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Identifying California *Microtus* species using geometric morphometrics documents Quaternary geographic range contractions

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Paleontology can provide a deep-time dimension to observations about recent reactions of small mammals to climate change. Obtaining this perspective for voles (*Microtus*), a common and important constituent of North American mammal communities, has been difficult because species identification based on their dental remains is problematic. Here I demonstrate that geometric morphometrics and discriminant analyses can use commonly fossilized dental features to identify the 5 extant species of *Microtus* in California: *M. californicus* (California vole), *M. longicaudus* (long-tailed vole), *M. montanus* (montane vole), *M. oregoni* (Oregon vole), and *M. townsendii* (Townsend's vole). Analyses of landmarks on the lower 1st molar (m1) provide more accurate identification than those of the 3rd upper molar (M3), and it is important to use jackknife misidentification metrics to assess the precision of discriminant analyses. Addition of semilandmark curves on m1 does not improve accuracy. The utility of these techniques is demonstrated by identifying *Microtus* specimens from 2 California fossil localities, Pacheco 2 and Prune Avenue, which provides the first evidence for extralimital presence of *M. longicaudus* at both localities. The presence of *M. longicaudus* at these low-elevation sites indicates that pronounced geographic range shifts in this species that have been observed in California over the last 100 years also occurred during previous climate changes. Eventually it might be possible to ascertain whether current range shifts are exceeding those that typified responses to past climate changes.

Key words: California, geometric morphometrics, identification, jackknife, *Microtus*, *Microtus longicaudus*, paleontology, Quaternary

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Small mammal species in California have shifted their ranges over the last 100 years as a result of climate change (Moritz et al. 2008), but the significance of these shifts relative to a background rate of range shifts is not well known. Such knowledge can be gained only by setting recent movement in a longer temporal context using the fossil record (Hadly and Barnosky 2009). Although we know that in general small mammals shifted their ranges in response to climatic changes throughout the Quaternary (Graham et al. 1996; Guralnick 2007; Lyons 2003), and almost certainly earlier, few studies have documented range shifts in California (Blois and Hadly 2009; Blois et al. 2010), and none have confidently documented shifts in the species of one of the most abundant taxa, voles of the genus *Microtus.*

Rodents of the genus *Microtus* can provide essential information about how small mammals reacted to historic environmental changes, because they are abundant now, well represented in the Quaternary fossil record, and known to have experienced range shifts in response to recent and past environmental changes. For example, Moritz et al. (2008) reported that over the last 100 years in California *M. longicaudus* (long-tailed vole) has experienced an approximately 600-m upward contraction of its lower range boundary in Yosemite National Park, and *M. californicus* (California vole) experienced an approximately 500-m expansion of its upper elevational range into the same mountains. No recent range shift is recorded for a 3rd species of *Microtus* present in Yosemite National Park, *M. montanus* (montane vole). At the generic level *Microtus* in northern California weathered a major ecosystem shift at Samwell Cave during the glacial–interglacial transition at the Pleistocene–Holocene boundary that marked the end of the Last Glacial Maximum 21 thousand years ago (kya—Blois et al. 2010). At other fossil localities researchers found that members of this genus demonstrated

dramatic range movements when faced with similar ecosystem shifts (Bell and Bever 2006; Graham et al. 1996; Guilday 1962; Repenning 1987; Wood and Barnosky 1994). Major range contractions have been reported since the Pleistocene in 2 species, *M. montanus* and *M. longicaudus*, from lower-elevation regions east of their modern range extending into the Great Plains (Hoffman and Jones 1970; Stewart 1987; Turner 1974; Wallace 2001); however, these specimen identifications are uncertain, and not all have been validated.

Few species-level range shifts are documented in *Microtus*, despite the abundance of specimens in the fossil record, because they are preserved primarily as isolated teeth that have frustrated attempts to assign them to species. It is difficult to identify isolated *Microtus* teeth because intraspecific variation in dental characters is considerable (Barnosky 1990; Bell and Repenning 1999; Graham and Semken 1987). Some previously recognized characters commonly used for specific identification in *Microtus* have since been found unreliable as diagnostic features because they sometimes occur in other species (e.g., the posterolingual dentine field on the 2nd upper molar [M2]—Bell and Repenning 1999). Past work has demonstrated, however, that it is possible to distinguish between vole species if one considers tooth shape as a whole rather than individual characters (Smartt 1977; Wallace 2006), especially using discriminant analyses. For example, Smartt (1977) identified fossil *Microtus* species in New Mexico by quantifying tooth length, tooth width, and reentrant angle depths (Fig. 1) for each molar. He included *M. pennsylvanicus* (meadow vole), *M. mexicanus* (Mexican vole), *M. montanus*, and *M. ochrogaster* (prairie vole) in his analysis. He noted that, of the molars examined, the 1st lower molar (m1) most often correctly identified to species (Smartt 1977). Wallace (2006) demonstrated that geometric morphometrics could be used in conjunction with discriminant analyses to distinguish between 2 other species of *Microtus*, *M. pennsylvanicus* and *M. xanthognathus* (taiga vole), also using m1.

I used geometric morphometrics and discriminant analyses to distinguish between the 5 extant species of the genus *Microtus* common to the Pacific Coast region of the United States by examining a single molar. Those species include *M. californicus*, *M. longicaudus*, *M. montanus*, *M. oregoni* (Oregon vole), and *M. townsendii* (Townsend's vole). I report the results from discriminant analyses using the landmarks established by Wallace (2006) on m1, landmarks and semilandmark curves on m1, and landmarks on the 3rd upper molar (M3). Further, I discuss the importance of using jackknife results to determine whether the sample size of the training set is large enough to build a precise discriminant analysis. The methods described herein allow accurate identification of many more fragmentary specimens than were previously recognizable to species. Once I established discriminant methods for identifying *Microtus* species, I applied the function to specimens from 2 fossil localities in Northern California, Pacheco 2 and Prune Avenue (Fig. 2) to assess whether California *Microtus* species' ranges were as mobile as the purported movement of the species east of the Rocky Mountains (Hoffman and Jones 1970; Stewart 1987; Turner 1974; Wallace 2001). Pacheco 2 is presumed to be late Quaternary in age (10 kya to the present), and Prune Avenue is from the Holocene (836–4,283 calibrated years ago). Both California localities are within grassland habitats of the Central Valley. These techniques permit the discovery of previously undetectable past geographic range contractions. I finally compared the rate of range shifts in the past to those that have occurred over the last 100 years to give a deep-time perspective to these species' reactions to current environmental changes.

