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Karyotype and meiosis analysis of four species of Cameroonian Pygomorphidae (Orthoptera)

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In this article, the karyotypic features (chromosome number, morphology, size and length and length of X chromosome), and meiosis in *Atractomorpha lata*, *Dictyophorus griseus, Taphronota thaelephora and Zonocerus variegatus* (Orthoptera: Pyrgomorphidae: Pyrgomorphinae) were analysed in order to determine similarities and differences amongst them. All four species were cytogenetically similar in relation to chromosome number, morphology and sex mechanism. They revealed karyotypes that comprised of acrocentric chromosomes with complement number 2n = 19 (male) and the XX♀–XO♂ sex mechanism. Chromosomes in the four species occurred in size groups of long, medium and short. The number of chromosomes in the size groups varied with species. Cluster analysis revealed chromosomes 4, 5 and 9 to be comparable in length in all four species and it is suggested that these chromosomes could be marker chromosomes for the subfamily Pyrgomorphinae. The meiotic process in the four species was normal and chiasmate. Similar bivalent shapes were recognized for both Diplotene and first meiotic Metaphase in the four species. Mean chiasma frequency was not significantly different (P>0.05) for *A. lata*, *D. griseus, T. thaelephora* but was significantly higher (P< 0.05) for *Z. variegatus* compared to the other three species.

Key words: Pyrgomorphidae, Pyrgomorphinae, Comparative Karyotype, Comparative Meiosis.

INTRODUCTION

An overview of chromosome data from a cytogenetic viewpoint revealed that the African Pyrgomorphidae have been studied sporadically. Of the over 79 described species, (Mestre and Chiffaud, 2009) less than 20% have been examined cytogenetically. African species are therefore the most neglected. Available information indicates that only six African species, *Pyrgomorpha rugosa*, *P. granulata*, *Pyrgomorpha* spp (unclassified), *Zonocerus variegatus, Dictyophorus griseus* and *Taphronota thaelephora,* have been cytogenetically characterized (Faluyi and Olorode, 1988; Fossey et al., 1989; Seino et al, 2007, 2012a, b).

Karyotypic information from African Pyrgomorphidae revealed variation in diploid chromosome number from 2n=11 to 2n=19♂. Chromosome number variations in this family came from autosome –autosome centric fusions, X - autosome fusions and the presence of supernumerary chromosomes (White, 1973; Faluyi and Oyidi, 1988; Fossey et al., 1989; Seino et al., 2007, 2012a, b). The variation in chromosome number was accompanied by variation in chromosome morphology. As expected, the centric fusions introduced not only a reduction in chromosome number but also submetacentric chromosomes in the overwhelming acrocentric Pyrgomorphidae karyotype of 2n =19♂ (White, 1973; Fossey et al., 1989). In spite of these variations, Pyrgomorphidae are known to exhibit

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Table 1. Morphometric characters of karyotypes of the four species studied.

Serial Number	Species	Sub- family	Total number of chromosomes per cell in the male	Sex determining mechanism ♀ - ♂	Number of chromosome per size group			Ratio of longest to shortest chromosome	Total chromosome length (µm) (Haploid set)	Morphology of chromosomes	Length (µm) of X chromosome	Size of X chromosome
					Long	Medium	Short					
1	*A. lata*	Pyrgomorphinae	19	XX-XO	3	5	1	3.42 : 1	56.39	All Acrocentric	5.63	Medium
2	*D. griseus*	Pyrgomorphinae	19	XX-XO	1	4	4	2.31: 1	43.87	All Acrocentric	8.40	Long
3	*T. thaelephora*	Pyrgomorphinae	19	XX-XO	2	6	1	3.25: 1	54.46	All Acrocentric	6.75	Long
4	*Z. variegatus*	Pyrgomorphinae	19	XX-XO	2	6	1	2.19 : 1	55.00	All Acrocentric	6.77	Long

karyotypic stability in diploid chromosome number and morphology. The vast majority of them possess a fundamental chromosome complement of 2n = 19♂ acrocentrics (Hewitt, 1979; Santos et al., 1983; Fossey et al., 1989; Seino et al., 2002, 2012a,b).

