CARYOLOGIA Vol. 39, n. 2: 131-142, 1986

KARYOLOGICAL STUDIES OF SOUTH AMERICAN RODENTS (RODENTIA:CRICETIDAE). I. COMPARATIVE CHROMOSOMIC ANALYSIS IN *SCAPTEROMYS* TAXA

N. BRUM-ZORRILLA, G. OLIVER, T. GENTILE DE FRONZA * and R. WAINBERG *

División Citogenética, Instituto de Investigaciones Biológicas Clemente Estable, Av. Italia 3318, Montevideo, Uruguay; and * Cátedra de Biología General, Facultad de Ciencias Exactas, Universidad de la Plata, La Plata, Argentina.

SUMMARY — South American rodents of the genus *Scapteromys*, are know to exist in a number of different chromosomal forms. *S. tumidus* has $2n = 24$ from Uruguay while three different Brazilian taxa, all referred to as *S. tumidus* have respectively $2n = 36,34$ and 24. *S. aquaticus* from Argentina has $2n = 32$. By G- band comparisons we find that the $2n = 24$ *S. tumidus* from Uruguay and Brazil are chromosomically more closely related to *S. aquaticus* from Argentina than to alleged *S. tumidus* with $2n = 34$-36 from Brazil. These results suggest that the latter may represent a new, still undescribed, species.

INTRODUCTION

Scapteromys tumidus is a common rodent of the plains in Uruguay and South of Brazil, whereas *S. aquaticus* is found in the Centre-East of Argentina, and in the Delta region the «marginal forest» of the Rio de La Plata. The genus *Scapteromys*, together with *Kunsia* and *Bibiamys*, belongs to the tribe Scapteromyini (REIG 1981, 1984), the relationships of which to other Sudamerican Sigmodontinae is currently a matter of debate.

Previous cytogenetic studies on *S. tumidus* from Uruguayan populations (BRUM 1965 and BRUM-ZORRILLA *et al.* 1972) have demonstrated that this species has a $2n = 24$: NF = 40. By contrast *S. aquaticus* from Argentina studied by FRONZA (1971-1974) and FRONZA *et al.* (1976), indicated a karyotype of $2n = 32$; NF = 40. More recently (FREITAS *et al.* 1984) reported three different diploid numbers ($2n = 36,34$ and 24) in specimens of *S. tumidus* collected at different localities in Southern Brazil. These result suggest that species distinctions in *Scapteromys* may need to be redefined in terms of chromosome data. Stimulated by these results we have compared chromosome morphology between the species of *Scapteromys* karyotyped to date using G-

banding. We have also carried out a comparative analysis of the distribution and amount of heterochromatin and the localisation of nucleolus organiser regions (NORs) between populations of *S. tumidus* and Argentinian populations of *S. aquaticus*. This paper reports the results of these comparisons.

MATERIAL AND METHODS

Ten wild caught specimens (6 ♀ and 4 ♂) of *S. tumidus* (Waterhouse 1837) and (6 ♀ and 6 ♂) of *S. aquaticus* (Thomas 1920), were karyotyped. The material came from four different localities in Uruguay: Arroyo del Cordobés, Isla Paredón, Barra de San Juan and Martín Chico. Specimens of *S. aquaticus* were obtained from Boca Cerrada, Ribera de Punta Lara in Argentina.

Chromosome preparations were made by bone marrow technique. Metaphase configurations were photographed and karyotype constructed from enlarged prints of 20 cells of *S. tumidus* and 20 cells of *S. aquaticus* using the nomenclature of LEVAN *et al.* (1964).

Skin and skull voucher specimens of the Uruguayan individuals have been deposited in the collection of Mammals at the Department of Zoology, Facultad de Humanidades y Ciencias, Universidad Mayor de la República Oriental del Uruguay, Montevideo. The specimens from Argentina were deposited in the Mammal Collection of the La Plata Museum.

To obtain well differentiated C-bands in *S. tumidus*, the technique described by ARRIGHI and HSU (1971) was used with modifications. In the case of *S. aquaticus*, SUMMER's (1972) procedure was employed. In both species CHIARELLI *et al.*'s (1972) technique was used to induce G- bands and that of HOWELL (1977) to identify nucleolar organizer regions (NOR's).

