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Cytogenetic Characterization of the Trinidad Endemic, *Arachnocoris trinitatus* Bergroth: the First Data for the Tribe Arachnocorini (Heteroptera: Cimicomorpha: Nabidae)

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As an extension of the ongoing cytogenetic studies of the bug family Nabidae (Heteroptera: Cimicomorpha), the first evidence for the tribe Arachnocorini (the subfamily Nabinae), with reference to the Trinidad endemic, *Arachnocoris trinitatus* Bergroth, is provided. This is an attempt to gain a better insight into the evolution, systematics and within-family relationships of the family Nabidae. The studies were conducted using a number of cytogenetic techniques. The male karyotype (chromosome number and size; sex chromosome system; NOR location; C-heterochromatin amount, distribution and characterization in terms of the presence of AT-rich and GC-rich DNA), and male meiosis with particular emphasis on the behavior of the sex chromosomes in metaphase II are described. Also investigated are the male and female internal reproductive organs with special reference to the number of follicles in a testis and the number of ovarioles in an ovary. *A. trinitatus* was found to display a number of characters differentiating it from all hitherto studied nabid species placed in the tribe Nabini of the subfamily Nabinae, and in the tribe Prostemmatini of the subfamily Prostemmatinae. Among these characters are chromosome number 2n = 12 (10 + XY), the lowest within the family, nucleolus organizer regions (NORs) situated on the autosomes rather than on the sex chromosomes as is the case in other nabid species, and testes composed of 3 follicles but not of 7 as in other nabids. All the data obtained suggest many transformations during the evolution of *A. trinitatus*.

Key words: Heteroptera, Nabidae, Arachnocorini, *Arachnocoris trinitatus*, karyotype, C-heterochromatin, NOR, CMA_3, DAPI, meiosis, internal reproductive organs, evolution, karyosystematics.

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Nabidae are one of the primitive families in the large infraorder Cimicomorpha (Heteroptera). The family shows Holarctic distribution and includes approximately 400 species belonging to 20 genera and 4 subfamilies: the very small subfamilies Velocipedinae and Medocostinae, which are sometimes considered separate families, and the sister subfamilies Nabinae (includes more than 2/3 of the species of the family) and Prostemmatinae. The systematics of Nabinae is not sufficiently advanced. The subfamily is classified into 4 tribes: Arachnocorini, Carthasini, Gorpini and the largest tribe, Nabini. The subfamily Prostemmatinae is divided into the tribes Phorticini and Prostemmatini, each with two genera only. Prostemmatini considered to be more primitive (KERZHNER 1981, 1996). Data on chromosome numbers, sex chromosome systems and patterns of male meiosis (reviewed by KUZNETSOVA *et al.* 2004) are presently available only for the tribes Nabini (25 species karyotyped from the genera *Nabis*, *Lasiomerus*, *Himacerus*, and *Hoplistoscelis*) and Prostemmatini (2 species karyotyped from the genera *Pagasa* and *Prostemma*). The tribes are distinguished

by chromosome numbers and behaviour of the sex chromosomes in male meiosis. Among common characters are holokinetic chromosomes, XY sex chromosome system, the nucleolus organizers (NORs) positioned on the sex chromosomes, and achiasmatic male meiosis with the post-reduction of sex chromosomes (UESHIMA 1979; NOKKALA & NOKKALA 1984; KUZNETSOVA & MARYAŃSKA-NADACHOWSKA 2000; GROZEVA & NOKKALA 2003; KUZNETSOVA *et al*. 2004; GROZEVA *et al*. 2004). In addition, the tribes are characterized by similar structure of male testes and female ovaries, consisting of 7 follicles and 7 ovarioles respectively in all the species studied (CARAYON 1950, 1951; MIYAMOTO 1957, 1959; KERZHNER 1981; KUZNETSOVA *et al.* 2004).

Although the above-mentioned data have allowed a number of preliminary taxonomical conclusions (KUZNETSOVA *et al.* 2004), it is necessary to examine more representatives of Nabidae before an adequate appraisal of the value of the characters studied in the classification and phylogeny of the family can be made.

In the present paper the first data on the tribe Arachnocorini (Nabinae), with reference to the endemic of Trinidad, *Arachnocoris trinitatus* Bergroth, are provided. Arachnocorini occur in the New World and represent a morphologically and biologically highly specialized group, with only two genera. Of these, the genus *Arachnocoris* Scott, 1881 includes about dozen species, all of which inhabit spider's web nests, whereas the taxonomic position of the monotypic genus *Pararachnocoris* Reuter, 1908 is not conclusively established (KERZHNER 1981).

The studies of *A. trinitatus* were conducted using a number of cytogenetic techniques. We have analyzed the male karyotype (with reference to chromosome number and size; sex chromosome system; NOR location; C-heterochromatin amount, distribution, and molecular characterization in terms of the presence of AT-rich and GC-rich DNA) and male meiosis with particular emphasis on the behavior of sex chromosomes in MII. Also the male and female internal reproductive organs have been investigated with special reference to the number of follicles in a testis and the number of ovarioles in an ovary.

