# A comparative phylogenetic analysis of full-length *mariner* elements isolated from the Indian tasar silkmoth, *Antheraea mylitta* (Lepidoptera: saturniidae)

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Mariner like elements (MLEs) are widely distributed type II transposons with an open reading frame (ORF) for transposase. We studied comparative phylogenetic evolution and inverted terminal repeat (ITR) conservation of MLEs from Indian saturniid silkmoth, *Antheraea mylitta* with other full length MLEs submitted in the database. Full length elements from *A. mylitta* were inactive with multiple mutations. Many conserved amino acid blocks were identified after aligning transposase sequences. Mariner signature sequence, DD(34)D was almost invariable although a few new class of elements had different signatures. *A. mylitta* MLEs (*Anmmar*) get phylogenetically classified under cecropia subfamily and cluster closely with the elements from other Bombycoidea superfamily members implying vertical transmission from a common ancestor. ITR analysis showed a conserved sequence of AGGT(2-8N)ATAAGT for forward repeat and AGGT(2-8N)ATGAAAT for reverse repeat. These results and additional work may help us to understand the dynamics of MLE distribution in *A. mylitta* and construction of appropriate vectors for mariner mediated transgenics.

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## 1. Introduction

Mariner transposons also called as mariner like elements (MLEs) are members of a large, diverse and widespread superfamily of transposable elements comprising prokaryotic and eukaryotic members. Tn7, IS2, IS3, IS4, IS30, IS630 and bacteriophage *Mu* are some of the prokaryotic members while *Tc1* is an example of eukaryotic members (reviewed in Hartl *et al* 1997a). MLE was first isolated from *Drosophila mauritiana* (Haymer and Marsh 1986; Jacobson *et al* 1986) and later similar elements were identified in a wide range of vertebrates and invertebrates, and in plant species (Robertson 1993; Jarvik and Lark 1998; Feschotte and Wessler 2002).

MLEs are short elements of about 1280 bp coding for a transposase of 350 amino acids enclosed by short inverted terminal repeats (ITRs) of 28 to 32 bp. Transposase consists of two domains, an N-terminal ITR binding domain and a C-terminal catalytic domain. These elements jump in their host genome by a cut and paste transposition mechanism, similar to that described for *Tc1* and several bacterial insertion sequences (reviewed in Hartl *et al* 1997a). This process occurs via a DNA intermediate catalyzed by transposase with a TA duplication at the insertion site (Hartl 1989; Lohe *et al* 1996). The mariner transposase contains two highly conserved motifs WVPHEL and YSPDLAP separated by about 150 amino acids, as well as a specific D,D(34)D signature motif (Robertson 1993; Doak *et al* 1994), which is assu-

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Abbreviations used: ITRs, Inverted terminal repeats; MLE, mariner like elements; ORF, open reading frame.

med to be a part of active site serving as divalent cation binding domain required for catalysis.

Except a few, all MLEs were found to be inactive because of the multiple inactivating mutations like stop codons, deletions, insertions and substitutions (Maruyama *et al* 1991; Robertson 1993; Lohe *et al* 1997). The functional elements found so far are *Mos1* from *D. mauritiana*, *Mos1* like elements from *D. simulans* and *Himar1* from *Haematobia irritans* (Maruyama *et al* 1991; Capy *et al* 1992; Lampe *et al* 1996).

Presence and perpetuation of a large number of inactive elements is thought to be because of vertical inactivation during the course of mariner life cycle to limit the cause of deleterious effects on host genome. The proposed model for mariner life cycle in the host genome involves invasion by horizontal transmission, immediate multiplication in number, vertical inactivation by accumulating mutations and stochastic loss by random genetic drift (Lohe *et al* 1995; Hartl *et al* 1997b,c).

Mariner elements are classified into seven distinct subfamilies namely *mauritiana*, *cecropia*, *mellifera*, *irritans*, *capitata*, *elegans* and *mori*. The elements from different subfamilies share 40% to 56% identities at nucleotide level (Robertson and MacLeod 1995). Gene transfer between species, a phenomenon known as horizontal gene transfer, appears to have played an important role in the evolution of mariner elements. Horizontal transmission is inferred from the presence of very similar mariner transposon sequences in phylogenetically distant species and from the presence of *mariner* elements of diverse subfamilies in a single species (Robertson 1993). Recently Yoshiyama *et al* (2001) found mariner elements of 97·6% nucleotide identity in parasitoid insect and its host wasp inferring that the element is possibly horizontally transferred from one to another.

The presence of different copy number of distinct mariner subfamily elements in the same genome suggests that they may be regulated differently, as presence of one element in the genome may restrict/regulate the multiplication of a new horizontally transferred element in the same genome. Recently Lampe *et al* (2001) have shown that a relatively small amount of divergence in ITR sequence is sufficient to substantially reduce the interactions between these elements.

Presence of MLEs in a broad host range indicate the host independent transposition. This has helped researchers to undertake genetic manipulation of many species. Recent reports have indicated the potential of *mariner*-based germline transformation for making transgenic fruitfly, mouse cells, chicken, mosquito, zebrafish, and leishmania (reviewed in Plasterk *et al* 1999).

To know the distribution and dynamics of mariner elements in *Antheraea mylitta*, we studied full-length mariner elements and their phylogenetic status compared with other full-length mariner transposase sequences submitted in the database. We also report the ITR comparison and its conservation across different MLEs belonging to different mariner subfamilies.

