Provided by Biblioteca Digital da Produção Intelectual da Universidade de São Paulo (BDPI/USF





### Universidade de São Paulo Biblioteca Digital da Produção Intelectual - BDPI

Departamento de Biologia Celular e do Desenvolvimento - ICB/BMC

Artigos e Materiais de Revistas Científicas - ICB/BMC

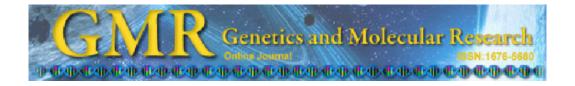
2013-08-02

# Molecular characterization of a mariner-like element in the Atta sexdens rubropilosa genome

GENETICS AND MOLECULAR RESEARCH, RIBEIRAO PRETO, v. 11, n. 2, supl. 1, Part 3, pp. 1475-1485, FEB, 2012

http://www.producao.usp.br/handle/BDPI/36587

Downloaded from: Biblioteca Digital da Produção Intelectual - BDPI, Universidade de São Paulo



## Molecular characterization of a *mariner*-like element in the *Atta sexdens rubropilosa* genome

P. Rezende-Teixeira, J.B. do Amaral, F. Siviero and G.M. Machado-Santelli

Departamento de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil

Corresponding author: P. Rezende-Teixeira

E-mail: paularez@usp.br

Genet. Mol. Res. 11 (2): 1475-1485 (2012) Received March 28, 2011 Accepted August 1, 2011 Published May 21, 2012 DOI http://dx.doi.org/10.4238/2012.May.21.4

ABSTRACT. Mobile elements are widely present in eukaryotic genomes. They are repeated DNA segments that are able to move from one locus to another within the genome. They are divided into two main categories, depending on their mechanism of transposition. involving RNA (class I) or DNA (class II) molecules. The mariner-like elements are class II transposons. They encode their own transposase, which is necessary and sufficient for transposition in the absence of host factors. They are flanked by a short inverted terminal repeat and a TA dinucleotide target site, which is duplicated upon insertion. The transposase consists of two domains, an N-terminal inverted terminal repeat binding domain and a C-terminal catalytic domain. We identified a transposable element with molecular characteristics of a mariner-like element in Atta sexdens rubropilosa genome. Identification started from a PCR with degenerate primers and queen genomic DNA templates, with which it was possible to amplify a fragment with mariner transposableelement homology. Phylogenetic analysis demonstrated that this element belongs to the *mauritiana* subfamily of *mariner*-like elements and it was named Asmar1. We found that Asmar1 is homologous to a transposon described from another ant, Messor bouvieri. The predicted transposase sequence demonstrated that Asmar1 has a truncated transposase ORF. This study is part of a molecular characterization of mobile elements in the *Atta* spp genome. Our finding of *mariner*-like elements in all castes of this ant could be useful to help understand the dynamics of *mariner*-like element distribution in the Hymenoptera.

**Key words:** Transposable element; *Mariner*-like element; Leaf-cutter ant; *Atta sexdens rubropilosa* 

#### INTRODUCTION

Mobile elements are present in all organisms in multiple copies; they have occupied different genomes for millions of years. There is an important division of classes involving these elements, which consider mainly the transposition mechanism. Class I elements transpose via a RNA intermediate, while Class II elements transpose via a cut and paste mechanism.

Class II elements are important tool for genetic transformation in many different organisms. A special transposable element widely used for transformation is the *mariner* element (Jacobson et al., 1986; Berghammer et al., 1999; Coates et al., 2000; Moreira et al., 2000). This element possesses common characteristics, such as: inverted terminal repeat (ITR) sequence at the extremities; ITR sites flanked by TA nucleotides, duplication of two base pairs in the insertion site (direct repeats); catalytic domain, which contains the D,D(34)D catalytic triad and the transposase domain; DNA-binding domain, which contains the nuclear localization signal and the helix-turn-helix motif, and finally a short size of about 1300 bp.

The *mariner* elements were first identified in *Drosophila melanogaster*, but are extensively distributed in nature, and can be found in a wide variety of insects and other arthropods. *Mariner* family members have also been identified in several organisms such as nematodes, marine species, fungi, plants, and mammals, including humans (Robertson, 1993; Capy et al., 1996; Jarvik and Lark, 1998; Leroy et al., 2003; Mandrioli, 2003; Halaimia-Toumi, et al., 2004).

Currently, these elements are members of a large transposon family, known as *mariner* and *mariner*-like elements (MLEs) (Lampe et al., 1996). There are over 13 subfamilies known of *mariner* elements, which typically contain approximately 40-56% identity in nucleotide and 23-45% identity in amino acids between the subfamilies and 25-100% identity in amino acids with a particular subfamily. The phylogenetic history of the *mariner* element family is known for extensive horizontal transfer between species, some separated by great phylogenetic distances (Robertson and Zumpano, 1997; Robertson and Martos, 1997; Lampe et al., 2003).