Though the family Pyrgomorphidae has for sometime been under constant cytogenetic investigation, cytotaxonomic classifications and phylogenetic relationships in African Pyrgomorphidae are yet to be initiated. In this paper, we comparatively analyzed karyotypic features and meiosis in four Pyrgomorphidae species: *A. lata*, *D. griseus, T. thaelephora* and *Z. variegatus* collected in Cameroon, so as to bring out some similarities and differences that could be pointers to phylogenetic relationships.

MATERIALS AND METHODS

Forty (40) adult male individuals of *A. lata, D. griseus, T. thaelephora* and *Z. variegatus* used for this study were captured in the North – West and West Regions of Cameroon. Chromosome analysis were made from testes fixed in 3:1 ethanol acetic acid and squashed in 2% Lactic acetic Orcein with the method of Seino et al. (2010). The grasshoppers were treated with colchicine in order to easily obtain mitotic chromosomes. However, some individuals were not treated with colchicine in order to obtain meiotic cells (Tepperberg, 1997). The chromosome smears thus prepared were examined using the 40x objective of a Fisher laboratory microscope and photographed using the 100x oil immersion objective of a Lietz photomicroscope.

Mitotic metaphase chromosomes were measured directly from the microscope (Magnification X40) with the help of ocular and stage micrometers. Ten individuals were examined for each of the four species studied.

The structure of bivalents in Diplotene and Metaphase 1 were compared for similarity and chiasmata were counted at Diplotene / Diakinesis from five cells per individual.

RESULTS AND DISCUSSION

In spite of the large number of Pyrgomorphidae species on the African continent, only six species have their conventionally stained karyotypes described so far (Seino et al., 2012a). The lack of karyotype information has hampered cytotaxonomic, phylogenetic and evolution studies on the African species of this family. This study therefore attempts to analyse the similarities and differences in karyotype and meiosis of four African Pyrgomorphidae.

A perusal of Table 1 reveals all the morphometric characters of the four species studied. All of them belonging to the subfamily Pyrgomorphinae showed similar karyotypes composed of 9 somatic chromosomes and an X, corresponding to 2n=19, XO diploid karyotype in males. Therefore *A. lata, D. griseus, T. thaelephora and Z. variegatus* had an XX♀-XO♂ sex determining mechanism commonly reported for most Orthoptera species (White, 1973; Hewitt, 1979). In this study, it was ascertained that the chromosome morphology was acrocentric for all the chromosomes in the species and all four species were cytogenetically similar in relation to chromosome number, morphology and sex mechanism. Faluyi and Olorode (1988) reported similar results for *Zonocerus variegatus* from Nigerian while Fossey et al. (1989) reported similar results among three South African species of *Pyrgomorpha*. These reports reveal agreement in the cytogenetics of Pyrgomorphidae species from different regions of Africa. Similar results have also been reported for Neotropical species (Mesa and Fontanetti, 1983), European species (John and King, 1983) as well as Russian and Central Asian species (Bugrov, 1996). So the Pyrgomorphidae of different regions of the world show cytogenetic uniformity regarding karyotype (chromosome number and morphology) and sex –mechanism. Figure 1 shows the haploid karyotype arrangement of chromosomes in *A. lata, D. griseus, T. thaelephora* and *Z. variegatus* and the chromosomeswere classified according to three size groups of long, medium and short, another common characteristic of Orthoptera karyotypes (Burgrov and Warchalowska–Silva, 1997; Bugrov et al., 1999; Turkoglu and Koca, 2002;

