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Natural nidality in Bolivian hemorrhagic fever and the systematics of the reservoir species

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Abstract

Zoonoses within wild reservoir host populations often occur focally obeying Pavlovskii's rules of "natural nidality". What appears to be a clear example is Bolivian hemorrhagic fever (BHF), a disease endemic to northeastern Bolivia. The etiological agent is Machupo virus (MACV, Arenaviridae). The vertebrate reservoir, identified 30 years ago, was *Calomys callosus* a wild rodent common to open biomes in the lowlands of southeastern South America. The lack of concordance between the occurrence of MACV and the range of its rodent host has puzzled cadres of researchers and could be used as an exemplar of natural nidality. Here, we show that the populations of rodents responsible for the maintenance and transmission of MACV are an independent monophyletic lineage, different from those in other areas of South America. Therefore a clearer understanding of the systematics of the host species explains the apparent natural nidality of BHF. Similar studies may prove to be informative in other zoonoses.

Keywords: Calomys; Machupo; Bolivian hemorrhagic fever; Systematics; Infection; Bolivia; Beni; Arenaviridae

1. Introduction

A characteristic of a number of zoonoses is their apparent natural nidality (Pavloskii, 1966), a concept that describes the localized or nested occurrence of zoonoses. Several hemorrhagic fevers of arenaviral origin in the Americas appear to demonstrate this phenomenon (Vainrub and Salas, 1994), as most pathogenic arenaviruses have an incomplete pattern of overlap with the host species range, and at least two well-documented examples exist. Junin virus, the etiological agent of Argentine hemorrhagic fever (AHF) is found primarily in the murid rodent *Calomys musculinus*, whose area of distribution is larger than the endemic area of AHF (Mills and Childs, 1998). Similarly, Guanarito virus, the etiological agent of Venezuelan hemorrhagic fever (VHF) is found in *Zygodontomys brevicauda* whose area of distribution is larger than the endemic area of distribution of VHF (Fulhorst et al., 1997; Voss, 1991). The only known disease-causing arenavirus endemic to Africa (Lassa virus) appears to demonstrate a somewhat similar pattern: the natural host of the virus is common in all Africa south of the Sahelian zone (Granjon et al., 1997), but Lassa fever appears to be restricted to west Africa.

Another eloquent example of this lack of complete overlap is Bolivian hemorrhagic fever (BHF), a severe acute disease caused by Machupo virus (MACV), isolated from humans (Johnson et al., 1965) and wild murid rodents identified as *Calomys callosus* (Johnson et al., 1966). The distribution of *C. callosus*, as currently understood (Olds, 1988), includes the lowlands of Bolivia, Brazil, Paraguay, and Argentina, whereas BHF is known and endemic to only a handful of localities in northeastern Bolivia (Fig. 1). This scenario begs the question: why is BHF found in only a certain restricted geographic area if the apparent reservoir rodent is widespread in several South American countries? This study examines the phylogenetic relationships of the "*callosus* clade" (Salazar-Bravo et al., 2001) of *Calomys*, in an effort to understand the perceived natural nidality of this hemorrhagic fever.

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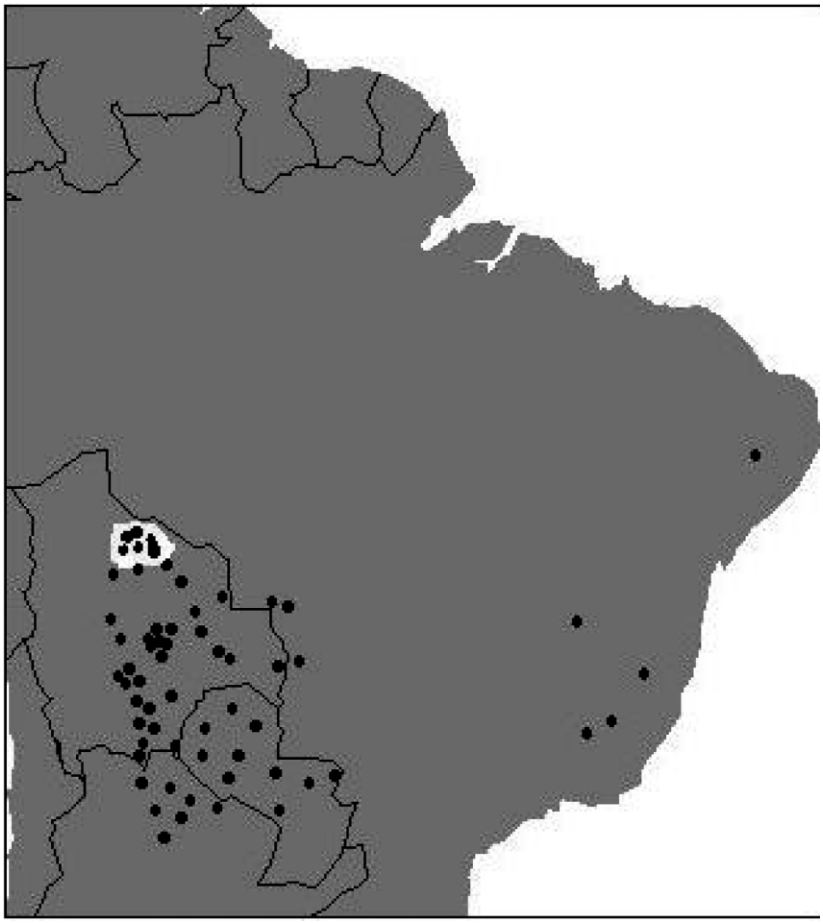

