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Jan ZIMA, Miloš MACHOLÁN, Boris KRYŠTUFEK, Svetozar PETKOVSKI:

Karyotypes of certain small mammals (Insectivora, Rodentia) from Macedonia

Kariotipi nekaterih malih sesalcev (Insectivora, Rodentia) Makedonije

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Karyotypes of certain small mammals (Insectivora, Rodentia) from Macedonia

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ABSTRACT – Standard and banded karyotypes are reported for 14 species of insectivores and rodents. Results are based on 99 specimens collected at 5 localities in western Macedonia:. *Sorex araneus, S. minutus, Neomys fodiens, Talpa stankovici, Dinaromys bogdanovi, Clethrionomys glareolus, Chionomys nivalis, Microtus arvalis, M. subterraneus, Nannospalax leucodon, Apodemus sylvaticus, A. flavicollis, A. mystacinus,* and *Dryomys nitedula.*

Key words: karyotypes, small mammals, Insectivora, Rodentia, Macedonia

IZVLEČEK – KARIOTIPI NEKATERIH MALIH SESALCEV (INSECTIVORA, RODENTIA) IZ MAKEDONIJE – Predstavljeni so standardni in pasasto obarvani kariotipi 14 vrst žužkojedov in glodalcev. Rezultati so osnovani na 99 primerkih, zbranih v zahodni Makedoniji: *Sorex araneus, S. minutus, Neomys fodiens, Talpa stankovici, Dinaromys bogdanovi, Clethrionomys glareolus, Chionomys nivalis, Microtus arvalis, M. subterraneus, Nannospalax leucodon, Apodemus sylvaticus, A. flavicollis, A. mystacinus in Dryomys nitedula.*

Ključne besede: kariotipi, mali sesalci, žužkojedi, glodalci, Makedonija

Introduction

During a mammal survey of Macedonia we also made an attempt to karyotype insectivores and rodents. We supposed that chromosomal data would be of help in evaluating taxonomic positions of certain taxa as well as in understanding phyletic relations among them. Thus, we karyotyped 99 individuals of 14 species of insectivores and rodents; three taxa were analysed from their type localities. The aim of this report is to summarise synoptically all the data obtained until now.

Material and Methods

The material originates from five localities (Fig. 1): 1 – Mt. Pelister, Magarevo (1000 m above sea level); 2 – Mt. Pelister, Rotinska reka stream (1590 m a.s.l.); 3 – Mt. Pelister, Lake Golemo Ezero (2218 m a.s.l.); 4 – Mt. Galičica, between Leskoec and Trpejca (1600 m a.s.l.); 5 – Mt. Bistra, Careva češma, i.e. between Mavrovo and Galičnik (1580 m a.s.l.).

Small mammals were collected by live-traps or by digging (moles and mole rats). The animals examined are listed in Table 1.

Chromosome preparations from somatic cells of bone marrow and spleen were made in the field by a modified flame-drying technique (Ford & Hamerton 1956). G- and C-banded karyotypes were prepared in selected specimens by modified methods of Seabright (1971) and Sumner (1972). In a male mole rat, meiotic chromosomes were prepared from testes by a standard technique.

Table 1. Survey of species and individuals examined. See text and Fig. 1 for identification numbers of collecting sites.

Species	no. individuals	locality no.
Sorex araneus	10	2
Sorex minutus	1	2
Neomys fodiens	3	2
Talpa stankovici	1	2
	1	5
Dinaromys bogdanovi	2	5
Clethrionomys glareolus	11	2
Microtus arvalis	4	5
Microtus subterraneus	7	2
Chionomys nivalis	7	3
	2	4
Nannospalax leucodon	2	1
	1	3
	4	5
Apodemus sylvaticus	2	5
Apodemus flavicollis	36	2
	2	3
	1	4
Apodemus mystacinus	1	4
Dryomys nitedula	1	2

Specimens are preserved as standard skins and skulls; they are deposited in the collection of B. Kryštufek.

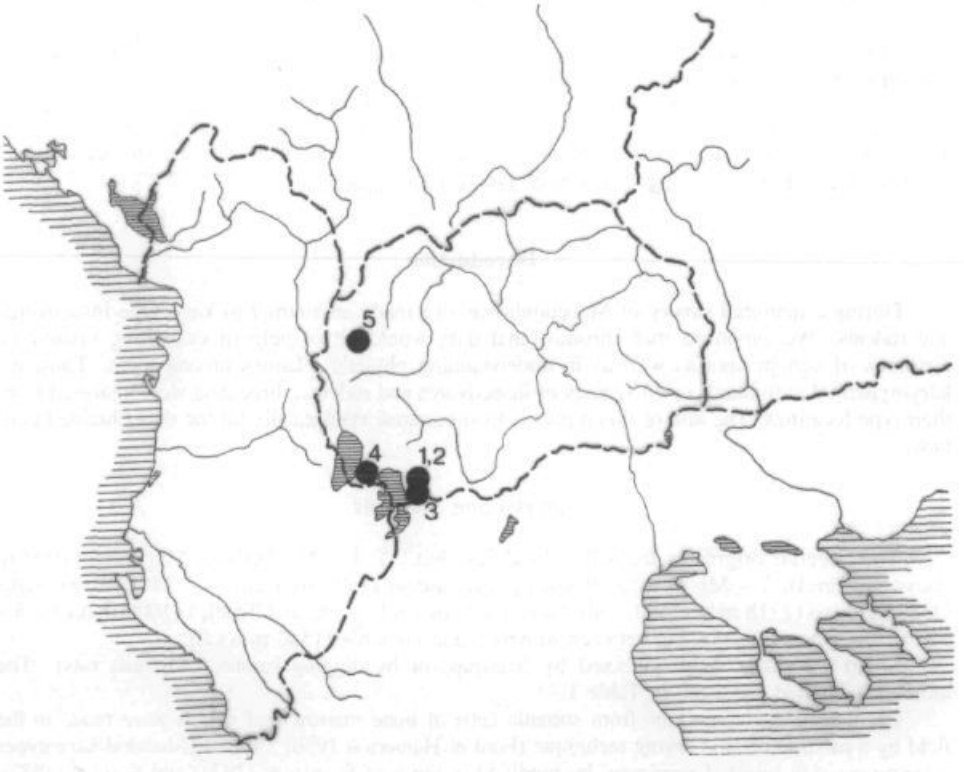

Fig. 1: Map of Macedonia with collecting sites. See text for locality identifications.

