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Cytogenetic Studies on Three Species of Genus *Burmagomphus* of Family Gomphidae (Odonata: Anisoptera) from India

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Abstract: Male germ cell chromosomes of *Burmagomphus divaricatus*, *Burmagomphus pyramidalis* and *Burmagomphus sivalikensis* of family Gomphidae have been investigated by using conventional staining, C-banding, silver nitrate staining and sequence specific staining. The species were collected from Punjab and Himachal Pradesh, India. All the species possess the chromosome number $2n = 23$ which is the type number of the family. Terminal C bands and NOR's are present at the autosomal bivalents and X chromosome is C positive and NOR rich in all the three species, while m bivalents show variation in distribution of C- heterochromatin and NOR's. In the sequence specific staining, whole complement shows bright DAPI signals in *B. divaricatus*, bright CMA₃ signals in *B. pyramidalis* and both DAPI and CMA₃ signals in *B. sivalikensis*.

Keywords: Gomphidae, *Burmagomphus*, Conventional staining, C- banding, Silver nitrate staining, Sequence specific staining

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Introduction

Taxonomically, 1004 species of family Gomphidae are known worldwide, while 83 species are present in India. Cytogenetic data is available only for 71 species which also includes 21 species 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). Majority of the

species possess $2n = 23$, which is the type number of the family, while variation due to presence/absence of m chromosomes, presence of neo-XY sex determination resulted by the fusion of an autosome with sex element have been observed in few species. Genus *Burmogomphus* contains 30 species, while only 3 species are studied cytogenetically (Tyagi, 1977, 1978b, 1982; Chahal, 2013). In this study, structure and behaviour of the chromosomes during meiosis, detection of constitutive heterochromatin, location of NORs' and presence of AT and GC rich region across the chromosomes of *B. divaricatus*, *B. pyramidalis* and *B. sivalikensis* of genus *Burmogomphus* have been studied and compared. Among these, chromosome complements of *B. divaricatus* and *B. sivalikensis* have been described for the first time.

Materials and Methods

The live and adult specimens were collected from the areas of Punjab and Himachal Pradesh, India. Adult specimens were dissected in saline solution (0.69%), testes were extirpated, kept for 45 minutes in sodium citrate (0.9%) then fixed in Carnoy's fixative and tapped on grease free slides. Prepared slides were treated for different cytogenetic staining techniques like 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

In all the three species, diakinesis possesses 12 elements, among these, 11 are autosomal bivalents, including small sized m bivalent and one large univalent, showing bipartite

behaviour is X chromosome. The m bivalent is achiasmatic in *B. divaricatus* and *B. sivalikensis*, while showing terminal chiasma is *B. pyramidalis* (Figs. 1a, 2a, 3a). At metaphase I, all the autosomal bivalents are rod shaped with central constriction due to condensation and X chromosome shows bipartite behaviour (Figs. 1b, 2b, 3b).

Fig. 1: *Burmogomphus divaricatus*– 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, h) (DAPI: metaphase-I, CMA₃: metaphase-I).

During the diakinesis and metaphase I, all autosomal bivalents show terminal C-bands and bipartite X chromosome is C-positive, while m bivalent is C- positive in *B. divaricatus* and *B. pyramidalis* and C-negative in *B. sivalikensis* (Figs. 1c, 1d, 2c, 2d, 3c, 3d). At diakinesis and metaphase I, all the autosomal bivalents show terminal NOR's, while m bivalent possesses dim NOR's due to small size, whereas X chromosome possesses

Fig. 2: *Burmagomphus pyramidalis* – 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, h) (DAPI: metaphase-I, CMA₃: metaphase-I).

bipartite behaviour and NOR's rich in all the species (Figs.1e, 1f, 2e, 2f, 3e, 3f).

At metaphase I, all the autosomal bivalents and X chromosome are DAPI bright than CMA₃ while m bivalent possesses light DAPI and CMA₃ signals due to its small size in *B. divaricatus* (Figs. 1g, 1h). In *B. pyramidalis*, all the autosomal bivalents including m bivalent and bipartite X chromosome show more CMA₃ bright signals (Figs. 2g, 2h) and in *B. sivalikensis*, all the autosomal bivalents including m bivalent and X chromosome

Fig. 3: *Burmagomphus sivalikensis*– 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, h) (DAPI: metaphase-I, CMA₃: metaphase-I).

reveals both DAPI and CMA₃ signals (Figs. 3g, 3h).

Discussion

The modal number of the family Gomphidae is $2n = 23$ as it is present in majority of the species. The reduced chromosome number in the family is originated from the primary complement $2n = 25$ of the suborder Anisoptera by fusion of autosomes or fusion between an autosome with sex element (Kiauta, 1969). XO/XX type of sex mechanism is present in 67 cytogenetically studies species, while 4 species show with neo-XY sex determining mechanism.

Table 1: Earlier reports on *Burmagomphus*

S.No.	Name of the species	Locality	Chromosome number	Reference
1.	<i>Burmagomphus arboreus</i> Lieftinck, 1940	India	n = 12 m	Tyagi, 1978b, 1982
2.	<i>Burmagomphus pyramidalis</i> Laidlaw, 1922	India	n = 12 m	Tyagi, 1977, 1982 Present Study
3.	<i>Burmagomphus williamsoni</i> Forster, 1914	India	n = 12 m	Chahal, 2013
4.	<i>Burmagomphus divaricatus</i> Lieftinck, 1964	India	n = 12 m	Present Study
5.	<i>Burmagomphus sivalikensis</i> Laidlaw, 1922	India	n = 12 m	Present Study

In India, only 13 species, out of 71 cytogenetically studied species of family Gomphidae have been described (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).

Genus *Burmagomphus* contain 30 species, while only 3 species have been described cytogenetically from India (Tyagi, 1977, 1978b, 1982; Chahal, 2013). All the species possess $2n=23m$ with XO-XX sex mechanism. In the present study, *B. divaricatus*, *B. pyramidalis* and *B. sivalikensis* also possess $2n=23$ with XO-XX sex mechanism which is in accordance to the earlier reports (Table 1). All the species of genus *Burmagomphus* show the presence of large X chromosome, which might be originated by the fusions. This process is divided into three stages, with an intermediate neo-X neo-Y type of sex determination and a final stage with reduction in two elements in diploid stage (Kiauta, 1969). Among these, *B. divaricatus* and *B.*

sivalikensis have been studied for the first time.

C- banding and silver nitrate staining have been done on all the 3 species of the genus. C-bands and NOR's are present mostly at the terminal ends of autosomal bivalents and X chromosome is C- positive and NOR rich, while m bivalent show variations. Sequence specific staining shows the presence of overlapping AT and GC rich regions which are in accordance to the results of C-banding and silver nitrate staining.

Conclusion

Cytogenetic analysis on *B. divaricatus*, *B. pyramidalis* and *B. sivalikensis* of genus *Burmagomphus* 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 NOR's are present on all the autosomal bivalents, while m bivalent shows variation in

C-heterochromatin and NOR's. Sequence specific staining shows the presence of overlapping AT and GC rich regions which are in accordance to the results of C-banding and silver nitrate staining.

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