



## Original investigation

# The phylogenetic position of southern relictual species of *Microtus* (Muridae: Rodentia) in North America

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## Abstract

Climatic fluctuation led to isolation of some populations of temperate species on southern mountaintops during warming trends. The most southern species of arvicoline rodents (*Microtus guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus*) in North America may be relicts, isolated in the mountains of Mexico and Guatemala at the end of the Pleistocene. We used parsimony and likelihood analyses of complete mitochondrial cytochrome b gene sequences of 28 species of *Microtus*, including eight Eurasian species, holarctic *M. oeconomus*, and all extant North American species except the island endemic *M. breweri*. North American species of *Microtus* were monophyletic under the maximum-likelihood criterion, but polyphyletic under parsimony. Likelihood ratio tests and bootstrapping indicated a rapid basal radiation with short intervals between cladogenic events. However, several sister taxon relationships were robust to bootstrapping or consistent between methods. We found that *M. quasiater* was sister to *M. pinetorum*, and these taxa were sister to a clade of *M. oaxacensis* and *M. guatemalensis*. The phylogenetic position of *M. umbrosus*, however, was unclear. Monophyly of the relicts was rejected by a likelihood ratio test, suggesting multiple southern invasions by arvicoline rodents. Phylogenetic data for these and other co-distributed taxa should be used in conservation efforts for these remote areas.

**Key words:** *Microtus*, Arvicolinae, historical biogeography, conservation, molecular systematics, Mexico

## Introduction

Climatic fluctuations have been linked to the spatial expansion and contraction of species (GRAHAM and GRIMM 1990; GRAHAM et al. 1996). Isolation during contraction phases may have stimulated allopatric diversification, particularly at the southern edge of ranges. For example, the mountains of Mexico and Guatemala host a highly en-

demic flora and fauna (RAMAMOORTHY et al. 1993) that includes a diverse set of organisms associated with mesic environments. These organisms apparently invaded the region during cooler periods and then became isolated at higher elevations as conditions at lower elevations became drier and warmer. This invasion and isolation

cycle may have occurred numerous times during the Pleistocene and has contributed to the complex biogeographic history of the region (SULLIVAN et al. 1997).

The genus *Microtus* is holarctic and north temperate in distribution and in the New World reaches its southern limit in Central America. At higher latitudes, up to five species (e.g. *M. longicaudus*, *M. miurus*, *M. oeconomus*, *M. pennsylvanicus*, and *M. xanthognathus* in Yukon Territory) may be found in close proximity. However, species tend to be more allopatrically distributed at southern latitudes. For example, *M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* are endemic to separate mountains in the cloud and pine forests of Mexico and Guatemala (Fig. 1). *M. quasiater*, the Jalapan vole, is found in the southern Sierra Madre Oriental in Central Mexico. *M. oaxacensis*, the Tarabundi vole, is isolated in the Sierra de Juarez of Oaxaca, and occurs at elevations of 1,600 (SANCHEZ et al. 1996) to 2,499 m (JONES and GENOWAYS 1967). *M. umbrosus*, the Zempoaltepec vole, is restricted to approximately 80 km<sup>2</sup> at elevations ranging from 1,829 to 3,000 m (FREY and CERVANTES 1997) around Mt. Zempoaltepec in Oaxaca. *M. guatemalensis*, the most southern species, occurs from the mountains of central Chiapas south to central Guatemala. These species may be the result of peripheral isolation of ancestors that were more widely distributed during cool periods

of the early to middle Pleistocene (HOFFMANN and KOEPL 1985).

In contrast to these four disjunct species, *M. mexicanus* is widespread in Mexico and occurs in limited sympatry or parapatry with *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* with a discontinuous distribution extending from New Mexico and Arizona to southern Mexico (HALL 1981). Its fossil record is limited to the late Wisconsinan from San Josecito in northeastern Mexico and localities farther north (ZAKRZEWSKI 1985). Phylogenetic relationships between *M. mexicanus* and other species of *Microtus* are unclear, but there is little indication that this species shares a common ancestor with other Mesoamerican species. Thus, its presence in Mexico probably reflects an independent colonization.

The aim of our study therefore is to investigate the phylogenetic relationships of the southern species of *Microtus* in order to test hypotheses regarding their ancestry in North America and their taxonomic relationships to other species.

