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C-banding Karyotype and Relationship of the Dipodids *Allactaga* and *Jaculus* (Mammalia: Rodentia) in Egypt

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The C-banding karyotype of the jerboas *Allactaga tetradactyla*, *Jaculus jaculus jaculus*, and *Jaculus orientalis* was described and interspecific relationships were discussed. Despite the conservation of a relatively small amount of C-heterochromatin located at the centromeric region of some chromosomes in all karyotypes, a striking loss of C-heterochromatin was clearly observed in *J. orientalis*. C-bands were totally absent in 33 of the 48 chromosomes of *J. orientalis*, compared to only 7 for *J. j. jaculus* and 11 for *A. tetradactyla*. The differences in C-banding amongst karyotypes of the three species were attributed either to transformation of heterochromatin into euchromatin or *vice versa*, deletion of heterochromatic segments resulting from pericentric inversions, or to variation of euchromatin content and its correlation with the chromosome size and arrangement of heterochromatin. The present findings are consistent with the main hypotheses derived from morphological, chromosomal, and biochemical data that the genera *Allactaga* and *Jaculus* have independently developed from a common ancestral form and that *J. jaculus* and *J. orientalis* are both distinct congeneric species, but revealed that the C-banding karyotypes of both *J. j. jaculus* and *J. orientalis* are distantly related to each other. Therefore, it is concluded that the karyotype of *J. j. jaculus* may be ancestral and that of *J. orientalis* may have derived from it.

Key words: C-bands, jerboas, Dipodidae, *Allactaga tetradactyla*, *Jaculus jaculus jaculus*, *Jaculus orientalis*.

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Considerable attention has been paid to the relationship of the dipodids (jerboas) *Allactaga tetradactyla*, *Jaculus jaculus*, and *Jaculus orientalis* during the last half century as a consequence of their earlier taxonomic controversy (POCOCK 1922; VINOGRADOV 1930; SIMPSON 1945; ELLERMAN 1948). WASSIF (1960) examined osteological traits and found that both *J. jaculus* and *J. orientalis* are more similar to each other than either of them are to *A. tetradactyla*. OSBORN and HELMY (1980) studied both morphological and osteological traits and accepted their classification into the genera *Allactaga* and *Jaculus*. The genus *Allactaga* comprised only *A. tetradactyla*, while *Jaculus* included two species, *J. jaculus* and *J. orientalis*. In addition, OSBORN and HELMY (1980) recognized four subspecies of the lesser jerboa, viz. *Jaculus jaculus butleri*, *Jaculus jaculus flavillus*, *Jaculus jaculus jaculus*, and *Jaculus jaculus schlueteri*, based on morphological variation. Afterwards, studies on sperm morphology revealed that these dipodids are part of a single evolutionary radiation (SHAHIN & IBRAHEEM 1998). Additionally, SHAHIN (1999), based on molar and soft palate characters, hypothesized that the genera *Allactaga* and *Jaculus* may represent two offshoots from a single dipodid ancestor and that *J. jaculus* and *J. orientalis* are both distinct congeneric species. Chromosomal studies (ATA & SHAHIN 1999; ATA *et al.* 2001; SHAHIN & ATA 2001) confirmed SHAHIN's (1999) hypothesis and interpreted the dissimilarity in the G-bands of the morphologically different chromosome pairs, either between *A. tetradactyla* and the two congeneric species *J. orientalis* and *J. j. jaculus* or between the two latter species, as pericentric inversions. However, G-banding heterogeneity in morphologically similar chromosomes is attributed to variation in heterochromatin content and its correlation with chromosome size (ATA & SHAHIN 1999). Moreo-

ver, biochemical studies (SHAHIN 2003) demonstrated that *J. orientalis* appears to have shared a more recent common ancestor with *J. jaculus* than *A. tetradactyla* and divergence of these species had occurred by the Miocene (ca. 9.6 to 18.7 million years ago).

C-heterochromatin has been proven to differentiate between very similar karyotypes (FREDGA & MANDAHL 1973; MIURA 1995; SPASIC-BOSKOVIC *et al*. 1997; BRAGGIO *et al*. 1999; FORMAS & CUEVAS 2000; OSHIDA *et al*. 2000a, b; CUEVAS & FORMAS 2003; LEITE-SILVA *et al*. 2003). A pair of homologous chromosomes may be heteromorphic when one member has more heterochromatin material than the other, i.e. an addition or deletion making the homologues unequal (MANDAHL 1978, 1979; WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1995; OSHIDA *et al*. 2000b). In the present investigation, C-heterochromatin was used to verify the relationships between dipodid species obtained from morphological, biochemical, and karyotypic data and to describe karyotype evolution in these species that possess a similar karyotype of 2n=48 and FN=95-96 (SHAHIN & ATA 2001).

