

CHROMOSOME CHARACTERIZATION OF THREE SPECIES OF GENUS *ICTINOGOMPHUS* OF THE FAMILY GOMPHIDAE (ODONATA: ANISOPTERA)

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ABSTRACT

Chromosomal investigations on *Ictinogomphus angulosus*, *Ictinogomphus pertinax* and *Ictinogomphus rapax* of genus *Ictinogomphus* of family Gomphidae by conventional staining, C- banding, silver nitrate staining and sequence-specific staining have been done. All the species possess $2n = 23$ with XO-XX sex determination. Terminal C-bands and NORs are present on all the autosomes, while the X chromosome is C-positive and NOR rich, whereas autosomes show brighter DAPI signals than CMA₃ and the X chromosome is bright for both the dyes in all three species. Among these, chromosome complements of *Ictinogomphus angulosus* and *Ictinogomphus pertinax* have been described for the first time.

Keywords: Gomphidae, *Ictinogomphus*, Conventional Staining, C- Banding, Silver Nitrate Staining, Sequence-Specific Staining

INTRODUCTION

Family Gomphidae with 1004 species is the second largest family of suborder Anisoptera, while 83 species under 29 genera are present in India (Subramanian and Babu, 2018). Cytogenetically 71 species have been described around the world, among these, 15 species are from India (Asana and Makino, 1935; Oksala, 1945; Das, 1956; Dasgupta, 1957; Cumming, 1964; Cruden, 1968; Kiauta, 1969, 1972, 1975, 1979; Tyagi, 1977, 1978 a,b, 1982, 1985, 1986; Ferreira *et al.*, 1979; Zhu and Wu, 1986; Suzuki and Saitoh, 1988; Walia and Sandhu, 1999; Perepelov and Bugrov, 2001; Perepelov *et al.*, 1998, 2001, Walia *et al.*, 2006, Chahal, 2013; Walia and Chahal, 2014, 2020). The majority of the species possess $2n = 23$ which is considered as the type number of the family. This number is secondarily originated from the primary complement ($2n = 25$) by the fusion between the autosomes or between autosomes and the sex chromosome (Kiauta, 1969). Genus *Ictinogomphus* consists of 18 species worldwide, while only two species, *Ictinogomphus decorates* and *Ictinogomphus rapax* have been described cytogenetically (Kiauta and Kiauta, 1982; Asana and Makino, 1935; Makino, 1935; Kichijo, 1942; Omura, 1949, 1952, 1953; Dasgupta, 1957; Tyagi, 1982). Presently, chromosomes of *Ictinogomphus angulosus*, *Ictinogomphus pertinax* and *Ictinogomphus rapax* of genus *Ictinogomphus* based on detection of constitutive heterochromatin, NOR's and AT and GC regions have been characterized.

MATERIALS AND METHODS

Live adult gomphid specimens were collected from the areas of Jammu and Kashmir and Meghalaya during the years 2015-2017. Male gonads were fixed in Carnoy's fixative for the chromosomal preparations. Prepared slides were treated for conventional staining (Carr and Walker, 1961), C-banding (Sumner, 1972), Silver nitrate staining (Howell and Black, 1980) and Sequence-specific staining (Rebagliati *et al.*, 2003).

RESULTS

Spermatogonial metaphase of all the species includes 23 elements, among these, 22 are autosomes, one large element is the X chromosome and m chromosomes are absent (Fig. 1a, 2a, 3a). At metaphase -I, autosomal bivalents are rod-shaped with central constriction because of condensation, while the bipartite X chromosome is distinct in all the species (Fig. 1b, 2b, 3b).