Fig. 1. The distribution of *C. callosus* as currently recognized. Black dots represent localities based on museum specimens. The white area is the region where BHF has been reported in the Beni Department of Bolivia.

2. Methods

DNA from tissue (frozen or alcohol preserved) was isolated by cell lysis followed by organic solvent purification from 36 specimens of the *callosus* clade and two specimens of *C. laucha* (outgroup). Animals from the *callosus* clade of *Calomys* were obtained from throughout the range of this group of species. The entire sequence of cytochrome *b* gene was obtained using a combination of primers and protocols slightly modified from the literature (Anderson and Yates, 2000). Additionally, we amplified the entire displacement loop region in the mitochondrial genome of *Calomys* using the following combination of primers. For the light strand DF434 (5′-CAT GAA TCG GAG GAC AA-3′), DF465 (5′-ATA CCC CTC TTC TCG CT-3′), and for the heavy strand DR678 (5′-TTG ACG GCT ATG TTG AGG-3′), and DR1117 (5′-GAC CAA ACC TTT CGG TG-3′). The approximate locations of these primers in the mitochondrial genome of *Mus* are: DF434 at L15245, DF465 at L15719, DR678 at H15899, and DR1117 at H80.

Relationships among members of the *callosus* clade of *Calomys* and the outgroup were assessed by the use of the maximum parsimony (MP) and maximum likelihood (ML) methods using PAUP* 4.0b6 (Swofford, 2000). For the MP analysis all characters were treated as unordered and equally weighted. The data set was subjected to a heuristic search of 100 random addition replicates with TBR branch swapping. Bootstrap values were calculated using 1000 bootstrap replicates with heuristic searches, with 10 random addition replicates, and TBR branch swapping. Bremer decay indices (Eriksson, 1998) values were calculated as surrogates for branch support. In the ML analysis, sequences were analyzed based on a model (HKY + Γ) that optimizes base composition, variable rates of change at different sites, and the ratio of transitions to transversions.

3. Results

The sequence of the cytochrome *b* gene sequence (1140 base pairs), plus 76 base pairs (bp) of the Phe^{tRNA} and 996 bp of the displacement loop were obtained from all specimens analyzed. The topology of the trees in both (MP and ML) analyses was similar (Fig. 2). In the MP analysis there were