RESULTS

1. *Karyotypes.*

a) *Scapteromys aquaticus.* — All the specimens analyzed showed a diploid number of $2n = 32$; NF = 40. The karyotype (Fig. 1a) includes one submetacentric (sm), (pair 1) and four pairs of metacentric (m), (pairs 2, 3, 6 and 7) autosomes. The remaining autosomes are acrocentric (t). The X is a telocentric (T) and the Y is a small submetacentric.

b) *Scapteromys tumidus.* — All the specimens analyzed showed a diploid number of $2n = 24$; NF = 40. The karyotype is made of three pairs of submetacentric (sm) (pairs 1, 4 and 5); three pairs of metacentric (M), (pairs 6, 7 and 9); three pairs of metacentric (M), (pairs 2, 3 and 8) an two pairs of acrocentric (t), (pair 10 and 11) autosomes. Both the X and the Y sex chromosomes are telocentric (T) (Fig. 1b).

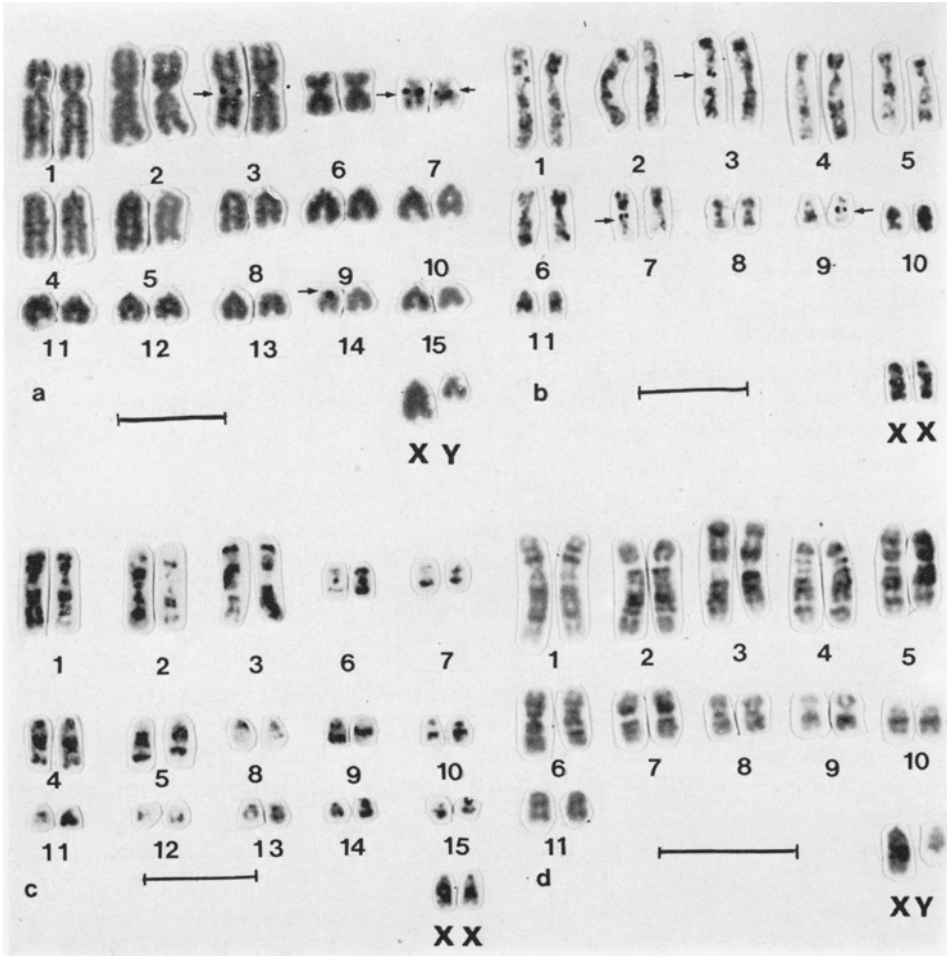

Fig. 1a. — Karyotype of *Scapteromys aquaticus*. Localization of the NORs in the pairs 3, 7 and 14 (arrows).
Fig. 1b. — Karyotype of *S. tumidus*. Location of the NORs in the pairs 3, 7 and 9 (arrows).
Fig. 1c. — G-banding karyotype of *S. aquaticus* and 1d. G-banding pattern of *S. tumidus*.
The bar represent 10 μm.