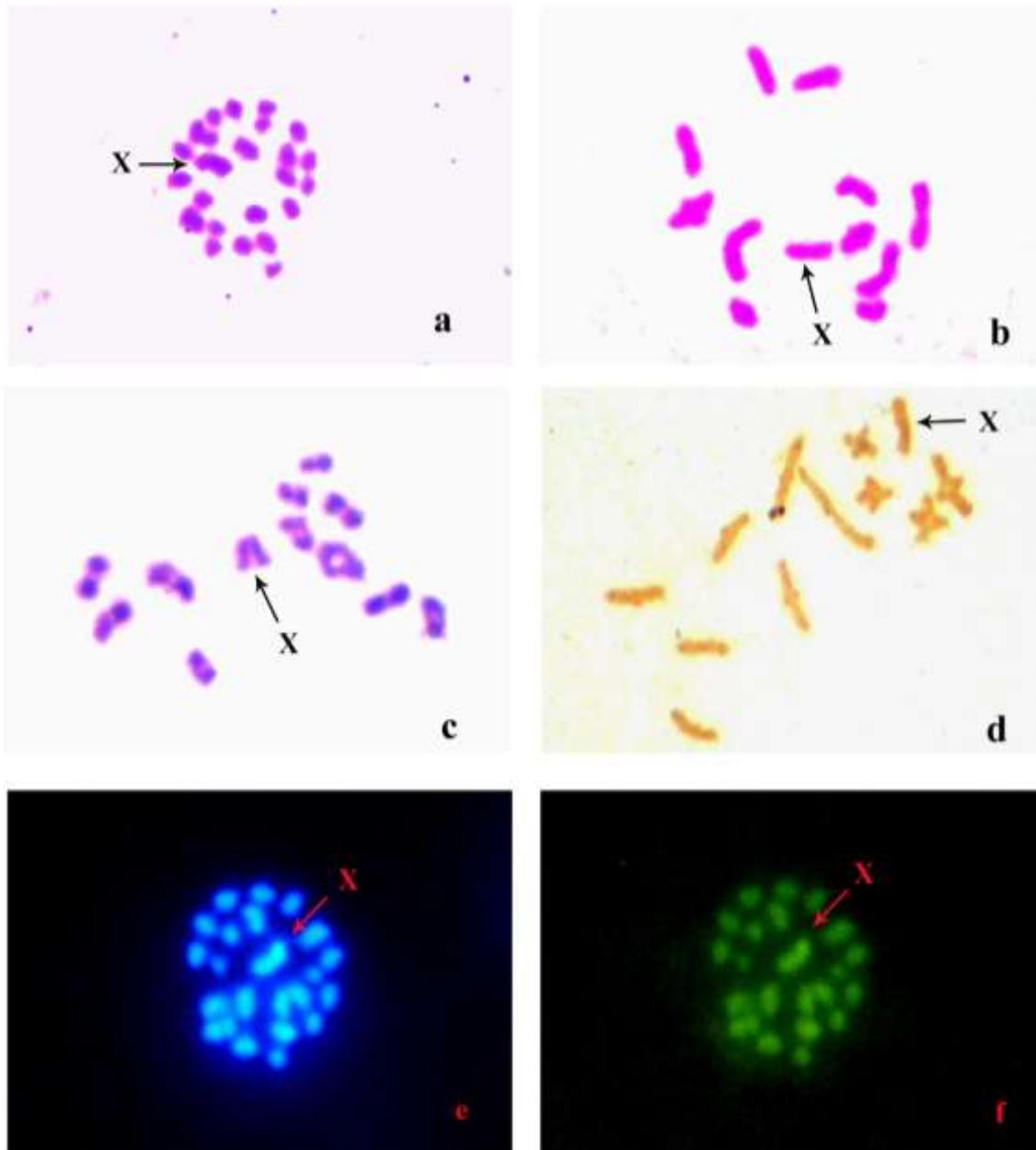


Figure 1. *Ictinogomphus angulosus*. Conventional staining (spermatogonial metaphase 1a, metaphase-I 1b), C-banding (metaphase-I 1c), silver nitrate banding (Diakinesis 1d) and DAPI (spermatogonial metaphase 1e) CMA₃ (spermatogonial metaphase 1f), Bar= 0.01mm.

