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The first cytogenetic report of *Hoplopoderus gemmeatus* (Attelabidae: Coleoptera: Insecta) from Indian fauna

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Abstract
The chromosomal analysis of beetle *Hoplopoderus gemmeatus* Thunberg. of subfamily Attelabidae and tribe Hoplop Soderini was done using standard staining, C-banding and $AgNO_3$ banding. Tribe Hoplopoderini was first time mapped cytogenetically. The cytological information of these beetles is useful in many ways. Theoretically, it is important to understand the mechanism governing the transmission of genetic information, hence speciation and for classification on taxonomic levels. It is also useful in the field of applied economic entomology for the improvement of species. The karyotype of *Hoplopoderus gemmeatus,* is comprised of 30 chromosomes with meioformula, 14AA+Xyp. The analysis of constitutive heterochromatin (CH) revealed small blocks located in the centromeric regions of chromosomes, whereas silver staining revealed the silver dark spots corresponding to the nucleolar organizing region. The increased in chromosome number from 20 (Modal number) to 30 and with conserved sex chromosome mechanism in *Hoplopoderus gemmeatus* as compared to other species of this family is suggestive of the Robertsonian fission of autosomes having played some role in the evolution of karyotype in this family.

Keywords: Attelabidae, hoplopoderini, *Hoplopoderus gemmeatus*, karyotype, chromosomal analysis, C-banding, $AgNO_3$ banding

Introduction
Coleoptera means "sheath wings", a reference to the hardened forewings which cover the insect's body. Most people can easily recognize members of this order-the beetles. Coleoptera experienced countless speciation processes and today it is the most successful insect order having the maximum number of species. Arnett (1968) [1] recorded 3,50,000 described species of Coleoptera. Moreover, about 1000 new species are being added annually to this number. We may number beetles among our friends as well as foes and in either case they deserve and receive scientific study. Prowazek (1902) [5] and Stevens (1906) [7] initiated studies into the chromosomal cytology of beetles. Smith and Virkki (1978) [9] listed the chromosomal data of 2160 species, subspecies and parthenotes referable to 45 families of Coleoptera investigated upto 1975. However, there has been an explosive expansion in our knowledge of the chromosome cytology during the last three decades. The order Coleoptera has the highest species diversity within the animal kingdom, yet cytogenetic data using specific banding techniques are still scarce. C-banding data have revealed a preferential localisation of constitutive heterochromatin (CH) in centromeric area and less so observed in interstitial and telomeric areas. Sex chromosomes also show a variable CH distribution, as it has been observed in the pericentric region or along the entire chromosome.

The Attelabidae is a widespread family of weevils which includes more than 2000 species. These are primitive weevils, because of their straight antennae which are inserted near the base of the rostrum. Some members of this family have long neck and some of are known as Giraffe weevils. These are known as true leaf rollers and some of these species are minor agricultural pests. Out of these 24 species of Attelabidae from Japan (Takenouchi 1973) [20] and one species from Slovakia were examined karyologically (Rozek *et al.* 2004) [6]. Karyotype characterisation of *Apoderus coryli* using C-banding technique was reported by Rozek *et al.* (2004) [6]. Tribe Hoplopoderini was first time brought to the cytological map by investigating *Hoplopoderus gemmeatus,* during the present investigations, which exhibited haploid number n=15 with chromosomal formula 14+Xyp.

Materials and methods
Sexually mature male specimens of *Hoplapoderus gemmatus* Thunberg. Were collected from Kurukshetra University campus, Kurukshetra (Haryana, India) in months of August and September. The beetles were sacrificed in 0.56% KCL solution. The testicular material on removal was treated with 0.001% colchicine for 20 minutes. Then it was kept in 1% sodium citrate solution for 20 minutes at room temperature. After the hypotonic treatment the material was fixed in cold 1:3 acetic-methanol for 20 minutes giving 2 or 3 changes. Fixed material was used for the preparation of slides by air drying method described by Yadav and Lyapunova (1983) [27]. Air dried slides were incubated for 2-4 days at 37° C. C-banding was done according to BSG (Barium hydroxide / Saline / Giemsa) technique of Sumner (1972) [8] with slight modifications. The silver staining technique of Bloom and Goodpasture (1976) [2] was followed for staining the nucleolar organizer regions.

