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Comparative chromosome analysis in three *Sorex* species: *S. raddei*, *S. minutus* and *S. caecutiens*

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High-resolution G-band analysis allowed detailed comparison between the karyotypes of *Sorex raddei* Satunin, 1895 (2n = 36, NF = 68), *S. minutus* Linnaeus, 1766 (2n = 42, NF = 56) and *S. caecutiens* Laxmann, 1788 (2n = 42, NF = 68). Extensive homology was revealed. The major chromosome rearrangements involved in the evolutionary divergence of these species were identified as centric and tandem fusions as well as centromeric shifts. A chromosomal phylogeny of the species studied is presented and a presumed ancestral karyotype, containing mainly acrocentric chromosomes, is proposed.

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Introduction

Shrews of the genus *Sorex* have frequently been used as an evolutionary model for the study of the mammalian karyotype. However, such studies are limited to only certain species and/or species groups within the genus (for review see Zima *et al.* 1998). In this respect, the most detailed information about the karyotype structure and phylogenetic relationships has been obtained for the species of the

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Sorex araneus group (Aniskin 1987, Wójcik and Searle 1988, Volobouev 1989, Volobouev and Catzeflis 1989, Volobouev and Dutrillaux 1991). For other members of the genus *Sorex* there is less information. Halkka and Halkka (1974) compared Q- and G-banded chromosomes of *S. caecutiens, S. isodon* and *S. minutus*. Tada and Obara (1988) used G-banding to demonstrate the close relationships of certain Japanese taxa (*S. shinto, S. unguiculatus, S. gracillimus*). A comparison of the chromosome banding pattern in certain shrews and other species of insectivores was performed by Graphodatsky *et al.* (1993). Dannelid (1994) studied G-band homology in five shrew species: *S. samniticus, S. araneus, S. alpinus, S. caecutiens* and *S. minutus*. He suggested close chromosome relationships between *S. caecutiens* – *S. minutus* and *S. samniticus* – *S. araneus*, respectively, while *S. alpinus* appeared in an isolated position. Finally, Volobouev and van Zyll de Jong (1994) showed extensive chromosomal homologies between the two American species, *S. haydeni* and *S. cinereus*.

In this study, the karyotypes of *S. raddei* Satunin, 1895, *S. minutus* Linnaeus, 1766 and *S. caecutiens* Laxmann, 1788 were examined and compared. These species are currently considered to belong to separate karyotypic groups (Zima *et al.* 1998). Previous studies on the chromosomes of *S. caecutiens* and *S. minutus* are supplemented here by a detailed analysis of the G-banding pattern to reveal additional important homologies. The karyotype of *S. raddei*, a species endemic to the Caucasus and adjacent parts of eastern Turkey, has so far only been studied with the use of conventional chromosome staining (Kozlovsky 1973, Zima *et al.* 1998). *S. raddei* is one of few species of *Sorex* with a low diploid number, which makes it an attractive model in the study of karyotypic relationships within the genus.

Material and methods

One female and two males of *S. raddei* were captured in the northern part of the Lagonack plateau, 40 km S of the Maikop city, Krasnodar Region, Caucasus (44°12'N, 40°06'E); another male was collected in Sumela, Trabzon Province, eastern Turkey (40°45'N, 39°45'E). One male and one female of *S. caecutiens* and one male and two females of *S. minutus* were collected in the suburbs of the city of Novosibirsk (54°49'N, 83°06'E). Finally another male of *S. minutus* originated from the Šumava Mts., Czech Republic (49°06'N, 13°34'E). Mitotic chromosome spreads were prepared in the field from bone marrow and spleen cells, with the use of a flame-drying method after fixation with methanol and glacial acetic acid. For G-banding, slides were treated with trypsin solution according to Seabright (1971), with the use of a technique modified after Graphodatsky and Radjabli (1988).

The numbering of individual chromosomal pairs in the karyotypes studied followed basically that used by Graphodatsky *et al.* (1993) for *S. minutus*, and Tada and Obara (1988) for *S. shinto* and other species. However, slight modifications of these systems were introduced if differences appeared between our results and those previously published in the assessment of the centromeric position for certain chromosomal pairs. The individual chromosomal pairs from the karyotypes of the species studied were designated with the chromosome number and an abbreviated version of the species name (r = *raddei*, m = *minutus*, c = *caecutiens*).

