

# Karyotypes of five species of jewel-beetles and presumptive ancestral state of karyotypes of the subfamilies Polycestinae, Chrysochroinae and Buprestinae (Insecta: Coleoptera: Buprestidae)

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**Abstract.** The male karyotypes of 5 buprestid species belonging to 4 genera, 4 tribes and 3 subfamilies – Polycestinae, Chrysochroinae, Buprestinae from Armenia are described. In *Acmaeodera flavolineata* Laporte et Gory, 1835, *Sphenoptera tamarisci beckeri* Dohrn, 1866, *Anthaxia olympica* Kiesenwetter, 1880, *A. amasina* Daniel, 1903 and *Chrysobothris affinis tetragramma* Ménétrière, 1832 diploid chromosome numbers range between 16 and 30. Three variants of autosomal chromosome numbers (14, 18 and 28) and two types of sex chromosome systems ( $Xy_p$  and neo-XY) were revealed. The available karyological data on the subfamilies Polycestinae, Chrysochroinae and Buprestinae are discussed.

**Key words:** Coleoptera, Buprestidae, karyotypes, meiosis.

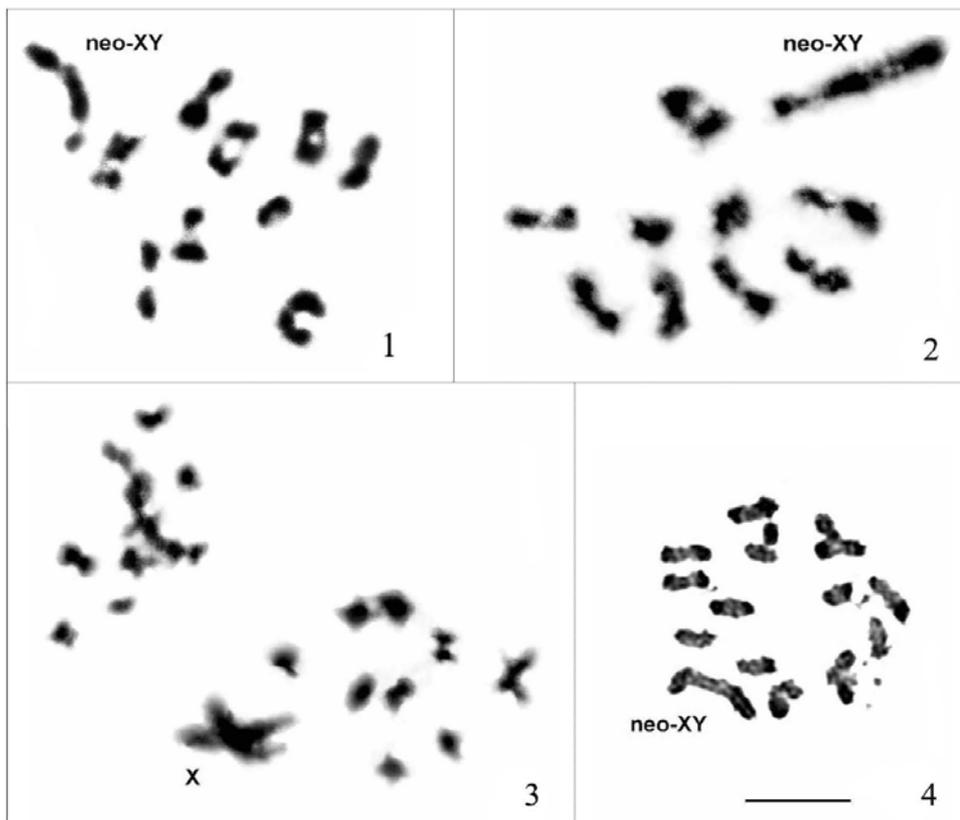
## INTRODUCTION

The family Buprestidae is a large group of Polyphagan beetles which comprises about 14500 nominal species in the world fauna (Volkovitsh, 2001; Bellamy, 2003). More than 160 buprestid species belonging to 27 genera, 15 tribes and 5 subfamilies are present in the Armenian fauna (M. Kalashian, personal communication). Chromosome numbers have up till now been published for only 83 species from 22 genera, 14 tribes of the subfamilies Julodinae, Polycestinae, Chrysochroinae, Buprestinae and Agrilinae (Smith, Virkki, 1978; Karagyan, Kuznetsova, 2000; Karagyan, 2001; Karagyan et al., 2004). The diploid chromosome numbers in the family vary between  $2n=12$  recorded for *Melanophila acuminata*