Evaluation of chromosomal morphology was based on ten spermatogonial metaphases. Selected stages were microphotographed using oil immersion objective (100X) and digital compact camera (Olympus, C-7070). Chiasma frequency per bivalent was calculated from randomly scored

diakinetic/ metaphase I stages in each species by applying the formula as follows:
Chiasma frequency = Total number of chiasmata per cell/ no. of bivalents per cell.

Results and discussion

***H. gemmatus* Thunberg**

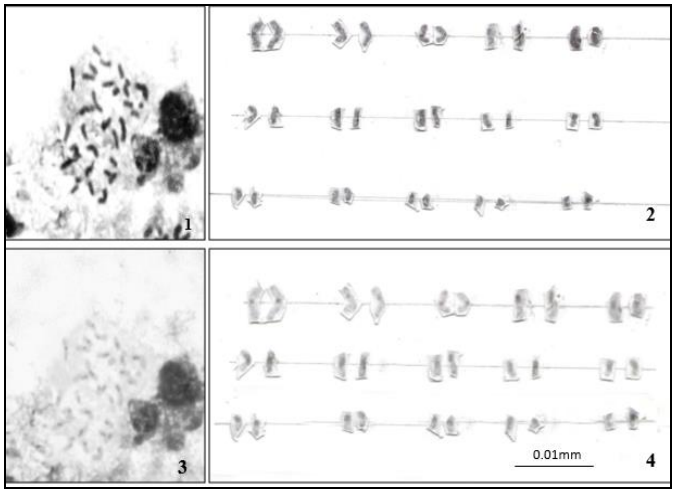
Spermatogonial metaphase exhibited the diploid number of 30 chromosomes (Fig. 1). The karyotype comprised of six pairs of metacentric (Pairs 1-6), three pairs of sub metacentric (Pairs 7-9) and five pairs of acrocentric (Pairs 10-14) autosomes, metacentric X and dot shaped y chromosomes (Fig. 2). Percentage relative length of autosomes varied from 4.01 to 11.34, whereas that of X and y was 3.90 and 3.26, respectively (Fig. 14). C-banded spreads of Spermatogonial metaphase (Fig.3) were also obtained and the karyotype of banded chromosomes depicted the centromeric localisation of heterochromatic regions in all autosomes and X chromosome, while y being euchromatic (Fig. 4). Pachynemal thick thread like chromatids were seen to overlap each other at pachytene stage (Fig. 5).

$AgNO_3$ banding: Application of silver staining revealed the silver spots corresponding to the nucleolar organiser region, in the pachynemal threads along with densely stained sex pseudobivalent (Fig. 6).

More condensed granulated threads forming two rings and other 12 rods along with sex parachute were observed during diplotene (Fig. 7). At diakinesis the bivalents became separately visible, forming 11 rings and three rods along with a sex parachute (Fig. 8). As many as 14 rod shaped autosomal bivalents and sex parachute Xyp marked the prometaphase-I (Fig. 9). Terminalisation of chiasmata was complete by metaphase-I, which showed 14 highly condensed rods of autosomal bivalents and one sex parachute Xyp (Fig. 10). Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase-I was 1.0 and 1, respectively. The meio-formula of this species is 14AA+Xyp. C-banded spreads of metaphase-I (Fig. 11) were obtained which show the C- bands placed distally on the bivalents.

As a result of first reductional division, two classes of secondary spermatocytes were produced, with respect to the distribution of X and y. Hence two types of metaphase-II plates one with X (Fig. 12) and other with minute y (Fig. 13) chromosome in addition to seven pairs of autosomes. Due to separation of chromatids, centromeric positions of all the chromosomes were clearly marked.