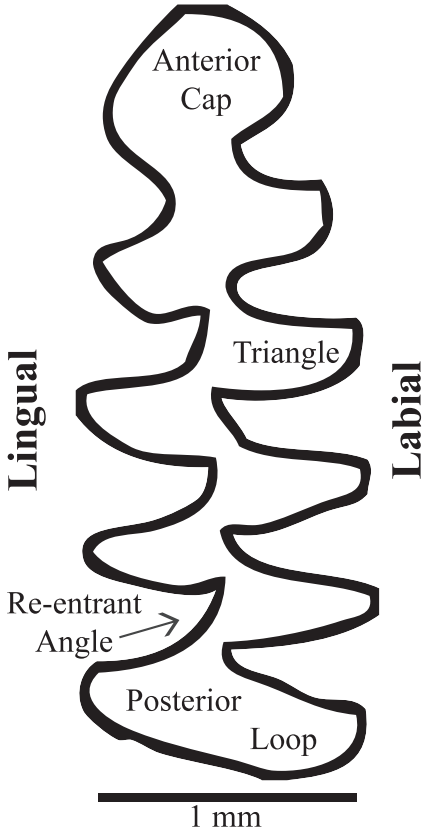


Fig. 1.—First lower molar (m1) of *Microtus* showing the morphological terminology used.

Bell and Bever (2006) reported that fossil specimens of California *Microtus* historically have been assigned to species based on current geographic affinity (Miller 1971; Savage 1951) when the specimens could be confidently assigned only to genus based on morphological characters. The 5 species included here are the only *Microtus* currently present in California: the range of *M. californicus* covers the majority of the state, whereas *M. montanus* and *M. longicaudus* are restricted to the higher elevations of the Sierra Nevada and Cascade Mountains (*M. montanus* is also in the Klamath and Northern Coastal Ranges and *M. longicaudus* in the Transverse Ranges), and *M. oregoni* and *M. townsendii* are present only in the very northern portion of the state (Fig. 2). Geography-based species identifications are clearly problematic if we want to understand species-level range shifts, and observations by Bell and Bever (2006) about California's

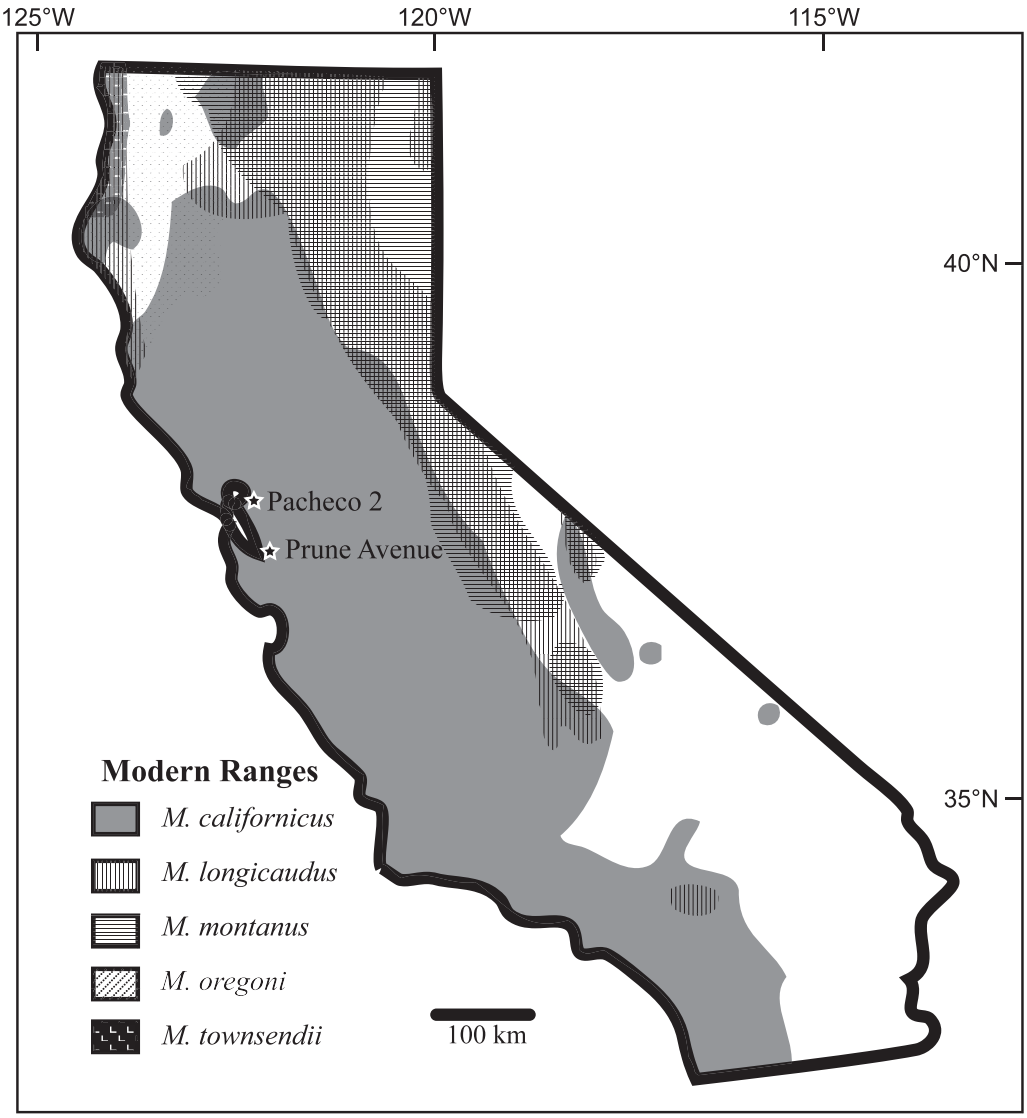


Fig. 2.—Map of California showing the modern distribution of *Microtus californicus*, *M. longicaudus*, *M. montanus*, *M. oregoni*, and *M. townsendii*. The fossil localities Pacheco 2 and Prune Avenue also are shown.

Microtus identifications are accurate. Few *Microtus* fossil identifications in California are indisputable, and all are identifications for species whose ranges overlap with the locality where the fossil was collected.

Materials and Methods

Specimens.—Discriminant functions were built using training sets of 241 to 271 modern specimens of known identity from the Museum of Vertebrate Zoology (University of California, Berkeley) collections (Table 1; Appendix I), depending upon the analysis. The total number of specimens examined varied among the 3 analyses performed because an increasing number of specimens had to be discarded due to breakage as the number of variables (landmarks) and surface area of the tooth perimeter examined increased. Specimens were selected evenly from throughout the geographic range of each species and included an approximately equal number of males and females from each species. Only jaws with fully erupted molars were used, although specimens with juvenile skull sutures were included. All specimens used were curated as skin and skull preparations, and all field identifications were confirmed (Fig. 3), with ambiguous specimens excluded. Toothrows were photographed digitally using a Nikon D70s and AF Micro-NIKKOR 60 mm f/2.8D lens (Nikon Inc., Melville, New York).