## 2. Materials and methods

### 2.1 *Species sample and DNA isolation*

Liquid nitrogen-frozen moths of *A. mylitta* (Indian tropical tasar silkmoth, Bihar, India) were crushed to fine powder, mixed with 10 ml of 2 PK buffer (200 mM Tris-Cl, 25 mM EDTA, 300 mM NaCl, 2% SDS; pH 7·5), and incubated at 55°C for 2 h in the presence of proteinase K (150 μg/ml), followed by extraction once with Tris-saturated phenol, twice with saturated phenol : chloroform (1 : 1), and once with chloroform. DNA solution was then treated with 1 μg/ml of RNase A for 2 h at 37°C and extracted once with phenol : chloroform (1 : 1) and once with chloroform. DNA was ethanol precipitated and spooled out, washed with 70% alcohol, dried and dissolved in TE buffer.

### 2.2 *PCR amplification, cloning and sequencing*

Approximately 50 ng of genomic DNA was taken and PCR was done as per Robertson (1993). Amplification products were analysed in 1·2% agarose/TAE gels containing 0·5 mg/ml of ethidium bromide. PCR amplification product of 490 bp was excised from gel and DNA was eluted using a Bio 101 Bioclean kit, then cloned into pCR2·1 plasmid (Invitrogen, USA). Clones were checked for inserts of expected size by colony PCR. Three clones were randomly picked and sequenced in an ABI Prism 311 automated sequencer using the Big-Dye terminator chemistry.

### 2.3 *Genomic library screening*

Genomic DNA from *A. mylitta* was used to construct a genomic library in *l*ZAPII vector (Stratagene, USA) using DNA digested to completion with *Eco*RI. The library was screened with a 450 bp *A. mylitta mariner* fragment generated by PCR (see above, accession No.: AF125219). Screening of approximately 20,000 plaques yielded about 50 positives. From secondary and tertiary screens, three positive plaques were randomly chosen. The inserts were excised *in vivo* as the pBlueScript plasmid clones, and sequenced using the *mariner*-specific primers 450f (TCGCCAGCGCGAAGTACGC), 450r (CGGATGACGGAGTACTTCCAAC), RC1 (GCGTACTTCGCGCTGGCGA), HRC2 (CACGACAACGCGCGACCTCAT),

fTRP (AATTGCCGTTCTGCAGTACT), and rTRP (GAAATTGCCGTTTTGTATGA).

### 2.4 *Southern hybridization*

*A. mylitta* genomic DNA (20 µg) was digested with *Eco*RI, *Bam*HI, and *Hind*III individually and fractionated in 0·8% agarose/TAE gel. DNA was transferred on to Hybond N+ membrane (Amersham Pharmacia) using a vacuum blotter and UV crosslinked to the membrane in UVP CL-1000 crosslinker. Probe comprising the *A. mylitta* MLE *Anmmar1* was radiolabelled with $\alpha^{32}$P using random primer labelling kit supplied by BRIT, India, and hybridized as per the standard methodology under high stringency conditions, followed by washes in 0·1X SSC, 0·1% SDS at 65°C.

### 2.5 *Sequence analysis*

Sequences of the three mariner elements from *A. mylitta* were conceptually translated with judicious introduction of frameshifts. These were then aligned with the multiple mariner transposase sequences downloaded from Genbank (all amino acid sequence submissions) (table 1) with the help of CLUSTAL X (Thompson *et al* 1994). The aligned sequences were then used in Boxshade to obtain a shaded format to highlight the similarities in the sequences. Using the aligned sequences, a phylogenic tree was constructed using PAUP software (Swofford 2002). ITR sequences defined by the authors among the accessions mentioned in table 1 were taken and aligned using clustal W. Later the alignment was optimized by manual alignment. Sequence logo was generated using the WebLogo server (http://www.bio.cam.ac.uk/seqlogo/) and computed using makelogo algorithm (Schneider and Stephens 1990).

## 3. Results and discussion

### 3.1 *Full length elements from* A. mylitta

To obtain full length copies of the *A. mylitta* mariner elements, a genomic library of *A. mylitta* was screened and three random clones were sequenced. The sequences were submitted to Genbank under the accession numbers AF126011, AF126012 and AF125249. Genomic sequences flanking the three full-length *A. mylitta* mariner elements, named as *Anmmar4*, *Anmmar5* and *Anmmar6*, as per the convention proposed by Robertson and Asplund (1996) do not show any consensus, although all of them are AT rich regions. A direct TA duplication at the site of insertion of the transposable element was identified. None of the three sequenced clones had any functional open reading frame (ORF) because of the presence of multiple stop codons, frameshift mutations and deletions. One of the clones, *Anmmar4*, was truncated at the 3′ end by about seventy amino acid residues. Among the three elements, identity of conceptually translated transposase was from 49% to 67% at amino acid level. The signature sequence, D,D(34)D, which is a characteristic of *mariner*-like elements, was identified in the sequences, although in two clones (*Anmmar4* and *Anmmar6*) the last aspartic acid residue was deleted.

Both the partial elements (Prasad *et al* 2002) and the full length *A. mylitta* MLEs sequenced were inactive with multiple stop codons and frameshift mutations indicating that they have accumulated mutations in the process of their vertical transmission (Maruyama *et al* 1991; Capy *et al* 1992; Lampe *et al* 1996). These mutations show that transposition event of cecropia family element in *A. mylitta* is not a recent event which is supported by the recent study showing non-occurance of inter-subfamily excisions because of divergence in the transposase target sequence (Lampe *et al* 2001). Although these elements were found to be inactive, there appears to be selective constraint for conservation of functional domains of transposase (Prasad *et al* 2002).

Southern blot analysis showed an intense streak (data not shown), indicating that the elements are present at high copy number. To determine the copy number of *Anmmar* elements in *A. mylitta* genome, we did dot blot analysis using *Anmmar1* as probe (same as the one used against genomic library) as described by Prasad *et al* (2002). The copy number was about 2000 assuming that the genome size of *A. mylitta* is equivalent to *Bombyx mori*. This copy number is a moderate number compared to varying copy number from 70–5000 in different species of silkmoths (Prasad *et al* 2002).

