

Normal and defective *mariner*-like elements in *Rhynchosciara* species (Sciaridae, Diptera)

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ABSTRACT. *Mariner*-like elements are widely present in diverse organisms. These elements constitute a large fraction of the eukaryotic genome; they transpose by a “cut-and-paste” mechanism with their own transposase protein. We found two groups of mobile elements in the genus *Rhynchosciara*. PCR using primers designed from *R. americana* transposons (*Ramar1* and *Ramar2*) were the starting point for this comparative study. Genomic DNA templates of four species: *R. hollaenderi*, *R. millerii*, *R. baschanti*, and *Rhynchosciara* sp were used and genomic sequences were amplified, sequenced and the molecular structures of the elements characterized as being putative *mariner*-like elements. The first group included the putative full-length elements. The second group was composed of defective *mariner* elements that contain a deletion overlapping most of the internal region of the transposase open reading frame. They were named *Rmar1* (type 1) and *Rmar2* (type 2), respectively. Many conserved amino acid blocks were identified, as well as a specific D,D(34)D signature motif that was defective in some elements. Based on predicted transposase sequences, these elements

encode truncated proteins and are phylogenetically very close to *mariner*-like elements of the *mauritiana* subfamily. The inverted terminal repeat sequences that flanked the *mariner*-like elements are responsible for their mobility. These inverted terminal repeat sequences were identified by inverse PCR.

Key words: *Rhynchosciara*; Polytene chromosome; Transposon; *Mariner*-like element; Transposase; Inverted terminal repeat sequence

INTRODUCTION

Transposons are also called “selfish DNA” or “jumping genes”; these mobile elements copy themselves to different locus forming additional copies inside the genome. There are a variety of mobile genetic element families that belong to class II such as the *mariner*-like elements. These elements constitute a large fraction of the eukaryotic genome; they transpose by a “cut-and-paste” mechanism with their own transposase protein.

These elements were first identified in *Drosophila mauritiana* (Jacobson et al., 1986) as *mariner* (*Mos1*) elements, and were initially described as a small gene (~1.3 kb) that encodes a single protein (*mariner* transposase) flanked by short sequences of inverted terminal repeats (ITRs) of 28 bp. The transposase consists of two domains; an N-terminal is the ITR binding domain and a catalytic domain in the C-terminal (Hartl et al., 1997a). The transposable mechanism is mediated by the ability of transposase to recognize the ITR sequence (Hartl et al., 1997b; Lohe et al., 1997). The two functions of the transposase, the binding in the DNA specific sequence and the excision and insertion of the transposable element, are mediated by DNA binding and catalytic domains separately. The *mariner* transposase contains two highly conserved motifs: WVPHEL and YSPDLAP, and the D,D(34)D signature motif (Robertson, 1993; Doak et al., 1994). The transposable process is mediated by the transposase’s ability to recognize the ITR sequence. It occurs via a DNA intermediate catalyzed by the transposase with a duplication of TA nucleotides in the insertion site (Lohe et al., 1997).

Mariner transposable elements are widely distributed in nature and can be found in a variety of insects and other arthropods. *Mariner* family members have also been identified in various 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). The *mariner*-like element family is subdivided into more than 13 subfamilies; the different subfamilies are present in the same genome (Robertson and MacLeod, 1993).

Rhynchosciara americana, an important and typically Brazilian model system used to study molecular and developmental biology, has become the target of new findings internationally (Santelli et al., 2004; Siviero et al., 2006; de Andrade et al., 2009). The search for a mobile element that can be used as a transforming vector could represent an important advance in understanding crucial aspects in the development of this species (Rezende-Teixeira et al., 2008a,b, 2009). In the present study, we identified two different groups of the *mariner* elements in the genus *Rhynchosciara*: the full-length and defective elements. They were called *Rmar1* or type 1 and *Rmar2* or type 2. Some characteristics that identify the *mariner*-like elements and some conserved motifs were present. *In situ* hybridization in the salivary gland of the polytene chromosome showed a similar localization in the genus *Rhynchosciara*.

