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ORIGINAL ARTICLE

Studies on chromosomal characteristics of *Ctenus indicus* (Gravely 1931) (Araneae: Ctenidae)

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Abstract The karyological information gathered for the Indian spiders taxa thus far were cytologically derived from only few species but none for the representatives belonging to the genus *Ctenus*. *Ctenus indicus* (Gravely 1931), an Indian ctenid spider was cytogenetically analyzed following conventional, C- and NOR-banding techniques so as to gather substantial data for future course of understanding of karyotypic evolution among spider species. The karyotypic data for *Ctenus indicus* revealed the complement consisting of (2n = 28) 26AA + X_1X_2♂ and (2n = 30) 26AA + $X_1X_1X_2X_2$♀ acrocentric chromosomes. A closer scrutiny of meiotic progression disclosed many male pachytenic cells displaying the occurrence of 'bouquet' formation. The results of C-banding enabled in identifying centromeric constitutive heterochromatin locales, and in some chromosomes also the distal ends of telomeric regions. Silver nitrate stained NOR-specifications were noticed at the distal telomeric regions of two pairs of chromosomes (#8 and #10) in the complement. Cytological evidence procured from the present study not only adds to the ever-growing list of the spider cytogenetic assessments but also offers as a baseline data towards establishing evolutionary relationships within this important group.

Keywords Mitotic and meiotic chromosomes · Multiple sex chromosomes · Pachytenic 'bouquet' formation · Centromeric C-heterochromatin · NOR impregnation

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Introduction

Worldwide fauna on spiders (Araneae) include about 46,000 nominate species distributed among 3988 genera and 114 families [43]. Giving primary importance to the monophyletism as the basis for spider classification, it was possible to infer essentially orienting upon chromosomal biology into two broader groupings: viz., Mesothelae and Ophisthothelae [13]. Among the majority of spiders contributing towards their elucidation of the global level diversity observed based on the morphological specifications are the taxa belonging to entelegynes of the infraorder Aranaeomorphae that could offer as an opportunistic subject of phylogenetic importance. Within the superfamily Lycosoidea, ctenids provide an ideal clade consisting of about 500 species within 41 genera projected to be of worldwide geographical distribution [43]. Ctenid chromosomes offer as an attractive source of genetic material for cytogenetic research since chromosomal information gathered thus far came from only 8 species belonging to 7 genera with the diploid chromosome range 2n = 22–29 [6, 11, 31].

In spite of their persistent nature of exhibiting exuberant types of morphological plasticity among spiders, it was appalling to realize that only about 2% of them have been subjected to karyological studies [25]. The impressive part of these analyses has driven to an understanding that most spider karyotypes seemed to reflect in possession of acrocentric chromosomes in the complement [5]. However, some primitive spiders could be drawn to recognize containing sub-meta and metacentrics in addition to acrocentrics in their respective karyotypes [2]. Interestingly, based on the available chromosomal informations for the arachnids as a group that reveal a prevalence of exhibiting a broader range of the identifiable basic chromosomes

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(within 2n = 7–128) for each such said species; rather a unique chromosomal feature bestowed with Araneae from among the chromosomally scrutinized other insect examples [3, 5, 23]. This situation seemed reflecting upon their compliance with the current opinions since spiders are known for their occurrence of worldwide geographical and cosmopolitan dispersal. As such, they provide a subjective material and thus driving them in the considerations and in proclivity of its implicit nature upon population dynamicities.

Another diagnostic cytogenetic feature that frequently encountered during the course of chromosomal studies of spiders had been their prevalence towards inclusive nature of multiple sex chromosome polymorphisms [4]. Earlier studies have documented their phylogenies eliciting evolutionary trend following XX–XO sex determining mechanisms. This particular mode of sex chromosomes seems to prevail in the case of some haplogyne spiders examined thus far. However, in the derived forms it becomes evident to have evolved towards the attainment in the range of X_1X_20–$X_1X_2X_30$ [24, 37].