2. *Nucleolar organizer regions (NORs).*

In *S. aquaticus*, silver staining demonstrated two black silver spots close to the centromere in only one homologue of pair 3. Additionally pair 7 showed similar black spots in both homologues, while in pair 14 the silver precipitate is again located at the centromere region of only one homologue (Fig. 1a). In *S. tumidus* positive staining NORs were present on one homologue of each of chromosomes 3, 7 and 9.

3. *G-banding: comparisons.*

G-banded karyotypes of *S. aquaticus* and *S. tumidus* are shown in Figs. 1c and 1d respectively. A comparison of the G-banding pattern between the two taxa is shown in Fig. 2. Using the terminology of SPOTORNO (1977) and WALKER *et al.* (1979) several degrees of correspondence is evident between chromosome of both species. Pairs 1, 2 and 3 of both share either total and partial correspondence. Three biarmed autosomes belonging to *S. tumidus* (pairs 4, 5 and 6) agree in banding pattern with six of the uniarmed chromosome of *S. aquaticus* (pairs 4, 5, 8, 13, 14 and 15). The submetacentric chromosome 4 of *S. tumidus* has a similar banding sequence to the combined acrocentric chromosomes 4 and 14 of *S. aquaticus*. Chromosome 5 of *S. tumidus* corresponds with chromosome 5 and 15 of *S. aquaticus*. Chromosome 6 of *S. tumidus* shares G-banding characteristics with chromosomes 8 and 13 of *S. aquaticus*.

Chromosome 8 of *S. tumidus* has a similar G-banding pattern to chromosome 6 of *S. aquaticus*. The remaing pairs 7, 9, 10 and 11 of *S. tumidus* are not readly comparable with chromosomes 7, 9, 10, 11 and 12 of *S. aquaticus* since no correspondence is apparent in their bands. The X chromosome of both species show similar G-banding.

In the idiogram presented in the Fig. 3 and in the data of Table 1, we summarise the results of a comparative analysis of G-banding pattern available for all the material of *Scapteromys* studied to date. In constructing Fig. 3 we have incorporated the nomenclature proposed by FREITAS *et al.* (1984) for the identification of individual chromosomes. An analysis of Fig. 3 and Table 1 shows that acrocentric chromosome 1 of ($2n$ = 36-34) *S. tumidus* corresponds with the long arm (A) of the submetacentric chromosome 1 of *S. aquaticus* and ($2n$ = 34) *S. tumidus*.

Chromosome 2, is shared only by the Brazilian *Scapteromys*. The long arm (F) of chromosome 3 of ($2n$ = 36-34) *S. tumidus* matches the long arm (F) of chromosome 2 of both *S. aquaticus* and ($2n$ = 24) *S. tumidus*.

Chromosomes 4 and 5 of ($2n$ = 36 and 34) *S. tumidus*, have a similar banding pattern. There is also homology between chromosome 5 of ($2n$ = 36) *S. tumidus*, chromosome 6 of ($2n$ = 34) *S. tumidus* and the short arm (D) of chromosome 2 of *S. aquaticus* and ($2n$ = 24) *S. tumidus*.

Chromosomes 6 and 7 of ($2n$ = 36 and 34) *S. tumidus* share common G-band with the long arm (E) of chromosome 3 of *S. aquaticus and* ($2n$ = 24) *S. tumidus*.

Similarity is also evident between chromosome 7 *(G) of* ($2n$ = 36) *S. tumidus*, the long arm (G) of chromosome 4 of ($2n$ = 34) *S. tumidus* and the short arm (G) of chromosome 3 of *S. aquaticus* and ($2n$ = 24) *S. tumidus*.

In chromosome 8 banding homology exist only in the brazilian forms. Chromosome 9 of ($2n$ = 36-34) *S. tumidus*, chromosome 6 of *S. aquaticus* and