### 3.2 *Similarity analysis*

We aligned all the transposase sequences of different full length MLEs available in the database (table 1, figure 1). We observed that the WVPHEL, DEKW, H/QDNAP and HPPYSPDLAPSD sequences are highly conserved in all the mariner elements, with the last one being the largest and most conserved block. These blocks are dispersed all along the MLEs. The plant MLEs [*Soymar1* (DD39D) and *Osmar* (DD39D)], mosquito MLEs [*Aatmar* (DD37E) and *Agmar*ITm (DD37E)], an ancient MLE from *B. mori* [*Bmmar1* (DD37D)] and Natal fruit fly MLE [*Crmar2*\5 (DD41D)] do not share relatively high similarity with rest of the sequences and they share different signature sequence as indicated in the parenthesis. With these exceptions, DD34D signature sequence is almost invariable. The differences in these elements were also depicted in the phylogenetic tree (figure 2), where

**Table 1.** Accession number, clone name and nomenclature (as per Robertson and Asplund 1996) of analysed sequences.

| Species | Clone | Nomenclature | Accession number |
|---|---|---|---|
| *Drosophila mauritiana* | Mos1 | DmMos1 | A26491 |
| *Mayetiola destructor* | | *Desmar1* | AAA66077 |
| *Girardia tigrina* | | *Gtmar1* | 1917335A |
| | | *Gtmar2* | CAA56763 |
| *Dugesia tigrina* | | DtMLE | S35068 |
| | | *Dtmar0* | CAA56855 |
| | | *Dtmar1* | CAA50801 |
| | | *Dtmar3* | CAA56859 |
| | | *Dtmar5* | CAA56858 |
| | | *Dtmar8* | CAA56857 |
| | | *Dtmar9* | CAA56856 |
| *Homo sapiens* | | *Hsmar1*cons | AAC52010 |
| | | *Hsmar2*cons | AAC52011 |
| *Glossina palpalis* | | Gpamar1 | U18308 |
| *Drosophila erecta* | p19 | *Demar*p19 | U08094 |
| *Chrysoperla plorabunda* | | *Cplmar* | S35520 |
| | | *Cplmar1* | AAA28265 |
| | Cp8 | *Cplmar*Cp8 | AAC46948 |
| | Cp4 | *Cplmar*Cp4 | U11653 |
| | Cp3 | *Cplmar*Cp3 | AAC46947 |
| | Cp2 | *Cplmar*Cp2 | AAC46946 |
| | Cp1 | *Cplmar*Cp1 | AAC46945 |
| *Drosophila simulans* | Mos6a | *Dsmar*Mos6a | AAC16610 |
| | Mos6b | *Dsmar*Mos6b | AAC16611 |
| | L8 | *Dsmar*L8 | AAC16612 |
| | L14 | *Dsmar*L14 | AAC16613 |
| | MadB | *Dsmar*MadB | AAC16614 |
| | Sev2 | *Dsmar*Sev2 | AAC16615 |
| | Pr1 | *Dsmar*Pr1 | AAC16616 |
| | PrA | *Dsmar*PrA | AAC16617 |
| | BordA | *Dsmar*BordA | AAC16618 |
| *Drosophila sechellia* | PA2 | *Dsemar*PA2 | AAC16608 |
| | 228C | *Dsemar*228C | AAC16609 |
| *Drosophila teissieri* | DtBz3 | *Dtemar*DtBz3 | AAC28261 |
| | DtBz4 | *Dtemar*DtBz4 | AAC28262 |
| *Apis cerana* | | *Acemar1* | BAB86288 |
| *Attacus atlas* | | AaMLE | BAA21826 |
| *Mamestra brassicae* | | *Mbmar1* | AAL69970 |
| *Anopheles gambiae* | ITm | *Agmar*ITm | AAL16724 |
| | Ag8 | *Agmar*Ag8 | U11659 |
| | Ag5 | *Agmar*Ag5 | U11658 |
| *Aedes atropalpus* | | *Aatmar* | AAL16723 |
| *Blattella germanica* | BGMA1 | *Bgmar*BGMA1 | AAK40118 |
| *Ceratitis rosa* | | *Crmar2ι5* | AAK61417 |
| *Ceratitis capitata* | | *Ccmar1ι18* | AAB17945 |
| *Musca domestica* | MDMA1 | *Mdmar*MDMA1 | AAK54758 |
| *Mantispa pulchella* | Mp1 | *Mpmar*Mp1 | U11649 |
| *Drosophila ananassae* | Da9 | *Danmar1ι1* | U11648 |
| | Da14 | *Danmar1ι2* | U11646 |
| *Haematobia irritans* | Hi6 | *Himar1ι6* | U11645 |
| | Hi5 | *Himar1ι5* | U11644 |
| | Hi4 | *Himar1ι4* | U11643 |
| | Hi3 | *Himar1ι3* | U11642 |
| | Hi2 | *Himar1ι2* | U11641 |
| *Glycine max* | | *Soymar1* | AAC28384 |
| *Bombyx mori* | | *Bmmar1* | AAB47739 |
| | | *Bmmar2* | BAA23532 |
| *Glossina palpalis* | | *Gpamar1* | U18308 |

**Table 1.** *Contd.*

| Species | Clone | Nomenclature | Accession number |
|---|---|---|---|
| *Oryza sativa* | | *Osmar1›1* | BAA88208 |
| | | *Osmar1›2* | BAA88172 |
| | | *Osmar1›3* | AAK98713 |
| *Caenorhabditis elegans* | | *Cemar1›1* | NP_497296 |
| | | *Cemar1›2* | NP_497120 |
| | | *Cemar1›3* | AAB63925 |
| *Hyalophora cecropia* | | HcMLE | M63844 |
| *Antheraea mylitta* | Anm1·2 | *Anmmar4* | AF125249 |
| | Anm3·2 | *Anmmar5* | AF126011 |
| | Anm4·2 | *Anmmar6* | AF126012 |
| *Apis mellifera* | | AmMLE | U19902 |

these elements come out as basal branches from the main cluster. Based on the phylogenetic clustering, elements from flowering plants, two elements (*Aatmar* and *Agmar* ITm) from mosquito, and *Bmmar1* were proposed to be classified as different family of IS630-Tc1 superfamily (Shao and Tu 2001).

