 2014.

PAUCHET, Y.; HECKEL, D. G. The genome of the mustard leaf beetle encodes two active xylanases originally acquired from bacteria through horizontal gene transfer. **Proceedings. Biological sciences / The Royal Society**, [s. l.], v. 280, n. 1763, p. 20131021, 2013.

RATCLIFFE, B. C.; JAMESON, M. L.; SMITH, A. B. T. SCARABAEIDAE Latreille 1802. In: ARNETT, R. et al. (Eds.). **North**. 2. ed. [s.l.] : American Beetles, 2002. p. 39–81.

RICHARDS, S. et al. The genome of the model beetle and pest *Tribolium castaneum*. **Nature**, [s. l.], v. 452, n. 7190, p. 949–955, 2008.

RIVERA-VEGA, L.; MITTAPALLI, O. Molecular characterization of *Mariner*-like elements in emerald ash borer, *Agrilus planipennis* (Coleoptera, Polyphaga). **Archives of Insect Biochemistry and Physiology**, [s. l.], v. 74, n. 4, p. 205–216, 2010.

ROBERTSON, H. M. The *Tcl-mariner* superfamily of transposons in animals. **Journal of Insect Physiology**, [s. l.], v. 41, n. 2, p. 99–105, 1995.

ROBERTSON, H. M. et al. The mariner transposons of animals: horizontally jumping genes. In: SYVANEN, M.; KADO, C. I. (Eds.). **Horizontal gene transfer**. 2. ed. San Diego, California: Academic Press. A Division of Harcourt, Inc., 2002. p.

173–185.
ROBERTSON, H. M.; LAMPE, D. J. Recent horizontal transfer of a mariner transposable element among and between Diptera and Neuroptera. **Molecular biology and evolution**, [s. l.], v. 12, n. 5, p. 850–862, 1995. a.
ROBERTSON, H. M.; LAMPE, D. J. Distribution of transposable elements in arthropods. **Annual review of entomology**, [s. l.], v. 40, p. 333–357, 1995. b.
ROBERTSON, H. M.; MACLEOD, E. G. Five major subfamilies of *Mariner* transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. **Insect Molecular Biology**, [s. l.], v. 2, n. 3, p. 125–139, 1993.
SARMIENTO-GARCÊS, R.; AMAT-GARCÍA, G. Escarabajos del género *Dichotomius* Hope 1838 (scarabaeidae: scarabaeinae) en la amazonía colombiana. **Rev. Acad. Colomb. Cienc.**, [s. l.], v. 33, n. 127, p. 285–296, 2009.
SCHERER, A.; MARASCHIN-SILVA, F.; BAPTISTA, L. R. de M. Florística e estrutura do componente arbóreo de matas de Restinga arenosa no Parque Estadual de Itapuã, RS, Brasil. **Acta Botanica Brasilica**, [s. l.], v. 19, n. 4, p. 717–726, 2005.
SILVA, F. A. B. et al. Study of the Dung Beetle (Coleoptera: Scarabaeidae) Community at Two Sites: Atlantic Forest and Clear-Cut, Pernambuco, Brazil. **Environmental Entomology**, [s. l.], v. 39, n. 2, p. 359–367, 2010.
SILVA, J. C. et al. A Potentially Functional Mariner Transposable Element in the Protist *Trichomonas vaginalis*. **Mol Biol Evol.**, [s. l.], v. 1, n. 22, p. 126–134, 2005.
SILVA, J. C.; LORETO, E. L.; CLARK, J. B. Factors that affect the horizontal transfer of transposable elements. **Current issues in molecular biology JID - 100931761**, [s. l.], p. 57–72, 2004.
VALOIS, M. C.; VAZ-DE-MELLO, F. Z.; SILVA, F. A. B. Taxonomic revision of the *Dichotomius sericeus* (Harold, 1867) species group (Coleoptera: Scarabaeidae: Scarabaeinae). **Zootaxa**, [s. l.], v. 4277, n. 4, p. 503–530, 2017.
VAZ-DE-MELLO, F. Z. et al. **A multilingual key to the genera and subgenera of the subf amily Scarabaeinae of the New World (Coleoptera: Scarabaeidae)**. [s.l: s.n.]. v. 2854
VAZ-DE-MELLO, F. Z. **Scarabaeidae**. 2017. Disponível em: <http://fauna.jbrj.gov.br/fauna/faunadobrasil/126713>.
VAZ-DE-MELLO, F. Z.; LOUZADA, J. N. C.; GAVINO, M. Nova espécie de *Dichotomius* Hope, 1838 (Coleoptera, Scarabaeidae) do Espírito Santo, Brasil. **Revista Brasileira de Entomologia**, [s. l.], v. 45, n. 2, p. 99–102, 2001.
VIEIRA, L. et al. New records, threatens and conservation status for *Dichotomius schiffleri* Vaz-de-Mello, Louzada & Gavino (Coleoptera: Scarabaeidae): an endangered dung beetle species from Brazilian atlantic forest ecosystems. **Neotropical entomology**, [s. l.], v. 40, n. 2, p. 282–284, 2011.
VIEIRA, L.; LOUZADA, J. N. C.; SPECTOR, S. Effects of degradation and replacement of southern Brazilian Coastal sandy vegetation on the dung beetles (Coleoptera: Scarabaeidae). **Biotropica**, [s. l.], v. 40, n. 6, p. 719–727, 2008.
WALLAU, G. L.; ORTIZ, M. F.; LORETO, E. L. S. Horizontal transposon transfer in eukarya: Detection, bias, and perspectives. **Genome Biology and Evolution**, [s. l.], v. 4, n. 8, p. 689–699, 2012.
WARREN, I. A. et al. Evolutionary impact of transposable elements on genomic diversity and lineage-specific innovation in vertebrates. **Chromosome Research**, [s. l.], v. 23, n. 3, p. 505–531, 2015.
WICKER, T. et al. A unified classification system for eukaryotic transposable

elements. **Nature Reviews Genetics**, [s. l.], v. 8, n. 12, p. 973–982, 2007.

XAVIER, C.; CABRAL-DE-MELLO, D. C.; MOURA, R. C. Heterochromatin and molecular characterization of *Dsmar*MITE transposable element in the beetle *Dichotomius schiffleri* (Coleoptera: Scarabaeidae). **Genetica**, [s. l.], v. 142, n. 6, p. 575–581, 2014.

YANG, G. et al. Tuned for Transposition: Molecular Determinants Underlying the Hyperactivity of a Stowaway MITE. **Science**, [s. l.], v. 325, n. 5946, p. 1391–1394, 2009.

YUAN, Y.-W.; WESSLER, S. R. The catalytic domain of all eukaryotic cut-and-paste transposase superfamilies. **Proceedings of the National Academy of Sciences**, [s. l.], v. 108, n. 19, p. 7884–7889, 2011.