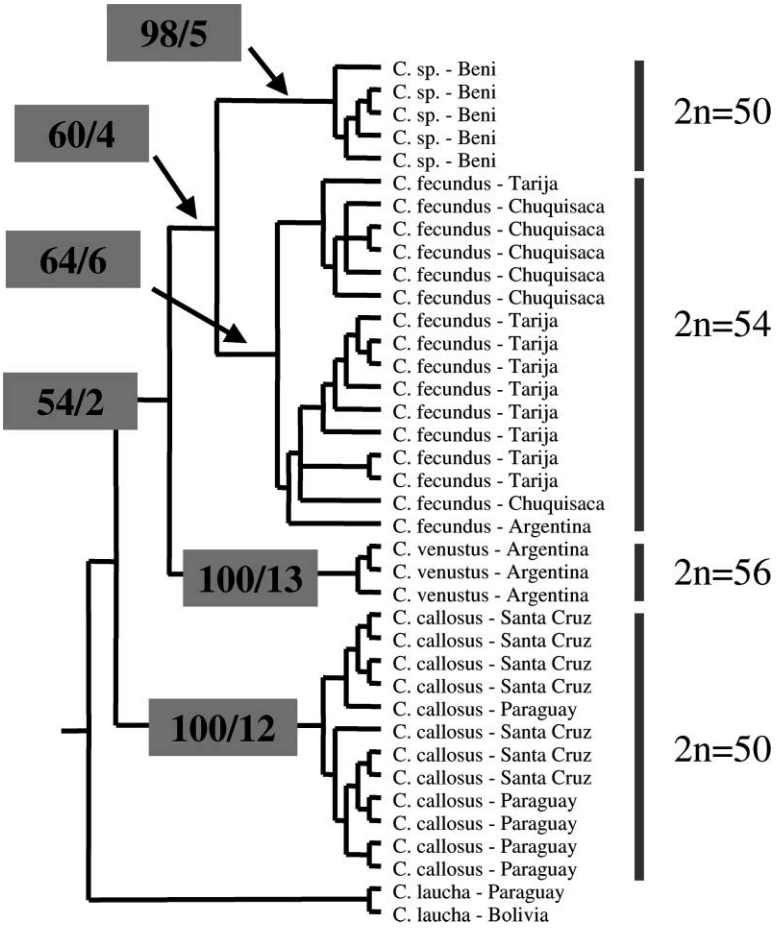

Fig. 2. Phylogenetic relationships among populations of *Calomys* based on both cytochrome *b* gene and displacement loop sequence, a total of 2212 bp shown is the most parsimonious tree obtained when all nucleotide substitutions among cytochrome *b* and displacement loop DNA sequences in 38 specimens on *Calomys*, were analyzed. A similar topology was obtained with ML analysis. The 336 sites were parsimony-informative. Tree length is 641, CI = 0.872, HI = 0.128, and RI = 0.9013. Bootstrap support from 1000 replicates where it is 50% or greater are included above the branches, followed by Bremer decay indices (under the branch) calculated with the program autodecay. *C. callosus*: *Callomys callosus*; *C. venustus*: *Callomys venustus*; *C. laucha*: *Callomys laucha*. All localities denote departments in Bolivia, unless otherwise noted. Diploid chromosome counts are included for the different groups.

336 parsimony informative sites, and the tree length was 641. Only one most parsimonious tree was found with a consistency index (CI) of 0.872, homoplasy index of 0.128 and retention index of 0.9013. The ln-likelihood value under the HKY + Γ model for the tree in Fig. 2 is −6700.37771, with empirical base frequencies ($A = 0.30830$, $C = 0.24326$, $G = 0.12769$, $T = 0.32075$), a transition-to-transversion ratio of 1.771525 ($\kappa = 3.7108$), and the value of the gamma shape parameter, $\alpha = 0.2727$.

In all analyses, a clade formed by specimens assigned to *C. callosus* from Paraguay and the Bolivian Department of Santa Cruz was basal to other groups (Fig. 2). The resolution offered by the analysis of these data indicates the existence of three reciprocally monophyletic lineages within currently recognized *C. callosus* s.l. supporting at the same time the differentiation of *Calomys venustus*. The *C. callosus* s.l. clade is paraphyletic consisting of three different natural groups. The first group is formed by populations assigned to this species from the lowlands of central, south and southeast Santa Cruz Department in Bolivia, and west and northeast Paraguay. The second group is formed by specimens from populations in the Beni Department and in the third group is composed of animals from intermediate elevations in the Bolivian Departments of Tarija and Chuquisaca (southern Bolivia) and northern Argentina.

4. Discussion and conclusions

The taxonomy and systematics of several groups of zoonotic agent hosts is in disarray. For example, most murid rodent genera have not been critically revised since they were first described, in many cases at the end of the 19th century. Zoonoses with wild animal reservoirs often occur focally, obeying Pavloskii's rule of natural nidality and controlling the transmission to humans of such zoonoses

depends on the accurate identification and a clear understanding of the ecology of the reservoir host. In order to design appropriate ecological studies that will result in predictive models for control, it is critical that the reservoir be accurately identified and placed in a phylogenetic context.

4.1. Taxonomic implications

The rodent genus *Calomys* (Sigmodontinae, Phyllotini) is a widespread member of the neotropical rodent fauna and along with other members of the tribe Phyllotini, it is common to abundant in South American landscapes. The genus shows a disjunct distribution. One species (*Calomys hummelincki*) inhabiting the Llanos of northern South America and islands off the Venezuelan coast north of the Amazon basin, and several more ranging south of the Amazon basin in the grasslands, savannas and forest fringes of Brazil, Bolivia, Peru, Argentina, Paraguay, Uruguay and portions of northern Chile. This peculiar distribution suggests that the evolution of the genus is tightly associated to that of the non-forested biomes of South America (Salazar-Bravo et al., 2001).

