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Linear differentiation of chromosomes of *anisogomphus bivittatus* selys, 1854 from India (odonata: anisoptera: gomphidae)

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Abstract
Live adult male specimens of *Anisogomphus bivittatus* of family Gomphidae have been collected from Andretta, Himachal Pradesh (India). Male germ cell chromosomes of the species have been described on the basis of conventional staining, C-banding, silver nitrate staining and sequence specific staining. The species possesses 2n (♂) =23m, as the chromosome number and X0 (♂)/XX (♀) type sex determination. Dark terminal C-bands are present on all the autosomal bivalents, while m bivalent is C-negative and X chromosome is C-positive throughout the length. Terminal light/dark NOR's are present on all autosomal bivalents including m bivalent, while X chromosome possesses terminal dark NOR on one side and light NOR on other side. During sequence specific staining, all the autosomal bivalents except m bivalent show more CMA_3 bright signals than DAPI signals at chiasmatic ends and X chromosome is also more CMA_3 bright than DAPI.

Keywords: odonata, anisoptera, gomphidae, conventional staining, c-banding, silver nitrate staining, sequence specific staining

1. Introduction
Taxonomically, 1010 gomphid species belonging to 101 genera have been reported all over the world, while 85 species belonging to 29 genera are known in India (Subramanian and Babu, 2017) [12]. Out of 85 Indian gomphid species, cytogenetical data is available only on 12 species (Asana and Makino, 1935; Das, 1956; Dasgupta, 1957; Tyagi, 1977, 1978a, b, 1982, 1985, 1986; Walia and Sandhu 1999, Walia *et al*., 2006, Chahal, 2013 and Walia and Chahal, 2014) [1, 3, 4, 5, 14, 15, 16, 17, 18, 19, 20, 21, 22]. Majority of the species possess 2n=23 which is considered as type number of the family. This number is secondarily originated from the primary complement 2n=25 by the fusion of autosome with autosome or with sex element. The data mostly pertains to chromosome number, sex determination and few reports on the distribution of constitutive heterochromatin. Worldwide, 17 species of genus *Anisogomphus* are present, while only 4 species are available in India. Cytogenetic data is available only on two species. These are *Anisogomphus bivittatus*, 2n=23m (Das, 1956; Kiauta, 1975; Tyagi, 1982) [4, 7, 17] and *Anisogomphus occipitalis*, 2n=23m (Kiauta, 1975; Tyagi, 1978b, 1982) [7, 16, 17]. In the present study, *Anisogomphus bivittatus* possesses the same chromosome number (2n=23m, X0) as reported earlier, while linear characterization of the chromosomes has been done by using C-banding, silver nitrate staining and sequence specific staining.

2. Materials and Methods
Live adult male specimens of the species were collected from Andretta, Himachal Pradesh in the month of June, 2017. Specimens were dissected in 0.67% saline solution in the field and testes were taken out. Subsequently, the testes were put in sodium citrate (0.9%) for 45 minutes then fixed in freshly prepared Carnoy's fixative (3: 1, absolute alcohol: acetic acid glacial) for 15 minutes. Two more changes in the fixative, each of 15 minutes duration were given. After this, testes were teased on the grease free slides and slides were air dried.
Prepared slides were stained in Carbol fuchsin for 3-4 hours (Carr and Walker, 1961)[2] to study the chromosome complement, detection of constitutive heterochromatin (Sumner, 1972)[13], localization of Nucleolar Organiser Regions (NOR's) (Howell and Black, 1980)[6], sequence specificity (Rebagaliati *et al*., 2003)[10]. Relevant meiotic and mitotic stages were microphotographed.

