Folia biologica (Kraków), vol. **52** (2004), No 3-4

New Cytogenetic Data on Armenian Buprestids (Coleoptera, Buprestidae) with a Discussion of Karyotype Variation within the Family*

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Accepted September 6, 2004

KARAGYAN G., KUZNETSOVA V. G., LACHOWSKA D. 2004. New cytogenetic data on Armenian buprestids (Coleoptera, Buprestidae) with a discussion of karyotype variation within the family. Folia biol. (Kraków) **52**: 151-158.

As a part of ongoing cytogenetic studies on the jewel-beetles (Buprestidae, Coleoptera) of Armenia, the male karyotypes and meiosis of nine species (5 genera, 4 tribes, 2 subfamilies) are described, figured and discussed. In *Ovalisia nadezhdae* Sem., *Sphenoptera artemisiae* Reitt., *Coraebus rubi* L., *C. sinuatus* Creutz., *Meliboeus caucasicus* Reitt., *Agrilus angustulus* Ill. Men., *A. obscuricollis* Kiesw., and *A. araxenus* Khnz. diploid chromosome numbers vary in a narrow range from 20 to 24. In *Sph. glabrata* Men. a high chromosome number of 2n=40 was discovered. All the species have a XY sex chromosome system, which is however of different types. The data available on the buprestid karyotypes and karyotype variation at different taxonomic levels within the family are discussed.

Key words: Coleoptera, Buprestidae, karyotypes, meiosis.

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The Buprestidae represent one of the only two families referred to the superfamily Buprestoidea of the series Elateriformia, though the family Schizopodidae is sometimes considered as a subfamily of the Buprestidae (VOLKOVITSH 2001). The Buprestidae comprise about 14500 described species grouped in 494 genera, 47 tribes, and 6 subfamilies, namely Julodinae, Polycestinae, Galbellinae, Chrysochroinae, Buprestinae, and Agrilinae (VOLKOVITSH 2001; BELLAMY 2003). The family is cosmopolitan and includes many species that are agricultural and forestry pests.

Within the Buprestidae, karyotypes were first described in two unidentified species collected from spruce (STEVENS 1906) and subsequently in 74 other species (reviewed by SMITH & VIRKKI 1978 and KARAGYAN & KUZNETSOVA 2000).

The diploid chromosome number ranges between 12 and 46, the most frequent sex chromosome determining system is XX/XY, mainly of the Xy_p type. In two species multiple X and Y chromosomes and a XX/X0 system were described (MESA & FONTANETTI 1984; SMITH 1953).

In all but one paper only a conventional cytogenetic technique was applied. KARAGYAN (2001) has studied 6 buprestid species using an AgNOR-technique to reveal the NORs in their karyotypes.

In the present work, the meiotic karyotypes of *Ovalisia nadezhdae* Sem., *Sphenoptera artemisiae* Reitt., *Sph. glabrata* Men., *Coraebus rubi* L., *C. sinuatus* Creutz., *Meliboeus caucasicus* Reitt., *Agrilus angustulus* Ill., *A. obscuricollis* Kiesw., and *A. araxenus* Khnz. are analysed by a conventional technique. The variation in karyotype constitution presently known to occur at different taxonomic levels within the Buprestidae is discussed.

Material and Methods

The material was collected in 1999-2001 from several localities in Armenia. The locality and number

*Supported by a grant to G. KARAGYAN and V. G. KUZNETSOVA from the Russian Foundation for Basic Research (Nr 02-04-48649).

Table 1

Studied species, collection data, number of specimens examined, number of chromosomes (2n), and sex chromosome systems

N	Taxa	Collection site and date (number examined)	Meioformula (metaphase I)	2n and sex chromosome system
	Chrysochroinae: Poecilonotini			
1	*Ovalisia* (*Scintillatrix*) *nadezhdae* Sem.	Khosrov 7.07.00 (3)	9AA + Xy_p	2n = 20 (18+Xy_p)
Sphenopterini				
2	*Sphenoptera* (*Chrysoblemma*) *artemisiae* Reitt.	Meghry 24.07.99 (2)	11AA + Xy	2n = 24 (22+Xy)
3	*Sph.* (*s. str.*) *glabrata* Men.	Gorovan 25.05.99 (3)	19AA + XY	2n = 40 (38+XY)
Agrilinae: Coraebini				
4	*Coraebus rubi* L.	Meghryb 24.07.99 (2)	10AA + Xy_p	2n = 22 (20+Xy_p)
5	*C. sinuatus* Creutz.	Kajaran 21.07.99 (2)	11AA + XY	2n = 24 (22+XY)
6	*Meliboeus* (*s. str.*) *caucasicus* Reitt.	Khosrov 26.05.99 (3)	10AA + Xy_p	2n = 22 (20+Xy_p)
Agrilini				
7	*Agrilus* (*Quercuagrilus*) *angustulus* Ill.	Kajaran 22.07.99 (2)	10AA + Xy_p	2n = 22 (20+Xy_p)
8	*A.* (*Q.*) *obscuricollis* Kiesw.	Lichk 18.06.00 (2)	9AA + Xy_p	2n = 20 (18+Xy_p)
9	*A.* (*Aridagrilus*) *araxenus* Khnz.	Khosrov 7.07.00 (2)	9AA + XY	2n = 20 (18+XY)

