# Phylogenetic Relationship among Several Japanese Odonate Species Inferred from Mitochondrial DNA Sequences

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**Abstract.** Using mitochondrial DNA sequences, phylogenetic relationships were studied on several odonate species occurring in Honshu, Japan. A calopterygid damselfly, *Mnais pruinosa* was roughly classified into two groups:subspecies *nawai* and others which were subdivided into subsp. *pruinosa* and *costalis.* On the other hand, the nucleotide sequences of COI region in *Somatochlora viridiaenea* were identical between subsp. *viridiaenea* and *atrovirens.* The sequence of *Sympetrum frequens* differed from that of *S. depressiusculun* in a single nucleotide, but this change was synonymous, and thought to be within individual variation or polymorphism.

**Key words**: Odonata, DNA sequence, phylogeny.

About 210 odonate species have been known from Japan. Classification of these damselflies and dragonflies, based mainly on morphology, has left several problems concerning intra- or inter-specific relationships. In order to find a clue to the problems and to support biodiversity conservation, we have analyzed nucleotide sequences of about 60 Japanese odonate species, using a region of mitochondrial cytochrome oxidase subunit I (COI) gene (reported in the 22nd Annual Meeting of the Molecular Biology Society of Japan, Fukuoka, 1999). Based on additional data, here we investigate phylogenetic relationships among three *Mnais pruinosa* subspecies, two *Somatochlora virideaenea* subspecies and two allied sympetrine species.

## Materials and Methods

**Specimens analyzed.** *Mnais pruinosa pruinosa* f. *strigata* ♂, Ishikawa Pref. (No.1), Fukui(2),Okayama(3), Saga(4); f.*sieboldi* ♀, Ishikawa(5): *M. p. costalis* ♂, Niigata( 6 ), ♀,Niigata(7); f. *ogumai* ♂, Niigata(8): *M. p. nawai* f. *nawai* ♂, Ishikawa (9), Fukui (10), f. *taketoi* ♀, Toyama (11), Ishikawa (12), Ishikawa (13), Okayama (14), f. *kadowakii* ♂, Okayama (15): *Calopteryx cornelia* ♂, Ishikawa (16): *Somatochlora viridiaenea viridiaenea* ♂, Ishikawa (17): *S. v.atrovirens* ♂, Ishikawa (18): *Somatochlora clavata* ♂, Miyazaki (19): *Sympetrum frequens* ♂, Ishikawa

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(20), Fukui (21): *Sympetrum depressiusculum* ♂, Ishikawa (22), Ishikawa (23), Ishikawa (24): *Sympetrum striolatum imitoides* ♂, Ishikawa (25). *Epiophlebia superstes* ♂, Ishikawa (Ep). All of these specinens were captured by A. T.. Although more individuals were analyzed, their data were omitted here.

**Other methods.** Extraction of DNA, amplification of COI region by PCR, purification, sequencing and data analysis were carried out as described previously[1]. Phylogenetic analysis was performed using MEGA 3.1 soft ware.

## Results and Discussion

The nucleotide sequences (total 554 residues) of the COI region of 7 odonate species were compared with those of *Epiophlebia superstes* Selys ( a "living fossil"), and substituted nucleotides were shown in Fig. 1. A calolpterygid damselfly *Mnais pruinosa* has been commonly classified into 3 subspecies *pruinosa, costalis* and *nawai*[2]. Recently, however, Hayashi et al. devided Japanese *Mnais* into two species *M. strigata* and *M. costalis*[3]. Present sequencing data indicated that this zygopteran species was divided into *pruinosa-costalis* group and *nawai* group. Distribution of subsp. *costalis* is limited to north of the Chubu district, whereas *pruinosa* and *nawai* inhabit south of the Central Japan. Although the latter two subspecies occasionally coexist in border regions, their main microhabitat differs each other (e.g. rapid upper vs. slow middle reaches of a stream). In addition, quantity of pectral muscle is larger in *nawai* than in *pruinosa*, reflecting their flight ability (A.T., unpublished observation). Within the regions sequenced, 4,-5 nucleotides (2-3 transitions, 0-2 transversions) were different between *pruinosa* and *costalis*, whereas 7-8 substitutions (6-7 transitions, 0-1 transversion) were found between *pruinosa* and *nawai*, but these changes were synonymous and did not cause amino acid substitution. Number of substituted nucleotides among the individuals in each subspecies was 0-2. On the other hand, 97 nucleotides (59 transitions, 38 transversions) and 8 amino acids were different between *pruinosa* and *Calopteryx cornelia*, a damselfly belonging to the same family. Probably, Japanese *Mnais pruinosa* is still under evolution, and subsp. *nawai* might be equivalent to a semispecies. Relationship between *nawai* and *pruinosa* is, however, somewhat complicated in Kyushu district (data not shown). Thus, classification into 3 subspecies is operationally not improper at present stage. Although several forms have been described among these subspecies, nucleotide sequence specific to each forma was not detectable in this study.

