J. Adv. Zool. 2021: 42(1) : 107-117 **ISSN-0253-7214**

CHROMOSOME CHARACTERIZATION OF FOUR CALOPTERYGID DAMSELFLIES WITH CYTOGENETIC REVIEW OF FAMILY CALOPTERYGIDAE (ODONATA: ZYGOPTERA)

Gurinder Kaur Walia* and Neha Katnoria**
*****Associate Professor in Department of Zoology and Environmental Sciences,
Punjabi University, Patiala-147 002, INDIA
E-mail : gurinderkaur_walia@yahoo.co.in [*corresponding author]
******Department of Zoology and Environmental Sciences,
Punjabi University, Patiala, INDIA.
E-mail : neha29katnoria@gmail.com

ABSTRACT: Taxonomically, in family Calopterygidae, 183 species under 21 genera have been reported worldwide. Out of these, cytogenetic data pertains to only 22 species which is only 12% of the known species. In India, 9 species under 6 genera are present, while only 2 species has been studied cytogenetically. The present study has been conducted to linearly characterize the chromosomes of 4 species (*Matrona nigripectus*, *Neurobasis chinensis*, *Vestalis apicalis* and *Vestalis gracilis*) of family Calopterygidae by conventional staining, C-banding, silver nitrate staining and sequence-specific staining and also compiled the cytogenetic data of the family. The species were collected from Meghalaya, Goa, Kerala, Himachal Pradesh states of India. All the species possesses 2n=25m as the diploid chromosome number with XO-XX sex determination except *Neurobasis chinensis* with 2n=23, characterized by the presence of two equal sized large autosomal bivalents originated by the autosome fusion. C-banding and silver nitrate staining results depict the presence of C-bands and NOR's on the terminal positions of autosomal bivalents, while X chromosome and m bivalent show variation in distribution of C-heterochromatin and NOR's. Sequence-specific staining represents the complement of all the species as AT-rich due to more DAPI bright signals. All the cytogenetically studied species have been catalogued including the presently studied species and the list has been updated to 23 species.

KEYWORDS: Odonata; Zygoptera; Calopterygidae; Conventional staining; C-banding; Silver nitrate staining; Sequence-specific staining.

INTRODUCTION

The damselflies of this family are called as birds of paradise because of their beautiful metallic coloured wings. They are also known as broad-winged damselflies having long and slender in shape and usually found near flowing water. *Neurobasis chinensis* is the only species of the genus, present in most of the regions of the Asia continent. So far, 22 species under the genera *Calopteryx*, *Hetaerina*, *Matrona*, *Mnais*, *Neurobasis*, *Phaon* and *Vestalis* of the family Calopterygidae have been reported cytogenetically, among these, *Neurobasis chinensis chinensis* (2n=23m) and *Vestalis apicalis* (2n=25m) are from India (Table-1). Majority of the species except *Neurobasis chinensis* possess n=13 which is considered as the type number of the family Calopterygidae. However, 2 species show variation in the complement due to fragmentation as *Calopteryx virgo meridionalis* with n=13m and $14m^{16}$ and *Hetaerina rosea* with n=13 and $14m^{6}$. During the present study, chromosome

Table 1. Cytologically studied species of family Calopterygidae

S. No.	Taxa	Locality	Chromosome number	References
	Subfamily-Calopteryginae			
1	*Atrocalopteryx atrata* (Selys, 1853)	Japan	n=13(m)	Reported as *Calopteryx atrata* Selys, 1853; Oguma (1930), Kichijo (1942a), Omura (1957)
2	*Calopteryx aequabilis* (Selys, 1839)	USA	n=13(m)	Cruden (1968)
3	*Calopteryx cornelia* (Selys, 1853)	Japan	n=13(m)	Reported as *Anaciagrion cornelia* (Selys, 1853); Oguma (1930), Kichijo (1942b)
4	*Calopteryx dimidiata* Burmeister, 1839	Florida, United States	n=13m	Kiauta and Van Brink (1978)
5	*Calopteryx japonica* Selys, 1869	Japan	n=13m	Kichijo (1942b), Hirai (1956), Omura (1957)
6	*Calopteryx maculata* (Beaurois, 1805)	Bolivia USA	n=13m	Cumming (1964), Cruden (1968)
7	*Calopteryx splendens* (Harris, 1780)	Turkey	n=13m	Reported as *C.s. amasina* Bartenev, 1912; Kiauta (1972)
		Italy	n=13m	Reported as *C.s.caprai* Conci, 1956; Kiauta (1971 b)
		Former USS	n=13	Reported as *C.s.splendens* (Harris, 1782); Makalowskaja (1940),
		Finland Italy France	n=13	Oksala (1945), Kiauta (1969, 1971a), Kiauta (1973),
		Russia	n=13m	Pereplov *et al.* (1998), Kuznetsova *et al.* 2020
8	*Calopteryx virgo* (Linnaeus, 1758)	Spain	n=13m n=14m	Reported as *C.v. meridionalis* Selys (1873); Kiauta (1971a)
		Slovenija	n=13m	Reported as *C. v. padana Conci*, 1956; Kiauta (1967, 1968a, c),
		Austria Belgium Finland Germany Netherlands	n=13m	Kiauta (1967, 1968 c) Reported as *C.v.virgo* (Linnaeus, 1758) Carnoy (1885), Oksala (1945), Kiauta (1968a, b), Kiauta (1972),
		Russia	n=13m	Kuznetsova *et al.* (2020)

