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A Cytogenetic Study on Three Chilean Species of Chrysomelinae (Coleoptera, Chrysomelidae)

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Three species of Chilean leaf beetles were chromosomally analyzed. The endemic *Araucanomela wellingtonensis* displays a male meioformula of 13 + Xy_p with 2n =28 chromosomes and an asymmetric karyotype with two large autosome pairs and 12 medium/small pairs of autosomes and sex-chromosomes, a diploid number which had not been found among the other species of the subtribe Paropsina *sensu lato* studied to date. *Strichosa eburata* presents a meioformula of 11 + Xy_p, 2n = 24 chromosomes, as occurs in many species of chrysomelines belonging to different subtribes. Furthermore, *Phaedon cyanopterum* has a 16 + Xy_p meioformula, that is 2n = 34 chromosomes, of small size mostly, also in agreement with the karyological findings obtained in all the other congeneric species so far examined. These cytogenetic data are discussed with respect to the previous ones in this subfamily and with other characters of taxonomic and evolutionary value.

Key words: Coleoptera Chrysomelidae, chromosomes, cytotaxonomy.

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The subfamily Chrysomelinae is one of the largest among the 20 so far described in leaf beetles (Chrysomelidae *sensu lato*), with some 3000 species in 133 genera (DACCORDI 1994, 1996; RILEY *et al.* 2002). Cytogenetic analyses on this group have been performed in roughly 240 species (PETITPIERRE *et al.* 1988; LACHOWSKA *et al.* 1996; PETITPIERRE 1999; PETITPIERRE & GARNERÍA 2003; GÓMEZ-ZURITA *et al.* 2004; PETITPIERRE & GROBBELAAR 2004; PETITPIERRE *et al.* 2004; PETITPIERRE, unpublished). Nevertheless, very few species from the Neotropical region have been subjected to chromosomal studies, only 15 species of 11 genera (VIRKKI 1964; ALBIZU DE SANTIAGO 1968; VAIO & POSTIGLIONI 1974; VIDAL 1984; PETITPIERRE 1988), in spite of the fact that nearly 24 % of the endemic genera of chrysomelines belong to this biogeographic region (DACCORDI 1996). The Chilean fauna of Chrysomelinae holds six endemic genera (DACCORDI 1994), and a good number of endemic species (BLACKWELDER 1944), which have never been examined from karyological standpoints.

The aims in this paper are to improve the poor cytogenetic knowledge on the Neotropical fauna and, more in particular, contribute with this first report to the study of the Chilean endemic leaf beetles. The present findings will be discussed together with the relatively large amount of karyological data which have been published for this subfamily.

Material and Methods

The three species and their geographical sources are given in Table 1. They were collected during two short campaigns in December 2004. The chromosome analyses were conducted from male living individuals brought from Chile to our laboratory, where they were killed with ethyl acetate before dissecting their testes. Slide preparations were performed by adding a few drops of 45% acetic acid in distilled water for 10-15 min to the tissue and later squashing under a cover slip. Then, they were dipped into liquid nitrogen for 15-25 sec and the frozen cover slip immediately removed by a sharp blade. Finally, the slides were stained in 4% Giemsa diluted in tap water for 10-15 min and excessive staining briefly rinsed in

Table 1

Surveyed species and their geographic and biological sources in Chile

Araucanomela wellingtonensis Bechyné et Bechyné, 1973	Parque Nacional Vicente Pérez Rosales: road to volcano Osorno, región X, on *Nothofagus betuloides* (Mirbel) Blume (Fagaceae)
Strichosa eburata Blanchard, 1851	Parque Nacional Puyehué, región XI, on *Berberis buxifolia* Lamarck (Berberidaceae)
Phaedon cyanopterum Guérin, 1844	Rangüe, Maipú, región Metropolitana, on *Baccharis marginalis* DC (Asteraceae)

tap water also. The micrographs were obtained by a ZEISS AXIOPHOT photomicroscope and subsequently enlarged to print at 2000 ×.

