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Karyotypes and Chromosome Rearrangements in Two Tribes of Weevils (Coleoptera, Curculionidae: Sciaphilini and Brachydrini)*

Dorota LACHOWSKA

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A description of karyotypes in two tribes of weevils, Sciaphilini and Brachyderini, was carried out with a discussion on the main trends of chromosomal evolution occurring in these groups. Some important cytological characteristics, such as chromosome morphology, sex determination type, pattern of male meiosis, B chromosome occurrence, as well as C-heterochromatin, NOR localization, and fluorochrome AT and GC specific staining are presented. The chromosome numbers and morphology in the two tribes of weevils are highly conserved. With the exception of one species, all possessed a diploid number of 22 chromosomes or triploid number of 33 chromosomes. Constitutive heterochromatin was observed in the pericentromeric regions of the majority of the chromosomes. In some species, additional constitutive heterochromatin was also observed in interstitial positions. The study of meiotic cells revealed the occurrence of total synapsis between autosomes, the presence of one terminal, interstitial or two chiasmata, reductional behaviour and regular segregation of all chromosomes, as well as the formation of associations of the Xy_p type in sex chromosomes. Testicular cells impregnated with silver nitrate demonstrated NORs localized on autosomes and argentophilic material in the space between the X and y chromosomes. The use of CMA_3/DAPI staining showed that centromeric heterochromatin is AT-rich, whereas CMA_3 bands were probably conincident with NOR sites.

Key words: Sciaphilini, Brachyderini, Curculionidae, chromosomes, meiosis, fluorochrome banding.

Dorota LACHOWSKA, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Science, Sławkowska 17, 31-016 Kraków, Poland. E-mail: lachowska@isez.pan.krakow.pl

The importance of cytogenetic studies for taxonomy has long been recognized because most speciation events are accompanied by karyotypic changes (WHITE 1978). Cytogenetic methods have become a widespread and powerful tool for the delineation and identification of many insects, particularly in groups in which species may be morphologically cryptic. The value of karyotype for taxonomy and phylogenetic relationships has been demonstrated in many beetle species, e.g. Carabidae, Chrysomelidae, and Curculionidae (ANGUS 1982; SERRANO 1986; PETITPIERRE 1997; ROŻEK *et al.* 2004).

Curculionidae is the most speciose family among beetles. Over 50,000 species have been described in the world fauna and more than 6,000 in the Palaearctic region (LAWRENCE & NEWTON 1995). So far, the karyology of about 600 species of Curculionidae has been investigated, but the great majority of cytogenetic findings on weevils have only referred to the male chromosome numbers and sex determination system at meiotic metaphase I. Chromosome banding techniques such as C-banding allow for a better characterization of beetle karyotypes and selectively reveal chromosome regions consisting of constitutive heterochromatin, therefore offering much more information on karyotype architecture. Unfortunately, most of the karyotypic data were obtained by standard analysis, only a minor part of the literature describes the banded karyotypes of curculionids (HSIAO & HSIAO 1984; HOLECOVÁ *et al.* 1997, 2002, 2008; ROŻEK & HOLECOVÁ 2000; ROŻEK *et al.* 2004; LACHOWSKA *et al.* 2004, 2005, 2006a, b, 2008a, b). Knowledge of the karyology of Curculionidae varies greatly from genus to genus and from

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subfamily to subfamily. Many species-rich genera exist for which the karyologic makeup has not yet been determined.

The tribe Sciaphilini includes 76 species distributed mainly in the Palaearctic region (SMRECZYŃSKI 1966) from which 17 species hitherto have been karyologically examined (MIKULSKA 1953; PETRYSZAK 1972; LACHOWSKA *et al.* 1998, 2005; LACHOWSKA & HOLECOVÁ 2000; HOLECOVÁ *et al.* 2002, 2005). This tribe is not very rich in species but is characterized by the presence of some endemics which show some morphological and chromosomal differences, and also the occurrence of parthenogenetic forms (HOLECOVÁ *et al.* 1999; LACHOWSKA *et al.* 2008a). The tribe Brachyderini contains 13 mostly Palaearctic genera with the nominal genus *Strophosoma* represented in Central Europe by four bisexual and one parthenogenetic species (JELÍNEK 1993; WANAT & MOKRZYCKI 2005). Parthenogenesis in weevils is geographic, apomictic and occurs in connection with polyploidy (LACHOWSKA *et al.* 2008a).

