VOLUME 8 ISSUE 2 2022

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, No. 2, 944-956 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Karyotypic Studies on Seven Chlorocyphid Species Based on C-Bands, AgNOR's and AT-GC Regions with Review of the Hitherto Cytologically Examined Data of Family Chlorocyphidae (Odonata: Zygoptera)

Katnoria Neha* and Walia Gurinder Kaur

Department of Biosciences, Division: Zoology, Career Point University, Hamirpur 176041, Himachal Pradesh, India

**Corresponding Author*

Received: 16th November, 2022; **Accepted:** 17th December, 2022; **Published online:** 24th December, 2022

https://doi.org/10.33745/ijzi.2022.v08i02.111

Abstract: Cytogenetic characterization on seven species of family Chlorocyphidae by carbol-fuchsin staining, C-banding, silver nitrate staining and DAPI and CMA_3 staining has been done. Among these, *Aristocypha quadrimaculata, A. trifasciata, Heliocypha biforata, H. bisignata, H. perforate* and *Paracypha unimaculata* are of subfamily Rhinocyphinae and *Libellago lineata* is of subfamily Libellagininae. All the species of subfamily Rhinocyphinae possess n=12m, which is the type number of the subfamily and complement is characterized by the presence of one large autosomal bivalent, originated by the autosomal fusion. But *L. lineata* with n= 13m is the only species of subfamily Libellagininae.

C-bands and NOR's are mostly detected on terminal ends of autosomal bivalents in all the 7 species and also at interstitial regions of large bivalent. X chromosome is entirely C-positive in all the species, while it is NOR-positive in both the species of genus *Aristocypha, P. unimaculata, H. perforata*, and shows NOR on one terminal end in *H. bisignata* and *L. lineata*. m bivalent is C-negative in *H. biforata* and in both the species of genus *Aristocypha*, while shows C-bands on both the terminals in *H. perforata*, *P. unimaculata* and *L. lineata*, whereas possesses C-band on one terminal end in *H. bisignata*. m bivalent shows NOR on one terminal end in *P. unimaculata* and *L. lineata,* while NOR-negative in all the species of genera *Aristocypha* and *Heliocypha.* Complement of all the species shows bright DAPI and weak CMA_3 signals which depicts the presence of more AT rich regions than GC regions.

Keywords: Chlorocyphidae, Libellagininae., Rhinocyphinae, Carbol-fuchsin staining, C-heterochromatin, AgNOR's

Citation: Katnoria Neha and Walia Gurinder Kaur: Karyotypic studies on seven Chlorocyphid species based on C-Bands, AgNOR's and AT-GC regions with review of the hitherto cytologically examined data of family Chlorocyphidae (Odonata: Zygoptera). Intern. J. Zool. Invest. 8(2): 944-956, 2022.
https://doi.org/10.33745/ijzi.2022.v08i02.111

Introduction

Globally, more than 1000 described species under 26 families are present in the superfamily Calopterygoidea, while 51 species referable to 4 families (Calopterygidae, Chlorocyphidae, Euphaeidae and Philogangidae) are present in India. Damselflies of family Chlorocyphidae are

commonly known as jewels because of their beautiful coloured wings. They are present around freshwater streams and flowing water bodies. Taxonomically, family Chlorocyphidae includes 162 species under 20 genera worldwide, while 22 species under 8 genera are known from India. So far, cytogenetic data is available only on 9 species of family Chlorocyphidae (Chatterjee and Kiauta, 1973; Tyagi, 1978 a, b; Handa and Kochhar, 1980; Kiauta and Kiauta, 1980, 1982; Sandhu *et al*., 1994; Sandhu and Walia, 1996; Walia *et al*., 2018). The list has been updated to 11 species by including the presently studied species. Presently, structure and behaviour of chromosomes during meiosis, detection of C-bands, presence of NOR's and AT-GC rich regions have been studied for 7 species under 4 genera of two subfamilies, Rhinocyphinae (*Aristocypha quadrimaculata, A. trifasciata, Heliocypha biforata, H. bisignata, H. perforata, Paracypha unimaculata*) and Libellagininae (*Libellago lineata*) of family Chlorocyphidae. Among these, *H. bisignata* and *H. perforata* have been described cytogenetically for the first time. C-banding and Silver nitrate staining on six species of subfamily Rhinocyphinae and DAPI and CMA_3 staining in all the seven species of family Chlorocyphidae have been performed for the first time. Review of cytologically studied species has also been done. Earlier, variation in chromosome complement of *A. quadrimaculata,* n=12m from Nepal (Kiauta and Kiauta, 1982) and n=13m from India (Chatterjee and Kiauta, 1973; Sandhu *et al*., 1994; Sandhu and Walia, 1996) and of *Libellago lineata* n=14 (Kiauta and Kiauta, 1980) from Thialand, n=12, 13 (Walia *et al*., 2018) from Himachal Pradesh, India have been reported. Presently, these two species from different localities possess stable chromosome complement n=12m in *A. quadrimaculata* and n=13m in *Libellago lineata*.

