

Notes on Chromosome Numbers and C-banding Patterns in Karyotypes of Some Weevils from Central Europe (Coleoptera, Curculionoidea: Apionidae, Nanophyidae, Curculionidae)

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Chromosome numbers and C-banding patterns of sixteen weevil species are presented. The obtained results confirm the existence of two groups of species with either a small or large amount of heterochromatin in the karyotype. The first group comprises twelve species (Apionidae: *Oxystoma cerdo*, *Eutrichapion melancholicum*, *Ceratapion penetrans*, *Ceratapion austriacum*, *Squamapion flavimanum*, *Rhopalapion longirostre*; Nanophyidae: *Nanophyes marmoratus*; Curculionidae: *Centricnemus* (= *Peritelus*) *leucogrammus*, *Sitona humeralis*, *Sitona lineatus*, *Sitona macularis*, *Sitona suturalis*). In weevils with a small amount of heterochromatin, tiny grains on the nucleus during interphase are visible, afterwards appearing as dark dots during mitotic and meiotic prophase. The second group comprises four species from the curculionid subfamily Cryptorhynchinae (*Acalles camelus*, *Acalles commutatus*, *Acalles echinatus*, *Ruteria hypocrita*) which possess much larger heteropycnotic chromosome parts visible during all nuclear divisions. The species examined have pericentromeric C-bands on autosomes and on the X chromosome.

Key words: Curculionoidea, Apionidae, Nanophyidae, Curculionidae, C-bands, chromosome number.

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The cytogenetic distribution of heterochromatin can be visualized by the C-banding technique. Chromosome banding analysis is useful in establishing the nature of chromosomal differences, but unfortunately most karyotypic data have been obtained by standard analysis, only a minor fraction of papers have analysed banded karyotypes of weevils (HOLECOVÁ *et al.* 1997, 2002; HSIAO & HSIAO 1984; ROŽEK & HOLECOVÁ 2000; ROŽEK *et al.* 2004).

The present paper is a continuation of investigations on the C-banding patterns of Palaearctic weevils. The aim of our study is (1) to describe chromosome numbers for 8 weevil species examined for the first time, (2) to analyse the C-banding patterns on chromosomes of 16 species, and (3) to investigate whether the small amount of heterochromatin is characteristic for genus, tribe or family.

The systematics of Curculionoidea are based on STREJČEK (1993) and ALONSO-ZARAZAGA and LYAL (1999).

Material and Methods

For the cytogenetic study, adult males were collected in forest and grassland habitats of Slovakia and Poland from June to October 2003. Their gonads were dissected and used as material for squashes. The gonads were fixed according to the method described by ROŽEK (1994) with minor modification (ROŽEK & HOLECOVÁ 2000; ROŽEK & LACHOWSKA 2001). C-bands were determined using modifications of the procedure described by ROŽEK *et al.* (2004). The squashed slides were treated with a 0.3 N HCl at 20-23°C for 1 min and with a freshly prepared solution of 5% barium hydroxide at 20-23°C for 2-3 min. Next, they were rinsed with distilled water and incubated in 2xSSC at 50°C for 1 h. Dry slides were stained with 4% Giemsa for 10 to 20 min. Observations of chromosomes and photomicrographs were made using a Jenaval light microscope (C. Zeiss, Jena). The ma-

terial is deposited in the Institute of Systematics and Evolution of Animals (Kraków).

Results and Discussion

Chromosome numbers were analysed in sixteen weevil species from the families Apionidae, Nanophyidae and Curculionidae (Table 1). The chromosome numbers of eight species – *Rhopalapion longirostre*, *Oxystoma cerdo*, *Acalles camelus*, *Acalles echinatus*, *Ruteria hypocrita*, *Centricnemus* (= *Peritelus*) *leucogrammus*, *Sitona lineatus*, *Sitona humeralis*, were described earlier (TAKENOUCHI 1974; PETRYSZAK 1977; HOLECOVÁ *et al.* 1999a, b; LACHOWSKA *et al.* 1999, 2001).