DeGeer, 1774 and  $2n=46$  for *Sphenoptera scovitzi* Faldermann, 1835 (Smith, 1953; Karagyan, 2001). The modal numbers are  $2n=20$  (found in 15 species, 7 genera, 4 tribes, 3 subfamilies) and  $2n=22$  (in 36 species, 6 genera, 3 tribes and 2 subfamilies). Most species have XX/XY chromosome system, but in two of them, *Euchroma gigantea* Linnaeus, 1764 and *Chalcophora lacustris* LeConte, 1860, multiple X and Y chromosomes, and an XX/XO system were respectively described (Smith, 1953; Mesa, Fontanetti, 1984). The XY system of males is very diverse: neo-XY, XY,  $Xy_p$ ,  $Xy_r$ . The  $Xy_p$  type is modal and occurs in 61 species, 15 genera, 10 tribes, 4 subfamilies.

The present paper reports new data on meiotic karyotypes of *Acmaeodera flavolineata*





**Figs 1-4.** 1-3 - *Acmaeodera flavolineata*,  $n = 9AA + \text{neo-XY}$ ; 1, 2 - late diakinesis –prometaphase I; 3 - metaphase II. 4 - *Sphenoptera tamarisci beckeri*,  $n = 14AA + \text{neo-XY}$ , metaphase I. Bar = 10  $\mu\text{m}$ .

Laporte et Gory, 1835, *Sphenoptera tamarisci beckeri* Dohrn, 1866, *Anthaxia olympica* Kiesenwetter, 1880, *A. amasina* Daniel, 1903 and *Chrysobothris affinis tetragramma* Ménériés, 1832. The taxonomic data are given according to Bellamy (2003) and a check list of world Buprestoidea ([www.fond4beetles.com/Buprestidae/WorldCat/Classif/webcat.html](http://www.fond4beetles.com/Buprestidae/WorldCat/Classif/webcat.html) on 3 November 2007, C.L. Bellamy).

#### MATERIAL AND METHODS

The beetles were collected in 2004 in Southern Armenia (Vayotsdзор and Syunik Provinces). Males were fixed in the field in 3:1 ethanol-acetic acid mixture. Karyological preparations were made from the testes by the method described by Rozek

(1994). Slides were stained with Schiff's reagent (Grozeva, Nokkala, 1996) and then by 4% Giemsa solution in phosphate buffer (pH 6.8).

The preparations were analyzed and photographed with a Nikon Eclipse 400 light microscope and CCD DS-U1 camera using the software Lucia Image 5.0.

