Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

New Mariner Elements in *Anastrepha* Species (Diptera: Tephritidae)

HS Marcon[1], DS Domingues[2], VE Coscrato[1], D Selivon[3], ALP Perondini[3], CL Marino[1]

[1]Depto de Genética, Instituto de Biociências, Univ Estadual Paulista Júlio de Mesquita Filho, Botucatu, SP, Brasil
[2]Lab de Biotecnologia Vegetal, Instituto Agronômico do Paraná, Londrina, PR, Brasil
[3]Depto de Genética e Biologia Evolutiva, Instituto de Biociências, Univ de São Paulo, São Paulo, SP, Brasil

Keywords
Fruit fly, *mellifera*, tephritid, transposon, *rosa*

Correspondence
Helena S Marcon, Instituto de Biociências, Departamento de Genética, Botucatu – UNESP, Distrito de Rubião Junior, s/n, 18618-970; marcon@ibb.unesp.br

Edited by Fernando B Noll – UNESP

Received 19 November 2010 and accepted 29 March 2011

Abstract

Mariner-like elements (MLE) are members from class II of transposable elements also known as DNA transposons. These elements have a wide distribution among different groups of organisms, including insects, which can be explained by horizontal and vertical gene-transfer. MLE families have been described in tephritid flies and other genera. During screening for *Wolbachia* bacteria in fruit flies of the genus *Anastrepha*, we discovered two sequences related to *mariner*-like elements. Based on these sequences, we designed primers that allowed us to isolate and characterize two new *mariner*-like elements (*Anmar1* and *Anmar2*) in *Anastrepha* flies. These elements, which belong to the *mellifera* and *rosa* subfamilies have a low nucleotide diversity, and are probably inactive and acquired by vertical transfer. This is the first report of *mariner*-like transposons in flies found in South America.

Introduction

Transposable elements are repetitive DNA segments that can change position within the genome and account for a large portion of the genetic material of eukaryotes (Bowen & Jordan 2002), e.g., 45% of the genome in humans and 50-80% of the genome in plants (as reviewed by Feschotte *et al* 2002). Traditionally, transposable elements have been classified into two classes: retrotransposons (class I) and DNA transposons (class II). Class I elements (or retrotransposons) are widely distributed among eukaryotes and their transposition involve a RNA intermediate, in a "copy and paste" transposition. Class II transposable elements occur in prokaryotes and in almost all eukaryotes as segments inserted in the genome or as part of complex structures (Wicker *et al* 2007). DNA transposons usually transpose through a "*cut-and-paste*" mechanism, i.e., under normal conditions the copy number of these elements does not increase exponentially. Based on the classification proposed by Wicker *et al* (2007), *mariner*-like elements (MLE) are DNA transposons belonging to subclass I that have terminal inverted repeats and a transposition mechanism that involves a "classic" transposase with a DDE motif (Silva *et al* 2005). *Mariner*-like elements are divided into 15 subfamilies that have been classified phylogenetically based on their sequence similarity (Rouault *et al* 2009).

The first MLE was identified in *Drosophila mauritiana* (Tsacas & David), where it occurred as an insertion within a gene (Jacobson & Hartl 1985). *Mariner*-like elements have since been described in plants, fungi, vertebrates and prokaryotes (Bigot *et al* 1994, Auge-Gouillou *et al* 1995, Robertson 1995, Plasterk *et al* 1999, Feschotte & Wessler 2002). The distribution of *mariner*-like elements among species of different phyla can be explained by two ways: vertical transfer (elements evolved from a common ancestor) and horizontal transfer (recent transference among phylogenetically unrelated species)

(reviewed by Sperb *et al* 2009).

Among insects, *mariner*-like elements have been described in tephritid flies (Torti *et al* 1997, Gomulski *et al* 2001). *Anastrepha* are considered pests because of the damage they cause to commercially important fruits. *Anastrepha* is endemic to the Americas, and 103 species are reported to occur in Brazil (Zucchi 2008). *Anastrepha* species from different localities in Brazil are usually positive for the presence of the bacterial endosymbiont *Wolbachia* (Mascarenhas 2007, Coscrato *et al* 2009, Marcon 2009), which is known to transfer DNA segments into eukaryotic hosts (Kondo *et al* 2002, Hotopp *et al* 2007).