A cordulid dragonfly *Somatochlora viridiaenea* includes two subspecies *viridiaenea* and *atrovirens* [4]. Generally, subsp. *viridiaenea* occurs at northern or mountainous regions, and subsp. *atrovirens* inhabits rather southern or hilly regions. These two subspecies are morphologically indistinguishable, and only hind-wing length has been used as the key for identification. As shown in Fig. 1, the nucleotide sequences of the COI region were identical between the two subspecies, whereas 43 nucleotides ( 39 transitions, 4 transversions) were substituted in *S. clavata* that is closely related to *S. viridiaenea.* Unlike *atrovirens* , subsp. *viridiaenea* is rather rare in Hokuriku district, but both subspecies have been found in the same microhabitat. Change in the hind-wing length was continuous, and no difference was observed in their behavior. These results indicate that subsp. *viridiaenea* is within individual variation, at least in Hokuriku district. Thus, it seems proper to refer this cordulid dragonfly simply as *Somatochlora viridiaenea* (Uhler).

The most popular "akatombo" *Sympetrum frequens* closely resembles *S. depressiusculum* in morphology and behavior[5]. Distribution of *S. depressiusculum* covers the Continental Eurasia, and mature adults of this species occasionaly arrive several regions of this country facing the Sea of Japan, after autumn northern gale. In certain years, mature *S. depressiusculum* was more frequent than *S. frequens* in the Noto penninsula, but progeny or teneral insect of this immigrant species was extremely rare there. On the other hand, mating behavior was sometimes observed between *S. frequens* and *S. depressiusculum.* Using the specimens captured at the Noto penninsula, nucleotide sequences were determined and compared with those of *S. frequens* and *S. striolatum imitoides.* A few variations were detected in individuals of *S. frequens* and *depressiusculum* : 71st residue T or A, 92nd A or G , 104th G or A, 191st T or A, 254th G or A, 317th T or C, 458th G or A, and 542nd T or C. The sole specific substitution was at the 290th residue (T in *frequens,* C in *depressiusculum),* but this change did not alter amino acid sequence. Between *S. frequens* and *S. striolatum imitoides* , however, 90 nucleotides and 5 amino acids were different. Thus it seems probable that *S. frequens* is a subspecies of *S. depressiusculum.*

References

1) Fukuda H., Takegawa Y., and Taketo A. 2006. Memoirs Fukui Univ. Technol. 36, 163-170
2) Asahina, S. 1976. Tombo, 19, 2-16.
3) Hayashi, F., Dobata, S., and Futahashi, R. 2004. Odonatologica, 33, 399-412.
4) Asahina, S. 1957. Shinkonchu, 13(12), 51-57.
5) Asahina, S. 1961. Insecta Japonica. Series 1, Part 1. Hokuryukan.

Fig. 1. Nucleotide sequences of odonate species as compared with *Epiophlebia superstes* (Ep). Residues varied from those of Ep were shown selectively.

