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Chromosomal characteristics of *Orthezia insignis* browne (Hemiptera: Coccoidea: Ortheziidae)

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Abstract
Ortheziidae is one of the primitive extant families of scale insects (Coccoidea). One of the species like *Ortehzia insignis* Browne, commonly known as *Lantana* bug/ *Jacaranda* bug was subjected to cytological investigations through advanced air-drying, C-, and NOR banding techniques. The cytological preparation has revealed the chromosome number for the species was 2n = 8. The somatic metaphase chromosomal complement consisting of two longer metacentric chromosomes (#1 and 2) in which each appear distinct and could easily be identifiable, whereas third was a metacentric and the fourth an acrocentric chromosome. It was difficult to differentiate individual sexes based on the chromosomal morphology. No discernible sex chromosomes were found, and males and females appear to have the same chromosomal constitution and no heterochromatization. The cytological study on this species is of its first kind and this provides a baseline data for further studies. Dark C- bands were noticed in the metacentric region of the 1st and 2nd pair of the chromosomes, while in 3rd pair it was located from one of the telomeric regions. Silver nitrate staining preparation revealed the presence of a prominent NOR-band at the penultimate end of one of the telomeric regions of fourth pair of Chromosome. The cytological study on this species is of its first kind and this provides a baseline data for further studies.

Keywords: Scale insects, *Orthezia insignis*, polyphagous, sap suckers

Introduction
Ortheziidae is one of the primitive extant families of the super family Coccoidea, includes about 198 species and 17 genera (Ben-Dov *et al*. 2017) [1]. The species are cosmopolitan in distribution. In many tropical, subtropical and temperate countries some of which are highly polyphagous pests, and their host plants range includes woody, herbaceous to graminaceous plant species (Epila, 1986). Species like Ortehzia insignis Browne, commonly known as *Lantana* bug/*Jacaranda* bug or greenhouse Orthezia was seriously threatened the endemic gumwood, *Commidendrum robustum* in St. Helena (Fowler, 2004) [5]. On the other hand, in Hawaiin ranches Ortehzia insignis was used to check the spread of *Lantana* camara (Epila, 1986).
Only three species of the family are cytogenetically studied, the diploid number of chromosomes ranges from 14 to 16 (Gavrilov, 2007) [7]. These scale insects show the ancestral characters like holocentric chromosomes and inverse meiosis.
The chromosome system offered by Orthezia species is of special interest because it is characterized by XX-XO in Newsteadia sp. and 2n(♀)-2n(♂) in Praelongorthezia praelonga (Douglas) (Gavrilov, 2007) [7]. The conventional type XX(♀)–XO(♂) mechanism was deviated with a manifestation in the form of acquisition of complete male diploidy – 2n(♀)-2n(♂) system, as there were no differential sex chromosomes. Male meiosis is apparently of achiasmatic type. While a limited study by Brown (1958) [2] it was found to act as a representative model for all the taxa belonging to Ortheziidae. Orthezia species are well known as pests of many greenhouse crops, but the knowledge of their cytology is very scanty. So, the purpose of the present study was to acquire chromosomal data on *Orthezia insignis* Browne genome.

