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Patterns of Differentiation and Disparity in Cranial Morphology in Rodent Species of the genus *Megadontomys* (Rodentia: Cricetidae)

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Rachel M. Vallejo, José Antonio Guerrero, and Francisco X. González-Cózatl (2017) The genus *Megadontomys* is a Mexican endemic group of rodents with allopatric populations occurring in fragmented patches of cool-humid forest. In this study we used geometric morphometrics methods to assess patterns of morphological variation and differentiation in skull and mandible among and within species of the genus. ANOVA showed that sexual dimorphism was significant for skulls size ($P < 0.01$) but not for mandibles, and MANOVA indicated that both structures did not differ in shape between sexes. ANOVA reveled a significant difference among the three species ($P < 0.01$), *M. nelsoni* exhibit the largest skull. Canonical variate analyses and Goodall's test found differences in both skulls and mandibles shape among species, being *M. cryophilus* and *M. thomasi* the most divergent. The comparison between phylogroups within *M. thomasi* also revealed significant differences in shape for both structures. Disparity assessment showed that *M. thomasi* is the species that contributed the most to the overall shape disparity (51.80% for skull and 38.29% for mandible). The permutation test of phylogenetic signal in morphometric data was significant for the skull but not for the mandible. Morphometric data support the recognition of three morphotypes whitin the genus. The sister species *M. nelsoni* and *M. thomasi* displayed a grater shape similarity in the skull and mandible shape between them. In contrast, *M. cryophilus* exhibited the greatest shape divergence relative to the other species. The morphological evidence supports the existence of the two different phylogroups within *M. thomasi*, supporting their recognition as Evolutionary Significant Units previously suggested on molecular data. The lack of phylogenetic signal in the mandible corresponds with the environmental plasticity of this structure as compared with the skull.

Key words: Disparity, Evolutionary history, Geometric morphometrics, *Megadontomys*, Morphological differentiation.

BACKGROUND

Morphological structures constitute fundamental features for identification and description of new taxonomic groups (Arnold and Ahearn 1972; Rzhavsky 1993; Martin et al. 1996; Scotland et al. 2003; Solari 2004; González-Sponga 2009) and for understanding rates of species diversification (Abramov et al. 2016). Also, morphological characters have been used for reconstructing phylogenetic patterns, under the assumption that the phenotype is the result of evolutionary history (Caumul and Polly 2005). However, it is clear that environmental features may also play an important role in molding phenotypic attributes, which in some cases, may result in homoplastic characteristics (Collard and O'Higgins 2001; Caumul and Polly 2005; Gilbert and Rossie 2007). Therefore, the use of morphological characters in phylogenetic reconstruction should be carefully considered because the establishment of homology in phenotypic attributes may become complicated, and ultimately, may lead to an imprecise genealogical reconstruction (Scotland

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et al. 2003; Wiens 2004; Collard and Wood 2007; Cardini and Elton 2008).

With the development of new approaches, the use of morphological characters has been reconsidered, and even, these have been employed under a different context. A relatively novel technique with the potential of formally dissociating size and shape is geometric morphometrics. This approach is based on the analysis of landmark coordinates or Cartesian geometric coordinates of morphological structures under rigorous statistical methods (Adams et al. 2004; Mitteroecker and Gunz 2009; Lawing and Polly 2011). Geometric morphometrics allows the description of patterns of shape variation within and among groups, and graphically display changes or differences among morphological characters (Adams et al. 2004). Even though geometric morphometrics is a promising approach for phylogenetic reconstruction (Catalano et al. 2010, 2014), it has been pointed out that several practical and theoretical issues should be considered in the implementation of any method that seeks to recover historical patterns (Scotland et al. 2003; Adams et al. 2011). For now, geometric morphometrics analysis has been employed to assess patterns of morphological variation (Hernández-Romero et al. 2015) or to study morphological evolution and to detect phylogenetic signal on previously recognized monophyletic groups as defined for phylogenetic analysis based on alternative kind of characters (*i.e.* molecular data; Cardini 2003; Adams et al. 2004). The application of this strategy has allowed the quantification of the diversity of forms within a group, using the disparity as a measure of morphological variation (Foote 1997; Collar et al. 2005). Particularly, disparity estimation may play an important role, from a conservation viewpoint, in the identification of the diversity of forms within a species that may eventually lead to the recognition of Evolutionary Significant Units (ESUs). According to Crandall et al. (2000) the categorization of population distinctiveness as ESUs, should include genetic and ecological evidence. Thus, diagnosis of distinct populations must emphasize variation in phenotypes, allowing preservation of important adaptative characters and their associated underlying genetic variation. Genetically, this variation can be shaped by gene flow, and ecologically, genetic drift and natural selection are mainly responsible for variation in phenotypes. Certainly, although morphological variation may be the result of only one or several forces, it is clear that assessment of disparity may contribute to identification of potential ESUs within species.

The genus *Megadontomys* is a Mexican endemic group of rodents with allopatric populations occurring in fragments of cool-humid forest in the highlands of the states of Guerrero, Hidalgo, Oaxaca, Puebla and Veracruz (Fig. 1; Musser, 1964; Heaney and Birney 1977; Werbitsky and Kilpatrick 1987; Ceballos and Oliva 2005; Vallejo and González-Cózatl 2012). Recently, Vallejo and González-Cózatl (2012) reevaluated the systematics of the genus *Megadontomys* based on mitochondrial cytochrome *b* sequence data, and found support for the recognition of three species within the genus: *M. cryophilus* (Sierra de Juárez, Oaxaca), *M. nelsoni* (Sierra Madre Oriental/Sierra Mazateca) and *M. thomasi* (Sierra Madre del Sur/Sierra Mixteca). Their data also support a closer evolutionary relationship between *M. nelsoni* and *M. thomasi*, relative to *M. cryophilus*, a view compatible with Musser (1964) and Werbitsky and Kilpatrick (1987; Fig. 2). At the intraspecific level, *M. thomasi* is formed by two genetically differentiated lineages that are proposed as distinct Evolutionary Significant Units (Vallejo and González-Cózatl 2012; Fig. 2).

Although previous taxonomic decisions and hypotheses on the evolutionary relationship within the genus *Megadontomys* have been addressed using morphological characters (Merriam 1898; Musser 1964; Carleton 1980, 1989), treatment of data has not included the use of formal phylogenetic or morphometric methods. Additionally, overall sample size and geographic representation have been limited. Therefore the amplification of geographical sampling and the use of morphometric techniques are justified and, even more provide finer resolution on the degree of relatedness and degree of divergence within this group. In this context, the goal of this study was to examine patterns of variation and differentiation in skull and mandible morphology among and within species of *Megadontomys* to assess whether these patterns are consistent with previous views on the evolutionary history of the genus, particularly those depicted by phylogenetic analyses of molecular evidence. Also, we were interested in assessing if the degree of morphological disparity is congruent with levels of molecular differentiation among species and between phylogroups within *M. thomasi* as pointed out by Vallejo and González-

Cózatl (2012). To this end, we substantially increased the number of sampling localities and the total number of specimens for each species, comparing with previous studies, and analyzed morphological attributes using geometric morphometric methods.

