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Identification of two sibling species, Ephemera formosana and E. sauteri (Ephmeroptera: Ephemeridae), based on Micochondrial DNA sequence analysis【Research report】

從粒腺體去氧核糖核酸序列鑑定蜉蝣屬(蜉蝣目：蜉蝣科). Ephemera formosana及E. sauteri【研究報告】

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Abstract

Ephemera formosana and E. saiteri are the only 2 recorded species of Ephmeri in Taiwan. They are sibling species that are not easily distinguished from each other using only morphological characters. Therefore, we sued PCR and DNA sequencing methods to obtain the nucleotide sequences of the mitochondrial 16S rDNA gene yo identify the species of Ephemera in Taiwan. Sequence comparison of the 486 nases at the 3'-end of the gene from 7 E. formosana and 4 E. sauteri specimens (Ef1, Ef5) were different from formosana and sauteri. The nucleotide sequence divergence among the specimens examined tanged from 1.65% to 3.5%, which was distant enough to consider Ef1 and Ef5 (from Sofong, Hualien Co. and Pahsienshan, Tichung Co., respectively) to be another 2 species. Multidimnsional scaling analysis also confirmed that Ef1, Ef5, E. formosana and E. sauteri were far away one another. Nucleotide data also suggested that the specimen Ef4 from Lona (Nantou Co.), formerly described as a member of E. formosana, should be a member of E. sauteri as it was grouped consistently with the members of E. sauteri in both phylogenetic reconstruction and multidimentional scaling analysis. Furthermore, since both adults and nymphs of the same species from different localities were consistently grouped together, the molecular apprach is good for both nymphal and adult classification.

摘要

Ephemera formosana及E. sauteri為同胞種（sibling species）蜉蝣，也是台灣目前記錄中僅有的2種Ephemera蜉蝣，其形態極難區分，因此本研究運用PCR的技術取得粒線體核糖體基因的去氧核糖核酸序列，鑑定台灣的（Ephemera）昆蟲。從該基因3’ 端486個鹽基比對來自全省各地11個標本的結果顯示，除了E. formosana及 E. sauteri外，可能有另外兩個種也分佈在台灣，其中一個採自花蓮縣壽豐鄉（Ef1），另一個採自台中縣八仙山（Ef5），因為此二標本與其它標本之間的差異範圍1.65 % -3.5 % 已遠超過種內變異；多尺度空間分析亦顯示Ef1, Ef5, E. formosana及E. sauteri彼此分離，各成一群。類緣分析及多尺度空間分析的結果可知，採自南投縣羅娜的標本Ef4,原本列為E. formosana一員，應該是E. sauteri種類，因其去氧核糖核酸序列與E. sauteri一群相似。另外，不同時期採自不同地點的標本，無論是成蟲或稚蟲，同種類的成員分別聚類在一起，據此而推，核酸序列的數據應可運用於成蟲及稚蟲分類體系的結合。

Key words: mayfly, Ephemera, mitochondrial DNA, 16S rDNA.

關鍵詞: 蜉蝣、蜉蝣屬、粒線體去氧核糖核酸、16S去氧核糖核酸。

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Identification of Two Sibling Species, *Ephemera formosana* and *E. sauteri* (Ephemeroptera: Ephemeridae), Based on Mitochondrial DNA Sequence Analysis

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ABSTRACT

Ephemera formosana and *E. sauteri* are the only 2 recorded species of *Ephemera* in Taiwan. They are sibling species that are not easily distinguished from each other using only morphological characters. Therefore, we used PCR and DNA sequencing methods to obtain the nucleotide sequences of the mitochondrial 16S rDNA gene to identify the species of *Ephemera* in Taiwan. Sequence comparison of the 486 bases at the 3'-end of the gene from 7 *E. formosana* and 4 *E. sauteri* specimens showed that 2 specimens (*Ef1, Ef5*) were different from *formosana* and *sauteri*. The nucleotide sequence divergence among the specimens examined ranged from 1.65% to 3.5%, which was distant enough to consider *Ef1* and *Ef5* (from Sofong, Hualien Co. and Pahsienshan, Taichung Co., respectively) to be another 2 species. Multidimensional scaling analysis also confirmed that *Ef1, Ef5, E. formosana*, and *E. sauteri* were far away from one another. Nucleotide data also suggested that the specimen *Ef4* from Lona (Nantou Co.), formerly described as a member of *E. formosana*, should be a member of *E. sauteri* as it was grouped consistently with the members of *E. sauteri* in both phylogenetic reconstruction and multidimensional scaling analysis. Furthermore, since both adults and nymphs of the same species from different localities were consistently grouped together, the molecular approach is good for both nymphal and adult classifications.