Materials and Methods

Male damselflies of family Chlorocyphidae were collected from running water bodies and from plants twigs around water bodies present in different states (Meghalaya, Goa, Kerala and Himachal Pradesh) of India, during the years 2016-2018 (Table 1). Live damselflies were dissected in 0.67% saline solution to remove the testes. After that, testes were fixed in freshly prepared Carnoy's fixative (3:1 absolute alcohol: glacial acetic acid). Testes were teased on clean and dust-free slides and stored in the refrigerator for Carbol-fuchsin staining, (Carr and Walker, 1961), C-banding (Sumner, 1972), Silver nitrate staining (Howell and Black 1980) and DAPI and CMA_3 staining (Rebagliati *et al*., 2003).

Results

Carbol-fuchsin staining:

In the diakinesis of *A. quadrimaculata*, *A. trifasciata*, *H. biforata*, *H. bisignata, H. perforata* and *P. unimaculata*, 12 elements are visible, among these, 11 are autosomal bivalents and one oval shaped element is X element. One large autosomal bivalent and m bivalent are clearly distinguishable in the plates (Figs. 1a, 2a, 3a, 4a, 5a, 6a). In *L. lineata,* chromosome complement is n=13, including minute m bivalent and one X chromosome (Fig. 7a). During metaphase-I, all the elements are dumbbell shaped because of further condensation and terminalization of chiasmata, while X chromosome and m bivalent are distinct (Figs. 1b, 2b, 3b, 4b, 5b, 6b, 7b). Karyotypes of metaphase -1 plates of all the species have been prepared for comparison (Fig. 8) and their metrical analysis is given in the Table 2.

C- banding:

By diakinesis and metaphase-I, in both the species of genus *Aristocypha*, C-bands are present on both the terminal ends of 7 bivalents, while 2 bivalents possess C-band on one side in *A. trifasciata* and are C-negative in *A. quadrimaculata*. Large bivalent possesses C-band only on one side in *A. quadrimaculata*, while it shows C-bands on terminal as well as on interstitial regions in *A. trifasciata*. X chromosome is C-positive for the entire length, while m bivalent is C-negative in both the species (Figs. 1c, d, 2c, 2d). In genus *Heliocypha,* 10 bivalents in *H. biforata*, 8 bivalents in *H. bisignata* and all the bivalents including large

Table 1: List of species of family Chlorocyphidae collected during the present study

S. No.	Name of the species	Place of Collection	Altitude (Meters) Amsl	Latitude	Longitude	Date of collection
Subfamily-Rhinocyphinae						
1	*Aristocypha trifasciata* (Selys, 1853)	Chamba, Andretta, Kullu, (Himachal Pradesh)	996 m 1301 m 1279 m	32°34′12″N 32° 3′ 50.04″ N 31° 57' 28.2636'' N	76° 7′48″ E 76° 33′ 46.8″ E 77° 6' 34.0524'' E	9-6-2017 30-6-2017 3-7-2017
2	*Aristocypha quadrimaculata* (Selys, 1853)	Chamba, Andretta, Kullu (Himachal Pradesh), Neora valley, Lataguri (West Bengal), Dehradun (Uttrakhand)	996 m 1301 m 1279 m 3200 m 800 m 652 m	32°34′12″N 32° 3′ 50.04″ N 31° 57' 28.2636'' N 27° 3′ 36″ N 26° 42′ 22.62″ N 30°18'59.3856'' N	76° 7′48″ E 76° 33′ 46.8″ E 77° 6' 34.0524'' E 88° 42′ 0″ E 88° 45′ 57.91″ E 78° 1' 55.8768'' E	9-6-2017 30-6-2017 3-7-2017 24-8-2016 29-8-2016 25-6-2015
3	*Heliocypha biforata* (Selys, 1859)	Noghyllum (Meghalaya)	580 m	25°34′ 27.228″ N	91°18′ 35.5248″ E	18-10-2017
4	*Heliocypha bisignata* (Hagen in Selys, 1853)	Jowai , Noghyllum, (Meghalaya)	1380 m 580 m	25° 18′ 0″ N 25°34′ 27.228″ N	92° 9′ 0″ E 91°18′ 35.5248″ E	29-9-2017 18-10-2017
5	*Heliocypha perforata* (Percheron, 1835)	Jaintia Hills (Meghalaya)	1200 m	25° 21' 33.8688"N	92° 22' 52.9032" E	22-9-2017
6	*Paracypha unimaculata* (Selys, 1853)	Chamba , Kullu, Andretta (Himachal Pradesh)	996 m 1279 m 1301 m	32°34′12″N 31° 57' 28.2636'' N 32° 3′ 50.04″ N	76° 7′48″ E 77° 6' 34.0524'' E 76° 33′ 46.8″ E	9-6-2017 3-7-2017 30-6-2017
Subfamily-Libellagininae						
7	*Libellago lineata* (Burmeister, 1839)	Sirmour (Himachal Pradesh) Noghyllum (Meghalaya) Kollam (Kerala)	672 m 580 m 3 m	30° 36' 21.59" N 25°34′ 27.228″ N 8° 52′ 48″ N	77° 27' 17.99" E 91°18′ 35.5248″ E 6° 36′ 0″ E	18-05-2017 18-10-2017 22-04-2018