MATERIALS AND METHODS

Specimens

The skulls and mandibles were obtained from Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos

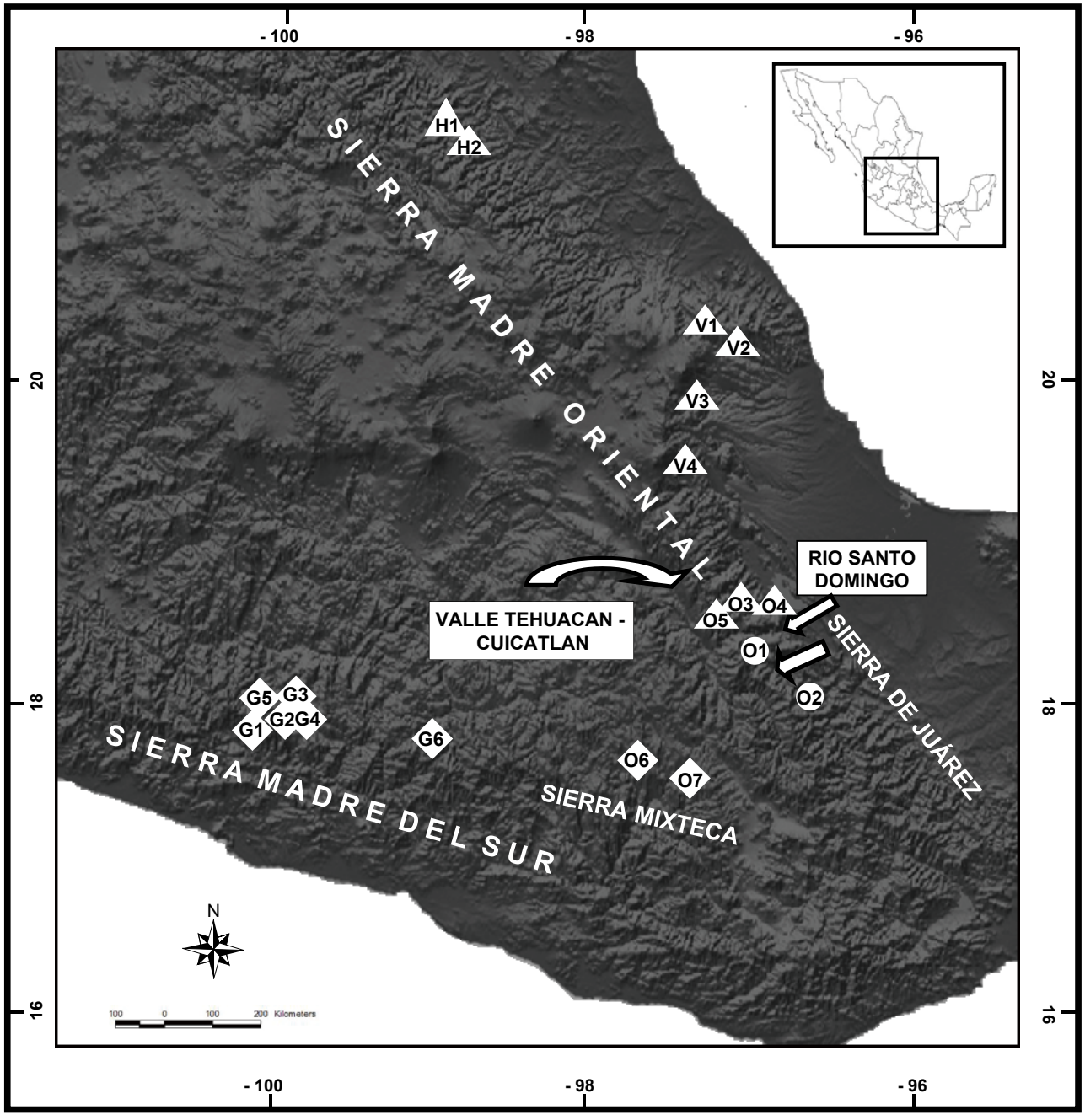

Fig. 1. Map of México showing sampling localities of the 3 species of *Megadontomys* (modified from Vallejo and González-Cózatl 2012*). M. cryophilus* = circles; *M. nelsoni* = triangles*; M. thomasi* = diamonds. The locality code corresponds to those listed in Appendix I, where the letter stands for the respective State in México (H = Hidalgo; V = Veracruz; O = Oaxaca; G = Guerrero).

(CMC), and Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA). A total of 153 adults from 19 localities, representing the three species of the genus, were examined (*M. cryophilus* = 61 individuals, *M. nelsoni* = 51 individuals, *M. thomasi* = 41 individuals; Fig. 1; Appendix I). Also, 24 specimens of *Peromyscus mexicanus* were exclusively included for comparison purposes in the phylogenetic signal test (see below; Appendix I). Adults were categorized based on the wear of cusps of teeth, the presence of the third molar, and hair color (Hoffmeister 1951).

Digital images, landmarks, and geometric morphometrics

The ventral view of 153 skulls and lateral view of 127 mandibles (26 had broken structures and were not included in the analysis) were photographed with a digital camera Canon Power Shot A300, with a resolution of 300 pixels per inch. The coordinates X and Y of 37 landmarks for the ventral view of the skull (Fig. 3a, Table 1) and 18 landmarks for the lateral view of the mandible (Fig. 3b, Table 1), which are assumed to be homologous from one individual to another, were digitized using the tpsDig 2.15 software (Rohlf 2010). For the ventral view of the skull, only landkmarks on the left side were registered to avoid redundant information due to the bilateral symmetry condition of this structure. For each structures (skull and mandible), a Generalized Procrustes Superimposition procedure was implemented in CordGen module of IMP software (Sheets 2002) to eliminate all variation that is not related to shape, additionally, it produced an overall average shape that was used as a reference in subsequent analyses (Zelditch et al. 2004). Moreover, the coordinates of the superimposed configurations

