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Cytological Study of Family Aeshnidae (Odonata: Anisoptera) From India: A Review

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Cytological review of 59 aeshnid species and cytogenetic investigations on Anax ephippiger, Anax immaculifrons, Anax indicus, Anax nigrofasciatus nigrolineatus, Anax parthenope, Gynacantha subinterrupta of the family Aeshnidae by carbol fuchsin staining and C - banding have been under taken. All the species posses 2n = 27m with X0 - XX sex determination except Anax ephippiger with 2n = 14 + neo XY, resulted by the 13 simultaneous fusions among the autosomes and between autosome and sex chromosome. The structure and behaviour of chromosomes, variation in size of m chromosomes and X chromosome and distribution of C - heterochromatin have been studied and compared among the species. C - bands are mostly present at the terminal regions of autosomal bivalents, while Anax ephippiger and Anax parthenope also possess C - bands at the interstitial and sub-terminal regions of the bivalents. Moreover, sex chromosome and m bivalent show variation in distribution of C-heterochromatin in the species. Out of these, chromosome complement of Anax indicus Lieftinck, 1942 and C - banding on Anax ephippiger and Anax indicus have been investigated for the first time. List of cytologically studied species of family Aeshnidae has been updated to 60 species.

Keywords: Anisoptera; Aeshnidae; Chromosome complement; C - heterochromatin; Micro chromosomes (m); Sex determination.

Family Aeshnidae (Anisoptera) includes large and vigorous dragonflies known as hawkers or darners or aeshnids. Aeshnids (Greek: '*aeschna*' means 'ugly') are homogeneous in shape and their markings are nonmetallic with variable colors. Taxonomically, family Aeshnidae includes 54 genera, 480 species all over world, while 13 genera representing 49 species are available in India (Subramanian and Babu, 2017). Cytogenetic data pertaining to 59 species under the genera *Aeshna*, *Anaciaeschna*, *Anax*, *Andaeschna*, *Austroaeschna*, *Basiaeschna*, *Boyeria*, *Caliaeschna*, *Castoraeschna*, *Cephalaeschna*, *Coryphaeschna*, *Gynacantha*, *Gynacanthaeschna*, *Oplonaeschna*, *Planaeschna*, *Remartinia, Rhionaeschna* and *Staurophlebia* have been reviewed which also includes 11 species from India (Table 2). Presently, cytogenetic investigations on *Anax ephippiger*, *Anax immaculifrons*, *Anax indicus*, *Anax nigrofasciatus nigrolineatus*, *Anax parthenope* and *Gynacantha subinterrupta* by carbol fuchsin staining and C - banding have been attempted. All the species posses 2n = 27m with X0 - XX sex determination except *Anax ephippiger* with 2n = 14 + neo XY sex determination. Distribution of C - heterochromatin has been observed and compared

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among the species. Chromosome complement of *Anax indicus* (Lieftinck, 1942) and C - banding on *Anax ephippiger* (Burmeister, 1839) and *Anax indicus* (Lieftinck, 1942) has been analysed for the first time. List of cytologically studied species of family Aeshnidae has been updated to 60 species.

MATERIALS AND METHODS

Male specimens of *Anax ephippiger*, *Anax immaculifrons*, *Anax indicus*, *Anax nigrofasciatus nigrolineatus*, *Anax parthenope* and *Gynacantha subinterrupta* were captured from different localities from India (Table 1). Alive specimens were dissected in 0.67 % saline solution (Sodium chloride in distilled water) in the field and testes were removed from the abdomen. Subsequently, the testes were put in sodium citrate (0.9 %) for 45 minutes and then fixed in freshly prepared Carnoy's fixative (3 parts absolute alcohol : 1 part glacial acetic acid) and tapped on grease - free slides. Slides were proceeded for carbol fuchsin staining (Carr and Walker, 1961) and C - banding (Sumner, 1972). Relevant meiotic stages were micro- photographed for further cytogenetical investigations.

