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Research Article

The Evolutionary Relationship of Indian Skimmer Dragonflies (Odonata: Libellulidae): A Molecular Perspective

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ABSTRACT

Background: This study addresses the challenging classification of the diverse Libellulidae (skimmer dragonflies) using molecular systematics. **Materials and Methods:** By analyzing the mitochondrial COI gene for 21 Indian species, including the first Indian sequences for *Hylaeothemis apicalis* and *Onychothemis testacea* and combining them with 84 public sequences, a robust 80-species dataset was assembled. **Results:** A clear DNA barcoding gap was confirmed, with low intraspecific (0.1-1.9%) versus high interspecific (2.5-24.1%) genetic divergence. Maximum Likelihood phylogenetic analysis resolved the family into six major clades and one distinct lineage. While the monophyly of genera like *Sympetrum* (Clade I) and *Orthetrum* (Clade II) was strongly supported, and some clades matched recent molecular studies, the recovered groups showed no correspondence with traditional, morphology-based subfamilies. This profound discordance was exemplified by the distant relationship between *Diplacodes* and *Palpopleura*. **Conclusion:** The study validates DNA barcoding for species identification in Indian libellulids, suggests potential cryptic diversity, and provides a phylogenetic framework necessitating a comprehensive taxonomic revision using integrative approaches.

Keywords: DNA barcoding, Molecular systematics, Odonata, Taxonomic revision.

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INTRODUCTION

The order Odonata, encompassing dragonflies and damselflies, comprises a diverse group of insects with 6,407 extant species distributed globally.[1] This order is primarily divided into two suborders: Anisoptera (dragonflies), with 3,011 species, and Zygoptera (damselflies), with 2,941 species.[1] Among the 11 families within Anisoptera, the Libellulidae (skimmers) is the most species-rich, playing a significant ecological role in aquatic and terrestrial ecosystems. Libellulidae is a cosmopolitan family, containing 1,035 species across 144 genera worldwide, of which 91 species from 40 genera are recorded in India.[1-3] Members of this family exhibit considerable diversity in size, shape, and coloration, with body lengths ranging from 20-60 mm and wingspans of 30-100 mm.[4] They are characterized by a broadened abdomen, often possess brightly colored and patterned wings, and feature a well-developed, foot-shaped anal loop on the hindwing, a key morphological synapomorphy for the family.[5,6]

The internal classification of Libellulidae has been historically challenging. Early taxonomic systems relied on wing venation to define eight subfamilies.[7] This was later expanded to 13 subfamilies.[8,9] However, morphological characters like wing venation and male genitalia are often homoplastic, proving insufficient for resolving the family's complex phylogenetic relationships.[10]

Molecular techniques, particularly DNA barcoding using the mitochondrial Cytochrome c Oxidase Subunit I (COI) gene, have become a powerful tool for species identification and phylogenetic inference.[11,12] The COI gene, approximately 650 base pairs in length, evolves at a rate suitable for discriminating between closely related species and has robust universal primers.[13,14] Since the first mitochondrial gene was sequenced for a libellulid,[15] molecular studies based on the COI gene have grown, molecular analysis has been done till now on 73 libelluid species which also include 24 species from India.[16-23] These studies generally support the monophyly of Libellulidae, though relationships among its genera and subfamilies remain partly unresolved. Given the taxonomic complexities within Libellulidae and the relative scarcity of comprehensive molecular data from the Indian subcontinent, this study aims to contribute new genetic insights. We conducted molecular analyses based on

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the COI gene for 21 libellulid species collected from North and South India, two of which represent the first submissions of their sequences to the NCBI database.

MATERIALS AND METHODS

Adult libellulid dragonflies (21 species) were collected from freshwater habitats across India and preserved in ethanol (Figure 1). Morphological identification used a standard taxonomic reference.[4] Genomic DNA was extracted from thoracic muscle, and a ~650 bp fragment of the COI gene was amplified via PCR with universal primers.[24] The PCR reactions were carried out in a 25 µL reaction mixture containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 1 unit of Taq DNA polymerase, and approximately 50 ng of genomic DNA. The thermocycling conditions were as follows: initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 48-52°C for 45 sec, and extension at 72°C for 1 min; with a final extension at 72°C for 7 min. The PCR products were purified and sequenced bidirectionally using the Sanger dideoxy method at a commercial sequencing facility (Biologia, New Delhi). The resulting sequences were submitted to the GenBank database, and their accession numbers were obtained (Table S1). These sequences, plus 84 additional COI sequences from GenBank (Table S2), were aligned, creating a final dataset of 112 sequences (80 libellulid species, one gomphid outgroup) trimmed to 430 bp.

Genetic divergence was calculated using the Kimura 2-Parameter (K2P) model,[25] assessing both intra- and interspecific variation. Phylogenetic relationships were reconstructed using Maximum Likelihood (ML) and Neighbor-Joining (NJ) methods in MEGA v11.[26] The best-fit model for ML was K2+G. Tree robustness was evaluated with 1,000 bootstrap replicates. All sequences were deposited in GenBank.

RESULTS

Out of 430 bp final dataset of mitochondrial COI gene show 250 bp conserved sites (55.55%), 180 bp conserved sites (40%) and 140 bp parsimony informative sites (31.11%). Highly variable regions reflect the variations in the species and the conserved area shows phylogenetic relationship in species.

Genetic Divergence

Analyses show high level of inter-specific divergence as compared to intraspecific divergence in all the species of family Libellulidae.