MATERIAL AND METHODS

Animals

Larvae of *R. americana* were collected in the region of Ubatuba, State of São Paulo, Brazil, and bred in the laboratory, using the conditions established by Lara et al. (1965).

PCR, inverse PCR and DNA sequencing

Polymerase chain reaction (PCR) amplifications were made with specific primer to amplify the *Ramar1* (accession number: DQ784570) and *Ramar2* (accession number: DQ784571) element internal regions (pRamar1: 5'-TTTGACAACAAGTTCAATTT-3' and 5'-TTTCTGGCAATTTACGGAT-3'; pRamar2: 5'-TTCGGTTAGCTTTCGCAGAT-3' and 5'-CTTTTCCCATCTCTCAGGCAG-3'). The protocol of DNA circularization was described in Rezende-Teixeira et al. (2008a). Primers were designed for inverse PCR (iRmar1R 5'-ATCAAACTTCCCGCAAATG-3', iRmar1F 5'-GATGGGAAATTCTGGTTCA-3'; iRmar2R 5'-TGACGCTTACTTGGCTCAAA-3' and iRmar2F 5'-CCGTCCGATTATCACCTGTT-3') based on the internal sequences of *Ramar1* and *Ramar2*. The PCR amplifications were performed using the Platinum Taq DNA polymerase (Invitrogen Life Technologies) according to manufacturer instructions. Cycle conditions were 94°C for 2 min, 35 cycles [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 T3 primers. The nucleotide sequences were analyzed in a Linux workstation with Phred, Phrap Crossmatch 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 *mariner*-like element sequences of *Rhynchosciara* have been deposited in the GenBank database and have the following accession numbers: *R. hollaenderi* *Rhmar1* (GU442124) and *Rhmar2* (GU442125), *R. millerii* *Rmlmar1* (GU442126) and *Rmlmar2* (GU442127), *R. baschanti* *Rbmar1* (GU442122) and *Rbmar2* (GU442123) and *Rhynchosciara* sp *Rspmar1* (GU442128) and *Rspmar2* (GU442129).

In situ hybridizations

Salivary gland chromosomes fixed with ethanol-acetic acid (3:1) were squashed in 45% acetic acid. The protocol of *in situ* hybridization was described in Rezende-Teixeira et al. (2008b). The preparations were analyzed in a laser scanning confocal microscope, LSM-510 (Zeiss), and positive regions were considered those labelled in most of the chromosome optical sections.

RESULTS

PCR amplifications were done using genomic DNA of different species of *Rhynchosciara* and primers designed from internal regions of *Ramar1* and *Ramar2* *mariner*-like elements (Rezende-Teixeira et al., 2008a). The species used in this comparative study were: *R. hollaenderi*, *R. millerii*, *R. baschanti*, and *Rhynchosciara* sp (a species not yet described). Ini-

tially, the amplification of the internal region was done for all species and then the flanks were identified for three of the four species studied using the inverse PCR technique. The general molecular structures of *mariner* elements identified in the genus *Rhynchosciara* are shown in Figure 1, except for *R. baschanti*, whose ITR sequences could not be identified.

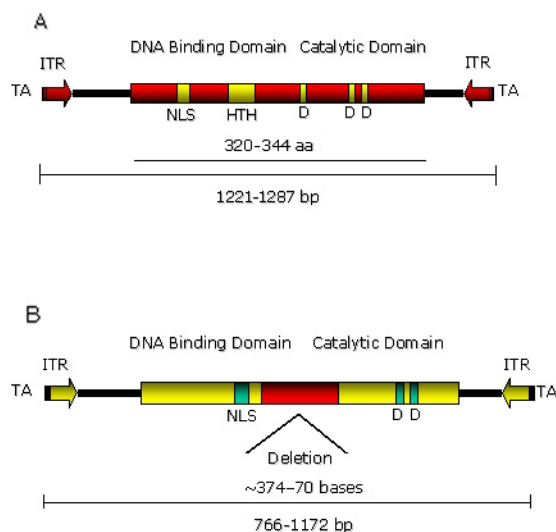


Figure 1. Molecular structure of *mariner* elements studied in the *Rhynchosciara* species. **A.** The putative full-length *mariner* element (*Rmar1*). **B.** The putative defective *mariner* element (*Rmar2*). ITR = inverted terminal repeat sequence; NLS = nuclear localization signal; HTH = helix-turn-helix motif; aa = amino acids.

