Karyology and morphology of *Zygodontomys* (Rodentia, Sigmodontinae) from the Brazilian Amazon, with a molecular appraisal of phylogenetic relationships of this genus

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SUMMARY

We analysed karyologic data and qualitative morphologic characters of a sample assigned to the genus *Zygodontomys* from the middle course of the Rio Negro in the Brazilian Amazon and the karyotype of a specimen from Misión Tukuko, Venezuela. The samples from the Rio Negro showed 2n = 82, FN = 94 while the Venezuelàn specimen showed 2n = 82, FN = 116. The sample from the Rio Negro could not be allocated to any currently recognised species of *Zygodontomys* on the basis of karyologic and qualitative morphologic characters. A comparison of this new karyotype with others assigned to *Zygodontomys brevicauda* suggests that samples currently allocated to this taxon based on morphologic grounds are representative of a species group. Cytochrome *b* DNA sequence analyses of the Rio Negro sample support previous suggestions that *Zygodontomys* is more closely related to taxa belonging to the tribe Oryzomyini than to genera belonging to the tribes Akodontini and Phyllotini.

KEY WORDS
Zygodontomys, morphology, karyotype, molecular phylogeny, Brazilian Amazon.

RESUMÉ

Nous présentons les données caryologiques et morphologiques de plusieurs spécimens du genre *Zygodontomys*, provenant du cours moyen du Rio Negro, Amazonas, Brésil. Leur caryotype est comparé avec celui des spécimens reconnus comme appartenant à l'espèce *Zygodontomys brevicauda*, ainsi que le caryotype d'un spécimen de Misión Tukuko, Vénézuéla, qui est aussi décrit. Les spécimens du Rio Negro présentent 2n = 82, FN = 94, tandis que le spécimen du Vénézuéla montre 2n = 82 et un FN = 116. Les données caryologiques et morphologiques ne permettent pas d'assigner les spécimens du cours moyen du Rio Negro aux espèces de *Zygodontomys* décrites jusqu'à présent, soulignant le caractère polytypique de *Zygodontomys brevicauda*. Les séquences du gène cytochrome *b* (DNA) suggèrent que *Zygodontomys* est plus apparenté aux taxons de la tribu Oryzomyini qu'aux genres des tribus Akodontini et Phyllotini.

INTRODUCTION

The taxonomy and phylogenetic affinities of the genus *Zygodontomys* have been inconsistent. Over the last century, this genus was historically allocated to at least three different suprageneric groups, namely (1) the " Akodont " group of Thomas (1916), (2) the " South and Central American oryzomine genera " of Tate (1932), (3) the " phyllotine " group of Hershkovitz (1962), and also as *sigmodontinae incertae sedis* (Reig 1987). More recently, the genus has been considered a member of the tribe Oryzomyini *sensu stricto* (Voss and Carleton 1993 ; Steppan 1995). Although part of this controversy was clarified by the removal of species belonging to the genus *Bolomys* based on karyologic and morphologic attributes (Gardner and Patton 1976 ; Reig 1987, Voss and Linsey 1981 ; Maia and Langguth 1981 ; Voss 1991), several points still remain to be clarified regarding species identification and limits within *Zygodontomys* and the phylogenetic relationships of this genus. Although molecular studies have been recently carried out for determining phylogenetic relationships of other sigmodontine genera (Smith and Patton 1993, 1999 ; Patton *et al.* 2000), the genus *Zygodontomys* has not been included in any analyses reported so far.

The latest comprehensive review of the genus *Zygodontomys* considered only two species : *Z. brevicauda* and *Z. brunneus*, both inhabitants of conserved or altered open vegetation formations in Central America and northern South America (Voss 1991). Despite being locally abundant, a comprehensive analysis of the morphologic variation of this genus has been only recently reported (Voss 1991) while karyologic data are still scarce. So far, only the chromosome complement of *Z. brevicauda* has been reported while the karyotype of *Z. brunneus* remains undescribed. The chromosome complement of *Z. brevicauda brevicauda* was found to be variable, with a diploid number of 84 or 88 and a fundamental number ranging from 116 to 118 (Gardner and Patton 1976 ; Perez-Zapata *et al.* 1984, Reig *et al.* 1990). The chromosome complement associated to specimens of *Z. brevicauda cherriei* was also found to be variable, with a diploid number ranging from 82 to 84 (Gardner and Patton 1976 ; Voss 1991). The karyotype of *Z. brevicauda microtinus* has not been described, although Tranier (1976) reported a diploid number of 78 in specimens from French Guiana.

We herein describe two karyotypes of *Zygodontomys* specimens from the middle course of the Rio Negro in the Brazilian Amazon and northern Venezuela. In order to identify these samples we analysed them based on descriptions and diagnoses reported by Voss (1991) and compared our karyologic descriptions with previous data in the literature. Phylogenetic relationships of *Zygodontomys* were inferred by comparisons of cytochrome *b* mt DNA sequences of samples from the middle course of Rio Negro (Brazil) and French Guiana with representative taxa of distinct sigmodontine lineages.

MATERIAL AND METHODS

We collected seven *Zygodontomys* specimens from two localities along tributaries of the Rio Aracá, a left - bank tributary of the middle Rio Negro, Municipality of Barcelos, Amazonas state, Brazil (Fig. 1) :

1) Igarapé Tucunaré, 00°09′72′′N, 63°30′72′′W (GPS), a right - bank affluent of the Rio Curuduri (females CRB 1514, 1517 and males CRB 1509, 1516, 1519) ;

2) Serra do Aracá, 00°54'05.7"N, 63°26'02.1"W (GPS), near Igarapé da Anta, a right - bank affluent of the Rio Aracá (female CRB 2035 and male CRB 2036).