This study aims to gain a better insight into the evolution, systematics and within-family relationships of Nabidae.

Material and Methods

Specimens of *A. trinitatus* have been repeatedly collected by J. N. SEWLAL in 2004 and 2005 in Arena Forest Reserve (10^{o}34N 61^{o}14W), Trinidad, W.I. (Table 1). The bugs were found to inhabit the webs of the spider *Mesabolivar aurantiacus* (Mello-Leitao, 1930) (Araneae: Pholcidae), where they are suggested to catch prey and find mates (SEWLAL 2005) The freshly picked specimens were fixed in the field in a 3:1 ethanol-glacial acetic acid solution.

Table 1

Arachnocoris trinitatus: the material examined, with place and date of collection

Collection place	Collection date	Samples examined
Arena Forest Reserve, Trinidad W.I., 10^{o}34'N 61^{o}14'W	08.04.05	5 ♂, 3♀
	19.05.05	2♂
	24.06.04	3♂
	13.07.05	3♂, 3♀
	08.08.05	4♂, 4♀, 2♂ larvae

For examining the internal reproductive organs, the abdomen of a male or a female was open on a slide in a drop of 45% acetic acid. In males, gonads were removed dorsally with fine-tipped needles through an incision between the fourth and ninth abdominal segments and spread on the slide to allow tracing all the parts and calculating number of testicular follicles in every testis. In females, structure of gonads was not studied in detail, except for the number of ovarioles in every ovary.

For cytogenetic studies, testicular follicles were squashed on the slide under the cover slip in a drop of 45% acetic acid. The preparations were examined initially by a phase contrast. The cover slips were removed by a dry ice-technique. The preparations were then dehydrated in freshly prepared 3:1 fixative for 20 min, and air-dried. Several methods were applied to stain the preparations:

(1) S t a n d a r d s t a i n i n g. To study the standard karyotype and course of meiosis a *Feulgen-Giemsa* – method (GROZEVA & NOKKALA 1996) was applied. The preparations were subjected to hydrolysis in 1 N HCl first at room temperature for 20 min and then at 60^{o}C for 8 min, and stained in Schiff's reagent for 20 min. After rinsing thoroughly in distilled water, the slides were additionally stained by 4% Giemsa in Sörensen's buffer pH 6.8 for 20 min. The slides were rinsed briefly in distilled water, air-dried and made permanent with Entellan.

(2) C-b a n d i n g. To detect the amount and distribution of constitutive heterochromatin

(C-bands), a procedure described in an earlier paper of this series (GROZEVA & NOKKALA, 2003) was used.

(3) AgNOR-banding (Silver nitrate staining). For visualization of nucleolar organizer regions (NORs), the 1-step method with colloidal developer (HOWELL & BLACK 1980) was followed.

(4) To reveal the presence of AT-rich and GC-rich DNA of C-heterochromatin, the preparations were treated with fluorochromes CMA_3 and DAPI, which are known to show the affinity for DNA regions enriched in GC and AT respectively (SCHWEIZER 1976; DONLON & MAGENIS 1983 with modifications by KUZNETSOVA *et al*. 2001) as described below. The preparations were immersed into 45% acetic acid for 30 min, rinsed in distilled water and incubated in saturated solution of $Ba(OH)_2$ at room temperature for 6 min. To stop the action of $Ba(OH)_2$, the preparations were dipped in 0.2 N HCl and rinsed in distilled water. The preparations were then sequentially treated in 2xSSC at 60°C for 40 min, rinsed in distilled water, and air-dried. Then they were immersed in Methyl Green solution (28 mg Methyl Green in 75ml McIlvaine buffer, pH 7.0) for 5 min, and rinsed in McIlvaine buffer at pH 7.0. After this, the preparations were stained first with CMA_3 at a final concentration of 5 μg/ml (in 10 mM McIlvaine buffer, pH 7.0; CMA_3 solution contained additionally 2.5 mM $MgCl_2$ in 10 mM McIlvaine buffer) for 25 min, rinsed in the same buffer, and then stained with DAPI at a final concentration of 0.4 μg/ml (in 10 mM McIlvaine buffer, pH 7.0) for 5 min. After staining, the preparations were rinsed in the buffer and mounted in an anti-fade medium (700 μl of glycerol, 300 μl of 10 mM McIlvaine buffer at pH 7.0, and 10 mg of N-propyl gallate).