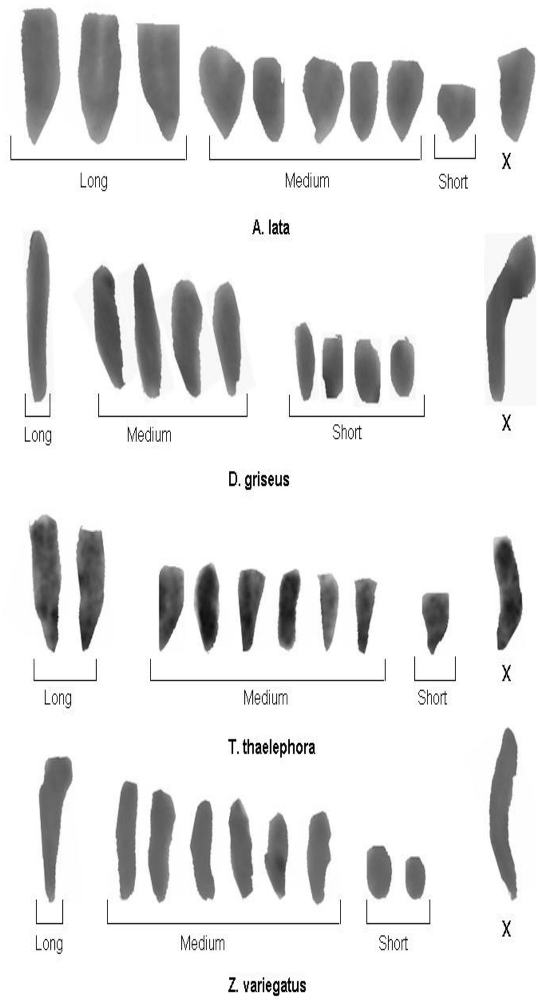

Figure 1. Composite karyotypes of *A. lata*, *D. griseus*, *T. thaelephora*, and *Z. Variegates.*

Warchalowska-Silva et al., 2002; Ren et al., 2008; Seino et al., 2012). Figure 1 and Table 1 reveal that *A. lata* had three long pairs (L_1 – L_3) including the X chromosome, five medium sized pairs (M_4 – M_8) and one short pair (S_9). *D. griseus* had one long pair (L_1) including the X chromosome which was the longest chromosome in the genome, four medium sized pairs (M_2 – M_5) and four short pairs (S_6 –S_9). *T. thaelephora* had two long pairs (L_1 – L_2) including the X chromosome, six medium sized pairs (M_3 – M_8) and one short pair (S_9). *Z. variegatus* had one long pair (L_1) including the X chromosome, six medium sized pairs (M_2 – M_7) and two short pairs (S_8 – S_9). Figure 1 and Table 1 further reveal, that the total chromosome length varied among the species and occurred in the series *A. lata* > *Z. variegatus* > *T. thaelephora* > *D. griseus* confirming the specific nature of each of the karyotypes. However, these total chromosome lengths were not significantly different (P>0.05) for the four species. The X chromosome in *A. lata* corresponded to the medium size group while in *D. griseus*, *T. thaelephora* and *Z. variegatus* it corresponded to the long size group (Figure 1). The X chromosome could therefore not be used as a marker chromosome for these species as has been the case in comparative karyotype studies involving some Tryxalinae (Acrididae) grasshoppers (Chadha and Mehta, 2011).

Cluster analysis of chromosome lengths revealed that the chromosomes in the four species could be grouped into four clusters (Figure 2). In cluster 1, chromosomes 4, 5 and 9 were comparable in all four species; chromosomes 3 was comparable in *D. griseus*, *T. thaelephora* and *Z. variegatus*; chromosome 6 was comparable in *A. lata*, *D. griseus*, and *Z. variegatus;* chromosome 7 was comparable in *A. lata*, *T. thaelephora* and *Z. variegatus;* Chromosome 8 was comparable in *A. lata*, *D. griseus* and *T. thaelephora*. In clusters 2 and 3, there were no comparable chromosomes for the four species here studied. In cluster 4, chromosome 1 was comparable for *A. lata* and *D. griseus;* chromosome 2 was comparable for *A. lata*, *T. thaelephora* and *Z. Variegates.* The X-chromosome was comparable for *T. thaelephora* and *Z. variegatus*. The closest relationships with respect to chromosome lengths involved chromosomes 4, 5 and 9 in all four species studied. These chromosomes could be marker chromosomes for the subfamily Pyrgomorphinae.

