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KARYOTYPE ANALYSIS OF *NANNOSPALAX XANTHODON* (SPALACIDAE, RODENTIA) AT THE EASTERNMOST PART OF ITS DISTRIBUTION RANGE

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ABSTRACT. Chromosomal differentiation can play a crucial role in speciation. In order to reveal a complete picture of the speciation process within a taxon, it is important to determine the geographic distribution of all its chromosomal forms. The blind mole rats (subfamily Spalacinae) are one of the best models for studying chromosomal speciation due to extremely rich chromosomal variation across their large geographic distribution range. To fill the gap in our knowledge of the distribution of chromosomal races of small-bodied blind mole rats (*Nannospalax* sp.), we collected individuals from the region of Javakheti, Georgia – one of the easternmost localities known for *N. xanthodon*. We determined that this population has 9 meta- or submetacentric and 15 acrocentric chromosomes (2n=50, NFa=66, NF= 70). The same chromosomal formula is known for the blind mole rats from the nearby Erzurum-Kars plateau in Turkey. We compare our results with the other chromosomal races with the same diploid number 2n=50 found in Anatolia and Eastern Europe.

1. Introduction

The family of subterranean rodents Spalacinae originated from a Muroid-Cricetoid ancestor in Asia Minor or its vicinity in Oligocene or early Miocene and adaptively radiated in the Balkans, Ponto-Caspian steppes, and the Middle East, extending into North Africa [1]. The taxonomy within the group is uncertain, in part because of extremely high amount of cytogenetic variation in the genus *Nannospalax*, i.e. small bodied blind mole rats, with > 60 different, parapatric chromosomal forms described

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to date [2-3], with some authors arguing for separate species status for the most distinct cytotypes [4]. Molecular DNA variation suggest the presence of several deep phylogenetic clades within Spalacinae, but with no obvious geographic barriers between their respective geographic ranges [5-7].

Despite multiple karyological studies elsewhere (see references with in Arslan et al. 2016), there are relatively few reports on the mole rat karyology in the Caucasus and Armenia, with the diploid chromosome numbers in north-western Armenian mole rats determined to be 2n= 50 [2,8]. At the same time, several distant mole rat populations with identical diploid chromosome numbers (2n= 50) are known, which however still differ by the number of chromosome arms (NFa= 66-68, NF 70-72 etc., reviewed in Arslan et al 2016). The different NF variation of 2n= 50 of *N.* xanthodon were recorded in the Norht-West, South and Eastern Turkey [2, 9-16]. The karyotype 2n= 50 is also known for the Balkan mole rat *N. leucodon* in Romania [17] and Hungary [1, 18-20], which is closely related to *N. xanthodon* [5,7].

Identification of all chromosomal forms and their distribution ranges is necessary to understand the evolutionary history of the entire group, as well as to construct a more realistic taxonomy. For the first time, we describe the karyotypic characteristics of the blind mole rat populations on the Javakheti plateau, Georgia, and compare their similarities with other chromosomal races that have the same number of diploid chromosomes 2n=50.

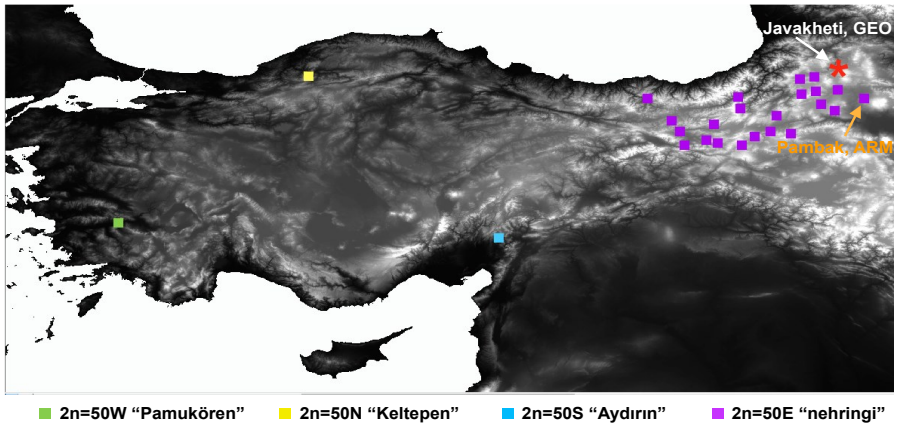
2. Materials and Methods

In this study, 10 (4 males, 6 females) blind mole rats were studied from two close localities on Javakheti plateau in Georgia: Agara (N41°22`55`, E43°12`36``) and Myasnikyani (N41°18`11``, E41°19`12``). Both these localities are of similar altitude (~1800 m a.s.l) and are characterised by similar alpine meadow / pasture habitat, but separated geographically by the deep Kura river canyon (Fig 1 a and b). The number of individuals analysed and karyological results are presented in Table 1.