The elements were called as *Rhmar1* and *Rhmar2* from *R. hollaenderi*, *Rmlmar1* and *Rmlmar2* from *R. millerii*, *Rbmar1* and *Rbmar2* from *R. baschanti*, and *Rspmar1* and *Rspmar2* from *Rhynchosciara* sp. The consensus nucleotide sequence to elements of type 1 showed a characteristic length of 1221 to 1287 bp, corresponding to the typical size of a *mariner*-like element, while the elements of type 2 had a length ranging from 766 to 1172 bp. The predicted translation of the transposase open reading frame (ORF) was derived from defective copies of elements encoding 320 to 344 amino acid proteins to a type 1 element. In the elements of type 2, the deleted internal region showed a range of 374 to 70 bp, varying markedly for different species. This may be indicative that the five *Rhynchosciara* species, including *R. americana*, evolved independently.

It is possible to identify two main domains in the scheme: 1) in the amino-terminal region, known to contain the DNA binding domain, it is possible to identify the nuclear localization signal and the helix-turn-helix motif, responsible for the ITR binding domain, and 2) the catalytic domain is located in the carboxy-terminal, represented by the D,D(34)D catalytic triad proposed by Doak et al. (1994) and Robertson and Lampe (1995). The two main conserved motifs of the *mariner* transposases (WVPHEL and YSPDLAP) described by Robertson (1993) are present but with some modifications. The consensus motif identified was YVPYEL and YSPDLAP for all species. This can be an indication that this motif is specific to *Rhynchosciara*, and may be a selective mutation that generates this sequence that is perpetuated every generation.

Tables 1 and 2 show the comparison between the consensus sequences of *Rmar1* and *Rmar2* elements of different species; a high identity in nucleotide sequences is also observed. The *Rmar1* consensus transposase sequences share 63-86% amino acid identity and 73-92.7% conservative replacements, while *Rmar2* transposase share 35.5-76.8% and 44-81% amino acid identity and conservative replacements.

Table 1. Comparison between the studied *mariner*-like element sequences amplified in the *Rhynchosciara* species.

	<i>Ramar1</i>	<i>Rspmar1</i>	<i>Rhmar1</i>	<i>Rbmar1</i>
<i>Rspmar1</i>	68.1/76.5 (91.8)	-	-	-
<i>Rhmar1</i>	80.0/85.2 (87.4)	63.1/73.4 (88.0)	-	-
<i>Rbmar1</i>	86.0/89.5 (77.8)	65.7/73.0 (75.4)	73.0/77.9 (70.6)	-
<i>Rmlmar1</i>	87.2/92.7 (91.0)	66.0/74.7 (93.3)	74.8/82.0 (86.4)	81.4/86.3 (74.7)

Data are reported as percent of amino acid identity/conservative replacements with nucleotide identity in parentheses. For abbreviations, see Figure 4.

Table 2. Comparison between the studied *mariner*-like element sequences amplified in the *Rhynchosciara* species.

	<i>Ramar2</i>	<i>Rspmar2</i>	<i>Rhmar2</i>	<i>Rbmar2</i>
<i>Rspmar2</i>	42.0/53.3 (61.0)	-	-	-
<i>Rhmar2</i>	76.4/79.2 (90.1)	35.5/44.4 (60.4)	-	-
<i>Rbmar2</i>	76.8/81.0 (76.1)	37.7/47.0 (48.6)	76.5/78.5 (73.8)	-
<i>Rmlmar2</i>	63.0/73.0 (76.0)	37.5/44.4 (55.5)	61.5/70.0 (78.6)	64.1/73.7 (60.0)

Data are reported as percent of amino acid identity/conservative replacements with nucleotide identity in parentheses. For abbreviations, see Figure 4.