Many of these transposable elements belonging to the *mariner* family are non-functional; they accumulated some kind of mutations during evolution and thus transcribe inactive proteins. The number of MLEs per genome can vary tremendously from species to species (Hartl et al., 1997). Several species that had characterized parcial or full-length elements showed inactive MLEs, with multiple stop codons, deletions or frameshifts. They were observed in the genomes of insects: *Rhynchosciara americana* (Rezende-Teixeira et al., 2008, 2010), *Messor bouvieri* (Palomeque et al., 2006), *Musca domestica* (Yoshiyama et al., 2000), *Hessian* fly (Russell and Shukle, 1997), *Bombyx mori* (Robertson and Asplund, 1996; Robertson and Walden, 2003; Kumaresan and Mathavan, 2004), *Bactrocera tryoni* (Green and Frommer, 2001), *Ochlerotatus atropalpus* (Zakharkin et al., 2004), in Lepidoptera: *Antheraea mylitta* (Prasad and Nagaraju, 2003), *Mamestra brassicae* (Mandrioli, 2003), in nematode:

*Meloidogyne chitwoodi* (Leroy et al., 2003), and several other species. The prevalence of inactive copies of *mariner* elements in many genomes suggests that the vertical inactivation by mutation had an important, probably dominant, role in the evolution dynamic of *mariner* elements (Lohe et al., 1995).

The present study characterized a transposable element of *mariner* element family in the leaf-cutter ant genome, *Atta sexdens rubropilosa*. This insect social behavior and responses to pesticides have been well studied, but there is little data on other aspects of its biology. Recent data comparing the morphology and cytoskeleton organization of the salivary glands (the post-pharyngeal, hypopharyngeal, mandibular, and thoracic salivary gland) of different castes show a lack of molecular markers in this system (do Amaral and Machado-Santelli, 2008). It was the starting point for molecular characterization of mobile elements, which nowadays are considered important molecular markers. As observed in many invertebrates the element identified presented defective open reading frame (ORF), stop codons within the ORF and imperfect ITR sequences. However, the study of this element in this system can provide important information on the evolution and function of mobile elements, which always appear to collaborate with a variety of biological functions (revised by Khurana and Theurkaf, 2010; George et al., 2010).

#### MATERIAL AND METHODS

#### **Animals**

Ants of *A. s. rubropilosa* were collected at the Universidade de São Paulo campus, in the Brazilian State of São Paulo, and the wild ants bred in the laboratory.

#### Nucleic acid

Genomic DNA was extracted from an A.s. rubropilosa queen ant, added to 300  $\mu L$  TMD (10 mM Tris-Cl, pH 8, 5 mM EDTA, and 0.3 M NaCl), and homogenized. This was then mixed with 12  $\mu L$  20% SDS and incubated at 65°C for 1 h in the presence of proteinase K (100  $\mu g/mL$ ), followed by one extraction with phenol:chloroform. DNA was precipitated in ethanol, washed with 70% ethanol, dried and dissolved in TE buffer (10 mM Tris-HCl, pH 8, and 1 mM EDTA). DNA was quantified in a NanoDrop ND1000 Spectrophotometer.

#### PCR, inverse PCR and DNA sequencing

PCR amplifications were made with degenerated primer to amplify the *Asmar1* element internal regions (pmar1R: 5'-TTTGCACAACAAGTTCAATTT-3' and pmar1F: 5'-TTTCTGGCAATTTACGGAT-3'). Primers were designed for inverse PCR (iAsmar1R: 5'-TCCAATTAAAGCAGAAAATCAA-3', iAsmar1F: 5'-TGGTGGGACTAAAAGGATCT-3') based on the internal sequences of *Asmar1*. The protocol of DNA circularization was described in Rezende-Teixeira et al. (2010). The PCR amplifications were performed using Platinum Taq DNA polymerase (Invitrogen Life Technologies) according to manufacturer instructions. Cycle conditions were 94°C for 2 min, 35 cycles of 94°C for 30 s; 55°C for 30 s; 72°C for 2 min and a final extension at 72°C for 7 min. The PCR products were cloned in pGemT-easy vector (Promega). Clones were sequenced using the BigDye terminator (PerkinElmer) and run

on an ABI-3100 sequencer (PerkinElmer), using T7 and SP6 primers. The nucleotide sequences were analyzed in a Linux workstation with Phred, Phrap Crosmatch and Consed 17 programs (Ewing and Green, 1998; Ewing et al., 1998; Gordon et al., 1998). The BLAST analyses were done in the non-redundant GenBank database (Wheeler et al., 2000). The MLE sequences of *A. s. rubropilosa* were deposited in the GenBank database and have the following accession No.: *A. s. rubropilosa Asmar1* (JF717775).

