

## Cytogenetic data of subfamily Disproneurinae (Odonata: Zygoptera: Platycnemididae) based on localization of C-heterochromatin, AgNOR's and AT-GC regions

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### Abstract

Cytogenetic analyses have been carried on *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla*, *Prodasineura nigra* and *Prodasineura verticalis* of subfamily Disproneurinae by conventional staining, C-banding, silver nitrate staining and sequence specific staining. All the species possess  $n=13$  as haploid chromosome number with X0-XX sex determining mechanism, while m chromosomes are present only in the two species of genus *Prodasineura*. Mostly, dark terminal C-bands are present on the autosomal bivalents of all the species, while 3 bivalents in *Disparoneura quadrimaculata* and 4 bivalents in *Esme cyaneovittata* possess light terminal C-bands. X chromosome shows terminal C-band on one side in *Disparoneura quadrimaculata*, while it is C-positive in *Esme cyaneovittata*, *Esme longistyla* and *Prodasineura verticalis*. Whereas in all the species, dark terminal NOR's are seen on the autosomal bivalents, while 1 bivalent of *Prodasineura verticalis* and 3 bivalents of *Disparoneura quadrimaculata* show dark terminal NOR only on one side. X chromosome possesses terminal NOR's in *Disparoneura quadrimaculata* and *Prodasineura verticalis* and it is NOR rich in both the species of genus *Esme*. Whole chromosome complement of *Esme longistyla* possesses both DAPI and CMA<sub>3</sub> bright signals, while it is more DAPI bright than CMA<sub>3</sub> in *Disparoneura quadrimaculata*, *Esme cyaneovittata* and *Prodasineura verticalis*.

**Keywords:** zygoptera, platycnemididae, disparoneurinae, c-banding, silver nitrate staining, sequence specific staining

### 1. Introduction

Family Platycnemididae is divided into six subfamilies viz., Onychargiinae, Allocnemidinae, Idiocnemidinae, Calicnemiinae, Disparoneurinae and Platycnemidinae. Damselflies of subfamily Disparoneurinae are commonly known as Bambootails and are small to medium sized slender shaped damselflies. They are usually black coloured and marked with blue, red, yellow or rarely iridescent colours. Wings are transparent and rounded or pointed at the tips. Abdomen is long but not twice the length of hind wing. All the species breed in running water and are restricted to forest landscapes. Taxonomically, subfamily Disparoneurinae comprises 125 species referable to 9 genera all over the world, while 22 species of 7 genera are present in India (Dijkstra *et al.* 2014, Subramanian and Babu 2017) [3, 15]. So far, *Caconeura autumnalis* (Fraser 1922) ( $2n=25m$ ,  $n=13m$ ) is the only species of subfamily Disparoneurinae has been cytologically described from Dehradun, India (Tyagi 1978) [17]. During the present study, cytogenetic characterization of *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla*, *Prodasineura nigra* and *Prodasineura verticalis* of subfamily Disproneurinae has been done. All the species possess  $n=13$  as haploid chromosome number with X0-XX sex determining mechanism. Distribution of C-heterochromatin, localization of NOR's and AT-GC rich specificity have also been studied in all the species except *Prodasineura nigra*.

### 2. Materials and Methods

#### 2.1. Sample collection

Live adult male specimens were captured in the field from the states of Himachal Pradesh and Kerala during pre-

Monsoon and post-monsoon seasons of the years 2016-2019. Geographical distribution and collection details of the species are given in the Table 1.

#### 2.2. Chromosome preparation

Specimens were dissected in 0.67% saline solution in the field. Testes were taken out with the help of fine forceps. Subsequently, testes were put in sodium citrate (0.9 %) for 45 minutes and were fixed in freshly prepared Carnoy's fixative (3:1, absolute alcohol: glacial acetic acid). After that, testes were teased on the grease free slides with the help of sharp forceps and slides were air dried.

