Zoological Studies **50**(3): 385-393 (2011)

Zoological Studies

Comparative Analysis of the Mitochondrial Control Region in Orthoptera

Ling Zhao[1], Zhe-Min Zheng[2], Yuan Huang[2,*], Zhijun Zhou[3], and Li Wang[1]

[1]*College of Agronomy, Xinjiang Agricultural Univ., Urumqi 830052, China*

[2]*College of Life Science, Shaanxi Normal Univ., Xi'an 710062, China*

[3]*College of Life Science, Hebei Univ., Baoding 071002, China*

(Accepted December 17, 2010)

Ling Zhao, Zhe-Min Zheng, Yuan Huang, Zhijun Zhou, and Li Wang (2011) Comparative analysis of the mitochondrial control region in Orthoptera. *Zoological Studies* **50**(3): 385-393. The entire sequence of the mitochondrial (mt)DNA control region (CR) in 3 new grasshopper species, *Euchorthippus fusigeniculatus*, *Mekongiana xiangchengensis* and *Mekongiella xizangensis*, consisting of 875, 733 and 1063 bp, respectively, were determined and subjected to a comparative analysis with the mtDNA CRs of 25 other orthoptera species obtained from GenBank. In this study, we stressed the comparative analysis of the stem-loop secondary structure in the A+T-rich region of all orthoptera species available to date, and it showed that the stem-loop secondary structure can be classed into 3 different types. Furthermore, we also reported new findings which may facilitate further investigations of this secondary structure and a better understanding of it. Finally, using these sequences of the secondary structure, we reconstructed a phylogeny of the Caelifera as a vehicle to examine the phylogenetic usefulness of stem-loop secondary structure data in resolving relationships within the suborder. Our results showed that the short sequences of the stem-loop secondary structure provided good resolution at the intra-subfamily level within the Caelifera, whereas it poorly resolved family- and subfamily-level relationships. http://zoolstud.sinica.edu.tw/Journals/50.3/385.pdf

Key words: Comparison, mtDNA, Control region, Stem-loop secondary structure, Phylogeny.

The mitochondrial(mt)DNA control region (CR) is the largest non-coding portion of the mt-genome, and it contains a number of regulatory elements responsible for replication and transcription of mtDNA (Wolstenholme 1992, Shadel and Clayton 1997). In insects, the CR is also known as an AT-rich region because of its extremely high adenine and thymine contents. However, Hua et al. (2008) proposed that the term "AT-rich" should not be used, because this region is not always the most AT-rich part of the mt-genome.

The size of the CR in insects varies considerably in different taxa. For instance, the CR size in insects can range from 70 base pairs (bp) in katydids (Zhou et al. 2007) to 13 kilo-base pairs (kb) in bark weevils (Boyce et al. 1989). The structure of the CR varies among animal groups. In mammals and birds, the CR is organized into 3 major regions or domains, including the extended terminal-associated sequence (ETAS), central, and conserved-sequence block domains (Sbisà et al. 1997, Randi and Lucchini 1998, Matson and Baker 2001). In insects, on the other hand, there are apparently 2 main types of CRs (Taylor et al. 1993, Zhang et al. 1995, Zhang and Hewitt 1997, Vila and Björklund 2004): group 1, in which a conserved domain is followed by a variable domain, as found in fruitflies; and group 2, found in grasshoppers, locusts, butterflies, and mosquitoes, characterized by a lack of distinct conserved regions. In arthropods, the CRs often have some or all of these 4 motifs: a long sequence of thymines, tandemly repeated sequences, a subregion of an even-higher A+T content, and stem-loop structures (Cook 2005). In a detailed analysis based on

*To whom correspondence and reprint requests should be addressed. Tel: 86-29-85307819. Fax: 86-29-85310097.
E-mail:yuanh@snnu.edu.cn

comparisons of dipteran and orthopteran control regions, Zhang et al. (1995) and Zhang and Hewitt (1997) pointed out 5 so-called structural elements, which are apparently conserved, and their relative locations on mtDNA are the same: a poly-T stretch at the 5' end of the control region, a (TA(A)) n-like sequence between the poly-T stretch and a secondary structure, a stem and loop structure, conserved 5' and 3' flanking regions of the stem and a G+A-rich sequence block downstream of the secondary structure. These structural elements of the CR were analyzed in Diptera (Clary and Wolstenholme 1985, Monnerot et al. 1990, Monforte et al. 1993, Lewis et al. 1994, Brehm et al. 2001, Rondan Dueňas et al. 2006, Sugihara et al. 2006), Lepidoptera (Taylor et al. 1993, Snäll et al. 2002, Vandewoestijne et al. 2004, Vila and Björkund 2004), Plecoptera (Schultheis et al. 2002, Stewart and Beckenbach 2006), Orthoptera (Rand and Harrison 1989, Zhang et al. 1995), Isoptera (Cameron and Whiting 2007), and other orders of insects. In 2005, Saito et al. (2005) successfully determined the precise position of the replication origin of mtDNA in several insect species.