Recent attempts to understand the systematics and taxonomy of the genus *Calomys* has evidenced the difficulties associated with such a task (e.g. Salazar-Bravo et al., 2001). There is no agreement on the identity and number of species involved in the genus (Table 1) and even the monophyly of the genus has been questioned (e.g. Steppan, 1995). Olds (1988) revised the genus and based on external characters, skull and body morphometry and scattered chromosomal information, recognized 10 species: *C. bimaculatus*, *C. callosus*, *C. hummelincki*, *C. laucha*, *C. lepidus*, *C. murillus*, *C. musculinus*, *C. sorellus*, *C. tener*, and *C. venustus*. Musser and Carleton (1993) recognized nine species with this and Olds' (1988) lists sharing only seven species. One of the points of disagreement is the number of species recognized of the "*callosus–venustus*" species group. Recent work in our laboratory (Salazar-Bravo, unpublished results) suggests that *Calomys* is indeed monophyletic and composed of at least 12 recognizable species (see below).

The analyses presented in this paper suggest that there are four phylogenetic units within our sample of what is currently recognized as the *callosus–venustus* clade. Whilst bootstrap values and decay indices do not strongly support deeper branches they do argue for cohesive units at terminal branches, most notably the *C. callosus* s.s. and the *C. venustus* clades.

The analysis of chromosomal and natural history information of the species analyzed herein provides additional support to the recognition of these as independent lineages. For example, all animals from the four Beni populations (see Appendix A for a list of animals used in karyotypic analyses) present a chromosomal complement of $2n = 50$ and a fundamental number (FNa = 66; Salazar-Bravo, unpublished results). This chromosomal complement is identical to the one presented by Olds (1988) for *C. callosus* and by Pearson and Patton (1976) for *Calomys fecundus*. In that same paper, Pearson and Patton (1976) presented a chromosomal complement for what the considered *C. callosus* from Paraguay with a diploid and fundamental number much lower than

Table 1
Species of *Calomys* recognized in the primary literature and in some subsequent synopses

Primary literature	Cabrera (1961)	Hershkovitz (1962)	Olds (1988)	Musser and Carleton (1993)	Espinosa et al. (1997)
C. bimaculatus	*C. callosus*	*C. callosus*	*C. bimaculatus*	*C. bolivae*	*C. boliviae*
C. laucha	*C. dubius*	*C. laucha*	*C. callosus*	*C. callidus*	*C. callidus*
C. m. musculinus	*C. expulsus*	*C. lepidus*	*C. hummelincki*	*C. callosus*	*C. callosus*
C. m. cortensis	*C. frida*	*C. sorellus*	*C. laucha*	*C. hummelincki*	*C. hummelincki*
C. c. callosus	*C. gracilipes*		*C. lepidus*	*C. laucha*	*C. laucha*
C. c. bolivae	*C. laucha*		*C. murillus*	*C. lepidus*	*C. lepidus*
C. gracilipes	*C. lepidus*		*C. musculinus*	*C. musculinus*	*C. musculinus*
C. expulsus	*C. muriculus*		*C. sorellus*	*C. sorellus*	*C. sorellus*
C. lepidus	*C. tener*		*C. tener*	*C. tener*	*C. venustus*
C. tener	*C. venustus*		*C. venustus*		
C. venustus					
C. sorella					
C. ducilla					
C. carillus					
C. c. marcarum					
C. c. argurus					
C. murillus					
C. m. cordovensis					
C. frida					
C. f. miurus					
C. muriculus					
C. fecundus					
C. hummelincki					

Subspecific treatments are included only for the primary literature. Primary literature is arranged chronologically and alphabetically thereof.

those reported here (i.e. $2n = 36$, FNa = 48). We have suggested elsewhere (Salazar-Bravo et al., in preparation) that, due to technical difficulties, the ideogram published by Pearson and Patton represents a subset of the complete karyotype of *C. callosus*. Animals from *C. venustus* show the typical chromosomal complement of $2n = 56$ (FNa = 66) that Tiranti (1996), and Espinosa et al. (1997) have reported for this species. The populations from Tarija and Chuquisaca show a $2n = 54$ (FNa = 66) similar to the chromosomal data that De Catalfo and Wainberg (1974) reported for animals from Department of Faimalla in the Argentinean province of Tucuman. Populations from Santa Cruz and Paraguay show a $2n = 50$ (FNa = 66). Karyotypes similar to those reported here for populations in Tarija/Chuquisaca and Santa Cruz were also reported by Malygin et al. (1998). We acknowledge that the chromosomal information for the Beni form and that of *C. callosus* s.s. being similar in diploid and fundamental number lends weak support to the chromosomal diagnosis of the Beni form. We have begun the analyses of both morphometric data and differentially banded karyotypes in all of these species and will be reporting them elsewhere.

