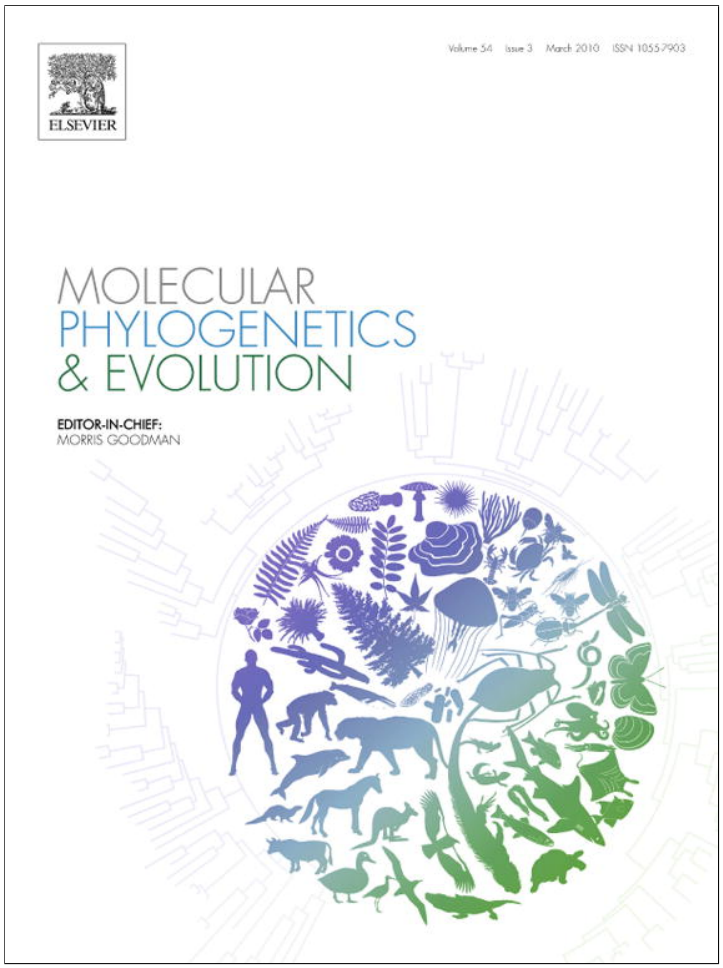

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Phylogenetic structure of the *Thomomys bottae–umbrinus* complex in North America

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ABSTRACT

The phylogeography of the *Thomomys bottae–umbrinus* complex in the United States and Mexico was assessed with sequences of the mitochondrial cytochrome *b* gene. These sequences were obtained from 225 individuals representing 108 locations over the range, including 56 sequences from GenBank. 110 (500 bp) sequences were used for Bayesian inference and neighbor-joining analyses, and 34 (1140 bp) specimens from the main clades obtained from the Bayesian inference were used in maximum-parsimony and maximum-likelihood analyses. The different analyses indicate significant variation within the species complex that averages 13% among major groups of genetic differences among *Thomomys bottae–umbrinus*. The overall pattern of geographic variation is not concordant with the current taxonomy. To the contrary, eight monophyletic groups are supported by all analyses and can be considered phylogenetic species. Overall divergence among these groups appears influenced by historical biogeographic events active during the Pliocene and Pleistocene.

1. Introduction

Pocket gopher *Thomomys bottae–Thomomys umbrinus* complex exhibit numerous morphotypes associated with distinct ecological conditions (Davis, 1938; Hadly, 1997; Smith and Patton, 1984) ranging from desert to alpine environments (Patton and Brylsky, 1987). Pocket gophers (Geomyidae) typically exhibit strong genetic structure among populations (Steinberg and Patton, 2000), which contributes to their evolutionary and taxonomic diversity. For the *umbrinus–bottae* complex, this diversity is extremely high (e.g., 213 subspecies recognized by Hall (1981)), led to numerous taxonomy evaluations (Anderson, 1966, 1972; Hall, 1981; Hall and Kelson, 1959; Hoffmeister, 1969, 1986; Patton, 1973, 1993; Patton and Dingman, 1968; Patton and Smith, 1981; Thaeler, 1980).

The entire *bottae–umbrinus* complex ranges from southern Oregon in the United States to Veracruz in Mexico, and from the Pacific Ocean to the Rocky Mountains in western North America. Originally, *T. baileyi* and *T. townsendii* were considered different species from *T. umbrinus* (Hall, 1981). Later, on the basis of limited hybridization (Patton, 1973; Patton et al., 1972) and differences in chromosomal fundamental chromosome (Patton and Dingman, 1968), *T. bottae* was considered distinct from *T. umbrinus*.

Much is known about the genetic architecture of pocket gopher populations. This knowledge is based on numerous studies of morphological and allozymic variation, including detailed geographic surveys of local populations (e.g., Álvarez-Castañeda and Patton, 2004; Daly and Patton, 1986; Patton and Feder, 1981; Patton and Smith, 1990; Wickliffe et al., 2005). Genetic structure, as revealed by mitochondrial DNA sequences (matrilineally inherited), is particularly evident in pocket gophers, because female pocket gophers are strongly phylopatric (Daly and Patton, 1986). Under such conditions, mitochondrial sequences exhibit more detailed levels of geographic structure than nuclear genes.

A number of cytogenetic, protein electrophoretic, and DNA analyses have drawn attention to the presence of several geographic groups in the *Thomomys bottae–umbrinus* complex. This study is designed to address the current taxonomy within the *T. bottae–T. umbrinus* complex by the inclusion of populations throughout the range of the complex and the incorporation of an extensive amount of nucleotide sequence data.

2. Materials and methods

2.1. Geographical sampling

A total of 225 specimens from 110 named subspecies (110 localities) from the *Thomomys bottae–umbrinus* complex are either deposited in the Centro de Investigaciones Biológicas del Noroeste (CIB) or Museum of Vertebrate Zoology, University of California (MVZ). These specimens are distributed throughout the range of the complex (Fig. 1; Table 1). The specimens that were examined were representative samples of the populations from all the range of *Thomomys bottae–umbrinus.*

2.2. Laboratory and sequence protocols

Genomic DNA was extracted from liver tissue originally preserved in 95% ethanol or frozen and maintained in the laboratory

