New contributions to the cytotaxonomy of tiger beetles (Coleoptera, Cicindelidae) from the Afrotropical Region: cytogenetic characterization of *Prothyma concinna, Elliptica lugubris* and *Ropaloteres cinctus*

Proença Sónia Jesus Ramalho, Maria João Collares-Pereira* and Artur Raposo Moniz Serrano

Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, Campo Grande, Edifício C2 - 3° Piso, 1749-016 Lisboa, Portugal. Tel: 00 351 217500000 - Ext. 24309; fax number: 00 351 217500028; e-mail: soniajr@um.es, mjpereira@fc.ul.pt, aserrano@fc.ul.pt.

Abstract — Metaphases from germinal tissue of three Afrotropical species of tiger beetles occurring in Guinea-Bissau were analysed using conventional Giemsa-staining. The species showed distinct karyotypes and multiple and morphologically diverse sex chromosome systems: $2n = 20 + X_1X_2X_3X_4Y$ for *Prothyma concinna*, $2n = 18 + X_1X_2X_3Y/X_1X_1X_2X_2X_3X_3$ for *Elliptica lugubris* and $2n = 22 + X_1X_2X_3X_4Y$ for *Ropaloteres cinctus*. The existence of a higher number of autosome pairs in *P. concinna* and *R. cinctus* (10 and 11 respectively) when compared with the stability of 9 autosome pairs in most species of genus *Cicindela* (*s.l.*) supports the hypothesis of a gradual reduction in the number of autosomes in the karyotype evolution of cicindelids. This reduction might have been achieved by centric fusions considering the almost general mediocentric morphology of autosomes and the existence of acrocentric chromosomes in some basal species of Cicindelini. The existence of one chromosome in *P. concinna* assigned to the sex system with strong blocks of heterochromatin in the short arm and in the distal region of the long arm could favour such an assumption and may question the hypothesis of a complete independent evolution for autosomes and heterosomes.

Key words: Afrotropical Region, Cicindelidae, Cytogenetics, Guinea-Bissau, Multiple sex chromosome systems.

INTRODUCTION

Cytogenetic studies in tiger beetles have shown the presence of an unique and diverse multiple sex chromosome system, XnY, where n varies from two to four, that forms a non-chiasmatic multivalent during meiosis linked by telomeric connections (for revision see Galián *et al.* 1990; Serrano and Galián 1998; Proença *et al.* 1999b).

In the last few years, the number of tiger beetles karyotyped has increased and, placing this karyotypic information in a phylogenetic context, a hypothetical pattern of chromosome evolution within this family can be defined (Vogler and Pearson 1996; Galián and Hudson 1999; Proença *et al.* 1999a, b; 2002a, b; Galián *et al.* 2002). Multiple sex chromosomes are found in representatives of the closely related tribes Collyrini and Cicindelini but not in the most basal ones, such as Manticorini, Omini and Megacephalini, described with simple sex chromosome systems of the X0 or XY type. This suggests an old and single origin for the multiple heterosomes in a common ancestor of Collyrini and Cicindelini before the splitting of these two tribes (Galian and Hudson 1999; Galián *et al.* 2002). Some exceptions to this pattern have been reported with the description of single sex systems in some species of Cicindelini: *C. germanica* (XY) (Giers 1977), *C. paludosa* (X0) (Serrano *et al.* 1986), *Odontocheila confusa* (XY), *O. nodicornis* (X0) (Proença *et al.* 2002b) and *Pentacomia* sp. (X0) (Galián *et al.* 2002) but they were considered to represent independent secondary losses of the multiple systems. The number of autosome pairs seems to gradually decrease from the most basal taxa (Manticorini and Omini) to the most derived ones, with an apparently stability at 9 pairs of autosomes in

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* Corresponding author.

the large genus *Cicindela* (*s.l.*) (SERRANO and GALIÁN 1998; PROENÇA *et al.* 1999b).

Although the mechanisms involved in the chromosome evolution of cicindelids and in the development of the multiple sex systems remain an open question, Robertsonian fusions have been proposed to explain major autosome reduction in some species (PROENÇA *et al.* 2002a).