of individuals of the species that were cytogenetically studied are presented in Table 1. Males of all the species were fixed in the field in a 3:1 ethanol-acetic acid mixture. Chromosomes in spermatogenesis, being the most informative and easiest to obtain, were analysed. In the laboratory, gonads of males were dissected and preparations were made by the method of ROŻEK (1994). Slides were stained using methodology from GROZEVA and NOKKALA (1996), with little modification. This method includes a soft hydrolysis of chromosomal DNA in 1N HCl followed by staining with Shiff's reagent and then by 4% Giemsa solution in phosphate buffer (pH 6.8).

The systematics mainly followed BELLAMY (2003); in this work due to nomenclatural reasons some names were changed, namely Chrysochroinae instead of Chalcophorinae and Dicercini instead of Psilopterini.

Chromosome spreads were analysed using a Jenaval light microscope. The photo magnifications are shown by a bar representing 10 μm.

Results

Chrysochroinae Lap.

Poecilonotini Jacobs.

1. *Ovalisia* (*Scintillatrix*) *nadezhdae* Sem.

The meioformula (haploid karyotype formula) of the species is 9AA + Xy_p; the male diploid complement is 2n=20 (18 + Xy_p).

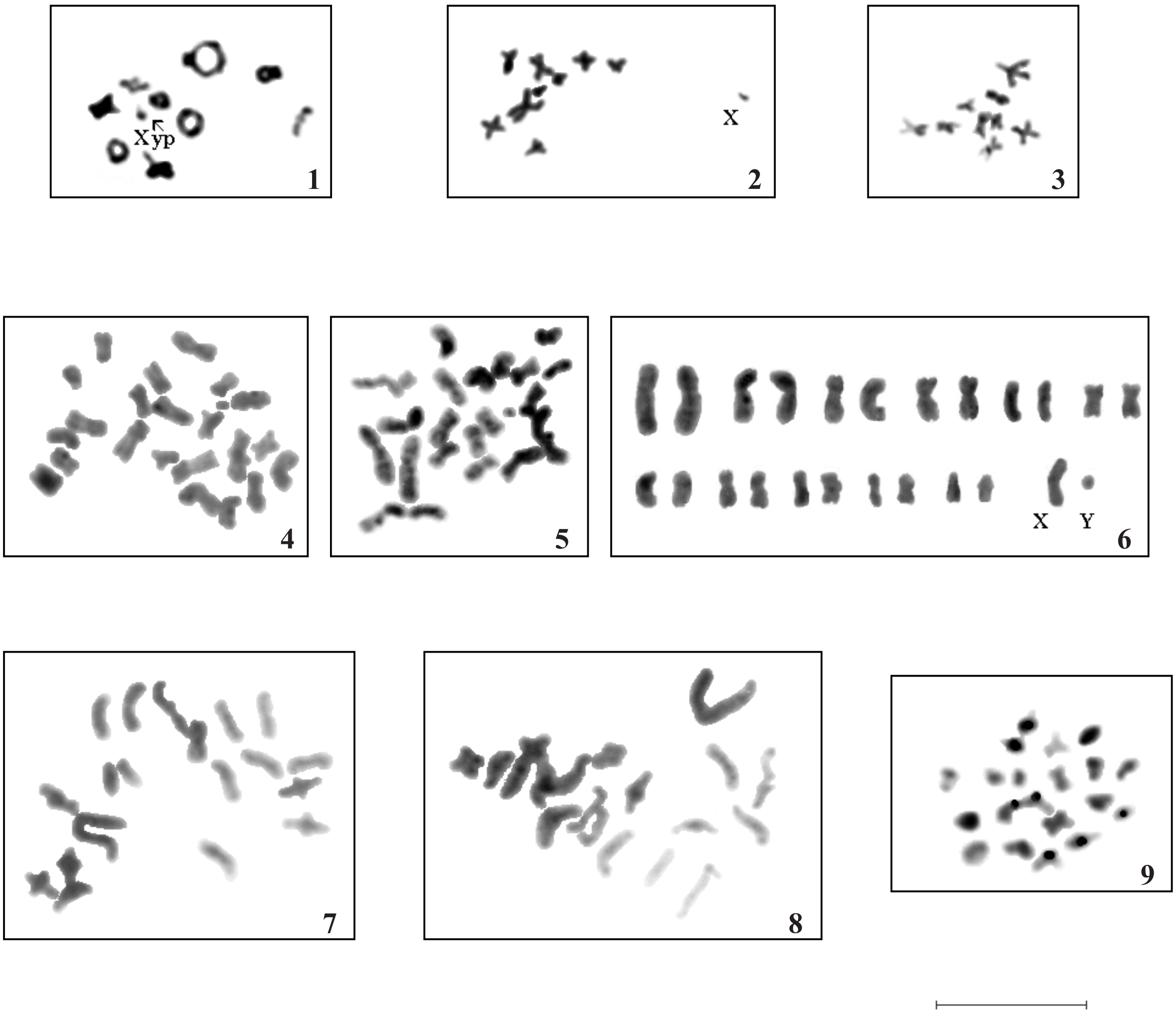
At diakinesis, 9 autosomal bivalents and sex heterochromosomes X and Y associated to form a typical parachute configuration traditionally designated by the symbol Xy_p were discovered (Fig. 1). Rod-shaped (with a terminal chiasma), cross-shaped (with an interstitial chiasma) and ring-shaped (with two terminal chiasmata) bivalents were observed. The ring-shaped bivalents prevail. The heterovalent Xy_p is very small when compared to the autosomal bivalents. The MII nuclei display 10 chromosomes, including 9 autosomes and either larger X (Fig. 2) or very small and faintly visible Y-chromosome (Fig. 3). At this stage the individual chromatids of each chromosome are repelled from each other while remaining connected at the centromeric area, allowing assessment of the size and morphology of the chromosomes. As for the size, the autosomes make up a gradually decreasing row. Six autosomes are metacentric, two autosomes are submetacentric and one autosome is subtelocentric. The X-chromosome is two-armed, probably submetacentric, and smaller than the smallest autosome. Thus, the total number of chromosome arms (FN) in the set is 38 (without considering the Y-chromosome of obscure morphology).

