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The First Cytogenetic Report on *Laena reitteri* Weise, 1877 (Coleoptera, Tenebrionidae, Lagriinae) with Notes on Karyotypes of Darkling Beetles

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The karyotype structure of *Laena reitteri* is described for the first time. The chromosome number 2n=18+1-3B and meioformula n♂=8+Xy_p+1-3B deviates from the modal tenebrionid number. The karyotype exhibits low variation in morphology and length. The diploid set consists of four long (subtelocentric and acrocentric), twelve medium-sized acrocentric autosomes and sex heterochromosomes Xy. The X chromosome is submetacentric, while the y is acrocentric and the smallest element of the set. On mitotic and meiotic plates 1-3 small additional elements are also visible, and probably represent B-chromosomes. The NORs are very active at mitotic prophase and early meiotic stages.

Key words: Coleoptera, Tenebrionidae, *Laena reitteri*, karyotype, C-bands, NORs, flurochrome staining.

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The darkling beetles are a large coleopteran family with almost 25,000 species (DAJOZ 1984; CROWSON 1981; LAWRENCE & NEWTON 1995) that show extreme morphological variation. Cytogenetically they are conservative in chromosome number because more that 60% of examined species show a 9+Xy_p meioformula which is considered ancestral for Coleoptera - Polyphaga (SMITH & VIRKKI 1978). To date, 200 species of Tenebrionidae have well characterized karyotypes; i.e. the diploid number, the chromosome morphology and the type of the sex chromosome determination (JUAN & PETITPIERRE 1991). The range of diploid numbers in this family ranges from 2n=14 in *Diaperis boleti* Linnaeus and *Scotobius miliaris* Billberg to 2n♂=37 in *Blaps lethifera* Marsham. The most frequent sex-chromosome system is the "parachute" Xy_p shared by 67% of species while the remaining species have mostly chiasmatic systems like neo XY, Xy_r, or rarely X0 (SMITH & VIRKKI 1978; JUAN & PETITPIERRE 1991). In spite of the fact that the family includes 10 subfamilies, 96 tribes and 61 subtribes, the vast majority of species examined cytogenetically (96%) belong to two subfamilies: Pimeliinae and Tenebrioninae (BOUCHARD *et al.* 2005). The cytogenetic data on species from the other eight subfamilies are very sparse.

The present paper brings the first karyological report on a typical forest species *Laena reitteri* Weise, 1877 (Lagriinae, Laenini).

Material and Methods

For the cytogenetic study, specimens of *L. reitteri* were collected in thermophilous oak-hornbeam forests (Querco-Carpinetum caricetosum pilosae) in Czaszyn (49°27'N, 22°13'E), Beskid Niski Mts. (SE Poland), Medzilaborce – Kamenná hill (49°16'N, 21°53'E) and Udavské near Humenné (48°57'N, 21°56'E), Laborecká vrchovina hills (NE Slovakia) in June 12 2005, April 24 and July 4 2007. Vouchers were deposited in the Institute of

Systematics and Evolution of Animals Polish Academy of Sciences. Gonads of males 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). C-banding was performed using the procedure described by SUMNER (1990) with some modifications (LACHOWSKA *et al.* 2006). For the NOR silver staining the method described by HOWELL & BLACK (1980) was used with some modifications (LACHOWSKA *et al.* 2005). Chromosomes were classified according to LEVAN *et al.* (1964). Spermatogonial metaphase, meiotic stages and interphase nuclei were analyzed and photographed with a Nikon Eclipse 400 light microscope and CCD DS-U1 camera (Nikon) using the programe NIS-Elements BR 2.30 (Nikon).

Results and Discussion

The diploid complement of *Laena reitteri* males consists of 16 autosomes and the sex heterochromosomes X and y. The karyotype exhibits little variation in the morphology and length of individual autosomes and X chromosome. Male mitotic plates include two pairs of long (subtelocentric, acrocentric) and six pairs of medium-sized acrocentric autosomes. The X chromosome is submetacentric and slightly shorter than medium-sized acrocentric autosomes while the y is acrocentric and the smallest element of the set. In a large number of mitotic metaphase plates the y chromosome was more spiralised and assumed a dot -like shape (Fig. 1).

The sex chromosome system of non-chiasmatic parachute type (Xy_p) is clearly distinguishable in meiotic stages from the beginning of pachytene to metaphase I (Fig. 2). Examination of diakinesis shows that autosomal bivalents possess two terminal chiasmata, one terminal and one interstitial chiasmata, or only one chiasma either interstitial or terminal. Thereby they form rod-shaped figures, crosses and rings (Fig. 2). On mitotic and meiotic plates also 1-3 small additional elements are visible, probably representing B-chromosomes (Figs 1 & 2).