The measuring of chromosome areas in the well-spread metaphases I (MI) was performed with the aid of a Videotest Image Analyzer (Ista-Videotest, St. Petersburg, Russia: STEIN *et al.* 1998) with a digital camera attached. True and relative areas of the chromosomes (since autosomes are represented by bivalents in MI, the measured area of every bivalent was divided by 2) were calculated. Relative chromosome area is the area of each chromosome expressed as a percentage of the total area of the complement in the nucleus. In the aggregate, 32 MI plates, 27 from one male and 5 from another male, were measured. Likewise, the chromosomes from 7 MI plates of *Nabis* (*Aspilaspis*) *viridulus* Spinola, 1837 were measured for comparison.

The preparations were analysed by means of an Olympus BX 51 light microscope at 1000x, and the photomicrographs taken using Camera Nikon DS-U1. The fluorochrome-labeled preparations were analysed using a fluorescence microscope Dialux 22 at 1000 ×, and the photomicrographs were taken using Camera Nikon DS-U1.

In *A. trinitatus*, a total of 19 males and 10 females, and about 150 nuclei at different stages of male meiosis were examined.

Results

Internal reproductive organs

The internal reproductive organs in males include paired testes arranged longitudinally in the abdomen. Each testis consists of 3 elongated colourless follicles (testis tubes) and a tubular *vas deferens* with a well developed vesicula seminalis. Two sister *vasa deferentia* enter the common ductus ejaculatorius. There are two pairs of highly branched accessory glands. The upper glands (mesadenia) open into vasa deferentia where they enter the ductus ejaculatorius. The lower glands (ectodenia) empty into the bulbus ejaculatorius, which represents a strongly expanded part of ductus ejaculatorius. In females, each ovary includes 7 elongated colourless ovarioles.

Male karyotype and spermatogenesis

Standard staining. Meiotic stages are found in males, collected in different months, April through August, whereas no spermatogonial mitoses are found in adult males and only few spermatogonial mitoses in male larvae, probably indicating an early maturation of the gonads. The spermatocyte condensation stage and MI display 5 autosomal bivalents and univalent X and Y chromosomes (Figs 1a, 2), thus the meioformula of male *A. trinitatus* is n = 5A + X + Y suggesting 2n = 12 (10 + XY). The bivalents form a gradual size series, except for the first bivalent, which stands out because of its very large size. The X is the second largest chromosome, whereas the Y is the smallest chromosome of the complement. The true and relative areas of chromosomes are presented in Table 2, the middle-sized bivalents (2-4) being placed in one group, as they could not be satisfactory distinguished from one another in the majority of nuclei examined.

The chromosomes show no localized centromeres, thus they are holokinetic. In meiotic prophase I, diplotene and diakinesis stages are absent. In prophase and MI, bivalents consist of parallel-aligned chromosomes without any traces of chiasmata between them. However, at condensation stage, which precedes MI, the homologous chro-