Material and Methods

Adult individuals of *Allactaga tetradactyla* Lichtenstein 1823 (3 ♂♂ and 4 ♀♀), *Jaculus jaculus jaculus* Linnaeus 1758 (3 ♂♂ and 3 ♀♀), and *Jaculus orientalis* Erxleben 1777 (3 ♂♂ and 5 ♀♀) were captured in Egypt from Al Salum, Abu Rawash, and Marsa Matruh, respectively, either with the use of butterfly nets or with live traps placed in tracks made in sand by vehicles. The animals were intraperitoneally injected with 0.05% colchicine solution (0.01 ml/g body weight) and one hour later killed with chloroform. Mitotic chromosome spreads from the femoral bone marrow cells were prepared by the flame drying technique using the method of YOSIDA (1973). C-bands were obtained according to the standard protocol of SUMMNER (1972), with major modifications. The slides were immersed in 0.2 N HCL at 10°C for one hour, rinsed in distilled water, and then kept in a saturated $Ba(OH)_2$. $8H_2O$ solution for about 20-30 min at 60-65°C. Subsequently, the slides were rinsed in 70% and 95% ethanol, incubated in 2X SSC at 60°C for 30 min, and rinsed in 2X SSC, 70%, and 95% ethanol. The slides were then stained in buffered 4% Giemsa solution (1:10, pH 7.0) for 3-5 min at room temperature. About 20 to 100 metaphaseplates from both males and females of each species were examined and good spreads (about 20-30) from each species were scored and photographed using Olympus BX 51 microscope with a C-4040 zoom digital camera. The karyotype was determined on the basis of five to ten well-spread metaphase cells for each species. Chromosomes were measured under a 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), with a slight modification as described by SHAHIN and ATA (2001).

Results

The karyotype of dipodid species has previously been described by SHAHIN and ATA (2001). All species have a diploid number of 2n=48 chromosomes and a fundamental number (FN) of 95 in males and 96 in females. The X chromosome is submetacentric, while the Y is telocentric (acrocentric). In addition, *A. tetradactyla* has six subtelocentrics (pairs no. 2, 4, 5, 6, 7, and 9), while the remainders are submetacentrics, except for pairs no. 10, 11, 18, and 19, which are metacentrics. Nevertheless, *J. orientalis*, and *J. j. jaculus* have only three subtelocentrics (pairs no. 2, 21, and 22) and the others are submetacentrics, except for pair no. 18 in *J. orientalis* and 20 in *J. j. jaculus*, which are metacentrics.

As a rule, the dipodid species examined in this study had a relatively small amount of constitutive heterochromatin and their C-banding pattern was characterized by the presence of a centromeric band in a variable number of chromosomes of each of the three species. Although the position of C-bands was frequently similar in all species, significant variation in size and occurrence of C-bands was observed between the three species (Table 1; Figs 1-3).

Allactaga tetradactyla

All chromosomes of the complement have C-bands except for pairs no. 1, 4, 5, 6, 8, and the X chromosome. In these C-banding stained pairs, a small centromeric C-band was observed only in autosomes no. 3, 9, 11, 12, and 15, while the remaining 13 pairs and the Y chromosome possessed a large centromeric C-band (Fig. 1).

Jaculus jaculus jaculus

Three autosomes (pairs no. 9, 16, and 23) and the X chromosome have no C-bands. Nonetheless, six pairs, no. 7, 8, 10, 15, 17, 20, and the Y chromo-

Table 1

Distribution of C-blocks into different size categories among chromosomes of the three dipodids examined. L = large, S = small, – = no C-blocks

Chromosome no.	Species (Number of cells examined in both males & females)		
	A. tetradactyla (37 &100)	*J. j. jaculus* (31 & 23)	*J. orientalis* (29 & 63)
1	–	L	L
2	L	L	L
3	S	L	–
4	–	L	S
5	–	L	–
6	–	L	S
7	L	S	–
8	–	S	–
9	S	–	–
10	L	S	–
11	S	L	–
12	S	L	S
13	L	L	–
14	L	L	–
15	S	S	–
16	L	–	–
17	L	S	–
18	L	L	–
19	L	L	–
20	L	S	–
21	L	L	L
22	L	L	L
23	L	–	–
X	–	–	–
Y	L	S	S

some have a small centromeric C-band, while the remaining 14 autosomes exhibit a large centromeric C-band. In addition, pair no. 11 has a heteromorphic feature (Fig. 2).

