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Bionomics and cytogenetics of *Anomala bengalensis* (Coleoptera: Scarabaeidae: Scarabaeinae) from Haryana

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Abstract
Anomala bengalensis belongs to subfamily Rutelinae in family Scarabaeidae. These are widely distributed scarab beetles also known as leaf chafers with ecological and economic importance in agriculture ecosystem. Adult beetles were collected during monsoon season by using Light traps fitted with halogen bulb and installed in open agriculture fields. Specimens were identified by using the standard keys and then get proceeded for ecological, economical and cytogenetic analysis. Diploid number 20 was revealed and karyotype exhibit metacentric or submetacentric type of chromosomes. Mean chiasma frequency and terminalisation coefficient per bivalent has also been calculated to confirm the recombination frequency and revealed conservation of chromosomal architecture of *Anomala bengalensis* with other species of family Scarabaeidae, while variation of diploid number in specimens of *Anomala bengalensis* suggested the important role of centric fusion and centric fission in evolutionary trend of karyotypic asymmetry.

Keywords: *Anomala*, chromosomal architecture, chiasma frequency, dung beetles, rutelinae

Introduction

The family Scarabaeidae includes subfamilies Scarabaeinae and Aphodiinae which are Laparosticti or dung beetles for housing in the dung, while Rutelinae, Melolonthinae, Dynastinae, Cetoninae, Sericinae, Euchirinae, Hoplinae, Valginae and Trichinae are Pleurosticti or phytophagous which feed on plants and are pests of crops (Chandra 1986) [7]. Rutelinae are a group of beetles of considerable economic importance. All members are phytophagous and are recognized as major agricultural pests affecting a wide range of cultivated crops. Both the larval and adult stages feed on plants. The larvae damage crops by cutting and feeding on roots, often causing plant death, while adults feed on foliage and reproductive parts, leading to significant yield losses. *Anomala bengalensis* (Blanchard) is a widely distributed scarab beetle belonging to the subfamily Rutelinae, commonly referred to as shining leaf chafers. Members of the genus *Anomala* are known for their economic importance as root-feeding larvae and foliage-feeding adults in agricultural ecosystems (Arrow 1917; Chandra and Gupta 2012) [3, 8].

In subfamily Rutelinae diploid number varies from 16 to 22 in 46 cytologically known species, out of which all species of genus *Adoretus* and one species of *Adorrhinyptia* possess the higher number 2n=22: 10+Xyp (Joneja 1960; Kacker 1970, 1971; Yadav and Pillai 1975, 1976 a, b, 1979; Mittal *et al.* 1987; Yadav and Dange 1988; Yadav *et al.* 1989) [9, 10, 11, 17, 32, 35, 38, 39], whereas one unidentified species of genus *Adorrhinyptia* show polymorphic nature (Saha and Manna 1971; Saha 1973) [22, 23]. Although most of the species of genus *Anomala* (Yosida 1949; Joneja 1960; Agarwal 1960, 1962; Lahiri and Manna 1969; Manna and Lahiri 1972; Kudoh *et al.* 1973; Yadav and Pillai 1974, 1975, 1979; Smith and Virkki 1978; Mittal *et al.* 1985) [1, 2, 9, 12, 13, 14, 16, 26, 34, 35, 38, 41] depicted basic karyotype 2n=20, yet two types of diploid number 2n=18 and 20 were reported in *Anomala bengalensis* (Yadav *et al*. 1993) [40] and *A. rufocuprea* (Saha and Manna 1971; Yadav *et al.* 1993; Kudoh *et al.* 1973; Yosida 1949) [12, 23, 40, 41] explicating dimorphic nature of both the species. Cytogenetic Catalogue of subfamily Rutelinae has also been displayed along with meioformula in Table 1.

Table 1: Cytogenetic Catalogue of beetles belonging to Subfamily: Rutelinae (Family: Scarabaeidae)