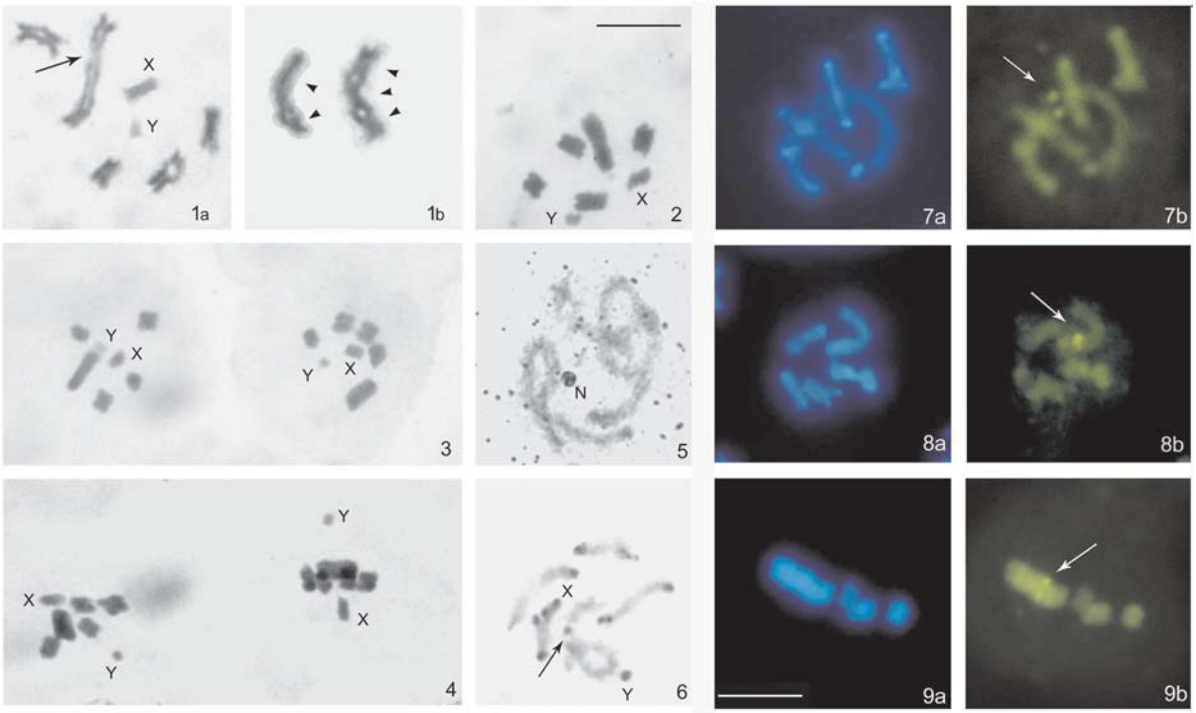
Table 2

True and relative chromosome areas of *Arachnocoris trinitatus* karyotype

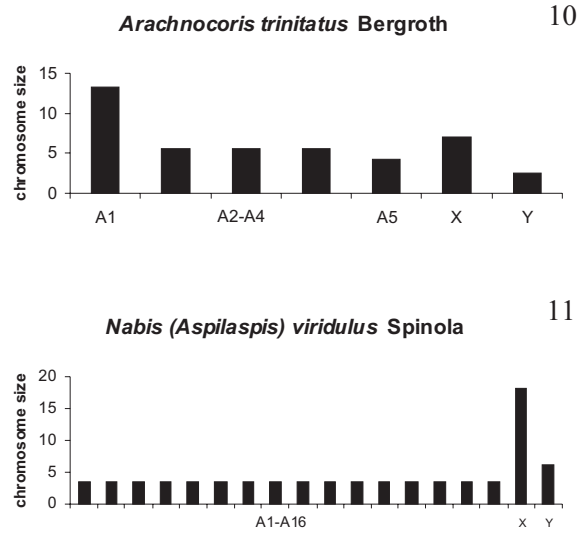
Chromosomes	True areas (μm^2): means and limits	Relative areas (% of total area of chromosomes): means and limits	Number of males/cells/measurements
A1	13.29 3.79-20.65	17.09 13.46-19.93	2/32/32
A2-A4	5.52 1.50-8.25	7.56 5.67-10.05	2/32/96
A5	4.21 1.09-6.75	5.38 3.76-6.70	2/32/32
X	6.99 1.62-11.3	8.95 6.45-11.41	2/32/32
Y	2.43 0.68-3.88	3.26 1.7-6.59	2/32/32

mosomes are physically associated in several sites via some kind of connection (Figs 1a, b). The largest bivalent displays, besides these connections, a gap, which divides the bivalent into two unequal parts (Fig. 1a). At diffuse stage sex chromosomes could be seen both separated or associated with each other (not shown). At condensation stage and in MI, X and Y chromosomes lie separately in all the cells examined. The first division is reductional for the autosomal bivalents, but the sex chromosomes undergo equational separation in anaphase I (AI) and segregation in anaphase II (AII), that is the sex chromosomes undergo post-reduction. Each MII cell therefore contains both X and Y chromosomes (Figs 3, 4). When viewed from the pole, MII plates are "radial" with the sex chromosomes lying in the center of a ring formed by autosomes (Fig. 3). Viewed from the side, sex chromosomes show a bipolar co-orientation and are located near the opposite poles, showing the so-called "distance pairing" (Fig. 4).

A g N O R-b a n d i n g. At silver-stained condensation stage, a nucleolus organizer (NOR) is visible associated with the largest bivalent, and the site of NOR divides the bivalent into two unequal parts (Fig. 5) as also does the above-mentioned gap (Fig. 1a). The NORs are not noticeable in the highly condensed chromosomes in mature MI.



Figs 1-9. Meiotic chromosomes of male *Arachnocoris trinitatus* Bergroth (Heteroptera, Nabidae), 2n = 10 + XY. Fig. 1. Standard staining of condensation stage. (a) The male meioformula is n = 5 + X + Y. Arrow indicates the gap on the largest bivalent; (b) The largest bivalent with "collochores". Arrowheads point to the collochore regions. Fig. 2. Standard staining of MI showing 5 bivalents and univalent X and Y chromosomes. Figs 3-4. Standard staining of the two sister MII nuclei, each with n = 5A + X + Y. (3) View from the pole; (4) View from the side; X and Y chromosomes show "distance-pairing". Fig. 5. AgNOR-staining of early condensation stage. A nucleolus organizer (NOR) is associated to the largest bivalent, N – nucleolus. Fig. 6. C-banding of condensation stage. The only interstital C-band in the largest bivalent is situated at the NOR site. Arrow points to NOR-connected C-band. Figs 7-9. DAPI-staining (a) and CMA_3-staining (b) of condensation stage (Figs 7a, b), prometaphase I (Figs 8a, b), and MI (Figs 9a, b). Arrow points to CMA_3-positive signal in the NOR- site in the largest bivalent. Bar = 10 μm for Figs 1-9.



