



New Chromosomal Form of Mole-rat *Nannospalax leucodon* (Rodentia: Spalacidae) from Western Bulgaria. Synaptonemal Complex Karyotyping

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Abstract: Blind mole-rats of the genus *Nannospalax* have wide chromosome variability. Ten chromosomal forms in lesser mole-rats *N. leucodon* (Nordmann, 1840) have been described in Bulgaria between 1978 and 1983. No karyological studies have been carried out since then. In this study, we present new karyotype from Ravno Pole Village near Sofia. Karyotyping was carried out based on immunocytochemical analysis of spread synaptonemal complexes (SC). An SCs study of *N. leucodon* has been conducted for the first time. It was found that the animals had $2n=56$, $NFa=84$ and $NF=88$. Fifteen banded chromosomes [2 metacentrics (M), 3 submetacentrics (Sm) and 10 subtelocentrics (St)] and 12 acrocentrics (A) were identified in the SC's karyotype. The X chromosome is a medium Sm. The Y chromosome is a small St. A similar mole-rat's karyotype was previously described in other study from Novo Selo near Plovdiv, however with a different chromosome combination. We suppose that these two populations with same $2n$, NFa and NF but different chromosome morphology are most likely independently separated chromosomal forms.

Key words: Lesser mole rat, chromosome variability, synaptonemal complexes, meiosis, new chromosomal form.

Introduction

In recent years, blind mole-rats (subfamily Spalacinae Gray, 1821) have become a valuable research object because of their specific biological features as a stable habitat environment, low migration mobility, high inbreeding rates, and limited reproduction (NEVO 2013). Due to their solitary, territorial and aggressive behaviour, their geographic distribution is further fragmented (KRYŠTUFEK & VOHRALÍK 2009). The numerous adaptations as a result of their underground life determine their phenotype convergence. Moreover, opposing the convergence of morphological traits, they have developed extremely high karyotype variability. Thus,

74 chromosomal forms have been described so far (SAVIĆ et al. 2017).

The accumulated data in molecular phylogenetics and cytogenetics reflect the number of newly-described species among blind mole-rats. The fragmented distribution pattern is believed to support speciation events (ARSLAN et al. 2016). Often, these are morphologically similar but genetically and/or karyologically divergent cryptic species with specific karyotype evolution (BAKER & BRADLEY 2006).

The genus *Nannospalax* Palmer, 1903 consists of small-bodied species with varying diploid chromosome numbers (NEVO et al. 2001, SÖZEN et al. 2006) and proliferating chromosomal speciation (TOPACHEVSKII 1969, LYAPUNOVA et al. 1974).

Among the 26 species of Palaearctic mammals, this genus has the highest karyotype variability (ZIMA 1993). *Nannospalax* species and populations show significant $2n$ and NF diversity but it is not yet clear which chromosomal rearrangements have formed the existing karyotypes because the cytogenetic studies that use banding techniques or molecular approaches are hitherto rather rare.

In Bulgaria, studies of *Nannospalax* (super-species *leucodon*) have not been conducted for almost 40 years. The first data on chromosomes of the mole-rats were obtained by WALKNOWSKA (1963), which reported the diploid number of 54 chromosomes. SOLDATIVIĆ & SAVIĆ (1978) described the chromosomal form *thracious* form Novo selo (near Plovdiv) with $2n=56$. The studies of PESHEV (1981,1983) covered eight chromosome forms with $2n$ from 46 to 54 chromosomes: *tranensis* (Tran, Western Bulgaria), *softiensis* (Sofia-East, Cherven Briag, Western Bulgaria), *rhodopiensis* (Dobrostan near Asenovgrad, Southern Bulgaria), *bulgaricus* (Veliko Tarnovo and Sliven, Central Bulgaria), *srebarnensis* (Srebarna, Nord Eastern Bulgaria), *lom* (Lom, Northern Bulgaria), *varna* (Varna, Nord Eastern Bulgaria) and *pazardzhik* (Pazardzhik, South Bulgaria).

