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Molecular characterization and genetic divergence among the species of families Aeshnidae and Macromiidae (Odonata: Anisoptera) based on mitochondrial COI gene

Dalveer Singh Somal[1*], Gurinder Kaur Walia[2]
[1] Assistant Professor, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India
[2] Professor, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India

Abstract
DNA barcoding based on 23 COI sequences referable to 16 sequences of 10 species (Anaciaeschna jaspidea, Anax ephippiger, Anax guttatus, Anax immaculifrons, Anax indicus, Anax nigrofasciatus nigrolineatus, Anax parthenope, Gynacantha bainbriggei, Gynacantha bayadera, Gynacantha subinterrupta) of family Aeshnidae and 7 sequences of 4 species (Epophthalmia vittata, Macromia ellisoni, Macromia flavicincta, Macromia moorei) of family Macromiidae have been done. Tramea carolina species of the family Libellulidae is considered as out-group. Genetic divergence among the species of both the families based on variable, parsimony informative, conserved sites, nucleotide base composition of COI gene fragment and transversion/transistional bias have been calculated. COI gene sequences of three species Anax immaculifrons, Anax indicus and Gynacantha bainbriggei of family Aeshnidae and four species of Epophthalmia vittata, Macromia ellisoni, Macromia flavicincta and Macromia moorei of family Macromiidae have been submitted for the first time to NCBI, while sequences of 5 species Anax ephippiger, Anax guttatus, Anax nigrofasciatus nigrolineatus, Anax parthenope and Gynacantha subinterrupta matched with corresponding sequences of the species of family Aeshnidae have been submitted for the first time from India.

Keywords: Odonata, Aeshnidae, Macromiidae, Mt COI gene, Genetic divergence.

Introduction

Molecular techniques provide useful information regarding the divergence of closely related species and genetic variation among them. Several DNA markers have been developed for the identification of genetic polymorphism prevalent in living world. Nuclear and mitochondrial genomes have been used for phylogenetic studies to trace the evolutionary history. Mitochondrial genome is better than the nuclear genome for molecular analysis because it lacks introns, limited exposure to recombination, and haploid mode of inheritance (Saccone *et al*., 1999) [24]. Mitochondrial COI gene possesses a greater range of phylogenetic signals than any other mitochondrial gene and is considered the best barcoding gene for generating molecular data of biological diversity (Herbert *et al*., 2003). Standard barcoding region of mitochondrial cytochrome c oxidase (COI) is 650 base pairs.

The family Aeshnidae is known by 54 genera comprising 480 species from the world including 13 genera representing 49 species present in India. The family Macromiidae is known by 4 genera and 123 species, worldwide, including 2 genera, Epophthalmia Burmeister, 1839 and Macromia Rambur, 1842 representing 17 species present in India (Subramanian and Babu, 2017) [25]. So far, molecular analysis based on COI gene has been done on 36 species of family Aeshnidae (Galimberti *et al*., 2020 [10]; Bybee *et al*., 2021 [3]; Maggioni *et al*., 2021 [21]; Mehmood *et al*., 2021) [22] and on 5 species of family Macromiidae (Kim *et al*., 2014 [14], 2018) [15] including only one species Anaciaeschna jaspidea (Aeshnidae) done from India (Karthika *et al*., 2012) [13]. The objective of this study was to record the phylogenetic relationships among the species and genera based on 23 COI sequences referable to 16 sequences of 10 species under family Aeshnidae and 7 sequences of 4 species under family Macromiidae.

Materials and methods

Live specimens of 10 species of the family Aeshnidae and 4 species of the family Macromiidae were captured from different localions from India (Table 1) using sweep net from lentic and lotic water bodies with vegetation. Stretched samples were identified morphologically by consulting "The Fauna of British India including Ceylon and Burma" Volume III (Fraser, 1936) [9]. Some specimens of these species were also preserved in absolute alcohol (100%) in deep freezer at - 20º C for molecular studies.