Given that Smartt (1977) demonstrated that m1 was superior for *Microtus* identification, this tooth initially was analyzed to determine whether the inclusion of the semilandmark curves along the anterior loop—referred to hereafter as m1-SL analysis, as opposed to m1 analysis, which does not include semilandmark curves (Fig. 1; Table 1)—would improve identification rates. Because any single fossil locality has a limited number of specimens with differing preservation quality, M3 also was examined to see whether this tooth could provide reliable species-level identifications (M3 analysis;

TABLE 1.—Comparison of the 3 discriminant analyses—M3, m1, and m1-SL—with landmark and semilandmark placements depicted. Landmarks are black-and-white points on the molars, and semilandmark curves are black lines along the anterior cap and posterior loop. Scale bars represent 1 mm. n = sample size. Subscripts *c*, *l*, *m*, *o*, and *t* represent *Microtus californicus*, *M. longicaudus*, *M. montanus*, *M. oregoni*, and *M. townsendii*, respectively.

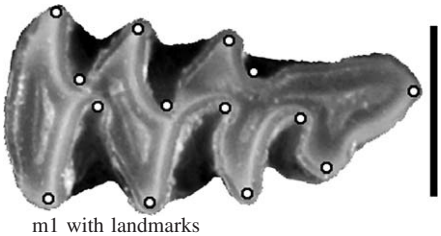
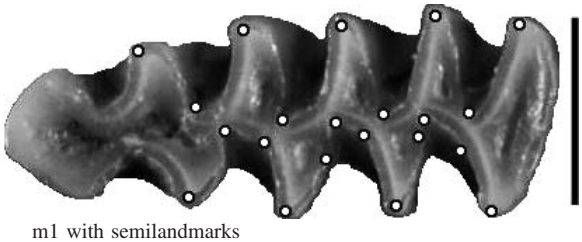
Anterior Posterior	Total specimens examined	Centroid size included?	Percent misclassification	Jackknife percent misclassification
M3 with landmarks 	100: $n_{c,l,m,o,t} = 20$	No	19	32
		Yes	23.6	22
	271:	No	18.8	29.5
	$n_c = 52$	Yes	15.1	24
	$n_l = 58$			
	$n_m = 55$			
	$n_o = 54$			
	$n_t = 52$			
m1 with landmarks 	100: $n_{c,l,m,o,t} = 20$	No	1	39
		Yes	1	40
	251:	No	9.1	20.7
	$n_c = 50$	Yes	5.2	12.7
	$n_l = 50$			
	$n_m = 50$			
	$n_o = 50$			
	$n_t = 51$			
m1 with semilandmarks 	100: $n_{c,l,m,o,t} = 20$	No	0	42
		Yes	0	42
	241:	No	3.7	19.1
	$n_c = 46$	Yes	1.2	12.9
	$n_l = 48$			
	$n_m = 49$			
	$n_o = 49$			
	$n_t = 49$			

Table 1). M3 is the 2nd most abundantly preserved fossil *Microtus* molar, is highly variable, and has excellent potential for identification purposes (Barnosky 1990; Bell and Bever 2006; Bell and Jass 2004; Guilday 1982; Semken and Wallace 2002; Smartt 1977; Zakrzewski 1985). Only left molars were used, although no significant differences were found between left and right molars, and right fossils could likely be identified reliably, judging from the discriminant analyses (Wallace 2006).

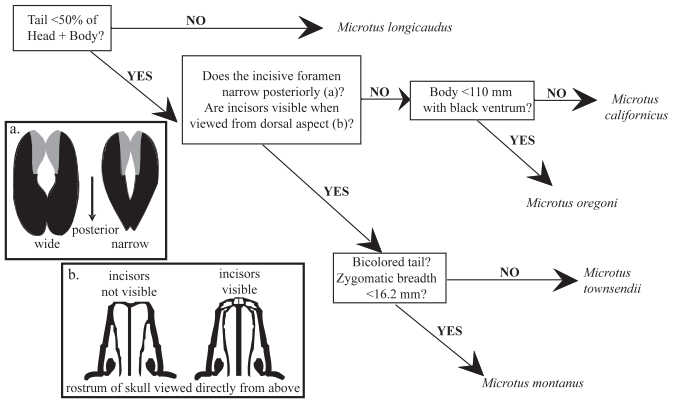


FIG. 3.—Key used to identify extant *Microtus* museum specimens from California. a) Appearance of incisive foramina that do and do not narrow posteriorly. b) View from directly above the rostrum of the skull where incisors are and are not visible. The key is a compilation based on Hall (1981), Ingles (1965), Jameson and Peeters (2004), Maser and Storm (1970), and Verts and Carraway (1998).

Landmark selection.—Two-dimensional landmark coordinates were digitized on Tiff images using tpsDig 2.10 (Rohlf 2006; Table 1). Landmarks are points that can be described anatomically and are homologous between specimens (Bookstein et al. 1985). Landmarks for m1 and m1-SL were based upon those selected by Wallace (2006), with the exception of the 4 landmarks that anchor semilandmark curves, which were moved to the outside of the enamel band (Table 1). Landmarks on M3 are similar to those on m1, with the exception of the posteriormost landmark, which is a type II landmark (Bookstein 1991) representing the apex of the curve on the posterior loop.

Semilandmarks are points along a feature, such as a curve, that are defined relative to their positions on that feature (Zelditch et al. 2004). Semilandmarks in the m1-SL analysis were placed equidistant along the tooth's posterior loop and anterior cap (Table 1). Three semilandmarks and 2 anchor landmarks were placed on the posterior loop to delimit the tooth and measure the relative width of the posterior loop. Twelve semilandmarks and 2 anchor

landmarks were placed along the anterior cap (Fig. 1; Table 1), an important feature that represents the highest amount of variation in this tooth (Bell and Bever 2006). Semilandmark curves were drawn and initially subsampled in tpsDig 2.10 (Rohlf 2006), slid using bending energy in tps Relative Warps 1.45 (Rohlf 2007), and subsequently subsampled in tps Utility 1.40 to the semilandmarks reported herein (Rohlf 2008).

Geometric morphometric and discriminant analyses.—Kendall (1977) defines shape as ''all the variation that remains in the configurations of landmarks after removing differences in location, size and orientation.'' Geometric morphometrics directly compares shape differences among specimens. Generalized Procrustes analysis was performed to superimpose landmark configurations and correct for nonshape variation (Rohlf and Slice 1990). In a generalized Procrustes analysis specimens are superimposed by translating the centroid of each specimen (x_L, y_L) to that of a mean specimen (x_c, y_c). Next, centroid size (CS), defined as $CS = \sqrt{\sum \left[(x_L - x_c)^2 + (y_L - y_c)^2\right]}$, was normalized across specimens. Finally, specimens were rotated to minimize the overall summed squared distances between landmarks. The resulting size- and orientation-corrected landmark coordinates, now known as shape coordinates, then could be used to compare shape differences among specimens.