In a manner analogous to Carr (2008), who accidentally identified *mariner* transposable elements in stalk-eyed flies during screening for the sex determination gene *dsx*, we also serendipitously discovered *mariner*-like elements during screening for *Wolbachia* in *Anastrepha* fruit flies. Based on the sequences obtained, we designed specific primers to investigate the occurrence and diversity of *mariner*-like elements in *Wolbachia*-infected fruit flies. In this report, we describe the isolation and characterization of two *mariner*-like elements families (*Anmar1* e *Anmar2*) in three subspecies of *Anastrepha* that belong to the *A. fraterculus* complex of cryptic species (Selivon *et al* 2004, 2005), and analyze their diversity and phylogenetic relationship to other *mariner*-like elements.

Material and Methods

Fly samples and mariner-like elements isolation

Samples of *Anastrepha* sp.1 *affinis fraterculus, Anastrepha* sp.2 *aff. fraterculus* and *Anastrepha* sp.3 *aff. fraterculus* were collected in five regions of São Paulo state, southeastern Brazil (Table 1). Genomic DNA was extracted from the abdomen of individual adult flies according to the protocol of Jowett (1998). *Wolbachia* were detected using the *wsp* primers (F1: 5'TGAAATTTTACCTCTTTTC 3' and R1: 5´AAAAATTAAACGCTACTCCA 3'; and F2: 5'TGGTCCAATAAGTGATGAAGAAAAC 3' and R2: 5´ACCAGCTTTTGCTTGATA 3´), as proposed by Zhou *et al* (1998). Polymerase chain reactions (PCRs) followed the conditions described in Coscrato *et al* (2009). The ~600 bp amplicons obtained were cloned into the pGEM®-T Easy Vector (Promega) and plasmids obtained from positive clones were subjected to bidirectional sequencing in order to obtain consensus sequences. Using this approach we identified two sequences related to *mariner*-like elements (see Results) that were amplified using the *wsp* primers indicated above.

Based on the sequences of the two *mariner*-like elements identified, we designed the following specific primers: Anmar1 forward (5' CCTGCTCGAACGACTGATTT 3') and Anmar1 reverse (5' CCACGATTTATTGGGTGGTC 3'), Anmar2 forward (5' AGGCGGAAAAGCTGAAGAGT 3') and Anmar2 reverse (5' AAGCCGGCCAAACATTAAC 3'). These primers were subsequently used to identify *Anmar1* and *Anmar2* elements, respectively, in 11 samples from the three *Anastrepha fraterculus* (Wiedemann) subspecies. The amplification reaction followed Coscrato *et al* (2009), with some adaptations in annealing temperature. It consisted of an initial cycle at 94°C for 4 min, 55°C for 1 min and 72°C for 2 min, followed by 37 cycles of denaturation at 94° C for 15 s, annealing at 55°C for 1 min and extension at 72°C for 2 min, a further cycle of 95°C for 15 s and 58°C for 1 min and a final extension at 72°C for 7 min. PCR products were analyzed in 1% agarose gels and PCR products were purified with Exosap enzyme mixture (GE), according to the manufacturer´s protocol. The purified products were sequenced in a Genetic Analyzer 3100 sequencer (Applied Biosystems) and the similarity of the *mariner*-like elements was confirmed by BLAST (Altschul *et al* 1997). All sequences obtained were deposited in GenBank with accession numbers HM773359 to HM773366 and HM775148 to HM775156.

Table 1 Sample collection locality and number and identification of *mariner* transposons *Anastrepha* flies from five regions in São Paulo state, southeastern Brazil. The GenBank accession numbers correspond to the first hit obtained in BLASTN sequence analyses.

Species/subspecies (sp)	Locality	Quantity	BLASTN first hit
Anastrepha sp. 1 *affinis fraterculus*	São Paulo (23°32´S, 46°37´W)	1[1]	AY034623.1
Anastrepha sp. 1 *affinis fraterculus*	Jacareí (23°17´S, 46°01´W)	3[1]	AF349134.1
Anastrepha sp. 1 *affinis fraterculus*	Jacareí (23°17´S, 46°01´W)	4	AY034623.1
Anastrepha sp. 2 *affinis fraterculus*	Boiçucanga (23°45´S, 45°51´W)	1[1]	AF349134.1
Anastrepha sp. 2 *affinis fraterculus*	Caraguatatuba (23°37´S, 45°24´W)	1[1]	AY034623.1
Anastrepha sp. 3 *affinis fraterculus*	Caraguatatuba (23°37´S, 45°24´W)	1[1]	AF349134.1
Anastrepha sp. 1 *affinis fraterculus*	Serra Negra (22°35´S, 46°50´W)	3	AY034623.1

1: *Anmar1* and *Anmar2* were initially idetified using the wsp primer.

