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Evolutionary and systematic relationships among tuco-tucos of the *Ctenomys pundti* complex (Rodentia: Octodontidae): a cytogenetic and morphological approach

Sergio I. TIRANTI
Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Uruguay 151, 6300. Santa Rosa, La Pampa (Argentina)

Fernando J. DYZENCHAUZ
Universidad CAECE, Dpto. de Ciencias Biológicas, Tte. Gral. Juan D. Perón 2933, 1198 Buenos Aires (Argentina)

Esteban R. HASSON
GIBE (Grupo de Investigación en Biología Evolutiva), Dpto. de Ciencias Biológicas, FCEyN, Universidad de

Alicia I. MASSARINI
Buenos Aires, Pabellón II, 4º piso, Cdad. Universitaria, Nuñez, 1428 Buenos Aires (Argentina)
alicia@biolo.fcen.uba.ar

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ABSTRACT
We present new cytogenetic, morphometric, and sperm morphology data of eight populations belonging to the *C. pundti* complex from Southern Córdoba and Eastern La Pampa Provinces in Argentina. The diploid numbers ranged from 2n = 44 to 2n = 50, and C- bands revealed a pattern of centromeric and pericentromeric heterochromatin. Comparisons of G-banded karyotypes revealed that the 2n = 44 (Holmberg, Santa Catalina, Sampacho), 2n = 46 (Realicó), 2n = 48 (El Guanaco, Guatraché), 2n = 46-48 (Vicuña Mackenna), and 2n = 50 (Puente Olmos) karyotypes, are closely related. In addition, these karyotypes show a high degree of homology (95%) with *C. talarum talarum*, despite the fact that five chromosomal rearrangements differentiate both taxa. Discriminant Function Analysis of morphometric data allows to distinguish three clusters: i) the *C. mendocinus* species group, ii) *C. t. talarum*, and iii) populations of the *C. pundti* complex proposed herein. The close phylogenetic relationship between *C. talarum* and the *C. pundti* complex, which undoubtedly belong to the same evolutionary lineage, is well supported by two different kinds of evidence: the extensive chromosomal homology and the same symmetric type of sperm. The morphological and chromosomal differences show that these two forms have diverged recently.

KEY WORDS
Ctenomys,
cytogenetics,
morphometric,
systematics,
evolutionary relationships.

RÉSUMÉ

Relations évolutives et systématiques dans le complexe Ctenomys pundti *(Rongeurs : Octodontidae) : une approche cytogénétique et morphologique.*

Nous présentons des données originales sur la cytogénétique, la morphométrie et la morphologie spermatique de huit populations de *C. pundti* du sud de la province de Córdoba et de l'est de la province de La Pampa, en Argentine. Les nombres diploides varient entre 2n = 44 et 2n = 50, et les bandes C- ont révélé une distribution d'hétérochromatine centromérique et péri-centromérique. Les comparaisons faites avec les bandes G- montrent que les caryotypes 2n = 44 (Holmberg, Santa Catalina, Sampacho) , 2n = 46 (Realicó), 2n = 48 (El Guanaco, Guatraché), 2n = 46-48 (Vicuña Mackena) et celui 2n = 50 (Puente Olmos), sont étroitement corrélés. Un degré d'homologie très élevé (95 %) a été observé entre le caryotype 2n = 48 de El Guanaco et ceux de *C. talarum talarum*, malgré le fait que cinq réarrangements chromosomiques différencient les deux taxa. Une analyse des fonctions discriminantes basée sur des données morphométriques a permis de distinguer trois groupes ou clusters : le group des populations de *C. mendocinus*, des populations de *C.t. talarum* et le complex de populations de *C. pundti*. La relation étroite entre *C. talarum* et le complexe *C. pundti*, appartenant certainement à la même lignée évolutive, est supportée par deux sortes différentes de preuve : l'homologie chromosomique et le même type symétrique de sperme. Les différences morphologiques et chromosomiques indiquent que ces deux formes ont divergé récemment.

MOTS-CLÉS
Ctenomys, cytogénétique, morphométrie, systématique, relations évolutives.

INTRODUCTION

The fossorial rodents of the genus *Ctenomys*, commonly known as tuco-tucos, are characterized by an extensive karyotypic heterogeneity, ranging from 2n = 10 in *C. steinbachi* to 2n = 70 in *C. pearsoni* and *C. dorbignyi* (Reig & Kibliski 1969; Anderson *et al.* 1987; Cook *et al.* 1990; Ortells *et al.* 1990; Reig *et al.*, 1990; Gallardo 1991; Ortells 1995). However, some species from western and central Argentina, referred to as the *C. mendocinus* group (Massarini *et al.* 1991; Freitas 1994; D'Elía *et al.* 1999), show a similar karyotype of 2n = 47 to 48, and recently has been described de 2n = 46 karyomorph for two population of *C. azarae* (Massarini *et al.* 1998). Moreover, some species exhibit a wide array of chromosomal polymorphisms, and interpopulation variation (Massarini *et al.* 1991; Freitas 1995; Massarini *et al.* 1998). The significance of this extensive intra and interspecific chromosomal variation has been the subject of considerable debate (Baker *et al.* 1983; Reig 1989; Nevo 1991; Ortells & Barrantes 1994; Freitas 1994; Freitas 1997).