ZENGER, J. T.; GIBB, T. J. Identification and impact of egg predators of *Cyclocephala lurida* and *Popillia japonica* (Coleoptera: Scarabaeidae) in turfgrass. **Environmental Entomology**, [s. l.], v. 30, n. 2, p. 425–430, 2001.

APÊNDICE A - ARTIGO PUBLICADO NA REVISTA GENETICS AND MOLECULAR BIOLOGY

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Research Article

Characterization and chromosomal mapping of the *Dgmar*MITE transposon in populations of *Dichotomius* (*Luederwaldtinia*) *sericeus* species complex (Coleoptera: Scarabaeidae)

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Abstract

Transposable elements are dispersed repetitive DNA sequences that can move within the genome and are related to genome and chromosome evolution, adaptation, and speciation. The aim of this study was to characterize and determine the chromosomal location and accumulation of a *Mariner*-like element in populations of four phylogenetically related species of the *Dichotomius* (*Luederwaldtinia*) *sericeus* complex. Mapping of the isolated element was performed by fluorescent *in situ* hybridization in different populations of analyzed species. Characterization of the isolated element revealed a degenerated transposon, named *Dgmar*MITE. This transposon is 496-bp-long, AT rich (57%), and contains 24 bp terminal inverted repeats. *In situ* mapping revealed presence of this element only in two out of four species analyzed. *Dgmar*MITE sites were located in heterochromatic and euchromatic regions and varied in location and number on the karyotypes of *Dichotomius* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* across different populations. These results demonstrate differential accumulation of the *Dgmar*MITE in genomes of these species, which is probably due to the occurrence of ectopic recombination and cross-mobilization of the element mediated by the transposase of closely related or unrelated transposable elements.

Keywords: Mariner-like elements, cross-mobilization, chromosome evolution.

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Introduction

Mariner transposable elements are DNA transposons that exhibit broad diversity in their structure. *Mariner* elements are characterized by a size of about 1,300 bp, a single ORF (open reading frame) encoding a transposase, a conserved catalytic domain [DD(34)D] necessary for transposition, and two terminal inverted repeats (TIRs) of 28-30 bp flanked by a TA dinucleotide resulting from target site duplications (Robertson, 1995; Robertson and Lampe, 1995; Plasterk *et al.*, 1999). During transposition, the encoded transposase recognizes the TIRs and catalyzes excision of the two DNA strands at the donor site and fusion of the element at another site in the genome (Lampe *et al.*, 1996).

The *Mariner* elements are probably the most widely distributed family of transposable elements (TEs) in nature, being represented in diverse taxa, such as rotifers, fungi, plants and vertebrates. Their wide distribution in metazoan species, including arthropods (Robertson and Lampe, 1995; Wallau *et al.*, 2014), is probably related to horizontal transfer events (Robertson 1995; Robertson and Lampe, 1995; Lampe *et al.*, 2003) which, for example, account for the presence of the *Mariner*_Tbel and *Mariner*1_BT families in phylogenetically distant species such as insects and mammals (Oliveira *et al.*, 2012). These elements have been found in a wide range of insects from different orders, including Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Coleoptera (Robertson and Lampe, 1995; Palacios-Gimenez *et al.*, 2014).

The existence of nonfunctional *Mariner* elements is common, including a large number of inactive copies in different genomes (Lohe *et al.*, 1995). Some of those inactive elements, the miniature inverted repeat transposable elements (MITEs), do not encode the enzyme necessary for their transposition and therefore require the transposase of other elements for their mobilization (Kidwell, 2005). The origin of these TEs is related to the internal degeneration of autonomous elements (Deprá *et al.*, 2012). MITEs are distinguished from their autonomous counterparts by their high copy number, compact structure, short terminal in-

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verted repeats, genic preference, and DNA sequence identity (Feschotte *et al.*, 2002, Feng, 2003).

Regarding the speciose order Coleoptera, *Mariner* elements have so far been described in only a few species belonging to the families Chrysomelidae, Buprestidae, Cerambycidae, Laemophloeidae, Meloidae, Scarabaeidae, Staphylinidae and Tenebrionidae (Robertson, 1993; Robertson and Macleod, 1993; Robertson *et al.*, 2002; Lampe *et al.*, 2003; Richards *et al.*, 2008; Rivera-Vega and Mittapalli, 2010; Oliveira *et al.*, 2013; Xavier *et al.*, 2014). However, data from chromosome mapping of *Mariner* TEs in Coleoptera are limited to two species of *Coprophanaeus*, one of *Diabroctis* (Oliveira *et al.*, 2013) and one *Dichotomius* (Xavier *et al.*, 2014), all genera belonging to the family Scarabaeidae. Despite the small number of studies, TEs have been associated with important evolutionary processes in Scarabeidae, such as chromosome rearrangements (Oliveira *et al.*, 2013), dispersion of 18S rDNA sites (Cabral-de-Mello *et al.*, 2011a,b), and dynamics of the repetitive DNA fraction that composes the constitutive heterochromatin (CH) in the genomes of *Dichotomius* species (Cabral-de-Mello *et al.*, 2011c).

Cytogenetic studies have been carried out in only 18 of the 165 described *Dichotomius* species, including molecular cytogenetics studies in 15 species (Cabral-de-Mello *et al.*, 2008, 2011a,b; Silva *et al.*, 2009; Korasaki *et al.*, 2012; Xavier *et al.*, 2014). This genus presents groups of closely related species (Sarmiento-Garcés and Amat-Garcia, 2009), including *Dichotomius* (*Luederwaldtinia*) *sericeus* complex (Coleoptera: Scarabaeidae). This complex was recently taxonomically revised by Valois *et al.* (2017), raising the number of species from five to eight. More specifically, *D. sericeus var. aterrimus* (Luederwaldt, 1929) was synonymized with *D. sericeus* and four new taxa, *D. guaribensis*, *D. gilletti*, *D. iannuzziae*, and *D. catimbau* have been described.

Species of the genus *Dichotomius* present the derived karyotype $2n = 18, Xy_p$, with meta-submetacentric chromosome morphology and presence of a large metacentric pair (Silva *et al.*, 2009; Cabral-de-Mello *et al.*, 2011a). The constitutive heterochromatin, located in pericentromeric regions of all autosomes, show similar patterns of highly and moderately repeated DNAs (C0t-1 DNA fraction) distribution in the six analyzed species (Cabral-de-Mello *et al.*, 2011c). Furthermore, the 45S rDNA is predominantly located in the distal region of the third autosome pair, whereas the 5S rDNA and H3 histone were co-located in the proximal region of the second pair in 14 analyzed species (Cabral-de-Mello *et al.*, 2011a,b).

The aim of this study was to access whether distinct populations present differential patterns of location and accumulation of *Mariner*-like elements. Therefore, we characterized and mapped *Dgmar*MITE sequences in chromosomes of phylogenetically related species of the *Dichotomius* (*Luederwaldtinia*) *sericeus* complex belonging to different populations.