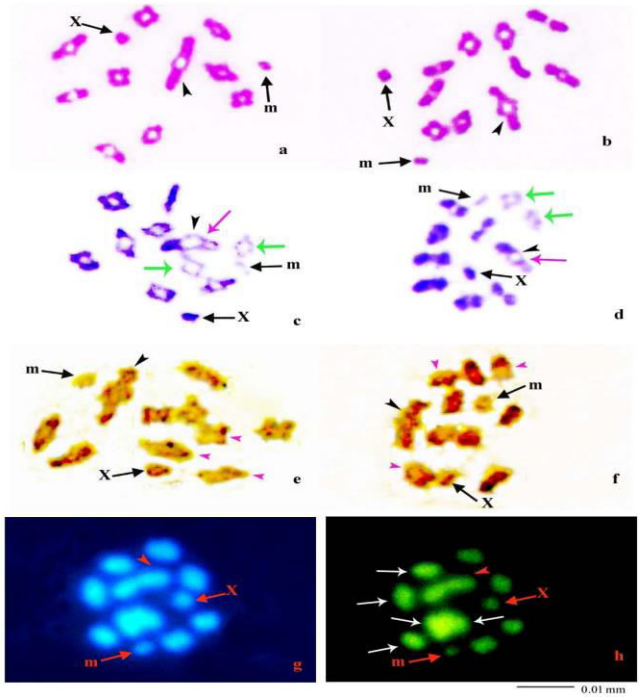

Fig. 1: *Aristocypha quadrimaculata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining),(h) Diakinesis (CMA_3 Staining).

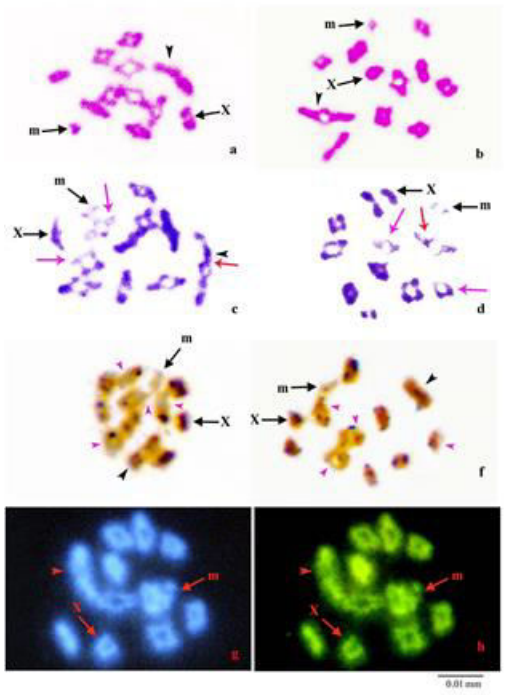

Fig. 2: *Aristocypha trifasciata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining),(h) Diakinesis (CMA_3 Staining).

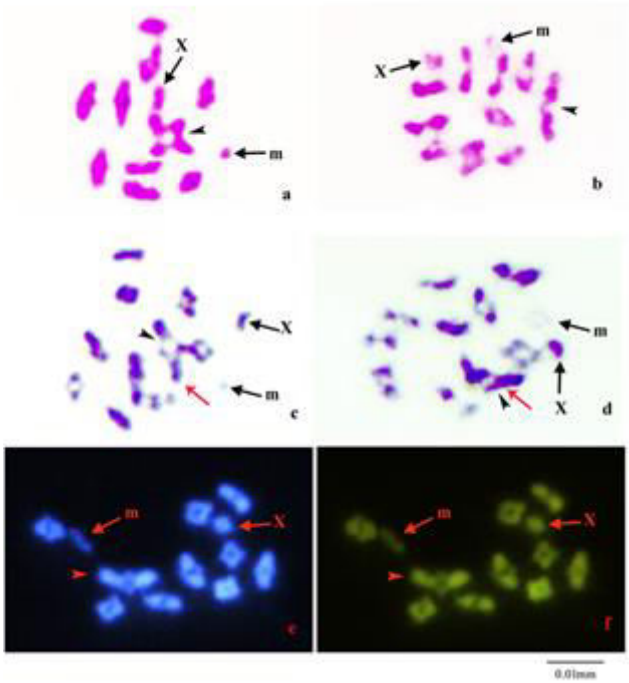

Fig. 3: *Heliocypha biforata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Sequence specific staining: (e) Diakinesis (DAPI Staining), (f) Diakinesis (CMA_3 Staining).

