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# Molecular and phylogenetic analysis of the genus *Orthetrum* (Odonata: Anisoptera: Libellulidae) using mitochondrial CO1 gene

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### **ABSTRACT**

Molecular phylogenetic relationships among members of the odonate genus *Orthetrum* (Odonata: Anisoptera: Libellulidae) were examined using 403 bp of mitochondrial COI. The support for monophyly of the *Orthetrum* was found in some studies with unresolved complexity. The *O. sabina, O. serapia* and *O. trincaria* formed a seperate and distinct group from the morphological analysis. We analysed the COI sequences of 22 species of *Orthetrum* using MEGA6. The p-distance between the members and the rate of transitional and transversional substitution was generated. The analysis indicated that the *Orthetrum* are monophyletic and *O. sabina* and *O. trinacria* formed a distinct and a seperate group.

Key words: Molecular phylogeny; mitochondrial COI; monophyly; Orthetrum; p-distance.

### INTRODUCTION

The genus *Orthetrum* includes about 60 species, of which one-half is distributed in tropical Africa and the rest extends across Eurasia to Australia. The genus is diverse in appearance in the Oriental region, such as red, boldly patterned and wingmarked species. 1 14 species of *Orthetrum* are reported from India 2 and in Mizoram 4 species have been recorded. Mor-

Corresponding author: Lalṭanpuii Phone: +91-9436152033 E-mail: laltetei@yahoo.co.in phological analyses indicated that *O. trinacria* forms a distinct group with *O. sabina* and *O. serapia.*<sup>2</sup> *O. sabina* and *O. trincaria* are well known for feeding on other odonate species, sometimes of greater size than their own.<sup>3</sup> It has been found that the African *O. trinacria* has spread out into Madagascar, Mesopotamia and Europe, while tropical Asia's most dominant species, *O. Sabina*, has migrated to northern Africa, Turkey and Europe.<sup>1</sup> Some support for the monophyly of *Orthetrum* have been found, but the inclusion of other species suggests some unresolved complexity.<sup>4,5</sup>

In the present study we infer a phylogeny for the genus *Orthetrum* utilising the cytocrome *c* oxydase subunit 1 (CO1) gene of the mitochondrial DNA. The mitochondrial DNA is a good choice for phylogenetic study because of its fast mutational rate which gives a significant variation between species, lack of introns, limited exposure to recombination and its haploid mode of inheritance. CO1 universal primer is very robust, enabling recovery of its 5' end from most of the animal phyla and it possesses a great phylogenetic signal.<sup>6</sup>

### **MATERIALS AND METHODS**

Taxon sampling

Table 1. List of taxa for the present study.

The taxon sample for the present study included 18 *Orthetrum* species retrieved from Gen-Bank (National Centre for Biotechnology Information) with the locality and the accession numbers. The 4 species generated from Mizoram were also included in the present study (Table 1). The gomphidus, *Davidus lunatus* was selected as outgroup.

## MOLECULAR EVOLUTIONARY GENETIC ANALYSIS (MEGA)

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6.7 All the 23 sequences of were aligned and edited using clustalW implimented in MEGA6.

Taxon name	Country	Genbank Accession no.
Davidius lunatus (Bartenev, 1914)	Republic of Korea	EU591677
O. pruinosum (Burmeister,1839)	Mizoram, India	KC122236
O. sabina (Drury, 1770)	Mizoram, India	KC122234
O. triangulare (Selys, 1878)	Mizoram, India	KC287152
O. glaucum (Brauer, 1865)	Mizoram, India	KC122232
O. triangulare (Selys, 1878)	Republic of Korea	KF257074
O. lineostigma (Selys, 1886)	Republic of Korea	KF257071
O. albistylum (Selys, 1848)	Republic of Korea	KF257070
O. japonicum (Uhler,1858)	Republic of Korea	KF257061
O. triangulare (Selys, 1878)	Japan	AB781568
O. sabina (Drury, 1770)	Japan	AB781554
O. pruinosum (Burmeister,1839)	Japan	AB781552
O. melania (Selys, 1883)	Japan	AB781551
O. luzonicum (Brauer, 1868)	Japan	AB781544
O. glaucum (Brauer, 1865)	Japan	AB781542
O. trinacria (Selys, 1841)	Germany	KC912286
O. julia falsum (Longfield, 1955)	Germany	KC912281
O. coerulescens (Fabricius, 1798)	Germany	KC912271
O. chrysostigma (Burmeister, 1839)	Germany	KC912262
O. brachiale (Palisot de Beauvois, 1805)	Germany	KC912258
O. poecilops (Ris, 1916)	Japan	AB709089
O. internum (McLachlan, 1894)	Japan	AB709025
O. japonicum (Uhler,1858)	Japan	AB709026