#### RESULTS AND DISCUSSION

To amplify *mariner*-like transposable elements in the *A. s. rubropilosa* genome the primers initially used were designed to amplify *mariner* elements of *Rhynchosciara* diptera (Rezende-Teixeira et al., 2010). Given the high identity observed between these elements, the primers functioned perfectly, and the ORF internal region of a *mariner*-like family element was amplified, as expected (pmar1R: 5'-TTTGCACAACAAGTTCAATTT-3' and pmar1F: 5'-TTTCTGGCAATTTACGGAT-3'). To perform all PCRs queen ant DNA of *A. s. rubropilosa* was used.

Since the ORF internal region was known, a new primer pair was drawn from the amplified sequence of *A. s. rubropilosa*. These primers point to the ends of the transposon to carry out an inverse PCR to provide data of the complete sequence, flanking regions and target site.

The amplified sequence of element in *A. s. rubropilosa* was named *Asmar1*, and the consensus element is 1267 nucleotides in length and has the typical structure of an MLE; however, the ITRs are imperfect (Figure 1). An ATG starts at nucleotide 162, one defective ORF of 1035 bp encoding a putative transposase with 345 amino acid protein and 5 internal stop codons. The translation start site, which has been described in some full-length *mariner* elements, is located in a non-canonical Kozak's box (PuXXATGPu), which was also observed in the element *Asmar1* of *A. s. rubropilosa*. The last position of the subject contains a pyrimidine nucleotide (thymidine) in the place of one purine.

The D,D(34)D signature-sequence, which is also a characteristic of *mariner* elements, defines the second functional domain of the transposase, which is the catalytic domain (129 amino acids in *A. s. rubropilosa*). This domain is responsible for site-specific cleavage and junction in the transposition process (van Luenen et al., 1994; Craig, 1995). The active site of this domain is defined by three amino acid motifs, consisting of two aspartic acid (D) residues separated by 94 amino acids, followed by another aspartic acid residue separated by 34 amino acids (Robertson, 1993; Doak et al., 1994). The D,D(34)D catalytic triad that makes up the active site serves as a binding domain for the divalent cation (Mg<sup>2+</sup>) required for catalysis (Prasad et al., 2003). The motif D,D(34)D was identified in the *Asmar1* transposase of *A. s. rubropilosa* with 81 residues, which were 100% aligned.

It was noted that some sequences (WVPHEL, DEKW, H/QDNAP, HPPYSPDLAPSD), highly conserved in the MLE family (Robertson, 1993; Prasad and Nagaraju, 2003), are also present in the *Asmar1* element, but some amino acids appear altered. The sequences found in *A. s. rubropilosa* are: WVPMNL, DEMW, HDNAR, and HPPYSPDVAPSD. The underlined amino acids are shifts found in *Asmar1*. These sequences correspond to regions of aspartic acid residues that comprise the D,D(34)D catalytic triad and two motifs (WVPHEL and YSPDLAP), typical of *mariner* transposases described by Robertson (1993).

The ITRs are short sequences of about 28 bp flanking the mobile element, responsible