The vegetation of the first sampled area, locally known as " campinarana ", grows on seasonally flooded white sand and is composed of natural grassland of approximately one meter in height and sparse scrub vegetation up to three metres high. In this locality, 127 traps-nights (Sherman live traps) were placed on the ground. The neighbouring vegetation, flooded and non-flooded rain forest, was sampled with 332 traps-nights. In the second locality, situated at an altitude of 1,300 m and characterised by fields with native grasses and other herbaceous vegetation, 10 traps-nights (Sherman traps) were placed on the ground.

Five of the above mentioned specimens were karyotyped. Chromosome preparations were obtained in the field from bone marrow cultures in RPMI 1640 medium supplemented with 20 % fetal calf serum, ethidium bromide (5 µg/ml) and colchicine 10^{-6} M for two hours. Estimates of fundamental number were restricted to autosome pairs. Skins and skulls were deposited in the mammal collection of the Museu Nacional (MN, Universidade Federal do Rio de Janeiro). We also examined slides, with conventional Giemsa staining, of a male specimen (USNM 448668, original number RVS 1136) from Misión Tukuko, Venezuela, and skins and skulls of two specimens (AMNH 79401, 79402) from Tabocal, upper Rio Negro, Amazonas state, Brazil.

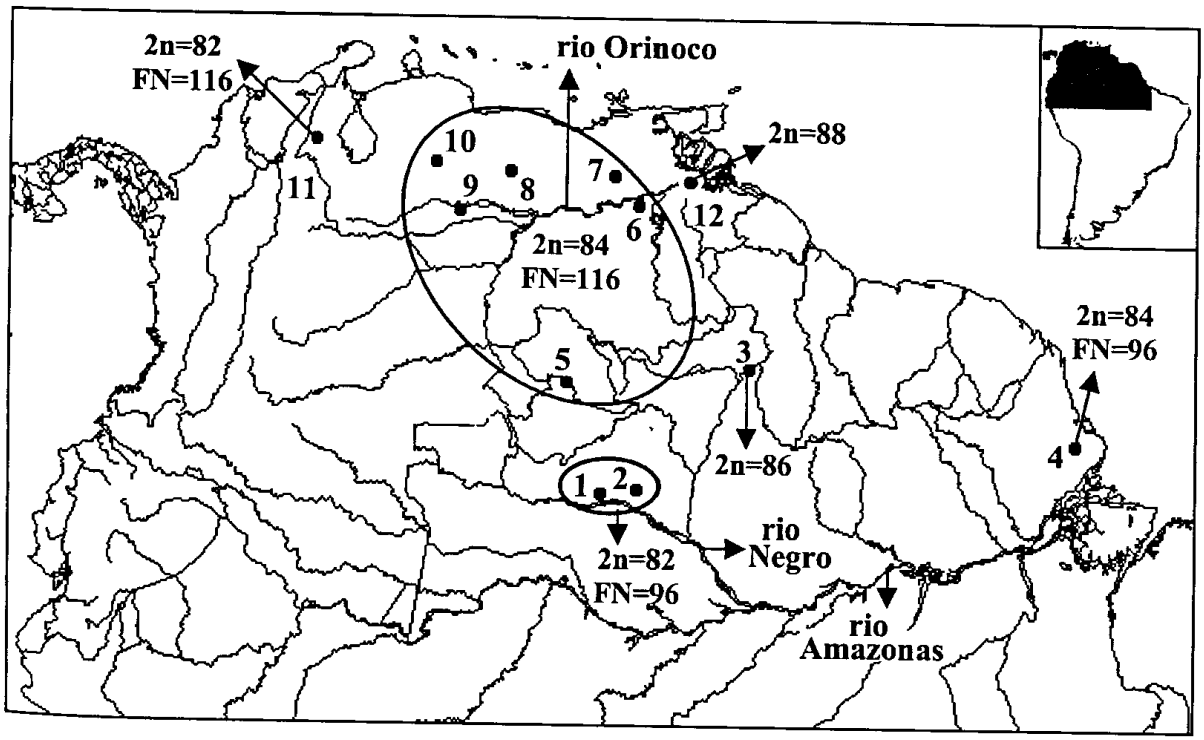

Fig. 1. – Localities of Zygodontomys with known karyotype. 2n = diploid number, FN = fundamental autosome number. Brazil : Igarapé Tucunaré, tributary of Rio Curuduri, tributary of Rio Aracá (1) and Serra do Aracá (2), Amazonas state ; Surumú (3), Roraima state ; Tartarugalzinho (4), Amapá state. Venezuela : La Esmeralda (5), Amazonas Federal Territory ; Ciudad Bolívar (6), Bolívar state ; Río Pao-Hato Sano Antonio (7), Anzoátegui state ; Calabozo (8), Guárico state ; Hato «El Frío» (9), Apure state ; Tierra Buena (10), Portuguesa state ; Misión Tukuko (11) and Isla Guara (12), Monagas state.

DNA samples of two *Zygodontomys* specimens (CRB 2036 and 2035) were isolated from livers preserved in ethanol following the procedures of Sambrook *et al.* (1989). Cytochrome *b* mt DNA (*ca.* 762 bp) was amplified with primers MVZ 05 and MVZ 16 and sequenced with the same primers in an ABI Prism™ 377 automatic DNA sequencer following Smith and Patton (1993). Sequences were edited using the Sequence Navigator software (Applied Biosystems, Inc. 1994) and deposited in GenBank (Table 1). We included sequence data from two other *Zygodontomys*

TABLE 1. – List of sigmodontine specimens used in the phylogenetic analyses. GenBank = GenBank accession number ; Number = Museum or field number ; asterisk (*) indicates specimens sequenced in the present work.

