

Karyotype Analysis of Three Weevil Species from the Genera *Brachysomus* (Boheman, 1845) and *Strophosoma* Billberg, 1820 (Coleoptera, Curculionidae)*

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Karyotypes of three weevil species, viz. *Brachysomus dispar* ($2n=22$, $n\sigma=10+Xy_p$), *Brachysomus hirtus* ($3n=33$, $n\sigma=10+X$) and *Strophosoma faber* ($2n=22$, $n\sigma=10+Xy_p$) belonging to the tribes Sciaphilini and Brachyderini, were studied using the C-banding technique. The karyotype structures of the two bisexual and one parthenogenetic species are described for the first time. Most chromosomes are meta- or submetacentric. In the two species of the genus *Brachysomus*, the chromosomes resemble one another in having a rather small amount of heterochromatin restricted to the pericentromeric region, visible as dark stained blocks mainly during the early stages of nuclear division. Larger bands at mitotic metaphase and diakinesis occur only in *Strophosoma faber*. Geographic parthenogenesis in *Brachysomus hirtus* is briefly discussed.

Key words: Coleoptera, Curculionidae, *Brachysomus*, *Strophosoma*, karyotype, C-bands.

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The genus *Brachysomus* comprises about 48 apterous, terricolous species of minute adelognathan weevils distributed mainly in the Western Palearctic. Their ranges are usually geographically restricted. Numerous species live in the Balkans. A considerable number of species are concentrated along the lower altitudes of the Carpathians, many are very local, being restricted to particular calcareous ranges or steppe and forest-steppe refuges in Central and Eastern Europe. Several endemic species are found only in Asia Minor and the Caucasus, four *Brachysomus* species inhabit the mountains of Crimea. Only *B. echinatus* has a Holarctic range. Second in distribution size is *B. hirtus*, occurring in southern parts of Europe to the Northwestern Caucasus. *B. echinatus* and *B. hirtus* have vast ranges owing to the expansions of parthenogenetic populations. *B. echinatus* is known only as a parthenogenetic form, while a bisexual

form of *B. hirtus* was discovered in the Dinaric Alps (FORMÁNEK 1905). No parthenogenetic form of any of the other species of *Brachysomus* is known. All members of this group are probably polyphagous, possibly even saprophagous, feeding on decaying leaves. Many have cryptic life habits. Several species have been very recently discovered in Europe, or resurrected from synonymy after revisions of museal material (KOŠTÁL 1991a, b, 1992; BENEDIKT 2001; WANAT & MAZUR 2005a; YUNAKOV 2006). The cytogenetic makeup of *Brachysomus* is poorly known. So far the karyotypes of only 2 species of this genus, *B. echinatus* and *B. setiger*, have been studied (LACHOWSKA *et al.* 2006a and in press).

The genus *Strophosoma* includes over 45 species inhabiting the Palearctic region, mainly the Iberian Peninsula and the western part of North Africa. Six species have been found to date in

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Central Europe (SMRECZYŃSKI 1966; DIECKMANN 1980; LUCHT 1987). Adult beetles are mostly generalists feeding on various herbaceous and woody plants although several species are associated with plants of the genera *Calluna* and *Erica*. Alpha-karyological studies concerning this genus have been carried out only on the parthenogenetic triploid species *Strophosoma melanogrammmum* and the bisexual species *S. capitatum* (SUOMALAINEN 1940a; 1940b; 1947; 1954; 1966). C-banding patterns in chromosomes of the aforementioned species have been described in previous papers (ROŽEK & HOLECOVÁ 2000; LACHOWSKA *et al.* in press).

The present paper is a continuation of investigations on the karyology of Palaearctic weevils. The authors report on the karyotype of *Brachysomus dispar*, *B. hirtus* and *Strophosoma faber* using the C-banding technique.

