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Karyotypic Characterization of Three Weevil Species (Coleoptera: Curculionidae, Brachyderini)*

Dorota LACHOWSKA, Maria ROŻEK and Milada HOLECOVÁ

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Karyotypes of three species, *Brachyderes incanus, Brachysomus setiger* and *Paophilus afflatus,* belonging to the tribe Brachyderini, were studied using C-banding technique. The species share the same chromosome number 2n=22 and meioformula n=10+Xy_p at all metaphase I plates of spermatid division. Some differences between karyotypes were observed in terms of centromere positions and C-band sizes. Most chromosomes are meta- or submetacentric and form a graded series in respect to length. The chromosomes resemble one another in having a rather small amount of heterochromatin restricted to the pericentromeric region and visible as dark stained blocks mainly during early stages of nuclear division. Only in *Brachyderes incanus* do larger bands occur at mitotic metaphase and diakinesis. These cytogenetic data are in agreement with karyological findings obtained in other species of Brachyderini so far examined.

Key words: Coleoptera, Curculionidae, karyotype, C-bands.

Dorota LACHOWSKA, Maria ROŻEK, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.
E-mail: lachowska@isez.pan.krakow.pl
rozek@isez.pan.krakow.pl
Milada HOLECOVÁ, Department of Zoology, Comenius University, Mlynská dolina B-1, 842-15 Bratislava, Slovakia.
E-mail: holecova@fns.uniba.sk

The Curculionidae is one of the largest beetle families with some 50,000 described species. So far, about 600 species of Curculionidae have been karyologically investigated, but the great majority of cytogenetic findings on weevils refer to the male chromosome numbers and sex determining 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 papers present the banded karyotypes of curculionids (HSIAO & HSIAO 1984; HOLECOVÁ *et al.* 1997, 2002; ROŻEK & HOLECOVÁ 2000; ROŻEK *et al.* 2004; LACHOWSKA *et al.* 2004, 2005). Knowledge of the karyology in Curculionidae varies greatly from genus to genus and from subfamily to subfamily. Many species-rich genera exist in which karyology has not been examined. The tribe Brachyderini includes 76 species distributed mainly in the Palaearctic region from which 13 species hitherto have been karyologically examined (MIKULSKA 1953; PETRYSZAK 1972; LACHOWSKA *et al.* 1998, 2005; LACHOWSKA & HOLECOVÁ 2000; HOLECOVÁ *et al.* 2002, 2005).

The present paper is a continuation of investigations concerning the karyology of Palaearctic weevils. The authors have studied the karyotype of *Brachysomus setiger* (Gyllenhal, 1840), *Brachyderes incanus* (Linnaeus, 1758) and *Paophilus afflatus* (Boheman, 1833) C-banding technique. The aim of this study was to analyse the C-banding patterns in the karyotypes of three species and to describe chromosome numbers, meiotic behaviour, and sex determining systems for two bisexual curculionids examined for the first time.

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Table 1

Chromosomally examined species of weevils

Species	Geographic source and date of collection	Chromosome number	References
Brachyderes incanus (Linnaeus, 1758)	SW Slovakia, Borská nížina lowland, Moravský Sv. Ján, August 6, 2005	2n=22 n♂=10+Xy_p	LACHOWSKA & HOLECOVÁ (2000), Present study
Brachysomus setiger (Gyllenhal, 1840)	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla Nature Reserve, May 20, 2005	2n=22 n♂=10+Xy_p	Present study
Paophilus afflatus (Boheman, 1833)	SE Poland, Pogórze Przemyskie, Żurawica, June 2, 2005	2n=22 n♂=10+Xy_p	Present study