Jaculus orientalis

The chromosome complement of this species exhibited a characteristic C-banding distribution, which is somewhat different from the other two species. Sixteen autosomal pairs and the X chromosome are completely devoid of C-bands, however, centromeric C-bands were scored in the remainder seven pairs and in the Y chromosome. In these latter chromosomes, a small C-band was shown in pairs no. 4, 6, 12, and in the Y chromosome, while a large band was scored in nos. 1, 2, 21, and 22 (Fig. 3).

C-banding comparison

A comparison of C-banding size and occurrence in the chromosome complement of the three dipodids is given in Table 1. In the 48 chromosomes, only 9 (18.8%) possessed C-bands in all taxa, 38 (79.2%) chromosomes possessed C-bands, but exhibited intertaxon variation, and only one (2.1%) chromosome, the X chromosome, was entirely devoid of C-bands. In this regard *J. j. jaculus* has the lowest percentage of absence of C-blocks, 14.6%, compared to 22.9% in *A. tetradactyla* and 68.8% in *J. orientalis*. Further, C-blocks were heterogeneous in eight pairs (33.3%) of chromosomes and completely absent in the X chromosome between *J. j. jaculus* and *A. tetradactyla*, while they were variable in 17 pairs (70.8%) and absent in pairs no. 5 and 8 and in the X chromosome between the latter species and *J. orientalis*. Nevertheless, there was heterogeneity of C-band occurrence in 13 pairs (54.2%) and 3 pairs, no. 9, 16, and 23, and the X chromosome exhibited no C-bands between *J. j. jaculus* and *J. orientalis*.

Discussion

Chromosome examination of the dipodid species *A. tetradactyla*, *J. j. jaculus* and *J. orientalis* revealed a diploid number of 2n=48 chromosomes and a fundamental number (FN) of 95 in males and 96 in females (SHAHIN & ATA 2001). Of these 48 chromosomes, both *J. j. jaculus* and *J. orientalis* are different from *A. tetradactyla* in the morphology of ten pairs (nos. 4, 5, 6, 7, 9, 10, 11, 19, 21, and 22), while they are in turn different from each other in pair no. 20, only. ATA and SHAHIN (1999), based on G-banding analysis, pointed out that the polymorphism of the G-banding pattern in the morphologically different chromosome pairs no. 4, 5, 6, 7, 9, 10, 11, 19, 20, 21, and 22 is due to pericentric inversions that occurred during karyotype evolution of these species. Moreover, they interpreted the heterogeneity of G-bands in morphologically similar pairs no. 1, 2, 3, and 14 as variation of heterochromatin content and its correlation with chromosome size and arrangement of G-bands.

Many explanations have been put forward to account for variation in C-bands among karyotypes of related species. For example, it has been attributed to transformation of heterochromatin into euchromatin or *vice versa* (KING 1980, 1991;