Cytological enunciation made in respect of spiders in general and of the Indian fauna in particular, is highly limited and fragmentary. Only a limited information is available upon karyo- and biosystematics of Indian fauna, whereas none on the chromosomes of the family Ctenidae [8, 14, 30, 33, 39, 40]. Oliveira et al. [31] presented the first karyotype for a South American ctenid viz., *Ctenus ornatus*, (Cteninae) depicting $2n♂ = 28$ $(26AA + X_1X_20)$ chromosomes. Subsequently, some important contributions were made in the elucidation of chromosome informations for the other taxa involving members of other subfamilies of ctenid species. A Taiwanese ctenid species (*Anahita fauna*) karyotype was described representing $2n♂ = 29$ $(26AA + X_1X_2X_30)$ chromosomes [11]. Besides, two more representative karyotypes were described for the South American taxa (*Nothroctenus* sp. and *Viracucha andicola*) both belonging to the subfamily Acanthocteninae, bearing $2n♂ = 29$ $(26AA + X_1X_2X_30)$, while *Phoneutria nigriventer* and *Parabatinga brevipes* (Cteninae) both depicting $2n♂ = 28(26AA + X_1X_20)$, whereas *Asthenoctenus borellii* (Viridasiinae) exhibiting $2n♂ = 22(20AA + X_1X_20)$ chromosomes [6].

Recently, there occurred a revision of phylogenetic revaluation of Ctenidae [35]. Until now, the sole representative examples of three ctenid subfamilies (Acanthocteninae, Cteninae and Viridasiinae) had been chromosomally known. All chromosomal data point toward an existence of close relationship between *Ctenus* and *Phoneutria*, the placement of *P. brevipes* with Cteninae, the placement of *Anahita* in a separate branch within Cteninae and the inclusion of *A. borelli* in a distinct group within Ctenids (Viridasiinae). Whereas the two genera, *Vulsor* and *Viridasius* are found elevated to a family level status and is excluded to a family from the Ctenidae and thereby inserted into Dionycha group [6, 34–36, 41]. These results seem projecting as supportive towards the demonstration and maintaining of a common basal chromosome number ($2n♂ = 28$) that include variable sex chromosome composition.

A considerable amount of cytogenetic data has been gathered for the Indian forms that were drawn from several representative families of araneomorphs, but none for any representatives belonging to the family Ctenidae [8, 14, 33, 39].

The present report entails upon chromosomal characteristics of *Ctenus indicus* based on the karyotype, meiotic progression, C- and NOR- banding profiles.

Materials and methods

Details of collection of specimens of *Ctenus indicus* from five selected geographical locations of South India are given in Table 1. The collected specimens were separated by identifying male and female specimens of *Ctenus indicus* species following the keys of Sebastian and Peter [38]. The voucher specimens preserved in 70% ethanol are deposited at the Natural History Museum of Department of Zoology, Bangalore University, Bengaluru, India.

Conventional air-drying technique of Chowdaiah and Venkatachalaiah [12] with appropriate modifications was adopted for the preparation of (1) mitotic chromosomes from gut epithelium and (2) meiotic chromosomes from testes and ovaries of male and female specimens respectively of *Ctenus indicus* species. Diluted Giemsa solution

Table 1 Details of the collection of *Ctenus indicus* species

Locality	Habitat	Geographical coordinates	No. of animals used
Kolar Gold Fields, Kolar, Karnataka, India	Dry forest floor	12°56′18.48″N, 78°14′28.55″E	4♂, 2♀
Bengaluru city, Karnataka, India	Dry forest floor	12°56′50.15″N, 77°30′31.24″E	3♂, 2♀
Tirupati, Andhra Pradesh, India	Dry deciduous forest floor	13°37′54.88″N, 79°23′37.41″E	2♂, 1♀
Vellore, Tamil Nadu, India	Dry forest floor	12°53′34.52″N, 77°30′31.24″E	4♂, 3♀
Kasaragodu, Kerala, India	Backyard Garden	12°29′55.75″N, 75° 0′3.68″E	3♂, 3♀

(3%) was used for conventional staining of the chromosomal preparations. Mitotic and meiotic chromosomes were subjected to C-banding [42] and NOR staining [20] with minor modifications. Chromosome preparations were observed using Zeiss Axioskop 2 plus Microscope and well spread complements were photographed. The karyotypes were constructed from somatic metaphase chromosomes essentially based on aligning them in the decreasing order of their total length [26].

Results

Mitotic chromosomal complement and Karyotype of *Ctenus indicus*

The somatic metaphase complement of *Ctenus indicus* consists of $2n = 28$ ($26 + X_1X_20$) chromosomes in males and $2n = 30$ ($26 + X_1X_1X_2X_2$) in females respectively. In the karyotype of males (Fig. 1a), all the chromosomes were acrocentrics and the two smallest non-homologous pairs were considered as the sex chromosomes. In females, the karyotype (Fig. 1b) is represented by thirteen homomorphic acrocentric autosomal pairs and a smaller two differentiated sets of sex chromosomes that are non-homologous acrocentrics.