Material and Methods

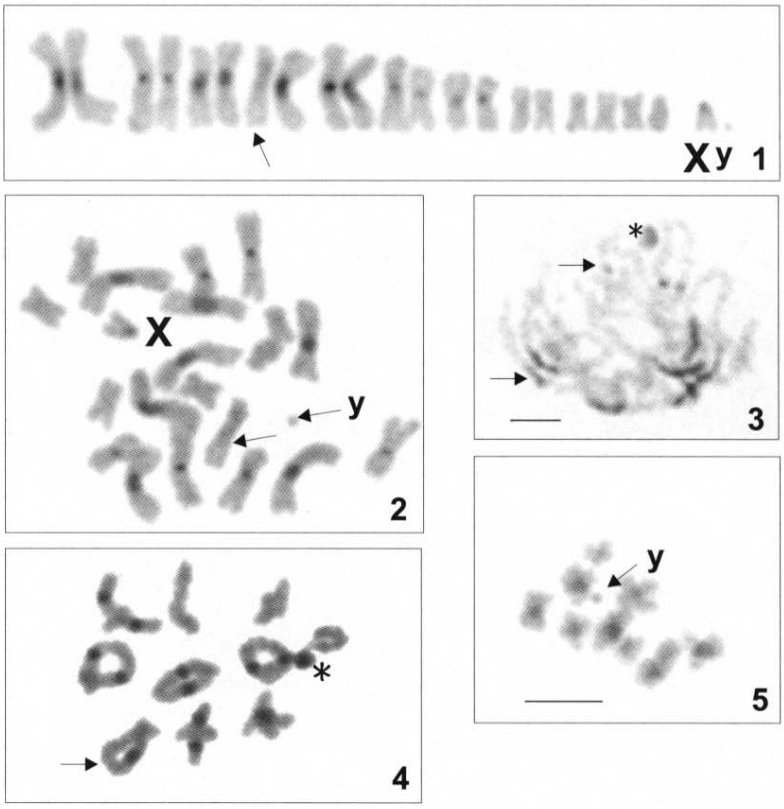
For the cytogenetic study, adults of both sexes were collected in SW Slovakia and SE Poland in May and June 2005 (Table 1). The systematics of Curculionidae was assumed after ALONSO-ZARAZAGA & LYAL (1999). Gonads were dissected under a stereomicroscope in several drops of hypotonic 0.9 % sodium citrate 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 according to the method described by ROŻEK (1994) with minor modification (ROŻEK & LACHOWSKA 2001). C-banding was performed 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 air-drying. 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 2xSSC at 50°C for 1h and again air-dried. Then the slides were stained with 4% Giemsa phosphate buffer (pH 6.8) for 10 to 20 min. Spermatogonial metaphases, mitotic and meiotic stages were analyzed and photographed with a Nikon Eclipse 400 light microscope and CCD DS-U1 (Nikon) camera using the software Lucia Image version 5.0 (Laboratory Imaging, Prague, Czech Republic). The material is deposited in the Institute of Systematics and Evolution of Animals Polish Academy of Sciences (Kraków).

Results and Discussion

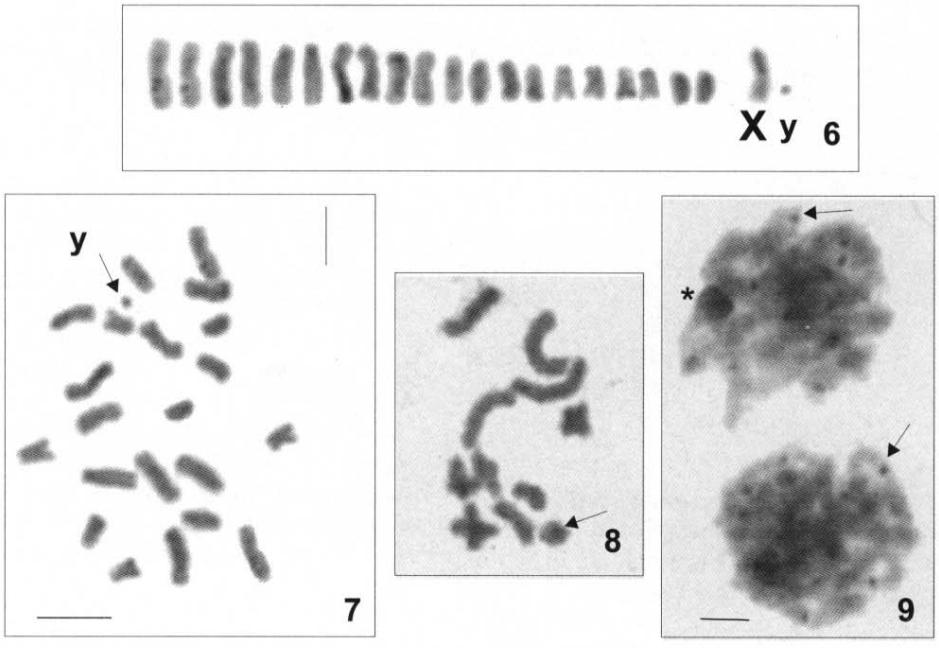
In three examined species the same chromosome number 2n=22 was observed at spermatogonial metaphases. The meioformula n = 10+Xy_p was identical at all metaphase I plates of spermatid division. During meiotic stages, from late prophase to metaphase I, the large X chromosome and small y chromosome form non-chiasmatic associations of the parachute type (Figs 3, 8, 9, 12). Some differences between karyotypes are related to the morphology of chromosomes, eg. the locations of centromeres or C-band patterns.