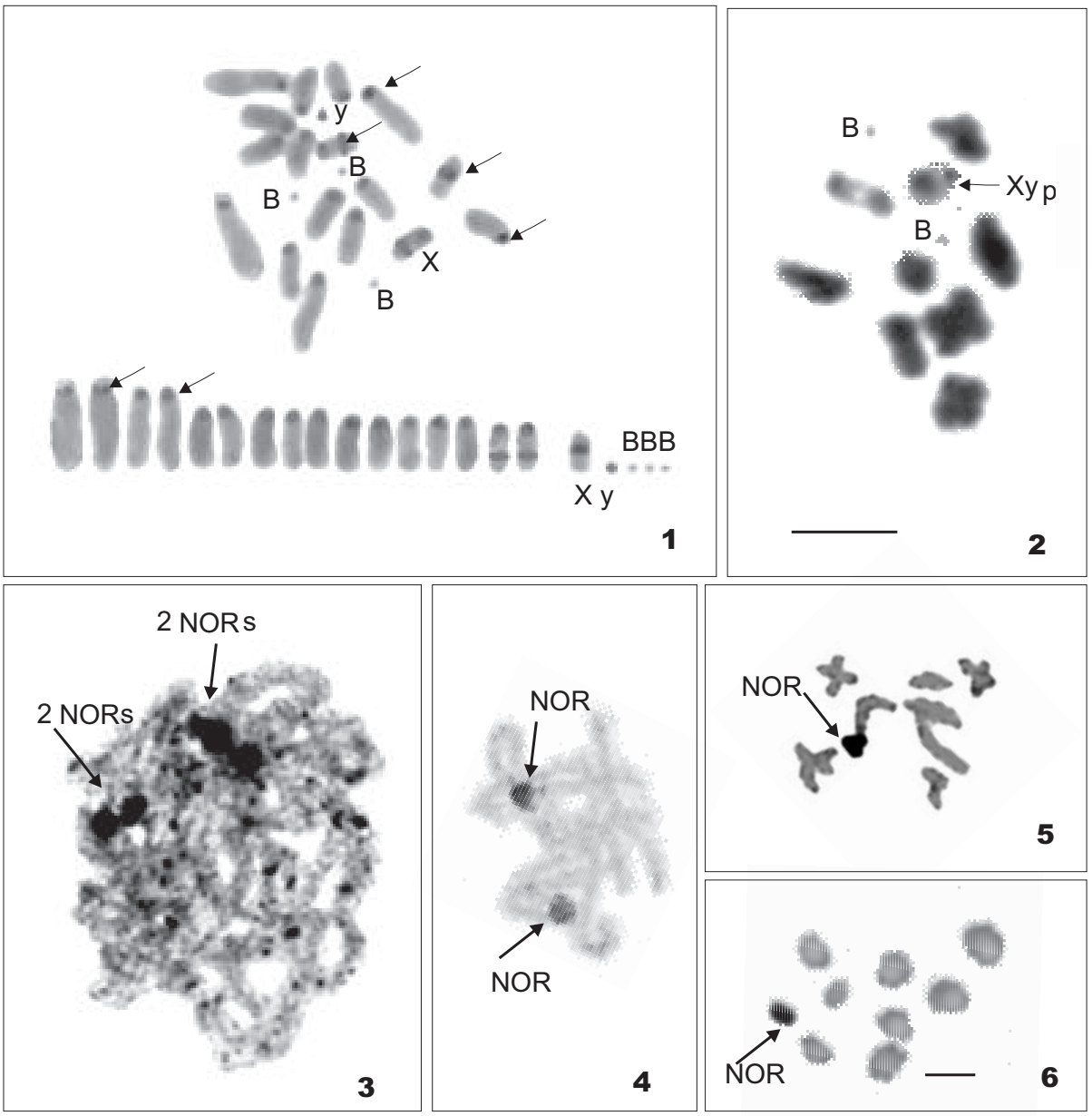
According to the present state of knowledge six types of changes are responsible for the evolution of the tenebrionid karyotype: (1) Centric fusion: autosome – autosome fusion and/or autosome – X fusion. (2) Centric fission of autosomes. (3) Increase or decrease in the size of X chromosome. (4) Increase in the size of Y chromosome. (5) Elimination or loss of Y chromosome. (6) Polyploidy (SMITH 1952; YADAV & PILLAI 1976; SMITH & VIRKII 1978; JUAN & PETITPIERRE 1990; PALMER & PETITPIERRE 1997). The karyotype of *Laena reitteri* presumably arose from the fusion of a pair of non-homologous autosomes. Pericentric inversions may be responsible for the origin of acrocentric chromosomes predominating in the complement. Moreover the presence of supernumerary or B-chromosomes confirms this sort of karyotype modification.

The constitutive heterochromatin occurs in centromeric regions in all autosomes and sex chromosomes, moreover autosomes from the 8th pair possess also one short intercalary band. The heavily stained pericentromeric block of heterochromatin on the X chromosome is twice as long as C bands on the other chromosomes of the set (Fig. 1).