Spermatogonial and oogonial meiosis

During spermatogonial meiosis I, thirteen autosomal bivalents and two non-homologous, heteropycnotic sex univalents were observed from pachytene to diakinesis (Fig. 2a–c). During the early pachytene stage (Fig. 2a), the bouquet-like arrangements of the bivalents were found to be more frequent. Whereas, analysis of female meiosis revealed comprising of thirteen autosomal bivalents and two bivalent sex chromosomes during early pachytene to diakinesis stage (Fig. 2d–f).

C-banding

The C-staining pattern in both male (Fig. 1c) and female somatic metaphase karyotypes (Fig. 1d) indicates that constitutive heterochromatin is not only confined to the centromeric regions but also occur occasionally at distal telomeric regions of some chromosomal pairs. Intensely stained discrete C-heterochromatic bands were observed at the centromeric region of all the chromosomes of pachytene (Fig. 2g) and diakinesis (Fig. 2h) stages of male meiosis.

Silver nitrate impregnation

Somatic metaphase chromosomal preparations subjected to NOR staining exhibit consistent NOR bands on two autosomal pairs (#8 and #10) (Fig. 2k). Whereas, interphase nuclei (Fig. 2l) exhibit a minimum of one and a maximum of four nucleolar spots. Correspondingly, the meiotic chromosomal preparations following NOR staining revealed silver impregnation at the distal telomeric regions upon two chromosomes (#8 and #10) (Fig. 2i, j).

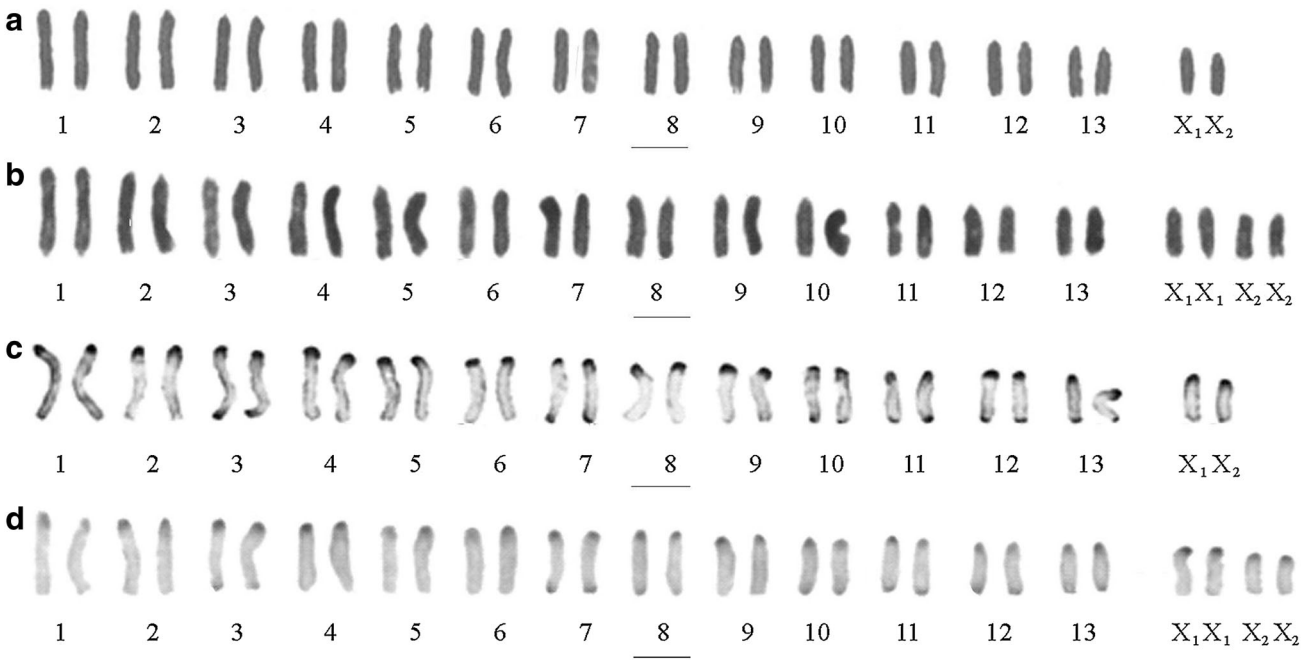

Fig. 1 *Ctenus indicus* somatic chromosomes: conventional Geimsa-stained. **a** Male ($2n = 26AA + X_1X_2$) and **b** female ($2n = 30AA + X_1X_1X_2X_2$) karyotypes; C-banded metaphase **c** male and **d** female karyotypes **Scale bar* 5 µm