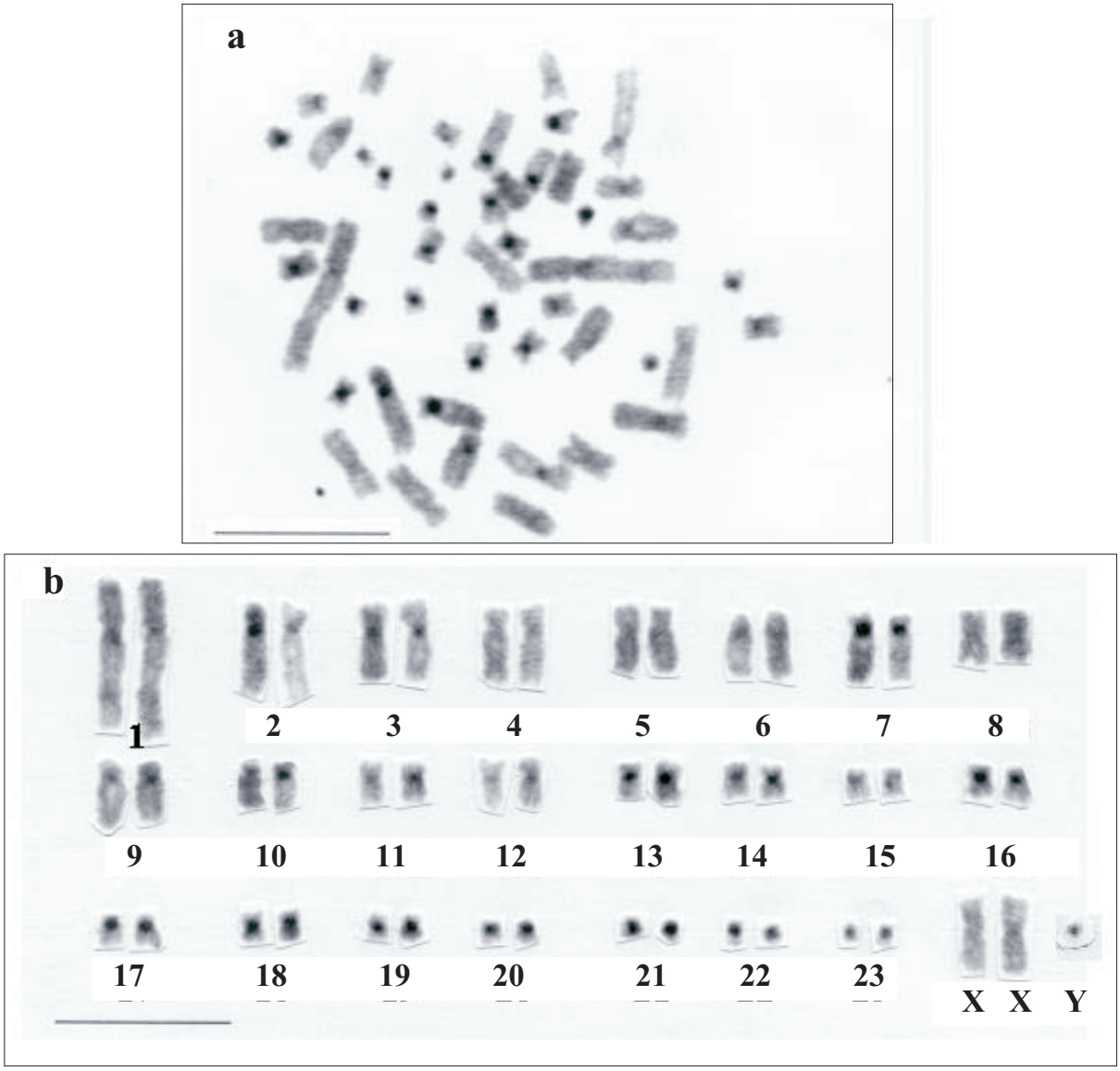

Fig. 1. C-banded karyotype of *Allactaga tetradactyla*. (a) Metaphase cell from a female; (b) Karyotype of a female, in addition to the Y chromosome from a male. Bar = 10 μm.

KING & JOHN 1980; CABRERO *et al*. 1985; CUEVAS & FORMAS 2003) or to deletion or duplication of heterochromatic segments (WHITE 1973; CABRERO *et al*. 1985). It is evident that the heterogeneity of C-banding in morphologically different chromosome pairs no. 4, 5, 6, 7, 9, 10, 11, 19, and 20 either between *A. tetradactyla* and both *J. j. jaculus* and *J. orientalis* or between the two latter species was due to transformation of heterochromatin into euchromatin or *vice versa* or to deletion of heterochromatic segments resulting from pericentric inversions that occurred during karyotype evolution. However, the heterogeneity of C-banding distribution in morphologically similar pairs no. 1, 3, 8, 13, 14, 15, 16, 17, 18, and 23 could be attributed to variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin. Moreover, the similarity of C-banding in pairs no. 21 and 22 could be interpreted as pericentric inversion of heterochromatin arms without any change in the arrangement of both euchromatin and heterochromatin content. Nevertheless, the similarity of the C-banding pattern in pairs no. 2, 12, and the Y chromosome could be explained just as well in terms of variation of euchromatin content and its correlation with chromosome size and amount of heterochromatin. This interpretation was also confirmed in a G-banding analysis carried out by ATA and SHAHIN (1999).

