Cytogenetics of the genus *Arvicanthis* (Rodentia, Muridae). 2. The chromosomes of three species from Ethiopia: *A. abyssinicus*, *A. dembeensis* and *A. blicki*.

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**Abstract**

The karyotypes of three Ethiopian species of *Arvicanthis*, the lowland *A. dembeensis*, the highland *A. abyssinicus*, and the endemic mountain species *A. blicki* are described. Their diploid numbers and autosomal Fundamental Numbers (aFN) are $2n = 62$ (aFN = 62), $2n = 62$ (aFN = 64), and $2n = 48$ (aFN = 64), respectively. Except for a large submetacentric (no. 4) in *A. abyssinicus* and a small metacentric in *A. dembeensis* and *A. abyssinicus*, all their chromosomes are acrocentric, while the karyotype of *A. blicki* is characterized by having seven pairs of Rb metacentric chromosomes. C- and G-banding patterns and Ag-NORs positions were used to identify chromosomal rearrangements (Rb translocations, pericentric inversions and heterochromatin additions). Phylogenetic hypotheses are suggested based upon the comparisons among karyotypes investigated and with the previously described karyotype of *A. niloticus* from West Africa (Benin).

**Introduction**

*Arvicanthis*, the unstriped grass rat, is a rodent very common to the South and East of the Sahara and North of the Zambezi (Kingdon 1974). The genus comprises of few named species, with *A. niloticus* (Desmarest, 1822) being the most common in the Nile basin and in central and western sub-Saharan regions, and a group of species present in the Horn of Africa and East Africa, i.e. *A. abyssinicus* Rüppel, 1842, *A. dembeensis* Rüppel, 1842, *A. somalicus* Thomas, 1902, and *A. blicki* Frick, 1914. According to Corbet and Hill (1991), or *A. abyssinicus*, *A. blicki*, *A. somalicus*, and *A. nairobae* Allen, 1909, according to Musser and Carleton (1993). However, cytogenetic findings (Capanna and Civitelli 1988; Civitelli et al. 1995; Granjon et al. 1992; Matthey 1965; Viegas-Pequignot et al. 1983; Volobouev et al. 1987, 1988) suggested that *A. niloticus* represents rather a species complex with different karyomorphs.

*A. abyssinicus* is adapted to the highlands between 2000 and 3000 m above sea level and it reaches 3700 m in Simien. *A. blicki* has a restricted range occurring at the higher altitudes of the Bale mountains between 2750 and 4100 m (Yalden and Largen 1992). Finally, the range of *A. dembeensis* extends from sea level up to 2000 m (Yalden et al. 1976).

*A. abyssinicus* and *A. blicki* are genetically related (they diverged during recent Pleistocene), as shown by Nei’s genetic distances, and have an older divergence from *A. dembeensis* (Capula et al. 1996).
A multivariate investigation of morphometric traits (Afework Bekele et al. 1993) has shown that *A. dembeensis* and *A. abyssinicus* are distinct and that there is a clear pattern in morphometry related to altitudinal variation suggesting trends in adaptation to these very different environments. Nonetheless, the taxonomy of *A. dembeensis* is still puzzling: Corbet and Hill (1991) and Yalden et al. (1976) consider it as a separate species, while Musser and Carleton (1993) include it within *A. niloticus*. There is an apparent correspondence between the karyotypes of the two (at least for the Egyptian populations of *A. niloticus*, Viegas-Pequignot et al. 1983), so that *A. dembeensis* could be a geographic variant of *A. niloticus*. However, to solve its taxonomic status, a clarification of the entire ‘*niloticus*’ complex is needed.

In the present study we describe the karyotypes of three species reported by De Winton (1900) and Yalden and Largent (1992) in their study of the Ethiopian fauna, i.e. *A. abyssinicus*, *A. dembeensis*, and *A. blicki*. Moreover, based upon chromosomal rearrangements, we suggest their phylogenetic relationship.