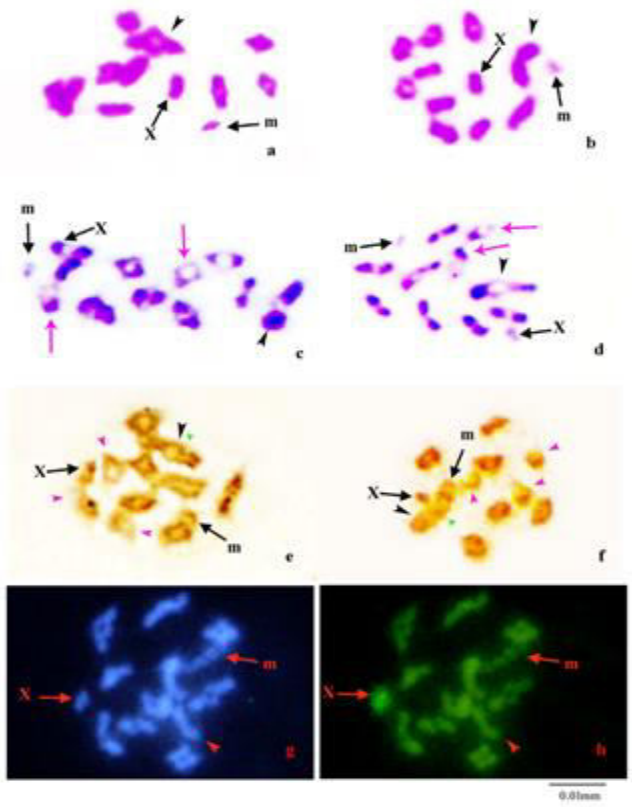

Fig. 4: *Heliocypha bisignata* : Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining),(h) Diakinesis (CMA_3 Staining).

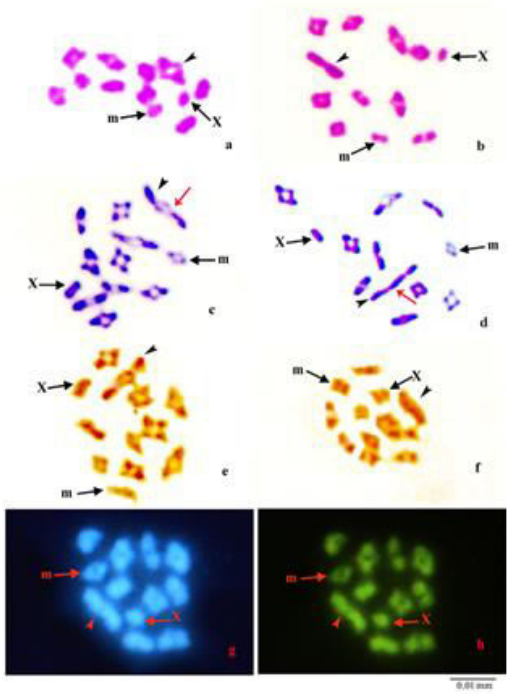

Fig. 5: *Heliocypha perforata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining),(h) Diakinesis (CMA_3 Staining).

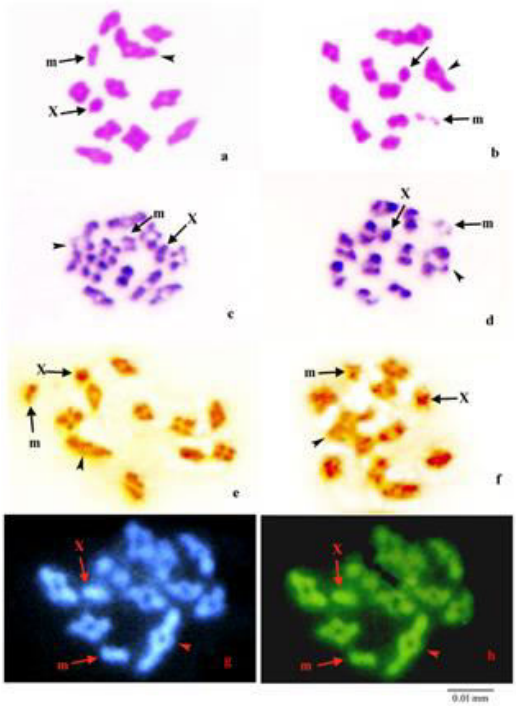

Fig. 6: *Paracypha unimaculata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining),(h) Diakinesis (CMA_3 Staining).

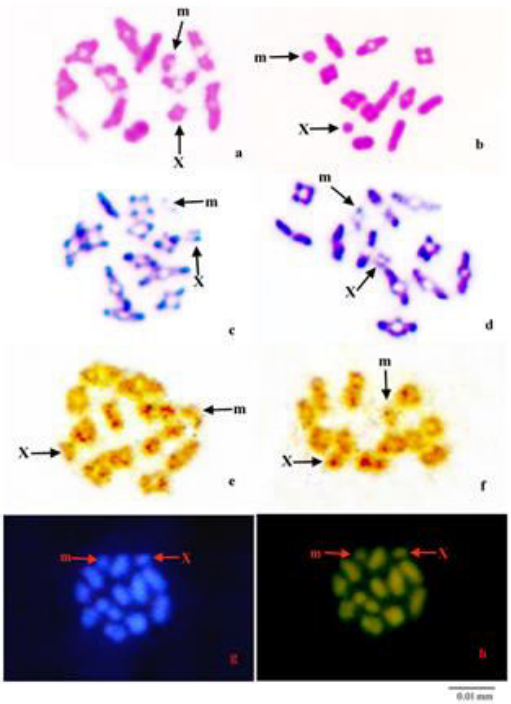

Fig. 7; *Libellago lineata*: Conventional staining: (a) Diakinesis, (b) Metaphase-I, C-banding: (c) Diakinesis, (d) Metaphase-I, Silver nitrate staining: (e) Diakinesis, (f) Metaphase-I, Sequence specific staining, (g) Diakinesis (DAPI Staining), (h) Diakinesis (CMA_3 Staining).