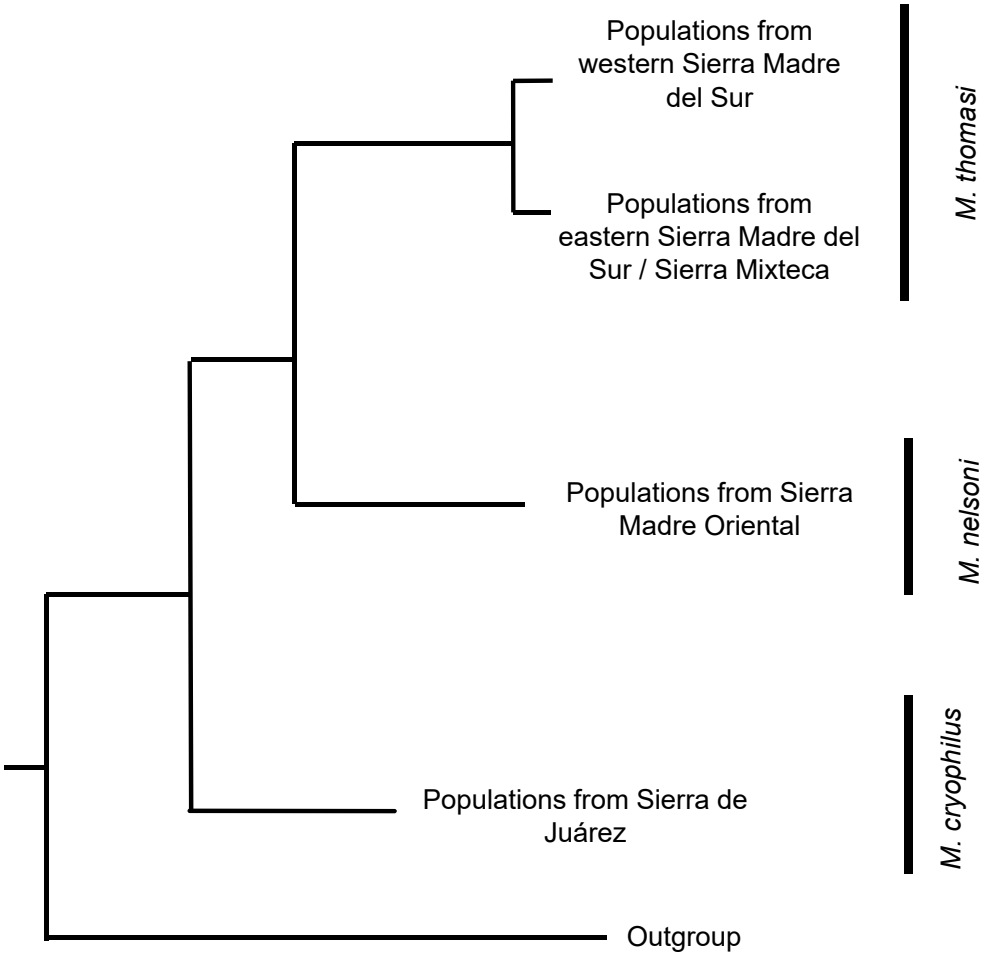

Fig. 2. Phylogenetic relationships of the genus *Megadontomys* based on cytochrome *b* sequence data (modified from Vallejo and González-Cózatl 2012*)*.

were transformed into shape variables (partial warps) through a Thin-plate spline analysis (Bookstein 1991). This analysis produces a geometric description of shape using the partial warps to detect deformations relative to a general consensus to explain the shape change within and between species (Singh et al. 1997; Rosas and Bastir 2002).

Statistical analysis

Two-way univariate analysis of variance (ANOVA) for centroid size and multivariate for shape variables (MANOVA) were performed to test for the differences between sexes and among species. Statistical analyses were executed using Statistica 6.0 software (Statsoft 2001).

Canonical Variate Analysis (CVA) was employed to analyze among and within species shape variation using CVA Gen6 (IMP series; Sheets 2002). This method extracts a number of axes (canonical variables) from a multidimensional space, which explain the higher proportion of the variance between the groups (Klingenberg et al. 2003). The choice of the canonical variate axes was based on the Wilks' λ value, which is the sum of squares within groups divided by the total sum of squares within and between groups (IMP series; Sheets 2002). To graphically visualize the shape changes associated with the canonical variables, we generated deformation grids with the Thin-plate spline interpolation function (Bookstein 1991), considering only the extreme points of each axis on the CVA plot and magnifying the changes three times. Moreover, we evaluated differences in mean centroid size among the three species of *Megadontomys* implementing a one-way ANOVA with a Bonferroni correction as implemented in Statistica 6.0 software (Statsoft 2001). Additionally, although this not a traditional morphometric study, we performed an ANOVA on standard measures (Total length, tail length, hind foot, ear from notch, and weight) to test if there is an unequal pattern of differentiation in size, among these species. Data were obtained from specimens deposited at the CMC (Appendix I).

A permutation test was performed on Procrustes distances to examine the shape differences among species and between phylogroups of *M. thomasi*, as recognized by Vallejo and González-Cózatl (2012). Goodall's *F* statistical test is specifically designed to the coordinates produced by Procrustes superimposition (Goodall 1991). The Procrustes

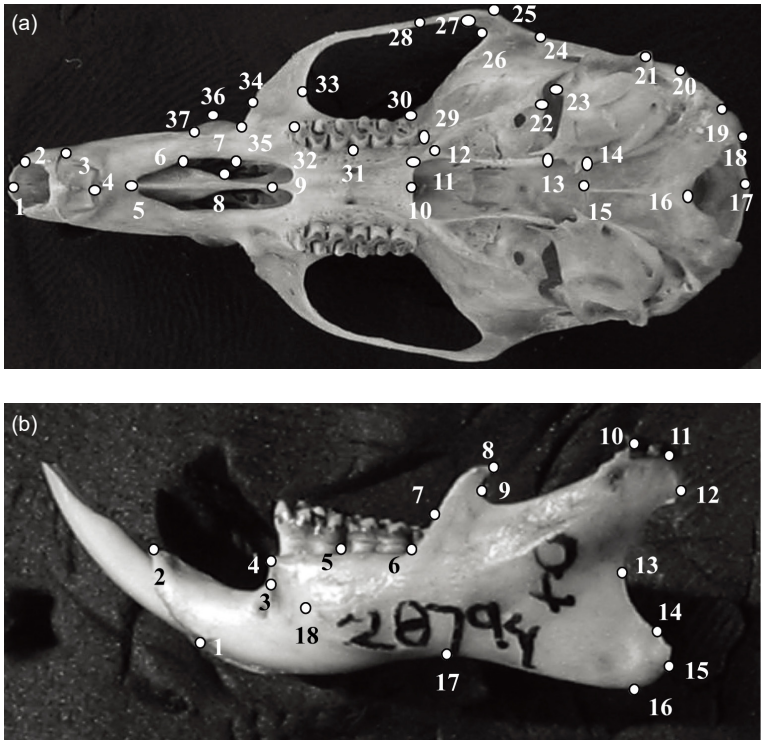

Fig. 3. Locations of landmarks for (a) ventral view of the skull and (b) lateral view of the mandible. Landmarks numbers corresponded to those listed in Table 1.