Mitochondrial DNA extraction was done from alcohol preserved samples. 650 bp long segment of COI gene was extracted by the method of Kambhampti and Rai (1991) with minor modifications and amplified with the help of universal primers LCO 1490 and HCO 2198 (Folmer *et al*., 1994) [8]. Amplified products were purified and sequenced from Biologia, New Delhi by Sanger dideoxy method. The obtained sequences were used to calculate the various genetic divergence parameters to draw the phylogenetic relationships among the species of both the families by using MEGA X software (Kumar *et al*., 2018) [19].

Twenty three COI sequences referable to 16 sequences of 10 species of family Aeshnidae and 7 sequences of 4 species of family Macromiidae were submitted to GenBank and their accession numbers were obtained (Table 1). The COI gene sequences of 10 conspecific species of family Aeshnidae and 3 conspecific species of family Macromiidae were downloaded from GenBank (Table 2). There were no stop codon and frameshift in all sequences (23 present and 13 from GenBank) which indicated the absence of NUMTs.

COI gene sequences (507 - 1147 bp) of 27 species belonging to families Aeshnidae (10 present study + 10 from GenBank) and Macromiidae (4 present study + 3 from GenBank) were analysed for proportion of variable sites, nucleotide composition, transition/transversion bias, nucleotide divergence and construction of phylogenetic tree. Maximum Composite Likelihood (MCL) method was used to estimate the pattern of nucleotide substitutions. The analysis involved 36 nucleotide sequences. Codon positions 1st+2nd+3rd+Noncoding were included. All positions containing gaps and missing data were eliminated. There are 386 positions in the final dataset.

Evolutionary analyses have been conducted by the Neighbour Joining method and nucleotide interspecific divergence of species was calculated with Kimura 2 - parameter (K2P) substitution model (Kimura, 1980) by using MEGA X software. Neighbour Joining method is best for phylogeny analysis. Unpublished sequences and short sequence (500bp) were not included in the analysis.

Table 1: Collection details of species of the two families with GenBank accession numbers of COI sequences and number of base pairs

S. No.	Species	Common Name	Locality	Latitude	Longitude	Altitude (metres)	Month/Year	Accession No.	Length of COI (bp)
	Family Aeshnidae								
1	Anaciaeschna jaspidea (Burmeister, 1939)	Rusty Darner	Punjabi University campus, Barnala (Punjab)	30º 36’ 13” N 30º 38’ 25” N	76º 44’ 92” E 75º 54’ 65” E	257 229	May, 2017 May, 2018	MN182751 MW228325	643 532
2	Anax ephippiger (Burmeister, 1839)	Vagrant emperor	Sangrur (Punjab)	30º 36’ 95” N	75º 86’ 13” E	240	July, 2020	MW602634 MW729731	529 527
3	Anax guttatus (Burmeister, 1839)	Pale - spotted emperor	Nagpur (Maharashtra)	21º 14’ 58” N	79º 08’ 82” E	310	September, 2019	MW880926	541
4	Anax immaculifrons Rambur, 1842	Magnificent emperor	Andretta (Himachal Pradesh) Kuttiady river, Wayanad (Kerala)	32º 03’ 50” N 11º 39’ 03” N	76º 33’ 46” E 75º 45’ 00” E	1301 122	May, 2018 May, 2018	MG544869 MW233047	583 551
5	Anax indicus Lieftinck, 1942	Lesser Green Emperor	Nagpur (Maharashtra)	21º 14’ 58” N	79º 08’ 82” E	310	September, 2018	MZ014028	525
6	Anax nigrofasciatus nigrolineatus Fraser, 1935	Blue - spotted Emperor	Andretta Dal lake, (Himachal Pradesh)	32º 03’ 50” N 32º 24’ 71” N	76º 33’ 46” E 76º 18’ 38” E	1301 1775	May, 2018 June, 2018	MG544870 MW239175	572 544
7	Anax parthenope (Seyls, 1839)	Lesser emperor	Patiala (Punjab)	30º 30’ 95” N	76º 31’ 76” E	257	May, 2019	MN207192 MW238344	574 552
8	Gynacantha bainbriggei Fraser, 1922	Dusk hawker	Neora valley (West Bengal)	27º 08’ 24” N	88º 70’ 07” E	1203	September, 2017	MZ148316	507
9	Gynacantha bayadera Selys, 1891	Parakeet darner	Melghat, Nagpur (Maharashtra)	21º 40’ 60” N	77º 14’ 87” E	1149	September, 2019	MZ203544	604
10	Gynacantha subinterrupta Rambur, 1842	Dingy dusk hawker	Andretta (Himachal Pradesh)	32º 03’ 50” N	76º 33’ 46” E	1301	September, 2017	MN242689 MN308079	553 539
	Family Macromiidae								
11	Epophthalmia vittata Burmeister, 1839	Common torrent hawk	Nagpur (Maharashtra) Kollam (Kerala)	21º 14’ 58” N 08º 52’ 48” N	79º 08’ 82” E 76º 36’ 00” E	310 3	September, 2019 April, 2018	MW509733 MW729758	646 642
12	Macromia ellisoni Fraser, 1924	Coorg torrent hawk	Kuttiadi river (Kerala)	11º 39’ 03” N	75º 45’ 00” E	122	April, 2018	MW911301	527
13	Macromia flavicincta Selys, 1874	-	Kuttiadi river (Kerala)	11º 39’ 03” N	75º 45’ 00” E	122	May, 2018	MW494964 MW730517	647 643
14	Macromia moorei Selys, 1874	River cruiser	Andretta (Himachal Pradesh) Kuttiadi river (Kerala)	32º 03’ 50” N 11º 45’ 00” N	76º 33’ 46” E 75º 46’ 12” E	1301 1150	September, 2019 April, 2018	MW454917 MW730533	544 532

