

Karyotype and X–Y chromosome pairing in the Sikkim vole (*Microtus (Neodon) sikimensis*)

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Abstract

Pairing of the X and Y chromosomes during meiotic prophase and the G- and C-banding patterns were analysed in *Microtus sikimensis*. The karyotype of *M. sikimensis* from central Nepal, has $2n = 48$ and $FN_a = 56$. It has been derived from the all single-armed karyotype of *M. carruthersi* through a unique course of evolution. There is a synapsis between the X and Y chromosomes at pachytene and end-to-end association from diakinesis to metaphase I. *Microtus sikimensis* has no close karyological or morphological relationship to the other synaptic species studied so far. These data confirm that several species or lineages with synaptic condition exist in the genus *Microtus*, and that the ancestor of *Microtus* had synaptic sex chromosomes.

Key words: *Microtus sikimensis*, karyotype, synaptic sex chromosomes, *Microtus* phylogeny

INTRODUCTION

Nowhere is the explosiveness and recency of Arvicolinae evolution more dramatically highlighted than in the specious genus *Microtus* (Musser & Carlton, 1993). Various authors studying palaeontological, morphological, cytogenetical, molecular, and biochemical data sets (Modi, 1987b, 1990, 1996; Chaline & Graf, 1988; Gromov & Poliakov, 1992; Conroy & Cook, 1999, 2000) have proposed systematic relationships within *Microtus*. There is no consensus concerning the morphological limits or monophyly of many of these taxa. These classification difficulties have been caused by diversification that occurred too quickly to produce transition forms, and by the remnants of ancestral polymorphism that still exist, because insufficient time has elapsed since speciation for the fixation of alternative character states in different taxa (Modi, 1996; Conroy & Cook, 1999).

Sikkim voles *M. sikimensis* (Hodgson, 1849) are fairly common in bushes growing at the edges of rhododendron and coniferous forests, and in the alpine meadow, in central Nepal (Abe, 1971). The biology of

M. sikimensis is poorly known, and consequently, their systematic status is unclear. *Microtus sikimensis* that inhabits the Himalayan region belongs to the subgenus *Neodon*. *Neodon* is the representative of such variability and inconsistency of systematic opinion in the so-called 'pine voles' with a pitymoid structure of the first lower molar. They have been treated as the independent genus *Pitymys* (Ellerman & Morrison-Scott, 1966; Corbet, 1978), or as a subgenus of the genus *Microtus* (Musser & Carlton, 1993). At times, the taxon *Neodon* has been allocated as a subgenus in each genus.

The Palaearctic species within the genus *Microtus* studied so far can be classified into two types by the pattern of XY chromosomes pairing at meiotic prophase: (1) a group, with asynaptic sex chromosomes; (2) a synaptic group, those species that undergo sex chromosome pairing in the male. The former group includes *M. arvalis* (Ashley, Jaarola & Fredga, 1989a; Borodin, Sablina, Zakiyan *et al.*, 1991), *M. agrestis* (Wolf, Baumgart & Winking, 1988; Ashley, Jaarola & Fredga, 1989b), *M. cabreriae* (Jimenez *et al.*, 1991), *M. duodecimcostatus* (Carnero *et al.*, 1991), *M. rossiaemerdionalis*, *M. kirgisorum*, *M. transcaspicus* (Borodin, Sablina, Zakiyan *et al.*, 1991; Borodin, Sablina & Rodionova, 1995), *M. guentheri*, *M. socialis*, *M. afghanus*, *M. bucharicus*, *M. majori* (Borodin,

