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C-Heterochromatin and its Base Composition in Holokinetic Chromosomes of Three Species of Scutelleridae (Hemiptera:Heteroptera)

Patial Nisha and Kaur Harbhajan*

Department of Zoology and Environmental Sciences, Punjabi University, Patiala 147002, Punjab, India

**Corresponding Author*

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Abstract: Scutelleridae is a small family of Heteroptera comprising economically important bugs that feed on grasses, herbs, fruits, and flowers. In this study, three Indian species of Scutelleridae, *Chrysocoris stollii*, *Poecilocoris hardwickii* and *Poecilocoris interruptus*, all possessing the same complement of 2n =12 (10A+XY), have been differentiated based on distribution pattern of heterochromatin and its base composition.

C-heterochromatin is located at terminal and subterminal regions in *Poecilocoris interruptus*, at terminal and interstitial regions in *Chrysocoris stollii* but is homogeneously distributed in *Poecilocoris hardwickii.* Sex chromosomes are C-positive in *Poecilocoris interruptus* and *Chrysocoris stollii* and C-negative in *Poecilocoris hardwickii*. C-heterochromatic bands on both autosomes and sex chromosomes are DAPI/CMA3 bright in *Chrysocoris stollii* and *Poecilocoris interruptus* but only DAPI bright in *Poecilocoris hardwickii*.

Keywords: Heteroptera, Scutelleridae, C-banding, Fluorescent banding, *Poecilocoris interruptus*, *Chrysocoris stollii*, *Poecilocoris hardwickii*

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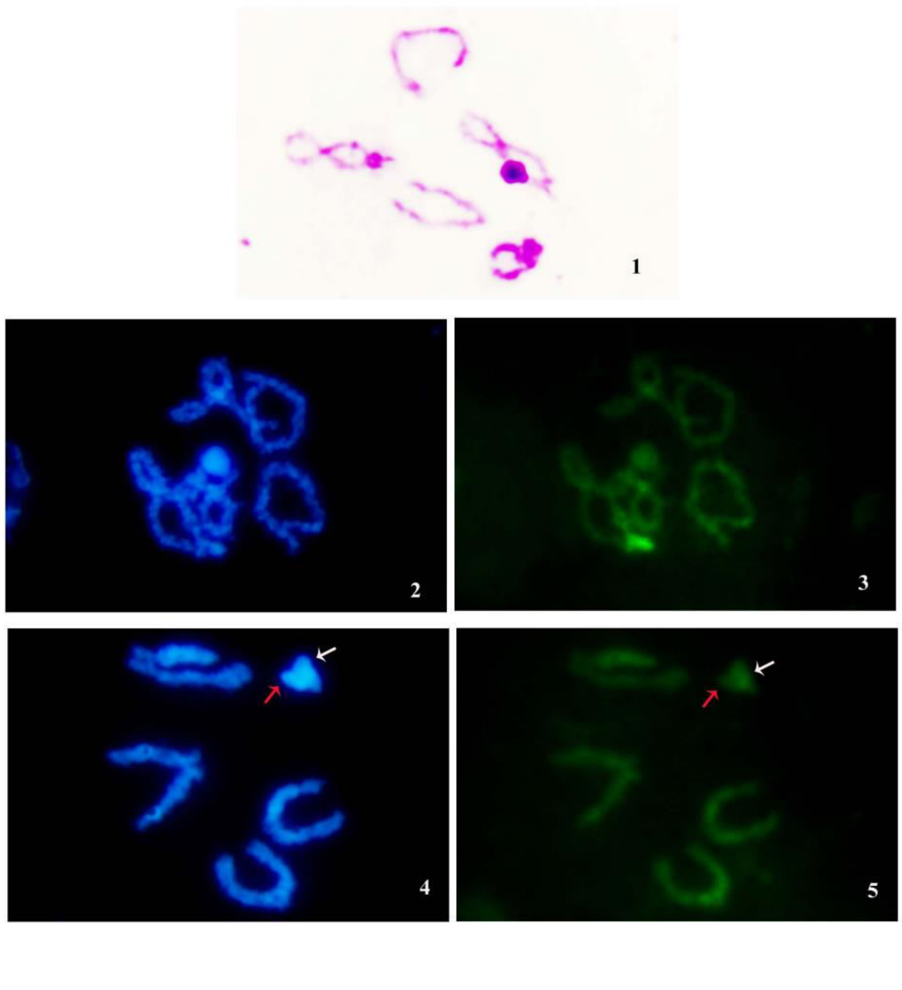
Introduction