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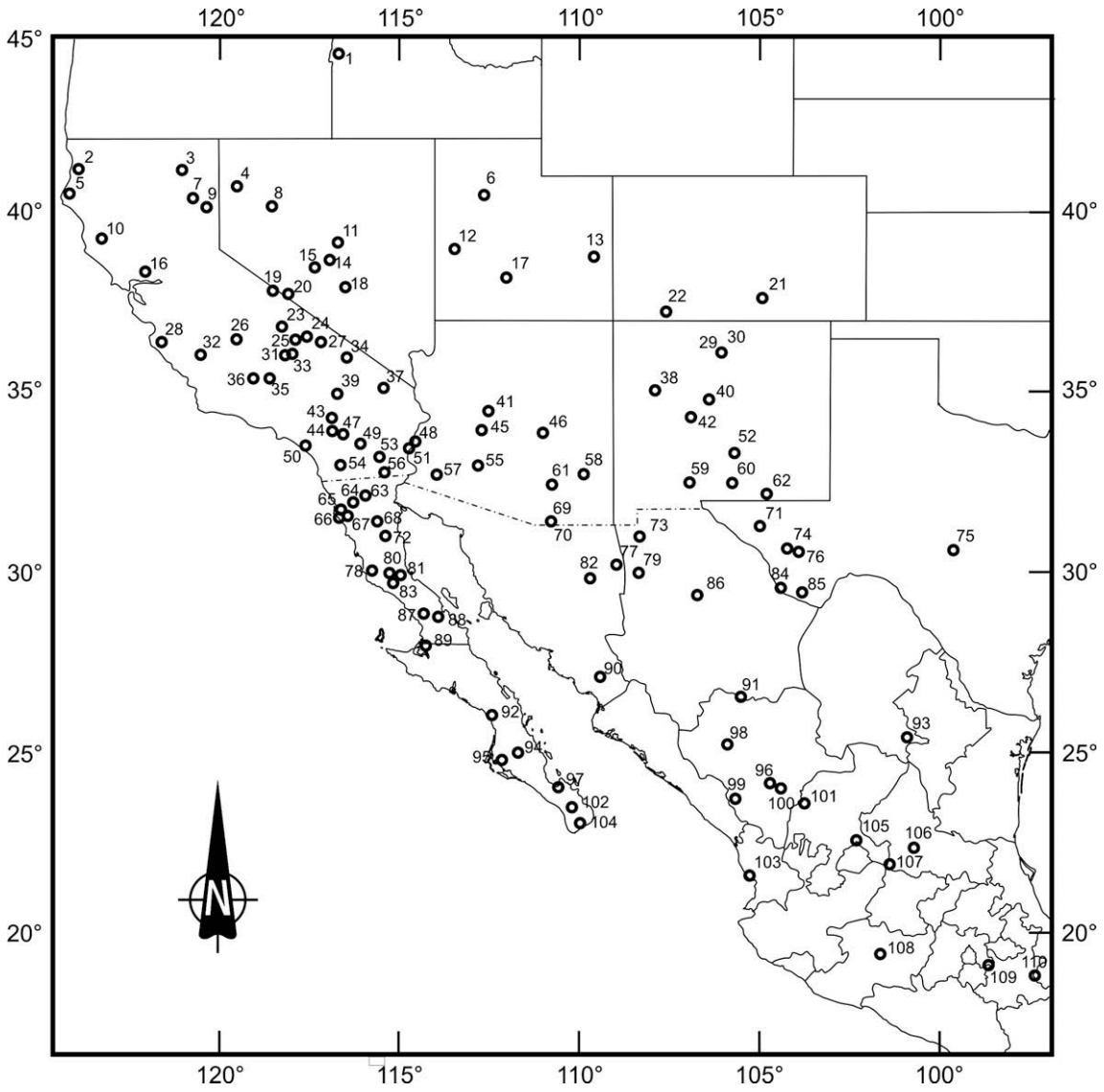

Fig. 1. Location of specimens examined for sequencing of *Thomomys*. The numbers of locality, latitude and longitude, catalog number of the specimen, and museum in which the specimens are housed are in Table 1.

at −80 °C using the DNeasy kit (QIAGEN Inc., Valencia, California). The chelex method was used to extract DNA from dried skins of one population. Samples were placed in 5% chelex, incubated at 55 °C for 20 min, and boiled for 10 min. Two phases were implemented. First, 500 bp were obtained for 110 individuals, and second, 1140 bp were obtained for 37 individuals. All PCR was performed with the primer pairs MVZ05/MVZ16, MVZ69/MVZ16, MZV127/MVZ14 (primer sequences given in Smith, 1998). The following conditions for initial double-strand amplifications were used: 12.5 μl of template (10 ng), 4.4 μl of ddH_2O, 2.5 μl of each primer (10 μM concentration), 0.474 μl (0.4 nM) dNTPs, 0.5 μl (3 μM) $MgCl_2$, 0.125 μl of (5 U/μl) Taq polymerase (platinum, invitrogen, Carlsbad, California), and 1× Taq buffer to a final volume of 25 μl. Amplification conditions consisted of 3 min of initial denaturation at 94 °C followed by 37 cycles of denaturation at 94 °C for 45 s, 1-min annealing at 50 °C, and 1-min extension at 72 °C. Amplicons were purified using the QIAquick PCR purification kit (Qiagen, Inc., Valencia, California), and primers MVZ05, MVZ69 or MVZ127 were used with Big Dye terminator chemistry (Applied Biosystems Inc., Foster City, California) to sequence all amplicons on an ABI 377 automated sequencer. Representative haplotypes of each main branch of the phylogenetic analyses generated from this study have been deposited in GenBank (Accession Numbers in Table 1).

Nucleotide sequences were aligned using Sequencher ver. 3.1 software (Gene Codes Corp., Ann Arbor, Michigan), checked by eye, and translated into amino acids for confirmation of alignment.

2.3. Phylogenetic analyses

Two separate analyses were conducted. One based on a 500 bp fragment from 110 specimens (sequences beginning at base pair 81 in relation to the start codon, Table 1) to improve as much as possible the phylogenetic resolution of the largest number of populations. A second analysis was undertaken based on 1140 bp from 34 individuals representing the major lineages identified in trees derived from the shorter sequences.

Previously published sequences were obtained from GenBank (Álvarez-Castañeda and Patton, 2004; Patton and Smith, 1990; Smith, 1998; Wickliffe et al., 2005). The catalog number, locality, GenBank Accession Numbers, and geographic locations of the specimens used in this study are in Table 1.

Table 1
List of specimens used in the study (each specimen has a unique haplotype). The localities are north–south. Inside each locality are the subspecies recognized for that population, the official acronym of the states in USA and Mexico represent the locality, latitude and longitude, catalog number of the specimen, museum in which the specimens are housed, and the GenBank is the Accession Number of the Cyt *b* sequence. Pop, population, Cl, group, Cat No, number of catalog, Mus, museum. The museum acronyms are: Centro de Investigaciones Biológicas del Noroeste (CIB), Museum of Vertebrate Zoology, University of California Berkeley (MVZ); Texas Tech University (TTU).