Among the Pyrgomorphidae, available data on meiosis is scarce even though meiosis has been aptly described for some genera that include *Atractomorpha, Taphronota* and *Zonocerus* (Oyidi, 1967; Faluyi and Olorode, 1988; Seino et al, 2002). In this study Prophase 1, Metaphase 1, Anaphase 1, Metaphase 2 and Anaphase 2 were recorded in the meiotic processes in *A. lata, D. griseus, T. thaelephora and Z. variegatus* (Figure 3). The meiotic processes in these four species were observed to be normal and chiasmate, a characteristic common to Orthopteran species (White, 1973; Hewitt, 1979). Although there are no reviews dedicated to meiosis in the Pyrgomorphidae, it can be supposed that chiasmate meiosis is ancestral, dominant and probably the only type of meiotic pattern in the family. This was confirmed during this study by comparing Diplotene and first meiotic Metaphase bivalents. It was possible to recognize similar Diplotene and first meiotic Metaphase bivalent shapes for each chromosome in the karyotypes of the four species (Figures 4 and 5). Furthermore, the X chromosome in all four species investigated exhibited the reversal type of heteropycnosis. It was positively heteropycnotic in first meiotic Prophase and negatively heteropycnotic in first meiotic Metaphase and Anaphase. This heteropycnotic nature and behaviour of the X chromosome is a characteristic of Orthoptera grasshoppers (White, 1973; Turkoglu and Koca, 2002).

Chiasma frequency varied among the species (Table 2). Mean chiasma frequency was in the series *Z. variegatus* > *A. lata* > *D. griseus* > *T. thaelephora.* Mean chiasma frequency was found to be significantly different (P < 0.05) among the species. This was expected since it been reported that chiasma is under the control of genes

