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CHROMOSOMAL CHARACTERIZATION OF *IRENOMYS TARSALIS* (RODENTIA, CRICETIDAE, SIGMODONTINAE)

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ABSTRACT. The systematics of *I. tarsalis* is far from being understood. Morphological and molecular analyses have given contradictory results, pointing towards its inclusion or exclusion from the Phyllotini. The karyotype of *Irenomys tarsalis*, as well as its C-bands are reported for the first time. The species has 2n = 64 chromosomes (FN = 98) and a C-banding pattern characterized by small amounts of centromeric heterochromatin. Although the high diploid number and the C-bands of *I. tarsalis* support the notion of the species´ ancestral karyotypic condition, its affiliation within the sigmodontines cannot be discerned by the data.

RESUMEN. Caracterización cromosómica de *Irenomys tarsalis* (Rodentia, Cricetidae, Sigmodontinae). Las relaciones sistemáticas de *I. tarsalis* están poco comprendidas. Los análisis morfológicos y moleculares han dado resultados contradictorios, que indican su pertenencia o exclusión de la tribu Phyllotini. Se describe por primera vez el cariotipo de *Irenomys tarsalis*, así como la distribución de la heterocromatina C-positiva. La especie tiene 2n = 64 cromosomas (FN = 98) y un patrón de bandas C pequeñas y centroméricas. Si bien el alto número diploide y la distribución de la heterocromatina apoyan la condición cariotípica ancestral de *I. tarsalis*, su afiliación dentro de los sigmodontinos no puede ser discernida solamente por estos datos.

Key words: *Irenomys tarsalis*, karyotype, Sigmodontinae, Phyllotini.

Palabras clave: *Irenomys tarsalis*, cariotipo, Sigmodontinae, Phyllotini.

INTRODUCTION

The Neotropical sigmodontine rodents have been extensively studied cytogenetically (Gardner and Patton, 1976; Pearson and Patton, 1976; Spotorno et al., 2001). The Sigmodontinae is composed of eight tribes (Akodontini, "Abrothricines", Ichthyomyini, Oryzomyini, Phyllotini, Sigmodontini, Thomasomyini, Wiedomyini) and several genera without clear phylogenetic relationships among them (Smith and Patton, 1999; D'Elía, 2003). The Chilean tree mouse or "laucha arbórea", *Irenomys tarsalis* (Philippi, 1900) is the only representative of this monotypic genus, mainly restricted to temperate forests of South-western South America (Kelt, 1993). The phylogenetic relationships of *Irenomys* to other sigmodontines are controversial. *Irenomys* was excluded from the phyllotines due to the lack of morphological characters common to the group (Hershkovitz, 1962). Nevertheless, it was included in such a tribe in a later revision by Olds and Anderson (1989) as well as by Braun (1993) and Steppan (1993, 1995). Subsequent phylogenetic analyses of mitochondrial sequences depicted *Irenomys* outside the phyllotine clade but associated to

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a variety of divergent taxa depending on the algorithm used (Smith and Patton, 1999). Phylogenetic analyses based on mitochondrial and nuclear genes depict *Irenomys* as an isolated taxon outside the Phyllotini, close to *Scolomys juruaense* and *Rhipidomys macconnelli* (D'Elía, 2003).

In the present paper, the karyotype and the C-bands of *I. tarsalis* are described for the first time. This data set is compared with that of the phyllotines, aiming to shed some light into the phylogenetic relationships of *Irenomys*.

MATERIAL AND METHODS

Four specimens of *I. tarsalis* (three males and one female) were collected from the San Martín woods, Valdivia province, Chile (39°38'S; 73°07'W). Chromosome preparations were obtained from bone marrow following the conventional colchicine-hypotonic solution technique (Lee and Elder, 1980; Baker et al., 1982). C-bands were induced by the barium hydroxide technique (Sumner, 1971). Ten to fifteen metaphase spreads were counted for each specimen. Nomenclature for chromosome morphology and fundamental arm number (FN) follows Patton (1967). The specimens analysed were deposited in the Collection of Mammals of Instituto de Ecología y Evolución, Universidad Austral de Chile.