In one of the best studied species, *Ctenomys talarum*, two subspecies are currently recognized, *C. t. recessus* and *C. t. talarum* which include several populations distributed along the Atlantic coast of Buenos Aires Province, varying in diploid number from 2n = 46 to 2n = 50, as well as other populations in central and western areas of the species' distribution range, whose karyotypes have not been yet studied (Contreras & Reig 1965; Vidal Rioja 1985).

Moreover, *C. talarum recessus* is, in certain areas, sympatric with *C. australis,* which belongs to the *C. mendocinus* group.

Sperm morphology has also been used to discriminate between species groups in the genus.

TABLE 1. — Collection localities of *Ctenomys* used for morphometric analysis.

Species	Locality	Coordinates	males	females
C. australis	Claromecó	38° 52' S 60° 02' W	5	
	Monte Hermoso	38° 42' S 60° 45' W	8	
	Necochea	38° 32' S 58° 46' W	16	23
C. azarae	Anguil	36° 32' S 64° 01' W	4	
	Santa Rosa	36° 37' S 64° 17' W	1	4
C. mendocinus	C. San Isidro	32° 53' S 68° 49' W	12	16
C. porteousi	Bonifacio	36° 49' S 62° 13' W	18	20
C. pundti	Puente Olmos	33° 25' S 63° 06' W	6	
	Realicó	34° 59' S 64° 16' W	3	1
	Santa Catalina	33° 04' S 64° 32' W	10	3
	Vicuña Mackenna	33° 54' S 64° 23' W	7	
	El Guanaco	36° 19' S 66° 17' W		6
C. t. talarum	Magdalena	34° 52' S 57° 53' W	22	22
	Santa Clara	37° 58' S 57° 34' W	21	23

Number of males and females used in morphometric analysis, localities of collection with its coordinates, and species to which they belong.

Three types of sperm have been described: symmetric, simple-asymmetric and complex-asymmetric (Vitullo *et al.* 1988; Vitullo & Cook 1991; Freitas 1995). *C. talarum* has symmetric sperm whereas species of the *C. mendocinus* group have simple asymmetric sperm.

In Northern Córdoba province, new species with assymetric sperm have recently been described (Giménez *et al.* 1999), but in Southern Córdoba and Eastern La Pampa provinces, there is a suite of *Ctenomys* populations with symmetric sperm that has not been cytogenetically characterized yet. Although, *C. azarae* was described from material collected in La Pampa Province (Thomas 1903), in southern Córdoba the only described taxa is *Ctenomys pundti* (Nehring, 1900, type locality: Alejo Ledesma, Marcos Juárez Department). These two species can be easily distinguished in the field by external morphology. *Ctenomys azarae* is larger and more robust, having a bigger hindfoot, than *Ctenomys pundti*, which is more similar to *C. talarum recessus*. The skull of *C. azarae* is larger with wide auditory bullae, while *C. pundti* and *C. talarum* have narrower bullae.

Here we report new cytogenetic, morphometric, and sperm type data of *Ctenomys* populations from Southern Córdoba and Eastern La Pampa, which may help to interpret the phylogenetic relationships among these populations and other taxa of the genus of central Argentina.

MATERIAL AND METHODS

A morphometric comparative study based on 24 craniometric variables of 241 adult specimens from a total of 14 localities, 10 in male samples and 12 in female samples, was performed on data from Ortells (1990) and Massarini (1992) (Table 1). The studied craniometric variables are shown in Fig. 1. The data were analyzed by means of Discriminant Function Analysis (DFA), using the program STATISTICA. This kind of analysis allows to find a linear combination of variables that maximize the differences among previously defined groups. Groups were defined according to the geogrphapic sampling locality, which corresponds to local populations.

Chromosomal preparations of *C. pundti* and *C. t. talarum* specimens were obtained from bone marrow of animals injected with yeast one day before sacrifice (Lee & Elder 1980), and G- and C- banded karyotypes by the methods of Seabright (1971) and Hsu (1974), respectively. Chromosomal nomenclature follows Levan *et al.* (1964), and nomenclature relating to the chromosomal size and arrangement of chromosome