The taxonomic nomenclature followed that proposed by Wolsan and Hutterer (1998).

Results and discussion

The basic karyotypic characteristics

The karyotype of *S. raddei* consisted of 36 chromosomes with NF = 68 (Fig. 1). Ten pairs of autosomes were metacentric, six pairs submetacentric and one pair acrocentric, with distinct satellites in the short arm. The X chromosome was the largest acrocentric chromosome. The acrocentric Y chromosome was slightly larger than the smallest acrocentric autosome.

The chromosomal set of *S. minutus* consisted of 42 chromosomes with NF = 56 (Fig. 2). Seven autosomal pairs were meta- and submetacentric, the remaining autosomes and the X chromosome were acrocentric. A secondary constriction was observed in the long arm of the acrocentric autosome No. 19.

The karyotype of *S. caecutiens* contained 42 chromosomes with NF = 68 (Fig. 3). Six autosomal pairs were metacentric, seven pairs submetacentric, the remaining autosomes and the X chromosome were acrocentric.

Comparison of G-banded karyotypes

The G-banded prometaphase chromosomes of *S. minutus* and *S. caecutiens* are compared in Fig. 4. An almost identical banding pattern was observed in twelve autosomal pairs (m1 – c7, m2 – c2, m3 – c1, m5 – c3, m6 – c6, m7 – c13, m12 –c14, m13 – c15, m14 – c16, m15 – c17, m18 – c18, m20 – c20), and in the X chromosome. The banding pattern in six other autosomal pairs was also identical but the centromeric position differed (m8 – c9, m9 – c4, m10 – c10, m11 – c5, m16 – c11, m17 – c12) indicating tandem or centric fusions or centromeric shifts. For the autosomes m8 – c9 and m19 – c19 the causes of incomplete homology were uncertain. The m4 and c8 autosomes differed by a large pericentric inversion. The similarity in the banding pattern of m4 and r13 indicates the derived state of the autosome c8 of *S. caecutiens* and the autapomorphic character of the rearrangement.

Extensive homology between the G-banded karyotypes of *S. minutus* and *S. caecutiens* was reported by Dannelid (1994), who also noted the polymorphic banding pattern in one of the pairs of large biarmed chromosomes. The biarmed autosomes c4, c5, c9 and c10 were also identified in the complements of *S. gracillimus, S. unguiculatus* and *S. shinto* but the autosome c12 was considered acrocentric in all these species (Tada and Obara 1988). Intra- and interspecific variation in the centromeric position, attributed to a pericentric inversion, was described in *S. shinto* and *S. unguiculatus* in the autosome corresponding to the c10 element (Tada and Obara 1988).

High-resolution chromosome analysis of the G-banded karyotypes of *S. minutus* and *S. raddei* revealed a considerable degree of homology between individual chromosomal arms and segments (Fig. 5). An identical banding pattern and centromeric position was recorded in two large biarmed autosomes (m1 – r11, m4 – r13), two acrocentric autosomes (m6 – r10, m20 – r17) and in the X chromosomes