Pop	Cl	State	Locality	Lat	Long	Cat No.	Mus	Subspecies	GenBank
1	SR	ID	Weiser	44.2601	−116.9564	163685	MVZ	*t. townsendii*	U65282
2	NC	CA	Coyote Peak	41.1585	−123.8504	160661	MVZ	*silvifugus*	EU240734
3	NC	CA	Adin	41.1573	−120.9906	160751	MVZ	*leucodon*	U65248
4	PG	NV	Deep Hole	40.7192	−119.4822	175674	MVZ	*canus*	U65265
5	NC	CA	Rio Dell	40.4993	−124.1043	160618	MVZ	*laticeps*	U65247
6	PG	UT	South Willow Creek	40.4832	−112.5958	148825	MVZ	*stansburyi*	EU240765
7	NC	CA	Susanville	40.3788	−120.7023	160759	MVZ	*saxatilis*	U65250
8	PG	NV	Lovelock	40.1431	−118.4903	136074	MVZ	*t. bachmani*	U65281
9	PG	CA	Milford	40.1303	−120.3180	175659	MVZ	*t. relictus*	U65280
10	NC	CA	Redwood Valley	39.2640	−123.2057	160579	MVZ	*acrirostratus*	EU240735
11	PG	NV	Monitor Valley	39.1544	−116.6936	163235	MVZ	*concisor*	U65264
12	SR	CA	Skull Rock Pass	38.9625	−113.4180	179591	MVZ	*centralis*	U65266
13	SW	UT	Moab	38.7309	−109.5339	150383	MVZ	*osgoodi*	EU240737
14	PG	NV	Meadow Creek	38.6753	−116.9059	163307	MVZ	*vescus*	EU240749
15	PG	NV	Big Smoky Valley	38.4608	−117.3028	163290	MVZ	*curtatus*	EU240750
16	NC	CA	Lagoon Valley	38.3372	−122.0176	160299	MVZ	*agricolaris*	EU240736
17	PG	UT	Kingston	38.1615	−112.0235	150406	MVZ	*lenis*	EU240751
18	SR	NV	Kawich Mts.	37.9157	−116.4742	144245	MVZ	*brevidens*	EU240752
19	PG	CA	Benton	37.8185	−118.4761	166383	MVZ	*amargosae*	EU240753
20	PG	NV	Fish Lake Valley	37.7207	−118.0416	163317	MVZ	*lacrymalis*	EU240754
21	SW	CO	Walsenburg	37.5898	−104.8716	150313	MVZ	*internatus*	EU240738
22	PG	CO	Bayfield	37.2256	−107.5975	150358	MVZ	*aureus*	EU240755
23	PG	CA	Independence	36.8252	−118.2176	165118	MVZ	*melanotis*	U65263
24	PG	CA	Jackass Spring	36.5425	−117.5184	166344	MVZ	*argusensis*	EU240756
25	PG	CA	Keeler	36.4597	−117.8445	175631	MVZ	*operarius*	U65262
26	PG	CA	Kingsburg	36.4423	−119.4800	162868	MVZ	*pascalis*	U65255
27	PG	CA	Harrisburg Flats	36.3951	−117.1354	166334	MVZ	*scapterus*	EU240757
28	PG	CA	Hastings Res.	36.3809	−121.5617	166821	MVZ	*bottae*	U65253
29	SW	NM	Des Moines	36.0889	−106.0531	150298	MVZ	*cultellus*	U64980
30	SW	NM	Alcalde	36.0889	−106.0531	150272	MVZ	*pervagus*	U64979
31	PG	CA	Coso junction	36.0444	−117.9502	175637	MVZ	*perpes*	U65256
32	PG	CA	Parkfield	36.0379	−120.4722	156196	MVZ	*angularis*	EU240758
33	PG	CA	Fork Kern River	36.0241	−118.1313	164670	MVZ	*alpinus*	EU240759
34	PG	CA	Salsberry Pass	35.9260	−116.4265	166293	MVZ	*oreoecus*	EU240760
35	BC	CA	Walker Basin	35.3800	−118.5500	164137	MVZ	*piutensis*	EU240776
36	SR	CA	Bakersfield	35.3648	−119.0177	146970	MVZ	*ingens*	EU240761
37	PG	CA	Gold Valley	35.0914	−115.4003	155968	MVZ	*providentialis*	EU240762
38	SW	NM	San Rafael	35.0507	−107.8683	158511	MVZ	*morulus*	EU240739
39	PG	CA	Harvard	34.9366	−116.6639	175587	MVZ	*mohavensis*	U65261
40	SW	NM	Tajique	34.7916	−106.3765		MVZ	*actuosus*	U64970
41	SW	AZ	Bradshaw Mts	34.4547	−112.4564	146880	MVZ	*fulvus*	U65269
42	SW	CA	La Joya	34.3180	−106.8661	158634	MVZ	*connectens*	U65270
43	BC	CA	San Bernardino Mts.	34.2739	−116.8133	165053	MVZ	*altivallis*	EU240777
44	BC	CA	Cabazon	33.9151	−116.8046	166264	MVZ	*cabezonae*	EU240778
45	SW	AZ	Wickenberg	33.9141	−112.6726	156002	MVZ	*patulus*	EU240740
46	SW	AZ	Sierra Ancha	33.8322	−110.9701	147002	MVZ	*mutabilis*	EU240741
47	BC	CA	Palm Springs	33.8284	−116.5334	166255	MVZ	*perpallidus*	EU240779
48	PG	AZ	Ehrenberg	33.6042	−114.5244	154192	MVZ	*chrysonotus*	EU240763
49	SW	CA	Mecca	33.5728	−116.0668	156074	MVZ	*boregoensis*	EU240742
50	BC	CA	San Juan Capistrano	33.5143	−117.5688	164070	MVZ	*pallescens*	EU240780
51	PG	CA	Ripley	33.4403	−114.6557	148289	MVZ	*riparius*	EU240764
52	SW	NM	Otero	33.3317	−105.6725	TK49858	TTU	*ruidosae*	AF445062
53	SW	CA	Niland	33.1842	−115.5175	156105	MVZ	*crassus*	EU240743
54	BC	CA	Julian	32.9819	−116.5976	164091	MVZ	*nigricans*	U65257
55	SW	CA	Gila Bend	32.9534	−112.7813	156025	MVZ	*cervinus*	U65267
56	SW	CA	Holtville	32.7835	−115.3796	156116	MVZ	*albatus*	U65260
57	SW	AZ	Tacna	32.7111	−113.9532	156062	MVZ	*phasma*	EU240744
58	SW	CA	Graham Mts.	32.6666	−109.8754	146961	MVZ	*grahamensi*	U65268
59	SW	NM	Radium Springs	32.4805	−106.9162	150202	MVZ	*opulentus*	U64981
60	SW	TX	Otero	32.4801	−105.7476	TK51802	TTU	*tularosae*	AF445053
61	SW	AZ	Santa Catalina Mts.	32.4301	−110.7368	146822	MVZ	*catalinae*	EU240745
62	SW	NM	Whites City	32.1940	−104.7731		MVZ	*guadalupensis*	U64978
63	BC	BC	Juárez	32.1100	−115.9258	8260	CIB	*juarezensis*	EU240781
64	BC	BC	Ojos Negros	31.9333	−116.2500	8271	CIB	*jojobae*	EU240782
65	BC	BC	Punta Banda	31.7167	−116.5667	8274	CIB	*sanctidiegi*	EU240783
66	BC	BC	San Isidro	31.5672	−116.4290	8277	CIB	*aphrastus*	EU240784
67	BC	BC	Santo Tómas	31.5543	−116.6081	8298	CIB	*proximarinus*	EU240785
68	BC	BC	Trinidad	31.4000	−115.5700	153689	MVZ	*xerophilus*	U65275
69	SW	AZ	Patagonia Mts.	31.3870	−110.7545	184977	MVZ	*modicus*	EU240786
70	MX	AZ	Patagonia Mts	31.3869	−110.7437	148306	MVZ	*intermedius*	U65283
71	SW	TX	Culberson;	31.2763	−104.9102	TK54190	TTU	*scotophilus*	AF445055