Figs 10-11. Idiograms of diploid male karyotypes: 10 – *Arachnocoris trinitatus*; 11 – *Nabis* (*Aspilaspis*) *viridulus.*

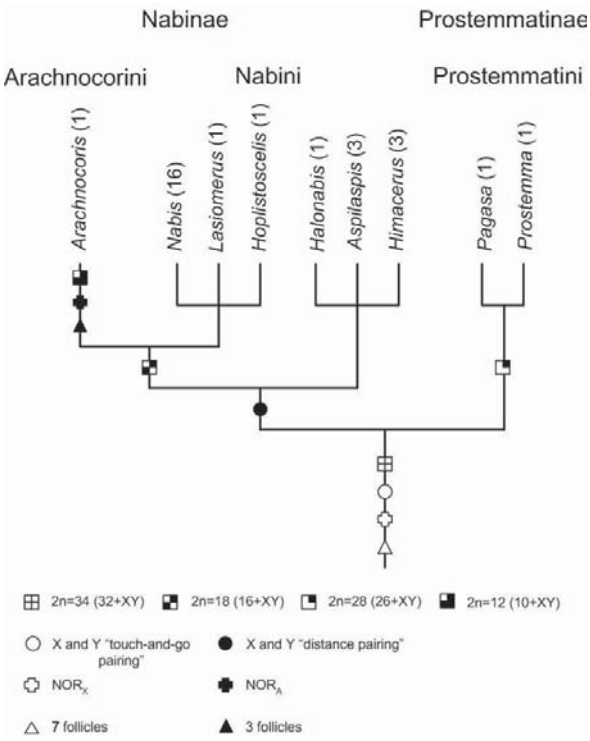


Fig. 12. Distribution of chromosome numbers, male meiotic patterns, number of testicular follicles, and the relationships between some genera of the tribes Nabini, Arachnocorini (the Nabinae), and Prostemmatini (the Prostemmatinae).

C-b a n d i n g. At condensation stage, C-bands are revealed in every bivalent and in both sex chromosomes (Fig. 6). The bivalents and X and Y sex chromosomes each show a particular C-banding pattern. The largest bivalent display no bands except for an interstitial C-band situated at the NOR site and dividing the bivalent into two unequal parts. The smallest bivalent and one of the middle-sized bivalents have only terminal bands, whereas two other middle-sized bivalents display each three bands, two terminal and one subterminal. X chromosome shows C-bands on both telomeres, whereas Y chromosome bears a major portion of heterochromatin throughout its extension. In highly condensed chromosomes of MI no prominent C-bands are distinctive.

Table 3

True and relative chromosome areas of *Nabis* (*Aspilaspis) viridulus* karyotype

Chromosomes	True chromosome areas (μm^2): means, limits	Relative chromosome areas (% of total area of chromosomes): means, limits	Number of males/cells/measurements examined
A1-A16	3.46 1.52-5.6	4.33 2.04-6.46	1/7/112
X	18.1 15.5-18.8	22.69 22.62-24.64	1/7/7
Y	6.35 5.72-6.89	7.9 6.45-8.94	1/7/7

D A P I- a n d C M A $_3$-b a n d i n g. At DAPI-stained condensation stage, the distribution of signals is similar to that of C-bands except for the DAPI-negative interstitial C-band in the largest bivalent (Fig. 7a). When stained by CMA_3, the same chromosome plate reveals signals in none of the chromosomes except for the largest bivalent showing a bright interstitial signal (Fig. 7b). In a prometaphase, successively labeled with DAPI and CMA_3, the chromosomes show very small, mainly telomeric DAPI-positive signals and a single bright CMA_3-positive signal in the largest bivalent (Figs 8a, b). The Figures 9a and 9b demonstrate a MI plate after DAPI and CMA_3 respectively. No signals are evident in the former, whereas the only interstitial signal in the largest bivalent is seen in the latter.

Discussion

In this paper the results of the study of karyotype, male meiosis, and some features of male and female internal reproductive organs of *A. trinitatus* are presented. This is the first studied representative of the highly specialized tribe Arachnocorini (of the subfamily Nabinae), which occurs in the New World only and includes about a dozen species, all of which inhabit the spiders' web nests.