The Attelabidae is a widespread family of weevils which includes more than 2000 species. Only 36 species are known cytologically by different workers. Out of these, 24 species of Attelabidae are from Japan, one species from Slovakia and others from India have been examined karyologically by Takenouchi (1954, 1955a, 1958 a, b, 1963, 1969, 1970 a, b, 1973, 1974 b, c, 1976a, 1979) [10-26]; Yadav *et al.* (1992 a) [28]; Rozek *et al.* (2004) [6] and the present study. There is a wide range of haploid chromosome number in this family from 9 to 19 and male sex chromosome Xyp, Xy and Neo-XY (Takenouchi 1981 and Holecova *et al.* 1999) [26, 3]. Haploid number 11 and 13 showed the maximum peaks in this family with 6 species each, whereas subsidiary peaks for n= 12, 14, 15, 16 and 19 were also found (Fig. 15). Tribe Hoplopoderini was first time brought to the cytological map by investigating *Hoplopoderus gemmeatus*, during the present investigations, which exhibited haploid number n=15 with chromosomal formula 14+Xyp. Only two other species *Paroplapoderus ulmi* and *Paratrachelophorus longicornis* of this family possess meioformula 14+Xyp (Takenouchi 1958b, 1963) [14, 15]. For the first time in this family, karyotype characterisation of *Apoderus coryli* using C-banding technique was reported by Rozek *et al.* (2004) [6]. A small amount of heterochromatin was found in this species. In contrast to it, under present investigations, *H. gemmeatus* was also studied by applying the banding techniques, prepared C-banded karyotype showed the centromeric localisation of heterochromatic regions on autosomes and X chromosome whereas y being euchromatic in nature. Nucleolar organiser regions were found on pachynemal threads along with densely stained sex pseudobivalent by applying silver nitrate staining.