(*continued on next page*)

Table 1 (*continued*)

Pop	Cl	State	Locality	Lat	Long	Cat No.	Mus	Subspecies	GenBank
72	BC	BC	Mártir	31.0101	−115.3406	8303	CIB	*martirensis*	EU240787
73	SW	CHIH	Janos	30.9669	−108.1450	150183	MVZ	*toltecus*	EU240746
74	SW	TX	Jeff Davis	30.6448	−104.1627	TK84860	TTU	*texensis*	AF445060
75	SW	TX	London	30.6331	−99.6096	TK26996	TTU	*confinalis*	AF445048
76	SW	TX	Wild Rose Pass	30.5881	−103.8942	TK75201	TTU	*limpiae*	AF445058
77	SW	SON	Huachinera	30.2189	−108.9497	146861	MVZ	*divergens*	EU240747
78	BC	BC	Rosario	30.0833	−115.6833	8319	CIB	*abbotti*	EU240788
79	MX	BC	Colonia García	30.0030	−108.3206	150606	MVZ	*madrensis*	U65284
80	BC	BC	San Fernando	29.9694	−115.2343	8322	CIB	*brazierhowelli*	EU240789
81	BC	BC	Catavina	29.9193	−114.9417	8337	CIB	*catavinensis*	EU240790
82	MX	SON	Moctezuma	29.8014	−109.6883	147093	MVZ	*sonoriensis*	EU240772
83	BC	BC	Catarina	29.7000	−115.1333	8332	CIB	*ruricola*	EU240791
84	SW	TX	Big Bend Ranch	29.5606	−104.3717	TK46425	TTU	*pervarius*	AF445052
85	SW	TX	Brewster	29.4439	−103.7814	TK54879	TTU	*limitaris*	AF445057
86	MX	BC	Canon Santa Clara	29.3669	−106.5722	147083	MVZ	*juntae*	EU240773
87	BC	BC	El Rosarito	28.8377	−114.1022	153706	MVZ	*cactophilus*	U65276
88	BC	BC	San Borja	28.7385	−113.7516	7719	CIB	*borjasensis*	EU240792
89	BC	BCS	Guerrero Negro	27.9532	−114.0567	7721	CIB	*russeolus*	EU240793
90	SW	SON	Navojoa	27.1136	−109.4439	146813	MVZ	*camoae*	EU240748
91	MX	DGO	Las Nieves	26.5377	−105.4925	150465	MVZ	*nelsoni*	EU240774
92	BC	BCS	Los Laureles	26.0512	−112.1210	7680	CIB	*incomptus*	AY589039
93	SW	COAH	Bela Unión	25.4406	−100.8171	158017	MVZ	*analogus*	U65273
94	BC	BCS	Cd. Constitución	25.0356	−111.7169	153727	MVZ	*magdalenae*	U65278
95	BC	BCS	San Carlos	24.7916	−112.1113	6159	CIB	*litoris*	AY589036
96	MX	BCS	Morcillo	24.1479	−104.7088	150454	MVZ	*durangi*	EU240775
97	BC	BCS	La Paz	24.1418	−110.4339	6665	CIB	*imitabilis*	AY589017
98	MM	Dgo	Buenos Aires	23.7048	−104.2794	12542	CIB	*chihuahuae*	EU240794
99	MM	Dgo	La Ciudad	23.7322	−105.6760	150425	MVZ	*chihuahuae*	U65289
100	MM	Dgo	Durango	23.7048	−104.2794	12543	CIB	*chihuahuae*	EU240795
101	MX	ZAC	Sombrerete	23.6166	−103.7302	153746	MVZ	*crassidens*	EU240766
102	BC	BCS	La Laguna	23.5394	−109.9713	6516	CIB	*alticolus*	AY589022
103a	MP	Nay	San Blas	21.5884	−104.8298	12548	CIB	*atrovarius*	EU240796
103b	MP	Nay	San Blas	21.5884	−104.8298	12552	CIB	*atrovarius*	EU240797
104	BC	BCS	Santa Anita	23.1748	−109.7177	6220	CIB	*anitae*	AY589021
105	MX	ZAC	Ojocaliente	22.5971	−102.2514	153778	MVZ	*zacatecae*	EU240767
106	MX	SLP	Ventura	22.3397	−100.8045	153792	MVZ	*potosinus*	EU240768
107	MX	SLP	Arriaga	21.9192	−101.3743	153810	MVZ	*arriagensis*	EU240769
108	MX	MICH	Pátzcuaro	19.4212	−101.6094	153825	MVZ	*pullus*	EU240770
109	MX	MEX	Amecameca	19.0775	−98.6314	153851	MVZ	*vulcanius*	EU240771
110	MX	PUE	Esperanza	18.8303	−97.3289	153877	MVZ	*umbrinus*	U65286
Outgroups								*Thomomys monticolus*	AF215813
Outgroups								*Geomys personatus*	AY393959
Outgroups								*Geomys texensis*	AY393965
Outgroups								*Cratogeomys fulvescens*	AY649459
Outgroups								*Cratogeomys merriami*	AY649466

Non-redundant haplotypes were identified using the Collapse software (ver. 1.1, Posada, 2004, available from http://darwin.uvigo.es). The General Time Reversible model with a fraction of invariable sites and gamma-distributed among-site rate variation (GTR+I+G; Tavaré, 1985) was shown to be the most appropriate for this dataset using the model comparison software MrModeltest ver. 2 (Nylander, 2004) under the Akaike Information Criterion (AIC). A Bayesian analysis was performed using MrBayes ver. 3.1.1 software (Ronquist and Huelsenbeck, 2003). Four independent runs were performed with Markov chain Monte Carlo simulations starting from a random tree. Each run was conducted with 5 million generations and sampled at intervals of 1000 generations. The first 5000 trees (10% burn-in) were discarded as a conservative measure to avoid the possibility of including random, suboptimal trees. The remaining sampled trees were analyzed to find the posterior probability of clades. A consensus tree was generated with the 50% majority-rule algorithm in PAUP 4.0b10 (Swofford, 2001), and the percentage of samples recovered in a particular clade was assumed to be that clade's posterior probability.