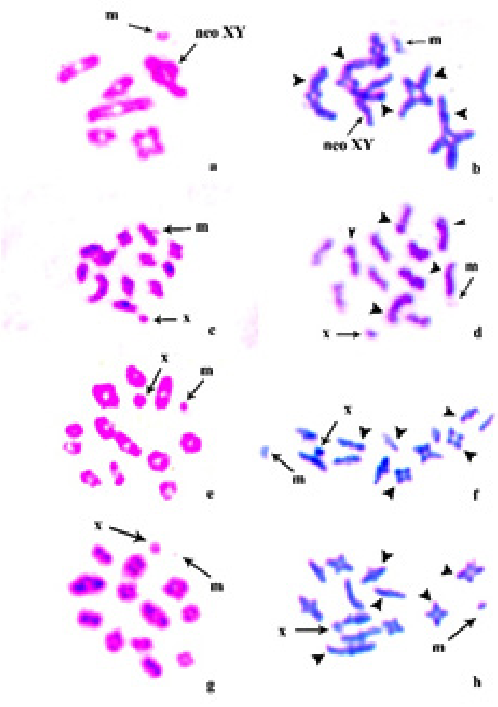

Fig. 1. *Anax ephippiger* Normal complement 1a. Diakinesis, C - banding 1b Diplotene. *Anax immaculifrons* Normal complement 1c. Diakinesis, C - banding 1d Metaphase - I. *Anax indicus* Normal complement 1e. Diakinesis, C - banding 1f. Diakinesis. *Anax nigrofasciatus nigrolineatus* Normal complement 1g. Diakinesis, C - banding 1h Diakinesis. Arrows show the neo XY, X chromosome and m bivalent. Arrowhead shows C - bands. Bar = 0.01 mm.

RESULTS

Carbol fuchsin staining

Chromosome complement of *Anax immaculifrons* (Fig. 1c), *Anax indicus* (Fig. 1e), *Anax nigrofasciatus nigrolineatus* (Fig. 1g), *Anax parthenope* (Fig. 2a), *Gynacantha subinterrupta* (Fig. 2c) show 2n (B&) = 27 (24A+2m+X0), while the only exception is *Anax ephippiger* with 2n (B&) = 14 (10A + 2m + neo - XY) resulted by the 13 simultaneous fusions in the complement (Fig. 1a). Moreover, variation in the size of sex chromosome and m chromosomes is observed. X chromosome is 2nd smallest in *Anax nigrofasciatus nigrolineatus*, *Gynacantha subinterrupta*, while it is medium sized in *Anax immaculifrons*, *Anax parthenope* and is of large sized in *Anax indicus* and *Anax ephippiger* (neo - XY). The size of m bivalent is slightly smaller than X chromosome in *Anax parthenope* (Fig. 2a), minute in *Anax*

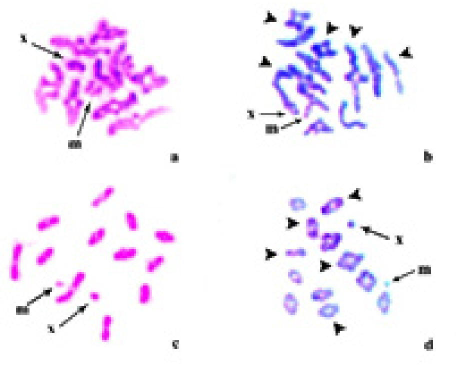

Fig. 2. *Anax parthenope* Normal complement 2a. Diakinesis, C - banding 2b Diakinesis. *Gynacantha subinterrupta* Normal complement 2c. Diakinesis, C - banding 2d. Diplotene. Arrows show X chromosome and m bivalent. Arrowhead shows C - bands. Bar = 0.01 mm

Table 1. Collection details of species of family Aeshnidae

S. No.	Species	Common Name	Locality	Latitude	Logitude	Altitude	Month/Year
1	*Anax ephippiger* (Burmeister, 1839)	Vagrant emperor	Sangrur(Punjab)	30° 36' 95" N	75° 86' 13" E	240m	July, 2020
2	*Anax inmmaculifrons*Rambur, 1842	Magnificent emperor	Andretta (Himachal Pradesh)	32° 03' 50" N	76° 33' 46" E	1301m	May, 2018
3	*Anax indicus* Lieftinck, 1942	Lesser Green Emperor	Nagpur (Maharashtra)	21° 14' 58" N	79° 08' 82" E	310m	September, 2018
4	*Anax nigrofasciatusnigrolineatus* Fraser, 1935	Blue - spotted Emperor	Dal lake, (Himachal Pradesh)	32° 24' 71" N	76° 18' 38" E	1775m	June, 2018
5	*Anax parthenope*(Seyls, 1839)	Lesser emperor	Patiala (Punjab)	30° 30' 95" N	76° 31' 76" E	257m	May, 2019
6	*Gynacantha subinterrupta* Rambur, 1842	Dingy dusk hawker	Andretta (Himachal Pradesh)	32° 03' 50" N	76° 33' 46" E	1301m	September, 2017

immaculifrons (Fig. 1c), *Anax indicus* (Fig. 1e), *Anax nigrofasciatus nigrolineatus* (Fig. 1g), *Gynacantha subinterrupta* (Fig. 2c) and small in *Anax ephippiger* (Fig. 1a) (Table 3).