Table 2: List of COI gene sequences of species of families Aeshnidae and Macromiidae downloaded from GenBank

S. No.	Species	Accession Number	Length (bp)	Country	Reference
		Family Aeshnidae			
1	Anaciaeschna donaldi Fraser, 1922	LC466163	451	Japan	Conniff *et al*., 2019 [5]

2	Anaciaeschna jaspidea (Burmeister, 1939)	JX306649	624	India	Karthika *et al*., 2012 [13]
3	Anaciaeschna martini Selys,1897	LC466165	451	Japan	Conniff *et al*., 2019 [5]
4	Anax congoliath Fraser 1953	KU565910	658	Netherlands	Dijkstra *et al*., 2015 [7]
5	Anax gladiator Dijkstra and Kipping, 2015 [7]	KU565914	658	Netherlands	Dijkstra *et al*., 2015 [7]
6	Anax rutherfordi McLachlan, 1883	KU565919	658	Netherlands	Dijkstra *et al*., 2015[7]
7	Anax speratus Hagen, 1867	KU565930	658	Netherlands	Dijkstra *et al*., 2015 [7]
8	Anax tristis Hagen, 1867	KU565931	658	Netherlands	Dijkstra *et al*., 2015 [7]
9	Gynacantha bullata Karsch, 1891	KU566143	658	Netherlands	Dijkstra *et al*., 2015 [7]
10	Gynacantha usambarica Sjostedt, 1909	KU566138	658	Netherlands	Dijkstra *et al*., 2015 [7]
		Family Macromiidae			
11	Epophthalmia elegans Brauer, 1865	KF257073	1147	Republic of Korea	Kim *et al*., 2014 [14]
12	Macromia amphigena Selys, 1871	KF257059	1147	Republic of Korea	Kim *et al*., 2014 [14]
13	Macromia manchurica Asahina, 1964	KF257082	1147	Republic of Korea	Kim *et al*., 2014 [14]

Results

All the species of this study possessed distinct barcodes and matched with sequences of congeneric species procured from GenBank by the BLAST search which shows that COI marker is useful for the identification of the species.

Proportion of variable sites, nucleotide base composition, trasition/ transversion bias (r)
and codon usage bias

Overall aligned data of 386 bp length shows 158 bp variable sites (40.9%), 131 bp parsimony informative sites (33.9%) and 228 bp conserved sites (59.0%) in species of Aeshnidae and 133 bp variable sites (34.4%), 108 bp parsimony informative sites (27.9%) and 253 bp conserved sites (65.5%) in species of Macromiidae.