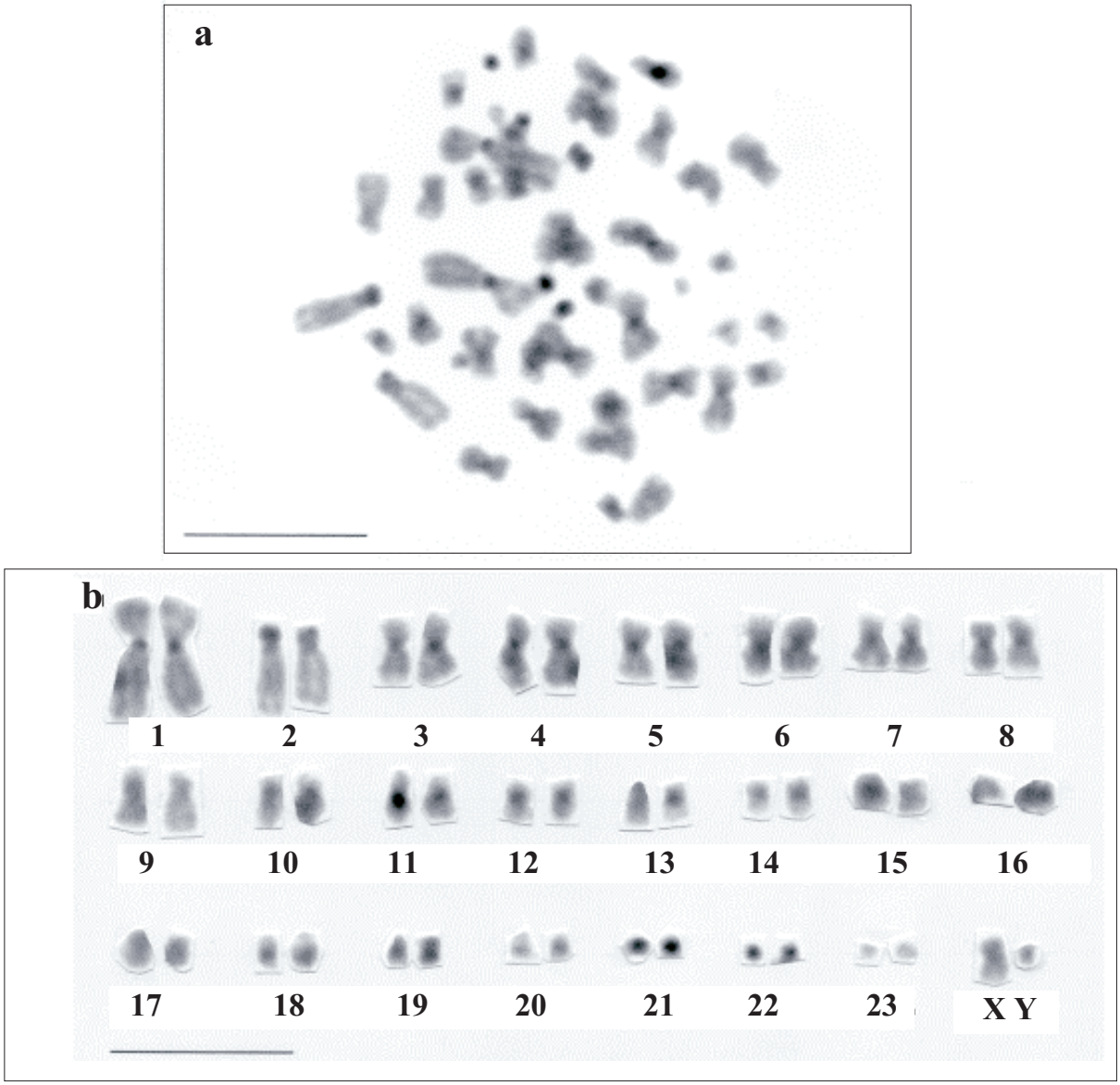

Fig. 2. C-banded karyotype of *Jaculus jaculus jaculus*. (a) Metaphase cell from a male; (b) Karyotype of a male. Bar = 10 μm.