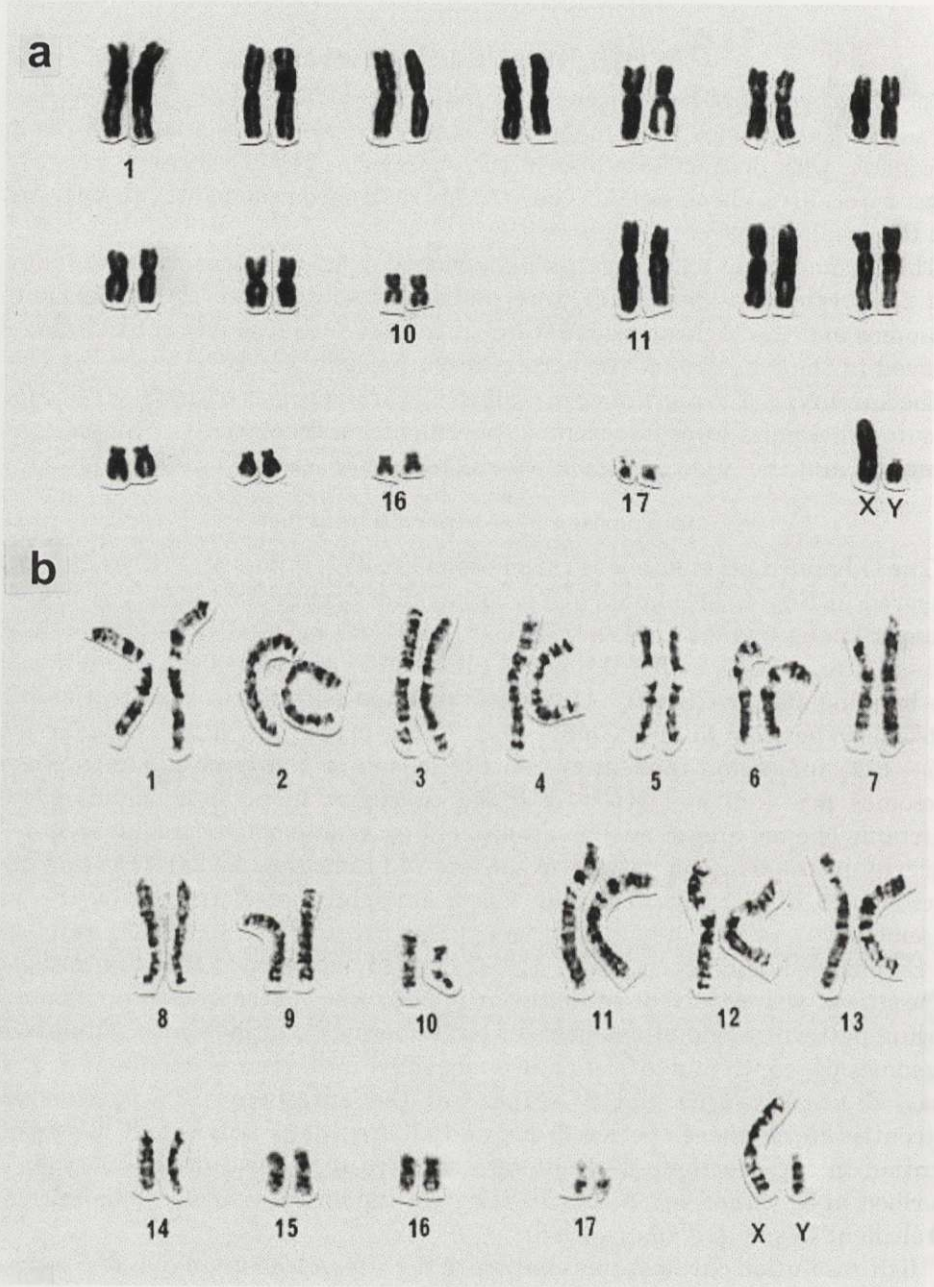

Fig. 1. Conventionally stained (a) and G-banded (b) karyotype of *Sorex raddei*.