### 3.3 *Phylogenetic analysis*

To test the phylogenetic distribution and evolutionary status of the full length mariners, we constructed a phylogenetic tree using CeTc1 as outgroup. The tree obtained was classified into 5 subfamilies based on their branching pattern (figure 2). The subfamilies are irritans, mellifera, elegans, cecropia and mauritiana. *Bmmar1* and *Crmar2›5* come out as basal elements which is expected because of their divergence, the former having DD(37)D and latter with DD(41)D signatures (figure 2). Two of the mosquito elements having signature of DD(37)E and plant elements carrying signature DD(39)D come out as separate clusters. *Anmmar4*, *Anmmar5* and *Anmmar6* were grouped under cecropia subfamily closely branching with *Bmmar2*, AaMLE and HcMLE elements. Host species of these elements, *A. mylitta*, *B. mori*, *A. atlas* and *Hyalophora cecropia* respectively, taxonomically belong to Bombycoidea superfamily thus indicating that these elements probably predate the speciation event. This is also supported by the non-identification of any active element from *A. mylitta*. All the mariner elements of *A. mylitta* identified until today belong to cecropia subfamily whereas elements of different subfamilies were found in *B. mori*, *B. mandarina* and *Phylosamia cynthia ricini* (Prasad *et al* 2002). This indicates that *A. mylitta* may have elements from few subfamilies although other subfamily elements cannot be ruled out. The limited presence of elements may be explained by the presence of *A. mylitta* in a few geographically isolated pockets of India. Thus, it has lesser chances of horizontal transfer compared to a widely occurring species interacting with a wide array of other species. A similar tree based on distance matrix indicated that *Bmmar1* was one of the oldest elements, which is obvious with the kind of divergence it possesses. This divergence is caused because of the mutations accumulated by the element over a long time, ultimately leading to its divergence into a different class of element.

### 3.4 *ITR analysis*

The ITRs were used to generate a sequence logo. This is a graphical way of representing aligned sequences and is easier to interpret than a strict consensus sequence. The upper and lower ITRs were imperfect ranging between 33–35 bp in length. Upper and lower inverted repeats were analysed separately and are shown in figure 3. Although the actual average ITR length is 28–32 bp, the length shown in our figure is greater because of the gaps introduced to maintain an optimum alignment. ITR analysis showed marked conservation of certain bases although elements come from different subfamilies and diverse species. The upper repeat has conserved residues GTTTAAT at positions 10, 12, 13, 17, 22, 23 and 25 respectively (considering bit value of > 3). Similarly, lower repeat has conserved residues ACGATTTACCT at positions 8, 10, 12, 15, 17, 18, 21 and 30–34 respectively. We identified a conserved ITR of AGGT(2-8N)ATAAGT, with 2Ns, 6Ns and 8Ns in *DmMos1*, *Himar1* and consensus respectively. The reverse consensus AGGT(2-8N) AT**G**AA**A**T has a variation in two bases represented in bold letters. This consensus fits very well with the consensus reported earlier from ITR sequences of six mariner family elements (Lampe *et al* 2001). There are more reports on transposase mutations to identify both inactivating and hyperactivating mutations but not much is known on the influence of ITR sequence variation on transposase activity (Lohe *et al* 1997; Lampe *et al* 1999,