**Material and methods**

Animals were collected from the following localities: *A. abyssinicus*: Sululta (9° 15′ N–38° 43′ E, 2 700 m a.s.l.), cropland, 1 ♀ and 4 ♂; Managhesha (9° 00′ N–38° 35′ E, 2 200–2 300 m a.s.l.), savanna and forest limits, 5 ♀. *A. dembeensis*: Koka (08° 24′ N–39° 0′ E, 1 650 m a.s.l.), open savanna and cropland, 2 ♀ and

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Fig. 1. Map of Ethiopia demonstrating distribution of *A. dembeensis* (open circles), *A. abyssinicus* (closed circles) and *A. blicki* (open triangles) (redrawn from Yalden et al. 1976), with the location of trapping sites. 1 = Koka; 2 = Sululta; 3 = Managhesha; 4 = Bale. Isohypses represent 1 600 m asl. Ethiopia and Benin are shown in dark in the inset.
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1♀, *A. blicki*: Sannetti Plateau, Bale Mts. (6°52’N–39°52’E, 4000 m a.s.l.), alpine moorland. 1♂. The karyotypes of samples of *A. niloticus* from Benin (Civitelli et al. 1995) were used for outgroup comparison and are from Lokossa (1°37’E–6°43’N), open savanna, 4♀ and 5♂; Attogon (2°10’E–6°43’N), cropland; 1♀ and 2♂, and Toffo (2°6’E–6°49’N), forest, 1♀ (all at sea level) (Fig. 1).

Metaphases were obtained from bone marrow preparations (Hsu and Patton 1969) at Addis Ababa University. Slides and cell suspensions in fixative solution (3:1 methanol/acetic acid) were then transported at 4°C to the Dipartimento di Biologia Animale e dell’Uomo, Rome, for differential staining.

Metaphases standard staining was performed by Giemsa 4% in phosphate buffer pH7. G-bands were obtained following Seabright (1971) and C-bands according to Bickham (1979). Nucleolus Organizer Regions (NORs) were enhanced by means of silver reaction following Howell and Black (1980). To identify chromosomes carrying Ag-NORs, the silver stained metaphases were also treated with trypsin to highlight the G-banding.

To construct phylogenetic relationships between species, homologies in chromosomal elements were identified and then coded into binary form (additive binary coding, Snead and Sokal 1973). To find the shortest tree (Wagner parsimony) a global swapping procedure was used and character state trees were polarized using, as an outgroup, *A. niloticus* from Benin.

Besides geographic considerations, the use of this species as an outgroup is supported by our findings from multi-locus protein electrophoresis (Capula et al. 1996) which indicates a very high Nei’s genetic distance (D = 0.847) between the Benin and the Ethiopian species and a lower Nei’s genetic distance (D = 0.186) among the latter. Furthermore, there is also the advantage that this is the only population for which C- and G-banding and Ag NOR staining are available (Civitelli et al. 1995), and this allows proper character state tree polarization.

Chromosomes carrying Ag-NORs were used cautiously (see Schubert and Wobus 1985) and then only when a peculiar localization of ribosomal cistrons was found and/or if shared by two or more branches.

### Results

**Karyotype descriptions**

*A. dembeensis*. The diploid number is 2n = 62, the autosomal Fundamental Number (aFN) is 62. All autosomes are acrocentrics of decreasing size, except a metacentric pair of small size (Fig. 2). The X-chromosome is a large submetacentric (the largest of the complement) and the Y-chromosome is a metacentric of smaller size (Fig. 2). All autosomes are characterized by a large centromeric heterochromatic region (Fig. 3). The Y-chromosome is entirely C-positive. The short arm of the X-chromosome is completely heterochromatic and the long arm shows a C-positive region near the centromere and a smaller one before the distal end (Figs. 3 and 4). A total of 7 chromosomes carrying NORs was found. These NORs have a telomeric position in two pairs of large chromosomes (nos. 2 and 5) and are paracentromeric in two other pairs of medium-size chromosomes (probably nos. 18 and 19) (Fig. 3).