Materials and methods
The materials used for chromosomal preparations were collected from different host plants like *Duranta* and *Lantana* periodically 2018 to 2019.
Chromosome plates were obtained by adapting a conventional air-drying technique (Venkatachalaiah & Chowdaiah, 1986) [11]. The embryonic tissues of different developmental stages of the material were minced in 0.125M KCl solution. After brief incubation, the cell suspension was washed twice with fresh carnoy's fixative and the same was dropped on to the pre-chilled slides and were stained with 2% Giemsa solution.
The prepared chromosomal plates were further used for various banding techniques like: NOR- and C- bandings. NOR banding - the destained/ unstained chromosomes were exposed to 2or 3 drops of AgNO3 (50% AgNO3 in 18 to 20 drops of 3% formaldehyde fixative) solution, incubated at 50oc in a moist chamber till it turns golden yellow and counter stained with 2% Giemsa solution (Goodpasture & Bloom 1975) [8]. C-banding - the unstained chromosomes were treated with 0.2N HCl followed by 5% Ba(OH)2 and 2XSSC. The treated chromosomes were stained with 5% Giemsa solution (Sumner 1972) [10].
A conventional squashing technique (with LAO stain) based on the procedure of French *et al*. (1962) [4] was followed for the preparation of linear banding sequences.
Karyotypes of both female and male were prepared by arranging the metaphase chromosomes in decreasing order of their length. The length of each chromosome was measured by using Image J software. Idiograms of both female and male individuals were constructed based on haploid complements.
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Results and discussion

The chromosome complement for the current studied species was found to be 2n=8 (Figs. 1a - 1d). The distribution of the 4 pairs of chromosomes in the prophase and of metaphase stages were of common sight. The somatic metaphase chromosomal complement consisting of two longer metacentric chromosomes (#1 and 2) in which each appear distinct and could easily be identifiable, whereas third was a metacentric and the fourth an acrocentric chromosome. It was difficult to differentiate individual sexes based on the chromosomal morphology.

As per the available data from around the world, about 465 species belonging to 14 families of scale insects were cytologically been studied. This comprises of 6% of total coccoid species in the world fauna (Gavrilov, 2007) [7]. The chromosome number varies from 2n=4 to 2n~192. Each of these family known for having basal (model number) chromosome number (2n=4, 8, 10, 18) and specific genetic system i, e., Lecanoid system, Comstockiella system, Diaspidid system, XX-XO system, 2n(♀)-2n(♂) system, Parthenogenesis and Hermophroditism. For several families only partial data are available. One such family is Orthiziidae, the chromosome number (2n) and genetic system is known only from three species *viz*., Newsteadia sp. with 2n=14/13 and XX-XO system (Nur, 1980) [9], Orthezia urticae (Linnaeus) with 2n=18 (Gavrilov, 2004a) [6], Praelongorthezia praelonga (Douglas) with 2n=16 and 2n(♀)-2n(♂) system (Brown, 1958) [2]. The present studied species O. insignis has shown to be the least number of chromosomes with 2n=8 and 2n(♀)-2n(♂) genetic system without any identifiable sex-chromosomes.

LAO-stained chromosomes showed longitudinal dark and light bands along their length and that helps in identification of individual chromosome in the complement (Fig.2. a). Dark C- bands were noticed in the metacentric region of the 1st and 2nd pair of the chromosomes, while in 3rd pair it was located from one of the telomeric regions (Fig.2. b). Silver nitrate staining preparation revealed the presence of a prominent NOR-band at the penultimate end of one of the telomeric regions of fourth pair of chromosomes (Fig.2.c).

Conclusion

In the primitive coccoid family Orthiziidae, the chromosome numbers and sex-determination systems were derived from the least number of species cytologically known. At present, relaying on this precarious situation wherein affixing the basal numbers for this family becomes premature and a difficult task. The present investigation might be the baseline data for further research in finding the basal chromosome number and genetic system of the family Ortheziidae.