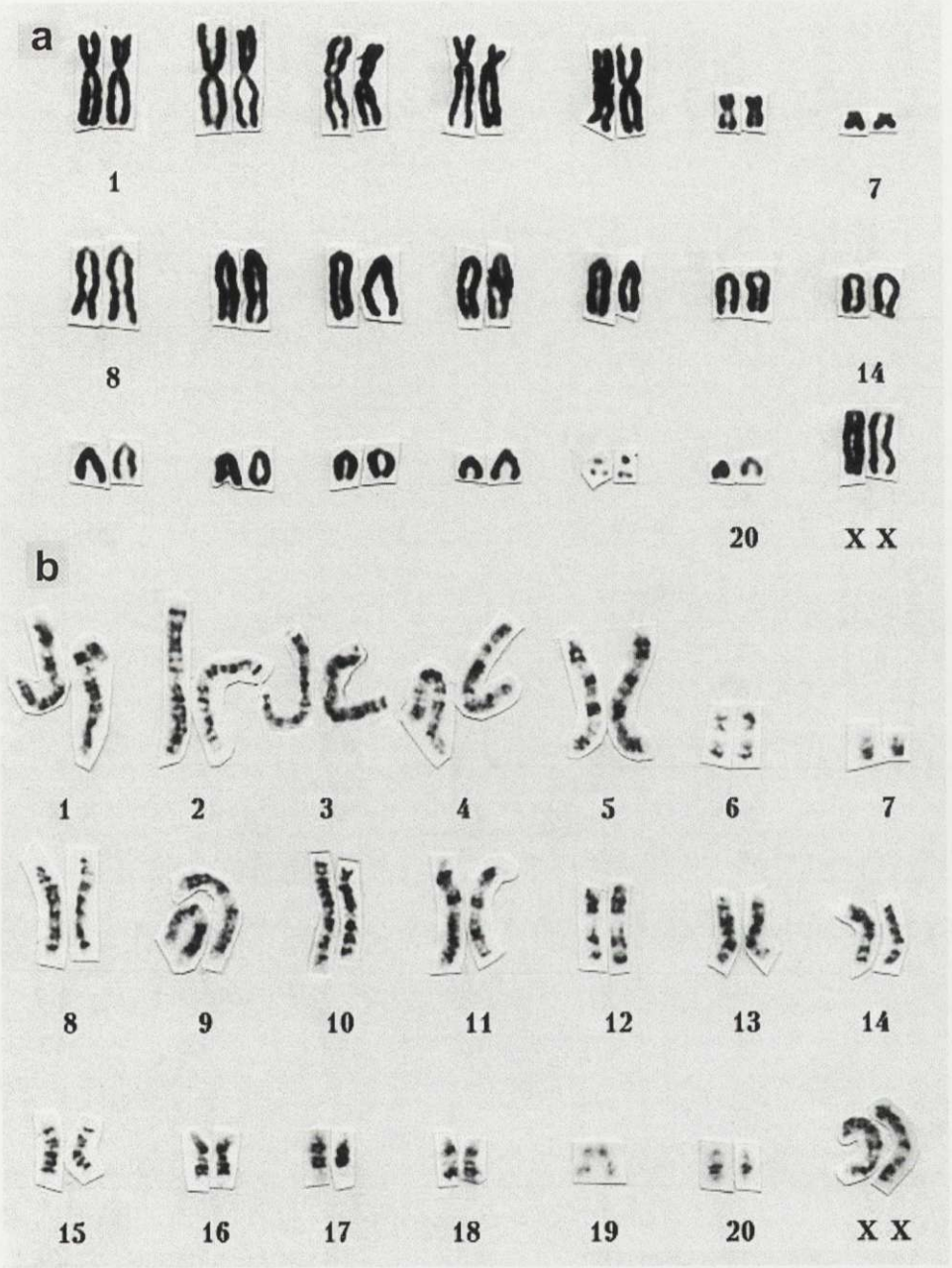

Fig. 2. Conventionally stained (a) and G-banded (b) karyotype of *Sorex minutus*.