The organization of constitutive heterochromatin is usually specific and its distribution pattern in chromosomes is an important tool in taxonomic and evolutionary studies in Heteroptera (Gabriela *et al*., 2013). Scutelleridae comprises a small family of Heteroptera with 80 genera and 450 species distributed worldwide. Insects of this family vary in size from medium to large that appear more like beetle because of an extended scutellum which covers the entire abdomen including wings. These insects feed on grasses, herbs, fruits, and flowers and are of immense economic importance. However, genetic analyses of these insects are scarce, and are restricted to only the description of the karyotype. The diploid chromosomes number is consistently 12 with XY



Figs. 1-5: *Chrysocoris stollii.* C-banding (Fig. 1) autosomal bivalents with terminal and interstitial C-positive bands at diplotene. Fluorescent banding (Figs. 2-5) autosomal bivalents show DAPI/CMA3 bright signals at diplotene. X and Y chromosomes appear bright for DAPI/CMA3. White arrows denote X chromosome; Red arrows denote Y chromosome; denotes fused XY chromosome. Bar- 0.01mm

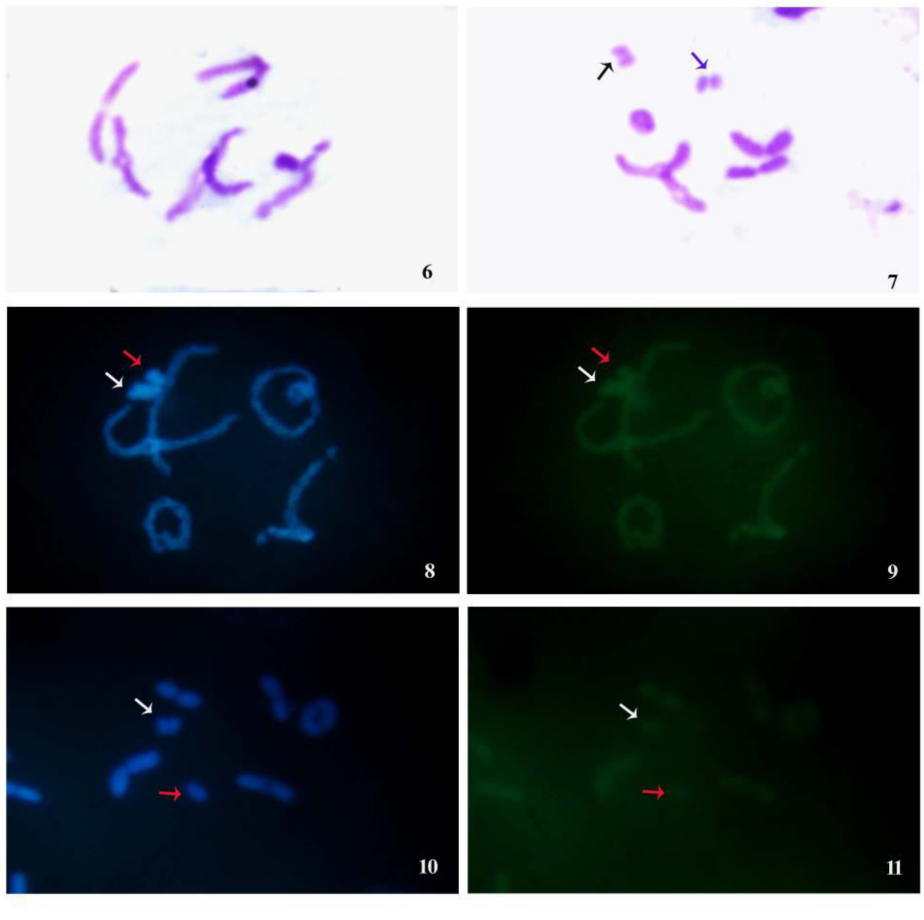
sex chromosome system (Souza *et al*., 2014). Distribution pattern of heterochromatin has been studied only in one species i.e., *Pachycoris torridus* (Souza-Firmino 2015). In this study, we describe heterochromatin and its characterization in three Indian species of Scutelleridae.