The processes of translation, resizing, and rotation each constrain the data, removing degrees of freedom and creating a mismatch between the number of variables and the degrees of freedom (Rohlf and Corti 2000; Zelditch et al. 2004). Therefore, the discriminant analysis used partial warp and uniform component scores calculated using PCAGen 6p (Sheets 2001), rather than Cartesian coordinates, because these scores contain equal degrees of freedom and variables. This gives the same results as if the Cartesian coordinates were used and the degrees of freedom were corrected manually (Rohlf and Corti 2000; Zelditch et al. 2004). Discriminant analyses were performed on the partial warp scores, uniform components, or centroid sizes, or a combination of these, from the training set using JMP 7.0 (SAS Institute Inc., Cary, North Carolina), and the precision of each discriminant analysis was described with and without the inclusion of centroid size (Table 1). Centroid sizes were produced by Coordgen 6h (Sheets 2000) and, in the case of the m1-SL, were calculated prior to semilandmark sliding and subsampling.

A discriminant analysis determines the canonical axes that maximally separate designated training groups. Standard and jackknifed misidentification rates are both reported. The specimens were jackknifed by removing each specimen, coding it as an unknown, and then determining whether it was identified correctly by the discriminant analysis before replacing it and repeating for all specimens. Additionally, training sets were subsampled to 20 specimens per species to determine the effects of sample size on standard misidentification rates and jackknife misidentification rates. Jackknife misidentification rates were calculated using CVAGen 6n (Sheets 2005) and the R package MASS (R Development Core Team 2009; Venables and Ripley 2002).

Identification of fossil specimens.—Both Pacheco 2 and Prune Avenue are fossil localities from the East Bay region of Northern California (Fig. 2) that were salvaged by members of the University of California Museum of Paleontology during construction activities. Specific locality information can be attained through the University of California Museum of Paleontology. Pacheco 2 is associated with another locality, Pacheco 1, which contains extinct megafauna, indicating that it is late Quaternary in age (Tomiya et al., in press). Prune Avenue has been radiocarbon dated to 836–4,283 calibrated years ago (Holocene) at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry facility (Livermore, California) using preparation procedures described by Brown et al. (1988) and Bronk Ramsay et al. (2004). Once the discriminant analysis was established, 26 fossil *Microtus* specimens from Pacheco 2 and 19 specimens from Prune Avenue were included as unknowns in the m1-SL discriminant analysis (Appendix II).

Identification confidence indicates with which of the 5 species a specimen is most closely associated. If a specimen were to fall midway between 2 species—for example, *M. californicus* and *M. longicaudus*—it would have 50% identification confidence for each species; however, if a specimen fell the same distance away from *M. californicus* but not in the direction of another species, it still would have high confidence for *M. californicus* but might also have a large Mahalanobis distance (squared distance to the centroid of the *M. californicus* group). Therefore, if a specimen is a representative of a species not included in the training set, it would not necessarily be detected by identification confidence but would have a high Mahalanobis distance and potentially have other purported unidentifiable specimens clustered around it in discriminant shape space. Only specimens with $\geq$95% confidence in identification were considered, and the Mahalanobis distance of the specimen must have fallen within 2 *SD*s of the species mean shape.

Results

Discriminant analyses.—In all cases, except the subsampled analyses, the inclusion of centroid size improved the discriminant analyses. Therefore, all results refer to those analyses that include centroid size. The m1-SL analysis did not show an improvement over the m1 analysis (m1 = 12.7; m1-SL = 12.9; Table 1). The m1-SL analysis showed the same pattern as the m1 analysis in that the standard misclassification metric indicated that the analysis with fewer specimens in the training set was more accurate than the one with more specimens ($m1\text{-}SL_{100}$ = 0; $m1\text{-}SL_{251}$ = 1.2); whereas the jackknife misclassification rates indicated the opposite ($m1\text{-}SL_{100}$ = 42.0; $m1_{251}$ = 12.9; Table 1). In addition, geometric morphometrics and discriminant analyses were better able to distinguish between the training set m1

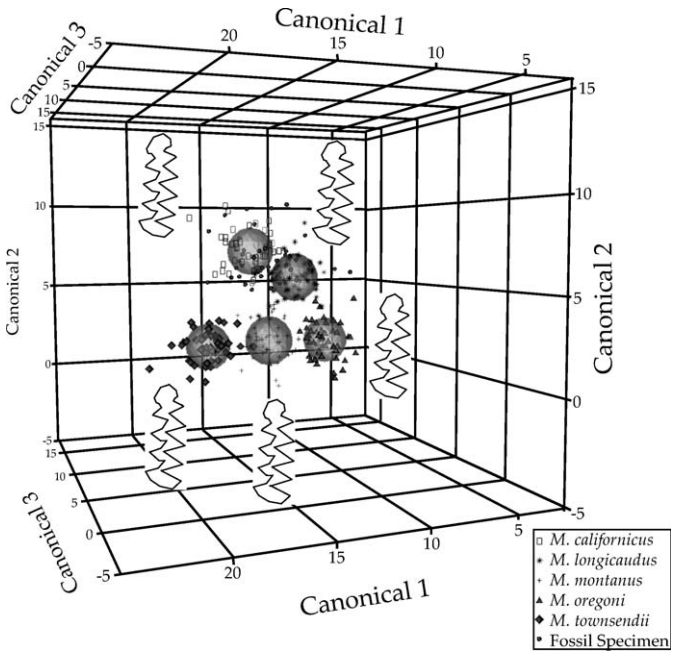


Fig. 4.—Plot of discriminant analysis of m1-SL for identification of *Microtus* spp. showing the first 3 canonical axes of training group separation. Fossil specimens are plotted as points. Spheres represent the 95% confidence interval for the group mean. Mean tooth shapes for each species indicate the shape changes along the canonical axes.

specimens than the M3 specimens, both according to the standard misidentification rates (m1 = 5.2; M3 = 15.1) and the jackknife misidentification rates (m1 = 12.7; M3 = 24.0). The first 4 canonical axes provide nearly complete separation of the training groups (first 3 most significant canonical axes shown in Fig. 4). Shape separation of m1s appears to be based upon different relative widths of lingual and labial triangles and reentrant angle size (Fig. 4).