Material and Methods

Specimens sampling

All species investigated herein belong to *Dichotomius* (*L.*) *sericeus* complex. *Dichotomius* (*Luederwaldtinia*) *gilletti* and *D.* (*L.*) *iannuzziae* were collected in Aldeia (7°53'48' S, 35°10''47'W) and Igarassu (7°48'37''S; 34°57'25''W), remnants of the Atlantic Rain Forest in the state of Pernambuco, Brazil. Additionally, individuals of *D.* (*L.*) *schiffleri* and *D.* (*L.*) *guaribensis* were collected in Maracaípe (8°31'26"S 35°1'31"W), Pernambuco. *D.* (*L.*) *guaribensis* was also collected in REBIO Guaribas (6°42'41"S 35°11'17"W), Paraíba, Brazil. The individuals were collected using pitfall traps, in compliance with IBAMA/SISBIO guidelines (Permanent license No. 16278-1 for the collection of zoological material, authorization No. 41761-4 for collection in a Federal Conservation Unit for scientific purposes, and the license No. 50438-1, specific for *D.* (*L.*) *schiffleri*). The specimens were identified by the taxonomist Dr. Fernando Silva, from the Universidade Federal do Pará, in Brazil.

DNA extraction and isolation of the transposable element

DNA samples of the four species of *Dichotomius* mentioned above were obtained from the pronotum tissue. Genomic DNA was extracted according to the protocol described by Sambrook and Russell (2001). *Mariner* elements were amplified by PCR using the MOS_N679 primer from *Drosophila* (5'GCCATATGTCGAGTT TCGTGCCA) (Zhang *et al.*, 2001).

The volume of each PCR assay was 25 µL containing 12 ng genomic DNA, 1x PCR buffer, 5 mM $MgCl_2$, 0.2 mM dNTP (Invitrogen), 1 pmol primer, and 1 U *Taq* polymerase (Invitrogen). The PCR conditions were 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 49 °C for 30 s and 72 °C for 1.20 s, and a final extension step at 72 °C for 5 min.

PCR products were separated by electrophoresis on 1% agarose gel. A band of approximately 500 bp obtained from *D.* (*L.*) *gilletti* (Supplementary Figure S1) was isolated from the gel using the Zymoclean™ Gel DNA Recovery Kit (Sinapse) according to the protocol of the manufacturer.

Cloning and sequencing

The isolated DNA fragment was cloned using the pGEM-T Easy Vector (Promega) according to manufacturer's instructions. The insert was isolated by PCR using the M13 primer (M13F 5'-GTAAAACGACGGC CAG/M13R 5'-CAGGAAACAGCATATGAC). Concentrations of PCR reagents were the same as those described above. The PCR conditions were 95 °C for 3 min, followed

by 30 cycles at 95 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. For sequencing, the M13 PCR product was purified with ExoSAP-IT (Affymetrix/USB) and sequenced in an ABI3730XL automated sequencer (Applied Biosystems) by Macrogen Inc.

Editing and analysis of the transposable element

The chromatograms of forward and reverse strands of the M13 PCR product were analyzed with the Pregap4 software of the Staden package (Bonfield *et al.*, 1995) in order to generate consensus sequences. Only bases with a Phred value of 20 or higher were considered in this analysis. Vector sequences were removed using the VecScreen tool. The sequence obtained (accession number: KX787885) was used as a query in GenBank Blast and RepBase Censor for correct identification and classification of the elements. In addition, the presence of ORFs was investigated using the ORFfinder tool.

Chromosome preparations, C-banding and fluorescent *in situ* hybridization (FISH)

Cytological preparations of our four target species were obtained by the classical testicular follicles squashing technique in 50% acetic acid. Two male individuals of each species were analyzed. C-banding was performed on *D.* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* karyotypes following Sumner (1972). FISH was performed according to the protocol of Pinkel *et al.* (1986), with modifications as proposed by Cabral-de-Mello *et al.* (2010). The probe of the transposable element was labeled with dUTP-digoxigenin (Roche) and detected with anti-digoxigenin-rhodamine (Roche).

Photodocumentation

Hybridization images were captured with a Leica DM 2500 epifluorescence microscope. Brightness and contrast of the images were optimized using the Photoshop CS5 program.

Results

The presence of fragments amplified by the MOS_N679 primer of *Mariner* elements (Figure S1) and hybridization signals of the *DgmarMITE* were observed in only two out of the four analyzed species, namely *Dichotomius* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis*. The element isolated from *D.* (*L.*) *gilletti* was 496 bp-long, rich in AT (57%), and had perfect TIRs of 24 bp. The consensus sequence used as a query sequence in GenBank and RepBase revealed 100% similarity with TIRs of the *AfMar*2 *Mariner*-like element of the grasshopper *Abracris flavolineata* (Figure 1) (accession number: KJ829354.1). The sequence between TIRs had no similarities to previously described elements. In addition, the largest identified ORF contained only 30 amino acids and showed no similarity to any transposase.

The species *Dichotomius* (*L.*) *guaribensis*, *D.* (*L.*) *gilletti*, *D.* (*L.*) *iannuzziae* and *D.* (*L.*) *schiffleri* presented similar karyotypes with $2n = 18$, and meta-submetacentric chromosomal morphology. However, distinct sexual determination systems were obverved: *D.* (*L.*) *gilletti*, and *D.* (*L.*) *iannuzziae* had a Xy_p system, whereas *D.* (*L.*) *schiffleri* and *D.* (*L.*) *guaribensis* presented a Xy_r sex bivalent configuration (Figure 2). C-banding revealed pericentromeric constitutive heterochromatin in all autosomes, and additionally, along the entire length of the seventh bivalent and X chromosome of *Dichotomius* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* (Figure 2a,b).

Mapping of *Dgmar*MITE probes on the karyotype of *D.* (*L.*) *gilletti* revealed signals in all chromosomes, except for pairs five and seven of the Igarassu population (Figure 3a), and pair five of the Aldeia population (Figure 3b). Overall, *Dgmar*MITE sequences were predominantly located in euchromatic regions in individuals from both populations, except in the Igarassu population, for which signals were detected at heterochromatic regions of chromosome pairs six and eight (Figure 3a). Similarly, in Aldeia population, *Dgmar*MITE was restricted to the heterochromatic region of pair two (Figure 3b). In addition, five heteromorphic pairs were observed in Aldeia individuals (Figure 3b).

