

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

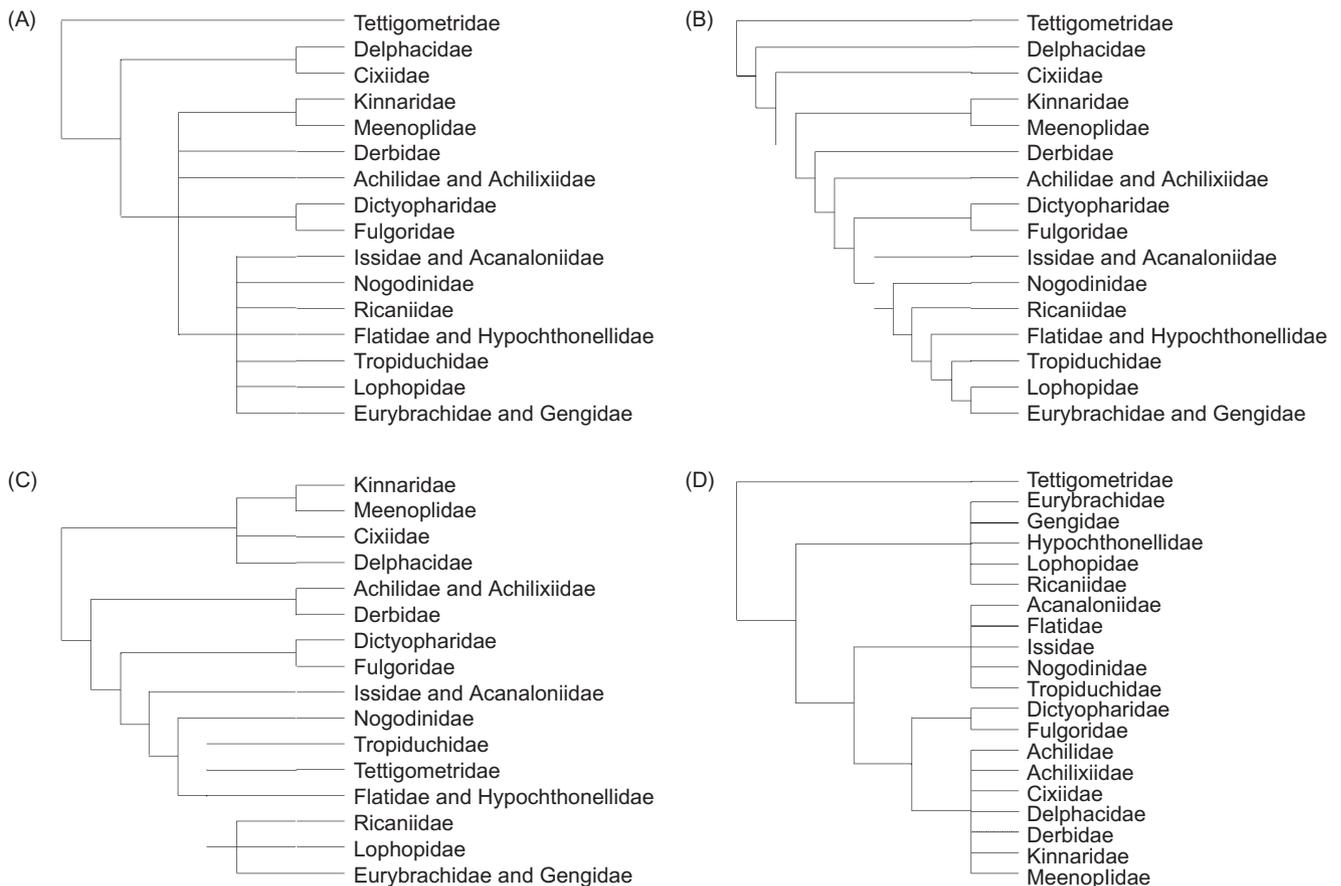


Fig. 1. Hypotheses of phylogenetic relationships of fulgoroid families. (A) Phylogenies proposed by Asche (1988), (B) Emeljanov (1991), (C) Bourgoin et al. (1993, 1997), and (D) Cheng and Yang (1995).

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 pachycephs</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 jocosus</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)
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
	Tettigometridae	Tettigometrinae	Tet2	<i>Tettigometra</i> sp.
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 Tropicuchidae 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'-CCGGTCTGAAC-TCAGATCA-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

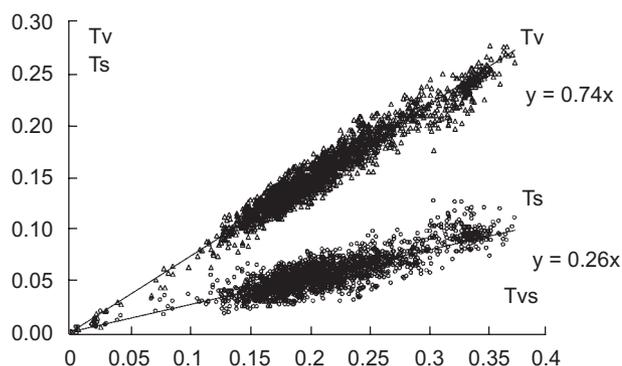


Fig. 2. Regression analysis of total substitutions (Tvs) versus transitions (Ts, \circ) and transversions (Tv, \triangle) in the 16S rDNA sequences of fulgoroids.

