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Cytogenetic analysis of two species of genus *Protosticta* from Himachal Pradesh, India

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Cytogenetically, out of 262 species, only three species of family Platystictidae has been reported worldwide. Present study has been undertaken to study chromosome complement and its characterization of more species of this family. Cytogenetic analyses of *Protosticta sanguinostigma* Fraser, 1992 and *Protosticta uncata* Fraser, 1931 of family Platystictidae, collected from Andretta, Himachal Pradesh, India have been carried out on the basis of conventional staining, C-banding and silver nitrate staining. Both the species possess n=13m as haploid chromosome number and X0-XX sex determining mechanism. One large bivalent is present in all the meiotic stages of *P. sanguinostigma* which is considered as the species specific character. Chromosome complement of both the species shows variation in distribution of C-bands and Nucleolar organizer regions (NORs). Cytologically, both the species have been described for the first time.

Keywords: C-bands, Chromosome complement, m-Chromosomes, Meiotic stages, Nucleolar organizer regions, Silver nitrate staining

Suborder Zygoptera of order Odonata includes 4 superfamilies: Lestoidea, Platystictoidea, Calopterygoidea, Coenagrionoidea. Superfamily Platystictoidea consist of family Platystictidae. Damselflies of this family are small black or brown in color marked with white, blue rarely iridescent markings and are commonly known as Reedtails. Transparent wings are slightly pointed at the tip. Abdomen is very long and doubles the length of hindwing. Family Platystictidae includes 262 species of 9 genera worldwide, while 15 species under 3 genera are known from India[1]. Worldwide, cytogenetic data pertains to only three species of family Platystictidae, which includes two species of genus *Drepanosticta*[2,3] and one species of genus *Palaemnema*[4] both with n=13m. In view of the paucity of cytogenetic studies in the family Platystictidae, here we studied the structure and behaviour of chromosomes during meiosis, detection of C-heterochromatic regions and location of Nucleolar organizer regions (NOR's) in *Protosticta sanguinostigma* and *P. uncata.*

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Materials and Methods

Damselflies were collected during the months of June and September, 2015 from Andretta, Himachal Pradesh, India. Specimens were dissected in 0.67% saline solution in the field. The gonads were kept in sodium citrate solution (0.9%) for 45 min. Then these were fixed in Carnoy's fixative (absolute alcohol 3: glacial acetic acid 1) for 30 min. The fixed gonads were teased on slides with the help of forceps. Slides were air dried and preceded for conventional staining[5], C-banding[6] and silver nitrate staining[7] with minor modifications in the laboratory. The identification of the specimens was done by consulting "The Fauna of British India including Ceylon and Burma, Odonata Vol-II (Fraser, 1934) and "A reclassification of the order Odonata" (Fraser, 1957).

Results and Discussion

Conventional staining

During present study, structure and behaviour of chromosomes during meiosis have been studied in *Protosticta sanguinostigma* and *P. uncata*. Both the species showed same chromosome complement (n=13m) in all the meiotic stages with XO sex determination. Moreover, *P. sanguinostigma* was characterized by the presence of large bivalent (shown by black arrow heads in Figs. 1 A-G) in all the stages. X chromosome and m bivalent were lying at the peripheral position in both the species. In the diplotene, all the autosomal bivalents including m bivalent possessed single chiasma per bivalent and gave 'X' or 'V' shapes to the elements and X chromosome and m bivalent were clearly distinguished (Fig. 2A). During diakinesis, 13 elements were seen, among these, 12 were autosomal bivalents, which also include m bivalent and one X univalent. X chromosome and m bivalent were clearly visible (Figs. 1 A & B and 2B). During metaphase-I, autosomal bivalents were found to be rod shaped due to extreme condensation and terminalization of chiasmata (Figs. 1C and 2C). In the prophase-II, chromosomes appeared ε- shape due to partial association of the chromatids, which is the characteristic feature of order Odonata. X and m chromosomes were clearly distinguished (Figs. 1D and 2D). During