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Ep -GG CAC CCC GAA GTA TAC ATT TTA ATT TTA CCA GGA TTT GGT ATA ATT TCA CAT ATT ATT  [ 60]
1  -.A ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
2  -.A ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
3  -.A ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
4  -.A ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
5  -.A ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
6  -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
7  -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
8  -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ..G ..C ..T ..C ... ..C  [ 60]
9  -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
10 -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
11 G.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
12 -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
13 -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
14 -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
15 -.C ... ..T ... ..C ..T ... C.. ... C.. ... ... ..C ... ... ..C ..C ..C ... ..C  [ 60]
16 -.C ... ..A ... ..G ... ... C.. ..C C.. ... ..G ... ..C ... ... ..C ..C ... ...  [ 60]
17 -.T ... ... ... ..T ... ... ... ... C.. ... ... ... ..A ... ..C ..C ..C ... ...  [ 60]
18 -.T ... ... ... ..T ... ... ... ... C.. ... ... ... ..A ... ..C ..C ..C ... ...  [ 60]
19 G.T ... ... ... ..T ..T ... ... ... C.. ... ... ... ..A ... ..C ..T ..C ..C ...  [ 60]
20 -.C ... ... ... ..T ..T ... ... ... ... ..G ... ... ... ... ... ..T ... ... ...  [ 60]
21 -.C ... ... ... ..T ..T ... ... ... ... ..G ... ... ... ... ... ..T ... ... ...  [ 60]
22 -.C ... ... ... ..T ..T ... ... ... ... ..G ... ... ... ... ... ..T ... ... ...  [ 60]
23 -.C ... ... ... ..T ..T ... ... ... ... ..G ... ... ... ... ... ..T ... ... ...  [ 60]
24 G.C ... ... ... ..T ..T ... ... ... ... ..G ... ... ... ... ... ..T ... ... ...  [ 60]
25 -.. ... ..T ... ... ... ... ... ... ..G ..T ..T ..C ..A ... ... ..T ... ... ...  [ 60]

Ep GCA CAA GAA AGA GGT AAA AAG GAA ACC TTT GGA GTA TTG GGT ATA ATT TAT GCT ATA ATT  [120]
1  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
2  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
3  ..C ... ... ... ..G ... ..A ... ... ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
4  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
5  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
6  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
7  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
8  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
9  ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
10 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
11 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
12 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
13 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
14 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... ..C  [120]
15 ..C ... ... ... ..G ... ..A ... ..A ... ... ... ..A ..A ... ... ... ..A ... G.C  [120]
16 ..C ... ... ... ... ... ..A ... ..G ... ... ... ..A ..A ... ... ... ..A ... ...  [120]
17 ..C ... ... ..T ... ... ... ... ..A ... ... ... ..A ..A ... ... ... ..A ... G.A  [120]
18 ..C ... ... ..T ... ... ... ... ..A ... ... ... ..A ..A ... ... ... ..A ... G.A  [120]
19 ..C ... ... ..T ... ... ... ... ..A ... ... ... ..A ..A ... ... ..C ..A ... G.A  [120]
20 ..T ... ... ... ... ... ... ... ..A ... ... ... C.A ..A ... ... ... ... ... G.A  [120]
21 ..T ... ... ..T ... ... ... ... ..A ... ... ... C.T ..A ..G ... ... ... ... G.A  [120]
22 ..T ... ... ..T ... ... ... ... ..A ... ... ... C.T ..A ... ... ... ... ... G.A  [120]
23 ..T ... ... ..T ... ... ... ... ..A ... ... ... C.T ..A ..G ... ... ... ... G.A  [120]
24 ..T ... ... ..T ... ... ... ... ..A ... ... ... C.T ..A ..G ... ... ... ... G.A  [120]
25 ... ... ... ... ... ... ... ... ..A ... ..G ... C.T ... ... ... ... ... ... G.A  [120]
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Fig. 1. continued

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Ep GCT ATT GGT ATT TTA GGA TTT GTA GTA TGA GCA CAT CAT AAA TTT ACA GTA GGA ATA GAT [180]
1  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
2  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
3  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
4  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
5  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
6  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
7  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
8  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
9  ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
10 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
11 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
12 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
13 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
14 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
15 ..A ... ..A G.. C.T ... ... ..G ... ... ... ..C ..C .TG ..C ... ..G ... ... ... [180]
16 ..A ..C ..G T.A C.T ... ... ... ..T ..G ... ... ... .T. ... ... ..G ..G ... ... [180]
17 ..A ... ... ... ... ..G ... ... ... ... ..T ..C ..C .T. ... ... ... ... ... ... [180]
18 ..A ... ... ... ... ..G ... ... ... ... ..T ..C ..C .T. ... ... ... ... ... ... [180]
19 ..A ... ... ... ... ... ..C ... ... ... ..T ..C ..C .T. ..C ... ... ... ... ... [180]
20 ..C ... ... ... C.. ... ... ..T ... ..G ..C ..C ... .T. ..C ... ... ... ..G ... [180]
21 ..C ... ... ... C.. ... ... ..T ... ..G ..C ..C ... .T. ..C ... ... ... ..G ... [180]
22 ..C ... ... ... C.. ... ... ..T ... ..G ..C ..C ... .T. ..C ... ... ... ..G ... [180]
23 ..C ... ... ... C.. ... ... ..T ... ..G ..C ..C ... .T. ..C ... ... ... ..G ... [180]
24 ..C ... ... ... C.. ... ... ..T ... ... ..C ..C ... .T. ..C ... ... ... ..G ... [180]
25 ... ... ..A ... ... ..T ... ..T ... ... ... ... ..C .T. ... ... ... ..T ... ... [180]