Results and Discussion

Sorex araneus LINNAEUS, 1758

In all animals from Mt. Pelister, 28 autosomes and the XY_1Y_2/XX sex chromosome system were found. The arm combination in the biarmed autosomes were *ab, bc, jl,* and *tu* (see SEARLE et al. 1991 for the chromosome nomenclature). The Y_1 was a small metacentric.

The autosomal set of shrews from Mt. Pelister is similar to that of populations from the western Alps (Acrocentric or Cordon race, Hausser et al. 1991). However, the metacentric Y_1 is a unique feature of the Pelister population (MACHOLAN et al. 1994).

Sorex minutus Linnaeus, 1766

The chromosomal complement of a female from Mt. Pelister included 40 autosomes and two acrocentric X chromosomes. There were five large and one small biarmed autosomal pairs, and 14 acrocentric autosomal pairs (Fig. 2).

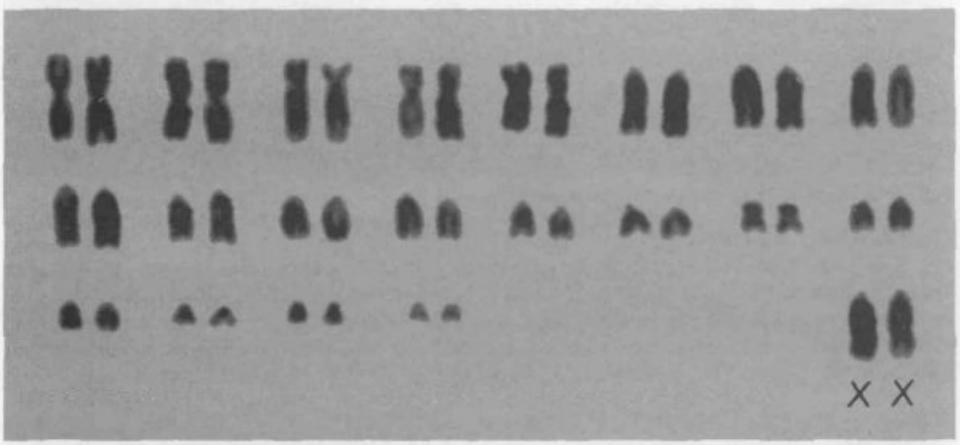

Fig. 2. Karyotype of *Sorex minutus* from Mt. Pelister.

This karyotype is the same as described from Mt. Pelister by PETROV et al. (1983) as well as from most other European localities (ZIMA & KRAL 1984).

Neomys fodiens (PENNANT, 1771)

The karyotype included 52 chromosomes. The size and centromeric position in the autosomes was similar to findings reported from other regions of Europe (ZIMA & KRAL 1984). The X chromosome was metacentric and the Y chromosome was acrocentric.

Talpa stankovici V. MARTINO and E. MARTINO, 1931

The diploid number of 34 chromosomes was found in two males; the specimen from Magarevo originated from the type locality of the species. All autosomes were biarmed, most of them being meta- and submetacentric. A large pair was subtelocentric, and a medium-sized pair was acro- or subtelocentric. The X chromosome was metacentric, and the Y chromosome was dot-like (Fig. 3).

This karyotype corresponds with those of *T. stankovici* described in other sites of western Macedonia (TODOROVIĆ et al. 1972, 1987). Specimens from NW Greece differ in the position of the centromere on 14th and 16th chromosome pair (SOLDATOVIĆ et al. 1986).

Fig. 3. Karyotype of *Talpa stankovici* from Magarevo.

Dinaromys bogdanovi MARTINO, 1922

Specimens were collected from the type locality of *D. b. grebenscikovi* MARTINO, 1943. The diploid number of 54 chromosomes was found in both individuals studied. All autosomal pairs but one were acrocentric and the smallest pair was metacentric. Distinct short arms were visible in most of the acrocentric autosomes. Compared to the other autosomes, the largest acrocentric pair was distinctly bigger. The presumable X chromosomes were large subtelocentrics (Fig. 4). G-

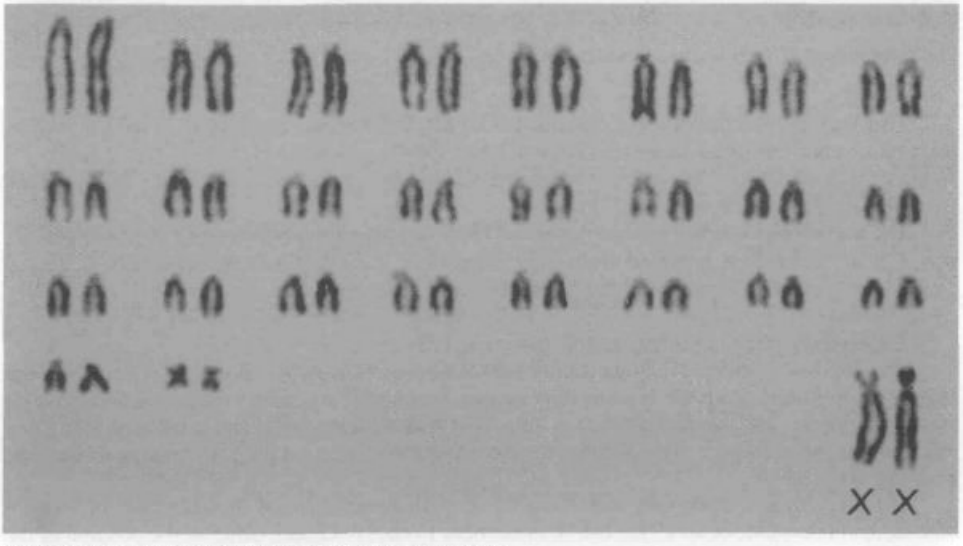

Fig. 4. Karyotype of *Dinaromys bogdanovi* from Mt. Bistra.

banding help to identify most of the homologous chromosomes (Fig. 5). C-banding showed the presence of dark centromeric bands in all acrocentric autosomes, with the exception of the largest pair. The short arms of acrocentrics stained apparently darkly in certain pairs. A distinct centromeric C-positive band was observed in the small metacentric autosomes. The proximal region of the long arm of the X chromosome was C-positive. This heterochromatic block comprised approximately one third of the long arm; the large size of the X chromosome is thus probably due to heterochromatic area (Fig. 6).