Key words: mayfly, *Ephemera*, mitochondrial DNA, 16S rDNA.

Introduction

Ephemera formosana and *E. sauteri* are 2 closely related species. The major method capable of classifying the sibling species is based on the locality elevation. *E. formosana* lives at low elevations (<1000 m) and *E. sauteri* lives in high mountains (Kang and Yang, 1994). It is rather difficult to distinguish them using either nymphal features or imaginal characters due to similar features on nymphs and overlapping characters on imagos. Minor variable morphological characters from nymphs can also be considered in distinguishing these 2 species; (1) in dorsal view, anterior gills distinctly larger than posterior ones in *E. sauteri* but

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subequal in *E. formosana*; (2) markings of terga; (3) frontal process of head; (4) molar of left mandible; and (5) canine teeth of maxillae (Kang and Yang, 1994).

Using morphological characters is the most convenient identification tool available but it also has shortcomings since the above characters are subject to environmental changes (Kang, pers. comm.). Alternative information indicates that the locality elevation is not an absolute criterion to distinguish *E. formosana* and *E. sauteri*. Genetic markers have been widely used for identification in insect populations (Hall and Smith, 1991; Bogdanowicz *et al.*, 1993; Vanlerberghe-Masutti, 1994; Tang *et al.*, 1995; Erney *et al.*, 1996; Estoup *et al.*, 1996; King *et al.*, 1996; Mcmichael and Hall, 1996). The aim of this study is to use molecular characters to identify *Ephemera* sibling species. Another motivation is to combine mayfly classification systems of nymphs and adults using molecular data, since each of these systems alone would result in the phenomenon of synonym.

Materials and Methods

DNA Isolation, Amplification, and Sequencing

Eleven specimens were studied including 7 *Ephemera formosana* (*Ef*) and 4 *Ephemera sauteri* (*Es*). The collection information which includes abbreviated number, locality, adult or nymph, and elevation is given in Table 1. Live insects were collected, and preserved in 70% alcohol at room temperature. Entire insect body or abdomen only (about 0.1 gram) was homogenized by glass homogenizer in 500 μl digestion buffer which contained 100 mM Tris-Cl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.5% SDS, 50 mM dithiothreitol, and 0.5 mg / l proteinase K. The mixture was left at 50°C overnight. A DNA template was generated using phenol-chloroform extraction of total nucleic acids (modified from Jean *et al.*, 1994). 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 DNA template in the following amplification reaction.

The polymerase chain reaction (PCR) was employed to amplify the part of the 3'end sequence of the mitochondrial 16S rDNA gene. The primers used to amplify the region were 5'-GCCTGTTTATCAAAAACAT-3' and 5'-CCGGTCTGAACTCAGATCA-3' which were designed according to the conserved nucleotide sequences of 16S rDNA of *Lymantria dispar* (Davis *et al.*, 1994), *Locusta migratoria, Drosophila yakuba, D. melanogaster, Aedes albopictus, Anopheles gambiae,* and *Apis mellifera*. The locations of the 2

Table 1. Locality data for mayfly sibling species of *Ephemera formosana* and *E. sauteri*

Species	Specimen	Locality	Elevation (m)	Date
	Ef1 (N*)	Sofong (Hualien Co.)	85	03-04-1991
	Ef2 (A)	Pahsienshan (Taichung Co.)	700	31-03-1990
Ephemera	*Ef3* (N)	Hsincheng (Hualien Co.)	90	01-04-1990
formosana	*Ef4* (A)	Lona (Nantou Co.)	750	19-05-1990
	Ef5 (A)	Pahsienshan (Taichung Co.)	700	31-03-1990
	Ef6 (A)	Pinglin (Taipei Co.)	365	31-05-1991
	Ef7 (A)	Pinglin (Taipei Co.)	365	31-05-1991
Ephemera	*Es1* (N)	Fenchihu (Chiayi Co.)	1,585	08-02-1991
sauteri	*Es2* (A)	Nanhutashan (Ilan Co.)	2,450	06-07-1991
	Es3 (N)	Nanhutashan (Ilan Co.)	2,900	10-05-1990
	Es4 (N)	Shoulin (Hualien Co.)	2,200	27-01-1991

*N: nymph; A: adult.

primers correspond to nucleotides 13416-13396 and 12866-12884, respectively, of the 16S rDNA gene of *Drosophila yakuba* (Clary and Wolstenholme, 1985). The amplification was conducted at 39 cycles in a final volume of 100 μl containing 100 mM Tris-Cl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton-X100, 2 units of SuperTaq polymerase (HT Biotechnology LTD), 0.2 mM of each dNTP, 20 pmoles of each primer, and a 2-μl DNA template. The reaction was carried out with the following temperature profile: denaturation for 50 s at 95℃, annealing for 1 min at 50℃, and extension for 2 min at 72℃ (RoboCycler gradient 96 Temperature Cycler). After electrophoresis, target DNA was recovered from the gel by Nucleotrap Kit, and DNA products were sequenced directly using the PCR Sequencing Kit (Perkin Elmer) for 29 cycles with the following temperature profile: 50 s for denaturation at 95℃, annealing at 50℃ and extension at 72℃.