## Material and methods

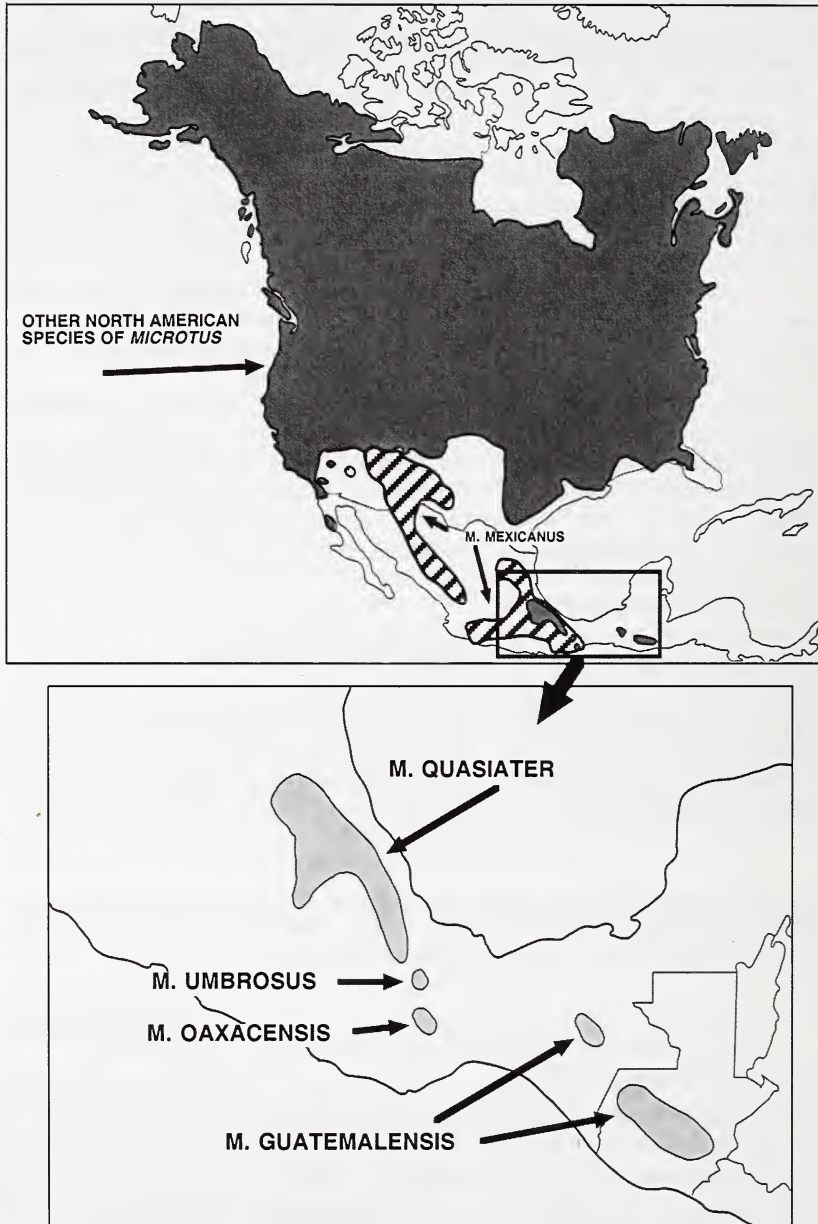
DNA was extracted from ethanol-preserved tissues of these four southern *Microtus* (Tab. 1) with methods described in CONROY and COOK (1999). The cytochrome b gene (hereafter cyt b) was amplified in two or three sections and sequenced in

**Table 1.** Specimens of Meso-American species of *Microtus* examined in this study (in addition to those reported in CONROY and COOK [2000]) including location from which each specimen was collected, and CNMA (Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México) catalog number.

Species	Collection location	CNMA Catalog Number
<i>M. umbrosus</i>	Mexico: Oaxaca Cerro Zempoaltepetl, 5 km N Sta Ma. Yacochi. Mpio. Tlahuitoltepec, 2450 m.	34890, 34894
<i>M. guatemalensis</i>	Mexico: Chiapas: Cerro Tzontehuitz, 13 km NE San Cristobal de las Casas, Mpio. Chamula, 2880 m. Mexico: Oaxaca: 11 km SW La Esperanza, Mpio. Santiago	35262
<i>M. oaxacensis</i>	Comaltepec, 2000 m.; Oaxaca: 11 km SE La Esperanza, Mpio. Santiago Comaltepec, 2000 m.	27415, 33815
<i>M. quasiater</i>	Mexico: Veracruz: 5 km W Naolinco, Mpio. Naolinco, 1650 m.	35282, 35274

both directions (Perkin-Elmer Prism<sup>®</sup> dye terminator kit; Fst-RR, 402119) on an ABI 373a automated sequencer. We included cyt b sequences

from 24 species of *Microtus* and two species of *Clethrionomys* (CONROY and COOK 2000). In the phylogenetic analysis we represented each species



**Fig. 1.** Map of the distribution of species of *Microtus* in North America and south into Guatemala (redrawn from HOFFMANN and KOEPL 1985).