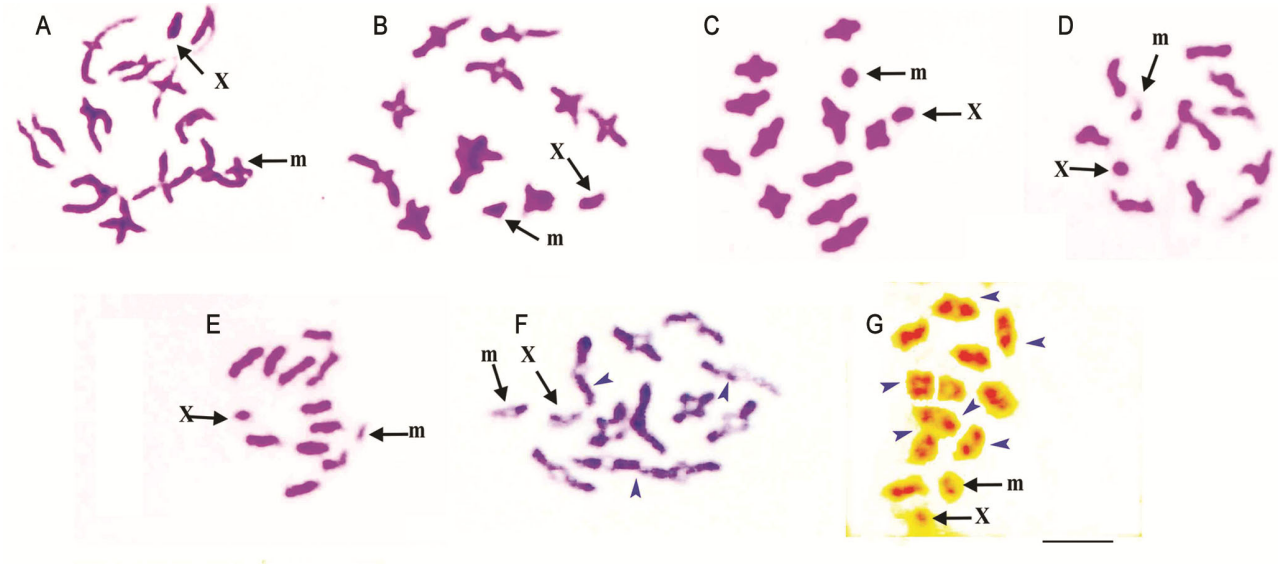

Fig. 1 — Normal complement, C bands and NOR's in *Protostictauncata* (A-E). Normal complement. (A) Diplotene (B) Diakinesis, (C) Metaphase-I, (D) Prophase-II, (E) Metaphase-II. (F) C-bands. (F) Diakinesis, (G) NOR's. (G) Diakinesis. [Bar line- 0.01 mm.; NOR-bands and interstitial C-bands are shown by blue arrow heads]