DNA Analyses

The partial 16S rDNA gene sequences were aligned using the Pileup program of the GCG software package (Genetic Computer Group, version 7.0) (Devereux *et al.*, 1991) and checked by eye. Aligned nucleotide sequences were analyzed using the MEGA program (Kumar *et al.*, 1993) for calculating (1) the proportions of nucleotide compositions of each specimens, and (2) the total substitution proportions and the ratio of transitions over transversions between all paired sequences.

Phylogenetic analysis was performed by the Neighbor-Joining (Saitou and Nei, 1987), UPGMA, and Maximum Parsimony methods as implemented in the MEGA program. In the clustering method, the proportion distances and the pairwise distance estimates were used based on the models of Jukes and Cantor (1969), Tamura (1992), Kimura 2-parameter (1980), and Tamura and Nei (1993). Bootstrap analysis was performed 1000 replications in the clustering method.

It is possible to find a representation of the taxa in few dimensions such that the interitem proximities would nearly match the original distances (Shepard, 1980). Multidimensional scaling techniques deal with a set of distances between every pair of taxa items representing their space distribution in 2 dimensions. The proportional pairwise distances of the 16S rDNA sequence were performed in multidimensional scaling analysis to infer the relative distribution of mayflies in 2 dimensions with the NTSYS-pc program (Rohlf, 1993).

Results

DNA Sequence Compositions

The partial 16S rDNA gene sequence data for all 11 individual insects are presented in Fig. 1. There are no gaps in the sequences and 459 of the 486 sites used in analysis are constant. Of 27 varied sites, 11 sites are informative in parsimony analysis. The average nucleotide compositions of guanine, adenine, thymine, and cytosine are 22%, 33.8%, 32.5%, and 11.7%, respectively. The proportion of adenine is similar to that of thymine, while the proportion of guanine is 2 times that of cytosine.

The ratio of transitions over transversions (Table 2) suggests that transition substitution evolved faster than did transversion substitution. The substitution proportions (Table 2) suggest the specimen, *Ef4*, from Lona (Nantou Co.), should be classified into the species of *Ephemera sauteri* since the nucleotide divergences between *Ef4* and the other members of *E. formosana* (*Ef1*~*Ef7*) are greater than the divergences between *Ef4* and the members of *E. sauteri* (*Es1*~*Es4*). While the average nucleotide divergence between *E. sauteri* and *E. formosana* is 2.15%, that among the members of *E.*