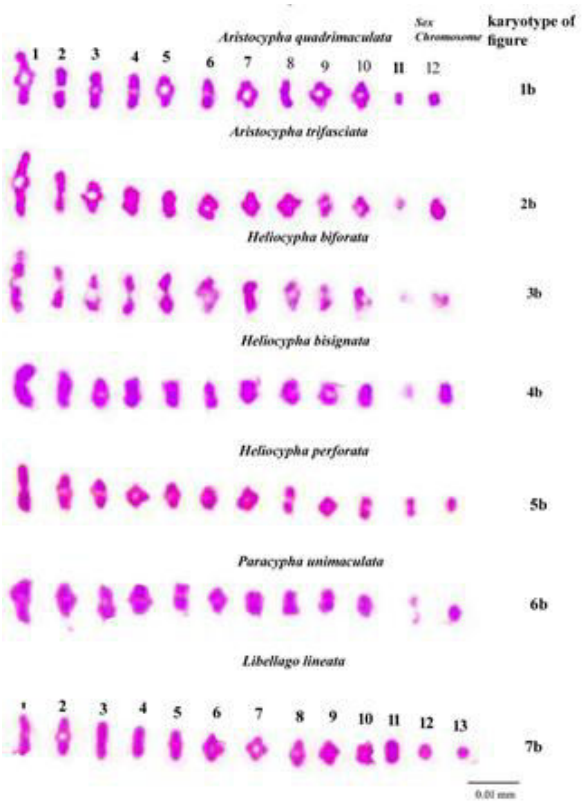

Fig. 8: Comparative karyotype of Metaphase-I chromosomes of all the seven species.

- Largest autosomal bivalent is shown by the black arrow head in all the figures except in sequence specific staining where it is shown by red arrow head**.**
- Green arrow shows the C-negative autosomal bivalent.
- Pink arrow shows the presence of C-band on one side only.
- Red arrow shows the presence of C-band on interstitial region.
- Pink arrow heads show the presence of NOR on one side.
- Green arrow head shows the presence of interstitial NOR's.
- White arrow shows the presence of bright CMA_3 regions.
-

Table 2: Comparative metrical analysis of Metaphase-I chromosomes of seven species

Homologus chromosome pairs [Length in (μm)]													
	Subfamily-Rhinocyphinae												
Species name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**X (Univ-alent**	
Aristocypha quadrimaculata	10	7.85	7.14	6.42	5.71	5.71	5.71	5.71	5.71	5.71	2.14	2.85	
Aristocypha trifasciata	11.4	7.85	6.42	5.71	4.71	4.71	4.71	4.28	4.28	3.57	1.42	4.28	
Heliocypha biforata	11.4	7.85	7.14	7.14	7.14	6.42	6	5.71	5.00	4.28	0.71	2.14	
Heliocypha bisignata	9.28	7.14	5.71	5.71	5	5	4.28	4.28	4.28	4.28	1.42	4.28	
Heliocypha perforata	8.57	7.14	5.71	4.28	4.28	4.28	4.28	4.28	3.57	3.57	2.14	2.14	
Paracypha unimaculata	8.57	6.42	6.42	5.71	5	4.28	4.28	4.28	4.28	4.28	1.42	2.85	
Subfamily-Libellagininae													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**X (Univ-alent**
Libellago lineata	7.14	7.14	7.14	5.71	5.71	5.00	4.28	4.28	4.28	4.28	4.28	2.14	2.00

bivalent in *H. perforata* show very prominent, dark C-bands on terminal regions, while large bivalent also possesses C-band on interstitial position in *H. biforata* and *H. perforata*, whereas 3 bivalents including m bivalent show C-band only on one terminal end in *H. bisignata*. X chromosome is C-positive for entire length in all the species, while m bivalent is C-negative in *H. biforata* (Figs. 3c, d, 4c, d, 5c, 5d). In *P. unimaculata* and *L. lineata*, all the autosomal bivalents are showing prominent terminal C-bands on both sides, while m bivalent shows light C-bands on both the terminals. X chromosome is C-positive throughout the length in *P. unimaculata*, while it is bipartite and shows dark C-bands on both terminal ends in *L. lineata* (Figs. 6c, d, 7c, d).

Silver nitrate staining:

During diakinesis and metaphase-I, in genus *Aristocypha*, 7 autosomal bivalents in *A. quadrimaculata* and 6 bivalents in *A. trifasciata* including largest bivalent are showing terminal NOR's, while 3 bivalents in *A. quadrimaculata* and 4 bivalents in *A. trifasciata* possess NOR only on one side. X is bipartite and NOR positive, while m bivalent is NOR-negative in both the species (Figs. 1e, f, 2e, f). In genus *Heliocypha,* 7 bivalents in *H. bisignata* and all the bivalents in *H. perforata* show NOR's on both the sides, while 3 bivalents possess NOR only on one terminal end in *H. bisignata* and largest bivalent also shows dark interstitial NOR's. X chromosome is entirely NOR-positive in *H. perforata*, while it show NOR on one terminal end in *H. bisignata*. m bivalent is NOR-negative in both the species (Fig. 3e, f, 4e, f, 5e, f). In *P. unimaculata* and *L. lineata*, all autosomal bivalents are showing NOR's at the terminal regions on both sides, while m bivalent shows dark NOR on one side. X chromosome is NOR rich in *P. unimaculata*, whereas it shows dark NOR on one side in *L. lineata* (Figs. 6e, f, 7e, f).