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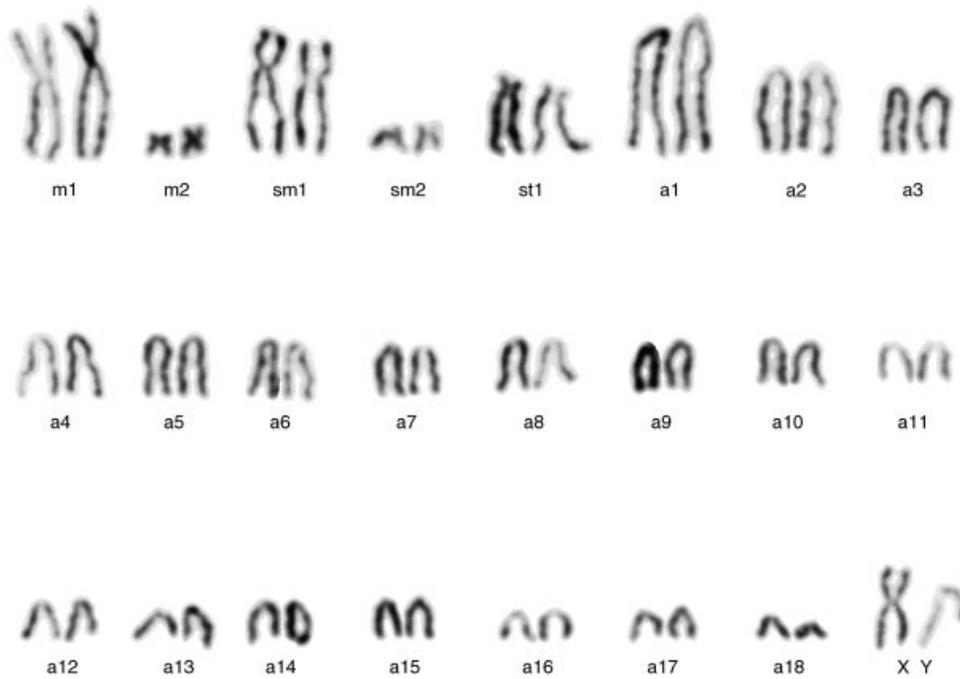


Fig. 1. Non-banded (conventional) karyotype of *Microtus sikimensis*, male.

Sablina & Rodionova, 1995), and *M. fortis* (Levenkova, Malygin & Safronova, 1997). The latter group includes *M. oeconomus* (Ashley & Fredga, 1994; Borodin, Sablina & Rodionova, 1995), *M. montebelli* (Iwasa & Obara, 1995; Borodin, Rogatcheva *et al.*, 1997), *M. kikuchii* (Mekada *et al.*, 2001), and *M. mandarinus* (Gu, Wang & Zhu, 1999). The former three synaptic species are related to each other karyologically (Fredga, Persson & Stenseth, 1980; Yamakage *et al.*, 1985; Harada *et al.*, 1991; Mekada *et al.*, 2001). These findings suggest that the synaptic and asynaptic behaviour of the XY chromosomes of *Microtus* species might prove to be a synapomorphic divide of *Microtus* lineages, and might help to reconstruct the confused systematic relationships within *Microtus* (Borodin, Sablina & Rodionova, 1995; Mekada *et al.*, 2001).

This study reports on the conventional, G- and C-banded karyotypes, and the pattern of X and Y chromosome pairing of *M. sikimensis*, and discusses phylogenetic relationships within *Microtus*.

MATERIAL AND METHODS

Four wild males collected from Ghorepai (Myagdi District) in central Nepal, in November 1994 and March 1999, were examined. Mitotic chromosomes were prepared from fibroblast cells obtained from primary lung or tail tissue cultures using standard techniques. The karyotype was then analysed using conventional Giemsa staining. The banding pattern analyses were performed using G-band staining (Summer, Evans & Buckland, 1971) and C-band staining (Modi, 1987a).

Surface-spread preparations of 2 of the specimens were made according to the method described by Chandley (1989) and stained with silver nitrate (Howell & Black, 1980). After light microscopic examination, spreads were transferred to specimen grids and examined and photographed with an electron microscope, JEM-100 (JEOL, Japan) at 80 kV. Seventy-six pachytene and 20 diakinesis spermatocytes were examined from these animals.

RESULTS

The diploid chromosome number ($2n$) of *M. sikimensis* was 48 and the number of major autosome arms (FNa) was 56, based on 220 metaphases analysed. The karyotype comprises two (large and small) metacentric (m) pairs, two (large and small) submetacentric (sm) pairs, one medium-sized subtelocentric (st) pair, and 18 large to small acrocentric (a) pairs. The X chromosome is a medium-sized metacentric element. The Y chromosome is a medium-sized acrocentric element (Fig. 1). The G-bands on *M. sikimensis* are distinct and each homologous pair was clearly identified (Fig. 2). In *M. sikimensis*, tiny C-bands are present only in the centromeric regions of the autosomes and X chromosome. The Y chromosome is totally heterochromatic (Fig. 3).