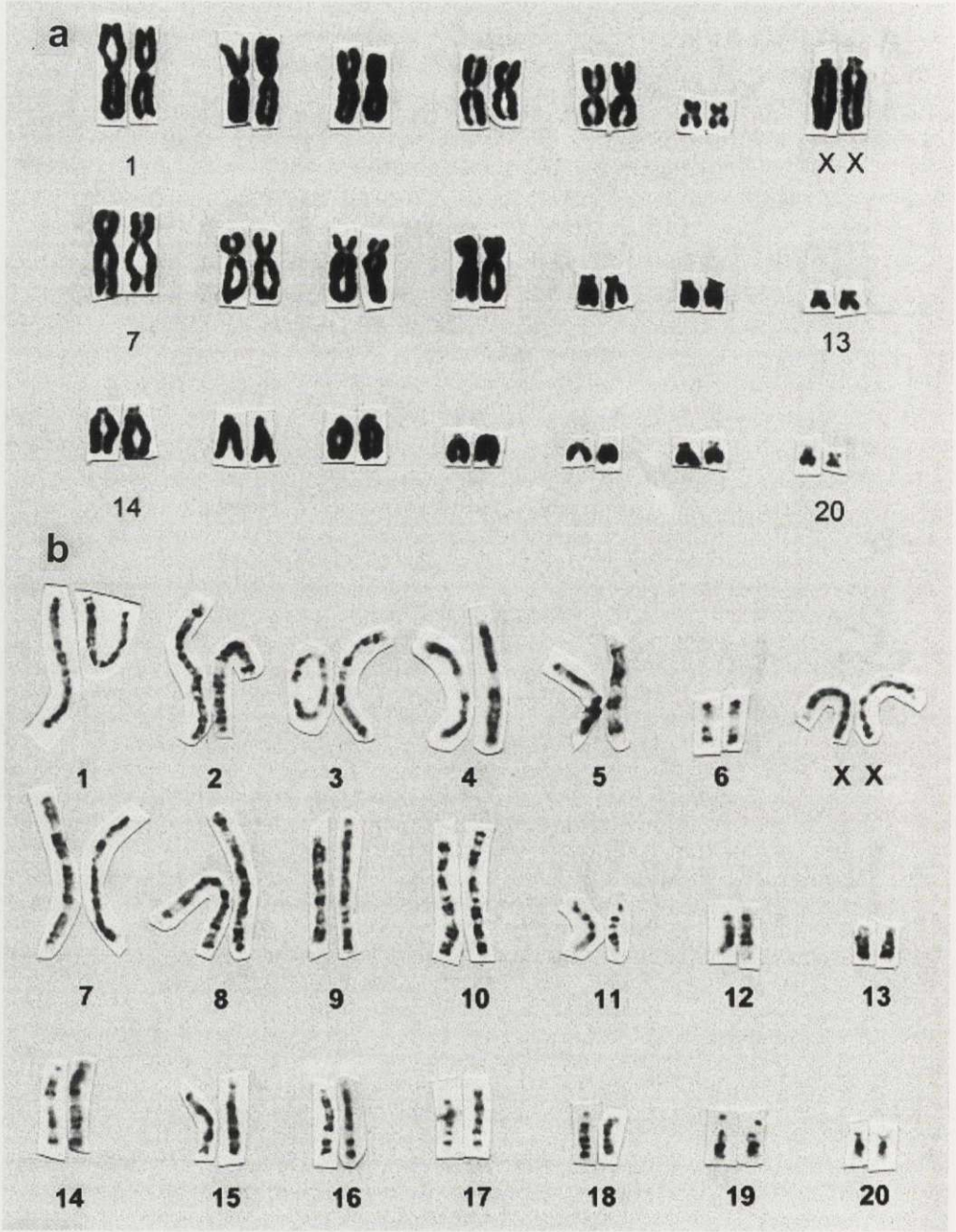

Fig. 3. Conventionally stained (a) and G-banded (b) karyotype of *Sorex caecutiens*.

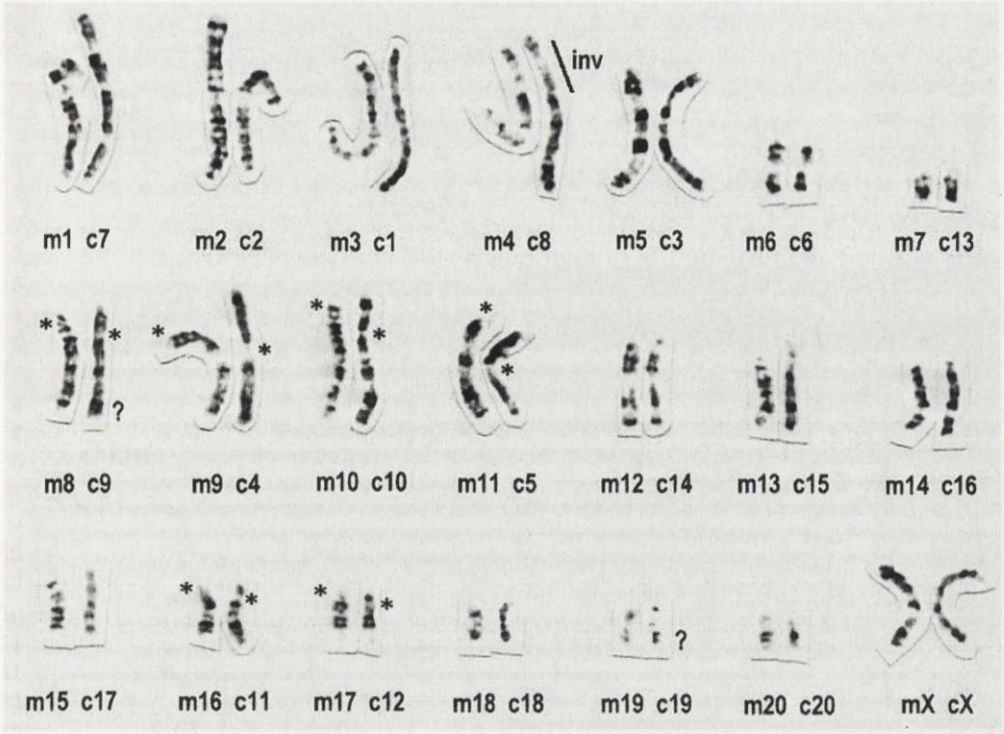

Fig. 4. Comparison of haploid complements of *Sorex minutus* (m) and *S. caecutiens* (c). Asterisks indicate centromere position and question-marks incomplete homology.