Intraspecific genetic distances

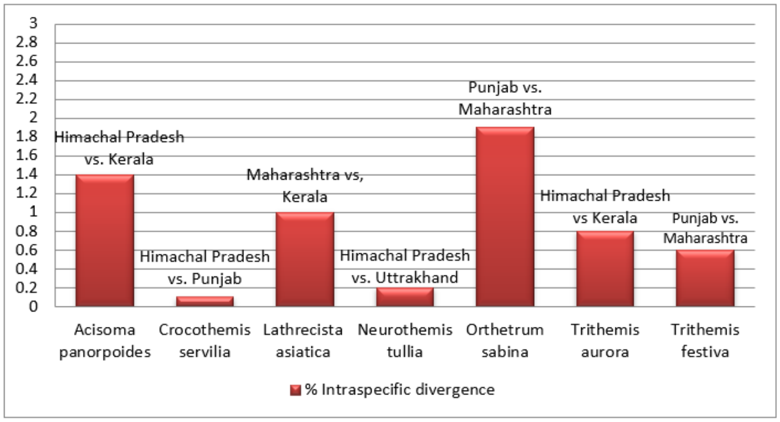
Intraspecific genetic divergence is calculated for 7 Indian species and it varies from 0.1% to 1.9 % for the samples collected from the different localities. The genetic divergence is <1% for species *Crocothemis servilia, Neurothemis tullia, Trithemis aurora* and *T. festiva*. It is 1.4% for *Acisoma panorpoides* and 1.9% for *Orthetrum sabina* which might be due to non-overlapping of geographical ranges of the species (Table S3).

Interspecific genetic distances

Presently, interspecific genetic distances between species range from 2.5 to 24.1 based on the mitochondrial COI gene (Table 1 and Table S4). The mean sequence divergence is 17.6% in the two species of genus *Brachydiplax*, 19.10% in the two species of genus *Diplacodes*, 11.20%-14.20% in the three species of genus *Neurothemis*, 3%- 21.3% in the fourteen species of genus *Orthetrum*, 2.7% -24.1% in the 33 species of genus *Sympetrum*, 2.5%-6% in the three species of genus *Tramea* and 9%-14% in the genus *Trithemis*.

Phylogenetic analysis

Phylogenetic analysis has been carried out for 112 sequences of 80 libellulid species to draw the evolutionary relationships among the family Libellulidae based Maximum Likelihood (Figure 2) and Neighbor Joining (Figure 3) phylogenetic trees.



Bar Graph For Intraspecific genetic distances.

Conspecific species recovered at the same node and congeneric species clustered together with high bootstrap values in both distance based and character based phylogenetic trees.

The Maximum Likelihood tree, presented as the preferred phylogeny (Figure 2), resolved the family Libellulidae into six major clades and one distinct lineage. The overall topology was largely consistent with the Neighbor Joining tree (Figure 3), with minor discrepancies in the relationships within Clades III and IV. The generic composition of these clades is almost similar in both Neighbor Joining as well as Maximum Likelihood phylogenetic trees except clade III and clade IV which split into two distantly related groups in Neighbor Joining. Presently, Maximum Likelihood phylogenetic tree is selected for the discussion. Clade I includes the 33 species of genus *Sympetrum* arranged into six clusters. Clade II includes 13 species of genus


Acisoma panorpoides
Brachythemis contaminata
Crocothemis servilia
Diplacodes trivalis
Hylaeothemis apicalis
Indothemis carnatica
Lathrecista asiatica
Neurothemis tullia
Onychothemis testacea
Orthetrum luzonicum
Orthetrum sabina
Palpopleura sexmaculata
Pantala flavescens
Rhyothemis varigata
Tholymis tillarga
Tramea limbata
Trithemis aurora
Trithemis festiva
Trithemis pallidinervis
Urothemis signata
Zyxomma petiolatum

Figure 1: Pinned specimens of libellulid species. Bar line = 1 cm.

