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Phylogenetic analysis of the Genus *Coeliccia* (Odonata: Zygoptera: Platycnemididae) using Mitochondrial 16S rRNA Gene

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Abstract

Order Odonata includes one of the ancient groups of insects and is divided into three suborders, Zygoptera, Anisozygoptera and Anisoptera. Suborder Zygoptera consists of 4 superfamilies: Coenagrionoidea, Calopterygoidea, Platysticoidea and Lestoidea. Superfamily Coenagrionoidea includes families Platycnemididae and Coenagrionidae. Family Platycnemididae holds 43 genera with 455 species worldwide, while 14 genera including 52 species are present in India. The 16S rRNA gene is 1368-1374 bp in length and is used in constructing phylogenies. Presently, phylogenetic analysis based on 16S rRNA gene of *Coeliccia bimaculata, Coeliccia chromothorax, Coeliccia fraseri* and *Coeliccia renifera* with 9 conspecific sequences of genus retrieved from GenBank and *Calicnemia chaseni* as outgroup of family Platycnemididae has been conducted using Neighbour-joining method. Interspecific divergence ranges from 4.4% to 10%. Presently studied species are placed in one group and are more closely related to each other than to other species in the tree. *Coeliccia fraseri* and *Coeliccia bimaculata* are placed on one node and *Coeliccia renifera* and *Coeliccia chromothorax* are on another node. *Coeliccia didyma* is distantly related to the other species of genus.

The order Odonata is one of the ancient groups of insects, represents 482 species and 50 subspecies under 150 genera and 18 families from India. Family Platycnemididae constitutes 43 genera referable to 455 species all over the world, while 52 species of 14 genera are present in India[10]. They are commonly known as white-legged damselflies and predominantly found at running waters. The 16S rRNA gene can be used as a molecular marker because of inefficient DNA repair system, haploid genome and high rate of mutations. Mitochondrial analysis based on the 16S rRNA gene has been reported on 64 species of the family Platycnemididae[2,4,8-10]. Presently, four species of genus *Coeliccia- Coeliccia bimaculata, Coeliccia chromothorax, Coeliccia fraseri* and *Coeliccia renifera* have been selected to decipher the phylogeny on the basis of 16S rRNA gene.

Preserved platycnemidid damselflies in the absolute alcohol were used. Presently, four species of genus *Coeliccia* of subfamily Calicnemiinae under family Platycnemididae were selected and collection data is summarized in the Table-1.

Table-1. Details of collection of species

S No.	Name of species	Place of collection	Altitude	Latitude/Longitude
1.	*Coeliccia bimaculata* (Laidlaw, 1914)	Nongkhyllem (Meghalaya)	580m	25°34'27.228" N/91°18'35.5248" E
2.	*Coeliccia chromothorax* (Selys, 1891)	Andretta (Himachal Pradesh)	673m	31°19'48" N/76°33'46.8" E
3.	*Coeliccia fraseri* Laidlaw, 1932	Nongkhyllem (Meghalaya)	580m	25°34'27.228" N/91°18'35.5248" E
4.	*Coeliccia renifera* (Selys, 1886)	Bilaspur (Himachal Pradesh)	1301m	32°3'50.04" N/76°45'0" E

Molecular Analysis :

Extraction, Amplification and Sequencing:

The extraction of DNA was done by following the method given by Kambhampti and Rai[6] with minor modifications. Agarose gel electrophoresis was used to check the integrity of extracted DNA. The amplification of extracted mitochondrial DNA was done by using Polymerase Chain Reaction (PCR) to generate millions of copies of target DNA. PCR amplifications were carried out with the help of primers ODO_12852F and ODO_13393R with a total volume of 30 µl, having PCR Water- 6.6 µl, Master Mix- 15 µl, ODO F primer- 1.2 µl, ODO R primer- 1.2 µl, BSA- 4 µl, DNA- 2 µl. Thermal cycling program for PCR reactions is 1 Cycle for initial denaturation at 95ºC for 5 minutes, 35 cycles for denaturation at 95º C for 1 minute**,** annealing at 52 ºC for 1 minute**,** extension at 72 ºC for 90 seconds and 1 Cycle for final extension at 72ºC for 7 minutes. All the PCR products were visualized in Agarose gel electrophoresis using Ethidium Bromide (EtBr) stain, under UV light by using Gel documentation system and gel images were captured. Amplified products were sequenced by following Sanger dideoxy method from Bioserve, Hyderabad.