Concerning multiple sex chromosomes, WHITE (1973) proposed that in most groups these systems can be derived from simple XY by fission of the X chromosomes, possibly after other chromosomal rearrangements have occurred, such as fusions and translocations between autosomes and heterosomes or between the heterosomes. However, in tiger beetles there are some evidences against interactions between autosomes and heterosomes for the origin of such multiple systems and several authors have suggested an independent evolution for autosomes and heterosomes (SERRANO and COLLARES-PEREIRA 1989; GALIÁN *et al.* 1990; GALIÁN and VOGLER 2003). These evidences concern the inability to produce chiasmata during meiosis, which may constrain translocations between the chromosomes and the fact that in some *Cicindela* species the autosome number remains unchanged while the number of heterosomes varies. This means that chromosomal rearrangements between autosomes and heterosomes for the production of multiple sex chromosomes, as proposed in other groups (WHITE 1973), are unlikely to be the mechanism involved in the evolution of the multiple sex chromosome systems in tiger beetles. Moreover, asymmetric heterosomes in *Cicindela* species as well as smaller X chromosomes when their number is high were not observed so that simple fissions and fusions are unlikely to be responsible for the changes in X-chromosome numbers in tiger beetles. Therefore other mechanisms might have been involved that balance the resulting asymmetry and size differences such as pericentric inversions and increase in the total amount of DNA as proposed by GALIÁN and VOGLER (2003).

Even so, the number of tiger beetles species karyotyped remains scarce and the majority of the analyses concerned almost exclusively representatives of the most derived genus *Cicindela* (*s.l.*). Also, there are some biogeographic regions still with very few available data, like the Australian (GALIAN and HUDSON 1999) and the Afrotropical (PROENÇA *et al.* 1999b) ones.

The aim of this work was to karyotype three new Afrotropical cicindelids, *Prothyma concinna* (Dejean, 1831), *Elliptica lugubris* (Dejean, 1825) and *Ropaloteres cinctus* (Olivier, 1790), in order to provide additional information for the study of chromosomal evolution within the family.

MATERIAL AND METHODS

Adult tiger beetles were collected in natural populations at Guinea-Bissau in the localities listed in table 1. They were identified by one of the authors (A. R. M. Serrano) and are deposited at the Department of Animal Biology of the Faculty of Sciences, University of Lisbon.

Gonads were extracted from anaesthetised beetles, subjected to a hypotonic treatment with distilled water and fixed using fresh ethanol-acetic acid solution (3:1), with several changes of the fixative solution. Small portions of the gonads were squashed in 70% acetic acid, air dried and stained in a phosphate buffered 4% Giemsa (pH = 6.8).

RESULTS

A diploid value of 25 was observed in all spermatogonial mitotic metaphases of the two males of *Prothyma concinna*, with 10 homomorphic pairs of metacentric and submetacentric chromosomes and five quite distinct unpaired chromosomes (Fig. 1a). The biggest unpaired chromosome had differences in chromatin distribution with strong blocks of heterochromatin in the short arm and in the distal region of the long arm with a large "gap" between. The remaining chromosomes were smaller with two metacentric, one submetacentric and one acrocentric chromosome.

Table 1 — Localities, number of individuals (in parenthesis the number of mitotic metaphases analysed), diploid number and meioformula of the studied species.

Species	No. of individuals	2n	Meioformula	Localities
Prothyma concinna	2 males (22)	25	$10 + X_1X_2X_3X_4Y$	Bissorã (Guinea-Bissau)
Elliptica lugubris	1 male (11)	22	$9 + X_1X_2X_3Y$	Bissorã (Guinea-Bissau)
	2 females (8)	24	$9 + X_1X_1X_2X_2X_3X_3$	
Ropaloteres cinctus	4 males (14)	27	$11 + X_1X_2X_3X_4Y$	Varela (Guinea-Bissau)

Spermatocyte metaphase I cells were not available but metaphases II spreads were of two types with 11 (Fig. 1e) and 14 (Fig. 1f) chromosomes, respectively. Considering these observations the karyotype of this species is likely $2n = 20 + X_1X_2X_3X_4Y$. The Y chromosome could not be identified among the heterosomes but by analysing the morphology of the chromosomes in metaphase II figures one may identify the biggest unpaired chromosome as a X chromosome (Fig. 1f, arrow).