Recently an influx of mt-genomes have provided new, large, diverse datasets which are useful in comparative and phylogenetic studies. Today there are currently 196 hexapod mt-genomes available on GenBank, including 25 orthoptera species with 16 from the suborder Caelifera and nine from the suborder Ensifera. Of the 16 Caelifera species, 15 are from the Acrididae and only 1 is from the Pyrgomorphidae. To help remedy this lack of data, especially data on the Pyrgomorphidae, and to facilitate comparative mt-CR analyses, we sequenced the mt-CR from 3 Caelifera species: *Euchorthippus fusigeniculatus* (subfamily Gomphocerinae, family Acrididae, superfamily Acridoidea), *Mekongiana xiangchengensis*, and *Mekongiella xizangensis*, both of which belong to the subfamily Pyrgomorphinae, family Pyrgomorphidae, superfamily Pyrgomorphoidea. All 3 species were previously reported (Jin and Zhang 1983, Yin 1984, Zheng et al. 2008).

In this article, based on 25 mt-CR sequences plus the 3 new sequences, we present a comparative analysis of the Orthoptera representing 6 families (Acrididae, Pyrgomorphidae, Gryllidae, Gryllotalpidae, Rhaphidophoridae, and Tettigoniidae) and 15 subfamilies belonging to 2 suborders in an effort to better understand its evolution, structure, and function. Finally, to examine the resolution of the phylogenetic tree from the primary sequence of the stem-loop secondary structure, we undertook a phylogenetic study of all Caelifera species available from GenBank to date using maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian (BA)-inference methods. We do not propose a definitive phylogenetic relationship for the Caelifera, but instead show that the short sequence of the stem-loop secondary structure may be a resourceful tool for elucidating some phylogenetic relationships within the Caelifera.

MATERIALS AND METHODS

Specimen collection and DNA extraction

Specimen information on *E. fusigeniculatus*, *Mekongianna xiangchengensis*, and *Mekongiella xizangensis* is listed in table 1. All specimens were preserved in 100% ethanol and stored at -4°C. Voucher specimens were deposited in the College of Life Science, Shaanxi Normal Univ., Xi'an, China.

Total DNA was isolated from leg muscle tissue using a routine phenol/chloroform method (Zhou et al. 2007). Before use, it was diluted to 50ng/μl with double-distilled water, and used as a template in a polymerase chain reaction (PCR).

PCR amplification and sequencing

The A+T-rich region was amplified in a MyCycler™ thermal cycler using primers SR-

Table 1. Information on *Euchorthippus fusigeniculatus*, *Mekongiana xiangchengensis*, and *Mekongiella xizangensis*

Family	Species	Locality	Date	Collector	GenBank acc. no
Acrididae	*E. fusigeniculatus* Jin et Zhang	Helongjiang , China	Aug. 2007	Shu-juan Xu	HM583652
Pyrgomorphidae	*Mekongiana xiangchengensis* Zheng*	Sichuang , China	Aug. 2007	Zhi-jun Zhou	HM583653
Pyrgomorphidae	*Mekongiella xizangensis* Yin	Tibet, China	July 2008	Zhi-jun Zhou	HM583654

**Mekongiana xiangchengensis* is a new species named by Zhe-min Zheng in 2008.

J14610 and TI-N18 (Simon et al. 2006). The cycling protocol contained an initial 92°C denaturation for 2 min, followed by 10 cycles of 20 s denaturation at 92°C, 30 s of annealing at 52°C, and elongation at 60°C for 180 s. These were followed by 30 cycles using the same steps, but with 20 s per cycle cumulatively added to the duration of the elongation step. The PCR products were directly sequenced from both strands using an ABI PRISM™ 3100-Avant Genetic Analyzer (Applied Biosystems, USA) after separation and purification.

Prediction of the stem-loop secondary structure

The stem-loop secondary structure in the CR of all orthoptera species in this study was determined by identifying the conserved sequence blocks, E1 and E2, as reported by Zhang et al. (1995). All stem-loop secondary structures were drawn by hand.

Phylogenetic analyses

Using the new CR sequences in addition to the previously published 18 CR sequences of the Caelifera, we reconstructed a preliminary phylogeny using *Gryllus firmus* as the outgroup under analyses with the MP, ML and BA inference methods to examine the resolution of the smaller stem-loop secondary structure.

MP analyses were conducted using PAUP (Swofford 2002), and bootstrap support was calculated from 1000 bootstrap replicates. ML analyses were also carried out in PAUP* 4.0b10 using the HKY+G model with parameter values as estimated by ModelTest (Posada and Crandall 1998). Models for the BA analyses were chosen using the Akaike information criterion (AIC) as implemented in ModelTest, and all BA analyses were performed with MrBayes Vers. 3.1.1 (Ronquist and Huelsenbeck 2003).

RESULTS

Comparison of the CR

The A+T-rich region in the 3 species was located in a conserved position between *srRNA* and *trnI*. The length and the A+T content of this region were 875 bp and 81.5% in *E. fusigeniculatus*, 1063 bp and 78.7% in *M. xizangensis*, and 733 bp and 82.8% in *M. xiangchengensis*, respectively.