S. No.	Species	Diploid Number	Meioformula	References
1.	*Adorrhinyptia dorsalis* (Burm.)	22	10+Xyp	Yadav and Pillai (1976b); Yadav and Pillai (1979) [38]
2.	*A*. sp.	16/18/20	7/8/9+Xyr	Rozek *et al.* (2004); Saha (1973) [21, 22]
3.	*Anomala bengalensis* Blanch.	18 20	8+Xy 9+Xyp	Saha 1973 [22] Yadav *et al.* (1993) [40]
4.	*A. biharensis* Arrow	20	9+Xyp	Mittal *et al.* (1985) [16]
5.	*A*. sp. nr. *Bilobata* Arrow	20	9+Xy	Lahiri and Manna (1969) [13]
6.	*A. cincta polychalca* Bates	20	-	Smith and Virkki (1978) [26]
7.	*A. corpulenta* Mots.	18	8+Xy	Yosida (1949) [41]
8.	*A. cuprea*	20	10II	Kudoh *et al.* (1973) [12]
9.	*A. dorsalis* F.	20	9+Xyp	Agarwal (1960, 1962) [1, 2]
10.	*A. lucens*	20	9+Xyr	Kudoh *et al.* (1973) [12]
11.	*A. vestigator* Arrow	20	9+Xyp	Yadav and Pillai (1975) [35]
12.	*A. polita* Blanch.	20	9+Xyp	Yadav and Pillai (1979) [38]
13.	*A. ruficapilla* Burm.	20	9+Xyp	Yadav and Pillai (1975,1979) [35, 38]
14.	*A. rufocuprea* Mots	18	8+Xy	Kudoh *et al.* (1973) [12]

		20	10II	Yosida (1949) [41]
15.	*A. superflua* Arrow	20	9+Xyp	Joneja (1960) [9]
16.	*A. varicolor* Gyll.	20	9+Xyp	Yadav and Pillai (1975) [35]
17.	*A*. sp.	-	9+Xyp	Rozek *et al.* (2004) [21]
18.	*A*. sp.	-	9+Xyp	Manna and Lahiri (1972) [14]
19.	*Rhinyptia indica* Burm.	-	9+Xyp	Yadav *et al.* (1989, 1993) [39, 40]
20.	*Mimela glabra* Hope	20	9+Xyp	Yadav and Dange (1988) [32]
21.	*Mimela* sp.	20	9+Xyp	Joneja (1960) [9]
22.	*Popillia japonica* Newn.	18	8+Xy	Yosida (1949) [41]
23.	*Strigodermella protea* (Burm.)	-	9+Xyr	Smith and Virkki (1978) [26]
24.	*Adoretus birmanus* Arrow	22	10+Xyp	Yadav and Pillai (1974) [34]
25.	*A. bombinator* Burm.	-	10+Xyp	Yadav and Dange (1988) [32]
26.	*A. decanus* Ohaus	-	10+Xyp	Yadav and Pillai (1975) [35]
27.	*A. duvaceli* Blanch.	22	10+Xyp	Yadav and Pillai (1975) [35]
28.	*A. epipleuralis* Arrow	22	10+Xyp	Yadav and Dange (1988) [32]
29.	*A. incurvatus* Ohaus	22	10+Xyp	Yadav and Pillai (1975) [35]
30.	*A. lasiopyqus* Burm.	-	10+Xyp	Yadav and Pillai (1975) [35]
31.	*A. limbatus* Blanch	22	10+Xyp	Yadav and Pillai (1975) [35]
32.	*A. minutus* Bk.	22	10+Xyp	Mittal *et al.* (1985) [16]
33.	*A. simplex* Sharp	22	10+Xyp	Yadav and Pillai (1974) [34]
34.	*A. versutus* Har.	22	10+Xyp	Yadav and Pillai (1976b)
35.	*Catalpa lanigera* L.	20	9+Xy 9+Xyp	Shaffer (1920) [24]
36.	*Ectinohoplia rufipes*	-	9+Xyr	Kudoh *et al.* (1973) [12]
37.	*Hoplia communis*	-	10II	Kudoh *et al.* (1973) [12]
38.	*Pelidnota punctata*	20	9+Xy	Shaffer (1920) [24]
39.	*P. virescens aurescens* Latr.	-	9+Xyp	Smith and Virkki (1978) [26]
40.	*Phyllopertha campestris* Latr.	20	9+Xy	Virkki (1954) [29]
41.	*Pocalta ursine* Horn	20	9+Xyp	Virkki (1960) [30]
42.	*Geniates borelli*	20	9+Xyp	Bione (1999, 2005) [4, 5]
43.	*Macraspis festiva*	18	8+Xyp	Bione (2005) [5]
44.	*M. tristis*	18	8+Xyp	Silva *et al*. (2009) [25]
45.	*Macraspis dichroa* ssp. Cribata Watr.	-	8+Xyp	Vidal (1984) [28]
46.	*Pelidnota pallidipennis*	20	9+Xyp	Bione (1999, 2005) [4, 5]

Materials and methods

Study Area: The present study was conducted in Village Jhansa, Kurukshetra district, Haryana, India (approx. 29°58′N, 76°52′E). The region is characterized by agricultural landscapes, dominated by crops such as wheat, rice, and sugarcane. The climate is subtropical, with hot summers, a monsoon season (July–September) and cool winters. The soil type is predominantly alluvial, which provides favourable conditions for the development of scarab larvae (Yadav and Sharma, 1995) [31].