Spermatogonial metaphases of *Elliptica lugubris* had 22 chromosomes and the oogonial metaphases 24 chromosomes. The male karyogram presented nine homomorphic pairs and four unpaired chromosomes (Fig. 1b) and the female karyogram showed 12 pairs (Fig. 1c), thus making a $X_1X_2X_3Y/X_1X_1X_2X_2X_3X_3$ sex determination system. The chromosomes were meta- and submetacentrics and gradually decreased in size. The heterosomes were also meta- and submetacentrics with the Y being the biggest in size. Spermatocyte metaphase I cells had nine bivalents plus a sex complex composed of four chromosomes with slight differences in size (Fig. 1g). Spermatocyte metaphases II cells were not observed.

The diploid number of *Ropaloteres cinctus* based on spermatogonial metaphases was 27 in all the four males. The male karyogram exhibited 11 homomorphic pairs and five unpaired chromosomes (Fig. 1d). The latter, according to meiotic observations were assigned to sex chromosomes, thus suggesting a $X_1X_2X_3X_4Y/X_1X_1X_2X_2X_3X_3X_4X_4$ sex determination system. The first, second, sixth and seventh pairs were acrocentric and the remaining meta- and submetacentrics. One of the heterosomes was the biggest chromosome of the complement. Spermatocyte metaphase I cells showed 11 bivalents and a sex vesicle with five elements (Fig. 1h). Metaphase II figures were not observed.

DISCUSSION

Our findings are generally consistent with data from the literature. All three species of Cicindelini here studied revealed the presence of multiple sex chromosome systems, 3Xs in *E. lugubris*, 4Xs in *R. cinctus* and possibly also 4Xs in *P. concinna*, reinforcing their establishment as a characteristic of the large clade that includes the Collyrinae and Cicindelini. The few exceptions to the description of multiple heterosomes in Cicindelini are the simple sex chromosomes found in species of *Odontocheila* and *Pentacomia* (GALIÁN et al. 2002; PROENÇA et al. 2002b) and *Cicindela* (GIERS 1977; SERRANO et al. 1986) "genera". The two taxa *Odontocheila* and *Pentacomia* grouped together in the recent phylogenetic analyses of Cicindelidae, based on mitochondrial 16S and nuclear 18S rRNA gene sequences (VOGLER and PEARSON 1996; GALIÁN et al. 2002), and it is possible that their karyotypic similarity is due to a common ancestry and, hence, the lost of multiple sex systems may be a trait of a larger *Pentacomia-Odontocheila* clade. Also, and according to molecular analysis, the two *Cicindela* species with simple sex chromosome systems are closely related species and such a pattern could represent a single evolutionary event that reversed the ancestral multiple system (PEARSON and VOGLER 2001).

Within tribe Cicindelini, the majority of species studied so far belong to the genus *Cicindela* (*s.l.*) with very few reports on other genera (PROENÇA et al. 1999a, b; GALIÁN et al, 2002; PROENÇA et al. 2002b). Nevertheless, the data presented here along with other available data have shown that genera such as *Prothyma*, *Euryarthron* and *Therates* have slightly higher numbers of autosome pairs (10 and 12 pairs) than most species of the derived *Cicindela* (PROENÇA et al. 1999b; GALIÁN et al. 2002), supporting a gradual reduction in autosome numbers from basal groups to derived ones.

