

Cytogenetical Aspects of Testicular Cells in Economically Important Species of Coreidae Family (Heteroptera)

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Received October 31, 2006; accepted November 18, 2006

Summary Some cytogenetical aspects of spermatozoa formation were studied in 9 Coreidae Brazilian species: *Anasa bellator*, *Athaumastus haematicus*, *Chariesterus armatus*, *Dallacoris obscura*, *Dallacoris pictus*, *Leptoglossus gonagra*, *Leptoglossus zonatus*, *Sphictyrtus fasciatus*, and *Zicca annulata*. Similarly to the other species described to date, all the species studied herein showed cystic spermatogenesis, a reddish membrane covering the testes, a X0 sex determining system, a pair of m-chromosomes, interstitial chiasmata in most autosomes, and autosomes dividing reductionally at first meiotic division and equationally in the second 1 while sex chromosomes, divide equationally and reductionally at first and second meiotic division, respectively. In addition, it was observed that the sex chromosome is heteropycnotic at prophase and that heteropycnotic chromosomal material is found in the nuclei at spermiogenesis. In the species studied, the diploid chromosome number ranged from 19 to 25. It was 19 in *S. fasciatus* ($16A+2m+X0$); 21 in *A. bellator*, *A. haematicus*, *D. obscura*, *D. pictus*, *L. gonagra*, and *L. zonatus* ($18A+2m+X0$); 23 in *Z. annulata* ($20A+2m+X0$); and 25 in *C. armatus* ($22A+2m+X0$).

Key words Heteroptera, Coreidae, Holocentric, Meiosis, Chiasma, M-chromosome.

Coreidae is a large Heteroptera family with many and diverse forms. They are widely distributed, but more abundant in tropical and subtropical regions, where they reach their largest size and strangest appearances. A number of species in this family is economically important (Schuh and Slater 1995). As all heteropterans studied to date, Coreidae are cytogenetically characterized by having holocentric chromosomes (no localized centromere), with meiotic cells without a kinetochoric structure (Buck 1967, Comings and Okada 1972, Motzko and Ruthmann 1984, Rufas and Giménez-Martin 1986, Wolf 1996). The kinetic activity is restricted to the chromosome ends, *i.e.* the telomeric regions (hence the term telokinetic chromosome, Motzko and Ruthmann 1984, Schrader 1935, 1940, Hughes-Schrader and Schrader 1961, González-García *et al.* 1996). Meiotic division is pre-reductional, *i.e.* bivalent autosomes segregate reductionally at anaphase I and equationally at anaphase II, while the sex chromosomes divide equationally and reductionally at first and second meiotic division, respectively (Ueshima 1979).

In Coreidae, the most common sex chromosome determining system is X0/XX (male/female), though $X_1X_2/X_1X_1X_2X_2$ (male/female) is also observed (Parshad 1957, Yoshida 1946, 1947, 1950). Another characteristic of this family is the presence of m-chromosomes whose meiotic behavior

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differs from the other autosomes in that they are achiasmatic and associate in a pseudobivalent at metaphase I. The m-chromosomes also undergo pre-reductional division. In the Coreidae species already described, the diploid chromosome number ranges from 13 ($10A+2m+X0$) to 28 ($24A+2m+X_1X_20$) (Ueshima 1979, Sands 1982, Manna 1984, Colombo and Bidau 1985, Dey and Wangdi 1988, Satapathy and Patnaik 1989, Cattani and Papeshi 2004).

Especially due to the diverse diploid chromosome number and different sex chromosome determining systems observed, the purpose of this study was to provide further cytogenetical information about the testicular cells of adult males of economically important Brazilian species belonging to the Coreidae family.

Materials and methods

This study included 10 adult males per each of the following species: *Anasa bellator*, *Athau-mastus haematicus*, *Chariesterus armatus*, *Dallacoris obscura*, *Dallacoris pictus*, *Leptoglossus gonagra*, *Leptoglossus zonatus*, *Sphictyrtus fasciatus*, and *Zicca annulata*, which were collected in the region of São José do Rio Preto, SP, Brazil, from the plants *Lycopersicone sculentum* L. (tomato), *Glycine max* (soybean), *Punica granatum* (pomegranate), *Malpighia glabra* (acerola), *Gossypium hirsutum* (cotton), *Luffa cylindrica* (loofah sponge), *Muntinginia calabura* L. (calabura), *Sechium edule* (chayote), *Helianthus annus* (sunflower), *Zea mays* (corn), *Eugenia uniflora* L. (surinan cherry). All specimens were cytogenetically analyzed in slides prepared by having seminiferous tubules squashed and stained with lacto-acetic orcein. Images were obtained under a Zeiss microscope using the image analyzer software AXIO VISION at the Morphology Laboratory, IBILCE/UNESP, São José do Rio Preto, SP.

