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Male meiotic behaviour and linear characterization of holocentric chromosomes of two species of Acanthosomatidae (Hemiptera: Heteroptera)

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Abstract
Acanthosomatidae is a small family of pests and decomposers, comprising around 230 species worldwide. Cytogenetic data for Indian Acanthosomatidae is available for only six species. In the present paper, cytogenetic details of two Acanthosomatid species, *Acanthosoma rufispinum* and *Sastragala esakii* collected from Himachal Pradesh have been described for the first time.
Both *A. rufispinum* and *S. esakii* possess 2n=10A+XY. Single chiasma per bivalent is the predominant feature in both the species. In *A. rufispinum*, all autosomal bivalents show thick terminal C-bands while in *S. esakii*, thin terminal bands are observed in four bivalents while one is C- negative. Y is C- positive and X is negative in both the species. All C-positive regions are DAPI/CMA3 bright. In *A. rufispinum*, each autosomal bivalent shows a specific distribution pattern of NORs while it is similar in all the bivalents in *S. esakii*. Linear characterization shows species specific distribution of C-bands, AT-GC rich DNA and NORs.

Keywords: heteroptera, acanthosomatidae, c-banding, sequence-specific staining, silver nitrate staining

Introduction
Acanthosomatidae is a small family of superfamily Pentatomoidea of suborder Heteroptera. It includes around 230 species of 57 genera distributed mainly in temperate regions or at high altitudes in the subtropics (Faúndez, 2014) [9]. Acanthosomatid bugs can be differentiated from rest of pentatomoids in having only two tarsal segments and by the presence of Pendergrast's organ in females. They are phytophagous feeding on young tissues of trees and shrubs belonging to families Aceraceae, Berberidaceae, Cuperessaceae, Cyperaceae, Pinaceae, Rosaceae and Tiliaceae (Schafer and Ahmad, 1987 [35]; Schaefer *et al*., 2000 [36]; Faunzed, 2007, 2008 [7, 8]; Osorio, 2009 [25]; Schwertner and Grazia, 2015) [37], and in scarcity of food, there are casual records of feeding on decaying organic matter. Some species are known to show maternal care (Tallamy and Schaefer, 1997) [39].

Cytogenetic data on Acanthosomatidae is very meagre and pertains to only 13 species. Linear characterization of chromosomes using differential banding techniques is altogether absent. Based on data available so far, the family is characterized by a modal diploid chromosome complement of 12= 10A+XY (Toshioka, 1935 [40]; Xavier, 1945 [41]; Yosida, 1944, 1946, 1947 [42, 43, 44]; Halkka, 1956 [15]; Mikolajski, 1968 [19]; Muramoto, 1973a,b, 1978 [21, 22, 23]; Mittal and Joseph,1981) [20]. The exceptions to this complement are observed in *Elasmucha recurvum* and *Acanthosoma* sp. which possess 2n=16 =14A+XY (Parshad, 1957 [32]; Mittal and Joseph, 1981) [20] and *Acanthosoma expansum* which has 2n= 11 =10A+X0 (Muramoto, 1973a) [21]. In the present paper, chromosome complement, course of meiosis and distribution of constitutive heterochromatin, AT- GC rich DNA and nucleolar organizer regions in two species of Acanthosomatidae, *Acanthosoma rufispinum* (Distant, 1887) and *Sastragala esakii* Hasegawa, 1959 have been described for the first time.

Material and Methods
Adult males of *Acanthosoma rufispinum* (Distant, 1887) and *Sastragala esakii* Hasegawa, 1959 were collected from wild vegetation in the areas falling in Shimla and Palampur (H.P.) during the month of June, July and August. Testes were removed and fixed in 3:1: ethanol: glacial acetic acid, and air dried chromosomal preparations were made. Slides were processed for conventional staining (Carr and Walker, 1961) [4], C-banding (Sumner, 1972) [38], DAPI/CMA3 staining (Rebagliati *et al*., 2003) [34] and Silver staining (Howell and Black, 1980) [16].