The aim of this paper is to present new cytogenetic characters regarding G-C specific and A-T specific sites, as well as to review the cytogenetic data from the literature and propose the main strategies of chromosomal evolution in these two groups of weevils.

Material and Methods

For the cytogenetic study, weevil adults were collected in Slovakia and Poland between 2005-2007. The species are classified according to ALONSO-ZARAZAGA and LYAL (1999) and WANAT and MOKRZYCKI (2005). Vouchers were deposited in the Institute of Systematics and Evolution of Animals Polish Academy of Sciences. Gonads (eight - ten from each species) were dissected under a stereomicroscope in several drops of hypotonic 0.9 % sodium citrate solution containing 0.005% colchicine. The gonads were transferred into a small volume of the same solution and incubated for 30-45 min at room temperature. Then the gonads were fixed according to the method described by ROŻEK (1994) with minor modification (ROŻEK & LACHOWSKA 2001). DNA binding fluorochromes including GC-specific chromomycin A_3 (CMA_3) and AT-specific 4'-6-diamidino-2-phenylindole (DAPI) were used according to the methods described in SCHWEIZER (1976) and DONLON and MAGENIS (1983), with minor modifications. The slides were first subjected to the C-banding procedure and, to improve the fluorochrome staining, 0.5% methanol was included in the fluorescent dye (KUZNETSOVA *et al.* 2003). After staining, the slides were mounted in anti-fade medium consisting of 1% n-propylgallate in a 10 M phosphate buffer solution with 70% glycerol, pH 7.0. Spermatogonial metaphase, meiotic stages and interphase nuclei were analyzed and photographed with a Nikon Eclipse 400 light microscope and CCD DS-U1 camera (Nikon, Tokyo, Japan) using the software Lucia Image, version 5.0 (Laboratory Imaging, Prague, Czech Republic).

Results

The chromosome characteristics of the Sciaphilini and Brachyderini are listed in Table 1. The chromosomal numbers and morphology found in eight examined species were in accordance with those reported previously. Application of fluorochrome staining gave positive results only in species which possess a substantial amount of heterochromatin. After DAPI staining, bright signals were visible on the centromeric regions of every chromosome (with the exception of heterochromosome y) in *Barypeithes liptoviensis, B. mollicomus, B. interpositus, B. formaneki, B. chevrolati, Strophosoma capitatum*, and *S. faber* (Fig. 1a- c, e, g-h). Application of CMA_3 revealed yellow signals on one autosomal pair in *B. interpositus* and *B. formaneki* (Fig. 1c, e).

Discussion

The representatives of two tribes, Sciaphilini and Brachyderini, exhibit remarkable karyotypic uniformity. The karyotypes are broadly similar to one another in the number of chromosomes and in sex determination. The diploid complement of 22 chromosomes, and the meioformula $n♂ = 10+Xy_p$, are considered as the basic karyotype for Curculionidae since about 42% of the 600 examined species concord to this pattern (LACHOWSKA *et al.* 1998). Only the endemic *Barypeithes liptoviensis* differs strikingly from the other bisexual species not only because of its higher chromosome number (2n=26) but also due to the asymmetry of its karyotype. The parthenogenetic species are represented by triploid forms with 33 chromosomes, confirming that triploidy is the most common level of ploidy within Curculionidae. Within all examined parthenogenetic weevils there are 43 triploid, only 4 diploid and 18 tetraploid forms, this suggests that the triploid forms are ancestral and the diploids and tetraploids derived (SAURA *et al.* 1993). Many parthenogenetic forms have separate chromosome set that form separate metaphase plates during meiosis (STENBERG *et al.* 2003; LACHOWSKA *et al.* 2008a). All karyological results show that most chromosomes in bisexual and parthenogenetic species are meta- or submetacen-