Phylogenetic analyses

For phylogenetic analyses, we compared the sequences of *Anmar1* and *Anmar2* with previously reported sequences for *mariner*-like elements from the subfamilies *mauritiana*, *cecropia*, *mellifera* and *rosa* (Torti *et al* 1997, Gomulski *et al* 2001, Sinzelle *et al* 2006, Bui *et al* 2008, Rouault *et al* 2009). Thirty-two sequences were aligned using the MUSCLE alignment tool (Edgar 2004) and cured by Gblocks (Castresana 2000), using the site www.phylogeny.fr (Dereeper *et al* 2008). The resulting alignment was used to draw a Maximum Likelihood tree with Kimura-2-parameters distance and based on 10,000 bootstrap replicates using MEGA 5.0 software (Tamura *et al* in press).

The sequences related to the MLE *mellifera* subfamily were aligned with the GenBank sequence corresponding to accession number AAO12862 and those related to the MLE *rosa* subfamily were aligned with the GenBank sequence corresponding to accession number AAK61417 using blast2seq (Tatusova & Madden 1999) in both cases. Based on this alignment, regions shared by the sequences and the two accession numbers indicated above were selected manually. From this selection, we calculated the mean diversity (Pi) using the software DnaSP 5.00.05 (Librado & Rozas 2009) and the pairwise distance (p) using MEGA 5.0 software (Tamura *et al* in press), after gap exclusion.

Results

Anmar1 *and* Anmar2 *identification*

In a BLASTN analysis of sequences obtained using *wsp* primers, we identified one sequence with similarity to a *mariner* transposase from *Bactrocera tryoni* (Froggatt) (GenBank accession number AF349134.1) and another sequence similar to a *Ceratitis rosa* (Karsch) *mariner* transposase (GenBank accession number AY426626.1). Since these samples were positive for *Wolbachia*, specific primers for the *Anastrepha* Internal Transcribed Spacer (ITS) region (Prezotto 2008) were used to confirm the presence of *Anastrepha* DNA in these preparations. The resulting PCR products confirmed the presence of *Anastrepha* DNA in all samples, as expected (data not shown), once it is impossible to dissociate bacterial cells from fly tissue in DNA extraction.

These sequences, designated as *Anmar1* (similar to AF349134.1) and *Anmar2* (similar to AY426626.1), were used to design specific primers for each new *mariner* element. The new primers were then used in PCR reactions with the MLE clones obtained in *wsp* amplification, which allowed confirmation of the primer specificity. We subsequently screened other *Wolbachia*-positive *Anastrepha* samples with the *Anmar1* and *Anmar2* primers and obtained 13 samples that yielded PCR fragments (Table 1). The sequences of these purified PCR products were similar to the *mariner*-like elements originally observed with *wsp* primers. All analyzed sequences were obtained using *Anmar1* and *Anmar2* specific primers.

Anmar1 *and* Anmar2 *characterization and phylogenetic analysis*

Initial BLAST results suggested that the *Anmar1* and *Anmar2* elements belonged to the *mellifera* and *rosa* subfamilies, respectively. To confirm this, we generated a phylogenetic tree with the *Anmar1* and *Anmar2* sequences and the sequences from four *mariner* subfamilies (*mauritiana*, *cecropia*, *mellifera* and *rosa*). The clade distribution and bootstrap values in the phylogenetic analysis confirmed that these elements belonged to the *mellifera* and *rosa* subfamilies (Fig 1).

Eight and nine sequences were obtained for the *Anmar1* (*mellifera*) and *Anmar2* (*rosa*) elements, respectively. Both *Anmar1* and *Anmar2* sequences had stop codons in their coding region, and manual analysis of these two sequences detected common regions of 201 (Fig 2a) and 115 nucleotides (Fig 2b), respectively; these regions were further used to analyze nucleotide diversity. *Anmar1* (*Anmar1.1* to *Anmar1.8*) sequences had a Pi diversity of 0.045 ± 0.01 and those for *Anmar2* (*Anmar2.1* to *Anmar2.9*) had a Pi of 0.068 ± 0.02, indicating a higher diversity in the *Anmar2* element than in the *Anmar1*. P distances among the *Anmar1* and *Anmar2* sequences indicated that both families had three identical sequences (Tables 2 and 3), although distinct distance values were observed: P distances in the *Anmar1* sequences ranged from 0.010 to 0.094 (Table 2), but were higher in *Anmar2*, ranging from 0.010 to 0.173 (Table 3). The p distance between *Anmar1.1* and *Anmar2.1* was 0.51 (Online Supplementary Material 1). Higher distances in *Anmar2.1* were also observed comparing this element to other sequences used for phylogenetic characterization (Fig 1, *Online Supplementary Material 1*): *Anmar1.1* distances ranged from 0.18 to 0.59 and from 0.48 to 0.62 in *Anmar2.1*.