Eight Apionidae species: *Eutrichapion melancholicum*, *Ceratapion penetrans*, *Ceratapion austriacum*, *Squamapion flavimanum*; Nanophyidae: *Nanophyes marmoratus*; Curculionidae: *Acalles commutatus*, *Sitona macularis*, *Sitona suturalis* were investigated for the first time.

In Apionidae meiotic stages were exclusively observed in the preparations. All species examined possess the same chromosome number: $n\sigma=10+Xy_p$, $2n=22$ (Figs 3-5, 7). The sex chromosomes were connected achiasmatically (Figs 3-5, 7). In *Oxystoma cerdo*, metaphase II was observed (Fig. 2). In this species the chromosomes were metacentric, with slight differences in length. The X chromosome was the longest, whereas the y chromosome was the smallest element in the set. These results

Table 1

Species of weevils in which chromosomes were examined

Family, subfamily, tribe Species	Geographic source and date of collection	Chromosome number	References
Family: Apionidae			
<i>Oxystoma cerdo</i> (Gerstaecker, 1854)	S Poland, Zawoja, June 13, 2003	$2n=22$ $n\sigma=10+Xy_p$	HOLECOVÁ <i>et al.</i> 1999b
<i>Eutrichapion melancholicum</i>	SW Slovakia, Borská ní ina lowland, Devínska Nová Ves, August 21, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
<i>Ceratapion penetrans</i> (Germar, 1817)	SW Slovakia, Borská ní ina lowland, Devínske Jazero, August 21, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
<i>Ceratapion austriacum</i> (Wagner, 1904)	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, August 18, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
<i>Squamapion flavimanum</i> (Gyllenhal, 1833)	SW Slovakia, Borská ní ina lowland, Devínske Jazero, August 21, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
<i>Rhopalapion longirostre</i> (Oliver, 1807)	SW Slovakia, Bratislava env., June 9, 2003	$2n=22$ $n\sigma=10+Xy_p$	HOLECOVÁ <i>et al.</i> 1999a
Family: Nanophyidae			
<i>Nanophyes marmoratus</i> (Goeze, 1777)	SW Slovakia, Borská ní ina lowland, Devínske Jazero, Juli 14, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
Family: Curculionidae			
Subfamily: Cryporrhynchinae			
Tribe: Cryptorhynchini			
<i>Acalles camelus</i> (Fabricius, 1792)	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, September 28, 2003	$2n=30$ $n\sigma=14+Xy_p$	LACHOWSKA <i>et al.</i> 2001
<i>Acalles commutatus</i> Dieckmann, 1982	SW Slovakia, Malé Karpaty Mts., Lozorno, October 19, 2003	$2n=28$ $n\sigma=13+Xy_p$	Present study
<i>Acalles echinatus</i> (Germar, 1824)	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, October 12, 2003	$2n=30$ $n\sigma=14+Xy_p$	LACHOWSKA <i>et al.</i> 2001
<i>Ruteria hypocrita</i> (Boheman, 1837)	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, October 4, 2003	$2n=30$ $n\sigma=14+Xy_p$	LACHOWSKA <i>et al.</i> 2001
Subfamily: Entiminae			
Tribe: Peritelini			
<i>Centricnemus</i> (= <i>Peritelus</i>) <i>Leucogrammus</i> Germar, 1824	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, August 15, 2003	$2n=22$ $n\sigma=10+Xy_p$	PETRYSZAK 1977, LACHOWSKA <i>et al.</i> 1999
Sitonini			
<i>Sitona humeralis</i> Stephens, 1831	SW Slovakia, Borská ní ina lowland, Devínske Jazero, August 21, 2003,	$2n=22$ $n\sigma=10+Xy_p$	HOLECOVÁ <i>et al.</i> 1999a
<i>Sitona lineatus</i> (Linnaeus, 1758)	SW Slovakia, Borská ní ina lowland, Závod-Abrod, August 15, 2003	$2n=22$ $n\sigma=10+Xy_p$	TAKENOUCHI 1974
<i>Sitona macularis</i> (Marsham, 1802)	SW Slovakia, Borská ní ina lowland, Závod-Abrod, August 15, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study
<i>Sitona suturalis</i> Stephens, 1831	SW Slovakia, Malé Karpaty Mts., Devínska Kobyla, August 18, 2003	$2n=22$ $n\sigma=10+Xy_p$	Present study