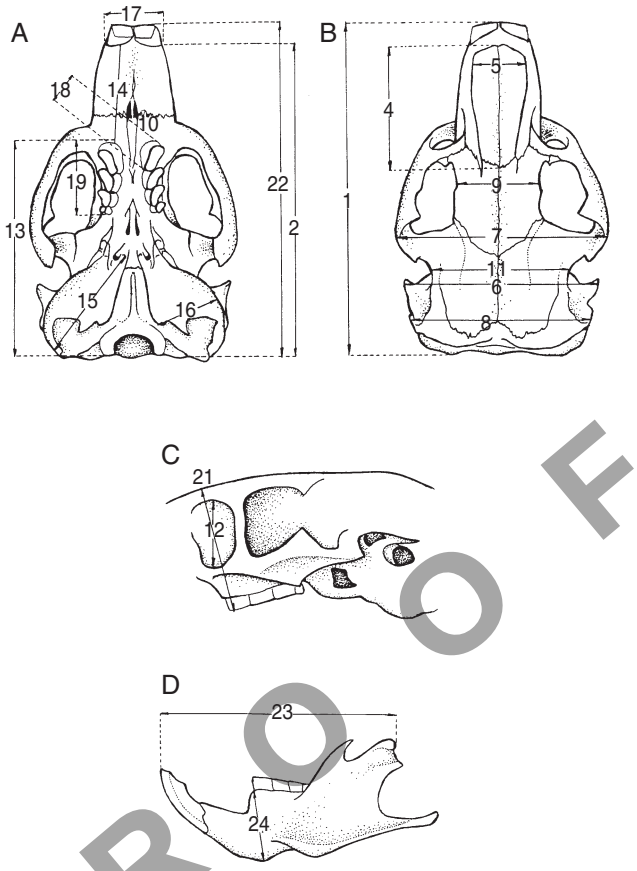

FIG. 1. — Craneometric variables used in morphometric analysis: skull lenght (1), basilar condyle lenght (2), nasal condyle length (3), nasal length (4), nasal width (5), width between meatuses (6), width between cygomatic arches (7), width between mastoid apophyses (8), frontal width (9), rostral width (10), braincase width (11), preorbital foramen width (12), premolar condyle length (13), diastema length (14), auditory bulla length (15), auditory bulla width (16), incisive width (17), P^4 length (18), upper molar series length (19), upper jaw width (20), height of skull (21), basal length (22), lower jaw length (23), lower jaw height (24).

pairs, follows Massarini *et al*. (1991). Sperm preparations were produced by squeezing a droplet of fluid from the epididymus and the seminal vescicles onto a slide, and fixed in 10% formalin. Geographic location of the populations sampled are shown in Fig. 2, whereas the coordinates of localities and samples sizes are given in Table 2. *Ctenomys t. talarum* specimens included in this study were collected at the type locality (Magdalena, Buenos Aires Province). Studied specimens were deposited in the Museo de Ciencias Naturales "Lorenzo Scaglia" from Mar del Plata, Buenos Aires Province, Argentina.

RESULTS

Ctenomys specimens from Córdoba localities showed variable karyotypes with diploid numbers ranging from 2n = 44 to 50 (Table 2).

The 2n = 44 karyotype has 19 pairs of biarmed chromosomes, the majority subtelocentric, except pair A15 which is submetacentric, and A16 and A17 which are metacentrics. Pair A15 presents secondary constrictions in the long arm. There are two pairs of small telocentrics, the X is a medium sized metacentric and the Y chromosome is a small subtelocentric (Fig. 3A).

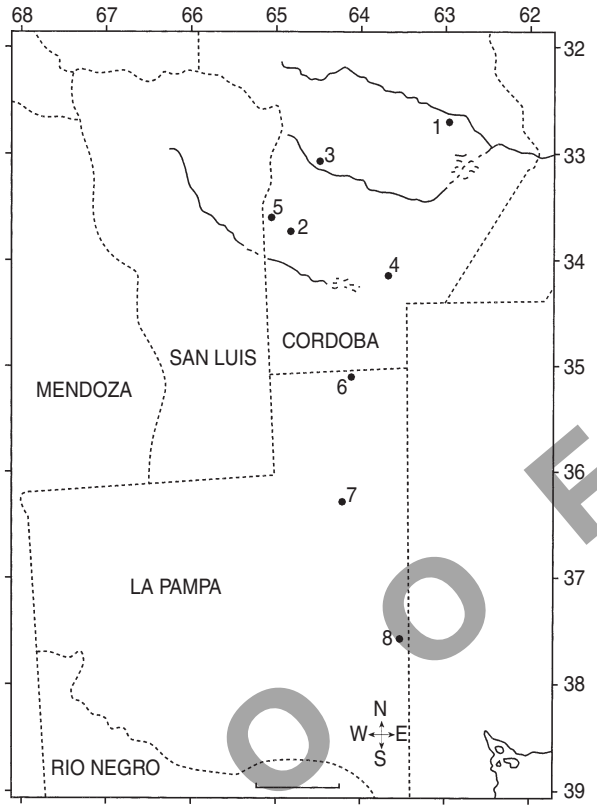

FIG. 2. — Geographic distribution of localities of collection included in this study. Puente Olmos (1), Santa Catalina (2), Holmberg (3), Vicuña Mackenna (4), Sampacho (5), Realicó (6), El Guanaco (7), and Guatraché (8). Bar represents 100 kms.