Discussion

Mariner-like elements are widely distributed among tephritids, and *Ccmar1* and *Tcmar1* elements have distinct distributions among related tephritids species due to their relatively recent transmission between species (Torti *et al* 1997, Green & Frommer 2001). Our findings therefore agree with these and other investigations (Robertson 1993, Robertson & MacLeod 1993) showing that *mariner*-like elements are widely distributed in insects.

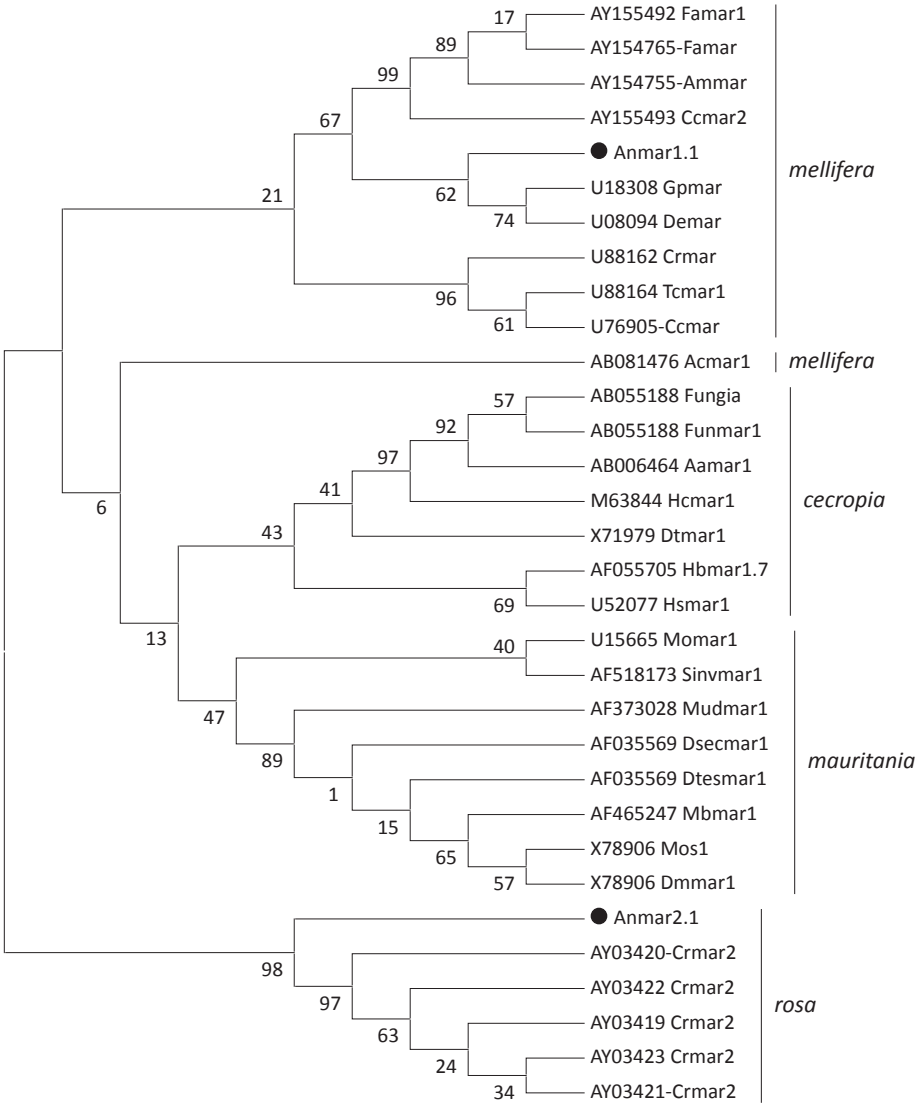

Fig 1 Phylogenetic tree of four MLE subfamilies inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model with 10,000 bootstrap replicates. The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic searches were obtained using BIONJ method with MCL distance matrix. The analysis involved 32 nucleotide sequences. Genbank access number is given to all sequences, except for *Anmar1.1* (HM773359) and *Anmar2.1* (HM775148). There were a total of 114 positions in the final dataset. Phylogenetic analyses were conducted in MEGA5 (Tamura *et al* in press). Sequences identified in this study are indicated with circles.

