Chromosomal and C-heterochromatin Characterization of
Arvicathanis niloticus (Rodentia: Murinae) in Egypt

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Abstract: The karyotype and C-banding pattern of the unstriped grass rat Arvicathanis niloticus from four localities in Egypt are presented. All individuals karyotyped, as a rule, have the same diploid number of 2n = 62 and autosomal Fundamental Number of aFN = 62. In addition, all chromosomes have a large centromeric block of fairly uniform size. However, an additional interstitial or telomeric small C-band is scored in some chromosomes. Nevertheless, frequent heteromorphism in the morphology and heterochromatin content of both the homologous chromosomes of the pair No. 1 and the X chromosome are scored in some individuals from the four localities and led to an aFN = 63. Accordingly, four forms or cytotypes, namely ANI-1, ANI-1, ANI-1 and ANI-1, are recognized based on this variation, which is mostly attributed either to addition or deletion of a heterochromatic segment as a result of pericentric inversions. Of these four forms, the ANI-1 is considered ancestral for A. niloticus in Egypt and is closely similar to that of the Ethiopian A. dembeensis, regardless the contradiction concerned with nomenclature of the X chromosome, while the cytotypes of the other forms are synapomorphy of the form ANI-1 and showed as well a relative resemblance to those of the Ethiopian A. abyssinicus and A. blicki. Therefore, it is concluded that the genus Arvicathanis would be represented by an Egyptian-Ethiopian radiation (A. niloticus, A. dembeensis, A. abyssinicus and A. blicki) and by a Central-Western African one, including the cytotypes described as A. centralis and A. solatus. Moreover, A. niloticus should be regarded no longer as a single species but as a cluster of several proper species.

Keywords: Arvicathanis niloticus, Murinae, Rodentia, karyotype, C-heterochromatin, Egypt

Introduction

The unstriped grass rat or Nile rat of the genus Arvicathanis (Lesson, 1842) has a widespread distribution throughout the African savannas south of the Sahara and extending along the Nile Valley to the Mediterranean sea and its taxonomy has long been the subject to repeated discussions. The difficulties of its taxonomy, particularly the species composition and their distribution limits, result primarily from the combination of large inter-individual variability in body measurements and pelage coloration and relatively low differentiation amongst population. On the basis of morphological variations, Allen (1939) recognized 37 subspecies among the following six species: Arvicathanis niloticus, A. lacernatus, A. tenebrosus, A. ochropus, A. abyssinicus and A. somalicus. However, Ellerman (1941) considered A. tenebrosus as a subspecies of A. abyssinicus and A. ochropus as a synonym of A. niloticus. Later on, a specific status is conferred to one subspecies of A. niloticus, which became A. blicki by Dorst (1972). Nevertheless, Misonne (1974) lumped all of the previously described subspecies into one species A. niloticus. Subsequently, Yalden et al. (1976) distinguished

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these subspecies as fairly five species: *A. niloticus*, *A. abysinnicus*, *A. blicki*, *A. dembeensis* and *A. somalicus*. Corbet and Hill (1980) counted also five species, but they conferred a specific rank to a subspecies of *A. niloticus*, *A. testicularis* and rejected the status of species for *A. dembeensis*. Moreover, Horaček et al. (1982) confirmed Misorne's (1974) assumption that the genus *Arvicacanthis* is monotypic with only one subspecies *A. niloticus*. Furthermore, Rousseau (1982) recognized four species: *A. niloticus*, *A. abysinnicus*, *A. blicki* and *A. somalicus*, but Nowak and Paradiso (1983) added to them a fifth species *A. dembeensis* as previously assumed by Yalden et al. (1976). Further, Musser and Carleton (1993) recognized five species, namely *A. abysinnicus*, *A. blicki*, *A. neumannii*, *A. nairobae* and *A. niloticus*, where the first three species are distinct and easily recognizable, while the definition and diagnosis of the other two species are still unsatisfactory.