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).

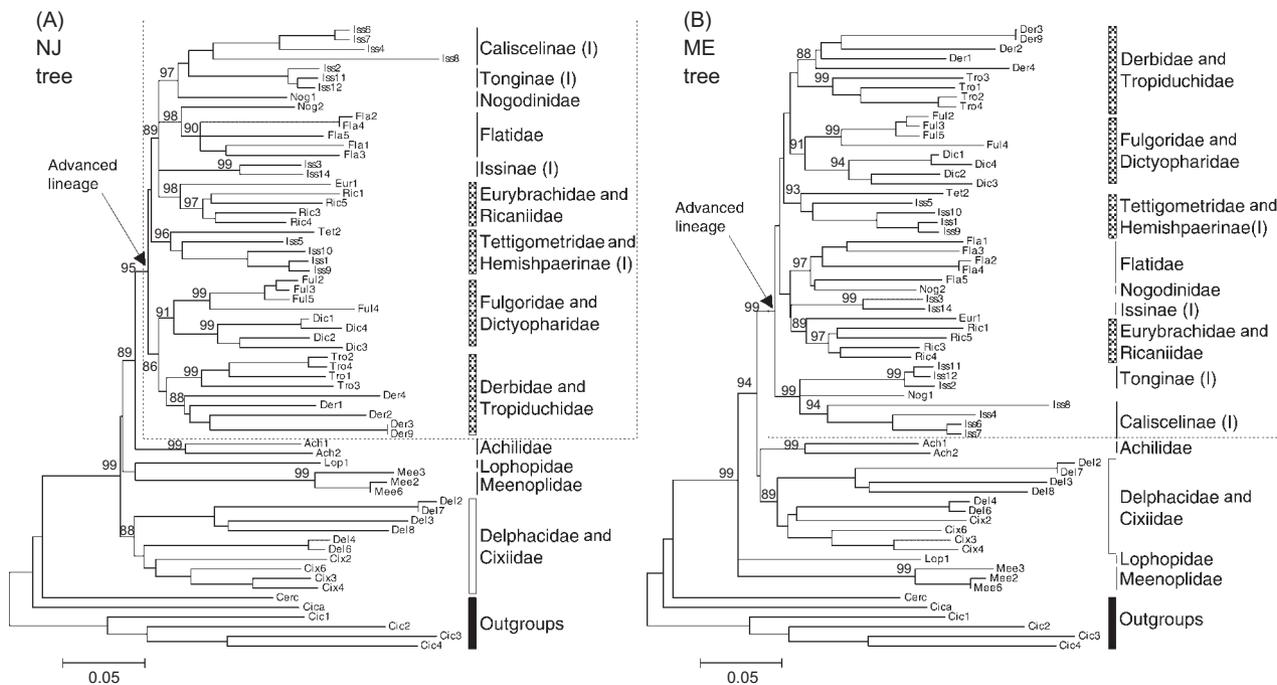


Fig. 3. Phylogeny of fulgoroids based on the partial mitochondrial 16S rDNA sequences by neighbor-joining (A) and minimum evolution (B) reconstructions based on the 3-parameter Tamura model. Bootstrap scores exceeding 80% from 1000 replications are given beneath the branches (not shown for branches below 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 subsfamily category.

