Folia Zool. – 58(4): 416–419 (2009)

Cytogenetic characteristic of the southern water shrew, *Neomys anomalus* (Insectivora: Soricidae), in the Strandzha Mountains (South-East Bulgaria)

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Received 18 March 2008; Accepted 24 February 2009

A b s t r a c t . The chromosome set of *N. anomalus* from Bulgaria was studied by means of C-, and NOR-banding. The diploid chromosome number of this species is known to be 2n=52 (NF = 98, NFa = 94). In all the studied individuals, the X chromosome appeared to be the second longest submetacentric chromosome, whereas the Y chromosome was a medium-sized submetacentric chromosome, consisting of heterochromatin. Such morphology of the Y chromosome was not described previously. The extent and localization of C-bands in the pericentromeric regions varied between the pairs of chromosomes. The nucleolar organizer regions (NOR's) were observed in four pairs of autosomes.

Key words: water shrews, karyotype, C-banding, NOR's

Introduction

The water shrews of the *Neomys* genus are represented by three species in the Palaearctic region: *Neomys fodiens* (Pennant, 1771), *N. anomalus* (Cabrera, 1907), and *N. teres* (Miller, 1908). In Europe *N. anomalus* and *N. fodiens* are largely sympatric (M i t c h e l l - J o n e s et al. 1999), but differ in their ecological requirements.

The cytogenetic studies of the species of the *Neomys* genus have shown that they alone in the Soricidae family have a stable karyotype, whereas most of the other members of the family display different polymorphic forms. The *Neomys* genus is notable for the fact that all three species, *N. Anomalus*, *N. fodiens*, and *N. teres,* have a similar karyotype: 2n = 52, the NF varying between 90 and 98. This karyotype contains 22 pairs of biarmed autosomes and three pairs of uniarmed autosomes (B o v e y 1949, M e y l a n 1964, F r e d g a & L e v a n 1969, Z i m a 1984). Only G r a f o d a t s k y et al. (1993) have observed a different phenomenon: they have described 21 pairs of biarmed autosomes and four pairs of uniarmed autosomes in a population of *N. fodiens* in Siberia (Novosibirsk). However, polymorphism, regarding the morphology of the sex chromosomes, is found in this genus. In the European range of the species subtelocentric, submetacentric and metacentric X chromosomes and subtelocentric, submetacentric and acrocentric Y chromosomes were described. (F r e d g a & L e v a n 1969, R i m s a et al. 1978, Z i m a 1984, J i m e n e z et al. 1984, I v a n i t s k a y a 1989, B e l c h e v a & K o l e v s k a 1992, G r a f o d a t s k y et al. 1993).

The studies of the *N. anomalus* karyotype in Europe are few. This is probably due to the fact that most authors assume it is identical to the karyotype of *N. fodiens*

(G r a f o d a t s k y et al. 1993). The two karyotypes seem to display similarity in the pattern of their G-bands (Z i m a et al. 1998). The karyotype of *N. anomalus* has been described in populations inhabiting Switzerland (M e y l a n 1966), Yugoslavia (R i m s a et al. 1978), Austria, Slovakia, and Romania (Z i m a et al. 1998), but the morphology of the sex chromosomes has only been determined in Spanish populations (J i m e n e z et al. 1984), in which the sex chromosomes of *N. anomalus* were defined as subtelocentric. In Bulgaria, the only karyotype that has been described is that of the northern water shrew (B e l c h e v a & K o l e v s k a 1992).

The aim of the present study is to characterize the karyotype, the distribution of heterochromatin, and the localization of NOR in the southern water shrew in Bulgaria.

Material and Methods

Five individuals (3 males and 2 females) of the southern water shrew from the Strandzha Mountains (outflow of the Veleka River – 42°4' N, 27°59' E) were studied. Morphological criteria were used in determining the species (P e s h e v et al. 2004).

The karyotype in bone marrow cells was analyzed, according to the standard method (R o t h f e l s & S i m i n o v i t c h 1958). The chromosomes in the karyotype were arranged according to their morphology and decreasing size. Differential C- and NOR-banding was performed by standard methods (S u m n e r 1972, G o o d p a s t u r e & B l o o m 1975). A total of 80 metaphases were analyzed in all five individuals.

Results and Discussion

The karyotype of all the shrews studied was represented by 52 chromosomes (NF = 98, NFa = 94): 10 pairs were metacentric, 10 pairs were submetacentric, 2 pairs were subtelocentric, and 3 pairs were acrocentric (Fig. 1). Based on these results, the recorded *N. anomalus* karyotype did not differ from the karyotype previously described in Europe (M e y l a n 1966, R i m s a et al. 1978, J i m e n e z et al. 1984, Z i m a et al. 1998).