#### 2.3. Chromosome staining and Microscopic analysis

Conventional staining (Carr and Walker 1961) [1], C-banding (Sumner 1972) [16], silver nitrate staining (Howell and Black 1980) [6] and sequence specific staining (Rebagliati *et al.* 2003) [14] with minor modifications have been attempted on air dried slides. Appropriate meiotic stages were photomicrographed for further cytogenetical investigations.

### 3. Results

#### 3.1. Conventional staining

In diakinesis of *Disparoneura quadrimaculata*, *Esme cyaneovittata* and *Esme longistyla*, 13 elements are present, out of these, 12 are autosomal bivalents (without m bivalent) and one univalent is X chromosome (Figs. 1a, 2a, 3a), whereas *Prodasineura nigra* and *Prodasineura verticalis* also possess  $n=13$  but autosomal bivalents include m bivalent (Figs. 4a, 4c). In metaphase I, autosomal bivalents show rod shaped structure due to condensation and terminalization of chiasmata, while X chromosome is oval shaped (Figs. 1b, 2b, 3b, 4b, 4d).

### 3.2. C-Banding

During diakinesis, dark terminal C-bands on both sides are present on most of the autosomal bivalents in *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla* and *Prodasineura verticalis* (Figs. 1c, 2c, 3c, 4e), while 3 bivalents in *Disparoneura quadrimaculata* and 4 bivalents in *Esme cyaneovittata* show light terminal C-bands (Figs.1c, 2c). X chromosome possesses terminal C-band only on one side in *Disparoneura quadrimaculata* (Fig.1c), while it is C-positive in *Esme cyaneovittata*, *Esme longistyla* and *Prodasineura verticalis* (Figs. 2c, 3c, 4e).

### 3.3. Silver nitrate staining

During diakinesis, all the autosomal bivalents in *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla* and *Prodasineura verticalis* (including m bivalent) are showing dark terminal NOR's on both sides (Figs. 1d, 2d, 3d, 4e), while in *Disparoneura quadrimaculata*, 3 bivalents show dark terminal NOR's only on one side (Fig.1d). X chromosome possesses terminal NOR's in *Disparoneura quadrimaculata* and *Prodasineura verticalis* (Figs.1d, 4f), while it is NOR rich in *Esme cyaneovittata* and *Esme longistyla* (Figs. 2d, 3d).

### 3.4. Sequence Specific Staining

In *Disparoneura quadrimaculata*, during pachytene, chromatin material shows more DAPI bright than CMA<sub>3</sub> signals but two dark CMA<sub>3</sub> bright regions are also present (Figs. 1e-f). During diakinesis, in *Esme longistyla*, all elements including X chromosome possess DAPI and CMA<sub>3</sub> bright signals (Figs. 3e-f), whereas in *Esme cyaneovittata* and *Prodasineura verticalis* (including m bivalent) autosomal bivalents possess DAPI bright and CMA<sub>3</sub> dull signals at their chiasmatic and non-chiasmatic ends and X chromosome is DAPI bright (Figs. 2e-f, 4g-h).

## 4. Discussion

Type number of the family Platycnemididae is 2n=25 (24A+XO) and it is present in all the species of genera *Calicnemia*, *Caconeura*, *Copera*, *Platycnemis* and *Coelicia* except in *Risioicnemis incisa* with 2n=21 and n=11, which was secondarily originated by the fusion of six elements of primary complement 2n=25 (Kichijo 1941, 1942a, b,

Dasgupta 1957, Kiauta 1971, Kiauta 1975, Tyagi 1978, Handa and Kochhar 1980, Kiauta and Kiauta 1981, Walia 2012, Gill 2014, Walia and Devi 2018) [10, 11, 12, 2, 7, 8, 17, 5, 9, 18, 4, 19]. So far, *Caconeura autumnalis* (Fraser 1922) (2n=25m, n=13m) is the only species of subfamily Disparoneurinae which has been cytologically reported from Dehradun, India (Tyagi 1978) [17]. During the present study, *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla*, *Prodasineura nigra* and *Prodasineura verticalis* also possess n=13 as haploid chromosome number which is considered as the type number of the subfamily Disparoneurinae. Pair of m chromosomes is present only in the two species of genus *Prodasineura* as earlier observed in the *Caconeura autumnalis* (Fraser 1922) (n=13m) by Tyagi (1978) [17], which is considered as the cytogenetic character of the genus.