In the pachytene stage, all the autosomal axes form complete synaptonemal complexes (SCs). The X and Y chromosomes pair at pachytene, forming a clear SC in their terminal regions (Fig. 4). At early pachytene, complete synapsis was regularly formed between the ends of the X and Y axes in *M. sikimensis*. During meiotic progression, the X and Y axes gradually

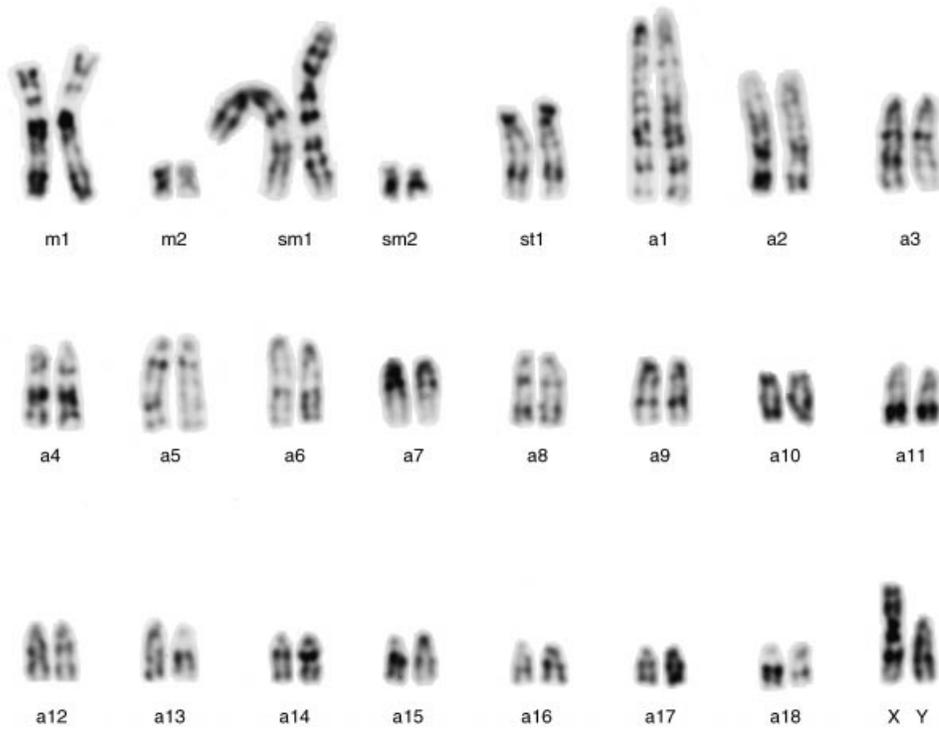


Fig. 2. G-banded karyotype of *Microtus sikimensis*, male.

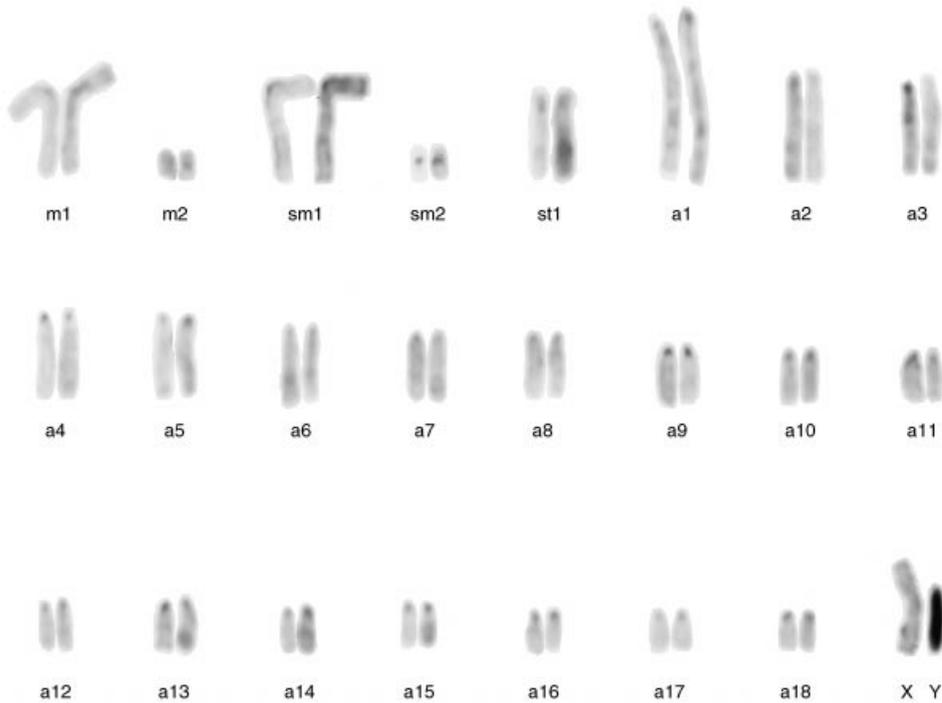


Fig. 3. C-banded karyotype of *Microtus sikimensis*, male.

desynapse, leaving an end-to-end association at late pachytene-diplotene. Univalents of X and Y were also observed at middle and late pachytene (Fig. 4e), but at a low frequency (7%). The average portions of the X and Y axes involved in pairing in pachytene are $6.6 \pm 2.9\%$ (SD) and $8.1 \pm 2.9\%$, respectively.