Genetic distances were calculated using the General Time Reversible (GTR+I+G, Tavaré, 1985) as the best-fit model of nucleotide substitution and the Kimura 2 parameter model. The latter is the most commonly used model for comparing levels of divergence among studies (Baker and Bradley, 2006). A neighbor-joining analysis was conducted in PAUP ver. 4.0b10 (Swofford, 2001). Support for nodes was assessed with bootstrap analyses, including a fast heuristic procedure with 1000 pseudo-replicates. Sequences from *Cratogeomys merriami* (AY649466), *Cratogeomys fulvescens* (AY649459), *Geomys personatus* (AY393959), *Geomys texensis* (AY393965), and *Thomomys monticola* (AF215813) were used as outgroups. The outgroup specimens were chosen following in part the study of Wickliffe et al. (2005). Only *T. monticola* was used as outgroup in the Bayesian inference because MrBayes ver. 3.1.1 software (Ronquist and Huelsenbeck, 2003) only accepted one species as outgroup, and the maximum-likelihood shows that *T. monticola* could be considered as the sister species of the *bottae–umbrinus* complex.

Maximum-parsimony (MP) and maximum-likelihood (ML) analyses were implemented in PAUP ver. 4.0b10 (Swofford, 2001). For maximum-parsimony analysis, all characters were equally weighted, and heuristic searches were performed with 1000 random additions of sequences, and tree-bisection reconnection (TBR) algorithm for branch swapping. For all analyses that resulted in multiple most parsimonious trees, consensus trees were constructed using the 50% majority rule. The GTR+I+G model was

then used for maximum-likelihood searches consisting of 100 random replicates with TBR branch swapping. Bootstrap values ⩾50% are reported for branch support.

3. Results

3.1. Sequence variation

There were 498 variable positions in the 1140-bp dataset (418 were phylogenetically informative), which defined 37 haplotypes. In the 500-bp dataset, there were 215 variable positions and 195 were phylogenetically informative.

3.2. Phylogenetic analyses

The most appropriate model of evolution found for the short and long fragments of Cyt *b* with software MrModeltest ver. 2 (Nylander, 2004) was the GTR+I+G. The model parameters for the long fragments were: I = 0.53 and G = 1.52, $-\ln L$ = 10,946.05, k = 9, AIC = 21910. Base frequencies were A = 0.344, C = 0.275, G = 0.087, T = 0.293 and relative substitution rates were A–C = 0.97, A–G = 11.47, A–T = 0.91, C–G = 0.17, C–T = 11.47, G–T = 1.0.

The Bayesian inference for the short fragments of pocket gophers shows eight monophyletic groups, all deeply divergent and strongly supported (Fig. 2). The neighbor-joining, maximum-parsimony and maximum-likelihood analyses produced a similar topology (not shown) to that obtained under Bayesian inference. The average of genetic distances (K2P) among the eight monophyletic groups of *Thomomys* were between 11.1% and 19.5% (Table 2).

The maximum-parsimony analysis of long sequences recovered one tree (CI = 0.33, RI = 0.63, length = 2,290 steps; not shown). The maximum-likelihood and Bayesian analyses of the long fragment supported the same eight monophyletic groups (Fig. 3) recovered by short-fragment analyses. The average percentage sequence distance (K2P) in the Mexico (MX) clade (10.3%) almost doubled those within the Southwest (SW, 5.6%), Peninsula of Baja California (BC, 3.8%), North of California (NC, 3.9%), Snake River (SR 3.87%) and Pacific group (PG, 5.8%) clades. The smallest average genetic distances are within the Pacific Mexico (MP, 0.4%) and Mountain Mexico (MM, 0.04%) clades (Table 2).

4. Discussion

4.1. Phylogeographic structure

All phylogenetic analyses (Bayesian inference, maximum-parsimony, maximum-likelihood and neighbor-joining) show similar topologies. I used the Bayesian analysis (Fig. 3a) as a reference for discussing relationships among clades. Additionally, I refer to each of the primary clades identified in the study as "groups".

A study of electromorphic allozyme variation of 25 polymorphic loci (Patton and Smith, 1990) showed that the *T. bottae–umbrinus* gophers of the United States and northwestern Mexico could be resolved into six main groups: Northern California (corresponding to NC in Fig. 2), Central California (corresponding to PG), Great Basin (corresponding to SW), Basin and Range (corresponding to SW), Baja California (corresponding to BC), and Sonora (corresponding to MX). Patton and Smith (1990) considered that genetic differences among these six groups are stronger than those found in separate species of many other mammal groups. These six electromorphic groups are phylogeographically structured according with the mitochondrial groups obtained from analyses performed in this study, with a few differences in the geographical borders among them (Patton and Smith, 1990). The major difference between the analyses is that the Basin and Range and Great Basin groups are distinct in the electromorphic analysis, but form one group in the mitochondrial analysis. The names assigned to these mitochondrial clades are based on their geographical range.

An analysis, based on a combination of allozymes and sequences from both mtDNA and nuclear DNA, that addressed the specific-level status of *T. townsendii* concluded that the following four species be recognized (Patton and Smith, 1994): (1) the northern California Group represented by *T.b. saxatilis* of the Patton and Smith (1994) study, equivalent to the Northern California Group of the present study; (2) the central and western Nevada (*T.b. canus* and *T.b. concisor*, including *T.t. nevadensis*), equivalent to the Pacific Group; (3) the eastern Great Basin (*T.b. latus* and *T.b. centralis*) equivalent to the Southwestern Group, and (4) the Snake River (*T. townsendii*), that considered the populations at the eastern side of the Snake River, following Fig. 1 of Patton and Smith (1994). Therefore, *T. townsendii* was considered as a different species of *T. bottae* because hybridization was essentially limited to the F_1 generation (Patton et al., 1984). *Thomomys t. townsendii* is in the same clade with *T. bottae centralis* (Figs. 3, 5 and 6, Smith, 1998), *T. townsendii* is nested in the *T. bottae* (Pacific Group of the present analysis), with the mtDNA genetic distance from *T. bottae* of 4.72% (0.80–7.43, Smith, 1998). Moreover, Smith (1998) mentions that *T. townsendii* may have had a separate origin from *T. bottae* of the nearby Great Basin, which could be supported by *T. townsendii* and *T. bottae* being in different allozymic genetic units (Patton and Smith, 1990).