The size and the A+T content of the CR in all 30 orthopteran species available at the time of this study ranged 70 (Ensifera: *Ruspolia dubia*) to 1512 (Caelifera: *Chorthippus parallelus*) and 67.4% (Ensifera: *Gampsocleis gratiosa*) to 88.2% (Caelifera: *Prumna arctica*) (Table 2), including earlier reported control regions of *Schistocerca gregaria* and *Chorthippus parallelus* (Zhang et al. 1995). As reported in table 2, A+T contents of the

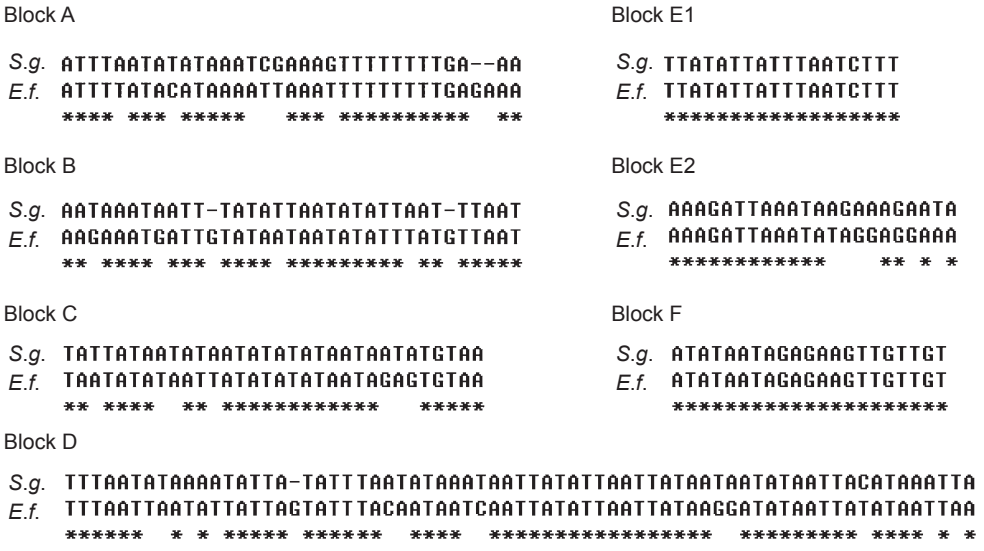

Fig. 1. Conserved sequence blocks in the control region of *Euchorthippus fusigeniculatus* and *Schistocerca gregaria*. The 7 blocks of A, B, C, D, EI, E2, and F are indicated; within each block, nucleotides identical in the 2 sequences are marked at the bottom with asterisks. The poly-thymidine stretch in the A+T-rich region of *E. fusigeniculatus* is shown in block A. *S.g.*, *S. gregari*; *E .f.*, *E. fusigeniculatus*.

CR of caeliferans were much higher than those of ensiferans with a mean A+T percent of 84.6% in caeliferans and 74.7% in ensiferans.

In this study, 7 conserved sequence blocks were identified between *E. fusigeniculatus* and *Schistocerca gregaria*: blocks A, B, C, D, EI, E2, and F with a poly-T stretch in block A (Fig. 1). A stem-loop secondary structure was also found in the A+T-rich region of all the 3 species (Figs. 2B-D).

Phylogenetic analyses

The different analyses based on the primary sequence of the stem-loop secondary structure resulted in similar topologies with an overall lower level of support. Three major clades were suggested: clade 1 included 9 species from 5 subfamilies; clade 2 was composed of 7 species from 4 subfamilies; and clade 3 consisted of 5 species of the Gomphocerinae (Fig. 3).

DISCUSSION

The CRs, including those of grasshoppers, mosquitoes and possibly butterflies, could not be divided into distinct conserved or variable domains, while tandem repetitions and conserved structural elements were observed (Zhang and Hewitt 1997). In this study, the A+T-rich region of *E. fusigeniculatus* contained a poly-T stretch

Table 2. Type, size and A+T content of the control region of all 30 Orthopteran species available thus far, including 3 newly sequenced species in this study

Suborder	Family	Subfamily	Species	A+T-rich region		
				Type*	Size (bp)	AT content%
Caelifera	Acrididae	Acridinae	*Acrida willemsei*	2	848	87.3
		Calliptaminae	*Calliptamus italicus*	2	783	Incomplete
		Catantopinae	*Ognevia longipennis*	2	775	87.6
			Prumna arctica	2	744	88.2
			Traulia szetschuanensis	2	922	82.5
		Cyrtacanthacridinae	*Schistocerca gregaria*	2	762	86.8
			Schistocerca gregaria gregaria	2	762	87.0
		Gomphocerinae	*Arcyptera coreana*	3	964	85.7
			Chorthippus chinensis	3	721	84.1
			Chorthippus parallelus	3	1512	85.1
			Euchorthippus fusigeniculatus[a]	3	875	81.5
			Gomphocerus licenti	3	712	83.7
			Phlaeoba albonema	2	728	83.0
		Oedipodinae	*Gastrimargus marmoratus*	2	1061	84.3
			Locusta migratoria	2	875	85.9
			Locusta migratoria migratoria	2	1189	84.9
			Oedaleus decorus asiaticus	2	1401	84.5
		Oxyinae	*Oxya chinensis*	2	562	86.8
	Pyrgomorphidae	Pyrgomorphinae	*Atractomorpha sinensis*	1	778	81.4
			Mekongiana xiangchengensis[b]	1	733	82.8
			Mekongiella xizangensis[c]	1	1063	78.7
Ensifera	Gryllidae	Gryllinae	*Teleogryllus emma*		940	73.9
		Myrmecophilinae	*Myrmecophilus manni*		789	74.5
	Gryllotalpidae	Gryllotalpinae	*Gryllotalpa orientalis*		920	74.9
			Gryllotalpa pluvialis		867	77.7
	Rhaphidophoridae	Rhaphidophorinae	*Troglophilus neglectus*		539	Incomplete
	Tettigoniidae	Bradyporinae	*Deracantha onos*		815	77.8
		Conocephalinae	*Ruspolia dubia*		70	71.4
		Tettigoniinae	*Anabrus simplex*		987	80.1
			Gampsocleis gratiosa		1111	67.4