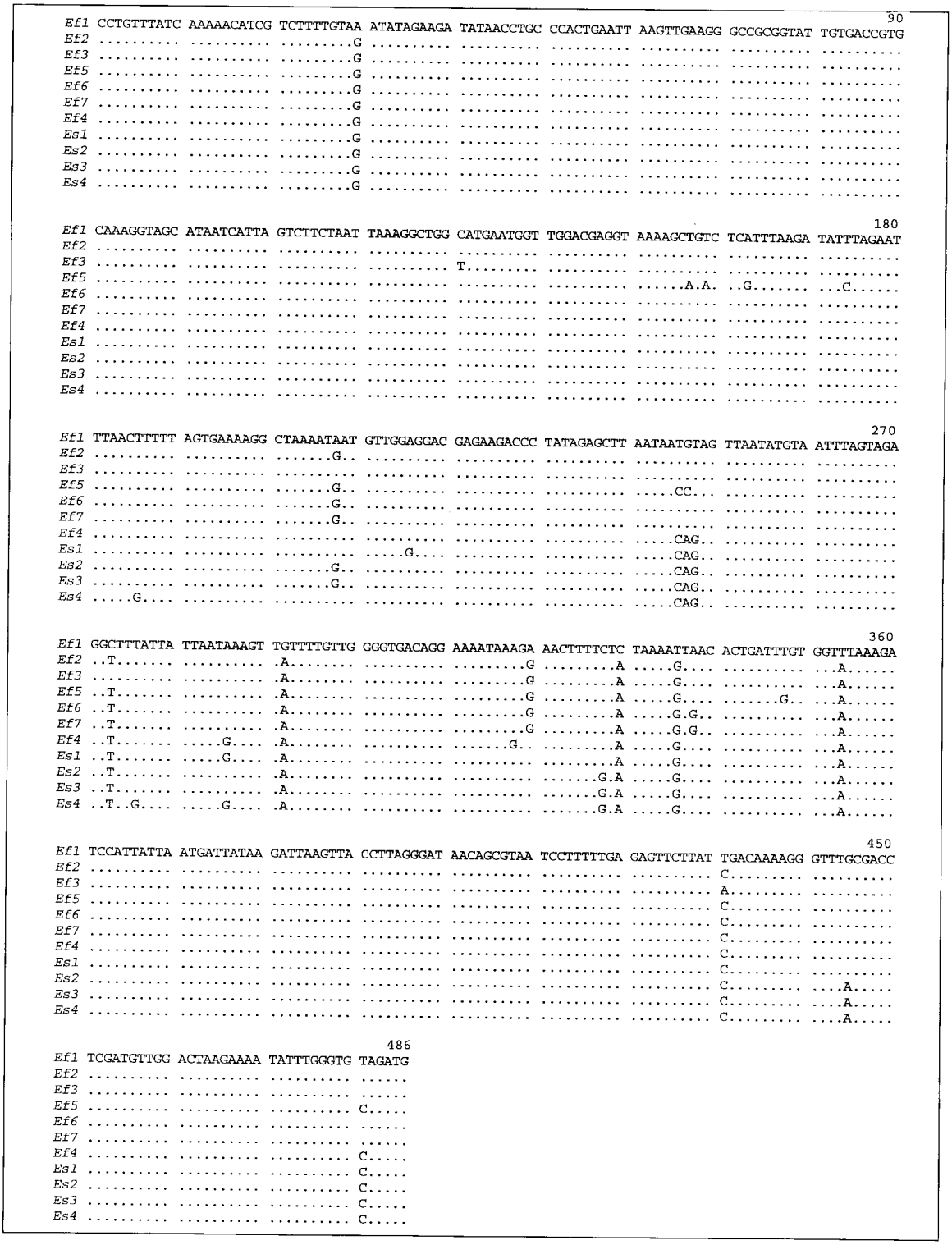

Fig. 1. Nucleotide sequence alignments of the partial 16S rDNA gene of mayflies. Dots indicate identical nucleotides. Abbreviations are the same as in Table 1.

Table 2. Pairwise proportional distances (lower-left) and ratio of transitions over transversions(upper-right) of the partial mitochondrial 16S rDNA sequence in mayflies. The values of nucleotide proportion distances are in percent (%)

	Ef1	*Ef5*	*Ef2*	*Ef3*	*Ef6*	*Ef7*	*Ef4*	*Es1*	*Es2*	*Es3*	*Es4*
Ef1	--	1.43	2.00	1.00	2.33	2.33	2.25	2.25	1.80	1.80	1.29
Ef5	3.50	--	1.00	1.40	1.25	1.25	1.20	1.20	0.67	0.67	0.75
Ef2	1.85	1.65	--	3.00	*	*	7.00	7.00	2.50	2.50	1.75
Ef3	1.65	2.47	0.82	--	4.00	4.00	4.00	4.00	2.67	2.67	1.60
Ef6	2.06	1.85	0.21	1.03	--	*	8.00	8.00	3.00	3.00	2.00
Ef7	2.06	1.85	0.21	1.03	0.00	--	8.00	8.00	3.00	3.00	2.00
Ef4	2.67	2.26	1.65	2.06	1.85	1.85	--	*	4.00	4.00	0.67
Es1	2.67	2.26	1.65	2.06	1.85	1.85	0.41	--	4.00	4.00	0.67
Es2	2.88	2.06	1.44	2.26	1.65	1.65	1.03	1.03	--	*	1.00
Es3	2.88	2.06	1.44	2.26	1.65	1.65	1.03	1.03	0.00	--	1.00
Es4	3.29	2.88	2.26	2.67	2.47	2.47	1.03	1.03	0.82	0.82	--

* The transversion substitution value was zero in this pairwise comparison.

