

A Molecular Phylogeny of Planthoppers (Hemiptera: Fulgoroidea) Inferred from Mitochondrial 16S rDNA Sequences

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Wen-Bin Yeh, Chung-Tu Yang, and Cho-Fat Hui (2005) A molecular phylogeny of planthoppers (Hemiptera: Fulgoroidea) inferred from mitochondrial 16S rDNA sequences. *Zoological Studies* 44(4): 519-535. Phylogenetic reconstruction of family relationships within the superfamily Fulgoroidea was conducted based on DNA sequences of the mitochondrial 16S rDNA gene. Sequences of 569 bases of the 3' end of the gene from 59 populations representing 53 species within 15 families were analyzed. Ranges of the 16S rDNA nucleotide divergences within species were 0%-0.6%, those among species of a given genus were 1.7%-7.8%, and those among genera of the same tribe or subfamily were 8.1%-19.5%. Scatterplots of total substitutions (Tvs) against transversions (Tv), or transitions (Ts) of the 16S rDNA gene revealed that the mutation rate of Tv was 3 times higher than Ts, and substitutional saturation has not yet been reached. Phylogenetic reconstruction and bootstrap confidence analysis revealed several basal lineages and 1 advanced group. The basal lineages included 5 families, where the Cixiidae exhibited close affinity to the Delphacidae, while the phylogenetic positions of the Achilidae, Meenoplidae, and Lophopidae were ambiguous. Monophyly of the remaining 10 advanced families showed several subdivided family groups with close affinities of the Derbidae to the Tropiduchidae, Dictyopharidae to the Fulgoridae, and Ricaniidae to the Eurybrachidae. The ancestral lineage of the Tettigometridae which exhibits many ancestral morphological characters was not supported by the 16S rDNA sequence data analysis, and the relationship of the families Flatidae and Nogodinidae was poorly resolved. In addition, the Issidae was not shown to be monophyletic, although issid members at the subfamily level were well supported. <http://zoolstud.sinica.edu.tw/Journals/44.4/519.pdf>

Key words: Molecular phylogeny, Mitochondrial 16S rDNA, Hemiptera, Fulgoroidea.

Planthoppers constitute a large group of phytophagous insects in the order Hemiptera and family Fulgoroidea including more than 9000 described species with division into 19 families distributed worldwide (O'Brien and Wilson 1985). These insects occupy extensive ranges of habitats (Denno and Roderick 1990), and some major agricultural pests are included (Wilson and O'Brien 1987). The Fulgoroidea consists of common herbivores in both agricultural and natural systems, often causing severe damage to their host plants. Attention has focused on a number of planthopper

species because of large damage incurred by the crops of maize, rice, wheat, and forage grasses. Several species of delphacids exhibit high reproductive potentials and dispersal capabilities which allow them to track changes in favorable resources and therefore predispose them to be agricultural pests (Denno and Roderick 1990). *Nilaparvata lugens* (Stål), for example, a delphacid, caused more than US\$1.23 billion in losses to rice in South and Southeast Asia annually (Herdt 1987).

The first phylogenetic hypothesis concerning

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the Fulgoroidea was proposed by Muir (1923). Thereafter, several such hypotheses were proposed based on adult or nymphal morphological characters (Asche 1988, Emeljanov 1991, Chen and Yang 1995). The Tettigometridae is usually considered the most-ancestral family in the Fulgoroidea since it exhibits many ancestral characters pertaining to the Cicadoidea (Muir 1923). Other popular relationships of the remaining fulgoroids are those proposed by Asche (1988) and Emeljanov (1991). Figure 1A shows a cladistic analysis based on 30 adult morphological characters. It suggests that the Cixiidae is a sister group to the Delphacidae, and both are more ancestral than the others which form several aligned lineages including 1 major group, but the affinity among these lineages is ambiguous (Asche 1988, Wilson et al. 1994). Asche's hypothesis was criticized by Emeljanov (1991) based on 50 morphological characters (Fig. 1B), and the Delphacidae has been suggested as being the next-most ancestral and not a sister group of the Cixiidae.

Each of the following lineages in Asche's hypothesis is further divided in an unequal succession of dichotomies. Both hypotheses suggest that the Meenoplidae is a sister group to the Kinnaridae, and the Fulgoridae is a sister group to the Dictyopharidae. These commonly cited fulgoroid phylogenies have frequently been challenged. The ancestral taxon of the Tettigometridae is questionable, and the primitive characters in tettigometrids are the result of convergence (Fig. 1C) (Bourgoin 1993). Further molecular evidence using 18S rDNA sequences confirms this controversial point (Bourgoin et al. 1997). Also, based on the ontogeny of metatarsal characters, Cheng and Yang (1995) suggested that the Delphacidae and Cixiidae constitute the most-advanced lineage of fulgoroids (Fig. 1D). Thus, the only conclusion generally accepted in fulgoroid systematics is that the Fulgoridae and Dictyopharidae are sister groups. Obviously, more studies of fulgoroid phylogeny using different characters are needed.

The 3' end sequences of the mitochondrial

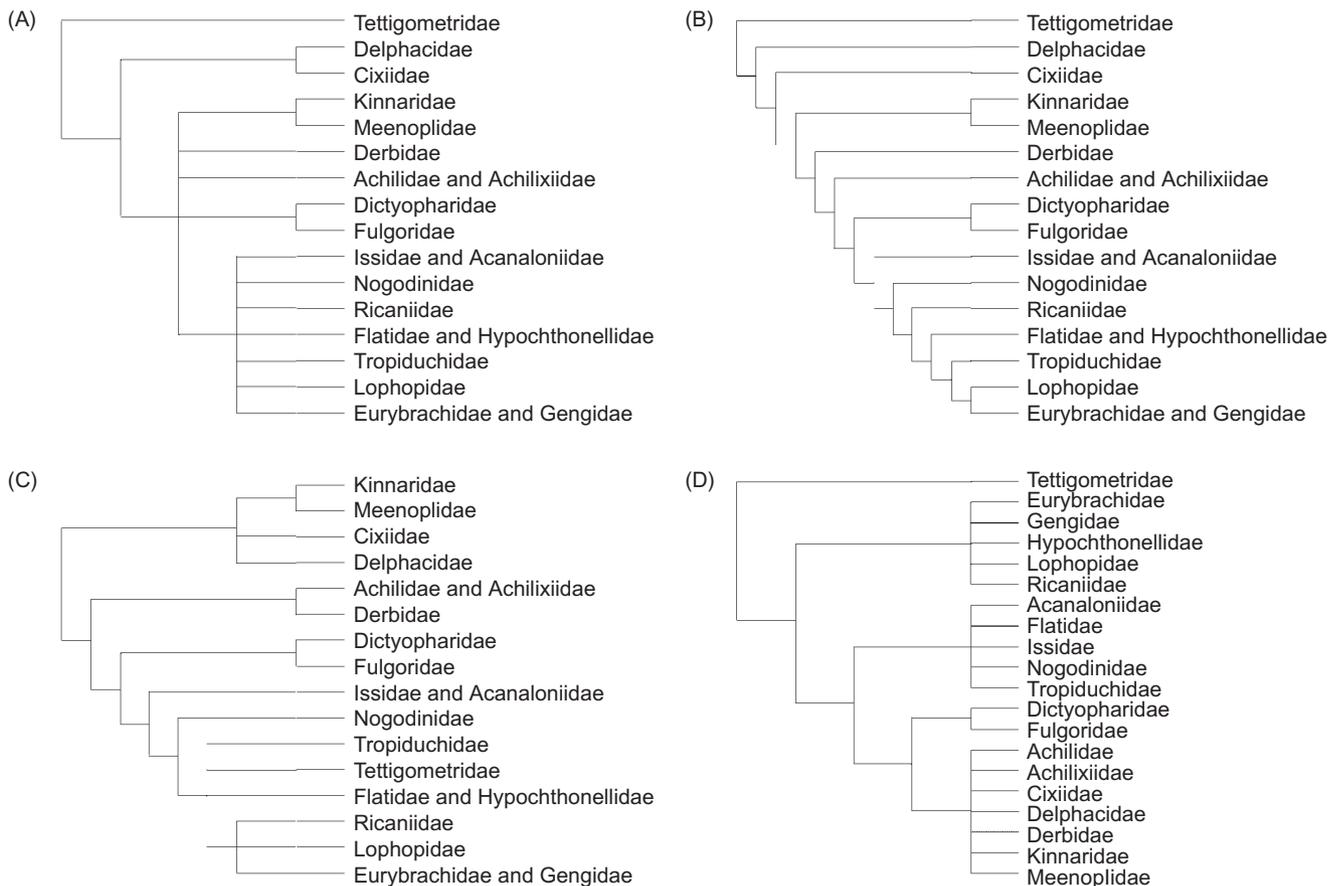


Fig. 1. Hypotheses of phylogenetic relationships of fulgoroid families. (A) Phylogenies proposed by Asche (1988), (B) Emeljanov (1991), (C) Bourgoin et al. (1993, 1997), and (D) Cheng and Yang (1995).

Table 1. Taxonomic status of fulgoroid taxa examined in this study with accession nos