*Type of stem-loop secondary structure in the A+T-rich region of all 21 Caeliferan species. [a], [b], and [c] represent the 3 species sequenced from this study.

that was highly conserved in the Orthoptera and Diptera (Fig. 1, block A), which may be involved in transcriptional control or may be the site for initiation of replication (Clary and Wolstenholme 1987, Lewis et al. 1994, Zhang et al. 1995, Cha et al. 2007). Seven conserved sequence blocks were identified in *E. fusigeniculatus* (Fig. 1). These conserved blocks were spread through the entire A+T-rich region and showed high sequence similarities with those of *Schistocerca gregaria*. In fact, block E1 was a partial inverse repeat of block E2; the sequences containing these 2 blocks can form a stem and loop (or hairpin) secondary structure (Zhang et al. 1995). The putative stem-loop secondary structure of *E. fusigeniculatus* is shown in figure 2D. The stem of this highly conserved secondary structure is formed of 16 nucleotide (nt) pairs with only 1 mismatch, and the terminal loop is 16 nt. In *Mekongiella xizangensis* and *Mekongiana xiangchengensis*, the corresponding stems are respectively formed by 17 and 16 nt pairs with only 1 mismatch in *Mekongiella xizangensis* and 2 mismatches in *Mekongiana xiangchengensis*; the respective terminal loops are 13 and 8 nt (Figs. 2B, C). By comparing the stem-loop secondary structures of all 21 Caeliferan species available to date, we found that the stems of all Caeliferan species were composed of 16 or 17 nt pairs and the 1st 13 nt pairs in the stem were almost identical in sequence, while the remaining pairs differed. Based on the remaining nucleotide pairs in the stem, there seemed to be 3 main types of stem-loop secondary structures in the CR of all 21 Caeliferan species: in type 1, the stem was composed of 16 or 17 nt pairs, and the remaining 3 or 4 nt pairs close to the loop were all A-T pairs, which was found in all 3 Pyrgomorphidae species thus far (*Atractomorpha sinensis*, *Mekongiana xiangchengensis*, and *Mekongiela xizangensis*) (Figs. 2A-C); in type 2, the stem was formed by a perfect match of 17 nt pairs (except in *Oxya chinensis* and *Calliptamus italicus*, both of which had 1 mismatch in the stem) with 1 C-G pair and 3 A-T pairs close to the loop, which was found in 13 Acrididae species (Figs. 2E-J); and type 3, found in all Gomphocerinae species except *Phlaeoba albonema*, was characterized by 16 nt pairs in the stem with only 1 mismatch and 1 A-T pair and 2 C-G pairs close to the loop (Fig. 2D). The minor difference between types 1and 2 was that the 14th bp is changed from a C-G pair in 13 Acrididae species to a T-A pair in 3 Pyrgomorphidae species. Type 3 showed big differences from type 2 in the last 3 bp which were in the order of A-T, C-G, and C-G in the 5 Gomphocerinae species and correspondingly changed to C-G, T-A, and T-A in the 13 Acrididae species. After comparing the stem-loop secondary structures of all 21 caeliferan species in table 2, we drew the following conclusions. (i) In contrast to conservation in the stems, the size of the terminal loop is highly divergent (8-16 nt), indicating that the loop region sequence in the conserved secondary structure has less functional importance. In addition, it was noteworthy that the 5' flanking sequences (TTATA) were identical in all caeliferan species and were more conserved than the 3' flanking sequences (Fig. 2). (ii) *Gastrimargus marmoratus*, *Locusta migratoria*, *L. migratoria migratoria*, and *Oedaleus decorus asiaticus* are all in the subfamily Oedipodinae and their stem-loop secondary structures plus flanking sequences were completely identical. The stem was formed by a perfect match of 17 nt pairs, including the 4 pairs next to the loop with 1 C-G pair and 3 A-T pairs, and the terminal loop was 11 nt (5'-ATTATTAGTGA-3'). The flanking sequences were highly conserved with a 5' consensus of TTATA and 3' consensus sequences of TAAAGAAAGAT (Fig. 2E). Phylogenetic analyses also showed that the 4 species formed a monophyletic group (Figs. 3A, B), consistent with newly published reports (Sun et al. 2010, Zhao et al. 2010, Zhou et al. 2010). (iii) *Arcyptera coreana*, *Chorthippus chinensis*, *C. parallelus*, *E. fusigeniculatus* and *Gomphocerus licenti* are in the subfamily Gomphocerinae and they had almost the same stem-loop secondary structures. It was observed that in these 5 species, all 16 nt pairs, including the 3 pairs close to the loop (1 A-T pair and 2 C-G pairs), with only 1 mismatch in the stem were identical in sequence, and that the terminal loop appeared to contain a sequence consensus of ATATAGTT(A)n except in *Arcyptera coreana* which had a G→A substitution and an insertion of A in the loop region (Fig. 2D). The stem-and-loop structure and flanking sequences in *Phlaeoba albonema* appeared to be more closely related to those found in *Acrida willemsei* (Acridinae) despite belonging to the Gomphocerinae (see Figs. 2F, G). Both secondary structures showed minor differences from the Oedipodinae in the loop and 3' flanking sequences.