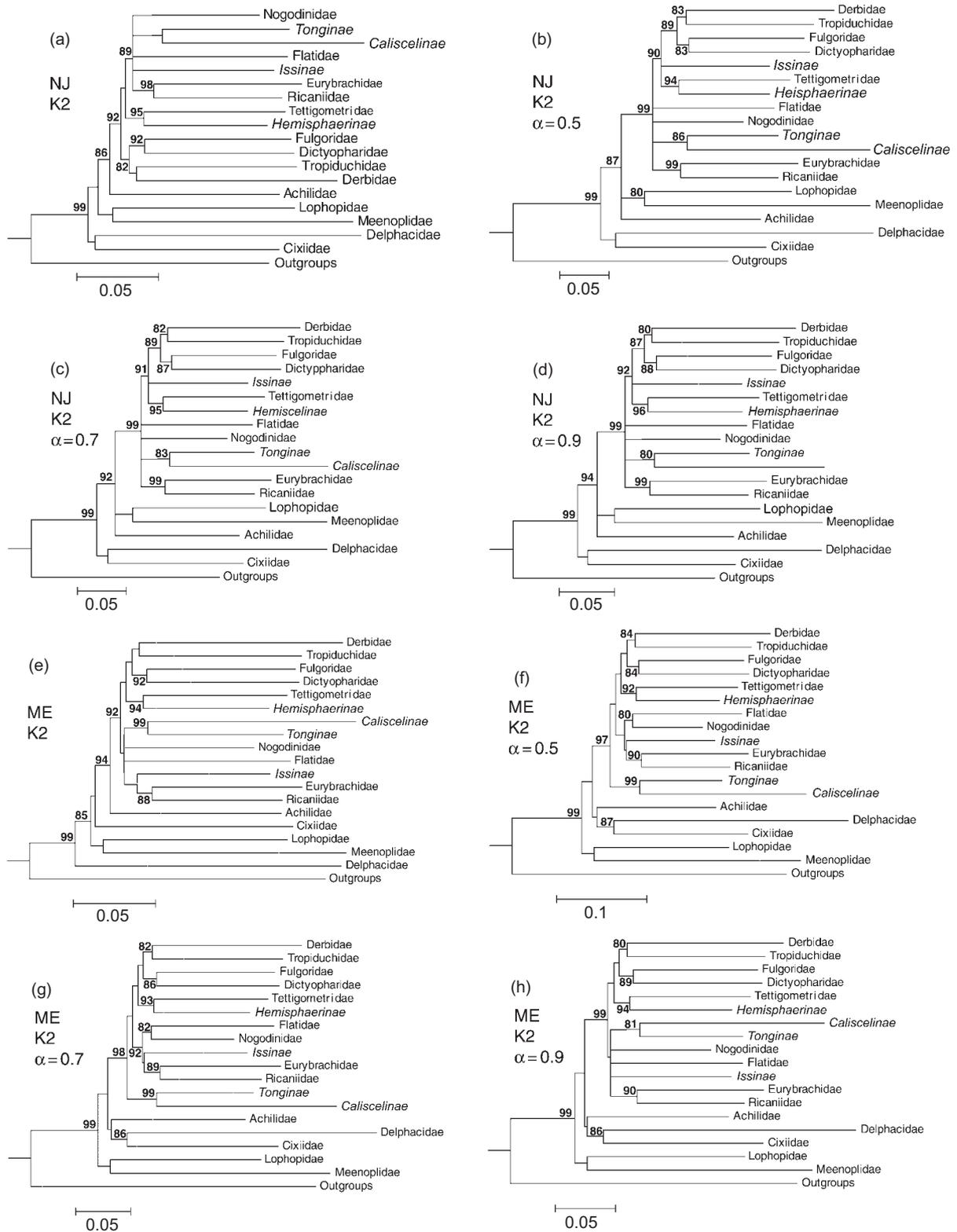


Fig. 4. Phylogenetic tree inferred from the partial mitochondrial 16S rDNA sequences by neighbor-joining (a-d) and minimum evolution (e-h) analyses based on the Kimura-2-parameter distance model. Correction for rate heterogeneity among sites with different values for the parameter α in the gamma distribution 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 Eurybrachidae. 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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APPENDIX I: Alignment of the fulgoroïd 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	CAGGGTTTAATTT-----	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--	ATTTAAAT
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--	ATTTAAAT
Der4	CGCC	TGTTT	ATCAA	AAACA	TGG	TTTT	-TAGTGTTTAATTT-----	AAAA	AT	T-TGCC	T	GCCCAATGA-	---T--	-CTAAAT
Der9	CGCC	TGTTT	ATCAA	AAACA	TGT	TTTT	-TTGTTTAAATTTA-----	AAAG	AT	TAAATC	T	GCTCAATGAA	ATTAAT	ATTTAAAT
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--	ATTTAAAT
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--	ATTTAAAT
Ric1	????	?????	?????	?????	???	????	????????????????????	????	??	???????	?	???????????	???????	???????
Ric3	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTGTTTAATTT-----	AAAG	TC	T-GACC	T	GCTCAATGAT	---TA--	TTTAAAT
Ric4	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTGTTTT-----	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--	ATTTAAAT
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--	ATTTAAAT
Iss10	CGCC	TGTTT	ATCAA	AAACA	TGT	CTTT	-AAGTGTTTAATTT-----	AAAG	TC	T-GGCC	T	GCTCAGTGAT	TTTTA--	ATTTAAAT
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--	ATTTAAAT
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	-ATGGGTTTTT-----	GAAG	TA	T-TCCC	T	GCCCATGAA	-TTA-T	TTTAAAT
Mec3	TGAA	TGTTT	ATCAA	AAACA	TTT	CTTC	-ATGTTTTTTTT-----	GAAG	TA	T-TCCC	T	GCTCTATGAA	-TTAGT	TTTAAAT
Mec6	TGAA	TGTTT	ATCAA	AAACA	TTT	CTTC	-ATGGGTTTTT-----	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	GCCCATGCGG	---AT--	ACTAAAT
Cic3	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTGTGTGTTA-----	AAAG	GT	ATCTTC	T	GCCCATGAT	-TATT--	ATTTAAAT
Cic4	CGCC	TGTTT	AACAA	AAACA	TTT	CTTT	-TTAGTTTTTAAATTT-----	AAAG	TA	C-TTTC	T	GCCCATGCGG	-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 AAATTA 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
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 Iss11 AGC CGCAGT ATTTTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGATT CA CG
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 Iss3 AGC CGCGGT AAATTA ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA GA CG
 Iss14 AGC CGCGGT ATTTTG ACTGTG CTA AGG TAGCATAATA ATT A GTCTTT TAATT GAGGTC TGGAAATGAAT GGTTA AA CG
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 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 GTTCTT TAATT GAAGGC TGGTATGAAT GGAAA TT TG
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APPENDIX I : (Cont.)