3. Results
3.1 Conventional staining
Spermatogonial metaphase plate possesses 23 elements, out of these, 22 are autosomes and one is X chromosome. Autosomes also include a pair of m chromosomes and X chromosome is large in size and clearly differentiated (Fig. 1a). During Pachytene, X chromosome is visible in the chromatin material (Fig. 1b). In the diplotene, 12 elements are visible, among these, 11 are autosomal bivalents which also include small sized m bivalent and one univalent is X chromosome (Figs. 1c, 1d). Diakinesis also includes 11 autosomal bivalents including m bivalent and large X chromosome which occupies the peripheral position (Figs. 1e, 1f). In the metaphase I, all the autosomal bivalents are showing central constriction. m bivalent is centrally placed, while X chromosome is present at the peripheral position (Fig. 1g). During metaphase II, all the chromosomes are dumbbell shaped having central constriction which confirms the presence of two chromatids, while X chromosome shows only a single chromatid because it divides equationally in the meiosis I and divide reductionally in the meiosis II (Fig. 1h).

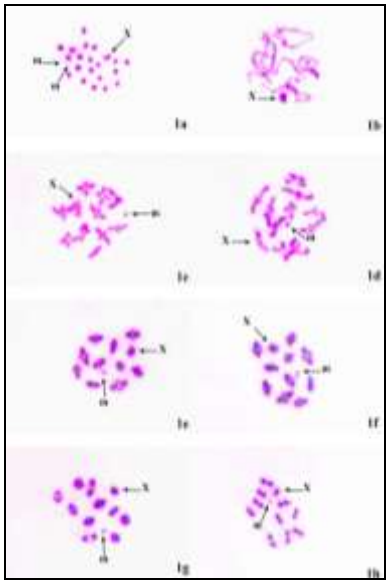

Fig 1: 1a-1h, Conventional staining, 1a spermatogonial metaphase, 1b pachytene, 1c,1d diplotene, 1e,1f diakinesis, 1g metaphase I, 1h metaphase II. X and m marked with arrows. Bar= 0.01mm

3.2 C-banding
During the diakinesis and metaphase I, all autosomal bivalents show large terminal C-bands, while m bivalent is C-negative and X chromosome is entirely C-positive and shows bipartite behaviour (Figs. 2a, 2b, 2c, 2d).

3.3 Silver nitrate staining
In the diakinesis and metaphase I, all 10 autosomal bivalents except m bivalent show terminal NOR's, while m bivalents possesses dim NOR's due to small size. X chromosome shows bipartite behaviour and possesses dark NOR on one side and light NOR on other side (Figs. 2e, 2f, 2g, 2h).

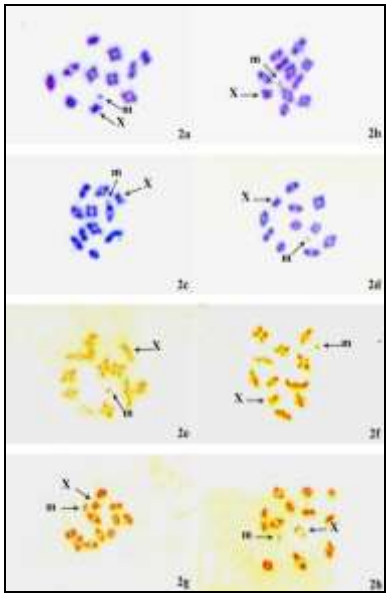

Fig 2: 2a-2d, C-banding, 2e-2h, silver nitrate staining 2a,2b diakinesis, 2c,2d metaphase I, 2e,2f diakinesis, 2g,2h metaphase I. X and m marked with arrows. Bar= 0.01mm

3.4 Sequence specific staining
During pachytene, X chromosome and 10 more CMA_3 bright signals as compared to DAPI signals are seen in the chromatin material (Figs. 3a, 3b). In the diakinesis, metaphase I and early anaphase I, all the autosomal bivalents except m bivalent show more CMA_3 bright signal as compared to DAPI at the chiasmatic ends, while X chromosome possesses more CMA_3 bright signals than DAPI signals (Figs. 3c, 3d, 3e, 3f, 3g, 3h).