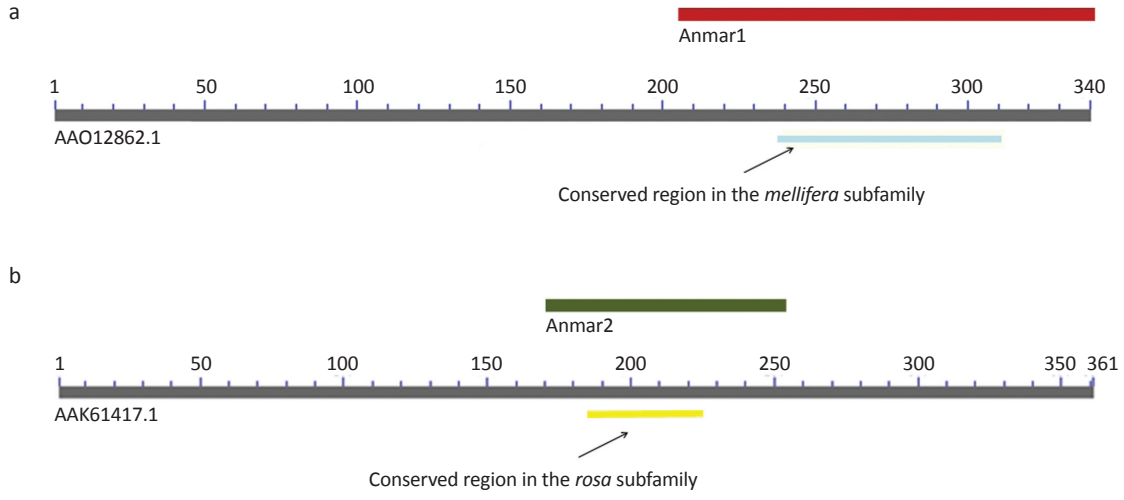

Fig 2 Shared sequence region among members of *Anmar1* (a. in red) and *Anmar2* (b. in green) *mariner*-like elements and conserved regions of the *mellifera* (in blue) and *rosa* (in yellow) subfamilies. The GenBank reference sequences used for comparison were from accession numbers AAO12862.1 in (a) and AAK61417.1 in (b).

Table 2 Distance values among sequences of *Anmar1* elements identified in this study.

	Anmar1.1	Anmar1.2	Anmar1.3	Anmar1.4	Anmar1.5	Anmar1.6	Anmar1.7	Anmar1.8
Anmar1.1								
Anmar1.2	0.000							
Anmar1.3	0.000	0.000						
Anmar1.4	0.039	0.039	0.039					
Anmar1.5	0.049	0.049	0.049	0.069				
Anmar1.6	0.010	0.010	0.010	0.049	0.049			
Anmar1.7	0.049	0.049	0.049	0.089	0.089	0.049		
Anmar1.8	0.054	0.054	0.054	0.084	0.094	0.054	0.054	

Sequence comparisons and phylogenetic analyses have classified *mariner*-like elements into various subfamilies. In the present work, we first describe *mariner*-like elements of the *rosa* and *mellifera* subfamilies in subspecies of *A. fraterculus*. The *Anmar2* element was classified as a member of the *rosa* subfamily, which also contains *Crmar2*, *Asmar1* and *Almar1* (Gomulski *et al* 2001). The *Anmar1* element is a member of the *mellifera* subfamily (Fig 2).

BLASTN analyses indicated that the *Anastrepha* sp. *mariner*-like elements shared similarity with transposases from *Bactrocera tryoni* and *C. rosa*, thus reinforcing the suggestion that *mariner* transposases have spread among tephritids over a long period of time; this finding also suggested that dispersal of the *Anmar1* and *Anmar2* elements did not involve horizontal gene transfer. Rather, these elements were probably present in an ancestral *Anastrepha* lineage that gave rise to the species analyzed here.

Although the *mellifera* and *rosa* subfamilies have been identified in other tephritids (Gomulski *et al* 2001, Green & Frommer 2001), the *Anmar1* and *Anmar2* elements are distinct from the other members of these subfamilies (Fig 2). This analysis indicated that *Anmar1* occurs in the same clade as *Demar1* and *Gpmar1* from the Drosophilidae and tsetse flies, respectively (Lohe *et al* 1995, Blanchetot & Gooding 1995). In contrast, *Anmar2* clustered with members of the *rosa* subfamily, all of which were originally identified in dipteran flies. Our data indicate that similar *mariner*-like elements identified here also occur in other genera of the same family, suggesting an acquisition by vertical transmission.

The *Anmar1* and *Anmar2* sequences showed a low level of diversity (4.5% and 6.8%, respectively) that has also been observed in *mariner* elements of *B. tryoni* (Green & Frommer 2001) and other insects (Carr 2008), which suggests that insect *mariner*-like elements have not been under pressure to diverge. The presence of a stop codon in all sequences suggested that they were inactive.