Family	Subfamily or Tribe	Abbrev.	Species name	Accession no.	
Achilidae	Plectoderini	Ach1	<i>Betatropis formosana</i> Matsumura	AF158028	
	Rhotalini	Ach2	<i>Rhotala formosana</i>	AF158029	
Cixiidae	Cixiinae	Cix2	<i>Pentastiridius pachyceps</i> (Matsumura)	AF158030	
	Cixiinae	Cix3	<i>Cixius circulus</i> Tsaur et Hsu	AF158031	
	Cixiinae	Cix4	<i>Cixius inflatus</i> Tsaur et Hsu	AF158032	
	Cixiinae	Cix6	<i>Betacixius ocellatus</i>	AF158033	
Delphacidae	Delphacinae	Del2	<i>Nilaparvata lugens</i> Stål	AF158034	
	Delphacinae	Del3	<i>Purohita taiwanensis</i> Muir	AF158035	
	Asiracinae	Del4	<i>Ugyops tripunctatus</i> (Kato)	AF158036	
	Asiracinae	Del6	<i>Ugyops vittatus</i> (Matsumura) ^a	AF158037	
	Delphacinae	Del7	<i>Nilaparvata lugens</i> Stål	AF158038	
Derbidae	Dlephacinae	Del8	Delphacinae sp.	AF158039	
	Zoraidini	Der1	<i>Zoraida kotoshoensis</i> Matsumura	AF158040	
	Zoraidini	Der2	<i>Diostrombus politus</i> Uhler	AF158041	
	Otiocerini	Der3	<i>Kamendaka aculeata</i> Yang et Wu	AF158042	
	Rhotanini	Der4	<i>Rhotana obaerata</i> Yang et Wu	AF158043	
Dictyopharidae	Otiocerini	Der9	<i>Kamendaka aculeata</i> Yang et Wu	AF158044	
	Dictyopharinae	Dic1	<i>Orthopagus splendens</i> (Germar)	AF158045	
	Dictyopharinae	Dic2	<i>Raivuna</i> sp.	AF158046	
	Dictyopharinae	Dic3	<i>Saigona gibbosa</i> Matsumura	AF158047	
Eurybrachidae	Dictyopharinae	Dic4	<i>Orthopagus</i> sp. ^a	AF158048	
	Platybrachinae	Eur1	<i>Platybrachys decemmacula</i> Walker	AF158049	
Flatidae	Flatini	Fla1	<i>Phylliana alba</i> (Jacobi)	AF158050	
	Flatini	Fla2	<i>Mimophantia maritina</i> Matsumura	AF158051	
	Nephesini	Fla3	<i>Geisha distinctissima</i> (Walker)	AF158052	
	Flatini	Fla4	<i>Mimophantia maritina</i> Matsumura	AF158053	
	Flatoidinae	Fla5	<i>Atracis</i> sp.	AF158054	
Fulgoridae	Aphaeninae	Ful2	<i>Lycorma meliae</i> Kato	AF158055	
	Aphaeninae	Ful3	<i>Lycorma olivacea</i> Kato	AF158056	
	Fulgorinae	Ful4	<i>Fulgora laternaria</i> Linne'	AF158057	
	Aphaeninae	Ful5	<i>Lycorma delicatula</i> White ^a	AF158058	
Issidae	Hemisphaerinae	Iss1	<i>Epyhemisphaerius tappanus</i> (Matsumura)	AF158059	
	Tonginae	Iss2	<i>Tonga botelensis</i> Kato	AF158060	
	Issinae	Iss3	<i>Eusarima astuta</i> Chan et Yang	AF158061	
	Caliscelinae	Iss4	<i>Mushya facia</i> Kato	AF158062	
	Hemisphaerinae	Iss5	<i>Hemisphaerius formosus</i> Melichar	AF158063	
	Caliscelinae	Iss6	<i>Bruchomorpha oculata</i> Newman	AF158064	
	Caliscelinae	Iss7	<i>Bruchomorpha jocosa</i> Stål	AF158065	
	Caliscelinae	Iss8	<i>Aphelonema histrionica</i> Stål	AF158066	
	Hemisphaerinae	Iss9	<i>Gergithus rotundus</i> Chan et Yang	AF158067	
	Hemisphaerinae	Iss10	<i>Gergithus yayeyamensis</i> Hori	AF158068	
	Tonginae	Iss11	<i>Tonga westwoodi</i> (Signoret) ^a	AF158069	
	Tonginae	Iss12	<i>Tonga westwoodi</i> (Signoret) ^a	AF158070	
	Issinae	Iss14	<i>Eusarima condensa</i>	AF158071	
	Lophopidae	Lophopinae	Lop1	<i>Lophops carinata</i> (Kirby)	AF158072
Meenoplidae	Kermesiinae	Mee2	<i>Nisia serrata</i> Tsaur	AF158073	
	Kermesiinae	Mee3	<i>Nisia lansunensis</i> Yang	AF158074	
	Kermesiinae	Mee6	<i>Nisia serrata</i> Tsaur	AF158075	
Nogodinidae	Varciini	Nog1	<i>Mindura subfasciata kotoshonis</i> Matsumura	AF158076	
	Pisachini	Nog2	<i>Pisacha naga</i> Distant	AF158077	
Ricanidae	Ric1	Ric1	<i>Ricania fumosa</i> (Walker)	AF158078	
	Ric3	Ric3	<i>Ricanula pulverosa</i> Stål	AF158079	
	Ric4	Ric4	<i>Ricania simulans</i> (Walker)	AF158080	
	Ric5	Ric5	<i>Euricania ocella</i> (Walker)	AF158081	
	Tet2	Tet2	<i>Tettigometra</i> sp.	AF158082	
Tettigometridae	Tettigometrinae	Tet2	<i>Tettigometra</i> sp.	AF158082	
	Tropiduchidae	Tambiniini	Tro1	<i>Kallitaxila sinica</i> (Walker)	AF158083
		Tambiniini	Tro2	<i>Ossoides lineatus</i> Bierman	AF158084
		Catulliini	Tro3	<i>Catullia subtestacea</i> Stål	AF158085
Tambiniini	Tro4	<i>Ossoides lineatus</i> Bierman	AF158086		

^aNymph material.

16S rDNA gene have been shown to be useful for examining insect relationships from the genus level to the family level (Han and McPheron 1997, Yeh et al. 1998, Marini and Mantovani 2002, Hypša et al. 2002, Whitfield et al. 2002, Ribera et al. 2003). Mitochondrial 16S rDNA sequences have been used to infer relationships of 6 fulgoroid families of the Tropicodidae group, and the results indicated that the 3' end sequences of the 16S rDNA are suitable for studying family relationships within the Fulgoroidea since nucleotide divergence increases with increasing taxonomic distance (Yeh et al. 1998). Nuclear 18S rDNA sequences have been used to evaluate 5 fulgoroid families and the affiliation of the Tettigometridae (Campbell et al. 1995, Bourgoïn et al. 1997). Nucleotide sequences of the 18S rDNA gene may be too conserved for analysis of closely related families since nucleotide divergences between fulgoroid families are in the range of 1.5% to 6.3% (Campbell et al. 1995, Bourgoïn et al. 1997). Also, too few families have been studied so far to obtain a general picture of fulgoroid phylogeny (Campbell et al. 1995, Bourgoïn et al. 1997, Yeh et al. 1998).

In this work, sequences of the 16S rDNA gene of 59 populations within 53 species representing 15 families of fulgoroids were analyzed. The primary aims of this study were to use molecular characters to infer phylogenetic relationships within the Fulgoroidea as well as to address 2 issues that are still being debated: the phylogenetic position of the Tettigometridae, and whether Cixiidae and Delphacidae are ancestral taxa. Results from fulgoroid 16S rDNA sequences revealed that transversion substitutions are accumulating 3 times faster than transition substitutions. Phylogenetic analysis showed that members of the same family are grouped together with high bootstrap values, and that the Tettigometridae is not an ancestral taxon. Finally, the Achilidae, Cixiidae, Delphacidae, Lophopidae, and Meenoplidae were found to constitute the basal lineages, with the remaining 10 families comprising advanced lineages.

MATERIALS AND METHODS

Taxa examined

In total, 59 populations representing 53 species of 15 families of fulgoroids were included in this study (Table 1). Specimens of *Bruchomorpha oculata*, *B. jocosus*, and *Aphelonema histrionica* of

the Issidae were provided by M. R. Wilson (International Institute of Entomology, London). Six species selected for outgroup comparisons were *Mogannia* sp. (Cicadidae, Cica, GenBank accession no.: AF158087), *Cosmoscarta kotoensis* (Cercopidae, Cerc, accession no.: AF158088), and 4 species of the Cicadellidae: *Macrosteles fascifrons* (Cic1), *Exitianus exitiosus* (Cic2), *Mocuellus caprillus* (Cic3), and *Amblysellus grex* (Cic4) (Fang et al. 1993).

DNA extraction, amplification, and sequencing

Live insects were collected and preserved in 95% ethanol at -20°C. The entire insect body or the leg of a large specimen was homogenized in a glass homogenizer in 500 µl digestion buffer that contained 100 mM Tris-Cl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.5% SDS, 50 mM dithiothreitol, and 0.5 mg/ml proteinase K. The mixture was incubated at 50°C overnight, then extracted with phenol-chloroform (modified from Yeh et al. 1998). Extracted crude DNA was dissolved in 50 µl TE buffer, and an aliquot of 10 µl crude DNA was diluted 10-fold and used as the DNA template in the following amplification reaction.

The polymerase chain reaction (PCR) was employed to amplify a partial sequence of the mitochondrial 16S rDNA gene. The primers used to amplify the region were 5'-GCCTGTTTATCAAAAACAT-3' and 5'-CCGGTCTGAACTCAGATCA-3' that correspond to nucleotides 13416-13396 and 12866-12884, respectively, of the 16S rDNA gene of *Drosophila yakuba* (Clary and Wolstenholme 1985). Amplification was carried out for 39 cycles in a final volume of 100 µl containing 10 mM Tris-Cl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 0.1% Triton-X100, 2 units of SuperTaq polymerase (HT Biotechnology, Taiwan), 0.2 mM of each dNTP, 20 pmoles of each primer, and 2 µl DNA template with the following temperature profile: denaturation for 50 s at 95°C, annealing for 1 min at 50°C, and extension for 1 min at 72°C. Amplified DNA fragments were separated by agarose gel electrophoresis and extracted from the gel using the Nucleotrap Kit (Macherey-Nagel, Germany). The resulting DNA product was directly sequenced using the Cycling PCR Sequencing Kit (Perkin Elmer, USA), and 29 cycles were carried out with the following temperature profile: 40 s for denaturation at 95°C, annealing at 50°C, and extension at 72°C (modified from Yeh et al. 1998).

DNA analysis

Initial alignment of the mitochondrial sequences was conducted using the Pileup program of the GCG software package (available at <http://bioinfo.nhri.org.tw>), then manually refined based on the secondary structures of the 16S rRNA sequences (Davis et al. 1994, Fang et al. 1993, Kambhampati et al. 1996). The proportion of the nucleotide composition of each taxon was calculated using the MEGA version 3 program (Kumar et al. 2004) with a variety of genetic distances. To correct for AT-richness of the mtDNA sequences and different substitution patterns of transition (Ts) and transversion (Tv), the parameters of nucleotide composition and substitution types were used in the sequence divergence estimation. The pair-wise distance estimates were based on models that included the proportional, Kimura 2-parameter, and Tamura 3-parameter distance measures. A statistical analysis system (SAS Institute 2001) was used to test for significant correlations between nucleotide base compositions and substitution patterns of Ts and Tv.

Following the sequence variation estimation, neighbor-joining (NJ) and minimum evolution (ME) implemented in MEGA3 (Kumar et al. 2004) were used for the phylogenetic reconstruction. Different values of the parameter of α in the gamma distribution were used to determine the effect of heterogeneity in substitution rates among sites. Sites 40-45, 69-74, 185-186, 279-280, 290-291, 375-377, and 496-497 of the mitochondrial sequence data were excluded from the phylogenetic analysis because they could not be aligned unambiguously. Bootstrap analyses of 1000 replications were carried out on the trees inferred from the NJ and ME methods.