9	*Matrona basilaris* Selys, 1853	Taiwan	n=13(m)	Kiauta (1968c)
10*#	*Matrona nigripectus*	India	n=13m	Present study
11	*Mnais costalis* Selys, 1869	Japan	n=13(m)	Oguma (1930), Kichijo (1942b)
12	*Mnais pruinosa* Selys, 1853	Japan	n=13(m)	Reported as *M. strigata* Selys, 1853; Oguma (1930), Kichijo (1942b), Omura (1957)
13*	*Neurobasis chinensis* (Linnaeus, 1758)	Nepal	n=12	Reported as *N. chinensis chinensis* (Linnaeus, 1758);
			n=13	Kiauta (1975)
		India	n=12	Tyagi (1982),
		Nepal	n=12m	Kiauta and Kiauta (1982),
		Thailand	n=12	Kiauta and Kiauta (1983),
		India	n=12m	Walia and Sandhu (2002),
		India	n=12	Walia *et al.* (2016), Walia and Katnoria (2018)
14	*Phaon iridipennis* (Burmeister, 1839)	Republic of South Africa	n=13m	Boyes *et al.* (1980)
15*	*Vestalis apicalis* Selys, 1873	India	n=13m	Walia and Kaur (2011)
16*	*Vestalis gracilis* (Rambur, 1842)	Thialand	n=13m	Kiauta and Kiauta (1983)
	Subfamily-Hetaerininae			
17	*Hetaerina americana* (Fabricius, 1798)	Bolivia, USA	n=13m	Cumming (1964), Cruden (1968)
18	*Hetaerina charka* Calvett, 1909	Bolivia	n=13m	Cumming (1964)
19	*Hetaerina longipes* (Hagen in Selys, 1853)	Brazil	n=13m	Reported as *H. carnifex* Hagen in Selys, 1853; Souza Bueno (1982)
20	*Hetaerina rosea* Selys, 1853	Bolivia	n=14m	Cumming (1964)
		Brazil	n=13, 14 m	Ferreira *et al.*(1978)
21	*Hetaerina sanguina* Selys, 1853	Bolivia	n=13	Cumming (1964)
22	*Hetaerina titia* (Drury, 1773)	Bolivia	n=13m	Cumming (1964)
		Mexico	n=13m	Reported as *H. tricolor* (Burmeister, 1839); Kiauta (1970)
23	*Hetaerina vulnerata* (Selys, 1853)	Mexico	n=13m	Kiauta (1970)

* Represents presently studied species.

\# Represent first cytogenetic report on the species.

complements of 4 species of subfamily Calopteryginae of family Calopterygidae (*Matrona nigripectus, Neurobasis chinensis, Vestalis apicalis* and *Vestalis gracilis*) have been analysed based on structure and behaviour of chromosomes during meiosis, distribution of C-heterochromatin and NOR's and AT-GC sequence specificity. Chromosome complement of *Matrona nigripictus* has been studied for the first time. Moreover, C-banding and sequence specific staining report on three species and silver nitrate staining on 2 species has been done for first time. Only conventional staining and C-banding have been performed on *Vestalis gracilis* because of limited number of specimens and slides.