confirmed an earlier observation (HOLECOVÁ *et al.* 1999b). In *Ceratapion austriacum* metaphase II was constituted by meta-, submeta- and subtelo-centric chromosomes. The X chromosome was submetacentric and the longest, while the y chromosome was subtelo-centric (Fig. 6).

The C-banding results revealed that species from the family Apionidae possess a small amount of heterochromatin. Short, heterochromatic bands were visible in prophase stages (Figs 1 & 8).

In Nanophyidae only prophase and metaphase I stages with meioformula $n\sigma=10+Xy_p$ were observed in *Nanophyes marmoratus*. A small amount of heterochromatin was visible as in Apionidae.

In Curculionidae three species from the genus *Acalles*, one from *Ruteria*, one from *Centricnemus* (= *Peritelus*) and four from *Sitona* were analysed.

In *Acalles* and *Ruteria*, 30 chromosomes with meioformula $n\sigma=14+Xy_p$ were observed. The karyotypes were asymmetric with meta-, submeta- and subtelo-centric chromosomes (Figs 17-20).

Acalles camelus – two long metacentric pairs of autosomes, one pair of long submetacentric, one pair of small metacentric, one pair of submetacentric, and nine pairs of subtelo-centric autosomes were observed. The X chromosome was long and metacentric, while the y chromosome was dot-like (Fig. 10). During diakinesis it was possible to distinguish 4 rings, 10 rods, and parachute type sex heterochromosomes (Fig. 17).

Acalles commutatus – the male diakinetik plates contained 5 rings, 2 crosses, 7 rods and Xy_p were visible (Fig. 19).

Acalles echinatus – the karyotype comprised two long pairs of metacentric autosomes, two long submetacentric pairs, two smaller pairs of metacentric, eight pairs of subtelo-centric autosomes and a submetacentric X sex chromosome. The y chromosome was dot-like and it was the smallest element in the set. In diakinesis two rings, one cross, 11 rods and Xy_p were clearly distinguishable (Fig. 20).

In all examined species, wide pericentromeric blocks of heterochromatin were observed during nuclear divisions (Figs 10-20). In *A. commutatus*, subterminal bands were also visible during diakinesis on the first pair of autosomes (Fig. 19).

Species from the genus *Acalles* were characterized by the presence of karyotypes with different numbers of metacentric, submetacentric, and subtelo-centric chromosomes – a lesser number of meta- and submetacentric autosomes, and a greater number of subtelo-centric autosomes. It seems that pericentric inversions and translocations played important roles during the karyotype evolution of

Acalles. The present karyological observations confirm the hypothesis that *A. commutatus* and *A. echinatus* are sibling species differing in morphology of male genitalia, habitat (microhabitat) preference and also in karyology.

From the genus *Centricnemus* only one species – *C. leucogrammus*, was analysed and the results confirmed earlier observations (PETRYSZAK 1977; LACHOWSKA *et al.* 1999). This species has a small C-bands in pachytene (Fig. 21).

Four examined species from the genus *Sitona*: *S. humeralis*, *S. lineatus*, *S. macularis*, and *S. suturalis*, possessed the same chromosome number and meioformula $n\sigma=10+Xy_p$, a small amount of heterochromatin was observed (Figs 21-24).