Karyological data, on the other hand, are not less confusing where several diploid numbers (2n) 46 (Capanna et al., 1985), 56 (Matthey, 1965), 58 (Volobouev et al., 1987, 2002) and 62 (Viéjas-Péquignot et al., 1983; Volobouev et al., 1988, 2002; Capanna et al., 1996) are described for *A. niloticus*, 62 for *A. abysinnicus* (Matthey, 1959; Orlov et al., 1992; Cittegelli et al., 1995; Capanna et al., 1996, Corti et al., 1996), 62 for *A. nairobae* and 53-54 for *A. neumannii* (Castiglia et al., 2003), 48 for *A. blicki* (Corti et al., 1995, 1996) and 62 for *A. dembeensis* (Capanna et al., 1996; Corti et al., 1996).

In Africa, the very slight external morphological differentiation displayed by *Arvicacanthis* samples in western and central regions encouraged most authors to consider them as belonging to the sole species *A. niloticus*. However, biochemical (Kaminski et al., 1984, 1987; Capanna et al., 1996; Capula et al., 1997), geometrical morphometric (Fadda, 1998; Corti et al., 1996; Ducroz et al., 1997), karyotypic (Volobouev et al., 1987, 1988; Capanna et al., 1996; Corti et al., 1996; Ducroz et al., 1997 and molecular (Ducroz et al., 1997, 1998) data contradicted such taxonomic arrangement. This is because the chromosome banding studies, for example, have revealed the existence of four chromosomal forms labeled as ANI-1, ANI-2, ANI-3 and ANI-4 (Ducroz, 1998). The differences between these forms have been interpreted via laboratory crossbreeding experiments (Ducroz et al., 1997) as a series of chromosomal arrangements resulting likely from reproductive isolation (Ducroz, 1998). Recently, Volobouev et al. (2002) supported the existence of these four karyotypic forms, or cytotypes and provided a distinct geographic distribution limit for each species throughout western and central Africa. In addition, they proposed that the three western African species ANI-1, ANI-3 and ANI-4 could be renamed as *A. niloticus*, *A. ansorgel* and *A. rufrinus*, respectively; however, the affinity and naming of ANI-2 is still uncertain whether it is *A. centrals* or *A. testicularis*. In Egypt, however, morphological (Fadda and Corti, 1998; Fadda et al., 2001), biochemical (Kaminski et al., 1984), cytogenetic (Viéjas-Péquignot et al., 1983; Volobouev et al., 1988) and molecular (Ducroz et al., 1998, 2001) data revealed the occurrence of a single species ANI-1, viz., *A. niloticus* which has been considered a geographic variant of the Ethiopian *A. dembeensis* (Yalden et al., 1976; Volobouev et al., 1988; Orlov et al., 1992; Musser and Carleton, 1993; Corti et al., 1996).

Despite these immense studies on the taxonomy of *Arvicacanthis* along all ranges of its distribution, additional morphological, chromosomal and molecular investigations are necessary to understand the connection between this karyotypic diversity and to evaluate their distribution limits and support the earlier suggestions of its taxonomy. In addition, earlier chromosome data varied among authors in the ordering of chromosome pairs within karyotypes, hence making comparisons of the available data quite difficult. As a consequence, the extent of intraspecific chromosomal variation and interspecific karyological differences remained to be assessed. In this context, the necessity of a standardized chromosome nomenclature and the study of larger samples from more widespread geographical areas have become crucial. Moreover, the cytogenetic conclusions concerned with the occurrence of a single form ANI-1, or cytotype, of *A. niloticus* in Egypt are primarily based in many cases on a single locality samples. Hence, it was found useful and crucial to carry out a detailed survey study of
Arvicathus populations in Egypt, with the major objectives of 1) scoring of all possible forms of this species throughout all parts of its distribution in Egypt, 2) identifying and characterizing the karyotypes of these forms, 3) assessing the karyotype evolution among these forms by examining the C-banding pattern and 4) comparing the present data with that available in the literatures on A. niloticus from Egypt and other countries and particularly on the Ethiopian species in an attempt to clarify their cytogenetic relationship.