TABLE 2. — Karyotypic variation of *C. pundti* complex studied populations.

Locality	males	females	Coordinates	Province	2n	FN
Puente Olmos	13	7	33° 25' S 63° 06' W	Córdoba	50	86
Santa Catalina	6	16	33° 04' S 64° 32' W	Córdoba	44	80
Holmberg	1	1	33° 13' S 64° 25' W	Córdoba	44	80
Vicuña Mackenna	0	6	33° 54' S 64° 23' W	Córdoba	46-47-48	80-82-84
Sampacho	5	4	33° 23' S 64° 44' W	Córdoba	44	80
Realicó	2	7	34° 59' S 64° 16' W	La Pampa	46	80
El Guanaco	1	6	36° 19' S 64° 17' W	La Pampa	48	84
Guatraché	0	1	37° 41' S 63° 31' W	La Pampa	48	84

Localities of collection of individuals, coordinates and provinces to which they belong, number of males and females of each sample, and diploid number (2n), and fundamental number (FN) found in each locality.

The 2n = 46 karyotype shows a similar chromosomal morphology as the 2n = 44, except that pair A19 is absent and there are two extra pairs of small telocentrics (B3 and B4) (Fig. 3B). The 2n = 48 karyotype is similar to the 2n = 46 karyotype except that pair A3 is absent, and there are two extra pairs of chromosomes, one of which is a medium subtelocentric (A8) and the other is a