Observations
Acanthosoma rufispinum (Distant, 1887)
Male diploid chromosome complement of *Acanthosoma rufispinum* is 2n=12=5AA+XY. X and Y chromosomes remain fused and lie at the periphery of the nucleus against the decondensed autosomes during the diffuse stage (Fig. 1a). In most of the diplotene/diakinesis plates (85.7%), autosomal bivalents show one terminal chiasma each while rest of the plates (14.3%) show one ring bivalent (Figs. 1b, 1c). During metaphase I, autosomes form a rough ring while X and Y chromosomes lie within the ring (Fig. 1d). At metaphase II, X and Y chromosomes associate forming a pseudobivalent which lies in the centre of ring formed by autosomes (Fig. 1e). During anaphase II, sex chromosomes divide reductionally resulting in two type of telophase plates, one with 5A+X and the other with 5A+Y chromosomes (Fig. 1f).

C banding of the chromosomes shows two autosomal bivalents with thick C bands at one or both terminal ends, two autosomal bivalents with interstitial bands and one completely

C- positive (Fig. 2a). All the autosomal bivalents appear bright with both DAPI and CMA3 (Figs. 2c, 2d, 2e, 2f).Y appears C-positive and DAPI/CMA3 bright while X is C-negative and DAPI/ CMA3 dull (Figs. 2b, 2e, 2f). With silver staining, two large nucleolar bodies are observed during diffuse stage (Fig. 2g). During diplotene, NORs have been found on all the chromosomes but at different locations. Two autosomal bivalents show silver positive bands at terminal positons, one shows interstitially located thin silver bands, one is completely silver positive and one is positive for three fourth part with terminal regions distinctly negative. X and Y chromosomes are silver positive (Fig. 2h).

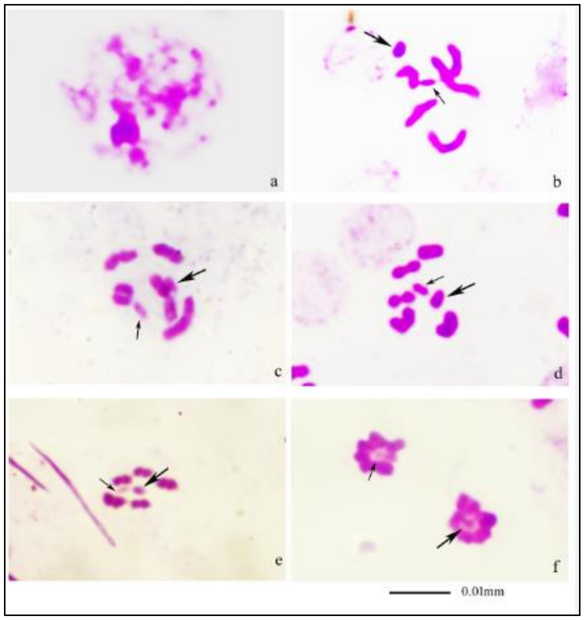

Fig 1: (a-f) Male meiosis – (a) Diffuse stage; (b&c); Diplotene/Diakinesis; (d) Metaphase I; (e) Metaphase II; (f) Telophase II.