DISCUSSION

Among other members of the subgenus *Neodon*, the karyotypes of *M. juldaschi* and *M. carruthersi* have been reported (Gileva *et al.*, 1982). Although the specific status of the latter is unclear, it has been treated as a

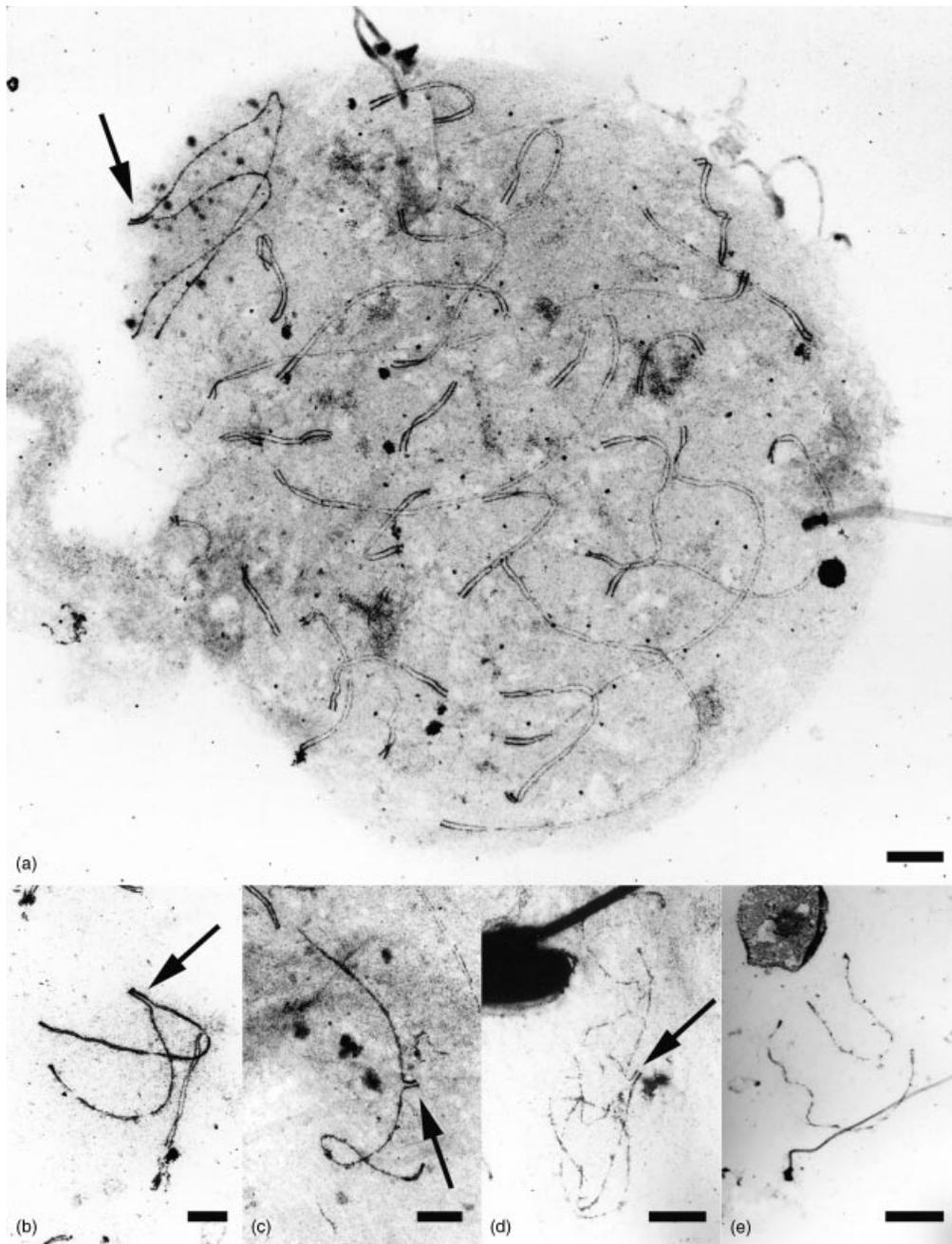


Fig. 4. (a) Electron micrograph of silver-stained surface-spread pachytene nuclei of *Microtus sikimensis* spermatocytes. (b), (c) Sex bivalent at meiotic prophase in *M. sikimensis*. Arrows, region of pairing between the X and Y elements. (a) Middle pachytene; (b) early pachytene; (c) middle pachytene; (d) late pachytene; (e) univalents of X and Y at late pachytene. Scale bar = 2 μ m.