RESULTS

Sequence variation

When gaps were added to the alignment, 569 bases (Appendix I) were analyzed. Length variations of the partial 16S rDNA sequences among taxa ranged from 534 to 548 bases. Of the 569 bases examined, 375 bases (66%) were variable. The average nucleotide composition proportions (\pm SD) for the fulgoroid sequences were: G, 16.7 (\pm 1.2); A, 30.6 (\pm 2.5); T, 43.1 (\pm 1.7); and C, 9.6 (\pm 0.5). A bias towards adenine and thymine is consistent with the base composition of the corre-

sponding 16S rDNA region of other insects (Fang et al. 1993, Dowton and Austin 1994, Kambhampati 1995, Vogler and Pearson 1996, Han and McPheron 1997). Variable sites were not randomly distributed over the examined 16S rDNA region, and nucleotide divergence patterns in the 3' region of this gene showed that there are 3 highly variable regions (positions 40-74, 279-291, and 372-393; Appendix I). This result suggests that substitution patterns might be constrained by the function of the gene. The pattern of substitutions showed the greatest bias with A \leftrightarrow T (11.5%), which was larger than changes of A \leftrightarrow G (3.2%), T \leftrightarrow C (1.8%), and T \leftrightarrow G (2.3%), while changes of A \leftrightarrow C (0.5%) and G \leftrightarrow C (0.1%) were rare. However, correlation analysis of the base composition and substitution patterns using Kendall's tau (τ) (Kendall 1938) showed no significant relationship between base composition and substitution patterns ($\tau = 0.6$; $\tau^* = 0.6$, $\alpha = 0.1$).

The uncorrected nucleotide divergences within species were 0%-0.6%, those among species of a given genus were 1.7%-7.8%, and those among genera of the same tribe or subfamily of fulgorids were 8.1%-19.5%. Regression analysis of total substitutions (Tvs) versus transversions (Tv) and transitions (Ts) revealed that the mutation rate of Tv (with a slope of 0.74; $R^2 = 0.94$) was 3 times higher than that of Ts (with a slope of 0.26; $R^2 = 0.71$), and substitutional saturation due to multiple hits was not yet observed in fulgoroids (Fig. 2). This information indicates that both Tv and Ts may provide phylogenetic information.

Phylogenetic analysis

Information on the differentiated nucleotide composition and Tv and Ts substitution patterns

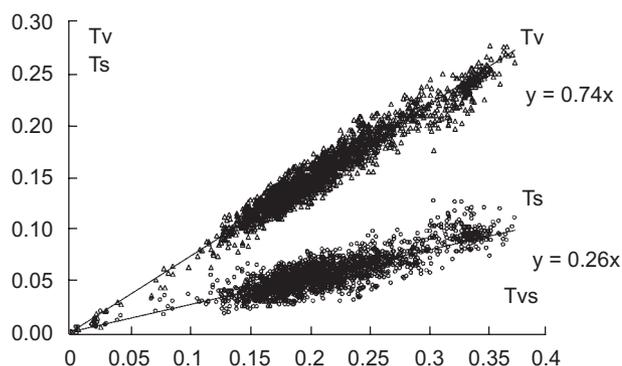


Fig. 2. Regression analysis of total substitutions (Tvs) versus transitions (Ts, \circ) and transversions (Tv, Δ) in the 16S rDNA sequences of fulgoroids.

allowed us to use the 3-parameter estimated distance model (Tamura 1992) in the NJ analysis, and the results are shown in figure 3A, for which the results of 1000 bootstrap replications are shown in the phylogenetic tree. Members of the same family were generally grouped together and received significant bootstrap possibilities of from 88% to 99%. The phylogenetic tree reveals the presence of 2 patterns of lineages. The basal lineages include 5 families of the Cixiidae, Delphacidae, Meenoplidae, Lophopidae, and Achilidae, and a close relationship is evident in the lineages of the Cixiidae and Delphacidae, although members Del4 and Del6 of the Delphacidae are cohesive to the Cixiidae lineage. After excluding the 5 basal families, the derived lineages can be divided into many groups and several independently aligned families: (i) Derbidae-Tropiduchidae; (ii) Fulgoridae-Dictyopharidae; (iii) Eurybrachidae-Ricaniidae, and the Flatidae, Nogodinidae, Tettigometridae, and Issidae. Bootstrap values suggest a robust relationship for each lineage of these groups. Furthermore, similar phylogenetic topologies were obtained when the data matrix was analyzed under other distance estimation models (Kimura 2-parameter with different values of α in the gamma dis-

tribution, Fig. 4a-d). These trees use family names since members of the same family consistently grouped together (Fig. 3). The trees support the 2 patterns of lineages but lack resolution among family groups in the advanced lineages.

The minimum evolution result constructed from the 3-parameter estimated distances exhibits the grouping pattern shown in figure 3B and is mostly consistent with that from the NJ analysis. The basal lineages include 5 families, and the relationship between the Delphacidae and Cixiidae is close, but it is necessary to further elucidate the paraphyletic resolution in the Delphacidae. Relationships among the Meenoplidae, Delphacidae-Cixiidae, Lophopidae, and Achilidae cannot be clearly resolved (Fig. 3B). Several groups among families in the derived lineages are revealed, including Derbidae-Tropiduchidae, Dictyopharidae-Fulgoridae, and Eurybrachidae-Ricaniidae, but relationships among the remaining families were poorly resolved. Furthermore, similar phylogenetic topologies were inferred in the minimum evolution analyses when using different values of α in the gamma distribution under the Kimura 2-parameter distance estimate model (Fig. 4e-h).

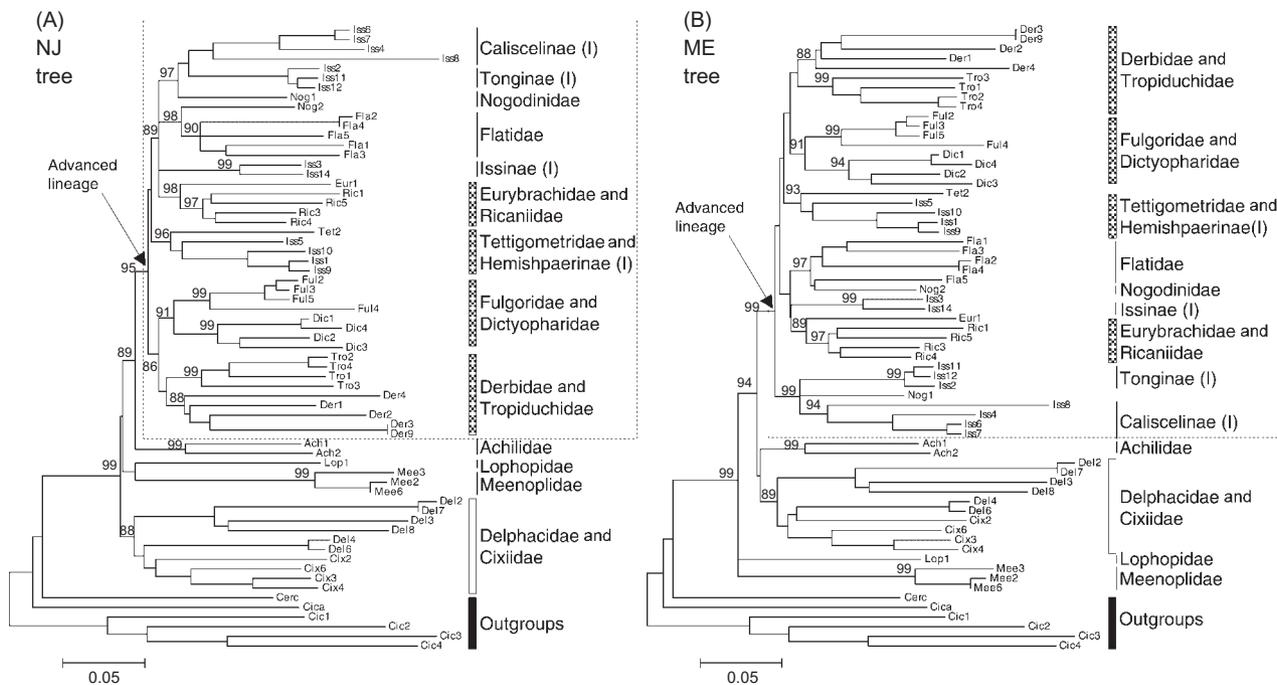


Fig. 3. Phylogeny of fulgoroids based on the partial mitochondrial 16S rDNA sequences by neighbor-joining (A) and minimum evolution (B) reconstructions based on the 3-parameter Tamura model. Bootstrap scores exceeding 80% from 1000 replications are given beneath the branches (not shown for branches below the family level). The black, white, and hatched rectangular boxes indicate clusters of the outgroups and two defined subdivided groups, respectively. The black lines indicate families whose phylogenetic relationships are ambiguous according to these analyses. The dotted-line box indicates advanced lineages. Taxa of the Issidae (I) are labeled in the subfamily category.