Collection Method: Adult specimens of *Anomala bengalensis* were collected during the monsoon and post-monsoon period (June–September), coinciding with their peak emergence (Veeresh, 1983) [27]. Collection was carried out using light traps (Mukhi 2002) [18]. Light traps fitted with a halogen bulb was installed in open agricultural fields and operated from evening to next morning. A glass jar was placed beneath the light source to facilitate easy collection of attracted beetles (Arrow, 1917; Mukhi 2002; Chandra and Gupta, 2012) [3, 8, 18].

Preservation and Handling: Adult beetles were killed using ethyl acetate soaked cotton in killing jars and subsequently pinned and oven-dried at 60^0C for morphological examination.

Identification: Specimens were identified using standard taxonomic keys and morphological descriptions (Arrow, 1917) [3].

Chromosomal analysis: Sexually mature male specimens of *Anomala bengalensis* 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. In which, the testicular material was taken in a small amount of 50% glacial acetic acid on clean grease free slide, which was immersed in dehydrated ethanol and cleaned by a piece of muslin cloth. The testes were macerated by means of dissecting needles. The slides were then allowed to dry in air and stained in 2% Giemsa stain. This method was given by Yadav and Lyapunova (1983) [33]. 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). The silver staining technique of Bloom and Goodpasture (1976) [6] was followed for staining the nucleolar organizer regions. Air dried slides were kept in moist air tight plastic box, flooded with silver nitrate solution covered with a cover slip. Distilled water was poured in the plastic box to maintain moisture taking care that it will not touch the slides. The slides were incubated at 37ºC for 20-40 hrs., rinsed in running distilled water and dried. The slides were examined either as such or after counter staining with 2% Giemsa solution (pH 6.8).

Chiasma frequency per bivalent was calculated from randomly scored diakinetic/ metaphase I stages in each species by applying the formula as follows:
Chiasma frequency per cell by applying the formula

Chiasma frequency per nucleus = Number of chiasmata per nucleus
Chiasma frequency per bivalent =

$$\frac{Total\ number\ of\ chiasmata\ per\ cell}{no.of\ bivalents\ per\ cell}$$

Percentage relative Length of each chromosome was calculated, which represented the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus.

Results and Discussion

1. Systematic Account

Family: Scarabaeidae
Subfamily: Rutelinae
Tribe: Anomalini
Name: *Anomala bengalensis* Blanchard, 1851
Distribution: India: West Bengal, Bihar, Karnataka, and **Tamil Nadu. Elsewhere:** Myanmar.
Feeding habit: Phytophagous.
Remarks : Recorded by Roy *et. al* (2014) [20].

2. **Morphology:** The adult beetle is oval, convex, and moderately robust, measuring approximately 10–18 mm in length. The body coloration varies from metallic green to coppery or yellowish-brown, often exhibiting iridescence, a characteristic feature of many ruteline beetles (Arrow, 1917) [3]. The head bears a broad clypeus, which is slightly emarginate anteriorly. The antennae are lamellate with a three-segmented club, adapted for sensory perception (Ritcher, 1966) [19]. The pronotum is smooth, shiny, and rounded laterally. Elytra are generally smooth with fine punctation and weakly developed striae. The legs are adapted for digging, with the fore tibiae distinctly tridentate, a common trait among scarab beetles. Sexual dimorphism is observed, with males typically having relatively larger antennal clubs compared to females (Arrow, 1917) [3]. The larva (white grub) is Scarabaeiform, C-shaped, with a creamy white body and a well-developed brown head capsule. The raster pattern on the last abdominal segment is species-specific and aids in identification (Ritcher, 1966) [19].

3. **Distribution:** *Anomala bengalensis* is widely distributed across the Indian subcontinent, particularly in regions such as West Bengal, Uttar Pradesh, Haryana, Punjab, and Bihar. It is commonly found in agricultural lands, grasslands, and forest margins, where soil conditions favour larval development (Chandra and Gupta, 2012) [8].