In conclusion, we have identified two new *mariner*-like elements in *A. fraterculus* subspecies that share similarities with other dipteran *mariner*-like elements. This finding indicates that the *Anmar1* and *Anmar2* families are present in a common ancestor of many dipterans and that there is no horizontal transfer. These results provide new perspectives for future studies on the distribution, transcription and functional character of these elements in *Anastrepha* sp. and other flies.

Table 3 Distance values among sequences of *Anmar2* elements identified in this study.

	Anmar2.1	Anmar2.2	Anmar2.3	Anmar2.4	Anmar2.5	Anmar2.6	Anmar2.7	Anmar2.8	Anmar2.9
Anmar2.1									
Anmar2.2	0.010								
Anmar2.3	0.010	0.000							
Anmar2.4	0.010	0.000	0.000						
Anmar2.5	0.019	0.010	0.010	0.010					
Anmar2.6	0.029	0.019	0.019	0.019	0.010				
Anmar2.7	0.115	0.106	0.106	0.106	0.115	0.125			
Anmar2.8	0.077	0.067	0.067	0.067	0.077	0.087	0.125		
Anmar2.9	0.135	0.125	0.125	0.125	0.135	0.135	0.173	0.087	

Acknowledgments

This research was supported by a grant from FAPESP (03/02698-3) to DS, ALPP, and CLM. HSM had fellowships from CAPES / FAPESP and VEC from FAPESP. DS is a fellow of CNPq.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403-410.

Auge-Gouillou C, Bigot Y, Pollet N, Hamelin M-H, Meunier-Rotival M, Perique G (1995) Human and other mammalian genomes contain transposons of the *mariner* family. FEBS Letters 68: 541-546.

Bigot Y, Hamelin MH, Capy P, Periquet G (1994) *Mariner*-like elements in hymenopteran species: insertion site and distribution. Proc Natl Acad Sci USA 91: 3408-3412.

Blanchetot A, Gooding RH (1995) Identification of a *mariner* element from the tsetse fly, *Glossina palpalis palpalis*. Insect Mol Biol 4: 89-96.

Bowen NJ, Jordan IK (2002) Transposable elements and the evolution of eukaryotic complexity. Curr Issues Mol Biol 4: 65-76.

Bui Q-T, Casse N, Leignel V, Nicolas V, Chénais B (2008) Widespread occurrence of *mariner* transposons in coastal crabs. Mol Phylogenet Evol 47: 1181-1189.

Carr M (2008) Multiple subfamilies of *mariner* transposable elements are present in stalk-eyed flies (Diptera: Diopsidae). Genetica 132: 113-122.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17: 540-552.

Coscrato VE, Braz ASK, Perondini ALP, Selivon D, Marino CL (2009) *Wolbachia* in *Anastrepha* fruit flies (Diptera: Tephritidae). Curr Microbiol 59: 291-301.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic acids res 1: 465-469.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids res 32: 1792-1797.

Feschotte C, Jiang N, Wessler SR (2002) Plant transposable elements: where genetics meets genomics. Nat Rev Genet 3: 329-341.

Feschotte C, Wessler SR (2002) *Mariner*-like transposases are widespread and diverse in flowering plants. Proc Natl Acad Sci USA 99: 1280-1285.

Green CL, Frommer M (2001) The genome of the Queensland fruit fly *Bactrocera tryoni* contains multiple representatives of the *mariner* family of the transposable elements. Insect Mol Biol 10: 371-386.

Gomulski LM, Torti C, Bonizzoni M, Moralli D, Raimondi E, Capy P, Gasperi G, Malacrida AR (2001) A new basal subfamily of *mariner* elements in *Ceratitis rosa* and other tephritid flies. J Mol Evol 53: 597-606.

Hotopp JCD, Clark ME, Oliveira DCSG, Foster JM, Fischer P, Torres MCM, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317: 1753-1756.

Jacobson JW, Hartl DL (1985) Coupled instability of two X-linked genes in *Drosophila mauritiana*: germinal and somatic mutability. Genetics 111: 57-65.

Jowett T (1998) Preparation of nucleic acids, p.347-372. In Roberts DB (ed) *Drosophila:* a pratical approach 2nd edn. Oxford, Oxford University Press, 389p.

Kidwell MG (1994) The evolutionary history of the *P* family of transposable elements. J Heredity 85: 339-346.

Kondo N, Nikoh N, Ijichi N, Shimada N, Fukatsu T (2002) Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc Natl Acad Sci USA 99: 14280-14285.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452.