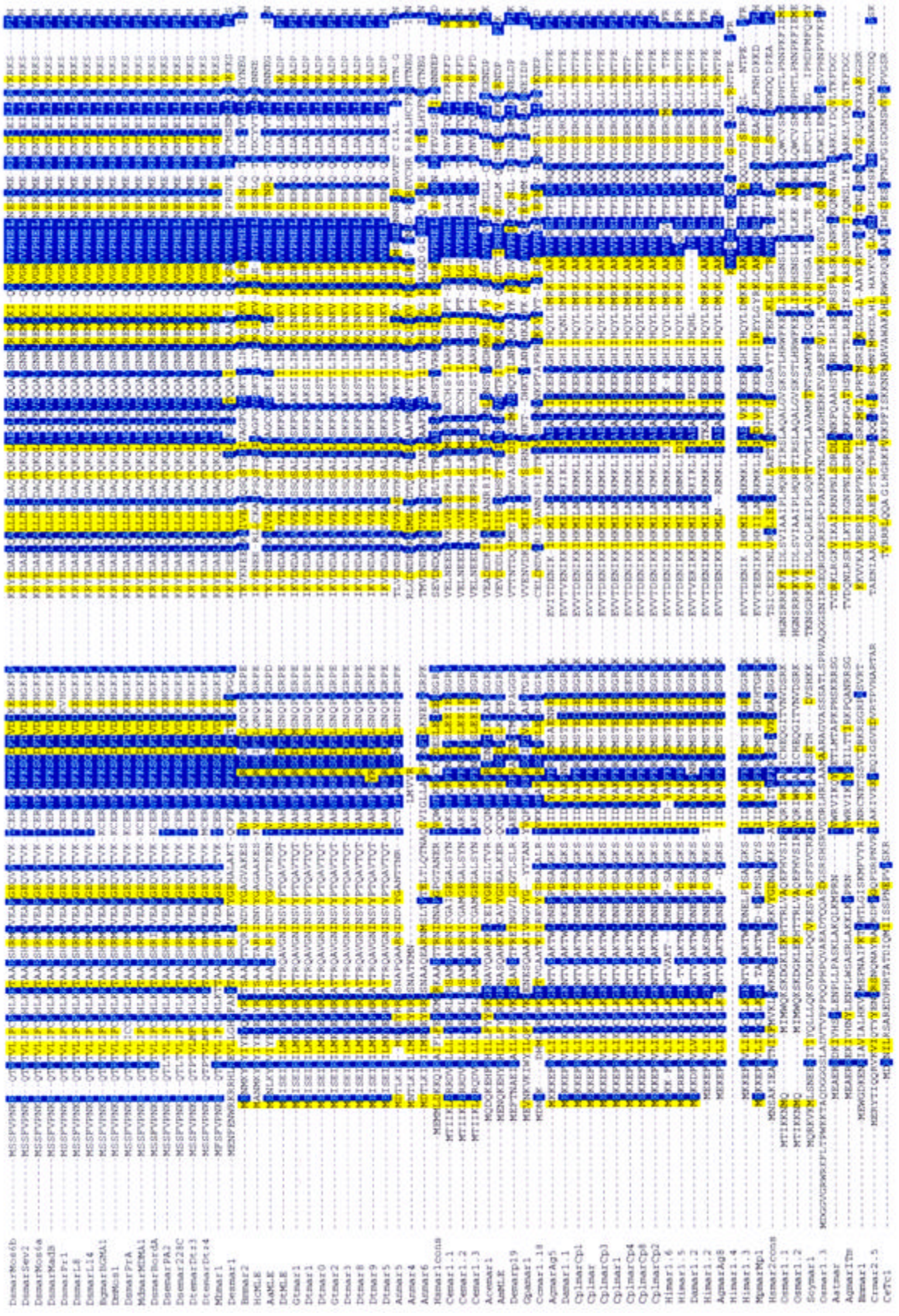

**Figure 1.** *Contd.*

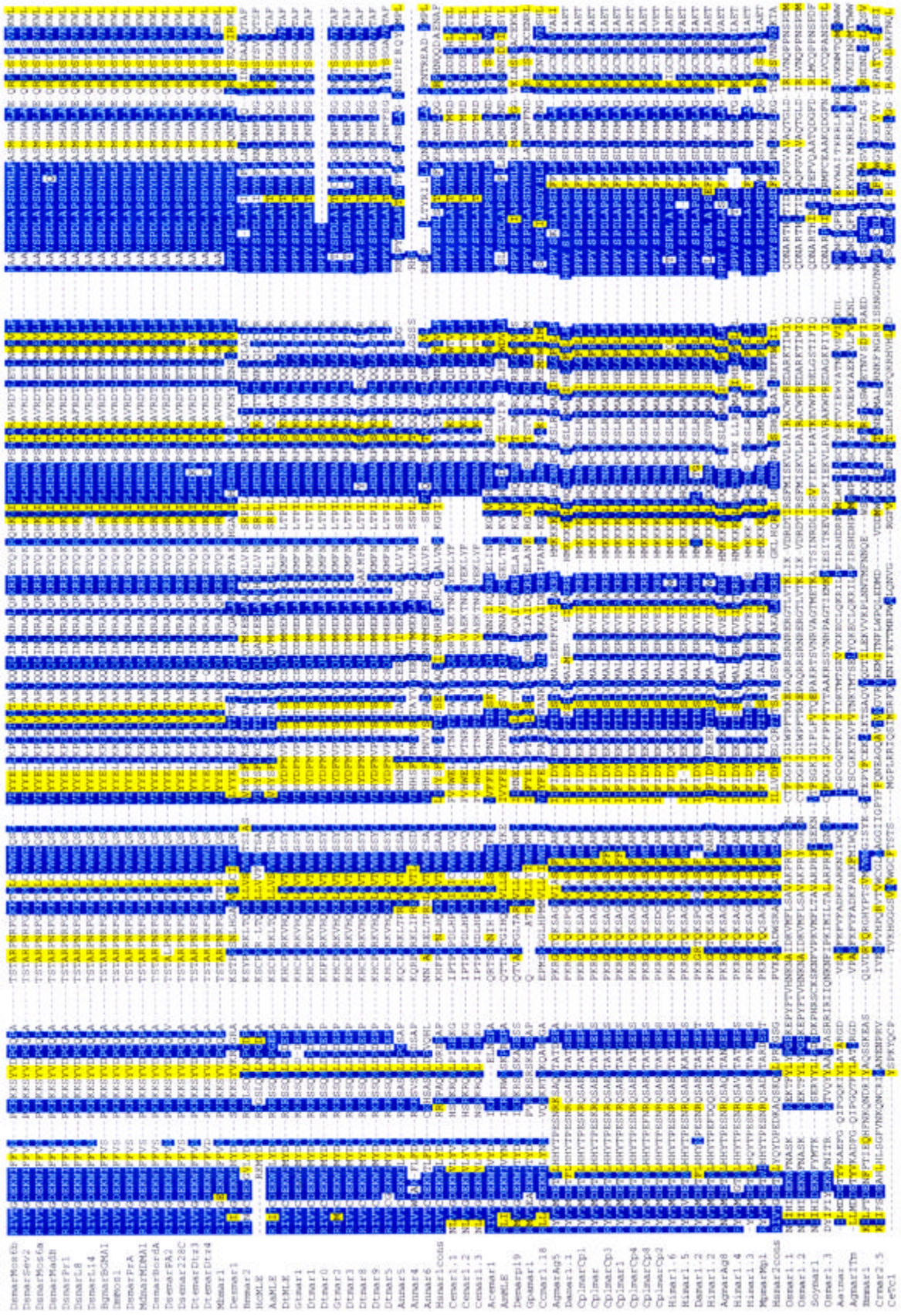

**Figure 1.** *Contd.*

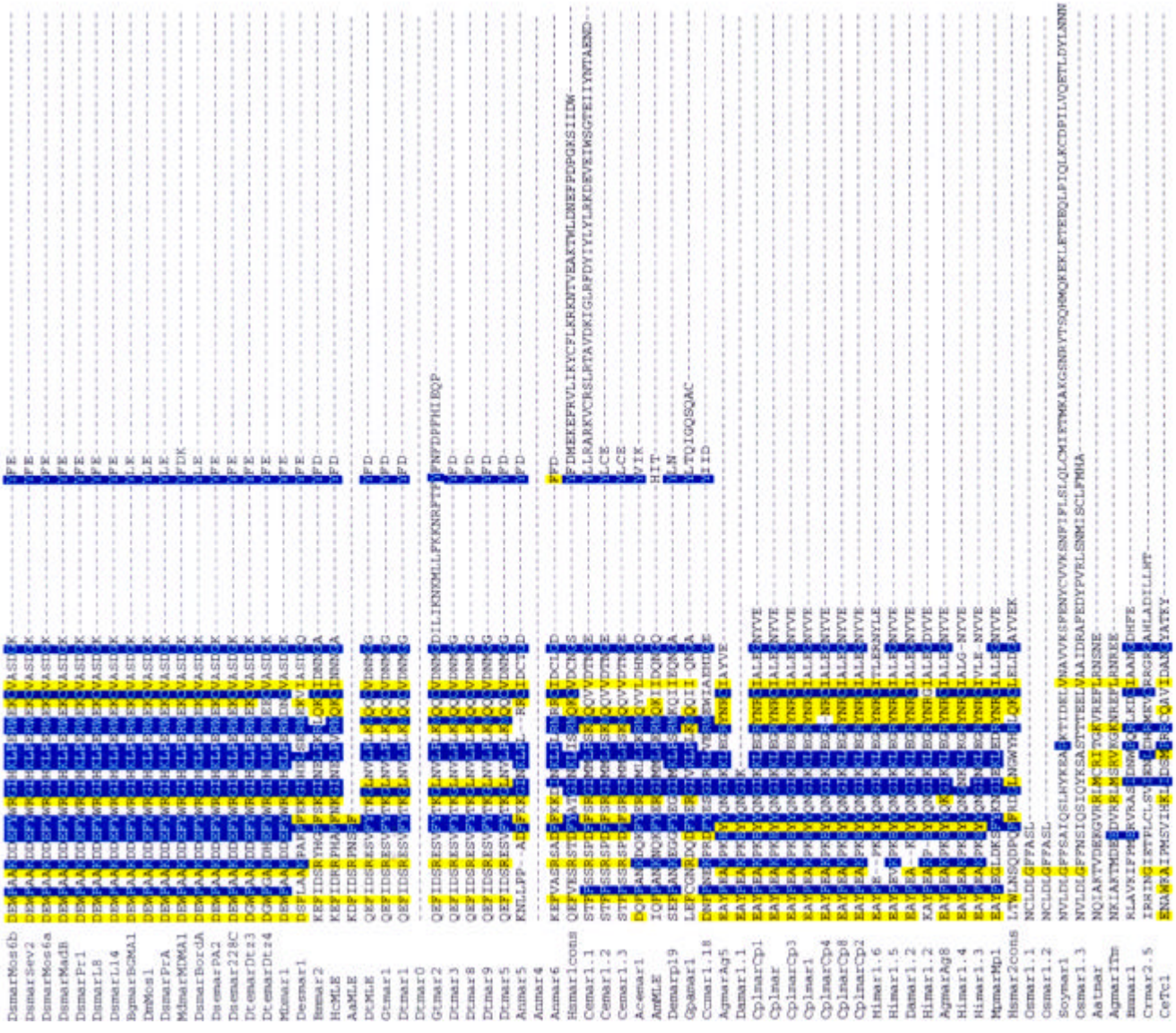

**Figure 1.** Alignment of full -length *mariner* sequences. The sequence name in relation to the species and accession numbers can be referred in table 1. "–" shows alignment gaps introduced to maintain alignment. Amino acid residues with blue background and yellow background indicate identical and similar residues, respectively.