Chromosomes were numbered according to their size (no. 1 being the largest). The karyotype of *A. dembeensis* was chosen as the reference point for comparison of other species, since its diploid and fundamental numbers are most similar to those of *A. niloticus* from terra typica (Viegas-Pequignot et al. 1983). The karyotypes of *A. abyssinicus* and *A. blicki* were consequently numbered based upon the homologies identified with the chromosome elements of *A. dembeensis*. The notation used by Civitelli et al. (1995) for the chromosomes of *A. niloticus* from Benin will be shown in brackets when the karyotypes are compared (Fig. 5).

*A. abyssinicus*. The diploid number is 2n = 62 and the aFN is 64. All autosomes are acrocentrics which decrease in size, except a submetacentric pair of medium size and a metacentric pair of small size (Fig. 1). All autosomes are characterized by a large centromeric heterochromatic region (Fig. 3). A variability in shape and size of the X-chromosome was observed: two females out of four have shown a heteromorphic condition for
Fig. 2. Karyotypes of *Arvicanthis dembeensis*, *Arvicanthis abyssinicus* and *Arvicanthis blicki*. The karyotype of *Arvicanthis dembeensis* has been arranged according to the chromosomes decrease in size. The lower diploid number of *Arvicanthis blicki* (2n = 48) is determined by seven pairs of Rb metacentrics. Note the fourth pair which is acrocentric in *Arvicanthis dembeensis* but submetacentric in *Arvicanthis abyssinicus* and *Arvicanthis blicki*. Note also the smallest metacentric (the first in each karyotype third row) which is common to all three species.
Fig. 3. Part A: Metaphase plates of the three Ethiopian species with, on the left, C-bands (arrows indicate sex chromosomes), and, on the right, Ag-NORs (arrows indicate chromosomes with Ag-NORs).
Part B: Chromosomes carrying Ag-NORs in the three Ethiopian species.
this chromosome that can be either subtelocentric or submetacentric, with the latter configuration similar to that found in *Arvicanthis dembeensis* (Fig. 4). The large submetacentric variant of the X-chromosome possesses large heterochromatic blocks in the short arm not present in the subtelocentric form (Figs. 3 and 4). All the six males analysed presented a submetacentric X-chromosome. The Y-chromosome is a metacentric of medium size (Fig. 2) and entirely C-positive (Figs. 3 and 4). A total of nine chromosomes with NORs were detected. Three acrocentric pairs (chromosomes nos. 1, 7 and 12) have NORs at the centromere, one pair (no. 2) has both telomeric and centromeric NORs, and another pair (no. 5) has telomeric NORs only (Fig. 3).

*A. blicki*. The species is characterized by $2n = 48$ and by aFN = 64. The autosomal set is composed by 14 pairs of acrocentrics and 9 pairs of biarmed chromosomes (metacentrics and submetacentrics) (Fig. 2). The X-chromosome is submetacentric (Fig. 2), and its G- and C-banding patterns are identical to those found in *A. dembeensis* and to the equivalent form described in *A. abyssinicus* (Fig. 4). As no males were analyzed, there is no information relative to the Y-chromosome. All biarmed chromosomes are characterized by C-positive centromeric regions; acrocentric chromosomes have centromeric heterochromatic spots except for a few pairs of medium size (Fig. 3). C-bands in *A. blicki* are less evident than in the two species described above. A total of six chromosomes have shown NORs. The NORs occupy a centromeric position on the largest chromosome pair (no. 1), a telomeric position on the smallest (no. 5) and have both a telomeric and centromeric position on chromosome no. 2 (Fig. 3).
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**Fig. 4.** The sex chromosomes of *A. blicki, A. dembeensis, A. abyssinicus,* and *A. niloticus.* Figure 4 represents the metacentric (X m), submetacentric (X sm) and subtelocentric (X st) shapes found for the X chromosome. For each condition the G- (left) and C-banding (right) patterns are shown, as well as for the Y chromosome. Arrows indicate the plausible rearrangement sequence for variants of the X chromosome; 1 indicates pericentric inversion, 2 and 3 indicate heterochromatin addition. Y chromosome has not been described for *A. blicki.*