Ful2	CTATAGATCTT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTAATT	--TTAAAAATTTTAAATTAATAAAT	TT	TGTTG	GGG	TGAC
Ful3	CTATAGATCTT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTTATT	--TTAAAAATTTTATTAATTAATAAAT	TT	TGTTG	GGG	TGAC
Ful4	CTATAGATCTT	T	ATTTTTTCCATTTTTTTTTTAITTT--	TAGTTTATT	--TTAGATTTTTTTTTGGTTATAAAAT	TT	TGCTG	GGG	TGGT
Ful5	CTATAGATCTT	T	ATTTTATTTTTGATTTTTTTTTTT--	TAGTTTATT	--TTAAAAATTTTATGAATTAATAAAT	TT	TGTTG	GGG	TGAC
Dic1	CTATAGATCTT	A	ATTTAAATTTATTTAAGTTTTTTTT--	TTGTTGCTT	--TTTTAAATTTATTTAATTTAAAT	TT	GGTTG	GGG	CGAC
Dic2	CTATAGATCTT	G	AAITTTATTTAAATTAATTTATTTTT--	TTGTTGTTT	--TTATTAATTTATTTTAAATTAATTT	TT	AGTTG	GGG	CGAT
Dic3	CTATAGATCTT	A	AAITTTTTTATAAATTTATTTTT--	TTGTGGTTT	--TTTTTAATTTGAAGTAATCAATTT	TT	GGTTG	GGG	CGAT
Dic4	CTATAGATCTT	A	ATTTAAATTTATTTAAGATAAAAAT--	TTGTTGTTT	--TTTTTAATTTATTTAATTTAAATTT	TT	GGTTG	GGG	CGAC
Fla1	CTATAGATCTT	T	ATAATTCCTATTTTTTTGTTTTTT--	TTGTTGTTT	--TAAGATTTATTTATTTATGATTTAT	TT	AGTTG	GGG	TGAT
Fla2	CTATAGATCTT	T	AAAAAAAATATTTATTTTTCTTTT--	TTGTTGTTT	--TTAAAAATTAAGATATTTTTTTTT	TT	TGTTG	GGG	TGAC
Fla3	CTATAGATCTT	T	ATAATATTTATTTATTTAGTTTTT--	TTGTTGTTT	--TTTTTATTTAGTATTTTTTTTAT	TT	AGTTG	GGG	TGAT
Fla4	CTATAGATCTT	T	AAAAAAAATATTTATTTTTCTTTT--	TTGTTGTTT	--TTAAAAATTAAGATATTTTTTTTT	TT	TGTTG	GGG	TGAC
Del8	CTATAGATCTT	A	AAATTTTTTATATTTGAAAATAATTT--	TATGGGTTA	ATTTAAATTTTGTTTGGGAAAATTT	TT	TGTTG	GGG	TGAC
Nog1	CTATAGATCTT	T	AAAAAATTTATTTATTTTTTTTTT--	TTGTTGTTT	--TTATTTTATTTATTTATTTTTTTT	TT	TGTTG	GGG	TGAT
Nog2	CTATAGATCTT	T	AAAAATGTTTTTTTTCTTTTTTT--	TTGATGTTT	--TTTTAGGAGAATTTTTCATTTTTT	TT	TGTTG	GGG	TGAT
Achl	???????????	?	???????????????????????????	??????????	????????TTTTTTAGGAAGTAAAAAT	TT	TGTTG	GGG	TGAC
Ach2	CTATAGATCTT	T	ATTATTAATTTTTATAAGATGTTTAG	TAGATTTTT	-----TTTTTTGTGAAAATTTATTA	TT	TGCTG	GGG	TGGC
Der1	CTATAGATCTT	T	ATTATTTGATTTTTTATTTTTTTG----	TTAATTTTTT	-----TATTTAATTTAATTAATAAT	TT	TATTG	GGG	TGAT
Der2	CTATAGATCTT	T	ATTATTTAGTTTTTATATTATTA----	TTATTTGTTT	--TTGATTTTTATAAATTTAAATAAT	TT	TATTG	GGG	TGAT
Der3	CTATAGATCTT	T	ATTATATTTATAAATTTATTTTTTTT--	TTGTTGTTT	--TTATTTATTTATAAATTTAAATTT	TT	TATTG	GGG	TGAT
Der4	CTAAAGATCTT	T	ATTTAATAATTTATTTTAAITTTTT--	TTTAA----	--TTTTTTTATTTTAAITTTTTAAATTT	TT	GGTTG	GGG	TGAT
Der9	CTATAGATCTT	T	ATTATATTTATAAATTTATTTTTTTT--	TTATTTGTTT	--TTTATTTATTTATAAATTTAAATTT	TT	TATTG	GGG	TGAT