The mtDNA results in the population of the continental part of Mexico (not including the Baja California Peninsula) are in relation to the chromosomal evidence and electrophoretical variation of proteins (Hafner et al., 1987), due that could be divided into three different species. In Hafner et al. (1987) related to different mtDNA groups found in the present study. The groups of the Mexican Pacific and Mexican Mountain are the only populations of the *bottae–umbrinus* complex with a karyotype of $2n$ = 76, different from all the others that have $2n$ = 78 (Hafner et al., 1987). Besides, the phenetic clustering of allozymic data (Fig. 3, Hafner et al., 1987:23) shows strong differences between the two groups. Therefore, the pocket gophers of the coast of Sinaloa are morphologically and electromorphically very different (Hafner et al., 1987:32). Hafner et al. (1987) still considered them as the same species because they do not have evidences to suggest that the two populations are reproductively incompatible. However, if I use the phylogenetic species concept (Cracraft, 1997) or the Genetic Species Concept (Bradley and Baker, 2001), those groups could be considered as different species.

The other karyotype $2n$ = 78, was divided into two different groups; the north group has the presence of microchromosomes, while in the southern group they are absent; therefore, the phonetic clustering of allozymic data supports the differences between the two groups (Hafner et al., 1987). The two karyological and allozymic groups have a strong relationship with the mtDNA group. The data of the northern group present the same subdivision as that from the Southwestern group and southward with the Mexican group.

The eight groups obtained from the analyses, where each one could be considered with the geographic structure that represents evolutionary units, and with well defined geographical boundaries along the *bottae–umbrinus* complex are:

4.1.1. North California group (NC)

This group includes the populations in the mountains north of the Central Valley of California (including the Coastal Range of California from San Francisco Bay to the north) extending to southern part of Oregon (including the Cascade Range in California and the McLoughin mountain region in Oregon) and south along the north-

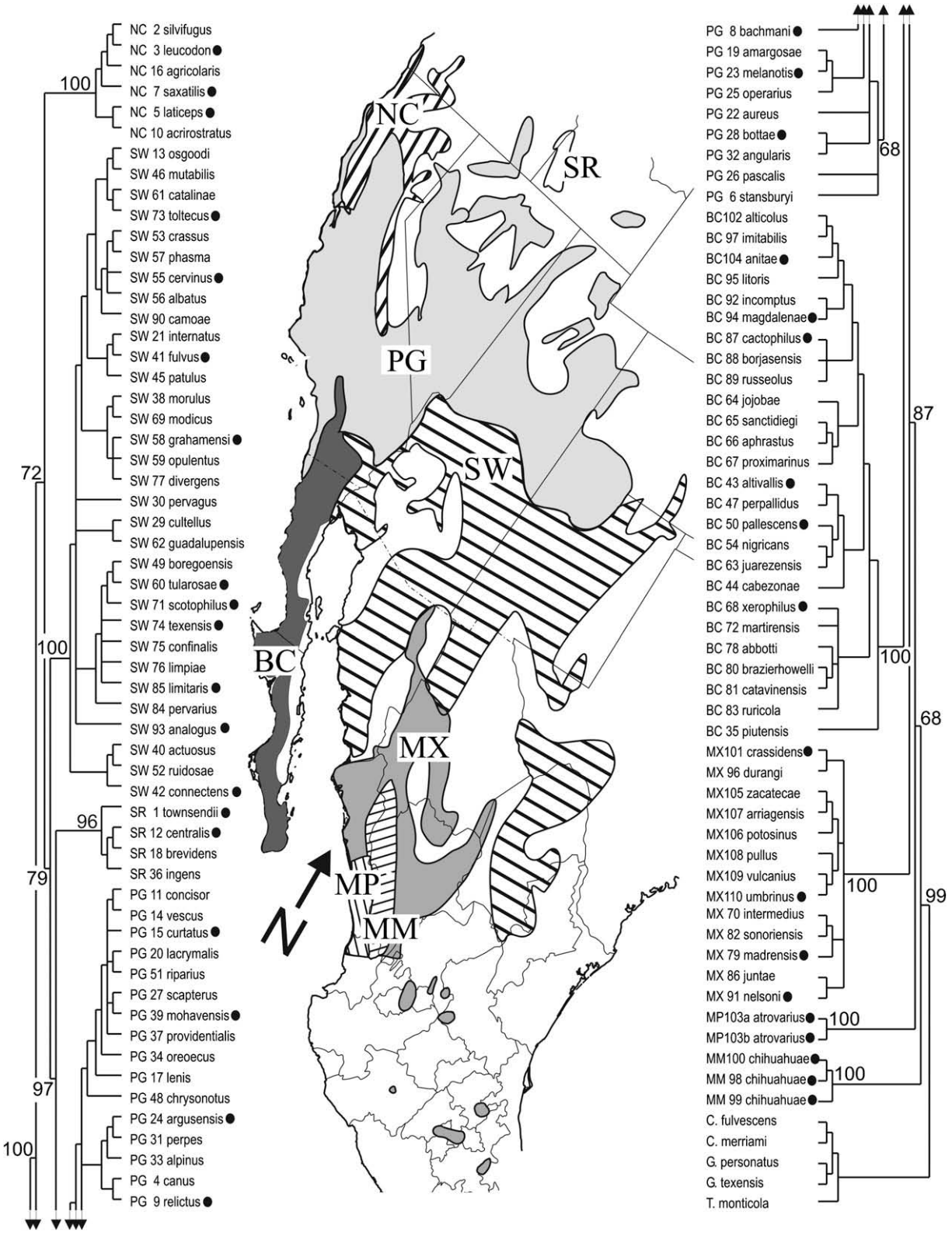

Fig. 2. Consensus of Bayesian inference from 110 specimens (500 bp) using cytochrome *b* gene sequences. The sequences represent individuals from different populations in the range of the *bottae–umbrinus* complex. This tree supports the monophyly of eight clades within the *bottae–umbrinus* complex. Each of the clades represents one geographical area: BC, Peninsula of Baja California; MM, Mountain Mexico; MX, Mexico; NC, North of California; PG, Pacific group; PM, Pacific Mexico; SR, Snake River; and SW, Southwestern. Black dots represent those specimens used in the 1140 bp analysis. The upper part of the cladogram is in the left side and the lower part is in the right side of the map. Bootstrap values are given for only the main nodes.

eastern slope of the Sierra Nevada of California. The mean percentage of genetic variation within the group is 3.94%, its average divergence from other groups of the *bottae–umbrinus* complex is 17.0% using the K2P algorithm. This group is the most geographically limited (Fig. 3) in the *bottae–umbrinus* complex and it includes only a few populations. Only two subclades were found,

Table 2
Average genetic distances (%) among (upper and lower matrices) and within (diagonal, bold) proposed species of *Thomomys* examined here. The upper-right matrix was generated under the GTR+I+G the best evolution model. Distances in the lower-left and diagonal matrix (bold) were generated using the Kimura 2 parameter model to allow comparison to traditional estimates of genetic distance in small mammals.