**Karyotype comparisons**

The complete comparison of homologies and chromosomal rearrangements as detected by G-banding is shown in figure 5. The identification of homologies has been possible without ambiguity for most of the chromosomes. There is a minor group of chromosomes
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**d** = *Arvicanthis dembeensis*

**a** = *A. abyssinicus*

**b** = *A. blicki*

**n** = *A. niloticus*
(at the bottom of Fig. 5) for which the small size and the limited number of G-bands do not allow the identification of homologies. Within this group, there is a medium sized metacentric of *A. blicki* whose arms were not identified.

Character state trees (CST) were constructed to describe changes in each chromosome in order to allow the identification of phylogenetic relationships between the species (*a* = *A. abyssinicus*, *b* = *A. blicki*, *d* = *A. dembeensis* and *n* = *A. niloticus*). The form in which character state trees are represented follows the standard representation as in Forey et al. (1992). In case of doubt, the choice was made assuming the most common rearrangement as the most primitive.

Comparisons are as follows: Chromosome 1 – the chromosome has the same acrocentric morphology and banding pattern in the three Ethiopian species, while *A. niloticus* shows a pericentric inversion; NORs are centromeric in *A. abyssinicus, A. blicki* and *A. niloticus* and absent in *A. dembeensis* (*cst = n – ab – d*). Chromosome 2 – the chromosome has the same acrocentric morphology and banding pattern in the three Ethiopian species, while it is a subtelocentric in *A. niloticus*; NORs have a telomeric and centromeric position in *A. abyssinicus* and *A. blicki*, while they are telomeric in *A. dembeensis* and *A. niloticus* (*cst = n – d – ab*). Chromosome 3 – the chromosome has the same morphology and banding pattern in the three Ethiopian species, while in *A. niloticus* it is submetacentric (*cst = n – abd*). Chromosome 4 – the chromosome is acrocentric in *A. dembeensis*, submetacentric in *A. abyssinicus* and *A. blicki*, and acrocentric with a deletion in *A. niloticus* (*cst = n – d – ab*). Chromosome 5 – the chromosome is an acrocentric with the same morphology, banding pattern and telomeric NORs in all four species. Chromosomes 6 and 8 – the chromosomes have the same acrocentric morphology and banding patterns in all four species. Chromosome 7 – the chromosome is acrocentric in all four species; two distinct banding patterns characterize the centromeric region in *A. dembeensis* and *A. abyssinicus* on one side, and *A. blicki* and *A. niloticus* on the other; *A. abyssinicus* possesses Ag-NORs in a centromeric location (*cst = bn – d – a*). Chromosome 9 – the chromosome is a large acrocentric in *A. niloticus*; it is a medium sized acrocentric in the Ethiopian species; *A. dembeensis* and *A. abyssinicus* show an additional centromeric band (*cst = n – ad – b*). Chromosomes 10 and 14 – chromosome 10 is a medium size acrocentric in *A. dembeensis, A. abyssinicus* and *A. niloticus*, with a dark centromeric band in the former two; chromosome 14 is an acrocentric shared by the same three species with the same banding; the two chromosomes form the 10/14 Robertsonian (Rb) metacentric in *A. blicki* (*cst = n – ad – b*). Chromosome 11 – chromosome 11 is a medium size acrocentric in *A. dembeensis, A. abyssinicus* and *A. niloticus*; this chromosome forms the 11/? Rb metacentric in *A. blicki* through fusion with an unidentified element (?) in *A. dembeensis* and *A. abyssinicus* but which, on the contrary, was identified by Civitelli et al. (1995) in *A. niloticus* and numbered as 21 (*cst = ad – n – b*). Chromosomes 12 and 13 – chromosome 12 is medium sized acrocentric in *A. dembeensis, A. abyssinicus* and *A. niloticus*; *A. abyssinicus* has centromeric NORs; chromosome 13 is an acrocentric shared by the same