Table 1. Morphological definition of landmarks for the occlusal view of the skull and the lateral view of the mandible

Cranial View	Number Landmarks	Definition
Skull	1	Anterior tip of the nasal
	2	Anterior tip of suture between nasal and premaxilla
	3	Base of the incisivor
	4	Meeting point between the incisivors
	5	Anterior tip of incisive forame
	6	Meeting point between incisive forame and palatine process
	7	Medium point of incisive forame
	8	Medium point of palatine process
	9	Posterior point of incisive forame
	10	Posterior point of palatine
	11	Posterior point lateral of palatine
	12	Meeting point between external and internal pterigoid processes
	13	Posterior point of internal pterigoid process
	14	Lateral point of tympanic bulla
	15	Anterior point of occipital
	16	Meeting point between occipital condyle
	17	Posterior point of foramen magnum
	18	Meeting point between foramen magnum and occipital condyle
	19	Posterior meeting point between occipital condyle and occipital
	20	Posterior lateral point of external auditory meatus
	21	Anterior lateral point of external auditory meatus
	22	Posterior point of external pterigoid process
	23	Anterior point of tympanic bulla close to external pterigoid process
	24	Posterior point of squamosal
	25	Lateral medium point of squamosal
	26	Anterior point of squamosal
	27	Meeting point between squamosal and jugal
	28	Meeting point between jugal and zygomatic
	29	Posterior point of third molar
	30	Lateral point of third molar
	31	Meeting point between 1st and 2nd molar
	32	Anterior point of 1st molar
	33	Posterior point of malar process
	34	Anterior point of malar process
	35	Meeting point between malar process and maxilla
	36	Lateral point of maxilla
	37	Anterior point of maxilla
Mandible	1	Base of the incisor
	2	Anterior point of diastema
	3	Inferior point of maxillary toothrow
	4	Posterior point of diastema
	5	Meeting point between 1st and 2nd molar
	6	Meeting point between 2nd and 3rd molar
	7	Base of the coronoid process
	8	Tip of the coronoid process
	9	Medium point of incisura mandibulae
	10	Anterior tip of the condyle
	11	Medium tip of the condyle
	12	Posterior tip of the condyle
	13	Medium point of the condyle
	14	Medium point of the angular process
	15	Posterior tip of the angular process
	16	Anterior point of the angular process
	17	Inferior medium point of mandible
	18	Anterior point of the masseteric ridge

distances express the differences between shapes from each group especially when sample size is unequal (Cardini 2003; Zelditch et al. 2004). Goodall's *F* test was applied employing the software TwoGroup6 (IMP series; Sheets 2002) with 2500 permutations.

Morphological diversity in the shape of both cranial structures among and within species was assessed using the Procrustes distances following the method proposed by Foote (1993). This approach considers that morphological disparity depends on a measure that reflects distances among points in morphological space. In this case, the variances are the measures used for explain the disparity because they have the property of being additive allowing the calculation of the partial disparity to obtain the overall disparity as the result of the partial contributions for each group (Foote 1993; Zelditch et al. 2004). Partial disparity estimations were obtained with the software PairDisparity6 (IMP series; Sheets 2002).

Phylogenetic signal test

The phenotype could be the resulted either from of phylogenetic history or adaptations to local environments (Caumul and Polly 2005). If the first case, the morphology of two groups that share a common ancestor will be more similar in comparison with that of most distant groups. Several studies have used a permutation test to evaluate the presence or absence of phylogenetic signal in the morphometric data (Figueirido et al. 2010; Meloro et al. 2011; Klingenberg et al. 2012). This test assumes that closely related forms tend to occupy the same portion of the morphometric space, because they share a common ancestor in comparison with distantly related species, which are found at different segments of the morphometric space. This approach is implemented by mapping morphometric traits onto know phylogenies by the method of squared-change parsimony to reconstruct ancestral shape of morphometric data. The test simulates the null hypothesis of the complete absence of phylogenetic structure by permutation of the shape data among the terminal taxa (Klingenberg and Gidaszewski 2010). In order to perform this test we selected *Peromyscus mexicanus* as outgroup. Although there is uncertainty about the sister group of the genus *Megadontomys*, several studies have pointed out a close phylogenetic affinity between these taxa (Rogers 1983; Rogers et. al. 1984; Vallejo and González-Cózatl 2012). The permutation test was implemented in MorphJ software with 50,000 random permutations (Klingenberg 2008).

RESULTS

Sexual dimorphism

For skulls, sexual dimorphism in size was significant. Males were, on average, larger than females in the three species. Interspecific differences in size were also significant, while sex by species interaction was not significant (Table 2). Differences in skull shape were, however, significant only between species (Wilks' $\lambda = 0.012$, $P < 0.05$, Table 3). For mandibles, neither size nor shape was significantly different between sexes ($P > 0.05$, Table 2 and Table 3) only significant differences were detected in shape among species (Wilks' $\lambda = 0.066$, $P > 0.05$, Table 3). For both skull and mandible, interaction between sex and species was not significant ($P > 0.05$, Tables 2 and 3); therefore, sexes were pooled in all subsequent analyses.

Table 2. Two-way ANOVA for sex, species, and sex × species interaction effects for the skull centroid size in *Megadontomys*. Significant *P* values ($P < 0.05$) are indicated in bold and with an asterisk

Cranial View	Effect	Sum of squares	*F*	*d.f.*	*P*-value
Skull	Sex	97.1	5.67	1	**0.0185***
	Species	177.2	5.18	2	**0.007***
	Sex × Species	34.5	1.01	2	0.367
	Error	2515.25		147	
Mandible	Sex	13.6	3.49	1	0.064
	Species	62.8	8.03	2	**0.000***
	Sex × Species	0.1	0.01	22	0.99
	Error	473		121	

CVA and interspecific morphometric variation

Canonical variates analyses, with species as the grouping variable, found differences in both skulls (Fig. 4) and mandibles (Fig. 5). In both cases, the first two canonical variates showed significant differentiation in the shape among three species: *M. cryophilus*, *M. nelsoni* and *M. thomasi* (Fig. 4 and Fig. 5; Skull: CV1: Wilks' λ = 0.0127, $P < 0.0001$, CV2: Wilks' λ = 0.1673, $P < 0.0001$; mandible: CV1 Wilk's λ = 0.0674, $P < 0.0001$, CV2 Wilks' λ = 0.3479, $P < 0.0001$). Shape differences detected by canonical variates are illustrated on TPS grids (Figs. 4 and 5). When comparing the skull between *M. cryophilus* and *M. thomasi*, CV1 shows that the former has a shorter malar process