Taxon	GenBank	Number	Locality of collection
Zygodontomys sp.*	AY029478	CRB 2035	Brazil : Amazonas, Serra do Jauari
Zygodontomys sp.*	AY029479	CRB 2036	Brazil : Amazonas, Serra do Jauari
Zygodontomys brevicauda	F. Catzeflis	V-979	French Guiana : Macouria
Zygodontomys brevicauda	F. Catzeflis	V-934	French Guiana : Kourou
Oecomys bicolor	U58382	MNFS1499	Brazil : Acre, Rio Juruá, Nova Vida
Oecomys roberti	U58384	JLP15241	Brazil : Amazonas, Penedo
Oecomys trinitatis	U58390	MNFS 1250	Brazil : Acre, Igarapé Porongaba
Oligoryzomys longicaudatus	U03535	MVZ15582	Argentina : Río Negro, Bariloche
Oligoryzomys microtis	U58381	MNFS1321	Brazil : Acre, Igarapé Porongaba
Oryzomys angouya	AF181281	CRB 1271	Brazil : Rio de Janeiro, Teresópolis
Oryzomys lamia	AF181273	CRB 968	Brazil : Goiás, 65km SSW Cavalcante
Oryzomys macconnelli	U58379	MNFS 156	Brazil : Amazonas, Alto Rio Urucu
Oryzomys megacephalus (1	AF251516	JLP16731	Brazil : Amazonas, Macaco, Rio Jaú
Oryzomys megacephalus (2)	AF251517	CM 76933	Suriname : Saramacca, Tafelberg
Oryzomys megacephalus (3)	AF251518	ROM 97979	Guyana : Rupununi, 30km NE Surama
Oryzomys nitidus	U58383	MNFS 1419	Brazil : Acre, righ bank Rio Juruá
Oryzomys perenensis	U03538	MVZ166676	Peru : Cuzco, Río Urubamba
Oryzomys russatus (1)	AF181272	ORG 67	Brazil : Rio de Janeiro, Guapimirim
Oryzomys russatus (2)	AF251523	ML 48	Brazil : São Paulo, Ilha Bela
Oryzomys subflavus	AF181274	CEG 42	Brazil : Minas Gerais, P.N. do Rio Doce
Oryzomys yunganus	U58380	MNFS 1101	Brazil : Acre, rio Juruá, Ig. Porongaba
Microryzomys minutus	U58387	MVZ173957	Peru : Cuzco, 3km E Amaybamba
Neacomys spinosus	J.Patton	MVZ 155015	Peru : Amazonas, Huampami
Nectomys garleppii	U03539	MVZ 166700	Peru : Cuzco, Kiteni
Nectomys squamipes	AF181283	CRB 540	Brazil : Mato Grosso do Sul, Maracajú
Akodon azarae	U03529)	UNMZ134443	Paraguay : Neembacu, 5.8km NE Pilar
Akodon lindberghi	AF184057	MN48026	Brazil : Minas Gerais
Akodon montensis	J. Patton	UMMZ133958	Paraguay : Dept Caaguazú
Blarinomys breviceps	AF108668	UFMG-MAS17	Brazil : Bahia, Una
Bolomys lasiurus	U03528	UMMZ134431	Paraguay : Dept. Pres. Hayes
Bolomys amoenus	AF159283	MVZ 172878	Peru : Puno, 12km S Santa Rosa
Brucepattersonius igniventris	AF108667	MVZ183250	Brazil : São Paulo, Capão Bonito
Oxymycterus delator	U03525	UMMZ133939	Paraguay : Dept Canendiyú
Calomys sorelus	U03543	MVZ171558	Peru : Puno, Putina
Calomys lepidus	AF159294	MSB57107	Bolivia : 1km E Iscayachi, R. Tomayapo
Eligmodontia puerulus	AF159289	MSB70538	Bolivia : La Paz, S. Andrés de Machaca
Eligmodontia morgani	AF108691	MVZ182670	Argentina : Río Negro, Bariloche
Phyllotis xanthopygus	AF108693	MVZ182703	Argentina : Río Negro
Phyllotis magister	U86824	FMNH107611	Peru : Tacna, Tarata
Rhipidomys leucodactylus	U03550	MVZ –RMW9	Peru : Madre de Dios, Albaque
Rhipidomys macconnelli	AF108681		Venezuela : Amazonas, Cerro Neblina
Rhipidomys mastacalis	AF108684		Brazil : Espírito Santo, 30km N Linhares
Thomasomys gracilis	AF108674	MVZ 166668	Peru : Cuzco, 90 km SE Quillabamba
Thomasomys aureus	U03540	MVZ 166714	Peru : Cuzco, 75km NE Paucartambo
Thomasomys ischyurus	AF108675	MVZ 181999	Peru : Cajamarca, Río Zana
Juliomys pictipes	AF108688	MAM 40	Brazil : São Paulo, Capão Bonito
Juliomys sp.	AF108689	MAM 139	Brazil : São Paulo, Cotia
Neotoma albigula	AF108704	MVZ147667	Mexico : Sonora
Scotinomys teguina	AF108705	UMMZ3373	Costa Rica : Cartago

specimens from French Guiana gently supplied by Dr. François Catzeflis, and GeneBank sequence data from 40 other sigmodontine species, as well as from *Neotoma* and *Scotinomys*, which were used as outgroups on the basis of previous phylogenetic analyses of sigmodontine genera (Smith and Patton 1999). Kimura's two-parameter distance estimates were used for constructing neighbor-joining trees using the software package Molecular Evolution Genetics Analyses (MEGA 2 ; Kumar *et al.* 1993). Maximum parsimony analyses were carried out by full heuristic procedure, with general search options stepwise addition with 10 random replicates (PAUP 4.0b, Swofford 1993). These analyses were carried out considering all positions with thansversions weighing 2 and 10 times higher than transitions, and excluding third position with transversions weighting 3 times higher than transitions. These weights were used in view that transversion : transition ratios estimated by MEGA fell between 1 and 2 when considering all positions or was approximately 3 when excluding third position. Maximum likelihood analyses were performed with a transversions weighing 2 times higher than transitions using DNAML (PHYLIP - version 3.5c ; Felsenstein 1995). In neighbor-joining and parsimony analyses considering all positions, bootstrap estimates were based on 1,000 replicates.