Materials and Methods

Adult males of *Chrysocoris stollii*, *Poecilocoris hardwickii* and *Poecilocoris interruptus* of Scutelleridae were collected mainly by hand picking from stems, leaves and seed pods of wild and cultivated vegetation. Live male specimens of bugs were dissected in hypotonic (0.67% NaCl) saline to take out gonads which were fixed in Carnoy's fixative (3:1:: absolute alcohol: glacial acetic acid) for 20 min followed by a second change of fresh Carnoy's fixative for another 15 min. The fixed material was teased on pre-cleaned labelled slides with fine forceps and was allowed to air dry. The prepared slides were stained and well spread stages were photographed using Nikon digital camera (DXM 1200C).

Results

All the three species, *Chrysocoris stollii*, *Poecilocoris hardwickii* and *Poecilocoris interruptus* possess a diploid chromosome complement of ten

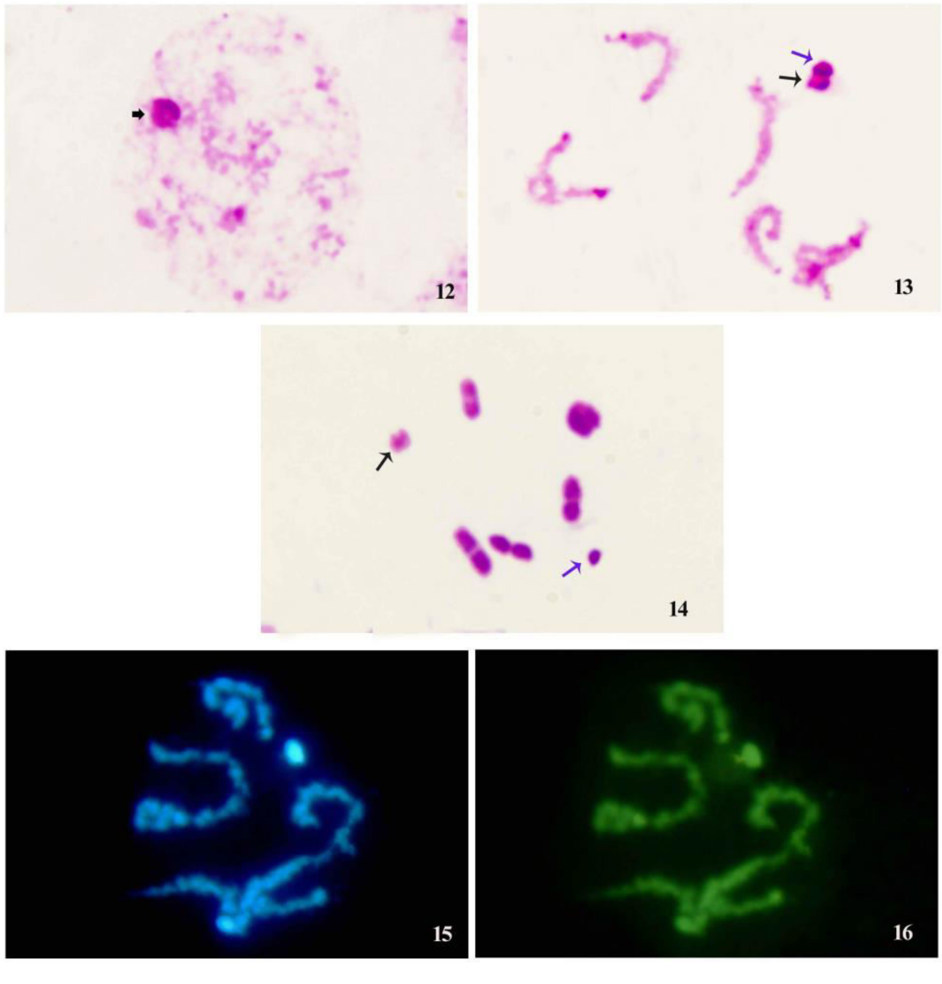


Figs. 6-11: *Poecilocoris hardwickii.* C-banding (Figs. 6, 7) autosomal bivalents stain homogeneously for C-banding. Sex chromosomes appear C-negative. Fluorescent banding (Figs. 8-11) autosomal bivalents and sex chromosomes show DAPI bright signals. White arrows denote X chromosome; Red arrows denote Y chromosome; denotes fused XY chromosome. Bar- 0.01mm

autosomes and two sex chromosomes, X and Y (2n=12=10A+XY). In *Chrysocoris stollii*, autosomal bivalents show terminal and interstitial C-positive bands which are DAPI /CMA3 bright. Sex chromosomes too appear C-positive and DAPI/CMA3 bright (Figs. 1-5). In *Poecilocoris hardwickii*, autosomal bivalents stain homogeneously for C-banding and show only DAPI bright signals while sex chromosomes appear C-negative and DAPI bright (Figs. 6, 7, 8-11). In *P. interruptus,* one autosomal bivalent shows weak terminal and prominent subterminal C-band while rest of the bivalents show only terminal C-bands. The fused XY body at diffuse stage appears partially C-positive (Fig. 12), and as the two sex chromosomes separate at diplotene, X appears C-negative while Y appears C- positive (Figs. 13,14). All the autosomal bivalents show DAPI /CMA3 bright bands at interstitial and terminal regions. X and Y chromosomes are DAPI/CMA3 bright (Figs. 15, 16).