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

Marcon HS (2009) Identificação da bactéria endossimbionte *Wolbachia* em populações de moscas-das-frutas do complexo *Anastrepha fraterculus* (Diptera: Tephritidae). M. Sc. Dissertation, Botucatu, Instituto de Biociências – UNESP, 110p.

Mascarenhas RO (2007) Endossimbionte *Wolbachia* em moscas-das-frutas do gênero *Anastrepha* (Tephritidae) e em vespas parasitóides (Braconidae) associadas. M. Sc. Dissertation, São Paulo, Instituto de Biociências - USP, 80p.

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

Prezotto LF (2008) Análise de ITS1 do DNA do ribossômico em espécies do complexo *Anastrepha frarterculus* (Diptera: Tephritidae). M. Sc. Dissertation, São Paulo, Instituto de Biociências - USP, 52p.

Robertson HM (1993) The *mariner* transposable element is widespread in insects. Nature 38: 241-245.

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

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.

Rouault J-D, Casse N, Chénais B, Hua-Van A, Filée J, Capy P (2009) Automatic classification within families of transposable elements: application to the *mariner* family. Gene 448: 227-232.

Selivon D, Perondini ALP, Morgante JS (2005) A genetic-morphological characterization of two cryptic species of the *Anastrepha fraterculus* complex (Diptera:Tephritidae). Ann Entomol Soc Am 98: 367-381.

Selivon D, Vretos C, Fontes L, Perondini ALP (2004) New variant forms in the *Anastrepha* complex (Diptera: Tephritidae), p.253-258. In Barnes BN (ed) Proceedings 6th International Symposium on Fruit Flies of Economic Importance. Isteg Scientific Publication, Irene, South Africa.

Silva JC, Bastida F, Bidwell SL, Johnson PJ, Carlton JM (2005) A potentially functional *mariner* transposable element in the protist *Trichomonas vaginalis*. Mol Biol Evol 22: 126-134.

Sinzelle L, Chesneau A, Bigot Y, Mazabraud A, Pollet N (2006) The *mariner* transposons belonging to the *irritans* subfamily were maintained in chordate genomes by vertical transmission. J Mol Evol 62: 53-65.

Sperb F, Schuck DC, Rodrigues JJS (2009) Occurrence and abundance of a *mariner*-like element in freshwater and terrestrial planarians (Platyhelminthes, Tricladida) from southern Brazil. Genet Mol Biol 32: 731-739.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (in press) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol doi: 10.1093/molbev/msr121.

Tatusova TA, Madden TL (1999) BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. FEMS Microbiol Lett 174: 247-250.

Torti C, Gomulski LM, Malacrida AR, Capy P, Gasperi G (1997) Characterization and evolution of *mariner* elements from closely related species of fruit flies (Diptera: Tephritidae). J Mol Evol 46: 288-298.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, San Miguel P, Schulman AH (2007) A unified classification system for eukaryotic transposable elements. Nat Rev Genet 8: 973-982.

Zhou W, Rousset F, O'Neill SL (1998) Phylogeny and PCR based classification of *Wolbachia* strains using wsp gene sequences. Proc R Soc Lond Ser B 265: 509-515.

Zucchi RA (2008) Fruit flies in Brazil - Anastrepha species and their host plants. (http://www.lea.eslq.usp.br/anastrepha/).