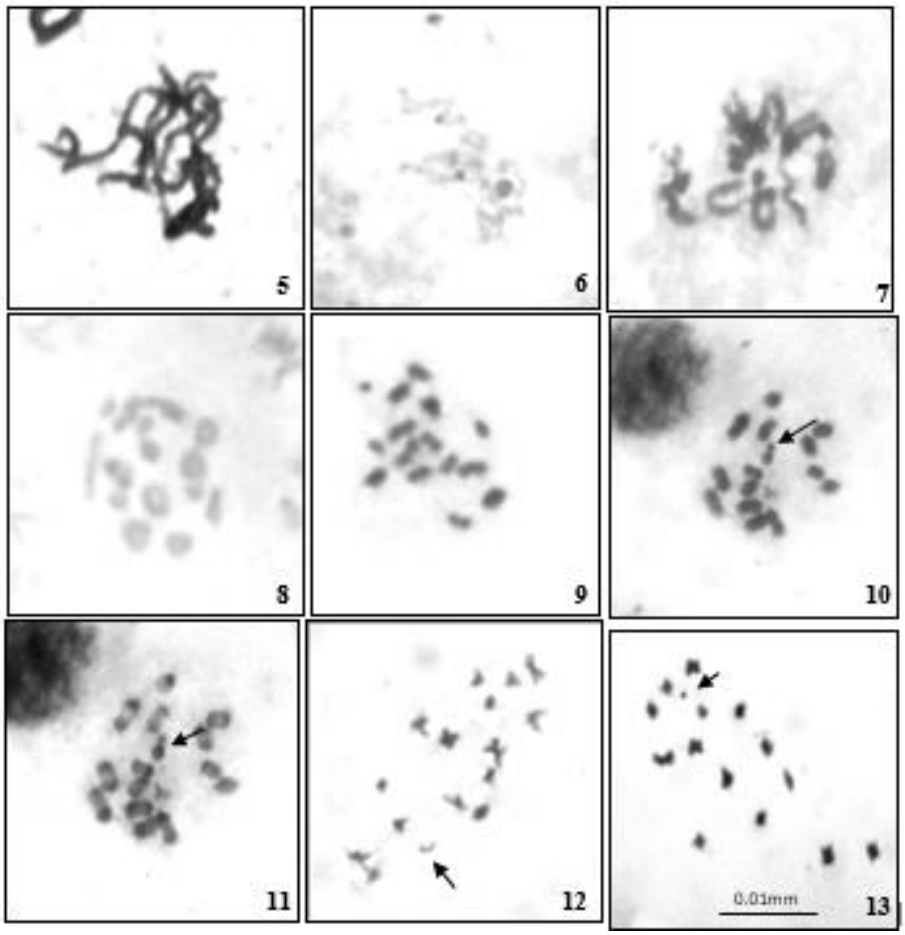


***Hoplapoderus gemmatus*:** Fig. 1: Spermatogonial Metaphase; Fig. 2: Karyotype; C- banded: - Fig. 3: Spermatogonial Metaphase; Fig. 4: Karyotype

Haploid chromosome varies from 9 to 19 in known species of family Attelabidae. Of these haploid numbers 11 and 13 are possessed by six species each. Increase of diploid number is prominent in this family. Only one species *Bysticus venustus* possess 2n=18. Whereas all other species have chromosome number more than 20 with Xy or Xyp sex mechanism. So, it was suggested that the autosome fission is responsible for the increase and X-autosome fusion with neo XY formation, for fresh decrease in chromosome number in two species of *Deporaus* (Takenouchi 1958a, b) [13]. Present investigation of *Hoplopoderus gemmetus*, a new cytological record, with 2n=30 and Xyp type of sex mechanism agreed with earlier reports given by Takenouchi (1958a, b, 1963) [13] on *Paroplapoderus* with 2n= 14+Xyp. C-banding revealed a small amount of heterochromatin on the autosomes which agreed with Rozek *et al.* (2004) [6].

In this family three types of sex chromosome mechanisms Xyp, Xy and neo-XY were encountered. Of the 32 sexually known species 29 species exhibit Xyp system while 2 species have neo XY and only one species possess Xy sex chromosome system. A perusal of literature (Fig. 15) Xyp seems to be a primitive sex mechanism in this family whereas

other two are the derived forms. As a little amount of cytological data is available of this family, so we cannot frame any opinion in connection to the evolution of sex chromosome mechanism.



***Hoplapoderus gemmatus*:** Fig. 5: Pachytene; Fig.6: Pachytene showing silver staining; Fig. 7: Diplotene; Fig. 8: Diakinesis; Fig. 9: Prometaphase I with Xyp (arrow indicates Xyp bivalent); Fig. 10: Metaphase I with Xyp; Fig. 11: Metaphase I with C- banding; Fig. 12: Metaphase II with X (arrow indicates X chromosome); Fig. 13: Metaphase II with y (arrow indicates y chromosome)