Materials and Methods

Adult individuals of the grass rat Arvicathus niloticus (Lesson, 1842) were collected from the following four localities in Egypt with the use of live traps baited with tomato and cucumbers and placed in the croplands: Al-Sharqiya (30°30-45' N-31°15-45' E, 35 m a.s.l.), Al-Minufiya (30°30-00' N-30°10-50' E 40 m a.s.l.), El-Faiyum (29°20-35' N-31°06-7' E, 15 m a.s.l.) and El-Minia (28°25-15' N-30°45-50' E, 35 m a.s.l.). The collecting sites and the corresponding samples sizes are indicated in Fig. 1. The animals were intraperitoneally injected with 0.05% colchicine solution (0.01 ml/g body weight) and one hour later killed with chloroform. Mitotic chromosomes spread from the femoral bone marrow cells were prepared by the air drying technique using the method of Yosida (1973), with a slight modification (Shahin and Ata, 2001). About 100 metaphase spreads from each animal in the different population localities were examined and the karyotype from each population was determined based on five to ten well-spread metaphase cells. Chromosomes were measured under Olympus BX 51 microscope using the Soft Imaging System (SIS) analysis program (Version 3.0) edited in 1999 by Soft Imaging System GmbH, Germany and classified according to the system proposed by Green and Sessions (1991) and as described by Shahin and Ata (2001). C-bands were attained by using the standard protocol of Summar (1972), with major modification (Shahin and Ata, 2004). About 100 to 200 metaphase plates from both males and females of each individual in each locality were examined.

Fig. 1: A map showing the geographic localities from which individuals of Arvicathus niloticus are collected. The localities and samples sizes are: Al-Sharqiya: 23, Al-Minufiya: 9, El-Faiyum: 9 and El-Minia: 19.
and good spreads (about 20-30) from each locality were scored and photographed using Olympus BX 51 microscope with a C-4040 zoom digital camera. The C-banding karyotype was determined and prepared as previously mentioned in the conventional preparation technique.

Results

Karyotype Description

Basically, the karyotype of *A. niloticus* (primarily designated as ANI-1) from the four localities surveyed in this study consists of a diploid number (2n) of 62 chromosomes and an autosomal Fundamental Number (aN) of 62. Of the 62 chromosomes, the X chromosome appears here as a large subtelocentric depending upon the arm ratio values; however, it is previously described by many authors as a large submetacentric (for references see discussion), while the Y chromosome is a medium-sized metacentric. The remaining 60 autosomes are variable-sized acrocentrics (telocentrics), except the pair no. 25 which is metacentrics and, therefore, they are arranged in a graded series according to their lengths (Fig. 2a). Nevertheless, frequent variation in the morphology of the homologous chromosomes of the pair no. 1 and the X chromosome is scored among individuals of some localities (Fig. 2b and 3, Table 1). Consequently, as the result of this heterozygosity and of the

![Image](image_url)

Fig. 2: Karyotype and C-banding pattern of males of *A. niloticus*. (a) Cytotype ANI-1* showing the basic karyotype of 2n = 62 and aFN = 62; note that the X chromosome is clearly subtelocentric (arrows), while the Y is metacentric. (b) Cytotype ANI-1* showing the heteromorphism in the chromosomes of the pair No. 1, one chromosome is telocentric, while the other is subtelocentric (arrow). Note, the female XX chromosomes are added.
### Table 1: A summary of chromosomal characteristics of the four forms or cytotypes scored for *Aristichthys niloticus* from the four localities surveyed in Egypt. Measurements are given in mm and data are presented as mean(SD).

**Abbreviations:** m = metacentric; sm = submetacentric; st = subteloentric; t = teloentric (acrocentric)

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<td>(0.16)</td>
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### Table 1 Continued

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<td>(0.65)</td>
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<td>2.15±</td>
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<td>0.00±</td>
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<td></td>
<td>(0.90)</td>
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<tr>
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<td></td>
<td>(0.90)</td>
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<td></td>
<td>1.34±</td>
<td>sm</td>
<td>0.00±</td>
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* Male individuals have the same chromosome morphology; - = Cytotypes are not scored in these localities

Relative sympathy between some populations, particularly between El-Faiyum and El-Minia localities, four forms or cytotypes could be recognized within the karyotype of the 60 individuals of *A. niloticus* examined from the four localities (Fig. 2 and 3, Table 1). A detailed description of the four karyotypic forms is as follows:

**ANI-I**

This type represents the basic karyotype of *A. niloticus* and it is recorded in a total of 39 individuals (15 $\sigma$ and 24 $\varphi$) from the 60 individuals collected from the four localities: Al-Sharqiya ($4 \sigma$ and $12 \varphi$), Al-Minufiya ($3 \sigma$ and $4 \varphi$), Al-Faiyum ($4 \sigma$) and El-Minia ($4 \sigma$ and $8 \varphi$). The karyotype consists of 2n = 62 chromosomes and aFN = 62. All the autosomes, including the pair No.1, are acrocentrics except the pair No. 25 which is metacentric. The X chromosome is a large subteloentric, while the Y chromosome is a medium-sized metacentric (Fig. 2a and Table 1).
Fig. 3: Karyotype and C-banding pattern of males of *A. niloticus*. (a) Cytotype ANI-1 showing the heteromorphism in the X chromosomes, one chromosome is subtelocentric, while the other is submetacentric (arrows). (b) Cytotype ANI-1 showing the heteromorphism in both of chromosomes of the pair No. 1, where one chromosome is telocentric, while the other is subtelocentric (arrow) and the X chromosomes where one chromosome is submetacentric (arrowheads), while the other is subtelocentric (arrow). Note, the female XX chromosomes are added.

ANI-1<sup>+</sup>

This karyotype is characterized by the presence of heteromorphism in the homologous chromosomes of the pair No. 1 and monomorphism in the X chromosome. Therefore, it is nearly identical to that of ANI-1<sup>-</sup> i.e., it has a 2n = 62 and aFN = 62, except that the homologous chromosomes of the pair No. 1 are heteromorphic, one chromosome is acrocentric, while the other is subtelocentric, thus resulting in an aFN = 63. This karyotype is not scored in animals from El-Faiyum locality; however, it is recognized in only 13 animals (3♂ and 10♀) of the 51 animals examined from Al-Sharqiya (2♂ and 5♀), Al-Minufiya (2♂) and El-Minia (1♂ and 3♀) localities (Fig. 2b and Table 1).

ANI-1<sup>-</sup>

This karyotype includes individuals, which displayed heteromorphism in the X chromosome, while the homologous chromosomes of the pair No. 1 are monomorphic. Likewise ANI-1<sup>+</sup>, the chromosome complement of this karyotype, consists of 2n = 62 chromosomes and aFN = 62 in individuals from all localities. The heterogeneity in the X chromosome, which is related to relative variation in length of the short arms, is scored in only two females from each of El-Faiyum and El-Minia localities where they are sorted as submetacentric (Fig. 3a and Table 1).
This karyotype comprises animals, which showed heteromorphism in both of the chromosomes of the pair No. 1 and the X chromosome. Accordingly, this form consists also of 2n = 62 chromosomes, but the aFN is either 63, as found in only three animals (1 ♀ and 2 ♂) from El-Faiyum and (1 ♂) from El-Minia where one chromosome of the pair No. 1 is acrocentric, while its homologue is subteloacentric (Fig. 3b and Table 1), or 62 as appeared in the remaining animals from the four localities. The morphology of the X chromosome is closely similar to that found in the karyotype of ANI-1'.

C-heterochromatin

As a rule, all autosomes of A. niloticus from the four localities surveyed in this study have a large centromeric block of fairly uniform size (Fig. 2 and 3). However, in the subteloacentric chromosome of the pair No. 1, the short arm is not entirely heterochromatic. This feature is detected in 13 animals: four females from Al-Sharqiya, two females from Al-Minufiya, five males from El-Minia and a single male and female from El-Faiyum (Fig. 2b and 3b). In addition, some autosomal pairs of both males and females from all localities show either interstitial (pair No. 1) or telomeric (pairs No. 2, 4, 5, 6, 10, 11, 14, 15 and 20) gray to black C-band, which likely indicate the location of the nucleolus organizer regions (Fig. 3). Moreover, the short arms of the X chromosome are completely stained intensively in the pericentromeric region, while the long arms show a relatively small, less stained, interstitial C-band located near the distal region. The Y chromosome is stained more intensively and thus it is almost entirely heterochromatic. Nonetheless, a heteromorphic feature related to variations in the amount of C-heterochromatin in the short arms of the X chromosome is recorded in three animals (1 ♀ and 2 ♂) from each of El-Faiyum and El-Minia localities due to relative differences in the length of short arms (Fig. 3).