distinct species (Ellerman & Morrison-Scott, 1951; Ognev, 1964) or synonymized with *M. juldaschi* (Corbet, 1978; Musser & Carlton, 1993). Their diploid number is $2n = 54$, but their FNa ranges from 52 to 60. The FNa of *M. juldaschi* is 58, and consists of three metacentric pairs, 23 acrocentric pairs, a metacentric X, and an acrocentric Y. On the other hand, *M. carruthersi* has three different karyotypes. The FNa of the first form is 52, and consists entirely of single-armed elements. The FNa of the second form is 56, and consists

of two metacentric and 24 acrocentric pairs, and acrocentric X and Y. The FNa of the third form is 60, and consists of four metacentric and 22 acrocentric pairs, and acrocentric X and Y. These three forms of *M. carruthersi* and a form of *M. juldaschi* differ from each other by three or four pericentric inversions. By the character of the banding pattern as well as by the results of laboratory crosses, they form a single group, regardless of whether they belong to *M. juldaschi* or *M. carruthersi*, except for the entirely single-armed

(FNa=52) form of *M. carruthersi*. The entirely single-armed form, on the other hand, occupies a separate position. It is presumed that the ancestral karyotype of the genus *Microtus* was $2n=56$, with all chromosomes single-armed (Matthey, 1957, 1973). Based on this hypothesis, the form of *M. carruthersi* comprised entirely of single-armed elements should be regarded as ancestral. Consequently, the other forms of *M. carruthersi* and *M. juldaschi* may have been derived through pericentric inversions.

This study clearly showed that the diploid chromosome number of *M. sikimensis* is $2n=48$ (FNa=56). Comparison with the published karyotypes of *M. juldaschi* and *M. carruthersi* (Gileva *et al.*, 1982), revealed homologous G-banding patterns on the chromosomal arms among these species. The G-banding pattern of m1 of *M. sikimensis* is similar to that of the large metacentric pair of *M. juldaschi*. The m1 of *M. sikimensis* might be caused from chromosome 2 of the entirely single-armed form by a single pericentric inversion like that of the metacentric pair of *M. juldaschi*. This is also true for sm2 (*M. sikimensis*) and chromosome 23 (all single-armed form). A Robertsonian rearrangement explains the homology of the short and long arms of st1 (*M. sikimensis*) with chromosome 26 and 5 (all single-armed form), respectively. This kind of rearrangement also explains the homology of the arms of sm1 and m2 with chromosomes 13 and 7, and chromosomes 25 and 24, respectively. For small autosomes, however, the identification of banding pattern homologies is rather difficult. The morphological difference between the X chromosome of *M. sikimensis* and that of the all single-armed form can be explained by a pericentric inversion. The chromosomal rearrangement of *M. sikimensis* from the entirely single-armed form is characteristic when compared to those of *M. juldaschi* and *M. carruthersi* (Gileva *et al.*, 1982), except for m1 and the X chromosome. This suggests that the karyotype of *M. sikimensis* was derived from that of the entirely single-armed form through a unique course of evolution.

It is possible that the loss of the ability of the sex chromosomes to pair at male meiosis is a derived character, which appeared in the common ancestor of most members of the genus *Microtus* living in the Palaearctic region (Borodin, Rogatcheva *et al.*, 1997).