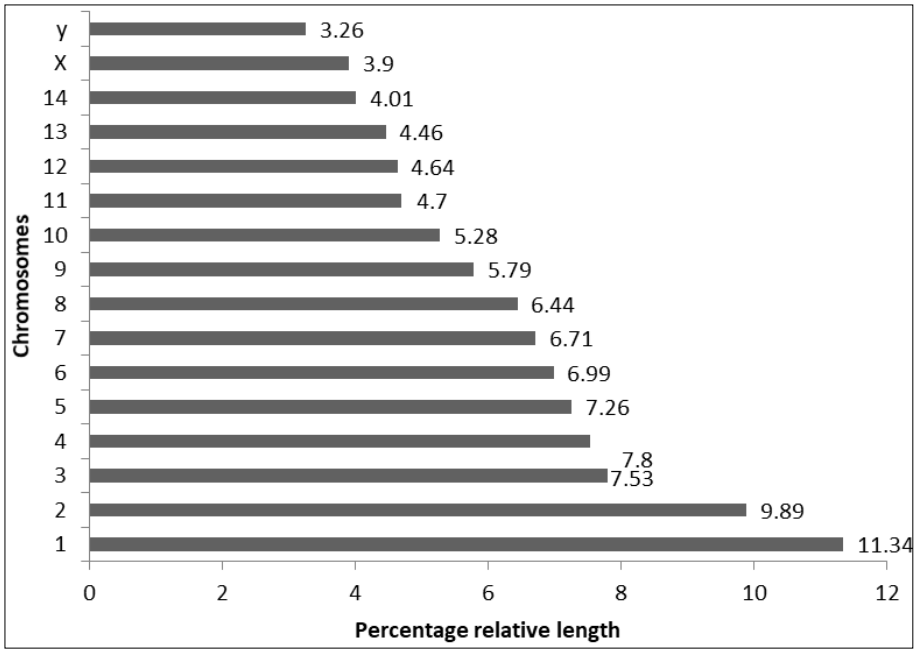


Fig 14: Percentage relative length of chromosomes of *Hoplopoderus gemmeatus*

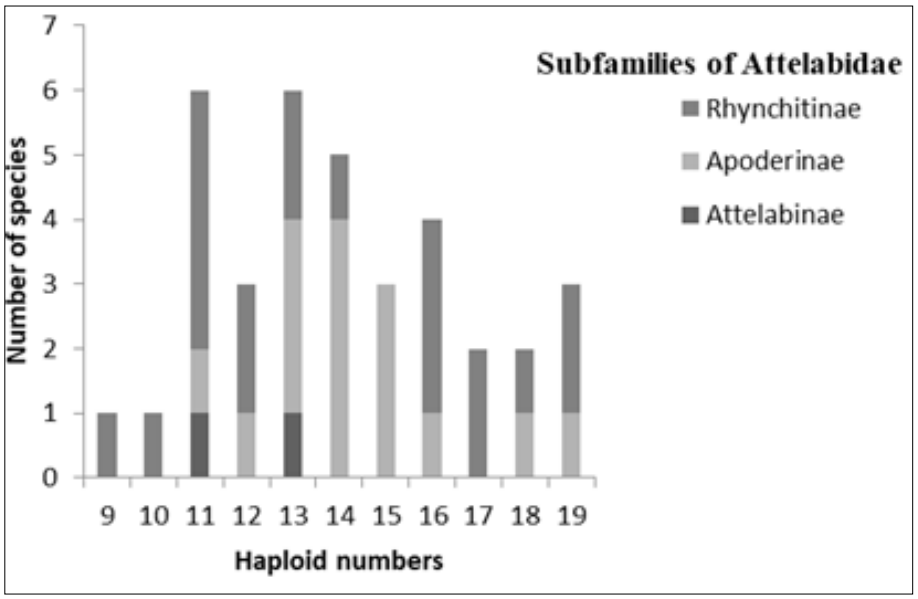


Fig 15: Histogram showing distribution of haploid number in family Attelabidae

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References

1. Arnett RH. The beetles of the United States. Amer. Entomol. Inst. Ann. Arbor. Mich. U.S.A, 1968.
2. Bloom SE, Goodpasture C. An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. Hum. Genet. 1976; 34:199-206.
3. Holecova M, Rozek M, Lachowska D. Meiotic chromosomes of 10 weevil species from Slovakia (Coleoptera, Curculionoidea: Attelabidae, Apionidae, Curculionidae). Acta Zool. Univ. Comenianae. 1999; 43:5-16.
4. Slovakia (Coleoptera, Curculinoidae: Attelabidae, Apuonidae, Curculionidae). Acta Zoo. Univ. Comenianae, 43:3-14
5. Prowazek S. Spermatologische studien II Arb. Zool. Inst. Univ. Wien. 1902; 13:223-236.
6. Roz˙ ek M, Lachowska D, Petitpierre E, Holecova M. C-bands on chromosomes of 32 beetle species (Coleoptera: Elateridae, Cantharidae, Oedemeridae, Cerambycidae, Anthicidae, Chrysomelidae, Attelabidae and Curculionidae). Hereditas. 2004; 140:161-170.
7. Stevens NM. Studies in spermatogenesis, II. Carneg. Inst. Wash., Pub. 1906; 36:33-74
8. Sumner AT. A simple technique for demonstrating centromeric heterochramatin. Exp. Cell. Research. 1972; 75:304-306.
9. Smith SG, Virkki N. *Animal Cytogenetics.* Insects 5 Coleoptera. Gebruder Borntraeger Berlin. Stuttgart, 1978.
10. Takenouchi Y. Chromosomes of 14 species of the Curculionidae (Coleoptera). J Hokkaido Gakugei Univ. 2nd ser. 1954; 5:85-92.
11. Takenouchi Y. A chromosome survey in thirty species of weevils (Curculionidae, Coleoptera). Jap. J Zool. 1955a; 11:425-441.
12. Takenouchi Y. Notes on cytology and hybridisation in two puzzling species of *Epilachna* (Coccinellidae: Coleoptera). Annot. Zool. Japan. 1955b; 28:238-243.
13. Takenouchi Y. A further chromosome survey in thirty species of weevils (Curculionidae, Coleoptera). Jap. J Zool. 1958a; 12:139-155.
14. Takenouchi Y. Further survey of the chromosomes in curculionid weevils (Coleoptera). Jap. J Genet. 1958b; 33:163-175.