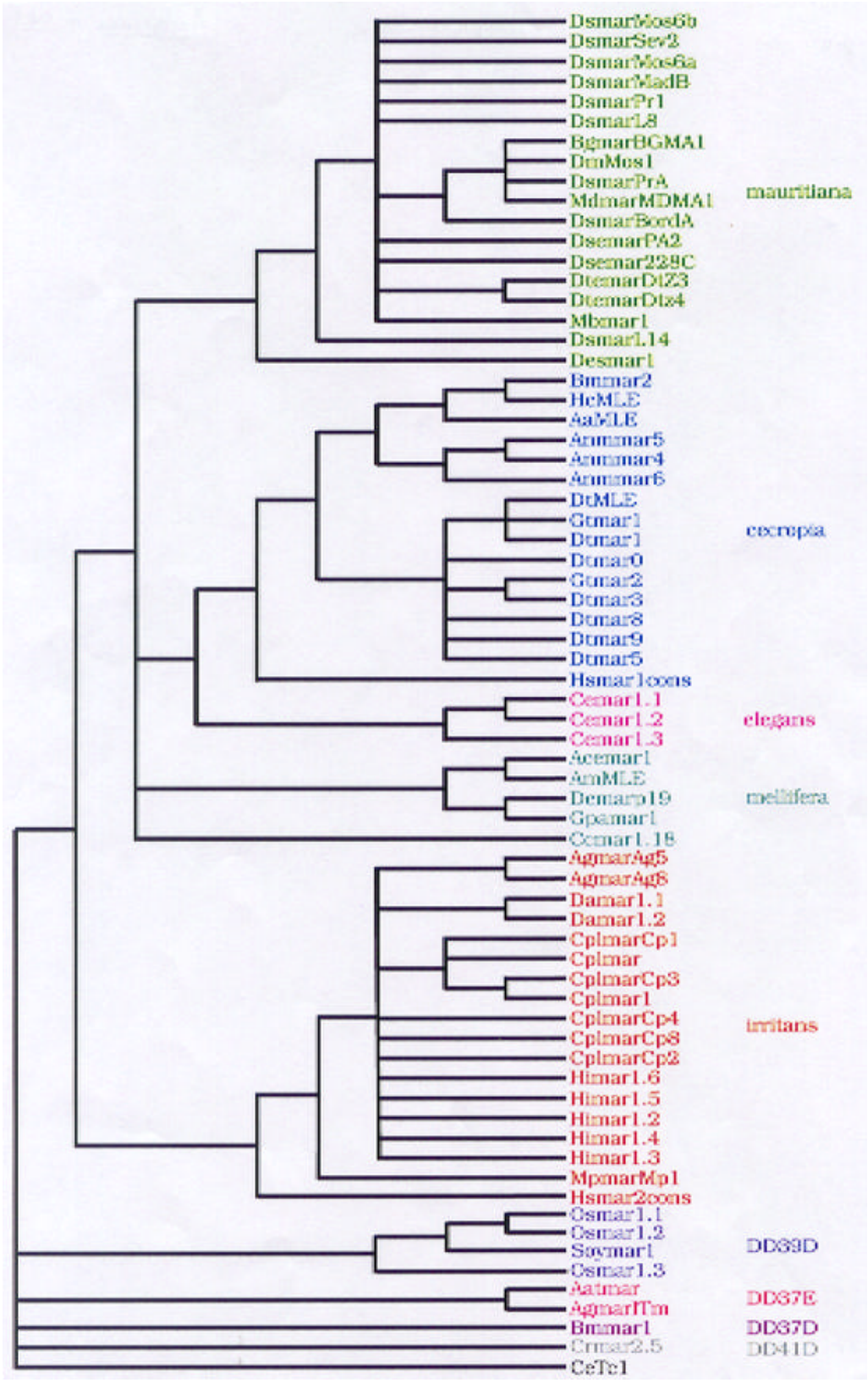


**Figure 2.** Parsimony tree based on Heuristic search constructed using PAUP. The tree is rooted with *CeTC1* as outgroup. Names written on the right side of the tree with similar colour indicate subfamilies (mauritiana, cecropia, elegans, mellifera and irritans) and mariner superfamiles (DD39D, DD37E, DD37D and DD41D).

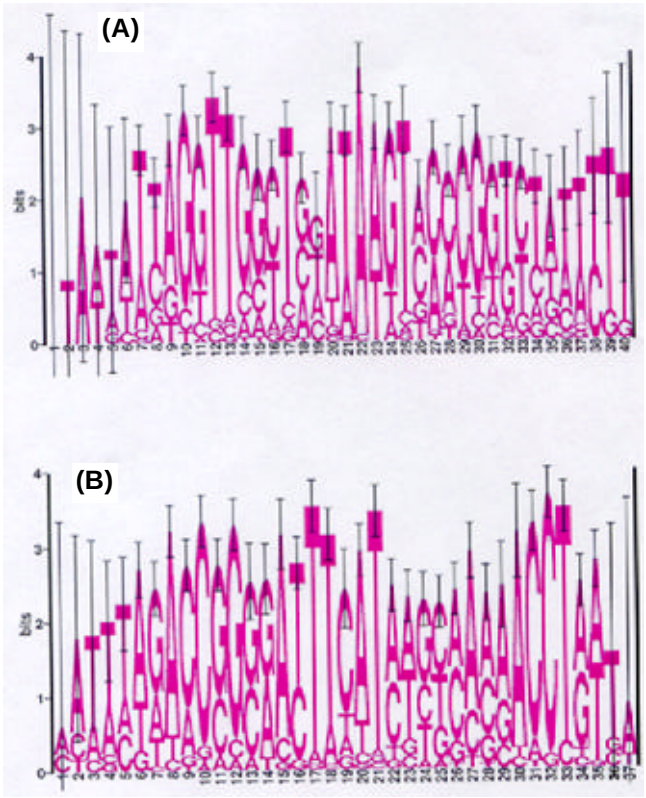


**Figure 3.** Sequence logo of inverted terminal repeats from different mariner family elements. The sequence logo was generated using the WebLogo server (http://www.bio.cam.ac.uk/seqlogo/). At each position the nucleotides are stacked one top of the other with the most frequent one on the top. It displays the frequency of bases at each position with height indicating the proportion of occurrence. The vertical scale is in bits with maximum of four bits possible at each position indicating that there can be a possibility of 4 different bases at each position. (**A**) Upper inverted terminal repeats. (**B**) Lower inverted terminal repeats.

2001). Brunet *et al* (1999), proposed that ITRs evolve faster than the putative catalytic sites modifying the interactions between MLEs. Thus, studying ITRs is important for understanding the interactions of different mariner elements. Our identification of consensus bases and the distance between the two blocks of consensus in ITRs may help us in identifying the most suitable ITR sequence for optimum activity of a particular transposase. A wider study of ITRs will help us to understand different cross-mobilizations that may occur after making mariner mediated transgenics as it is reported that relatively small amount of divergence is sufficient to substantially reduce transposition activity.