Sphenopterini Lacord.

2. *Sphenoptera* (*Chrysoblemma*) *artemisiae* Reitt.

The meioformula of the species is 11AA + Xy; the male diploid complement is 2n=24 (22 + Xy).

Mitotic metaphase comprises 24 chromosomes, including a quite large metacentric X chromosome and a tiny Y-chromosome of obscure morphology



Figs 1-9. Figs 1-3. *Ovalisia nadezhdae* Sem., n = 9AA + Xy_p. Fig. 1. Diakinesis. Figs 2, 3. Metaphase II. Figs 4-6. *Sphenoptera artemisiae* Reitt., n=11AA + Xy. Figs 4, 5. Mitotic metaphase. Fig. 6. Karyogram, 2n=24. Figs 7-9. *Sphenoptera glabrata* Men., n=19AA + XY. Figs 7, 8. Metaphase I. Fig. 9. Metaphase II. Bar = 10 μm.

(Figs 4-6). Autosomes form a more or less gradual size series and the X is close in size to the third pair. The autosomes are meta- and submetacentrics, the former prevailing (tentatively, 8m + 3sm). Thus, the total number of chromosome arms in the set is 46 (excluding Y-chromosome). Meiotic divisions were not observed, therefore the particular type of Xy is unknown.

3. *Sphenoptera (sensu stricto) glabrata* Men.

The meioformula of the species is 19AA + XY; the diploid male karyotype is 2n=40 (38 + XY).

In MI, the number of bivalents is determined tentatively at 19-20 (Figs 7, 8). Sex chromosomes were not detected. Because of the lack of a univalent, the sex chromosome determining system is suggested as of the XY type. Every MII displays 20 chromosomes, which form a more or less gradual size series (Fig. 9). Sex chromosomes are likewise indistinguishable at this stage. Because the very small Y-chromosome was not observed, both sex chromosomes are probably similar in size (likely "XY" according to SMITH 1953). Four chromosomes are clearly two-armed, metacentric/submetacentric, and two more chromosomes are acrocentric; the morphology of the rest of the chromosomes remained obscure.

Agrilinae Lap. & Gory

Coraebini Bedel

4. *Coraebus rubi* L.

The meioformula of the species is 10AA + Xy_p; the male diploid complement is 2n=22 (20 + Xy_p).

In diakinesis/prometaphase I, ten autosomal bivalents and a sex chromosome heterovalent of the Xy_p type were observed (Figs 10, 11). The bivalents form a more or less gradual size series. A large baculiform bivalent has a terminal chiasma. The ring-shaped bivalents, about 4 in number, have two chiasmata. The MII nuclei display 11 chromosomes, including either the X (undistinctive) or very small Y-chromosome (Figs 12, 13). About 6 chromosomes are two-armed (meta/submetacentric) and one chromosome is acrocentric. The morphology of the remaining chromosomes is obscure.