with a single individual, but we examined intra-specific variation where possible by including multiple representatives for 24 of the 28 species. We used unweighted parsimony (MP) and maximum likelihood (ML) with the software PAUP\*, version 4.0d64 (SWOFFORD 1998). We estimated parameters for likelihood models of increasing complexity (JC: JUKES and CANTOR 1969; HKY85: HASEGAWA et al. 1985; and GTR: YANG 1994a; HKY85 +  $\Gamma$ , and GTR +  $\Gamma$ : YANG 1994b). We tested these with likelihood ratio tests to determine significant differences among their likelihood scores. We used 1,000 random addition sequences to locate multiple tree islands in the heuristic MP searches. As tests of node strength, we bootstrapped the parsimony analysis 1,000 times and bootstrapped the final ML analysis 100 times. We tested several alternative phylogenetic topologies against the ML tree by forcing likelihood searches to find the ML topology with particular constraints and then comparing scores (KISHINO and HASEGAWA 1989). We tested monophyly of the four relictual Mesoamerican species, and the four shortest maximum parsimony trees. Besides examining relationships among these southern latitude taxa, several tests were conducted to ascertain the effects of adding these four species to more general hypotheses concerning the systematics of *Microtus* previously conducted (CONROY and COOK 2000). We tested the monophyly of subgenus *Stenocranius* (RAUSCH 1964). This subgenus includes the Asiatic species *M. gregalis* and the North American *M. miurus* and *M. abbreviatus*. It has been hypothesized that the species in this subgenus diverged relatively recently. Secondly, we tested the monophyly of a clade of taiga adapted voles (*M. richardsoni*, *M. xanthognathus*, *M. chrotorrhinus*) that was not supported in our previous study (CONROY and COOK 2000). And, third, we tested the monophyly of all North American taiga voles (HOFFMANN and KOEPL 1985), because previously we were able to reject this hypothesis with the Kishino-Hasegawa test. By including these tests, our investigation explores whether the addition of four southern taxa changes our interpretation of arvicoline history in North America. Prior to this study, the relationships of southern species to other North American species was unclear.