Material and Method

Weevil adults were collected in Slovakia in 2006 and 2007 (Table 1). The species were diagnosed

Table 1
Chromosomally examined species of weevils

Tribe Species	Geographic source and date of collection
Brachyderini	
<i>Brachysomus dispar</i> Penecke, 1910	C Slovakia, Strážovské vrchy Mts., Strážov Nature Reserve, June 9 and 16, 2006
<i>Brachysomus hirtus</i> (Boheman, 1845)	SW Slovakia, Malé Karpaty Mts., Naháč, June 1 and 6, 2007
Sciaphilini	
<i>Strophosoma faber</i> (Herbst, 1795)	SW Slovakia, Borská nížina lowland, Pernek, July 7, 2006

according to ALONZO-ZARAZAGA and LYAL (1999) and WANAT and MOKRZYCKI (2005b). Vouchers were deposited in the Institute of Systematics and Evolution of Animals PAS. Gonads (eight to 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 ROŽEK (1994) with minor modification (ROŽEK & LACHOWSKA 2001). C-banding was performed using the procedure described by SUMNER (1990) with some modifications (LACHOWSKA *et al.* 2006b). The slides were stained with 4% Giemsa phosphate buffer (pH 6.8) for 10 to 20 min. Evaluation of chromosome morphology was based on ten mitotic metaphases (Table 2). Chromosome lengths were calculated as the percentage of the total chromosome length of the haploid set (% TCL), which also includes the sex chromosome. 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 software Lucia Image version 5.0 (Laboratory Imaging, Prague, Czech Republic).