 $\mathtt{TA}\underline{TTGTGAGTACAAATTAATTCGGTCCGTT}\mathtt{TCTAACTGTTGTGAATGGTT}$ CATAGTGACAGCTGATTTTGGCGATTTCAGAAAGGTTAAACATCTACTAA TAA TATT CAGTTTATAT CACTTTGAG TTTCA TACA AAAACCTTGATTTTT ACTGTGTTTGAAATGTCGAATTTTGTGTTAACTTAGCAGCATTTGAGAGA MSNFVLT \* qHLRD TTTTGATTTTCTGCTTTAATTGGAAG AAAAG TGCGGCTGAAGCCCATCGA FDFLL\*L kks A A E A H R ATGCTTGTCGAAGTTTATGGTAACACTGCTCCAACTGGTAAATCATGTAG MLVEVYGNTAPTGKSCR GGAATGGTTTCGACGTTTCAAGGATGAGATTTCAGCGTTGAAGACAAGCC EWFRRFKDEISALKT TCGCTCTGGACAGCCAAAAAATTCCGAAGACAAAGAATTGAGACATTACT ALDSQKIPKTKN\*DIT CGAAGAAGATCAGAGTCAAACGCAAGAGGAGCTTGCAGAATCATTGGGGA RRRSESNARGACRIIGD TAACTCAAGCCGTATCTGTACGATTGAGAGCCATGAAGTCATGGGAATGA SSRICTIESHEVMG TTGAGAAACAAGGAAATTGGGTGCCT ATGAA CTTA AACCGAGAGACTTTG EKQGNWVPMNLNRETL AAAGGCGATTTTTCACTTGCGAACAGTTGATTCAAAGATAACAGAGAAAA K G D F S L A N S \* F K D N R E K GGTTTTTTGCATCGGATTGTGAGACGAGATGTGGATATTCTACGACAATC V F C I G L \* D E M W I F Y D N P CCAAGAAGAAAAAATACTACGCTAAGCCTGATCAATCGTTGCCATCGATC K K K Y Y A K P D Q S L P S I TCAACAT CAACA CCGAA CATT CATGA TTCAA AGAT CATGCTTTGTATCTG STSTPNIHDSKIMLCIW GTGGGACTAAAAGGATCTTGTTTACTATGAGCTGCTGAAACCTGGCGATT W D \* K D L V Y Y E L L K P G D CCATTACGGGCGATCGGTATCGGCTA CAATTGATTCGTTTGAGTCGTGTA ITGDRYRLQLIRLSRV TTGCAAGAAAA TGGCCGGAATACGA GCAAA GACA TGTGATTTTGCAGCA LQEKWPEYEQRHVILQH TGA CAAT GCTCGACCCCATGTTGCGA AAGTG GTCA AGACATACTTGGAAA DNARPHVAKVVKTYLE CGTTGAAATGGGAAGTCCTACCCCAT CCGCCGTAT TCTCCAGACGTTGCT L K W E V L P H P P Y S P D V A CCCTCTGACTAT CACTTGTTT CGATC AATGG CACA CGGTCTGGCTGACCA PSDYHLFRSMAHGLADQ GCACTTC CGGTTTTGTGAAGAAGTAA AAAAT TGGA TCGATTCGT GGATAG H F R F C E E I K N W I D S W I A CCT CAAAAGATG ACCAG TGTT TTCGA CGCGG GATT CGTAC GCTAC CCGAA SKYVLCFRRGVRTLPE AGATGGGAGAAAGTAGTGGCCAGCGATGGACAATACTTTGAATCATAAAT RWEKVVASDGQYFE GTATAAC CAGTTTTTTa cAAATTTCG AATTT CGGA AAAAAAACGGCGGAAGC **AAAGCTTGTACACCATA** 

**Figure 1.** Nucleotide sequence and conceptual translation of the consensus *Asmar1* element inserted at a duplicated TA dinucleotide site. The inverted terminal repeats (ITRs) and the positions of two conserved motifs described by Robertson (1993) are underlined. The aspartic acids (D) of the D,D(34)D catalytic triad are in red underlined and the catalytic domain in blue.

for the binding of transposase protein in the transposition mechanism. The binding domain of the ITR sequence of the Mos1 *mariner* transposase was defined by Augé-Gouillou et al. (2001) analyzing the interaction between the transposase and the ITR sequence. The transposase of MLEs specifically bind as a dimer in the inverted terminal repeat sequence of transposon that encodes it. Two binding motifs were localized in the ITR sequence (Bigot et al., 2005). These motifs are involved in the binding of *mariner* transposase in ITR. The ITR sequence found for *Asmar1* is imperfect 5'-TTGTGAGTACAAATTAATTCGGTCCGTT-3'. Thus, in the *A. s. rubropilosa* the transposase is probably non-functional, not only due to stop codons within the ORF, but also imperfect ITR sequences.

A comparison of the terminal repeats showed that there is a high degree of conservation among the sequences of different species. The *Asmar1* ITR sequence shares extensive identity with the inverted repeats of *mauritiana* subfamily members (Figure 2). High identity (64%) is observed between *Asmar1* from *A. s. rubropilosa* and *Snvmar1* from the fire ant *Solenopsis invicta* (Krieger and Ross, 2003). This is evidence of the relationship of these *mariner* elements within *A. s. rubropilosa* as well as its relationship to *mariner* elements found in other taxa. Besides, it is possible to observe that *Asmar1* ITR shares 57-27% identity with different subfamilies.

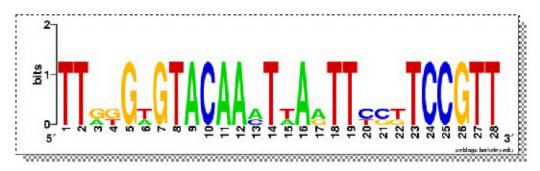
	ITR Sequence		
	1	<pre>% Identity</pre>	Subfamily
Asmar1	TTGTGAGTACAAATTAATTCGGTCCGTT	1	
Ramar1	TTGGGTGTACAACTTAATTCCTTCCGTT	82	
Ramar2	TTAGGTGTACAAATAAGTTTCTTCCGTT	72	mauritiana
Sinvmar1	TTAGGTGTTAAACTTAATTCCTGCCGCT	64	
Mos1	TCAGGTGTACAAGTATGAAATGTCGGTT	54	
Desmar1	TTGGGTGTACAACTTAAAAACCGGAATT	57	
Ammar1	TTGGGTTGGCAACTAAGTAATTGCGGATTT	43	mellifera
Bmmar1	CAGGTGTGTAAATATGAAACCGGAATT	32	mori
Hcmar1	TTAGGTCCTTACATATGAAATTAGCGTT	57	cecropia
Cemar1	TCAGGTTGTCCCATTTGTTTTTGCACTA	36	elegans
Himar1	ACAGGTTGGCTGATAAGTCCCCGGTCTGA	27	irritans