Table 3. Average nucleotide base compositions of the 3'-end portion of the 16S rDNA sequence of some insects

Species	G%	A%	T%	C%	Reference
Mayflies	22.0	33.8	32.5	11.7	Current study
Crickets	22.4	30.8	36.1	10.7	Yang *et al.* (unpub. data)
Grasshopper	17.9	31.3	40.7	10.1	Uhlenbusch *et al.*, 1987
Leafhoppers	16.5	33.9	41.8	7.8	Fang *et al.*, 1993
Planthoppers	16.3	30.7	44.0	9.0	Yeh *et al.* (unpub. data)
Moth	14.8	38.9	38.3	8.0	Davis *et al.*, 1994
Hymenoptera	11.4	41.4	40.1	7.1	Derr *et al.*, 1992
Drosophilids	14.1	36.7	40.0	9.2	DeSalle, 1992
Black flies	14.9	34.5	42.2	8.4	Xiong *et al.*, 1991

sauteri is 0.82%, and among the members of *E. formosana* is 1.48%. When *Ef1* and *Ef5* are excluded, average divergence among members of *E. formosana* is 0.55%. *Ef1* and *Ef5* are interesting members in this analysis, as their divergences from the other members of *E. formosana* give high divergence percentages. Thus, *Ef1* and *Ef5* should be excluded from both *E. formosana* and *E. sauteri*.

Relationship Analyses

The phylogenetic trees constructed by the Neighbor-Joining method using proportion distance and the pairwise distance models of Jukes-Cantor, Tamura, Kimura 2-parameter, and Tamura-Nei are all identical (Fig. 2A). The trees constructed from Maximum Parsimony and UPGMA show virtually equivalent topologies to the trees from Neighbor-Joining (Fig. 2). The mayflies in this analysis can be divided into 3 groups, i.e., *E. sauteri, E. formosana*, and *Ef5*. *Ef5* is a singular lineage in this analysis which implies that *Ef5* is different from the other specimens. The results of bootstrap analysis with distance analyses are shown in trees (Fig. 2A, B). There is 83%-95% support for monophyly of *E. sauteri* and 74%-75% for monophyly of *E. formosana* when *Ef1* and *Ef5* are excluded. The specimen *Ef4*, which was described as a member of *E. formosana* (Kang and Yang, 1994), should be a member of *E. sauteri*, since all 3 grouping methods place it with