Discussion

Chrysocoris stollii, *Poecilocoris hardwickii* and *Poecilocoris interruptus* have the same complement of 2n =12 (10A+XY) which is invariably present in all the species of Scutelleridae studied so far (Das Gupta, 1950; Manna, 1951; Manna and Deb-Mallick, 1981; Mittal and Joseph, 1981; Souza *et al*., 2014).



Figs. 12-16: *Poecilocoris interruptus.* C-banding (Fig. 12) the fused XY body appears partially C-positive at diffuse stage. Figs. 13, 14: All autosomal bivalents show terminal C-bands and one bivalent shows prominent subterminal C-band also. X appears C-negative while Y appears C-positive at diplotene. Fluorescent banding (Figs. 15,16) all the autosomal bivalents with DAPI/CMA3 bright bands at interstitial and terminal regions. X and Y chromosomes are DAPI/CMA3 bright. Black arrows denote X chromosome. Bar- 0.01mm

Autosomal bivalents show C-heterochromatin located at terminal and subterminal regions in *Poecilocoris interruptus,* at terminal and interstitial regions in *Chrysocoris stollii* and homogeneously in *Poecilocoris hardwickii.* X and Y chromosomes appear C-positive in *Poecilocoris interruptus* and *Chrysocoris stollii* and C-negative in *Poecilocoris hardwickii*. Earlier only one scutellerid species *Pachycoris torridus* has been studied which shows heterochromatic blocks only in the chromocenter formed by the sex chromosomes X and Y (Souza-Firmino *et al*., 2015). 1n other families of Heteroptera, heterogeneity with respect to C-heterochromatin has been reported. It is located terminally in autosomes in majority of the species (Muramoto, 1980; Dey and Wandgi, 1990; Panzera *et al*., 1992; Grozeva and Nokkala, 2001; Angus *et al*., 2004; Cattani *et al*., 2004; Ituarte and Papeschi, 2004; Bressa *et al*., 2005; Lanzone and Souza, 2006; Kaur *et al*., 2012; Suman *et al*., 2012; Suman and Kaur, 2013; Bansal and Kaur, 2015; Kaur and Patial, 2017), interspersed in some species (Mauldin, 1974; Muramoto, 1978, 1980; Camacho *et al*., 1985; Dey and Wangdi 1990; Panzera et al.

1997; Grozeva and Nokkala 2003; Ituarte and Papeschi 2004; Franco *et al*., 2006; Kaur *et al*., 2010; Suman *et al*., 2012) and homogeneous in only a few species (Suman *et al*., 2012; Bansal and Kaur, 2015).

The heterochromatin can vary in composition from highly AT to highly GC rich DNA. C-heterochromatic bands on both autosomes and sex chromosomes are DAPI /CMA3 bright in *Chrysocoris stollii* and *Poecilocoris interruptus* but only DAPI bright in *Poecilocoris hardwickii*. In Heteroptera, C bands are reported to be both DAPI and CMA3 bright in majority of the species (Grozeva and Nokkala, 2002; Rebagliati *et al*., 2003; Ituarte and Papeschi, 2004; Lanzone and Souza, 2006; Rebagliati and Mola, 2010; Kaur *et al*., 2012; Kaur and Patial, 2017), DAPI bright/CMA3 dull in some species (Papeschi *et al*., 2003; Rebagliati *et al*., 2003; Franco *et al*., 2006; Bressa *et al*., 2005, 2008) and uniformly fluoresced with DAPI/CMA3 in a few species (Rebagliati et al. 2003; Grozeva et al. 2007; Suman et al. 2012; Suman and Kaur 2013; Bansal and Kaur 2015; Bardella et al. 2016).

The two species of *Poecilocoris*-- *P. interruptus* and *P. hardwickii* differ in C-banding pattern as well as base composition of heterochromatin and thus can be clearly differentiated cytogenetically.

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Conflict of Interest

The authors declare no conflicts of interest.

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