Specimens of M3 appeared to exhibit higher rates of misidentification when fewer specimens were included in the analysis, according to the standard misidentification metric ($M3_{100}$ = 23.6; $M3_{271}$ = 15.1). However, the jackknife metric suggests that sample size does not strongly affect the misidentification rates ($M3_{100}$ = 22.0; $M3_{271}$ = 24.0). The m1 demonstrated a quite different effect. According to the standard misidentification metric, m1 specimens appeared to have higher misidentification when more specimens were included in the training set than when they were not ($m1_{100}$ = 1; $m1_{251}$ = 5.2). However, the jackknife misidentification rates illustrate that the m1 analysis with fewer specimens actually performed much more poorly than the one that included more specimens in the training set ($m1_{100}$ = 40.0; $m1_{251}$ = 12.7; Table 1).

Fossil identification.—Pacheco 2 specimens were identified as 15 (58.5%) specimens of *M. californicus*, 3 (11.5%) specimens of *M. longicaudus*, and 8 (30%) unknown (Appendix II). Prune Avenue specimens were identified as 7 (37%) specimens of *M. californicus*, 3 (16%) specimens of *M. longicaudus*, and 9 (47%) unknown (Appendix II). No specimens with extreme Mahalanobis distances were present at either fossil locality. The total percent of unidentifiable specimens among both localities was 38%.

Discussion

Prehistoric range shifts and the present.—The fossil *Microtus* identifications include the 1st extralimital *Microtus* fossils in California, demonstrating that *M. longicaudus* occurred at lower elevations and approximately 160 km farther west than the species occurs at a similar latitude today. The fossil localities are approximately 600 m lower than the lower elevation limit of *M. longicaudus* in Yosemite 100 years ago and about 1,200 m lower than its present known location in California. This corresponds with the observed range contractions of this species east of the Rocky Mountains over the same time period (Wallace 2001) and gives a deeper time extension to the modern range shifts (Moritz et al. 2008) of the species in California. Although the exact age of the fossil specimens is unknown, examination of palynological data from nearby Mono Lake indicates that this region was approximately 2°C cooler and received approximately 200 mm more precipitation 2,000 years ago than currently and was even cooler and moister during the Last Glacial Maximum of the late Pleistocene (Davis 1999). An upward, eastward range contraction of *M. longicaudus* is consistent with warming and drying since the Last Glacial Maximum. It also is consistent with range contraction due to anthropogenic landscape modification at the 2 fossil localities.

Given that elevational shifts in range appear to be in response to temperature changes, it is interesting to see if recently increased rates of climate change correspond with increased rates of range change. If the fossil range is assumed to be from the minimum age of the confidently dated extralimital fossils of *M. longicaudus*, 836 calibrated years ago, the rate of range contraction from that time until 100 years ago was 0.82 m/year. In comparison, the rate of elevation range contraction in Yosemite of 6 m/year is considerably more rapid. Although it is possible that past range change was more punctuated and had periods of faster and slower range change within them, this order of magnitude difference in rates points to accelerating range movement that is simultaneous with modern increased rates of climatic change. Such observations highlight the need for additional identifications of fossil specimens so that we can identify rates of range shifts along the entirety of species' range boundaries.

Although *M. montanus* is purported to have undergone a range contraction similar to that of *M. longicaudus* from east of the Rocky Mountains from the end of the Pleistocene to recent times (Hoffman and Jones 1970; Stewart 1978, 1987; Turner 1974; Wallace 2001), no evidence exists of *M. montanus* at low-elevation Central Valley fossil sites in California. This parallels the pattern of recent ranges reported for Yosemite, where *M. montanus* has not changed its distribution in the same time that *M. longicaudus* moved upslope. This difference in patterns of range shift between *M. montanus* and *M. longicaudus* suggests different responses to similar climatic changes. Comparative

physiology studies, species distribution models, and tests for competitive interactions could determine whether this disparity represents different life histories or the effects of interspecific interactions.

The m1 for discrimination of taxa.—These identifications and biogeographic observations were made possible by the establishment of improved identification methods using geometric morphometrics, discriminant analyses, and jackknife assessments to determine what morphological structures best discriminate the species. Separation of the modern taxa is strong under the m1-SL analysis. The jackknife results of the discriminant analysis indicate that the m1 analyses that include centroid size are superior to those without at distinguishing between the 5 extant California *Microtus* species and identifying unknowns. However, changes in the shape variables independent of size remain the most important factors in discriminating species. Despite the suggestion of Barnosky (1990) that the anterior cap of m1 is highly variable, the discriminant analysis is not improved significantly by using semilandmark curves describing this region. This is likely because the region remains too variable to reliably inform species identification. Therefore, no reason exists to include semilandmark curves on m1, and doing so might only weaken the analysis by introducing more uninformative variation. The M3 analysis has lower reliability than the m1 analysis, possibly because of fewer homologous structures, and therefore landmarks, on the former. However, it is also possible that little selection operates on this tooth or it exhibits greater variability within each species. The superiority of m1 to M3 for distinguishing between *Microtus* species parallels the results seen by Smartt (1977), indicating that this pattern might be consistent across North American *Microtus* species.

Jackknifing and discriminant analyses.—Subsampling the training sets used to establish the discriminant function has demonstrated the power of using jackknife metrics to compare the relative strengths of discriminant analyses rather than just using the standard misidentification metrics. When few specimens are included in each group of the training set, the overall variation within each group might not be well sampled. If this is the case, it will be easier to find canonical axes, which separate the groups well, than when enough specimens are included to reliably estimate the true variation in each group. The standard misclassification metric, which describes how many specimens are misclassified when the canonical axes of maximum group separation are applied, therefore can make an analysis with very few specimens superficially appear to discriminate more accurately than one in which more specimens are included. However, when these specimens are jackknifed, identifications of those unknowns are less accurate than the standard misclassification rate reported and consistently favor the analysis that includes more specimens. For this reason, simply reporting how many specimens of known identity are misclassified by the discriminant analysis is not sufficient to characterize the precision or repeatability of the analysis. To create a precise discriminant analysis that will assign identifications robustly to unknown specimens, enough specimens must be included in each group of the training set that the percent misclassified under jackknifing approaches a stable number as more specimens are included. This problem is exacerbated as more variables are included in the discriminant analysis, as in the case of the M3 analysis, which has very few variables and less difference between the accuracy of the subset and full set analyses.

Species identification using geometric morphometrics.—In traditional morphometrics, which use discrete and continuous morphological variables to identify specimens, the variables are considered both separately and in multivariate analyses to determine the best combination of variables with which to identify specimens. These data do not accurately capture the overall shape of the morphological feature, because some components of shape are redundantly sampled whereas others may not be captured by any of the measurements or classifications (Maderbacher et al. 2008). Traditional morphometric characters generally are based on the lengths or the relative sizes of traits, and as a result they often confound size and shape variables (Zelditch et al. 2004). Additionally, they artificially make the morphological element being analyzed discrete (Zelditch et al. 2004). As a result, convergence on one or several of the characters being used is common, and if a candidate species is not included in the training set, the relative importance of the individual characters in the analysis for identification purposes can be compromised.