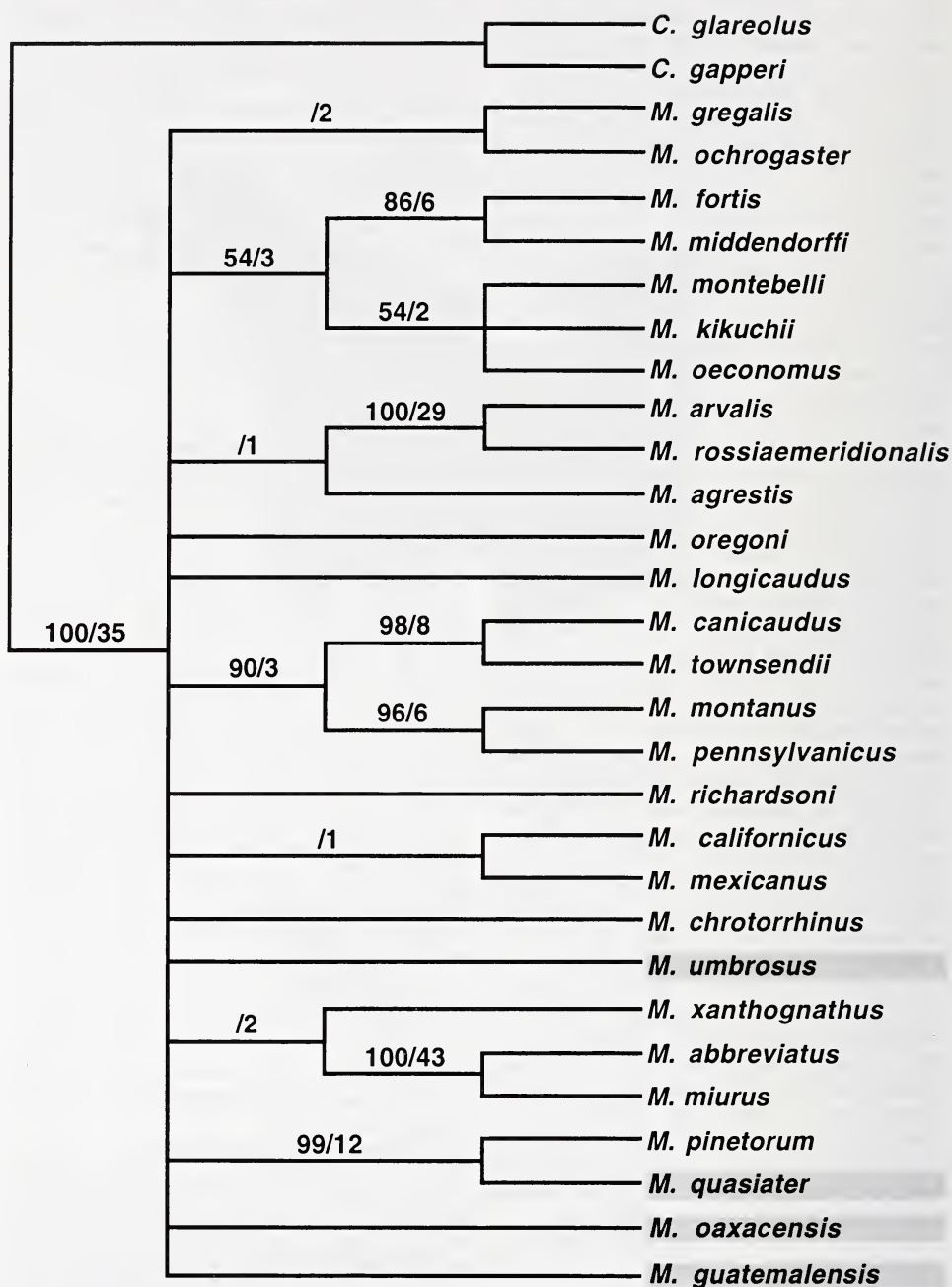
## Results

Base composition and distribution of variable sites of the cyt b gene was similar to other *Microtus* (CONROY and COOK 2000) as

well as other mammals (IRWIN et al. 1991). Of the 1143 base pairs, 472 were variable across 28 species of *Microtus* and two species of *Clethrionomys*. When outgroups were excluded, 460 sites were variable. Of these, 100 were in the first position, 23 in the second position, and 337 in the third position of codons. Of the 381 amino acids, 76 (20%) were variable across species of *Microtus* and the replacement pattern was consistent with structural models (e.g. IRWIN et al. 1991). There were 351 parsimony informative nucleotide sites and  $g_1$  statistics ( $g_1 = -0.325$ ) indicated phylogenetic signal in the data.

Maximum parsimony searches recovered four equally parsimonious trees (Fig. 2), each including a basal clade of the North American *M. ochrogaster* and Asian *M. gregalis*. A clade of *M. oeconomus*, *M. middendorffi*, *M. montebelli*, *M. kikuchii*, and *M. fortis* (hereafter the "Asian clade"), and the *M. pennsylvanicus* clade (i.e. *M. pennsylvanicus*, *M. montanus*, *M. townsendii*, and *M. canicaudus*) were present in the four trees. *M. pinetorum* and *M. quasiater* were sister in all trees, had high bootstrap support in MP and ML analyses (99%) and relatively high decay values (12). *M. oaxacensis* and *M. guatemalensis* were sister taxa in three of four MP trees, but the branch leading to this pair had weak bootstrap support (<50%). Other clades were found in three or four of these shortest trees, but bootstrap support was generally low across basal relationships. *M. umbrinus* stemmed from the polytomy at the base of the European and North American species. Random addition of taxa uncovered these four shortest trees, but simple addition (i.e. alphabetical by species name) led to an island of longer length trees.

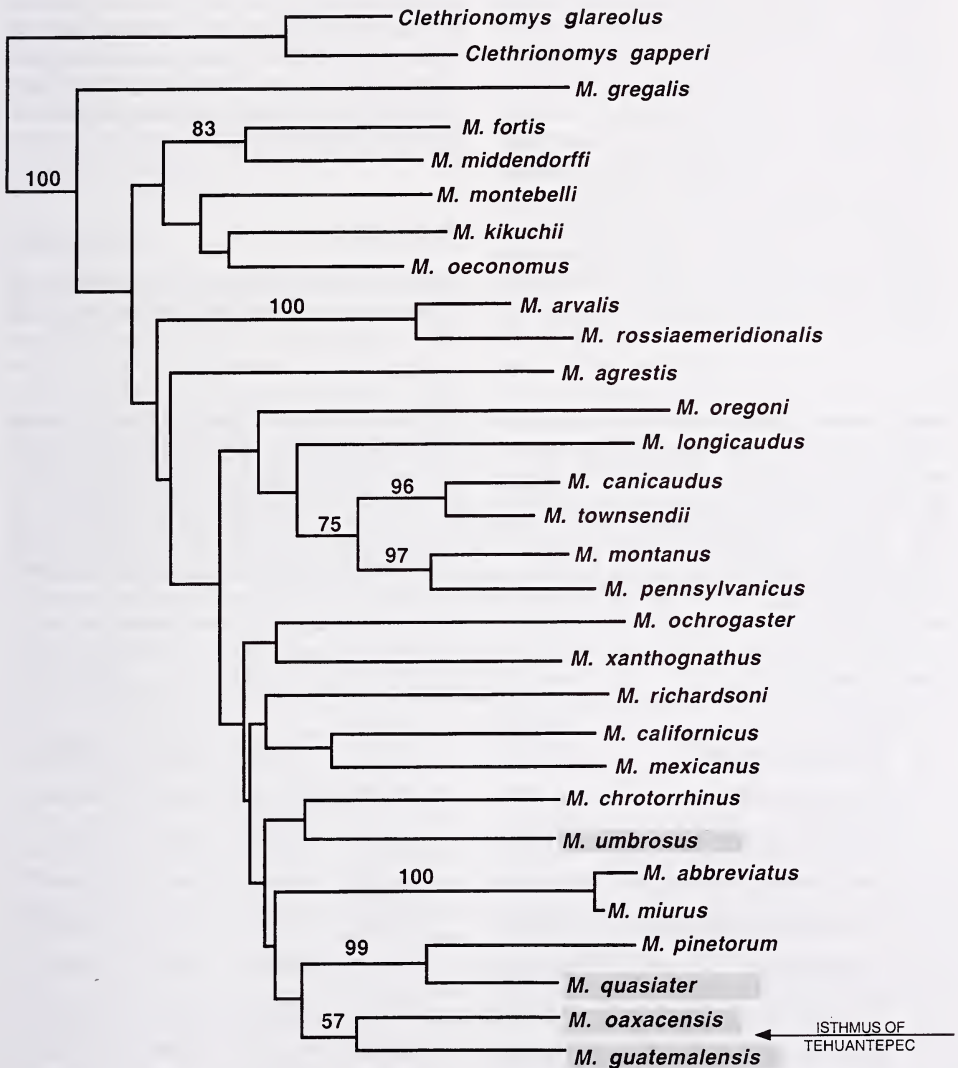
In the ML analysis, the HKY85 +  $\Gamma$  likelihood model (transition/transversion ratio = 3.43,  $\alpha = 0.213$ ) was chosen since more complex models (e.g. GTR +  $\Gamma$ ) were not significantly more likely and produced the same topology (not shown). As in other studies of cytochrome b (e.g. SULLIVAN et al. 1997), the addition of the gamma-distributed rate parameter, allowing among-site rate variation, contributed significantly to