Online Supplementary Material - 1

Overall distance values among MLEs used for phylogenetic classification of *Anmar1* and *Anmar2* (Fig 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1- Hbmar1.7																																		
2- Dtmar1	0.37																																	
3- Hsmar1	0.40	0.13																																
4- Aamar1	0.35	0.16	0.18																															
5- Funmar1	0.35	0.16	0.18	0.00																														
6- Hcmar1	0.43	0.27	0.24	0.13	0.13																													
7- Crmar	0.51	0.24	0.18	0.24	0.24	0.21																												
8- Ccmar	0.54	0.27	0.21	0.27	0.27	0.24	0.02																											
9- Famar	0.59	0.32	0.32	0.29	0.29	0.29	0.24	0.24																										
10- Ammar	0.62	0.29	0.35	0.32	0.32	0.32	0.27	0.27	0.02																									
11- Gpmar	0.54	0.37	0.35	0.35	0.35	0.32	0.29	0.32	0.27	0.29																								
12- Demar	0.64	0.35	0.37	0.32	0.32	0.35	0.35	0.37	0.29	0.29	0.27																							
13- Sinvmar1	0.56	0.40	0.43	0.43	0.43	0.37	0.43	0.45	0.45	0.43	0.37	0.40																						
14- Momar1	0.56	0.48	0.43	0.43	0.43	0.43	0.32	0.32	0.35	0.37	0.35	0.48	0.40																					
15- Mbmar1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.35	0.40	0.35	0.40																				
16- Dtesmar1.1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.32	0.37	0.32	0.40	0.02																			
17- Dsecmar1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.35	0.40	0.35	0.40	0.00	0.02																		
18- Mudmar1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.35	0.40	0.35	0.40	0.00	0.02	0.00																	
19- Dmmar1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.35	0.40	0.35	0.40	0.00	0.02	0.00	0.00																
20- Hsmar1.1	0.40	0.13	0.00	0.18	0.18	0.24	0.18	0.21	0.32	0.35	0.35	0.37	0.43	0.43	0.37	0.37	0.37	0.37	0.37															
21- Fungia	0.35	0.16	0.18	0.00	0.00	0.13	0.24	0.27	0.29	0.32	0.35	0.32	0.43	0.43	0.37	0.37	0.37	0.37	0.37	0.18														
22- Acmar1	0.45	0.24	0.24	0.21	0.21	0.21	0.21	0.24	0.21	0.24	0.24	0.29	0.37	0.35	0.32	0.32	0.32	0.32	0.32	0.24	0.21													
23- Famar1	0.59	0.32	0.32	0.29	0.29	0.29	0.24	0.24	0.00	0.02	0.27	0.29	0.45	0.35	0.48	0.48	0.48	0.48	0.48	0.32	0.29	0.21												
24- Ccmar2	0.59	0.32	0.32	0.29	0.29	0.29	0.21	0.21	0.02	0.05	0.27	0.27	0.45	0.35	0.51	0.51	0.51	0.51	0.51	0.32	0.29	0.24	0.02											
25- Mos1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.35	0.40	0.35	0.40	0.00	0.02	0.00	0.00	0.00	0.37	0.37	0.32	0.48	0.51										
26- Dtesmar1	0.40	0.35	0.37	0.37	0.37	0.37	0.40	0.43	0.48	0.45	0.32	0.37	0.32	0.40	0.02	0.00	0.02	0.02	0.02	0.37	0.37	0.32	0.48	0.51	0.02									
27- Crmar2.1	0.59	0.51	0.43	0.54	0.54	0.54	0.37	0.37	0.48	0.51	0.51	0.67	0.54	0.43	0.56	0.56	0.56	0.56	0.56	0.43	0.54	0.51	0.48	0.48	0.56	0.56								
28- Crmar2.2	0.59	0.51	0.43	0.54	0.54	0.54	0.37	0.37	0.48	0.51	0.51	0.67	0.54	0.43	0.56	0.56	0.56	0.56	0.56	0.43	0.54	0.51	0.48	0.48	0.56	0.56	0.00							
29- Crmar2.3	0.59	0.51	0.43	0.54	0.54	0.54	0.37	0.37	0.48	0.51	0.51	0.67	0.54	0.43	0.56	0.56	0.56	0.56	0.56	0.43	0.54	0.51	0.48	0.48	0.56	0.56	0.00	0.00						
30- Crmar2.4	0.59	0.51	0.43	0.54	0.54	0.54	0.37	0.37	0.48	0.51	0.51	0.67	0.54	0.43	0.56	0.56	0.56	0.56	0.56	0.43	0.54	0.51	0.48	0.48	0.56	0.56	0.00	0.00	0.00					
31- Crmar2.5	0.59	0.51	0.43	0.54	0.54	0.54	0.37	0.37	0.48	0.51	0.51	0.67	0.54	0.43	0.56	0.56	0.56	0.56	0.56	0.43	0.54	0.51	0.48	0.48	0.56	0.56	0.00	0.00	0.00	0.00				
32- Anmar1. 1	0.59	0.32	0.29	0.35	0.35	0.29	0.24	0.27	0.21	0.24	0.29	0.18	0.37	0.45	0.51	0.48	0.51	0.51	0.51	0.29	0.35	0.29	0.21	0.18	0.51	0.48	0.56	0.56	0.56	0.56	0.56			
33- Anmar2.1	0.62	0.54	0.54	0.54	0.54	0.56	0.48	0.51	0.59	0.62	0.51	0.51	0.45	0.45	0.48	0.45	0.48	0.48	0.48	0.54	0.54	0.45	0.59	0.59	0.48	0.45	0.54	0.54	0.54	0.54	0.54	0.51		
34- Tcmar1	0.51	0.24	0.24	0.24	0.24	0.27	0.05	0.08	0.27	0.29	0.32	0.37	0.45	0.35	0.40	0.40	0.40	0.40	0.40	0.24	0.24	0.24	0.27	0.24	0.40	0.40	0.43	0.43	0.43	0.43	0.43	0.29	0.48	