Figure 2. Comparison and % identity of inverted terminal repeats (ITR) sequences among Asmar1 from Atta sexdens rubropilosa and other elements. The elements represented are Ramar1 and Ramar2 from Rhynchosciara americana, Sinvmar1 from Solenopsis invicta, Mos1 from Drosophila mauritiana, Desmar1 from Mayetiola destructor, Ammar1 from Apis mellifera, Hcmar1 from Hyalophora cecropia, Cemar1 from Caenorhabditis elegans, Himar1 from Haematobia irritans, Bmmar1 from Bombyx mori.

Figure 3 shows a comparison among ITR sequences of *Asmar1*, *Ramar1* and *Ramar2*. These ITR sequences share a high identity (82-72%), including six continuous nucleotides (5'-GTACAA) in the 5'-extremity and the six late nucleotides (TCCGTT-3') of the sequence.

However, the presence of an imperfect ITR sequence, generated from mutations may have been the starting point for the inactivation of this element in A. s. rubropilosa. Asmar1, no longer able to undergo transposition, may have accumulated new mutations, creating a totally defective element. Some studies show important characteristics about the ITR sequences, such as conservation of a palindrome sequence and mirror motifs (Bigot et al., 2005), and a pos-

sible function, initially proposed by Pietrokrovski and Henikoff (1997) and later confirmed by Augé-Gouillou et al. (2001), where the ITR sequence could be a region involved in the *mariner* transposase binding by HTH motif present in the N-terminal, although the conservation and evolution of ITR sequences in the *mariner* elements represent a puzzle to be solved.



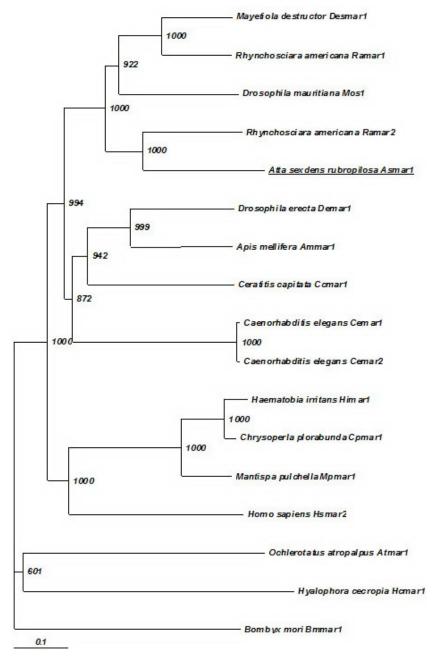
**Figure 3.** Comparison of inverted terminal repeat (ITR) sequences among *Asmar1* from *Atta sexdens rubropilosa* and *Ramar1* and *Ramar2* from *Rhynchosciara americana*. The logo was drawn in weblogo.berkeley.edu.

A TA dinucleotide immediately flanks the ITR sequence, as is typical for *mariner* insertion events. This finding suggests that the *Asmar1* element has a transposition mechanism similar to other *mariner* elements. The TA dinucleotide represents the target site of the element, which would then be duplicated upon insertion in the genome.

A phylogenetic tree based on transposase sequences was constructed to compare and investigate the phylogenetic distribution and evolutionary status of the known full-length mariners. The alignment was generated by the use of ClustalX with default parameters and the tree was elaborated by neighbor joining algorithm and constructed with the TreeView 1.6.6 software (Saitou and Nei, 1987; Page, 1996). The protein sequences used were downloaded from GenBank and the accession Nos. are: A. s. rubropilosa Asmar1 (JF717775), R. americana Ramar1 (DQ784570), R. americana Ramar2 (DQ784571), D. erecta Demar1 (U08094), Apis mellifera Ammarl (U19902), Ceratitis capitata Ccmarl (AAB17945), Caenorhabditis elegans Cemar1 (NP 497296), C. elegans Cemar2 (NP 497120), Mayetiola destructor Desmar1 (AAA66077), D. mauritiana Mos1 (AAA28678), Haematobia irritans Himar1 (U11645), Chrysoperla plorabunda Cpmarl (AAA28265), Mantispa pulchella Mpmarl (U11649), Homo sapiens Hsmar2 (AAC52011), O. atropalpus Atmar1 (AAL16723), Hyalophora cecropia Hcmar1 (M63844). A Bmmar1 sequence from B. mori (AAB47739) was used as an out-group (Shao and Tu, 2001). The tree obtained was classified into six subfamilies based on their branching pattern (Figure 4). The grouping of Asmar1 within the mauritiana subfamily of mariner elements was strongly supported in bootstrap analysis and amino acid identity and similarity with its sister clade.

