

A new diploid chromosome number in *Gymnopleurus Illiger, 1803* (Coleoptera: Scarabaeidae, Scarabaeinae) from Haryana, India

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Abstract: The chromosomes obtained from scarab beetle *Gymnopleurus mundus* Wied., 1819 of subfamily Scarabaeinae were studied using standard staining and C-banding. The karyotype is comprised of 14 chromosomes with meioformula, 6AA+Xyp. The analysis of constitutive heterochromatin (CH) revealed small blocks located in the centromeric region of all chromosomes. Some of the anomalies like polypoidy, decondensation, stickiness and fragmentation of the chromatids were also encountered. The reduction of chromosome number and with conserved sex chromosome mechanism in *G. mundus* as compared to other *Gymnopleurus* spp. is suggestive of the Robertsonian fusion of autosomes having played some role in the evolution of karyotype in this genus.

Keywords: Scarabaeinae, *Gymnopleurina*, *Gymnopleurus*, Karyotype, Chromosomal analysis

I. Introduction

The Scarabaeinae constitute a highly diverse subfamily that comprises about 5,000 described species belonging to 234 genera spread widely in the world [1]. Most of the 85 Scarabaeinae species which are known cytogenetically have a chromosome number varying from $2n=12$ to $2n=20$, with the Xyp type being the most prevalent sex chromosome mechanism [2, 3].

The genus *Gymnopleurus* Illiger, 1803 (Scarabaeinae: Scarabaeidae), whose distribution is Palearctic, Afrotropical and Oriental [4] comprises about 50 species [5], but only five of them have been partly investigated from karyological point of view. According to some of the reports haploid number $n=10$ for *G. koenigii* [6], the diploid number $2n=18$ for *G. sinatus* [7], $2n=20$ for *G. cyaneus* [7], $2n=20$ for *G. sturmi* [3] and $2n=18$ for *G. geoffroyi* [8] has been established. Since information on these insects as meagre as compared to other genera of polyphagan beetles, the present communication deals with the chromosomal analysis of *G. mundus* by means of Geimsa staining and C-banding. This is a new cytological record of the subtribe *Gymnopleurina*, whose phylogeny is still controversial [9].

II. Materials And Methods

Sexually mature male specimens of *Gymnopleurus mundus* Wied., 1819 were collected from Seonti forest, Kurukshetra (Haryana, India) in months of August and September, 2009. All the specimens were identified following the guidelines laid down by taxonomist Arrow [11]. Chromosomal preparations were obtained, using the air drying method [12] from male gonads. C-bands were determined using the procedure described by researcher Sumner [13]. Evaluation of chromosomal morphology was based on ten spermatogonial metaphases. Percentage relative length of chromosomes was also calculated. Spermatogonial metaphase and other meiotic stages were analysed, photographed and karyotypes were prepared.

III. Results And Discussion

Gymnopleurus mundus Wied. $2n=14$

Spermatogonial metaphase was characterised by the presence of 14 chromosomes (Fig.1). The karyotype comprised of six pairs of autosomes and sex chromosomes X and y (Fig. 2). The ideogram of the chromosomes represented the location of centromeres in chromosomes (Fig.11). Autosomal pairs 1 to 3 are metacentric, pairs 4 and 5 are submetacentric and remaining one pair 6 is acrocentric. The autosomes showed a gradual decrease in size. The X chromosome is subtelocentric whereas y is acrocentric. Percentage relative length of autosomes varied from 5.27 to 18.75 whereas that of X is 17.41 and y is 8.32 (Table 2). The analysis using C-banding technique allowed the identification of small constitutive heterochromatic blocks located in the centromeric region of all chromosomes (Fig. 3 & 4). During pachytene stage elongated thread like chromosomes appeared (Fig. 5). Stickiness and fragmentation of the chromatids was observed at pachytene stages (Fig.6). *G. mundus* also showed the polyploid nuclei at different stages of spermatogenesis like spermatogonial prophase and spermatogonial metaphase (Fig. 7). Metaphase I revealed 6 autosomal bivalents in the form of highly condensed rods and the sex parachute (Fig. 8 & 9). Due to chromatid separation the morphology of the chromosomes is very clear at metaphase II (Fig. 10). The haploid number at metaphase II and number of bivalents at metaphase I confirmed the diploid number counted at spermatogonial metaphase.

Scarabaeinae (Coprinae) is less conservative subfamily of Scarabaeidae [14]. Unlike other scarabs there are much more variations in the form and size of chromosome numbers. But as in most of other scarabs the sex chromosome mechanism in Scarabaeinae is mainly Xyp type.