15. Takenouchi Y. A further investigation on the chromosomes in twenty three species of weevils (Curculionidae: Coleoptera). J Hokkaido Gakugei Univ. 2nd ser. 1963; 13:160-175
16. Takenouchi Y. A chromosome survey in a weevil, *Deporaus unicolour* (Curculionidae: Coleoptera): An additional instance of the Xyp sex determining mechanism. Jap. J Genet. 1969; 44:379-380.
17. Takenouchi Y. Chromosome polymorphism in *Deporaus unicolour* Roelofs (Curculionidae: Coleoptera). Chrom. Inform. Serv. 1970a; 11:17-18.
18. Takenouchi Y. A bisexual race of the reportedly parthogenetic Japanese weevil *Catapionus gracilicornis* Roelofs (Coleoptera: Curculionidae). J Hokkaido Univ. Educ. IIB. 1970b; 22:1-17.
19. Takenouchi Y. A chromosome study of a new polyploidy parthenogenetic weevil, *Myllocerus nipponicus* Zumpt (Coleoptera: Curculionidae). Kontyu. 1972; 40:121-123.
20. Takenouchi Y. A revised study of the chromosomes of twenty-four species of Japanese weevils (Coleoptera: Curculionidae). Genetica. 1973; 44:621-632.
21. Takenouchi Y. Chromosomes of four species of scotylid beetles (Scotylidae: Coleoptera). Chrom. Inform. Serv. 1974a; 16:7-8.
22. Takenouchi Y. A study of the chromosomes of twenty-two species of Japanese weevils (Coleoptera: Curculionidae). Jap. J Genet. 1974b; 49:147-157.

23. Takenouchi Y. A study of the chromosomes of thirty four species of Japanese weevils (Coleoptera: Curculionidae). Genet. 1974c; 45:91-110.
24. Takenouchi Y. Chromosome survey in twelve species of bisexual weevils (Coleoptera: Curculionidae) in Kyushu, Japan I. La Kromosomo II. 1976a; 1:1-7.
25. Takenouchi Y. A diploid parthenogenetic race of the weevil species *Atapionus gracilicornis* from Japan (Coleoptera: Curculionidae). Entom. Gen. 1979; 6:367-369.
26. Takenouchi Y. A chromosomal study on two new polyploidy weevils in Kaushu, Japan. *Genetica.* 1981; 55:137-139.
27. Yadav JS, Lyapunova EA. A simple and rapid method for making karyological preparations from Coleopteran insects. Nucleus. 1983; 26:159-162.
28. Yadav JS, Lyapunova EA, Vorontsov NN, Koreablev VP, Baklushinskaya I Yu. Karyological investigations on five species of Polyphaga Coleoptera from Russia. J Cytol. Genet. 1992a; 27:11-17.