The nucleotide base composition in all 36 sequences (23 present study and 13 procured from GenBank) of 27 species of both the families showed that the average percentage of base composition in species of Aeshnidae are A = 29.9%, T = 35.7%, C = 18.0% and G = 16.4% with high AT content (64.9%) and in the species of Macromiidae are A = 30.3%, T = 33.2%, C = 21.0% and G = 15.4% with high AT content (63.5%) (Table 3).

Each entry shows the probability of substitution (r) from one base (row) to another base (column) (Table 4). For convenience, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. In the family Aeshnidae, nucleotide frequencies are 29.94% (A), 35.72% (T/U), 17.98% (C), and 16.36% (G). The transition/transversion rate ratios are k1 = 0.503 (purines) and k2 = 2.758 (pyrimidines). The overall transition/transversion bias is R = 0.811, where R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]. The analysis involved 26 nucleotide sequences. In family Macromiidae, the nucleotide frequencies are 30.31% (A), 33.24% (T/U), 21.04% (C), and 15.41% (G). The transition/transversion rate ratios are k1 = 1.37 (purines) and k2 = 3.002 (pyrimidines). The overall transition/transversion bias is R = 1.104, where R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]. The analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. The analysis indicates that the transitions are more than transversions (Table 4).

The codon UUA for Leusine is having maximum codon usage frequency of 9.2 followed by AUA (Isoleucine) 7.5, AUU (Isoleucine) 6.7 and GUA (Valine) 6.6 in the family Aeshnidae and maximum codon usage frequency for UUA (leucine) is 5.3 followed by GUA (Valine) 5, CUA (leucine) 3.6 and AUA (leucine) 3.4 in the family Macromiidae.

Nucleotide divergence of families Aeshnidae and Macromiidae

Interspecific divergence has been calculated for one sequence each of 3 species of genus Anaciaeschna (from GenBank) showed that A. jaspidea has high interspecific divergence at 8.0% with A. donaldi and at 8.3% with A. martini (Table 5).

Interspecific divergence of 15 sequences of 11 species of the genus Anax (A.
congoliath, A. gladiator, A. rutherfordi, A. speratus and A. tristis retrieved from GenBank and A. ephippiger, A. guttatus, A. immaculifrons, A. indicus, A. nigrofasciatus nigrolineatus and A. parthenope) was examined. Maximum interspecific divergence has been observed between A. ephippiger and A. congoliath (14.0%), while the minimum interspecific divergence has been seen between A. nigrofasciatus nigrolineatus and A. parthenope (2.1%), and A. ephippiger and A. indicus (2.1%) (Table 5).

Interspecific divergence calculated for 5 species of genus Gynacantha comprising of 2 sequences of G. subinterrupta and 1 sequence each of G. bainbriggei, G. bayadera (present study), G. bullata and G. usambarica (from GenBank) showed very high divergence at 22.7% between G. bainbriggei and G. usambarica; 22.5% for G. bainbriggei and G. bullata and a minimum distance observed at 15.0% between G. bayadera and G. bullata (Table 5).

Interspecific divergence calculated for 2 species of genus Epophthalmia comprising of 2 sequences of E. vittata (present study) and 1 sequence of E. elegans (from GenBank) showed divergence at 14.2% (Table 6).

Interspecific divergence calculated for 3 species of genus Macromia comprising 1 sequence of M. ellisoni, 2 sequences each of M. flavicincta and M. moorei (present study), and 1 sequence each of M. amphigena and M. manchurica (from GenBank) showed high divergence between the species with maximum of 23% observer M. moorei and M.
amphigena (Table 6).

