Scientific Note

Belostoma estevezae Ribeiro and Alecrim (Heteroptera: Belostomatidae) reveals a new karyotype complement in *Belostoma* Latreille from mitotic metaphases

Belostoma estevezae Ribeiro y Alecrim (Heteroptera: Belostomatidae) revela un nuevo complemento cariotípico en *Belostoma* Latreille a partir de metáfases mitóticas

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Resumen. *Belostoma* Latreille comprende 74 especies de insectos acuáticos depredadores, con 42 de ellas registradas en Brasil. Este grupo se caracteriza por presentar cromosomas holocéntricos con sistemas de determinación sexual múltiple o simple. En Belostomatidae Leach, unas pocas especies presentan microcromosomas. *Belostoma estevezae* Ribeiro y Alecrim, especie endémica de Brasil, pertenece al grupo *plebejum* y se parece mucho a *Belostoma micantulum* Stål. En este trabajo describimos el complemento cromosómico y el contenido y distribución de la heterocromatina C constitutiva en machos de *B. estevezae*. Las células mitóticas de *B. estevezae* fueron obtenidas a partir de embriones provenientes de tres masas de huevos. Las preparaciones cromosómicas fueron teñidas con Giemsa 2% para la caracterización cariotípica de la especie y para la técnica de bandas C implementada. El cariotipo masculino de *B. estevezae* fue estimado como 26 + 4m + XY (2n = 32), lo que representa un complemento cariotípico nuevo para *Belostoma*. El sistema de determinación sexual y los patrones de distribución de bandas-C registrados en está especie son similares a los observados en *B. plebejum* y *B. micantulum*. Diferentes eventos de fisión de autosomas pueden explicar el alto número diploide encontrado en *B. estevezae*.

Palabras clave: Cromosomas holocéntricos, Insecta, microcromosomas, insectos acuáticos.

Abstract. *Belostoma* Latreille comprises 74 species of ambusher predator true water bugs, with 42 species registered in Brazil. This group is characterized by presenting holocentric chromosomes with multiple or simple sexual determination systems. In Belostomatidae Leach, only a few species present microchromosomes. *Belostoma estevezae* belongs to the *plebejum* group and it is an endemic species to Brazil which is looking much like *B. micantulum* Stål. We describe the chromosomal complement and the content and distribution of constitutive heterochromatin C in males of *B. estevezae*. The mitotic cells of *B. estevezae* were obtained from embryos from three egg masses. The chromosomal preparations were stained with 2% Giemsa for the cariotypic characterization of the species and for the C banding technique implemented. The male karyotype of *B. estevezae* was estimated as 26 + 4m + XY (2n = 32), which represents a new karyotype complement for *Belostoma*. The system of sexual determination and distribution patterns of C-bands recorded in this species are similar to those observed in *B. plebejum* and *B. micantulum*. Different autosomal fission events may explain the high diploid number found in *B. estevezae*.

Key words: Holocentric chromosomes, Insecta, microchromosomes, water bugs.

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The genus *Belostoma* Latreille, 1807 comprises 74 true water bug species of ambush predators. Being the most diverse of the Belostomatidae Leach, 1815 family, its representatives occur mainly in South America (Lauck and Menke 1961). From 74 species distributed in the Neotropical region, 42 of them were recorded in Brazil until now (Moreira *et al*. 2011). This genus was subdivided by Lauck (1962) into 16 groups of species, from an evaluation of the general morphological similarity between these taxa. All species of *Belostoma* cytogenetically studied present holocentric chromosomes, without a localised centromere, with different number and size of chromosomes (Papeschi 1996; Papeschi and Bressa 2006; Bardella *et al*. 2012; Chirino *et al*. 2013; Chirino and Bressa 2014). The sex determination systems can be multiple (XnY/XnXn, male/female) or simple (XY/XX; male/female) with a huge interspecific variation in the diploid number (Bardella *et al*. 2012; Chirino and Bressa 2014; Gallo *et al*. 2017).