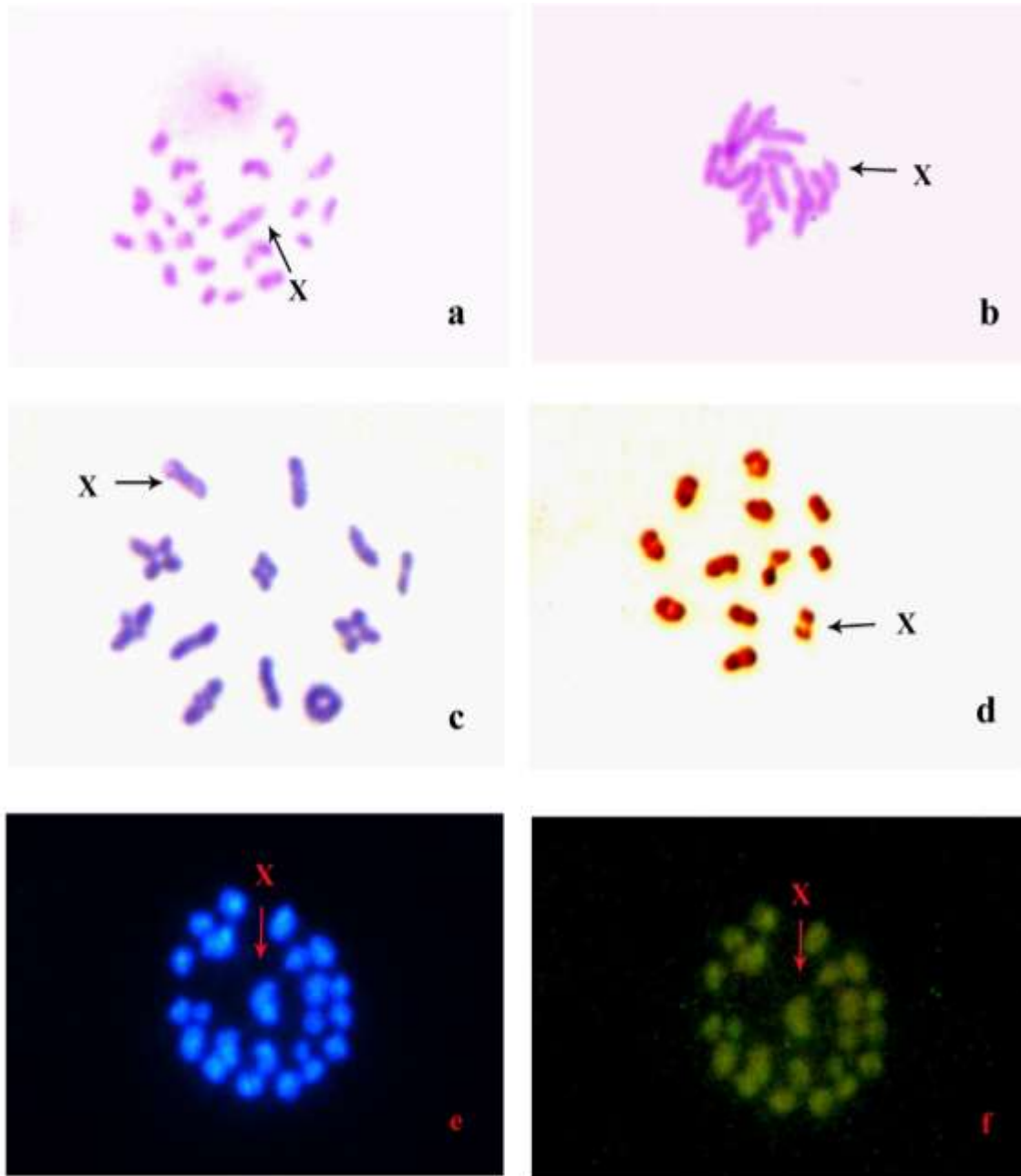


Figure 2. *Ictinogomphus pertinax*. Conventional staining (spermatogonial metaphase 2a, metaphase-I 2b), C-banding (diakinesis 2c), silver nitrate banding (metaphase-I 2d) and DAPI (spermatogonial metaphase 2e) CMA₃ (spermatogonial metaphase 2f), Bar= 0.01mm.