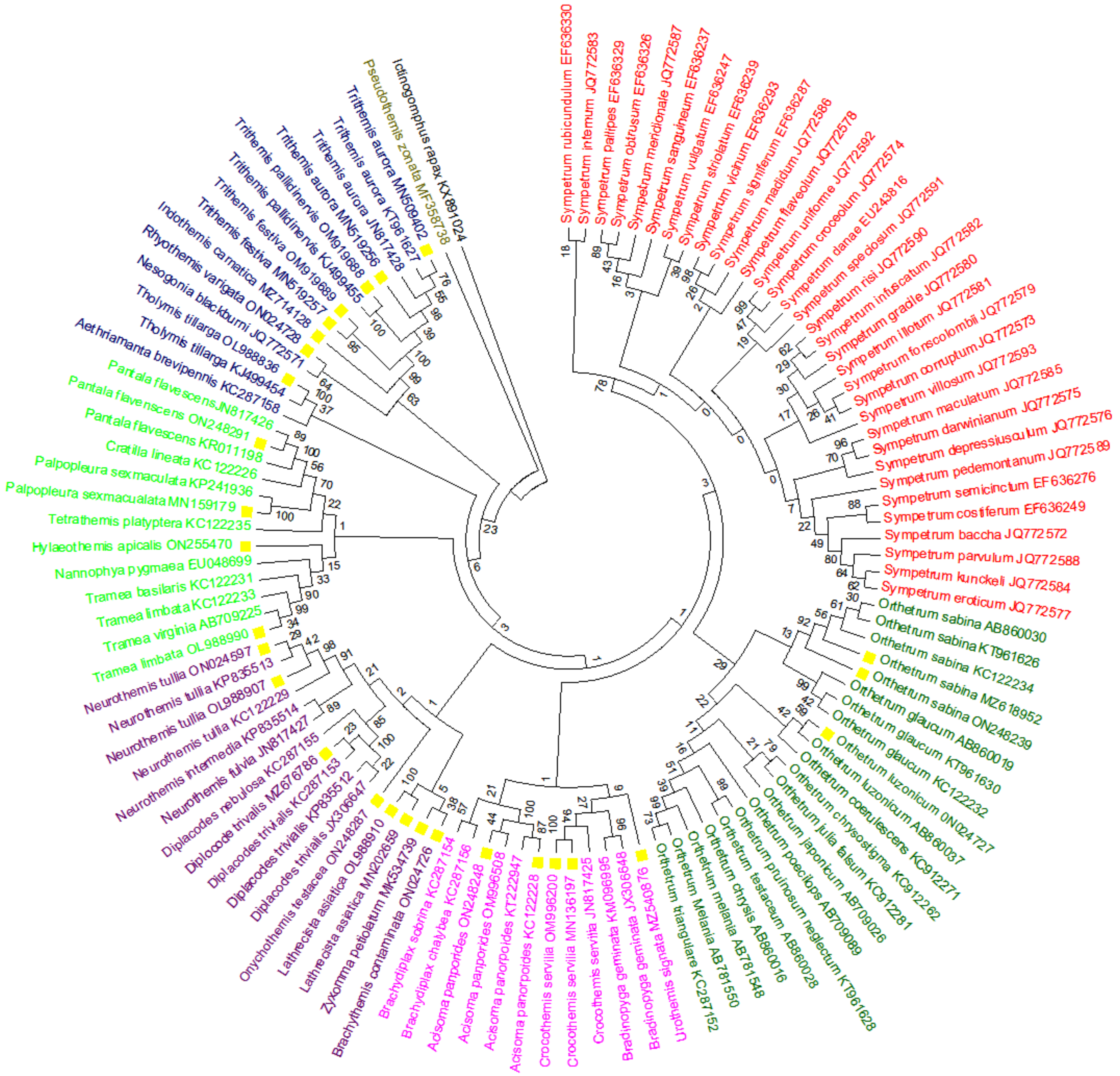


Figure 2: Maximum Likelihood phylogenetic tree (Clade I (Red), Clade II (Green), Clade III (Fuchsia), Clade IV (Purple), Clade V (Lime), Clade VI (Navy), Out group (Black)). Yellow mark shows presently studied species for molecular work.

Orthetrum which are grouped into two clusters. Species of genus *Orthetrum* (Clade II) shows close relationship with species of genus *Sympetrum* species (Clade I) in Maximum Likelihood phylogenetic tree, but in Neighbor Joining phylogenetic tree, these genera show distant relationships. Clade III includes 6 species under five genera-*Acisoma*, *Crocothemis*, and *Urothemis*. Earlier, Laltanpuii[21] reported that genera *Acisoma*, *Brachydiplax*, *Bradinopyga* and *Crocothemis* lie near to each other in the phylogenetic trees based on COI and NDI genes as observed in present study. Clade IV includes the 9 species of 6 genera-*Brachythemis*, *Neurothemis*, *Diplacodes*, *Onychothemis*, *Lathrcista* and *Zyxomma*. In the present study, species of genera *Diplacodes* and *Palpopleura* are found at different clades. On the other hand, clade V includes the 9 species of 7 genera-*Cratilla*, *Nannophya*, *Pantala*, *Palpopleura*, *Tetrathemis*, *Hyaleothemis* and *Tramea*. All nine species are divided into three clusters. Clade VI includes the 8 species of 6 genera-*Aethriamanta*, *Indothemis*, *Nesogonia*, *Rhyothemis*, *Trithemis* and *Tholymis* divided into four clusters.

DISCUSSION

This study provides a significant contribution to the molecular systematics of Indian Libellulidae, presenting new COI data for 21 species, two of which represent the first sequences from India submitted to international databases. Our phylogenetic analysis, based on a comprehensive sampling of 80 species, offers robust insights into generic relationships and challenges traditional classifications.

The Efficacy of COI Barcoding and Population-Level Signals

The clear distinction between low intraspecific and high interspecific genetic distances reaffirms the COI gene's power as a DNA barcode for libellulid dragonflies. The "barcoding gap" observed here is critical for species identification, especially in a diverse and potentially cryptic group. The elevated intraspecific divergence in *Acisoma panorpoides* and *Orthetrum sabina* (1.4% and 1.9%, respectively) is particularly intriguing. Like the 1% divergence in *Neurothemis tullia* across distant populations in