Table 1

Karyotypic data of *Sciaphilini* and *Brachyderini*

Species	Chromosomal formula	Chromosomal morphology	C-banding	References
Sciaphilini				
Sciaphilus asperatus (Bonsdorff, 1785)	3n=33, part. 3n=33, part.	majority M 18M, 9SM, 6ST	– centromeric	PETRYSZAK 1972 LACHOWSKA *et al*. 2008a
Eusomus ovulum Germar, 1824	3n=33, part. 3n=33, part.	majority M 18M, 3SM, 6ST, 6A	– small amount of heterochromatin	MIKULSKA 1953 LACHOWSKA *et al*. 2008a
Paophilus afflatus (Boheman, 1833)	2n=22, n♂=10+Xy$_p$	majority M and SM, y-dot	small amount of heterochromatin	LACHOWSKA *et al*. 2006a
Brachysomus dispar Penecke, 1910	2n=22, n♂=10+Xy$_p$	17M, 2ST, 2A, y-dot	small amount of heterochromatin	HOLECOVÁ *et al*. 2008
Brachysomus echinatus (Bonsdorff, 1785)	3n=33, part.	24M, 9A	small amount of heterochromatin	LACHOWSKA *et al*. 2008
Brachysomus hirtus (Boheman, 1845)	3n=33, part.	majority M or SM	small amount of heterochromatin	HOLECOVÁ *et al*. 2008
Brachysomus setiger (Gyllenhal, 1840)	2n=22, n♂=10+Xy$_p$	majority M and SM, y-dot	small amount of heterochromatin	LACHOWSKA *et al*. 2006a
Barypeithes albinae (Formánek 1903)	2n=22, n♂=10+Xy$_p$	majority M, y-dot	small amount of heterochromatin	LACHOWSKA *et al*. 2005
Barypeithes chevrolati (Boheman, 1843)	2n=22, n♂=10+Xy$_p$ 2n=22, n♂=10+Xy$_p$	majority M, y-dot 16M or SM, 4ST, X-ST, y-SM	– centromeric and intercalary	HOLECOVÁ *et al*. 2002 LACHOWSKA *et al*. 2005
Barypeithes formaneki (Fremuth 1971)	2n=22, n♂=10+Xy$_p$	16M or SM, 4ST,X-ST, y-SM	centromeric	LACHOWSKA *et al*. 2005
Barypeithes interpositus (Roubal, 1920)	2n=22, n♂=10+Xy$_p$	majority M and SM, y-M or SM	centromeric and intercalary	LACHOWSKA *et al*. 2005
Barypeithes liptoviensis (Weise 1894)	2n=26, n♂=12+Xy$_p$ 2n=26, n♂=12+Xy$_p$	– 23M or SM, 2ST, y-dot	– centromeric and intercalary	LACHOWSKA *et al*. 1998 LACHOWSKA *et al*. 2005
Barypeithes mollicomus (Ahrens, 1812)	2n=22 n♂=10+Xy$_p$	15M or SM, 6ST	centromeric	LACHOWSKA *et al*. 2005
Barypeithes pellucidus (Boheman, 1834)	2n=22+0-6B, n♂=10+Xy$_p$+0-6B 2n=22+0-6B, n♂=10+Xy$_p$+0-6B	– majority M and SM	– small amount of heterochromatin	HOLECOVÁ *et al*. 2005 LACHOWSKA *et al*. 2005
Barypeithes purkynei (Fremuth 1965)	2n=22, n♂=10+Xy$_p$	16M, 6ST	small amount of heterochromatin	LACHOWSKA *et al*. 2005
Foucartia liturata (Stierlin, 1884)	2n=22, n♂=10+Xy	–	not examined	PETRYSZAK 1972
Parafoucartia squamulata (Herbst, 1795)	3n=33, part. 3n=33, part.	majority M or SM 24M, 3SM, 3ST, 3A	– small amount of heterochromatin	PETRYSZAK 1972, LACHOWSKA *et al*. 2008a
Brachyderini				
Brachyderes incanus (Linnaeus, 1758)	2n=22, n♂=10+Xy$_p$ 2n=22, n♂=10+Xy$_p$	– 20 M, X-A, y-dot	– centromeric	LACHOWSKA & HOLECOVÁ 2000 LACHOWSKA *et al*. 2006a
Strophosoma capitatum (De Geer, 1775)	2n=22, n♂=10+Xy	–	centromeric	ROŻEK & HOLECOVÁ 2000
Strophosoma melanogrammum (Forster, 1771)	3n=33, part.	24M, 6SM, 3A	centromeric	LACHOWSKA *et al*. 2008a
Strophosoma faber (Herbst, 1784)	2n=22, n♂=10+Xy$_p$	18M, 2A, X-M, y-dot	centromeric	HOLECOVÁ *et al*. 2008

part. – parthenogenetic species, M – metacentric, SM – submetacentric, ST – subtelocentric, A – acrocentric, dot – dot-like chromosome.