DAPI and CMA_3 staining:

In genus *Aristocypha*, all the autosomal bivalents show DAPI and CMA_3 bright signals at chiasmatic ends in *A. trifasciata*, while they are DAPI bright at terminal position but in *A. quadrimaculata,* 5 bivalents possess bright CMA_3 regions and 5 are dull. X chromosome is DAPI as well as CMA_3 bright, while m bivalent shows dim DAPI and CMA_3 signals in both the species (Figs. 1g, h, 2g, h). In genus *Heliocypha*, all the species show terminal DAPI bright and CMA_3 light signals on autosomal bivalents, while X chromosome is DAPI and CMA_3 bright in *H. bisignata* and *H. perforata*, but more DAPI bright than CMA_3 in *H. biforata*. m bivalent shows bright DAPI and CMA_3 signals at terminal position in *H. perforata*, while it is dull for both the dyes in *H. bisignata* and *H. biforata* (Figs. 3g, h, 4g, h, 5g, h). In *P. unimaculata*, all the autosomal bivalents including m bivalent show terminal DAPI and CMA_3 bright signals except largest bivalent which is homogeneously stained with both the dyes. X chromosome is DAPI and CMA_3 bright (Figs. 6g, h). In *L. lineata*, autosomal bivalents including m bivalent show DAPI bright and weak CMA_3 signals at chiasmatic/nonchiasmatic ends. X chromosome possesses bright DAPI and weak CMA_3 signals (Figs. 7g, h).

Discussion

So far, 9 species under two subfamilies Rhinocyphinae and Libellagininae of family Chlorocyphidae have been studied cytologically. The list has been updated to 11 species including two presently studied species (Table 3). Majority of the species possess n=12m in subfamily Rhinocyphinae which is considered as the type number of the subfamily and complement is characterized by the presence of one large bivalent originated by the autosomal fusion. Moreover, variation in chromosome complement of *A. quadrimaculata,* n=12m from Nepal (Kiauta and Kiauta, 1982), n=13m from India (Chatterjee and Kiauta, 1973; Sandhu *et al*., 1994; Sandhu and Walia, 1996) and *Rhinocypha colorata* n=12m, 13m from Philippines (Kiauta and Kiauta, 1980) have been reported.

Presently, 6 species of subfamily Rhinocyphinae (*A. quadrimaculata, A. trifasciata, H. biforata, H. bisignata, H. perforata* and *P. unimaculata*) have been studied. All the species possess n=12m including one large autosomal bivalent originated by the autosomal fusion in the primary complement 2n=25 and is considered as the type number of the subfamily (Table 3).

In genus *Aristocypha* (earlier under the genus *Rhinocypha*), both the species *A. quadrimaculata* and *A. trifasciata* from Himachal Pradesh possess n=12m with chiasmatic m bivalent and bipartite X chromosome. However, size of the X chromosome varies in both the species as it is 2nd largest in *A. trifasciata*, while 4th largest in *A. quadrimaculata*. Earlier, variation in chromosome complement has been reported in *A. quadrimaculata* as n=12m by Chatterjee and Kiauta (1973) from the Darjeeling, Himalaya and by Kiauta and Kiauta (1982) with one distinctly large bivalent from Arun valley, Eastern Nepal, while Sandhu *et al.* (1994) and Sandhu and Walia (1996) noted n=13m, with large bivalent from Himachal Pradesh, India. Present results on the same species are in accordance to the earlier reports of Chatterjee and Kiauta 1973), Kiauta and Kiauta (1982). Similarly, n=12m is reported in *A. trifasciata* during present study, which is in accordance to earlier reports of Tyagi (1978a) from Dehradun; Kiauta and Kiauta (1982) from Nepal and Sandhu and Walia (1996) from Himachal Pradesh, India.

In the genus *Heliocypha* (earlier under the genus *Rhinocypha*), three species *H. biforata, H. bisignata, H. perforata* have been described with chromosome complement n=12m. In all the species, size of X chromosome varies as it is 2nd smallest in *H. biforata*, bipartite and 2nd largest in *H. bisignata* and bipartite and medium sized in *H. perforata*. m bivalent is chiasmatic in all the species. Present results on *H. biforata* are in accordance to earlier report (Tyagi 1978a, b) from Dehradun, India. *H. bisignata* and *H. perforata* have been studied for the first time.