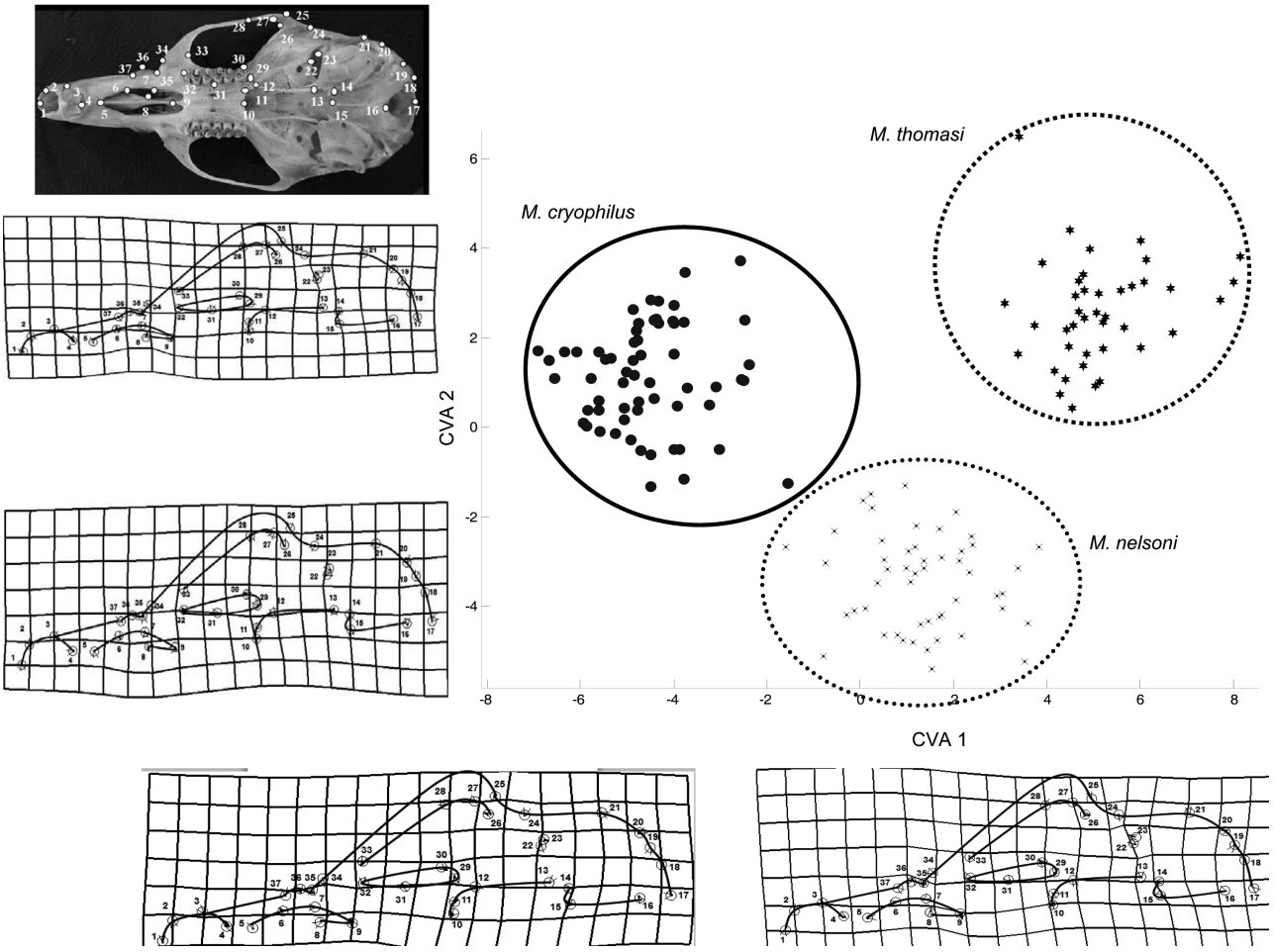

Fig. 4. Scatterplot of CV1 and CV2 scores for oclussal view of skull. TPS deformation grids for the extreme points of each axis are shown. Deformation grids were 3x exaggerated.

Table 3. Two-way ANOVA for sex, species, and sex × species interaction effects for the skull centroid size in *Megadontomys*. Significant *P* values ($P < 0.05$) are indicated in bold and with an asterisk

Cranial View	Effect	Wilks' λ	*F*	*d.f.*	*P*-value
Skull	Sex	0.526	1	70	0.49
	Species	0.012	8.745	140	**0.000***
	Sex × Species	0.294	0.938	140	0.648
Mandible	Sex	0.692	1.249	32	0.206
	Species	0.066	8.063	64	**0.000***
	Sex × Species	0.58	0.88	64	0.718

and maxilla (landmarks 35- 37), short and narrow incisive foramen (landmarks 5- 9), the region of tympanic bulla and occipital is short and narrow (landmarks 13- 15, 22- 23), closed zygomatic arch (landmarks 26- 27), narrow squamosal (landmark 24) and the region of the maxillary molars is long (landmarks 29- 32) (Fig. 4). Compared to *M. thomasi*, *M. nelsoni* has a large malar process and maxilla (landmarks 35- 37), long and wide incisive foramen (landmarks 5- 9), a long and narrow tympanic bulla and occipital region (landmarks 13- 15, 22- 23), opened zygomatic arch (landmarks 26- 27), wide squamosal (landmark 24) and the region of the maxillary molars is short (landmarks 29- 32) (Fig. 4).

Canonical variate 1 showed that the mandibles of *M. thomasi* and *M. cryophilus* are different (Fig. 5). *M. cryophilus* has a shorter mandible, a narrower condyle (landmarks 10, 12), a longer coronoid process (landmark 8), a deeper incisura mandibulae (landmark 9), a more opened posterior point of diastema (landmark 4), and a wider and longer angular process (landmarks 14- 16). Canonical variate 2 revealed that *M. nelsoni* has a large mandible, but it is narrow in the zone of processes and in the middle portion. Also, it possesses a wider condyle (landmarks 10, 12), shorter coronoid process (landmark 8), wider incisura mandibulae and longer molar region (landmarks 9, 4), and it is long and wide in the angular process (landmarks 14- 16) (Fig. 5), compared with *M. thomasi*.