Results

Araucanomela wellingtonensis Bechyné et Bechyné 1973, 2n=28, 13+Xy_p

In the six analysed male individuals possessed meiotic metaphases I with 12 autosomal bivalents plus the "parachute" Xy_p sex-chromosome pair (Figs 1 & 2) and some spermatogonial mitotic metaphases. Its karyotype is composed of 28 chromosomes, mostly metacentrics out of two submetacentric pairs (Figs 3 & 4). Their relative lengths and shapes of the chromosomes are given in Table 2, where it is shown that this karyotype is remarkably asymmetrical because the two large autosome pairs represent nearly 37% of the total complement length, whereas the remaining autosomes and the sex-chromosomes are medium or small elements whose relative lengths range from 9.4% to 2.6%, this last value corresponding to a tiny y-chromosome.

Strichosa eburata Blanchard 1851, 2n =24, n ♂ =11+Xy_p

Three male individuals supplied good meiotic cell spreads with a karyotypic meioformula of 11 + Xy_p, that is 2n = 24 chromosomes. A cell at diakinesis (Fig. 5), displays four bivalents of large/medium size with two or three chiasmata, plus seven smaller with only one chiasma and the sex-bivalent showing the parachute-like (Xy_p) association of a large X and a small y-chromosome, characteristic of many coleopterans (SMITH & VIRKKI 1978). The bivalents become much more condensed at metaphase I but the four large ones are still distinguishable (Fig. 6). At anaphase I the segregation of two groups of haploid chromosomes is apparent though the tiny y-chromosome cannot be visible (Fig. 7).

Phaedon cyanopterum Guérin 1844, 2n=34, n ♂ =16+Xy_p

Our cytogenetic findings on this species were obtained from three male individuals and all of them have shown meiotic cells with a meioformula made of sixteen autosomal bivalents plus the pair of sex-chromosomes. The autosomal bivalents are of medium and mostly small size, and among them the parachute sex-bivalent Xy_p is again clearly distinguishable (Figs 8 & 9).

Discussion and Conclusions

The monospecific genus *Araucanomela* Bech. & Bech., distributed in the southern half of Chile, is classified in the subtribe Paropsina close to the Australian monospecific genera *Novacastria* Selman and *Ewanius* Reid, with whom it also shares the southern beech *Nothofagus* as food-plants (DACCORDI, 1994; REID 2002; DACCORDI & DELITTLE 2003). Unfortunately, neither *Novocastria* nor *Ewanius* have been chromosomally examined for their possible comparison with *Araucanomela,* therefore, comparisons can only be made between the karyotype of *A. welling-*

Table 2

Percent of relative length and shape of the chromosomes in *Araucanomela wellingtonensis.* m = metacentric, sm = submetacentric

chr.	1	2	3	4	5	6	7	8	9	10	11	12	13	X	y
	22.8	14.3	9.4	7.3	7.0	6.8	4.9	4.5	4.2	3.9	3.4	3.0	3.0	8.2	2.7
	m	m	sm	sm	m	m	m	m	m	m	m	m	m	m	m