Figure 5 shows an alignment of *Asmar1* (Genbank accession No. JF717775) consensus with the *Ramar1* element of *R. americana* (Genbank accession No. DQ784570), *Mboumar* element of *M. bouvieri* (Genbank accession No. AJ781769.1) and *Desmar1* element of *M. destructor* (Genbank accession No. AAA66077). The *Asmar1* and *Mboumar* consensus transposase sequences share 44% amino acid identity and 58% conservative replacements, while *Desmar1* transposase shares 45 and 60% amino acid identity and conservative replacements.

The *Ramar1* element appears to be the closest *mariner* to *Asmar1* with 46 and 62% amino acid identity and conservative replacements, respectively.



**Figure 4.** Phylogenetic relationship among the consensus *Asmar1* and other *mariner* elements based on their transposase amino acid sequences, using *Bmmar1* as outgroup.

```
MS NEVLT---QHLRD--FDFLLLKKSAAEAHRMLVEVYGNTAPTGKS CREWFRRFKDEISALKTS LALD SQKI PKTKNDI 75
Asmar1
          MS SEVAN -- KIHLLRIEVILI ECEHWIKKSAAEAHOMUV EVYGDSAP SERFICREWEGREKS ODES VKDK ER PGOPKIK FEDIE-EL 77
Ramar 1
Mos1
          MS SEVPIN-KEOTR TVLI FOEH LIKKTAAE SHEMLVEAFGEOVP TVKK CERWEORFKSGDED VOOK ENGK PPKR YEDA-EL
         MEINFENWRKRICHLREVILL CHFFAKKTAAESHRILLVEVYGEHALAKTOCFEWFORFKS CDED TEDKERPGOPKK FEDE-EL 79
Desmar1
          TRIRISE SNAR CACR II CO -NISSRICTIE SHE VMCMIEKO CNW/PMNLNRETLK CODES LANS EKO - NREK VEC - - - IGLDE 150
Asmar1
          ET LLEO DSOO TOTE LAKS LGVTQQAI SKR LK AAGY IQKQ QYVV PYEL KERDVERRECMSEM LLER HKRK SELH R IV TGDE 157
Ramar1
          OA LLDE DDAQ TOKO LABO LEVS QOAVSNR LE EMGK IOKV GRWV PHELNEROMERRKNTCET LLSR YKRK SFLH RIVTGDE 157
          EA LLIDE DOOD TOBE LAKS LGVT QOALSKRIK AAGY IQKO ONW PHEL KPRDVERRECMSEMILLOR HKKK SELS RII TODE 159
          MW IFYDNPKK KKYY AKPDQS LPS ISTSTPNIHDSK IMLC IWWD-KDLVYYE LLKPGDSITGERYR LQLIRLSRVLQEKWP 229
Ramar1
          KL IHYD NPKR KRSY VKPGQ--PGTSTSKPNI HGAK VMLC IWWDQKGL IHYE LLKPQQ TINGDFYR QQWI RLKQ AVAEKRP 235
Mos1
          KWIFFVSPKRKKSYVDPGQ--PATSTARPNRFGKKTMLCVWWDQSGVIYYELLKRGETVNTARYQQQLINLNRALQRKRP 235
Desmar1
          KW IHYD NSKR KKSY VKRGG--RAKST PKSNL HGAK VMLC IWWD QRGV LYYELLEPGQTITGDLYR TQLI RLWQ ALAEKRP 237
Asmar1
          EY BORH -- VI LOHD NARP HVAKVVKTY LETLIKWEV LPHP PY SP DVAP SDYHLFRSMAHGLADOHF RFCE EI KN WID SWIA 307
Ramar1
          DY ATRICES I FRIND NARPHAAVQVKNY LKNS GWEI LWHP PY SPIDLAP SDYNLFRSMONALS GIRF TSEOGIKS WLNSFLA 315
Most
          EYOKROHRVI FILHDINAPS HTAR AVROT LETILINWEV LIPHA AY SPIDLAP SDYH LIFASMICHA LA KORFIDSYE SVKK WILDEWFA 215
          EY AKRH CAVI FHHD NARP HVAL PVKNY LENS GVEV LPHP PYSP DLAP SDYH LFRSMOND LACKRE TSED GIRK WLDS F LA 317
Desmar1
          SKYVLC FRRGVRTL PERWEKVVASDGQYFES 338
Asmar1
          SKIDERF FHIG TRKI, PERWEKVVA SDGOYF -- 244
Ramar1
          AK DDEF YWRG IHKL PERWEKCVASDGKY LE- 345
Mos1
Desmar1 AK PAKF FERG IHEL SERWEKVI ASDGQYFE- 347
```

Figure 5. Alignment of the conceptual translation of the consensus *Asmar1* transposase sequence with those of *Rhynchosciara americana Ramar1*, *Messor bouvieri Mboumar* and *Mayetiola destructor Desmar1* elements.

