INTRODUCTION

Calomys hummelincki is the only species of the genus Calomys living in a limited area of northern South America (Aruba, Curaçao and northern Venezuela); it has a disjunct distribution with respect to all the other species of this genus. Its presence was first noted in the 1940s in the Netherlands Antilles by W. Hummelinck, who tentatively identified this rodent as Hesperomys sp. (HUSSON 1960a). Husson (1960b) validated its status as a species, but assigned it to the genus Baiomys. Two years later, HERSHKOVITZ (1962) claimed that it should be identified as Calomys laucha, based on a report by BUTTERWORTH (1960) on specimens captured in Venezuela. In his surveys in Venezuela, HANDLEY (1976) found natural populations of C. hummelincki in at least four locations, and he established the use of this specific denomination. After that, no further information was obtained for this species until the late 1980s. Basic karyological information from one specimen of C. hummelincki captured in the Venezuelan llanos was provided by PÉREZ-ZAPATA et al. (1987), who established that it was karyologically different from C. laucha.

VITULLO et al. (1990) and ESPINOSA et al. (1997) proposed that, within the Calomys group, C. hummelincki belongs to the Calomys ancestral stock, together with C. sorellus and C. laucha, because all these species present 2N≥60. This hypothesis predicts that the C. hummelincki chromosome banding pattern should be similar to...
those of *C. sorellus* and/or *C. laucha* and share some chromosomes with the immediately derived species.

The present study had two objectives: first, to analyze the chromosomal composition of the various natural populations of this species within its distribution range; second, to describe the chromosome pattern, which would allow us to test the evolutionary relationship of *C. hummelincki* with other species of the *Calomys* group proposed by Vitullo et al. (1990).

**MATERIAL AND METHODS**

Animals were obtained by live trapping in five Venezuelan locations: Represa El Isiro (Falcón State, N=18), Curarigua (Lara State, N=13), Puerto Páez (Apure State, N=18), El Merey (Monagas State, N=5), Sipao (Bolívar State, N=3), and at Aruba Island (N=5).

Fig. 1 – Location of the sampled populations of *C. hummelincki*. 1: Aruba Island (N=5); 2: El Isiro (N=14); 3: Curarigua (N=12); 4: Puerto Páez (N=12); 5: Sipao (N=3); 6: El Merey (N=3). All places are in Venezuela, except Aruba.

Fig. 2 – Giemsa stained karyotype of *C. hummelincki* (2n=60, FN=64).
(Fig. 1). After capture, all animals were identified and transported to the laboratory to obtain bone marrow preparations, following HSU and PATTON (1969). Voucher specimens were deposited at Museo de la Estación Biológica de Rancho Grande (MARNR, Maracay, Venezuela), Museo de Historia Natural La Salle (Caracas, Venezuela) and Museo de Biología de la Universidad Simón Bolívar (Caracas, Venezuela). C- and G-banding were performed following SUMNER (1972) and SEABRIGHT (1971) with slight modifications. NOR regions were revealed by Silver Nitrate reaction (HOWELL and BLACK 1980). Chromomycin A3 (CMA) and 4,6-diamidino-2-phenyl-indol (DAPI) treatments were performed following SCHWEIZER (1976) with slight modifications.

RESULTS

All animals from all locations presented a stable diploid and fundamental number (2n=60, FN=64); they also had the following karyological characteristics: two pairs of large metacentrics, a small metacentric, a submetacentric X, while the remaining chromosomes and chromosome Y were acrocentrics (Fig. 2). We arranged the metacentrics in one group, calling them M1, M2 and M3, while the acrocentrics were numbered consecutively A1 to A26.