Six nucleolar organizer regions (NORs) impregnated with silver are visible. A total of 4 NORs occur in mitotic prophase (Fig. 3). Ag-positive NORs were not observed on chromosomes of mitotic metaphase. Two NORs are recognizable from leptotene to early diakinesis, 1 NOR in metaphase I (Figs 4, 5, 6).

The chromosome number and morphology of *Laena reitteri* deviates from the ancestral tenebrionid karyotype ($2n=20, n=18+Xy_p$) which is characteristic for 63% of the darkling beetles examined karyologically (SMITH 1952; SMITH & VIRKII 1978; YADAV & PILLAI 1974a, b, 1976; YADAV *et al.* 1980; JUAN & PETITPIERRE 1991). These results show that most chromosomes are acrocentric, a condition which is not typical for the karyotypic architecture of Polyphagan beetles. In the majority of darkling beetles studied cytogenetically, mitotic metaphases show a predominance of metacentric chromosomes (JUAN & PETITPIERRE 1988, 1989, 1990; JUAN *et al*. 1989; PALMER & PETITPIERRE 1997). On the other hand, the "parachute" Xy_p is the most frequent sex-chromosome system among tenebrionids. The y heterochromosome is usually minute and dot-shaped. The terminal or subterminal position of the centromere on the y is visible less frequently (YADAV & PILLAI 1976, the present study). The nature of the X chromosome in darkling beetles varies from acrocentric to metacentric (YADAV & PILLAI 1974a,b, 1976 and the present study).

The karyotype of *Laena reitteri* has also shown the presence of B chromosomes, clearly distinguishable from the regular members of the complement. In comparison to the y heterochromosome, these additional chromosomes are half-sized and euchromatic. Within the present state of knowledge of B-chromosomes in darkling beetles, it is difficult to



Figs 1-6. C-bands and NOR stained chromosomes of *Laena reitteri*. Fig. 1. Mitotic metaphase and karyotype, with 2n(♂)=16+X+y+3B, the arrows indicate segments of heterochromatin. Fig. 2. Late diakinesis with visible Xy_p. Fig. 3. Mitotic prophase with 4 NORs. Fig. 4. Diplotene. Fig. 5. Diakinesis. Fig. 6. Metaphase I. Bar = 10 μm , the scale bar in 2 refers to Figs 1 and 2, the scale bar in 6 refers to Figs 3-6.

comment on their genesis. Of the total number of up to 200 species of Tenebrionidae examined karyologically, only three species with supernumerary chromosomes were hitherto described (SMITH 1956; HALSTEAD 1969; VIDAL 1984).

The application of the C-banding technique evidences a defined pattern of bands. To date the present C-banding karyotype was occasionally used for identification of closely related species in some coleopteran groups e.g. Carabidae, Aphodiidae, Hydrophilidae etc. where conventional staining techniques often give insufficient information (ANGUS *et al*. 2000; WILSON & ANGUS 2004). In examined species, chromosomes resemble one another in having the C-bands restricted mostly to the area around the centromere, characteristic for the majority of insects (JUAN & PETITPIERRE 1989; IMAI 1991; ALMEIDA *et al.* 2000; PROENÇA *et al.* 2002; ROŻEK *et al.* 2004; ZACARO *et al.* 2004; LACHOWSKA *et al*. 2005; SCHNEIDER *et al*. 2006). An intercalary C-band was detected in only one pair of autosomes. Constitutive heterochromatin is also located in the centromeric region of the X sex chromosome, whereas the y is completely heterochromatic.

$AgNO_3$ staining of chromosomes has been useful for the analysis of nucleolar organizer regions (NORs), although in Coleoptera this technique stains functionally active NORs (VIRKKI *et al.* 1991; BIONE *et al.* 2005). In beetle families NORs may be located in the autosomal pairs and/or sex chromosomes, although most data show that the nucleolus organizer is widely distributed in one autosomal pair (MOURA *et. al.* 2003; BIONE *et al.* 2005). In the examined species the Ag-stained NOR is situated on sex chromosomes, as well as on one pair of autosomes. In Coleoptera NOR activity is observed at the beginning of the meiotic prophase, disappearing in the middle of the diplotene phase. The nucleolar masses produced can persist for a longer time in species with a prolonged diplotene (VIRKKI *et al.* 1991). This phenomenon was clearly observed in the species studied here, but the presence of NOR labeling in the sex chromosomes until the late phase of meiosis I may indicate that argentophilic material plays an adhesive role controlling the correct separation of the sex chromosomes during meiosis. Our data are in concordance with the hypothesis that an autosome pair functions as a nucleolus organizer, but only the employment of fluorescent in situ hybridization with a rDNA probe will definitively provide precise identification of the NORs in the analysed species.

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