Phylogenetic analysis and evolutionary relationships

Phylogenetic tree constructed by the NJ method consisted of two groups. One group has family Macromiidae which divides into two clusters, one has two species of genus Epophthalmia, among these. E. vittata species was collected from India (present study) and other species E. elegans was reported from Republic of Korea (Kim *et al*. 2014). Both the species share the same node but branched with 14.2% interspecific divergence in the phylogenetic tree. The second cluster is of genus Macromia which further divides into two sub-clusters, one has three species (M. ellisoni, M.

amphigena and M. manchurica), among these, M. ellisoni (present study) and M. amphigena (Republic of Korea) show 19.3% interspecific divergence, while M. ellisoni (present study) and M. manchurica (Republic of Korea) show 19.9% interspecific divergence. Other sub-cluster divides again into two groups, one group has two species (M. flavicincta, M. moorei) but present at the same node and branched with the minimum 7.6% interspecific divergence in the phylogenetic tree and are closely related. The other group has all the species of family Aeshnidae which show close relationship with the species of genus Macromia of family Macromiidae.

Family Aeshnidae divides into three clusters, cluster one composed of genus Gynacantha with five species, viz., G. bainbriggei, G. bayadera, G. subinterrupta collected from India and G bullata and G. usambarica reported from Netherlands (Dijkstra *et al*. 2015). All the species of genus Gynacantha share same node and branched different in the phylogenetic tree. G. bayadera and G. subinterrupta are closely related with minimum 15.0% interspecific divergence, while the G. bainbriggei and G. usambarica showed the maximum 22.7% interspecific divergence. The second cluster splits into two groups, one composed of genus Anaciaeschna with three species A. jaspidea collected from India, and A. donaldi and A. martini reported from Japan (Conniff *et al*. 2019) [5]. The maximum 8.3% interspecific divergence is found between A. jaspidea (present study) and A. martini (Japan), while minimum 8.0% interspecific divergence is seen in A. jaspidea (present study) and A. donaldi (Japan). Third cluster composed of genus Anax with eleven species, viz., A. ephippiger, A. guttatus, A. immaculifrons, A. indicus, A. nigrofasciatus nigrolineatus, A. parthenope collected from India, and A. congoliath, A. gladiator, A. rutherfordi, A. speratus and A. tristis reported from Netherlands (Dijkstra *et al*. 2015). The maximum 14.0% interspecific divergence is found between A. ephippiger (present study) and A. congoliath (Netherlands), while minimum 2.1% interspecific divergence is seen in A. ephippiger and A. indicus. In family Aeshnidae, genera Anaciaeschna and Anax show sister group relationships. Tramea carolina of family Libellulidae used as an out group, is found at the different cluster.

Table 3: Average nucleotide base composition of COI sequences of 27 species of this study and those accessed from GenBank; representing percent content of individual nucleotides and their total number

S. No.	Species (Accession No.)	T(U)	C	A	G	Total
Family Aeshnidae						
1	Anaciaeschna donaldi LC466163	36.3	17.9	30.1	16.1	386.0
2	Anaciaeschna jaspidea JX306649	37.0	17.1	29.5	16.3	386.0
3	Anaciaeschna jaspidea MW228325	36.3	117.4	29.5	16.8	386.0
4	Anaciaeschna jaspidea MN182751	36.3	117.4	29.5	16.8	386.0
5	Anaciaeschna martini LC466165	36.8	17.9	29.8	15.5	386.0
6	Anax congoliath KU565910	34.2	18.9	30.8	16.1	386.0
7	Anax ephippiger MW729731	35.2	18.7	30.1	16.1	386.0
8	Anax ephippiger MW602634	35.2	18.7	30.1	16.1	386.0
9	Anax gladiator KU565914	33.4	18.9	31.3	16.3	386.0
10	Anax guttatus MW880926	37.3	17.1	29.8	15.8	386.0
11	Anax immaculifrons MW233047	33.7	18.1	32.1	16.1	386.0
12	Anax immaculifrons MG544869	33.7	18.1	32.1	16.1	386.0
13	Anax indicus MZ014028	36.0	17.9	29.8	16.3	386.0
14	Anax nigrofasciatus nigrolineatus MG544870	36.0	17.9	30.1	16.1	386.0
15	Anax nigrofasciatus nigrolineatus	36.0	17.9	30.1	16.1	386.0

Table 4: Substitutions rate and transition/ transversion ratios denoted by different fonts determined from COI gene base composition analysis of 27 species