MATERIALS AND METHODS

Male damselflies of family were collected from running water bodies and from plants twigs around water bodies present in different states (Meghalaya, Goa, Kerala, Himachal Pradesh) of India, during the years 2016-2018 (Table-2). Live damselflies testes were removed by dissecting in 0.67% saline solution and were kept in sodium citrate (0.9%) for 45 minutes. After that, testes were fixed in freshly prepared Carnoy's fixative (3:1, absolute alcohol: glacial acetic acid) for 15 minutes then two more changes of the fixative each of 15 minutes was given. After that, testes were teased on clean and dust-free slides. Slides were air-dried and stored in the refrigerator for Conventional staining[3], C-banding[34], Silver nitrate staining[9] and Sequence specific staining[32].

RESULTS AND DISCUSSION

Conventional staining

At the diakinesis of *Matrona nigripectus*, *Vestalis apicalis* and *Vestalis gracilis*, 13 elements are seen, among these, 12 are autosomal bivalents including m bivalent and one oval shaped X chromosome (Figs. 1a, 3a, 4a), while in the diakinesis of *Neurobasis chinensis*, 12 elements are visible, among these, 11 are autosomal bivalents and one is oval shaped X chromosome. m bivalent is absent in this species and two large autosomal bivalents are seen in meiotic stages, among these, one bivalent shows terminal chiasma, while other possesses interstitial chiasma (Fig. 2a). By the metaphase-I, condensation and terminalization of chiasma make the bivalents dumbbell shaped, while X chromosome and m bivalent are distinct (Figs. 1b, 2b, 3b, 4b). In *N. chinensis*, X chromosome is mostly present at the periphery (Fig. 2b).

C- banding

In the diakinesis and metaphase-I, all the autosomal bivalents show C-bands at terminal position on both the ends in all the species (Figs. 1c, d, 2c, d, 3c, d, 4c, d), while in *N. chinensis* 2 large bivalents also possess interstitial C-bands (Figs, 2c, d). X chromosome is C-positive and m bivalent reveals less amount of C-heterochromatin in *M. nigripectus* and *Vestalis gracilis*, C-negative in *V. apicalis* and absent in *N.*

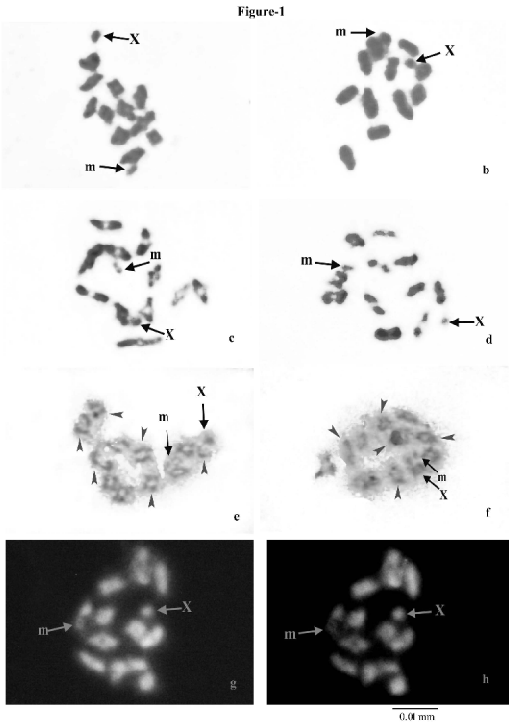

Fig. 1. ***Matrona nigripectus,*** **Conventional staining**: **a.** diakinesis. **b.** metaphase-I. **C-banding: c.** diakinesis. **d.** metaphase-I. **Silver nitrate staining**: **e.** diakinesis. **f.** metaphase-I. **Sequence specific staining**: **e. f.** diakinesis.