Our analyses include animals from Tucumilla a Tarija locality near the *terra typica* of *C. fecundus* (Tablada). These animals correspond closely to the description of *C. fecundus* (Thomas, 1926) and form a monophyletic entity with a diploid number of $2n = 54$. Therefore, it may be warranted to recognize such as a valid species name. Embedded into the clade of *C. callosus* s.s. are animals that are morphologically similar and come from near the type locality of *C. muriculus*. These specimens form part of the *C. callosus* s.s. clade which, by virtue of including specimens from Paraguay, may properly be referred to as *C. callosus*. The identity of *C. venustus* is well warranted based on chromosomal (Vitullo et al., 1990), and the genomic data reported herein. That leaves only one clade that has not yet received formal recognition, and that may deserve one: the Beni populations.

Habitat differentiation provides an alternative source of informative data. To convey this point we have relied on the most updated map of the vegetation of South America. This map, prepared by the Woods Hole Research Center (ttp://www.whrc.org/science/Globfor/globfor.ht) presents the land cover of South America at a resolution equal to 1 km based on satellite imagery from the NOAA AVHRR series. Fig. 3 depicts the general vegetation types that the species included in this report inhabit. As it can be seen, *Calomys* from Beni occurs in an isolated patch of grassland vegetation dubbed Pampas de Moxos or Llanos del Beni. This restricted Bolivian habitat is characterized by poorly drained and seasonally waterlogged soils that extend into smaller and isolated representatives in the Bolivia–Peru border (Pampas del Heath). It has been suggested (Sarmiento, 1990; Haase and Beck, 1989) that the vegetation in this habitat closely resembles that of the Venezuelan llanos and Guayanan savannas, with influences from the closer Cerrados of Brazil. A complete ecological characterization of this habitat can be found in Hanagarth (1993).

Our samples of *C. fecundus* come from several localities in the eastern flanks of the Andes of the Bolivian Departments of Tarija and Chuquisaca and the Argentinean Department of Salta. Populations from this species ($2n = 54$) occur in a belt of mountain forest at middle elevations (600–2000 m) along the eastern flanks of the Andres (Fig. 3). This phyogeographic region was called the southeast Coordilleta by Pacheco et al. (1994). These authors suggest that the Tucumano-Boliviano forest is one of the vegetation types included in this phytogeographic region.

Only three specimens of *C. venustus* from two localities in Argentina were included in our analyses. Our specimens come from localities in the "Espinal" biome in central Argentina, and we agree with Tiranti (1996) in suggesting this species as associated to this biome. We believe *C. callosus* s.s. is almost exclusively associated with Chacoan vegetation and enclaves of such in other habitats (e.g. east of the Paraguay River) or near the base of the Andes (e.g. the headwaters of the Grande and Itonama rivers). Myers (1982) has suggested a similar phenomenon for other rodent species (e.g. *Oligoryzomys chacoensis*). As representatives of this species have been collected from habitats associated to Chaco habitat in both Bolivia and Paraguay, it is likely that this species is also present in the Argentine Chaco.

Other species associated with the *callosus–venustus* clade but not included in these analyses due to the lack of specimens are *Calomys boliviae* and *C. callidus*. The association of *C. boliviae* with the remaining of the members in the *callosus–venustus* clade needs to be evaluated critically. The rather different habitat it occupies in central Bolivia and its rarity in museum collections suggests the need for further research and collecting in these areas. Another member of potential bearing in this group is *C. callidus* from the Argentine Mesopotamia. Based on chromosomal information ($2n = 48$; Vitullo et al., 1990) the specific identity of this species appears to be warranted. In summary, we recognize the following species as members of the *callosus–venustus* clade: *C. callosus*, *C. venustus*, *C. callidus*, *C. fecundus*, and *C. innom* (ex Beni).

The tree in Fig. 2 is composed of four reciprocally monophyletic clades. For three of them binomial names do exist. *C. venustus* from Argentina, *C. callosus* s.s. from Paraguay and Bolivia, a *C. fecundus* from the Andean foothills from southern Bolivia and northern Argentina and a monophyletic lineage from the Beni region of Bolivia. However, regardless of the taxonomic status of these lineages it is clear that the populations that host MACV, the causative agent of BHF, have had an independent evolutionary history, which helps explain why this disease only occurs in northeastern Bolivia and not throughout the species range. This result resolves the apparent lack of overlap between the distribution of MACV and *C. callosus*, and confirms the high degree of host specificity found between areanviruses and their mammalian reservoirs (Salazar-Bravo et al., 2002).