Brachyderes incanus – the male karyotype consists of 20 metacentric autosomes of which 12 are long and the remaining 8 are medium size. The X chromosome is acrocentric, similar in size to medium autosomes, whereas the y chromosome is dot-like and is the smallest element in the set (Figs 1, 2, 5). The number of chromosome arms (Fundamental Number) is FN = 42. The meiotic behaviour is in agreement with that previously described by LACHOWSKA & HOLECOVÁ (2000). Five ring-shaped bivalents, one rod-shaped bivalent, four cross-shaped bivalents and the sex heterochromosomes occur at the diakinesis plate (Fig. 4). Application of the C-banding technique revealed seven pairs of longer autosomes with pericentromeric heterochromatin bands of different size at spermatogonial metaphase. Within this group the fourth heteromorphic pair is clearly distinguishable because of a lack of a C-band on one chromosome (Figs 1-2). Probably the homologous autosome possesses a small amount of heterochromatin faintly stained during this stage of division. The autosomes of the next three pairs (8 to 10) also do not show C-bands, indicating that in these chromosomes the blocks of heterochromatin are also very short. The existence of longer and shorter heterochromatic blocks on chromosomes was confirmed by observations of spermatocyte division stages, eg. pachytene, diakinesis and metaphase II (Figs 3-5). The acrocentric X chromosome is heterochromatic only in the centromeric region, while the y chromosome is entirely euchromatic (Figs 1-2).

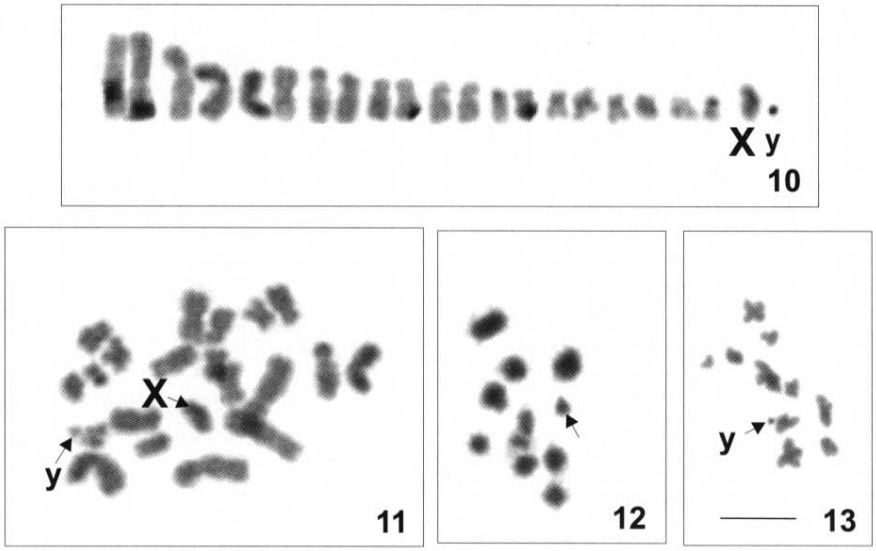
Brachysomus setiger – characterized by a symmetrical karyotype with chromosomes forming a series decreasing in size. Most autosomes possess the centromere at a medial or submedial position



Figs 1-5. Chromosomes of *Brachyderes incanus* after C-banding. Fig. 1. Karyotype of *Brachyderes incanus*, arrow indicates chromosome of heteromorphic pair. Fig. 2. Mitotic metaphase, arrow indicates a chromosome without C-bands. Fig. 3. Pachytene bouquet, asterisk indicates Xy_p, arrows indicate short and long bands of heterochromatin. Fig. 4. Diakinesis, asterisk indicates Xy_p, arrow indicates the heteromorphic pair of autosomes. Fig. 5. Metaphase II. Bar = 5 μm.