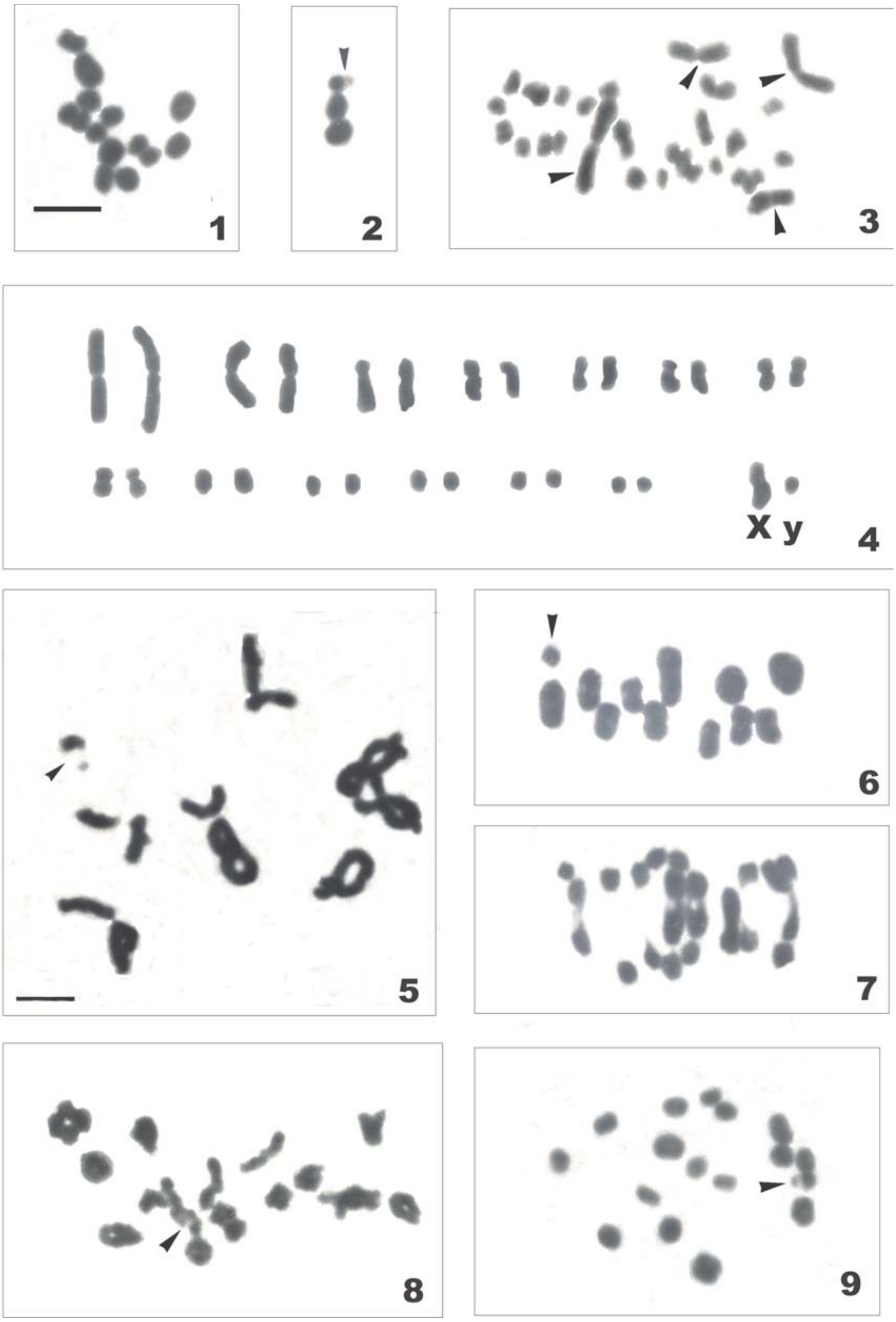

Fig. 1. Meiosis metaphase I of *Araucanomela wellingtonensis* showing 14 bivalents. Fig. 2. A group of metaphase I bivalents of the same species including the Xy_p pair arrowheaded. Fig. 3. Spermatogonial metaphase of *A. wellingtonensis* with 2n = 28 chromosomes, the two largest autosomes are arrowheaded. Fig. 4. Karyogram of *A. wellingtonensis* showing its remarkable asymmetry of chromosome sizes. Figs 5-7. Meiosis of *Strichosa eburata*; Fig. 5. Diakinesis showing 11 + Xy_p with some two and three chiasmata bivalents and the Xy_p pointed by an arrowhead; Fig. 6. Metaphase I with the same meioformula showing also the Xy_p arrowheaded; Fig. 7. Anaphase I with the two haploid sets migrating to the respective poles. Figs 8-9. Meiosis of *Phaedon cyanopterum* with 16 + Xy_p meioformulas showing small bivalents mostly and the Xy_p pointed by arrowhead; Fig. 8. Diakinesis; Fig. 9. Metaphase I. All figures × 2000, bars = 5 μm.