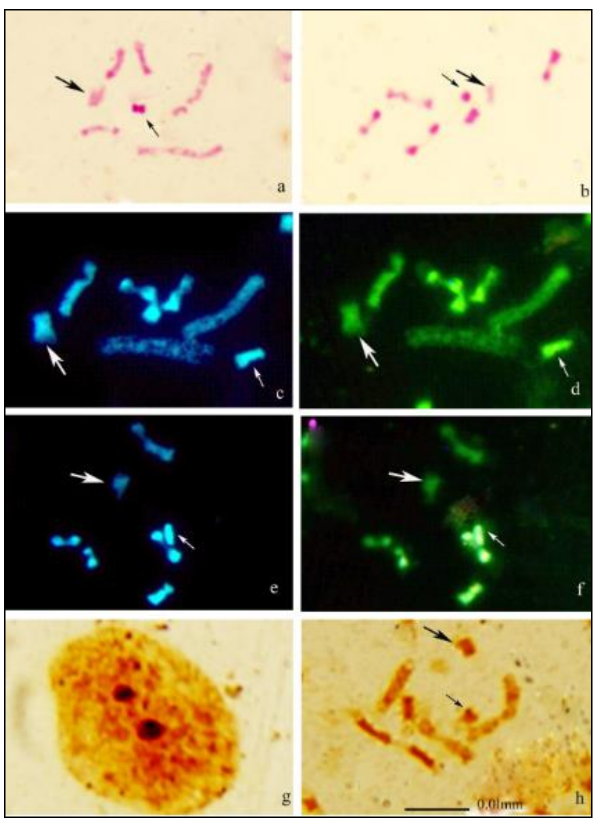

Fig 2: (a&b) C-banding showing distribution of C- bands at diplotene and diakinesis, X is C-negative; (c-f) Sequence specific staining showing DAPI/CMA3 bright bands in autosomes, DAPI/CMA3 dull X and DAPI/CMA3 bright Y; (g&h) Silver staining showing two nucleolar bodies at diffuse stage and distribution of NORs at diplotene, X and Y are silver positive.

Sastragala esakii **Hasegawa, 1959**

Male chromosome complement of *Sastragala esakii* is the same ie, 2n=12=5AA+XY. X and Y chromosomes remain associated at diffuse stage forming a positively heteropycnotic body that lies in the centre of the nucleus against decondensed autosomes (Fig. 3a). In 74% of the diplotene/diakinesis plates, single chiasma per bivalent is observed while in rest of the plates, 1 to 3 ring bivalents are observed (Figs. 3b, 3c). During metaphase I, autosomal bivalents and X chromosome form the ring while Y chromosome lies within the ring (Fig. 3d). At metaphase II, X and Y chromosomes associate forming a pseudobivalent which lies in the centre of the ring (Fig. 3e). During anaphase II, sex chromosomes divide reductionally resulting in two types of telophase plates, one with 5A+X and the other with 5A+Y chromosome (Fig. 3f).

Four autosomal bivalents show terminal thin C-bands which are DAPI and CMA3 bright while one is C-negative and DAPI/CMA3 dull (Figs. 4b, 4e, 4f). The fused X and Y body appears C-positive for one-third part during diffuse stage (Fig. 4a). At diplotene where the two are distinct, Y appears C-positive and DAPI/CMA3 bright (Figs. 4c, 4d, 4e) while X appears C-negative but DAPI/CMA3 bright (Figs. 4c, 4d, 4f). NOR regions have been observed scattered throughout the length of all autosomal bivalents (Fig. 4g). Y chromosome is silver positive but X is negative (Fig. 4h).

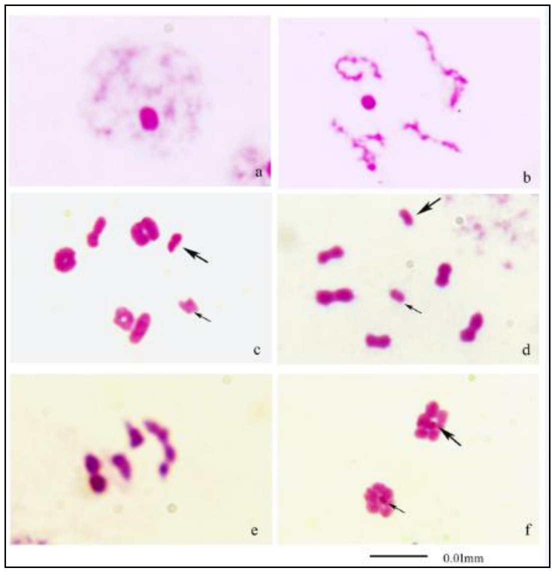

Fig 3: (a-f) Male meiosis - (a) Diffuse stage; (b&c) Diplotene/Diakinesis; (d) Metaphase I; (e) Metaphase II; (f) telophase II.