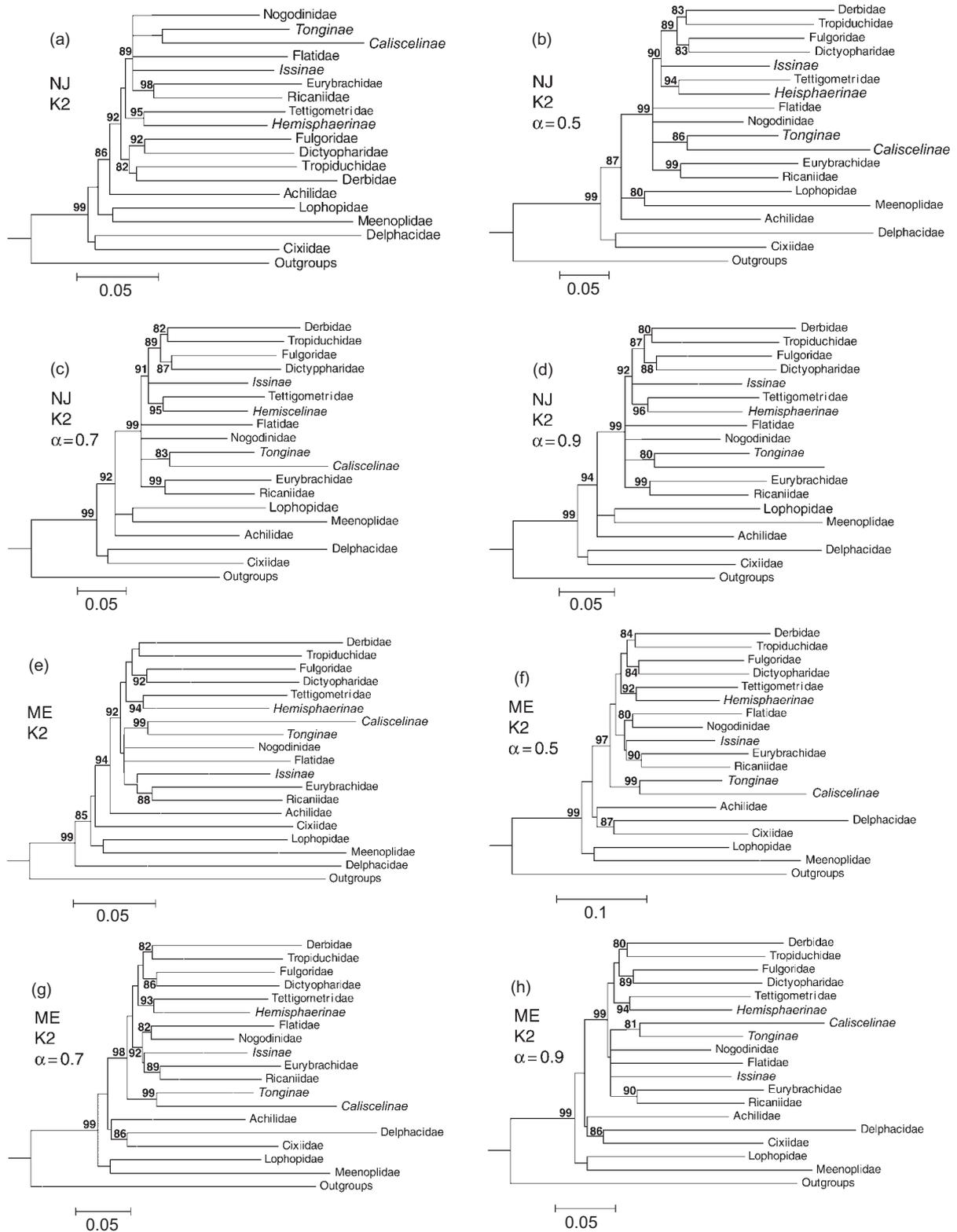


Fig. 4. Phylogenetic tree inferred from the partial mitochondrial 16S rDNA sequences by neighbor-joining (a-d) and minimum evolution (e-h) analyses based on the Kimura-2-parameter distance model. Correction for rate heterogeneity among sites with different values for the parameter α in the gamma distribution are shown, and bootstrap scores exceeding 80% from 1000 replications are given beneath the branches (not shown for branches below the family level). The trees use family names since members of the same family grouped together in figure 3.

However, monophyly of the Nogodinidae was not recovered by phylogenetic analysis, with Varciini (Nog1) and Pisachini (Nog2) falling into the Issidae and Flatidae, respectively. In addition, a non-monophyletic composition was seen in the family Issidae, particularly the subfamily Hemisphaerinae which formed a well-supported cluster with the family Tettigometridae.

DISCUSSION

The popular view among fulgoroid systematists that the Tettigometridae is the most-ancestral lineage among fulgoroids (Muir 1923, Asche 1988, Emeljanov 1991, Cheng and Yang 1995) was not supported by the 16S rDNA sequence data analyses. Average nucleotide sequence divergence between the tettigometrid and other fulgoroid families (19.3%) was small when compared to the 2 most-divergent families, i.e., the Meenoplidae (24.5%) and Delphacidae (23.8%). Also, phylogenetic analyses indicated that the Tettigometridae belongs to a more-advanced lineage of the Fulgoroidea and is grouped with the subfamily Hemisphaerinae of the Issidae. Based on the 18S rDNA sequence data, Bourgoïn et al. (1997) indicated that the Tettigometridae is not a basal family in the Fulgoroidea, and they depicted it as a sister group of the Tropiduchidae. However, Bourgoïn et al. also suggested that the sister taxon of tettigometrids still needed to be rigorously determined partly due to the weak sequence information in the 5' terminal region of 18S rDNA of the Tropiduchidae. Obviously, the sister group of Tettigometridae can be further clarified when additional numbers of related sequences are included.

Molecular phylogenies of planthoppers inferred from the 16S rDNA sequences indicate that fulgoroids are monophyletic. The relationships among families in these analyses are nearly identical: (i) The basal lineages are constituted by the Achilidae, Cixiidae-Delphacidae, Lophopidae, and Meenoplidae; (ii) Robust family groups of the Derbidae-Tropiduchidae, Dictyopharidae-Fulgoridae, and Eurybrachidae-Ricaniidae are aligned with other families in the advanced lineage; and (iii) The Issidae is a non-monophyletic group. It has been generally accepted that cixiids, delphacids, and meenoplids are relatively ancient families, but the relationships among these 3 families have not been resolved. For example, it was proposed that cixiids and delphacids are sister groups and are more ancestral than meenoplids based on a

cladistic analysis (Asche 1988) with delphacids being more ancestral than cixiids, and cixiids more ancestral than meenoplids (Emeljanov 1991); furthermore, 18S rDNA sequence analysis showed that cixiids and delphacids are sister groups and are more ancestral than the other 5 fulgoroid families (Bourgoïn et al. 1997). However, more-recent relationship reconstruction using cytochrome b (COB) sequences suggested that meenoplids are the most-ancestral family within the fulgoroids (Yeh et al. 1998). Phylogenetic inferences in these analyses indicated that the Cixiidae has a close affinity to the Delphacidae and constitutes one of the ancestral lineages within the fulgoroids.

Additional evidence from fossil records supports these basal lineages. The first fossil known of an extant member of the Fulgoroidea is of the Cixiidae, which appears at the beginning of the Jurassic period (210 Ma) (Shcherbakov 1996), and the next-oldest fossil is of the Achilidae (135 Ma) (Hamilton 1990). Most of the other fulgoroid families radiated out in the Cenozoic period (Shcherbakov 1993). However, sequence analyses revealed an unexpected phylogenetic position of the Lophopidae, which had been considered to be in the advanced lineage based on morphological characters (Asche 1988, Emeljanov 1991, Chen and Yang 1995). Soulier et al. (1996) pointed out that the Lophopidae is a paraphyletic family. Molecular results may have been biased from the sample size or the poor alignment of the AT-rich 16S rDNA. More than 1 taxon of lophopids or an additional sequence, such as 18S rDNA, must necessarily be included to help clarify the evolutionary relationship of the lophopids.

Excluding the 5 families (Achilidae, Cixiidae, Delphacidae, Lophopidae, and Meenoplidae), phylogenetic relationships confirm 1 major derived lineage although several phylogenetic relationships within it are poorly resolved. This polytomous result was possibly caused by the poor alignment of the 16S rDNA AT-rich regions or by the rapid radiation of fulgoroid families in the Cenozoic period (Shcherbakov 1993). However, some reliable conclusions can be drawn from the phylogenetic analyses. Molecular data confirm a close relationship between the Dictyopharidae and Fulgoridae, and depict the Derbidae as a sister group of the Tropiduchidae, and the Ricaniidae as a sister group to the Eubrybrachidae. Characters of female genitalia suggest that the family Achilidae has a close affinity to the Derbidae (Fig. 1C). However, according to the adult and nymphal morphological characters (Asche 1988, Chen and

Yang 1995), the phylogenetic affinity is ambiguous between the Achilidae and Derbidae. Asche (1988) proposed a polytomous relationship for the following 7 families: Eurybrachidae, Flatidae, Issidae, Lophopidae, Nogodinidae, Ricaniidae, and Tropiduchidae. Nymphal characters also reveal a polytomous relationship of the Eurybrachidae, Gengidae, Hypothonellidae, Lophopidae, and Ricaniidae (Chen and Yang 1995). Sequence data in this study further confirm the relationship of the Eurybrachidae and Ricaniidae, although the positions of the remaining Flatidae, Issidae, and Nogodinidae cannot be precisely defined. The non-monophyletic resolution of the Issidae and Nogodinidae highlights the difficulty in defining their phylogenetic position. It has been proposed that the Issidae is a nonhomogenous group (Yeh et al. 1998, Emeljanov 1999, Gnezdilov 2003), although many key morphological characters such as the tegmina, wing length, clavus, corium, and aedeagus shape in issids can effectively define the subfamily classification (Chan and Yang 1994). A revision of the Caliscelidae was described by Emeljanov (1999), whereas efficient molecular sequence is needed to delimitate the category of issid subfamilies. Furthermore, according to the characters of the tegmina, more than 10 species of issids were revised into the Nogodinidae (Fennah 1984). Analyses of 16S rDNA sequences elucidate many phylogenetic relationships within the Fulgoroidea, although many questions remain unresolved and more research effort is needed.

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REFERENCES

- Asche M. 1988. Preliminary thoughts on the phylogeny of Fulgoromorpha (Homoptera: Auchenorrhyncha). *In* Proceedings of the 6th International Auchen. Meet. Turin, Italy: Univ. of Turin Press, pp. 47-53.
- Bourgoin T. 1993. Female genitalia in Fulgoromorpha (Insecta, Hemiptera): morphological and phylogenetical data. *Ann. Soc. Entomol. Fr.* **29**: 225-244.
- Bourgoin T, JD Steffen-Campbell, BC Campbell. 1997. Molecular phylogeny of Fulgoromorpha (Insecta, Hemiptera, Auchenorrhyncha). The enigmatic Tettigometridae: evolutionary affiliations and historical biogeography. *Cladistics* **13**: 207-224.
- Campbell BC, JD Steffen-Campbell, JT Sorensen, RJ Gill. 1995. Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences. *Syst. Entomol.* **20**: 175-194.
- Chan ML, CT Yang. 1994. Issidae of Taiwan (Homoptera: Fulgoroidea). Taipei: Chen Chung Book Press. 168 pp.
- Chen S, CT Yang. 1995. The metatarsi of the Fulgoroidea (Homoptera: Auchenorrhyncha). *Chinese J. Entomol.* **15**: 257-269.
- 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.
- Davis RE, TJ Kelly, EP Master, BS Thyagaraja, CA Rote, RB Imberski. 1994. Complete base sequence for the mitochondrial large subunit ribosomal RNA of the gypsy moth *Lymantria dispar* (L.). *Insect Mol. Biol.* **3**: 219-228.
- Denno RF, GK Roderick. 1990. Population biology of planthoppers. *Annu. Rev. Entomol.* **35**: 489-520.
- Dowton M, AD Austin. 1994. Molecular phylogeny of the insect order Hymenoptera: apocritan relationships. *Proc. Natl. Acad. Sci. USA* **91**: 9911-9915.
- Emeljanov AF. 1991. An attempt to construct a phylogenetic tree for planthoppers (Homoptera, Cicadina). *Entomol. Rev.* **70**: 24-28.
- Emeljanov AF. 1999. Notes on delimitation of families of the Issidae group with description of a new species of Caliscelidae belonging to a new genus and tribe (Homoptera, Fulgoroidea). *Zoosyst. Ross.* **8**: 61-72.
- Fang Q, WC Black IV, HD Blocker, RF Whitcomb. 1993. A phylogeny of New World *Deltocephalus*-like leafhopper genera based on mitochondrial 16S ribosomal DNA sequences. *Mol. Phylogenet. Evol.* **2**: 119-131.
- Fennah RG. 1984. Revisionary notes on the classification of the Nogodinidae (Homoptera, Fulgoroidea), with descriptions of a new genus and a new species. *Entomol. Mon. Mag.* **120**: 81-86.
- Gnezdilov VM. 2003. A new tribe of the family Issidae with comments on the family as a whole (Homoptera: Cicadina). *Zoosyst. Ross.* **11**: 305-309.
- Hamilton KGA. 1990. Homoptera: insects from the Santana formation, Lower Cretaceous, of Brazil. *Bull. Am. Mus. Nat. Hist.* **195**: 82-122.
- Han HY, BA McPherson. 1997. Molecular phylogenetic study of Tephritidae (Insecta: Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. *Mol. Phylogenet. Evol.* **7**: 17-32.
- Herdt RW. 1987. Equity consideration in setting priorities for third world rice biotechnology research. *Development Seeds Change* **4**: 19-24.
- Hypša V, DF Tietz, J Zrzavy, RO Rego, C Galvao, J Jurberg. 2002. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. *Mol. Phylogenet. Evol.* **23**: 447-457.
- Kambhampati S. 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA* **92**: 2017-2020.
- Kambhampati S, KM Kjer, BL Thorne. 1996. Phylogenetic relationships among termite families based on DNA sequence of mitochondrial 16S ribosomal RNA gene. *Insect Mol. Biol.* **5**: 229-238.
- Kendall MG. 1938. A new measure of rank correlation. *Biometrika* **30**: 81-93.
- Kumar S, K Tamura, M Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* **5**: 150-163.