5. *Coraebus sinuatus* Creutz.

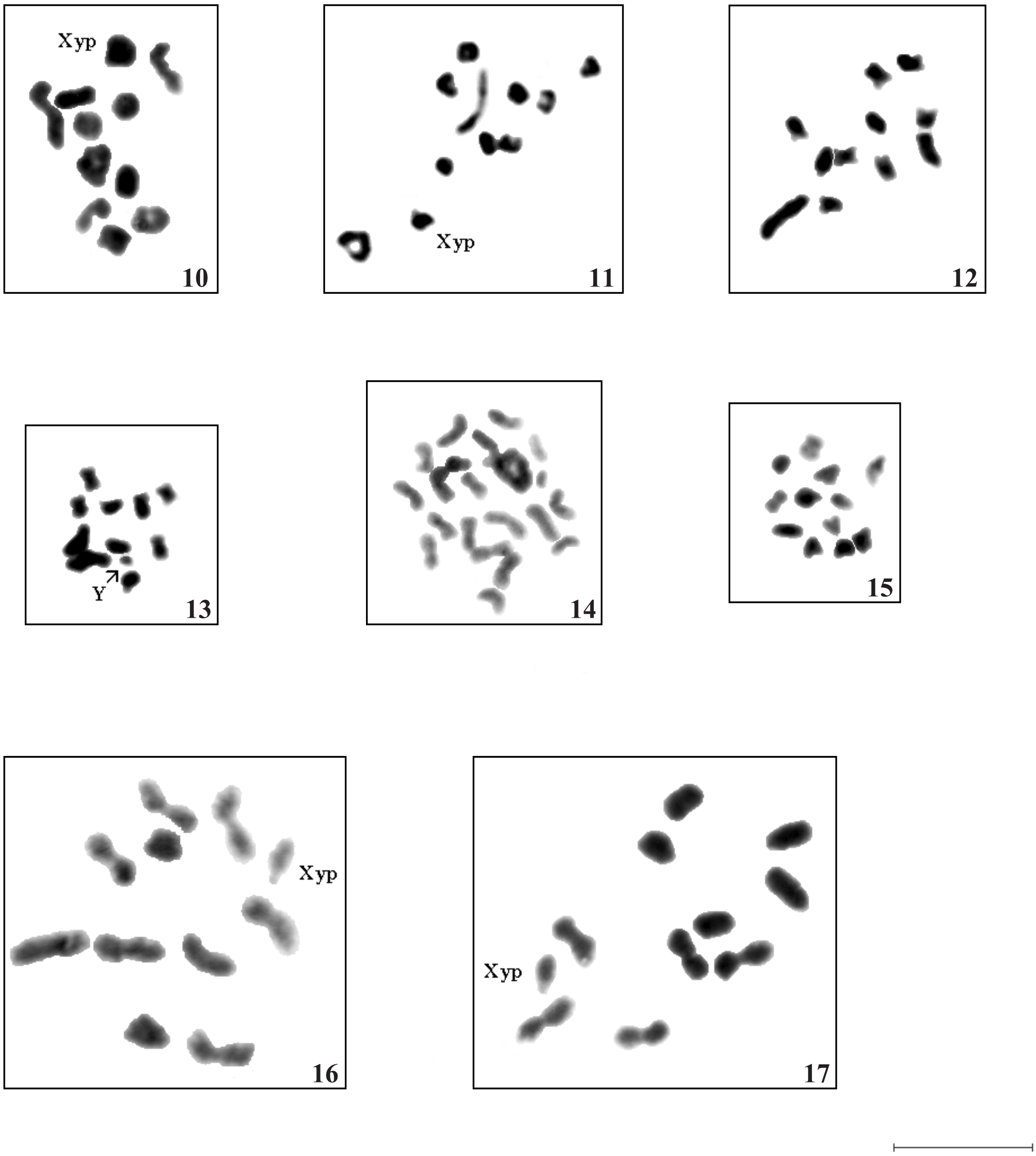
The meioformula of the species is 11AA + XY; the male diploid complement is 2n=24 (22 + XY).

In mitotic metaphase, the number of chromosomes was determined tentatively as 22-24 (Fig. 14). Most of the chromosomes are meta/submetacentrics. No MI stage was found in the studied males. In every MII nucleus, 12 chromosomes were observed, sex chromosomes are likewise indistinguishable. A very small Y-chromosome was not observed, therefore the sex chromosome system is determined as XY (probably, "XY" according to SMITH 1953) (Fig. 15). The chromosomes make up a gradually decreasing series. Two chromosomes are clearly acrocentric, whereas the majority of remaining chromosomes appear two-armed.