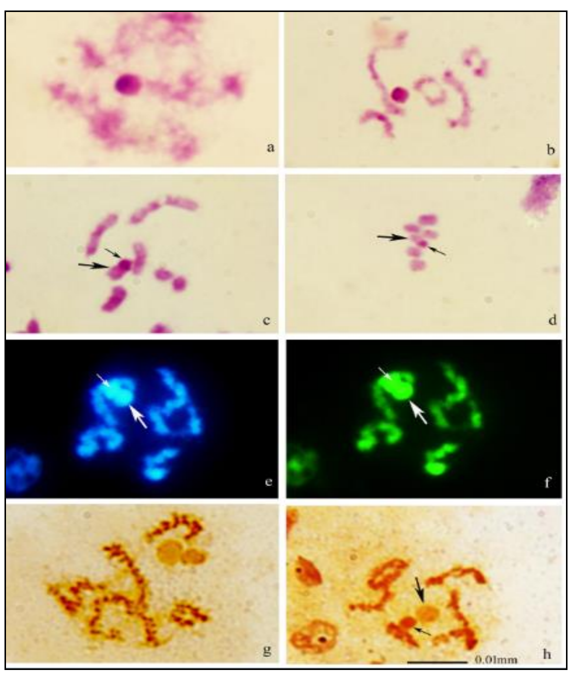

Fig 4: (a) Fused X and Y body appears C-positive for one-third part at diffuse stage; (b-d) C- banding showing distribution of C- bands at diplotene, diakinesis and metaphase II; (e&f) Sequence specific staining showing DAPI/CMA3 bright bands in autosomes and DAPI/CMA3 bright X and Y; (g&h) Silver staining showing distribution of NORs diplotene, Y chromosome is silver positive but X is negative**.**

Discussion

Both *Acanthosoma rufispinum* and *Sastragala esakii* possess 12 chromosomes with 2n=10A+XY which is present in all the cytologically known species of this genus except *Acanthosoma* sp. (Mittal and Joseph, 1981) [20]. Over all cytogenetic data on Acanthosomatidae pertains to sixteen species including presently added two species (Table 1). The diploid number of 12 is shown by thirteen species (Toshioka, 1935 [40]; Xavier, 1945 [41]; Yosida, 1944, 1946, 1947 [42, 43, 44]; Woodward, 1950 [45]; Halkka, 1956 [15]; Parshad, 1957 [32]; Mikolajski, 1968 [19]; Muramoto, 1973a, b, 1978) [21, 22, 23] while deviations are observed in three species with 16 in *Acanthosoma*. sp. and *Elasmucha*. *recurvum* (Parshad, 1957 [32]; Mittal and Joseph, 1981) [20] and 11 in *Acanthosoma expansum* (Muramoto, 1973a) [21].

Table 1: List of cytologically investigated species of Acanthosomatidae with their chromosome complements