- Marini M, B Mantovani. 2002. Molecular relationships among European samples of *Reticulitermes* (Isoptera, Rhinotermitidae). *Mol. Phylogenet. Evol.* **22**: 454-459.
- Muir F. 1923. On the classification of the Fulgoroidea (Hemiptera). *Proc. Hawaiian Entomol. Soc.* **5**: 205-247.
- O'Brien LB, SW Wilson. 1985. Planthopper systematics and external morphology. *In* LR Nault, JG Rodriguez, eds. *The leafhoppers and planthoppers*. New York: Wiley, pp. 61-102.
- Ribera I, DT Bilton, AP Vogler. 2003. Mitochondrial DNA phylogeography and population history of *Meladema* diving beetles on the Atlantic Islands and in the Mediterranean basin (Coleoptera, Dytiscidae). *Mol. Ecol.* **12**: 153-167.
- SAS Institute. 2001. PROC user's manual, version 6th. Cary, NC: SAS Institute.
- Shcherbakov DE. 1993. Geological history of Auchenorrhyncha. *In* Proceedings of the 8th International Auchenorrhyncha Congress. Delphi, Greece. pp. 3-4.
- Shcherbakov DE. 1996. Origin and evolution of the Auchenorrhyncha as shown by the fossil record. *In* T Say, ed. *Studies on Hemiptera phylogeny*. Lanham, Entomol. Soc. Am., pp. 31-45.
- Soulier AD, T Bourgoin, M Wilson. 1996. Are the Lophopidae a paraphyletic taxon? Proceedings of the 20th Congress of Entomology, Florence, Italy, 25-31 August. p. 485.
- Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Mol. Biol. Evol.* **9**: 678-687.
- Vogler AP, DL Pearson. 1996. A molecular phylogeny of the tiger beetles (Cicindelidae): congruence of mitochondrial and nuclear rDNA data sets. *Mol. Phylogenet. Evol.* **6**: 321-338.
- Whitfield JB, P Mardulyn, AD Austin, M Dowton. 2002. Phylogenetic relationships among microgastrine braconid wasp genera based on data from the 16S, COI, and 28S genes and morphology. *Syst. Entomol.* **27**: 337-359.
- Wilson SW, C Mitter, RF Denno, MR Wilson. 1994. Evolutionary patterns of hostplant use by delphacid planthoppers and their relatives. *In* RF Denno, TJ Perfect, eds. *Planthoppers: their ecology and management*. Chapman and Hall Press, pp. 7-113.
- Wilson SW, LB O'Brien. 1987. A survey of planthopper pests of economically important plants (Homoptera: Fulgoroidea). *In* MR Wilson, LR Nault, eds. *Proceedings of the 2nd International Workshop on Biotaxonomy, Classification, and Biology of Leafhoppers and Planthoppers (Auchenorrhyncha) of Economic Importance*. London: Commonwealth Institute of Entomology, pp. 343-360.
- Yeh WB, CT Yang, CF Hui. 1998. Phylogenetic relationships of the Tropiduchidae-group (Homoptera: Fulgoroidea) of planthoppers inferred through nucleotide sequences. *Zool. Stud.* **37**: 45-55.

APPENDIX I: Alignment of the fulgorooid 16S rDNA sequences. Secondary structure domains in the last line are based on previous models (see text)