#### **Concluding remarks**

Leaf-cutter ant colonies of *Atta* spp are responsible for the largest herbivory impacts in most habitats of the New World tropics (Wilson, 1980). They may account for the destruction of up to 17% of the total leaf production in tropical rainforests (Begon, 1996). The present study provides the first evidence of a full-length *mariner* element in the *A. s. rubropilosa* genome. This transposable element, although non-functional, shows the presence of the *mariner* element family in the *A. s. rubropilosa* genome. The high identity among these elements of different model systems led to the amplification of the *Asmar1* element. Thus, it is possible to relate this fact with the hypothesis of horizontal transfer, which provides that the *mariner* transposable elements would be inherited from related species, although similarity has already been observed between *mariner* elements of distant species. These results serve as a step toward understanding the dynamics of MLE distribution in *Atta sexdens rubropilosa*. Further studies will be important to better characterize the mobile elements in the different castes and to establish its possible association with some relevant cellular function.

#### **ACKNOWLEDGMENTS**

We would like to thank Dr. Paolo M.A. Zanotto, Juliana Velasco and Fernando Lucas Melo for sequencing support and FAPESP and CNPq for their financial support and fellowship.

#### REFERENCES

Augé-Gouillou C, Hamelin MH, Demattei MV, Periquet G, et al. (2001). The ITR binding domain of the *Mariner Mos-1* transposase. *Mol. Genet. Genomics* 265: 58-65.

Begon M, Harper JL and Townsend CR (1996). Ecology - Individuals, Populations and Communities. 3rd edn. Blackwell

- Science, Oxford.
- Berghammer AJ, Klingler M and Wimmer EA (1999). A universal marker for transgenic insects. Nature 402: 370-371.
- Bigot Y, Brillet B and Auge-Gouillou C (2005). Conservation of palindromic and mirror motifs within inverted terminal repeats of *mariner*-like elements. *J. Mol. Biol.* 351: 108-116.
- Capy P, Vitalis R, Langin T, Higuet D, et al. (1996). Relationships between transposable elements based upon the integrase-transposase domains: is there a common ancestor? *J. Mol. Evol.* 42: 359-368.
- Coates CJ, Jasinskiene N, Morgan D, Tosi LRO, et al. (2000). Purified mariner (Mos1) transposase catalyss the integration of marked elements into the germ-line of yellow fever mosquito, Aedes aegypti. Insect Biochem. Mol. Biol. 30: 1003-1008
- Craig NL (1995). Unity in transposition reactions. Science 270: 253-254.
- do Amaral JB and Machado-Santelli GM (2008). Salivary system in leaf-cutting ants (*Atta sexdens rubropilosa* Forel, 1908) castes: a confocal study. *Micron* 39: 1222-1227.
- Doak TG, Doerder FP, Jahn CL and Herrick G (1994). A proposed superfamily of transposase genes: transposon-like elements in ciliated protozoa and a common "D35E" motif. *Proc. Natl. Acad. Sci. U. S. A.* 91: 942-946.
- Ewing B and Green P (1998). Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* 8: 186-194.
- Ewing B, Hillier L, Wendl MC and Green P (1998). Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* 8: 175-185.
- George JA, Traverse KL, DeBaryshe PG, Kelley KJ, et al. (2010). Evolution of diverse mechanisms for protecting chromosome ends by *Drosophila* TART telomere retrotransposons. *Proc. Natl. Acad. Sci. U. S. A.* 107: 21052-21057.
- Gordon D, Abajian C and Green P (1998). Consed: a graphical tool for sequence finishing. Genome Res. 8: 195-202.
- Green CL and Frommer M (2001). The genome of the Queensland fruit fly *Bactrocera tryoni* contains multiple representatives of the *mariner* family of transposable elements. *Insect Mol. Biol.* 10: 371-386.
- Halaimia-Toumi N, Casse N, Demattei MV, Renault S, et al. (2004). The GC-rich transposon *Bytmar1* from the deep-sea hydrothermal crab, *Bythograea thermydron*, may encode three transposase isoforms from a single ORF. *J. Mol. Evol.* 59: 747-760
- Hartl DL, Lohe AR and Lozovskaya ER (1997). Regulation of the transposable element mariner. Genetica 100: 177-184.
  Jacobson JW, Medhora MM and Hartl DL (1986). Molecular structure of a somatically unstable transposable element in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 83: 8684-8688.
- Jarvik T and Lark KG (1998). Characterization of Soymar1, a mariner element in soybean. Genetics 149: 1569-1574.
- Khurana JS and Theurkauf W (2010). piRNAs, transposon silencing, and Drosophila germline development. JBC 191: 905-913.
- Krieger MJ and Ross KG (2003). Molecular evolutionary analyses of mariners and other transposable elements in fire ants (Hymenoptera: Formicidae). *Insect Mol. Biol.* 12: 155-165.
- Kumaresan G and Mathavan S (2004). Molecular diversity and phylogenetic analysis of *mariner*-like transposons in the genome of the silkworm *Bombyx mori. Insect Mol. Biol.* 13: 259-271.
- Lampe DJ, Churchill ME 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.
- Leroy H, Castagnone-Sereno P, Renault S, Auge-Gouillou C, et al. (2003). Characterization of *Mcmar1*, a *mariner*-like element with large inverted terminal repeats (ITRs) from the phytoparasitic nematode *Meloidogyne chitwoodi*. *Gene* 304: 35-41.
- 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.
- Mandrioli M (2003). Identification and chromosomal localization of *mariner*-like elements in the cabbage moth *Mamestra brassicae* (Lepidoptera). *Chromosome Res.* 11: 319-322.
- Moreira LA, Edwards MJ, Adhami F, Jasinskiene N, et al. (2000). Robust gut-specific gene expression in transgenic *Aedes aegypti* mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 97: 10895-10898.
- Page RD (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12: 357-358.
- Palomeque T, Antonio CJ, Munoz-Lopez M and Lorite P (2006). Detection of a *mariner*-like element and a miniature inverted-repeat transposable element (MITE) associated with the heterochromatin from ants of the genus *Messor* and their possible involvement for satellite DNA evolution. *Gene* 371: 194-205.
- Pietrokovski S and Henikoff S (1997). A helix-turn-helix DNA-binding motif predicted for transposases of DNA transposons. *Mol. Gen. Genet.* 254: 689-695.