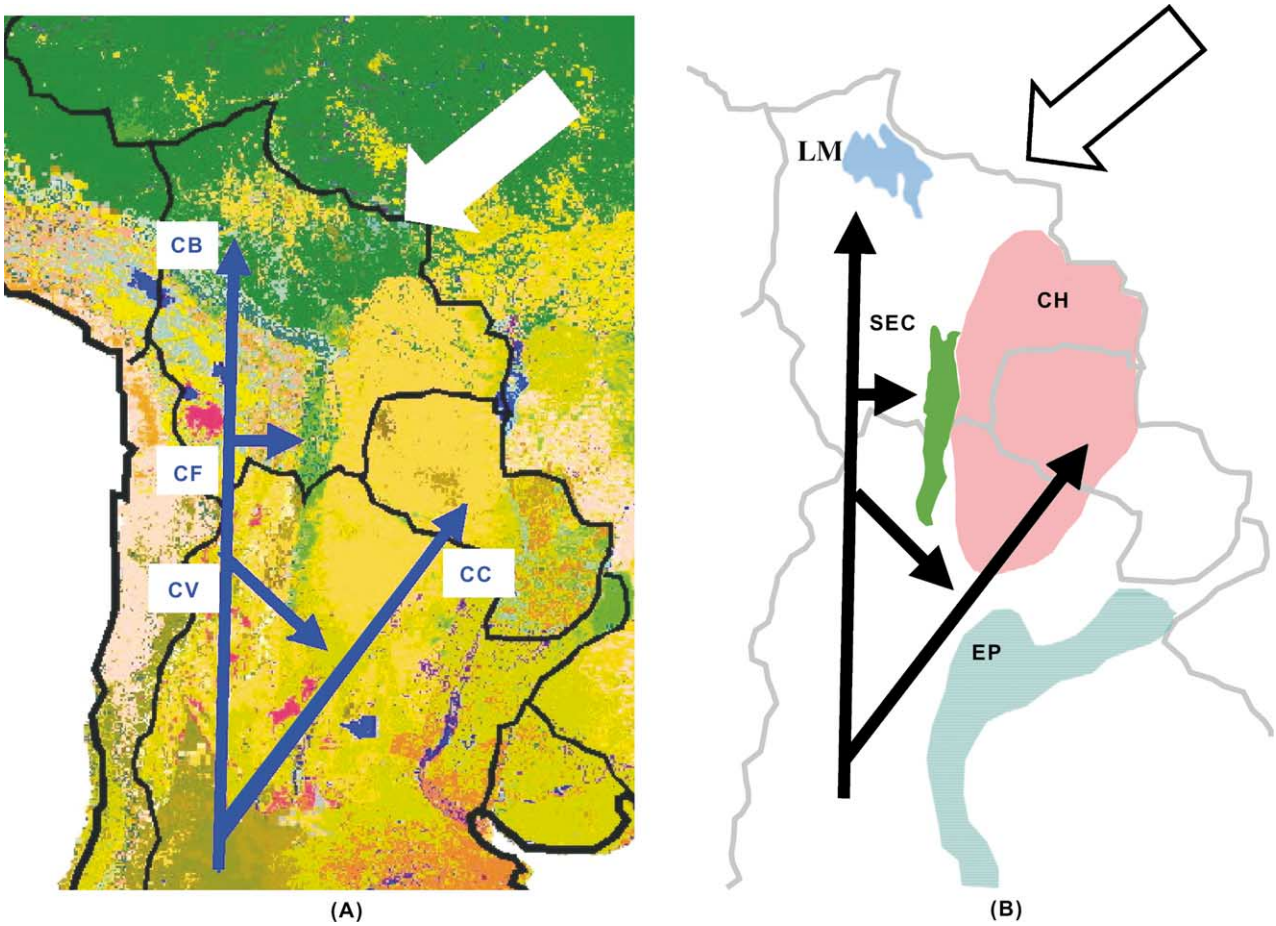

Fig. 3. Vegetation map of the area of interest, with the vegetation units discussed in the text marked. A: a "summary" cladogram of the taxa under study; CB: *Calomys* sp. ex Beni; CF: *C. fecundus*; CV: *C. venustus*; CC: *C. callosus*. The white arrow points to the forested area that separates the Llanos de Moxos from the Chaco region. B: distribution of vegetation units in the region of interest; LM: Llanos de Moxos; SEC: southeast Coordillera; CH: Chaco; EP: Espinal.

4.2. Coevolution between muroid rodents and arenaviruses

Current understanding of the Arenaviridae indicates that most of these viruses are associated with a particular species of the rodent family Muridae (e.g. Bowen et al., 1997; Gonzales and Duplantier, 1999, for a review see Salazar-Bravo et al., 2002). There are only two exceptions: Sabia virus for which no wild reservoir is known, and Tacaribe virus, which was isolated from bats of the genus *Artibeus*. Arenaviruses from the old world are known to be hosted by murid rodents of the subfamily Murinae (old world rats and mice), while members of the rodent subfamily Sigmodontinae (new world rats and mice) host arenaviruses in the Americas. This pattern indicates a virus–host association of at least 30 million years. No arenavirus is known to be hosted by members of the third largest subfamily of rodents (the Arvicolinae: voles, lemmings and muskrats), which could be construed as support to a closer evolutionary relationship between murines and sigmodontines. Recent discoveries of several new species of arenavirus in North America (e.g. Whitewater Arroyo in *Neotoma albigula* and an undescribed species in *Peromyscus* from Arizona) closely related to South American forms support a sigmodontine clade containing Neotomine/Peromyscine species, contra Engel et al. (1998).