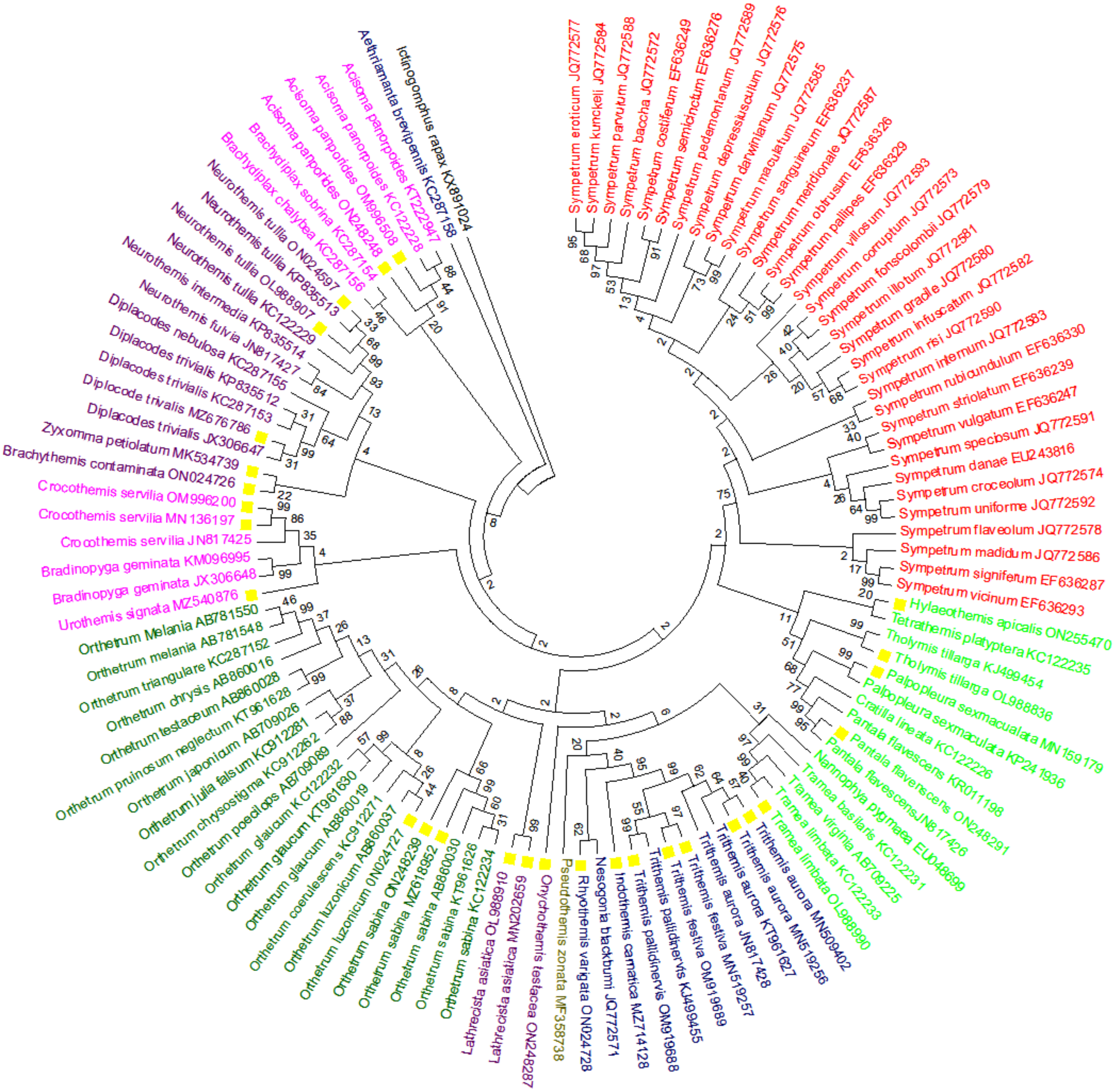


Figure 3: Neighbor joining phylogenetic tree (Clade I (Red), Clade II (Green), Clade III (Fuchsia), Clade IV (Purple), Clade V (Lime), Clade VI (Navy), Out group (Black)). Yellow mark shows presently studied species for molecular work.

Table 1: Interspecific genetic divergences (%) among the species of genus of family Libellulidae.

Genus Brachydiplax

Sl. No.	Taxa	1
1.	*B. chalybea*	
2.	*B. sobrina*	17.6

Genus Diplacodes

Sl. No.	Taxa	1
1.	*D. trivialis*	
2.	*D. nebulosa*	19.10

Genus Neurothemis

Sl. No.	Taxa	1	2
1.	*N. tullia*		
2.	*N. fulvia*	14.20	
3.	*N. intermedia*	10.40	11.20

Genus Tramea

Sl. No.	Taxa	1	2
1	*T. basilaris*		
2	*T. limbata*	6	
3.	*T. virginia*	6	2.5

Genus Trithemis

Sl. No.	Taxa	1	2
1.	*T. aurora*		
2.	*T. festiva*	9	
3.	*T. pallidinervis*	13	10

Genus Orthetrum

Sl. No.	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	*O. chrysis*													
2.	*O. chryostigma*	11.5												
3.	*O. corulescens*	13.2	9.6											
4.	*O. glaucum*	11.9	11	10										
5.	*O. japonicum*	14.4	9.6	10	11.1									
6.	*O. julia falsum*	12.0	5.7	9.2	9.9	8.7								
7.	*O. luzonicum*	16.0	18	12.8	18	19	16							
8.	*O. melania*	10.0	13	11.5	12	12.3	12	13.3						
9.	*O. poecilops*	11.1	10.4	12.0	10.3	11.3	12.4	14.6	11.3					
10.	*O. pruinosum negelectum*	9.2	9.5	8.8	8.7	9.1	8.8	12.5	8.4	10.4				
11.	*O. sabina*	20	15	20	15	20	19	18	17.5	16.9	14			
12.	*O. testaceum*	10	9.9	9.1	9.5	9.5	9.1	12	8.0	11.1	3	21.3		
13.	*O. triangulare*	10.4	11.1	11.1	10.3	11.1	10.4	12.5	2.5	10.4	6.9	21	7.2	
14.	*O. trinacria*	15.7	13.6	13.6	11.5	14.4	11.2	15.4	14.5	16.7	9.1	21	9.5	12

Mizoram and Kerala,[18] our findings suggest that geographic isolation across the Indian subcontinent may be driving population genetic structure and, in some cases, the early stages of speciation. Future studies employing more sensitive population genetic markers and denser geographical sampling are warranted to explore these patterns.

In stark contrast, interspecific divergence was substantially higher, ranging from 2.5% to 24.1% across the dataset. Mean congeneric sequence divergence was pronounced in all examined genera: *Brachydiplax* (17.6%), *Diplacodes* (19.1%), and *Neurothemis* (11.2-14.2%). The genus *Orthetrum* exhibited a wide range of divergence (3-21.3%), while *Sympetrum* showed the most extensive range (2.7-24.1%). These values are consistent with, and often exceed, those reported in previous studies, firmly establishing a significant molecular gap between congeneric species.[16,18]

Phylogenetic Relationships

Phylogeny demonstrates strong support for the monophyly of several large genera, notably *Sympetrum* (Clade I) and *Orthetrum* (Clade II). The consistent sister relationship between these two genera in our Maximum Likelihood analysis supports the earlier findings[27] and suggests a shared evolutionary history that may not be fully reflected in their morphology. The composition of several clades (III, V, and VI) shows remarkable congruence with recent multi-gene studies. The close relationship between *Acisoma, Brachydiplax, Bradinopyga*, and *Crocothemis* (Clade III) and the classic *Pantala-Tramea* grouping (within Clade V) are now well-supported molecular facts. Similarly, the association of *Trithemis, Nesogonia*, and *Rhyothemis* (Clade VI) provides a clear phylogenetic hypothesis for these taxa.

Taxonomic Implications and Incongruences

Perhaps the most significant finding of this study is the profound discordance between our molecular phylogeny and the traditional subfamilial classification of Libellulidae based on morphology.[8,28] The recovered Clades (I-VI) do not correspond to any recognized morphological subfamilies. This reinforces the conclusions of[17,29] strongly indicates that the current morphological subfamily system may not reflect the true evolutionary history of the group. A comprehensive revision, ideally using phylogenomic approaches, is urgently needed to establish a natural classification for the family.