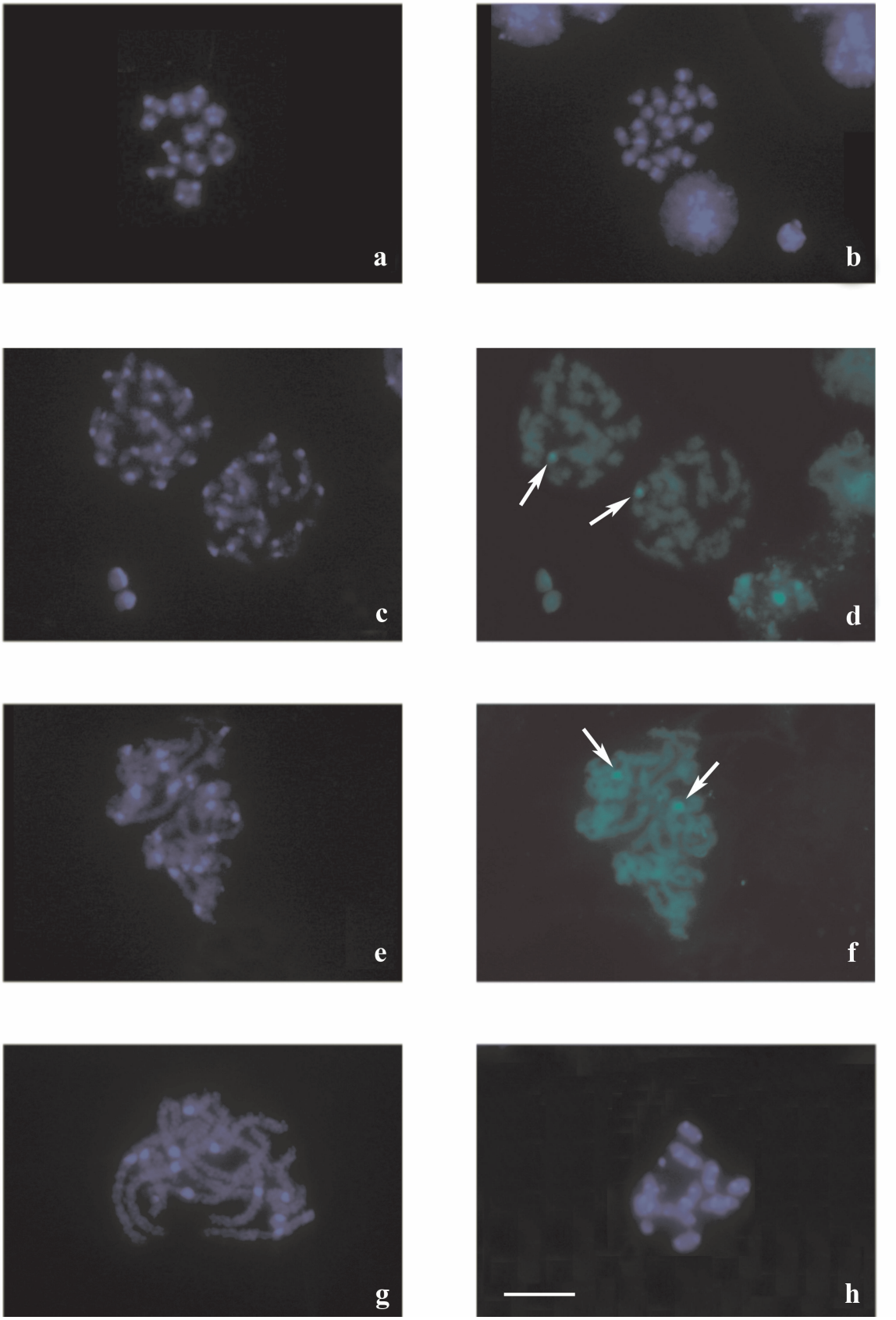

Fig. 1. Spermatocytes of eight weevil species. (a) metaphase I in *Barypeithes chevrolati* after DAPI staining; (b) mitotic plate in *B. mollicomus* after DAPI staining; (c) mitotic prometaphase in *B. interpositus* after DAPI staining; (d) mitotic prometaphase in *B. interpositus* after CMA_3 staining, arrows show positive signals; (e) early diplotene in *B. formaneki* after DAPI staining; (f) early diplotene in *B. formaneki* after CMA_3 staining, arrows show positive signals; (g) diplotene after DAPI staining in *Strophosoma capitatum*; (h) metaphase I after DAPI staining in *S. faber*. Bar = 10 μm.

tric, following the general rule of metacentry in the karyotypic architecture of weevils. Karyotypes are most often symmetrical with chromosomes forming a series decreasing in size. The presence of acrocentric chromosomes in some species suggests the occurrence of pericentric inversions that have converted meta/submetacentric chromosomes into acrocentric chromosomes.

The available data on the karyology of weevils suggest that B chromosomes are very rare in Curculionidae. Of the 600 species examined karyologically, only four species with supernumerary chromosomes have been hitherto described (ENNIS 1972; SMITH & BROWER 1974; DEY 1989; HOLECOVÁ *et al.* 2005; LACHOWSKA *et al.* 2008b). In *Barypeithes pellucidus* the number of B chromosomes ranges from one to six per cell. B chromosomes pair with the sex heterochromosomes, only rarely with autosomes. The sizes of these additional chromosomes are the same or slightly smaller than that of the y. It is generally assumed that B chromosomes appear as the result of structural rearrangements of some A chromosomes, however, detailed molecular investigations of synaptonemic complex may shed light on their specific origin.

The meiotic chromosomes of Sciaphilini and Brachyderini exhibit behavioural patterns similar to those encountered in the majority of beetle species (SMITH & VIRKKI 1978). All chromosomes display regular patterns of synapsis, orientation, and segregation. Diplotene and diakinesis cells show that the majority of the autosomal bivalents possess one interstitial or terminal chiasma (cross-shaped and rod shaped bivalents), however, bivalents with two chiasmata demonstrating a ring-shaped formation are also observed. According to JOHN (1990), the number and location of chiasmata is partly dependant on chromosome length, thus the large sized bivalents are those that always have a high number of chiasmata. The "parachute" configuration of the Xy_p bivalent during meiosis has been found in all examined bisexual species. This non-chiasmate association is the rule in beetles of the suborder Polyphaga, consisting of a large X and minute y chromosome (SMITH & VIRKKI 1978).

