

## Cytogenetic differences between *Peritelus familiaris* and *Centricnemus leucogrammus* (Coleoptera: Curculionidae: Entiminae: Peritelini)

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**Abstract.** Differences in the karyology of two species, *Centricnemus leucogrammus* and *Peritelus familiaris* (Coleoptera: Curculionidae), were investigated in order to elucidate their taxonomic position of the taxa. Previously both species were placed in one genus whereas the latest taxonomic revision puts them in separate genera. Cytogenetic analysis of *P. familiaris* and *C. leucogrammus* showed significant differences in karyotype structure and confirmed their present taxonomic status. The diploid set of *C. leucogrammus* consists of 22 chromosomes with a fundamental number of arms (FN) of 45 and little variation in morphology and length. *Peritelus familiaris* has 24 chromosomes with FN of 47 and a more diverse karyotype. The karyotype evolution might have occurred by centric fissions of autosomes. At pachytene and diplotene in spermatocytes, each chromosome bivalent showed a small band of pericentric heterochromatin. The bands were hardly visible or undetectable in other stages of spermatogenesis, namely mitotic metaphase, diakinesis, metaphase I and II. The nucleolar organizer regions (NORs) were active at premeiotic stages and early meiosis, but invisible at meiotic metaphase I, metaphase II, and mitotic metaphase. These results indicate the usefulness of cytogenetic methods in taxonomic evaluations.

### INTRODUCTION

The tribe Peritelini includes species of weevil indigenous to the Southwestern Palearctic, Nearctic and Ethiopian regions. Of the sixteen genera of Peritelini known from Europe, eleven occur in the Mediterranean area, and only five (*Caenopsis* Bach, 1854, *Centricnemus* Germar, 1827, *Peritelus* Germar, 1824, *Simo* Dejean, 1821 and *Stomodes* Schoenherr, 1826) inhabit Central Europe (Pierotti & Bellò, 1997, 1998; Alonso-Zarazaga & Lyal, 1999). Most species of Peritelini, either as adults and/or larva, are xerothermophilous and phytophagous, feeding on various plants. Numerous species are apterous, and nocturnal (Dieckmann, 1980; Hoffmann, 1950; Solari, 1955; Smreczyński, 1966). So far, only two species, *Centricnemus leucogrammus* Germar 1824 and *Simo hirticornis* (Herbst, 1795) have been examined karyologically (Suomalainen, 1954; Petryszak, 1977; Palm, 1995; Lachowska et al., 1999, 2004). The first species, *Centricnemus leucogrammus*, was originally described as *Peritelus leucogrammus* Germar, 1824, later transferred to the genus *Ctenochirus* by Seidlitz (1890) and subsequently classified as *Peritelus (Ctenochirus)* by Hoffmann (1950), Smreczyński (1966) and Dieckmann (1980). According to the latest taxonomic revision based on external morphology, this species belongs to the separate genus *Centricnemus* (Pierotti & Bellò, 1998). Regarding *Simo hirticornis*, Suomalainen (1954) confirmed the existence of triploid and tetraploid populations of this parthenogenetic species in Switzerland. Palm (1995) found that the taxon “*Simo hirticornis*” sensu various European authors was a mixture of two species, *S. hirticornis* (Herbst, 1795) and *S. variegatus* (Boheman, 1843). Both of these species occur in Switzerland.