Morphometric differentiation among and within species

ANOVA showed a significant difference in size among groups/species (skull: $F = 5.05$, $P = 0.007$; mandible: $F = 7.24$, $P = 0.001$). For both skull and mandible, *M. nelsoni* was the largest (skull centroid size (SCS) = 71.049, mandible centroid size (MCS) = 30.145), although there were not differences between *M. cryophilus* (CS = 68.531) - *M. thomasi* (CS = 69.462) and *M. nelsoni* (CS = 71.049) - *M.*

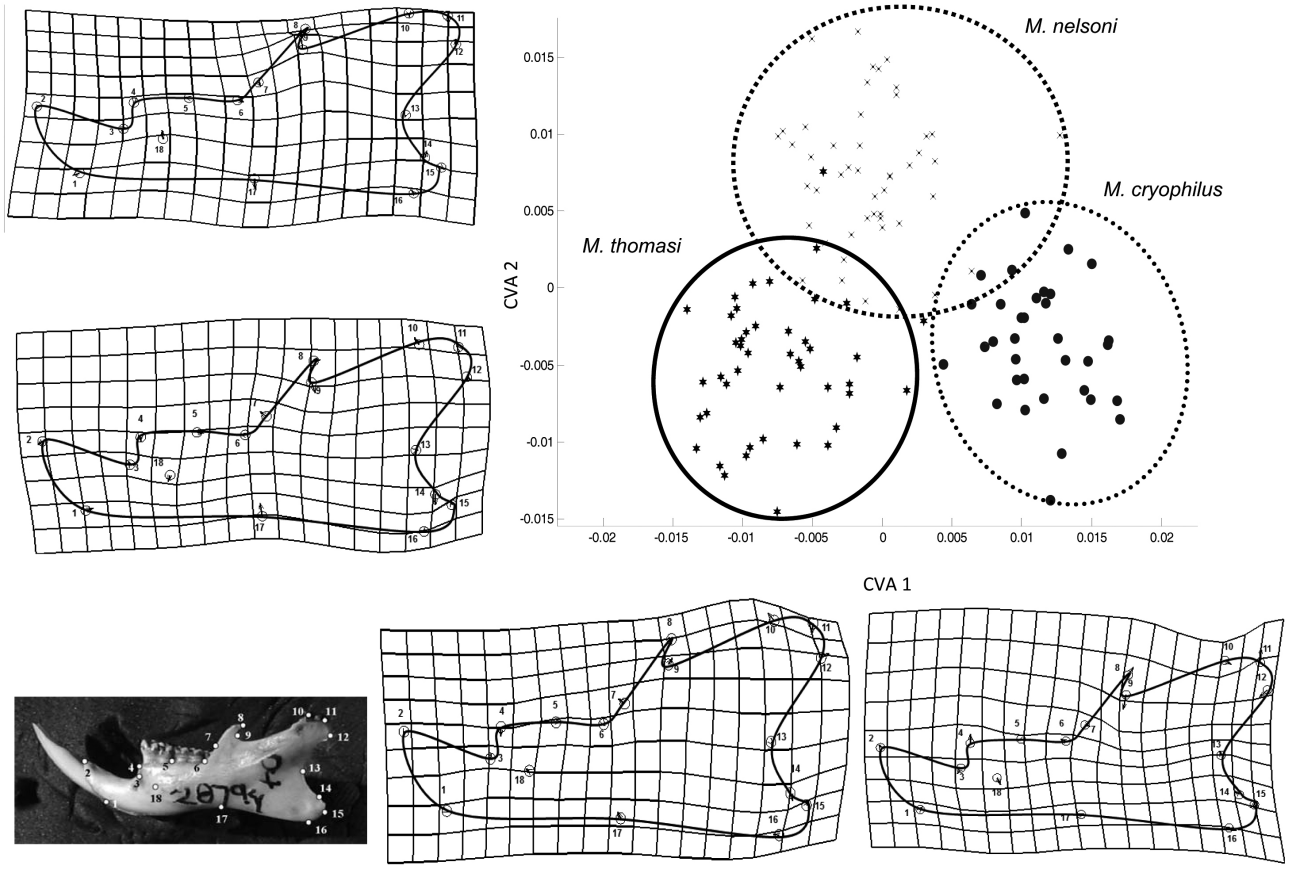

Fig. 5. Scatterplot of CV1 and CV2 scores for lateral view of the mandible. TPS deformation grids for the extreme points of each axis are shown. Deformation grids were 3x exaggerated.

thomasi (CS = 69.462) for skull (P = 0.819 and P = 0.220, respectively). For the mandible, significant differences were found between *M. nelsoni* and *M. thomasi* (P = 0.001; CS = 30.145 and CS = 28.699 respectively), but not between *M. cryophilus* and *M. thomasi* (P = 1.000; CS = 28.868 and CS = 28.699 respectively). In addition, ANOVA on standard measures showed that *M. nelsoni* was significantly larger only in total body length (333.89 mm; P = 0.02), and weight (79.88 g; P < 0.01). With respect to the shape, Goodall's F test revealed three different shapes (P < 0.0001) that correspond to each one of the species (see above). Additionally, the greatest morphological divergence was between *M. cryophilus* and *M. thomasi* (Procrustes distance for skull: 0.0306 and mandible: 0.0486), while the lowest value was between *M. nelsoni* and *M. thomasi* (Procrustes distance for skull: 0.0191 and mandible: 0.0289). The comparison between phylogroups within *M. thomasi* (western of the Sierra Madre del Sur and eastern of the Sierra Madre del Sur- Sierra Mixteca) revealed significant differences in shape for both structures (skull: F = 2.22, P < 0.0001; mandible: F = 2.72, P <0.0001).

Disparity analysis and permutation test for phylogenetic signal

Disparity assessment indicated that, for skulls, *M. thomasi* is the species that contributed the most to the overall disparity (skull: 51.80%), whereas *M. nelsoni* displayed intermediate values (26.89%), and *M. cryophilus* had relatively lower values (21.32%). With respect to the mandible, disparity values for *M. thomasi* and *M. cryophilus* were higher (38.29% and 36.21%, respectively) with respect to *M. nelsoni* (25.50%).

The permutation test of phylogenetic signal for unweighted squared-change parsimony found significant phylogenetic signal in morphometric data for the skull (Tree length= 0.0037, P = 0.0001) but not for the mandible (Tree length= 0.0816, P = 0.1236).

DISCUSSION

Overall, geometric morphometric analyses of skull and mandible (ANOVA, CVA and Goodall's F test on Procrustes distances) are consistent with the recognition of three general phenotypic shapes or morphotypes within the genus *Megadontomys.* These shapes correspond well with the three main lineages recognized in this genus, either at the subspecies level (Musser 1964; Carleton 1980; Werbitsky and Kilpatrick 1987) or as distinct species (Merriam 1898; Carleton 1989; Vallejo and González-Cózatl 2012). In addition, the overall similarities and differences in both, skull and mandible shape, as reveled by morphometric analyses, are, in general congruent with the evolutionary history of the genus as proposed by several authors (Musser 1964; Werbistky and Kilpatrick 1987; Vallejo and González-Cózatl 2012). Our results showed a great shape similarity in the skull and mandible between the sister species *M. nelsoni* and *M. thomasi*, as they had the lowest Procrustes distances. In contrast, *M. cryophilus* exhibits the greatest shape divergence relative to the other species, which is also consistent with the position of this taxon as a separate clade in the phylogeny (Fig. 2). Also, we confirmed the existence of an unequal degree of differentiation among the species, with respect to size, where *M. nelsoni* was the largest. Our results evidently do not support Musser's view (1964), who suggested that *M. thomasi* and *M. cryophilus* represent the extremes of morphological differentiation within the genus. Though, it is important to point out that sample size in Musser's work was limited to only one individual of *M. nelsoni* and, therefore, interpretation of this comparison is not strictly conclusive.