Mapping of *Dgmar*MITE probes on the karyotype of *D.* (*L.*) *guaribensis* revealed their location in heterochromatic regions of all autosomes and of the X chromosome in both populations (Figure 3c,d). Additional signal was observed on the y chromosome of the Guaribas population (Figure 3c). Furthermore, *Dgmar*MITE sites were observed in euchromatic regions of all autosomes, except pair eight, in specimens from Maracaípe (Figure 3d). Overall,

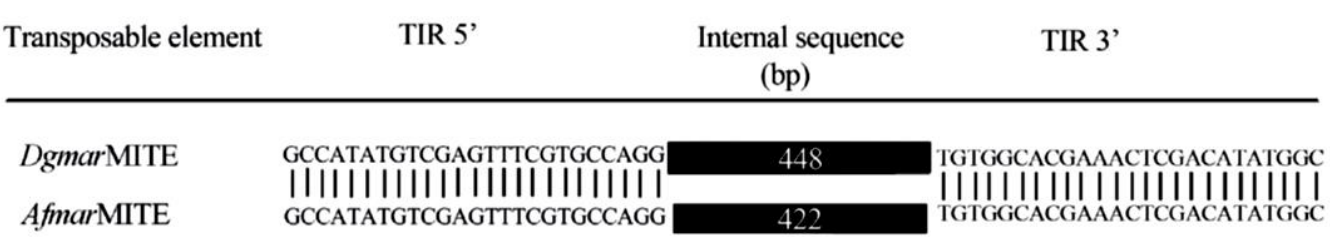

Figure 1 - Alignment of terminal inverted repeats (TIRs) of the elements *Dgmar*MITE and *AfMar*2.

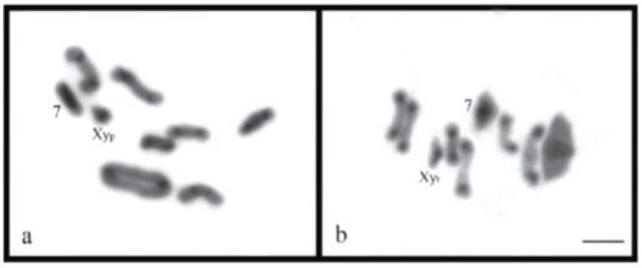

Figure 2 - C-banding in metaphase I of *Dichotomius* (*L.*) *gilletti* (a) and metaphase I of *D.* (*L.*) *guaribensis* (b). Bar = 5 µm.

stronger FISH signals were observed in the karyotypes of individuals from Guaribas when compared to specimens from Maracaípe (Figure 3c,d).

Discussion

The karyotype observed in *D.* (*L.*) *guaribensis* ($2n$ = 18) is considered derived from the ancestral number reported for the family Scarabaeidae ($2n$ = 20), but conserved in most species of *Dichotomius*. The configuration of the sexual bivalent (Xy_r), which has been reported so far only in *D. schiffleri*, also differs from the ancestral Scarabeidae Xy_p (Cabral-de-Mello *et al.*, 2008, 2011a; Silva *et al.*, 2009; Xavier *et al.*, 2014). The derived karyotypes of *D. gilletti* and *D. iannuzziae* observed in this study are similar to those described by Cabral-de Mello *et al.* (2011a) prior to the taxonomic revision by Valois *et al.* (2017). In Cabral-de Mello *et al.* (2011a), these species were referred to as *D. sericeus* and *D. laevicollis*, respectively. The presence of constitutive heterochromatic blocks in pericentromeric regions of all autosomes, as observed in *D.* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis*, is a common feature in the genus *Dichotomius* (Cabral-de-Mello *et al.*, 2011c), and has also been reported for the other two species investigated herein: *D.* (*L.*) *iannuzziae* (Cabral-de-Mello *et al.*, 2011c) and *D.* (*L.*) *schiffleri* (Xavier *et al.*, 2014).

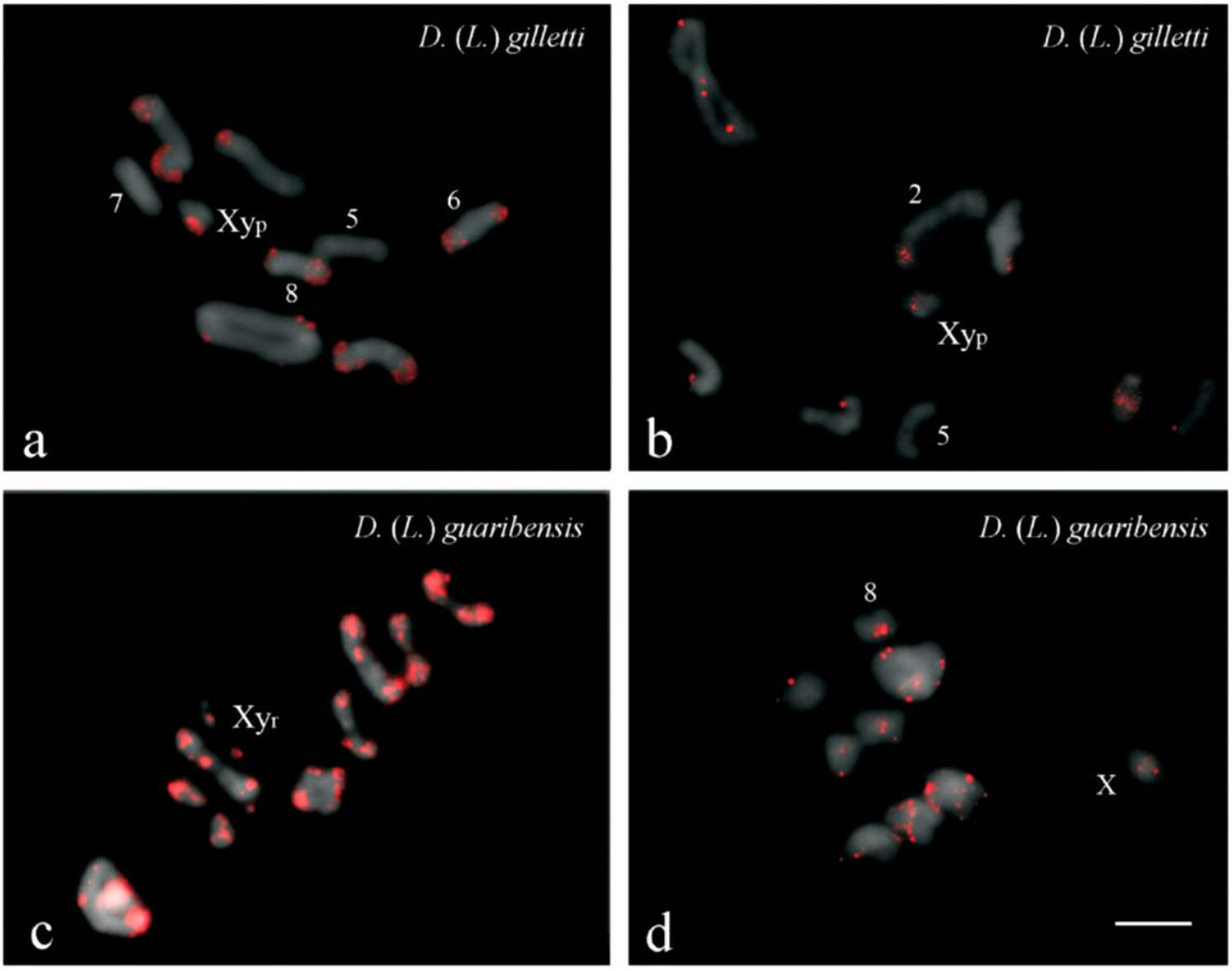

Figure 3 - Fluorescent *in situ* hybridization of the element *Dgmar*MITE in meiotic cells of two individuals of *Dichotomius* (*Luederwaldtinia*) *gilletti* (a, b) and *D.* (*L.*) *guaribensis* (c, d). (a) Metaphase I of an individual from Igarassu population; (b) diplotene of specimen from Aldeia population; (c) metaphase I of individual from Guaribas population; (d) metaphase II of specimen from Maracaípe population. Note the localization and the size of the signals between populations. Bar = 5 µm.