C banding

During diplotene, in *Anax ephippiger*, cross shaped autosomal bivalent are showing the terminal, sub - terminal and interstitial C - bands, m bivalent possesses less amount of C - heterochromatin, while neo - XY bivalent is cross shaped and showing interspersed C - bands in (Fig. 1b). Similarly, in *Anax parthenope*, bivalents show terminal and interstitial C - bands, while m bivalent possesses less amount of C - heterochromatin and X chromosome reveals terminal C - bands (Fig. 2b). During the diakinesis, *Anax indicus* (Fig. 1f), *Anax nigrofasciatus nigrolineatus* (Fig. 1h) and *Gynacantha subinterrupta* (Fig. 2d) and metaphase - I, *Anax immaculifrons* (Fig. 1d) autosomal bivalents possess terminal C - bands, while m bivalent is C - negative except in *Anax nigrofasciatus nigrolineatus* (Fig. 1h) with less amount of C - heterochromatin and X chromosome is entirely C - positive in all the species (Table 4).

DISCUSSION

Cytogenetic data pertaining to 60 species (including *Anax indicus* of present study) of family Aeshnidae have been reviewed. Chromosome number in males varies from 2n = 14 - 27, resulted by the fusion of chromosomes (Fig. 3). The species are differentiated on the basis of chromosome numbers as 2n = 14, 15, 19, 21, 23, 24 (1species each); 2n = 16 (2 species each); 2n = 25 (4 species each); 2n = 26 (4 species each) and 2n = 27 (44 species). Most frequent chromosome number is 2n = 27 which is present in 73.3% of the species and considered as the type number of the family (Table 2).

Kiauta (1967a - d) discussed the evolution of chromosome number in odonate species. He considered n = 9 as ancestral chromosome number and divided dragonflies' chromosomes into two groups, one with high - n complements (n = 9 to 15) and second with low - n complements (n = 3 to 7). He explained that size of chromosomes of high - n species is smaller than the size of low - n species. He found the low - n complement in tropical species, while high - n complement in

Table 2. List of cytogenetically examined species of the family Aeshnidae. Nomenclature is based on 'World Odonata List' by Paulson *et al*. (2022)

No.	Name of species	Locality	Chromosome complement	Sex	m chromosomes determination	References
1	*Aeshna caerulea*(Strom,1783)	Finland	n = 12	neo - XY	Absent	Oksala, 1943
2	*Aeshna canadiensis*Walker, 1908	U. S. A.	n = 14	X0	Present	Cruden, 1968
3	*Aeshna clepsydra*Say, 1839	U. S. A.	n = 14	X0	Present	Hung, 1971
4	*Aeshna crenata*Hagen, 1856	Finland	n = 14	X0	Present,	Oksala, 1943;Perepelov and Bugrov, 2002
		Russia	n = 14	X0	Absent	
5	*Aeshna cyanea*(Muller, 1764)	Finland	n = 14	X0	Present	Oksala, 1943;Kiauta, 1969b
		Netherlands	n = 14	X0	Present	
6	*Aeshna grandis*(Linnaeus, 1758)	U. S. S.	n = 14	X0	Present	Fuchsowna and Sawczynska, 1928;Makalowskaja,
		RU.	n = 13	X0	Present	1940;Oksala, 1939, 1943, 1944, 1945;Kiauta,
		S. S. R	n = 13	neo - XY	Present	1967a - d, 1968a - b, 1969b;Perepelov and
		Finland	n = 132	neo - XY	Present	Bugrov, 2002;Nokkala *et al*., 2002
		Netherlands	n = 26	neo - XY	Present	
		Russia	n = 13	X0	Absent	
		Finland				
7	*Aeshna isoceles* (Muller, 1767)	U. S. A.	n = 14	X0	Absent	Kiauta, 1978 under the name *Anaciaeschna isosceles* (Muller, 1767)
8	*Aeshna juncea*(Linnaeus, 1758)	U. S. S. R.	n = 13	neo - XY	Present	Makalovskaja, 1940;Oksala, 1943;Kiauta,
		Finland	n = 13	neo - XY	Present	1971;Perepelov and Bugrov, 2002
		Italy	n = 142	X0	Present	
		Russia	n = 26	neo - XY	Present	
9	*Aeshna mixta* Latreille, 1805	Netherlands	n = 14	X0	Present	Kiauta, 1969b;Sandhu and Malhotra, 1994;
		India	n = 13	X0	Present	Sharma and Durani, 1995;Perepelov
		India	n = 13	X0	Present	and Bugrov, 2002
		Russia	n = 14	X0	Present	
10	*Aeshna nigroflava*Martin, 1908	Japan	n = 14	X0	Present	Katatani, 1987;Perepelov and Bugrov, 2002
		Russia	n = 14	X0	Absent	
11	*Aeshna palmata*Hagen, 1856	U. S. A.	n = 14	X0	Present	Cruden, 1968
12	*Aeshna serrata*Hagen, 1856	Finland	n = 13	neo - XY	Present	Oksala, 1943 under the name *Aeshna osiliensis fennica*Mierzejewski, 1913 and *Aeshna serrata fennica*Valle, 1938
13	*Aeshna subarctica*Walker, 1908	U. S. A.	n = 14	X0	Present	Oksala, 1943, 1952 under the name *Aeshna subarctica*
		Switzerland	n = 14	X0	Present	*elisabethae*Djakonov, 1922;Kiauta and Kiauta, 1980
14	*Aeshna umbrosa*Walker, 1908	U. S. A.	n = 14	X0	Absent	Cruden, 1968 under the name *Aeshna umbrosa occidentalis*Walker, 1908 and *Aeshna umbrosa umbrosa*Walker, 1908
15	*Aeshna verticalis*Hagen, 1861	U. S. A.	n = 14	X0	Present	Hung, 1971
16	*Aeshna viridis*Eversman, 1836	Finland	n = 13	neo - XY	Present	Oksala, 1943;Perepelov *et al*., 1998
		Russia	n = 13	neo - XY	Present	
17	*Aeshna walkeri*Kennedy, 1917	U. S. A.	n = 14	X0	Present	Cruden, 1968
18	*Anaciaeschna jaspidea*	India	n = 13	X0	Present	Walia and Sandhu, 1999