C-banding revealed the presence of pericentric heterochromatin in almost all homologous chromosomes, but it was very weak or almost nil in the large metacentrics and the acrocentric pairs A1, A21, A22, A23 and A24. Pairs A3, A7, A9, A11, A14, A18 and X exhibited conspicuous C bands (Fig. 3). The Y chromosome was fully heterochromatic. NOR regions were localized on the short arms of the telocentric pairs A8 and A21, and in interstitial positions on acrocentric pairs A11 and A22 (Fig. 4). Fluorescent banding revealed strong euchromatic blocks in several pairs: A1, A3, A5, A7, A8, A9, A12, A13,
A17, A19 and A22. DAPI counter-staining showed a close complementary banding pattern with respect to CMA. Neither CMA nor DAPI revealed the pericentromeric heterochromatin (Fig. 5).

The G-banding allowed us to identify almost 90% of the chromosomal complement (Fig. 6), with only the very small chromosomes being difficult to discern. We compared the G-banding pattern with those published for other Calomys species: C. callidus, C. venustus (VITULLO et al. 1990) and C. laucha, kindly facilitated by Dr. Maria Susana Merani of Universidad de Buenos Aires (Argentina). This comparison (Fig. 7) showed the correspondence of C. hummelincki and

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Fig. 4 – Metaphase cell of C. hummelincki showing the position (arrowheads) of Nucleolar Organizing Regions (NOR).

Fig. 5 – Karyotype of C. hummelincki metaphase stained sequentially with Chromomycin (A3) (cr) and DAPI (dap).
for almost all chromosomes. Some discrepancies are evident because it was not possible to obtain a better matching of some chromosomes pairs, e.g. pairs A6, A7 and A8 of *C. hummelincki* with pairs A7, A6 and A5 of *C. laucha*. However, the following points must be mentioned: M1 of *C. hummelincki* seems be the product of fusion of A2 and A3 of *C. laucha*; A1 of *C. hummelincki* seems be the result of fusion and posterior pericentric inversion of pairs A4 and A23 of *C. laucha*; the same seems be true for A2 of *C. hummelincki* and pairs A14 and A25 of *C. laucha*. The long arm of *C. laucha*’s M2 is similar to *C. hummelincki*’s A4, and the short arm of the same chromosome is similar to *C. hummelincki*’s A24. In addition, there are probably two paracentric inversions on *C. laucha*’s acrocentric pairs A1 and A12, which produce *C. hummelincki*’s acrocentric pairs A5 and A14, respectively. Finally, it seems that *C. hummelincki*’s acrocentric A3 is the result of duplication of a portion of *C. laucha*’s acrocentric pair A8.

A similar comparison of G-band karyotypes showed good correspondence between *C. hummelincki* and *C. venustus* (Vitullo et al. 1990), with the sole exception of a few discrepancies in the matching of some chromosome pairs. It should be noted that the two large metacentrics of *C. hummelincki* match the two biggest metacentrics of *C. venustus*, and the small metacentric 19 of *C. venustus* matches M3 of *C. hummelincki*. In addition, pair 4 of *C. venustus* seems be the result of a pericentric inversion of *C. hummelincki*’s A1. The possible fusion of *hummelincki*’s A3 with A5 should result in metacentric 3 of *C. venustus*, and fusion of *C. hummelincki*’s A10 with A15 will form metacentric 5 of *C. venustus*.

We do not present the comparison with *C. callidus*, since the comparison of it with *C. venustus* is given in Vitullo et al. (1990); thus with this work it is easy to establish its correspondence with *C. hummelincki*.

**DISCUSSION**

The diploid and fundamental number and the beta karyological characteristics described for *C. hummelincki* are the same as those reported by Pérez-Zapata et al. (1987). Our results also indicate the lack of chromosomal polymorphism in the populations examined, which is likely the situation for the other populations spread throughout the distribution range.