*Dgmar*MITE, the TE characterized here, presented features shared by all MITEs such as a large copy number, which was observable with FISH resolution, lack of transposase coding, AT richness, and conservation of TIR structure (Kuang *et al.*, 2009). In most cases, sequence similarity between a MITE and its closest element is restricted to the TIRs (Feschotte and Pritham, 2007). The similarity between the TIRs of *Dgmar*MITE and the *AfMar*2 element of *A. flavolineata* indicates that the former belongs to the *Mariner* family.

The presence of *Dgmar*MITE in the taxonomically similar species *D.* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* [considering the genus revision of Valois *et al.* (2017)] and therefore possibly phylogenetically closer, suggests an origin of this element in the common ancestor of these species. An alternative hypothesis is that this element may have originated independently in the species *D.* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* by horizontal transfer events, which are frequently observed for *Mariner*-like elements (Robertson, 1995; Robertson and Lampe, 1995), including MITEs, as described previously for the Stowaway element in the plant family Pooideae (Minaya *et al.*, 2013). The *Dgmar*MITE origin may be recent or not however, since older TEs accumulate preferentially in heterochromatic regions (Junakovic *et al.*, 1998), as has been previously proposed for other TEs in Scarabaeidae (Oliveira *et al.*, 2013). Genome colonization of this MITE possibly occurred earlier in *D.* (*L.*) *guaribensis* than in *D.* (*L.*) *gilletti.* In *D.* (*L.*) *gilletti*, the preferential location in euchromatic regions, the presence of heteromorphic pairs in the population of Aldeia, and absence of the signal in one or two chromosome pairs, suggest that this element originated most recently.

In addition to *D.* (*L.*) *gilletti*, predominantly euchromatic signals have been reported for TEs in *D.* (*L.*) *schiffleri* (Xavier *et al.*, 2014), for the grasshopper species *Eyprepocnemis plorans* (Montiel *et al.*, 2012) and *A. flavolineata* (Palacios-Gimenez *et al.*, 2014). The occurrence of *Dgmar*MITE in euchromatic regions can influence gene expression and/or gene and chromosome mutations (Kidwell and Lish, 2000; Feschotte and Pritham, 2007). However, it is also possible that the element is inserted in pseudogenes or even other dispersed repetitive sequences in euchromatin, as proposed for *Mariner* family elements of *E. plorans* (Montiel *et al.*, 2012). On the other hand, the presence of MITEs in heterochromatic regions, as observed for *Dgmar*MITE in *D.* (*L.*) *guaribensis*, is not common, since these TEs are preferentially associated with genes (Lu *et al.*, 2011). However, heterochromatic enrichment of these elements has been described in other organisms, such as in the insect *Anopheles gambiae* (Quesneville *et al.*, 2006) and in the plants *Oryza sativa* (Lu *et al.*, 2011) and *Arabidopsis thaliana* (Guo *et al.*, 2017).

Mapping of *Dgmar*MITE in *D.* (*L.*) *gilletti* and *D.* (*L.*) *guaribensis* showed variation in the location and number of sites between species and populations. These findings suggest that this non-autonomous element may be cross-mobilized to different regions of host genomes using the transposase of either an older or a newly emerged transposon, in this latter case *Dgmar*MITE accumulation occurs by a process known as "snowball effect" (Feschotte *et al.*, 2005). The transposase used by *Dgmar*MITE could belong to a ,closely related TE as observed between the inactive *peach* element and the transposase of *Mariner*-like *Mos1* in *Drosophila melanogaster* (Garza *et al.*, 1991), or to a phylogenetically distant TE, as observed between an element of the Stowaway family and *Osmar* transposase, an autonomous *Mariner*-like element in the rice genome (Feschotte *et al.*, 2003; Yang *et al.*, 2009).

With respect to copy-number variation in heterochromatic regions of *D.* (*L.*) *guaribensis* chromosomes, an increase in *Dgmar*MITE copy number in the Guaribas population probably results from transposition-independent events, including ectopic recombination and concerted evolution. The latter has been observed for highly repetitive DNA sequences such as *Mariner* elements found in the heterochromatin of *Drosophila erecta* (Lohe *et al.*, 1995). An alternative hypothesis to explain this variation is that this element is undergoing a reverse process with quantitative and random copy loss in the genomes of individuals from Maracaípe population. In this scenario, *Dgmar*MITE would be undergoing senescence, the last stage of the transposable element "life cycle", as described by Kidwell and Lisch (2000).

Mapping of *Dgmar*MITE in species of the *Dichotomius* (*Luederwaldtinia*) *sericeus* complex contributed to increase our knowledge about the location and distribution of TEs in dung beetle genomes. This analysis also revealed the accumulation of *Dgmar*MITE in the karyotype of two species. Plausible mechanisms underlying such accumulation include the occurrence of cross-mobilization and/or ectopic recombination in heterochromatic regions. However, we cannot completely rule out the possible involvement of other molecular mechanisms discussed here. Therefore, further characterization and chromosome mapping should be extended to other species within this complex of species.

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References

Bonfield JK, Smith KF and Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Res 24:4992-4999.

Cabral-de-Mello DC, Oliveira SG, Ramos IC and Moura RC (2008) Karyotype differentiation patterns in species of the subfamily Scarabaeinae (Scarabaeidae, Coleoptera). Micron 38:1243-1250.

Cabral-de-Mello DC, Moura RC and Martins C (2010) Chromosomal mapping of repetitive DNAs in the beetle *Dichotomius geminatus* provides the first evidence for an association of 5S rRNA and histone H3 genes in insects, and repetitive DNA similarity between the B chromosome and A complement. Heredity 104:393-400.

Cabral-de-Mello DC, Moura RC and Martins C (2011a) Cytogenetic mapping of rRNAs and histone H3 genes in 14 species of *Dichotomius* (Coleoptera, Scarabaeidae, Scarabaeinae) beetles. Cytogenet Genome Res 134:127-135.