RESULTS

Conventional analysis indicates 2n = 64, FN = 98 for *I. tarsalis*. The autosomal complement consists of 5 pairs of metacentrics (2 large, 2 medium-sized and 1 small), 13 pairs of subtelocentrics (medium to small-sized) and 13 pairs of medium-sized acrocentrics (**Fig. 1A**). The X chromosome is the largest acrocentric present in pairs in the female complement, whereas only one element is observed in males. The Y chromosome is a medium-sized metacentric (**Fig. 1A**).

The C-bands of *I. tarsalis* are small, centromeric and vary in size across the karyotype (**Fig. 1B**). Discrete blocks of heterochromatin including part of the arms are observed in pairs 7, 8, 19, 20, 22 and 26. The rest of the autosomes show small centromeric C-bands whereas no banding pattern is observed in pair 2 (**Fig. 1B**). The X chromosomes exhibit clear C-bands in the centromeric region. The Y chromosome is totally heterochromatic (**Fig 1B**)

DISCUSSION

The systematics of *Irenomys* is far from being understood. The lack of diagnostic morphological characters have been the defining criterium for excluding *Irenomys* from the Phyllotini (Hershkovitz, 1962). Nevertheless, phylogenetic analyses of morphological traits depict *Irenomys* as an independent and enigmatic stem clade of phyllotines (Braun, 1993). This affiliation to the phyllotines was further emphasized by Steppan (1995) although a close relationship to *Andinomys edax* and *Chinchillula sahamae* is implicated by the data.

The extent and composition of the Phyllotini (formed by *Andalgalomys*, *Andinomys*, *Auliscomys*, *Calomys*, *Chinchillula*, *Eligmodontia*, *Galenomys*, *Graomys*, *Neotomys*, *Phyllotis* and *Salinomys*) has been recently studied by mitochondrial and nuclear DNA markers (D'Elía, 2003). Mitochondrial sequencing data excludes *Irenomys* from the phyllotines and suggest a relationship with *Scolomys juruaense* or *Sigmodon hispidus* (Smith and Patton, 1999). Nevertheless, the position of *Irenomys* within the Sigmodontinae is uncertain and depends on the algorithm used for phylogenetic inference. A recent revision based on nuclear and mitochondrial markers coincides with Smith and Patton (1999) in excluding *Irenomys* from the Phyllotini, and considers *I. tarsalis* as a Sigmodontinae *incertae sedis* (D'Elía, 2003).

The karyotypic studies conducted on the Neotropical sigmodontines have indicated extensive chromosomal multiformity and a chromosomal trend towards reduction in diploid number (Gardner and Patton, 1976; Pearson and Patton, 1976). The phyllotines also display an exceedingly broad range of karyotypic variation. In fact, diploid number ranges from 22 to 70 chromosomes, and the fundamental arm number, from 30 to 76 (Pearson and Patton, 1976). Assuming that chromosomal evolution within the phyllotines has proceeded through Robertsonian fusions, the high diploid number of *Neotomys ebriosus* (2n = 70, FN = 68) and