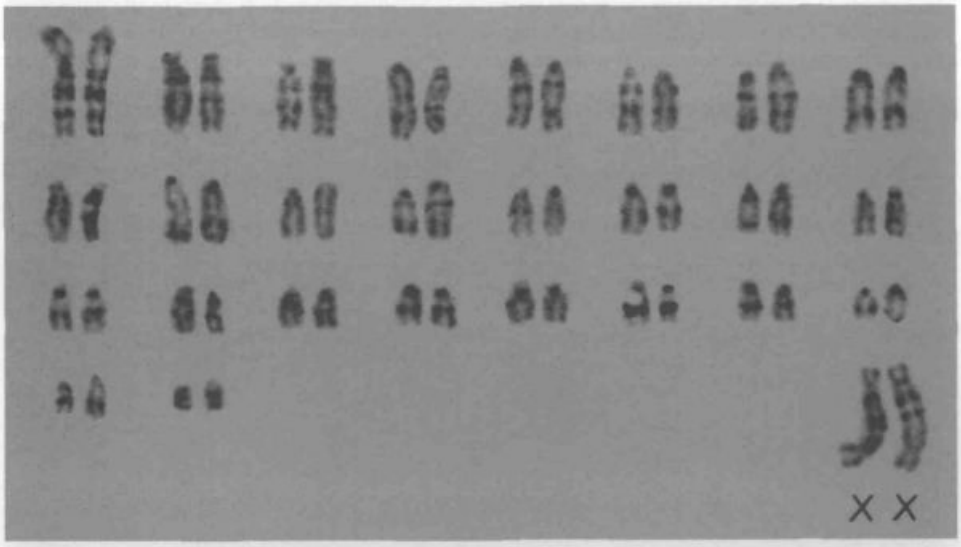

Fig. 5. G-banded karyotype of *Dinaromys bogdanovi*.

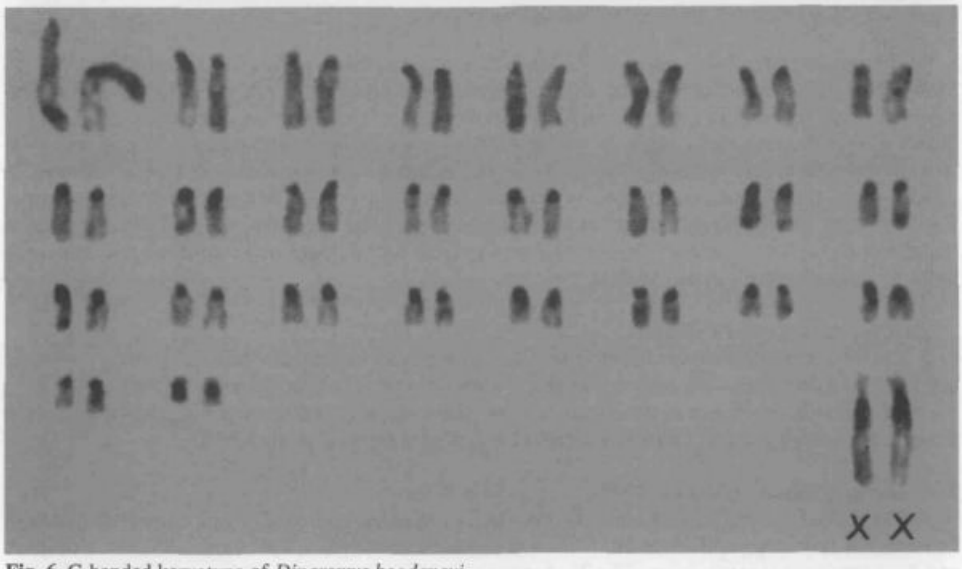

Fig. 6. C-banded karyotype of *Dinaromys bogdanovi*.

The conventionally stained karyotype appears similar to those described in *D. bogdanovi* from Macedonia (Šar planina), central Bosnia and Montenegro (MATTHEY 1956, SAVIĆ et al. 1966, 1967, DJULIĆ et al. 1971). Available data indicate a conservative character of the karyotype in this relict vole. Chromosomal banding pattern in *D. bogdanovi* is described here for the first time.

Clethrionomys glareolus (SCHREBER, 1780)

The material originates from the type locality of *C. g. macedonicus* FELTEN and STORCH, 1965. The karyotype with 56 chromosomes was ascertained in all individuals studied. In the autosomal complement, 26 acrocentric and one small metacentric pair was present. The X chromosome was acrocentric and the Y chromosome metacentric (Fig. 7).