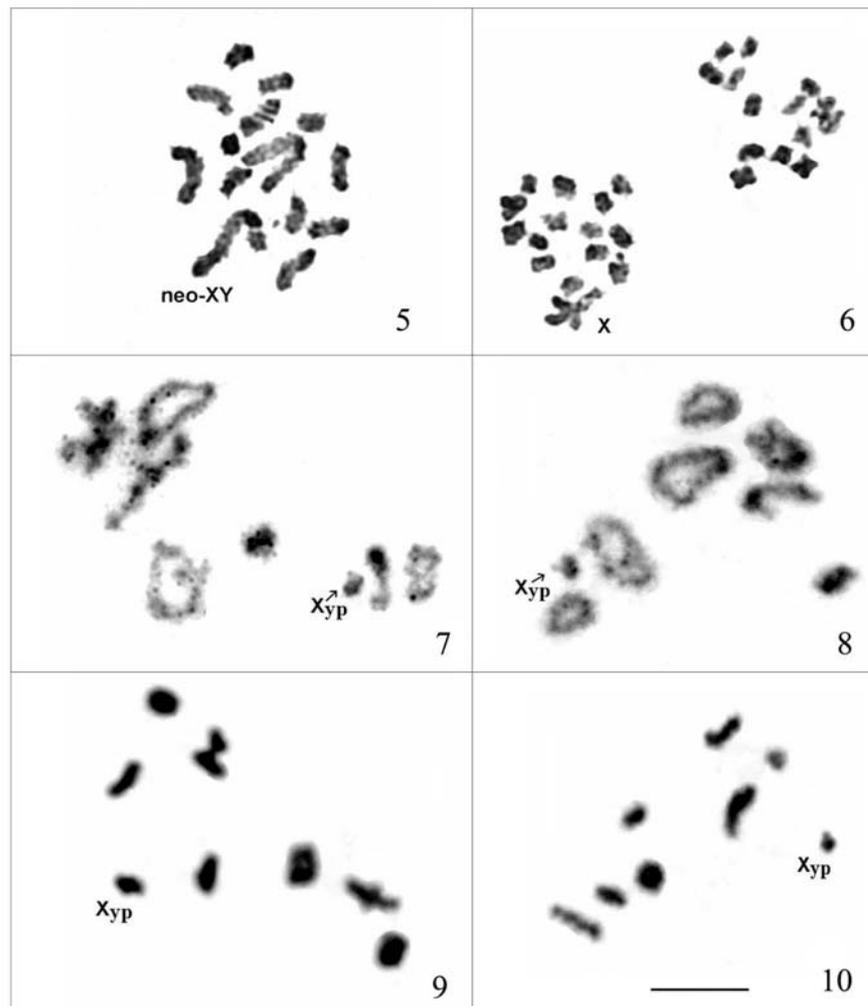
#### RESULTS

##### Subfamily Polycestinae

##### Tribe Acmaeoderini

*Acmaeodera flavolineata* ( $2n = 20$ ,  $n\sigma = 9 + \text{neo-XY}$ ).

In late diakinesis/prometaphase I nine autosomal bivalents and a sex chromosome heterovalent



**Figs 5-10.** 5-6 - *Sphenoptera tamarisci beckeri*,  $n = 14AA + \text{neo-XY}$ ; 5 - metaphase I, 6 - metaphase II. 7, 8 - *Anthaxia olympica*,  $n = 7AA + Xy_p$ , early diakinesis. 9, 10 - *Anthaxia amasina*,  $n = 7AA + Xy_p$ , late diakinesis – prometaphase I. Bar = 10  $\mu\text{m}$ .

of the neo-XY type were observed (Figs 1, 2). The bivalents gradually differ in size. The six rod-shaped bivalents have one terminal chiasma and three ring-shaped bivalents – two chiasmata. The nuclei of metaphases II displayed 10 chromosomes (Fig. 3). The majority of the chromosomes were biarmed of metacentric and submetacentric morphology. The X was the largest chromosome of the set and clearly metacentric, whereas the Y-

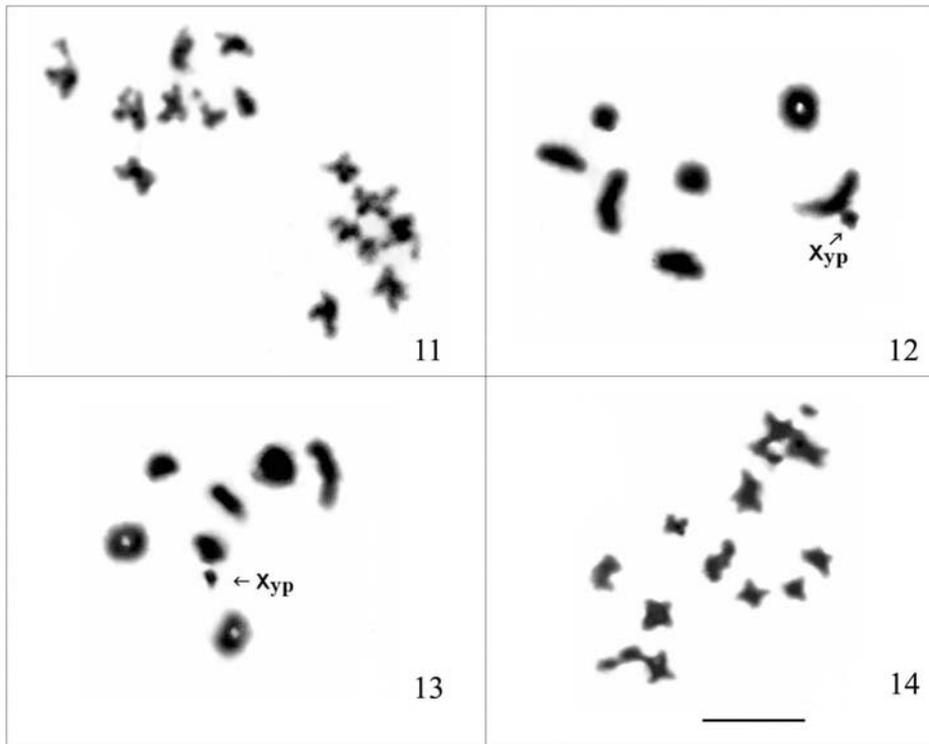
chromosome (not distinctive) was probably acrocentric and similar in size to the autosomes.

#### Subfamily Chrysochroinae

##### Tribe Sphenopterini

*Sphenoptera tamarisci beckeri* ( $2n = 30$ ,  $n\sigma = 14 + \text{neo-XY}$ ).