(Fig. 5a). An identical banding pattern and a different centromeric position was found in two autosomal pairs (m16 – r14, m7 – r16) suggesting occurrence of centromeric shifts. The submetacentric autosome r15 possessed an additional short arm compared to the partly homologous autosome m19 (Fig. 5b). The other chromosomes were involved in different centric and tandem fusions in the complements of both species. Possible homology of individual autosomal arms and their parts is shown in Fig. 5c.

Proposed chromosomal phylogeny

Centric and tandem fusions of various autosomes appear to be the major mechanism of chromosomal divergence between the species studied, although centromeric shifts are also important.

In an attempt to reconstruct the ancestral karyotype of the three species, we accepted the common view that a prevailingly acrocentric karyotype, found eg in *S. alpinus*, could be close to the actual ancestral complement of the Palaearctic shrews of the genus *Sorex* (Dannelid 1994). The tendency for a decrease in diploid

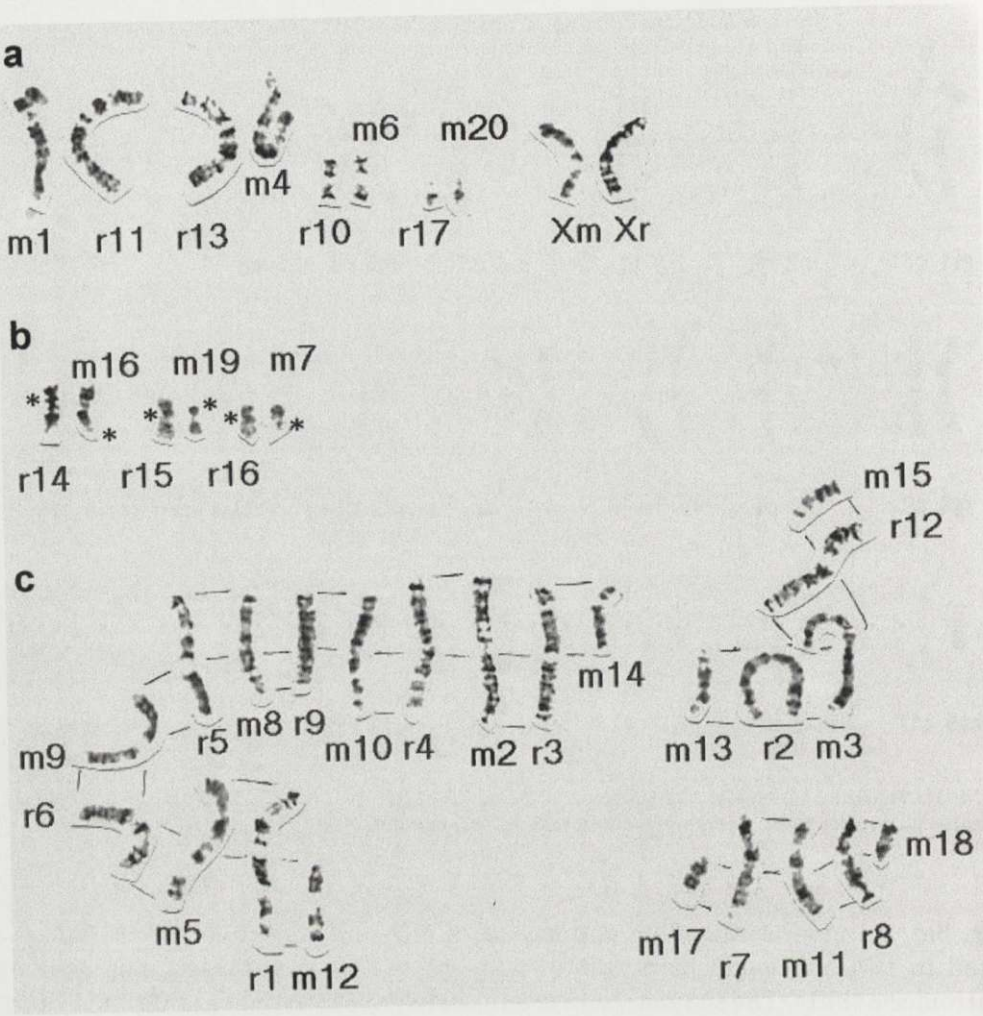

Fig. 5. Comparison of haploid complements of *Sorex minutus* (m) and *S. raddei* (r). Some chromosomes are identical (a), others differ because of centromeric shifts or the presence/absence of an additional arm [centromere position indicated by asterisks; (b)], or because chromosomal segments of the two species were involved in different fusions (c).

number has been suggested for the *S. araneus* group (Volobouev 1989, Zima 1991), and this is probably a prevailing mode also in other *Sorex* lineages. The basic descriptive data obtained in this study were summarised and compared with the results by Tada and Obara (1988) in Table 1, and a tentative phylogeny of the species under consideration was derived (Fig. 6). A possible scenario for the chromosomal evolution in these shrews is that acrocentric chromosomes of the