**Fig. 2.** Strict consensus of four trees from the MP search. Each tree had a length of 2038, a consistency index of 0.331, and retention index of 0.360. Values to the left of slash are bootstraps percentages greater than 50% from 1,000 searches with simple addition of taxa; values to the right of slash indicate decay indices calculated with 10 random-addition replicates for each search.

the likelihood. This model produced one tree (Fig. 3) in which *M. gregalis* was basal, followed by the Asian clade. North American endemic species formed a clade and the European species formed a sister clade to North American species. The Mesoamerican endemics were not basal within the clade of North American species. Three

Mesoamerican species (*Microtus guatemalensis*, *M. oaxacensis*, and *M. quasiater*) displayed the same branching pattern as three of the parsimony trees, while *M. umbrosus* was sister to *M. chrotorrhinus*. Other relationships were similar to previous analyses (CONROY and COOK 2000). For example, the *M. pennsylvanicus* and Asian clades were



**Fig. 3.** Maximum likelihood phylogenetic tree based on the HKY85 +  $\Gamma$  model (see text for parameter values). Values above branches are bootstrap percentages greater than 50% from 100 bootstraps.

supported and *M. mexicanus* was sister to *M. californicus*. In Kishino-Hasegawa tests (Tab. 2), few of the alternate topologies could be rejected. Those that were rejected were one of the MP trees and two ML trees constrained to monophyly of the four southern species.

Relative depth of divergence was estimated with pairwise likelihood distances between taxa estimated under the same model used for the ML phylogeny. *M. abbreviatus* and *M. miurus*, which differ by only 0.015, probably separated at the end of the Pleistocene when rising sea levels isolated *M. abbreviatus* on islands in the Bering Strait. Another late Pleistocene divergence may be *M. canicaudus* and *M. townsendii* which differ by 0.058. Other pairs that were relatively closely related included *M. arvalis* and *M. rosiaemeridionalis* (0.072) and *M. montanus* and *M. pennsylvanicus* (0.086). Divergence values between the southern species (*M.*

*quasiater* and *M. pinetorum* [0.094], *M. guatemalensis* and *M. oaxacensis* [0.113], and *M. chrotorrhinus* and *M. umbrosus* [0.137]) are greater than any of the preceding examples, suggesting older speciation events. These comparisons should be interpreted with caution. Demographic and historical differences among species can alter rates of evolution and species of *Microtus* probably have evolved at different rates. Also, genetic differentiation may not necessarily coincide with vicariant events, such as the rise of the Bering Sea. Finally, molecular estimates of divergence, particularly for a single locus, have substantial amounts of stochastic variation (AYALA 1999).

## Discussion

Previous investigations into the history of North American species of *Microtus* sug-

**Table 2.** Kishino-Hasegawa tests of tree topologies. The “*Stenocranius*” constraint forced *M. miurus*, *M. gregalis*, and *M. abbreviatus* to be monophyletic. The “Second taiga vole” constraint enforced *M. xanthognathus*, *M. chrotorrhinus*, and *M. richardsoni* monophyly. The “All taiga voles” constraint enforced the monophyly of the “Second taiga vole” species with a clade of *M. pennsylvanicus*, *M. montanus*, *M. townsendii*, and *M. canicaudus*. The “Meso-Americans” constraint enforced *M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* monophyly. Two trees were obtained from the ML search under this last constraint. The parsimony trees were derived from unweighted heuristic searches with ten random-addition replicates. Columns below indicate, from left to right, 1) -log likelihood score of that tree, 2) difference in score between that tree and the best tree, 3) the standard deviation of that comparison, 4) the associated T test statistic, and 4) the associated p value.