Tro1	CTATAGATCTT	T	ATTTTTTTTTTTTTTTTTTCTTTT--	TTGATGGTT	--TTTATTTAAATTTAATTTATAAATTT	TT	TGTTG	GGG	TGAC
Tro2	CTATAGATCTT	T	ATTTTTTTTATATTTTTTTTTTTTT--	TTGTTGATTT	--TTATATAAATTTAGTTTAAAAAATTT	TT	TGTTG	GGG	TGAT
Tro3	CTATAGATCTT	A	ATTAATTTTTTAATATTTTTTTTTT--	TTGTTGTTT	----TTTATTTGTTATTTAAATTTAAATTT	TT	TGTTG	GGG	TGAT
Tro4	CTATAGATCTT	T	ATTTTTTTT-CTATTTTTTTTTTTTT--	TTGTTGATTT	--TTATGTTATTTTAGTTTAAAAAATTT	TT	TGTTG	GGG	TGAT
Ric1	CTATAGATCTT	A	AAAAATTTTACTTTTTTATTTTTTTTTT--	TTGTTGTTT	--TTTATTTAAATTTATTTATTTTTTT	TT	AGTTG	GGG	TGAT
Ric3	CTATAGATCTT	A	AAAATTTTATTTATTTATTTTTTTTTT--	TTGTTGTTT	--TATTAATTTATTTATTTATTTTTTTT	TT	AGTTG	GGG	TGAT
Ric4	CTATAGATCTT	G	AGAAATTTTTTTTTTTTTTTTTTTTTT--	TTGTTGTTT	--TTTTATTTGGAATTTTAAITTTTCTT	TT	AGTTG	GGG	TGAT
Ric5	CTATAGATCTT	A	ATAATTTATTTTTTTTTTGTTTTTTTT--	TTGTTGTGA	--TTAATATATTTAAAAATTTTTTTTT	TT	TGTTG	GGG	TGAT
Eur1	CTATAGATCTT	-	ATAATAATTTTTTTTTTAAAAATTTT--	TAGTTGTTT	--ATAAATTTAAAAATAAAAATTTTAA	TT	TGTTG	GGG	TGAT
Lop1	CTATAGATCTT	A	ATAATATAAATAAATTAATAAATTTT--	TAGATTATA	--TATTGGTAATTTTTTTTTTTTTTAT	TT	TGTTG	GGG	TGAT
Tet2	CTATAGATCTT	T	ATAAATTAAGTTTATTTTTTTTTTTT--	TTGAATTTT	--TTTTATATTTAAATTTTTTAAATTTAT	TT	TGTTG	GGG	TGAT
lss1	CTGATAGATCTT	T	ATAATTTAAATAAATTTTATTTTTTTT	GTGTTGTTT	--TTTTTTGTTATTTATTTGATTTAT	TT	TGTTG	GGG	TGAC
lss5	CTATAGATCTT	T	ATAATAAAATTTTTTTTTTTTTTTTTT--	TTGATGTTT	--TTTTAAAAATTAATTTAAATTTAT	TT	TGTTG	GGG	TGAT
lss9	CTATAGATCTT	T	ATAATTTAAATAAATTTTATTTTTTTT--	TTGTTGTTT	--TTTTTTGTTATTTATTTGATTTAT	TT	TGTTG	GGG	TGAC
lss10	CTGATAGATCTT	T	ATAAATTAAGACTATTTTTATTTTTT--	TTGTTGTCT	--TTTTTTGTAATTTTTTATTTTTTAT	TT	TGTTG	GGG	TGAT
lss2	CTATAGATCTT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGGTTATTTAAATTTATTTTTTT	TT	TGTTG	GGG	AGAT
lss11	CTATAGATCTT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGTTTATTTAAATTTAATTTTTTT	TT	TGTTG	GGG	AGAT
lss12	CTATAGATCTT	T	AAAATTTTTTTTTTTTAAITTTTTTT--	TTGTTGTTT	--ATTGTTTATTTAAATTTAATTTTTTT	TT	TGTTG	GGG	AGAT
lss3	CTATAGATCTA	A	AGAAAGTTTATTTTATTTTTTTTTTTT--	TTGTTGTTT	--TTTTATTTAAATATAAATTTTTTTTT	TT	TGTTG	GGG	TGAC
lss14	CTATAGATCTT	T	AAATAGGTTTATTTTATTTTTTTTTT--	TTGTTGTTT	--TTAATTAATATTAATCTTTTTTTT	TT	TGTTG	GGG	TGAT
lss4	CTATAGATCTT	A	AAAATATTTATATTTATATATATTTT--	TTGTTGTTT	ATATTTATAAAAAATTTATATTTTTT	TT	TGTTG	GGG	TGAC
lss6	CTATAGATCTT	A	AAAATATTTTTTTTTTTATTTAAITTT--	TTGTTGTTA	AAITTTTTGTAATAAATTTATTTTTT	TT	TGTTG	GGG	TGAC