81

Fu12	CGCC	TGTTT	ATCAA	AAACA	TGT	CCTT	TTGGAATTTAATTT-----	AAGG	TT	T-GGCC	T	GCTCAATGA-	---T--	-TTAAAT
Fu13	CGCC	TGTTT	ATCAA	AAACA	TGT	CCTT	TTGGAATTTAATTT-----	AAGG	TT	TGGGCC	T	GCTCAATGA-	---T--	-TTAAAT
Fu14	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTC	CAGGGTTTAATTTG-----	GAGG	TT	T-GGCC	T	GCTCAATGCA	---T--	AGTAAAT
Fu15	CGCC	TGTTT	ATCAA	AAACA	TGT	CCTT	TTGTAATTTAATTT-----	AAGG	TT	T-GACC	T	GCTCAATGA-	---T--	-TTAAAT
Dic1	CGCC	TGTTT	AACAA	AAACA	TCT	TCTT	-TAGTGTTTAATTA-----	AAGA	TA	T-AACC	T	GCTCAATGAT	---T--	TTTAAAT
Dic2	CGCC	TGTTT	ATCAA	AAACA	TCT	CTTT	-TTGGTTTAAATTT-----	----	--	A-AACC	T	GCTCAATGAA	---A--	TTTAAAT
Dic3	????	?????	?????	?????	???	????	????????????????????	????	??	???????	?	???????????	???????	?????????
Dic4	CGCC	TGTTT	ATCAA	AAACA	TCT	TCTT	-TAGTGTTTAATTA-----	AAGA	TA	T-AACC	T	GCTCAATGAT	---T--	TTTAAAT
Fla1	TGCC	TGTTT	AACAA	AAACA	TGT	CTTT	-TAGTGTTTAATTT-----	AAAG	TC	T-AACC	T	GCTCAATGAT	-TTA--	TTTAAAT
Fla2	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGAATTTGTTA-----	AAAG	TT	G-ATTC	T	GCTCAATGAT	---AA--	TTTAAAT
Fla3	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGGTTTAAATTT-----	AAAG	TC	T-AACC	T	GCTCAATGAA	---G--	TTTAAAT
Fla4	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGAATTTGTTA-----	AAAG	TT	G-ATTC	T	GCTCAATGAT	---AA--	TTTAAAT
De18	CGCC	TGTTT	ATCAA	AAACA	TTT	CTTT	-TAGTGTTTAATCTTA----	AAGG	TT	G-GGCC	T	GCTCAATGAA	---T--	ATTAAAT
Nog1	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGTGTTTAATGTTA-----	AAAG	TC	T-ATCC	T	GCTCAATGAT	-TA--	TTTAAAT
Nog2	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGGTTTTT-TTA-----	AAAG	TC	T-GGCC	T	GCTCAATGAA	-TAT--	TTTAAAT
Ach1	CGCC	TGTTT	ATCAA	AAACA	TGT	TTTT	-TTGTTTAAATTTAATTT----	AAGA	TT	G-GCCC	T	GCTCAATGAT	---A--	TTTAAAT
Ach2	CGCC	TGTTT	ATCAA	AAACA	TGG	TTTT	-TAGTGTTTAATTTA-----	AAGA	TC	A-GACC	T	GCTCAATGAA	-TAT--	TTTAAAT
Der1	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TTGATTTTAAATTT-----	AAGG	TT	T-TACC	T	GCTCAATGAT	----T	TTTAAAT
Der2	CGCC	TGTTT	ATCAA	AAACA	TGT	TATT	-TTGATTTTAAATTT-----	TAAT	AT	TTAACC	T	GCTCAATGAT	---AA-	TTTAAAT
Der3	CGCC	TGTTT	ATCAA	AAACA	TGT	TTTT	-TTGTTTAAATTTA-----	AAAG	AT	TAAATC	T	GCTCAATGAA	A-AA-	ATTAAAT
Der4	CGCC	TGTTT	ATCAA	AAACA	TGG	TTTT	-TAGTGTTTAATTT-----	AAAA	AT	T-TGGC	T	GCCCAATGA-	--T--T-	CTAAAT
Der9	CGCC	TGTTT	ATCAA	AAACA	TGT	TTTT	-TTGTTTAAATTTA-----	AAAG	AT	TAAATC	T	GCTCAATGAA	ATTAAT	ATTAAAT
Tro1	CGCC	TGTTT	ATCAA	AAACA	TGT	CGTC	-TTGATTTTAAATTT-----	GATC	TT	T-AACC	T	GCTCAATGAT	--TA--	TTTAAAT
Tro2	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTC	-TTGATTTTAAATTT-----	GAAG	TC	T-GGCC	T	GCTCAATGAT	---TA--	ATTAAAT
Tro3	CGCC	TGTTT	ATCAA	AAACA	TCT	CTTC	-ATGAATTTAATTT-----	GAAG	TC	A-GGCC	T	GCTCAATGAG	--T--	TTTAAAT
Tro4	CGCC	TGTTT	ATCAA	AAACA	T-T	CTTA	---GGTTTAAATTT-----	GAAG	TC	T-A-CC	T	GCTCAATGAT	--TA--	ATTAAAT
Ric1	????	?????	?????	?????	???	????	????????????????????	????	??	???????	?	???????????	???????	?????????
Ric3	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTTTAAATTT-----	AAAG	TC	T-GACC	T	GCTCAATGAT	---TA-	TTTAAAT
Ric4	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTTTTTTTTT-----	AAAG	TC	T-GGCC	T	GCTCAATGAG	-AAT--	TTTAAAT
Ric5	CGCC	TGTTT	ATCAA	AAACA	TGT	CCTT	-GAGAGTTTAAATTT-----	AAGG	TC	T-GTCC	T	GCTCAATGAA	TTTTTT	TTTAAAT
Eur1	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-GAGAGTTTAAATTT-----	AAAG	TT	T-GTCC	T	GCTCAATGAA	-TTTA-	TTTAAAT
Lop1	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGATTTTAAATTT-----	AAAG	TC	T-AACC	T	GCTCAATGAA	---T--	TTTAAAT
Tet2	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGAGTTTAAATTT-----	AAAG	TC	A-AACC	T	GCTCAATGAT	-AATT-	TTTAAAT
Iss1	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-GAGAGTTTAAATTT-----	AAGG	TC	T-GACC	T	GCTCAGTGAT	TTT--	ATTAAAT
Iss5	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGAGTTTAAATTT-----	AAAG	TC	T-GGCC	T	GCTCAATGAA	TT---	TTTAAAT
Iss9	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-GAGGGTTTAAATTT-----	AAAG	TC	T-GACC	T	GCTCAGTGAT	-TAA-	ATTAAAT
Iss10	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTTGTTTAAATTT-----	AAAG	TC	T-GGCC	T	GCTCAGTGAT	TTTTA-	ATTAAAT
Iss2	CGCC	TGTTT	AACAA	AAACA	TGT	CTTG	-TAGATTTTAAATTT-----	TAAG	TC	T-GTCC	T	GCTCAGTGAT	---T--	TTTAAAT
Iss11	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTG	-TAGTGTTTTAAATTT-----	TAAG	TC	T-ATCC	T	GCTCAGTGAT	---T--	TTTAAAT
Iss12	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTG	-TAGTGTTTTAAATTT-----	TAAG	TC	T-ATCC	T	GCTCAGTGAT	---T--	TTTAAAT
Iss3	CGCC	TGTTT	AACAA	AAACA	TGT	CTTT	-TAGTGTTTTAAATTT-----	AAGG	TC	A-AGCC	T	GCTCAATGAT	ATATT-	TTTAAAT
Iss14	CGCC	TGTTT	AACAA	AAACA	TGT	CCTT	-TAGTGTTTTAAATTT-----	AAGG	TC	T-AGCC	T	GCTCAATGAT	GTTAA-	TTTAAAT
Iss4	CACC	TGTTT	ATCAA	AAACA	TGT	CTTA	-AAAAAATTTAATTT-----	TAAG	TT	T-AACC	T	GCTCAATGAA	TTAT--	TTTAAAT
Iss6	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTA	-ATGTTAATTTAATTT-----	TAAG	TC	T-AGCC	T	GCTCAATGAA	TTGT--	TTTAAAT
Iss7	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTA	-ATGTTAATTTAATTT-----	TAGG	TC	T-AGCC	T	GCTCAATGAA	TTAT--	TTTAAAT
Iss8	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTG	-TAGTGTTTTAAATTT-----	TAAG	TC	T-ATCC	T	GCTCAATGAA	GTGATT	TTTAAAT
Cix2	CGCC	TGTTT	ATCAA	AAACA	TGT	TCTT	-TAGATTTTAAATTT-----	AAGA	TT	T-AGCC	T	GCTCTATGAT	-TTAA-	ATTAAAT
Cix3	CGCC	TGTTT	ATCAA	AAACA	TGT	TCTT	-TAGATTTTAAATTT-----	AAGG	TT	T-AACC	T	GCTCTATGAT	---GA--	TTTAAAT
Cix4	CGCC	TGTTT	ATCAA	AAACA	TGT	TCTT	-TAGATTTTAAATTT-----	AAGG	TT	T-AACC	T	GCTCTATGAT	--TA--	TTTAAAT
Cix6	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGATTTTAAATTT-----	AAGG	TT	T-AACC	T	GCTCTATGAA	--TA--	TTTAAAT
De12	CGCC	TGTTT	ATCAA	AAACA	TTT	CTTT	-CAGGTATTATATTTAATTTG	AAAG	TT	T-AACC	T	GCTCAATGAA	-TT-T-	TTTAAAT
De13	TGAC	TGTTT	ATCAA	AAACA	TTT	TCTT	-TTGAT-TTAATTTAA----	AAGG	TA	A-AACC	T	GCTCACTGAA	-TTTT-	TTTAAAT
De14	TGCC	TGTTT	ATCAA	AAACA	TGT	TCTT	-TAGGTTTTAATTT-----	AAGG	TC	T-GGCC	T	GCTCAATGAT	-TGAT-	TTTAAAT
De16	TGCC	TGTTT	ATCAA	AAACA	TGT	TCTT	-TAGGTTTTAATTT-----	AAGG	TT	T-GGCC	T	GCTCAATGAT	-TAAT-	TTTAAAT
De17	CGCC	TGTTT	ATCAA	AAACA	TTT	CTTT	-CAGGTATTATATTTAATTTG	AAAG	TT	T-AACC	T	GCTCAATGAA	-TT-T-	TTTAAAT
Fla5	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TTGTTTAAATTT-----	AAAG	TC	T-GACC	T	GCTCAATGAA	-TTA--	TTTAAAT
Mec2	TGAA	TGTTT	ATCAA	AAACA	TTT	CTTC	-ATGGGTTTTTTTT-----	GAAG	TA	T-TCCC	T	GCCCATGAA	-TTA-T	TTTAAAT
Mec3	TGAA	TGTTT	ATCAA	AAACA	TTT	CTTC	-ATGTTTTTTTTT-----	GAAG	TA	T-TCCC	T	GCTCTATGAA	-TTAGT	TTTAAAT
Mec6	TGAA	TGTTT	ATCAA	AAACA	TTT	CTTC	-ATGGGTTTTTTTT-----	GAAG	TA	T-TCCC	T	GCCCATGAA	-TTA-T	TTTAAAT
Cerc	CGCC	TGTTT	ATCAA	AAACA	TTT	TTTT	-TAGTGTTTAATTTA-----	AAAA	AT	TTAATC	T	GCCCAATGAT	---T--	TTTAAAT
Cica	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-TAGATTATATTTA-----	AAAG	TC	T-AATC	T	GCCCAATGAT	---T--	TTTAAAT
Cic1	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTGATTATATAA-----	AAAG	TA	T-TTTC	T	GCCCAATGAT	ATTTA-	TTTAAAT
Cic2	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTGCTTTTATA-----	AAAG	GT	G-AGTC	T	GCCCATGGG	---AT--	ACTAAAT
Cic3	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTGTGTGTTA-----	AAAG	GT	ATCTTC	T	GCCCATGAT	-TATT-	ATTAAAT
Cic4	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTAGTTTTTTTTTA-----	AAAG	TA	C-TTTC	T	GCCCATGGT	-TTGT-	TCATAAT

<65a> <---> <65b> <-> <66a><-----> <66b><> <67a>- - <---68a--> <----> <--68b-

APPENDIX I : (Cont.)

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Ful2 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT GA CG
 Ful3 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT GA CG
 Ful4 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GGGGTC TAGGATGAAG GGTTA GA CG
 Ful5 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT GA CG
 Dic1 AGC CGCAGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG GA CG
 Dic2 AGC CGCGGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG AA CG
 Dic3 ?GC CGCGGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG AA CG
 Dic4 AGC CGCAGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG GA CG
 Fla1 AGC CGCAGT ATATTG ACTGTG CGA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTG GA CA
 Fla2 AGC CGCAGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GATTT GA CG
 Fla3 AGC CGCAGT ATATTG ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTG GA CG
 Fla4 AGC CGCAGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GATTT GA CG
 Del8 AGC TGCAGT ATTTTA ACTGTA CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT GA CA
 Nog1 AGC CGCGGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG GA CG
 Nog2 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTG AA CG
 Ach1 AGC CGCGGT AATTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT GA CA
 Ach2 AGC CGCGGT AACTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA GA CA
 Der1 AGC CGCAGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA GA CG
 Der2 AGC CGCGGT ATTTTG ACCGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAA GGTTG GA CG
 Der3 AGC CGCGGT ATATTG ACCGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GATTT GA CG
 Der4 AGC CGCAGG AATTTA ACTGTA CAA AGG TAGCATAATA ATT A GTCTTT TAATT TGAGTC TTGTATGAAT GGTTT GA CG
 Der9 AGC CGCGGT ATATTG ACCGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GATTT GA CG
 Tro1 AGC CGCGGT AATTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG GA CG
 Tro2 AGC CGCGGT AATTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TAGAATGAAT GGTTG GA CG
 Tro3 AGC CGCGGT AATTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GGGGTC TTGAATGAAT GGTTA GA CG
 Tro4 AGC CGCGGT AATTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TAGAATGAAT GGTTG GG CG
 Ric1 ??? ?????? ?????? ?????G CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA GA CG
 Ric3 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTG GA CG
 Ric4 AGC CGCGGT ATTTTG ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA GA CG
 Ric5 AGC CGCGGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTA GA CG
 Eur1 AGC CGCAGT ATTTTG ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAA GGTTA GA CG
 Lopol AGC CGCAGT ATTTTA ACTGTG TAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTG AA TG
 Tet2 AGC CGCAGT ATTTTA ACTATA CAA AGG TAGCATAAATC ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA AA CG
 Iss1 AGC CGCGGT ATATTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TAGAATGAAT GGTTG AA CG
 Iss5 AGC CGCGGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGGATGAAT GGTTG AA CG
 Iss9 AGC CGCGGT ATATTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GGGGTC TAGAATGAAT GGTTA AA CG
 Iss10 AGC CGCGGT ATATTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGTATGAAT GGTTA AA CG
 Iss2 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGATT CA CG
 Iss11 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGATT CA CG
 Iss12 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGATT CA CG
 Iss3 AGC CGCGGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGGATGAAT GGTTA GA CG
 Iss14 AGC CGCGGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA AA CG
 Iss4 AGC CGCAGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT AAGGTC TTGAATGAAT GGTTG GA TG
 Iss6 AGC CGCAGT AATTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAAGTC TTGAATGAAA GGTTG GA TG
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 Iss8 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT AAAGTC TTGAATGAAT GGTTG GG TG
 Cix2 GGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTTCTT TAATT AGGATC TGGAAATGAAT GGTTG GA CA
 Cix3 GGC CGCGGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTCT TAATT AGGGTC TTGAATGAAT GGTTG GA CG
 Cix4 GGC CGCGGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTCT TAATT GGGGTC TTGAATGAAT GGTTG AA CG
 Cix6 GGC CGCGGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTCT TAATT AGGGTC TTGAATGAAT GGTTG GA CG
 Del2 AGC TGCAGT AATTTA ACTGTA CAA AGG TAGCATAAGTA ATT A GTCTTT TAATT GAGGTC TAGAATGAAT GGTTT AA CA
 Del3 AGC CGCAGT ATTTTA ACTGTA CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTT AA CA
 Del4 GGC CGCGGT AATTTA ACTGTG CTA AGG TAGCGTGATA ATT A GTCTTT TAATT GAGGTC TTGTATGAAT GGTTG GA CA
 Del6 AGC CGCGGT AATTTA ACTGTG CTA AGG TAGCGTAAATA ATT A GTCTTT TAATT GGGGTC TTGTATGAAT GGTTG GA CA
 Del7 AGC TGCAGT AATTTA ACTGTA CAA AGG TAGCATAAGTA ATT A GTCTTT TAATT GAGGTC TAGAATGAAT GGTTT AA CA
 Fla5 AGC CGCAGT ATATTG ACTGTG CGA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GGTTA AA CG
 Mee2 GGC CGCGGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GGTTT AA CA
 Mee3 GGC CGCGGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GGTTT AA CA
 Mee6 GGC CGCGGT ATTTTA ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TTGAATGAAT GGTTT AA CA
 Cerc GGC CGCAGT AATTTG ACTGTG CAA AGG TAGCATAATA ATT A GTCTTT TAATT AAAGTC TTGTATGAAT GATTG GA TG
 Cic1 GGC CGCGGT AAAGTC ACCGTG CGA AGG TAGCATAATA ATT A GTCTTT TAATT GAAGGC TTGAATGAAT GATTG GA TG
 Cic2 GGC TGCAGT ATTTTG ACTGTG CAA AGG TAGCATAAGTA ATT A GTTCTT TAATT AGAAGC TGGTATGAAT GAATT AA TG
 Cic3 GGC TGCAGT ATATTG ACTGTG CAA AGG TAGCATAAGTA ATT A GTTCTT TAATT GGAAGC TTGTATGAAT GGATG TA TG
 Cic4 GGC TGCAGT AGCTTG ACTGTG CAA AGG TAGCATAATA ATT A GTTTT TAATT GAAGGC TGGTATGAAT GGAAA TT TG
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APPENDIX I : (Cont.)