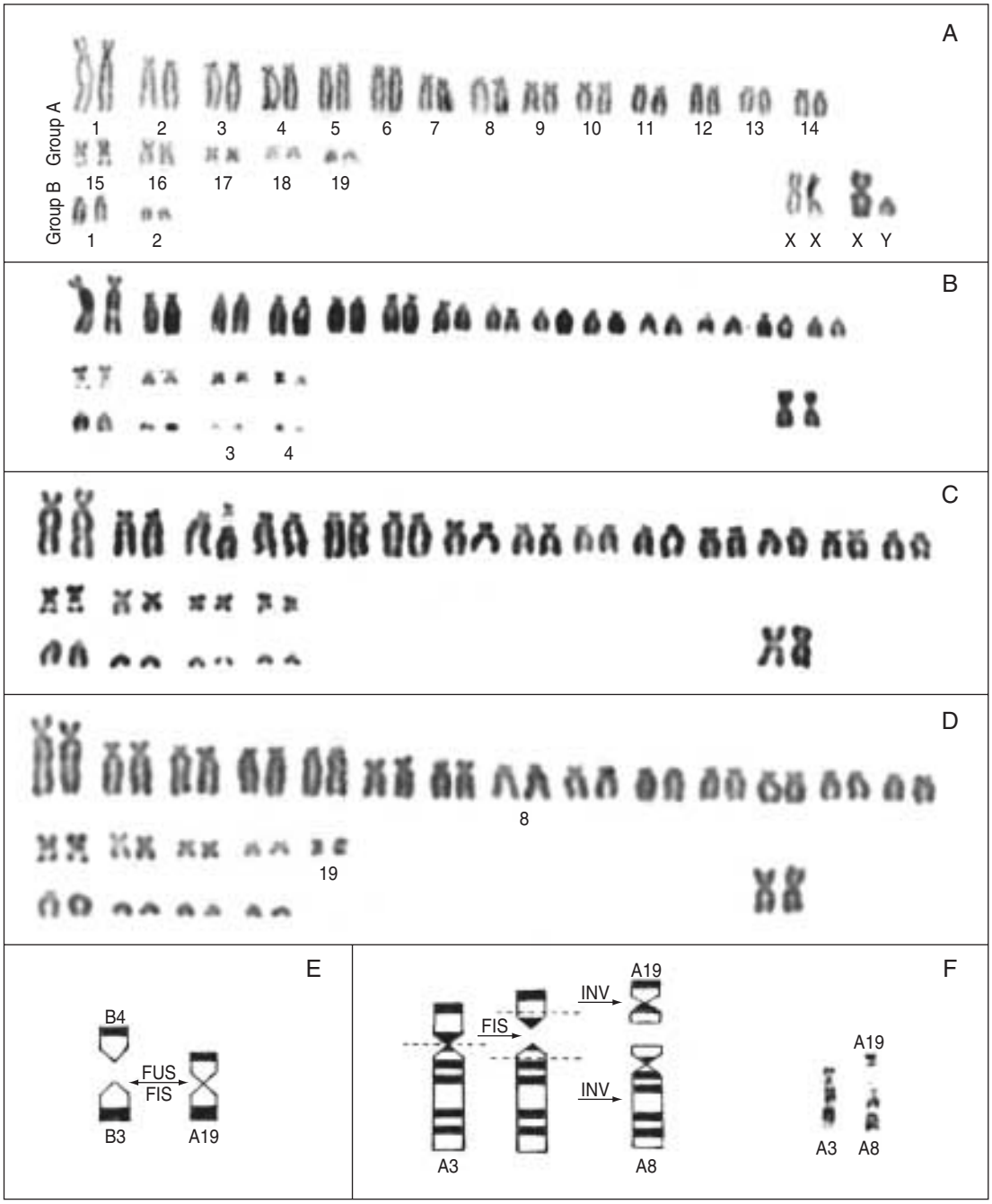

FIG. 3. — Nondifferentially stained karyotype of a female from Santa Catalina, and the sexual pair of a male, 2n = 44 (A); karyotype of a female from Realicó, 2n = 46 (B); heterokaryotype of a female from Vicuña Mackenna, 2n = 47 (C); karyotype of a female from Vicuña Mackenna, 2n = 48 (D); idiographic representation with G-bands of the chromosomal changes between the 2n = 44 and the 2n = 46 karyomorphs (E); idiographic representation with G-bands of the chromosomal rearrangements between the 2n = 46 and the 2n = 48 karyomorphs (F). Bar represents 10 µm.

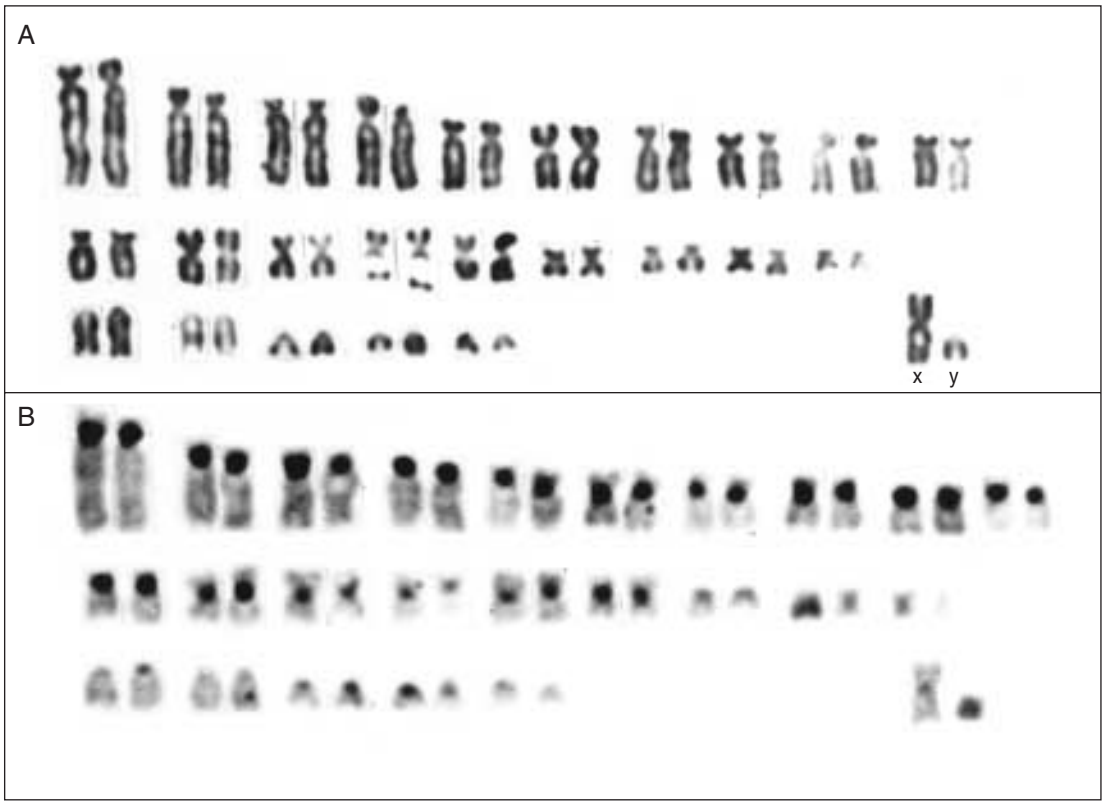

FIG. 4. — Nondifferentially stained karyotype of a male from Puente Olmos, with a diploid number of 2n = 50 (A); sequential C-band karyotype of same cell (B). Bar represents 10 µm.

small metacentric (A19) (Fig. 3D). Individuals collected in Vicuña Mackenna were either homozygous for the 2n = 46 or 2n = 48 karyomorphs, or heterozygous with a karyotype of 2n = 47. In the latter, there is an heteromorphic pair composed of a single A3 subtelocentric chromosome of the 2n = 46 karyomorph, and one homologue of each of the two extra biarmed pairs of the 2n = 48 karyomorph (A8 and A19) (Fig. 3C, 3F).