S. No	Taxa	Male Chromosome complement (♂)	Authors
1	*Acanthosoma denticauda*	12= 10A+XY	Toshioka (1935), Xavier (1945),Yosida (1944,1947), Muramoto (1973a)
2	*Acanthosoma expansum*	11= 10A+X0	Muramoto (1973a)
3	*Acanthosoma frater*	12=10A+XY	Muramoto (1978)
4	*Acanthosoma haemorrhoidale*	12= 10A+XY	Xavier, 1945
5	*Acanthosoma labiduroides*	12= 10A+XY	Yosida (1944, 1946, 1947), Xavier (1945), Muramoto (1973a)
6	*Acanthosoma sp.*	16= 14A+XY	Mittal and Joseph (1981)
7	*Acanthosoma rufispinum*	12= 10A+XY	Present study
8	*Elasmostethus humeralis*	12= 10A+XY	Yosida (1944, 1946, 1947), Muramoto (1973a,b)
9	*Elasmostethus interstinctus*	12= 10A+XY	Halkka (1956)
10	*Elasmucha grissae*	12= 10A+XY	Mikolajski (1968)
11	*Elasmucha putoni*	12=10A+XY	Muramoto (1973a,b)
12	*Elasmucha recurvum*	16= 14A+XY	Parshad (1957)
13	*Lindbergicoris gramineus*	12= 10A+XY	Muramoto (1973a,b)
14	*Microdeuterus dallasi*	12=10A+XY	Mittal and Joseph (1981)
15	*Sastragala heterospilla*	12=10A+XY	Mittal and Joseph(1981)
16	*Sastragala esakii*	12=10A+XY	Present study

Both *Acanthosoma rufispinum* and *Sastragala esakii* follow the course of meiosis typical of Heteroptera. Autosomes follow usual pre-reductional meiosis but division is post-reductional for sex chromosomes which divide equationally during anaphase-I and reductionally during anaphase-II. Single chiasma per bivalent is the predominant feature in both the species.

In *Acanthosoma rufispinum*, all autosomal bivalents show thick terminal C-bands at both the termini while in *Sastragala esakii*, thin terminal bands are observed at both the termini in four autosomal bivalents while one autosomal bivalent is altogether C- negative. Y chromosome is C- positive and X is negative in both the species. All the C-positive regions are DAPI/CMA3 bright indicating presence of both AT and GC rich DNA. For Acanthosomatidae, information on distribution pattern of C- heterochromatin, AT- GC rich DNA and NOR is altogether lacking. In other heteropterans, terminal or nearly terminal location of C- heterochromatin is most common (Muaramoto, 1980 [24]; Camacho *et al*., 1985 [3]; Papeschi, 1988, 1991 [27]; Panzera *et al*., 1997) [26] while interstial C-positive bands are described only in a few species (Camacho *et al*., 1985 [3]; Grozeva and Nokkala, 2001 [12]; Iturate and Papeschi, 2004 [17]; Grozeva *et al.*, 2006) [14]. Most of the reports referring to heterochromatin characterization describe C- bands as DAPI-bright/CMA3 dull (Perez *et al*., 2000 [33]; Papeschi and Bressa, 2002, 2006 [30]; Papeschi *et al*. 2003 [31]; Rebalgliati *et al*., 2003 [34]; Bressa, 2005) [2]. However, in a few species of Pentatomidae, Micronectidae. Miridae and Cimicidae, GC- rich regions are interspersed with AT rich regions (Rebagliati *et al*., 2003 [34]; Iturate and Papeschi, 2004 [17]; Bansal and Kaur, 2015) [1].

In *Acanthosoma rufispinum*, each autosomal bivalent shows a specific distribution pattern of NORs which are terminal and interstitial. On the other hand, in *Sastragala esakii*, NORs are located throughout the length of all the autosomal bivalents. Y chromosome is silver positive in both the species while X is positive in *Sastragala esakii* and negative in *Acanthosoma rufispinum.* In Heteroptera, heterogeneity has been observed with respect to NOR distribution. These have been reported at interstitial as well as terminal positions on autosomes and sex chromosomes (Camacho *et al*., 1985 [3]; Fossey and Liebenberg, 1995 [10]; Gonzalez-Garcia *et al*., 1996 [11]; Papeschi *et al*., 2003 [31]; Rebagliati *et al*., 2003 [34]; Cattani and Papeschi, 2004 [5]; Cattani *et al*., 2004 [6]; Grozeva *et al*. 2004) [13].

Conclusion

Both *Sastragala esakii* and *Acanthosoma rufispinum* possess the same chromosome complement but linear characterization shows species-specific distribution of constitutive heterochromatin, AT-GC rich DNA and nucleolar organizer regions.

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