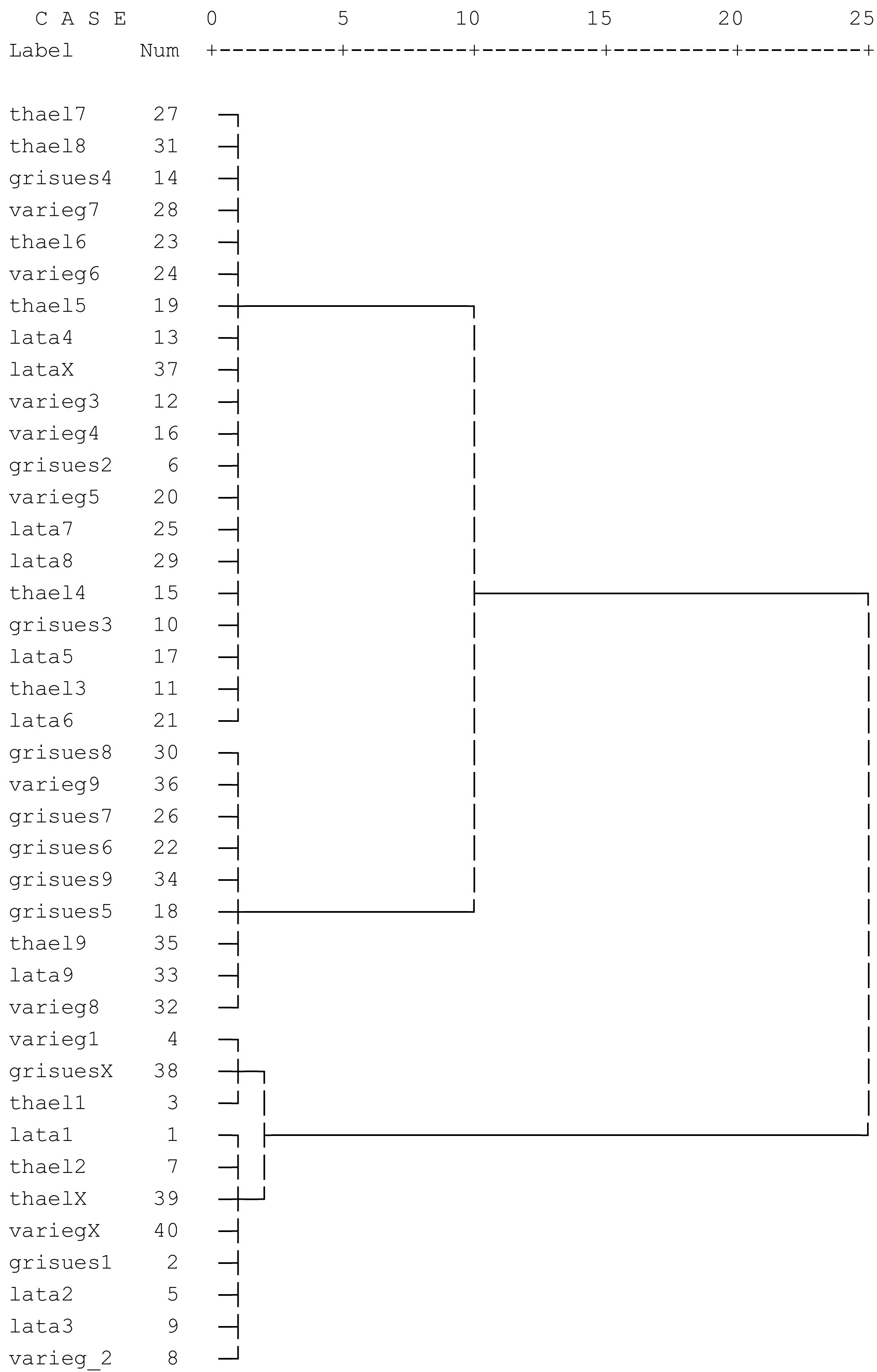

Figure 2. Dendrogram showing relationships among *A. lata*, *D. griseus*, *T. thaelephora*, and *Z. variegatus*. Letter abbreviations represent species and numbers represent individual chro-mosomes: Lata = *A. Lata*; griseus= *D. Griseus*; thael= *T. Thaelephora*; varieg = *Z. variegatus*; X = X chromosome.