The main causes of this variation, from 8 to 30 chromosomes, are the events of fission and fusion, when compared to the hypothetical karyotype of the ancestor, proposed with 2n = 26 + XY / XX (male/female) (Papeschi and Bressa 2006). This result is allied to the distribution of clusters of 18S rDNA and of telomere motifs in chromosomes (Bardella *et al*. 2012; Kuznetsova *et al*. 2012; Chirino *et al*. 2013; Chirino and Bressa 2014; Chirino *et al*. 2017). In addition, within Belostomatidae only a few species have been identified with microchromosomes (m), till this moment (Kuznetsova *et al*. 2012; Grozeva *et al*. 2013). Although there is a range of characteristics that make the study of *Belostoma* chromosomes interesting, only 17 species have been cytogenetically described so far (Chirino *et al*. 2017; Gallo *et al*. 2017).

The group *plebejum* sensu Nieser (1975) is monophyletic (J. R. I. Ribeiro, unpublished data), and its representatives are recorded from Central America (Honduras) to southern South America (Argentina) (Estévez and Polhemus 2007; Ribeiro and Alecrim 2008). From the nine known species of this group, only two have cytogenetic data described: *Belostoma micantulum* (Stål, 1858), which presented a diploid number of 14 + XY (Papeschi 1988; Papeschi and Bressa 2006) and *B. plebejum* (Stål, 1860) showed chromosomal polymorphism, varying from 2n=13 + XY or 2n=14 + XY (Papeschi 1994). *Belostoma estevezae* Ribeiro and Alecrim, 2008 (Fig. 1A), one species from this group, is apparently endemic to Brazil. In general, this species is similar to *B. micantulum*, since both are within the body length range of 10.5 to 13.0 mm and both present the diverticulum, in ventral view the male genitalia are more rounded (Ribeiro and Alecrim 2008).

Studying the chromosomal complement of *B. plebejum* group, which has few cytogenetic analyses, is important to better understand their cytogenetic diversity for future analyses of the relationships among this group. In this way, we describe for the first time the karyotype complement and the accumulation of constitutive heterochromatin in males of *B. estevezae* by means of embryo culture.

We obtained egg masses which were taken from three male specimens of *B. estevezae* caught with entomological sieves in the municipality of São Gabriel, in the Rio Grande do Sul, Brazil. The egg mass was removed from the back of the insects and the embryos underwent cleaning procedures, according to Mitsuhashi (2002). The embryo culture, chromosome preparation, and conventional 2% Giemsa staining followed the protocols by Popescu and Dutrillaux (2000). For better chromosome visualisation, the slide was treated using a magnetic stirrer with 40% acetic acid for about one minute to allow its evaporation, before staining. The constitutive heterochromatin content was verified using C-banding (Sumner 1972; Grozeva and Nokkala 2001). The karyotype was assembled using an optical microscope with increased 1000X.

Males of *B. estevezae* showed a diploid number of 32 chromosomes (26 + 4m + XY), with 26 autosomes, 4 autosomal microchromosomes, and the XY simple sexual system (Figs. 1 B-E). The X chromosome is much larger than the Y chromosome, which was identified as a microchromosome together with pairs 14 and 15 (Figs. 1 B-C).

The chromosomes did not present primary constrictions in any of the observed phases. The C-banding technique obtained positive marking on the whole chromosome pairs 2, 4, 5, 6, 7, 11 and 13 (Figs. 1D-E). Large C-positive blocks in chromosome pairs 1, 3, 8, 10, and in the X sex chromosome were observed (Figs. 1 D-E). The chromosome pairs 9 and 12 and the microchromosomes 14 and 15, as well as of the sexual chromosome Y, presented non-heterochromatic banding (C negative, euchromatic) (Figs. 1 D-E).