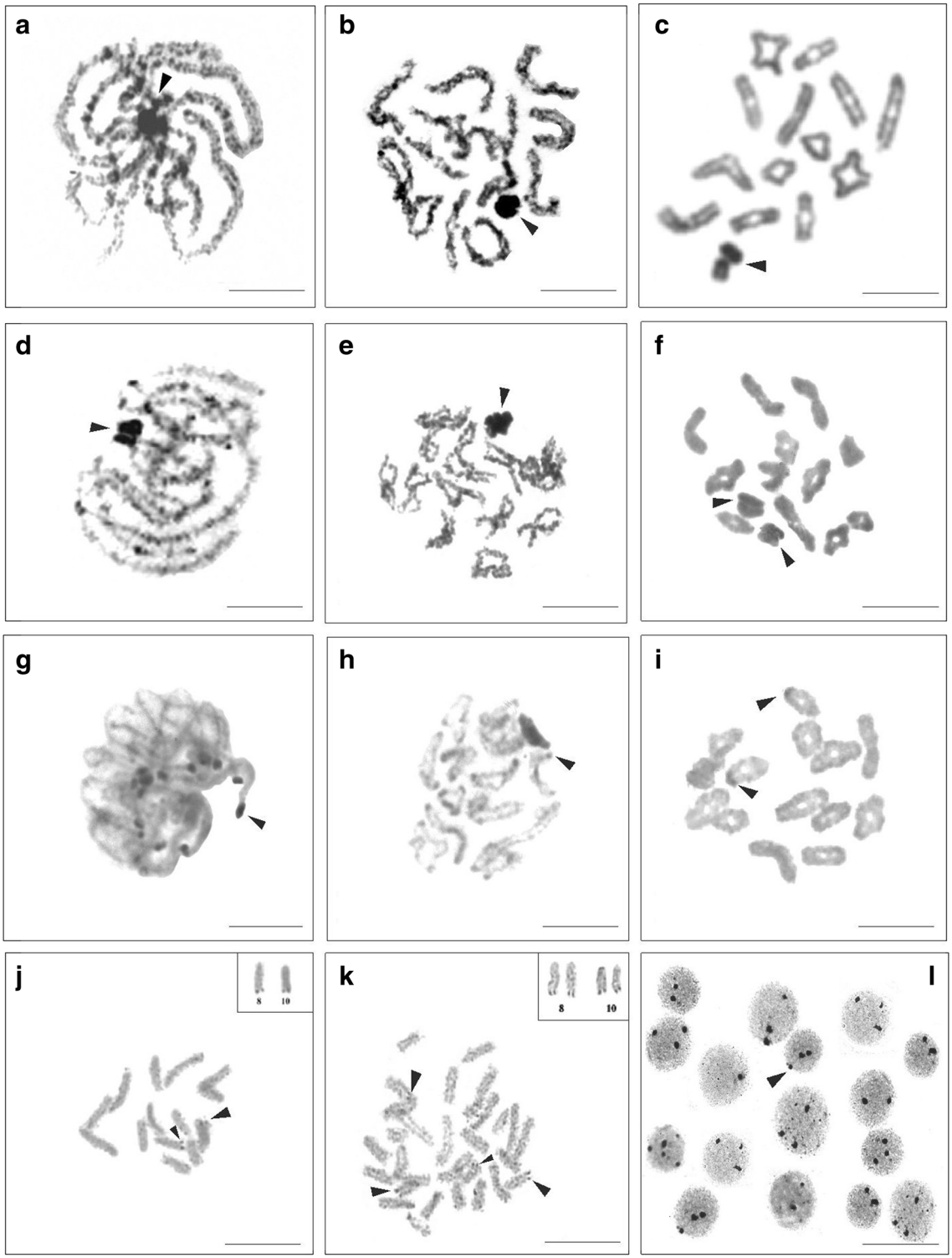

Fig. 2 *Ctenus indicus* meiotic stages: conventional Giemsa stained male. **a** Pachytene complement with a heteropycnotic chromocentre; **b** Diplotene complement with a heterochromatic sex bivalent; **c** Diakinetic configuration highlighting of heterochromatic dissociated sex bivalent partners; Conventional Giemsa stained female. **d** Pachytene complement with a highly compact heterochromatic sex bivalent; **e** Diplotene complement along with a compact heterochromatic sex bivalent; **f** Diakinetic configuration displaying of heterochromatic but almost dissociated two nonhomologous sex bivalents; C-banded male. **g** Pachytene complement; **h** Diplotene complement with a heterochromatic sex bivalent; Silver nitrate stained male. **i** Diakinetic and **j** Metaphase I complements displaying of NOR bands on two autosomal bivalents (#8 and #10); **k** Silver nitrate stained female somatic metaphase complement displaying of NOR bands at two chromosomal pairs (#8 and #10); **l** Interphase nuclei demonstrating of variable physiological features of NOR activity. **Scale bar* 5 μm

Discussion

Although of very limited in its extent, elucidation of the present results is in accordance with the earlier karyotypic formulation of ctenid cytogenetic assessment. Whereas, the karyological evaluations made in respect of other taxa belonging to Lycosoidea especially of Lycosidae clade, seem acceding to a distinctive pattern in which autosomes exhibit variability in basic chromosome numbers (2n = 18–28) and thus paving way for karyotypic differentiation [15] for this group.