Results

The cytogenetical analysis of spermiogenesis in the 9 Coreidae species studied revealed that some characteristics are shared by all the species, while others are shared by only some of the species, and others are species-specific. All species exhibit 7 testicular lobules covered by a reddish membrane, except *Zicca annulata* and *Chariesterus armatus*. These latter 2 species have 4 lobules, and the covering membrane is yellow in *Z. annulata* and translucent in *C. armatus*.

Fig. 1 shows the polyploid nuclei of the nutritive cells in all species studied. These nuclei are large and may be found at either interphase (*L. gonagra*, *L. zonatus*, *Z. annulata*, *C. armatus*, *S. fasciatus*, *A. bellator*) or division stage (*D. obscura* and *D. pictus*). Nuclear heteropycnotic regions are observed in *L. gonagra*, *L. zonatus*, *Z. annulata*, *C. armatus*. Polyploid nuclei at both interphase and division stages are seen exclusively in *A. bellator* and *A. haematicus* (Fig. 1a–d).

The diploid chromosome number ranges from $2n=19$ to $2n=25$. It is $19=16A+2m+X0$ in *S. fasciatus*; $21=18A+2m+X0$ in *A. bellator*, *A. haematicus*, *D. obscura*, *D. pictus*, *L. gonagra*, and *L. zonatus*; $23=20A+2m+X0$ in *Z. annulata*; and $25=22A+2m+X0$ in *C. armatus*. Thus, all species exhibit m-chromosomes and a X0 sex determining system for males.

In all species, spermatogenesis is cystic (Fig. 1e, f). At early prophase I (leptotene), there is always a very distinguishable heteropycnotic round-shaped body located at the periphery of the nucleus, which is likely to be the sex chromosome. In all species, this body remains visible throughout prophase I and disappears at diakinesis (Fig. 2a–h). In contrast, *A. bellator* displays a larger body and a variable number of smaller ones but they stain lighter (Fig. 2c).

At diakinesis I, all species exhibit m-chromosomes, which are negatively heteropycnotic and are generally far from each other (Fig. 2h). Usually, all species exhibit a single chiasma that may be terminally or interstitially located. This chiasma occurs in the majority of autosomes (Fig. 2h–j), but is not seen in the m-chromosomes or in the sex chromosomes. In *S. fasciatus* 2 chiasmata are