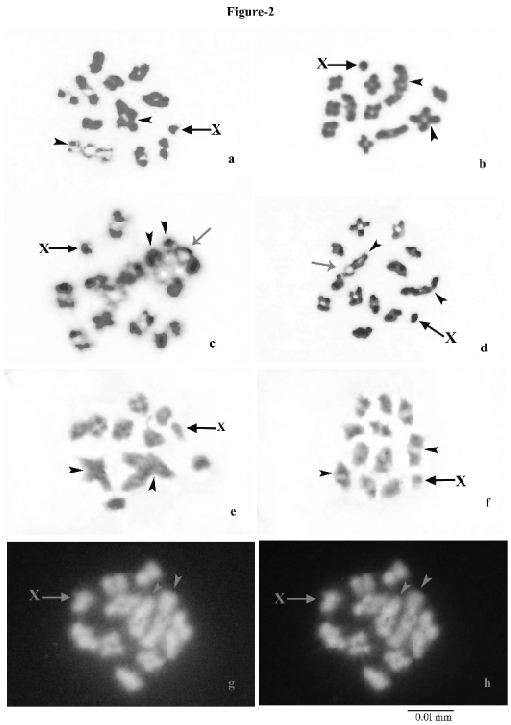

Fig. 2. ***Neurobasis chinensis,*** **Conventional staining**: **a.** diakinesis. **b.** metaphase-I. **C-banding: c.** diakinesis. **d.** metaphase-I. **Silver nitrate staining**: **e.** diakinesis. **f.** metaphase-I. **Sequence specific staining**: **e. f.** diakinesis.

chinensis.

Silver nitrate staining

At the diakinesis and metaphase-I, all the autosomal bivalents of *N. chinensis*, 6 bivalents of *M. nigripectus* and 7 bivalents of *V. apicalis* show NOR's on both the terminal ends, while 5 bivalents of *M. nigripectus* and 4 bivalents of *V. apicalis* possess NOR on the one terminal end (Figs. 1c, d, 3 c, d). m bivalent is NOR-negative in *M. nigripectus,* while NOR rich in *V. apicalis* and absent in *N. chinensis*. X chromosome is NOR rich in all the three species (Figs. 2 a, b).

Sequence specific staining

During diakinesis and metaphase-I, autosomal bivalents except m bivalent show both DAPI and CMA_3 bright signals in *Matrona nigripectus* (Figs. 1g, h), show DAPI bright signals than CMA_3 signals on chiasmatic as well as non-chiasmatic ends in *N. chinensis* (Figs. 2a, b) and both DAPI

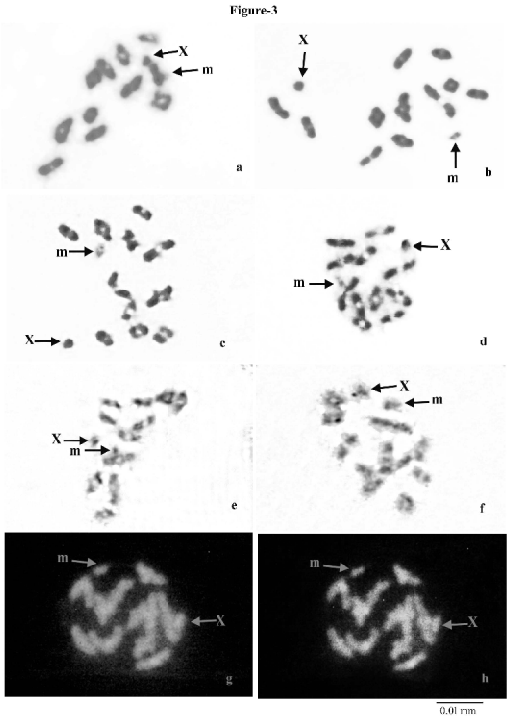

Fig. 3. ***Vestalis apicalis,*** **Conventional staining**: **a.** diakinesis. **b.** metaphase-I. **C-banding: c.** diakinesis. **d.** metaphase-I. **Silver nitrate staining**: **e.** diakinesis. **f.** metaphase-I. **Sequence specific staining**: **e. f.** diakinesis.

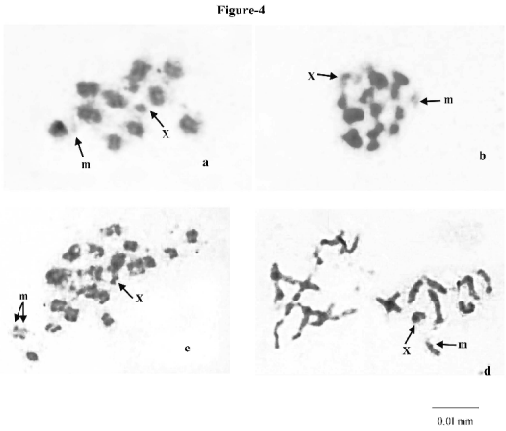

Fig. 4. ***Vestalis gracilis,*** **Conventional staining**: **a.** diakinesis. **b.** metaphase-I. **C-banding: c.** spermatogonial metaphase. **d.** diakinesis. **Bar = 0.01 mm**

and CMA_3 bright signals in *Vestalis apicalis* (Figs. 3a, b). Two large autosomal bivalents of *N. chinensis* also reveal overlapping bright DAPI than CMA_3 regions at terminal and interstitial positions as also evident in C- banding (Figs. 2a, 2b). X chromosome shows overlapping DAPI and CMA_3 bright signals in *M. nigripectus*, shows more DAPI bright signal than CMA_3 in *N. chinensis*, both DAPI and CMA_3 bright signals in *V. apicalis*. m bivalent is DAPI bright and CMA_3 dull in *M. nigripectus*, while both DAPI and CMA_3 bright in *V. apicalis* and absent in *N. chinensis.*