The position of constitutive heterochromatin on the autosomal and X chromosomes in *C. hummelincki* is similar to what has been found in other species of *Calomys*. The karyologically close *C. laucha* shows conspicuous pericentromeric
bands in almost all chromosomes (BRUM-ZORRILLA et al. 1990; SVARTMAN and ALMEIDA 1992). Nevertheless, the C-band pattern of *C. hummelincki* is similar to that of *C. lepidus* (ESPINOSA et al. 1997). In this species, the large metacentrics do not present pericentromeric heterochromatin like some acrocentrics. Other species of *Calomys*, e.g. *C. musculinus* and *C. lepidus*, show weak pericentromeric heterochromatic bands on the large metacentrics, which may indicate their Robertsonian origin (LISANTI et al. 1976; FORCONE et al. 1980; CICCIOLI 1991), while the other chromosomes present more or less conspicuous pericentromeric bands. The sex chromosomes show more variability: for example, the X chromosome of *C. lepidus* has a terminal heterochromatic band (ESPINOSA et al. 1997). Chromosome Y in *C. hummelincki* is fully heterochromatic, as in *C. lepidus* (ESPINOSA et al. 1997) and *C. callosus*, while this chromosome is not heterochromatic in *C. musculinus* (FORCONE et al. 1980; LISANTI et al. 1996).

The position of NOR in *C. hummelincki* is different from those observed in other *Calomys* species. In *C. laucha*, NOR are present in a centromeric position (BRUM-ZORRILLA et al. 1990), while in *C. callosus expulsus* they are present on the short arms of the acrocentric chromosomes (SVARTMAN and ALMEIDA CARDOSO 1992).

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Fig. 7 – Comparison of G-banded karyotypes of *C. laucha* (l), *C. hummelincki* (h) and *C. venustus* (v). Asterisks indicate the presence of paracentric inversions.
In *C. musculinus*, they are on the long arms of four metacentrics and the first submetacentric chromosome (Ciccioli 1991).

Chromomycin/DAPI fluorescent staining showed that *C. hummelincki* presents heavy fluorescent euchromatic regions with a weak R-banding pattern, which indicates a very rich concentration of C-G or A-T bases in some places depending on the fluorochrome used. The lack of evidence of pericentromeric heterochromatin with these dyes seems to indicate that the composition of constitutive heterochromatin is not the same in all chromosomes and it is included in the fluorescent block revealed by the fluorochrome used. In this case, it is necessary to carry out a more detailed analysis with DNA probes, which will permit a better characterization of this constitutive heterochromatin. A similar result was obtained in human chromosomes by Schweizer (1976), who found that the CMA procedure gave a weak R-banding pattern, while AMD/DAPI staining showed strong fluorescence blocks, complementary to the CMA pattern. Among *Calomys* species, this kind of banding has been performed only in *C. musculinus*: DAPI fluorescence revealed only pericentromeric heterochromatic regions, while Chromomycin showed an R-like banding pattern. In other rodents, e.g. *Thomomys bottae*, the pericentromeric C-bands were not revealed by CMA/DAPI fluorochromes, which only showed a weak R-like banding (Barros and Patton 1985). Meanwhile, in the chromosomes presenting heterochromatic regions, CMA and DAPI fluorochromes show different fluorescent patterns. In four species of *Microtus*, Burgos et al. (1990) found a differential response of heterochromatic fluorescent blocks, concluding that it could be due to modifications of the nucleotide composition or cluster organization, or to changes in the DNA-protein content which may alter the access of the fluorescent dyes to the DNA. A similar explanation of the results for the *Calomys* species is possible.