		Family Aeshnidae		
	A	T	C	G
A	-	9.62	4.84	2.22
T	8.06	-	13.35	4.41
C	8.06	26.53	-	4.41
G	4.06	9.62	4.84	-
		Family Macromiidae		
A	-	7.81	4.94	4.96
T	7.12	-	14.84	3.62
C	7.12	23.45	-	3.62
G	9.76	7.81	4.96	-

Table 5: Interspecific divergence (%) calculated in COI gene sequences of different species of Aeshnidae based on this study and of those that were sourced from GenBank

S. No.	Species (Accession No.)	Species (Accession No.)	Interspecific Divergence (%)
1	Anaciaeschna jaspidea (MN182751)	Anaciaeschna Donaldi (LC466163)	8.0
2	Anaciaeschna jaspidea (MN182751)	Anaciaeschna martini (LC466165)	8.3
3	Anax ephippiger (MW729731)	Anax congoliath (KU565910)	14.0
4	Anax ephippiger (MW729731)	Anax gladiator (KU565914)	11.8
5	Anax ephippiger (MW729731)	Anax guttatus (MW880926)	8.8

6	Anax ephippiger (MW729731)	Anax immaculifrons (MG544869)	9.7
7	Anax ephippiger (MW729731)	Anax indicus (MZ014028)	2.1
8	Anax ephippiger (MW729731)	Anax nigrofasciatus nigrolineatus (MW239175)	8.5
9	Anax ephippiger (MW729731)	Anax parthenope (MN207192)	9.4
10	Anax ephippiger (MW729731)	Anax rutherfordi (KU565919)	9.4
11	Anax ephippiger (MW729731)	Anax speratus (KU565930)	10.0
12	Anax ephippiger (MW729731)	Anax tristis (KU565931)	9.1
13	Anax guttatus (MW880926)	Anax congoliath (KU565910)	10.6
14	Anax guttatus (MW880926)	Anax gladiator (KU565914)	10.0
15	Anax guttatus (MW880926)	Anax immaculifrons (MG544869)	9.7
16	Anax guttatus (MW880926)	Anax indicus (MZ014028)	8.8
17	Anax guttatus (MW880926)	Anax nigrofasciatus nigrolineatus (MG544870)	4.6
18	Anax guttatus (MW880926)	Anax parthenope (MN207192)	6.0
19	Anax guttatus (MW880926)	Anax rutherfordi (KU565919)	7.5
20	Anax guttatus (MW880926)	Anax speratus (KU565930)	7.7
21	Anax guttatus (MW880926)	Anax tristis (KU565931)	3.5

Table 6: Interspecific divergence (%) calculated in COI gene sequences of different species of Macromiidae based on this study and of those that were sourced from GenBank

S. No.	Species (Accession No.)	Species (Accession No.)	Interspecific Divergence (%)
1	Epophthalmia vittata (MW509733)	Epophthalmia elegans (KF257073)	14.2
2	Macromia ellisoni (MW911301)	Macromia amphigena (KF257059)	19.3
3	Macromia ellisoni (MW911301)	Macromia flavicincta (MW730517)	20.5
4	Macromia ellisoni (MW911301)	Macromia manchurica (KF257082)	19.9
5	Macromia ellisoni (MW911301)	Macromia moorei (MW454917)	22.5
6	Macromia ellisoni (MW911301)	Macromia moorei (MW730533)	22.9
7	Macromia flavicincta (MW730517)	Macromia amphigena (KF257059)	20.3
8	Macromia flavicincta (MW730517)	Macromia manchurica (KF257082)	18.6
9	Macromia flavicincta (MW730517)	Macromia moorei (MW454917)	7.9
10	Macromia flavicincta (MW730517)	Macromia moorei (MW730533)	7.6
11	Macromia moorei (MW454917)	Macromia amphigena (KF257059)	23.3
12	Macromia moorei (MW454917)	Macromia manchurica (KF257082)	20.8
13	Macromia moorei (MW730533)	Macromia amphigena (KF257059)	23.0
14	Macromia moorei (MW730533)	Macromia manchurica (KF257082)	20.5