Discussion

As far as A. niloticus, labeled ANI-1 by Volobouev et al. (1988), is known to exhibit a discrete chromosomal variability, with diploid numbers varying from 2n = 44 to 2n = 62. Within this range of diploid numbers, five to six cytotypes are recognized: two of which with 2n = 62 exhibited additional chromosome polymorphism and three cytotypes are described as cryptic species with 2n = 58, 56 and 44 (Matheu, 1965; Volobouev et al., 1987, 1988; Capanna and Civitelli, 1988). Moreover, Ducroz et al. (1997) pointed out that the cytotype ANI-1, with 2n = 62, corresponds to A. niloticus sensu stricto (Nile Valley and northern Sahelian territory). Furthermore, the Egyptian A. niloticus has been proven to be similar to A. niloticus populations from East of Senegal (Volobouev et al., 1988; Corti et al., 1996) as well as it has been considered a geographic variant of the Ethiopian A. dembeensis (Viégas-Péquignot et al., 1983; Orlov et al., 1992; Musser and Carleton, 1993; Capanna et al., 1996; Corti et al., 1996). This relationship is suggested by the high similarity of their karyotypes which consist of 2n = 62 and aFN = 62 (Orlov et al., 1992; Capanna et al., 1996; Corti et al., 1996) and named ANI-1 by Volobouev et al. (1988). Nevertheless, it has been established that the Egyptian from ANI-1, differs from the other forms ANI-2 and ANI-3 from Burkina-Faso, Mali and Central African Republic by limited chromosomal rearrangements, mostly by a pericentric inversion and likely by a reciprocal translocation (Volobouev et al., 1988).

Concerning the karyotypes of the four forms scored here in this study, it is evident that the cytotype ANI-1* is closely consistent in its configuration and C-heterochromatin content with that of the Ethiopian lowland A. dembeensis (Capanna et al., 1996; Corti et al., 1996), without regard to variation in the nomenclature of the X chromosome. On the basis of the arm ratio values, the morphology of the X chromosome appeared herein as well as in the previous studies by
Velouchou et al. (1987, 1988) and Viégas-Péquignot et al. (1983) as large subtelocentric; however, the latter authors have sorted it as submetaacentric. Similarly, the X chromosome of the Ethiopian A. dombeensis has been designated by Capanna et al. (1996) and Corti et al. (1996) as large submetaacentric, although it appears in their cited photographs as subtelocentric. In addition, the form ANI-1<sup>*</sup> is quite similar to the cytotypes ANI-1 recognized by Volouchou et al. (1988) from Cairo and Senegal and ANI-1a described by Volouchou et al. (2002) from Niger, Chad and Mali, except the variation concerned with the occurrence of telomeric C-band in the pairs No. 4, 10, 11, 14, 15 and 20 scored in this study. On the contrary, there are quite variations between the four forms suggested here in this study and the other A. niloticus forms such as ANI-2 from Central African Republic and ANI-3 from Burkina Faso and Mali (Volouchou et al., 1988) as well as ANI-1b from Mali, Senegal, Burkina Faso and Mauritania (Volouchou et al., 2002) and A. niloticus from Benin (Civitelli et al., 1995). These variations have been attributed to numerous chromosomal rearrangements, most of which are due to pericentric inversions and heterochromatin additions/deletions and likely to reciprocal and Robertsonian translocations (Corti et al., 1996; Volouchou et al., 1988, 2002).

Generally, the variation in the amount of C-heterochromatin between a pair of homologue chromosomes or among chromosomes of the same karyotype or even among karyotypes of the closely related species has been attributed by many authors to transformation of heterochromatin into euchromatin or vice versa or to deletion or duplication of heterochromatic segments (Shahin and Atu, 2004).