4. **Life Cycle:** The species undergoes complete metamorphosis (holometabolous development), including egg, larval, pupal, and adult stages. Eggs are laid in moist soil, where larvae develop and feed on plant roots. The larval stage typically lasts 2–4 months, depending on environmental conditions such as soil moisture and temperature (Veeresh, 1983) [27]. Pupation occurs in earthen cells within the soil, and adults emerge mainly during the monsoon and post-monsoon seasons (Yadav and Sharma, 1995) [31].

5. **Ecology and Behaviour:** Adults of *A. bengalensis* are primarily nocturnal and are often attracted to artificial light sources. They feed on foliage of various host plants, while larvae remain subterranean and feed on roots. Their activity is strongly influenced by soil moisture, rainfall, and temperature, which regulate emergence and population dynamics (Veeresh, 1983; Yadav and Sharma, 1995) [27, 31].

6. **Economic Importance:** *Anomala bengalensis* is considered an important agricultural pest, particularly in its larval stage. The white grubs feed on roots of crops such as sugarcane, wheat, groundnut, and vegetables, leading to reduced plant vigour and yield losses (Yadav and Sharma, 1995) [31]. Adult beetles may also cause defoliation, although the damage is generally less severe compared to larval feeding (Veeresh, 1983) [27].

7. **Ecological Role:** Despite its pest status, the species contributes to soil aeration and nutrient cycling through larval burrowing activity. Scarabaeidae beetles, in general, play an important role in maintaining soil structure and fertility (Mittal, 1993) [15].

8. **Cytogenetic Analysis:** The diploid chromosome number 20 was revealed at spermatogonial metaphase (Fig. 1a). The chromosomes were categorised into six pairs of metacentric (pairs 1-3, 5, 7, 8) and three pairs of submetacentric (pairs 4, 6, 9) autosomes, metacentric X and acrocentric y chromosome (Fig. 1b).

Percentage relative length of autosomes varied from 5.54 to 18.51, whereas that of X and y was 9.38 and 3.77, respectively. Application of silver staining to the initial stages of prophase like zygotene revealed the densely silver stained two grains in the chromatids correspond to the nucleolar organiser region (Fig. 1c), while darkle stained rounded elements were identified as sex vesicles. Pachytene stage displayed intersecting chromatids along with densely stained sex chromatins (Fig. 1d). At diplotene rings and eight shaped elements were encountered (Fig. 1e). Metaphase-I presented ten elements consisting of nine dumbbell shaped autosomal bivalents and a heteromorphic sex parachute Xyp (Fig. 1f). Anaphase-I shows the bridges between two extended groups of homologous chromosomes (Fig. 1g). First reductional division results in the formation of two types of secondary spermatocytes with 9A+X (Fig. 1h) and 9A+y constitution. Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase I was 1.0 and 1, respectively (Table 17). The meio-formula of this species is 9AA+Xyp.
As we know that in subfamily Rutelinae, diploid number varies from 16 to 22 in 46 cytologically known species (Table 1), so the variations have taken place towards decrease and increase in chromosome number. 13 species of genus *Adoretus* and one species of *Adorrhinyptia* with higher diploid number 2n=22: 10+Xyp (Joneja 1960;

Kacker 1970, 1971; Yadav and Pillai 1974, 1976 a-b, 1979; Mittal *et al.* 1987; Yadav and Dange 1988; Yadav *et al.* 1989) [9, 10, 11, 17, 32, 34, 38, 39], suggested the autosome dissociation, whereas on the other hand one unidentified species of genus *Adorrhinyptia* show polymorphic nature with 2n= 16/ 18/ 20 (Xyr) (Saha and Manna 1971; Saha 1973) [22, 23] and *Popillia japonica*, *Macraspis* spp and *Anomala corpulenta* with 2n= 18 (Xyp) suggested the involvement of autosome – autosome fusion in karyotype rearrangements. Although most of the species of genus *Anomala* depicted basic karyotype 2n=20, yet two types of diploid number 2n=18 and 20 were reported in *Anomala bengalensis* (present report) and *A. rufocuprea* (Saha and Manna 1971; Yadav *et al.* 1993; Kudoh *et al.* 1973; Yosida 1949) [12, 23, 40, 41] explicating dimorphic nature of both the species. Thus, it seems probable that the trend of evolution in the genus is towards increasing asymmetry in the karyotype.