A noteworthy finding of this study is that most heterochromatic segments were conserved in the chromosomes of the species examined except for *J. orientalis*, where the corresponding segments found in both *J. j. jaculus* and *A. tetradactyla* have been lost or euchromatinized during karyotype evolution. Although such variability in heterochromatin content may generally alter the total DNA content for these species, it provides a considerable affinity between these species and in particular between the two congeneric species *J. jaculus* and *J. orientalis*. A more detailed analysis using comparative chromosome painting (ZOO-FISH) and gene mapping technology will be needed to determine the patterns of genome variation or conservation in these species, particularly in the genus *Jaculus*. Furthermore, the X chromosome, in contrast to many mammalian species, has no constitutive heterochromatin (e.g. FREDGA and MANDAHL 1973; MANDAHL 1978; GAMPERL *et al*. 1982; GRAVES and WATSON 1991; BRAGGIO *et al*. 1999; OSHIDA *et al*. 2000a, b; LEITE-SILVA *et al*. 2003; MEGIAS-NOGAELS *et al*. 2003). This means that the euchromatic X chromosome may have been shared in these dipodid species throughout the process of karyotype evolution. These results were also confirmed in meiotic preparations (ATA and SHAHIN, in preparation).

In conclusion, the present findings are consistent with the main hypotheses derived from morpho-

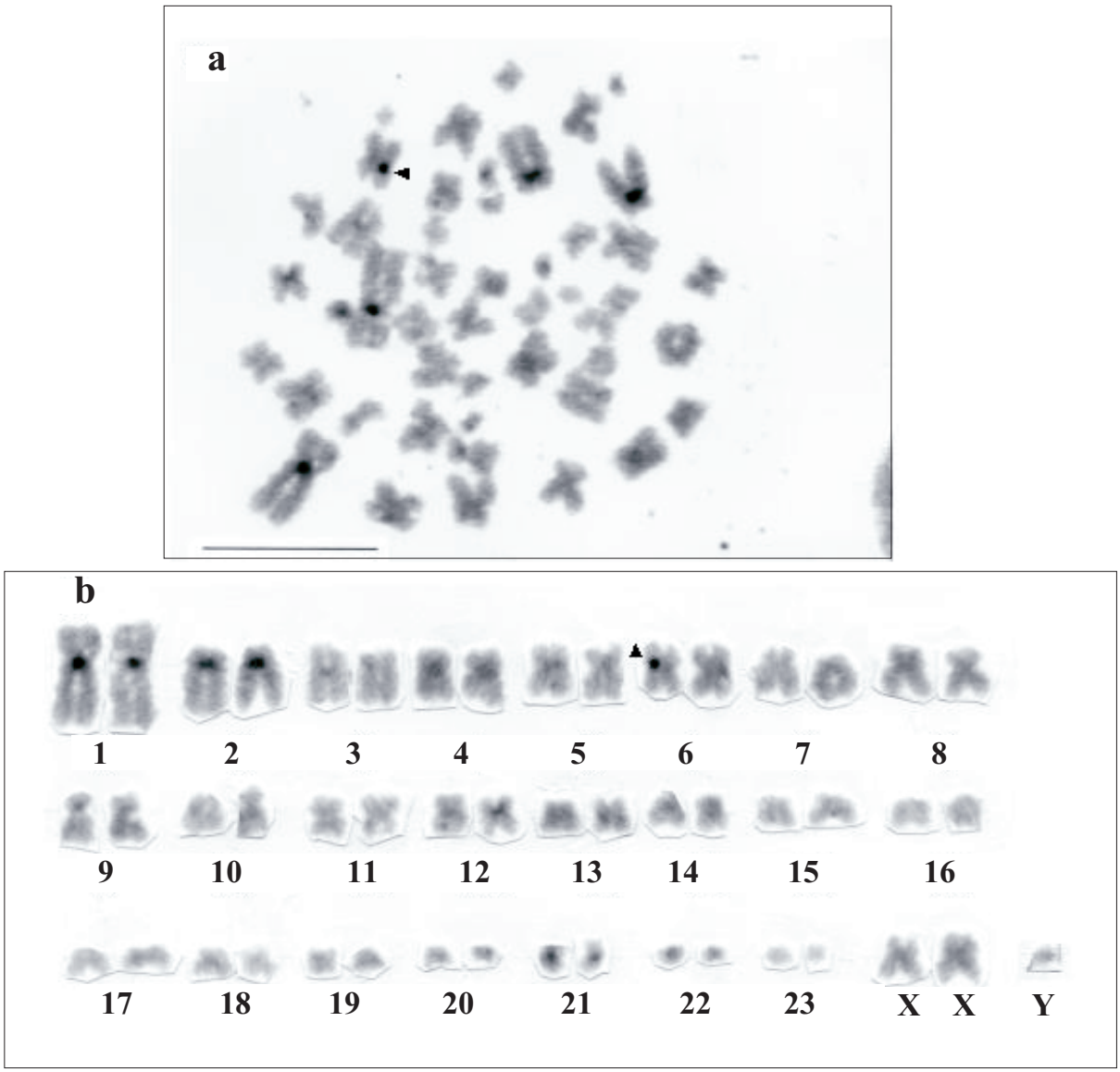

Fig. 3. C-banded karyotype of *Jaculus orientalis*. (a) Metaphase cell from a female; (b) Karyotype of a female, in addition to the Y chromosome from a male. Arrow heads refer to a staining artifact. Bar = 10 μm.