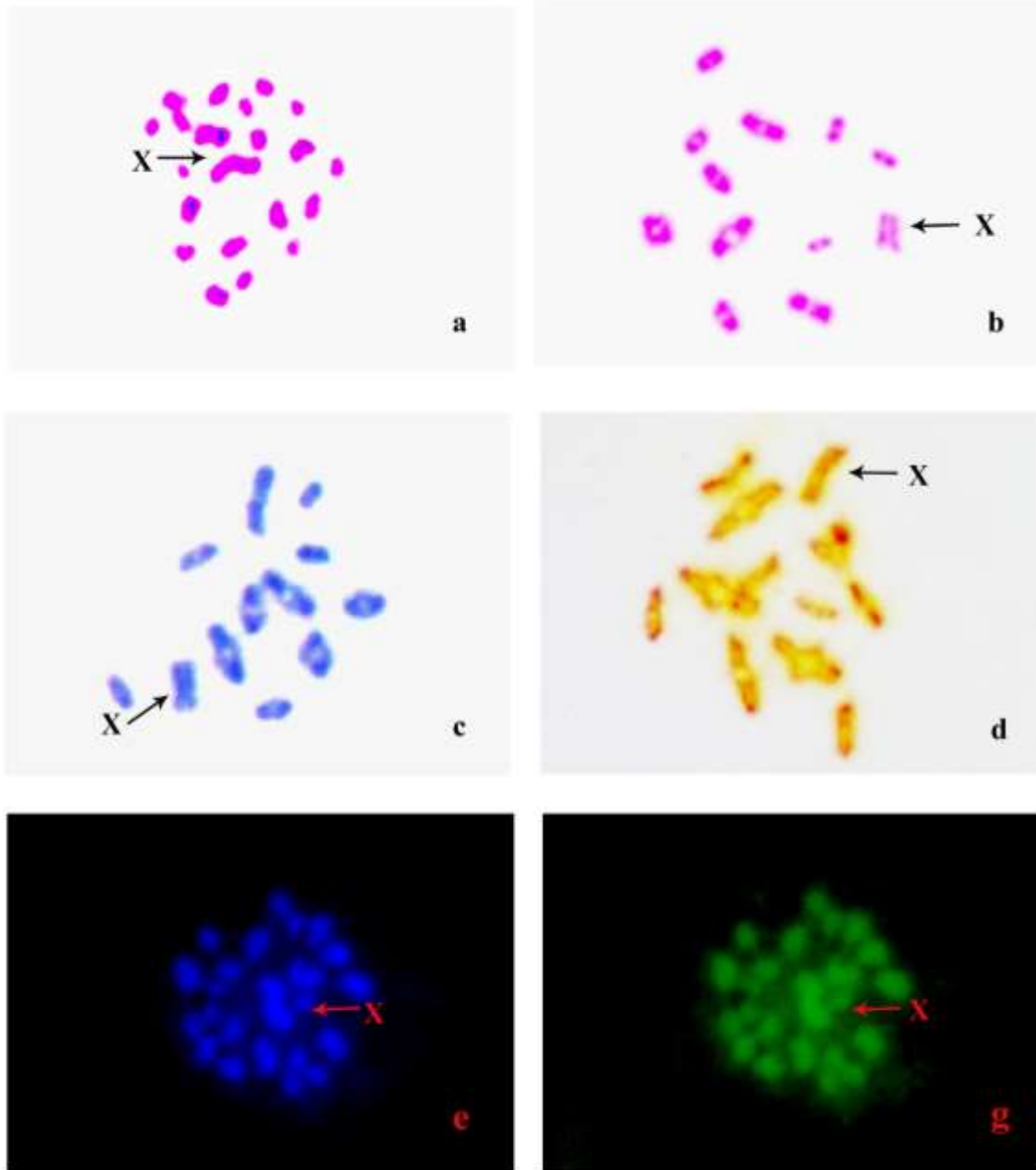


Figure 3. *Ictinogomphus rapax*. Conventional staining (spermatogonial metaphase 3a, metaphase-I 3b), C-banding (metaphase-I 3c), silver nitrate banding (Diakinesis 3d) and DAPI (spermatogonial metaphase 3e) CMA₃ (spermatogonial metaphase 3f), Bar= 0.01mm.

C-banded metaphase I of all three species shows the presence of terminal C-bands on autosomal bivalents, while both the chromatids of the bipartite X chromosome are C-positive (Fig. 1c, 2c, 3c). During AgNOR stained diakinesis/metaphase I, all the autosomal bivalents possess terminal NOR's on one end/both ends, while the bipartite X chromosome is NOR rich in all the species (Fig. 1d, 2d, 3d).

Spermatogonial metaphase of all the species shows autosomes as more DAPI bright than CMA₃, while the X chromosome is bright for both DAPI and CMA₃ (Fig. 1e, 1f, 2e, 2f, 3e, 3f).

DISCUSSION

The majority of the species of the family Gomphidae possess $2n = 23$, which is the modal number of the family. To date, the chromosome complement of 71 species has been described, Among these, 21 species are from India (Asana and Makino, 1935; Das, 1956; Dasgupta, 1957; Tyagi, 1977, 1978a, b, 1982, 1986; Walia and Sandhu, 1999; Walia *et al.*, 2006; Chahal, 2013; Walia and Chahal, 2014, 2020).

Taxonomically, 18 species of genus *Ictinogomphus* are known, out of these, chromosomes complement of *Ictinogomphus decoratus* and *Ictinogomphus rapax* have been reported (Kiauta and Kiauta, 1982; Asana and Makino, 1935; Makino, 1935; Kichijo, 1942; Omura, 1949, 1952, 1953; Dasgupta, 1957; Tyagi, 1982). Presently, *Ictinogomphus angulosus*, *Ictinogomphus