Phylogenetic analyses showed that the Gomphocerinae was never recovered as monophyletic, with *Phlaeoba albonema* always clustered into 1 clade with *A. willemsei* (Fig. 3). (iv) *Ognevia longipennis*, *Prumna arctica*, and *Traulia*

szetschuanensis are in the subfamily Catantopinae. The hairpin structure and its flanking sequences of the former 2 species were almost the same (Fig. 2H), while the stem-loop secondary structure of *T. szetschuanensis* showed a high similarity to that of the Oedipodinae, especially the loop (Fig. 2E). The phylogenetic analyses showed that the Catantopinae was not a monophyletic group, with

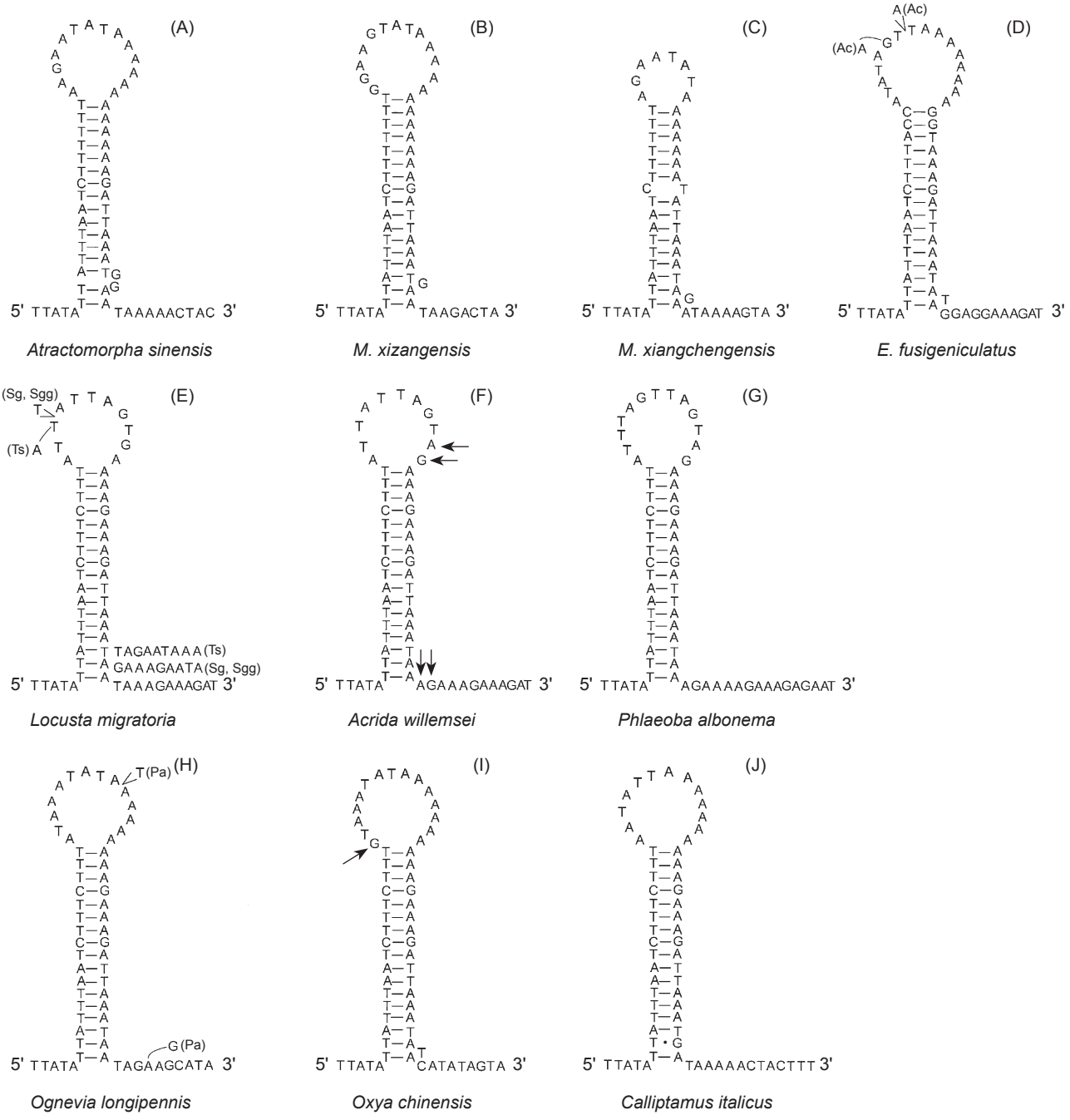

Fig. 2. Possible conserved secondary structures in the mitochondrial control regions of *Mekongiella xizangensis* (B), *Mekongiana xiangchengensis* (C), *Euchorthippus fusigeniculatus* (D), and 7 other caeliferan species. In D, E, and H, positions that differ in other species are shown in the corresponding places of the stem-loop secondary structure, with the particular species in which the change occurs listed in parentheses after the base change. Bases between wedges represent base insertions. In F, arrows indicate differences between *Acrida willemsei* and *Locusta migratoria*. In I, the arrow indicates a difference in the loop regions between *Oxya chinensis* and *Ognevia longipennis*. Ac, *Arcyptera coreana*; Sg, *Schistocerca gregaria*; Sgg, *Schistocerca gregaria gregaria*; Ts, *Traulia szetschuanensis*; Pa, *Prumna arctica*.

Traulia szetschuanensis always forming a separate clade far from *Ognevia longipennis* and *Prumna arctica* (Fig. 3). (v) *Schistocerca gregaria* and *S. gregaria* gregaria are in the Cyrtacanthacridinae. Both secondary structures showed only 1 difference from the Oedipodinae in the loop (Fig. 2E). (vi) *Oxya chinensis* and *Calliptamus italicus* are in the respective subfamilies Oxyinae and Calliptaminae. The stem-loop secondary structure of *O. chinensis* belonged to type 2 and its loop was highly