SAVIĆ & SOLDATOVIĆ (1979) assumed that the evolution of karyotypes of the Balkan Spalacinae was driven by Robertsonian (Rb) re-arrangements and most probably took the form of a decrease in the number of acrocentric autosomes and consequently of the diploid number of chromosomes. Similarly, IVANITSKAYA et al. (2005) and MATUR et al. (2011, 2013) considered chromosomal fusion as the major force of karyotype evolution in blind mole-rats.

Studies of meiosis, respectively of prophase I, were carried out three times for some blind mole-rats (WAHRMAN et al. 1985, GREENBAUM et al. 1990, MATVEEVSKY et al. 2018) but *Nannospalax* (super-species *leucodon*) has not been studied so far. Therefore, the studies of meiosis and chromosomal variability are of scientific interest.

Many questions remain unanswered regarding the karyotypic variability of the species in Bulgaria, the cytogenetic mechanisms of divergence and post-zygotic isolation of the chromosomal forms described. The number and taxonomic status of the described chromosomal forms in Bulgaria as well as the mechanism of chromosomal speciation in such polymorphic species are unclear. The phylogenetic position of the Bulgarian chromosomal races in relation to those described so far on the Balkan Peninsula must be revealed. Blind mole-rats are an appropriate model for studying the co-evolution of

sex chromosomes and highly variable autosomes as well as for evaluating the evolutionary consequences of chromosomal rearrangements.

The presence of different chromosomal forms in Bulgaria with completely stalled gene flow as well as the limited number of available recent records, determines the aim of the present study to describe new *Nannospalax* karyotypes from Bulgaria.

Materials and Methods

Animals

Two male mole-rats *N. leucodon* (1 adult and 1 sub-adult) were captured in a sunflower field near Ravno Pole Village in the eastern area of Sofia, Bulgaria ($42^{\circ}40.4440'N$, $23^{\circ}31.2830'E$, 541 m a.s.l.) in May 2019. Manipulations with animals were carried out according to the international rules (STOKES, 2000) and the rules of the Ethical Committee of Vavilov Institute of General Genetics RAS (order No. 3 of November 10, 2016).

Meiotic investigation

The suspensions and spreads of spermatocytes of two males of *N. leucodon* were made as described by KOLOMIETS et al. (2010). More than 300 spermatocytes at different stages of prophase I were analysed with focus on the pachytene stage. Immunostaining was designed as in our previous studies (MATVEEVSKY et al. 2016). Synaptonemal complexes (SC) and centromeres in pachytene spermatocytes were detected using antibodies to axial SC elements – SYCP3 (Abcam, UK) and the kinetochores (CREST, Fitzgerald Industries International, USA). The slides were analysed with an Axioimager D1 microscope (Carl Zeiss, Jena, Germany). Images were processed using Adobe Photoshop CS3 Extended.

SC karyotyping

Karyotyping was carried out based on immunocytochemical analysis of spread SCs. SC is a nucleoproteid skeleton formed between two homologous chromosomes in the meiotic prophase I. The number of SCs corresponds to the haploid number of the species chromosomes. Immunodetection of the centromere position allowed us to determine in which chromosomes, single-armed or biarmed, SC were formed. SC bivalents were arranged according to size from larger to smaller, starting with a biarmed chromosome, then a single-armed one (BOGDANOV & KOLOMIETS 1985). SCs measurements were performed using the MicroMeasure program (Colorado State University, CO, USA).

Results

Using immunodetection of the major SC protein - SYCP3 and kinetochore proteins as a marker of centromeric regions, mole-rat spermatocytes I were analysed. A complete set of SCs at the pachytene was shown: 27 bivalents and sex (XY) bivalent (Fig. 1a, b). Pachytene autosomes were fully synapsed. The XY body was found in the periphery of the nucleus. The X and Y chromosomes synapsed only in the homologous site, called pseudoautosomal region (PAR). X had a long asynaptic segment, while Y had a very short one. As a rule, the centromeres of sex chromosomes were not aligned.