Procurement of Accession numbers and Phylogenetic analysis : In NCBI, BLAST was used to check whether sequence had any conspecific sequence. Then 16S rRNA sequences of species under genus *Coeliccia* were downloaded from NCBI. All the sequences were then aligned, edited to remove mismatched bases and trimmed to remove gaps in MEGA X software. The DNA sequences of 16S rRNA gene were translated into proteins in order to check stop codons and frameshift mutations. Then sequences were submitted to GenBank to get accession numbers of species for 16S rRNA gene. MEGA X software was used to make phylogenetic tree for four

species by using Neighbour-joining method based on K2P distances. *Calicnemia chaseni* is taken as an outgroup.

During the present study, four species of genus *Coeliccia- Coeliccia bimaculata, Coeliccia chromothorax, Coeliccia fraseri* and *Coeliccia renifera* have been selected for extraction, amplification and sequencing of 16S rRNA gene. (Table-2).

Table-2. Details of sequences of studied species

S. No.	Species	Sequence length	Accession number
1.	*Coeliccia bimaculata* Laidlaw, 1914	461 bp	OK510779
2.	*Coeliccia chromothorax* (Selys, 1891)	546 bp	MZ157118
3.	*Coeliccia fraseri* Laidlaw, 1932	461 bp	OK486521
4.	*Coeliccia renifera* (Selys, 1886)	501 bp	MZ496957

Table-3. Nucleotide Base Composition

S. No	Species	Accession number	A	T	G	C
1.	*Coeliccia bimaculata* (Laidlaw, 1932)	OK510779	35.3	32.6	13.6	18.5
2.	*Coeliccia borneensis* (Selys, 1886)	KF369668	31.7	39.7	18.1	10.5
3.	*Coeliccia chromothorax* (Selys,1891)	MZ157118	33.9	39.1	16.5	10.5
4.	*Coeliccia cyanomelas* Ris, 1912	EU055081	33.9	39.3	16.5	10.3
5.	*Coeliccia cyaneothorax* Kimmins, 1936	KF369669	33.7	39.5	16.7	10.0
6.	*Coeliccia didyma* (Selys, 1863)	KF369671	33.5	39.7	17.0	9.8
7.	*Coeliccia flavicauda* Ris, 1912	AB707491	33.3	39.5	16.7	10.5
8.	*Coeliccia flavostriata* Laidlaw, 1918	KF369672	32.4	39.3	17.9	10.5
9.	*Coeliccia fraseri* Laidlaw, 1932	OK486521	35.0	32.8	13.6	18.5
10.	*Coeliccia nemoricola* Laidlaw, 1912	KF369673	33.3	39.5	17.2	10.0
11.	*Coeliccia poungyi* (Fraser, 1924)	KF369674	33.7	39.3	16.5	10.5
12.	*Coeliccia renifera* (Selys, 1886)	MZ496957	34.4	39.1	16.3	10.3
13.	*Coeliccia ryukuensis* Asahina, 1951	LC366431	34.2	38.8	17.0	10.0

Percentage value of nucleotide bases of 16S gene is
A= 35.3%, T= 32.6%, G= 13.6%, C= 18.5% for *Coeliccia bimaculata*
A= 33.9%, T= 39.1%, G= 16.5%, C= 10.5% for *Coeliccia chromothorax*
A= 34.4%, T= 39.1%, G= 16.3%, C= 10.3% for *Coeliccia renifera*
A= 35.0%, T= 32.8%, G= 13.6%, C= 18.5% for *Coeliccia fraseri*

Sequence Divergence :

Mean distances of 16S rRNA gene sequences among the species has been calculated for distance-based threshold analysis, using the Kimura 2-parameter (K2P) substitution model in MEGA X.