similar to that of *Ognevia longipennis* (Fig. 2I). In addition, we also found the intact secondary structure in the incomplete CR of *C. italicus*, and its stem-loop secondary structure belonged to type 2 and had a poly-A sequence close to the 3' end in the loop (see Fig. 2J). The phylogenetic analyses indicated that the Oxyinae, Calliptaminae, and Cyrtacanthacridinae had close relationships of (Oxyinae + (Calliptaminae + Cyrtacanthacridinae) (Zhou et al. 2010). Comparison of loop sequences of different species from same subfamily revealed that the loop sequences were also well conserved. Despite some differences, the conserved secondary structures in these caeliferan species were very similar. This can be seen not only from the conformation of the stem and loop structures itself but also from several other features, such as similarities in sequences flanking them and their relative locations in the CRs. This suggests that such a secondary structure in mitochondrial CR may be widely conserved in all caeliferan species. In the 9 Ensifera species from GenBank, putative stem-loop structures were identified in an A+T-rich region of *Gampsocleis gratiosa* (Zhou et al. 2008) and *Ruspolia dubia* (Zhou et al. 2007). In earlier days, the stem-loop structures of 1 cricket were reported (Zhang et al. 1995). All these secondary structures completely differed from those of caeliferan species in the conformation (except the cricket) and also the flanking sequences.

Seven acridid subfamilies and the Pyrgomorphidae were included in the phylogenetic analyses, and none of the results produced the separated Pyrgomorphidae and Acrididae clades. Furthermore, the phylogenetic relationships of the 7 acridid subfamilies were poorly resolved compared to other studies (Ma et al. 2009, Zhao et al. 2010, Zhou et al. 2010) based on mt-genomes. However, species belonging to the same subfamily were almost always grouped together despite having lower support. The poor resolution and lower support of the phylogenetic