lss7	CTATAGATCTT	A	AAAATATTTTTTTTTTTATTTAAITTT--	TTGTTGTTA	AAITTTTTGTAATAAATTTATTTTTT	TT	TGTTG	GGG	TGAC
lss8	CTATAGATCTT	T	AAAATTTTTTATATATTTTATTTTTT--	TTGTTGTTT	--TTAATAAATTTTTAAATAGATTTTT	TT	TATTG	GGG	TGAT
Cix2	CTATAGATCTT	A	AAAATATTTTTTATATAAATTTTTT--	TTGTTGGTAT	--TCITTTTTTTGTAGGAATTTATTTT	TT	AGTTG	GGG	TGAT
Cix3	CTGATAGATCTT	T	ATAATTAATTAATAAATAAATTTTTT--	TGGTTGTTT	--TATTTTTTTTTTTAGTTAATTTAT	TT	TGTTG	GGG	TGAC
Cix4	CTGATAGATCTT	T	ATAATTAATTTAAAAATAAATTTTTT--	TGGTTGATA	--TTTTTTTTTTTTTAAATTAATTTTT	TT	TGTTG	GGG	TGCC
Cix6	CTGATAGATCTT	A	AAATATTTAATAAGTTTCAITTTTTT--	TTGTTGTTA	TTATGTTTCTTATTTTAAACTTTTT	TT	TATTG	GGG	TGAT
Del2	CTTTAGATCTG	T	AAATTTTAAATGGAATAAATAATTTA--	ATGTTGGATT	--TAATTTTTTATTAATAAATAAATTT	TT	TGTTG	GGG	TGAC
Del3	CTATAGATCTT	A	AAITTTTTTTTATCTTAAITTTCT--	TGGTTAAATA	AAATGTTAAAAATTTAAAAAATAATTT	TT	TGTTG	GGG	TGAC
Del4	CTGATAGATCTT	T	AAAATTTGTTTTTTTTTAAITTTTTT--	TTGTTTAAIT	--TAAITTTTTATTTTTTAAITTTTTT	TT	GGTTG	GGG	TGAT
Del6	CTGATAGATCTT	T	AAAATTTGATTTTAAATAAATTTTTT--	TTGTTTAAIT	--TAGTTTTTATTTTTTAAITTTTTT	TT	GGTTG	GGG	TGAT
Del7	CTTTAGATCTT	T	AAATTTTAAATGGAATAAATAATTTA--	ATGTTGGATT	--TAATTTTTTATTAATAAATAAATTT	TT	TGTTG	GGG	TGAC
Fla5	CTATAGATCTT	A	AAAATATTTTTTTTATTTTTTTTTTTT--	TTGTTGTTT	--TTTTAAAAATTTATCTTTTTATTTTT	TT	TGTTG	GGG	TGAT
Mee2	CTATAGATCTT	A	AAAAATAAATTTCTTATT-----	TGTTTTTTT	--TATTTATAAATTAATTTATTTATTT	TT	AGTTG	GGG	AGAT
Mee3	CTATAGATCTT	A	AAAAATAAATTTTTTATATT-----	TAGTTTTTTTT	--TTATTTTTTAAAAATTTATTTTTTT	TT	AATTG	GGG	AGAT
Mee6	CTATAGATCTT	A	AAAAATAAATTTCTTATT-----	TGGTTTTTTTT	--TATTTATAAATTAATTTATTTATTT	TT	AGTTG	GGG	AGAT
Cerc	CTATAGATCTT	T	AAAATATTTTTTTTTTAAATTTTTTT--	AGAAITTTAT	--ATTTCTTATTTAAATTTATTTAATTTTT	TT	TGTTG	GGG	TGAT
Cica	CTATAGAACTT	G	AAATCTATAAATTTTAAATTTTATT--	TAGATAAAT	--ATTAATTTTAAATAAATTTATGAAATTT	TT	TGTTG	GGG	TGAC
Cic1	CTATAGAACTT	T	ACTAAATATAAATTTAGTTGGTTTTT--	TTTTTTTATA	----TAAATTTATTTAAATTTTATTTT	TT	AGTTG	GGG	TGAC
Cic2	CTATAGAACTT	T	ACATC-TAATTTCTAGTTGATTTTT--	AACTTTTTAT	--ACTCTCTAGGGTTTTTATGATGTA	TT	CGCTG	GGG	TGGT
Cic3	CTATAGAACTT	T	ATATTACCTAGTTTATAGTTTATTT--	TTGATTATA	--ATACTATCTTAAATTTACAGTACTT	TT	TGCTG	GGG	TGGT
Cic4	CTATAGAACTT	T	ACATTTGTTGTTTTTAAATGAATTTTT	TACATTTAAA	--TATTTATTTAAITTTAAGACTTTGTT	TT	TGCTG	GGG	TGGT
	---74a----	>	<-----75a----->	<----->	<-----75b----->	>	<80a>	<->	<80b