Type number of the family Calopterygidae is n=13 as it is present in majority of the species (Table-I). Out of cytogenetically studied species, 2 species show variation in the chromosome number as Kiauta[16] reported the chromosomal fragmentation and precocious segregation due to incomplete pairing in the chromosome complement of *Calopteryx virgo meridionalis* (n=13m, 14m) of subfamily Calopteryginae from northwestern Spain and Cumming[5] observed n=14m in *Hetaerina rosea* of subfamily Hetaerininae from Bolivia, while Ferreira *et al.*[6] noticed fragmentation (n=13, 14m) in the chromosome complement of the same species from Brazil.

Table- 2: List of species of family Calopterygidae collected during the present study:

S. No.	Name of the species	Place of Collection	Altitude (Meters) Amsl	Latitude	Longitude
1.	*Matrona nigripectus* Selys, 1879	Mukhla (Meghalaya)	1520 m	25° 21' 33.8688" N	92° 22' 52.9032" E
2.	*Neurobasis chinensis* (Linnaeus, 1758)	Solan, Andretta (Himachal Pradesh) Surla (Goa)	1550 m 1301 m 800 m	30° 54' 18" N 32° 32' 50.04" N 15° 26' 20.63" N	77° 52' 49.2" E 76° 33' 46.8" E 74° 15' 8.72" E
3.	*Vestalis apicalis* Selys, 1873	Surla (Goa) Kollam, Kozhikode (Kerala).	800 m 3 m 1 m	15° 26' 20.63" N 8° 52' 48" N 11° 15' 0" N	74° 15' 8.72" E 76° 36' 03" E 75° 46' 12" E
4.	*Vestalis gracilis* (Rambur, 1842)	Surla (Goa)	800 m	15° 26' 20.63" N	74° 15' 8.72" E

Neurobasis chinensis chinensis is the only species of the genus, present in most of the regions of Asia continent. Chromosome complement of the species is unstable because many variation in the complement has been reported by many workers as Kiauta[20] noticed n=12/13 from Nepal region and suggested that reduction in chromosome number due to the fusion of two autosomes. Later, Tyagi[35] reported n=12 without m chromosomes from Dehradun valley; Kiauta and Kiauta[21] observed n=12m from Arun valley, Eastern Nepal; Walia and Sandhu[38] noted n=12m from Himachal Pradesh, India and Walia *et al.*[40] reported n=12, without m bivalent from the Himachal Pradesh, India. Later, Walia and Katnoria[37] reported n=12 (without m bivalent) with karyomorphological variation in size of the two large bivalents from different localities of Himachal Pradesh as two equal sized, large autosomal bivalents with terminal and interstitial chiasmata have been observed in the Dehradun and Solan specimens, while one extra large autosomal bivalent and second smaller than large autosomal bivalent with single chiasma have been seen in the specimens of Bilaspur. During the present study, n=12 is observed in the *Neurobasis chinensis* from Himachal Pradesh and Meghalaya and complement is characterized by the presence of two equal sized large autosomal bivalents with two interstitial chiasmata originated by the fusion of autosomes, while remaining bivalents show single chiasma. These results are in accordance to the reports given by Walia and Katnoria[37] from the Dehradun

and Solan areas of Himachal Pradesh. In *Neurobasis chinensis,* fusions favour the survival value and reproductive capability of the species to adapt itself in the various localities. Moreover, the chromosome complement of the species from same locality (Himachal Pradesh) shows variation which depicts that species possess the unstable complement or under the process of karyotypic evolution.

In genus *Matrona* only one species *Matrona basilaris* from Taiwan with n=13m has been studied by Kiauta[13]. Present species, *Matrona nigripectus* also possesses the same chromosome number, n=13m from Meghalaya. This species has been studied for the first time.