The diploid number currently known within the genus *Belostoma* varies from 2n = 8 (6 + XY) in *Belostoma oxyurum* Dufour, 1863, to 2n = 30 (26 + $X_1X_2X_3Y$) in *B. testaceopallidum* Latreille, 1807 and *B. dilatatum* Dufour, 1863 (Papeschi 1988; Gallo *et al*. 2017). The species of the *plebejum* group have a low diploid number when compared to the other species of the genus, varying between 15 and 16 chromosomes. Furthermore, these species always present a system of simple sex chromosomes (Papeschi 1994, 1996; Papeschi and Bressa 2006). Males of *B. estevezae* presented a diploid number bigger than expected for the *plebejum* group. However, there is an inverse relationship between number and size chromosomes (Papeschi 1988, 1992; Chirino and Bressa 2014; Chirino *et al.* 2017). Therefore, the presence of two microchromosome pairs in this species possibly originated from successive fission events on the autosomal chromosomes of the males of *B. estevezae*, and it may explain the high diploid number.

Chromosomes fissions are extremely common in the sex chromosomes in *Belostoma.* Therefore, it is observed some species with multiple sex determination system with the X chromosomes smaller than the Y, whereas in others, both of them have similar sizes or the Xs are slightly larger than the Y (Bardella *et al*. 2012; Chirino *et al*. 2013, 2017; Gallo *et al*. 2017). However, in species with a simple sexual determination system, as in the hypothetical ancestor proposed (Papeschi and Bressa 2006), the Y chromosome was smallest during metaphases I and II of meiosis in several species of *Belostoma* (Papeschi 1991, 1994, 1995, 1996; Bardella *et al*. 2012; Chirino and Bressa 2014). The reduced Y chromosome found in males of *B. estevezae* follows the same patterns found for species with simple sex determination system in *Belostoma*.

The microchromosomes were described in the family Belostomatidae in *Abedus indentatus* Haldeman, 1854, *Lethocerus indicus* Lepeletier and Serville, 1775, *L. patruelis* Stål, 1855, *Diplonychus annulatus* Fabricius, 1781, *Benacus griseus* Say, 1832 and *Belostoma flumineum* Say, 1832 (Grozeva *et al*. 2013). Although the presence of microchromosomes may be considered rare to date for the genus *Belostoma*, only 17 of the 74 species of the genus (about 23%) had some karyotypic description (Gallo *et al*. 2017). The amplitude of variation in the diploid number of a maximum of 30 chromosomes and the "rare" presence of microchromosomes in the karyotype complement of the *Belostoma* species may reflect the limited karyotypic analyses for the group.

The constitutive heterochromatin accumulation at the telomeric positions is commonly found in *Belostoma* species, as well as the non-heterochromatic (C-negative) marking on the Y chromosome, being reported for several species of the genus (Papeschi 1991; Chirino *et al*. 2013). Although entirely heterochromatic chromosomes (C-positive) are not common within the genus, there are records for the Y chromosome in some species such as *B. oxyurum* (Papeschi 1995) and *B. candidulum* (Chirino and Bressa 2014).

Males of *Belostoma estevezae* presented a larger diploid number than expected for the *plebejum* group, but the sexual system and the C-banding patterns found for the species follow the patterns observed in the *plebejum* group. Despite microchromosomes being rare in *Belostoma*, probably due to successive fission events occurring on the autosomal chromosomes, since the X chromosome has a high size in relation to the Y, explaining the high diploid number found. This inference can be reinforced by the positive C-labels found in several autosomal pairs and in the two pairs of microchromosomes in this species. We emphasise that this is the first cytogenetic data in *Belostoma* obtained by means of embryo

culture, which adopted a rather simplistic methodology. Future analyses that allow for the visualisation, of which chromosomal pairs have undergone fission, are needed to clarify the phenomena involved in the increasing of the diploid number in males of *B. estevezae*.