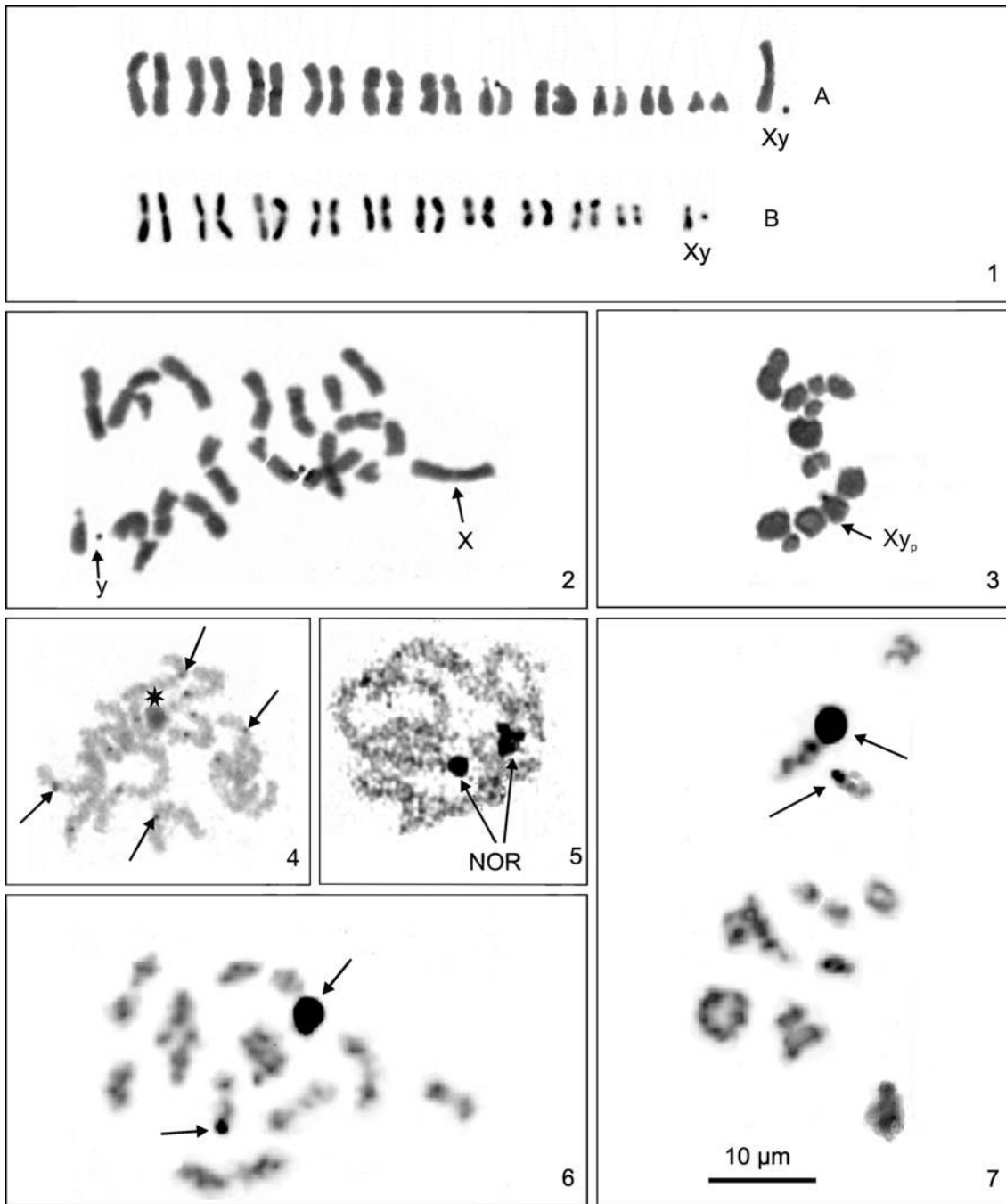
In the present study, differences in the karyology of two species of the tribe Peritelini from the family Curculionidae, *Centricnemus leucogrammus* (Germar 1824) and *Peritelus familiaris* Boheman, 1834, are described. The karyotype of *P. familiaris* is described and the differences between the species

in terms of chromosome number, sex determination system, the course of meiosis, the relative length of chromosomes, C-band patterns, and localization of active NORs, are analysed. The chromosomal data are discussed in relation to the taxonomic classification of the species.

### MATERIAL AND METHODS

For the cytogenetic study, adults of both sexes were collected in xerotherms of Poland and Slovakia in May and June 2004, 2005. Gonads 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 45–60 min at room temperature. Then the gonads were fixed using to the method described by Rožek (1994) with minor modifications (Rožek & Lachowska, 2001). C-banding was revealed using the procedure described by Sumner (1972) with some modifications. Briefly, the squashed preparations were treated with 0.3 N HCl for 1 min at 20–23°C, followed by thorough rinsing with distilled water and then air-dried. The slides were placed in a freshly prepared solution of 5% barium hydroxide at 20–23°C for 1–1.5 min. Next, they were rinsed with distilled water and incubated in 2× SSC at 50°C for 1 h and again air-dried. Then the slides were stained with 4% Giemsa in phosphate buffer (pH 6.8) for 10 to 20 min.