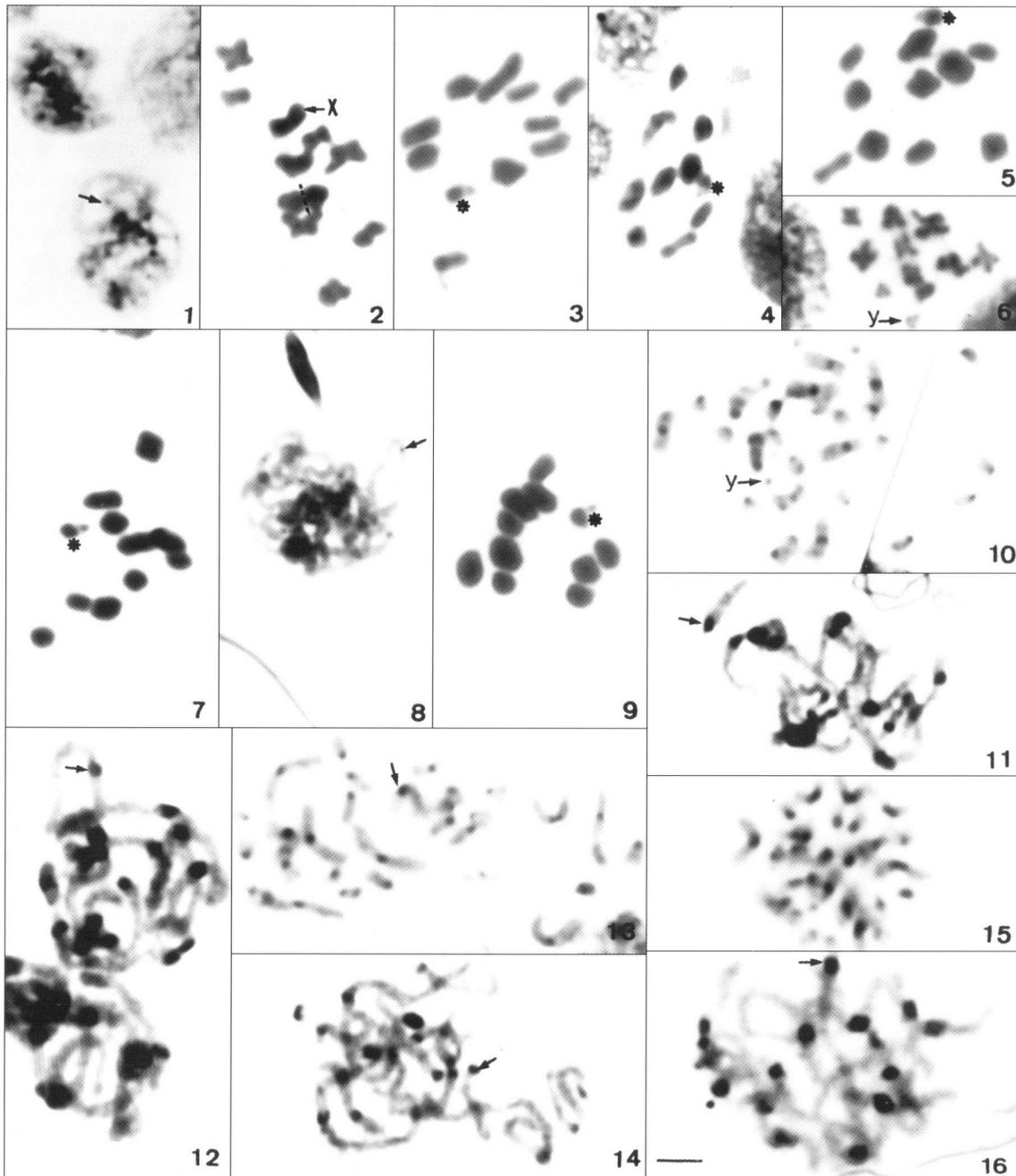
According to the authors' observations, twelve weevils surveyed here share the diploid complement of 22 chromosomes, and the meioformula $n\sigma=10+Xy_p$ (Table 1). This is the most characteristic chromosome number for weevils and seems to be ancestral for Apionidae and Curculionidae – group Adelognathi (SMITH & VIRKKI 1978; SHARMA *et al.* 1980; LACHOWSKA *et al.* 1998).