Relationships between individual taxa were assessed by maximum likelihood (ML) method with nucleotides distances (p-distance), transition/transversion rate ratios, and nucleotide diversity. The p-distance is the proportion (p) of nucleotide sites at which two sequences being compared are different. It is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared considering the proportion of nucleotide sites that are different.<sup>8</sup>

The overall ts/tv bias (R) was calculated by the formula:  $R = [A \times G k_1 + T \times C \times k_2] / [(A + G) \times (T + C)]$ , where  $k_1 =$  purine and  $k_2 =$  pyrimidine. For simplicity, the sum of r values is made equal to  $100.^9$  The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 403 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

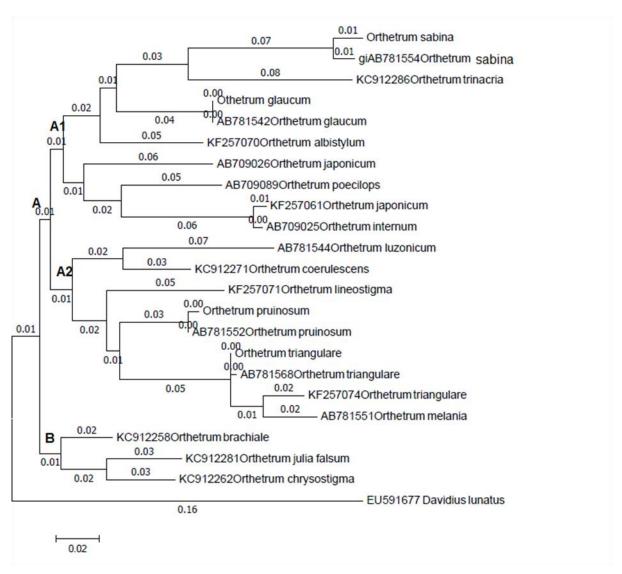


Figure 1. Molecular phylogenetic analysis by maximum likelihood method.

Table 2. The genetic distance (p-distance) between the Orthetrum species.