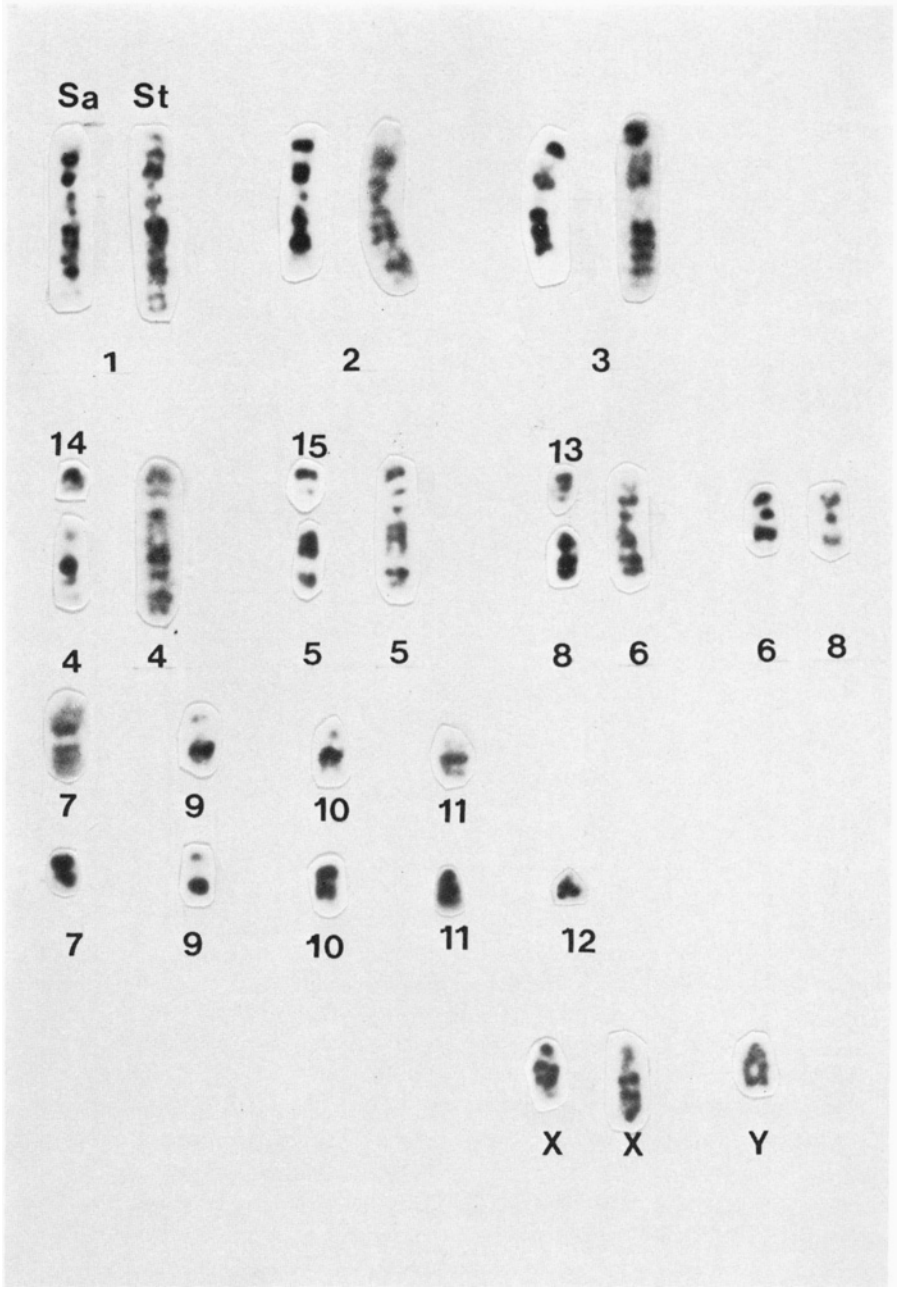

Fig. 2. — Chromosome homologies between *Scapteromys aquaticus* (s.a.) and *S. tumidus* (s.t.).

TABLE 1 - *G-band correspondence of chromosome pairs and chromosome arms in the different cytological forms of «Scapteromys».*

Diploid number (2*n*)	Chromosome pairs														
36	1	2	$3_p/3_q$	4	5	6	7	8	$9_p/9_q$*	$10_p/10_q$	11	12	13	14	15
34	1	2	$3_p/3_q$	5	6	7	$-/4_q$	8	$9_p/9_q$*	$10_p/10_q$	$4_p/-$	11	12	13	14
32	1_p	–	$-/2_q$	–	$-/2_q$	3_q	$-/3_p$	–	$6_p/6_q$*	–	$1_p/-$				
24	1_p	–	$-/2_q$	$-/2_p$	3_q	$-/3_p$	–	–	$8_p/8_q$*	$9_p/9_q$	$1_p/-$				

	Chromosome lacking homology						Chromosomes involved in the rearrangements		
36									
34									
32	7	9	10	11	12	XY	4-14	5-15	8-13
23	7	9	11			XY	4	5	6

p = short arm; q = long arm; asteriks indicate marker chromosomes.