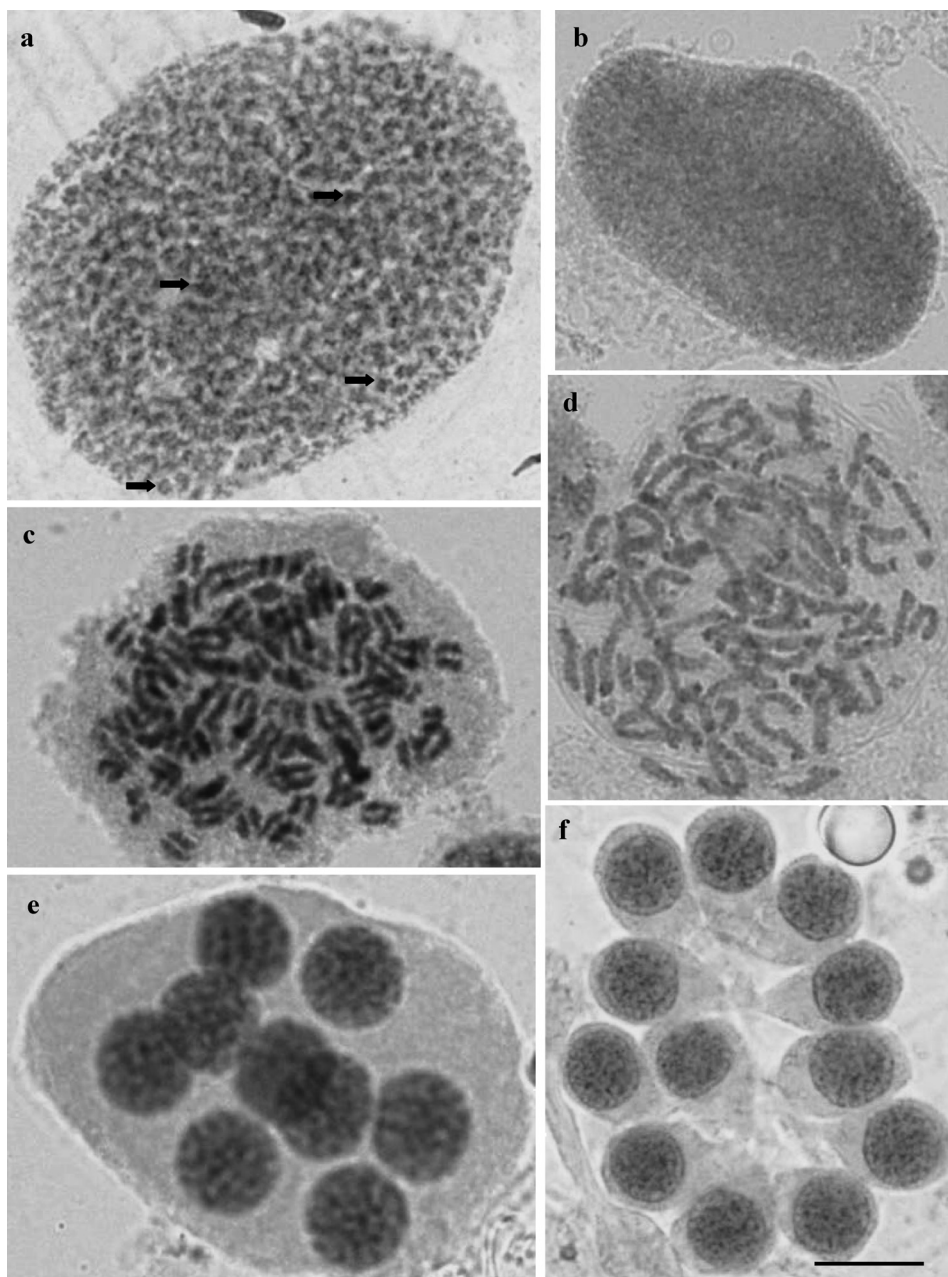


Fig. 1. Seminiferous tubules cells stained with lacto-acetic orcein. (a, b) interphasic polyploid nucleus in *Leptoglossus zonatus* (a) and in *Anasa bellator* (b) Heteropycnotic regions characteristic of the species *L. zonatus* (arrow); (c, d) polyploid nuclei division in *Athaumastus haematicus* (c) and in *A. bellator* (d); (e, f) spermatogonial cyst in *A. haematicus* (e) in *L. zonatus* (f). Bar=10 μ m (same amplification in all photos and figures).