Table 3: Data of cytologically studied species of family Chlorocyphidae

II. FAMILY: CHLOROCYPHIDAE				
1.	*Aristocypha fenestrella* Rambur, 1842	Thialand	n=12	Reported as *Rhinocypha fenestrella* Rambur, 1842; Kiauta and Kiauta (1983)
2. *	*Aristocypha quadrimaculata* (Selys, 1853)	India Nepal	n=12m	Reported as *Rhinocypha quadrimaculata* Selys, 1853; Chatterjee and Kiauta (1973), Kiauta and Kaiuta (1982),
		India	n=13m	Sandhu *et al.* (1994), Sandhu and Walia (1996),
3.*	*Aristocypha trifasciata* (Selys, 1853)	India	n=12m	Reported as *Rhinocypha trifasciata* Selys, 1853; Tyagi (1978a), Sandhu and Walia (1996)
		Nepal		Kiauta and Kiauta (1982)
4. *	*Heliocypha biforata* (Selys, 1859)	India	n=12m	Reported *as Rhinocypha biforata besoni* Selys, 1859; Tyagi (1978a,1982), Handa and Kochhar (1980)
5.	*Heliocypha biseriata (*Selys, 1859)	Thialand	n=12	Reported as *Rhinocypha biseriata biforata* Selys, 1859; Kiauta and Kiauta (1983)
6.*	*Paracypha unimaculata* (Selys, 1879)	Nepal India	n=12 m	Reported as *Rhinocypha unimaculata* Selys, 1879; Kiauta and Kiauta (1982), Tyagi (1982)
7.	*Rhinocypha colorata* Selys, 1869	Philippines	n=12, n=13	Kiauta and Kiauta (1980)
8.	*Rhinocypha ignipennis* Selys 1879	India	n=12	Sandhu and Walia (1996)
9.*	*Libellago lineata* (Burmeister, 1839)	Thialand India	n=14 n=12,13	Reported as *Libellago lineata lineata* (Burmeister, 1839); Kiauta and Kiauta (1983), Walia *et al.* (2018)

* Represents presently studied species; # Represent first cytogenetic report on the species.

Paracypha unimaculata (earlier under the genus *Rhinocypha*) with n=12m has been reported by Kiauta and Kiauta (1982) from Arun valley, Nepal and Handa and Kochhar (1980) from Punjab, India. Similarly in the present study, n=12m has been observed for the same species from Himachal Pradesh, India.

In subfamily Libellagininae, only one species *Libellago lineata* has been studied with unstable karyotype n=12, 13, 14 originated by autosomal fragmentation. Kiauta and Kiauta (1983) reported n=14 due to autosomal fragmentation from Thialand. Later, Walia *et al.* (2018) noticed two complements n=12 and n=13, without m chromosomes resulted by autosomal fragmentation from Himachal Pradesh, India. They described the chromosomes morphologically as 6 medium sized, 5 small sized, one largest

autosomal bivalent and X chromosome in n=13 complement, while 7 medium sized, 3 small sized, one largest autosomal bivalent and X chromosome in n=12 complement. They suggested that variation in the chromosome number is due to the fragmentation of the medium sized autosomal bivalent. In the present study, n=13m without large autosomal bivalent has been observed in the same species from Himachal Pradesh, India. These results indicated that karyotype of the species is unstable because species has been collected from the same locality (Himachal Pradesh) by Walia *et al.* (2018).

C-banding:

Presently, C-banding has been done on 6 species of subfamily Rhinocyphinae. C-bands are mostly found on terminal ends of bivalents and also present at interstitial regions of large bivalent, while amount of C- heterochromatin varies from species to species. X chromosome is entirely C-positive in all the species, while m bivalent show variation in distribution of C-heterochromatin as it is C negative in both the species of genus *Aristocypha*, shows C-bands on both the terminals in *H. perforata* and *P. unimaculata* and C-band on one terminal end in *H. bisignata*.

In *Libellago lineata* of subfamily Libellagininae, all the autosomal bivalents including m bivalent possess terminal prominent C-bands on both the sides and X chromosome also shows dark C-bands on both terminals. However, variation in C-heterochromatin in the same species has been observed by Walia *et al*. (2018). They found C-positive regions at chiasmatic and non-chiasmatic ends on 9 autosomal bivalents, while remaining 3 bivalents show less amount of C-heterochromatin but large autosomal bivalent reveals dark C- band only on one side, whereas X chromosome possesses less amount of C-heterochromatin.

The presence of terminal C-bands on the bivalents are due to the localization of centromeric activity at the terminal ends during the division, which is considered as the characterstic feature of holocentric chromosomes present in odonates, heteropteran and homopteran insects (Walia and Katnoria, 2017, 2018, 2021; Walia *et al*., 2018; 2022; Katnoria and Walia, 2022). C-banding in all the six species of subfamily Rhinocyphinae has been attempted for first time.

Silver nitrate staining:

Silver nitrate staining has been attempted on 5 species of subfamily Rhinocyphinae. All the autosomal bivalent show NOR's on one/both sides of terminal regions, while X and m bivalent show variation in loacalization of NOR's. X chromosome is NOR-positive in both the species of genus *Aristocypha, P. unimaculata, H. perforata*, while it shows NOR on one terminal end in *H. bisignata*. m bivalent shows NOR on one terminal end in *P. unimaculata,* while NOR-negative in all the species of genera *Aristocypha* and *Heliocypha*. NOR banding on *H. biforata* has not been attempted due to insufficient material.

In *Libellago lineata* of subfamily Libellagininae**,** all autosomal bivalents show terminal NOR's on both sides. X chromosome and m bivalent possess dark NOR on one side. Similarly, Walia *et al*. (2018) observed terminal NOR's on autosomal bivalents, while X chromosome show variation from the present results as it is bean shaped and NOR-negative. Silver nitrate staining in all the six species of subfamily Rhinocyphinae has been attempted for first time.