Discussion

Phyogenetic analysis based on COI gene on 36 species of family Aeshnidae (Rehn, 2003 [23]; Damm *et al*., 2010 [6]; Karthika *et al*., 2012 [13]; Bergmann *et al*., 2013 [2]; Kohli *et al*., 2014 [17], 2018; Dijkstra *et al*., 2015; Casas *et al*., 2017 [4]; Almansoori *et al*., 2019 [1]; Conniff *et al*., 2019 [5]; Ma *et al*., 2020; Galimberti *et al*., 2020 [10]; Bybee *et al*., 2021 [3]; Maggioni *et al*., 2021 [21]; Mehmood *et al*., 2021) [22] and on 5 species of family Macromiidae (Kim *et al*., 2014 [14], 2018) has been reported. Previously, only one species Anaciaeschna jaspidea of family Aeshnidae has been analysed on the basis of COI gene from India (Karthika *et al*., 2012) [13]. 23 COI sequences referable to 16 sequences of 10 species under family Aeshnidae and 7 sequences of 4 species under family Macromiidae have been submitted to GenBank. These include three species (Anax immaculifrons, Anax indicus and Gynacantha bainbriggei) of family Aeshnidae and four species (E. vittata, M. ellisoni, M. flavicincta, M. moorei) of family Macromiidae, while sequences of 5 species A. ephippiger, A. guttatus, A. nigrofasciatus nigrolineatus, A. parthenope, G. subinterrupta of family Aeshnidae have been submitted for the first time from India.

Genetic divergence based on variable, parsimony informative, conserved sites, nucleotide base composition of COI gene fragment and transversion/transistional bias have been calculated for both the families. Earlier, Kim *et al*. (2014) [14] reported the nucleotide base composition in suborder Anisoptera as A = 30.9%, T = 33.7%, C = 18.5% and G = 17% with high AT content (64.6%). Similarly, nucleotide base composition of family Aeshnidae as A = 29.9%, T = 35.7%, C = 18.0% and G = 16.4% with high AT content (65.6%) and in family Macromiidae as A = 30.3%, T = 33.2%, C = 21.0% and G = 15.4% with high AT content (63.5%) have been observed during the present study.

The intraspecific divergence as 0 - 0.7 in 11 species and the interspecific distance as 5.8 to 26.1 from Europe and Africa in family Aeshnidae (Bergmann *et al*., 2013) [2] and interspecific distance as 17.27 in family Macromiidae from Korea (Kim *et al*., 2014) [14] have been reported. In the family Aeshnidae maximum interspecific divergence 22.7% is found in the genus Gynacantha (G. bainbriggei and G. usambarica) and are distantly related, whereas minimum interspecific divergence 2.1% is observed in the genus Anax (A. ephippiger and A. indicus) and are closely related. In the genus Macromia (family Macromiidae), maximum interspecific divergence 23.3% is found between M. moorei and M. amphigena which are distantly related, whereas minimum interspecific divergence 7.6% is seen in M. moorei and M. flavicincta which shows close relationships. In phylogenetic analysis, Anaciaeschna and Gynacantha genera are closely related as compared to Anax and Gynacantha genera. Phylogenetic tree also depicts the monophyletic origin of both the families. Species of the two families of this study possess distinct barcodes and matched with sequences of congeneric species vindicating the usefulness of COI gene in the identification of species. The evolutionary analysis using 37 nucleotide sequence of species of families Aeshnidae and Macromiidae was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.73425567 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