Tree	Constraint	-log likelihood	Diff-lnL	s. d. (diff)	T	P*
1	None	9868.811	(best)			
2	Stenocranius Monophyletic	9891.258	22.447	16.723	1.342	0.180
3	Second taiga vole clade	9879.176	10.365	9.080	1.142	0.254
4	All Taiga voles Monophyletic	9897.044	28.233	18.904	1.494	0.136
5	Meso-Americans monophyletic I	9910.346	41.535	11.613	3.577	0.0004*
6	Meso-Americans monophyletic II	9910.346	41.535	11.613	3.577	0.0004*
7	Parsimony Tree 1	9908.109	39.298	22.586	1.740	0.082
8	Parsimony Tree 2	9908.743	39.932	20.492	1.949	0.052
9	Parsimony Tree 3	9908.493	39.682	20.581	1.928	0.054
10	Parsimony Tree 4	9919.839	51.027	20.528	2.486	0.013*

\*Significant at  $P < 0.05$



gested an evolutionary history closely tied to fluctuating boreal ecosystems (HOFFMANN and KOEPL 1985). The elevational distribution of species of *Microtus* in Mexico and Guatemala indicates Holocene isolation due to the contraction of cool, moist forests. The paucity of synapomorphic morphologic characters, but apparent abundance of autapomorphic characters has led to their characterization as ancient, highly divergent species; however, their biogeographic history has been enigmatic. This study reconsiders the biogeographic and evolutionary history of the southern relicts in light of molecular characters examined within a wider taxonomic sampling (28 species) for the genus.

### Biogeography

The Mesoamerican species of *Microtus* were not basal in the North American clade, nor were they monophyletic. *M. quasiater* is closely related to *M. pinetorum*, and *M. oaxacensis* and *M. guatemalensis* are sister to this pair, while the fourth (*M. umbrosus*) is a weakly-supported sister taxon to *M. chrotorrhinus*. Our data do not support the hypothesis of HOFFMANN and KOEPL (1985) that these southern species of *Microtus* may be relicts of an early colonization of North America prior to the arrival of the ancestors of the other species that are endemic to the higher latitudes of North America. Interspecific distances do, however, suggest they began to diverge earlier than suspected late-Pleistocene peripheral isolates such as *M. abbreviatus* and *M. miurus*. Assuming an equivalent rate of cyt b evolution across species of *Microtus* and *Clethrionomys*, Mesoamerican *Microtus* apparently fragmented into isolated populations prior to the colonization of North America by *Clethrionomys*, which is thought to have occurred during the early Pleistocene (REPENNING et al. 1990). The split between North American *Clethrionomys gapperi* and Eurasian *C. glareolus* (Tamura-Nei  $D = 0.079$ ) occurred about the time their common ancestor invaded North America. However, disparate levels of ancestral polymorphism and his-

tories of population fluctuation in these independent colonizers, among other variables, would impact estimates of ages of differentiation. Two topologies that constrained ML searches for monophyly of these southern species were significantly less likely than the best ML topology, refuting common ancestry of Mesoamerican species of *Microtus*.

SULLIVAN et al. (1997) and SULLIVAN et al. (2000) found that the Isthmus of Tehuantepec was a significant geographic barrier for other mesic rodents such as *Reithrodontomys* and the *Peromyscus aztecus* group in Mexico and Guatemala. They summarized divergence patterns in other co-distributed organisms (see RAMAMOORTHY et al. 1993) and recommended that the significance of the isthmus as a biogeographic barrier be tested with other mesic taxa. *M. guatemalensis* occurs east of the isthmus and is sister to *M. oaxacensis*, found west of the isthmus, suggesting that their common ancestor may have been distributed across the isthmus when conditions were cooler. Western and eastern populations subsequently diverged. *Microtus*, *Reithrodontomys*, and *Peromyscus* are widely sympatric in this region, and levels of genetic differentiation across the isthmus are surprisingly similar with  $D = 0.075\text{--}0.091$  in *Peromyscus* (SULLIVAN et al. 1997),  $D = 0.063\text{--}0.085$  in *Reithrodontomys* (SULLIVAN et al. 2000), and  $D = 0.100$  between *M. guatemalensis* and *M. oaxacensis*. If we assume similar rates of molecular evolution, these three clades may have diverged across the Isthmus of Tehuantepec at roughly the same time. High levels of biotic diversity in the region provide ample material to further test hypotheses regarding the timing and number of mammalian colonizations of the region. The biogeography of other organisms, such as reptiles (e.g. CAMPBELL 1984; CAMPBELL et al. 1989), may also suggest former connections across the Isthmus of Tehuantepec.