Because geometric morphometrics is the compilation of many variables that capture and characterize the overall shape of a morphometric structure, convergence on any overall shape axis is much more difficult. Although this might still be possible given strong selective pressure, it would require convergence of form across the entire tooth's shape rather than in an isolated feature. The discriminant analysis on m1 shape used herein establishes the nature of the geometric morphometric shape difference that maximally separates extant *Microtus* species in California. That component of m1 shape then is applied to unknowns. As a result of the conservative guidelines (e.g., consideration of Mahalanobis distance and 95% identification confidence) for determining unidentifiable specimens established herein, only specimens that are very similar to the mean for that species are positively identified, leaving little room for false positive identifications, as indicated by the jackknife analyses.

Although the analysis is unlikely to confuse the 5 modern taxa used in the training set, I cannot entirely discount the possibility of falsely identifying fossil species that either belong to an unsampled *Microtus* species or that are from fossil populations of 1 of the 5 sampled species that has past variation that overlaps that of a different extant species. Bell and Bever (2006) report that for North American *Microtus* species 12 candidate species with similar m1 five-triangle morphology exist. Although geometric morphometrics mitigates the potential for convergence by considering shape across the whole tooth, the possibility remains that the tooth shape for one of the included species is plesiomorphic, increasing the likelihood that a similar shape would appear in

other species that also retained the plesiomorphic state. Another scenario that could result in a misidentification is if variation in m1 of a species changed drastically through time, making it possible that the past shape of m1 in one species overlaps the modern shape of m1 in another used in the discrimination. Bell et al. (2010) and Bever (2005) demonstrated the importance of establishing phylogenetic polarity in characters that are used to discriminate species to help identify extinct species, detect when a character is plesiomorphic, and recognize when major shape change might have occurred within a lineage. Unfortunately, incomplete sampling across the *Microtus* genus, including species that fall within the phylogeny of the 5 species considered herein (Conroy and Cook 2000; Jaarola et al. 2004), preclude statistically confident polarization of the current shape axes used in this analysis. However, future inclusion of more *Microtus* species will allow for the polarization of m1 shape, which will determine the strength of m1 tooth shape as an identifying character across all North American *Microtus*. These issues are unlikely to affect the present study because of its geographic focus and consideration of relatively recent *Microtus* fossil specimens (<10,000 years old). However, caution should be taken when applying this identification method outside of the current geographic range or to ancient fossil specimens without the addition of other candidate *Microtus* species.

Unidentifiable fossil specimens.—Although the geometric morphometrics approach mitigates some concerns over false positives, my analyses did detect a significantly larger number of unidentified specimens than predicted by chance. The percent of unidentifiable fossil specimens from Pacheco 2 and Prune Avenue (38%) exceeds that predicted by the jackknife misidentification rate of the m1-SL training set (12.9%). This could represent 2 possible scenarios.

First, it is possible that some of the specimens included in the sample belong to species that were not included in this analysis. This is the benefit of having strong metrics for establishing unknowns. Once additional species are included in this analysis, these specimens likely will be identified correctly. Another possibility, as Wallace (2006) pointed out, is that the variation in the unknowns might exceed that of the training set. The training set consists of *Microtus* specimens collected over the last 100 years, whereas the fossil specimens represent a time-averaged sample of unknown duration. Additionally, it is difficult to know whether morphological variation was the same in past populations as currently exists. California *Microtus* species have weathered a large shift in grassland plant species in the Holocene (Dallman 1998), and this change in food type could have affected the amount of dental variation present in the species. Although the number of identifiable specimens is lower than expected, the identified specimens still strongly cluster with modern species, suggesting that mean shape might not have changed as drastically as variation in shape. As we expand our understanding of *Microtus* ecology in response to modern-day and Quaternary climate changes, we will begin to test these hypotheses of change in variation and range shifts through the fossil record.

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APPENDIX I

Museum of Vertebrate Zoology *Microtus* specimens used to create discriminant analyses. X indicates in which analysis each specimen was included.

Specimen no.	Species	m1	m1-SL	M3
13469	*M. longicaudus*	X	X	X
21099	*M. longicaudus*	X	X	X
21190	*M. longicaudus*	X		X
22388	*M. longicaudus*	X	X	X
23173	*M. longicaudus*	X	X	X
25014	*M. longicaudus*	X	X	X
25593	*M. longicaudus*	X	X	X
26409	*M. longicaudus*	X	X	X
26422	*M. longicaudus*	X	X	X
30816	*M. longicaudus*	X	X	X
30907	*M. longicaudus*	X	X	X
35063	*M. longicaudus*	X	X	X
35214	*M. longicaudus*			X
35224	*M. longicaudus*	X	X	X
37199	*M. longicaudus*			X
45858	*M. longicaudus*			X
46339	*M. longicaudus*	X	X	X
50909	*M. longicaudus*	X	X	X
51713	*M. longicaudus*	X	X	X
54419	*M. longicaudus*	X	X	X
54423	*M. longicaudus*	X	X	X
54823	*M. longicaudus*			X
54924	*M. longicaudus*	X	X	X
61270	*M. longicaudus*	X	X	X
64499	*M. longicaudus*	X	X	X
65221	*M. longicaudus*	X	X	X
65540	*M. longicaudus*	X	X	X
67060	*M. longicaudus*			X
69538	*M. longicaudus*	X	X	X
69558	*M. longicaudus*	X	X	X
71127	*M. longicaudus*	X	X	X
72400	*M. longicaudus*	X	X	X
74260	*M. longicaudus*	X	X	X
77535	*M. longicaudus*	X	X	X
79507	*M. longicaudus*	X	X	X
83922	*M. longicaudus*	X	X	X
86576	*M. longicaudus*	X	X	X
88687	*M. longicaudus*	X	X	X
96035	*M. longicaudus*	X	X	X
96036	*M. longicaudus*	X	X	X
96983	*M. longicaudus*	X	X	X
99283	*M. longicaudus*	X	X	X
103440	*M. longicaudus*	X	X	X
105523	*M. longicaudus*	X		X
109060	*M. longicaudus*	X	X	X
109327	*M. longicaudus*	X	X	X
118673	*M. longicaudus*	X	X	X
119354	*M. longicaudus*	X	X	X
122003	*M. longicaudus*	X	X	X
122005	*M. longicaudus*			X
126147	*M. longicaudus*	X	X	X
136903	*M. longicaudus*	X	X	X
148474	*M. longicaudus*			X
190188	*M. longicaudus*	X	X	X
196704	*M. longicaudus*	X	X	X
198766	*M. longicaudus*			X
202858	*M. longicaudus*	X	X	X
206525	*M. longicaudus*	X	X	X
15570	*M. montanus*	X	X	X
15633	*M. montanus*			X
24002	*M. montanus*	X	X	X
33722	*M. montanus*	X	X	X
36824	*M. montanus*	X	X	X
39748	*M. montanus*	X	X	X
40829	*M. montanus*	X	X	X
42048	*M. montanus*	X	X	X
45404	*M. montanus*	X	X	X
45416	*M. montanus*	X	X	X
45816	*M. montanus*	X	X	X
46590	*M. montanus*	X	X	X
50902	*M. montanus*	X	X	X
54789	*M. montanus*	X		X
59615	*M. montanus*	X	X	X
61280	*M. montanus*	X	X	X
64478	*M. montanus*	X	X	X
64711	*M. montanus*	X	X	X
64716	*M. montanus*	X	X	X
64922	*M. montanus*	X	X	
67671	*M. montanus*	X	X	X
68521	*M. montanus*	X	X	X
68750	*M. montanus*	X	X	X
68981	*M. montanus*	X	X	X
71110	*M. montanus*	X	X	X
72358	*M. montanus*	X	X	X
77464	*M. montanus*	X	X	X
77738	*M. montanus*	X	X	X
78051	*M. montanus*	X	X	X
78053	*M. montanus*	X	X	X
79421	*M. montanus*			X
79450	*M. montanus*			X
81522	*M. montanus*	X	X	X
83900	*M. montanus*	X	X	X
87790	*M. montanus*			X
88120	*M. montanus*	X	X	X
89964	*M. montanus*	X	X	X
93558	*M. montanus*	X	X	X
96372	*M. montanus*	X	X	X
96726	*M. montanus*	X	X	X
96735	*M. montanus*	X	X	X
112287	*M. montanus*	X	X	X
116451	*M. montanus*	X	X	X
116608	*M. montanus*	X	X	X
120809	*M. montanus*	X	X	X
122072	*M. montanus*	X	X	X
125219	*M. montanus*	X	X	X