tonensis with those of taxa belonging to other genera of paropsines, but much more distantly related. In this sense, both the 13 + Xy_p meioformula and the diploid complement of *A. wellingtonensis* with 2n = 28 chromosomes, of quite remarkable differences in size, is strikingly distinct with regard to those found in 14 species from five genera of the Australian *sensu strictu* paropsines, whose karyotypes of 2n = 24 chromosomes and 11 + Xy_p meioformulas are roughly similar to each other (PETITPIERRE 1978, 1982, 1988; PETITPIERRE *et al.* 1988). Moreover, this uniqueness of the *A. wellingtonensis* karyotype is also observed when it is compared with those of the Holarctic *Gonioctena* species group of Paropsina, almost all again with 2n = 24 chromosomes and 11 + Xy_p meioformulas (PETITPIERRE *et al.* 1988). Furthermore, the question posed by DACCORDI and DELITTLE (2003) whether the genus *Araucanomela* may be classified within the subtribe Paropsina or in a new subtribe, cannot be answered until the allied genera *Novocastria* and *Ewanius* are cytogenetically surveyed and molecular phylogenies address this issue.

The meioformula found in *Strichosa eburata,* 11 + Xy_p (2n = 24 chromosomes), is the modal one for the whole subfamily Chrysomelinae since about 41% of the 203 checked species show 12 haploid chromosomes (PETITPIERRE 1997). On the other hand, the karyotype of this species does not differ significantly from those reported in the congeneric *Strichosa nigripes* (*sensu* DACCORDI 1996, and named as *Desmogramma nigripes* by VIDAL 1984), or in other Neotropical species of genera related to *Strichosa*, such as *Platyphora aulica* and *Calligrapha polyspila,* all with 11 + Xy_p meioformulas (PETITPIERRE *et al.* 1988). Although the analysis in *S. eburata* did not provide spermatogonial metaphases, the meiotic configuration of its bivalents at diakinesis suggests that most chromosomes are meta- or submetacentrics, a condition which is almost the rule in the karyotypic architecture of chrysomelines (ROBERTSON 1966; PETITPIERRE 1976, 1983; HSIAO & HSIAO 1983; PETITPIERRE & SEGARRA 1985; PETITPIERRE 1999; PETITPIERRE & GARNERÍA 2003; PETITPIERRE *et al.* 2004; GÓMEZ-ZURITA *et al.* 2004).

The cytogenetics of *Phaedon cyanopterum,* a taxon of Central Chile and neighbouring regions in Argentina belonging to the subgenus *Orthosticha,* agrees and reinforces the previous findings published on four congeneric species of *Phaedon.* Two of these species are from Europe, one of the subgenus *Phaedon s.str.* and another of the subgenus *Paraphaedon*, and two from South America of the subgenus *Orthosticha,* having also the same meioformula of 16 + Xy_p, 2n = 34 chromosomes (PETITPIERRE *et al.* 1988). The food-plant of *Ph. cyanopterum* in Chile, *Baccharis marginalis* DC (Asteraceae), is not related to the Brassicaceae and Apiaceae plants taken by the two examined European species of *Phaedon,* respectively, nevertheless all of them agree cytogenetically. Thus, the genus *Phaedon* seems to be very conservative at this first karyological level, sharing the same number and with most chromosomes of rather small size, contrary to those of the present *A. wellingtonensis* and *S. eburata,* and many other species of chrysomelines in general. These main characteristics of the *Phaedon* karyotypes, which are composed of 34 small chromosomes mostly, are also found in species of further neighbouring genera within the subtribe Chrysomelina, such as *Chrysomela, Linaeidea, Prasocuris, Hydrothassa* and *Phratora* (PETITPIERRE 1988; PETITPIERRE *et al.* 1988; PETITPIERRE unpublished). The chromosomal resemblances among the species of the previous genera are also in accordance with their common secretion of isoxazolinone glucosides as defensive substances (PASTEELS & ROWELL-RAHIER 1989; PASTEELS *et al.* 2003), and even more significantly, with their molecular phylogenies based on the sequences of four mitochondrial gene markers (HSIAO 1994; TERMONIA *et al.* 2001).

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