Karyotypes were prepared from bone marrow according to Ford and Hamerton (1956) [21]. 25-30 metaphase cells, well-stained, and with chromosomes visibly separate and distinct, were examined from each animal. The diploid number of chromosomes (2n), the number of autosomal arms (NFa), the total number of chromosomal arms (NF) and the morphology of sex chromosomes were determined

from photographs of the metaphase plates according to the centromere position. Previously published karyotype data on *N. xanthodon* with diploid number 2n= 50 were compared with those of Georgian mole rats. We followed Matur et al. 2011 for classification of the corresponding cytopes (2n= 50S, 50N, 50E, 50W) [16].

a)



b)

FIGURE 1. a. Geographic distribution of 2n= 50 cytotypes of *N. xanthodon* and *N.leucodon*. Chromosomal races named according to Arslan et al. (2016). b. Locations of the two populations on the Javakheti plateau.

TABLE 1. The karyotype characteristics of known 2n= 50 cytotypes of *N. xanthodon* and *N. leucodon*. NF – fundamental number of chromosome arms, a – acrocentric, sm – small metacentric.

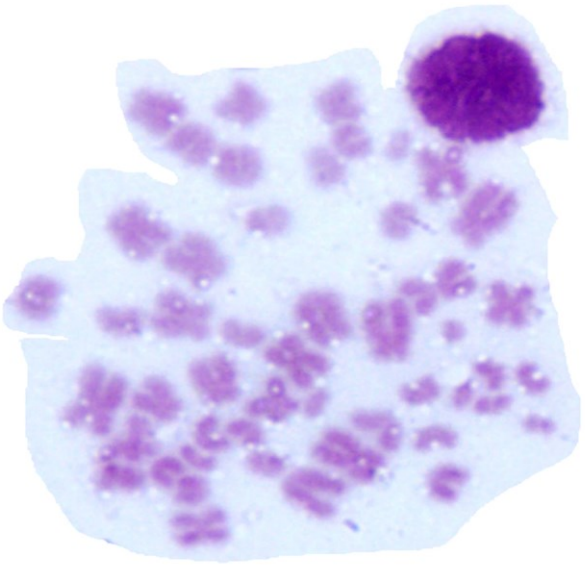
Species	**2n**	**NF**	**X**	**Y**	**Type locality**	**References**
N.xanthodon	**50Ge**	**70**	sm	a	**Javakheti, Georgia**	**This study**
N.xanthodon	50W	–	–	–	Aydın (Turkey)	Nevo et al. (1994, 1995)
N.xanthodon	50W	74			Alaşehir, Pamukören (Turkey)	Matur et al. (2011)
N.xanthodon	50	72			Maralik (Armenia)	Lyapunova et al. (1974)
N.xanthodon	50E	70	sm	–	Erzurum, Sarıkamış	Nevo et al. (1994), (1995)
N.xanthodon	50E	72	sm	a	Erzurum, Susuz, Ardahan	Sözen et al. (2000a)
N.xanthodon	50E	72			Eğribel Pass (Giresun), Ovid mount. (Rize), Demirözü (Bayburt), Yollarüstü (Erzincan)	Kankılıç et al. (2007b)
N.xanthodon	50E	70			Kandilli, Ilıca, Otlukbeli (Erzurum), Arpayazı (Erzincan)	Matur et al. (2011)
N.xanthodon	50E	70			Susuz, Selim (Kars)	Kankılıç et al. (2007b)
N.xanthodon	50N	70	sm	a	Karabük (Keltepe)	Sözen (2004), Matur et al. (2011)
N.xanthodon	50N	70	sm	a	Kahyalar	Sözen et al. (2006b)
N.xanthodon	50E	70	sm	a	Pasinler (Erzurum), Digor, Selim, Arpaçay (Kars), Hanak, Çıldır, Göle (Ardahan)	Coşkun (2003), Ulutürk et al. (2009)
N.xanthodon	50E	70			Göle (Erzurum)	Kankılıç et al. (2007)
N.xanthodon	50S	70			Andırın (Kahramanmaraş)	Matur et al. (2011)
N.xanthodon	50NE	72	sm	a	Başköy, Ovit pass	Sözen et al. (2006a)
N.leucodon	50	84			Transylvania, (Romania)	Raicu et al. (1968)
N.leucodon/ N.transsylvaticus	50	84			Hajduhadhaz, Hajdubagos (Hungary) Urziceni, Dabaca, Cluj-Napoca (Romania)	Raicu et al. (1968), Soldatovic (1977), Savic and Soldatovic (1977), Soldatovic and Savic (1983), Savic and Nevo (1990)

The specimens examined were deposited at Illia State University in Georgia, and the karyotype preparations were deposited in the Department of Biology, the Faculty of Sciences, Dokuz Eylül University in Turkey.