The 2n = 50 karyotype found in Puente Olmos, has 19 pairs of biarmed chromosomes and 5 pairs of telocentric chromosomes. Eleven pairs of the biarmed chromosomes are subtelocentric, three pairs are submetacentric and five pairs are metacentric. Secondary constrictions are located in pair A14 (Fig. 4A). This karyotype differs from the 2n = 48 karyotype in an extra pair of telocentrics, and in chromosome morphology of five pairs of biarmed chromosmes.

Sexual chromosomes in all studied karyotypes (2n = 44, 46, 47, 48 and 50) show the same morphology, being the X a medium metacentric chromosome, and the Y a small subtelocentric.

C-banding showed a typical pattern of centromeric and pericentromeric heterochromatin distribution in biarmed chromosomes, and centromeric in telocentrics. The X chromosome shows centromeric heterochromatin, and the long arm of the Y is totally heterochromatic (Fig. 4B).

Comparisons of G-banded karyotypes showed that the differences between 2n = 44 and 2n = 46 karyomorphs are due to a single Robertsonian

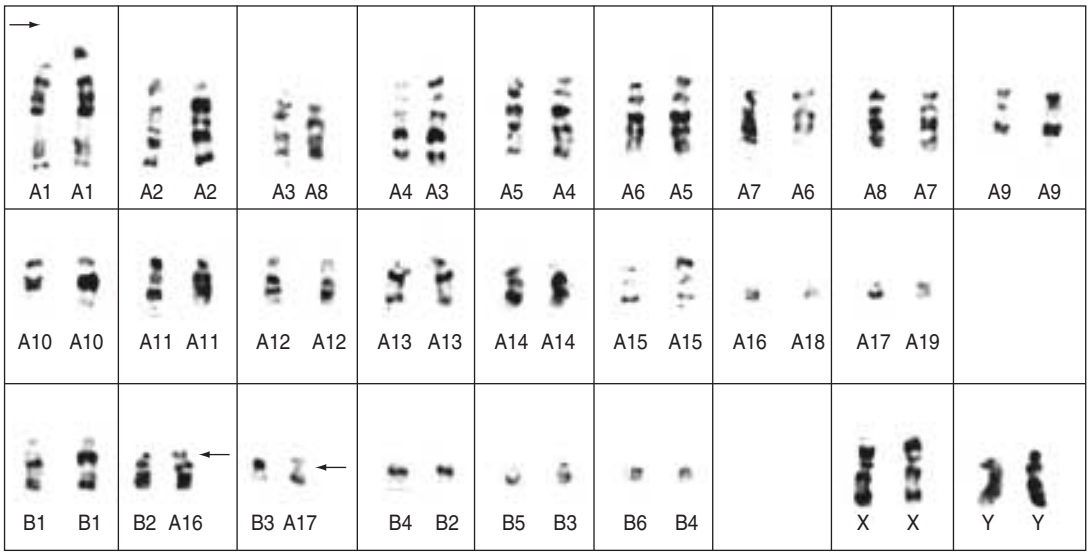

FIG. 5. — Arm to arm comparisons of G-banded chromosomes of a 2n = 48 male from El Guanaco (right chromosome of each pair), and a 2n = 48 *Ctenomys t. talarum* male from its type locality (left chromosome). Arrows show the chromosomal changes between these two karyomorphs. Bar represents 10 µm.

rearrangement involving pairs B3 and B4 of the 2n = 46 karyotype, and pair A19 of the 2n = 44 karyotype (Fig. 3E). However, a complex series of rearrangements, that include a fission and two inversions, differentiate the 2n = 46 karyomorph from the 2n = 48 (Fig. 3F). The chromosomal pairs involved in this rearrangement are A3 of the 2n = 46 karyotype and pairs A8 and A19 of the 2n = 48 karyotype. Arm to arm comparisons between 2n = 48 and 2n = 50 karyotypes showed an 83% homology. Nevertheless, four pairs of chromosomes of the 2n = 50 karyotype, did not show any homology with the chromosomes of the 2n = 48 karyotype, and neither did three pairs of the 2n = 48 karyotype with the 2n = 50 karyotype.

G-banding comparisons between the karyomorph of 2n = 48 from El Guanaco and the 2n = 48 karyotype of *Ctenomys t. talarum* from the type locality, shows 95% homology. Three inversions and two deletions affecting pairs A1, A3, B2 and B3 of *C. t. talarum* and pairs A1, A8, A16 and A17 of the 2n = 48 karyotype from El Guanaco, can account for the observed differences (Fig. 5).

All specimens analysed in this study, showed the symmetric type of sperm morphology.