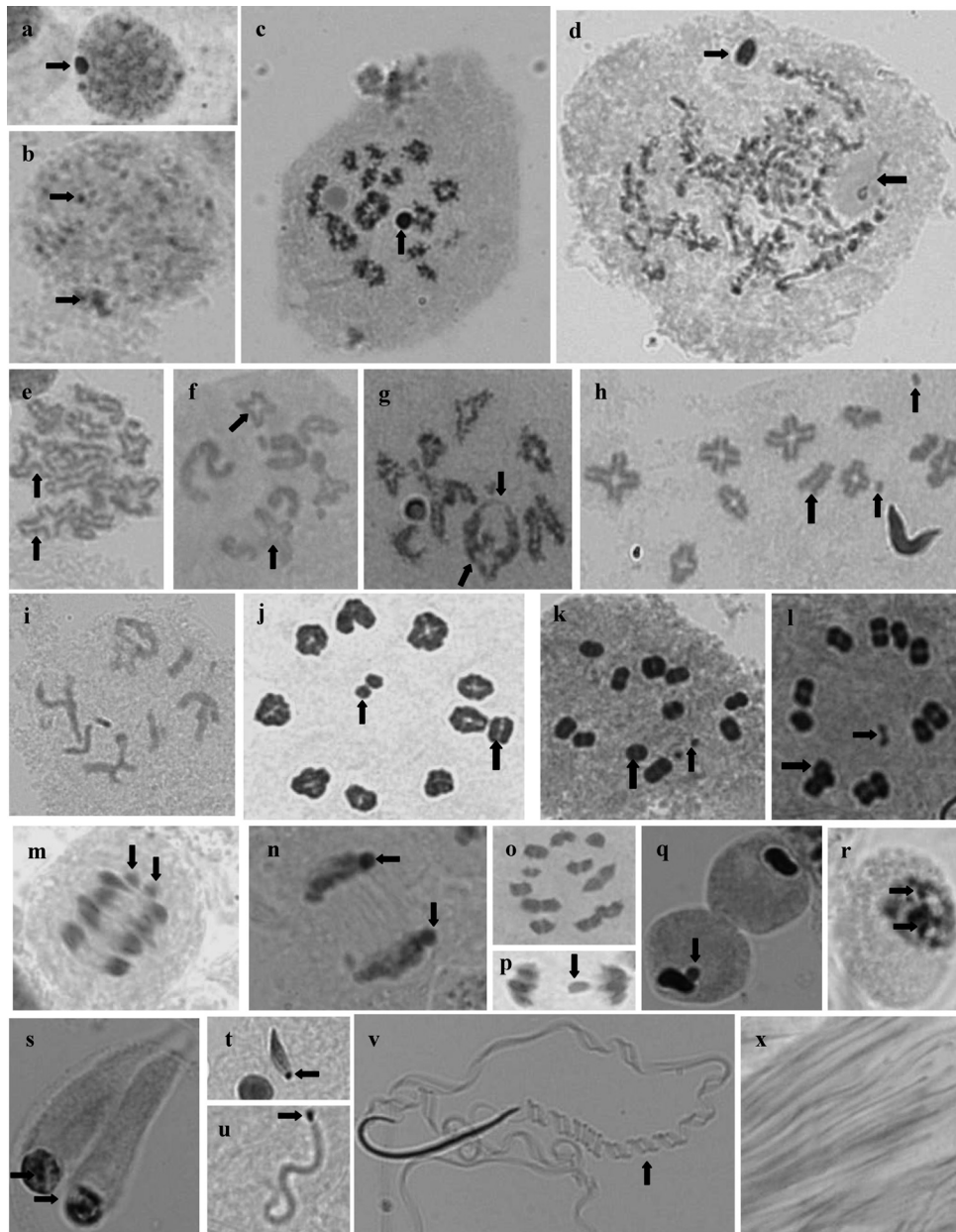


Fig. 2. Seminiferous tubules cells stained with lacto-acetic orcein. (a-j) prophase I: (a) (leptotene/zygotene) with a heteropycnotic body (arrow) in *Leptoglossus zonatus*; (b) a large body and smaller ones in *Anasa bellator* (arrows); (c) paquitene (the arrow points to a heteropycnotic body in *Zicca annulata*); (d) paquitene in *Dallacorisc obscura*: evident nucleolus (large arrow) and heteropycnotic body (small arrow); (e, f) dyplotene/diakinesis. Notice interstitial chiasmata (arrows) in *A. bellator* (e) and *L. gonagra* (f); (g) 2 chiasmata in *Spichtyrtus fasciatus* (terminal and interstitial, arrows); (h) *A. bellator*. Notice the m-chromosomes (small arrow), the sex chromosome (large arrow) and chiasmata; (i) chiasmata in *A. haematicus*; (j) nuclei at late diakinesis/early metaphase in *D. obscura* with m-chromosomes at the center of the ring (polar view, small arrow) and the sex chromosome lying outside it (large arrow); (k, l) metaphase I in *Z. annulata* (k) in *Chariesterus armatus* (l) m-chromosomes (small arrow) and sex chromosome (large arrow); (m) anaphase I in *L. zonatus* with late migration of sex chromosomes (arrows); (n) telophase I in *C. armatus* with heteropycnotic sex chromosomes (arrows); (o) metaphase II in *L. zonatus*; (p) anaphase II in *L. zonatus*; (q) telophase II in *C. armatus* (in p and q the arrow points to late migration of X); (r, s) spermatids with round nuclei in *D. pictus* (r) and in *C. armatus* (s) heteropycnotic regions in both (arrows); (t, u) elongate spermatid in *A. haematicus* with heteropycnotic material (arrow); (v) spermatid with spiral tail in *C. armatus*; (x) spermatozoon in *L. gonagra*. Bar=10 μ m.