The C-banding technique is a method of demonstrating the location of constitutive heterochromatin in chromosomes. This method supplies valuable data on variability and differentiation of karyotypes and helps in defining the types of rearrangements important in the evolution of a systematic group. The location and size of heterochromatin blocks permit identification of pairs of chromosomes in the set. The C-banding technique was occasionally used for identification of closely related species in some coleopteran groups, e.g. Carabidae, Aphodiidae, Hydrophilidae etc., where conventional staining techniques frequently give insufficient information (ANGUS 1998; ANGUS *et al.* 2000; WILSON & ANGUS 2004). In Sciaphilini and Brachyderini the chromosomes resemble one another in having the C-bands restricted mostly to the area around the centromere. The pericentromeric constitutive heterochromatin pattern is similar to that reported in most species of the beetle Polyphaga suborder (ROŻEK & HOLECOVÁ 2002; ROŻEK *et al.* 2004; SCHNEIDER *et al.* 2007; HOLECOVÁ *et al.* 2008; LACHOWSKA *et al.* 2008a, b). Additionally, intercalary bands which occur in some species probably arose by duplication of small repetitive DNA segments or from the transfer of constitutive heterochromatic material among equidistant sites of non-homologous chromosomes (SCHWEIZER & LOIDL 1987). In the majority of species the y was entirely C-band negative. In the Curculionidae, heterochromatin occurs mainly in a small proportion and often is weakly visible or cannot be discerned at all during chromosome condensation in mitotic metaphase, diakinesis or metaphases I and II (ROŻEK *et al.* 2004; LACHOWSKA *et al.* 2005). Chromosomes with a small amount of constitutive heterochromatin were observed in many coleopteran families, e.g. Anthicidae, Attelabidae, Cerambycidae, Chrysomelidae (ROŻEK *et al.* 2004). The lack of C-band positive regions can be attributed to the methodology used, because it does not reveal all types of heterochromatin (SUMNER 1990). A search for possible satellite DNA sequences would probably account for this pattern if they were not demonstrated.