The discriminant function analysis of morphometric data (Table 3A & 3B) showed that for both sexes it is possible to distinguish clusters of populations that correspond to different species. The first discriminant function, which accounts for 51 and 59% of total variance in males and females respectively, shows the presence of two clusters. One of the clusters includes populations of *C. t. talarum* and *C. pundti*, with negative values; and the second cluster, species of the *C. mendocinus* group, with values greater or equal to 0. In males, the second discriminant function (which explains 22% of total variance) separates, within the first group, populations of *C. t. talarum*, with negative values, from those of the *C. pundti* complex, with positive values. In females, *C. t. talarum* and *C. pundti* presented positive values and negative values, respectively, for the second discriminant function function (which accounts for 21% of total variance). In the *mendocinus* group the second function allows to distinguish in males, populations of *C. azarae*, with high positive values, from those of *C. porteousi* and

TABLE 3. — Morphometric data.

3A.

OTU Nº	Population	Species	Function 1	Function 2	Function 3
1.	Necochea	*C. australis*	9.7053	5.0685	1.6920
2.	San Isidro	*C. mendocinus*	2.6105	– 1.2180	4.1611
3.	Bonifacio	*C. porteousi*	1.4466	– 0.5644	1.8015
4.	Magdalena	*C. t. talarum*	– 5.2721	2.8381	0.6561
5.	Santa Clara	*C.t. talarum*	– 6.3524	1.4897	0.2659
6.	Santa Catalina	*C. pundti*	– 2.6156	– 2.6320	– 4.0353
7.	Puente Olmos	*C. pundti*	– 1.4203	– 3.4226	– 3.5179
8.	Vicuña Mackenna	*C. pundti*	– 2.0978	– 1.2531	– 3.7424
9.	Realicó	*C. pundti*	– 3.1352	– 2.2690	– 4.0646
10.	El Guanaco	*C. pundti*	– 3.2941	– 1.0408	– 2.9524
11.	Santa Rosa	*C. azarae*	3.2314	– 5.7145	– 0.0354
12.	Anguil	*C. azarae*	5.6099	– 3.9273	– 1.0431

3B.

OTU Nº	Population	Species	Function 1	Function 2	Function 3
1.	Claromecó	*C. australis*	4.9198	– 4.1121	0.2967
2.	Monte Hermoso	*C. australis*	4.4699	– 1.0612	0.2081
3.	Necochea	*C. australis*	4.4434	– 1.0385	– 1.9385
4.	San Isidro	*C. mendocinus*	– 0.0206	0.4028	– 3.6319
5.	Bonifacio	*C. porteousi*	1.1253	1.2356	– 2.0884
6.	Magdalena	*C. t. talarum*	– 2.4819	– 2.5446	0.3217
7.	Santa Clara	*C. t. talarum*	– 2.8949	– 1.5727	– 0.1595
8.	Santa Catalina	*C. pundti*	– 3.7032	1.7439	1.4665
9.	Puente Olmos	*C. pundti*	– 3.1502	2.4916	2.0713
10.	Santa Rosa	*C. azarae*	1.3934	4.0621	0.4882

Values correspond to the first three functions (Axis 1, 2, and 3) of Discriminant Function Analysis, based on the morphometric data of Females (3A) and: Males (3B).

C. mendocinus, with low positive values and *C. australis* with negative values. In females, the second function allows to distinguish within the *mendocinus* group, *C. azarae* populations with high negative values from *C. porteousi* and *C. mendocinus* with low negative values, and from *C. australis* which had positive values. The third discriminant function, which accounts for 11% of total variance in both sexes, allows to differentiate in *C. mendocinus* from *C. porteousi* (Fig. 6A & 6B). It is worth noting that the percentages of well classified specimens was100% in males and, on average, 96.5% in females. Misclassified females correspond to cases of individuals sampled in different populations of the same subspecies (3 out of 21 specimens of *C. t. talarum*), in different localities of the same species (1 out five specimens of *C. pundti*), and in individuals that belong to different species of the mendocinus group (2 out of 36 specimens of *C. porteusi* and *C. mendocinus*).

DISCUSSION

The analysis of chromosomal homologies by means of G-banded karyotypes show that the 2n = 46 (Realicó), 2n = 48 (El Guanaco and Guatraché) populations, and the polymorphic population of Vicuña Mackenna (2n = 46, 47, 48) are closely related. The 2n = 44 populations from Santa Catalina, Holmberg and Sampacho are karyologically undistinguishable, and differ from the rest of the forms by a single Robertsonian change. The rearrangements that explain the differentiation between the 2n = 46