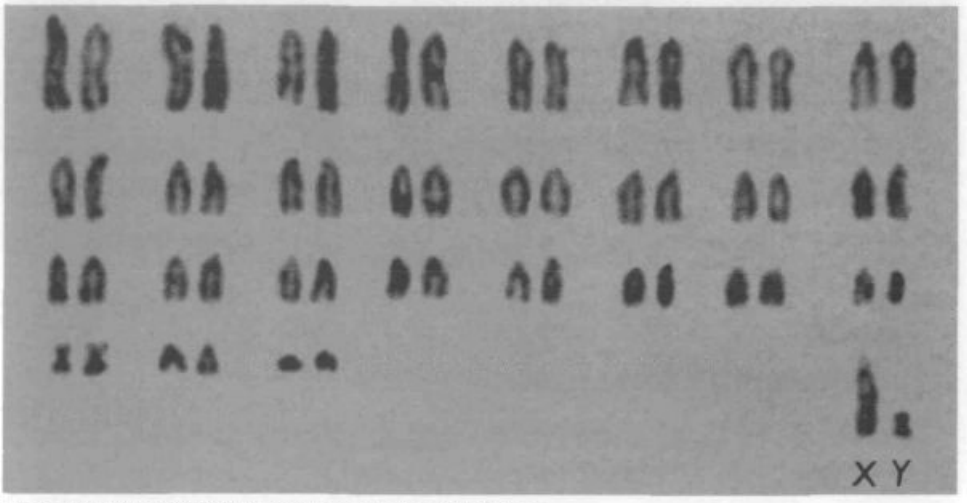

Fig. 7. Karyotype of *Clethrionomys glareolus* from Mt. Pelister.

The appearance of the autosomes is the same as described previously in various regions of former Yugoslavia (SAVIĆ et al. 1968, ŽIVKOVIĆ et al. 1975a, RADOSAVLJEVIĆ et al. 1990). Variations, however, were reported in the centromeric position in the Y chromosome. ŽIVKOVIĆ et al. (1975a) found acrocentric and metacentric Y chromosomes in populations from former Yugoslavia. The two chromosomal races were supposed to be separated by the Danube River, and the acrocentric Y chromosome was found only in the populations occurring on the left, i.e. southern riverside. RADOSAVLJEVIĆ et al. (1988) reported a case of intrapopulation variability of Y chromosome in a population from Mt. Jastrebac, Serbia. Our results from Mt. Pelister revealed the Y chromosome to be metacentric in all males studied.

Chionomys nivalis (MARTINS, 1842)

The diploid number of 54 chromosomes was found in all individuals studied. All the autosomes and the Y chromosome were acrocentric, the X chromosome was a large submetacentric (Fig. 8).

The same karyotype was described in most other localities (ZIMA & KRAL 1984), including those in former Yugoslavia (TODOROVIĆ et al. 1971, RADOSAVLJEVIĆ et al. 1990).

Microtus arvalis (PALLAS, 1779)

The diploid number was 46 chromosomes in four females studied. The autosomal complement consisted of four large and 13 small meta- and submetacentric pairs, one large subtelocentric pair, and four small acrocentric pairs. The X chromosomes were medium-sized metacentrics (Fig. 9).

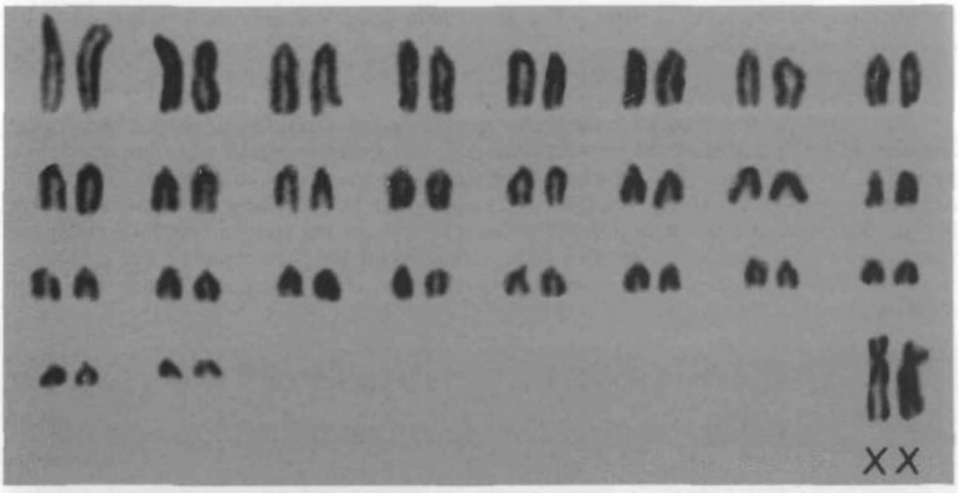

Fig. 8. Karyotype of *Chionomys nivalis* from Golemo Ezero, Mt. Pelister.

The karyotype is of standard type found in many areas in Europe (ZIMA & KRAL 1984), including former Yugoslavia (SAVIĆ et al. 1971, ŽIVKOVIĆ et al. 1975b, RADOSAVLJEVIĆ et al. 1990). A higher proportion of small acro- and subtelocentric autosomes was recorded in Popova Šapka, Šar planina Mts. (ŽIVKOVIĆ et al. 1975b). Both, the population of Šar planina Mts. and the population studied by us (Mt. Bistra), are at the very margins of the species distribution range (PETROV 1992). Macedonia lowlands are populated by a sibling species *Microtus rossiaemeridionalis* OGNEV, 1924 (ŽIVKOVIĆ & PETROV 1974, RUŽIĆ et al. 1975, ŽIVKOVIĆ et al. 1975b).

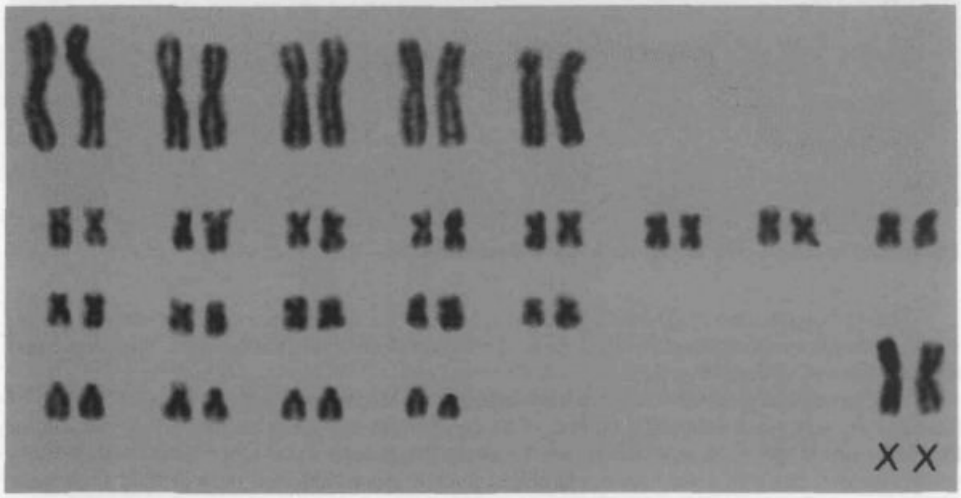

Fig. 9. Karyotype of *Microtus arvalis* from Mt. Bistra.