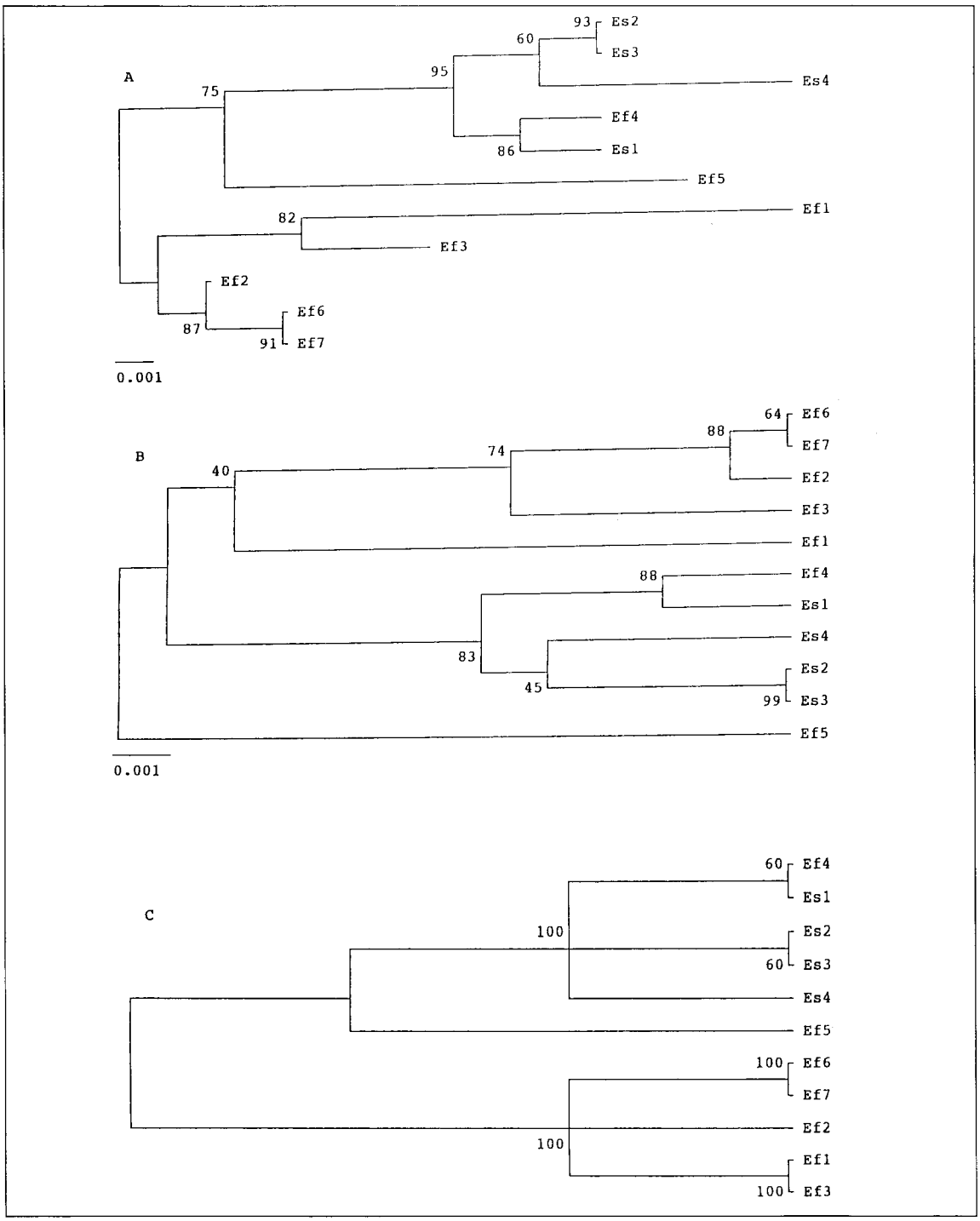

Fig. 2. Phylogenetic trees of *Ephemera* mayflies as inferred from nucleotide sequence of the partial 16S rDNA gene by the different grouping methods (A) Neighbor-Joining, (B) UPGMA, and (C) Maximum Parsimony. Bootstrap values in clustering methods are shown on trees (A, B), and the value on the parsimony tree is constructed from 30 trees (tree length = 30) by the 50% majority consensus method. Abbreviations are the same as in Table 1.

the members of *E. sauteri*.

The result of multidimensional scaling (Fig. 3) shows the space distribution of these specimens. There are 4 distinct groups, *Ef1, Ef5, Ef2-Ef3-Ef6-Ef7,* and *Es1-Es2-Es3-Es4-Ef4.* The stress in this analysis, 0.0018, suggests the results match perfectly according to the index of goodness of fit by Kruskal (1964). This result is generally congruent to the phylogenetic analysis.

Application to Mayfly Classification System

Six adults and 5 nymphs were used in this analysis, in which 2 adults, *Ef6* and *Ef7*, of *E. formosana* came from Pinglin (Taipei Co.) with identical sequences, and 1 adult (*Es2*) and 1 nymph (*Es3*) of *E. sauteri* collected from Nanhutashan (Ilan Co.) in different periods, were also identical. Two adults and 3 nymphs of *E. sauteri* from different localities were clustered together either in phylogenetic analysis or space distribution, and 3 adults and 1 nymph of *E. formosana* from different localities were grouped together when the 2 potential species of *Ef1* and *Ef 5* were excluded. Thus, nucleotide sequence data could be used to combine classification systems of both adult and nymph mayflies.

Discussion

It is well known that nucleotide sequence compositions of insect mtDNA are rich in adenine and thymine. When nucleotide sequence composition of the 3' end of the 16S rDNA gene from a number of insects are compared (Table 3), all of them are AT rich. However, there are some significant differences in base compositions of mayflies from other insects. The adenine plus thymine content of these insects is more than 70%, except in mayflies and crickets (~66%). The average proportion of guanine is 22% in mayflies and crickets, significantly higher than that in other insects (11.4%~