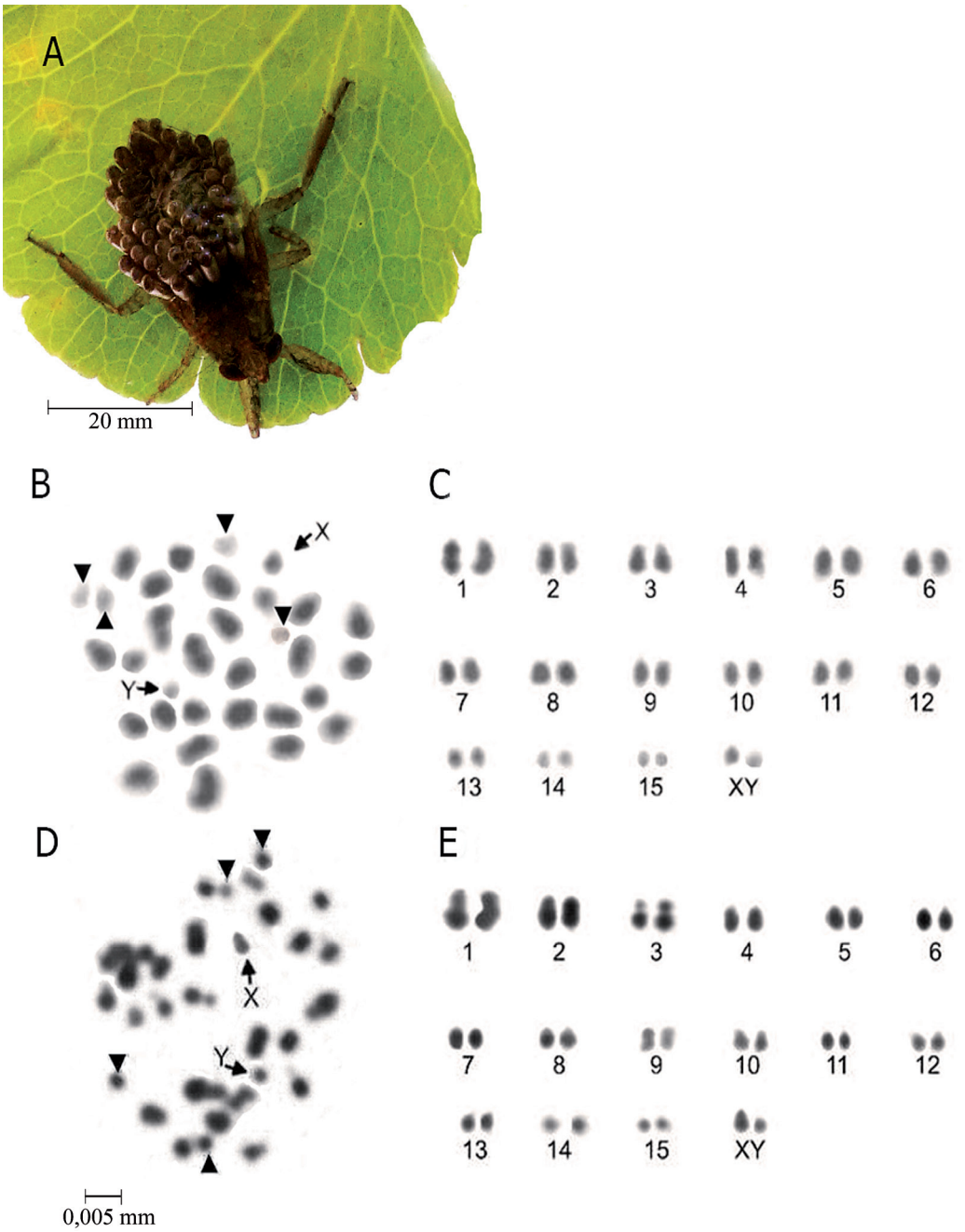

Figure 1. *Belostoma estevezae* and chromosomes. (A) Male of *B. estevezae* with eggs laid on its back. (B) Metaphase with conventional staining (2% Giemsa). (C) Male karyotype of *B. estevezae* with pairs 14 and 15 formed by microchromosomes. (D) C-banding in metaphase. (E) C-banding in chromosomes of *B. estevezae*. In (B) and (D) the arrows indicate the sex chromosomes X and Y and the arrow heads indicate the microchromosomes. Scale bar: 20 mm (A); 0,005 mm (B), (C), (D) and (E).

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