logical (SHAHIN & IBRAHEEM 1998; SHAHIN 1999), chromosomal (ATA & SHAHIN 1999; SHAHIN & ATA 2001; ATA *et al.* 2001), and biochemical (SHAHIN 2003) data that the genera *Allactaga* and *Jaculus* have independently developed from a common ancestral form and that *J. jaculus* and *J. orientalis* are both distinct congeneric species. Moreover, this cytogenetic information is crucial for explaining the relationship of these species and reveales that although the karyotypes of both *J. j. jaculus* and *J. orientalis* are morphologically more similar to each other than either of them are to *A. tetradactyla* (SHAHIN & ATA 2001), they have a quite variable C-banding pattern. Hence, a formal suggestion would be that the karyotype of *J. j. jaculus* may be ancestral and that of *J. orientalis* derived, although caution must be exercised in arriving at evolutionary judgments based on karyology alone.

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References

ATA A. M., SHAHIN A. B. 1999. Variation of G-bands in the chromosomes of *Allactaga tetradactyla*, *Jaculus jaculus jaculus* and *Jaculus orientalis* (Rodentia: Dipodidae) common in Egypt. J. Union of Arab Biologists **11**: 295-309.

ATA A. M., SHAHIN A. A. B., ALLAM H. Z. 2001. A Comparative analysis of the rate of meiosis, chiasma frequency and terminalization in the jerboas *Allactaga* and *Jaculus* (Rodentia: Dipodidae) in Egypt. Folia biol. (Kraków) **49**: 129-135.

BRAGGIO E., GIMENEZ M. D., CONTRERAS J. R., JUSTO E., BIDAU C. J. 1999. Karyotypic variation in populations of *Ctenomys* (Rodentia, Ctenomyidae) from La Pampa Province (Argentina). Caryologia **52**: 131-140.

CABRERO J., CAMACHO J. P. M., PASCUAL F. 1985. Cytotaxonomic studies on Pamphaagids genus *Eumigus* detection of two chromosomal races in *E. monticola* (Rambur) (Insecta, Orthoptera). Caryologia **38**: 1-12.

CUEVAS C. C., FORMAS J. R. 2003. Cytogenetic analysis of four species of the genus *Alsodes* (Anura : Leptodactylidae) with comments about the karyological evolution of the genus. Hereditas **138**: 138-147.

ELLERMAN J. R. 1948. Rodents of southwest Asia. Proc. Zool. Soc. London **118**: 765-816.

FREDGA K., MANDAHL N. 1973. Autosomal heterochromatin in some carnivores. Nobel (Chromosome identification) **23**: 104-117.

FORMAS J. R., CUEVAS C. C. 2000. Comparative cytogenetic analysis of the Chilean leptodactylid frog genus *Telmatobufo*, with the description of the chromosomes of *T. venustus*. Proc. Biol. Soc. Wash. **113** : 890-899.

GAMPERL R., EHMANN C. H., BACHMANN K. 1982. Genome size and heterochromatin variation in rodents. Genetica **58**: 199-212.

GRAVES J. A. M., WATSON J. M. 1991. Mammalian sex chromosomes: Evolution of organization and function. Chromosoma **101**: 63-68.

GREEN D. M., SESSIONS S. K. 1991. Nomenclature for chromosomes. (In: Amphibian Cytogenetics and Evolution. D. M. Green and S. K. Sessions eds. San Diego): 431-432.

KING M. 1980. C-banding studies on Australian hylid frogs: Secondary constriction structure and the concept of euchromatin transformation. Chromosoma **80**: 191-217.

KING M. 1991. The evolution of heterochromatin in the amphibian genome. (In: Amphibian Cytogenetics and Evolution. D. M. Green and S. K. Sessions eds. Academic Press): 359-381.