Microtus subterraneus (DE SELYS-LONGCHAMPS, 1836)

The karyotype of the Pelister population was identical with that of 52-chromosomal race of *M. subterraneus* in Europe. The Y chromosome was relatively large, because of the heterochromatin

amplification (KRYŠTUFEK et al. 1994). This result is in agreement with the previous study by PETROV & ŽIVKOVIĆ (1979).

Nannospalax leucodon (NORDMANN, 1840)

The karyotypes from all sites studied comprised 52 chromosomes. The autosomal complement consisted of two metacentric, seven submetacentric, seven subtelocentric, and nine acrocentric pairs. The X chromosome was a medium-sized submetacentric, the Y chromosome was one of small acrocentrics (Figs. 10, 11). The G-banded karyotype of the Mt. Bistra population is shown in Fig. 12. We found it difficult to differentiate exactly between the medium sized submeta- and subtelocentric autosomal pairs, so we cannot exclude possibilities of intra- or interpopulation variation within this group of chromosomes. However, no clear differences of this type were observed.

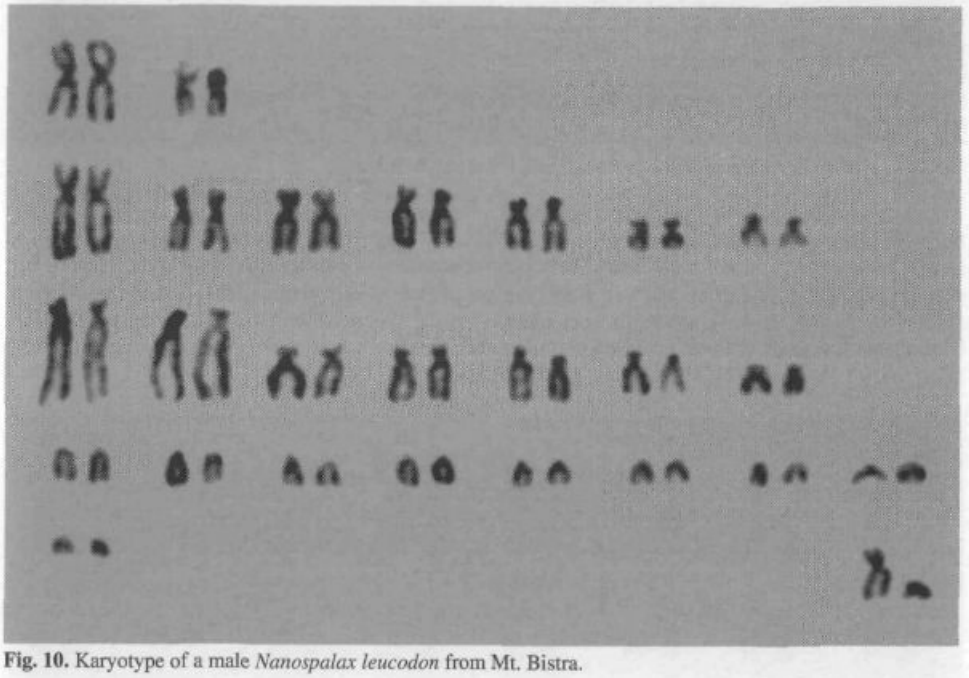

Fig. 10. Karyotype of a male *Nanospalax leucodon* from Mt. Bistra.

The karyotypes observed are identical with those described by SAVIĆ & SOLDATOVIĆ (1974, 1984) in the chromosomal form *macedonicus*. This form is distributed in Western Macedonia and adjacent parts of Greece (Fig. 13).

Two more chromosomal forms were described from Macedonia, both having 2n=54. One of these, a form with the fundamental number of 88 chromosome arms (*strumiciensis*) is restricted to the field Strumičko pole in extreme south-eastern Macedonia; the other form with NF=94 (*ovchepolensis*) has also a very restricted range, since it was established only in field Ovče pole (SOLDATOVIĆ & SAVIĆ 1973, SAVIĆ & SOLDATOVIĆ 1984).

Apodemus sylvaticus (LINNAEUS, 1758)

A standard karyotype with 48 chromosomes was ascertained in both animals; no B chromosomes were observed.