At the intraspecific level, patterns of morphological variation and differentiation of skull and mandible are also congruent with molecular data (Vallejo and González- Cózatl 2012). Populations of *M. thomasi* that are distributed in western Sierra Madre del Sur (Fig. 1; G1- G5) and those located in the eastern portion of the Sierra Madre del Sur and the Sierra Mixteca (Fig. 1; G6, O6 – O7) are morphologically differentiated. As pointed out by Vallejo and González- Cózatl (2012), within *M. thomasi* there are two clearly divergent phylogroups, restricted to these mountain ranges. Although genetic levels of differentiation are still below the threshold suggested for specific recognition (Bradley and Baker 2001), it is clear that the congruence between patterns of molecular and morphological differentiation suggests that these allopatric populations may be reproductively isolated. Although these divergent populations are not yet full species, the significant degree of differentiation should be considered in the designing of conservation strategies for this group (see below).

Disparity assessment showed that *M. thomasi* is the species that contributes the most

to the morphological richness of the genus. Interestingly, our data indicate that more than 50% of the morphological space configured by skull shape is occupied for this species. Morphological disparity assumes that taxonomic diversity is the consequence of evolutionary processes (Foote 1997), and therefore it can be interpreted that morphological differentiation displayed between phylogroups of *M. thomasi* is the result of evolution. This finding is supported by the Permutation test of phylogenetical signal in the skull, which indicates that this structure may provide reliable information on the evolution of this group. However, this was not the case for the mandible, since the test indicated that there is not significant phylogenetic signal on this morphological feature. Explanation on the lack of phylogenetic signal in the mandible is beyond the scope of this study, but it has been suggested that the mandible is more prone to ecophenotypic variance than skull, due to developmental aspects associated to physical and metabolic factors (Caumul and Polly 2005). Even though mandible shape may have not been molded by phylogeny it was efficient in detecting intraspecific variation within *M. thomasi*.

Although molecular (Vallejo and González-Cózatl 2012) and morphological evidence (this study) are consistent in detecting two different groups within *M. thomasi*, there were no sufficiently diagnostic characters to support the recognition of these units as valid species under the phylogenetic species concept (Cracraft 1992). Yet, in agreement with Vallejo and González- Cózatl (2012), we considered that the intermediate levels of genetic divergence along with the morphological differentiation documented here support the recognition of populations of *M. thomasi* occurring at the eastern of Sierra Madre del Sur and Sierra Mixteca, and those at western of Sierra Madre del Sur, as two different ESUs. Even though patterns of reciprocal monophyly displayed by mtDNA may be used as evidence to claim for the recognition of ESUs (Moritz 1994), it has been suggested that categorization of distinct populations as ESUs, should also include ecological evidence because genetic data may not be able to detect local adaptations that are reflected in distinct ecological requirements, morphologies, etc. (Crandall et al. 2000; Rader et al. 2005). As mentioned above, skull variation in these taxa does follow a historical pattern, as supported by the Permutation test of phylogenetic signal; however, the lack of the phylogenetic signal in the mandible may be due to local ecological adaptations or evolutionary constraints in mandible morphology. Although the second hypothesis is more parsimonious, further studies are needed to test them with empirical data. For now, our results support the categorization of the two divergent populations of *M. thomasi* as distinct ESUs.

CONCLUSIONS

Geometric morphometric analyses support the recognition of three morphotypes within the genus *Megadontomys*. These groups are distinguishable in both form and size and correspond to the species currently described. Patterns of morphometric variation support the sister phylogenetic relationship of *M. nelsoni* and *M. thomasi* proposed by others authors: skull and mandible shape is more similar between these two species than the shape of these structures between any of these groups and *M. cryophilus.* Disparity results identified the two different groups, within *M. thomasi*, that have been categorized as Evolutionary Significant Units, based on molecular data. The Permutation test of phylogenetic signal indicated that the skull may provide more reliable information on the evolution of this group than the mandible.

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Appendix I. Specimens examined are listed by taxon, collecting locality, museum acronym and voucher number. Collecting localities are preceded by a locality code (bold case) that corresponds to those in figure 1. Collection acronyms are as follows: Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos (CMC); Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA).

Megadontomys cryophilus - ***Oaxaca1*****:** Mpio. Concepción Pápalo, 14.4 Km NE Concepción Pápalo (camino a Santa Flor), 17°53'0.3444"N, 96°48'34.56"W, 2600 m (CMC 1312; CMC 1313; CMC 1314; CMC 1315). ***Oaxaca2*****:** Distrito de Ixtlán, 29 Km SW (by road) La Esperanza, 17°35'08"N, 96°30'41"W, 2950 m (CMC 89; CMC 90; CMC 92; CMC 93; CMC 98; CMC 99). ***Oaxaca2*****:** Distrito de Ixtlán, Mpio. Santiago Comaltepec, 11 Km SW La Esperanza Camino Nuevo, San Isidro 17°33'21"N, 96°26'51"W, 2000 m (CNMA 28325; CNMA 28785; CNMA 28787; CNMA 28788; CNMA 28789; CNMA 28792; CNMA 28794; CNMA 28795; CNMA 28797; CNMA 28799; CNMA 28801; CNMA 28803; CNMA 28804; CNMA 28805; CNMA 28806; CNMA 28807; CNMA 28808; CNMA 28809; CNMA 28810; CNMA 28811; CNMA 28812; CNMA 28816; CNMA 28817; CNMA 27818; CNMA 28819; CNMA 28820; CNMA 28821;CNMA 28822; CNMA 28823; CNMA 28824; CNMA 28828; CNMA 28829; CNMA 28827; CNMA 28830; CNMA 28833; CNMA 28834; CNMA 28835; CNMA 29185; CNMA 29186; CNMA 29187; CNMA 29188; CNMA 29189; CNMA 29196; CNMA 29200; CNMA 29204; CNMA 29819; CNMA 29820; CNMA 29821; CNMA 29822; CNMA 30697; CNMA 33841; CNMA 33847; CNMA 39854; CNMA 39855; CNMA 39857).