Cabral-de-Mello DC, Oliveira SG, Moura RC and Martins C. (2011b) Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: Insights into the evolutionary dynamics of multigene families and heterochromatin. BMC Genetics 12:1.

Cabral-de-Mello DC, Moura RC, Melo AS and Martins C (2011c). Evolutionary dynamics of heterochromatin in the genome of *Dichotomius* beetles based on chromosomal analysis. Genetica 139:315-325.

Deprá M, Ludwig A, Valente VL and Loreto EL (2012) Mar, a MITE family of hAT transposons in *Drosophila*. Mobile DNA 3:1.

Feng Y (2003) Plant MITEs: Useful tools for plant genetics and genomics. Genomics Proteomics Bioinformatics 1:90-100.

Feschotte C and Pritham EJ (2007) DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet 41:331-368.

Feschotte C, Zhang X and Wesslle SR (2002) Miniature inverted-repeat transposable elements and their relationship to established DNA transposons. In: Craig N, Craigie R, Gellert M and Lambowitz A (eds) Mobile DNA II. AMS Press, Washington, DC, pp 1147-1158.

Feschotte C, Swamy L and Wessler SR (2003) Genome-wide analysis of *Mariner*-like transposable elements in rice reveals complex relationships with Stowaway miniature inverted repeat transposable elements (MITEs). Genetics 163:747-758.

Feschotte C, Osterlund MT, Peeler R and Wessler SR (2005) DNA-binding specificity of rice *Mariner*-like transposases and interactions with Stowaway MITEs. Nucleic Acids Res 33:2153-2165.

Garza D, Medhora M, Koga A and Hartl DL (1991) Introduction of the transposable element *Mariner* into the germline of *Drosophila melanogaster*. Genetics 128:303-310.

Guo C, Spinelli M, Ye C, Li QQ and Liang C (2017) Genome-wide comparative analysis of miniature inverted repeat transposable elements in 19 *Arabidopsis thaliana* ecotype accessions. Sci Rep 7:2634.

Junakovic N, Terrinoni A, Di Franco C, Vieira C and Loevenbruck C (1998) Accumulation of transposable elements in the heterochromatin and on the Y chromosome of *Drosophila simulans* and *Drosophila melanogaster*. J Mol Evol 46:661-668.

Kidwell MG (2005) Transposable Elements. In: Gregory TR (ed) The Evolution of the Genome. Elsevier, San Diego, 165-221 pp.

Kidwell MG and Lisch D (2000) Transposable elements and host genome evolution. Trends Ecol Evol 15:95-99.

Korasaki V, Vaz-de-Mello FZ, Braga RF, Zanetti R and Louzada J (2012) Taxocoenosis of the Scarabaeinae (Coleoptera: Scarabaeidae) in Benjamin Constant, AM. Acta Amazon 42:423-432.

Kuang H, Padmanabhan C, Li F, Kamei A, Bhaskar PB, Ouyang S, Jiang J, Buell CR and Baker B (2009) Identification of miniature inverted-repeat transposable elements (MITEs) and biogenesis of their siRNAs in the Solanaceae: New functional implications for MITEs. Genome Res 19:42-56.

Lampe DJ, Churchill MEA and Robertson HM (1996) A purified *Mariner* transposase is sufficient to mediate transposition *in vitro*. EMBO J 15:5470-5479.

Lampe DJ, Witherspoon DJ, Soto-Adames FN and Robertson HM (2003) Recent horizontal transfer of mellifera subfamily *Mariner* transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer. Mol Biol Evol 20:554-562.

Lohe AR, Moriyama EN, Lidholm DA and Hartl DL (1995) Horizontal transmission, vertical inactivation, and stochastic loss of *Mariner*-like transposable elements. Mol Biol Evol 12:62-72.

Lu C, Chen J, Zhang Y, Hu Q, Su W and Kuang H (2011) Miniature inverted–repeat transposable elements (MITEs) have been accumulated through amplification bursts and play important roles in gene expression and species diversity *in Oryza sativa*. Mol Biol Evol 29:1005-1017.

Minaya M, Pimentel M, Mason-Gamer R and Catalan P (2013) Distribution and evolutionary dynamics of Stowaway Miniature Inverted repeat Transposable Elements (MITEs) in grasses. Mol Phylogenet Evol 68:106-118.

Montiel EE, Cabrero J, Camacho JPM and López-León DM (2012) Gypsy, RTE and *Mariner* transposable elements populate *Eyprepocnemis plorans* genome. Genetica 140:365-374.

Oliveira SG, Bao W, Martins C and Jurka J (2012) Horizontal transfers of *Mariner* transposons between mammals and insects. Mobile DNA 3:1-6.

Oliveira SG, Cabral-de-Mello DC, Moura RC and Martins C (2013) Chromosomal organization and evolutionary history of *Mariner* transposable elements in Scarabaeinae coleopterans. Mol Cytogenet 6:1-9.

Palacios-Gimenez OM, Bueno D and Cabral-de-Mello DC (2014) Chromosomal mapping of two *Mariner*-like elements in the grasshopper *Abracris flavolineata* (Orthoptera: Acrididae) reveals enrichment in euchromatin. Eur J Entomol 111:329-334.

Pinkel D, Straume T and Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A 83:2934-2938.

Plasterk RHA, Izsvák Z and Ivics Z (1999) Resident aliens the Tc1/*Mariner* superfamily of transposable elements. Trends Genet 15:326-332.

Quesneville H, Nouaud D and Anxolabéhère D (2006) P elements and MITE relatives in the whole genome sequence of *Anopheles gambiae*. BMC Genomics 7:214.

Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, Beeman RW, Gibbs R, Beeman RW, Brown SJ, Bucher G, *et al.* (2008) The genome of the model beetle and pest *Tribolium castaneum*. Nature 452:949-955.

Rivera-Vega L and Mittapalli O (2010) Molecular characterization of *Mariner*-like elements in emerald ash borer, *Agrilus planipennis* (Coleoptera, Polyphaga). Arch Insect Biochem Physiol 74:205-216.

Robertson HM (1993) The *Mariner* transposable element is widespread in insects. Nature 362:241-245.

Robertson HM (1995) The Tcl-*Mariner* superfamily of transposons in animals. J Insect Physiol 41:99-105.

Robertson HM and Macleod EG (1993) Five major subfamilies of *Mariner* transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. Insect Mol Biol 2:125-139.

Robertson HM and Lampe D (1995) Distribution of transposable elements in Arthropods. Annu Rev Entomol 40:333-357.

Robertson HM, Soto-Adames FN, Walden KK, Avancini RM and Lampe D (2002) The *Mariner* transposons of animals: Horizontally jumping genes. In: Kado CI (ed) Horizontal gene transfer. Academic Press, San Diego, CA, pp 173-185.