In metaphase I, 14 autosomal bivalents and a



**Figs 11-14.** 11 - *Anthaxia amasina*,  $n = 7AA + Xy_p$ , metaphase II. 12-14 - *Chrysobothris affinis tetragramma*,  $n = 7AA + Xy_p$ ; 12, 13 - late diakinesis – prometaphase I, 14 - metaphase II. Bar = 10  $\mu$ m.

large heteromorphic neo-XY sex-bivalent were discovered (Figs 4, 5). The rod-shaped bivalents (having each most likely a terminal chiasma) formed a more or less gradual size series. The metaphase II showed 15 chromosomes of which four or five autosomes and a large X-chromosome were metacentrics, whereas the morphology of other chromosomes was vague (Fig. 6). At this stage, the Y-chromosome, which was probably acrocentric, could not be identified.

### Subfamily Buprestinae

#### Tribe Anthaxiini

*Anthaxia olympica* ( $2n = 16$ ,  $n\sigma = 7 + Xy_p$ ).

In early diakinesis the karyotypes showed seven autosomal bivalents and the X and Y sex-

chromosomes, associated to form a typical “parachute”  $Xy_p$  (Figs 7, 8). Although it is hard to determine the morphology of chromosomes at this stage, the majority of them and also the X-chromosome, seemed to be biarmed and very probably meta- and submetacentrics. The largest four or five ring-shaped bivalents possessed two chiasmata each. The  $Xy_p$  sex-bivalent consisted of a relatively small X-chromosome and a hardly observable dot-like Y-chromosome.

*Anthaxia amasina* ( $2n = 16$ ,  $n\sigma = 7 + Xy_p$ )

In late diakinesis/prometaphase I seven autosomal bivalents and sex chromosome heterovalent of the  $Xy_p$  type were observed (Figs 9, 10). The autosomal bivalents formed a series of gradually decreasing sizes. Rod-shaped (with a terminal chiasma), cross-shaped (with an interstitial chiasma)

and ring-shaped (with two terminal chiasmata) bivalents were visible; the rod-shaped bivalents were most numerous. The heterovalent  $Xy_p$  was rather small. In metaphase II all chromosomes (except the dot-like Y whose morphology could not be determined) were meta- and submetacentrics of gradually decreasing sizes (Fig. 11). The X-chromosome was not recognized at this stage.

### Tribe Chrysobothrini

*Chrysobothris affinis tetragramma* ( $2n = 16$ ,  $n\sigma = 7 + Xy_p$ ).

In late diakinesis/prometaphase I seven autosomal bivalents and the sex-chromosomes forming a parachute-like heterovalent  $Xy_p$  were detected (Figs 12, 13). Rod-shaped and ring-shaped bivalents were observed; these first ones were more numerous. The sex heterovalent was very small in comparison with the autosomal bivalents which had gradually decreasing sizes. In metaphase II the majority of the chromosomes were biarmed, of metacentric and submetacentric morphology (Fig. 14). The sex-chromosomes were undistinguishable at this stage.

### DISCUSSION

Karyological data within the subfamily **Polycestinae** are known only for tribe Acmaeoderini, for 4 species of *Acmaeoderella* Cobos, 1955 and 2 species of *Acmaeodera* Eschscholtz, 1829 (Karagyan, Kuznetsova, 2000; Karagyan, 2001; present paper). All species of the genus *Acmaeoderella* have  $2n=18$  ( $16+Xy_p$ ). Two species from the second genus possess a neo-XY sex-chromosome system but they differ in chromosome number. The diploid complement of *Acmaeodera hepburni* LeConte, 1860 is  $2n=18$ , whereas in *A. flavolineata* is  $2n=20$  (Smith, 1960; present paper). If our assumption about the ancestral karyotype  $2n=20$  ( $18+Xy_p$ ) for the family Buprestidae (Karagyan et al., 2004) is correct, the presently reported species of *Acmaeoderella*

had probably undergone one autosomal fusion, while in *Acmaeodera* a loss of the  $Y_p$ -chromosome and a fusion of the X-chromosome with one of the autosomes led to the neo-XY mechanism. Furthermore, in the karyotype of *A. flavolineata* one autosomal division probably took place.