RESULTS

Karyotype description

Karyotypic analyses of the Rio Negro specimens showed 2n = 82, FN = 94. The autosome complement is composed by seven pairs of biarmed chromosomes (2 large and 5 varying in size from medium to small) and 33 pairs of acrocentric chromosomes varying in size from large to small (Fig. 2a). The X chromosome is large and submetacentric and the Y chromosome is small and submetacentric. Karyologic analysis of one specimen from Misión Tukuko showed 2n = 82, FN = 116 (Fig. 2b). The autosome complement is composed of 18 pairs of biarmed chromosomes (2 large pairs and 16 pairs varying in size from medium to small) and 22 acrocentric pairs, varying in size from medium to small. The X chromosome is a large submetacentric and the Y chromosome is a small metacentric.

Morphological characterisation

Skulls of specimens from Rio Negro collected by us exhibited a relatively short and narrow rostrum that is similar in width to the interorbital constriction. The interorbital region is hourglass shaped, with a weak supraorbital ridge that is more evident in old specimens, without postorbital ridges and with weakly developed lambdoidal ridges. The zygomatic plate is relatively broad, with a deep zygomatic notch. The posterior borders of the elongate incisive foramina extend posteriorly beyond the plane of the anteroconules of the first molars. The superior molar rows are parallel, though slightly convergent due to the small size of the third molars. The palatal bridge is broad and long, with large posterolateral fossae and posteropalatal pits, with the posterior limit of the palatal bridge extending beyond the plane of the third molars. The mesopterigoyd fossa lacks sphenopalatines vacuities. The stapedial circulation is incomplete, with a small stapedial foramen and a definite posterior opening of the alisphenoid canal, and lacking the squamosalalisphenoid groove and the sphenofrontal foramen. Examined specimens showed a small subsquamosal fenestra, a very large postglenoid foramen and lacked an alisphenoid strut. The tegmen tympani do not overlap the squamosal anteriorly, and the mastoid is not fenestrated.

The following morphological comparisons were performed on the basis of the descriptions of samples assigned by Voss (1991) to *Z. brevicauda brevicauda*, *Z. b. cherriei* and *Z. b. microtinus*. The sample collected by us differed from other samples assigned to *Z. b. brevicauda* by lacking a fenestrated mesopterygoid fossa and by a different chromosome complement. It differed from *Z. b. cherriei* by (1) an incomplete stapedial arterial circulation contrary to a predominantly complete stapedial circulation of this form ; (2) the absence of sphenopalatine vacuities, and (3) a different chromosome complement, that was also different from the one previously reported for *Z. b. microtinus* (Tranier 1976). The sample herein described differed from specimens from Tabocal, upper Rio Negro Basin (Voss 1991) by (1) the

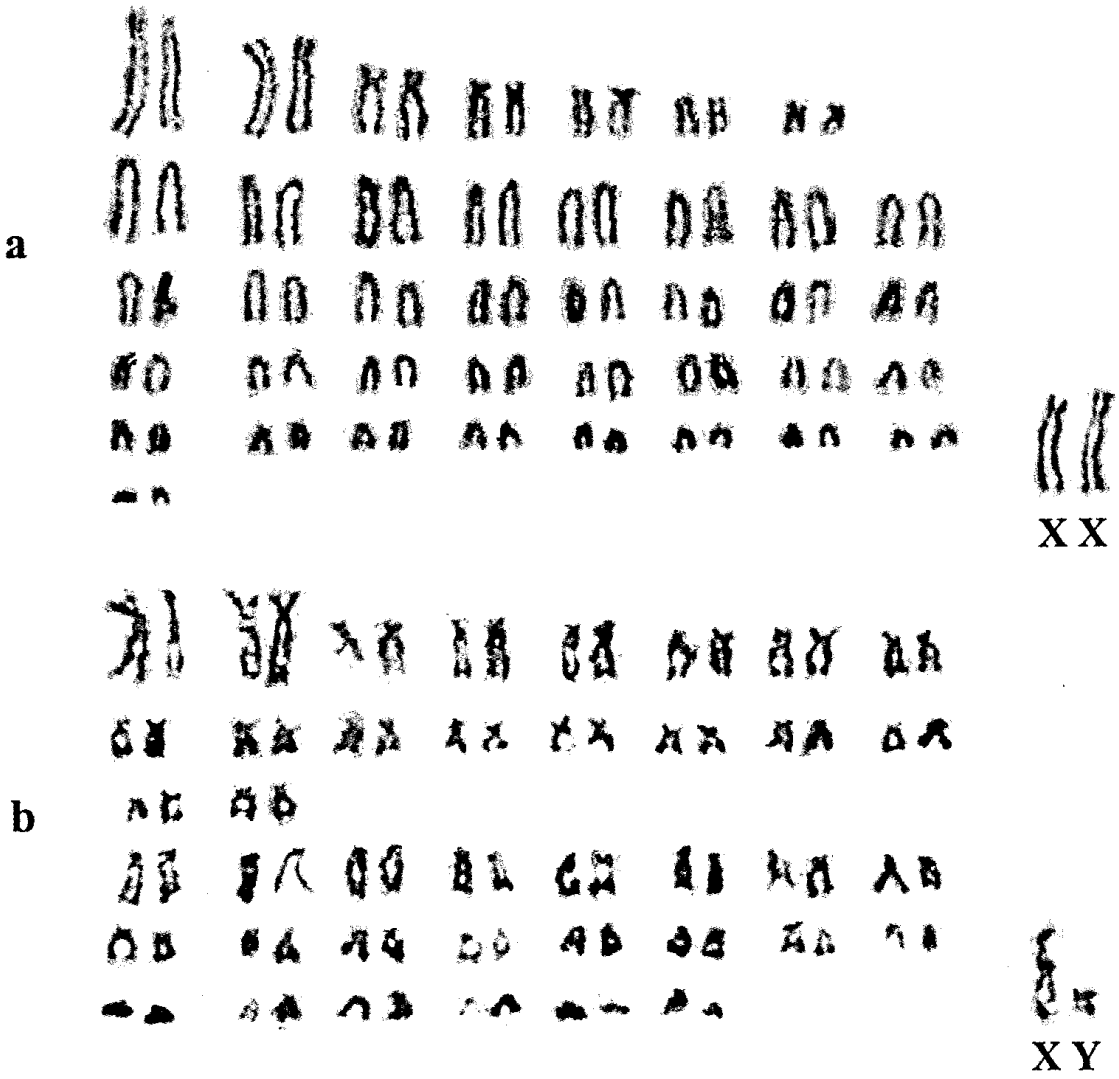

Fig. 2. – Conventionally stained karyotype of (a) *Zygodontomys* sp. (female CRB 1514) from Serra do Aracá, Barcelos, Amazonas state, Brazil and (b) *Zygodontomys brevicauda cherriei* (male USNM 448668) from Misión Tukuko, Venezuela.