Specific generic relationships also revealed points of incongruence. The separation of Diplacodes (Clade IV) and Palpopleura (Clade V) in our analysis conflicts with their grouping to earlier studies based on COI and NDI genes.[16] This discrepancy could be attributed to several factors, including differences in taxon sampling, the presence of homoplasy in the COI gene, or incomplete lineage sorting. It highlights the complex evolutionary dynamics within Libellulidae and underscores the need for cautious interpretation of relationships based on a single gene.

Finally, the isolated position of *Pseudothemis zonata* as a distinct lineage aligns with the multi-gene (mitochondrial COI and nuclear 18S rRNA, ITS, 5.8S rRNA, ITS2, 28S rRNA genes) results of earlier studies.[23] This consistent finding across independent studies provides compelling evidence that *Pseudothemis* may represent a unique, early diverging lineage worthy of separate subfamilial status.

CONCLUSION

In conclusion, this study significantly expands the molecular resources for Indian Odonata and delivers a well-resolved COI phylogeny that clarifies generic relationships within the Libellulidae. This study confirms the efficacy of DNA barcoding for species identification while revealing potential cryptic diversity. Most importantly, this study joins a growing consensus that the morphological subfamilial classification of Libellulidae is untenable. The phylogenetic framework presented here serves as a critical foundation for future taxonomic revisions, which should integrate dense taxon sampling with multi-locus or genomic data to resolve remaining uncertainties and build a stable, evolutionarily grounded classification for this magnificent family of dragonflies.

ACKNOWLEDGEMENT

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ABBREVIATIONS

COI: Cytochrome c Oxidase Subunit I; **CSIR:** Council of Scientific and Industrial Research; **dNTPs:** Deoxynucleotide triphosphates; **ITS:** Internal Transcribed Spacer; **K2P:** Kimura 2-parameter; **MEGA v11:** Molecular Evolutionary genetics analysis version 11; **ML:** Maximum Likelihood; **NCBI:** National Center for Biotechnology Information; **NET-JRF:** National Eligibility Test-Junior Research Fellowship; **NJ:** Neighbor-Joining; **PCR:** Polymerase Chain Reaction; **rRNA:** Ribosomal Ribonucleic Acid; **SRF:** Senior Research Fellow.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Dr. Gurinder Kaur Walia was responsible for the conceptualization and design of the study, provided continuous guidance and supervision throughout the research process, meticulously reviewed and edited the manuscript, and acts as the corresponding author; meanwhile, Dr. Hardeep Singh Hansra conducted all the practical experiments and field investigations, managed the resulting data and Dr. Gagandeep Kaur Dhillon performed the initial analysis, and prepared the original draft of the paper.

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SUPPLEMENTARY TABLES

Table S1: Detail of collected species with Accession numbers.

Sl. No.	Name of Species	Places of collection	Accession Numbers
1.	*Acisoma panorpoides* Rambur, 1842	Andhreeta (H.P)	ON248248
		Kuttivadi river (Kerala)	OM996508
2.	*Brachythemis contaminata* (Fabricius, 1793)	Bhadurgarh (Punjab)	ON024726
3.	*Crocothemis servilia* (Drury, 1773)	Jagraon (Punjab)	OM996200
		Andreeta (H.P.)	MN136197
4.	*Diplacodes trivialis* (Rambur, 1842)	Punjabi University Campus, Patiala (Punjab)	MZ676786
5.	*Hylaeothemis apicalis* Fraser, 1924	Nedumanged (Kerala)	ON255470
6.	*Indothemis carnatica* (Fabricius, 1798)	Khandala (Maharashtra)	MZ714128
7.	*Lathrecista asiatica* (Fabricius, 1798)	Sipana river (Maharashtra)	OL988910
		Neyaar (Kerala)	MN202659
8.	*Neurothemis tullia* (Drury, 1773)	Paonta sahib (H.P.)	ON024597
		Dehradun (U.K.)	OL988907
9.	*Onychothemis testacea* Laidlaw, 1902	Nedumanged (Kerala)	ON248287
10.	*Orthetrum luzonicum* (Brauer, 1868)	Khandala (Maharashtra)	ON024727
11.	*Orthetrum sabina* (Drury, 1773)	Dhindsa form, Patiala (Punjab)	ON248239
		Melghat (Maharashtra)	MZ618952
12.	*Palpopleura sexmaculata* (Fabricius, 1787)	Andhreeta (H.P.)	MN159179
13.	*Pantala flavescenes* (Fabricius, 1798)	Punjabi University, Patiala (Punjab)	ON248291
14.	*Rhyothemis variegata* (Linnaeus, 1763)	Narendranagar (Uttrakhand)	ON024728
15.	*Tholymis tillarga* (Fabricius, 1798)	Melghat (Maharashtra)	OL988836
16.	*Tramea limbata* (Desjardins, 1835)	Wayanad (Kerala)	OL988990
17.	*Trithemis aurora* (Burmeister, 1839)	Andhreeta (H.P.)	MN509402
		Neyyar (Kerala)	MN519256
18.	*Trithemis festiva* (Rambur, 1842)	Lonawala (Maharashtra)	MN519257
		Ropar (Punjab)	OM919689
19.	*Trithemis pallidinervis* (Kirby, 1889)	Andhreeta (H.P.)	OM919688
20.	*Urothemis signata* (Rambur, 1842)	Mannarkad (Kerala)	MZ540876
21.	*Zyxomma petiolatum* Rambur, 1842	Nedumbassery (Kerala)	MK534739

Table S2: List of COI gene sequences of species downloaded from GenBank.