There was no problem to determine the type of chromosomes by the immunoidentification of the

centromere position. Pachytene chromosomes were measured and then chromosomes were arranged according to their length (Fig. 1c). In mole-rat SC karyotype from Ravno Pole, the following set of chromosomes was observed:

- 15 biarmed chromosomes:
 - 2 medium metacentrics (№ 6, 11)
 - 3 submetacentrics (medium – №7, 10, small – 15)
 - 10 subtelocentrics (large – №1-5, medium – 8-9, 12-14)
 - 12 medium and small single-armed (acrocentrics) chromosomes (№16-27)
 - Sex chromosomes: X – medium submetacentric; Y – small subtelocentric
- This combination of chromosomes was defined as the general formula $2n=56$, $NFa=84$, $NF=88$.

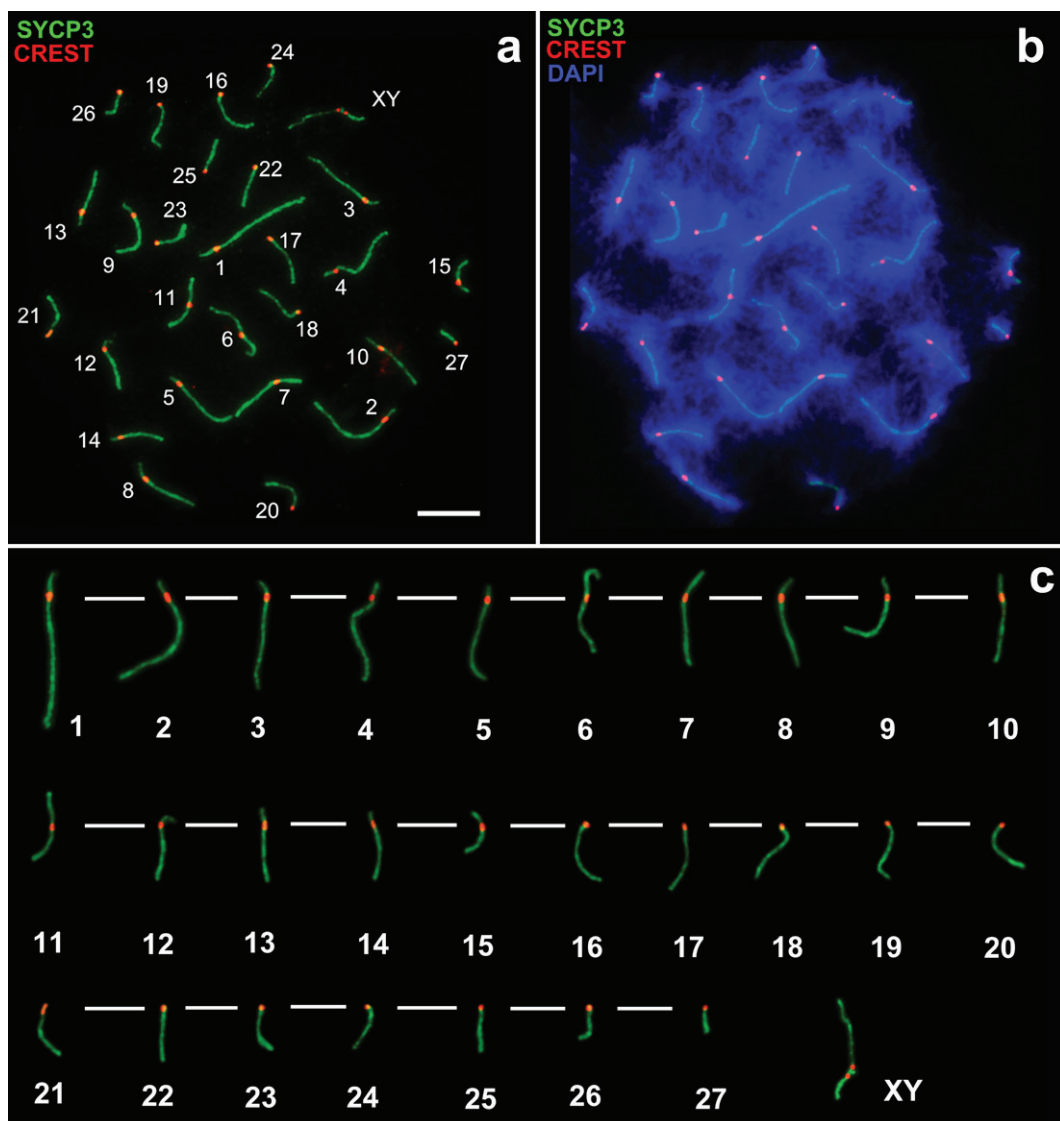


Fig. 1. Synaptonemal complexes (a, b) and SC karyotype (c) of *N. leucodon* from Ravno Pole (Sofia). SCs were immunostained using antibodies to