Another interesting feature in the karyotypes of these Cicindelini genera is the existence of a great variability of autosome numbers and X chromosomes, also within species of the same genus, in spite of the scarcity of data. Thus, comparing the three species of *Prothyma* karyotyped so far, the three types of multiple sex chromosomes (2Xs, 3Xs and 4Xs) and 10 and 12 pairs of chromosomes (*P. concinna*, $n = 10 + XXXXY$; *P. leprieurii*, $n = 12 + XXY$, PROENÇA 1999b; *Prothyma* sp., $n = 10 + XXXY$, GALIÁN et al. 2002) were found. Likewise, in *Ropaloteres*, the two species analysed have the same multiple sex chromosome system with 4Xs but different number of autosomal pairs (*R. cinctus*, $n = 11 + XXXXY$; *R. feisthameli*, $n = 10 + XXXXY$, PROENÇA et al. 1999b). This variability in autosome number and also in heterosome number in such genera may suggest the occurrence of extensive karyotypic reorganizations leading to a more stable karyotype in the genus *Cicindela* (*s.l.*) at least at the autosomal level. However, autosome numbers in *Cicindela* are not entirely fixed as some Oriental species have 10 pairs (SERRANO and GALIÁN 1998) and some Australian ones have 11 pairs (GALIÁN
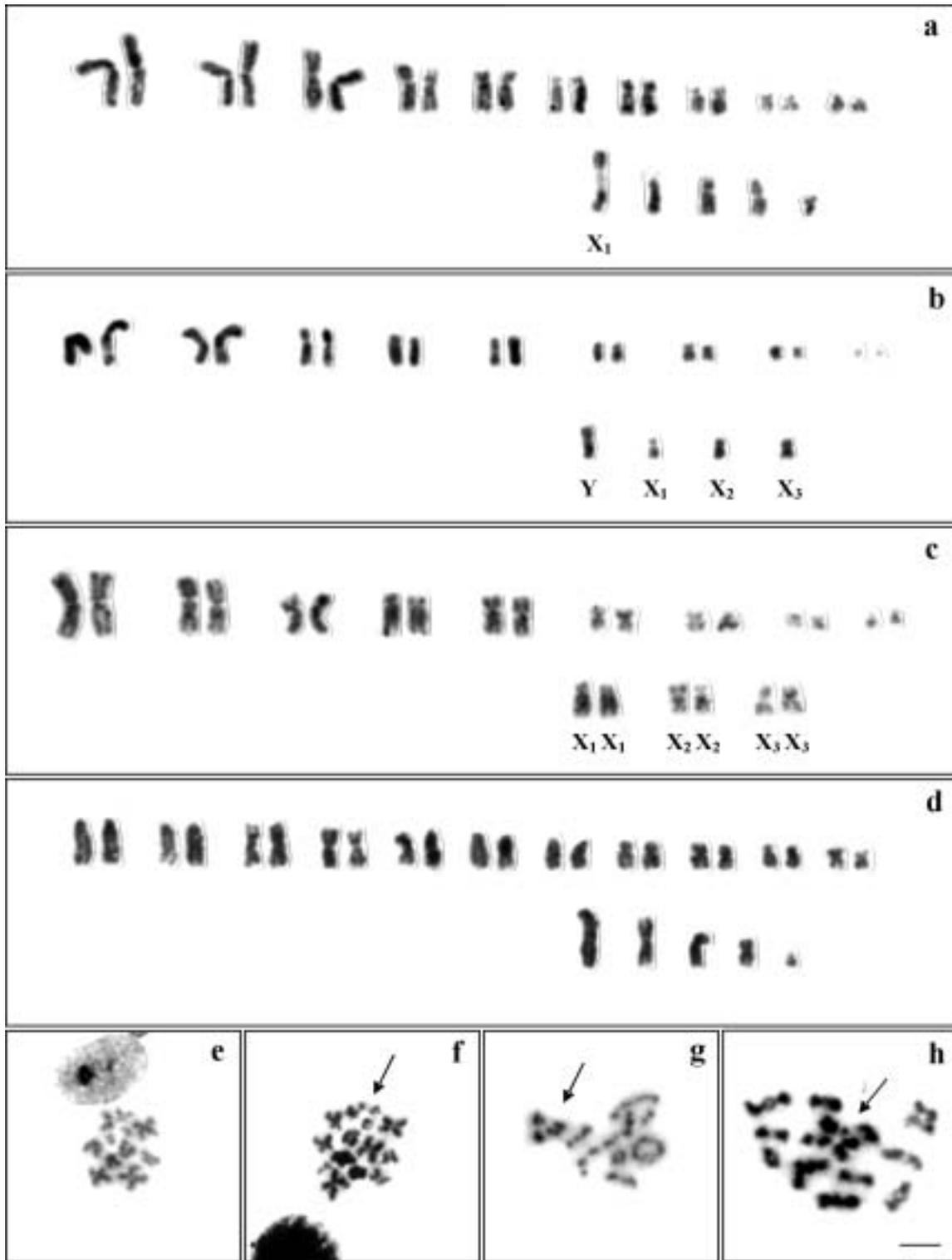
Fig. 1 — Mitotic and meiotic chromosomes of the species of tiger beetles studied. (**a**) Male karyogram of *Prothyma concinna*, $2n = 20 + X_1X_2X_3X_4Y$; (**b**) Male karyogram of *Elliptica lugubris*; $2n = 18 + X_1X_2X_3Y$; (**c**) Female karyogram of *Elliptica lugubris*; $2n = 18 + X_1X_1X_2X_2X_3X_3$; (**d**) Male karyogram of *Ropaloteres cinctus*, $2n = 22 + X_1X_2X_3X_4Y$; (**e**) *Prothyma concinna*, male metaphase II, $n = 10 + Y$, (**f**) *Prothyma concinna*, male metaphase II, $n = 10 + X_1X_2X_3X_4$ (arrow indicates the biggest X chromosome); (**g**) *Elliptica lugubris*, male metaphase I, $n = 9 + X_1X_2X_3Y$ and (**h**) *Ropaloteres cinctus*, male metaphase I, $n = 11 + X_1X_2X_3X_4Y$ (arrows indicate the multiple sex chromosomes). Bar = 4 μm.