APPENDIX I.—Continued.

Specimen no.	Species	m1	m1-SL	M3
126151	*M. montanus*			X
132861	*M. montanus*	X	X	X
134880	*M. montanus*	X	X	X
138668	*M. montanus*			X
147617	*M. montanus*	X	X	X
154039	*M. montanus*			X
154040	*M. montanus*	X	X	X
183846	*M. montanus*	X	X	X
206537	*M. montanus*	X	X	
206940	*M. montanus*	X	X	X
12348	*M. townsendii*	X	X	X
12428	*M. townsendii*	X	X	X
12435	*M. townsendii*	X	X	X
31069	*M. townsendii*	X	X	X
46453	*M. townsendii*	X	X	X
54432	*M. townsendii*	X	X	X
54433	*M. townsendii*	X	X	X
54434	*M. townsendii*	X	X	X
54435	*M. townsendii*	X	X	X
54436	*M. townsendii*	X	X	X
63495	*M. townsendii*	X	X	X
68823	*M. townsendii*	X		X
68824	*M. townsendii*	X	X	X
68825	*M. townsendii*	X	X	X
70435	*M. townsendii*	X	X	X
70436	*M. townsendii*	X	X	X
70437	*M. townsendii*	X	X	
70445	*M. townsendii*	X	X	X
83429	*M. townsendii*	X	X	X
83430	*M. townsendii*	X	X	X
83431	*M. townsendii*	X	X	X
88894	*M. townsendii*	X	X	X
94503	*M. townsendii*	X	X	X
94509	*M. townsendii*	X	X	X
94510	*M. townsendii*	X	X	X
94511	*M. townsendii*	X	X	X
94516	*M. townsendii*	X	X	X
94517	*M. townsendii*			X
94518	*M. townsendii*	X	X	X
94532	*M. townsendii*	X	X	X
94534	*M. townsendii*	X	X	X
94538	*M. townsendii*	X	X	X
94540	*M. townsendii*	X		X
94541	*M. townsendii*	X	X	X
94542	*M. townsendii*	X	X	X
94543	*M. townsendii*	X	X	X
96040	*M. townsendii*	X	X	X
96041	*M. townsendii*	X	X	X
96042	*M. townsendii*	X	X	X
96045	*M. townsendii*	X	X	X
96046	*M. townsendii*	X	X	X
101609	*M. townsendii*	X	X	X
134933	*M. townsendii*	X	X	X
134935	*M. townsendii*			X
134942	*M. townsendii*	X	X	X
134952	*M. townsendii*	X	X	X
183858	*M. townsendii*	X	X	X
183859	*M. townsendii*	X	X	X
190214	*M. townsendii*	X	X	X
216762	*M. townsendii*	X	X	X
216763	*M. townsendii*	X	X	X
216764	*M. townsendii*	X	X	X
216766	*M. townsendii*	X	X	X

APPENDIX I.—Continued.

Specimen no.	Species	m1	m1-SL	M3
20105	*M. oregoni*	X	X	X
20127	*M. oregoni*	X	X	X
20133	*M. oregoni*	X	X	X
20135	*M. oregoni*	X	X	X
20148	*M. oregoni*	X	X	X
44275	*M. oregoni*	X	X	X
46450	*M. oregoni*	X	X	X
46452	*M. oregoni*	X	X	X
54439	*M. oregoni*	X	X	X
54440	*M. oregoni*			X
54442	*M. oregoni*	X	X	X
57038	*M. oregoni*	X	X	X
60372	*M. oregoni*	X	X	X
87782	*M. oregoni*	X	X	X
87783	*M. oregoni*	X	X	X
87788	*M. oregoni*	X	X	X
88900	*M. oregoni*			X
88901	*M. oregoni*	X	X	X
94423	*M. oregoni*	X	X	X
94425	*M. oregoni*	X	X	X
94445	*M. oregoni*	X	X	X
94447	*M. oregoni*	X	X	X
94450	*M. oregoni*	X	X	X
94451	*M. oregoni*	X	X	X
94455	*M. oregoni*	X	X	X
94494	*M. oregoni*	X	X	X
94495	*M. oregoni*			X
94496	*M. oregoni*	X	X	X
96020	*M. oregoni*	X	X	X
96021	*M. oregoni*			X
96022	*M. oregoni*	X	X	X
96026	*M. oregoni*			X
96027	*M. oregoni*	X	X	
96028	*M. oregoni*	X	X	X
97516	*M. oregoni*	X	X	X
97518	*M. oregoni*	X	X	X
101862	*M. oregoni*	X		X
113249	*M. oregoni*	X	X	X
113251	*M. oregoni*	X	X	X
120608	*M. oregoni*	X	X	X
120609	*M. oregoni*	X	X	X
120610	*M. oregoni*	X	X	X
120612	*M. oregoni*	X	X	X
134901	*M. oregoni*	X	X	X
134902	*M. oregoni*	X	X	X
134905	*M. oregoni*	X	X	X
134906	*M. oregoni*	X	X	X
134910	*M. oregoni*	X	X	X
134915	*M. oregoni*	X	X	X
134918	*M. oregoni*	X	X	X
179087	*M. oregoni*	X	X	X
179088	*M. oregoni*	X	X	X
190202	*M. oregoni*	X	X	X
190203	*M. oregoni*	X	X	X
216761	*M. oregoni*	X	X	X
2790	*M. californicus*			X
2947	*M. californicus*	X	X	X
8991	*M. californicus*	X	X	
11586	*M. californicus*	X	X	X
12807	*M. californicus*			X
15760	*M. californicus*			X
15775	*M. californicus*	X	X	
15776	*M. californicus*			X

APPENDIX I.—Continued.