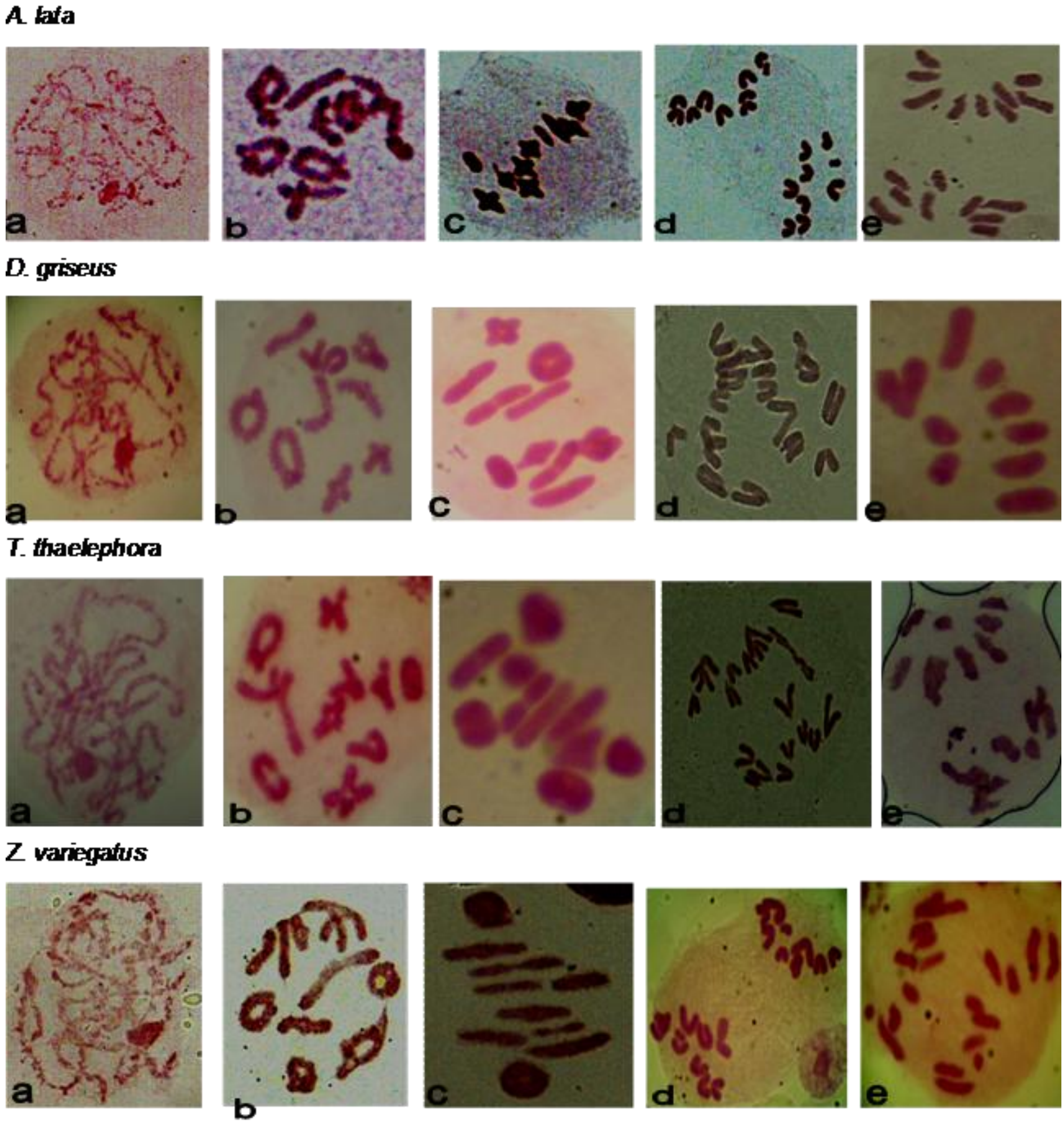

Figure 3. Meiotic stages in the four species. Similar meiotic prophase stages were observed in all four species. a= Zygotene; b= Diplotene; c= Metaphase -1; D= Anaphase -1; e = Anaphase -2.

and is dependent on the length of the chromosome Verma and Agarwal, 2005). However, Duncan's Multiple Range Test (DMRT) revealed that mean chiasma frequency was significantly higher ($P<0.05$) in *Z. variegatus* than in the other three species and was not significantly different ($P >0.05$) for *A. lata*, *D. griseus* and *T. thaelephora.* The cyto-genetic evidences here presented constitute an additional tool for the taxonomic characterization of these species.