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19 20																					860.0	0.138 0.148 0.113 0.153 0.065 0.102 0.130 0.074 0.094 0.076 0.172 0.128 0.116 0.113 0.098 0.162 0.140 0.127 0.122 0.107 0.108
28																				9700		0.076
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Species	Orthetrum_pruinosum	Orthetrum_sabina	Orthetrum_triangulare	Othetrum_glaucum	KF257074Orthetrum_triangulare	KF257071Orthetrum_lineostigma	KF257070Orthetrum_albistylum	KE257061Orthetrum janonicum	Los con or triction _ Japonican	B781568Orthetrum_triangulare	B781568Orthetrum_triangulare AB781554Orthetrum	8781568Orthetrum_triangulare AB781554Orthetrum 8781552Orthetrum_pruinosum	9781568Orthetrum_triangulare 8781554Orthetrum_pruinosum 8781552Orthetrum_pruinosum 8781551Orthetrum_melania	9781568Orthetrum_triangulare AB781554Orthetrum_pruinosum 8781552Orthetrum_pruinosum 8781551Orthetrum_melania 8781551Orthetrum_luzonicum	9781568Orthetrum_triangulare AB781554Orthetrum_pruinosum 9781551Orthetrum_melania 978154Orthetrum_luzonicum 978154Orthetrum_glaucum	3781568Orthetrum_triangulare 48781554Orthetrum_pruinosum 3781552Orthetrum_pruinosum 3781551Orthetrum_luzonicum 3781542Orthetrum_glaucum 5781542Orthetrum_glaucum	9781568Orthetrum_triangulare 8781554Orthetrum_pruinosum 8781551Orthetrum_pruinosum 8781551Orthetrum_luzonicum 878154Orthetrum_glaucum C912286Orthetrum_trinacria C912281Orthetrum_trinacria	9781568Orthetrum_triangulare 8781554Orthetrum_pruinosum 8781551Orthetrum_pruinosum 8781551Orthetrum_uzonicum 878154Orthetrum_glaucum 8781542Orthetrum_trinacria C912286Orthetrum_trinacria C912281Orthetrum_julia_falsum C912271Orthetrum_coerulescens	9781568Orthetrum_triangulare 8781554Orthetrum_pruinosum 9781552Orthetrum_pruinosum 8781551Orthetrum_luzonicum 878154Orthetrum_glaucum 6712286Orthetrum_glaicia 6912271Orthetrum_coerulescens 6912271Orthetrum_coerulescens	B781568Orthetrum_triangulare A8781554Orthetrum_triangulare B781552Orthetrum_pruinosum B781551Orthetrum_ulzonicum B78154Orthetrum_glaucum C912286Orthetrum_trinacria C912280Orthetrum_coerulescens C912262Orthetrum_coerulescens C912262Orthetrum_chrysostigm C912262Orthetrum_chrysostigm	B781568Orthetrum_triangulare B781554Orthetrum_triangulare B781551Orthetrum_pruinosum B781551Orthetrum_melania B78154Orthetrum_glaucum B781542Orthetrum_trinacria C912286Orthetrum_trinacria C912281Orthetrum_coerulescens C912251Orthetrum_chrysostigm C912258Orthetrum_brachiale B709089Orthetrum_proecilops	9 AB781558Orthetrum_triangulare 10 giAB781554Orthetrum_triangulare 11 AB781552Orthetrum_pruinosum 12 AB781551Orthetrum_melania 13 AB78154Orthetrum_luzonicum 14 AB78154Orthetrum_luzonicum 15 KC912286Orthetrum_trinacria 16 KC912281Orthetrum_trinacria 17 KC912281Orthetrum_coerulescens 18 KC912258Orthetrum_brachiale 19 KC912258Orthetrum_brachiale 20 AB709089Orthetrum_poecilops 21 AB709025Orthetrum_internum
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The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. 10 Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 403 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.7

#### **RESULTS**

The genetic distance between the 22 species of *Orthetrum* was generated (Table 2). The nucleotide distance (p-distance) between *O. lineostigma* and *O. sabina* was 0.180; O. triangulare and *O. sabina* was 0.180; O. melania and O. sabina was 0.183 and 0.186; O. japonicum and O. sabina was 0.179; O. trinacria and O. melania was 0.178 which were found to be highest p-distance. The p-distance between O. pruinosum from Mizoram and Japan was 0.005 and the p-distance between O. trinagulare from Mizoram against Korea and Japan was 0.033 and 0.002 and between Korea and Japan was found to be 0.035. The p-distance between O. glaucum from Japan and Mizoram was found to be zero.

The maximum likelihood estimate of transitional substitution matrix between A/G = 5.58, T/C = 14.44, C/T = 25.64 and G/A = 11.09 (Table 3). The nucleotide frequencies are A = 31.64%, T/U = 33.56%, C = 18.89%, and G = 15.91%. The transition/transversion rate ratios are  $k_1$  = 1.621 (purines) and  $k_2$  = 3.533 (pyrimidines). The estimated transition/transversion bias (R) is 1.225.