APPENDIX I : (Cont.)

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 Fu14 AAGATACCTTAGGG ATAACAG CGTGATAAATCTGGAGA GTTC-ATA TTGAT-AGATTTGTTTGCG ACCTCGATG TTGGATTAATA AT
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 Dic1 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-ATA TTGAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
 Dic2 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-TTA TTGAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA AT
 Dic3 TAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TAA TCAAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
 Dic4 AAGATACCTTAGGG ATAACAG CGTTATTTAGTTGGAGA GTTC-ATA TTGAT-AGCTAAGATTGCG ACCTCGATG TTGGATTAATA TT
 Fla1 AAGATACCTTAGGG ATAACAG CATAAATAAATCTGGAGA GTTC-TAA TCGAT-AGACTTGTTTGCG ACCTCGATG TTGGATTAATA TT
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 Nog1 AAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TAA TTGAT-AGATATGTTTGCG ACCTCGATG TTGGATTAATA TA
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 Der3 TAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TTA TTGAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
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 Der9 TAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TTA TTGAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
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 Tro2 AAGATACCTTAGGG ATAACAG CGTAATAATACTCTGGAGA GTTC-ATA TTGAT-AAATATACTCTGGAGA ACCTCGATG TTGGATTAATA TT
 Tro3 AAGATACCTTAGGG ATAACAG CGTAATAATACTCTGGAGA GTTC-AAA TTGAT-AAATAAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Tro4 AAGATACCTTAGGG ATAACAG CGTAATAATACTCTGGAGA GTTC-ATA TTGAT-AAATAAATTTGCG ACCTCGATG TTGGATTAATA TT
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 Ric3 AAGATACCTTAGGG ATAACAG CGTTATTTGTTTGGAAGA GTTC-TTA TTGAT-AAATGAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Ric4 AAGATACCTTAGGG ATAACAG CGTAATAATACTCTGGAGA GTTC-TTA TTGAT-AAATAAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Ric5 TAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TTA TCTAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
 Eur1 AAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TAA TTGAT-AAATAAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Lop1 AAGATACCTTAGGG ATAACAG CGTTATTTCACTCTGGAGA GTTC-ATA TCTGT-AAATGAGTTTGCG ACCTCGATG TTGGATTAATA TA
 Tet2 CAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-AAA TTGAT-AAATAAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Iss1 AAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TTA TTGAT-AAATTTGTTTGCG ACCTCGATG TTGGATTAATA TT
 Iss5 AAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-AAA TTGAT-AACTAAGATTGCG ACCTCGATG TTGGATTAATA TT
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 Iss2 AAGATACCTTAGGG ATAACAG CGTTATAAATCTGGAGA GTTC-TAA TTGAT-AGATTTGTTTGCG ACCTCGATG TTGGATTAATA TT
 Iss11 AAGATACCTTAGGG ATAACAG CGTTATAAAGTTGGAGA GTTC-AAA TTTAT-AGATTTGTTTGCG ACCTCGATG TTGGATTAATA TT
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 Iss8 AAGATACCTTAGGG ATAACAG CGTTATAAATTTGGAGA GTTC-TAA TCAAT-AAATTTGTTTGCG ACCTCGATG TTGGATTAATA TA
 Cix2 AAGATACCTTAGGG ATAACAG CGTAATAATACTCTGGAGA GTTC-TTA TTGAT-AGATATGTTTGCG ACCTCGATG TTGGATTAATA TT
 Cix3 AAGATACCTTAGGG ATAACAG CGTTATACATTTGGAGA GTTC-ATA TTGAT-AAATGTTTGCG ACCTCGATG TTGGATTAATA TT
 Cix4 AAGATACCTTAGGG ATAACAG CGTTATACATTTGGAGA GTTC-TTA TTGAT-AAATGTTTGCG ACCTCGATG TTGGATTAATA TT
 Cix6 AAGATACCTTAGGG ATAACAG CGTTATATACTCTGGAGA GTTC-TTA TTAAT-AAATATGTTTGCG ACCTCGATG TTGGATTAATA TT
 De12 AAGATACCTTAGGG ATAACAG CATAAATAAATTTGTAATA GTTC-ATA TATAT-AAATTTGATTGCG ACCTCGATG TTGAATTAATA TA
 De13 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGTAATA GTTC-ATA TTGAT-AAATTTGATTGCG ACCTCGATG TTGAATTAATA TA
 De14 AAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TTA TTGAT-AAATTTAATTTGCG ACCTCGATG TTGGATTAATA TA
 De16 AAGATACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TTA TTGAT-AAATTTAATTTGCG ACCTCGATG TTGGATTAATA TA
 De17 AAGATACCTTAGGG ATAACAG CATAAATAAATTTGCAAAA GTTC-ATA TATAT-AAATTTGATTGCG ACCTCGATG TTGAATTAATA TA
 Fla5 AAGATACCTTAGGG ATAACAG CATAAATAAATTTGCAAAA GTTC-AAA TCGAT-AAATATGTTTGCG ACCTCGATG TTGGATTAATA ??
 Mee2 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGAGA GTTC-ATA TTTAT-AAATTTGTTTGCG ACCTCGATG TTGAATTAATA TT
 Mee3 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGAGA GTTC-ATA TTTAT-AAATTTGTTTGCG ACCACGATG TTGAATTAATA TT
 Mee6 AAGATACCTTAGGG ATAACAG CGTAATAAATTTGGAGA GTTC-ATA TTTAT-AAATTTGTTTGCG ACCTCGATG TTGAATTAATA TT
 Cerc AAGTTACCTTAGGG ATAACAG CGTGATTTAATTTGGAGA GTTC-ATA TTTAT-AAATAAGTTTGCG ACCTCGATG TTGGATTAATA TT
 Caa1 AAGTTACCTTAGGG ATAACAG CGTTATTTAATTTGGAGA GTTC-TTA TTGATAAATTTAGATTGCG ACCTCGATG TTGAATTAATA ??
 Cic1 AAGTTACCTTAGGG ATAACAG CGTAATTTTAGTTGGAGA GTTC-ACA TCTAT-ACTAATTTTGCG ACCTCGATG TTGAATTAATA TA
 Cic2 TAGTTACCTTAGGG ATAACAG CGTAATTTTATTTGGAGA GTTC-ATA TCTAT-A-TTAAATTTTGCG ACCTCGATG TTGAATTAATA AA
 Cic3 AAGTTACCTTAGGG ATAACAG CGTAATTTTATTTGGAGA GTTC-TTA TTTAT-A-TAAGTTTTGCG ACCTCGATG TTGAATTAATA AA
 Cic4 AAGTTACCTTAGGG ATAACAG CGTAATTTTATTTGGAGA GTTC-ATA TTTAT-A-TTGAATTTTGCG ACCTCGATG TTGAATTAATA AA
 <-----74b-----> <-----> <-----89a-----> <-----> <-----89b-----> <-----> <-----90a-----> <----->