In the present chromosomal analysis of *G. mundus* is a new cytological record of Scarabaeinae. A perusal of literature on *Gymnopleurus* in TABLE 1, indicated the chromosome number, $2n=18$ for *G. geoffroyii* [9] and *G. sinatus* [7], whereas that of *G. koenigii* [6], *G. cyaneus* [8] and *G. sturmi* [3] is $2n=20$ which is the modal number of polyphagan beetles. But unlike all these species of *Gymnopleurus* cytogenetically analysed till date, the diploid number, $2n=14$ of *G. mundus* is lowest of all other species given in TABLE 1. But the sex chromosomal mechanism was conserved in all the species of Xyp type.

Six pairs of autosomes as subtelocentric and acrocentric in *G. sturmi* with $2n=20$ [3], whereas, four pairs of autosomes as acrocentric in *G. geoffroyi* with $2n=18$ [9] have been reported. But in the present study, *G. mundus* with $2n=14$ possessed only one pair of autosomes as acrocentric. Acrocentry being the primitive evolutionary behaviour of the chromosomes, this decrease in the diploid number, number of acrocentrics and subtelocentrics from *G. sturmi* [3] to the *G. mundus* (present study), with conserved sex chromosomes is probably the resultant of pericentric inversions followed by fusions [15] as observed in other species belonging to Scarabaeidae, for instance, *Bubas bubalus* $2n=18$, Xy [9], *Dichotomius geminatus* $2n=18$, Xyp [15], *Isocopriss inhiata* $2n=18$, Xyp [16], and *Macraspis festiva* $2n=18$, Xyp [17]. This occurrence of extensive karyotypic reorganisation leading to a more stable karyotype at least at autosomal level.

The centromeric C-banding pattern observed in *G. mundus* is quite common among Scarabaeidae representatives and also to Coleoptera as a whole [17, 18, 19, 20, 21, 22, 23, 24, 25]. Meanwhile, particular cases of CH distribution have been reported in some Scarabaeidae; *Cetonia aurata* and *Bubas bison* have CH blocks located in the terminal region of eight autosomal bivalents, in addition to the pericentromeric blocks [20, 26, 27]. In some species of Scarabaeidae, such as *Diabroctis mimas* and *Isocopriss inhiata*, the occurrence of diphasic chromosomes that present one heterochromatic and another euchromatic arm has been described [16]. In addition to these patterns, there are still some species with almost total heterochromatic chromosomes or with CH absence. In *Lygirus ebenus*, *Geniates borelli* and *Pelidnota pallidipennis*, the X chromosome is almost totally heterochromatic, while the Y do not show CH blocks [17]. CH distribution was centromeric and pericentromeric in *G. sturmi* [3] and *G. geoffroyi* [9]. CH bands also found in the metaphase I plate revealed the terminal localisation of heterochromatin. Using conventional staining, it has been proposed that the occurrence of autosome fusions is the main reason in reduction of chromosome number [15].

Polyploid nuclei in spermatogonial prophase and metaphase in *G. mundus* agree with some of the workers [28], who suggested that in all multicellular animals and plants certain tissues regularly consist wholly or in part of polyploid cells, whose nuclei contain multiples of the basic number of chromosomes. Sporadic occurrence of polyploidy nuclei in the germ cells of Coleoptera was observed by some of the researchers [29, 30, 31, 32]. Such polyploidy may have possibly originated either due to endomitosis [33] or by neighbouring nuclei [30] or by the loss of cytokinesis which follows the karyokinesis. Presence of polyploidy and stickiness of the chromatid material represents the anomalies in natural populations. This may be the reason for the variation in the karyotype reorganisation in different species of *Gymnopleurus*.

IV. Conclusion

The autosomal fusion events are probably the main rearrangement responsible for the chromosome differentiation in this species, acting in diploid number reduction. The present study provides a new cytological record of the genus *Gymnopleurus* which joined to future cytological data on other species of the same genus will provide a set of potentially informative characters suitable to understand the phylogeny of subtribe *Gymnopleurina* which according to recent literature [3, 9, 16, 17] is still unclear. So, this new data helps to draw the phylogeny of the genus *Gymnopleurus*.