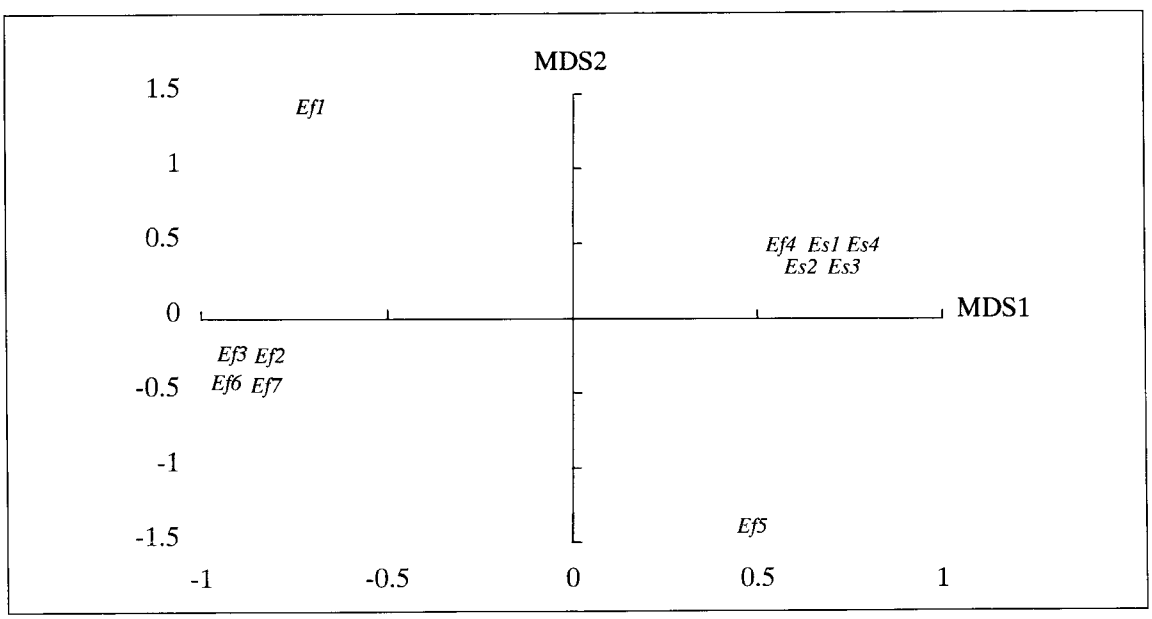

Fig. 3. Space distribution of 11 mayfly specimens by multidimensional scaling analysis. Abbreviations are the same as in Table 1.

17.9%). The proportion of guanine seems to decrease from hemimetabolous to holometabolous insects.

The specimen *Ef4* should be a member of *E. sauteri*, yet it was collected at an elevation of 750 m; whereas other members of *E. sauteri* were from high mountains (>1500 m). Apparently, elevation information alone is not a reliable characteristic to distinguish *E. sauteri* from *E. formosana*.

The amount of nucleotide sequence divergence which accompanies speciation is always an interesting subject. Molecular evidence from an allozyme survey of *Drosophila willistoni* complex (Ayala *et al.*, 1975) indicated genetic differentiation between sibling species was over 50%. But how much would be equivalent to nucleotide sequence divergence? The nucleotide sequence divergences at the species level of a number of insects and 1 crustacean are given in Table 4. Average sequence divergence in the COI-COII genes within aphid species is 2.30% (Sunnucks and Hales, 1996), while there is no divergence of the COI gene in bumblebees (Pedersen, 1996). The genes of the 12S (Taylor *et al.*, 1996) and 16S rDNA in mtDNA (Xiong and Kocher, 1993, Tang *et al.*, 1995, and Yang *et al.*, unpub. data), and the ITS1 region in genomic DNA (Kuperus and Chapco, 1994) are much more conserved than are the COI and COII genes. The sequence divergences in these conserved genes show 0.2%-0.8% nucleotide differences within species and 1.1%-1.4% within a species complex. The data based on the nucleotide sequence divergences in Table 4 could be used as a reference to infer whether specimens belong to the same species or not. Nucleotide sequence divergences in mayflies (Table 2) suggest that the specimens *Ef1* and *Ef5* each should be a species different from *E. formosana* since the divergences among *Ef1*, *Ef5*, and the other members of *E. formosana* ranged from 1.65% to 3.50%, which already have reached the species level. The result of multidimensional scaling also shows that the space distributions of *Ef1* and *Ef5* are far away from the members of *E. formosana* and *E. sauteri*. In Kang and Yang's (1994) description of the specimens of Ephemeroidea, there were only 2 species of *Ephemera* in Taiwan, i.e., *E. sauteri* distributed in high mountains, and *E. formosana* found at lower elevations (<1000 m). Ulmer (1912) recorded 2 species, *E. japonica* McLachalm and *E. supposita* Eaton from Taiwan; perhaps the specimens *Ef1* and *Ef5* belong to these 2 species. Further evidence is needed to support this possibility.

According to the phylogenetic ana-

Table 4. Nucleotide sequence divergences within species of some insects

Species (N)	Gene	Divergence (%)	Reference
Aphids (12)	COI-COII	2.30±(0.80)**	Sunnucks *et al.* 1996
Bumblebees (28)	COI	0	Pedersen 1996
Grasshoppers (3)	ITS1	0.67±(0.19)	Kuperus *et al.* 1994
Crustaceans (11)	12S rDNA	0.79±(0.46)	Taylor *et al.* 1996
Crickets (69)	16S rDNA	0.23±(0.22)	Yang *et al.* (unpulished)
Leafhoppers (4)	16S rDNA	0.18±(0.10)	Fang *et al.* 1993
Black flies	16S rDNA	0.47±(0.42)	Xiong *et al.* 1993
Black flies*	16S rDNA	1.09±(0.73)	Xiong *et al.* 1993
Black flies (15)	16S rDNA	1.38±(0.86)	Tang *et al.* 1995

N: The number of pairs in sequence comparisons of that taxa.

* The divergence value in this block was the comparison among species complex.