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**Fig. 5.** G-banding comparison of the autosomes of the three Ethiopian species (*A. dembeensis, A. abyssinicus, A. blicki, respectively in first, second and third position*) and *A. niloticus* from Benin (in fourth position). The chromosomes of *A. dembeensis* have been numbered according to their decrease in size. This species constitutes the reference point for the chromosome numbering of the others. Robertsonian metacentries are shown twice near the corresponding acrocentric elements. Question marks (?) indicate arms that were not identified. Chromosome numbering assigned by Civitelli et al. (1995) to *A. niloticus* is preserved and enclosed into brackets. Elements from number 20 onwards (chromosome 25 excluded) for which identification was difficult or impossible are shown in the four rows at the bottom of the figure.
three species with the same banding pattern; these two chromosomes are fused in *A. blicki* into the 12/13 Rb metacentric (cst = *nd - a - b*). Chromosomes 15 and 16 – chromosomes 15 and 16 are the same medium sized acrocentric in *A. dembeensis*, *A. abyssinicus* and *A. niloticus*; chromosome 16 shows in *A. niloticus* an addition at the pericentromeric region: the two chromosomes form the 15/16 Rb metacentric in *A. blicki* (cst = *n - ad - b*). Chromosome 17 – chromosome 17 is one of the smallest acrocentrics of intermediate size with the same morphology and banding described for *A. dembeensis* and *A. abyssinicus*; this chromosome corresponds to the small acrocentric numbered as 23 in *A. niloticus* by Ci-

Fig. 6. Phylogeny of Ethiopian *Arvicanthis*, with the most parsimonious trees found. Homoplasies in the tree in part A concern chromosome 7 and the two Rb metacentrics 11/? and 17/?; homoplasies in the tree in part B concern chromosomes 2, 4 and the Rb metacentric 12/13. Cladograms have been out-group rooted on *A. niloticus*. Letter a indicates the most primitive chromosome condition, b the intermediate and c the most derived.
vitelli et al. (1995); this chromosome forms the 17/? Rb submetacentric in A. blicki. The unidentified (?) arm corresponds to an acrocentric chromosome of A. niloticus numbered as 16 by civitelli et al. (1995), but not yet identified in A. dembeensis and A. abyssinicus (cst = ad - n - b). Chromosomes 18 and 19 – these chromosomes are acrocentrics in A. dembeensis, A. abyssinicus and A. niloticus; the former two carry centromeric AgNORs; the two chromosomes form the 18/19 Rb metacentric in A. blicki (cst = d - na - b). Chromosomes 20–30 – all these chromosomes are acrocentrics in A. dembeensis and A. abyssinicus, except one small metacentric (no. 25) which is common to the three Ethiopian species. Also this chromosome possibly corresponds to no. 30 in the karyotype of A. niloticus from Benin. Since it has not been possible to identify homologies in banding patterns from chromosome 20 onwards, these have been excluded from a phylogenetic assignment; for their general morphology, see the previous section.

A most parsimonious solution was computed using the character state trees of chromosomes 1, 2, 4, 7, 9, 10/14, 11, 12/13, 15/16, and 17. Two trees of equal length were produced (Fig. 6). The most primitive chromosome condition is indicated in the figure as a, the most derived as c and the intermediate as b. The two trees differ in the phylogenetic relationships of A. abyssinicus which, in one case, is connected to A. blicki (part A) and in the other to A. dembeensis (part B). The length of the trees is 25 and the consistency index is 0.88. Three homoplasies occur in the trees (indicated as filled rectangles); these concern chromosomes 7, 11/? and 17/? in the first tree, and chromosomes 2, 4 and 12/13 in the second tree (Fig. 6).