	(Burmeister, 1839)					
19	*Anax amazili*(Burmeister, 1839)	Argentina	n = 14	X0	Absent	Capitulo *et al*., 1991;Mola *et al*., 1999
		Argentina	n = 14	X0	Present	
20	*Anax concolor*Brauer, 1865	Suriname	n = 14	X0	Present	Kiauta, 1979
21	*Anax ephippiger* (Burmeister, 1839)	India	n = 7	XY	Present	Seshachar and Bagga, 1962 under the name *Hemianax ephippiger* (Burmeister, 1839); Present study
22	*Anax guttatus* (Burmeister, 1839)	Nepal	2n = 15	X0	Present	Kiauta and Kiauta, 1982
23	*Anax immaculifrons* Rambur, 1842	India	n = 14	X0	Present	Sangal and Tyagi, 1982;Walia *et al*., 2018
		India	n = 14	X0	Present	Present study
		India	n = 14	X0	Present	
24	*Anax imperator*Leach, 1815	France	n = 14	X0	Present	Kiauta, 1965, 1969b;Wasschner, 1985;Perepelov and Bugrov, 2002
		Kenya	n = 14	X0	Absent	
		Russia	n = 14	X0	Present	
25	*Anax indicus*Lieftinck, 1942	India	n = 142	X0	Present	Present Study
26	*Anax junius*(Drury, 1773)	U. S. A.	n = 14	X0	Present	McGill, 1904, 1907;Lefevre and McGill, 1908;
		U. S. A.	n = 14	X0	Present	Kichijo, 1942; Kiauta, 1972c;Cruden, 1968
		Japan	n = 14	X0	Present	
		U. S. A.	n = 14	X0	Present	
27	*Anax longipes*Hagen, 1861	U. S. A.	n = 14	X0	Present	Cruden, 1968
28	*Anax nigrofasciatus nigrolineatus* Fraser, 1935	Nepal	n = 14	X0	Present	Kiauta, 1975;Sandhu and Malhotra, 1994;
		India	n = 13	X0	Present	Walia and Sandhu, 1999;Walia *et al*., 2018;
		India	n = 14	X0	Present	Present study
		India	n = 14	X0	Present	
		India	n = 14	X0	Present	
29	*Anax papuensis*(Burmeister, 1839)	Australia	n = 14	X0	Present	Kiauta, 1968c, 1969b under the name *Hemianax papuensis*(Burmeister, 1839)
30	*Anax parthenope*(Selys, 1839)	Japan	n = 14	X0	Present	Omura,1957 under the name *Anax parthenope*
		India	n = 14	X0	Present	*julius*Brauer, 1865;Thomas and Prasad, 1986;
		China	n = 14	X0	Present	Zhu and Wu, 1986;Suzuki and Saitoh, 1990;
		Japan	n = 14	X0	Present	Sandhu and Malhotra, 1994;Present study
		India	n = 13	X0	Present	
		India	n = 13	X0	Present	
31	*Andaeschna unicolor* (Martin, 1908)	Bolivia	n = 14	X0	Present	Cumming, 1964 under the name *Aeschna cf. uncolor* Martin, 1908
32	*Austroaeschna anacantha* (Tillyard, 1908)	Australia	n = 14	X0	Present	Kiauta, 1968c under the name *Acanthaeschna anacantha* (Tillyard, 1908)
33	*Austroaeschna multipunctata* (Martin, 1901)	Australia	n = 14	X0	Present	Kiauta, 1968c under the name *Acanthaeschna Multipunctata* (Martin, 1901)
34	*Basiaescha janata*(Say, 1839)	U. S. A.	n = 13	X0	Absent	Cruden,1968
35	*Boyeria maclachlani*(Selys, 1883)	Japan	n = 14	X0	Present	Omura, 1957
36	*Boyeria vinosa*(Say, 1839)	U. S. A.	n = 14	X0	Absent	Cruden, 1968
37	*Caliaeschna microstigma* (Schneider, 1845)	Greece	n = 8	neo - XY	Present	Kiauta, 1972b
38	*Castoraeschna castor*	Brazil	n = 14	X0	Present	Kiauta, 1972a