APPENDIX I : (Cont.)

											569	
Ful2	--	TTCTGTGGG	GTAGGT	TTTACAATA	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ful3	--	TTCTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ful4	--	TTCTGTGGG	GCAGGT	TTTACAGTT	TA	GGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ful5	--	TTCTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Dic1	--	TTCTGTGGG	GTAGGT	TTTACAGTT	AA	GGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Dic2	--	TTCTGTGGG	GTAGGT	TTTACAGTT	TA	AGGTC	TGTTT	GACTT	ATAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Dic3	--	TTCTGTGGG	GTAGGT	TTTACAGTT	TA	AGGTC	TGTTT	GACTT	ATAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Dic4	--	TTCTGTGGG	GTAGGT	????????	??	????	????	????	????	????????????????	????	????
Fla1	T-	ATTTGTGGG	GTAGTT	TTTACAATT	TT	AGGTC	TGTTT	????	????	????????????????	????	????
Fla2	TA	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACC?	????	????????????????	????	????
Fla3	T-	ATTTGTGGG	GTAGTT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Fla4	TA	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Del8	A-	ATTTGTGGG	GCAGAA	GCTAGAATT	TT	AGGTC	TATAC	GACTT	TITAA	TATTACATGATCTGA	GTTCA	GACCCG
Nog1	A-	AGCTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Nog2	T-	TACTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ach1	--	AATTTGTGGG	GTAGGT	????????	??	????	????	????	????	????????????????	????	????
Ach2	T-	GTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Der1	--	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Der2	T-	ATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Der3	--	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Der4	--	ATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Der9	--	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Tro1	T-	TTTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Tro2	T-	TTTTGTGGG	GTAGGT	TTTACAATT	AT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Tro3	T-	GTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Tro4	T-	TTTTGTGGG	GTAGGT	TTTACAATT	AT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ric1	T-	GTTTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ric3	T-	TTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ric4	T-	GTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Ric5	T-	GTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Eur1	T-	GTTTGTGGG	GTAGGT	TTTACAATT	CT	AGGTC	TGTTT	????	????	????????????????	????	????
Lop1	A-	ATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACC?	????	????????????????	????	????
Te12	T-	AATTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss1	T-	TACTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss5	T-	TACTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss9	T-	TACTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss10	T-	TACTGTGGG	GTAGGT	TTTACAGTT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss2	T-	TACTGTGGG	GTAGGT	TTTACAGTT	GT	GGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss11	A-	TTTTGTGGG	GTAGGT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAT	AGTTACATGATCTGA	GTTCA	GACCCG
Iss12	T-	TACTGTGGG	GTAGGT	TTTACAGTT	GT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss3	T-	AATTTGTGGG	GCAGTT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss14	T-	AATTTGTGGG	GCAGTT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Iss4	A-	ATTTGTGGG	GTAGAT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	AATTACATGATCTGA	GTTCA	GACCCG
Iss7	A-	ATTTGTGGG	GTAGAT	TTTACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	AATTACATGATCTGA	GTTCA	GACCCG
Iss8	A-	TTTTGTGGG	GTAGGT	????????	??	????	????	????	????	????????????????	????	????
Cix2	A-	ATAATTGGG	GCAGAA	GATTATTTA	TT	AGGTC	TGTTT	GACTT	TAAAA	TCTTACATGATCTGA	GTTCA	GACCCG
Cix3	T-	AATTTGTGGG	GCAGAA	TATACAATA	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Cix4	T-	AATTTGTGGG	GCAGAA	TATACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Cix6	T-	AATTTGTGGG	GCAGAA	TATACAATT	TT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Del2	G-	AATTTGTGGG	GCAGAA	AA??????	??	????	????	????	????	????????????????	????	????
Del3	A-	AATTTGTGGG	GCAGAA	ACTTAAAGT	TT	AGGTC	TGTTT	GACTT	TAAAT	TTTTACATGATCTGA	GTTCA	GACCCG
Del4	A-	GTTTTTGGT	GTAGAT	GATAATTAA	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Del6	A-	GTTTTTGGT	GTAGAT	GATAATTAA	CT	AGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Del7	G-	AATTTGTGGG	GCAGAA	AA??????	??	????	????	????	????	????????????????	????	????
Fla5	??	????????	????	????????	??	????	????	????	????	????????????????	????	????
Mee2	T-	TGGGAGGGG	GAAGGT	TTTTCTAT	AT	TGGTC	TGTTT	????	????	????????????????	????	????
Mee3	T-	TAGGAAGGG	GAAGGT	TTTTCTAT	AT	TGGTC	TGTTT	????	????	????????????????	????	????
Mee6	T-	TGGGAGGGG	GAAGGT	TTTTCTAT	AT	TGGTC	TGTTT	GACTT	TAAAA	TTTTACATGATCTGA	GTTCA	GACCCG
Cerc	A-	GAATAAGGG	GTAGGT	TTTTTA???	??	????	????	????	????	????????????????	????	????
Cica	??	????????	????	????????	??	????	????	????	????	????????????????	????	????
Cic1	A-	AAAAAAGAA	GCAG??	????????	??	????	????	????	????	????????????????	????	????
Cic2	A-	GATTAAGAA	GCAGAA	TTCTTAAGT	CT	AACTT	TAAAA	TCCT?	????	????????????????	????	????
Cic3	--	TAGTTTGGG	TCTGTT	CGACTTTTA	AA	TTCTT	ACTTG	ATCT?	????	????????????????	????	????
Cic4	AA	TTTTTAGAG	TCTGTT	CGACTTTTA	AA	TCCCT	ACTTG	ATCT?	????	????????????????	????	????
	->	<-91a->	<----->	<-91b->	<>	<92a>	<----->	<92b>	<----->	<-----90b----->	<----->	<93a->