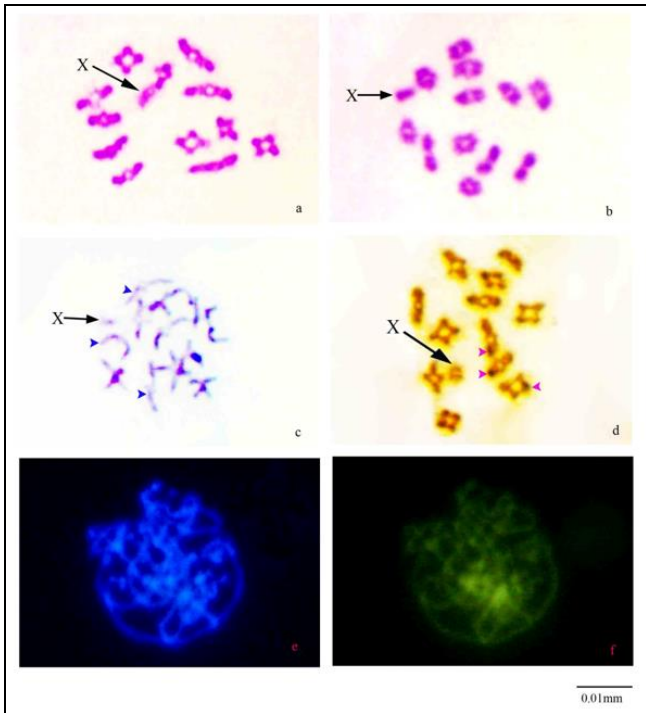
During the present study, C-banding, silver nitrate staining and sequence specific staining for the enrichment in AT and GC base pairs have been performed on four species except *Prodasineura nigra* because only 2 specimens of the species were collected. Dark/light terminal C-bands are present on the autosomal bivalents of all the species and X chromosome possesses terminal C-band only on one side in *Disparoneura quadrimaculata* and it is C-positive in *Esme cyaneovittata*, *Esme longistyla* and *Prodasineura verticalis*. Presence of C-bands on the terminal ends is due to the localization of centromeric activity at the terminal ends, which is the characteristics feature of holocentric chromosomes of odonates as well as of other insect groups. Variation in the size and stain intensity of the C-bands among the bivalents is due to the restriction of centromeric activity in response to chiasma formation, axial orientation of bivalents on spindle fibers during the first meiotic division (Prasad and Thomas 1992) [13].

Dark terminal NOR's on the both sides/one end of autosomal bivalents are present and X chromosome possesses terminal NOR's in *Disparoneura quadrimaculata* and *Prodasineura verticalis* and it is NOR rich in both the species of genus *Esme*. All the four species show more DAPI bright signals than CMA<sub>3</sub> signals which indicate that complements of the species are AT rich as compared to GC rich, which also correspond to the results of C-banding and silver nitrate staining.