Sl. No.	Name	Localities	Accession number	References
1	*Acisoma panorpoides* Rambur, 1842	Kerala (India)	KT222947	Krishnan and Sebastian (2016)
		Mizoram (India)	KC122228	Laltanpuii *et al.*, (2017)
2	*Aethriamanta brevipennis* (Rambur, 1842)	Mizoram (India)	KC287158	Laltanpuii *et al.*, (2017)
3	*Brachydiplax chalybea* Brauer, 1868	Mizoram (India)	KC287156	Laltanpuii *et al.*, (2017)
4	*Brachydiplax sobrina* (Rambur, 1842)	Mizoram (India)	KC287154	Laltanpuii *et al.*, (2017)
5	*Bradinopyga geminata* (Rambur, 1842)	Kerala (India)	JX306648	Karthika *et al.*, (2012)
		Kerala (India)	KM096995	Krishnan and Sebastian (2016)
6	*Cratilla lineata* Kirby, 1900	Mizoram (India)	KC122226	Laltanpuii *et al.*, (2017)
7	*Crocothemis servilia* (Drury, 1773)	Mizoram (India)	JN817425	Laltanpuii *et al.*, (2017)
8	*Diplacodes nebulosa* (Fabricius, 1793)	Mizoram (India)	KC287155	Laltanpuii *et al.*, (2017)
9	*Diplacodes trivialis* (Rambur, 1842)	Kerala (India)	JX306647	Karthika *et al.*, (2012)
		Kerala (India)	KP835512	Krishnan and Sebastian (2015b)
		Mizoram (India)	KC287153	Laltanpuii *et al.*, (2017)
10	*Nannophya pygmaea* Rambur, 1842	Korea	EU048699	Kim *et al.*, (2007)
11	*Neurothemis intermedia* (Rambur, 1842)	Kerala (India)	KP835514	Krishnan and Sebastian, (2016)
12	*Neurothemis tullia* (Drury, 1773)	Kerala (India)	KP835513	Krishnan and Sebastian, (2015a)
		Mizoram (India)	KC122229	Laltanpuii *et al.*, (2017)
13	*Neurothemis fulvia* (Drury, 1773)	Mizoram (India)	JN817427	Laltanpuii *et al.*, (2017)
14	*Nesogonia blackburni* (McLachlan, 1883)	Hawaii	JQ772571	Pilgrim and Dohlen (2012)
15	*Orthetrum brachiale* (Palisot de Beauvois, 1817)	Germany	KC912258	Laltanpuii *et al.*, (2014)
16	*Orthetrum chrysis* (Selys, 1891)	University Malaya, (Malaysia)	AB860016	Yong *et al.*, (2014)
17	*Orthetrum chryostigma* (Burmeister, 1839)	Germany	KC912262	Laltanpuii *et al.*, (2014)
18	*Orthetrum corulescens* (Fabricius, 1798)	Germany	KC912271	Laltanpuii *et al.*, (2014)

Sl. No.	Name	Localities	Accession number	References
19	*Orthetrum glaucum* (Brauer, 1865)	University Malaya (Malaysia)	AB860019	Yong *et al.*, (2014)
		Mizoram (India)	KC122232	Laltanpuii *et al.*, (2017)
		Andreata (Himachal Pradesh)	KT961630	Kaur (2016)
20	*Orthetrum japonicum* (Ulher, 1858)	Japan	AB709026	Laltanpuii *et al.*, (2014)
21	*Orthetrum julia falsum* (Kirby, 1900)	Germany	KC912281	Laltanpuii *et al.*, (2014)
22	*Orthetrum luzonicum* (Brauer, 1868)	Pahang (Malaysia)	AB860037	Yong *et al.*, (2014)
23	*Orthetrum melania* (Selys, 1883)	China	AB781548	Yong *et al.*, (2014)
		Japan	AB781550	
24	*Orthetrum poecilops* Ris, 1911	Japan	AB709089	Laltanpuii *et al.*, (2014)
25	*Orthetrum pruinosum negelectum* (Burmeister, 1839)	Andreeta (Himachal Pradesh)	KT961628	Kaur (2016)
26	*Orthetrum sabina* (Drury, 1773)	Kampar, Perak (Malaysia)	AB860030	Yong *et al.*, (2014)
		Mizoram (India)	KC122234	Laltanpuii *et al.*, (2017)
		Andreeta (Himachal Pradesh)	KT961626	Kaur (2016)
27	*Orthetrum testaceum* (Burmeister, 1839)	University Malaya (Malaysia)	AB860028	Yong *et al.*, (2014)
28	*Orthetrum trinacria* (Selys, 1841)	Germany	KC912286	Laltanpuii *et al.*, (2014)
29	*Palpopleura sexmaculata* (Fabricius, 1798)	Mizoram (India)	KP241936	Laltanpuii *et al.*, (2017)
30	*Pantala flavescene* (Fabricius, 1798)	Kerala (India)	KR011198	Krishnan and Sebastian (2016)
		Mizoram (India)	JN817426	Laltanpuii *et al.*, (2017)
31	*Pseudothemis zonata* (Burmeister, 1839)	Japan	MF358738	Huang *et al.*, (2020)
32	*Rhyothemis variegata* (Linnaeous, 1763)	Mizoram (India)	KC287151	Laltanpuii *et al.*, (2017)
33	*Sympetrum baccha* (Selys, 1884)	Japan	JQ772572	Pilgrim and Dohlen (2012)
34	*Sympetrum corruptum* (Hagen, 1861)	USA	JQ772573	Pilgrim and Dohlen (2012)
35	*Sympetrum costiferum* (Hagen, 1861)	USA	EF636249	Pilgrim and Dohlen (2012)
36	*Sympetrum croceolum* (Selys, 1883)	USA	JQ772574	Pilgrim and Dohlen (2012)