6. *Meliboeus (sensu stricto) caucasicus* Reitt.

The meioformula of the species is 10AA + Xy_p; the male diploid complement is 2n=22 (20 + Xy_p).

In MI, ten autosomal bivalents and X and Y chromosomes, associated to form a typical parachute-



Figs 10-17. Figs 10-13. *Coraebus rubi* L., n=10AA + Xy_p. Figs 10, 11. Diakinesis/prometaphase I. Figs 12, 13. Metaphase II. Figs 14, 15. *Coraebus sinuatus* Creutz., n=11AA + XY. Fig. 14. Mitotic metaphase. Fig. 15. Metaphase II. Figs 16, 17. *Meliboeus caucasicus* Reitt., metaphase I: n=10AA + Xy_p. Bar = 10 μm.

like heterovalent Xy_p, were discovered (Figs 16, 17). The sex heterovalent is very small when compared to the autosomal bivalents. Autosomal bivalents make up a gradually decreasing series. Morphology of all the chromosomes remained obscure.

Agrilini Lap. & Gory

7. *Agrilus (Quercuagrilus) angustulus* Ill.

The meioformula of the species is $10AA + Xy_p$, the male diploid complement is $2n=22\ (20 + Xy_p)$.

In early MI ten autosomal bivalents and sex heterochromosomes X and Y, associated to form a typical parachute Xy_p, were discovered (Figs 18, 19). Autosomal bivalents are rather small and form a more or less gradual size series. Morphology of all the chromosomes remained obscure.

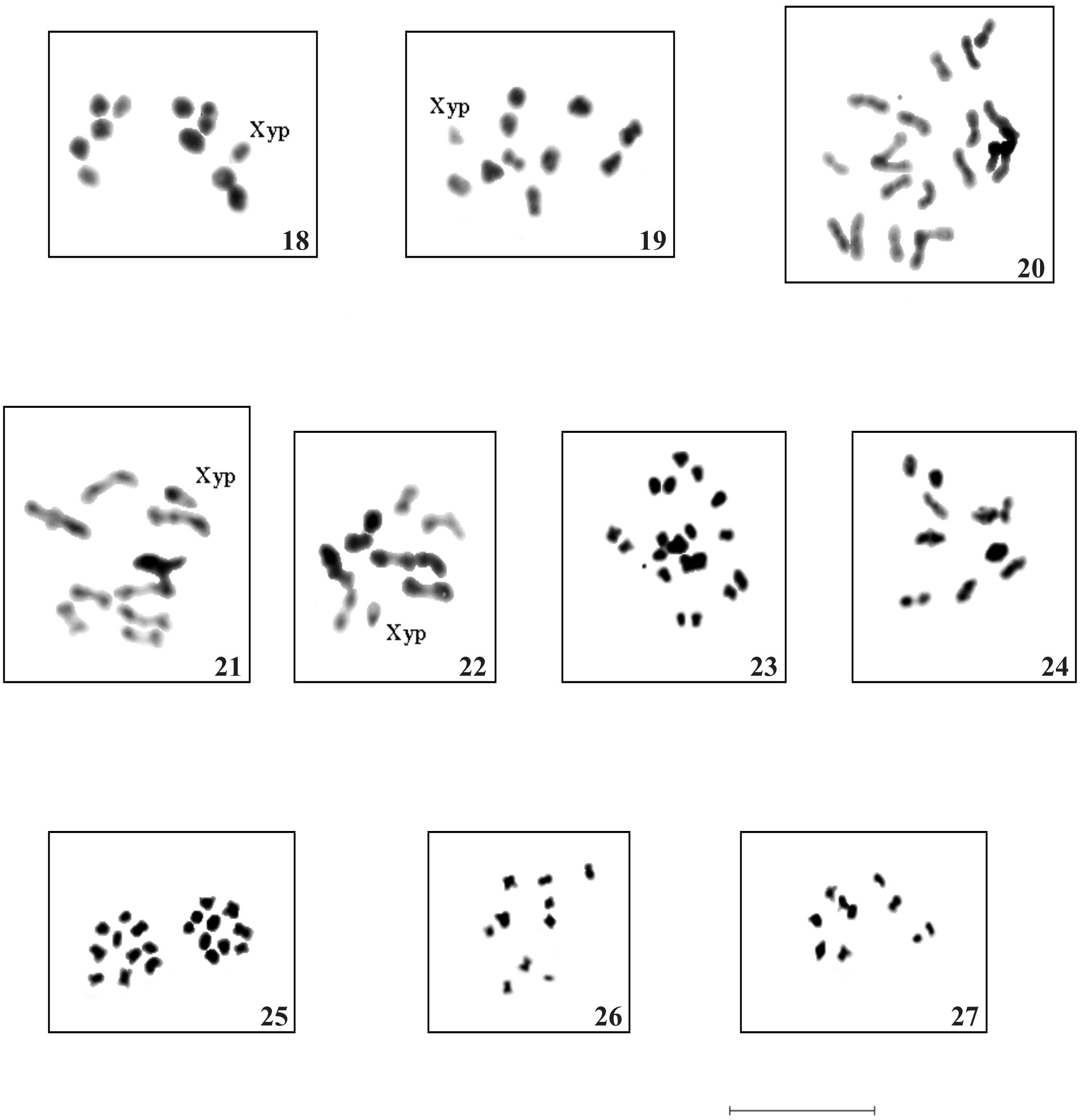
8. *Agrilus (Q.) obscuricollis* Kiesw.