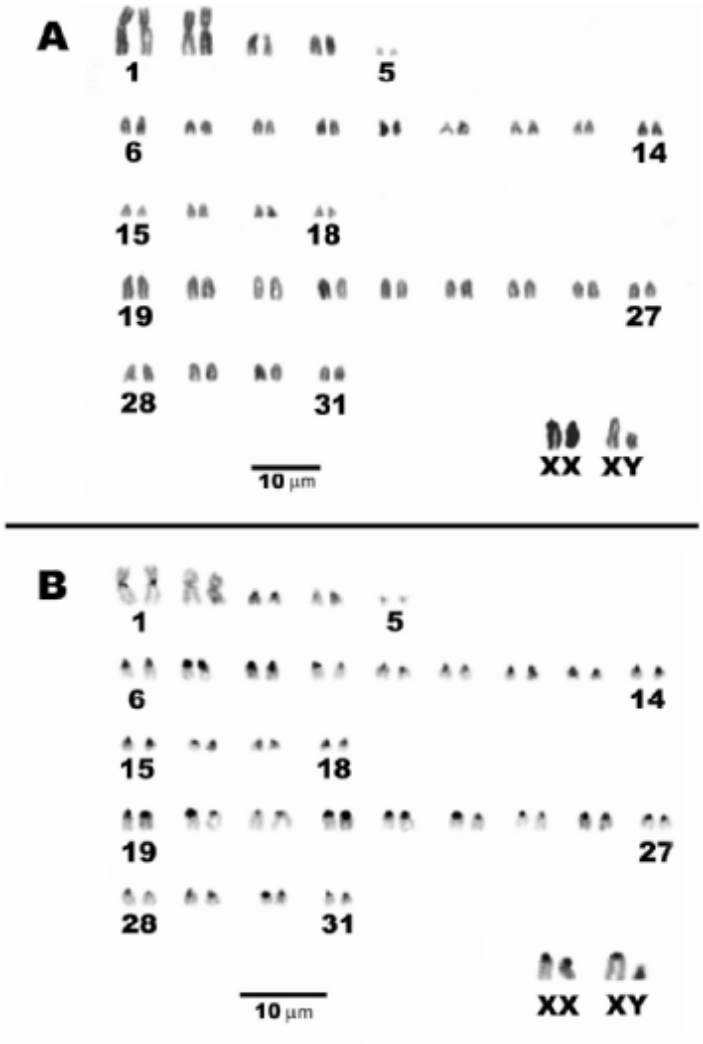

Fig. 1. **A**) Standard Giemsa-stained karyotype of *Irenomys tarsalis* (2n = 64, FN = 98). Biarmed chromosomes are arranged first, acrocentric chromosomes, last. Sex chromosomes (XY) are also indicated. **B**) C-banded karyotype of *Irenomys tarsalis* (2n = 64, FN = 98). Note the centromeric blocks of heterochromatin in most chromosomes, except pair 2.

A) *Cariotipo de* Irenomys tarsalis *con tinción estándar (2n = 64, FN = 98). Los cromosomas biarmadados se ubican al principio y los comosomas acrocentricos al final. También se indican los cromosomas sexuales (XY).* ***B)*** *Banda C de Irenomys tarsalis. Nótense los bloques de heterocromatina centromérica en la mayoría de los cromosomas, excepto en el par 2.*

Phyllotis osilae (2n= 70; FN = 68) represent karyotypic ancestrality whereas the totally-biarmed karyotypes of *Phyllotis darwini* and *P. caprinus* (2n = 38; FN = 72) represent the derived condition (Pearson and Patton, 1976).

The diploid number of *Irenomys´* (although from a single locality) suggests an association with the phyllotines, whereas its high FN is consistent with sequence-based cladograms that exclude it from such a group. These characteristics (2n and FN) show high variation within the phyllotines to infer detailed relationships

on their basis. Further analyses are necessary to clarify the relationship of *Irenomys*. The characteristic distribution and amount of heterochromatin in the karyotype contributes to genome size differences especially in rodents (Redi et al., 2001; Gallardo et al., 2003). Thus, the increase in heterochromatin content through the selfish replicative properties of short repetitive DNA sequences (Wallrath, 1998; Hennig, 1999) represents a recently evolved trait in rodents (Gamperl, 1982; Gallardo, 1992), insects (Baimai, 1998) and other taxa. On the other hand, the centromeric location and small blocks of heterochromatin of *I. tarsalis* and its high diploid number are likely ancestral conditions (Gallardo, 1992), and are therefore consistent with the proposed early divergence of *Irenomys* within the sigmodontine radiation (Smith and Patton, 1999). Further molecular analyses are needed to substantiate this view, since the karyotypic variation displayed by the phyllotines prevents chromosomal data from clarifying this issue.

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