For the NOR silver staining, the method described by Howell & Black (1980) was used with some modifications. A brief outline of the technique is as follows. Two solutions, A and B (A – a silver nitrate solution; 4 g of AgNO<sub>3</sub> dissolved in 8 ml of deionized water; B – a gelatin solution with formic acid; 1 g gelatin dissolved in 50 ml distilled water, with stirring and gentle heating; after the gelatin dissolves, 0.5 ml of formic acid is added), were prepared and mixed in a ratio of 2A : 1B on slides with squashed chromosomes. Then the slides were covered with a coverslip, placed into a dark humid box, and incubated at 50°C for 25 min, at which time the mixture turns a



Figs 1–7: Mitotic and meiotic chromosomes of *Peritelus familiaris* and *Centricnemus leucogrammus*. 1A – karyotype of *Peritelus familiaris* and B – *Centricnemus leucogrammus*. Chromosomes stained with 4% Giemsa in phosphate buffer after C-banding technique. 2 – male mitotic metaphase of *P. familiaris* with 24 chromosomes; arrows indicate the X and y sex chromosomes. 3 – male meiotic metaphase I of *P. familiaris* with 11 autosome bivalents and a heterovalent; arrow indicates the sex chromosomes of parachute type ( $Xy_p$ ). 4 – male diplotene of *P. familiaris*; arrows indicate C-bands, asterisk indicates the heteropycnotic sex chromosomes. 5 – pachytene complement after silver staining with two active NORs in *C. leucogrammus*. 6 – male diakinesis of *P. familiaris*; arrows indicate two active NORs. 7 – male diakinesis of *C. leucogrammus*; arrows indicate two active NORs.

golden brown colour. The preparations were then rinsed with tap water, then distilled water, air-dried and mounted in Euparal. Chromosomes were classified according to Levan et al. (1964). Evaluation of chromosome morphology was based on ten mitotic metaphases (Table 1). Chromosome lengths were calculated as a percentage of total chromosome length of the haploid set (% TCL), which also includes the sex chromosomes. Spermatogonial metaphase, meiotic stages and interphase nuclei

were analyzed and photographed using a Nikon Eclipse 400 light microscope and a CCD DS-U1 camera (Nikon, Tokyo, Japan), and the software Lucia Image version 5.0 (Laboratory Imaging, Prague, Czech Republic).

## RESULTS

Relative lengths and centromeric indexes of chromosomes are given in Table 1. The diploid complement of *Peritelus famil-*

TABLE 1. Relative length (% TCL) and centromeric index (AR) of particular chromosome pairs of *Peritelus familiaris* and *Centricnemus leucogrammus*.

Pair no.	<i>Peritelus familiaris</i> 2n = 24, n♂ = 11 + Xy <sub>p</sub>		<i>Centricnemus leucogrammus</i> 2n = 22, n♂ = 10 + Xy <sub>p</sub>	
	%TCL	AR	%TCL	AR
1	10.92	1.25	12.57	1.43
2	10.91	1.33	12.68	1.08
3	10.52	1.27	11.75	1.17
4	9.72	1.25	11.53	1.09
5	8.27	1.24	9.35	1.26
6	8.16	1.77	8.45	1.26
7	7.62	3.72	8.05	1.24
8	6.22	1.23	7.35	1.28
9	5.41	3.29	6.78	1.60
10	4.92	3.52	4.25	1.76
11	3.02	7.21	–	–
X	12.93	1.82	5.75	1.23
y	1.92		1.50	

*iaris* males consisted of 22 autosomes and X and y sex chromosomes. The fundamental number of arms was FN = 47. The karyotype was asymmetric with 6 metacentric pairs of autosomes, one submetacentric pair, 3 subtelocentric pairs, and one acrocentric pair. The submetacentric X chromosome was the longest element in the set while the y chromosome was the smallest and dot like (Figs 1A, 2, 3) (Table 1). In meiotic prophase I, the sex chromosomes formed a parachute association (Xy<sub>p</sub>) and showed strong heteropycnosis (Fig. 4). Meiotic diakinesis was composed of large and medium size bivalents, five to seven rod-shaped, and one to two cross-like with one chiasma, three to five ring-shaped with two chiasmata, and Xy<sub>p</sub> (Fig. 6). The relative lengths of autosomes and heterochromosomes ranged from 12.93% to 1.92% (Table 1).