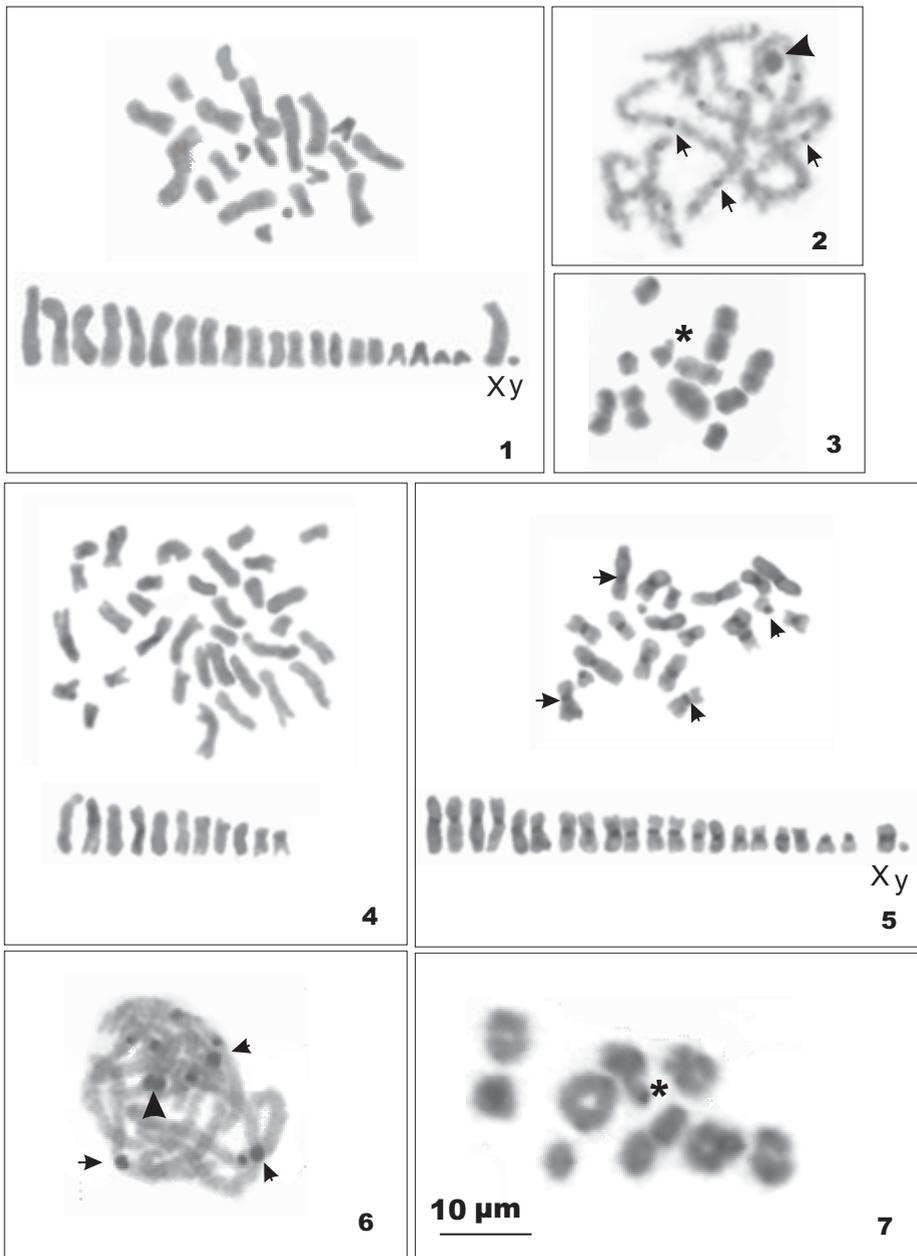
Results and Discussion

The karyotypes of the two bisexual and one parthenogenetic species are characterized by the prevalence of meta- and submetacentric chromosomes, a condition which is almost the rule in the karyotypic architecture of weevils (LACHOWSKA *et al.* 2006a).

Table 2

Relative length (%TCL) and centromeric index (AR) of particular chromosome pairs

Pair No.	<i>Brachysomus dispar</i> – ♂ 2n = 22 n = Xy _p		<i>Strophosoma faber</i> – ♂ 2n = 22 n = Xy _p		Pair No.	<i>Brachysomus hirtus</i> – ♀ parthenogenetic species 3n = 33	
	mitotic metaphase		mitotic metaphase			mitotic metaphase	
	%TCL	AR	%TCL	AR		%TCL	AR
1	15.04	1.27	14.95	1.55	1	13.68	4.48
2	11.72	1.08	12.87	1.58	2	13.21	3.17
3	11.52	1.16	10.09	1.03	3	11.54	3.23
4	9.90	1.20	9.38	1.05	4	10.84	1.28
5	8.11	1.05	9.03	1.10	5	9.51	1.27
6	7.09	1.02	8.96	1.11	6	8.52	1.01
7	6.94	1.07	7.45	1.01	7	7.36	1.44
8	6.28	1.03	6.94	1.09	8	7.19	1.21
9	5.12	2.76	6.62	1.15	9	6.26	1.45
10	2.78	–	4.54	–	10	6.14	1.60
X	13.27	2.96	7.00	1.05	11	5.74	1.41
y	2.23	–	2.14	–	–	–	–



Figs 1-3. Chromosomes of *Brachysomus dispar* after C-banding. Fig. 1. Mitotic metaphase and karyotype. Fig. 2. Diplotene, small arrows show short segments of heterochromatin, large arrow shows heteropycnotic sex-chromosomes. Fig. 3. Metaphase I, star shows Xy_p association. Fig. 4. Mitotic metaphase and haploid set of chromosomes of *Brachysomus hirtus* after C-banding. Figs 5-7. Chromosomes of *Strophosoma faber* after C-banding. Fig. 5. Mitotic metaphase and karyotype. Fig. 6. Pachytene, small arrows show wide segments of heterochromatin, large arrow shows the heteropycnotic sex-chromosomes. Fig. 7. Diakinesis, star shows Xy_p association. Bar = $10\mu\text{m}$.

Brachysomus dispar ($2n=22$, $n^\sigma=10+Xy_p$). The male diploid complement consists of 20 autosomes and X and y sex chromosomes. The karyotype is symmetric with chromosomes forming a series decreasing in size. Eight pairs of autosomes are metacentric, one is submetacentric, and one acrocentric. The X chromosome is metacentric and similar in size to the longest autosomes, whereas the y chromosome is dot-like (Fig. 1). The relative length of the autosomes is 2.23%-15.04% (Table 2). During male meiotic stages autosomal bivalents

are connected by one terminal or interstitial chiasmata forming rod-shaped elements, the sex chromosomes form non-chiasmatic associations of the parachute type (Fig. 3). All chromosomes display short bands of heterochromatin visible during the early stages of nuclear division but are undetectable at spermatogonial metaphase (Fig. 2).