3. Results and Discussion

The chromosomal complement of the Javakheti mole rats included nine bi-armed and 15 acrocentric autosomes (NFa = 66: Fig. 2a,b, Fig. 3). NF = 70, X is a large sub-metacentric, and Y is a small acrocentric chromosome. Matur et al. (2011) [16] compared four 2n= 50 from Turkey, and named them according to their geographical distribution in Turkey; of these 2n= 50E is found in North-Eastern Anatolia [16]. Two NF types were described previously within 2n=50E: First is NFa = 68, NF = 72 from Pambak, Maralik (Armenia) and Erzurum, Erzincan, Susuz, Ardahan, Rize, Giresun, and Bayburt provinces in eastern Turkey [2, 11-12, 15, 22]. The second is NFa = 66, NF = 70 from Erzurum, Kars and Karabük provinces in Anatolia [4, 9-11, 13-16, 23-24]. It is apparent that the cytotype from Javakheti is more similar to the second described type of 2n=50E (NFa = 66, NF = 70), with the closest reported location less than 100 km away in the Eastern Turkey, but is different from the another nearby location in Armenia (Pambak, Fig. 1). Previously, the small-bodied blind mole rats were described in Georgia and Armenia as *Nannospalax nehringi* Satunin, 1898 - Nehring's Mole Rat [25-27], now synonymous with *N. xanthodon*.
Our results thus confirm that the cytotype 2n=50 is found at the north-eastern edge of *N. xanthodon* distribution range in Georgia. Both of its NF types (NF=70 and NF=72) were classified as a single chromosomal race "*nehringi*" by Arslan et al. (2016) [3], due to their continuous and distinct distribution ranges in the Eastern Turkey, Georgia and Armenia. At the same time, other *N. xanthodon* populations with the same diploid number 2n=50 are found in West, South and North of Anatolia (Matur et al. 2011) [16], and the cytotype 2n=50 of closely related *N. leucodon* is known from the Carpathian basin in Eastern Europe [18] (Raicu et al. 1968, Table 1). There is a substantial variation of NF types among these geographically distant populations (Fig. 2, Fig. 3), thus providing a support to the hypothesis that the same diploid number must have evolved independently in different populations. The cytogenetic variation in the blind mole rats corresponds only partly to their molecular DNA phylogeny, and sometimes even contradicts the accepted species taxonomy within the *Nannospalax* genus, as revealed, for example, by Matur et al. 2019 [7]. Further studies of phylogenetic relationship among the various chromosomal races of all species in the subfamily Spalacinae (both genera *Nannospalax* and *Spalax*), combining both molecular DNA and cytogenetic analyses, are needed to reveal the evolutionary history in this unique group.

a)



b)

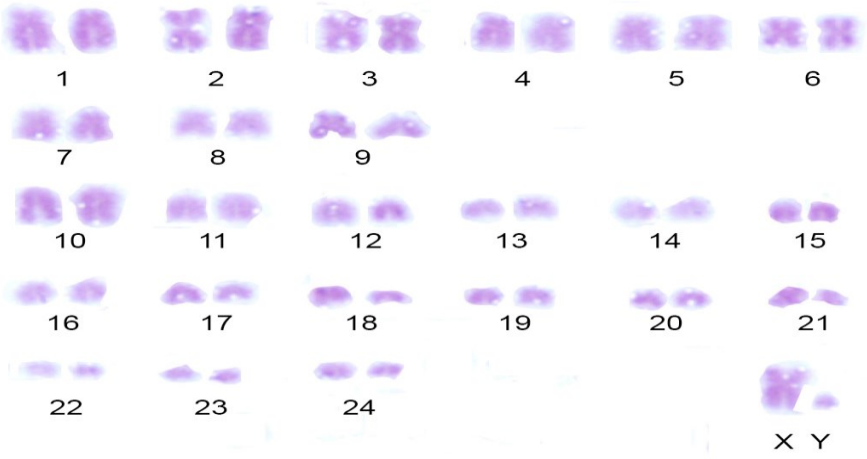


FIGURE 2. Karyotype analysis of Georgian Mole rats. (a) chromosome plate and (b) karyotypes of the samples.

FIGURE 3. Per chromosome comparison of 2n= 50 karyotypes in different populations of mole rats. The corresponding figures from the published sources shown in Table 1 were used. The comparison shows that 2n=50Ge from Javakheti is similar to the nearby 2n=50E in Turkey and 2n= 50leu (*N. leucodon*) has the largest number of bi-armed chromosomes among the other 2n=50.

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