Interspecific divergence :

Table-4. Interspecific divergence of 16S rRNA gene sequences in the species of genus *Coeliccia*

S. No.	Sequences of presently studied Species	Sequences of presently studied species and species from NCBI	Interspecific Divergence
1.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia borneensis* (KF369668) (Malaysia)	8.3
2.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia cyaneothorax* (KF369669) (Malaysia)	9.4
3.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia cyanomelas* (EU055081) (USA)	8.9
4.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia didyma* (KF369671) (Malaysia)	8.8
5.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia flavicauda* (AB707491) (Japan)	9.2
6.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia flavostriata* (KF369672) (Malaysia)	8.7
7.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia nemoricola* (KF369673) (Malaysia)	9.4
8.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia poungyi* (KF369674) (Thailand)	9.3
9.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia ryukuensis* (LC366431) (Japan)	8.7
10.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	8.5
11.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia fraseri* (OK486521) (Meghalaya)	7.0
12.	*Coeliccia bimaculata* (OK510779) (Meghalaya)	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	8.4
13.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia borneensis* (KF369668) (Malaysia)	7.8
14.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia cyaneothorax* (KF369669) (Malaysia)	8.5

15.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia cyanomelas* (EU055081) (USA)	5.3
16.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia didyma* (KF369671) (Malaysia)	7
17.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia flavicauda* (AB707491) (Japan)	6.5
18.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia flavostriata* (KF369672) (Malaysia)	10
19.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia nemoricola* (KF369673) (Malaysia)	9
20.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia poungyi* (KF369674) (Thailand)	8.3
21.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia ryukuensis* (LC366431) (Japan)	6
22.	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	9
23.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia borneensis* (KF369668) (Malaysia)	8.5
24.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia cyaneothorax* (KF369669) (Malaysia)	9.1
25.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia cyanomelas* (EU055081) (USA)	9.1
26.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia didyma* (KF369671) (Malaysia)	9.0
27.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia flavicauda* (AB707491) (Japan)	9.2
28.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia flavostriata* (KF369672) (Malaysia)	8.5
29.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia nemoricola* (KF369673) (Malaysia)	9.1
30.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia poungyi* (KF369674) (Thailand)	9.4
31.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia ryukuensis* (LC366431) (Japan)	8.9
32.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	8.6

33.	*Coeliccia fraseri* (OK486521) (Meghalaya)	*Coeliccia chromothorax* (MZ157118) (Himachal Pradesh)	8.7
34.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia borneensis* (KF369668) (Malaysia)	7.3
35.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia cyaneothorax* (KF369669) (Malaysia)	7.8
36.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia cyanomelas* (EU055081) (USA)	4.4
37.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia didyma* (KF369671) (Malaysia)	6.1
38.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia flavicauda* (AB707491) (Japan)	5.6
39.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia flavostriata* (KF369672) (Malaysia)	9.5
40.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia nemoricola* (KF369673) (Malaysia)	8.3
41.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia poungyi* (KF369674) (Thailand)	7.5
42.	*Coeliccia renifera* (MZ496957) (Himachal Pradesh)	*Coeliccia ryukuensis* (LC366431) (Japan)	5.1

Lowest distance value is noted between *Coeliccia renifera* and *Coeliccia cyanomelas*, which is 4.4%. Highest distance value is noted between *Coeliccia chromothorax* and *Coeliccia flavostriata* which is 10%. Present results are in accordance to the report of Lim *et al*.[8], they inferred interspecific distance based on combined COI, COII, 16S rRNA and 28S rDNA in the species of family Platycnemididae and found 13.90% to 15.39% between the genera *Copera* and *Coeliccia* and 14.43% to 14.46% between the genera *Coeliccia* and *Prodasineura*.