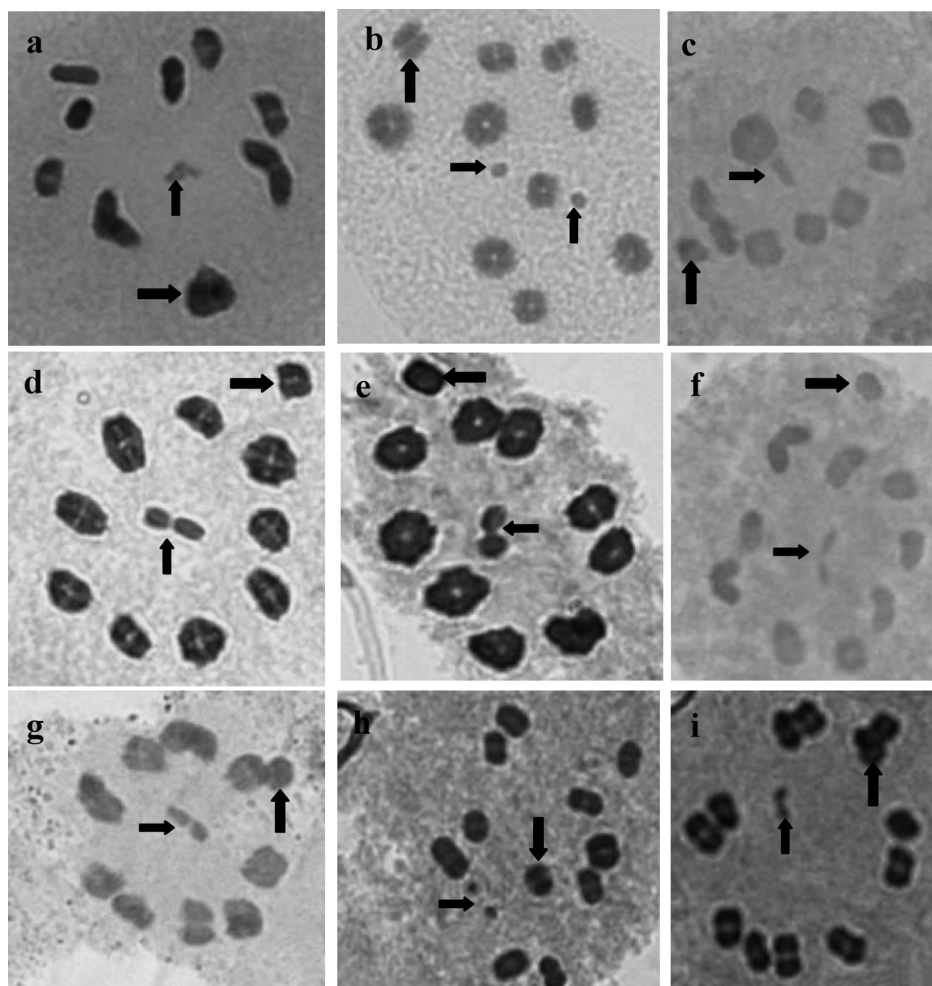


Fig. 3. Polar view of male seminiferous tubules cells stained with lacto-acetic orcein at late prophase I (diakinesis) and early metaphase I, to note each one's karyotype. (a) *Sphictyrtus fasciatus* with 19 chromosomes ($16A+2m+XO$), (b–g) in sequence, *Anasa bellator*, *Athaumastus haematicus*, *Dallacoris obscura*, *Dallacoris pictus*, *Leptoglossus gonagra* and *Leptoglossus zonatus* with 21 chromosomes ($18A+2m+XO$); (h) *Zicca annulata* with 23 chromosomes ($20A+2m+XO$) and (i) *Chariesterus armatus* with 25 chromosomes ($22A+2m+XO$). Bar=10 μ m.

observed, one at the terminal region and the other at the interstitial region of the same autosome (Fig. 2g). In all species, but *A. haematicus*, chiasmata are cross-shaped (Fig. 2i).