	GROUP	1	2	3	4	5	6	7	8	9	10
1	NC	**3.94**	16.56	16.36	17.34	20.16	17.43	18.37	19.88	25.82	17.64
2	SW	16.06	**5.64**	12.92	14.37	17.72	15.78	16.95	18.55	23.11	15.91
3	SR	15.90	12.65	**3.87**	11.33	16.97	15.94	16.23	16.93	22.70	14.39
4	PG	16.83	14.03	11.14	**5.84**	17.33	16.11	17.36	17.95	24.52	16.95
5	MX	19.53	17.23	16.57	16.97	**10.30**	18.44	17.50	17.56	25.21	17.97
6	BC	16.61	15.25	15.37	15.59	17.72	**3.84**	15.92	18.19	23.90	16.45
7	PM	17.71	16.39	15.87	16.91	17.07	15.57	**0.40**	15.99	24.81	17.14
8	MM	19.39	17.93	16.54	17.54	17.30	17.64	15.51	**0.04**	24.65	18.14
9	*monticola*	24.88	22.40	22.06	23.71	24.17	23.27	23.68	23.86		
10	Complex	17.06	15.45	14.04	16.52	17.42	15.97	16.46	17.57		

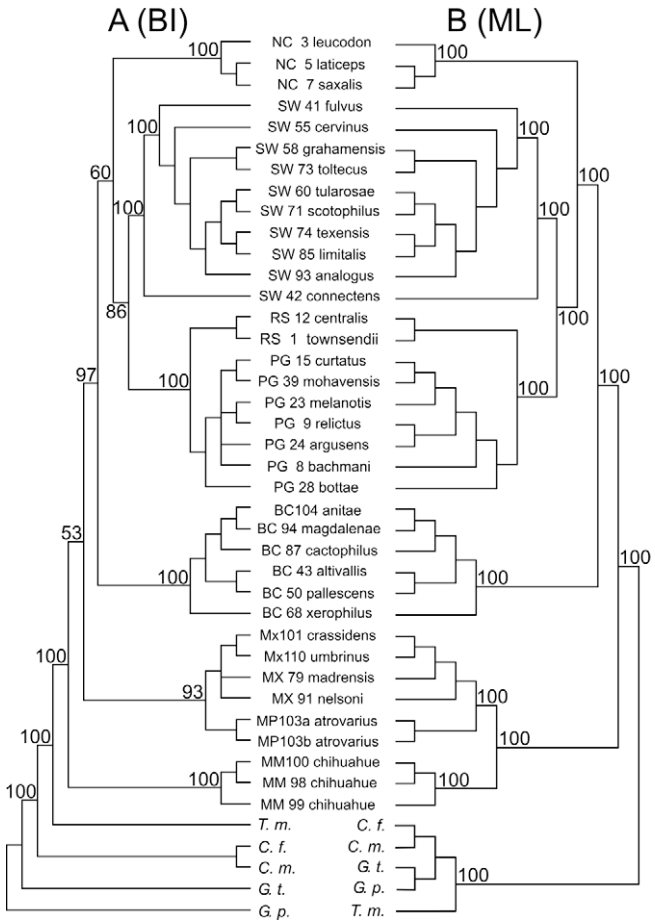

Fig. 3. Trees from 1140 bp from the cytochrome *b* gene of 34 representative individuals. These trees support eight different monophyletic groups. The model used was GTR+I+G. (A) Bayesian inference (BI). (B) Maximum-likelihood analyses (ML – Score = 10,939). At the tip of each branch, the number of locality and subspecies indicates the group following Table 1. The acronyms used for outgroup are: *Cratogeomys merriami* (C.m.), *Cratogeomys fulvescens* (C.f.), *Geomys personatus* (G.p.), *Geomys texensis* (G.t.), and *Thomomys monticola* (T.m.). Bootstrap values are given for only the main nodes.

both with strong Bayesian inference (Fig. 2). Its range includes the Coastal Range of California from San Francisco Bay to the north and throughout the southern part of Oregon, the Cascade Range in California.

4.1.2. Pacific group (PG)

It includes populations from the Central Valley of California south through the Mojave Desert and eastward north of the Colorado River to southwestern Colorado and northwestern New Mexico. The mean percentage of genetic differences within the group is 5.84%; its average divergence from other groups of *bottae–umbrinus* is 16.52% with the K2P. This clade includes some isolated populations from the northern part of the *bottae–umbrinus* complex range. The clade shows three subclades. The range is from the central valley in California to the east along the northern side of the Colorado River, with the exception of the area around the Salton Sea and the highlands of northern California. It includes the states of California, east of Idaho, Nevada, Oregon, New Mexico, Colorado and Utah in the United States.

4.1.3. Snake River Group (SR)

It includes populations from the eastern part of the Snake River in Idaho and specimens of California, Nevada and Utah. The mean percentage of genetic differences within the group is 3.87%; its average divergence from other groups of *bottae–umbrinus* is 14.04% with the K2P. The populations eastern of Snake River have been considered part of a different species, *T. townsendii* (Patton and Smith, 1994). However, the populations of California, Nevada and Utah have been considered as part of *T. bottae*.

4.1.4. Southwestern group (SW)

It includes populations in a large area of the southwestern United States and northwestern Mexico. The westernmost populations are in the Colorado Desert of California and adjacent Baja California and Sonora. In Arizona it occurs south of the Colorado River and extends south through most of Sonora west of the Sierra Madre. The range includes much of New Mexico and continues south into western Texas and Chihuahua east of the Sierra Madre Occidental. A possible disjunctive part of the range includes the Big Bend region of Texas south through much of Coahuila and reaching into Nuevo León. The mean genetic distance within the group is 5.64%; its average distance from other groups in the *bottae–umbrinus* complex is 15.45%. The clade includes three main subclades, each with its own geographic distribution. I did not find a geographical overlap among the subclades. The SW group represents an incongruity between the Cyt *b* and the electromorphic analysis (Patton and Smith, 1990). According to the electromorphic analysis (Patton and Smith, 1990), some of the populations of NW New Mexico and NE Arizona belong to the Pacific group (PG), but are part of the Southwestern group (SW) from the Cyt *b* data. I follow the Cyt *b* pattern until more detailed analyses are made. The distribution of the species includes two main ranges, one that continues from the southwestern part of the United States and northwestern Mexico and a second one in Texas, Coahuila, and Nuevo León from the Colorado River to the south and east, including the Sierra Madre Occidental and the northern part of the Sierra Madre Oriental. The second part includes the states: Arizona, SE California, New

Mexico, and Texas in the United States, and NE Baja California, Chihuahua, Coahuila, Nuevo León, and Sonora in Mexico.

4.1.5. Mexican group (MX)

It includes all the populations from north and central mainland Mexico. The mean genetic distance within the group is 10.30%; its average distance from other groups of the *bottae–umbrinus* complex is 17.42%. The clade has three main subclades that are geographically distinct. This species has a fragmentized distribution including the mountain range of the Sierra Madre Occidental, many isolated populations on the tops of high volcanoes in Central Mexico, and areas in the southwestern Mexican plateau. It includes the states of Aguascalientes, Chihuahua, Distrito Federal, Durango, Guanajuato, Jalisco, Morelos, Nayarit, Puebla, San Luis Potosí, Sinaloa, State of Mexico, Sonora, Veracruz, and Zacatecas.