The karyotype of *Brachysomus dispar* is similar to its congeneric species *Brachysomus setiger* examined by LACHOWSKA *et al.* (2006a). The congeners are characterized by the same diploid

complement of 22 chromosomes and the meioformula $n=10+Xy_p$ which seems ancestral for all adelnathan curculionids (SMITH & VIRKKI 1978; SHARMA *et al.* 1980; LACHOWSKA *et al.* 1998) and confirms the karyological conservatism of the examined genus. Both studied species have a symmetric karyotype with a predominance of meta- and submetacentric chromosomes; sex determination as well as the small amount of heterochromatin are visible only during prophase stages.

Brachysomus hirtus ($3n=33$). During oögonial division in this parthenogenetic species 27, 28, 29, 30 or 33 chromosomes were observed, but the haploid set consists of 11 chromosomes. The karyotype is symmetric with a majority of metacentric (pairs 4th-11th) and submetacentric chromosomes (1st-3rd) of relative length ranging between 5.74%-13.68% (Fig. 4, Table 2). Sex chromosomes are likewise three in number, however, they could not be distinguished from the autosomes. C bands were not observed in the chromosomes of mitotic metaphase plates.

According to the present state of knowledge a haploid number of $n=11$ occurs in bisexual congeners of *Brachysomus hirtus* (LACHOWSKA *et al.* 2006a). The present study confirms that triploidy is the most common level of ploidy within Curculionidae, as well as in other insect groups having apomictic parthenogenesis. Excluding the Polish and Finnish populations of *Polydrusus mollis* and the Japanese curculionid *Scepticus insularis* with 22 chromosomes, parthenogenetic weevils are comprised of 53 triploids having 33 or 30 chromosomes, 18 tetraploids with 44 chromosomes, 6 pentaploids with 55 chromosomes, three hexaploids carrying 66 chromosomes and one decaploid (SAURA *et al.* 1993; LACHOWSKA *et al.* in press). Substantial evidence shows that related bisexual and parthenogenetic forms have different geographic distributional patterns. Parthenogens quite often show a tendency to inhabit "marginal" environments, including high latitudes and altitudes, xeric environments and islands, when compared to their close bisexual counterparts, representing the so-called "geographic parthenogenesis", first noted by VANDEL (1928). This pattern is often assumed to reflect an association between parthenogenesis and environments that were strongly affected by the Late Pleistocene glacial cycles (SUOMALAINEN *et al.* 1987; KOROTYAEV & MELESHKO 1995; NOKKALA *et al.* in press). A characteristic example of geographic parthenogenesis is *Brachysomus hirtus* analysed in the present paper. Parthenogenetic races of this xerothermophilous species inhabit territory from England to the Northwestern Caucasus, while its bisexual form lives in a restricted area and at higher altitudes (YUNAKOV 2006).

Strophosoma faber ($2n=22$, $n\sigma=10+Xy_p$). The symmetric karyotype is composed of 9 pairs of metacentric and one pair of acrocentric chromosomes, a metacentric X chromosome, and dot-like y chromosome (Fig. 5). The chromosomes make up 2.14%-14.95% of relative length (Table 2). During diakinesis the majority of autosomes form eight or nine ring-shaped bivalents connected by two chiasmata. The remaining, smaller bivalents are rod-shaped with one chiasma, the sex chromosomes present the "parachute" association. The C-banding patterns are characterized by the presence of pericentromeric C-bands in all autosomes and the X chromosome (Figs 5-6). The karyotype and behaviour of sex chromosomes of *Strophosoma faber* agrees with the modal curculionid formula and previously analysed karyotypes of species belonging to the subgenus *Strophosoma* s. str. (ROŽEK & HOLECOVÁ 2000; LACHOWSKA *et al.* in press).

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