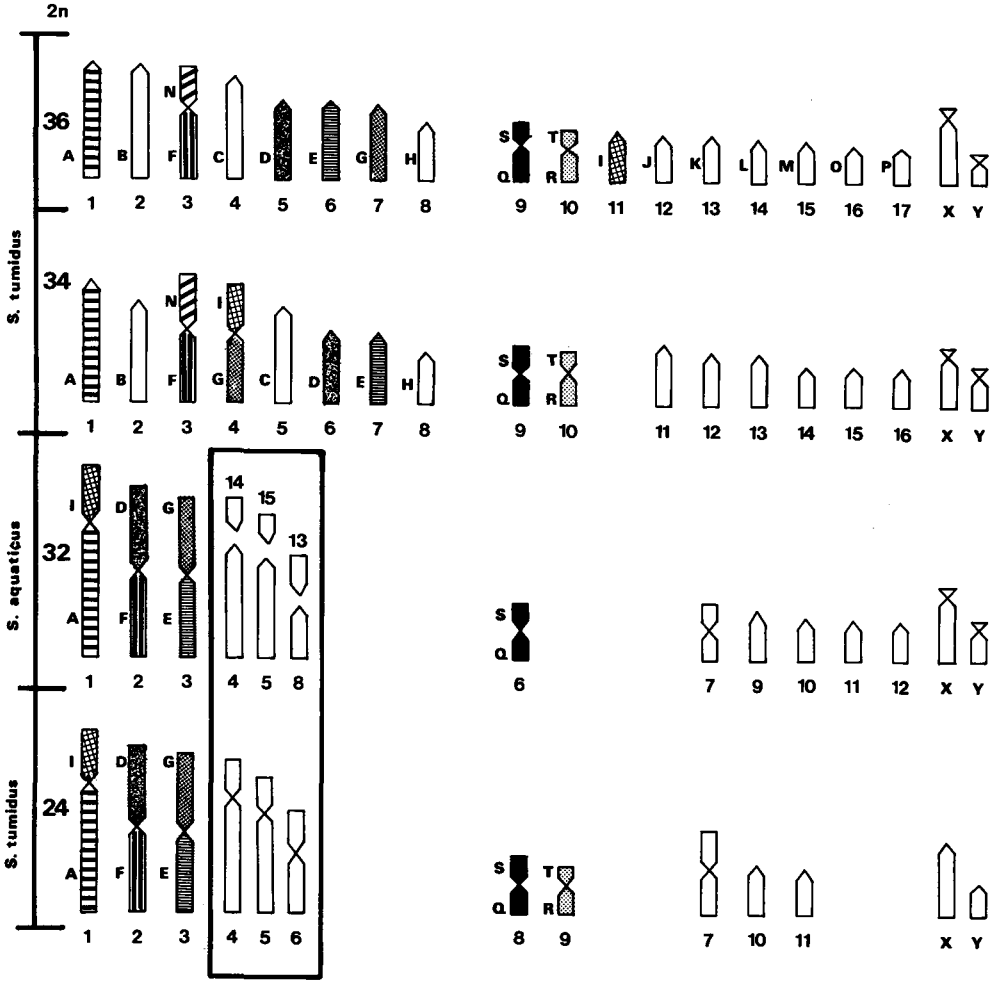

Fig. 3. — An idiogram incorporating a comparative analysis of the chromosomes and chromosome arms shared by the different cytological forms of *Scapteromys*.

chromosome 8 of (2*n* = 24) *S. tumidus* have similar morphology and banding pattern, and we consider them to be «marker» chromosomes for the genus *Scapteromys*. Chromosome 10 of (2*n* = 36-34) *S. aquaticus* appeares only in the (2*n* = 24) form of *S. tumidus*.

Chromosome 11 (I) of (2*n* = 36) *S. tumidus* corresponds with the short arm (I) of chromosome 4 of (2*n* = 34) and the short arm (I) of chromosome 1 of *S. aquaticus* and (2*n* = 24) *S. tumidus*.