### Systematics

*Microtus quasiater* is a member of the subgenus *Pitymys* (MUSSEY and CARLETON



1993) and shares dental morphology with extinct *Microtus* (*Pitymys*) *meadensis*, a widespread species of mid-Pleistocene North America and Mexico (REPENNING 1983). *M. pinetorum* has often been classified as a member of *Pitymys* based on shared dental morphology (ZAKRZEWSKI 1985). Thus, it is not surprising that morphological characters (MUSSEY and CARLETON 1993) and these DNA sequences place *M. quasiater* sister to *M. pinetorum*. *M. quasiater* previously was considered sister to *M. ochrogaster* (MOORE and JANECEK 1990) in an allozyme study, but only nine of 19 North American species and no Palearctic species were examined.

The evolutionary relationships of *M. guatemalensis* and *M. oaxacensis* have not been addressed in detail, although they have not been suspected to be closely related (MUSSEY and CARLETON 1993). *Microtus guatemalensis* is in the monotypic subgenus *Herpetomys*, but may have affinities with *Pitymys* (MARTIN 1987). Our analyses contradict its placement in *Herpetomys*, or suggestions that it is related to *Phenacomys* (HINTON 1926). The relationship between *M. oaxacensis* and other species has also been obscure (MUSSEY and CARLETON 1993), but it also was considered a part of an early pitymyine invasion (HOFFMANN and KOEPL 1985; MARTIN 1974). A widespread ancestor (e.g. *M. meadensis*) may have given rise to *M. pinetorum*, *M. guatemalensis*, *M. quasiater*, and *M. oaxacensis*, prior to peripheral isolation in the eastern deciduous forests and southern cloud forests (HOFFMANN and KOEPL 1985). The mitochondrial phylogeny suggests isolation occurred first between an ancestor of *M. pinetorum*–*M. quasiater* and an ancestor of *M. guatemalensis*–*M. oaxacensis* although the branching order among these clades is weakly supported. The latter pair may have diverged after invasion across the Isthmus of Tehuantepec, while the former pair diverged following an episode of range retraction induced by climatic warming. Significant portions of the temperate flora on mountain tops in Mesoamerica are as old as the late Cenozoic (GRAHAM

1999), however, we do not know of a comparable phylogenetic assessment of vegetation.

A sister relationship between *M. umbrosus* and *M. chrotorrhinus* was weakly supported in the ML tree and was unexpected because they differ morphologically. *Microtus umbrosus* is the sole member of the subgenus *Orthiomys* (MUSSEY and CARLETON 1993) and has been considered a relict from an early invasion from Asia during the mid-Pleistocene by the extinct *Phaiomys* (MARTIN 1987). Though previously considered closely related to *M. xanthognathus* (HALL and KELSON 1959), *M. chrotorrhinus* was later distinguished based on chromosomal complement (RAUSCH and RAUSCH 1974). The lack of similarity between *M. chrotorrhinus* and *M. umbrosus* and the “pitymyine” species suggests an independent invasion of the southern latitudes by a common ancestor of *M. umbrosus* and *M. chrotorrhinus*.

#### North American monophyly and other phylogenetic hypotheses

Our assessment of *Microtus* includes 28 of approximately 65 species of *Microtus* (MUSSEY and CARLETON 1993), with the addition of Mesoamerican taxa potentially contributing to more accurate estimations of relationships (HILLIS 1996). This larger study did not significantly alter the ML topology previously obtained based on 24 species of *Microtus* (CONROY and COOK 2000). Monophyly of all North American species included in this study and of the *M. pennsylvanicus* clades was supported. The expanded analysis suggested two differences based on Kishino-Hasegawa tests. Without Mesoamerican species, we rejected the topology ( $p = 0.026$ ; CONROY and COOK 2000) which constrained all North American taiga voles as monophyletic (sensu HOFFMANN and KOEPL 1985). However, with the inclusion of Mesoamerican species, this hypothesis was not rejected ( $p = 0.136$ ). The expanded analysis also rejected a topology placing *M. chrotorrhinus* basal to all species of *Microtus* sampled and *M. gre-*

*galis* (Russia) within a clade of North American species ( $p = 0.013$ ).