	(Brauer, 1865)					
39	*Cephalaeshna orbifrons* Selys, 1883	Nepal	n = 13	X0	Present	Kiauta, 1975
40	*Cephalaeshna sp.*	India	n = 13	X0	Present	Sandhu and Malhotra, 1994
41	*Coryphaeschnaadnexa* (Hagen, 1861)	Bolivia	n = 14	X0	Absent	Cumming, 1964
42	*Coryphaeschnaperrensi* (McLachlan, 1887)	Argentina	n = 13	X0	Absent	Capitulo *et al*., 1991;Mola *et al*., 1999; De Gennaro *et al*., 2008
		Argentina	n = 14	X0	Present	
		Argentina	n = 14	X0	Present	
43	*Coryphaeschnaviriditas* Calvert, 1952	Suriname	n = 12	X0	Absent	Kiauta, 1979
44	*Gynacantha bayadera* Selys, 1891	India	n = 14n = 13	X0	Present	Walia, 2007 under the name *Gynacantha milliardi*Fraser, 1936
45	*Gynacantha hyalina*Selys, 1882	India	n = 14	X0	Present	Tyagi, 1978a, b
46	*Gynacatha interioris* Williamson, 1923	Suriname	n = 13	neo - XY	Present	Kiauta, 1979;Ferreira *et al*., 1979
		Brazil	n = 13	neo - XY	Present	
47	*Gynacantha japonica* Bartenev, 1909	Japan	n = 14	X0	Present	Omura, 1957
48	*Gynacantha subinterrupta* Rambur, 1842	India	n = 14	X0	Present	Walia and Somal, 2019;Present study
		India	n = 14	X0	Present	
49	*Gynacanthaeschna sikkima* (Karsch, 1891)	India	n = 13	X0	Present	Walia *et al*., 2016
50	*Oplonaeshna armata* (Hagen, 1861)	Mexico	n = 14	X0	Present	Kiauta, 1970
51	*Planaeschna milnei* (Selys, 1883)	Japan	n = 14	X0	Present	Kiauta, 1968c, 1969b
52	*Remartinia luteipennis* (Burmeister, 1839)	Suriname	n = 13	X0	Present	Kiauta, 1979 under the name *Coryphaeschnaluteipennis luteipennis* (Burmeister, 1839)
53	*Rhionaeschna bonariensis* (Rambur, 1842)	Argentina	2n = 26	neo - XY	Absent	Mola and Papeschi, 1994;Mola, 1995 as *Aeschna bonariensis*Rambur, 1842
		Argentina	n = 13	neo - XY	Present	
54	*Rhionaeshna californica* (Calvert, 1895)	Canada	n = 14	X0	Present	Kiauta, 1973 under the name *Aeshna californica* (Calvert, 1895)
55	*Rhionaeshna confusa* (Rambur, 1842)	Argentina	n = 14	X0X0	Present	Mola and Papeschi, 1994 under the name *Aeshna confusa*Rambur, 1842Mola, 1995
		Argentina	n = 14		Present	
56	*Rhionaeshna diffinis* (Rumbur, 1842)	Bolivia	n = 11	X0	Present	Cumming, 1964 under the name *Aeshna diffinis diffinis*Rumbur, 1842
57	*Rhionaeshna intricata* (Martin, 1908)	Bolivia	n = 10	X0	Present	Cumming, 1964 under the name *Aeshna intricata*Martin, 1908
58	*Rhionaeshna peralta*(Ris, 1918)	Bolivia	n = 14	X0	Present	Cumming, 1964 under the name *Aeshna peralta*Ris, 1918
59	*Rhioneschna planaltica* (Calvert, 1845)	Argentia	n = 8	neo - XY	Present	Mola and Papeschi, 1994 under the name *Aeschna cornigera planaltica* Calvert, 1952
60	*Staurophlebia reticulata* (Burmeister, 1839)	Brazil	n = 14	X0	Present	Souza Bueno, 1982 under the name*Staurophlebia reticulata reticulata* (Burmeister, 1839)

Table 3. Morphological characterization of chromosome complements in the species of families Aeshnidae