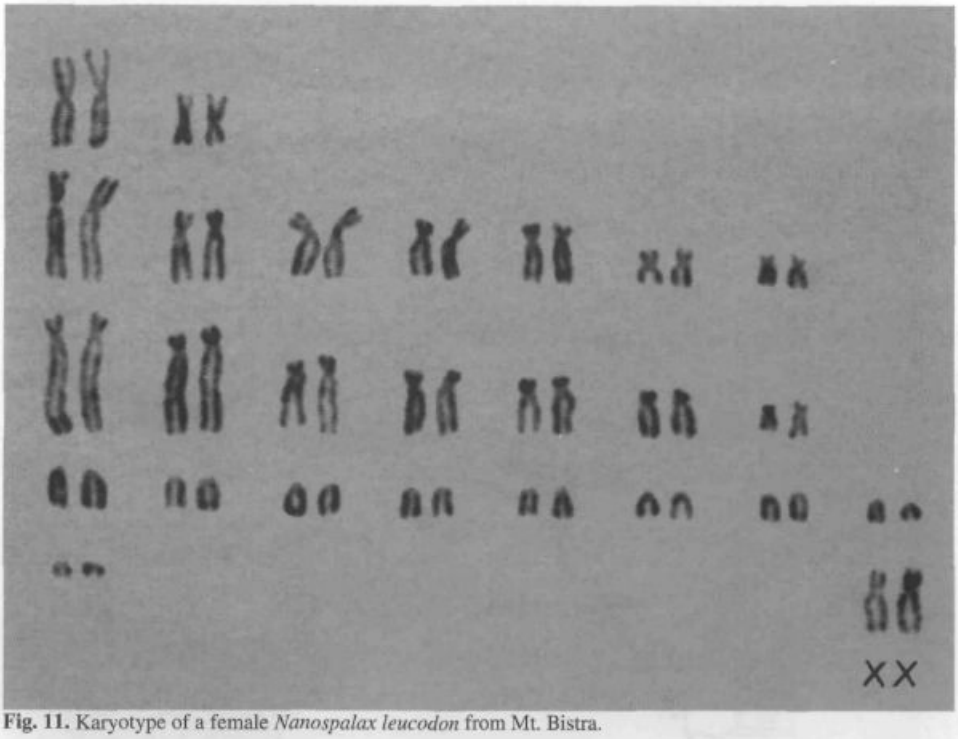

Fig. 11. Karyotype of a female *Nanospalax leucodon* from Mt. Bistra.

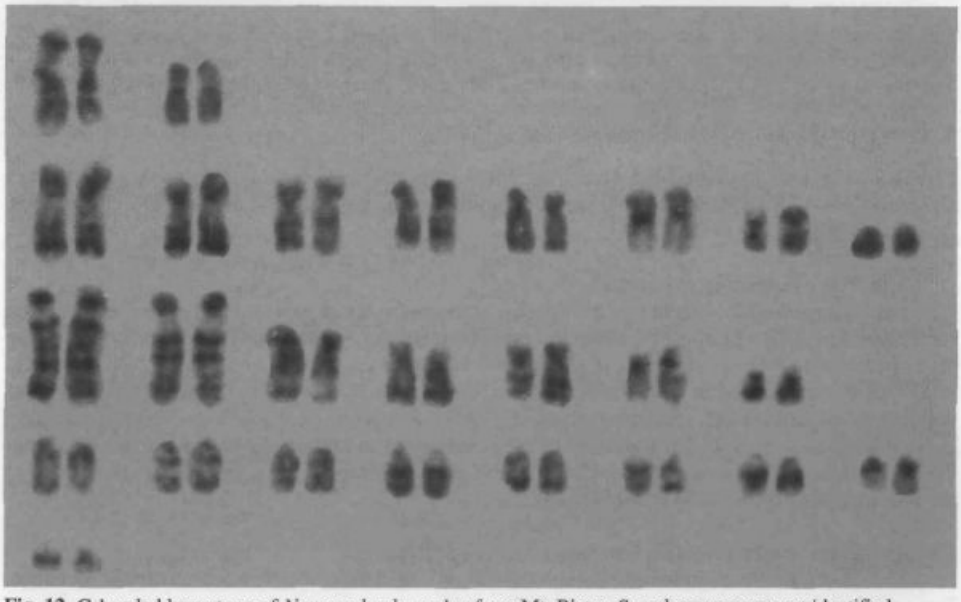

Fig. 12. G-banded karyotype of *Nanospalax leucodon* from Mt. Bistra. Sex chromosomes not identified.

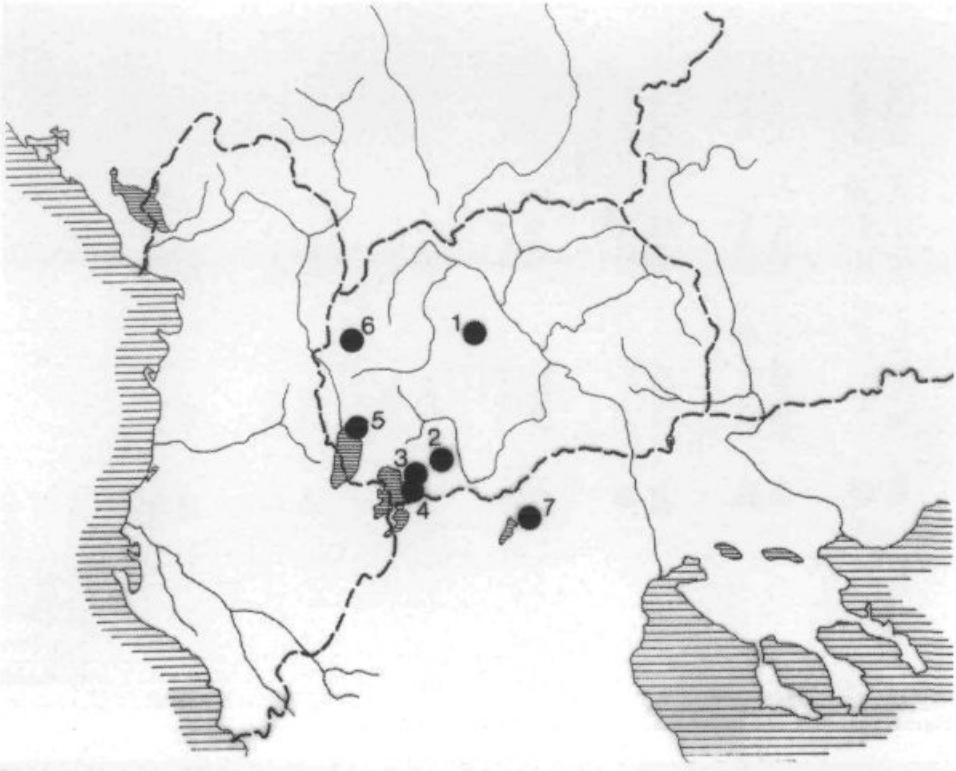

Fig. 13. Distribution of the *macedonicus* chromosomal form of *Nannospalax leucodon*. Macedonia: 1 – Mt. Jakupica, below Solunska glava (altitude 2200 m); 2 – between Bitola and Kukurečani (500-600 m); 3 – Magarevo (1000 m); 4 – Mt. Pelister, Golemo Ezero (2218 m); 5 – between Ohrid and Struga (700 m); 6 – Mt. Bistra, between Mavrovo and Galičnik (1580 m). Greece: 7 – Arnissa. Localities nos. 1, 2, 5, and 7 are from Savić & Soldatović (1984).