- Prasad MD and Nagaraju J (2003). A comparative phylogenetic analysis of full-length *mariner* elements isolated from the Indian tasar silkmoth, *Antheraea mylitta* (Lepidoptera: Saturniidae). *J. Biosci.* 28: 443-453.
- Rezende-Teixeira P, Siviero F, Andrade A, Santelli RV, et al. (2008). *Mariner*-like elements in *Rhynchosciara americana* (Sciaridae) genome: molecular and cytological aspects. *Genetica* 133: 137-145.
- Rezende-Teixeira P, Lauand C, Siviero F and Machado-Santelli GM (2010). Normal and defective mariner-like elements in *Rhynchosciara* species (Sciaridae, Diptera). *Genet. Mol. Res.* 9: 849-857.
- Robertson HM (1993). The mariner transposable element is widespread in insects. Nature 362: 241-245.
- Robertson HM and Asplund ML (1996). *Bmmar1*: a basal lineage of the mariner family of transposable elements in the silkworm moth, *Bombyx mori. Insect Biochem. Mol. Biol.* 26: 945-954.
- Robertson HM and Martos R (1997). Molecular evolution of the second ancient human *mariner* transposon, Hsmar2, illustrates patterns of neutral evolution in the human genome lineage. *Gene* 205: 219-228.
- Robertson HM and Zumpano KL (1997). Molecular evolution of an ancient mariner transposon, Hsmar1, in the human genome. *Gene* 205: 203-217.
- Robertson HM and Walden KK (2003). *Bmmar6*, a second mori subfamily *mariner* transposon from the silkworm moth *Bombyx mori. Insect Mol. Biol.* 12: 167-171.
- Russell VW and Shukle RH (1997). Molecular and cytological analysis of a mariner transposon from *Hessian* fly. *J. Hered.* 88: 72-76.
- Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.
- Shao H and Tu Z (2001). Expanding the diversity of the *IS630-Tc1-mariner* superfamily: discovery of a unique DD37E transposon and reclassification of the DD37D and DD39D transposons. *Genetics* 159: 1103-1115.
- van Luenen HG, Colloms SD and Plasterk RH (1994). The mechanism of transposition of Tc3 in *C. elegans. Cell* 79: 293-301.
- Wheeler DL, Chappey C, Lash AE, Leipe DD, et al. (2000). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 28: 10-14.
- Wilson EO (1980). Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). *Behav. Ecol. Sociobiol.* 7: 143-156.
- Yoshiyama M, Honda H, Shono T and Kimura K (2000). Survey of *mariner*-like elements in the housefly, *Musca domestica*. *Genetica* 108: 81-86.
- Zakharkin SO, Willis RL, Litvinova OV, Jinwal UK, et al. (2004). Identification of two *mariner*-like elements in the genome of the mosquito *Ochlerotatus atropalpus*. *Insect Biochem. Mol. Biol.* 34: 377-386.