SYCP3 protein (green), centromeres – antibodies to proteins of kinetochores (CREST, red). DAPI stained chromatin (blue). Scale bar: 5 μ m.

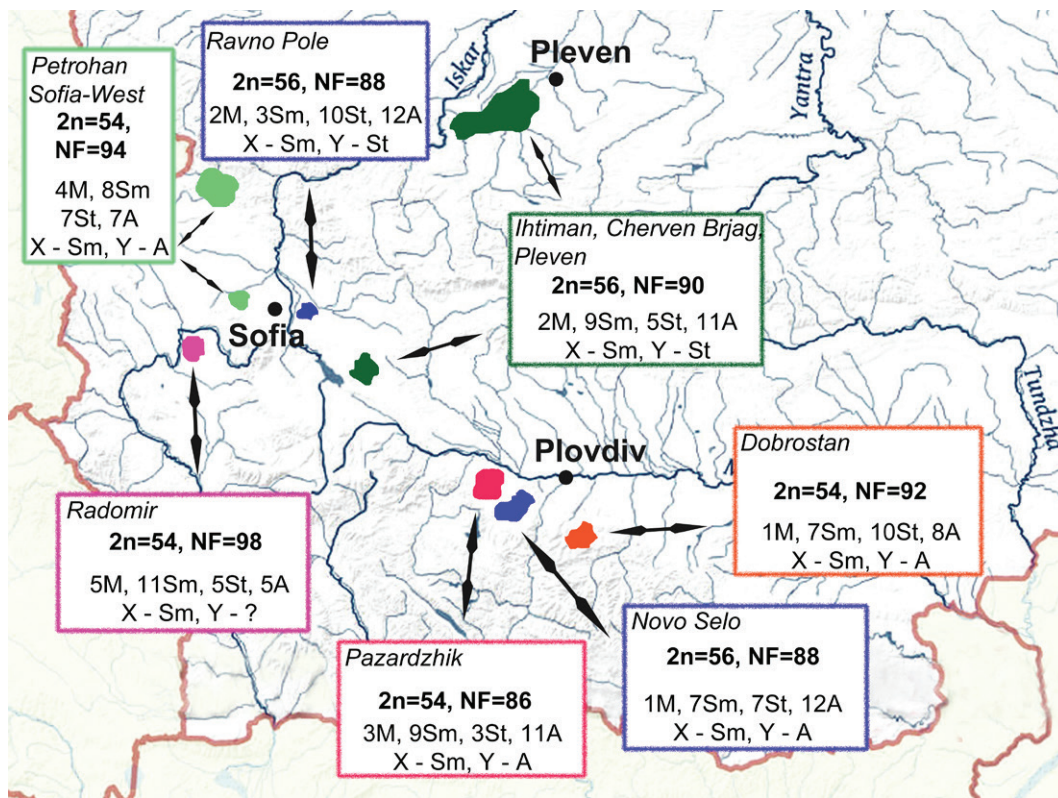


Fig. 2. Distribution of some chromosome forms of *N. leucodon* in Western Bulgaria (our data and data from SAVIĆ & SOLDATOVIĆ 1978, PESHEV 1981, 1983). Chromosomes: M – metacentric, Sm – submetacentric, St – subtelocentric, A – acrocentric.

Discussion

Karyotypic polymorphism among animals (mammals inclusive) is well known (KING 1993, DOBIGNY et al. 2015). This chromosome variability is often based on fusions of two acrocentric chromosomes, named Rb translocations. The most noticeable examples are the house mouse (CROPP & WINKING 1981), the common shrew (SEARLE et al. 2010, MATVEEVSKY et al. 2012), the eastern mole-vole (BAKLOUSHINSKAYA & MATVEEVSKY 2018) and the three *Nannospalax* superspecies (NEVO et al. 2001).