Specimen no.	Species	m1	m1-SL	M3
17385	*M. californicus*			X
18671	*M. californicus*	X	X	
23121	*M. californicus*			X
25001	*M. californicus*			X
26374	*M. californicus*			X
26383	*M. californicus*	X	X	
26385	*M. californicus*			X
28269	*M. californicus*	X	X	
28270	*M. californicus*	X	X	X
28922	*M. californicus*	X	X	
28923	*M. californicus*	X	X	
28933	*M. californicus*	X	X	X
29336	*M. californicus*	X	X	X
35863	*M. californicus*	X	X	X
35884	*M. californicus*	X	X	
35887	*M. californicus*			X
36132	*M. californicus*			X
36136	*M. californicus*			X
36137	*M. californicus*	X	X	
36320	*M. californicus*	X	X	X
36865	*M. californicus*			X
44510	*M. californicus*	X	X	X
54927	*M. californicus*			X
59095	*M. californicus*	X	X	X
60289	*M. californicus*			X
65428	*M. californicus*	X	X	X
66806	*M. californicus*	X	X	X
69510	*M. californicus*	X	X	X
69522	*M. californicus*	X		X
70129	*M. californicus*	X		X
70130	*M. californicus*	X	X	X
73074	*M. californicus*	X	X	X
74697	*M. californicus*	X	X	X
83519	*M. californicus*	X	X	
84924	*M. californicus*	X	X	X
89453	*M. californicus*	X	X	
89904	*M. californicus*	X	X	
90271	*M. californicus*	X	X	
93895	*M. californicus*			X
96275	*M. californicus*	X	X	X
99652	*M. californicus*	X	X	X
101859	*M. californicus*	X		X
102696	*M. californicus*	X	X	X
103904	*M. californicus*	X	X	X
108768	*M. californicus*			X
108784	*M. californicus*	X	X	X
108816	*M. californicus*	X	X	X
113456	*M. californicus*			X
121605	*M. californicus*	X		
123685	*M. californicus*			X
129075	*M. californicus*	X	X	
132724	*M. californicus*	X	X	X
132727	*M. californicus*	X	X	X
148489	*M. californicus*	X	X	X
149766	*M. californicus*	X	X	X
154399	*M. californicus*	X	X	X
182146	*M. californicus*	X	X	
200047	*M. californicus*	X	X	
200053	*M. californicus*	X	X	X
200876	*M. californicus*	X	X	X
206897	*M. californicus*	X	X	
206898	*M. californicus*			X

APPENDIX II

Fossil *Microtus* specimens from the University of California Museum of Paleontology and their identifications based upon m1 tooth shape using geometric morphometrics and discriminant analyses. X indicates specimens with confident identifications based on prediction probability ($\geq$0.95) and Mahalanobis distances.

Specimen no.	Locality	Predicted species	Prediction probability	Confident
v_190249	Pacheco 2	*M. longicaudus*	0.81	
v_190246	Pacheco 2	*M. californicus*	1.00	X
v_190244	Pacheco 2	*M. californicus*	0.99	X
v_190255	Pacheco 2	*M. longicaudus*	0.86	
v_190243	Pacheco 2	*M. californicus*	1.00	X
v_190242	Pacheco 2	*M. californicus*	1.00	X
v_190240	Pacheco 2	*M. californicus*	0.99	X
v_190251	Pacheco 2	*M. californicus*	0.99	X
v_190248	Pacheco 2	*M. longicaudus*	0.94	
v_190247	Pacheco 2	*M. californicus*	0.54	
v_190237	Pacheco 2	*M. californicus*	1.00	X
v_190236	Pacheco 2	*M. californicus*	1.00	X
v_190249	Pacheco 2	*M. californicus*	0.93	
v_190239	Pacheco 2	*M. californicus*	1.00	X
v_190463	Pacheco 2	*M. californicus*	0.85	
v_190465	Pacheco 2	*M. californicus*	1.00	X
v_190184	Pacheco 2	*M. californicus*	1.00	X
v_190199	Pacheco 2	*M. californicus*	1.00	X
v_190205	Pacheco 2	*M. californicus*	0.63	
v_190207	Pacheco 2	*M. californicus*	0.99	X
v_190209	Pacheco 2	*M. longicaudus*	0.99	X
v_190211	Pacheco 2	*M. californicus*	1.00	X
v_190241	Pacheco 2	*M. longicaudus*	1.00	X
v_190245	Pacheco 2	*M. longicaudus*	1.00	X
v_190460	Pacheco 2	*M. californicus*	0.99	X
v_197576	Prune Avenue	*M. californicus*	1.00	X
v_197577	Prune Avenue	*M. californicus*	1.00	X
v_197578	Prune Avenue	*M. longicaudus*	0.97	X
v_197579	Prune Avenue	*M. californicus*	0.83	
v_197580	Prune Avenue	*M. californicus*	0.97	X
v_197581	Prune Avenue	*M. californicus*	0.88	
v_197582	Prune Avenue	*M. californicus*	1.00	X
v_197583	Prune Avenue	*M. longicaudus*	0.53	
v_197584	Prune Avenue	*M. longicaudus*	0.99	X
v_197585	Prune Avenue	*M. californicus*	1.00	X
v_197586	Prune Avenue	*M. longicaudus*	1.00	X
v_197587	Prune Avenue	*M. californicus*	1.00	X
v_197589	Prune Avenue	*M. longicaudus*	0.86	
v_197590	Prune Avenue	*M. californicus*	1.00	X
v_197591	Prune Avenue	*M. longicaudus*	0.74	
v_197592	Prune Avenue	*M. californicus*	0.86	
v_197593	Prune Avenue	*M. longicaudus*	0.97	X
v_197594	Prune Avenue	*M. californicus*	0.84	
v_197595	Prune Avenue	*M. californicus*	0.79	