All the species possess a number of distinctive morphological characters (e.g. the absence of ovipositor in females), which are due to their way of living (KERZHNER 1981). This species was found to differ from all the hitherto studied representatives of the family Nabidae also in a number of cytogenetic characters, such as chromosome number, karyotype structure, location of NOR, and, in addition, testis structure. *A. trinitatus* males have 2n = 12 (10 + XY), the lowest chromosome number so far known in Nabidae. As was mentioned in the Introduction, a total of 27 species belonging to the tribes Prostemmatini (2 species) of the subfamily Prostemmatinae and Nabini (25 species) of the subfamily Nabinae have been karyotyped until the present time (reviewed by KUZNETSOVA *et al.* 2004). In Prostemmatini, two studied genera, *Pagasa* and *Prostemma*, share 2n = 28 (26 + XY). In Nabini, 3 values of chromosome number were found: 2n = 18 (16 + XY); 2n = 34 (32 + XY); 2n = 38 (36 + XY). The karyotype 2n = 18 (16 + XY) predominates and occurs in the genera *Lasiomerus* (1 species studied), *Hoplistoscelis* (1 species studied), and in 16 representatives of the genus *Nabis*. The most common chromosome number of a group is usually referred to as the modal number, therefore at present the karyotype 2n = 18 (16 + XY) can be taken as the modal karyotype in Nabinae, at least in the genus *Nabis*. It is noteworthy that in the same genus the subgenera *Aspilaspis* (3 species studied) and *Halonabis* (1 species studied) display 2n = 34 (32 + XY), and the main peculiarity of this karyotype is that it shows a precise doubling of the autosome number compared with the modal 2n = 18 (16 + XY). These subgenera were previously treated as separate genera (KERZHNER 1981), and the validity of this opinion is supported by their karyotype different from that of other subgenera of *Nabis* (KUZNETSOVA *et al.* 2004). It is of interest that the karyotype 2n = 34 (32 + XY) also occurs in *Himacerus mirmicoides* (O. Costa), whereas one further studied representative of this genus, *H. maracandicus* (Reuter), shows a higher number, 2n = 38 (36 + XY).

The rearrangements resulting in chromosome number differences in Nabidae are open to question. One hypothesis is that the karyotype 2n = 16 + XY is ancestral for the family, at least for the subfamily Nabinae, and that 2n = 32 + XY is a result of polyploidy (LESTON 1957; THOMAS 1996) or autosomal polyploidy (KUZNETSOVA & MARYAŃSKA-NADACHOWSKA 2000). The polyploid hypothesis has been recently questioned by JACOBS (2002). Another view holds that the ancestral nabid karyotype was 2n = 32 + XY, and all other karyotypes had originated from it by a series of autosome fusions and fission (KUZNETSOVA *et al.* 2004). This hypothesis seems to be in better agreement with the common mechanisms of karyotype evolution operating in groups with holokinetic chromosomes and also with the data on the related to Nabidae families Miridae, Anthocoridae and Cimicidae, which are characterized with high chromosome numbers, close or equal to 34. To decide between these hypotheses further studies are needed. In particular, comparison studies of chromosome size in species with different chromosome numbers can make possible testing the hypotheses, and a start has been made in the present work.

The true and relative chromosome areas for *A. trinitatus* are presented in Table 2. Since the middle-sized bivalents (2-4) could not be satisfactory distinguished from one another in the majority of cells examined, they are placed in one size group. The largest autosome AI is double the size of A2-A4. Of the sex chromosomes, the X is fairly large however approximately two times shorter than A1, whereas the Y is about a third as large as the X and represents the smallest element of the complement (Fig. 10). *A. trinitatus* differs in karyotype structure from the nabid species with other chromosome numbers, for example, from *Nabis* (*Aspilaspis*) *viridulus* having 2n = 34 (32 + XY). The true and relative chromosome areas of this species are given in Table 3, and the ideogram of its karyotype is presented in Fig. 11. In *N. viridulus*, the great majority of bivalents could not be satisfactory distinguished from one another, then to avoid errors, they are placed in one size group, 1-16. In this species, in contrast to *A. trinitatus*, the conventional largest autosome A1 is similar in size to A2, whereas the X is the largest chromosome of the set and three times as large as A1. Due to our preliminary estimations (unpublished), differences in chromosome size are also characteristic of the nabid species with the same chromosome number that is an evidence for many karyotype transformations in the evolution of the family.

If the chromosome formula 2n = 32 + XY is primitive in the Nabidae, and all other karyotypes (except for 2n = 36 + XY) have originated from this initial state via autosome fusions (KUZNETSOVA *et al.* 2004), then the change to the 2n = 10 + XY shown by *A. trinitatus* is most likely the result of fusion of 11 pairs of autosomes. The fact that the largest chromosome pair is considerably longer than the remaining chromosomes of the karyotype provides strong evidence for fusions. In A1 there is an unstained gap, or constriction having a submedian location, and the silver impregnation technique confirms that it is the site where the nucleolar organizer region (NOR) is situated. An important point is that in all other previously studied nabid species the NORs are associated to sex chromosomes, both to X and Y (NOKKALA & NOKKALA 1984; GROZEVA *et al.* 2004). It allows

suggestion that in Nabidae the NOR is originally located in sex chromosomes and that there has been a translocation of the NOR material since *A. trinitatus* speciation.