pertinax* and *Ictinogomphus rapax* of genus *Ictinogomphus* have been studied and all the species possess $2n = 23$ which is the type number of family. The presence of a large X chromosome in all the species depicts that the X chromosome originated by the fusion of autosome pair with X chromosome with an intermediate stage of neo-XY. m chromosomes are present in previously studied species, while these are absent in all the presently studied species.

Linear characterization of chromosomes based on C-banding, silver nitrate staining and sequence-specific staining has been done for the first time on all three species of genus *Ictinogomphus*. C-bands and NORs are present at the terminal end of the autosomes, while the X chromosome is C-positive and NOR-rich. These results are confirmed by the sequence-specific staining as the chromosome complement is more DAPI bright than CMA, while the X chromosome possesses interspersed AT and GC regions in all the species.

CONCLUSION

Cytogenetic analysis on *I. angulosus*, *I. pertinax* and *I. rapax* of genus *Ictinogomphus* of family Gomphidae have been done by using conventional staining, C-banding, silver nitrate staining and sequence-specific staining. All the species possess the chromosome number $2n = 23$ with XO-XX sex determination. Terminal C-bands and NORs are present on all the autosomal bivalents, while m bivalent show variation in C- heterochromatin and NORs. Sequence-specific staining shows the presence of overlapping AT and GC-rich regions which are in accordance with the results of C-banding and silver nitrate staining.

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