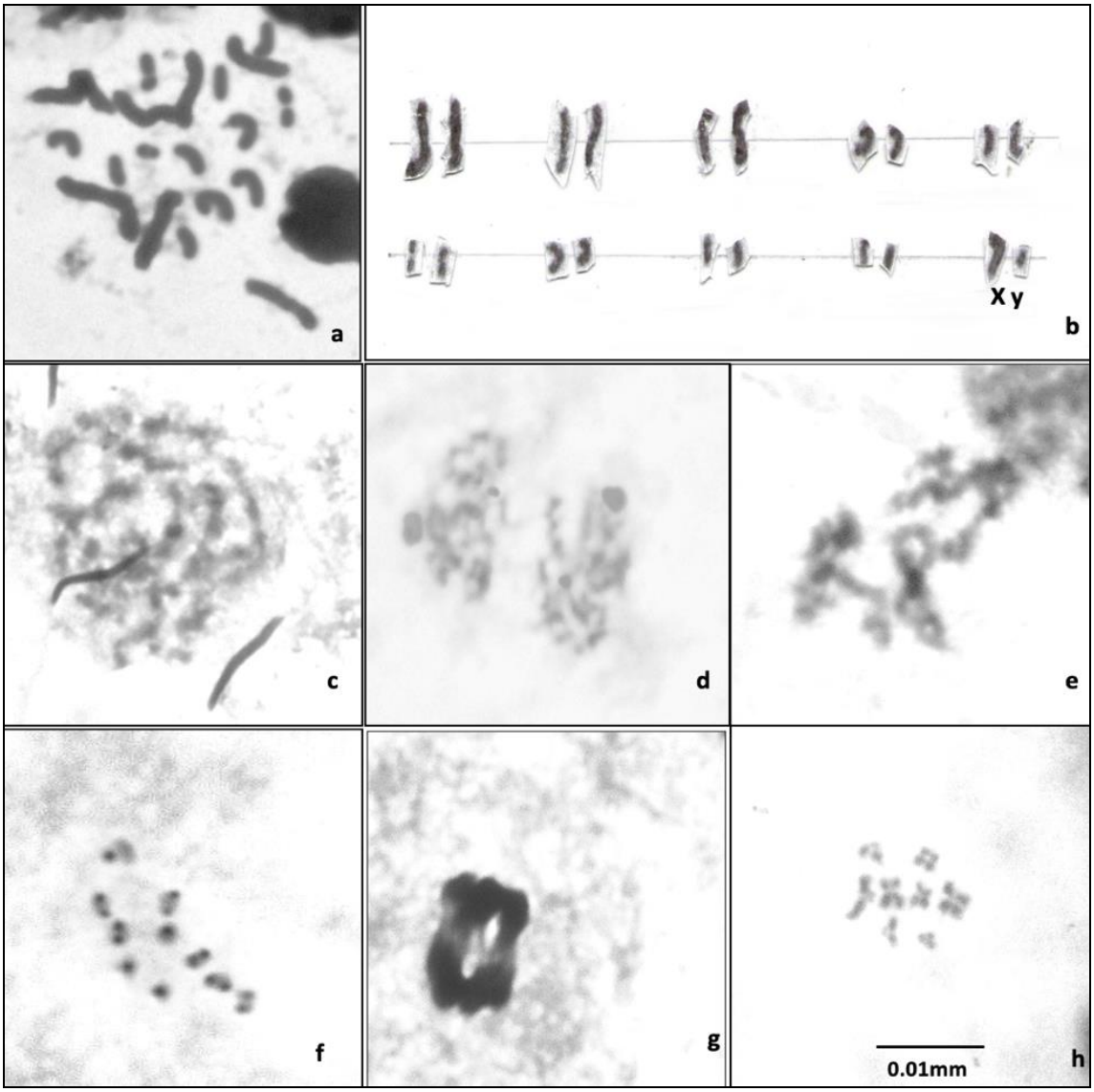

Fig 1: *Anomala bengalensis* Blanch. A) Spermatogonial Metaphese; b) Karyotype; Zygotene with silver staining; d) Pachytene; e) Diplotene; f) Mataphase-I with C- banding; Anaphase-I; h) Metaphse-II

Conclusion

The present study on the bionomics and cytogenetics of *Anomala bengalensis* provides valuable insights into the species' life history traits, morphological characters, seasonal activity and reproductive biology, along with its chromosomal architecture. The investigation revealed species-specific patterns in ecological role, economic strategies and population dynamics that are closely associated with environmental conditions. Cytogenetic analysis established the diploid chromosome number 2n=20 and highlighted structural features important for understanding karyotype organization and sex determination mechanisms. Collectively, these findings contribute to the taxonomic characterization and evolutionary understanding of *A. bengalensis* and offer baseline information useful for future ecological studies and the development of effective management strategies for this economically significant scarab beetle.

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