In genus *Vestalis*, only two species, *Vestalis apicalis* and *Vestalis gracilis* have been studied. Walia and Kaur[38] described the chromosome complement of *Vestalis apicalis* (2n=25m) from Mangalore (Karnataka) and present results on the same species from Kerala are in accordance to the earlier report. Kiauta and Kiauta[22] reported n=13m in *V. gracilis* from Thialand and present results on the same species from Goa are also in accordance to this report. Chromosome complement of both the species is stable. Moreover, structure and behaviour of the chromosomes has been studied, as both the species of this genus possess very small pair of achiasmatic m chromosomes and 2nd smallest X chromosome, which is considered as the cytogenetical marker at the genus level.

C-banding

C-banding has been reported only on two species *Calopteryx* splendens[38] and *Calopteryx virgo*[26] Both the species show variation in distribution of C-heterochromatin as Pereplov *et al.*[38] observed terminal C-bands on the autosomes of *Calopteryx splendens*, while X chromosome shows dark C-heterochromatin throughout the length. However, Kuznetsova *et al.*[26] noticed wide C-heterochromatic bands at the telomeres on four bivalents including m bivalent, while all other bivalents with tiny telomeric C- bands and two distinct dark C-bands on X chromosome at each ends in *Calopteryx virgo*.

During present study, C-banding has been done on all the four species (*Neurobasis chinensis, Matrona nigripectus, Vestalis apicalis* and *Vestalis gracilis*). Moreover, variation in C-banding pattern of autosomal bivalents has been reported in *Neurobasis chinensis* as Walia *et al.*[40] observed presence of terminal C-bands on 8 autosomal bivalents including largest bivalent, while remaining bivalents are C-negative. Later, Walia and Katnoria[37] reported C-bands on terminal ends of all the bivalents, while large bivalents possess terminal as well as interstitial C-bands. During present study, all the autosomal bivalents including large bivalents are showing terminal C-bands, while 2 large bivalents also possess interstitial C-bands. Moreover, X chromosome is C-positive in all the earlier reports as well as during the present study, which depicts that X

chromosome remains stable and does not participate in the fusions occurred in the species.

In *Matrona nigripectus* and *Vestalis apicalis and Vestalis gracilis*, all the autosomal bivalents except m bivalent possess terminal C-bands, while m bivalent possesses variation in C-band pattern as it shows less amount of C-heterochromatin in *M. nigripectus* and *V. gracilis* but it is C-negative in *V. apicalis*. However, X chromosome is C-positive for all the species.

In all the species, presence of terminal C-bands on the bivalents has been observed because centromeric activity is localized at the terminal ends during the division in case of holocentric chromosomes of order Odonata. Moreover, amount of C-heterochromatin varies species to species and species of the same genus/different genera.

Silver nitrate staining

Presently, silver nitrate staining has been attempted on all the species except *V. gracilis*. Earlier, only one species *N. chinensis* has been studied by Gill[7] and Walia *et al.*[40]. They observed terminal NOR bands on all the autosomal bivalents, while X chromosome is lacking NOR's. Present results on the same species are in accordance to the earlier reports as terminal NOR's are present on all the autosomal bivalents. However, variation in localization of NOR's in the X chromosome has been observed as it is completely NOR rich.

Similarly, NOR's are mostly present at the terminal regions of autosomal bivalents, while X chromosome is NOR rich in *M. nigripectus* and *V. apicalis*. m bivalent is NOR-negative in *M. nigripectus*, while it is NOR rich in *V. apicalis*.

Sequence specific staining

So far, sequence specific staining has not been reported on any species of family Calopterygidae. During the present study, all the species except *Vestalis gracilis* has been studied to detect the presence of AT-GC rich regions. In all the species, more DAPI bright signals at the terminal regions of autosomal bivalents are seen, while in *N. chinensis*, two large autosomal bivalents also possess overlapping bright DAPI and dull CMA_3 regions at terminal and interstitial points. X chromosome also possesses overlapping DAPI/ CMA_3 signals. m bivalent is DAPI bright and CMA_3 dull in *M. nigripectus*, while it shows overlapping DAPI and CMA_3 signals in *V. apicalis*.

Sequence specific staining has been performed for the first time on all the three species. DAPI bright signals correspond to the AT regions, which also correspond with C-banding, while CMA_3 bright signals are GC rich regions which relate with the NOR's. The results of sequence specific staining depict that complement of the species is AT rich as found in other insect orders.