A similar karyotype was reported from Macedonia and the neighbouring areas (Živković et al. 1969, Kral 1972, Soldatović et al. 1972, Vujošević et al. 1984, Giagia et al. 1985, Vujošević & Živković 1987).

Apodemus flavicollis (Melchior, 1834)

The representative sample of this species from Macedonia revealed high incidence of the supernumerary or B chromosomes (46.2% of individuals are with B's). This frequency is fairly high compared to other populations studied in south-eastern Europe (Giagia et al. 1985, Vujošević & Živković 1987, Zima & Macholan 1995). One or two B chromosomes were found in our specimens. Supernumerary chromosomes were either acrocentric or dot-like. C-banding pattern showed all the acrocentric chromosomes of the standard set to possess dark centromeric bands but the B chromosomes stained rather uniform. The X chromosome had a large C-positive segment in the proximal area of the long arm (Fig. 14).

Apodemus mystacinus (Danford and Alston, 1877)

The chromosome complement comprised 48 chromosomes, which were mostly acrocentric. Two smaller autosomal pairs were biarmed (Fig. 15).

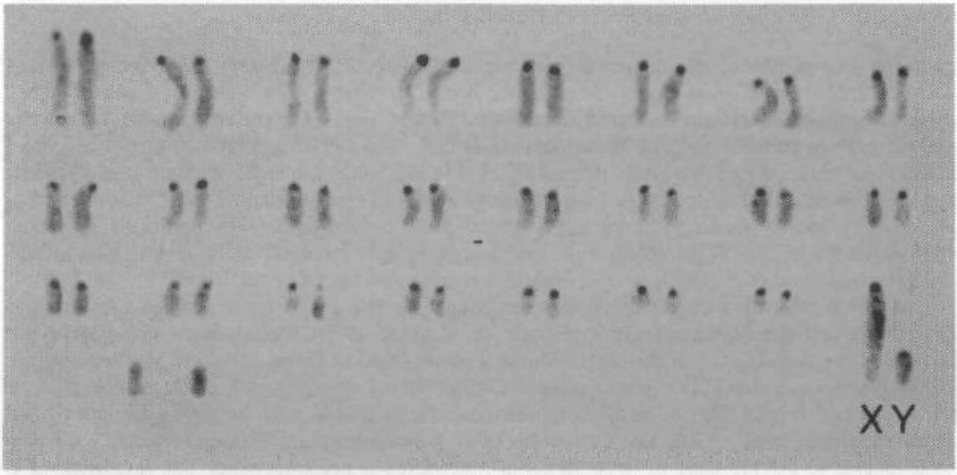

Fig. 14. C-banded karyotype of *Apodemus flavicollis* from Mt. Pelister. Two B chromosomes are present (lower row).

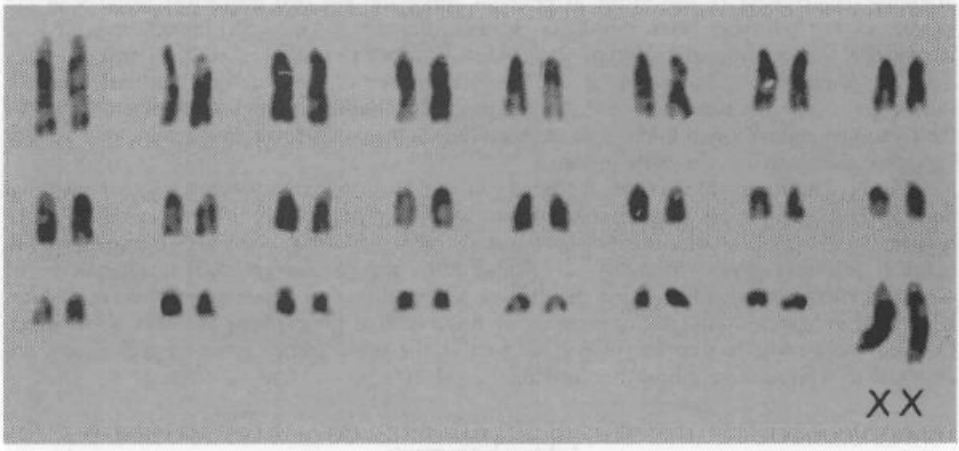

Fig. 15. Karyotype of *Apodemus mystacinus* from Mt. Galičica.

Identical karyotype was reported from various regions of former Yugoslavia (Soldatović et al. 1969, 1975). A population with only one biarmed autosomal pair was described from Greece (Giagia et al. 1985), and a B chromosome was found in an individual from Bulgaria (Belcheva et al. 1988).

Dryomys nitedula (Pallas, 1779)

The karyotype of a single male had 48 chromosomes. The poor quality of preparation did not allow detailed description of chromosome morphology, but no apparent differences were observed in comparison with the karyotypes described in other regions (Zima et al. 1996).

Conclusions

The area of Macedonia comprises rich mammalian fauna, consisting of species of various zoogeographic origin. Many mammals reach the border of their distribution in this area. Besides, a high degree of endemism is also characteristic (PETROV 1992). On the other hand, karyotypic variation seems to be limited in Macedonian mammals, and only few chromosomally divergent forms were found. Apart from the findings reported in this study, numerous data on karyotypes of small mammals of Macedonia were published, e.g. for *Talpa europaea* and *T. caeca* (TODOROVIĆ et al. 1972), *Microtus guentheri* (ŽIVKOVIĆ & PETROV 1975), *M. rossiaemeridionalis* (RUŽIĆ et al. 1975, ŽIVKOVIĆ et al. 1975b), *M. felteni* (ŽIVKOVIĆ & PETROV 1974, ŽIVKOVIĆ et al. 1975c), *Mus domesticus* (DJULIĆ et al. 1980), and *Apodemus agrarius* (SOLDATOVIĆ et al. 1969).