Ful2	CTATAGATCCT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTAATT	--TTAAAAATTTTAAATTAATAAAT	TT	TGTTG	GGG	TGAC
Ful3	CTATAGATCCT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTTATT	--TTAAAAATTTTATTAATTAATAAAT	TT	TGTTG	GGG	TGAC
Ful4	CTATAGATCCT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTTATT	--TTAGATTTTTTTTTGGTTATAAAAT	TT	TGCTG	GGG	TGGT
Ful5	CTATAGATCCT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTTATT	--TTAAAAATTTTATGAATTAATAAAT	TT	TGTTG	GGG	TGAC
Dic1	CTATAGATCCT	A	ATTTAAATTTATTTAAGTTTTTTTT--	TTGTTGCT	--TTTTAAATTTATTTAATTTAAAT	TT	GGTTG	GGG	CGAC
Dic2	CTATAGATCCT	G	AAITTTATTTAAATTAATTTATTTTT--	TTGTTGTTT	--TTATTAATTTATTTTAAATTAATTT	TT	AGTTG	GGG	CGAT
Dic3	CTATAGATCCT	A	AAITTTTATTTAATAATTTATTTTT--	TTGTTGGTTT	--TTTTTAATTTGAAGTAATCAATTT	TT	GGTTG	GGG	CGAT
Dic4	CTATAGATCCT	A	ATTTAAATTTATTTAAGATAAAAAT--	TTGTTGTTT	--TTTTTAATTTATTTAATTTAAATTT	TT	GGTTG	GGG	CGAC
Fla1	CTATAGATCCT	T	ATAATTCCTATTTTTTTGTTTTTT--	TTGTTGTTT	--TAAGATTTATTTATTTATGATTTAT	TT	AGTTG	GGG	TGAT
Fla2	CTATAGATCCT	T	AAAAAAAATATTTATTTTTCTTTT--	TTGTTGTTT	--TTAAAAATTAAGATATTTTTTTTT	TT	TGTTG	GGG	TGAC
Fla3	CTATAGATCCT	T	ATAATATTTATTTATTTAGTTTTT--	TTGTTGTTT	--TTTTTATTTAGTATTTTTTTTAT	TT	AGTTG	GGG	TGAT
Fla4	CTATAGATCCT	T	AAAAAAAATATTTATTTTTCTTTT--	TTGTTGTTT	--TTAAAAATTAAGATATTTTTTTTT	TT	TGTTG	GGG	TGAC
Del8	CTATAGATCCT	A	AAATTTTTTATATTTGAAAATAATTT--	TATGGGTTA	ATTTAAATTTTGGTTGGGAAAATTT	TT	TGTTG	GGG	TGAC
Nog1	CTATAGATCCT	T	AAAAAATATTTATTTATTTTTTTT--	TTGTTGTTT	--TTATTTTATTTATTTATTTTTTTT	TT	TGTTG	GGG	TGAT
Nog2	CTATAGATCCT	T	AAAAATGTTTTTTTTCTTTTTTT--	TTGATGTTT	--TTTTAGGAGAATTTTTCATTTTTT	TT	TGTTG	GGG	TGAT
Achl	???????????	?	???????????????????????????	??????????	????????TTTTTTAGGAAGTAAAAAT	TT	TGTTG	GGG	TGAC
Ach2	CTATAGATCCT	T	ATTATTAATTTTTATAAGATGTTTAG	TAGATTTTT	-----TTTTTTGTGAAAATTTATTA	TT	TGCTG	GGG	TGGC
Der1	CTATAGATCCT	T	ATTATTTGATTTTTTATTTTTTTG----	TTAATTTTTT	-----TATTTAATTTAATTAATAAT	TT	TATTG	GGG	TGAT
Der2	CTATAGATCCT	T	ATTATTTAGTTTTTATATTATTA----	TTATTTGTTT	--TTGATTTTTATAAATTTAAATAAT	TT	TATTG	GGG	TGAT
Der3	CTATAGATCCT	T	ATTATATTTATAATATTTATTTTTTT--	TTGTTGTTT	--TTATTTATATTATAAAATTTTTTT	TT	TATTG	GGG	TGAT
Der4	CTAAAGATCCT	T	ATTTAATAATTTATTTTAAITTTTT--	TTTAA----	--TTTTTTTATTTTAAITTTTTAAATTT	TT	GGTTG	GGG	TGAT
Der9	CTATAGATCCT	T	ATTATATTTATAATATTTATTTTTTT--	TTATTTGTTT	--TTTATTTATATTATAAAATTTAAT	TT	TATTG	GGG	TGAT
Tro1	CTATAGATCCT	T	ATTTTTATTTTTTTTTTTTTCTTTT--	TTGATGGTT	--TTTATTTAAATTTAATTTATAAAAT	TT	TGTTG	GGG	TGAC
Tro2	CTATAGATCCT	T	ATTTTTTTTATATTTTTTTTTTTTT--	TTGTTGATTT	--TTATATAAATTTAGTTTAAAAAATTT	TT	TGTTG	GGG	TGAT
Tro3	CTATAGATCCT	A	ATTAATTTTTAATATTTTTTTTTTT--	TTGTTGTTT	----TTTATTTGTTATTTAAATTTAAT	TT	TGTTG	GGG	TGAT
Tro4	CTATAGATCCT	T	ATTTTTTTT-CTATTTTTTTTTTTTT--	TTGTTGATTT	--TTATGTTATTTTAGTTTAAAAAATTT	TT	TGTTG	GGG	TGAT
Ric1	CTATAGATCCT	A	AAAAATTTACTTTTTTATTTTTTTTTT--	TTGTTGTTT	--TTTATTTAAATTTATTTATTTTTTT	TT	AGTTG	GGG	TGAT
Ric3	CTATAGATCCT	A	AAAATTTTATTTATTTATTTTTTTTT--	TTGTTGTTT	--TATTAATTTATTTATTTAATTTTTTT	TT	AGTTG	GGG	TGAT
Ric4	CTATAGATCCT	G	AGAAATTTTTTTTTTTTTTTTTTTTT--	TTGTTGTTT	--TTTTATTTGGAATTTTAAITTTTCT	TT	AGTTG	GGG	TGAT
Ric5	CTATAGATCCT	A	ATAATTTATTTTTTTTTTGTTTTTTT--	TTGTTGTGA	--TTAATATATTTAAAAATTTTTTTT	TT	TGTTG	GGG	TGAT
Eur1	CTATAGATCCT	T	ATAATAATTTTTTTTTTAAAAATTT--	TAGTTGTTT	--ATAAATTTAAAAATAAAAATTTTAA	TT	TGTTG	GGG	TGAT
Lop1	CTATAGATCCT	A	ATAATATAAATAAATAAATAAATTT--	TAGATTATA	--TATTGGTAATTTTTTTTTTTTTTAT	TT	TGTTG	GGG	TGAT
Tet2	CTATAGATCCT	T	ATAAATTAAGTTTATTTTTTTTTTT--	TTGAATTTT	--TTTTATATTAAATTTTTTAAATTTAT	TT	TGTTG	GGG	TGAT
lss1	CTGATAGATCCT	T	ATAATTTAAATAAATTTTATTTTTTTT	GTGTTGTTT	--TTTTTTGTTATTTATTTGATTTAT	TT	TGTTG	GGG	TGAC
lss5	CTATAGATCCT	T	ATAATAAAATTTTTTTTTTTTTTTTTT--	TTGATGTTT	--TTTTAAAAATTAATTTAAATTTAT	TT	TGTTG	GGG	TGAT
lss9	CTATAGATCCT	T	ATAATTTAAATAAATTTTATTTTTTT--	TTGTTGTTT	--TTTTTTGTTATTTATTTGATTTAT	TT	TGTTG	GGG	TGAC
lss10	CTGATAGATCCT	T	ATAAATTAAGACTATTTTTATTTTTT--	TTGTTGTCT	--TTTTTTGTAATTTTTTATTTTTTAT	TT	TGTTG	GGG	TGAT
lss2	CTATAGATCCT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGGTTATTTAAATTTATTTTTTT	TT	TGTTG	GGG	AGAT
lss11	CTATAGATCCT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGTTTATTTAAATTTAATTTTTTT	TT	TGTTG	GGG	AGAT
lss12	CTATAGATCCT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGTTTATTTAAATTTAATTTTTTT	TT	TGTTG	GGG	AGAT
lss3	CTATAGATCCT	A	AGAAAGTTTATTTTATTTTTTTTTTT--	TTGTTGTTT	--TTTTATTTAATATAAAATTTTTTTT	TT	TGTTG	GGG	TGAC
lss14	CTATAGATCCT	T	AAATAGGTTTATTTTATTTTTTTTTTT--	TTGTTGTTT	--TTAATTAATATTAATCTTTTTTTT	TT	TGTTG	GGG	TGAT
lss4	CTATAGATCCT	A	AAAATATTTATATTTATATATATTTT--	TTGTTGTTT	ATATTTATAAAAAATTTATATTTTTTT	TT	TGTTG	GGG	TGAC
lss6	CTATAGATCCT	A	AAAATATTTTTTTTTTTTAAITTTTT--	TTGTTGTTT	AAITTTTTTGTAAA-AAATTTATTTTT	TT	TGTTG	GGG	TGAC
lss7	CTATAGATCCT	A	AAAATATTTTTTTTTTTTAAITTTTT--	TTGTTGTTT	AAITTTTTTGAATTAATATTTATTTTT	TT	TGTTG	GGG	TGAC
lss8	CTATAGATCCT	T	AAAATTTTTTATATATTTTATTTTTTT--	TTGTTGTTT	--TTAATAAATTTTTAAATAGATTTTT	TT	TATTG	GGG	TGAT
Cix2	CTATAGATCCT	A	AAAATATTTTTTATATAAAATTTTTT--	TTGTTGGTAT	--TCITTTTTTTGTAGGAATTTATTTTT	TT	AGTTG	GGG	TGAT
Cix3	CTGATAGATCCT	T	ATAATTAATTAATAAATAAATTTTTT--	TGGTTGTTT	--TATTTTTTTTTTTAGTTAATTTAT	TT	TGTTG	GGG	TGAC
Cix4	CTGATAGATCCT	T	ATAATTAATTTAAAAATAAATTTTTT--	TGGTTGATA	--TTTTTTTTTTTTTAAATTAATTTTT	TT	TGTTG	GGG	TGCC
Cix6	CTGATAGATCCT	A	AAATATTTAATAAGTTTCAITTTTTT--	TTGTTGTTT	TTATGTTTCTTATTTTAAACTTTTTT	TT	TATTG	GGG	TGAT
Del2	CTTTAGATCCT	T	AAATTTTAAATGAAATAAATAATTTA-	ATGTTGGATT	--TAATTTTTTATTAATAAATAAATTT	TT	TGTTG	GGG	TGAC
Del3	CTATAGATCCT	A	AAITTTTTTTTATCTTAAITTTCT--	TGGTTAAATA	AAATGTTAAAAATTTAAAAAATAATTT	TT	TGTTG	GGG	TGAC
Del4	CTGATAGATCCT	T	AAAATTTGTTTTTTTTTAAITTTTTTT--	TTGTTTAAIT	--TAAITTTTTATTTTTTAAITTTTTTT	TT	GGTTG	GGG	TGAT
Del6	CTGATAGATCCT	T	AAAATTTGATTTTAAATAAATTTTTTT--	TTGTTTAAIT	--TAGTTTTTATTTTTTAAITTTTTTT	TT	GGTTG	GGG	TGAT
Del7	CTTTAGATCCT	T	AAATTTTAAATGAAATAAATAATTTA-	ATGTTGGATT	--TAATTTTTTATTAATAAATAAATTT	TT	TGTTG	GGG	TGAC
Fla5	CTATAGATCCT	A	AAAATATTTTTTTTATTTTTTTTTTTT	TTGTTGTTT	--TTTTAAAAATTTATCTTTTTATTTTT	TT	TGTTG	GGG	TGAT
Mee2	CTATAGATCCT	A	AAAAATAAATTTCTTATT-----	TGTTTTTTT	--TATTTATAAATTAATTTATTTATTT	TT	AGTTG	GGG	AGAT
Mee3	CTATAGATCCT	A	AAAAATAAATTTTTTATATT-----	TAGTTTTTTTT	--TTATTTTTTTAAAAATTTATTTTTTT	TT	AATTG	GGG	AGAT
Mee6	CTATAGATCCT	A	AAAAATAAATTTCTTATT-----	TGGTTTTTTTT	--TATTTATAAATTAATTTATTTATTT	TT	AGTTG	GGG	AGAT
Cerc	CTATAGATCCT	T	AAAATATTTTTTTTTTAAATTTTTTT--	AGAAITTTAT	--ATTTCTTATTTAAATTTATTTAATTTTT	TT	TGTTG	GGG	TGAT
Cica	CTATAGAACTT	G	AAATCTATAAATTTAAATTTTATT--	TAGATAAAT	--ATTAATTTAATAAATTTATGAAATTT	TT	TGTTG	GGG	TGAC
Cic1	CTATAGAACTT	T	ACTAAATATAAATTTAGTTGGTTTTT--	TTTTTTTATA	----TAAATTTATTTAAATTTTATTTT	TT	AGTTG	GGG	TGAC
Cic2	CTATAGAACTT	T	ACATC-TAATTTCTAGTTGATTTTT--	AACTTTTTAT	--ACTCTCTAGGGTTTTTATGATGA	TT	CGCTG	GGG	TGGT
Cic3	CTATAGAACTT	T	ATATTACCTAGTTTATAGTTTATTT--	TTGATTATA	--ATACTATCTTAAATTTACAGTACT	TT	TGCTG	GGG	TGGT
Cic4	CTATAGAACTT	T	ACATTTGTTGTTTTTAAATGAATTTTT	TACATTTAAA	--TATTTATTTAAITTTAAGACTTTG	TT	TGCTG	GGG	TGGT
	---74a----	>	<-----75a----->	<----->	<-----75b----->	>	<80a>	<->	<80b