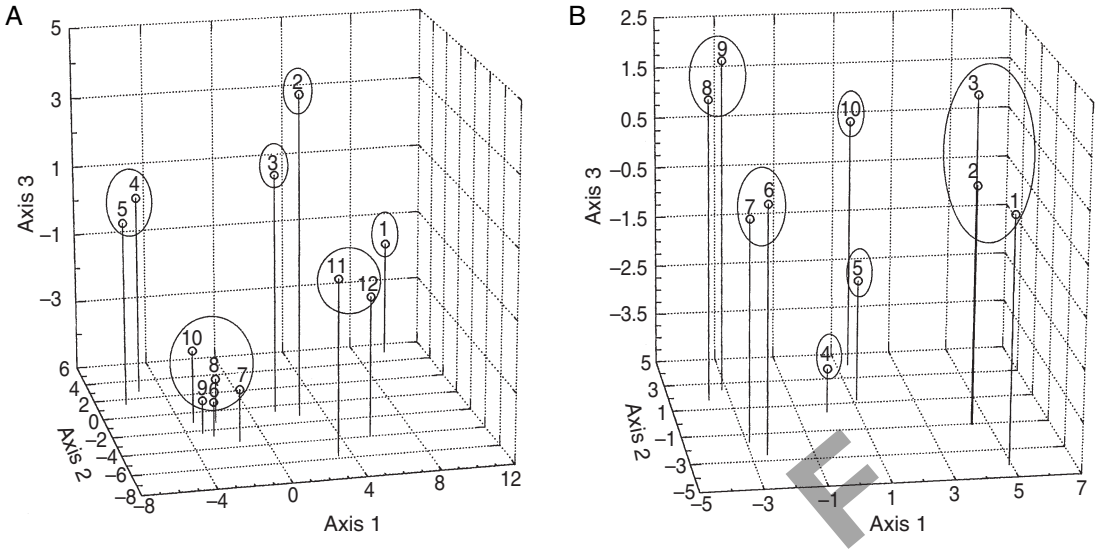

FIG. 6. — Graphical representation of the first three functions of Discriminant Canonical Analysis based on morphometric data for females (A) and males (B). Only centroids of each a priori defined group are plotted. Circles assemble populations belonging to the same species. In females (A) population 1 belongs to *C. australis*, 2 to *C. mendocinus*, 3 to *C. porteusi*, 4-5 to *C. t. talarum*, 6-10 to *C. pundti* and 11-12 to *C. azarae*. In males (B) populations 1-3 belong to *C. australis*, 4 to *C. mendocinus*, 5 to *C. porteusi*, 6 to *C. talarum*, 8-9 to *C. pundti* and 10 to *C. azarae*.

and 2n = 48 karyotypes, involve a complex set of mutation events that suggests a significant distinction between these two forms. However, the existence of the 2n = 47 heterokaryotype in Vicuña Mackenna points out that this incipient chromosomal differentiation does not involve reproductive isolation.

The 2n = 50 karyotype from Puente Olmos is the more divergent. With the data presented so far, it was not possible to establish the nature of the chromosomal rearrangements involved in karyotypic divergence, but it is expected that high resolution G-banding would provide an answer to this question.

The close phylogenetic relationships between *C. talarum* and the *C. pundti* complex, is well supported by two different kind of evidence: the great chromosomal homology and the sharing of the same type of sperm. Although, they undoubtedly belong to the same evolutionary lineage, morphological and cytogenetic differentiation suggest that these are recently diverged species.

Cytogenetic and sperm morphology evidence along with the morphometric analysis support the homogeneity within lineages of *Ctenomys*. On one side the *C. mendocinus* group shares the asymmetric sperm type, and a high degree of chromosomal uniformity, but these taxa are morphologically differentiated from each other. On the other side, the populations of the *C. pundti* complex and *C. talarum*, also share the same symmetric sperm morphology and a significant degree of chromosomal homology. Morphometric analysis allows to distinguish *C. t. talarum* from the *C. pundti* complex, but populations of the latter, though variable in chromosome number, cannot be discriminated by means of morphometric criteria. In this sense, it is noteworthy that the level of morphological differentiation that exists between populations of the *C. pundti* complex, is comparable to differentiation among *C. azarae* populations.

Based solely on morphology, Justo (1992) described a new subspecies of *C. talarum* (*C. t. occidentalis*) from La Pampa and included samples from El Guanaco. However, our present study shows that the latter is better referred to as belonging to the *C. pundti* complex rather than

to *C. t. talarum*, on grounds of cytogenetic and morphometric criteria . Similarly, we propose that rest of the populations studied by Justo should be assigned to the pundti complex, which conform a closely related but distinguishable unit from *C. talarum*.

Despite the growing amount of information available for these forms, there exists a nomenclatorial problem regarding *C. pundti*. Nowadays, in the type locality of this species (Alejo Ledesma), there are no tuco-tucos, due to the intense ecosystem modification brought upon by agriculture. The nearest population of tuco-tucos is in Puente Olmos, which is 50 km W of Alejo Ledesma (Reig *et al.* 1992). Therefore, we propose to use the name *C. pundti* for the 2n = 50 population from Puente Olmos. Additionally, this one and the rest of the populations (2n = 44, 46, 47, 48) should be included in the *C. pundti* complex until more information is available to further solve the taxonomy and systemtics of these comlex forms from Central Argentina.

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