Comparison of the chromosomes morphology and C-banded karyotypes of the recognized four forms showed that all autosomes are identical, except that the subtelocentric chromosome of the pair No. 1 categorized in ANI-1<sup>*</sup> and ANI-1<sup>4</sup> and which acquired a short heterochromatic arm is involved in a deletion of this arm as well as a pericentric inversion in ANI-1<sup>*</sup>. In addition, the subtelocentric chromosome of the X chromosome in ANI-1<sup>3</sup> and ANI-1<sup>1</sup>, particularly in females from El-Minia locality, is involved also in an addition of segment as well as a further pericentric inversion in ANI-1<sup>1</sup> and ANI-1<sup>4</sup> and thus acquired a heterochromatic short arm. However, the acquisition of a relatively short heterochromatic short arm is due to the deletion of a heterochromatic block to the distal part of the short arm as a result of a pericentric inversion. Similar findings of polymorphism in the X chromosome (with metaacentric, submetaacentric and subtelocentric configuration) both in homozygous and heterozygous state have been found in the South of Benin for the cytotype ANI-3, without any apparent reduction in relative fertility of structural heterozygotes (Civitelli et al., 1995). This heterozygosity observed among the four cytotypes in both of the homologues of the pair No. 1 and the X chromosome as well as the presence of a limited number of chromosomal rearrangements amongst these forms could likely be interpreted as crossbreeding resulting from reproductive association between these forms, but the postulation of such hypothesis needs further laboratory crossbreeding experiments.

As regards, the karyotype of the form ANI-1<sup>*</sup> represents the ancestral karyotype of A. niloticus in Egypt, which has previously been described by many authors as ANI-1 from Cairo and terra typica, i.e. the Nile delta (Viégas-Péquignot et al., 1983; Corti et al., 1996; Volouchou et al., 1987, 1988, 2002). This karyotype is apparently quite homologous to that of the Ethiopian A. dombeensis populations (Orlov et al., 1992; Corti et al., 1996), regardless the contradiction concerned with the morphology of the X chromosome. This similarity, however, could be due to convergence, as both species occur at low altitudes and are adapted to lowlands. On the other hand, the karyotypes of the other forms are synapomorphy, i.e., they are derived from the cytotype ANI-1<sup>*</sup> and produced by limited chromosomal rearrangements, mostly by addition or deletion of chromosomal segments as a result of pericentric inversions. This conclusion is supported as well by G-banding analyses of which data are under preparation. Moreover, the heteromorphism observed in the shape and size of the X chromosome that can be either subtelocentric or submetaacentric and also its heterochromatin content, particularly in the
forms ANI-1<sup>a</sup> and ANI-1<sup>b</sup> is similar to that found in the Ethiopian A. abyssinicus and A. blicki (Corti et al., 1996). Furthermore, the subtelocentric chromosome of the pair No. 1 found in the forms ANI-1<sup>a</sup> and ANI-1<sup>b</sup> is homologous to the chromosome No. 4 in both A. abyssinicus and A. blicki (Corti et al., 1996), with regard to their differences in size.

As a consequence, it could be concluded that the Egyptian A. niloticus is just a geographic variant of the Ethiopian A. dembeensis as assumed by Viégas-Péquignot et al. (1983), Volobouve et al. (1988), Orlov et al. (1992) and Corti et al. (1996). This hypothesis supports also the inclusion of A. dembeensis in A. niloticus (Masser and Carleton, 1993) and contradicts the consideration of Yalden et al. (1967) and Corbet and Hill (1991) who classified A. dembeensis as a separate species. Moreover, it is apparent as suggested by Corti et al. (1996) that the genus Arvicanthis would be represented by an Egyptian-Ethiopian radiation (A. niloticus synonym A. dembeensis), A. abyssinicus and A. blicki and by a Central-Western African one (Volobouve et al., 1987, 1988), including the karyotypes described by Civitelli et al. (1995), Grajon et al. (1992) and by Volobouve et al. (1988) as A. centralis and A. solatus.

Finally, as assumed by many authors on the basis of chromosomal data, the genus Arvicanthis is polytypic (Volobouve et al., 1988). This assumption, although it is proposed also by morphological data (Rousseau, 1982; Nowak and Paradiso, 1983), it contradicts the earlier opinion of Misserne (1974) and Honacki et al. (1982). Moreover, A. niloticus, as pointed out by Volobouve et al. (1988), should be regarded no longer as a single species but as a cluster of several proper species.

References