A specifically distinct karyotype was found only in *Microtus felteni*, a species endemic to Macedonia and the adjacent regions (PETROV & ŽIVKOVIĆ 1979). Populations with karyotypes different from those found in the other regions were reported in *Sorex araneus* (MACHOLAN et al. 1994), *Nannospalax leucodon* (forms *macedonicus, ovchepolensis, strumiciensis*; SAVIĆ & SOLDATOVIĆ 1984; this paper), and *Mus domesticus* (Robertsonian populations with 2n=36 in Katlanovo and Strumica; DJULIĆ et al. 1980). The karyotypes of all these species are extremely variable through their ranges, so the presence of local forms in Macedonia is not surprising. The incidence of B chromosomes in the Pelister population of *Apodemus flavicollis* is relatively high in comparison to other populations from the Balkans. The large Y chromosome found in the Pelister population of *Microtus subterraneus* is also rather exceptional. Since the last two distinctive features of the karyotypes resulted from heterochromatin changes, they are not likely to include any phyletic information. An exceptional karyotype was further described in an individual of *Microtus arvalis* from Šar planina Mts. (ŽIVKOVIĆ et al. 1975b). Examination of the additional material from the same population is necessary to establish more precisely the chromosomal divergence pattern. All the remaining chromosomal findings in the Macedonian mammals revealed the same or a similar karyotype as reported in the other regions.

Further chromosomal research in Macedonia should establish karyotypes in other marginal *Sorex araneus* populations (e.g. from Mt. Kožuv and Šar planina Mts.), reconsider relationships between various mole species (cf. SOLDATOVIĆ et al. 1986, TODOROVIĆ et al. 1987), investigate the status of *Microtus arvalis* from the Šar planina Mts., and analyse in detail the karyotype of *Microtus felteni*, the heterochromatin distribution and frequency of supernumerary chromosomes in *Apodemus* species, and the occurrence of Robertsonian populations of *Mus domesticus*. Detailed analyses of banded karyotypes, as well as the use of other genetical techniques, are desirable to achieve more thorough knowledge.

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We thank Mr. Shimon Simson (Rome) for help in collecting material. Logistic support during field work was provided by the Slovenian Museum of Natural History (Ljubljana) and Macedonian Natural History Museum (Skopje).

Povzetek

V želji, da bi razjasnili taksonomski položaj nekaterih vrst sesalcev na ozemlju Makedonije, so bila ob favnističnih raziskavah opravljena tudi kariološka. Kariotipirano je bilo 99 primerkov žužkojedov in glodalcev, ki pripadajo 14 vrstam. Gradivo je bilo zbrano na 5 lokalitetah (sl. 1) v zahodni Makedoniji. Živali so bile ujete z živolovkami ali izkopane (krti, slepa kužeta). Takoj po pridobitvi gradiva so bili na terenu izdelani kromozomski preparati. Uporabljena je bila neposredna metoda, ki jo priporočata FORD & HAMERTON (1956). Preparati so bili pripravljeni iz somatskih

celic kostnega mozga, v nekaterih primerih pa je bila uporabljena vranica. Kasneje je bilo v laboratoriju opravljeno selektivno barvanje za dosego G- in C-pasov.

Pri vrsti *Sorex araneus* s Pelistra je bilo ugotovljenih 28 avtosomov in XY_1Y_2/XX spolni kromosomi. Metacentrični Y_1 je značilen za pelistersko populacijo, ki se tako odlikuje z edinstvenim kariotipom. Pri vrsti *Sorex minutus* je potrjen kariotip, kakršnega so ugotovili PETROV et all (1983): 40 avtosomov in dva akrocentrična X kromosoma. Populacija vrste *Neomys fodiens* s Pelistra ima standardno kromosomsko garnituro z 52 kromosomi. Od dveh primerkov vrste *Talpa stankovici* je eden dobljen na tipski lokaliteti. Kariotip (2n=34), ki je ugotovljen pri naših krtih, je enak kot v literaturnih podatkih, ki se nanašajo na zahodno Makedonijo. V tem pogledu se ta vrsta iz severozahodne Grčije razlikuje od nominatne oblike *T.* stankovici iz Makedonije. Endemična voluharica *Dinaromys bogdanovi* je bila dobljena na tipskem nahajališču za podvrsto *grebenscikovi*. Kariotip se ne razlikuje od ostalih populacij te vrste (2n=54). V delu so prvič opisani C-pasovi. Za topotipski material *Clethrionomys glareolus macedonicus* je značilno diploidno število 2n=56, medtem ko je Y kromosom metacentričen. Pri vrsti *Chionomy nivalis* s Pelistra in Galičice je ugotovljen kariotip, kakršen je znan z ostalega areala vrste (2n=54). Na Bistri so bile ugotovljene poljske voluharice z 2n=46, ki pripadajo vrsti *Microtus arvalis* in ne *M. rossiaemeridionalis*. Vrsta *Microtus subterraneus* s Pelistra pripada obliki z 52 kromosomi, značilen zanjo pa je veliki kromosom Y, kar je posledica pomnoženega heterohromatina. Vsi pregledani primerki vrste *Nannospalax leucodon* pripadajo kromosomski obliki *macedonicus* z diploidnim številom 2n=52. Razširjenost te vrste je prikazana na sl. 13. Pri vrsti *Apodemus sylvaticus* je potrjen standardni kariotip z 2n=48, pri vrsti *A. flavicollis* so našli visoko incidenco "nadštevilnih" kromosomov B. Ti so ugotovljeni pri 46,2 % primerkov. Pri vrstah *Apodemus mystacinus* in *Dryomys nitedula* so dobljeni standardni kariotipi.

Za večji del raziskanih vrst so značilni standardni kariotipi. Značilni kariotipi so, razen pri ozko razširjenih endemitih (*Talpa stankovici, Dinaromys bogdanovi*), ugotovljeni pri vrstah, ki so tudi širše poznane po variabilnem kromosomskem kompletu (*Sorex araneus, Nannospalax leucodon, Microtus subterraneus*). V delu so poudarjena odprta vprašanja, ki zahtevajo nadaljnja raziskovanja.

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