Ep GTA GAT ACA CGA GCC TAC TTT ACA TCT GCA ACT ATA GTA ATT GCC GTC CCT ACT GGA ATT [240]
1  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
2  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
3  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
4  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
5  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
6  ... ..C ..C ... ... ... ... ... ..A ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
7  ... ..C ..C ... ... ... ... ... ..A ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
8  ... ..C ..C ... ... ... ... ... ..A ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
9  ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
10 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
11 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
12 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
13 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
14 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
15 ... ..C ..C ... ... ... ... ... ..C ..C ... ... ... ... ..T ..G ..A ..A ... ..C [240]
16 ... ..C ..C ... ..A ..T ..C ..T ..A ..T ..A ... ... ... ... ... ..A ..A ... ... [240]
17 ... ..C ... ... ..A ..T ..C ... ..A ... ... ... ... ... ..T ..A ... ..A ..T ... [240]
18 ... ..C ... ... ..A ..T ..C ... ..A ... ... ... ... ... ..T ..A ... ..A ..T ... [240]
19 ... ... ... ... ..A ... ... ... ..A ... ... ... ..G ... ..T ..A ..C ..A ... ... [240]
20 ... ... ..T ... ..A ... ... ..T ..C ... ..A ... ... ... ..T ..G ..G ..A ... ... [240]
21 ... ... ..T ..T ..A ... ... ..T ..C ... ..A ... ... ... ..T ..G ..G ..A ... ... [240]
22 ... ... ..T ..T ..A ... ... ..T ..C ... ..A ... ... ... ..T ..G ..G ..A ... ... [240]
23 ... ... ..T ..T ..A ... ... ..T ..C ... ..A ... ... ... ..T ..G ..G ..A ... ... [240]
24 ... ... ..T ..T ..A ... ... ..T ..C ... ..A ... ... ... ..T ..G ..G ..A ... ... [240]
25 ... ... ..T ..T ..A ... ..C ..C ... ..C ..A ..G ... ... ..T ..A ..A ..G ... ..C [240]
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Fig. 1. continued

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Ep AAA ATT TTT AGT TGA CTA GCA ACA TTA CAC GGA ACC CAA CTA TCT TAT AGC CCA TCA CTC [300]
1  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
2  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
3  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
4  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
5  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
6  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
7  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
8  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
9  ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
10 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
11 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
12 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
13 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
14 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
15 ..G ... ... ..C ... T.. ..G ..T ... ... ..G ... ... T.C CAA ..C ... ... ..T ..T [300]
16 ... ... ... ... ... T.. ..C ... ..G ... ..C ... ... T.C A.A ..C TC. ... ... ..A [300]
17 ... ... ..C ..A ... ..T ..C ..T C.C ..T ..T ..A ... T.C A.A ... ..T ..T ..T ... [300]
18 ... ... ..C ..A ... ..T ..C ..T C.C ..T ..T ..A ... T.C A.A ... ..T ..T ..T ... [300]
19 ... ..C ..C ..A ... ..T ..T ..T C.T ..T ..T ..A ... T.C A.A ..C ..T ..T ..T ..T [300]
20 ... ... ..C ... ..G ... ..T ... C.T ..T ... ..A ... T.C ..A ... ..T ... ... T.A [300]
21 ... ... ..C ... ..G ... ..T ... C.T ..T ... ..A ... T.C ..A ... ..T ... ... T.A [300]
22 ... ... ..C ... ... ... ..T ... C.T ..T ... ..A ... T.C ..A ... ... ... ... T.A [300]
23 ... ... ..C ... ... ... ..T ... C.T ..T ... ..A ... T.C ..A ... ... ... ... T.A [300]
24 ... ... ..C ... ..G ... ..T ... C.T ..T ... ..A ... T.C ..A ... ... ... ... T.A [300]
25 ..G ... ... ..A ..G T.G ..T ... C.T ..T ..C ..T ... T.C ... ... ... ... ... ..A [300]