The synapctic group, which consists of *M. oeconomus*, *M. montebelli* and *M. kikuchii*, is considered closely related phylogenetically (Yamakage *et al.*, 1985; Conroy & Cook, 2000; Mekada *et al.*, 2001) and belongs to the *oeconomus* species lineage (subgenus *Pallasinus*). However, the new synapctic species, *M. sikimensis* is thought to be not closely related to the *oeconomus* species lineage, based on both karyological and morphological evidence. This suggests that several species or lineages with the synapctic condition exist in the genus *Microtus*, and that the ancestors of *Microtus* had synapctic sex chromosomes (Mekada *et al.*, 2001). It was recently reported that *M. mandarinus* has synapctic XY chromosomes (Gu *et al.*, 1999). This species belongs to the taxon *Lasiopodomys*, which is often treated as a full

genus (Gromov & Poliakov, 1992; Musser & Carleton, 1993; Nowak, 1999) or subgenus of *Microtus* (Corbet, 1978; Corbet & Hill, 1991). Moreover, it is thought that the taxa *Neodon* and *Lasiopodomys*, which include *M. sikimensis* and *M. mandarinus*, respectively, appeared early in *Microtus* diversification (Zakrzewski, 1985).

Recently, Conroy & Cook (2000) reported the phylogenetic relationships among some *Microtus* species, based on mitochondrial cytochrome-*b* gene sequences. *Microtus gregalis* was placed basal to all the other *Microtus* species studied. However, several species stemmed from a polytomy, and there were generally weak relationships across the basal branches. These basal relationships most likely reflect rapid diversification (Modi, 1996; Conroy & Cook, 2000). The taxa *Neodon* and *Lasiopodomys* may be located outside the *Microtus* clade or be part of the polytomy. According to Conroy & Cook (2000), the asynapctic *M. fortis* and *M. middendorffi* (subgenus *Alexandromys*) form the Asian clade with the synapctic *oeconomus* species lineage (*M. oeconomus*, *M. montebelli*, and *M. kikuchii*). If we accept the phylogenetic tree of Conroy & Cook (2000), then loss of the ability of XY chromosome pairing in *M. fortis* must have occurred independently from the other asynapctic species, such as *M. agrestis*, *M. arvalis*, and *M. rossiaemeridionalis*. It is also possible to assume that the ability of the sex chromosomes to pair at male meiosis in the *oeconomus* species lineage may be a derived character in the common ancestor of this lineage, as proposed by Ashley & Fredga (1994), although this possibility is not thought to be parsimonious (Borodin, Rogatcheva *et al.*, 1997). Knowledge of the pattern of XY chromosome pairing during meiotic prophase in *M. gregalis* will help to clarify this point.

From biochemical data, Chaline & Graf (1988) regarded the subgenus *Pitymys* as polyphyletic and suggested that the Nearctic *M. pinetorum*, on which *Pitymys* was originally based, did not belong to the same systematic group as the Palaearctic *Pitymys*. Consequently, the European species were assigned to the subgenus *Terricola*. *Terricola* species studied so far, i.e. *M. majori* and *M. duodecimcostatus*, have asynapctic sex chromosomes. The synapctic condition of *M. sikimensis* rules out the possibility that this species was derived from the common ancestor of European *Pitymys* species (subgenus *Terricola*), and that it is the extreme eastern member of the European *Terricola* lineage, as suggested by Gromov & Poliakov (1992). However, it is unlikely that the taxon *Terricola* is monophyletic. Morphological, electrophoretic and karyological data suggest that this taxon is either paraphyletic or polyphyletic. (Chaline & Graf, 1988; Brunet-Lecomte & Chaline, 1991; Zagorodnyuk & Zima, 1992). It is still possible that this taxon contains species with both asynapctic and synapctic sex chromosomes.

On the other hand, it is clear that the taxon *Blanfordimys* should be treated as a subgenus of *Microtus* because *M. afghanus* and *M. bucharicus*, the members of *Blanfordimys*, have asynapctic sex chromosomes

(Borodin, Sablina & Rodionova, 1995). Systematic opinion on the taxon *Blanfordimys* is also variable and inconsistent. Some authors treat it as a subgenus of *Microtus* (Golenishchev & Sablina, 1991; Gromov & Poliakov, 1992); some include it in the subgenus *Neodon* (Corbet, 1978); and the others consider it a separate genus (Ellerman & Morrison-Scott, 1966; Musser & Carlton, 1993).

Thus, knowledge of whether species have the synaptic or asynaptic condition should help to reconstruct the confused systematic relationships within the genus *Microtus*, especially the position of *Pitymys*. The pattern of X–Y chromosome pairing at meiotic prophase of the Nearctic *Pitymys* remains unclear. Since the Nearctic *Microtus* are thought to be monophyletic (Conroy & Cook, 2000), it is reasonable to assume that most of the Nearctic *Microtus*, including *Pitymys*, will exhibit the same pattern of X–Y chromosome pairing. Knowledge of this pattern will throw light on the origin of the Nearctic *Microtus*.

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