The elements amplified and sequenced have a typical structure of a *mariner*-like element, the inverted terminal repeats with 28 bp and TA dinucleotide immediately flanking the ITRs, as is typical for *mariner* insertion events (Figure 2). Only in *R. baschanti* was it not possible to identify the ITR sequence. It may suggest that these elements are fully defective and therefore the ITR sequences have been lost from the genome of this species.

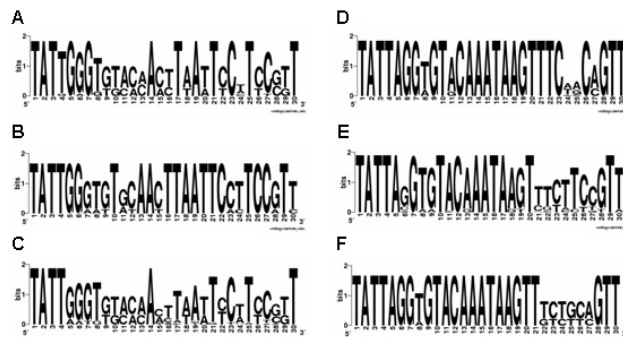


Figure 2. Sequence logo of the region flanking and inverted terminal repeats of the *mariner*-like elements of three *Rhynchosciara* species. The sequence logos were generated using the WebLogo server (<http://weblogo.berkeley.edu/>), and showed the consensus ITR sequence and the insertion site in genomic DNA to *Rmlmar1* (A) and *Rmlmar2* (B); *Rhmar1* (E) and *Rhmar2* (F); *Rspmar1* (C), and *Rspmar2* (D). The vertical axis has a maximum value of two, which is proportional to the level of sequence conservation at each position. For abbreviations, see Figure 4.

The ITR sequences found for other species: *R. millerii*, *R. hollaenderi* and *Rhynchosciara* sp are highly defective. They exhibited imperfect terminal repeats with many mismatches and no specific mutation site. The opposite was observed for *R. americana* where the *Ramar1* and *Ramar2* elements shared high identity (82%) in the ITR sequences, including nine continuous nucleotides (5'-GGTGTACAA) at the 5' end and the last eight nucleotides (CTTCCGTT-3') of the sequence (Rezende-Teixeira et al., 2008a).

However, the mismatches identified in the ITR sequences of *Rhynchosciara* could inactivate these *mariner* elements by losing the capacity for mobility. Some studies show important characteristics about the ITR sequences, such as conservation of a palindrome sequence and motif mirror (Bigot et al., 2005), and a possible function, initially proposed by Pietrokovski and Henikoff (1997) and then confirmed by Augé-Gouillou et al. (2001). The ITR sequence could be a region involved in the binding of the *mariner* transposase by helix-turn-helix motif present in the N-terminal portion. However, the conservation and evolution of ITR sequences in the *mariner* elements represent a puzzle to be solved.

In situ hybridizations of a *mariner*-like element in the salivary gland of polytene chromosomes of *R. baschanti*, *R. hollaenderi* and *Rhynchosciara* sp appeared as a single band. The same labeling was observed in *R. americana* using the *Ramar2* probe. The chromosomes were propidium iodide stained and the arrows showed the positive regions. The labeling appeared in the same and in a very similar region of polytene chromosome A in all the *Rhynchosciara* species studied (Figure 3).

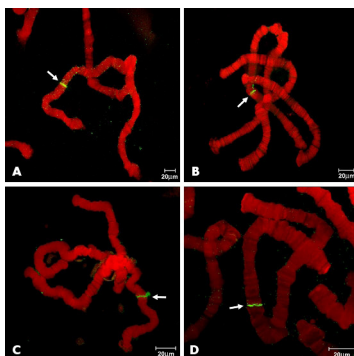


Figure 3. Laser scanner confocal microscope images of the *in situ* hybridization of a *mariner*-like element in salivary gland chromosomes using the *Rhynchosciara americana* *mariner*-like element as a probe. **A.** *R. baschanti*. **B.** *R. hollaenderi*. **C.** *Rhynchosciara* sp. **D.** *R. americana*.

Currently, it is known that these elements are members of a large family of transposons, which became known as *mariner* or *mariner*-like elements (Lampe et al., 1996). Over 13 subfamilies of *mariner* elements are known, which typically have about 40-56% nucleotide identity and 23-45% amino acid identity between the subfamilies and 25-100% amino acid identity with a particular subfamily (Robertson and MacLeod, 1993).