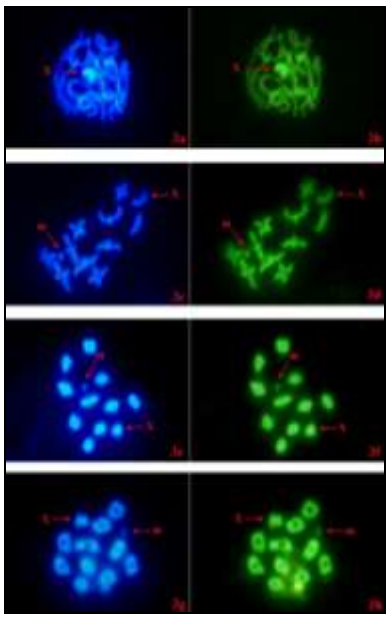

Fig 3: 3a-3h, sequence specific staining 3a,3c,3e,3g, DAPI staining, 3b,3d,3f,3h, CMA3 staining, 3a,3b pachytene, 3c,3d diakinesis, 3e,3f metaphase I 3g,3h early anaphase II. X and m marked with arrows. Bar= 0.01mm

4. Discussion
In the family Gomphidae, diploid chromosome number varies from19-23. Majority of the species possess 2n=23 which is considered as type number of the family. This number is secondarily originated from the primary complement 2n=25 by the fusion of autosome with autosome or with sex element. These fusions are responsible for the reduction in chromosome number and origin of Neo-XY sex determining mechanism. Both X0 and Neo-XY sex determining mechanism are seen in gomphid dragonflies.

Cytogenetical data is available only on 12 Indian gomphid species (Asana and Makino, 1935; Das, 1956; Dasgupta, 1957; Tyagi, 1977, 1978a, b, 1982, 1985, 1986; Walia and Sandhu 1999, Walia *et al*., 2006, Chahal, 2013 and Walia and Chahal, 2014) [1, 3, 4, 5, 14, 15, 16, 17, 18, 19, 20]. Among these, out of 4 species of genus *Anisogomphus*, only two species *Anisogomphus bivittatus*, 2n=23m (Das, 1956; Kiauta, 1975; Tyagi, 1982)[4, 8, 18] and *Anisogomphus occipitalis*, 2n=23m (Kiauta, 1975; Tyagi, 1978b, 1982)[7, 16, 17] have been described. In the present study, *Anisogomphus bivittatus* also possesses 2n (♂) =23m, with X0 (♂)/XX (♀) type sex determination which is in accordance to the earlier reports on the same species.

C-banding has been done on 9 species of family Gomphidae (Suzuki and Saitoh, 1988; Perepelov and Bugrov, 2001; Perepelov *et al*., 2001; Chahal, 2013; Walia and Chahal, 2014) [11, 8, 9, 3, 20]. Majority of the species possess terminal C-bands, while banding patterns might be useful for the elucidation of sex chromosome system in the family Gomphidae because of fusions. Similarily, in *Anisogomphus bivittatus*, all autosomal bivalents show large terminal C-bands, while m bivalent is C-negative and X chromosome is entirely C-positive and shows bipartite behaviour. Localization of NOR's in four species of family has been

reported (Chahal, 2013; Walia and Chahal, 2014) [3, 20] and observed terminal NOR's. During the present study, in *Anisogomphus bivittatu,* all 10 autosomal bivalents except m bivalent show terminal NOR's, while m bivalents possesses dim NOR's due to small size. X chromosome shows bipartite behaviour and possesses dark NOR on one side. Sequence specific staining has been performed on the *Anisogomphus bivittatus* of the family Gomphidae for the first time. The complement of the species possesses more CMA_3 bright signals than DAPI signals which depicts that heterochromatin regions are showing interspersed AT and GC rich regions.

5. Conclusions

Cytogenetic characterization of chromosomes of *Anisogomphus bivittatus* (2n=23m with X0/XX) has been done for the first time. All autosomal bivalents show large terminal C-bands and NOR's, while m bivalent is C-negative and possesses dim NOR's due to small size. X chromosome is entirely C-positive, while possesses dark NOR on one side and dim on other side and also shows bipartite behaviour. All the autosomal bivalents except m bivalent and X chromosome show more CMA_3 bright signal as compared to DAPI which depicts that heterochromatin regions are showing interspersed AT and GC rich regions.

6. Acknowledgment

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7. References

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