Megadontomys nelsoni - ***Hidalgo1*****:** Mpio. Agua Blanca, 5 Km ENE Crucero los Tules, camino a Xuchitl, 20°23'0.004"N, 98°21'0.884"W, 2455 m (CMC 1879; CMC 1880). ***Hidalgo2*****:** Mpio. Tenango de Doria, 21 Km NE Metepec (by road), 20°18'54.78" N, 98°14'25.02"W, 2200 m (CMC 1040; CMC 1041; CMC 1042; CMC 1043). ***Veracruz1*****:** Mpio. Acajete, 3.4 Km SW de la desviación a Mazatepec (Carretera Xalapa- Perote) Mesa de la Yerba, 19°33'33.48"N, 97°1'6.6"W, 2040 m (CMC 1316; CMC 1317). ***Veracruz2*****:** Mpio. Xico, Matlalapa, Cerro de la Cruz, 19°28'18.78"N, 97°4'42.36"W, 2070 m (CMC 1452; CMC 2119; CMC 2120; CMC 2121). ***Veracruz3*****:** Mpio. La Perla, Xometla (cañada por el puente), 18°58'39.9"N, 97°11'27.9"W 2615 m (CMC 767; CMC 768; CMC 769; CMC 770; CMC 771). ***Veracruz4*****:** Mpio. Acultzingo, 2.9 Km E Puerto del Aire (by road), 18°40'41.16"N, 97°19'36.78"W, 2440 m (CMC 766). ***Veracruz4*****:** Mpio. Acultzingo, 3.1 Km S Puerto del Aire (by road), 18°40'40.998"N, 97°21'39.6"W, 1300 m (CNMA 34249; CNMA 34250). ***Oaxaca3*****:** Distrito Teotitlán, Mpio. Santa María Teopoxco, 2.5 Km SW Plan de Guadalupe, 18°7'42"N, 96°58'19.992"W, 2300 m (CNMA 38104; CNMA 38105; CNMA 38131). ***Oaxaca4*****:** Mpio. Huautla de Jiménez, Puente de Fierro, 18°9'10.998"N, 96°51'9.993"W, 1200 m (CNMA 35329). ***Oaxaca5*****:** Mpio. Teotitlán de Flores Magón, Puerto de la Soledad, 18°9'56"N, 96°59'54.024"W, 2350 m (CNMA 21666). ***Oaxaca5*****:** Mpio. Teotitlán de Flores Magón, 1.5 Km S Puerto de la Soledad, 18°9'6"N, 96°59'54"W, 2600 m (CNMA 33848;CNMA 33849; CNMA 33850; CNMA 33851; CNMA 33852; CNMA 38310; CNMA 38741; CNMA 38742; CNMA 38746; CNMA 39309; CNMA 39572; CNMA 39573; CNMA 39574; CNMA 39743; CNMA 39859; CNMA 39860; CNMA 39861; CNMA 39862; CNMA 39863; CNMA 39864; CNMA 39865; CNMA 39866; CNMA 39867; CNMA 39869; CNMA 39870; CNMA 39871; CNMA 39872; CNMA 39873).

Megadontomys thomasi - ***Guerrero1*****:** Mpio. General Heliodoro Castillo, El Iris, Tlacotepec., 17°29'6"N, 100°13'0.984"W, 2200 m (CNMA 24577; CNMA 24578). ***Guerrero2*****:** Mpio. General Heliodoro Castillo, 1.1 Km E (by road) Cruz Nueva, 17°30'48.54"N, 100°1'46.26"W, 2650 m (CMC 1406; CMC 1407). ***Guerrero3*****:** Mpio. Leonardo Bravo, 3.4 Km W (by road) Carrizal de Bravo, 17°36'1.5"N, 99°49'35.4"W, 2480 m (CMC 597; CMC 1408; CMC 1409; CMC 1410; CMC 1411). ***Guerrero4*****:** Mpio. Chilpancingo, 6.1 Km SW (by road) Omiltemi, 17°32'57"N, 99°43'15.6"W, 2490 m (CMC 430; CMC 431; CMC 433; CMC 436; CMC 438; CMC 439; CMC 440; CMC 441; CMC 445; CMC 617). Guerrero4**:** Mpio. Chilpancingo, 0.5 Km NW (by road) Omiltemi, 17°33'24"N, 99°41'7.998"W, 2200 m (CNMA 30705; CNMA 40084; CNMA 40261; CNMA 40262; CNMA 40264; CNMA 40268; CNMA 40269; CNMA 40270; CNMA 41850; CNMA 41851; CNMA 41852). ***Guerrero5*****:** Mpio. Atoyac de Álvarez, Puerto de Gallo, 17°29'360"N, 100°13'59.04"W, 2200 m (CNMA 24579). ***Guerrero6*****:** Mpio. Malinaltepec, 3 Km E El Tejocote, 17°18'17.52"N, 98°39'4.02"W,

2620 m (CMC 1536; CMC 1537; CMC 1538; CMC 1540). ***Guerrero6*:** Mpio. Malinaltepec, 4.8 Km S El Tejocote, 17°18'19.38"N, 98°40'2.16"W, 2455 m (CMC 1544; CMC 1545; CMC 1547; CMC 1548; CMC 1549). ***Oaxaca6*:** Mpio. San Isidro, 20 Km N Putla de Guerrero 17°12'27.997"N, 97°55'45"W 1800 m (CNMA 39243). ***Oaxaca7*:** Mpio. Tlaxiaco, 2 Km SE Llano de Guadalupe, 17°9'15.998"N, 97°36'29.988"W, 2850 m (CNMA 39568; CNMA 39569; CNMA 39570).

Peromyscus mexicanus - Oaxaca, Mpio. Putla Villa de Guerrero, Concepción Guerrero, 17°4'37.26"N, 97°51'55.38"W 1050 m (CMC 1625). Oaxaca: Distrito de Teotitlán, Mpio. Huautla de Jiménez, 1 Km N Huautla de Jiménez, 18°9'43"N, 96°51'9.99"W, 1300 m (CNMA 38135; CNMA 38137; CNMA 38138; CNMA 38139; CNMA 38140; CNMA 38142; CNMA 38143). Oaxaca: Distrito de Teotitlán, Mpio. Huautla de Jiménez, 2 Km N Huautla de Jiménez, 18°10'16"N, 96°51'9.99"W, 1400 m (CNMA 39647; CNMA 39648; CNMA 39649; CNMA 39650; CNMA 39653). Oaxaca: Mpio. Santa María Pápalo, 3 Km N Santa María Pápalo, 17°52'14.995"N, 96°44'35.016"W, 2200 m (CNMA 39646). Oaxaca: Mpio. Santa María Chilchotla, 6 Km Carretera Puente de Fierro- Santa María Chilchotla, 18°11'38"N, 96°50'20.994"W, 1000 m (CNMA 38327; CNMA 38328; CNMA 38329; CNMA 38330; CNMA 38331; CNMA 38332; CNMA 38333).