No.	Name of species	Chromosomal complement	Conventional staining Size of X chromosome	Size of m chromosomes	Variation in complement
1	*Anax ephippiger* (Burmeister, 1839)	2n (B&) = 14 (10A+2m+ neo - XY)	X is largest Y is medium sized	Small sized	2n = 14 with neo - XY complement originated by the 13 simultaneous fusions between autosomes and sex chromosome with an autosome.
2	*Anax immaculifrons* Rambur, 1842	2n (B&) = 27 (24A+2m+X)	Medium sized	Minute sized	
3	*Anax indicus* Lieftinck, 1942	2n (B&) = 27 (24A+2m+X)	Largest	Minute sized	
4	*Anax nigrofasciatus nigrolineatus* Fraser, 1935	2n (B&) = 27 (24A+2m+X)	2nd smallest	Minute sized	
5	*Anax parthenope* (Seyls, 1839)	2n (B&) = 27 (24A+2m+X)	Medium sized	Slightly smaller than X chromosome	
6	*Gynacantha subinterrupta* Rambur, 1842	2n (B&) = 27 (24A+2m+X)	2nd Smallest	Minute sized	

Table 4. Distribution of C - heterochromatin in the species of family Aeshnidae

No.	Name of species	Distribution of C - heterochromatin Autosomes	m chromosome	Sex chromosomes
1	*Anax ephippiger* (Burmeister, 1839)	Dark terminal, subterminal and interstitial C - bands on 5 bivalents.	less amount of C - heterochromatin	Cross shaped neo - XY bivalent with interspersed C - bands
2	*Anax immaculifrons* Rambur, 1842	Dark terminal C - bands on 9 bivalents.Light terminal C - bands on 3 bivalents.	C - negative	C - positive
3	*Anax indicus* Lieftinck, 1942	Dark terminal C - bands on 8 bivalents.Light terminal C - bands on 4 bivalents.	C - negative	C - positive
4	*Anax nigrofasciatus nigrolineatus* Fraser, 1935	Dark terminal C - bands on 9 autosomal bivalents.Light terminal C - bands on 3 bivalents.	less amount of C - heterochromatin	C - positive
5	*Anax parthenope* (Seyls, 1839)	Dark terminal C - bands on 7 bivalents.Light terminal C - bands on 5 bivalents.	less amount of C - heterochromatin	Tserminal C - bands
6	*Gynacantha subinterrupta* Rambur, 1842	Dark terminal C - bands on 6 bivalents.Light terminal C - bands on 6 bivalents.	C - negative	C - positive

temperate region species based on geographical distribution. He also explained that in Odonata breaks lead to haploid numbers 10 to 15 and fusions lead to haploid numbers 3 to 8 from the ancestral chromosome number n = 9.

During the present study, all the species of family Aeshnidae show 2n = 27, (26A+X) which is the type number of the family as earlier reported (Table - 2). The only exception is *Anax ephippiger* with 2n = 14, (10A+2m+neo - XY) which originated by the 13 successful fusions between autosomes and autosome with sex chromosome. The complement of the species is stable because same complement in the species has been reported by Seshachar and Bagga (1962).

Micro chromosomes and their size

Micro chromosomes (m) are considered as cytogenetic marker of the order Odonata. Absence or presence of m chromosomes depicts the taxonomic status of a species. McGill (1904) observed the presence of m chromosomes in chromosome complement of *Anax junius* for the first time in the family Aeshnidae. Later, Oguma (1930) proposed the "m chromosome theory" and considered m chromosome as an autosome which undergoes gradual diminution in volume until they disappear. Later, Dasgupta (1957) and Cumming (1964) supported the theory, but Kiauta (1968a) discarded the theory and considered m chromosomes as fragment of autosome which is present in 80% of the odonate species. He further explained that accidental breaks can occur at any time in the holocentric chromosomes which is responsible for the variation in the size of m chromosomes.

In the family Aeshnidae, out of 60 cytogenetically studied species, m chromosomes are present in 45 species, while they are absent in 15 species. Presently, all the 6 species of the family show the presence of m chromosomes in the complement (Table 2). Variation in the size of m chromosomes can serve as the identifying feature of odonate species and to differentiate closely related species of the same genus. Presently, variation in the size of m chromosomes has been recorded. Size of m bivalent is slightly smaller than X chromosome in *Anax parthenope* (Fig. 2a), minute in *Anax immaculifrons* (Fig. 1c), *Anax indicus* (Fig. 1e) *Anax nigrofasciatus nigrolineatus* (Fig. 1g), *Gynacantha subinterrupta* (Fig. 2c) and small in *Anax ephippiger* (Fig. 1a) (Table - 3).

Size and behaviour of sex chromosomes

In Odonata, majority of the species possess the XX(@&)/X0(B&) sex determining mechanism (Fig. 4). In the family Aeshnidae, Out of 60 species, 50 species possess XX(@&)/X0(B&) sex mechanism, while 10 species show neo - XY sex determining mechanism, originated by the fusion of sex chromosome with an autosome (Fig. 4). 16.6% species possess neo - XY sex determining mechanism which is very high in the family Aeshnidae as compared to other families of the order.