Ep TTA TGG GCT TTA GGA TTT GTA TTT TTA TTC ACT ATT GGG GGA TTA ACT GGA GTA GTC TTA [360]
1  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
2  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
3  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
4  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
5  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
6  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
7  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
8  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
9  A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
10 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
11 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
12 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
13 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
14 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
15 A.. ..A ..A ... ... ... ... ... C.. ..T ..A G.G ..A ..C ... ..A ... ..G ... ... [360]
16 A.. ..A ... C.. ... ..C ... ... ... ... ..A G.G ..T ..T ... ..A ... ..C ..A ... [360]
17 C.. ..A ..A ... ..T ... ... ... ... ..T ..A ... ..T ..T ... ..A ..T ... ..T C.. [360]
18 C.. ..A ..A ... ..T ... ... ... ... ..T ..A ... ..T ..T ... ..A ..T ... ..T C.. [360]
19 C.. ..A ..C C.. ..T ... ... ..C ... ... ..C ... ..A ..T ... ..A ..G ... ..T C.. [360]
20 ... ..A ..A C.. ..T ..C ... ... C.. ..T ... ... ... ... C.G ..C ... ... ..T C.. [360]
21 ... ..A ..A C.. ..T ... ... ... C.. ..T ... ... ... ... C.G ..C ... ... ..T C.. [360]
22 ... ..A ..A C.. ..T ... ... ... C.. ..T ... ... ... ... C.G ..C ... ... ..T C.. [360]
23 ... ..A ..A C.. ..T ... ... ... C.. ..T ... ... ... ... C.G ..C ... ... ..T C.. [360]
24 ... ..A ..A C.. ..T ... ... ... C.. ..T ... ... ... ... C.G ..C ... ... ..T C.. [360]
25 ... ..A ..A ... ... ... ... ..C ... ..T ..C ... ... ..T ... ..A ..T ..T ..A ... [360]
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Fig. 1. continued

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Ep GCT AAT TCT TCA ATT GAC ATT GCT ATA CAC GAT ACC TAT TAT GTA GTA GCA CAC TTC CAC  [420]
1  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
2  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
3  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
4  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
5  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
6  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
7  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
8  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
9  ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ..C ... ... ... ... ... ...  [420]
10 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ..C ... ... ... ... ... ...  [420]
11 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ..C ... ... ... ... ... ...  [420]
12 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ..C ... ... ... ... ... ...  [420]
13 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ..C ... ... ... ... ... ...  [420]
14 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
15 ..A ..C ..A ... ... ..T ..C ..G ..G ..T ... ... ..C ... ... ... ... ... ... ...  [420]
16 ..A ... ..A ... ... ... ... ..A ..G ..T ..C ... ... ..C ... ... ..C ... ... ...  [420]
17 ..A ... ..A ..T ..C ..T ... ... ..G ..T ... ..T ... ... ... ... ..C ... ... ..T  [420]
18 ..A ... ..A ..T ..C ..T ... ... ..G ..T ... ..T ... ... ... ... ..C ... ... ..T  [420]
19 ..A ... ..A ..T ... ..T ... ... ... ..T ..C ..T ... ..C ... ... ..T ... ... ..T  [420]
20 ..A ... ..A ... ... ..T ... ... T.. ..T ..C ..A ... ... ..G ... ..T ..T ..T ..T  [420]
21 ..A ... ..A ... ... ..T ... ... T.. ..T ..C ..A ... ... ..G ... ..T ..T ..T ..T  [420]
22 ..A ... ..A ... ... ..T ... ... T.. ..T ..C ..A ... ... ..G ... ..T ..T ..T ..T  [420]
23 ..A ... ..A ... ... ..T ... ... T.. ..T ..C ..A ... ... ..G ... ..T ..T ..T ..T  [420]
24 ..A ... ..A ... ... ..T ... ... T.. ..T ..C ..A ... ... ..G ... ..T ..T ..T ..T  [420]
25 ..A ..C ..A ... ... ..T ... ... T.. ... ..C ..A ..C ... ..C ..G ..T ..T ..T ...  [420]