APPENDIX I : (Cont.)

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 Fu15 AAGATACCTTAGGG ATAACAG CGTTATAAACTCTGGAGA GTTC-TTA TTGAT-AGATTTGTTTTCGG ACCTCGATG TTGGATTAATA GT
 Dic1 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-ATA TTGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
 Dic2 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-TTA TTGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA AT
 Dic3 TAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TAA TCAAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
 Dic4 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-ATA TTGAT-AGCTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
 Fla1 AAGATACCTTAGGG ATAACAG CATAATAAATCTGGAGA GTTC-TAA TCGAT-AGACTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Fla2 AAGATACCTTAGGG ATAACAG CATAAATTTAATTTGGAGA GTTC-AAA TCGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
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 Ach1 AAGATACCTTAGGG ATAACAG CGTTATATATTTGGAGA GTTC-AAA TTGAT-AAATATGTTTTCGG ACCTCGATG TTGGATTAATA TT
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 Der2 AAGATACCTTAGGG ATAACAG CGTTATATAACTGGAGA GTTC-TTA TTGAT-AGTTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Der3 TAGATACCTTAGGG ATAACAG CGTTATATAAATTTGGAAA GTTC-TTA TTGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
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 Tro1 AAGATACCTTAGGG ATAACAG CGTAATATATTTGGAGA GTTC-TTA TTGAT-AAATATTTTTCGG ACCTCGATG TTGGATTAATA TT
 Tro2 AAGATACCTTAGGG ATAACAG CGTAATTTAATTTGGAGA GTTC-ATA TTGAT-AAATATATTTTCGG ACCTCGATG TTGGATTAATA TT
 Tro3 AAGATACCTTAGGG ATAACAG CGTAATTTGTTTGGAGA GTTC-AAA TTGAT-AAATAAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Tro4 AAGATACCTTAGGG ATAACAG CGTAATTTAATTTGGAGA GTTC-ATA TTGAT-AAATAAATTTTCGG ACCTCGATG TTGGATTAATA TT
 Ric1 AAGATACCTTAGGG ATAACAG CGTTATTCACCTGGAGA GTTC-TAA TCGAT-AGTGAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Ric3 AAGATACCTTAGGG ATAACAG CGTTATTTGTTTGGAGA GTTC-TTA TTGAT-AAATGAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Ric4 AAGATACCTTAGGG ATAACAG CGTAATTTAATTTGGAGA GTTC-TTA TTGAT-AAATAAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Ric5 TAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TTA TCTAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
 Eur1 AAGATACCTTAGGG ATAACAG CGTTATATAATTTGGAGA GTTC-TAA TTGAT-AAATATGATTTCGG ACCTCGATG TTGGATTAATA TT
 Lop1 AAGATACCTTAGGG ATAACAG CGTTATTCATTTGGAAGA GTTC-ATA TCTGT-AAATGAGTTTTCGG ACCTCGATG TTGGATTAATA TA
 Tet2 CAGATACCTTAGGG ATAACAG CGTTATATAATTTGGAGA GTTC-AAA TTGAT-AAATAAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Iss1 AAGATACCTTAGGG ATAACAG CGTTATAGATTTCGGAGA GTTC-TTA TTGAT-AAATTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Iss5 AAGATACCTTAGGG ATAACAG CGTTATATAGTTGGAGA GTTC-AAA TTGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
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 Iss2 AAGATACCTTAGGG ATAACAG CGTTATAAACTCTGGAAA GTTC-TAA TTGAT-AGATTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Iss11 AAGATACCTTAGGG ATAACAG CGTTATAAAGTCTGGAAA GTTC-AAA TTTAT-AGATTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
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 Iss14 AAGATACCTTAGGG ATAACAG CGTAATATAATTTGGAGA GTTC-AAA TTGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA TT
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 Iss8 AAGATACCTTAGGG ATAACAG CGTTATAAATTTGGAGA GTTC-TAA TCAAT-AAATTTGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Cix2 AAGATACCTTAGGG ATAACAG CGTAATATAATTTGGAGA GTTC-TTA TTGAT-AGATATGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Cix3 AAGATACCTTAGGG ATAACAG CGTTATACATTTGGAAA GTTC-ATA TTGAT-AAATGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Cix4 AAGATACCTTAGGG ATAACAG CGTTATACATTTGGAAA GTTC-TTA TTGAT-AAATGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Cix6 AAGATACCTTAGGG ATAACAG CGTTATATAATTTGGAGA GTTC-TTA TTAAT-AAATATGTTTTCGG ACCTCGATG TTGGATTAATA TT
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 Fla5 AAGATACCTTAGGG ATAACAG CATAATAATAGTTGGAGA GTTC-AAA TCGAT-AACTAAGATTTCGG ACCTCGATG TTGGATTAATA ??
 Mee2 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGGA GTTC-ATA TTTAT-AAATTTGTTTTCGG ACCTCGATG TTGAATTAATA TT
 Mee3 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGGA GTTC-ATA TTTAT-AAATTTGTTTTCGG ACCACGATG TTGAATTAATA TT
 Mee6 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGGA GTTC-ATA TTTAT-AAATTTGTTTTCGG ACCTCGATG TTGAATTAATA TT
 Cerc AAGTTACCTTAGGG ATAACAG CGTGATTTAATTTGGAAA GTTC-ATA TTTAT-AAATAAGTTTTCGG ACCTCGATG TTGGATTAATA TT
 Caa1 AAGTTACCTTAGGG ATAACAG CGTTATTTAATTTAAAAA GTTC-TTA TTGATAAATTTAGATTTCGG ACCTCGATG TTGAATTAATA ??
 Cic1 AAGTTACCTTAGGG ATAACAG CGTAATTTTTCGGAAA GTTC-ACA TCTAT-ACTAATTTTTCGG ACCTCGATG TTGAATTAATA TA
 Cic2 TAGTTACCTTAGGG ATAACAG CGTAATTTTTCGGAAA GTTC-ATA TCTAT-A-TTAAATTTTTCGG ACCTCGATG TTGAATTAATA AA
 Cic3 AAGTTACCTTAGGG ATAACAG CGTAATTTTTCGGAAA GTTC-TTA TTTAT-A-TAAGTTTTCGG ACCTCGATG TTGAATTAATA AA
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