To investigate the phylogenetic distribution of the known full-length *mariner*, a phylogenetic tree based on transposase sequences was constructed. The elements used were downloaded from GenBank and the accession numbers are: *R. americana* *Ramar1* ([DQ784570](https://www.ncbi.nlm.nih.gov/nuccore/DQ784570)),

R. americana Ramar2 (DQ784571), *Drosophila erecta* Demar1 (U08094), *Apis mellifera* Ammar1 (U19902), *Ceratitis capitata* Ccmar1 (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* Cpmar1 (AAA28265), *Mantispa pulchella* Mpmar1 (U11649), *Homo sapiens* Hsmar2 (AAC52011), *Ochlerotatus atropalpus* Atmar1 (AAL16723), *Hyalophora cecropia* Hcmar1 (M63844). A *Bmmar1* sequence from *Bombyx mori* (AAB47739) was used as an out-group (Shao and Tu, 2001). The alignment was generated by the use of ClustalX with default parameters and the tree was elaborated using a neighbor-joining algorithm and was constructed with the TreeView 1.6.6 software (Saitou and Nei, 1987; Page, 1996). The tree obtained was classified into six subfamilies (*mellifera*, *elegans*, *mauritiana*, *irritans*, *cecropia*, and *mori*) based on their branching pattern (Figure 4).

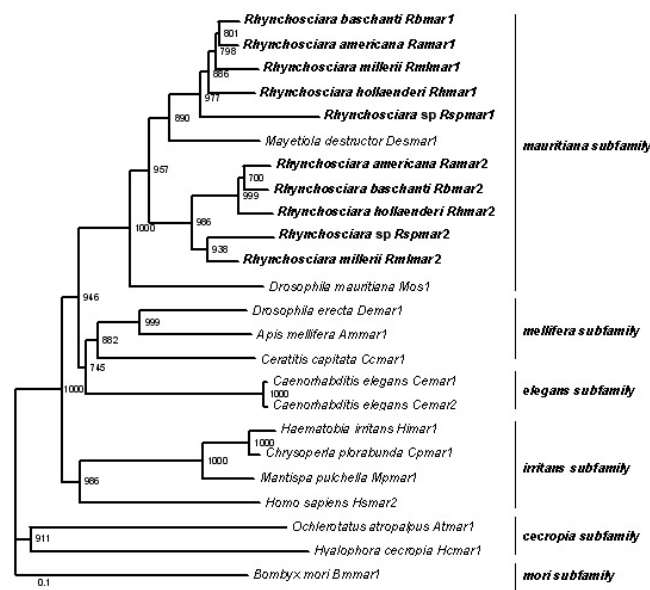


Figure 4. Phylogenetic relationship among the consensus sequences of the elements of *Rhynchosciara* species and other *mariner* elements based on their transposase amino acid sequences, using *Bmmar1* as an outgroup.

The grouping of *mariner* elements of the genus *Rhynchosciara* within the *mauritiana* subfamily was strongly supported in bootstrap analysis and amino acid identity and similarity with its sister clade. However, two different sub-clades could be identified that represented the *mariner*-like elements type 1 and type 2.

CONCLUSION

The transposable elements can be autonomous or defective with respect to their own transposition. Defective elements often exhibit deletions in ORFs or in the sequence of terminal repeats necessary for its insertion. The autonomous elements are able to move non-

functional elements, since they belong to the same subfamily. So just a functional element is necessary for the event of transposition to occur. The ability to identify an ORF for the transposase from multiple non-functional copies would indicate that the ancestor of the element *Rmar1* could have been a hyperactive transposase.

The characterization of *mariner*-like elements in the genus *Rhynchosciara* can be an indication that these elements populated the *Rhynchosciara* genome for many years. Nevertheless, these elements still represent a puzzle in the evolutionary history of this model system. Furthermore, these results represent the principle for understanding the dynamics of *mariner* elements and their distribution in the genome of *Rhynchosciara*.

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