Table 1. Proposed chromosomal homologies among certain Palaearctic shrew species. Data concerning *Sorex shinto, S. unguiculatus* and *S. gracillimus* adapted from Tada and Obara (1988). # – pericentric inversion, * – centromeric shift.

S. minutus	*S. caecutiens*	*S. shinto shinto*	*S. shinto saevus*	*S. unguiculatus*	*S. gracillimus*	*S. raddei*
1	7	8	8	8	9	11
2p	2p	2p	2p	2p	2p	4q
2q	2q	2q	2q	2q	2q	3p
3p	1p	1p	1p	1p	1p	12q
3q	1q	1q	1q	1q	1q	2p
4	8#	9	9	9	12	13
5p	3p	3p	3p	3p	4p	6p
5q	3q	3q	3q	3q	4q	1p
6	6	7	7	7	7	10
7	13	12	12	12	–	16*
8 prox	9p+9q prox	10p+10q prox	10p+10q prox	10p+10q prox	11p+11q prox	9p
8 dist	9q dist	10q dist	10q dist	10q dist	11q dist	5q
9 prox	4p	4p	4p	4p	8q prox	5p
9 dist	4q	4q	4q	4q	8q dist	6q
10 prox	10p+10q prox	5p+5q prox	5p	5p	5p	9q
10 dist	10q dist	10q dist	5q	5q	5q	4p
11 prox	5p	6p	6p	6p	6p	7p
11 dist	5q	6q	6q	6q	6q	8q
12	14	13	13	13	10q	1q
13	15	14	14	14	3q	2q
14	16	15	15	15	3p	3q
15	17	16	16	16	8p	12p
16	11*	11*	11*	11*	10p	14*
17	12*	17	17	17	–	7q
18	18	18	18	18	14	8p
19	19+?	19+?	19+?	19+?	–	15+?
20	20	20	20	20	17	17
X	X	X	X	X	X	X

hypothetical common ancestor fused to metacentrics or submetacentrics in two different ways, one found in *S. raddei* and the other in all other species with 42 chromosomes. The species with 42 chromosomes were included in the same clade because of a shared set of autosomal fusions. *S. raddei* is thus not an ancestor, but rather a sister lineage to the combined *S. minutus* – *S. caecutiens* group. Further evolution within the latter group included tandem and centric fusions leading to the karyotype of *S. gracillimus*, centromeric shifts responsible for the difference between *S. minutus* and remaining species (if this is also responsible for the *minutus*-like chromosomal condition in *S. buchariensis* and *S. volnuchini* remains to be seen). Other centromeric shifts differentiated the 42 chromosome species with a higher number of autosomal arms and, finally, pericentric inversions appeared in