and HUDSON 1999), but these changes seem to be rare and restricted to particular species groups.

Metacentric chromosomes appear to be more common in beetles than telo- and acrocentric morphologies. In most species of *Cicindela* (*s.l.*), the mediocentric morphology of the chromosomes seems to prevail with meta- and submetacentrics, whereas acro- and subtelocentrics appear almost exclusively in more basal species such as in the primitive Manticorini, Omini and Megacephalini (GALIAN *et al.* 2002). Regarding our results, *P. concinna* and *E. lugubris* both have chromosomes with mediocentric morphology with meta- and submetacentrics, whereas *R. cinctus* has four pairs of clear acrocentric chromosomes. Acrocentrics have also been reported in other Afrotropical Cicindelini species such as *P. leprieuri* and *Hipparidium interruptum* (PROENÇA *et al.* 1999b). The existence of acrocentric chromosomes in these Cicindelini genera with more than 9 autosome pairs might suggest that processes of Robertsonian fusions between autosomes occurred in the progressive reduction of autosome pairs in the chromosome evolution of tiger beetles. The presence of one conspicuous unpaired chromosome in *P. concinna*, identified as a X chromosome, with a large "gap" of heterochromatin in the proximal region of the long arm could also be considered a further evidence of the involvement of fusion processes, although of different kind. Considering the evolutionary direction of chromosome changes in tiger beetles, one can suggest that some fusion processes, either only between autosome pairs or between heterosome and autosome pairs occurred, leading to the production of one unpaired chromosome or in the second case one unpaired autosome and one X-autosome. The segregation patterns of heterozygous rearrangements at metaphase and anaphase I in meiosis would determine whether such mutations will be fixed in the population as a balanced or transient polymorphism, or as a negatively heterotic change related to cladogenesis. Also, heterozygosity may result in the production of multivalents at meiosis by the association of unpaired chromosomes such as the sex chromosomes (KING 1993). In this case, the unpaired chromosome or chromosomes could easily be incorporated in meiosis as sex chromosomes, more easily if the autosomes involved in the fusion contained genes responsible for sex determination. This evolutionary mechanism for the development of such unique multiple chromosome system disagree with the most accepted hypothesis of an independent evolution of autosomes and het-

erosomes in cicindelids mainly based on *Cicindela* autosome number stability. Another possible and perhaps more plausible explanation for the origin of the fourth X chromosome would be to invoke multiple processes of fissions and fusions between the heterosomes. Thus, if we compare the karyotypes of *P. concinna* and *Prothyma* sp. (GALIAN *et al.* 2002), both species have 10 autosome pairs, similar in size and shape, with three large, five medium-sized and two small pairs. However, while *P. concinna* has 5 heterosomes, one large, three medium and one small, *Prothyma* sp. lacks the smallest heterosome. Also, the biggest heterosome observed in *P. concinna* with the large heterochromatic blocks, identified as a X chromosome, resembles the smallest X chromosome in *Prothyma* sp. which appear to have also a "gap" of heterochromatin in the pericentromeric region. Though, the analysis of meiosis I of *P. concinna* would be of extreme importance to identify the Y chromosome and the behaviour of the unpaired chromosomes at meiosis.