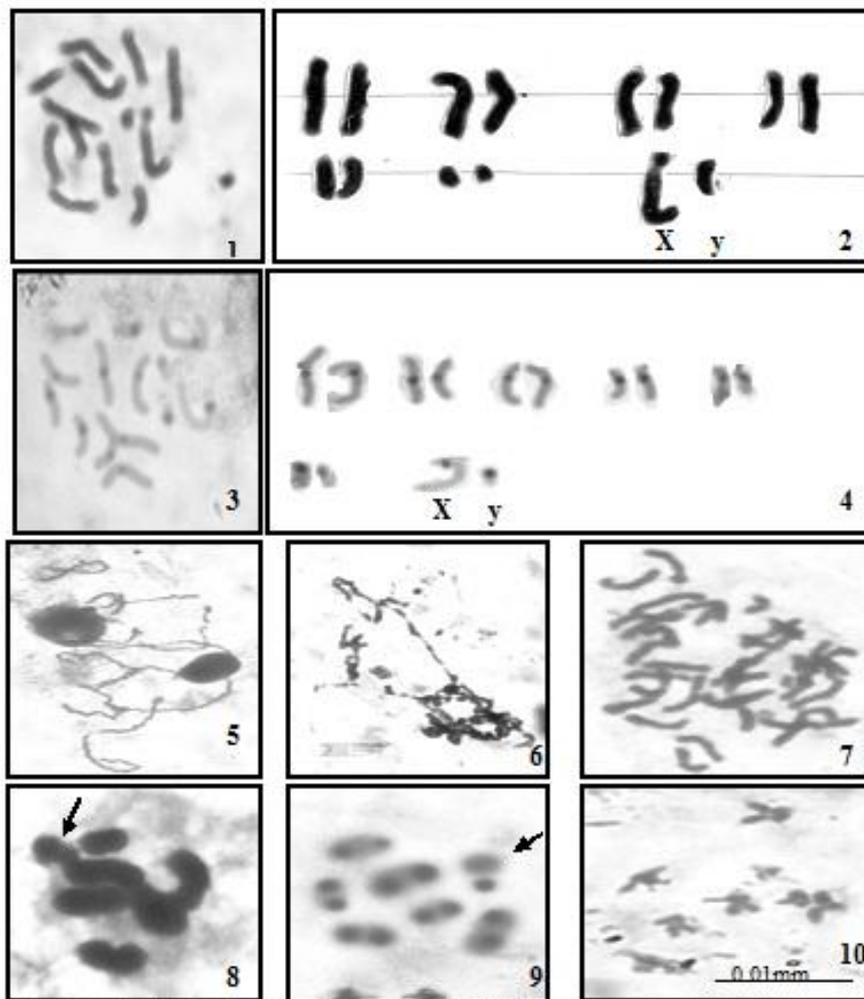
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Figures (1 – 10) *Gymnopleurus mundus* Wied.

1. Spermatogonial metaphase; 2. Karyotype; 3. C- banded Spermatogonial metaphase; 4. C- banded Karyotype ; 5. Pachytene ; 6. Stickyness in Pachytene; 7. Polyploidy in spermatogonial metaphase; 8 Metaphase-I, arrow indicates Xyp; 9. C-banded Metaphase-I; 10. Metaphase-II

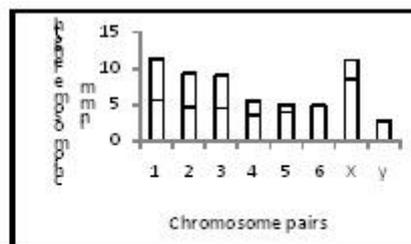


Fig. 11 . Diagrametic representation of average karyotype of *G. mundus*

Table 1. Chromosomal analysis of *Gymnopleurus* spp.

| Sr.No. | Species | Diploid number (2n) | Meioformula | Reference |
|--------|---------------------|---------------------|-------------|------------------------------|
| 1 | <i>G. koenigii</i> | 20 | 9AA+Xyp | Dasgupta, 1963 |
| 2 | <i>G. cyaneus</i> | 20 | 9AA+Xyp | Kacker, 1976 |
| 3 | <i>G. sturmi</i> | 20 | 9AA+Xyp | Colomba <i>et al.</i> , 2000 |
| 4 | <i>G. sinatus</i> | 18 | 8AA+Xyp | Manna & Lahiri, 1972 |
| 5 | <i>G. geoffroyi</i> | 18 | 8AA+Xyp | Angus <i>et al.</i> 2007 |
| 6 | <i>G. parvus</i> | 18 | 8AA+Xyp | Unpublished |
| 7 | <i>G. miliaris</i> | 36 | 17AA+Xyp | Unpublished |
| 8 | <i>G. mundus</i> | 14 | 6AA+Xyp | Present report |

Table 2. Percentage relative length of chromosomes of *Gymnopleurus mundus*

| Chromosomal pairs | percentage relative length of chromosomes |
|-------------------|---|
| Pair no. | |
| 1 | 18.75 |
| 2 | 14.3 |
| 3 | 13.1 |
| 4 | 12.5 |
| 5 | 10.2 |
| 6 | 5.27 |
| X | 17.41 |
| y | 8.32 |