Figs 6-9. Chromosomes of *Brachysomus setiger* after C-banding. Fig. 6. Karyotype of *Brachysomus setiger*. Fig. 7. Mitotic metaphase. Fig. 8. Diakinesis, arrow indicates Xy_p. Fig. 9. Pachytene, small arrows indicate short bands of heterochromatin, asterisk indicates Xy_p. Bars = 5 μm.



Figs 10-13. Chromosomes of *Paophilus afflatus* after C-banding. Fig. 10. Karyotype of *Paophilus afflatus*. Fig. 11. Mitotic metaphase. Fig. 12. Metaphase I, arrow indicates Xy_p. Fig. 13. Metaphase II. Bar = 5 μm.

with the exception of the third and ninth pairs which are subtelocentric. The metacentric X chromosome belongs to the group of long elements, the tiny y chromosome represents the smallest component in the karyotype (Figs 6-7). The Fundamental Number is FN = 43. During diakinesis seven rod-shaped bivalents with one chiasma, three cross-shaped bivalents also with one chiasma, and the non-chiasmata sex bivalent Xy_p can be observed (Fig. 8). All chromosomes display short bands of heterochromatin visible only during the initial stages of nuclear division (Fig. 9).

Paophilus afflatus – diakinesis and meiotic metaphase I plates include nine unichiasmate bivalents (3 crosses and 6 rods), one bichiasmate ring, and a nonchiasmate Xy_p association (Fig. 12). The spermatogonial metaphases consist of 20 meta- and submetacentric autosomes forming a graded series in respect to length, a metacentric X chromosome of size similar to medium autosomes, and a dot-like y chromosome (Figs 10-11). The Fundamental Number is FN = 43. All chromosomes possess a small amount of heterochromatin visible during early stages of nuclear division (as in Fig. 9) and undetectable at spermatogonial metaphase (Figs 10-13).

The karyotypes of these three species are broadly similar to one another in the number of chromosomes and in sex determination. The diploid complement of 22 chromosomes and the meioformula $n = 10+Xy_p$ is ancestral for all of Curculionidae since about 42% of 600 surveyed species show this karyotype (LACHOWSKA *et al.* 1998). Among all examined species from the tribe Brachyderini, only the endemic *Barypeithes liptoviensis* possesses two additional pairs of autosomes. Also, the parthenogenetic *Eusomus ovulum*, *Foucartia squamulata* and *Sciaphilus asperatus* differ from the modal value of 22 chromosomes and represent triploid forms with 33 elements. In weevils there is a very wide array of magnitudes from the smallest dot-like y chromosomes to the largest X heterochromosomes or autosomes. The present results show that the size and morphology of chromosomes do not differ significantly from those reported in other Brachyderini, most chromosomes are meta- or submetacentric, a condition which is almost the rule in the karyotypic architecture in weevils. The application of the C-band technique evidences a defined pattern of bands. In *Brachyderes incanus, Brachysomus setiger,* and *Paophilus afflatus* the chromosomes resemble one another in having C-bands restricted to the area around the centromere, characteristic for the majority of insects (IMAI 1999). In Curculionidae heterochromatin occurs mainly in small proportions and very often when the chromosomes become more condensed during the mitotic metaphase, diakinesis, metaphase I and II, these short segments are weakly or not visible (ROŻEK *et al.* 2004; LACHOWSKA *et al.* 2005). Therefore in this group of beetles C-banding patterns cannot always be used in taxonomic investigations and for differentiation of similar karyotypes, explaining the scarce data concerning C-banded chromosomes of weevils.

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