Many authors including Loidl [27] and Haaf et al. [19] have fortuitously argued that centromeric associations (or also called as distributive pairings) are being facilitated by large blocks of constitutive heterochromatin with an inclusive nature of both centromeric and paracentromeric heterochromatin for such a cumulative effect. This situation appropriates inclusiveness imploring especially of acrocentric chromosomes as perceived in some examples examined during the processes of dynamicities of meiotic progression [1, 9, 16, 21, 28]. In accordance with these authors contention that a particular type of meiotic chromosomal association found between and among homologous and non-homologous chromosomes is probably mediated by the presence of highly reiterated loci consisting of 18S and 21S cistrons and C-heterochromatin surrounding these areas that may have been offering as a format for the consideration of chromosomal 'flanking effect'. Evidently, in some specific cases, during the courses of earlier spermatogonic meiosis that the centromeric zones of some non-homologous chromosomal bivalents may show a strong tendency to arrange in tandem and propel in a sort of 'bouquet formation'.

The results of the present report pertaining to our observations of certain pachytenes seem involving association of non-homologous bivalents that was primarily localizable at their centromeric regions. It also appears probable that these situations are in line with several of earlier observations and the notions incorporated for the justification [23, 24]. The association of centromeric regions of non-homologous pachytene bivalents may be also to establish proximity between heterochromatinized zones, as is currently demonstrated [1]. Moreover, a closer perusal of Synaptonemal Complex (SC) formation during the meiosis specifically at pachytene stages studied for the spider species *Tibellus* sp. and *Pardosa* sp. by Gorlov et al. [18] and studies of Dolejš et al. [15] seem fully endorsing towards their persistence nature observed in the form of bouquet formation. These observations have relevance to the present study, in which a good number of pachytenes were encountered in such a process.

Generally, the C-bands are shown confining to the centromeric and/or at telomeric regions and in certain cases at nucleolar regions and rarely to the intercalary regions of the chromosomes [42]. The presence of the copious and a predominant but cumulative nature of C-staining profile enabled in representing the constitutive heterochromatin at the centromeric zones that may also include pericentromeric region for extrapolation [7]. The present results on the mitotic metaphase and other stages of meiosis (Fig. 2g, h) show similarity in localizing C-heterochromatin at the centromeric regions in all the acrocentric chromosomes, while inconsistently also at the distal telomeric regions of some chromosomes (Fig. 1c, d). On the contrary, sparsely represented C-banding profiles observed in respect of those taxa belonging to certain Lycosidae surveyed depicting perhaps of chromosome morphological entity and thus highlighting inclination of respective centromeric region alone (perhaps of kinetochore regions) [10].

Results of NOR specifications (Fig. 2k) in the chromosomes of somatic cells (chromosome #8 and #10) were found dictating as an ideal cytological representative. Justifiably, there are earlier reports endorsing towards this effect in respect of other examples cited of some ctenid species (for example, *Ctenus ornatus, Phoneutria nigriventer* and *Viracucha andicola*) [6]. It is interesting to note that those of haplogyne genomes were when exposed for such a privilege for the demonstration of NOR specificities it was obvious to find them over the autosomal and sex chromosomal counterparts [17, 22].

The appearance of prominent nucleolus in the early part of the first prophase in many earlier studies, it is implied that the nucleolar cistrons are active in early part of meiotic progression and they are likely to get switched off as the prophase advances [29]. It was opined earlier by several workers including Oliveira et al. [32] and Araujo et al. [6] that in the majority of the entelegyne spiders screened for NOR activation, it was found that they were generally recognizable cytologically on two pairs of autosomal chromosomes.

Cytological evidence emerged from the current report would certainly add to the ever-growing list of the spider cytogenetic assessments. Studies using chromosome banding techniques may also seem limited in extent but would help appraising better elucidation for the current understandings of karyotypic evolution in the order Araneae. Thereby, indenting to undertake more chromosomal analyses in future. Thus, the current cytogenetic information acquired offer as a baseline data towards establishing phylogenetic relationships within this important group.

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