During the present study, *Anax immaculifrons* (Fig. 1c), *Anax indicus* (Fig. 1e), *Anax nigrofasciatus nigrolineatus* (Fig. 1g), *Anax parthenope* (Fig. 2a) and *Gynacantha subinterrupta* (Fig. 2c) show XX/X0 sex mechanism, while *Anax*

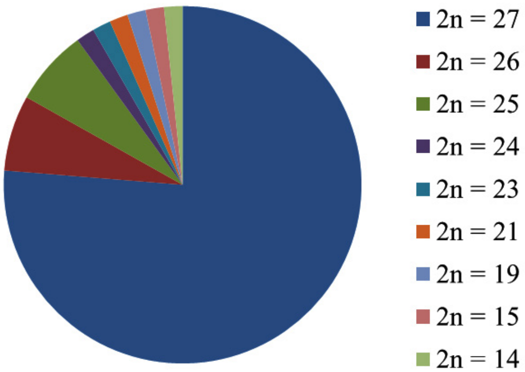

Fig. 3. Different chromosome numbers present in the species of family Aeshnidae

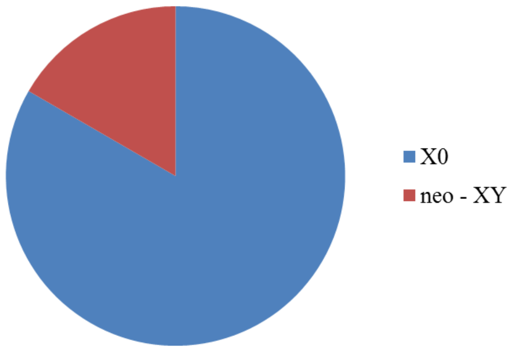

Fig. 4. Sex determining mechanism in species of family Aeshnidae

ephippiger possesses neo - XY (Fig. 1a) as earlier reported (Seshachar and Bagga, 1962). They observed largest X chromosome and medium sized Y chromosome in the chromosome complement and explained that almost 13 centric fusions occurred in *Hemianax ephippiger*, which decreased the chromosome number from 27 to 14 and one fusion occurred between X chromosome and an