Ep TAC GTT CTT TCA ATG GGA GCA GTA TTT GCA ATT ATA GGG GGA TTC GTC CAT TGA TTC CCT  [480]
1  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
2  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
3  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
4  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
5  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
6  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
7  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
8  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
9  ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
10 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
11 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
12 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
13 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
14 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
15 ..T ..C ..A ..C ..A ..G ... ... ..C ..T ... ..G ..T ... ..A ..G ..C ... ..T ..C  [480]
16 ..T ..A ... ... ..A ... ..C ... ... ..C ..C ... ..A ..C C.A A.T ..C ... ... ..C  [480]
17 ..T ... T.A ..T ..A ... ..T ... ... ... ... ... ..A ... C.A ... ..A ... ... ..C  [480]
18 ..T ... T.A ..T ..A ... ..T ... ... ... ... ... ..A ... C.A ... ..A ... ... ..C  [480]
19 ..T ..A T.A ..T ... ... ..T ... ..C ... ... ... ..A ..G ..G ..T ..A ... ... ...  [480]
20 ... ..C T.A ..T ..A ..T ..T ... ... ..T ... ... ..A ..T C.A ..A ... ... ..T T.C  [480]
21 ... ..C T.A ..T ..A ..T ..T ... ... ..T ... ... ... ..T C.A ..A ... ... ..T T.C  [480]
22 ... ..C T.A ..T ..A ..T ..T ... ... ..T ... ... ..A ..T C.A ..A ... ... ..T T.C  [480]
23 ... ..C T.A ..T ..A ..T ..T ... ... ..T ... ... ... ..T C.A ..A ... ... ..T T.C  [480]
24 ... ..C T.A ..T ..A ..T ..T ... ... ..T ... ... ... ..T C.A ..A ... ... ..T T.C  [480]
25 ..T ..A ..A ... ..A ..T ..T ..G ... ..T ... ... ..A ..G ..A ..T ..C ... ... T.C  [480]
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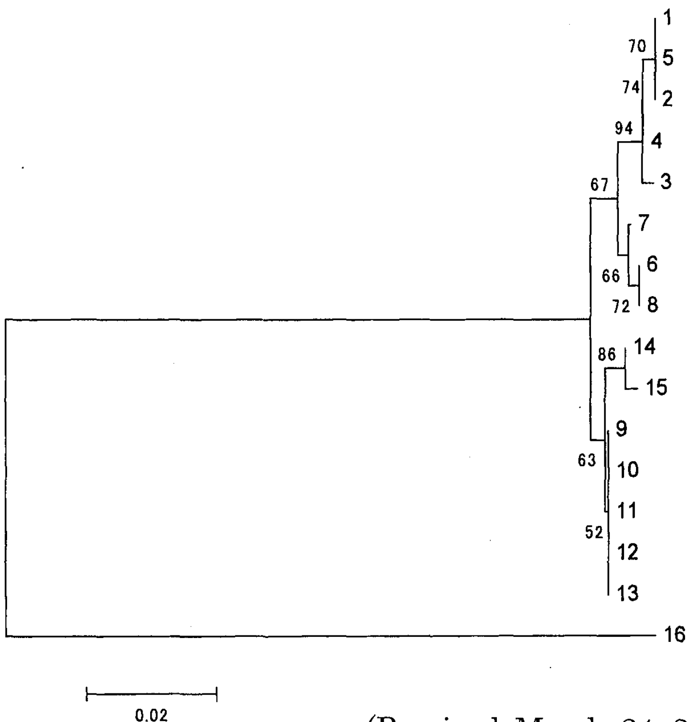
Fig. 1. continued