DAPI and CMA_3 staining:

DAPI and CMA_3 staining has been attempted on all the six species of subfamily Rhinocyphinae. In both the species of genus *Aristocypha*, all the autosomal bivalents and X chromosome show DAPI/CMA_3 bright regions which depict overlapping AT/GC rich regions, while m bivalent is DAPI and CMA_3 dull in *A. quadrimaculata*, while it is DAPI as well as CMA_3 bright in *A. trifasciata*. In all the species of genus *Heliocypha*, autosomal bivalents possess bright DAPI and weak CMA_3 signals at terminal positions, while X chromosome is more DAPI bright than CMA_3 signals, but m bivalent possesses variation as DAPI and CMA_3

dull in *H. biforata* and *H. bisignata*, while it is DAPI and CMA_3 bright in *H. perforata*. In *Paracypha unimaculata*, all the autosomal bivalents show terminal bright DAPI and CMA_3 signals, except largest bivalent which is homogeneously stained with both the dyes. X chromosome and m bivalent are both DAPI and CMA_3 bright. In *Libellago lineata* of subfamily Libellagininae, all the autosomal bivalents including m bivalent and X chromosome show bright DAPI and weak CMA_3 signals.

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

Conclusion

Chromosomes investigation has been done on seven species of family Chlorocyphidae by different staining techniques. Two types of chromosome complement have been seen in the family. Subfamily Rhinocyphinae possess n=12m and subfamily Libellagininae possess n=13m chromosome complement. Family Chlorocyphidae is characterized by the presence of one large autosomal bivalent, originated by the autosomal fusion. Results of C-banding, NOR banding and sequence specific banding has been compared which depicts the presence of more AT rich regions than GC regions and review of cytologically studied species have also been done.

Acknowledgements

Authors are grateful to the Head, Department of Zoology and Environmental Sciences, Punjabi University, Patiala for providing necessary lab facilities for the research work and Rajiv Gandhi National Fellowship, UGC New Delhi (India) for financial support.

References

Carr DH and Walker JE. (1961) Carbol-fuchsin as a stain for human chromosomes. Stain Technol. 36: 233-236.

Chatterjee K and Kiauta B. (1973) Male germ cell chromosomes of two Calopterygoidea species from the Darjeeling, Himalaya (Zygoptera: Chlorocyhidae, Euphaeidae). Odonatologica 2(2): 105-108.

Handa SM and Kochhar N. (1980) Cytology of eight species of damselflies (Zygoptera: Odonata). In: Proceedings of 67th Indian Science Congress, Chandigarh. Part III: p. 104.

Howell M and Black DA. (1980) Controlled silver staining of nucleolar organiser regions with protective colloidal developer: I- step method. Experientia 36: 104-105.

Katnoria N and Walia GK. (2022) A cytological study of euphaeid damselflies (Odonata: Zygoptera) from India. Chromosome Sci. 24 (3-4): 55-61.

Kiauta B and Kiauta MAJE. (1980) On a small collection of dragonflies karyotype from the Philipines. Odonatologica 9(3): 237-245.

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

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

Rebagliati PJ, Papeschi AG and Mola LM. (2003) Meiosis and fluorescent banding in *Edessa meditabunda* and *E. rufomarginata* (Heteroptera: Pentatomidae: Edessinae). Eur J Entomol. 100 (1): 11-18.

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

Sandhu R and Walia GK. (1996). Karyological studies of the genus *Rhinocypha* (Zygoptera: Chlorocyphidae). Fraseria 3: 15-19.

Sandhu R, Walia G and Gulati S. (1994) Chromosomal studies of three abundantly occurring damselflies from Himachal Pradesh (India). In: Advances in Oriental Odonatology, (ed.) Srivastva V.K., p. 93-100.

Tyagi BK. (1978a) The chromosome numbers and sex-determining mechanisms newly recorded in thirteen Indian dragonflies (Odonata). Chromosome Inf Serv. 25: 5-7.

Tyagi BK. (1978b) Studies on the chromosomes of Odonata of Dun Valley (Dehradun, India), Ph. D.

Thesis, University of Garhwal, Srinagar, India.

Tyagi BK. (1982) Cytotaxonomy of the Indian dragonflies. Indian Rev Life Sci. 2: 149-161.

Walia GK and Katnoria N. (2017) Linear characterization of chromosomes in two species of family Euphaeidae (Odonata:Zygoptera). Indian J Entomol. 2(4): 75-78.

Walia GK and Katnoria N. (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.

Walia GK and Katnoria N. (2021) Chromosome characterization of four Calopterygid damselflies with cytogenetic review of family Calopterygidae (Odonata:Zygoptera). J Adv Zool. 42(1): 107-117.

Walia GK, Katnoria N and Gill JK. (2018) Chromosome of *Libellago lineata lineata* (Chlorocyphidae: Odonata). Int J Entomol Res. 80 (3): 737-740.

Walia GK, Katnoria N and Devi M. (2022) Cytogenetic analysis of two species of genus *Protosticta* from Himachal Pradesh, India. Indian J Exp Biol. 60(05): 367-369.