The meioformula of the species is $9AA + Xy_p$; the male diploid complement is $2n=20\ (18 + Xy_p)$.

Mitotic metaphase comprises 20 chromosomes, however sex chromosomes were not identified (Fig. 20). The chromosomes form a more or less gradual size series. The majority of chromosomes are metacentrics. In MI, there are 9 autosomal bivalents (baculiform, having most likely a terminal chiasma each) and a sex chromosome heterovalent, which is of the "parachute-like" type (Figs 21, 22).

9. *Agrilus (Aridagrilus) araxenus* Khnz.

The meioformula of the species is $9AA + XY$; the male diploid complement is $2n=20\ (18 + XY)$.



Figs 18-27. Figs 18, 19. *Agrilus angustulus* Ill., early metaphase I, $n=10AA + Xy_p$. Figs 20-22. *Agrilus obscuricollis* Kiesw., $n=9AA + Xy_p$. Fig. 20. Mitotic metaphase. Figs 21, 22. Metaphase I. Figs 23-27. *Agrilus araxenus* Khnz., $n=9AA + XY$. Fig. 23. Mitotic metaphase. Fig. 24. Metaphase I. Figs 25-27. Metaphase II. Bar = 10 μm.

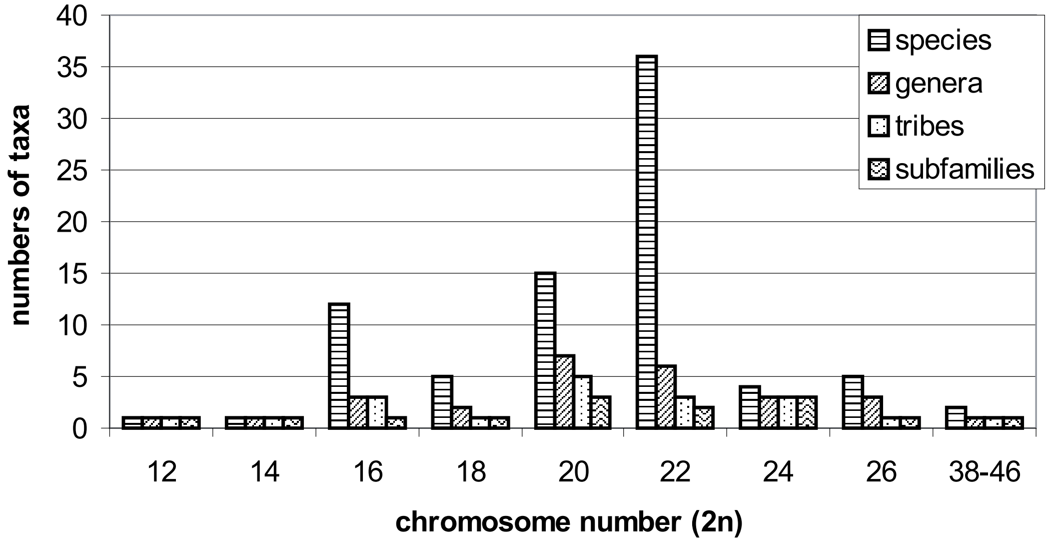


Fig. 28. Distribution of diploid chromosome numbers in the Buprestidae.