Unfortunately, we did not directly perform G-banding in the other species, resulting in a different elongation and resolution of the karyotypes of the various species compared. Thus we found some discrepancies in the comparison of the chromosome pairs. However, despite this limitation, it was possible to establish the correspondence of almost all chromosome pairs of *C. hummelincki* with those of *C. venustus* and *C. laucha*. Indeed, 22 pairs of chromosomes are similar among the three species (Fig. 7), suggesting a close relationship among them. With *C. hummelincki*’s chromosome position as reference, these chromosomes are two metacentric pairs (one large and one small), the acrocentric pairs A6 to A9, A11, A13 to A23, A25, A26 and X. It is interesting that the small metacentric pair in the *C. hummelincki* karyotype (equivalent in size to the medium to small acrocentric pairs) is observed in almost all species of *Calomys* with known karyotype (Ciccioli 1991; Gardenal et al. 1977; Brum-Zorrilla et al. 1990; Hurtado de Catalfo and Wainberg 1974; Lisanti et al. 1976). The presence of this particular chromosome, which presents pericentric heterochromatin in all species studied, suggests that the ancestral diploid number of the *Calomys* group was 68 instead of 70 as originally proposed by Pearson and Patton (1976).

Reig (1986) postulated that the phyllotine rodents, the tribe to which *Calomys* belongs, differentiated in the south-central Andes area and from there colonized the highlands and low open lands. The *Calomys* group has species present in puna areas (*C. lepidus*, Espinosa et al. 1997) and in low-altitude areas (*C. hummelincki*, Handley 1976; Martino 1997). Pearson and Patton (1976) postulated that *C. sorellus* should have the most primitive karyotype of the *Calomys* group, and they proposed an evolutionary derivation of the other species from *C. sorellus*. Vitullo et al. (1990), with new karyological evidence, modified the Pearson and Patton (1976) hypothesis, suggesting a more or less direct derivation of *C. musculinus* from the ancestral stock. In this hypothesis, *C. hummelincki* is intermediate between the *C. laucha*/C. sorellus (2n=64) stock and the *C. venustus* (2n=56) stock. However, our results do not suggest a direct derivation of *C. hummelincki* from *C. laucha*, as suggested by Vitullo et al. (1990). Indeed, to obtain a *C. hummelincki* karyotype from that of *C. laucha* would require three fusions, one fission, two pericentric inversions plus another two paracentric inversions. Considering the law of parsimony, it seems that all these events would have had a low probability of occurring at one time. On the other hand, it is probable that a *C. hummelincki* ancestor gave rise to the *C. venustus-C. lepidus* group, since it was possible to identify the two fusions and inversions necessary to convert a *C. hummelincki* karyotype into a *C. venustus* form. These results indicate that the chromosomal characteristics of *C. hum-
C. hummelincki and indicates no close or direct relationship between mosome number, like chromosomes from species with a higher chromosome number, like C. laucha. This evidence indicates no close or direct relationship between C. venustus-C. lepidus and found that Calomys DNA characteristics of three species of Calomys and that C. musculinus, which is also supported by the poor matching of the G-band karyotypes in a preliminary comparison of the two species.

It would be interesting to study the chromosomal characteristics of C. sorellus, a key species in this evolutionary pattern, which would permit us to establish if it was the antecedent of C. laucha or C. hummelincki. Nevertheless, studies on morphology and cytochrome b DNA indicate that the Calomys group could have a polyphyletic origin (STEPAN 1995, 1998), in which case C. sorellus should be assigned to a new genus within the phyllotine group. Corach et al. (1988) analyzed the DNA characteristics of three species of Calomys and found that C. callidus has a different DNA composition from that of C. laucha and C. musculinus. These authors proposed that the three species derived from different evolutionary lines. Recently, from sequences of cytochrome b DNA, Salazar-Bravo et al. (in prep.) concluded that Calomys species are organized into two clusters, one including C. venustus, C. lepidus and C. sorellus, and the other C. fecundus, C. venustus, C. callidus, C. callous, C. laucha and C. hummelincki. The last species seems to be the oldest, splitting in an independent branch from the other species. The chromosomal characteristics described for C. hummelincki indicate that it did not derive from C. laucha directly and that C. musculinus did not derive, at least directly, from C. hummelincki or C. laucha. However, it is necessary to conduct similar chromosome characterization studies on other species of Calomys for a clearer understanding of the evolutionary patterns of this interesting phyllotine group.

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