It is generally agreed that holokinetic chromosomes contain only a small amount of C-heterochromatin, the distribution of which is mainly confined to the telomeres (BLACKMAN 1985; BRESSA *et al.* 2005). It is apparent however, that neither structural nor behavioral features of holokinetic chromosomes in fact set limits on the heterochromatin accumulation. In support of this assertion one can argue that a great deal of heterochromatin occurs in some species (PAPESCHI 1991; KUZNETSOVA *et al.* 1997; ANGUS *et al.* 2004; BRESSA *et al.* 2005) and in some specialized chromosomes like sex chromosomes and B chromosomes, which are sometimes completely or almost completely heterochromatic (BLACKMAN 1990; PAPESCHI 1995; MARYAŃSKA-NADACHOWSKA 2004; NECHAYEVA *et al.* 2004; ANGUS *et al*. 2004; GROZEVA *et al.* 2004). The wide prevalence of telomeric localization of heterochromatin in holokinetic chromosomes allows suggestion that this pattern is not occasional but is evolutionarily fixed and caused by the biological advisability.

The Nabidae bugs characteristically display small C-bands in their karyotypes, the bands showing both telomeric and interstitial localization and variously distributing in different species (GROZEVA & NOKKALA 2003; GROZEVA *et al*. 2004). This is true even for closely related species, such as *Nabis* (*Aspilaspis*) *indicus* (Stal) and *N.* (*A*.) *viridulus* Spinola, and suggests some chromosome rearrangement events during speciation. These species share the same chromosome number, 2n = 34 (32 + XY), however demonstrate respectively telomeric and interstitial C-bands (GROZEVA *et al*. 2004). It is likely that the inversions have played a role in the redistributing C-blocks along the chromosomes of *N. viridulus.* The analysis of constitutive heterochromatin content and distribution in *A. trinitatus* has revealed the presence of conspicuous C-positive bands of the terminal or sub-terminal location in all the bivalents except for A1, where a single interstitial C-band is connected to NOR. All the bands are DAPI-positive except for the NOR-related band, which is CMA_3-bright indicating an excess of GC base pairs. The correspondence of CMA-bright bands with NORs has also been reported in other heteropteran species (GONZÁLEZ-GARCÍA *et al*. 1996; REBAGLIATI *et al.* 2003), including Nabidae (GROZEVA *et al.* 2004). In *A. trinitatus*, heterochromatin is available in both sex chromosomes, the X showing C-bands on both telomeres, whilst the Y looking as if it is totally heterochromatic or at least contains a very great amount of heterochromatin. With the Y, the same pattern has been reported in many heteropteran species (PAPESCHI *et al.* 1995; PÉREZ *et al.* 1997; GROZEVA & NOKKALA 2001), other representatives of Nabidae included (GROZEVA & NOKKALA 2003; GROZEVA *et al.* 2004). Constitutive heterochromatin is known to be gene-impoverished (PARDUE & HENNIG 1990). It is very tempting to assume that the Y in the Heteroptera contains no major genes, at least those required for sex determination or male fertility. This provides an insight into why in the evolution of Heteroptera, having the XY sex chromosome system as an ancestral condition, the Y chromosome is not infrequently lost bringing into existence the co-generic species with both XY and X0 systems (NOKKALA & NOKKALA 1984; BLACKMAN 1995). This clearly suggests that the heteropteran Y chromosome is transcriptionally inert, but probably fulfils the role of a pairing partner for the X chromosome, thereby providing high accuracy in segregation.

In common with other hitherto studied nabid species, male meiosis in *A. trinitatus* is achiasmatic, as exemplified by prophase I, where diplotene and diakinesis are absent due to the lack of chiasmata, as well as by M I, where in the bivalents the homologues are aligned in parallel to each other with no traces of chiasmata between them. There is no evidence as to whether chiasmata are formed in females, however they are most likely formed. It is well known, that the abolition of chiasmata, when it occurs, is confined to one sex of a species, more often to the male (WHITE 1973), and its genetic control may be related to heterogamety (NOKKALA & NOKKALA 1983). However at condensation stage of *A. trinitatus* the homologous chromosomes are physically associated in several sites via a kind of connection, a phenomenon, which has never been found before in Nabidae. Till now, two basic types of achiasmatic meiosis have been reported in Insecta, including Heteroptera. By the description of NOKKALA and NOKKALA (1986a), in the most widespread type, the alignment of homologous chromosomes attained early in meiosis remained externally unchanged until the beginning of anaphase, whereas in the other type, after synapsis, the so-called "collochores" are formed, and homologous chromosomes are connected with each other at specific positions via these tenacious structures. Within Heteroptera, the "alignment" type occurs in the families Saldidae, Anthocoridae, Microphysidae, and Nabidae (NOKKALA & NOKKALA 1983, 1984, 1986, b; KUZNETSOVA & MARYAŃSKA-NADACHOWSKA 2000; NOKKALA & GROZEVA 2000; KUZNETSOVA *et al.*2004), whereas the "collochore" type is encountered in the families Miridae and Cimicidae (NOKKALA & NOKKALA 1986a; GROZEVA & NOKKALA 2002). The "alignment" type is consid-