| Ep | TTA | TTT | ACT | GGA | GTT | ACT | ATA | AAT | AAT | TAT | AGT | TTA | AAA | ATT | CAA | TTC | GCA | GTA | ATA | TTC | [540] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 2 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 3 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 4 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 5 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 6 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 7 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 8 | ... | ... | ..A | ... | A.A | ... | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 9 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 10 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 11 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 12 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 13 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TG | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 14 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TA | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 15 | ... | ... | ..A | ... | A.A | ..C | ... | ... | ... | ACA | .TA | C.T | ..G | GCA | ... | ... | ... | ACT | ... | ..T | [540] |
| 16 | ... | ... | ..A | ... | ..A | ..A | ... | ... | ..C | AC. | .TG | C.C | ... | .G. | ... | ... | .T. | ..T | ... | ..T | [540] |
| 17 | ... | ... | ..A | ... | ..A | ..A | T.. | ..C | ..C | ... | TTA | ... | ... | ... | ... | ..T | A.. | ... | ... | ..T | [540] |
| 18 | ... | ... | ..A | ... | ..A | ..A | T.. | ..C | ..C | ... | TTA | ... | ... | ... | ... | ..T | A.. | ... | ... | ..T | [540] |
| 19 | ... | ... | ..A | ... | ..A | ..A | T.. | ..C | ..C | ... | CTA | ... | ... | ... | ... | ..T | A.. | ... | ... | ..T | [540] |
| 20 | C.. | ..C | T.. | ..T | ..A | ..A | T.. | ... | ... | ..C | TTA | ... | ..G | ... | ... | ..T | CTT | A.T | ... | ... | [540] |
| 21 | C.. | ..C | T.. | ..T | ..A | ..A | T.. | ... | ... | ..C | TTA | ... | ..G | ... | ... | ..T | CTT | A.T | ... | ... | [540] |
| 22 | C.. | ..C | T.. | ..T | ..A | ..A | T.. | ... | ... | ..C | TTA | ... | ..G | ... | ... | ..T | CTT | A.T | ... | ... | [540] |
| 23 | C.. | ..C | T.. | ..T | ..A | ..A | T.. | ... | ... | ..C | TTA | ... | ..G | ... | ... | ..T | CTT | A.T | ... | ... | [540] |
| 24 | C.. | ..C | T.. | ..T | ..A | ..A | T.. | ... | ... | ..C | TTA | ... | ..G | ..C | ... | ..T | CTT | A.T | ... | ... | [540] |
| 25 | C.T | ... | ..C | ... | ... | .GC | T.. | ... | .GA | ... | TTA | ... | ..G | G.A | ... | ..T | CTT | A.T | ..G | ..T | [540] |

| Ep | ATC | GGA | GTA | AAT | CTA | [555] |
|---|---|---|---|---|---|---|
| 1 | G.G | ..G | ..G | ... | ... | [555] |
| 2 | G.G | ..G | ..G | ... | ... | [555] |
| 3 | G.A | ..G | ..G | ... | ... | [555] |
| 4 | G.A | ..G | ..G | ... | ... | [555] |
| 5 | G.G | ..G | ..G | ... | ... | [555] |
| 6 | G.A | ..G | ... | ..C | ... | [555] |
| 7 | G.A | ..G | ..G | ..C | ... | [555] |
| 8 | G.A | ..G | ... | ..C | ... | [555] |
| 9 | G.A | ..G | ..G | ..C | ... | [555] |
| 10 | G.A | ..G | ..G | ..C | ... | [555] |
| 11 | G.A | ..G | ..G | ..C | ... | [555] |
| 12 | G.A | ..G | ..G | ..C | ... | [555] |
| 13 | G.A | ..G | ..G | ..C | ... | [555] |
| 14 | G.A | ..G | ..G | ..C | ... | [555] |
| 15 | G.A | ..G | ..G | ..C | ... | [555] |
| 16 | G.A | ... | ... | ... | A.. | [555] |
| 17 | ..T | ... | ..G | ... | ... | [555] |
| 18 | ..T | ... | ..G | ... | ... | [555] |
| 19 | ..T | ... | ... | ... | ... | [555] |
| 20 | ... | ... | ... | ... | T.. | [555] |
| 21 | ..T | ... | ... | ... | T.. | [555] |
| 22 | ..T | ... | ... | ... | T.. | [555] |
| 23 | ..T | ... | ... | ... | T.. | [555] |
| 24 | ... | ... | ... | ... | T.. | [555] |
| 25 | ..T | ..G | TG. | ... | T.. | [555] |

Fig. 2. Phylogenetic tree based on mitochondrial COI sequences (neighbor joining method). As the out group, *Calopteryx cornelia* was used.



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