Sambrook J and Russell DW (2001) Molecular Cloning. Cold Spring Harbor Laboratory Press, New York, 2100 p.

Sarmiento-Garcés R and Amat-García G (2009) Escarabajos del género *Dichotomius* Hope 1838 (Scarabaeidae: Scarabaeinae) en la amazonía colombiana. Rev Acad Colomb Cienc 33:285-296.

Silva GM, Bione EG, Cabral-de-Mello DC, Moura RC, Simões ZLP and Souza MJ (2009) Comparative cytogenetics of three species of Dichotomius (Coleoptera, Scarabaeidae). Genet Mol Biol 32:276-280.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304–306.

Valois MC, Vaz-de-Mello FZ and Silva FBA (2017) Taxonomic revision of the *Dichotomius sericeus* (Harold, 1867) species group (Coleoptera: Scarabaeidae: Scarabaeinae). Zootaxa 4277:503–530.

Wallau GL, Capy P, Loreto E and Hua-Van A (2014) Genomic landscape and evolutionary dynamics of *Mariner* transposable elements within the *Drosophila* genus. BMC Genomics 15:727.

Xavier C, Cabral-de-Mello DC and Moura RC (2014) Heterochromatin and molecular characterization of *Dsmar*MITE transposable element in the beetle *Dichotomius schiffleri* (Coleoptera: Scarabaeidae). Genetica 142:575-581.

Yang G, Nagel DH, Feschotte C, Hancock CN and Wessler SR (2009) Tuned for transposition: molecular determinants underlying the hyperactivity of a Stowaway MITE. Science 325:1391-1394.

Zhang L, Dawson A and Finnegan DJ (2001) DNA-binding activity and subunit interaction of the *mariner* transposase. Nucleic Acids Res 29:3566-3575.

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Supplementary material

The following online material is available for this article:

Figure S1 – Amplification of *Dgmar*MITE in four phylogenetically related species of the *Dichotomius* (*Luederwaldtinia*) *sericeus* complex.

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Supplementary Material to "Characterization and chromosomal mapping of the *Dgmar*MITE transposon in populations of *Dichotomius* (*Luederwaldtinia*) *sericeus* species complex (Coleoptera: Scarabaeidae)"

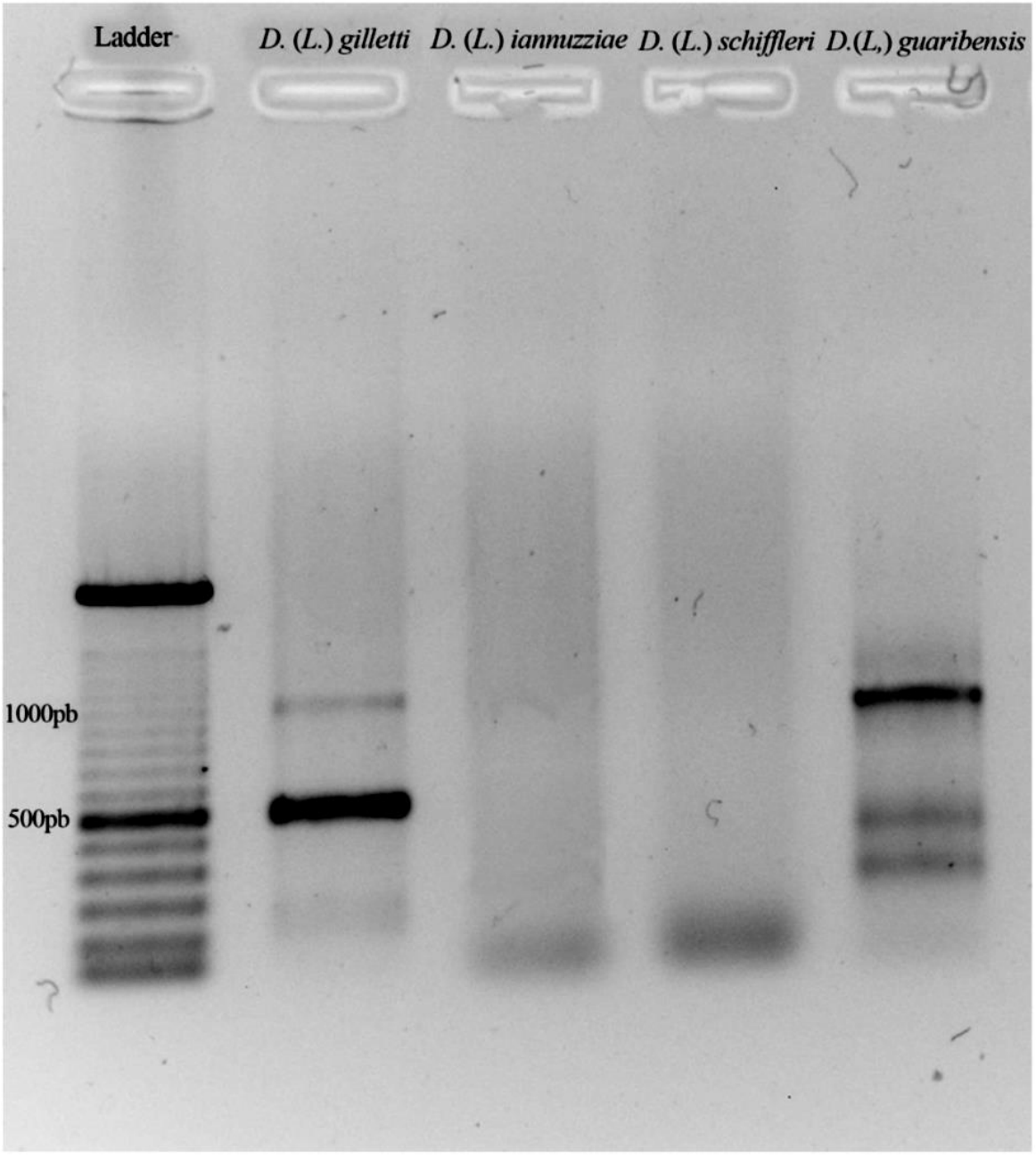

Figure S1 – Amplification of *Dgmar*MITE in four phylogenetically related species of the *Dichotomius* (*Luederwaldtinia*) *sericeus* complex.

APÊNDICE B – PRODUÇÃO ASSOCIADA

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Dichotomius (*Luederwaldtinia*) *schiffleri* (Coleoptera: Scarabaeidae) mitochondrial genome and phylogenetic relationships within the superfamily Scarabaeoidea

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ABSTRACT

The mitochondrial DNA of *Dichotomius* (*Luederwaldtinia*) *schiffleri* was characterized and its phylogenetic position was reconstructed in Scarabaeoidea. This mitogenome presented 14,802 bp-long, richness in AT of 77.4% and 37 genes, including 13 protein-coding, 22 transfer RNAs, and two ribosomal RNAs. In addition, it was observed intergenic spacers and reading frame overlaps. The phylogenetic trees reconstructed from protein sequences provided best resolution, indicating Scarabaeinae and Aphodiinae as a sister groups, as previously reported in other molecular phylogenies.