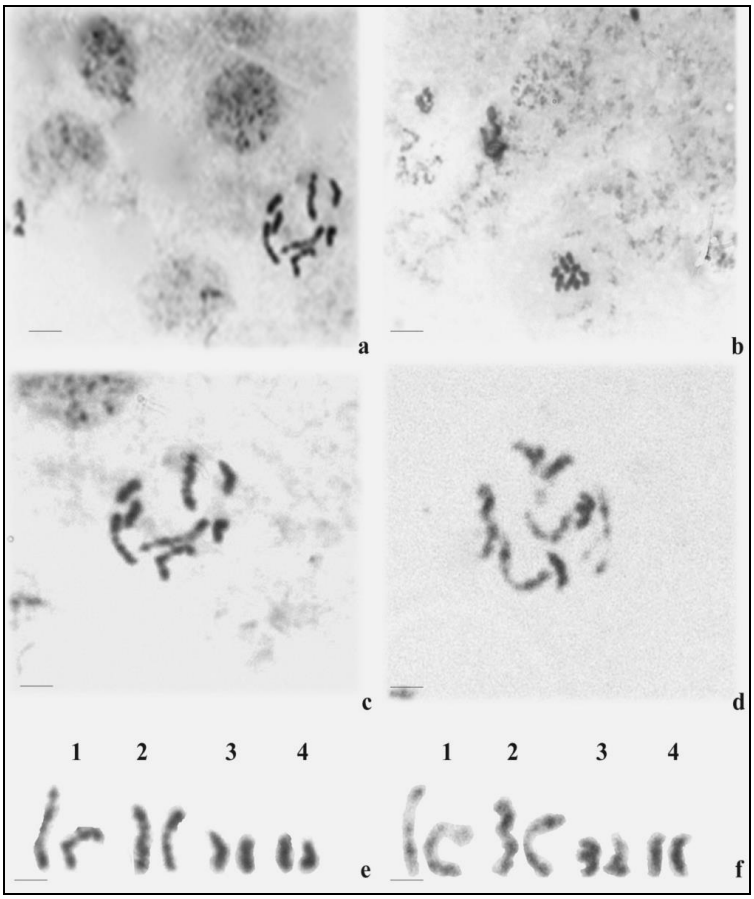

Fig 1: Somatic chromosomal complement and karyotype: *O. insignis*

a. Giemsa -stained female midgut region showing various stages of mitosis
b. Giemsa- stained male embryonic body wall showing various stages of mitosis
c. Female somatic complement showing 2n = 8;
d. Male somatic complement showing 2n = 8
e. Female somatic chromosomal karyotype; f. Male somatic chromosomal karyotype

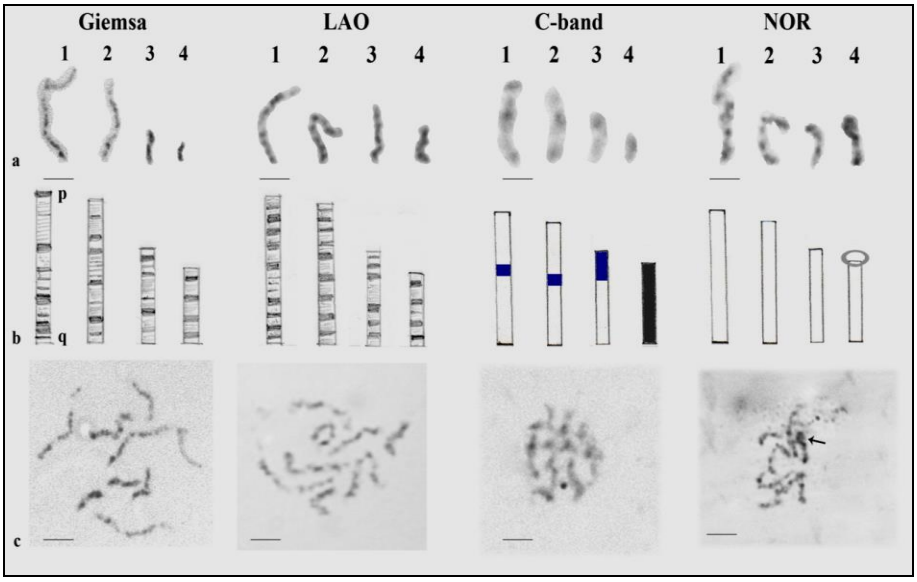

Fig. 2: Differentially stained female metaphase chromosomal karyotype: *O. insignis*.
a. Haploid karyotype b. Haploid ideogram c. Diploid complement

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