Sl. No.	Name	Localities	Accession number	References
37	*Sympetrum danae* (Sulzer, 1776)	USA	EU243816	Pilgrim and Dohlen (2012)
38	*Sympetrum darwinianum* (Selys, 1883)	Japan	JQ772575	Pilgrim and Dohlen (2012)
39	*Sympetrum depressiusculum* (Selys, 1841)	Netherland	JQ772576	Pilgrim and Dohlen (2012)
40	*Sympetrum eroticum* (Selys, 1883)	Japan	JQ772577	Pilgrim and Dohlen (2012)
41	*Sympetrum flaveolum* (Linnaeus, 1758)	Netherland	JQ772578	Pilgrim and Dohlen (2012)
42	*Sympetrum fonscolombii* (Selys, 1841)	Greece	JQ772579	Pilgrim and Dohlen (2012)
43	*Sympetrum gracile* Oguma, 1915	Japan	JQ772580	Pilgrim and Dohlen (2012)
44	*Sympetrum illotum* (Hagen, 1861)	USA	JQ772581	Pilgrim and Dohlen (2012)
45	*Sympetrum infuscatum* (Selys, 1883)	Japan	JQ772582	Pilgrim and Dohlen (2012)
46	*Sympetrum internum* Mongomery, 1943	USA	JQ772583	Pilgrim and Dohlen (2012)
47	*Sympetrum kunckeli* (Selys, 1884)	Japan	JQ772584	Pilgrim and Dohlen (2012)
48	*Sympetrum maculatum* Oguma, 1922	Japan	JQ772585	Pilgrim and Dohlen (2012)
49	*Sympetrum madidum* (Hagen, 1861)	USA	JQ772586	Pilgrim and Dohlen (2012)
50	*Sympetrum meridionale* (Selys, 1841)	Turkey	JQ772587	Pilgrim and Dohlen (2012)
51	*Sympetrum obtrusum* (Hagen, 1867)	USA	EF636326	Pilgrim and Dohlen (2012)
52	*Sympetrum pallipes* (Hagen in Hayden, 1874)	USA	JQ636329	Pilgrim and Dohlen (2012)
53	*Sympetrum parvulum* (Bartenev, 1912)	Japan	JQ772588	Pilgrim and Dohlen (2012)
54	*Sympetrum pedemontanum* (Muller in Allioni, 1766)	Netherland	JQ772589	Pilgrim and Dohlen (2012)
55	*Sympetrum risi* Bartenev, 1914	Japan	JQ772590	Pilgrim and Dohlen (2012)
56	*Sympetrum rubicundulum* (Say, 1840)	USA	JQ636330	Pilgrim and Dohlen (2012)
57	*Sympetrum sanguineum* (Muller, 1764)	Netherland	JQ636237	Pilgrim and Dohlen (2012)
58	*Sympetrum semicinctum* (Say, 1840)	USA	JQ636276	Pilgrim and Dohlen (2012)

Sl. No.	Name	Localities	Accession number	References
59	*Sympetrum signiferum* Cannings and Garrison, 1961	USA	JQ636287	Pilgrim and Dohlen (2012)
60	*Sympetrum speciosum* Oguma, 1915	Japan	JQ636291	Pilgrim and Dohlen (2012)
61	*Sympetrum striolatum* (Charpentier, 1840)	Netherland	EF636239	Pilgrim and Dohlen (2012)
62	*Sympetrum uniforme* (Selys, 1883)	Japan	JQ636292	Pilgrim and Dohlen (2012)
63	*Sympetrum vicinum* (Hagen, 1861)	USA	JQ636293	Pilgrim and Dohlen (2012)
64	*Sympetrum villosum* Ris, 911	Chile	JQ772593	Pilgrim and Dohlen (2012)
65	*Sympetrum vulgatum* (Hagen, 1861)	Netherland	EF636247	Pilgrim and Dohlen (2012)
66	*Tetrathemis platyptera* Selys, 1878	Mizoram (India)	KC122235	Laltanpuii *et al.*, (2017)
67	*Tholymis tillarga* (Fabricius, 1778)	Mizoram (India)	KJ499454	Laltanpuii *et al.*, (2017)
68	*Tramea basilaris* (Palisot de Beauvois, 1817)	Mizoram (India)	KJ122231	Laltanpuii *et al.*, (2017)
69	*Tramea limbata* (Desjardins, 1835)	Mizoram (India)	KC122233	Laltanpuii *et al.*, (2017)
70	*Tramea virginia* (Rambur, 1842)	Japan	AB709225	Huang *et al.*, (2020)
71	*Trithemis aurora* (Burmeister, 1839)	Mizoram (India)	JN817428	Laltanpuii *et al.*, (2017)
		Bilaspur	KT961627	Kaur (2016)
72.	*Trithemis festiva* (Rambur, 1842)	Mizoram (India)	KJ817429	Laltanpuii *et al.*, (2017)
		Andreeta (Himachal Pradesh)	KT961627	Kaur (2016)
73.	*Trithemis pallidinervis* (Kirby, 1891)	Mizoram (India)	KJ499455	Laltanpuii *et al.*, (2017)

Table S3: **Intraspecific distances (%) between species of family Libelluldae.**

Sl. No.	Name of the species with localities		% Intraspecific divergence
1.	*Acisoma panorpoides,* Rambur, 1842		1.4
	Himachal Pradesh ON248248	Kerala OM996508	
2.	*Crocothemis servilia* (Drury, 1773)		
	Punjab OM996200	Himachal Pradesh MN136197	0.1
3.	*Lathrecista asiatica* (Fabricius, 1798)		1.0
	Maharashtra OL988910	Kerala MN202659	
4.	*Neurothemis tullia* (Drury, 1773)		0.2
	Himachal Pradesh ON024597	Uttrakhand OL988907	
5.	*Orthetrum sabina* (Drury, 1773)		1.9
	Punjab ON248239	Maharashtra MZ618952	
6.	*Trithemis aurora* (Burmeister, 1839)		0.8
	Himachal Pradesh MN509402	Kerala MN519256	
7.	*Trithemis festiva* (Rambur, 1842)		0.6
	Maharashtra MN519257	Punjab OM919689	

Table S4: Interspecific genetic divergence (%) among the species of genus *Sympetrum*.