Hugot et al. (2001) presented evidence that supports the "diffuse coevolution" hypothesis between old word arenaviruses and their rodent hosts. This model proposes as the most common mechanisms of transmission a parallel phylogeny (cophylogeny) between viruses and rodent hosts, while allowing for host switches between closely related taxa. Ongoing analyses in our laboratory support a similar model for the new world arenavirus.

Phylogenetic relationships of rodent hosts and arenaviruses have not been found to be as concordant as those among hantaviruses and their reservoirs, although several clades of both viruses and their rodent hosts did show support for a co-evolutionary scenario (Bowen et al., 1997; Schmaljohn and Hjelle, 1997). It is quite possible, however, that this fact may have resulted, at least in part, from the still incipient state of knowledge of the species phylogenies of the rodent hosts. The same is true even at the genus level within some of these groups, for example, several species of

Peromyscus harbor highly host-specific hantaviruses (Monroe et al., 1999). We submit that a clear understanding of the evolutionary histories of these groups is essential to develop predictive models involving human health regardless of whether the evolution of these groups has been branch for branch or not.

It is possible to use the information available on the evolution of the Arenaviridae and the phylogenetic relationships of the rodent host, as a "predictive tool" to find and study viruses of potential human importance. Fulhorst et al. (1997) have outlined the phylogenetic relationships of the new world arenaviruses (NWA). These authors suggest that NWA can be grouped into three lineages A, B, and C. Among the members of the "B" lineage are viruses highly pathogenic to humans (e.g. Guanarito, Junin, Sabiá, and Machupo), but also other that are not known to be causative agents of disease (e.g. Tacaribe, and Amapari). Because Guanarito virus, the causative agent of Venezuelan hemorraghic fever is closely related to Amapari virus, we suggest that there exist the possibility that Amapari virus still may be shown to be pathogenic. Both mammalogists and public health workers in northeastern Brazil—where Amapari virus was originally identified—need to be aware of the presence of this potential disease-causing agent whose reservoirs are rodents of the genus *Neacomys* (Peters, 1997). However, other arenaviruses are known to have caused laboratory infections even though they do not belong to the group B of arenaviruses. For example, Flexal virus a member of the A group of NWA is known to have caused two laboratory infections with disease (Peters, 1997).

1.1. Co-speciation and coevolution

The results presented herein strongly support a pattern of host specificity already postulated elsewhere (Salazar-Bravo et al., 2002, and references therein). As further support of this co-evolutionary pattern, Bowen et al. (1998) have suggested that the name Cupixi should be assigned to a virus strain previously assigned to Amapari, but isolated from a species of *Oryzomys*. Therefore the arenavirus Amapari is known to infect *Neacomys spinosus* whereas *Oryzomys megacephalus* harbors Cupixi (Salazar-Bravo et al., 2002).

Regardless of the taxonomic status of the Beni *Calomys* it is clear that these populations, reservoirs of Machupo virus, have had an independent evolutionary history, which helps explain why this disease only occurs in northeastern Bolivia and not throughout the species range. This result resolves the apparent lack of overlap between the distribution of MACV and *C. callosus*.

The results reported here support earlier claims (Baker and Yates, 1998) of the value of research collections and storage of properly vouchered specimens, given that most of the specimens used in this study were collected for other purposes. This study further supports the need for multidisciplinary research and underscores the importance of understanding the evolutionary history of reservoir species, as well as their ecology and natural history. The need to combine all of these in a concerted effort to address the increasing threat of emerging infections should be underscored.

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Appendix A

Specimens sequenced in this study, identification numbers, GeneBank accession codes and locality information of the samples studied. All NK numbers were obtained from the Museum of Southwestern Biology, AK numbers from the Sam Noble Museum of Natural History, and TK numbers from Texas Tech University. All localities are in Bolivia unless otherwise specified. Animals used in karyotypic analyses are marked with an asterisk.