Chromosome studies of the family Spalacidae have clarified that $2n$ varies between 36-62 and the number of chromosome arms (NF) between 70-124 (NEVO et al. 2001). Mole-rat karyotypic differences are a result of different chromosome rearrangements such as Rb translocations, heterochromatin additions, pericentric inversions (SAVIĆ & SOLDATOVIĆ 1984, WHARMAN et al. 1985, IVANITSKAYA et al. 1997, 2008). The unique Y chromosome alteration in southern cytotypes of *N. ehrenbergi* has been also described (MATVEEVSKY et al. 2018).

Nannospalax leucodon ($2n=46-58$, $NF=76-98$) superspecies includes 25 cytotypes (SAVIĆ & SOLDATOVIĆ 1984, SAVIĆ et al. 2017). However,

there are many areas of the species' range that have not been studied yet. Moreover, new results can be found even in already studied areas as the karyotype from Ravno Pole.

Several cytotypes were identified (PESHEV 1981, 1983) in the area near Sofia (Fig. 2). To the west of the city, the forms Sofia-West ($2n = 54$, $NF = 94$) and Radomir ($2n = 54$, $NF = 98$) were established. The closest research point to the east of Sofia is approximately 45 km near Ihtiman Town ($2n=56$, $NF=90$) (PESHEV 1981, 1983). In the new locality, near the Ravno Pole Village, a 56-chromosomal form was found, but with a different NF ($2n = 56$, $NF = 88$). A similar karyotype ($2n=56$, $NF=88$) was found in Novo Selo (SOLDATOVIĆ & SAVIĆ 1978), 100 km southeast of Sofia. It has a chromosome combination 1M, 7Sm, 7St, 12A, X-Sm, Y-St. A comparative analysis of these karyotypes reveals their differences in the composition of meta-, submeta-, subtelocentrics and acrocentrics. In addition, Pazardzhik ($2n = 54$, $NF = 86$) and Ihtiman ($2n = 56$, $NF = 90$) karyofoms are located between Novo Selo and Ravno Pole. Thus, forms with identical $2n$ and NF, most likely, have an independent origin.

A similar situation has been described for *N. nehringi* forms with the same chromosome formula

from different localities in Turkey, $2n = 50$, $NF = 70$ (MATUR et al. 2011) and $2n = 56$, $NF = 72$ (ARSLAN et al. 2014). According to the assumption of MATUR et al. (2011), most of the central Anatolia area is occupied by the 60-chromosome form, and chromosome forms with different $2n$ and NF variations are formed on its boundaries. Moreover, there are forms with the same chromosomal formula but located far from each other. In the chromosomal evolution of *N. leucodon*, not only Rb translocations, but also centromere shifts, and inversions were observed, like in the closely related species *N. ehrenbergi* (WAHRMAN et al. 1985). Analysis of the distribution of mole-rat's chromosomal forms (SAVIĆ & SOLDATOVIĆ 1984, SAVIĆ et al. 2017) shows that 54- and 56-chromosome karyotypes occupy most of the central Balkans. We suppose that ancestral forms used to have 54 or 56 chromosomes, but this assumption requires further research.

An attempt to combine studies of karyotypes, craniometry, animal morphology, and some other features of mole-rat's populations led to the construction of the dendrogram (SAVIĆ & SOLDATOVIĆ 1984). According to this scheme, mole-rats from Western and Central Bulgaria belong to two groups: The South Balkan and Serbicus Branches. The karyotype from Ravno Pole can be attributed to the South Balkan Branch. Of course, this classification is somewhat artificial, since adjacent chromosome forms belong to different branches, such as Novo Selo and Dobrostan or Ravno Pole and Ihtiman. However, this division is a good attempt to understand the evolution of chromosomal diversity in the *N. leucodon* superspecies.

Undoubtedly, the most complete picture of the cytogenetic differentiation and karyotype evolution of the *N. leucodon* can be estimated after extensive karyological and phylogenetic studies over the entire range of species, including the Bulgarian part.

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