absence of sphenopalatine vacuities, sphenofrontal foramina and squamosal alisphenoid grooves, ; (2) a proportionally smaller palatal bridge, and (3) a relatively smaller zygomatic notch.

Molecular analyses

Cytochrome *b* data of the *Zygodontomys* specimens reported in this study were deposited in GenBank (Table 1). Sequence data showed that the two *Zygodontomys* from Brazilian Amazon were identical while inter-individual variation was observed in *Zygodontomys* specimens from French Guiana, probably because they were from different localities.

Comparison of nucleotide data (all positions) showed 407 variable sites, with 341 informative sites for parsimony analysis. Maximum parsimony (MP) analysis with a 10 :1 weighing-scheme replicates resulted in one most-parsimonious tree with a length of 12,490, consistency index (CI) of 0.279, retention index (RI) of 0.514 and re-scaled consistency index (RC) of 0.112. MP analysis with a 2 :1 weighing scheme resulted in three most-parsimonious trees with length = 3,837, CI = 0.234, RI = 0.506 and RC = 0.118. Comparison of nucleotide data

excluding third positions showed 143 variable sites with 91 informative sites for parsimony analyses. Analysis without third positions with 3 :1 transversion : transition ratio resulted in five most-parsimonious trees with length = 671, CI = 0.465, RI = 0.660 and RC = 0.307. In the MP analyses (all positions) with different transversion : transition ratios (10 :1 and 2 :1), the topologies differed in respect to the position of *Juliomys* that collapsed in the former arrangement and appeared as the most basal offshoot in the latter, and in the position of specimens within the *Oecomys* clade which appeared as a trichotomy in the latter.

The MP tree estimated with a 10 :1 transversion : transition ratio was selected for further comments and comparisons with other topologies. Neighbor - joining (NJ), MP and maximum likelihood (ML) trees are shown in figures 3, 4, and 5 respectively. NJ and ML analyses were similar in respect to the position of *Juliomys* in one clade and the remaining sigmodontine species as its sister group. In MP analysis, the genus *Juliomys* collapsed with the other sigmodontine genera in a polytomy. The internal arrangement of the remaining sigmodontine species presented some variation between the three analyses but some clades were conserved in all of them. These comprise : (1) the akodontine species, with bootstrap values of 83 % in NJ and 56 % in MP analyses ; (2) the phyllotine species, with bootstrap values of 71 % in NJ and MP analyses ; and (3) the thomasomyine forms, with bootstrap values of 50 % in NJ and of 79 % in the MP analyses. In the NJ analysis, *Zygodontomys* clustered with a poor bootstrap support within a monophyletic oryzomyine group (*sensu* Voss and Carleton 1993) but the relationships of *Zygodontomys* were unresolved in the MP tree where oryzomyine monophyly was not supported. In the ML tree, *Zygodontomys* clustered with some species of *Oryzomys*. This analysis also showed that oryzomines were paraphyletic.

The MP analysis without third positions showed a similar topology to NJ analysis in showing Oryzomyini as a monophyletic clade. However, in this MP analysis, *Zygodontomys* appeared as the most basal offshoot of this tribe, *Juliomys* grouped with the Oryzomyini and phyllotine taxa were not monophyletic (data not shown). The remaining MP topology was similar to NJ in showing the Akodontini as a sister group of a monophyletic thomasomyine group.

DISCUSSION

Karyologic and morphologic variation

The 2n = 82, FN = 94 karyotype here described is different from any previously reported in *Zygodontomys* (Table 2). Our specimens from Rio Negro were collected near the southernmost known distribution of *Z. brevicauda brevicauda,* which includes the drainage of the upper Rio Branco (Voss 1991). The Venezuelan populations of this subspecies showed variation in diploid number (84 or 88) but they were considered to belong to the same taxon due to lack of morpho-

TABLE 2. – Data on *Zygodontomys* karyotypes. Names are as cited in original references. All taxa have been considered *Zygodontomys brevicauda* by Voss (1991). n = sample size, 2n = diploid number, FN = autosome fundamental number. Bra = Brazil, Vem = Venezuela.

Taxon	n	2n	FN	locality	Reference
Zygodontomys sp.	3	82	94	Bra : Amazonas state, Barcelos	This study
Z. brevicauda cherriei	1	82	116	Ven : Misión Tukuko	This study
Z. microtinus thomasi	33	84	116-118	Ven : Bolívar, Apure, Guárico, Sucre, Carabobo, Portuguesa, Anzoátegui states and Amazonas Fed. Territory	Reig et al. 1990
Z. brevicauda	7	84	94-96	Bra : Amapá, Tartarugalzinho	Barreto et al. 1999
Z. brevicauda		84	?	Costa Rica : Parrita	Gardner and Patton 1976
Z. brevicauda	12	86	96-100	Bra : Roraima state, Surumú	Barreto et al. 1999
Z. microtinus	9	88	116-118	Ven : Isla Guara	Reig et al. 1990
Z. microtinus thomasi		88	?	Ven : Delta Amacuro	Perez-Zapata et al. 1984
Z. microtinus		88	?	Colombia : Villavicencio	Gardner and Patton 1976