Genus Sympetrum																																	
Sl. No.	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	S. baccha																																
2	S. corruptum	15.3																															
3	S. costiferum	8.4	14.9																														
4	S. croceolum	16.2	22.7	13.2																													
5	S. danae	14.1	19.3	9.6	8																												
6	S. darwinianum	13.6	16.7	11.9	15.4	11.2																											
7	S. depressiusculum	12.4	15.8	10	13.3	10.8	6.1																										
8	S. eroticum	9.2	20.3	11.2	14.9	14.2	11.2	11.7																									
9	S. flaveolum	16.2	17.9	14.9	14	12.8	14.1	13.7	16.8																								
10	S. fonscolombii	16	14.5	16.9	17.4	17.5	15.4	17.1	18.8	16.2																							
11	S. gracile	15.3	17.3	12.3	12.8	12.5	11.2	13.3	17.1	15.4	13.3																						
12	S. illotum	17.3	14.9	16.5	14.8	14.8	16.5	16.2	17.8	16.5	15.7	12.4																					
13	S. infuscatum	17.1	18	13.6	15.3	13.6	14.9	13.7	14.9	16.3	17.1	11.2	14.9																				
14	S. internum	16.2	15.7	13.1	15.8	10.8	12.8	13.2	14.0	13.3	16.6	16.3	19.2	15.8																			
15	S. kunckeli	9.3	20.7	12	16.2	15.4	15.4	15.0	5.7	18.1	21.0	17.1	17.8	15.3	15.8																		
16	S. maculatum	13.6	14.5	2.7	17.6	13.2	3.5	8.8	14.6	14.1	15.0	11.7	16.6	14.5	14.0	16.2																	
17	S. madidum	16.1	22.2	14.9	15.9	15.0	16.2	14.9	15.3	15.3	20.5	20.4	20.3	17.8	18.1	18.9	17.1																
18	S. meridionale	12.7	17.9	12.8	16.2	13.3	14.1	14.5	14.5	14.5	15.7	19.0	19.7	15.8	15.0	15.4	14.9	15.3															
19	S. obtrusum	15.7	17.1	14.4	17.2	12.9	13.3	12.0	15.4	14.2	18.4	16.8	19.6	15.8	7.6	16.2	15	19	7.6														
20	S. pallipes	15.3	16.6	14	17.2	12.0	13.3	12.4	15.0	13.8	18.4	16.8	19.2	15.4	7.3	15.8	15	17.6	7.6	10													
21	S. parvulum	9.6	20.7	9.2	13.6	13.7	14.6	13.7	9.2	17.2	20.2	14.0	19.2	14.4	17.2	7.7	15.4	16.6	12.4	15.8	15.8												
22	S. pedemontanum	11.2	15.7	9.6	15.9	14.3	10.4	10.8	8.8	12.5	18.8	17.7	18.8	17.2	10.0	11.2	12.4	12.0	11.6	12.0	11.2	12.1											
23	S. risi	14.5	15.8	13.3	14.9	13.2	12.8	12.0	15.1	15.1	14.9	10.0	15.7	9.7	15.8	16.3	12.0	17.4	16.2	17.6	17.2	13.2	14.6										
24	S. rubicundulum	15.4	14.1	13.6	15.8	12.8	13.7	12.4	14.5	12.9	18.0	13.3	15.2	13.2	8.0	14.5	13.7	18	15.0	8.4	8	12.9	12.9	11.2									
25	S. sanguineum	11.9	16.1	9.1	12.3	10.3	13.2	12.4	12.4	13.7	17.0	15.3	15.6	14.1	12.0	14.9	14.5	15.3	8.9	10.9	10.9	11.9	12.4	13.7	12								
26	S. semicinctum	10.7	17.1	4.3	13.7	11.2	10.3	8.7	10.3	18.4	19.2	14.4	17.9	12.3	13.1	11.6	11.9	14.5	12.4	13.2	12.7	11.2	9.5	14.5	14	11.6							
27	S. signiferum	14	18.8	11.9	13.7	16.8	10.7	10.7	15.4	16.2	17.0	16.3	19.8	16.6	16.2	16.7	10.7	14.1	15.3	17.6	17.6	15.4	11.1	15.3	17.7	15.4	10.7						
28	S. speciosum	11.5	14	8.3	11.7	7.6	10.7	8.0	11.2	14.5	17.0	12.8	14.8	12.3	10.4	12.8	12.7	14.5	14.5	13.3	12.8	14.1	10.8	12.8	12.8	10.3	8	10.7					
29	S. striolatum	20.3	24.1	17.2	14.5	15	16.7	19	17.1	18.2	21.6	19.6	20.7	16	16.8	19.4	18.9	17.6	17	18.7	18.7	18.5	17.7	16.9	20	13.9	15.8	21	16.8				
30	S. uniforme	15.3	21.7	12.8	10	9.2	14.9	12.9	14.5	14.5	17	12.3	14.4	15.8	15.4	15.7	17.1	16.4	15.8	16.7	16.7	13.2	15.4	14.5	14.5	11.1	13.2	16.4	11.2	15.9			
31	S. vicinum	16.2	20.1	12.3	12.1	12	12.8	12.8	17.6	13.2	17	14	17.5	17.5	15.4	19.0	13.6	13.7	16.2	18.6	18.6	16.3	11.6	14	16.7	13.6	15.3	3.9	10.8	20.1	11.6		
32	S. villosum	17.4	18.9	16.1	17.8	16.9	16.6	16.6	17.5	17.4	17.6	16.1	18.7	16.5	18.3	19.7	17.0	16.9	17	18.3	17.9	20.1	15.7	17	19.2	15.2	15.2	17	16.1	20.6	17.8	17	
33	S. vulgatum	140	18.8	10.7	13.3	10	11.5	11.9	15	12.1	18.8	14.5	18.8	15.4	10.8	16.7	12.7	13.3	13.8	14.3	13.9	14.1	10.8	12	12.9	9.2	14.1	14.1	9.6	13.1	12.9	10	18.8