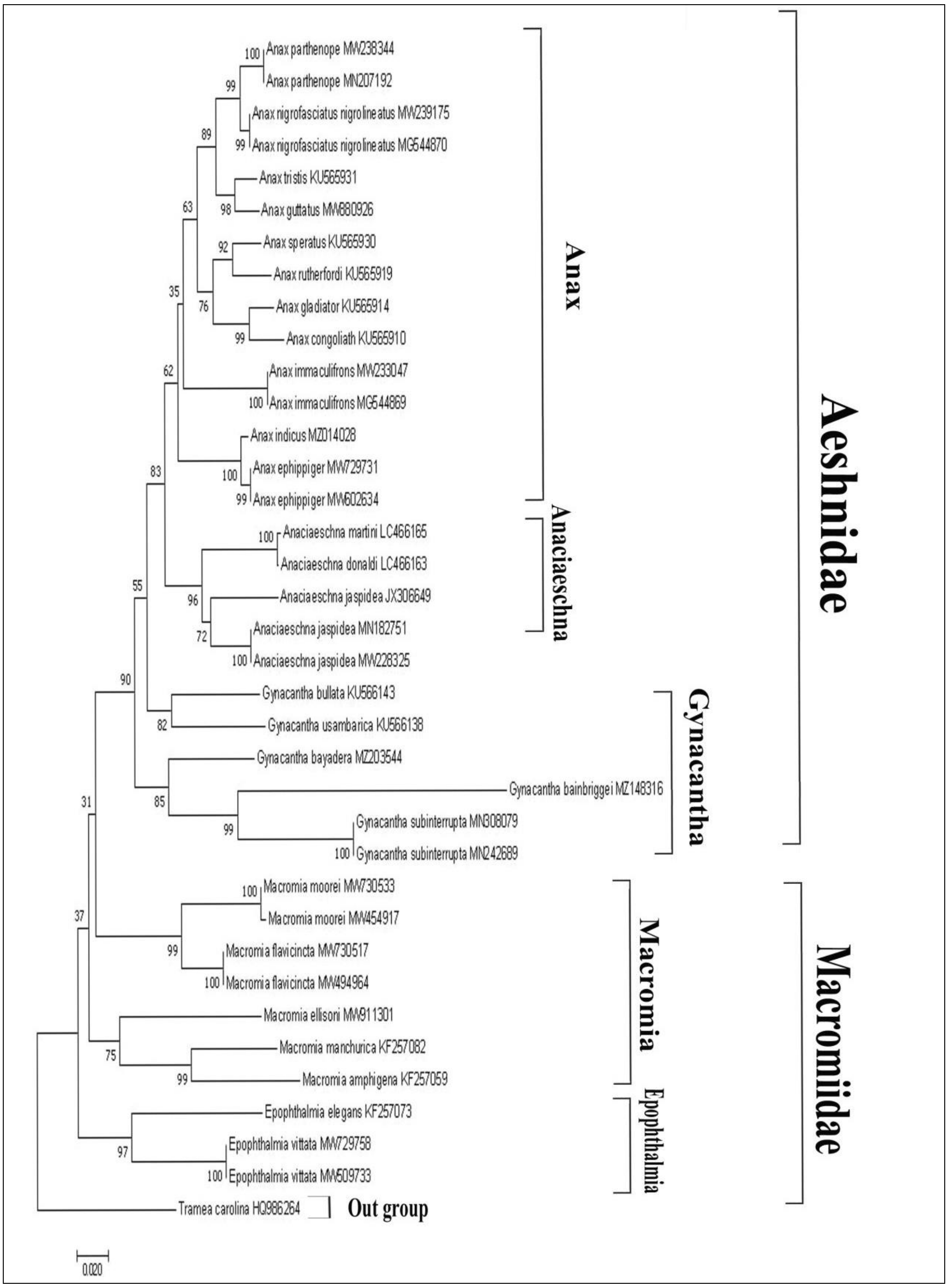

Fig 1: Evolutionary relationships of taxa

Conclusion

The objective of this study was to document the evolutionary relationships among the species and genera using 23 COI sequences, 16 of which correspond to 10 species in the family Aeshnidae and 7 sequences to 4 species in the family Macromiidae. The COI gene fragment's nucleotide base composition, transversion/transistional bias, variable, parsimony informative, conserved sites, and nucleotide divergence have all been calculated to identify closely related or distantly related species in both families. The phylogenetic tree also shows that both families are monophyletic in origin. The COI gene is helpful in the identification of species because all currently investigated species have unique barcodes that can be matched with congeneric species sequences.

Contribution

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