logic differences (Reig *et al.* 1990). Nevertheless, their data suggested that more than one taxon might be present in this population because heterozygotes could not be found among the 42 karyotyped specimens (Reig *et al.* op. cit.). Specimens with 2n = 88 were restricted to Isla Guara, in isolation from continental populations with 2n = 84, suggesting that each of these karyotypes was fixed rather than polymorphic. Reig *et al.* (1990) suggested that differences between these two karyotypes could be explained by two alternative hypotheses, either by two Robertsonian fissions affecting pairs 15 and 19 of the 2n = 84 karyotype or by a supernumerary chromosome complex with entirely, C-band positive, small minute autosomes in the 2n = 88 karyotype. These two hypotheses, however, were weakly supported because neither fission heterozygotes nor intermediate numbers of b chromosomes were ever found. As the type locality of *Z. b. brevicauda* is an island (Trinidad) and the only island from which specimens were karyotyped showed a different chromosome complement from the mainland population, further studies are necessary to clarify the pattern of karyotypic variation in this taxon.

The 2n = 82, FN = 94 karyotype herein described also differs from the ones attributed to the two other subspecies of *Z. brevicauda.* The karyotype of *Z. brevicauda microtinus* (Thomas) is unknown and only its diploid number (2n = 78) has been reported for specimens from French Guiana (Tranier 1976). Reig *et al.* (1990), however, reported a diploid number of 84 for specimens collected in the same locality. The other currently recognised subspecies, namely *Z. brevicauda cherriei* (Allen), also showed variation in diploid number, with 2n = 84 in one locality of Costa Rica (Gardner and Patton 1976) and 2n = 82 in another locality in the same country (Voss 1991). These diploid numbers (82 and 84) were also reported in specimens from Venezuela (in Misión Tukuko ; Voss 1991). However, all of these reports were restricted to diploid numbers without karyotypic descriptions or illustrations. Analysis of slides from one of these specimens from Misión Tukuko showed 2n = 82, FN = 116 (Fig. 2b), which is very different from the Brazilian 2n = 82 karyotypes herein reported (Fig. 2a).

In respect to morphological attributes, the Brazilian specimens here analysed resemble more closely *Z. b. microtinus,* despite differences in diploid number. Karyologic and morphologic data showed that the middle Rio Negro population is different from any other and unlikely to belong to any previously described form of *Zygodontomys.* Despite occurring in the same river basin, the middle Rio Negro population differed with respect to morphologic characters (*e.g.*, incomplete stapedial circulation) from that of Tabocal (upper Rio Negro), which exhibits a complete stapedial circulation. Voss (1991) suggested that this latter population might represent a genetically divergent group associated with distinctive habitats of the Rio Negro basin. Similarly, the middle Rio Negro population here studied is karyotypically and morphologically distinct, probably representing a different evolutionary lineage.

The *Zygodontomys* specimens that we collected were trapped only in open vegetation despite most traps having been set in flooded and non-flooded rain forest. Their absence in rain forest, even within the limits of its typical habitat, has been previously noted (Voss 1991). Conversely, Handley (1976) found *Zygodontomys* in forest habitats along the borders of grassy openings. The campinarana vegetation where our specimens were trapped is completely flooded in the rainy season reinforcing the postulation that *Z. brevicauda* is a colonist of temporally unstable habitats (Voss 1991). Similarly to other inhabitants of flooded vegetation of the middle Rio Negro, such as *Proechimys quadruplicatus, Z. brevicauda* seems capable of repeatedly abandoning and re-invading local habitats in response to seasonal environmental changes.

Our findings extend the distribution of *Zygodontomys brevicauda* (*sensu* Voss 1991) southward to the middle Rio Negro. However, karyologic data and morphologic characters here analysed suggest that *Z. brevicauda* (*sensu* Voss 1991) may be a species group with distinct karyotypes and representing different evolutionary lineages.

Molecular analyses

The genus *Zygodontomys* has been historically allocated to different tribes (Akodontini, Oryzomyini, Phyllotini) or listed as *incertae sedis* (Voss 1991). To evaluate its phylogenetic relationships we compared its cytochrome *b* DNA sequence data with those of several genera belonging to

these tribes as well as with selected " thomasomyine ", peromyscine and neotomine genera. NJ, MP and ML analyses clearly exclude *Zygodontomys* from the akodontine, phyllotine and " thomasomyine " assemblages (Figs. 3-5). NJ analysis placed *Zygodontomys* within the oryzo-