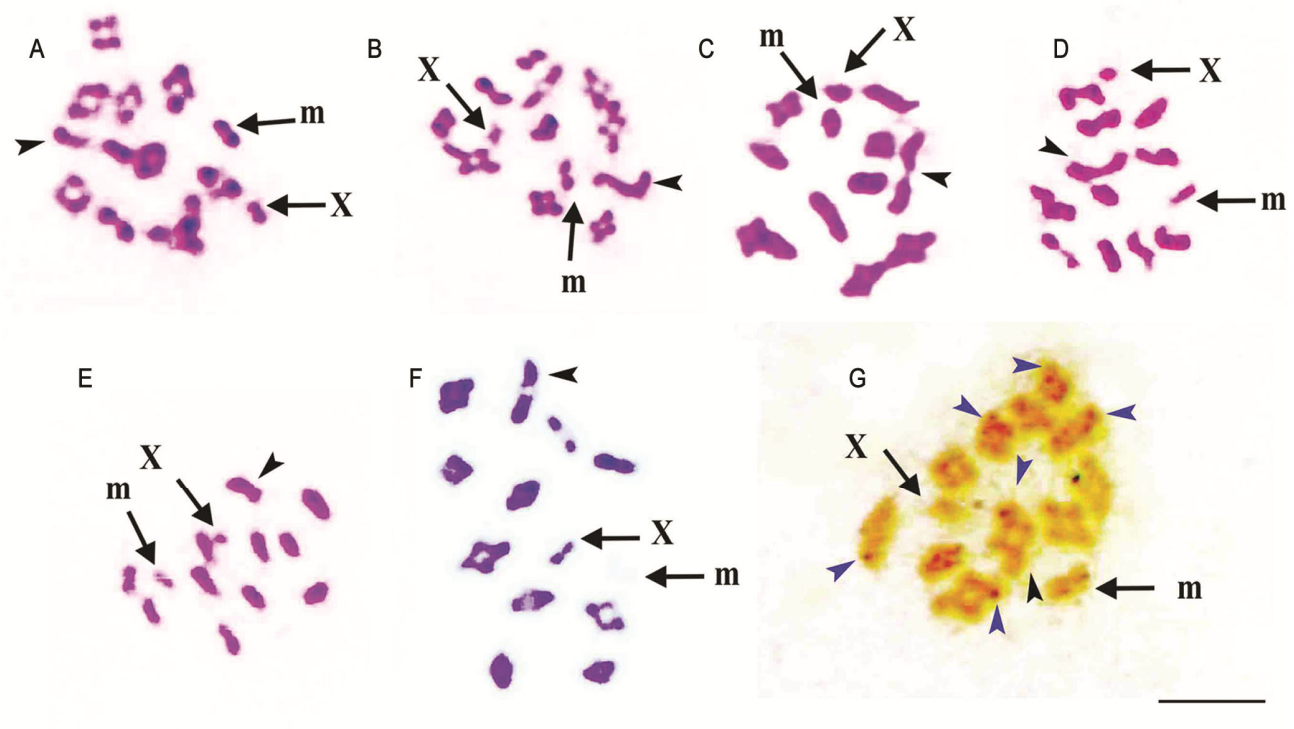

Fig. 2 — Normal complement, C bands and NOR's in *Protostictauncata* (A-E). Normal complement. (A) Diplotene (B) Diakinesis, (C) Metaphase-I, (D) Prophase-II, (E) Metaphase-II. (F) C-bands. (F) Diakinesis, (G) NOR's. (G) Diakinesis. [Bar line- 0.01 mm. Largest autosomal bivalent is shown by black arrow head. NOR-bands are shown by blue arrow heads]

metaphase- II, the size of the chromosomes was found half the size of metaphase-I chromosomes, while m and X chromosomes were clearly distinct (Figs. 1E and 2E).

Chromosome number n=13 is considered as the type number of the family as it is present in *Palaemnema paulina* from Costa Rica, Central America[4], in *Drepanosticta* sp. from Lantang valley, Central Nepal[2] and in *Drepanosticta* sp. from Dehradun valley, India[3], whereas the m chromosomes are absent in both the species of genus *Drepanosticta.* Similarly, n=13m, is

observed in *P. uncata* and *P. sanguinostigma*, while later species is characterized by one large autosomal bivalent which is the species specific character.

C-banding

In *P. uncata*, 3 autosomal bivalents showed one terminal and one interstitial band (shown by blue arrow heads in Fig. 2F) and 8 autosomal bivalents possessed terminal C-bands on both ends. X chromosome and m bivalent revealed C-band on one terminal end (Fig. 2F). In *P. sanguinostigma*, all the autosomal bivalents except m bivalent were showing terminal C-bands on one side/both sides, while X chromosome was C-positive for the entire length and m bivalent was C-negative (Fig. 1F).

Silver nitrate staining

In the diakinesis, all the autosomal bivalents including m bivalent were showing terminal NOR's on both sides (shown by blue arrow headsin Fig. 2G) in *P. uncata* and X chromosome was NOR-positive (Fig. 2G), while variation in NOR's was seen in *P. sanguinostigma* as 5 autosomal bivalents showed NOR's on both the terminal ends, while 3 bivalent possessed NOR on one end and 3 bivalents lacked NOR's. X chromosome was NOR-positive, while m bivalent showed light NOR's (Fig. 1G).

Variation in distribution of C-heterochromatin and NOR's have been observed in both the species. C-bands are mostly present at the terminal ends of autosomal bivalents in both the species, while interstitial C-bands are seen on 3 autosomal bivalents of *P. uncata.* m bivalent and X chromosome possess C-band at one terminal end in *P. uncata*, while X chromosome is C-positive and m bivalent is C-negative in *P. sanguinostigma.* Terminal NOR's on both sides are present on all the autosomal bivalents in *P. uncata* and X chromosome is also NOR-positive, while in *P. sanguinostigma* 5 bivalents show NOR's on both the terminal ends, while 3 bivalents reveal NOR on one end and 3 bivalents lack NOR's. X chromosome is NOR-positive, whereas m bivalent shows light NOR band. Presence of C-bands and NOR's on the terminal ends of bivalents indicates that both C-heterochromatin and nucleolar organizer regions in the species are co-localized. In Odonata, presence of heterochromatin regions on the terminal ends depict centromeric activity, which is localized at the terminal ends during the meiosis and helps in segregation and movement of chromosomes on the spindle[8-11]. This behaviour of localization of C-heterochromatin on terminal ends is mostly seen in other insect orders having holocentric chromosomes. Cytogenetically, both the species have been studied for the first time.

Conclusion

Both the species of genus *Protosticta* possess n=13m as haploid chromosome number and X0-XX sex determining mechanism. One large bivalent is present in all the meiotic stages of *P. sanguinostigma*, which is considered as the species specific character. Presence of C-bands and NOR's on the terminal ends of bivalents indicates that both C-heterochromatin and nucleolar organizer regions in the species are co-localized. Cytologically, both the species have been described for the first time.

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Conflict of interest

Authors declare no competing interests.

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