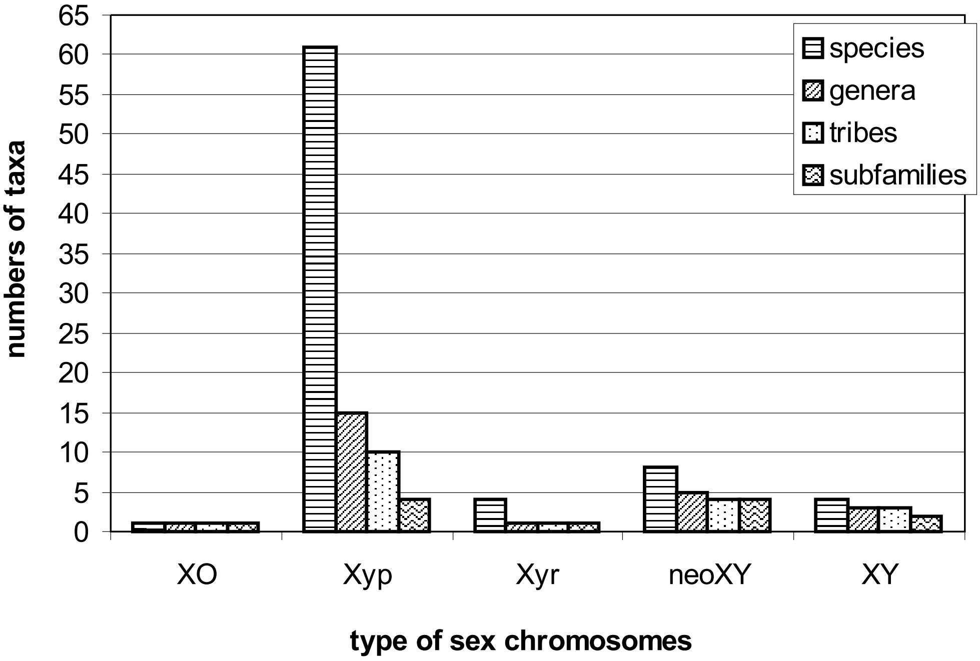


Fig. 29. Distribution of sex chromosome systems in the Buprestidae.

Table 2

Chromosome numbers and sex chromosome systems in subfamilies of the Buprestidae*

Taxa	Number ** of described		Number of karyologically studied		Chromosome numbers (2n)	Sex chromosome systems
	genera	species	genera	species		
1. Julodinae	6	170	3	6	24, 26	neoXY; Xy_p
2. Polycestinae	64	1500	2	5	18	neoXY; Xy_r
3. Chrysochroinae	95	2800	7	14	14, 20, 21, 24 (26), 38 – 46	XO; neoXY; Xy_p; XnYn
4. Buprestinae	95	3000	7	48	12, 16, 20, 22	Xy_p
5. Agrilinae	175	5550	3	10	20, 22, 24	Xy_p; neoXY; "XY"

* For extended information, see Table 2 and Discussion in KARAGYAN & KUZNETSOVA 2000, Results in KARAGYAN 2001, and Table 1 and also Discussion in the present paper.

** The number of species is given roughly.

Mitotic metaphase comprises 20 chromosomes, which are mainly meta/submetacentrics (Fig. 23). Sex chromosomes were not identified. In MI, ten bivalents are observed (Fig. 24). The sex chromosome bivalent is likewise unidentified. MII nuclei show 10 chromosomes, seven of which are metacentric, whereas the morphology of the rest is vague (Figs 25-27). A very small Y-chromosome was not observed, most likely both sex chromosomes are of very close size. Therefore the sex chromosome system is determined as XY (probably "XY" according to SMITH 1953).

Discussion

Chromosome numbers have been published for 74 species of the Buprestidae (SMITH & VIRKKI 1978; KARAGYAN & KUZNETSOVA 2000; KARAGYAN 2001) and in the present paper 9 species are added, bringing the total to 83. The studied species belong to 22 genera, 14 tribes of the subfamilies Julodinae, Polycestinae, Chrysochroinae, Buprestinae, and Agrilinae, and represent about 1% of the described species (about 5% of the accepted genera) of the worldwide buprestids. One third of the species, namely 29, all from Armenia (about 18% of the known buprestid species of this region (KALASHIAN, pers. comm.), have been studied by the senior author.

The lowest chromosome number, 2n=12, was recorded for *Melanophila acuminata* DeG. from the subfamily Buprestinae, tribe Melanophilini (SMITH 1953). The highest numbers were discovered in the genus *Sphenoptera* (Chrysochroinae, Sphenopterini), in which *Sph. glabrata* has 2n=40 (present paper) while *Sph. scovitzi* Fald. 2n=38-46 (KARAGYAN 2001*), whereas two more studied species, *Sph. artemisiae* (present paper) and *Sph. mesopotamica* Mars. (KARAGYAN 2001) have 2n=24.