Recently, *Mos1* mariner transposon from *D. mauritiana* was successfully used to tranform Bm5 *B. mori* cells (Wang *et al* 2000) indicating that mariner based vectors could be attempted for genetic transformation of silkmoth. A full length mariner element with functional ORF has also been isolated from emperor moth, *Attacus atlas* (Nakajima *et al* 1998). The ORF of this element along with the ITRs of silkmoth MLEs are being tested for their efficiency as transformation vectors in silkmoths. Thus information on MLEs in silkmoths and recent advancement in the mariner-mediated transgenesis will help us to test the feasibility of transforming silkmoths with a foreign gene of economic importance using mariner-based vectors.

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## References

Brunet F, Godin F, Bazin C and Capy P 1999 Phylogenetic analysis of *Mos1*-like transposable elements in the *Drosophilidae*; *J. Mol. Evol.* **49** 760–768

Capy P, Koga A, David J R and Hartl D L 1992 Sequence analysis of active mariner elements in natural population of *Drosophila simulans; Genetics* **130** 499–506

Doak T G, Doerder F P, Jahn C L 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. USA* **91** 942–946

Feschotte C and Wessler S R 2002 Mariner-like transposons are widespread and diverse in flowering plant; *Proc. Natl. Acad. Sci. USA* **99** 280–285

Hartl D L 1989 Transposable element *mariner* in *Drosophila* species; in *Mobile DNA* (eds) D E Berg and M W Howe (Washington DC: *American Society for Microbiology*) pp 531–536

Hartl D L, Lohe A R and Lozovskaya E R 1997a Modern thoughts on an ancyent marinere: function, evolution, regulation; *Annu. Rev. Genet.* **31** 337–358

Hartl D L, Lohe A R and Lozovskaya E R 1997b Regulation of the transposable element *mariner*; *Genetica* **100** 177–184

Hartl D L, Lozovskaya E R, Nurminsky D I and Lohe A R 1997c What restricts the activity of mariner like transposable elements; *Trends Genet.* **13** 197–201

Haymer D S and Marsh J L 1986 Germ line and somatic instability of a *white* mutation in *Drosophila mauritiana* due to a transposable genetic element; *Dev. Genet.* **6** 281–291

Jacobson J W, Medhora M M and Hartl D L 1986 Molecular structure of somatically unstable transposable element in *Drosophila; Proc. Natl. Acad. Sci. USA* **83** 8684–8688

Jarvik T and Lark K G 1998 Characterization of Soymar1, a mariner element in soybean; *Genetics* **149** 1569–1574

Lampe D J, Akerley B J, Rubin E J, Mekalanos J J and Robertson H M 1999 Hyperactive transposase mutants of the Himar1 mariner transposon; *Proc. Natl. Acad. Sci. USA* **96** 11428–11433

Lampe D J, Churchill M and Robertson H M 1996 A purified mariner transposase is sufficient to mediate transposition *in vitro*; *EMBO J.* **15** 5470–5479

Lampe D J, Walden K K and Robertson H M 2001 Loss of Transposase-DNA interaction may underlie the divergence of mariner family transposable elements and the ability of more than one mariner to occupy the same genome; *Mol. Biol. Evol*. **18** 954–961

Lohe A R, De Aguiar D and Hartl D L 1997 Mutations in the mariner transposase: the D,D(35)E consensus sequence is nonfunctional; *Proc. Natl. Acad. Sci. USA* **94** 1293–1297

Lohe A R, Moriyama K, Lidholm D A and Hartl D L 1995 Horizontal transmission, vertical inactivation and stochastic loss of mariner-like transposable elements; *Mol. Biol. Evol*. **12** 62–72

Lohe A R, Sullivan D T and Hartl D L 1996 Subunit interaction in the mariner transposase; *Genetics* **144** 1087–1095

Maruyama K, Schoor K D and Hartl D L 1991 Identification of nucleotide substitutions necessary for trans-activation of *mariner* transposable elements in *Drosophila*: analysis of naturally occurring elements; *Genetics* **128** 777–784

Nakajima Y, Hashido K, Shiino T, Hayashi T, Tsuchida K, Nagamine M and Maekawa H 1998 Isolation of a mariner-like sequence containing a complete open reading frame for transposase from *Attacus atlas* and its phylogenetic relationships within the Ditrysia of Lepidoptera; *J. Seric. Sci. Jpn*. **67** 271–278

Plasterk R H, Izsvak Z and Ivics Z 1999 Resident aliens: the Tc1/mariner superfamily of transposable elements; *Trends Genet*. **15** 326–332

Prasad M D, Nurminsky D L and Nagaraju J 2002 Characterization and molecular phylogenetic analysis of mariner elements from wild and domesticated species of silkmoths; *Mol. Phyl. Evol*. **25** 210–217

Robertson H M 1993 The mariner transposable element is widespread in insects; *Nature* (*London*) **362** 241–245

Robertson H M and MacLeod E G 1995 Five major subfamilies of mariner transposable elements in insects, including the Mediterranean fruit fly, and related arthropods; *Insect. Mol. Biol*. **2** 125–139

Robertson H M and Asplund M L 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

Schneider T D and Stephens R M 1990 Sequence logos: a new way to display consensus sequences; *Nucleic Acids Res*. **18** 6097–6100

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

Swofford D L 2002 *PAUP Phylogenetic Analysis Using Parsimony*, version 4. Sinauer Associates, Sutherland, Massachusetts, USA

Thompson J D, Higgins D G and Gibson T J 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice; *Nucleic Acids Res*. **22** 4673–4680

Wang W, Swevers L and Iatrou K 2000 Mariner (Mos1) transposase and genomic integration of foreign gene sequences in *Bombyx mori* cells; *Insect Mol. Biol*. **9** 145–155

Yoshiyama M, Tu Z, Kainoh Y, Honda H, Shono T and Kimura K 2001 Possible horizontal transfer of a transposable element from host to parasitoid; *Mol. Biol. Evol*. **18** 1952–1958

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