The polar view of metaphase I shows that autosomes arrange in a ring with the m-chromosomes at its center forming a pseudobivalent, while the sex chromosome displays sister-chromatids that are either paired or form a pseudobivalent (Fig. 2j–l). At metaphase II, the autosomes show a telomeric association and form a pseudobivalent, in all species (Fig. 2o). At anaphase I and II the late migration of the sex chromosomes can be observed (Fig. 2m, p). This process is confirmed at telophase I and II (Fig. 2n, q).

At the onset of spermiogenesis, spermatids are round and contain heteropycnotic material apparently associated with the nuclear envelope (Fig. 2r, s). As the nucleus elongates, heteropycnotic regions can be seen near the anterior region of the head (Fig. 2t, u). Exclusively in *C. armatus*, a

spiral tail is observed during spermatogenesis (Fig. 2v). In all species, spermatozoa have a small head and a long tail (Fig. 2x).

To favor the visualization of chromosome number in all species, Fig. 3 shows cells at late diakinesis and early metaphase I.

Discussion

All Coreidae studied to date are characterized by holocentric chromosomes with kinetic activity in the telomeric region; a sex determining system of the X0 type; a pair of achiasmatic m-chromosomes that associate in a pseudobivalent at the center of the metaphase I plate and undergo pre-reductional division; and, in general, an interstitial autosomal chiasma (Bressa *et al.* 2001, Ueshima 1979, Manna 1984, Papeschi and Mola 1990, Wilson 1905). These observations were confirmed in the 9 Brazilian species studied herein.

Furthermore, all the species analyzed in this work exhibit large polyploid nuclei (undergoing division or not), that carry heteropycnotic regions of variable size and number. The chiasmata observed are either interstitially or terminally located in most autosomes. The chromosomes at diakinesis have a well defined morphology and chiasmata that can be clearly visualized (except in *Athaumastus haematicus*). The membrane covering the testis is red in *Leptoglossus gonagra*, *Leptoglossus zonatus*, *Sphictyrtus fasciatus*, *Anasa bellator*, *Dallacoris obscura*, *Athaumastus haematicus* and *Dallacoris pictus*; yellow in *Zicca annulata*; and translucent in *Chariesterus armatus*. The meiotic behavior of *A. haematicus* males has been previously described by Bressa *et al.* (2005). In our study, a similar meiotic behavior and the same chromosome complement were observed. Nonetheless, our overall findings include other characteristics which were not reported by those authors.

In the species studied herein, the sex chromosome usually lies outside the autosomal ring at metaphase I, found in polar view. At anaphase II, the sex chromosomes undergo reductional division and thus form 2 types of cells, one containing the sex chromosome and the other does not. At this phase, the X chromosome decondenses asynchronously with the autosomes, which decondense later. In addition, the migration of the X chromosome also occurs at a later time.

In most Coreidae species described to date, the diploid chromosome number is 21 (18A+2m+XO) (approximately 48% of the 100 species analyzed) (Ueshima 1979, Sands 1982, Manna 1984, Colombo and Bidau 1985, Dey and Wangdi 1988, Satapathy and Patnaik 1989, Cattani and Papeschi 2004). Of the nine Brazilian species studied here, 6 (67%) exhibit this diploid chromosome number (*A. bellator*, *A. haematicus*, *D. obscura*, *D. pictus*, *L. gonagra*, and *L. zonatus*). The remaining species display 19, 23 and 25 chromosomes (*S. fasciatus*, *Z. annulata*, and *C. armatus*, respectively).

The literature suggests that the chromosome complements constituted by higher numbers of chromosomes are originated by the duplication or fragmentation of an autosomal pair (Ueshima and Ashlock 1980). According to Jacobs (2004), as all Heteroptera possess holocentric chromosomes, fragmentation is the process more likely to occur because it originates fragments that regularly move to the poles at anaphase and survive for many cell generations. Likewise, chromosome fusion is also theoretically easier in organisms with holocentric chromosomes.

Based on these grounds, it may be assumed that the species *Z. annulata* (23 chromosomes) and *C. armatus* (25 chromosomes) have both originated from an ancestor species possessing 21 chromosomes by autosomal fragmentation and chromosome fusion, respectively. Further studies are needed to confirm this hypothesis.

Acknowledgements

Special thanks to Dr. Sonia Maria Oliani of the Department of Biology-IBILCE/UNESP, for allowing us to use the cell image capturing equipment of her laboratory, and to FUNDUNESP and CNPq for their financial support.

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