**Table 1:** Geographical distribution and collection details of the species.

Sr. No.	Name of Species	Place of Collection	Latitude	Longitude	Months of collection
1.	<i>Disparoneura quadrimaculata</i> (Rambur, 1842)	Khandala (Maharashtra)	18° 3' 28.188'' N	74° 1' 34.356'' E	13-Sep.-2019 14-Sep.-2019
2.	<i>Esme cyaneovittata</i> Fraser, 1922	Thiruvananthapuram (Kerala)	8° 29' 15.756'' N	76° 55' 26.184'' E	25-Apr.-2018 26-Apr.-2018
3.	<i>Esme longistyla</i> Fraser, 1931	Wayanad (Kerala)	11° 42' 33.336'' N	76° 6' 18.9'' E	10-May. - 2018 11-May. - 2018
4.	<i>Prodasineura nigra</i> Fraser, 1922	Bilaspur (Himachal Pradesh)	31° 20' 28.32'' N	76° 45' 47.16'' E	8-Sep. - 2016 9-Sep. - 2016
5.	<i>Prodasineura verticalis</i> (Selys, 1860)	Thiruvananthapuram (Kerala)	8° 29' 15.756'' N	76° 55' 26.184'' E	29-Apr. - 2018 30-Apr. - 2018

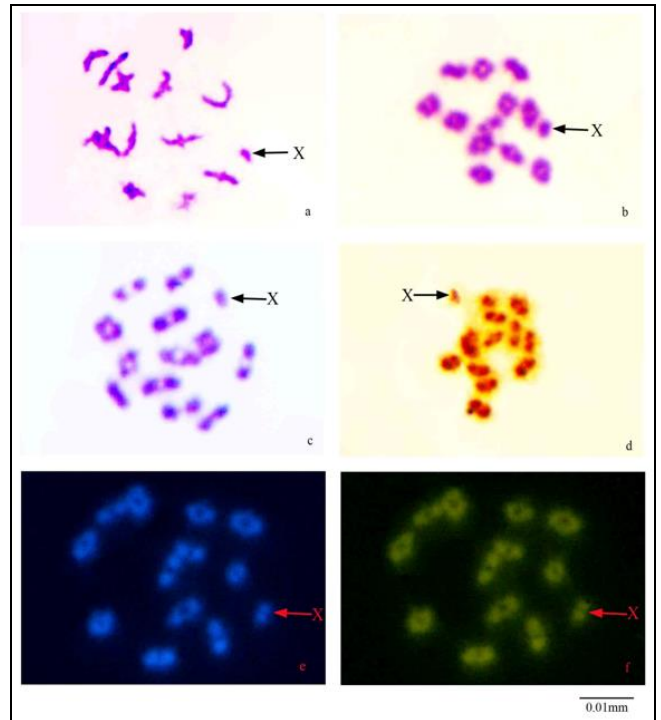
**Figure Captions**



**Fig 1:** *Disparoneura quadrimaculata*

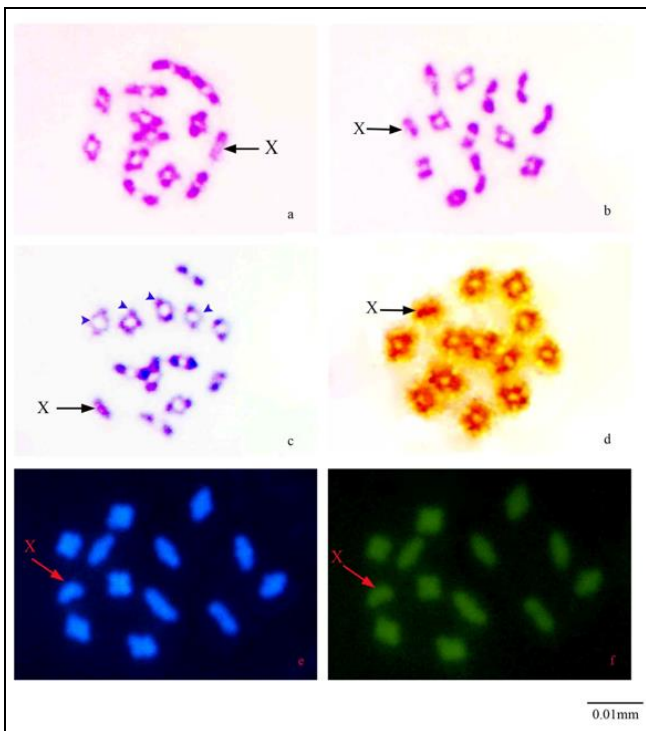
**Conventional staining:** a- Diakinesis, b- Metaphase-I  
**C-banding:** c- Diakinesis  
**Silver nitrate staining:** d- Diakinesis  
**Sequence specific staining:** e- (DAPI Staining) Pachytene  
 f- (CMA<sub>3</sub> Staining) Pachytene