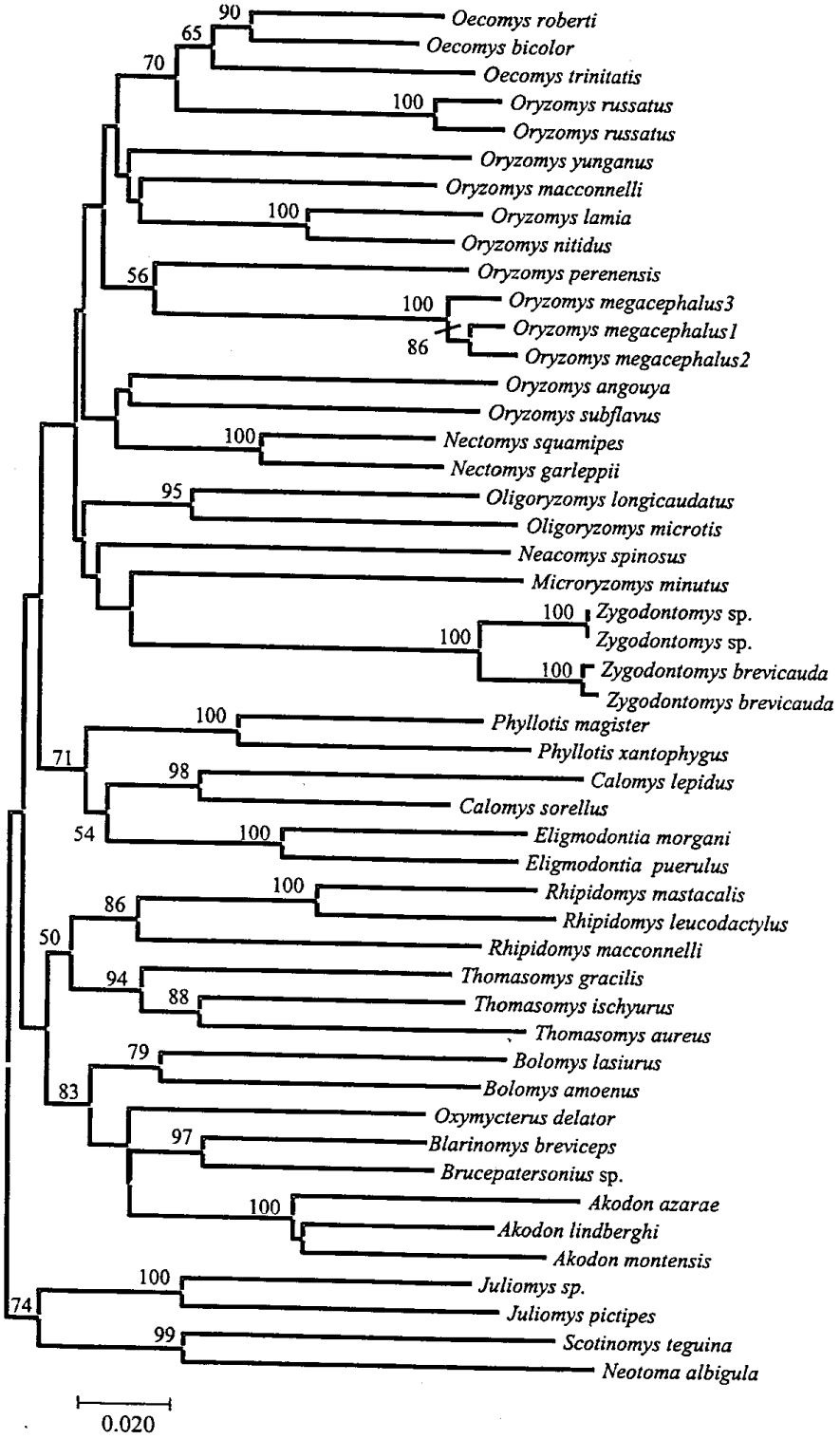

Fig. 3. – Neighbor-joining tree with distances estimated by the Kimura´s two-parameter method. Numbers near nodes are bootstrap values estimated with 1,000 replicates.

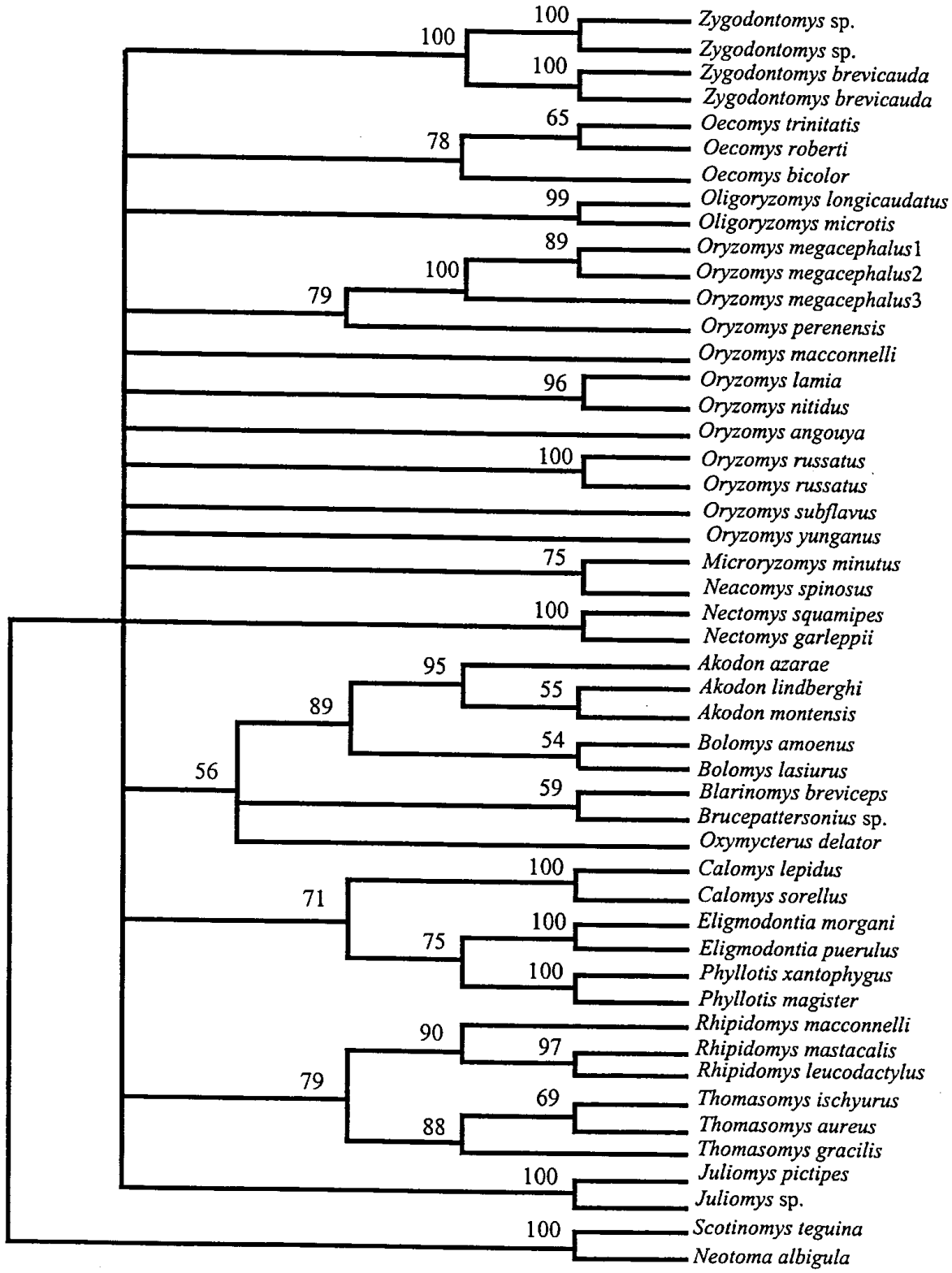

Fig. 4. – Maximum Parsimony tree obtained after booststrap analyses with 1,000 replicates (number near nodes) with 10 :1 weight of transversions : transitions ; length = 12490 ; CI = 0.219 ; RI = 0.514 ; RC = 0.112.