The unsolved questions about the evolution of multiple systems in Cicindelidae would certainly benefit from new data on autosome-heterosome relationships in the still undescribed karyotypes of the most basal groups. Moreover, only the use of chromosome microdissection tools and specific molecular chromosome markers will allow comparative genome analyses in order to establish karyotype evolutionary trends within the family.

REFERENCES

GALIÁN J., HOGAN J.E. and VOGLER A.P., 2002 — *The origin of multiple sex chromosomes in tiger beetles.* Molecular Biology and Evolution, 19: 1792-1796.

GALIÁN J. and HUDSON P., 1999 — *Cytogenetic analysis of Australian tiger beetles (Coleoptera: Cicindelidae): chromosome number, sex-determining system and localisation of rDNA genes.* Journal of Zoological Systematics and Evolutionary Research, 37: 1-6.

GALIÁN J., ORTIZ A.S. and SERRANO J., 1990 — *Karyotypes of nine species of Cicindelini and cytotaxonomic notes on Cicindelinae (Coleoptera, Carabidae).* Genetica, 82: 17-24.

GALIÁN J. and VOGLER A.P., 2003 — *Evolutionary dynamics of a satellite DNA in the tiger beetle species pair Cicindela campestris and C. maroccana.* Genome, 46: 213-223.

GIERS E., 1977 — *Die Nicht-Homologen-Assoziation multipler Geschlechtschromo-somen in der Spermatogenese von Cicindela hybrida (Coleoptera)*. Ph.D. Thesis, University of Münster.

KING M., 1993 — *Species evolution. The role of chromosome change*. Cambridge University Press.

PEARSON D.L. and VOGLER A.P., 2001 — *Tiger Beetles. The evolution, Ecology and Diversity of the Cicindelids*. Cornell University Press, Ithaca, NY.

PROENÇA S.J.R., COLLARES-PEREIRA M.J. and SERRANO A.R.M., 1999a — *Karyological study of Cicindelidia trifasciata (Coleoptera, Cicindelidae) from Cuba: Evidence of B chromosomes*. Genetics and Molecular Biology, 22: 45-48.

PROENÇA S.J.R., SERRANO A.R.M. and COLLARES-PEREIRA M.J., 1999b — *First record on the cytotaxonomy of cicindelids (Insecta, Coleoptera) from an Afrotropical region*. Caryologia, 52: 37-47.

PROENÇA S.J.R., SERRANO A.R.M. and COLLARES-PEREIRA M.J., 2002a — *An unusual karyotype with low chromosome number in Megacephalini, a basal group of tiger beetles (Coleoptera, Cicindelidae): cytogenetic characterisation by C-banding and location of rDNA genes*. Hereditas, 137: 202-207.

PROENÇA S.J.R., SERRANO A.R.M. and COLLARES-PEREIRA M.J., 2002b — *Cytogenetic variability in genus Odontocheila (Coleoptera, Cicindelidae): karyotypes, C-banding, NORs and localisation of ribosomal genes of O. confusa and O. nodicornis*. Genetica, 114: 237-245.

SERRANO A.R.M. and COLLARES-PEREIRA M.J., 1989 — *Cytotaxonomic study of Cephalota hispanica (Gory, 1833) and Spiralia maura (Linnaeus, 1758), two cicindelids from Portugal (Coleoptera)*. Genetica, 79: 69-75.

SERRANO J. and GALIÁN J., 1998 — *A review of karyotypic evolution and phylogeny of carabid beetles (Coleoptera, Carabidae)*. In: G.E. Ball and A. Casale (Eds), "Phylogeny and Classification of Caraboidea". Museo Regionale di Scienze Naturali, p. 191-228, Torino.

SERRANO J., GALIÁN J. and ORTIZ A., 1986 — *Cicindelid beetles without multiple sex chromosomes (Coleoptera, Caraboidea)*. Canadian Journal of Genetics and Cytology, 28: 235-239.

VOGLER A.P. and PEARSON D.L., 1996 — *A molecular phylogeny of the tiger beetles (Cicindelidae): Congruence of mitochondrial and nuclear rDNA data sets*. Molecular Phylogenetics and Evolution, 6: 321-338.

WHITE M.J.D., 1973 — *Animal cytology and evolution*. 3rd ed. Cambridge University Press, Cambridge.