The results clearly show that there are two groups of species with either a small or large amount of heterochromatin in the karyotype. The first group is more numerous and comprises twelve species – all examined Apionidae, Nanophyidae and the tribes Peritelini and Sitonini (Curculionidae, Entiminae) (Table 1).

The C-banded technique revealed that in beetles with a small amount of heterochromatin in interphase, tiny grains on the nucleus are visible. When the chromosomes become more condensed – in mitotic metaphase, diakinesis, metaphase I and II, these short heterochromatic segments localized in the centromeric regions are weakly or not visible under a light microscope.

The second group comprises four species of the Cryptorhynchinae subfamily (*Acalles camelus*, *Acalles echinatus*, *Acalles commutatus*, *Ruteria hypocrita*) which possess much larger heteropycnotic parts of chromosomes visible during all nuclear divisions. The examined species have pericentromeric C-bands on autosomes and the X chromosome, except for *A. camelus*, which also has subterminal bands on the first pair of autosomes. The y heterochromosome is dot-like and entirely euchromatic in *A. camelus*, *A. commutatus* and *Ruteria hypocrita*, while in *A. echinatus* it is partly heterochromatic (Figs 17-20).

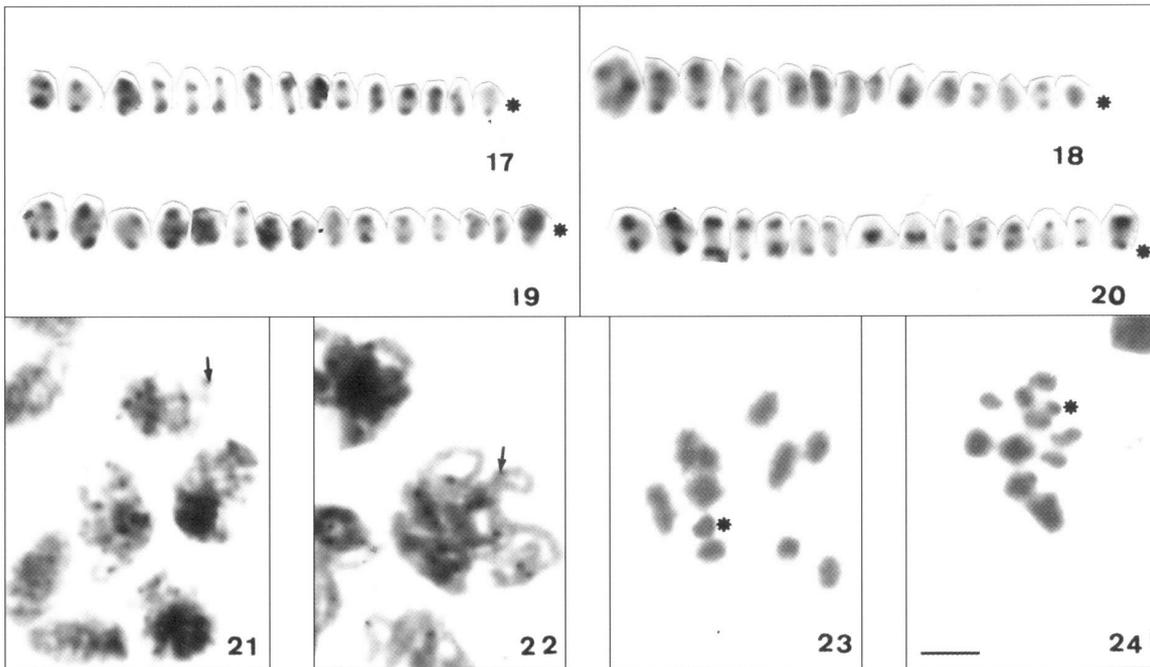
Up to now C-banding patterns have been described on chromosomes of 37 weevil species (HSIAO & HSIAO 1984; HOLECOVÁ *et al.* 1997; ROŽEK & HOLECOVÁ 2000, 2002, ROŽEK *et al.* 2004, and the present study). Only eleven exam-