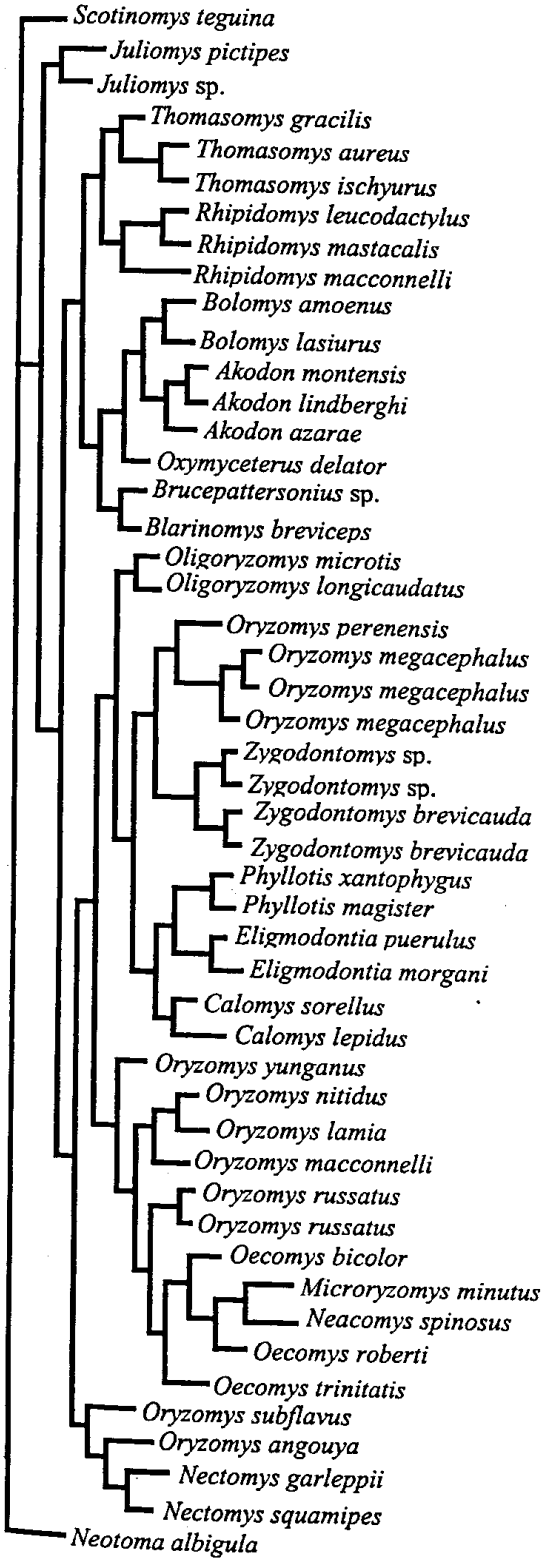

Fig. 5. – Maximum likelihood analysis with 2 :1 weight of transversions : transitions. Ln Likelihood = - 14793.93.

myine genera (Fig. 3), suggesting their closer association while, in MP and ML analyses, the oryzomyine genera did not appear as a monophyletic group. Previous analyses that did not include *Zygodontomys* have also found the oryzomyine genera to be consistently associated despite low bootstrap support (Smith and Patton 1999). Our results are in agreement with previous suggestions showing *Oryzomys* as a non-monophyletic genus (Bonvicino and Moreira 2001), and in showing the association of *Thomasomys* and *Rhipidomys* in a clade (Smith and Patton 1999). Previous reports excluding *Zygodontomys* have also revealed the monophyly of *Oligoryzomys, Thomasomys, Rhipidomys, Juliomys, Akodon, Bolomys, Calomys, Eligmodontia* and *Phyllotis* as well as the monophyly of the tribes Akodontini and Phyllotini (Myers *et al.* 1995 ; Smith and Patton 1993, 1999).

The association between *Zygodontomys* and the oryzomyine genera is supported by the observation that very high diploid numbers occur only in the Oryzomyini but not among the Akodontini or the Phyllotini. Furthermore, this association has been reinforced by several character states shared by *Zygodontomys* and the oryzomyine genera like a long palate with prominent posterolateral pits, absence of gall bladder, possession of pectoral mammae, lack of overlap between tegmen tympani and squamosal, lack of an alisphenoid strut and possession of 12 ribs (Voss and Carleton 1993, Steppan 1995). On the other hand, the lack of developed mesolophs and mesolophids in *Zygodontomys* has historically been cited as a criterion for excluding this taxon from the tribe Oryzomyini (Voss 1991). Despite lack of bootstrap support in phylogenetic analyses of cytochrome *b* sequence data our results point to a closer affinity between *Zygodontomys* and oryzomyine rodents rather than with any other sigmodontine group.

Distance estimates between the *Zygodontomys* sp. from Rio Negro and the two specimens of *Zygodontomys brevicauda* from French Guiana is low (K2p = 0.05) but not very different from estimates between *Nectomys* species (K2p = 0.08) or *Oecomys* species (K2p = 0.08). Patton *et al.* (2000) also found low distance estimates (K2p = 0.07-0.09) between *Oecomys* species from Rio Juruá and suggested that diversification in this genus occurred relatively recently. The K2p

distance found by us between species of *Zygodontomys* suggests that they are closely related to one another despite the wide geographic distance between the studied samples.

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