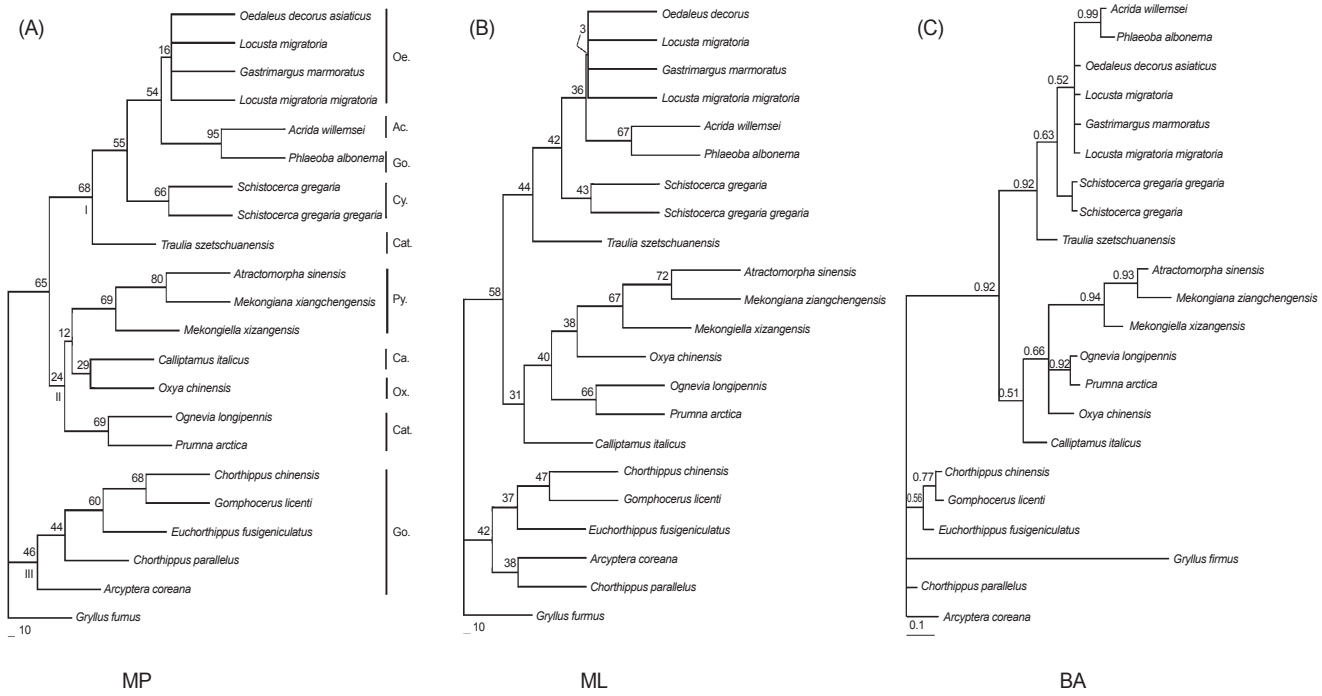

Fig. 3. Phylogenetic reconstruction of the Caelifera based on the stem-loop secondary structure in the control region using different inference methods. (A) Maximum-parsimony (MP) results, (B) maximum-likelihood (ML) results, and (C) Bayesian (BA)-inference results. Numbers near the nodes represent bootstrap support (percent values, 1000 for MP and 100 for ML) and Bayesian posterior probabilities.

tree were due to the few phylogenetic signals that the primary sequence contained. Therefore, our results showed that the short sequences from the stem-loop secondary structure poorly resolved in family- and subfamily-level relationships, whereas they had a good resolving capacity at the intra-subfamily level, despite their smaller sizes.

Acknowledgments: S.J. Xu and Z.J. Zhou kindly provided specimens. This research was supported by grants from the National Natural Science Foundation of China (no.30670279) and Foundation of Xinjiang Agricultural Univ. (no. XJAU 200901).

REFERENCES

Boyce TM, ME Zwick, CF Aquadro. 1989. Mitochondrial DNA in the bark weevils: size, structure and heteroplasmy. Genetics **123:** 825-836.

Brehm A, DJ Harris, M Hernandez, VM Cabrera, JM Larruga, FM Pinto, AM Gonzalez. 2001. Structure and evolution of the mitochondrial DNA complete control region in the *Drosophila subobscura* subgroup. Insect Mol. Biol. **10:** 573-578.

Cameron SL, MF Whiting. 2007. Mitochondrial genomic comparisons of the subterranean termites from the Genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). Genome **50:** 188-202.

Cha SY, HJ Yoon, EM Lee, MH Yoon, JS Hwang, BR Jin et al. 2007. The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). Gene **392:** 206-220.

Clary DO, DR Wolstenholme. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. J. Mol. Evol. **22:** 252-271.

Clary DO, DR Wolstenholme. 1987. Drosophila mitochondrial DNA: conserved sequences in the A+T-rich region and supporting evidence for a secondary structure model of the small ribosomal RNA. J. Mol. Evol. **25:** 116-125.

Cook CE. 2005. The complete mitochondrial genome of the stomatopod crustacean *Squilla mantis*. BMC Genomics **6:** 105.

Hua JM, M Li, PZ Dong, Y Cui, Q Xie, WJ Bu. 2008. Comparative and phylogenomic studies on the mitochondrial genomes of Pentatomomorpha (Insecta: Hemiptera: Heteroptera). BMC Genomics **9:** 610.

Jin XB, FL Zhang. 1983. A new species of genus *Euchorthippus* from China (Orthoptera: Acridoidea). Zool. Res. **4:** 377-380. (in Chinese)

Lewis DL, CL Farr, AL Farquhar, LS Kaguni. 1994. Sequence, organization, and evolution of the A+T region of *Drosophila melanogaster* mitochondrial DNA. Mol. Biol. Evol. **11:** 523-538.

Ma C, C Liu, P Yang, L Kang. 2009. The complete mitochondrial genomes of two band-winged grasshoppers, *Gastrimargus marmoratus* and *Oedaleus asiaticus*. BMC Genomics **10:** 156.

Matson CW, RJ Baker. 2001. DNA sequence variation in the mitochondrial control region of red-baked voles (Clethrionomys). Mol. Biol. Evol. **18:** 1494-1501.

Monforte A, E Barrio, A Latorre. 1993. Characterization of the length polymorphism in the A+T-rich region of the *Drosophila obscura* group species. J. Mol. Evol. **36:** 214-223.

Monnerot M, M Solignac, DR Wolstenholme. 1990. Discrepancy in divergence of the mitochondrial and nuclear genomes of *Drosophila teissieri* and *Drosophila yakuba*. J. Mol. Evol. **30:** 500-508.