Morphological material for interspecific comparison is abundant, but the phylogenetic utility of some morphological characters for arvicolines (e.g. tooth pattern) has been criticized due to high variation within and between species (GUTHRIE 1965; ZAKRZEWSKI 1985). Despite the availability of standard karyotypes for many species of *Microtus*, non-differentially stained chromosomes have minimal phylogenetic information because the rate of chromosomal evolution varies greatly among species (CERVANTES et al. 1997; MODI 1987). Indeed, our phylogeny suggests a complicated series of events are needed to explain chromosomal rearrangements in *Microtus*. Species with low diploid numbers are not sister to each other. For example, the mitochondrial data indicate *M. oaxacensis* ( $2N = 30$ ) is sister to *M. guatemalensis* ( $2N = 52$ ), and *M. canicaudus* ( $2N = 24$ ) is sister to *M. townsendii* ( $2N = 50$ ). These relationships support Cervantes et al.'s (1997) contention that the interpretation of chromosomal evolution based only on standard karyotypes will be difficult.

### Conservation

Mexico has one of the richest mammalian faunas due partially to an overlap of neotropical and nearctic biomes (FA and MORALES 1993). Conservation efforts are complicated by this high diversity and a variety of threats (CEBALLOS and NAVARRO 1991). Species of *Microtus* in the region are nearctic relicts that are dependent on high elevation, mesic habitats. Protection of habitat in the mountains of Oaxaca, a region of high mammalian diversity (ARITA et al. 1997), could help conserve three endemics, *M. oaxacensis*, *M. umbrosus*, and *M. quasiater*. Whether conservation criteria focus on rarity, diversity, or degree of endemism, these southern relicts warrant conservation concern.

Molecular systematic studies of other endemic taxa should be considered in planning conservation efforts in this region (BAKER

et al. 1995; Cook et al. 2001). Our analysis suggests that temporal scale may be a crucial component to interpreting the significance of biogeographic barriers. Detection of patterns at different temporal scales could help resolve shared histories of taxa in the region (AVISE 1994) and be used to conserve historical associations of flora and fauna.

Species of *Microtus* in Mexico and Guatemala are not monophyletic but instead are the result of at least three colonizations of this region during the Pleistocene: one by the ancestors of *M. oaxacensis*, *M. quasiater*, and *M. guatemalensis*, a second by the ancestor of *M. umbrosus*, and a third colonization apparently gave rise to *M. mexicanus*. A lack of fossils inhibits the dating of cladogenic events among these species; however, depth of divergence relative to other splits within *Microtus* suggest mid-Pleistocene divergence. Morphological similarity between extant species (e.g. *M. pinetorum* and *M. quasiater*) and formerly widespread (mid to late Pleistocene) taxa that are now extinct (e.g. *M. meadensis*) suggest isolation by range retraction is a viable hypothesis. Morphological studies also support the shared history of several of the pitiomyine species. The weakly-supported sister relationship between *M. umbrosus* and *M. chrotorrhinus* has not been predicted previously and should be tested further. Phylogenetic analysis of other Mesoamerican organisms may help identify regions of shared evolutionary history and the role of significant biogeographic barriers in promoting diversification in this biologically rich region.

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## Zusammenfassung

### Die phylogenetische Stellung südlicher Reliktarten von *Microtus* (Muridae Rodentia) in Nordamerika.

Klimatische Fluktuationen führten in Zeiten der Erwärmung zur Isolation einiger Populationen von Arten aus gemäßigten Zonen auf südlichen Bergen. Die südlichsten Arten arvicoliner Nager (*Microtus guatemalensis*, *M. oaxacensis*, *M. quasiater* und *M. umbrosus*) in Nordamerika mögen Relikte darstellen, die am Ende des Pleistozäns in den Bergen von Mexico und Guatemala isoliert wurden. Wir untersuchten mittels Parsimony- und Likelihood-Analysen die kompletten mitochondrialen Cytochrom b-Gen-Sequenzen von 28 Arten der Gattung *Microtus*. Darunter befanden sich acht eurasische Arten, holarktische *M. oeconomus* und alle heute lebenden Arten Nordamerikas außer der inselendemischen *M. breweri*. Die nordamerikanischen Arten waren in der Maximum-Likelihood-Analyse monophyletisch, in der Maximum-Parsimony-Analyse jedoch polyphyletisch. Likelihood-Ratio-Tests und Bootstrap-Analysen wiesen auf eine rasche adaptive Radiation mit kurzen Intervallen zwischen Kladogenese-Ereignissen hin. Die Analysen von Schwestertaxa zeigten jedoch Konstanz in Bootstrap-Analysen oder unterschiedlichen phylogenetischen Auswertemethoden. Nach unseren Ergebnissen war *M. quasiater* die Schwestergruppe von *M. pinetorum* und beide bildeten eine Schwestergruppe zu einer Klade aus *M. oaxacensis* und *M. guatemalensis*. Die phylogenetische Stellung von *M. umbrosus* blieb unklar. Die Monophylie der Relikte erwies sich nach Likelihood-Ratio-Tests als unhaltbar, was auf multiple südliche Invasionen arvicoliner Nager hinweist. Die phylogenetischen Daten für 17 diese und andere Taxa sollten in Arbeiten zur Arterhaltung in diesen abgelegenen Gebieten berücksichtigt werden.

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