The remaining pairs of (2*n* = 36-34) *S. tumidus* from Brazil show complete correspondence but we have not able to recognize any homology in the

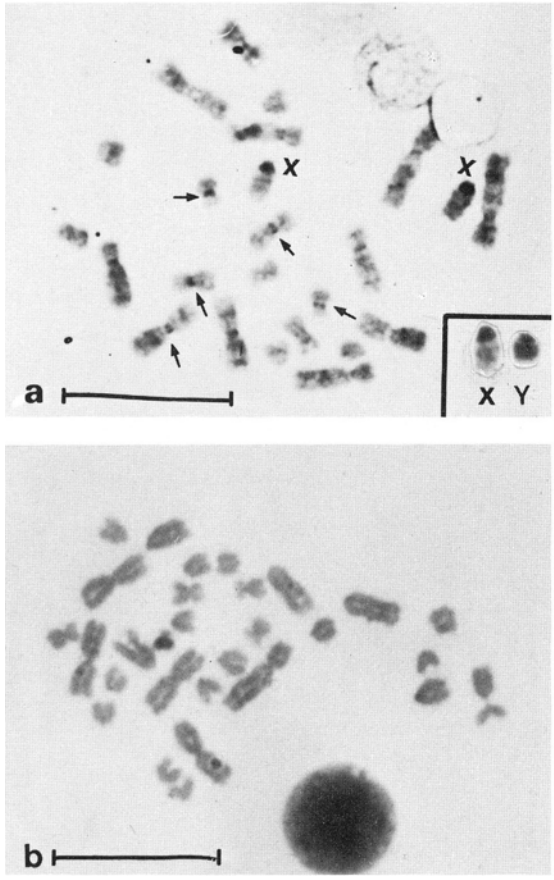

Fig. 4a. — C-banded metaphase of *Scapteromys tumidus*. Arrows indicate small heterochromatic blocks.
Fig. 4b. — C-banded metaphase of *S. aquaticus* male. Only the Y chromosome (arrow) is positively heterochromatic.

The bar represent 10 μm.

other chromosomes of *S. aquaticus* and ($2n = 24$) *S. tumidus*. The chromosomes of ($2n = 24$) *S. tumidus* and *S. aquaticus* with similar banding pattern which have been involved in evident rearrangementes are outlined in Fig. 3.

4. *C-banding.*

In *S. tumidus* C-banding indicates that small heterochromatic blocks are present around the centromere regions of several chromosome whereas others lack, or at least have only a trace, of heterochromatin (Fig. 4a). The X's, chromosome, however, has large heterochromatic blocks which contrasts with

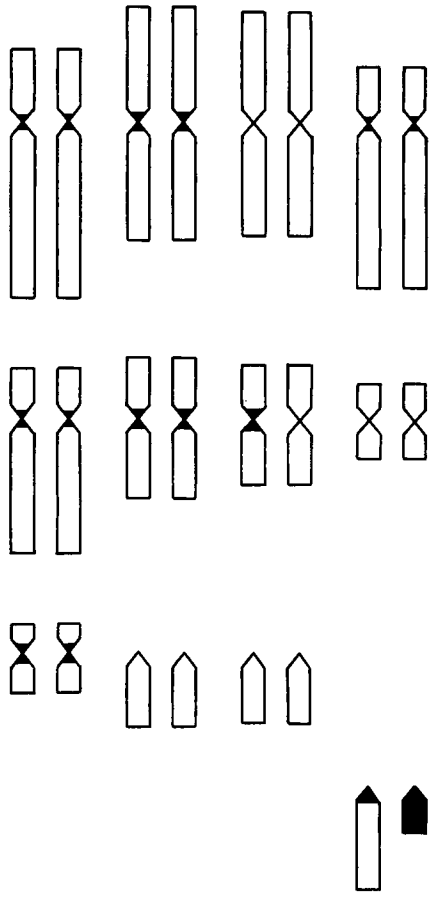

Fig. 5. — Idiogramatic representation of the distribution of C-band material in *S. tumidus*.

the small amount of the heterochromatin observed in the autosomes. While the Y appeared fully heterochromatic.

S. aquaticus, by contrast showed C-band material only on both sex chromosomes XY. The long arm of the Y chromosome appeared fully heterochromatic, while the X chrosome has small heterochromatic blocks in the centromeric regions (Fig. 4b).

DISCUSSION

Karyotypic analysis of *S. tumidus* from Uruguay showed that all specimens examined in this paper and in a previous report (BRUM 1965; BRUM-ZORRILLA *et al.* 1972) have a similar diploid number ($2n = 24$) and chromosome morpho-

logy. The only observed difference was in the Y chromosome. While in former papers the Y is reported to be a submetacentric, in the specimens examined in the present paper we find it to be telocentric. Variation in the sex chromosomes of *Scapteromys* species has also been seen by FRONZA *et al.* (1976) in two different populations of *S. aquaticus* from the Delta del Paraná (Argentina).