ered to be more primitive as compared with the "collochore" one, and the occurrence of these types was used for the determination of phylogenetic affinities within Cimicomoprha (NOKKALA & NOKKALA 1986a). However the data are presently available for solely separate representatives of all the above-mentioned families, so the conclusions can be accepted just as preliminary. For example, NOKKALA & NOKKALA (1984) reported achiasmatic meiosis of the "alignment" type for Anthocoridae based on a study of *Anthocoris nemorum* (L.), however, according to the assertion of WANG *et al.* (2003), while not provided by any proofs, "two kinds of achiasmatic meiosis exist in *A. montanus* Zheng". Because of connections observed between homologous chromosomes, the achiasmatic meiosis of *A. trinitatus* is reminiscent of the "collochore" type. It is however significant, that these connections are visible during meiotic prophase only, whereas in MI homologous chromosomes show parallel alignment along all their length. This evidence suggests that "collochore" and "alignment" types are derivable from each other, and hence, these terms can be considered descriptive. The "alignment" and "collochore" types seem to differ in such a way that numerous collochores occur between the pairing chromosomes in the first case, whereas single (one or two) collochores occur in the second case. Thus, achiasmatic meiosis encountered in *A. trinitatus* can be considered as an intermediate one between "collochore" and "alignment" types. At present, little is known about the nature of collochores, primarily if they are the remnants of synaptinemal complexes or represent some independent structures destined for holding the homologous chromosomes together in meiotic bivalents when chiasmata are absent (COOPER 1964; NOKKALA 1986). Undeniably, a closer look at the nature of collochores and at the problem of achiasmatic meiosis as a whole is called for.

In *A. trinitatus*, as in the overwhelming majority of Heteroptera (UESHIMA 1979), sex chromosomes in male meiosis are subjected to postreduction so that they separate equationally in AI while segregate reductionally in AII. Likewise, typical for Heteroptera is a so-called "touch-and-go pairing" of sex chromosomes, that is they associate transiently as a pseudo-bivalent in MII and segregate (poleward) in AII (UESHIMA 1979). In Nabidae this, most likely ancestral, pattern is retained in the primitive tribe Prostemmatini (KUZNETSOVA *et al.* 2004). However, the tribe Nabini is characterized by another pattern which is known as "distance pairing" of sex chromosomes, where they do not associate but are located near the opposite poles, forming a "distance bivalent" in MII followed by their segregation in AII (NOKKALA & NOKKALA 1984; KUZNETSOVA & MARYAŃSKA-NADACHOWSKA 2000; KUZNETSOVA *et al.* 2004), and the same pattern is also found in *A. trinitatus*. This provides evidence in support of a recent assumption (KUZNETSOVA *et al.* 2004) that this meiotic pattern is an autapomorphy of the subfamily Nabinae within the family Nabidae.

On the basis of the morphology of the male and female internal reproductive apparatus in a number of heteropteran families, PENDERGRAST (1957) has identified several characters, which could provide phylogenetic indications at various taxonomic levels. He refers, in particular, to the number of seminal follicles per testis in males and the number of ovarioles per ovary in females. In many families male testes and female ovaries were shown to consist of 7 follicles and 7 ovarioles respectively (WOODWARD 1950; PENDERGRAST 1957), and this number is taken as the generalized or plesiomorphic condition in Heteroptera (AKINGBOHUNGBE 1983). Other numbers, within a range from 1 to 8, also occur, and in most species for which the numbers are known they are the same in both sexes (WOODWARD 1950). However, in the case when the numbers are different, the number of follicles in males is generally lower as compared to that of ovarioles in females (see Table 1 in GROZEVA & KUZNETSOVA 1992). In Nabidae, male testes and female ovaries (concerning the females, the data are however fewer in number) are invariably composed of 7 lobes (CARAYON 1950, 1951; MIYAMOTO 1957, 1959; KERZHNER 1981; KUZNETSOVA *et al.* 2004), except for *A. trinitatus*, in which males display only 3 follicles in a testis, even if females retain the plesiomorphic state of ovaries, each with 7 ovarioles.

Thus, *A. trinitatus* displays a number of characters differentiating it from all hitherto studied nabid species. Among these characters are chromosome number, 2n = 12 (10 + XY), the lowest within the family; NORs situated on the autosomes rather than on the sex chromosomes; testes composed of 3 seminal follicles but not of 7. These distinctive characters indicate many transformations during evolution of *A. trinitatus* and probably represent, at least the reduced follicle number, a result of extreme specialization shown by this species if not by the entire tribe Arachnocorini, all representatives of which are known to be morphologically and biologically highly specialized. Clearly further studies of this group could be useful.

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