If the Buprestidae as a whole are taken into account, the statistically prevailing chromosome numbers are 22 and 20, found respectively in 36 and 15 species. However these chromosome numbers are not in fact widely distributed within the family. They have not been discovered in the subfamily Julodinae, nor in the subfamily Polycestinae, whereas in the remaining subfamilies these numbers are confined only to a subset of the taxa. Thus, the karyotype of 2n=22 is characteristic for the Australian tribe Stigmoderini (Buprestinae), in which all but two studied species from the genera *Themognatha* (14 species), *Stigmodera* (6), and *Castiarina* (12) have this chromosome number (GARDNER 1988). This number seems to be also characteristic for the subfamily Agrilinae, in which it occurs in each of the genera studied, namely in *Agrilus* (Agrilini), *Coraebus* and *Meliboeus* (Coraebini). In the Chrysochroinae only *Chalcophora lacustris* Lec. (Chrysochroini) has 2n=22, however, only in females since this species displays an uncommon sex chromosome system XX/X0. The karyotype of 2n=20 is characteristic of the tribe Dicercini (Chrysochroinae), in which data are available for the genera *Capnodis*, *Perotis* and *Dicerca*. This chromosome number, and also 2n=22, occurs in the genera *Agrilus* (Agrilini, Agrilinae) and *Themognatha* (Stigmoderini, Buprestinae). The subfamilies Julodinae and Polycestinae show deviating chromosome numbers. The Julodinae demonstrate a rather high level of specialization (VOLKOVITSH 2001) and display relatively high chromosome numbers of 24 and more often of 26 in the three studied genera *Julodis*, *Julodella* and *Sternocera*. In the Polycestinae, species of two studied genera *Acmaeodera* and *Acmaeoderella,* both in the tribe Acmaeoderini, have 2n=18. Thus, there is a clear trend for chromosome number stabilization at different taxonomic levels of the Buprestidae. The genus *Anthaxia* (Anthaxiini, Buprestinae) is another taxon showing a stable chromosome number, namely 2n=16, found in all 8 studied species. The range of variation of diploid chromosome numbers in the family is presented in Table 2 and in Fig. 28.

The variety of sex chromosome systems in the Buprestidae is fairly significant and adequately accounts for that known in the Coleoptera as a whole (SMITH 1953). The range of variation of sex chromosomes in the family is presented in Table 2 and in Fig. 29. The systems of XX/X0 and multiple sex chromosomes are rare, the former described only in *Chalcophora lacustris* (Chrysochroinae, Chrysochroini), while the latter of the $X_1X_2X_3/Y_1Y_2Y_3$ type (also $X_1X_2X_3/Y_1Y_2$ probably as a polymorphism) in *Euchroma gigantea* L. (Chrysochroinae, Hypoprasini) (SMITH 1953; MESA & FONTANETTI 1984). The rest of the buprestid species have the XY - system, however the latter is not in fact the same pattern in different cases. The system is designated as "XY" where both sex chromosomes are of the same size or very close in size as in *Agrilus politus pseudocoryli* Fish. (SMITH 1953) and, probably, in *Sphenoptera glabrata, Coraebus sinuatus* and *Agrilus araxenus* from this study. If X is large whereas Y is very small, they are noted respectively by a capital "X" and a small "y". SMITH (1953) has distinguished several patterns of Xy in relation to configuration, which sex chromosomes form in MI. In the Buprestidae, as in the Coleoptera as a whole, the most characteristic pattern is Xy_p, which was discovered in 61 buprestid spe-

* The chromosome number of 24 reported by KARAGYAN and KUZNETSOVA (2000) is invalid (KARAGYAN 2001)..

cies, including 5 species from the present study, and is combined with different numbers of autosomes in different species. One more pattern symbolized preliminarily as Xy_r is found in all four studied species of the genus *Acmaeoderella* (KARAGYAN & KUZNETSOVA 2000; KARAGYAN 2001). Quite often, the pattern of Xy remains obscure as in *Sphenoptera artemisiae*. In the Buprestidae, a common pattern is neo-XY in many genera such as *Julodis*, *Sternocera* (Julodinae), *Acmaeodera* (Polycestinae), *Capnodis* (Chrysochroinae) and *Agrilus* (Agrilinae) (KARAGYAN & KUZNETSOVA 2000).

It is not possible at present to infer the ancestral karyotype constitution for the Buprestidae. However, it may be 2n=20 (18 + Xy_p), which was presumed to be ancestral for the Coleoptera as a whole, at least for the Polyphaga (SMITH 1950; SMITH & VIRKKI 1978; VIRKKI 1984). As noted above, this karyotype occurs in the buprestid subfamilies Chrysochroinae (the genera *Capnodis*, *Perotis*, *Dicerca* and *Ovalisia*), the Buprestinae (2 species of *Themognatha*), and Agrilinae (*Agrilus*), but does not occur in Julodinae and Polycestinae. The cytogenetic data available are not sufficient for use in practical taxonomy and to assess the evolutionary implications of the buprestid karyotypes. Of special note is the absence of data on the subfamily Galbellinae, and also on the family Schizopodidae, which is very small and is sometimes considered to be a subfamily of the Buprestidae (VOLKOVITSH 2001).

Acknowledgements

The authors are very grateful to Dr. Mark KALASHIAN, Institute of Zoology, Armenian Academy of Sciences, for help in identification of the buprestid specimens.

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