The X and Y chromosomes were submetacentrics, the X being the second longest in the submetacentric chromosome group, while the Y chromosome was a medium-sized

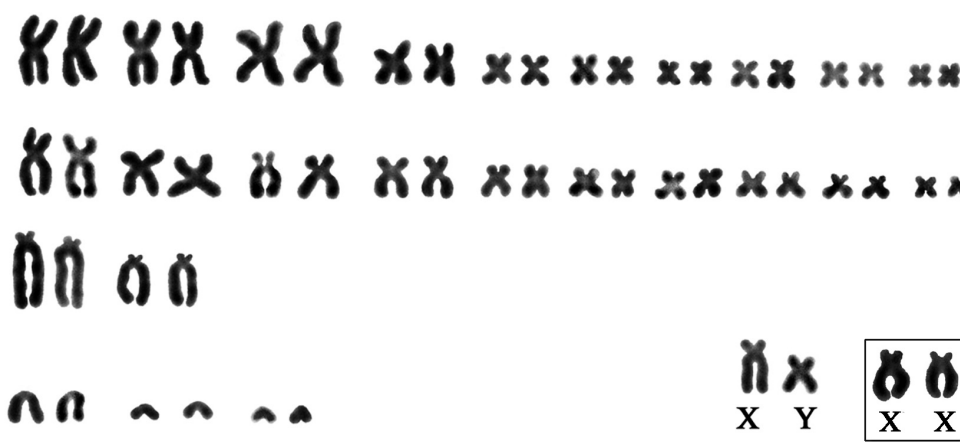

Fig. 1. Karyotype of the Southern water-shrew from the Strandzha Mountains (South-East Bulgaria).

submetacentric, as long as the long arm of the X chromosome (Fig. 1). The morphology of the Y chromosome differed from the one described in the Spanish populations' subtelocentric Y-chromosome (J i m e n e z et al. 1984). No sex chromosome polymorphism was recorded.

Staining for structural heterochromatin showed pericentromeric C-bands in most autosomes. The pattern of C-banding in the first six metacentric pairs was similar in all the studied shrews: dark C-bands in the pericentromeric regions (Fig. 2). The remaining metacentric chromosome pairs were variable with respect to the content and localization of C-bands in the pericentromeric regions. Two of these pairs were heteromorphic for the localization of heterochromatin (+/–), while the others were C-negative (Fig. 2). Most of the submetacentric chromosome pairs had dark C-bands in the pericentromeric regions, but the smallest pair was heteromorphic. The largest subtelocentric chromosome pair contained no C-bands possibly because pericentric inversion was followed by the loss of heterochromatin. The two acrocentric chromosome pairs were heteromorphic as well, whereas the middle-sized pair demonstrated an intensively stained pericentromeric C-band.

Fig. 2. Male C-banded karyotype of the southern water-shrew from the Strandzha Mountains (South-East Bulgaria).

The X chromosome had a dark C-band in the pericentromeric region, whereas the Y chromosome was characterized by dark C-banding across its entire length.

The NORs were localized in two middle-sized submetacentric autosome pairs (within the short arms) and in two small acrocentric autosome pairs (within telomeric regions)

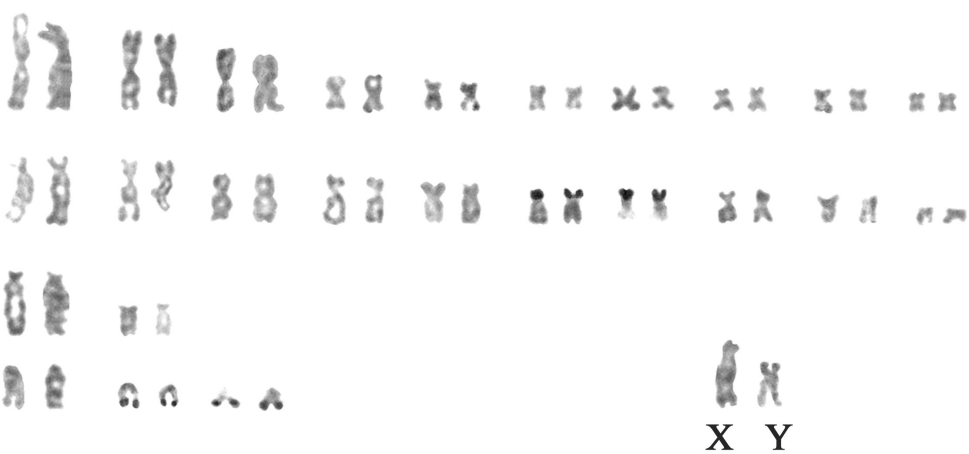

Fig. 3. Male Ag-banded karyotype of the southern water-shrew from the Strandzha Mountains (South-East Bulgaria).