$AgNO_3$ staining of chromosomes has been a very useful approach for the analysis of the structure and variability of nucleoli, nucleolar organizer regions (NORs) and kinetochores (VIRKKI *et al.* 1991). Although in Coleoptera this technique has been considered unsuitable for the identification of active rDNA clusters, it is generally accepted that silver impregnation is indicative of rRNA synthesis and that it stains functionally active NORs (COLOMBA *et al.* 2000; BIONE *et al.* 2005). Data on localization of NORs in Coleoptera suggest that an autosome pair function as a nucleolus organizer (BIONE *et al.* 2005; SCHNEIDER *et al.* 2007). NOR activity at the beginning of the meiotic prophase is widely observed in Coleoptera species, however this activity was observed during a restricted period of time only, declining rapidly and disappearing in the middle of the diplotene phase. The examination of NORs in Curculionidae showed that even when the NORs are autosomal, the lumen of the sex bivalents is filled with a substance with an affinity for silver from diakinesis to anaphase I (BIONE *et al.* 2005; LACHOWSKA *et al.* 2008b). It has been suggested that this substance

has an adhesive role, controlling the correct separation of the sex chromosomes (VIRKKI *et al.* 1991). The observation that the sex bivalents of species from these two tribes can be labelled with silver confirms that the Xy_p association is not due to the NOR, but to the presence of argentophilic proteins. In some species silver impregnation does not reveal NORs because of the placement of ribosome cistrons in small groups on the chromosomes and, consequently, the proteins responsible for silver precipitation may be so small that NORs are not detected (GOODPASTURE & BLOOM 1975). The application of fluorescence *in situ* hybridization with an rDNA probe permits the precise identification of NORs in weevils.

Chromosome staining by DNA base specific fluorochromes is still rarely used in cytogenetic studies of Coleoptera (VITTURI *et al.* 1999; COLOMBA *et al.* 2000; MOURA *et al.* 2003), and remains incompletely investigated in Curculionidae (LACHOWSKA *et al.* 2008b). In the studied species of *Barypeithes* and *Strophosoma,* C-blocks fluoresced brightly after DAPI staining, suggesting the occurrence of a large amount of AT base pairs in heterochromatic DNA. Some differences in fluorescent intensity may be explained by the degree of condensation, i.e. the more the chromosomes are elongated, the weaker the signal. Fluorochrome CMA_3 staining labels NORs independently of their activity, the fluorescence is associated with G-C content typical of genes coding for ribosomal RNA (rDNA) (ANOKHIN & NOKKALA 2004). This correlation between CMA_3 bands and NORs is quite common among insects (BRITO *et al.* 2003). In *B. interpositus* and *B. formaneki* fluorescent signals were obtained in one chromosome pair, and are probably connected with NORs. In other species a lack of positive signals after CMA_3 staining suggests a small number of rDNA genes, alternatively, the absence of CMA_3 bands might be due to technical reasons because sometimes this band disappeared when C banding was applied before sequential staining with chromomycine (BRITO *et al.* 2003).

In conclusion, the chromosome numbers and morphology in two tribes of weevils are highly conserved, predominantly presenting the diploid number of 2n=22, a sex chromosome mechanism of the "parachute" type, and a triploid karyotype in parthenogenetic species of 3n=33. The only detected numerical chromosomal variation involves the occurrence of an additional two pairs of autosomes in *Barypeithes liptoviensis* and the occurrence of supernumerary chromosomes in *B. pellucidus.* Most chromosomes possess the centromere at a medial or submedial position, forming a symmetrical karyotype. Karyotypic evolution probably occurred by chromosomal rearrangements of some type of pericentric inversion and/or the addition of constitutive heterochromatic material.

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