REFRENCES

1. Boyes, J.W., J.M. Van Brink & B. Kiauta, 1980. Sixteen dragonfly karyotypes from the republic of South Africa and Swaziland, with

evidence on the possible hybrid nature of *Orthetrum julia falsum* Longfeild (Anisoptera: Libellulidae). *Odonatologica*, 9 (2): 131-145.

2. Carnoy, B.J., 1885. La cytodierese chez les arthropods IV, Pseudo-Neuropteres. *Cellule*, 1: 279-282
3. Carr, D.H. & J.E. Walker, 1961. Carbol-fuchsin as a stain for human chromosomes. *Stain Technol,* 36: 233-236.
4. Cruden, R.W., 1968. Chromosome number of some North American dragonflies (Odonata). *Can. J. Genet. Cytol.*, 10 (1): 200-214.
5. Cumming, R.B., 1964. *Cytogenetic studies in the order Odonata,* Ph.D. thesis, University Texas, Austin.
6. Ferreira, A., B. Kiauta & A. Zaha, 1978. Male germ cell chromosomes of thirty-two Brazilian dragonflies. *Odonatologica,* 8 (1): 5-22.
7. Gill, J.K., 2014. *Cytogenetical studies on some damselflies species from Himachal Pradesh, India (Odonata: Zygoptera),* M. Phil. Dissertation, Punjabi University, Patiala.
8. Hirai, H., 1956. Chromosomes of six species of dragonflies. *Zoological Magazine,* 65: 198-202.
9. Howell, M. and D.A. Black, 1980. Controlled silver staining of nucleolar organiser regions with protective colloidal developer: I- step method. *Experientia*, 36: 1014-1015.
10. Kiauta, B., 1967. Evolution of the chromosome complement in Odonata. *Genen Phaenen*, 11 (4): 56-61.
11. Kiauta, B., 1968a. Variation in the size of m chromosome of dragonfly, *Calopteryx virgo* (L.) and its significance for the chorogeography and taxonomy of the *Calopteryx virgo* superspecies. *Genen Phaenen*, 12 (1): 11-16.
12. Kiauta, B., 1968b. Variation in the dragonfly m chromosome, with considerations on its significance for the chorogeography and taxonomy of the order Odonata and notes on the validity of the rule of Reinig. *Genetica*, 39 (1): 64-74.
13. Kiauta, B., 1968c. The chromosome numbers of eight old World dragonflies (Odonata). *Chromosome Information service*. Tokyo, 9: 3-4.
14. Kiauta, B., 1969. Sex chromosomes and sex determining mechanisms in Odonata, with review of the cytological conditions in the family Gomphidae and references to the karyotypic evolution in the order. *Genetica*, 40 (2): 127-157.
15. Kiauta, B., 1970 The chromosome numbers of four neotropic dragonflies from Mexico. *Chromosome Information Service.* 11: 8-9.
16. Kiauta, B., 1971a. An unusual case of precocious segregation and chromosome fragmentation in primary spermatocytes of the damselfly *Calopteryx virgo meridionalis* Selys, 1873, as evidence for a possible hybrid character for some populations of the *Calopteryx virgo complex* (Odonata: Zygoptera: Calopterygidae). *Genen Phaenen*, 14 (2): 32-40.
17. Kiauta, B., 1971b. Studies on the germ cell chromosome cytology of some cytotaxonomically interesting or hitherto not studied Odonata from the autonomous region Friuli-Venezia Giulia (Northern Italy). *Estratto dagli atti del Museo Civico di Storia Naturale*, Trieste, 27 (2): 65-127.
18. Kiauta, B., 1972. Synopsis on the main cytotaxonomic data in the order Odonata. *Odonatologica*, 1 (2): 73-102.
19. Kiauta, B., 1973. Notes on new or little known dragonfly karyotypes. IV. Spermatocyte chromosomes of *Calopteryx splendens splendens* Harris (Zygoptera: Calpterygidae), *Gomphus pulchellus* Selys, *Libellula depressa* Linnaeus (Anisoptera: Gomphidae, Libellulidae) from northern France. *Genen Phaenen*, 16 (2): 55-60.
20. Kiauta, B., 1975. Cytotaxonomy of dragonflies with special reference to the Nepalese fauna. Nepal Research Centre, Kathmandu. 1-78.