(Fig. 3). The density of NOR-banding proved to be variable, which may be explained by the differential activity of ribosomal gene clusters between homologous chromosomes.

The carried out karyological analysis confirmed the karyotype similarity between *N. anomalus* and *N. fodiens* in Bulgaria. The intraindividual polymorphism, regarding the size and morphology of sex chromosomes, described in Bulgarian populations of *N. fodiens* (B e l c h e v a & K o l e v s k a 1992), has not been found in the studied southern water shrews. The sex chromosomes reported in *N. fodiens* in Bulgaria should be specified.

A c k n o w l e d g e m e n t s

The present study was supported by MES-NSF – project No B-5-2005.

L I T E R A T U R E

Belcheva R. & Kolevska N. 1992: Cytogenetical study on some Soricidae (Insectivora, Mammalia). *Annuaire de l' Universite de Sofia "Kl. Ohridski", Faculte de Biologie, livre1-Zoologie 80: 144–156.*

Bovey R. 1949: Les chromosomes des Chiropteres et des Insectivores. *Rev. Suisse Zool. 56: 371–460.*

Fredga K. & Levan A. 1969: The chromosomes of the European water shrew (*Neomys fodiens*). *Hereditas 62: 348–356.*

Goodpasture K. & Bloom S.E. 1975: Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. *Chromosoma 53: 37–50.*

Graphodatsky A.S., Radjabli S.I., Zaitsev M.V. & Sharshov A.A. 1993: Urovni chromosomnogo konservatisma v razlichnikh grupakh nasekomoyadnykh mlekopitayushchikh (Mammalia, Insectivora) [The levels of chromosome conservatism in the different groups of insectivores (Mammalia, Insectivora)]. In: Zaitsev M.V. (ed.), Questions of systematic, faunistics and paleontology of small mammals. *Trudy zoologicheskogo instituta 243: 47–57.* (*in Russian with English summary*)

Ivanitskaya E. 1989: Strukturnyj heterokhromatin i yadrishkoobrazuiushchie raiony v kariotipakh nekotorikh zemleroek (Soricidae, Insectivora) [Constitutitive heterochromatin and nucleolar organizer regions in karyotypes of some shrews (Soricidae, Insectivora)]. *Genetika 25: 1188–1198.* (*in Russian with English summary*)

Jimenez R., Burgos M. & Diaz de la Guardia R. 1984: Meiotic behavior of sex chromosomes and polymeiosis in three species of insectivores. *Genetica 65: 187–192.*

Meylan A. 1964: Le polymorphisme chromosomique de *Sorex araneus* L. (Mamm.-Insectivora). *Rev. Suisse Zool. 71: 903–983.*

Meylan A. 1966: Donneés nouvelles sur les chromosomes des insectivores Europeéns (Mamm.). *Rev. Suisse Zool. 73: 548–558.*

Mitchell-Jones A.J., Amori G., Bogdanowicz W., Kryštufek B., Reijnders P.J., Spitzenberger F., Stubbe M., Thissen J.B., Vohralík V. & Zima J. 1999: Atlas of European mammals. *Academic Press, London.*

Peshev Ts., Peshev D. & Popov V. 2004: Fauna Bulgarica, 27 (Mammalia). *Editio Academica "Marin Drinov", Sofia.* (*in Bulgarian with English summary*)

Rimsa D., Zivkovic S. & Petrov B. 1978: The results of cytogenetical study of shrews (Soricidae, Insectivora, Mammalia) in Yugoslavia. *Biosystematika 4: 209–215.*

Rothfels K. & Siminovitch L. 1958: Air drying technique for flattening chromosomes in mammalian cells grown in vitro. *Stain. Technol. 33: 73–75.*

Sumner A.T. 1972: A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res. 75: 304–306.*

Zima J. 1984: Chromosomes of certain small mammals from Southern Bohemia and the Šumava Mts. (ČSSR). *Folia Zool. 33: 133–141.*

Zima J., Lukáčová L. & Macholán M. 1998: Chromosomal evolution of shrews. In: Wójcik J.M. & Wolsan M. (eds.), Evolution of shrews. *Mammal Research Institute, Polish Academy of Sciences, Bialowieza*: *175–218.*