Species	Identification code	GeneBanks accession code		Locality
		Cytb	D-loop	
Calomys spp.	NK 27668*	AY033153	AY033191	Beni; El Valle, 13°39′S, 64°26′W
Calomys spp.	NK 37800	AY033154	AY033192	Beni; Villa Olga, 13°38′S, 64°43′W
Calomys spp.	NK 37735	AY033155	AY033193	Beni; La Republica, 13°10′S, 64°10′W
Calomys spp.	NK 37787	AY033156	AY033194	Beni; Chumano, ca. 13°37′S, 64°49′W
Calomys spp.	NK 37739	AY033157	AY033195	Beni; La Republica, 13°10′S, 64°10′W
C. fecundus	NK 23834*	AY033158	AY033196	Tarija; Padcaya, 21°47′S, 64°40′W
C. fecundus	NK 21508	AY033159	AY033197	Chuquisaca; Monteagudo, 19°49′S, 63°58′W

Appendix A. (*Continued*)

Species	Identification code	GeneBanks accession code		Locality
		Cytb	D-loop	
C. fecundus	NK 21330	AY033160	AY033198	Chuquisaca; Monteagudo, 19°49′S, 63°58′W
C. fecundus	NK 21355	AY033161	AY033199	Chuquisaca; Monteagudo, 19°49′S, 63°58′W
C. fecundus	NK 21357	AY033162	AY033200	Chuquisaca; Monteagudo, 19°49′S, 63°58′W
C. fecundus	NK 21923*	AY033163	AY033201	Chuquisaca; Chuhuayacu, 19°43′S, 63°51′W
C. fecundus	NK 23650*	AY033164	AY033202	Tarija; Tucumilla, 21°27′S, 64°49′W
C. fecundus	NK 23695*	AY033165	AY033203	Tarija; Tucumilla, 21°27′S, 64°49′W
C. fecundus	NK 23697*	AY033166	AY033204	Tarija; Tucumilla, 21°27′S, 64°49′W
C. fecundus	NK 23707	AY033167	AY033205	Tarija; Tucumilla, 21°27′S, 64°49′W
C. fecundus	NK 23705*	AY033168	AY033206	Tarija; Tucumilla, 21°27′S, 64°49′W
C. fecundus	NK 25116	AY033169	AY033207	Tarija; Carapari, 21°48′S, 63°47′W
C. fecundus	NK 23354	AY033170	AY033208	Tarija; Camatindy, 21°00′S, 63°23′W
C. fecundus	NK 23361	AY033171	AY033209	Tarija; Camatindy, 21°00′S, 63°23′W
C. fecundus	NK 12564	AY033172	AY033210	Tarija; Porvenir, 20°45′S, 63°13′W
C. fecundus	AK 15276	AY033173	AY033211	Argentina; Tucuman, ca. 26°49′S, 65°22′W
C. venustus	TK 49115*	AY033174	AY033212	Argentina; Cordoba, ca. 33°01′S, 64°21′W
C. venustus	TK 49116*	AY033175	AY033213	Argentina; Cordoba, ca. 33°01′S, 64°21′W
C. venustus	AK 15337	AY033176	AY033214	Argentina; Stgo del Estero, 9°26′S, 63°34′W
C. callosus	NK 12308*	AY033177	AY033215	Santa Cruz; Stgo de Chiquitos, 18°18′S, 59°36′W
C. callosus	NK 12310	AY033178	AY033216	Santa Cruz; Stgo de Chiquitos, 18°18′S, 59°36′W
C. callosus	NK 21176	AY033179	AY033217	Santa Cruz; Las Cruces, 17°47′S, 63°22′W
C. callosus	NK 23320	AY033180	AY033218	Santa Cruz; Zanja Honda, 18°16′S, 63°11′W
C. callosus	NK 72351	AY033181	AY033219	Paraguay; Monte Palma, 22°36′S, 59°31′W
C. callosus	NK 13009*	AY033182	AY033220	Santa Cruz; San Ramón, 16°36′S, 62°42′W
C. callosus	NK 11590	AY033183	AY033221	Santa Cruz; San Miguel Rincón, 17°23′S, 63°32′W
C. callosus	NK 23306	AY033184	AY033222	Santa Cruz; Zanja Honda, 18°16′S, 63°11′W
C. callosus	NK 22532	AY033185	AY033223	Paraguay; Cerro Cora NP, 22°39′S, 56°01′W
C. callosus	NK 22523	AY033186	AY033224	Paraguay; Cerro Cora NP, 22°39′S, 56°01′W
C. callosus	NK 72344*	AY033187	AY033225	Paraguay; Monte Palma 22°36′S, 59°31′W
C. callosus	NK 72378	AY033188	AY033226	Paraguay; Boqueron, Filadelfia 22°20′S, 60°02′W
C. laucha	NK 25158	AY033189	AY033227	Tarija; Estancia Bolivar, 21°38′S, 62°34′W
C. laucha	NK 72376	AY033190	AY033228	Paraguay; Boqueron Filadelfia, 22°20′S, 60°02′W

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