Posada D, KA Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics **14:** 817-818.

Rand DM, RG Harrison. 1989. Molecular population genetics of mtDNA size variation in crickets. Genetics **121:** 551-569.

Randi E, V Lucchini. 1998. Organization and evolution of the mitochondrial DNA control region in the avian genus *Alectoris*. J. Mol. Evol. **47:** 449-462.

Rondan Dueñas JC, CN Gardenal, GA Llinas, GM PanzettaDutari. 2006. Structural organization of the mitochondrial DNA control region in *Aedes aegypti*. Genome **49:** 931-937.

Ronquist F, JP Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572-1574.

Saito S, K Tamura, T Aotsuka. 2005. Replication origin of mitochondrial DNA in insects. Genetics **171:** 1695-1705.

Sbisà E, F Tanzariello, A Reyes, G Pesole, C Saccone. 1997. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene **205:** 125-140.

Schultheis AS, LA Weigt, AC Hendricks. 2002. Arrangement and structural conservation of the mitochondrial control region of two species of Plecoptera: utility of tandem repeat-containing regions in studies of population genetics and evolutionary history. Insect Mol. Biol. **11:** 605-610.

Shadel GS, DA Clayton. 1997. Mitochondrial DNA maintenance in vertebrates. Annu. Rev. Biochem. **66:** 409-435.

Simon C, TR Buckley, F Frati, JB Stewart, AT Beckenbach. 2006. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. **37:** 545-579.

Snäll N, K Huoponen, ML Savontaus, K Ruohomäki. 2002. Tandem repeats and length variation in the mitochondrial DNA control region of *Epirrita autumnata* (Lepidoptera: Geometridae). Genome **45:** 855-861.

Stewart JB, AT Beckenbach. 2006. Insect mitochondrial genomics 2: the complete mitochondrial genome sequence of a giant stonefly, *Pteronarcys princeps*, asymmetric directional mutation bias and conserved plecopteran A+T-region elements. Genome **49:** 815-824.

Sugihara K, R Yui, Y Ibaragi, ET Matsuura. 2006. Complete nucleotide sequence of the A+T-region of *Drosophila mauritiana* mitochondrial DNA. Genes Genet. Syst. **81:** 21-28.

Sun HM, ZM Zheng, Y Huang. 2010. Sequence and phylogenetic analysis of complete mitochondrial DNA genomes of two grasshopper species *Gomphocerus rufus* (Linnaeus, 1758) and *Primnoa arctica* (Zhang and Jin, 1985) (Orthoptera: Acridoidea). Mitochon. DNA **21:** 115-131.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Vers. 4. Sunderland, MA: Sinauer Associates.

Taylor MFJ, SW McKechnie, N Pierce, M Kreitman. 1993. The lepidopteran mitochondrial control region: structure and evolution. Mol. Biol. Evol. **10:** 1259-1272.

Vandewoestijne S, M Baguette, PM Brakefield, IL Saccheri. 2004. Phylogeography of *Aglais urticae* (Lepidoptera) based on DNA sequences of the mitochondrial COI gene and control region. Mol. Phylogenet. Evol. **31:** 630-646.

Vila M, M Björklund. 2004. The utility of the neglected mitochondrial control region for evolutionary studies in Lepidoptera (Insecta). J. Mol. Evol. **58:** 280-290.

Wolstenholme DR. 1992. Animal mitochondrial DNA: structure and evolution. Int. Rev. Cytol. **141:** 173-216.

Yin XC. 1984. Grasshoppers and locusts from Qinghai-Xizang Plateau of China. Beijing: Science Press, pp. 34. (in Chinese)

Zhang DX, FM Hewitt. 1997. Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. Biochem. Syst. Ecol. **25:** 99-120.

Zhang DX, JM Szymura, GM Hewitt. 1995. Evolution and structural conservation of the control region of insect mitochondrial DNA. J. Mol. Evol. **40:** 382-391.

Zhao L, ZM Zheng, Y Huang, HM Sun. 2010. A comparative analysis of mitochondrial genomes in Orthoptera (Arthropoda: Insecta) and genome descriptions of three grasshopper species. Zool. Sci. **27:** 662-672.

Zheng ZM, Y Huang, ZJ Zhou. 2008. New genus and new species of grasshoppers from Henduanshan region, China (Orthoptera: Acridoidea). Acta Zootaxon. Sin. **33:** 363-367. (in Chinese)

Zhou ZJ, Y Huang, FM Shi. 2007. The mitochondrial genome of *Ruspolia dubia* (Orthoptera: Conocephalidae) contains a short A+T-rich region of 70 bp in length. Genome **50:** 855-866.

Zhou ZJ, FM Shi, Y Huang. 2008. The complete mitogenome of the Chinese bush cricket, *Gampsocleis gratiosa* (Orthoptera: Tettigonioidea). J. Genet. Genomics **35:** 341-348.

Zhou ZJ, HY Ye, Y Huang, FM Shi. 2010. The phylogeny of Orthoptera inferred from mtDNA and description of *Elimaea cheni* (Tettigoniidae: Phaneropterinae) mitogenome. J. Genet. Genomics **38:** 315-324.