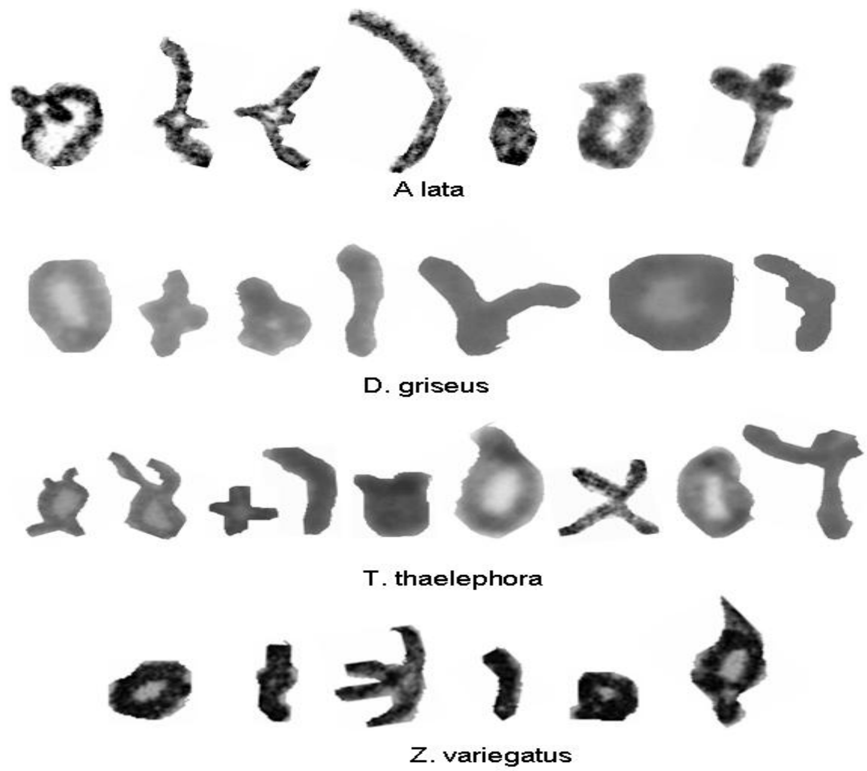

Figure 4. Composite Diplotene figures in *A. lata*, *D. griseus*, *T. thaelephora*, and *Z. Variegates*

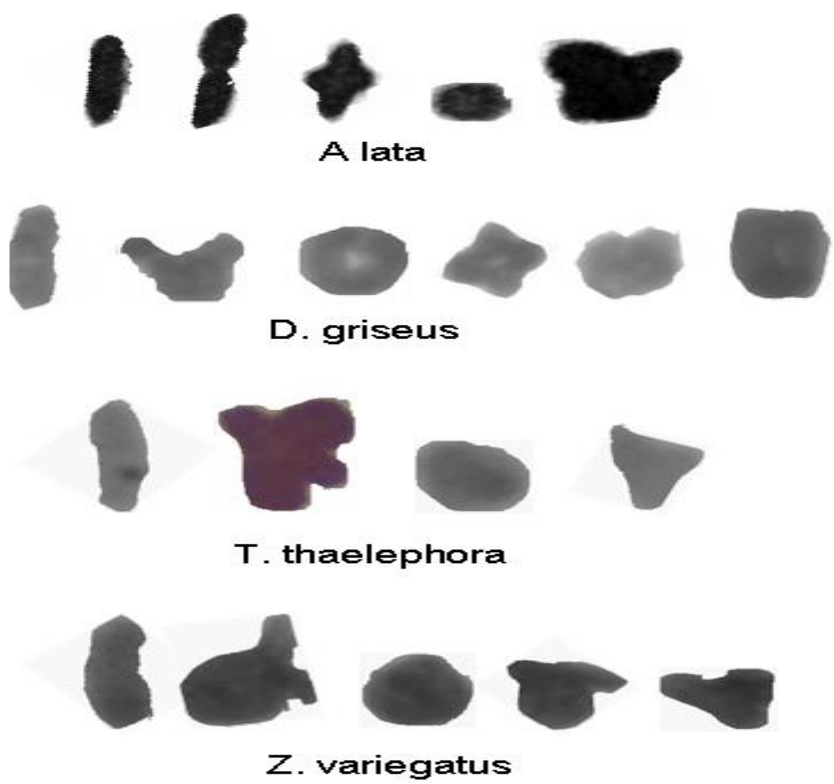

Figure 5. Composite Metaphase -1 bivalents in *A. lata*, *D. griseus*, *T. thaelephora*, and *Z. Variegates*.

Table 2. Mean chiasma frequency per species.

Specie	Individual grasshopper										Specie mean chiasma frequency
	1	2	3	4	5	6	7	8	9	10	
A. lata	12.8	13.0	13.2	12.2	12.6	12.6	12.8	13.0	13.0	13.2	12.84 ± 0.29
D. griseus	11.2	11.4	13.0	12.0	12.4	12.4	12.4	12.6	11.8	11.8	12.10 ± 0.53
T. thaelephora	12.0	10.0	11.4	11.0	11.6	12.2	12.4	12.2	12.8	12.6	11.82 ± 0.80
Z. variegatus	12.6	15.4	13.6	13.6	14.0	13.2	12.6	12.6	14.8	12.6	13.50 ± 0.94

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