Discussion

A. niloticus from Benin belongs to the group of the three or more species identified by volobouev et al. (1988). granjon et al. (1992) and civitelli et al. (1995) in Central and West Africa. There is a series of chromosomal rearrangements characterizing this lineage that are different from the karyotype of A. niloticus from terra typica (volobouev et al. 1988). The Benin population is therefore the sister group of the Ethiopian species and is the proper outgroup for character polarization. Furthermore, its divergence from the Ethiopian group has been placed back at the Early Pliocene (about 4.2 Myr), as estimated from nei’s genetic distances (capanna et al. 1996; see also yalden and largent 1992).

The three Ethiopian species have karyotypes which are more similar to each other and constitute a monophyletic group. A. dembeensis and A. abyssinicus differ only for one pericentric inversion and for some of the chromosomes carrying Ag-NORs. A. blicki has the karyotype showing the greatest differences, with a diploid number reduced by robertsonian fusions, as evidenced by the nFA (64) which is equal to that of A. abyssinicus. The isolation of A. blicki and its range restricted to the Afroalpine habitat of the Bale Mountains account for its peculiar diversity (corti et al. 1995).

It is still an open question which one of the two alternative equivalent trees may represent the true phylogeny of Ethiopian Arvicanthis, i.e. whether A. abyssinicus is the closest relative to A. dembeensis or to A. blicki. The similar adaptation to the highlands in A. abyssinicus and A. blicki, together with Ag-NORs position, would favour the second hypothesis. Indeed, the two pairs of chromosomes carrying Ag-NORs in A. blicki (nos. 1 and 2) are the same as in A. abyssinicus. In particular, the peculiar position of Ag-NORs in the second pair of these two species, i.e. the nucleolus organizer regions which are both telomeric and centromeric, constitutes a synapomorphy (i.e. it derived from their direct common ancestor) rather than a parallelism. Furthermore, the pattern of genetic relationships as deduced from nei’s genetic distances (capula et al. 1996) points in favour of the hypothesis of a close phylogenetic relationship between A. abyssinicus and A. blicki.
The sex chromosomes provide some insight into the systematics of these species. They show very similar hetero- and euchromatic patterns in the three Ethiopian species, and this reinforces the monophyletic hypothesis for the Ethiopian group. A high intra-populational variability has been described by CIVITELLI et al. (1995) in the Benin population with three different variants, i.e. subtelocentric, submetacentric, and metacentric. Rearrangements involved are, respectively, a pericentric inversion and addition of heterochromatin. The same subtelocentric form occurs in *A. abyssinicus* with the same G- and C-band pattern. However, there is a second variant of the X chromosome in *A. abyssinicus* which is submetacentric and differs due to an addition of heterochromatin in the short arm. This condition is shared also by *A. dembeensis* and *A. blicki*. Due to the low number of individuals examined, the occurrence of a polymorphism in the X chromosome cannot be excluded a priori for *A. dembeensis* and *A. blicki*.

Only the comparison with the ‘true’ *A. niloticus* from terra typica, i.e. the Nile delta, would solve the systematics of these species (see MUSSER and CARLETON 1993). If *A. dembeensis* was a geographic variant or a subspecies of *A. niloticus*, then the genus would be represented by an Egyptian-Ethiopian radiation (*A. niloticus*, ‘A. dembeensis’, *A. abyssinicus*, and *A. blicki*) and by a Central-Western African one (VOLOBOUEV et al. 1987, 1988), including the karyotypes described by CIVITELLI et al. (1995), GRANJON et al. (1992) and by VOLOBOUEV et al. (1988) as ‘*A. centralis*’ and ‘*A. solatus*’.

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Zusammenfassung

*Cytogenetik der Gattung Arvicanthis (Rodentia, Muridae). 2. Die Chromosomen von drei Arten aus Äthiopien: A. abyssinicus, A. dembeensis und A. blicki*


References

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