f-(CMA<sub>3</sub> Staining) Diakinesis



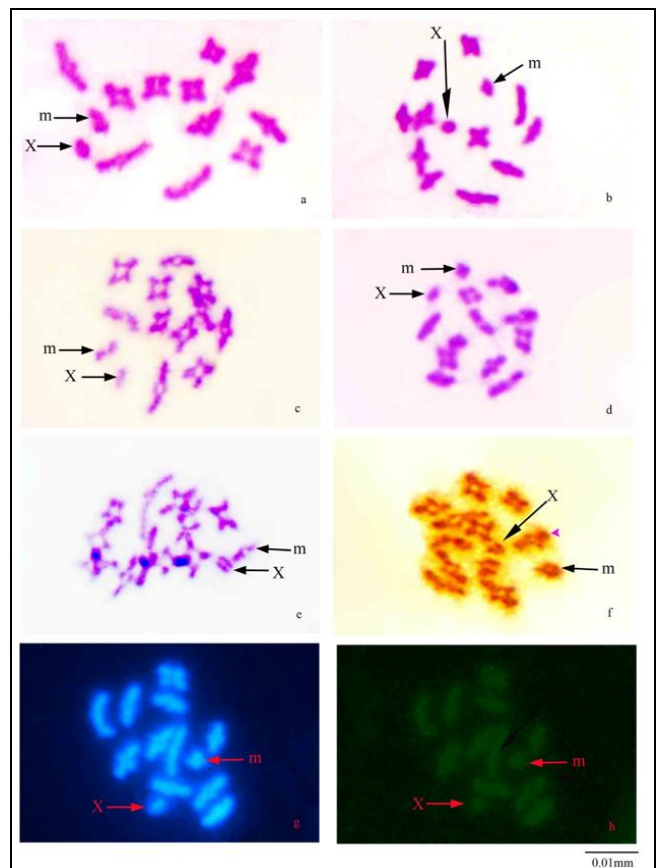
**Fig 3:** *Esmé longistyla*

**Conventional staining:** a- Diakinesis, b- Metaphase-I  
**C-banding:** c- Diakinesis  
**Silver nitrate staining:** d- Diakinesis  
**Sequence specific staining:** e- (DAPI Staining) Diakinesis  
 f- (CMA<sub>3</sub> Staining) Diakinesis



**Fig 2:** *Esmé cyaneovittata*

**Conventional staining:** a- Diakinesis, b- Metaphase-I  
**C-banding:** c- Diakinesis  
**Silver nitrate staining:** d- Diakinesis  
**Sequence specific staining:** e- (DAPI Staining) Diakinesis



**Fig 4:** *Prodasineura nigra*

**Conventional staining:** a- Diakinesis, b- Metaphase-I  
*Prodasineura verticalis*

**Conventional staining:** c- Diakinesis, d- Metaphase-I

**C-banding:** e- Diakinesis

**Silver nitrate staining:** f- Diakinesis

**Sequence specific staining:** g-(DAPI Staining) Diakinesis  
h-(CMA<sub>3</sub> Staining) Diakinesis

Black and Red arrows show the X chromosome.

Pink color arrowheads show presence of dark terminal NOR only on one side.

Blue color arrowheads show presence of light terminal C-bands.

## 5. Conclusion

During the present study, chromosomal complement of *Disparoneura quadrimaculata*, *Esme cyaneovittata*, *Esme longistyla*, *Prodasineura nigra* and *Prodasineura verticalis* and linear characterization of four species except *Prodasineura nigra* have been described for the first time.

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## 7. Conflict of Interest

Author confirmed no any conflict of interest.

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