21. Kiauta, B. and M. Kiauta, 1982. The chromosome numbers of sixteen dragonfly species from the Arun Valley, Eastern Nepal. *Notulae Odonatologicae*, 9 (1): 143-146.

22. Kiauta, B. and M. Kiauta, 1983. The chromosome number of some Odonata from Thailand. *Notulae Odonatologicae*, 2 (2): 27-28.

23. Kiauta, B. and J.M. Van Brink, 1978. Male chromosome complements of some Florida dragonflies, United States. *Odonatologica*, 7 (1): 15-25.

24. Kichijo, H., 1942a. A comparative study of seven species of Zygoptera from Japan. *Acta. Medica. Nagasakiensia*, 3 (2): 95-97.

25. Kichijo, H., 1942b. Insect chromosomes. IV. Order of dragonflies, Pt. 2. *Nagasaki medical Journal*, 20 (10): 1639-1648.

26. Kuznetsova, V. G., A. Maryañska-Nadachowska, B. A. Anokhin, N. A. Shapoval & A. P. Shapoval, 2020. Chromosomal analysis of eight species of dragonflies (Anisoptera) and damselflies (Zygoptera) using conventional cytogenetics and FISH: insights into the karyotype evolution of the ancient insect order Odonata. *Journal of Zoological Systematics and Evolutionary Research*., 58 (1): 1–13.

27. Makalowskaja, W.N., 1940. Comparative karyological studies of dragonflies (Odonata). *Arch. Russ. d' anat., d' histol. etd'embryol.*, 25 (1): 24-39.

28. Oguma, K., 1930. A comparative study of the spermatocyte chromosome in allied species of the dragonfly. *J. Fac. Sci.*, 1 (1): 1-32.

29. Oksala, T., 1945. Zytologische Studien an Odonaten III. Die Ovogense. *Anales Academiae Scientiarum Fennicae*. (A, IV, Biologica), 9: 1-32.

30. Omura, T., 1957. A comparative study of the spermatogenesis in the Japanese dragonflies. II. Families Aeschnidae, Gomphidae and Calopterygidae. *Biol. J.* Okayama Univ., 3 (1-2): 1-86.

31. Pereplov, E., A.G. Burgov & E. Warchalowska-Sliwa, 1998. C-banded karyotypes of some dragonfly species of Russia. *Folia Biol.*, 46 (3): 137-142.

32. Rebagliati, P.J., A.G. Papeschi & L.M. Mola, 2003. Meiosis and fluorescent banding in *Edessa meditabunda* and *E. rufomarginata* (Heteroptera: Pentatomidae: Edessinae). Eur. J. *Entomol.*, 100 (1): 11-18.

33. Souza Bueno, A.M., 1982. *Estudos cromossomicos na ordem Odonata*, M. Sc. Thesis, Universi-dad Estatal Paulista.

34. Sumner, A.T., 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.*, 75 (1): 304-306.

35. Tyagi, B.K., 1978. *Studies on the chromosomes of Odonata of Dun Valley* (*Dehradun, India*), Ph. D. thesis, University of Garhwal, Srinagar.

36. Tyagi, B.K., 1982. Cytotaxonomy of the Indian dragonflies. *Indian Rev. Life Sci*., 2: 149-161.

37. Walia, G.K. and N. Katnoria, 2018. Morphological variation in the chromosome complement of *Neurobasis chinensis chinensis* of family Calopterygidae (Odonata: Zygoptera). *Int. J. Life Sci. Res.*, 6 (4): 260-266.

38. Walia, G.K. & J. Kaur, 2011. Karyological study on ten odonate species from Mangalore (Karnataka), India. *Hislopia Journal*, 4 (1): 83-88.

39. Walia, G.K. and R. Sandhu, 2002. Cytogenetical data on *Neurobasis chinensis chinensis* (Zygoptera: Calopterygidae). *Fraseria*, 7: 13-16.

40. Walia, G.K., J.K. Gill & H.K. Hallan, 2016. C-banding and Ag- NOR staining in *Neurobasis chinensis chinensis* (Linnaeus) of family Calopterygidae from Himachal Pradesh, India (Odonata: Zygoptera). *Cytologia*, 81(2): 175-178.

41. Walia, G.K., N. Katnoria & J.K. Gill, 2018. Chromosome of *Libellago lineata lineata* (Chlorocyphidae: Odonata). *Indian J. Entomol*., 80 (3): 737-740.