More recently FREITAS *et al.* (1984) found that the Y chromosome in three cytotypes of *S. tumidus*, with 34, 36 and 24 chromosomes, could be either subtelocentric or submetacentric. It is evident that in *Scapteromys*, as in other cricetids (i.e. *Akodon*, BIANCHI 1971, 1973; *Holochilus*, VIDAL *et al.* 1976 and RIVAS *et al.* 1977), polymorphism among sex chromosomes is a common occurrence.

Comparative studies carried out by several authors (TRANTRAVAHI *et al.* 1976; OLERT and SCHMID 1978; MANDAHL 1979 and YOSIDA 1979), have shown that the number and distribution of the NORs can be used to differentiate populations and species. *Scapteromys* shows three of four NORs. Thus, *S. tumidus* from Brazil has fours NORs located in pairs 3, 6, 7 and 8 (FREITAS *et al.* 1984), while *S. tumidus* from Uruguay shows only three NORs, in pairs 3, 7 and 9, and always in a heterozygous condition. *S. aquaticus*, on the other hand, has NORs in pairs 3, 7 and 14. From an analysis of the NOR distribution we believe that these taxa share identical NORs on chromosomes 3 and 7 but that the others pairs are variable.

G-banding clearly demostrates that according the terminology proposed by SPOTORNO (1977), WALKER *et al.* (1979), all forms of *S. tumidus* ($2n = 36$, 34, 24) and *S. aquaticus*, have homology among several chromosomes or chromosome arms.

MASSOIA and FORNES (1964) have demonstrated that *S. aquaticus* and *S. tumidus* from Uruguay are clearly different species. Cytogenetic studies on *Scapteromys* from Argentina and Uruguay (BRUM 1965; BRUM-ZORRILLA *et al.* 1972; FRONZA 1971, 1972; FRONZA *et al.* 1974) also identified these rodents as distinct, plain, species. The present results indicate a very definitie phylogenetic relation between these several taxa but we believe that *S. tumidus* from Brazil may represent a distinct species.

The C-banding pattern is different in all the forms of *Scapteromys* studied. FREITAS *et al.* (1984) found C-bands only on autosome pair n. 10 and on the X chromosome in the $2n = 36$ and $2n = 34$ Brazilian forms, whereas ($2n = 24$) *S. tumidus* lacked C-bands on all autosomes. In the material of *S. tumidus* which we examined small positive C-bands were presented on a mayority of the autosomes, while in *S. aquaticus* only heterochromatic blocks on the sex chromosome, were observed. Such difference suggest the existence of intra specific geographical variation in C-banding pattern.

Preliminary DNA analysis of our material had showed the presence of a small amount of satellite DNA, MUSTO *et al.* (1981), which agree with the existence of small heterochromatic blocks.

Acknowledgements. — The authors wish to thank Dr. O. REIG for their valuable suggestions and critical reading of the manuscript. We thank also Drs. Norberto ZWINER, Andrés ZAMBELLI and Anibal RAMOS from Facultad Ciencias Exactas of La Plata, Argentina for aid in the field collection of *S. aquaticus* specimens and Dr. A. LANGGUTH and S. VALLEJO from Dto. Zoology Facultad Humanidades y Ciencias - Montevideo for the systematic classifications of *S. tumidus* specimens. We are also gratefull to Br. A.M. CAZENAVE, G. SELUJA and C. DEGIOVANANGELO for technical assistance in the laboratory and in the preparation of the photographic reproduction.

REFERENCES

ARRIGHI F.E. and HSU T.C., 1971. — *Localization of heterochromatin in human chromosomes.* Cytogenetics, 10: 81-86.

BIANCHI N.O., REIG O.A., MOLINA O.J. and DULOUT F.N., 1971. — *Cytogenetics of South American Akodonts rodents (Cricetidae). I. A progress report of Argentinian and Venezuelan forms.* Evolution, 25: 724-736.

BRUM N., 1965. — *Investigaciones citogenéticas sobre algunas especies de Cricetidae (Rodentia) del Uruguay.* Annais II Congr. Lat. Amer. Zool., 2: 315-320.