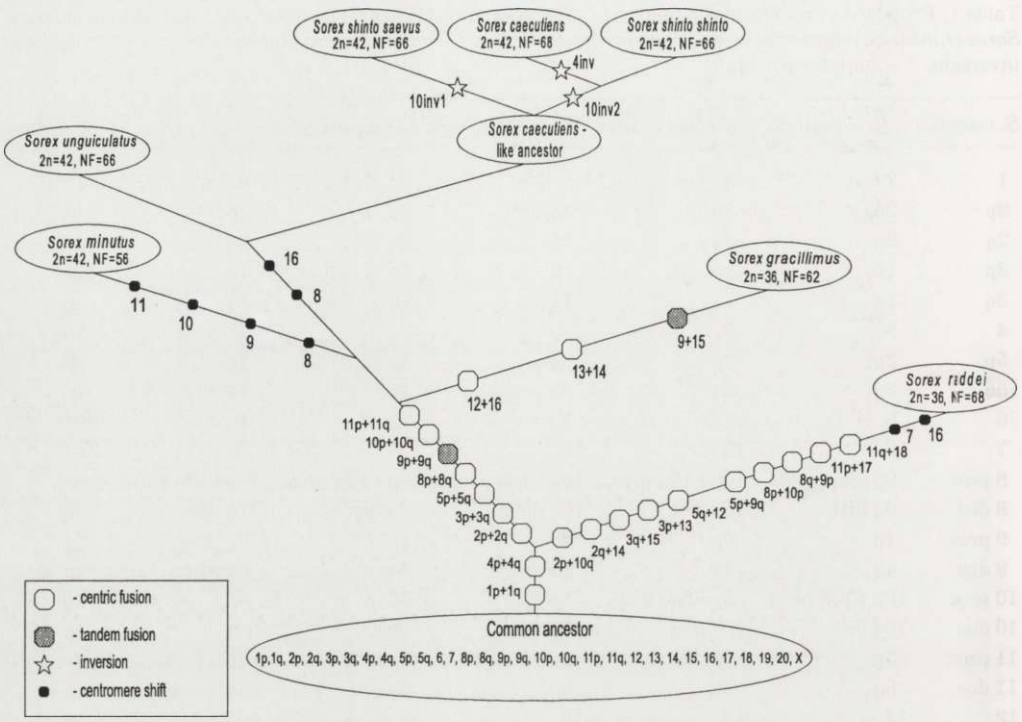

Fig. 6. Proposed phylogeny of certain Palaearctic species of the genus *Sorex*. The numbering of individual chromosomal pairs is the same as for *S. minutus*.

the *S. caecutiens* – *S. shinto* lineage. The chromosomal phylogeny suggests that *S. shinto* may be a paraphyletic taxon.

The G-banding homologies described in this paper do indeed contribute to the knowledge of the relationships of Old World *Sorex* species. Zima *et al.* (1998) divided Eurasian *Sorex* species into six chromosomal groups (not counting the East Asian members of the *S. cinereus* group which belongs to a North American radiation, rather than to an Eurasian one), which were named after *S. araneus, S. alpinus, S. caecutiens, S. minutus, S. raddei* and *S. samniticus,* respectively. Similarly, Dannelid (1994) indicated a division of '42-chromosome' shrews (a term used also for species with a diploid number close to 42) between a chiefly western *S. minutus* group and a chiefly eastern *S. caecutiens* group. However, this paper shows that variation among the '42-chromosome' shrews is actually smaller than within the *S. araneus* group, and all the fusions observed in the species of the *S. caecutiens* group are identical with those found in *S. minutus*. Therefore, a division between a *S. minutus* group and a *S. caecutiens* group seems no longer acceptable. The close relationship between the *S. caecutiens* and *S. minutus* groups

is also supported by results of mtDNA and protein studies (Fumagalli *et al.* 1996, Ruedi 1998).

Thus Old World *Sorex* species could be divided into three main karyotypic groups. The first consists only of *S. alpinus* with a presumed primitive karyotype made up of mainly acrocentric chromosomes. The relationships between this species and the remaining Eurasian *Sorex* is still not well understood. The second is the *S. araneus* group characterized by XX/XY_1Y_2 sex chromosomes. To this group *S. samniticus* is clearly allied, though it cannot be assigned to it, due to lack of the rearranged sex chromosomes. The third group is made up of the '42-chromosome' species, which can be named the *S. minutus* group after the first described species. *S. raddei* seems to occupy a similar position to this group as *S. samniticus* has to the *S. araneus* group. The phylogeny of Old World *Sorex* species should be further clarified by reliable G-banding of species like, for instance, *S. isodon*, *S. minutissimus*, *S. mirabilis*, *S. buchariensis* and *S. volnuchini*.

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