Genetic distances within the Mexican group and the genetic differences among the specimens from Central Mexico, compared to those from the mountain range of Northwest Mexico and to those of south of the Mexican tableland, could indicate that more than two different groups are included under *Thomomys umbrinus.*

4.1.6. Baja California group (BC)

It includes populations from southwestern California (southern Salton Sea) south throughout most of the Baja California peninsula. The mean genetic distance within the group is 3.84%; its average distance from other groups of the *bottae–umbrinus* complex is 15.97%. The clade shows two subclades with a strong geographic pattern. The range of one subclade includes from San Bernardino Mountains to Southern California, and the range of the other subclade is the Baja California Peninsula.

4.1.7. Mexican-Pacific group (MP)

It includes populations from the Sinaloa-Nayarit costal plains. Only 2 haplotypes were found in 15 sequenced specimens. The mean genetic distance within the group is 0.4 %; its average distance to other groups in the *bottae–umbrinus* complex is 16.46%. This group only presents one clade and can be considered from only 1 population along the Sinaloa–Nayarit costal plain. Its range includes from the San Lorenzo River in Sinaloa through the costal plains continuing southward to Nayarit.

4.1.8. Mexican Mountain group (MM)

It includes populations from the western part of Durango. The mean genetic distance within the group is 0.04%; its average distance from other groups in the *bottae–umbrinus* complex is 17.57%. This group only presents one clade and can be considered from only one population along the Sierra Madre Oriental western to the city of Durango. Its range includes the highlands of the Sierra Madre Oriental from the Fuerte River Canyon in Sonora to the south, including the states of Chihuahua, Sonora, Durango and the eastern part of Sinaloa.

4.2. Haplotype diversity, genealogy, and population history

All groups defined in the phylogenetic analyses contain unique haplotypes and distributions. The Mexican (MX) group has the most genetic variation (Table 2). This is probably due to the strong isolation among populations that were range-restricted, mainly to the tops of volcanoes in central Mexico. In contrast, the Mexican Mountain group has the lowest pairwise genetic distances among populations, and is probably the most isolated population. The Southwestern and Pacific groups have similar pairwise genetic distances, similar range sizes, and continuous distributions. Thus, I suggest that similar ecological conditions can favor the genetic flow between populations, which can result in similar levels of genetic divergence.

4.3. The geography of differentiation in pocket gophers

No morphological analyses have been made to distinguish the eight monophyletic groups identified by the Cyt *b* analysis. However, the capacity of pocket gophers to exhibit great variation in their morphotypes in response to local ecological conditions could mean that any differences that exist among the major clades merely represent local differentiation among populations (Davis, 1938; Hadly, 1997; Ingles, 1950; Smith and Patton, 1984, 1988). Morphological variation has been associated with nutrition and habitat quality, so specimens from alfalfa fields are significantly larger that those specimens adjacent to agricultural fields (Patton and Brylsky, 1987; Smith and Patton, 1988). Such variation at the population level makes it impossible to determine which morphological characteristics of the eight groups might be used to differentiate clades. In some populations of Baja California, morphological and genetic variation within localities is as great as or greater than that between populations (Álvarez-Castañeda and Patton, 2004; Rios and Álvarez-Castañeda, 2007; Trujano-Álvarez and Álvarez-Castañeda, 2007).

4.4. Taxonomic implications

The species concepts applied to pocket gophers have changed over the last 150 years. In the early years each population was described as a different species (e.g., *Thomomys altivallis, T. bottae, T. townsendii, T. umbrinus*) mainly by C. H. Merriam. Merriam (Merriam, 1901) described 22 gopher taxa as different species (Poole and Schantz, 1942). Early in the 20th century, the point of view changed and mammalogists began to recognize differences among the populations at subspecific level. An example is Goldman who described 76 subspecies (Poole and Schantz, 1942) or Huey who described 25 (Bond, 1969).

Virtually all populations in the *bottae–umbrinus* complex were considered subspecies of *Thomomys umbrinus* in the first revision of Mammals of North America, except for *T. baileyi* and *T. townsendii*, which were retained as separate species (Hall and Kelson, 1959). Anderson (1966, 1972), Hoffmeister (1969, 1986), Patton and Dingman (1968), Patton and Smith (1981) and Patton (1973) demonstrated that *T. bottae* is a different species from *T. umbrinus*. Thaeler (1968), Patton et al. (1984) and Patton and Smith (1990) confirmed the specific status of *T. townsendii* and *T. bottae*. However, Hall (1981) combined all the species of the *bottae–umbrinus* complex as *T. umbrinus*. In the current taxonomy (Patton, 1993, 1999, 2005; Patton and Smith, 1990) *T. umbrinus* and *T. bottae* are recognized as different species. However, the present study shows that the *bottae–umbrinus* complex could be an assemblage of at least eight different species.

4.5. Conclusion

Following the phylogenetic species concept, congruent results of mtDNA and allozyme analyses show that the *bottae–umbrinus* complex consists of at least eight monophyletic groups with no apparent diagnostic morphological differences among them. In contrast to the usual pattern of cryptic species in mammals, wherein populations that are genetically distinct appear morphologically identical, pocket gophers display high levels of anatomical variation among populations within species. Whether such fine-scale variation in morphology is due to local adaptation or random drift, it is likely accentuated by the strong site-fidelity and consequent lack of gene flow in these burrowing rodents. In such a case, patterns of genetic differentiation are likely to be a more accurate guide to species boundaries. I therefore suggest that eight groups, each one corresponding to a monophyletic mtDNA haplogroups, be recognized in the *bottae–umbrinus* complex. To recognize these

eight groups as different species, an analysis of the nuclear DNA needs to be made. However, the specific analyses between *T. bottae* and *T. townsendii* combination of allozymes, sequences from both mtDNA, and hybridization between populations show that both species could be considered different with a percentage of 4.72% in the mtDNA.

Considering the fact that each one of the 8 groups is monophyletic, the presence of chromosomal evidence, electrophoretic variation of proteins among some of them, and a high percentage of genetic distance in the Cytochrome *b* analysis, the *bottae–umbrinus* complex may be assembled into the following eight species: *T. townsendii* eastern of the Snake River (Patton and Smith, 1994): *T. laticeps* from northern California; *T. bottae* from the rest of California, north and west of the Colorado River; *T. fulvus* from south and east of the Colorado River to the Sierra Madre Occidental in Sonora; *T. anitae* from south of the Salton Sea through the Baja California peninsula; *T. atrovarius* from the coastal lands of Sinaloa-Nayarit; *T. chihuahue* from western Chihuahua and Durango; and *T. umbrinus* from elsewhere in Mexico. The *umbrinus* group could be a complex of two species that needs to be analyzed in more detail.

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