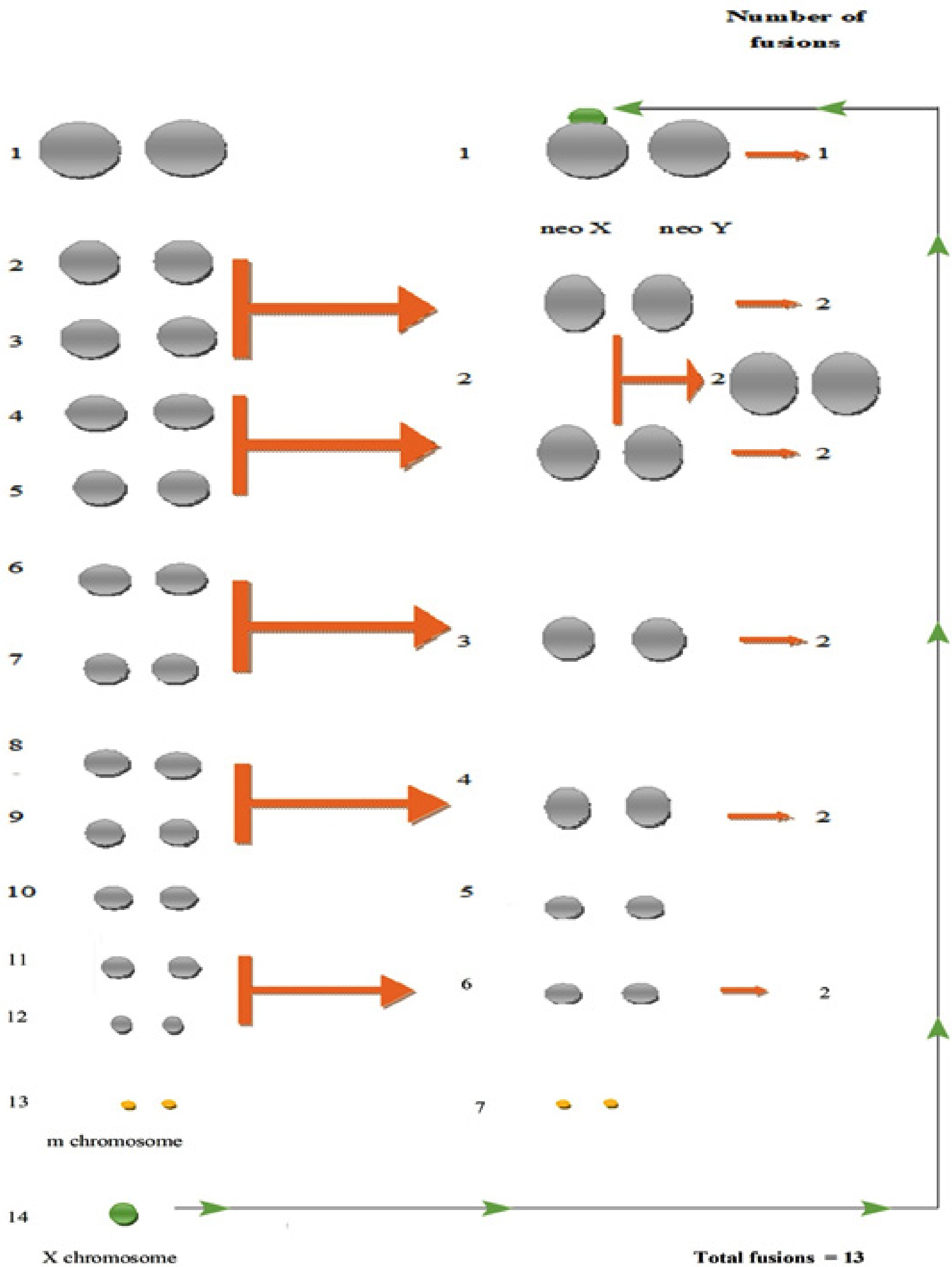

Fig. 5. Schematic presentations for the evolution of chromosome number and sex determining mechanism in *Anax ephippiger*

autosome. Similar results have been found in the species. Schematic presentations for the evolution of chromosome number and sex determining mechanism in *Anax ephippiger* (Burmeister, 1839): 2n = 14, (10A+2m+neo - XY) is established (Fig. 5).

Size of the X chromosome is peculiar feature of the species and is variable in different species. Majority of the researcher are silent as to the size of X chromosome, but few reports have recorded the size of X chromosome as smallest element in *Anax amazili* (Mola *et al*., 1999) and in *Aeshna nigroflava* (Pereplov and Bugrov, 2002), while as second largest element in *Aeshna crenata* (Pereplov and Bugrov, 2002). Presently, X chromosome is 2^{nd} smallest in the complement of *Anax nigrofasciatus nigrolineatus*, *Gynacantha subinterrupta*, while it is medium sized in *Anax immaculifrons*, *Anax parthenope* and large sized in *Anax indicus* and neo - XY of *Anax ephippiger* (Table 3).

C - banding

In the family Aeshnidae, C - banding has been reported on 11 species (Thomas and Prasad, 1986; Perepelov *et al*., 1998; Perepelov and Bugrov, 2002; Nokkala *et al*., 2002; Walia *et al*., 2016, 2018; Walia and Somal, 2019). They found terminal C - bands on autosomal bivalents and X chromosome is mostly C - positive. Presently, C - banding on 6 species of family Aeshnidae have been under taken. C - bands are mostly present at the terminal regions, while amount of C - heterochromatin varies in the species. Moreover, distribution of C - heterochromatin in m bivalent and X chromosome shows variations. The m bivalent is C - negative in *Anax immaculifrons*, *Anax indicus* and *Gynacantha subinterrupta*, while possesses less amount of C - heterochromatin in *Anax ephippiger*, *Anax nigrofasciatus nigrolineatus* and *Anax parthenope*. On the other hand, X chromosome is C - positive in *Anax immaculifrons*, *Anax nigrofasciatus nigrolineatus*, *Anax indicus* and *Gynacantha subinterrupta*, while shows terminal C - bands in *Anax parthenope*. *Anax ephippiger* and *Anax parthenope* possess different C - banding pattern as C - bands are present on the sub-terminal and interstitial regions of autosomal bivalents, while cross shaped neo - XY bivalent in *Anax ephippiger* shows interspersed C - bands (Table 4).

Presence of C-heterochromatin on the terminal regions is due to the localization of centromeric activity at the terminal regions of the bivalents which is necessary for the segregation of chromosomes during division and is peculiar feature of holocentric chromosomes present in Odonata and in other insect groups. Cytogenetic analysis on *Anax indicus* (Lieftinck, 1942) has been attempted for the first time and C - banding of *Anax ephippiger* (Burmeister, 1839) and *Anax indicus* (Lieftinck, 1942) has been studied for the first time. List of cytologically studied species of family Aeshnidae has been updated to 60 species.

CONCLUSION

Chromosome complement and C - banding of six species of family Aeshnidae have been done and list of cytologically studied species of family has been updated to 60 species. All species have 2n = 27m with X0 - XX sex determination except *Anax ephippiger* with 2n = 14 + neo XY resulted by the 13 simultaneous fusions between the autosomes and autosome with sex chromosome. C-heterochromatin distribution has been compared among the species. C - bands are primarily seen at the terminal regions of autosomal bivalents, while *Anax ephippiger* and *Anax parthenope* also have C - bands in the interstitial and sub-terminal sections of the bivalents. Additionally, the distribution of C-heterochromatin for sex chromosome and m bivalent varies in the species.

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