The tree with the highest log likelihood (-

2745.6358) is shown (Figure 1). The Maximum Likelihood tree generated shows the monophyly of *Orthetrum* with respect to the outgroup having 2 distinct groups A and B. The Clade B consisted of *O. brachiale*, *O. julia falsum* and *O. chysostygma*, and the rest of the taxa are included in clade A. Clade A can be divided into clade A1 and A2. Within clade A1 the 2 *O. sabina* and *O. trinacria* formed a sister clade with *O. glaucum* and *O. albistylum*; these two sister clades formed a sister clade with *O. poecilop*, *O. japonicum* and *O. internum*. In clade A2 *O. coerulescens* and *O. luzonicum* formed a sister clade with 2 *O. glaucum*, 3 *O. tringulare*, *O. lineostigma* and *O. melania*.

Table 3. Maximum composite likelihood estimate of the pattern of nucleotide substitution.

	Α	Т	С	G
Α	-	7.26	4.09	5.58
т	6.84	-	14.44	3.44
С	6.84	25.64	-	3.44
G	11.09	7.26	4.09	-

NOTE: Each entry shows the probability of substitution (r) from one base (row) to another base (column). Rates of different transitional substitutions are shown in **bold** and those of transversional substitutions are shown in *italics*.

### **DISCUSSIONS**

The genus *Orthetrum* having more than 60 species are suggested to be monophyletic from the previous morphological and molecular studies but with a complex resolution. Morphological analyses indicated that *O. trinacria* forms a distinct group with *O. sabina* and *O. serapia*. <sup>1,3,4</sup> In the present study the COI gene for *O. serapia* is not available; we analysed 22 species of *Orthetrum* using the COI gene. The phylogenetic tree showed the *Orthetrum* are monophyletic in relation to the outgroup *Davidus lunatus*. The *O. sabina* and *O. trinacria* were found to be most distantly related to the rest of the *Orthetrum* ana-

lysed having a genetic distance of approximately 18%. In the phylogenetic analysis *O. sabina* from oriental region and O. trinacria from Europe were forming a separate and distinct sister clade eventhough the genetic distance between them was found to be 15%. The O. sabina and O. trinacria forming a sister clade and a distinct group from the rest of the Orthetrum analysed is in congruent with the behaviour and morphological characters indicated by Silsby<sup>3</sup> and Dijkstra and Kalkman.<sup>1</sup> The genetic distance of individuals of O. pruinosum, O. glaucum, O. triangulare and O. sabina sampled from different regions (all from oriental region) were found to be very low. The nucleotide composition of Orthetrum COI was A + T rich, which is typical for arthropods. 11 The rate of transitional substitution is higher than transversional substitution in the COI sequence analysis.

The present analysis indicated that the *Orthetrum* are monophyletic and *O. sabina* and *O. trinacria* formed a disticut and a seperate group. The inclusion of more species, more molecular markers and other phylogenetical analysis methods will further resolve the complexity of the genus *Orthetrum*.

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### **REFERENCES**

- Dijkstra KDB & Kalkman VJ (2012). Phylogeny, classification and taxonomy of European dragonflies and damselflies (Odonata): a review. Org Divers Evol, 12, 209–227.
- Subramanian KA (2014). A Checklist of Odonata of India (Version 2.0.2014). Zoological Survey of India. URL: <a href="http://zsi.gov.in/check\_list.html">http://zsi.gov.in/check\_list.html</a> (30 July 2014).
- Silsby J (2001). Dragonflies of the World. Smithsonian Institution Press. Washington, D. C, USA, pp. 1–216.
- Ware JL, May ML & Kjer KM 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. *Mol Phylogenet Evol*, 45, 289–310.
- Pilgrim EM & Von Dohlen CD (2008). Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. Syst Entomol, 33, 159–174.
- Hebert PD, Cywinska A, Ball SL & Dewaard JR (2003). Biological identifications through DNA barcodes. Proc R Soc B, 270, 313–321.
- Tamura K, Stecher G, Peterson D, Filipski A & Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Phylogenet Evol, 30, 2725–2729.
- Nei M & Kumar S (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York, pp. 33–36.
- Tamura K, Nei M, and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighborjoining method. *Proc Natl Acad Sci (USA)*, 101, 11030– 11035.
- Tamura K & Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*, 10, 512–526.
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H & Flook P (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am*, 87, 651–701.