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KEYWORDS
Mitogenome; nucleic acid and protein phylogeny; mtDNA organization

Dichotomius (*Luederwaldtinia*) *schiffleri* Vaz-de-Mello, Louzada, Gavino 2001 is an Endangered species, endemic to Brazilian coastal ecosystems (Vieira et al. 2011). Here it was characterized the mtDNA of *D.* (*L.*) *schiffleri* and established its position in the superfamily Scarabaeoidea based on phylogenomic methods.

The specimen analyzed was collected in Ipojuca (8°31'26" S 35°1'31" W), Pernambuco, Brazil and deposited in the Laboratório de Biodiversidade e Genética de Insetos (accession number: LBGI_M9032) at the Universidade de Pernambuco, Brazil. The collection of this species was authorized by IBAMA/SISBIO (Licence No. 50438-1). The DNA was extracted according to the protocol of Sambrook and Russel (2001). The mitogenome was sequenced on Solexa-Illumina HiSeq 2000 (San Diego, CA). The genome assembly was performed with the Oligonucleotide Analysis Package, screened through a reference mitogenome and characterized on the MITOS WebServer. For phylogenetic analysis, the complete or partially mitogenome sequence of species of Scarabaeoidea superfamily from GeneBank was used. The Staphyliniformia species were used as outgroup. The phylogenetic trees of nucleic acid and protein sequences were reconstructed by Bayesian inference and maximum likelihood methods.

The mitogenome of *D.* (*L.*) *schiffleri* comprised a coding region of 14,802 bp (accession number: KY100258), with high percentage of A-T (77.4%). The characterization revealed the presence of 37 genes, including 13 protein-coding (cox1-3, cob, nad1-6, nad4L, atp6, and atp8), 22 tRNA, and two rRNA genes (rrnS and rrnL). Regarding the orientation, 23 genes are located on the positive chain and 14 genes on negative chain. Mitogenome size, nucleotide composition, number, orientation, and order of genes are similar to most of the insects (Sheffield et al. 2008, 2009; Cameron 2014). In the mitogenome of *D.* (*L.*) *schiffleri* 23 intergenic spacers and six reading frame overlaps were observed, including an overlap of 36 bp between the *rrnL* and *trnL1* genes, corresponding to more than half of the sequence of this tRNA (67 bp). The presence of intergenic spacers and reading frame overlaps was also observed in other Coleoptera species (Sheffield et al. 2008).

Regarding the phylogenetic analysis the Bayesian and maximum likelihood trees presented similar topology. However, the trees reconstructed from protein sequences showed a better resolution and correctly positioned the species/family (Figure 1 and Supplemental Figure S1), what was previously reported in other analyses in Coleoptera (Du et al. 2016; Timmermans et al. 2016). Therefore, here it was discussed only the protein tree. The phylogeny showed *D.* (*L.*) *schiffleri* on the same clade as *Sarophorus* sp. with the subfamily Aphodiinae as a sister group (Figure 1, subclade A). The position of Aphodiinae as a sister group of Scarabaeinae was observed in other phylogenies based on nuclear and mitochondrial genes (Bocak et al. 2014; Timmermans et al. 2016). In turn, the superfamily Scarabaeoidea formed a monophyletic group (Figure 1, clade I). The characterization of the mitogenome of *D.* (*L.*) *schiffleri* provides useful knowledge for future studies aimed to investigate the genetic diversity on population level and develop strategies for the management and conservation of this species.

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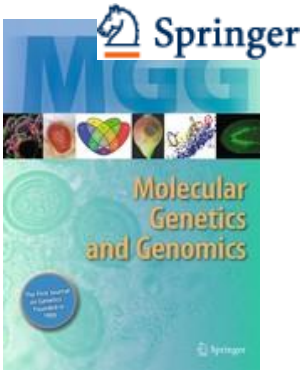
Supplemental data for this article can be accessed here.

ANEXO A – NORMAS DAS REVISTAS



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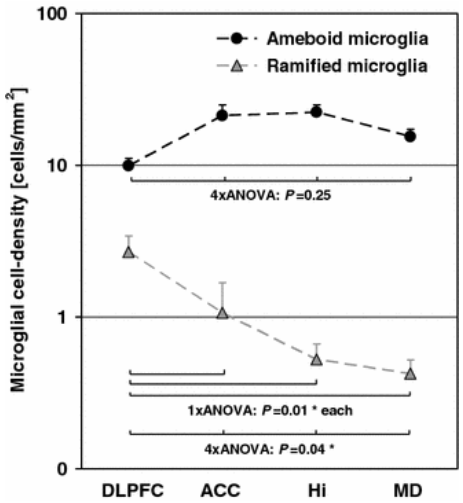

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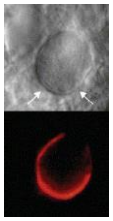

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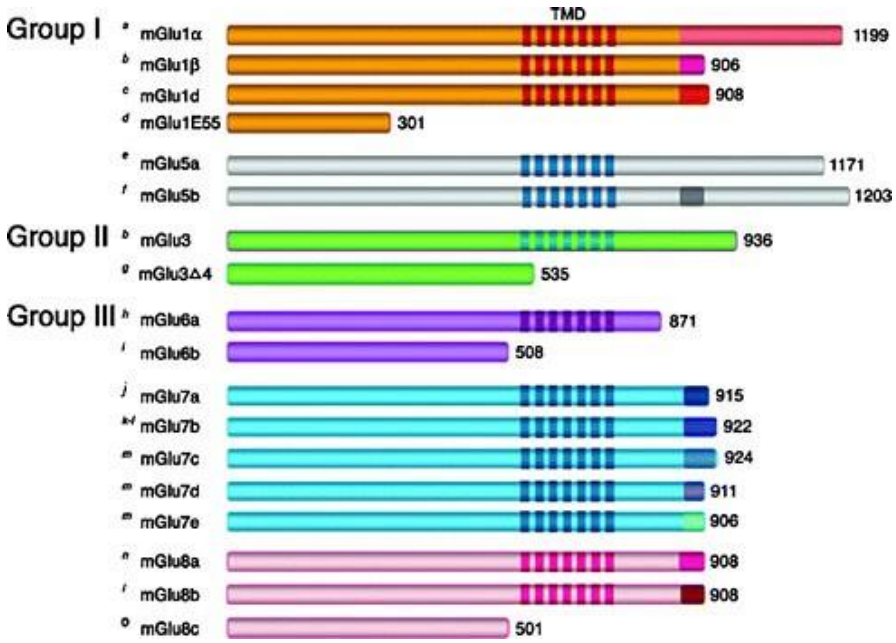

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