BRUM-ZORRILLA N., LAFUENTE N. and KIBLISKY P., 1972. — *Cytogenetics studies in the cricetid rodent Scapteromys tumidus (Rodentia: Cricetidae).* Experientia, 28: 1373.

CHIARELLI B.A., SARTI CHIARELLI N. and SHAFER D., 1972. — *Chromosome banding with trypsin.* Genetica, 43: 190-194.

FREITAS T.R.O., MATTEVI M.S. and OLIVEIRA L.F.B., 1984. — *Unusual G-band patterns in three karyotypically rearranged forms of Scapteromys (Rodentia: Cricetidae) from Brazil.* Cytogenetic Cell. Gen., 38: 39-44.

FRONZA DE G.T.M., 1974. — *Variación del cromosoma X en Scapteromys (Rodentia: Cricetidae) de Punta Lara (Prov. B. Aires).* Tesis.

FRONZA DE G.T.M., WAINBERG R.L. and LLORENTE B.E., 1976. — *Polimorfismo del cromosoma X y significacion filogenética del cariotipo de la «Rata acuática» Scapteromys aquaticus (Rodentia: Cridetidae) de la ribera de Punta Lara (Argentina).* Mendeliana, 1: 41-48.

GALLIMORE P.H. and RICHARDSON C.R., 1973. — *An improved banding technique exemplified in the karyotype analysis of two strains of rat.* Chromosoma, 41: 259-263.

HOWELL W.M., 1977. — *Visualization of ribosomal gene activity: silver stains proteins associated with rRNA transcribed from oocyte chromosome.* Chromosoma, 62: 361-367.

LEVAN A., FREDGA K. and SANBERG A.A., 1964. — *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 1-11.

MASSOIA E. and FORNES A., 1964. — *Notas sobre el género Scapteromys (Rodentia: Cricetidae).* Physis, 24: 279-297.

MUSTO H., HEGUY A. and WETTSTEIN R., 1981. — *Análisis del ADN de cinco especies de la familia Cricetidae.* Arch. Biol. Med. Exp. Soc. Biol. Chile, V Congr. Lat. Amer. Gen., 14: 73.

OLERT J. and SCHMID M., 1978. — *Comparative analysis of karyotypes in European shrew species.* Cytogen. Cell. Gen., 20: 308-322.

TANTRAVAHI R., MILLER D.A., DEV V.G. and MILLER O.J., 1976. — *Detection of nucleolus organizer regions in chromosome of human, chimpanzee, gorilla, orangután and gibbon.* Chromosoma, 56: 15-27.

REIG O.A., 1981. — *Teoría del origen y desarrollo de la fauna de mamíferos de América del Sur.* Monogr. Naturae Mus. Munic. Cienc. Nat. «Lorenzo Scaglia», 1: 1-161.

—, 1984. — *Distribución geográfica e historia evolutiva dos roedores muroideos sulamericanos (Cricetidae: Sigmodontinae). (Geographic distribution and evolutionary history of South american muroids, Cricetidae: Sigmodontinae).* Rev. Brasil Genet., 7: 333-365.

RIVAS R., VIDAL O.R. and BARO N.I., 1977. — *Los cromosomas del género Holochilus. II. El cariotipo de H. brasiliensis vulpinus.* Physis, 36:215-218.

SPOTORNO A.E., 1977. — *Phylogenetics partitioning of banded karyotypes in mammals. A model of cladistic analysis.* III Latin Amer. Congr. Genet., Montevideo, Uruguay (Ed. Drets M.E., Brum-Zorrilla N. and Folle G.), pág. 179-187.

SUMMER A.T., 1972. — *A simple technique for demostrating centromeric heterochromatin.* Exp. Cell Res., 75: 304-306.

VIDAL O.R., RIVA R. and BARO N.I., 1976. — *Los cromosomas del género Holochilus. I. Polimorfismo de H. chacarius Thomas (1906).* Physis, 35: 75-85.

WALKER L.I., SPOTORNO A.E. and FERNANDEZ-DONOSO R., 1979. — *Conservatism of whole arms during chromosomal divergence of phyllotine rodents.* Cytog. Cell Genet., 24: 209-216.

YOSIDA T.H., 1979. — *A comparative study on nucleolus organizer regions (NORs) in 7 Rattus species with special emphasis on the organizer differentiation and species evolution.* Proc. Jap. Acad. Serv. B, 10: 481-486.

Received 27 February 1985; revision accepted 7 August 1985