KING M., JOHN B. 1980. Regularities and restrictions governing C-band variation in acridoid grasshoppers. Chromosoma **76**: 123-150.

LEITE-SILVA C., SANTOS N., FAGUNDES V., YONENAGA-YASSUDA Y., DE SOUZA M. J. (2003). Karyotypic characterization of the bat species *Molossus ater*, *M. molossus* and *Molossops planirostris* (Chiroptera, Molossidae) using FISH and banding techniques. Hereditas **138**: 94-100.

MANDAHL N. 1978. Variation in C-stained chromosome regions in European hedgehogs (Insectivora, Mammalia). Hereditas **89**: 107-128.

MANDAHL N. 1979. Localization of nucleolar organizing regions in European hedgehogs (Insectivora, Mammalia). Hereditas **91**:149-161.

MEGIAS-NOGAELS B., MARCHAL J. A., ACOSTA M. J., BULLEJOS M., DIAS DE LA CUARDIA R., SANCHEZ A. 2003. Sex chromosomes pairing in two Arvicolidae species: *Microtus nivalis* and *Arvicola sapidus*. Hereditas **138**: 114-121.

MIURA I. 1995. Two differentiated groups of the Japanese toad, *Bufo japonicus japonicus*, demonstrated by C-banding analysis of chromosomes. Caryologia **2**: 123-136.

OSBORN D. J., HELMY I. 1980. The contemporary land mammals of Egypt (including Sinai). Fieldiana Zoology **5**: 1-579.

OSHIDA T., OBARA Y., LIN L. K., YOSHIDA M. C. 2000a. Comparison of banded karyotypes between two subspecies of the red and white giant flying squirrel *Petaurista alborufus* (Mammalia, Rodentia). Caryologia **53**: 261-267.

OSHIDA T., YANAGAWA H., TSUDA M., INOUE S., YOSHIDA M. C. 2000b. Comparisons of the banded karyotypes between the small Japanese flying squirrel, *Pteromys momonga* and the Russian flying squirrel, *P. volans* (Rodentia, Sciuridae). Caryologia **53**: 133-140.

POCOCK R. I. 1922. The external characters of *Scarturus* and other jerboas compared with those of *Zapus* and *Pedetes*. Proc. Zool. Soc. London **1922**: 659-682.

SHAHIN A. A. B. 1999. A comparative study of the molar and soft palate characters of the genera *Allactaga* and *Jaculus* (Mammalia: Rodentia) in Egypt. Zoology in the Middle East **18**: 17-32.

SHAHIN A. A. B., ATA A. M. 2001. A comparative study on the karyotype and meiosis of the jerboas *Allactaga* and *Jaculus* (Rodentia: Dipodidae) in Egypt. Zoology in the Middle East **22**: 5-16.

SHAHIN A. A. B., IBRAHEEM M. H. 1998. Sperm morphology of the dipodid rodents (Jerboas) common in Egypt. Belgian J. Zool. **128**: 189- 200.

SHAHIN A. A. B. 2003. Genetic differentiation and relationship of the dipodids *Allactaga* and *Jaculus* (Mammalia, Rodentia) in Egypt based on protein variation. Acta Theriologica **48**: 309-324.

SIMPSON G. G. 1945. The principles of classification and a classification of mammals. Bull. Amer. Mus. Nat. Hist. **85**: 1-350.

SPASIC-BOSKOVIC O., TANIC N., BLAGOJEVIC J., VUJOSEVIC M. 1997. Comparative cytogenetic analysis of European brown frogs: *Rana temporaria*, *R. dalmatina* and *R. graeca*. Caryologia **2**: 139-149.

SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

VINOGRADOV B. 1930. On the classification of the Dipodidae (cranial and dental characters). Bull. Acad. Sci. USSR **1930**: 331-350.

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A. 1995. Cytogenetic studies of the genus *Tettigonia* (Orthoptera, Tettigonioidea, Tettigoniinae). II. Heteromorphism of C-bands. Folia biol. (Kraków) **43**: 99-106.

WASSIF K. 1960. Studies on the osteology of Egyptian jerboas. Bull. Zool. Soc. Egypt **15**: 71-92.

WHITE M. J. D. 1973. Animal Cytology and Evolution. 3 ed. Cambridge University Press.

YOSIDA T. H. 1973. Evolution of karyotypes and differentiation in 13 *Rattus* species. Chromosoma **40**: 285-297.