Figs 1-16. Meiotic, mitotic chromosomes and C-bands of Apionidae, Nanophyidae and Curculionidae. Fig. 1. Pachytene of *Oxytoma cerdo*. Fig. 2. Metaphase II of *O. cerdo*. Fig. 3. Metaphase I of *Eutrichapion melancholicum*. Fig. 4. Metaphase I of *Ceratapion penetrans*. Fig. 5. Metaphase I of *C. austriacum*. Fig. 6. Metaphase II of *C. austriacu*. Fig. 7. Metaphase I of *Squamapion flavimanu*. Fig. 8. Pachytene of *Rhopalapion longirostre*. Fig. 9. Metaphase I of *Nanophyes marmoratus*. Fig. 10. Mitotic metaphase plate of *Acalles camelus*. Fig. 11. C-bands on chromosomes in diplotene of *A. camelus*. Fig. 12. C-bands on chromosomes in pachytene of *A. commutatus*. Fig. 13. Mitotic metaphase plate of *A. echinatus*. Fig. 14. C-bands on chromosomes in diplotene of *A. echinatus*. Fig. 15. Mitotic metaphase plate of *Ruteria hypocrita*. Fig. 16. C-bands on chromosomes in diplotene of *R. hypocrita*. The stars show Xy_p configurations, arrows indicate the C-bands. Bar = $5\mu\text{m}$.

ined species have wide C-bands in a centromeric position, clearly visible also on condensed chromosomes. A small amount of heterochromatin is characteristic of all studied Attelabidae, Apionidae, Nanophyidae and Curculionidae, the tribes Cionini (*Cionus*), Peritelini (*Centricnemus*), Phyl-

lobiini (*Phyllobius*), Polydrusini (*Liophloeus*), Sitonini (*Sitona*), Tanymecini (*Tanymecus*), Hyperini (*Hypera viciae*), Lixini (*Larinus*), Molytini (*Liparus*) and Pissodini (*Pissodes*). Wide C-bands (mainly in centromeric regions) were only observed in Curculionidae – Ceutorhynchini (*Nedyus*), Cryp-



Figs 17-24. Meiotic chromosomes of Curculionidae. Fig. 17. Meiotic karyotype of *Acalles camelus*. Fig. 18. Meiotic karyotype of *Ruteria hypocrita*. Fig. 19. Meiotic karyotype of *A. commutatus*. Fig. 20. Meiotic karyotype of *A. echinatus*. Fig. 21. C-bands in pachytene of *Centricnemus leucogrammus*. Fig. 22. C-bands in pachytene of *Sitona humeralis* (similar pictures were observed in all examined species of *Sitona*). Fig. 23. Metaphase I of *S. macularis*. Fig. 24. Metaphase I of *S. suturalis*. Bar = 5 μ m. Stars show Xy_p configurations.

torhynchini (*Acalles*, *Ruteria*), Otorhynchini (*Otorhynchus*), Polydrusini (*Polydrusus*), Brachyderini (*Strophosoma*), Sciaphilini (*Barypeithes*) and Hyperini (*Hypera postica* in terminal and subterminal position). This study confirmed that in weevils, differences of heterochromatin content are characteristic of individual genera and/or tribes but further comparative studies are necessary to elucidate basic evolutionary trends within higher taxons of Curculionoidea.

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