In the subfamily **Chrysochroinae** the following taxa have been karyologically studied: Chalcophorini (one species of *Chalcophora* Dejean, 1833 – Smith, 1953), Hypoprasini (one species of *Euchroma* Solier, 1833 – Mesa, Fontanetti, 1984), Poecilonotini (one species of *Lamprodila* Motschulsky, 1860 – Karagyan et al., 2004, as *Ovalisia* Kerremans, 1900), Sphenopterini (five species of *Sphenoptera* Dejean, 1833 – Karagyan, 2001; Karagyan et al., 2004; present paper), and Dicerini (two species of *Capnodis* Eschscholtz, 1829 and two species of *Perotis* Dejean, 1833 – Karagyan, Kuznetsova, 2000; three species of *Dicerca* Eschscholtz, 1829 – Smith, 1953; Smith, Virkki, 1978). The diploid chromosome numbers within this subfamily vary from 14 to 46. The modal karyotype is  $2n=20$  ( $18+Xy_p$ ), mainly reported in the species of Dicerini with the exception of *Capnodis tenebrionis* Linnaeus, 1761, which has  $2n=14$  ( $12+neo-XY$ ). This karyotype differs significantly in its chromosome number and probably was formed from the initial karyotype  $2n=20$  ( $18+Xy_p$ ) through a number of evolutionary events including two autosomal fusions, the loss of  $Y_p$ -chromosome and one X – autosomal fusion, giving rise to the neo-XY sex-chromosomes. In addition, biarmed chromosomes are prevalent in the karyotypes of the majority of the studied species, in agreement with the findings for most beetles (Smith, Virkki, 1978). The male sex-determining system of *Chalcophora lacustris* is XO, which could apparently derive from an ancestral species with  $Xy_p$  sex-chromosome system where the Y-chromosome was lost. The ways of formation of the unique karyotype of *Euchroma gigantea* with its

peculiar autosomal polymorphism, multiple sex chromosomes and B-chromosomes, are not so far known. In the tribe Sphenopterini one “group” represented by the species of *Sphenoptera mesopotamica* Marseul, 1865 and *S. artemisiae* Reitter, 1889 is characterized by a diploid chromosome number  $2n=24$ . The second “group” shows higher chromosome numbers: in *S. tragacanthae* Klug, 1829 (as *S. glabrata* Ménériés, 1832 – Karagyan et al., 2004)  $2n=40$ , in *S. scovitzii*  $2n=38-46$ , and *S. tamarisci beckeri* has an intermediary chromosome number  $2n=30$ . Probably, the chromosome numbers in the species of *Sphenoptera* originated from a putative ancestral species with a low chromosome number, presumably  $n=12$ , through chromosome divisions. Although the morphology of all chromosomes of the set was not completely identified in any of the studied species of *Sphenoptera*, it is certain that in two species with  $2n=24$  the most of the chromosomes are biarmed, whereas the karyotypes of the species with higher chromosome numbers include more acrocentric chromosomes. In the latter case, the chromosome divisions probably took place. The type of sex chromosomes was determined only for *S. mesopotamica* and *S. tamarisci beckeri* as  $Xy_p$  and neo-XY, respectively.

In the subfamily **Buprestinae**, the karyotypes are known for species of the tribes Buprestini (one species of *Buprestis* Linnaeus, 1758 – Smith, 1953), Stigmoderini (34 species of 3 genera – Gardner, 1988), Anthaxiini (10 species of *Anthaxia* Eschscholtz, 1829 – Smith, 1960; Karagyan, Kuznetsova, 2000; present paper), Melanophilini (3 species of *Melanophila* Eschscholtz, 1829 – Smith, 1953, 1960; Smith, Virkki, 1978), and Chrysobothrini (3 species of *Chrysobothris* Eschscholtz, 1829 – Smith, 1953, 1960). The diploid chromosome numbers within the subfamily vary from 12 to 22. The  $Xy_p$  sex-chromosome system was found in all of the studied species, except for *Buprestis fasciata* Fabri-

cius, 1787 ( $2n=20$ ) where the sex-chromosomes were not identified. In this subfamily, the statistically prevailing karyotype is  $2n=22 (20+Xy_p)$ , which is found in 32 species belonging to three genera of Stigmoderini. However, karyotype  $2n=16 (14+Xy_p)$  was discovered in three out of the five studied tribes of the subfamily and so, it is the modal at the higher taxa level. This modal karyotype was found in all studied species of Anthaxiini (10 species), Chrysobothrini (3 species) and Melanophilini (two species out of three). If it is assumed that  $2n=16 (14+Xy_p)$  was the evolutionarily original karyotype in the subfamily of Buprestinae, the karyotype  $2n=12 (10+Xy_p)$  in *Melanophila acuminata* could have been formed as a result of two autosomal fusions. All other karyotypes, including those with higher chromosome numbers  $2n=20 (18+Xy_p)$  and  $2n=22 (20+Xy_p)$ , could have resulted through two and three autosomal divisions, respectively.

Overall, the karyotypes of 88 species (34 from Armenia) of jewel-beetles belonging to 22 genera, 14 tribes of the subfamilies Julodinae, Polycestinae, Chrysochroinae, Buprestinae and Agrilinae are presently known, still a poor number for the family containing roughly 14500 described species.

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