Phylogenetic Analysis :

Phylogenetic analysis has been done for the 13 species of genus *Coeliccia* of family Platycnemididae and the outgroup species of genus *Calicnemia* of same family by Neighbour-Joining method. It has been studied by Bootstarp Consensus, N-J tree inferred from 1000 replicates. Data includes 14 sequences of 16S rRNA gene fragment including outgroup species.

The phylogenetic tree depicts that the presently studied species *Coeliccia fraseri* and *Coeliccia bimaculata* are placed on same node and *Coeliccia renifera* and *Coeliccia chromothorax* are on same node as earlier observed on the basis of COI gene[3]. The outgroup *Calicnemia chaseni* is separated from the other species of *Coeliccia*. *Coeliccia*

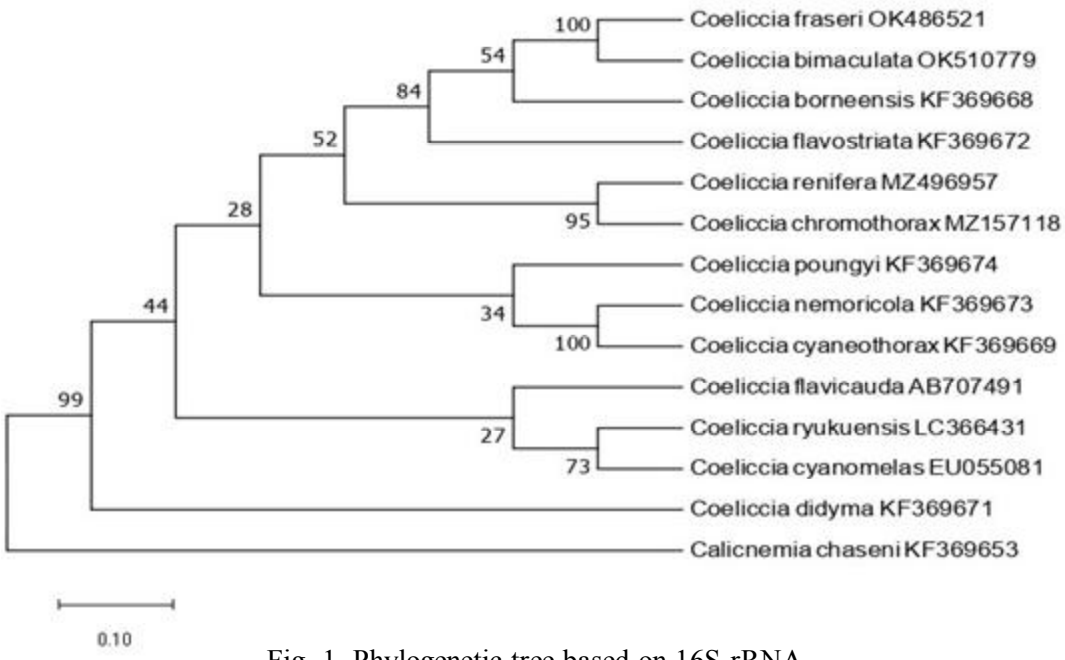

Fig. 1. Phylogenetic tree based on 16S rRNA

didyma is distantly related to the other species of genus *Coeliccia*. Presently studied species are in one group and are more closely related to each other than to other species in the tree. *Coeliccia nemoricola* and *Coeliccia cyaneothorax* are closely related to each other than to *Coeliccia poungyi*. *Coeliccia cyanaomelas* and *Coeliccia ryukyuensis* are closely related to each other than to *Coeliccia flavicauda*.

The bootstrap values range from 27% to 100% except some nodes. *Coeliccia borneensis* is a neighbour of *Coeliccia flavostriata,* as earlier observed on the basis of mitochondrial (16S rRNA and COI) and nuclear (28S) genes as by Dijkstra *et al*.[4].

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