** Following by divergence ±SD, $P=0.05$.

lysis, these specimens are divided into 3 major groups, *E. sauteri*, *E. formosana*, and *Ef5*, by the Neighbor-Joining, UPGMA, and Maximum Parsimony methods. The taxonomic position of the members of *E. sauteri* is clear because they are always grouped into the same lineage by these 3 methods, and *Ef4* is a member of *E. sauteri*. *Ef5* is a single lineage in the analysis, in congruence with the data of multidimensional scaling and sequence divergence comparisons.

It is curious that *Ef1* groups with *Ef3* in the Neighbor-Joining and Maximum Parsimony methods, and yet, it is a singular lineage in the UPGMA method. Proportional distance between *Ef1* and *Ef3* is 1.65%, and distances between *Ef3* and the other *E. formosana* specimens are 0.82%~1.03%. Thus *Ef3*'s grouping with *Ef1* should be due to the effect of algorithm calculation in different reconstruction methods. In the Neighbor-Joining method, a modified distance matrix is constructed, in which distance is adjusted on the basis of its average divergence from all other distances. The result of *Ef3*'s grouping with *Ef1* should be due to the adjusted effect from the other distances such as 2.47%, 2.06%, 2.06%, 2.26%, 2.26%, and 2.67% between *Ef3* and the other individuals, *Ef5*, *Ef4*, *Es1*, *Es2*, *Es3*, and *Es4*, respectively. The tree-constructing algorithm in Maximum Parsimony is absolutely different from cluster analysis. The parsimony principle states that the best tree is the shortest, i.e., having the smallest number of evolutionary changes, and autapomorphic character has no influence in phylogenetic construction. There are only 27 varied sites in mayfly sequence comparisons, and only 11 sites have phylogenetic information, with the remaining 16 sites as autapomorphic sites. *Ef1* has 5 autapomorphic sites which is more than the other specimens, and it is the reason for *Ef1*'s grouping with *Ef3* in the Maximum Parsimony construction. The above analyses confirm that *Ef3*, but not *Ef1*, should be closer to members of *E. formosana*.

The resemblance species of *Ephemera* genus in Taiwan are difficult to distinguish (Kang and Yang, 1994) by their nymphs. While in adult stage, the diagnostic character, venation used to distinguish the 4 *Ephemera* species, become unreliable since an examination of a large number of specimens reveals that the venation patterns in these 4 species are with confounding overlap (Kang, pers. comm.). Our studies based on the nucleotide sequence of the partial 16S rDNA gene offer some information different from previous studies: (1) the proportion of the base guanine is higher in mayflies than in the other insects; (2) the specimens from Lona (Nantou Co.) should be a member of *E. sauteri*, and habitat elevation is not an absolute criterion for distinguishing *E. sauteri* from other closely related species; (3) there might be 4 distinct species of the *Ephemera* genus in Taiwan; and (4) the data from nucleotide sequence may apply simultaneously to the classification of both adult and nymph mayflies.

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從粒線體去氧核糖核酸序列鑑定蜉蝣屬(蜉蝣目：蜉蝣科)同胞種 *Ephemera formosana*及 *E. sauteri*

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摘 要

*Ephemera formosana*及*E. sauteri*為同胞種(sibling species)蜉蝣，也是台灣目前記錄中僅有的2種*Ephemera*蜉蝣，其形態極難區分，因此本研究運用PCR的技術取得粒線體16S核糖體基因的去氧核糖核酸序列，鑑定台灣的蜉蝣屬(*Ephemera*)昆蟲。從該基因3'端486個鹽基比對來自全省各地11個標本的結果顯示，除了*E. formosana*及*E. sauteri*外，可能有另外兩個種也分佈在台灣，其中一個採自花蓮縣壽豐鄉(*Ef1*)，另一個採自台中縣八仙山(*Ef5*)，因為此二標本與其它標本之間的差異範圍1.65%-3.5%已遠超過種內變異；多尺度空間分析亦顯示*Ef1*, *Ef5*, *E. formosana*及*E. sauteri* 彼此分離，各成一群。類緣分析及多尺度空間分析的結果可知，採自南投縣羅娜的標本*Ef4*，原本列為*E. formosana*一員，應該是*E. sauteri*種類，因其去氧核糖核酸序列與*E. sauteri*一群相似。另外，不同時期採自不同地點的標本，無論是成蟲或稚蟲，同種類的成員分別聚類在一起，據此而推，核酸序列的數據應可運用於成蟲及稚蟲分類體系的結合。

關鍵詞：蜉蝣、蜉蝣屬、粒線體去氧核糖核酸、16S去氧核糖核酸

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