For *Centricnemus leucogrammus*, the chromosome number and meiotic behaviour is described by Petryszak (1977) and Lachowska et al. (1999, 2004). The diploid male complement consists of 20 autosomes and X and y sex chromosomes (Fig. 1B). The relative lengths of chromosomes ranged from 12.57% to 1.50% and the fundamental number of arms (FN) was 45 (Table 1).

The heterochromatin visualized by C-banding was found in the pericentromeric region of all autosomes and the X chromosome, while the y chromosome was completely heterochromatic. In *P. familiaris* and *C. leucogrammus*, all the chromosomes obtained from male germ cells showed heavily stained but small heterochromatin segments visible during pachytene and diplotene (Fig. 4) and hardly visible or undetectable during the mitotic metaphase (Figs 1 and 2), diakinesis (not shown), metaphase I (Fig. 3) and metaphase II (not shown).

Males of both species exhibited the same pattern of silver precipitation during the cell cycle. During meiotic stages two Ag-positive nucleoli could be recognized at early prophase but faded rapidly during middle prophase and disappeared completely by late prophase. In mitotic metaphase plates, the silver-impregnated blocks became invisible. One NOR was located in an autosome pair, the other NOR in the sex chromosomes (Figs 5–7).

## DISCUSSION

Cytogenetic methods have become a widespread and powerful tool for the delineation and identification of many insects, particularly in groups, in which species might be morphologically cryptic. Species-specific karyotypes, defined by chromosome number, size, morphology and sex chromosomes are usually constant and can be considered a definite character for taxonomic purposes. The value of karyotype for taxonomy and phylogenetic relationships has been demonstrated in many beetle species, e.g. Carabidae, Chrysomelidae, and Curculionidae (Serrano, 1986; Petitpierre, 1997; Rožek et al., 2004). The cytogenetic differences between the two species examined are chromosome number and morphology of the karyotypes. The diploid complement of *Centricnemus leucogrammus* consists of 22 chromosomes with little variation in morphology and length of autosomes. Karyotypes with a prevalence of meta- and submetacentric chromosomes are the rule in the karyotypic architecture of curculionids (Lachowska et al., 1998). The second species, *Peritelus familiaris*, possesses 24 chromosomes, and the karyotype is more diverse and asymmetric with a very wide array of sizes from the small dot-like y chromosome to the large X chromosome. The course of meiosis in both species is similar. The metaphase I plates consist of 10 autosomal bivalents in *C. leucogrammus* or 11 autosomal bivalents in *P. familiaris*, respectively, and the sex chromosomes form a typical parachute. The parachute association (Xy<sub>p</sub>) of the large X and small y chromosomes is characteristic for many coleopterans (Smith & Virkki, 1978). It can be concluded from all available data that the diploid chromosome number 2n = 22 is characteristic for weevils, occurring in 34% of species examined karyologically and seems to be ancestral for Curculionidae as a whole. Therefore, the karyotype evolution in *P. familiaris* might occur by centric fission of autosomes, because such rearrangements would increase the chromosome number and change chromosome morphology. In this sense, the diploid complement of *P. familiaris* is strikingly distinct from that of *C. leucogrammus* and the majority of weevils. C-banding pattern on chromosomes of *C. leucogrammus* and *P. familiaris* shows a small amount of heterochromatin as dots located around centromeres. The karyotypes of many examined members of the family Curculionidae, described from gonial cells, are characterized by a small proportion of heterochromatin, located mainly in a centromeric position (Holecová et al., 2002; Rožek et al., 2004; Lachowska et al., 2005).

Cytogenetic analysis of *Peritelus familiaris* and *Centricnemus leucogrammus* showed significant differences in karyotype structure and confirmed their present taxonomic affiliation to two separate genera (Pierotti & Bellò, 1998; Alonso-Zarazaga & Lyal, 1999). Currently, the chromosome number in conservative groups, such as the Entiminae subfamily (having the ancestral karyotype), is a very important attribute. Recent phylogenetic and evolutionary studies of beetles mainly rely on DNA analysis (Gómez-Zurita & Galián, 2005). In the future, mtDNA markers may provide additional information about the genetic relationship of both genera.

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